CELL BIOLOGY, GENETICS, EVOLUTION AND ECOLOGY

(For B Sc., B. Sc. (Hons.), and M. Sc. Classes of All Indian Universities)

By

P. S. VERMA
M.Sc., Ph.D., F.E.S.I., F.A.Z.

V. K. AGARWAL
M.Sc.

Department of Zoology,
Meerut College, Meerut.

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to

Our Kids

Anurag, Prashant, Surabhi,

Sameer & Guddan

With love

P.S.V. & V.K.A.

PREFACE TO THE FOURTH EDITION

It gives us immense pleasure in releasing the thoroughly revised, improved and enlarged third edition of *Cell Biology, Genetics, Evolution and Ecology* amidst the hope that it will serve the purpose of our generous readers more adequately. Present edition exhibits two salient features—firstly each and every topic has been studded with latest available informations and certain new illustrations, and secondly, the chapters on Evolution and Ecology have been entirely written afresh keeping in view the recent advancements in these fields. During the course of revision, our basic aim and conscious efforts were that a penetrating interest and a irresistable curosity be aroused amongst the readers for having a vital interest in these modern biological fields so that they may understand and appreciate the different fantastic natural phenomena occurring at the level of

and shortcomings of variable nature in the text for that we regret in anticipation. However, it will be highly appreciated, if such errors may be brought to our notice so that we may eradicate them in the next edition.

In the end, we thank to the generous readers for taking pains in drawing our attention towards certain shortcomings in the last edition. Our thanks are also due to generous readers, friends and family members whose continuous inspirations always put us in right tune. Constructive suggestions for improvement of the book shall be highly appreciated and incorporated in the next edition.

Department of Zoology,
Meerut College, Meerut.

P. S. Verma
V. K. Agarwal

PREFACE TO THE FIRST EDITION

Recently the biologists have become crazy about the cell and they have tried to find out the possible causes of every biological phenomena, such as metabolism, growth, pathological diseases, heredity, reproduction and ecological interactions at the level of cell and molecules. This is true that cell is a vital unit of organisms and it can be considered as the anatomic and physiologic substrate of biologic phenomena. Most of the Indian Universities have recognized the importance of cell in due time and have incorporated the Cell Biology, Molecular Biology, Genetics, Evolution and Ecology in their syllabi for *under-graduate, honours and post graduate* classes. Although several excellent text books of Cell Biology, Genetics, Evolution and Ecology written by foreign authors are available in the market, but there does not exist a complete book which could give a brief account of classical and recent developments in these branches of biology. By visualizing the genuine need of such a text book authors have attempted to provide a well-illustrated, concise account of Cell Biology, Genetics, Evolution and Ecology under one cover. At every step the authors remain conscious about the needs and difficulties of students. Complicated issues have been explained by well-suited illustrations and examples.

The authors are grateful to Shri Shyam Lal Gupta and Shri T. N. Goel, M/s. S. Chand & Company Ltd., New Delhi for their whole hearted cooperation in the publication of this book. Thanks are also due to late Prof. Vimal Sharma for preparing the illustrations.

Suggestions for the improvement of the book shall be welcomed from colleagues as well as from the students.

Department of Zoology,
Meerut College, Meerut.

P. S. Verma
V. K. Agarwal

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CELL BIOLOGY

CHAPTER 1

INTRODUCTION

The cell biology or cytology (Gr., *kytos*—hollow vessel or cell, *logous*—to discourse) is a biological science which deals with the study of cells from morphological, biochemical, cytochemical, physiological, genetical and developmental point of views. It is a young biological science and it has taken its individual entity by the end of the nineteenth century.

HISTORY

The study of cell biology has been inseparable from the evolution of the microscope. The beginning of the cell biology can be visualized in the 15th century when **da Vinci** in 1483 has stressed upon the use of lenses in viewing small objects. The simple microscope was invented by **Galileo** in 1610. The first compound microscope was made by **Robert Hooke** (1665). He examined thin slices of cork under his microscope and observed that the cork was composed of box-like compartments which were termed as the cells (L., *cella* –hollow space) by him. **Antone V. Leeuwenhoek** (1723) made a simple microscope and studied the structures of bacteria, Protozoa, spermatozoa, red blood cells and nuclei of red corpuscles of salmon blood.

The 19th century was an era in which the cell was investigated extensively with the microscope. In the beginning of the 19th century it was known that all the various organs of animals were made up of tissues, such as muscle, bone, cartilage and fat and similarly, that the stems, roots, leaves and other organs of higher plants were also composed of distinct tissues. In 1824, a Frenchman, **H. J. Dutrochet** stated that "all organic tissues are actually globular cells of exceeding smallness which appear to be united only by simple adhesive forces; thus all tissues, all animal and plant organs are actually only a cellular tissue variously modified."

CELL THEORY

In 1838, a German botanist, **M. I. Schleiden** studied the plant cells and emphasized that "c[illegible]

like parts namely of cells." The deductions of the two microscopists (Schleiden and Schwann) formed the basis of what came to be known as the **cell theory**. The cell theory holds that all living matter, from the simplest of unicellular organisms to very complex higher plants and animals, is composed of cells and that each cell can act independently but functions as an integral part of the complete organism.

Objections to cell theory—The cell theory enjoyed immense success and was at once adopted by zoologists and botanists. It explained most of the biological enigmas of those days, but it was not free from errors. **Schwann** for instance believed that cell could be spontaneously generated by a process analogous to crystal formation. But studies of developing embryos showed that during growth, cells duplicated themselves by cell division. These observations were summarized by **Rudolf Virchow** in 1858 and he stated "where a cell exists there must have been a pre-existing cell", [illegible] from an animal and the plant only fr[illegible] thirty years, many cytologists such as [illegible], **Mohl, Max Schultze, Ranvier,** and **Nageli,** corrected various other errors of cell theory.

Exceptions to cell theory—Various cytological investigations have shown that all living organisms are not cellular as considered by the cell theory and some organisms are without any true cell or acellular. A true cell has been defined as a mass of protoplasm having a distinct nucleus and limited by plasma membrane; but, most viruses have no protoplasm and nucleus but only DNA or RNA as the [illegible] material. The bacteria and blue green algae also have no true [illegible] the [illegible]ism. Moreover certain algae such as *Vaucheria* and certain fungi, such as [illegible] because their [illegible]lasm in which many nuclei remain scattered. These examples form the exceptions to cell theory.

PROTOPLASM THEORY

In the middle of 19th century, various biologists started to recognize the importance of 'jucy' or 'slimy' contents of the cells. **Pur**[illegible] (1839) [illegible] protoplasm (Gr. *protos*-primitive or first [illegible] wig[illegible] by [illegible] at all living matter, out of which animals and plants are formed, is

protoplasm. According to this theory, the cell is an accumulation of living substance or protoplasm which is limited in space by an outer membrane and possess a nucleus.

In 19th century many improvements have been made in light microscopy and various cellular components have been observed. In 1867 G[illegible] In 1871 Miescher discovered nuclei[illegible] an ovum[illegible] the spliti[illegible] cell divis[illegible] a correlation between nucleic acid and chromatin. The cell division [illegible] and the term cyto- [illegible] n 1883 Metchnikoff The term chromosome was introduced by Waldeyer in 1888. Benda (1898) named the mitochondria. In 1898 Golgi described the Golgi complex as an internal reticular apparatus. Because in the last quarter of nineteenth century many important investigations have been made in cell biology, therefore, that period generally considered as "classical period" of cytology.

In the 20th century various modern microtechniques have been employed in cytological investigations. For instance, new histochemical and cytochemical methods have been worked out, various cellular [illegible] gists to investigate minute details of cell and its components. Due to th[illegible] f cc[illegible] cc[illegible] placed by another new theory called organismal theory.

ORGANISMAL THEORY

The organismal theory holds that the body of all [illegible] protoplasm into subordinate, semi-independent compartments, the so called cells. Further, this theory holds that the ontogenetic embryo-

logical development of a multicellular individual includes only growth and progressive internal differentiation of a small single protoplasmic individual (egg). But the organismal theory also fails in ascertaining the position of viruses, certain algae and fungi.

CELL BIOLOGY AND OTHER BIOLOGICAL SCIENCES

The cell biology has helped the biologists to understand various complicated life-activities such as metabolism, growth, differentiation, heredity and evolution at the cellular and molecular levels. Due to its wide application in various branches of biological science, many new hybrid biological sciences, have sprung up. Some of them are as follows :

1. Cytotaxonomy (Cytology and Taxonomy)

Each plant and animal species has a definite number of chromosomes in its cells and the chromosomes of the individuals of a species resemble closely with one another in shape and size. These characteristics of the chromosomes help a taxonomist in determining the taxonomical position of a species. Further, cell biology furnishes strong support to the manner of origin of certain taxonomic units. Therefore, the cytotaxonomy can be defined as a cytological science which provides cytological support to the taxonomic position of any species.

2. Cytogenetics (Cytology and Genetics)

Cytogenetics is that branch of cell biology which is concerned with the cytological and molecular bases of heredity, variation, mutation, phylogeny, morphogenesis and evolution of organisms. The Weismann's germ plasm theory, Mendel's laws of inheritance and the concept of gene could be well understood only after the application of cytological concept to the genetics.

3. Cell physiology (Cytology and Physiology)

The cell physiology is the study of life activities, *viz.*, nutrition, metabolism, excitability, growth, reproduction or cell division and differentiation of the cell. The cell physiology has helped in understanding various complicated physiological activities at cellular level.

4. Cytochemistry (Cytology and Biochemistry)

The cytochemistry is that branch of cytology which deals with the chemical and physico-chemical analysis of living matter. For example, the cytochemical analysis has revealed the presence of carbohydrates, lipids, proteins, nucleic acids and other organic and inorganic chemical compounds in the cells.

5. Ultrastructure and Molecular biology

These are the most modern branches of biology in which the merging of cytology with biochemistry, physico-chemistry and especially macromolecular and colloidal chemistry becomes increasingly complex. Knowledge of the submicroscopic organization or ultrastructure of the cell is of fundamental importance because practically all the functional and physico-chemical transformations take place with the molecular architecture of the cell and at a molecular level. The recent discoveries in molecular biology such as the discovery of molecular model of DNA by Watson and Crick in 1953, molecular interpretation of protein synthetic [illegible] an extraordinary impact on moder [illegible]

6. Cytopathology (Cytology [illegible]

The application of molecular biology to pathological science has helped in understanding various human diseases at molecular level. Because most diseases are caused due to disorder of genetic codes in DNA molecule which alter the synthetic process of enzymes and ultimately disturbs metabolic activities of the cell.

7. Cytoecology (Cytology and Ecology)

The cytoecology is the science in which one studies the effects of ecological changes on the chromosome number of the cell. The cytological studies on plants and animals have revealed that the ecological habitat and geographical distribution have the correlation with chromosome numbers.

Thus, the cell biology has given a great impetus to early and modern biologists to explore new vistas in biological sciences. The modern cytological studies are helpful in understanding various life activities in the terms of molecules, in the curing of various chronic human diseases and in improving the breeds of plants and animals.

UNIT OF MEASUREMENT OF CELL

The cells of most animals, plants and bacteria have microscopic size and these are measured by the fractions or multiples of **standard units.** The standard units are metres, kilograms and seconds. The **metre** is the standard unit of measurement of length and is now defined as 1,650,763·73 times the wavelength of a specified orange-red line in the light emitted by an isotope of Krypton (86 Kr). The standard unit of mass is the international **kilogram,** a cylinder of platinum -iridium kept in the vault in Paris. It is equal to the mass of 1000 cm^3 of water at 4°C. The standard unit of time is **second** which is the duration of 9,192,631, 770 periods of the radiation corresponding to the transition between two hyperfine levels of the fundamen-

tal state of an atom of ^{133}cesium. Thus, the new standard second is based upon an "atomic clock". Several of the units which have been given names of their own are listed in following table :

Table 1·1—Fractions and multiples of units.

Fraction or multiple	Prefix	Symbol
10^{6}	mega—	M
10^{3}	kilo—	k
10^{0}	unit	—
10^{-2}	centi—	c
10^{-3}	milli—	m
10^{-6}	micro—	μ
10^{-9}	nano—	n
10^{-12}	pico—	p

A number of fractions or multiples of metres are commonly used in measurement of length of cell and its components. These units are chosen to permit the use of small whole numbers, rather than very large numbers or extremely small fractions. The centimetre, and millimetre follow the prefix system described in table 1·1, but smaller units have names of their own. One-thousandth of a millimetre (10^{-6}m) is called a **micron** and is given the symbol μ. One-thousandth of a micron (10^{-9}m) is the **millimicron** (m μ), and a tenth of this (10^{-10} m) is the Angstrom unit (A or A°). However, some workers use the term "**nanometre**" for "millimicron".

The gram (10^{-3} kg) is the most commonly used unit of mass in cell biology. The prefixes and symbols of table 1·1 apply quite directly to mass units. The microgram has sometimes been given the symbol gamma (γ), but this is being discouraged.

CHAPTER 2

CELL

The cell is the basic unit of organization or structure of all living matter. Within a selective and retentive semipermeable membrane, it contains a complete set of different kinds of units necessary to permit its own growth and reproduction from simple nutrients. A. G. Loewy and P. Siekevitz (1963) have defined a cell as *"a unit of biological activity delimited by a semipermeable membrane and capable of self-reproduction in a medium free of other living systems"*. This definition of cell does not include the viruses because they lack [illegible] they are capable of self-repro[illegible]. But, because viruses are primitive and simpler units of life, therefore, they should be discussed prior to other cells.

VIRUSES

The viruses (Latin-venom or poisonous fluid) are the simplest forms of life which instead of having cellular organization (*viz.*, plasma membrane, cytoplasm and nucleus) similar to bacteria, blue green algae, plants and animals, contain definite genetically determined macromolecular organization, genetic material and characteristic mode of inheritance.

The viruses are submicroscopic entities ranging between 10 to 250mμ or 100A° to 2500A° in size. Most viruses are smaller than bacteria, but some viruses (*e.g.*, the psittacos virus, 0·75μ diameter), are larger than bacteria (*e.g.*, PPLO bacterium, 0·25μ diameter). The viruses possess a regular geometrical and macromolecular organization. Basically, all viruses consist of a core of only one type of [illegible] of [illegible] to [illegible] o- [illegible] -tagonal, lobular, or any other shape. The arrangement of capsomeres determines the shape of the virus particle or virion. Thus, the viruses may contain three types of symmetry, such as cubic (*e.g.*, Bacteriophage×174, turnip yellow mosaic virus, Adenovirus, etc.), helical (*e.g.*, Bacteriophages and tobacco mosaic virus) and complex (*e.g.*, pox virus, vaccinia virus, etc.). Some viruses (*e.g.*, potato spin-

dle tuber virus, etc.) have no capsid around the nucleic acid core or **viral chromosome,** while certain highly specialized viruses, such as influenza virus and mumbs contain a membranous envelope around the capsid. The capsid protects the viral chromosome during the extracellular phase of the virus life cycle.

The viruses contain only one kind of nucleic acid as the hereditary material. The **DNA viruses** have single DNA molecule which may be either linear or circular in shape. Mostly, the linear DNA molecule is double-stranded and occurs in T-bacteriophages, P_{22} bacteriophage, etc The circular DNA molecule may be either single stranded as in certain bacteriophages (*e.g.*, $\phi \times 174$, S_{13}, M_{13} bacteriophages), or double-stranded as in most animal viruses. The **RNA viruses** are the only biological systems known in which RNA and not DNA is the hereditary material. Often the RNA is present in its usual single-stranded form (*e.g*, plant viruses, some animal viruses such as poliomyelitis, influenza virus, etc., and bacteriophages *such as* MS_2, f_r, R_{17}, F_2, etc.) *but some viruses such as* Reovirus, have a core of double-stranded RNA similar in properties to DNA

The viruses lack in necessary energy yielding and synthetic enzyme systems, therefore, they cannot lead a free living mode of life. For the performance of fundamental life activities such as reproduction, genetically determined structures and functions, they have to lead a parasitic mode of existence. All viruses are, therefore, invariably intracellular parasites of specific hosts which may be bacteria, plants or animals. Their dependence on cells for reproduction argues against the consideration of viruses as primitive in the evolutionary sense, that is, as the ancestors of cellular life. Perhaps the simplest notion is that they arose as a sort of parasite at the same time as or subsequent to the origin of cellular life, possibly some are degenerate

structures that were once capable of

and Holtzman, 1970).

ied into following three groups according to the type of host :

A. BACTERIAL VIRUSES OR BACTERIOPHAGES

Viruses that parasitize bacterial cells are called **bacteriophages** or **phages** (phage– to eat). They were discovered in 1917 by a French Scientist **d' Herelle.** The bacteriophages have specific hosts, and variable shapes, sizes and structures. Some phages may be spherical or comma-shaped or may have a tail and resemble a tadpole. The most widely studied bacteriophage is **T_4 bacteriophage** t infects the colon bacillus, *Escherichia coli.* T_4 phage contains a

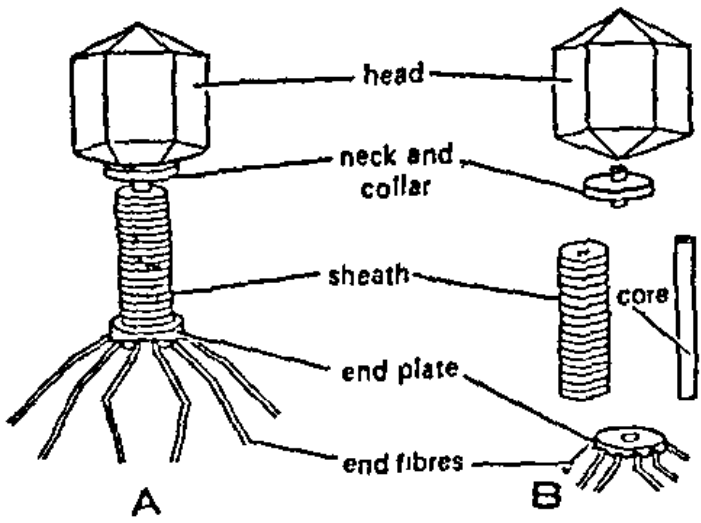


Fig. 2·1. A bacteriophage. A—external structure, B—parts

than 75 genes and remain highly coiled and tightly packed within a protein envelope (capsid) of polyhedral head. The head has a length of 1250A° and width of 850A°. The capsid of head is made up of about 2000 similar subunits or capsomeres of protein molecules, each of which has a molecular weight about 80,000. A short neck containing collar connects the head with a tail. The tail of a T_4 phage consists of a central hollow core through which phage DNA molecule passes on its way into the host cell. Around the core occurs a spring-like **contractile sheath** or **tube.** The contractile sheath is formed by 144 subunits, which are arranged in a hollow cylinder consisting 24 rays of six subunits each. The contractile sheath and core of the tail remain attached to a hexagonal end plate. Six slender fibres and six short spikes protrude from the end plate. Each of the tail-fibres and tail-spikes is a bundle of polypeptide chains of a molecular weight of about 100,000.

The mode of infection of T_4 bacteriophage is highly mechanised. At the time of infection, a T_4 bacteriophage becomes anchored to the bacterial cell wall by its tail-fibres and tail-spikes, so that the tail and head are perpendicular to the surface. Within a few minutes after attachment, the

than half its original

phage digests the part of the bacterial cell wall so as to produce a hole and finally, the phage DNA is injected into the bacterial cell. The capsid (protein coat of head and tail) of phage remains outside the bacterial cell.

T_4 phage may affect the bacterial cell in two ways : (1) It may act as a **virulent** or **lytic** phage by growing and lysing the bacterial cell. In such a case, the DNA molecule of host cell becomes disturbed, manufacture of bacterial proteins stops and the viral genes take over. Among the first proteins to be made are the enzymes

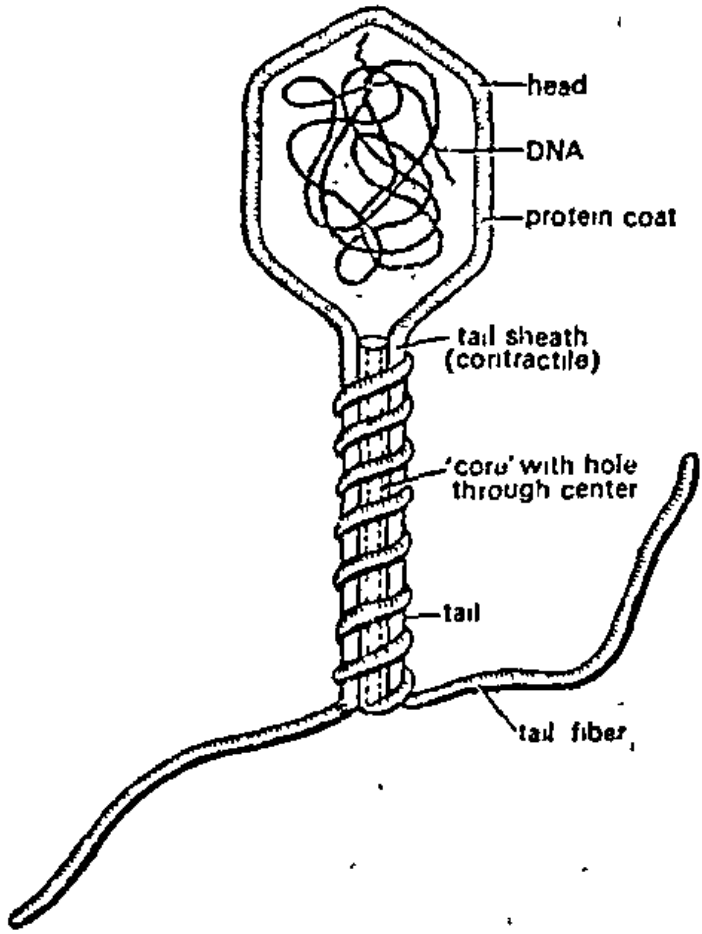


Fig. 2·2. Internal structure of a T bacteriophage.

needed for viral DNA replication. This happens within the first 5 minutes after break through. Three minutes later another set of (viral) genes directs the synthesis of the structural proteins that will form the head components and tail components. The first completed virus particle appears 13 minutes after infection, and the process continues for another 12 minutes until about 200 such particles are completed and the raw materials of the bacterial cell are virtually exhausted. A viral enzyme, a lysozyme, then appears and attacks the cell wall, liberating the new viral particles, (2) A T_4 bacteriphage may also act as a **temperate, non-virulent** or **lysogenic** phage when its genetic material becomes incorporated into the genetic material of the bacterium (host cell) and there that may remain in an inactive or masked state for long time. In this stage, viral chrome (DNA molecule) becomes **prophage** to replicate in synchrony

with the bacterial chromosome. However, such a phage alters the hereditary charac[illegible] t phage at some st

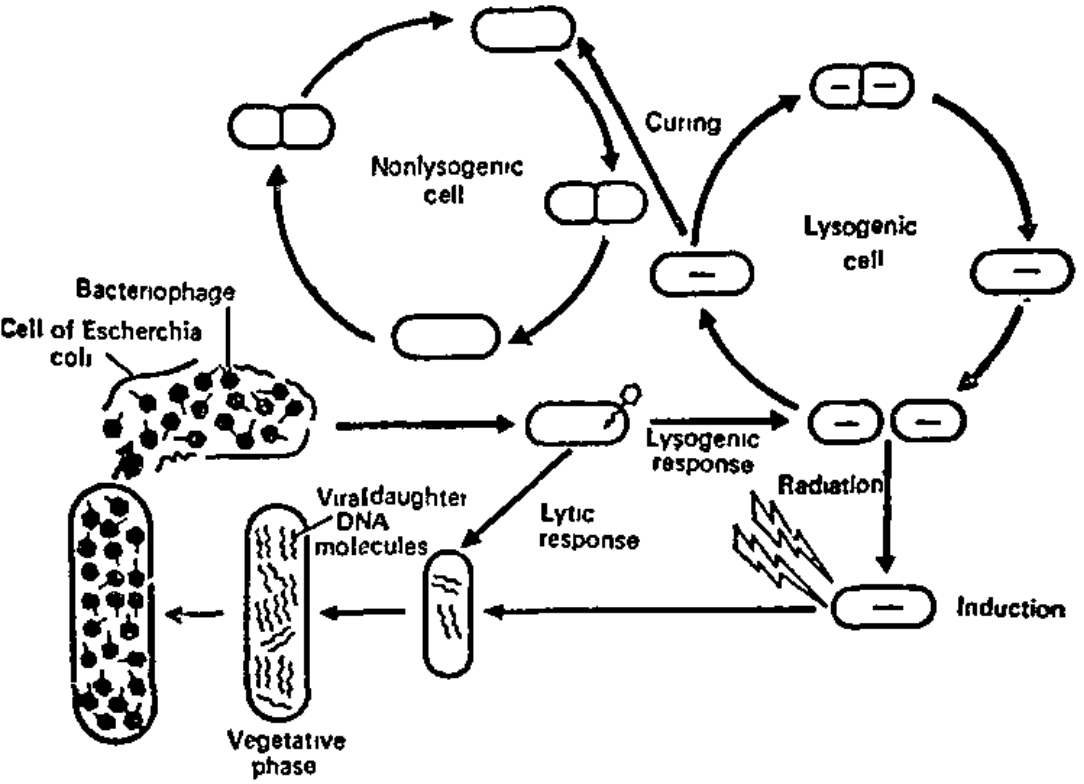


Fig. 2·3. Life cycle of T_4 bacteriophage.

B. PLANT VIRUSES

The plant viruses parasitize the plant cells and disturb their metabolism and cause severe diseases in them. All plant viruses consist of ribonucleoproteins in their organization. The important plant viruses are tobacco rattle virus (TRV), tobacco mosaic virus [illegible] ean mosa- [illegible] ich is dis- [illegible] y Stanley (1935). It is a helically, rod-shaped virus having the length of 3000A° and diameter of 180A°. TMV contains a nucleic acid core of a elongated, spirally coiled, single-stranded RNA molecule which has 6500 nucleotides. The RNA molecule of TMV remains surrounded by 2130 identical protein subunits or **capsomeres**, each with a molecular weight of 18,000. The capsomeres are elliptical and remain arranged helically around the RNA to form the capsid of TMV.

C. ANIMAL VIRUSES

The animal viruses infect the animal cells and cause different [illegible] have a poly- [illegible] orm of DNA [illegible] is surroun-

ded by an envelope. The important animal viruses are small pox virus, vaccinia virus, herpes virus, adeno virus, influenza virus, mumps, poliomyelitis, reo virus, etc.

Poliomyelitis is a most extensively studied animal virus. The polio virus has comparatively very simple organization. It consists of a protein shell built up of 60 structurally equivalent asymmetric protein subunits of approximately 60A° diameter, packed together in such a way that they form a spherical shell of about 300A° in diameter. The shell or capsid encloses a single stranded RNA molecule of 5,200 nucleotides. During infection, a poliomyelitis virus get attached with animal cell and is engulfed by the latter by phagocytosis. Inside the host cell, it drastically alters the metabolism of the host cell and ultimately leads to quick death of that.

CELLS OF CELLULAR ORGANISMS

The body of all living organisms (bacteria, blue green algae, plants and animals) except viruses has **cellular organization** and may contain one or many cells. The organisms with only one cell in their body are called **unicellular organisms** (*e.g.*, bacteria, blue green algae, some algae, Protozoa, etc.). The organisms having many cells in their body are called **multicellular organims** (e.g., most plants and animals). Any cellular organism may contain only one type of cell from the following types of cells

A. Prokaryotic cells.

B. Eukaryotic cells.

PROKARYOTIC CELLS

The prokaryotic (Gr., *pro*—primitive, *karyon*—nucleus) cells are the most primitive cells from morphological point of view. They occur in bacteria and blue green algae. A prokaryotic cell is essentially a **one-envelope system** organized in depth. It consists of central nuclear components (*viz.*, DNA molecule, RNA molecules and nuclear proteins) surrounded by cytoplasmic ground substance, with the whole enveloped by a plasma membrane. Neither the nuclear apparatus nor [illegible] enclosed by membra[illegible]sma membrane itself ma[illegible]lasm of a prokaryotic cell lacks in well defined cytoplasmic organelles such as endoplasmic reticulum, Golgi complex, mitochondria, centrioles, etc. Few important prokaryotic cells are following :

I. PLEUROPNEUMONIA-LIKE ORGANISMS OR PPLO

The pleuropneumonia-like organisms or PPLO are the bacteria-like organisms which differ with the latter only in lacking the cell

wall and mesosomes and in having very simple organization, small size and a deformable plasma membrane around their prokaryotic cells. W. V. Iterson (1969) has placed PPLO in the group Mycoplasmateeae of bacteria (Schizomycetes), but Novikoff and Holtzman (1970) have considered them as simplest known cells and have excluded them from the bacteria. The diameters of the smallest PPLO are 0·1-0·3μ (*e.g., Mycoplasma laidlawii* (0·1μ diameter) and *Mycoplasma gallisepticum* (0·25μ diameter). The prokaryotic cells of

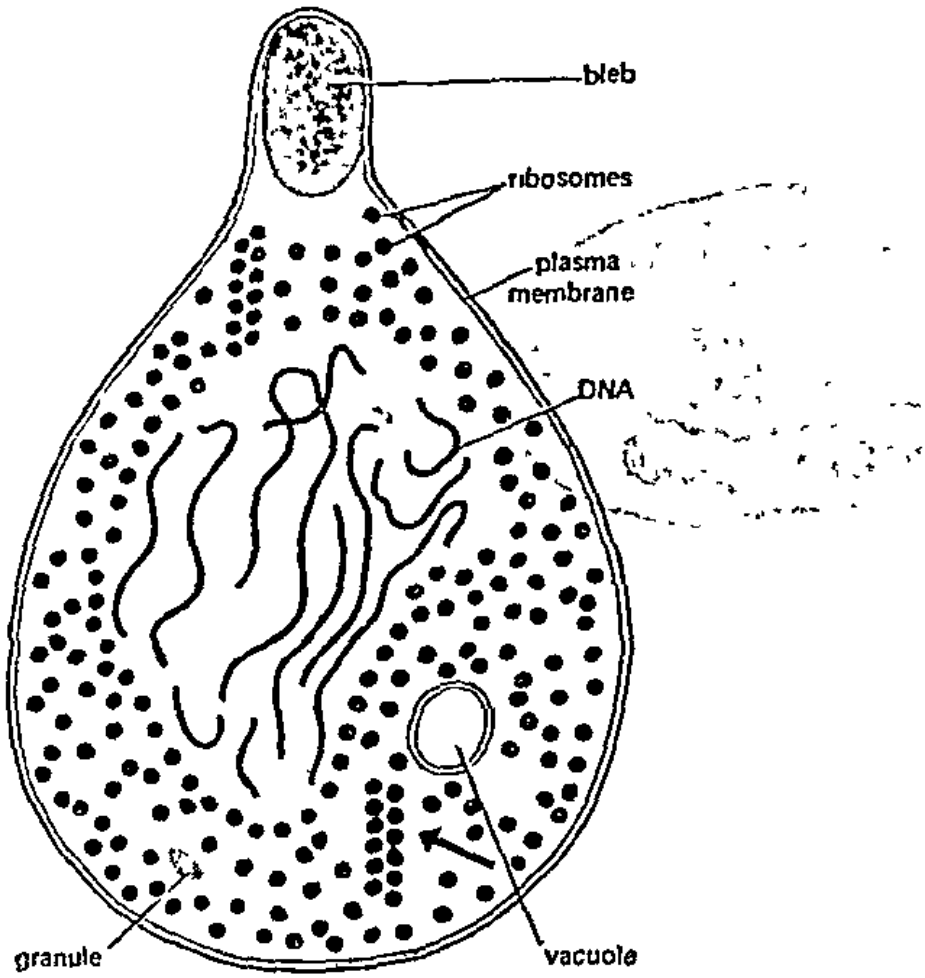


Fig 2·4. A schematic diagram of a typical PPLO cell.

PPLO are bounded by a 75A° thick plasma membrane. The circular, helically, double-stranded DNA molecule is contained in a nuclear region which lacks nuclear membrane and is surrounded by many ribosomes and other components involved in protein synthesis.

The PPLO cells contain many enzymes which may be required for DNA replication, the transcription of different kinds of RNA molecules and translation involved in protein synthesis, and also in the biosynthesis of adenosine triphosphate (ATP) by anaerobic breakdown of sugars. Unlike viruses, they are free living and do not req-

uire host cells for their duplication. PPLO reproduce by binary fission, budding, formation of small spore-like bodies and by growth of large branched filaments that ultimately fragment.

2. BACTERIA

The bacteria are microscopic, unicellular, achlorophyllous, asexually reproducing prokaryotes which occur in fresh and salt water, in soil and in plants and animal. They lead either a saprophytic or parasitic mode of existence. The saprophytic species of bacteria are of great economic significance for man. Some parasitic species of bacteria are pathogenic (disease producing) to plants, animals and man.

Size of bacteria—The bacterial cells are very small and range between 0·15μ to 15μ. The smallest bacterium is *Dialister pneumosintes* (0·15 to 0·3μ in length). The largest bacterium is *Spirillum volutans* (13 to 15μ in length).

Form of bacteria—There are three basic shapes of the bacteria the spheroidal (**coccus**), rod-shaped or cylindrical (**bacillus**) and spiral (**spirillum** and **spirochetes**). The spheroidal bacteria occur either singly (called **micrococcus**), in groups of two (called **diplococcus**, *e.g.*, *Diplococcus pneumoniae*, pneumonia causing bacterium) ; in long chains (called **streptococcus**, *e.g.*, *Streptococcus phyegens*, sore throat causing bacterium) ; or in irregular clumps, resembling bunches of grapes called **staphylococci**, *e.g.*, *Staphylococcus aureus*, boils causing bacterium). The rod-shaped bacteria or bacilli may occur as single rods (*e.g.*, the bacillus causing anthrax) ; or as long chains of rods joined together. Certain bacilli cause many notorious diseases such as tuberculosis (*Mycobacterium tuberculosis*), diphtheria (*Corynebacterium diptheriae*), typhoid fever (*Salmonella typhosa*), etc,, in man. The spiral-shaped bacteria may occur in two forms : the **spirilla**, which are less coiled and sometimes resemble a comma (*e.g. Vibrio comma*, a cholera producing bacterium); and the **spirochetes**, which are highly coiled and resemble a corkscrew (*e.g.*, syphilis causing bacterium).

Structure of bacteria—The typical prokaryotic cell of bacteria contains an **outer covering** and **cytoplasm**. The outer covering of a bacterial cells includes following three layers—A. **Plasma membrane**—The cytoplasm of bacterial cells is bounded by a thin plasma membrane of lipid and protein molecules. The plasma membrane of bacteria contains oxidative or respiratory chain enzymes and multi-enzyme complexes and performs the functions which are usually done by the mitochondria of eukaryotic cells. The bacterial plasma membrane may become differentiated to form varied structu-

res. For example, the plasma membrane of bacterium *Thiovulum majus* sinks deep into the cytoplasm to form multilayered structures called the **desmosomes.** In some bacteria (*e.g.*, *Bacillus subtilis*) the plasma membrane gives out certain invaginations or infoldings of complex whorls of convoluted membranes, called **mesosomes,** in the cytoplasm. The mesosomes have varied functions. They act as the respiratory organs and also synthesize material for extracellular transport and for the formation of cell wall. The mesosomes receive the transforming DNA molecule from other bacterial cells during conjugation. B. **Cell wall.** The [illegible] and is covered by [illegible] cell wall [illegible] carbohydrates, [illegible] organic salts, contains a specific amino acid **diaminopimelic acid** and a derivative of glucose called **muramic acid.** Outside the cell wall of most bacteria occurs a **slimy capsule** which mainly contains the polysaccharides.

The **cytoplasm** of bacterial cells is dense, colloidal and contains granules of glycogen, proteins and lipids. It also contains poly-β-hydroxybutyric acid, granulose (a polymer of glucose), volutin (metaphosphate granules) and elemental sulphur as the stored food materials. It lacks most of the important membrane bounded [illegible] **bacteriochlorophyll.**

The genetic informations of bacterial cells are carried by DNA molecules which are called **bacterial chromosomes** or **genophores** and occur in distinct nuclear regions called **nucleoids.** The nucleoids lack membranes. Cocci usually contain one nucleoid per cell, while the rod-like bacilli usually contain two or more nucleoids per cell.

Many bacteria are able to swim about by beating of whip-like cellular outgrowths called **flagella.** A bacterial flagellum consists of a single fibril of 100 to 200A° thickness and several micron length, protruding from an intracellular "basal granule" through the plasma membrane and cell wall. The fibril is made largely of the protein **flagellin.** The cell of some bacteria contain hair-like extracellular outgrowths called **pili** or **fimbriae,** for the attachment of the bacterial cell to solid surfaces. Each of the pili is composed of helically arranged subunits of "**pilin**" protein.

Escherichia coli—*E. coli* is a gram-negative bacillus (*i.e.*, unstainable by a stain developed by C. Gram in 1885) which is non-pathogenic and found in the mammalian digestive tract. It is a favourite experimental organism for cell biologists, geneticists, biochemists and molecular biologists.

An *E. coli* cell is about 2μ long and 0·8 to 1μ thick. It has an outer cell wall of 100A° thickness. Its cell wall and plasma membrane have typical structure of the bacterial cells. The cytoplasm of *E coli* is colloidal containing roughly 5000 distinguishable chemical components, ranging from water to DNA molecule and other molecules, such as RNA, enzymes and glycogen. It contains only 20,000 to 30,000 ribosomes of 200A° diameter as the main cellular organelles. All the genetic informations of an *E. coli* cell are carried by a single,

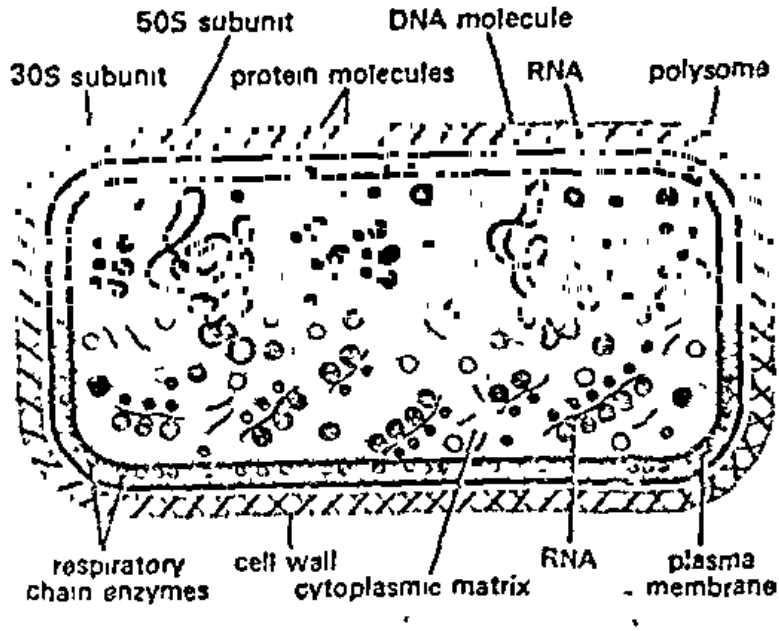


Fig 2.5. A prokaryotic cell of *Escherichia coli*.

double-stranded, circular and 1000–1500μ long molecule of DNA. DNA molecule of *E. coli* does not remain wrapped in histone proteins like the DNA of eukaryotes, but certain polyamines may be bound to some of its phosphate groups. It forms bacterial chromosome or **genophore** and remains confined to two clear central regions of cytoplasm which may lack any membrane and called **nucleoids**.

3. BLUE GREEN ALGAE

The blue green algae form another group of prokaryotes which includes about 2000 species resembling bacteria in many details. A few blue green algae are unicellular and grow as single separate cells, but most species are multicellular and forming multicellular colonies of prokaryotic cells.

A typical cell of a blue green alga is composed of outer cellular coverings and cytoplasm. The outer cellular coverings include an [illegible] iddle cell wall and an inner-

The cell wall of blue green algae resembles to the cell wall of bacteria and contains molecules of lipoproteins, lipopolysaccharides, and mucoproteins. The cytoplasm of blue green algae lacks endoplasmic reticulum, Golgi complex, mitochondria and lysosomes. It contains numerous 70 S ribosomes which are freely distributed in the cytoplasm and form polyribosomes during protein synthesis.

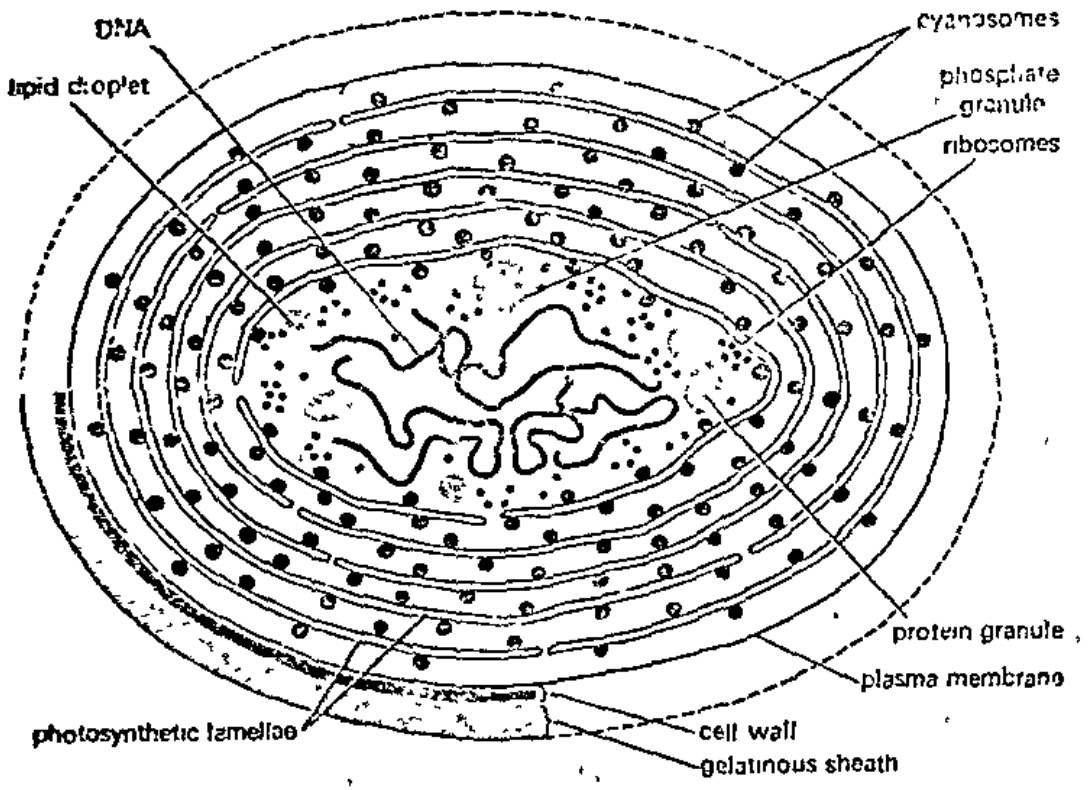


Fig. 2·6. Diagram of a blue-green alga cell.

Because the metabolism of the blue green algae is based on photosynthesis, there [illegible] pigments, *viz.*, the [illegible] pigments, these alga[illegible] called phycobilin ; one of the phycobilin is blue and called phycocyanin, while other type of phycobilin is red and called phycoerythrin. The photosynthetic pigments (chlorophylls and carotenoids) occur in flattened sacs called lamellae which remain arranged in parallel arra[illegible] In between the lamellae occur certain granules of 450A° diame[illegible] These granules contain phycobilin pigments and are called [illegible] somes or phycobilisomes.

As in all prokaryotes, the DNA molecule of blue green algae is circular, double-stranded helix and it does not remain segregated into a nucleus separated from the cytoplasm by a nuclear envelope. The cells of blue green algae though lack cilia or flagella, but have the capacity to do movement by gliding and rotatory motions.

EUKARYOTIC CELLS

The eukaryotic cells (Gr., *eu*-good, *karyotic*-nucleated) are essentially two envelope systems and they are very much larger than prokaryotic cells. Secondary membranes envelope the nucleus and other internal organelles and to a great extent they pervade the cytoplasm as the endoplasmic reticulum. The eukaryotic cells are the true cells which occur in the plants (from algae to angiosperms) and the animals (from Protozoa to mammals). Though the eukaryotic cells have different shape, size, and physiology but all the cells are typically composed of plasma membrane, cytoplasm and its organelles, *viz.*, mitochondria, endoplasmic reticulum, ribosomes, Golgi complex, etc., and a true nucleus. Here the nuclear contents, such as, DNA, RNA, nucleoproteins and nucleus remain separated from the cytoplasm by the thin, perforated nuclear membranes. Before going into the details of cell and its various components, it will be advisable to consider the general features of different types of eukaryotic cells which are as follows :

SHAPE

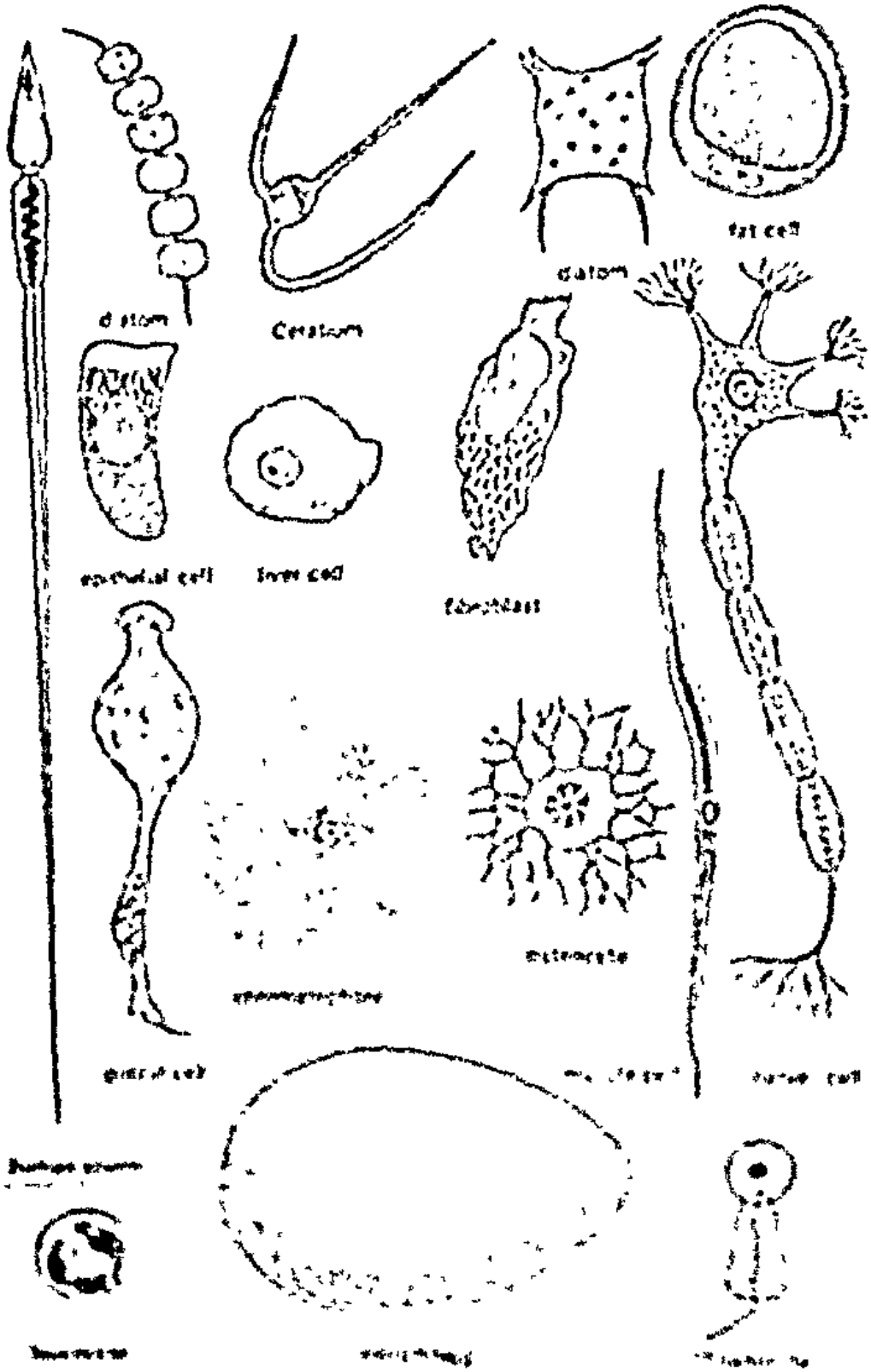
The plant and animal cells exhibit various forms and shapes. Typically the animal cell is spherical in shape but the shape of the cell may be irregular, triangular, tubular, cuboidal, polygonal, cylindrical, oval, rounded or elongated in different animals and plants. The shape of the cell may vary from animal to animal and from organ to organ. Even the cells of the same organ may display variations in the shape. Generally the shape of the cell is correlated with its functions. For example, the epithelial cells have flat shape and the muscle cells are elongated. Moreover, external or internal environment may also cause shape variations in the cell due to internal or mechanical stress or pressure and surface tension, etc.

SIZE

Mostly the eukaryotic cells are microscopic in size but definitely they are larger in size than the bacterial cells. The size of cells varies from 1μ to 175000μ (175 mm). The ostrich egg cell is usually considered as largest cell (175 mm diameter) but certain longest nerve cells have been found to have the length of 3 or 3·5 feet.

NUMBER

The body of unicellular or acellular organisms (Protozoa and Protophyta) consist of single cell. Most of the animals and plants



have many cells in the body and are known as multicellular animals or plants. The number of cells in the multicellular organisms usually remains correlated with size of the organisms and, therefore, small-sized organism has less number of cells in comparison to large-sized organisms.

STRUCTURE

An eukaryotic cell consists of the following components :

A. Cell wall and plasma membrane, B. Cytoplasm, and C. Nucleus.

CELL WALL AND PLASMA MEMBRANE

(i) Cell wall—The protoplasm of plant cells is separated from the external world by the cell wall which is entirely lacking in animals. The cell wall is a semi-rigid, laminated, external and non-living covering of the cell The cell wall is secreted by the cell itself and primarily it contains a complex polysaccharide carbohy-

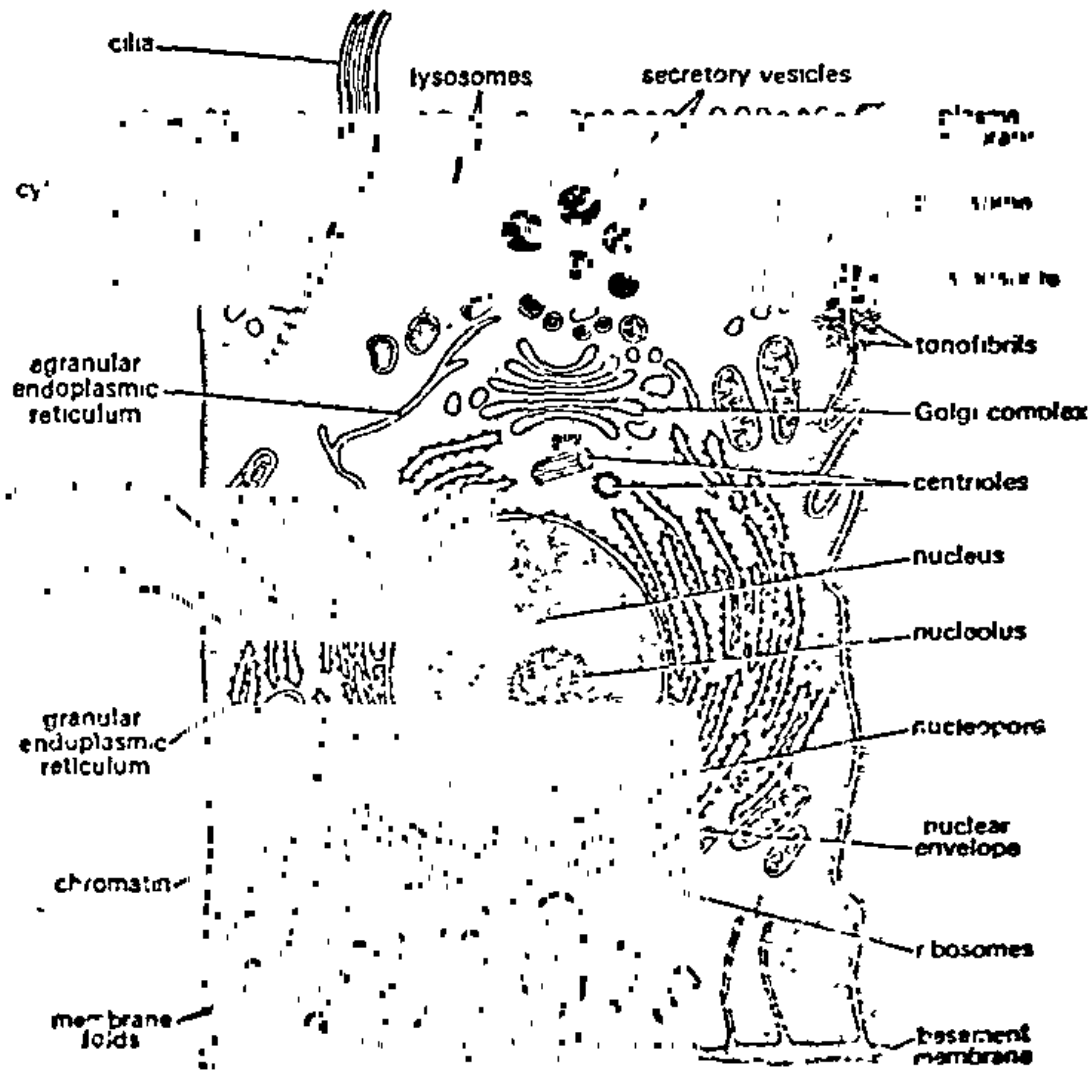


Fig 2.8. Structure of a typical eukaryotic animal cell.

drate, the **cellulose**. The cell wall provides protection and support to the plasma membrane and the cytoplasm, both of which lie beneath it. The cell wall of some plant cells has some canal-like or pit-like minute apertures by which the cells remain connected with adjacent cells. These canals of cell wall are known as **plasmodesmata.**

(ii) Plasma membrane—Most plant and animal cells have an external covering known as plasmalemma, cell membrane, or plasma membrane. The plasma membrane is a living, ultra-thin, elastic, porous, semipermeable membranous covering of cell. Primarily the plasma membrne provides mechanical support and external form to the protoplasm (cytoplasm + nucleus) and it also delimits the protoplasm from the exterior, checks the entry or exit of undesirable substances and due to its semipermeability it transmits necessary materials to and from the cell. It consists of an outer and inner layers of proteins and a middle layer of lipids. It is found to contain many pores through which exchange of molecules may occur.

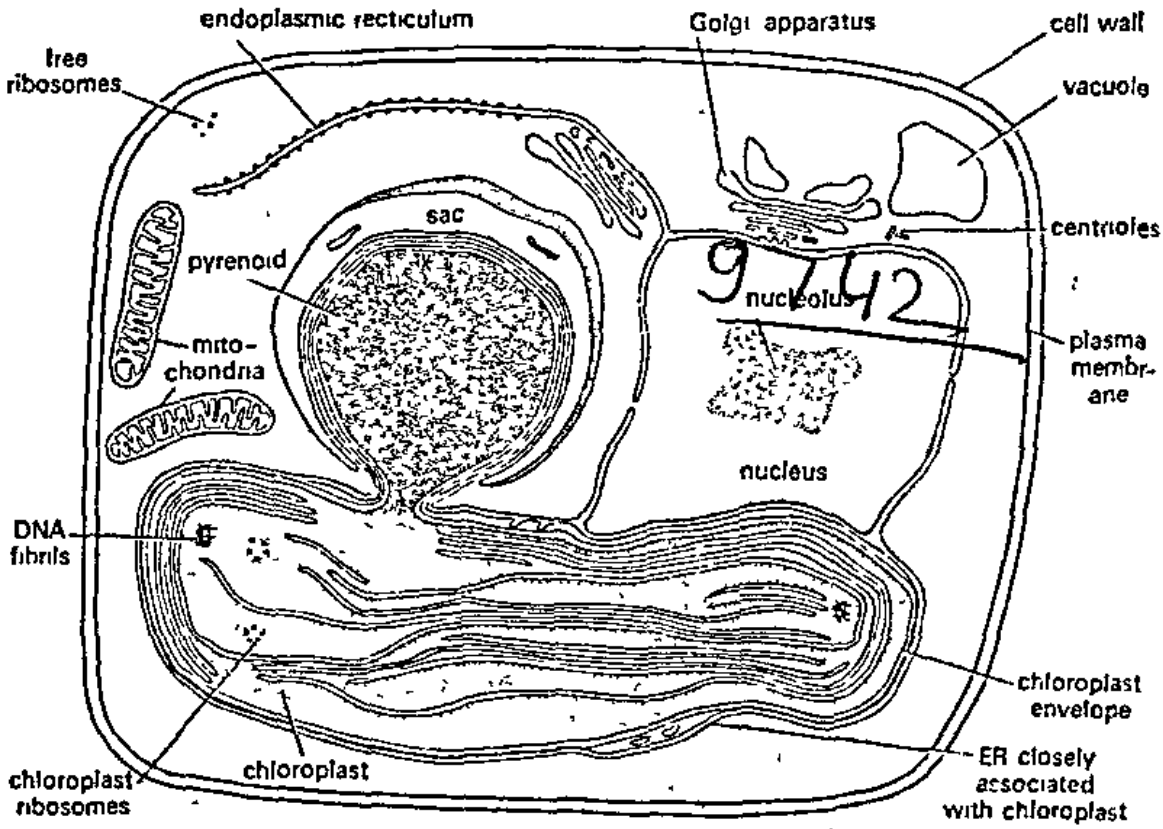


Fig. 29. n alga. exten- stroma

CYTOPLASM

The plasma membrane is followed by the cytoplasm which is distinguished into following structures :

A. Cytoplasmic matrix—The space between the plasma membrane and the nucleus is filled by an amorphous, transluscent, homogeneous colloidal liquid known as **hyaloplasm** or **cytoplasmic matrix.** The cytoplasmic matrix consists of various inorganic molecules such as water, salts of Na, K and other metals and various organic compounds, *viz.*, carbohydrates, lipids, proteins, nucleoproteins, nucleic acids, (RNA and DNA) and a variety of enzymes. The peripheral layer of cytoplasmic matrix is relatively non-granular, viscous, clear and rigid and is known as the **plasmagel, cortex** or **cortical layer** or **ectoplasm** (Gr., *ecto*-outside, *plasm*-form). The inner portion of the cytoplasmic matrix is granular, less viscous and known as **endoplasm** (Gr., *endo*-linear, *plasm*-form).

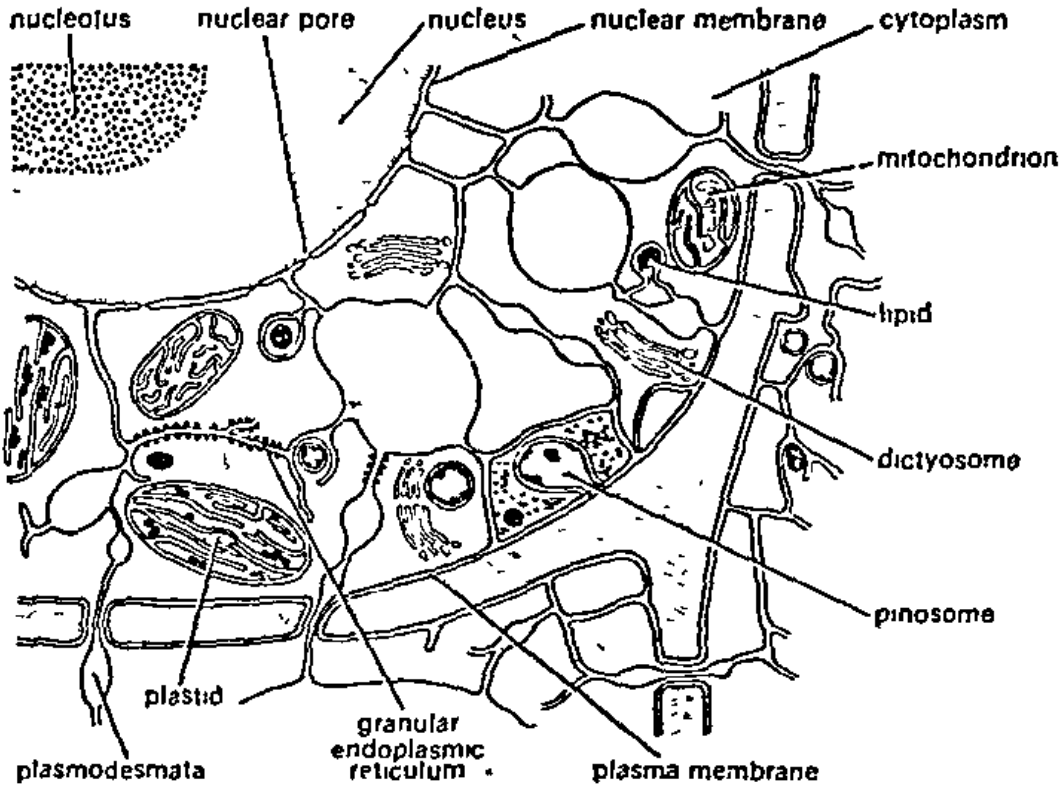


Fig 2.10. Ultrastructure of plant cell (showing small portion of a cell).

B. Cytoplasmic structures—In the cytoplasmic matrix certain non-living and living structures remain suspended. The non-living structures are called **paraplasm, deutoplasm** or **inclusions,** while the living structures are membrane bounded and are called **organoids** or **organelles** Both kinds of cytoplasmic structures can be studied under the following headings :

(a) Cytoplasmic inclusions—The stored food and secretory substances of the cell remain suspended in the cytoplasmic matrix in the form of refractile granules and forming the cytoplasmic inclu-

sions. The cytoplasmic inclusions include oil drops, yolk granules, pigments, secretory granules, and glycogen granules.

(b) Cytoplasmic organoids or organelles—The organoids or organelles are living structures of the cytoplasm. They perform various important biosynthetic and metabolic activities such as respiration, biosynthesis, transportation, support, storage and reproduction. The most important cytoplasmic organelles are microtubules, centrosome, basal granules, cilia and flagella, endoplasmic reticulum, Golgi complex, lysosomes, cytoplasmic vacuoles, ribosomes, microbodies, mitochondria and plastids.

1. Microtubules and microfilaments—The cytoplasm of plant and animal cells is traversed by numerous ultrafine tubules of **tubulin** protein, called **microtubules**. The function of microtubules is the transportation of water, ions or small molecules, cytoplasmic streaming (cyclosis), and the formation of fibres or asters of the mitotic or meiotic spindle during cell division. Moreover, they form the structural units of the centrioles, basal granules, cilia and flagella.

The cytoplasm of most animal cells also contains many ultrafine, proteinous, *solid microfilaments which maintain the structure* of cell and form contractile components of muscle cells.

2. Centrosome—The centrosome contains dense cytoplasm and is located near the nucleus of animal cells. During the cell division the centrosome is found to contain two rod-shaped granules known as **centrioles**. The centrioles consist of nine fibrillar units and each fibrillar unit is found to contain three microtubules. At the time of cell division the centrioles form the spindle of microtubules which help in the separation and movement of chromosomes during concluding stages of cell divisions.

3. Basal granules or kinetosomes—The animal or plant cells which are having locomotory organelles such as the cilia or flagella, contain spherical bodies known as **basal granules** or **kinetosomes**, at the base of the cilia or flagella. The kinetosomes remain embedded in the ectoplasm and are composed of nine fibrils. Each fibril consists of three microtubules, out of which two enter in the cilia or flagella. The basal granules may contain both DNA and RNA. The evidence is questionable.

4. Cilia and flagella—The cells of many unicellular organisms and ciliated epithelium of multicellular organisms consists of some hair-like cytoplasmic projections outside the surface of the cell. These are known as **cilia** or **flagella**. They help in locomotion of the cell. The cilia and flagella consist of nine outer fibrils around the two large central fibrils. Each outer fibrils consists of two micro-

tubules. The cilia and flagella are originated from the basal granules and chemically consist of proteins and adenosine triphosphate (ATP).

5. **Endoplasmic reticulum**—The cytoplasmic matrix is traversed by a vast reticulum or network of inter-connecting tubules and vesicles which is known as **endoplasmic reticulum** or ER. The endoplasmic reticulum is having a single vast and inter-connected cavity which remains bounded by a single unit membrane. The membrane of endoplasmic reticulum is supposed to be originated by inpushings of plasma membrane in the matrix because like the plasma membrane it has an outer and an inner layer of protein molecules sandwiching the middle layer of lipid molecules.

The membranes of the endoplasmic reticulum may be either smooth when they do not have attached ribosomes or rough when they have attached ribosomes with them. The membrane of endoplasmic reticulum is found to be continuous with the nuclear membrane and plasma membrane. The endoplasmic reticulum forms the ultrastructural skeletal framework of the cytoplasmic matrix and it provides mechanical support to it. It also acts as an intracellular circulatory system and it circulates various substances into and out of the cells by the membrane flow mechanism. Further, the endoplasmic reticulum acts as a storage and synthetic organ. For example, it synthesizes lipids, glycogen, cholesterol, glycerides, hormones, etc.

6. **Golgi complex**—In cytoplasmic matrix, a stack of flattened, membrane bounded, parallely arranged organelles occur in the association of endoplasmic reticulum and known as **Golgi complex** Each Golgi complex is composed of many lamellae, tubules, vesicles and vacuoles. The membranes of Golgi complex are of lipoproteins and these are supposed to be originated from the membranes of endoplasmic reticulum The function of the Golgi complex is storage of proteins and enzymes which are secreted by ribosomes and transported by endoplasmic reticulum to them. Further the Golgi complex has most important secretory function. It secretes many secretory granules and lysosomes. In plant cells the Golgi complex is known as **dictyosome** and secretes necessary materials for cell wall formation during cell division.

7. **Lysosomes**—The cytoplasm of animal cells contains many tiny, spheroid or irregular-shaped, membrane bounded vesicles known as **lysosomes**. The lysosomes are originated by Golgi complex and contain many digestive enzymes. Their function is the digestion of food material which comes in the cell by pinacocytosis and phagocytosis. The lysosomes of plant cells are membrane-bounded storage

granules containing hydrolytic digestive enzymes and are comprised of **spherosomes, aleurone grain** and **vacuoles.**

8 Cytoplasmic vacuoles—The cytoplasm of many plant and some animal cells (*i.e.*, ciliate protozoans) contains numerous small or large-sized, hollow, liquid-filled structures, the **vacuoles.** These vacuoles are supposed to be greatly expanded endoplasmic reticulum or Golgi complex. The **vacuoles** of animal cells are bounded by a lipoproteinous membrane and their function is the storage, transmission of the materials and the maintenance of internal pressure of the cell

The vacuoles of the plant cells are bounded by a single, semi-permeable membrane known as **tonoplast.** These vacuoles contain water, phenol, flavonols, anthocyanins (blue and red pigment), alkaloids and storage products, such as sugars and proteins.

9. Microbodies—The cytoplasmic matrix of many kinds of cells, *viz.*, Yeast, Protozoa, cells of higher plants, hepatocytes (liver cells) and kidney cells, contains certain roughly spherical, membrane bounded particles (0·3–1·5 μ diameter). These particles have a central granular or crystalloid core containing some enzymes, occur in intimate relation with endoplasmic reticulum, mitochondria and chloroplasts and are called **microbodies.** The microbodies use molecular oxygen like mitochondria, but instead of having cytochromes and capacity of ATP synthesis like mitochondria, they contain enzymes for hydrgen peroxide metabolism, purine metabolism, gluconeogenesis (conversion of fat into carbohydrate) and photorespiration.

10. Ribosomes—Many minute, spherical structures known as ribosomes are remain attached with the membrane of endoplasmic reticulum and forms granular or rough type of ER. The ribosomes are originated in the nucleolus and consist of mainly the ribonucleic acids (RNA) and proteins. Each ribosome is composed of two structural units, a smaller subunit known as **40 S subunit** and a larger snbunit known as *60 S subunit*. The ribosomes remain attached with the membranes of endoplasmic reticulum by the 60 S subunits. The 40 S subunits occur on the larger unit and form a cap-like structure. The ribosomes consists of 3 types of RNA's known as **ribosomal** RNA's or rRNA, *viz.*, 5 S, 18 S and 28 S rRNA's. The 28 S and 5 S rRNA's occur in large (60 S) subunit, while 18 S rRNA occurs in the smaller ribosomal subunit. The ribosomes also occur freely in the cytoplasm. They are the sites of protein synthesis.

11. Mitochondria—In the cytoplasm of most cells occur many large-sized, rounded or rod-like structures known as **mitochondria.** The mitochondria occur singly or in groups and their shape

and size vary from cell to cell. The mitochondria are bounded by two membranes of lipoproteins. The outer membrane forms a bag-like structure around the inner membrane which gives out many finger-like folds in the lumen of the mitochondria. The folds of inner mitochondrial membrane are known as **cristae**. The space between outer and inner mitochondrial membrane as well as the central space is filled up by a viscous **mitochondrial matrix**. The matrix, outer and inner membranes are found to contain many oxidative enzymes and coenzymes. The functions of mitochondria, are respiration, oxidation of food, and metabolism of energy. The mitochondria also contain a circular DNA molecule and ribosomes and are capable of synthesis of certain proteins.

12. Plastids—The plastids occur commonly in plant cells and their diameter varies from 4μ to 6μ. They may be colourless or coloured. The colourless plastids are known as **leucoplastids** or **leucoplasts** and coloured plastids as **chromoplastids** or **chromoplasts**. The leucoplasts usually have storage function and store starch and lipids and may be called **amyloplasts** and **lipoplasts** respectively. The chromoplasts may have many forms and pigments, but those having the chlorophyll are most important. These are known as **chloroplasts**. The chloroplasts have complicated organization. They contain DNA, ribosomes and complete protein synthetic machinery. The chloroplasts help in the biosynthesis of food stuffs by the process of photosynthesis.

NUCLEUS

The nucleus is a centrally located and spherical cellular component which controls all the vital activities of the cytoplasm and carries the hereditary material the DNA in it. The nucleus consists of three structures : (i) nuclear membranes, (ii) nucleoplasm and chromosomes, and (iii) the nucleolus.

(i) Nuclear membranes—The nucleus is bounded by two membranes of lipoprotein which are known as **nuclear membrane**. The nuclear membranes form a kind of envelope around the nucleus and this envelope is known a **nuclear envelope**. The nuclear envelope contains many pore-like octagonal perforations through which to and fro movement of chemical substances takes place. The outer nuclear membrane of nuclear envelope remains continuous with the membranes of endoplasmic reticulum and plasma membrane.

(ii) Nucleoplasm and Chromosomes—The space between the nuclear membrane and nucleolus is filled by a watery substance known as **nucleoplasm** or **karyolymph**. The nucleoplasm contains dissolved phosphorus, ribose sugars, proteins, nucleotides and nu-

cleic acids. The nucleoplasm contains certain thread-like elongated structures known as **chromosomes**. The chromosomes appear only during cell division otherwise they occur in the form of ultrathin chromatin threads. The chromatin threads or chromosomes fundamentally consist of large molecules of deoxyribonucleic acid (DNA) which remains wrapped in RNA and nucleoproteins.

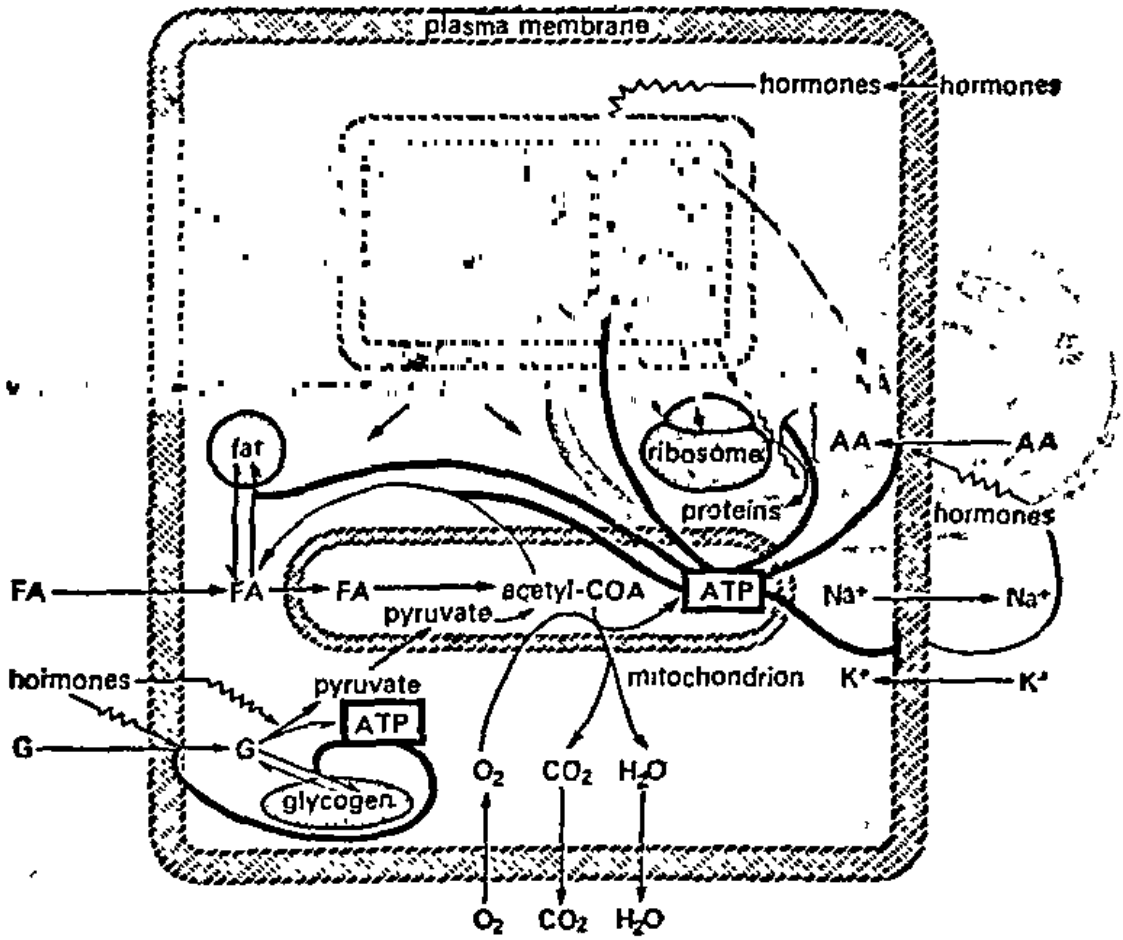


Fig 2·11. Diagram showing functions of different organelles of cell. (AA—amino acids, FA—fatty acids, G—glucose, tRNA—transfer RNA, mRNA—messenger RNA, rRNA—ribosomal RNA).

(iii) Nucleolus—The nucleoplasm contains a conspicuous, darkly stained spherical body known as the **nucleolus**. Chemically nucleolus is composed of large amount of ribosomal proteins and ribosomal RNA. The nucleolus stores the rRNA which is synthesized by nucleolar organizer region of DNA and it provides raw materials such as different types of rRNA'S and ribosomal proteins for the biogenesis of ribosomes.

FUNCTIONS OF CELLULAR ORGANELLES

The cellular organelles perform various important biological functions which have been shown in the figure No. 2·11.

CHAPTER 3

CYTOPLASM

The part of the cell which occurs between the plasma membrane and nuclear envelope is known as cytoplasm (Gr, *kytos*-hollow, *plasma*-form). The cytoplasm forms the most essential part of the cell because it is the seat of all biosynthetic and bioenergetic functions. It is composed of two distinct type of structures, the matrix (L., *matrix*-womb) and the organelles (Gr., *organon*-instrument).

MATRIX

After the removal of all the organelles from the cytoplasm, there remains a transparent, homogeneous and colloidal substance called **matrix. Purkinje** (1840) has described the matrix by the name of **protoplasm.** The matrix is also known by various other names such as the ground cytoplasm, fundamental cytoplasm, hyaloplasm and kinoplasm, etc.

PHYSICAL NATURE OF MATRIX

In past, there has been a lot of controversy about the physical nature of the matrix. Different workers advanced different theories about the physical characteristics of the matrix. Their theories can be represented as follows :

1. **Reticular theory** suggests that the matrix is composed of reticulum of fibres or particles in the ground substances(Fig. 3·1 A).
2. **Alveolar theory** was proposed by **Butschili** in 1892 and according to it, the matrix consists of many suspended droplets or alveoli or minute bubbles resembling the foams of emulsion. (Fig. 3·1 B).
3. **Granular theory** was propounded by **Altmann** in 1893. This theory supports the view that the matrix contains many granules of smaller and larger size arranged differently. These granules were known as bioplasts (Fig. 3·1 C)
4. **Fibrillar theory** was proposed by **Fleming** and it holds that the matrix is fibrillar in nature (Fig. 3·1 D).
5. **Colloidal theory** has been forwarded very recently after the electron microscopical observations of the matrix. According to the recent concept, the matrix is partly a true solution, partly a colloidal system.

A solution is a mixture of liquid called solvent and any chemi-l substance in solid or liquid state, called solute. In a solution the rticles of solute should be less than 1/10,000 milimetre in diameter. ne solution part of the matrix consists of water as solvent in which rious solutes of biological importance such as glucose, amino acids, tty acids, electrolytes, minerals, vitamins, hormones and enzymes main dissolved

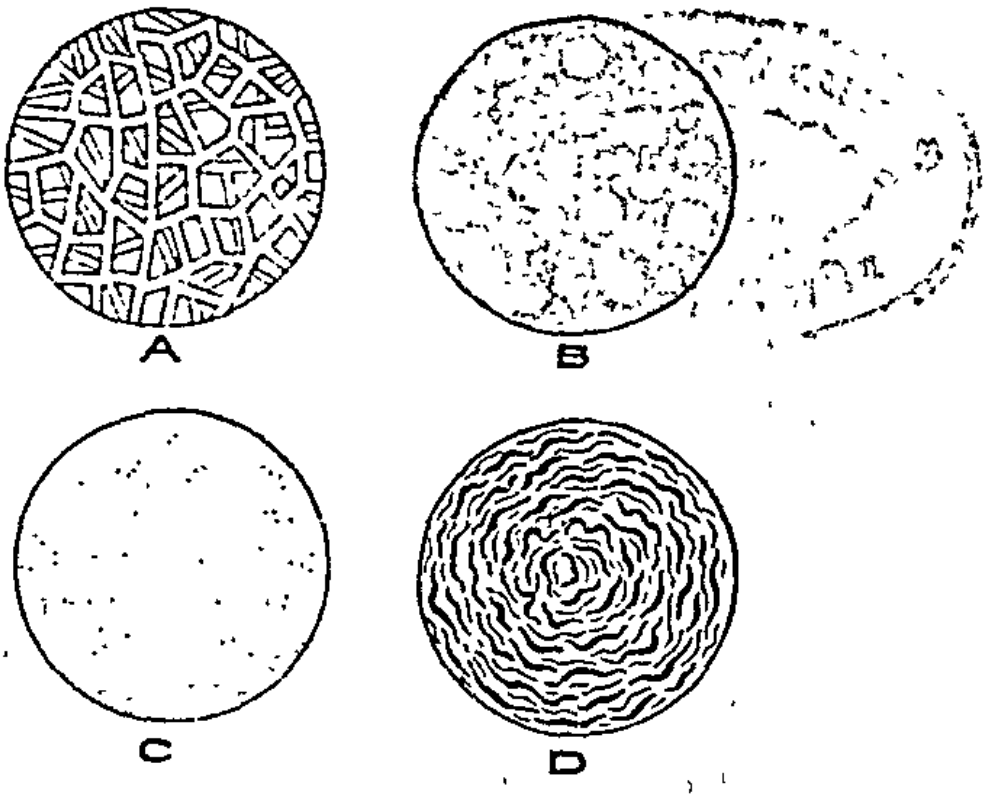


Fig. 3 1. Physical appearance of protoplasm. A—Reticular, B—Alveolar, C—Granular, and D—Fibrillar.

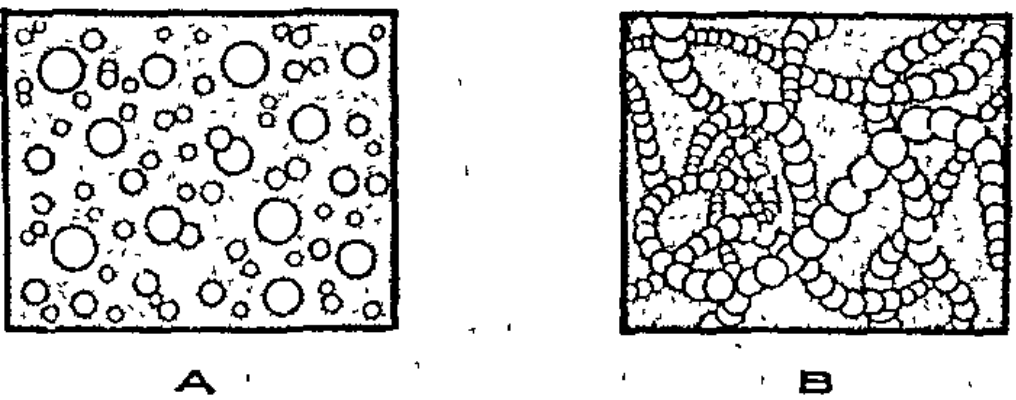


Fig. 3 2. Cytoplasm in sol (A) and gel (B) state.

A colloidal system can be defined as a system which contains a liquid medium in which the particles ranging from about 1/1,000,000 to 1/10,000 millimetre in diameter, remain dispersed. In other

words, a colloid system composed of two phases namely liquid phase and dispersed phase. The colloidal part of the matrix is polyphasic and consists of a liquid phase in the form of water and dispersed phase in the form of macromolecular solids such as proteins and nucleic acids and liquids such as oily fats (lipids).

Phase Reversal—The colloidal system of the matrix may occur either in a semisolid or liquid state. The semisolid state is known as gel phase or gel state, while the liquid state is known as sol phase or sol state. In gel state the molecules of colloidal substances remain held, together by various types of chemical bonds or bond between H—H, C—H or C—N. The stability of gel depends on the nature and strength of chemical bonds.

Both the states of colloidal matrix are interchangeable according to the various physiological, mechanical and biochemical activities of the cell. Thus, a gel can change into sol by **solation** and a sol can change into gel by **gelation**. This property of colloidal matrix is called **phase reversal**.

CHEMICAL ORGANIZATION OF MATRIX

Chemically, the cytoplasmic matrix is composed of many chemical elements in the form of atoms, ions and molecules.

Chemical elements—According to **John Dalton** (1766–1844), a **chemical element** is a fundamental substance which cannot be broken down into two or more simpler substances. Uptill now, about 104 elements have been discovered in the universe. Out of these 104 elements of universe, the cytoplasmic matrix consists of about 36 elements. The 36 elements occur in different percentage in the matrix and perform various important biological and chemical activities for the cell. Generally, they are grouped in the following three categories according to their percentage in the matrix and in molecules.

(i) **Major constituents**—The elements which occur predominantly in the matrix are called **major constituents**. The major consituents of the matrix are oxygen, (O 62%), carbon (C, 20%), hydrogen (H, 10%) and nitrogen (N, 3%). These elements occur in the wide array of chemical molecules (inorganic and organic) of great biological importance.

(ii) **Trace elements**—The elements which occur in low percentage in the matrix are called **trace elements**. The trace elements of the matrix are: calcium (Ca, [illegible]
chlorine Cl, 0·16), sulphur (S, 0·14
(Na, 0·10%), magnesium (Mg, 0·[illegible]
(Fe, 0·10%). These elements occ[illegible]

biologically important molecules for [illegible]

trace elements required by the cell for its various physical and biochemical activities such as osmosis, diffusion, impulse conduction and other various physico-chemical activities.

(iii) Ultrastructure elements : The rest 23 elements such as copper (Cu), cobalt (Co), manganese (Mn), zinc (Zn) molybdenum (Mo), boron (B), silicon (Si), etc., occur in the matrix in 0·756%. Most of them required by the cell as cofactors for various chemical reactions.

Atoms : The elements exist in the form of exceedingly small units called **atoms.** The atoms of all elements are constructed out of **subatomic particles, namely neutrons, protons** and **electrons.** The neutrons are electrically neutral, while protons carry one unit of positive charge. Both of these subatomic particles occur in the centre of atom, called **atomic nucleus.** The electrons carry one unit of negative electrical charge and there are exactly as many electrons present as there are protons in the nucleus. The electrons are maintained in orbit around the nucleus because the positively charged nucleus attracts the negatively charged electrons. Since the total positively charge equals the total negative charge, so the atom as a whole remain electrically neutral.

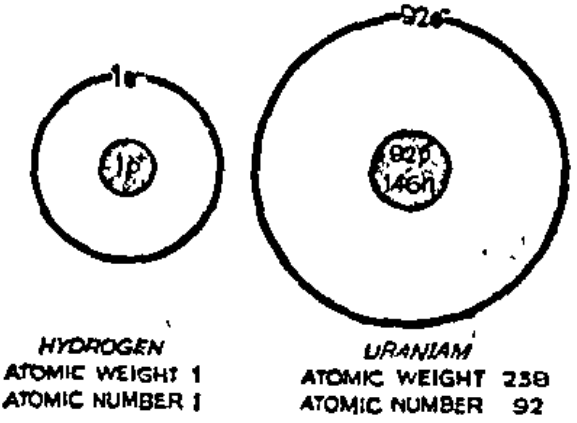


Fig. 3·3. Atomic structure of hydrogen and uranium.

Atomic number : The number of protons present in the atomic nucleus of an atom of an element forms the **atomic number** of that atom. The atomic number of an element is specific. Thus, the atomic number of hydrogen is 1, that of carbon is 6 and that of sodium is 11.

Atomic weight : The weight of a quantity of the element in question relative to the weight of an equal quantity of carbon, which is taken as 12·01, is called the **atomic weight** of that element. Thus, the atomic weights of some biologically important elements are following : nitrogen (14·01), oxygen (16·00), sodium (23·00), chlorine (35·46), potassium (39·10) and iron (55·85).

Ions : If two atoms 'A' and 'B', come extremely close to one another and if the nucleus of atom 'A' attracts the electrons strongly,

then, it may attract not only its own electrons but also those of atom 'B'. If atom 'B' happens to attract electrons rather less strongly, it may then happen that one or more electrons of the 'B' are pulled away from 'B' and are added to 'A'. Atom 'A' now possesses additional electrons, hence additional negative electrical charge. It is, therefore, no longer neutral but electrically negative. At the same time, atom 'B' which has lost electrons and thus, negative charges, is no longer electrically neutral either. The positive charges in the 'B' nucleus are no longer fully neutral, but, electrically positive. Atoms or groups of atoms that have gained or lost one or more electrons are called **ions**. The negatively charged ions are called **anion** and positively charged ions are called **cations**. The process of transfer of electrons among atoms is called **ionization of atoms.**

The cytoplasmic matrix consists of various kin of ions. The ions are important in maintaining osmotic pressure and acid-base balance in the cells. Retention of ions in the matrix produces an increase in osmotic pressure and thus, the entrance of water in the cell. The concentration of various ions in the intracellular fluid (matrix) differs from that in the interstitial fluid. For example, in the cell K^+ and Mg^{++} can be high, and Na^+ and Cl^- high outside the cell. In muscle and nerve cells a high order of difference exists between intracellular K^+ and extracellular Na^+. Free calcium ions (Ca^{++}) may occur in cells or circulating blood. Silicon ions occur in the epithelium cells of grasses. The free ions of phosphate (primary, $H_2PO_4^-$ and secondary, HPO_4^-) occur in the matrix and blood. These ions act as a buffering system and tend to stabilize pH of blood and cellular fluids. The ions of different cells also include sulphate (SO_4^-), carbonate (CO_3^-), bicarbonate (HCO_3^-), magnesium (Mg^{++}) and amino acids.

Compounds and molecules. Under specific conditions most atmos can become attached to certain other atoms. Such combinations of two or more atoms are called **compounds**. In a compound, the atoms are held together by specific bonding forces or chemical bonds. The fundamental or smallest unit of a compound is a **molecule**. A molecule is not a mere mixture of atoms of same or different types of elements, but in a molecule the atoms of different or same elements are bound in different proportions and by different kinds of chemical bonds. A molecule has a definite shape and properties. There are following two possible ways by which atoms of different elements may join each other to form a molecule of a compound :

(i) **Formation of an ionic compound :** When in a group of atoms of different types of elements, due to ionization some atoms

become anions (electronegative) and other atoms become cations (electropositive), both electronegative and electropositive atoms start to attract each other. Subsequently, both atoms become united by a chemical bond, called **ionic or electrovalent bond** and form a compound. Such compounds which are formed by a transfer of electrons between atoms and a mutual attraction of the resulting ions are called **ionic compounds.**

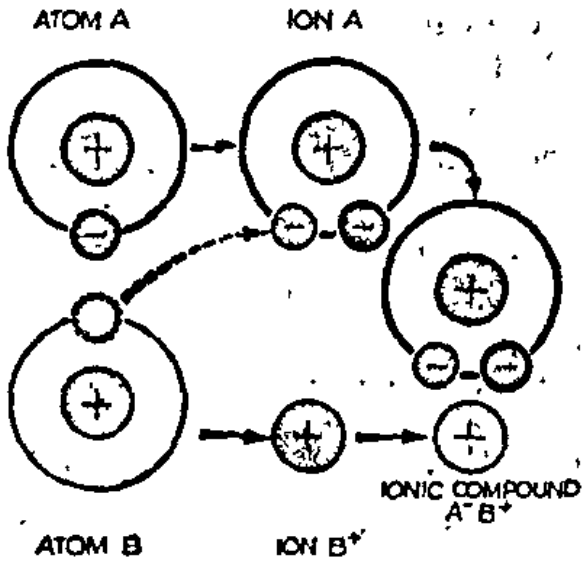


Fig. 3·4. Electron transfer and formation of ionic compound.

The number of the ionic bonds to a ionic compound depends on the number of transferring electrons between two ionized atoms and that varies compound to compound. For example, NaCl is ionic compound. Its single molecule contains a cation (Na^+) and a anion (Cl^-) both of which remain united by a single ionic bond. In such electron transfers, atom that tend to lose electrons and thereby becomes positively charged ions are otherwise called **metals,** and atoms that gain electrons and become negatively charged ions are **non-metals.** Sodium is metal and chlorine is non-metal.

(ii) Formation of molecular compounds : In the previous paragraph, we *have observed that the ionic compounds are typically* formed when in a group of atoms, some (non-metal) attract electrons very strongly whereas others (metals) attract electrons quite weakely. But, when two or more atoms attract electrons with more or less equal force, another type of situation comes in existence. For example, when two atoms of oxygen (A and B) occur in close proximity, then the nucleus of oxygen atom A will attract some of the electrons of atom B, but the nucleus of oxygen atom B now also attracts some of electrons of atom A with equal force and thus, an electronic "tug of the war" will, therefore, take place and neither atom can "win". This mutual tugging will continue indefinitely and as a result the electrons atoms will remain linked together. In this case, thus, no transfer of occurs and also no ion is formed. Instead, as each atom pulls on electrons of the other, some electrons of both atoms are shared in common.

Electron sharing represents a second major way in which compounds are formed. Compounds of this type are called **molecular compounds** or **simply molecules.** The chemical bonds within molecules are called **covalent bonds.** The number of covalent bonds in a molecular compound is related directly to the number of electrons shared.

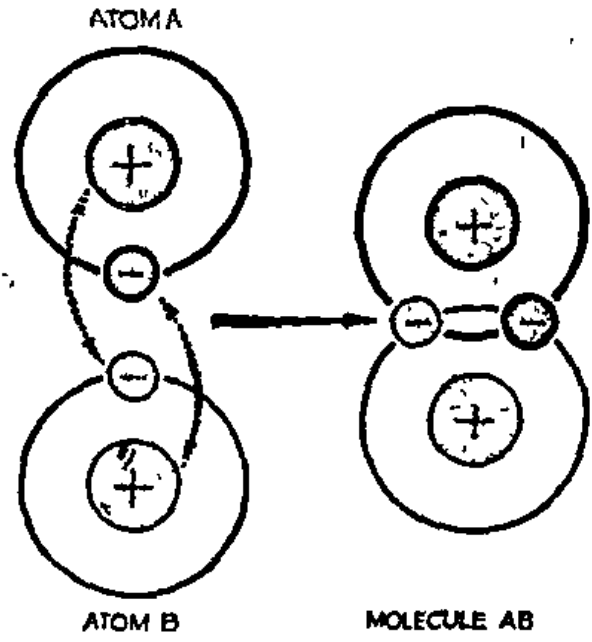


Fig. 3·5. Electron sharing and formation of molecular compounds.

Dissociation : All chemical compounds of biological importance occur in the water medium of the matrix. When ionic compounds are dissolved in water, they generally exist as free ions physically separate from one another, water molecules become interposed between the ions of the compound. The process by which the atoms of a compound become separated as cations or anions is called **dissociation**. Similarly, when the molecular compounds are dissolved in water, one of two events may occur. Some types of molecular compounds simply continue to exist as whole, intact molecules with water interposed between individual ones. Ordinary sugar is a good example of such molecular compounds. By contrast, other types of molecular compounds do not remain intact in water, instead become dissociated into ions like an ionic compound, such as follows :

$$\underset{\textit{Sodium hydroxide}}{NaOH} \xrightarrow{H_2O} \underset{\textit{Sodium ion}}{Na^+} + \underset{\textit{Hydroxyl ion}}{OH^-}$$

$$\underset{\text{Acetic acid}}{CH_3COOH} \xrightarrow{H_2O} \underset{\text{Acetate ion}}{CH_3COO^-} + \underset{\text{Hydrogen ion}}{H^+}$$

Electrolytes and non-electrolytes—In as much as dissociation produces equal amounts of positive and negative electric charges (or cations and anions), solutions containing dissociated ionic or molecular compounds remain electrically neutral. However, the presence of free ions permits passage of electric current through such solutions. Dissociable compounds are therefore, also called **electrolytes** and undissociable compounds are called **non-electrolytes.** The matrix consists of both electrolytes and non-electrolytes.

(i) Electrolytes—The electrolytes play a vital role in the maintenance of osmotic pressure and acid base equilibrium in the matrix. Certain ions such as the magnesium (Mg^+), etc., are essential for many enzymatic activities because these act as cofactor. The phosphate (PO_4) ion is the important constituent of adenosine triphosphate (ATP) which is the chief supplier of energy for most of the life processes.

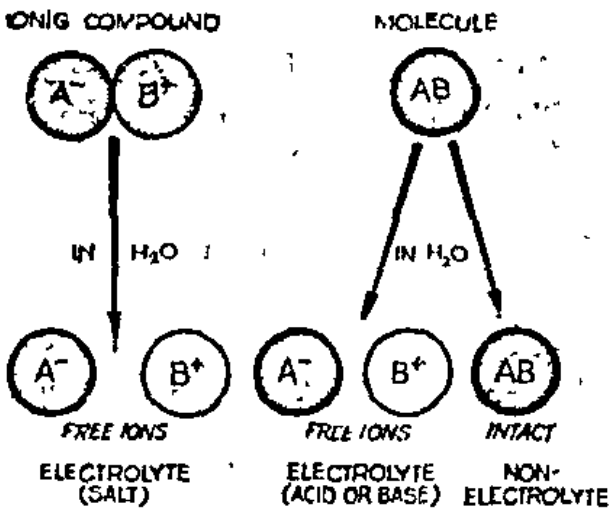


Fig. 3·6. Electrolytes and non-electrolytes.

(ii) Non-electrolytes—Some of minerals occur in matrix in non-ionizing state. The non-electrolytes of the matrix are Na, K, Ca, Mg, Cu, I, Fe, Mn, Fl, Mo, Cl, Zn, Co, Ni, etc. The iron (Fe) occurs in the haemoglobin, ferritin cytochromes and some enzymes as catalase and cytochrome oxidase. The calcium (Ca) occurs in the blood, matrix and the bones. The copper (Cu), manganese (Mn), molybdenum (Mo) and zinc (Zn) are useful as cofactors for enzymatic actions. The iodine and fluorine are essential for the thyroid and the enamel metabolism, respectively.

Acids, bases, and salts—The ionic and molecular compounds of the matrix generally belong to one of the three general classes : acids, bases or salts. An **acid** is a compound containing hydrogen which is replaceable by a metal or in other words an acid is a compound which in solution yields hydrogen ions :

HCl	$\rightarrow$	H^+	$+$	Cl^-
Hydrochloric acid		Hydrogen ion		Chloride ion
H_2SO_4	$\rightarrow$	$2H^+$	$+$	SO_4^-
Sulphuric acid		Hydrogen ion		Sulphate ion

A base is a compound in which a metal is combined with a hydroxyl radical (OH^-) or in other words a base is a compound in which solution yields hydroxyl ions :

KOH	$\rightarrow$	K^+	$+$	OH^-
Potossium hydroxide		Potassium ion		Hydroxyl ion

The salts are composed of metals and non-metals combined in ways which do not qualify them as either acid or bases ; that is, in solution salts yield neither hydrogen ions nor hydroxyl ions :

$NaCl$	$\rightarrow$	Na^+	$+$	Cl^-
Sodium chloride		Sodium ion		Chloride ion

5. The water has high surface tension.

6. The water has greater importance for the various metabolic functions because most of them require exclusively the aqueous media.

7. The water is used by the cell as a transporting media for the food, nitrogen wastes and other necessary substances.

8. The water is immiscible with the non-polar liquids as the lipids, so its molecules remain stable and unmixed with the lipid contents of the plasma membrane and other intracellular membranes.

9. The water is transparent to light, enabling the specialized photosynthetic organelles, the chloroplast, inside the plant cell to absorb the sunlight for the process of photosynthesis.

ORGANIC COMPOUNDS

T[illegible] contain carbon (C) in combi-[illegible]nts as hydrogen (H), nitrogen [illegible] **organic compounds.** The organic [illegible] cules which are formed by the similar or dissimilar unit structures known as the **monomers.** A monomer (Gr., *mono*-one, *meros*-part) is the simplest unit of the organic molecule which can exist freely. Some organic compounds such as carbohydrates occur in the matrix as the monomers. The monomers usually link with other monomers to form **oligomers** (Gr., *oligo*-few or little, *meros*-part) and **polymers** (Gr., *poly*-many, *meros*-part). The oligomers contain small number of monomers, while the polymers contain large number of monomers. The oligomers and polymers contain large-sized molecules or macromolecules. When a polymer contains similar kinds of monomers in its macromolecule it is known as **homopolymer** and when the polymer is composed of different kinds of monomers it is known as the **heteropolymer.**

The main organic compounds of the matrix are the carbohydrates, lipids, proteins, vitamins, hormones and nucleotides.

CARBOHYDRATES

The carbohydrates (L., *carbo*-carbon or coal, Gr., *hydro*-water) are the compounds of the carbon, hydrogen and oxygen. They form the main source of the energy for all living beings. Only green [illegible]e power of synthesizing [illegible] in the presence of sun-[illegible]hotosynthesis. All the [illegible] stem, root, etc), non-[illegible] depend on green parts of plants for the supply of carbohydrates.

Chemically the carbohydrates are polyhydroxy aldehydes or ketones and they are classified as follows :

A. *Monosaccharides (Monomers),*

B. Oligosaccharides (Oligomers), and

C. Polysaccharides (Polymers).

A. Monosaccharides—The monosaccharides are the simple sugars with the empirical formula $C_n (H_2O)_n$. They are classified and named according to the number of carbon atoms in their molecules as follows :

(i) Trioses contain three carbon atoms in their molecules, *e.g*, glyceraldehyde and dihydroxy acetone.

(ii) Tetroses contain four carbon atoms in their molecules, *e.g.*, erythrulose.

(iii) Pentoses contain five carbon atoms in their molecules, *e.g.*, ribose, deoxyribose, arabinose and xylulose.

(iv) Hexoses contain six carbon atoms in their molecules, *e.g*, glucose, fructose and galactose.

(v) Heptoses contain seven carbon atoms in their molecules, *e.g.*, sedoheptulose.

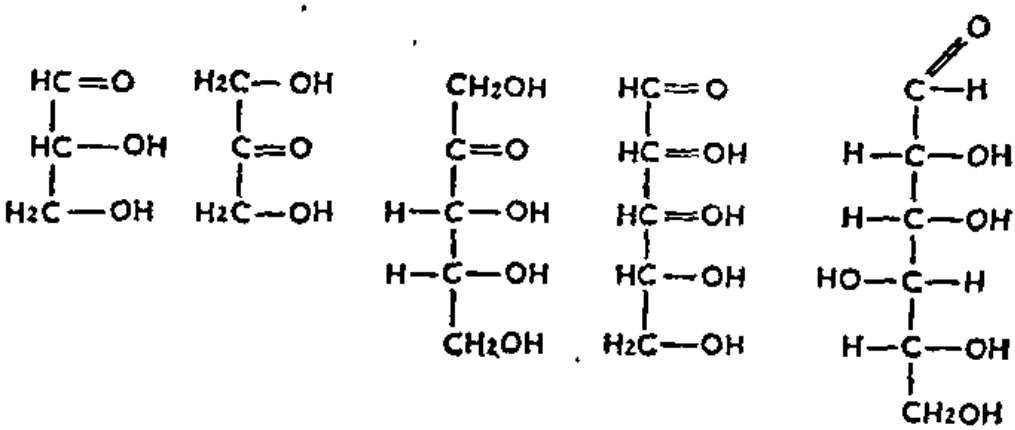


Fig. 3·7. Structure of some monosaccharides.

The monosaccharides are the monomers and cannot split further or hydrolysed into the simpler compounds. The pentoses and hexoses are the most abundantly occurring monosaccharides of the matrix.

The pentose sugar, **ribose** is the important constituent molecule of the ribonucleic acid (RNA) and certain coenzymes as nicotinamide adenine dinucleotide (NAD), NAD phosphate (NADP), adenosine triphosphate (ATP) and coenzyme A (COA) Another pentose sugar the **deoxyribose** is the important constituent of the deoxyribonucleic

acid (DNA). The **ribulose** is a pentose sugar which is necessary for photosynthetic mechanism.

The **glucose**, a hexose sugar is the primary source of the energy for the cell. The other important hexose sugars of the matrix are the fructose and galactose.

B. Oligosaccharides—The oligosaccharides consist of 2 to 10 monosaccharides (monomers) in their molecules. The monomers remain linked with each other by the glycosidic linkages. Certain important oligosaccharides are as follows :

(i) Disaccharides contain two monomers, *e.g.*, sucrose, maltose, lactose, etc.

(ii) Trisaccharides contain three monomers, *e.g.*, reffinose, mannotriose, rabinose, rhaminose, gentianose and melezitose.

(iii) Tetrasaccharides contain four monomers, *e.g.*, stachyose, and scordose.

(iv) Pentasaccharides contain five monomers, *e.g.*, verbascose.

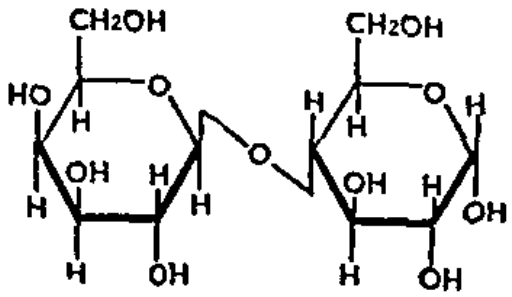


Fig 3·8. Chemical formula of Lactose

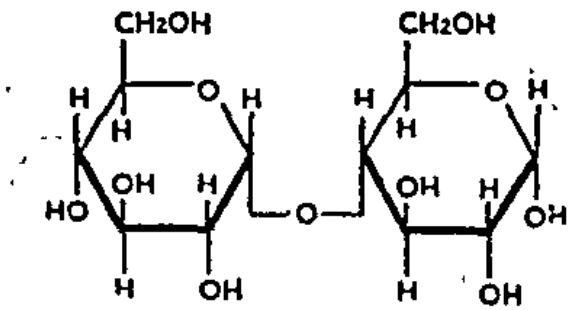


Fig. 3·9. Chemical formula of Maltose.

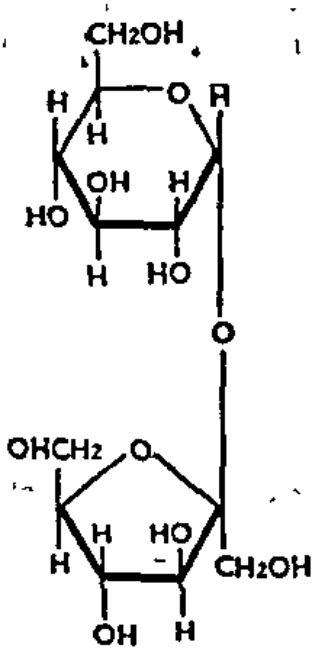


Fig. 3·10. Chemical formula of Sucrose.

The most abundant oligosaccharides of the animal and plant cells are the disaccharides such as sucrose, maltose and lactose. The sucrose and maltose occur mainly in the matrix of plant cells, while the lactose occurs exclusively in the matrix of animal cells. The molecules of sucrose are composed of D-glucose and D-fructose. The molecules of maltose consist of D-glucose. The molecules of lactose are composed of two monomers, *viz.*, D-glucose and D-galactose.

3. *Polysaccharides*—The polysaccharides are composed of ten to many thousands monosaccharides as the monomers in their macromolecules. Their empirical formula is $(C_6H_{10}O_6)_n$. The molecules of the polysaccharides are of colloidal size having high molecular weights. The polysaccharides can be hydrolysed into simple sugars.

The matrix contains two kinds of polysaccharides, *viz.*, homopolysaccharides and heteropolysaccharides.

Homopolysaccharides—The homopolysaccharides contain similar kinds of monosaccharides in their molecules. The most important homopolysaccharides of the matrix are the starch, glycogen and cellulose.

(a) Starch—The molecules of the starch composed of several units of amyloses and amylopectins. The starch is the important storage food material of the plant cells.

(b) Glycogen—The glycogen is a polymer of glucose molecules. It is the important storage food material of animal cells. The glycogen though occurs in most animal cells in smaller amount but the cells of molluses, liver and muscles are rich in glycogen contents.

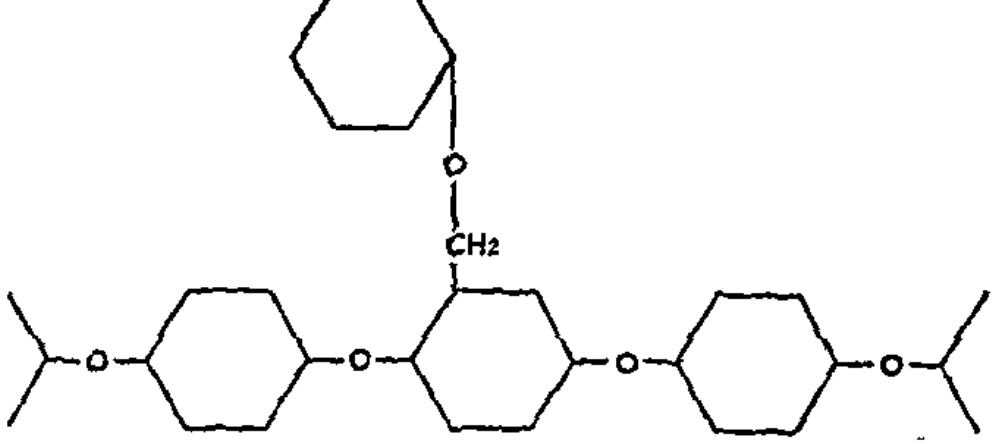


Fig. 3·11. Chemical formula of Glycogen.

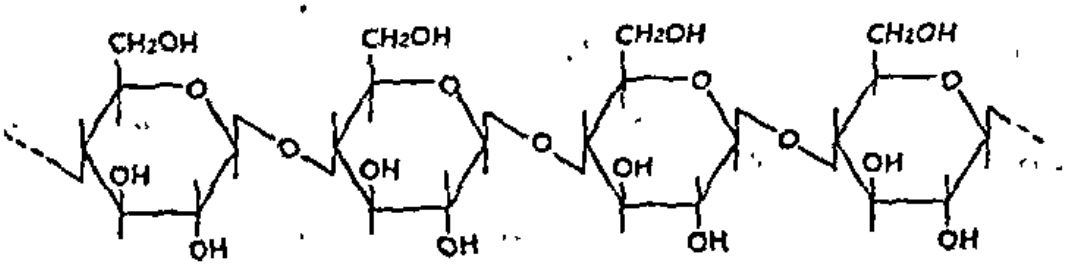


Fig 3 12. Chemical formula of Cellulose.

(c) Cellulose—The cellulose is the polymer of the cellobiose ($C_{12}H_{22}O_{11}$) which in turn contains many glucose molecules in its

molecule. The cellulose forms the cell wall of the plant cells and provides mechanical support to the cell. The plant cells besides containing starch and the cellulose contain other polysaccharides such as xylan, alginic acids (algae), pectic acids, inulin, agar-agar and hemicellulose. Of these, some polysaccharides provide mechanical support to the cell, while others are used as stored food material.

Heteropolysaccharides—The polysaccharides which are composed of different kinds of the monosaccharides and amino-nitrogen or sulphuric or phosphoric acids in their molecules are known as heteropolysaccharides. The most important heteropolysaccharides are as follows :

(a) Neutral heteropolysaccharides—The neutral heteropolysaccharides contain monosaccharides and the acetylated amino-nitrogen in their molecules and are known as **acetyl glucosamines.** The most important acetyl glucosamine of the animal cells is the **chitin** which is an important constituent of the cells of the crustaceans and some insects. The chitin provides mechanical support to the cells.

(b) Acidic heteropolysaccharides—The acidic heteropolysaccharides contain different kinds of monosaccharides and sulphuric or other acids in their molecules. The most important acidic heteropolysaccharides of the matrix of the animal cells are the hyluronic acid, chondroitin sulphate and heparin.

The **hyluronic** acid forms the cementing material of the connective tissues. It occurs in the skin, connective tissues and synovial fluid of the joints. The **chondroitin sulphate** occurs in the cells of the cartilage, skin, cornea, umbilical cord and it serves as a matrix for the bone formation, The **heparin** is a blood anticoagulant and found in the liver, lung, thymus, spleen and blood.

(c) Mucoproteins and Glycoproteins—When the acetyl glucosamines, monosaccharides and proteins unite together, they form **mucoproteins** and **glycoproteins.** These include the blood group polysaccharides and occur in the red blood corpuscle, saliva, gastric mucin, ovomucoids, serum and albumins, etc.

LIPIDS (FATS)

The lipids (Gr., *lipos*-fats) are the organic compounds which are insoluble in the water but soluble in the ether, chloroform, benzene, hot alcohols and petroleum ether. They contain long chains of aliphatic hydrocarbons or benzene ring in their molecules. The lipids are non-polar and hydrophobic. They are important constituents of the cellular membranes, hormones, and vitamins of the cells. They also form the source of energy for the cells. The lipids are classified as follows :

(1) Simple lipids, (2) Compound lipids.

1. Simple lipids—Simple lipids are the esters of the alcohols or the triglycerides containing fatty acids and alcohols.

$$\text{Triglyceride} \xrightarrow[H_2O]{\text{Lipase}} \text{Glycerol} + 3 \text{ Fatty acids.}$$

The constituent fatty acids may be saturated, *e.g.*, palmatic, stearic acids, or unsaturated, *e.g.*, oleic, linoleic, linolenic, arachedonic and clupanadonic acids. The lipids of the matrix of animal cells contain fatty acids, *viz.*, palmatic, stearic palmitoleic, oleic, linoleic and linolenic acids. The simple lipids of the matrix are as follows:

(i) Natural fats—The natural fats are the naturally occurring fats which occur in animal and plant cells as stored food substances.

(ii) Wax—The waxes are the simple lipids and are the esters of fatty acids of high molecular weight with the alcohol except the glycerol. The most important constituent alcohol of the molecules of waxes is the **cholesterol**, *e g.*, bees wax.

2. Compound lipids—The compound lipids contain fatty acids, alcohols and other compounds as phosphorus, amino-nitrogen carbohydrates, etc., in their molecules. The most important compound lipids of the matrix of animal and plant cells are as follows :

(i) Steroids—These lipids contain a common cyclic nucleus of cyclopentano-perhydro-phenanthrene. The common steroids of the matrix of animal cells are the sex hormones, adeno-cortical hormones, vitamin D, and bile acids.

The steroids which possess—OH groups in their molecules are known as **sterols**. The sterol alcohol **cholesterol** occurs in the bile, brain, adernal gland, blood and other animal cells. The plant cells usually contain no cholesterol in there cellular matrix.

(ii) Phospholipids—The phospholipids or phosphotids contain in their molecules, the glycerol, fatty acids and phosphoric acids. The phospholipids are the main components of the lipids of the unit

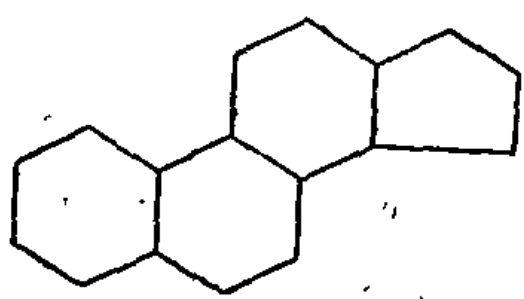


Fig. 3·13. Cyclopentano-perhydro-phenanthrene nucleus of the steroids.

membranes of the cells. In animal cells the phospholipids occur along with the choline, ethanolamine or serine. For instance, the phosphotidyl-cholines or lecithins occur in most animal cells and

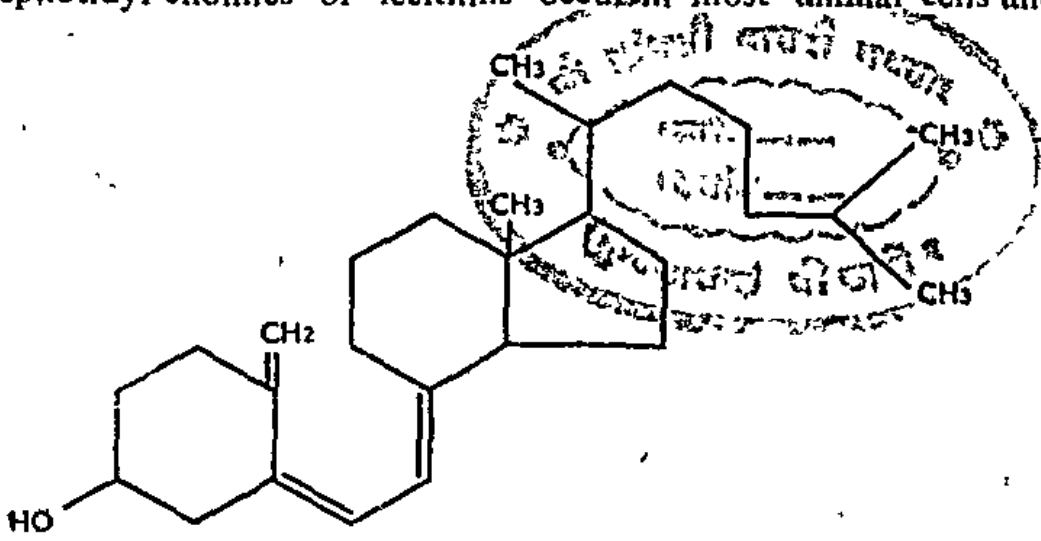


Fig. 3·14. Chemical formula of Cholesterol.

are important for the metabolism of fats in the liver cells. The phosphotidyl ethanolamine and phosphotidyl serine are commonly called as **cephalins.** The other important phospholipids of the matrix are the phosphoinositides (occur mostly in the cells of liver, brain, muscle and soyabean), plasmologens and isositides.

(iii) Sphingolipids—The sphingolipids occur mostly in the cells of the brain. Instead of the glycerol, they contain in their molecules amine alcohol (sphingol or sphingosine). For instance, the myelin sheaths of the nerve fibres contain a lipid known as **sphingomyelin** which contains sphingosine and phospholipids in its molecules.

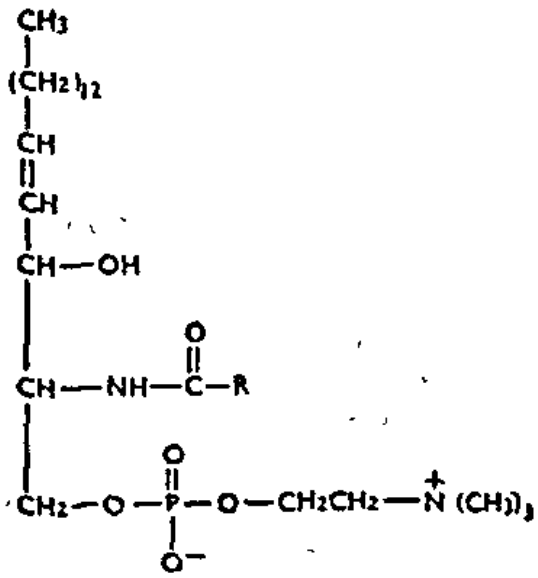


Fig. 3·15. General chemical formula of Sphingomyelin.

(iv) Glycolipids—The glycolipids contain in theirmoleculesthe carbohydratesand the lipids. The matrix of the animal cells contain two kinds of glycolipids, *viz.*, cerebrosides and gangliosides.

(a) Cerebrosides—The cerebrosides contain in their molecules sphingosine, fatty acids and galactose or glucose. The cerebrosides are the important lipids of the white matter of the cells of brain and the myelin sheath of the nerve. The important cerebrosides are the kerasin, cerebron, nervon and oxynervons.

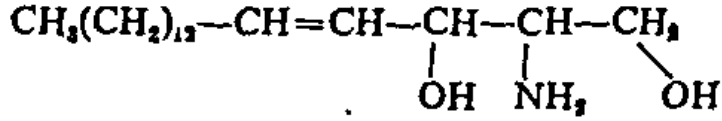


Fig. 3·16. Chemical formula of Sphingosine.

(b) Gangliosides—The gangliosides have complex molecules which are composed of sphingosine, fatty acids and one or more molecules of glucose, lactose, galactosamine and neuraminic acid. The gangliosides occur in the grey matter of the brain, membrane of erythrocytes and cells of the spleen.

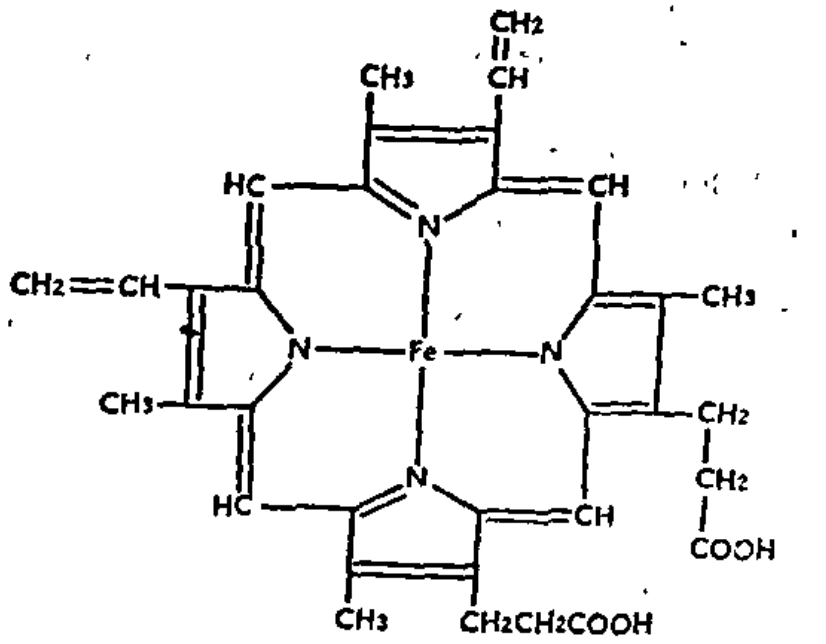


Fig. 3.17. Chemical formula of heme portion of haemoglobin

(v) Lipoproteins—The lipoproteins contain lipids and proteins in their molecules and occur in blood of mammals in association with plasma proteins. In lipoproteins, cholesterol and α and β globulins are found.

(vi) Carotenoids—The carotenoids are the compound lipids and they form the pigments of the animal and plant cells. There are about 70 carotenoids occurring in both type of cells. The important enoids of cells are the α, β and γ carotenes, retineve, xantho-

phylls, lactoflavin in milk, riboflavin (vitamin B_2), xanthocyanins, coenzyme Q, anthocyanins, flavones, flavonols and flavonones, etc. Chemically carotenoids are porphyrins. The porphyrin (Gr. *porphyra*-purple) are the cyclic compound formed by four pyrole $(CH)_4NH$ rings linked by methylene bridges. The porphyrins linked with metals and proteins and forming the important pigments of animal and plant cells such as the chlyrophyll and haemoglobin. The chlorophyll is a porphyrin which contains magnesium in its molecule. Many pigments of animal cells such as haemoglobin, myoglobin and cytochromes contain porphyrins in their molecules.

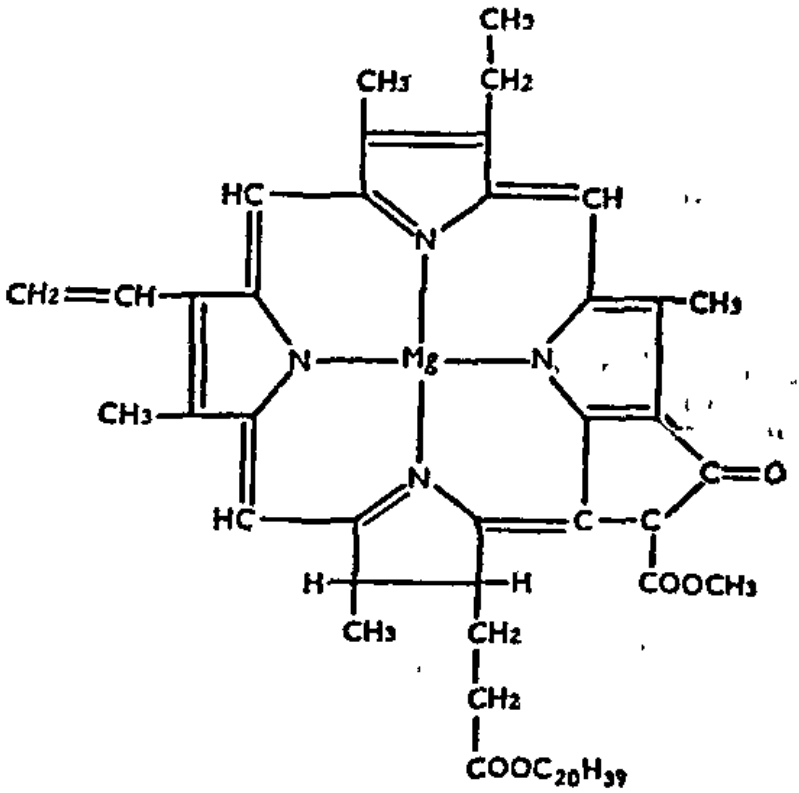


Fig 3 18. Chemical formula of Chlorophyll.

PROTEINS

The proteins (Gr., *proteno*-occupy first place) are the important constituents of the matrix. They are composed of carbon (C), hydrogen (H), oxygen (O) and nitrogen (N) in their molecules. The proteins are the polymers of amino acids.

Amino acids—An org[illegible] or more amino groups ($-NH_2$) and [illegible] ($-COOH$) is known as amino acid. T[illegible] the cytoplasmic matrix and constitute the so called **amino acid pool**. The amino acids are derived from the organic acids in which the hydrogen in alpha position is replaced by the amino groups. For example,

the amino acid **glycine** is formed by the acetic acid and **alanine** derived from propionic acid, is shown by following reactions :

Acetic acids + Amino group → Glycine

Propionic acids + Amino group → Alanine

The general molecular formula of amino acids is

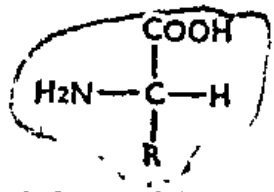
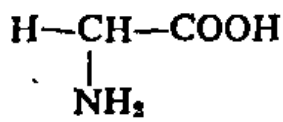
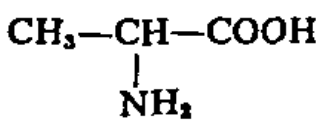
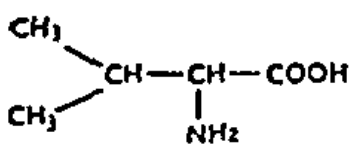
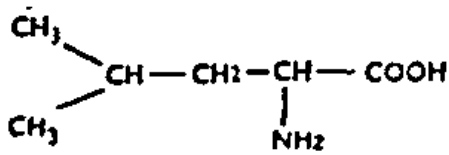
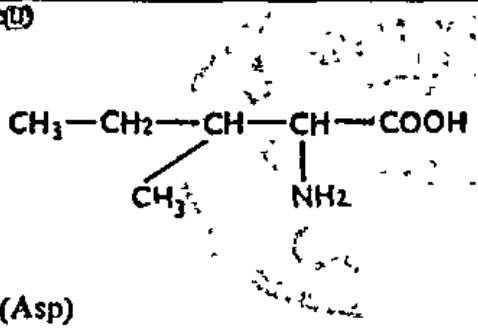
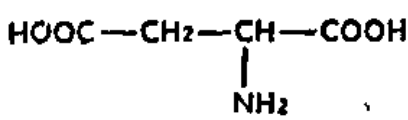
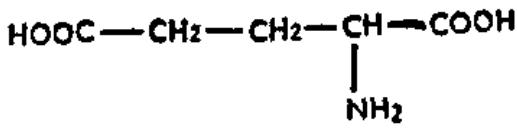
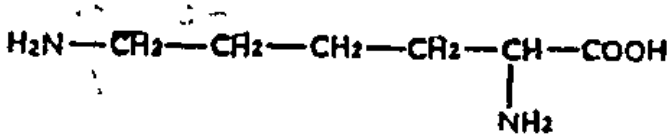
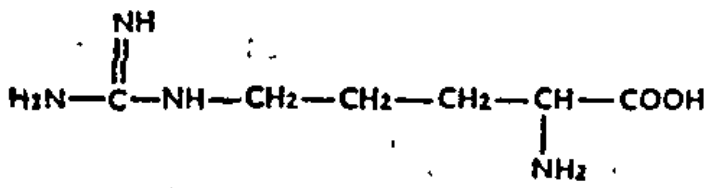
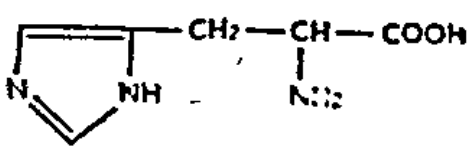
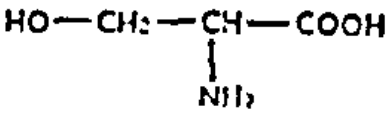
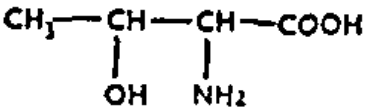
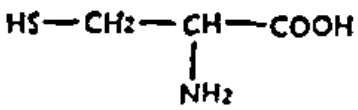
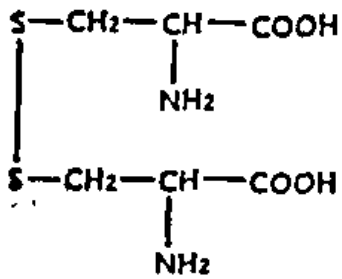
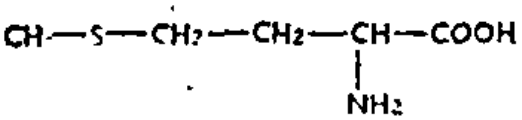
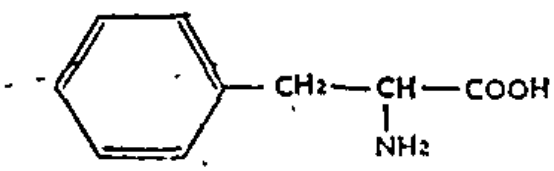


Fig. 3·19. General chemical formula of amino acids.

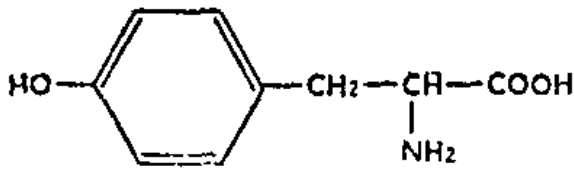
where the NH_2 is the amino group, —COOH is the carboxyl group while the 'R' represents variety of chemical combinations which occur in different types of amino acids. The matrix contains about 22 amino acids which can be tabulated as follows :

Group of amino acid	Name of the amino acids, symbols and chemical formulae
A. Aliphetic amino acids.	
1. Monoamino-monocarboxylic amino acids or simple amino acids.	1. Glycine (Gly)  2. Alanine (Ala)  3. Valine (Val)  4. Leucine (Leu) 

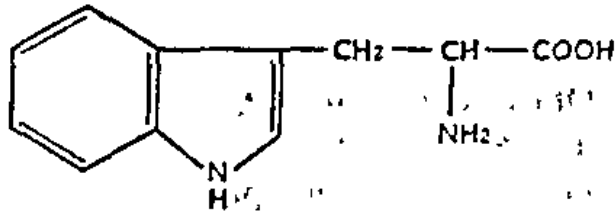
Group of amino acid	Name of the amino acids, symbols and chemical formulae
	5. Isoleucine (Ileu) 
II. Mono—amino-dicarboxylic or acidic amino acids	6. Aspartic acid (Asp) 
	7. Glutamic acid (Glu) 
III. Diamino-mono-carboxylic or basic amino acids	8. Lysine (Lys) 
	9. Arginine (Arg) 
	10. Histidine 

Group of amino acid	Name of the amino acids, symbols and chemical formulae
IV. Hydroxyl containing amino acids.	11. Serine (Ser) 
	12. Threonine (Thr) 
V. Sulphur containing amino acids.	13. Cysteine 
	14. Cystine (Cys) 
	15. Methionine (Met) 
B. Aromatic amino acids.	16. Phenylalanine (Phe) 

Group of amino acid	Name of the amino acids, symbols and chemical formulae
	17. Tyrosine

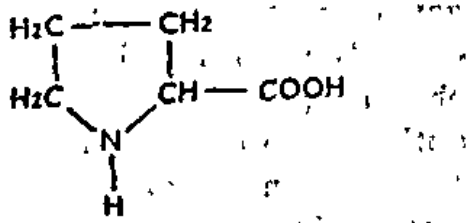


18. Tryptophan (Tryp)

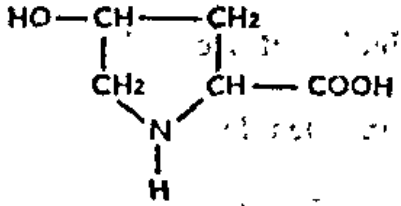


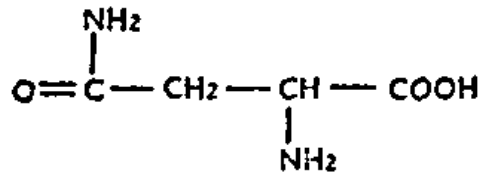
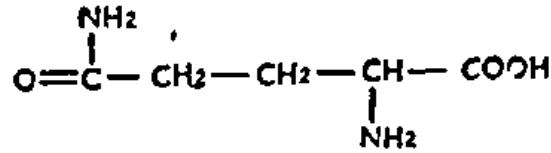
C. Secondary amino acids.

19. Proline (Pro)



20. Hydroxyproline (Hpro)



Group of amino acid	Name of the amino acids, symbols and chemical formulae
D. Amino acid amides.	21. Aspargine  22. Glutamine 

Other amino acids—According to **Mazur** and **Harrow** (1971) following amino acids are not found as part of the usual protein structure; they are either products of metabolism and found in the free state or part of the structure of special peptides—

1. **β—Alanine** is a constituent of carnosine, anserine, panthothenic acid and muscle.
2. **Phosphoserine** is found in casein, and in a number of enzymes.
3. **Ergothionine** is found in ergot and blood.
4. **Hydroxylysine** is found in collagen and in calf embryo in the form of phosphate ester.
5. **Ornithine** is an intermediate in urea biosynthesis.
6. **Citrulline** is an intermediate in urea biosynthesis.
7. ***Homocysteine** is an intermediate in methionine metabolism.*
8. **Cystathionine** is an intermediate in methionine metabolism.
9. **γ—Aminobutyric acid** is found in bacteria, green plant, yeast and brain.
10. **β—Aminoisobutyric acid** is an intermediate of thymine metabolism.
11. **Dihydroxyphenylalanine** is an intermediate of phenyl-alanine metabolism.
12. **Creatine** is found in urea.
13. **Taurine** is found in bile salts.

Formation of Proteins—Because a molecule of the amino acid contains both amino ($-NH_2$) and acidic or carboxyl ($-COOH$) group, it can behave as an acid and base at a time. The molecules of such organic compounds which contain both acidic and basic properties are known as **amphiteric molecules.** Due to amphiteric molecules, the amino acids unite with one another to form complex and large protein molecules. When two molecules of amino acids are combined then the basic group ($-NH_2$) of one amino acid molecule combines with the carboxylic ($-COOH$) group of other amino acid and the loss of a water molecule takes place. This sort of condensation of two amino acid molecules by —NH—CO linkage or bond is known as **peptide linkage or peptide bond.** A combination of two amino acids by the peptide bond is known as **dipeptide.**

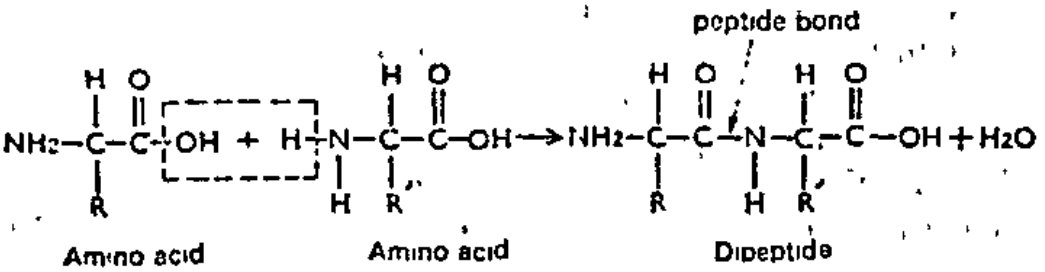


Fig. 3·20. A chemical reaction showing formation of a dipeptide.

When three amino acids are united by two peptide bonds, they form **tripeptide.** Likewise, by condensation of few or many amino acids by the peptide bonds the **oligopeptides** respectively. The various molecules of the **peptones, proteases and proteins.** are the polymers of many amino acid monomers. The macromolecules of proteins comparatively have high molecular weights. The molecular weight of certain animal proteins are given in the following table :

Table : Molecular weight of some animal proteins.

S. No.	Name of the protein	Molecular weight
1.	Insulin	12,000
2.	Cytochrome (Horse heart)	12,100
3.	Trypsin	23,800
4.	Pepsin	35,500
5.	Ova albumin	44,000

S. No.	Name of the protein	Molecular weight
6.	Serum albumin (Human)	65,000
7.	Haemoglobin (Human)	67,000
8.	Y-globulin (Human)	100,000
9.	Catalase	250,000
10.	Collagen	345,000
11.	Thyroglobin (Pig)	650,000

TYPES OF PROTEINS

The proteins are the organic compounds of immense biological significance. They are of following two types :

A. Simple proteins—The proteins which contain only amino acids in their molecules are known as simple proteins, *e.g.*, albumins, *globulins, histones, prolamine and protamine.*

B. Conjugated proteins—The proteins which contain amino acids and other chemical compounds in their molecules are known as conjugated proteins. Some important conjugated proteins are following :

1. Glycoproteins and mucoproteins contain carbohydrates and proteins in their molecules, *e.g.*, musin of saliva.

2. Lipoproteins contain amino acids and lipids in their molecules, *e.g.*, lipovitellin of egg yolk, serum, protein of brain and nerve tissues.

3. Nucleoproteins contain amino acids and nucleic acids in their molecules, *e.g.*, viruses and nucleic acid proteins.

4. Chromoproteins contain amino acids and pigments in their molecules, *e.g.*, haemoglobin, haemocyanin, flavoproteins and cytochromes.

5. Phosphoproteins contain amino acids and phosphate groups in their molecules, *e.g.*, casein of the milk.

6. Metalloproteins contain amino acids and metallic ions in their molecules, *e.g.*, carbonic anhydrase enzyme contains zinc ions and amino acids in its molecule.

The simple and conjugated proteins may have different types of molecular structures and on the basis of arrangement of polypeptide chains in the protein molecules following kinds of proteins have been recognized :

1. Primary proteins—The molecules of primary proteins contain many amino acids which remain arranged in a single linear chain by the peptide bonds, *e.g.*, insulin, ribonuclease.

2. Secondary proteins—The secondary proteins contain spirally or helical [illegible] in their molecule [illegible] or scleroproteins [illegible].

3. Tertiary proteins—The tertiary proteins are composed of many polypeptide chains which remain held together by —S—S— bonds in their molecules. These molecules contain compactly arranged helix which assumes ellipsoid or other form, *e.g.*, globular proteins.

4. Quaternary proteins—The quaternary proteins contain two or many polypeptide chains of similar or different nature which remain bound together by weak covalent bonds, *e.g.*, haemoglobin.

NATURE OF THE PEPTIDE BONDS OF PROTEIN MOLECULE

The different kinds of the proteins contain various types of peptide bonds in their molecules which are as follows :

1. Chemical or covalent bonds—These bonds occur in the protein molecules containing amino acids of similar nature, *e.g.*, –S–S– bond in cystine of insulinand ribonuclease.

2. Ionic or electrostatic bonds—These are weaker bonds which occur in secondary and tertiary proteins.

3. Hydrogen bonds—The hydrogen bonds are weaker than [illegible]

value of the solvent. 3. The proteins can bind salts by forming ionic bonds. 4. The proteins are precipitated by salts of heavy metals and by high temperature. 5. Certain proteins are specific for a particular [illegible]

[illegible] :wo types of proteins can be recognised :

1. The structural proteins which form various structures of the cell, **2. The functional proteins** or **enzymes** which function as specific catalysts in most biosynthetic and metabolic activities of the cell.

ENZYMES

The cytoplasmic matrix and many cellular organelles contain very important organic compounds known as the **enzymes**. The word enzyme (Greek. "in yeast") has been proposed by **Khune** in 1878. The enzymes are the specialized proteins and they have the capacity

to act as catalysts in chemical reactions. Like the other catalysts of chemical world, the enzymes are the catalysts of the biological world and they influence the rate of a chemical reaction, while themselves remain quite unchanged at the end of the reaction. The substance on which the enzymes act is known as substrate. The enzymes play a vital role in various metabolic and biosynthetic activities of the cell such as synthesis of DNA, RNA and protein molecules and metabolism of carbohydrates, lipids, fats and other chemical substances. The enzymes of the matrix and cellular organelles are classified as follows :

1. Oxireductases—The enzymes catalyzing the oxidation and reduction reactions of the cells are known as oxireductases. These enzymes transfer the electrons and hydrogen ions from the substrates, *e.g.*, hydrogenases or reductases, oxidases, oxygenases and peroxidases.

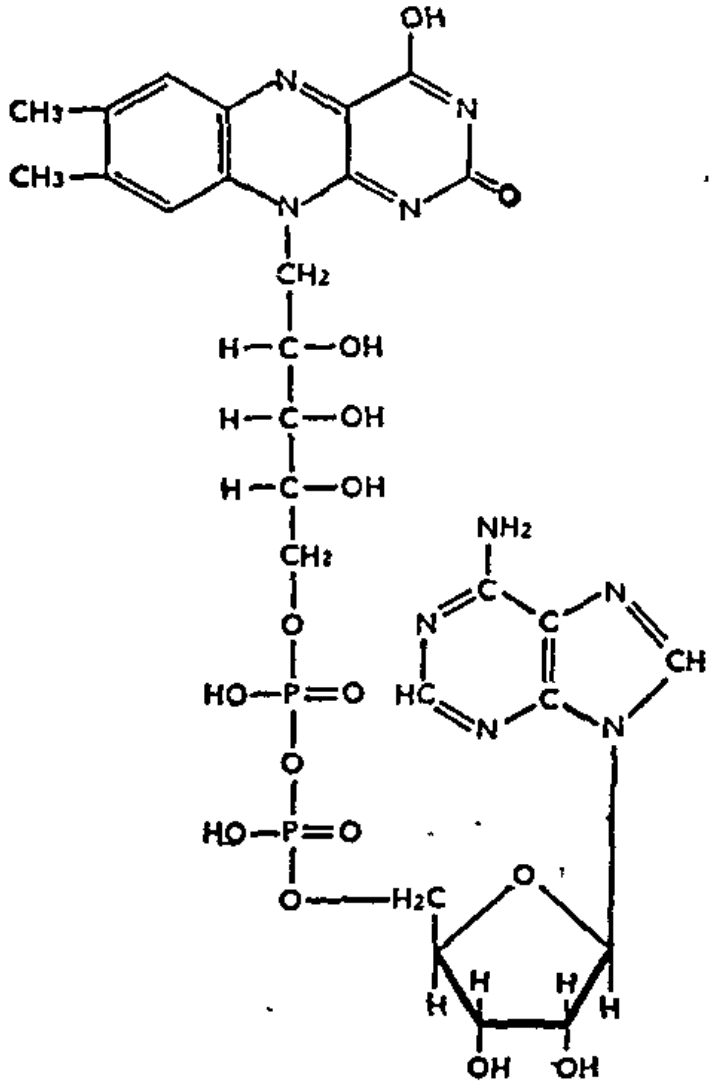


Fig. 3·21. Chemical formula of flavin adenine dinucleotide (FAD).

2. Transferases—The enzymes which transfer following groups from one molecule to other are known as transferases : one carbon, aldehydic or ketonic residues, acyl, glycosyl, alkyl, nitrogenous, phosphorus containing groups and sulphur containing groups.

3. Hydrolases—These enzymes hydrolyse a complex molecule into two compounds by adding the element of the water across the bond which is cleaved. These enzymes act on the following bonds ester, glycosyl, ether, peptide, other C—N bonds, acid anhydride, C–C, halide and P–N bonds. Certain important hydrolase enzymes are the profeases, esterases, phosphatases, nucleases and phosphorylases.

4. Lysases—The lysase enzymes add or remove group to or from the chemical compounds containing the double bonds. The lysases act on C—C, C—O, C—N, C—S and C—halide bonds.

5. Isomerases—These enzymes catalyze the reaction involving in the isomerization or intramolecular rearrangements in the substrates, *e.g.*, intramolecular oxidoreductases, intramolecular transferases, intramolecular lysases, cis-trans-isomerases, racemases and epimerases.

6. Ligases or synthetases—These enzymes catalyze the linkage of the molecules by splitting a phosphate bond. The synthetase enzymes form C—O, C—S, C—N and C—C bonds.

According to the chemical nature of the substrate the enzymes have also been classified as follows :

1. Carbohydrases, 2. Proteases (endopeptidases and exopeptidases), 3. Amylases, 4. Esterases, 5. Dehydrogenases, 6. Oxidases, 7. Decarboxylases, 8. Hydrases, 9. Transferases, and 10. Isomerases.

The enzymes are specific in action and many factors such as pH, temperature and concentration of the substrate effect the rate of the activity of enzymes. Certain enzymes, *viz.*, **zymogens**, occur in the inactive form and these are activated by other enzymes known as **kinases** to perform catalytic activities. Likewise, the ezymes **trypsinogen** of the pancreatic cells is activated in the intestine by the enzyme **enterokinase** and the enzyme **pepsinogen** of the chief cells of the stomach is activated by the **hydrochloric acid** which is secreted by parietal cells.

PROSTHETIC GROUPS AND COENZYMES

Certain enzymes such as **cytochromes** are the conjugated proteins and contain prosthetic group as **metalloporphyrins** complex in their molecules.

Certain enzymes cannot function singly but they can function only by the addition with the small molecules of other chemical substance which are known as **coenzymes**. The inactive enzyme

(which cannot function singly) is known as the apoenzyme. The apoenzyme and coenzyme are collectively known as holoenzyme. For instance, the enzyme hydrogenases is an apoenzyme which can function either with the coenzyme NAD^+ or NADP.

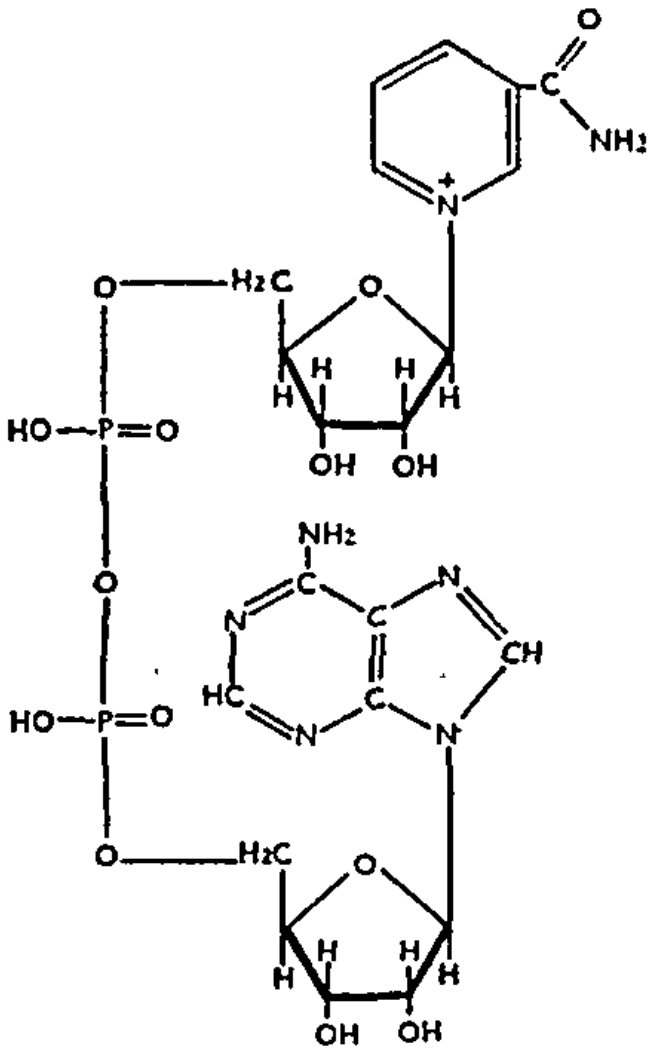


Fig. 3 22. Chemical formula of Nicotinamide adenine dinucleotide (NAD+).

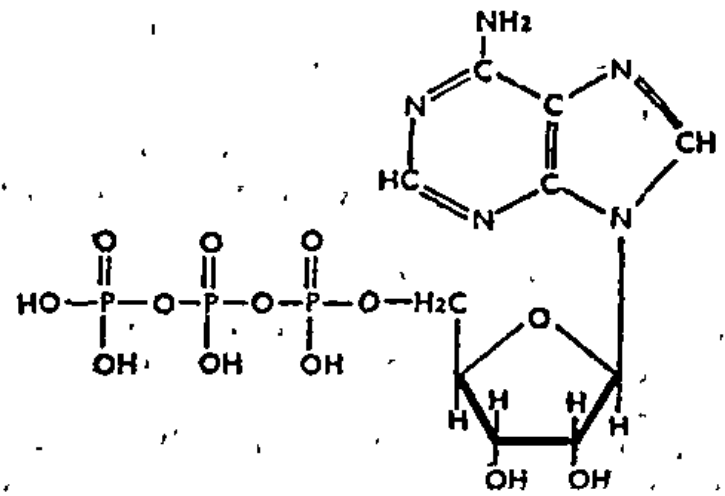


Fig. 3·23. Chemical formula of Adenosine triphosphate (ATP).

Some important coenzymes or cofactors are as follows :

1. Nicotinamide adenine dinucleotide (NAD) or Diphosphopyridine nucleotide (DPN), 2. Nicotinamide adenine dinucleotide phosphate (NADP) or *Triphosphopyridine nucleotide* (TPN), 3. Flavin adenine mononucleotide (FAM), 4. Flavin adenine dinucleotide (FAD), 5. Ubiquinone (coenzyme Q or Q), 6. Lipoic acid (LIP or S_2), 7. Adenosine triphosphate (ATP), 8. Pyridoxyl phosphate (PALP), 9. Tetrahydrofolic acid (CoF), 10. Adenosylmethionine. 11. Biotin, 12. Coenzyme A (CoA), 13. Thiamine pyrophosphate (TPP), 14. Uridine diphosphate (UDP).

ISOENZYMES

Recently, it has been investigated that some enzymes have similar activities and almost similar molecular structures. These enzymes are known as **isoenzymes.** The isoenzymes have relation with the heredity (**Latner and Skillen,** 1969, and **Weyer** 1868) There are about 100 isoenzymes in the cell, *e. g.*, lactic dehydrogenase (LDH) occur in the form of five identical isoenzymes.

VITAMINS

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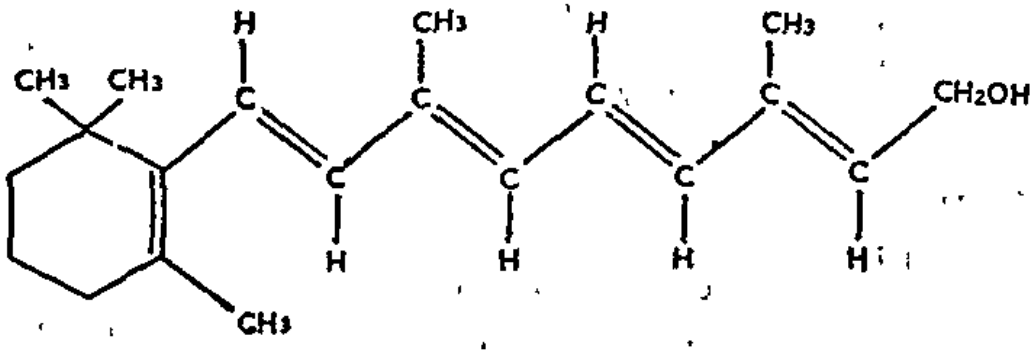


Fig. 3 24. Chemical formula of Vitamin A.

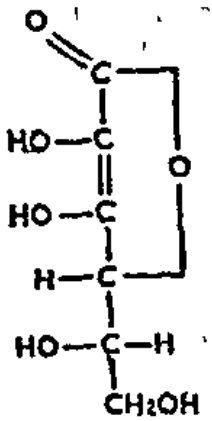


Fig 3 25. Chemical formula of Vitamin C or ascorbic acid.

the cellular metabolism and act as the enzymes or other biological catalysts in the various chemical activities of the cell. Their importance for the animals has been reported by **Hopkins, Osborne, Mendal,** and **McCollum** (1912—1913). **Funk** (1912) demonstrated the presence of basic nitrogen in them and give the name "vitamins" meaning vital amines to them.

The cell cannot synthesize the vitamins from

Table : Classification and other characters of vitamins

Vitamin	Chemical nature	Sources	Diseases due to deficiency	Cellular functions
Fat Soluble Vitamins				
A	Carotenoid	Milk, butter, liver, fish, carrots and vegetables.	Xeropthalmia, hardening of cornea, night blindness and growth interference.	Maintains the integrity of epithelial cells, forming the part of the visual purple (rhodopsin) of retinal cells and necessary for growth.
D	Sterol	Fish liver oils steroid containing food, ultraviolet light, butter, eggs.	Bone defects, *e.g.*, rickets and osteomalacia.	Regulates calcium and phosphorus metabolism.
E	Tocopherol	Green salad, vegetable oils, egg yolk and meat.	Sterility in male rats.	Rapid cell proliferation.
K	1-4 napinthoquinone acetate	Green leafy vegetables, spinach, liver and eggs.	Haemorrhage	Forms prothrombin in the liver which is necessary for clotting of blood.
Water Soluble Vitamins				
B_1	Thiamine	Unpolished cereal grains, yeast, egg yolk, liver, nuts, and pork.	Beriberi and loss of appetite.	Coenzyme for pyruvate metabolism in cellular respiration and metabolism of carbohydrates. Promotes appetite.
B_2	Riboflavin	Liver, cheese, milk, egg-white, meat, kidney and green leaves.	Catract, form lesions in the mouth, tongue, nose, and eyes, cheiosis.	Cellular respiration and growth.
Niacin	Nicotinic acid	Milk, liver, kidney, meat, legumes, coffee, tea, eggs, vegetables, etc.	Pellagra.	Part of dehydrogenase coenzymes (NAD and $NADA_2$). Controls cellular functions
B_6	Pyridoxine	Yeast, polished rice, cereal grains egg-yolk, royal jelly (bee)	Anaemia, skin lesions.	Coenzyme in the metabolism of amino acids

Vitamin	Chemical nature	Sources	Diseases due to deficiency	Cellular functions
Pantothenic acid	Pantothenic acid	Yeast, liver, kidney, eggs-wheat, rice. bran, peanuts and peas.	Dermatitis and spectacle eye.	Part of coenzyme A.
Biotin (H)	Biotin	Egg-yolk, liver, kidney, milk, yeast and royal jelly (bee).	Dermatitis (rat, pig, fowl), paralysis, muscle pain.	Coenzyme in CO_2 fixation.
Folic acid	Pteroylglutamic acid	Green leafy vegetables, yeast, beef, wheat and soya-bean	Anaemia	Part of coenzymes of nucleic acid metabolism and essential for growth and formation of blood cells.
B_{12}	Cyanocobalamin	Liver, eggs, milk, meat, and fish	Pernicious anaemia.	Coenzyme of an enzyme involved in methyl transfer and nucleic acid metabolism and red blood cell formation.
Protogen	Thioctic acid or α-Lipoic acid	Yeast and liver		Coenzyme in pyruvate oxidation.
C	Ascorbic acid	Tomatoes, citrus fruits, green vegetables, flowers, and pepper.	Scurvy	Maintains optimal oxidation reduction potential, capillary walls and normal growth of bones and teeth.
P	Eriodictin, hesperidin, rutin.	—	Capillary fragility.	Maintains cement of capillary walls.

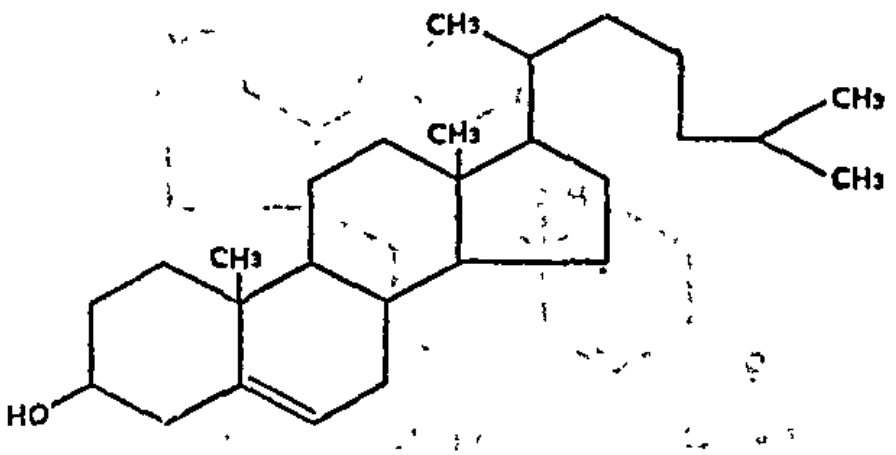


Fig. 3·26. Chemical formula of vitamin D.

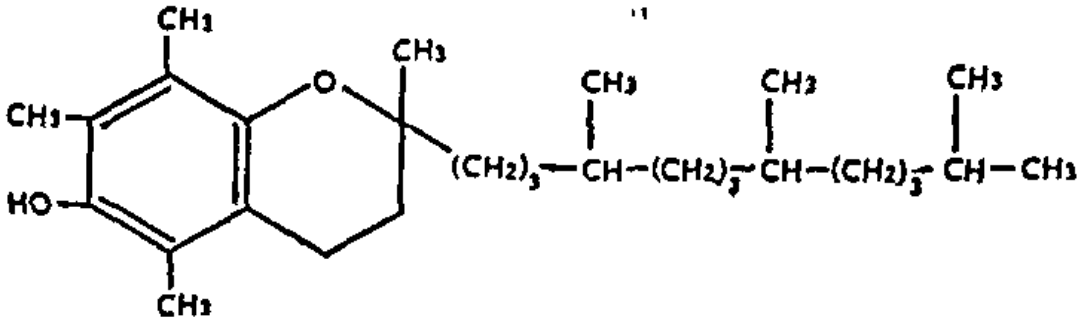


Fig. 3·27. Chemical formula of vitamin E (alphatocopherol).

HORMONES

Hormones are the complex organic compounds which occur in traces in the cytoplasm and regulate the synthesis of mRNA, enzymes and various other intracellular physiological activities The most important hormones are growth hormones, estrogen, androgen, insulin, thyroxine, cortisone, and adrenocortical hormones, etc. These hormones are synthesized by the ductless glands and transported to various cells of multicellular organisms by blood vascular system. In cells they regulate various metabolic activites. For example, the ecdysone hormone has been found to form puffs (Balbiani rings) in the giant chromosomes of insects. The hormones activate or depress the gene at the particular locus on the chromosome. Thus, hormones serve to coordinate the various activities concerned with a particular function, *e.g.*, the hormone ecdyosone control moulting and metamorphosis in insects (Beermann, 1965).

In [illegible] cells, the [illegible] convert glucose [illegible] which is synthe- [illegible] ncreas. Moreover, [illegible] nd, activates the enzyme phosphorylase to form glucose phosphate from the glycogen.

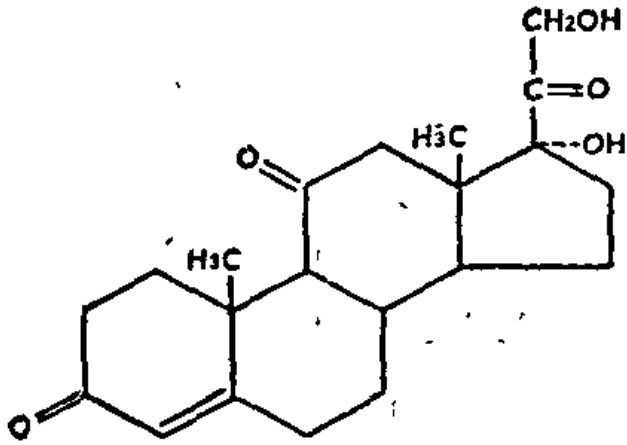


Fig. 3·28. Chemical formula of Cortisone.

NUCLEIC ACIDS

The nucleic acids are the complex macromolecular organic compounds of immense biological importance. They control the important biosynthetic activities of the cell and carry hereditary informations from generation to generation. There occur two types of nucleic acids in living organims, *viz.*, **Ribonucleic acid (RNA)** and

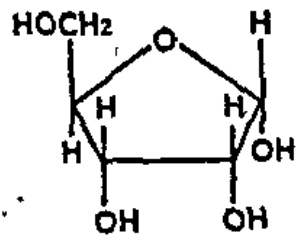


Fig. 3·29. Chemical formula of ribose sugar.

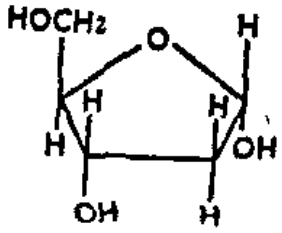


Fig. 3·30. Chemical formula of deoxyribose sugar.

Deoxyribonucleic acid (DNA). Both types of nucleic acids are the polymers of the nucleotides. [illegible] and phosphoric acid. Even th[illegible] tose sugars (Ribose or Deoxyribose) and nitrogen bases (Purines or Pyrimidines). The **purines** are **adenine** and **guanine** and the **pyrimidines** are the **cytosine, thymine** and **uracil.** The cytoplasmic matrix contains only RNA, while DNA exclusively remains concentrated in the nucleus.

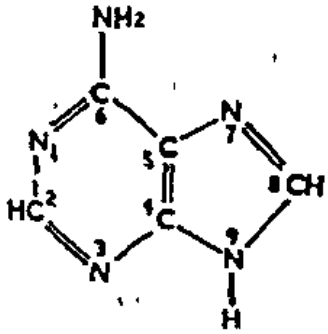


Fig 3·31. Chemical formula of Adenine.

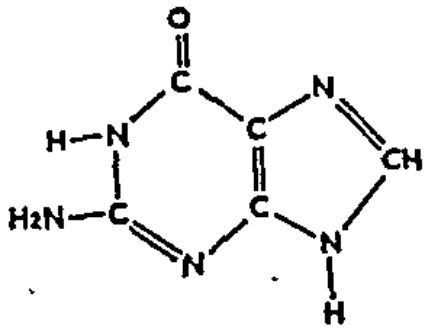


Fig. 3·32. Chemical formula of Guanine.

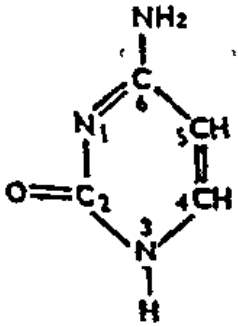


Fig 3·33. Chemical formula of Cytosine.

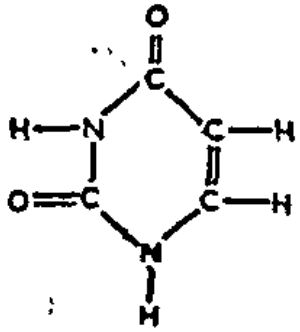


Fig. 3·34. Chemical formula of Uracil.

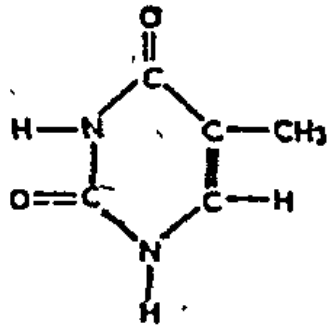


Fig. 3·35. Chemical formula of Thymine.

The DNA and RNA have almost similar chemical compositions except a few differences. Both can be compared as follows :

DNA	RNA
1. It contains pentose sugar known as deoxyribose.	1. It contains pentose sugar called the ribose.
2. The molecule contains the phosphoric acid (phosphate) molecule which connects various sugars with one another.	2. The molecule contains the phosphoric acid (phosphate) molecule which connects various sugars with one another.
3. The nitrogen bases are : (i) Purines—adenine and guanine. (ii) Pyrimidine—cytosine and thymine.	3. The molecule contains following nitrogen bases in its molecule : (i) Purines—adenine and guanine. (ii) Pyrimidines—cytosine and uracil.
4. Molecules have four nucleotides as deoxyadenosine monophosphate, deoxyguanosine monophosphate, deoxycytidine monophosphate and thymidine monophosphate.	4. Molecules have four nucleotides as , adenosine monophosphate, guanosine monophosphate, cytidine monophosphate and uridine monophosphate.
5. The molecule contains a double *stranded helix structure in* which many nucleotides remain arranged in pair.	5. The molecules consist of single *chain of polynucleotides.*
6. DNA is a genetic material and occurs in chromosomes, nucleoplasm and mitochondria, etc.	6. RNA is a carrier of genetic informations and it plays very significant role in the mechanism of protein synthesis. It mostly occurs in nucleolus, nucleoplasm and cytoplasm.

PROPERTIES OF CYTOPLASMIC MATRIX

The matrix is a living substance and it has following physical and biological properties :

PHYSICAL PROPERTIES

The most of the physical properties of the matrix are due to its colloidal nature and these are as follows :

1. **Tyndall's effect**—when a beam of strong light is passed through the colloidal system of the matrix at right angles in the dark room, the small colloidal particles which remain suspended in the colloidal system, reflect the light. The path of the light appears like a cone. This light cone is known as Tyndall's cone because this phenomenon has been first of all reported by Tyndall (1820-1893) in colloids.

2 Brownian movement. The [illegible]ed colloidal particles of [illegible] This movement of molecules which strike with the colloidal molecules to provide motion to them. This type of movement was first of all observed by Scottish botanist **Robert brown** in 1827 in the colloidal solution. Therefore, such movements are known as **Brownian movement**. The Brownian movement is the peculiarity of all colloidal solutions and depends on the size of the particles and temperature.

[illegible] —Due to the phase re[illegible] the intracellular streaming or movement of the matrix takes place. This property of intracellular movement of matrix is known as the **cyclosis**. The cyclosis usually occurs in the sol-phase of the matrix and is effected by the hydrostatic pressure, temperature, pH, viscocity, etc. The intracellular movements of the pinosomes, phagosomes and various cytoplasmic organelles such as the lysosomes, mitochondria, chromosomes, centrioles, etc., occur only due to cyclosis of the matrix. The cyclosis has been observed in most animal and plant cells.

The amoeboid movement depends directly on the cyclosis. The ameoboid movement occurs in the protozoans, leucocytes, epithelial, [illegible]

[illegible] molecules equally in all directions. At the surface of the liquid where it touches air or some other liquid, however, they are attracted downward and sideways or inward, more than upward; consequently they are subjected to unequal stress and are held together to form a membrane. The force by which the molecules are bound is called the **surface tension** of the liquid. The cytoplasmic matrix being a [illegible] have high surface tension, therefore, occur in deeper part of the matrix.

5. **Adsorption**—The increase in the concentration of a substance at the surface of a solution is known as adsorption (L.,*ad*-to, *sorbex*-to draw in). The phenomena of adsorption helps the matrix to form protein boundaries.

6. **Other mechanical or physical properties of matrix**—Besides surface tension and adsorption the matrix possesses other mechanical properties, *e g.*, elasticity, contractility, rigidity and viscosity which provide to the matrix many physiological utilities.

7. **Polarity of the egg**—The colloidal system due to its stable phase determines the polarity of the cell matrix which cannot be altered by centrifugation or other mechanical means

8. **Buffers and pH**—The matrix has a definite pH value and it dose not tolerate significant variations in its pH balance. Yet various metabolic activities produce small amount of excess acids or bases. Therefore, to protect itself from such pH variation the matrix contains certain chemical compounds as carbonate—bicarbonate system known as buffers which maintain a constant state of pH in the matrix.

BIOLOGICAL PROPERTIES

The matrix is a living substance and it has following biological properties .

1. **Irritability**—The irritability is the fundamental and inherent property of the matrix. It possesses a sensitivity to stimulation, an ability to transmission of excitation and ability to react according to stimuli. The heat, light, chemical substances and other factors stimulate the cytoplasmic matrix to contract.

2. **Conductivity**—The conductivity is the process of conduction or transmission of excitation from the place of its origin to the region of its reaction. The matrix of nerve cells possesses the property of the conductivity.

3. **Movement**—The cytoplasmic matrix can perform movement due to cyclosis. The cyclosis depends on the age, water contents, heredity factors and composition of the cells.

4. **Metabolism**—The matrix is the seat of various chemical activities. These activities may be either constructive or destructive in nature. T[illegible] teins, lipids, [illegible] processes, w[illegible] stuffs, etc., are known as **catabolic processes**. The anabolic and catabolic processes are collectively known as metabolic process.

5. **Growth**—Due to the secretory or anabolic activities (Gr., *anabolism*—a throwing up) of the cell, new protoplasm continuously increases in its volume. The increase in the volume of the matrix causes into the growth of the cell which ultimately divides into daughter cells by the cell division.

6. **Reproduction**—The cytoplasm has the property of asexual and sexual reproduction.

CHAPTER 4

PLASMA MEMBRANE

The light microscopic observations of the animal cells have clearly established that around the cells occurs a limiting membrane which separates the cellular protoplasm with their external environment. This limiting membrane has been named as the **plasmalemma** or **plasma membrane**. The plasma membrane is a very thin, elastic, permeable and living structure The cells of bacteria and plants also possess the plasma membrane in between the cell wall and the cytoplasm.

The ultrastructure and molecular composition of plasma membrane of various cells, *e.g.*, *Amoeba proteus*, striated muscles, liver cells, eggs of sea urchins, medullated nerve fibres and red blood cells or erythrocytes have been investigated by various workers who have

The plasma membrane is a trilaminar (three layered) membrane of lipid and protein molecules. The trilaminar nature of plasma membrane has been proposed by **Danielli** and **Davson** in 1935 by certain indirect evidences. In 1938 **Harvey** and **Danielli** produced a hypothetical model of

Robertson found wide occurrence of such trilaminar composition in most of the membranes of cellular organelles and in 1959, he propounded the concept of the **"unit membrane"**. A unit membrane has the protein-lipid-protein arrangement. The unit membrane has been found in plasma membrane, endoplasmic reticulum, Golgi complex, lysosomes and plastids. Further, both the outer and inner membranes of the mitochondria and the nucleus are the unit membranes.

The plasma membrane of most cells varies from 100 to 215°A in thickness. The plasma membrane of most cells of intestinal epithelium has been found 105A° thick. It is composed of an outer

and inner dense layers of 40 A° thicknesses enclosing a middle less dense layer of 25 A° thickness. The plasma membrane of erythrocytes or ghosts is 215 A° in thickness.

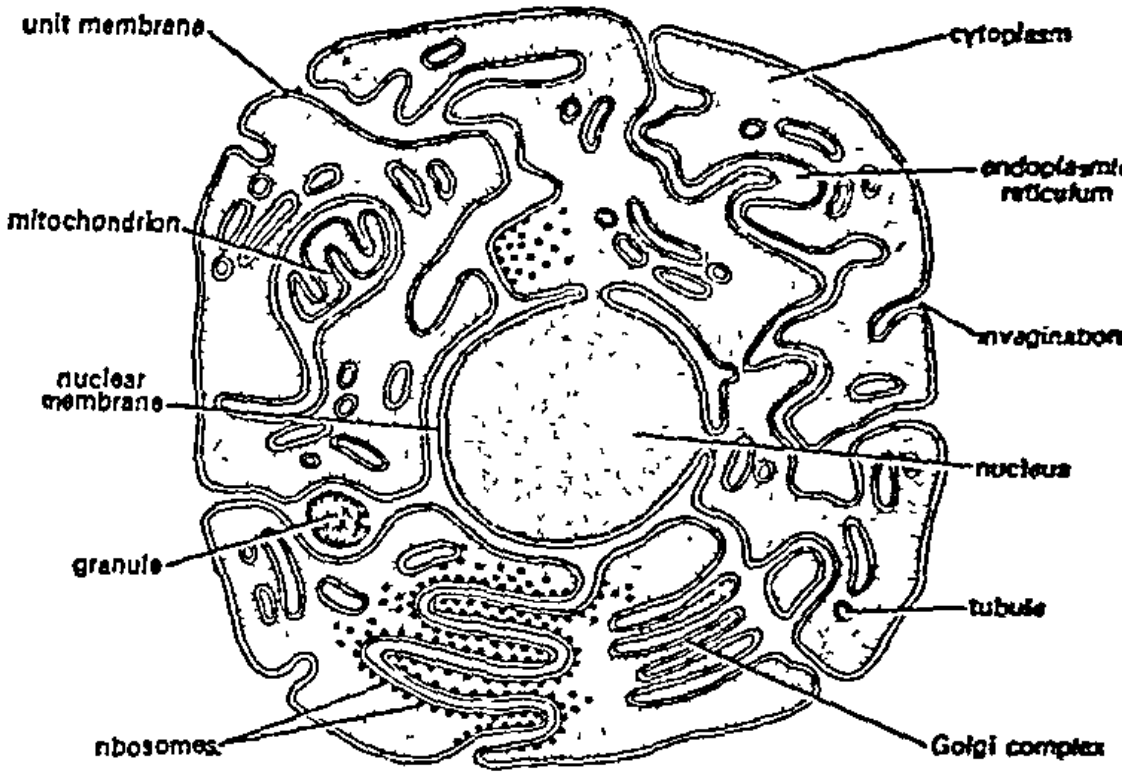


Fig. 4 1. Diagram showing the origin and development of membranous structures of the cell from the unit membrane.

The plasma membrane is not a continuous layer but contains certain minute pores of 8 to 50 A° diameter. The opening and closing of these pores depend on the metabolic phase of the cell.

CHEMICAL COMPOSITION

The plasma membrane of most cells is composed mainly of carbohydrates, lipids and proteins.

(i) **Carbohydrates**—Bell (1962) has suggested the presence of carbohydrates in the plasma membrane. The plasma membrane of erythrocytes and liver cells has been found to contain 5% carbohydrates. T[illegible] the hexose, hexosamine, fucose a[illegible] of purple sides or [illegible] plasma membranes of nerve endings.

(ii) **Lipids**—The plasma membrane contains about 20 to 40 per cent of lipids. The main constituent lipids are the phospholipids such as lecithin, cephalin and sphingomyelin. Other lipids are cholesterol and galactolipids The lipids chemically composed of a [illegible] of fatty acids. For instance, the plasma membrane of

erythrocytes of mammals has been found to contain many fatty acids such as palmitic, stearic, oleic, linoleic and arachidonic acids.

(iii) Proteins—The proteins form the main bulk of the plasma membrane. The plasma membrane of erythrocytes contains 60 to 80 per cent proteins. The proteins of erythrocytes plasma membrane have high molecular weights and have been named as "tectins" by **Mazia and Ruby** in 1968 because these proteins resemble with the protein "actin" of muscle cells and actin-like protein of microtubules. The common constituent amino acids of these proteins are arginine, lysine, histidine, tyrosine, tryptophan, methionine and cysteine. But the kind of protein may vary from cell to cell and from animal to animal.

(iv) Enzymes—About thirty enzymes have been reported from the isolated plasma membrane. Some important enzymes of plasma membrane are 5'-nucleotidase, Mg^{2+} ATPase, Na^{+}—K^{+} activated —Mg^{2+} ATPase, alkaline phosphatase, acid-phosphomono-esterase and RNAase.

MOLECULAR STRUCTURE

The plasma [illegible] molecules and two [illegible] occur in [illegible] remain [illegible] imolecular or double layered-s [illegible] ced with each other

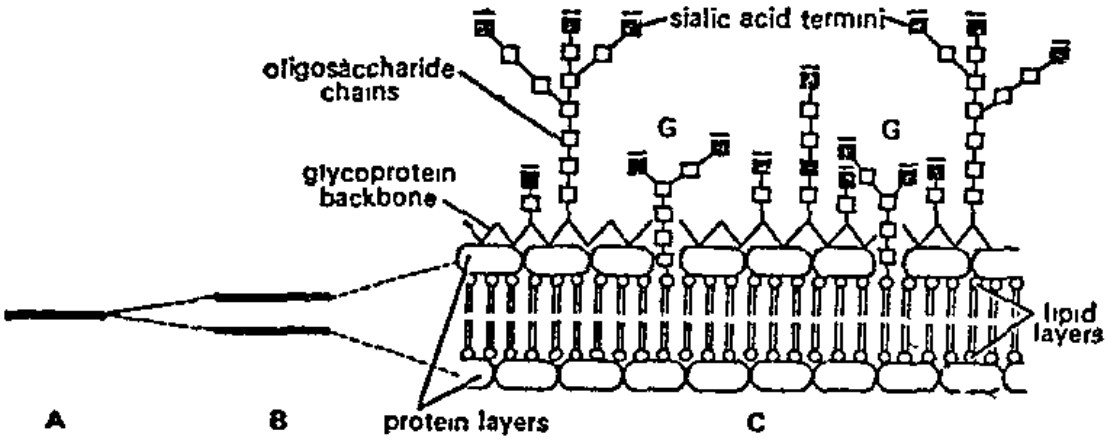


Fig. 4·2. Diagram showing the structure of plasma membrane as seen at A—Low magnification compound microscope, B—High magnification, C—Ultrastructure in electron microscope.

by the inner ends of lipid molecules which are non-polar and hydrophobic (Gr., *hydr*-water, *phobe*-hate) in nature. Both the layers of lipids are able to be held together due to Vanderwaal's forces at these non-polar ends.

The lipid layers are enclosed by an outer and inner monomolecular layers of non-lipids, *i.e.*, the proteins. The lipid molecules remain linked with the molecules of protein layers by their outer, polar and hydrophilic (Gr., *hydra*-water, *phil*-loving) ends. In the hydrogen bonds, ionic linkages or electrostatic forces bind the molecules of lipids and proteins together. According to some workers, it is possible that different types of component lipids and phospholipids may be to membrane structure what amino acids are to protein structure, namely that, the secondary, tertiary, and quaternary structure of membranes is determined by the structure and space-filling properties of the building blocks, their ratio, and their two dimensional patterns, (see **Berrill, 1971**).

The carbohydrate molecules occur in the association of protein molecules and provide stability to lipoprotein complex. The protein layers provide elasticity and mechanical resistant to the plasma membrane.

The inter-cellular space—In the tissues of multicellular animals, the plasma membrane of two adjacent cells usually remain seperated by a space of 10 to 150 A° wide. This inter-cellular space is uniform and contains a material of low electron density which can be considered as a cementing substance. The exact chemical nature of this substance is still unknown.

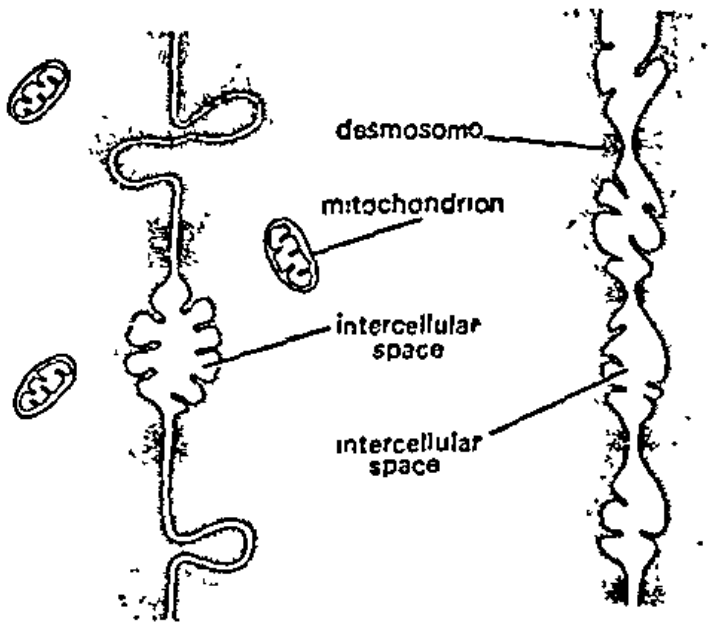


Fig 4 3. Inter-cellular space.

SPECIALIZED STRUCTURES OF PLASMA MEMBRANE

The cell surface of certain cells perform various physiological activities such as absorption, secretion, transportation, etc. To per-

form such specialized functions certain modifications are inevitable in the plasma membrane of such cells. The most important specialized structures of plasma membrane are as follows :

A. SPECIALIZATIONS DUE TO OUTPUSHING OR EVAGINATION OF PLASMA MEMBRANE

Microvilli—The columnar epithelial cells of intestine have to perform active absorption of digested food. The free surfaces of these epithelial cells give out many minute, finger-like, cytoplasmic processes of plasma membrane which are known as the **microvilli**. These microvilli are 0·6 to 0·8 μ long and have the diameter of 1000 A°. A single cell may contain about 3000 microvilli. The narrow spaces between the microvilli form a kind of sieve through which substances may pass during the process of absorption. These microvilli increase the absorptive surface of the intestinal epithelial cell. Such microvilli also occur in hepatic cells, mesothelial cells of the convoluted tubules of the kidney and in the epithelial cells of gall-bladder, uterus and yolk sac.

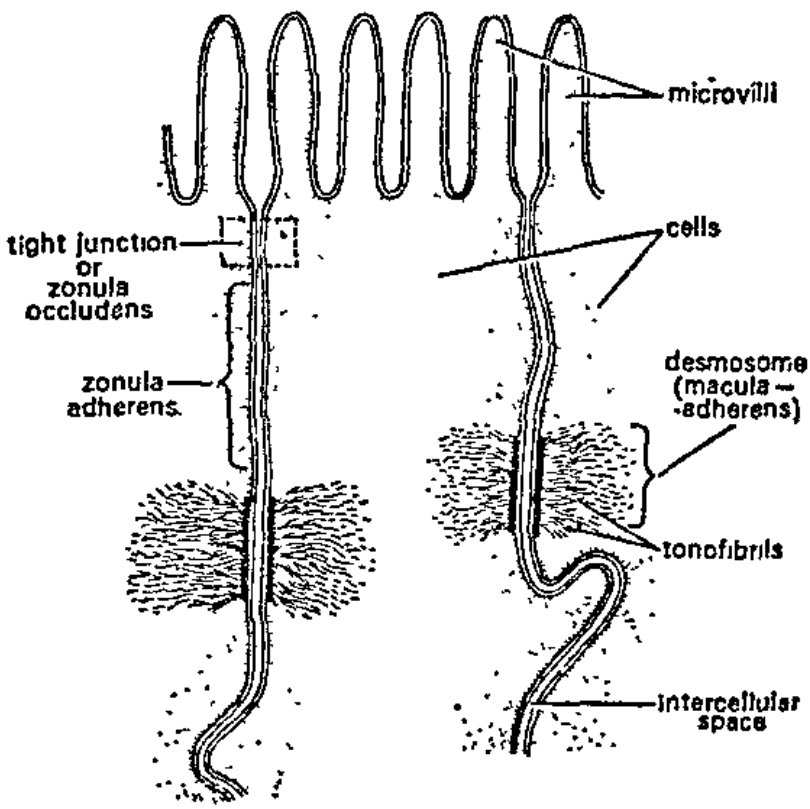


Fig 4.4. Microvilli of the plasma membrane.

B. SPECIALIZATION DUE TO INPUSHING OR INVAGINATIONS OF PLASAM MEMBRANE

The bases (inner ends) of certain cells, such as the cells of the kidney, perform active transportation and contain many invagina-

tions or infoldings of the plasma membrane. At the base of these folds there develops a septa and thus narrow compartments of basal cytoplasm are formed. These infoldings contain many mitochondria. These mitochondria along with the enzymes of plasma membrane possibly provide energy rich compound, *viz.*, ATP to the plasma membrane for the active transportation of solutes.

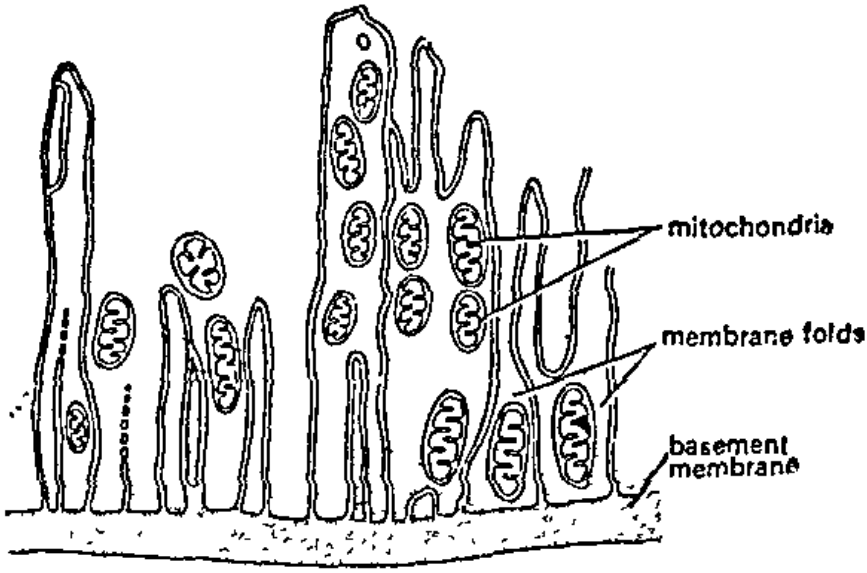


Fig 4.5 Infoldings of plasma membrane.

C. SPECIALIZATION IN PLASMA MEMBRANE DUE TO CONTACT

Though the plasma membrane of adjacent cells often remains separated by a uniform inter-cellular space but at certain places the plasma membrane of adjacent cells may come too close or may go too far This type of specializations in plasma membrane are of following types :

1. Desmosomes or Maculae adhaerentes—In certain epithelial cells (especially abundant in stratified squamous epithelium), the plasma membrane of adjacent cells becomes thicker in certain regions and from these thickened areas (or plaques) many fine filaments known as **tonofilaments** or **tonofibrils** are radiated towards the interior of the cell. Such thickened areas of plasma membrane are known as **desmosomes** or **Maculae adhaerentes**. The desmosomes are formed by a circular area of 0·5 μ in diameter of the plasma membranes of two adjacent cells which are separated by a distance of 300 to 500A°. The intercellular space between the desmosomes contains a coating material which provides cellular adhesion to the cell. The tonofibrils provide intra-cellular mechanical support. Desmosomes appear to be limited to vertebrate tissues (**J. P. Trinkaus, 1969**).

2. Hemidesmosomes—**Kelley** (1966) has reported half portion of the desmosome at the basal surfaces of certain epithelial cells and named it as **hemidesmosome**. The hemidesmosomes resemble with

desmosomes in finer details but their outer side remain coated with collagen fibrils.

3. **Septate desmosomes—Wood** (1959), **Locke** ((1965) and **Gauranten** (1967) have reported different types of desmosomes in the epithelia of invertebrates and named them as **septate desmosomes.** In septate desmosomes, the two plasma membranes remain separated by a distance of 150 to 200A° and both remain joined by many transverse parallel septa. These septa are found to be continuous upto outer proteinous layers of plasma membranes. There are no intracellular deposits (cementing substance) backing up septate desmosomes (as in the vertebrate desmosome) and no intra-cellular fibrils (tonofibrils) converge on the site. The septate desmosomes are universally considered as adhesive or attachment devices. They also permit intercellular communication and electrical coupling.

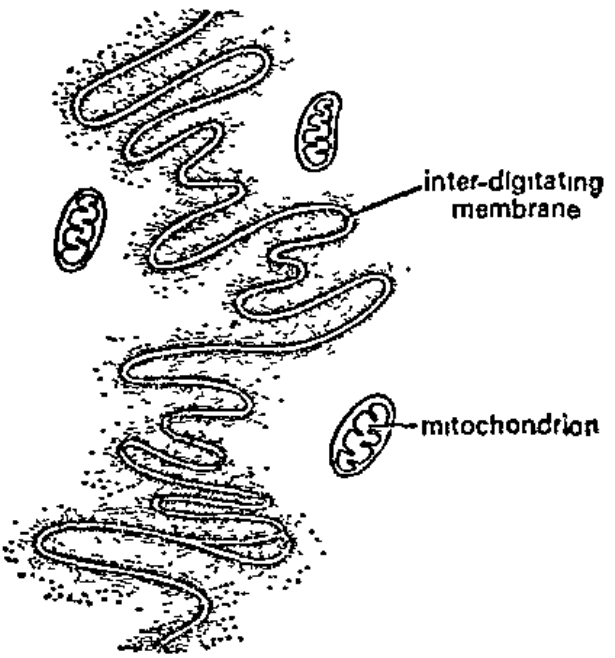


Fig. 4·6. Inter-digitation of plasma-membrane.

4. **Terminal bars**—The terminal bars are also krown as **inter·mediary junctions** or **zonula adhaerens.** The terminal bars are similar to desmosomes except they lack in the tonofibrils. In terminal bar the plasma membrane is thickened and the cytoplasm of thickened area is dense. The terminal bars occur in the intermediary portion of the plasma membrane of columnar cells just below the surface. The correct identity of zonula adhaerens is still questionable (**J. P Trinkaus, 1969**).

5. **Inter-digitations**—At some places the plasma membrane of two adjacert cells gives out finger-like projections known as **inter-digitations.** The inter-digitations may become further complicated by the development of desmosomes and terminal bars. etc.

6. **Tight junctions** or **zonulae occludentes**—At certain places, the plasma membranes of two adjacent cells fuse so intimately that the inter-cellular space becomes wanting. Such fusions of the plasma membrane are known as **tight junctions** or **zonulae occludentes.** The tight junctions act as barriers for diffusion of the large-sized molecules through these regions. The tight junctions occur in between epithelial

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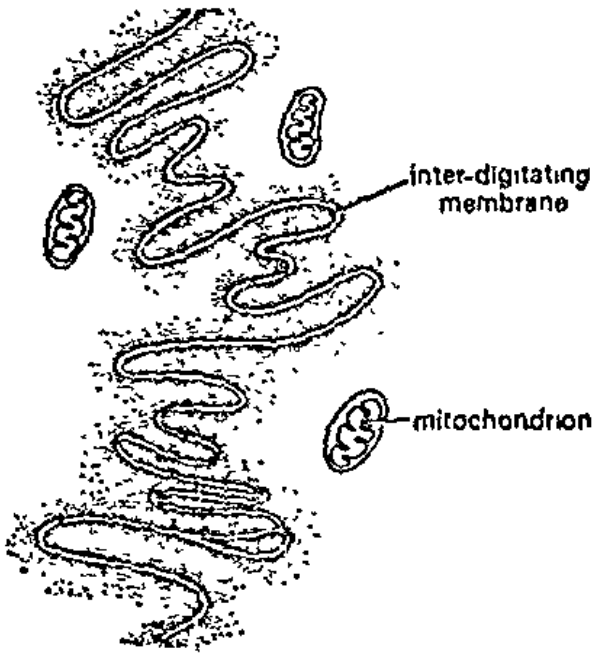


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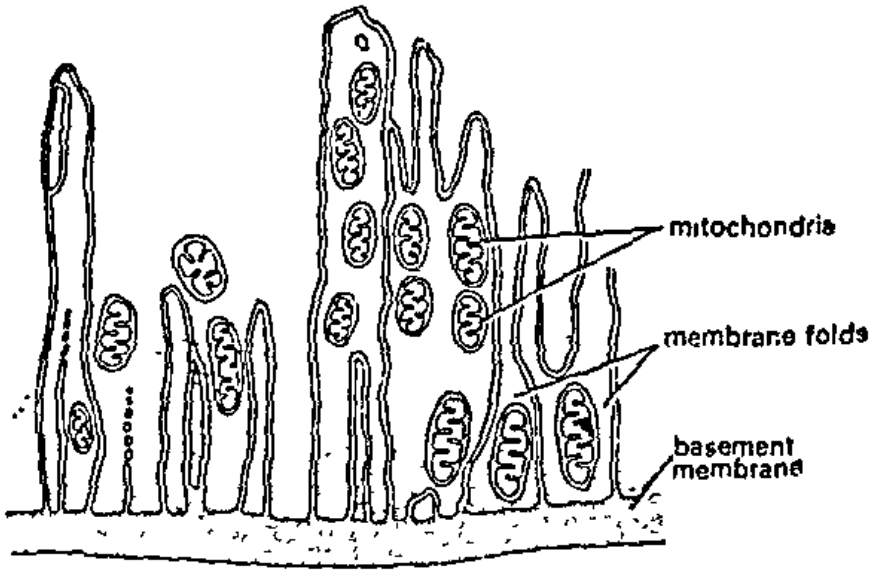


Fig 4·5. Infoldings of plasma membrane.

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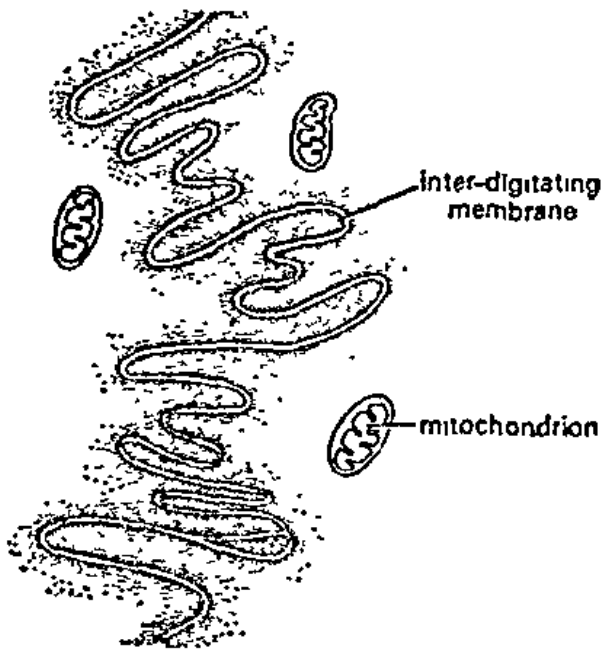


Fig. 4-6. Inter-digitation of plasma-membrane.

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cells and brain cells and act as blood-brain barriers and the synaptic barriers. The tight junctions permit inter-cellular electrical communication and also inter-cellular chemical communication of inorganic ions.

7. Gap junctions—The cells of myocardial tissue contain a inter-cellular gap of 20 A° wide between their plasma membranes. These gaps are found to be hexagonal (70 to 75A° wide) in tangential sections. These hexagonal gaps are known as **gap junctions** when inter-cellular gap remain 40–70A°, then such junctions are called **close junctions.**

PHYSIOLOGY OF PLASMA MEMBRANE

The plasma membrane acts as a thin barrier which separates the intra-cellular fluid or the cytoplasm from the extra-cellular fluid in which the cell lives. In case of unicellular organisms (Protophyta and Protozoa) the extra-cellular fluid may be fresh or marine water, while in multicellular organisms the extra-cellular fluid may be blood, lymph or interstitial fluid. Though the plasma membrane is a limiting barrier around the cell but it performs various important physiological functions which are as follows :

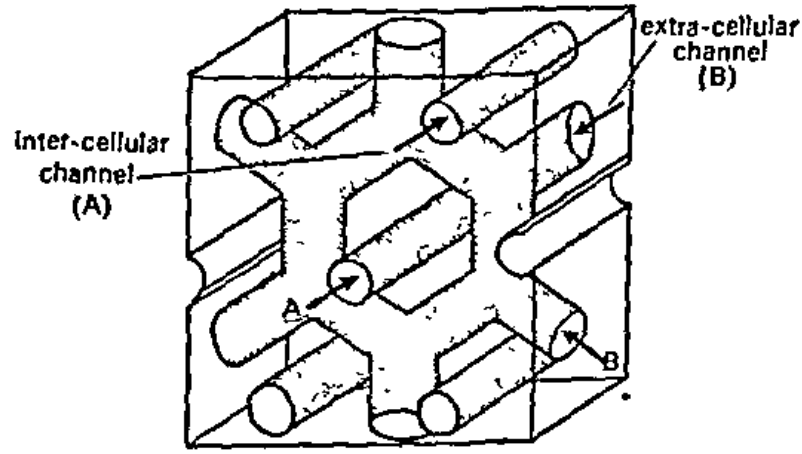


Fig. 4·7. The model shows possible distribution of intercellular channels (A) and extracellular channels (B) with the junctional membrane complex.

1. Permeability—The plasma membrane is a thin, elastic membrane around the cell which usually allows the movement of small ions and molecules of various substances through it. This nature of plasma membrane is termed as permeability. According to permeability following types of the plasma membranes have been recognised :

(I) Impermeable plasma membranes—The plasma membrane of the unfertilized eggs of certain fishes allows nothing to pass through

it except the gases. Such plasma membranes can be termed as impermeable plasma membranes.

(ii) **Semi-permeable plasma membrane**—The membranes which allow only water but no solute particle to pass through them are known as semi-permeable membranes. Such a membrane has not to

brane and other intra-cellular membrane are very selective in nature. Such membranes allow only certain selected ions and small molecules to pass through them.

(iv) **Dialysing plasma membrane**—The plasma membranes of certain cells have certain extraneous coats around it. The basement membranes of endothelial cells are the best examples of extraneous coats. This type of plasma membrane having extraneous coats around it, acts as a **dialyzer**. In these membranes the water molecules and crystalloids are forced through them by the hydrostatic pressure forces

2. **Osmosis**—The plasma membrane is permeable to water molecules. The to and fro movement of water molecules through the plasma membrane occurs due to the differences in the concentration of the solutes on its either sides. The process by which the water molecules pass through a membrane from a region of higher water concentration to the region of lower water concentration is known as **osmosis** (Gr., *osmos*–pushing). The process in which the water molecules enter into the cell is known as **endosmosis**, while the reverse process which involves the exit of the water molecules from the cell is known as **exosmosis**. In plant cells due to excessive exosmosis the cytoplasm along with the plasma membrane shrinks away from the cell wall. This process is known as **plasmolysis** (Gr., *plasma*-molded, *lysis*–loosing).

A cell contains variety of solutes in it, for instance, the mammalian erythrocytes contain the ions of potassium (K^+), calcium (Ca^+), phosphate (PO_4^-), dissolved haemoglobin and many other substances If the erythrocyte is placed in a 0·9% solution of sodium chloride (NaCl), then it neither shrinks nor swells. In such case, because the intra-cellular and extra-cellular fluids contain same concentration and no osmosis takes place. This type of extra-cellular solution or fluid is known as **isotonic solution or fluid.** If the concentration of NaCl solution is increased above 0·9% then the erythrocytes are shrinked due to excessive exosmosis. The solutions which have higher concentrations of solutes than the intracellular fluids are known as **hypertonic solutions.** Further, if the concentration of

NaCl solution decreases below 0·9% the erythrocytes will swell up due to endosmosis. The extra-cellular solutions having less concentration of the solutes than the cytoplasm are known as **hypotonic solutions**.

Due to endosmosis or exosmosis the water molecules come in or go out of the cell. The amount of the water inside the cell causes a pressure known as **hydrostatic pressure**. The hydrostatic pressure which is caused by the osmosis is known as **osmotic pressure**. The plasma membrane maintains a balance between the osmotic pressure of the intra-cellular and inter-cellular fluids.

3. **Diffussion** or **passive transport**—When molecules of two kinds are placed together they tend to mix with each other by a process known as the **diffusion**. The diffussion of certain solutes or substances takes place through the plasma membrane. Such diffusing solute particles require no energy for the diffussion process through the plasma membrane. The diffussion of ions through the plasma membrane depends on the concentration and electrical gradients.

4. **Active transport**—When the molecules or ions move through the plasma membrane from low concentration to higher concentration they require energy for such movement. The energy is provided by adenosine triphosphate (ATP) which is produced by oxidative phosphorylation in the mitochondria. The active transport of the molecules occurs in nerve cells and kidney cells

Through the pores of the plasma membrane some chemical compounds such as urea, formamide and glycerol could pass actively. **Brachet (1957)** has shown that sometimes large molecules of certain proteins penetrate the cell. The enzyme ribonuclease (a protein) has been fourd to pass actively through the plasma membrane of living plant cells, eggs, flagellates and ascitic tumors

5. **Endocytosis** and **exocytosis**—Usually the cells of unicellular or multicellular organisms take their food in the form of solution. For instance, in a multicellular animal the food substances are digested by certain enzymes in the digestive tube. [illegible] by the intestine and pass to the internal body fluid such as lymph and interstitial fluid. The internal body fluids contain food substances in the molecular state. From [illegible] molecular food substances (*viz.*, [illegible] acids, glycerol, vitamins and various other [illegible] through plasma membrane [illegible] of diffussion and active transp[illegible] cell,

substances are oxidised partially in cytoplasm and completely in the mitochondria.

But sometimes the plasma membrane participates actively in the digestion of certain large-sized foreign food substances. The process by which the foreign substances are taken in and are digested is known as **endocytosis** (Gr., *endon*–within+*kytos*–cell). The endocytosis differs with another process of the cell known as exocytosis (Gr., *exo*–outside+*kytos*–cell). In the process of exocytosis the cells which have secretory function, such as pancreatic cells, pass out their enzymatic secretions outside the cell. According to the nature of food or foreign substances, the endocytosis may be classified into two types, pinocytosis and phagocytosis.

(i) [illegible] fluid material in bulk takes place by the cell through the plasma membrane, the process is known as **pinocytosis** (Gr., *pinein*–to drink). The process of pinocytosis was first of all observed by **Edward** in *Amoeba* and by **Lewis** (1931) in the cultured cells.

In the process of pinocytosis, the particle free globules of fluid are surrounded and ultimately engulfed by the clasping folds of the cytoplasm. The plasma membrane forms membranous vacuoles around the fluid globules. Such membrane bound vacuoles are called **pinosomes** (Lewis, 1931). The pinosomes, later on, are transported to the interior of the cell where they are fused with the secretory granules or lysosomes. In the food vacuoles the digestion of food substances takes place and the digested food is diffused to the surrounding cytoplasm. The pinocytosis can be observed ordinarily in the cells by light microscopy.

Micropinocytosis—Electron microscopic observations have been made on the pinocytotic process at sub-cellular or sub-microscopic level in the cells. The pinocytosis which occurs at sub-microscopic level is known as **micropinocytosis**. In the process of micropinocytosis, the plasma membrane invaginates to form small vesicles of 650A° diameter. These vesicles have opening on both outer and inner surfaces which suggest the possible transportation of fluids through these vesicles to the cell. The micropinocytosis have been observed in endothelial cells, Schawn and satellite cells of nerve ganglion, macrophages, muscle cells and reticular cells, etc.

(ii) **Phagocytosis**—Sometimes the large-sized solid food or foreign particles are taken in by the cell through the plasma membrane. The process of ingestion of large-sized solid substances by the cell is known as **phagocytosis** (Gr., *phagein*–to eat, *kytos*–cell or hollow vessel).

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substances are oxidised partially in cytoplasm and completely in the *mitochondria*.

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Occurrence of phagocytosis—The process of phagocytosis occurs in most protozoans and certain cells of multicellular organisms. In multicellular organisms such as mammals, the phagocytosis occurs very actively in granular leucocytes and in the cells of mesoblastic origin. The cells of the mesoblastic origin are collectively known as the cells of **macrophagic** or **reticuloendothelial system.** The cells of macrophagic system are histiocytes of the connective tissue, the

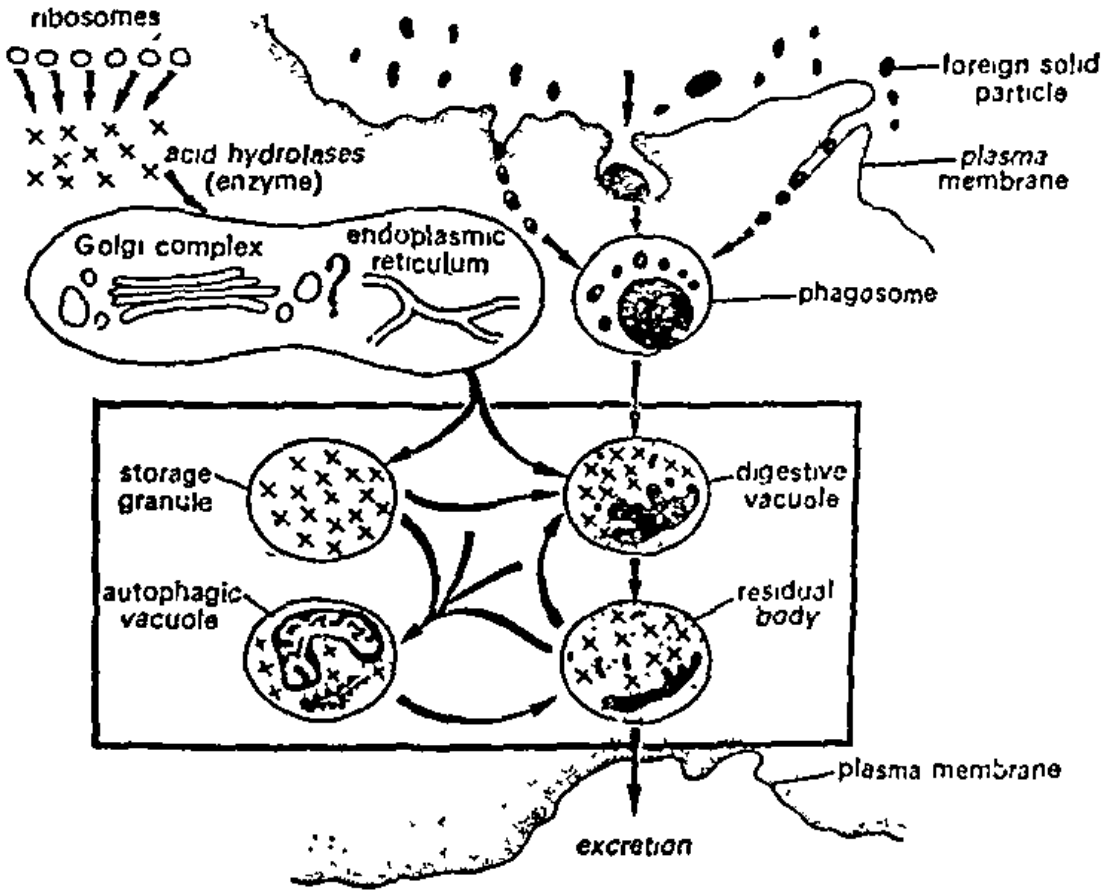


Fig. 4 8. Phagocytosis in the cell.

reticular cells of the hematopoietic organs (bone marrow, lymph nodes and spleen) and the endothelial cells which form the lining of capillary sinusoid of the liver, adrenal gland and hypophysis. The cells of macrophagic system can ingest bacteria, Protozoa, cell debris or even colloidal particles by the process of phagocytosis.

Process of phagocytosis—*In* the process of phagocytosis the external solid substances are captured by an area of the plasma *membrane which ultimately pirches off from the cell surface to form* a vesicle or vacuole around the solid particle. The process of vacuole formation can be termed as vacuolization. The membrane bounded vesicles or vacuoles are known as phagosomes. The phagosomes migrate to the interior of the cell and fuse with the pre-exist-

ing lysosomes. The food is digested by the hydrolytic enzymes (acid hydrolase) of the lysosomes and the digested food is ultimately diffused to the surrounding cytoplasm. The undigested food is expelled from the plasma membrane by the process of ephagy or egestion.

Kinds of phagocytosis—According to the physical and chemical nature of foreign substance following types of phagocytosis have been recognised ;

(i) **Ultraphagocytosis** or **colloidopexy**—The process in which plasma membrane ingests smaller colloidal particles is known as **colloidopexy** or **ultraphagocytosis**, *e g*, leucocytes and the macrophagic cells of mammals.

(ii) **Chromopexy**—When the cell ingests colloidal chromogen particles phagocytotically the process is known as **chromopexy**, *e.g.*, some mesoblastic cells.

EMEIOCYTOSIS OR CELL VOMITING

In certain cells as in the cells of pancreas, a process reverse to the endocytosis occurs. In such cells the vacuoles which have secretory products (enzymes) move from the interior of the cytoplasm towards the surface of the cytoplasm. At the surface these vacuoles fuse with the plasma membrane and discharge their stored products to the exterior of the cell. This process is known as **emeiocytosis** or **cell vomiting** or **exocytosis**.

ORIGIN OF PLASMA MEMBRANE

The problem of origin of plasma membrane is still little understood. Following concepts have been forwarded by different workers concerning its origin :

(1) According to some workers, the plasma membrane is an indepedent organelle and it enlarges with cell growth autonomously.

(2) According to many workers, the plasma-membrane is originated by **self-assembly** of its conponent chemical molecules. Thus, small complexes of membrane are organized in the cytoplasm and are inserted as blocks into the pre-existing membrane.

It is suggested that self-assembly of plasma-membrane is not a one-step process, but it includes many steps which occur in sequential order. First step includes polymerization of building-blocks or monomers into lipid and protein molecules. Next step includes self-assembly of lipid and protein molecule into a basic membrane. The final step includes the insertion of specialized functional components (*i.e.*, enzymatic proteins) into the membrane to form a mature plasma-membrane or any membrane of cellular organelles. The sequ-

ential order in assembly pathway of a membrane is supposed to be imposed entirely by structural features of the intermediate gene products. The products which are used in the early and late stages of assembly are produced at one time and cannot do otherwise than assemble in certain sequence (see Berrill, 1971).

EXTRANEOUS COATS OF PLASMA MEMBRANE

The plasma membrane of plant cells and some animal cells is covered by one or more thick protective layers of non-living substances. The outer protective layers around the plasma membrane are known as **extraneous coats** The extraneous coats of the cells of animals and plants can be studied under separate headings as follows :

A. EXTRANEOUS COATS OF ANIMAL CELLS

The extraneous coats of the plasma membrane of animal cells are also known as basement membranes, basal laminae, boundry layer, glycoprotein mantle, external lamina and glycocalyx. The extraneous coats of animal cell are mainly composed of following organic substances :

(i) Glycoproteins—The most common glycoprotein which occurs as the extraneous coat in the animal cells is the **mucin.** The mucin occurs in the eggs of amphibians and most marine animals and the epithelial cells of gastro-intestinal tract.

(ii) Polysaccharides—The polysaccharides occur in the from of **chitin** (the cells of crustaceans and insects), **cuticle** (epithelial cells of insects) and **zona pellucida** (ova).

Glycocalyx—Some animal cells have both glycoproteins and polysaccharides in the extraneous coats. Such extraneous coats of glycoproteins and polysaccharides are known as **glycocalyx.** For instance, the glycocalyx occurs in the from of **hyaluronic acid** in most epithelial cells, blood capillaries and inter-cellular spaces.

Functions of extraneous coats in animal cells—The electron microscopic observations have proved that the extraneous coats of the animal cells are the by-products of the cell surfaces and their substances are not neccessary for the integrity of the cell. However, these coats have certain important functions for the cells which are as follows :

(i) Filteration—The extraneous coats sometimes act as filters, For instance, the extraneous coats surrounding the blood capillaries of most vertebrates, especially the kidney glomerulus act as filter ard regulate the passage of molecules through it. The extracellular coats of connective tissues contain the chemical compound **hyaluronate** which controls the diffusion.

(ii) **Maintenance of the micro-environment of the cell**—The extraneous coats of animal cells can affect the concentrations of different substances at the surface of the cell. For example, a muscle cell with its excitable plasma membrane which is surrounded by a glycocalyx is found to maintain the micro-environment of muscle cell by trapping the sodium ions.

3. [illegible]ts—Some substances [illegible]perties to the cell. [illegible]alian erythrocytes is found to contain some specific, genetically determined substances (carbohydrate and proteins) corresponding to the A, B and O blood groups.

B. EXTRANEOUS COATS OR CELL WALL OF PLANT CELLS

[illegible]

Due to the shape of cell walls many types of plant cells as the paren-[illegible]cognised.

[illegible]valls of plant cells are [illegible] Besides the cellulose various chemical substances such as **hemicellulose, pectin, liginin, cutin** and **chitin** also occur singly or along with the cellulose. The cell wall also contains certain minerals such as calcium and magnesium in the form of carbonates and silicates. The **cellulose** is a poly-[illegible]chemical substance [illegible]ly consists of long [illegible]the structural unit of the cellulose and about 3000 glucose molecules are linked together to form the chains of cellulose molecules. Many chains of cellulose molecules lie parallel to each other to form the bundles. A bundle of 100 chain molecules of cellulose forms the elementary fibril known as the **micelle**. The 20 micelle when get arranged parallely, form the fibrils of 250 A° thick known as **microfibrils**. These microfibrils form large-sized bundles of cellulose fibres to form the **macrofibrils**. The macrofibrils consisting of many cellulose fibrils form the main frame work of the cell walls. The **hemicellulose** is composed of monosaccharide units such as arabinose, xylose; mammose and galactose. It occurs sometimes in between the macrofibrils of the cellulose. The **pectin** chemically composed of glucuronic and galacturonic acid. The **liginin** chemically composed of coniferyl alcohols. The **cutin** composed of many fatty acids. The **chitin** is a polymer of glucosamine.

Structure of cell wall—The cell wall is complex in nature and is differentiated in the following layers :

(i) Primary cell wall,

(ii) Secondary cell wall,

(iii) Tertiary cell wall.

(i) Primary cell wall—The first formed cell wall is known as primary cell wall. It is the outer most layer of the cell and in the immature meristmatic and parenchymatous cells it forms the only cell wall. The primary cell is comparatively thin and permeable. Certain epidermal cells of the leaf and the stem also possess the cutin and cutin waxes which make the primary cell wall impermeable. The primary cell wall of the yeast and the fungi is composed of the chitin.

(ii) Secondary cell wall—The primary cell wall is followed by *secondary cell wall. The secondary cell wall is thick, permeable and* lies near the plasma membrane or the tertiary cell wall, if the latter occurs. It is composed of three concentric layers which occur one after another. Chemically the secondary cell wall composed of compactly arranged macrofibrils of the cellulose, in between which sometimes occurs lignin as a inter-fibrillar material.

(iii) Tertiary cell wall—In certain plant cells, there occurs another cell wall beneath the secondary cell wall which is known as tertiary cell wall. The tertiary cell wall differs from the primary and secondary cell walls in its morphology, chemistry and staining properties. Besides the cellulose, the tertiary cell wall consists of another chemical substance known as the xylan.

MIDDLE LAMELLA

The cells of plant tissues generally remain cemented together by an inter-cellular matrix known as the middle lamella. The middle lamella is mainly composed of the pectin, lignin and some proteins.

PLASMODESMATA

The cell walls and the middle lamella of plants never occur in the form of continuous layers but have many minute apertures through which the cells of a tissue maintain cytoplasmic relations with each other. Such cytoplasmic junctions or bridges between the adjacent cells are known as plasmodesmata.

The plasmodesmatas of meristmatic cells have been found to be continuous with the plasmodesmatas of adjacent cells. Through endoplasmic reticulum probably through the of solutions containing nutritional products, dissolved gases, ions or other substances takes place

ORIGIN OF CELL WALL

The cell walls are f
plasmic organelles such
etc., play a very import
The process of new cell w
ding cell immediately after the nuclear divisions.

The cell wall formation is started by the formation of phragmoplast which in lateral view appears as a barrel-shaped plasma body in the equational plane of the div
plast is formed by the small vesicl
cut off from the endoplasmic retic
phery to the equator of the cell. In the equator region of the dividing cells, the vesicles of endoplasmic reticulum get arrange on the equator and thus separate the two daughter parts of the cytoplasm. Later on all the vesicles, except a few which form the plasmodesmata, fuse with one another to form a discontinuous cell plate. The cell plate in later stages develops pectin and changes into the middle lamella. The formation of the middle lamella is also accompanied by some large vesicles known as phragmosomes and the vesicles of Golgi complex which provide non-cellulose material. Soon the fibrils of cellulose are deposited on both sides of the middle lamella and form the primary cell wall. The secondary cell wall develops later on by the deposition of cellulose, hemicellulose and pectin beneath the primary cell wall. The plasma membrane is formed by the Golgi complex beneath the primary cell wall.

Lignification—The structure of cell wall is stabilized by the *deposition of lignin. Such a process of lignification was required in* connection with the transition from aquatic to the terrestrial plant life during evolution of plants.

The lignified cell wall consists of microfibrils embedded in matrix consisting of lignin. Usually the primary cell wall becomes more lignified than secondary cell wall.

cell wall in plant
latter. Like the
acts as skeletal
ligher plants having vascular
supporting framework.

CHAPTER 5

ENDOPLASMIC RETICULUM (ER)

The cytoplasmic matrix is traversed by a complex network of inter-connecting membrane bound vecuoles or cavities. These vacuoles or cavities often remain concentrated in the endoplasmic portion of the cytoplasm, therefore, known as **endoplasmic reticulum.**

The endoplasmic reticulum was first of all reported by **Porter** in 1945. *Fawcett* and *Ito (1958), Thiery (1958)* and **Rose** and **Pomerat** (1960) have made various important contributions on the endoplasmic reticulum.

OCCURRENCE

The occurrence of the endoplasmic reticulum varies from cell to

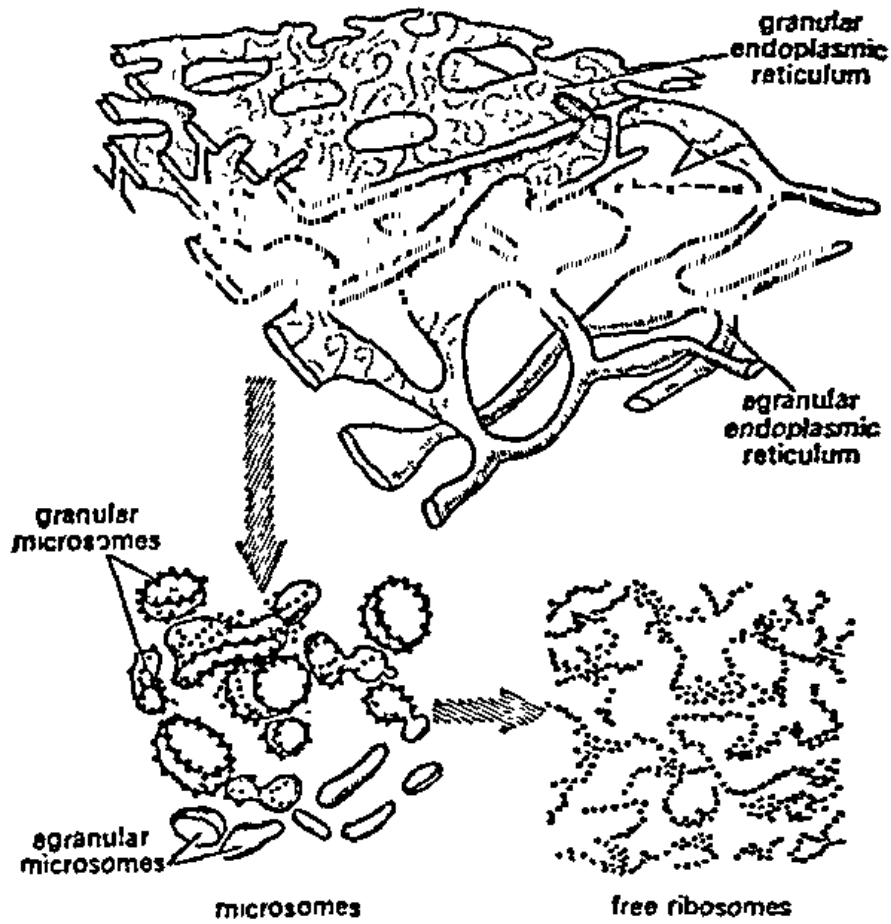


Fig 5·1. Three dimensional structure of endoplasmic reticulum showing microsomes and ribosomes.

cell. The egg and embryonic cells lack in endoplasmic reticulum. The spermatocytes have poorly developed endoplasmic reticulum. The adipose tissues, brow [illegible] of [illegible] [illegible] synthesis of proteins, as the pancreas and other endocrine glands are found to contain highly developed endoplasmic reticulum and most endoplasmic reticulum is of granular type. The liver cells possess both types of endoplasmic reticulum.

MORPHOLOGY

Morphologically the endoplasmic reticulum is composed of following three kinds of structures, *viz.*, 1. cisternae, 2. vesicles and, 3. tubules.

1. CISTERNAE

The cisternae are long, flattened, sac-like, unbranched tubules having the diameter of 40 to 50 *mμ*. They remain arranged parallely in bundles or stakes. The cisternae usually occur in those cells which have synthetic roles as the cells of pancreas, notochord and brain.

2. VESICLES

The vesicles are oval, membrane-bound vacuolar structures having the diameter of 25 to 500 *mμ*. They often remain isolated in the cytoplasm and occur in most cells but especially abundant in the pancreatic cells.

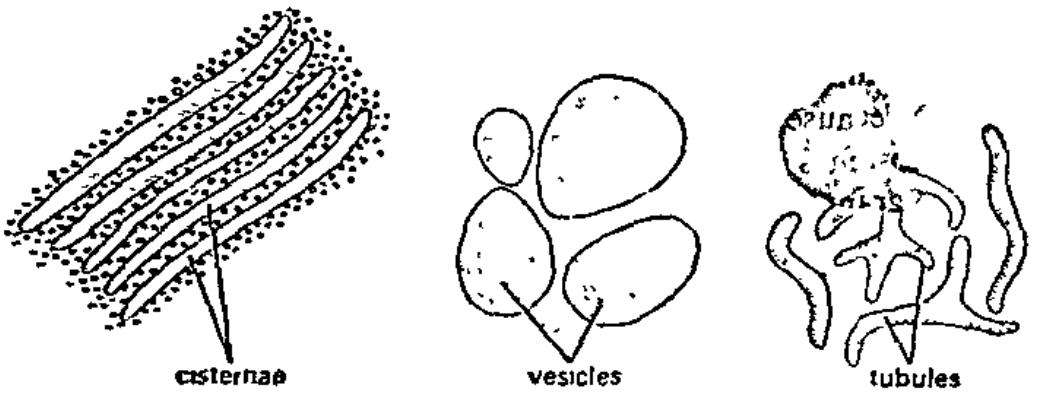


Fig. 5·2. Various components of the endoplasmic reticulum

3. TUBULES

The tubules are branched structures forming the reticular system along with the cisternae and vesicles. They usually have the diameter from 50 to 190 *mμ* and occur almost in all the cells.

ULTRASTRUCTURE

The cavities of cisternae, vesicles and tubules of the endoplasmic reticulum are bounded by a thin membrane of 50 to 60 A° thick.

ness The membrane of endoplasmic reticulum is trilaminar like the unit membrane of the plasma membrane, nucleus, Golgi complex, etc. The membrane, thus, is composed of an outer and inner dense layers of the protein molecules sandwiching two thin and transparent layers of phospholipids. The membrane of endoplasmic reticulum remains continuous with the membranes of plasma membrane, nuclear membrane and Golgi complex The cavity of the endoplasmic reticulum is well developed and acts as a passage for the secretory products. Palade (1956) has observed secretory granules in the cavity of endoplasmic reticulum

TYPES OF ENDOPLASMIC RETICULUM

Two types of endoplasmic reticulum have been observed in same or different types of cells which are as follows :

1. AGRANULAR OR SMOOTH ENDOPLASMIC RETICULUM

This type of endoplasmic reticulum possesses smooth walls because the ribosomes are not attached with its membranes. The smooth type of endoplasmic reticulum occurs mostly in those cells, which have no active participation in the synthesis of proteins. The smooth endoplasmic reticulum is generally found in adipose cells, interstitial cells, glycogen storing cells of the liver, spermatocytes and leucocytes. The muscle cells are also rich in smooth type of endoplasmic reticulum and here it is known as **sarcoplasmic reticulum.** In the pigmented retinal cells it exists in the form of tightly packed vesicles and tubes known as **myeloid bodies.**

2. GRANULAR OR ROUGH ENDOPLASMIC RETICULUM

The granular or rough type of endoplasmic reticulum possesses rough walls because the ribosomes remain attached with its membranes. Ribosomes play a vital role in the process of protein synthesis. The granular or rough type of endoplasmic reticulum is found abundantly in those cells [illegible] such as pancreatic cells, plasma

The granular type of endoplasm

due to its RNA contents of ribosomes. The region of the matrix containing granular type of endoplasmic reticulum takes basiophilic stain and is named as **ergastoplasm, basiophilic bodies, chromophilic substances** or **Nissl bodies** by early cytologists.

ANNULATE LAMELLAE

Usually the endoplasmic reticulum has no pores or annuli in it but in certain cases the pores or annuli have been reported, *e.g.*, ER of invertebrates, ovocytes and spermatocytes of the vertebrates. These annuli resemble with the pores or annuli of the nuclear membranes.

Like the annuli of nuclear membranes it contains a diaphragm across it (Ward and Ward, 1968) and possesses a octagonal symmetry (Maul, 1968). The annulate lammellae (pores) of the ER arise by the evagination from the nuclear envelope and have their association with the ribosomes (Merriam 1959 ; Kessel, 1963).

ORIGIN OF ENDOPLASMIC RETICULUM

The exact process of the origin of endoplasmic reticulum is still unknown. But because membranes of ER resemble with the nuclear membrane and plasma membrane and also at the telophase stage the ER membranes are found to form the nuclear envelope. Therefore, it is normally assumed that the ER is originated by evagination of the nuclear membranes. Seikevitz and Palade (1966) have reported that the granular type of ER is originated first and later it synthesizes the agranular or smooth type of endoplasmic reticulum.

ENZYMES OF THE ER MEMBRANES

The membranes of the endoplasmic reticulum are found to contain many kinds of enzymes which are needed for various important ost important enzymes are the stearases, uctase, NADH diaphorase glucose-6-phosphotase-and-Mg^{++} activated ATPase. Certain enzymes of the endoplasmic reticulum such as nucleotide diphosphate are involved in the biosynthesis of phospholipid, ascorbic acid, glucuronide, steroids and hexose metabolism. The enzymes of the endoplasmic reticulum perform the following important functions :

1. Synthesis of glycerides, *e.g.*, triglycerides, phospholipids, glycolipids and plasmalogens.
2. Metabolism of plasmalogens
3. Synthesis of fatty acids.
4. Biosynthesis of the steroids, *e.g.*, cholesterol biosynthesis, steroid hydrogenation of unsaturated bonds.
5. $NADPH_2+O_2$—requiring steroid transformations : Aromatization and hydroxylation.
6. $NADPH_2+O_2$—requiring steroid transformations : Aromatic hydroxylations, side-chain oxidation, deamination, thio-ether oxidations. desulfuration.
7. L-ascorbic acid synthesis.
8. UDP-uronic acid metabolism.
9. UDP-glucose dephosphorylation.
10. Aryl-and steroid sulfatase.

FUNCTIONS OF ENDOPLASMIC RETICULUM

The endoplasmic reticulum acts as secretory, storage, circulatory and nervous system for the cell. It performs following important functions :

A. COMMON FUNCTIONS OF GRANULAR AND AGRANULAR ENDPLASMIC RETICULUM

1. The endoplasmic reticulum provides an ultrastructural skeletal framework to the cell and gives mechanical support to the colloidal cytoplasmic matrix.

2. The exchange of molecules by the process of osmosis, diffusion and active transport occurs through the membranes of endoplasmic reticulum.

3. The endoplasmic membranes contain many enzymes which perform various synthetic and metabolic activities. Further the endoplasmic reticulum provides increased surface for various enzymatic reactions.

4. The endoplasmic reticulum acts as a circulatory or transporting system. Various secretory products of granular endoplasmic reticulum are transported to various organelles as follows :
Granular ER→agranular ER→Golgi membrane→lysosomes or secretory granules.

5. The ER membranes are found to conduct intra-cellular impulses. For example, the sarcoplasmic reticulum transmits impulses from the surface membrane into the deep region of the muscle fibres.

6. The ER membranes form the new nuclear envelope after each nuclear division.

7. The ER membranes protect the cell by the toxic effects of various substances by a process of detoxification. **Jones** and **Fawcett** (1966) have reported that when a toxicant known as **phenobarbitol** is injected in the animal cell, the enzymes of endoplasmic reticulum such as $NADPH_2$ become active and hypertrophy (Gr., *hyper*-over, *tropho*-nourish, abnormal increase in size) of endoplasmic reticulum takes place.

B. FUNCTIONS OF GRANULAR ENDOPLASMIC RETICULUM

The granular endoplasmic reticulum possesses attached ribosomes with its membranes. The ribosomes synthesize new proteins according to the direction of the nuclear DNA. The ribosomes synthesize proteins either for intra-cellular organelles or for the extracellular use. For instance, the ribosomes of certain cells sometimes synthesize the proteins such as haemoglobin and fibrous proteins which are usually stored in the cytoplasmic matrix. The endoplasmic

reticulum has no concern with such secretory proteins. But some times the ribosomes synthesize the proteins as trophocollagen serum proteins, enzymatic proteins for the extra-cellular export. These proteins are transported by the endoplasmic reticulum to the exterior of the cell via smooth endoplasmic reticulum, Golgi complex and secretory granules.

C. FUNCTIONS OF AGRANULAR ENDOPLASMIC RETICULUM

The agranular or smooth type of endoplasmic reticulum synthesizes and stores various substances.

1. Synthesis of lipids—The cells in which active lipid metabolism takes place are found to contain large amount of the smooth type of endoplasmic reticulum. According to some workers as **Christensen** (1961) and **Claude** (1968) the smooth type of endoplasmic reticulum is related with the synthesis and metabolism of the lipids.

2. Synthesis of glycogen—The agranular endoplasmic reticu[illegible] of the glycogen storing cell of the liver and the cells of certain pl[illegible] bc[illegible] Porter [illegible] Porter [illegible] su[illegible] glycogenolysis (digestion of glycogen) and not to glycogenesis (synthesis o[illegible]

r[illegible]

glycerides, hormones, testosterone and progesteron. Further, the smooth endoplasmic reticulum of the pigmented epithelial cells of the retina synthesizes certain visual pigments from vitamin A.

CHAPTER 6

GOLGI COMPLEX

For the performance of certain important cellular functions such as biosythesis of polysaccharides, packaging (compartmentalizing) of cellular synthetic products (proteins), production of exocytotic (secretory) vesicles and differentiation of cellular membranes, there occurs a complex organelle called **Golgi apparatus** or **Golgi complex** in the cytoplasm of animal and plant cells. The *Golgi complex, like the endoplasmic reticulum, is a canalicular system with sacs, but unlike the endoplasmic reticulum it has parallely arranged, flattened, membrane-bounded vesicles which lack* ribosomes and stainable by osmium tetraoxide and silver salts. It was by these staining reactions that **Camilo Golgi** (1891) first of all recognised it in the nerve cells of the barn owl and the cat, long *before the discovery of endoplasmic reticulum. After* the name of its discoverer Golgi complex was variously named as **Golgiosome, Golgi bodies, Golgi material, Golgi membrane,** etc. The Golgi complex of the cells of plants and lower invertebrates is usually referred as **Golgi body** or **dictyosome.**

OCCURRENCE

The Golgi complex occurs in all cells except the prokaryotic cells (*viz.*, mycoplasmas, bacteria and blue green algae) and eukaryotic cells of certain fungi, sperm cells of bryophytes and pteridiophytes, cells of mature sieve tubes of plants and mature sperm and red blood cells of animals. Their number per plant cell can vary from several hundred as in tissues of corn root and algal rhizoids (*i.e.*, more than 25,000 in algal rhizoids, Sievers, 1965), to a single organelle in some algae. In animal cells, there usually occurs a single Golgi complex, however, developing oocytes of chordates may contain many Golgi bodies.

DISTRIBUTION

In the cells of higher plants, the Golgi bodies or dictyosomes are usually found scattered throughout the cytoplasm and their distribution does not seem to be ordered or localized in any particular manner (**Hall** *et al.*, 1974). However, in animal cells the Golgi complex is a localized organelle. For example, in the cells of ecto-

dermal origin, the Golgi complex remains polar and occurs in between the nucleus and the periphery, and *in* the nerve cells is occupies a circum-nuclear position.

MORPHOLOGY

The Golgi bodies of plant cells are about 1–3μ in length and about 0·5μ high. The Golgi bodies of plant cells and Golgi complex of animal cells basically have a similar morphology. Each type of organelle is disc-shaped and consists of. central, flattened, plate-like compartments or cisternae, peripheral network of interconnecting tubules, and peripherally occurring vesicles and Golgian vacuoles.

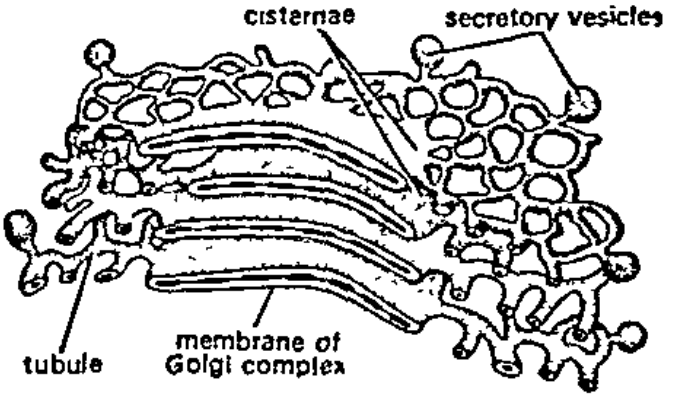


Fig 6·1. Three dimensional view of Golgi complex.

1. CISTERNAE

A cisterna is a sac or cavity filled with fluid contents within a cell or organism (Morre *et al.*, 1971). A Golgi body or Golgi complex usually consists of four to seven (Hall *et al.*, 1974) or three or twelve (Northcote, 1973) flat, tubular or filamentous cisternae which are closely held in parallel bundle or stack one above the other. The number of cisternae per Golgi body may exceed upto 30 or more in lower organisms All the cisternae of a stack of Golgi body or Golgi complex are slightly curved, so as to give a concave and a

enclosed by the usual three-layered unit membrane of 6 $m\mu$ thickness. In the stack, the cisternae are separated from each other by an inter-cisternal space of about 100–150 A° width. In some cells, a layer of parallel fibres, called inter-cis[illegible] ross each inter-cisternal spaces and may [illegible] nts maintaining the regular spacing bet- [illegible] (Hall *et al.*, 1974). Further, each cisterna consists of a central plate-like region ("saccule") of 0·5–1μ diameter which is fenestrated at its peripheral margins due to its extension into network of tubules

The cisternae have some definite polarity. One pole of each of them is associated with the nuclear envelope or with endoplasmic reticulum. This is **proximal pole** or **forming face**. The membranes of cisternae near this pole are morphologically and histochemically similar to endoplasmic reticulum. Toward the opposite pole (**distal pole** or **maturing face**), the cisternal membranes become progressively more like plasma membrane. The secretory vesicles are formed from the distal poles of cisternae.

2. TUBULES

From the peripheral area of cisternae arise a complex, anastomosing flat network of tubules of 300 to 500 A° diameter. **Clowes** and **Juniper** (1969) have compared this tubular network to a disc of lace.

3. VESICLES

The vesicles are small droplet-like sacs which remain attached to tubules at the periphery of the cisternae. They are of following two types :

1. **Smooth vesicles**—The smooth vesicles are of 20 to 80$m\mu$ diameter. They contain secretory material (so often are called **secretory vesicles**) and are budded off from the ends of cisternal tubules within the net. Often more than one tubule connect to, and presumably fill, a single forming vesicle.

2. **Coated vesicles**—The coated vesicles are spherical protuberances, about 50 $m\mu$ in diameter and with a rough surface. They are found at the periphery of the organelle, usually at the ends of [illegible] quite distinct from the secre-

The Golgian vacuoles are large, spacious, rounded sac-like structures occurring at the distal ends of cisternae. They are produced by vesiculation of saccules of cisternae.

ZONES OF EXCLUSION

A Golgi body or Golgi complex is surrounded by a differentiated region of cytoplasm where ribosomes, glycogen, and organelles such as mitochondria and chloroplasts are scarce or absent. This is called **zone of exclusion** (**Morre *et al.*, 1971**) or **Golgi ground substance** (**Sjostrand** and **Hanzon, 1954**). Endoplasmic reticulum within the zone of exclusion has a smooth surface (lacking ribosomes), and coated vesicles of the Golgi complex are restricted to this region. Similar zone of exclusions are associated with microtubules (**Porter, 1966**), centrioles (**Bainton** and **Farquhar, 1966**), and regions of centriole formation (**Sorokin, 1968**).

CHEMICAL COMPOSITION

Chemical analysis of the membranes of the Golgi complex has shown that they have a composition between that of the ER and of the plasma membrane (Keenan and Morre, 1970). The Golgi membranes are rich in phospholipids (*e.g.*, cephalin and lecithin), proteins and enzymes, as ADPase, ATPase, CTPase, thiamine pyrophosphatase, NADH-cytocrome C reductase, NADPH-cytochrome C reductase, UDP–N–acetyl glucosamine transferase, galactosyl transferase, glucose–6–phosphatase and other enzymes. The Golgi complex also contains some carotinoids, fatty acids and vitamin C.

RELATIONSHIP OF GOLGI COMPLEX WITH ENDOMEMBRANE SYSTEM

In conjunction with the endoplasmic reticulum, membrane-bounded vesicles and lysosomes, the Golgi complex forms an extensive inter-communicating membrane system called endomembrane system within the cell. The lumen enclosed by this membrane system is made up by that of the perinuclear space ; the ER ; the cisternae, tubules and vesicles of the Golgi complex ; the lysosomes ; the pinocytotic and autophagic vesicles ; and in plant cells, the vacuole contained by the tonoplast. The region outside the plasma membrane is also included in the endomembrane system by Northcote (1973). All these spaces are connected either by a functional continuity or by a direct morphological connection, so that they together form a distinct channel which is separated from the rest of the cytoplasm and nucleoplasm of the cell and in which material can be synthesized, modified, and transported. The movement of material can take place either within the cell or from the inside to the outside of the cell and also from the exterior back into the vesicles and lysosomes. The Golgi complex occupies a key position in the functioning of this membrane system, since not only specific synthetic functions are carried out within the organelle and its associated vesicles, but also, as part of the transport route, it presents an irreversible connection between the perinuclear space and ER on the one hand and the vesicles and plasma membrane on the other De Duve, (1969).

ORIGIN

Variour cytolc [illegible] that the membrane[illegible] membranes of the s[illegible] rough ER. Certain [illegible] bodies arose fro[illegible] cisternae. Further,

lized close to the cell plate after mitosis in plant cells, it has been suggested that the Golgi arise *de novo* from the phragmoplast. But, both of these theories have been discarded now.

FUNCTIONS

The Golgi complex is mainly concerned with the formation and packaging of material for export from the cell across the plasma membrane by a process of reverse pinocytosis. In this way cell wall material is deposited in plants (Northcote, 1969), and secretory proteins, digestive enzymes (Jamieson and Palade, 1967), and the matrix of the connective tissue (Neutra and Leblond, 1966) are transported in animal tissues. Some of the most important functions of Golgi complex or Golgi body are following :

1. Formation of secretory vesicles—The formation of secretory vesicles by the Golgi complex has been studied best in pancreatic cells by Palade and his co-workers (1964, 1967). They have shown that the digestive enzymes (*e.g.*, α-amylase) or their precursors (*e.g.*, α-chymotrypsinogen), which are synthesized on the ribosomes at the

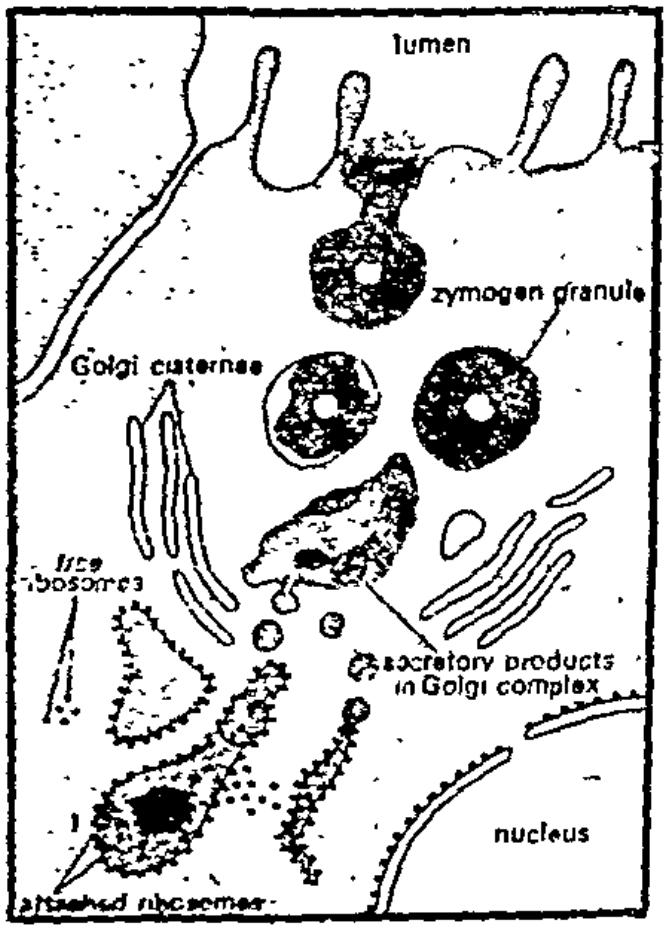


Fig. 6-2. The formation of secretory vesicles by Golgi complex of pancreatic cells.

outer surface of the rough ER, are transferred across the reticulum membrane into the lumen by some mechanism unknown at present. Once in the lumen they are transported to elements of smooth ER where they are packed into small vesicles and carried into the proximal poles forming faces of a Golgi cisternae. In the Golgi cisternae, the protein is concentrated into the membrane-bounded zymogen granules (inactivated state of pancreatic enzymes) which arise from the maturing faces of Golgi cisternae. These granules (secretory vesicles) migrate to the cell apex and discharge their contents into the pancreatic ducts where they pass to the intestine The passage of the secretory proteins to the outside of the cell is brought about by reverse pinocytosis in which the membrane of secretory vesicles fuses with and becomes part of the plasma membrane

goblet
1969).

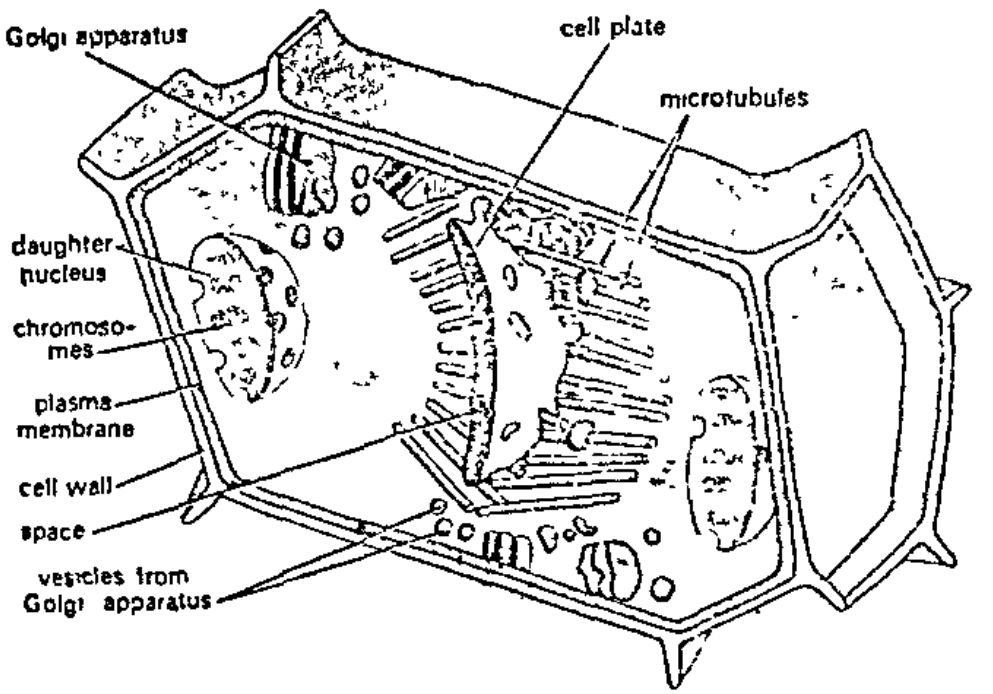


Fig 6·3. Schematic representation of a cell of a higher plant as seen at telophase in mitosis. In the phragmoplast region, a region of membranes and microtubules, a cell plate forms and grows until it separates the cytoplasm into two daughter cells The cell plate develops as a membrane-delimited structure enclosing a space in which new cell-wall will form The Golgi apparatus contributes many vesicles to the phragmoplast membrane ; the vesicle membranes apparently are incorporated into the membrane of the cell plate and the vesicle contents enter the forming cell wall.

to the Golgi complex where it is conjugated with carbohydrate, and the resultant mucopolysaccharide is transported as mucigen granules to become the mucus of the colon.

2. **Formation of plasma membrane**—As the membranes of secretory vesicles are morphologically similar to those of plasma membranes and are capable of fusing with them. Therefore, when they discharge their contents outside the cell, their membranes are incorporated into the plasma membrane, and thus the size of plasma membrane gradually increases.

3. **Formation of cell wall of plant cells**—All the polysaccharides as pectin, hemicellulose and microfibrils of α-cellulose of cell wall of plant cells are synthesized by the Golgi bodies of these cells. The Golgi bodies of plant cell forms a cell plate at the centre of the miotic spindle during mitosis and this cell plate is gradually enlarged and thickened due to deposition of matrix of pectic substances, hemicellulose and microfibrils of α cellulose by the Golgi bodies. Microfibrils of α-cellulose are later on woven into the cell wall of cellulose.

4. **Other functions**—The Golgi complex also forms certain intracellular structures as **acrosome** of spermatozoa of animals, certain **yolk substances** and **cortical granules** of oocytes, **tubular inclusions** of endothelial cells, **lysosomes** and certain vacuoles. It also stores certain proteins and lipids. In endocrine cells, it has a key role in the secretion of hormones.

CHAPTER 7

LYSOSOMES

The lysosomes (Gr., *lyso*-digestive, *soma*-body) are tiny, membrane-bounded, vesicular structures of the cytoplasm which perform intra-cellular digestion of the cell. Lysosomes were first of all reported by de Duve in 1955.

OCCURRENCE

Usually the lysosomes occur in most animal cells and few plant cells. The animal cells which are secretory in function such as the pancreatic cells, leucocytes, liver cells, spleen cells and kidney cells contain large number of the lysosomes. The lysosomes are particularly numerous and large in cells such as macrophages which perform special digestive functions. The lysosomes often remain distributed evenly in the cytoplasm.

MORPHOLOGY

SHAPE AND SIZE

The lysosomes are generally spherical in shape but certain meristamatic cells of plant roots contain irregular-shaped lysosomes. The size of the lysosomes usually ranges from 0·2 to 0·8μ but may be exceptionally large in the cells of mammalian kidney, *i.e.*, upto 5μ and even more in phagocytes and leucocytes.

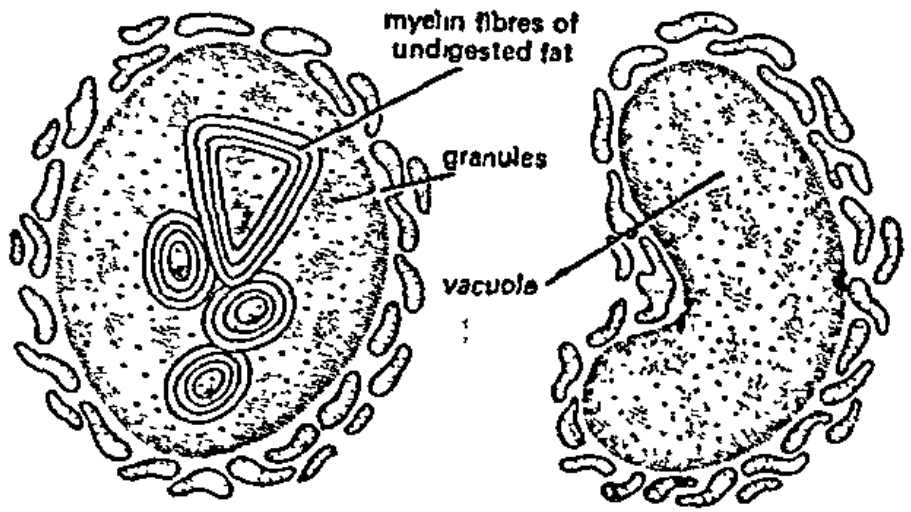


Fig. 7·1. Two different kinds of lysosomes of the kidney cells of rat.

STRUCTURE

The lysosomes are round vacuolar structure which remain filled with dense material and acid phosphatase enzymes. The lysosomes are bounded by a unit membrane of the lipoprotein. The space or vacuole of the lysosomes contains dense, granular, vacuolated and homogeneous material.

CHEMICAL COMPOSITION

The lysosomes have been found to contain molecules of about 24 enzymes. These are β-galactosidase, β-glucuronidase, β-N-acetyl-glucosaminidase, α-glucosidase, α-mannosidase ; cathespin A (Acid protease), cathespin B (Acid protease), aryl sulphatase A, aryl sulphatase B, acid ribonuclease, acid deoxyribonuclease, acid phosphatase, acid lipase, phospholipase A, phosphotidic acid phosphatase hyaluronidase, phosphoprotein phosphatase, amino peptidase A, dextranase, saccharase, lysozyme (muramidase), Mg^{++} activated

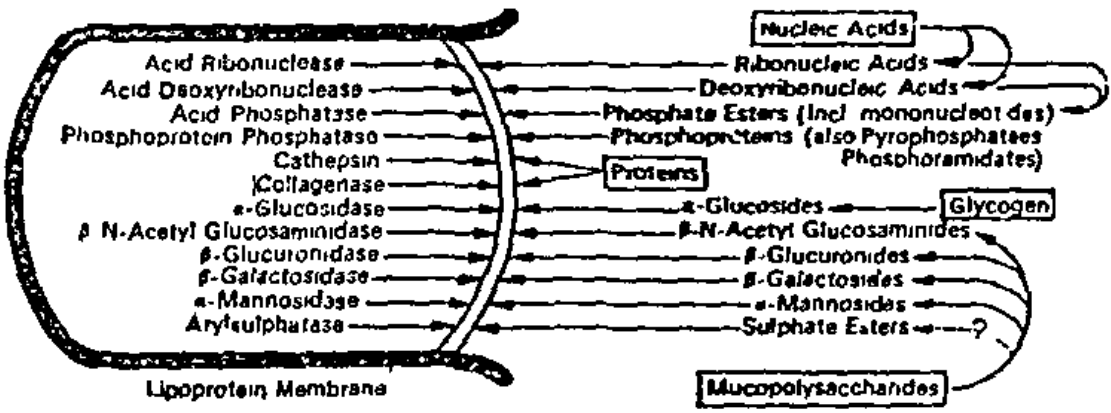


Fig. 7·2. The enzymes of lysosomes and their substances.

ATPase, indoxylacetate, esterase and plasminogen activator. All these enzymes of the lysosomes are enclosed within the single lipoprotein membrane which can be ruptured easily by progesterone, endotoxins, testosterone, vitamin A and E, proteases, digitonin, X-irridation, ultra-violet irridation and bile salts. But the lysosomes remain stable in living cells. The stability to the lysosomes is provided by certain chemical substances such as cholesterol, cortisone, cortisol and chloroquine. These substances stabilize the membrane of the lysosomes.

KINDS OF LYSOSOMES
(POLYMORPHISM IN LYSOSOMES)

In different types of cells and at different times in a same cell four types of lysosomes have been observed which are as follows :

1. Primary lysosomes.
2. Secondary lysosomes.

3. Residual bodies.
4. Autophagic vacuole or cytolysosome.

1. PRIMARY LYSOSOMES

The primary lysosomes or pre-lysosomes are small sac-like bodies having many enzymes. These are either secreted directly by the granular endoplasmic reticulum or by the cisternae of the Golgi complex.

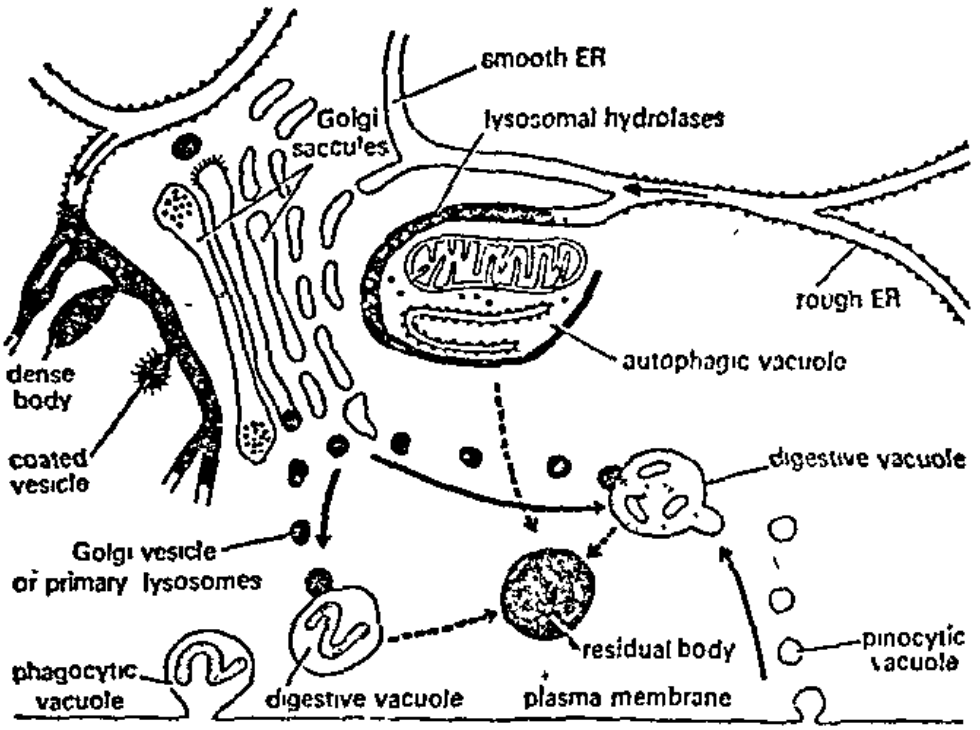


Fig. 7·3. Diagram suggesting probable interrelations of some organelles in the transport of lysosomal hydrolases and the formation of lysosomes.

2. SECONDARY LYSOSOMES

The secondary lysosomes are also known as heterophagosomes or digestive vacuoles. When the cells [illegible] or extracellular substances [illegible] cytosis (heterophagy), then the plasma membrane forms the membrane-bound vesicles known as pinosomes or phagosomes around these substances. The [illegible]

[illegible] takes place by the enzymatic [illegible] the secondary lyso[illegible] weight and [illegible]

The secondary lysosomes are now known as **residual bodies.** The debris, which is generally lipid, is ejected from the cell by exocytosis into the external environment. In certain cells the residual bodies remain in the cells for long time and play important role in the aging process. These residual bodies are formed due to the lack of certain enzymes in the lysosomes and they may cause about twelve pathological diseases in man. The disease such as fever, congested heart failure, hepatitis, pylonephritis, hypertension, mitosis, joint injury, leucocyte granules and tissue injury have been related with lysosomes by various pathological studies.

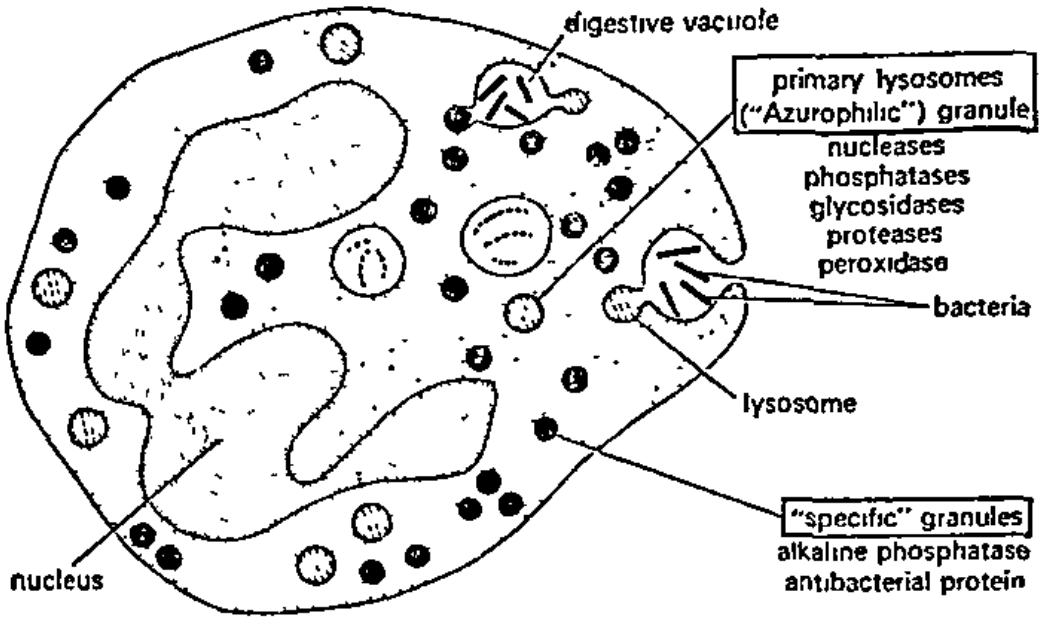


Fig 7·4. Diagram of a white blood cell (neutrophil) ingesting bacteria. Two types of granules fuse with the phagocytosis vacuoles and contribute digestive enzymes and other components.

4. AUTOPHAGIC VACUOLES

The autophagic vacuoles are also known as **autophagosomes** or **cytolysosomes.** The autophagic vacuoles are formed when the cell [illegible] mitochondria and [illegible] such cases, [illegible] ntra-cellular [illegible] acuoles are [illegible] tions. C. de [illegible] starvation of the organisms many autophagic vacuoles develope in the liver cells which feed on the cellular components.

ORIGIN OF LYSOSOMES

The origin of lysosomes is not known with any amount of certainly. Some workers have suggested that the lysosomes have

It seems likely

the granular endoplasmic reticulum.

FUNCTIONS OF LYSOSOMES

The important functions of lysosomes are as follows :

1. Digestion of large extracellular particles—The lysosomes digest the food contents of the phagosomes or pinosomes. The lysosomes of leucocytes enable the later to devour the foreign proteins bacteria and viruses,

2. Digestion of intracellular substances—During the starvation, the lysosomes digest the stored food contents, *viz.*, proteins, lipids and carbohydrates (glycogen) of the cytoplasm and supply to the cell necessary amount of energy.

3. Autolysis—In certain pathological conditions the lysosomes start to digest the various organelles of the cells and this process is known as autolysis or cellular autophagy. In the process of metamorphosis of amphibians, es, by th

4

rms.

and

process of extracellur digestion.

5. The lysosomes

6. Altered Mitochondria

and the r enzymes may be involved in carcinogenesis.

7. The lysosomes form a built-in mechanism of the cell and enable the cell to adopt metabolically rapidly changing conditions of food supply, oxygen concentration and other environmental factors which occur in many living systems.

LYSOSOMES IN PLANTS

It has become clear now that plant cells though have no lysosome exactly similar to lysosomes of animal cells, but many membrane-bounded storage granules of them are found to contain certain digestive enzymes, and these granules are considered as lysosomes of plant cell (Gahan, 1972). According to Matile (1969) the plant lysosomes can be defined as membrane-bounded cell compartments containing hydrolytic digestive enzymes. Following storage granules of plant cells are found to possess lysosome-like activities :

1. Spherosomes.
2. Aleurone grain.
3. Vacuoles.

1. SPHEROSOMES

The spheromsomes are [illegible] cles of 0·5 to 2·5μ diameter, occu [illegible] e a fine granular structure internally which is rich in lipids and proteins. They originate from the endoplasmic reticulum (ER). Oil accumulates at the end of a strand of ER and a small vesicle is then cut off by constriction to form particles, called prospherosomes. The prospherosomes grow in size to form spherosomes. Basically, the spherosomes are involved in lipid synthesis and storage. But, the spherosomes of maize root tips (Matile, 1968) and spherosomes of tobacco endosperm tissue (Spichiger, 1969) have been found rich in hydrolytic digestive enzymes and so have been considered as lysosome. Like lysosomes they are not only responsible for the accumulation and mobilization of reserve lipids, but also for the digestion of other cytoplasmic components incorporated by phagocytosis.

2. ALEURONE GRAIN

The aleurone grains or protein bodies are spherical membrane bounded stora[illegible] cotyledons of [illegible] ripening and [illegible] store protein [illegible] Matile (1968) [illegible] contain a wid[illegible] phosphatase which are required for the mobilization of stored protein and phosphate, although the presence of other enzymes such as β-amylase and RNAase suggest that other cell constituents may also be digested. Thus, like spherosomes, aleurone grains store reserve materials, mobilize them during germination and in addition form

a compartment for the digestion of other cell components (Hall *et al.*, 1974). The aleurone grains are derived from the strands of the endoplasmic reticulum.

3. VACUOLES

The plant cells also contain particles called vacuoles that are approximately [illegible] usually show lit[illegible] enzymes and m[illegible] are derived from endoplasmic reticulum which gives out provacuoles. The provacuoles are analogous to the primary lysosomes of animal cells and develop by fusion to form larger vacuoles.

CHAPTER 8

MITOCHONDRIA

The mitochondria (Gr., *mito*-thread, *chondrion*-granule) are filamentous or granular cytoplasmic organelles of all aerobic cells of higher animals and plants and also of certain micro-organisms including Algae, Protozoa and Fungi. These are absent in bacterial cells. T
e
c
mes for the protein synthesis.

HISTORICAL

The mitochondria were first of all observed in 1880 by Kolliker who teased them out of muscle cells of insects. He noted that the granules (mitochondria) possessed a membrane and that they swelled in water. In 1882 Flemming named them as fila. Later on Altmann (1890) made systematic observations on them and named them as bioblast. The present name mitochondria was assigned by Benda (1897—98) to them. He stained mitochondria with alizarin and crystal violet. Michaelis (1900) first of all stained the mitochondria by janus green. Lewis and Lewis (1914) demonstrated the possibility of metabolic activities in the mitochondria of cultured cells. In 1934 Bensley and Hoerr first of all isolated the mitochondria from the liver cells. Hogeboom (1948) has shown that mitochondria is the site for the cellular respiration. Chevremont (1956, 1957) suspected the presence of DNA molecule in the mitochondria. Nass (1963) actually observed and proved the presence of the DNA molecule in the mitochondria.

Previously the mitochondria have been known by various names such as **fuchsinophilic granules, parabasal bodies, plasmosomes, plastosomes. fila, vermicules. bioblasts and chondriosomes.**

DISTRIBUTION OR LOCALIZATION

The mitochondria move autonomously in the cytoplasm, so they generally have uniform distribution in the cytoplasm, but in many cells their distribution is very restricted. For example, in the rod and cone cells of the retina all the mitochondria are located in a portion of the inner segment, in certain muscles of the diaphragm the

mitochondria are concentrated around the I bands of the myofibril and forming a ring, and in the cells of renal distal tubules the mitochondria lie near and perpendicularly to the cell membranes. The concentration of the mitochondria in such cells has suggested their utility in providing more energy to these cells for active metabolic functions.

In some cells, the mitochondria are concentrated in the peripheral cytoplasm or cytoplasm just beneath the plasma membrane as in *Paramecium*, pancreatic cells, cells of the Malpighian tubules of the insects and young spermatids of the rat. While in certain cells the mitochondria remain concentrated around the nucleus. During the cell division (mitosis and meiosis) the mitochondria get concentrated around the spindle.

ORIENTATION

The mitochondria have definite orientation. For example, in cylindrical cells the mitochondria usually remain orientated in basal apical direction and lie parallel to the main axis. In leucocytes, the mitochondria remain arranged radially with respect to the centrioles. The orientation of the mitochondria is said to depend on the nature of the cytoplasmic matrix, vacuolar system and the direction of the diffussion currents of the cell.

MORPHOLOGY

NUMBER

The number of mitochondria in a cell depends on the type and functional state of the cell. It varies from cell to cell and from species to species Certain cells contain exceptionally large number of the mitochondria, *e.g.*, the *Amoeba, Chaos chaos* contain 50,000 ; eggs of sea urchin contain 140,000 to 150,000 and oocytes of amphi bians contain 300,000 mitochondria. Certain cells, *viz.*, liver cells of rat contain only 500 to 1600 mitochondria. The cells of *green* in comparison to animal mitochondria is taken over contain only one mitochondrion.

SHAPE

The mitochondria may by filamentous or granular in shape and may change from one form to another depending upon the physiological conditions of the cells. Thus, they may be of club, racket, vesicular, ring or round-shape.

SIZE

The size of the mitochondria may vary from cell to cell. The diameter of the mitochondria ranges from 0·2μ to 2μ and the length

may range from 0·3μ to 40μ. The pancreatic cells of mammals contain mitochandria of 10μ langth and the oocytes of amphibian *Rana pipens* contain 20 to 40μ long mitochondria.

STRUCTURE

The mitochondria are bounded by a double membrane envelope which provides good tensile strength. stability and flexibility to them. The outer and inner mitochondrial membranes are 60—70 A° thick and trilaminar in nature. Each of them is composed of an outer, and an inner dense, osmiophilic layer of protein molecules of 20 to 25 A thickness and a middle 25 A° thick bimolecular layer of lipids. The

diameter and spaced rather irregularly.

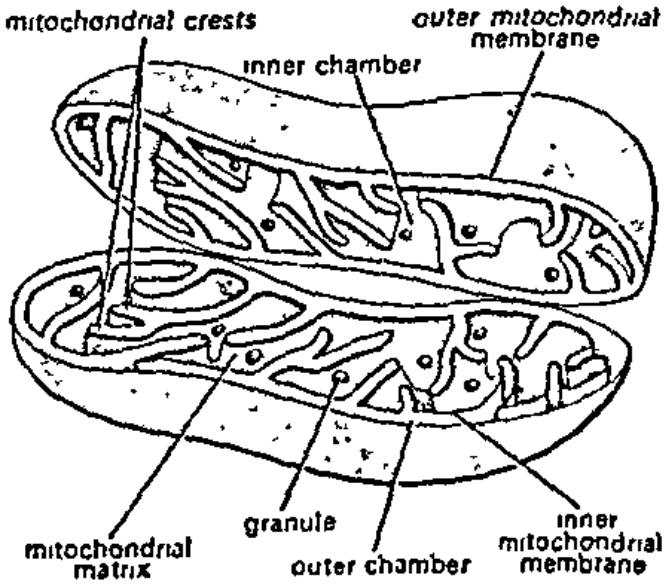


Fig 8 1 Detailed structure of a typical mitochondrion.

innermost com-
chondrial matrix
70S ribosomes
of mitochondria

to accumulate ions. Granules are prominent in the mitochondria of cells concerned with the transport of ions and water, including kidney tubule cells, epithelial cells of the small intestine, and the osteoblasts of bone-forming cells. Further, the inner membrane has an outer **cytosol** or **C face** toward the perimitochondrial space and an inner **matrix** or **M face** toward matrix.

The inner mitochondrial membrane increases its surface area by giving out plate-like or tubular invagination called **mitochondrial**

crests or cristae mitochondriales, which penetrate the mitochondrial matrix. In general, the cristae of plant mitochondria are tubular, while those of animal mitochondria are lamellar or plate-like (**Hall, Flowers** and **Roberts, 1974**), but, in many Protozoa and in steroid synthesizing tissues including the adrenal cortex and corpus luteum, they occur as regularly packed tubules (**Tyler, 1973**). The space within the cristae which is continuous with the perimitochondrial space is known as **intracristal space.**

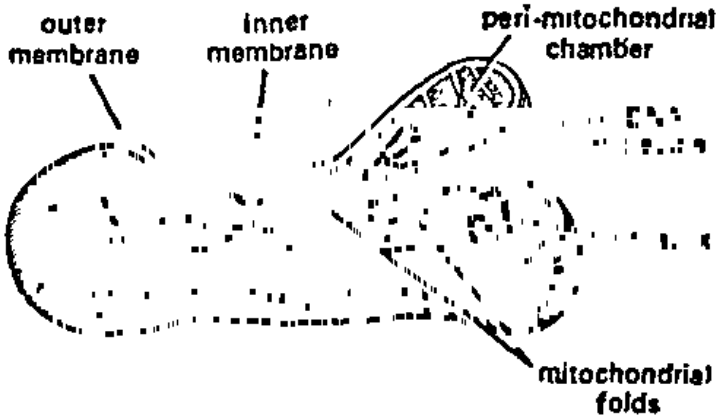


Fig 8·2. A mitochondrion showing its various components and the position of DNA molecules inside it.

Inner membrane subunits or elementary particles—Under high resolving power of the electron microscope the inner membrane of [illegible] particles at membrane of the mitochondria by a short stem, stalk or pedicle of 35 to

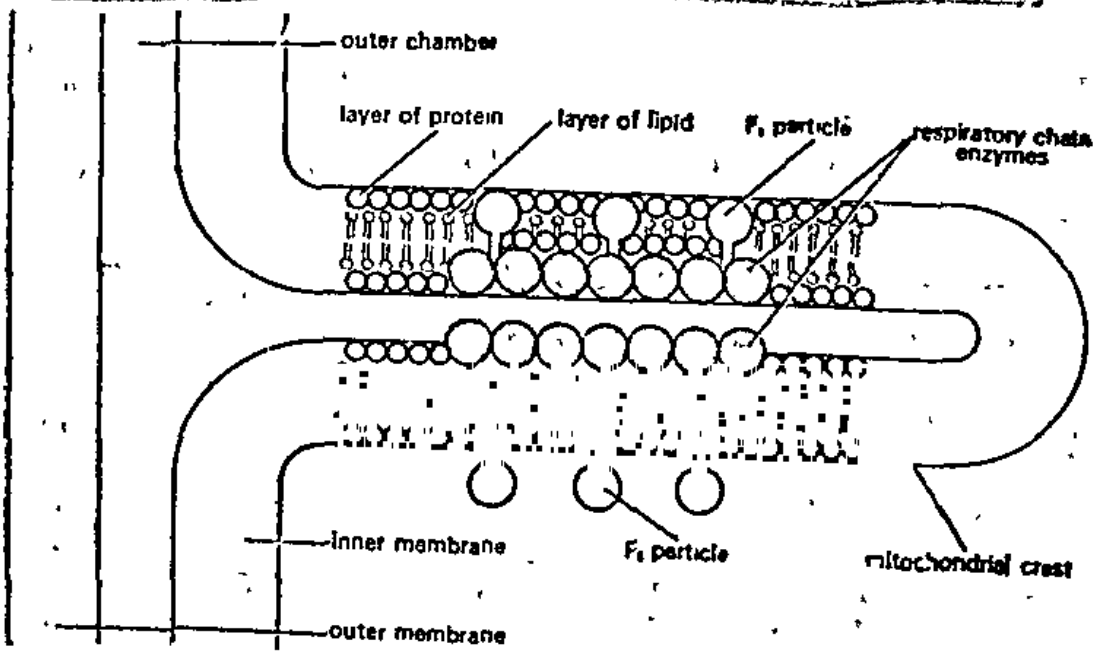


Fig. 8·3. Ultrastructure of a mitochondrial crest

50 A° length. These particles may be 10^4 to 10^5 in number in each mitochondrion and are known as elementary particles, F_1 particles or inner membrane subunits. Usually the inner membrane subunits occur in the thickness of inner mitochondrial membrane but in certain special conditions of mounting only these particles are coming out of the membrane.

Previously the inner membrane subunits were thought to contain all the enzymes for electron transport system and for oxidative phosphorylation and, therefore, named as electron transport particles or ETP (Parsons, 1963). But recently Racker (1967) has shown that the F_1 particles represent a special ATPase or ATPsynthetase and are involved in the process of oxidation and phosphorylation.

CHEMICAL COMPOSITION

The gross chemical composition of the mitochondria varies in different animal and plant cells. However the mitochondria are found to contain 65 to 70 per cent proteins, 25 to 30 per cent lipids, 0·5 per cent RNA and small amount of the DNA. The lipid contents of the mitochondria composed of 90 per cent phospholipids (lecithin and cephalin), 5 per cent or less cholesterol and 5 per cent free fatty acids and triglycerides. The outer mitochondrial membrane contains comparatively large amount of phospholipids and cardiolipin proteins.

ENZYME SYSTEM OF MITOCHONDRIA

The mitochondria contain more than 70 enzymes and co-enzymes which act in a orderly fashion along with several co-factors. These enzymes are distributed in the matrix, outer and inner membranes of the mitochondria. Lehninger (1969) has tabulated following important mitochondrial enzymes :

A. The enzymes of outer mitochondrial membrane are monoamine oxidase, rotenone-insensitive NADH-cytochrome-C-reductase, kynurenine hydroxyalase, and fatty acid COA ligase.

B. The enzymes of outer mitochondrial chamber are adenylate kinase and nucleoside diphosphokinase.

C. The enzymes of inner mitochondrial membrane are the [illegible] adenine [illegible] diphosphopyridine nucleotide (DPN) dehydrogenase, four cytochromes (Cyt. b, Cyt. c, Cyt. a and Cyt. a_3), ubiquinone Q_{10} or Coenzyme Q_{10}, non-heme copper and iron, succinic dehydrogenase, ATP synthetase, succinate dehydrogenase : β-hydroxybutyrate dehydrogenase ; carnitive fatty acid acyl transferase.

D. The enzymes of the mitochondrial matrix are malate dehydrogenase, isocitrate dehydrogenase, fumarase, aconitase, citrate synthetase, α-keto acid dehydrogenase, β-oxidation enzymes. Moreover, the mitochondrial matrix contains different nucleotides, nucleotide coenzymes and inorganic electrolytes—K^+, HPO_4^{--}, Mg^{++}, Cl^- and SO_4^{--}.

FUNCTIONS

The mitochondria perform most important functions such as [illegible] oxidative phosphorylation and respi- [illegible] heir structure and enzymatic system are [illegible] fferent functions. They are the actual respiratory organs of the cells where the food stuffs, *i.e.*, carbohydrates and fats are completely oxidised into CO_2 and H_2O. During the biological oxidation of the carbohydrates and fats large amount of energy is released which is utilized by the mitochondria for synthesis of the energy rich compound known as adenosine triphosphate or ATP. Because mitochondria synthesize energy rich compound ATP, they are also known as "power houses" of the cell.

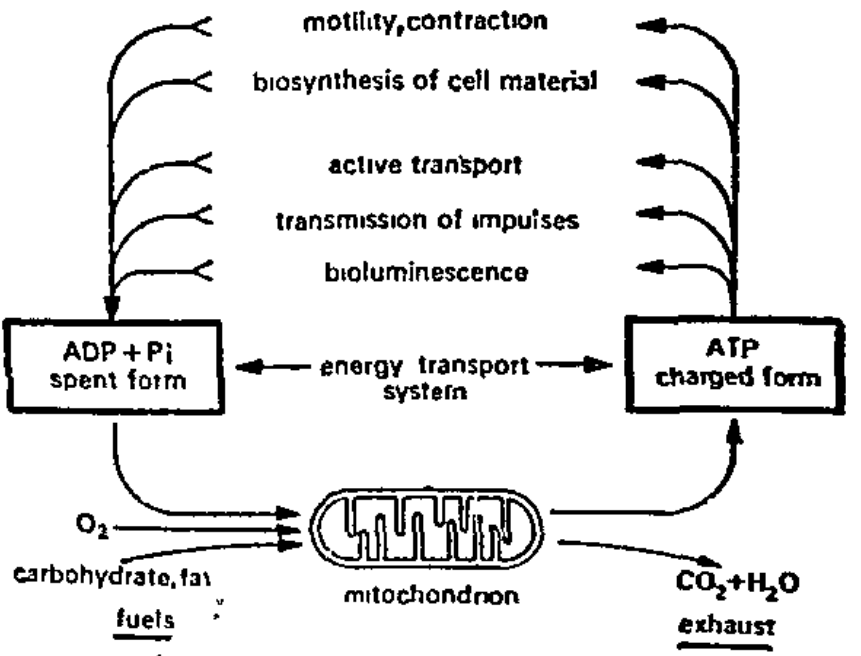


Fig. 8.4. Diagrammatic representation of the energy liberation in mitochondrion and its utilization in various cellular functions.

[illegible]

the nucleoside, adenosine which by having one, two or three phosphate groups forms the adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP) respectively.

In ATP the last phosphate group is linked with ADP by a special bond known as "energy rich bond" because when the last phosphate group of the ATP is released the large amount of energy is released as shown by the following reaction.

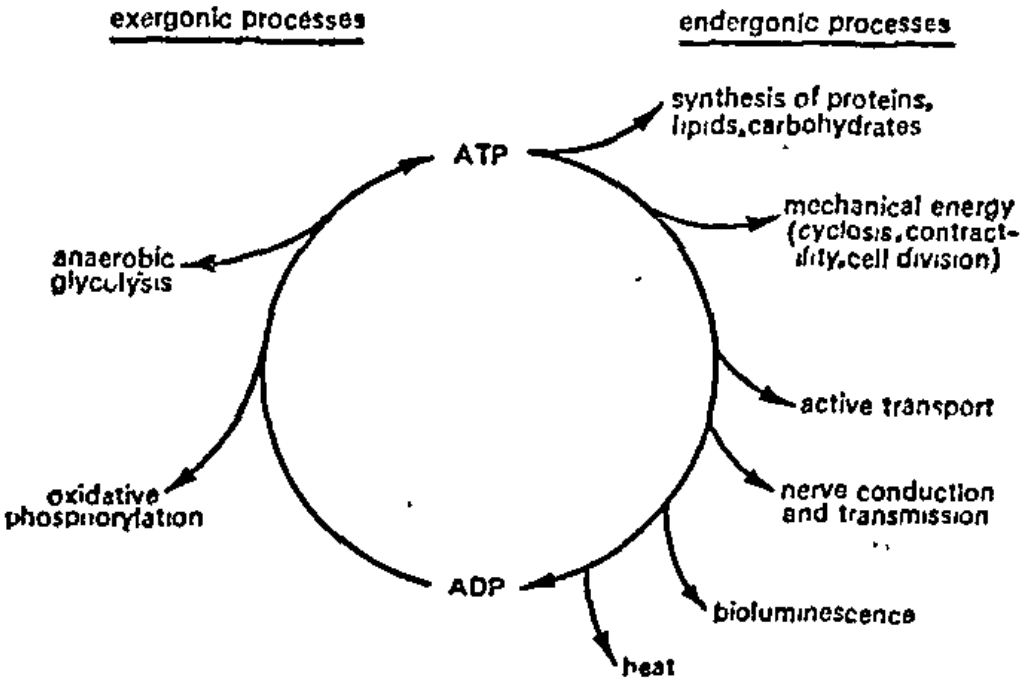
$$\underset{\text{(ATP)}}{A-P\sim P\sim P} \rightleftharpoons \underset{\text{ADP}}{AP\sim P} + \underset{\text{Phosphate group}}{P_i} + 7000 \text{ calories}$$

In the above reaction, we have seen that by the breaking of the energy rich bond about 7000 calorie
common chemical bond releases c
chemical reactions which synthesize
require great amount of energy, wh
of the food stuffs in the mitochondria. The utility of energy rich phosphate bond (~P) of the ATP is that great amount of energy is kept stored in the ready state in a very limited space of the cell. The stored chemical energy is disposed off very quickly at the time of the need in various cellular functions such as respiratory cycle, protein and nucleic acid syntheses, nervous transmission, cell division, transportation and bioluminescence, etc.

Recently, besides ATP, certain other energy rich chemical compounds have been found to be active in the cellular metabolism. These are **cytosine triphosphate (CTP), uridine triphosphate (UTP)** and **guanosine triphosphate (GTP).** These compounds, however, derive the energy from the ATP by nucleoside diphosphokinases.

OXIDATION OF CARBOHYDRATES

The carbohydrates enter in the cell in the form of monosaccha-



8·5. Diagrammatic representation of uses and synthesis of ATP.

rides such as glucose or glycogen. These hexose sugars are first broken down into 3-carbon compound (pyruvic acid) by a series of chemical reactions known by many enzymes. The pyruvic acid enters in the mitochondria for its complete oxidation into CO_2 and water. The reactions which involve in the oxidation of glucose into CO_2 and water are known to form the metabolic pathways and they can be grouped under the following heads :

(i) [illegible]rhof pathways.
(ii)
(iii) Krebs cycle.
(iv) Respiratc

(I) GLYCOLYSIS C

In the proces

the six carbon chain glucose or glycogen molecule is hydrolysed into two molecules of 3 carbon chain pyruvic acid molecules. The hydrolysis af glucose takes place in various steps and each step is catalyzed by a specific enzyme. These steps have been worked out by two German biochemists, Embden and Meyerhof and, therefore, known as Embden-Meyerhof pathways after the name of its discoverers. The Embden-Meyerhof pathways takes place in the cytoplasm and it does not require any amount of oxygen. The various steps of anaerobic degradation or hydrolysis of glucose have been shown in the figure 8·6.

[illegible] during the Embden-Meyerhof pathways the glucose mole-

anaerobic cells such as yeast and muscle cells or enter in the mitochondria of the aerobic cells and oxidized into CO_2 and water. The conversion of pyruvic acid into ethyl alcohol or lactic acid in the yeast and muscle cells have been shown by following reactions :

(In yeast cells) Pyruvic acid $\xrightarrow{-CO_2}$ Acetaldehyde $\rightarrow$ Ethyl alcohol + Energy

(In muscle cells) Pyruvic acid $\xrightarrow[+NADH]{\text{Lactic dehydrogenase}}$ Lactic acid + NAD + Energy

(II) OXIDATIVE DECARBOXYLATION

In the aerobic cells the pyruvic acid enters in the mitochondria

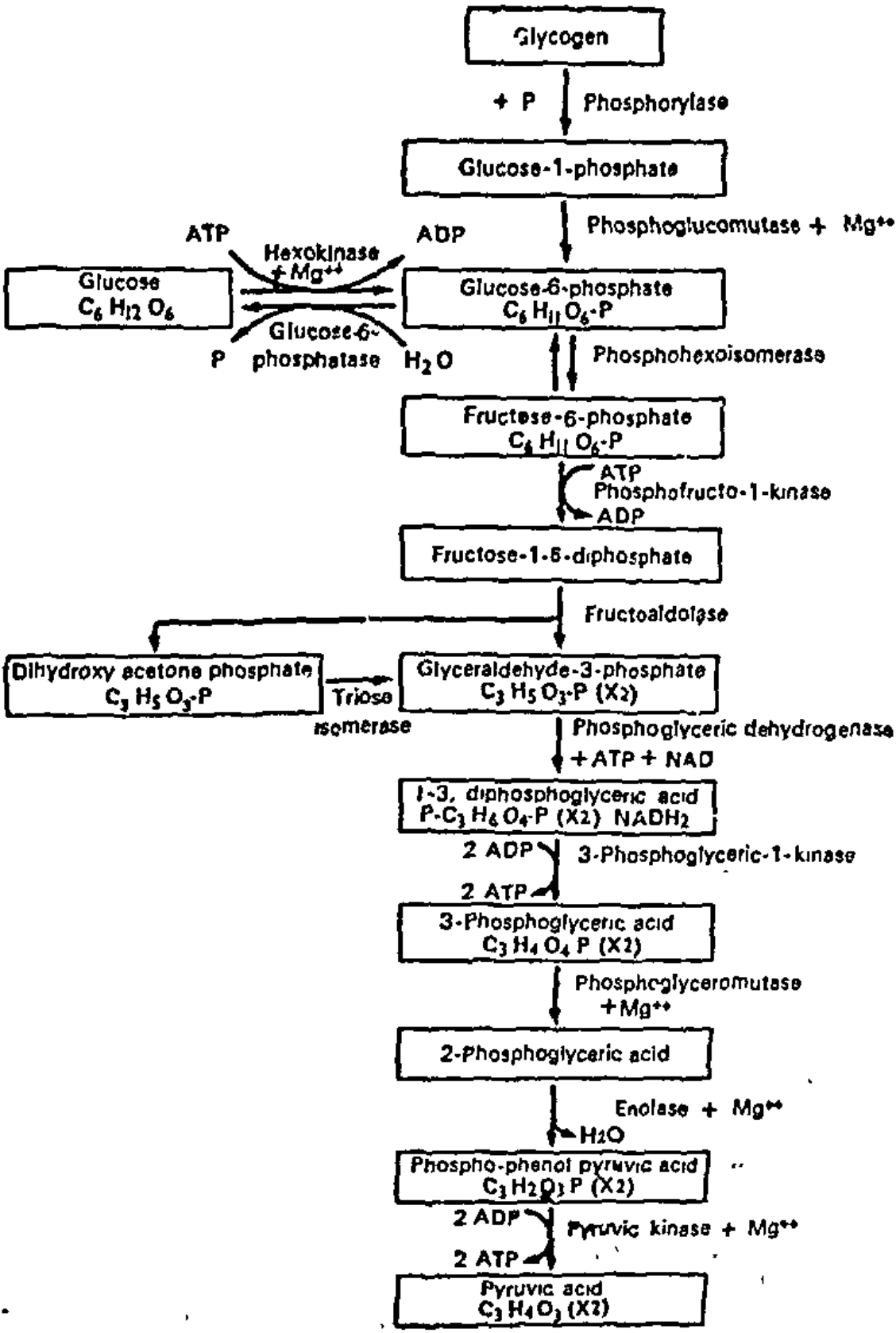


Fig. 8·6. Glycolysis or Embden-Meyerhof pathways showing the step-wise degradation of glucose to pyruvic acid.

acid. The oxidative decarboxylation process involves a complex system of three enzymes, *viz.*, pyruvic acid decarboxylase, dihydroxylipoyl transacetylase and dihydroxylipoyl dehydrogenase and five cofactors-coenzyme A, NAD, lipoic acid, Mg^{++}, and thiamine pyrophosphate Acetyl COA is formed as the acetyl moiety of pyruvic acid, is transferred to coenzyme A ; concomitantly, the carbon of the carboxyl group is liberated as carbon dioxide. Two hydrogen atoms remain; one from the pyruvate carboxyl group and other from the sulfhydryl coenzyme A. These hydrogen molecules are transferred to NAD. The reactions of oxidative decarboxylation have been shown in fig. 8·7.

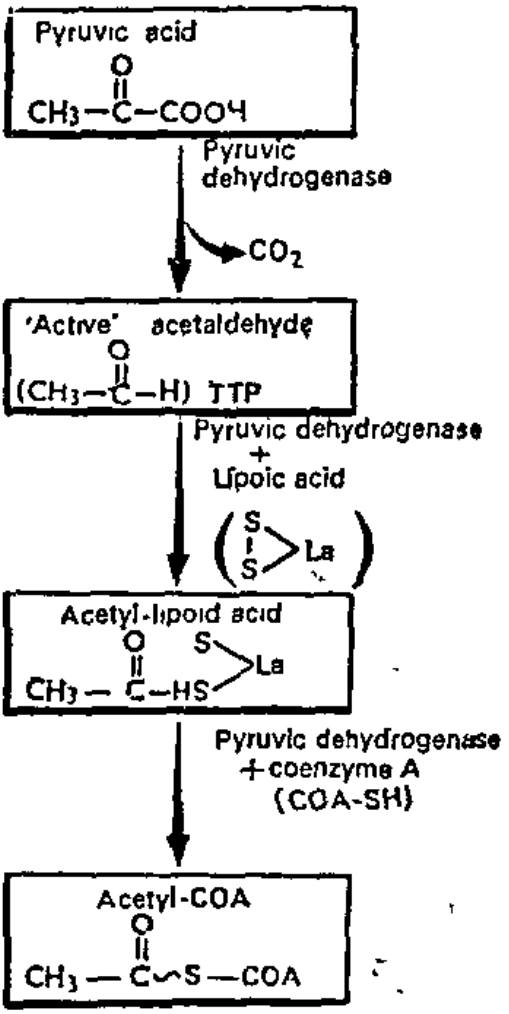


Fig. 8·7. The oxidative decarboxylation of pyruvic acid to acetyl coenzyme A.

(III) KREBS CYCLE

The acetyl coenzyme A enters in the Krebs cycle. The Krebs cycle occurs in the matrix of mitochondria. The Krebs cycle is also known as **citric acid cycle** or **tricarboxylic acid cycle** and involves many enzymes and coenzymes. The Krebs cycle first begins to turn properly when the acetyl CoA gives its acetyl group to oxalo aceticacid to form citric acid. Citrate synthetase catalyzes this reaction which is also called condensing enzyme. The citric acid passes through various stages, involving a variety of enzymes and coenzymes and ultimately oxidised into oxaloacetate as shown in the figure 8·8.

(IV) RESPIRATORY CHAIN AND OXIDATIVE PHOSPHORYLATION

During the oxidation of one molecule of acetyl coenzyme A by the Krebs cycle occurring in the mitochondrial matrix, one molecule of flavoprotein (FP or FAD) and three molecules of nicotinamide adenine dinucleotide (NAD) are oxidized by molecular oxy coenzymes called **respiratory**

occurring in the inner mitochondrial membrane. During this oxidation process enormous amount of free energy is released, some of which is utilized by inner membrane subunits or F_1 particles containing three coupling factors and ATPase enzyme, in the synthesis of ATP molecules. The formation of ATP molecules during oxidation is called **oxidative phosphorylation.**

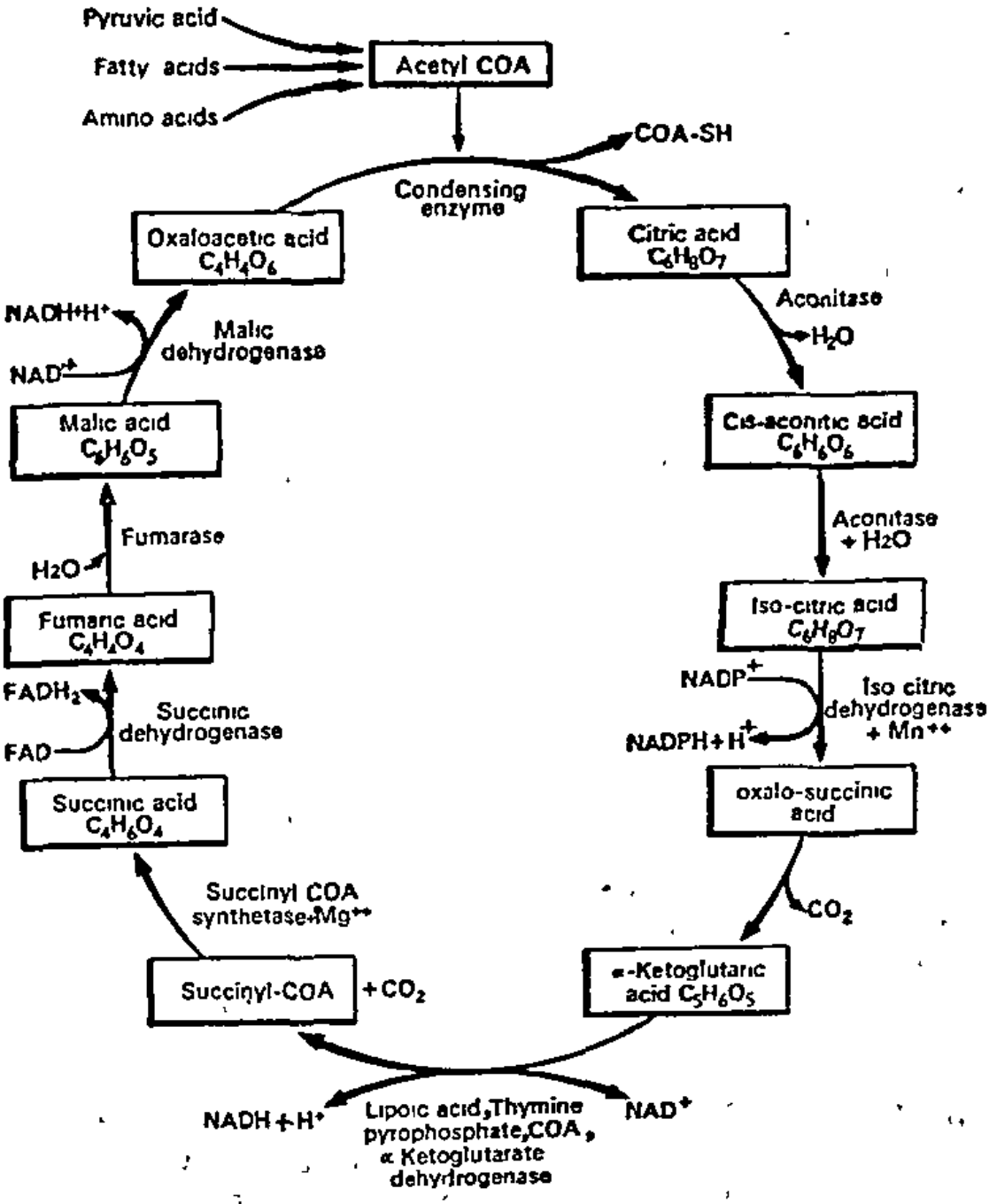


Fig. 8 8 Krebs cycle or tricarboxylic acid cycle.

According to the widely accepted **chemi-osmotic hypothesis** of oxidative phosphorylation of **Mitchell (1968)**, various components of respiratory chain or electron transport system are arranged in the

inner mitochondrial membrane in following sequence—succinic dehydrogenase (SDH) which remain associated with coenzyme NAD' flavoprotein (FP or FAD), non-haem iron protein or iron-sulphur protein, flavoprotein (FP or FAD), cytochrome b, ubiquinone or coenzyme Q_{10}, cytochrome c_1, cytochrome c, cytochrome a, cytochrome a_3 and three coupling sites (the sites where phosphorylation is coupled oxidation in the presence of ATPase enzyme (Hall *et al.*, 1974). The respiratory chain manufactures the ATP molecules during the oxidation of reduced coenzymes in following steps :

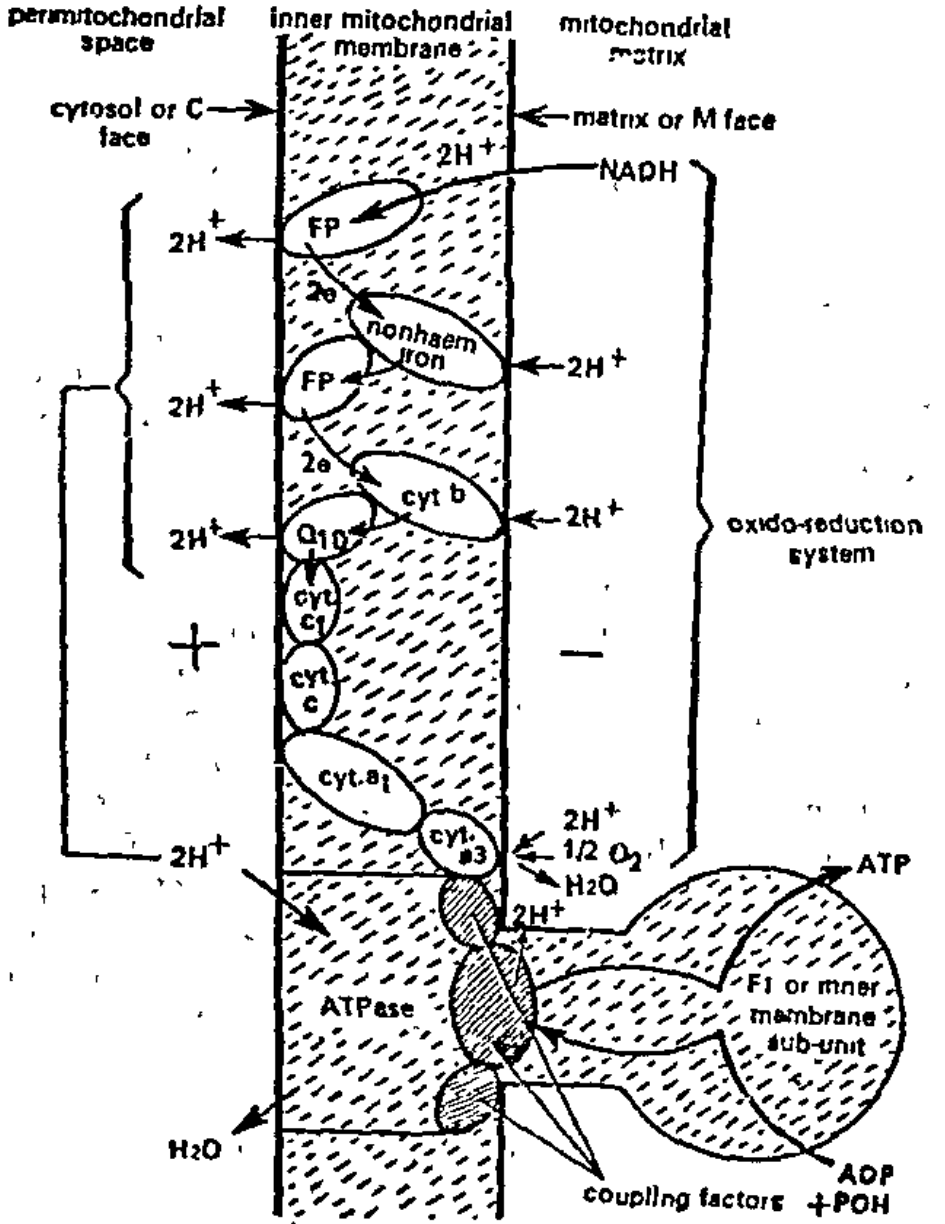


Fig. 8-9. Diagram showing the possible arrangement of respiratory chain enzymes and coenzymes in the inner mitochondrial membrane according to the chemi-osmotic hypothesis of oxidative phosphorylation and the process of oxidative phosphorylation. [Mitchell (1968) and Racker (1972)].

1. The dehydrogenase enzyme removes the hydrogen from the substrate. The hydrogen atom becomes ionized into protons (+) and electrons (e^-) :

$$2H \longrightarrow \underset{\text{Protons}}{2H^+} + \underset{\text{Electrons}}{2e^-}$$

The protons or hydrogen ions reduce the coenzyme NAD :

$$NAD + 2H^+ \longrightarrow NADH + H^+.$$

The NADH molecule passes through the M-side of inner mitochondrial membrane and enters in the respiratory chain system.

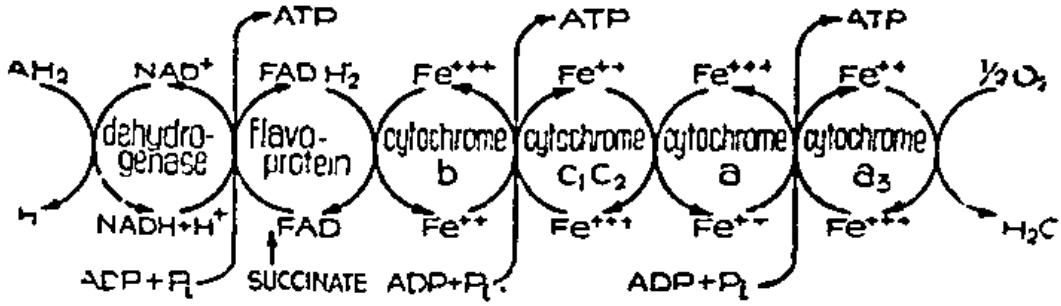


Fig 8·10 Diagram showing cycle of electrons through respiratory chain and synthesis of ATP molecules (A) is oxidized substrate.

2 The NADH is oxidized into NAD^+ by transferring its proton (hydrogen ion) to the flavoprotein (FP or FAD) which acts as a hydrogen carrier. From the flavoproteins each proton or hydrogen ion is discharged into perimitochondrial space through the C-side of inner mitochondrial membrane and electrons are passed on to non-haem iron protein, from which they cycle through different components of respiratory chain and ultimately form the water molecules.

3 The inner mitochondrial membrane is postulated to be mostly impermeable to protons and the respiratory chain is arranged within this membrane in such a way that the oxido-reduction reactions alternately involve $2H^+$ and 2e (see fig. 8·9). Thus for the reduction of one atom of oxygen by malic acid (malate), six protons are translocated from the matrix (or M-side) to the perimitochondrial space side (or C side). This creates a pH difference across the membrane and an electrical potential difference (positive on C side and negative on M side) in the membrane. In other words, when protons or electrons pass down the respiratory chain, an imbalance of protons occurs across the inner membrane and to maintain a balance of protons across the inner membrane, the protons tend to return towards the M side of the inner membrane. During their return journey to the mitochondrial matrix, they often pass through the coupling factors of F_1 particles During their passage through coupling factors, ATP synthesis occurs by following method :

$$ADP + POH \xrightarrow{\text{ATPase}} ATP + H^+$$

Net amount of ATP synthesis from a molecule of glucose. The
k cule
o etyl
be
produced by oxidative phosphorylation would result in nine molecules from the NADH and two molecules from the reduced flavoprotein In total 12 molecules of ATP may be produced from the Krebs cycle oxidation of one molecule of acetyl coenzyme A, the other molecule of ATP being produced in the succinyl coenzyme A synthetase reaction, either directly by the ADP specific enzyme or indirectly by nucleoside diphosphate kinase (GTP+ADP=GDP+ATP). Thus, from one molecule of pyruvic acid, a total of 15 molecules of ATP are produced. Of these 38 molecules, 8 molecules arise from glycolysis The complete reaction can be represented as follows :

$$C_6H_{12}O_6 + 6O + 6H_2O + 38ADP + 38P_i \xrightarrow[\text{enzymes}]{\text{Respiratory}} 6CO_2 + 12H_2O \rightarrow$$
$$+38ATP \text{ or } 456000 \text{ calories}$$

OXIDATION OF FATS

The lipids, before entering the cell, are hydrolyzed by lipase enzyme into glycerol and fatty acids. The process of hydrolyzation of fats takes place in the alimentary canal, from which the molecules of glycerol and fatty acids diffuse into blood of that system. The blood transports these molecules to the cells. In cell, glycerol is phosphorylated and converted into glyceraldehyde-3-phosphate which ultimately oxidized into CO_2 and water by the enzymes of Embden-Meyerhof-pathwayas, oxidative decarboxylation and Krebs cycle and liberate various molecules of ATP. The fatty acids are oxidised by enzymes of β-oxidation chain and liberate various molecules of ATP

OXIDATION OF PROTEINS

The proteins like the lipids are hydrolysed into amino acids by protease enzymes of alimentary canal. The amino acids are transported to cells by the blood. In cell, sometimes, they are used for the synthesis of ATP molecules. The amino acids are first deaminated into keto acids by oxidase enzymes :

$$\text{Amino acids} + CO^{-} \xrightarrow{O^{-}} \text{keto acids}$$

$$\alpha\text{-amino acids} + H_2O \xrightarrow{\text{Oxidase enzymes}} \text{keto acids} + NH_3$$

The keto acids in later stages are converted into pyruvic acids and α-keto glutaric acids. Both of these are oxidised and oxidised.

Thus, by oxidation of carbohydrates, fatty acids and amino acids, mitochondria synthesize many molecules of ATP which are utilized by the cell for its different activities (Fig. 8·5).

The mitochondrial membranes are also capable of active transport and they often accumulate ions of Ca^{-}, Mg^{++} and PO_4^{-} against the concentration gradient. Sometimes, the mitochondria assume storage function, *e.g.*, the mitochondria of ovum store large amounts of yolk proteins and transform into yolk platelets.

MITOCHONDRIAL DNA

The mitochondria contain one or two molecules of DNA according to its size. The mitochondrial DNA molecule is highly twisted, double stranded and circular in shape. The length of mitochondrial DNA molecule has been measured as 5μ and it resembles with the bacterial DNA which also contains circular molecule. Mitochondrial DNA, besides its circular shape, differs from the nuclear DNA in many respects. Rabinowitch (1968) has shown that the mitochondrial DNA contains more guanine and cytosine (G—C) contents than the nuclear DNA. The mitochondrial DNA is shorter than the nuclear DNA, therefore, contains few coded informations.

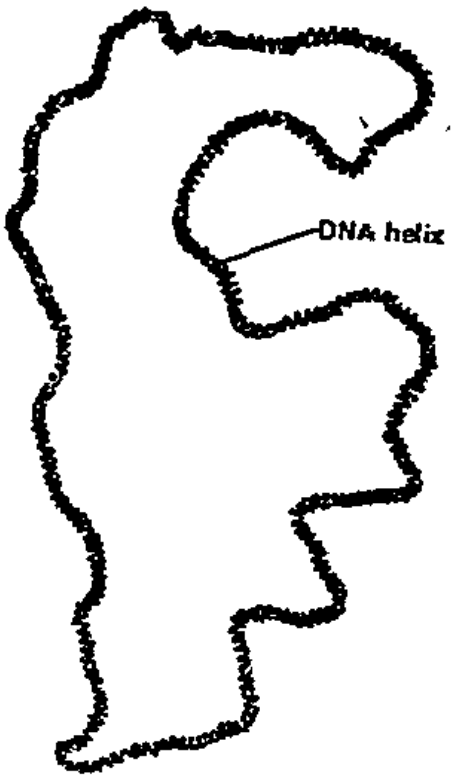


Fig. 8·11. Mitochondrial DNA molecule.

The mitochondrial DNA behaves like a chromosome and duplicates in the usual manner. Parson and Simpson (1967) have shown that the mitochondria contain the enzyme DNA polymerase which helps in the duplication of mitochondrial DNA themselves. Due to DNA molecule the mitochondria are capable to undergo self-reproduction by the division and they may carry biological informations to exhibit a type of cytoplasmic inheritance.

MITOCHONDRIAL RIBOSOMES

Mitochondrial ribosomes and protein synthesis—The mitochondria also contain 70S ribosomes similar to the bacteria in their matrix and they are called mitoribosomes. The mitochondria thus contain several components required for protein synthesis, including DNA, DNA-dependent RNA polymerase, RNA, ribosomes and amino acid-

activating enzymes (Ashwell and Work, 1970). It has been proved [illegible] several specific [illegible] dehydroge- [illegible] ructural and enzymatic proteins of mitochondria are synthesized on cytoribosomes (ribosomes of ER) according to the genetic informations of nuclear DNA.

ORIGIN OR BIOGENESIS OF MITOCHONDRIA

Regarding the origin of the mitochondria, several hypotheses have been postulated which are as follows :

1. "de novo" origin,
2. Origin from the endoplasmic reticulum or plasma membrane.
3. Origin by division of pre-existing mitochondria.
4. Prokaryotic origin.

1. "de novo" origin—According to this hypothesis, the mitochondria are originated "de novo" (L. anew) from the simple building blocks such as amino acids and lipids. But, there is no direct evidence in support of "de novo" hypothesis for the origin of the mitochondria therefore, it is discarded now.

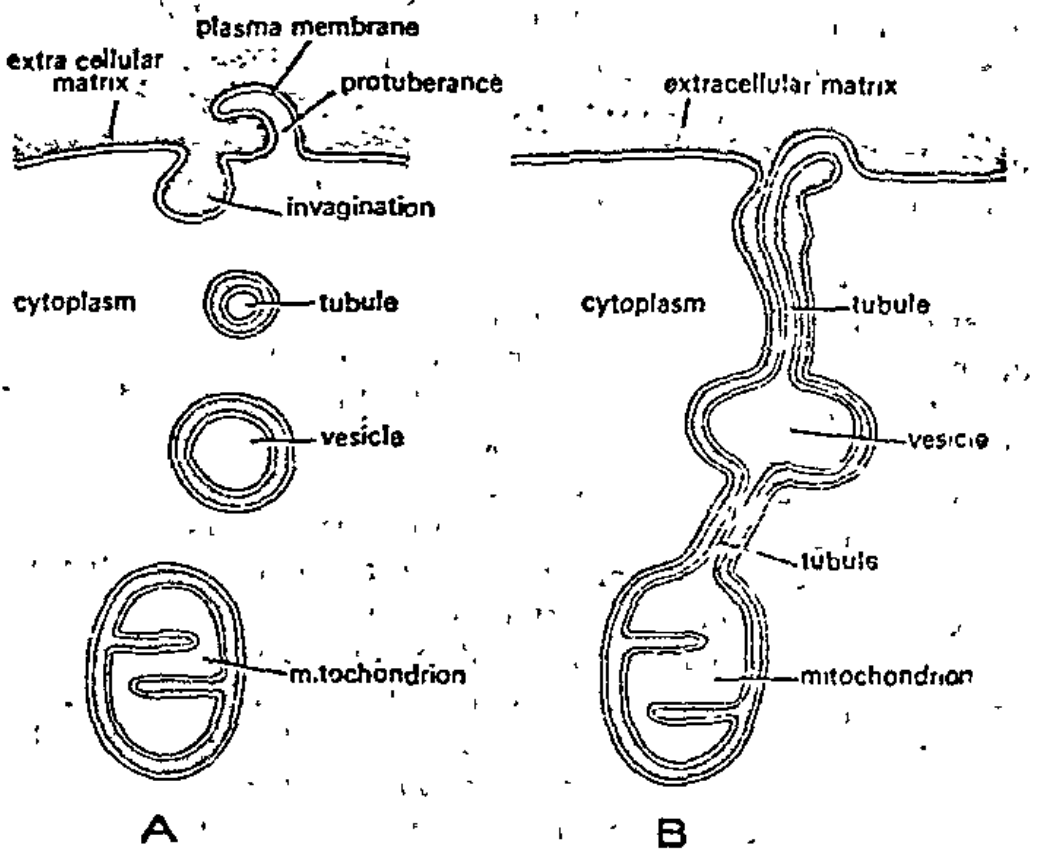


Fig. 8-12. Hypothetical diagrams showing the origin of mitochondria from plasma membrane.

2. Origin from the endoplasmic reticulum or plasma membrane—According to Morrison (1966) the new mitochondria might have been originated from the endoplasmic reticulum or plasma membrane. This hypothesis also could not provide direct evidences, therefore, it is not well accepted at present time.

3. Origin by division of pre-existing mitochondria—The electron microscopic and radio-autographic observations of the culture cells have shown clearly that the new mitochondria are originated by the division of the pre-existing mitochondria. It has been observed that the mitochondria sometimes become elongated and fragmented into small pieces. Each fragment forms a new mitochondria in later stages. Moreover, during the cell division, the mitochondrion divides transversely into two and each daughter mitochondrion develops into a mature mitochondrion.

4. Prokaryotic origin or Symbiont hypothesis—Early cytologists such as **Altmann** and **Schimber (1890)** have suggested the possibility of origin of the mitochondria from the prokaryotic cells. According to their hypothesis, the mitochondria and chloroplasts may be considered as intra-cellular parasites of the cells which have entered in the cytoplasm of eukaryotic cells in early evolutionary days, and have maintained the symbiotic relations with the eukaryotic cells. The mitochondria are supposed to be derived from the bacterial cells, while chloroplasts are supposed to be originated from the blue green algae. Due to these reasons **Altmann** suggested the name **"bioblasts"** to the mitochondria and he also hinted about their self-duplicating nature.

Recent cytological findings have also suggested many homologies between the mitochondria and the bacterial cells. The similarities between the two can be summarised as follows :

1. Similarity in inner mitochondrial membrane and bacterial plasma membrane—(i) In the mitochondria the enzymes of the respiratory chain are localized on the inner mitochondrial membrane like the bacteria in which they remain localized in the plasma membrane. The bacterial plasma membrane resembles with the inner mitochondrial membrane in certain respects.

(ii) The plasma membrane of certain bacterial cells gives out finger-like projections in the cytoplasm known as mesosomes The mesosomes can be compared with mitochondrial crests. **Salton (1962)** has reported respiratory chain enzymes in the mesosomes.

(iii) Because the outer mitochondrial membrane resembles with the plasma membrane, therefore, it may be assumed that the mitochondrial matrix and the inner mitochondrial membrane represent

the symbiont which might be enclosed by the membrane of the cellular origin (outer mitochondrial membrane).

2. Similarity in DNA molecule—The DNA molecule of the mitochondrial is circular like the DNA molecule of the bacterialcells. Further the replication process of the mitochondrial DNA is also similar to bacterial DNA.

3. Similarity in ribosomes—The mitochondrial ribosomes are small in size and resemble the ribosomes of the bacteria.

4 Similarity in the process of protein synthesis—The process of protein synthesis of both mitochondria and bacteria is fundamentally same because in both, the process of protein synthesis can be inhibited by same inhibitor known as chloramphenicol.

Further, the mitochondria for the process of protein synthesis depend partially on the mitochondrial matrix and DNA and partially on the nucleus and cytoplasm of the eukaryotic cells. This shows the symbiotic nature of the mitochondria.

Due to the above mentioned similarities between the bacteria and mitochondria, the symbiont hypothesis postulated that the host cell (eukaryotic cell) represented an anaerobic organism which derives the required energy from the oxidations of food by the process of glycolysis. While the mitochondria represent the symbionts which respire **aerobically** and contain the enzymes of **Krebs cycle** and **respiratory chain**. The symbionts seem to be capable to get the energy by **oxidative phosphorylation** from the partially oxidised food (pyruvic acid) of the host cell.

CHAPTER 9

PLASTIDS

The plastids are the cytoplasmic organelles of the cell of plants and some protozoans such as *Euglena*. They perform most important biological activities as the synthesis of food and storage of carbohydrates, lipids and proteins. The plastids occur in most plant cells and few protozoans [illegible] algae, fungi and animals [illegible] the plastids.

CHROMATOPHORES

The chromatophores (Gr., *chrom*-colour, *phor*-to bear) are the plastid-like colouring bodies of the cells of the bacteria, blue green algae and fungi. In chromatophores the pigments are arranged in concentric rings or plates. The chromatophores are of various types and are as follows :

[illegible]

the t

and

(iii) **Carotenoids**—Certain bacteria, fungi and red-coloured ripe tomato, pepper or flower contain the carotenoids. The carotenoid pigment lycopene gives red colour to the ripe tomato and ripe pepper. The bacteria and fungi contain the pigment **capsanthin** in their carotenoids. The other carotenoid pigments as **fucoxanthin, xanthophyll and carotene, etc.**, occur singly or along with the chlorophyll in the algae and other plant cells.

CLASSIFICATION OF THE PLASTIDS

The term 'plastid' is derived from the Greek word "*plastikas*" (=formed or moulded) and was used by **A.F.W. Schimper** in 1885. Schimper classified the plastids into following types according to their structure, pigments and the functions :

A. **Chromoplasts**—The chromoplasts (Gr., *chroma*-colour, *plast*-living) are the coloured plastids of the plant cells. The chromo-

plasts contain variety of pigments and synthesize the food by the process of photosynthesis. The most common chromatoplasts of micro organisms and plant cells are as follows :

(i) **Chloroplast**—The chloroplast (Gr., *chlor*-green, *plast*-living) is most widely occurring chromoplast of the plants. It occurs mostly in the green algae and higher plants. The chloroplast contains the [illegible] NA and RNA.

[illegible] *haeo*-dark or brown, [illegible] which absorbs the light. The phaeoplasts occur in the diatoms, dinoflagellates and brown algae.

(iii) **Rhodoplast**—The rhodoplast (Gr , *rhode*-red, *plast*-living) contain the pigment phaeoerythrin which absorbs the light. The rhodoplast occur in the red algae.

B. **Leucoplasts**—The leucoplasts (Gr., *leuco*-white, *plast*-living) are the colourless plastids which store the food material as carbohydrates, lipids and proteins. The leucoplasts are rod-like or spheroid in shape and o[illegible] in the embryonic cells, sex cells and meristematic cells. The mos[illegible] cells are as follows :

(i) **Amyloplasts**—The amyloplasts (L., *amyl*-starch ; Gr., *plast* living) are those leucoplasts which synthesize and store the starch. The amyloplasts occur in those cells which store the starch.

(ii) **Elaioplasts**—The elaioplasts store the lipids (oils) and [illegible] oteoplasts are the [illegible] s and contain few

According to Schimper, different kinds of plastids can transform into one another, as shown in following figure :

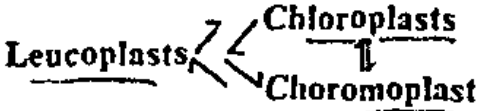


Fig. 9 1. Interconversion of three kinds of plastids.

CHLOROPLASTS

We will describe here only the chloroplasts in detail because of two reasons. Firstly they are most common plastids of [illegible] cells and second [illegible] test biological i[illegible] chloroplast synthesizes the carbohydrates which contain energy in the form of chemical energy. The chemical energy is utilized by all living beings to perform various life activities.

DISTRIBUTION

The chloroplasts remain distributed homogeneously in the cytoplasm of plant cells. But in certain cells, the chloroplasts become concentrated around the nucleus or just beneath the plasma membrane. The chloroplasts have a definite orientation in the cell cytoplasm.

MORPHOLOGY

SHAPE

Higher plant chloroplasts are generally biconvex or plano-convex. However, in different plants cells, chloroplast may have various shapes, *viz.*, filamentous, saucer-shaped, spheroid, ovoid, discoid or club-shaped. They are vesicular and have a colourless centre.

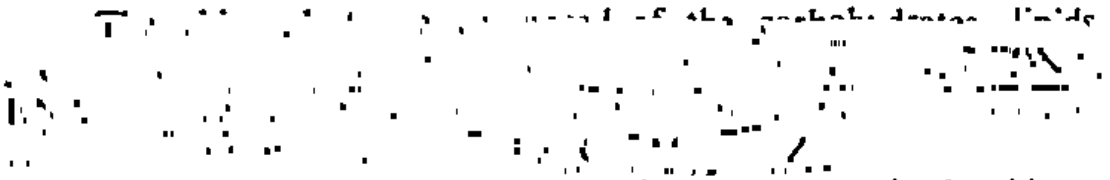
SIZE

The size of the chloroplasts varies from species to species. The chloroplasts generally measure 2–3μ in thickness and 5–10μ in diameter (**Hall** *et al.*, 1974). The chloroplasts of polyploid plant cells are comparatively larger than the chloroplasts of the diploid plant cells.

NUMBER

The number of the chloroplasts varies from cell to cell and from species to species and is related with the physiological state of the cell, but it usually remains constant for a particular plant cell. The algae usually have a single huge chloroplast. The cells of the higher plants have 20 to 40 chloroplasts.

CHEMICAL COMPOSITION



The carbohydrates occur in very low percentage in the chloroplasts. The most common carbohydrates of the chloroplasts are the starch and sugar phosphates.

The chloroplasts contain 20–30 per cent lipids of its dry weight. The lipids are composed of 50 per cent fats, 20 per cent sterols, 16 per cent waxes and 7 to 20 per cent phospholipids. The most common alcohols of the lipids are the choline, inositol, glycerol, ethanolamine.

The proteins constitute 35 to 55 per cent of the chloroplast About 80 per cent proteins are insoluble and forming the unit membranes of the chloroplasts along with the lipids. The 20 per cent proteins are soluble and occur in the form of the enzymes.

The chlorophyll is a green pigment of the chloroplasts. The chlorophyll contains an assymetrical molecule which has a hydrophilic

head of four rings of the pyrols and a hydrophobic tail of the phytol chain. Chemically the chlorophyll is a porphyrin like the animal pigment haemoglobin and cytochromes except besides the iron (Fe), it contains Mg atom in between the rings of the pyrols which remain connected with each other by the methyl groups. The chlorophyll consists of 75 per cent *chlorophyll a* and 25 per cent *chlorophyll b*.

The carotenoids are carotenes and xanthophylls, both of which are related to vitamin A. The carotenes have hydrophobic chains of unsaturated hydrocarbons in their molecules. The xanthophylls contain many hydroxy groups in their molecules.

ULTRASTRUCTURE

The chloroplasts are bounded by two unit membranes like the mitochondria. Each membrane of the chloroplast is trilaminar, lipoproteinous and 50 A° thick. Both unit membranes of plastids are separated by one another by a distinct space known as the "periplastidial space". In *Nicotiana*, this periplastidial space is 100 A°—300 A° wide. The inner contents of the chloroplasts are heterogeneous and composed of following structures :

1. The matrix or stroma.
2. The grana.

1. **The matrix or stroma**—The inner periplastidial space of the chloroplasts is filled ent substance known as the the chloroplasts the unit st and intergrana connecting membranes remain embedded.

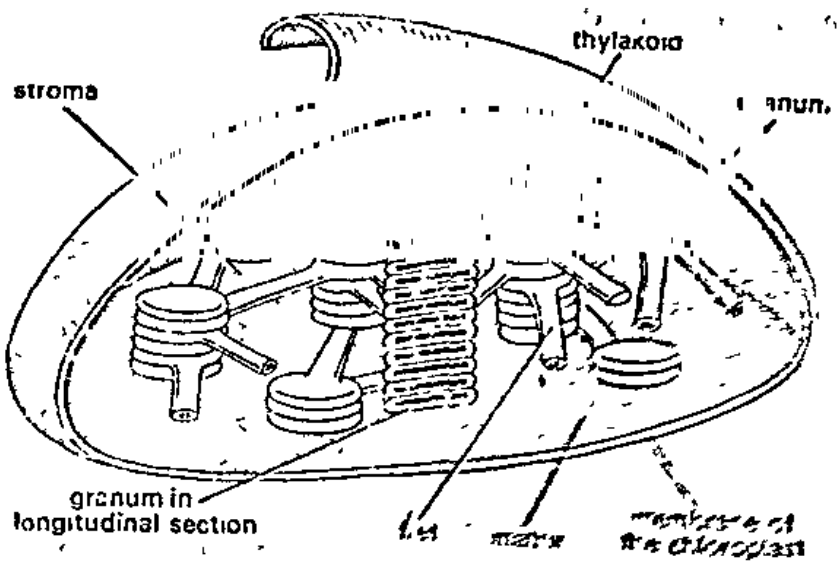
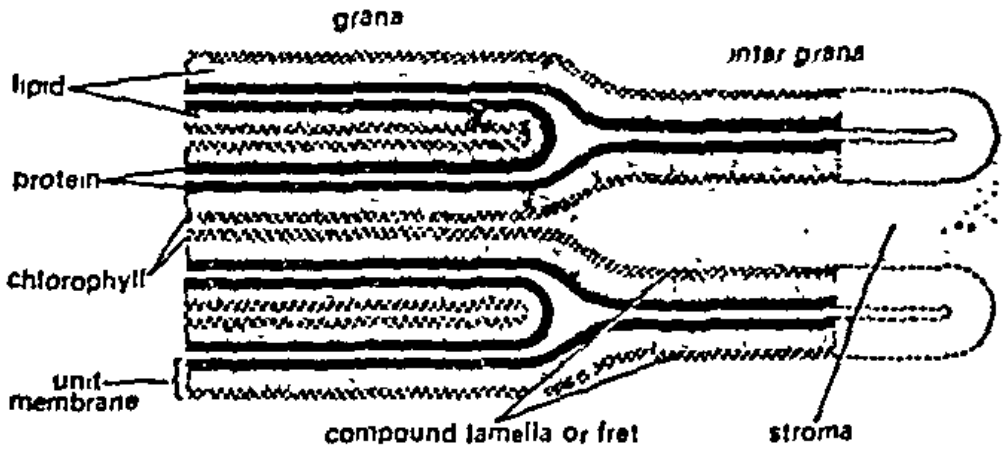


Fig 9·2 Submicroscopic

2. **The grana**—The membranous, granular and

the grana. The size of the grana may range from 0·3 to 1·7μ. Th chloroplasts may contain 40 to 60 grana in their matrix.

Each granum of the chloroplast of a higher plant cell composed of 10 tc ments known as However, in the of thylakoids per granum varies. Thus, there may be single thylakoi (*e.g.*, red alga), paired thylakoids (*e.g.*, chrysophyta), triple thylakoid and multiple thylakoids (*e.g.*, green algae and higher plants). Eac thylakoid remains separated from the stroma or the matrix of th chloroplast by its unit membranes. In a granum these thylakoid remain arranged parallely to form the stack.



9·3. Macromolecular structure of grana of a chloroplast.

ter-connected by a network out by the membranes of interconnecting membranes of the grana are known as the **stromal lamellae** or **frets**. The chlorophyll occurring in the membranes of the thylakoids and frets often remain associated with their lipidous layers.

QUANTOSOME CONCEPT

d of many para- *crysta* *tosomes (Park and* Pon, Å by 180 A° and about 100 A° thick. **Sauer** and **Calvin** (1962) have shown that 3 to 6 quantosomes may aggregate together to form a large particle of 500 × 100 A°. [illegible] that each *quantosome is con* *to Howell* and **Moundrianakis** allest units capable of carrying out the photochemical reaction and each of them contains about 200 chlorophyll molecules.

According to the size, nature and arrangement of quantosomes in the membrane of thylakoids and stromal lamellae, **Branton** and **Park** (1967) have recognised three types of membranes which are as follows :

1.
2.
3. a few or no particles.

The chloroplasts, thus, act as elaborate biochemical machines and tr el.

organization of chloroplas ed due to mutations. **D. Von Wettstein** (1956) reported that the plastids of normal barley plant have a well organized system of grana and storma But the plastids of an albino mutant of barley, fail to develop beyond a particular stage and there occurs no differentiation of grana and stroma. Further, the plastids of a yellow-green mutant of barley develop somewhat further than plastids of an albino plant.

DNA OF CHLOROPLAST

Recently the chloroplasts of the algae and higher plants are found to contain DNA molecules. First of all **Ris** and **Plant** (1962) have reported DNA molecule in the chloroplast of the *Chlamydomonas* Later on DNA molecule has been reported from the chloroplasts of other algae and higher plants. **Woodcock** and **Fernandez** (1968) have isolated 150μ long segments of the DNA molecules from the chloroplasts. The DNA of the chloroplasts differs from the nuclear DNA in many respects and it resembles closely with the bacterial DNA
chloroplast hav

The chlo

and 16S rRNA (**Stutz** and **Noll**, 1967, **Bager** and **Hamilton**, 1967). **Lyttleton** (1962) has also separated polyribosomes or polysomes from the chl yl —tRNAs, am

FU

1. **Photosynthesis**—The most important and fundamental function of the chloroplast is the photosynthesis. In the photosynthesis the chlorophyll of the chloroplast traps the radiant energy of

l transforms it into
ed in the chemical
xose sugars which
ynthesis of various
macromolecules such as polysaccharides (starch, cellulose), lipids, proteins and nucleic acids.

The process of photosynthesis occurs in the presence of chlorophyll, sunlight, CO_2 and H_2O and it includes following three steps :

(i) Photophosphorylation in which ATP is formed :

$$ADP + P_{(i)} \xrightarrow{\text{Light}} ATP$$

(ii) Photolysis of the water in which TPN^+ is reduced to *TPNH (triphospho-pyridine-nucleotide)* :

$$H_2O \xrightarrow{\text{Light}} 2H^+ + O^- \qquad (a)$$

$$TPN + 2H^+ \xrightarrow{\text{Ferredoxin}} TPNH + H^+ \qquad (b)$$

(iii) Carbon dioxide fixation and reduction to carbohydrates. This includes following reactions :

(a) Transfer of CO_2 from air to a C_5 sugar compound (ribulose 1, 5-diphosphate) and splitting or cleavage of the resulting C_6 compound into two molecules of phosphoglyceric acid.

(b) Hydrogenation of phosphoglyceric acid into phosphoglyceraldehyde, a triose which can again polymerize into a hexose sugar.

The first and second steps take place in the sunlight and are included in light or photochemical or Hill reaction of the photosynthesis. The third step of photosynthetic process occurs in the darkness and is known as dark or thermochemical reaction. The
al reaction take place
the phosphorylation
resence of the quanto-
somes containing chlorophyll, the hydrogen transfer system is perhaps located in the stroma. The chloroplast contains various enzymes for the photosynthetic process, *e.g.*, ferradoxins, vitamin K (phylloquinone), plastoquinone, carboxydismutase, etc.

2. *Protein synthesis*—*According to most recent studies (see* Hill. [illegible] for
[illegible] esis
[illegible] controlled by
[illegible] last are found to require Mg^{++} for their stability and also have a requirement for

N-formyl methionyl—tRNA in chain initiation process of protein

ORIGIN AND DEVELOPMENT

The choroplasts are never originated *de novo*. About the origin of chloroplasts following concepts have been postulated :

1. The origin from the mature chloroplast.
2. The origin from the proplastid.
3. Nuclear origin.
4. Monotropic development.
5. Symbiotic origin.

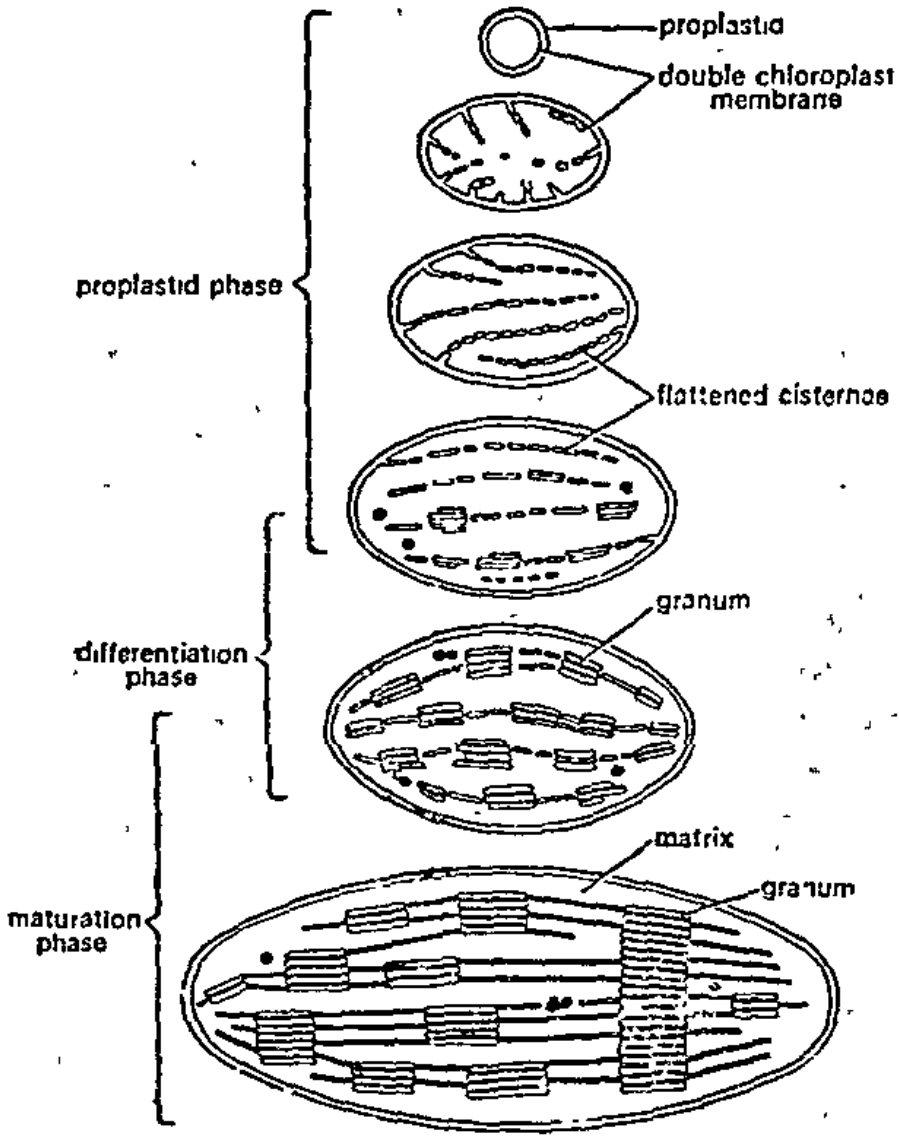


Fig. 9·5. Development of a chloroplast from a submicroscopic proplastid in the presence of light.

1. The origin from the mature chloroplast—In algae and ferns the new chloroplast originates by the division of the mature chloroplast. Under certain abnormal conditions (*viz.*, regeneration of plant from dissected leaves) the new chloroplasts originate by budding from the mature plastids.

2. The origin of chloroplast from the proplastids—In higher plants, the new chloroplast develops from the proplastids. The proplastids are the spheroid structures of 0·5μ diameter and each remains bounded by two unit membranes and contains dense stroma. The development of the thyalkoids and frets takes place according to the synthesis of the chlorophyll in the sunlight. The inner membrane of the chloroplast gives off vesicles which arrange themselves parallely in the matrix. These vesicles fuse together to form large discs or lamellae. At certain places the discs or lamellae vesicles arrange themselves in closely packed bundles of thylakoids to form a grana. Some thylakoids, however, remain connected with each other by the tubules or stromal lamellae or frets in the mature chloroplast. This lamellation takes place only in the presence of light (Fig. 9·5). In the absence of light these structure start disorganizing (Fig. 9·6).

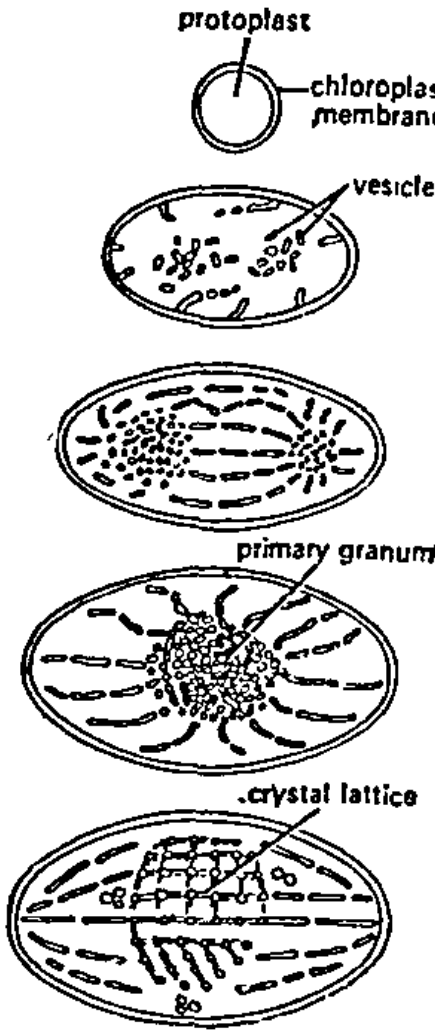


Fig. 9·6. Development of a chloroplast from *submicroscopic* proplastid in dark,

3. *Nuclear origin*—A large number of building blocks (membranes, etc.) or initials of proplastids have been observed under special conditions around the nucleus. This suggested that the initials originated due to evagination of the double nuclear membrane and gave rise to mitochondria and proplastids.

4. Monotropic development—According to the theory of monotropic development of chloroplast, the plastids are autonomous structures, which can change from one form to another but can never originated de novo. Although in certain cases the change from one form to the other is reversible, at other times irreversible changes

can also be brought about. For example, if chloroplasts become sufficiently yellow due to absence of light, they may not become green again and will therefore only degenerate. Similarly, in the transformation of chloroplasts into chromoplasts, the chlorophyll disappears and this change may be irreversible. It is for this reason that the chromoplasts are considered as degenerating forms of plastids (chloroplasts). The mode of monotropic development of plastid can be illustrated by following figure :

Initlals } Young stage
↓ } (Growth)
Proplastids
↓ differentiation
Leucoplasts } Active stage
Chloroplasts } (Metabolism)
↓ degeneration
Chromoplasts—Gerontic or old stage
(passive degenerated form)

Fig. 9·7. Monotropic development of plastids.

5. **The symbiotic origin of the chloroplast**—In certain characteristics, the chloroplasts are comparable with that of a semiautonosmous or symbiotic organism living with in the plant cells. They divide, grow and differentiate ; they contain circular DNA, ribo-

[illegible]

appears very justified but Kirk (1966) has shown that certain important enzymes which are necessary for the development of the chlorophyll and for the photosynthetic mechanism are synthesized according to the codes of the nuclear DNA. There still exists certain doubt about the symbiotic origin of the chloroplast.

AMYLOPLASTS

Amyloplasts or starch granules are leucoplasts which lack any visible pigment and are involved in the synthesis and storage of various kinds of carbohydrates.

STRUCTURE AND FUNCTION

The amyloplasts resemble proplastids and differ from them only in size. They contain less number of lamellae, but, can build up thylakoid structure found in chloroplast, in the presence of light. In the dark they synthesize and store starch. The conversion of

glucose into starch is a chief characteristic of amyloplasts. However, chloroplasts can also synthesize starch and store it in stroma region. But, this starch in chloroplasts disappears quickly and is, therefore, known as transitory starch. The starch of amyloplasts on the other hand can be stored for longer periods and is therefore called reserve starch.

ORIGIN OF AMYLOPLASTS

The starch development begins with the formation of a particle in the stroma. This particle consists of several tubuli and thylakoids and is surrounded by additional rings of starch. It grows till the amyloplast is filled with starch. Ultimately, the tubuli are pressed against the wall and gradually, starch granule exceeds the size of amyloplast. Finally, the membraneous wall of amyloplasts ruptures and withers away, so that only the starch granules can be seen.

CHROMOPLASTS

The plastids which co
are the chromoplasts. The
and also from leucoplasts.
from chloroplasts can be observed in the petals which are green

that the roots have no colour in the beginning but become coloured at a later stage.

STRUCTURE AND FUNCTION

Chromoplasts appear to be products of degeneration or disintegration of chloroplasts or leucoplasts. Their specific functions in plant
ever, their presence in flowers
for pollination and propagation.

The chromoplasts can originate either from chloroplasts or from leucoplasts :

(i) When a chloroplast transforms into a chromoplast, it has been observed that some yellow coloured droplets called **globulins** appear in the former. In the course of development chlorophyll and starch of chloroplasts gradually decrease, large globuli are formed, the lamellar structure breaks down and stroma is disorganized. Ultimately, globuli are arranged along plastid membrane and the centre of the plastid appears empty due to disintegration of stroma.

(ii) During the transformation of leucoplasts into chromoplasts, certain fibrils appear which give rise to crystals filling up the whole plastids. These crystals are normally found in the form of sheet-like structures containing large quantities of carotenoids.

CHAPTER 10

MICROBODIES-PEROXISOMES AND GLYOXYSOMES

The cells of Protozoa, fungi, plants, liver and kidney of vertebrates contain certain membrane-bounded, spherical bodies of 0·2 to 1·5μ diameter in close association of endoplasmic reticulum, and mitochondria or chloroplast, or both. These organelles have a central granular or crystalloid core containing some enzymes and are called **microbodies.** Microbodies use molecular oxygen like mitochondria, but instead of having cytochromes and capacity of ATP synthesis like them, they contain flavin-linked oxides and catalases for the hydrogen peroxide metabolism and also enzymes for fatty acid metabolism.

TYPES OF MICROBODIES

Recent biochemical studies have distinguished two types of microbodies, namely **peroxisomes** and **glyoxysomes.** These two organelles differ both in their enzyme complement and in the type of tissue in which they are found. **Peroxisomes** are found in animal cells and in the leaves of higher plants. In both they participate in the oxidation of substrates, producing hydrogen peroxide which is subsequently destroyed by catalase activity :

1. Reduced substrate → $FADH_2$ → H_2O_2
   Oxidized substrate ← FAD ← O_2
2. $H_2O_2 \longrightarrow H_2O + \frac{1}{2}O_2$

In plant cells, peroxisomes remain associated with ER, chloroplasts and mitochondria and are involved in photorespiration. **Glyoxysomes** occur only in plant cells and are particularly abundant in germinating seeds which store fats as a reserve food material. They remain intimately associated with lipid bodies, the spherosomes and contain enzymes for fatty acid metabolism and glyconeogenesis (*i.e.*, formation of glucose from various non-carbohydrate precursors as succinate in this case). Apart from peroxisomes and glyoxysomes, a number of other terms have been used to describe microbodies, including **cytosomes, phragmosomes** and **crsytal-containing bodies.** A detailed discussion of each type of microbody can be made as follows :

PEROXISOMES

Peroxisomes occur in many animal cells and in a wide range of plants. They are present in all photosynthetic cells of higher plants in etiolated leaf tissue, in caleoptiles and hypocotyls, in tobacco stem and callus, in ripening pear fruits and also in Euglenophyta, Protozoa, brown algae, fungi, liverworts, mosses and ferns.

STRUCTURE

Peroxisomes are variable in size and shape, but usually appear circular in cross section having diameter between 0·2 and 1·5μ. They have a single limiting membrane of lipid and protein molecules, which encloses their granular matrix. In some cases (*e.g.*, in the festuciod grasses) the matrix contains numerous threads or fibrils, while in others they are observed to contain either an amorphous nucleoid or a dense inner core which in many species shows a regular crystalloid structure (*e.g.*, tobacco leaf cell, **Newcomb and Frederick** (1971). Little is known about the function of the core, except that it is the site of the enzyme urate oxidase in rat liver peroxisomes and much of the catalase in some plants (see **Hall *et al.*, 1974**).

BIOCHEMICAL ACTIVITY

Peroxisomes are found to perform following two types of biochemical activities :

A. Hydrogen peroxide metabolism—Peroxisomes contain catalase enzyme together with one or more enzymes which produce hydrogen peroxide. The primary electron donor for these latter enzymes is usually glycolate, L-lactate or another L-hydroxy acid, and one mole of hydrogen peroxide is produced with the uptake of one mole of oxygen. Subsequeatly, the action of catalase causes peroxidations such as that of ethanol to acetaldehyde ($CH_3CH_2OH + H_2O_2 \rightarrow CH_3CHO + 2H_2O$), formic acid to carbon dioxide and water ($HCOOH + H_2O_2 \rightarrow CO_2 + 2H_2O$) or of a further mole of peroxide to oxygen and water ($2H_2O_2 \rightarrow 2H_2O + O_2$). In every case there is a net uptake of oxygen.

B. Glycolate cycle—Peroxisomes of plant leaves contain catalase together with the enzymes of glycolate pathway, as glycolate oxidase, glutamate glyoxylate, serine-glyoxyiate and asparate-α-ketoglutarate aminotransferases, hydroxy pyruvate reductase and malic dehydrogenase. They also contain FAD, NAD and NADP coenzymes. The glycolate cycle is thought to bring about the formation of the amino acids glycine and serine from the non-phosphorylated intermediates of photosynthetic carbon reduction cycle, *i.e.*, glycerate to serine, or glycolate to glycine and serine in a sequence of reactions which involve chloroplasts, peroxisomes and mitochondria (**Tolbert,**

1971, Bird *et al.*, 1972). There is a net uptake of oxygen and evolution of CO_2 during the operation of the glycolate cycle; this is known as physiologically as **photorespiration** since it is stimulated in the light.

COMPARISON BETWEEN PEROXISOMES AND MITOCHONDRIA

Peroxisomes resemble with mitochondria only in one respect that they use molecular oxygen, otherwise, they differ with them in following two objects :

(i) The rate of peroxisomal oxygen uptake is almost directly proportional to the oxygen concentration, while mitochondrial oxygen up take is almost independent of oxygen concentration (above about 5%) due to extremely high affinity of cytochrome oxidase for oxygen.

(ii) In mitochondria the respiration is involved in ATP synthesis, but the peroxisomal respiration in animals is not involved in ATP synthesis and in this respect it appears be a wasteful process.

FUNCTIONS

The peroxisomes have following functions :

(i) In plants, they are involved in the synthesis of glycine and serine and possibly of C—1 donor from the primary products of photosynthesis.

(ii) They protect the cellular organelles from the toxic effect of hydrogen peroxide.

GLYOXYSOMES

Glyoxysomes are found to occur in the cells of yeast, *Neurospora*, and oil rich seeds of many higher plants. They resemble with peroxisomes in morphological details, except that, their crystalloid core consists of dense rods of 6·0$m\mu$ diameter. They have enzymes for fatty acid metabolism and gluconeogenesis, *i.e.*, conversion of stored lipid molecules of spherosomes of germinating seeds into the molecules of carbohydrates.

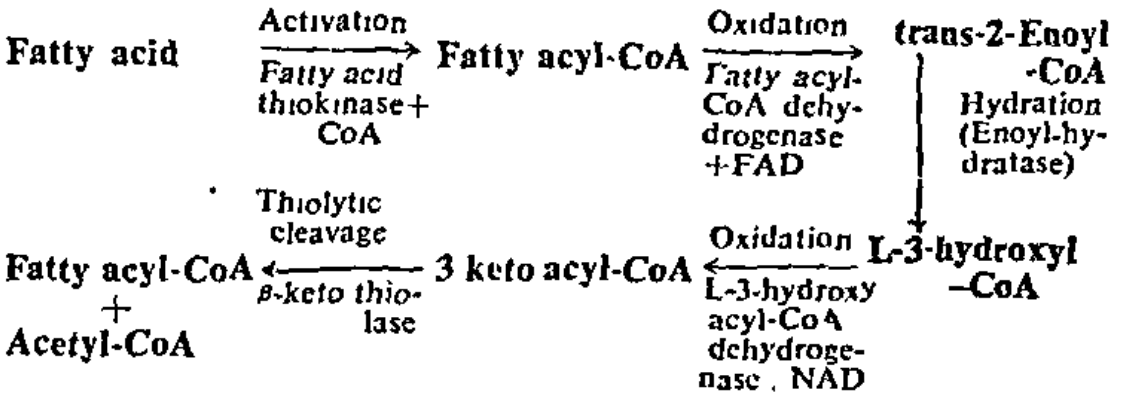
BIOCHEMICAL ACTIVITIES

Glyoxysomes perform following biochemical activities of plant cells :

1. Fatty acid metabolism—During germination of oily seeds, the stored lipid molecules of spherosomes are hydrolysed by the enzyme lipase (glycerol ester hydrolase) to glycerol and fatty acids. The phospholipid molecules are hydrolysed by the enzyme phospholipase. The long chain fatty acids which are released by the hydrolysis are then broken down by the successive removal of two carbon or C_2 fragments in the process of **β-oxidation.**

β-Oxidation—During β-oxidation process, the fatty acid is first activated by enzyme fatty acid thiokinase to a fatty acyl-CoA which

is oxidized by a FAD linked enzyme *fatty acyl-CoA dehydrogenase* into **trans-2-enoyl-CoA.** Trans-2-enoyl-CoA is hydrated by an enzyme enoyl hydratase or crotonase to produce the **L-3-hydroxy-acyl-CoA,** which is oxidized by a NAD linked L—3—hydroxyacyl-CoA dehydrogenase to produce **3-Keto acyl-CoA.** The 3-keto acyl -CoA looses a two carbon fragment under the action of the enzyme thiolase or β-keto thiolase to generate an **acetyl-CoA** and a new **fatty acyl-CoA** with two less carbon atoms than the original. This new fatty acyl-CoA is then recycled through the same series of reactions until the final two molecules of acetyl-CoA are produced. The complete β-oxidation chain can be represented as follows :



In plant seeds β-oxidation occurs in glyoxysomes (Cooper and Beevers, 1969). But in other plant cells β-oxidation occur in glyoxysomes and mitochondria. The glyoxysomal β-oxidation requires oxygen for oxidation of reduced flavoprotein produced as a result of the *fatty-acyl-CoA dehydrogenase activity. In animal cells β-oxidation* occurs in mitochondria.

In plant cells, the acetyl-CoA, the product of β-oxidation chain is not oxidized by Krebs cycle, because it remains spatially separated from the enzymes of Krebs cycle, instead of it, acetyl-CoA *undergoes the glyoxylate cycle* to be converted into succinate.

Glyoxylate cycle—The glyoxylate pathway occurs in glyoxysomes and it involves some of the reactions of the Krebs cycle in that **citrate** is formed from **oxaloacetate** and **acetyl-CoA** under the action of citrate synthetase enzyme. The citrate is subsequently converted into **isocitrate** by aconitase enzyme. The cycle then involves the enzymatic conversion of isocitrate to **glyoxylate** and **succinate** by isocitratase enzyme :

$$\text{Isocitrate} \xrightarrow{\text{Isocitratase}} \text{Glyoxylate} + \text{Succinate}$$

The glyoxylate and another mole of acetyl-CoA form a mole of malate by malate synthetase:

$$\text{Acetyl CoA} + \text{Glyoxylate} \xrightarrow{\text{Malate synthetase}} \text{Malate}$$

This malate is converted to oxaloacetate by malate dehydrogenase for the cycle to be completed. Thus, overall, the glyoxylate pathway involves :

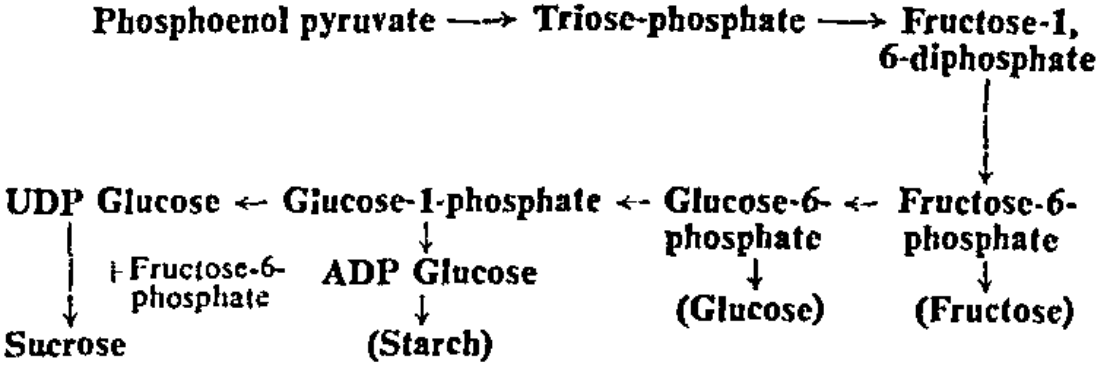
$$2\ \text{Acetyl-CoA} + NAD^+ \longrightarrow \text{Succintiate} + NADH + H^+$$

Succinate is the end product of the glyoxysomal metabolism of fatty acid and is not further metabolized within this organelle.

The synthesis of hexose or gluconeogeneis involves the conversion of succinate to oxaloacetate, which presumably takes place in the mitochondria, since the glyoxysomes do not contain the enzymes fumarase and succinic dehydrogenase. Two molecules of oxaloacetate are formed from four molecules of acetyl-CoA without carbon loss. This oxaloacetate is converted to phosphoenol pyruvate in the phosphoenol pyruvate carboxykinase reaction with the loss of two molecules of CO_2 :

$$2\ \text{Oxaloacetate} + 2ATP \rightleftharpoons 2\ \text{Phosphoenol pyruvate} + 2CO_2 + 2ADP$$

The phosphoenol pyruvate is converted into monosaccharides (*e.g.*, glucose, fructose), disaccharide (sucrose) and polysaccharide (starch) by following reaction :



OTHER BIOCHEMICAL ACTIVITIES OF GLYOXYSOMES

Glyoxysomes are found to contain enzyme urate oxidase and allantoinase. Urate oxidase (uricase) converts uric acid to allantoin and allantoinase hydrates allantoin to allantoic acid during the degradation of purines.

ORIGIN OF MICROBODIES

The microbodies or peroxisomes and glyoxysomes lack ribosomes and nucleic acids. Little is known about their origin. Their close association with the rough ER had led to the speculation that they are produced from that membrane. According to Trelease *et al.*, (1971), the glyoxysomes appear first during germination of seed and later on, are conveted into peroxisomes by changing their enzymal complement. However, Kagawa and Beevers (1970) and Vigil (1970) have found that the glyoxysomes are destroyed following fat utiliza-

tion, so, there arises no question of conversion of glyoxysomes into peroxisomes. Further, because Trelease *et al.*, 1971 could not provide electron micrographic evidence to support their hypothesis, so, the controversy is still awaiting resoultion (see Hall *et. al.*, 1974).

SYMBIOTIC ORIGIN OF MICROBODIES

It has been speculated (see de Duve, 1969) that microbodies arose as respiratory particles during the increase in oxygen levels occurring in the earth's atmosphere. This oxygen was and still is able to react with certain metabolic intermediates to produce toxic amounts of hydrogen peroxide. The peroxisomes could therefore have served a dual role both in destroying the peroxide and also acting as a terminal oxidase for the products of fermentation, thus providing a sink for electrons which would otherwise produce a pool of reduced metabolites. Mitochondria were envisaged as having originated from parasitic bacteria which had since evolved to a state of symbiosis with the host cell. Once both mitochondria and microbodies were established in the cell, competition between the two must have taken place. The fact that the microbodies have survived such competition indicates that they may well have functions in addition to those already postulated, particularly in animal cell.

CHAPTER 11

MICROTUBULES AND MICROFILAMENTS

For the performance of two major cellular functions namely production of cell shape and motility, cytoplasm of most eukaryotic cells contains two types of slender, proteinaceous threads, the **microtubules** and **microfilaments**, both of which having following structure and functions :

MICROTUBULES

Microtubules were first of all observed in the axoplasm of the myelinated nerve fibres by **Robertis** and **Franchi** (1953). They called them **neurotubules**. The exact nature of microtubules was brought into light when **Sabatini, Bensch** and **Barnett** (1963) made use of the glutarldehyde fixative in the electron microscopy. Microtubules of plant cells were first described in detail by **Ledbetter** and **Porter** (1963).

OCCURRENCE

In the cytoplasm of animal and plant cells, microtubules occur at following seven sites :—1. cilia and flagella, 2. centrioles and basal bodies, 3. nerve processes, 4. the mitotic apparatus, 5. the cortex of meristematic plant cells, 6. elongating cells such as during the formation of the lens or during spermatogenesis of certain insects, 7. selected structures in Protozoa such as the axostyle of parasitic flagellates, the axoneme of *Echinosphaerium*, the fibre systems of *Stentor*, and the cytopharyngeal basket of *Nassula*.

STRUCTURE

Microtubules constitute a class of morphologically and chemically related filamentous rods which are common to both plant and animal cells. A microtubule consists of a long, unbranched, hollow tube of several microns length and 210 to 250 A° (or 21–25 $m\mu$) diameter. Its wall is composed of globular sub-units, each 40 A° or $4m\mu$ in diameter, and the sub-units are aligned in 11–13 longitudinal strands.

Although the cytoplasmic microtubules are usually isolated from each other, there are examples of precise associations of several tubules into more or less permanent structures. The axonemata of

cilia and flagella, for example, have a characteristic 9+2 arrangement of modified tubules. On occasions, thin thread-like bridges have also been observed between microtubules of cilia and flagella ; axostyle of flagellates ; spermatids of fowl ; mitiotic apparatus ; axons ; axonemes of *Echinosphaerium ; Stentor* and *Nossula ;* and cell plate of dividing plant cells. The function of these bridges is still unknown, however, there is evidence, that they give pattern to clusters of microtubules as well as playing a major role in motility (**Tilney, 1971**).

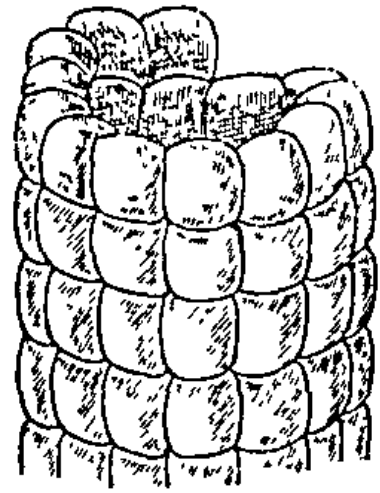


Fig 11 1. Diagram showing the sub-unit structure of a microtubule

CHEMICAL COMPOSITION

The protein chemistry of microtubules of animal cells has been worked out by the help of a drug called **colchicine** which inhibits microtubule assembly, because it binds tightly to the subunit proteins and preventing their polymerization. Each microtubule subunit has a sedimentation coefficient of about 6S and consists of two globular polypeptides of molecular weight about 60,000 with an amino acid composition quite similar to that of the muscle protein actin (see **Hall *et al*** , 1974). The subunit will bind a single molecule of GTP, suggesting that this nucleotide might play some role in the assembly of the microtubules

ASSEMBLY AND DISASSEMBLY OF MICROTUBULES

The microtubules of cilia, flagella and axostyle are stable and under normal conditions, they do not break down and reassemble. But the microtubules of cytoplasm are labile, and they readily undergo reversible assembly and disassembly. It has been observed that during mitosis, a constant flux between the polymer and subunits (monomers) occurs at the tip of each microtubule of cytoplasm of dividing cells. Further, there exists a delicate equilibrium between subunits (monomers) and polymer of microtubules. Colchincine influences this equilibrium by binding monomers and removing them from the system so that the microtubules dissociate by a mass action effect. It has also become evident that the same monomeric units can be assembled into different forms depending upon local conditions of pH, cation concentration and temperature and this may account for the diversity of fibril dimensions that has been noted in *different organisms.*

Nucleating centres—The control of distribution of microtubules cells involves the loci which affect the nucleation of microtubules.

These loci are thought to include the basal body, the centriole, satellites associated with the basal body and centriole, the kinetochore, and a host of other dense particles, many of which are membrane-associated. By activating and repressing specific loci the cell can control the distribution of microtubules, providing of course that the equilibrium for assembly is favourable and that the requisite molecules are available.

FUNCTIONS

Microtubules perform following important functions of cells :

1. Microtubules are specifically related with movement such as undulation of cilia, flagella and axostyle.

2. Microtubules form the mitotic apparatus (or spindle) during cell division and get attached with the kinetochore of the chromosomes. Their contraction causes the movement of the chromosomes toward the opposite poles of the cells.

3. In the protozoan *Nassula*, microtubules drive the food in the gullet.

4. Microtubules are also associated with cytoplasmic streaming (cyclosis) in plant cells, the mitotic apparatus, neurous, and the axopodia of *Echinosphaerium*.

5. They serve as **cytoskeletal** agents of some kind, maintaining local areas of stress and rigidity and defining cytoplasmic channels.

6. Microtubules also function like a **microcirculatory system** for the intracellular transport of small molecules of water and inorganic ions.

7. During embryonic development, microtubules play a vital role in the process of differentiation.

MICROFILAMENTS

Microfilaments are much narrower than microtubules, being only about 5–6 $m\mu$ in diameter. They usually occur in bundles and have been observed in the path of streaming cytoplasm in plant cells, just below the cleavage furrow of mammalian cells during cytokinesis and in a number of other instances when cell invagination occurs (**Hall** *et. al.*, 1974). Just as colchicine provided a tool to investigate microtubules, another drug, **cytochalasin B**, appears to have a specific and reversible effect on microfilaments. The use of this drug has demonstrated a clear correlation between the integrity of the microfilament system and the contractility of cytoplasm. It becomes evident now that microfilaments form the internal contractile machinery of cells.

CHAPTER 12

CENTRIOLES AND BASAL BODIES

Cytoplasm of some eukaryotic cells contains two cylindrical, rod-shaped, microtubular structures, called centrioles, near the nucleus. Centrioles lack limiting membrane and DNA or RNA and form a spindle of microtubules, the mitotic apparatus during mitosis or meiosis and sometimes get arranged just beneath the plasma membrane to form and bear flagella or cilia in flagellated or ciliated cells (Fulton, 1971). When a centriole bears a flagellum or cilium, it is called basal body. There are many synonyms for basal body, including kinetosome, blepharoplast, basal granule, basal corpuscle, and *proximal centriole*.

OCCURRENCE

Centrioles occur in algae, fungi, bryophytes, ferns, gymnosperms, protozoans (ciliates and flagellates) and most of the multicellular animals. They are absent in prokaryotes, diatoms, yeast and most higher plants (conifers and angiosperms). Centrioles are most often found associated in pairs and a pair of centrioles is called diplosome. Usually during interphase these are next to the nuclear envelope, though in some cell types they are near the cell surface (Fawcett, 1966), sometimes these pairs are arranged end to end, but more commonly they come together to form a right angle. Often the area in which a centriole or a diplosome lies is relatively devoid of cellular constituents as mitochondria, chloroplasts, ribosomes and glycogen granules. This area has different appearance than the surrounding cytoplasm and contains a fine fibrous material and is called centrosphere, (Robbins *et al.*, 1968; Kalnins and Porter, 1969).

STRUCTURE

Centrioles and basal bodies are cylindrical structures which are 0·15—0·25μ in diameter usually 0·3—0·7μ in length, though, some are as short as 0·16μ and others are as long as 8μ (See Fulton, 1971). Both have following ultrastructural components :

1. CYLINDER WALL

The most striking and regular ultrastructural feature of centrioles and basal bodies is the array of nine triplet microtubules equally spaced arround the perimeter of an imaginary cylinder (fig. 12·1). The space between and immediately around the triplets

is filled with an amorphous, electrone-dense material. In transverse section the triplets are arranged like the vanes of a pinwheel. The

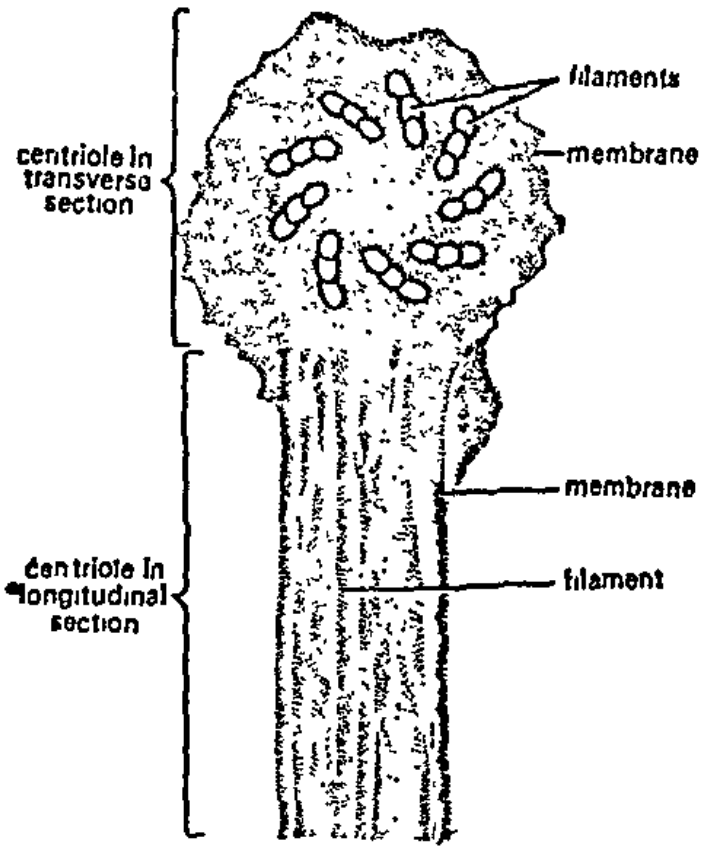


Fig. 12·1. A centriole in transverse and longitudinal section.

vanes are tilted so they form an angle of roughly 40° to the radius of the cylinder. Since centrioles have no outer membrane, the triplets are considered to form the wall of the cylinder, and arbitrarily define the inside and outside of the centriole.

2. TRIPLETS

The nine triplets that make up the wall are indistinguishable from one another, and seem basically similar in centrioles and basal bodies. The three subunit microtubules have been designated A, B, and C, with the innermost tubule being A. Individual tubules are 200—260A° in diameter. Only the A tubule is round ; the other are partial and share their wall with the preceding tubule. At both ends the C tubule often terminate before the A and B tubules.

The substructure of A, B and C tubules, is similar to the structure of other microtubules (Stephens, 1970). The A tubule has 12 to 13, 40—45A° globular subunits around its perimeter. Three or four of these subunits are shared with the B tubules, which in turn shares several of its subunits with the C tubules.

Often the triplets are thought to run parallel to one another and to the long axis of the cylinder, but this is not always the case.

In the basal bodies of some organisms, the triplets get closer toward the proximal end, so the diameter of the cylinder gets smaller. In some centrioles the triplets are parallel to one another but turn in a long-pitched helix with respect to the cylinder axis (Fulton, 1971).

3. LINKERS

The A tubule of each triplet is linked with C tubule of neighbouring triplet by a dense link and these A—C linkers do not run the full length of centrioles or basal bodies. It has been suggested that the A—C linkers are responsible for the radial tilt of the triplets (see, Fulton, 1971).

4. FEET AND CARTWHEEL

There are dense condensations from the A tubules toward the centre of the cylinder, forming a series of 'feet'. Further, at the proximal ends of centrioles and basal bodies, occur connections between tubules in the form of hub-and-spokes cartwheel. The cartwheel consists of a central hub and 9 radial spokes which run toward the A tubules. These spokes end in the dense "feet".

5. VACUOLE

A small, membrane-bounded vacuole or vesicle has been found inside the centriolar pinwheel. Some centrioles lack this vacuole.

6. ASSOCIATED STRUCTURES

There occur nine amorphous plaques or spheres of electron-dense material, with poorly defined outer limits, near centroiles. These are called **massules** or **pericentriolar satellites**. These satellites are found to change their positions, shapes, etc , during spermatogenesis and mitosis.

Basal bodies also have a remarkable variety of associated dense plaques and masses, and of **rootlets**, rhizoplasts and other filaments. The rootlets are fine, tube-like structures which arise from basal bodies and end near the nucleus. The rootlets are of two types :

1. **Striated root fibrils**—Striated root fibrils contain regular cross bands, each of which remain separated by a distance of 550 to 700A°.

2. **Tubular root fibrils**—The tubular root fibrils have the diameter of 200A°.

CHEMICAL COMPOSITION

The microtubules of centrioles and basal bodies contain the structural protein, **tubulin**, along with lipid molecules (**Fulton**, 1971). The centrioles and basal bodies contain a high concentration of ATPase enzyme. There exists a controversy that whether centrioles and basal bodies have DNA and RNA. **Fulton** (1971) has doubted presence of nucleic acids in these organelles.

ORIGIN OF CILIA, BASAL BODIES AND CENTRIOLES

The origin of cilia, basal bodies and the centrioles is interrelated. The centriole originates first and later on differentiates into the basal bodies which ultimately give rise to the cilia or flagella.

According to Gall (1961), Mizukanni and Gall (1966) and Sorokin (1968) centriole may be originated by the following two methods :

1. During the cell division the pre-existing centrioles give rise daughter centrioles or procentrioles. Procentrioles arise as annular structures which become lengthened into cylinders. From each tubule of the procentriole originates three subfibrils and the maturation of the centriole takes place in the cytoplasm. Such method of centriole formation occurs only during the cell division. Sometimes these centrioles form the primary cilia which are transitory and are short-lived.

2. When centriole is to form basal bodies or kinetosomes and cilia or flagella, then origin of centriole takes place by the different method. In this method the centriole originates from the fibrogranular material which lies in it. The granules are formed, increased in size and aggregate to form the spheroid bodies. These spheroid bodies give rise to the procentrioles. Procentrioles after maturation form centrioles which get arranged in rows just beneath the plasma membrane. Each centriole produces other procentrioles from its lateral side and itself converted into the basal bodies Basal bodies produce the ciliary rootlets from their basal ends and the cilia or flagella from their apical ends. Such sort of differentiation occurs in the ciliary epithelium. The portion of the cytoplasm in which centriole formation takes place lies near the nucleus and is known as centrosomes.

The formation of the cilia from the basal bodies is started by the formation of a vesicle-like structure of the cytoplasm towards the distal end of the basal bodies. The walls of the vesicle are invaginated due to rapid growth of ciliary shaft. The walls of the vesicle are temporary and are replaced by the new and permanent ciliary sheats.

FUNCTION

Centrioles and basal bodies have following functions :

(i) Centrioles serve as foci for the production of new centrioles and basal bodies.

(ii) Centrioles are foci at the poles of the mitotic apparatus, where they are surrounded by microtubules.

(iii) The microtubules of cilia and flagella originate and bear by basal bodies.

CHAPTER 13

CILIA AND FLAGELLA

The cilia (L., *cili*-eye lash) and flagella (L., little whip) are microscopic, contractile and filamentous [illegible] which create food currents, act as sense [illegible] *mechanical functions for the cell.* Morphologically and physiologically, the cilia and flagella are identical structures but even then both can be distinguished from each other by their number, size and functions. Their distinguishing features are as follows :

1. The flagella are less (1 or 2) in number than the cilia which may be numerous (3000 to 14000 or more) in number.
2. The flagella occur at one end of the cell, while the cilia may occur throughout the surface of the cell.
3. The flagella are longer (upto 150μ) processes, while the cilia are short (5 to 10μ) appendages of the cytoplasm.
4. The flagella usually beat independently, while the cilia tend to beat in a coordinated rhythm.
5. The flagella exhibit undulatory motion, while the cilia move in a sweeping or pendular stroke.

STEROCILIA AND KINOCILIA

The cell sometimes [illegible] known as sterocilia. The [illegible]

[illegible] OF CILIA AND FLAGELLA

The flagella occur in the protozoans of the class *Flagellata*, choanocyte cells of the sponges, spermatozoa of the Metazoa and among plants in the algae and gamete cells. The cilia occur in the protozoans of the class Ciliata and members of other classes and ciliated epithelium of the Metazoa. The cilia may occur on external body surface and may help in the locomotion of such animals as the larvae of certain Platyhelminthes, Nemertines, Echinodermata, Mollusca and Annelida. The cilia may line the internal cavities or passages of the metazoan bodies as air passage of the respiratory system. The nematode worms and arthropods have no cilia.

sheath. Gibbons (1967) has reported that the sheath of the central fibrils gives out nine radially oriented links or spokes to each subfibril A.

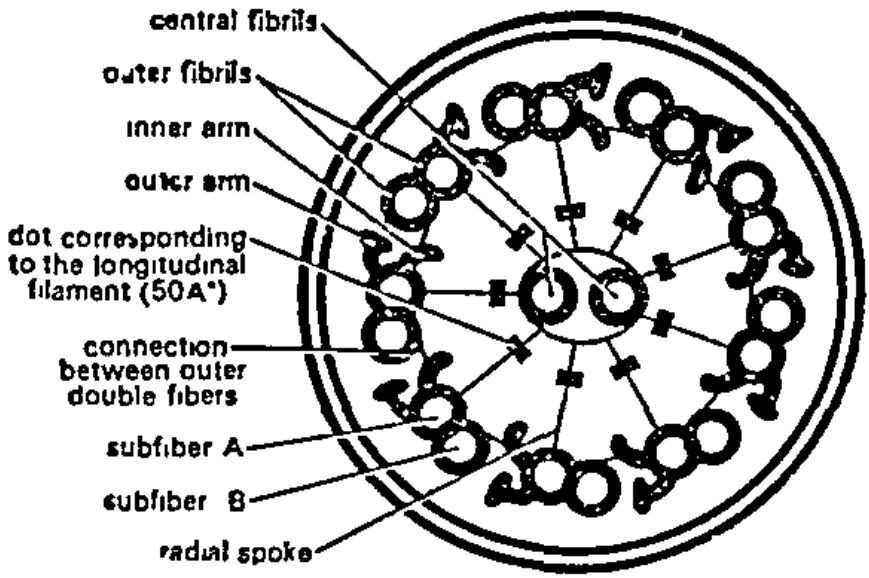


Fig. 13·2. Diagrammatic transverse seciton of a flagellum.

The high resolution electron microscopy has revealed that each of the peripheral and central fibrils of the cilia and flagella is composed of ten to twelve (10 to 12) filaments of 40°A thickness. Each filament is beaded, Each bead remains arranged in the lattices of 40 by 50A° in the plane of the wall of the tubule. These beads are considered as the basic subunit of the tubule structure. The flagellum of spermatozoon tail has an additional element, a helical fibril around the periphery of the flagellum. Fin-like structures have also been found projecting in the direction of central fibres in spermatozoon tails of some species (Fawcett and Porter, 1954).

The flagellum of bacteria is somewhat different in plan of organization, consisting of single fibre instead of 9+2 filaments. The fibres are cylindrical and composed of globular molecules of 40A° in diameter, arranged in a hexagonal packing with a helical twist. These molecules are of a fibrous protein, known as flagellin which belongs to the keratin myosin-elastin-fibrinogen group.

CHEMICAL COMPOSITION OF CILIA AND FLAGELLA

Both the cilia and flagella are chemically composed of 70 to 84 per cent proteins, 13 to 23 per cent lipids, 1 to 6 per cent carbohydrates and 0·2 to 0·4 per cent nucleotides. The arms of the subfibril A and central fibrils contain the protein dynein which contains enzyme ATPase of the cilia. The outer fibrils contain a protein which resembles closely with the acitin of the muscle cells.

FUNCTIONS OF CILIA AND FLAGELLA

The cilia and flagella serve many purposes and their movements propel the organism. The cilia are contractile structures and in them two types of rhythms known as **metachronic** and **isochronic** or **synchronous rhythms** produce the wave of contractions in the cilia. In the **metachronic** type of rhythm the cilia of a row beat one after the other, while in the **synchronous** or isochronic rhythm, all the cilia of a row beat simultaneously. In contrast to the cilia, the flagella exhibit undulant motion and beat independently.

The beating of cilia or flagella is caused by the intraciliary excitation which is followed by the interciliary conduction. **Hayashi** (1961) has reported that the two inner filaments of a cilium transmit excitation and the nine outer filaments are the seat of ATP splitting. The movement of cilia may be under nervous or cytoplasmic control. In a few invertebrate embryos the cilia are probably under nervous control since their movement may be stopped upon stimulation of the embryo. In ciliates they are thought to be coordinated by a neuro-motor centre near the mouth since destruction of the fibres connecting the centre to the cilia results in uncoordinated movements (**Taylor**, 1920). However, **Okajima** (1966) reported coordinated movements in *Euplotes* even after complete dissection of the neuro-motor fibres. Recent studies have shown that cytoplasm is necessary for the ciliary movements. The ATP provides necessary amount of energy for the motion of the cilia and flagella. Four types of ciliary movements have been recognized which are as follows :

1. **The pendulus ciliary movement**—The pendulus type of ciliary movement is carried out in a single plane. It occurs in the ciliated protozoans which have rigid cilia. In such cases the movement of the cilia is carried out by a flexion at its base.

2. **The uniform ciliary movement**—The uniform ciliary movement occurs commonly in the metazoan cells. In such type of movement, when the cilia contract it becomes double and acquires a hook-like shape.

3. **The infundibuliform ciliary movement**—The infundibuli form ciliary movement occurs due to the rotatory movement of the cilium and flagellum. In this case, the cilium or flagellum is passed through three mutually perpendicular planes in the space and makes conical or funnel-shaped shape.

4. **The undulant movement**—The undulant movement is the characteristic of the flagellum. In undulant movement the waves of the contraction proceed from the site of implantation and pass to the border.

OTHER FUNCTIONS OF THE CILIA AND FLAGELLA

1. The ciliary or flagellar movement provides the locomotion to the cell or organism.
2. The cilia create food currents in lower aquatic animals.
3. In the respiratory tract, the ciliary movements help in the elimination of the solid particles from it.
4. The eggs of amphibians and mammals are driven out from the oviduct by the aid of vibratile cilia of the latter.

Thus, the cilia and flagella serve many physiological processes of the cell, such as locomotion, alimentation, circulation, respiration, excretion and perception of sense.

DERIVATIVES OF CILIA

The cilia are modified into a variety of structures such as the rods and cones of the retina, crown cells of saccus vasculosus of third ventricle of fishes primitive sensory cells of the pineal eye and enidocil of the nematocysts of the coelenterates.

CHAPTER 14

RIBOSOMES

The ribosomes are small, dense, rounded and granular particles of the ribonucleoprotein. They occur either freely in the matrix of mitochondria, chloroplast and cytoplasm (*i.e.*, cytoplasmic matrix) or remain attached with the membranes of the endoplasmic reticulum and nucleus. They occur in most prokaryotic and eukaryotic cells and are known to be the sites of protein synthesis where amino acids are assembled in a specified sequence to produce polypeptide chain.

The ribosomes were first noted in plant cells by **Robinson** and **Brown** in 1953 when studying bean roots with the electron microscope and shortly afterward **Palade** (1955) observed them in animal cells He isolated the *ribosomes and detected the RNA in them in 1956* The present knowledge about the ribosomes have been gained by the cell fractionation, ultra-centrifugation, radioisotopy and electron microscopy.

OCCURRENCE AND DISTRIBUTION

The ribosomes occur in cells, both prokaryotic and eukaryotic cells. In prokaryotic cells the ribosomes often occur freely in the cytoplasm. In eukaryotic cells the ribosomes either occur freely in the cytoplasm or remain attached to the outer surface of the membrane of endoplasmic reticulum. The yeast cells, reticulocytes or lymphocytes, meristamatic plant tissues, embryonic nerve cells and cancerous cells contain large number of ribosomes which often occur freely in the cytoplasmic matrix. The cells in which active protein synthesis takes place, the ribosomes remain attached with the membranes of the endoplasmic reticulum. Such cells are the pancreatic cells, plasma cells, hepatic parenchymal cells, Nissls bodies, osteoblasts, serous cells, or the submaxillary gland, chief cells of the glandular stomach, thyroid cells and mammary gland cells. The cells which synthesize specific proteins for the intracellular utilization and storage often contain large number of free ribosomes. Such cells are the erythroblasts, developing muscle cells, skin and hair.

METHOD OF ISOLATION

The ribosomes are usually isolated from the cell by ths differential centrifugation method in which a analytical centrifuge is em-

ployed. The sedimentation coefficient of the ribosomes is determined by the various optical and electronic techniques. The sedimentation coefficient is expressed in the svedberg unit, *e.g*, S unit. The S is related with the size and molecular weight of the ribosomal particles.

TYPES OF RIBOSOMES

Recently according to the size and the sedimentation coefficient (S) two types of ribosomes have been recognised.

1. 70S Ribosomes : The 70S ribosomes are comparatively smaller in size and have sedimentation coefficient 70S and the molecular weight $2{\cdot}7 \times 106$ daltons. (Dalton is the unit of molecular weight (MW) ; one dalton equals the weight of a hydrogen atom. For example, a water molecule weighs 18 daltons, see **De Robertis *et al.*,** 1970). According to the data of electron microscopy the dimension of the dry particles of 70S ribosomes are $170 \times 170 \times 200$A° (**Hall** and **Stayter,** 1959, **Huxley** and **Zubay,**1960). They occur in the prokaryotic cells of the blue green algae and bacteria and also in mitochondria and chloroplast of eukaryotic cells.

2. 80S Ribosomes : The 80S ribosomes have the sedimentation coefficient of 80S and the molecular weight 40×10^6 daltons. The 80S ribosomes occur in eukaryotic cells of the plants and animals.

STRUCTURE OF RIBOSOMES

The ribosomes are oblate spheroid structures of 150 to 250A° in diameter. Each ribosome is porous, hydrated and composed of two subunits. One ribosomal subunit is larger in size and has a dome-like shape, while the other ribosomal subunit is smaller in size and occurring above the larger subunit and forming a cap-like structure.

The 70S ribosome consists of two subunits, *viz.*, 50S and 30S. The 50S ribosomal subunit is larger in size and has the size of 160A° to 180A°. The 30S ribosomal subunit is smaller in size and occurs above the 50S subunit like a cap.

The 80S ribosome also consists of two subunits, *viz.*, 60S and 40S. The 60S ribosomal subunit is dome-shaped and larger in size. In the ribosomes which remain attached with the membranes of endoplasmic reticulum and nucleus, etc., the 60S subunit remains attached with the membranes. The 40S ribosomal subunit is smaller in size and occurs above the 60S subunit forming a cap-like structure. Both thesubunitsremain separated by a narrow cleft.

The two ribosomal subunits remain united with each other due to low concentration of the Mg^{++}(·001M) ions. When the concentration of Mg^{++} ions reduces in the matrix, both ribosomal subunits

get separated. Actually in bacterial cells the two subunits are found to occur freely in the cytoplasm and they unite only during the pro-

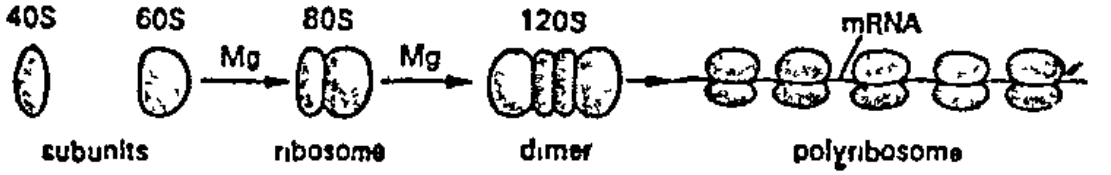


Fig. 14·1. Diagram of the subunit structure of the ribosome and the influence of Mg^{++}ions

cess of protein synthesis. At high concentration of Mg^{++}ions in the matrix, the two ribosomes become associated with each other and known as the dimer. Further, during protein synthesis many ribosomes are aggregated due to common messenger RNA and form the polyribosomes or polysomes.

ULTRASTRUCTURE

The fine structure of the ribosome is still less worked out. However, Nauninga (1967) has observed that the 50S subunit of the 70S ribosome is pentagonal in shape and is a compact particle of 160 to 180 A°. In the centre of this subunit occurs a round area of 40 to

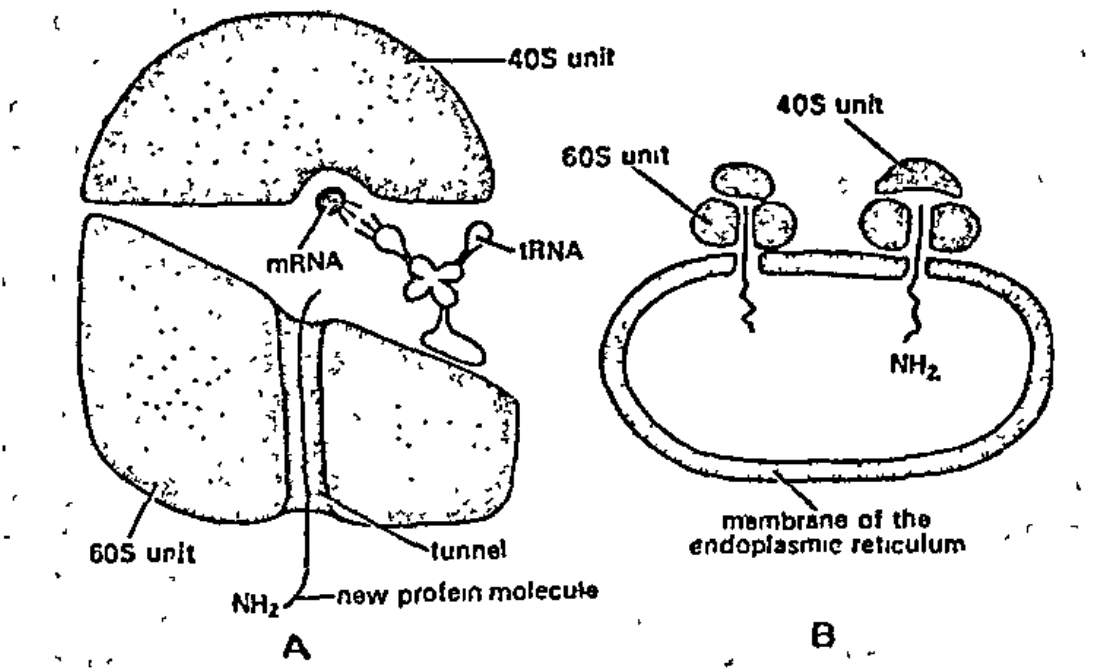


Fig. 14·2. Ultrastructure of the ribosome A—Diagram of a ribosome showing the two subunits and the probable position of mRNA and tRNA. B—Diagram showing the relationship between the ribosomes and the membrane of endoplasmic reticulum and the entrance of the polypeptide chain into the centre of endoplasmic reticulum during the process of protein synthesis.

60A°. Florendo (1968) has observed a pore-like transparent area in the 50S subunit which does not allow the entrance of the proteolytic

Table : Protein factors of ribosomes. (Lippmann, 1969).

Factors	Source	Function
F_1, F_2, F_3+GTP	30S ribosomal unit	Initiation
Tu, Ts T+GTP	?	Binding of aminoacyl tRNA to ribosome, translation and elongation of polypeptide chain
tyl transfe-	50S ribosomal unit	Peptidyl transfer to aminoacyl-tRNA
	?	Translocation of ribosome over mRNA and transfer of tRNA residue from one site to other.
	?	Termination (release of polypeptide from tRNA and from ribosome)
	unit	Dissociation of 70S ribosomes after completion of process of protein synthesis

remain loosely attached with the ribo-
rich compound, guanosine tripho-
functioning.

of nomenclature for translation
formulated by many cell biologists. It
:

for translation factors (From : Science,

Nomenclature replaced	
F_1 (1), A (2), FI (3)	
F_2 (2), C (2), FIII (3)	
F_3 (3), B (2), FII (3)	
T_u (4), FI_u (5), S_3 (6)	
T_s (4), FI_s (5), S_1 (6)	
G (4), FII (5) S_2 (6)	
Translocase (7)	
R (8), R_1 (9)	
R_2 (9)	
α (10), S (11)	
Transferase I (12),	TF—1 (13)
Transferase II (12),	TF—2 (13)

enzymes or the enzyme ribonuclease. Such pores have also been observed in the 60S subunit of 80S ribosomes. The smaller subunits of the ribosomes possess no regular shape and often remain divided into two portions. Each portion remains inter-connected with each other by a strand of 30 to 60A° thickness.

CHEMICAL COMPOSITION

The ribosomes are chemically composed of ribosomal ribonucleic acid (rRNA), proteins, lipids and certain metallic ions. However, rRNA and proteins are the major constituents of ribosomes and in both types of ribosomes, *viz.*, 70S and 80S, the proportion of rRNA and protein often remains variable. The 70S ribosomes contain more rRNA (60 to 64%) than the proteins (36 to 37%), *e.g.*, the ribosomes of *E. coli* contain 63% rRNA and 37% proteins. In contrast to 70S ribosomes, the 80S ribosomes consist of less rRNA (40 to 44%) than the proteins (60 to 56%), *e.g.*, yeast ribosomes have 40 to 44% rRNA and 60 to 56% proteins; ribosomes of pea seedling contain 40% rRNA and 60% proteins ; ribosomes of rat liver contain 40% rRNA, 50 to 55% proteins and 5 to 10% lipids.

1. RIBOSOMAL PROTEINS

According to **Garrett and Wittmann (1973)**, the 70S ribosomes of *E.coli* contain about 55 ribosomal proteins. Out of these 55 proteins, 21 proteins occur in smaller 30S subunit, while, 50S ribosomal subunit contains 34 proteins. The proteins from small subunit are prefixed by an S and those from large subunit as L. The molecular weight of S-proteins (from S_{21} to S_1) range from 13000 to 65000, while the molecular weights of L-proteins (from L_{35} to L_1) range from 9000 to 22000.

2. RIBOSOMAL ENZYMATIC PROTEINS

Most of the ribosomal proteins act as enzymes which catalize the process of protein synthesis at various steps. For example, the proteins called **"transfer factors"**, *viz.*, G-Factor, Ts factor, etc., are required for the translocation of ribosomes over the mRNA, for transfer of a tRNA residue from one site of the ribosome to the other site, for translation of codes of mRNA, for binding of tRNA carrying amino acid to the mRNA, etc., during the process of protein synthesis. Likewise, certain enzymatic proteins such as F_1 and F_2 proteins initiate the process of protein synthesis and are called **"initiation factors"**. Certain other proteins, such as R_1 and R_2 proteins terminate and release completed polypeptide chain from the ribosomes after the termination of translation and are called **termination factors.** These protein factors have been isolated only from 70S ribosomes of *E. coli.* and can be tabulated in following table :

Table : Protein factors of ribosomes. (Lippmann, 1969).

Factors	Source	Function
F_1, F_2, F_3+GTP	30S ribosomal unit	Initiation
$\left.\begin{matrix}Tu\\Ts\end{matrix}\right\}$ T+GTP	?	Binding of aminoacyl tRNA to ribosome, translation and elongation of polypeptide chain
Peptidyl transferase	50S ribosomal unit	Peptidyl transfer to aminoacyl-tRNA
G+GTP	?	Translocation of ribosome over mRNA and transfer of tRNA residue from one site to other.
R_1, R_2	?	Termination (release of polypeptide from tRNA and from ribosome)
F_3	30S ribosomal unit	Dissociation of 70S ribosomes after completion of process of protein synthesis

These protein factors remain loosely attached with the ribosomes and often require energy rich compound, guanosine triphophate (GTP) for their normal functioning.

Recently, a uniform pattern of nomenclature for translation factors of ribosomes has been formulated by many cell biologists. It is tabulated in following table :

Table : Uniform nomenclature for translation factors (From : Science, Vol. 176, 14 April, 1972)

Factors	Abbreviations	Nomenclature replaced
A. Prokaryotic forms		
Initiation factor 1	IF—1	F_1 (1), A (2), FI (3)
Initiation factor 2	IF—2	F_2 (2), C (2), FIII (3)
Initiation factor 3	IF—3	F_3 (3), B (2), FII (3)
Elongation factor T_u	EF—T_u	T_u (4), FI_u (5), S_3 (6)
Elongation factor T_s	EF—T_s	T_s (4), FI_s (5), S_1 (6)
Elongation factor G	EF—G	G (4), FII (5) S_2 (6) Translocase (7)
Release factor 1	RF—1	R (8), R_1 (9)
Release factor 2	RF—2	R_2 (9)
Release factor 3	RF—3	α (10), S (11)
B. Eukaryotic cell		
Elongation factor 1	EF—1	Transferase I (12), TF—1 (13)
Elongation factor 2	EF—2	Transferase II (12), TF—2 (13)

3. RIBOSOMAL RNA

The 70S ribosomes contain three types of rRNA, *viz.*, 23S rRNA, 16S rRNA and 5S RNA. The 23S and 5S rRNA occur in the larger 50S ribosomal subunit, while the 16S rRNA occurs in the smaller 30 S ribosomal subunit. The 23S rRNA consists of 3200 nucleotides 16S rRNA contains 1600 nucleotides and 5S rRNA includes 120 nucleotides in it (Brownlee, 1968 ; Fellner, 1972).

The 80S ribosomes also contain three types of rRNA, *viz.*, 28S rRNA, 18S rRNA and 5S rRNA. The 28S and 5S rRNA occur in the larger 60S ribosomal unit, while the 18S rRNA occurs in the smaller 40S ribosomal subunit.

The 28S rRNA has the molecular weight $1{\cdot}6\times10^6$ daltons and its molecule is double stranded and having nitrogen bases in pairs. The 18S rRNA has the molecular weight $0{\cdot}6\times10^6$ daltons. The molecule of 5S rRNA has a clover leaf shape and a length equal to 120 nucleotides (Forget and Weissmann, 1968).

4. METALLIC IONS

The most important low molecular weight components of ribosomes are the divalent metallic ions such as Mg^{++}, Ca^{++} and Mn^{++}

BIOGENESIS OR SYNTHESIS

The ribosomal RNAs of 70S ribosomes of prokaryotes are transcribed by particular cistrons (genes) of the genome, called **cistron of ribosomal DNA** and are immediately released. Thus, in bacteria biogenesis of ribosomes takes place in cytoplasm.

In contrast to biogenesis of 70S ribosomes the biogenesis of 80S ribosomes takes place in nucleolus. The nucleolus is synthesized by a specific region of a chromosome (autosome or sex chromosome) called **nucleolar organizer** region. Certain cistrons (genes) of nucleolar organizer region contain the codes for biogenesis of 28S rRNA and 18S rRNA of the 80S ribosomes and that portion of nucleolar organizer is called *ribosomal DNA or rDNA.* The ribosomal DNA or rDNA of nucleolar organizer transcribes a large molecule of 45S rRNA which is the precursor of both the 28S and 18S rRNAs. The transcription of 45S rRNA takes place in nucleolus and completes in 2·3 minutes. The 45S rRNA is stored in the nucleolus as fibrils of 50 A° length (Bernhard and Granboulad 1968). The 45S rRNA remains in the nucleolus for 20 minutes and get methylated. Soon it loses some non-methylated part and cleaves into 32S and 18S rRNAs. The 18S rRNA is wrapped in certain ribosomal and accessory proteins of the 'pool of proteins' of nucleolus and immediately transported to the cytoplasm, where it forms the 40S ribosomal subunit of the ribo-

some. The 32S rRNA remains intact in the nucleolus for 40 minutes and is then cleaved again to form the 28S rRNA. The 28S rRNA is wrapped in proteins which are synthesized on the cytoplasmic ribosomes (see Hall *et al.*, 1974), and after 30 minutes is transported to the cytoplasm.

The 5S rRNA is transcribed by the cistrons outside the nucleolar organizer and before it leavas the nucleus for the cytoplasm it becomes incorporated with the ribonucleoprotein particle (RNP) having 28S rRNA. The 28S RNP granule and 5S rRNA form the 60S ribosomal subunit in the cytoplasm. All the RNP granules of the nucleolus are transported to the cytoplasm via the nucleopores (Robertis, 1964).

FUNCTIONS

The ribosomes are the fundamental apparatuses of cytoplasm which provide necessary space and protein factors (enzymes) for the process of protein synthesis. The role of ribosomes will be discussed in a separate chapter.

CHAPTER 15

NUCLEUS

The nucleus (L., *nux*-nut) is the most significant component of the cell which controls various metabolic activities of the cell and contains the genetic material DNA. The nucleus is discovered by Robert Brown in 1831. Since its discovery the cytologists became interested in it more and more because of its extra-ordinary participation in the cellular metabolism and heredity mechanism.

OCCURRENCE AND POSITION

The nucleus is found in all the eukaryotic cells of the plants and animals. However, certain eukaryotic cells such as the mature sieve tubes of higher plants and mammalian erythrocytes contain no nucleus. In such cells nuclei are present during the early stages of development. The prokaryotic cells of the bacteria do not have true nucleus, *i.e.*, the single, circular and large DNA molecule remains in direct contact with the cytoplasm. The position or location of the nucleus in a cell is usually the characteristic of the cell type and it is often variable. Usually the nucleus remains located in the centre. But its position may change from time to time according to the metabolic states of the cell. For example, in the embryonic cells the nucleus generally occupies the geometric centre of the cell but as the cells start to differentiate and the rate of the metabolic activities increases, the displacement in the position of the nucleus takes place. In certain cells such as the glandular cells the nucleus remains located in the basal portion of the cell.

MORPHOLOGY

NUMBER

Usually the cells contain single nucleus but the number of the nucleus may vary from cell to cell. According to the number of the nuclei following types of cells have been recognised.

1. **Mononucleate cells**—Most plant and animal cells contain single nucleus, such cells are known as mononucleate cells.

2. **Binucleate cells**—The cells which contain two nuclei are known as binuleate cells. such cells occur in certain protozoans such as *Paramecium* and cells of cartilage and liver.

3. Polynucleate cells—The cells which contain many (from 3 to 100) nuclei are known as polynucleate cells. The polynucleate cells of the animals are termed as syncytial cells, while the polynucleate cells of the plants are known as coenocytes. The most common example of the syncytial cells are the osteoblast (polykaryocytes of the bone morrow) which contain about 100 nuclei per cell and striated muscle fibres contain many hundred nuclei. The siphonal algae *Vaucheria* contains hundreds of nuclei and certain fungi are the best example of the coenocyte plant cells.

SHAPE

The shape of the nucleus normally remains related with the shape of the cell, but certain nuclei are almost irregular in shape. The spheroid, cuboid or polyhedral cells (isodiametrical cells) contain the spheroid nuclei. The nuclei of the cylindrical, prismatic or fusiform cells are ellipsoid in shape. The cells of the squamous epithelium contain the discoidal nuclei. The leukocytes, certain infusoria, glandular cells of some insects and spermatozoa contain the irregular shaped nuclei.

SIZE

The size of the nucleus of various cells is also variable. The size of the nucleus is directly proportional to that of the cytoplasm. R. Hertwig has given the following formula for the deduction of the size of the nucleus of a particular cell.

$$NP = \frac{V_n}{V_c - V_n}$$

where NP is the nucleoprotein index, V_n is the volume of the nucleus and V_c is the volume of the cell.

Moreover, the size of the nucleus is related with the number of the ch

of the DNA and proteins and metabolic phase of the cell.

ULTRASTRUCTURE

The nucleus is composed of following structures :

1. The nuclear membrane or karyotheca.
2. The nuclear sap or nucleoplasm.
3. The chromatin fibres.

and 4. The nucleolus.

NUCLEAR MEMBRANE

The nuclear membrane or karyotheca forms an envelope-like structure around the nuclear contents and commonly known as the

nuclear envelope. The nuclear envelope separates the nucleoplasm from the cytoplasm.

The electron microscopic studies of the nuclear envelope have shown that it is composed of two unit membranes, *viz.*, an outer membrane and an inner membrane. Each membrane is about 75 to 90 A° thick and lipoproteinous in nature. The outer and inner nuclear membranes ren ain separated by a space of 100 to 150 A°

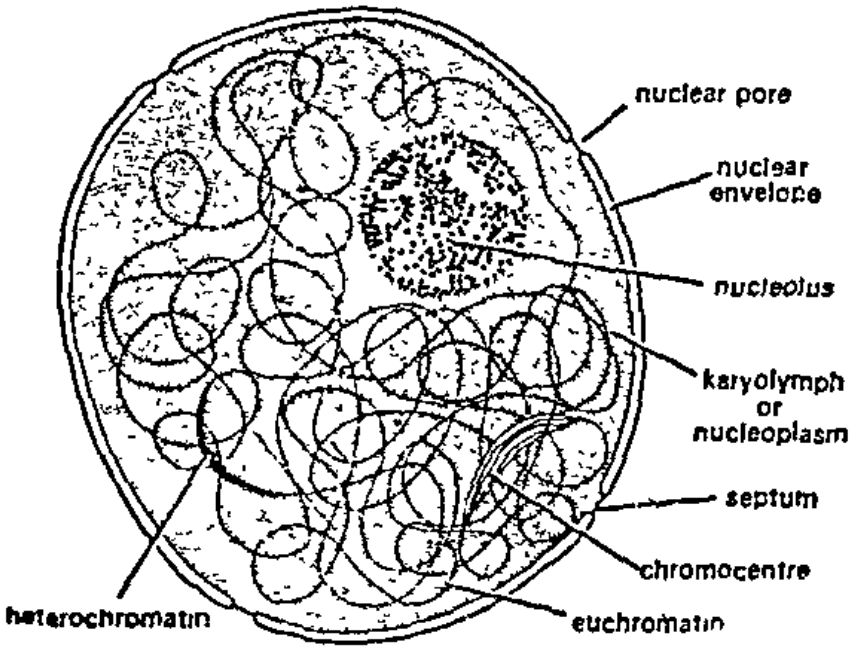


Fig. 15·1. Structure of a metaphase nucleus.

(Robertis *et al.*, 1970), 100 to 300 A° (Cohn, 1970) or 400 to 700A° (Burke, 1970). This inter-membranous space is known as perinuclear space or cisterna. The outer nuclear membrane often remains rough due to attached ribosomes with it. Sometimes it remains continuous with the membranes of the endoplasmic reticulum, Golgi complex, mitochondria, etc. The inner nuclear membrane contains no ribosomes and sometimes it also remains associated with the chromatin. Further, the nuclear membrane is followed by a supporting membrane of a uniform thickness known as fibrous lamina which is 300 A° in thickness.

NUCLEAR PORES

The nuclear membranes are not altogether continuous but at several places they are broken by the nuclear openings or pores. The number of the pores for a particular nucleus is variable and often depends on the species and the type of the cell. For instance, the oocytes of the sea-urchin have 40 to 80 nuclear pores per square micron of the nucleus. Watson (1959) has calculated the nuclear pores of the mammalian cells as the 10 per cent of the total surface area of the nucleus.

The nuclear pores are octagonal in shape and have the diameter of 600 A°. These are enclosed by a circular structures known as annuli. The pores and the annuli are collectively known to form the pore complex. The pore complex remains arranged hexagonally on the surface of the nuclear envelope. The annuli have an outer diameter of 1200A° and an inner diameter between the 0 and 400A°.

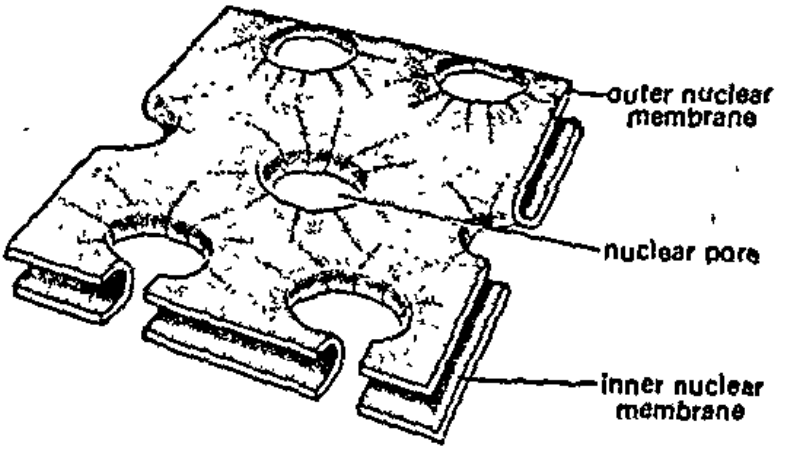


Fig. 15·2. Three dimensional structure of nuclear envelope.

In certain cases, the pore complex remains covered by a thin membrane or septum which may serve as a special structure for the selective permeability.

Recently **Roberts** and **Northcote** (1970) have proposed a model based on thin-sectioning and freeze-etching of a variety of higher plant species. They consider that the pores have a circular boundary and that previous reports of an octagonal shape as seen by negative staining are artefects. The pore has eight inner electrone-dense peripheral granules, while the annulus is bounded by two rings of eight granular subunits attached to the membrane on the nuclear and cytoplasmic sides of the pore. In many pores a central granule is observed which may be connected to the pore margin by thin filaments. The pore complex is filled with an amorphous material of different electron density from that of the cytoplasm and nucleus and which may act as a barrier to free diffusion. The com-

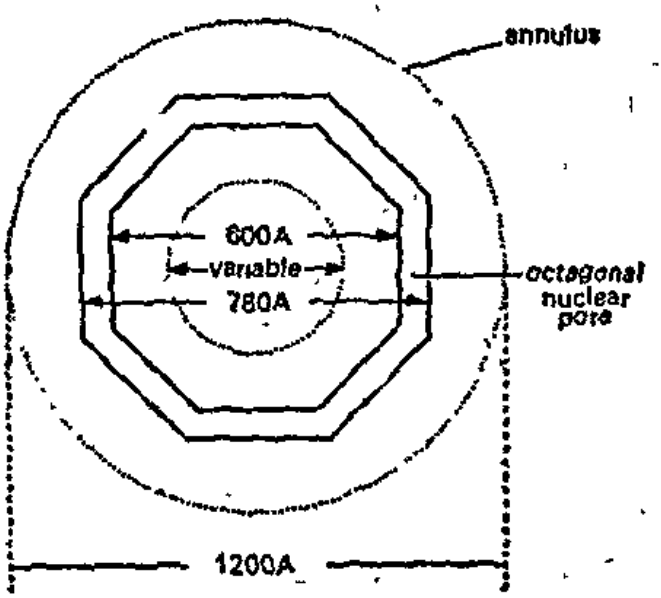


Fig. 15·3. Structure of a nuclear pore.

position and function of the granules is not clear, although it has been suggested that the peripheral granules are composed of RNA and may, in fact, be ribosomes (Thair and Wardrop, 1971).

ORIGIN OF NUCLEAR ENVELOPE

The nuclear envelope is the derivative of the membrane of the endoplasmic reticulum. In telophase of the mitosis, it has been observed that the cisternae of the endoplasmic reticulum collect around the chromosomes and by fusing together form the nuclear envelope.

FUNCTION OF NUCLEAR MEMBRANE AND NUCLEAR PORES

The nuclear membranes perform various important functions for the nucleus, such as, in certain cells (oocyte) the nuclear membranes allow free exchange of ions between the nucleus and the cytoplasm, while certain nuclear membranes as of the cells of the salivary glands of the *Drosophila* act as barrier for the diffusion of substances and even the ion of K^+, Na^+ and Cl^-.

The nuclear pores are the pathways for the exchange of the macromolecules Th[illegible] particles, etc., have [illegible] from the nucleopl[illegible] exchange of the macromolecules through the pore complex according to their chemical nature and the size. The permeability of the pore complex depends on the type and metabolic state of the cell because Feldher (1969) has reported that such differential properties of the cell have an effect on the nature of the annuli.

NUCLEOPLASM

The space between the nuclear envelope and the nucleolus is [illegible] slightly acidophilic [illegible] clear sap or nucleo- [illegible] such as the chromatin threads and the nucleolus remain suspended in the nucleoplasm.

The nucleoplasm has a complex chemical composition. It is composed of mainly the nucleoproteins but it also contains other inorganic and organic substances, *viz.*, nucleic acids, proteins, enzymes and minerals.

1. Nucleic acids—The most common nucleic acids of the nucleoplasm are the DNA and RNA. Both may occur in the macromolecular state or in the form of their monomer nucleotides.

2. Proteins—The nucleoplasm contains many types of complex proteins. The nucleoproteins can be categorized into following two types :

(i) Basic proteins—The proteins which take basic stain are

low molecular weight (about 4000 daltons). The most abundant amino acid of these proteins is arginine (pH 10 to 11). The protamines usually remain bounded with the DNA molecules by the salt linkage. The protamines occur in the spermatozoa of the certain fishes. The nucleohistones have high molecular weight, *e.g.*, 10,000 to 18,000 dal
as arginine. I
ted with t
most organisms. According to the composition of the amino acids following types of histone proteins have been recognised, *e.g.*, histones with rich lysine, histones with arginine and histones with poor amount of the lysine.

(ii) N
occur in the
acidic protei
phosphoproteins.

3. Enzymes—The nucleoplasm contains many enzymes which are necessary for the synthesis of the DNA and RNA. Most of the nuclear enzymes are composed of non-histone (acidic) proteins. The most important nuclear enzymes are the **DNA polymerase, RNA polymerase, NAD synthetase, nucleoside triphosphatase, adenosine diaminase, nucleoside phosphorylase, guanase, aldolase, enolase, 3-phosphoglyceraldehyde dehydrogenase** and **pyruvate kinase.** The nucleoplasmalso contains certain cofactors and coenzymes such as *ATP and acetyl CoA.*

4. Lipids—According to **Stoneburg (1937)** and **Dounce (1955),** the nucleoplasm contains small lipid content.

5. Minerals—The nucleoplasm also contains several inorganic compounds such as phosphorus, potassium, sodium, calcium and magnesium. The chromatin comparatively contains large amount of these minerals than the nucleoplasm.

CHROMATIN FIBRES

The nucleoplasm contains many thread-like, coiled and much elongated structures which take readily the basic stains such as the

cell divisions (mitosis and meiosis) chromatin fibres become thick ribbon-like structures which are known as the chromosomes.

The fibres of the chromatin are twisted, fine anastomose and uniformly distributed in the nucleoplasm. Two types of chromatin material have been recognised, *e.g.*, heterochromatin and euchromatin.

A. Heterochromatin : The darkly stained, condensed region of the chromatin is known as heterochromatin. The condensed portions of the nucleus are known as **chromocenters** or **karyosomes** or **false nucleoli.** The heterochromatin occurs around the nucleolus and at the periphery. It is supposed to be metabolically and genetically inert because it contains comparatively small amount of the DNA and large amount of the RNA.

B. Euchromatin : The light stained and diffused region of the [illegible]. The euchromatin contains [illegible] of DNA

NUCLEOLUS

The nucleus contains a large, spherical and acidophilic dense granule known as the **nucleolus.** The nucleoli were discovered by **Fontona** in 1781. It was well established in 19th century that the size of the nucleolus is related with the synthetic activity of the cell. Therefore, the cells with little or no synthetic activities, *e.g.*, sperm cells, blastomeres, muscle cell, etc., are found to contain smaller or no nucleoli, while the oocytes, neurons and secretory cells which synthesize the proteins or other substances contain comparatively large sized nucleoli. The number of the nucleoli in the nucleus depends on the species and the number of the chromosomes. The number of the nucleoli in the cells may be one, two or four. The position of the nucleolus in the nucleus is eccentric.

FINE STRUCTURE OF NUCLEOLUS

In certain cells the nucleolus may exist in a fragmented or diffused form. But most nuclei contain compact nucleoli. The nucleolus composed of the following three regions :

1. **Granular region :** The granular region occurs at the periphery of the nucleolus and composed of dense granules of 150 to 200 A° diameter. The granular region consists of ribonucleic proteins.

2. **Fibrillar region:** The fibrillar region is known as the **nucleolonema.** The nucleolonema is composed of many fibrils of 50 to 80 A° long. Sometimes very long as 500 A° fibrils have been reported in certain nucleoli. The fibrils are composed of ribonucleic proteins.

3. **Amorphous region :** The amorphous region has low electron density and it is found only in certain nucleoli. It is composed of

certain proteins which can be hydrolysed easily by the pepsin enzyme.

The nucleolus possesses no limiting membrane and the calcium ions are supposed to maintain the intact organisation of the nucleolus. The nucleolus is surrounded by heterochromatin which enters in the nucleolus and forms intra-nucleolar ramification. The nucleolus also contains vacuoles which have no limiting membranes and contain certain proteins.

CHEMISTRY OF THE NUCLEOLUS

The nucleolar is chemically composed of RNA and proteins. The nucleolur RNA resembles very closely with the ribosomal RNA in its base composition. The nucleolus contains large amount of the proteins. Among the proteins the most abundant are phospho-proteins. The nucleolus also contains orthophosphates which supply the phosphorus for the rRNA synthesis. It also contains certain enzymes for the biosynthesis of some coenzymes, nucleotides and the ribosomal RNA. The most important enzymes of the nucleolus are the acid phosphatase, nucleoside phophorylase, DPN and RNA methylase.

MITOTIC CYCLE

The nucleolus shows the cyclic changes in itself during the

informations for the synthesis of the nucleolus and ribosomal RNA.

FUNCTION OF THE NUCLEOLUS

The main function of nucleolus is the biogenesis of ribosomes. It stores all the proteins of ribosomes and also rRNA which is transcribed

18S rRNA—both of which become wrapped by nucleolar proteins of cytoplasmic origin (*i.e.*, proteins formed on cytoplasmic ribosomes) and are transported to cytoplam to form the ribosome.

CHAPTER 16

CHROMOSOMES

The chromosomes are the nuclear components of special organisation, individuality and function. They are capable of self reproduction and play a vital role in heredity, mutation, variation and evolutionary development of the species.

HISTORICAL

W. Fleming (1879) first of all described the splitting of chromosomes and coined the term chromatin for stainable material of the nucleus. W. Roux (1883) suspected the involvement of the chromosomes in the mechanism of inheritance. Benden and Bovery (1887) [illegible] each species was constant. [illegible] *chromi*-colour, *soma*-body) was [illegible] stained bodies of nucleus. W. S. Sutton and T. Boveri in 1902 suggested that chromosomes were the physical structures which acted as messengers of heredity. Morgan (1933) discovered the function of chromosomes in the transmission of heredity traits. Heitz (1935), Kuwanda (1939), Geitler (1940), and Kaufmann (1948) have described the morphology of chromosomes. The molecular organization of chromosomes have been recently worked out.

CHROMOSOME NUMBER

The number of the chromosomes is constant for a particular species. Therefore, these are of great importance in the determination of the phylogeny and taxonomy of the species. The organism with the lowest number of the chromosomes is the nematode, *Ascaris megalocephalus univalens* which has only two chromosomes in the somatic cells. The chromosome number of some animals and plants is tabulated as follows :

Table : Chromosome number of some organisms.

Group	Common name	Scientific name	Chromosome number
Animals :			
Protozoa	Paramecium	*Paramecium aurelia*	30—40
Cnidaria	Hydra	*Hydra vulgaris*	32

Group	Common name	Scientific name	Chromosome number
Nematoda	Round worm	*Ascaris lumbricoides*	24
Arthropoda	House fly	*Musca domestica*	12
	Mosquito	*Culex pipiens*	6
Chordata	Frog	*Rana esculenta*	26
	Pigeon	*Columba livia*	80
	Rabbit	*Oryctolagus cuniculus*	44
	Gorilla	*Gorilla gorilla*	48
	Man	*Homo sapiens*	46
Plants :			
Algae	Chlamydomonas	*Chlamydomonas reinhardii*	10 ?, 12 ? 16 ? (Haploid sets)
Fungi	Bread mold	*Mucor heimalis*	2
Gymnosperm	Yellow pine	*Pinus ponderosa*	24
Angiosperm	Cabbage	*Brassica oleracea*	18
	Coffee	*Coffea arabica*	44
	Potato	*Solanum tuberosum*	48

of most set or genomes and are known as the diploid cells. The diploid cells achieve the diploid set of the chromosomes by the union of the hapliod male and female gametes in the sexual reproduction.

MORPHOLOGY

SIZE

The size of the chromosomes varies from species to species and relatively remains constant for a particular species. The length of the chromosomes may vary from 0·2 to 50μ. The diameter of the chromosomes may be from 0·2 to 20μ. For instance, the human chromosomes are to 6μ in length. Moreover, the organisms with less number of somes than somes.

large-sized chromosomes in general have large-sized mals. Further, the chromo- some may be exceptionally large and other may be too small. The largest chromosomes are lampbrush chromosomes of certain vertebrate oocytes and polytene chromosomes of certain dipteran insects.

SHAPE

The shape of the chromosomes is changeable from phase to phase in the contiunous process of the cell growth and cell division.

In the resting phase or interphase stage of the cell, the chromosomes occur in the form of thin, coiled, elastic and contractile, thread-like stainable structures, the chromatin [illegible] the anaphase, the chromosomes bec[illegible] chromosome contains a clear zone, [illegible] core, along their length. The centromere divides the chromosomes into two parts, each part is called chromosome arm. The position of centromere vary from chromosome to chromosome and it provides different shapes to the latter which are following :

1. **Telocentric**—*The rod-like chromosomes which* have the centromere on the proximal end are known as the **telocentric chromosomes.**

2. **Acrocentric**—The acrocentric chromosomes are also rod-like in shape but these have the centromere at one end and thus giving a very short arm and an exceptionally long arm. The locusts (Acrididae) have the acrocentric chromosomes.

3. **Submetacentric**—The submetacentric chromosomes are J- or L-shaped. In these, the centromere occurs near the centre or at medium portion of the chromosome and thus forming two unequal arms.

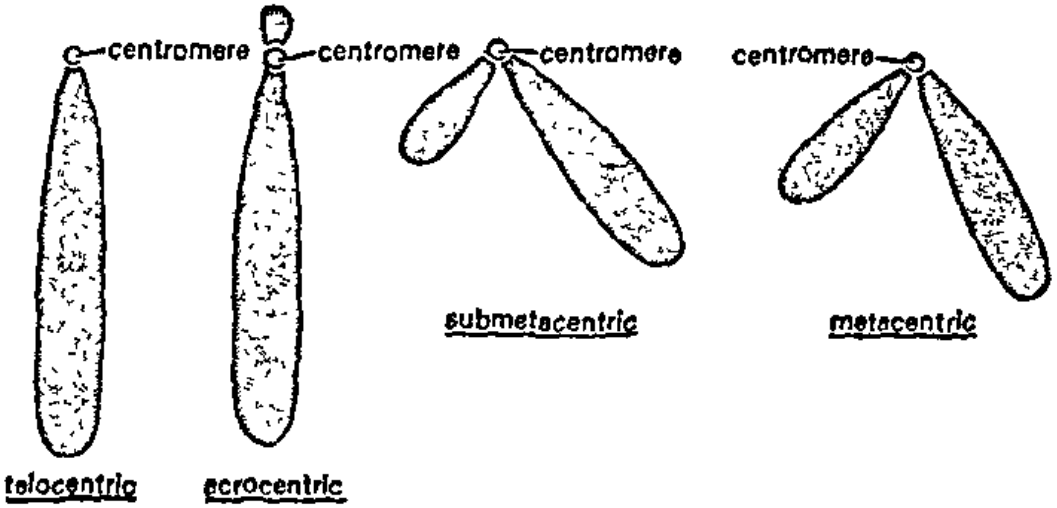


Fig. 16·1. The four morphological types of chromosomes according to the position of the centromere.

4. **Metacentric**—The metacentric chromosomes are V-shaped and in these chromosomes the centromere occurs in the centre and forming two equal arms. The amphibians have metacentric chromosomes.

KARYOTYPE AND IDIOGRAM

[illegible] identif[illegible], [illegible] karyotypes [illegible] species [illegible] idiogram.

STRUCTURE

According to classical cytology the chromosomes are composed of the chromonema and the centromeres.

CHROMONEMA

In less compact chromosomes, under the optical microscope, a coiled filament is seen in the chromosome. The coiled filament was called **chromonema by Vejdovsky in 1912.**

The chromonema may be composed of two, four or more fibres according to the species Further, the number of threads in the chromonema may depend on the different phases because at one phase the chromonema may contain one thread and at other phase it may contain three or four threads. The threads or fibrils of the chromonema remain coiled with each other. The coils may be of the following two types :

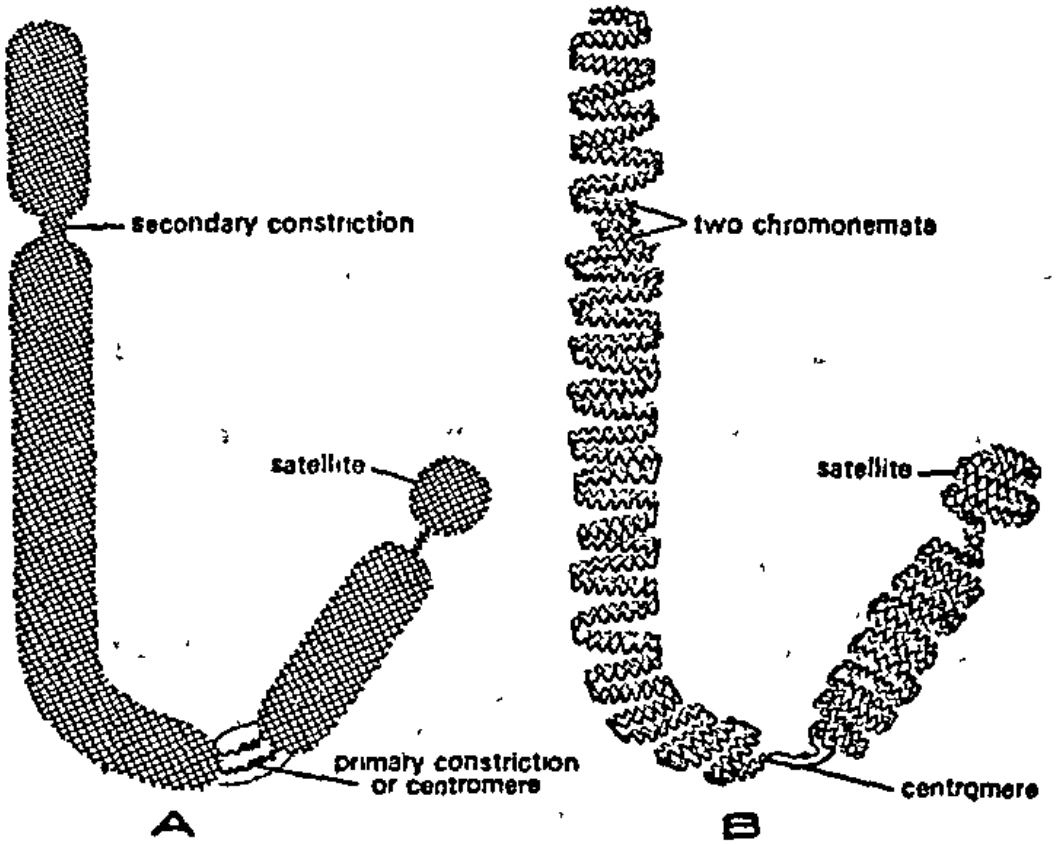


Fig. 16·2. Diagram of a metacentric chromosome. A—External structure. B—Internal structure.

...units or the chromonemal ... their coils then such coils ...

(ii) **Plectonemic coils**—When the chromosomal threads remain inter-twined so intimately that they can not be separated easily. This type of the coils are known as plectonemic coils.

The degree of the coiling in the chromonemal threads at mitosis and meiosis is variable and often depends on the length of the chromosmes. The chromosomes at the time of meiosis and mitosis contain three peculiar types of coils which are as follows :

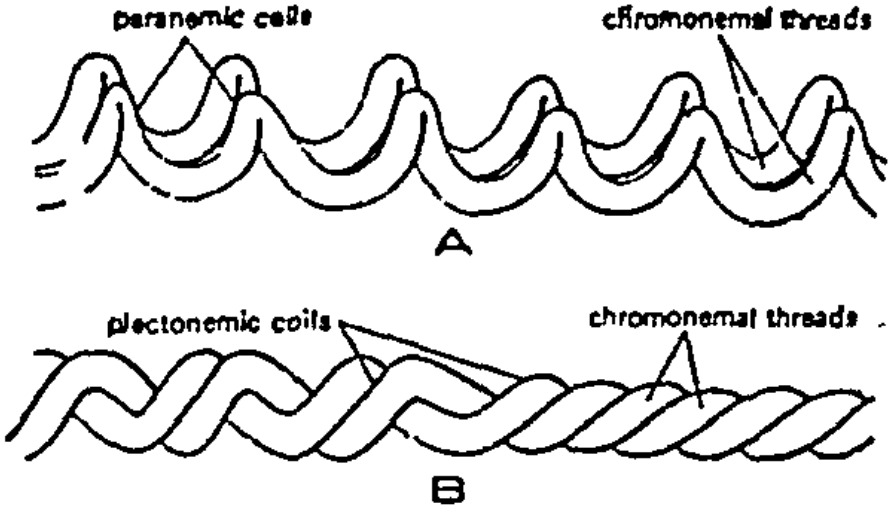


Fig. 16·3. Two types of coils of chromonemal threads.
A—Paranemic coils. B—Plectonemic coils.

1. **Major coils**—The major coils of the chromonema contain 10 to 30 gyres.

2. **Minor coils** –The minor coils of the chromonema lie perpendicularly to the major coils and contain many gyres in comparison to the major coils.

3. **Standard or somatic coils**—The chromonema of the mitosis has also been reported to contain helical structures which resemble with the major coils of the meiotic chomosomes. Such coils are known as standard or somatic coils.

CHROMOMERES

The chromonema of thin chromosomes of mitotic and meiotic prophases have been found to contain alternating thick and thin regions and thus giving the appearance of a necklace in which several beads occur on a string. The thick or bead-like structures of the

gion in
s. The
e cons-

tant for a given chromosome.

The cytologists have given various interpretations about the chromomeres. Some consider chromomeres as condensed nucleoprotein material, while other postulated that the chromomeres are regions of the super-imposed coils. The latter view has been confirmed by the electron microscopic observations. For long time most geneticists considered these chromomeres as genes, *i.e.*, the units of heredity.

The early concept that the chromosome consists of the limiting membrane (pellicle) and the amorphous matrix between the coils of the chromonema, now altogether has been rejected by the recent electron microscopic observations of the chromosomes.

CENTROMERE

The centromere consists of small granules or spherules. The centromere of the chromosome of the *Trillium* has the diameter of 3μ and the spherules have the diameter of 0·2μ. The chromonema remains connected with the spherules of the centromere.

The chromosomes of most organisms contain only one centromere and are known as **monocentric** chromosomes. Certain chromosomes may have two or more centromeres and can be termed as **dicentric** and **polycentric** chromosomes respectively. The chromosomes of the *Ascaris megalocephala* and hemipterans have diffused type of the centromere.

Secondary constriction—The chromosomes besides having the

nucleolar zone or nucleolar organizers. Usually in a nucleus only two chromosomes possess such nucleolar zone and therefore, known as the **nucleolar chromosomes.**

Telomere—(Gr. *telo*-for ; *meros*-part). Each extremity of the

erefore, it prevents other chromo-
The chromosomal ends are known

Satellite—Sometimes the chromosomes bear round elongated or knob-like appendages known as **satellites.** The satellite remains connected with the rest of the chromosome by a thin chromatin filament. The chromosomes with the satellite are designated as the **sat chromosomes.** The shape and size of the satellite and the chromatin filament remain constant.

MATERIAL OF THE CHROMOSOMES

The composing material of the chromosomes is the **chromatin** substance which may be of the following two types :

1.

chromatin

. In the inter-phase the

heterochromatin forms condensed chromosomal regions which are known as the **chromocenters** or **false nucleoli.** The heterochromatin remains closely associated with the nucleous. The heterochromatin is considered as genetically inert substance but it controls the metabolism of the chromosome, biosynthesis of the nucleic acids and the energy metabolism. The sex chromosomes of the animals have been found rich in the heterochromatin. During the mitosis, the heterochromatin if takes dark stain than the euchromatin, then it is known as the **positive heteropicknosis** and when it takes light stain than euchromatin then such heterochromatin is known as the **negative heteropicknosis.**

MOLECULAR COMPOUNDS OF CHROMOSOMES

The major molecular constituents of the eukaryotic chromosomes are DNA, RNA, *histone and non-histone proteins* and metallic ions. Most recent studies have established that the chromosomes consist of a single, double strande

transported to the cytoplasm but some RNA remain associated with DNA along with pro-

proteins and contain basic amino

their molecules. They remain associated with DNA and serve as repressors of gene activity.

etc., which make the organization of chromosomes intact.

ULTRASTRUCTURE OF CHROMOSOMES

There exists a lot of confusion and controversy regarding the exact nature of the chromosomes. However, electron microscopic studies have revealed that basically the chromosomes possess the fibrillar constitution. The inter-phase chromatin fibres consist of the fibril of 230 A°, while the metaphase chromosomal fibrils have the diameter of 300 A° (Dupraw, 1965, 66). In the chromosomes of the spermatocytes and ovocytes, the thin chromosomal fibrils have the diameter of 100A°. Two views have been expressed about the composition of the chromosomes clarifying that whether chromosomes are composed of many chromonemal fibrils or single chromonemal fibril. These views are as follows :

I. MULTISTRANDED VIEW

This view holds that the smallest unit of the chromosome is the 100 A° thick fibril. Each fibril consist of two DNA double helix molecules at the distance of 25 A°. Now a chromosome has two chromatids and each chromatid in its turn is composed of two half chromatids. A half chromatid consists of four fibrils and thus eight DNA

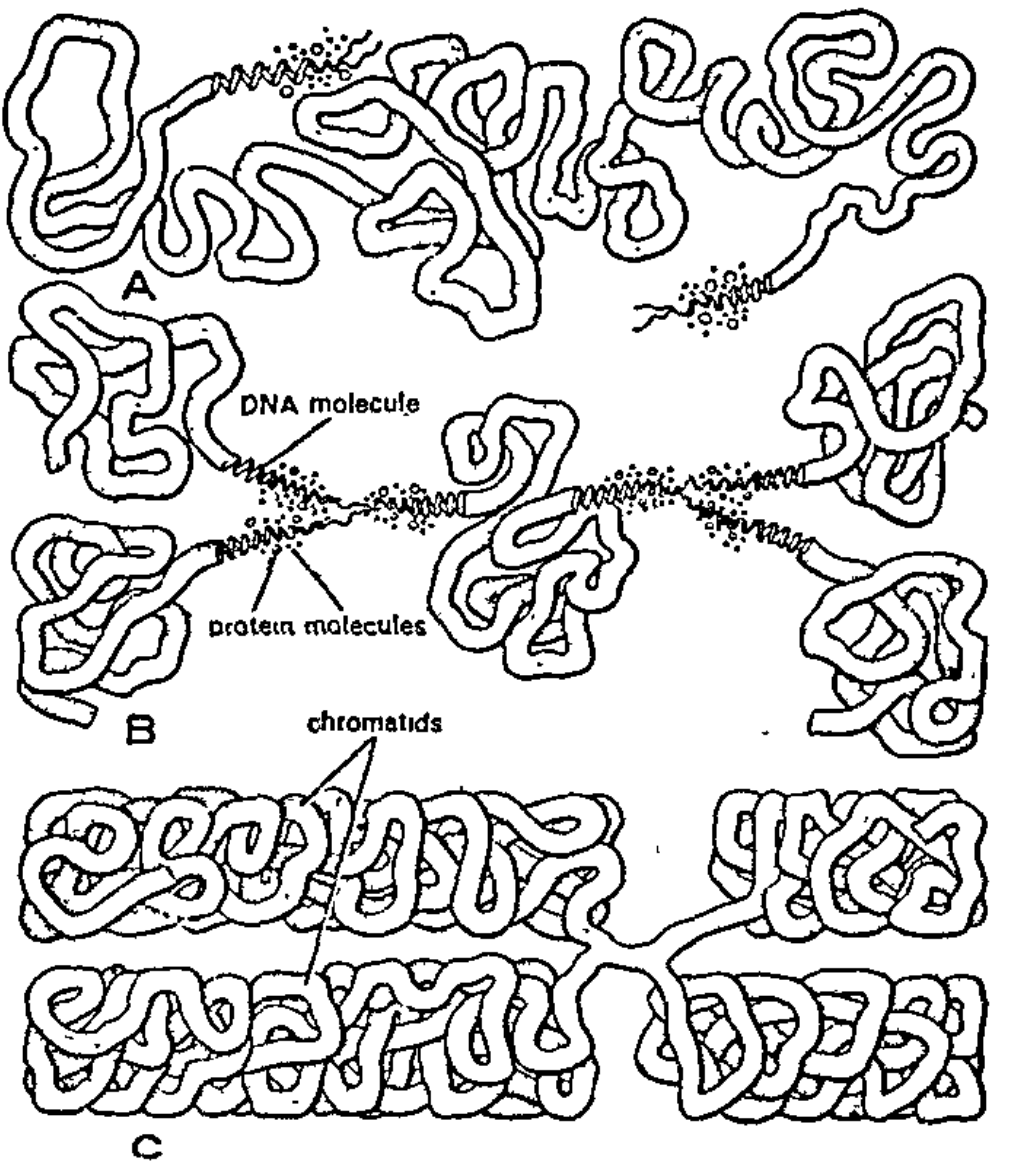


Fig. 16·4. Dupraw's folded fibre model of chromatin in interphase (A and B) and in metaphase (C).

double helix molecules. According to this view a chromosome of 1600 A° thickness consists of 16 chromonemal fibrils and 32 DNA double helix molecules. But this view could not be proved adequately by electron microscopic observations, therefore, it could get less support from the modern cytologists.

2. UNISTRANDED VIEW

The unistranded view holds that the chromosome is composed of a single microfibril which consists of a single DNA double helix molecule and nucleoproteins. Actually **Dupraw** (*1965*) has proposed a "**folded fibre model**" of the chromatid. He suggested that the DNA molecule is first wrapped in the nucleoproteins and then this DNA protein fibre is coiled tightly to form the 250 to 300 A° fibre. The DNA protein molecule of both the chromatids of the chromosome remain held at the centromere by a unreplicated fibre segment of the DNA until the anaphase. This model also suggested that the replication of the chromosome during DNA synthesis occurs by the uncoiling of the DNA molecule at certain point and the formation of new DNA polynucleotide chain.

The **folded fibre model** of the chromosome has been proved by various cytochemical analysis and electron microscopic observations, **Solari** (1965) has reported about 50 to 90μ long DNA molecule in the interphase chromosome of the sperm of sea urchin. Further, **Cairus** (1963) and **Sasaki** (1966) have calculated the length of the DNA molecule of the mammalian nuclei and found it 1 mm to 2·2 cm long. The controversy between multistranded view and unistrandid view is still unresolved (see **Ord** and **Stocken, 1973; Hall,** *et al.*, 1974).

ULTRASTRUCTURE OF THE CENTROMERE

Electron microscopic observations have shown that about seven chromatin fibres, each 230 A° in diameter, pass through the centromere (**Dupraw,** *1968*). The spherules of the centromere are found to act as an adhesive to join the microtubules of the spindle to the chromosomes. These spherules are trilaminar structures and four to seven microtubules of the mitotic or meiotic spindle get attached to each spherule during the cell division (**Jokelainen,** *1967*).

GIANT CHROMOSOMES

Some cells at certain particular stages contain large nuclei with giant or large-sized chromosomes. The giant chromosomes are the **polytene** and **lampbrush** chromosomes.

1. POLYTENE CHROMOSOMES

The polytene chromosomes occur in the tissues of the salivary glands, gut, trachea, fat body cells and Malpighian tubules of many insects of the order Diptera. The polytene chromosomes were first of all reported by **E. G. Balbiani** in 1881 and the name **polytene** was suggested by **Kollar** due to the occurrence of many chromonemae in them. Various cytologists such as **Carnoy, Alverdes, Costoff, Painter,**

Heitz, Baner and Bridges have investigated the cytogenetical importance of the polytene chromosomes.

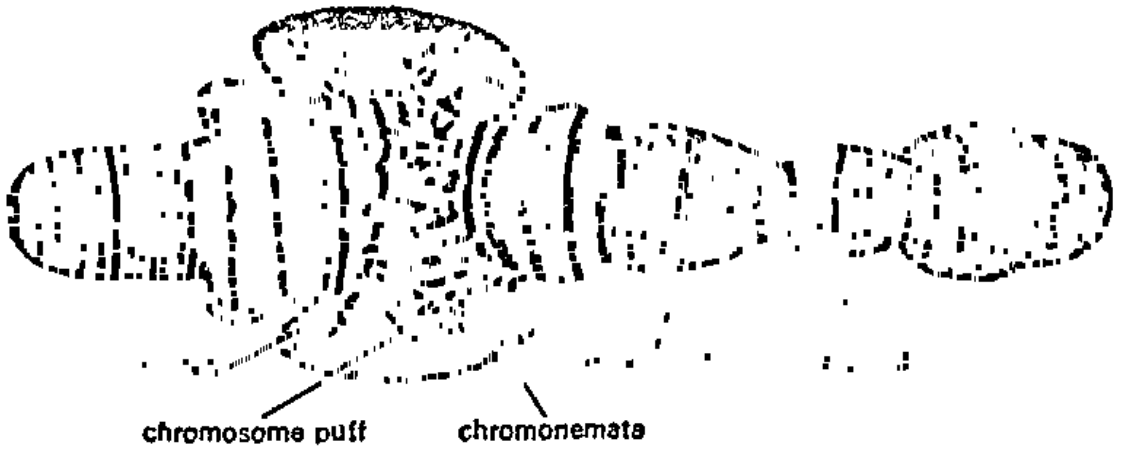


Fig. 16·5. Polytene chromosome of an insect.

The polytene chromosomes are larger in size in comparison to other somatic chromosomes. For instance, the four stranded polytene chromosomes of the *Drosophila melanogaster* have the total length about 200μ which is much larger than the length 7·5μ of a somatic chromosome. This enormous size of the polytene chromosome is achieved by the duplication of the chromonema for nine or ten times and the duplicated chromonemae are not separated. Moreover, the polytene chromosomes consist of closely coiled or associated homologous pairs of the chromosomes. This sort of association is known as the **somatic pairing**. The polytene chromosomes are the permanent prophase chromosomes which contain 1,000 times more DNA than the somatic chromosomes.

Bands and inter-bands of the polytene chromosomes : The polytene chromosomes bear alternating dark and light bands along their length. The cytogeneticists have attributed great significance to these bands The dark bands take deep stain by the basic chromosomal stains. These are disc-shaped structures and occupy the whole diameter of the chromosome. The dark bands may be narrow or broad. These are composed mainly of the euchromatin which chemically contains large amount of DNA, small amount of the RNA and certain basic proteins. The light bands or inter-bands take light chromosomal stains. These are fibrillar and composed of the heterochromatin which contains small amount of DNA, large amount of RNA and acidic proteins.

Polytene chromosomes and endomitosis : The multistranded or polytenic structure of the polytene chromosomes is caused by the process of the endomitosis. In endomitosis the chromonemae of the chromosomes become duplicated and the resulted chromonemae do

not separate to form new chromosomes but remain closely associated with each other. In the process of endomitosis, the nuclear membrane does not rupture and no spindle formation takes place.

Puffs and Balbiani rings of the polytene chromosome : During certain developmental stages the polytene chromosomes of the larvae of certain dipterous insects develop swellings at a particular point of the dark band or the inter-band. Such chromosomal swellings are known as the **chromosome puffs or bulbs.** The process of puff formation is cyclic and is controlled by certain specific genes. The puffs are related with the synthesis of RNA and proteins. **Edstrom** (1962) found that the chromosomal RNA differs from the nucleolar and cytoplasmic RNA and even the RNA of different puffs differ from each other in the chemical composition. The process of puffing involves several processes such as the accumulation of acidic proteins, despiralization of DNA, synthesis of mRNA and storage of newly synthesized mRNA in the chromosomes.

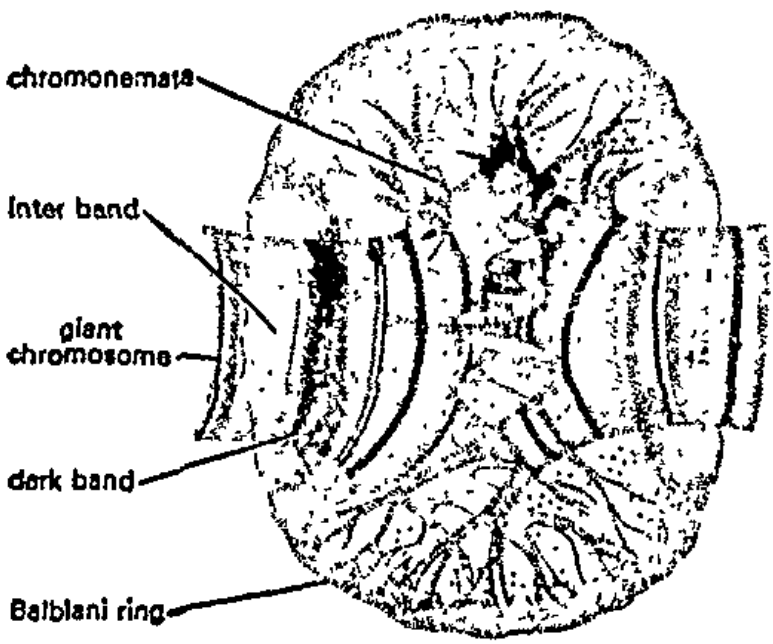


Fig. 16·6. A Balbiani ring of a polytene chromosome

The chromonema of the polytene chromosomes gives out many series of the loops laterally. These rings are known as the **Balbiani rings** and they are rich in DNA and mRNA. The formation and function of the Balbiani rings are similar to the chromosomal puffs.

2. LAMPBRUSH CHROMOSOMES

In diplotene stage of the meiosis, the yolk rich oocytes of many vertebrates such as fishes, amphibians, reptiles and birds contain exceptionally large-sized chromosomes known as the **lampbrush chromosomes.** The lampbrush chromosomes were discovered by **Ruckert** in 1892.

The lampbrush chromosomes are much larger than the polytene chromosomes. The length of the lampbrush chromosomes of certain urodele oocytes may reach upto 5900 μ or three times longer than the polytene chromosomes. The lampbrush chromosomes are composed of the **main axis** and the **lateral loops**. The main axis is composed of four chromatids or two bivalent chromosomes. The chromonema of these chromatids gives out fine loops at the lateral sides

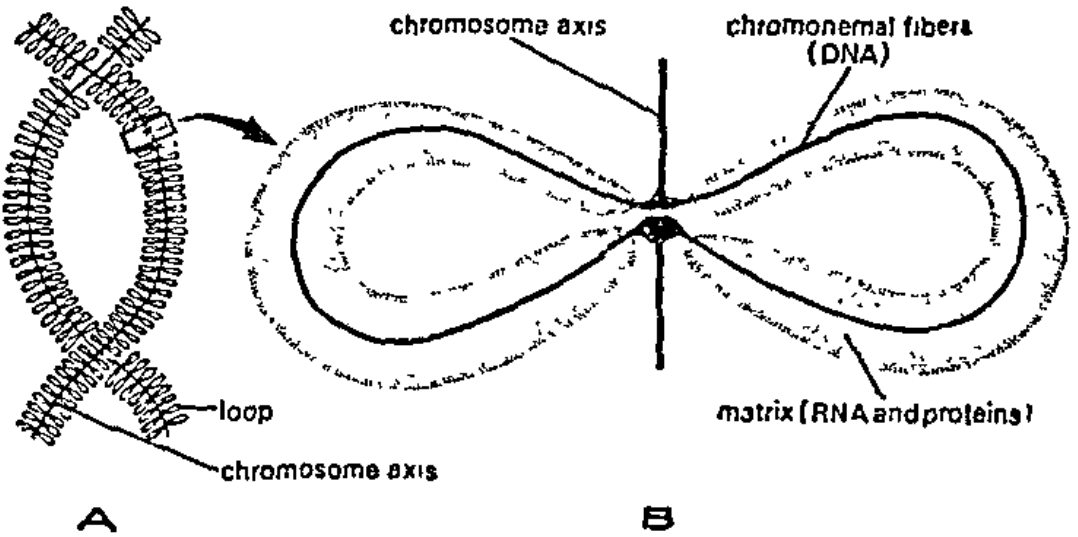


Fig. 16·7. Lambrush chromosome. A—At low magnification. B—loop magnified.

giving the appearance of lampbrush or test tube brush. Ris (1957) by electron microscopic observations of loops has revealed that they are composed of many submicroscopic fibrils. In method of loop formation, the lampbrush chromosomes resemble greatly with the polytene chromosomes.

The loops of lampbrush chromosomes are rich in RNA and proteins. The RNA appears to be mRNA. The synthesis of the proteins and the yolk takes place near the loop and when these synthesized materials are given off the loops are collapsed or reduced (Gall and Callan, 1963).

CYTOGENETICAL FUNCTIONS OF CHROMOSOMES

The chromosomes are the most significant components of the cell. They control most of the cell biological and genetical activities of a species. They contain the genetical material, the DNA which ultimately influe[illegible] physiologicaland [illegible] an attempt shall [illegible] logical and genetical role of chromosomes.

CHAPTER 17

CHROMOSOMAL VARIATIONS

The chromosomes of each species has a characteristic morphology (structure) and number. But, sometimes due to certain accidents or irregularities at the time of cell division, crossing over or fertilization some alterations in the morphology and number of chromosomes take place. The slightest variation in the organisation of chromosomes is manifested phenotypically and is of great genetical interest. We can study both types of variations separately under the following headings :

A. Variation in chromosome number.
B. Variation in chromosome morphology.

VARIATION IN CHROMOSOME NUMBER

Each species has a characteristic number of chromosomes in the nuclei of its gametes and somatic cells. The somatic chromosome number constitutes a [illegible] gamete cell contains si[illegible]loid gametes of both sexes (male and female) unite in the process of fertilization a diploid zygote with two genomes is formed. The diploid zygote undergoes embryological development and forms an adult animal which upon attaining sexual maturity produce haploid gametes. And this alternation of generation continues between haploidic and diploidic generation in most species. But, sometimes the size of haploidic or diploidic genome or number of chromosomes in a haploid or diploid cell does not remain constant (same) but alters variously. Variation in number of sets of chromosomes (ploidy) is commonly encountered in nature and may be of following two types :

1. Euploidy.
2. Aneuploidy.

1. EUPLOIDY

The term euploidy (Gr., *eu*-even or true; *ploid*-unit) designates genomes containing whole sets of chromosomes. The euploids are those organisms which contain balanced set or sets of chromosomes in any number. The euploids may be of following types :

(i) Monoploid.—The monoploid organisms have one set of chromosomes or one genome (n) in the nuclei of their body cells.

pteran insects such as wasps and bees.

Cytology of monoploids—In a monoploid or haploid set, the chromosomes are non-homologous and lack any homologue to pair-with, therefore, they are found as univalents at metaphase I of meiosis. During anaphase I, these univalents distribute at random. For instance, a haploid in maize (2n=20) will have 10 chromosomes and the number of chromosomes in a gamete can range from 0—10. Consequently, considerable sterility will be found in a monoploid maize. Further, some gametes will contain complete haploid set of chromosomes, and thus, will act in a normal fashion.

(ii) Triploid—The triploid organisms have three sets of chro-
body cells. A trip-
gamete (n) with a
s of the triploid is
distributed in various combinations to the germ cells, resulting in the genetically unbalanced gametes. Triploids are usually sterile, *e.g.*, some cells of birds, reptiles, man and other vertebrates (Ohno, 1963 Pennock 1965).

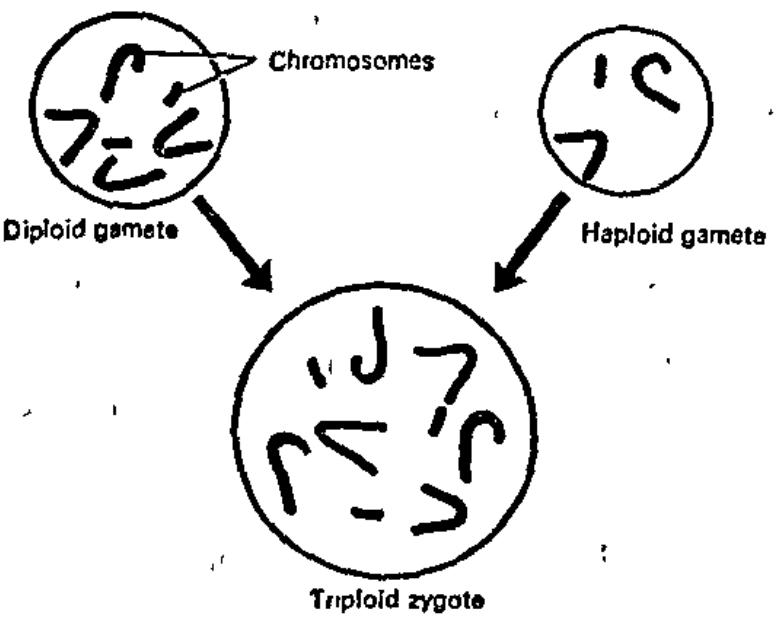


Fig 17 1. Diagram showing the origin of a triploid.

(iii) Tetraploid—The organisms with four genomes (4n) in the nuclei of their somatic cells are called tetraploids. The tetraploidy is arised by somatic doubling of the chromosome number. Doubling is accomplished either spontaneously or it can be induced in high frequency by exposure to chemicals such as colchicine, acenaphthene and veratrine and also by exposure to heat or cold, *e g.*, wheat, *Chrysanthemum*, *Solanum*, man (Carr, 1967), salamander, etc.

(iv) Polyploid—Any organism with more than two genomes (2n) is called a polyploid. Ploidy levels higher than tetraploid are not commonly encountered in natural populations, but our most important crops and ornamental flowers are polyploids, *e.g.*, wheat (Hexaploid, 6n), strawberries (octaploid, 8n), many commercial fruits and ornamental plants, liver cells of man, etc.

Types of polyploids—The polyploids may be of following two types :

(a) Autopolyploids—The autopolyploids are those polyploids, which consist of same basic set of chromosomes multiplied. For example, if a diploid species has two similar sets of chromosomes or genomes (AA), an autotriploid will have three similar genomes (AAA), and an autotetraploid, will have four such genomes (AAAA). Since an autotriploid remains sterile and cannot produce seeds, therefore, it (autopolyploidy) has great commercial value in producing seedless varieties of economic plants. For example, in Japan, **H. Kihara** produced seedless watermelons, which were autotriploids, common 'doob' grass of U. P. and Bihar is an autotriploid. Other seedless autopolyploids are grapes, sugerbeet, banana, etc. Whenever autopolyploids, orginate in nature, these would be eliminated due to natural selection. In *O. lamarckiana*, the giant mutant described by **de Vries** was later discovered as an autotetraploid.

Induced autopolyploidy—The autopolyploidy have been induced in many plant and animal cells by artificial means such as chemical (*e.g.*, chloral hydrate, cholchine, sulfanil amide, mercury chloride, hexachlorcyclohexane, etc.), radioactive substances, *e.g.*, radium and X-ray) and temperature shocks. These inducers usually disturb the mitotic or meiotic spindle and cause non-segregation of already duplicated chromosomes, during cell divisions.

(a) Allopolyploids—When the polyploidy results due the doubling of chromosome number in a F_1 hybrid which is derived from two

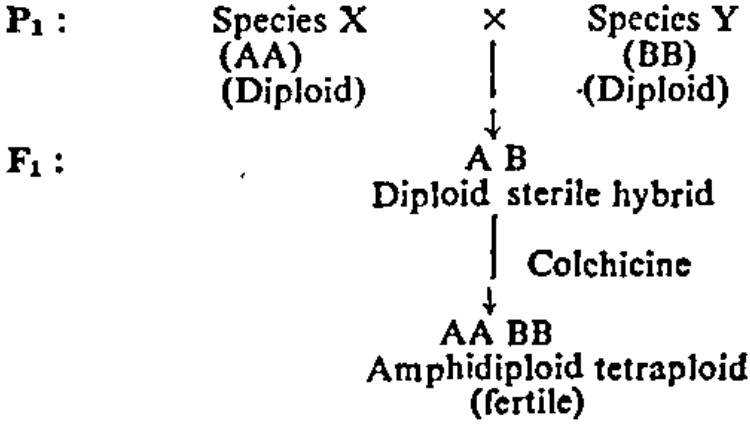


Fig. 17 2. Formation of a autotetraploid or amphidiploid tetraploid.

distinctly different species, then, it is called **allopolyploidy** and the resultant species is called an allopolyploid. Let A represent a set of chromosomes (genome) in species X, and let B represent another genome in a species Y. The F_1 hybrids of these species then would have one A genome and another B genome. The doubling of chromosomes in the F_1 hybrids will give rise to allotetraploids with two A and two B genomes (see fig. 17·2).

Raphanobrassica is a classical example of allopolyploidy or amphipolyploidy. In 1927, a Russian geneticist, **G. D. Karpechenko** performed a cross between radish (*Raphanus sativum*, 2n=18) and cabbage (*Brassica oleracea*, 2n=18) and in F_1 got sterile (diploid) hybrids. Among these sterile F_1 hybrids, he found certain fertile plants which were found to contain 36 chromosomes. These fertile tetraploids were called *Raphanobrassica.*

SYNTHESIZED ALLOPOLYPLOIDS

To find out the origin of naturally occurring allopolyploids some cytogeneticists produced certain allopolyploids in laboratory by employing artificial means. Common hexaploid wheat and tetraploid cotton furnish two such examples.

(i) *Triticum spelta*, is a hexaploid wheat which was artificially synthesized in 1946 by **E. S. McFadden** and **E. R. Sears** and also by **H. Kihara.** They crossed an emmer wheat, *Triticum dicoccoides*, (diploid ; hybrids as found to be similar to the primitive wheat *T. spelta.* When the synthesized hexaploid wheat was crossed with naturally occurring *T. spelta*, the F_1 hybrid was completely fertile. This suggested that hexaploid wheat

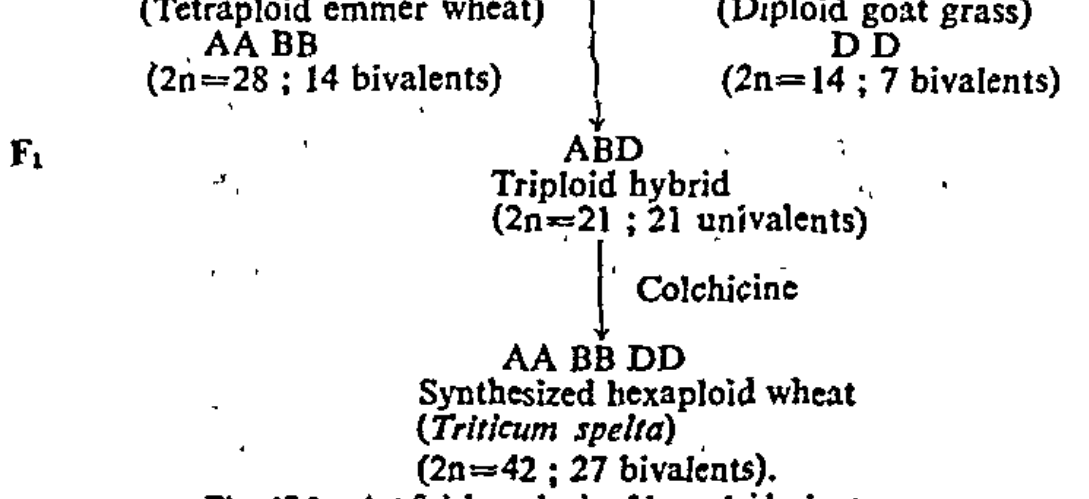


Fig. 17·3. Artificial synthesis of hexaploid wheat.

must have originated in the past due to natural hybridization between tetraploid wheat and goat grass followed by subsequent chromosome doubling.

(ii) *Gossypium hirsutum*, the New world cotton plant, is another interesting example of allopolyploidy. Old world cotton, *Gossypium herbaceum*, has 13 pairs of chromosomes, while, American or "upland cotton" also contains 13 pairs of chromosomes. J. O. Beasley crossed the old world and American cottons and doubled the chromosome number in the F_1 hybrids. The allopolyploids thus produced resembled the cultivated New world cotton and when crossed with it gave fertile F_1 hybrids (Fig. 17·4). These results, thus, suggested that tetraploid *Gossypium hirsutum* originated from two diploid species, namely *G. herbaceum* (2n=26) and *G. raimondii* (2n=26).

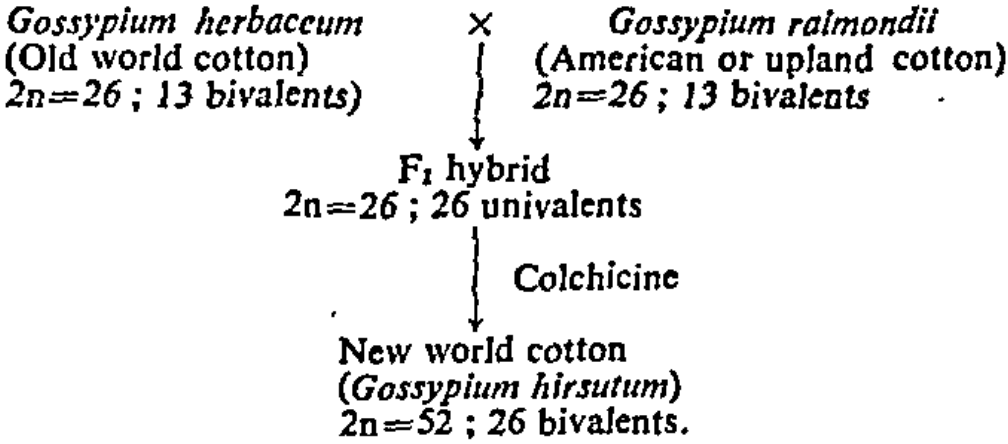


Fig. 17·4. Artificial synthesis of New world cotton.

PHENOTYPIC CONSEQUENCES OF POLYPLOIDY

The increase in the genome's size beyond the diploid level is often caused following detectable phenotypic characteristics in a polyploid organism :

(i) Morphological effect of polyploidy—The polyploidy is invariably related with **gigantism**. The polyploid plants have been found to contain large-sized pollen grains, cells, leaves, stomata, xylem, etc. The polyploid plants are more vigorous than diploids.

(ii) Physiological effect of polyploidy—The ascorbic acid content has been reported to be higher in tetraploid cabbages and tomatoes than in corresponding diploids. Likewise corn meal of a tetraploid maize seed contain 40 per cent more vitamin A than cornmeal from a diploid plant.

(iii) Effect on fertility of polyploidy—The most important effect of polyploidy is that it reduces the fertility of polyploid plants in variable degrees.

Polyploidy and speciation—Polyploidy has its great evolutio-

nary significance, because it is a mean of origin of new species, especially in plants. The polyploid plants have more morphological, genetical and physiological advancements over a normal diploid plant and a polyploid plant often faces the ecological hazards more boldly. These variations subsequently lead the polyploid plant toward speciation (formation of new species).

2. ANEUPLOIDY

Variation in chromosome number may occur which do not involve whole sets of chromosome, but only parts of a set. The term **aneuploid** (Gr., *aneu*-uneven; *ploid*-unit) is given to variations of this nature, and the suffix "—somic" is a part of their nomenclature. The aneuploid may be of following types :

(i) Monosomic—Diploid organisms which are missing one chromosome of a single pair are monosomic with the genomic formula 2n—1. A monosomic individual forms gametes of two types, (n) and (n—1). The n—1 gametes do not survive in plants, but, in animals that may cause genetic imbalance, which is manifested by *high mortality or reduced fertility of resulted organism.*

(ii) Nullosomic—An organism which has lost a chromosome pair is a nullosomic. The nullosomic organism has the genomic formula (2n—2). A nullosomic diploid often does not survive, however, a nullosomic polyploid (*e.g.*, hexaploid wheat, 6X—2) may survive but exhibit reduced vigour and fertility.

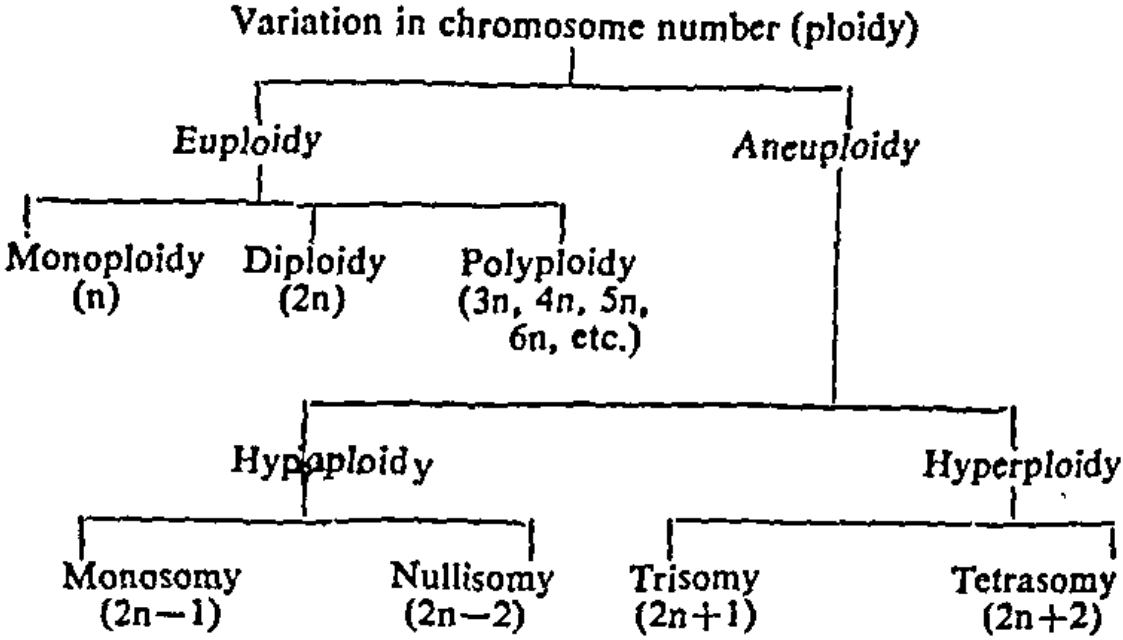
(iii) Trisomic—Diploids which have one extra chromosome are represented by the chromosomal formula 2n+1. One of the pairs of chromosomes has an extra number, so that a trivalent structure may be formed during meiotic prophase. If two chromosomes of the trivalent go to one pole and the third goes to the opposite pole, then gametes will be (n+1) and (n) respectively. Trisomy can produce different phenotypes, depending upon which chromosome of the complement is present in the triplicate. In man, trisomy has very deleterious effects resulting in **Down's syndrome**, formerly called **mongolism**. A mongole person is mentally retarded, short in stature, exhibits characteristic abnormalities of palm prints and certain other malformations, especially of heart, ear, hands and feet. In them sexual maturity and fertility are rarely attained.

(iv) Double trisomic—In a diploid organism when two different chromosomes are represented in triplicate, the double trisomic is resulted. The double trisomic causes great genetic imbalance and has the genomic formula 2n+1+1.

(v) Tetrasomic—The diploid organisms having two extra chro-

mosomes are known as tetrasomic. They have the genomic formula 2n+2. All the 21 possible tetrasomics are available in wheat.

Different types of ploidies can be summarized in following chart :



VARIATION IN CHROMOSOME MORPHOLOGY OR CHROMOSOMAL ABERRATIONS

Chromosomes are the structures with definite organization. They carry genes which remain arranged in them in linear order. In ordinary conditions, though the chromosomes remain unchangeable, but, under certain natural or artificial conditions some structural changes may occur in them. These structural changes are collectively called **chromosomal aberrations.**

Most chromosomal aberrations are caused due to accidental, natural, or induced breakage of chromosomes. The induced chromosome breakage may be caused by radiation, and chemicals such as Lysergic acid diethylamide (LSD), etc. The chromosomal aberrations may remain confined to a single chromosome, or may extend to both of the members of a pair or may involve two or more pairs of chromosomes. The chromosomal aberrations, may be of following two kinds :

1. Intrachromosomal aberrations.
2. Interchromosomal aberrations.

1. INTRACHROMOSOMAL ABERRATIONS

The intrachromosomal or homosomal aberrations occur in one and the same chromosome and are of following kinds :

(i) **Deficiency** or **deletion**—When a chromosome lacks in a **intercalary** or **terminal** segment, the deficiency or deletion occurs.

The lost chromosomal segment, if lacks in a centromere, lag in anaphasic movement and is lost from reorganizing nuclei. A loss of any considerable portion of chromosome is usually lethal to a diploid organism because of genetic imbalance, *e.g.*, maize, *Drosophila*, man, etc.

(ii) Duplication—Duplication occurs when a segment of the chromosome is represented two or more times in the chromosomes. The extra segment may be attached to the chromosome whose loci are repeated or to a different linkage group, or even be present as a separate fragment. The duplications of chromosomes are not deleterious to the organism like the deficiency and they usually protect the organism from the effect of a deleterious recessive gene or from otherwise lethal deletions. Moreover, the duplication is useful in the evolution of new genetic material and in altering the phenotype due to re-allocation of chromosomal material (position effect), *e.g.*, *Drosophila*, etc.

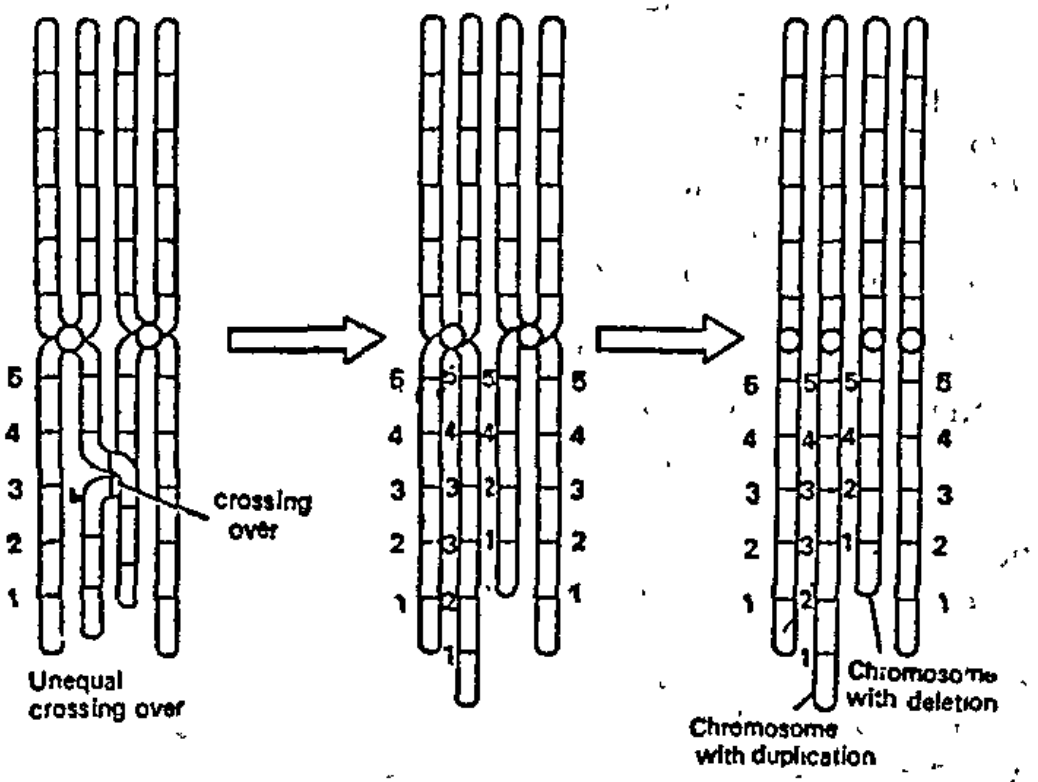


Fig. 17·5. Production of deletion and duplication in chromosomes due to unequal crossing over.

(iii) Inversion—An inversion is a chromosomal aberration in which a segment is inverted at 180 degree. Inversions are called pericentric when the segment includes the centromere and paracentric the centromere is located outside the segment. When a crossing

occurs within the inverted segment of a paracentric inversion, dicentric and acentric chromatids are formed. The dicentric chromatids form a bridge that breaks when the anaphase chromosomes separate towards the poles. If crossing over occurs within the loop of a pericentric inversion, the chromatids are produced with a deficiency and a duplication.

Isochromosomes : An isochromosome is a chromosome in which both arms are identical. It is thought to arise when a centromere divides in the wrong plane, yielding two daughter chromosomes, each of which carries the information of one arm only but present twice.

2. INTERCHROMOSOMAL ABERRATIONS

When breaks occur in non-homologous chromosomes and resulting fragments are interchanged by both of the non-homologous chromosomes, the interchromosomal aberration occurs. The inter-chromosomal aberrations include following one type :

Translocation : Translocation involves the shifting of a part of one chromosome to another, non-homologous chromosome. If two non-homologous chromosomes exchange parts, which need not be of same size, the result is a **reciprocal translocation.** The reciprocal translocation may be of following types :

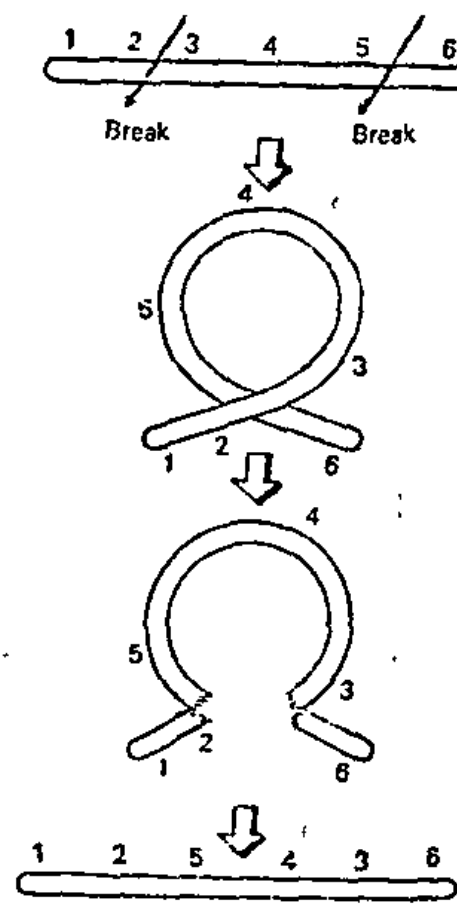


Fig. 17·6. Origin of an inversion in a chromosome.

(i) Homozygotic translocation : In homozygotic translocation normal meiosis occurs and that cannot be detected cytologically. Genetically they are marked by altered linkage groups and by the fact that a gene with "new neighbours" may produce a somewhat different effect in its new location.

(ii) Heterozygotic translocation: In heterozygotic translocation a considerable degree of meiotic irregularity occurs. Typically a cross-shaped formation is seen in prophase I; this often opens out into a ring as chiasmata terminalize. The heterozygous translocation

produces semisterile organisms because between half and two third of gametes (in animals) or meiospores (in plants) fail to receive the full

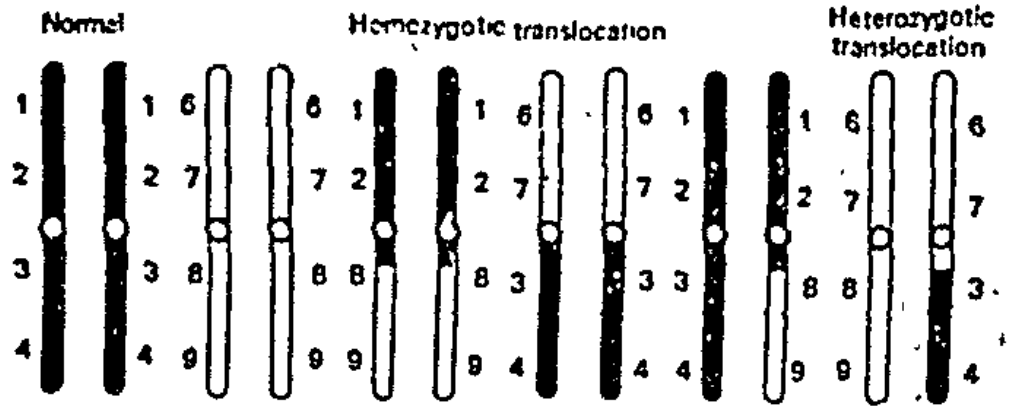


Fig. 17·7. Schematic representation of homozygotic and heterozygotic reciprocal translocations compared with the normal arrangement.

complements of genes required for normal development of sex. The heterozygotic translocation is common in maize plant.

CHAPTER 18

CELL DIVISION

The growth and development of every organism depend exclusively on the multiplication and enlargement of its cells. The development of a multicellular organism from the unicellular zygote is achieved by the cell division, growth and differentiation. The asexual and sexual reproduction of the organisms also depend on the cell division.

The division of the nucleate cells is achieved by two integral activities such as the division of the nucleus (**karyokinesis**) and the division of the cytoplasm (**cytokinesis**). Usually the karyokinesis is followed by the cytokinesis but sometimes the cytokinesis does not follow the karyokinesis and results into the multinucleate cells.

In animals and plants following three types of cell division have been distinguished :

1. Direct cell division or amitosis.
2. Indirect cell division or mitosis.
3. Reduction division or meiosis.

AMITOSIS

The amitosis or direct cell division is the means of asexual repro-

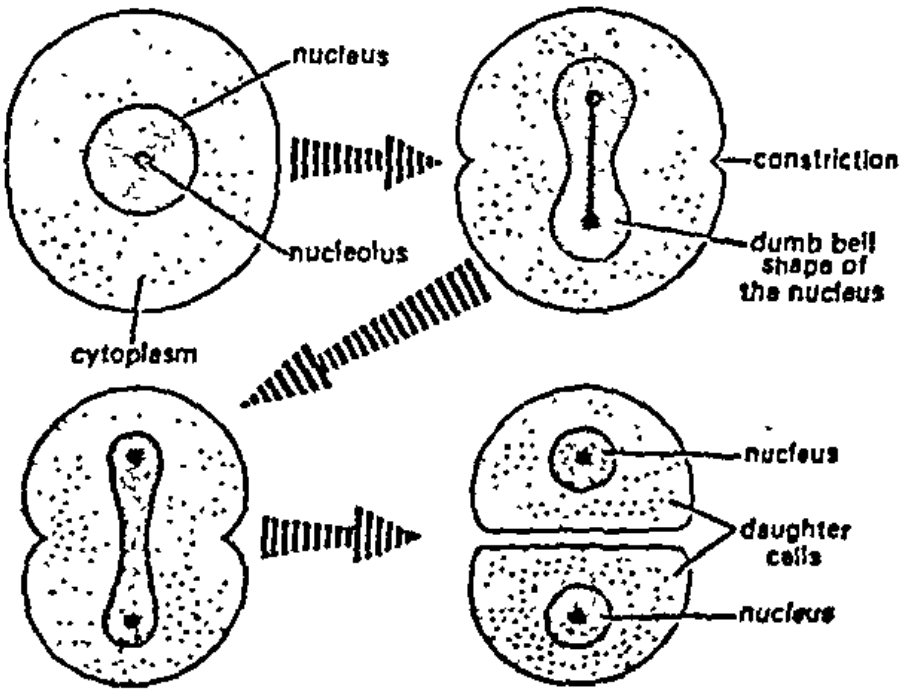


Fig. 18·1. Diagrammatic representation of amitosis.

duction in unicellular organisms like bacteria and protozoans and also a method of multiplication or growth in foetal membranes of some vertebrates. In amitosis type of cell division the splitting of nucleus is followed by cytoplasmic constriction.

During amitosis the nucleus elongates first and then assumes dumb-bell-shaped appearance. The depression or constriction increases in size and ultimately divides the nucleus into two nuclei. The division of nucleus is followed by the constriction of cytoplasm which divides the cell into two equal or approximately similar halves. Therefore, without the occurrence of any nuclear event two daughter cells are formed.

MITOSIS AND CELL CYCLE

The mitosis (Gr., *mitos*-thread) occurs in the somatic cells and it is meant for the multiplication of cell number during embryogenesis and blastogenesis of plants and animals. Fundamentally, it remains related with the growth of an individual from zygote to adult stage.

One of the basic characteristic of mitotic cell division which is meant for growth due to multiplication is that it gives rise to two daughter cells, which resembles each other and also the parent cell qualitatively and quantitatively. The basic outline of mitosis remains the same in all living organisms. It includes four phases namely G_1 phase, S phase, G_2 phase and mitotic phase which occur in succession and forming the so called **cell-cycle**. The G_1 phase, S phase and G_2 phase are collectively forming the interphase. Thus, in continuously dividing cells, an individual cell passes through following two main phases of cell or mitotic cycle :

A. Interphase
B. Mitotic phase

A. INTERPHASE

The resting phase or stage between the two mitotic divisions is called **intermitotic phase** or **interphase** (*L.. inter*-between, *Gr., phasis*-appearance). In interphase no division of chromosomes or cytoplasm occurs, but the nucleus and cytoplasm remain metabolically active and due to that an increase in volume of nuclear as well as cytoplasmic substances takes place. The interphase is the longest phase of the mitotic cycle, taking one or two days in its completion and including following three sub-phases : 1. G_1 phase ; 2. S phase and 3. G_2 phase. **G_1 phase** includes the synthesis and organization of the substrate and enzyme necessary for DNA synthesis. Therefore, G_1 is marked by the transcription of rRNA, tRNA, mRNA and synthesis of different types of proteins. During S phase synthesis (replica-

tion and duplication) of chromosomal DNA molecules takes place. G_2 phase is the post-DNA synthesis phase during which all the metabolic activities concerning the growth of cytoplasm and of its constituent organelles and macromolecules are performed. Thus, during interphase following events occur :

1. The nuclear envelope remains intact.
2. The chromosomes occur in the form of diffused, long, coiled and indistinctly visible chromatin fibres.
3. The DNA amount becomes double.
4. Due to accumulation of ribosomal RNA (rRNA) and ribosomal proteins in the nucleolus, the size of the latter is greatly increased.

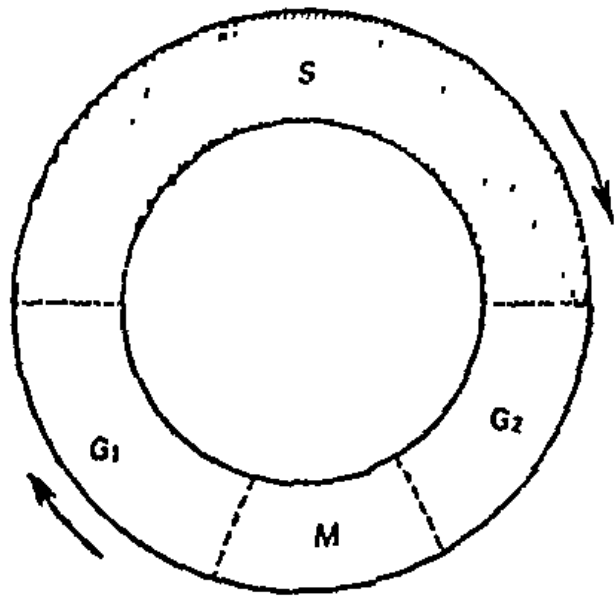


Fig. 18·2. The cell cycle or mitotic cycle, showing relative duration of phases (*e.g*, interphase and mitotic phase) in a growing cell. S, synthesis of DNA ; G_1 the pre-DNA-synthesis phase ; G_2, post DNA-synthesis phase ; and M, the period occupied by mitotic phase.

5. A daughter centriole is originatid near the already existing centriole and thus, a interphase cell has two centrioles or a diplosome. (Fulton, 1971).

B. MITOTIC PHASE

The main mitotic cell division occurs during mitotic phase or M phase, which includes following phases :

PROPHASE

The prophase (Gr., *pro*-before;*phasis*-appearance) is the actual first phase of the mitosis. During the prophase following events take place:

1. The cell becomes spheroid, refractive and viscous.
2. The disintegration of nuclear envelope starts.
3. Due to the DNA duplication in the interphase each chromosome now possesses two chromatids. Each chromatid consists of a single DNA molecule wrapped in the nucleoproteins.
4. Both chromatids remain connected with each other by the centromere and both remain closely associated along their entire lengths.

5. The chromatids become shortened and thickened.
6. The nucleolus starts to disappear.
7. Each centriole separates and migrates towards the opposite poles of the cell. Each centriole duplicates, so that both poles of the cell contain paired centrioles or a diplosome.
8. The centrosome forms an elongated body or bridge known as the **centrodesmus** in between the two centrioles.
9. From the centriodesmus delicate filaments (microtubules) or asters arise and form the spindle.

PROMETAPHASE

In prometaphase (Gr., *pro*-before, *meta*-after; *phasis*-appearance) following changes usually occur :

1. The nuclear membrane completely disintegrates.
2. A clear zone known as the equator appears in between the mid-line of the spindle and the nucleus.
3. The chromosomes move towards the equator.

METAPHASE

The metaphase (Gr., *meta-after; phasis*-appearance) follows the prometaphase and during this phase following events occur in the cell :

1. Each chromosome reaches to the equator and all chromosomes arrange themselves radially at the periphery of the spindle.
2. The smaller chromosomes usually remain towards the interior, while the larger chromosomes remain at the periphery.
3. Some of the fibres or microtubules of the spindle attach

o-

in

re

known as **continuous fibres.**

5. Certain fibres occur in between the chromosomes and are known as ***interzonal fibres*** or ***interchromosomal fibres.***

ANAPHASE

In the anaphase (Gr., *ana*-up ; *phasis*-appearance) following changes occur inthe cell :

1. The centromere of each chromosome divides into two.
2. The chromatids of the each chromosome are separated and

and thicker and migrate

hromosomes towards the opposite poles is achieved by the contraction of chromosomal fibres

and the stretching of interchromosomal or interzonal fibres. The *interzonal fibres push the daughter chromosomes towards the opposite poles.*

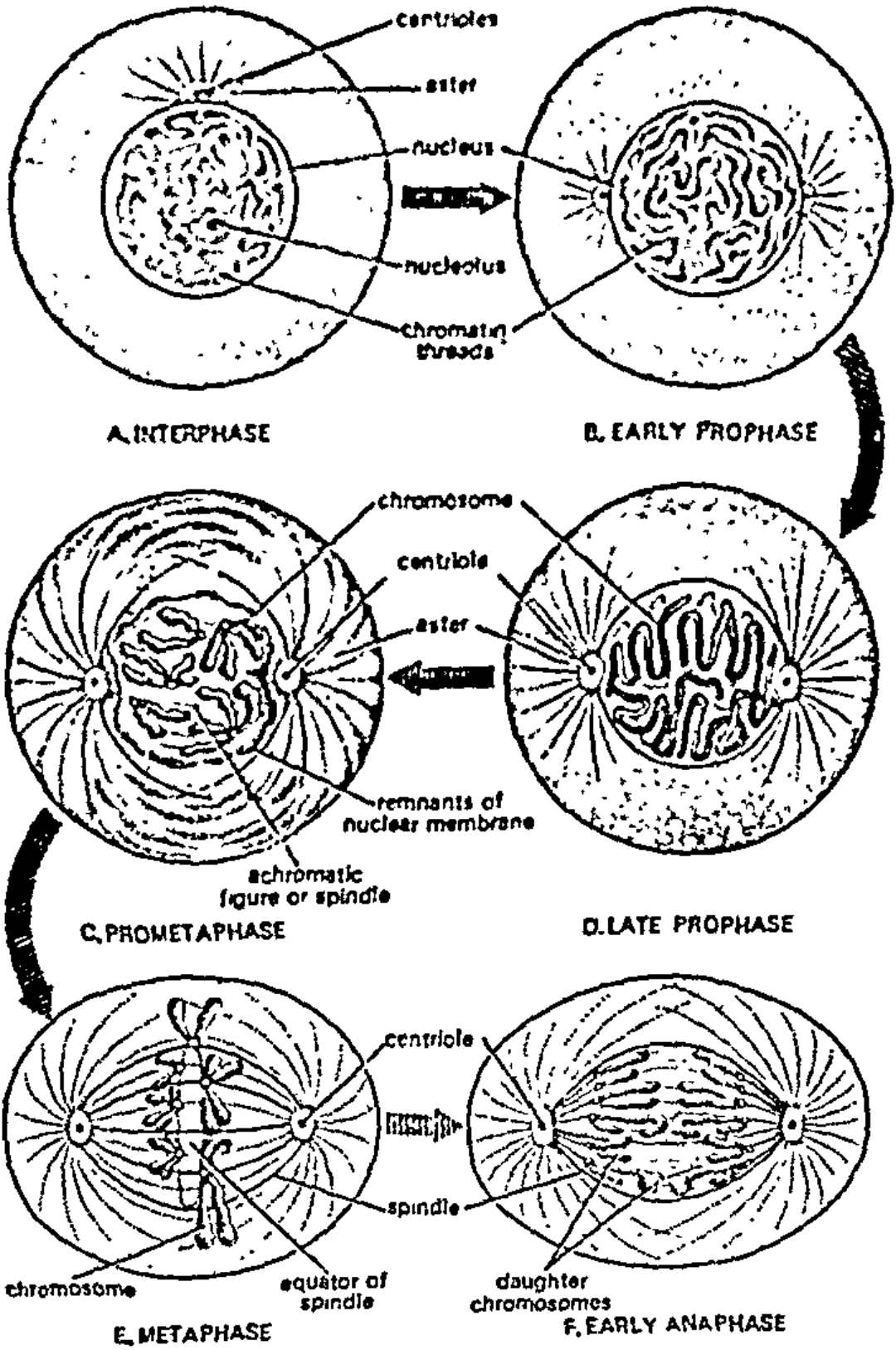


Fig. 18 3. Some early stages of mitosis.

TELOPHASE

The telophase is the final stage of mitosis and during this phase following events occur :

1. The chomosomes which reach at the opposite poles of the cell now elongate, the coils of DNA protein fibres loosen and the chromosomes become thread-like.

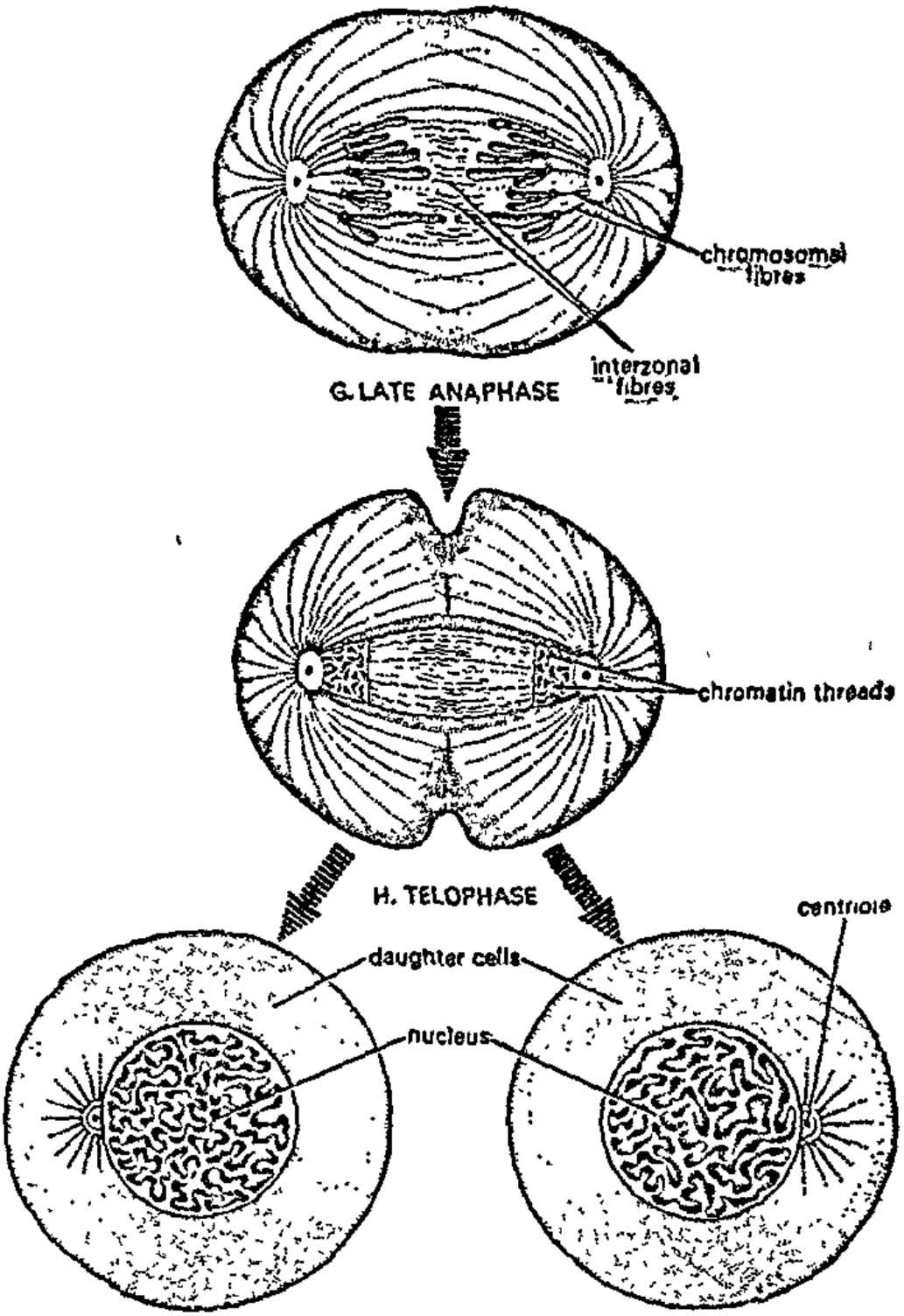


Fig 18 4. Diagram of late stages of mitosis.

2. The nucleolus reappears.

3. The endoplasmic reticulum forms the new nuclear envelope around the chromosomes and the nucleolus.

4. The microtubules of the aster and mitotic spindle rearrange and disappear.

Thus, after the telophase two daughter nuclei are formed due to the karyokinesis. The karyokinesis is followed by the cytokinesis.

CYTOKINESIS

In the process of cytokinesis the cytoplasm splits from the equatorial region and the two daughter halves of the cytoplasm are separated. Soon after a unit membrane of lipoprotein develops in between the two daughter cells. The cytokinesis of animal cells involves the cyclosis of the cytoplasm, formation of a contractile ring, the expansion of the plasma membrane, ATP and interaction of the spindle and asters with the cell surface, while in the plant cells the cytokinesis involves the movement of the endoplasmic reticulum and dicytosomes to the equator where they fuse to form the primary cell wall.

SIGNIFICANCE OF MITOSIS

The importance of the mitosis for the organisms has been summarized in the following points :

1. The mitosis helps the cell in maintaining its proper size.

2. Through the process of the mitosis an equilibrium is maintained in the amount of DNA and RNA contents.

3. The mitosis provides the opportunity for the growth and bevelopment to organs and the body of organisms.

4. Due to the mitosis the old, decaying and dead cells are replaced by the new cells.

5. The mitosis helps the organisms in the asexual reproduction.

6. The gonads and the sex cells also depend on the mitosis for the increase in their number.

MEIOSIS AND REPRODUCTIVE CYCLE

The term meiosis (Gr., *meioum*-to reduce) was coined by J. B. Farmer in 1905. The mitotic division is of utmost importance for those organisms in which the union of the haploid gametes takes place during the sexual reproduction. By reducing the number of chromosomes of the diploid germ cells into the haploid gametes the meiosis m
species. T
and diplc
meiosis maintains the regularity of reproductive cycle of sexually reproducing micro-organisms (*e.g., Chlamydomonas, Neurospora,* etc.)

and macro-organisms (*e.g.*, Bryophytes and higher plants and animals.) For instance, the reproductive cycle of *Chlamydomonas* includes a long haploid generation and a short diploid generation which involve the zygote formation. The zygote undergoes reduction division resulting in the formation of haploid spores.

In the process of meiosis the chromosomes divide once and the ... the meiosis four haploid ...

The process of meiosis is fundamentally same in all the animals and plants but certain biologists recognised following three types of meiotic divisions according to their occurrence at different stages of the life cycle of the organisms.

1. **Sporogenetic meiosis**—The meiosis occurring at the time of spore formation is referred as the sporogenetic meiosis. This type of meiosis is commonly found in plants.

2. **Gametic meiosis**—In most animals and lower plants the meiosis occur at the time of gametogenesis (spermatogenesis and oogenesis) and is known as gametic meiosis.

3. **Zygotic meiosis**—In certain lower plants, sometimes the meiosis occurs immediately after the fertilization of the egg by a sperm. This type of meiosis is known as zygotic meiosis.

Meiocytes—The cells in which the meiosis takes place are known as **meiocytes**. The meiocytes of the testis or male gonads are known as the **spermatocytes** and the meiocytes of the ovaries or female gonads are termed as **oocytes**. The meiocytes of the plant sporangium are known as **sporocytes**.

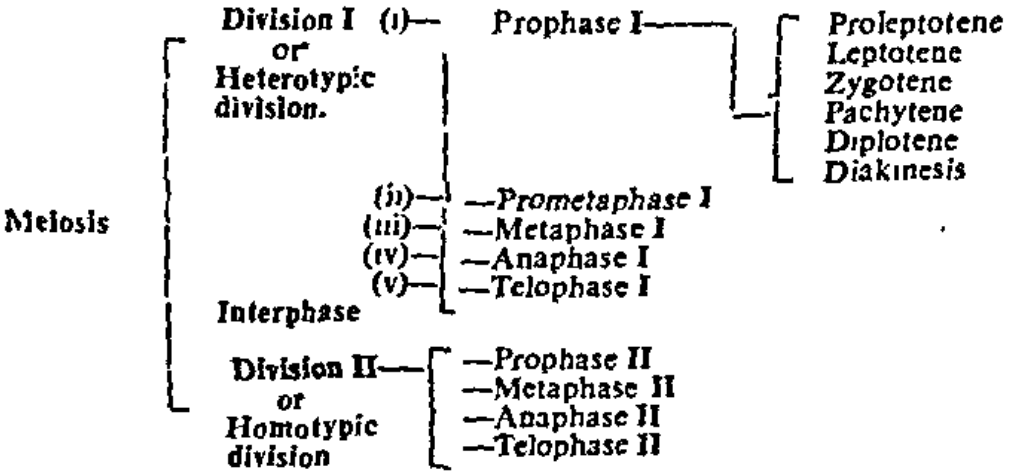
Chemical substances initiating meiosis—The process of meiosis seems to depend on the balance of nucleic acids in the nucleus or on certain hormones. For instance, insect moulting hormone, the ecdysone is found to initiate the meiosis in certain parasitic flagellate protozoans. Further, when in the cell the amount of the DNA material is increased in comparison to the RNA, meiosis is found to occur in the cell. However, till now no information is available about the exact chemical substances which may initiate the meiosis.

PROCESS OF MEIOSIS

The meiotic division includes two complete divisions of a diploid cell resulting into four haploid cells. The meiotic first division includes a long prophase in which the homologous chromosomes become closely associated to each other and interchange of hereditary material takes place between them. Further, in the first meiotic division the reduction of chromosome number takes place and thus two haploid cells are resulted by this division. The first meiotic division is

also known as the **heterotypic division.** In the second meiotic division, the haploid cell divides mitotically and results into four haploid cells. The second meiotic division is also known as the **homotypic division.** In the homotypic division pairing of chromosomes, exchange of the genetic material and reduction of the chromosome number do not occur.

Both the meiotic divisions occur continuously and each includes the usual stages of the mitosis, *viz.*, prophase, metaphase, anaphase and telophase. The prophase of first meiotic division is very significant phase because the most cytogenetical events such as synapsis, crossing over, etc., occur during this phase. The prophase is the longest meiotic phase, therefore, for the sake of convenience it is divided into six substages, *viz.*, proleptonema (proleptotene), leptonema (leptotene), zygonema (zygotene), pachynema (pachytene) diplonema (diplo tene) and diakinesis. The successive meiotic subtages can be represented as below :



HETEROTYPIC DIVISION OR FIRST MEIOTIC DIVISION

In the beginning of the first meiotic division the nucleus of the meiocyte starts to swell up by absorbing the water from the cytoplasm and the nuclear volume increases about three folds. This increase in the volume of the nucleus causes modification of nuclear components. After these changes the cell passes to the first stage of first meiotic division which is known as prophase.

FIRST PROPHASE

The first prophase is the longest stage of the meiotic division. During this stage the amount of DNA becomes double. Most of the synthesis of DNA occurs at the beginning of this phase. It includes following substages :

1. **Proleptotene or Proleptonema**—The proleptotene stage closely resembles with the early mitotic prophase. In this stage the

chromosomes are extremely thin, long, uncoiled, longitudinally single and slender thread-like structures.

2. **Leptotene or Leptonema**—In the leptotene stage the chromosomes become more uncoiled and assume a long thread-like shape. The chromosomes at this stage take up a specific orientation inside the nucleus ; the ends of the chromosomes converge toward one side of the nucleus, that side where the centrosome lies (**the bouquet stage**). The centriole duplicates and each daughter centriole migrates towards the opposite poles of the cell. On reaching at the poles, each centriole duplicates and thus, each pole of cell possesses two centrioles or a single diplosome.

3. **Zygotene or Zygonema**—In the zygotene stage, the pairing

synapsis-union). The synapsis begins at one or more points along the length of the homologous chromosomes. Three types of synapsis have been recognised.

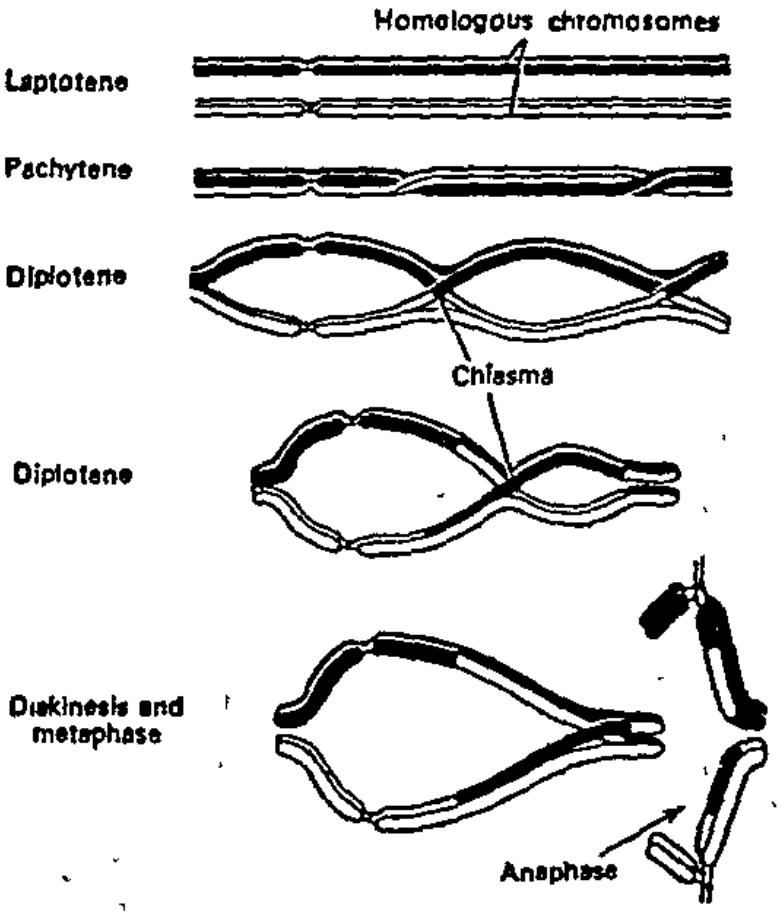


Fig. 18·5 Diagram of chromosomal exchange in the four-strand stage and of terminalization during first meiotic division.

(i) **Proterminal synapsis**—In proterminal type of synapsis the pairing in homologous chromosomes starts from the end and continues towards their centromeres.

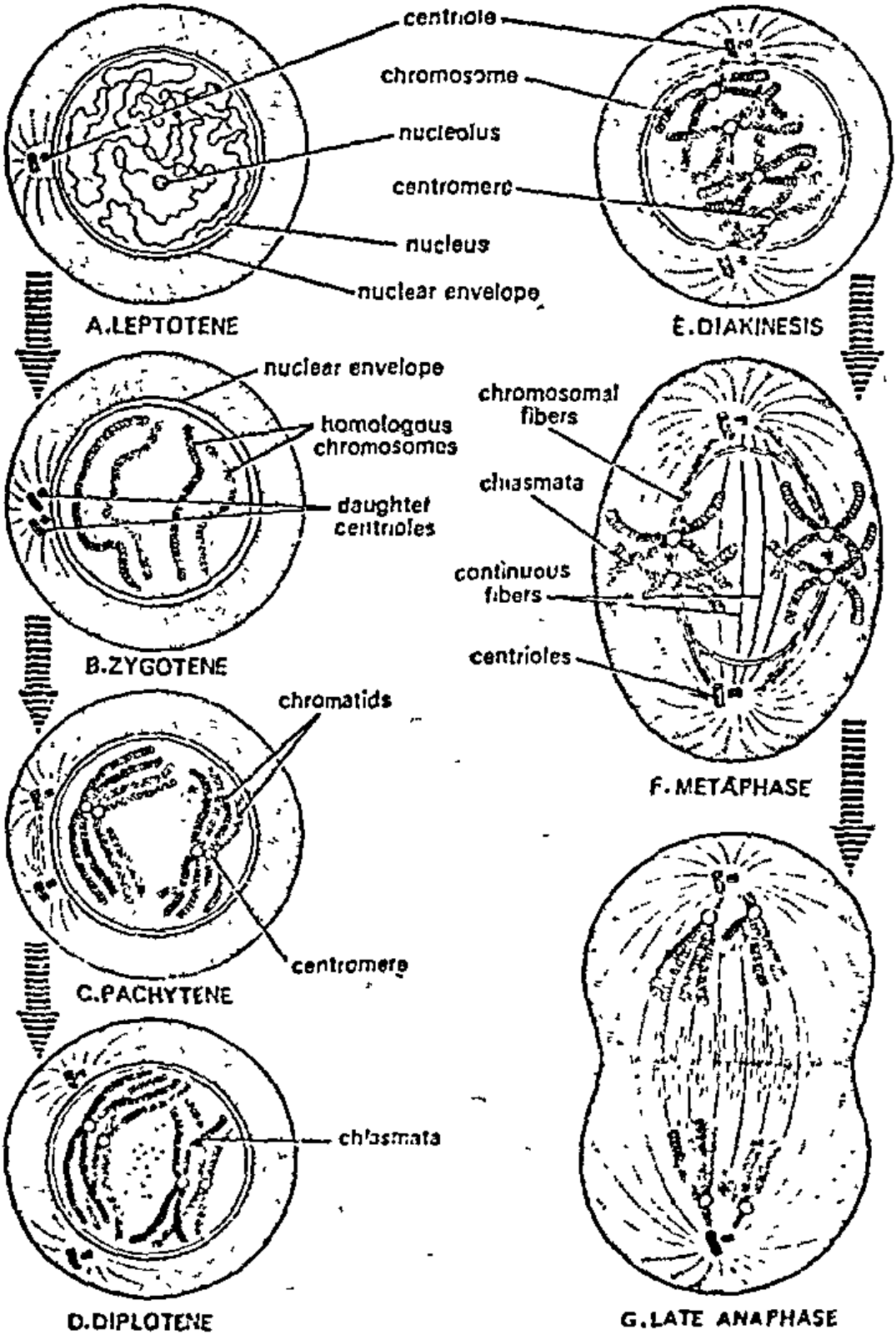


Fig 18 6. Different stages of first meiotic division.

(ii) Procentric synapsis—In procentric synapsis the homologous chromosomes start pairing from their centromeres and the pairing progresses towards the ends of the homologues.

(iii) Localized pairing or Random synapsis—The random type of synapsis occurs at various points of the homologous chromosomes.

The pairing of the homologous chromosomes is very exact and specific. The bouquet is supposed to maintain a regularity in the synapsis mechanism.

4. Pachytene or Pachynema—In the pachynema stage the pair c each other and can- n the pachynema stage each homologus chromosome splits lengthwise to form two chromatids. Actually, the doubling of the DNA molecule strands which is necessary for the subsequent duplication of chromosomes occurs earlier, before the beginning of meiotic prophase. Through the earlier part of the meiotic prophase, however, the DNA molecule in each chromosome behaves as a single body. In the pachynema stage, this is now changed, the two chromatids of each chromosome containing half of the DNA present in the chromosome at start, become partially independent of one another, although they still continue to be linked together by their common centromere. The pachynema chromosome, thus, consists of four chromatids closely joined together in one complex unit called a **bivalent**, because it actually contains a pair of chromosomes.

During pachynema stage an important genetic phenomena called "crossing over" takes place. The crossing over involves re- shuffli redistribution and mutual exchange of hered of tw to rec a biv donuc stage by Stern and Hotta (1969). After the division of chromatids, the interchange of chromatid segments takes place between the non- omosomes. The broken chro- omatids due to the presence 1969). This process of interchange of chromatin material between one non-sister chromatids of each homologous chromosome is known as the **crossing over** which is accompanied by the **chiasmata formation.**

Stern and Hotta (1969) have reported that during the pachytene and zygotene stages, synthesis of small amount of DNA takes place. This DNA amount is utilized in the repairing of broken DNA mole-

cules of the chromatids during the chiasmata formation and crossing over.

The nucleolus remains prominent upto this stage and it is found to be associated with the nucleolar organizer region of the chromosome.

5. **Diplotene or Diplonema**—In diplonema stage, the homologous chromosomes repel each other because the force of attraction between the two homologous chromosomes decreases. The two homologous chromosomes thus separate from each other, however not completely because both remain united at the point of interchange or chiasmata.

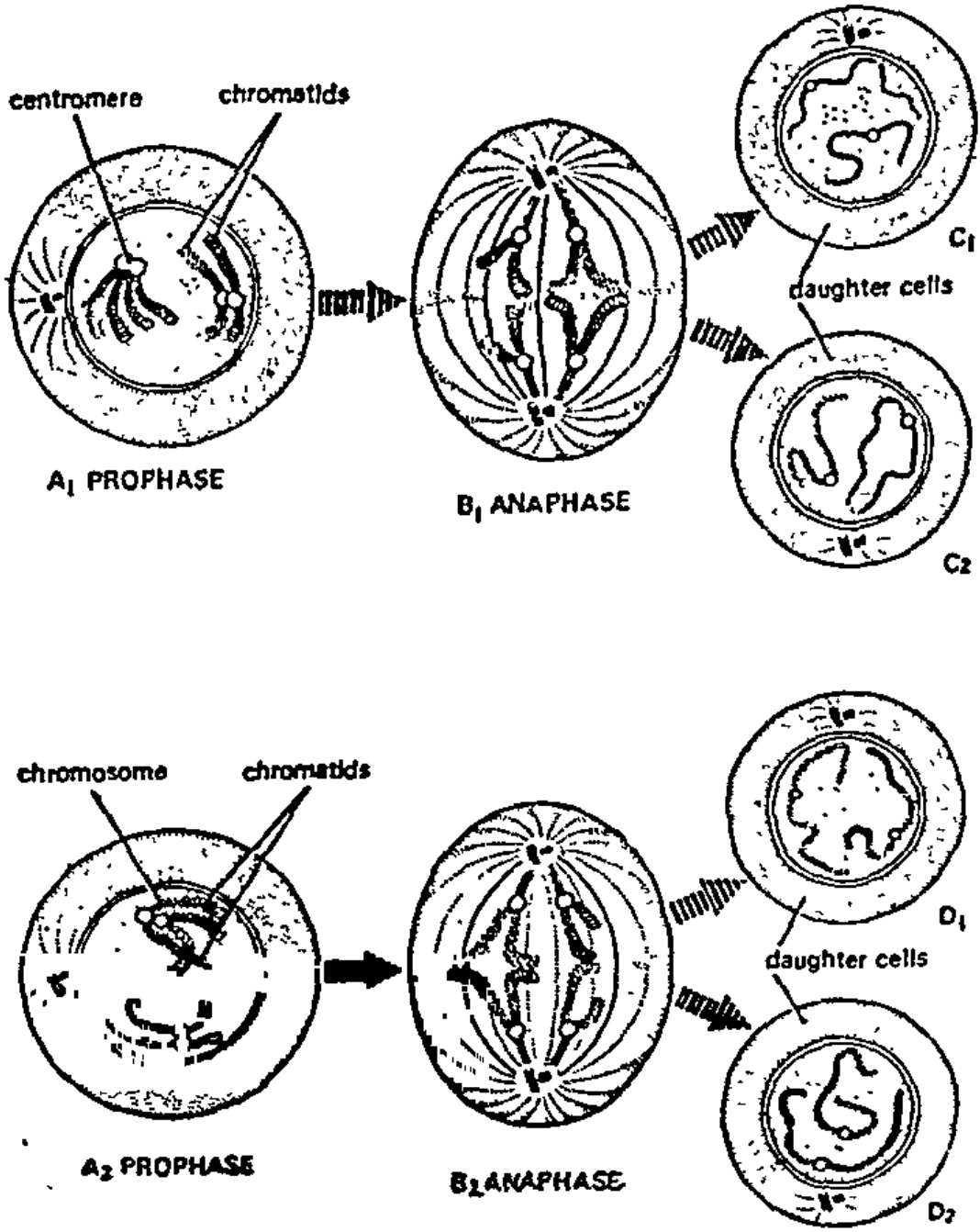


Fig. 18 7. Different stages of second meiotic division.

6. **Diakinesis**—In the diakinesis stage the bivalent chromosomes become more condensed and evenly distributed in the nucleus. The nucleolus detaches from the nucleolar organizer and ultimately disappears. The nuclear membrane disintegrates into numerous vesicles. The centrioles migrate towards the opposite poles and initiate the formation of spindle fibres. The chromosomes are held by the terminal chiasma and these exist upto the metaphase.

PROMETAPHASE

In the prometaphase the nuclear envelope disintegrates and the microtubules get arranged in the form of spindle in between the two centrioles which occupy the position of two opposite poles of these cell. The chromosomes become greatly coiled in the spiral manner and get arranged on the equator of the spindle.

METAPHASE I

In the metaphase I, the microtubules of the spindle are attached with the centromeres of the homologous chromosomes of each tetrad. The centromere of each chromosome is directed towards the opposite poles. The repulsive forces between the homologus chromosomes increase greatly and the chromosomes become ready to separate.

ANAPHASE I

Due to the contraction of chromosomal fibres or microtubules each homologous chromosome with its two chromatids undivided centromere moves towards the opposite poles. The shorter chromosomes move more frequently than the longer chromosomes containing many chiasma.

The actual reduction occurs at this stage. Here it should be carefully noted that the homologous chromosomes which move towards the opposite poles are the chromosomes of either paternal or maternal origin. Moreover, because during the chiasma formation out of two chromatids of a chromosme, one has change its counter part, therefore, the two chromatids of a chromosome do not resemble with each other in the genetical terms.

TELOPHASE I

In telophase I, the endoplasmic reticulum forms the nuclear envelop around the chromosomes and the chromosomes become uncoil. The nucleolus reappears and thus two daughter chromosomes are formed. After the karyokinesis, cytokinesis occurs and two haploid cells are formed.

Both cells pass through a short resting phase or interphase. In case of *Trillium* telophase I and interphase I do not occur and the anaphase I is followed by prophase II directly.

HOMOTYPIC OR SECOND MEIOTIC DIVISION

The homotypic or second meiotic division is actually the mitotic division which divides each haploid meiotic cell into two haploid cells. The second meiotic division includes following four stages :

PROPHASE II

In the prophase second each centriole divides into two and thus, two pairs of centrioles are formed. Each pair of centrioles migrates to the opposite pole. The microtubules get arranged in the form of spindle at the right angle of the spindle of first meiosis. The nuclear membrane and the nucleolus disappear. The chromosomes with two chromatids become short and thick.

METAPHASE II

During metaphase II, the chromosomes get arranged on the equator of the spindle The centromere divides into two and thus each chromosome produces two monads or daughter chromosomes. The microtubules of the spindle are attached with the centromere of the chromosomes.

ANAPHASE II

The daughter chromosomes move towards the opposite poles due to the contraction of chromosomal microtubules and stretching of interzonal microtubules of the spindle.

TELOPHASE II

The chromatids migrate to the opposite poles and now known as chromosomes. The endoplasmic reticulum forms the nuclear envelope around the chromosomes and the nucleolus reappears due to synthesis of ribosomal RNA (rRNA) by rDNA and also due to accumulation of ribosomal proteins.

After the karyokinesis, in each haploid meiotic cell, the cytokinesis occurs and thus, four haploid cells are resulted. These cells have different types of chromosomes due to the crossing over in the prophase I.

SIGNIFICANCE OF MEIOSIS

The meiosis have the greatest significance for the biological world because of its following uses :

1. The meiosis maintains a definite and constant number of the chromosomes in the organisms.

2. By crossing c
the exchange of the ge
among the species. Th
tionary process.

Thus the meiosis has a peculiar taxonomic, genetical, and evolutionary process.

COMPARISON BETWEEN MITOSIS AND MEIOSIS

Mitosis	Meiosis
1. Mitosis occurs continuously in the body or somatic cells.	1. Meiosis occurs in the germ cells (the cells of the testes or ovaries) during the process of gametogenesis.
2. The whole process completes in one sequence or phase.	2. The whole process completes in two successive divisions which occur one after the other.
Prophase	
3. The prophase is of short duration and includes no substage.	3. The prophase is of longer duration and it completes in six successive stages, *viz.*, proleptotene, leptotene, zygotene, pachytene, diplotene and dikinesis.
4. The homologous chromosomes (paternal and maternal) duplicate into two chromatids. The two chromatids separate and form new chromosomes. Each daughter cell receives the daughter chromosome or chromatids of each homologous chromosome and thus having the chromosome number like the parental cells.	4. Out of two homologous chromosomes only one type of chromosome either maternal or paternal move to the daughter cells. A daughter cell thus receives only a
5. No pairing or synapsis takes place between the homologous chromosomes.	5. Pairing or synapsis occurs between the homologous chromosomes.
6 Duplication of chromosomes takes place in the early prophase.	6. Duplication or spliting of chromosomes takes place in the late prophase (pachytene stage).
7. No chiasma formation or crossing over takes place.	7. Chiasma formation or crossing over takes place.
8. The exchange of the genetic material between the homologous chromosomes does not occur.	8. The exchange of the genetic material takes place between the non-sister chromatids of homologous chromosomes.
Metaphase	
9. The chromatids occur in the form of dyads.	9. The chromatids of two homologous chromosomes occur as the tetrads.
wards the poles.	10. The centromeres of the chromosomes remain directed towards the poles and the chromosomal arms remain directed towards the equator.

Mitosis	Meiosis
Anaphase	
11. The chromosomes are the monads, *i.e.*, having single chromatid.	11. The chromosomes are the biads, *i.e.*, having two chromatids and single centromere.
12. The chromosomes are long and thin.	12. The chromosomes are short and thick.
Telopase	
13. The telophase always occurs.	13. The first telophase is sometimes omitted.
Significance	
14. The chromosome number in each daughter cell remains the same like the parent cell.	14. In meiotic division the chromosome number is reduced to half in the daughter cells than the parental cells.
15. A diploid cell produces four diploid cells by a mitotic division.	15. A diploid cell produces four haploid cells by a meiotic division.

CHAPTER 19

REPRODUCTION

This is the inherent property of the living organisms to continue their race by the mechanism of reproduction. The reproduction is a process by which the living beings propagate or duplicate their own kinds. The reproduction may be of following two types :

1. Asexual reproduction.
2. Sexual reproduction.

ASEXUAL REPRODUCTION

The development of new individuals without the fusion of the male and female gametes is known as **asexual reproduction**. The asexual reproduction usually includes amitotic or mitotic division of the body (somatic) cells, therefore, it is also known as **somatogenic** or **blastogenic reproduction**. The asexual reproduction is common only in lower plants and animals and it may be of following types :

1. Fission.
2. Budding.
3. Gemmule formation.
4. Regeneration.

1. Asexual reproduction by the fission—The fission is the most widely occurring type of asexual reproduction of the protozoans and various metazoans. In this method of reproduction the nuclear and cytoplasmic contents of the cell divide or split completely into smaller sized daughter individuals. The fission itself may be of following types :

A. Binary fission—In the binary fission the animal body splits or divides in such a plane that two equal and identical halves are produced. It is most common in protozoans but it also occurs in certain lower metazoans. First of all the nucleus divides by amitotic or mitotic division and the division of the nucleus is followed by the division of the cytoplasm. According to the plane of fission following types of binary fission have been recognized in the organisms :

(i) **Simple or orthodox type of binary fission**—The simple or orthodox type of binary fission occurs in the irregular-shaped organisms, *e.g.*, *Amoeba* in which the plane of division is difficult to observe.

(ii) Transverse binary fission—The transverse binary fission occurs in some protozoans, *e.g.*, *Paramecium* and some metazoans such as certain coelenterates, turbellarians and annelids. In transverse binary fission the plane of the division is always transverse to the longitudinal axis of the body of the organisms.

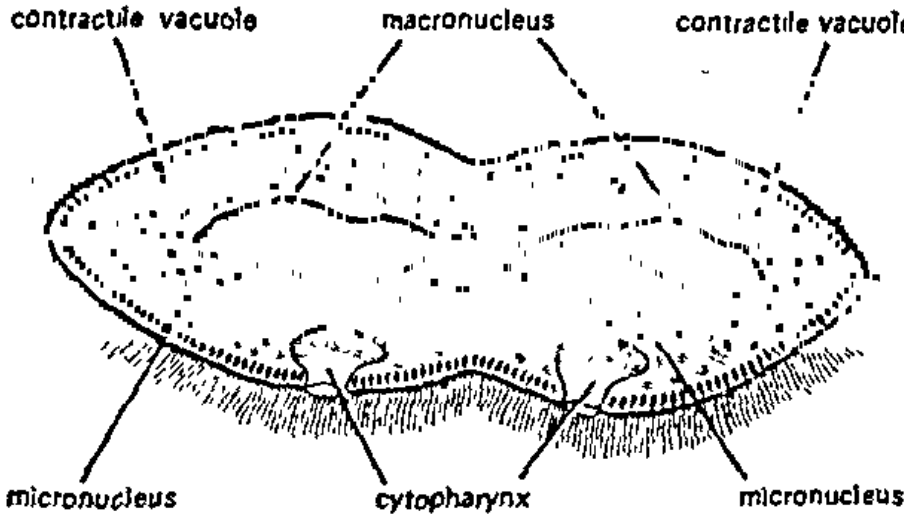


Fig 19·1. Transverse binary fission in *Paramecium.*

(iii) Longitudinal binary fission—The longitudinal binary fission occurs in certain ciliates and flagellates, *e.g.*, *Vorticella* and *Euglena* (Protozoa) and some corals (Anthozoa). In longitudinal binary fission the nucleus and the cytoplasm divide in the longitudinal plane.

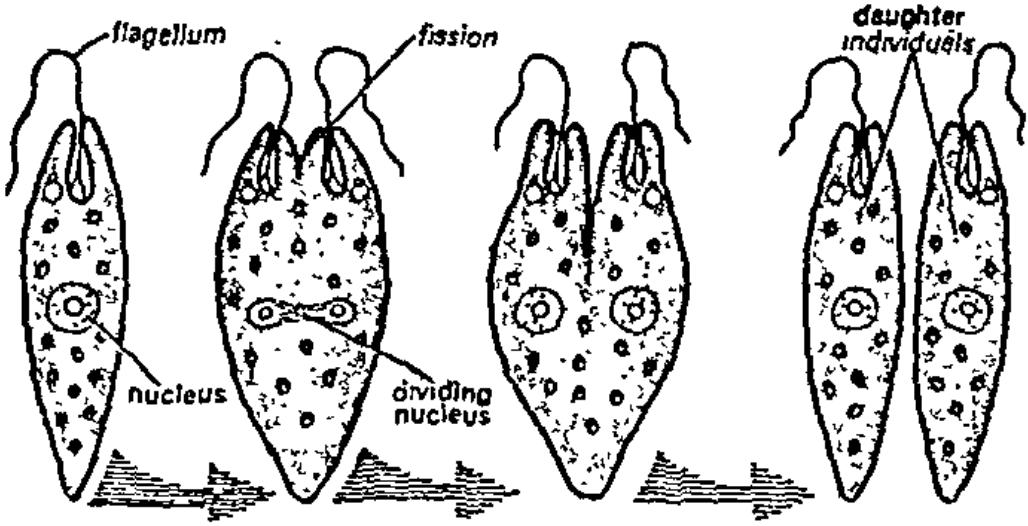


Fig. 19 2. Longitudinal binary fission in *Euglena.*

(iv) Oblique binary fission—The oblique binary fission occurs in most dinoflagellates. In this type of fission the cell or body of the organism divides by the oblique division.

(v) Strobilation—In certain metazoan animals a special type of transverse fission known as the strobilation occurs. In the process of the strobilation several transverse fissions occur simultaneously

and giving rise to a number of individuals which often do not separate from each other immediately. The strobilation occurs in the scyphozoan (*Aurelia*), certain polychaets and ascidians. In *Aurelia*, for instance, the strobilation occurs during the formation of ephyra larva.

B. Asexual reproduction by multiple fission—In the multiple fission, the nucleus of the cell divides very rapidly into many nuclei. Each daughter nucleus in later stage is surrounded by the little mass of the cytoplasm and forms the asexually reproducing body such as schizogont, gamont, spore, etc. The multiple fission occurs in most algae, fungi and some protozoans, *e.g.*, *Amoeba*, *Plasmodium* and *Monocystis*, etc.

2. Asexual reproduction by budding or gemmation—In certain multicellular animals such as *Hydra* (coelentrates) and certain tunicates, the body gives out a small out growth known as the **bud**. The bud is supported by the parent body and it ultimately develops into a new individual. The process of development of a bud into an adult animal is called **blastogenesis**. The developing individual gets its food from the body of the parent and when it becomes fully mature it is detached from the body of the parent and leads an individual existence.

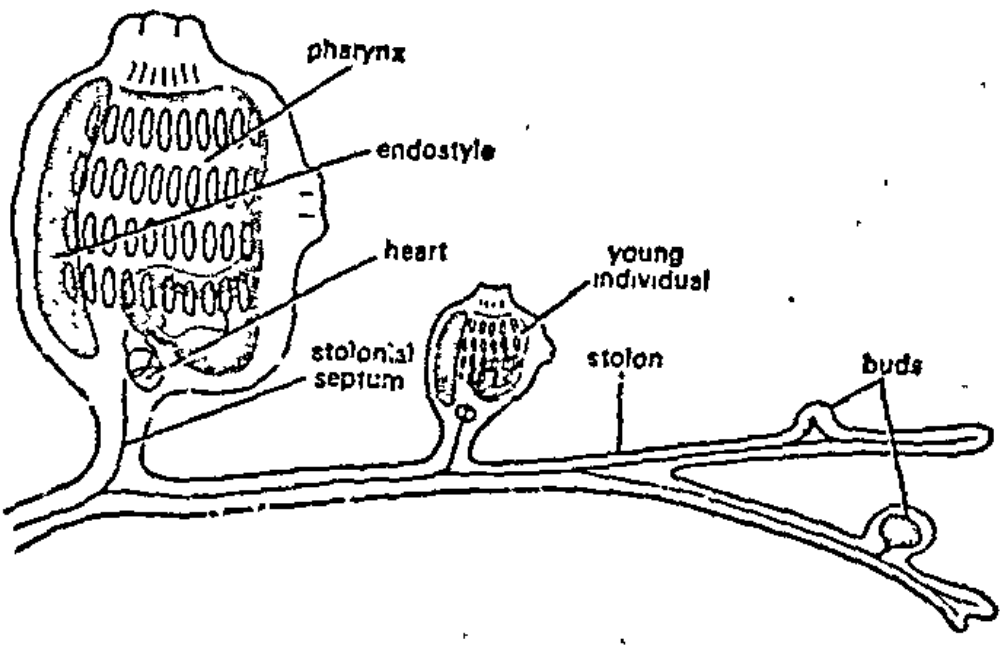


Fig. 19 3. Budding in a tunicate.

3. Asexual reproduction by gemmule formation—In certain metazon animals the asexual reproduction is carried on by certain peculiar asexual bodies known as the gemmules and statoblasts. The gemmules occur in fresh water sponges (family Spongilidae) and the statoblasts occur in the bryozoans.

The gemmules and the statoblasts are composed of a group of undifferentiated cells which contain stored food material. These cells are enclosed and protected by the monaxon spicules in the gemmules and by the chitinous covering in the statoblasts. Both (gemmules and statoblasts) are set free by the destruction of the parental body and they develop into the new individuals in the favourable conditions.

4 **Asexual reproduction by regeneration**—The regeneration is a process by which the organisms develop or regenerate their lost or worn out parts. The regeneration is the best means of asexual reproduction in certain protozoans, sponges, coelenterates planarians and echinoderms.

SEXUAL REPRODUCTION

In the sexual reproduction, the development of the new individual takes place by the fusion of the sex cells or male and female gametes. The sexual reproduction is the most common type of reproduction among the plants and animals. It may be of following types :

1. Syngamy—The syngamy is the most common type of sexual reproduction in the plants and animals. In syngamy (Gr., *syn*-together; *gam*-marriage) the fusion of two gametes takes place completely and permanently. Following kinds of syngamy is prevalent among the living organisms :

(i) Autogamy—In autogamy (Gr., *auto*-self; *gam*-marriage) the male and female gametes are produced by the same cell or organisms and both gametes fuse together to form a zygote, *e.g.*, *Actinosphaerium* and *Paramecium*.

(ii) Exogamy—In exogamy (Gr. *exo*-external; *gam*-marriage) the male and female gametes are produced by different parents and both unite to from a zygote.

(iii) Hologamy—In the lower organisms, sometimes the entire mature organisms start to act as gametes and the fusion of such mature individuals is known as the **hologamy.**

(iv) Paedogamy—Paedogamy is the sexual union of young individual produced immediately after the division of the adult parent cell by mitosis.

(v) Merogamy—In the merogamy (Gr., *meros*-part; *gam*-marriage), the fusion of smaller-sized and morphologically different gametes (merogametes) takes place.

(vi) Isogamy—In isogamy (Gr,, *is*-equal; *gam*-marriage) the fusion of morphologically and physiologically identical gametes (isogametes) takes place.

(vii) Anisogamy—Some organisms produce two types of gametes. Both types of gametes differ from each other in their shape, size and behaviour and are collectively known as the **anisogametes** or **heterogametes**. The male gametes are motile and small in size and known as the **microgametes**. The female gametes are passive and have comparatively large size and known as the **macro-or megagametes**. The union of micro-and macrogametes is known as the **anisogamy** (Gr., *an*-without ; *is*-equal ; *gam*-marriage). The anisogamy occurs in higher animals and plants but it is customary to use the term fertilization in them instead of the anisogamy or syngamy.

(viii) Macrogamy : The syngamy or fusion of the macrogamates is known as **macrogamy** (Gr., *macro*-large; *gam*-marriage).

(ix) Microgamy : The microgamy (Gr., *micro*-small; *gam*-marriage) is common in certain protozoans, *e.g.*, foraminiferans and *Arcella*. In microgamy the fusion of microgametes takes place.

2. Conjugation : The conjugation is the temporary union of the two individuals of the same species. During the union both individuals known as **conjugants** exchange certain amount of nuclear (DNA) material and after which conjugants are separated. The conjugation is most common among the ciliates, *e.g.*, *Paramecium* and bacteria.

3. Automixis : When the gamete nuclei of the same cell unite together to form new individuals this phenomenon is known as the **automixis**.

4. Parthenogenesis : The parthenogenesis (Gr., *parthenos*-virgin, *genesis*-birth) is the special type of sexual reproduction. In parthenogenesis, the eggs of an organism develop into the young individual without the fertilization of the eggs by the sperms. The parthenogenesis occurs in certain insects (wasps and bees, etc.) and rotifers.

CHAPTER 20

GAMETOGENESIS

The gametogenesis (Gr., *gamos*-marriage; *genesis*-origin) is the process of gamete formation in the sexually reproducing animals. The sexually reproducing animals contain two types of cells in their body, *e.g.*, somatic cells and the germinal cells. Both types of cells have diploid number of chromosomes but each type has its different destiny. The somatic cells form various organs of the body and provide a phase for the maturation, development and formation of the germinal cells. The somatic cells always multiply by mitotic division.

The germinal cells form the gonads (testis and ovaries) in the animal body. These cells produce the gamete cells by successive mitotic and meiotic divisions. The male gamete is known as **spermatozoon** or sperm and the female gamete is known as **ovum** or **egg**. The process of sperm production is known as the **spermatogenesis** (Gr., *sperma*-sperm or seed; *genesis*-origin) and the process of production of ovum is known as **oogenesis** (Gr., *oon*-egg; *genesis*-origin). Both the processes can be studied in detail under separate headings.

SPERMATOGENESIS

The process of spermatogenesis occurs in the male gonads or testis. The testis of the vertebrates are composed of many seminiferous tubules which are lined by the cells of germinal epithelium. The cells of the germinal epithelium form sperms by the process of spermatogenesis. But in certain animals, *e.g.*, mammals and Mollusca etc., there are somatic cells lying in between germinal cells, these somatic cells are known as **Sertoli cells**. The Sertoti cells anchor the differentiating cells and provide nourishment to the developing sperms. The insects do not possess Sertoli cells. The spermatogenesis is a continuous process and for the sake of convenience this process can be studied in two different stages.

1. Formation of spermatids.
2. Spermiogenesis.

1. FORMATION OF SPERMATIDS

The male germinal cells which produce the sperms are known as the **primary germinal cells** or **primordial cells**. The primordial cells pass through following three phases for the formation of spermatids :

(I) **Multiplication phase**—The undifferentiated germ cells or primordial cells contain large-sized and chromatin-rich nuclei. These cells multiply by repeated mitotic divisions and produce the cells which are known as the *spermatogonia* (*Gr., sperma-sperm or seed; gone*-offspring). Each spermatogonium is diploid and contains 2X number of chromosomes.

(II) **The growth phase**—In the growth phase, the spermatogonial cells accumulate large amount of nutrition and chromatin

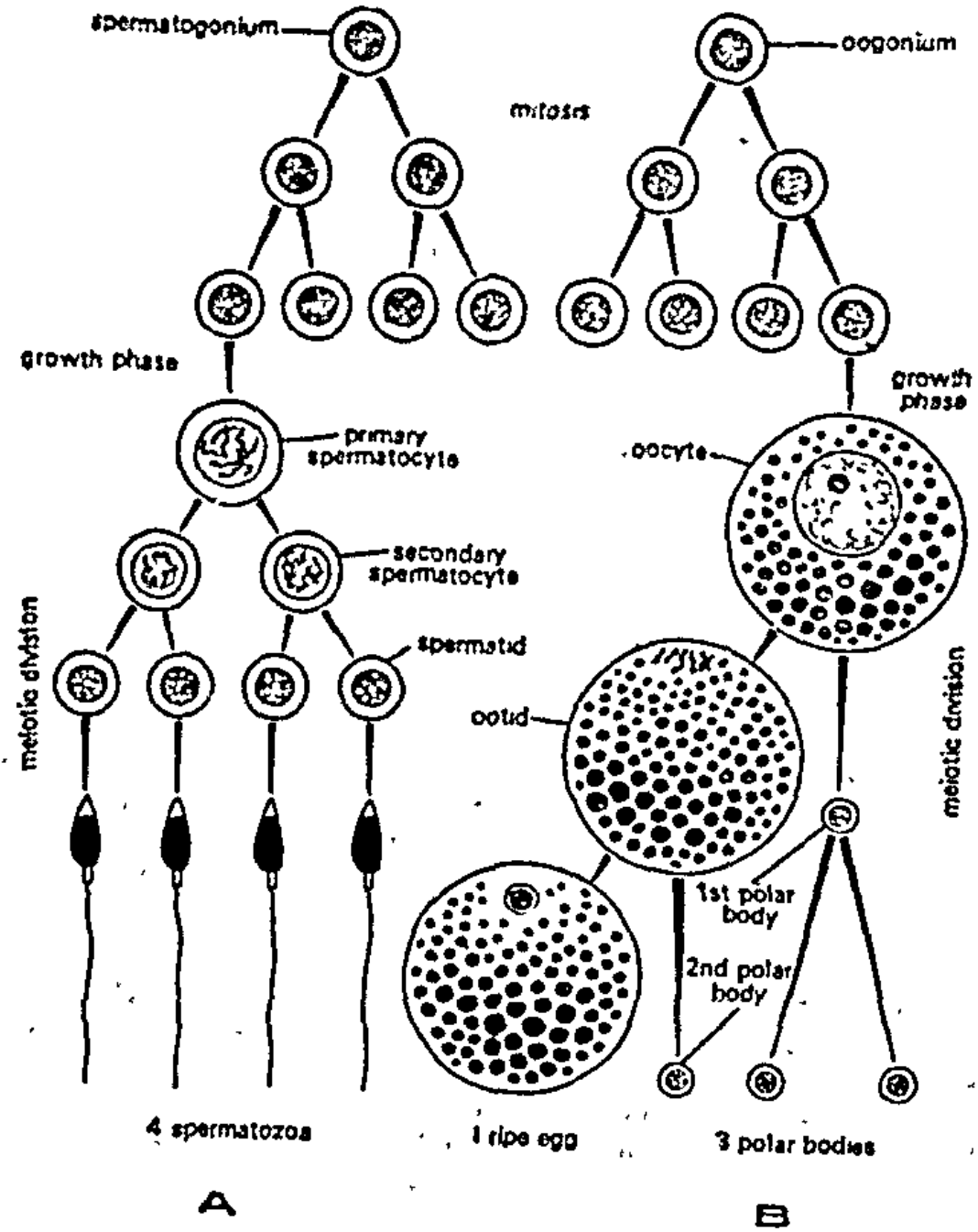


Fig. 20·1. Spermatogenesis and oogenesis,
A—Spermatogenesis. B—Oogenesis.

material. Now each spermatogonial cell is known as the **primary spermatocyte.**

(iii) The maturation phase—The primary spermatocytes are ready for first meiotic or maturation division. The homologous chromosomes start pairing (synapsis), each homologous chromosome splits longitudinally and by the chiasma formation the exchange of genetic material or crossing over takes place between the chromatids of the homologous chromosomes. The DNA amount is duplicated in the beginning of the division. By first meiotic division or homotypic division two **secondary spermatocytes** are formed. Each secondary spermatocyte is haploid and contains X number of chromosomes. Each secondary spermatocyte passes through the second maturation or second meiotic or heterotypic division and produces two **spermatids.** Thus by a meiotic or maturation division a diploid spermatogonium produces four haploid spermatids. These spermatids cannot act directly as the gametes so they have to pass through the next phase, the **spermiogenesis.**

2. SPERMIOGENESIS

The metamorphosis of differentiation of the spermatids into the sperms is known as **spermiogenesis.** Because the sperm or spermatozoon is a very active and mobile cell so to provide great amount of mobility to the sperm, the superfluous material of the developing sperms is discarded. For the reduction of the weight of the sperms following changes occur in the spermatids :

(i) Changes in the nucleus—The nucleus loses water from the nuclear sap, shrinks and assumes different shapes in the different animals. The sperm nucleus in man and bull becomes ovoid and laterally flattened. In rodents and amphibians the sperm nucleus becomes scimitar-shaped with pointed tip. In birds and molluscs the nucleus becomes spirally twisted like a cork screw. The bivalve molluscs have the round sperm nucleus. The shape of the nucleus also determines the shape of the sperm head which becomes fully adopted for the active propulsion through the water. The RNA contents of the nucleus and the nucleolus are greatly reduced. The DNA becomes more concentrated and the chromatin material becomes closely packed into small volume.

(ii) Acrosome formation—The acrosome occurs at the anterior side of the sperm nucleus and contains proteoses enzymes which help its easy penetration inside the egg. The acrosome is formed by the Golgi complex. The Golgi complex is concentrated near the anterior end of the sperm nucleus to form the acrosome. One or two vacuoles of the complex become large and occupy the place between

the tubules of Golgi complex. Soon after a dense granule known as the **proacrosomal granule** develops inside the vacuole. **Leblond** (1955) found the proacrosomal granule rich in the mucopolysaccharides. The proacrosomal granule attaches with the anterior end of the nucleus

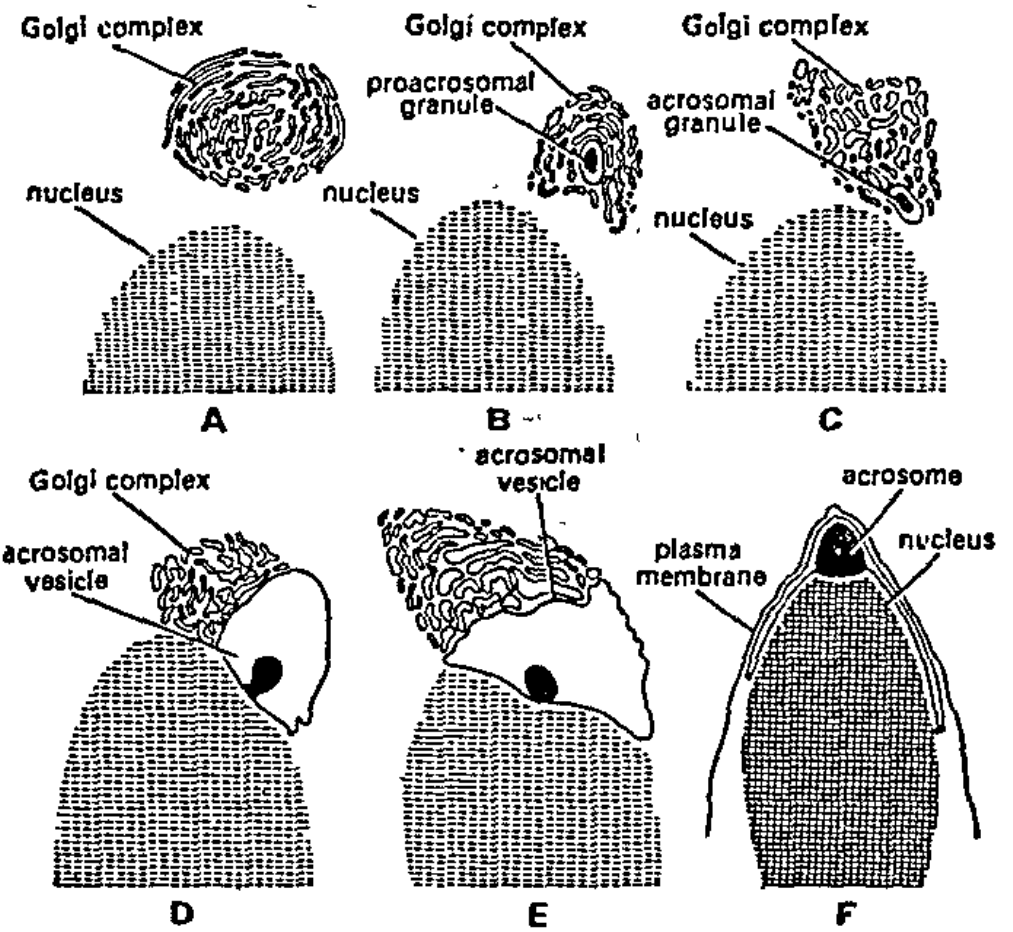


Fig. 20·2. Formation of acrosome of sperm.

and enlarges into the **acrosome.** The membranes of Golgi vacuoles form the double membrane (unit membrane of lipoprotein) sheath around the acrosome and forms the cap-like structure of the spermatozoa. The rest of the Golgi complex becomes reduced and discarded from the sperm as **Golgi rest.** In the sperms of certain animals an **acrosomal cone** or **axial body** also develops in between the acrosome and the nucleus.

(iii) The centrioles—The two centrioles of the spermatids become arranged one after the other behind the nucleus. The anterior one is known as the **proximal centriole** and the posterior one is known as the **distal centriole.** The distal centriole changes into the basal bodies and gives rise to the **axial filament** of the sperm. The axial filament or the flagellum is composed of a pair of central longitudinal fibres and nine peripheral fibres. The distal centriole and the basal part of the axial filament occur in the middle piece of the sper-

matozoa. The mitochondria of the spermatids fuse together and twist spirally around the axial filament.

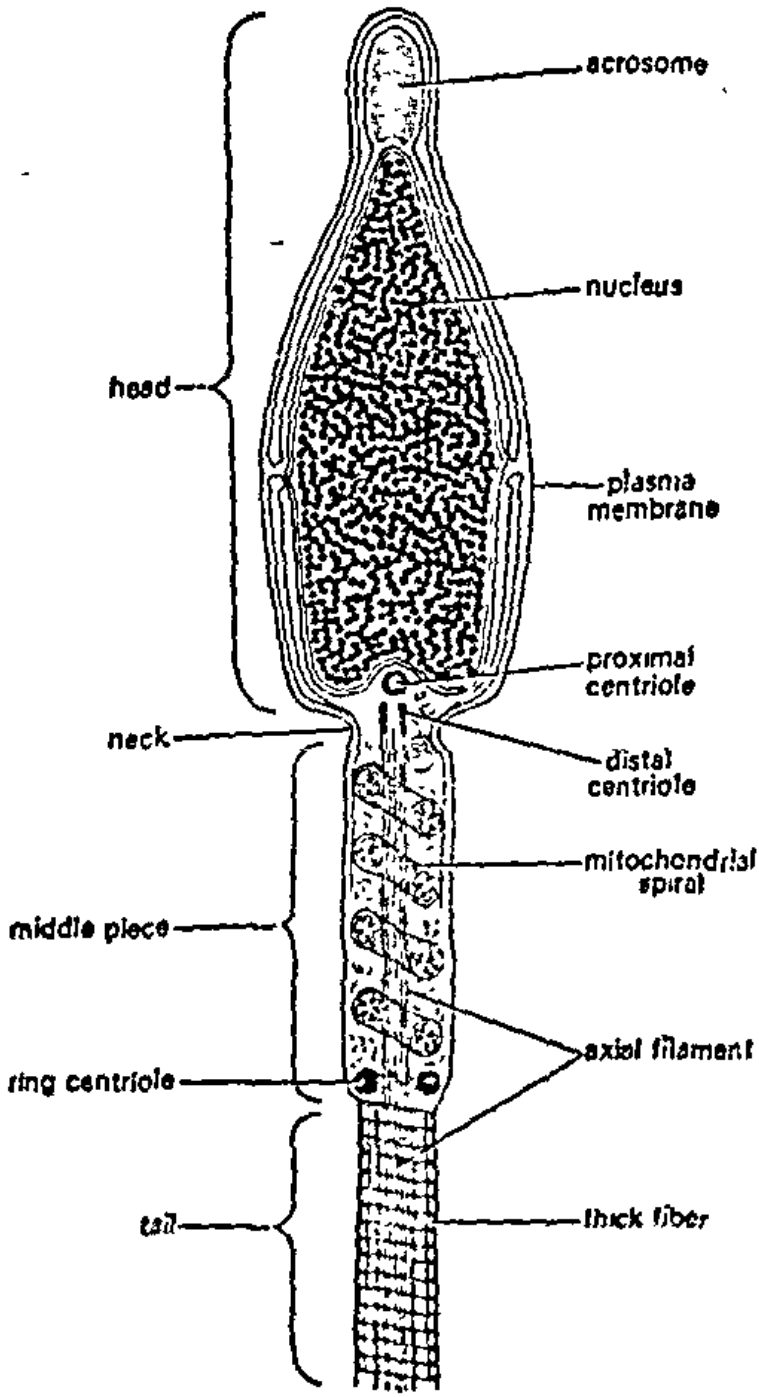


Fig. 20-3. Electron microscopic view of a flagellate spermatozoon.

Thus, most of the cytoplasmic portion of the spermatid except the nucleus, acrosome, centriole, mitochondria and axial filament is discarded during the spermiogenesis.

OOGENESIS

The process of oogenesis occurs in the cells of the germinal epithelium of the ovary, such cells are known as primordial germinal

cells. The oogenesis is completed in the following three successive stages :

1. Multiplication phase.
2. Growth phase.
3. Maturation phase.

1. MULTIPLICATION PHASE

The primordial germinal cells divide repeatedly to form the **oogonia** (Gr., *oon*-egg). The oogonia multiply by the mitotic divisions and form the **primary oocytes** which pass through the growth phase.

2. GROWTH PHASE

The growth phase of the oogenesis is comparatively longer than the growth phase of the spermatogenesis. In the growth phase, the size of the primary oocyte increases enormously. For instance, the primary oocyte of the frog in the beginning has the diameter about 50μ but after the growth phase the diameter of the mature egg reaches about 1000μ to 2000μ. In the primary oocyte, large amount of fats and proteins become accumulated in the form of yolk and due to its heavy weight (or gravity) it is usually concentrated towards the lower portion of the egg forming the **vegetal pole**. The portion of the cytoplasm containing the egg pronucleus remains often separated from the yolk and occurs towards the upper side of the egg forming the **animal pole**.

The cytoplasm of the oocyte becomes rich in RNA, DNA, ATP and enzymes. Moreover, the mitochondria, Golgi complex, ribosomes, etc., become concentrated in the cytoplasm of the oocyte. In certain oocytes (Amphibia and birds) the mitochondria become accumulated at some place in the oocyte cytoplasm and forming the **mitochondrial clouds.**

During the growth phase, tremendous changes also occur in the nucleus of the primary oocyte. The nucleus becomes large due to the increased amount of the nucleoplasm and is called **germinal vesicle.** The nucleolus becomes large or its number is multiplied due to excessive synthesis of ribosomal RNA by rDNA of nucleolar organizer region of chromosomes. Thus, the nucleus or germinal vesicle of primary oocyte of *Triturus* has 600 nucleoli, of *Siredon* has 1000 nucleoli and of *Xenopus* has 600 to 1200 nucleoli due to the synthesis of ribosomal RNA. The chromosomes change their shape and become giant lampbrush chromosomes which are directly related with increased transcription of *m*RNA molecules and active protein synthesis in the cytoplasm. When the growth of the cytoplasm and

nucleus of the primary oocyte is completed it becomes ready for the maturation phase.

3. MATURATION PHASE

The maturation phase is accompanied by the maturation or meiotic division. The maturation division of the primary oocyte differs greatly from the maturation division of the spermatocyte. Here after the meiotic division of the nucleus, the cytoplasm of the oocyte divides unequally to form a single large-sized haploid egg and three small haploid **polar bodies** or **polocytes** at the end. This type of unequal division has the great significance for the egg. If the equal divisions of the primary oocyte might have been resulted, the stored food amount would have been distributed equally to the four daughter cells and which might prove insufficient for the developing embryo. Therefore, these unequal divisions allow one cell out of the four daughter cells to contain most of the cytoplasm and reserve food material which is sufficient for the developing embryo.

(i) First maturation division—During the first maturation division or first meiosis, the homologous chromosomes of the primary oocyte nucleus pass through the pairing or synapsis, duplication, chiasma formation and crossing over. Soon after the nuclear membrane breaks and the bivalent chromosomes move towards the opposite poles due to contraction of chromonemal fibres. A new nuclear envelope is developed around the daughter chromosomes by the endoplasmic reticulum.

After the karyokinesis the unequal cytokinesis occurs and a small haploid polar body or polocyte and a large haploid **secondary oocyte** or **ootid** are formed.

(ii) Second meiotic division—The haploid secondary oocyte and first polocyte pass through the second meiotic division. Due to the second meiotic division the secondary oocyte forms a mature egg and a second polocyte. By the second meiotic division the first polocyte also divides into two secondary polocytes: These polocytes ooze out from the egg and degenerate while the haploid egg cell becomes ready for the fertilization.

STRUCTURE OF MATURE EGG

The mature egg has a cell-like structure and composed of the following parts :

1. Plasma membrane—The mature egg is covered by a plasma membrane which is the unit membrane. It is composed of an outer and an inner layer of protein. Both the layers are 50A° in thickness. Between the proteinous layers there occurs a lipodous layer of 60° thickness.

2. **Primary egg membranes**—In addition to the plasma membrane the eggs of most animals except the sponges and certain coelenterates consist of certain other additional egg membranes. These membranes are known as the primary and secondary egg membranes. The primary egg membrane is secreted around the plasma membrane by the oocyte itself. In the insects, molluscs, amphibians and birds the primary egg membrane is known as the **vitelline membrane,** while in tunicates and fishes this membrane is known as the **chorion.** The mammalian eggs contain similar membrane and in them this is known as the **zona pellucida.** The vitelline membrane is composed of mucoproteins and fibrous proteins. The vitelline membrane usually remains closely adhered to the plasma membrane but in later stages a space is developed between the plasma and the vitelline membrane and this space is known as the **perivitelline space.**

3. **Secondary egg membranes**—The secondary egg membranes are secreted by the ovarian tissues around the primary egg membranes. They are composed of either jelly-coats in amphibians or chitinous shells in insects, ascidians and cyclostomes.

4. **Tertiary egg membranes**—The tertiary egg membranes are formed by the oviduct or other accessory parts of female reproductive system. They may be composed of either jelly coats in amphibians, albumen and hard horny capsule in elasmobranch fishes, or albumen, shell membranes and calcareous shell in birds.

5. **The ooplasm**—The cytoplasm of the egg cell is known as the ooplasm. The ooplasm consists of large amount of reserve food material in the form of **yolk.** It is also composed of a lipoprotein, pigment granules, water, RNA, ribosomes, mitochondria and various other cellular inclusions. The peripheral layer of the ooplasm is known as the **cortex** and it contains many microvilli and cortical granules. The microvilli are formed by the out pushings of the plasma membrane and they help in the transportation of the substances from the follicle cells to the egg during the development of the egg. The cortical granules are spherical bodies of various diameters, *e.g.*, 0·8μ in the sea urchin eggs and 2·0μ in the eggs of frog. The cortical granules are surrounded by the unit membranes and are originated from the Golgi complex. They contain homogeneous and granular acid mucopolysaccharides and are present in the eggs of sea urchins, frogs, fishes, bivalve molluscs, some annelids and certain mammals. But they do not occur in the ova of man, rat, guinea pig, gastropod molluscs, urodele amphibians, insects and birds.

Yolk contents of the ooplasm—The amount of the yolk in the ooplasm varies from species to species. According to the amount of the yolk following types of egg cells have been recognised.

1. **Alecithal :** The eggs without the yolk are known as alecithal eggs, *e.g.*, Eutherian mammals.

2. **Microlecithal :** The eggs with very little amount of yolk are known as the microlecithal eggs, *e.g.*, *Amphioxus*.

3. **Macrolecithal :** The eggs with large amount of the yolk are known a the macrolecithal eggs. The macrolecithal eggs can be grouped into two types on the basis of the distribution of the yolk in the ooplasm.

A. **Homolecithal :** The eggs with evenly distributed yolk contents in the ooplasm are known as the homolecithal eggs, *e.g.*, eggs of echinoderms.

B. **Heterolecithal :** The eggs in which the yolk is not evenly distributed in the ooplasm are known as the heterolecithal eggs. The heterolecithal eggs may be of following types.

(I) **Telolecithal :** When the amount of the yolk is concentrated in the one half of the egg to form the vegetative pole of the egg, then this condition is known as the telolecithal, *e.g.*, eggs of amphibians, etc.

(II) **Meiolecithal :** In the meiolecithal eggs the amount of the yolk is very large and it occupies the largest portion of the egg except a small disc-shaped portion of the cytoplasm. The cytoplasm contains the zygote nucleus and is known as the germinal disc, *e.g.*, eggs of fishes, reptiles and birds.

(III) **Centrolecithal :** In the centrolecithal eggs the yolk accumulates in the centre of the ooplasm, *e.g.*, eggs of insects.

CHAPTER 21

FERTILIZATION

The union of the cytoplasm and pronuclei of the male and female gametes is known as the **fertilization** (L., *fertilis*-to bear) The fertilization is the most commonly used method for the production of the diploid zygotes in the sexually reproducing organisms of Metazoa and Metaphyta.

In the process of the fertilization the haploid male gamete (spermatazoa or pollen), which carries the paternal genetic information, unites with the haploid female gamete (ovum or egg), which carries genetic informations of female parent, to form a diploid zygote. The zygote ultimately, produces a diploid multicellular organism by the several repeated and organised mitotic divisions and cellular differentiation.

EXTERNAL AND INTERNAL FERTILIZATION

The fertilization always occurs in the aquatic media such as sea water, fresh water or intra-somatic (body) fluid of the maternal individual. If the fertilization occurs outside the body of the organism it is known as **external fertilization** and if it occurs inside the body of the organism then it is termed as **internal fertilization.** The external fertilization is common in various invertebrates and chordates, while the internal fertilization occurs only in those animals which possess specialized sex organs for receiving and transmitting the sperms, *e.g*, reptiles, birds, mammals and angiosperms, etc.

FERTILIZIN AND ANTIFERTILIZIN

The process of fertilization is very specific. The sperms of one particular species fertilize the ova of the same species. This type of specificity of male and female gametes is of utmost biological importance and is achieved by the help of certain chemical compounds. It is found that the egg contains a chemical substance known as **fertilizin (Lillie, 1919).** The fertilizin is a glycoprotein which is composed of different types of amino acids and monosaccharides (glucose, fucose, fructose and galactose) according to the species. The molecular weight of the fertilizin is 300,000 and it contains large molecules.

The surface layer of sperm contains another proteinous substance known as **antifertilizin.** The antifertilizin is a protein which is

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(iii) **Centrolecithal :** In the centrolecithal eggs the y mulates in the centre of the ooplasm, *e.g.*, eggs of insects.

filament touches egg membrane (plasma membrane), various important morphological and physiological changes are started in the egg.

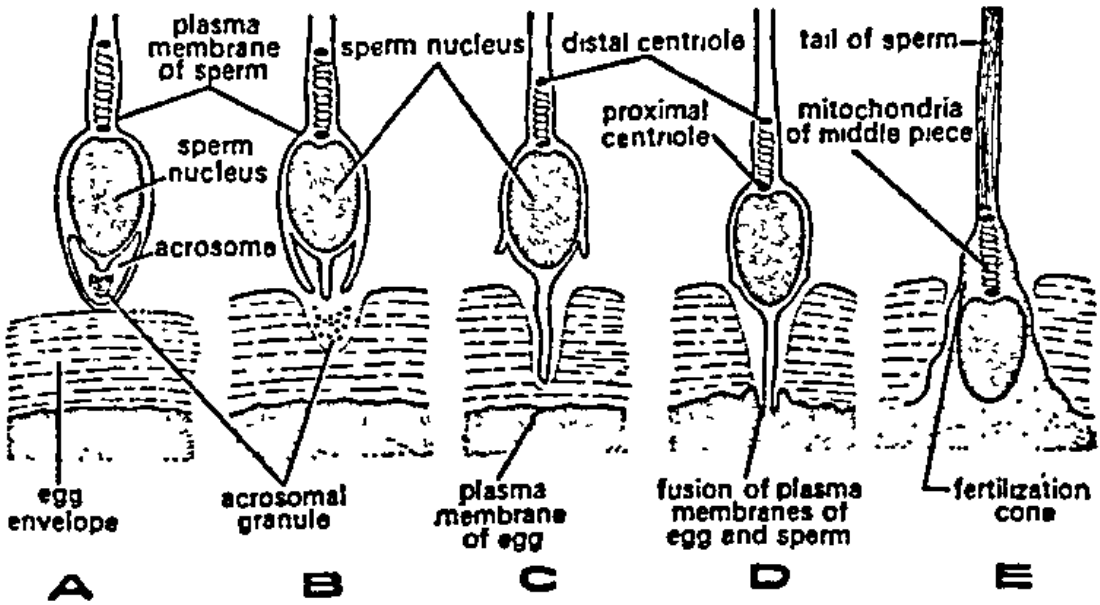


Fig. 21·1. Process of fertilization in animals

(iii) The activation of egg and insemination—As soon as the acrosomal filament touches the egg surface the ooplasm protrudes out at the point of contact into a cone-like process known as the **fertilization cone.** The fertilization cone may be conical, cylindrical or irregular. When the fertilization cone is irregular in shape it contains many pseudopodia-like processes of the ooplasm. The fertilization cone is composed of the plasma membrane and hyaline cytoplasm. The fertilization cone engulfs the sperm and the sperm which is surrounded by the hyaline cytoplasm move inwards. The penetration of the sperm in the egg is known as the **insemination.** Immediately after the insemination a thin membrane known as the **fertilization membrane** is formed around the plasma membrane of the egg. The fertilization membrane prevents the entrance of further sperms in the egg.

2. AMPHIMIXIS

During the insemination the entire sperm may enter in the egg such as in the mammals or the tail of the sperm remains out side the ch as in the echinoderms. In certain cases as in *Nereis*, the iddle piece of the sperm remain outside the egg and only d centrosome enter in the egg.

cleus the sperm is known as the **male pronucleus.** The us by absorbing the water from the surroun- es vesicular. The compactly arranged le pronucleus becomes finely granular.

composed of acidic amino acids. It has small molecules and the molecular weight is about 10,000.

The fertilizins of the eggs are supposed to attract the sperms which contain a particular type of antifertilizin. It has been found that egg fertilizin of any species reacts efficiently with the sperm antifertilizin of the same species. It has also been reported that the fertilizin in egg-water attracts the sperms of the same species and many sperms adhere together. This type of mutual adhesion of the sperms is known as the **agglutination.**

PROCESS OF FERTILIZATION

The process of the fertilization includes two successive steps which are as follows :

1. The activation of the egg.
2. The amphimixis.

1. ACTIVATION OF THE EGG

1. The process of activation of eggs is completed in following stages :

(i) Movement of the sperm towards the egg—The sperms which occur in the external or internal fluid media around the egg, swim towards the egg at randomly. They collide with the egg by chance. The chance of colliding of the sperms with the egg occurs regularly in the nature and remains fruitful only due to the large number of the sperms and enoromously large size of the ovum. The fertilizins and antifertilizins become active after the chance collision of the sperms with the ova. The egg fertilizin usually occurs in the jelly surrounding the egg. It gradually dissolves in the surrounding water of the egg and forms the so called egg water.

(ii) Activation of the sperms—When a sperm with a specific antifertilizin comes in contact with the egg water of its own species then certain significant changes occur in the acrosome of the sperm. The peripheral portion of the acrosome of sperm collapses and its enzymes the **lysins** are extruded and dissolve in the water. The central portion of the acrosome elongates and forms a 1 to 75μ long, thin tube known as the **acrosomal filament.** The acrosomal filament is the rigid tube which protrudes out from the sperm head. When the sperm possesses such an acrosomal filament it is said to be activated for the ready penetration in the unfertilized egg. When the activated sperms reach to the egg the acrosomal filaments penetrate into the egg jelly and vitelline membrane by the help of dissolving action of the sperm lysins. As soon as the tip of the acrosomal

filament touches egg membrane (plasma membrane), various important morphological and physiological changes are started in the egg.

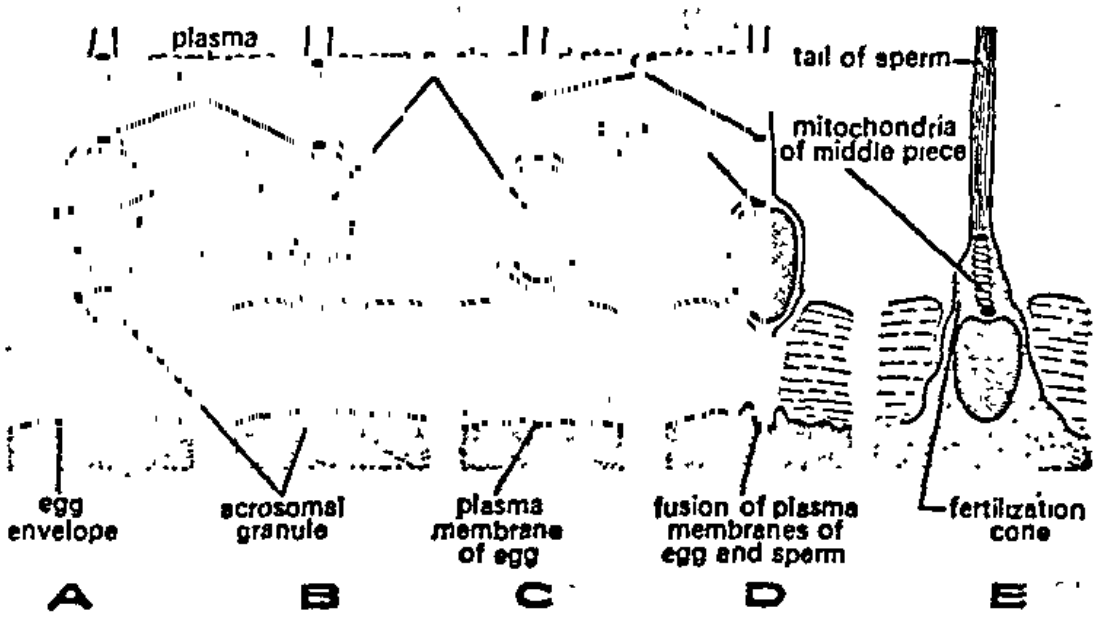


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During the insemination the entire sperm may enter in the egg such as in the mammals or the tail of the sperm remains out side the egg such as in the echinoderms. In certain cases as in *Nereis*, the tail and middle piece of the sperm remain outside the egg and only the head and centrosome enter in the egg.

The nucleus of the sperm is known as the **male pronucleus.** The male pronucleus swells up by absorbing the water from the surrounding ooplasm and it becomes vesicular. The compactly arranged chromatin material of the male pronucleus becomes finely granular.

The centriole of the sperm is surrounded by the centrosome and microtubules which form aster rays. The male pronucleus and the centriole move towards the egg pronucleus.

In the case of sea urchins and vertebrates, the two pronuclei (male and female) come close to each other and the close contact takes place between the two. The nuclear envelope is broken at the point of contact and the nuclear contents of both pronuclei are intermingled. The endoplasmic reticulum forms a new common nuclear envelope around the both pronuclei and thus forms a zygote nucleus. In case of *Ascaris*, annelids and molluscs this type of fusion of two pronuclei do not occur. In these animals the centrioles form the achromatic spindle from the microtubules of the ooplasm and both male and female pronuclei come close to each other, their nuclear envelopes are dissolved. The paternal and maternal homologous chromosomes get arranged on the equator of the achromatic figure and the first cleavage (mitotic) division of the egg occurs. After this division, the nuclear envelope is formed around the chromosomes of the daughter nuclei.

POST-FERTILIZATION CHANGES IN THE EGG

After the fertilization following changes occur in the egg :

1. The zygote becomes ready for the cleavage and for the formation of the embryo.

2. The oxygen consumption of the zygote increases enormously.

3. The metabolic rate of the zygote increases greatly. For instance, the amount of amino acids and the permeability of the plasma membrane of the egg increases the volume of the egg, decreases the exchange of phosphate and sodium ions between the zygote and the surrounding media. Further, diffusion of the calcium ions from the egg starts and the hydrolysing activities of the proteolytic enzymes increase.

4. The protein synthesis is started.

KINDS OF FERTILIZATION

In the organisms following types of fertilization occur :

1. Monospermic fertilization—In most animals usually only one sperm enters in the egg, this type of fertilization is known as **monospermic fertilization**. The monospermic fertilization is common in the coelenterates, annelids, echinoderms, bony fishes, frogs and mammals.

2. Polyspermic fertilization—When many sperms enter in the egg, the fertilization is known as the **polyspermic fertilization**. It may be of two types :

(i) **Pathological polyspermy**—Under certain abnormal conditions when in a monospermic type of egg many sperms enter in the egg, the condition is known as the **pathological polyspermy.** This type of egg does not develop further and dies soon.

(ii) **Physiological polyspermy**—In the animals with large yolky eggs such as molluscs, selachians, urodels, reptiles and birds, the polyspermic fertilization usually occurs. Such polyspermic fertilization is known as **physiological polyspermy.** In these cases, many sperms enter in the egg but the pronucleus of only one sperm unites with the pronucleus of the egg and rest are degenerated soon. Such eggs are viable and develop further.

3. **Polyandry**—When two male pronuclei unite with a female pronucleus, the union is known as polyandry, *e.g.*, man and rat.

4. **Polygamy**—When two egg pronuclei unite with single male pronucleus, the phenomenon is known as polygamy, *e.g.*, sea urchins, polychaete worm, urodels and rabbits.

5. **Gynogenesis**—When only sperm activates the egg but its pronucleus does not unite with the egg pronucleus, *e.g.*, planarians and nematodes.

SIGNIFICANCE OF FERTILIZATION

1. The fertilization ensures the usual specific diploidy of the organisms by the fusion of the male and female pronuclei.
2. The fertilization establishes definite polarity in the eggs.
3. The fertilization provides new genetic constitution to the zygote.
4. The fertilization activates the egg for the cleavage.
5. The fertilization increases the metabolic activities and rate of the protein synthesis of the egg.
6. The fertilization initates the egg to start cleavage and embryogenesis.

CHAPTER 22

PARTHENOGENESIS

Usually an unfertilized ovum develops into a new individual only after the fertilization but in certain cases the development of the egg takes place without the fertilization. This peculiar mode of sexual reproduction in which egg development occurs without the fertilization is known as the **parthenogenesis** (Gr., *parthenos*-virgin; *genesis*-origin). The phenomenon of the parthenogenesis occurs in different groups of the animals as in certain insects (Hymenoptera, Homoptera, Coleoptera), crustaceans and rotifers. The parthenogenesis may be of two types :

1. Natural parthenogenesis.
2. Artificial parthenogenesis.

NATURAL PARTHENOGENESIS

In certain animals the parthenogenesis occurs regularly, constantly and naturally in their life cycles and is known as the **natural parthenogensis**. The natural parthenogenesis may be of two types, *viz.*, complete or incomplete.

(i) Complete parthenogenesis—Certain insects have no sexual phase and no males. They depend exclusively on the parthenogenesis for the self reproduction. This type of parthenogenesis is known as the **complete parthenogenesis or obligatory parthenogenesis.**

(ii) Incomplete parthenogenesis—The life cycle of certain insects includes two generations, the sexual generation and parthenogenetic generation, both of which alternate to each other. In such cases, the diploid eggs produce females and the unfertilized eggs produce males. This type of parthenogenesis is known as the **partial** or **incomplete or cyclic parthenogenesis.**

The complete and incomplete type of natural parthenogenesis may be of following two types :

(a) Haploid or arrhenotokous parthenogenesis.

(b) Diploid or thelytotokous parthenogenesis.

(a) Haploid or arrhenotokous parthenogenesis—In the arrhenotokous parthenogenesis, the haploid eggs are not fertilized by the sperms and develop into the haploid individuals. In these cases the haploid individuals are always males and the diploid individuals are

the females, *e.g.*, 1. Insects : (i) Hymenoptera (Bees and Wasps) (ii) Homoptera, (iii) Coleoptera (*Micromalthus debilis*), (iv) Thysanoptera (*Anthothrips verbasi*). 2. Arachnids, *e.g.*, ticks, mites and certain spiders (*Pediculoidas ventricusm*). 3. Rotifers, *e.g.*, *Asplanchne amphora.*

(b) Diploid or thelytokous parthenogenesis—In the diploid parthenogenesis, the young individuals develop from the unfertilized diploid eggs. Following types of the thelytoky have been recognised :

(i) Ameiotic parthenogenesis—Sometimes during the oogenesis, first meiotic or reduction division does not occur but second meiotic division occurs as usual. Such eggs contain diploid number of chromosomes and develop into new individuals without the fertilization. This type of parthenogenesis is known as **apomiotic** or **ameiotic parthenogenesis** and occurs in *Trichoniscus* (Isopoda), *Dapnia pulex* (Crustcea) *Compelona rufum* (Mollusca), weevils and long-horned grasshopper.

(ii) Meiotic parthenogenesis—Certain eggs develop by the usual process of oogenesis but at certain stages **diplosis** or doubling of chromosome number and production of diploid eggs occur. Such eggs develop into the diploid individuals and this phenomenon is known as the **meiotic parthenogenesis.**

The **diplosis** of the diploid thelytoky may occur by the following methods :

(a) By autofertilization—In certain cases the oocyte divides meiotically upto the formation of ootid and secondry polocyte. But the ootid and the secondary polocyte unite together to form a diploid egg which develops into a new individual, *e.g.*,.*Artemia salina* (Crustacea) and various other organisms.

(b) By restitution—Sometimes in primary oocyte karyokinesis forms a nucleus of the secondary oocyte and a nucleus of the first polocyte. But the karyokinesis is not followed by the cytokinesis. The chromosomes of both daughter nuclei are arranged on the equator and undergo second meiotic division to form a diploid ootid and a diploid polocyte. The diploid ootid or ovum develops into a parthenogenetic diploid individual. This type of diplosis is known as the restitution, *e.g.*, insects of order Hymenoptera (*Nemertis conesceus*) and Lepidoptera

ARTIFICIAL PARTHENOGENESIS

The eggs which always develop into the young individuals by the fertilization sometimes may develop parthenognetically under

certain artificial conditions. This type of parthenogenesis is known as **artificial parthenogenesis.** The artificial parthenogenesis may be induced by various physical and chemical means.

A. Physical means—The following physical means cause the parthenogenesis.

(i) Temperature—The range of temperature may induce parthenogenesis in the eggs. For instance, when the egg is transferred from the 30°C to 0–10°C the parthenogenesis is induced.

(ii) Electrical shocks can cause parthenogenesis.

(iii) Ultraviolet light can cause parthenogenesis.

(iv) When the eggs are pricked by the fine glass needles the development of young ones takes place parthenogenetically.

B. Chemical means—The following chemicals have been found to cause parthenogenesis in the normal eggs :

1. Chloroform 2. Strychuine 3. Hypertonic and Hypotonic sea waters 4. Chlorides of K^+, Ca^{++}, Na^{++}, Mg^{++}, etc. 5. Acids as butyric acid, lactic acid, oleic acid and other fatty acids. 6. Fat solvents, *e g.*, toulene, ether, alcohol, benzene and acetone. 7. Urea and sucrose.

The artificial parthenogenesis has been induced by above mentioned physical and chemical means by various workers in the eggs of most echinoderms, molluscs, annelids, amphibians, birds and mammals.

SIGNIFICANCE OF PARTHENOGENESIS

1. The parthenogenesis serves as the means for the determination of sex in the honey bees, wasps, etc., and it supports the chromosome theory of inheritance.

2. The parthenogenesis is the most simple, stable and easy process of the reproduction, *e.g.*, aphids (insects).

3. The parthenogenesis eliminates the variation from the population, but encourages development of the advantageous mutant characters.

4. The parthenogenesis causes the polyploidy in the organisms

5. Due to the parthenogenesis, there is no need for the organisms to waste their energy in the process of mating but it allows them to utilize that amount of energy in the feeding and reproduction.

GENETICS AND EUGENICS

CHAPTER 1

INTRODUCTION

This is an evident fact that the living beings have unique individuality. Each person is recognised from other persons in a variety of ways. Similarly the dogs of the same breed are distinguishable each other by certain structural differences. The characters (structural differences) which provide individuality to a species are said to cause variation among the species. Among the species, the variations may be of two kinds, *viz.*, environmental and hereditary or genetical.

1. ENVIRONMENTAL VARIATIONS

The variations caused by the environmental factors such as [illegible] other ecological factor are known [illegible] ample, the differences between [illegible] person are environmental because [illegible]

2. HEREDITARY VARIATIONS OR GENETICAL VARIATIONS

Among the sexually reproducing organisms, no two individuals have the same heredity. The differences in the hereditary constitutions of the individuals of a species are known as **hereditary or genetical variations.**

Thus, the species differ from each other in many respects but when we observe various individuals of a species, it becomes evident that how much the individuals of same species may differ from each other but basically they have a fundamental plan of construction. For example, although the dogs differ more or less from one another, they do not differ in ways that permit them to be confused with the elephants or some other animal or with plants. Moreover, we are already aware that the offsprings of dogs are always dogs never cats or elephants. These examples clearly indicate that the parents transmit their characters to their offsprings and thus maintain the continuity of their race in the evolutionary process. The process of trans-

variations in living beings (viruses, bacteria, plants and animals) is known as Genetics. The word genetics was derived from the Greek root *gen* which means to become or to grow into and it was coined by Bateson in 1906 for the study of physiology of heredity and variations.

HISTORICAL

The science of genetics is a very young science in comparison to other biological sciences and its origin can be traced in the works of Mendel in the nineteenth century. But before Mendel's work men throughout the ages had some vague knowledge about genetics and more often have tried to explain the causes of heredity. About six thousand years ago men kept records of pedigrees of domestic animals such as horse and food plants as rice. The ideas or theories which have been forwarded time to time explain the phenomenon of inheritance can be categorized under ther following headings :

1. Vapour and fluid theories.
2. Preformation theories.
3. Particulate theories.

1. VAPOUR AND FLUID THEORIES

Early Greek philosophers such as Pythagoras (500 B. C.) proposed that every organ of animal body gives out some type of vapours. These vapours unite and form a new individual.

Further, Aristotle thought that the semen of man has some "vitalizing" effect and he considered it as the highly purified blood. According to him the mother furnishes inert matter and the father gives the motion to the new life.

2. PREFORMATION THEORIES

Leonardo da Vinci (1452-1519) proposed a theory that the male and the female parents contribute equally to the heredity of the offspring. The German botanist Kolreuter (1733-1806) after getting fertile hybrid by artificially crossing two species of

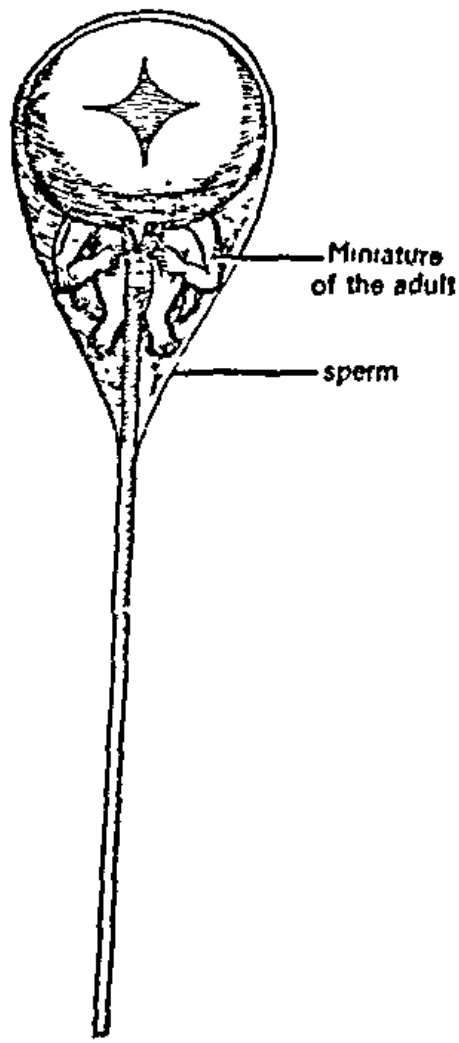


Fig. 1·1. Diagram of homunculus.

tobacco concluded that gametes are the physical basis of heredity. After the discovery of sperm and egg in eighteenth century the Dutch Scientist Swammerdum propounded the theory that the sex cells have the miniature copy of adults which were named as mankins. N. Hartsoecker (1965) has actually produced a drawing of a sperm containing a little man in it and named it as homunculus. This type of theories which advanced the concept of preformed embryo in the sex cells are known as preformation theories. The preformation theories persisted upto nineteenth century until these were rejected by a German investigator Wolff by offering experimental evidences that no preformed embryo exists in the egg of the chick

3. PARTICULATE THEORIES

French biologist Maupertuis (1689-1759) has proposed that the body of each parent gives rise minute particles. In sexual reproduction, the particles of both individuals unite together to form a new individual. He thought that in certain cases the particles of the male parent might dominate on those of the female parent and produce the male individual. In the production of female individual the particles of female might dominate on particles of male.

The great biologist Lamarck (1744-1829) believed in the phenomenon of "inheritance of acquired characters" among living organisms. But he failed to provide convincing evidences in support of his concepts.

In the 19th century the well known naturalist Charles Darwin has given his famous theory of pangenesis which exclusively depends on the particulate theory. The central idea of pangenesis theory has been given first of all by Hippocrates (400 B. C.). According to the pangenesis theory of Darwin each part of the animal body produces many minute particles known as gemmules. These gemmules are at first collected in the blood and later on are concentrated in the reproductive organs. When the animal reproduces into new individual, these gemmules pass on to it and it has blending of both parents. By this mechanism acquired characters would also be inherited because as the parts of the body changed so did the pangenes or gemmules they produced.

The theory of pangenesis was disapproved by Galton (1823-1911) and Weismann (1835-1934). Weismann postulated the theory of germplasm to explain heredity. According to this theory the body of organisms contain two types of cells namely somatic cells and reproductive cells. The somatic cells form the body and its various organ systems, while the reproductive cells form sperm and ova. The somatic cells contain the somatoplasm and germinal or reproductive

cells contain the **germplasm**. According to Weismann the germplasm can form somatoplasm but somatoplasm cannot form germplasm. Thus, the changes in the structure of somatic cells or somatoplasm which are caused by the environment (acquired characters) can not influence the reproductive cells or germplasm. By cutting the tails of mice for many generations, Weismann always got tailed mice. So, by such experimental evidences he rejected the Lamarckism and pangenesis theory.

Though the particulate theory faced many problems in its beginning but its basic concept has formed the central core of the modern understanding of the genetics.

Augustinian Monk **Gregor Mendel** was the first investigator who laid the foundation of our modern concept of the particulate theory. He could understand the heredity problems more clearly than any one in the past, because his approach was simple, logical and scientific. By his famous experiments on pea plant he concluded that the inheritance is governed by certain factors which occur in the cells of each parent. He thought that each parent has two such factors, while their sex cells (sperm or pollen and ovum or egg) have only one factor. However, he failed to explain the exact process by which these factors pass on to the sex cells.

The further progress of the genetical science was enhanced by various cytological investigations. **Fleming** (1882) investigated the proces
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total of heredity, while, phenotype of an individual represents the observable structural and functional properties which are produced by the interaction between genotype and environment. **T. H. Morgan** (1866-1945) made investigations on the nature of genes and received Nobel Prize in 1934. Few years later **H. J. Muller** got Nobel Prize in 1946 for the discovery of the induction of mutation in *Drosophila* by X-rays.

G. W. Beadle and **J. Lederberg** made various contributions to biochemical aspects of genetics and got Nobel Prize in 1958. The chemistry of DNA and RNA has been worked out by **A. Kornberg** and **S. Ochoa**; both got Nobel Prize in 1959. **Watson, Crick** and **Wilkins** got Nobel Prize in 1962 for the discovery of double helix

model of DNA which opened the new vistas in the genetical world. In 1965 Jacob, Monod and Lwoff made the discovery of a class of genes which regulate the activity of other genes. In 1968 Nirenberg and Khorana discovered the complicated DNA code known as Genetic code. Both scientists along with Holly received the Nobel Prize in 1968.

IMPORTANCE OF GENETICS

The cultural evolution of man has deep impact of the genetics on it. Because the genetics has helped man at various stages in improving the breed and varieties of various food plants and domestic animals which might have been necessary for his survival and culture.

In modern era the genetics has revolutionised the agriculture, horticulture, animal husbandry and many other branches of science. The science of genetics has proved worthy in removing many faulty concepts of man about the inheritance. Further it has opened a new era in the science of [illegible] of various inherited [illegible] betes, anemia and fe[illegible]

BRANCHES OF GENETICS

The modern geneticists have divided the science of genetics into many branches. Some of the important brances of genetics are following :

1. **Microbial genetics**—It deals with the genetics of microorganisms (*viz.*, viruses, bacteria, unicellular plants and animals).
2. **Plant genetics**—The genetics of plants.
3. **Animal genetics**—The genetics of animals.
4. **Human genetics**—The genetics of man.
5. **Cytogenetics**—It provides the cytological explanations of different genetical principles.
6. **Molecular genetics**—It interprets most genetical phenomena in terms of chemical molecules.

CHAPTER 2

GENETICAL TERMINOLOGY

Like other sciences, the science of genetics has its specific terminology which minimizes the chances of confusion, inconvenience and unnecessary repetition of full sentences. We are giving here certain most common terms which are used more frequently in genetics.

Acquired character—The alteration in the morphology or physiology of an organisms in response to its ecological factors (environment) is known as acquired character. Acquired characters are usually not heritable.

Albino—The animal without pigmentation in skin, hairs and eyes is called albino.

Allele (Allelomorph)—Two or more alternative forms of a gene are called **allelomorph** or **alleles**. For example, gene for the length of the pea stem may occur in two alternative forms . allele (T) for the tallness of the stem and allele (t) for the dwarfness of the stem.

Autosome—The chromosomes which are not associated with sex are known as autosomes. Except the sex chromosomes (X) and (Y) other chromosomes are the autosomes.

Back cross—The cross of a progeny individual with its parents is known as back cross.

Bivalent—A pair of synapsed ,homologous chromosomes is known as a bivalent.

Carrier—A heterozygous individual, which carries an unexpressed recessive sex-linked gene in it, is known as a carrier.

Chromatin—A DNA, RNA, histone and non-histone protein containing thread-like coiled structure of interphase nucleus is called chromatin.

Chromosome—The nucleoprotein structure which are generally more or less rod-like during nuclear division. The genes are arranged on the chromosomes in a linear fashion. Each species has a characteristic number of chromosomes. Chromosomes play most important role in inheritance.

Codominance—When both the alleles (dominant and recessive) are equally expressed in the hybrid, the phenomenon is known as codominance, *e.g.*, when the red and white cattles are crossed, they produce a roan offspring which possess both red and white hairs on the skin.

Crossing over—Crossing over is a phenomenon in which exchange of chromosomal segment or genetic material occurs between the non-sister homologous chromosomes.

Deoxyribonucleic acid (DNA)—It is a genetic material of many viruses, bacteria, plants and animals. It is a double stranded, helically coiled, macromolecule which is composed of phosphoric acid, deoxyribose sugar, two pyrimidenes (cytosine and thymine) and two purines (adenine and guanine). It is found to be most stable biological molecule which contains encoded genetic informations.

Dihybrid—An individual which is hybrid or heterozygous in two pairs of alleles or allelomorphs is known as dihybrid. For instance, the cross between pea plants with yellow round seeds and green

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sets of chromosomes is known as diploid.

Dominance—A phenomenon in which one member of a pair of allelic genes expresses itself as a whole (complete dominance) or in part (incomplete dominance).

Dominant trait—When out of two contrasting characters or traits only one expresses or appears in a generation. That trait is known as dominant trait, *e.g.*, in pea, round character of seed is dominant over wrinkle character of seed.

F_1 or First filial generation—The word filial is derived from the Latin word *filii*, meaning the son. The first generation of a given cross is known as F_1 generation.

F_2 or Second filial generation—The second generation which

selfing of F_1 offsprings is known as

g haploid set of chromosomes and arising due to meiotic cell division of diploid germ cell is known as gamete. The male gamete is known as pollen or sperm and female gamete is known as ovum or egg.

Gene—A particular segment of DNA molecule which determines the heredity of a particular trait is called gene.

Genome—A complete set of chromosomes which is inherited as a unit from one parent is known as genome.

Genotype—The genetic make up or genic constitution of an individual is known as its genotype. A pure red flower pea plant has genotype RR.

Gynandromorph or gynander—When the body of an individual exhibits both the male and female characters, this type of individual is known as gyanandromorph.

Haploid (Monoploid)—An individual or cell containing a single complete set of chromosomes is known as haploid.

Haemophilia—A metabolic disorder characterized by free bleeding even from slight wounds because of the lack of clot forming substances. It is linked with a gene occurring in sex chromosomes.

Heterogametic sex—A sex containing either only one or two different sex chromosomes such as XO, XY or ZW produces two types of gametes half with X or Z chromosome and other half with Y or W or no chromosomes. This type of sex is known as heterogametic sex.

Heterozygote (Heterozygous)—An individual containing both dominant and recessive genes or traits or characters of a allelic pair is known as heterozygous or hybrid.

Holandric gene—A gene which occurs only in Y chromosome is known as holandric gene.

Homogametic sex—The sex which possesses two identical sex chromosomes (XX or ZZ) and produces single type of gametes each with X or Z chromosome is known as homogametic sex.

Homologous chromosomes (Homolog)—The identical male and female parent chromosomes occur in pairs and are known as homologous chromosomes. Each chromosome of a homologous pair is known as homolog.

Homozygote (Homozygous)—The organism having two similar genes for a particular character in a homologous pair of chromosomes is known as homozygous or genetically 'pure' for that particular character.

Independent segregation—The independent behaviour of genes occurs on different pairs of chromosomes. This phenomenon is known as independent segregation.

Linkage—The occurrence of many different genes in a single chromosome is known as linkage and the genes are known as linked genes.

Meiosis—Meiosis is the reduction division in which the diploid or somatic chromosome numbers are reduced to half. The meiosis often produces haploid gametes or individuals.

Monohybrid—When the cross takes place between the parents differing in a single pair of contrasting characters resulted into a monohybrid individual.

Monohybrid cross—The cross between the two parents differing in a single pair of contrasting characters is known as monohybrid cross.

Phenotype—The external appearance of an organism for a parti-

cular contrasting character is the phenotype. It is expressed in words such as "tallness", "dwarfness", etc.

[illegible] phenotype

phenotype, in

y colour

is brown and a hereditary variant has yellow colour, when the larvae of wild type *Drosophila* with brown body colour are raised on food containing silver salts, they develop into yellow bodied flies. Thus, these flies are the phenocopies of yellow mutant, but would give rise to wild type brown flies in normal environment.

Progeny—Offspring individuals.

Recessive trait—Out of two contrasting traits when a single trait fails to express itself in the successive generation is known as recessive character, or trait, *e.g.*, in the cross between tall and dwarf pea plant, the dwarf trait remains recessive.

Sex chromosome—The chromosomes which do not occur in identical pairs and determines the sex of the individual are known as sex chromosomes, *e.g.*, X and Y chromosomes.

Sex-linked gene—The genes occurring only on the sex chromosomes are known as sex-linked genes.

Siblings or Sibs—The individuals having same maternal and paternal parents, *e.g.*, brother sister relationships.

Spermatogenesis—The process of sperm formation is known as spermatogenesis.

Syngamy—The union of sex cells or gametes in reproduction is known as syngamy.

Test cross—The cross of a F_1 hybrid with its recessive parent is known as a test cross.

Trihybrid—The individual which is heterozygous for three pair of alleles is known as trihybrid.

Wild type [illegible] phenotype among the memb[illegible]

[illegible] e union of male and female sex cells, *i.e.*, sperm and ova, is known as zygote.

SYMBOLS IN GENETICS

An organism with diploid (2X) cells has paired chromosomes and out of two contrasting characters each chromosome contains only one gene or trait for a single character. According to the classical method of symbolization, the dominant character is expressed in capital letter as the tall character is represented by 'T' and the rece-

ssive character is represented by 't'. Now homozygous tall plant will contain the genotype 'TT' and likewise a homozygous dwarf plant will have the genotype "tt'. Because the sperm or ova contains only one chromosome of a homologous pair, therefore, it contains only single gene, e.g., T or t. A heterozygous has both dominant and recessive characters, therefore, its genotype can be expressed by 'Tt' letters.

Recently, the classical method of symbolization has been modified a little. In the **modified classical method of symbolization**, like classical method, capital and small letters are commonly used to designate dominant and recessive alleles, but in contrast to that, the genetic symbol corresponds to the first letter in the name of the abnormal, recessive or mutant trait. For example, in man the recessive trait of albino is represented by letter 'a' while the normal trait is represented by capital letter 'A'.

In modern genetical literature one more method of symbolization called **"wild type symbolism"** is widely used. When out of two phenotypes, one phenotype is of much more common occurrence in the population than its alternative phenotype, the former is usually referred to as **wild type**. The phenotype which is rarely observed in the population is called the **mutant type**. In this system, the symbol plus (+) is used to indicate the normal allele for wild type. The base letter for the gene usually is borrowed from the name of the mutant or abnormal trait. If the mutant gene is recessive the symbol would be a lower case letter corresponding to the initial letter in the name of the trait. Its wild type or normal dominant would have the same lower case letter but with a^+ as a superscript. For example, black body colour in *Drosophila* is governed by a recessive gene b and wild type (gray body) by its dominant allele b^+.

However, if the mutant trait is dominant, the base symbol would be an upper case letter without a super script and its recessive wild type allele would have the same upper case letter symbol with + as a superscript. For example, lobe-shaped eyes in *Drosophila* are governed by a dominant gene L, and wild type (oval type) by its recessive allele L^+.

CHAPTER 3

MENDEL'S LAWS OF INHERITANCE

The Austrian monk **Gregor Mendel** is considered as the pioneer of modern genetics. He was born in July 22, 1822 in Heinzendorf in Austrian Silesia. He graduated from the Gymnasium in 1840. In his youth, he led a disastrous, poor, difficult and sad life. During 1854 to 1868, Mendel taught physics and natural sciences in a higher secondary school of Brunn and besides teaching, performed certain hybridization experiments on fruit trees, flowers, vegetables and more especially on garden peas. He published his conclusion in 1866 in the **"Annual Proceedings of the Natural History Society"** of **Brunn.** Unfortunately his remarkable piece of work remained unattended for many years probably because the biologist of that time were busy in removing the confusions and controversies which might have been arisen by the publication of **Darwin's "Origin of Species".** It might also be possible that biologists of his time could not understand his statistical approach and could not appreciate his work. It was in the beginning of 20th century that three botanists namely **Hugo de Vries** of Holland (working on *Oenothera*); **Karl Correns** of Germany (working on *Xenia*, peas and maize) and **Eric Von Tschermak** of Austria (working on various flowering plants) independently drawn the conclusion like Mendel. Later, these botanists came across the research paper of Mendel and rediscovered it in 1900. Mendel's original paper was republished in **Flora**, 89,364 (1901). **Bateson** confirmed Mendel's work by a series of hybridization experiments.

The approach of Mendel was simple, logical, scientific, mathematical and analytical, probably, because he was well aware about the failures of his predecessor hybridists. The pre-Mendelian hybridists made observations on plants and animals as a whole and studied all variations in the hybrid at a time. Mendel however, concentrated on a particular character and at a time studied the pattern of inheritance of only one or two character or characters.

MENDEL'S CONSIDERATIONS

For his hybridization experiments Mendel had certain considerations in his mind about the choice of a suitable material, Mendel's considerations about the material were as follows :

1. Variation—The organisms which are to be chosen for the genetical experiments, should have a number of detectable differences and at a time only single detectable character should be considered.

2 Reproduction—The chosen organisms should be sexually reproducing (*i.e.*, by fusion of male and female sex cells) because only then the offsprings will be able to receive different characters from both the male and female parents.

3. Controlled mating—The chosen organisms should be able to mate in controlled or well-planned conditions. Because in genetical experiments sometimes we have to rear genetically pure parents by methods of controlled mating. One should maintain careful records of the offsprings of many generations.

4. Short life cycle—The chosen organisms should have very short life cycles.

5. Large number of offsprings—The organisms which have been chosen for the genetical experiments should produce large number of offsprings after each successive mating because it will help in deducing the correct conclusions.

6. Convenience in handling—The experimental species should be of a type that can be raised and maintained conveniently and inexpensively in the laboratory. For instance, the elephant will prove

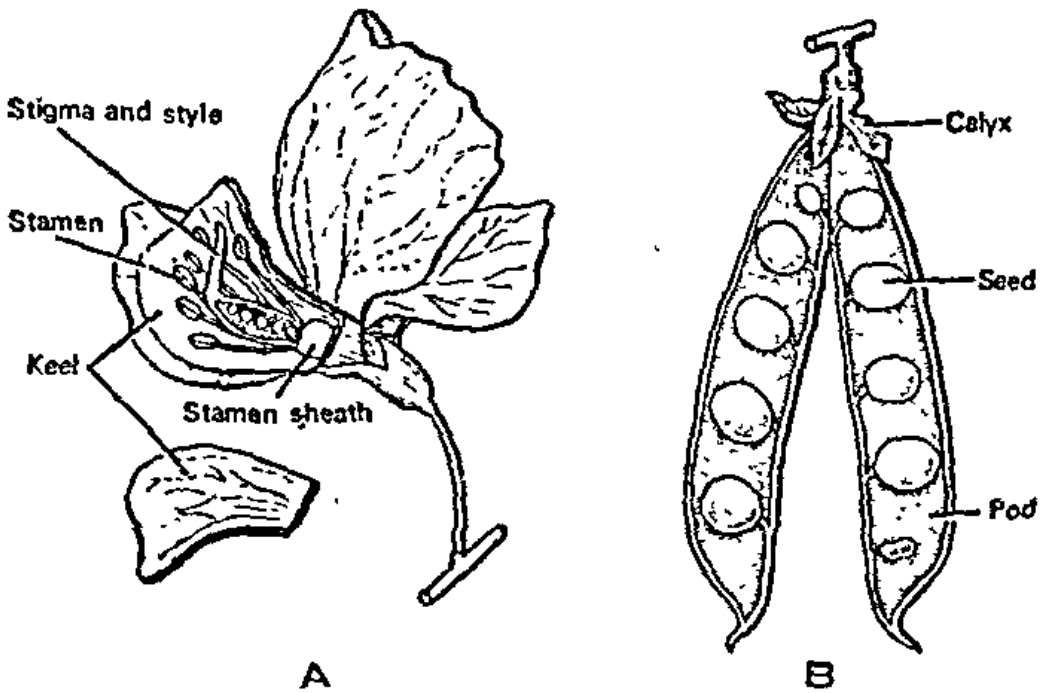


Fig 31. A—A self-fertilizing flower of *Pisum sativum*. A—Portion of the keel which encloses the reproductive organs has been cut open to show the stamen and stigma B—The mature fruit (pod) containing seeds that develop from the fertilized pea flower.

entirely useless material for genetical experiments than the *Drosophila*, pea plants, tomato, rats, guinea pigs, etc., which have been generally used and are still used in hybridization experiment.

MENDEL'S MATERIAL

Mendel found edible pea (*Pisum sativum*) a best material for trasting *tall* or *dwarf*, cotyledons may be *green* or *yellow*; seeds may be *round* or *wrinkled*, seed coat may be *coloured* or *colourless*; the unripe pods may be *green* or *yellow*; the ripe pods may be *inflated* or *constricted* between the seeds, flowers may have *axial* or *terminal* positions and the colours of flowers may be *red* or *white*. Besides these contrasting characters, the pea plant is a very satisfactory material for the hybridization experiments due to its flower structure. The flowers of pea plants are so constructed that the pollens of a flower normally fall on the stigma of the same flower and thus effects self-pollination or self-fertilization. Mendel easily prevented self-fertilization by removing the stamens from the flower buds. Because, for genetical experime ssity, so Mendel c a particular pa of one flower on the stigma of other flower whose stamens have already been removed. Further to prevent artificially pollinated flower from the contamination by pollens of unknown source by insects, Mendel covered the flowers by the bags.

MENDEL'S PROCEDURE

For getting the exact results in the breeding experiments, it was for Mendel to raise genetically pure varieties of

for dwarf pea plants. Mendel cross pollinated these two varieties of pea plants which were differing in a pair of contrasting characters, *viz.*, tallness and dwarfness of the stem. When he made observations on the offsprings of first generation he found only tall plants. He allowed the self pollination in the offsprings of first generation and made further observations on the offsprings of second generation. He was astonished to note both tall and dwarf offsprings in the second generation. This showed him that the character of dwarfness disappeared in first generation but again reappeared in second generation.

Further, the tall and dwarf plants of second generation were always in the ratio of 3:1 (3 tall : 1 dwarf). He self-pollinated the dwarf offsprings of second generation and found only dwarf plants of third generation. But when he self-pollinated the tall plants of second gene-

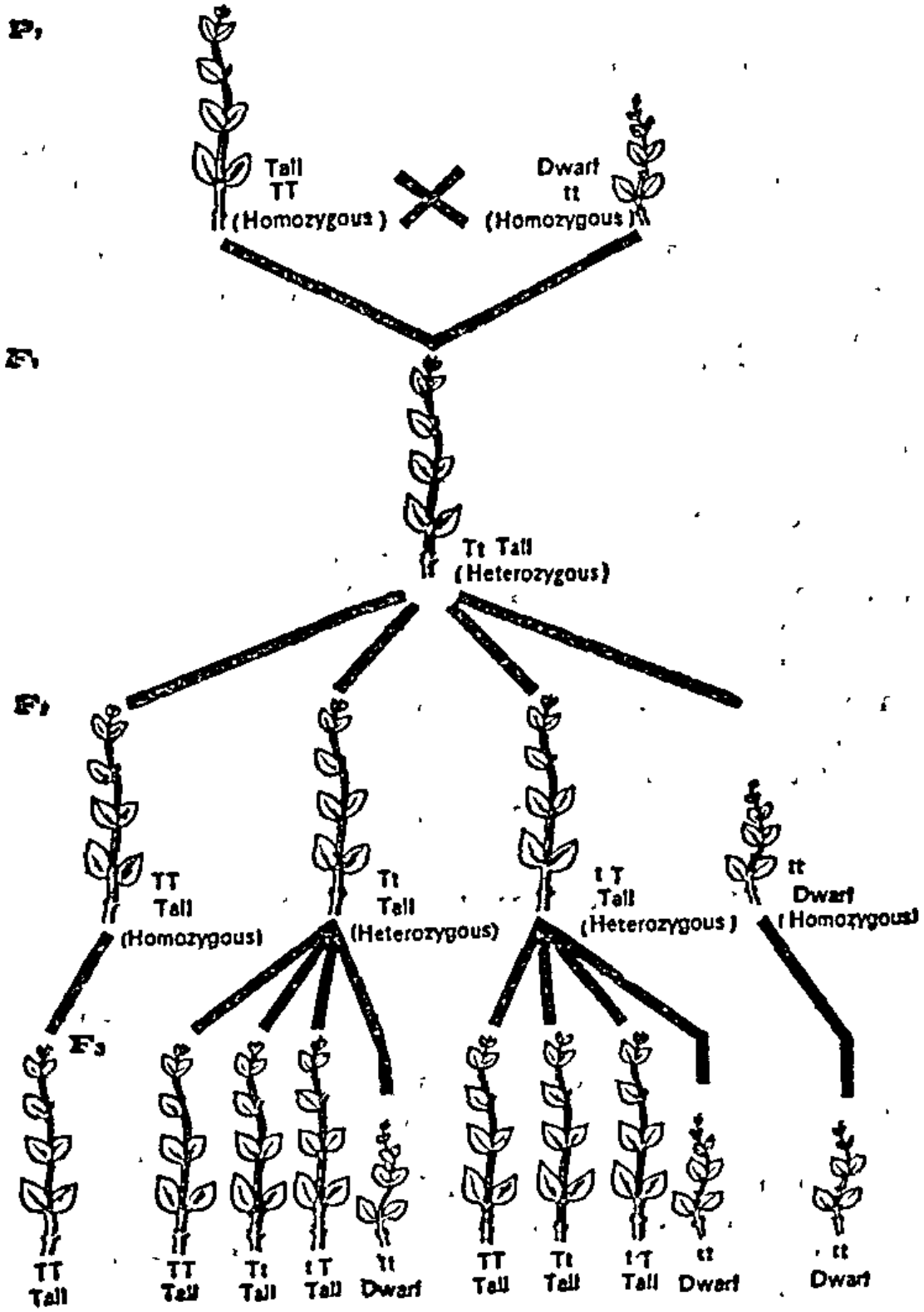


Fig. 3·2. A cross between a tall (TT) and a dwarf (tt) pea plant and their offsprings of F_1 and F_2 generations.

ration then he found that one-third (1/3) tall plants yield only tall plants in third generation, while rest two-third (2/3) tall plants yield tall and dwarf plants in the ratio of 3 : 1.

On the basis of the results of his experiments Mendel formulated following three laws :

1. Law of dominance.
2. Law of segregation.
3. Law of independent assortment.

Actually Mendel himself did not postulated any genetical principle or laws, he simply gave conclusive theoretical and statistical explanations for his hybridization experiments in his research paper. However, it was Correns, the discoverer of Mendel's work, who thought that Mendel's discovery could be represented by these fundamental laws of heredity.

LAW OF DOMINANCE

T[illegible]
rasting
noticed
ar[illegible]
fil[illegible]
[illegible] appeared in the second filial generation or F_2. This shows that in F_1 hybrid the character of tallness dominates or conceals the character of dwarfness and so the character of d[illegible] could not express itself in F_1 generation. The character whi[illegible] called by Mendel a[illegible] h re-

Table :

Character studied	Dominant	Recessive
1. Stem length	Tall	Dwarf
2. Flower position	Axial	Terminal
3. Flower colour	Red or purple	White
4. Pod colour	Green	Yellow
5. Pod shape	Inflated	Constricted
6. Seed form	Round or smooth	Wrinkle
7. Cotyledon colour	Yellow	White

mained unexpressed or latent had been called recessive. According to these results Mendel formulated the law of dominance which states that *in crossing between pure (homozygous) organisms for contrasting characters of a pair, only one characters of the pair appears in the first filial generation.*

In pea plant Mendel found following characters to be dominant or recessive in various pairs of contrasting characters :

CERTAIN EXAMPLES OF LAW OF DOMINANCE

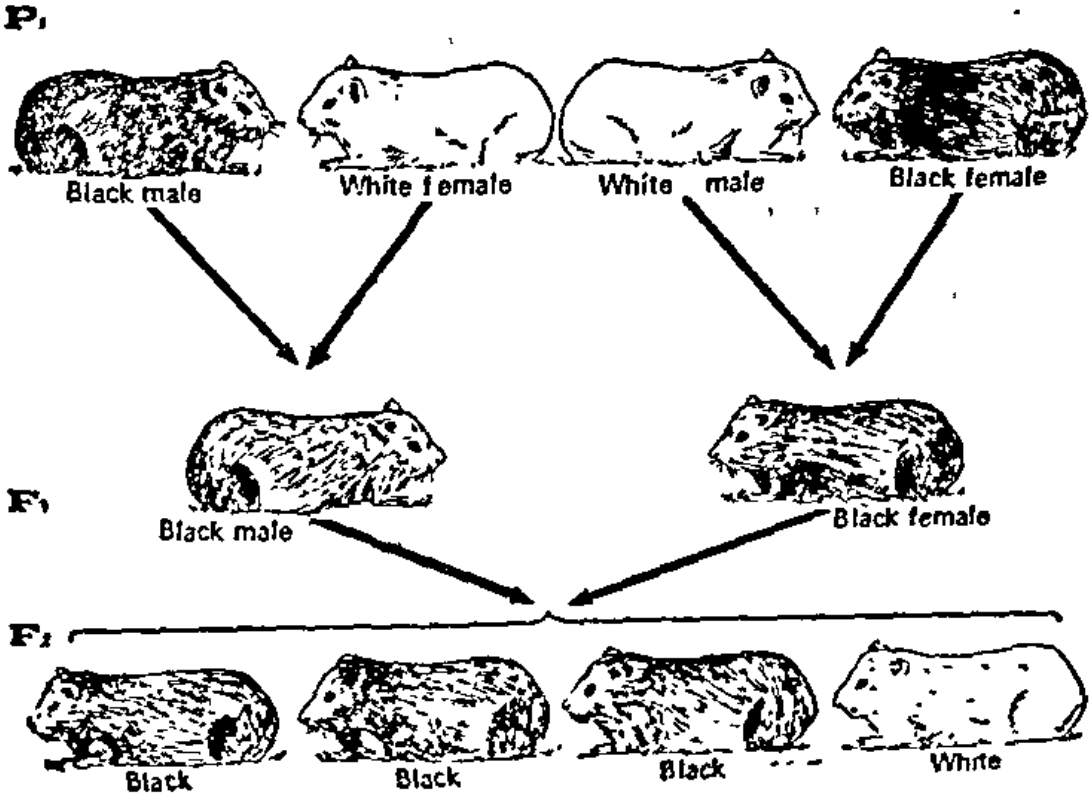
After Mendel several geneticists tested the validity of the law of dominance on several plants and animals. They found its wide application in various plants and animals. A few important examples are listed below.

1. LAW OF DOMINANCE IN PLANTS

Besides pea plant the law of dominance has also been observed in the following plants :

2. APPLICATION OF LAW OF DOMINANCE IN ANIMALS

A. The law of dominance is also applicable well to the animals. For instance, when a homozygous black guinea pig is crossed with a homozygous white guinea pig (fig. 3·3) then all hybrids of first filial generation (F_1) are found to be black. The black hybrids of F_1 when mated among themselves they produced black and white off-



springs in 3 : 1 ratio. This shows that black coat colour dominates over white coat colour.

Table : The dominant and recesssive characters in plants.

Name of the plant	Dominant	Recessive
1. Nettle	Serrated leaves	Smooth margined leaves
2. Sunflower	Branched habit	Unbranched habit
3. Cotton	Coloured lint	White lint
4. Maize	Round starchy kernel	Wrinkle, sugary kernel
5. Snapdragon	Red flower	Non-red flower
6. Barely	Beardlessness	Beardness
7. Wheat	Susceptibility to rust	Immunity to rust
8. Tomato	Two celled fruit	Many celled fruit

B. Certain other examples of law of dominance in animals— The dominant and recessive traits or characters of some animals can be tabulated in following table :

Table : The dominant and recessive characters in animals.

Name of animal	Body character	Dominant	Recessive
1. Cat	Skin colour Length of hair	Tabby Short hairs	Black or blue Long hairs (Angora)
2. Dog	Skin colour Tail	Grey Stumpy	Black Normal tail
3. Cattle	Colour of face Horn	White Polled or hornless	Coloured Horned
4. Horse	Skin colour *Movement*	Black *Trotting*	Red *Pacing*
5. Sheep	Hair or wool or fleece	White	Black
6. Swine or Pig	Skin colour Hoof	Black Uncleft	Red Normal
7. Salamander	Body colour	Dark	Light
8. Fruit fly (*Drosophila*)	Eye colour Wings Body colour	Red Flat and yellow Grey	White Curled and white Black
9. Land snail	Shape of shell	Unbanded shell	Banded shell

C. Dominant and recessive characters in man—In man various types of dominant and recessive characters have been reported. According to their nature these can be divided into normal, abnormal, and sex-linked characters.

(i) **Normal characters**—These characters always behave as dominant and recessive. These are tabulated as follows :

Body part	Character	Dominant	Recessive
1. Hair	Form Colour	Curly Dark	Straight Light
2. Skin	Colour Pigment	Dark Normal (with melanin)	Light None (albinism without melanin pigment)
3. Eyes or Iris	Colour	Brown	Blue

(ii) **Abnormal characters**—The abnormal characters occur unusually and can be tabulated as follows :

Body part	Dominant	Recessive
1. Hair	Absent	Present
2. Skin epidermis	Thickened	Normal
3. Fingers	Short Webbed Extra digits	Normal Normal Normal
4. Ear	Normal hearing	Deaf mutism
5. Ear lobe	Free ear lobe	Attached ear lobe
6. Eyes	Opaque lens Glaucoma	Normal Normal
7. Teeth	Absent	Present
8. Tongue	Ability to role	Inability to role
9. Taste of phenyl thiocarbamic (PTC)	Taste bitter to PTC	Tasteless to PTC

(iii) **Sex-linked characters**—Certain dominant and recessive characters depend on the sex of individual and are sex-linked. These are as follows :

1. Colour vision: Normal (Dominant) and Colour blind (Recessive).

2. Blood clotting: Normal (dominant) and Haemophilia (Recessive).

MECHANISM OF DOMINANCE

Mendel carefully studied the results of his experiments and it became evident to him that there is a clear cut difference in between the actual visible character and that something which caused its production. Because the character cannot be present as such in sex cells (sperm and ova) which form the only link between a new individual and the parents. Therefore, there must be something which represent the characters and is responsible for their production. This something of Mendel is now called the factor or gene. The gene thus can be considered as the unit of inheritance that is transmitted in a gamete and determines or controls the development of a character by interaction with the other genes, the cytoplasm and the environment.

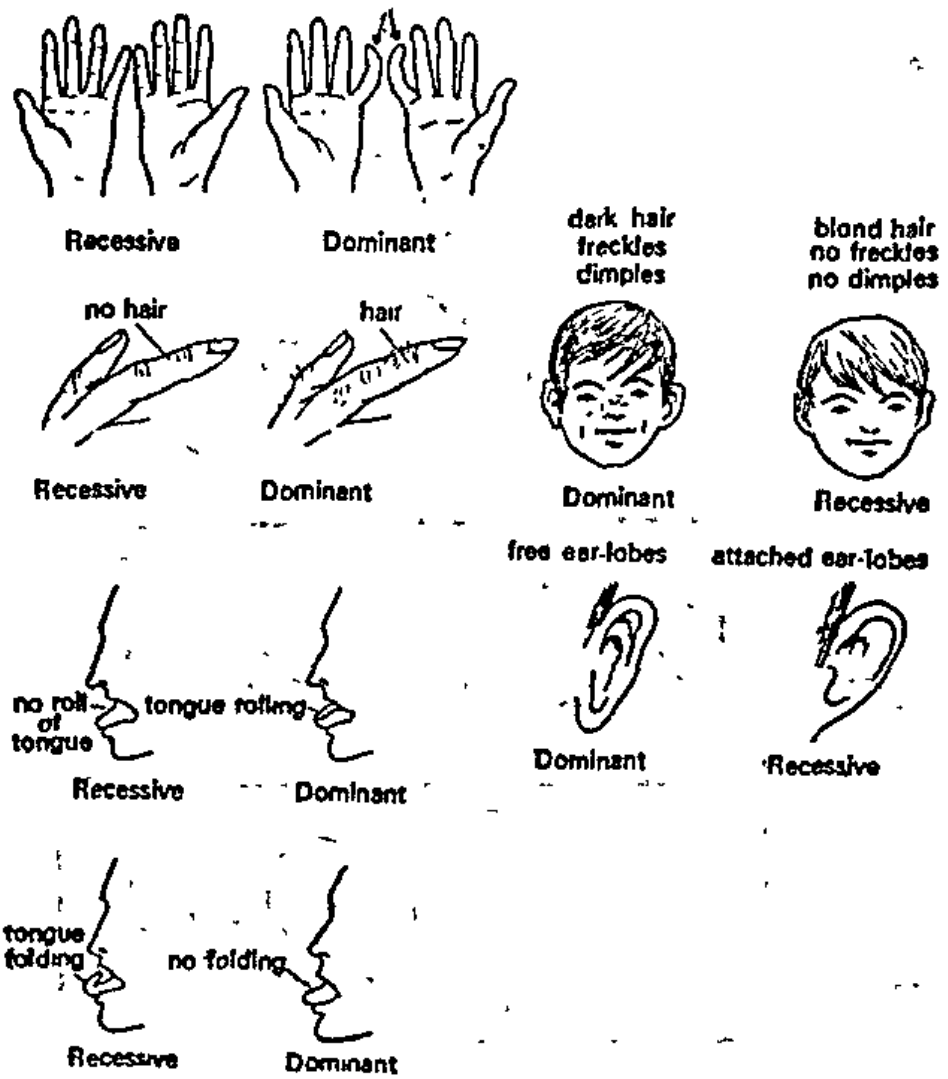


Fig. 3·4. Certain abnormal recessive and dominant characters of man.

The cytological investigations have now established that the genes are the units of deoxyribonucleic acid (DNA) which along with ribonucleic acid (RNA) and other nucleoproteins constitute the thread-like stainable structures the chromosomes. The chromosomes are specific in number, shape and size to a particular species.

A diploid cell has two sets of chromosomes which come from two different parents (male and female) via gametes (sperm and ova).

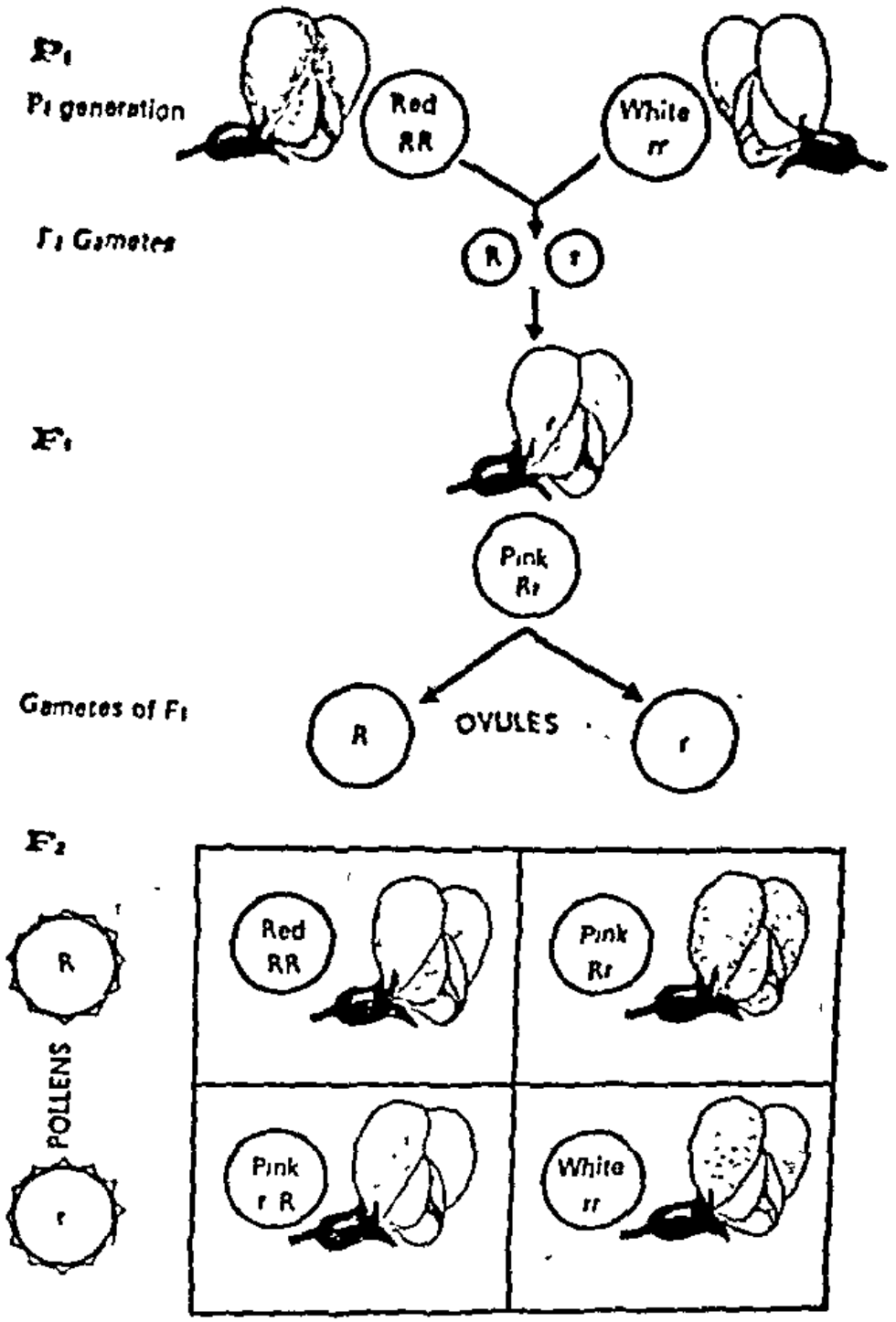


Fig. 3.5. A cross between a red flowered and a white flowered pea plant showing incomplete dominance.

The chromosomes of similar size and nature often form pairs during meiotic cell division and such identical chromosomes are known as homologous chromosomes. Each character of a pair of contrasting

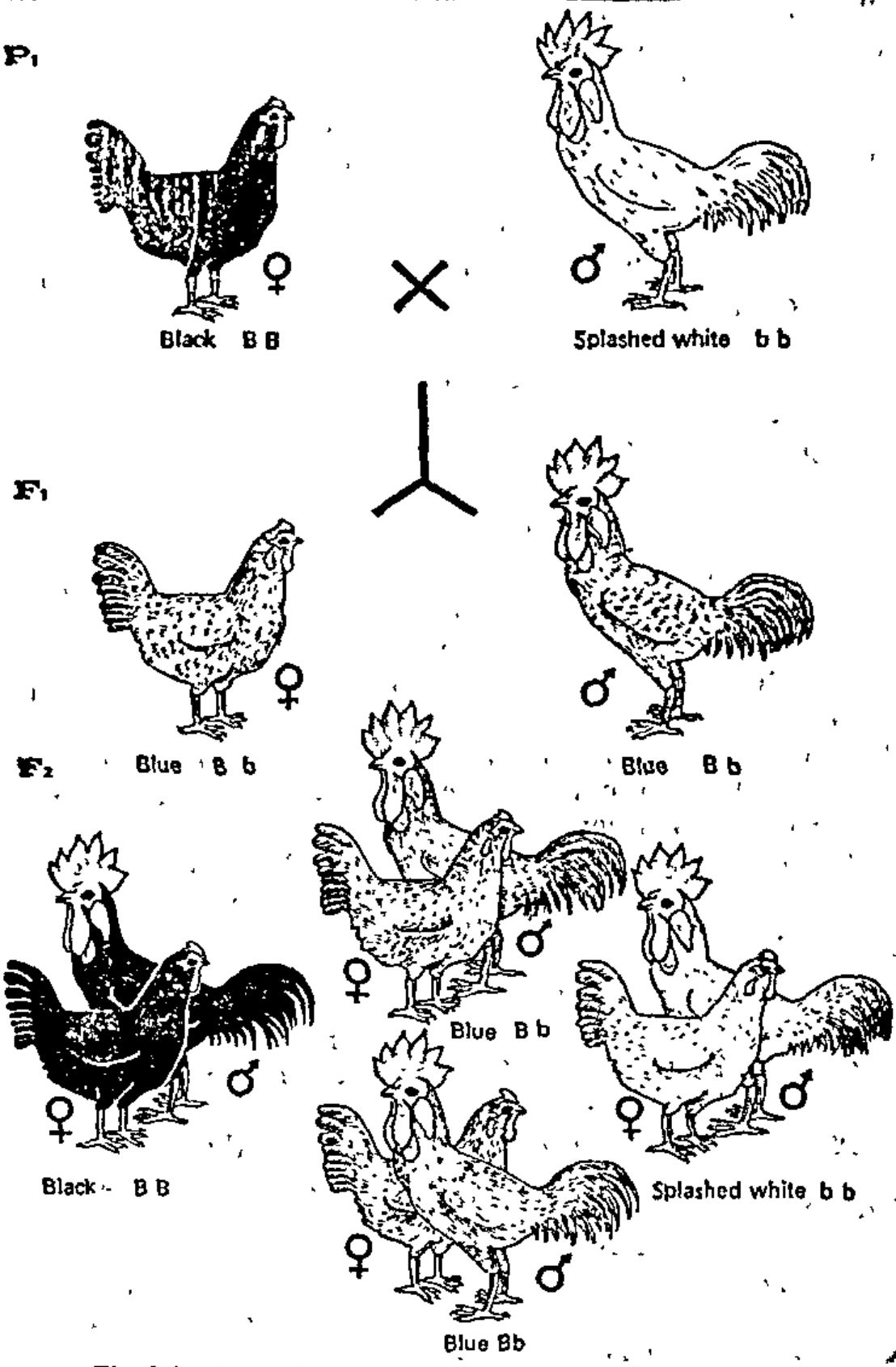


Fig. 3 6. A monohybrid cross between black and splashed white Andalusian fowl showing the incomplete dominance.

characters is represented by a single gene. If the character for tallness is represented by the gene T and because each homologous chromosome of a pair can have single gene for tallness so the character of tallness in a homozygous tall pea plant will be represented by a pair of genes 'TT' Likewise the homozygous dwarf pea plant contains the gene 'tt' for the character of dwarfness.

During the gametogenesis, the homologous chromosomes with TT or tt genes are separated and each chromosome with T or t gene is passed to the gamete. The [illegible] the process of fertilization an[illegible] both tall (T) and dwarf (t) [illegible] generation (F_1) contains two [illegible] characters, therefore, it is known as heterozygote or hybrid. Because the hybrids of F_1 have tall stems so the character of tallness (T) is considered as dominant and because the character for dwarfness could *not express itself in F_1 generation, therefore, it is considered as recessive.*

INCOMPLETE DOMINANCE

The law of dominance does not occur universally. For example, when a red flowered pea plant (RR) is crossed with white flowered pea plant (rr) then the F_1 hybrid pea plants are found to have pink flowers. It shows that gene for red colour could not dominate the gene for white colour as shown in the figure 3·5. The appearance of this intermediate character in F_1 generation in known as *incomplete dominance.* This type of incomplete dominance has also been reported in snapdragons and many animals. For example, when a homozygous Andalusian fowl with black feathers is crossed with a homozygous fowl with splashed white feathers the F_1 hybrids are found to contain blue feathers (Fig. 3·6).

CODOMINANCE

In codominance both the dominant and recessive characters occur side by side in F_1 hybrids to give the mixed characters. The best example of codominance has been observed in cattles. when a cattle of red coat is crossed with the cattle of white coat, the F_1 hete*rozygote or hybrid is found to possess roan coat (Fig. 3·7) In roan* [illegible] has

[illegible] ie-

tes. The law states that *the hybrids or heterozygotes of F_1 generation have two contrasting characters or allelomorphs of dominant and rece-*

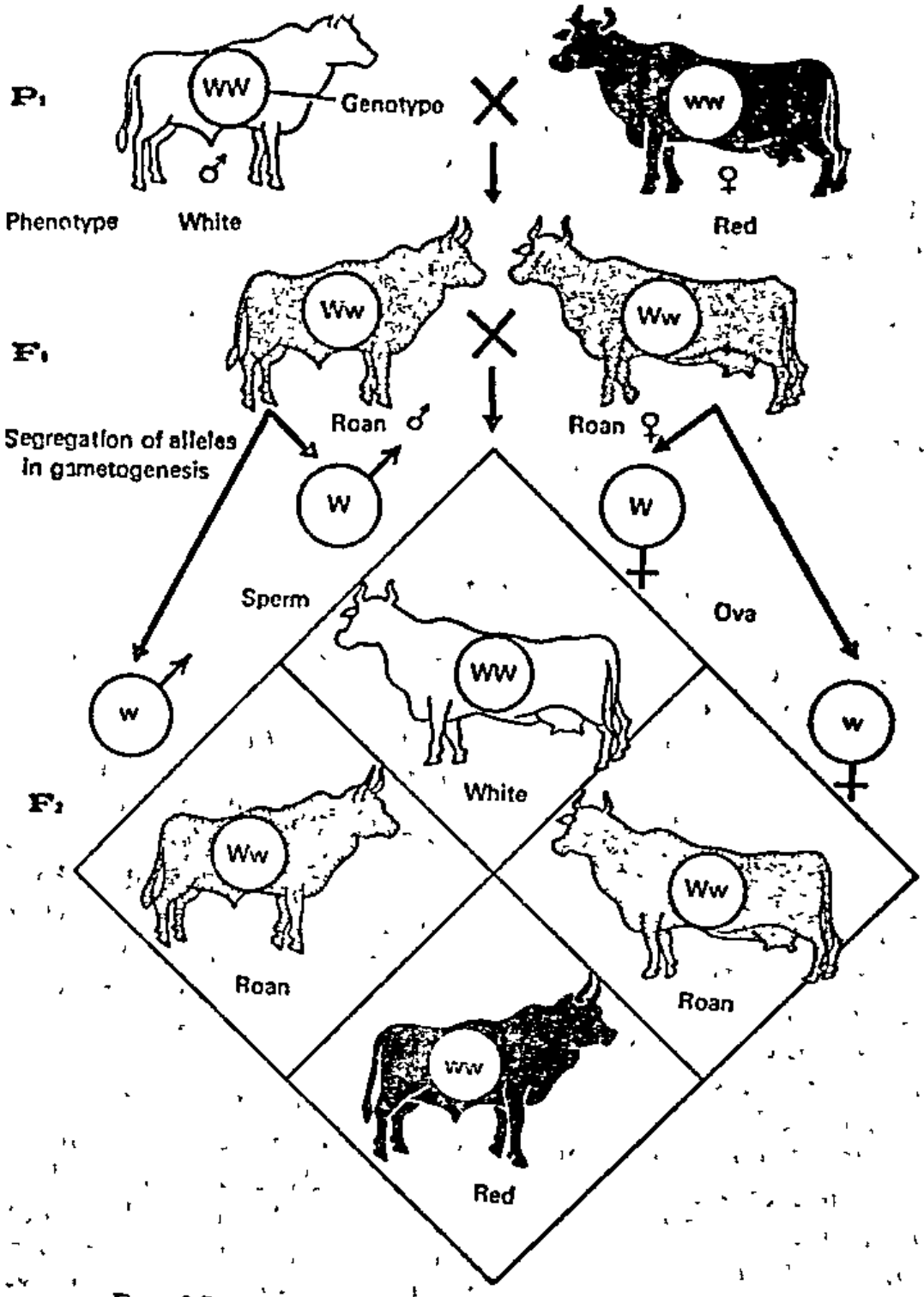


Fig. 3·7. A monohybrid cross in a white [illegible] coa

For the proper [illegible] egrega-tion it will be helpful to study one of the Mendel's monohybrid cross (Fig. 3·5). Mendel crossed a homozygous red flowered pea plant with

a homozygous white flowered pea plant. The F_1 heterozygotes or hybrids were found to be pink or purple flowered thus, showing the incomplete dominance of red colour over white colour. When the F_1 hybrids were allowed to be self fertilized, they produce both coloured (red or purple) and white flowered pea plants in F_2 generation in the ratio of three and one (3 : 1). The [illegible] in F_2 generation

MECHANISM

The mechanism of segregation in above mentioned monohybrid cross between red and white flowered pea plants can be understood by assuming that the homozygous red flowered pea plant has the allele RR for redness and white flowered pea plant has the alleles rr for whiteness. The pea plant with RR alleles produces the gametes with single allele R and pea plant with rr alleles produces the gametes with the allele r. The gametes of both united to form a hybrid or heterozygote having the alleles Rr both for redness and whiteness. Due to the phenomenon of incomplete dominance the allele R for red colour partially expresses itself in hybrids of F_1, while the allele r for white colour remains latent or recessive. Both the allele R and r remain together for long time but they do not effect each other. Neither they mix nor they contaminate each other. Because a gamete can contain only one chromosome of a homologous pair, therefore, each gamete can carry single allele R or r. At the time of gametogenesis two types of gametes are produced by F_1 hybrids in equal numbers. Half of the gametes carry the allele R and other half carry the allele r. These gametes during the process of fertilization can unite in three [illegible] RR, R[illegible] [illegible] types [illegible] colo[illegible] colo[illegible] whit[illegible] mix[illegible] ing gametogenesis.

CERTAIN OTHER EXAMPLES OF LAW OF SEGREGATION

The law of segregation is universal in its application and it has been found to occur in both plants and animals. The examples which are cited in law of dominance can also be considered for the law of segregation. However, to understand the mechanism of segregation more clearly in animals it will be helpful to consider an original experiment of well known genetist T. H. Morgan on *Drosophila*.

Morgan crossed a homozygous long winged (wild type) *Drosophila* with a homozygous vestigial winged *Drosophila*. The F_1 hetero-

zygotes or hybrids were found to be long winged. When the F_1 hybrids were allowed to mate among themselves, they produced long-winged and vestigial-winged hybrids in three and one ratio (3 : 1) in the F_2 generation (Fig. 3·8).

In this cross the mechanism of segregation can be well understood by assuming that the homozygous long winged *Drosophila* has

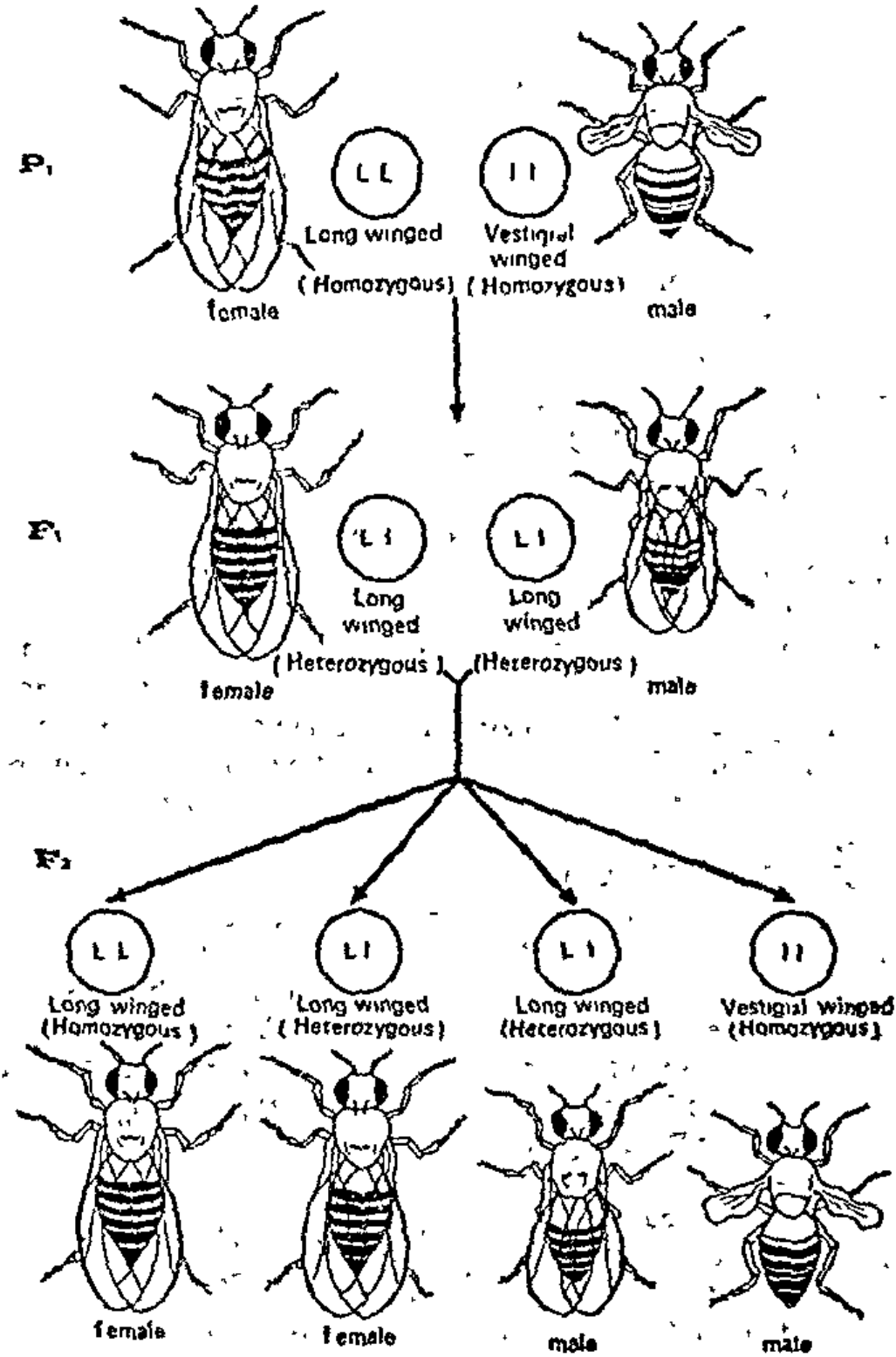


Fig. 3 8. Cross between long-winged vestigial winged *Drosophila*.

a pair of alleles LL for longness of wing and similarly homozygous vestigial-winged *Drosophila* has the alleles ll for vestigial nature of wings. The long-winged *Drosophila* fly thus produces the gametes (sperm or ova) with the single allele L and the vestigial-winged fly produces the gametes with the single allele l. The gametes of both parents unite in the process of fertilization and produce a hybrid with long wings in F_1 generation. The genotype of F_1 hybrid is Ll and in it the allele L is dominant over the alleles l which is recessive. At the time of gametogenesis these alleles (L and l) are separated along with chromosomes to form two types of gametes, half of the gametes have the allele L and other half have the allele l. These gametes unite in three possible combinations, *viz.*, LL, Ll and ll, to produce genotypically three types of individuals LL, Ll and ll in 1 : 2 : 1 ratio in F_2 generation. Phenotypically here occur only three long-winged and one vestigial-winged *Drosophila*. Thus the dominant and recessive alleles remain together for long time without contaminating or mixing with one another and segregate during gametogenesis.

LAW OF INDEPENDENT ASSORTMENT

To formulate the law of dominance and law of segregation Mendel considered monohybrid crosses in which single pairs of contrasting characters were considered at a time. But he tried to find out how different characters would behave in relation to each other in their inheritance from generation to generation. For this purpose [illegible] plants which were differing in [illegible]. Because such crosses yielded [illegible] trasting characters had been considered in them, therefore, these crosses were known as dihybrid crosses.

MENDEL'S DIHYBRID CROSS

In one of his hybridization experiment Mendel crossed a homozygous pea plant having yellow round seeds with the homozygous pea plant having green wrinkle seeds (Fig. 3·9). The F_1 hybrids were found to have yellow round seeds. When the F_1 hybrids were allowed to cross among themselves they produced four types of seeds in the ratio of 9 : 3 : 3 : 1 given as follows :

1. Yellow Round—9
2. Yellow Wrinkle—3
3. Green Round—3
4. Green Wrinkle—1.

Beside getting the ratio of 3 : 1 of the monohybrid crosses Mendel got the ratio of 9 : 3 : 3 : 1. This irregularity in the ratio of F_2 offsprings was explained by Mendel stating that *"when the*

parents differ from each other in two or more pairs of contrasting characters or factors then the inheritance of one pair of factors is independent to that of the other pair of factors". This is the Mendel's law of independent assortment.

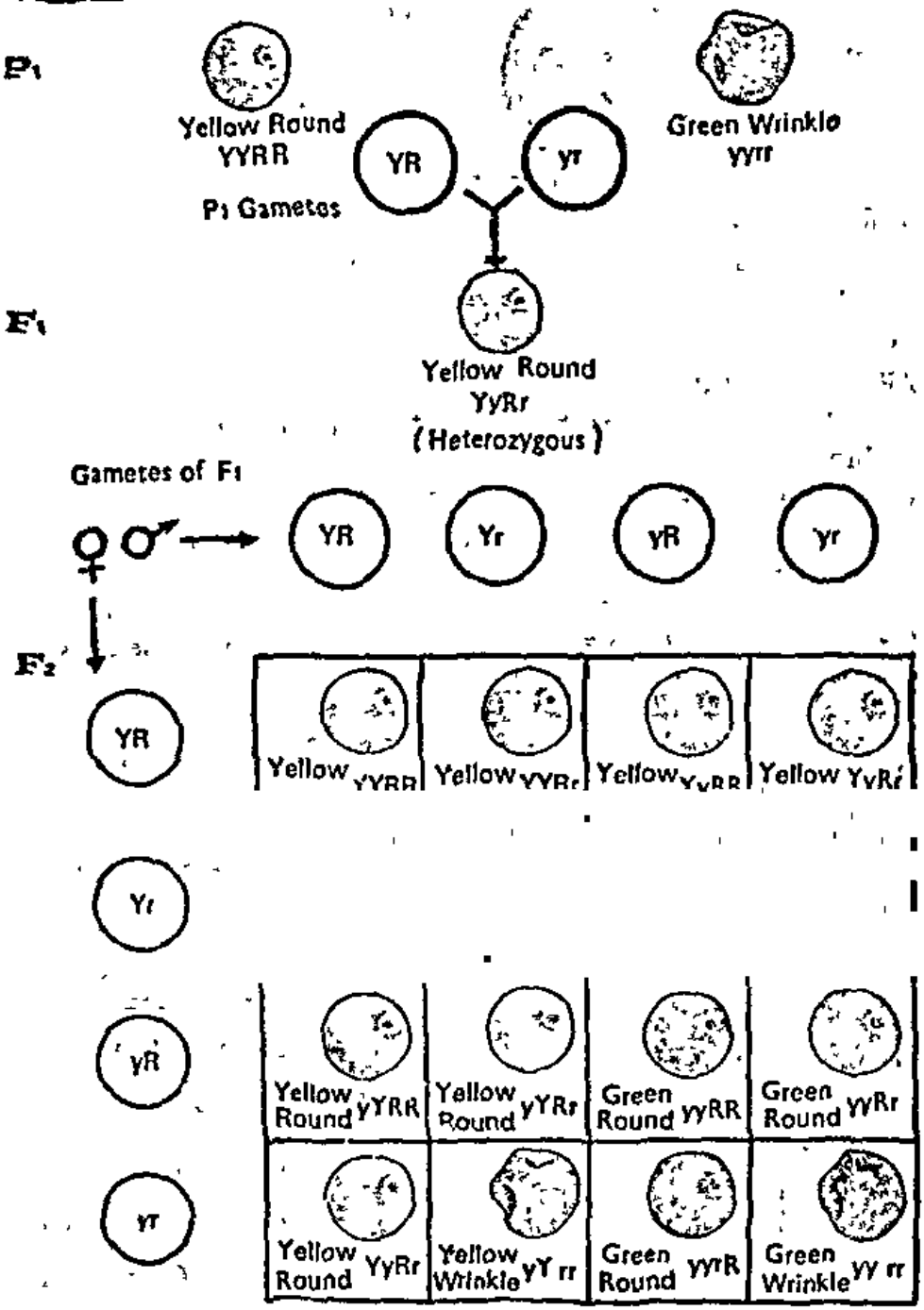


Fig. 39. A dihybrid cross between a yellow round and a green wrinkle pea plant.

MECHANISM OF INDEPENDENT ASSORTMENT

The mechanism of independent assortment can be understood easily by assuming that the homozygous pea plant with yellow round seeds has the alleles YY and RR for the yellow colour and roundness of the seed, respectively. Similarly the homozygous pea

with green wrinkle seeds contains the alleles yy and rr for the green colour and wrinkleness of seeds. The gametes which are produced by YYRR and yyrr plants are YR and yr types respectively. When both parent, are crossed the union of both types of gametes takes place to give the F_1 hybrid (Yy Rr). The F_1 hybrid have been found to contain yellow round seeds showing the dominance of allele Y for yellow colour over the recessive allele y for green colour and the dominance of allele R for roundness over the recessive allele r for wrinkleness of seed. Now the F_1 hybrids have four types of alleles, *viz.*, Y for yellow colour, y for green colour, R for round shape and r for winkleness of seed. During gametogenesis these four alleles, may combine in following four combinations :

1. The allele Y may be associated with the allele R to give rise YR combination.
2. The allele Y may be associated with the allele r to give rise Yr combination.

Table : The phenotype and genotype of F_2 generation of a dihybrid cross between pea plants with yellow round and green wrinkle seeds

Number of individuals	Genotype class	Phenotype class
1	YY RR	Homozygous Yellow Round
2	YY Rr, YY Rr	Heterozygous Yellow Round
2	Yy RR, Yy RR	
4	Yy Rr, Yy Rr, Yy Rr, Yy Rr	
		Yellow Round=9
1	YY rr	Homozygous Yellow Wrinkle
2	Yy rr, Yy rr	Heterozygous Yellow Wrinkle
		Yellow Wrinkle=3
1	yy RR	Homozygous Green Round
2	yy Rr, yy rR	Heterozygous Green Round
		Green Round=3
1	yy rr	Homozygous Green Wrinkle
		Green Wrinkle=1
16	—	9 : 3 : 3 : 1

3. The allele y may be associated with the allele R to give rise yR combination.

4. The allele y may be associated with the allele r to give rise yr combination.

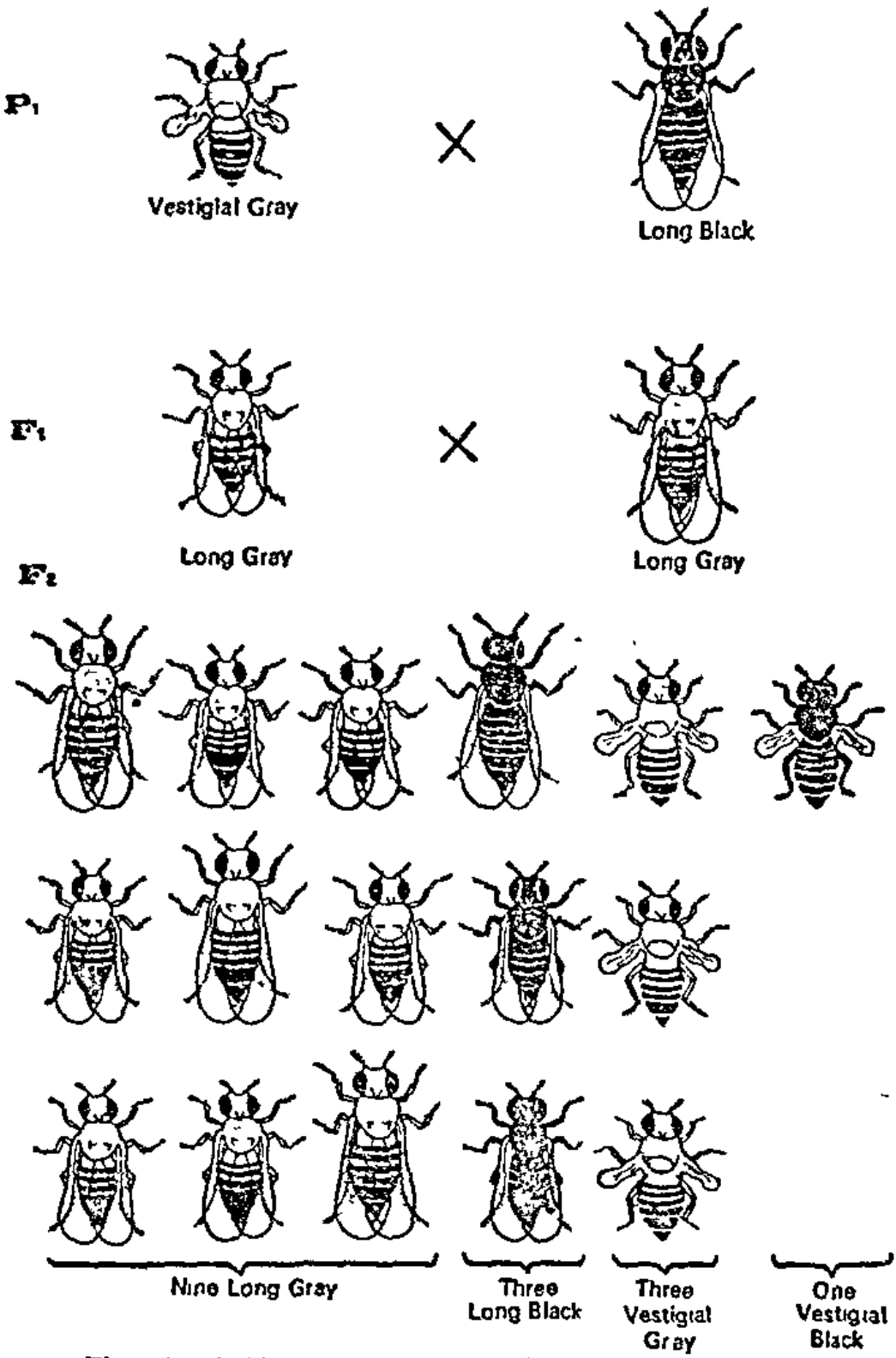


Fig. 3·9. A dihybrid cross between the vestigial-gray and long black *Drosophila*.

Thus, four types of alleles are assorted independently to produce four types of gametes, *viz.*, YR, Yr, yR and yr. These four types of gametes (pollens or ovules) of F_1 hybrid unite at random in the process of fertilization and produce sixteen types of individuals in F_2 generation which are given in the table on page 256.

Thus the sixteen F_2 individuals have the ratio of 9 yellow round : 3 yellow wrinkle : 3 green round : 1 green wrinkle. These results have proved the law of independent assortment and showed that each pair of contrasting characters behaves independently and bears no permanent association or relation with particular character. The allele Y was associated with allele R in parent but it does not always remain associated with it and it is also associated with the allele r.

DIHYBRID CROSS IN DROSOPHILA

In a dihybrid cross homozygous long-winged and black-bodied *Drosophila* fly is crossed with a vestigial-winged, gray-bodied fly, they produced in F_1 long-winged gray-bodied *Drosophila*. The hybrids of F_1 generation after mating with each other produced in F_2 generation 9 long-winged gray-bodied, 3 long-winged black-bodied. 3 vestigial-winged gray-bodied and 1 vestigial-winged black-bodied *Drosophila* (Total=16). Therefore, the 16 individuals of F_2 have the ratio of 9 : 3 : 3 : 1 (Fig. 3·9).

BACK CROSS AND TEST-CROSS

When F_1 individuals are crossed with one of the two parents [illegible] such a cross is called back cross

crosses are back crosses, only the cross with the recessive parent is known as test cross. It is called a test cross, because it is used, to [illegible]) or heterozygous remains 1 : 1, but,

EXAMPLE FOR MONOHYBRID BACK CROSS AND TEST CROSS

In a monohybrid cross of homozygous tall (DD) and homozygous dwarf (dd) pea plants, when a F_1 haterozygous tall (Dd) plant is crossed either with its dominant parent to perform a back cross or with its recessive parent to perform a test cross following results are obtained :

P_1 : Homozygous Tall (DD) × Homozygous Dwarf (dd)

↓

F_1 : Dd — Heterozygous Tall

A. Back cross : F_1 Tall × P_1 Tall
Dd ↓ DD
Back cross progeny : ½ DD : ½ Dd or 1 : 1
Homozygous tall : Heterozygous tall

B. Test cross : F_1 Tall × P_1 Dwarf
Dd ↓ dd
Test cross progeny : ½ Dd : ½ dd or 1 : 1
Heterozygous tall : Homozygous dwarf

EXAMPLE FOR DIHYBRID TEST CROSS

The test cross of a heterozygous yellow round seeded pea plant with a double recessive parent (green wrinkle, yyrr), yields the test cross genotypic and phenotypic ratio of 1 : 1 : 1 : 1 as follows :

Parents : F_1 Heterozygous yellow round Yy Rr × F_1 Homozygous green wrinkled yyrr

Gametes : YR, Yr, yR, yr ; yr

Test cross progeny : ¼ Yy Rr : ¼ Yy rr : ¼ yy Rr : ¼ yy rr
or ¼ yellow round : ¼ yellow wrinkle : ¼ green round : ¼ green wrinkle or 1 : 1 : 1 : 1.

MULTIHYBRID CROSS

The parents which differ in more than two pairs of contrasting characters then the cross between them is known as **polyhybrid** or **multihybrid cross.** The F_1 hybrids in these cases are known as **polyhybrids** or **multihybrids.** The law of independent assortment is also applicable to these crosses.

EXAMPLE OF A MULTIHYBRID CROSS

For example, we can consider a trihybrid cross of pea plant. The mating of a homozygous yellow, round, and tall pea plant (YY RR TT) with a homozygous green, wrinkle, and dwarf pea plant (yy rr tt) produces in F_1 yellow, round and tall (Yy Rr Tt) trihybrids. These F_1 trihybrids when self-crossed or crossedaamong themselves, they produce a F_2 progeny including 64 individuals in the phenotypic ratio of 27 yellow, round, tall : 9 yellow, round, dwarf : 9 yellow, wrinkle, tall : 9 green, round, tall : 3 yellow, wrinkle, dwarf : 3 green, wrinkle, tall : 3 green, round, dwarf : 1 green, wrinkle, dwarf or 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1. The possible genotypes and phenotypes of this trihybrid cross have been summarized in table on page 260.

DEVIATION FROM MENDEL'S DIHYBRID PHENOTYPIC RATIO.

The Mendelian dihybrid phenotypic ratio of 9 : 3 : 3 : 1 is obtained only when the alleles at both gene loci display dominant and

Table : Genotypic and phenotypic ratios of a trihybrid cross in between a yellow, round, tall (YY RR TT) and a green, wrinkled, dwarf (yy,rr, tt) pea plants :

Genotype	Genotypic ratio	Phenotype	Phenotypic ratio
YYRRTT	1	Yellow, Round, Tall	27
YYRrTT	2		
YyRRTT	2		
YyRrTT	4		
YYRRTt	2		
YYRrTt	4		
YyRRTt	4		
YyRrTt	8		
YYrrTT	1	Yellow, Wrinkled, Tall	9
YyrrTT	2		
YYrrTt	2		
YyrrTt	4		
yyRRTT	1	Green, Round, Tall	9
yyRrTT	2		
yyRRTt	2		
yyRrTt	4		
YYRRtt	1	Yellow, Round, Dwarf	9
YYRrtt	2		
YyRRtt	2		
YyRrtt	4		
yyrrTT	1	Green Wrinkled, Tall	3
yyrrTt	2		
YYrrtt	1	Yellow, Wrinkled, Dwarf	3
Yyrrtt	2		
yyRRtt	1	Green, Round, Dwarf	3
yyRrtt	2		
yyrrtt	1	Green, wrinkled, Dwarf	1

recessive relationships. If one or both gene loci have incompletely dominant alleles, or codominant alleles or lethal alleles, the dihybrid ratio becomes modified variously, such as follows :

1. 3 : 6 : 3 : 1 : 2 : 1 RATIO

When the dihybrid parent have dominant and recessive alleles at one gene locus and codominant alleles at second gene locus, the F_2 9 : 3 : 3 : 1 phenotyic ratio becomes 3 : 6 : 3 : 1 : 2 : 1.

Example : *In cattles, hornless or polled (P) condition is dominant* to horned (p) condition, and trait of white (W) coat is codominant to the trait for red (w) coat colour. The mating of homozygous white polled (PP WW) cattle with a homozygous red horned (pp ww) cattle produces F_1 heterozygotes (dihybrids) with the phenotype of hornless, roan and genotype of Pp Ww. These hornless roan F_1 dihybrids produce a F_2 progeny in the ratio of 3 : 6 : 3 : 1 : 2 : 1 as illustrated in following diagram :

P_1 : Phenotype Hornless White × Horned Red
Genotype PP WW pp ww
↓ ↓
P_1 Gametes : PW pw
F_1 : PpWw
Hornless Roan
F_2 : 3 Hornless White : 6 Hornless Roan : 3 Hornless Red : 1 Horned White : 2 Horned Roan : 1 Horned Red.

2. 1 : 2 : 1 : 2 : 4 : 2 : 1 : 2 : 1 RATIO

When each parent of a dihybrid cross has incompletely dominant alleles at both gene loci, then in F_2 large number of phenotypic classes are produced.

Example—In snapdragons, red flower colour (R) is incompletely dominant to white flower colour (r) and trait for broadness of leaf (B) is incompletely dominant to the trait for narrowness of leaf (b). The dihybrid cross between red, broad plant (RR BB) and white, narrow plant (rr bb) produces heterozygotes (dihybrids) having pink flowers and leaves of intermediate width and genotype of Rr Bb. These F_1 dihybrids produce the F_2 progeny in 9 phenotypic classes as follows :

P_1 : Phenotype Red broad × White Narrow
Genotype RR BB rr bb
↓ ↓
P_1 gametes RB rb
F_1 : Phenotype Pink Intermediate
Genotype Rr Bb
F_2 : 1 Red Broad : 2 Red Intermediate : 1 Red Narrow : 2 Pink Broad : 4 Pink Intermediate : 2 Pink Narrow : 1 White Broad : 2 White Intermediate : 1 White Narrow.

3. 3 : 1 : 6 : 2 RATIO

When the F_1 dihybrids have dominant-recessive alleles at one gene locus and recessive lethal alleles at second gene locus, the F_2 offsprings manifest the phenotypic ratio of 3 : 1 : 6 : 2.

4. 1 : 2 : 1 : 3 : 4 : 2 RATIO

When the F_1 dihybrids contain codominant alleles at first gene locus and recessive-lethal alleles at second gene locus, then their F_2 progeny display the phenotypic ratio of 1 : 2 : 1 : 2 : 4 : 2.

5. 4 : 2 : 2 : 1 RATIO

This phenotypic ratio is obtained when at both gene loci of F_1 dihybrids occur the recessive lethal alleles.

CHAPTER 4

INBREEDING, OUTBREEDING AND HYBRID VIGOUR

Among living organisms fundamentally following two systems of matings occur :

INBREEDING

The process of mating of individuals which are more closely related than the average of the population to which they belong, is [illegible], the self-fertilization in plants is an [illegible]ile the marriage between brothers and [illegible]ther familiar example of inbreeding

[illegible]DING

The continuous inbreeding results, genetically, in homozygosity. It produces homozygous stocks of dominant or recessive genes and eliminate heterozygosity from the inbred population. For example, if we start with a population containing 100 heterozygous individuals

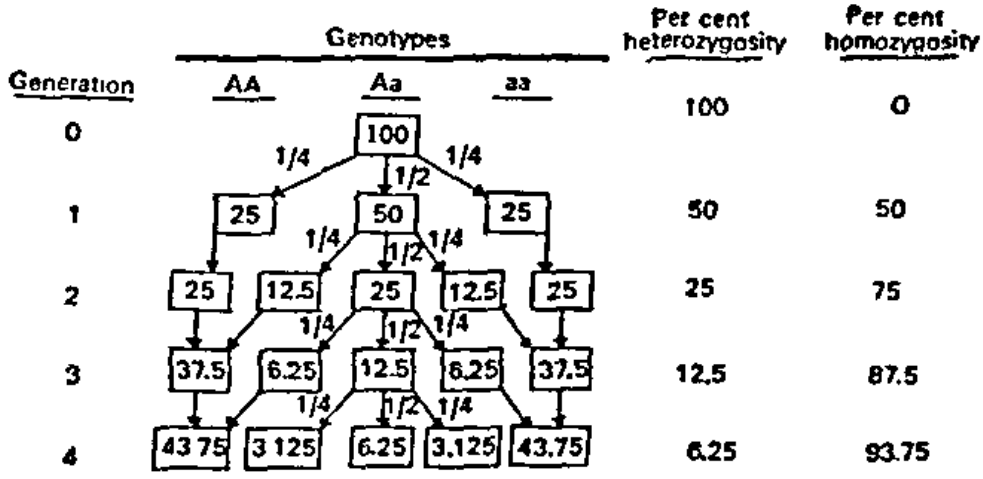


Fig. 4·1. Expected increase in homozygosity due to inbreeding.

(Aa) as shown in figure (Fig. 4·1), the expected number of homozygous genotypes is increased by 50% due to selfing or inbreeding in each generation.

Thus, due to inbreeding in each generation the heterozygosity is reduced by 50% and after 10 generations we can expect the total elimination of heterozygosity from the inbred line and production of

two homozygous or pure lines. But, because a heterozygous individual possesses several heterozygous allelic pairs, therefore, we can conclude that interbreeding will operate on all genes loci to produce totally pure or homozygous offsprings. In man if inbreeding continued over a number of generations it results in increasing homozygosity, but some what slowly. The different types of inbreedings and their corresponding increase in homozygosity have been graphically illustrated as follows :

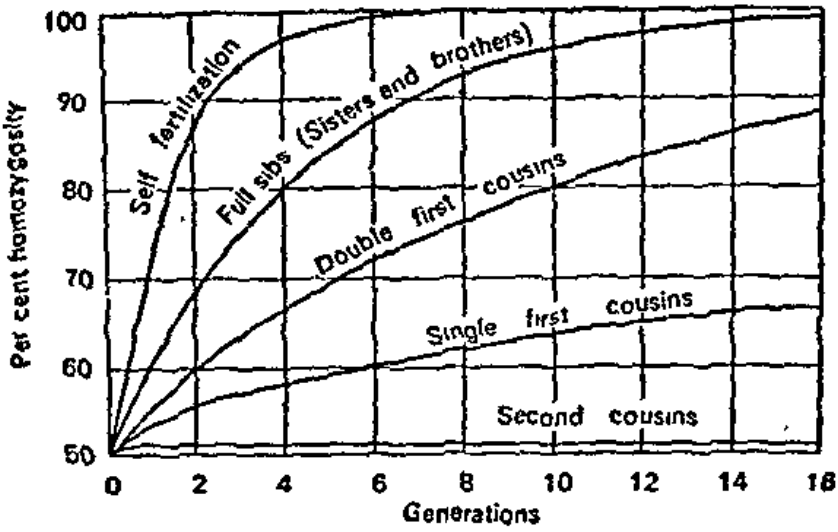


Fig. 4·2. Increase in percentage of homozygosity under various systems of inbreeding.

PRACTICAL APPLICATIONS OF INBREEDING

The correlation of inbreeding and homozygosity exhibits that how inbreeding may cause deleterious effects. As we already know that in a heterozygous individual, the harmful recessive alleles remains marked by their normal dominant alleles. If a heterozygous individual undergoes inbreeding for various generations, there will be equal chances of homozygosity for dominant as well as recessive alleles. In homozygous condition recessive alleles will be able to express their deleterious phenotypic effects on the individual. On the other hand, the homozygosity for dominant alleles has equal opportunity to express their beneficial phenotypic effects on inbred races. These considerations have helped the human society to protect it from the deleterious effects of continued inbreeding and to improve its various domestic animals. The practical applications of inbreeding are following :

1. Because inbreeding causes homozygosity of deleterious recessive genes which may result in defective phenotype, therefore, in human society, the religious ethics unknowingly and modern social norms consciously have condemned and banned the marriages of

brothers and sisters. Further, the plant breeders and animal breeders too avoid inbreedings in the individuals due to this reason.

2. The inbreeding because, results in the homozygosity of dominant alleles, therefore, it is a best mean of mating among hermaphrodites and self-pollinating plant species of several families. The animal breeder have employed the inbreeding to produce best races of horses, dogs, bulls, cattles, etc. The modern race horses, for example, are all descendents of three Arabian stallions imported into England between 1689 and 1730 and mated with several local mares of the slow, heavy type that had carried the medieval knights in heavy armour. The fast runners of F_1 were selected and inbred and stallions of the F_2 appear as beginning points in the pedigrees of almost all modern race horses. This sort of inbreeding is also called **line breeding** which has been defined as the mating of animals in such a way that their descendents will be kept closely related to an unusually desirable individual.

INBREEDING DEPRESSION

In a heterozygote the inbreeding increases the probability of homozygosity of deleterious recessive alleles in a inbred population. In other wc
vigour (*i.e.*
commonly
inbreeding depression.

OUTBREEDING AND HYBRID VIGOUR

The crosses between two unrelated pure breeding (inbred) popu-

has been termed as **hybrid vigour or heterosis.**

Examples—(i) When inbred bulls of Hereford cattles were crossed with unrelated cows which were sired by other inbred bulls, significant improvement was found in the hybrids. For example, F_1 hybrids showed an increase of 12 per cent body weight.

(ii) Outbreeding in sheeps produces improved strains of sheep which provide increased amount of meat and wool than inbreds.

(iii) The hybrids of maize plant which have been received by outbreeding have been found to have great vigorosity. Beside maize the hybrid vigour has been shown by various other plant species such as pea, tobacco, tomato, cucumbers, sqash, sweet corn, pine trees, many grasses, onion, sugar beets and many other plants.

CROSS BREEDING

produced
62) with
spicuous
patient beast of burden since time immemorial.

GENETICAL BASIS OF HETEROSIS

The genetical basis of heterosis is still a subject of controversy and following two theories have been propounded to explain it :

1. Over dominance theory of heterosis—The over dominance theory or **physiologic stimulation hypothesis of Shull (1874—1954)** considers heterozygosity to have stimulating effect on the heterosis of hybrid individual. Heterozygosity itself was thus considered to be the controlling factor.

Example—If we suppose that four gene loci are contributing to a quantitative trait, homozygous recessive genotype contribute 1 unit to the phenotype, heterozygous genotypes contribute 2 units to the phenotype and homozygous dominant genotypes contribute 1½ units. Then the results can be represented as follows :

Parents : AA BB cc dd × aa bb CC DD

Phenotypic value : $1\frac{1}{2}+1\frac{1}{2}+1+1=5$ | $1+1+1\frac{1}{2}+1\frac{1}{2}=5$

↓

F_1 : Aa Bb Cc Dd

$2+2+2+2=8$

2. Dominance theory of heterosis—The dominance theory of *heterosis holds that increased vigour and size in a hybrid is* due to combination of favourable growth genes by crossing two inbred races. In other words, the hybrid vigour is a result of action and interaction of dominant or fitness factors or cumulative (polygenic) effect of dominant genes.

Example—If we suppose, that a quantitative trait is governed by four genes. Each recessive genotype contributes one unit to the phenotype and each dominant genotype contributes two units to the phenotype. A out cross (out breeding) between two inbred lines can produce more heterotic F_1 individuals than the parents, in following manner :

Parent : AA bb CC dd × aa BB cc DD

Phenotypic value : 2+1+2+1=6 | 1+2+1+2=6

↓

F_1 Aa Bb Cc Dd

2+2+2+2=8

EVOLUTIONARY SIGNIFICANCE OF INBREEDING AND OUTBREEDING

The inbreeding and outbreeding, both provide raw material to natural selection [illegible] on recessive genes, [illegible] tions from [illegible] With [illegible] the accu[illegible] or line. [illegible] terious r[illegible] ding and [illegible] may be good or bad for the natural selection.

CHAPTER 5

GENETIC INTERACTION

During the discussion of Mendel's monohybrid and dihybrid crosses, we encountered with the fact that for the determination of single phenotypic trait of an organism, two alleles or allelomorphs of a single gene interacted in various ways. Such as, out of two allelomorphs of a single gene, one allelomorph might show simple (complete) dominance over the action of other which was recessive ; or both allelomorphs might have partial or incomplete dominant relationship or both allelomorphs might have equal expression or

netic interactions

type of gene and

ietic interactions.

al ratios of 3 : 1

and 9 : 3 : 3 : 1. But, in addition

non-allelic or inter-allelic

inter-allelic genetic inter

genes located on the san

one another for the ex

organism. The discovery of the inter-allelic genetic interactions has been made afte

dering the way

not only ecological factors such as temperature and the amount or quality of light, but also internal factors such as hormones and enzymes. The enzymes are proteins and the specific molecular organization of protein is determ

nt steps of a chemical reaction constitute the biosynthetic pathway.

Thus a simplest biosynthetic pathway includes various steps each st
matic p
For ex
transforms a precursor substance 'P' into the end product 'C' in following three subsequent steps :

$$P \text{ (precursor)} \xrightarrow{g_1^+} A \xrightarrow{g_2^+} B \xrightarrow{g_3^+} C \text{ (end product)}$$

In this biosynthetic pathway each metabolite (A, B, C) is produced by the catalytic action of different enzymes (e_1, e_2, e_3.....e_x specified by different wild type genes (g_1^+, g_2^+, g_3^+.....g_x^+). When more than two or more genes become involved in the specification of enzymes for different steps of a common biosynthetic pathway, the phenomenon of genetic interaction occurs. If substance C is essential for the production of a normal phenotype and the recessive mutant alleles g_1, g_2, g_3 produce defective enzymes, then a mutant or abnormal phenotype would result from a genotype homozygous recessive at any of the three loci. If wild gene g_3^+ becomes mutant, the conversion of metabolite B to C does not occur and substance B tends t antity.; if g_2^+ becomes mutant, subs s, the mutant genes caused "metabolic

An organism with a mutation only in gene g_2^+ could produce a normal phenotype, if it was given either substance B or C, but an organism with a mutation in gene g_3^+ has a specific requirement for substance C for the production of normal phenotype. Thus, gene g_3^+ becomes dependent upon g_2^+ for its expression as a normal phenotype. If the genotype is homozygous for the recessive g_2 allele, then the biosynthetic pathway ends with substance A. Neither g_3^+ nor its recessive allele g_3 has any effect on the phenotype. Thus, genotype (g_2 g_2 can hide or mask the phenotypic expression of alleles at the g_3^+ locus. Originally a gene or locus which suppressed or masked the action of a gene at another locus was termed epistatic gene. The gene or locus which was suppressed by a epistatic gene was called hypostatic gene. Later studies revealed the fact that both loci or genes (*i.e.*, epistatic and hypostatic) could be epistatic to one another. Presently, the term epistasis (Greek, standing upon) is used for almost any type of allelic genetic interaction.

DIFFERENCE BETWEEN DOMINANCE AND EPISTASIS

The phenomenon of dominance involves intra-allelic gene suppression, or the masking effect which one allele has upon the expre-

ssion of another allele at the same locus, while the phenomenon of epistasis involves inter-allelic gene suppression or the masking effect which one gene locus has upon the expression of another. The clas-

observed in the progeny of dihy-

stasis into ratios which are vari-

groupings.

KINDS OF EPISTATIC INTERACTION

When in dihybrid crosses, the epistatic interactions occur between two genes, less than four phenotypes appear in F_2. Such bigenic (two gene) epistatic interactions may be of following six types :

I. DOMINANT EPISTASIS (12 : 3 : 1)

When out of two genes, the dominant allele (*e.g.*, A) of one gene masked the activity of alleles of another gene (*e.g.*, B) and expressed itself phenotypically, then A gene locus is said to be epistatic to the B gene locus. Because, the dominant allele A can express itself only in the presence of either B or b allele, therefore, such type of epistasis is termed as **dominant epistatis**. The alleles of hypostatic locus or gene B will be able to express themselves phenotypically only when gene locus A may contain two recessive alleles (aa). Thus, the genotype AA BB or Aa Bb and AA bb or Aa bb produce the same phenotype whereas the genotype aa BB or Bb and aa bb produce two additional phenotypes. The dominant epistasis modify the classical ratio of 9 :3 : 3 : 1 into 12 : 3 : 1 ratio.

EXAMPLE

Dominant epistatis in dogs—Among dogs, the colours of coats depend upon the action of two genes. One gene locus has a dominant epistatic inhibitor allele (I) of coat colour pigment. The allele I prevents the expression of colour allele at another independently assorting, hypostatic gene locus (B or b) and produces white coat colour. The alleles of hypostatic gene locus (BB, Bb, or bb) express only when two recessive alleles (ii) occur on the epistatic locus, *i.e.*, ii BB or ii Bb produces black and ii bb produces brown individuals. When two such white coat colour dogs are crossed in F_1 the white, black and brown coat colours appear in 12 : 3 : 1 ratio as shown in the following table :

	White (Male)		White (Female)
P :	Ii Bb	×	Ii Bb

P Male gametes → / P Female gamete ↓	(IB)	(Ib)	(iB)	(ib)
(IB)	II BB White	II Bb White	Ii BB White	Ii Bb White
(Ib)	II Bb White	II bb White	Ii Bb White	Ii bb White
(iB)	Ii BB White	Ii Bb White	ii BB Black	ii Bb Black
(ib)	Ii Bb White	Ii bb White	ii Bb Black	ii bb Brown

F_1 :

F_1 Phenotypic ratio : 12/16 White : 3/16 Black : 1/16 Brown or 12 : 3 : 1 .

2. RECESSIVE EPISTASIS (9 : 3 : 4)

Sometimes the recessive alleles of one gene locus (aa) masked the action (phenotypic expression) of alleles of another gene locus (BB, Bb or bb alleles). This type of epistatis is called **recessive epistasis.** The alleles of B locus express themselves only when epistatic locus-A has dominant alleles (*e.g.*, AA or Aa). Due to recessive epistasis the phenotypic ratio 9 : 3 : 3 : 1 becomes modified into 9 : 3 : 4 ratio.

EXAMPLE

Recessive epistasis in mice—In mice various types of epistatic genetic interactions have been reported. The most interesting case is of recessive epistasis in coat colours. The common house mouse occurs in a number of coat colours, *i.e*,, agouti, black and albino. The agouti colour pattern is commonly occurred one (wild type) and is characterized by colour banded hairs in which the part nearest the skin is gray, then a yellow band and finally the distal part is either black or brown. The albino mouse lacks totally in pigments and has white hairs and pink eyes.

When a homozygous agouti or gray (BB AA) is crossed with a homozygous albino (bb aa) in F_1 all agouti offsprings appear. When, the F_1 agouti are crossed among themselves in F_2 agouti, black and albino offsprings appear in the ratio of 9 : 3 : 4 as shown in the following diagram :

P : Agouti (gray) BB AA × Black bb aa

P gamete : (BA) (ba)

F_1 : Agouti Bb Aa

F_1 cross : (Male) Bb Aa × (Female) Bb Aa

F_2 :

F_1 Male gametes → / F_1 Female gametes ↓	BA	Ba	bA	ba
BA	BB AA Agouti	BB Aa Agouti	Bb AA Agouti	Bb Aa Agouti
Ba	BB Aa Agouti	BB aa Albino	Bb Aa Agouti	Bb aa Albino
bA	Bb AA Agouti	Bb Aa Agouti	bb AA Black	bb Aa Black
ba	Bb Aa Agouti	Bb aa Albino	bb Aa Black	bb aa Albino

Phenotypic ratio : 9/16 Agouti : 3/16 Black : 4/16 Albino or 9 : 3 : 4.

3. DUPLICATE GENES WITH CUMULATIVE EFFECT (9 : 6 : 1)

Certain phenotypic traits (*e.g.*, coat colouration) depend on the dominant alleles of two gene loci. When the dominant condition (homozygous or heterozygous) at either locus (but not both) produces the same phenotype, the F_2 ratio becomes 9 : 6 : 1.

EXAMPLE

Cumulative effect in coat colour of pigs : In the Duroc-jersey breed of pigs, coat colour is influenced by two pairs of genes that interact in peculiar manner. Sandy coat colour results from a dominant gene S, and the homozygous recessive (ss) is white in colour. Sandy coat colour may also result from a non-allelic dominant gene R ; its homozygous recessive (rr) is also white, When a sanday pig (SS rr) is crossed with a second sandy pig (ss RR), the F_1 offsprings were found with red colour coats. Such interactions are said to be the result of **mutually supplementary genes.** When F_1 red coated pigs cross bred among themselves they produce red, sandy and white coats in the ration of 9 : 6 : 1 as shown in the following table :

P : Sandy SS rr × Sandy ss RR

P gametes : (Sr) (sR)

F_1 : Red Ss Rr

F_1 cross : Male Ss Rr × Female Ss Rr

F_1 Male gametes →
F_2 Female gametes ↓

F_2 :

	SR	Sr	sR	sr
SR	SS RR Red	SS Rr Red	Ss RR Red	Ss Rr Red
Sr	SS Rr Red	SS rr Sandy	Ss Rr Red	Ss rr Sandy
sR	Ss RR Red	Ss Rr Red	ss RR Sandy	ss Rr Sandy
sr	Ss Rr Red	Ss rr Sandy	ss Rr Sandy	ss rr White

F_2 Phenotypic ratio : 9/16 Red : 6/16 Sandy : 1/16 White or 9 : 6 : 1.

4. DUPLICATE DOMINANT GENES (15 : 1)

If the dominant alleles of both gene loci produce the same phenotype without cumulative effect, the 9 : 3 : 3 : 1 ratio is modified into a 15 : 1 ratio.

EXAMPLE

The seed capsules of shepherd's purse (genus *Capsella*) occur in two different shapes, *i.e.*, triangular and top-shaped. When a plant with triangular seed capsule is crossed with one having top-shaped capsule, in F_1 only triangular, character appears. The F_1 offsprings by self crossing produced the F_2 generation with the triangular and top-shaped seed capsules in the ratio of 15 : 1. Two independently segregating dominant genes (A and B) have been found to influence the shape of the capsule in the same way. All genotypes having dominat alleles of both of these genes (A and B) would produce plants with triangular-shaped capsules. Only those with the genotype aa bb would produce plants with top-shaped capsules. The results of this example are as follows :

P :	Triangular		Top-shaped
	AA BB	×	aa bb
P gamete :	(AB)	↓	(ab)
F_1 :	Triangular		Triangular
	Aa Bb	×	Aa Bb

F_2 :

Male gametes → / Female gametes ↓	AB	Ab	aB	ab
AB	AA BB Triangular	AA Bb Triangular	Aa BB Triangular	Aa Bb Triangular
Ab	AA Bb Triangular	AA bb Triangular	Aa Bb Triangular	Aa bb Triangular
aB	Aa BB Triangular	Aa Bb Triangular	aa BB Triangular	aa Bb Triangular
ab	Aa Bb Triangular	Aa bb Triangular	aa Bb Triangular	aa bb Top-shaped

F_2 phenotypic ratio : 15/16 Triangular : 1/16 Top or 15 : 1.

DUPLICATE RECESSIVE GENES (9 : 7)

If both gene loci have homozygous recessive alleles and both of em produce identical phenotypes, the F_2 ratio 9 : 3 : 3 : 1 would ecome 9 : 7. In such case, the genotypes aa BB, aa Bb, AA bb, Aa b, and aa bb produce one phenotype. Both dominant alleles when resent together, complement each other and are called complementary genes and produce a different phenotype. A case of such omplemental inheritance, resulting from the combined action of omplemental genes is known in sweet peas.

XAMPLE

When a pure line variety of white-flowered sweet pea (*Lathyrus doratus*) was crossed with another pure line variety of white flowered weet pea, in F_1 purple or red flowered plants were produced. The $_1$ plants when self-pollinated or crossed among themselves, produed the F_2 generation with the phenotypic ratio of 9 coloured and 7 hite flowers.

These surprising results could be understood by analysing the echanism of colour production in flowers. A given enzyme (genecally controlled as to absence or presence in a given individual) cts upon chromogen (a colourless colour base whose absence or resence is also genetically controlled) to produce the purple or red olour of flowers. The dominant allele or alleles (CC or Cc) of gene

C are responsible for the presence of chromogen, while the homozygous recessive alleles (cc) of this gene are responsible for the absence of chromogen. Likewise, the dominant alleles of gene E in homozygous (EE) or heterozygous (Ee) conditions caused the production of an enzyme which is necessary for colour production from chromogen, while homozygous recessive (ee) condition does not produce any such enzyme.

The appearance of 9 : 7 ratio besides 9 : 3 : 3 : 1 ratio from the cross of two white flowered sweet pea plants (CC ee × cc EE) can be illustrated as follows :

P : White flowers CC ee × White flower cc EE

P gametes : (Ce) × (cE)

F_1 : Purple flower Cc Ee × Purple flower CcEe

F_1 Male gametes → / F_1 Female gametes ↓	CE	Ce	cE	ce
CE	CC EE Purple	CC Ee Purple	Cc EE Purple	Cc Ee Purple
Ce	CC Ee Purple	CC ee White	Cc Ee Purple	Cc ee White
cE	Cc EE Purple	Cc Ee Purple	cc EE White	cc Ee White
ce	Cc Ee Purple	Cc ee White	cc Ee White	cc ee White

F_2 :

F_2 phenotypic ratio : 9/16 purple : 7/16 white or 9 : 7.

6. DOMINANT AND RECESSIVE INTERACTIONS (13 : 3)

Sometimes, the dominant alleles of one gene locus (A) in homozygous (AA) and heterozygous (Aa) condition and the homozygous recessive alleles (bb) of another gene locus (B) produce the same phenotype, the F_2 phenotypic ratio becomes 13 : 3 instead of 9 : 3 : 3 : 1. In such case, the genotype AA BB, Aa Bb, AA bb, Aa bb and aa bb produce one phenotype and the genotype aa BB or aa Bb another phenotype.

EXAMPLE

In Leghorn type of fowl the white colour of feather is caused

by the dominant genotype CC II, similarly the white colour of feathers of Plymouth Rock breed is caused by the recessive genotype cc ii. When both white varieties of fowl are crossed, in F_1 white coloured hybrids appear. The F_1 hybrids in F_2 produced the white and coloured offsprings in the ratio of 13 : 3, illustrated as follows :

P : White Leghorn × White Plymouth Rock

CC II × cc ii

P gametes : (C I) × (c i)

F_1 : White × White

Cc Ii × Cc Ii

F_1 male gametes → CI Ci cI ci

F_1 female gametes ↓

F_2 :

	CI	Ci	cI	ci
CI	CC II White	CC Ii White	Cc II White	Cc Ii White
Ci	CC Ii White	CC ii Coloured	Cc Ii White	Cc ii Coloured
cI	Cc II White	Cc Ii White	cc II White	cc Ii White
ci	Cc Ii White	Cc ii Coloured	cc Ii White	cc ii White

F_2 phenotypic ratio : 13/16 white 3/16 coloured or 13 : 3.

The various epistatic ratios can be summarized in the following table :

Genotypes	A— B— (AA BB, Aa BB, AA Bb, Aa Bb)	A—bb (AA bb, Aa bb)	aa B— (aa BB, aa Bb)	aa bb
Classical ratio	9	3	3	1
Dominant epistasis	12		3	1
Recessive epistatis	9	3	4	
Duplicate gene with cumulative effect	9	6		1

Genotype	A— B— (AA BB, Aa BB, AA Bb, Aa Bb)	A—bb (AA bb, Aa bb)	aa B— (aa BB, aa Bb)	aa bb
Duplicate dominant' genes	15			1
Duplicate recessive genes	9	7		
Dominant and recessive interaction	13		3	

NON EPISTATIC INTER-ALLELIC GENETIC INTERACTIONS

In certain cases, two pairs of genes determine a same phenotype but assorted independently, produce new phenotypes by mutual non-epistatic interactions and the F_2 phenotypic ratio 9 : 3 : 3 : 1 remains unaltered. Two pairs of genes which interact to affect comb size and shape but are independently transmitted exist in chicken.

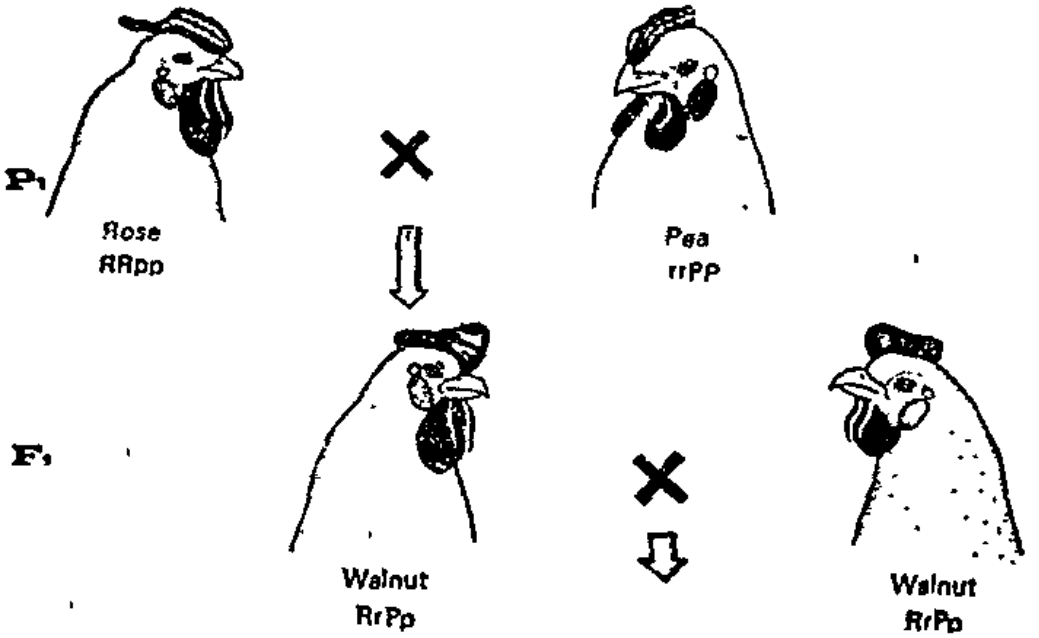
EXAMPLE

Combs in fowl (9 : 3 : 3 : 1) : The classical case of genetic interaction of two genes is discovered by **Bateson and Punnett** (1905-1908) in fowls. There are many different breeds of domestic chicken. Each breed possesses a characteristic type of comb. The wyandotte breed has a comb called "rose", the Brahmas breed has a comb called "pea", and the Leghorns have a comb called 'single'. Each of these type can be bred true.

A cross of chicken with a rose comb to one with a single comb produces $\frac{3}{4}$ rose and $\frac{1}{4}$ single, showing dominance of rose over single. Another cross between pea combed and single combed chickens produces pea and single combed chickens in the ratio of 3 : 1, showing dominance of pea over single. But, when a rose combed chicken is crossed with that of pea combed, the F_1 progeny was found with a different type of comb known as 'walnut'. When the F_1 walnut combed chickens were bred together, in F_2 all four types of combs, *i.e.*, 9/16 walnut, 3/16 rose, 3/16 pea and 1/16 single appeared.

These peculiar results were interpreted by **Bateson and Punnett** as follows : The rose comb is caused by the combination of homozygous recessive genes "pp" and a homozygous or heterozygous dominant genes 'RR' or 'Rr'. The pea comb is supposed to be produced by combination of a homozygous recessive condition (rr) and homo-

zygous or heterozygous dominant condition (PP or Pp). While, the single type comb is produced by the double recessive, rrpp, genes. Thus, R gene determines the shape of rose comb and P gene determines the shape of pea comb, but when both genes happen to come together in single individual due to cross between rose and pea combed chickens, they interact to produce a walnut comb in F_1. In the



F_2 analysis

Phenotypes	Walnut	Rose	Pea	Single
Genotypes	RRPP RRPp RrPP RrPp	RRpp Rrpp	rrPP rrPp	rrpp
Ratio	9	3	3	1
Explanation	Supplemental interaction of R and P	Dominance of R over r	Dominance of P over p	Recessiveness of rr and pp

Fig. 5·1. Supplementary inheritance. Inheritance of the shape of the comb in chickens.

cross of two walnut chickens, two genes interact variously and produce four types of offsprings in F_2.

In the inheritance of combs in fowls, the genes themselves do not determine the development of a character (presence or absence of comb) and simply modify a character determined by a basic gene and therefore, known as **supplementary or modifying genes.**

ATAVISM OR REVERSION

While we were discussing the cross between two white flowered sweet pea plants we observed that F_1 hybrid had purple colour unlike their immediate parents but like their remote ancestors. The appearance of such offsprings which resemble with their remote ancestors are called **throwbacks, atavisms or reversions.**

LETHALITY

There are certain ge[illegible] phenotypic traits, and at the same time also [illegible] the individuals. For example, *Drosophila* [illegible] ial wings have lower viability than wild type flies. There are still other genes which have no effect on the phenotype of their carrier individual but influence [illegible] individ [illegible] carryin [illegible] action is called lethality.

Example : In *Drosophila* genes like curly wings (Cy), plum eyes (P,) and [illegible] (Sb) influence the viability of the flies [illegible] mice an incomp[illegible] found lethal in homozygous condition. In man homozygous recessive genes for Amaurotic idiocy are found to be lethal genes.

PENETRANCE

The ability of a given gene or gene combination to be expressed phenotypically to any degree is called **penetrance.** It is of following two kinds :

1. COMPLETE PENETRANCE

Most dominant and recessive genes in homozygous conditions and many completely dominant genes even in heterozygous conditions give their complete phenotpyic expressions. Such genes are called to have **complete penetrance.**

EXAMPLES OF COMPLETE PENETRANCE

1. In pea the alleles (RR) for red flowers and the alleles (rr) for white flowers have complete penetrance in homozygous conditions.

2. In *Drosophila* the recessive alleles for vestigial wings in homozygous conditions have complete penetrance.

3. In guinea pigs the dominant allele 'B' for black coat has complete penetrance both in homozygous and heterozygous conditions.

2. INCOMPLETE PENETRANCE

(i) Polydactyly in man is thought to be produced by a dominant gene P. The normal condition with five digits on each limb is produced by the recessive genotype (pp). Some heterozygous individuals (Pp) are not polydactylus and therefore has a penetrance of less than 7 %.

(ii) In man, the tendancy to develop diabetes mellitus (a condition in which there is an excess of sugar in the blood) is controlled by certain genes. However, not everyone carrying the genes for diabetes actually develops the condition, for the genes have incomplete penetrance.

Effects of environment on penetrance—The environmental fac- nite effect on the deg- n various twins which is found that the disease appears only in those cases which ate more carbohydrate foods (starch and sugars).

EXPRESSIVITY

A trait though penetrant, may be quite variable in its phenotypic expressions. The degree of effect produced by a penetrant genotype is called **expressivity.**

Example of expressivity—In man the polydactylous condition may be penetrant in the left hand (6 fingers) and not in the right (5-fingers); or it may be penetrant in the feet and not in the hands.

Effects of environment on expressivity—The expressivity of a given gene is often influenced by environmental conditions. For instance, the expressivity of completely penetrant gene for vestigial wings in *Drosophila* is influenced by the temperature at which the fly develops, with the effect being most obvious at lower temperature.

Other examples of environmental effects on the experessivity of a gene includes such cases as the differences in the severity of symptoms of an inheritable allergy, or the differences in height of identical twins who have been raised in different home (with different

diets), or who have had different medical histories (one with a serious childhood disease, the other escaping this disease).

PLEIOTROPISM

effect
(allele
the ca
trait. However, it may be that
marked expression of some p
others with less evident phenotype (secondary effect). Most genes have their multiple effects and are called **pleiotropic genes.** The phenomenon of multiple effect (multiple phenotypic expressions) of a single gene is called **pleiotropism.**

EXAMPLES OF PLEIOTROPISM

1. In *Drosophila* the recessive gene for vestigial wings cause
v
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l
c
longevity is reduced.

2. In hum
tropic effect and
tively called sync
excessive quantity of amino acid phenylalanine in their urine, cerebrospinal fluid and blood. They become short stature, mentally deficient, with widely spaced incisors, with pigmented patches on skin, with excessive sweating, and with non-pigmented hairs and eyes.

CHAPTER 6

LINKAGE

The hereditary units or genes which determine the characters of an individual are carried in the chromosomes and an individual usually has many genes for the determination of various different [illegible] then the chromosomes, it can be [illegible] tains more than one gene. The [illegible] be either situated in the same chromosome or in different chromosomes. When the genes are situated in different chromosomes, the characters they control appear in the next generation either together or apart, depending on the chance alone. They assort independently according to Mendel's law of independent assortment. But if the genes are situated in the same chromosome and are fairly close to each other, they tend to be inherited together. This type of coexistence of two or more genes in the same [illegible] known as linkage. It is different from independent [illegible] following two examples :

Example 1 : Genes on different chromosomes assort independently giving a 1 : 1 : 1 : 1 test cross ratio which is as follows :

P_1 : AABB × aabb
P_1 gametes : (AB) ↓ (ab)
F_1 : Aa Bb
Test cross : Aa Bb × aa bb
Gametes : (AB) (Ab) (aB) (ab) (ab)
F_2 : ¼ Aa Bb : ¼ Aa bb : ¼ aa Bb : ¼ aa bb or 1 : 1 : 1 : 1.

Example 2 : Linked genes do not assort independently but tend to stay [illegible] parents. In [illegible] (/) are on [illegible] logous chromosome.

P_1 : AA/AB × ab/ab
P_1 gametes : (AB) | (ab)
↓
F_1 : AB/ab
Test cross : AB/ab × ab/ab
Gamates : (AB) (ab) (ab)
F_2 : ½ AB/ab : ½ ab/ab or 1 : 1.

This type of large deviation of F_2 results of example 2 from a 1 . 1 . 1 : 1 ratio in the test cross progeny of dihybrid is an evidence for linkage.

HISTORICAL

The hypothesis that linked genes tend to remain in their original combinations because of their location in the same chromosome was advanced by **T. H. Morgan** in 1911. But prior to Morgan, **Sutton** (1903) and **Bateson** and **Punnet** (1906) had given some hints about the phenomenon of linkage. Mendel could not notice the phenomenon of linkage because fortunately the seven pairs of factors or alleles studied by him in pea, were located on seven different pairs of chromosomes. The views of various geneticists about the phenomenon of linkage can be represented as follows :

1. SUTTON'S VIEW ON LINKAGE

Sutton predicted that the chromosomes are the bearers of the units of heredity or genes and since the number of hereditary units is much larger than the number of chromosomes, therefore, each chromosome must contain a number of genes. He further stated that since the chromosomes move as units during meiosis to the gametes, all the genes which are situated in same chromsome will be linked together. As a result each species of animals and plants would have a specific number of groups of linked genes which would correspond with the number of chromosomes found in that species. Unfortunately Sutton could not prove his predictions experimentally.

2. COUPLING AND REPULSION HYPOTHESIS

W. Bateson and Punnet in 1906 formulated the hypothesis of coupling and repulsion to explain the unexpected F_2 results of a dihybrid cross between a homozygous sweet pea (*Lathyrus odoratus)* having dominant alleles for blue flowers (BB) and long pollen grains (LL) with another homozygous double recessive plant with red flowers and round pollen grains (bbll). The F_1 individuals (BbLl) had blue flowers and long pollen grains. When they test crossed a F_1 dihybrid, (blue long-BbLl) with it double recessive parent (red round, bb ll), then besides getting the expected 1 : 1 : 1 : 1 test cross ratio, they received phenotypic ratio of 7 : 1 : 1 : 7 as has been illustrated in following diagram :

Parents : Blue Long BB LL × Red Round bb ll

P_1 gametes : BL bl

F_1 : Bb Ll
Blue Long

Test cross : F_1 Blue Long × P_2 Red Round

Test cross progeny : $\frac{7}{16}$ Blue Long (Bb Ll) : $\frac{1}{16}$ Blue Round (Bb ll) : $\frac{1}{16}$ Red Long (bb Ll) : $\frac{7}{16}$ Red Round (bb ll) or 7 : 1 : 1 : 7.

The 7 : 1 : 1 : 7 test cross ratio of this cross clearly indicated
to remain together.
his deviation was,
ateson and Punnett.
Similarly, it was ob
alleles come from
This was called repulsion. To understand the phenomenon of repulsion we can consider another test cross performed by Bateson and Punnett. In another test cross they took a sweet pea plant with blue flowers and round pollens (BB ll) and another plant with red flower and long pollens (bb LL) and obtained the ratio of 1 : 7 : 7 : 1 in F_2 as has been illustrated in following figure :

P_1 : Blue Round × Red Long
BB ll bb LL
↓ ↓
P_1 gametes : Bl bL
F_1 : Bb Ll
Blue long
Test cross : F_1 Blue long × P_1 Red Round
Bb Ll ↓ bb ll
Test cross progeny : $\frac{1}{16}$ Blue long (Bb Ll) : $\frac{7}{16}$ Blue Round (Bb ll) : $\frac{7}{16}$ Red Long (bb Ll) : $\frac{1}{16}$ Red Round (bb ll) or 1 : 7 : 7 : 1.

Bateson and Punnett could not explain the exact reasons of coupling and repulsion, and it was T. H. Morgan, who while performing experiments with *Drosophila*, in 1910, found that coupling or repulsion was not complete. He suggested that the two genes are found in coupling phase or in repulsion phase, because they are present on the same chromosome (coupling) or on two different
). Such genes are then called
f inheritance of linked genes is
E
f genes of homozygous parent tend to enter in same gametes and to remain together, whereas same genes from heterozygous parent tend to enter in different gametes and remain apart from each other. He further stated that the tendency of linked genes remaining together in original combination is due to their loc
degree or streng
linked genes in
developed the theory of linear arrangement of genes in the chromo-

somes which helped the cytogeneticists in the construction of genetic or linkage maps of chromosomes.

4. CHROMOSOME THEORY OF LINKAGE

Morgon along with Castle formulated the chromosome theory of linkage which is as follows :

1. The genes which show the phenomenon of linkage are situated in the same chromosomes and these linked genes usually remain bounded by the chromosomal material so that they can not be separated during the process of inheritance.

2. The distance between the linked genes determines the strength of linkage. The closely located genes show strong linkage than the widely located genes which show the weak linkage.

3. The genes are arranged in linear fashion in the chromosomes.

KINDS OF LINKAGE

T. H. Morgan and his co-workers by their investigations on the *Drosophila* and other organisms have found two types of linkage, *viz.*, complete linkage and incomplete linkage.

1. COMPLETE LINKAGE

The complete linkage is the phenomenon in which parental combinations of characters appear together for two or more generations in a continuous and regular fashion. In this type of linkage genes are closely associated and tend to transmit together.

Example—The genes for bent wings (bt) and shaven bristles (svn) of the fourth chromosome mutant of *Drosophila melanogaster* exhibit complete linkage.

2. INCOMPLETE LINKAGE

The linked genes do not always stay together because homologous non-sister chromatids may exchange segments of varying length with one another during meiotic prophase. This sort of exchange of chromosomal segments in between homologous chromosomes is known as crossing over. The linked genes which are widely
separation by crossing
nd the phenomenon of

Example—The incomplete linkage have been reported in female *Drosophila* and various other organisms such as tomato, maize, pea, mice, poultry and man, etc. Here, the examples of incomplete linkage have been considered only for *Drosophila* and maize.

1. INCOMPLETE LINKAGE IN DROSOPHILA

The wild type *Drosophila* has gray body and long wings ($b^+b^+v^+v^+/b^+b^+v^+v^+$), where alleles for gray b^+ and long v^+ dominate over the

mutant alleles for black b and vestigial v. When a gray long fly (b^+v^+/b^+v^+) is crossed with a black-bodied and vestigial-winged fly (bv/bv), the F_1 heterozygote is found with gray-long phenotype and b^+v^+/bv genotype. The F_1 heterozygote (b^+v^+/bv) when test crossed with double recessive parent (bv/bv), instead of occuring of two class of phenotypes in the ratio of 1 : 1, occur four classes of phenotypes as shown in following diagram :

Parents : Gray Long b^+v^+/b^+v^+ × Black Vestigial bv/bv

F_1 : Gray Long b^+v^+/bv

Test cross : F_1 Gray Long b^+v^+/bv × Black Vestigial bv/bv

Test cross ratio :	b^+v^+/bv :	b^+v/bv :	b v^+/bv :	bv/bv
	Gray Long	Gray Vestigial	Black Long	Black Vestigial
	41·5%	8·5%	8·5%	41·5%

The test cross results are clearly showing that parental combinations (gray-long and black-vestigial) are those expected from

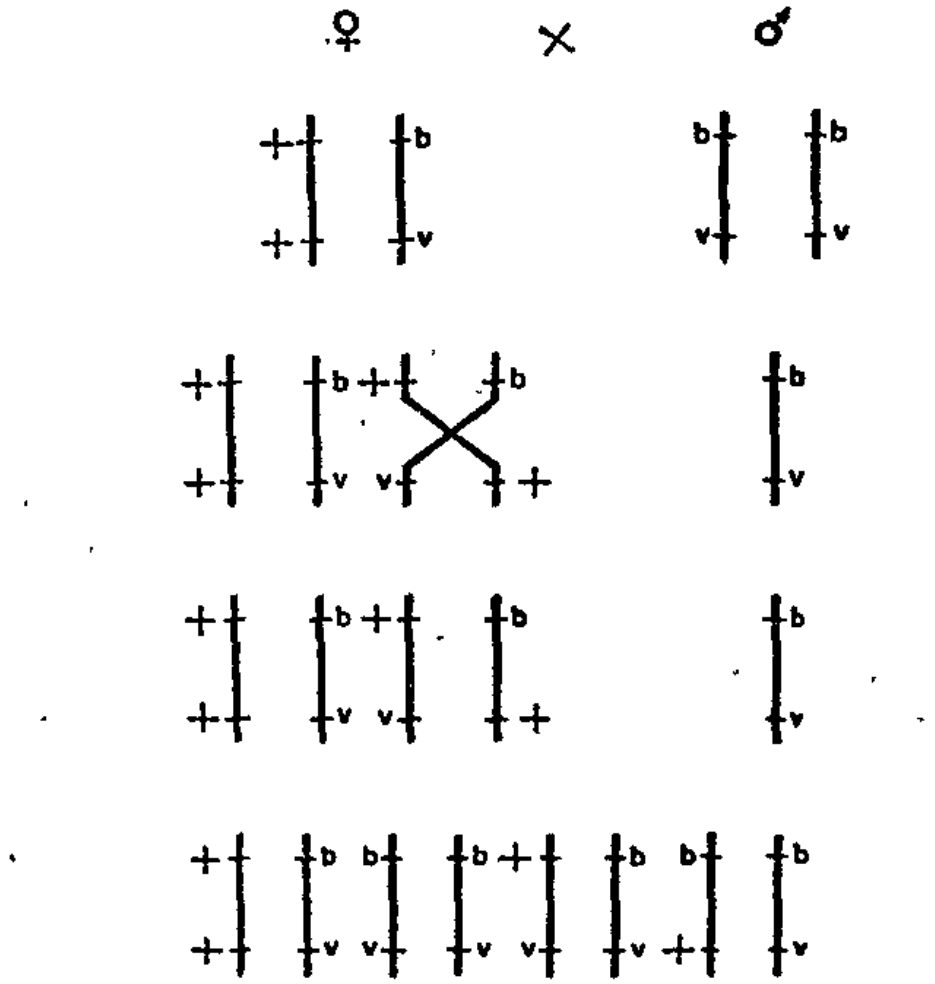


Fig. 6·1. Diagram showing incomplete linkage in linked genes colour and wing shape in *Drosophila*. b=black body, glal wings, +=gray body and +=long wings.

complete linkage and appeared in 83% cases. The other two (gray-vestigial and black-long) are new combinations and appeared in 17% cases. Thus, in 17% cases crossing over has occurred.

2. INCOMPLETE LINKAGE IN MAIZE

In *Zea mays* (maize) a case of incomplete linkage between the alleles for colour and shape of the seed has been observed by Hutchison. When a maize plant with seeds having colour and full endosperm (CS/CS) is crossed with another plant having recessive alleles for colourless, shrunken seeds (cs/cs), the F_1 heterozygotes are found with the phenotype of coloured full and genotype of CS/cs. When F_1 hybrid is test crossed with double recessive parent (cs/cs) four classes of descendants are obtained instead of two as shown in following figure :

Parents : Coloured Full × Colourless Shrunken
CS/CS cs/cs

F_1 : Coloured Full
(CS/cs)

Test cross : F_1 Coloured Full × Colourless Shrunken
CS/cs cs/cs

Test cross results :

Coloured Full	Coloured Shrunken	Colourless Full	Colourless Shrunken
CS/CS	Cs/cs	cS/cs	cs/cs
48%	2%	2%	48%

The test cross results are clearly showing that parental combination of alleles (*e.g.*, CS/CS and cs/cs) are those expected from complete linkage and appear in 96% cases, the other two are new combinations (*e.g.*, Cs/cs and cs/cs) and appear in 4% cases. Thus, in 4% cases crossing over has occurred between linked genes.

LINKAGE GROUPS

All the linked genes of a chromosome form a **linkage group.** Because, all the genes of a chromosome have their identical genes (alleomorphs) on the homologous chromosome, therefore linkage groups of a homologous pair of chromosome is considered as one. The number of linkage group of a species, thus corresponds with haploid chromosome number of that species.

Example : 1. *Drosophila has 4 pairs of chromosomes and 4 linkage groups.*
2. Man has 23 pairs of chromosomes and 23 linkage groups.

SIGNIFICANCE OF LINKAGE

The phenomenon of linkage has one of the great significance for the living organisms that it reduces the possibility of variability in gametes unless crossing over occurs.

CHAPTER 7

CROSSING OVER

In the chapter of linkage, we have stated that the genes located in the same chromosome show linkage. These linked genes may [illegible] of inheritance and thus [illegible] segregated or separated [illegible] the incomplete linkage. [illegible] to the occurrence of new [illegible] genes. The recombination in its turn is accomplished through a process known as **crossing over** in which the non-sister chromatids of homologous chromosomes exchange the chromosomal parts of segments. In another words "*the crossing over is a process that produces new combinations (recombinations) of genes by interchanging of corresponding segments between non-sister chromatids of homologous chromosomes*". The chromatins resulting from such interchanges of chromosomal parts are known as **cross overs.** According to its occurrence in the somatic or germ cells following two types of crossing over have been recognised :

1. SOMATIC OR MITOTIC CROSSING OVER

When the process of crossing over occurs in the chromosomes of body or somatic cells of an organism during the mitotic cell division it is known as **somatic or mitotic crossing over.** The somatic crossing [illegible]

Usually the crossing over occurs in germinal cells during the gametogenesis in which the meiotic cell division takes place. This [illegible] **germinal or meiotic crossing over.** [illegible] its occurrence and is of [illegible]

MECHANISM OF MEIOTIC CROSSING OVER

The process of crossing over includes following stages in it, *viz.*, synapsis, duplication of chromosomes, crossing over and terminalization.

1. SYNPASIS

During zygotene, stage of prophase –1 of meiosis occurring in mature sex cells the homologous chromosomes come close to each other and pairing of them takes place. The phenomenon of pairing of homologous chromosomes during prophase–I of meiosis is called synapsis. Synapsis is an event of prime importance in meiosis which provides the mechanical basis of heredity and variation. It started during zygotene when homologous chromosomes are held to make contact with each other at one or more points from which synapsis extends into adjacent regions and it ends or reaches its maximum in pachytene after which the homologs fall apart except the regions of chiasmata. Thus, synapsis is the phase of prolonged and close contact
two exactly id
resultant pair

Precocity theory and molecular biology of synapsis : To explain the question that why do homologous chromosomes, during synapsis, approach each other from a considerable distance and become closely associated, a British cytologist **C. D. Darlington** in 1937, proposed the **precocity theory of meiosis** which embraces well the cause of synapsis in it. According to the precocity theory of meiosis, chromosomes enter into meiotic prophase I, in contrast to mitotic prophase, as unreplicated structures, each of which consisting of a single chromatid which he considered as unbalanced or unsaturated state in electrostatical relations. In order to become staturated or balanced the chromosomes must pair. Thus the sequences of meiotic pairing or synapsis are d

The precocity theor fied only after 1955, when it was clearly shown that the main bulk of

DNA and histone proteins in *Grillus domesticus* during spermatogenesis by quantitative cytophotometric techniques, reported that DNA synthesis that converts the 2C into 4C amount occurs prior to leptotene, while histone synthesis is delayed by about 25% in zygotene and is completed only by pachytene. Thus, chromosomes enter prophase-I

with almost complete synthesis of DNA but with incomplete synthesis of histones. It was, therefore proposed that the shortage of 0·3% DNA and 2·5% histone protein in chromosomes during meiosis in fact, is nothing but a modernized version of Darlington's precocity theory.

SYNAPTINEMAL COMPLEX

Recently, **Montrose J. Moses** (1955) has revealed a highly organized structure of filaments called **synaptinemal complex** in between the paired chromosomes of zygotene and pachytene stages in crayfish by electron microscopy. Synaptinemal complex has also been observed in a wide variety of species of plants and animals.

In electron-micrographs the synaptinemal complex appears as three parallel dense lines that lie equally spaced in a plane and are flanked by chromatin. The elements of two lateral lines usually appear densest, while the element of central line is of variable prominence. Some fine transverse strands also cross between lateral elements, connecting them with the central element. Though, the morphology of lateral and central elements may vary from species to species, but the basic structure and the spacing of the synaptinemal complex is constant within the species.

Chemically, the synaptinemal complex contains DNA and a specific material known as the synaptinemal complex material of proteinaceous nature.

Functions of synaptinemal complex—The synaptinemal complex is found to be concomitant of both chiasma formation and crossing over. For instance, the synaptinemal complex may serve crossing over by facilitating effective synapsis in one or more of the follo-

may orient the non-sister chromatids of homologs in a manner to facilitate enzymatically induced exchanges between their DNA molecules. More recently, **D. A. Comings** and **T. A. Okada** (1971), have shown electron microscopically that synapsis occurs at two levels—one at chromosomal level and the other at the molecular level.

2. DUPLICATION OF CHROMOSOMES

The synapsis is followed by duplication of chromosomes. Each

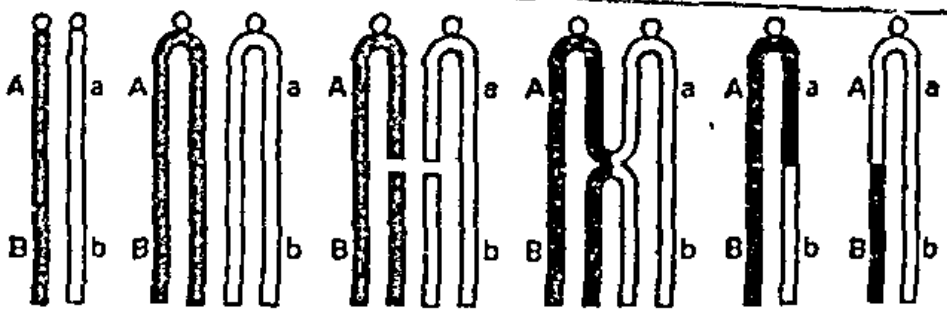


Fig 7 1. Diagram showing the mechanism of crossing over.

DNA molecules along with certain chromosomal proteins. At this stage each bivalent contains four chromatids, so it is known as tetrad.

3. CROSSING OVER

The

over, two non-sister chromatids first

and Hotta, 1969). Then a segment on one side of each break connects with a segment on the opposite side of the

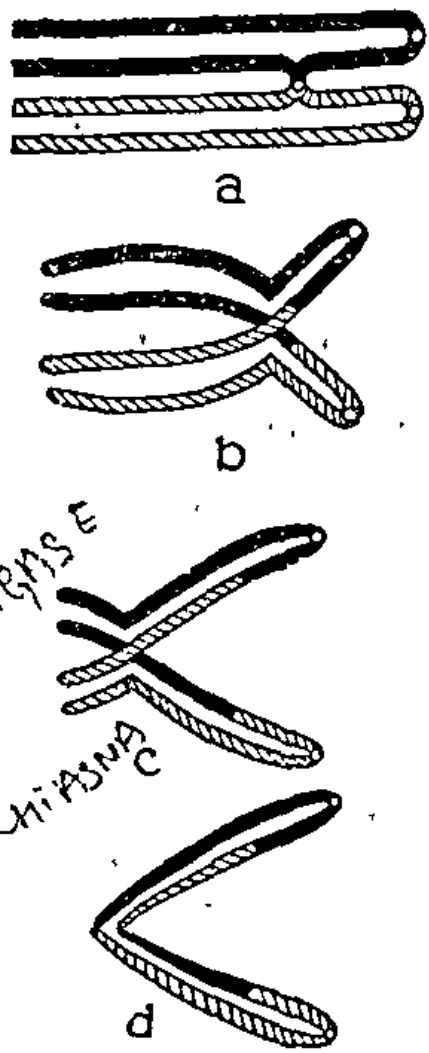


Fig. 7 2. Diagram showing the terminalisation

over may take place at several points in one tetrad and may result in the formation of several chiasmata. The

number of chiasmata depends on the length of the chromosomes because the longer the chromosome the greater the number of chiasmata. In a sepecies each chromosome has a characteristic number of chiasmata. The frequency by which a chiasmata occurs between any two genetic loci has also a characteristic probability. The more apart two genes are located on a chromosome, the greater the opportunity for a chiasma to occur between them. The closer two genes are linked lesser the chances for a chiasma occurring between them.

4. TERMINALISATION

After the occurrence of process of crossing over, the non-sister chromatids start to repel each other because the force of synapsis attraction between them decreases. The chromatids separate progre-
and the chiasma
of the tetrad. The
Due to the termi-
ated completely.

/ER

In *Drosophila* the factors for gray body (+) and long wings (+) are dominant, while the factors for black body (b) and vestigial wings (v) are recessive. When a gray-long (++/++) fly is crossed with a black-vestigial (bv/bv), the F_1 hybrids have gray body and long wings (++/bv). When a F_1 female hybrid (++/bv) is test crossed with a double recessive male with black body and vestigial wings (bv/bv) the F_2 test cross generation consists of 82% offsprings of parental ratio (*i.e.*, 41% gray-long, ++/bv and 41% black-vestigial, bv/bv) and 18% offsprings of new combination (*i.e.*, 9% gray-vestigial, +v/bv and 9% black-long, b+/bv). The occurrence of 18% new combinations in F_2 test cross represent that in 18% cases crossing over has occurred.

KINDS OF CROSSING OVER

According to the number of chiasma following types of crossing over have been described.

1. **Single crossing over**—When the chiasma occurs only at one point of the chromosome pair then the crossing over is known as **single crossing over.** The single crossing over produces two cross over chromatids and two non-cross over chromatids.

2. **Double crossing over**—When the chiasma occurs at two points in the same chromosome, the phenomenon is known as **double crossing over.** In the double crossing over, the formation of each chiasma is independent of the other and in it four possible classes of

recombination occur. In the double crossing over following two types of chiasma may be formed :

(i) **Reciprocal chiasma**—In the reciprocal chiasma the same

The reciprocal chiasma occurs in two strand double crossing over in which out of four chromatids only two are involved in the double crossing over.

(ii) **Complimentary chiasma**—When both the chromatids taking part in the second chiasma are different from those chromatids

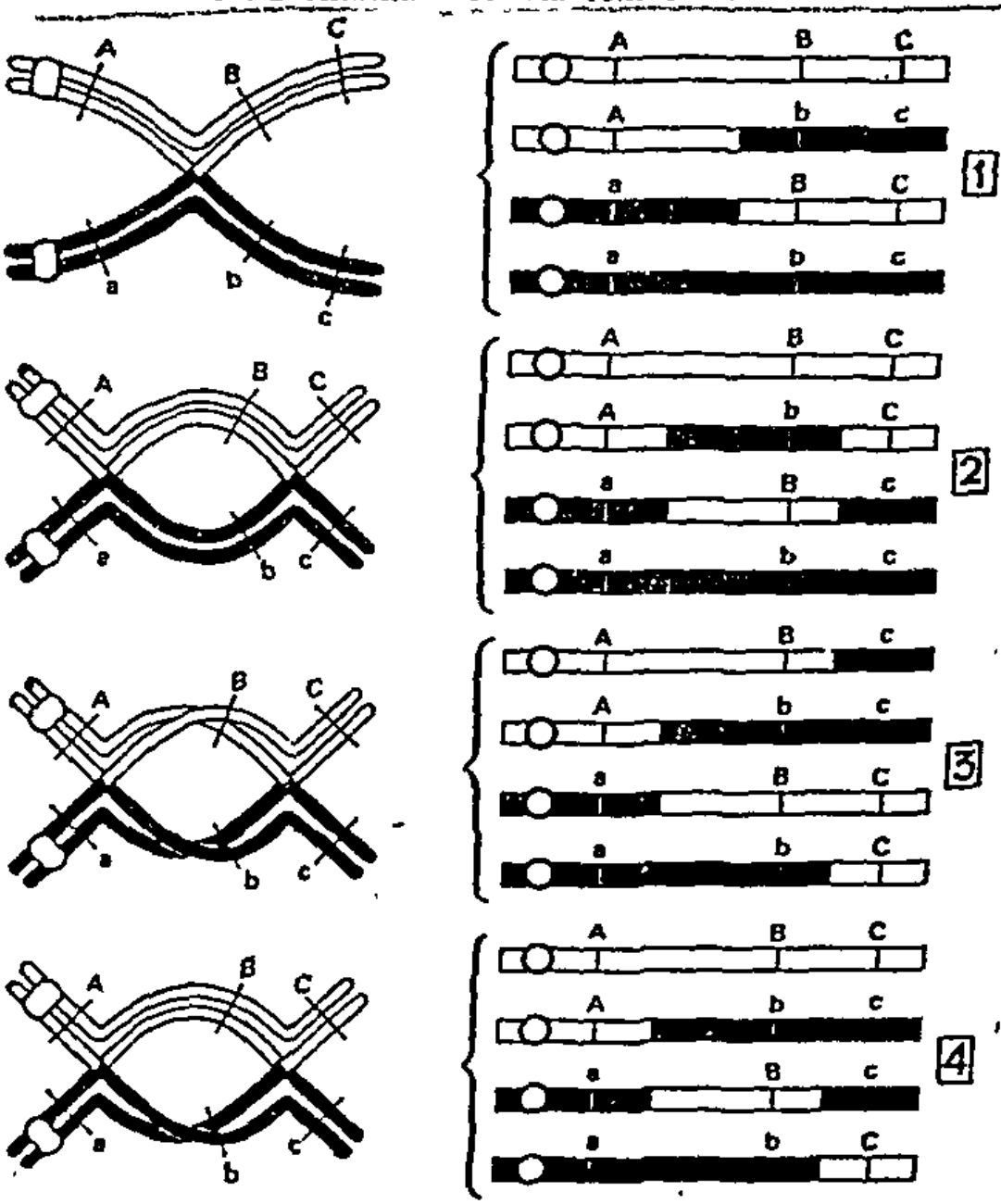


Fig. 7·3. Diagram showing the results of single and double crossing over 1. Single chiasma. 2. Two strand double cross over. 3. Four strand double cross over. 4. Three strand double cross over.

involved in the first chiasma, the chiasma is known as **complimentary chiasma.** The complimentary chiasma produces four single cross overs but no non-cross over. The complimentary chiasma occurs when three or four chromatids of the tetrad undergo the crossing over.

3. **Multiple crossing over**—When crossing over takes place at more than two places in the same chromosome pair then such crossing over is known as **multiple crossing over.** The multiple crossing over occurs rarely.

THEORIES ABOUT THE MECHANISM OF CROSSING OVER

Following theories have been propounded to explain the mechanism of crossing over :

1. **Belling's hypothesis**—J. Belling proposed that crossing over is really brought about due to novel attachments formed between newly synthesized genes. According to him chromosome duplication consists of two stages—(a) formation of new genes, and (b) the formation of new connections between these newly synthesized genes. This results in the formation of recombinants or cross overs.

2. **Copy choice theory**—To explain recombination in microorganisms, J. Laderberg (1955) proposed a modified version of Belling's hypothesis. This is called copy choice mechanism of recombination or crossing over, where it is speculated that a newly synthesized daughter chromatid is derived due to copying of one homologous chromosome up to a certain distance and then switching to the other homologous chromosome for copying the remaining distance or region of the chromosome.

Objections to copy choice theory—Some objections have been raised against the copy choice theory. One of the objection against this theory is that it proposed the involvement of only two chromatids in the process of crossing over, while, cytological investigations have made it clear that crossing over occurs at four strand stage. Second objection against this theory is that in higher organisms a little DNA synthesis occurs during the crossing over and that DNA is utilized in the repairing of broken DNA segments, and not used in forming new chromosomal segments.

3. **Break and exchange theory**—The break and exchange theory is the most accepted theory to explain the process of crossing over. This theory states that in the crossing over breaks occur in the non-sister chromatids of the tetrad and between the non-sister chromatids the exchange of chromosomal segments occurs. Recently the cytological findings of Stern and Hotta (1969) have shown that crossing over is performed by two nuclear enzymes namely end nuclease

and ligase. The endonuclease helps in the breaking of the chromatids and ligase restores the broken segments to the broken non-sister chromatids. Further, it is well established now that during the course of crossing over synthesis of small amount (about 3%) of DNA takes place which is used in the repairing of the broken chromatids during the crossing over.

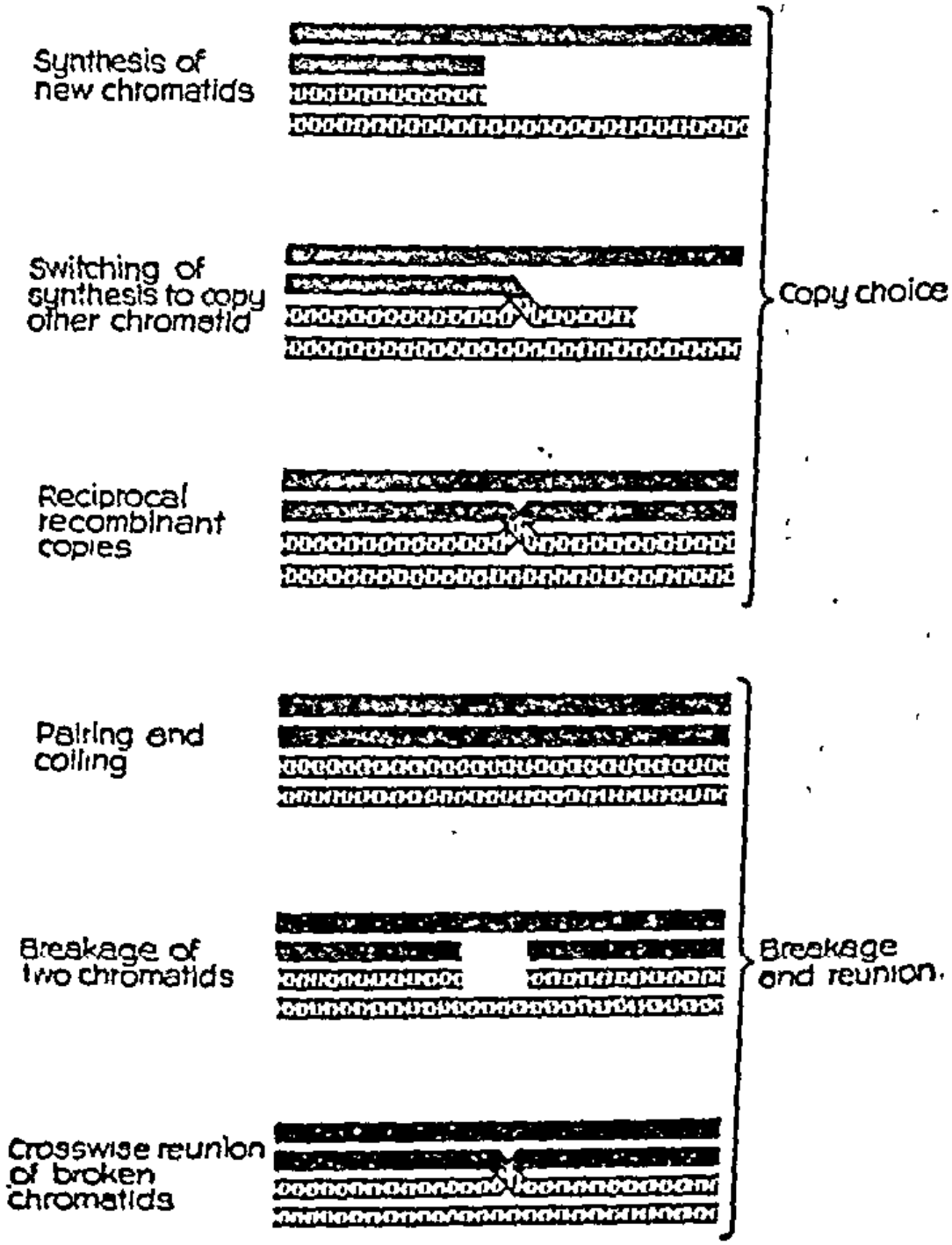


Fig. 7·4. Diagrammatic representation of two possible mechanisms of crossing over.

There exists a lot of controversy about the manner and time of the break of chromatids during the crossing over. Following theories

have been advanced by different workers to explain the process of breaking of chromatids :

1. **Serebrovsky's contact first theory**—According to this theory two chromatids of a tetrad first touch and cross each other and then at the point of cross the breakage of chromatids occur. The chromosome segments then unite to form new combinations.

2. **Muller's breakage first theory**—This theory holds that before the occurrence of chiasma and crossing over two chromatids or chromosomes break into two fragments and then the union of chromosomal segments takes place.

3. **Darlington's strain or torsion theory**—**Darlington** (1935) propounded that chromosomal breakage occurs as a result of strain during pairing. According to this theory during the late pachytene of meiosis, the homologous chromosomes pair and become spirally twisted around each other. Due to twists the coils are formed and these are known as **relational coil.** Then there develops a sort of strain on one chromatid. As a result of the strain due to torsion of chromosomes, the chromatids of the chromosomes break at the point of contact and thus recombination results.

SIGNIFICANCE OF CROSSING OVER

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CHAPTER 8

GENETIC AND CYTOLOGICAL MAPPING OF CHROMOSOMES

Our discussion of linkage and crossing over has made clear so far, that 1. Because the number of genes usually exceeds the number of chromosomes in different species, therefore, many genes have to be located on same chromosomes. 2. The genes remain arranged linearly on the chromosomes [illegible] except to behave according t[illegible] genesis and inheritance, there [illegible] *inherit together* and are said linkage groups. 3. The numbe[illegible] the number [illegible] species. 4. occasionall[illegible] by crossing [illegible] departure [illegible] genes. 6. And each gene has definite order and location in a linkage group or chromosomes, as the crossing over frequency has been found constant for two given linked genes of a species. For example various genetic crosses have shown that in *Drosophila*, among three mutant sex-linked genes (*viz.*, genes for white eyes, yellow body and cut wings), the genes for white and yellow are always found to have 1% crossing over and similarly, white and cut genes are found to have 20% crossing over. The percentage or frequency of crossing over, thus, appears to be closely related to physical distance between genes. When, one knows all the genes, linkage groups and number of linkage groups of a species, it becomes possible for him that by adopting the crossing over as a tool, he may determine the relative [illegible] their order of crossing over. Such a diagrammatic, graphical representation of relative distances between linked genes of a chromosome is called

linkage or genetic map. Because, such a linkage or genetic map is the outcome of crossing over studies, therefore, is also called cross-over map.

A. CONSTRUCTION OF A LINKAGE MAP OR GENETIC MAPPING

The method of construction of genetic maps of different chromosomes is called genetic mapping. The genetic mapping includes following processes :

1. DETERMINATION OF LINKAGE GROUPS

Before starting the genetic mapping of the chromosomes of a species, one has to know the exact number of chromosomes of that species and then, he has to determine the total number of genes of that species by undergoing hybridization experiments in between wild and mutant strains. By the same hybridization techniques, it can also be easily determined that how many phenotypic traits remain always together or linked and consequently their determiners or genes during the course of inheritance. And thus, the different

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TANCE

genes in each linkage group of a species, the relative distances between each linked gene have to be determined. The distance between two given genes is calculated

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1. If a F_1 hybrid having the genotype Ab/aB produces 8% of cross over gametes AB and ab, then the distance between A and B is estimated to be 16 map units or centimorgan.

2. If the map distance between the gene loci B and C is 12 centimorgan, then 12% of gametes of genotype BC/bc should be cross-over types, *i.e.*,6% Bc and 6%bC.

Because, each chiasma produces 50% crossover products, 50 percent crossing over is equivalent to 50 map units or centimorgans. If the mean number of chiasmata is known for a chromosome pair, the total length of the map for that linkage group may be predicted :

Total length = mean number of chiasmata × 50

TWO POINT TEST CROSS

The percentage of crossing over between two linked genes is calculated by test crosses in which a F_1 dihybrid is crossed with a double recessive parent. Such crosses because involved crossing over at two points, so called **two point test crosses**. For example, a dihybrid having the genotype Ac/ac is test crossed with a double recessive parent (ac/ac), then among F_2 test cross hybrids we may get 37% dominant genes at both gene loci (AC/ac), 37% recessive genes at both gene loci (ac/ac), 13% dominant gene at first gene locus and recessive gene at the second gene locus (Ac/ac), and 13% recessive gene at first gene locus and dominant gene at second gene locus (aC/ac). The last two groups (*i.e.*, 13% Ac/ac and 13% aC/ac) were produced by crossover gametes (13+13) from the dihybrid parent. Thus, 26% of all gametes (13+13) were of cross over types and the distance between the loci A and C is estimated to be 26 centimorgans. Because, double crossovers usually do not occur between genes less than 5 centimorgans apart, so for genes further apart, the three point test crosses are used.

THREE POINT TEST CROSS

A three point test cross or trihybrid test cross (involving three genes) gives us information regarding relative distances between these genes, and also shows us the linear order in which these genes should be present on chromosome. Such a three point test cross may be carried out if three points or gene loci on a chromosome pair can be identified by marker genes. If, in addition to genes A and C indicated above, a third marker gene B is located in fairly close proximity in the same linkage group, all three markers may be used together in conducting a more precise analysis of the map distance and the relative position of the three points.

Suppose that we testcross trihybrid individuals of genotype ABC/abc and find in the progeny the following :

36% ABC/abc 36% abc/abc	9% Abc/abc 9% aBC/abc	4% ABc/abc 4% abC/abc	1% AbC/abc 1% aBc/abc
72% Parental type	18% Single crossovers between A and B. (region I)	8% Single Crossovers between B and C. (region II)	2% Double crossover

To find the distance A—B we must count all crossovers (both singles and doubles) that occurred in region I=8%+2% or =20% or 20 map units between the loci A and B. To find the distance B—C we must again count all crossovers (both singles and doubles) that occurred in region II=8%+2%=10% or 10 map units between the loci B and C.

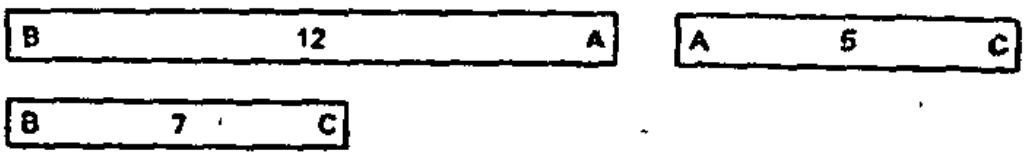
The A—C distance is therfore 30 map units when double crossovers are detected in a three point linkage experiment and 26 map units when double crossovers are undetected in the two-point linkage experiment above.

Without the middle marker (B), double crossovers would appear as parental types and hence we under estimate the true map distance (cr
would ap
parental
linked genes whose distances are known, the amount of detectable crossovers between the two outer markers A and C when the middle marker B is missing is; (A—B crossover percentage) plus (B—C crossovers percentage) minus (2×double crossover percentage).

3. DETERMINATION OF GENE ORDER

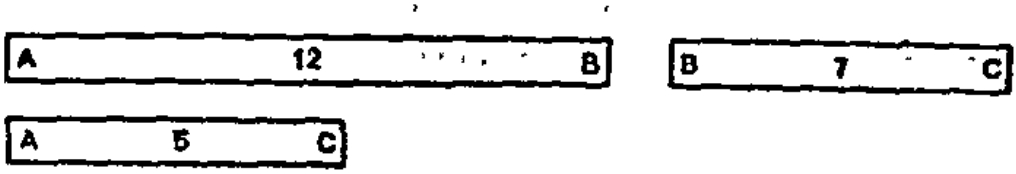
After determining the relative distances between the genes of a linkage group, it becomes easy to place genes in there proper linear order. For example, if the linear order of three genes ABC is to be determined, then these thre
rent orders depending upo
the time being we may
double crossovers do not occur, map distances may be treated as completely additive units. Now, if we suppose that the distance between the genes A—B=12, B—C=7 and A—C=5, we can determine the order of genes correctly in the following manner.

Case I. Let us assume that gene A is in the middle (*e.g.*, B—A—C) :



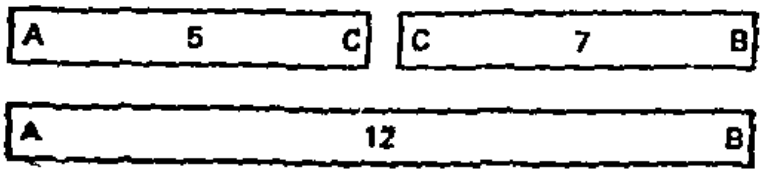
In this case because, the distances between B—C are not equitable, genes A cannot be in the middle.

Case II. Let us assume that gene B is in the middle (*e.g.*, A—B—C) :



In this case, because the distances between A —C are not table, therefore, gene B cannot be in the middle.

Case III Let us assume that gene C is in the middle (*e.g.*, A—C—B) :



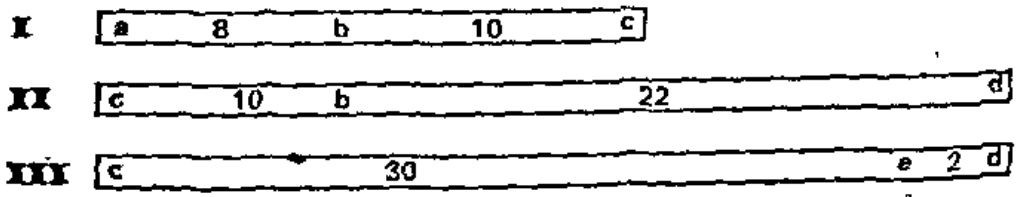
In this case, because the distances between A—B are equitable, therefore, gene C must be in the middle.

Thus, the relative distances and ordering of genes in a linkage group are determined in separate segments by two point test crosses or three point crosses, as the case may be.

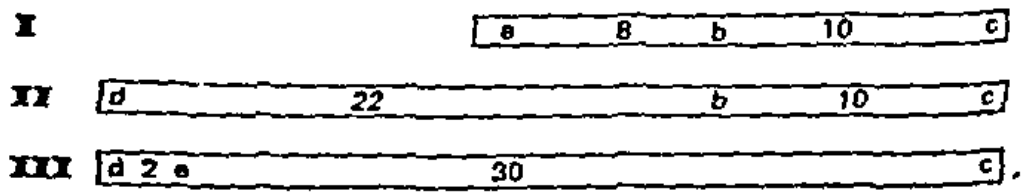
4. COMBINING MAP SEGMENTS

Finally, the different segments of maps of a complete chromosome are combined to form a complete genetic map of 100 centimorgans long for a chromosome.

Example : For example, suppose we have to combine following three map segments.



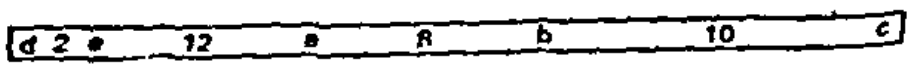
We can superimpose each of these segments by aligning the genes shared in common.



Then finally we may combine the three segments into one map—

The a to d distance=(d to b)—(a to b)=22—8=14

The a to e distance=(a to d)—(d to e)=14—2=12



INTERFERENCE AND COINCIDENCE

In most higher organisms it has been found that one chiama formation reduces the probability of another chiasma formation in an immediately adjacent region of the chromosome, probably because of physical inability of the chromatids to bend back upon themselves within certain minimum distances. The tendency of one crossover to interfere with the other crossover is called **interference.** The net result of this interference is the observation of fewer double crossover types than would be expected according to map distances. The str[illegible] of interference varies in different segments of the chromosom[illegible] efficient of coincidence, [illegible] expected double crossovers.

$$\text{Coefficient of coincidence} = \frac{\%\text{ observed double crossovers}}{\%\text{ expected double crossover}}$$

The coincidence is the complement of interference, so :.

Coincidence + Interference = 1·0

When interference is complete (1·0), no double crossovers will be observed and coincidence becomes zero. When, interference decreases, coincidence increases. Coincidence values ordinarily vary between 0 and 1. Coincidence is generally quite small for short map distance. There is no interference across centromere.

EXAMPLE

For explaining interference and coincidence, we can consider the results of one of the experiment of Hutchison (1922). He reported the map distances for three genes, c (colourless aleurone), Sh (shrunken grains), and wx (waxy endosperm) of corn and observed following crossing over frequencies between these genes :

Table : Crossing over frequencies between genes c, Sh and wx of corn.

'Regions'	Genes	Percentage crossovers	Map-distances (in map units)
I	c—Sh	3·4	3·4+0·1=3·5
II	Sh—wx	18·3	18·3+0·1=18·4
Double crossover	c—Sh—wx	0·1	

If crossing over in region I and II were independent, we should predict 0·035×0·184=0·6 per cent double crossovers, where as only 0·1 per cent was observed

$$\text{So, coincidence} = \frac{0{\cdot}1}{0{\cdot}6} = 0{\cdot}167$$

LINKAGE MAPS OF DIFFERENT ORGANISMS

By adopting the above mentioned techniques, geneticists have constructed the linkage or genetic maps of various organisms, such as, viruses, bacteria, fungi, tomato, barley, wheat, rice, sorghum, morning glory, garden pea, maize, *Drosophila*, chickens, mice, man, etc. The first linkage map has been constructed for two chromosomes of *Drosophila* by Strutevant in 1911. The linkage maps of other chromosomes of *Drosophila* have been constructed by **C. B. Bridges.** The linkage or genetic mapping in maize has been done by **McClintoek** under the leadership of **R. A. Emerson.**

B. CHROMOSOME. CYTOLOGICAL OR PHYSICAL MAPPING

It has been found that linkage map distances between genes are not necessarily proportional to physical linear measurements. Special cytological techniques have been used to determine the physical locations of a gene in a chromosome. Localization has been accomplished by identifying a gene locus with relation to some visible landmark such as a chromomere or cross band. In this process of **chromosome, physical or cytological mapping,** the polytene chromosome of salivary glands of *Drosophila* have been found very useful. **T.H. Painter** was the first geneticist who used polytene chromosomes of *Drosophila* in the cytological verification of genetic data. He related the bands on the giant chromosomes to genes, but he was more interested in the morphology of the chromosomes and applications concerning speciation than in the association of chromosome sections with particular genes. **Bridges,** beginning in 1934, made extensive and detailed investigation of the salivary gland chromosomes and, in the course of his investigations, developed a tool of practical usefulness in reating genes to chromosomes. In applying this method to *Drosophila melanogaster*, he prepared a series of cytological or chromosome maps to correspond with the linkage maps already available.

CYTOLOGICAL MAPPING OF CHROMOSOMES OF DROSOPHILA

About 5000 single cross bands have been noted on thefour pairs of salivary gland chromosomes in *D. melanogaster*. This number is considered a minimum approximation of the number of genes in that animal. Some genes have been associated with individual bands. Bridges system of designating parts of chromosomes with numbers, subdivision with letters, and numbering bands within subdivisions

had made it possible for investigators to discuss precise locations. In this system, fairly uniform divisions are numbered in order throughout each entire chromosome set from O at the beginning of the X chromosome to 102 at the end of chromosome 4. Subdivisions within the areas are identified with letters from A to F, and bands within subdivisions are numbered from left to right. For example, the gene (w) for white eyes is in bands 3C2. In linkage units this gene is located at 1·5 in the X chromosome. Linkage data do not correspond exactly with cytological locations, but the linear sequence of genes can be verified from salivary preparations

DIFFERENCES BETWEEN GENETIC AND CHROMOSOME MAPS

The frequency of crossing over usually varies in different segments of the chromosome, but is a highly predictable event between any two genes loci. Therefore, the actual physical distances between linked genes bears no direct relationsnip to the map distance calculated on the basis of crossover percentages. However, the linear order of linked genes is identical in both maps.

USES OF GENETIC MAPS

brid crosses.

CHAPTER 9

MULTIPLE ALLELES

So far, it has been observed that a given phenotypic trait (character) of an individual depends on a single pair of genes, each of which occupies a specific position called the gene locus on a homologous chromosome. Moreover, a [illegible] occur in two alternative forms. For [illegible] of *Drosophila* wings may occur in [illegible] gene (L^+) for normal development of wings and allele or gene (L^{vg}) for vestigial wings. Because, most flies have normally developed wings, so, it can be easily concluded that gene L^+ is the original form of gene or allele from which the other form of gene or allele (L^{vg}) might have originated by certain mutational event at sometime in past. The gene L^+ for normal development of wing is called the normal or wild type allele of the gene L and usually symbolized as L^+, while the mutated gene L^{vg} for vestigial wing is called reduced type or mutant allele of the gene L. A fly with normal wings, thus, has two wild type alleles (L^+L^+) and the vestigial winged fly has two mutant alleles (L^{vg} L^{vg}). Both of these allelic forms (L^+ and L^{vg}) of gene L occur at corresponding positions on genetically identical (homologous) chromosomes of same or different individual.

But, now there are ample evidences that a gene for particular character, besides occurring in two alternative forms or alleles or variants, may occur in several alternative forms or alleles. All the variants or alleles of a given gene are supposed to be originated by mutation of a single wild type gene. Out of several allelic forms of a gene, a given locus may bear any one allele, so that, a diploid individual possesses any two alleles of the allelic series. *When any of the* [illegible] *forms in a given*

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The most important and distinguishing features of multiple alleles are summarized below :

1. Multiple alleles of a series always occupy the same locus in the chromosome.

2. Because all the alleles of multiple series occupy same locus within the alleles

3. Multiple alleles always influence the same character

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be

4. When any two of the mutant multiple alleles are crossed,

The dominance hierarchy is defined at the beginning of each problem involving multiple alleles. A capital letter is commonly used to designate the allele which is dominant to all other alleles in the series. The corresponding lower case letter designates the allele which is recessive to all others in the series. Other alleles which are intermediate in their degree of dominance between these two extremes, are usually assigned the lower case letter with some suitable supper script.

EXAMPLES

The best examples of multiple allelic system have been observed in coat colour of rabbits, wings of *Drosophila* and blood groups in man.

1. COAT COLOUR IN RABBITS

The coat of rabbit may have following different colours :

(i) Full colour—The coat of the ordinary (wild type) rabbit is referred to as **"agouti" or full colour,** in which individuals have banded hairs, the portion nearest the skin being gray, succeeded by a yellow band, and finally a black or brown tip. The genes for full colour may be respresented by capital letter C.

(ii) Chinchilla—In some individuals, the coat lacking the yellow pigment and due to the optical effect of black and gray hairs, have the appearance of silvery-gray. The gene for chinchilla is represented as C^{ch}.

(iii) Himalyan—The himalyan type coat is white except for black extremities (nose, ears, feet and tail). The eyes are pigmented. The gene for himalyan coat is represented by C^h.

(iv) Albino—The albino coat totally lacks in pigmentation and the eyes of a albino also remain pink due to lack of pigment in iris of eye. The gene for albino is represented by c.

When crosses are made between rabbits with different coat colours, then it is revealed that full colour (C) is dominant to chinchilla (C^{ch}), himalayan (C^h) and albino (c). Further, the allele for chinchilla (C^{ch}) is found recessive to allele C but dominant to gene C^h and c. Likewise, the allele for himalayan (C^h) is found recessive to allele C and C^{ch}, but dominant to allele c and allele c for albino coat is found to be recessive to all alleles of this multipe allelic series. These results clearly indicate that the allele C^{ch} (chinchilla), C^h (himalyan) and c (albino) are mutant or allelic forms of wild type gene C. The dominance heirarchy may be symbolized as follows :

$$C > C^{ch} > C^h > c$$

The possible phenotypes and their associated genotypes, for this series of multiple alleles in rabbit, are as follows :

Phenotypes	Genotypes
Full colour (agouti)	CC, CC^{ch}, CC^h, Cc.
Chinchilla	C^{ch} C^{ch}, C^{ch} C^h, C^{ch}c.
Himalayan	C^h C^h, C^hc.
Albino	cc

It should be noted here that in this case four allelic forms of genes may produce at least ten genotypes, whereas in case of single pair of alleles at a given locus only three genotypes were produced where dominance is complete. Thus, as the number of genes in a series of multiple alleles increases, the variety of genotypes rises still more rapidly, such as examplified here :

Alleles in series	Genotype
2	3
3	6
4	10
5	15
n	$\frac{n}{2}(n+1)$

2. WING-SIZE IN DROSOPHILA

Drosophila has three alleles of gene L for size of wings—(i) L^+ for normal or wild type wings. (ii) L^{vg} for vestigial wings and (iii) L^a for antlered wings. The wild type gene L^+ is dominant to L^{vg} and L^a, while both recessive alleles (L^{vg} and L^a) have incomplete dominant recessive relationships, therefore, their cross gives a new mutant form as given below :

P : Vestigial-winged fly $L^{vg}\ L^{vg}$ × Antlered-winged fly $L^a\ L^a$

↓

F_1 : $L^{vg}\ L^a$ Intermediate

F_2 : $L^{vg}\ L^{vg}$, *Vestigial* ; $L^{vg}\ L^a$, $L^{vg}\ L^a$, *Intermediate* ; $L^a\ L^a$, *Antlered*

Thus, in *Drosophila*, the gene L occurs in three allelic forms (L^+, L^{vg} and L^a) and their possible genotypes can be summarized as follows :

Phenotypes	Genotypes
Long-wings	$L^+\ L^+$, $L^+\ L^{vg}$, $L^+\ L^a$
Vestigial-wings	$L^{vg}\ L^{vg}$
Antlered-wings	$L^a\ L^a$
Intermediate-wings	$L^{vg}\ L^a$

The dominance heirarchy of multiple allelic series of gene L can be symbolized as follows :

$$L^+ > L^{vg} > L^a$$

3. A B O BLOOD GROUPS IN MAN

The A B O blood group system of man furnishes a best example of multiple allelism, In man the gene I (for antigen) may occur in three possible allelic forms—I^A, I^B and i. The allele I^A for the A antigen is codominant with the allele I^B for the B antigen. But, I^A and I^B are completely dominant to the allele i which fails to specify any detectable antigenic structure. The dominance and recessiveness relationships between these alleles can be symbolized as $(I^A = I^B) > i$. Two antisera (anti-A and anti-B) are required for the detection of four phenotypes :

Genotypes	Reaction with		Phenotypes
	Anti—A	Anti—B	
$I^A I^A$, $I^A i$	+	–	A
$I^B I^B$, $I^B i$	–	+	B
$I^A I^B$	+	+	AB
ii	–	–	O

PSEUDOALLELES

In previous section of multiple allelism, we have observed that multiple alleles occupied same gene locus in both homologous chromosomes and never undergo crossing over, but there is one possibility that certain gene loci may be so closely linked to each other that they may inherit together and may have minimum chances of crossing over. Then, such genes which are actually different type of gene, may behave like alleles of same gene and erroneously may be considered as multiple alleles. Such genes which have two separate gene loci, but often inherit together due to close linkage, and have very rare chance of crossing over, are called pseudoalleles. There is a lot of controversy about the exact definition of pseudoalleles. According to Lewis (1955) and Green (1963), the pseudoalleles may be regarded as distinct genes with their own particular functions which must be carried out in sequence at the same intracellular location. Pontecorvo (1958) considered pseudoalleles as different sites within the same gene. The concept of pseudoallelism has two fold significance : (i) It shows the possibility of division of classical gene into a structural and a functioual unit and (ii) It provides a clue for intragenic units of recombination.

EXAMPLE OF PSEUDOALLELISM

The best example of pseudoallelism has been observed in sex-linked genes for eye colour in *Drosophila*. The colour of *Drosophila* eye is governed by a series of sex-linked genes which cause the hue to vary from red or wild type (w^+) through coral (w^{co}), blood (b^N), eosin (w^e), cherry (w^{ch}), appricot (w^a), honey (w^h), buff (w^{bf}), tinged (w^t), pearl (w^p) and ivory (w^i), to white (w) All of these were considered, on the basis of F_2 ratios, to form a multiple series, wild being dominant to all others and white recessive to all—$w^+ > w^{co} > w^{bl} > w^e > w^{ch} > w^a > w^{bf} > w^t > w^p > w^i > w$.

All are located at about 1·5 on the X chromosome map. However, *in crosses that originally produced only apricot and white progeny,* about in 10,000 individuals had wild type red eyes. By using certain techinques of chromosome mapping (*i.e.*, by using the "marker gene" y yellow body colour), at locus 1·0 and spl (split bristles) at locus 3·0, it was possible to show that genes for apricot and white occupied separate, but extremely close loci with a crossover frequency of approximately 0·01.

Surprisingly, the two possible kinds of heterozygous females turn out to have different phenotypes. Thus, w^+w/w^+w^{ap} is pale apricot, and w^+w^+/ww^{ap} is wild type. Thus, w and w^{ap} genes are allelic in functional sense but not in the structural sense.

CHAPTER 10

DETERMINATION OF SEX

The sexually reproducing organisms may be classified into two types, *viz.*, **monoecious** or **hermaphrodite** and **dioecious** In the monoecious organisms both male and female gametes or sex cells are produced by a single individual, *e.g.*, most higher plants and some animals of lower groups. In the majority of cases, however, the species is split up into two sections, from which we derive the word sex from the Latin *sexus* meaning section or separation. The male section has the testes or male gonad and the female the ovaries or female gonad. This kind of separation of the sexes is called **gonochorism**. The organisms in which both male and female gametes are produced by different individuals are called **dioecious**. The sex cells and reproductive organs form the **primary sexual characters** of male and female sexes. Besides these primary sexual characters, the male and female sexes differ from each other in many somatic characters known as **secondary sexual characteres**. The phenomenon of molecular, morphological, physiological or behavioral differentiation between male and female sexes is called **sexual dimorphism**.

The phenomena of sexual dimorphism has been a biological riddle for the thinkers and biologists of all time. People always tried to know those factors which determine the male and female sexes of a species. Literally hundreds of mistaken hypotheses and wild guesses were proposed before 1900 in vain attempts to find out a solution to the problem of determination of sex. Modern geneticists have reported many different mechanisms of determination of sex in living organisms. Some important and common mechanisms of sex determination are following :

A. GENETICALLY CONTROLLED SEX DETERMINING MECHANISMS

Most of the mechanisms of the determination of the sex are under genetic control and they may be classified into following categories :

1. Sex chromosome mechanism or Heterogamesis.
2. Genic balance mechanism,
3. Male haploidy or haplodiploidy mechanism.
4. Single gene effects.

SEX CHROMOSOME MECHANISMS (HETEROGAMESIS)

Discovery of sex chromosomes—In sexually dimorphic dioecious organisms besides morphological and behavioural differences between both sexes, the sexual diversity also occurs at the level of chromosomes. The chromosomal differences between the sexes of several dioecious species were found earlier in the course of cytological investigations. A German biologist, **Henking** in 1891 while studying spermatogenesis of the squash bug, *Pyrrhocoris*, noted that half of the spermatozoa contained an extra chromosome which was called by him as "**X body**" The significance of X body was not immediately understood, but in 1902 an American geneticist **McClung** who had made extensive observations of gametogenesis in grasshoppers, suggested that the X body was involved in some way with the determination of sex. He reported that the somatic cells of the female grasshopper (*Xiphidium fasciatum*) contained 24 chromosomes whereas those of the male had only 23. Three years later, **Miss Stevens** and **Wilson** succeeded in following both oogenesis and spermatogenesis in several insects and it was realized that the X body was a chromosome, so the X body became known as **X chromosome.** Because the X chromosome has been found to have relation with determination of sex of an individual by its presence or absence so it is called **sex chromosome. Stevens** and **Wilson** while working with the milkweed bug, *Lygaeus turicicus* found same number of chromosome in both the sexes, (*i.e.*, 14). In female all chromosomes were paired and the homologues were equal in size. In the male, all the chromosomes were paired, but the chromosome identified as the homologue to the X chromosome was distinctly smaller and was called the **Y chromosome.** In dioecious organisms thus two types of chromosomes were recognised which are as follows :

1. **Autosomes**—The chromosomes which have no relation with the sex and contain the genes which determine the somatic characters of the individuals are known as **autosomes** (A).

2. **Sex chromosomes**—The chromosomes which are responsible for the determination of sex are known as **sex chromosomes**, *e.g.*, X and Y chromosomes.

Structure of sex chromosomes—The X and Y sex chromosomes exhibit structural differences. The cytological studies have shown that the X chromosomes of most organisms are straight, rod-like and comparatively larger than Y chromosomes The Y chromosome is smaller in size with one end slightly curved or bent to one side in *Drosophila ;* in man and *Melandrium* no such curvature of Y chromosome occurs. The X chromosomes have large amount of euchromatin

and small amount of heterochromatin. The euchromatin has large amount of DNA material, hence, much genetic informations. The Y

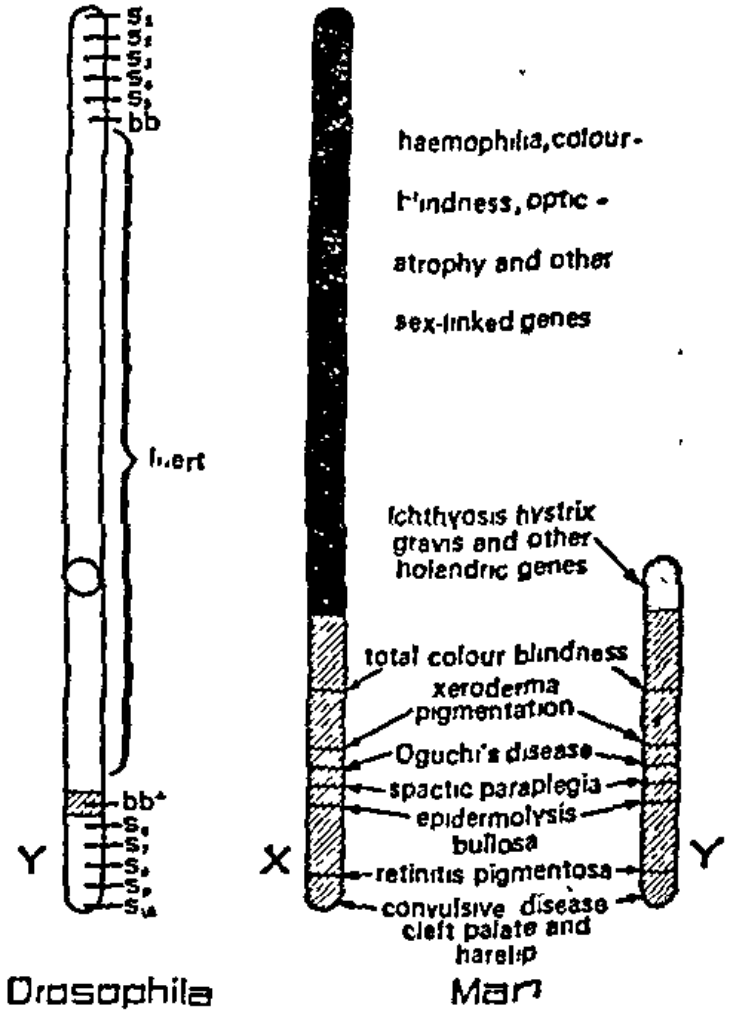


Fig 10.1 Sex chromosomes of *Drosophila* and man.

chromosome contains small amount of euchromatin and large amount of heterochromatin. The Y chromosome has little genetic information, therefore, sometimes it is referred as genetically inert or inactive.

TYPES OF SEX CHROMOSOMAL MECHANISM OF SEX DETERMINATION

In dioecious diploidic organisms following two systems of sex chromosomal determination of sex have been recognized :

A. Heterogametic males.

B. Heterogametic females.

A. HETEROGAMETIC MALES

In this type of sex chromosomal determination of sex, the female sex has two X chromosomes, while the male sex has only one X chromosome. Because, male lacks a X chromosome, therefore, during gametogenesis produces two types of gametes, 50% gametes carry the X choromosomes, while the rest 50% gametes lack in X chromosomes. Such a sex which produces two different types of

gametes in terms of sex chromosomes, is called **heterogametic sex.** The female sex, because, produces similar type of gametes, is called, **homogametic sex.** The heterogametic males may be of following two types :

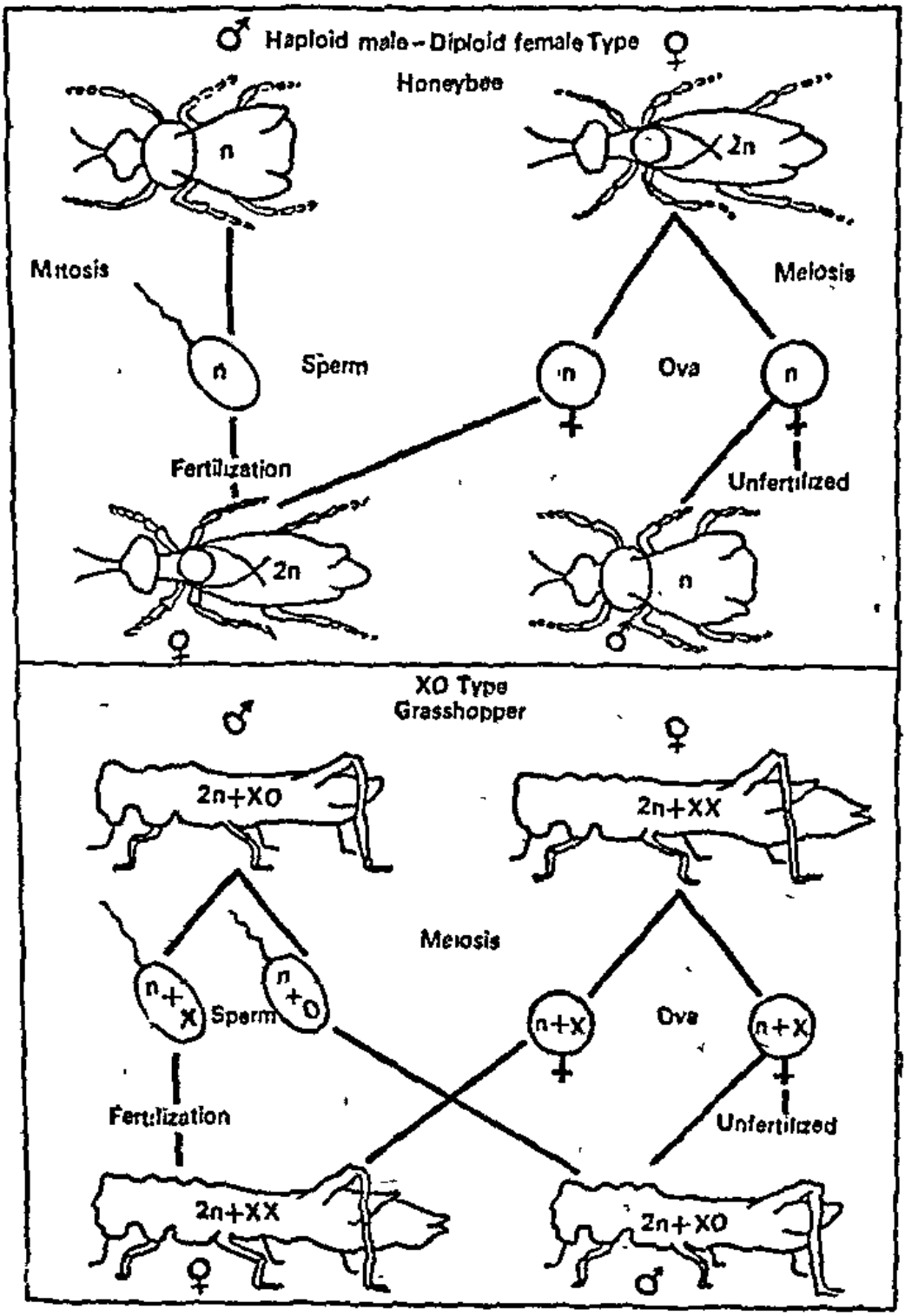


Fig. 10·2. Haploid male, diploid female and XX-XO type sex determination mechanism of insects.

(i) **XX-XO type**—In certain insects specially those of the orders Hemiptera (true bugs) and Orthoptera (grasshoppers and roaches), the female having two X chromosomes (hence referred as XX) and are thus homogametic, while the male having only one X chromosome (hence referred as XO). The presence of an unpaired X chromosome determines the masculine sex. The male lacking in one X chromosome produces two types of sperms : half with X chromosome and half without X chromosome. The sex of the offspring depends upon the sperm that fertilizes the egg (each of which carries a single X chromosome) as shown in fig. 10·2.

(ii) **XX-XY type**—In man, other mammals certain insects including *Drosophila* and *Lygaeus turicicus* and in certain angiospermic plants such as *Melandrium*, the female possesses two homomorphic X chromosomes in their body cells (hence referred as XX) and they being homogametic, produce one kind of eggs, each with one X chromosome. The males of these organisms possess one X chromosome and one Y chromosome (hence referred as XY). The males having two heteromorphic sex chromosomes produce two kinds of sperm : half with X chromosome and half with Y chromosome. The sex of embryo depends on the kind of sperm. An egg fertilized by a X-bear-

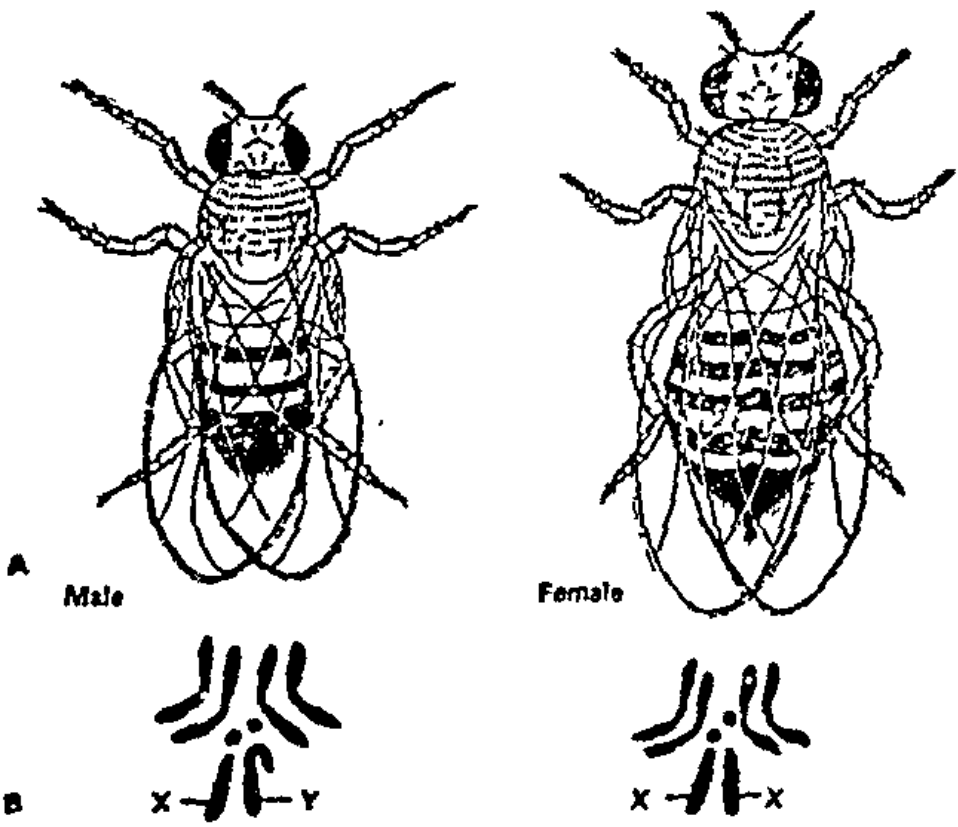


Fig. 10·3. Male and female *Drosophila melanogaster* and their chromosomes.

ing sperm, produces a female, but, if fertilized by a Y-bearing sperm, a male is produced. (See fig. 10·4).

HETEROGAMETIC FEMALES

In this type of sex chromosomal determination of sex, the male sex possesses two homomorphic X chromosomes, therefore,

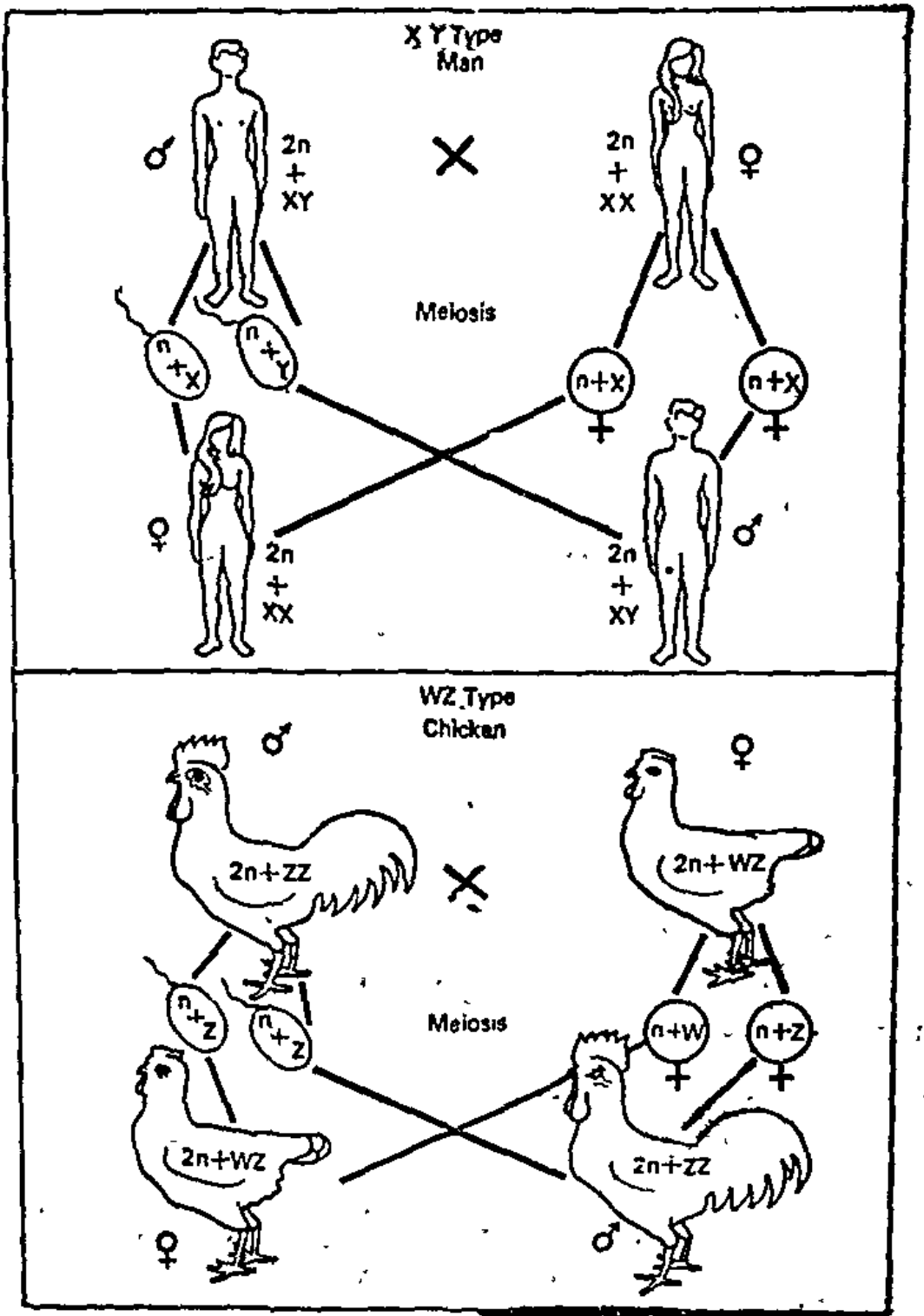
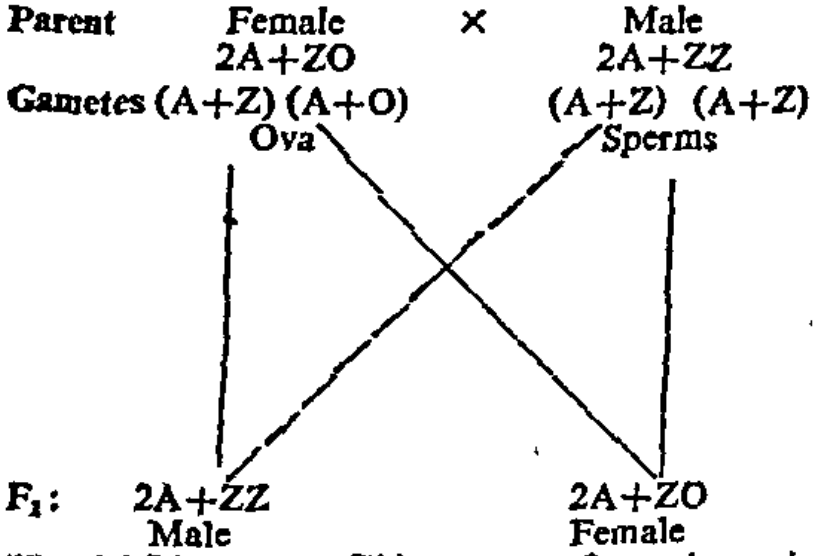


Fig. 10·4. The XX-XY and ZW-ZZ (WZ-ZZ) type determination of sex in man and chicken.

is homogametic and produces single type of gametes, each carries a single X chromosome The female sex either consist of single X chromosome or one X chromosome and one Y chromosome. The *female sex is, thus, heterogametic and produces two types of eggs,* half with a X chromosome and half without a X chromosome (with or without a Y chromosome). To avoid confusion with that of XX-XO and XX-XY types of sex determining mechanisms, instead of the X and Y alphabets, Z and W alphabets are generally used respectively.

The heterogametic females may be of following two types :

(i) ZO-ZZ system—This system of sex determination is found in certain moths, butterflies and domestic-chickens. In this case, the female possesses single Z chromosome in its body cells (hence, is referred as ZO) and is heterogametic, producing two kinds of eggs, half with a Z chromosome and half without any Z chromosome. The male possesses two Z chromosomes (hence referred as ZZ) and is homogametic, producing single type of sperms, each of which carries a *single Z chromosome. The sex of the offspring* depends on the kind of egg as shown below :



(ii) ZW-ZZ system—*This system of sex determination* occurs in certain insects and vertebrates such as fishes, reptiles and birds. Here the female sex has one Z chromosome and one W chromosome. It is heterogametic and produces two types of ova, 50% ova carry the Z chromosomes, while rest 50% ova carry W chromosomes. The *male sex has two homomorphic Z chromosomes and is homogametic* producing single type of sperms, each carries a Z chromosome. The sex of the offspring depends on the kind of egg, the Z bearing eggs produces males but the W bearing eggs produce females as shown in Fig. 10·4.

2. GENIC BALANCE MECHANISM

By studying sex chromosomal mechanism of sex determination, it may appear at first glance that some genes carried by the sex chromosomes (X and Y) were entirely responsible for sex. But this is not the case. Extensive experimentation of different workers (**Wilson, 1909 ; Bridges, 1921** and **Goldschmidt, 1934**) on different organisms have revealed the fact that most organism generally have inherent potentialities for both sexes and each individual is found to be more or less intermediate between male and female sexes (Hence may be referred as **intersex**). There seems to exist a delicate **balance** of masculine and feminine tendencies in the hereditary compliment of an individual and mechanisms like the XY, ordinarily serve to trip the balance in one direction or another. Such **genic balance** mechanism of determination of sex was first of all studied in *Drosophila* by **C. B. Bridges** in 1921.

Sex determination in Drosophila—In *Drosophila*, the presence of Y chromosome has been found essential for the fertility of male sex but that has nothing to do with the determination of male sex. In this fly, the sex is determined polygenically. The sex determining genes were so distributed that the net effect results in the autosomes determining maleness and the X chromosomes femaleness. The sex of an individual then depends upon the ratio of X chromosomes to autosomes. If each haploid set of autosomes carries factors with a male-determining value equal to one (1), then each X chromosome carries factors with a female determing value of one and half ($1\frac{1}{2}$). Let A represent a haploid set of autosomes. In a normal male (AAXY) the male and female determinants are in the ratio of 2 : $1\frac{1}{2}$ and, therefore, the genic balance is in the favour of maleness. A normal female (AAXX) has a male and female ratio of 2 : 3 and, therefore, the balance is in the favour of femaleness, (See Fig. 10·5).

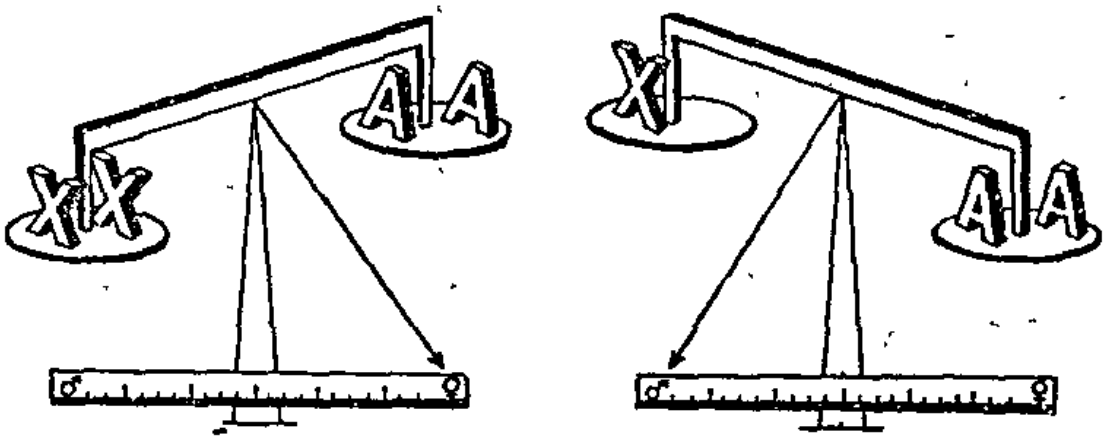


Fig. 10 5. A diagrammatic representation of genic balance theory in *Drosophila*.

SEX DETERMINATION IN MAN

In man like *Drosophila* XX-XY type sex determining mechanism occurs but here the Y chromosome contains potent male-determining genes which can almost completely overcome the feminizing action of the rest of the genotype. The evidences that Y chromosome is a determiner of fertility and sex of male individual comes from following sources :

1. Barr body—**Barr** and **Bertram** (1949) reported a deeply stained chromatin body (*i.e.*, a chromocentre) in the nerve cells of female cat which was absent in the male. This chromatin body is called **sex chromatin** or **Barr body** after the name of its discoverer.

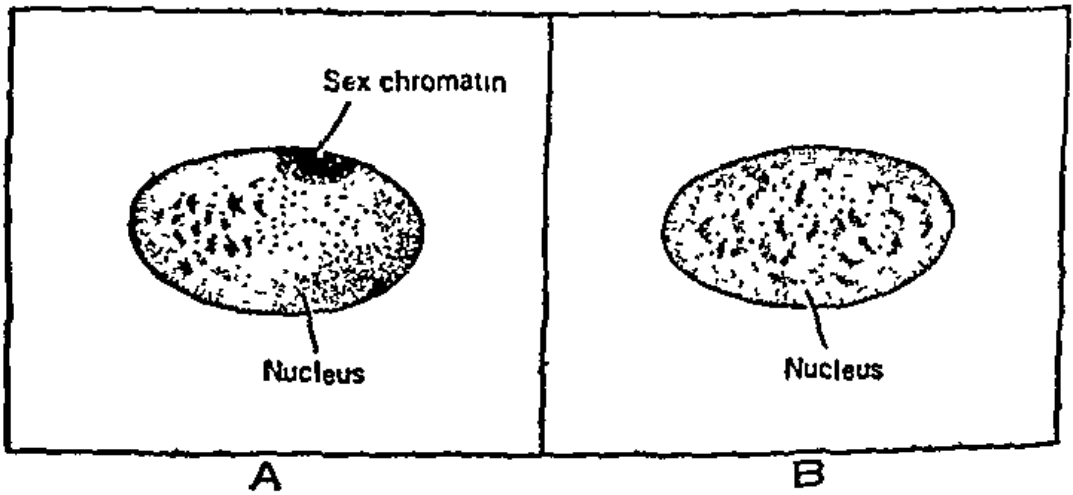


Fig. 10-6. Sex chromatin in the nucleus of female cat (A). Male cat (B) has no sex chromatin in its nucleus.

Such Barr body has also been observed in most of the body cells (*e.g.*, skin, oral epithelium and blood cells) of man and other mammals. Human females have the Barr body in the nuclei of their body cells in higher proportion than males and are therefore, referred as

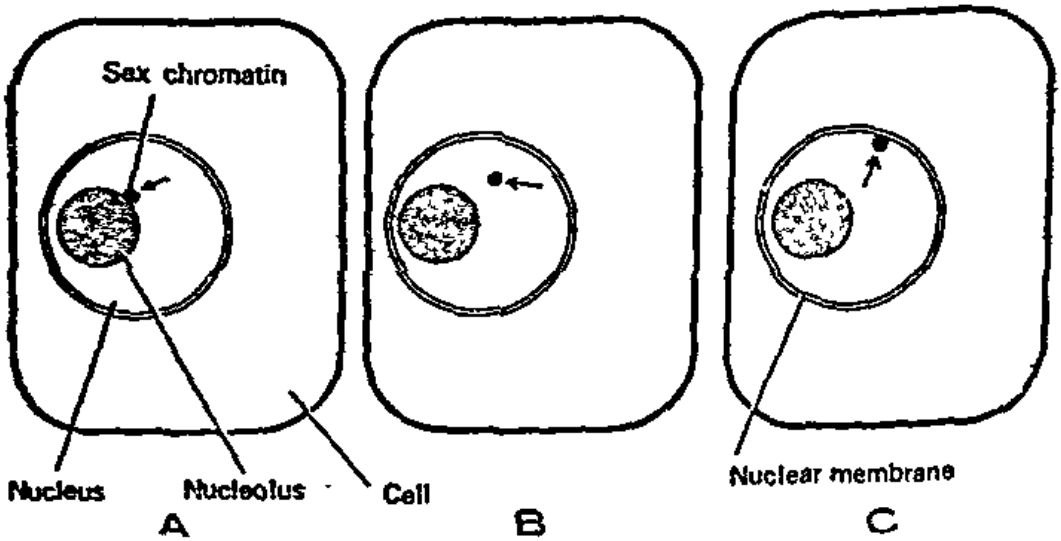


Fig. 10.7. A—Position of sex chromatin near nucleus, B—in

sex chromatin positive. The human males are called sex chromatin negative.

The sex chromatin appears in the interphase nucleus as a small chromocentre, heavily stained with basic dyes. It gives a positive Feulgen reaction and has a relatively constant position in each tissue and species. It can be found in four positions : (i) attached to the nucleus as in nerve cells of certain species ; (ii) attached to the nuclear membrane as in cells of epidermis or of the oral mucosa; (iii) free in the nucleoplasm as in neurons after electric stimulation, and (iv) as a nuclear expansion. The best known example of nuclear expansion is that of the neutrophil leukocyte of female in which the sex chromatin (Barr body) appears as a small rod called the drum stick.

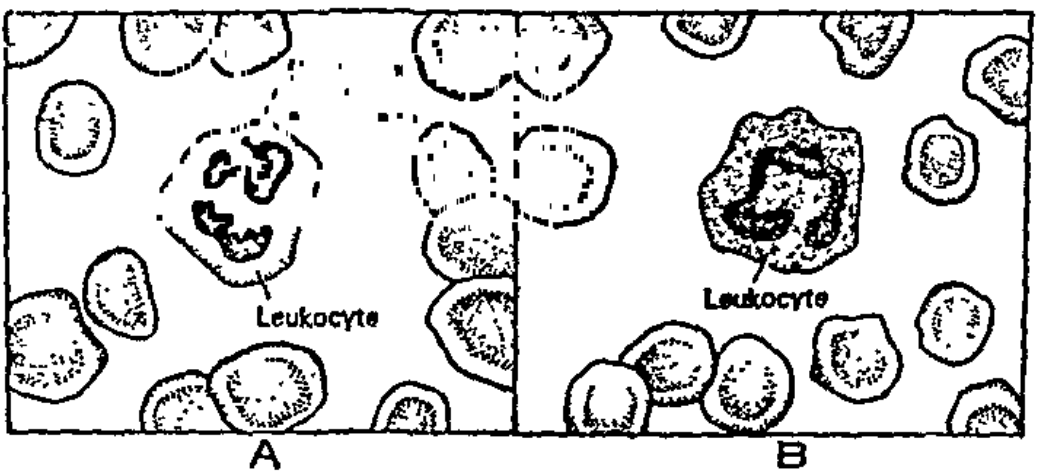


Fig. 10·8. A—Leukocyte of human female with drumstick-like sex chromatin. B—Leukocyte of human male without any drumstick.

The relationship between sex chromatin and sex chromosomes has recently been elucidated (**Ohno, Kaplan** and **Kinosita** 1959). It has been demonstrated that the sex chromatin is derived only from one of the two X-chromosomes. The other X chromosome behaves like a autosome and is not heteropycnotic at interphase. The number of corpuscles of sex chromatin at interphase is equal to nx–1. It means that there is one Barr body less than the number of X-chromosomes. Besides its genetical significance, Barr body has a wide field of medical application and offers the possibility of relating the origin of certain congenital diseases to chromosome anomalies.

Role of X and Y chromosomes in determination of sex in man— In the foregoing paragraphs we have observed that Barr body seems to have some relation with female sex. Besides the Barr body, the sex chromosomal aberrations have provided strong evidences that the Y chromosome in man have some definite role in the determination

and fertility of male sex. For instance, the individuals having at least one Y chromosome are males at least as to external genitalia though they may be sterile. Contrawise, persons with one or more

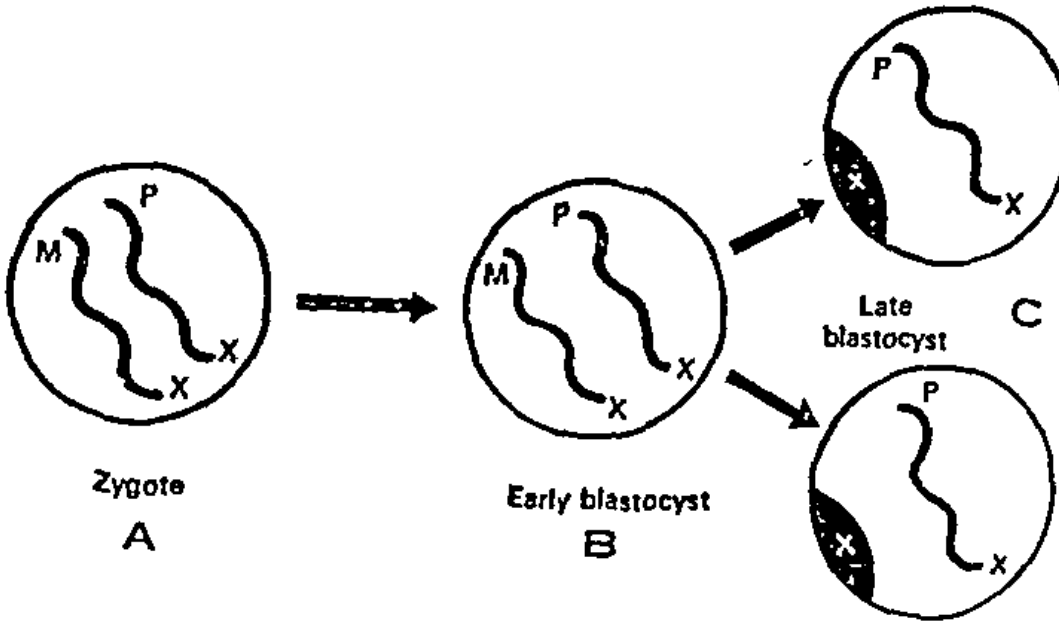


Fig 10 9 Diagram of the evolution of XX chromosome, A—In the zygote both paternal (P) and maternal (M) X-chromosome are euchromatic. B—In the early blastocyst the same is true as in A. C—In late blastocyst 50% of the cells have a *maternal heterochromatic* X chromosome and the other 50% have the paternal heterochromatic X chromosome. In diagram C formation of sex chromatin by one of the X-chromosome has been shown

X chromosomes are phenotypically female as long as no Y chromosome is present, though, again sometimes infertile. Therefore, it is concluded that in man genes for maleness reside in Y chromosome and those for femalenss on the X chromosome. The autosomes have no sex determining role in man unlike *Drosophila* and the Y chromosome determines maleness, even a single one outweighing the number of X chromosomes. The X chromosome determines thefemalesex only in the absence of Y chromosome.

3. MALE HAPLODY OR HAPLOIDIPLOIDY MECHANISM

Male haploidy or haplodiploidy or arrhenotokous parthenogenesis is particularly common in the hymenopterous insects such as ants, bees, sawflies and wasps, (*e.g., Bracon hebetor*) In these insects, since, fertilized eggs develop into diploid females and unfertilized ones into haploid males; so arrhenotoky is both a form of reproduction and a means of sex determination. Meiosis is normal in females, but crossing over and reduction in chromosome number fail to occur during spermatogenesis in males due to their haploidy. (See fig. 10·10).

Thus, arrhenotokous parthenogenesis determines the sex in hymenopterans and sex chromosomes have no identity here like the

Drosophila. During experiments, it has been found that the quantity and quality of food available to the diploid larva determines whether that female will become a sterile worker or a fertile queen.

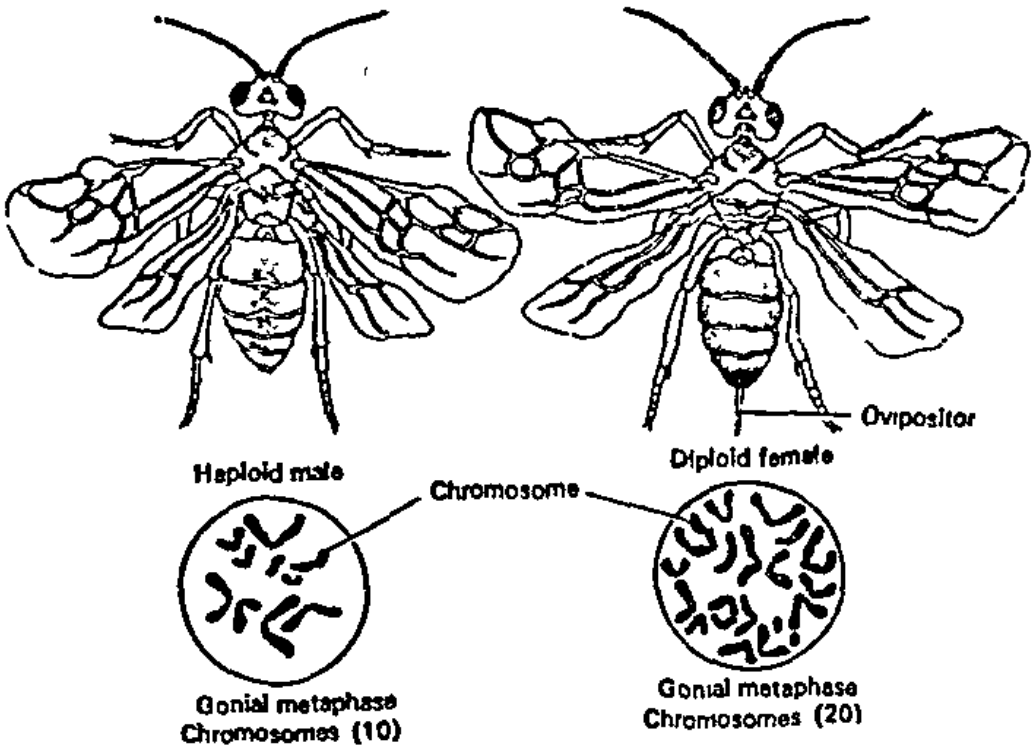


Fig. 10 10. Male and female *Bracon hebetor* and their gonial metaphase chromosomes.

Thus, environment here determines sterility or fertility but does not alter the genetically determined sex. The sex ratio of the offspring is under the control of the queen. Most of the eggs laid in the hive will be fertilized and developed into worker females. Those eggs which the queen chooses not to fertilize (from her store of sperm in the seminal receptacle) will develop into fertile haploid males. Queen bees usually mate only once during their life time.

4. SINGLE GENE CONTROL OF SEX

In certain organisms, for example *Chlamydomonas, Neurospora*, yeast, *Asparagus*, maize, *Drosophila* etc., individual single genes are found to be responsible for the determination or expression of sex, following cases examplified the single gene control of sex :

(a) Sex-determination in Asparagus—*Asparagus* is a dioecious plant, however, sometimes the female flowers bear rudimentary anthers and the male flowers bear rudimentary pistils. Thus, sometime it may happen that a rare male flower with poorly developed pistil may set seeds. In one of the experiment when the seeds of such a rare male flower were raised into plants, then, the male and female plants were found to be present in 3 : 1 ratioWhen the male plants raised

thus were used to pollinate the female flowers on female plants, only two third of them showed segregation indicating that the sex is controlled by a single gene.

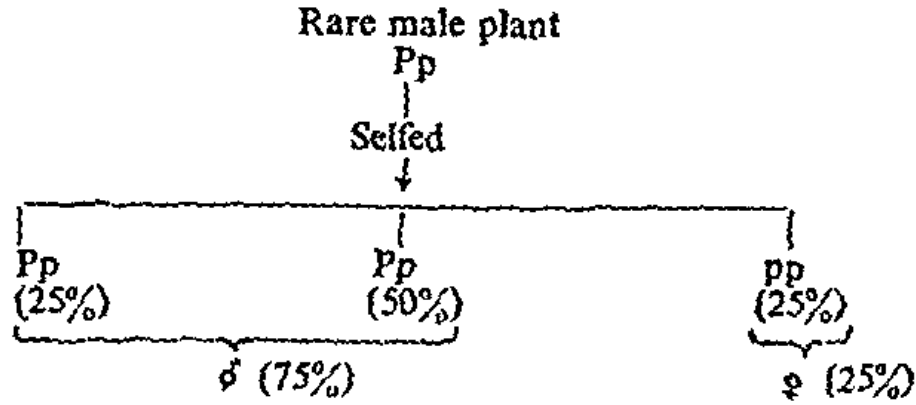


Fig. 10 11. Segregation for sex in seed obtained from a rare bisexual flower in Asparagus showing monogenic control.

(b) Sex-determination in Neurospora—*Neurospora* has two sexes exactly equivalent and designated A and a. Mating occurs only between gametes of unit sex, (*e.g.*, A×a). The mating type, A or a is determined by a pair of autosomal alleles and follows simple Mendelian inheritance.

(c) Monogenic sex determination in maize—Maize is a monoecious plant with male inflorescence (tassel) and the female inflorescence (silk) located on the same plant. A gene called tassel seed its, converts the tassel into seed bearing inflorescence, while, another gene called silkness, sk, is responsible for the absence of silks. Therefore, a plant with genotype sk/sk will be effectively a male plant and a plant with genotype ts/ts will be effectively a female plant. Thus, individual single genes (*viz.*, sk or ts) can impose bisexuality in maize.

(d) *Monogenic sex determination in Drosophila*—In *Drosophila*, a transformer gene (tra) has been recognized which when present in homozygous condition (tra/tra) transforms a female fly into a sterile male, but, it does not act upon normal male individuals. Thus, a XX female with tra/tra genotype will be a sterile male, but, a XY male with tra/tra genotype will still be a normal male fly.

B. METABOLICALLY CONTROLLED SEX DETERMINING MECHANISM

Certain workers have seen the possibility of sex determination in the phenomenon of metabolism. Crew suggested that sex is a physiological equitable division between anabolic and catabolic individuals. A. F. Shull and D. D. Whitney have shown that by increasing metabolic rate in rotifers the occurrence of male individuals increases than females. Likewise, Riddle found that metabolism had some definite

role in the determination of sex in pigeons and doves, because, increased rate of metabolism developed the male potency, while decreased rate of metabolism caused femaleness.

C. HORMONALLY CONTROLLED SEX DETERMINING MECHANISM

In many cases it has been observed that sexual differentiation is controlled by hormones. Following classical examples would make clear that how hormones control the sex of an individual :

(i) **Sex reversal in hen :** In birds only one gonad of a normal female develops into a functional ovary. The other gonad remains rudimentary If the functional ovary of a hen is destroyed, the rudimentary gonad develops into a testis (Crew, 1923). Thus, the female sex is reversed into male sex due to the phenomenon called **sex reversal.** Such a sex reversed hen can even be a father chick which will be expected to show a sex ratio of two females to one male, since in birds the female is the heterogametic sex. The case of sex reversal of hen can be interpreted as follows :

During embryonic development, the XY (ZW) genotype stimulates the pituitary gland to produce female hormones that cause thegonad of the hen to develop into an ovary. After the development of ovary, the pituitary ceases to produce female hormones, due to inhibition of the pituitary by hormones produced by the ovary, thus acting as **developmental feed-back system.** The high level of female hormones secreted sequentially by the pituitary and the ovary is sufficient to suppress the action of male hormone producing cells of the body such as the steroid producing cells of the adrenal glands. When the ovary of a hen is removed, the steroid cells of adrenal become active and provoked the development of rudimentary gonad into testis which itself is an endocrine gland. Both endocrine glands (*e.g*, adrenal and testis) produce large amount of male hormones which sufficiently suppress the action of the female hormone cells of pituitary. Such cases of sex reversals have also been observed in amphibians (*e.g.*, in salamander by E. Witschi).

(ii) **Hormones and free martin :** Another classical example of hormonal control of sex determination has been found in cattles. In cattle, when twin calves of different sexes occur, the female member is usually a sterile intersex called a **freemartin** The freemartin has external female genitalia but internal sex organs are more or less like those of male. The male twin is usually normal. **F. R. Lillie** (1917) has suggested that the formation of a freemartin was due to a fusion of the foetal membranes of the twin calves, while they were in uterus of the mother. The fusion of the foetal membranes permitted the

blood of each twin to circulate in the blood vessel of the other. The male hormones produced by the male twin are presumed to suppress the differentiation of the female internal sex organs of the co-twin.

D. ENVIRONMENTALLY CONTROLLED SEX DETERMINING MECHANISM

In some organisms, the environment determines the sexual phenotype of the individuals. Such environmentally controlled sex determining mechanism has been reported in the variety of animal species which belong to different groups such as Rotifera, Annelida, Arthropoda, Echiuroidea, Mollusca, etc. The environmental control of sex can be studied in following animals.

(a) Sex in Bonellia—An excellent example of environmental *determination of sexual phenotype is afforded by Bonellia viridis,* a marine echiuroid worm studied extensively by **F. Baltzer, (1935).** The adult female is about an inch long and has fairly complex anatomical organization. Male is of the size of large Protozoa and has rudimentary organs. The males live as parasites in the uterus of the females. All larvae of *Bonellia* are genetically and cytologically similar. If a particular larva settles on the proboscis of an adult female, it becomes a male individual. On the other hand, if a larva develops in isolation (*i.e.*, in water) it develops into a female. Further, if a incompletely developed male is detached from the proboscis

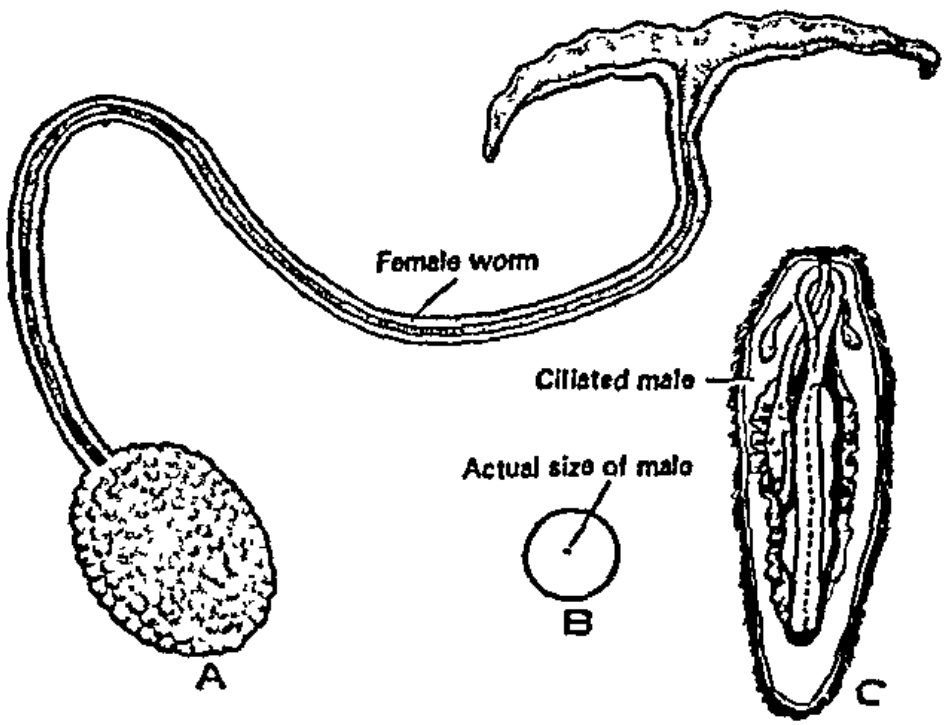


Fig. 10·12. The female and male of the echiuroid *Bonellia viridis*

of female, it becomes an intersex. Thus, it becomes evident that proboscis of adult female secretes some hormone-like substance which suppresses femaleness and initiating maleness in the larvae which are attached with it.

(b) Sex in Crepidula—Coe (1943) demonstrated in mollusc genus *Crepidula* that its every zygote contains all the genes which are necessary for the development of male and female reproductive systems. But, in this species it has been found that young *Crepidula* remains functional male and produce only sperms but no ov... As it matures the male phase is changed by transitional phase to a functional femele phase. The female phase continues for the remaineder of the animal's life. It is suggested that environment somehow regulates the activity of certain genes to produce only male phenotype during young stages, while inhibits them during adult stages and simultaneously initiating other genes to produce female phenotype.

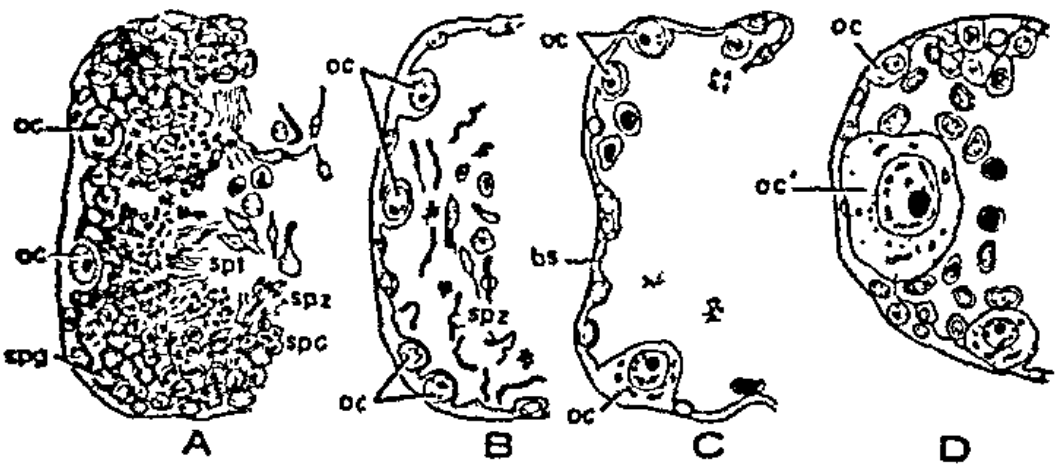


Fig. 10·13. Changes in the gonad of the mollusc *Crepidula* experiencing consecutive sexuality. A—Male phase, B, C—Transitional phases ; D—Female phase. OC—Immature oocyte, OC'—Mature oocyte : Spg—Spermatogonia, Spt—Spermatids, Spz—Spermatozoa, Spc—Spermntocytes.

SEX DETERMINATION IN PLANTS

Most flowering plants are monoecious and, therefore, have no sex chromosomes. The plant cells possess bipotentiality and develop both types of reproductive organs without fail. Well known examples of dioecious plants are usually under the genetic control of a single gene locus. However, chromosomal sexuality has been reported in two plant species namely *Melandrium* and *Spaerocarpos*. The *Melandrium* of pink family (Caryophyllaceae) has XX-XY chromosomal mechanism of sex determination like the man. Here, the pistilate plants are XX and staminate plants are XY.

The sex chromosomal mechanism of sex determination has also been observed in certain monoploid bryophytes (*i.e.*, *Spaerocarpos*). For example, Allen (1919) found that the sporophyte of *Spaerocrapos* contains two sex chromosomes (XY) and it produces two kinds of meiospores—some with X and other with Y chromosomes. The meiospores with X chromosome germinate into sexually reproducing female gametophyte and the meiospores with Y chromosomes germinate into sexually reproducing male gametophyte.

CHAPTER 11

SEX-LINKED INHERITANCE

In Mendel's crosses we have already observed that the progeny of a cross between two individuals of pure lines is the same regardless of which individual is female or which individual is male or we can say that sex makes no difference in Mendel's crosses. But the Mendel's laws are not applicable on those genes which are located in X and Y chromosomes. It has been observed that the genes occurring only in the X chromosomes are represented twice in female (because female contains 2X chromosomes) and once in male (because male has only one X chromosome). Moreover, if the recessive type of genes occur in X chromosomes of males, they express themselves phenotypically. Because in such case Y chromosome contains no dominant allelomorph or gene to overcome the recessive gene of X chromosome. The genes which occur exclusively on the X chromosome, (mammals, *Drosophila*, *Melandrium*, etc.) or on the analogous Z chromosome (in birds and other species with ZO or ZW mechanism

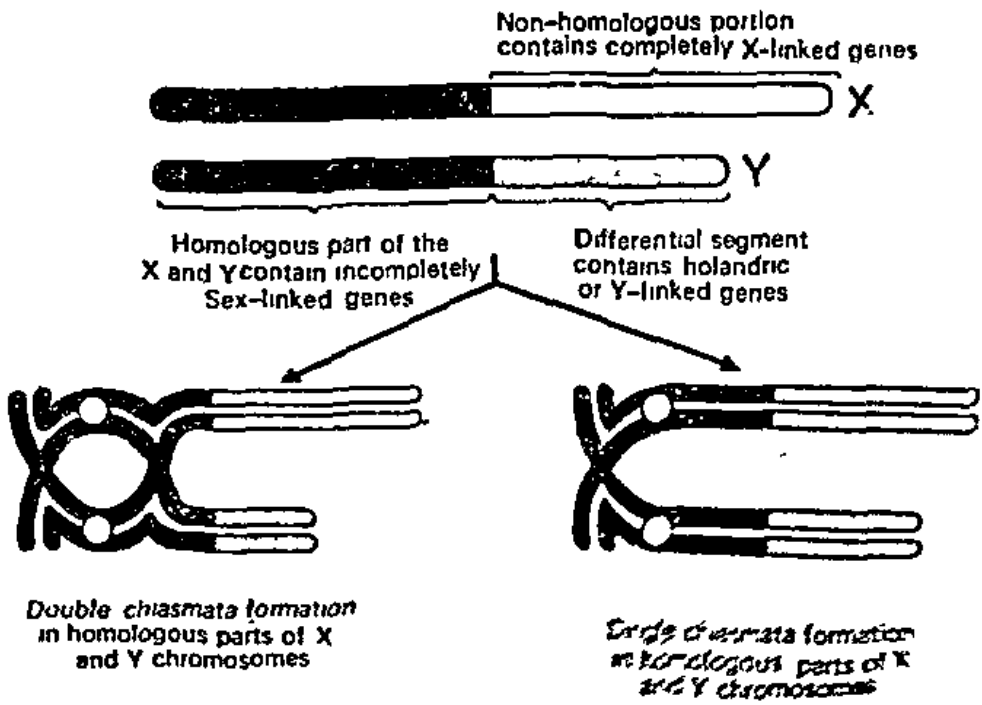


Fig. 11·1. X and Y chromosomes of mammals showing their differential segments and behaviours of their homologous parts in crossing over.

of sex determination) are called X- or **Z-linked genes.** The genes which exclusively occur in Y chromosomes are called **holandric genes.** The inheritance of X- or Z-linked and holandric genes is called **sex-linked inheritance.** In XX—XY type organisms, sex-linked genes (see fig. 11·1) can be classified into following three types :

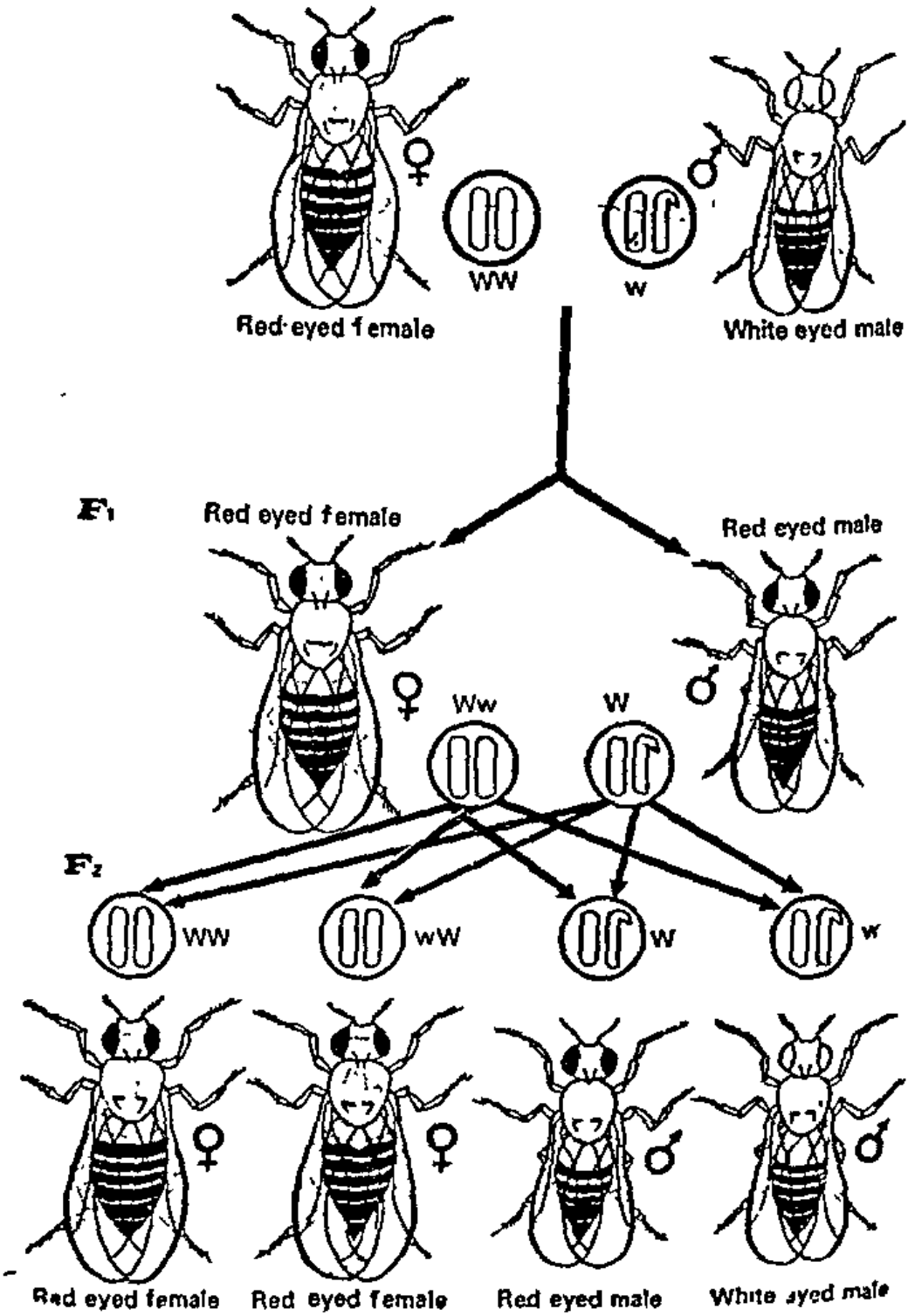


Fig. 11·2. A cross between red-eyed female and white-eyed male showing sex-linked inheritance in *Drosophila.*

A. X-linked—The X-linked type sex-linked inheritance is performed by those genes which are localized in the non-homologous sections of X-chromosomes and that have no corresponding allele in Y chromosome. The X-linked genes are commonly known as **sex-linked genes.**

B. Y-linked—The Y-linked type sex-linked inheritance is performed by those genes which are localized in the non-homologous section of Y chromosome, and that have no alleles in X chromosome. The Y-linked genes are commonly known as **holandric genes** (Greek, *holos*, whole, and *andros*, man).

C. XY-linked—The XY-linked type sex-linked inheritance is performed by those genes which are localized in homologous sections of X and Y chromosomes.

A. INHERITANCE OF X-LINKED (SEX-LINKED) GENES

A recessive X-linked or sex-linked gene typically has a characteristic pattern of inheritance called **criss-cross inheritance.** In criss-cross inheritance, a X-linked recessive gene is transmitted from P_1 male parent (father) to F_2 male progeny (grandsons) through its F_1 heterozygous females (daughters), which are called **carriers.** The criss-cross inheritance of recessive X-linked genes can be well understood by following classical examples in *Drosophila*, man and chikens etc. :

1. INHERITANCE OF X-LINKED GENE FOR EYE COLOUR IN DROSOPHILA

In *Drosophila*, the gene for white eye colour is X-linked and recessive to another X-linked, dominant gene for red-eye colour. It is discovered by **Morgan** in 1910. Following crosses between white eyed and red eyed *Drosophila* will make clear the characteristic criss-cross inheritance of gene for white eyed colour in it :

(a) Red eyed ♀ × White eyed ♂—If a wild red eyed female *Drosophila* is crossed with a mutant white eyed male *Drosophila*, all the F_1 individuals irrespective of their sex have red eyes (Fig. 11·2). When the red eyed male and red eyed female individuals of F_1 are intercrossed, the F_2 progeny is found to include a exclusively red eyed female population and a male population with 50% red eyed individuals and 50% white eyed individuals. Thus, F_2 generation includes red eyed and white eyed individuals in the ratio of 3 : 1.

(b) White eyed ♀ × Red eyed ♂—When a white eyed female *Drosophila* is crossed with a red eyed male *Drosophila*, all the female individuals in the F_1 generation are red eyed and all the male individuals are white eyed (Fig. 11·3). When these red eyed female individuals and white eyed male individuals of F_1 are intercrossed, the

female population of F_2 generation is found to include 50% red-eyed and 50% white eyed flies. Similarly, the male population of F_2 includes 50%, red eyed and 50% white eyed flies.

The inheritance of X-linked recessive gene for white eyes can be understood more properly by considering the behaviour of X and Y chromosome, such as follows :

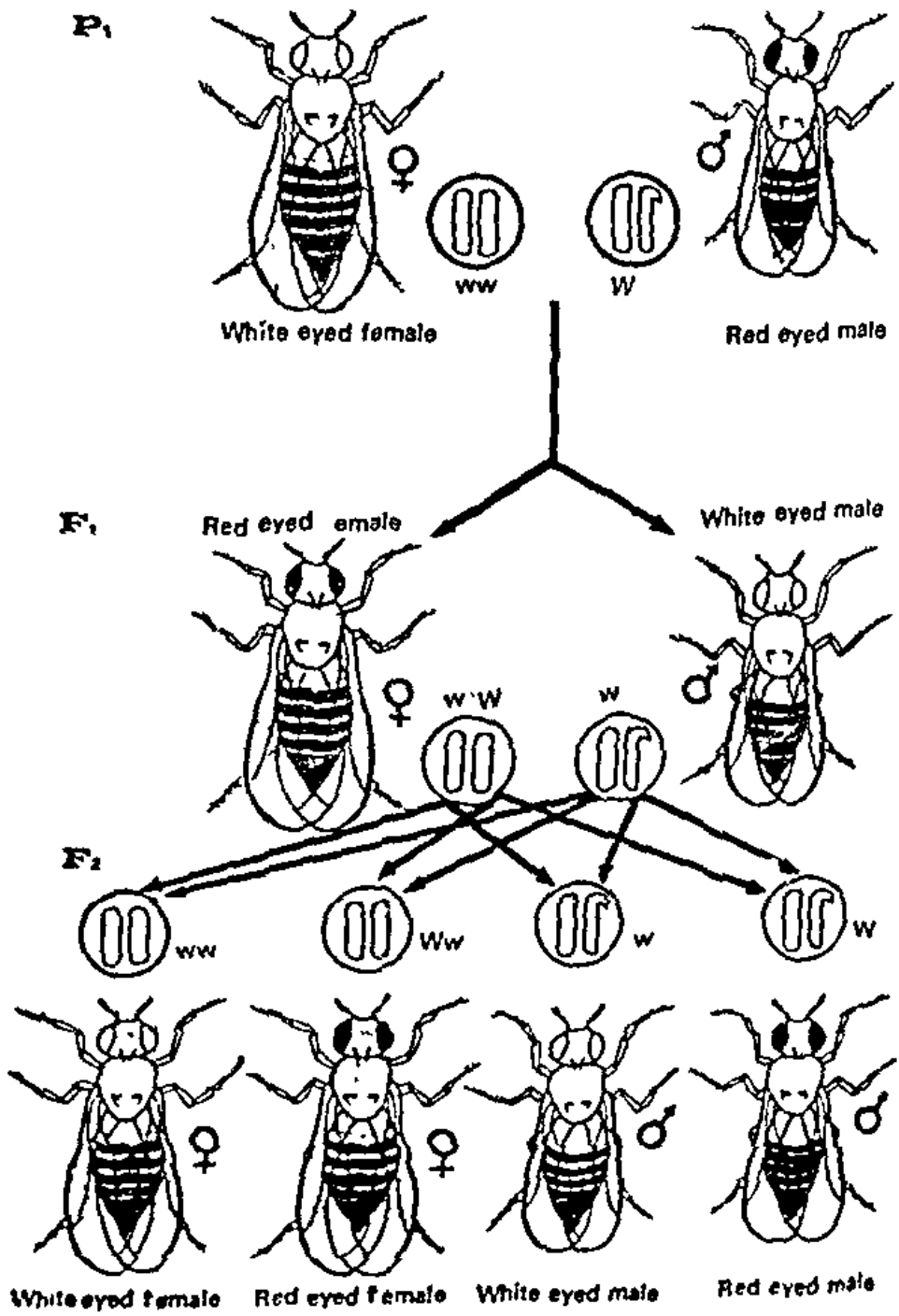


Fig. 11·3. A cross between white-eyed female and red-eyed male showing X-linked inheritance in *Drosophila*.

In the cross between red-eyed female and white-eyed male *Drosophila*, the red-eyed female contains the gene 'WW' for red colour of eye. The white-eyed male contains a single gene 'w' for white colour of eye which remains located in X chromosome. This one allelic condition of male is termed hemizygous condition in contrast to the homozygous or heterozygous possibilities in the female. The female being homogametic produces only one type of gametes or eggs each with the gene 'W' for red-coloured eyes. The male being heterogametic produces two types of gametes, 50% sperms with X chromosome containing 'w' gene, 50% sperms with 'Y' chromosome without w or W gene. The gametes of both parents unite in fertilization to produce F_1 progeny. The F_1 hybrids which receive a X chromosome with 'W' gene from the female and a X chromosome with 'w' gene from the male becomes red-eyed female because gene 'W' is dominant over gene 'w'. Further the hybrids which happens to receive a gene 'W' from mother and Y chromosome from father produce red-eyed males.

The F_1 red-eyed female with the gene 'Ww' when crossed with F_1 red-eyed male having the gene 'W'. The female hybrids produce two types of eggs, 50% eggs carry the gene 'W' and the remaining 50% carry the gene 'w'. The males produce two types of sperms, half carry the gene 'W' and half carry no such gene on Y chromosomes. The union of sperms and ova of F_1 offsprings may produce four possible types of F_2 individuals.

1. The eggs with 'W' genes if fertilized by sperms with 'W' genes produce homogametic red-eyed females.
2. The eggs with 'W' gene if fertilized by the sperms with 'Y' chromosomes produce the red-eyed males.
3. The eggs with the gene 'w' when fertilized by the sperms having the gene 'W' produce heterogametic red-eyed females.
4. The eggs with the gene 'w' when fertilized by the sperms having the Y chromosome white-eyed males are produced.

Likewise in other experiment in which a white-eyed female is crossed with red-eyed male, similar X-linked inheritance of recessive gene for white eye colour is revealed. The white-eyed female contains the gene 'ww' located on both X chromosomes. The red-eyed male contains the gene 'W' located on single X chromosome. The female being homogametic produces single type of eggs with single 'w' gene for whiteness, while the male being heterogametic produces two types of sperms, 50% sperms carry the gene 'W' and remaining 50% sperms carry no such genes on the Y chromosomes. In the mating of both parents by the union of these eggs and sperms, two kinds of F_1 hybrids are produced. The eggs with 'w' genes by union with the sperms

having 'W' genes produce heterogametic red-eyed female and the eggs with 'w' gene by union with the sperms having Y chromosome produce white-eyed male. When the F_1 brothers and sisters with the genes 'w' and 'Ww' breed together they produce four types of individuals in F_2 such as white-eyed female (ww), red-eyed female (Ww), white-eyed male (w) and red-eyed male (W).

The results of these experiments thus, are clearly indicating that the trait located on a sex chromosome alternates the sex from one generation to the next generation, *i.e.*, the trait of white eyes transfer from P_1 father to F_1 daughter and from F_1 daughter to F_2 son.

The trait of barred eye in *Drosophila* is another sex-linked trait which is dominant over the normal eye shape but display inheritance pattern like eye-colour.

2. INHERITANCE OF X-LINKED GENES IN MAN

In man about fifty sex X-linked genes have been reported. The most important and common X-linked genes of man are (*i*) colour or red-green blindness, (ii) haemophilia, (*iii*) anhidrotic ectoderma (non-functional sweet glands), (*iv*) night blindness, (*v*) myopia (short sightedness) (vi) juvenile glaucoma (hardening of eye ball), (*vii*) white fore lock, etc. These diseases are associated with X-linked recessive genes and are most common in man. Certain X-linked diseases such as defective tooth enamel (which results in early wearing down of the teeth) however, is caused by dominant genes.

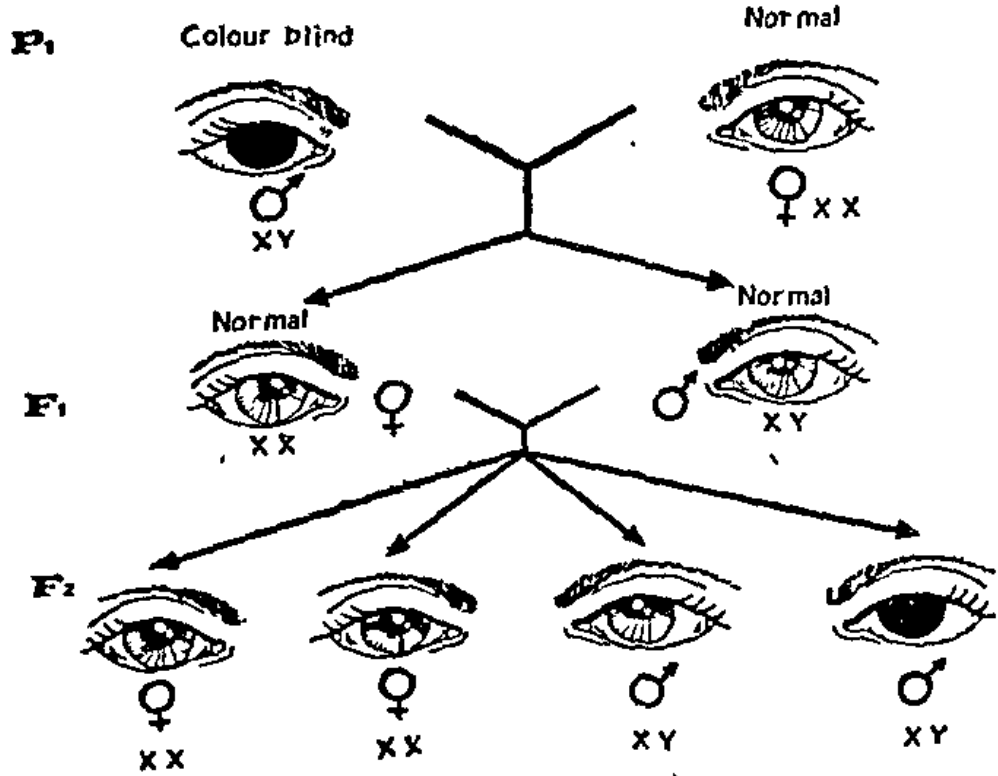


Fig. 11·4. A marriage between a red-green colour blind male and normal female showing X-linked recessive in man.

1. Inheritance of X-linked gene for colour blindness in man—The retina of eyes of man contain colour sensitive cells which are necessary for the distinction of red and green colours. These colour sensitive retinal cells are formed and controlled by certain genes which remain located in X chromosomes. The man having the recessive genes of above mentioned genes remain unable to distinct the red and green colour so are known as **colour blind or red green blind** man. This X-linked recessive gene is able to express itself in male due to absence of its any allelic gene in the Y chromosome.

The inheritance of colour blindness can be studied in the following two types of marriages :

(i) Marriage between colourblind man and normal visioned woman—When colour blind man marries with a normal visioned woman, then they will produce normal visioned male and female individuals in F_1. The marriage between a F_1 normal visioned woman and normal visioned male, will produce in F_2 two normal visioned female, one normal visioned male and one colourblind male (Fig. 11·4).

(ii) Marriage between normal visioned male and colourblind female—If a woman (XX) is colour blind and she happens to marry a normal visioned male (XY), then all F_1 sons will be colour blind

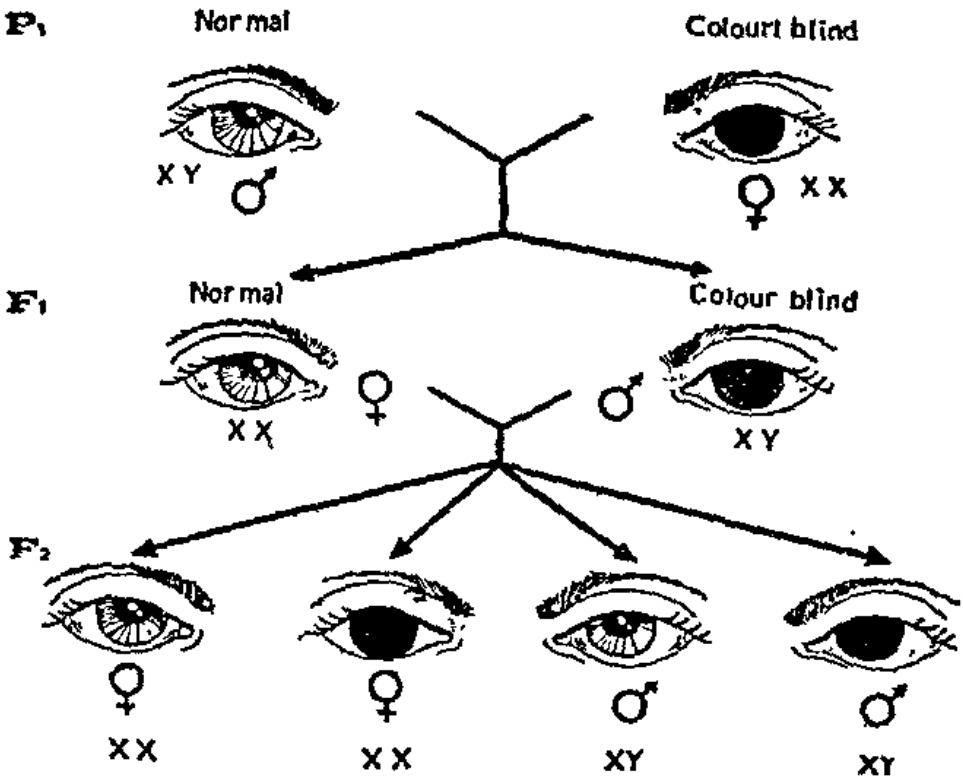


Fig. 11·5. A marriage between a red-green colour blind female (XX) and a normal male (XY) showing X-linked inheritance in man.

and daughters will be normal visioned. Because male receives one X-linked recessive gene for colour blindness from colourblind mother. The daughter receives one X-linked dominant gene for normal vision from father and one X-linked recessive gene for colour blindness from the mother. The F_1 brother and sisters, if inbred or married they will produce in F_2 a colourblind homozygous daughter, a normal visioned heterozygous daughter, a normal visioned hemizygous son and a hemizygous colourblind son (Fig. 11·5).

2. **Sex-linked inheritance of haemophilia in man**—Haemophilia is the most serious and notorious disease which is more common in men than women. This is also known as bleeder's disease. The person which contains the recessive gene for haemophilia lacks in normal clotting substance in blood so minor injuries cause continuous bleeding and ultimate death of the person due to haemorrhages. This hereditary disease was first reported by John Cotto of Philadelphia in 1803 in man. The haemophilia had been most common in royal families of Russia and England, *e.g.*, Queen Victoria and her further generations were having the haemophilic disease. The recessive X-linked gene of haemophilia show characteristic criss-cross inheritance like the gene of colour blindness.

3. INHERITANCE FOR Z-LINKED GENE FOR COLOURED (BLACK OR RED) FEATHERS IN CHICKEN

In case of birds, moths, butterflies, etc., the females are heterogametic and males are homogametic quite unlike that of *Drosophila* and man. Here also sex-linked genes follow the "crisscross" pattern but from mother through heterozygous F_1 sons to grand-daughters of F_2. The common example of ZW-ZZ sex-linkage is plymouth rock chicken.

In plymouth rock chicken the gene for barred feathers is dominant and the gene for black or red unbarred feathers is recessive Both the genes are Z-linked. A barred male chicken contains two genes for barring because it has two sex chromosomes (ZZ) When this barred male with the gene BB is crossed with unbarred female containing single recessive gene 'b' in its Z chromosome (W chromosome contains no genes) produce in F_1 only barred males and females (Fig. 11·6). These F_1 barred males and females when inbred produce in F_2, a hemizygous non-barred female, a hemizygous barred female a heterozygous barred male and a homozygous barred male.

In another cross when barred hens and non-barred cocks are crossed, the F_1 have half barred male and half non-barred females. (Fig. 11·7). These by inbreeding produce in F_2 half barred males and females and half non-barred males and females.

Besides fowl, many birds such as pigeon, duck, canary; insects such as moths, butteflies; all reptiles; some fishes and amphibians which have ZZ male and ZW or ZO female type of sexes showing sex-linked inheritance of (ZW-ZZ) type.

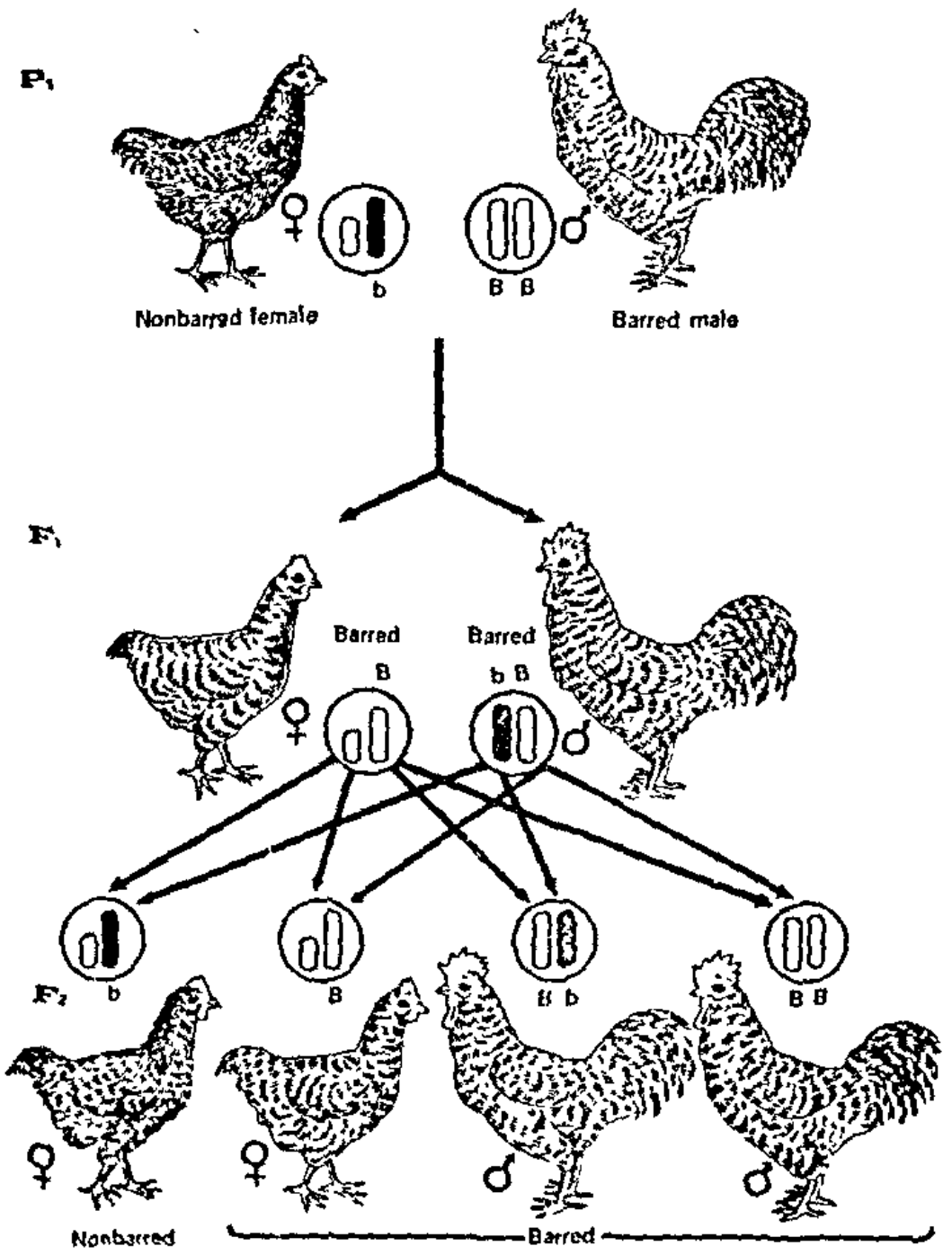


Fig 11·6. A cross between a barred and a non barred cock showing sex-linked (Z-linked) inheritance.

B. INHERITANCE OF Y LINKED GENES

Genes in the non-homologous region of the Y chromosome pass directly from male to male. In man, the Y-linked or holandric genes

such as ichthyosis hystrix gravis hypertrichosis (excessive development of hairs on pinna of ear) are transmitted directly from father to son.

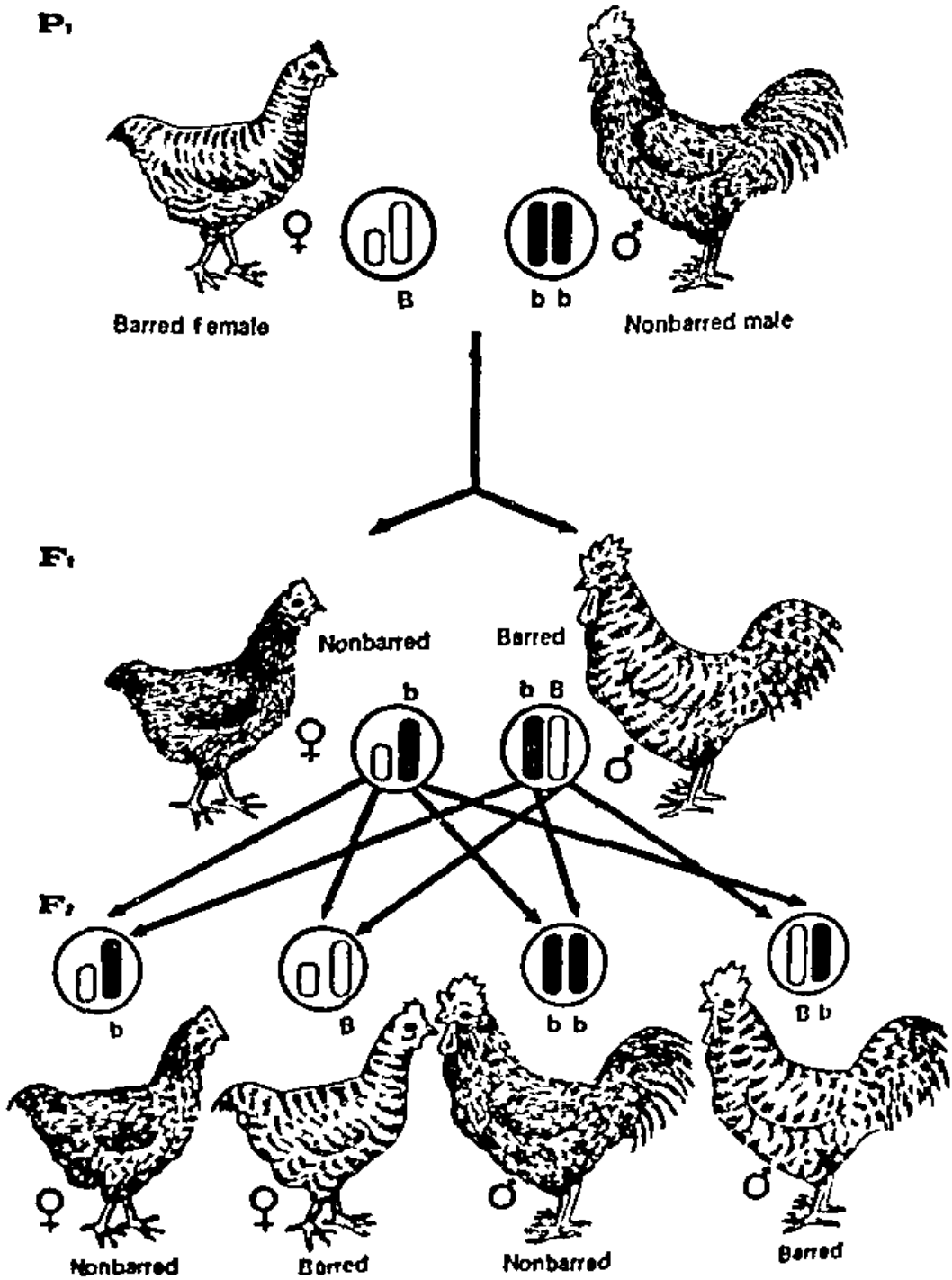


Fig. 11·7 A cross between a barred cock and a non-barred hen showing sex linked (Z-linked) inheritance.

C. INHERITANCE OF X-Y-LINKED GENES

The genes which occur in homologous section of X and Y chromosome have inheritance like the autosomal genes. The X-Y linked genes are partially or incompletely sex-linked, because, sometime, the crossing over may occur in the homologous sections of X and Y

chromosomes, (see fig. 11·1). In human several diseases are XY-linked. Certain XY-linked genes of man are total colour blindness, two skin diseases (*Xeroderma pigmentosum* and *Epipermolysis bullosa*), *Retinitis pigmentosa*, *Spastic paraplegia*, etc.

SEX-INFLUENCED GENES

The expression of certain genes which may reside on any of the autosomes or on the homologous portions of the sex chromosomes is influenced by the sex of the bearer, *e.g.*, baldness in man.

In man the baldness may occur due to disease, radiation or thyroid defects but in some families baldness is found to be inherited trait. In such inherited baldness the hairs gradually become thin on head top, leaving ultimately a fringe of hair low on the head and commonly known as pattern baldness. The gene B for baldness is found to be dominant in males and recessive in females. In heterozygous condition it expresses itself only in the presence of male hormones (in male sex) :

Genotype	Phenotypes	
	Men	Women
BB	Bald	Bald
Bb	Bald	Non-bald
bb	Non-bald	Non-bald

In sheep, the genes for the devlopment of horns is dominant in males and recessive in female. Further in cattles also sex-influenced genes occur as spotting in cattle. The genes for mahogany and white spotting is dominant in males and the genes for red and white spotting is dominant in females.

SEX-LINKED LETHALS

Certain sex-linked genes are lethal and may cause death from egg upto sexually mature adult stage. The sex-linked lethals of man are the genes for haemophilia and pseudohypertrophic muscular distrophy.

SEX-LIMITED GENES

some genes may only come for expression in one of the sexes either because of differences in their internal hormonal environment or because of anatomical dissimilarities. For example, bulls have genes for milk production which they transmit to their daughters,

but they or their sons are unable to express this trait. The production of milk is, therefore, limited to variable expression only in the female sex. This type of genes which could express in one sex and could not express in other sex are called **sex-limited genes.**

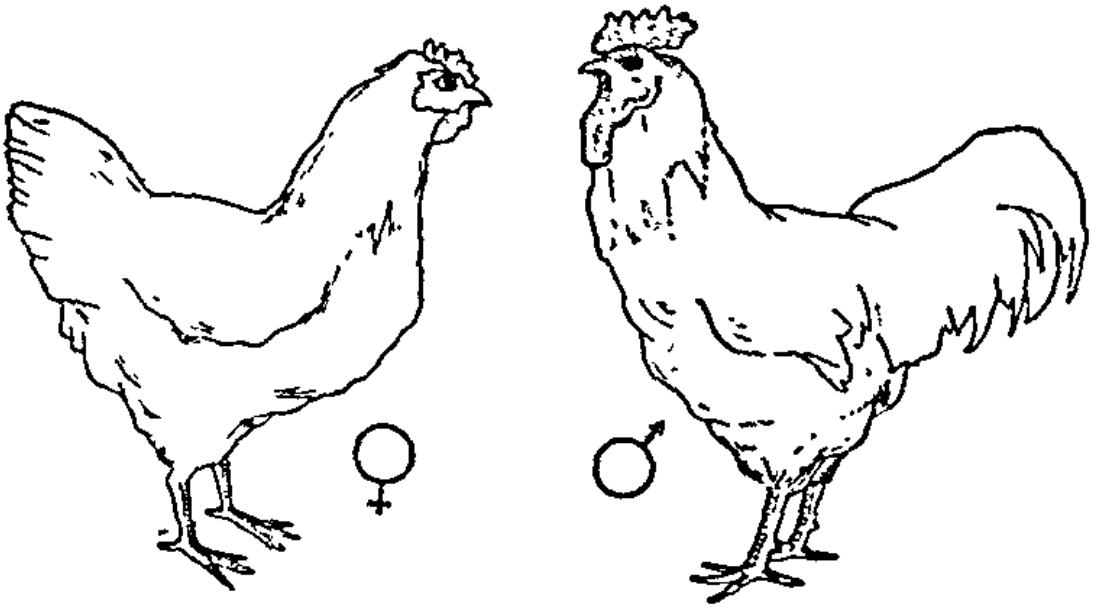


Fig. 11·8. The hen and cock feathering.

In chicken the recessive gene (h) for cock feathering is male sex-limited :

Genotypes	Phenotypes	
	Males	Females
HH	Hen-feathering	Hen-feathering
Hh	Hen-feathering	Hen-feathering
hh	Cock-feathering	Hen-feathering

CHAPTER 12

MUTATION

In a species variations may be caused by changes in the envi-
nore commonly
when the envi-
d, it is not heri-
table and as it is not built into the genotype, it does not provide a
permanent step on which e
are phenotypic variations a
defined as phenotypic differ
type. If, however, changes occur which are independent of the environment and heritable, they are liable to provide a permanent step
occur in
iation or
s no re-
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ure sudden
lterations
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lled chro-
mosomal mutations, or

HISTORICAL BACKGROUND

The earliest record of point mutations dates back to 17[illegible], when
Seth W[illegible] ock
of she[illegible] ged
sheep, he produced a flock of sheeps, each of which having short legs, by employing artificial breeding techniques. The short legged breed of sheep was known as **Ancon** breed. Later on, the trait of

evening primrose, *Oenothera lamarckiana*. Many mutations describe

followed by a extensive search of other mutants of *Drosophila* by Morgan and his co-workers and other geneticists. Consequently about 500 mutants of *Drosophila* have been reported by geneticists all over the world. Later on, sev

in a variety of micro-orgar

and animals, *i.e.*, rodents, fowls, man, etc.).

OCCURRENCE

Mutations occur frequently in the nature and have been reported in many organisms, *e.g.*, *Drosophila*, mice and other rodents, rats, rabbits, guinea pigs and man. In the *Drosophila* mutation causes white and pink eyes, black and yellow body colours, and vestigial wings. In rodents the mutuations are responsible for black, white and brown coats. In man, the mutations cause variation in hair colour, eye colour, skin pigmentation and several somatic malformations.

KINDS OF MUTATIONS

There exists a lot of controversy about the possible kinds of mutations among geneticists. They have been classified variously according to different criteria as follows :

1. CLASSIFICATION OF MUTATION ACCORDING TO TYPE OF CELLS

According to their occurrence in somatic and germinal cells following types of mutations have been classified :

occurring in non-repro-

duct

nutations. The genetical

and

mutations are insigni-

ficant, since only single cells and their daughter cells are involved. If, however a somatic mutation occurs early during embryonic life,

the

the

mut

Exa

lamarckiana (Hugo de Vries) and several other cases including

In man somatic mutation causes several fatal diseases such as *pera-*

oxysmal nocturnal hemoglobinura circumscribed neurofibroma, unilateral retinoblastoma and *heterochromia of the iris.*

B. Gametic mutations—The [illegible] gamete cells (*e.g.*, sperms and o[illegible] mutations are heritable and o[illegible] genetic mutations only form the [illegible]

2. CLASSIFICATION OF [illegible] SIZE AND QUALITY

According to size following two types of mutations have been recognised :

A. Point mutation—When heritable [illegible] y small segment of DNA molecule, *i.e* [illegible] tide pair, then this type of mutation [illegible] The point mutations may occur due t[illegible] tide changes in the DNA and RNA.

1. Deletion mutation—[illegible] due to [illegible] triplet [illegible] tion mu[illegible] (Phage [illegible]

2. Insertion [illegible] point mutations which [illegible] nucleotides to a gene or [illegible] insertion mutations can [illegible] nces called mutagens [illegible] avin molecule, it is [illegible] DNA strand, thereby stretching the strand lengthwise [illegible] would [illegible] [illegible] added to th[illegible]

3. Substitution mutation—[illegible] tide of a [illegible] tution mu[illegible] triplet c[illegible] a differe[illegible] the production of a protein with a single amino acid substitution. The substitution mutations alter the phenotype of an organism variously and are of great genetical significance. They may be of following types :

(i) **Transition**—When a purine (*e.g.*, adenine) base of a triplet codon of a cistron is substituted by another purine base (*e.g.*, guanine) or a pyrimidine (*e.g.*, thymine) is substituted by another pyrimidine base, (*e.g.*, cytosine) then such kind of substitution is called **transition**. The transitional substitution mutations occur due to tautomerization.

TAUTOMERIZATION

In a DNA molecule, normally, the purine, adenine (A) is linked to the pyrimidine, thymine (T), by two hydrogen bonds, while the

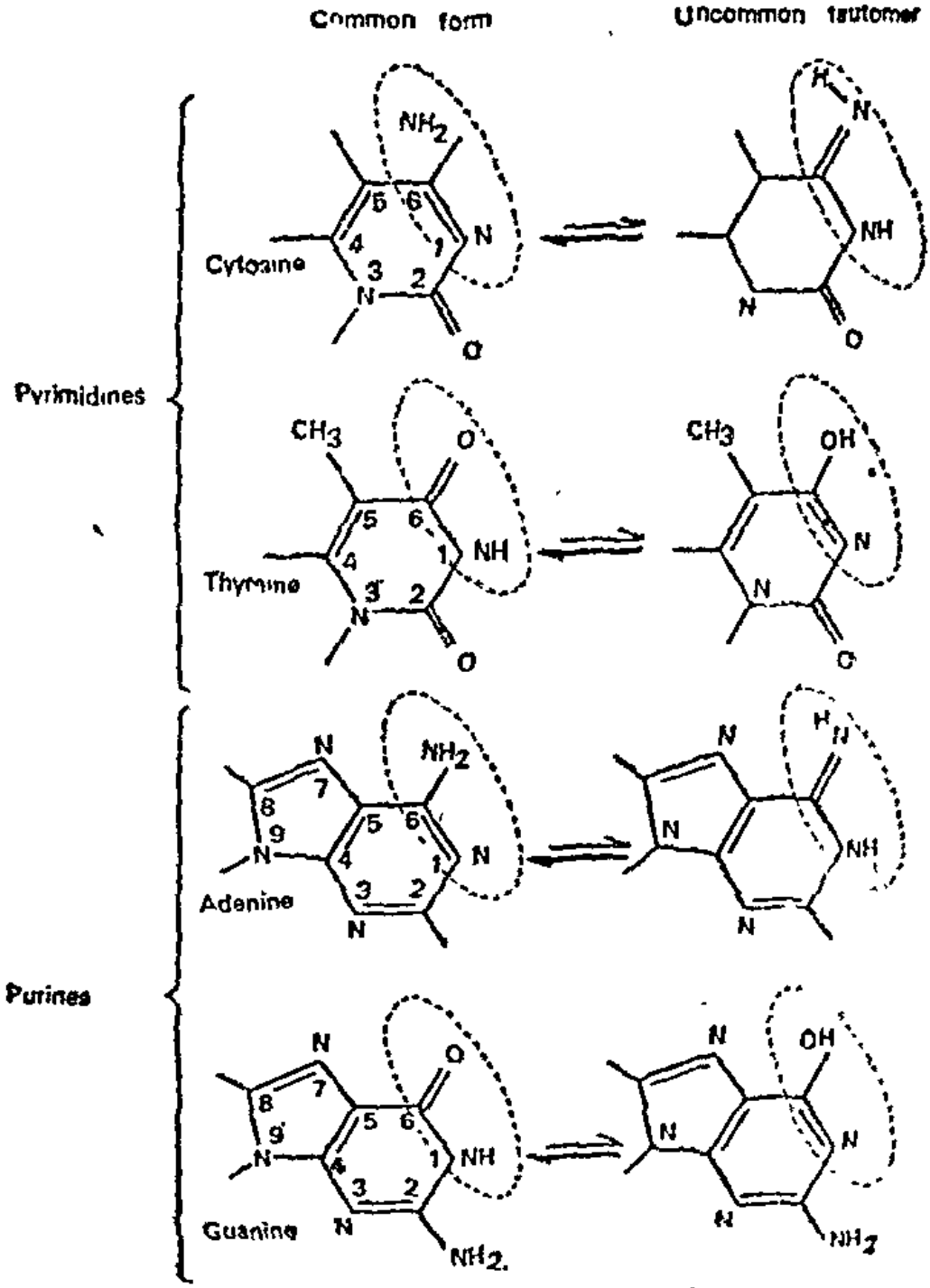


Fig. 12·1. The uncommon forms of DNA bases.

purine guanine (G) is linked to the pyrimidine, cytosine (C) by three hydrogen bonds. Besides the common molecular configurations, each DNA base may have some altered uncommon molecular configuration, as has been shown in the figure 12·1.

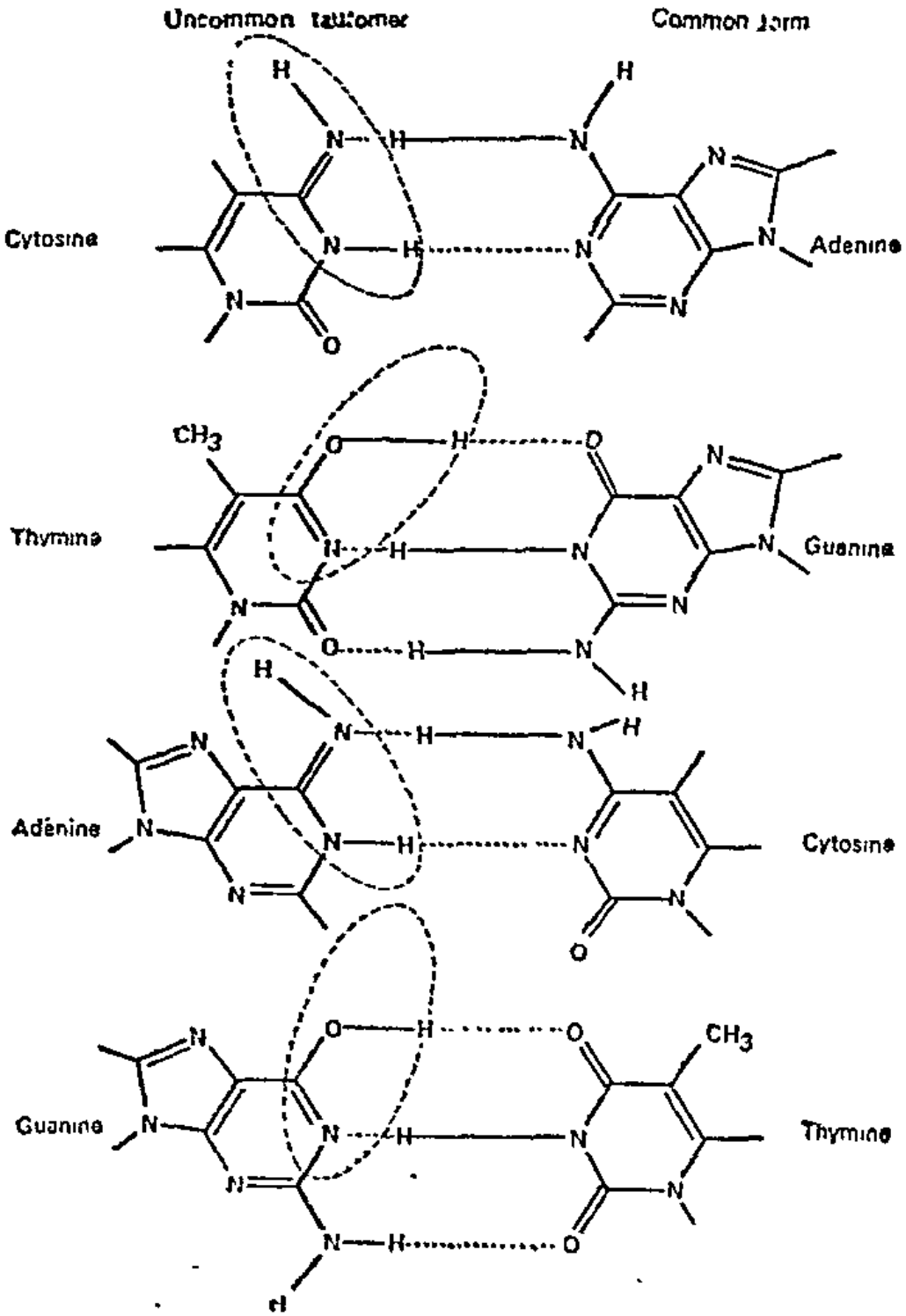


Fig. 12·2. Pairing qualities of rare tautomers of four DNA bases.

occur in thymine changing it from the keto (C=O) form to the rare enol (COH) form. When a base occurs in its rare or tatuomeric state, it cannot be linked to its normal partner. However, a purine, such as adenine can in its rare state forms a bond with cytosine (besides thymine), provided the cytosine is in its normal state.

Watson and Crick, (1953) hypothesized that the occurrence of the bases in their rare states provides a mechanism for mutation during DNA replication. If, for example, adenine in an old chain is in its rare state at the moment that the complementary new chain reaches it, cytosine can pair with it (adenine) and be added to the growing end of the new chain. The result of this type of pairing is the formation of a DNA molecule that contains an exceptional base pair. This situation is not stable and at the next replication adenine is expected to return to its common state and to pair with thymine. Cytosine introduced into the complementary strand due to tautomeric shift in adenine, would then pair with guanine. Thus, there would be formed two kinds of DNA molecules, one that is identical to the original DNA and another that has undergone a base pair substitution of G-C for A-T. This transitionally substituted DNA molecule has altered coding at a point and results in recognizable mutation. Such mutations which formed during DNA replication are called **copy error mutations.** Such copy error mutations have been shown by following figure.

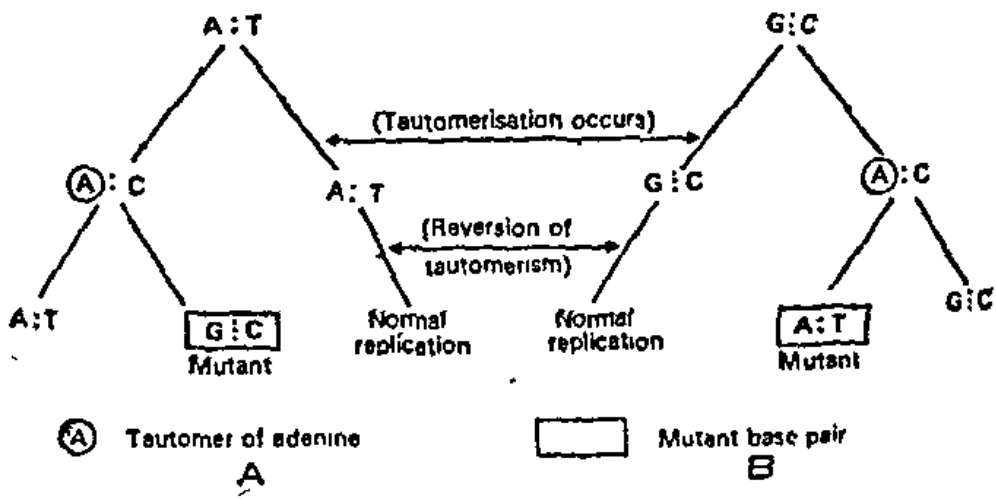
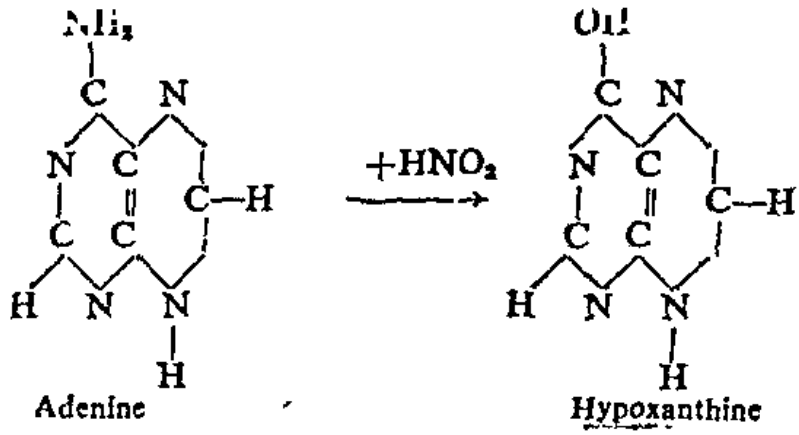


Fig. 12·3. Copy error mutation due to tautomerization of adenine.

The abnormal pairing due to transitional substitution may also occur due to ionization of a base at the time of DNA replication. Ionization involves the loss of the hydrogen from the number one

nitrogen of a base. For example, in its ionized state, thymine can pair with guanine, *if the guanine is in its common form. In similar* fashion, guanine in its ionized state can pair with thymine in its common form. From any such unstable base pair, a transition will result following the steps outlined in fig. 12·3 for A-T to G-C and G-C to AT.

EXPERIMENTAL EVIDENCES FOR TRANSITION



By *tautomeric shift the hypoxanthine (HX) is converted into* more common or keto-tautomer which pairs with cytosine. The A : T pair, thus, can be converted to a G : C pair.

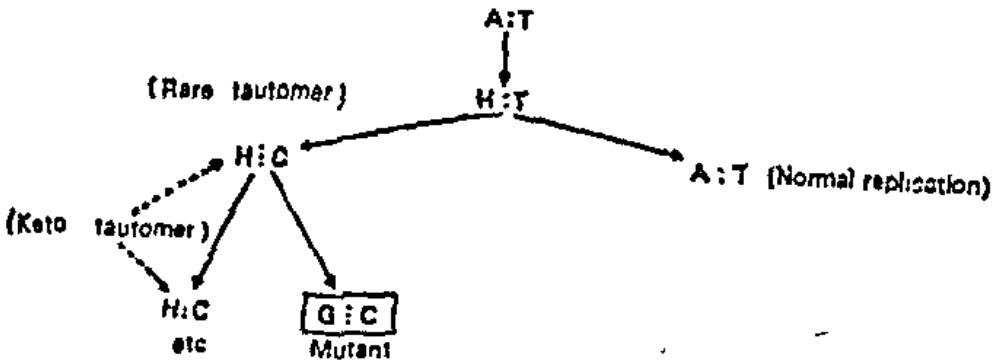


Fig. 12·4. Conversion of A : T pair into G : C pair due to keto-tautomer of adenine.

Similarly, deamination converts cysosine to uracil, which has pairing properties similar to thymine and in such a case G : C pair would be changed into A : T pair.

2. **Base analogs**—Certain chemical substances have molecular structure similar to the usual DNA bases that, if they are available, such analogs may be incorporated into a replicating DNA strand. For example, **5-bromouracil** (5BU) or its *nucleoside* **5-bromodeoxyuridine** (5-BUDR) in its usual (keto) form is a structural analogue of thymine (5-methyl uracil) and it will substitute for thymine. Thus, an A-T pair becomes and remains A-BU. There is some *in vitro* evidence to indicate the BU immediately adjacent to an adenine in one of DNA strands causes the latter to pair with guanine. But, in its rare (enol) state, 5BU behaves similar to the tautomer of thymine and pairs with guanine. This converts A : T to G : C as shown in the following figure.

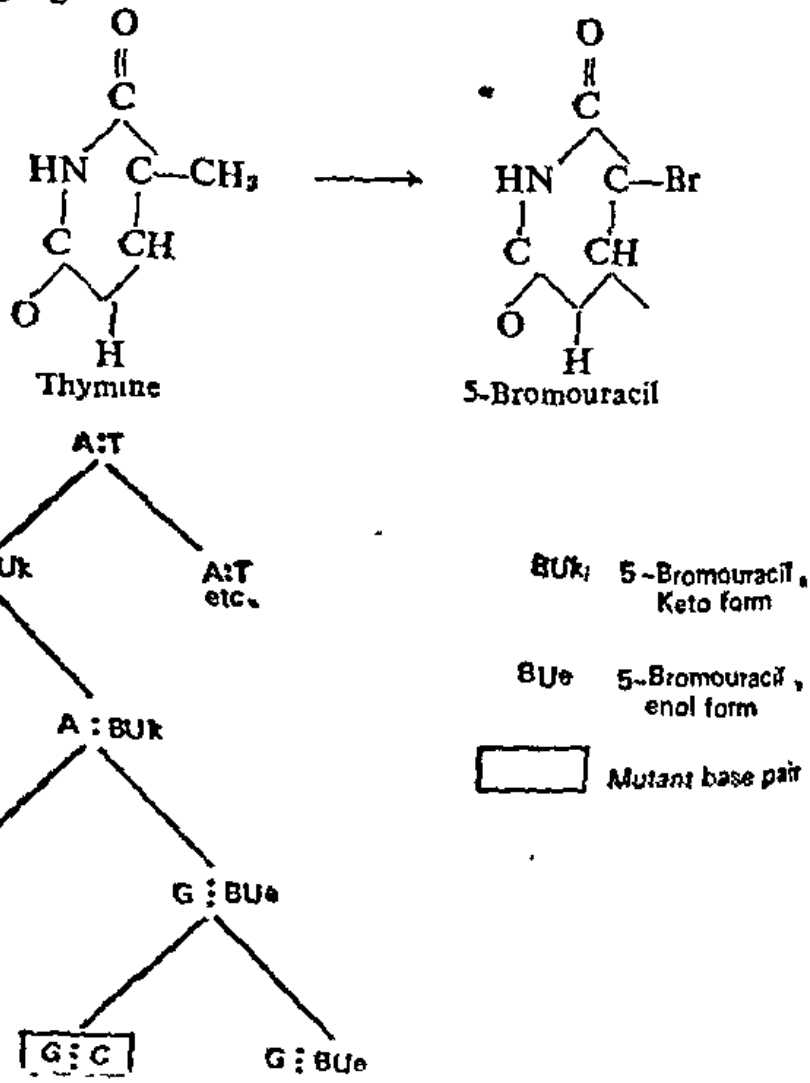


Fig. 12.5. Conversion of A : T base pair into G : C base pair due to keto and enol forms of bromouracil.

2—Aminopurine (2-AP) is another base analog which is a relatively undifferentiated purine that apparently can pair with cytosine and thymine. It is thought that 2-AP acts by "switching" pyrimidines : for example, it may be incorporated opposite thymine during one round of replication and then pair with a cytosine at the next

round to produce an AT→GC transition (see Goodenough and Levine, 1974).

3. Transversion—The substitution mutation when involves the substitution or replacent of a purine with a pyrimidine or vice versa then that type of substitution mutation is called **transversion mutation.** The existance of transversion mutation was first of all postulated by E. Freese in 1959. We have still poor information about the mechanism of induction, identification and characterization of transversion mutations. Moreover, it is extremely difficult to recognize transversion mutations genetically. However, they can be recognized only by analysis of amino acid substitutions in proteins.

High temperature and low pH value are known to effect depurination or loss of purine bases. The removal of a purine from a strand of DNA leaves a gap at that point. At the time of replication, it would be possible for any of the four bases to insert in the complementary newly formed strand. If the inserted nucleotide contained a purine, the complementary strand would contain a trans version.

B. Gross mutation—When changes involving more than one nucleotide pair, or entire gene, then such mutations are called **gross mutations.** The gross mutations occur due to rearrangements of genes within the ganome and may be of the following types :

1. The rearrangement of genes may occur within a gene. Two mutations within the same functional gene can produce different effects depending on gene whether they occur in the cis or transposition.

2. The rearrangement of gene may occur in number of genes per chromosome. If the numbers of gene replicas are non-equivalent on the homologus chromosomes, they may cause different types of phenotypic effects over the organisms.

3. Due to movement of a gene locus new type of phenotypes may be created, especially when the gene is relocated near heterochromatin. The movement of gene loci may take place due to following method :

(i) Translocation—Movement of a gene may take place to a non-homologous chromosome and this is known as **translocation.** (see chapter of chromosome aberration).

(ii) Inversion—The movement of a gene within the same chromosome is called **inversion** (see chapter of chromosome aberration).

3. CLASSIFICATION OF MUTATION ACCORDING TO THE ORIGIN

According to the mode of origin following two kinds of mutations, have been recognised :

A. Spontaneous mutations : The spontaneous mutations occur suddenly in the nature and their origin is unknown. They are also called "**background mutation**" and have been reported in many organisms such as, *Oenothera*, maize, bread molds, micro-organisms, (bacteria and viruses), *Drosophila*, mice, man, etc.

B. Induced mutations—Besides naturally occurring spontaneous mutations, the mutations can be induced artificially in the living organisms by exposing them to abnormal environment such as radiation, certain physical conditions (*i.e.*, temperature) and chemicals. The substances or agents which induce artificial mutations are called '**mutagenes or mutagenic agents.**'

Mutagenic agents—The mutagenic agents are of the following kinds :

A. Radiations—The radiations which are important in mutagenesis are of two categories : one type is **ionizing radiations** such as X-rays and gamma rays ; alpha and beta rays ; electrons, neutrons, protons and other fast moving particles. The second type is **non-ionizing radiations** such as ultraviolet and visible light. Both types of radiations induce mutations by following methods :

(i) Ionizing radiations as mutagens—*Relatively little* is known about the mechanism by which ionizing radiation cause

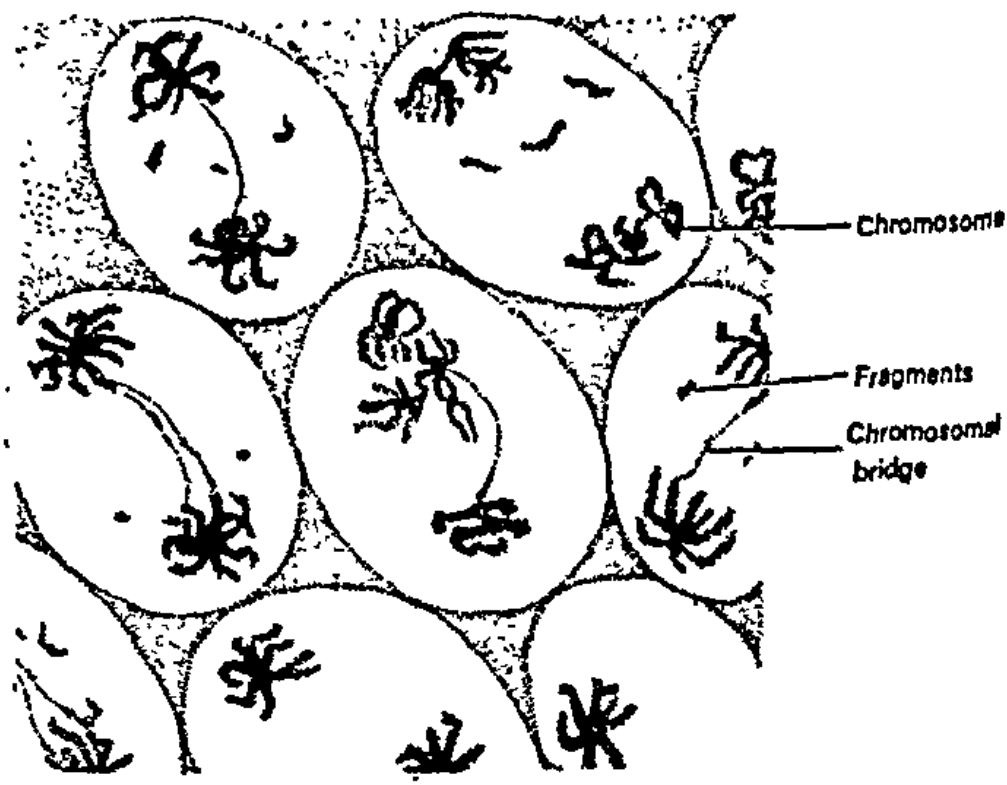


Fig. 12·6. Radiation-induced chromosomal bridges and fragments in cells of X-rayed anthers of *Trillium*.

mutation. As, we are already familiar that matter composed of atoms and atoms, in their turn, are made up of a positively charged atomic nucleus (with neutrons, protons) and a surrounding constellation of negatively charged electrons. The charges of atomic particles remain so balanced that normal atoms are electrically neutral. When ionizing radiations pass through matter, they dissipate their energy in part through the ejection of electrons from the outer shell of atoms and the loss of these balancing, negatively-charged particles (electrons) leaves atoms which are no longer neutral but are positively charged. The positively-charged atom is called **ion**. The ejected electrons move at high speed, knock other electrons free from their respective atoms and when their energy is dissipated, become attach to other atoms and convert the ion into negatively charged ions. To achieve their stable configuration (*i.e.*, neutral charge) ions undergo many chemical reactions and during these chemical reactions ionizing radiation is thought to cause mutation.

Further, ionizing radiations cause breaks in poly-sugar phosphate backbone of DNA and thus causing chromosomal mutations such as break, deletion, addition, inversion and translocation. During breakage of DNA molecule due to ionizing radiation the active role of oxygen is predicted. Because, oxygen is important in the formation of H_2O_2 and HO_2 in irradiated water and these products may induce breaks in DNA molecule.

(ii) Non-ionizing radiations as mutagens—The ultraviolet (UV) light is a non-ionizing radiation which may cause mutation. The most effective wave length of ultraviolet for inducing mutations is about 2,600 A°. This is a wave length that is best absorbed by DNA and a wave length at which proteins absorb little energy. When a substance absorbs sufficient energy from the ultraviolet light, some of their electrons are raised to higher energy levels, a state called **excitation**. The excited molecule becomes reactive and mutated and is called **photoproducts**.

Dimerization—The ultraviolet radiation produce several effects on DNA, one being the formation of chemical bonds between two adjacent pyrimidine molecules in a polynucleotide and particularly, between adjacent thymine residues as shown in fig. 12·7. As the two thymine residues associate, or dimerize to form a dimer, their position in the DNA helix becomes so displaced that they can no longer form hydrozen bonds with the opposing purines and thus the regularity of the helix becomes distorted. Thus dimerization interferes with the proper base pairing of thymine with adenine, and may

result in thymine's pairing with guanine. This will produce a T-A to C-G transition.

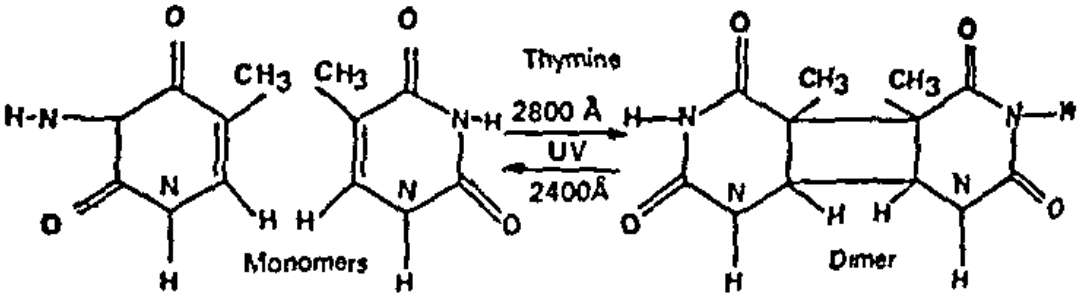


Fig. 12·7. Formation of a dimer of thymine.

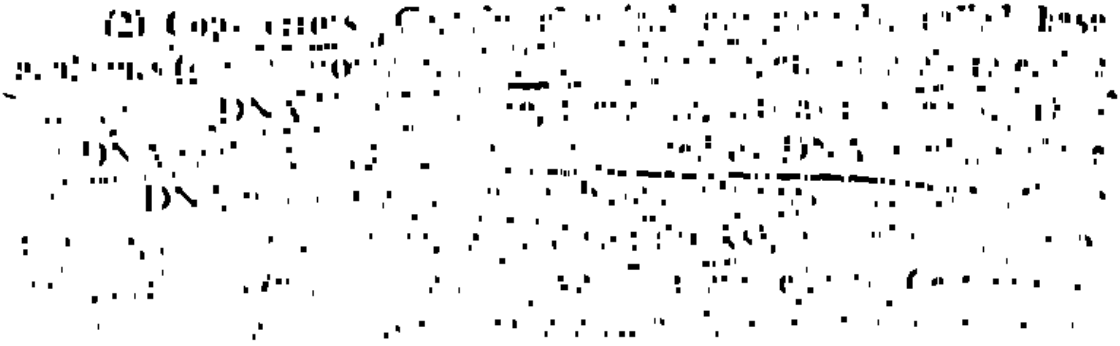
B. Temperature as mutagens—The rate of all chemical reactions are influenced by temperature. It is not surprising that temperature can be mutagenic. It is reported that the rate of mutation is increased due to increase in temperature. For example, an increase of 10°C temperature increases the mutation rate two or three fold. Temperature probably affects both thermal stability of DNA and the rate of reaction of other substances with DNA.

A study of swedish nudist indicated that the scrotal temperature of human males in ordinary clothing is about 3°C higher than that of nude male. The higher temperature could well increase the mutation rate nearly two fold, leading the investigators to suggest that the wearing of pants has possibly been much more dehygenic than fall out from testing of nuclear devices threatens to be. They suggested the wearing of kilts as one solution.

C. Chemical mutagens—Many chemical substances have been responsible to increase the mutability of genes. The ability of chemicals to induce mutation was first of all demostrated by **Auerbach** and **Robson** in 1947 using mustard gas and related compounds as the nitrogen and sulphur mustards, mustard oil and chloracetone in experiments with male *Drosophila melanogaster*. Since then many chemical compounds which are ordinarily considered to be non-toxic have been found to be mutagenic in certain specific situations. Any chemical substance th[illegible]omosomes is likely to infl[illegible]DNA and its ability to repl[illegible] can cause mutation only when it enters in the nucleus of the cell. It can affect the chromosomal DNA by following two ways :

(1) Direct gene change—Certain chemical mutagens affect DNA directly. They affect the constituents of DNA only when DNA is not replicating. For example, nitrous acid converts adenine into hypoxanthine and cytosine to uracil by deamination. Like the nitrous acid, nitrogen mustard, formaldehyde, epoxides, dimethyl

and diethyl sulfonate, methyl and ethyl methanesulfonate (MMS and EMS) and nitrosoguanadine (NG) also have direct mutagenic effect on the DNA molecule.



organisms, as, they are the compounds which bind calcium and thus interfere with the integrity of the chromosome structure.

4. CLASSIFICATION OF MUTATION ACCORDING TO THE DIRECTION

According to their mode of direction following types of mutations have been recognised :

(A) Forward mutations—In an organism when mutations create a change from wild type to abnormal phenotype, then that type of mutations are known as **forward mutations.** Most mutations are forward type.

(B) Reverse or back mutations—The forward mutations are often corrected by error correcting mechanism, so that an abnormal phenotype changes into wild type phenotype. They may be of the following types :

(i) Single site mutation—Some reverse mutations change only one nucleotide in the gene and are called **single site mutations.** For example, due to forward mutation the adenine is changed into guanine and backward mutation change guanine into adenine :

$$\text{Adenine} \xrightarrow{\text{forward}} \text{Guanine} \xrightarrow{\text{reverse}} \text{Adenine}$$

(ii) Mutation suppressor—When a mutation occurs at a different site from the site where already primary mutation occurred and that mutated gene reverse the effects of primarliy mutated gene, then such (secondary) mutations are called **mutation suppressors.** They may be of following types :

(a) Extragenic suppressor—The extragenic suppressor mutation occurs in a different gene from that of the mutant gene. In *E. coli*, a gene mutation suppressor gene called rec A (*rec.* for *recombination*) is known which is necessary for recombination and is found to repair ultraviolet-induced thymine dimers of a gene by a process called **postreplication recombinational repair** (see. **Goodenough and Levine, 1974**).

(b) **Intragenic suppressor**—The intragenic suppressor mutation occurs in a different nucleotide within the same gene and shift the reading frame back into register.

(c) **Photoreactivation**—In photoreactivation type reverse mutation reversal of ultraviolet induced thymine dimers takes place by specific enzymes in the presence of visible light waves. During ultraviolet radiation a particular enzyme is selectively bound to the bacterial DNA. During photoreactivation the enzyme is activated by visible light and that cleaves the pyrimidine or purine dimers into monomers and restores their original forms.

(d) **Excision repair or Dark reactivation**—In an ultraviolet (UV) induced mutation, the reverse mutation may also occur in the absence of light According to Howard Flanders and Boyce (1964) dark reactivation includes following stages : (i) An enzyme possibly endonuclease makes a cut in the polynucleotide strand on either side of the dimer which may be formed due to ultraviolet radiation and excises a short, single strand segment of the DNA. (ii) Another enzyme, possibly exonuclease widens the gap produced by the action of the endonuclease. (iii) DNA polymerase resynthesizes the missing segment, using the remaining opposite strand as a template ; and (iv) the final gap is closed by some enzymatic rejoining process, as yet unknown,

5. CLASSIFICATION OF MUTATION ACCORDING TO MAGNITUDE OF PHENOTYPIC EFFECT

According to their phenotypic effects following kinds of mutations may occur :

1. **Dominant mutation**—The mutation which have dominant phenotypic expression are called dominant mutations. For example, in man the mutation disease anfridia (absence of iris of eyes) occurs due to a dominant mutant gene.

2. **Recessive mutations**—Most types of mutations are recessive in nature and so they are not expressed phenotypically immediately. The phenotypic effects of mutations of a recessive gene is seen only after one or more generations, when the mutant gene is able to recombine with another similar recessive gene.

3. **Isoalleles**—Some mutations alter the phenotype of an organism so slightly that they can be detected only by special techniques Mutant genes that give slightly modified phenotypes are called isoalleles. They produce identical phenotypes in homozygous or heterozygous combinations.

4 **Lethal mutations**—some mutations result in the death of the organism and are called lethal mutations.

6. CLASSIFICATION OF MUTATION ACCORDING TO THE TYPES OF CHROMOSOMES

According to the types of chromosomes, the mutations may be of following two kinds :

1. Autosomal mutations—This type of mutation occurs in autosomal chromosomes.

2. Sex chromosomal mutations—This type of mutation occurs in sex chromosomes.

MUTATION RATE

The frequency with which genes mutate spontaneously is called **mutation rate.** Most genes are relatively stable and mutation is a rare event. The great majority of genes have mutation rate of 1×10^{-5} to 1×10^{-6}, *viz.*, one gamete in 100,000 to one gamete in million would contain a mutation at a given locus. The mutation rate is influenced by various factors which are as follows :

1. Genetic control of mutation rate—There are ample evidences which show that mutation rate is under genetic control, *viz.*, certain genes called *mutator genes may increase the mutation rate in Drosophila* (Demere, 1937), maize (Rhoades, 1938) and *E. coli* (Goldstein. 1955). However, certain suppressor genes may decrease the rate of mutation.

2. Viral control of mutation rate—Virus reportedly affect the mutability of host's genes Sprague (1963) experimented with maize suggested that virus may cause mutation. Baumiliar (1967) reported that viruses increase the mutation rate in *Drosophila melanogaster.* But, still we do not know how viruses increase the mutability of host genes.

3. Environmental control of mutation rate—There are three major environmental factors that affect mutation rates, *viz*,. temperature, certain radiations and chemicals.

METHOD FOR DETECTION OF SEX-LINKED LETHAL MUTATION

H. J. Muller devised an easy method for detecting lethal mutations in the sex chromosomes of *Drosophila.* This is called a CIB method in which a special type of female fly is employed which carries a normal X chromosome and an abnormal X chromosome. The abnormal X chromosome contains an inversion mutation C (which prevents the chromosome to do crossing-over with the normal X chromosome, therefore, called **crossover suppressor**) a recessive lethal mutational gene, l and a dominant gene B for bar-eye. In Muller's CIB technique these CIB female flies are mated with males

which are previously treated with some mutagenic agent (such as X-rays) to cause mutation in some of their sperms. The resulting zygotes are of four types and one of these, the CIB male, fail to survive because such embryos contain a recessive lethal which expresses itself when hemizygous. Thus, only one class of male (with CIB X chromosome) male remains to fertilize the F_1 females. Each heterozygous CIB female results from the fertilization of a CIB egg and an radiated X-bearing sperm and some of these sperms will contain mutated X-chromosomes. Mated F_1 heterozygous CIB females are distributed individually into culture tubes in which each lays fertile

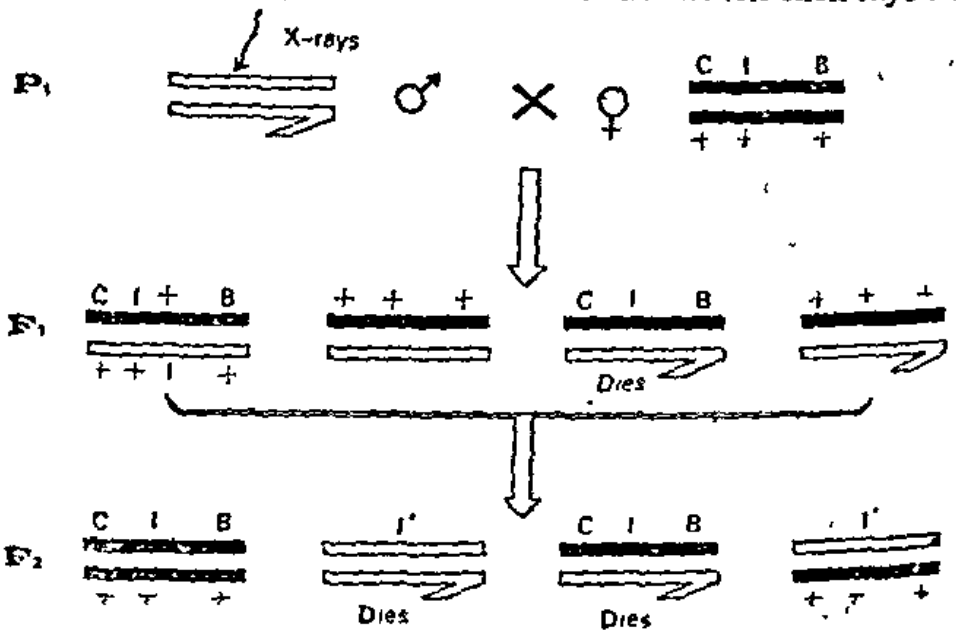


Fig. 12·8 Diagram of Muller's CIB technique for detecting sex-linked (X-linked), recessive lethal mutations.

eggs and so produces a single F_2 culture . The culture produced by females bearing an induced lethal mutation contain only females; whereas female bearing irradiated X-chromosomes in which no recessive lethal has been induced yield cultures containing some wild type males. Thus, if in a population of 1000 cultures 990 contained some males and 10 contained only females, the induced rate of sex-linked, recessive lethal mutations would be 1 per cent.

SIGNIFICANCE OF MUTATION

The vast majority of mutations [illegible] to the organism

CHAPTER 13

FINE STRUCTURE OF GENE

Mendel while explaining the results of his monohybrid and dihybrid crosses, first of all conceived of the genes as particulate units and referred them by various names such as hereditary factors or hereditary elements. But his concept about the gene was entirely hypothetical and he remained ignorant about the physical and chemical nature of gene.

Even before the rediscovery of Mendel's laws in 1900, it was already established that chromosomes have a definite role in the inheritance because it was found that chromosomes were the only link between one generation and the next generation and a diploid chro-

discovered.

Earlier workers proposed various hypotheses to explain the

the recessive character has no such determiner. But all these theories

consider the both classical as well as modern definition of gene.

CLASSICAL DEFINITION OF GENE

The definition of gene is an operational one and has different meanings according to different view points. Classically, a gene was assumed to be a genetic unit by the following three criteria :

(i) A gene is a **unit of physiological function** that occupies a definite locus in the chromosome and is responsible for a specific phenotypic character, *e.g.*, vestigial or long wings and white or yellow eyes in *Drosophila*.

(ii) A gene is a **unit of transmission** or **segregation**, because it can be segregated and exchanged at meiosis by way of crossing over.

(iii) A gene is a **unit of mutation**, because by a spontaneous or induced change it can give rise to a different phenotypic expression.

These classical definitions of genes have been revised thoroughly due to recent discoveries in the field of molecular genetics.

MODERN DEFINITION OF GENE

After the discovery of DNA, its parallel behaviour with that of chromosomes and proper understanding of most of the molecular phenomena which may interplay in the determination of a phenotypic trait, the gene has been defined as follows :

1. CISTRON

The portion of DNA specifying a single polypeptide chain is termed as a **cistron** which is a synonym for the term, the gene of physiological function. The term cistron has been coined by **Seymour Benzer.** Haemoglobin, therefore, would require two cistrons for its globin protein fraction, one each for the α and β chains. A cistron for α-chain has at least $141 \times 3 = 423$ nucleotides and the cistron for the β chain $146 \times 3 = 438$ nucleotides.

2. MUTON

There are many positions or sites within a cistron where muta- [illegible] smaller, [illegible] he al ic chan[illegible] f- mate[illegible] e ect.[illegible] s part[illegible] are called **homoalleles.** For example, in bacteria muton may be [illegible] cleotide pair and in cistron for haemoglobin the muton may be single nucleotide.

5. RECON

Sometimes crossing over or recombination occurs in a cistron and this provides still other subdivisional concept of the cistron.

namely the **recon.** A recon is the smallest unit of DNA capable of recombination or of being integrated by transformation in bacteria. Recombinationally separable forms of a cistron are called **hetero-alleles.**

with these definitions of
g
may serve our purpose.
ce of nucleotides (provid-
ing numerous sites for intragenic mutation and/or recombination)
which specifies the amino acid sequence of a specific polypeptide
chain."

CHAPTER 14

EUGENICS, EUPHENICS AND GENETIC ENGINEERING

Eugenics deals with the application of the laws of genetics to the improvement of humen race. The term eugenics (Gr. *eugenes*-well born) was coined by an English scientist Francis Galton in 1885. The science of eugenics can also be defined as a science of well born, improving the inborn qualities of race and obtaining the better heritage by judicious breeding.

EUGENICS AND EUTHENICS

The betterment of human society can be achieved by following two inter-related methods :

1. By one of the method we can deal with the already existing human beings. The improvement of already existing human beings can be achieved by improving the environmental conditions, *e.g.*, by subjecting them to better nutrition, better unpolluted ecological conditions, better education and sufficient amount of medical facilities. This type of method of improving the human race is known as **euthenics.**

2. By another method we can improve the future generations by improving the germplasm of existing individuals. The type of method is known as **eugenics.** Eugenics believes in artificial selection of physically and mentally sound individuals and discouragement of defective individuals for the inheritance of their defective germplasm to the future generations. In other words, eugenics seeks the measures to preserve the best type of germplasm and to eliminate defective germplasm from the human society by applying the laws of inheritance to man.

HISTORY

The primary aim of many of the ancient systems of eugenics was to produce a race of physically perfect human beings. The Greeks had definite ideas regarding eugenics. In Sparta a physically perfect manhood was the chief aim, where as the Atheniens carried more for the intellectual achievements.

Following the doctrine of Greeks and until the 19th century there was little interest in the eugenics. Of the particular importance was the eugenics movement in England in the last part of the nineteenth century. The movement, spearheaded by persons of outstanding intellect such as **Francis Galton** and **Karl Pearson**, had its objective the application of biologically sound principles to human populations. Since the biological basis of heredity was unknown, the first objective was to establish the nature of heredity.

Galton and **Pearson** choose to work with human beings and with what they considered important human traits such as intelligence, stature and special abilities. We now know that these characters are very complex traits and are under the control of many genes interacting with environmental variations. So, quite naturally the early investigators made little progress. However, **Francis Galton** should be credited with being the real founder of the modern movements of eugenics. He defined the eugenics as the study of all the agencies under social control which may improve or impair the inborn qualities of future generations of man either physically or mentally. **Darwin** also attached great importance to the eugenics and he compared it to a signpost with three directions. One of these indicates the influence of heredity on the fate of nations. Another point to the rules that an individual should strive to carry out in regard to parenthood based on the law of human heredity. The third arm indicates the regulations to be adopted by the society to encourage racial progress.

BASIS OF EUGENICS

The science of eugenics roots largely on the fact that the human being is an animal subject to the same laws and principles that govern the rest of the animal kingdom. It has been observed that the basic genetic mechanism of man, mice, higher plants and molds appear to be the same and in principle the genetics of man is not unlike the genetics of a virus.

For a long time man was considered a peculiarly awkward object of genetic investigation because his long life cycle and comparatively small individual progenies are indeed unfavourable to the use of certain standard research techniques of the geneticists. Moreover, we are neither able to subject ourselves to rigorous experimental conditions nor do we desire to do so. In the standard genetic approach, closely controlled matings and standardized environment are prerequisite. Where man is concerned, these experimental ideas are not feasible. Taking into consideration of above mentioned difficul-

ties, the geneticists have evolved the technique of studying the family known as pedigree analysis.

PEDIGREE ANALYSIS

Studies of human families have revealed the fact that desirable or undesirable traits depend for their expression on the genes carried by the germ cells of the parents. By pedigree analysis the mode of inheritance of a number of diseases and traits have been studied. Various diseases and defects like haemophilia, red green colour blindness, syphilis, insanity, rheumatism, hysteria, crenitism, polydactylism, syndactylism, etc., are all inherited from the parents. Besides the defects and diseases the good qualities such as memory, intelligence, ability of speech, mechanical skill, musical ability and various bodily characters are transmitted from one generation to another.

NEED FOR EUGENICS

The development of all organisms including the human individuals depends on both heredity and environmental factors. For the best development both good heredity and good-environment are essential. Even in the best environment there is little possibility for change of defective hereditary traits. The need of eugenics is apparent from the stand point of education, sociology and civilization The aim of geneticists should be of increasing the normal and gifted population and at the same time decreasing the abnormal and deficient populations.

EUGENICS AND HUMAN BETTERMENT

Both hereditary and environmental factors play a significant role in the development of the organisms. For better type of development both good heredity and suitable environment are necessary. When we consider the future welfare of the human race then the following two factors alarm us greatly : (i) the declining birth rate among the normal and superior people (those having best germplasm) (ii) a relative rapid increase of the abnormal and defective individuals (those having defective germplasm). For the betterment of future generation, it is necessary to increase the population of outstanding people and to decrease the population of abnormal and defective people by applying the principle of eugenics. The eugenics can be applicable by adopting following two methods :

(A) By encouraging the marriages between desirable persons (constructive method or positive eugenics).

(B) By discouraging the marriages between undesirable persons (restrictive method or negative eugenics).

A. POSITIVE EUGENICS

The positive eugenics attempts to increase consistantly better or desirable germplasm and thus to preserve best germplasm of the society. The percentage of desirable traits can be increased by adopting following measures:

1. Early marriage of those having desirable traits — It is most commonly observed fact that the highly placed persons of the society often have great ambitions for the future life. In achieving their ambitious goals, they often devote the best part of their youth and they are able to marry in their mature age (*i. e.*, 30 to 35 years). The biological and psychological investigations have revealed that the aged persons often lack in necessary amount of emotional warmth for the sexual activities and moreover, their germplasm also lost its vigour. Therefore, some laws should be formulated to prevent the late marriages of highly endowed persons by applying high taxation on them and at the same time the young persons having best hereditary traits should be encouraged for early marriages.

2. Subsidizing the fit—Because the highly endowed persons lead a well-planned life and to avoid unnecessary difficulties in nursing the children they often prefer to have small number of children. Therefore, the selected young men and women of best eugenic value should be encouraged to increase their birth rate. Moreover, **H.J. Muller** has suggested that such persons not only should increase their family size but through artificial insemination the outstanding man can serve as fathers to many more children than would be otherwise possible. The artificial insemination is already widely practiced to permit those women whose husbands are sterile or have some serious hereditary afflictions to bear children. The sperms and eggs of outstanding persons can be stored for future use by quick freezing and storing them in deep freeze. These germ cells thus can be stored for 100 or more years.

Very recently the scientists felt the urgent need of establishment of sperm and egg banks to protect these precious germ cells from the radiation. The germ cells could be collected during early adulthood and stored in lead lined containers in the deep freeze. In this state the germ cells would not be subjected to radiation expossure which might affect the donors.

3. Education—For the eugenically oriented reforms in the society, the people should be educated about the basic principles of human biology, human genetics, eugenics and sex. The children should be instructed about basic laws of health and they should be encouraged to develop a physically and mentally healthy body.

Moreover, the sex should be free from the wide spread confusion, narrow-minded concepts and religious and ethical bindings because that is a natural biological instinct. The children ignorant about the facts of sex may do more harm to society then otherwise.

4. **By avoiding germinal waste**—The wastage of best type of germplasm can be avoided by adopting following measures :

(i) The selection of marriage partners should be made with intelligence.

(ii) The social hinderance which do not allow the teachers, nuns and priests to get married, must be removed. By adopting such measures the wastage of best type of germplasm due to lack of opportunities can be prevented.

(iii) The wars must be avoided because in wars the best germplasm of the society is wasted.

5 **Genetic counselling**—Genetic counselling can do great benefit to human society The role of the genetic counsellor is to inform concerned individuals of the nature of the mutant condition that concern them. If it is inherited in a Mendelian fashion, then probability of producing affected offspring can be calculated. The final decision for taking a risk, is entirely the responsibility of the individual involved, it cannot be made by the counsellor.

6. **Improvement of environmental conditions**—Both heredity and environment have inter-related role in the development of eugenically better persons. Therefore, every person should get better food, living conditions, education and medical guidance, etc., so his or her hereditary traits can do their best development.

7. **Promotion of genetic research**—Our knowledge about the genetics is not sufficient enough because we still have little informations about various human diseases and metabolic disorders which are generally related with the genes. Therefore, the research in the field of cytogenetics should be increased so that we can learn more and more about the man.

B. NEGATIVE EUGENICS

The negative eugenics attempts to eliminate the defective germplasm of the society by adopting following measures :

1. **Sexual separation of the defective**—The defective persons may have various sex-linked diseases such as night blindness, haemophilia, colour blindness, etc., and various other defective traits which may be regulated by dominant or recessive genes. The increase of germplasm of the persons having such defective traits in the population can be checked by keeping them away and separated from the society. Different states have wisely adopted the restricted mea-

sures in segregation the mental defectives from the society and to place them in mental hospitals.

2. **Sterilization**—The sterilization is the best means to deprive an individual from his power of reproduction without inteferring with any of his normal functions. The sterilization method is based on surgical operation of sperm duct or vas deferens in males and oviducts or fallopian tubes in females. The former is known as **vasectomy** and the later is **splengectomy.** The family planning movement in India has adopted the sterilization as the tool for controlling the rate of vastly increasing population and in that case the sterilization is euthenical in its application than the eugenical.

3. **Control of immigration**—Through immigration there are enough chances that undesirable or defective genes of different races and nationalities may intermingle with the normal germplasm of the populations. Therefore, the immigration rules must be strict and the persons with undesirable hereditary traits must not be allowed to migrate from one place to another.

4. **Regulation of marriage**—Presently most human societies are money-minded and for the marriage relationship the wealthy or higly placed persons which, however, may contain several defective genetical traits, are more preferred than those which have eugenically sound hereditary traits but having no money and which fail to reach to highest status of the society due to lack of opportunities. Some rule must be enacted to encourage the marriages among desirable mates.

Recently human geneticists have devised following two eugenically sound methods (*i.e.*, euphenics and genetic engineering) for the improvement of human race .

EUPHENICS

The symptomatic treatment of genetic diseases of man is called **euphenics** (see **A.C. Pai, 1974**). The euphenics deals with the control of several inherited human diseases, especially inborn errors of metabolism in which the missing or defective enzyme has been identified. One example of this is the condition known as **phenylketonuria** or **pku**, determined by an autosomal recessive gene. Babies with this defect are unable to properly metabolize an amino acid, phenylalanine, the resulting chemical imbalance causes severe mental retardation. Now it is possible to distinguish pku homozygotes from normal individuals by testing the urine of all newborn babies with ferric chloride. In affected children, the metabolic imbalance caused by the mutation will turn the urine green. Once such a child is detected, a diet free of phenylalanine is imposed and the child can develop normally.

Although a number of inherited diseases can be treated in a similar euphenic manner, but these constitute only a small fraction of known inherited diseases. For the most part, biochemical geneticists could not identified the biochemical errors of many genetic diseases. In other cases, such as albinism, even though the metabolic block leading to an abnormality is known, but, it is not possible to correct it. However, in future following euphenic measures can get rid of man from certain fatal genetic diseases—

1. **Intake of missing enzyme ?** One possible euphenic measure for the future would be to supply the known missing enzyme to individuals that would allow their cells to complete the required biochemical reaction. Some attempts to do this have been made without much success. Immunological difficulties are encountered, since the enzymes being supplied are antigenic and the body produces antibodies against them.

2. **Cure for inherited anemia ?** Scientists studying the two anemias of man, **Cooley's** and **Lepore anemias**, resulting from abnormally low haemoglobin production in individuals homozygous for mutations at the beta-chain locus, hope that they may someday discover the factors regulating the beta gene activity and thereby increase the amount of beta chain synthesis. Furthermore, if the mechanism regulating haemoglobin synthesis in the faetus and the adult can be discerned, they might be able to cure lethal conditions, such as **thalassemia major** and **sickle cell anemia**, by suppressing synthesis of the abnormal beta chains and allowing fetal or gamma chains to be produced instead. In fact, individuals have been discoverd whose β chain locus for some unknown reason never becomes active. Such individuals function normally with fetal haemoglobin even as adults.

3. **Increasing role of genetics to medicine**—The increasing number of human diseases that are being discovered to have a genetic basis lend great importance to the development of such euphenic measures. Three per cent of all human have hereditary diseases which are transmitted in a Mendelian fashion. Two diseases which account for the deaths of hundreds of thousands a year, cancer and heart disease, are thought to have some heritable component. The future works of immunogenetics can suggest the ways by which an individual having genes for cancer may develop resistance for this diseases.

A recent study has shown that some forms of heart disease may be inherited as an autosomal dominant trait (L.K. Altman, 1972). An understanding of the genetic basis of such heart diseases would alert persons from families with an incidence of the disease to the possibility of incuring heart conditions and perhaps cause them to alter their diets and life habits accordingly. For example, those who

may inherit genes for heart trouble would then refrain from smoking and avoid high fat diets.

Harmful evolutionary effect of euphenics—To restore affected individuals to normalcy by euphenic measures is the only compassionate goal for scientists to maintain but to do so is to counter the forces of natural selection that are the basis for the evolutionary strength of a species. pku homozygotes, for example, would normally not reproduce and transmit the harmful mutations to future generations. Selection aganist them would be total; however when they do develop normally and produce progeny, every member of the next generation would be a carrier of the mutation, assuming that the wife of such person being normal. This has to add to our genetic load and to weaken the human species from the evolutionary point of view.

GENETIC ENGINEERING

The artificial synthesis of new genes and the subsequent alteration of genome of an organism is called **genetic engineering** (see **A.C. Pal, 1974**). The genetic engineering has evoked great public interest because of projections of someday being able to create genes, beneficial mutations, and various other processes that constitute the creation of life itself. Many of these have already been achieved by using microorganisms by certain microbial geneticists.

1. ARTIFICIAL SYNTHESIS OF DNA

Based on the double helix concept of the structure of a gene, experiments of Kornberg and his associates (1961) have led to the synthesis of DNA molecules that showed biological activity. They mixed together, deoxyribonucleotides, enzymes, ions and a piece of "primer DNA (*viz.*, DNA from a virus) and got new DNA molecules. These new DNA molecules when inserted into bacteria, caused the formation of new virus particles.

This experiment has profound implications. If it is possible to synthesize artificial DNA in a test tube, then it may also be possible to create specific genes which may then be inserted into cells to cause transformation. One can foresee the possibility of correcting mutant genes in this manner. For example, if the mutation for galactosemia diseases can be identified, then a normal gene which codes for the missing enzyme preventing the homozygotes from metabolizing galactose, a subunit of a sugar found in milk, could be synthesized and introduced into the individuals cells. This would supply the cells with the needed enzyme and would in effect be an artificially created back mutation. Some geneticists even have suggested culturing mutant human cells and screening them for back mutations which could be reintroduced into the individual.

2. TRANSDUCTION AND GENETIC ENGINEERING

A phenomenon in micro-organisms which may serve in genetic engineering is transduction, the process by which virus carries genetic material from one cell to another, causing the second host cell to be changed after incorporating the genetic material into its own genome. It has been found that some viruses are gene-specific with regard to transduction. For example, some derivatives of lambda bacteriophages are known to transduce the lac operon in bacteria. Very recently J. Shapiro and his coworkers utilized this fact in isolating some of the lac operon genes, including the promoter, operator and one of the structural genes, the β-galactosidase. Though single genes had been visualized before (*e.g.*, in *Triturus* by O. Miller and B. Beatty, 1969), but this was the first time that a specific gene had been intentionally isolated and transferred from cell to cell. One immediate possible application of the isolation of genes is to use specilized viruses to transduce normal genes into the cells of mutant individuals.

3. GENE INSERTION IN MAMMALIAN CELLS

For the treatment of human galactosemia disease by genetic engineering, C.R. Merril, M.R.Geier and J.C. Petricciani (1971) have treated human fibroblast cells from a galactosemia homozygote in tissue culture with phage bearing the gal operon from bacterial cells, and found what appeared to be enzyme activity in the fibroblasts which persisted through eight cell divisions. This indicates that bacterial and bacteriophage DNA can enter human cells and at least some of the DNA can undergo replication and transcription in human cells.

Further, Qasba and Aposhian (1971) have also shown that polyoma pseudovirions (particles containing viral protein coat and DNA from other cells) carrying DNA from mouse cells can insert this DNA into human cells in tissue culture systems. It is possible that DNA inserted in this manner may become incorporated into the chromosomes of the cell, although evidence for this has not been found.

Thus, in the future viruses may be found or produced that transduce into humans different genes to be used for the correction of various mutant conditions resulting from mutations at those loci. In addition to the lambda virus, phages have been found that transduce the tryptophan, maltose, and arabinose operons from chromosomes of the bacteria *Escherichia coli* and *Salmonella*.

Thus, by employing eugenics, euphenics and genetic engineering measures we can improve human race and can create future gererations with less or no genetic abnormalities and can exploit the nature fully.

CHAPTER 15

CYTOPLASMIC OR EXTRA-NUCLEAR INHERITANCE

In preceding chapters, we have discussed different roles of genes of the nuclear chromosomes in inheritance, cellular metabolism, development and mutation of the organisms in which they occur. Though, the genes of nuclear chromosomes have a significant and key role in the inheritance of almost all traits from generations to generations, but they altogether cannot be considered as the sole vehicles of inheritance, because certain experimental evidences suggest the occurrence of certain extranuclear genes or DNA molecules in the cytoplasm of many prokaryotic and eukaryotic cells. For example, bacterial cells such as *E. coli* possess a single main chromosome in the nucloid and often extra DNA elements called plasmids in the cytoplasm; the eukaryotic cells possess a main complement of chromosomes in the nucleus and extra-DNA-molecules or chromosomes in their mitochondria and chloroplasts. Qualifying also as extra hereditary elements are certain viruses, bacteria, and algae that take up residence within other cells and often aquire a permanent and mutually dependent relationship with their hosts (endosymbiosis). Finally, properties of certain eukaryotic cells suggest that their surfaces may possess genetic information that can be modified by the environment and inherited independently of the main chromosomes. These cytoplasmic extra-nuclear genes or DNA molecules of plasmids, mitochondria, chloroplasts, endosymbionts and cellular surfaces have a characteristic pattern of inheritance which does not resemble with that of genes of nuclear chromosomes and is known by different terms such as **non-Mendelian, non-chromosomal, uniparental, maternal, extra-chromosomal, cytoplasmic and extra-nuclear inheritance** (Goodenough and Levine, 1974).

COMPARISON OF EXTRA-NUCLEAR AND NUCLEAR GENETIC SYSTEMS

The inheritance of genes of nuclear chromosomes is characterized by the fact that the genes from male and female parents contribute equally to the genetic constitution of the progeny. There-

fore, in it, the reciprocal crosses between parents of different homozygous genotypes will yield offsprings of identical phenotype except for sex-linked genes, while in extra-nuclear inheritance, male and female parents though contribute equally the nuclear genes to the progeny but they do not make equal contributions of extra-nuclear genes to the progeny as pollens or sperms carry little or no cytoplasm or extranuclear genes, while eggs or ova contribute large amount of ooplasm and many extra-nuclear genes in the form of inactive mRNA, rRNA and tRNA and also in the form of DNA of mitochondria, chloroplasts and endosymbionts. Hence, the reciprocal crosses in the latter case yield different or non-Mendelian results.

A. EXTRA-NUCLEAR INHERITANCE IN EUKARYOTES

Many geneticists have studied various cases of extra-nuclear inheritance in different eukaryotes. Certain most important examples of extra-nuclear inheritnnce in eukaryotes are following :

I. MATERNAL INHERITANCE

In certain cases, it has been observed that certain characteristic phenotypic traits of F_1, F_2 or F_3 progeny are not the expressions of their own genes, but rather those of the maternal parents. Such phenotypic expressions of maternal genes (genotype) may be short-lived or may persist throughout the life-span of the individual. The substances which produce the maternal effects in the progeny are found to be transcriptional products (*i.e.*, mRNA, rRNA and tRNA) of maternal genes which have been manufactured during oogenesis and which exist in the ooplasm of unfertilized eggs in the form of inactive protein coated mRNA molecules (informosomes) or inactivated rRNA and tRNA. These transcriptional products of maternal genes produce their phenotypic effects during early cleavage and blastulation when there occur little or no transcription since, maternal and paternal genes of zygote remain engaged in mitotic replication or duplication of DNA. There may be other reasons of maternal effect which are still little understood. The maternal inheritance has been studied in some of following cases :

(a) Shell coiling in Limnea—In the snails (gastropods), the shell is spirally coiled. In most cases the direction of coiling of the shell is clockwise, if viewed from apex of the shell. This type of coiling is called **dextral**. However, in some snails the coiling of shell may be counter clockwise or **sinistral**. Both types of coilings are produced by two different types of genetically controlled cleavages, one being **dextral cleavage**, another being **sinistral cleavage**.

There are some species of gastropods in which all the individuals are sinistral but the main interest attaches to a species in which

sinistral individuals occur as a mutation among a population of normal dextral animals. Such a mutant was discovered in the freshwater snail *Limnaea peregra* (Boycott *et al.* 1930). Breeding and cross breeding of dextral and sinistral snails showed that the difference between the two forms is dependent on a pair of allelomorphic genes, the gene for sinistrality being recessive (S), and the gene for the normal dextral coiling being dominant (S^+). The two genes are inherited according to Mendelian laws, but the action of any genetic combination is manifested only in the next generation after the one in which a given genotype is found.

The eggs of a homozygous sinistral individual (SS) are fertilized by the sperm of a dextral individual (S^+S^+), the eggs cleave sinistrally and all the snails of this F_1 generation show a sinistral coiling of the shell. Thus, the genes of sperm do not manifest themselves, although the genotype of the F_1 generation is S^+S If a second generation (F_2) is bred from such F_1 sinistral individuals, it is all dextral, instead of showing segregation as would be expected in normal Mendelian inheritance. In fact, segregation does take place in the F_2 generation so far as the genes are concerned, but the new genic combinations fail to manifest themselves, since the coiling is determined by the genotype of the mother. The genotype of F_1 mother being S^+S, the gene for dextrality dominates and is responsible for the exclusively dextral coiling of the second generation. Only in the F_2 generation

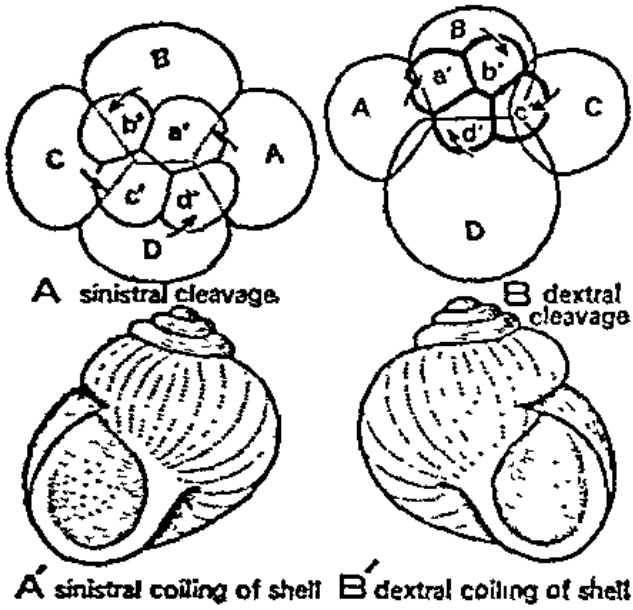


Fig. 15·1. Correlation of sinistral(A) and dextral(B) cleavage with sinistral (A') and dextral (B') coiling of the shell in gastropods.

does segregation in the ratio of 3 : 1 become apparent. Since the individuals of the F_2 generation had the genotypes—1 S^+S^+ ; 2 S^+S, 1 SS, three quarters of them, on the average, produce eggs developing into sinistral individuals.

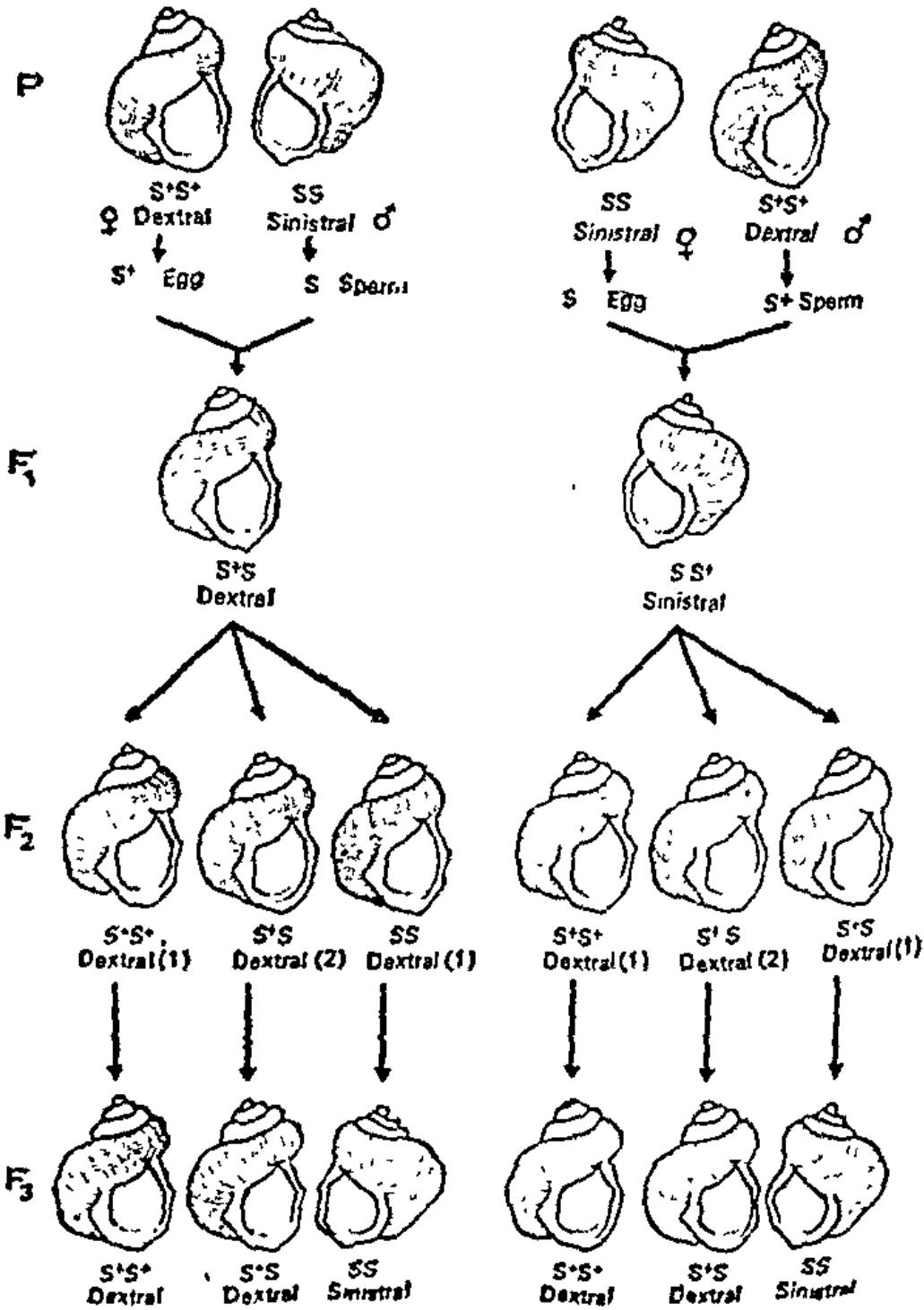


Fig. 15·2. Maternal effect in direction of coiling of the shell in *Limnaea.*

It is easy to understand that the results of a reciprocal cross—that is, of the fertilization of the eggs of a homozygous dextral individual (S^+S^+) by the sperm of a sinistral individual (S^+S)—will lead

to a somewhat different type of pedigree : the F_1 generation will be dextral (with genotype S^+S) and the F_2 generation again all dextral (with genotypic ratio of $1S^+S^+ : 2S^+S : 1SS$). The F_3 generation will show segregation among broods, just as in the cross examined st.

The whole case become clear if it is realized that the type of cleavage (sinistral or dextral) depends on the organization of the egg which is established before the maturation divisions of the oocyte nucleus. The type of cleavage is, therefore, under the influence of the genotype of the maternal parent. The sperm enters the egg after this organization is already established. Lastly, the direction of coiling of shell depends upon the orientation of the mitotic spindle of first cleavage of the zygote. If the spindle is tipped toward the left of the median line of the egg cell, the sinistral pattern will develop ; conversely if the mitotic spindle is tipped toward the right of the median line of the cell, the dextral pattern will develop. The spindle orientation is thus controlled by the organization of ooplasm which becomes established during oogenesis and before fertilization.

(b) Maternal inheritance in Ambystoma—In axolotl (*Ambystoma meicanum*) a lethal gene "O" has been discovered which has a maternal effect. The gene 'O' is recessive, so that heterozygote individuals (with genotype +O) are completely normal. Homozygote animals (with genotype OO) produced by a cross between two heterozygote parents (+O)develop normally in the early stages but in later lifeshow a slight retardation of growth. Their regeneration capacity is severly reduced so that amputated legs are not regenerated as would occur in normal axolotls. The homozygote (OO) males are sterile—their testes are poorly developed and spermatogenesis does not go beyond the spermatogonial stages. The homozygote (OO) females on the other hand, produce eggs capable of fertilization and normal cleavage, but at the on set of gastrulation the development is retarded and embryos normally die. This abnormal course of development is in no way affected by the genotype of the spermatozoon : the spermatozoon may carry the normal +gene or a mutant lethal O gene (if the male is heterozygote +O). The arrest in development is in both cases exactly the same : this shows that the particular type of abnormal early development does not depend on the genes present in the cells of the developing embryo (+O), but exclusiveliy on the OO genes of the mother (maternal effect), Briggs and Justus (1967) found that eggs of a OO female lack a protein-like substance the "corrective factor" which is necessary for normal development and

is synthesized by normal genes ++ of homozygous or heterozygous normal females (++ or +O) during oogenesis.

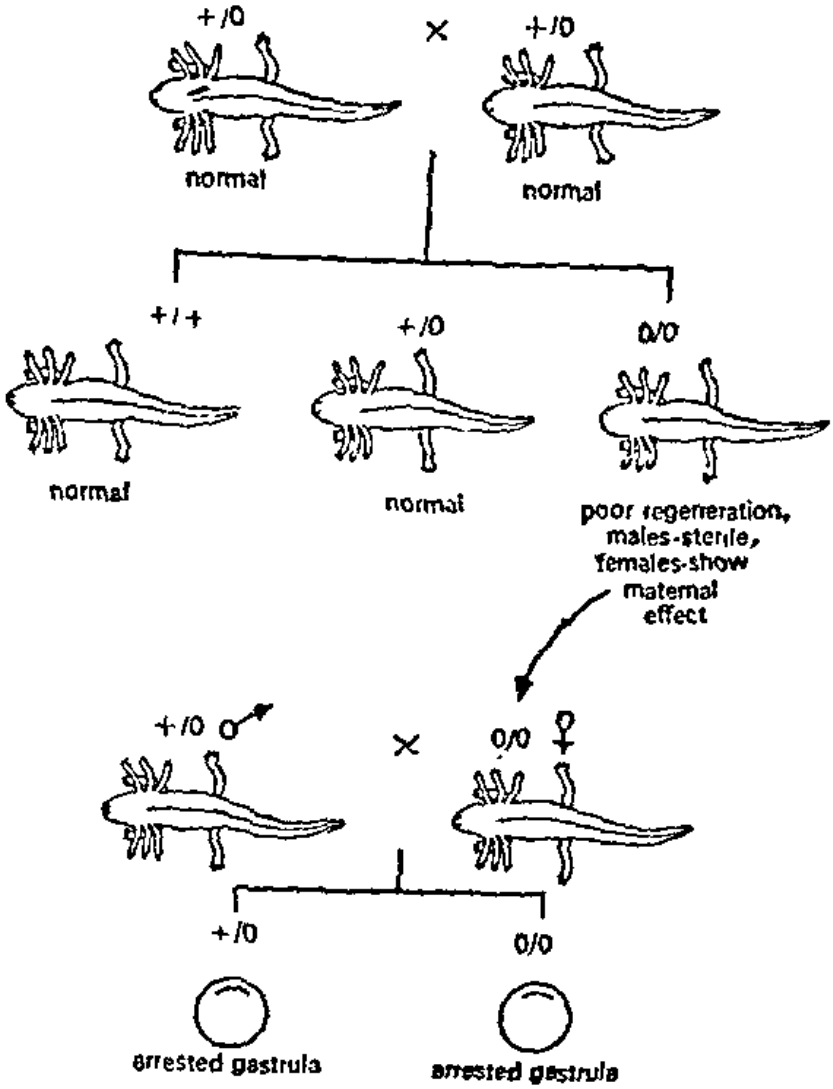


Fig. 15·3. Maternal inheritance and manifestation of the *O* gene in the axolotl.

(e) Eye pigmentation in water fleas and flour moths—Like the *Limnaea* and *Ambystoma mexicanum*, the maternal effect has also been observed in at least two very different invertebrates, the water flea (*Gammarus*) and the flour moth (*Ephestia kuhniella*). The normal colour of the eye in both invertebrates is dark due to the dominant gene (AA or KK) in which the dominant gene K directs the production of a hormone-like substance called kynurenine which is involved in the pigment synthesis. The recessive mutants do not possess pigment in the eye (*viz.*, kynurenineless) and have the genotype aa or kk. When aa or kk female is crossed with heterozygous with Aa or Kk male, only half of the larvae show dark pigment in the eye. But, a cross between Aa or Kk female and aa or kk male produces all larvae with dark eyes. On reaching the adult stage, half of the off-

springs (those of the genotype aa) become light eyed. This indicates that some kynurenine diffuses from the Aa mother into all young (larvae), enabling them to manufacture pigment regardless of their genotype. The aa progeny, however, has no means of continuing the supply of kynurenine, with the result that their eyes eventually become light. This example suggests an ephemeral type of maternal effect.

2. EXTRA-NUCLEAR INHERITANCE BY CELLULAR ORGANELLES

Chloroplasts and mitochondria are organelles that contain their own DNA and protein-synthesizing apparatus. A widely held theory concerning their origin proposes that they were once infectious endosymbiotic prokaryotes that evolved such a dependence on the gene products of the host that they are no longer able to function autonomously.

This theory has been supported by the fact that the genetic components of these organelle are often similar to those found in prokaryotes. For example, the chloroplasts of certain algae and *Euglena* contain 70S ribosomes and "naked" chromosomes or DNA which is circular Their protein synthesis begins with the amino acid N-formyl methionine, as does prokaryotic protein synthesis, and their DNA-dependent RNA polymerase is the sensitive to the inhibitor **rifampicin.** However, mitochondrial ribosomes of fungi are larger (77S) than bacterial ribosomes, while, mammalian mitochondrial ribosomes are smaller (60S) than bacterial ribosomes. Chloroplast chromosomes are found to contain more DNA than the average mitochondrial chromosomes, and thus can potentially carry more genetic information. The genetic materials of chloroplasts and mitochondria will betransmitted to offspring almost exclusively via the egg. Maternal inheritance due to chloroplast and mitochondria is well illustrated by following examples :

(a) Chloroplast inheritance in variegated four o' clock—The cytoplasmic or extra-nuclear inheritance of colour in plant plastids was first of all discribed by **C. Correns** in 1908 in the four o'clock plant, *Mirabilis jalapa*. In contrast to other higher plants, *Mirabilis* contain three types of leaves and parts : (1) Full green leaves or parts having chloroplast, (2) White (pale) leaves and branches having no chloroplast, (3) Variegated branches having leukoplast in white (pale) areas and chloroplast in green patches. Because, the chlorophyll pigment of chloroplast is related with photosynthetically prepared food and leukoplasts are incapable to perform photosynthesis, so the white or pale parts of plant survive by receiving nourishment from green parts. Correns reported that flowers on green branches produced only

green offsprings, regardless of the genotype and phenotype of pollen parent and likewise, flowers from the white or pale branches produced only white or pale seedings regardless of genotype and phenotype of pollen parent. The plants developing from the white or pale seedings die because they lack chlorophyll and cannot carry on photosynthesis. Correns further reported that flowers from the variegated branches yielded mixed progeny of green, white (pale) and variegated plants in widely varying ratios. These results are summarized in following table :

Table : Chloroplast inheritance in variegated four o'clocks.

Branch of origin of the male parent	Branch of origin of the female parent	Progeny
Green	Green Pale or white Variegated	Green Pale or white Green, pale or white, Variegated
Pale or white	Green Pale or white Variegated	Green Pale or white Green, pale or white, Variegated
Variegated	Green Pale or white Variegated	Green Pale or white Green, pale or white, Variegated

The irregularity of transmission from variegated branches could be understood by considering cytoplasmic genes (plasmagenes) of plastids. A study of the egg during oogenesis in *Mirabilis* reveals that the ooplasm contains plastids like cytoplasm of other plant cells. If the egg cell is derived from green plant tissues, its ooplasm will contain coloured plastids; if derived from white plant tissues, its ooplasm will contain white plastids ; if derived from variegated tissuse, its cytoplasm may contain coloured plastids only, white plastids only or a mixture of coloured and white plastids. A study of the pollenogenesis, however, reveals that pollen contains very little cytoplasm which in most cases is devoid of plastids. Without the plastids, the pollen cannot affect this aspect of the offspring's phenotype. In this type of inheritance because maternal cytoplasm has active participation in the determination of the phenotype of future generation, therefore, this is also known as **maternal inheritance.**

(b) Maternal inheritance by iojap gene of corn—Another example from higher plants also suggests the existence of plastid genes

controlling plastid integrity. A gene in corn plant called *iojap* (*ij*) has been mapped by M. Rhoades (1946) to nuclear chromosomes vii. Plants homozygous for ij are either inviable white seedings or variegated with a characteristic white striping, the phenotype being known as striped. When the variegated plants serve as females in a cross, they give rise to green, white, and striped progeny, regardless of the nuclear genotype of the paternal parent, Thus, if the pollen derives from a normal green Ij/Ij plant as in figure 15·4 b, the resulting progeny will be Ij/ij heterozygotes, but many will exhibit abnormal plastid pigmentation : the presence of the "normal" Ij gene has no curative effect *In* the reciprocal Ij/Ij ♀ ×ij/ij ♂ cross (*fig.15·4* a) on the other hand, the Ij/ij progeny are all normally pigmented.

The iojap trait, thus, exhibits classical maternal inheritance once it has become established in an ij/ij plant. Moreover, once established, it becomes independent of the ij gene, as can be demonstrated by crossing F_1 Ij/ij variegated females to Ij/Ij normal males. As shown in figure (15·4c), a mixture of green, striped and white progeny again results, even though some of the striped and white plants now have an Ij/Ij genotype. Thus the iojap trait, once established, is permanent.

The *iojap* phenomenon has been explained by two hypotheses. One hypothesis holds that the ij/ij genetic constitution could bring about or permit, frequent mutations in the chloroplast genome that

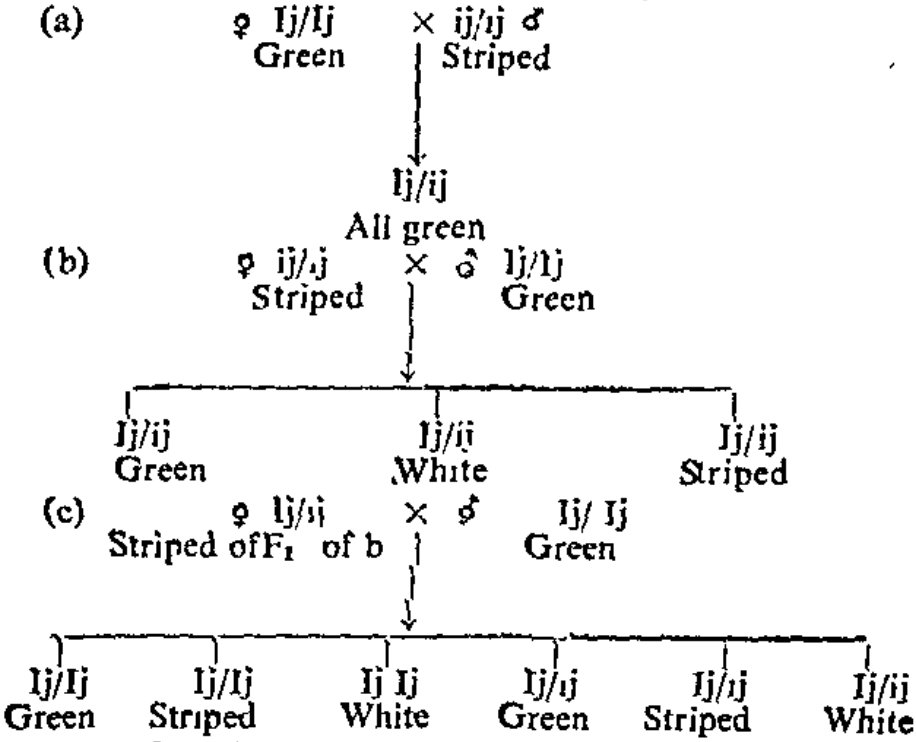


Fig. 15 4. (a) Cross between green (normal) and striped *iojap*) plants. (b) Reciprocal of cross (a) C. Cross of F_1 striped females (of cross b) to normal (green) males.

result in the production of lines of abnormal plastids. Another hypothesis suggests that certain cytoplasmic elements other then chloroplast mutations come into being or residence in *ij/ij* cells, are later inherited in the absence of this "susceptible" or" permissive" genotype, and bring about the bleaching of chloroplasts.

This type of maternal inheritance by plasmagenes of chloroplasts has been aslo studied in many other higher plants such as barley, *Oenothera* sp., rice etc.

(c) Extra-nuclear inheritance by mitochondria of yeast—Yeast, *Saccharomyces cerevisiae*, are single-celled ascomycetes fungi. In the life cycle, diploid and haploid adult alternates, the former reproducing by asexual meiospores called **ascospores**, the latter by **isogametes**. Certain strains of yeast are comparatively small-sized and slow growing, producing tiny colonies on agar and so known as "littles". The slow growth of "littles" has been found to be due to the loss of cells aerobic respiratory system **(Ephrussi, 1953)** and the utilization by the cell of less efficient fermentation process. When individuals from tiny colony (littles) are crossed with the individuals from a normal-sized colony, in F_1 "little" and normal offsprings are in the ratio of 1 : 1. Such tiny strains are evidently due to mutant nuclear genes and are called **segregational petites**. The nuclear genes of normal yeast cell for the formation of normal-sized colony can also be mutated artificially into segregational petites or littles by a certain chemical mutagens such as **acridine** dye or **euflavine**. These artificially mutated strains are called **vegetative petites**. When vegetative petite cells are crossed with the cells of normal colonies, the petite phenotype does not segregate normally. It shows that nuclear genes segregate in normal Mendelian fashion, but the "little" phenotype depends upon the kind of cytoplasm and mitochondria present.

(d) Extra-nuclear inheritance by mitochondria of poky strain of Neurospora : In fungi, *Neurospora crassa* a number of mutations of mitochondria are inherited via the female parent. The best studied of these is the **poky strain** of *N. crassa*, first isolated by Mitchell and Mitchell (1952) which grows very slowly and exhibits poorly differentiated mitochondria. The mitochondria are deficient in the membrane-bound cytochromes b and a_1 and a_3, essential proteins of the respiratory electron transport chain, and also possess greatly reduced numbers of the small ribosomal units. The **poky strain** thus may carry a mutant gene or genes for such mitochondrial deficiencies. However, the mitochondrial DNA of **poky strain** is found to be biochemically similar to mitochondrial DNA of wild type *N. crassa* (see **Goodenough and Levine, 1974**). **Poky mutation** is found to be a

suppresive mutation in which the mutant phenotype comes to take over or suppress the normal.

The poky trait is found to be inherited in a fashion of maternal inheritance. If the protoperitheceum derives from a poky hypha and the male conidium from a wild type hypha, the resulting eight ascospores are all poky, whereas in reciprocal crosses the ascospores are generally all wild type.

3. EXTRA-NUCLEAR INHERITANCE BY ENDOSYMBIONTS

Certain intra-cellular parasites such as bacteria and virus particles maintain symbiotic relationship with host cells. They are self-reproducing and look like the cytoplasmic inclusions. Sometimes they exhibit an infection like transmission with a hereditary continuity of their own. Generally such symbionts are coined by letters of the Greek alphabets (sigma, kappa, mμ, etc.). The various types of infective symbionts are as follows :

(i) Sigma virus in Drosophila—**L. Heritier** and **Teissier** found that a certain strain of *Drosophila melanogaster* shows a high degree of sensitivity to carbon dioxide, where as the wild type strain can be exposed for long periods to pure CO_2 without permanent damage, the sensitive strain quickly becomes uncoordinated in even brief exposure to low concentrations. This trait (extra-sensitivity) is transmitted primarily, but not exclusively, through the maternal parent. Tests have disclosed that CO_2 sensitivity is dependent upon an infectious DNA virus called sigma, found in the cytoplasm of CO_2 sensitives *Drosophila*. These infective particles are transmitted normally via the egg's larger amount of cytoplasm but occasionally through the sperms as well. Carbon dioxide sensitivity may even be induced in normal flies by injections of cell free extracts of sigma particles from CO_2 sensitive flies.

(ii) Spirochaetes and maternal sex ratio in Drosophila—Females of many *Drosophila* species can harbour a population of Spirochaetes known generally as SR. When SR Spirochaetes infect the eggs of the host and when these eggs are fertilized, virtually all XY zygotes are killed early in embryonic development and XX zygotes survive. Thus the Spirochaete can be considered as an endosymbiont of female but not of male *Drosophila*, and its presence in the female gives rise to the condition called **maternal sex ratio**, in which the progeny are exclusively or almost entirely female.

The SR Spirochaete is infectious, for when isolated from the haemolymph of female carriers and introduced into normal females

result in the production of lines of abnormal plastids. Another hypothesis suggests that certain cytoplasmic elements other then chloroplast mutations come into being or residence in ij/ij cells, are later inherited in the absence of this "susceptible" or" permissive" genotype, and bring about the bleaching of chloroplasts.

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the latter become carriers. Why the female genotype permits their retention, and conversely, why XY cells are sensitive to their presence is not yet known. **K. Oishi** and **D. Poulson** (1970) have found that the spirochaetes from a number of different species of *Drosophila* harbour DNA-containing viruses. Other species of *Drosophila* exhibit the maternal sex ratio condition but give no evidence of harbouring a Spirochaete. Thus, it becomes evident that the toxic principle affecting XY embryos may derive from a virus rather than from a Spirochaete.

(iii) Kappa particles—In 1938, **T. M. Sonneborn** reported that some races (known as "killers" or killer strain) of the common ciliate protozoan, *Paramecium aurelia* produce a poisonous substance called **paramecin** which is lethal to other individuals called "sensitives". The paramecin is water soluble, diffusible and depends for its production upon cytoplasmically located particles called **kappa**. Electron microscopic observations have shown that kappa particles are about 0.4μ long like the rickettsia (bacterium) or viruses. They are bounded by a membrane and possess a small amount of their own cytoplasm. They have diffusely distributed DNA and RNA. Kappa particles thus appear to be either parasites or more possibly symbionts, since they do not harm their hosts. A killer *Paramecium* may contain hundreds of kappa particles. The presence of kappa particles in the killer *Paramecium* is dependent for their maintenance and replication on the chromosomal dominant gene K. Paramecia with nuclear genotype kk are unable to harbour kappa particles.

When a *Paramecium* of killer strain having the genotype KK or (K^+) conjugates with the *Paramecium* of non-killer strain having the genotype kk, the exconjugants are all heterozygous for Kk genes. The kk genotype suggests that both exconjugant should be killers. But this is not the case. If conjugation is normal, *i.e*, lasts only for a short time, and no exchange of cytoplasm takes place between the two, both killers and non-killers (sensitive) are produced. However, rare or prolonged conjugation (*i.e*., lasting for long time) permits mixing of cytoplasm of both conjugants and results killers only. The killer trait is stable only in killer strain with KK genotype and is suitable in sensitive strain with kk genotype.

A killer *Paramecium* can be transformed into a sensitive one by exposure to high temperature, X-rays, nitrogen mustard and chloromycetin, showing the mutability of kappa (K) gene.

Recent studies of *kappa* bacterium have revealed a further fascinating complexity to the story. It turn out that when populations of kappas are examined about 20 per cent prove to possess a refrac-

tile, protein-containing body called an **R body** ; such kappas are known as "**brights.**" Brights also typically possess virus-like particles associated with R body. Finally, an analysis of the killing process indicates that the toxic particles liberated by killer strains are identical to bright kappas. This evidence suggests that the kappa endosymbionts are infected with a virus, one that retains a provirus state in the "nonbrights,' but dictates the synthesis of viral protein and an R body in brights. It is speculated that the virus-like elements act as the toxin in the killing response and the R body aids the penetration of the toxin at the time a sensitive paramecium is attacked.

(iv) **mμ particle**—Another type of killer trait known as **mate killer** has been reported in *Paramecium* by **R. W. Siegel** in *1952.* The mate killer trait is imparted by a cytoplasmic m^{μ} particle and a *Paramecium* with a mμ particle is called **mate killer** because when it conjugates with a *Paramecium* without any mμ particle is called **mate sensitive,** then it kills the latter. The mμ particles exist only in those cells whose micronucleus contains at least one dominant gene of either of two pairs of unlinked chromosomal genes (M_1 and M_2). The m^{μ} particles are composed of DNA, RNA and other substances and are symbionts.

Metagon in Paramecium—It is pointed out earlier that the maintenance of the m^{μ} symbiont in a *Paramecium* is dependent upon the genotype of the *Paramecium.* If a mate killer animal becomes m_1 m_1, m_2m_2, the m^{μ} particle will disappear. However, the m^{μ} symbiont does not disappear immediately. In fact, they maintain their normal number of particles for about seven generations. Then starting with the eighth generation, the particles suddenly and completely disappear from a small fraction of the cells. The fraction of cells, lacking the mμ symbionts, increases thereafter, until, only a very small fraction (7%) of cells containing them, (*i.e.*, mμ symbionts can be found by the fifteenth cell generation. This pattern of loss of cytoplasmic mμ particles of mate killers was investigated by **Gibson** and **Beale.** In 1962, they postulated that the maintenance of mμ particle in *Paramecium* was due to the presence of another cytoplasmic particle called metagon. The metagon was hypothesized to be a product of one or the other of the genes M_1 and M_2. Scientific investigations have confirmed the view about the occurrence of metagon and it is chemically found to be long-lived messenger RNA (informosome) which persists indefinitely through many cell generations and one metagon may be necessary for the maintenance of hundreds of m^{μ} particles.

(v) **Milk factor in mice**—**Bittner** found that females of certain lines of mice are highly susceptible to mammary cancer and this trait

was found to be maternally transmitted trait. Results of reciprocal crosses between these and animals of low-cancer-incidence strain depend on the characteristic of the female parent. When the young mice of a low-incidence strain are allowed to be nursed by susceptible foster mothers produces a high rate of cancer in them. Apparently this is a case of infective agent transmitted in the milk. This so called milk factor resembles in many respects with a virus and has been discovered to be transmissible also by saliva and semen. The presence of milk factor also depends on nuclear genes.

4. UNIPARENTAL INHERITANCE IN CHLAMYDOMONAS REINHARDI

R. Sager (1954, 1970) and **N. Gilham** (1968) have reported various cases of extra-chromosomal inheritance in green alga *Chlamydomonas reinhardi* which reproduces asexually as well as sexually. During sexual reproduction, two cells of opposite sexes fuse to form zygote. Sex is determined by a single chromosomal gene, two of the four daughter cells form female progeny, while the other two give rise to male progeny. Although both of the sexes contribute equally to the zygote, there is primarily maternal inheritance of certain traits that are due to cytoplasmic factors (DNA molecules of chloroplasts and mitochondria).

The uniting gametes of the single-celled alga, *Chlamydomonas reinhardi* are morphologically indistinguishable. One strain of the alga which is streptomycin resistant (Sr) and of the "plus" mating type (mt^+) is crossed to a cell of "negative" mating type (mt^-) which is streptomycin sensitive (SS). All the progenies are resistant but the nuclear genes for mating type segregate as expected : $\frac{1}{2}mt^+ ; \frac{1}{2}mt^-$. The reciprocal cross, $SSmt^+ \times Srmt^-$, again shows the expected segregation for mating type, but all progenies are sensitive. Repeated back crosses of $Srmt^+$ to $SSmt^-$ fail to show segmentation for resistant. It appears as though the plasmagenes of mt^- strain are lost in a zygote of mt^+. The mechanism which inactivates the plasmagenes of mt^- in the zygote is unknown.

B. EXTRA-NUCLEAR INHERITANCE IN PROKARYOTES

In prokaryotes (*i.e.*, bacteria) many cases of extra-nuclear inheritance have been reported. In them, the extra-nuclear inheritance is performed by (i) episomes and (ii) plasmids.

1. EPISOMES

During the discussion of extra-chromosomal inheritance by normal cell constituents (*e.g.*, chloroplast, mitochondria, etc.) and

infective symbiotic micro-organism which appear as cellular inclusions (*e g.*, sigma, kappa, mμ particles), we have observed that the genetic system and both the extra-chromosomal genetic systems are physically distinct. But, there have been reported certain genetic elements or particles which sometimes become the part of either of the two genetic systems. Such genetic elements which can participate in a cell either as extra-chromosomal or as chromosomal element and which altogether are non-essential to the life of the host cell are called episomes. The concept of episome was forwarded by Jacob and Wollman in 1958, to explain some of their findings on bacteriophages.

The best example of episome is provided by certain bacteriophages For example, when the bacterium, *Salmonella typhimurium*, is infected by P_{22} *bacteriophage*, then following two conditions may emerge—(i) the virus becomes virulent phage and its DNA replicate by taking precursor substances from host cytoplasm to form its own progeny. A host cell having a virulent phage soon bursts (lyses) and releases several hundred new virus particles or (ii) it may remain in the cell without killing the host (temperate state). Among the many descendents of the latter bacterium, from time to time, one of the daughter cell will break open and will yield a crop of new virus particles. This indicates that the bacterial host did not destroy the invading phage. In some strains when the descendents of the originally infected bacterium are exposed to ultra-violet light, X-rays or active chemicals such as nitrogen mustard or organic peroxides, the whole bacterial population will lyse within an hour, releasing tremendous number of viruses. This finding demonstrates that the invading phage particles were transmitted in some inactive form to all the cells of the bacterial culture. Cells that carry inactive viruses and are thus potentially subject to lysis are said to be lysogenic. The phages involved are called temperate, in their inactive form, they are called provirus or prophage and are said to be in an integrated state.

To explain the mechanism of transmission of temperate phage, it was postulated that the DNA or genome of temperate virus becomes attached to the genome of bacterial cell and then behaves as a chromosomal constituent of the host. In the prophage state (inactive state), the genome of temperate phase replicates along with the bacterial chromosome and is transmitted to all daughter bacterial cells. In some of the daughter cells, it enters the extra-chromosomal state, by detaching from the host's genome and becomes virulent. In the extra-chromosomal phase, the genetic material of virus has two functions : replication and transcription, leading to the production of the

protein material of the virus. In the chromosomal phase the transcription is not exercised. In fact, in its integrated state, the virus confers an immunity on the host to new infection (super infection) by identical or closely related phage.

TEMPERATE PHAGE AND ONCOGENESIS

The temperate phages have helped in the understanding of certain kinds of human cancers. oncogene (Gr. *oncos*-tumor) theory of cancer suggests that the integrated copies of virus chromosomes are present within the chromosomes of eukaryotes susceptible to cancer. These copies are transmitted from generation to generation and from cell to cell in the usual manner. Their presence may be unremarkable, or they may even bring to the organism certain genes that are beneficial or even essential In either case, they also bring to the cell genes that are potentially disastrous. Some stimulus (aging, a chemical carcinogen, an environmental insult) usually affecting a single somatic or vegetative cell, can induce the viral chromosome to abandon its repressed state. Release permits additional viral genes to be transcribed, and their gene products bring about changes in the cell's metabolism that ultimately encourage unregulated or cancerous growth. In certain cases the cell transformation may be accompanied by the synthesis of mature virus particles.

2. PLASMIDS

Plasmids are extra genetic elements found in bacterial cells A plasmid differs from most temperate bacteriophages in that it is capable of enjoying an autonomous, self-replicating status in the host cell without lowering host-cell viability. Most temperate phages, on the other hand, will kill the host if allowed to replicate, and their genetic information must therefore be maintained in the repressed prophage state if viability is to be preserved. A prominent exception to this generalization is given by phage P_1, whose prophage replicates free of the bacterial chromosome.

Most plasmids can be transmitted from cell to cell by infection without bringing about the death of the host. Certain plasmids can also become integrated into the host chromosome and thereby lose their independent status. The F element of *E coli* is a plasmid which show above discussed characteristics of plasmids.

KINDS OF PLASMIDS

In bacteria three general and often overlapping classes of plasmids have been discovered. They are following :

(a) Transfer agents—The first class of bacterial plasmids is called **sex factors, fertility agents or transfer agents.** *The sex factor* or **F element or factor or agent** plays a key role in the sexuality

of bacteria. *E. coli* cells that carry an F element are known as F^+ and such cells occur rarely in a natural population. In F^+ cells perhaps 2 per cent of the cell's DNA is found in the F element, which takes the form of a covalently closed circular molecule with a molecular weight of approximately 35×10^6 daltons. F element is found to contain 11 genes, eight of which control the elaboration of F pili —long appendages that extend from the surface of F^+ cells. Cell that possess F pili are sensitive to infection, by such single-stranded RNA phages as QB and R17 ; F^- cells which lack an F element and pili and are insensitive to these RNA phages.

A F^+ cell usually does not conjugates with a F^+ cell, but it readily establishes contact, probably via an F pilus, with a F^- cell. Once contact is made the pilus is believed to become modified and serves as a protoplasmic channel between the two cells and is referred to as a **conjugation tube.** Under ordinary circumstances the only genetic element transferred through the conjugation tube is the F element itself. In a mixture of F^+ and F^- cells each F^+ donor will pass a copy of F on to an F^- recipient while retaining at least one copy for itself, so that eventually virtually every cell in the population becomes an F^+ cell.

Inside a F^- cell, the F element first inserts itself into the bacterial chromosomes. In the insertion process, the circular F element breaks at a particular point and becomes a linear segment of the bacterial chromosome. An F^+ cell that carries such an integrated F element is known as an **Hfr cell** The integrated F element is ordinarily replicated passively along with the bacterial chromosome, and in this way is transmitted from one Hfr generation to the next. When a Hfr cell conjugates with a F^- cell, the latter inherits an incomplete copy of the F element and remains F^-. If transfer is complete, the recipient will inherit a complete F element and the Hfr property ; its descendants can act as Hfr cells. Those F^- cells that have received a part of the donor chromosome (of Hfr cells) are called **partial zygotes** or **hemizygotes,** since they possess copies of one or more genes that derive from two different parents.

Thus, F element acts a infectious factor which transform F^- bacterial cells into F^+ or Hfr cells. Because of this property it is called transfer agent.

(b) Resistance and colicins gene : Plasmids of second class are usually defined by the fact that they do not carry information for infectious transfer. Two types of plasmids fall into this class. The first carry genes that confer resistance to antibiotics. The second carry genes that dictate the synthesis of colicins. Colicins being hetero-

geneous array of proteins that are not toxic to the cell that elaborates them but are toxic to cells of related bacterial strains.

(c) R agents and colicinogenic agents : The third class of plasmid is a composite of first two : transfer agents become covalently associated with genetic elements that carry resistance or colicinogeny -determining genes. Thus one speaks of resistance agents or R agents as possessing a **resistance transfer factor (RTF)** unit and a resistance determining unit. For one well studied R agent, a circular *DNA* molecule 33μ in diameter, its RTF unit is about 26 to 31μ long and is resistance-determining unit about 4 to 6μ. Colicinogenic agents that possess transfer units dissociable from genes involved in colicin production are also known. Certain colicinogenic agents, such as *col v* and *col B*, infact possess transfer units that are indistinguishable from F elements.

Though, the evolutionary origin of bacterial plasmids is not known but present speculation holds that the transfer elements are probably very highly modified forms of bacteriophages and that the resistance genes, whose ancestry is unknown, came to be conserved as a package because of the enormous advantages they confer on their hosts in our modern environment replete with antibiotics. The association between transfer genes and resistance genes to form infectious R agents is of even greater advantage to the survival of the host and its progeny. Further, plasmids carrying resistance genes or colicinogenic genes may themselves have episomes, the transfer factor, that enter or leave by a reciprocal recombinational mechanism.

Medical significance of R agents : The infectious resistance (R) are of such importance in public health that they merit particular attention. R agents can be transmitted not only from cell to cell within a species but also across species lines. Thus, for example, *E. coli*, *Proteus*, and other non-pathogenic members of the intestinal flora often harbour R agents that have permitted the host cell lines to survive high doses of antibiotics ; these R agents can subsequently be transferred to infecting pathogenic bacteria such as *Shigella* (dysentery causing bacteria) and *Salmonella* so that they too become resistant to the same spectrum of antibiotics. Actually, the large-scale and indiscriminate application of antibiotics has created a pool of drug-resistant enterobacteria that can transmit their resistance genes to infecting pathogens. And the drug-resistant pathogens may create serious epidemics in such vulnerable places as hospitals or crowding living area.

INHERITANCE OF PREFORMED STRUCTURES

Besides the heredity of traits related with endosymbionts and cytoplasmic organelles, there are hereditary traits whose origin is not easily ascribed to nuclear or extranuclear DNA. The best studied of these characteristics pertain to the cortex of ciliated protozoa such as *Paramecium*. The mouth and the contractile vacuole are two prominent features of the cortex of *Paramecium*, and T. Sonneborn and his co-workers (1963, 65, 70) have shown that these existing, or preformed structures can be transmitted from one cell generation to the next, independent of the transmission of nuclear genes and cytoplasmic genetic factors.

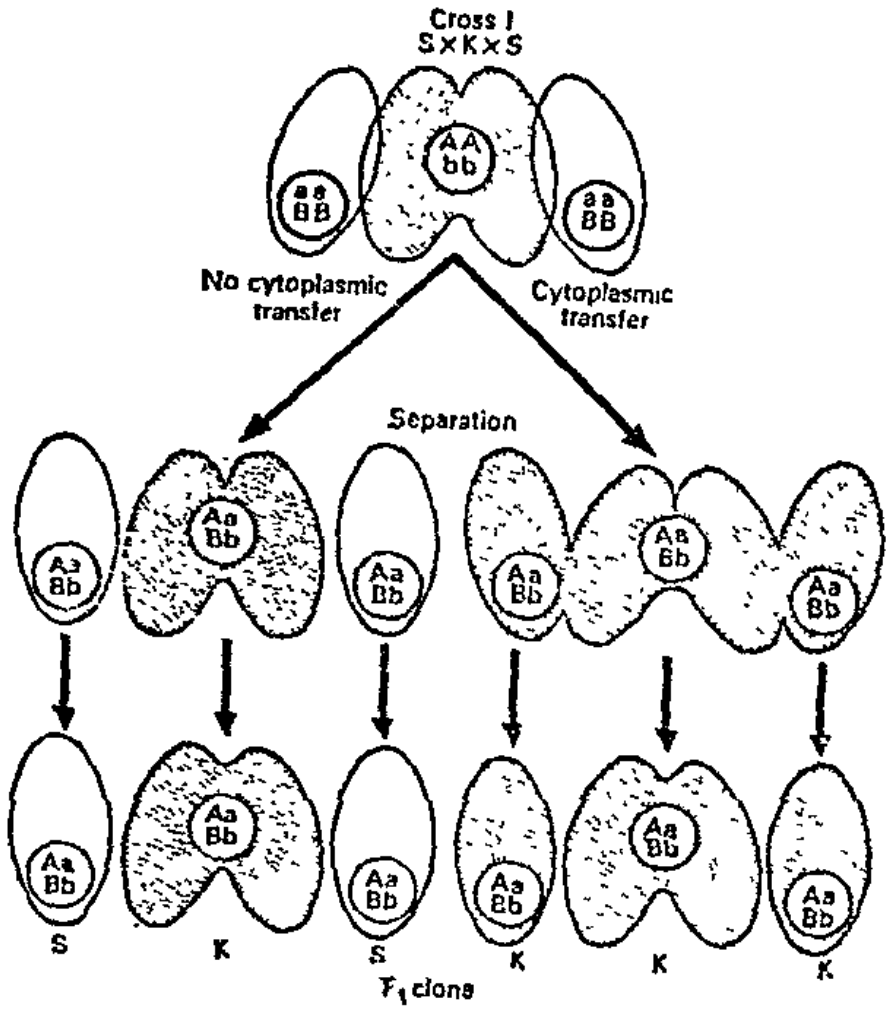


Fig. 15·4. Inheritance of the doublet phenotype in *Paramecium aurelia* cross I (S×K×S): killer (K) doublet sensitive (S) singlets. A and a, B and b represent two pairs of allelic nuclear genes. S=sensitive, K=killer, dots represent kappa in cytoplasm.

During normal sexual reproduction in *Paramecium* two cells conjugate and then separate after they have exchanged nuclei. At certain rare occasion, however, two conjngants fase together and

form a double animal (a doublet) with two sets of cortical structures. When doublets reproduce asexually by fission they give rise to doublets, showing that the double nature of the cortical structures has a genetic basis. When doublets are mated with normal (singlet) animal, the progeny of the doublet exconjugant are doublets and the progeny of the singlet exconjugauts are singlets. Also when the progeny go through autogamy, they maintain their doublet or their singlet properties. In these crosses it is found that the nuclear genes (A, B, a, b, etc.) of doublets and singlets inherited in normal Mendelian fashion.

The mode of inheritance of the duplicated cortical structures of *Paramecium*, therefore, appears to be independent of the mode of inheritance of nuclear genes. It is also independent of cytoplasmic hereditary factors. To study this fact, endosymbionts such as *kappa* can be used to mark the cytoplasm of either doublet or singlet animals, and matings can be made between killer doublets that have

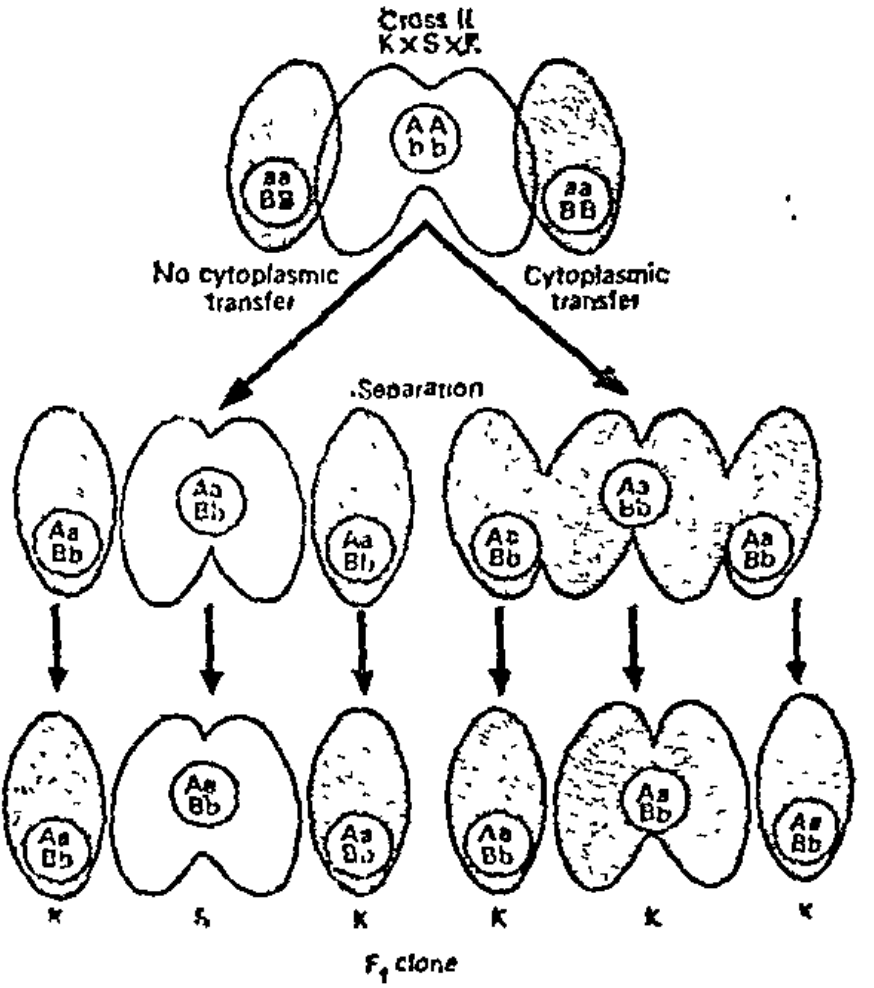


Fig. 15·5. Inheritance of the doublet phenotype in *Paramecium aurelia*. cross II (K×S×K) : sensitive (S) doublet×killer (K) *singlets*. A and a, B and b represent two pairs of allelic nuclear genes. S—sensitive, K—killer, dots represent kappa in cytoplasm.

kappa and sensitive singlet animals that lack *kappa*. The reciprocal cross can also be made. When conditions are correct for cytoplasmic exchange, its occurrence is marked by the transfers of *kappa* bacteira. The results of these matings show that the transmission of *kappa*. (as well as that of the killer and sensitive phenotypes, follows the expected pattern, yet the doublet and singlet exconjugants reproduce true to type among their progeny. These in turn reproduce true to type following autoamy. Such observations reveals that the inheritence of cortical structures is no more affected by exchange of cytoplasm than it is by nuclear genotype.

Further, the transplantation studies reveal the fact that cortical structures are autonomous. They are maintained by cell division, they appear to be essential for their own reproduction and their inheritance is not under the control of nuclear genes or cytoplasmic genetic factors. Sonneborn has suggested that different parts of the cortex might serve as sites for the "specific absorption and orientation of molecules derived from the milieu and genetic action". In addition, pre existing cortical structures could act by "determining where some gene products go in the cell, how these combine and orient, and what they do."

UTILITY OF EXTRA-NUCLEAR GENETIC SYSTEMS

The advantages of many self-reproducing cellular organelles in eukaryotes are (i) It prevents total loss of organelles due to a single mutation in nuclear genes. (ii) It provides a reservoir of cytoplasmic mutations which could become useful under adverse environmental conditions.

MOLECULAR BIOLOGY

CHAPTER 1

INTRODUCTION

The molecular biology is a scientific discipline which attempts to explain the morphology, anatomy, physiology, heredity, embryonic development and evolution of organisms in the terms of chemical molecules. More specially, it picturizes the molecular structure of genes and their ultimate functions in the determination of phenotype of the organisms. The roots of this discipline were established in 1953 when an Englishman, **Francis Crick** and young American, **James Watson** working at Medical Research Council Unit, Cavendish Laboratory, Cambridge, proposed a double helical model for the structure of DNA (deoxyribonucleic acid) molecule which was well known as the chemical bearer of genetic informations of certain micro-organisms (bacteria, bacteriophages, etc.) due to pioneer discoveries made by **Grifith** (1928) ; **Avery, Macleod** and **McCarthy** (1944) and **Hershey** and **Chase** (1952). This discovery was followed by a thorough search of occurrence of DNA as the genetic material in other micro-organisms, plants and animals and also by investigations of the molecular and atomic nature of different reactions of living cells From all these studies has emerged the realization that the basic chemical organization and the metabolic processes of all living things are remarkably similar despite their morphological diversity and that the physical and chemical principles governing living systems are similar to those governing non-living systems.

The present understanding of molecular biology is that in most organisms the phenotype or the body structure and function ultimately depend for their determination on the structural and functional (*i.e.*, enzymatic) proteins or po[illegible] tides is specified, directed an[illegible] tion of nitrogen bases in DNA molecule and are copied down by the process of transcripton. During transcription stage copies (that is, transcripts) of an individual gene or genes are synthesized. These copies are molecules of RNA that include such familiar classes as ribosomal RNA,

messenger RNA and transfer RNA. The biochemical interplay of these RNA copies which leads to the synthesis of a polypeptide chain, is called **translation**, meaning, literally, that the genetic message encoded in a messenger RNA molecule is translated into the linear sequence of amino acids in a polypeptide. The polypeptides in its turn determines the phenotype of the organism.

HISTORICAL BACKGROUND

The molecular biology is a very young biological discipline and has a very short history. Certain notable accomplishments of molecular biologists can be summarized as follows :

1928 F. Grifth discovered the phenomenon of transformation in bacteria.

1934 [illegible] e com-

1941 [illegible] study on the biochemical genetics of *Neurospora.*

1944 O. T. Avery, C. M. MacLeod and M. McCarthy recognized the DNA nature of transforming principle of pneumococcus bacteria. The fact suggested that it is DNA and not protein which is the heredity chemical.

1948 A. Boivin, R. Vendrely and C. Vendrely showed that in the different cells of an organism the quantity of DNA for each haploid set of chromosome is constant.

1950 E. Chargaff demonstrated that in DNA the numbers of adenine [illegible] so are the numbers

1952 [illegible] that only the DNA [illegible] *Escherichia*

1953 [illegible] or DNA [illegible] gether by

1956 [illegible] A is the genetic material of tobacco mosaic virus (TMV).

1957 H. Fraenkel-Contrat and B. Singer separated RNA from the protein of TMV viruses, produced hybrid RNA viruses and confirmed the view that RNA is the genetic material of some viruses.

1958 G. Beadle and E. Tatum received Nobel prize for their contribution in biochemical genetics of fungus.

1958 J. Laderberg got Nobel prize for the discovery of bacterial recombination.

1959 R. L. Sinsheimer isolated single-stranded DNA from a small virus ϕ X—174 which attacks *Escherichia coli*.

1959 S. Ochoa ; A. Kornberg received Nobel prize for artificial synthesis of nucleic acids.

1961 M. W. Nirenberg and J. H. Matthaei cracked the messenger RNA code.

F. H. C. Crick and his colleagues showed that the genetic language is made up of three-letter words (*i.e.*, triplet codons).

1961 F. Jacob and J. Monod put forward the operon concept.

1962 J. Watson and F. Crick ; M. Wilkens got Nobel prize for the discovery of molecular nature of DNA.

1965 F. Jacob., A. Lwoff, and J. Monod received Nobel prize for the discovery of protein synthesis mechanism in virus.

1968 R. W. Holley ; H. G. Khorana and M. W. Nirenberg got Nobel prize for deciphering the genetic code.

1969 M. Delbruck ; H. Hershey ; S. Luria received Nobel prize for the investigation of sexual reproduction and genetics of bacteriophages.

1970 Knippers ; Kornberg and Gefter ; Moses and Richardson isolated DNA-polymerase-II enzyme.

1972 R. Porter ; G. M. Edelman received Nobel prize (physiology and medicine) for the discovery of chemical structure of antibodies.

C. B. Anfinsen ; S. Moore and W. H. Stein got Nobel prize (chemistry) for the discovery of chemical structure and activity of the enzyme ribonuclease.

1974 A. Claude, G. E. Palade and C. de Duve were awarded Nobel prize for their innovations that started the modern science of cell biology nearly 30 years ago and for their major contributions to the understanding of the inner working of living cell.

1975 R. Dulbecco, H. M. Temin and D. Baltimore were awarded Nobel prize for their discoveries concerning the interaction between tumour viruses and the genetic material of the cell.

MATERIAL AND METHODS IN MOLECULAR BIOLOGY

Different molecular biologists have made a intensive use of a variety of the micro-organisms such as bacteriophages and other viruses; *Escherichia coli* and other bacteria ; unicellular green algae, yeast, *Neurospora* and other fungi ; protozoans, etc., in their investigations of varied nature. However, many significant discoveries in molecular biology have been made by using the bacteriophages, chromosomes of *Drosophila*, etc.

animals. To understand different biochemical events of prokaryotic and eukaryotic cells at molecular level, a wide array of bio-physico-chemical techniques such as electron m[illegible] colorimetry, spectrophotometry, ch[illegible] X-ray crystallography, electrophore[illegible] biology.

BASIC REQUIREMENTS TO BE MET BY GENETIC MATERIAL

According to the molecular biologists certain requirements must met by any molecule if it is to be qualified as the substance that transmits genetic information from one generation to next. These requirements extend directly from what is known about the continuity of species and the process of evolutionary change.

1. The genetic material must contain biologically useful information that is maintained in a stable form.

2. T[illegible] faithfully [illegible]

3. [illegible] other bio[illegible] be produced and maintained. Implicit in this requirement is that some mechanism be [illegible] mation contained in t[illegible] A narrow, but impor[illegible] cule that can generate only its own kind and a molecule that can also generate new kinds of molecules. For example, a salt crystal can "seed" a salt solution so that new salt crystals are formed, but this is the extent of its influence over its surroundings.

4. Genetic material must be capable of variation. Two sources of change have been recognized in present day genetic systems: mutation and recombination. A mutation changes the nature of information [illegible] offspring, and thus it represents a [illegible] about variation. Recombination is [illegible] g variation. It occurs during the [illegible] ocess, and it involves the precise [illegible] new combinations [illegible] the offspring.

[illegible] can study in forthcoming chapters that how the genetic material is discovered? What is the physical and chemical properties of it? How it carries genetic information from one generation to next? How a genetic material determines the phenotype of an organism? and many problems of similar nature.

CHAPTER 2

IDENTIFICATION OF THE GENETIC MATERIALS

When it became evident that the chromosomes were the organs of heredity, because (i) they formed the only link between two generations ; (ii) they carried linearly arranged genes ; (iii) they occurred in every organism in specific number and had specific morphology for a particular species ; and (iv) any variation in their number or morphology affected the phenotype of the species. Then, various attempts were made by early molecular geneticists to identify the physical and chemical nature of genes. But, the genes were found so minute structures that their physical identity remained almost impossible. [illegible] molecular geneticists have assigned the informational roles of genes to the chromosomal proteins, because, they found nucleic acids too simple to carry genetic informations. The controversy about the assignmet of genetic role either to chromosomal proteins or to chromosomal DNA, existed upto 1949, when A. Mirsky and H. Ris had found [illegible]

DNA or [illegible] ganisms has been developec :

A. DIRECT EVIDENCES FOR DNA AS THE GENETIC MATERIAL

The most conclusive evidences in support of DNA as the genetic material came from following three avenues of approach on microorganisms ; tranformation of bacteria, mode of infection of bacteriophages and conjugation in bacteria.

1. BACTERIAL TRANSFORMATION AND GRIFFITH EFFECT

In 1928, Frederick Griffith encountered a phenomenon now known as genetic transformation. Colonies of virulent strain (pathogenic) of pneumonia causing bacterium, *Diplococcus pneumoniae* grown on nutrient agar, have a smooth (S) glistering appearance

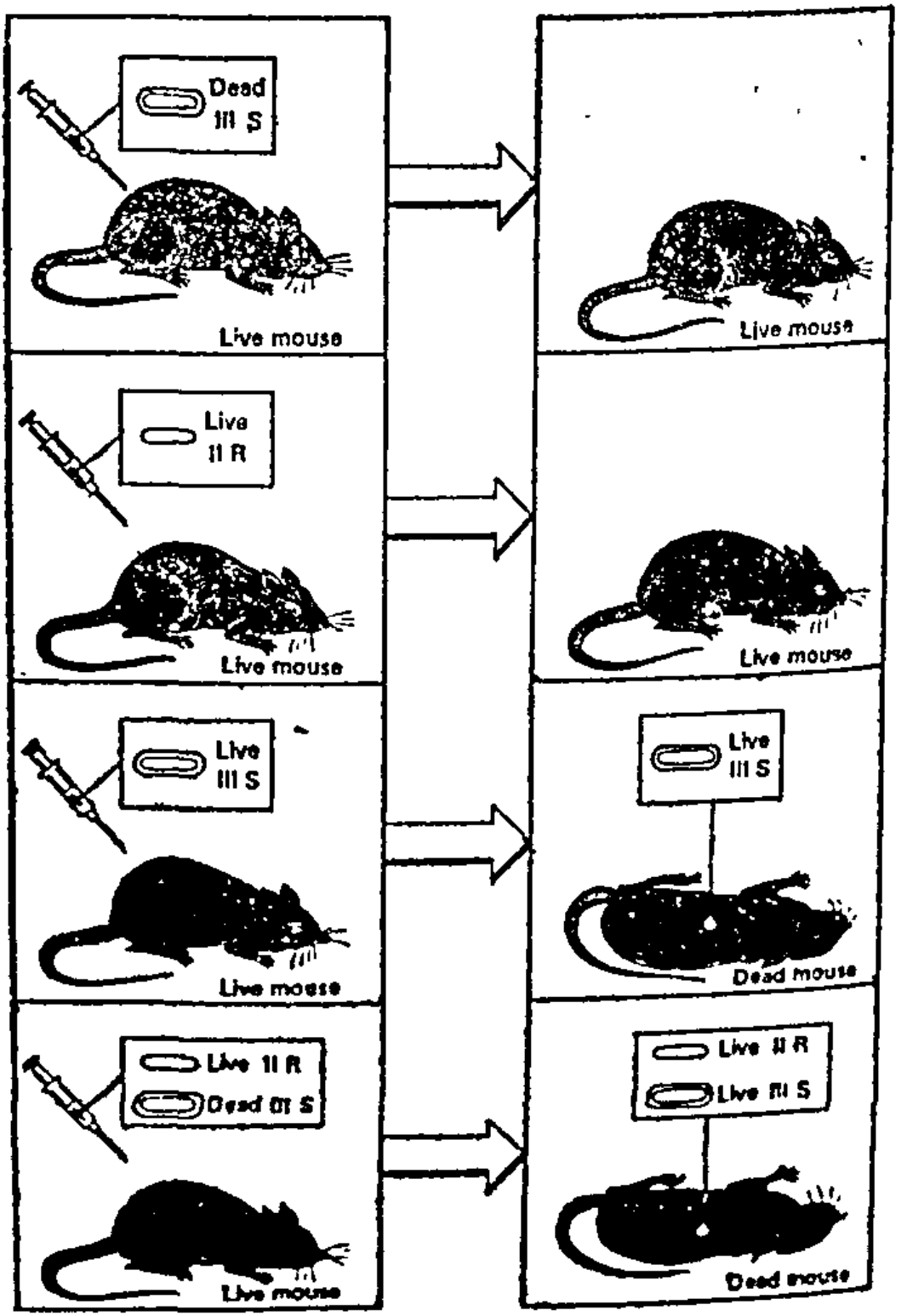


Fig. 2·1. Griffith experiment that demonstrated the principle of transformation.

owing to the presence of a type specific, polysaccharide (a polymer of glucose and glucuronic acid) capsule. The avirulent (non-pathogenic) strains, on the other hand, lack this capsule and they produce dull, rough (R) colonies. Smooth (S) and rough (R) characters are directly related to the presence or absence of the capsule and this trait is known to be genetically determined. Both S and R forms occur in several types and are designated as S–I, S–II, S–III, etc., and R–I, R–II, R–III, etc., respectively. All these subtypes of S and R bacteria differ with each other in the type of antigens, they produce. The kind of antigen produced is likewise genetically determined. Smooth (S) forms sometimes mutate to rough (R) forms, but this change have not been found reversible.

In the course of his work, Griffith injected laboratory mice with live R-II pneumococci ; the mice suffered no illness because R-II pneumococci was avirulent. But when the mice were injected with virulent S–III pneumococci the mice suffered with pneumonia and died. However, when he injected the heat killed S–III bacteria in mice, they did not suffered by pneumonia. But when the mice were injected with the mixture of living avirulent R–II and heat killed S–III virulent, the unexpected symptoms of pneumonia appeared and high mortality was resulted in them. By postmortoming the dead mice, it was found that their heart blood had both R–II and S–III pneumococci. From these results, Griffith concluded that the presence of the heat-killed S–III bacteria must have caused a transformation of the living R-II bacteria, so as to restore to them the capacity formation they had earlier lost by gene mutation. This was called **"Griffith effect"** or more popularly as **"bacterial transformation"**.

Identification of the "transforming principle or substance"—Griffith could not understand the cause of bacterial transformation and that is first of all identified by **Avery, MacLeod and McCarthy** (1944). They tested a fraction of heat killed S–III bacteria for the transforming ability. They could remove proteins, lipids, polysaccharids and ribonucleic acids from the S–III extract by a variety of chemical and enzymatic methods without seriously diminishing its power to transfer R–II mutants into the S–III wild types. They ultimately concluded that because a cell free and highly purified DNA extract of S–III bacteria could bring about transformation of R–II bacteria into S–III, therefore, DNA is the genetic material of pneumococci.

Later on, such a transforming substance, the DNA, was found in a variety of bacteria (*e.g., Hemophilus Influenzae, Bacillus subtilus. Shigella paradysenteriae.* etc.) and several other organisms.

2. MODE OF INFECTION OF BACTERIOPHAGES AND HERSHEY-CHASE EXPERIMENTS

By using radioactive tracers, A. Hershey and M. Chase (1952) provided further direct proof that DNA is the genetic material in cer-

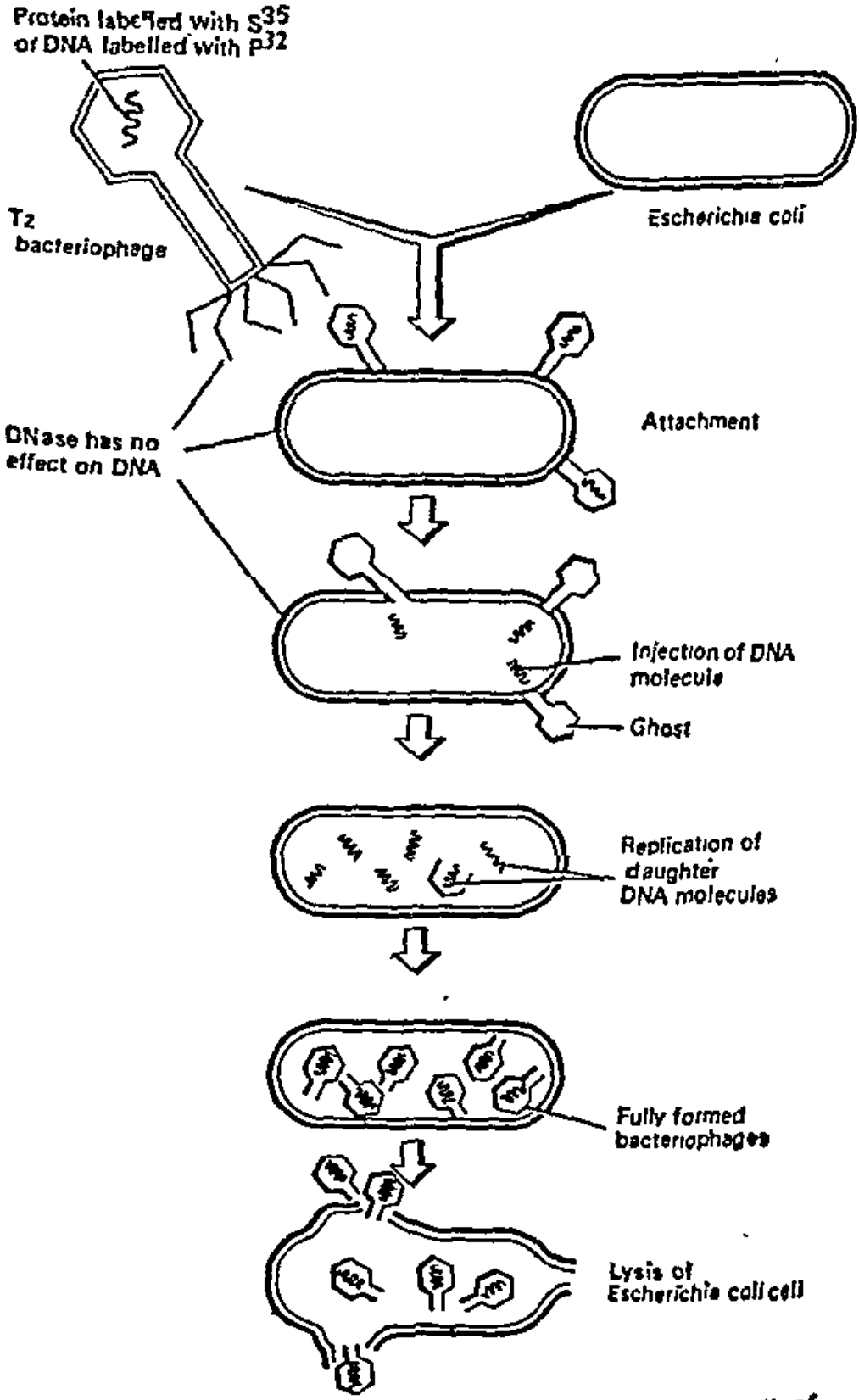


Fig. 2-2. Diagram of life cycle of a virulent phage showing details of Hershey-Chase experiment.

tain bacterial viruses (bacteriophages). These investigators were studying the bacteriophages that attack the bacterium, *Escherichia coli*. They prepared a chemically defined culture medium (for *E. coli*) containing phosphoric acid and sulphuric acid as the only sources of phosphorus and sulphur Known quantities of radioactive isotopes of phosphorus (p^{32}) and sulphur (S^{35}) were added to the culture medium. *E. coli* grown on such a culture medium incorporated the P^{35} and S^{35} into their chemical constituents. When T_2 bacteriophage particles infected the radioactive bacteria, they incorporated DNA. Phage-capsid proteins did not contain appreciable amounts of phosphorus and only the DNA was labeled with p^{32}. Similarly, the protein envelope around the T_2 phage was selectively labeled with S^{35}. DNA contains virtually no sulphur and was not labeled with S^{35}. By this method it was possible to differentially label the phage DNA and proteins of phage-capsid.

After phage growth, the phage particles were separted from the host cells by centrifugation. The radioactive phages were next introduced to non-radioactive bacterial cultures where they attacked the bacteria. Subsequently, the viruses were separated from the host cells by agitation and the content of P^{32} and S^{35} in the host (*E. coli*) and parasite (bacteriophage) was determined. The phosphorus label was found to be associated with the bacterial cells and the sulphur label was in the proteins coats (capsids) left in the medium. This indicated that the DNA had penetrated the cells but that the protein coat or capsid of the phage was left outside the wall of the bacterium. The *labeled DNA was found to reproduced in the host cells. These experi*ments, thus, clearly demonstrated the genetic informations of these bacteriophages resided in their DNA molecules and not in the protein molecules of their capsids.

3. BACTERIAL CONJUGATION

Another conclusive evidence for DNA as the genetic material came from the phenomenon of conjugation of bacteria. **Laderberg** and Tatum (1946) found that when a F^+ ('male') *E. coli* cell conjugated with a F^-('female') *E. coli* cell, a unidirectional tranfer of F^+ factor of 'male' cell to F^- or 'female cell took place, so that, the latter was converted into a F^+ or 'male' strain. The F^+ factor was found to be a fragment of DNA molecule which occurred in the cytoplasm of bacterial cell.

B.- INDIRECT EVIDENCES FOR DNA AS THE GENETIC MATERIAL

The indirect evidences in favour of DNA as the genetic material came from higher organisms which are not as easy to manipulate

as bacteria and viruses. The fact that DNA is the genetic material of higher organisms has been supported by following facts :

1. The Feulgen techniques have shown that DNA entirely remains restricted to the chromosomes and it forms one of the major component of chromosomes.

2. Various quantitative measurements of amount of DNA in different cells have shown that there is a correlation between the amount of DNA and the number of chromosome sets (ploidy). Diploid cells contain twice as much DNA as do haploid cells of the same species as shown in following table :

Table : DNA content and ploidy (Pollister *et. al.*, 1951)

Cells	Mean DNA Feulgen content (Picograms)	Presumed chromosome set (Ploidy)
Spermatids	1·68	Haploid (2n)
Liver	3·16	Diploid (2n)
Liver	6·30	Tetraploid (4n)
Liver	12·80	Octoploid (8n)

3. The diploid amount of DNA is constant within the species but varies from one species to another, as shown in following table :

Table : DNA contents in picograms (gram $\times 10^{-12}$) of various cells.

S. No.	Organism	Kidney	Liver	Erythrocytes	Sperm
1.	Chicken	2·4	2·5	2·5	1·3
2.	Bovine	6·4	6·4	—	3·3
3.	Carp	—	3·0	3·3	1·6
4.	Human	5·6	5·6	—	2·5

4. In 1950 Swift found the amount of DNA per resting nucleus (interphase nucleus) in mitotically active tissues of animals ranged from twice (2 n) the value found in the gametes (1 n) to four times (4 n) this value. He concluded that DNA synthesis occurred during the interphase.

This parallelism of behaviour in DNA and chromosomes clearly indicates that DNA is the genetic material of higher animals.

C. EVIDENCES FOR RNA AS THE GENETIC MATERIAL OF SOME VIRUSES

The demonstration that RNA is the genetic material in RNA-containing viruses came in 1956, when A. Gierer and G. Schramm showed that tobacco plants could be inoculated with purified RNA from the tobacco mosaic virus (TMV) and TMV-like lesions could later be identified on the tobacco leaves. A different approach was taken by H. Fraenkel Conrat and B. Singer in experiments published in 1957. They first separated the RNA from the protein of TMV viruses, much as Hershey and Chase had done with the DNA and the protein of T_2 bacteriophage. They then developed techniques for forming "reconstituted" viruses containing the protein from one mutant strain of TMV and the RNA from another, or vice-versa. Such hybrid viruses were allowed to infect tobacco leaves, and the progeny were examined. In all cases the progeny were the parental RNA type and not the parental protein type.

In a much improved technique N. Pace and S. Spiegelman in 1966 purified RNA from two different mutant strains of the RNA Phage QB which had quite distinct base compositions. The isolated RNAs were then incubated separately in the presence of an *E. coli* cell extract containing an enzyme capable of RNA replication. The new RNA synthesized was in each case identical in base composition to the particular phage RNA presented to the *in vitro* system, thus indicating that the phage RNA can serve as a template for its self-replication.

CHAPTER 3

CHEMICAL NATURE AND REPLICATION OF GENETIC MATERIALS (i.e., DNA AND RNA)

DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), the principal genetic materials of living organisms, are chemically called nucleic acids and are complex molecules larger than most proteins and contain carbon, oxygen, hydrogen, nitrogen and phosphorus. The nucleic acids were first isolated by F. Miescher in 1868 from the nuclei of pus cells and gained their name from the fact that they are acidic and were first identified in nuclei. Because, DNA is the only genetic material of most living organisms, so deserves priorty in discussion.

DEOXYRIBONUCLEIC ACID OR DNA

Highly purified DNA extracted from a wide variety of plants, animals, bacteria and viruses, has been found to be complex macromolecular or polymeric chemical compound which contains four kinds of smaller building blocks (monomers) called deoxyribotids or deoxyribonucleotides. Each deoxyribonucleotide is made up of three moieties : a phosphosic acid molecule (biologically called phosphate and discovered by Levene in 1910's); a pentose sugar called 2 deoxyribose (discovered by Levene in 1910's); and pyrimidine and purine nitrogenous bases (discovered by Fischer in 1880's). Four major kinds

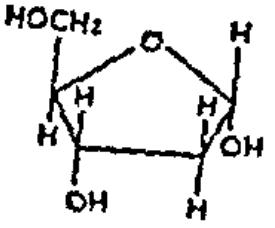


Fig. 3 1. Chemical formula of deoxyribose.

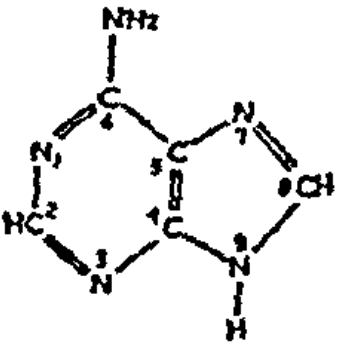


Fig. 3·2. Chemical formula of adenine (6–aminopurine, mw=135 13 daltons)

of nitrogenous bases have been found in four kinds of deoxyribonucleotides of DNA : two are heterocyclic and two-ringed purines, adenine (A) and guanine (G), and two are one ringed pyrimidines, cytosine (C) and thymine (T)

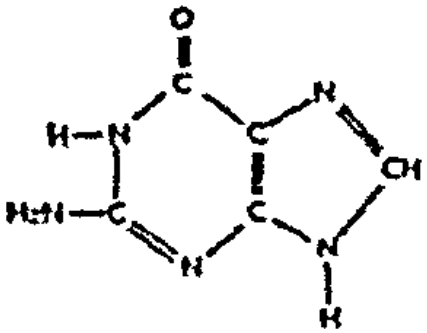


Fig. 3·3. Chemical formula of guanine (2-amino—6-hydroxypurine, mw—151.13 daltons).

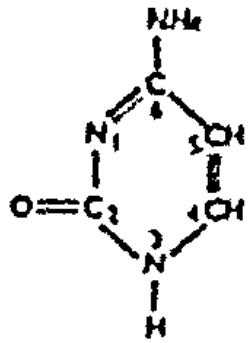


Fig. 3·4. Chemical formula of cytosine (2-hydroxy-6-aminopyrimidine, mw—111·10 daltons)

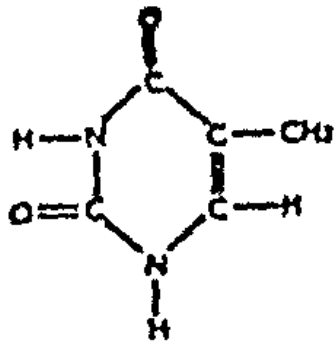


Fig. 3·5. Chemical formula of thymine (2,6-dihydroxy-5-methylpyrimidine, mw—126·12 daltons).

That part of each nucleotide which contains a nitrogenous base and deoxyribose is called deoxyribonucleoside. The four kinds *of deoxyribonucleosides and deoxyribonucleotides can be tabulated as follows :*

Table : Four nitrogen bases, *nucleosides* and *nucleotides* of DNA molecule.

Nitrogen base	Base + deoxyribose = deoxyribonucleoside.	Deoxyribonucleoside + Phosphoric acid = Deoxyribonucleotide.	Abbreviation for nucleotide.
1. Adenine (A)	Deoxyadenosine	Deoxyadenylic acid (Deoxyadenosine monophosphate)	dAMP
2. Guanine (G)	Deoxyguanosine	Deoxyguanylic acid (Deoxyguanosine monophosphate)	dGMP
3. Cytosine (C)	Deoxycytidine	Deoxycytidylic acid (Deoxycytidine monophosphate)	dCMP
4. Thymine (T)	Thymidine	Thymidylic acid (Thymidine monophosphate)	TM

The four deoxyribonucleotides besides occurring in DNA molecule, occur also in nucleoplasm and cytoplasm, but in

triphosphate forms such as deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP) and thymidine triphosphate (TTP). The significance of occurrence of deoxy ribonucleotides in triphosphate forms lies in the fact that during DNA replication, the DNA polymerase enzyme can act only on triphosphates of deoxyribonucleotides.

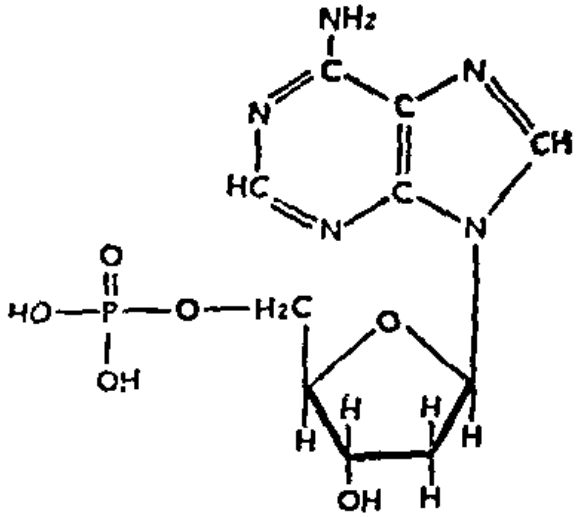


Fig. 3·6. Chemical formula of deoxycytidylic acid.

MOLAR RATIOS OF NITROGEN BASES IN DNA MOLECULE

When many samples of DNA were isolated, purified and analysed by various techniques, such as paper chromatography, etc., it was found by **Hotchkiss** and **Chargaff** in 1948 that contrary to **Levene's** (1920's) tetranucleotide theory which considered DNA as a monotonous polymer having four DNA bases in approximately equal molar proportions, the four nucleotide bases are not necessarily present in DNA in exactly equal proportions. Thus, Chargaff reported that the DNA extracted from calf-thymus nuclei contains the four bases in following molar proportions : 28% adenine, 24% guanine, 20% cytosine and 28% thymine. When he analyzed the DNA samples of varied animals, he found that the exact base composition of DNA differs according to its biological source. The relative amounts of purines and pyrimidines in samples of DNA of different living organisms can be tabulated as follows :

Table : Relative amounts of nitrogen bases in different samples of DNA (Chargaff and Davidson, 1955)

S. N.	Source	Adenine	Guanine	Cytosine	Thymine	$\frac{A+T}{G+C}$
1.	Beef sperm	28·7	22·2	22·0	27·2	1·26
2.	Human thymus	30·9	19·9	19·8	29·4	1·52

S. No.	Source	Adenine	Guanine	Cytosine	Thymine	$\frac{A+T}{G+C}$
3.	Human liver	30·3	19·5	19 9	30·3	1·53
4.	Human sperm	30·7	19·3	18 8	31·2	1·62
5.	Hen red cells	28 8	20·5	21·5	29·2	1·38
6.	Rat bone marrow	28·6	21·4	21·5	28·4	1·33
7.	Herring sperm	27·8	22 2	22·6	27·5	1·23
8.	*Paracentrotus lividus* (sea urchin) sperm	32 8	17·7	18·4	32·1	1·85
9.	Salmon	29·7	20 8	20 4	29·1	1·43
10.	Wheat germ	26·5	23 5	23·0	27 0	1·19
11.	Yeast	31·3	18 7	17·1	32·9	1·79
12.	*Diplococcus pneumoniae*	29·8	20·5	18 0	31·6	1·59
13.	*K-12 Escherichia coli*	26 0	24 9	25·2	23·9	1·00
14.	*Mycobacterium tuberculosis*	15·1	34·9	35·4	14·6	0·42
15.	Bacteriophage T_2	32·5	18·2	16·7	32·6	1 86

These different values of A, G, C, and T in different samples of DNA are suggesting that DNA rather than being a monotonous polymer, carries genetic information in the form of specific nucleotide base sequences.

The equivalence rule—Chargaff in 1950 discovered the equivalence rule which suggested that despite wide compositional variations exhibited by different types of DNA, the total amount of purines equaled the total amount of pyrimidines (A+G = T+C), the amount of adenine equaled the amount of thymine (A = T) and the amount of guanine equaled the amount of cytosine (G = C). Chargaff's equivalence rule has been found to apply almost universally in different organisms (viruses, bacteria, plants and animals). However, DNA isolated from higher plants and animals was in general rich in adenine and thymine (A : T) and relatively poor in guanine and cytosine (G : C) (*e.g.*, AT/GC ratio of DNA of calf was 1·43 : 1), where as, DNA isolated from micro-organisms (viruses, bacteria, lower plants and animals) was in general rich in guanine and

and relatively poor in adenine and thymine (*e.g.*, AT/GC ratio of DNA of *Mycobacterium tuberculosis* was 0·60 : 1). These differences in AT/GC ratios of micro organism and higher organisms undoubtedly reflect difference in genetic information carried by these hereditary molecules and also phylogenetic, evolutionary and taxonomical significance of them.

PHYSICAL, MOLECULAR OR GEOMETRICAL ORGANIZATION OF DNA

The first person to give any thought to the three dimensional structure of DNA was **W. T. Astbury** who by his X-ray crystallographic studies of DNA molecule concluded in 1940's that because DNA has high density, so, its polynucleotide was a stack of flat nucleotides, each of which was oriented perpendicularly to the long axis of the molecule and was situated every 3·4 A° along the stack. The X-ray crystallographic studies of Astbury were continued by **Wilkins** and his associates who managed to prepare highly oriented DNA fibres that allowed them to obtain an X-ray diffraction photograph. One of his female associate **Rosalind Franklin** obtained a superior X-ray diffraction photograph of DNA which confirmed Astbury's earlier inference of 3·4 internucleotide distance and suggested a helical configuration for DNA molecule. **Watson** and **Crick** who were already engaged in constructing some suitable model for DNA structure, when observed Franklin's picture of DNA molecule, they immediately utilized that information in constructing a molecular model for DNA. In April 1953 **Watson** and **Crick** published their conclusions about the structure of the DNA in the same issue of 'Nature, in which **Wilkins** and his colleagues presented the X-ray evidence for that structure.

Considerations of Watson and Crick in the construction of double helical structure of DNA molecule—**Watson** and **Crick** concluded directly from the X-ray diffraction photograph of DNA taken by Franklin that (1) the DNA polynucleotide chain has the form of a regular helix, (2) the helix has a diameter of about 20A° and, (3) the helix makes one complete turn every 34A° along its length, and hence, since the internucleotide distance is 3·4A°, consists a stack of ten nucleotides per turn. Considering the known density of the DNA molecule, **Watson** and **Crick** next concluded that the helix must contain [illegible] or two stacks of ten nucleotides each per [illegible] 0A° in diameter and 34A° [illegible] single stack of ten, and to [illegible] of ten nucleotides each. Before trying to arrange these two polynucleotide

chains into a regular helix of the required dimensions, however, Watson and Crick placed a further restriction on their model-a restriction that derived from their knowledge that DNA is, after all, the

they felt the need of construction of such a regular helix that though, it composed of two polynucleotide chains containing an arbitrary sequence of nucleotide bases every 3·4A° along their length, would nevertheless has a constant diameter of 20A°. Since the dimension of the purine ring is greater than that of the pyrimidine ring Watson and Crick hit upon the idea that the two-chain helix could have a constant diameter if there existed a complementary relation between the two nucleotide stacks, so that at every level one stack harbours a purine base and the other a pyrimidine base. Finally, to endow the helix with thermodynamics stability, the structure would have ample opportunities for the formation of hydrogen bonds between amino or hydroxyl-hydrogens and keto-oxygens or immino-nitrogens of the purines and pyrimidine bases. These considerations led them to construct a double helix model for the molecular structure of DNA molecule.

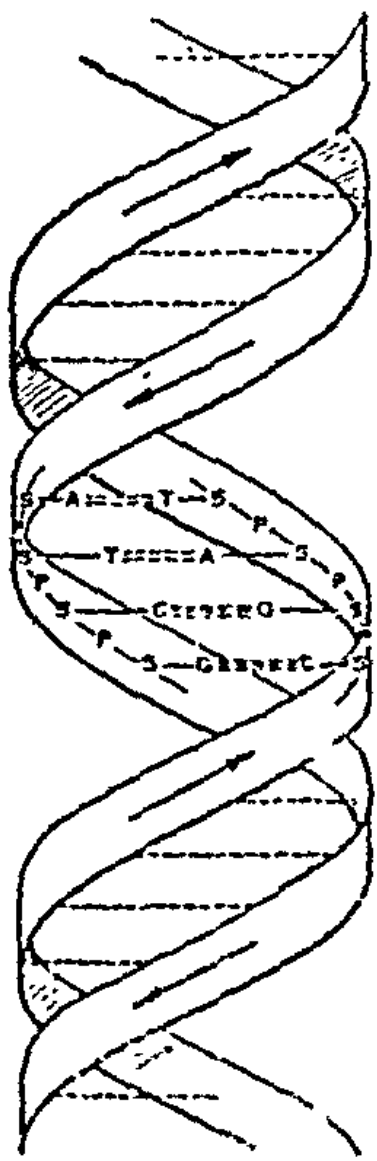


Fig. 3·7. Watson-Crick double helical DNA molecule

WATSON AND CRICK'S STRUCTURAL MODEL OF DNA

In DNA molecule the adjacent deoxyribonucleotides are joined in a chain by phospho-diester bridges or bonds which link the 5' carbon of the deoxyribose of one mono-nucleotide unit with the 3' carbon of the deoxyribose of the next mononucleotide unit. According to Watson and Crick DNA molecule consists of two such polynucleotide chains wrapped helically

around each other, with the sugar-phosphate chain on the outside (forming ribbon-like back bone of double helix) and purines and pyrimidines on the inside of the helix (projecting between two sugar phosphate backbones as transverse bars). The two polynucleotide strands are held together by hydrogen bonds between specific pairs of purines and pyrimidines.

The hydrogen bonds between purines and pyrimidines are such that adenine can bond only to thymine by two hydrogen bonds, and guanine can bond only to cytosine by three hydrogen bonds and no other alternative is possible between them. The specificity of the kind of hydrogen bonds that can be formed assures that for every adenine in one chain there will be a thymine in the other. For every guanine in first chain there will be a cytosine in the other and so on. Thus, the two chains are complementary to each other ; that is, the sequence of nucleotides in one chain dictates the sequence of nucleotides in the other. The two strands runs anti-parallely—that is, have opposite directions. One strand has phosphodiester linkage in 3′—5′direction while other strand has phosphodiester linkage in just reverse or 5′—3′ direction. Further, both polynucleotides strands remain separated by 20 A° distance. The coiling of double helix is right

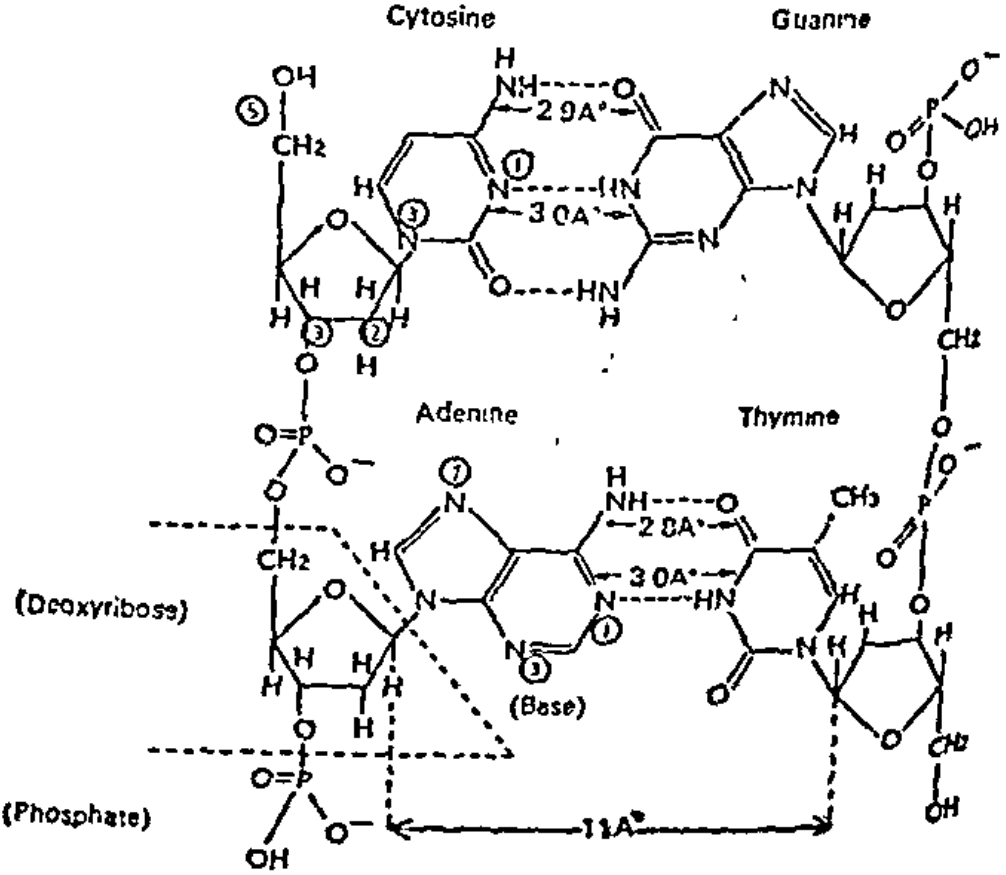


Fig. 3·8. Segment of a DNA molecule.

handed and a complete turn occurs every 34 A°. Since each nucleotide occupies 3·4 A° distance along the length of a poly ucleotide strand, ten mononucleotides occurs per complete turn.

REPLICATION OF DNA

As a carrier of genetic information DNA has following two important functions: *1. Heterocatalytic function*—When DNA directs the synthesis of chemical molecules other than itself (*e.g.*, synthesis of RNA, proteins etc.), then such functions of DNA are called

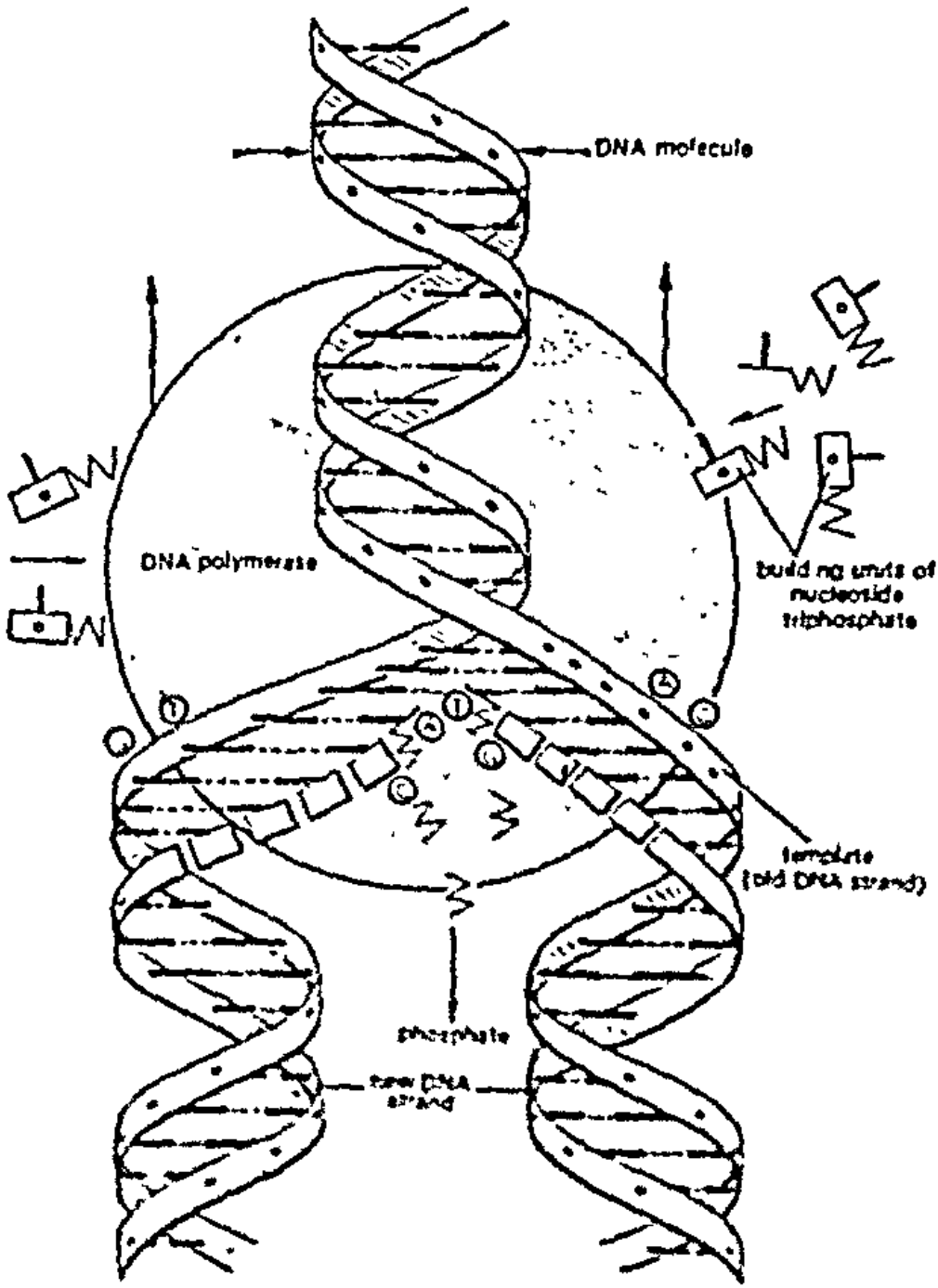


Fig. 39. The replication of DNA according to Watson and Crick's model.

heterocatalytic functions ; and ? [illegible] tions of DNA which directs the [illegible] autocatalytic functions. Here, [illegible] talytic function of DNA.

WATSON AND CRICK'S MODEL FOR DNA REPLICATION

The double helix model of DNA molecule of Watson and Crick beautifully embodied a built in template system for self-replication or autocatalytic function. Because of the specificity of base pairing the sequence of bases along one chain automatically determines the base sequence along the other. Thus, each chain of the double helix can serve as template for the synthesis of the other. For the replication of DNA molecule, Watson and Crick proposed that replication involved the disruption of hydrogen bonds followed by a rotation and separation of the two polynucleotide strands. Each

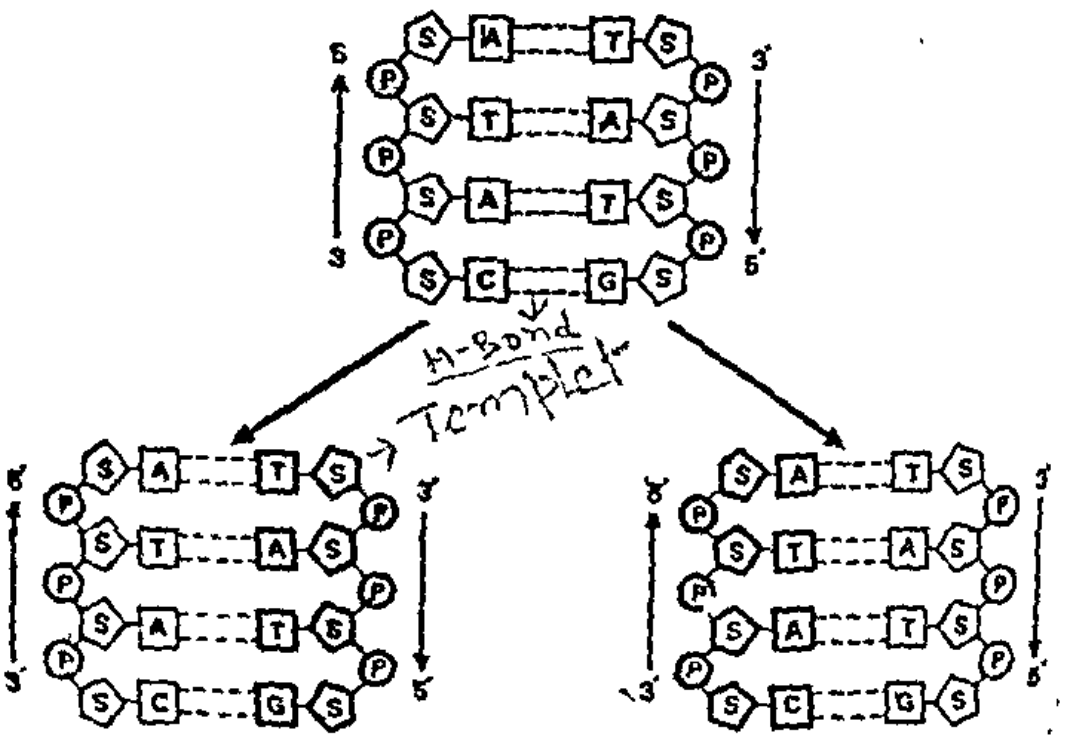


Fig. 3·10. Semiconservative model of DNA Replication

strand is thought [illegible] ble for polymeri- [illegible] zation in cell and to hold it in place by means of the specific hydrogen bonds. Once held in place on the parent template chain, the free nucleotides were sewn together by formation of the phosphate diester bonds that linked adjacent deoxyribose residues, forming a new polynucleotide molecule of predetermined base sequence. Thus, two double helical molecules, identical with each other are formed.

Watson-Crick's model of DNA replication later on verified by experiments of different sorts, and as following discussion will show, it was found to be correct.

ENZYMES OF DNA METABOLISM

Different prokaryotic and eukaryotic cells have been found to contain three kinds of nuclear enzymes or enzymatic activities that act on DNA namely **nucleases, polymerases and ligases.**

(I) NUCLEASE ENZYMES

The nuclease enzymes act to hydrolyze or break down a polynucleotide chain into its component nucleotides. A polynucleotide is held together by 3′, 5′ phosphodiester bonds and a nuclease enzyme will attack either the 3′ or the 5′ end of this linkage. The nuclease enzymes may be of following two kinds :

(a) **Exonuclease enzymes :** A nuclease enzyme which begins its attack from a free end of a polynucleotide is called **exonuclease.** Therefore, depending on the specificity of the enzyme, an exonuclease will either begin at a free 3′—OH end of a polynucleotide and progressively cleave the bonds on the 3′—OH side of the phospho-

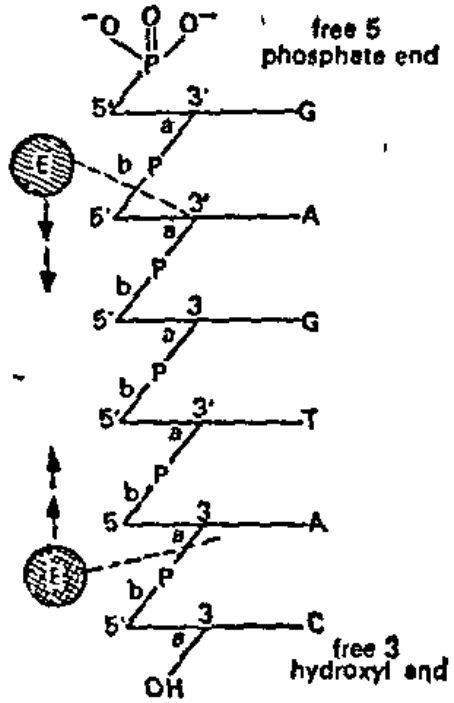


Fig. 3·11. Exonuclease action on a polynucleotide chain. The 3′ side of each phosphodiester linkage is labeled *a*, the 5′ side is labeled *b*. An exonuclease that cleaves *a* linkages, starting from a free 3′—OH end (*e.g.*, snake venom phosphodiesterase) is shown in check. An exonuclease that cleaves *b* linkages, starting from a free 5′—P end (*e.g.*, bovine spleen phosphodiestrase) is shown in line shade. (From Lehninger, 1970).

diester backbone or it will begin at a free 5′—P end and digest the polynucleotide in a 5′→3′ direction. In both cases the enzyme travels along the chain in a stepwise manner, liberating single nucleoside monophosphate molecules and eventually digesting the entire polymer.

(b) **Endonuclease enzyme** : Endonuclease enzyme also attacks one of the two sides of phosphodiester linkages, but they react only with those bonds that occur within the interior of a polynucleotide chain. If the polynucleotide chain is single-stranded (*e.g.*, viral DNA), such an attack will obviously cut the chain into two pieces. If however, the polynucleotide strand is a member of a DNA helix (*e.g.*, prokaryotic and eukaryotic DNA), a single endonucleolytic cut will create a nick in the helix ; the helix remain in one piece but it now possesses a gap that contains two free ends, which can serve as substrates for exonucleases. A nicked double helix suffers a localized disruption of its secondary structure. The molecules becomes free to bend or rotate around its intact strand and the two broken ends are free to dangle. The increased molecular motion in the region of the nick will very likely interrupt hydrogen bonding between bases in the vicinity of the nick, thus effecting a limited "unraveling" of the helix.

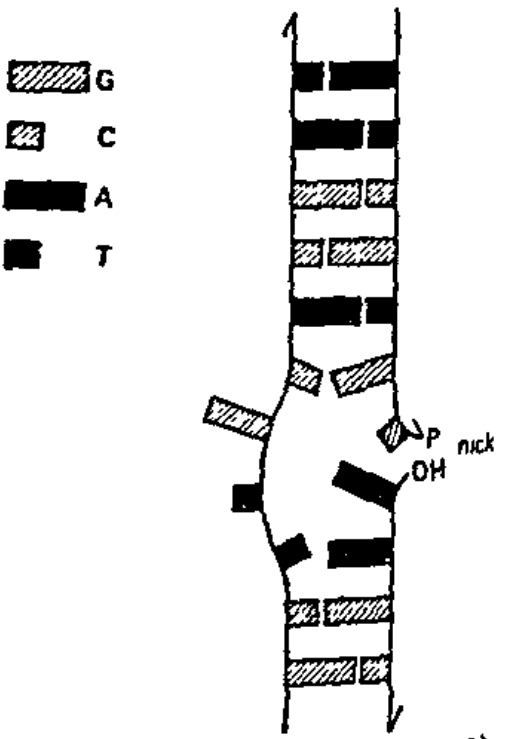


Fig. 3·12. A nicked duplex DNA molecule such as is formed by an endonuclease enzyme

(II) POLYMERASE OR REPLICASE ENZYMES

A polymerase enzyme catalyses the formation of a polymer and cellular polymerase enzymes of genetic interest are those that bring about the synthesis of one polynucleotide chain that is a copy of another. A polymerase enzyme is called **replicase enzyme** when the copy of polynucleotide chain so produced are inherited by daughter cells or viruses, that is, when the enzyme brings about chromosome replication (see **Goodenough and Levine, 1974**).

In vitro DNA polymerization—To understand the mechanism of DNA-replication inside the living cell (*in vivo*), molecular biolo-

gists tried *in vitro* polymerization of DNA and found that in addition to DNA polymerase enzyme, three classes of organic molecules are essential for an *in vitro* reaction. The first are deoxynucleoside triphosphates. These are the familiar deoxynucleotide monophosphates (*i.e.*, d AMP, d CMP, d GMP, and dTMP) with two additional phosphate groups attached to the initial or α-phosphate group (e.g., dATP, dCTP, dGTP, and dTTP). The second class of

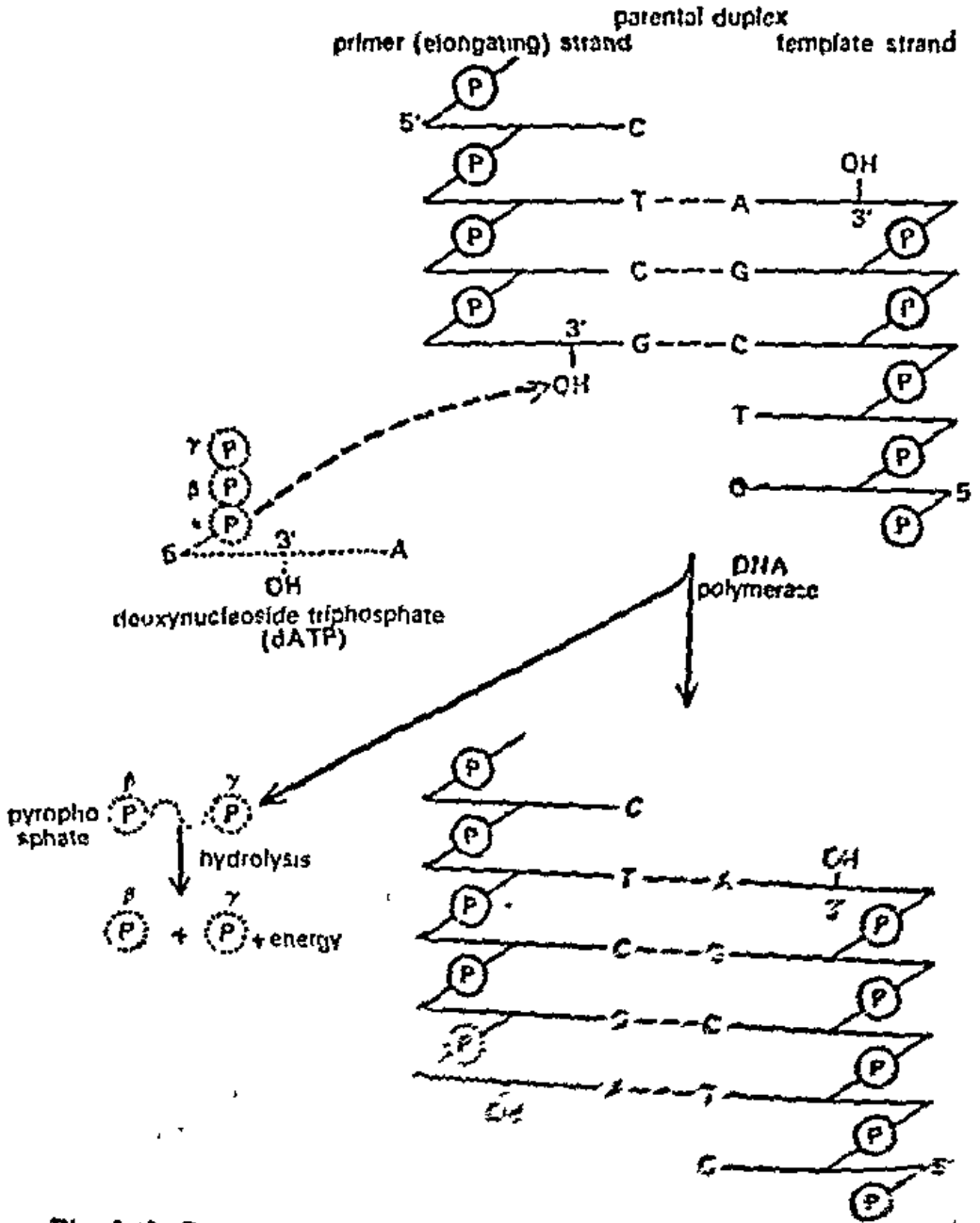


Fig. 3·13. DNA polymerization. A dATP molecule is shown being added to a parental DNA duplex or helix at its 3'—OH end. DNA polymerase catalyzes the formation of the phosphodiester bond. Pyrophosphate (PP) is hydrolyzed into two phosphate esters.

essential molecules for many DNA polymerase enzymes are polynucleotide chains with free 3'—OH ends. Many DNA strands (often called primer strands), meaning that they cannot initiate the *de novo* synthesis of a new strand. The third class of essential molecules for DNA polymerase enzymes are template strands. All biologically important DNA polymerases possess a critical property : they will add nucleotides to a primer strand only in response to the base sequence found on a second template strand. Just as Watson and Crick suggested in their original model of DNA replication, the polymerase enzymes observe the rules of complementary base pairing. Thus, if a free 3'—OH group on a primer strand lies opposite a thymine on a template strand (see fig. 3·13), a polymerase enzyme will add only an adenine group to the primer, even when d CTP, dTTP and dGTP are also present in the reaction mixture.

Once the appropriate molecules are present and the appropriate ionic conditions are maintained, *in vitro* polymerization reaction summarized in fig. 3·13 will occur. The α-phosphate of a nucleoside triphosphate molecule forms a 3', 5' phosphodiester bond with a free 3'—OH in the growing polynucleotide chain, and a molecule of pyrophosphate (P~P), is simultaneously released. Pyrophosphate contains a "high-energy" or "~" bond, meaning that when the released pyrophosphate is hydrolyzed into two phosphate molecules energy is liberated which drives the polymerization process forward. The resultant polymerization will always proceed in a net 5'→3' direction, meaning that the nucleotide at the 3' end is always the most recently added to the chain.

The Kornberg enzyme—**A. Kornberg** (1969) first of all used **DNA polymerase I** of *E. coli* which later on named as **Kornberg enzyme** or **Kornberg polymerase** after its discoverer, in *in vitro* polymerization of DNA. His early experiments which indicated that the polymerase enzyme makes faithful complementary copies of DNA molecules made use of artificially synthesized template strands alternating A and T [Poly d(AT)]. When such template strands were incubated with Kornberg polymerase and with the four nucleoside triphosphates carrying radioactive label, radioactive DNA that contained only alternating A and T was synthesized. In other words, even though a great deal of dGTP and dCTP was present in the solution, none was ever incorporated into DNA in response to poly d (AT) strand. When more complex biological DNAs with heterogenous base sequence were used, then by a molecular biological technique called **nearest neighbour analysis**, it was found

that the Kornberg polymerase catalyzes the synthesis of complementary copies of template DNA.

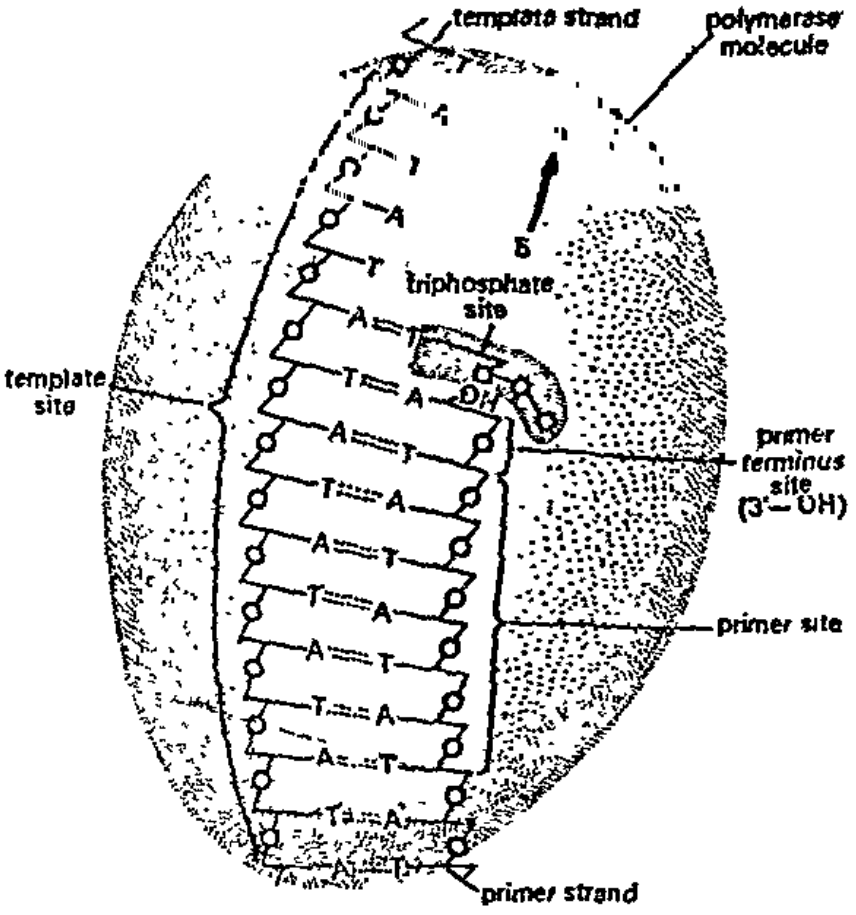


Fig 3·14 Diagram of the active centre of the DNA polymerase I *E. coli.*

Chemically the Kornberg enzyme is presently known to be a single polypeptide chain with a molecular weight of 109,000—one of the largest single-chain globular protein known (Fig. 3·14). Only one site on the enzyme appears capable of interacting with free nucleoside triphosphates. It is supposed, therefore, that once the polymerase enzyme encounters a given base in the template it can catalyze phosphodiester bond formation in the daughter strand only when the correct complementary triphosphate comes to occupy this triphosphate site. Presumably only the complementary base will have the correct geometry to align properly with the elongating daughter strand and so be in the correct position to form an ester bond; if, for instance, a pyrimidine is called for, a purine might "bulge out" too far. Once an ester bond is formed, the Kornberg enzyme is presumably free to move along to the next base in the template, where a new complementary triphosphate enters the site, another bond is formed, and so on, until the entire template is faithfully copied. The Kornberg

enzyme, therefore, like most DNA polymerases, stimulates 5'→3' synthesis of DNA by adding complementary nucleoside triphosphates to the 3'—OH end of a DNA strand. The Kornberg enzyme also possesses two exonuclease activities but such activities have not investigated so far for any DNA polymerase (Goodenough and Levine, 1974).

DNA polymerase III—For many years, Kornberg polymerase was believed to be the enzyme responsible for the replication of the *E.coli* chromosome, but genetic analysis has recently indicated other wise. **P. Delucia and J. Cairns** (1969) isolated a mutant strain called *pol A⁻ (polymerase I negative)* that contains no active form of the Kornberg enzyme and can apparently still replicate its DNA normally. This discovery has led to a search for the true replicase in *E. coli*.

Presently, **DNA polymerase III or DNA polymerase E** is considered a true replicase of *E.coli*. The molecular properties of DNA polymerase III are not yet well known, but it shares many features with the Kornberg enzyme : it synthesizes DNA in a 5'→3' direction, requires all four nucleoside triphosohates *in vitro*, and copies a template strand. Where the two enzymes differ is that polymerase *III* appears capable of initating the synthesis of a new daughter strand, whereas the Kornberg enzyme can only elongate a pre-existing strand.

(III) DNA LIGASES

DNA ligase enzymes are capable of catalyzing phosphodiester bond formation between free 3'—OH and free 5'—P groups of a nick of DNA which is created by endonuclease enzyme, thereby restoring an intact DNA duplex. Many DNA ligases have already been discovered. The ligase enzyme from *E. coli* requires the presence of oxidized nicotinamide adenine dinucleotide (NAD^+) a cofactor, whereas, the ligase enzyme specified by T_4 bacteriophage requires ATP to bring about the joining reaction.

EXPERIMENTAL EVIDENCE FOR WATSON-CRICK'S MODEL FOR DNA REPLICATION

The Watson–Crick's model of DNA structure and replication suggested that once DNA replication is initiated, the two original polynucleotide strands of the duplex or helix will unwind, at least locally, so that each can serve as a template for a new strand. An immediate prediction follows from this proposal : both duplexes that result from replication should be hybrid in nature, each containing an old strand derived from the orginal molecule and a new strand which has been formed during the replication process. This prediction is diagrammed in figure 3·15, in which old DNA is shown

have shown that the replication of the *E. coli* chromosome is probably more commonly **bidirectional**; after initiation two growing points appear to travel in opposite directions around the circular chromosome. Since the entire *E. coli* chromosome can be copied *in vivo* in 40 minutes and since the chromosome is 1100μ long, 30μ of chromosome must be copied per minute; this corresponds to 5000 base pairs added to each growing point per minute.

Initiation of DNA replication in E. coli:—Replication of *E. coli* DNA is found to be initiated at a fixed unique location in the chromosome. However, the mechanism by which replication is initiated at the unique origin is unknown. There is no evidence that small primer strands of DNA provide the replicating enzymes with free 3'—OH groups from which to elongate daughter strands. Therefore, daughter strand synthesis must be initiated by some mechanism other than that used by the Kornberg enzyme. According to a current view, short fragments of RNA may "prime" the *de novo* initiation of DNA synthesis. Another view holds that the true replicase enzyme which is undiscovered as yet, may not require priming like other polymerases of *E. coli*.

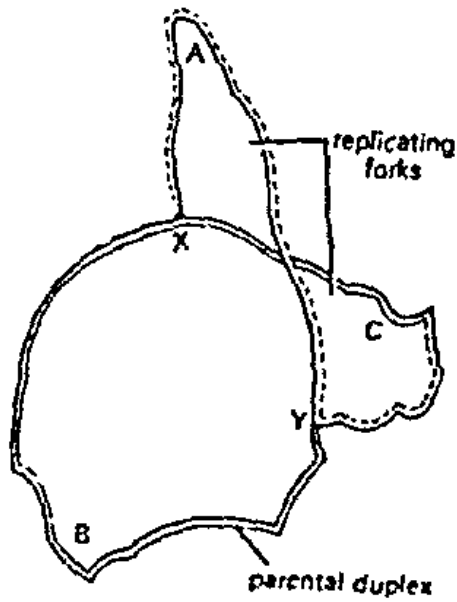


Fig. 3·16. Replicating DNA of *E. coli* K_{12}, as diagrammed from a autoradiograph visualized by Cairns (1963). Replicating DNA is divided into three sections (A, B, and C) that arise at the two forks (X and Y).

Models of DNA replication for E. coli—According to Goodenough and Levine (1974) following two models have been proposed by molecular biologists for explaining DNA replication in *E. coli*.

(i) Discontinuous model for DNA replication—This model suggested that the parent helix undergoes a localized unwinding or denaturation so that its internal bases are exposed for replication. It is assumed that at least one parental strand in the remaining helix

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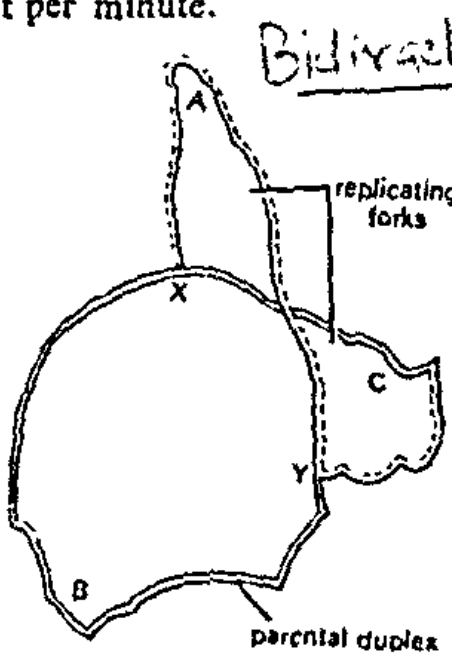


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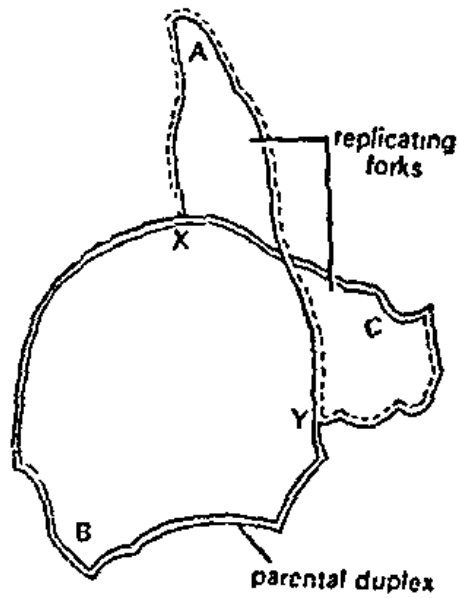


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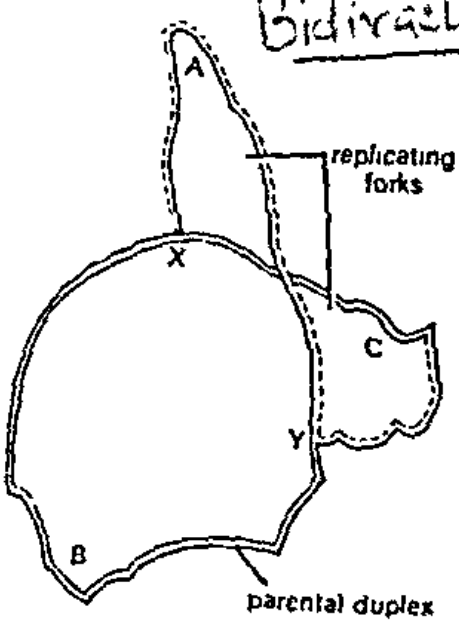


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C. Thomas (1970), a single DNA polymerase first copies one strand, then jumps across the replicating fork and, continuing to elongate the same DNA molecule, copies the other strand in the opposite direction until it encounters the barrier of already replicated DNA. Two events now occur: ligase joins the free ends of the second daughter strand as before and an endonuclease (the "knife") cut the newly synthesized strand in the forked region, thus creating two DNA molecules. A second polymerase now enters the fork, elongate one chain, jumps to the other side of the fork, and so on.

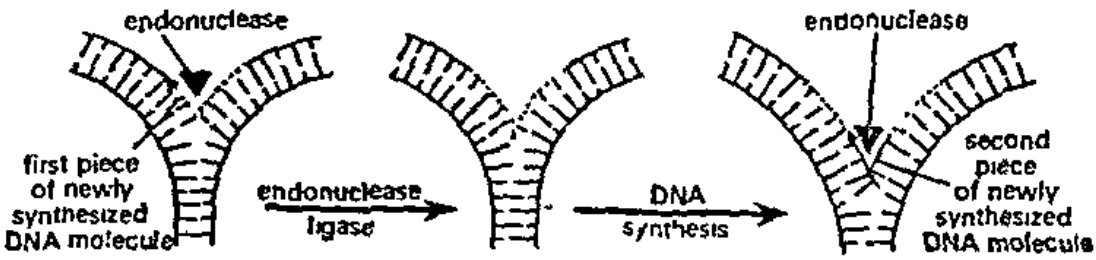


Fig. 3·18. "Knife and fork" model of DNA replication. The triangle represents an endonuclease.

Both models, however, predict that at least half of the newly synthesized DNA of a cell will experience a transient existence in the form of discontinuous pieces. This prediction has been verified by R. Okasaki *et. al.* (1968, 1971).

DNA segregation in E. coli—The final event in the replication of a bacterial DNA is the separation, or segregation, of the two daughter DNA molecules (each containing one old and one new strand) into two daughter cells at the time of cell division. This segregation is presumably mediated by an ingrowing shelf of bacterial membrane called a mesosome, which ultimately forms a septum through the middle of a cell and thus divides it into two cells. The simplest model of segregation which has not been proved experimentally, proposes that the two daughter DNA molecules become attached to either side of an ingrowing mesosome and thus come to reside in separate cells.

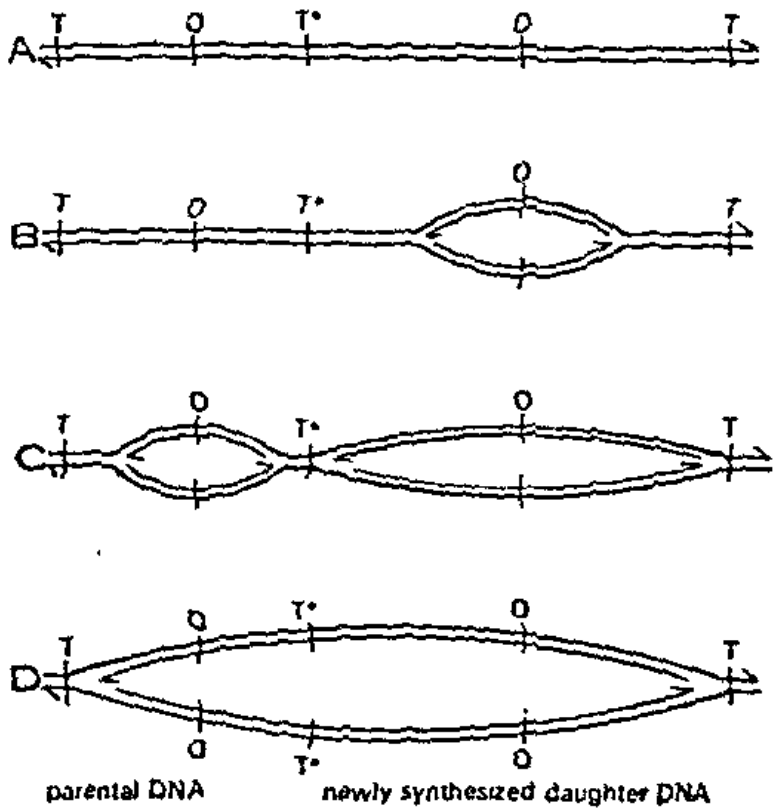
DNA REPLICATION IN EUKARYOTES

Most molecular biologists believe that at a molecular level the DNA in a nuclear chromosome is replicated by the same kinds of enzymes (*e.g.*, exonucleases, endonucleases, polymerases, and ligases) and by the same sorts of steps as DNA replication in bacterial chromosomes. *In most eukaryotes the molecular study of DNA replication* remains difficult due to the fact that their DNA exists as heterogenous chromatin, and the chromatin is aggregated in a veritable jungle of twisted fibers whose fundamental pattern of organization is unknown.

growth and metabolic activity) and M (mitosis) phases of the cell cycle. The nature of this mechanism is totally unknown.

EUKARYOTIC DNA REPLIACTION AT A MOLECULAR LEVEL

The detailed mechanism of eukaryotic DNA replication is no better understood than that of prokaryotic DNA. According to well accepted **bidirectional model** of eukaryotic DNA replication, DNA synthesis within a given replication unit is initiated some where at or near the midpoint of the unit at a site termed the **origin** (O). Two *replicating forks are then believed to travel in opposite directions* until they reach either end of that unit, the two end points being called **termini** (T). A given replication unit may or may not undergo bidirectional synthesis in synchrony with contiguous units. In either case the newly replicated strands in adjacent units will eventually meet. These strands are then linked, perhaps by a DNA ligase to *form long, continuous daughter* DNA strands.



3·19. *Bidirectional model for mammalian chromosomal DNA replication. Origins are indicated by O and termini by T (A) DNA prior to replication ; (B) replication started in right-hand replication unit ; (C) replication started in left-hand replication unit and completed at termini of right-hand unit ; (D) replication completed, sister duplexes joined at common terminus T.*

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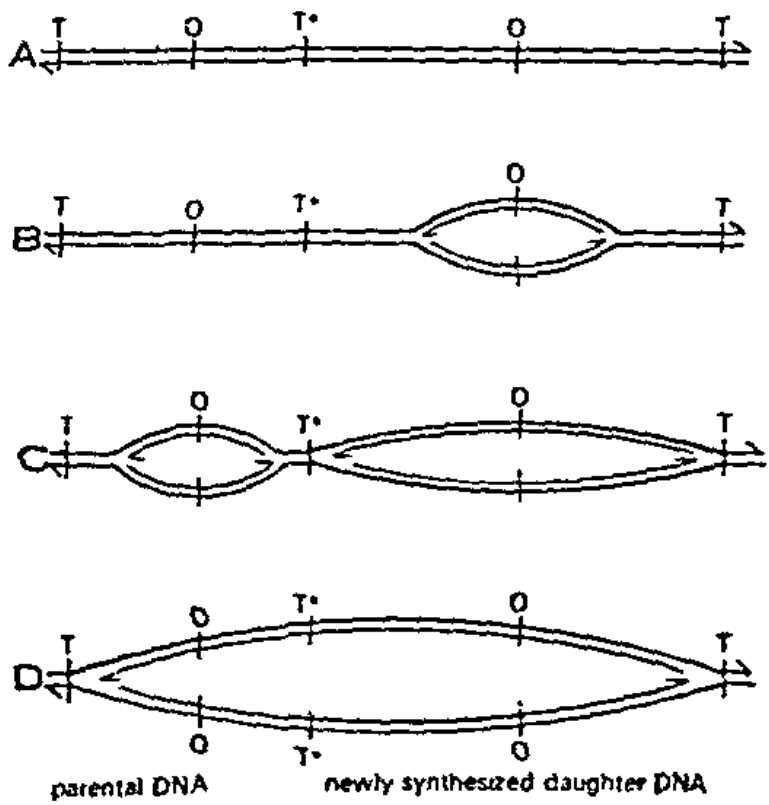


Fig. 3 19. Bidirectional model for mammalian chromosomal DNA replication. Origins are indicated by O and termini by T (A) DNA prior to replication ; (B) replication started in right-hand replication unit ; (C) replication started in left-hand replication unit and completed at termini of right-hand unit ; (D) replication completed, sister duplexes joined at common terminus T.

However, some information about DNA replication in eukaryotes has been received from dipteran polytene chromosomes, lampbrush chromosomes of amphibian oocytes, and chromosomes of some eukaryotes.

Time of DNA replication in eukaryotes—In rapidly growing bacteria DNA synthesis occurs from the time a cell originates at division to the time it gives rise to daughter cells by division. A similar pattern is observed in simple eukaryotes such as yeast, in which DNA synthesis takes place throughout the interphase and ceases only during the brief period of nuclear and cell division. In higher plants and animals, however, DNA replication occurs only during a discrete interval of the interphase known as synthesis or S period. Since histones are also synthesized during this time, the S period can be considered a time of chromosomal as well as DNA replication. The length of a S phase is a constant property of any one cell type.

Replication units—For studying DNA replication in eukaryotes, in one of the experiment, diploid cells are grown in a tissue culture medium containing H^3-thymidine for brief periods of time; large DNA fragments are spread onto a surface and subjected to autoradiography. J. Huberman and A. Riggs (1968) measured the lengths of individual radioactive chromosomal segments in preparations derived from a human cell line known as HeLa, and calculated that the DNA is replicated at about 1μ/min. (in contrast to 30μ/min for *E. coli*), or about 60μ/hr. When we consider that an average chromosome contains about 30,000μ of DNA, it becomes clear that if replication were to initiate at one end and proceed sequentially to the other end, it would take 500 hours. Instead, replication typically occurs within six to eight hours. This and a number of other considerations have led to the conclusion that during a typical S phase each chromosome is "worked on" by replication enzymes at many different places at once. In other words, each chromosome contains a number of different replication units. Autoradiographical studies have shown that each replication units functions independently of one another.

Further, all the DNA in a cell must be replicated in advance of mitosis, or meiosis if daughter cells are to receive complete sets of genetic information. Since individual replication units behave independently, some generalized kind of mechanism must exist which takes cognizance of the fact that DNA replication has been completed throughout the genome and which then triggers the onset of the G_2 (Gap period of interphase which follows S phase and includes

growth and metabolic activity) and M (mitosis) phases of the cell cycle. The nature of this mechanism is totally unknown.

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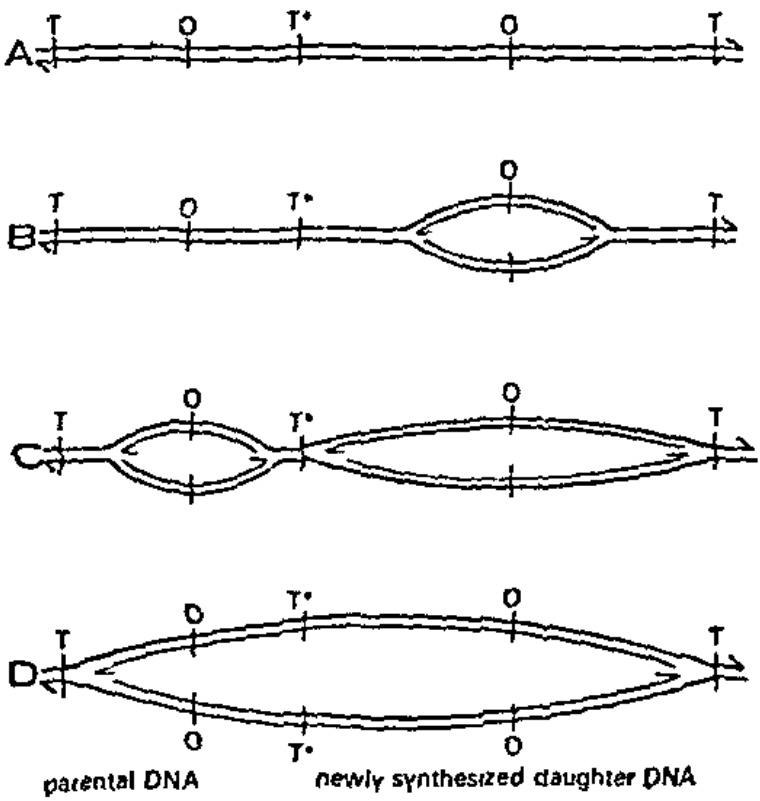


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The model shown in Fig. 3·19 describes DNA replication in eukaryotes as being semiconservative, and extensive evidence supports this assumption. Specifically, a number of investigators have performed experiments similar to those of Meselson and Stahl (1958) and have found that heavy DNA from eukaryotes becomes hybrid in density after one round of replication in light medium.

RIBONUCLEIC ACID (RNA)

Some plant viruses (*e.g.*, TMV, turnip yellow mosaic viruses, wound tumour viruses, etc.,), animal viruses (*e g.*, influenza viruses, foot and mouth viruses; rous sarcoma viruses, poliomyelitis viruses reoviruses, etc.) and bacteriophages (*e.g.*, MS_2, etc.) contain ribonucleic acid (RNA) as their genetic material. Like DNA, RNA is polymeric nucleic acid of four monomeric ribotids or ribonucleotids.

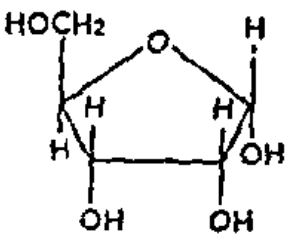


Fig. 3 20. Chemical formula of D-ribose

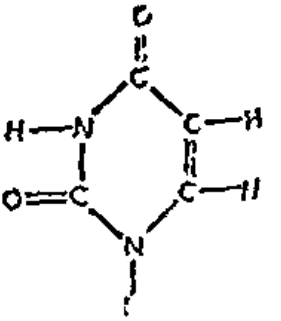


Fig. 3·21. Chemical formula of Uracil.

Each ribonucleotide contains a pentose sugar (**D-ribose**); a molecule of phosphate group and a nitrogen base. The nitrogen bases of RNA are two purines, adenine and guanine and two pyrimidines, cytosine and uracil. The four bases, ribonucleosides and ribonucleotides of RNA can be tabulated as follows :

Base	Ribonucleoside	Ribonucleotides	Abbreviation for ribonucleotide
1. Adenine (A)	Adenosine	Adenylic acid (Adenosine monophosphate)	AMP
2. Guanine (G)	Guanosine	Guanylic acid (Guanosine monophosphate)	GMP
3. Cytosine (C)	Cytidine	Cytidylic acid (Cytidine monophosphate)	CMP
4. Uracil (U)	Uridine	Uridylic acid (Uridine monophosphate)	UMP

The four ribonucleotides also occur freely in nucleoplasm but in the form of triphosphates of ribonucleosides such as adenosine

triphosphate (ATP), guanosine triphosphate (GTP) cytidine triphosphate (CTP), and uridine triphosphate (UTP).

MOLECULAR STRUCTURE OF RNA

RNA molecule may be either single stranded or double stranded but not helical like DNA molecule. Single stranded RNA occurs as genetic material in plant viruses (*e.g.*, TMV, TYM), animal viruses *e g.*, influenza viruses, foot and mouth viruses, rous sarcoma viruses, poliomyelitis viruses and bacteriophages (*e. g.*, MS_2). The non-genetic RNAs except tRNA of prokaryotes and eukaryotes, also have single stranded RNA molecules. The double stranded but non-helical RNA occurs as the genetic material in some plant viruses (*e. g.*, reoviruses). The transfer or soluble RNA (tRNA or sRNA) which is non-genetic RNA of prokaryotes and eukaryotes, is double stranded but non-helical structure.

Each strand of RNA is polynucleotidic, that is, made up of many ribonucletides. In the polynucleotide strand of RNA, the ribose and phosphoric acids of nucleotides remain linked by phosphodiester bonds. The organisms which have only RNA, employ RNA in their genetic mechanisms and that type of RNA is called **genetic RNA.** While, the organisms which have DNA along with RNA, they use the RNA in carrying the orders of DNA and in them because RNA has no genetic role, so called **non-genetic RNA.** The non-genetic RNA is heterogeneous and includes following three genera : ribosomal RNA (rRNA), transfer RNA (tRNA) and messenger RNA (mRNA). Each genera of non-genetic RNA has **DNA-dependent replication** of itself, that is, it is not self-replicating like DNA and is transcribed by DNA

REPLICATION OF GENETIC RNA

The genetic RNA of viruses is self-replicating, that is, it can produce its own replica by itself. So, its mode of replication is called **RNA-dependent RNA synthesis.** The genetical research on genetic RNA has revealed following facts about it :

(1) The viral RNA functions directly as a messenger RNA which, in association with the ribosomal apparatus of the host, directs the synthesis of both the RNA polymerase enzyme (require for RNA replication) and the proteins of the viral coat.

(2) With the mediation of RNA polymerase, and on the standard base-pairing principles, the viral RNA serves as a template in the synthesis of a complementary RNA chain and thus a double stranded structure is produced.

CHAPTER 4

GENETIC CODE

As DNA is a genetic material, it carries genetic informations from cell to cell and from generation to generation. At this stage, an attempt will be made to determine that in what manner the genetic informations are existed in DNA molecule ? Are they written in articulated or coded language on DNA molecule ? If in the language of codes what is the nature of genetic code ?

A DNA molecule is composed of three kinds of moieties : (i) phosphoric acid, (ii) deoxy- [illegible] The genetic informations may be [illegible] of DNA. But the poly-sugar-phosphate backbone is always the same, and it is therefore unlikely that these moieties of DNA molecule carry the genetic informations. The nitrogen bases, however vary from one segment of DNA to another so the informations might well depend on their sequences The sequences of nitrogen bases of a give segment of DNA molecule, actually has been found to be identical to linear sequence of amino acids in a protein molecule. The of proof such a colinearity between DNA nitrogen base sequence and amino acid sequence in protein molecules has first obtained from an analysis of mutants of head protein of bacteriophage T_4 (Sarabhai *et al.*, 1964) and the A protein of tryptophan synthetase of *Escherichia coli* (Yanofski *et. al*, 1964). The colinearity of protein molecules and DNA polynucleoti- [illegible] of four nitrogen

DNA [illegible] should be

or coded language ? If genetic informations might have occurred in an articulated language, the DNA molecule might require various alphabets, a complex system of grammer and ample amount of space on it. All of which might be practically impossible and troublesome too for the DNA. Therefore, it was safe to conclude for molecular biologists that genetic informations were existed in DNA molecule in the form of certain special language of code words which might

utilize the four nitrogen bases of DNA for its symbols. Any coded message is commonly called **cryptogram.**

BASIS OF CRYPTOANALYSIS

The basic problem of such a genetic code is to indicate how information written in a four letter language (four nucleotides or nitro-

of "mRNA language" [mRNA is a complementary molecule which copies the genetic informations (cryptogram of DNA) during its transcription] can be illustrated as follows :

Table: Possible singlet, doublet and triplet codes of mRNA.

Singlet Code (4 Words)	Doublet Code (16 Words)				Triple Code (64 Words)			
A	AA	AG	AC	AU	AAA	AAG	AAC	AAU
G	GA	GG	GC	GU	AGA	AGG	AGC	AGU
C	CA	CG	CC	CU	ACA	ACG	ACC	ACU
U	UA	UG	UC	UU	AUA	AUG	AUC	AUU
					GAA	GAG	GAC	GAU
					GGA	GGG	GGC	GGU
					GCA	GCG	GCC	GCU
					GUA	GUG	GUC	GUU
					CAA	CAG	CAC	CAU
					CGA	CGG	CGC	CGU
					CCA	CCG	CCC	CCU
					CUA	CUG	CUC	CUU
					UAA	UAG	UAC	UAU
					UGA	UGG	UGC	UGU
					UCA	UCG	UCC	UCU
					UUA	UUG	UUC	UUU

The first experimental evidence in support to the concept of triplet codes is provided by Crick and coworkers in 1961. During

their experiment, when they added or deleted single, or double base pairs in a particular region of DNA of T_4 bacteriophages of *E. coli*, they found that such bacteriophages ceased to perform their normal functions. However, bacteriophages with addition or deletion of three base pairs in DNA molecule, had performed normal functions From this experiment, they concluded that a genetic code is in triplet form because the addition of one or two nucleotides has put the reading of the code out of order, while the addition of third nucleotide resulted in a return to the proper reading of the message.

METHODS OF CRYPTOANALYSIS OF GENETIC CODE, OR METHODS OF CRACKING OF GENETIC CODE

The genetic code has been cracked or deciphered by following kinds of approaches :

1. IN VITRO CODON ASSIGNMENTS

Nirenberg in 1961, firsts of all, provided the means for a much more rapid decipherment of the genetic code. He made *in vitro* studies on the incorporation of radioactive amino acids in cell free protein synthetic systems containing artificial (synthetic) ribopolynucleotides or mRNA. A cell free system for protein synthesis includes ribosomes, enzymes, tRNA, etc. To produce such a cell free protein synthetic system, the protein synthesizing components of broken cell usually *E. coli*) were separated from the remainder by gentle centrifugation. During these manipulations natural mRNA is subjected to mechanical and enzymatic break down. Artificial messenger RNA were then introduced. The artificial mRNA was prepared for each nitrogen base with the aid of a polynucleotide phosphorylase enzyme obtained from *Azobacter vinelandii* or *Micrococcus lysodeikticus*. The enzyme differed from RNA polymerease enzyme in not requiring the presence of DNA as a primer or template for the synthesis of

acids) in different cell free protein synthetic systems of *E. coli*

coded for phenylalanine. Likewise because polycytidylic acid was found to direct synthesis of polyproline hence, the sequeuce of CCC codes designates proline and because, polyadenylic acid directs synthesis of polylysine, so the sequence of AAA designates lysine. Later on, the sequence of GGG was found to specify glycine.

In this way four of 64 codes or codons were easily accounted for. In order to gain some insight into the meaning of the remaining 60 codons containing more than one kind of nucleotide, Nirenberg continued these experiments by using artificially synthesized random ribopolynucleotides containing two, three or four different nucleotide constituents. By using artficial mRNAs, *viz.*, poly UA, poly UC, and poly UG, he cracked the codes for arginine, alanine, serine, proline, tyrosine, isoleucine, valine, leucine, cysteine, tryptophan, glycine, methionine and glutamic acid.

After learning of Nirenberg's first success with poly U, Ochoa and his collaborators carried out entirely analogous sets of decoding experiments in which they used artificial random ribopolynucleotides. Through the parallel efforts of these two research groups the empirical formula of many amino acid codes or codons became known within a year or so. But this work could not establish the actual sequence of the nucleotides within any codon, except for UUU, CCC, AAA and GGG.

In order to ascertain that which structural isomer of a codon of a given emperical formula designates which amino acid, artificial mRNAs of known sequences were employed. H. G. Khorana first of all, succeeded in synthesizing polyribonucleotides or mRNA of known sequences and he synthesized an alternating copolymer such as-UGUGUG-UGU- Use of this particular alternating polynucleotide as a mRNA in the *in vitro* protein-synthesizing system gave rise to the formation of the alternating polypeptide-(*e.g.*, valine-cys-

rected by the codon sequence-UGU-

ablished that the UUG (lucine) codon

UUG (tryptophan) nor the UGG (glycine) codons is GUG, but, UUG (valine) is UGU, and GUG is a cysteine synonym, or that UUG ((cysteine) is UGU and GUG is a

exact sequence of

scovered either by

simple trinucleotides of known base sequences to ribosomes would cause these ribosomes to bind that, and only that, aminoacyl-tRNA which carries the anticodon complementary to the trinucleotide added to the reaction mixture. For example, the trinucleotide GCC was found to be active in promoting the binding of alanyl-tRNA^{ala} but not of any other aminoacyl-tRNA and it was therefore concluded that GCC is a codon for alanine. These studies enabled the preparation of the complete genetic code dictionary.

2. IN VIVO CODON ASSIGNMENTS

The cell free protein synthetic systems, though have proved of great significance in decipherment of the genetic code, but, they could not tell us whether the genetic code so deciphered is used in the living systems of all organisms also. Three kinds of techniques are used by different molecular biologists to determine whether the same code is also used *in vivo* (a) amino acid replacement studies (*e.g.*, triptophan synthetase synthesis in *E.coli*(Yanofsky *et al.* 1963) and haemoglobin synthesis in man), (b) frame shift mutations (*e.g.*, investigations of Terzaghi *et al.* 1966, on lysozyme enzyme of T_4 bacteriophages, and (c) comparison of a DNA or mRNA polynucleotide cryptogram with its corresponding polypeptide clear text (*e.g.*, comparison of amino acid sequence of the R_{17} bacteriophage coat protein with the nucleotide sequence of the R_{17} mRNA in the rigion of the molecule that dictates coat-protein synthesis by S. Cory *et al.* 1970).

Thus, *in vitro* and *in vivo* studies so far described, gave the way to formulate a code table for twenty amino acids as follows :

Table : The genetic dictionary. The trinucleotide codons are written in the 5'→3' direction.

First base	Second base U	Second base C	Second base A	Second base G	Third base
U	UUU, UUC — Phe	UCU, UCC, UCA, UCG — Ser	UAU, UAC — Tyr	UGU, UGC — Cys	U
					C
	UUA, UUG — Leu		UAA, UAG — Non-sense codon	UGA — Non-sense codon; UGG — Try	A
					G
C	CUU, CUC, CUA, CUG — Leu	CCU, CCC, CCA, CCG — Pro	CAU, CAC — His; CAA, CAG — Gln	CGU, CGC, CGA, CGG — Arg	U
					C
					A
					G

First base	Second base U	C	A	G	Third base
A	AUU Ileu	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ileu	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ileu	ACA Thr	AAA Lys	AGA Arg	A
	AUG* Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GUC Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

* AUG-Met or chain initiation codon.

Patterns to the genetic code—A notable pattern emerged when the genetic code of above discuss table was studied. First, amino acids with similar structural properties tend to have related codons. Thus the aspartic acid codons (GAU and GAC) are similar to glutamic acid codons (GAA and GAG); similarly, the codons for the aromatic amino acids phenylalanine (UUU, UUC), tyrosine (UAU, UAC) and tryptophan (UGG) all begins with uracil. This feature of the code is thought to have evolved to minimize the consequences of mistakes made during translation or of mutagenic base substitutions. If an amino acid in a protein is erroneously replaced by one with similar properties, the protein may still be functional.

CHARACTERISTICS OF TRIPLET CODON

The genetic dictionary of mRNA codons reveals following important features of triplet codons :

almo … that
exam … For … each

have six synonymous codons. However, for many of the synonym codons specifying the same amino acid the first two bases of the triplet are constant, whereas the third can vary; for example, all codons starting with CC specify proline (CCU, CCC, CCA and CCG) and all codons starting with AC specify threonine This flexibility in the third nucleotide of a codon may well help to minimize the consequences of errors.

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C	CUU, CUC, CUA, CUG — Leu	CCU, CCC, CCA, CCG — Pro	CAU, CAC — His; CAA, CAG — Gln	CGU, CGC, CGA, CGG — Arg	U, C, A, G

First base	Second base U	Second base C	Second base A	Second base G	Third base
A	AUU } Ileu	ACU } Thr	AAU } Asn	AGU } Ser	U
	AUC } Ileu	ACC } Thr	AAC } Asn	AGC } Ser	C
	AUA } Ileu	ACA } Thr	AAA } Lys	AGA } Arg	A
	AUG* Met	ACG } Thr	AAG } Lys	AGG } Arg	G
G	GUU } Val	GUC	GAU } Asp	GGU	U
	GUC } Val	GCC } Ala	GAC } Asp	GGC } Gly	C
	GUA } Val	GCA } Ala	GAA } Glu	GGA } Gly	A
	GUG } Val	GCG } Ala	GAG } Glu	GGG } Gly	G

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The genetic dictionary of mRNA codons reveals following important features of triplet codons :

1. Degeneracy—The code contains many synonyms i.e. that almost all a · or example, th · ch have six synonymous codons. However, for many of the synonym codons specifying the same amino acid the first two bases of the triplet are constant, whereas the third can vary; for example, all codons starting with CC specify proline (CCU, CCC, CCA and CCG) and all codons starting with AC specify threonine This flexibility in the third nucleotide of a codon may well help to minimize the consequences of errors.

2. **Non-overlapping**—The code is non-overlapping, meaning that no single base can take part in the formation of more than one codon.

3. **Ambiguity :** The genetic code is ambiguous, that is, same codon may specifies more than one amino acid. For example, UUU codon usually code for phenylalanine but in the presence of streptomycin, may also code for isoleucine, leucine or serine.

[illegible] means th[illegible]

[illegible]ain ini[illegible]e chain.

6. **Non-sense codons:** The UAA (also called ochre), UAG (also called amber because an investigator who studied the properties of this codon belonged to the Bernstein family, and Bernstein means "amber" in German) and UGA [illegible] so, are called non-sense codons [illegible] dons, because, these codons are [illegible] end of translation of a particular polypetide. However, their inclusion in any mRNA results in the abrupt termination of the message at the point of their location even though the polypeptide chain has not been completed.

[illegible] The genetic code has been found to be uni[illegible] living systems.

CHAPTER 5

NON-GENETIC RIBONUCLEIC ACID (RNA)

In the organisms (*viz.*, prokaryotes and eukaryotes), where coded genetic informations are contained in the DNA molecule, different genetically controlled functions of their cells are performed by a different kind of nucleic acid, called **non-genetic ribonucleic acid (RNA)**. In the cells of such organisms, the DNA molecule occurs in the chromosomes of nucleus or nucleoids of eukaryotes or prokaryotes respectively and process of protein synthesis occurs in the cytoplasm. It is investigated that a DNA molecule does not leave the nucleus to participate directly in the process of protein synthesis, but employs different types of non-genetic ribobucleic acid molecules for carrying its genetic informations from the nucleus to the site of protein synthesis, *i.e.*, ribosomes. Thus, the DNA molecule acts as a template for the synthesis of different non-genetic RNA molecules which copy down the coded genetic informations of DNA molecule during their DNA dependent synthesis (*i.e.*, **transcription**).

CHEMICAL COMPOSITION OF NON-GENETIC RIBONUCLEIC ACID (RNA)

Chemically the non-genetic RNA is closely related with DNA. However, the non-genetic RNA is a single strand polymer of ribonucleotide units and is composed of phosphoric acid, ribose (pentose) sugar, and nittogen bases, which are purines (adenine and guanine) and pyrimidines (cytosine and uracil). The molecular weights of the various RNA molecules vary from about 25,000 to over one million. Its base composition does not follow the base composition of DNA, although, in general, the proportion of A+C roughly equals G+U, as has been shown in the table on page 434.

Further, some RNA molecules contain significant proportions of some methylated bases and an unusual nucleoside known as pseudouridine (ψU), in which the glycosidic bond is associated with position 5 of uracil rather than position z.

Table : Base ratios of RNA from various sources (as molar percentages) (From, Hall *et al*, 1974)

Source	Adenine	Guanine	Cytosine	Uracil
1. *Allium cepa* (onion seed)	24 9	29 8	24·7	20 6
2. *Phaseolus vulgaris* (bean seed)	24·9	31·4	24·1	19·6
3. *Cucurbita pepo* (pumpkin seed)	25·2	30 6	24·8	19 4
4, Ox liver	17·1	27·3	33·9	21·7
5. Yeast	25 4	24·6	22·6	27 4
6. *E coli*	25·3	28·8	24·7	21·2

COMPARISON BETWEEN DNA REPLICATION AND TRANSCRIPTION

The replication and transcription are two chief activities of DNA and they should be compared at the outset in following manner :

1. In DNA replication an enzyme (or enzyme complex) matches up complementary deoxyribonucleoside triphosphates with a DNA template according to Watson-Crick pairing rules and the bases are polymerized to form a daughter DNA molecule. In DNA transcription a different enzyme, called **DNA-dependent RNA polymerase** mediates a similar process, except that complementary ribonucleoside-triphosphates are matched with the DNA template and RNA polymers are formed. The base uracil (U) replaces thymine in RNA but, otherwise the DNA strand is faithfully copied. The RNA copy has the opposite polarity from the template so that for example the sequence $\overrightarrow{\text{TACAAC}}$ in DNA is transcribed as $\overleftarrow{\text{AUGUUG}}$ in RNA.

2. The RNA transcripts do not ordinarily remain hydrogen-bonded with their DNA templates as DNA daughter strands do. Instead, they "peel off" the template as they are formed, thus becoming available to participate in protein synthesis.

3. DNA of prokaryotes (*E. coli*) and eukaryotes has one or few initiation points for DNA replication, so that, at least large portions of genome are copied into single, enormous daughter DNA molecules. In contrast, the synthesis of RNA molecules is initiated at close intervals along the DNA and thus relatively short RNA copies are produced.

4. Lastly, replication and transcription have to serve two different functions. The purpose of replication is to conserve the entire genome for next generation, whereas the purpose of transcription is to make RNA copies of individual genes that the cell can use in the biochemistry. The copies are themselves not endowed with the same kind of permanence as the genetic material ; instead, they are typically degraded by cellular nucleases once their functional usefulness has been spent.

RNA POLYMERASE ENZYMES

DNA-dependent RNA polymerase enzymes are remarkable enzymes. They recognize individual bases in DNA, select the appropriate complementary ribonucleotides, catalyze phophodiester bond formation between ribonucleotides, pick the correct DNA strand to be transcribed, and initiate transcription at appropriate promoter sites along the chromosome. RNA polymerases of prokaryotes and eukaryotes have following characteristics :

(a) RNA POLYMERASES FROM PROKARYOTES AND ORGANELLES

The isolated polymerase enzyme from *E. coli.*, is one of the largest soluble proteins yet studied. It measures about *100A°* in diameter and has a molecular weight of about *490,000* daltons. RNA polymerase of *E. coli* is a **holoenzyme** and is composed of two main subunits, a smaller subunit called **sigma factor** (σ) and a large unit called **core polymerase** which is composed of two **alpha** (α) **units, two beta** (β) **units** and a **omega** (ω) **unit.** The core enzyme alone is capable of making an RNA copy of DNA, but it begins transcription at random along the template and copies both DNA strands. When sigma factor is added back to core enzyme, transcription begins at the true promoter sites and on the correct strand. The precise role of sigma is by no means clear, but at present core enzymes and sigma factors are believed to become dissociated in the cell. A sigma factor is thought to initiatethe transcription process by (1) recognizing a promoter region, possibly by its base sequence or possibly by its tertiary structure, and (2) mediating the binding of a core enzyme to the promoter. After a core enzyme is bound and begins to transcribe the contiguous sequence into RNA the sigma is thought to be released so that it can associate with another core enzyme.

Out of 5 subunits of core enzyme, only β' subunit is directly involved in the binding of RNA polymerase to DNA. The others are thought to play still undefined roles in transcription, perhaps in its regulation. Lastly, the RNA polymerase of *E. coli* and of certain chloroplasts and mitochondria is sensitive to the antibiotic **rifampicin**

which probably combines with the β subunit of the *E. coli* enzyme. However, RNA polymerases from higher plant chloroplasts and T_4 bacteriophages are found to be rifampicin-resistant.

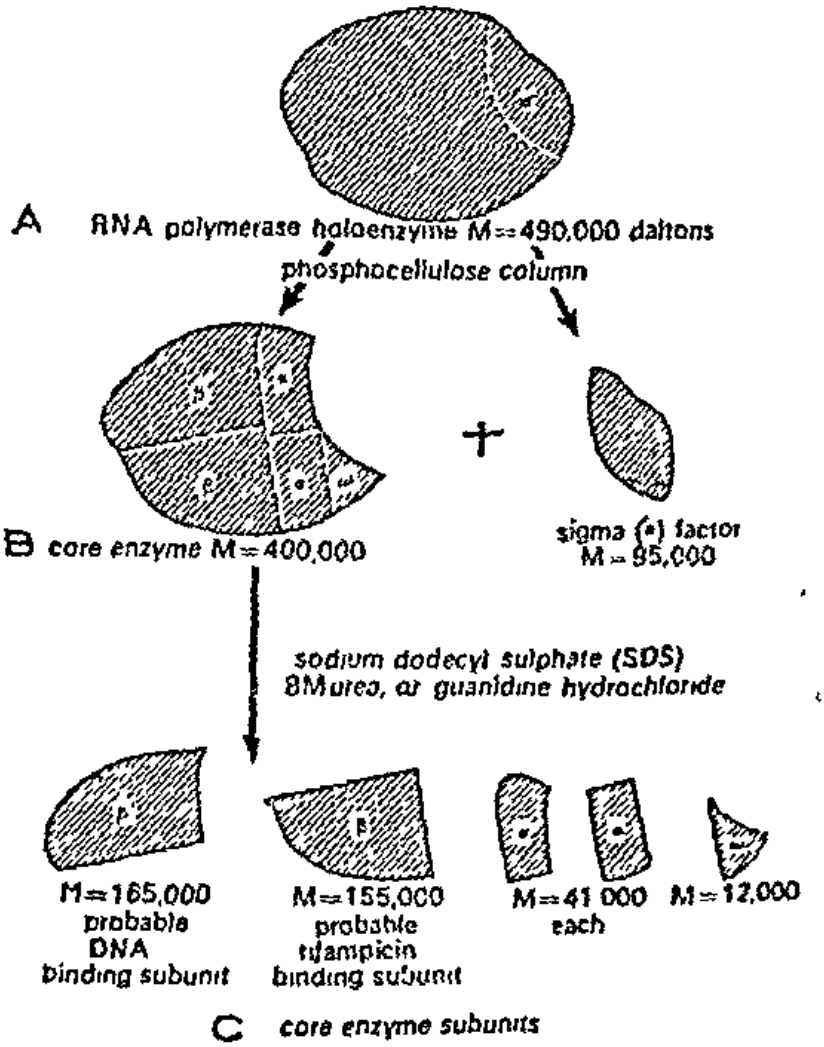


Fig. 5·1. The *E. coli* DNA-dependent RNA polymerase. All subunits are highly schematic ; (A) Intact molecule or holoenzyme. (B) The separated core enzyme and sigma units, (C) Subunits of the core enzyme.

(b) RNA POLYMERASES FROM EUKARYOTES

The nuclei of different eukaryotes are found to contain three different types of RNA polymerase, called I, II, and III RNA-polymerases. RNA polymerase I is believed to be localized exclusively in the nucleolus and involved in the synthesis of ribosomal RNA. Polymerase II and III are found outside the nucleolus and carry out the rest of nuclear transcription. All three enzymes have molecular weight greater than 500,000 and are presumed to be composed of subunits, but their molecular structures are not known like the prokaryotic enzymes.

RNA SYNTHESIS AND CHAIN TERMINATION

For transcription process entire chromosome (DNA) does not unwind and rewind but instead a local denaturation of the DNA helix

takes place so that only those bases are exposed that constitute the gene to be transcribed. Once RNA synthesis is initated, the first nucleoside triphosphateto be incorporated into the RNA chain is usually either ATP or GTP. This means (since the first base in the transcript is a purine) that the first base of a gene will be a pyrimidine, either T or C in DNA. Growth of the RNA chain then proceeds in a 5'→3' direction, just as the growth of a DNA chain does. The initial purine is, therefore, found as a ribonucleoside 5-triphosphate, and ribonucleotides are progressively added to free 3'-hydroxyl groups. One initated, RNA chains grow at a rapid rate—40 to 50 nucleotides per second in *E. coli* at 37°C.

The final event that must occur in the transcription process is the **termination of chain elongation** at a genes end. Genetic signals that dictate this event are presumed to be encoded in the DNA, and there is evidence that a protein factor called **Rho** (ρ) and discovered by **J. Roberts (1969)**, may participate as well. The molecular events that bring about initiation and termination of transcription are not well understood one.

TIME OF TRANSCRIPTION

In most eukaryotes most transcription occurs only during interphase, a time when the chromosomes are in their highly diffuse state. However, in case of dipteran polytene chromosomes and lampbrush chromosomes of amphibian oocytes, condensed chromosomes are transcribed but, in them transcription occur only in that portion of chromosome which becomes diffuse due to puff formation in polytene chromosomes or loop formations in lampbursh chromosomes.

TYPES OF NON-GENETIC RNA

According to their specific functions during the process of protein synthesis, following kinds of non-genetic RNA molecules have been recognized in prokaryotic and eukaryotic cells :

(1) RIBOSOMAL RNA (rRNA)

Ribosomal RNA (rRNA) or insoluble RNA constitutes the large [illegible] in [illegible] al[illegible] with a slight degree of methylation, and shows differences in the relative proportions of the bases between species. Its molecules appear to be single polynucleotide strands which are unbranched and flexible. At low ionic strength, rRNA behaves as a random coil, but with increasing ionic strength the molecule shows helical regions produced by base pairing between adenine and uracil and guanine and cytosine.

which probably combines with the β subunit of the *E. coli* enzyme. However, RNA polymerases from higher plant chloroplasts and T_1 bacteriophages are found to be rifampicin-resistant.

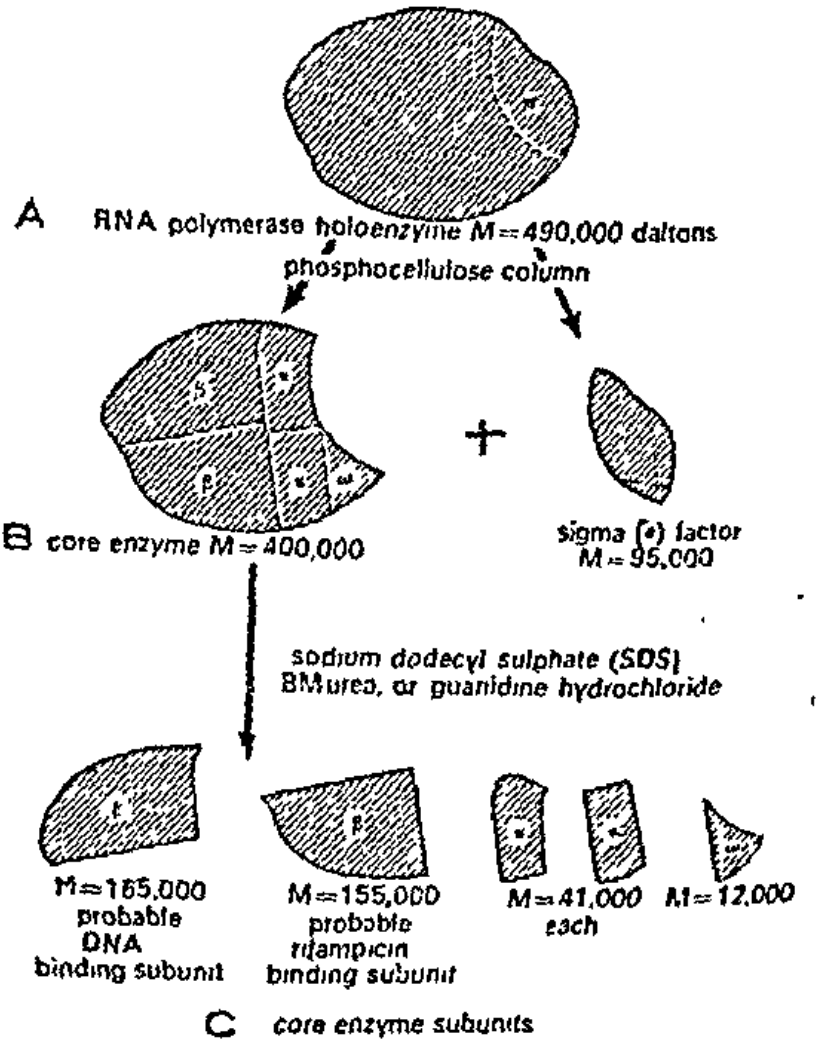


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Types and synthesis of rRNA : The eukaryotic cells have three kinds of rRNA molecules, namely **28S rRNA** (the sedimentation constant varies between 25S and 30S depending on the species), **18S rRNA** and **5S rRNA**. The 28S rRNA and 5S rRNA occur in 60S ribosomal snbunit, while 18S rRNA occurs in 40S ribosomal subunit of 80S ribosomes of eukaryotes. The prokaryotic cells also contain three kinds of rRNA molecules, namely **23S rRNA, 16S rRNA** and **5S rRNA**. The 23S rRNA and 5S rRNA occur in 50S ribosomal subunit, while 16S rRNA occur in 30S ribosomal subunit of 70S ribosomes of prokaryotes. In eukaryotic cells of plants and animals the 18S and 28S rRNA molecules are transcribed by rDNA of nucleolar organizer (No) region of chromosome and nucleolus acts as a site of synthesis and maturation of these rRNA molecules. 5S rRNA is transcribed by rDNA which occur outside the nucleolus organizer region (For details see the chapter of ribosomes.)

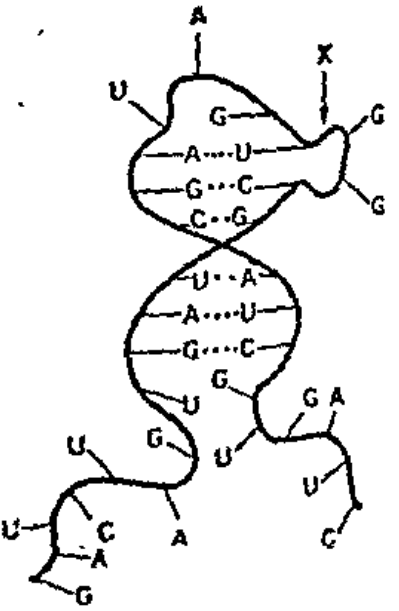


Fig. 5·2. The molecular (i.e., secondary) structure of rRNA showing a helical region with complementary base pairing and a looped outer region of the helix at X (Davidson, 1972)

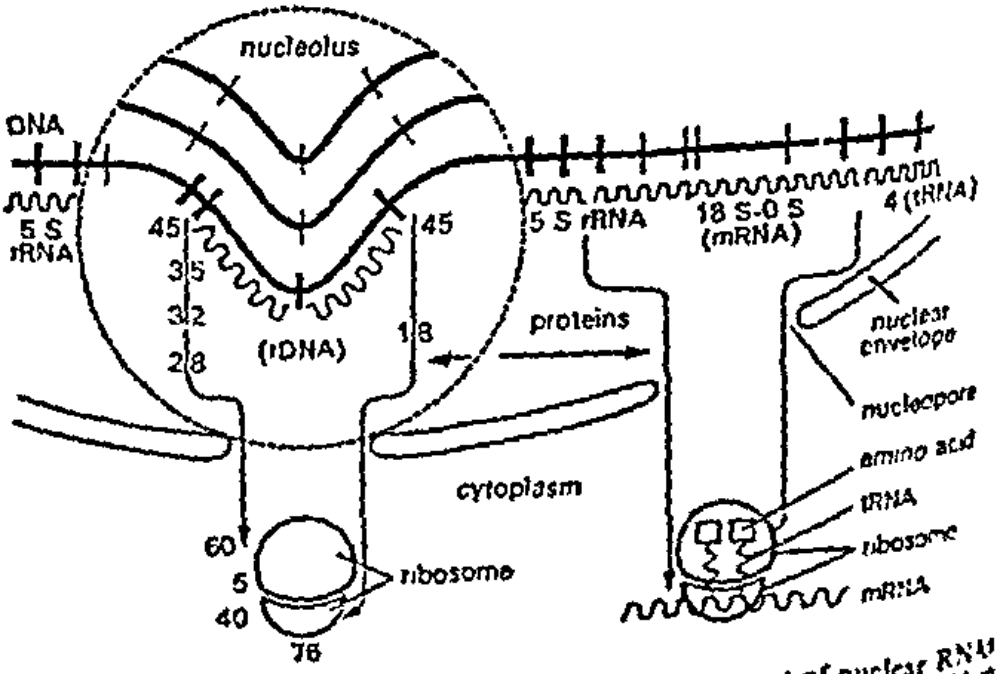


Fig. 5·3. Diagram of the transcription and transport of nuclear RNA in a eukaryotic cell and participation of tRNA and mRNA in protein synthesis.

The functions of rRNA molecules are still little understood. However, they are supposed to have some definite role in protein synthetic process, besides being the major constituent molecules of ribosomes.

(II) MESSENGER RNA (mRNA)

The RNA molecules which are transcribed from large number of genes of the total genome (*i.e.*, 99 per cent genes of the total genome of *E coli*), and have base sequence complementary to DNA,

associated to par

are called inform

messenger RNA has been proposed by **Jacob** and **Monod** (1961). The molecule of a mRNA is single-stranded like the rRNA molecule and it is DNA-like in its base composition so that GC contents of mRNA correspond to the GC contents of the genomes total DNA.

HETEROGENEITY AND TYPES OF mRNA

When the total mRNA population of an organism is considered, it is found to be heterogenous in size, showing a wide range of S values of 6 to30 This property of mRNA reflects the fact that the size or length of the mRNA molecule is directly related with the size of the codons for different protein molecules, the sizes of which may be quite variable. According to the size, following two types of mRNA molecules can be recognized :

(a) Monocistronic mRNA : Mostly the mRNA carries the codons of single cistron (*i.e.*, codes for one complete protein molecule) of the DNA. Such mRNA molecule is called **monocistronic mRNA**. For example, for the synthesis of a polypeptide chain of 300 to 500 amino acid residues, a monocistronic mRNA of *E.coli* contains 900 to 1500 nucleotides in its molecule.

(b) Polygenic or polycistronic mRNA: Sometimes a mRNA molecule carries the codes from several adjacent DNA cistrons and become much longer in size. This type of mRNA is called **polygenic or polycistronic mRNA**. For example, for the metabolism of the histidine protein the cell synthesizes about 10 specific enzymatic proteins and a mRNA in this case may carry codons for all the 10 enzymes.

LIFE-SPAN OF mRNA

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zyme. So that, at most times, mRNA makes up only 5% of the total cellular RNA. Likewise, in most eukaryotes the average life span of mRNA is one to four hours. However, in both bacteria and eukaryotes mRNAs are known that are apparently resistant to nucleases and survive for long period of time. For example, mRNA with life time of six hours has been detected in the bacterium *Bacillus cerus* at a time when the cells are induced to become long lived spores. Likewise, in differntiating eukaryotic cells mRNAs with a life time of days have been detected. For example, in the immature red blood cells (reticulocytes) of the mammals the mRNA is synthesised originally by the nucleus in early stages and expelled to the cytoplasm. In later stages the nuclei of maturing reticulocytes degenerate but the mRNA exists upto 2 days for prolong utilization in the synthesis of globin protein of hemoglobin. Further, in extreme cases, such as in the state of dormancy adopted by many animal eggs and plant seeds, mRNA is maintained in a stable form for months or even years.

Informosomes—In the eukaryotic cells, the stability to the mRNA is provided by certain proteins. Several investigators, *e.g.*, **Spirin, Beltisina** and **Lerman** (1965), **Perry** and **Kelley** (1968) and **Henshaw** (1968) have reported that in certain eukaryotic cells the mRNA does not enter in the cytoplasm as a naked RNA strand but often remains ensheathed by certain proteins. **Spirin** has coined a new term **informosome** to this mRNA and protein complex. The informosome is used by the cell when there is a delay in the translation. For instance, in the embryo the genetic expression is manifested late during organogenesis. In such cases mRNA occurs in the form of informosomes. The proteins of informosomes protect the mRNA from the degrading actions of the enzyme, ribonuclease. These proteins may also control the synthesis at the level of the translation and thus may regulate or modulate the protein synthetic process.

(III) TRANSFER RNA (tRNA)—

The RNA which [illegible] o combine specifically ted by a set of amino synthetases ; transfers to the site of protein NA is known as the soluble RNA (sRNA) or transfer RNA (tRNA). Thus, tRNA molecule has to perform several highly complex functions during protein synthesis—it interacts with a specific synthetase enzyme, possesses a site for binding an amino acid, possesses a second site for interacting

with a ribosome, and contains an anticodon that must be exposed to the codons of mRNA.

STRUCTURE AND MATURATION OF tRNA

The tRNA molecules that perform all these functions are relatively small : they contain only 70 to 80 nucleotides so that each has a molecular weight of about 30,000 and a sedimentation coefficient of 4S. Like the rRNA molecules, the molecules of tRNA are found to be matured or tailored in the nucleus prior to their movement to the cytoplasm. For example, in *E. coli* the precursors of tRNA molecules have been isolated, each of which has about 40 extra-nucleotides, principally at 5′ end but also at the 3′ end. These extra nucleotides are subsequently cleaved off by still unidentified enzymes to yield a molecule of the final 70 to 80 nucleotide size.

Following such large scale tailoring each tRNA molecule is more delicately modified before it becomes fully active. Three bases, 5′-CCA-3′ are added to the 3′ end of every tRNA molecule, regardless of its amino acid affinity, by an enzyme called **tRNA phosphorylase.** In addition, specific nucleotides in a given tRNA are transformed into what are called **minor** or **unusual bases** by specific chemical modifications : for example, methyl groups are added at certain positions to yield 3-methyl-cytosine or 1-methyl-guanosine ; certain uracils are reduced to dyhydrouracil or rearranged into a form, known as pseudouracil ; and adenine is deaminated to yield inosine.

The significance of these unusual bases of tRNA was understood well by molecular biologists during the construction of two-dimensional model from the the primary-sequences of nucleotides of known tRNA. Thus, it was realized that most bases of tRNA pair according to Watson-Crick's pairing rule, but, unusual bases fail to do so because they carry substitutions or alterations in those positions that usually participate in hydrogen bonding. Consequently, the presence of these bases forces the model builder to construct several non-base-paired loops in the tRNA molecule. By working on these lines, R. Holley (1968) first of all proposed a **clover leaf model** for yeast $tRNA^{ala}$. The clover leaf model of tRNA because accommodated several of the known functions of tRNA, so, it gained general acceptance. A typical clover-leaf model of tRNA (Fig. 5·4) depicts following structural peculiarities :

(i) All tRNA molecules contain the same terminal sequence of 5′-CCA-3′ bases at 3′-end of the polynucleotide chain. The last residue, adenylic acid (A), is the **amino acid attachment site.**

(ii) All tRNAs have a loop called Tψ arm of seven unpaired bases including pseudouridine. The Tψ arm is involved in the binding of tRNA molecules to the ribosomes.

(iii) All tRNA molecules contain a site for the recognition of the amino acid activating synthetase enzymes. This is the function of dihydrouridine loop or DHU arm which is made up of 8 to 12 unpaired bases.

(iv) There is one nucleotide triplet in the tRNA molecule which is different in all tRNAs examined. This is the codon recognition site or anticodon and it is complementary to the corresponding triplet codon of mRNA.

(v) Some tRNA with long chains may form a short, extra arm.

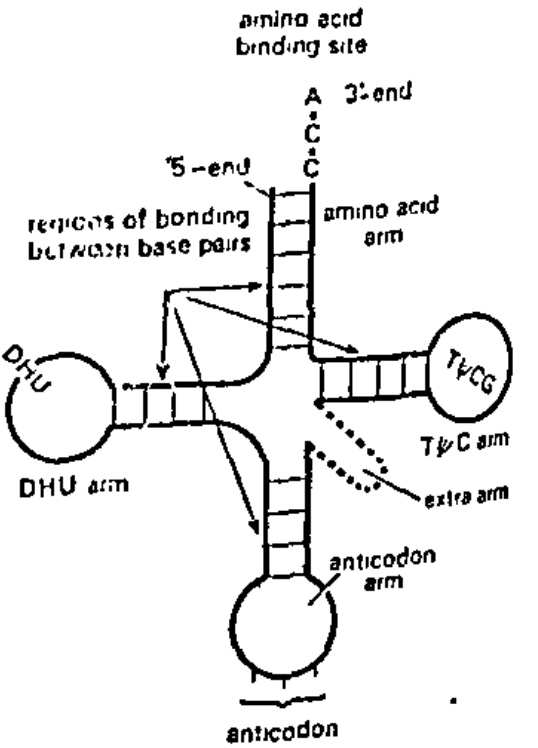


Fig. 5·4. Diagrammatic representation of clover leaf model of yeast alanine tRNA. (ψ—pseudouridine ; DHU—dihydrouridine).

As the clover leaf model is in two-dimensions, whereas the tRNA-synthetase enzyme interaction occurs between molecules that are three dimensional. Therefore, very recently S. H. Kim and coworkers (1973) have proposed a three-dimensional L-shaped configuration for a phenylalanine tRNA molecule of yeast cells. According to this model, the molecule of tRNA forms two main portions that are more or less at right angles to each other, with each portion doubled over by bonds holding complementary base together.

Genes for tRNA—There are probably at least 30 to 40 different tRNA genes and tRNA molecules in *E. coli*. Higher organisms are found to contain 60 tRNA molecules and 60 tRNA genes. Since the cell uses only 20 amino acids in protein synthesis (and probably only 20 synthetase enzymes), it follows that several tRNAs will often have an affinity for the same amino acid For example, *E. coli* cells contain five species of tRNA for leucine amino acid.

All the tRNA genes constitute for less than 1% of the total genome in both *E. coli* and eukaryotic cells, yet some 10 to 15% of

each cell's RNA may be in the form of tRNA. This discrepancy between the number of tRNA genes and gene transcripts occurs because of following facts—(1) The tRNA molecules are relatively stable compared with many kinds of RNA. (2) The tRNA molecules are transcribed continuously and more quickly by tRNA genes than other RNAs because they are needed in plentiful amounts.

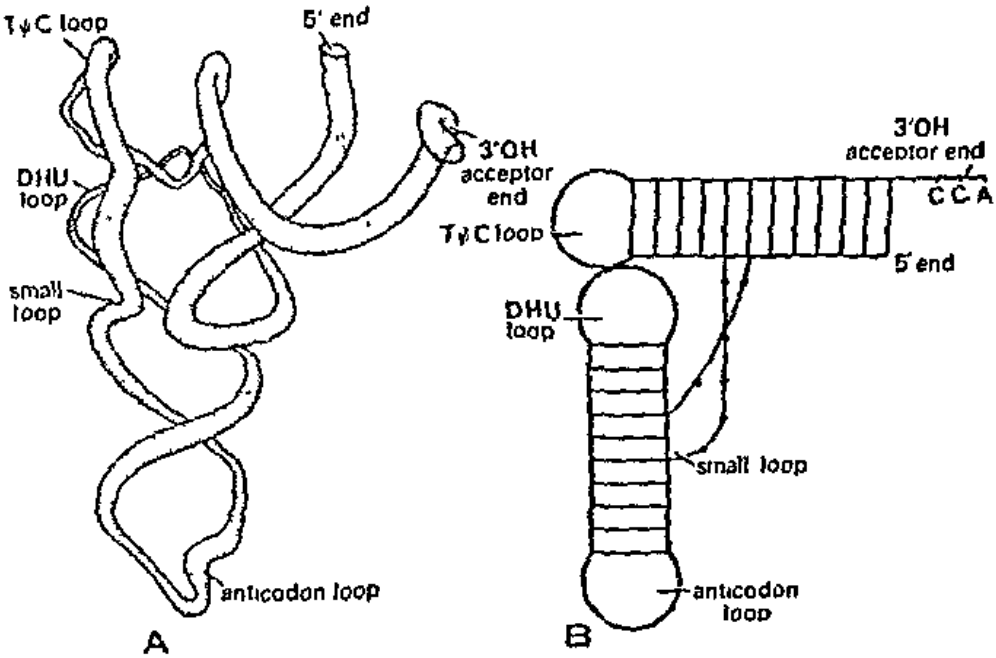


Fig. 5 5. Three dimensional structure of yeast phenylalanine tRNA. A—Actual appearance of the folding of the molecule. The polynucleotide chain is represented as a continuous coiled tube. B—The way that the cloverleaf representation must be transformed in order to show the physical connections between various parts of the molecule. Two double-stranded helical regions are seen, each oriented at right angles to the other produce an L-shaped structure.

(IV) HETEROGENEOUS NUCLEAR RNA

The eukaryotic neucleus synthesizes numerous, enormous, and *heterogeneous* RNA *molecules that are degraded shortly after they* are synthesized; and which do not engage in protein synthesis, remain restricted to the nucleus and are called heterogeneous nuclear RNA *het* RNA. *het* RNA shares several properties with mRNAs: it commonly has a GC content similar to that of genome DNA; it is heterogeneous in size and it hybridizes with a considerable fraction of the genome. Unlike mRNA, it varies in size from 10 to 200S and lar ge fraction of it do not leave nucleus.

het RNA molecules are supposed to serve as precursors for mRNA. Just as there is a tailoring of precursor rRNA and tRNA

molecules, so there is believed to be a tailoring of large RNA transcripts, in the eukaryotic nucleus to produce mature mRNA molecules. By this hypothesis, then, *het* RNA would represent the original large transcripts plus the non-message fragments that are cleaved off. Recent experiments of **J. Darnell** (1971) indicate that about 200 adenine residues **(polyadenylic acid or poly A)** are attached to the 3′—OH end of several eukaryotic mRNAs, similar sequences are also attached to the 3′—OH end of much *het* RNA. **Darnell** and others (1970, 1971) therefore suggested that the following sequence of events occur in the eukaryotic nucleus : an mRNA precursor molecule is first synthesized ; a poly A segment is then some how added to its 3′—OH end, triggering the separation of mRNA (with the poly A attached) from the rest of the molecule, which remains in the nucleus as *het* RNA. Poly A is speculated to serve in the migration or translation of mRNA.

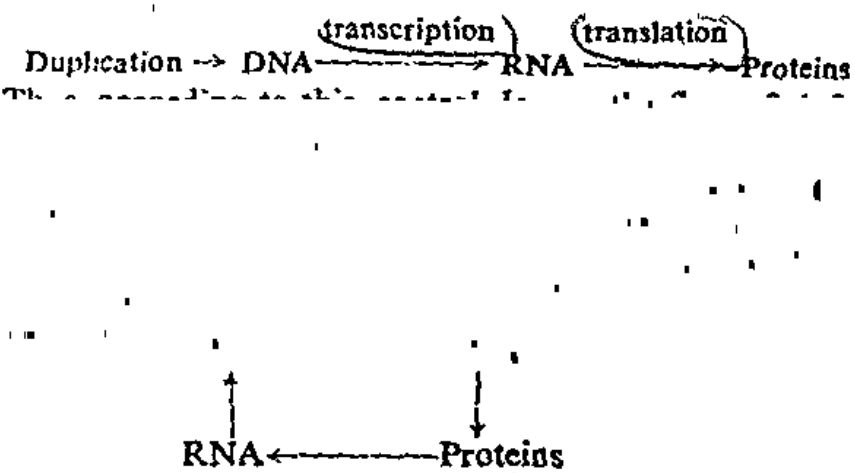
CHAPTER 6

BIOSYNTHESIS OF PROTEINS

The proteins have significant role in the structural and functional organisation of the cell. The structural proteins constitute various cellular components and some extracellular parts such as cuticle and fibres, etc. [illegible] control [illegible] regulating, proteins wh[illegible] synthesized by the cell itself intracellularly.

A cell, however, cannot synthesize all the required proteins of multicellular individual, therefore, different kinds of proteins are synthesized by different types of cell. Thus, the liver cells are specialized for the synthesis of liver and plasma proteins, the β-cells of pancreatic islets are specialized for synthesis of proteins of insulin hormone and cells of pituitary are specialized for synthesis of protein component of pituitary hormone and so on.

CENTRAL DOGMA AND CENTRAL DOGMA REVERSE



Very recently Temin (1970) reported the existence of an enzyme "RNA dependent DNA polymerase" (inverse transcriptase) which could synthesize DNA from a single stranded RNA template. D. Baltimore (1970) also reported the activity of this enzyme in certain RNA tumour viruses. This exciting finding in molecular biology gave rise to the concept of "central dogma reverse" suggesting that the sequence of information flow is not necessarily from DNA to RNA to protein but can also take place from RNA to DNA (see Mathur in Science Reporter, Vol. 12, No. 4, April 1975). The central dogma reverse can be illustrated as follows :

$$\text{DNA} \underset{\text{inverse transcription}}{\overset{\text{transcription}}{\rightleftharpoons}} \text{RNA} \xrightarrow{\text{translation}} \text{Proteins}$$

COMPONENTS OF PROTEIN SYNTHETIC MACHINERY

The process of protein synthesis is complicated and it employs following molecules and cellular organelles :

1. Amino acids,
2. DNA,
3. Non-genetic ribonucleic acids (RNAs),
4. Ribosomes and enzymes.

1. AMINO ACIDS

Because, the proteins are the polymers of amino acids therefore, protein synthetic process requires the amino acids as the raw material. As we have already discussed in the chapter of cytoplasmic matrix, all the naturally occurring proteins of living organisms fundamentally are the polymers of about 22 amino acids. These amino acids occur in the matrix forming an "amino acid pool" and are readily available for the process of protein synthesis.

2. DNA

The chromosomal, mitochondrial or chloroplastic DNA is the master macromolecule of the cell. Its immediate function is the determination of the kind of the protein which a cell has to manufacture In other words, the process of protein synthesis is initiated, guided, regulated, and controlled by the DNA molecule.

3. NON-GENETIC RIBONUCLEIC ACIDS (RNAs)

In the previous chapter three kinds of non-genetic RNAs, (*i.e.*, rRNA, mRNA and tRNA) have been described. All these three kinds of RNAs take an active part in protein synthesis. Thus, mRNA carries coded informations for protein synthesis—from DNA to the site of protein synthesis, the ribosomes; tRNAs transport activated amino acids from 'amino acid pool' to the site of protein synthesis where they recognize the triplet codons of mRNA and get attached

with that and lastly, rRNA though having no specific function at its disposal like mRNA and tRNA, but there are ample-evidences which suggest that rRNA has a general function to perform.

4. RIBOSOMES AND ENZYMES

The ribosomes play a significant role in the process of protein synthesis, as they provide the site or place to the latter and they contain required amount of enzymes and protein factors (about 50 species of ribosomal proteins) which are needed time to time by protein synthetic process.

Evidence for ribosomes as sites of protein synthesis—The first direct evidence relating ribosomes and protein synthesis was described by Zamecnik and coworkers in a series of investigations from 1952 onwards. Radioactive amino acids were injected into rats and at various time intervals after the injection the liver was removed, homogenized and centrifuged to separate the various cell organelles, which were studied for evidence of incorporation of amino acids into proteins. If examined shortly after injection, only the microsomal (ER) fraction contained labelled protein whereas at later times labelled protein was found in all cell fractions. These results suggested that proteins were first synthesized in the microsomes and then transferred to other parts of the cell. It was later found that isolated microsomal fractions, which were supplemented with ATP, catalysed the incorporation of amino acids into proteins in the presence of the unsedimentable, soluble fraction which provided essential cofactors. Since this pioneer work, cell free systems capable of catalysing amino acid incorporation into protein have been isolated from a variety of species and have been shown to require the following components : ribosomes, the twenty common amino acids, tRNAs, mRNA and ATP generating system, GTP, Mg^{++} and K^{+} ions and a supernatant enzyme fraction.

OTHER FUNCTIONS OF RIBOSOMES

The functions and other activities of the ribosomes and their subunits are given as follows :

1. Association and dissociation of the ribosomal subunits : The two ribosomal subunits may exist separately and freely in the cytoplasm before and after the process of protein synthesis. In the beginning of the process of protein synthesis both the subunits become associated and at the end of the process both the subunits become dissociated. This type of association and dissociation in the ribosomal subunits have been studied in various types of cells by different workers, *e.g.*, in *E. coli* and in yeast cells by Kaempfer (1968), in the reticulocytes by Colombo, Vesco and Baglioni (1968) and in the liver cells by Farber (1969).

2. Function of 30S subunit—The 30S subunit provides the site for the binding of the mRNA to it. The mRNA binds with the 30S subunit by its first codon and forms a **initiation complex.** The initiation complex is composed of 30S-formylmethionyl tRNA, guanosione triphosphate (GTP) and AUG or GUG triplet codons of mRNA.

The 30S subunit contains many protein factors called **initiation factors, *viz.*,** F_1, F_2, and F_3 which help in the initiation of the process of protein synthesis. These protein factors also help in the elongation and termination of the protein molecules. The molecular weights of the protein factors F_1, F_2 and F_3 are 8,000, 80,000 and 30,000 respectively. The F_1 factor is used in the binding of N formylmethionyl -tRNA with the AUG or GUG codes of mRNA, the F_2 factor binds with the GTP and F_3 factor binds the mRNA with the 30S subunit and is involved with the dissociation of ribosomes into subunits.

3. Function of 50S subunit—The formation of polypeptide chain occurs in the 50S ribosomal subunit. The 50S subunit is supposed to contain two binding sites, *viz.*, **amino acyl tRNA site** ('A' site) and **peptidyl tRNA site** ('P' site) for the tRNA. The tRNAs which carry activated amino acids along them enter in the 50S subunit from one side and attach with the amino acyl tRNA site. After leaving the amino acids to peptidyl tRNA site, the tRNAs exist from other side The peptidyl tRNA site contains the tRNA along with growing polypeptide chain. **W. D. Stansfield** (1969) has presumed three sites in the ribosome (i) a **decoding site** which binds the loaded tRNA with the mRNA by base pairing, (ii) a **condensing site** which joins the amino acid to the growing polypeptide chain and (iii) an **exit site** ('E'site) at which the tRNA datackes from the polypeptide, mRNA and ribosome. The 50S subunit contains an enzyme known as the **peptidyl synthetase** which helps in the formation of the peptide bond **(Monro, 1967).**

4. Formation of polysomes or polyribosomes—During the process of protein synthesis because the codes of mRNA are read by many ribosomes, therefore, as soon as a ribosome has translated perhaps 25 codons of mRNA, the 5′ end of the mRNA becomes free to form a second initiation complex and a second ribosome begins moving along the messenger, mediating the synthesis of a second polypeptipe chain. A third ribosome follows, and so on. The resulting structure, called a **polysome or polyribosome,** consists of an mRNA molecule which is being simultaneously translated by ribosomes into several polypeptide chains.

In a polysome each ribosome lies at a distance of 340A° from each other. The number of the ribosome in a polysome depends on the length of the mRNA. For instance, a polysome with the mRNA of 900 nucleotides contains 8 to 12 ribosomes in it and synthesizes a protein molecule of 35,000 molecular weight. The polysomes of *E. coli* and cells of chick embryos composed of 50 unit ribosomes (Rich, 1967). The polysomes may occur freely in the cytoplasm or may remain attached with the endoplasmic reticulum or other membranes.

5. Function of 60S subunit—It has been reported by **Halliman, Murty and Grant** (1968) that the ribosomes which remain attached with the endoplasmic reticulum synthesize proteins more efficiently than the free ribosomes.

In the 80S ribosome of eukaryotic cells, the large subunit 60S often remains attached with the membrane of the endoplasmic reticulum. The 60S subunit remains separated from the 40S by a groove. The 60S ribosomal subunit contains a central channel or space by which this groove remains connected with the canal of endoplasmic reticulum. Now it is supposed that the protein synthesis occurs in the groove between the two subunits and the newly formed polypeptide chains are transported to endoplasmic reticulum via the channel or tunnel of 60S subunit where these proteins remain well protected from the enzymatic actions of proteolytiy enzymes of the cytoplasm.

MECHANISM OF PROTEIN SYNTHESIS

The mechanism of protein synthesis has been investigated most throughly in bacteria (usually *E. coli*), therefore, the process of protein synthesis of *E. coli* which contains 70S ribosomes will be described in more detail and then this will be discussed in relation to our present knowledge of protein synthesis on 80S ribosomes of plants and animals.

A. PROTEIN SYNTHESIS ON 70S RIBOSOMES

The mechanism of protein synthesis on 70S ribosomes includes following stages :

1. THE TRANSCRIPTION

The process of protein synthesis is started by the uncoiling of strands of DNA molecule. One strand of DNA molecule acts as a template for the formation of mRNA. The mRNA is formed according to the triplet codes of DNA by the copying or transcription process. As soon as the mRNA is formed it leaves the nucleus and reaches in the cytoplasm where it attaches with the 30S subunit of the riobosomes. The mRNA carries the triplet codons for the synthesis of the proteins.

2. THE ATTACHMENT OF mRNA WITH 30S RIBOSOMES AND FORMATION OF POLYRIBOSOME

In prokaryotic cells it has been observed that before the process of protein synthesis the ribosomes occur in dissociated and inactive state. The mRNA binds with the 30S ribosomal subunit in the presence of F_2 protein factor. Soon the N-formylmethionyl-tRNA (F-met $tRNA_f$ comes from the cytoplasmic "amino acid pool" and

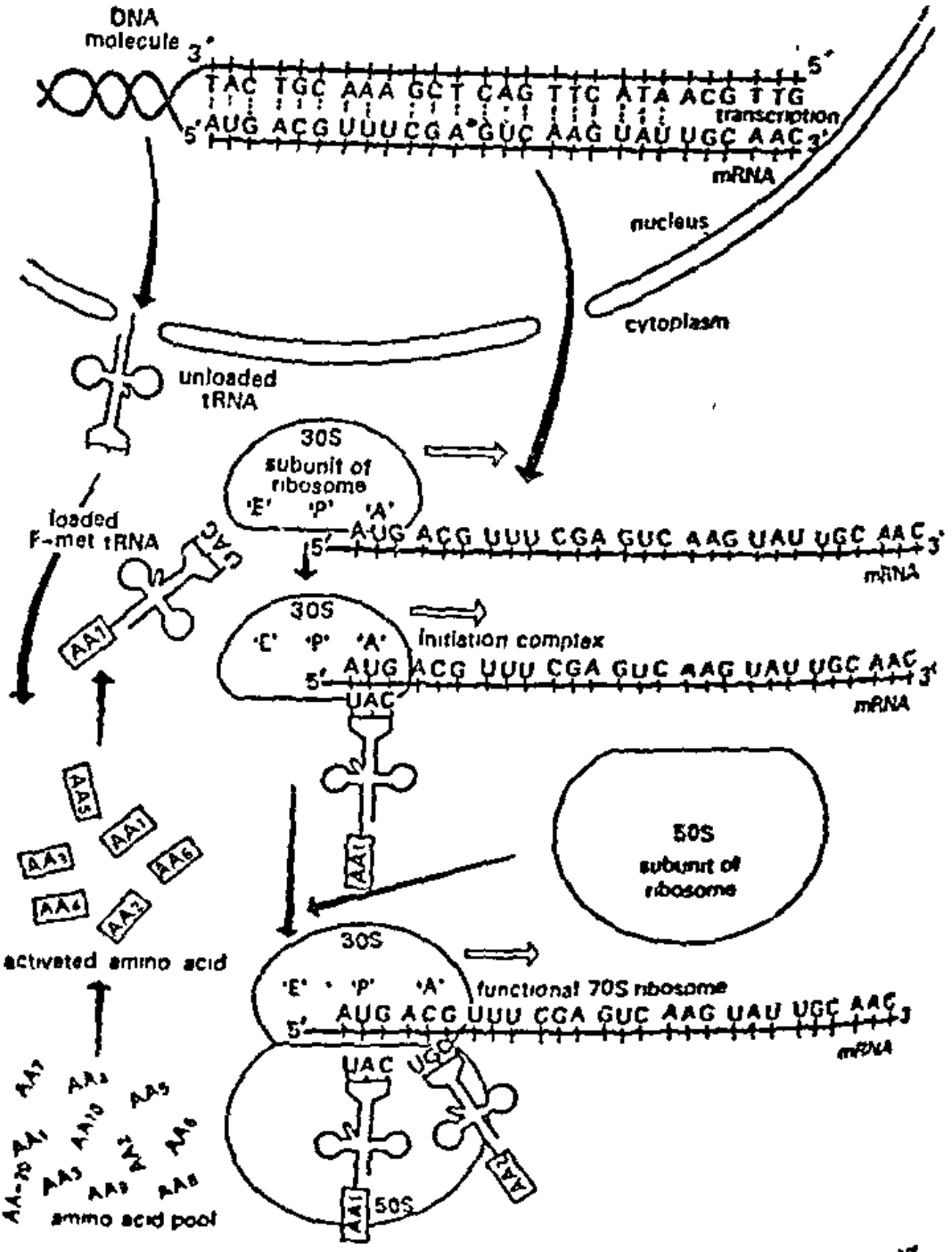


Fig 6.1. [illegible] steps of the process
A [illegible] site or decoding
[illegible] exit site.

binds with the first triplet codon (AUG or AUA) of the mRNA to initiate the process of protein synthesis and to form the **initiation complex.** The formation of initiation complex is aided by the GTP (guanosine triphosphate) and 3 protein factors (F_1, F_2 and F_3). After the formation of initiation complex the 30S ribosomal subunit unites

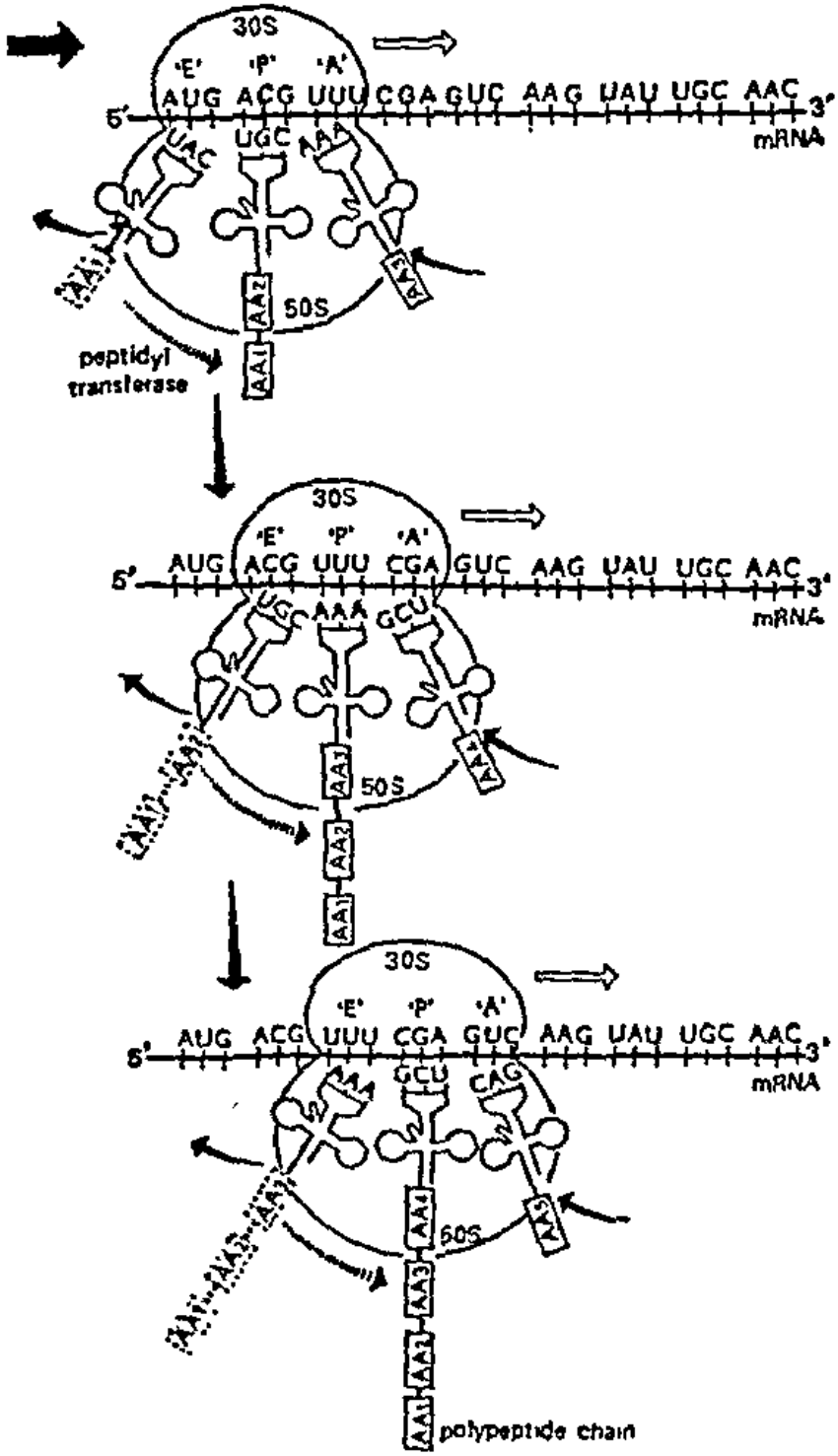


Fig. 6 2. Diagrammatic representation of some later stages of process of protein synthesis of *E. coli*. AA_3=phenylalanine, AA_4=arginine AA_5=valine, AA_6=lysine, (for rest of the abreviations consult Fig. 6·1).

with 50S ribosomal subunit to form the 70S ribosome. The union of ribosomal subunits occurs in the presence of Mg^{++} ions and F_1 and F_2 factors. The codes of mRNA are not read only by one ribosome but many ribosomes move and read the codes of the mRNA. When many ribosomes bind with the mRNA the formation of polysome or polyribosome occurs.

3. THE TRANSFER OF AMINO ACIDS TO THE SITE OF PROTEIN SYNTHESIS

The amino acids are transferred from the intra-cellular amino acid pool to the ribosomes by the tRNA. The transfer processes occur in various stages which are as follows :

(a) Activation of amino acids—Each of the 20 amino acids occur in the cytoplasm in an inactive state. Each amino acid before its attachment with its specific tRNA is activated by a specific activating enzyme known as the **aminoacyl synthetase** and ATP. The free amino acids react with ATP, resulting in the production of aminoacyl adenylate and pyrophosphate :

$$AA + ATP + Enzyme \longrightarrow AA \sim AMP\text{--}Enzyme + PP$$

Amino acid	Aminoacyl synthetase	Aminoacyl adenylate enzyme complex	Pyrophosphate

The reaction product aminoacyl adenylate is bound to the enzyme in the form of a monocovalent complex. This aminoacyl adenylate enzyme complex then esterifies to specific tRNA molecule.

The cell has at least 20 aminoacyl synthetase enzymes for the 20 amino acids. Each enzyme is specific and it attaches with the specific amino acid without any error.

(b) Attachment of activated amino acid to tRNA—The aminoacyl adenylate remains bounded with enzyme until it collides with the specific tRNA molecule and its synthetase is recognized by dihydrouridine (DHU) loop of specific tRNA. Then, amino acid residue of aminoacyl adenylate is transferred to amino acid attachment site of tRNA where its carboxyl group forms bond or linkage with the 3-OH group of the ribose of the terminal adenosine at CCA end of tRNA. As a result AMP and enzyme are released and a final product aminoacyl tRNA is formed by the following method :

$$AA\text{—}AMP\text{—}Enzyme + tRNA\text{—}AA \rightarrow tRNA + AMP + Enzyme$$

Aminoacyladenylate and enzyme Aminoacyl–tRNA

The aminoacyl-tRNA moves towards the site of protein synthesis, *i.e.*, ribosomes with mRNA.

4 INITIATION OF PROTEIN SYNTHESIS

As we have already mentioned that the initiation of protein synthesis in the bacterium *Escherichia coli* (*E.coli*) involves the for-

mation of 70S complex. In it the mRNA always has first triplet codon as AUG at its beginning (*i.e., 5'* end). The AUG codons are the codes for the amino acid methionine. The methionine remains formylated ard it has a very important role in initiating the process of protein synthesis. In every type of protein the formyl methionine occupies the first place in the molecule and when the protein molecule is completely synthesized then the formyl methionine often detaches from the newly synthesized protein molecule by the activity of a hydrolytic enzyme.

Since in protein synthesis the peptide chain always grows in a sequence from the free terminal amino (—NH_2) group towards the carboxyl (—COOH) end, the function of formyl methionine—tRNA (AA_1—tRNA in fig. 6·1 and 6·2) is to ensure that proteins are synthesized in that direction. In the formyl methionine—tRNA, the amino (—NH_2) group is blocked by the formyl group leaving only the —COOH group available to react with the—NH_2 group of the second amino acid (AA_2). In this way the synthesis of protein chain follows in the correct sequence.

5. ELONGATION OF POLYPEPTIDE CHAIN

With the formation of functional 70S ribosome (*i.e.*, 70S—mRNA—F met tRNA), the elongation of polypetide chain is brought about by the regular addition of amino acids and relative movements of ribosome and mRNA in the presence of GTP molecules, so that a new triplet codon remains available for new aminoacyl tRNA at the decoding or 'A' site of ribosome in each step. Thus, F-met tRNA must move from decoding site ('A' site) to peptidyl site or 'P' site, before the second aminoacyl-tRNA (*i.e.*, AA_2 tRNA) can bind to the next triplet codon occurring at decoding or 'A' site of ribosome. The aminoacyl-tRNA (AA--tRNA) binds with the codon of 'A' site in the presence of GTP and two proteins, called **transfer factors** (designed Tn and Ts) which remain associated with ribosome. During this binding process, a complex is formed between GTP, the transfer factors and the aminoacyl-tRNA (*viz.*, AA_2-tRNA), which ultimately deposits aminoacyl-tRNA at the 'A' site of ribosome with the release of transfer factors-GDP complex and inorganic phosphate.

In the next step, due to relative movement of ribosome and mRNA in the presence of single GTP molecule, the next triplet codon (*i,e.*, UUU codon in fig. 6·1 and 6·2) becomes available for next aminoacyl tRNA (*viz*, AA_3-tRNA) at the 'A' site of ribosome. At this stage, f-met-tRNA occurs at exit or 'E' site, while, AA_2-tRNA occurs at peptidyl or 'P' site. Now, an enzyme known as

transferase I kicks off tRNA from formyl-methionine (f-met or AA_1) and flips the formyl-methionine to the aminoacyl-tRNA (AA_2-tRNA) bound at the peptidyl or 'P' site. The 'G' factor is supposed to release the discharged or deacylated tRNA from the 'E' site of the ribosome.

It follows the next stage of elongation process which involves the synthesis of a peptide bond by a reaction (peptidyl transferase reaction) between the free amino group of the incoming amino acid (*i.e.*, AA_2) and the carboxyl group of the first amino acid (AA_1) which is esterified to tRNA. The enzyme which catalyses this reaction is called peptidyl transferase (or peptide synthetase) and is an integral part of the 50S sub-unit. The energy for peptide bond synthesis is derived from cleavage of the ester link between an amino acid and its tRNA.

Thus, during the elongation of polypeptide chain each charged or loaded tRNA (aminoacyl tRNA) enters the decoding or 'A' site, moves to the condensing or 'P' site, transfers its amino acid to the carboxyl end of the polypeptide, moves to the exit site, where the polypeptide chain is transferred to the adjacent tRNA on the condensing site, and tRNA is then released from the ribosome. This sequence of events involved in elongation must take place very rapidly since it has been calculated that, in *E. coli* growing under optimal conditions, a polypeptide chain of about 40 amino acids can be produced in 20 seconds.

6. TERMINATION AND RELEASE OF POLYPEPTIDE CHAIN

The synthesis of a polypeptide chain is concluded when a given ribosome in a polysome encounters a genetic signal encoded in the mRNA which specifies that the C-terminal amino acid of a polypeptide has been added to the chain. At least one of the termination signals is the presence of one or more terminator codons in the mRNA. A terminator codon is not recognized by the anticodons of any of the normally occurring aminoacyl-tRNAs, and its presence in the decoding or aminoacyl site precludes the addition of any further amino acids to the chain. In *E. coli*, its phages and in eukaryotes, the RNA triplet UAA, UGA and UAG all function as terminator codons.

When a terminator codon moves into an aminoacyl site, the following events are thought to occur. The terminator first interacts with one of two specific protein factors called R, and the R-terminator codon-ribosome complex effectively blocks further chain elongation. With the aminoacyl site so clogged, the completed polypeptide remains esterified to the final tRNA occupying the peptidyl

site. This linkage is then broken by
ted by still another protein factor, a
and a complete polypeptide are rele
ribosome then dissociates into its large and small subunits, an event that may be mediated by F_3 protein factor. The dissociated subunits are now free to form new initiation complexes and participate in another round of polypeptide synthesis.

7. MODIFICATION OF RELEASED POLYPEPTIDE

The released polypeptide chain contains the formylated methionine at its one end. An enzyme deformylase removes the formyl group of methionine. The exopeptidase enzyme may remove some amino acids from N-terminal end or the C-terminal end of polypeptide chain. At this stage the polypeptide (protein) possesses its primary and probably its secondary structures. The linear sequence of amino acids forms the primary structure ; at least some portion of many proteins have a secondary structure in the form of an alpha-helix. The protein chain may then fold back upon itself, forming internal bonds (including strong disulfide bonds) which stabilize its tertiary structure into a precisely and often intricately folded pattern. Two or more tertiary structures may unite into a functional quarternary structure. For example, haemoglobin consists of four polypeptide chains, two identical α-chains and two identical β-chains. A protein does not become an active enzyme until it has assumed its tertiary or quarternary pattern.

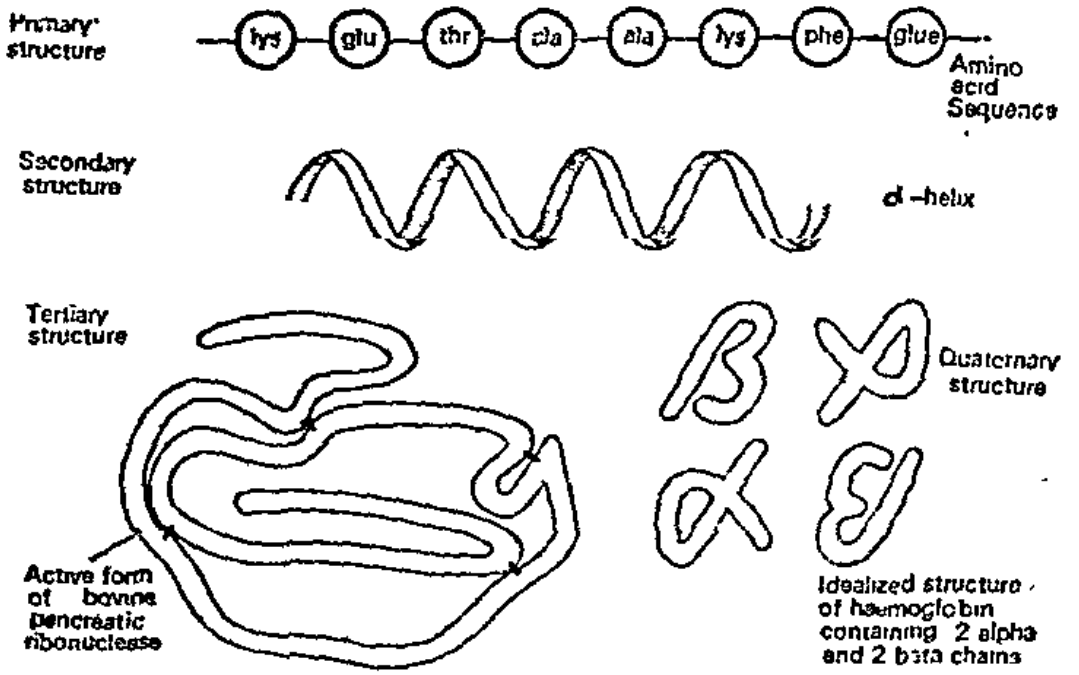


Fig. 6·3. Stages in the development of a functional protein.

3. PROTEIN SYNTHESIS ON 80S RIBOSOMES

The process of protein synthesis of eukaryotes is found to be similar to the mechanism of protein synthesis on 70S ribosomes of prokaryotes (see Allende, 1969 ; Boulter, Ellis and Yarwood, 1972 ; Hall *et al.*, 1974 for full details of protein synthesis on 80S ribosome). However, the process of initiation of polypeptide of 80S ribosomes of eukaryotes, differs with that of prokaryotes, in following two aspects :

1. In eukaryotes, formylation of methionine does not take place.
2. In eukaryotes, the smaller ribosomal subunit associates with met-tRNA$_f^{met}$ without the help of mRNA.

The initiation process of eukaryotes(*e.g.*, mammals, wheat and bean) is found to include following stages :

(i) 40S+met-tRNA$_f^{met}$ ⟶ 40S-met-tRNA$_f^{met}$

(ii) 40S−met-tRNA$_f^{met}$+mRNA→40S-mRNA-met-tRNA$_f^{met}$

(iii) 40S−mRNA-met-tRNA$_f^{met}$+60S→80S-mRNA-met−tRNA$_f^{met}$

RELATING TO GENES PROTEINS

It often becomes useful in genetic analyses to be able to determine by quick, rule-of-thumb estimates how much DNA corresponds to a gene, how many triplets are needed to specify a protein of a certain molecular weight and so on. In following table (after Goodenough and Levine, 1974) we have summarized some figures for maping such estimates :

Table : Method of relating genes with proteins.

1. Average nucleotide=600 daltons molecular weight.
2. Typical gene=1000 nucleotides $\simeq 0{\cdot}7\times10^6$ daltons of single-stranded DNA.
3. 1μ duplex DNA $\simeq 2\times10^6$ daltons of DNA $\simeq 1\times10^6$ daltons single strand DNA.
4. Typical mRNA=1000 nucleotides=300 triplet codons→polypeptide with 333 amino acids and a molecular weight of about 40,000 daltons.

A GENETIC PERSPECTIVE OF PROTEIN SYNTHESIS

Our account of the mechanisms of protein synthesis in prokaryotes and eukaryotes has conveyed that translation of a mRNA sequence into a polypeptide requires exquisitely elaborate interactions between a host of nucleic acids and proteins. If we include the 50-odd ribosomal proteins and the rRNAs, the tRNAs and aminoacyl tRNA synthetases, the GTP molecules and the various protein factors that promote initiation, elongation and termination events, the tally comes to well over 100 different kinds of macromolecules in-

volve. Clearly, a substantial proportion of a cell's genetic information must be devoted to the synthesis and regulation of its protein-synthesizing apparatus alone. Moreover this apparatus must be carefully designed to maximize the accurate translation of mRNA into protein : for example, the initiation of protein synthesis requires the interaction of two ribosomal subunits, two nucleic acids, three protein factors, and GTP and these multiple components presumably fit together in a productive manner only in a specific way, one then results in the correct alignment of the mRNA with respect to the aminoacyl and peptidyl sites and ribosome.

CHAPTER 7

REGULATION OF GENE ACTION OR GENETIC CONTROL OF GENE ACTION

DNA [illegible] almost all the pro-
[illegible]
DNA molecule [illegible]
are many evidences which show that many proteins which play an important role in an adult animal are not synthesized during embryonic development and certain other proteins may be synthesized primarily during embryonic development but not during adulthood Therefore, the flow of information from DNA (gene) to protein is regulated in some manner. The mechanism of regulation of gene action has been extensively studied in certain micro-organism such as *Escherichia coli* and in certain higher organisms. In both, the mechanisms of gene regulation is somewhat different, therefore, should be discussed separately.

REGULATION OF GENE ACTION IN PROKARYOTES

The mechanism of gene action in prokaryotes or micro-organisms has been best studied in the bacteria *Escherichia coli*. Among micro-organisms especially in *E. coli* following types of regulatory mechanisms of gene action have been reported :

A. ENZYME REGULATION OF GENE ACTION

The enzyme regulation of gene action can be well understood by knowing enzyme system and their mode of synthesis in *E. coli*. The synthesis of enzymes system in *E. coli* may be of following two types :

1. Unregulated enzyme production—Certain enzymes are produced continuously by an organism regardless of the presence or absence of its substrate in the environment. Such enzymes are called **constitutive enzyme** and they require no regulation. The constitutive enzymes are generally those that are needed at all times Thus the enzymes involved with glucose metabolism—enzymes of glycolysis

and the hexose monophosphate pathway-are constitutive, and one can imagine that the metabolism of glucose is sufficiently central to the physiology of *E. coli* that these pathways should be continuously functional.

2. **Regulated enzyme production**—The regulated enzymes are those enzymes which are synthesized in certain specific conditions and require one or other type of gene regulation. For example, the metabolism of less common sugars such as lactose, arabinose, and galactose, is effected by regulated enzymes, and it is clearly economical to elaborate such enzymes only on those occasions when their substrates are present. Regulated genes also specify enzymes of most biosynthetic pathways—pathways for the synthesis of amino acids, purines, pyrimidines, and so on—and these enzymes are synthesized according to the demand for their biosynthetic products. The regulated enzymes are of following two kinds :

(i) **Inducible enzyme**—An inducible enzyme is produced only when its substrate (inducer) is present in the environment. The phenomenon involved in the production of new enzyme due to a substrate or inducer is called **enzyme adaptation** or **induction**. This adaptation can be a reversible change, since in the absence of the inducer (substrate) the enzyme content may decrease **(deadaptation)**.

EXAMPLE

Lactose utilization in Escherichia coli—*E. coli* requires two enzymes, *viz.*, **β-galactoside permease** and **β-galactisidase** for lactose metabolism. The enzyme, β-galactoside permease is responsible for transport of lactose into the cell and its concentration there, while the enzyme β-galactosidase catalyzes the hydrolysis of lactose to galactose and glucose. One strain of *E. coli* produces both enzymes whether lactose is present or not ; this is a **constitutive strain**. Another strain produces these enzymes only in the presence of lactose ; this is an **inductive** strain. Thus the lactose induces the synthesis of an enzyme in the inductive mutant strain. Lactose, the substrate, is acting here as an enzyme inducer or effector (Monod, 1956).

However, it has been demonstrated that an inducer of an enzyme and the substrate on which its acts may or may not be identical. In fact, not all inducers are substrates and not all substrates are inducers. For example, methyl-β-D-thiogalactoside is an inducer but not substrate and phenyl-β-D-galactoside is a substrate but not an inducer.

(ii) **Repressible enzyme** : Some enzymes are normally present in the cell but cease to be synthesized when high concentrations of their end product are present. Such enzymes are called **repressible**

zymes; while the end product is called corepressor. A gene called regulator gene produces a substance called aporepressor which unites with corepressor to form a functional repressor molecule. This repressor molecule or substance inhibits synthesis of mRNA by all genes specifying enzymes in the synthetic pathway. Hormones may exert their phenotypic effects by derepressing genes previously repressed.

EXAMPLE

In *E. coli*, when the amino acid, histidine occurs in high concentration in the medium, that start to act as corepressor. As a corepressor, this amino acid terminates the synthesis of 10 enzymes which are required in pathway to histidine. This kind of repression is called **coordinate repression.**

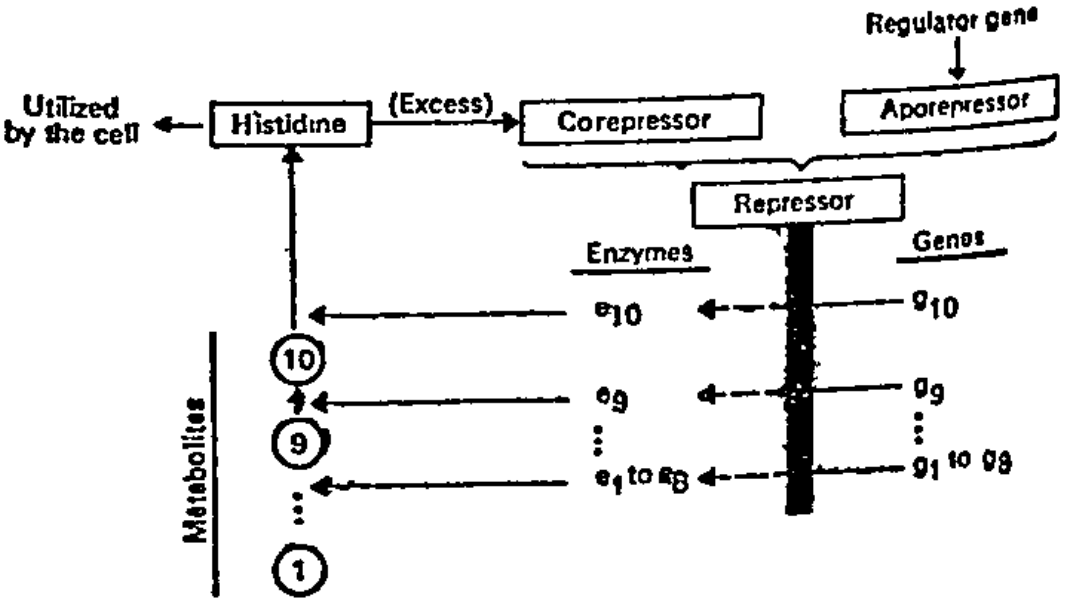


Fig. 7·1. Coordinate repression in *E. coli*.

(iii) Feedback inhibition (End product inhibition)—Feedback inhibition is a regulatory mechanism which does not effect enzyme synthesis, but rather inhibits enzyme activity. The end product of a biosynthetic pathway may combine loosely (if in high concentration) with the first enzyme in the pathway. This union does not occur at the catalytic site, but it does modify the tertiary structure of an enzyme and hence inactivates the catalytic site. This allosteric transition of protein molecule blocks its enzymatic activity and prevents overproduction of end products and their intermediate metabolities.

EXAMPLE

The studies on isoleucine synthesis in *E. coli*, (Umbarger 1961) demonstrated that addition of isoleucine (the end product of a five step conversion of threonine) to a culture of the bacteria resulted in

immediate blocking of the threonine→isoleucine pathway. In the presence of added isoleucine, ths cells preferentially use this exogenous end product (*i.e.*, isoleucine) and their own isoleucine synthesis become ceased. Moreover, the production of each of the five enzymes is not interfered with, but action of an enzyme responsible for deamination of theronine to α ketobutyrate is inhibited by the end product, isoleucine.

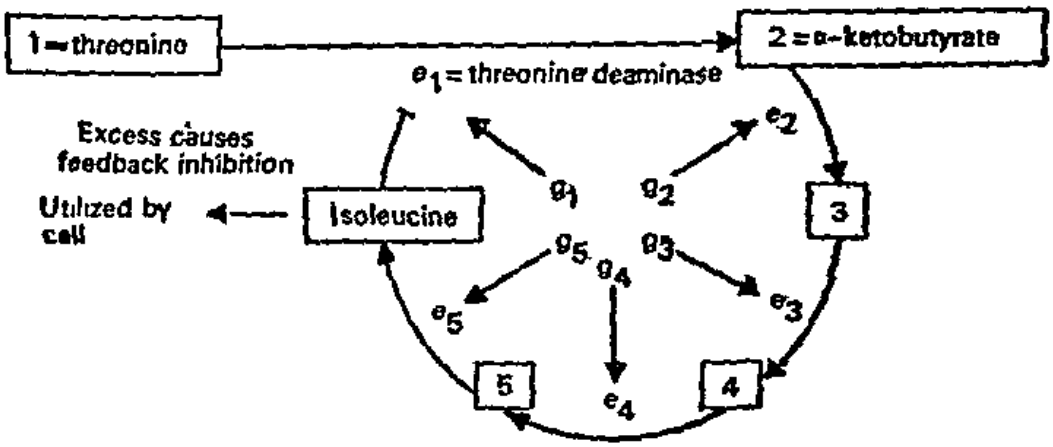


Fig 7·2. Feedback inhibition in *E. coli.*

B. GENE REGULATION OF GENE ACTION (REGULATORY GENES)

Studies in bacterial genetics indicate that not all genes act by

scriptional level, Jacob and Monod forwarded a hypothesis called operon hypothesis.

OPERON HYPOTHESIS

According to recent version of operon hypothesis, the genetic regulation in bacteria and bacteriophages is performed by following five key elements : 1.) operon or regulated structural genes; 2. the

mine structure of those enzymatic proteins which control sequential metabolic steps of a biochemical reaction.

EXAMPLES OF BACTERIAL OPERONS

In *E. coli* and *Salmonella typhimurium*, a variety of operons have been investigated. Few important operons of *E. coli* can be considered here as examples :

(i) *Gal* operon of *E. coli*—In the *gal* operon of *E. coli*, the sequence of genes (*e.g.*, gal E, gal T, and gal k) in the operon directly mirrors the sequence followed by the gene products (*e.g.*, galactokinase, galactose-1-phosphate uridyl transferase, and uridinediphospho-galactose-4-epimerase) as they carry out the degradation of galactose into glucose-1-phosphate and UDP-glucose.

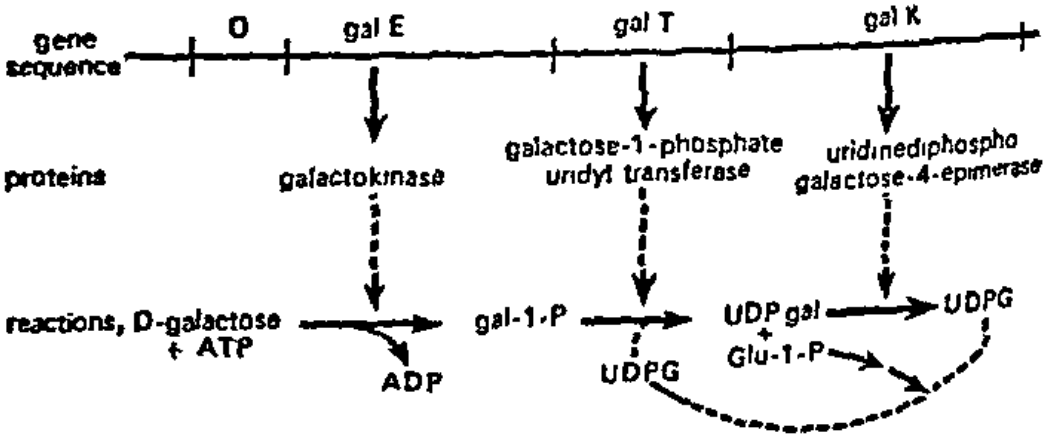


Fig. 7·3. The galactose (*gal*) operon of *E. coli*. Abbreviations : O=operator, Gal-1-P=galactose-1-phosphate, UDPG=uridine diphosphoglucose ; UDP Gal=uridine diphosphogalactose ; Glu-1-P=glucose-1-phosphate.

(ii) *Lac* operon of *E. coli* :—The *lac* operon of *E. coli* includes three structural genes (z,y, and a) and among its three structural genes, only the *z* gene specifies an enzyme known to be directly involved in the metabolic pathway for lactose utilization. The z gene product in a single long polypeptide chain (134,000 daltons) ; this combines with three other like chains to form the tetrameric enzyme β-galactosidase which catalyzes the hydrolysis of lactose (or other β-galactosides) into galactose and glucose. The y gene product, on the other hand, does not specify a catabolic enzyme at all but rather a molecule known as **M protein** or **galactoside permease** which is located in the bacterial cell membrane and acts to facilitate the specific uptake of lactose (and similar molecules) from the external medium. The last gene in the operon, gene a, specifies the enzyme **thiogalactoside transacetylase** which can, *in vitro*, transfer the acetyl group from acetyl coenzyme A to isopropyl-D-thiogatactoside (IPTG)

a synthetic galactoside derivative. The function of this enzyme *in vivo* is unknown, but since bacterial strains carrying deletions of of the *a* gene behave normally in all respects the gene appears not to specify an enzyme essential for lactose utilization.

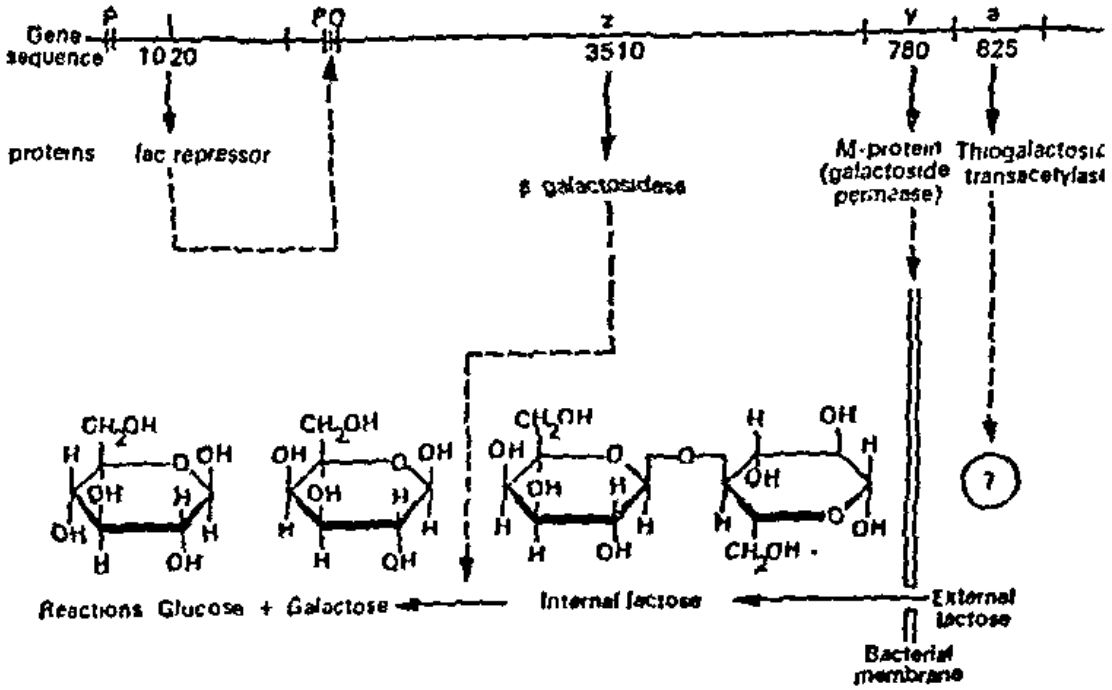


Fig. 7·4. The lactose (*lac*) operon of *E. coli* Numbers represent the estimated number of base pairs of each gene. Abbreviations P=promoter ; O=operator ; O=regulatory gene.

GENETIC PROPERTIES OF BACTERIAL OPERONS

A bacterial operon always consists of two or more distinct genes. These are "ordinary" genes in the sense that a missense mutation in one gene is usually without effect on the expression of other genes in the operon. The operon genes are unlike ordinary genes in that they are all transcribed into *a single mRNA molecule*, often called a polygenic or polycistronic messenger, starting from a single promoter site. This has been shown most clearly by examining the mRNA produced by individual operons. The mRNA from the *lac* operon, for example has a molecular weight of $1{\cdot}7 \times 10^6$ daltons, far greater than that needed to encode the sequence of β-galactosidase alone. This polygenic messenger contains several distinct translation "start" signals, each presumably heralded by an AUG. At a given time, therefore one ribosome attached to such a messenger may be involved in the synthesis of β-galactosidase and the next in the synthesis of M protein.

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2. The genes of an operon are independent in complementation tests but are cotranscribed into a single mRNA.

3. Nonsense mutations in promotor-proximal portions of the operon typically exert polar effects on the translation of distal genes.

4. The phenotypic expression of component genes of an operon is regulated by promotor, operator and regulator genes.

2. PROMOTOR GENE

The promotor gene represents a sequence of bases that are recognized by a DNA-dependent RNA polymerase enzyme and promotor-polymerase binding **initiates transcription** of the neighboring structural genes of an operon. The initiation properties of a promotor gene can be modified by temporary changes in the environment. Specifically, certain promotors are regulated by certain specific proteins known as **regulator proteins or repressors,** which are specified by genes called **regulatory genes.** In the presence of a regulator protein, initiation of transcription by a regulated promotor is blocked. When other regulated promotors interact with other regulator molecules, initiation is stimulated.

3. OPERATOR GENE

An operator gene also represents a sequence of bases that interacts with a protein. The protein is also called a regulator molecule, and operator-regulator interaction will either prevent or promote transcription of the regulated gene, depending on which operator is being considered. The major distinction between an operator and a promotor is that *the operator is not normally the site of attachment of an RNA polymerase.* Instead, it ordinarily lies between the promotor and the beginning of a regulated gene of an operon and thus it comes to influence whether a polymerase is able to progress from its attachment site to the gene itself.

4. REGULATORY GENE AND REGULATOR PROTEIN

The operator and promotor of an operon are under the control of yet another segment of DNA called a **regulatory gene,** which is situated apart from the operator, promotor and structural genes of an operon. The regulatory gene specify a protein called a **repressor** or **regulator protein** which has the potential to bind specifically to an operator or a promotor region, apparently because of some specificity in its three-dimensional configuration. Obviously, if this binding is to regulate gene transcription, some additional factor must exist which governs whether binding in fact takes place. This additional factor is known as **inductor** or **effector molecule.**

5. INDUCER OR EFFECTOR MOLECULE

An inducer or effector molecule is classically a small molecule—a sugar, an amino acid, or a nucleotide—that can bind to a regulator protein and thereby change its ability to interact with an operator or promotor. Jacob and Monod have suggested that a regulator protein will bind to an operator and inhibit operon expression as long as the effector or inducer is not present, but that if the effector molecule appears in the intracellular environment it will bind to the regulator and thereby "pull" the regulator off the operator, thus allowing operon expression to proceed. These relationships are diagrammed in figure 7·5.

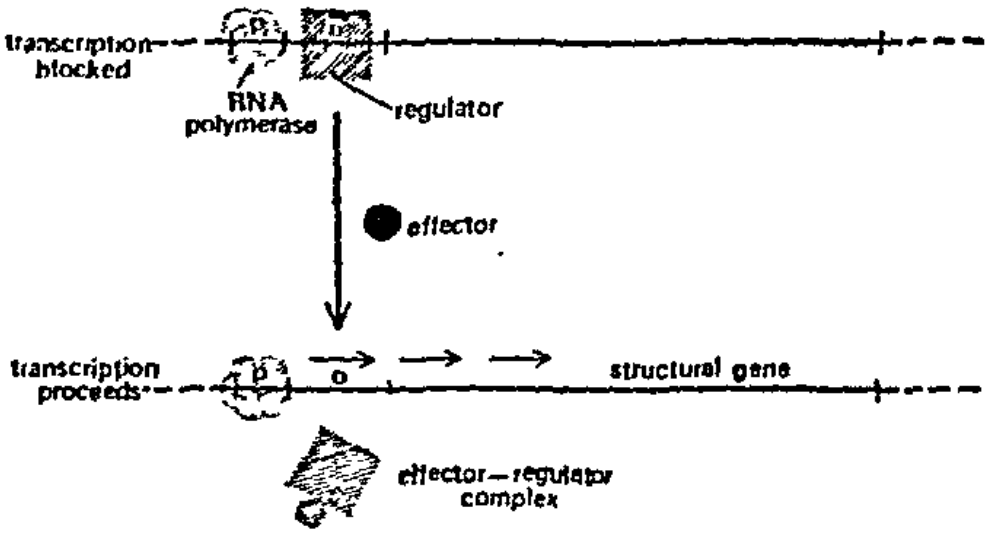


Fig. 7·5. Relationships between promotor (p), operator (o), and regulated structural gene (operon), showing RNA polymerase attachment to promotor, a regulator protein binding to the operator, and, in the presence of inducer or effector, the effector-regulator binding which removes the regulator from the operon. This diagram presents the essence of the 1961 Jacob-Monod modle for *lac* operon regulation.

REGULATION OF THE LAC OPERON OF E.COLI

As described in a preceding section, lactose utilization in *E.coli* involves the products of the three structural genes that constitute the *lac* operon : B-galactosidase, a lactose-splitting enzyme specified by gene z, M protein, a molecule necessary for lactose uptake and specified by gene y, and transacetylase, a poorly understood enzyme specified by gene a. The molecular basis for regulation of the *lac* operon is well understood. Briefly stated, the operon possesses a promotor locus that is under positive control, meaning that when it interacts with its regulator protein operon transcription is stimulated. The operon also possesses on operator locus that is under negative

control, meaning that when it interacts with its regulator protein operon transcription is repressed. The regulator protein for the *lac* operon locus is commonly referred to as the *lac* repressor, and it is specified by the i gene that maps just outside the operon.

Experiments have demonstrated that the repressor binds to the operator in an uninduced cell and that it comes to leave the operator when lactose is introduced, thereby leaving the operon open to transcription. In other words, the molecular details of *lac* regulation conform to the pattern suggested by the Jacob-Monod model of gene regulation (Fig. 7·5). The further molecular details of *lac* regulation were worked out with a number of mutant strains of *E. coli* whose genetics have been extensively analyzed.

MOLECULAR BIOLOGY OF *lac* OPERON

All available genetic and biochemical evidences in the mid-1960s indicated that an effector molecule such as IPTG (isopropylthi--ogalactose) interacted with a *lac* repressor protein to relieve some block in the transcription of the *lac* operon. W. Gilbert and B. Muller-Hill (1967) have isolated the *lac* repressor protein and a purified repressor protein proved to be a tetramer with a molecular weight of 150,000 daltons. The four subunits of the protein have since been shown to be identical, each having a molecular weight of 38,000 daltons, and each can bind a molecule of IPTG, meaning that the tetramer is capable of binding four IPTGs.

To study the mode of action of the repressor a preparation of *lac* operator DNA was obtained by incubating wild type *E. coli* DNA fragments with radioactively labeled repressor, centrifuging the mixture in a density gradient, and reserving that fraction of the DNA with labeled protein bound to it. A very small fraction of the total DNA exhibited such binding. The DNA-protein complex were judged to be operator-repressor complexes, and by digesting away the protein pure preparations of operator DNA were obtained.

During sensitive biochemical analyses of the *in vitro* interaction between purified *lac* repressor and purified *lac* operator DNA, when repressor-operator complexes are presented with IPTG the complexes fall apart, leaving free DNA and free repressor molecules which remain bound to IPTG. The *lac* operator is no more than 20 to 30 nucleotides long and these nucleotides must be in duplex form since *in vitro* studies have demonstrated that single-stranded operator DNA cannot bind repressor. Thus we come to a central question in gene regulation : how does a regulator protein find, among the millions of nucleotides in an *E. coli* chromosome, those few nucleotides that constitute the *lac* operator and lie buried within a DNA double helix ? The

answer is still unknown, but A. Riggs and S. Bourgeosis (1970) have proposed an attractive model. They suggested that the repressor is able to find specifically to the *lac* operator by recognizing the edges of the bases exposed in the large and-or small grooves of the DNA helix. They note that the shape of the edge of an AT pair will be quite different from the shape of a GC pair and, since the repressor will presumably bind to the DNA with a fixed orientation, that the grooves can potentially contain four types of edges : the A edge of an AT pair, the T edge of an AT pair, the G edge of a GC pair and the C edge of a GC pair. Assuming that a protein of the repressor can fit into the grooves and can establish specific hydrogen bonds only with the particular sequence of nucleotide edges found in the *lac* operator region, the specific repressor-operator binding can be explained. whether or not the model is correct for the *lac* operon, it will undoubtedly prove important to give greater consideration to the information that a double-stranded DNA might carry within its grooves (Goodenough and Levine, 1974).

C. CATABOLITE REPRESSION

B. Magasaine (1957) and others have shown that the presence of glucose inhibits the synthesis of enzymes involved with the utilization of such sugars as lactose, arabinose or galactose. This phenomenon is variously known as **catabolite repression** or the **glucose effect** and occurs in most micro-organisms including *E. coli* and yeast. For example, when a culture of *E. coli* cells is maintained on lactose and the growth medium is then supplemented with glucose. it was found that the synthesis of B-galactosidase become permanently repressed as long as glucose remains in the medium. This inhibitory effect of glucose remained poorly understood until it was noted that the presence of glucose leads to a marked drop in intracellular levels of cyclic AMP (3', 5'–cyclic adenylic acid), the small nucleotide. This discovery was followed by studies made by G. Zubay and J. Beckwith (1970) who used the isolated *lac* operon DNA of *E. coli* and hypothesized that glucose by depressing cyclic AMP levels, effectively suppresses the transcription of the *lac* operon.

Further *in vitro* studies of L. Eron *et al.* (1971) have indicated that *lac* operon transcription requires not only cyclic AMP but also a protein called CAP (catabolite activator protein). CAP is a molecule of about 45,000 molecular weight and binds to cyclic AMP. Thus, a two step reaction is supposed to take place during catabolite repression—cyclic AMP first binds to CAP, and the CAP cyclic AMP complex then exerts its stimulatory effect on the transcription process.

D. STRINGENT CONTROL

In addition to the finely tuned regulation that bacterial cells have developed for individual genes and operons, a number of more generalized regulatory systems exist that affect many aspects of cellular metabolism. For instance, the stringent control is designed to conserve the resources of a cell in the "hard times' incurred during amino acid starvation. Such hard times exist, for example, when an auxotrophic bacterium is deprived of a required amino acid. They also exist in a *shift down* experiment : prototrophic bacteria are first grown on a medium rich in amino acids to inhibit transription of all repressible genes concerned with amino acid biosynthesis ; they are then shifted to a medium free of amino acids so that they are forced to synthesize essential biosynthetic enzymes in the absence of a pool of exogenous amino acids.

Bacterial cells respond stringently to meet such emergencies : they greatly curtail their rates of synthesis of RNA, particularly rRNA and tRNA, and modify their rates of nucleotide biosynthesis phosphorylation, protein turnover, and many other processes, presumably to conserve their resources and thus maximize their chances of survival. Ryan and Borek (1971) have reported that *rel* (relaxed) gene of *E. coli* exerts a critical control over the stringent response and guanosine tetraphosphate or ppGpp molecules accumulated in response to amino acid starvation of *rel*$^+$ strain of *E. coli*.

CONTROL OF GENE EXPRESSION IN EUKARYOTES

In eukaryotes following two kinds of controls or regulations of gene expression occur : 1. Short-term or reversible regulation corresponds to the kind of regulation we studied in bacteria and it represents a cell's response to fluctuations in the environment, specifically, it involves changes in activities or concentrations of enzymes as particular substrates and or hormone levels rise and fall. The changes a cell experiences during a cell cycle, particularly the fluctuations in rates of DNA, RNA, and protein synthesis that regularly occur with respect to the time of mitosis can also be placed in this category. 2. Long-term or irreversible regulation includes the phenomena associated with determination, differentiation, or, more generally development : it is involved in the numerous steps by which a fertilized egg becomes an organism of perhaps trillions of cells with diverse and ultimately quite permanent roles to play in the maintenance of the whole. Short-term regulations also occur in developing and differentiating eukaryotic cells side by side of long-term regulation.

A. SHORT-TERM GENETIC REGULATION IN SIMPLE EUKARYOTES

In most simple eukaryotes as fungi, algae and Protozoa several cases of short-term genetic regulations which correspond to bacteria have been reported. For example, the fungi are most like bacteria in their ability to "turn on" and "turn off" particular enzymes in response to the presence or absence of their substrates in the growth medium. Enzymes associated with galactose utilization and with leucine and pyrimidine biosynthesis, for example, have been shown to be variously induced or repressed in several species of fungus. Inducible enzyme systems have been studied in eukaryotic algae, yeast, *Neurospora* and *Aspergillus*. Typically in most fungi, a group of regulated enzymes responds to more than one "effector molecule". For example, J. Pateman and D. Cove (1969) have found that the *de novo* synthesis of the nitrate reductase enzyme in *Aspergillus nidulans* is induced by nitrate (the substrate) and repressed by ammonium (the end product). Further, in most fungi gene regulation at transcription level by operon-mechanism of Jacob-Monod, has not been reported, however, some kind of post transcriptional control of gene activity in fungi have been recognized in certain fungi (see Goodenough and Levine, 1974).

B. SHORT-TERM GENETIC REGULATION IN COMPLEX EUKARYOTES

Significance of blood and hormones in short term genetic regulation in complex eukaryotes : Unlike simple eukaryotes, the complex eukaryotes have one predominant and fundamental property called **cell specialization** in which a given cell type comes to perform particular functions and is concomitantly unable to perform other functions. The extreme forms of cell specialization found in mature complex animals are possible because of the remarkable properties of blood (or blood like fluid such as hemolymph). The composition of the blood is so stabilized that each cell receives the proper balance of ions, sugars, amino acids, and other essential molecules. Consequently, most cells in a complex eukaryote never experience the drastic fluctuations in the quality of the environment that simple eukaryotes and bacteria must be prepared to face, and they are thereby free to focus on particular metabolic activities.

The constancy of the blood is effected by a number of tissues. For instance, in a vertebrate organism, certain tissues such as the intestine, liver and kidney exert a direct effect on the blood, composition by adding the appropriate concentrations of ions and substrates and removing any excesses or waste products. Other tissues respond to a change in blood composition more indirectly : they sec-

rete a hormone that ultimately acts to restore the equilibrium condition. As an example, certain cells of parathyroid gland secrete parathyroid hormone when blood calcium levels are below the optimum, and other cells of parathyroid secrete calcitonin when calcium levels are above the optimum. Parathyroid hormone ultimately promotes calcium resorption from the bone, increases calcium permeability in the intestine and reduces calcium excretion in the kidney. Calcitonin has opposite effect. In other words, kidney, liver, and bone cells do not respond to blood calcium levels per se but rather to hormonal signals that dictate what the levels of calcium should be. From this discussion, it can be concluded that the short-term regulation in complex eukaryotes is largely mediated by hormones.

I. MECHANISMS OF SHORT-TERM HORMONAL REGULATION

A given hormone, may have one or several target cells. Parathyroid hormone, for example, has target cells in the kidney, intestine, and bone, and each cell type ultimately responds quite differently to the same hormonal stimulus. Hormones are known to elicit new biosynthetic or metabolic processes in their various target cells and at this stage an attempt shall be made that how this is accomplished. Does a hormone enter the nucleus and act to turn on a new set of genes ? Does a hormone exert a post-transcriptional

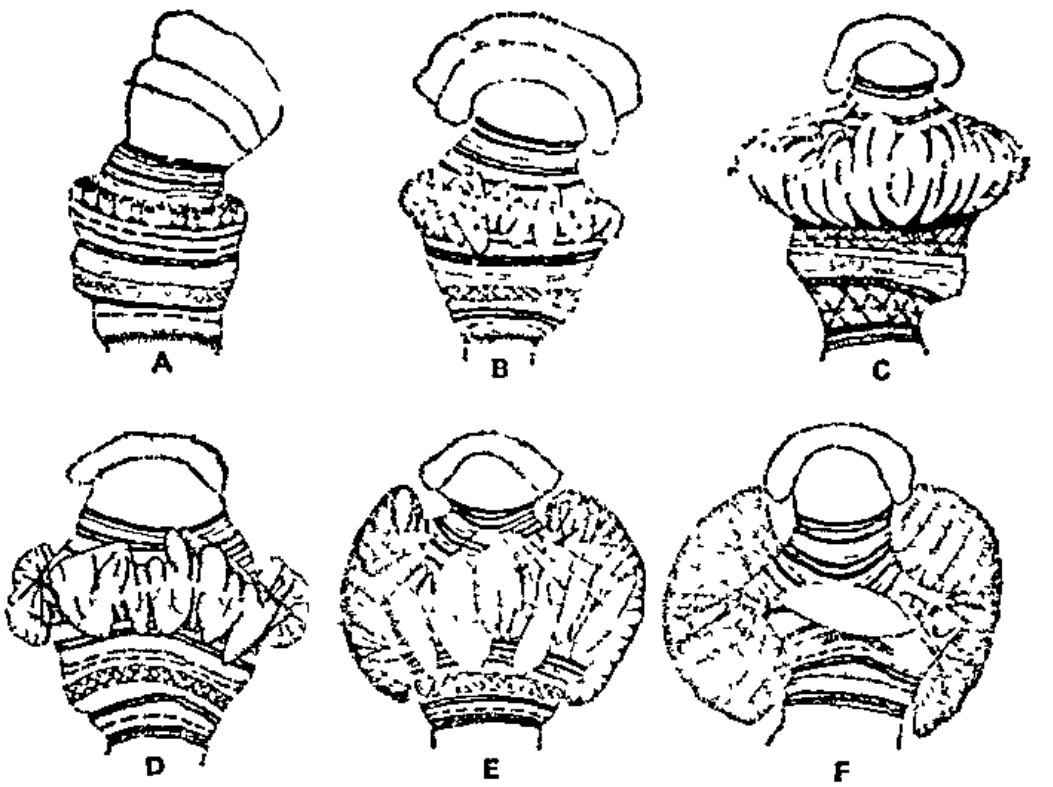


Fig. 7 6. The development of a chromosomal puff in a larval salivary gland cell nucleus of *Chironomus tentans.*

effect by activating some pre-existing mRNAs or stimulating some new translation mechanisms? E. Sutherland and his coworkers (1968) have shown that many hormones exert their initial effects on their target cells at the cell periphery. Hormones of this class interact with, and there by greatly stimulate the activity of, adenylate cyclase molecules located in the plasma membrane of their target cells. Each adenylate cyclase is stimulated by only certain target-specific hormones, thus the liver enzyme will be greatly stimulated by adrenaline but not by parathyroid hormone, the bone enzyme will be greatly stimulated by parathyroid hormone, but not by adrenaline. Once stimulated each adenylate cyclase will rapidly convert ATP into cyclic AMP (cAMP) which is found to activate pre-existing enzymes in the cell. Whether it stimulates gene transcription in eukaryotes like in *E. coli* cells is still not clear and is being actively investigated.

Some hormones, however, have been found to have a more direct effect on genes. The first report of a specific hormonal effect at the chromosomal level in any organism was made by U. Clever in the early 1950s for the dipteran *Chironomus tentans*. He first studied carefully the patterns of polytene chromosome puffing at various stages of larval development and established that these patterns followed a fixed sequence, with certain bands puffing at one stage and regressing at a later stage. He then took intermolt larvae, in which puffing of band 1-1 A was known to be particularly active, and injected the animals with ecdysone, an insect steroid hormone that triggers moulting. He found that within 10 to 15 minutes puff 1-19A regressed, band 1-18C began to puff, and band 1V-2B followed suit. This was exactly the puffing sequence known to take place just before moulting, a time at which the insect spontaneously releases ecdysone into the hemolymph. On the reasonably certain assumption that puffing corresponds to the transcription of one or several genes these experiments correctly shown that ecdysone somehow turns on the transcription of particular genes.

The hypothesis that hormones can stimulate transcription directly is supported by more recent studies of mammalian cells in which such steroid hormones as androgens, estrogens, and aldosterone appear to act, at least in part, at a transcriptional level. These studies have shown first, that the steroids (like ecdysone) are without effect on their target cells when actinomycin D is present to inhibit DNA transcription, where as the hormones that act via cAMP are generally insensitive to actinomycin D. Second bursts of RNA synthesis in the nucleus–the mammalian equivalent of puffing, it is assumed are commonly observed when steroids are presented to their target

cells. Third, it has been shown by autoradiography that labeled steroid hormones actually enter the cytoplasm of their target cells, once in the cell they appear to combine with specific receptor molecules and proceed to migrate into the nucleus.

Thus, it can be concluded that certain hormones (*e.g.*, many steroids) exert an effect on gene transcription and that certain other hormones (*e.g.*, many polypeptides) act by altering cAMP levels in their target cells. However, certain hormone such as insulin exerts its effect at the cell membrane, most of which do not involve cAMP. Likewise, certain other hormones exert their effect both at transcriptional and post-transcriptional levels.

2. SHORT-TERM REGULATION BY HISTONES

As histone poteins remain intimately associated with the phosphate goups of DNA in chromatin of a eukaryote, therefore, it has been proposed that they might somehow alter the transcriptional properties of the polynucleotides with which they associate. The *in vitro* studies have confirmed that histones inhibit the transcription of RNA from DNA. Further, Weissman and Hirschhorn (1969) have shown that a portion of chromosome becomes active and start transcription of RNA due to the digestion of histone coat of DNA by a lysosomal enzyme called histonase. About the possible mechanism of histones in regulating transcription of DNA, certain workers have hypothesized that certain type of histone (*e.g.*, histone A) remains abundant in one cell type and thereby shields from transcription all but those few genes which have little affinity for their specific histone (*i.e.*, histone A). But, as the histone composition of most nuclei in a complex eukaryote is found relatively constant, so this hypothesis has been held non-valid.

The current molecular biological understanding holds that local differences in histone distribution may exist along a chromosome, that hormones or other influences may affect this distribution and that transcription may be modified accordingly. Histones are known to be modified by phosphorylation, acetylation, and methylation, and some of these modifications might alter DNA–histone interactions and allow certain segments of the genome to be transcribed (see Goodenough and Levine, 1974).

3. SHORT-TERM REGULATION BY ACIDIC PROTEINS

As non-histone or acidic proteins are also intimately associated with chromatin of eukaryotes, so, these proteins are considered by some to be more attractive candidates for regulatory molecules than histones in that their distribution is claimed to vary widely from one tissue to another in the same organism. Moreover, it has been repor-

ted that a particular acidic protein is synthesized at the time that cortisone, a steroid hormone produced by the adrenal gland, is administered to adrenalectomized rats (*i.e.*, rats whose adrenal gland is cut and taken out). There are reports also that acidic proteins may bind to steroids at the time they enter the nucleus.

It is still not clear that how acidic proteins bring about regulation. According to **Goodenough** and **Levine** (1974) the DNA–histone complex itself is inert to transcription and that the acidic proteins interact with the basic histones, pulling the histones off certain critical genes or promotors so that the genes can be transcribed. But this view has not been confirmed by experiments.

4. SHORT-TERM REGULATION BY HETEROCHROMATIZATION

The heterochromatin is a condensed intercoiled state of chromatin, containing two to three times more DNA than euchromatin. However, it is genetically inert as it does not direct synthesis of RNA (*i.e.*, transcription) and protein and is often replicated at a different time from the rest of the DNA. Recent molecular biological studies have identified three kinds of heterochromatins ; namely **constitutive, facultative** and **condensed heterochromatin.** The **constitutive** heterochromatin is present at all times and in the nuclei of virtually all the cells of an organism. In a interphase nucleus, it tends to clump together to form chromocenter. In *Drosophila*, for example, most pupal, larval and adult cells contain large blocks of constitutive heterochromatin that lie adjacent to the centromeres. These blocks of heterochromatin are found to be the sites in which highly repetitive satellite DNA is located. This DNA is **late replicating** : it fails to replicate until late in the S phase and is then replicated during a brief period just before the G_2. The **facultative heterochromatin** reflects the existence of a regulatory device designed to adjust the "dosages" of certain genes in the nucleus. It is originated during a process called **facultative heterochromatization** : a chromosome or a set of chromosomes becomes heterochromatic (turned off) in the cells of one sex while remaining euchromatic (turned on) in the cells of the opposite sex. In other words, it remain indirectly related to sexual differentiation. The **condensed heterochromatin** is deeply staining and tightly coiled chromatin that is found in many interphase nuclei and that corresponds to facultative nor costitutive heterochromatin. It is reported to have some specific roles in gene regulation.

First, it is far mor[illegible] differentiated cells, cells commi[illegible] proteins, than it is in relatively [illegible], normal human white blood cells are found to contain large masses of con-

densed heterochromatin, than leukemic white blood cells which lack condensed heterochromatin and synthesize many different proteins at uncontrolled rates. The second relevant feature of condensed heterochromatin is that it appears to be relatively in gene transcription. When H^3—uridine is administered to cells and autoradiographs are made of specimens prepared for electron microscopy, then radioactive uridine was found to be associated with euchromatin than condensed heterochromatin.

These observations suggest that regulation might involve some mechanism by which specific genes are either "shunted into" or "released from" condensed physical conformation that cannot be transcribed. Some recent studies by **J. Frenster** have compared preparations of isolated heterochromatin and euchromatin and differences were found between their acidic protein contents but not between their histone contents. However, it is still not clear that how and why acidic proteins cause heterochromitization of certain genes.

5. SHORT-TERM REGULATION BY HETEROGENOUS NUCLEAR RNA

The heterogenous nuclear RNAs which act as precursor molecules for mRNA molecules in eukaryotic nucleus are found to function as short-term regulatory molecules to influence gene transcription in cleaved or uncleaved hetRNA states. They are degraded shortly after they are synthesized so that a new spectrum of regulatory RNA molecules could be rapidly elicited with the proper stimulus.

6. POST-TRANSCRIPTIONAL MECHANISMS

Histones, acid proteins, hetRNA and physical conformation (i.e., heterochromatization) are the most likely condidates for transcriptional control molecules, all of which are difficult to visualize at a molecular lavel as long as eukaryotic chromosome structure remains a mystery. Post-transcriptional control mechanisms, however, are far easier to postulate. It is suggested, for example, that short-term regulator molecules such as hormones might facilitate diffusion of particular mRNAs out of the nucleus, stabilize their lifetimes, increase the frequency with which they associate with ribosomes and become translated, and so on. It is also suggested that different classes of ribosomes translate different mRNAs or that regulator molecules induce the formation of special translation factors.

C. LONG-TERM GENETIC REGULATION IN COMPLEX EUKARYOTES

The long-term genetic regulation occurs in multicellular eukaryotes during the oogenesis and differentiation phases of embryo genesis and it is found to occur at transcriptional as well as post-trans-

criptional level. Many cases of such genetic regulation have been studied in amphibia, echinoderms and insects.

(I) GENE REGULATION DURING OOGENESIS

During differentiation of an oocyte cell into a mature egg (*i.e.*, oogenesis) of amphibia and echinoderms, it is found that the oocytes are engaged in the transcription of large amount of mRNA and rRNA molecules, which have some regulatory effects.

(a) Informational "lampbrush RNA" synthesis : During the diplotene stage of prophase I the oocyte chromosomes of an amphibian such as *Xenopus* assume the lampbrush configuration and transcribe so much mRNA molecules that they might synthesize 20,000 to 40,000 different kinds of proteins. Much of this mRNA is not translated but has some regulatory function. Much of the lampbrush mRNA is selectively transcribed and is maintained in stable form throughout the remaining stages of egg maturation. Once the egg is fertilized and cleavage begins, the "lampbrush mRNA" direct many of the ensuing developmental events. It has been shown that the presence of actinomycin D has no or little effect on protein synthesis or normal development until the blastula stage, at which time the embryo consists of 6000 to 15,000 cells. The embryonic nuclei, moreover, seem virtually inactive in RNA synthesis during this period. The eggs of amphibia, Insecta and echinoderms, therefore, accumulate well in advance the informational RNA or mRNA which is transcribed from maternal genotype and is used during early developmental stages.

(b) Ribosomal RNA synthesis : The second kind of RNA synthesized in the amphibian oocyte and stored for use by the embryo is ribosomal RNA. Its synthesis occurs in the pachytene stage of prophase I, the stage preceding the lampbrush stage. For example the nucleolar organizer DNA of *Xenopus* is a long stretch of tandemly repeated copies of rRNA genes interspersed with spacer DNA. During oogenesis this DNA undergoes repeated rounds of replication at a time when the rest of the genome is not being replicated so that 1000 to 1500 additional copies of nucleolar organizer DNA are sequestered in a region known as the **nuclear cap**. At diplonema this structure disperses and the nucleus comes to contain 1000 to 1500 tiny independent nucleoli, each actively engaged in the rapid synthesis of rRNA. The nucleoli function until the first meiotic division, and the rRNA that is synthesized is stored in the egg in the form of **maternal ribosomes**. At meiosis I the extra nucleolar DNA is discarded into the cytoplasm. Little is known about the mechanism of this gene amplification or how it is restricted to a particular period

in amphibian development. The storage of maternal ribosomes for use by the young zygote is a conspicuous activity of some egg cells and not others, and only fish and amphibia are found to undertake amplification of their ribosomal DNA in order to increase their synthetic potential for rRNA.

(II) GENE REGULATION DURING CELLULAR DIFFERENTIATION

Differentiation begins with a fertilized egg which undergoes a series of mitotic divisions (cleavage) and a process called blastulation to form blastula, in which all cells are morphologically similar and tissues are not yet defined. At various times during this mitotic period individual cells becomes so determined that they and their clones become committed to forming particular cell types–liver, heart kidney, or whatever. Following the determinations event and often many cell generations later, individual cells undergo differentiation into their specialized forms. Differentiation may involve mitosis, cell fusion, cell migration and-or intercellular interactions. These events occur in an elaborate, self-reinforcing chain. Self-reinforcing in the sense that event A must be completed before event B can possibly begin, the onset of event B triggers a biosynthetic event or cellular migration necessary for event C, and so on. Thus, cellular differentiation begins during late blastulation and continues through the process of tissue formation (histogenesis), which is followed by organogenesis.

Recent molecular biological studies of differentiation process have shown that (i) During embryogenesis, cells of organisms differentiate both physiologically and morphologically, but their genome (DNA contents) remain the same ; (2) Differentiation involves limited DNA transcription. Thus, cell differentiation implies the preferential synthesis of some specific proteins, *e.g.*, haemoglobin in erythrocytes, gamma globulin in plasmocytes, actin and myosin in muscles ; and (iii) Differentiation involves self-reinforcing changes in the cytoplasm. The cytoplasm is of critical importance in maintaining the differentiated state. It is imagined that when certain determined genes turned on, certain proteins are synthesized ; some

collection of regulatory molecules that reinforce the differentiated condition.

EVOLUTION
(Evolutionary Biology)

CHAPTER 1

INTRODUCTION

Diversity is the rule of the nature. There exist a wide array of species of micro-organisms, plants and animals which differ with each other in structure, function and behaviour and which have occupied almost all ecological niches existing on the planet earth (*viz.*, in its atmosphere, hydrosphere, and lithospere). Out of this preponderant diversity of living beings have emerged two overriding themes or concepts which give unity to the sciences of life and throw light upon them all. Out of these two unifying concepts or principles of modern biology, one is the **concept of organization**. This tells us that at every level, from the molecule through the supra-molecular organelle, the cell, the tissue, the organism, the individual, and upto the population or the society, the properties of life depend only to a small degree upon the substances of which living matter is composed. To a much greater degree living things owe their nature to the way in which the components are organized into orderly patterns, which are far more permanent than the substances themselves. The other unifying concept of modern biology is that of the **continuity of life through heredity and evolution**. This tells us that organisms resemble each other because they have received some common ancestor hereditary elements, chiefly the chromosomes of their nuclei, which are alike both in respect to the substances which they contain and the way in which these substances are organized. When related kinds of organisms differ from each other, this means that in the separate lines of descent from their common ancestor changes in the hereditary elements have taken place, and these changes have become established in whole populations.

Evolution is the development of organisms through time. The term evolution (Latin, *evolutio*–an unfolding or unrolling) means a gradual orderly change from one condition to another. There are ample geological evidences which suggest that the planets and stars, the earth's topography, the chemical compounds of the universe, and even the chemical elements and their subatomic particles, have undergone gradual, orderly changes during the long history of the universe. This kind of evolution which includes evolution from atoms and molecules to simple and then com-

plex substances, and from these to still more complex ones capable of self-duplication, has been termed as inorganic or chemical or molecular evolution. The most significant outcomes of chemical evolution are the origin of biologically important macromolecules (*viz.*, proteins and nucleic acids-DNA and RNA), origin of life and an environment to sustain life on the planet earth. Further, it is biologically evident that all the various prokaryotes and eukaryotes (*viz.*, viruses, bacteria, plants and animals) existing on the earth at the present time have descended from other, usually simpler organisms by gradual modifications which have accumulated in successive generations. This kind of evolution is started from the culmination point of chemical evolution into the origin of life, and still is in process and is called **biological or organic evolution.** Thus, the concept of organic evolution holds that *all the varied kinds of animals and plants which now known have developed out of earlier types by completely natural changes during the passage of time.*

The branch of biology which incorporates in it, the studies concerning the problems of chemical evolution and origin of life and organic evolution and origin of man and other present day organismal species of planet earth is called **evolutionary biology** (Mayr, 1970; Salthe, 1972).

The concept of evolution is based on detailed comparisons of the structures of living and fossil forms, on the sequences of appearances and extinction of species in past ages, on the physiological and biochemical similarities and differences between species and an analysis of the genetic constitution of present day plants and animals. It is an indubitable fact which has been accepted by all but one or two of those who are accredited experts in the study of biology. Yet as a scientific theory, evolution cannot really be said to have begun until about 1800, and it was not definitely established until the decades after 1859, the year when Charles Darwin published "*On the origin of species*" a master work which laid down the principles of evolution in a form which they are still largely accepted.

Though much knowledge has been gained about the different evolutionary processes during post-Darwinian phase, yet our knowledge is far from complete. Much remains to be discovered, much is to be learned. The applications of new techniques, such as the use of high-speed digital computers, and the application of comparative biochemistry to elucidate developmental pathways or to discover evolutionary relationships at the chemical level (*viz.*, chemotaxonomy) promise to open grand new vistas in the field of evolutionary biology.

THE FACT OF EVOLUTION

At the outset, it should be clear that most biologists accept as demonstrated the fact that organisms have evolved. To be sure, no biologist has actually seen the origin by evolution of a major group of organisms. Nevertheless, races and species have been produced by duplicating in the laboratory and garden some of the evolutionary processes known to take place in nature. The reason that major steps in evolution have never been observed is that they require millions of years to be completed. The evolutionary processes which gave rise to major groups of organisms, such as genera and families, took place in the remote past, long before there were people to observe them. Nevertheless, the facts (*i.e.*, evidences of organic evolution) we know about these origins provide very strong circumstantial evidence to indicate that the processes which brought them about were very similar to those found in modern groups of animals and plants.

EVOLUTION COMPARED WITH ANCIENT HISTORY

Certain authors of evolutionary biology such as **G L Stebbins** (1971), has compared the evolution with the ancient history. According to them, the state of our knowledge of the major steps of evolution which took in the past is much like that of our knowledge of ancient history. We do not have any reliable eye-witness accounts of the events which produced the rise and fall of the civilizations of ancient India, Egypt, Sumeria, Babylon and Crete, and the contemporary written records of these ancient times are fragmentary and unreliable. Nevertheless, the indirect evidences that these civilizations existed are so strong that it is accepted by scholars and laymen alike. Moreover, we teach as historical fact many of the probable events connected with their rise and fall. The evidence which biologists now have about the rise and extinction of major groups of animals in past geological eras is of a very similar nature, and carries with it about the same degree of high probability.

A PREVIEW OF EVOLUTION

Evolution as a process of gradual development of organisms through time includes following characteristics :

1. Basically evolution is the result of the **differential survival** in each generation of the progeny of individuals with certain special characteristics. In turn these adaptive characteristics that in part account for the differential survival.

2. **Mechanism of inheritance** is the foremost important element which plays an important role in evolution. The ways in

which genes determine the expression of characters in an organism and the manner in which genes are transmitted to the offspring, shape the whole evolutionary picture. Evolution is often defined, and quite rightly, so, as changes in the frequency of genes in a population. In turn the organization of genes in chromosomes and the behaviour of these during cell division affect the mechanisms of inheritance and evolution.

3. Normally sexual reproduction has far much evolutionary significance than asexual reproduction. Sexual reproduction is a mechanism that tends to combine the genetic materials of individuals and produce new and novel combinations. Its effect is a tremandous increase in variability, and the advantages of this are so great that the phenomenon has become almost universal in all plants and animals. Sexuality apparently developed very early in the evolutionary history of organisms.

4. Without sexuality and interbreeding, species as we know them today would not exist. But just as important for the evolution, particularly the multiplication of species has been the development of barriers (*viz.*, isolating mechanisms) to the free exchange of genes, be they geographical, ecological, behavioral, or genetical. The very simple earliest organisms may have been able to mix their genes with others of the same level of organization, but present-day organisms, with elaborate and complicated developmental pathways, cannot exchange genes with drastically different organisms. When they do the result of these exchanges is lethality or at best sterility. The selective advantage of barriers that prevent gene exchange in such instances is obvious.

5. In a slightly different sense, it also can be said that evolution is shaped by environment. Differential survival is always partly due to capacity to adopt to the environment, particularly the physical environment. Chemical evolution could occur only after our planet changed from the original "ball of fire" to a body where water could accumulate. Organic evolution, which has produced the tremandous number of organisms, is in part a consequence of the adaptation of these organisms to the infinite types of environments found on earth.

Thus, the evolution is the result of the interplay of many and diverse factors. These factors are themselves subjected to change. Early in the history of each lineage of plants and animals, structures or processes have developed which have profoundly influenced the evolutionary history of that group. So, for example, a segmented body and an exoskeleton have been major factors in the success of

the insects, but in turn these same factors have restricted the size and habits of the members of the class Insecta.

CERTAIN MISCONCEPTIONS OF EVOLUTIONARY BIOLOGY

The misconceptions about evolution are many and following two ideas are so fixed in the popular mind that they deserve to be considered separately. The first of them is the equation of evolution with struggle. The phrase "survival of the fittest" is Darwin's, but it is clear from his writings that he did not mean it literally. It was the British philosopher **Herbert Spencer** (*1820-1903*) who popularized the idea in his works and in his concept of social evolution. In the historical context of the times, the idea of struggle between individuals with the winner, the fittest, surviving to get the spoils was appealing to certain groups. In translating organic evolution to the social scene, Spencer misinterpreted the main idea—that is, differential survival of the progeny. He replaced it with one element of natural selection, differential mortality due to better adaptations to the environment, and interposed the idea of direct competition as responsible for differential mortality. The ideas as presented are entirely his, and have no scientific backing. Likewise, almost a hundred years later, the Soviet geneticist **Lysenko**, in the name of Darwinism, was to denounce several aspects of modern genetics, and cytology and accept the Lamarckian theory of the inheritance of acquired characters. In the Stalinist Russia, these ideas gave an apparent scientific backing to the efforts of the State to mould the individuals into an obedient servant, so that his descendants might become still more docile and obedient. In this case too, abandonment of scientific accuracy has created confusion.

We must keep always in mind that by the "fittest" **Darwin** meants the one with the largest surviving progeny. This can be and often is a comparatively weak individual. In this sense rabbits are "fitter" than lions, inspite of man, than lions, which are fighting a losing battle against man.

The second important point to understand is the no moral judgement of any sort can be read into evolution. Evolved organisms are not "better" in a moral sense, they are only better adapted to the environment they occupy compared to their extinct ancestors. Every living species, by this very fact, is adapted to the environment it occupies presumably as effectively as any other living organism irrespective of their phylogenetic position. Evolution is as blind as justice is supposed to be : those with the largest surviving progeny

will multiply regardless of how good or bad humans may consider them (**O. T. Solbrig**, 1966).

SIGNIFICANCE OF EVOLUTIONARY BIOLOGY

The theory of evolution is quite rightly called the greatest unifying theory in biology. The diversity of organisms, similarities and differences between kinds of organisms, patterns of distribution and behaviour, adaptation and interaction, all this was merely a bewildering chaos of facts until given meaning by the evolutionary theory. There is no area of biology in which that theory does not serve as an ordering principle. On the contrary, genetics, morphology, biogeography, systematics, palaeontology, embryology, physiology and other branches of biology, all have illuminated some special aspect of evolution and have contributed to the total explanation where other special fields failed In many branches of biology, one can become a leader even though one's knowledge is essentially confined to an exceedingly limited area. This is unthinkable in evolutionary biology (see **Mayr**, 1970). A specialist can make valuable contributions to special aspects of the evolutionary theory, but only he who is well versed in most of the branches of biology listed above can present a balanced picture of evolution as a whole. Whenever a narrow specialist has tried to develop a new theory of evolution, he has *failed.*

CHAPTER 2

MOLECULAR EVOLUTION AND ORIGIN OF LIFE

The fundamental issues of evolutionary biology are the problems of chemical or molecular evolution and its culmination into the origin of life and the organic evolution and its product, the origin of species. Though the problems of molecular evolution and origin of life which (Fox and Dose, evolutionary aspects than the phenomena of organic evolution and origin of species, yet they demand priorty in discussion, since they have occurred much before the organic evolution and origin of species. Further, for proper understanding of the phenomenon of protobiogenesis (origin of life), it is necessary to analyze the essence of the term 'life', before going into the details of protobiogenesis and molecular evolution.

DEFINITION OF LIFE

From prehistorical time, men of different walks of life have tried to define and explain the essence of life in their own ways and even every biologist at some time attempted to define life, yet no agreeable definition of life has been got till the present time. Anyhow, the life has been defined by following two entirely different approaches :

A. PHILOSOPHICAL DEFINITION OF LIFE

According to Russian evolutionist, Oparin (1968), ancient philosophers of two different camps namely idealism and materialism, have defined life in two entirely contradictory terms. The idealists thought of life to possess some ethernal supernatural source which is

lian" *universal reason*", the "*life force*" of vitalists, the dominting ide

naturalists begin their investigative work from a materialistic perception of living nature. Some scientists of mechanistic materialism denies any sort of qualitative difference between organisms and objects of inorganic nature, which also directs all phenomena occurring in specific biological

living bodies.

In contrast to this, dialectical materialism regards life

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motion is inherent only in livi

definition of Oparin (1968), the

of the matter, arising in a reg

development of matter.

B. BIOLOGICAL DEFINITION OF LIFE

Biologists instead of defining life by certain exact words, provide an operational definition of life (Korn and Korn, 1971). They differentiate living systems with non-living objects according to certain characteristic features and they have employed these characteristic features of living systems to define life. The set of properties that characterize living systems are following :

1. The capacity for synthesis : The living systems convert different energy forms into the chemical energy of ATP, which is then used to derive reactions for the synthesis of macromolecules for maintenance and growth.

2. The capacity for self-regulation : The living systems contain information content in the form of DNA, which specifies the polymeric synthesis and monomaric degradation that can occur through the regulation of protein synthesis.

3. The capacity to adapt genetically : The living systems retain an identity apart from, yet within, their environment while being inherently able to adapt to new environments or improve their efficiency in the same environment by random changes in their information content. In short, living systems undergo evolutionary changes.

Living systems can then be described as *chemical systems capable of elaborate polymeric syntheses, directed in specific patterns*

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ORIGIN OF LIFE

The question of origin of life on our planet, the earth, has remained a most complicated and puzzling problem for thinkers, philosophers and naturalists of modern as well as ancient times. Different views have been forwarded concerning the origin of life on earth by thinkers of different ages. Most of them are quite bizarre and merely having a historical significance. Certain most important hypotheses and theories concerning the phonomenon of protobioge-

ation theory, the life is myst-

The

ding

to Bible, for instance, world was created within six natural days. Plants were created on third day, fish and fowl on the fifth day, and

2. HINDU CONCEPT OF ORIGIN OF LIFE

Different schools of Indian philosophy and Hindu religion has

sufficient amount of

his book. However.

s here some ideas

"Mundaka Upanishad", the life arose from food and food arose the mind. Most of the early upanishadic philosophers considered brahman as not being something transcendental, superhuman, supernatural or other wordly. They did not recognise any God as the creator, controller and designer [illegible] but the infinite totality [illegible] (1970)—"*Man and Soc* [illegible] House, New Delhi, India)

3. THEORIES OF SPONTANEOUS GENERATION OR ABIOGENESIS

For centuries, the concep[t] [illegible] inanimate material was a prin[ciple] [illegible] The spontaneous generation o[f] [illegible] beginning with either inorganic materials or with putrefying organic matter. In China for example, even from the earliest times, there was belief in the spontaneous generation of aphids or other insects under the influence of heat and moisture. In sacred books of India there are indication of the sudden emergence of various parasites, flies, and beetles from sweat and manure. It has been deciphered from Babylonian cuneiform texts that worms and others creature, were formed from the mud of canals. In ancient Egypt the conviction prevailed that the layer of humus deposited by the Nile in its flood stage could give birth to living creatures. When it was warmed by the sun ; frog's, toads, snakes and mice were formed thus. We find a repitition of these tales in the writings of the ancient Greeks like Thales (624-547 B.C.). Empedocles (485-425 B.C.), Democritus (460-370 B.C.), Plato (427-347 B.C.), Aristotle (384-322 B.C.), etc. According to Aristotle in addition to the birth of living creatures from like [illegible] occurred. All s[ome] plete[ra] tion of living things. By the middle of the seventeenth century the theory of spontaneous generation of living things had not been doubted by ony one. William Harvey (1578-1657) despite his brilliant contributions to physiology and despite his important statement that all life comes from the egg, was not able to tear himself from the concept that lower froms of life, such as worms and insects may also originate by spotaneous generation from non-living precursors.

Disproof of the spontaneous generation theory—During the late seventeenth century, an Italian doctor Francesco Redi (1626-1698) performed what are now considered classical experiments to test the validity of the theory of spontaneous generation and

had the honour of being the first to come forward with experimental [illegible] lished for many [illegible] *nerazione degli* which he placed meat or fish (eel) under clean muslin coverings and demonstrated that while flies laid eggs on muslin, maggots or larvae appeared only when those eggs were transferred to the meat and allowed to hatch. He concluded that maggots come only from pre-existing flies and were not spontaneously generated by any other form of material.

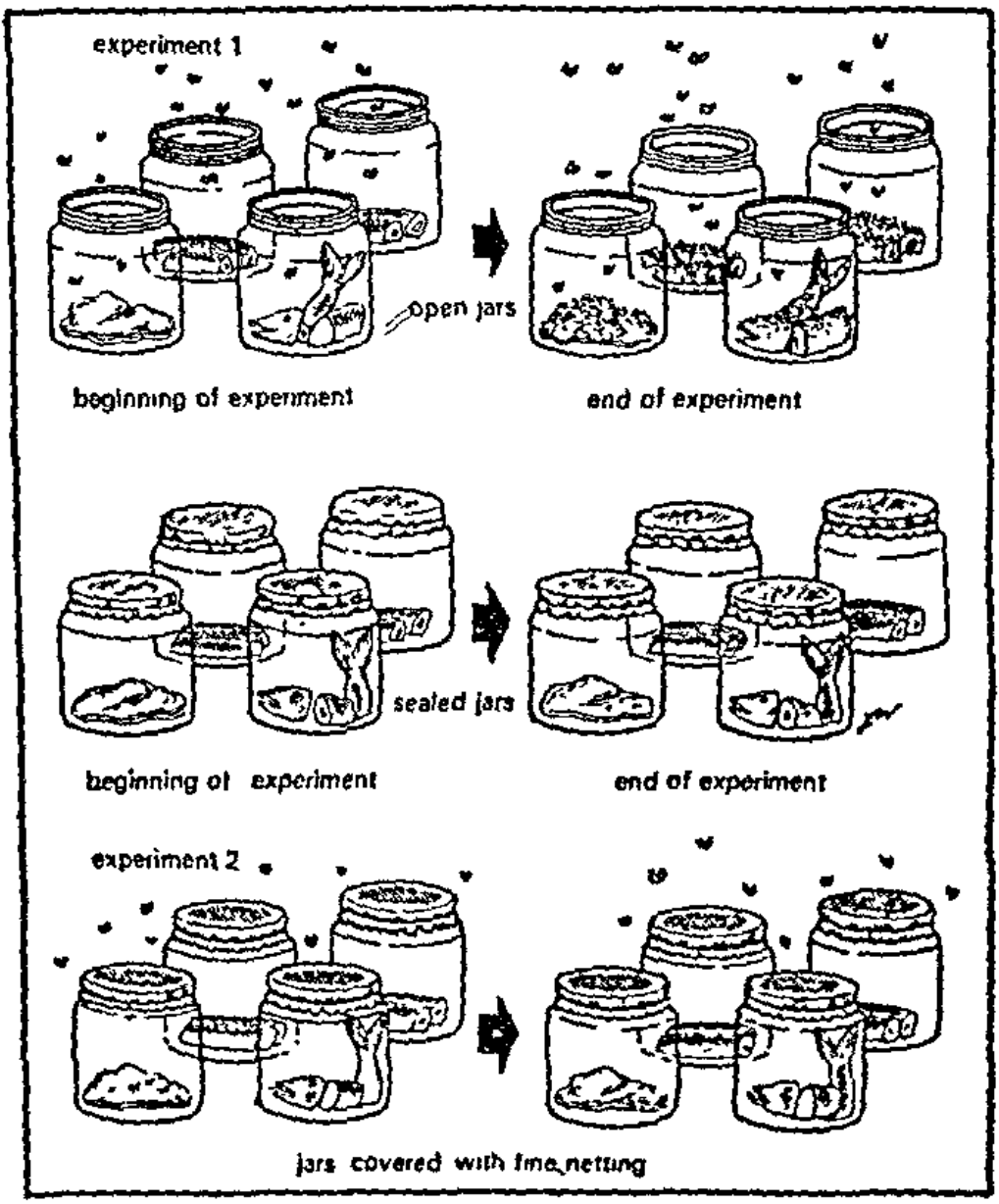


Fig. 2·1. Redi's experiments.

While Redi's experiment proved that even the simple forms of life known in the seventeenth century did not arise by spontaneous generation, the discovery of microbes by **Van Leeuwenhoek** (1632-1723) brought the question into the fore front of biological thought once again. After all, maggots are far more complex than those little spherical, rod, or corkscrew-shaped bacterial forms observed under the microscope. So it was considered that micro-organisms arose by spontaneous generation. This idea was very authoritatively maintained by the noted German philosopher **Leibniz** (1646-1716) French naturalist **Buffon** (1707-1788) and Englishman **John Ne dham** (1713-1781).

THE DECLINE AND FALL OF THE THEORY OF SPONTANEOUS GENERATION

[illegible] ration of microbes. He prepared flasks of meat broth which were boiled for several hours and then sealed. The broth remained clear for months, and when the seals were broken and the broth tested, it was shown to be free of microbes.

[illegible] contemporary scientists. Some claimed that by boiling he had driven out a "vital force" necessary for spotaneous generation. When Priestly proclaimed oxygen

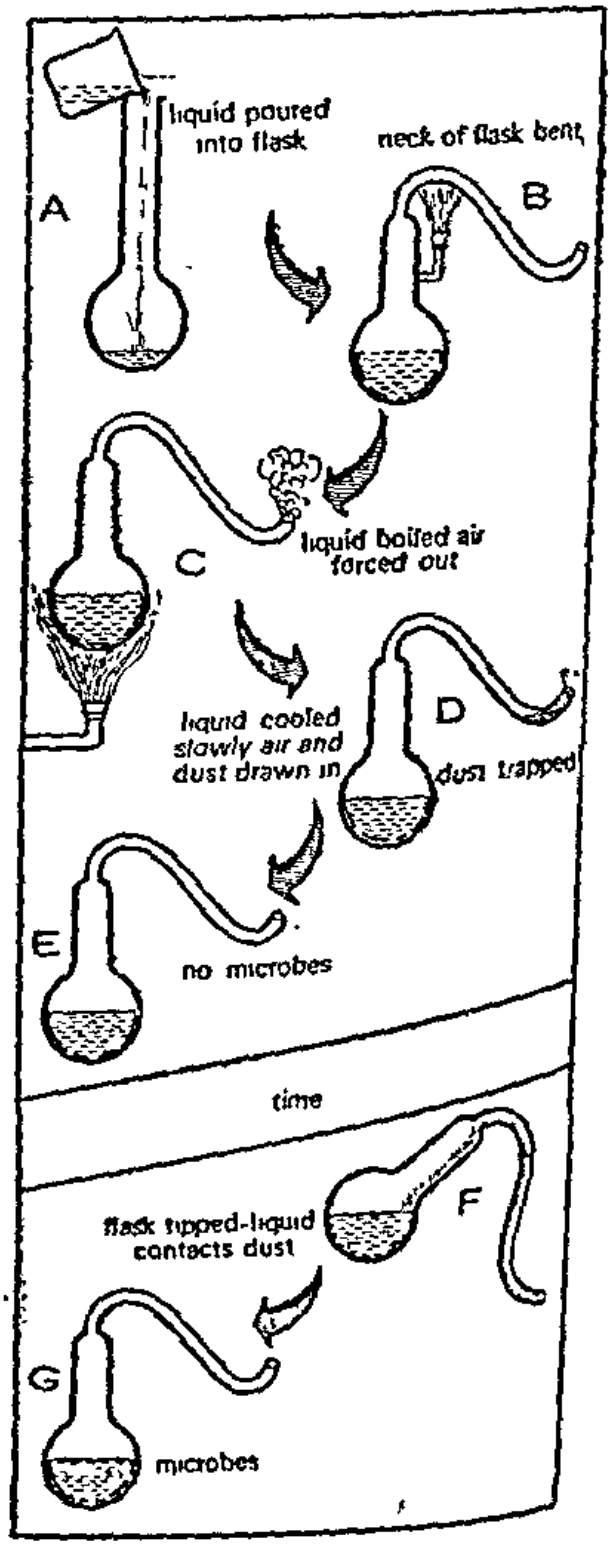


Fig. 2·2. Pasteur's experiment.

as a necessary factor for life and experiments of Gay-Lussac demonstrated that such boiled and sealed flasks contained no oxygen, than these were sized upon as evidence that the vital principle of life required oxygen. Many of Spallanzani's successors modified the experimental procedure in attempt to quell the criticisms, but each [illegible] gave rise to new criticisms. The death blow to the [illegible]obes was finally dealt by [illegible]enth century, some two [illegible] of Spallanzani.

The period of Pasteur : Pasteur devised several experimental means by which the spontaneous generation of microbes was disproved. The simplest and most sophisticated one was with the use of a swan-neck flask. He prepared a meat broth in this flask, and boiled it for several hours. He then left the flask unsealed on a labo- [illegible] the mere boi- [illegible].ty, so the ex- [illegible] The flask was [illegible] *the environ-* [illegible] e swan-neck for months, because, viable microbial particles fter several months when ontamination by air and proliferation of micro-organisms in the fluid ensued. This simple experiment thus altogether disproved the concept of spontaneous generation.

4. THEORY OF ETERNITY OF LIFE

When the concept of spontaneous generation had been given up, the scientific world turned to the theory that life must have existed forever changing only in form. It was generally accepted, even long ago, that the earth had not always existed. Therefore if life is ethernal it must have existed before the formation of this planet. Such reasoning gave rise to theories of the spread of life throughout the universe.

5. HYPOTHESIS OF PANSPERMIA

Some scientists of nineteenth century assumed that this planet was 'seeded' from sp [illegible] theory or hypothesis [illegible] and then supported b [illegible] (1903) [illegible]

inhabiting meteorites or the planets from which they were formed were preserved alive in the interior of meteorites. Such primitive organisms thus thought to arrived through meteorites, finding fertile soil on earth, and grew and evolved to produce all the species now present.

Another hypothesis of panspermia which developed by Arrhenius (1911) holds that spores of life together with particles of cosmic dust could be transformed from one heavenly body to another under the pressure of stellar rays. But it is doubtfull that whether such spore remained viable because as especially serious danger for living embryos is short wavelength ultraviolet radiations, penetrating interplanetary and interstellar space and destructive to all life.

With the end of the spontaneous generation theory and of hypothesis of panspermia and of the intellectual dissatisfaction with the notion of special creation, there appered to be a perplexing problem: there were no acceptable ideas on the origin of life left for one to consider.

About the same time that **Louis Pasteur** was dealing the final death blow to the already dying theory of spontaneous generation, new idea had come to the fore front-natural selection. Almost a century later biologists began to ask: could not biogenesis have occurred by natural selection on a molecular level? Certain biologists like **A. Weismann, Haeckel**, etc., advanced the idea that most primitive organisms would have to be generated spontaneously at some time from inorganic matter as a result of the formative action of some sort of special external physical force which is altogether absent presently. Several post Haeckelian scholars supported the idea of spontaneous generation of life from inorganic substance by some physical force as electrical charge, ultraviolet radiation etc. and even some workers namely **Traube, O. Bütschli, Dubois** (1904), **Kuckuk** (1904) **Leduc**, (1907), **Herra** (1942) attempted to generate life from inorganic substances in laboratory conditions, and in fact, they created some crude living things. Thus, theory of spontaneous generation, like the phoenix of antiquity, died, and rose again to resume its original position.

From 1870 to 1940, most areas of science progressed toward a level of maturity where their firmly established principles collectively created a unified picture of the world. Biology had brought life down to the chemical level through physiology and biochemistry. Chemistry had raised the elementary structure of matter to large synthetic molecules. Physics had provided the energy considerations on which reactions were based. Geology had measured the age of

earth as a few billion years and that of the universe as close to ten billion years. The stage was set for the idea of spontaneous generation to be given a more rigorous inspection. The first form of life was not to be a maggot, or bacterium, or even a virus but a collec-

[illegible] the time was in terms of several billion years, and the experimental procedure was natural selection.

6. THEORY OF CHEMICAL EVOLUTION AND SPONTANEOUS ORIGIN OF LIFE AT MOLECULAR LEVEL

[illegible] A. J. Oparin (1894—) was the first to [illegible] of chemical substances occurred before [illegible] s book "The origin of life on Earth", in 1924 he provided a biochemical explanation of origin of life. According to him, origin of life occurred along the origin and evolution of earth and its atmosphere. The primordial atmosphere of earth had water, methane and ammonia from which as a result of a series of changes, a colloidal body called coacervate and containing a mixture of biologically important macromolecules like proteins, lipids, nucleic acids, etc., were formed. These coacervates though were not living, but th[illegible] subjec[illegible] havin[illegible] J. B. S *Haldane (1929) about the origin of life. Both of these scientists* got the ideas from the studies of biochemistry made by *Sir F. G.* Hopkins, and both were of the opinion that the evolution is purely chemical, the gradual transmutation of inorganic compounds into organic ones.

EXPERIMENTAL SUPPORT OF OPARINS HYPOTHESIS

Oparin's ideas created a rash of excitement, but it was not until 1953 that some experimental evidence was gathered to support the Oparins hypothesis of spontaneous generation of life at molecular level. However, in 1942 a Mexican scientist **A. L. Herrera** synthesized sulphobes" (organized *microstructures with the appear*ance of cells) from ammonium thiocyanate and formalin and he also reported the formation of two amino acid and a condensation product plus pigments, but he failed to correlate his findings with the problem of origin of life.

Miller and Urey's experiment Stanley Miller, in collaboration with Harold Urey, in 1953, created conditions of the surface of the prebiological earth and analyzed the molecular forms that arose. The apparatus consisted of exposing water vapour to electric shock of 75,000 volts (to represent the lightening of thunder storms) in an ammonia and methane atmosphere (the atmosphere of the early earth). After two weeks of treatment, Miller chromatographed the final solution and found many organic compounds, including both carbohydrates and amino acids. Many of the compounds were important metabolic intermediates of present-day organisms.

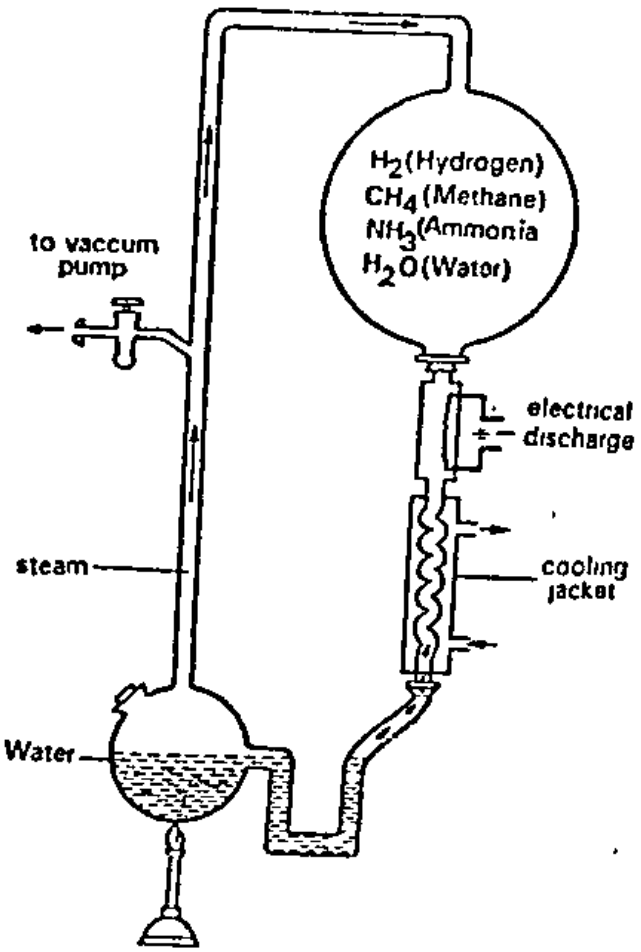


Fig. 2-3. The spark-charge apparatus of Miller for producing organic compounds from a reducing atmosphere.

Besides Miller and Urey many other biologists attempted for prebiotic synthesis in laboratory conditions. Their attempts can be summarized in following table :

Table : Representative model experiments demonstrating prebiotic synthesis of biologically important micromolecules (Fox and Dose, 1972).

Reactants	Reaction phase	Energy	Products	Authors
		Aliphatic Compounds		
1. CO_2, H_2O	Gaseous	UV (1470A°)	Formaldehyde, glyoxal	Groth and Suess (1938)
2 CO_2, H_2O, (Fe^{2+})	Gaseous/ aqueous	α-rays	Formaldehyde, formic acid, succinic acid	Calvin, Garrison *et. al*, (1951)
3 CH_4, NH_3, H_2, H_2O	Gaseous	Electric discharges	Various amino acids, hydroxy acids, HCN, Urea	Miller and Urey (1953, 1955)
4. Ammonium fumarate	Dry solid	Heat	Aspartic acid, alanine	Fox *et al*, (1955)
5. CO_2, NH_3, H_2, H_2O	Gaseous	Electric discharges	Various amino acids	Abelson (1956)
6 CH_4, NH_3, H_2O, H_2, Co_2, Co, N_2	Gaseous	X-rays	Amino acids	Dose and Ejewsky (1957)
7. Ammonium acetate	Aqueous	β rays	Glycine, aspartic acid, diamino-succinic acid	Hasselstrom *et al*, (1957)
8 Ammonium Carbonate	Aqueous	γ-rays	Glycine	Paschke *et. al*, (1957)
9. CH_4, NH_3, H_2O	Gaseous	UV (1165-1850 A°)	Simple amino acids and fatty acids	Groth and Weyssenhoff (1957)
10. NH_3, HCN, H_2O	Aqueous	Heat (70°C)	Amino acids	Oro and Kamat (1961)
11. CH_4, NH_3, H_2O	Gaseous	β-rays	Simple aliphatic compounds including amino acids	Palm and Calvin (1962)
12 HCHO, CH_3 CHO, CH_2 OH-CHOH-CHO Ca-$(OH)_2$	Aqueous	Heat (50°C)	Various sugars including 2-deoxyribose, 2-deoxyxylose	Oro and Cox (1962)
13. HCHO	Aqueous	UV(2537 A°)	Various sugars including ribose and deoxyribose	Ponnamperuma (1965)
14. CH_4, NH_3, H_2O (Silica contact)	Gaseous	Heat (850°C)	Amino acids	Harada and Fox (1964)

Reactants	Reaction phase	Energy	Products	Authors
		Aliphatic Compounds		
15. CH_4	Gaseous	Electric discharges	Higher hydrocarbons (including aromatic hydrocarbons)	Ponnamperuma and Woeller (1964)
16. CH_4 (silica contact)	Gaseous	Heat (1000°C)	Higher hydrocarbons (Mostly aromatic hydrocarbons)	Ora and Hao (1966)
17. $HC{\equiv}C{-}CN$, HCN, NH_3, H_2O	Aqueous	Heat (1000°C)	Aspartic acid	Sanchez *et. al.* (1966)
		B. Aromatic Compounds		
18. HCN, NH_3, H_2O	Aqueous	Heat (70°C)	Adenine	Oro and Kimball (1961, 1962)
19. Malic acid, Urea, Polyphosphoric acid	Liquid anhydrous	Heat (130°C)	Uracil	Fox and Harada (1961)
20. CH_4, NH_3, H_2O	Gaseous	α-rays	Adenine	Ponnamperuma, Lemmon, *et. al*, (1963)
21. Adenosine, polyphosphate ester	Liquid anhydrous	UV (2537A°)	AMP, ADP, ATP	Ponnamperuma, Sagan *et. al.*, (1963)
22. CH_4	Gaseous	Electric discharge	Mostly aromatic hydrocarbons	Ponnamperuma, and Woeller (1964)
23 Nucleoside phosphate	Solid anhydrous, hypohydrous	Heat (160°C)	Nucleotides	Ponnamperuma and Mack (1965)
24. CH_4 (Silica-gel contact)	Gaseous	Heat (1000°C)	Mostly aromatic hydrocarbons	Oro and Han (1966)
25. Nucleosides, Polyphosphoric acid	Liquid anhydrous	Heat (22°C)	Nucleotides, Nucleoside tri-phosphates	Waehneldt and Fox (1967)
26 CH_4, NH_3, H_2O	Gaseous	Electric discharges	Porphyrins	Hodgson and Ponnamperuma (1968)

The results of Miller's classical experiment were significant in ssible to pass from the inorganic to a ic level. But Oparin had postulated large natural-selection pressures that favour them rather than simple molecules. Could such large molecules (coacervates or some other associates) be produced in the laboratory under conditions that resembled the early earth ?

Sidney Fox a number of nd heated them The surface of) and allowed to cool. When the residue was examined under a microscope, millions of small spheres were seen, similar in size and shape to yeast cells. Fox designated them *proteinoids* or *microspheres* because of their chemical nature. These microspheres were found to possess polypeptides ; a double-layered surface with semipermeable properties ; a capacity to carry out enzymatic reactions, notably oxidations, and power of reproduction by budding.

7. MODERN SELF-ASSEMBLY THEORIES

Modern self-assembly theory holds that protobiogenesis

tically active complexes. Another example of intermacromolecular assembly is the combination of proteins and nucleic acids, as in tobacco mosaic virus (**Fraenkel-Conrat** and **Ramachandran, 1959**) ; **Caspar, 1963**) and in the ribosomes (**Nomura** and **Traub 1968** ; **Spirin, 1968**).

The modern researches of protobiogenesis by self-assembly method besides suggesting certain most convincing ideas concerning possible mechanism of origin of life and evolution of first living-being, gy suggested by enesis suggests amino-acids in

the geochemical matrix to protein in the first organism that separated from the environment. The primordial information might have flowed from protein to nucleic acid (Fox *et al.* 1959; Woese, 1968; Lacey and Pruitt, 1969; Fox and Dose. 1972) as well as in the reverse or contemporary direction.

With such a historical background about the speculated modes of protobiogenesis, now we are in better position to discuss the present (current) understanding of molecular evolution and its culmination into the origin of life. The pre-requisite for the treatment of the problem of protobiogenesis is the origin of the raw materials (matter, elements, stars, planets, etc.) for the molecular evolution, *viz.*, a brief introduction of cosmology.

1. THE STRUCTURE OF COSMOS

The universe or cosmos is made up of countless aggregations of stars organized into systems of various shapes and types, called galaxies. These galaxies, each of which includes hundreds billions of stars with their accompanying planets and satellites, are distributed

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2. ORIGIN OF MATTER

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Modified version of big-bang hypothesis is that the primary explosion and subsequent expansions of cosmos were caused by the annihilation of colliding particles of matter and anti-matter (Alfven, 1966, 1967).

3. EVOLUTION OF ELEMENTS

Most astrophysicists agree that stars are formed by gradual condensation of huge dark clouds of cool light elements, predominantly hydrogen. These clouds yield primarily large and diffuse proto stars (Fowler *et al.* 1967) that continue to condense by their own gravitational pull, causing their central regions to grow hotter. As the temperature increases, more atoms of hydrogen become ionized and as a consequence the collisions between the bare hydrogen atoms, that is the resulting protons, become more fast-...re
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At still higher temperatures, in the presence of nucleus of C^{12}, another more violent process of making helium from hydrogen is activated. This is called carbon cycle (Bethe, 1939, 1968) and it produce energy at a faster rate. The carbon cycle moves through a series of six steps during which four protons are captured by a carbon nucleus, forming a helium nucleus. In the final step a carbon nucleus reappears. In this process, thus, carbon acted only as a catalytic agent being used repeatedly.

...
more massive star may rise above 100,000,000°C. At such a temperature, helium nuclei and hydrogen nuclei fuse to make nuclei, chiefly of carbon, oxygen, and neon. At temperatures about 1,000,000,000°C. ...
...
of energy.

4. ORIGIN OF EARTH AND SOLAR SYSTEM

The sun and its planetary bodies have evolved from a common source. According to **Kuiper** (1949, 1951), a part of a huge cloud of dust and gas condensed to form primarily a **proto-sun** of low density surrounded by a rotating cloud disk. The outer zones of disk broke up into eddies of irregular size and arrangement, moving at various radii from the suns centre. The eddies in any radial zone merged into a single cluster of condensing gas and dust. These clusters have been called the **proto-planets.**

Kuiper further suggested that the **proto-earth** probably was a low-temperature body of gases and solids of about 500 times the earth's present mass. The matter of the sun had not condensed sufficiently at an early stage to be hot enough to radiate heat to the protoplanets. The gaseous part of the proto-earth consisted mainly of hydrogen and helium plus neon, methane ammonia and some water vapour, the solid part consisted principally of condensed water and minerals. The heavier material (such as iron, uranium, copper, etc.,) were eventually spiralled into the centre, while the lighter elements (such as hydrogen, and helium, concentrated on the surface. During such a process of aggregation the proto-earth is believed to have heated very pronouncedly due to the release of gravitational energy. Further the pressures generated such as heat that the core liquified and many radioactive materials were produced in the several million-degree heat.

As the sun condensed, it became hotter and brighter by conversion of gravitational energy into heat which was radiated into space, thus, further increasing the temperature of the protoplanets. Probable huge amounts of lighter gaseous components escaped from the gravitational fields of the proto-earth to form the proto-or primary atmosphere of earth. Eventually, the earth slowly cooled over a period of a million years to the point where small molecules maintained their stablet states.

Modern hypothesis of Urey (1962) about the aggregation and condensation of primitive earth, however, contradicts to Kuiper's hypothesis in certain respects. For example, he postulates the accumulation of cold solid, the planetesimals, to be the main process in the formation of primitive earth. In Urey's view, the size of these particles ranged in between the size of asteroids and that of dust particles. He agreed with Kuiper in postulating strong surface heating during the aggregation of smaller particles but, for largest bodies he postulated to remain cool and not to melt during aggregation. Today most geologists agree with Urey's concept that the temperature

of earth's surface during the aggregation probably was never high enough to bring about a general and complete melting of the lithosphere (>900°C). On the other hand, the temperature in the interior of the earth is believed to have been at some thousands of degrees after the earth was formed, because of the heat flow originating in its interior.

STRUCTURE OF PRIMITIVE EARTH'S LITHOSPHERE

The earth's lithosphere may be classified, into the core, the mantle, and the crust. Geochemistry has shown the following chemical composition of three components of lithosphere:

Table: The geochemical composition of the inorganic lithosphere (Fox and Dose, 1972)

Components	Composition	Physical state
Crust	Silicates of Al, Fe, Ca, Mg, Na, K	Mainly solid but flows under tectonic stress.
Mantle	Mainly silicates of Mg, and Fe Probable FeS margins	Solid but flows under stress.
Core	Fe—Ni only	Outer part liquid, central part possibly solid.

STRUCTURE OF PRIMITIVE EARTH'S ATMOSPHERE

It is generally assumed that upon its agglomeration the primitive earth was without an atmosphere (the gaseous constitiuents of the materials from which it was formed —the primary atmosphere having mostly escaped into outer space) and that its present atmosphere (the secondary atmosphere) evolved from volcanic outgassings (see Fox and Dose, 1972). Volcanic gases might be consisted of mainly juvenile water vapour (*i.e.*, deuterium-poor water) which is released from water of crystallization, and variable amounts of CO_2, N_2, SO_2, HCl, B_2O_3 and lesser amounts of H_2, CH_2 CO, NH_3 and HF but no oxygen.

The absence of oxygen in the earth's earliest atmosphere has been confirmed by many experiences—1. [illegible], 1962). [illegible] oxygen 3. Both the primary [illegible] of the evolving earth must have been free of oxygen for thermodynamic reasons. 4. Laboratory experiments show that chemical evolution would have largely inhabited by oxygen. 5. Organic compounds that according to Oparin (1965) and Haldane (1965) and others, have accumulated on the surface of the earth in the course of chemical evolution are not stable over geological times in the

presence of oxygen.. 6. Biochemical studies indicate that "primitive" forms of metabolism are anaerobic (Haldane, 1929, 1965). 7. The evolution of the contemporary oxygen atmosphere is correlated with photosynthesizing life forms although less with direct photodissociation of H_2O vapours. Lastly, geological and paleontological evidences suggest that the average oxygen level to sustain some primitive animal life prevailed towards the end of the precambrian (0·6–1 0 billion years ago) (Fischer, 1965).

Some evolutionist have reasoned that the primordial or primitive atmosphere of the earth was a reducing atmosphere consisting mainly of hydrogen, methane, ammonia, water vapour and noble gases (Russel, 1935, Oparin, 1938, Urey, 1952, Miller and Urey, 1959) In fact, hydrogen makes up more than 80 pre cent of cosmic matter and hydrogen and helium together make more than 99 per cent. Carbon, nitrogen, and oxygen are the most abundant bioelements in the universe besides hydrogen and helium. If hydrogen and helium had not escaped into space during the formation of earth, and if the temperature of the primordial atmosphere never significantly exceeded 25°C, the formation of a methane-ammonia-water atmosphere would have been favoured, according to the following equations :

$$C + 2H_2 \longrightarrow CH_4$$
$$NH_2 + 2H_2 \longrightarrow 2NH_3$$
$$H_2 + \tfrac{1}{2} O_2 \longrightarrow H_2O$$

However a variety of geological evidences have shown that the strongly reducing primary atmosphere consisting of methane, ammonia, hydrogen and water has already disappeared *before* a significant accumulation of organic compounds (formed from the constituents of the secondary atmosphere) took place (Urey, 1962: Abelson, 1966 ; Fox and Dose, 1972).

7. STRUCTURE OF PRIMITIVE HYDROSPHERE

The primitive ocean, like the present oceans, were cradled in basins which were great rift valleyes (Rittmann, 1962). It contained water mass less than one-tenth of the volume of water in the contemporary oceans, the remaining nine-tenth having been later supplied by outgassing from the interior (Rubey, 1955). The volume of the oceans increased significantly during pre-cambrian times and approached its present value at the beginning of cambrian (Weyl, 1968). For the origin of water following mechanism has been suggested(see Korn and Korn, 1971)—vast quantities of hydrogen and oxygen under great pressure and heat below the surface formed water. This water is escaped through fissures of the rocks and is known as

juvenile water and it formed an atmosphere of vapour. Gradually the temperature of the earth's surface lowered sufficiently for the condensation of this vapour to occur and enormous rains poured down. It has been estimated that the rains continued unceasingly for thousands of years.

During these storms, lightening discharged great quantities of electrical energy. The rains were down the rocks and the mountains were lowered. The water flowed down to the lower levels, eroding surfaces and carrying dissolved minerals with it. The oceans slowly formed, creating great seas of sterile, inorganic media. Subsequent evaporation of the water led to more rain, and more wear of rocks as this water found its way to the sea, bringing with it dissolved salts. The concentration of these inorganic materials began to approach the now prevailing 3% salt content of the sea. Most of the minerals common today were now present. Oxides and sulfates however, were not formed until free oxygen (a product of photosynthesis) was available in the atmosphere.

8. ENERGIES AVAILABLE ON THE PRIMITIVE EARTH

Those constituents of the primitive lithosphere, hydrophere and atmosphere, that contained such bioelements as carbon, hydrogen, oxygen, nitrogen, sulfur, and phosphorus were the raw materials for the evolution of organic compounds. In order to yield molecules of biological significance, the simple starting materials required energy in the form of free energy or activation energy. The possible sources of energies available on the primitive earth are following :

(i) **Solar radiations**—Different kinds of solar radiations were the most abundant type free energies of primitive earth. Visible light remained effective during a later phase of molecular evolution, however, ultraviolet radiation might have remained effective during early phase of molecular evolution.

(ii) **High energy radiations**—Most of the high-energy radiations originated from the interior of primitive earth. The major sources of high energy radiations on the earth 4—4·5 billion years ago were probably the unstable isotopes ^{238}U, ^{235}U, ^{232}Th, and ^{40}K. On decay, the first three isotopes, which are heavier, give off α-particles as well as γ-rays and β-particles ; ^{40}K gives off γ-rays and α-particles only. Horowitz and Miller (1962) have postulated that high-energy radiation and volcanic heat were available in small amounts on primitive earth, therefore, had little significance in molecular evolution.

(iii) Electric discharges—Electric discharges were the violent form of energies and they contributed significantly to the evolution of micromolecules.

In addition, some other forms of energy such as heat of volcanic emissions, heat of hydrothermal veins, microwaves, and supersonic shock waves, might had contributed to molecular evolution.

9. FORMATION OF MICROMOLECULES

The [illegible] daltons are called [illegible] omolecules are amino acids, fatty acids, monosacharides and other metabolites or building stones of organisms. Various experiments which

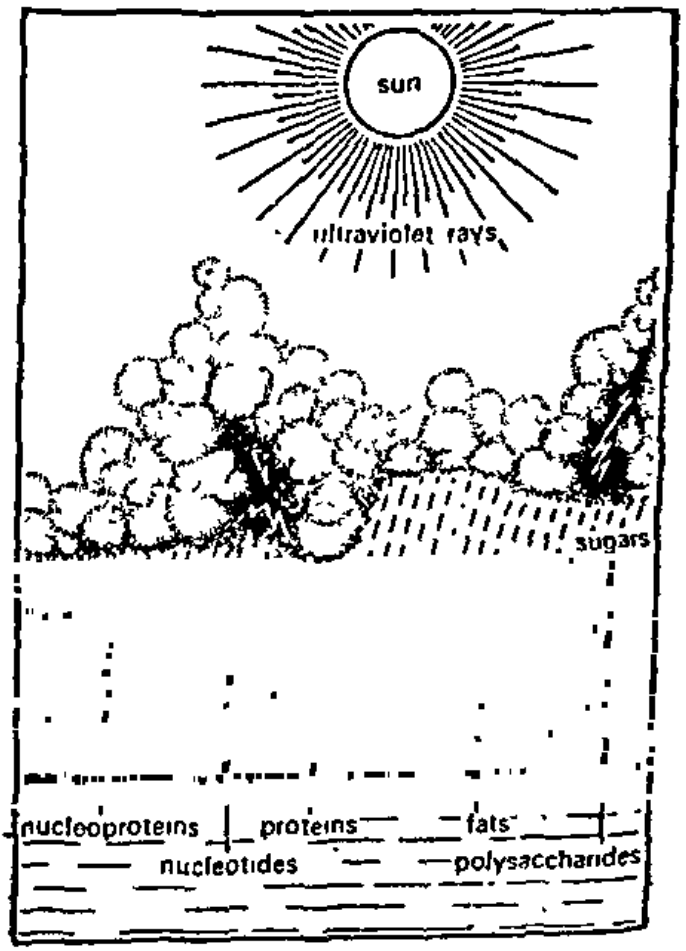


Fig. 2·4. Primitive sea and formation of organic molecule.

simulated pre-biotic conditions of earth and which have produced a variety of micromolecules such as amino acids, fatty acids, adenine and pyrimidine bases, ribose and other sugars, ATP etc. suggest a possible evolutionary sequence of micromolecular evolution. Some important experiments of such type have been tabulated in the table of page 19—20. The possible mode of formation of some biologically important micromolecules is of following type :

1. **Formation of amino acids**—The primitive terrestrial atmosphere and hydrosphere had a variety of organo-elements such as CH, NH, CO, H$_2$CO, HCN, H$_2$O, etc. These biochemicals became

electric discharge according to following reaction—

$$R\text{-}CHO + NH_3 + HCN \longrightarrow R\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}CN + H_2O$$

$$R\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}CN + 2H_2O \rightarrow R\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}COOH + NH_3$$

2. **Formation of hydrocarbons and fatty acids :** A variety of hydrocarbons and fatty acids are thought to be formed in primitive earth due to condensation of methane, ammonia water vapour hydrogen, etc., in the presence of some source of free energy. **Miller** (1955) demonstrated that **formic acid, acetic acid, propionic acid** and certain oxygen derivatives, *e.g.*, glycolic acid, and lactic acid, are formed when a gaseous mixture of methane, ammonia, water vapour and hydrogen is exposed to a spark discharge. Likewise, formaldehyde or glyceraldehyde has been reported to condense in a variety of sugars such as **fructose, cellobiose, sorbose, xylulose,** and **glycolaldehyde,** under prebiotic conditions. **Pfeil** and **Rueckert** (1961) reported the formation of glycolaldehyde, glyceraldehyde hydroxyacetone, and some higher monosaccharides from alkali-induced condensation of formaldehyde. During these reactions molecules of formaldehyde condensed in following ways to produce first aldol then trioses, tetroses, pentoses, hexoses, etc.

$$\underset{\text{Formaldehyde}}{2H_2CO} \xrightarrow{\text{slow}} \underset{\displaystyle CH_2OH}{\underset{|}{CHO}}$$

$$\underset{\displaystyle CH_2OH}{\underset{|}{CHO}} + H_2CO \xrightarrow[\text{fast}]{\text{base}} \underset{\displaystyle CH_2OH}{\underset{|}{\underset{\displaystyle CH_2OH}{\underset{|}{CHOH}}}}\text{-}CHO$$

Aldol condensation

Formaldehyde itself is reported to produced in abundance when gaseous mixtures consisting in part of methane, and water are subjected to an electric discharge (Miller, 1955), to γ-irradiation (Palm and Calvin, 1962), or to β-irradiation Ponnamperuma and Flores, 1965). Ponnamperuma (1965) claimed that sugars (ribose and deoxyribose) are directly formed by electron irradiation of methane

ammonia, and water. UV irradation or γ-irradiation of aqueous formaldehyde induce the formation of ribose, deoxyribose and other sugars.

3. Formation of purines and pyrimidines : Purines and pyrimidines are thought to be originated from hydrogen cyanide (HCN) in the primitive earth due to condensation reactions in the presence of heat (Oro *et al.*, 1960, 1965) or radiations (Ponnamperuma, 1965; Palm and Calvin; 1962), β irradiated hydrogen rich atmospheres of methane, ammonia, hydrogen and water vapour and reported the formation of 4—aminoimidazole—5—carboxamide (AICA) and adenine. AICA is a intermediate which by reacting with guanidine and formamidine can form guanine, xanthine, or hypoxanthine (Oro and Kimball, 1962).

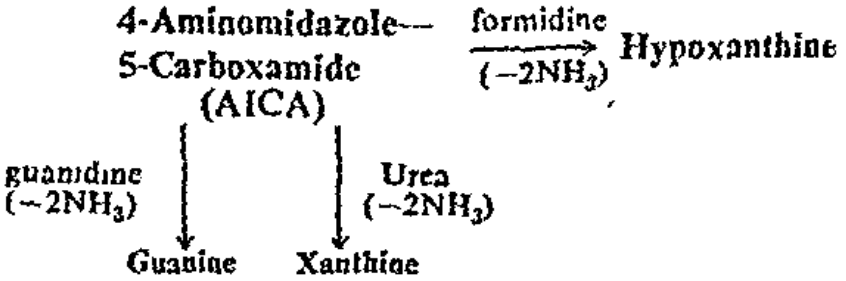


Fig. 2·5. Proposed mechanism for the synthesis of purines on purines on the primitive earth.

Likewise, following mechanisms for the synthesis on the primitive earth of pyrimidines from B-aminoacrylonitrile or α-methyl -β-aminoacrylonitrile have been proposed (Ferris *et. al.*, 1968) :

(i) $C_3 + C_1 \rightarrow$ β-aminoacrylonitrile $\xrightarrow[+NH_3]{H_2O}$ + Uracil or Cytosine

(ii) $C_3 + C_1 \rightarrow$ α-methyl-β-amino-acrylonitrile $\xrightarrow[Urea]{H_2O}$ Thymine

4. Formation of nucleosides and related compounds: Ponnamperuma (1965) demonstrated the production of adenosine by heating dilute aqueous solutions of adenine, ribose and phosphoric acid. He also synthesized adenosine monophosphate (AMP), adenosine diphosphate (ADP) and ATP by exposing an aqueous solution of adenosine and phosphoric acid to ultraviolet light. Besides phosphoric acid, ethyl metaphosphate was used as phosyhorylating agent by him. Waehneldt and Fox (1967) demonstrated that the nucleosides-adenosine, cytidine, guanosine, uridine, and thymidine-are phosphorylated with polyphosphoric acid at temperatures in the range of 0-22°C. All biologiclaly significant mono, di-and triphosphates of nucleosides have been obtained by this procedure. The nucleosides and

deoxynucleosides evolved during later phase of molecular evolution and before their origin, their function of coenzyme and constituents of genetic material was taken by other compounds. Inorganic pyrophosphate has been considered to be primitive group carrier in biological systems (**Lipmann**, 1965, **Kulaev**, 1970).

5. **Orgin of porphyrins**—Most protobiogenists have concluded that porphyrins (chlorophylls, haemoglobin, cytochromes, etc., contain porphyrin rings in their chemical composition) have been required in at least two stages of evolution of living systems during chemical evolution. One stage was the evolution of porphyrin-dependent photosynthesis which must have come into existence early in the evolution of living systems (2×10^9 years ago) as evidenced by fossils of vanadium-porphyrin complexes (**Fox** and **Dose**, 1972). The second stage was the evolution of porphyrin-dependent respiration which must have developed when the atmospheric oxygen level exceeds the Pasteur point, 6×10^8 years ago (**Mahler** and **Cordes**, 1967).

Pyrroles, the probable precursors of porphyrins, were likely formed at an early stage of chemical evolution due to speculated reaction between either acetylene and hydrogen cyanide, (**Meyer** (1913), or acetylene and ammonia (**Chichibabin**, 1915). **Szutka** (1965) have prepared porphyrin-like substances by irridating either the mixtures of pyrrole, benzaldehyde, pyridine, and zinc acetate by γ—irradiation or the mixture 3 ml freshly distilled pyrrole with 6 ml of benzaldehyde in 4 ml of water by ultra-violet irradiation. However, **Hodgson** and **Ponnamperuma** (1968) have suceessfully generated porphyrin-like compounds from a mixture of methane, ammonia, water vapour in the absence of hydrogen atmosphere and according to them formaldehyde and pyrrole were the precursors for porphyrin in primitive earth.

10. FORMATION OF MACROMOLECULES

The phase of origin and evolution of bio-micromolecules of molecular evolution was followed by the phase of origin and evolution of macromolecules during which many bio-macromolecules such as proteins, nucleic acids, lipids, polysaccharides, etc., were originated and evolved in a evolutionary sequence. The proteins were the first macromolecules to be originated during prebiotic molecular evolution (**Moody**, 1970; **Fox** and **Dose**, 1972). The initial prebiotic proteins were non-random, contained some enzymatic properties and are called **protoproteins**. These protoproteins were originated without getting any sort of information from the nucleic acids because the latter were not originated upto that stage.

Rather they, originated atrandomly due to polymerization of diverse amino acids of primitive environment of earth by getting required energy for such polymerization from thermal energy of the environment. Further, these precursor amino acids of protoproteins are thought to had some information called **protoinformation**, for polymerization in the latent form. At an later stage, the protoproteins acted as informational molecules for the polymerization of other kinds of amino acids to form different kinds of protoproteins.

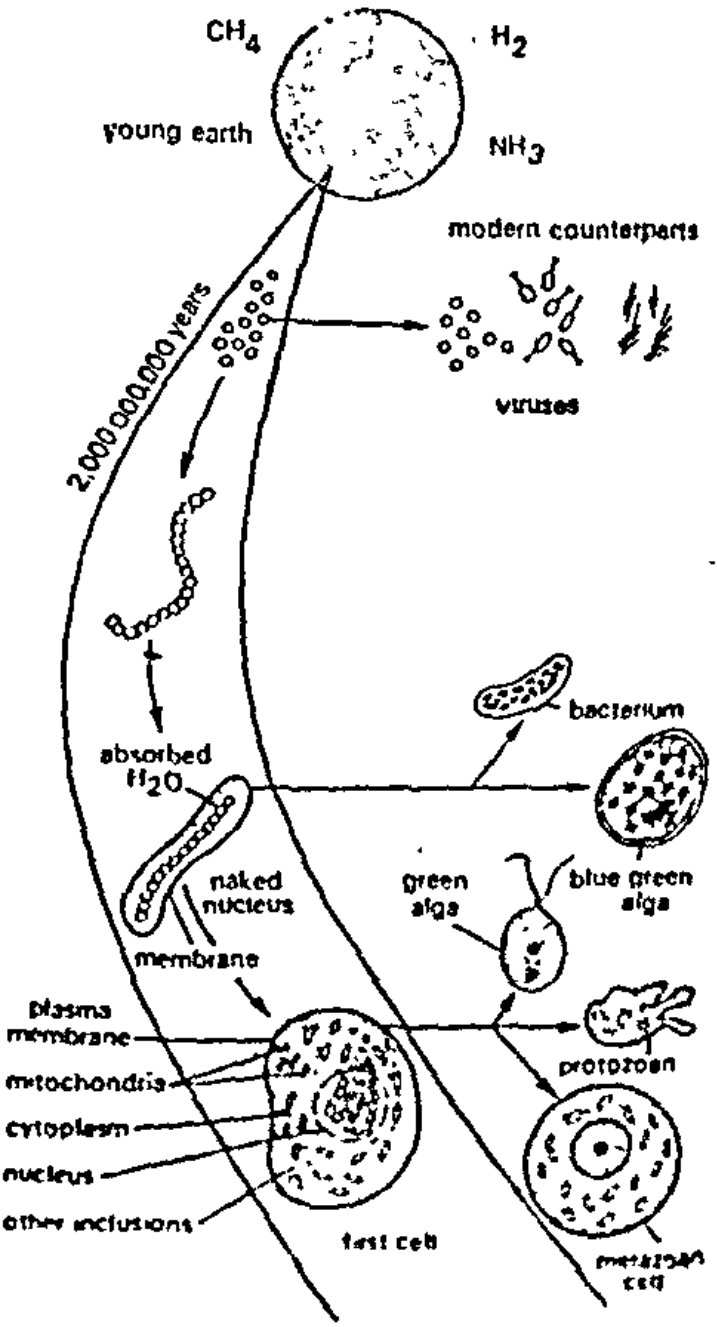


Fig 2·5. Origin of first organism.

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also for environmentally synthesized proteins (protenoids). The instructions for the behaviour in the protocellular life-cycle were in the protenoids, and were derived from the mixture of diverse precursor amino acids and their differing reactivities. Perhaps the emergence of the growing polyamino acid chain was essential, however, for a first feedback mechanism, originating from the interactions of the growing peptide chain with the condensing amino acids. This nucleic
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otrophic replication which was an evolutionary precursor of the nucleic acid-controlled genetic system, there existed in the environment self-ordered protenoids around the protocells. Variation and molecular selection would have occurred within the limited array of types in one protenoid, and as a result of variations in precusor mixtures of amino-acids to yield varied protenoids. Such prebiotic molecular evolution had proceeded on a pathway that was narrow due to the favouring by selection of some of the molecular processes.
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protobionts would have participated in the inheritance by the offsprings of the diversity of forms possible. This kind of life and protoheredity could have continued indefinitely for eons until the greater freedom from the environment was attained through an inter-macromolecular code.

12. ORIGIN OF LIFE

Thus, the protobionts were the complicated little creatures which did not come about by reproduction but were spontaneously generated through the combination of monomers available in the environment into polymeric sequences that cumulatively endowed

The protobionts were much simple than orary life entities the PPLO bacterial h have been considerered as the candi-

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genetic machinery and requirements of macromolecules.

It is suggested that the protobionts were formed not as a single chance event, but as the outcome of a stepwise accumulation of traits, directed by the selection pressures of the total environment. Life took about one billion years to progress from the stage of small aggregates of molecules to the biological level of the primitive PPLO type cell.

13. EVOLUTION OF PROTOBIONTS INTO CONTEMPORARY CELLS

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of matter produced. For the first stage of independence the biota had to develop the ability to trap energy from the sun. This energy trapping ability was achieved by the origin of porphyrins and their combinations with certain protenoids. Nor did all cells need this ability ; the other cells would obtain their energy through heterotorphic activity. A more independent and adaptable cell arose.
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These
which
the instructions from one generation to a descendant generation were channelled through the parents as individuals rather than being drawn again from the monomers and their polymers in the environment. In other words, the ability to produce structural polymer independent of the environment emerged and evolved with the ability of the cell newly generated from a parent to receive its instructions from that parent instead of from the environment For such protein synthesis, entirely independent of environment, protocells could have evolved some internal mechanism of protein biosynthesis. When the instructions were coded and transferred to

storage macromolecules, further independence would have been attained.

Thus, evolution of protocells into contemporay cell is thought to have included following stages :

During the first stage certain protocells became fully heterotrophic to draw from the environment all amino acids, nitrogen bases, cofactors, coenzymes, inorganic ions, and energy-rich molecules (such as pyro
somewhat later stage.
rins) were associated
able to trap solar energy during photosynthesis to synthesize the chemical molecules such as glucose containing chemical energy in
proteins.

Further advancement of life was achieved when these protobionts acquired the informational macromolecules the DNA, according to following suggested mechanisms or possibilities : (i) One possibility is that the adenylic acid, and perhaps other mononucleotides, polymerize simultaneously with the amino acids residues (Nakashima *et al.*, 1970). (ii) Another possibility is that the amino acid condensation product, under appropriate circumstances, has itself polymerase enzymatic activity and (iii) The third is that a thermal proteinoid *in situ* had polymerase activity.

In subsequent stages, the DNA-containing protobionts acquired other components of protein-synthesis mechanism such as RNA, molecules, decoding molecules (activating enzymes for each amino acid), ribosomes, etc.

14. ORIGIN OF CELL

Once the protobiont was formed and the DNA-RNA-protein system established, a variety of control mechanisms became essential. Genes cannot read and
ld regulate the synthesis of proteins selectively, according to the environmental contribution of substrates. The integration of organismal activities into a genetically mediated pattern took the form of the contemporary cell.

All the major steps of molecular evolution and origin of life can be summarized in following flow chart :

Origin of the universe or cosmos

↓

Evolution of stars and elements

↓

Formation of organoelements (*e.g.*, CH_4, NH_3, CO, H_2CO, HCN, H_2O, etc.) from first simple molecules in intergalactic clouds.

↓

Condensation of an intergalactic cloud of dust and simple gases (primary atmosphere) to form the solar system

↓

Formation of primitive earth

↓

Accumulation of a secondary atmosphere of NH_3, CH_4, H_2O H_2, CO_2, N_2, H_2S, CO, etc. of volcanic origin

↓

Pr[illegible] N-bases, various p[illegible] ion)

upon the components of the terrestrial atmosphere and hydrosphere. The diverse amino acids of environment contained proto-information for their polymerization

Heat ↓ — Unsuccessful polymers ↓

Formation of macromolecules (*e.g.*, polypeptides, polysaccharides, polynucleotides, etc.) by condensation of micromolecules by thermal energy. Formation of informational protoprotein which were non-ran dom and enzymic

Prebiotic molecular evolution

H_2O as triggering agent ↓

Beginning of primitive life — Self-assembly of polypeptides into protoproteinous protocells (origin of first protobiont and life).

Replication

↓

Some did not survive — Numerous protocells, including variants provided by the environment and by replication using preformed proto-protein from the environment : these undergo selection

↓

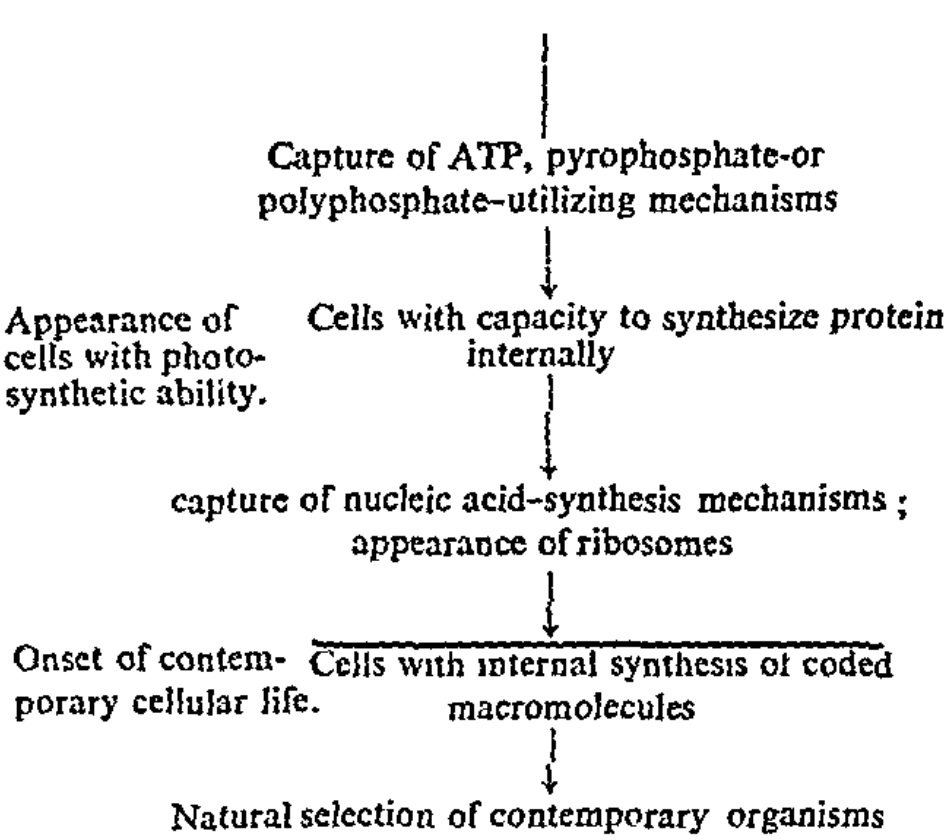


Fig. 2·6. Flow chart of principal stages of mol-cular evolution and the origin of life.

EXPERIMENTAL EVIDENCES SUPPORTING THE IDEA OF SPONTANEOUS ORIGIN OF PROTO-CELLS

The first attempt to describe the origin of life by natural selection on molecules in the pristine seas was made by Oparin in 1936. He proposed that [illegible] would

tion of a type of colloidal body well known in physical chemistry as [illegible] icro-[illegible] the [illegible] Oparin

[illegible]

The coacervate droplets were introduced as models for the contemporary cells by the Dutch colloid chemist de Jong and his colleagues in 1930. Oparin (1936, 1965, 1966) and his students namely Evreinova and Bailey (1968) and Serebrov Skaya (1968) have extensively studied coacervate droplets as models of precellular organization.

Synthesis of coacervate droplets—The coacervate droplets have been made typically by combining solutions of oppositely charged colloids, *e.g.*, gelatin+gum arabic, or, serum albumin+gum; or, clupein+gum, or histone+gelatin, histone+RNA, or gelatin+gum +DNA Each solution is uniform, but when the two are brought together they interact to form 'swarms' or 'clusters'. When these clusters reach a certain size they sep rate from the solution in the form of coacervate droplets.

Characters of coacervate droplets—Oparin's coacervate droplets can take up materials from the external medium and some of them show enzymatic activities. For example, Oparin and his associates have made coacervate droplets from gum arabic and histone at pH 6 2 and have included phosphorylase enzyme during the formation of droplets. When glucose-1-phosphate is dissolved in the equilibrium liquid, the droplets are found to store starch. In another experiment, coacervate droplets of some sort have been prepared with phosphorylase and β-amylase. In this case, the starch that formed from the entering glucose-1-phosphate then decomposed into maltose which diffused into the external medium where it was detected.

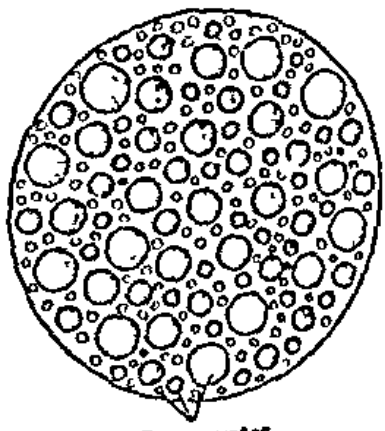


Fig. 2·7. Coacervate droplets.

Similarly, polynucleotides were synthesized enzymatically within coacervate droplets. These experiments with coacervate droplets thus show some sense of the special benefit of encapsulation of enzymes in living entities. Oparin proposed that although the coacervates which might be originated during chemical evolution, were not living, they behaved in a manner similar to biological systems (1) by being subjected to natural selection (in terms of a positive selection for stability as expressed through persistant size and constant chemical properties, (2) by being chemically directive (selective accumulation of material) and (3) by reproduction (by fragmentation).

But since coacervate droplets lack stability, they fall apart on standing and they were synthesized from polymers obtained from living systems, they no longer served as the models of protocells. The widely accepted model of protocell or protobiont was provided by Fox by preparing proteinoid microspheres from monomers and by presenting these microspheres as the models of protocells.

PROTENOID MICROSPHERES

[illegible] twenty amino acids together and heated them on vo[illegible] the r[illegible] thermal polyamino acids might came in contact with water). When the residue was examined under a microscope, millions of small spheres called protenoids or microspheres were visualized. The polypeptides in protenoids consisted of about eighteen amino acid residues, but because they were produced spontaneously, each had a different amino-acid sequence.

Physical properties of protenoid microspheres—The protenoid microspheres were spherical, microscopic and of uniform diameter. Their size remained in between the range of 0·5–0·7mμ diameter. They had a number of properties in common with coccoid bacteria and microfossils found in ancient geological strata (Fox, 1969), in containing uniformity in size, stability in centrifugation, electron microscopy, extreme pH variations, etc.

Structural properties of protenoid microspheres—Electron microscopic studies of protenoid microspheres have revealed concen-

[illegible] stains, others by gram-negative stain.

Enzyme like activities of protenoid microspheres—They are [illegible], such as degradation of glucose and [illegible] is partially lost during heating.

[illegible] microsphere for protocells is widely accepted because of its following significances—(1) Such protenoid microspheres arise from monomers, rather than from polymers obtained from organisms already in the biota, as is true for the usual experiments with coacervate droplets. (2) This model suggests that protenoids are informational and it shows the origin of communication which may be intercellular or intergenerational communication. In fact, it suggests the simultaneous origin of both types of communication.

Besides coacervate, and proteinoid microspheres, many models have been suggested by different workers. For example, Herrera (1858–1942) synthesized Sulphobes; K. Bahadur (1966) synthesized Jeewanu; Goldacre (1958) prepared vesicles from proteins and lipids and Grossenbacher and Knight (1965) produced spherules, but all

these protocellular models, do not explain the origin of protobiont, therefore, are discarded now.

GEOLOGICAL EVIDENCES FOR MOLECULAR EVOLUTION

Certain microfossils and biochemical fossils of Precambrian have evidently proved the occurrence of molecular evolution and origin of life.

FOSSILS OF THE PRECAMBRIAN

The gap in knowledge about the earth from its beginning (in Azoic era, approximately 4·5 to 5 billion years ago) through the Precambrian which included the Proterozoic and Archeozoic eras and ended 0 6 billion years ago, is tremandous (Tilton and Steiger, (1965). The fossil-poor Precambrian era spans about 90 per cent of the earth's history. With respect to molecular and early biological evolution only tiny bits of information are available for the first third of the earth's history, that is, the first 1·5–2 billion years. However, it is evident that biological evolution emerged from prebiotic evolution 3 billion years ago (Fox and Dose, 1972). The fossils of Precambrian can be studied under following sub-heading :

FOSSILS OF THE EARLY AND MIDDLE PRECAMBRIAN

[illegible] things—(1) During [illegible] The earth era by the predecessors of contemporay micro-organisms in various stages between protolife and life.

The most ancient and oldest (more than 3·2 billion years old) unmetamorphosed sedimentary terrestrial rocks that contain the fossils of micro-organisms belong to the **onverwacht series** (Swaziland system) of South Africa (**Engel** *et. al.*, 1968). The microfossils of onverwacht series are spheroid and also filamentous, alga-like. Gas chromatographic studies of these rocks detected in them irregularly distributed mixture of abiogenic and biogenic hydrocarbons (isoprenoid and alkanes). While the ancient rocks of (3·1 billion years old) of fig tree series, near Baberton, South Africa, are found to contain entrapped and petrified remains of minute bacterium-like structure (**Barghoorn** and **Schopf**, 1966). These rod-shaped microfossils are about 0·5μm long and 0·25 μm in diameter and have been named as *Eobacterium isolatum*. Electron microscopy of *E. isolatum* has revealed the presence of a two-layered cell-wall in it. Further, besides hydrocarbons, a significant amount of the amino acids, such as, glycine, alanine, and valine were also extracted from the Fig Tree rocks by **Pflug** *et. al.*, (1969).

The existence of photosynthetic organisms about 400 million years after Fig Tree sediments were deposited has been indicated by fossils of photo- in lime stone (2·7 a. The precursors of chlorophyll, solanosane has been detected in Soudan rocks (2·7 billion years old) of upper Michigan and Minnesota (Cloud *et. al.*, 1965, Calvin, 1969). These rocks also contain the microfossils which resemble with *Nostoc*, the blue green alga. The sedimentary rocks (2·7 billion years old) of the Witwater strand system of South Africa, contains the fossils of various microstructures resembling monocellular organisms with complicated inner structures (Saager, 1968). In the same material, Pflug *et. al.*, (1969) found significant amounts of leucine, isoleucine, threonine, and serine, besides alanine, glycine and valine.

More conclusive data are available about some middle Precambrian microorganisms whose remains are preserved in black cherts of Gunflint Formation (1·9 billion years old) at the northern shore of Lake Superior. Its microfossil assemblage has been studied by Barghoorn and Tyler (1965) and it is found to consist largely of photosynthetic algae. Eight genera and twelve species of morphologically distinct micro-organisms have been recognized in the assemblage. Some of the filamentous fossils of Gunflint rocks resemble the modern blue green alga *Oscillatoria* in being 0·5 to 6.0μm in diameter and several hundred μm long. Some other spherical fossils of these rocks contain diameter from 1 to 16μm and resemble with the blue green algae. In addition, the presence of well-preserved fossils of rod-shaped and coccoid bacteria was revealed by electron microscopy. One of such fossil called *Eoasterion*, resembles a contemporary manganese-and iron-oxidizing bacterium found at several Karelian lakes (Barghoorn and Tyler, 1965). A second kind of fossils called *Kakabekia umbellata* is found to contain the photosynthetic activity.

ORGANISMIC FOSSILS OF THE LATE PRECAMBRIAN

ate Precambrian rocks (abou posited earlier. Pflug (1964, microfossils of blue-green algae, aquatic fungi, spore-like cysts of primitive flagellate protozoans or unicellular algae and microscopic multi-chambered fossils of forminiferans present in organically rich rocks of the 1·1 billion years old Belt Series of South-Central Montana. Likewise, the microfossil assemblage which is discovered in black cherts from 0·8 billion year old Bitter Spring Formation of Central Australia

(Barghoorn and Schopf, 1965), is found to consist of fossil remains of filamentous blue-green algae, spheroidal primitive green algae, aquatic fungi and rod-shaped and coccoid bacteria. Various chlorophyll derivatives such as optically active oils, vanadium-porphyrin complexes associated with pristane and phytane, have been detected chromatographically in the organic material of the billion year old Nonesuch Shale of northern peninsular Michigan (White and Wright, 1964).

THE EMERGENCE OF LATE PRECAMBRIAN INVERTEBRATES

It is concluded from the paleontological evidences that the first metazoan invertebrate animal evolved from flagellated protozoan ancestors towards the close of Precambrian, about 0·6 billion years ago. This evolution of animal life has been correlated to a simultaneous increase of the atmospheric oxygen to a level above the "Pasteur point" (Berkner and Marshall, 1964). The Pasteur point is the minimal oxygen partial pressure above which oxidative metabolism can take place in aerobic organisms.

CONCLUDING REMARK ABOUT PROTOBIOGENESIS

At last, it can be conceived that selective pressures operating on large molecular forms in the pristine seas could initiate a building evolutionary process that some three billion years later carpeted the earth with a wide variety of living cellular forms.

CHAPTER 3

DEVELOPMENT OF THE IDEA OF ORGANIC EVOLUTION

The idea that the present forms of life have arisen from earlier, simpler ones was far from new when Charles Darwin published his book "*The origin of species*" in 1859. The development of the idea of organic evolution through pre-Darwinan, Darwinian, and post-Darwinian eras can be studied under following periods :

I. PERIOD OF OBSCURITY

The oldest speculation about evolution are found in the classical mythologies of different centres of ancient cultures, such as, India, China, Babylon and Egypt. For instance, the classical Indian (Hindu) mythology has forwarded the idea that universe and the body of all living beings are made up of five elements or Panch Bhuta" namely kshiti (earth), ap (water), teja (fire), marut (air) and vyoum (Void or sky). Further, the Padam Puran conceived ten incarnations or "Dashvatarahs" for explaining the evolution of of vertebrates. The sequence of 'Avatarahs, includes Matsya, fish-like appearance ; Kurma reptile like ; 'Varaha' mammal-like ; 'Nara simha' half-man and half-lion ; 'Vamana", the dwarf man-like appearance (anthropoid) ; "Parshurama',, a *Homo sapiens* of Pleistocene ; "Shri Rama", "Balrama" ; "Shri Krishna" and Kalpi"—the latter four incarnations depict the formation of clans and communities.

Similar to anceient Indians, the ancient Chinese conceived the world at the beginning as made up of five primary elements—water, wood, fire, soil, and the precious metal gold. According to their mythology, the positive and negative interactions of these primary five elements were responsible for the origin of life and different kinds of adaptations and diversities of organisms or "things" were originated from a single source through gradual unfolding and branching.

The Egyptian and the Babylonians gave the idea of cosmic evolution which included the following aspects (i) a Divine Intelligence and creator, (ii) water as a primitive and boundless

tance, (iii) the potentiality of all things in the Divine Intelligene, and (iv) Creation as the process of becoming actual by stages.

During this period, because the speculation on evolution idea was much shrouded in mystery and mythology, so, this period is come to be known as the *period of obscurity.*

2. FROM GREEKS TO 18TH CENTURY PERIOD

The classical writings of certain Greek philosophers, such as **Thales** (624-548 B.C.), **Anaximander** (611-547 B.C.), **Anaximenes** (588-524 B.C.), **Empedocles** (495-435 B.C.), and **Epicurus** (341-270 B.C.), contain the vague ideas and speculations about the organic evolution. However, Empedocles is considered as the 'Father of evolution idea" by many evolutionist for his ideas which he forwarded in his "*Poem of Nature*". According to him life arose spontaneously but gradually and change (evolution) would result when twin forces-the attraction and repulsion interact with each other. Based on it, he further explained that first living things were cast out in the form of separate parts and by chance these parts were united by attraction force and as a result plants and animals were born. The sex originated later and through reproduction the developing embryo resulted from both paternal and maternal substances and in that fashion distinct species were formed. He believed that when reproduction was established, earth felt no need of special creation of organism.

Aristotle discarded Empedocles views and he observed that organisms could be arranged in graded series from lower to higher. From this taxonomic fact, he drew the correct inference that one higher group evolved from other lower one in the taxonomic series. However, he had the metaphysical belief that the gradual evolution of living things occurred because nature strives to change from the simple and imperfect to the more complex and perfect. An evolutionary explanation of the origin of plants and animals was given by the Roman poet **Lucretius** (99-55 B.C.) in his poem *De Rerum Natura.*

The medieval period is characterized as dark age. During this period, superstition and blind following of ancient thinkers prevailed and Aristotalean reasoning and the spirit of scientific inquiry were died out. Thus, no progress occurred in the idea of evolution during medieval period.

With the Renaissance, interest in the natural sciences quickened and the increasing knowledge of the many kinds of animals led more and more scientists to consider the concept of evolution favourably. Among these were **Robert Hooke** (1635-1703), **John Ray** (1627-1705), **Buffon** (1707-1788), **Erasmus Darwin** (1731-1802), and **Lamarck** (1744-1829). Even before the Renaissance men had discovered shells,

teeth, bones, and other parts of animals buried in the ground (*i.e.*, fossils). Some of these fossils corresponded to the parts of familiar living animals, but others were strangely unlike any known form. Many of the objects (fossils) found in rocks high in the mountains, far from the sea, resembled parts of marine animals. In the fifteenth century, the versatile artist and scientist, Leonardo da Vinci, gave the correct explanation of these curious finds, and gradually his conclusion, that they were the remains of animals that had existed at one time but had become extinct, was accepted. This evidence of former life led to the formulation of the theory of catastrophism by the founder of palaeontology and a versatile naturalist-Georges Cuvier (1769-1832). The theory of catastrophism holds that a succession of catastrophes, fires and floods, have periodically destroyed all living things, followed each time by the origin of new and higher types by the acts of special creation.

The concept of catastrophism was replaced by the theory of uniformitarianism due to the landmark investigations of three geologists namely James Hutton, John Playfair and Sir Charles Lyell in the eighteenth and early nineteenth centuries. James Hutton (1785) developed the concept that the geologic forces at work in the past were the same as those operating now. He arrived this conclusion after a careful study of the erosion of valleys by rivers and the formation of sedimentary deposits at the mouth of rivers. He demonstrated that the processes of erosion, sedimentation, disruption and uplift, carried on over long periods of time, could account for the formation of fossil-bearing rock strata. The publication of the book—"*Illustrations of the Huttonian theory of the Earth*" by John Playfair (1802) gave further explanation and examples of the idea of uni - formitarianism in geologic processes. Finally, the publication of C. Lyell's book—"*Principles of Geology*" (1932), had firmly established the concept of uniformitarianism. A necessary corollary of the idea that slowly acting geologic forces have worn away mountains and filled up seas is that geologic time has been immensely long. This idea, completely revolutionary at the time, paved the way for the acceptance of the theory of organic evolution, for the process of evolution requires an extremely long time.

During the middle of 18th century, a definite concept of evolution idea is formed. The pioneers of evolution, during this period are Carol Linnaeus (1707-1778) and Lamarck (1744-1829). Linnaeus though was not an evolutionist, nor was he greatly concerned with explanations of nature, but he devoted his life industriously in arranging the kinds of animals and plants known to him in a convenient

system. This system he took to be actual and fixed; to discover it and use it was the major task of natural history. He broke the tradition in placing man, "the knowing one; *Homo sapiens*, in the animal kingdom, the class Mammalia and the order Primates. Of many categories employed today in classification, it is interesting that Linnaeus used only the kingdom, class, order, genus, and species, but other naturalists soon found it advantageous to divide the animal and plant kingdoms into phyla and to divide orders into families. However, when Linnaeus established his idea of species, he created a dilemma that a species is real, objective and retains its identity in nature instead of shifting haphazardly or spontaneously changing its characteristics. Thus, his idea of special creation and of immutability of species caused a serious hindrance to the development of the evolution idea.

The earliest theory of organic evolution to be logically developed was that of **Jean Baptiste de Lamarck**, (1744-1829), whose **Philosophic zoologique** was published in 1809. He was first in presenting the picture of the way in which different species of animals could be related to each other by descent along branching lines of a "family tree". He like most biologists of his time, believed that all living things are endowed with a vital force that controls the development and functioning of their parts and enables them to overcome handicaps in the environment. Lamarck believed that any trait acquired by an organism during its life time was passed on to succeeding generations—that acquired characters are inherited. By developing the notion that new organs arise in response to the demands of the environment, he postulated that the size of the organ is proportional to its use or disuse. The changes produced by the use and disuse of an organ are transmitted to the offspring and this process, repeated for many generations, would result in marked alterations of form and function.

Both Buffon and Erasmus Darwin had similar ideas about the role in evolution of the direct response of the organism to its environment but had not expressed them so clearly. In his book *Zoonomia* E. Darwin, the grand father of Charles Darwin, put forward his belief in the descent of organisms by modifications of one living filament [small mass of protoplasm (Osborn) or spermatozoan (Singer)] which the creator endowed with life and to which, he gave an inherent power to respond to its own activities. However, he was the first to express clearly that millions of years have been required for the process of organic evolution and that all life arose from one primordial protoplasmic mass. Lamarckism was supported by E. Geoffroy

St. Hilaire (1772-1844), Robbert Chambers (1802-1871) and Herbert Spencer (1802-1903), but bitterly opposed by G. Cuvier (1769-1832) and Weismann (1887).

3. DARWINIAN PERIOD

During the later half of 19th century, two most significant events occurred in the history of evolutionary biology—(i) Charles Darwin formulated and advanced his natural selection theory to explain the mechanism of organic evolution and to show that organic evolution had occurred, he presented a wealth of detailed evidences and cogent arguments. (ii) The theoretical distinction between somatoplasm and germplasm made by Weismann (1887) refuted all theories of evolution based on the inheritance of acquired characters, and also Darwin's pangenesis theory. He, thus, made it clear that acquired characters were present only in the body cells (somatoplasm) and not in the germ cells (germ plasm), and only traits present in the germ plasm were transmitted to the next generation.

Charles Darwin (1809-1882) and Alfred Russel Wallace (1823-1913), both spent many years in collecting and exploring South America and certain islands of its sea and both independently drew similar conclusions about the possible mechanism of organic evolution due to natural selection, after studying the essay on population by Malthus (1798) which suggested that human populations continually tend to out strip the availability of food supply, but are kept in check by natural diseases, and famine, and wars made wars. According to Darwin-Wallace theory of natural selection which was published in Journal of the Linnean Society in July, 18[illegible]

aspect of natural selection which was not fully appreciated by Darwin, is that the divergence of species from a common ancestral stock is brought about by some form of segregation in the original stock, so that the long-term effects of selection in one part of the population are different from those in other parts and isolation prevents a remingling of the hereditary traits thus changed. Without barriers to interbreeding, races or species could not become distinct, but with such barriers, they would eventually, inevitably, do so.

4. POST-DARWINIAN PERIOD

With the powerful support of **T. H. Huxley** (1825–1895), the idea became widely established among biologists that evolution had occurred and that it could be explained by processes observable in nature. Besides, **T. H. Huxley**, many noted German evolutionists such as **Karl Gegenbaur** (1826–1903), **Ernst Haeckel**, **A. Weismann** and many other biologists of different fields provided anatomical, embryological, taxonomical and palaeontological evidences in favour of Darwin's theory. **Gegenbaur** and English anatomist **Richard Owen** (1804–1892) illustrated the transition from pre-evolutionary to evolutionary interpretations of structural patterns in animals. **Haeckel** constructed phylogeny of vertebrates on their embryological development. In his **biogenetic law**, he suggested that various successional stages of embryo development would reappear in the same pattern in all related vertebrate classes (*i.e.*, ontogeny repeats its phylogeny). **A Weismann** was a experimental evolutionist and he condemned the Lamarckian and Darwinian inheritance mechanisms on the one hand and supported Mendelian hereditary mechanism on the other hand by suggesting the theory of the "*continuity of the germplasm*" (1892).

Thus, new lines of investigations developed rapidly in the latter half of the nineteenth century to support Darwinism. Further, besides natural selection, many additional mechanisms and causes were sought by different evolutionist of later part of nineteenth century for explaining organic evolution. For instance, **Wagner** emphasized the effects of different environments on isolated forms. **Gulik** pointed out the inevitability of divergence even without difference in environment. **Karl Nageli** suggested that an inner directive guides the course of evolution mainly independent of the environment. **Hugo de Vries** (1848–1935) believed that he had found, in 1900, the method by which new species originate not in natural selection, but in the rare occurrence of spontaneons variations (mutations) that could be inherited.

During first three decades of the twentieth century—different specialized branches of biology such as palaeontology, embryology, anatomy, genetics, biochemistry, ecology, etc., provided sound explanations to different evolutionary problems. For instance contributions of E. D. Cope (1840–1897), Othniel C. Marsh (1831–1899), H. F. Osborn (1857–1935), W. D. Matthew, W. K. Gregory, A. S. Romer, and G. Simpson in America; A. S. Woodward (1864–1944) and D. M. S. Watson in England; R. Broom (1866–1951) in South Africa, have provided a sound background to vertebrate palaeontology. Likewise, certain population geneticists namely J. B. S. Haldane, R. A. Fisher and Sewall Wright—provided mathematical evidence that natural selection can, after all, account for genetic changes in a population, given the mutability of genes as a source of selectable variations. However, evolution shows so many facets that it looks alike to no two persons. The more different the backgrounds of two biologists, the more different their attempts at casual explanation. At least, so it was, until the 1930's, when the many dissenting evolutionary theories were fused into a broad unified theory, the **"modern synthesis".**

5. THE MODERN SYNTHESIS PERIOD

The synthesis of a coherent theory of evolution which takes into account all the pertinent facts of modern biology, has been the work of several biologists during the past forty years. The first edition of **Theodosius Dobzhansky's** now classic book, *Genetics and the origin of species*', which appeared in 1937, set the stage, and stimulated biologists in several fields to contribute to the synthetic evolutionary theory. Books by zoologist **Julian Huxley** (1942) and **Ernst Mayr** (1942, 1963) showed how the modern theory could explain the origin of variation patterns in higher animals and **G.L. Stebbins** attempted to do the same for higher plants. A leading palaeontologist **George Simpson** (1951, 1953) showed in two books that the fossil record of higher animals is best explained by assuming that throughout the evolutionary history of living things those same processes took place which were being experimentally demonstrated by many workers in populations of contemporary animals. In 1960, **Bernhard Rensch** has made a strong case for the belief that the evolution of genera, families and higher categories of animals has taken place through an extension into long periods of time of those same processes which at any one time level govern the origin of races and species.

According to Mayr (1970), most of the earlier evolutionary theories were characterized by heavy emphasis on a single factor, as has been tabulated in following table :

Table : Theories of evolutionary changes (E. Mayr, *1970*).

A. Monistic (single-factor explanations)
1. ***Ectogenetic*** : Changes directly induced by the environment.
   (a) Random response (for example, radiation effects)
   (b) Adaptive response (Geoffroyism)
2. **Endogenetic** : Changes controlled by intrinsic forces
   (a) Finalistic (orthogenetic)
   (b) Volitional (genuine Lamarckism)
   (c) Mutational limitations
   (d) Epigenetic limitations.
3. **Random events ("accidents")**
   (a) Spontaneous mutations
   (b) Recombination
4. **Natural selection**

B. Synthetic (multiple-factor explanations)
1b+2a+2b= most "Lamarckian-type" theories
1b+2b+2c+4=some recent "Lamarckian" theories
1b+3+4=Late Darwinian, most non-mutationists during first three decades of 20th century
3+4=early "modern synthesis"
1a+2c+2d+3+4=recent "modern synthesis"

The modern synthetic theory selected the best aspects of the earlier hypotheses and combined them in a new and original manner. In essence a two-factor theory, it regards the diversity and harmonious adaptation of the organic world as the result of a steady production of variation and of the selective effects of the environment.

Attempting to explain evolution by a single-factor theory was the main weakness of the pre-Darwinian and most nineteenth-century evolutionary theories. For instance, Lamarckism with its internal self-improvement principle, Geoffroyism with its induction of genetic change by the environment, Cuvier's catastrophism, Wagner's evolution, De Vries' mutationism, all tried to explain evolution by a single principle, excluding all others. Even Charles Darwin occasionaly fell into this error, yet on the whole he was the first to make a serious effort to present evolutionary events as due to a balance of conflicting forces. The current theory of evolution the "modern synthesis", as **Huxley** (1942) has called it—owes more to Darwin than to any other evolutionist and is built around Darwin's essential concepts. Yet it incorporates much that is distinctly post-Darwinian. The concepts of mutation, variation, population, inheritance, isolation, and species, all of which were quite nebulous in Darwin's days, but now are much better defined and understood, have been incorporated in recent "modern synthesis" (see **Mayr**, 1970).

PRESENT STATE OF EVOLUTION IDEA

The current synthetic theory of evolution recognizes five basic types of processes—**Gene mutation, Changes in chromosome number and structure, Genetic Recombination, Natural Selection,** and **Reproductive isolation.** The first three provide the genetic variability without which change cannot take place; natural selection and reproductive isolation guide populations of organisms into adaptive channels. In addition, three accessory processes affect the working of these five basic processes. **Migration** of individuals from one population to another, as well as **Hybridization** between races or closely related species both increase the amount of genetic variability available to a population. The effects of **chance,** acting on small populations, may alter the way in which natural selection guides the course of evolution

Table : Theories of evolutionary changes (E. Mayr, *1970*).

A. **Monistic** (single-factor explanations)
   1. **Ectogenetic** : Changes directly induced by the environment.
      (a) Random response (for example, radiation effects)
      (b) Adaptive response (Geoffroyism)
   2. **Endogenetic** . Changes controlled by intrinsic forces
      (a) Finalistic (orthogenetic)
      (b) Volitional (genuine Lamarckism)
      (c) Mutational limitations
      (d) Epigenetic limitations.
   3. **Random events** ("accidents")
      (a) Spontaneous mutations
      (b) Recombination
   4. **Natural selection**

B. Synthetic (multiple-factor explanations)
   1b+2a+2b= most "Lamarckian-type" theories
   1b ; 2b+2c+4=some recent "Lamarckian" theories
   1b+3+4=Late Darwinian, most non-mutationists during first three decades of 20th century
   3+4=early "modern synthesis"
   1a+2c ; 2d+3+4=recent "modern synthesis"

The modern synthetic theory selected the best aspects of the earlier hypotheses and combined them in a new and original manner. In essence a two-factor theory, it regards the diversity and harmonious adaptation of the organic world as the result of a steady production of variation and of the selective effects of the environment.

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CHAPTER 4

EVIDENCES FOR BIOLOGICAL EVOLUTION

Darwinian evolution permits two general predictions—(i) if there were an evolution from simple forms to more complex ones, there must be certain structural, developmental and chemical similarities between different forms of life, and (2) there must be a means by which variation in populations arise, and are transmitted from generation to generation. This chapter will deal only with the evidences that have been found to support the idea of a relationship between existing organisms and also of a relationship between extinct and existing organisms, while certain forthcoming chapters will deal with the way in which change can initiate and bring about an evolutionary process such as that proposed by Darwin.

The evidence that organic evolution has occurred is so overwhelming that no one who is acquainted with it has any doubt that new species are derived from previously existing one by descent with modification. The fossil record (palaeontology) provides **direct evidence** of organic evolution and gives the details of the evolutionary relationships of many lines of descent, while different biological disciplines like comparative anatomy, taxonomy, embryology, physiology, biochemistry, genetics and biogeography provide **indirect evidence,** in suppot of biological evolution. All of these direct and indirect evidences for biological evolution can be discussed as follows :

I. PALAEONTOLOGICAL EVIDENCES

The science of palaeontology which deals with the finding, cataloguing and interpretation of fossil remains of ancient plants and animals, has aided immensely in our understanding of the lines of descent of many invertebrate and vertebrate animals and certain Protista, Bryophyta and Tracheophyta plants. A **Fossil** (Latin *fossilium*, something dug up) *is some evidence of animal or plant which* is seen in the stratum layers of earth's crust and which lived a long time ago. Thus, the fossils not only include the bones, shells, teeth and other hard parts of an animal body which may survive, but also any impression or trace left by previous organisms.

KIND OF FOSSILIZATION

In the main, the hard parts of organisms (teeth, skeletal parts and shells) are preserved as fossils In some of these fossils, the original hard parts or more rarely the soft tissues of the body, have been replaced by minerals, a process called **petrifaction.** Iron pyrites, silica and calcium carbonate are some of the common petrifying minerals. For example, the petrified muscle of a shark more than 300,000,000 years old was so well preserved by petrifaction that not only individual muscle fibres, but even their cross striations, could be observed in thin sections under the microscope. However some animals are preserved without petrifaction but with little or no change from the time of death. For example, a Russian worker described the finding of a frozen mammoth (an extinct form related to elephants) in Siberia It was estimated that the animal had been preserved in frozen state for approximately 25,000 years, yet the flesh was so well preserved that it could be, and was eaten by dogs. Further, numerous specimens of insects, spiders, and mites have been found preserved in amber in the Baltic region of Europe. During the Oligocene period, about 38 million years ago, Northern Europe was covered with coniferous forests (forests of gymnosperms) The trees in these forests exuded a sticky resin that trapped the spiders, mites and insects. The resin ultimately hardened into amber, with the arthropods embedded in it. In some cases the preservation is so good that the colours have not been changed. Land animals occasionally have been covered with wind-blown sand or volcanic ash, or have been trapped in bogs, quicksand or asphalt pits, and their hard parts have been preserved.

Footprints or trails made in soft mud, which subsequently hardened, are a common type of fossil. For example, the tracks of of an amphibian from the pennsylvanian period, discovered in 1948 near Pittsburgh, revealed that the animal moved by hopping rather than by walking, for the footprints lay opposite each other in pairs.

Still other fossils are in the form of **molds and casts,** both of which are superficially similar to petrified fossils but are produced in a different way. **Molds** are formed by the hardening of the material surrounding a buried organism, followed by the decay and removal of body of the organism. The mold may subsequently be filled by minerals which harden to form **casts** which are exact *replicas of the original structure.*

Conditions for fossilization—The formation and preservation of a fossil require that some structure be hard and be buried immediately. It should be clear that when a dead animal or plant is left

exposed, it soon disappears as the result of the activity of scavengers and the bacteria and fungi of decay. Even the bones of horses and cattle that die on the plains soon disappear. The chance of any exposed organism becoming fossilized is very slight indeed. Immediate burial is a prerequiste to the process of fossilization; by far the most common kind of burial favourable for fossilization is that provided by water-borne sedimentary rocks.

FORMATION OF ROCKS

Geologists envision the earth's crust as having gone through a series of changes involving the alterations of land masses by glacier movements or land shifts that resulted in the rise of mountain ranges, and the levelling of other land when one land mass came to rest upon another, the tremendous pressure is exerted, and the lack of available water, caused the conversion of the original land mass into **rock strata**. Through the geological ages, then, land mass over land mass formed layers of rock strata, each of which had at some time, been exposed to the atmospheric environment. The lowest strata solidified some two to three billion years ago.

The rock strata of earth includes **sedimentary** and **igneous rocks**, the formation of both of which is remain significant in the process of fossilization. To understand the nature of the formation of sedimentary rocks, one must take into account certain natural processes that are occurring in the world today and that have been occurring since the crust of the earth was formed By the process of **erosion**, through the action of wind and rain, and of freezing and thawing, rocks are gradually broken into the small porticles that form **soil**. Through the action of rain, the particles of the soil are carried into streams and rivers and ultimately into lakes or oceans. This sedimentary material carries with it the bodies of many aquatic organisms and also the bodies of terrestrial forms that happen to be swept along by the streams and rivers. The hard parts of some of these organisms may be preserved, and in the course of time the sedimentary deposit, owing to the pressure of water above it and also to chemical reactions, is converted into sedimentary rock. The nature of the sedimentary material determines the kind of sedimentary rock formed—**limestone**, **sandstone**, and **shale** are familiar kinds. The method of formation of sedimentary rocks clearly distinguishes them from **igneous rocks**, which were formed by the solidification of molten material when the earth cooled, and are being formed by cooling of magma expelled from active volcanoes.

Another natural processes called **submergence** and **emergence** have occurred in the past and are still occurring between land and

sea. Slow and gradual changes in level between land and sea are now in process. For instance, it is now recognized that the land on the eastern coast of United States is gradually sinking into the sea, where as the land on the Pacific coast is gradually rising The sinking of the land below the sea is called **submergence**, and the rising of land above the sea is called **emergence**. In the geologic past, many regions of the earth have undergone a series of submergences and emergences. As a conseqnence, in many places (*e g*, Strata of Gand Cranyon of the Colorado), a whole series of different layers of sedimentary rocks is found.

Further, the **law of superposition** of Geology states that the deepest layer of earth strata were deposited first and other layers in succession at later periods of time. Consequently, more superficially located fossils will be considered to be of recent origin. Moreover, if each formed layer of rock were left undisturbed until another layer was deposited on top of it, one would have perfect series for the study of fossil forms. The rock record of the earth, however, is not so complete. Layers deposited under water can emerge as land and be partially or completely eroded away If a new submergence then occurs and a new layer of sedimentary rock is formed, there will be an unconformity between the two strata. This lack of sequence between layers makes the study and identification of strata difficult. Also, fossils that were formed may be destroyed in the formation of **metamorphic rocks.** As a result of great pressure and heat, deep layers may melt. When this material later solidifies again, the fossils originally present will usually be lost. Limestone is a form of sedimentary rock rich in fossils, when limestone melts and crystallizes into metamorphic rock, the result is marble. Despite the paucity of fossil formation and destruction of fossils by several reasons, the story of rocks is a very convincing one with *reference to evolution.*

DETERMINATION OF AGE OF ROCKS AND FOSSILS

In the past, geologists and palaeontologists were able to make fairly accurate estimations of the age of different rock strata and their fossil record by using the known rate of the accumulation of salt in the oceans. Persently, rock deposits are dated largely by taking advantage of the fact that certain radioactive elements are transformed into other elements at rates which are slow and essentially unaffected by the pressures and temperature to which the rock has been subjectd. For example, geologists standardly use uranium dating to estimate the time of solidification of rock. Radioactive

uranium decays spontaneously to lead. The half-life of uranium is about 4,500,000,000 years.

Since the uranium of the earth was formed four to five billion years ago when the great pressure of a contracting dust cloud created thermonuclear heat, why should not all uranium measurement give the same age, four to five billion years? The answer lies in the difference between the radioactive decay in a solid and in a liquid. In a liquid, the uranium is diluted, washed away, etc. But when liquid solidifies, the uranium it contains is not free to move, and undergoes its decay into lead in a highly localized region. The ratio of uranium to its stable product, lead, indicates the time of solidification of the liquid material and, therefore, the time at which it was added to the earth's crust.

For the determination of the age of fossils, other radioactive materials are analyzed. **Radioactive carbon** (C^{14}) is a natural radioactive form, produced in the atmosphere from the contact of naturally occurring C^{12} with UV light. It passes down as $C^{14}O_2$ and enters plants and then animal material. The ratio of C^{14} to C^{12} remains constant during life, because of the constant interaction of biological organisms with the environment Upon death and fossilization, this ratio decreases as the C^{14} undergoes decay. It is possible therefore, to determine when the fossilized individual lived by comparing its present C^{14} to C^{12} ratio with that usually maintained during life. Radioactive carbon has a half-life of about $5,568 \pm 30$ years, and can only be measured upto 25,000 years or about 5 half-lives This limitation results because the amount of C^{14} in organisms is so small to begin with. **Radioactive-carbon dating** is excellent for the anthropological studies of early tribal civilizations, but not for the earlier strata

Recently, the transformation of radioactive potassium (k^{40}) to argon and rubidium to strontium has been used in a similar way for dating fossil-bearing rocks of any age and type. K^{40} has a half-life of 1·3 billion years. Also because of its greater concentration in most rocks, it is more accurate method of dating fossils than uranium, a relatively rare element. A bed of prehuman fossils in South Africa dated by rock composition gave a result of 500,000 years, whereas radioactivepotassium dating method indicated an age of 1,750,000 years—a most important difference, considering the nature of the fossils.

Relatively short periods of geologic time are estimated by measuring the rate at which waterfalls recede upstream as they wear away the rocks over which they tumble or by counting the annual deposits of clay on the bottom of ponds and lakes.

THE GEOLOGICAL TIME TABLE

Geologists, as a result of their studies of the strata of sedimentary rocks in the different regions of the world, have classified geologic history into six eras. The oldest era with fossils is the **Archeozoic** (era of primitive life) and this is followed in turn by the **Proterozoic** (era of early life), the **Paleozoic** (era of ancient life), the **Mesozoic** (era of medieval life), and the **Cenozoic** (era of modern life) The Paleozoic, Mesozoic, and Cenozoic eras are divided into **periods** and the periods of the Cenozoic into **epochs.** There is evidence that between the different eras there were widespread geologic disturbances, called **revolutions** which raised or lowered vast regions of the earth's surface and created or eliminated shallow island seas. These revolutions produced great changes in the distribution of sea and land organisms and wiped out many of the previous forms of life For instance, the Paleozoic era ended with the revolution that raised the Appalachian mountain and it is believed, killed all but 3 per cent of the forms of life existing then. The **Rocky mountain revolution** (which raised the Andes, Alps and Himalayas as well as the Rockies) annihilated most reptiles of the Mesozoic. Following table of geologic time-table shows the eras and some of their subdivisions, the approximate duration of each era, some of the important geological features, and the characteristic animals and plants (see table on page 58).

CONCLUSIONS DRAWN FROM FOSSIL RECORD

The most important features of the fossil record which is tabulated in the following table can be summarized as follows :

(i) There existed a multitude of diversified animal groups, similar to the animal groups that exist today.

(ii) All fossils did not appear at a time, but appeared during different great spans of times, and

(iii) The most primitive forms of life are found in the oldest rocks.

(iv) Moving up through the various strata, from older to more recent formations, there is a succession of higher and more complex forms of life (geologic succession).

(v) In many instances, a group arose in one period or era and remained scarce, but in the next period or era it became dominant after undergoing adaptive radiation.

(vi) There have been many extinction of large groups, but after the establishment of all major phyla, some species of each phylum have persisted down to the present.

(vii) None of the past forms of life are exactly like any of the now living.

Table : Geological time-table.

Millions of years ago†			Geological feature	Plants	Invertebrates	Vertebrates
Eras	Periods	Epochs				
Cenozoic (70)	Quaternary (2)	Recent (0·011)	End of last ice age; climate warmer.	Decline of woody plants; rise of herbaceous ones	—	Age of man
		Pleistocene (2)	Repeated glaciation; four ice ages.	Great extinction of species.	—	Extinction of great mammals; first human social life.
	Tertiary (68)	Pliocene (10)	Climate warm in the beginning but gradually cooling, formation of Alps and Himalayas.	Development and spread of *modern* flowering plants. Rise of grasses and of herbs.	Arthropods and molluscs most abundant Appearance of modern invertebrates type.	Archaic mammals declined after Eocene. Modern mammals evolved in the latter epochs. Rise of anthropoids.
		Miocene (25)				
		Oligocene (35)				
		Paleocene (70)				
Rocky Mountain Revolution (Little destruction of fossils)						
Mesozoic (230)	Cretaceous (135)		Great swamps in early part, Rocky Mountain and Andes formed.	Rapid development of angiosperms (first monocotyledons), gymnosperms declined.	Extinction of ammonites, spread of insects.	Extinction of *dinosaurs* and *toothed birds*; spread of birds; rise of primitive mammals.

Millions of years ago†			Geological feature	Plants	Invertebrates	Vertebrates
Eras	Periods	Epochs				
	Jurassic (180)		Continents fairly high, shallow seas over some of Europe and western U S.	Increase of dicotyledons; conifers and cycads dominant	Maximum of ammonites. Insects abundant, including social insects	Dominance of dinosaurs, first toothed birds; early mammals.
	Triassic (230)		Climate warm, great desert areas.	Spread of cycads and conifers, seeds ferns disappear.	Limulus present; Marine invertebrates decline in numbers	First dinosaurs. Mammal-like reptiles.
Appalachian Revolution (some loss of fossils)						
Paleozoic (600)	Permian (280)		Appalachians and Urals formed. Glaciation and aridity.	First cycads and conifers; decline of lycopods and horse tails.	Last of trilobites; expansion of ammonites; modern insects arose.	Expansion of reptiles, mammal-like reptiles arose.
	Pennsylvanian (320)		Mountain building. Great coal swamps.	Great forests of seeds ferns and gymnosperms	Insect common; first insects fossils.	First reptiles; spread of ancient amphibians
	Mississippian (345)		Warm humid climate. Shallow island seas.	Lycopsids, horsetails, and seed ferns dominant. First coal deposits.	Culmination of crinoids (echinoderms)	Spread of sharks. Rise of amphibians.

Millions of years ago†			Geological feature	*Plants*	Invertebrates	*Vertebrates*
Eras	Periods	Epochs				
	Devonian (405)		Emergence of land, some arid regions and glaciation	First forests, lands plants well established, first gymnosperms	Brachiopods flourishing, decline of trilobites.	First amphibians Rise of fishes—lungfishes, shark *abundant.*
	Silurian (425)		Extensive continental seas; lowlands increasingly arid as land rose.	First definite evidence of land plants, algae dominant	Corals, brachiopods, eurypterids. Marine arachnids *dominant*; first (wingless) insects.	Rise of ostracoderms (primitive fishes).
	Ordovician (500)		Great submergence of land; warm climates even in Arctic.	Marine algae abundant.	Trilobite abundant, diversified molluscs.	First vertebrates *armored* fishes
	Cambrian (600)		Lands low, climate mild; earliest rocks with abundant fossils.	Algae, especially marine forms.	Trilobites, brachiopods dominant, all phyla *represented.*	
			Second Great Revolution (*Considerable loss of fossils*)			
Proterozoic (1200)			Great sedimentation; volcanic activity later; extensive erosion, repeated glaciations.	Few fossils (sponges, radiolarian protozoans, worm burrows and algae) Thallophyta evolved. Fossils of blue green algae.	Most invertebrate phyla probably evolved.	—

First Great Revolution (Considerable loss of fossils)						
Millions of years age†			Geological features	Plants	*Invertebrates*	Vertebrates
Eras	Periods	Epochs				
Archeozoic (3500)			Great volcanic activities; some sedimentary deposition; extensive erosion; rocks mostly igneous or metamorphosed.	Indirect evidence of life from graphite and limestone, but no recognizable fossils except bacteria, (microfossils).	—	—
Azoic (4500)			Origin of earth. Igneous rocks.	Organic material found in rock, origin of life.		

† Numbers given in parentheses indicate approximate time since beginning of era, period or epoch.

All these significant aspects of fossil record substantiate the Darwins theory of evolution by showing the increasing complexity of *organismal structures with time Besides many other interesting features*, the fossil record helps in drawing correct conclusions about the possible origin of certain modern vertebrates (birds and horse) as follows :

1 Transitional forms –The critics of palaeontological evidence for the idea of evolution, sometimes maintain, that the fossil record lacks transitional *forms between one major group and another. In* fact fossil record does contain some fossil transitional link forms. One such link is *Archaeopteryx*, transitional fossil between birds and

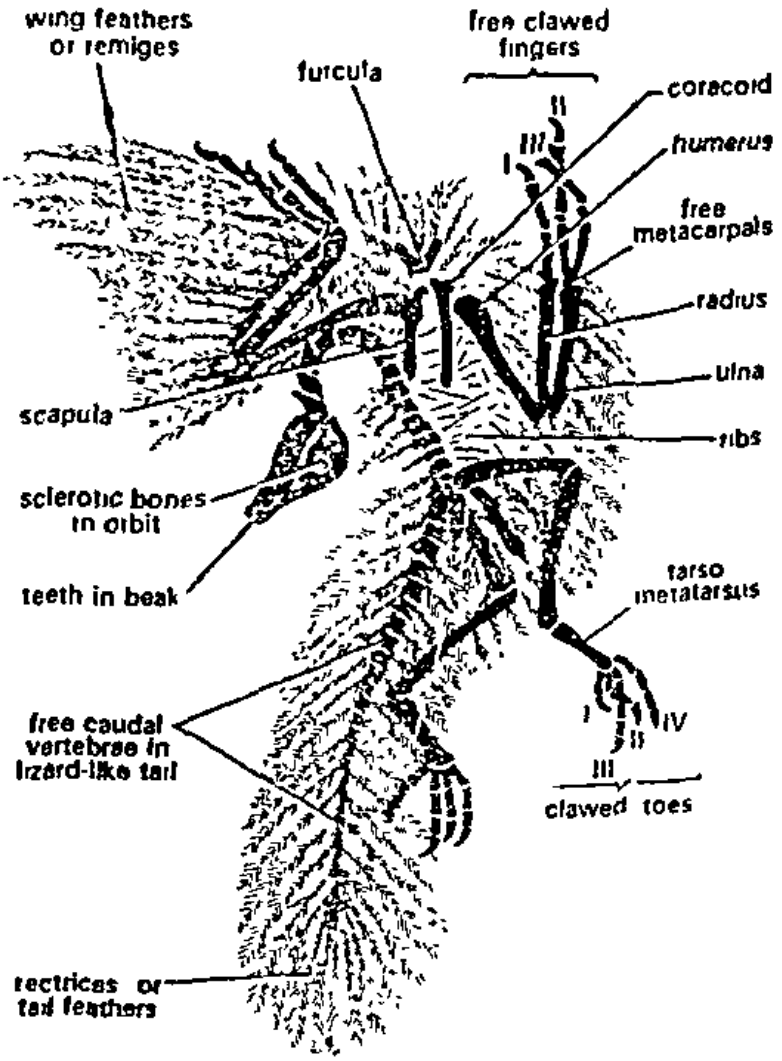


Fig 4·1. Fossil of *Archaeopteryx*.

reptiles. *Archaeopteryx* was found in the rocks of the Jurassic Period and it was about the size of a crow and in certain respects was quite like a reptile Like birds it had wings with feathers, but they were small in size. It's tail, quite unlike that of modern birds,was long with a row of feathers on each side. Further, its jaws were equipped with

conical teeth and three of the digits of each forelimb persisted, armed with claws. It is probable that the forelimbs were used for climbing as well as for flying. However, skeleton framework of *Archaeopteryx* resembled more with the reptiles than the birds. Thus, *Archaeopteryx* provides a connecting link between the reptiles and birds, suggesting that birds were evolved from animals like reptiles.

2. Forms showing serial evolution—Fossil records sometimes, allow us to construct the phylogeny of an animal. Such phylogeny or serial evolution of a animal such as horse, elephant, camel, etc., provides a strong evidence in favour of idea of biological evolution.

Phylogeny of horse : The history of horse dates back to about 60 million years in the Eocene period, involving about 20 genera. The horse phylogeny starts with *Eohippus* (*Hyracotherium*) a member of Equidae. The *Eohippus* lived in the Eocene Epoch. It was about the size of a fox terrier dog, but with a longer head. Its legs were short, with four toes on each front foot and three on each

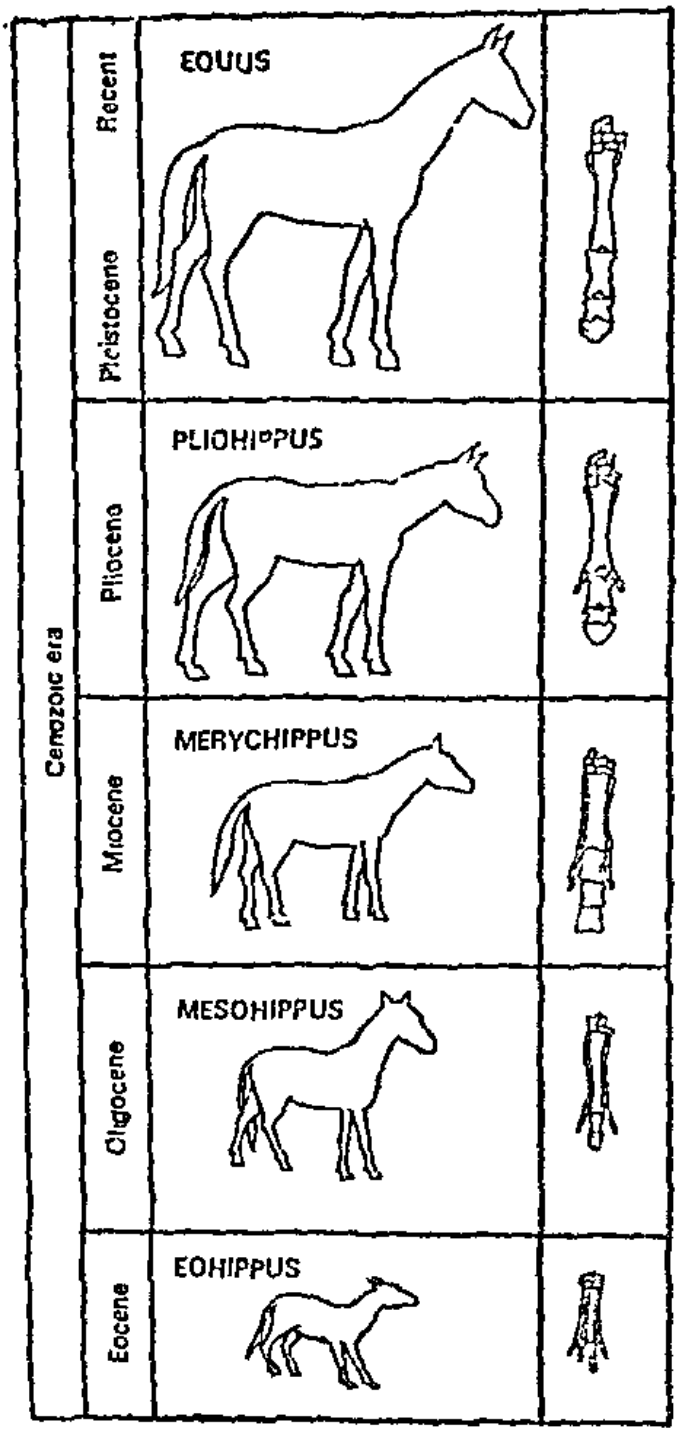


Fig. 4 2. Some stages in evolution of horse: 1. *Eohippus*, 2. *Mesohippus*. 3 *Merychippus*, 4 *Pliohippus*, 5. *Equus*.

foot. The third digit was somewhat longer than the others. All of the toes were placed on the ground and used in walking. *Eohippus* was a forest dweller and browser, feeding on soft vegetation. The lines of descent perhaps passed from *Eohippus* through *Mesohippus*, *Miohippus*, *Parahippus*, *Merychippus*, and *Pliohippus* to *Equus*, which represents the modern horse and its allies. Modern horse is plains dweller and a grazer. The most important changes in the evolution of the modern horse from *Eohippus* are the following— 1. The enlargement and elongation of the third digit, with a loss of the other digits. 2 The elongation of distal parts of the limbs so that the wrist and the ankle are high up. 3. The elongation of the fore part of the skull. 4. The increase of length and mobility of neck 5. The development of the premolars and molars into high-crowned, continuously growing grinders, and 6 A general increase in body size.

The phylogeny of horses is not a freak of nature, as opponents of evolution idea think, but a constantly progressive adaptation history to changing geological changes which compelled horses to undergo modification. Thus, phylogeny of horse and other animals provides evidence in favour of biological evolution. Further, paleontological evidence provides the most direct indication of evolution by showing that through the years organismal types became more complex, it only implies that the simpler forms gave rise to more complex ones.

2. EVIDENCES FROM TAXONOMY

Taxonomy is the science of classification of organisms. It began long before the doctrine of evolution was accepted. Indeed the founders of scientific taxonomy, Ray and Linnaeus, were firm believers in the fixity, the unchangingness, of species. Since the development of the theory of descent with modifications by Lamarck (1809) and Darwin (1859), the organisms were classified on the basis of their inter-relationships. Present-day taxonomists are concerned with the naming and describing of species primarily as a means of discovering or clarifying evolutionary relationships. This is based upon the assumption that the degree of resemblance in homologous structures is a measure of the degree of relationship. The characteristics of living things are such that they can be fitted into a hierarchical scheme of categories, each more inclusive than the previous one-species, genera, families, orders, classes and phyla. Thus very closely related animals or plants are kept in the same species which shows that they descended from a common ancestor. The species

which are much alike, are included in one genus which is distinct from other genera. In the same manner, all the like genera are included in one family which shows again a sign of affinity. Likewise, similar families form orders, and orders are grouped into classes, in which diverse members share only very fundamental characteristics. Lastly, all the related classes are included in a phylum. This system of classification of organisms can be best interpreted as proof of evolutionary relationship. If the kinds of animals and plants were not related by evolutionary descent, their chracteristics would be present in a confused, random pattern and no such hierarchy of forms could be established.

Further, the scheme of classification is itself a proof of descent from a common ancestor as shown by the phylogenetic tree. The forms which are associated together in species, genera, classes or phyla are supposed to have descended from a single common ancestor. For example, the vertebrates like fish, frog, lizard, pigeon rabbit and man look very different from each other, but they all resemble in having dorsal hollow central nervous system, a notochord and gill-slits. This similarity in the outwardly different vertebrate animals indicates their common ancestry.

The basic unit of taxonomy is the species, a population of closely similar individuals, which are alike in their morphologic, embryologic, and physiologic characters, which in nature breed only with each other and which have a common ancestry. It is difficult to give a definition of species that is universally applicable. The definition must be modified slightly to include species whose life cycles includes two or more quite different morphological forms (many coelenterates, parasitic worms, larval and adult insects and amphibians) A population that is spread over a wide territory may show local or regional differences which may be called **subspecies**. Many examples are known in which a species is broken up into a chain of subspecies, each of which differs slightly from its neighbours but interbreeds with them. The subspecies at the two ends of the chain, however, may be so different that they cannot interbreed Such a series of geographically distributed subspecies is called a **Rassenkreis (German, racecircle).**

Lastly, the classification of living organisms into well-defined groups is possible because most of the intermediate forms have become extinct. If representatives of every type of animal and plant that have ever lived were still living to day, there would be many series of intergrading forms and the division of these into neat taxonomic categories would be difficult. Certain taxonomists have com-

pared the present-day species to the terminal twigs of a tree whose main branches and trunk have disappared. The main problems of modern taxonomists are to reconstruct the missing branches and to put each twig on the proper branch.

3. EVIDENCES FROM COMPARATIVE ANATOMY

A comparative anatomical study of any particular organ system in the diverse members of a given phylum reveals a basic similarity of form which is varied to some extent from one class to another. The skeletal, muscular, circulatory, and excretory systems of the vertebrates provide especially clear illustrations of this principle, but this is generally true of all systems in all phyla, such studies in comparative anatomy provide many evidences of biological evolution by showing that (i) anatomical similarities become more and more complex progressively as one proceeds from a lower animal to a higher animal; and (ii) all the diverse animals inherited the anatomical similarity from a common ancestor.

Examples of anatomical similarities could be multiplied largely, but we resrict ourselves to few following examples :

1. HOMOLOGY AND ANALOGY

Homology is the correspondence or relationship between structures of different organisms due to their inheritance of these structures from the same ancestry. Such structures known as **homologous structures** and have a genetic basis of their similarities. Further, the homologous organs or structures are basically similar in their cell composition and gross anatomical features, in their relation-

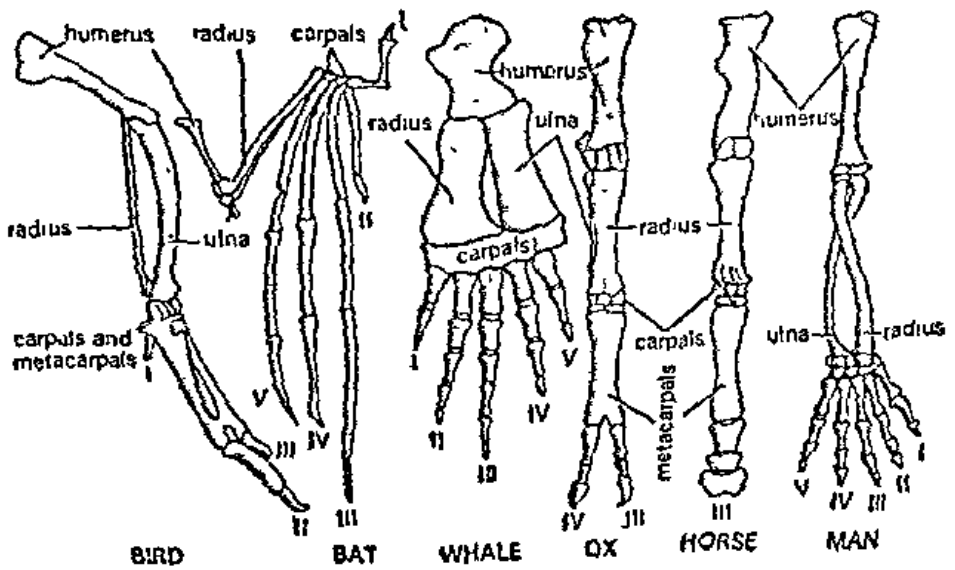


Fig 4.3. Comparision of fore limbs of some vertebrates showing homology.

ship to adjacent structures, in their embryonic development and in their nerve and blood supply. For example, a whale's or seal's front flipper, a bat's wing, a cat's paw, a horse's front leg, a human hand and even a birds wing, though superficially dissimilar and adapted for quite different functions (*viz* , grasping, running, flying, swimming, etc , nevertheless are homologous structures. Each consists of almost the same number of bones, muscles, nerves, and blood vessels arranged in the same pattern, and their mode of development is very similar. The existence of such homologous organs implies a common evolutionary origin of birds and mammals from some ancient reptilian ancestor.

All insect legs are composed of five parts that always occur in the same order. Starting at their attachment on the thorax, these parts are : coxa, trochanter, femur, tibia, and tarsus. These parts are single segments except the tarsus, which varies from one to five segments There have been many adaptive changes in insect legs during the evolution of the group. Many insects have a simple type of walking legs, such as the first and second legs of the grasshopper. The hind leg of the grasshopper, however is modified for jumping. The

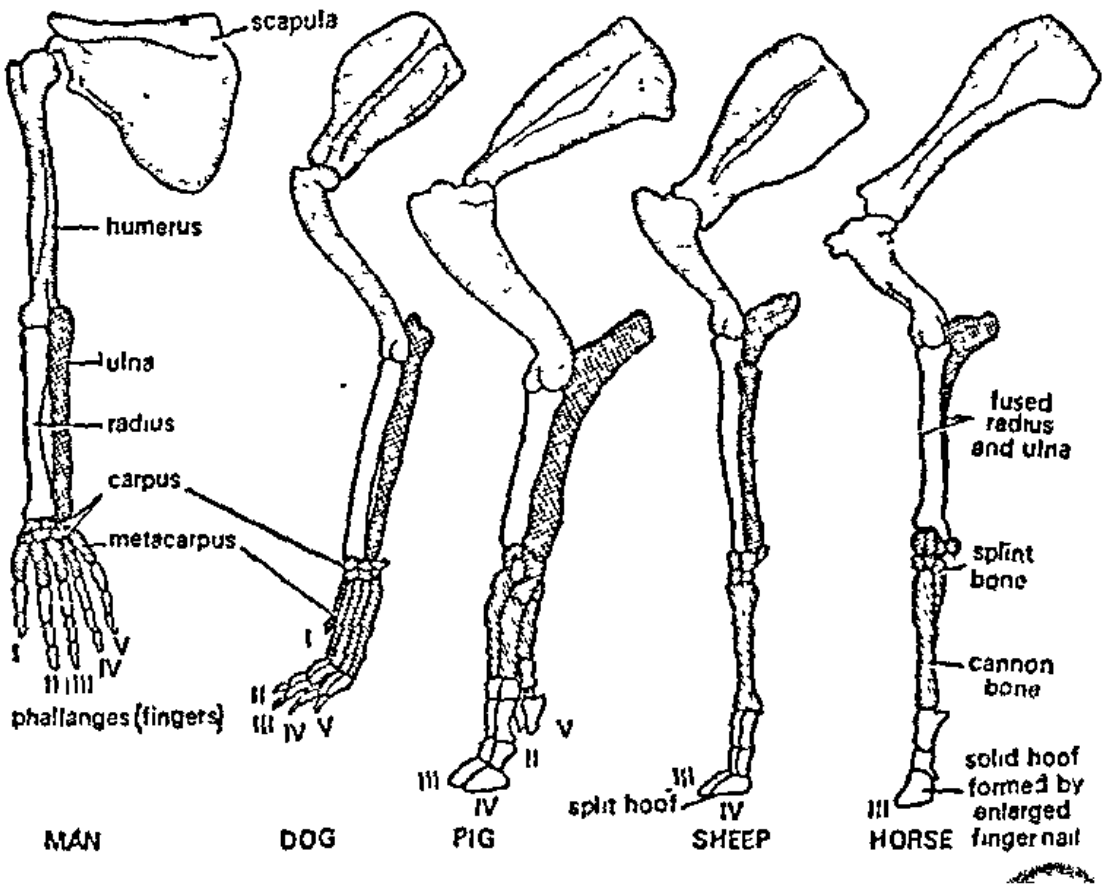


Fig 4 4. Fore-limbs of certain mammals showing serial homology.

forelimbs of a mole cricket are modified for digging; the legs of a hog louse are adopted for clinging to hairs; the legs of a diving beetle are constructed for swimming; and the claws, pulvilli, and hairs of the tarsi of the common housefly make it possible for the fly to walk upside down.

Among plants also, there are many examples of homologous structures. In all seed plants, for example, leaves are homologous structures, as are stems and roots. The many variations in their size, shape, and function indicate the many adaptive modifications that have taken place throughout the group. Leaves modified as thorns, tendrils, and the parts of flowers (sepals, petals, stamens, and carples) are examples of homologous structures that provide evidence of evolution.

In the study of comparative anatomy, structures are often found that have the same function and are superficially alike, such as the wing of a bird and wing of a butterfly, yet are quite different in origin and in structural design. Such structures which are non-homo-

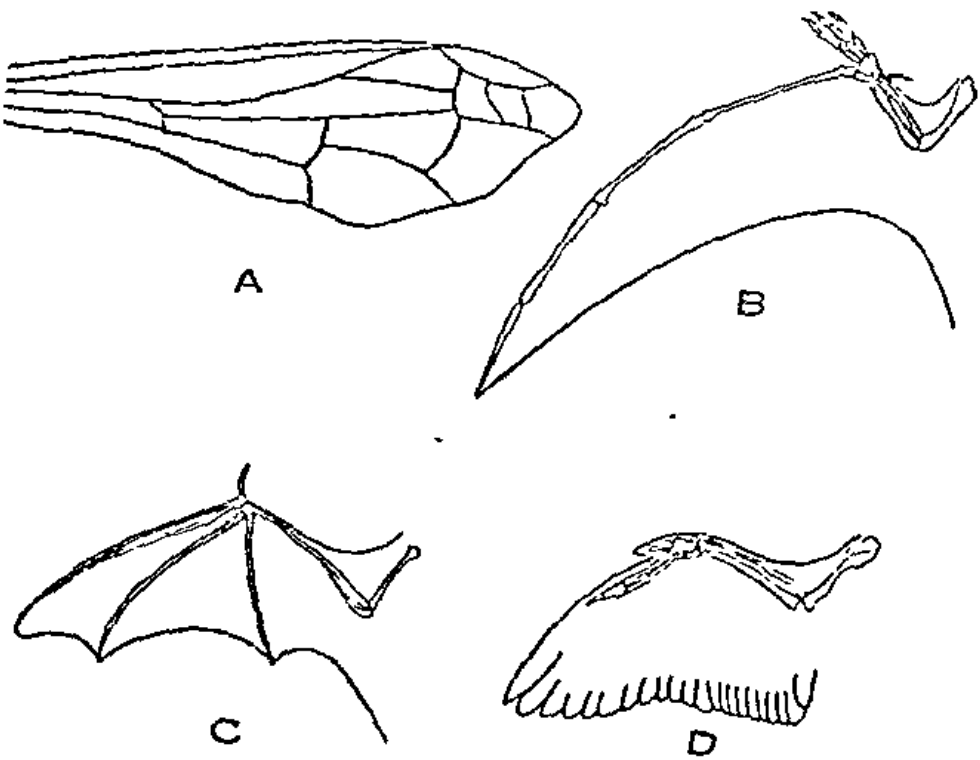


Fig. 45. Wings of a (A) insect, (B) pterodactyl, (C) bat and (D) bird The bones of the wings of the pterodactyle, bird and bat are homologous to each other. The insect's wing is similar to vertebrate wings in function, but it has only a superficial structural resemblance to them. It is therefore analogous and homoplastic

logous but with similar functions are said to be analogous organs or structures. They have arisen in the evolutionary process through adaptations of quite different organisms to similar modes of life. The analogy, thus, refers to the relationship between structures which though differ anatomically but would have superficial similarity due to similar functions. Often analogous organs have little gross structural resemblance to each other; for example, the gills of *Palaemon* and the lungs of man. Other analogous organs, such as the wings of insects, aves (birds) and flying mammals (bats) may have a superficial resemblance.

Their common function, flight, has only one structural solution the evolution of some sort of an airfoil. Analogous organs with a structural resemblance are said to be homoplastic.

There are many instances where different organisms that are not very closely related have developed similar adaptations for life in similar habitats. This phenomenon is often referred to as convergent evolution. The development of same fusiform type of body for life in the water by *Palaemon* (arthropod), shark (fish), *Ichthyosaurus* (reptile), and a dolphin (mammal) illustrates the convergent evolution well. The development of a limbless, cylindrical-snake like body by *Ophiosaurus* (lizard), *Uraeotyphlus* (caecilian amphibian) and *Typhlops* (blind snake) for life in the burrows, is another interesting example of convergent evolution in animals. Further, the development of a cactus-like body by members of several different families of plants is another good example of convergent evolution.

2. VESTIGIAL ORGANS

Another source of evidence stemming from the studies of comparative anatomy is the presence of organs in various animals that have no apparent function and are useless and often small or lacking some essential part; however, in related organisms, these organs remain full-sized, complete and functional. Such organs are called vestigial or rudimentary organs. There are more than 100 vestigial organs in human body, including the vermiform appendix, the coccyx (fused tail vertebrae), the wisdom teeth, the nictitating membrane of the eye, body hair, nipple in male, and the muscle that move the ears. Such organs are the remnants of ones which were functional in the ancestral forms, but when some change in the environment rendered the organ no longer necessary for survival it

gradually became reduced to a vestige. In other animals, there occu many cases of vestigial organs.

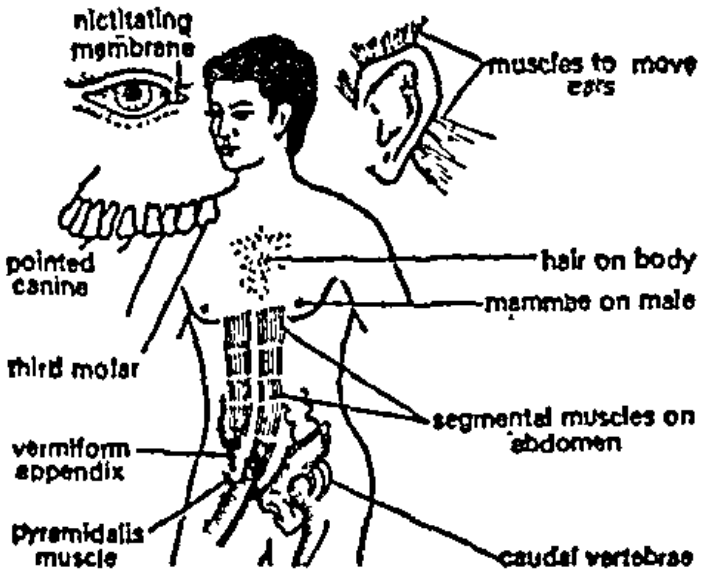


Fig. 4·6. Vestigial organs of man.

For example, python, has tiny rudiments of the hind limbs; whale possess vestiges of pelvic girdle (ilium) and leg bones (femur),

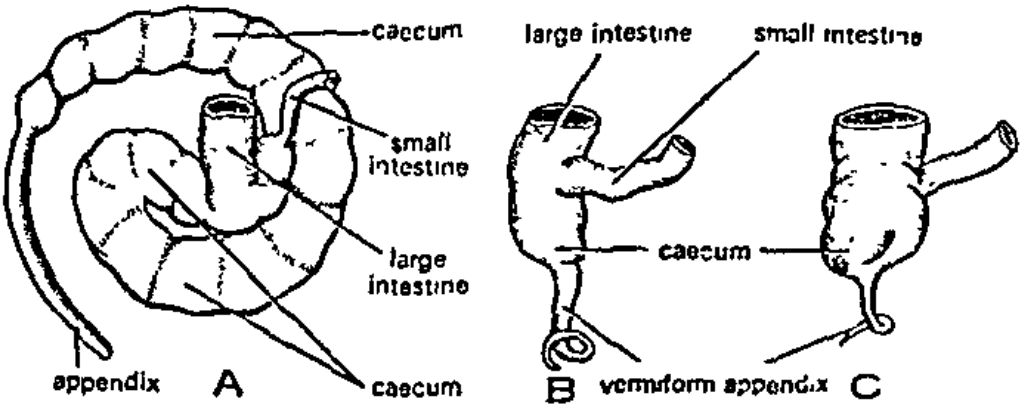


Fig. 4·7. Caecum and vermiform appendix :
A—Rabbit, B—Man embryo, C—Man.

and third metacarpal of the foot of horse remain reduced and occur as splint bone.

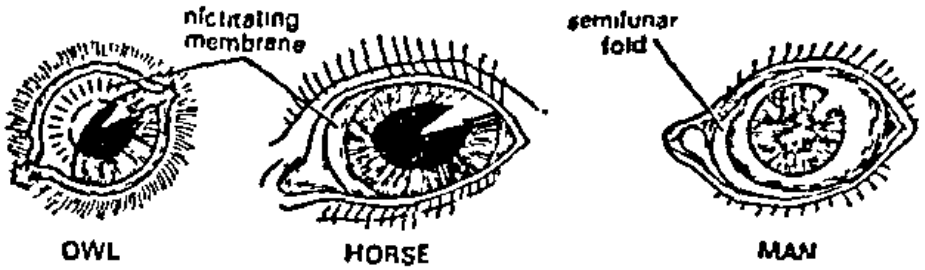


Fig. 4·8. Eyes of owl, horse, and man showing nictitating membrane or semilunar fold.

Visualizing numerous examples of vestigial organs in animals, one may conclude at the first glance that these rudimentary organs are the result of application of Lamarckian "use and disuse" concept to them. But, the underlying mechanism is quite different. Mutations for the decrease in the size and functional importance of an organ are occurring constantly; as long as the organ is necessary for survival, such mutations are lethal and eliminate their possessors. But if the organ is no longer needed for survival, such mutations will not be lethal, and they may accumulate and lead to the reduction of the organ (Villee, Walkers and Barnes, 1973).

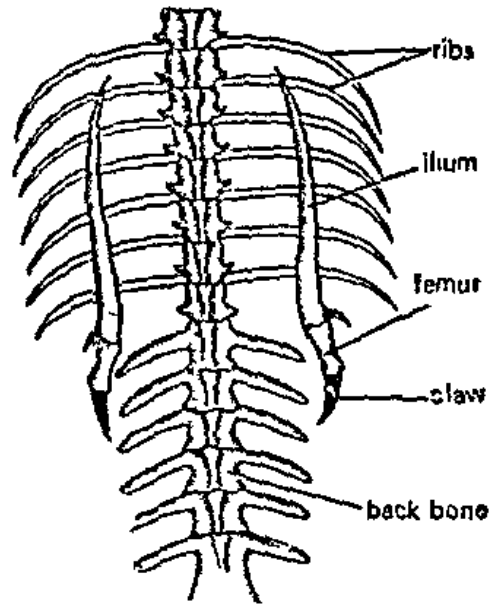


Fig 4·9. Vestigial hind-limbs of Python (snake).

4. EVIDENCES FROM COMPARATIVE EMBRYOLOGY

One of the most fascinating sources of evidence for the validity of Darwin's theory of evolution has come from studies of comparative embryology. The suspicions of a common ancestry for very diverse sets of organisms from comparative anatomical studies have been supported by embryological data.

As Darwinian evolution suggested that more complex organisms would have achieved their state by elaborating on the existing developmental patterns of more primitive forms, so, one would expect to find that certain relatively simple organisms and more complex ones have many initial developmental steps in common. In fact, the more developmental steps two species have in common, the more closely related they are to a common ancestral form.

Comparative embryological studies have revealed that there was one developmental pattern that could be viewed as having undergone a series of branchings. All multicellular animals start their development as a single zygote, and through a series of mitotic divisions, increase in cell number until a blastula is formed. The developing embryo elaborates upon the blastula stage by forming two fundamental germ layers, ectoderm and endoderm, during the course of gastrulation. After the differentiation of the ectoderm and endoderm in the gastrula, the third germ layer, mesoderm, is formed. There are three distinct pattern by which developing embryo of different

species produce a mesodermal layer. For instance, in annelids, molluscs and certain other invertebrates, the mesoderm develops from special cells which are differentiated early in cleavage. These cells migrate to the interior and come to lie between the ectoderm and endoderm. They then multiply to form two longitudinal cords of cells which develop into sheets of mesoderm between the ectoderm and endoderm. In primitive chordates (*i.e.*, *Amphioxus*, etc.) the mesoderm arises as a series of bilateral pouches from the endoderm. These lose their connections with gut and fuse one with another to form a connected layer. The mesoderm in amphibia, reptilia, birds and mammals is formed from the cells which remain associated with either ectoderm or endoderm or both and which get disassociated from them to migrate and come to lie in between both germinal layers in the form of mesoderm.

Despite some differences in developmental patterns, comparative embryological studies overwhelmingly support the concept of Darwinian evolution. Two species that follow the same pathway for several steps after the gastrula produce adult forms that are more similar than two species whose pathways diverge after that stage. The different developmental patterns are thought to be indications of different lines of evolution.

Another feature of embryological development that serves to link all multicellular animals is the embryological source of organ systems. Regardless of the way in which the developing animals are programmed for further differentiation of mesoderm they exhibit similar adult structures derived from the two primary germ layers. The outer covering of all multicellular animals, be it skin, scales, or gelatinous material, is derived from ectoderm. The universal features of ectodermally and endodermally derived tissues also indicates the presence of a common ancestral type early in evolutionary history.

Haeckelian idea though is wrong in the eyes of modern embryologists yet, it has stimulated research in embryology and focussed attention on the general resemblance between embryonic development and the evolutionnry processes. According to biogenetic law of Haeckel, the

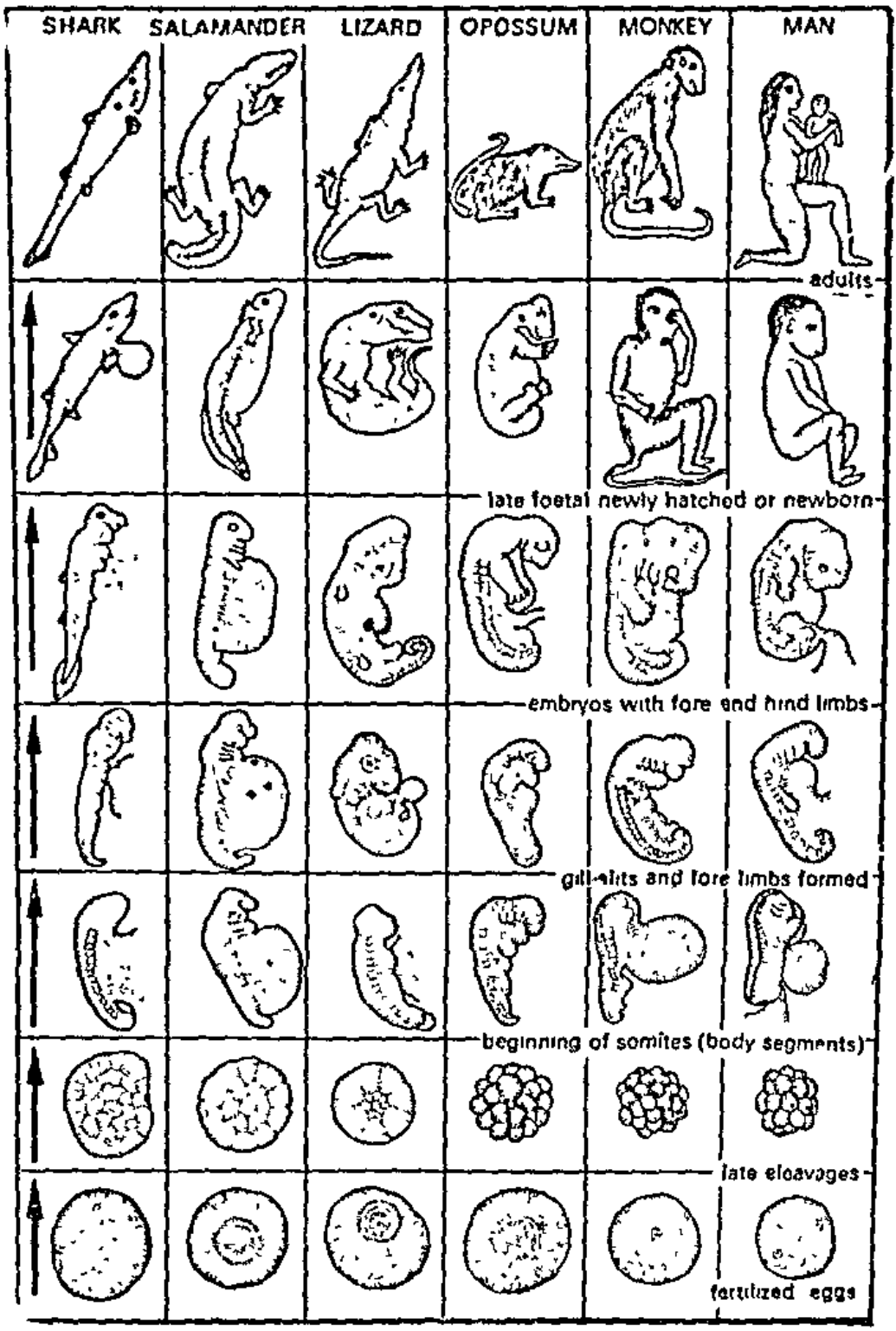


Fig. 4·10. Comparative embryology from fish to man

fertilized egg can be compared to the putative single-celled flagellate ancestor of all animals, and the blastula can be compared to a colonial protozoan or to some hypothetic blastula-like animal which has been postulated to be the ancestor of all Metazoa. Haeckel believed that the ancestor of coelenterates and all the higher animals was a gastrula like organism with two layers of cells and a central cavity connected by a blastopore to the outside. Modern scientists prefer Von Baer's recapitulation concept than biogenetic law of Haeckel.

There are numerous examples of recapitulation phenomenon during embryonic development, but one of the best is afforded by a comparison of different vertebrate embryos at comparable stages in development (see fig. 4·10). During the early embryonic stage in the series, all the embryos look very much alike. All have similar **pharyngeal arches** and **pharyngeal clefts**. At some what later stage, in the series, the **limb-bud** primordia of fore and hind limbs are forming on all embryos in a similar way and all of them have embryonic tails. The embryos of the lizard, chick, opossum, monkey, and man have strong resemblances, yet those of the fish and salamander are beginning to assume recognizable forms. At this stage, gills have formed from the tissues lining the gill clefts of both the fish and salamander. Later, each embryo has developed features that indicate fairly clearly its definitive nature.

Further, in the development of any mammalian embryo, the heart is a four-chambered in series structure, as it is in fish embryo; then it develops partitions of the auricle (atria) similar to those of amphibian embryos, followed by ventricular division that is incomplete for a period, as it is in the embryos of reptiles. A similar example of recapitulation is found in mammalian embryos in the formation of pronephric, mesonephric and metanephric kidneys. The existence of a common trochophore larval stage in the development of many molluscs and many annelids is also typical of this process.

Plants provide a few examples of recapitulation. One good example is found in the *Acacia* tree. This tree has well-developed compound leaves, yet the seedling has simple leaves like those found in all stages of development of its ancestors.

GENETIC BASIS OF RECAPITULATION

Our increasing understanding of physiological genetics provides us with an explanation of the phenomenon of recapitulation. All chordates have in common a certain number of genes which regulate the processes of early development. As mammalian ancestors

evolved from fish, through amphibian and reptilian stages, they accumulated mutations for new characteristics but kept some of the original "fish" genes, which still control early development. Later in development the genes which the human or any other mammal shares with amphibians influences the course of development so that the embryo resembles a frog embryo. Subsequently, some of the genes which have in common with reptiles come into control. Only after this stage, most of the peculiarly mammalian genes exert their influence, and these are followed by the action of genes human has in common with other primates. The anthropoid apes, which have the most immediate ancestors in common with man, have the most genes in common with him and their development is identical with him (human) except for some fine details. In general, during development the general characteristics that distinguish phyla and classes appear before the special characteristics that distinguish genera and, finally, species. Within each phylum, the higher forms pass through a sequence of developmental stages which are similar to those of lower forms but achieve a different final form by adding changes at the end of the original sequence and by altering certain of the earlier embryonic stages they share with the lower forms (Villee *et al.*, 1973).

5. EVIDENCES FROM COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY

Basically, evolution is a biochemical phenomenon and it is, therefore, natural that physiology and biochemistry have given some of the following most important and dependable evidences to suport the idea of evolution :

1. PROTOPLASM CHEMISTRY

Biochemical analysis of the living matter in the protoplasm which is considered as "the physical basis of life", suggests that protoplasm from a variety of sources (*i.e.*, bacteria, blue green algae, plants and animals) has the same biochemical constitution. It mainly consists of substances like proteins, lipids, carbohydrates, water, etc. This would suggest that during evolution the most fundamental property of living things has remained intact, while variations in certain essential respects produced the variability according to the needs of differential forms.

2. CHROMOSOME CHEMISTRY

Like protoplasm, another remarkable similarity at the biochemical level is found in the chemistry of chromosomes. The chromosomes of all living organisms basically consist of nucleic acids (DNA, and RNA) and proteins (histones and protamines). The molecules of

these chemical substances remain arranged in all chromosomes in, a almost identical fashion. Such a uniformity in the composition of chromosome again suggests a common origin of most living beings.

3. ENZYME SIMILARITIES

A large number of animals and plants contain identical enzymes. Several enzymes found in the digestive tract are common in a variety of animals. For example, trypsin and amylase are found from sponges to mammals. A number of enzymes used in photosynthesis are common in a variety of green plants. Such common enzymes and consequently a common mechanism of process of photosynthesis suggest acommon ancestry of green plants.

4. HORMONAL SIMILARITIES

Like enzymes, hormonal similarities are also found in all vertebrates. For example, thyroid hormone is commonly found in all vertebrates and this hormone from one class of organism can be substituted for that in another class of organisms. For example, in frogs deficiency of thyroid hormone can be corrected by feeding them on mammalian thyroid tissues. Likewise, another commonly occurring hormone of vertebrates is melanophore expanding hormone. It is concerned with the pigmentation of the skin to expand, thus, rendering the skin colour dark. This hormone is found in amphibians and mammals. In the latter it is a vestigial hormone, but if it is grafted into the amphibian skin, the skin pigmentation expands. The presence of these hormones in vertebrates is understandable only on the basis of descent from an ancestor to which these hormones were useful.

5. COMPARATIVE SEROLOGY

When a foreign protein is inoculated into the blood of an animal, the latter produces a complex protein compound against that foreign protein inoculated. These compounds are familiarly known as **antibodies** and the foreign inoculated protein is known as **antigen**. When a reaction occurs between antibody and antigen, a soft white precipitate will be formed. The strength of precipitate depends upon the concentration of antigen. The precipitate is the **precipitin** and the test is **precipitation test**. One of the remarkable features of this test is, that the antibodies formed, against one antigen, can also react with antigens of other source, provided the latter is chemically similar to the first antigen. Antibodies containing serum is known as antiserum. Antiserurm of antigen of one animal can be tested with antigens of other animals in order to show their relationships. The test can be interpreted that if precipitate results with more diluted antigen of one animal against the test animal, then the former is

more closely related to the latter ; if precipitate results with less diluted antigen of that animal, then it is distantly related to the test animal. Such precipitin tests have conducted to resolve the disputed relationships of organisms, in recent years. Of scores of examples, here we give two illustrations.

Till recently, it is believed that whales have relationship with fishes. It is because, almost all of their anatomy are so strongly modified to aquatic fish-like life. Only few anatomical clues to show their relationships to other mammals, remained. However comparative serology of whales with other mammalian groups indicates that their serum proteins are most like those of the even-toed hoofed (Order Artiodactyla) mammals. This might suggest that whales sprang from primitive artiodactyl stock.

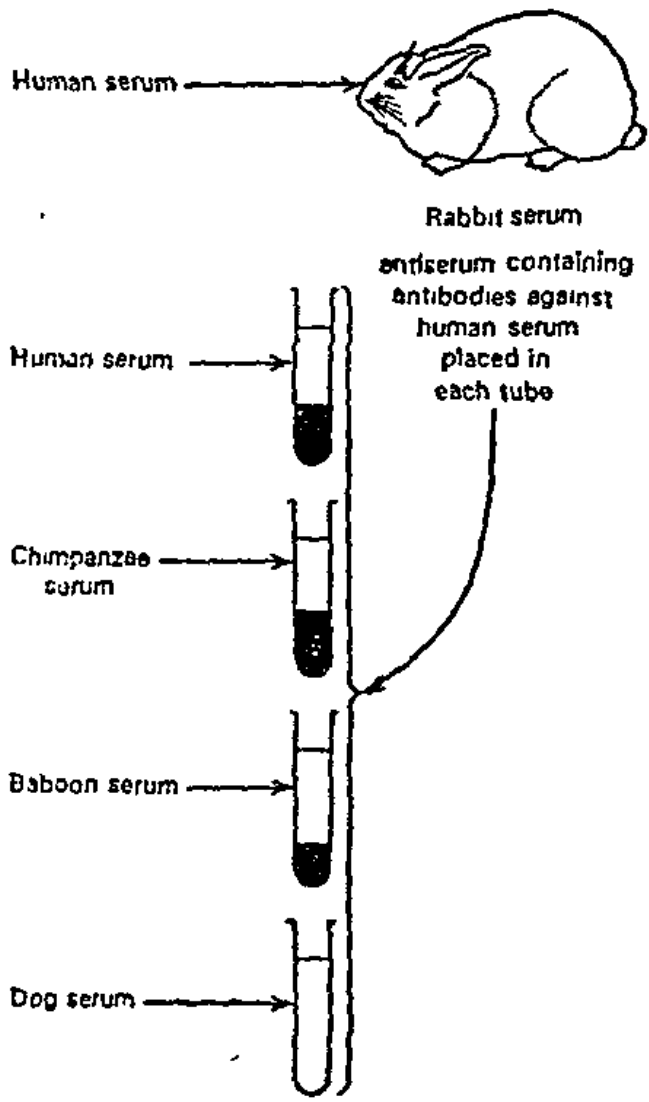


Fig. 4·11. Principle of precipitin test applied to investigation of animal relationship.

The same serological tests when performed in slighly modified form among the members of primates—man, an anthropoid ape, an old world-monkey, a new world-monkey and a lemur, the amount of precipitate of serum proteins would decrease in descending order. that is, the anthropoid ape is more closely related to man than other organisms. Even among anthropoid primates, tests done according to onchterlony technique, reveal that the serum proteins of chimpanzee are more alike to man's serum proteins than the serum proteins of asiatic apes, gorilla and baboon.

Similar comparative serological tests reveal the fact that cats, dogs, and bears are closely related. Cows, sheep, goats, dear and antelopes constitute another closely related groups in terms of "blood

relations." Serological tests also suggests that there is a closer relationship among the modern birds than among the mammals, for all of the several hundred species of birds tested give strong and immediate reactions with serum containing antibodies for chicken

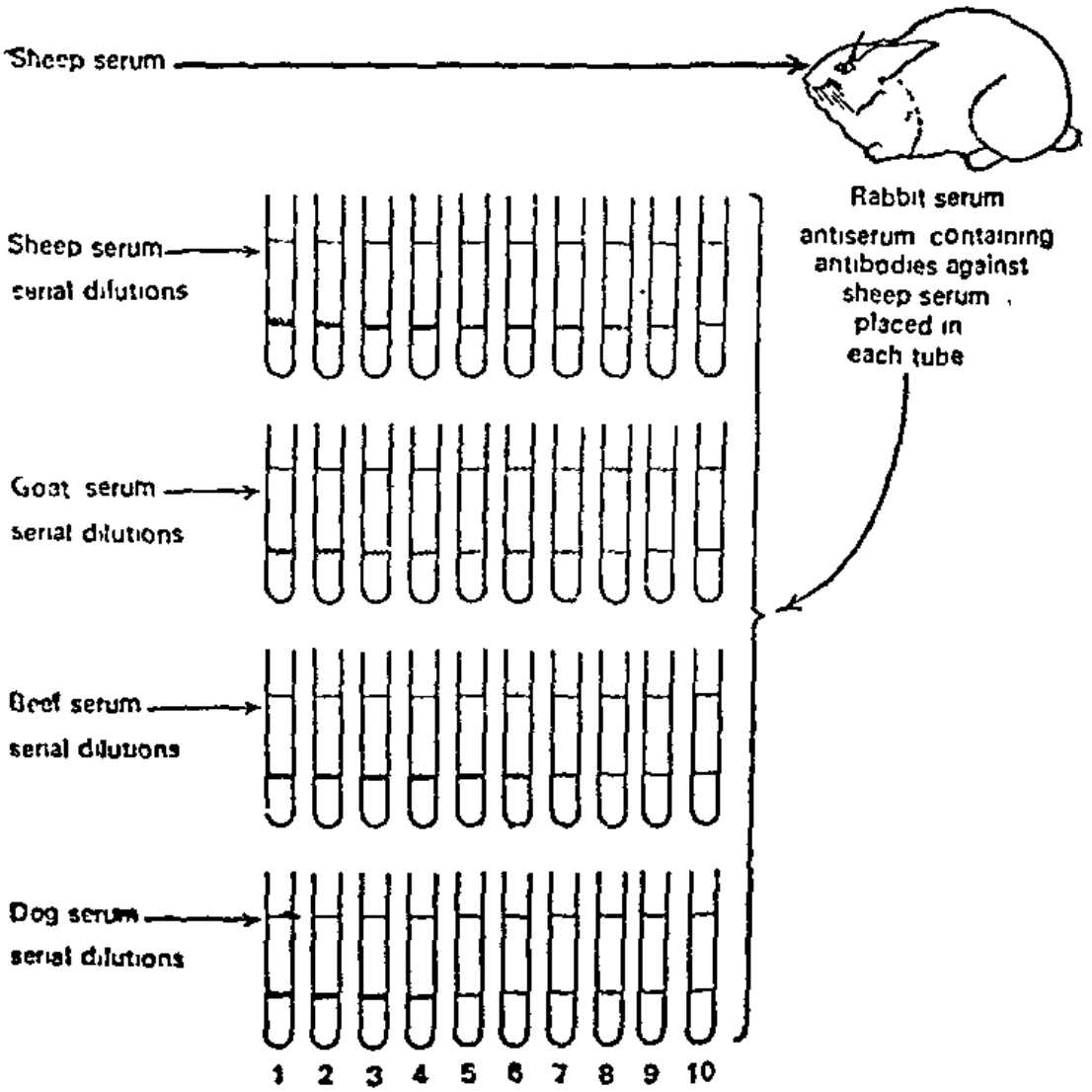


Fig. 4 12. Principle of precipitin test employing serial dilutions of antigen and the interfacial reaction.

serum. From other tests it was concluded that birds are more closely related to the crocodile line of reptiles than to the snake-lizard line, which corroborates the palaeontological evidence. Similar tests of the sera of crustaceans, insects and molluscs have shown that forms regarded as being closely related from morphologic and palaeontologic evidence also show similarities in their serum proteins.

6. AMINO-ACID SEQUENCE ANALYSES

Molecular biological investigations of the sequence of amino acids in the α and β chains of haemoglobins from different species have revealed great similarities, of course, and specific differences, the

pattern of which demonstrates the order in which the underlying mutations, the changes in nucleotide base pairs, must have occurred in evolution. The evolutionary relationships inferred from these studies agree completely with those based on morphologic studies. Analyses of the amino acid sequence in the protein portion of the cytochrome enzyme provide further concurring evolutionary relaships. Further the pattern and rates of reactions of lactate dehydrogenase and certain other enzymes with the normal pyridine nucleotide coenzyme (NAD) and with analogoues of NAD can be used to demonstrate evolutionary relationships.

7. EXCRETORY PRODUCT ANALYSES

An analysis of the urinary wastes of different species provides the evidence of evolutionary relationship. The kind of nitrogenous excretory waste depends upon the particular kinds of enzymes present, and the enzymes are determined by genes, which have been selected in the course of evolution. The waste products of the metabolism of purines, adenine and guanine, are excreted by man and other primates as uric acid, by other mammals as allantoin, by amphibians and most fishes as urea, and by most invertabrates as ammonia. Vertebrate evolution has been marked by the successive loss of enzymes for the stepwise degradation of uric acid. J. Needham made the interesting observation that the chick embryo in the early stages of development excretes ammonia, later it excretes urea and finally it excretes uric acid. The enzyme uricase, which catalyzes the first step in the degradation of uric acid, is present in the early chick embryo but disappears in the later stages of development. The adult frog excretes urea, but its tadpole larva excretes ammonia. These biochemical examples are the repitition of the principle of recapitulation.

8. PHOSPHAGENS

The phosphagens play a key role in muscle contraction and are the sources of energy for the resynthesis of ATP, once they are brokendown. In the muscles of most vertebrates, phosphagen is always a specific compound called creatine phosphate, while in most invertebrates it is arginine phosphate. Hemichordates, the most primitive chordates, have both the phosphagens, the creatine phosphate as well as argine phosphate. such a situation is also found in echinoderms and on morphological grounds echinoderms have been considered close to the ancestor of chordates.

6. EVIDENCES FROM COMPARATIVE CYTOLOGY

Another type of evidence that indicates that all forms of life are related comes form the cellular level. The very fact that the cell

is the unit of structure for all living organisms (except viruses) is thought to reflect the basic relationship among living forms. This relationship is even further emphasized by the fact that it has been possible for biologists to consruct a picture of the "generalized" cell from which all other types can be inferred.

Moreover, all cells that have been examined thus far have a DNA–RNA—protein information and communication system. All forms contain membranes that are made up of double-layered lipoproteins. All cells (except few bacterja) utilize the glycolytic pathway. Most bacterial forms, and all uni-and multicellular organisms, have a Krebs cycle and an electron transport system. All are based on ATP as an energy donor. Certainly these factors provide an overwhelming demonstration of the interrelatedness of biological forms.

7. EVIDENCES FROM GENETICS

Genetics, the science of heredity, deals the variability of plants and animals. Hereditary variations provide the raw material of evolution. There are mainly two sources of hereditary variations namely, **recombination** and **mutation**. While recombinations, after hybridization yield new combinations, mutations will create new genetic material which never existed earlier.

For the past several thousand years man has been selecting and breeding (*i.e.*, hybridizing) animals and plants for his own uses, and a great many varieties, adapted for different purposes, have been estabished. These results of artificial selection provide striking models of what may be accomplished by natural selection. All of our breeds of dogs have descended from one, or perhaps a very few, species of wild dog or wolf, yet they vary so much, in colour, size and body proportions that if they occurred in the wild they would undoubtedly be considered separate species. They are all interfertile and are known to come from common ancestors, so they are regarded as varieties of a single species. A comparable range of varieties has been produced by artifical selection in cats, chickens, sheep, cattle and horses. Plant breeders have established by selective breeding a tremandous variety of plants. From the cliff cabbage, which still grows wild in Europe, have come cultivated cabbage, cauliflower, Kohlrabi, Brussels, sprouts, brocoli and kale.

Further, cytogeneticists have been able to trace the ancestry of certain modern plants by a combination of cytologic techniques in which the morphology of the chromosomes is compared and by breeding techniques which compare the kinds of genes and their order in particular chromosomes in a series of plants. In this way, the present

cultivated tobacco plant, *Nicotiana tabacum*, was shown to have arisen from two species of wild tobacco, and corn was traced to teosinte, a grass-like plant which grows wild in the Andes and Mexico. Moreover, the cytologic details of the structure of the giant chromosomes of the salivary glands of fruit flies have been of prime importance in unrevealling the evolutionary history of many species of *Drosophila*,

Mutations have also played a very significant role in evolution. Different kinds of mutations-namely gross mutation due to variation in chromosome number (polyploidy and aneuploidy), and point mutations (see chapter of mutation) introduce different kinds of variations in plants and animals and consequently result in speciation. Plant breeders have employed induced polyploidy methods in producing numerous economically important varieties of plants. Geneticists have produced many new strains of micro-organisms, plants and animals (*Drosophila*) by artifiicially inducing point mutations in them.

Thus, the artificial selection methods due to recombination or polyploidy and induced methods of mutations have suggested the fundamental processes which may be involved in organic evolution.

8. EVIDENCES FROM BIO-GEOGRAPHICAL RELATIONS

Bio-geography deals with the manner in which plants and animals are distributed over our planet. On the basis of similarities in the existing fauna, found in different regions of the earth, following six bio-geographical regions have been distinguished :

(a) Nearctic—North America down to the Maxican Plateau.

(b) Palearctic—Asia North of the Himalayas, Europe and Africa, North of Sahara Desert.

(c) Neotropical—Central South of America.

(d) Oriental—Asia, south of Himalayas.

(e) Ethiopian—Africa, south of Himalayas.

(f) Australian—Australia and associated islands.

Any one who is familiar with biogeography of South American and African regions, immediately be convinced by the fact, that similar habitats are populated with similar animals. These two are extensive tropical regions and crossed by the equator. They both have similar habitats in that—have lowland jungles; extensive river systems and mountain regions. Both regions extends southward to temperate zones. Yet surprisingly they have dissimilarities in fauna more greatly than similarities. The characteristic fauna of Africa includes Lions, Elephants, Rhinoceroses,

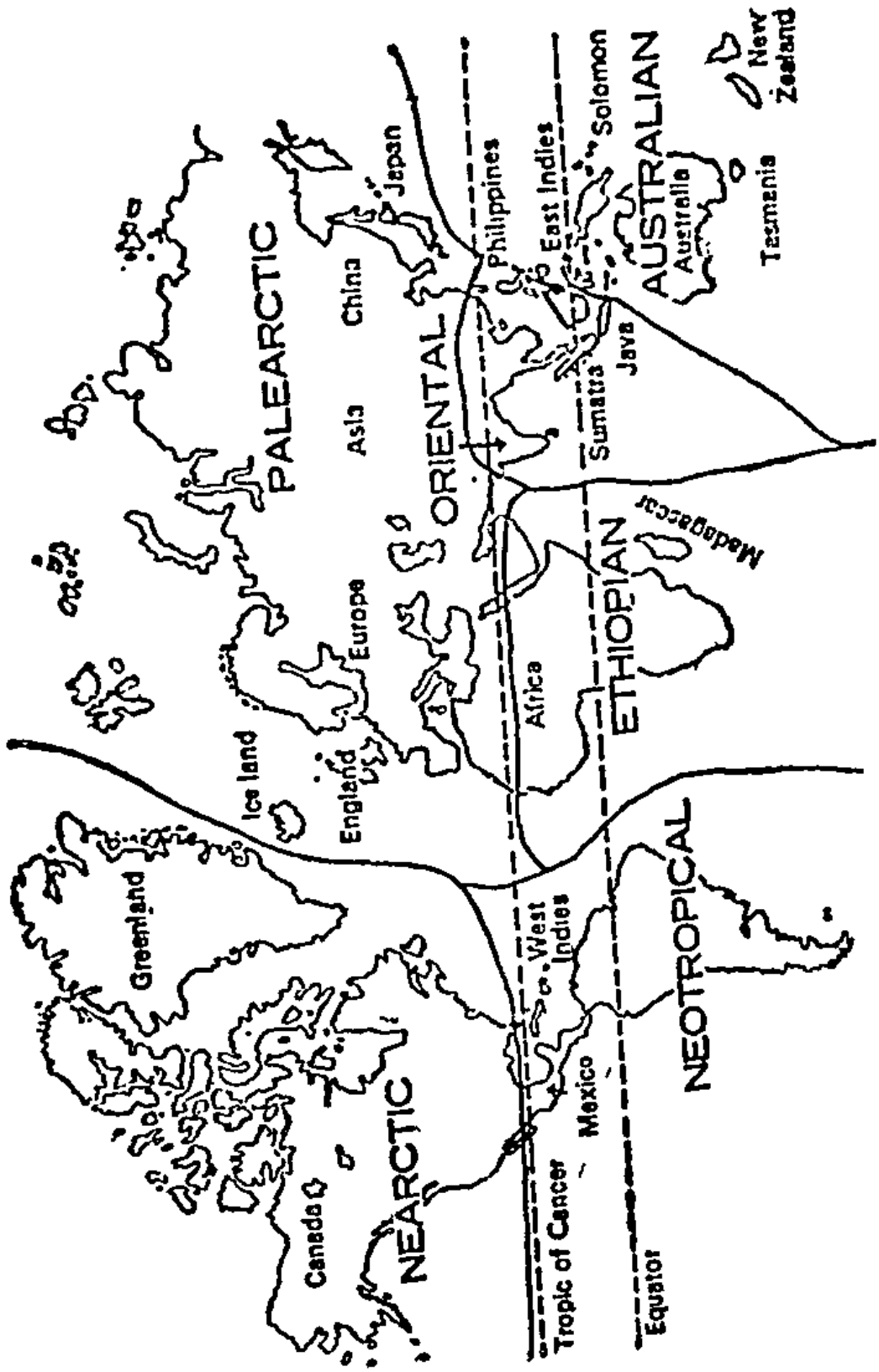


Fig. 4·4. Different zoogeographical regions of world.

kinds of Antelopes, Giraffes, Zebras, Hyenas, Lemurs, Baboons, Monkeys with narrow noses and non-prehenslie tailed Chimpanzees and Gorillas.

In South America, occur Monkeys with broad noses, Tapirs, Odd-toed hoofed mammals, rodents like Capybara. Agouti, Chinchilla and Paca, Mountain lions like panthers—Ocelots Jaguars of cat family, well known Uamas Gaunacos, Vicunas, and Alpacas, Armadillos, many opossums, Giant Anteaters, Raccoons, spectacled Bears and Sloths and many others.

Thus, if both Africa and S. America have similar varying habitats, then how it could have happened, to them to possess different fauna instead similar fauna ? We cannot simply satisfy with such an answer that suitability of habitat is important. Because, we do find similarities in fauna of both regions. They both have such widely ranging animals, bats, rats, squirrels, hares and rabbits, etc. The other alternative answer might be the accessibility of these two regions to the animals found in them. Let us examine this point here more briefly.

Simpson has established a relationship "faunal stratification" between the separateness of animals and their period of length of appearance in S. America. Based on it, he concludes that armadillos and sloths are the oldest inhabitants of S. America since early coenozoic times. They have not appeared in other part of the world. Monkeys and field mice have formed part of S. American fauna during mid and late coenozoic times. While the rest of the fauna have independently evolved. These facts have been ascertained by fossil record.

The record reveals that during long periods S. American forms have no contact with those on other continents. Occasionally animals might have reached the continent by island hopping across the intervening sea. This type of dispersal is best seen in fauna on continental islands. For the most part the isthums of Panama, that links S. America to N. America was submerged and thereby the two continents are isolated. When once geographical isolation take place local fauna along with invaders might evolve into distinct species, on many different times and have become unique to S. America.

Thus, this, is the only possibility for the same habitats in different parts of earth have been populated by different animals and plants. This fact suggests that animals today we see in different biogeographical regions, have descended from their predecessors with different structures, and have migrated from their place of

origin to their new areas, but have failed to return to their place of origin, because it is separated geologically.

CONTINENTAL ISLANDS

These islands are located on continental shelves of continents. These islands sometimes are connected to mainland when ocean water recedes or the level of island land rises.

The fauna and flora of continental islands characteristically resembled those of continents, to which they are formerly joined. One intersting feature is the presence of amphibians and mammals on cotinental islands. Island hopping in these animals is less likely and hence they might have arrived these island through land connections.

Thus, these evidences have estabilished that biological evolution is a fact but not a dogma. Consequently, the biologists conviction that, through a series of changes resulting from natural selective processes, life came to the state known today, is of such magnitude that entire science of biology has been oriented according to the evolutionary doctrine. Organisms have been reclassified according to proposed evolutionary relationships. Geneticists interpret their results as possible mechanisms of evolution or sources of variation. So powerful the idea is this today, that only a text organization based on evolutionary doctrine would truely represent the science of biology.

CHAPTER 5

THEORIES OF ORGANIC EVOLUTION

Two explanations have been offered for the origin of the different forms of life—special creation and evolution. Many evidences have been presented in previous chapters to support the concept of evolution, and almost without exception modern biologists are convinced of evolution. Uptill now, following convincing theories have been forwarded to explain the mechanism of evolution. The first of these theories, which is now only of historical significance, was that of **Jean Baptiste de Lamarck** (1744-1829).

1. THEORY OF INHERITANCE OF ACQUIRED CHARACTERS (LAMARCKISM)

The theory of inheritance of acquired characters states that *modifications which the organism acquires in adaptation to the environments which it meets during its life time are automatically handed down to its descendants, and so become part of heredity.* This theory was propounded by a renowned French naturalist, **Lamarck,** shortly after the occurrence of French Revolution. Lamarck spent the early part of his life as a botanist. Then at the age of 50 he turned his attention to zoology, particularly to the study of invertebrates (The term "invertebrate" and biology" have been coined by Lamarck). As a result of his systematic studies he became convinced that species were not constant but rather were derived from pre-existing species. This idea was in total conflict with the view of the period—that of fixity of species. As a result Lamarck's views were challenged by most of the biologists of that time, particularly by **Georges Cuvier.** In 1809, Lamarck published **Philosophie Zoologique,** which included his theory explaining the changes that occur in the formation of new types. Although, his views on evolutionary mechanism are outmoded now, he still occupies a very important place in the history of evolutionary thought. He was the first evolutionist to conclude that evolution is a general fact covering all forms of life. His evolutionary ideas can be discussed in brief as follows :

LAMARCK'S EVOLUTIONARY PROPOSITIONS

The Lamarckian theory may be summarized in four propositions—(1) Living organisms and their component parts tend continually to increase in size. (2) Production of a new organ resulted from new need and from the new movement which this need starts and maintains. (3) If an organ is used constantly, it will tend to become highly developed, whereas disuse results in degeneration. (4) Modifications produced by the above principles during the life time of an individual will be inherited by its offspring, with the result that changes are cumulative over a period of time.

Thus, Lamarck believed that organic changes seen in animals were resulted by the influence of environment, on the gradual changes of species due to their tendency to become more and more perfect. According to him, when an animal's environment change, its needs change, and this leads to special demands on certain organs. Organs used more extensively would enlarge and become more efficient. Conversely, an organ or organs, no longer used would degenerate and atrophy. He postulated that such changed characteristics (acquaired traits) would be transmitted to the offspring.

Examples of Lamarckism : To explain his theory which was, based on the ideas **direct environmental effect over living organisms use and disuse** and the **inheritance of acquired traits** Lamarck used examples like long neck of giraffe, limblessness in snakes, *webbed feet of ducks, blindness of moles,* and also dimorphism of submerged and aerial leaves in aquatic plants. He visualized the

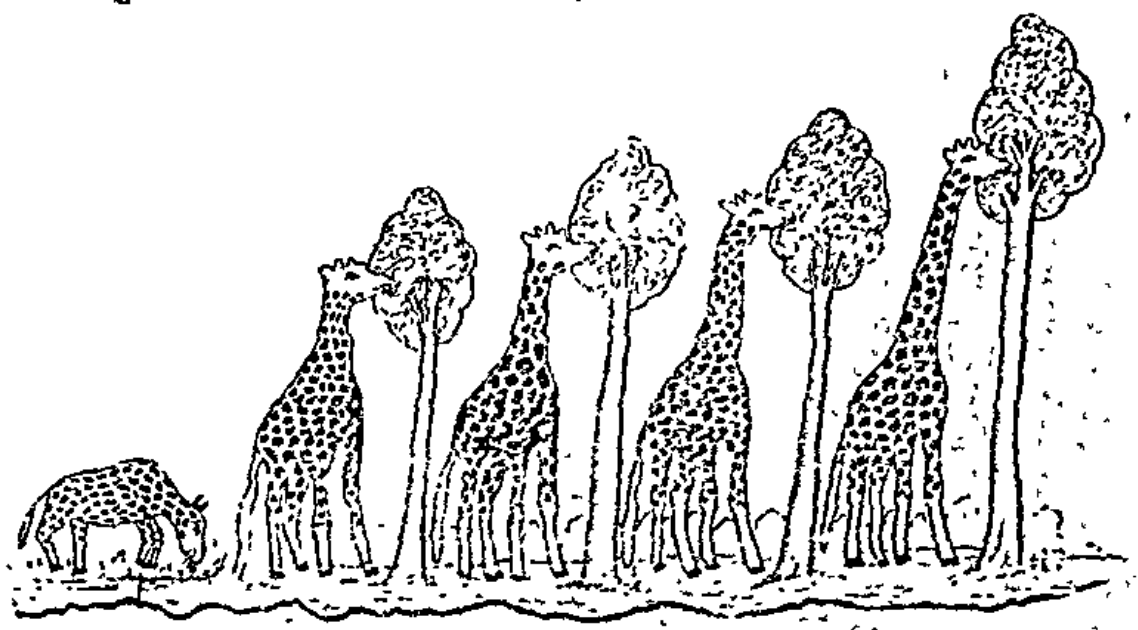


Fig. 5·1. Evolution of giraffe according to Lamarck.

evolution of the giraffe as follows : An origininal deer-like animal finding the supply of grass and herbs inadequate, started to feed on the leaves of trees. It needed greater height to reach the higher leaves, and in the process of reaching, its neck became longer and longer. In the course of generations the long neck became a more accentuated feature, and our modern giraffe was the result. Likewise, according to Lamarckian evolutionary theory, the webbed foot of an aquatic foul such as a duck would have developed in the following way : The duck would stretch its toes apart to give more push during swimming. This new characteristic would be inherited, and the subsequent generation of duck would upon stretching their toes form a more defined web. Each generation would do the same until the webbed foot seen on ducks today was fully formed. This would then be passed on form generation to generation, essentially unchanged once the perfected state was attained. For plants. Lamarck accepted the theory of his compatriot St. Helaire, who developed the notion that plant form is shaped by the combined effects of the environment.

Significance of Lamarckism—Lamarckian theory was simple and it had some appeal, as it provided a way in which changes in organisms could come about. It was the first completely comprehensive mechanistic theory that was offered. Further more, it was theory that lent itself to predictions and therefore, to testing. Thus, Lamarckian theory enjoyed popular acceptance near about 70 years because, it was examplified by many common examples. Most persons know that exercise results in larger muscles, deep-sea fishes have rudimentary eyes, carnivorous animals possess claw, and ventral side of the body possess sensitive skin and tactile points. The theory also afforded a means of explaining reduced or vestigial structures. Further, through disuse, the eyes of a cave animal might become functionless and might even disappear.

CRITICAL ANALYSIS OF LAMARCK'S PREPOSITIONS

Lamarck defended his evolutionary theory vigorously until his death. For it, he suffered both social and scientific ostracism, but he had the courage of his convictions However, he was criticized for following reasons by the contemporary scientist durings his lifetime and afterwards.

1. The first preposition of Lamarck suggests the tendency to increase in size. While the evolutionary trend in a certain groups of organisms may be associated with increase of size, there are many cases, where evolution proceeded not only without any increase in size but rather through a reduction in size. Many plants contradicts

this Lamarckian principle by showing such a reduction in size during their evolution. Many ferns and conifers which became extinct were gigantic trees and the more highly evolved flowering plants are really much smaller in size

2. The second Lamarckian principle, that new organs result from new needs, is quite manifestly false. In the case of plants, Lamarck believed that the environment acted directly upon the plant, causing the production of such new characters as might adapt the plant to its environment. In the case of animals, he believed that the environment acted through the nervous systems ; in other words, the desire of the animal leads to the formation of new structures. In its crudest form this would mean that the man who mused, "*Birds can fly, so why can't I ?* should have sprouted wings and taken to the air.

3. The third Lamarckian principle that organs will develop due to use and degenerate due to disuse, may be correct as far as growth *of an organ within the lifetime of an individual is concerned. For* example, it is a commonly observed fact that if muscles are put to use these would develop. However, this principle is meaningful only when it is studied in relation to following fourth principle.

4. The fourth and final preposition of Lamarck was that the inheritance of characters acquired during the life time of the individual. This principle has been tested by many biologists who have devised many types of experiments for it and have found it entirely uncorrect. Certain experiments which have discredited it are following:

1. The noted German scientist August Weismann was the first person who for the first time made a definite distinction between heritable changes and those which can not be inherited. In 1890, he performed some experiments to test if characters may disappear due to disuse. This he did by cutting the tails (mutilation) of white mice for more than 20 generation to see if this has any effect on tail length. The measuring of tail length of the offsprings of 20 successive generations, revealed that on average, the tail were not shorter. It means that acquired character (cut tail) was not inherited.

2. Castle and Phillips performed transplantation experiments to show that environment has no effect on heredity. In one of the experiment they transplanted the ovary of a black female guinea pig into the body of white female guinea pig and the recipient female was mated with a white male guinea pig. They found that all the individuals from this pair were black. This shows that the environment does not effect the heredity as has been suggested by Lamarck.

3. Loeb artificially fertilized the eggs of sea urchin by certain chemical stimuli and these parthenogenetically fertilized eggs produced the generations, the members of which possessed no parthenogenesis.

4. Boaring of ears and nostrils in Indian women has been continued as the tradition from centuries but their offsprings do not show any trace of holes in ears and nostrils.

5. The Chinese bound the feet of their women for many generations, yet this has not resulted in any modification of the feet of present-day Chinese women.

6. Jewish boys (and also muslim boys) have been circumcised for thousands of years, but this has not resulted in a tendency toward the reduction of the prepuce in them.

Besides these negative experimental evidences for discrediting Lamarckian theory, there are certain experimental evidences which tries to support it. Some of the important positive experimental supports for Lamarckism are following :

1. McDougall conducted learning experiments in rats and from the results obtained he tried to suggest that learning as an acquired character can be inherited. During his experiments, the rats were dropped into a tank of water from which there were two exits, one lighted and one dark, but, not always the same one. A rat leaving by the lighted exit received an electric shock, while one leaving by the dark exit received no shock. Thus the number of trials required for a particular rat to learn always to select the dark exit constituted a measure of the speed of learning. These rats were then bred, and their descendants were similarly studied. It appeared that the speed of learning increased from generation to generation, and so McDougall concluded that learning, an acquired trait *par excellence*, is inherited.

Some serious criticisms have been raised against McDougall's experiments It is suggested that genetic constitution of rats was not controlled. Moreover, in the progeny of control rats not subjected to these learning tests, the learning habit was found to change the same way as in the treatments. So, it was said that some unanalysed changes in the technique may be responsible for the recorded increase in the speed of learning Lastly, the most strange aspect about McDougall's experiment is, that when the same experiments were repeated in other laboratories never gave similar results.

2. F.B. Summer reared the white mice at 20°C to 30°C, because of which they developed longer bodies, tail and feet, when these mice were bred at lower temperatures their offsprings showed their normal proportions of size.

3. **Lindsey** subjected certain cold-blooded animals, warm-blooded animals and plants to unusual environmental conditions and found that environment affect certain changes in these organisms and these acquired modifications were inherited upto some extent in their offsprings.

Lastly, we can say that though Lamarck failed to conceive of what is now accepted to be the correct mechanisms of evolution, he did provide a complete and inter-related theory that set a standard for any theory that followed.

NEO-LAMARCKISM

Future followers of Lamarck namely **Cope** (1840-1897), **Giard** (1846-1908), **Packard, Spencer** and **McBride** tried to modify Lamarckism in order to make it acceptable. These neo-Lamarckians considered that adaptation is universal. It arises as a result of casual relationship of structure, function and environment. Changed environmental conditions alter habits of organisms, hence in response to new habits, organisms acquire new structures in place of old structures. Consequently, variations among animals have become distinct. These variations have finally become engrained in the heredity of the race. The kind of argument is infact a modified version of Lamarckian principles, because, it has omitted Lamarck's view that of a general perfecting tendency in evolution. It stressed direct action af environment on organic structure. For example, according to Neo-Lamarckians, the development of fur on skin by animals as protection against cold weather, is the consequence of changed environment from warmer to colder state only. But if the environment reverts to normal state, the fur would also disappear. To account it, Neo-Lamarckism included also the effects of use and disuse. Based on this, Neo-Lamarckians have rejected natural selection as the sole mechanism of evolution. On the other hand they believed in the interplay of structure, function and environment is the whole truth of evolution. However, no evolutionist of to-day is adherent to Neo-Lamarckism.

2. THEORY OF NATURAL SELECTION (DARWINISM)

In 1858, two Englishmen published jointly, in the *Proceedings of the Linnaen Society*, papers on a new concept of evolution. This concept, **the theory of the natural selection,** was destined to influence greatly the thinking of modern man in many different fields and to become the basis of most modern biological concepts of evolution.

One of these men, **Alfred Russel Wallace** (1823-1913) was a naturalist who had travelled widely in tropical South America and Southeastern Asia, for studying the flora and fauna of these regions. In early 1858 Wallace was working on the island of Ternate in

Indonesia, when in February, he became ill with a fever. During an attack of the fever he happened to think of an "*Essay on Population*" by Thomas Malthus who was a mathematician, an economist, and a minister, and the large diversity of plants and animals that he had been observing, when, as he said, "suddenly there flashed upon me the idea of the survival of the fittest." He thought about the theory throughout the fever and wrote a rough draft of the concept that same evening. Soon afterward he finished his short essay. "*On the tendency of varieties to depart indefinitely from the original type,*" and sent its manuscript to Charles Robert Darwin (1809-1882) in England and asked him, if he thought of sufficient interest, to present it to the Linnaean Society.

Charles Robert Darwin was born on February 12,1809 in Shrewsbury, England. His personality was not the type that one associates with a successful man, let alone a great one. While his family held a distinguished reputation (his grandfather Erasmus Darwin was a noted poet scientist, his father Robert, was an eminent physician, and his mother was a Wedgwood, the family of pottery fame), Charles was a singularly undistinguished individual whose contemporaries viewed him as just a normal, gentle, cautious, morally upright English clad. After completing his early education at Shrewsbury, Charles Darwin was sent at the age of fifteen to study medicine at the University of Edinburg. His medical interests soon dissipated, and he became something of an academic rover, studying a little science, but finding it too formal for his tastes.

His father then suggested him to become a clergyman, which appealed him. So, he transferred, after two years, to Christ's college, Cambridge University, to study theology. There too he spent his energy in card-playing and drinking. He was obviously not ready for clerical commitments.

He did manage, however, to find some constructive interest in natural history and in collection of natural objects of all kinds. His friendship with Professor John Stevens Henslow, botanist at Combridge, stimulated an interest in botany, while his exposure to the geologist Sedgwick stimulated an interest in geological explorations. Through the recommendation of Dr. Henslow, Darwin accepted a most inauspicious position as a naturalist without pay aboard the H.M.S. *Beagle*. The ship was to spend five years in exploration, mostly of south America, Drawin was to provide a collection of material representing the natural composition of the areas visited.

The ship *Beagle* left Plymouth on December 27, 1831 and visited many islands of the Atlantic ocean, some coasts of south America

and some islands of south pacific, of which Galapagos Islands are the most important. During his five years on the *Beagle*, Darwin recorded almost everything he observed and sent an enormous amount of material back to England. He was a competent observer of plant, animal, and geological specimens (*i e.*, fossils), but he was not a professional in any of these disciplines. During the course of his trip, he became convinced that the theory of special creation and idea of fixity of species were inadequate and consequently, he developed an acute sense of natural balances. Gradually he was transformed from just another observer of nature into an evolutionarily oriented thinker.

He did not consciously develop an evolutionary theory during his voyage, but the seeds of interest were sown. He observed many kinds of natural balances, catastrophic changes, and fossil remains of many communities. He saw nature as a dynamic force, constantly achieving and retaining equilibria, and felt the need for all these factors to be put into a causative pattern. Further, he was especially impressed with the striking adaptations found in the animals and plants that he collected and with the progressive changes in the different species of a given genus as the *Beagle*-expedition moved along the South American coast. In the Galapagos Island he found many species of a given genus confined to a single island, yet resembling other species on the mainland. Such facts, Darwin reasoned could be explained only by the supposition that species may become modified.

Upon his return home in 1836, he spent two years writing a book of his experiences, The *Voyage of the Beagle.* In 1838, he got married and later had two daughters and five sons. From 1838 to 1841, Darwin acted as the secretary of Geological society, where he came in contact with an eminent geoiogist Charles Lyell. He had already arrived independently at the concept of natural selection like Wallace and as early as 1844 had written out a summary of the theory but did not publish it. Rather, Darwin had proceeded to gather evidence, both from original research and from the writings of others, to support the concept. Perhaps he was well aware of how Lamarck's earlier theory had been received and wanted all possible supporting evidence before publishing his own new theory. In any case, we can easily imagine Darwin's consternation upon receiving Wallace's essay. Upon the insistence of the geologist Charles Lyell and the botanist Joseph Hooker, Darwin prepared an abstract of his conclusions for joint publication with Wallace's essay. These papers were published in 1858.

The following year, 1859, Darwin published his findings in full in a book, "*The Origin of Species by natural selection, or the preservation of favoured races in the struggle for life*". This book has been judged by many as the most important book of the nineteenth century, since it contained overwhelming evidence of the fact that evolution has and does occur, and presented a logical theory regarding the major mechanism of evolution—**natural selection** When Darwin's book, the "*Origin of Species...*" was published in 1859, Darwin became the centre of much controversy. The fact that this book, written by Darwin alone, exposed the public at large to the theory of natural selection, and that Wallace was still in Indonesia at the time of the controversy and could not champion Darwin's defense by adding his own views, brought the public to associate only Darwin with this theory. Thus the theory is called the **Darwinian**, rather than the **Darwin-Wallace theory of evolution.**

Darwin died on April 19, 1882, when he was 73 years old. During his life, he wrote numerous books, Journals, etc. Some of them can be listed in following table :

Table : Some publications of Charles Darwin.

	Title of book	Year of Publication
1.	Journal of researches	1839
2.	The structure and distribution of coral reefs.	1844
3.	Geological observations on South America	1846
4	A monograph on Cirripedia (Barancles, living and fossil, 4 volumes)	1851, 1854
5.	Origin of species	1859
6.	The fertilization of orchids	1862
7.	The variation of plants and animals under domestication.	1868
8.	The descent of man	1871
9.	The expression of the emotions in men and animals.	1872
10.	Insectivorous plants	1875
11.	The effects of cross and self fertilization in the vegetable kingdom.	1876
12.	Different forms of flowers on plants of the same species.	1877
13.	The power of movement in plants	1880
14.	The formation of vegetable mould through the action of worms.	1881

FACTS THAT INFLUENCED DARWIN'S THOUGHTS

During the period in which Darwin massed his evidence and developed his natural selection theory, many things affected his thinking, of which three in particular deserve mention here :

1. He had taken with him on the voyage of the *Beagle* a copy of Charles Lyell's *Principles of Geology*, which had been published only a short time before. This book attempted to explain the past changes in the earth's surface on the basis of processes now in operation. It extended greatly the older ideas about the age of the earth, and it gave, Darwin a basis for appreciating some of the fossil material that he found. In addition, Lyell's insistence on explaining the past history of the earth in terms of processes still operating affected Darwin's thinking about the causes of evolution.

2. Darwin was aware of the similarities of many domesticated animals and plants to wild forms and realized that men, for centuries, have selected individual plants and animals with desirable traits for breeding. In this way the various lines of domesticated forms have been developed. He found himself asking the question : Might not *some sort of selection operate in nature to produce the various highly* adapted forms found on the earth ?

3. Darwin obtained a suggestion that was to serve as the basis for his explanation of evolution from the essay of Thomas Malthus entitled *"An Essay on Population"*. Malthus' ideas were of sociological importance. He was concerned with problems of the increasing human population. He pointed out that human population, as well as plant and animal populations, tends to increase at such rates that its numbers outstrip its resources, and because there is not food or space enough for all, there is a struggle for existence. Malthus argued that in time the human population would outstrip the possible food supply and therefore that man should take steps to control his reproduction. He proposed late marriage and sexual restraint as a means of doing this and stated that unless steps were taken by *man* to limit the hman population, checks apparently beyond his control *in the form of famine, disease, and war—would limit instead.*

Darwin was familiar with the struggle for existence that occurs everywhere in nature. He reasoned that in such situations, favourable variations would be preserved and unfavourable ones destroyed, and the result would be the formation of new species.

DARWIN WALLACE THEORY OF NATURAL SELECETION

Darwin-Wallace explanation of the way in which evolution occurs may be generalized as follows —*"The change in species by the survival of an organismal type exhibiting a natural variation that gives it an adaptive advantage in an environment, thus leading to a new environmental equilibrium, is evolution by natural selection"*. Thus, natural selection is a continuous process of trial and error on a gigantic scale, for all of living matter is involved. It includes following elements :

1. The universal occurrence of variation—Variation is the characteristic of every group of animals and plants and there are many ways in which organisms may differ. (Darwin and Wallace did not understand the cause of variation, and assumed it was one of the innate properties of living things. We now know that inherited variations are caused by mutations).

2. An excessive natural rate of multiplication—Every species, in the absence of environmental checks, tends to increase in a geometrical manner. If a population of a given species doubles in one year and if there are no checks on its increase, it will quadruple the next year, and so on. Such a great reproductive potential of different species may be easily observed in nature. It has been estimated that a common Atlantic coast oyster may shed as many as 80 million eggs in one season. A salmon produces 28,000,000 eggs in a season, A single pair of English sparrows would be the ancestors of over 275 billion individuals in 10 years if they and their descendants could reproduce at their natural rate without any check. Darwin calculated that even a pair of elephants which are about the slowest breeding animals known, could, in the absence of any checks, have 29 million descendants at the end of 800 years.

Thus, more organisms of each kind are born than can possibly obtain food and survive. Since the number of each species remains fairly constant under natural conditions, it must be assumed that most of the offspring in each generation perish. If all the offspring of any species remained alive and reproduced they would soon crowd all other species from the earth.

3. Struggle for existence—Since more individuals are born than can survive there is a *intraspecific or interspecific* or environmental struggle for survival, a competition for food, mates, and

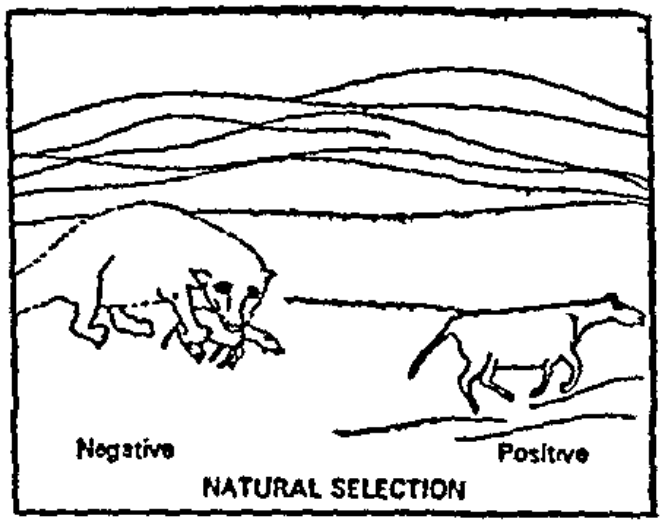


Fig. 52 Diagram showing negative and positive natural selection.

space. This contest may be an active kill-or-be-killed struggle, or one less immediately apparent but no less real, such as the struggle of plants or animals to survive drought or cold.

4. The consequent elimination of the unfit and the survival of only those that are satisfactorily adapted—Some of the variations exhibited by living things make it easier for them to survive; others are handicaps which bring about the elimination of their possessors, This idea of "the survival of the fittest" is the core of the theory of natural selection.

5. The *inheritance of the mutations or recombinations that* make for success in the struggle for existence—The surviving individuals will give rise to the next generation and, in this way, the "suceessful" variations are transmitted to the succeeding generations. The less fit will tend to be eliminated before they have reproduced.

Successive generations in this way tend to become better adapted to their environment; as the environment changes, further adaptations occur. The operation of natural selection over many generations may produce descendants which are quite different from their ancestors, different enough to be separate species. Furthermore, certain members of a population with one group of variations may become adapted to the environment in one way, while others, with a different set of variations, become adapted in a different way, or become adapted to a different environment. In this way two or more species may arise from a single ancestral stock.

CRITICAL ANALYSIS OF DARWINISM

With the appearance of Darwin's book in 1859 a veritable storm of controversy broke out. Many people became interested in his presentation; the arguments for and against that developed were often heated. The concept of natural selection was opposed primarily on ethical and religious grounds. But the sheer weight of evidence and the logic of Darwin's presentation finally convinced a majority of the educated people. The concept of evolution affected many aspects of man's thinking and it will continue to do so. However, the idea of "the survival of the fittest" of Darwinism has been erroneously interpreted by many to mean survival only by "tooth and claw", an interpretation that has led in many cases to rationalizations for the attitude of "every man for himself" in social and economic affairs. In fact, the process of evolution is not all "tooth and claw". Plants have evolved, too, and there is no bloody competition between them. In all forms, plants and animals alike, a part of the selective process involves the action of inanimate nature—factors such as drought, storm, moisture, temperature, etc. Active

competition between organisms is a part of the process, but as Walder Allee and others have pointed out, *cooperation* has in many cases been involved in survival. Natural selection results in the survival of those forms that are best integrated with the various factors of the environment in which they live.

The greatest weakness of Darwinism is that it did not explain the origin and transmission of variations. Although Darwin criticized many aspects of Lamarck's theory, he did not deny that acquired characteristics can be transmitted. He realized that the nature of inheritance was unknown, but he devised a working hypothesis, called hypothesis of pangenesis in 1868 to explain how acquired variations may be transmitted.

PANGENESIS HYPOTHESIS

Darwin's pangenesis hypothesis assumed that all the organs, and perhaps all the cells, in the body of an animal produced miniatures of themselves. These miniatures, called gemmules or pangenes, were shed into the blood stream and carried to the sex glands (*viz.*, reproductive organs the testes and ovaries), where they were assembled to form sex cells—the eggs or sperms Later, when a fertilized egg undergoes development, according to the concept, the pangenes present are responsible for the particular features of the new individuals. In this manner, environmental changes would produce modified organs, which in turn would produce modified pangenes that would transmit the change to the next generation.

In year 1875, Galton made pangenesis hypothesis untenable by presenting several experimental proofs. He and others at later time made a series of experiments involving blood transfusions and later, transplants of ovaries between black and white varieties of rabbits and chickens. Gametes produced by the transplanted ovaries were consistent with the phenotype of the individual in which the ovary originated and not with the animal currently carrying the ovary. Blood transfusions had no effect on the gametes produced. These experiments readily demonstrated that the pangenesis hypothesis was incorrect.

Certain other demerits of Darwinism—Besides its numerous merits, Darwinism has following demerits :

1. Darwinism or natural selection theory does not account for the beginning of organs, which may appear at first as the veriest rudiments having as yet no selection value. In other words, it remains concerned with the survival of fittest, but not for the arrival of the fittest. Thus, to give rise to such specializations as elaborate mimicry, or the electric organ of the torpedo, etc., which are of

apperent advantage only in the perfected state, natural selection, acting only upon minute gradations toward perfection, seems inadequate. The same is true of so complex a specialization as the eye and its function in the vertebrates or in the insects and crustaceans.

2. Over specialization in certain cases like extinct Irish deer in which huge antlers outweigh the entire skeleton, or the immense spiral tusks of the Jefferson mammoth, or the minute fidelity of certain mimicking insects such as *Kallima*, or huge dinosaurs of Mesozoic—all cannot be explained on the basis of continuous variations and natural selection. These organs or body structures should not have reached such a harmful stage, if natural selection was operating. However, such cases of over specializations have been explained by Darwin on the basis of discontinuous variations or "sports" which according to him do not play any role in evolution.

3. Natural selection cannot account for degeneracy. To say an organ is no longer useful and hence disappears, is to state the effect and not the cause. If under changed conditions a character built up by natural selection becomes a menace, the reversal of selection can accomplish its removal but this will not suffice where the characteristic is an indifferent ones.

4. One of classical objection to natural selection is that new variations would be lost by "dilution" as the individuals possessing them bred with others without them. We now know that although the phenotypic expression of a gene may be altered when it exists in combination with certain other genes, but the gene itself is not altered and is transmitted to succeeding generations.

5. Darwin indirectly accepted the lamarckian idea of inheritance of acquired characters in the form of **pangenesis hypothesis**, which cannot be accepted in the light of knowledge of genetics made available in the present century.

6. Lastly some persons have objected natural selection because it is essentially a materialistic doctrine, depending as it does purely on the laws of chance.

NEO-DARWINISM

Neo-Darwinism is a modified form of Darwinism. The Neo-Darwinians like **T.H. Huxley** and **Herbert spencer** of England, **D.S.Jordan** and **Asa Gray** of United States, and **E. Haeckel** and **A.Weismann** of Germany believed that natural selection has accounted every thing that is involved in evolution. In their dogmetic belief they went further to secure enough proof towards natural selection.

Certain Neo-Darwinians, such as Weismann and his followers rejected Darwin's theory except its principal element of natural selec-

tion. These Neo-Darwinians, though distinguished between germplasm and somatoplasm of living organisms in their germ-plasm theory, but they could not appreciate the role of mutations in evolution, while Darwin believed that the adaptations result mainly by a single source, *i.e.*, natural selection, Neo-Darwinians thought that adaptations result from multiple forces and natural selections is only one of these many forces. Neo-Darwinians also believed that characters are not inherited as such but there are character determiners, the **determinants** or **biophores**, which control only the development. The ultimate character would result out due to the interaction of the determiners, activity of the organism and the environment during development. This, Neo-Darwinism was incomplete and partly wrong because it lacked present understanding of genetics.

MATURATION OF NEO-DARWINISM INTO MODERN SYNTHESIS

During later half of 19th century and at the beginning of 20th century, many important ideas have been forwarded for explaining the inheritance of variations from one generation to successive generations and also for explaining evolution. Thus, the rediscovery of **Mendel's** laws of heredity by **Correns, de Vries,** and **Tschermak** in 1900 made it clear that (1) the factors given to the offspring by the parents do not "mix" but are segregated, and (2) if more than one pair of contrasting characters are considered in the same cross, the factors responsible for these are inherited independently. Likewise, **mutation theory of evolution** of **Hugo de Vries** (1886, 1887) stated that new species arise by sudden changes or steps called **mutations** rather than by gradual processes. According to **de Vries,** it was mutations and not selection that should be considered as the primary factor in evolution. Further, the studies of **Wagner** (1868) suggested the role of **geographic** or **spatial isolation** in the formation of every species, race, or tribe of animals or plants on the earth. Even more, certain **population geneticists** realized that the actual physical struggle between animals for survival or the competition between plants for space, sun and water is much less important as an evolutionary force than Darwin believed. The evolution of any given kind of organism occurs over many generations during which individuals are born and die, but the population has a certain continuity. Thus, the unit in evolution is recognised not the individual but rather a **population** of individuals.

A population of similar inviduals living within a circumscribed area and interbreeding is termed a **deme** or a **genetic population.** The next larger unit of population in nature is the **species,** composed of a series of inter-grading demes. Further, in a population, the relative

frequencies of the genes will remain constant from one generation the next (1) if the population is large, (2) if there is no selection for or against any specific gene or allele, *i.e.*, if mating occurs at random, (3) if no mutations occur, and (4) if there is no migration of individuals into or out of the population. The operation of the Hardy-Weinberg Principle will result in maintaining a given gene frequency in a population. Thus, a population undergoing evolution is one in which the gene pool is changing from generation to generation. The gene pool is the sum total of all the allelic genes in a population, and the gene pool of a given population may be changed (1) by mutation, (2) by hybridization, that is, by introduction into the population of genes from some outside population, or (3) by natural selection. Recombination brought about by crossing over and by the assortment of chromosomes in meiosis may also lead to new combinations of genes and phenotypes with some specific advantage or disadvantage for survival that would be reflected in a change in the gene pool.

All these modern understandings in cytology, genetics, cytogenetics, population genetics, and evolution gave a way for the formulation of a coherent theory called modern synthesis around 1930's, by **S. Wright, H.J. Muller, Th. Dobzhansky, R.B. Goldschmidt, J.S. Huxley, R.A. Fisher, J.B.S. Haldane, Ernst Mayr and G L. Stebbins.**

3. MODERN SYNTHETIC THEORY

According to **G.L. Stebbins** (1971), the modern synthetic theory of evolution recognizes **five basic types of processes—Gene mutation, Changes in chromosome number and structure, Genetic recombination, Natural selection and Reproductive isolation.** The first three provide the genetic variability without which change cannot take place ; natural selection and reproductive isolation guide populations of organisms into adaptive channels. In addition three accessory processes affect the working of these five basic process. **Migration** of individuals from one population to another, as well as **hybridization** between races or closely related species both increase the amount of genetic variability availabe to a population. The effects of chance acting on small populations, may alter the way in which natural selection guides the course of evolution.

However, according to **Ernst Mayr** (1970) recent modern synthesis theory includes **adaptive response, mutational limitations, epigenetic limitations, mutations, recombinations and natural selection.**

CHAPTER 6

ISOLATION OR ISOLATING MECHANISMS

The pre-requisite for speciation is **reproductive isolation.** Mechanisms that prevent successful reproduction between members of two or more populations (*viz.*, closely related species) that have descended from the same original population are called **isolating mechanisms.** The significance of isolating mechanisms (*i.e.*, reproductive isolation) in speciation first of all recognised by the post-Darwinian evolutionist, **Dobzhansky** in 1937. Since that time a great amount of literature has accumulated about isolating mechanisms.

TYPES OF ISOLATING MECHANISMS

Most modern evolutionists such as **Mecham** (1961), **Mayr** (1948, 1970), **Stebbins** (1966, 1971), etc., have classified the reproductive isolating mechanisms into two classes namely **premating or prezygotic isolating mechanisms** and **postmating or postzygotic isolating mechanisms.** Both types of isolating mechanisms differ fundamentally with each other in following manner : premating or prezygotic isolating mechanisms prevent wastage of gametes (germ cells) and so are highly susceptible to improvement by natural selection; postmating or postzygotic isolating mechanisms do not prevent wastage of gametes and their improvement by natural selection is indirect (Mayr, 1970). Both types of isolating mechanisms includes many subtypes, all of which have been listed in the following table :

Table : Types of isolating machanisms that prevent gene exchange between population of related species of organisms (Stebbin's 1976, Mayr 1971).

A. Premating or prezygotic isolating mechanisms—Mechanisms that prevent interspecific crosses (*i.e.*, fertilization and zygote formation).	
1. Habitat Isolation :	The populations live in the same regions, but occupy different habitats, so that potential mates do not meet.
2. Seasonal or temporal isolation :	The populations exist in the same regions, but are sexually mature at different times, so that, potential mates remain unable to mate.

3. Ethological isolation : (in animals only)	The populations are isolated by different and incompatible behaviour before mating, so that, potential mates meet but do not mate.
4. Mechanical isolation :	Cross fertilization or pollination is prevented or restricted by differences in structure of reproductive organs (genitalia in animals, flowers in plants), so that, copulation is attempted but no transfer of sperm takes place.

B Postmating or postzygotic isolating mechanisms—Fertilization takes place and hybrid zygotes are formed, but these are inviable, or give rise to weak or sterile hybrids.

1. Gametic mortality :	Sperm transfer takes place but egg is not fertilized
2. Zygotic mortality :	Egg is fertilized but zygote dies.
3. Hybrid inviability or weakness :	Zygote produces an F_1 hybrid of reduced viability.
4. Developmental hybrid sterility :	Hybrids are sterile because gonads develop abnormally, or meiosis breaks down before it is completed.
5. Segregational hybrid sterility :	Hybrids are sterile because of abnormal segregation to the gametes of whole chromosomes, chromosome segments, or combination of genes.
6. F_2 breakdown :	F_1 hybrids are normal, vigorous, and fertile, but F_2 contains many weak or sterile individuals.

It is further recognized that for these reproductive isolating mechanisms to evolve, the separated populations of an original single group must be separated spatially or geographically or by time. Therefore, before discussing the different subtypes of reproductive isolation, a discussion of following three types of isolations should be made :

1. ISOLATION BY TIME

The palaentological history of animals and plants suggest that the population at one time is always the descendant of the one living earlier. In case of straight evolution one species simply gets transferred gradually into a new species through accumulating genetic differences. If the changes are great enough than the biologists called the population at two different times as two different species. This may well be illustrated with the sequential fossil records of the evolution of horse family. According to G. G. Simpson horse evolved some 16 million years ago in Eocene. It underwent successive changes produced by thousands of favourable mutations of every gene involved in the evolutionary trend of each characteristic.

2. ISOLATION BY DISTANCE (SPATIAL ISOLATION)

Sheer distance apart may also act as an isolating factor for a species which occupies a great range of area, which is unbroken by effective barriers. Its example is wrens (birds) of South America. Wrens are found all over the continent but the wrens of one region differ from those of the other in colour patterns, size, proportions and habits. It shows that without any barrier, sheer distance apart tends to produce local races (subspecies).

3. GEOGRAPHICAL ISOLATION

It is most common type of isolation and occurs when an original population is divided into two or more groups by geographical barriers such as a river, desert, glacier, mountain or ocean, all of which prevent interbreeding between them, then in the course of time different mutations may become incorporated in the gene pools of the different groups. Often these differences are of such a nature that the separated groups, when they come in contact again, do not interbred, thus species have been formed by geographical isolation. Certain additional kinds of geographical barriers are the volcanic formation of a mountain on land, mountain ranges with deep valleys between and land masses as islands in sea.

The classic example of continuing evolution by geographical isolation is that described by Darwin for finches. Darwin found that there were 26 groups of finches among the Galapagos Islands (which lie a few hundred miles west of South America). Only five of these groups were the same as the finches found on the mainland. The other twenty-one were types peculiar to the groups of islands. Some of the twenty-one groups interbred quite freely, while others did not. Apparently, each of the groups became formed by migration. Each group evolved separately from the continental forms as well as from other isolated groups on the islands, forming a series of species and subspecies.

geographic or ecologic ranges separated from one another by spatial barriers they are called **allopatric populations**. The two kinds of elephant seals are **allopatric species** genetically isolated by an eco-geographic barrier.

Other examples of geographical isolation can be observed anywhere. It is well established, for example, that there are more different species of the same genus in mountainous country than in plains regions. For instance in the eastern part of U.S.A., there are eight species of cotton-tail rabbits, whereas in the mountainous regions of the west of U.S.A., there are 23 species of the rabbits. Often, in mountainous country many of the plants and animals found in deep valleys, which are separated by high peaks but which may be only a few miles apart, are peculiar to those valleys. Likewise, the isthmus of Panama provides another striking example of geographical isolation. On either side of the isthmus the phyla and classes of marine invertebrates are made up of different but closely related species. For some 16,000,000 years during the tertiary period, there was no connection between North and South America, and marine animals could migrate freely between what is now the Gulf of Mexico and the Pacific ocean. When the isthmus of Panama re-emerged, the closely related groups of animals were isolated and the differences between the fauna in the two regions represent the subsequent accumulation of hereditary differences.

Over the long haul, geographic isolation is seldom permanent. Changes in geography, migrations resulting from great population pressure, or chance dispersal during storms may bring separated groups in contact again. If they then interbreed and have fertile offspring, speciation has not occurred ; but if they do not interbreed, or if they interbreed and have sterile offsprings, new species have been produced, reproductive isolation has occurred.

The significance of geographical isolation in speciation first of all was understood by **Wagner** who in 1889 proposed "separation theory of isolation" which was held necessary for the formation of real variety. Darwin have admitted the important role of isolation in speciation. Many modern evolutionist such as **Jordan, Dobzhansky, Huxley, Stebbins, Mayr,** etc., consider geographical isolation as a pre-requisite for speciation.

A. PREMATING OR PREZYGOTIC ISOLATING MECHANISMS

Premating or prezygotic isolating mechanisms are those that prevent contact between the species when they are reproductively

active, or which prevent or restrict the union of gametes after mating or cross-pollination has occurred. It may be caused by following reasons :

1. HABITAT ISOLATION

Often two closely related species will thrive in different ecological conditions (habitats) within the same territory, but no hybrid between them will be found. However, when the members of the representative species are taken into the laboratory, hybrids between them can be obtained. Thus their respective gene pools are isolated physically, but not physiologically. Again, because of the ecological separation that inhibits the sharing of traits, the incorporation of new adaptations may lead to a functional separation of their gene pools, and speciation.

Habitat or ecological isolation is most common in plants because of their sedentary nature. In them it operates in two ways. The species may live in the same general area, but have such different habitat preferences that their populations are rarely close enough together to cross fertilize each other frequently. In addition, if hybrids are occasionaly formed, they may be either unable to grow maturity or leave few progeny under natural conditions, because no site to which they are adapted is available. American plant species of scarlet oak (*Quercus coccinea*) and black oak (*Q. velutina*) are sympatric throughout most of the Eastern United States and both are distinguishable by shapes of their leaves and acorns. **Sympatry** is the phenomenon of occurrence of two or more populations in the same area ; more precisely, the existence of a population in breeding condition within the cruising range of individuals of another population. Such populations which are related and share a portion of their ecologic ranges but remain isolated from one another not by space but through the physiologic expression of genetic difference, are called **sympatric populations** or **species.**

Habitat isolation is not a very effective isolating mechanism in mobile animals and have been observed most frequently in mouse, river fishes and certain water snakes. For example, two subspecies of the American mouse (*Peromyscus maniculatis*) inhabit regions that share a common boundary. While laboratory hybrids between these subspecies exist, none have been found in nature. The explanation for this isolation is that one subspecies lives in the forests, and the other on sandy beaches. Apparently members of the two groups rarely encounter each other.

The same situation has been found for another subspecies of mouse in Oregon, as well as for the water snake *Natrix sipedon*. One

subspecies of snake is a freshwater race, and the other is a saltwater race.

2. SEASONAL ISOLATION

Two groups may exist in exactly the same ecological area, but do not interbreed because they become sexually mature at different times of the year or under different conditions. Further, seasonal barriers are particularly frequent among aquatic animals, because, water temperatures are more stable than air temperatures and embryonic development is more closely harmonised to definite temperatures. These two factors combined and permit a close regulation of the breeding seasons.

Seasonal isolation is common in plants and occurs frequently among insects and other invertebrates and some vertebrates. For example, over ten subspecies of Cypress trees (*Cupressus*) are found in California. They do not interbreed because each race produces pollen at a different time of the year.

In animals it may be the time or condition for mating which may vary. In the north eastern United States, three species of frog, *Rana pipiens*, *R. sylvatica* and *R. clamitans* all mate in the same ponds at different times. *R. sylvatica* begins breeding when the water is 44°F and completes its mating season before the temperature reaches 55°F, the breeding temperature necessary for *R. pipiens*. *R. clamitans* must have temperatures of at least 60°F. By this time the temperature is reached, both *R sylvatica* and *R. pipiens* are past their breeding stages. Further, most closely related species of anurans (frogs and toads) have overlapping breeding seasons but in them, the isolation is provided by acoustic stimuli.

3. ETHOLOGICAL OR BEHAVIOURAL ISOLATION

Ethological (Gr., *ethos*=habit, custom) refers to behaviour pattern. Ethological isolating mechanisms are barriers to mating due to incompatibilities in behaviour. It is based on the production and reception of stimuli by the sex partners. The males of every species have specific courtship behaviour and females of the same species are receptive. Courtship involves an exchange of stimuli (visual, auditory, tactile, olfactory or chemical) between male and female continuing until both have reached a state of physiological readiness in which successful copulation can occur. The species specific stimuli which help two sexes for the simultaneous recognition of each other for courtship forms the ethological isolation.

For example, birds and insects embark on mating after highly standardized ritual dances. In some species of birds, plumage of the male stimulates the female sexual interest. Thus, the different colour

patterns of insects; different colour patterns of plumages of birds; light signals sent out by male fire flies (beetles of family *Lampyridae*); melodious songs produced by the males of insects, frogs, birds, etc., sex attractants (ectohormones or pheromones) produced by most insects, mammals including human female; are some of the examples of stimuli which have a significant role in ethological isolation.

In most animals it is the male that actively searches for a mate. He is usually rather easily stimulated to display to objects. Sometimes quite inappropriately, when he does not receive adequate response from his display partner, or is actively repulsed, his display derive eventually becomes exhausted. Consequently, if such a displaying male encounters an individual of a different species, or a male of his own species, he will break off his courtship sooner or later. If the male is displaying to a non-receptive female, the same will happen, perhaps after longer intervals.

In *Drosophila*, for example, it is shown that males act quite specifically to remove inhibitions, so that females will copulate with males of her own species. These actions of courtship include orientation of the body, display of wings and licking with tongue. Further, when the antennae, the most sensitive tactile organs of females, are etherised, the males can easily bring about copulation with females of different species.

4. MECHANICAL ISOLATION

Mechanical isolation is provided due to differences in the structure of flower of flowering plants or in the genital organs of different species in animals so that cross-pollination or copulation may not be easily brought about. In case of higher plants mechanical isolation provides the most effective and a major isolating mechanism. It is more effective in those plants where flower structures remain well specialized for insect pollination. For example, different species of *Asclepias* of *Asclepiadaceae* differ in the shape of pollinia (sacs carrying pollen) and that of the stigmatic slits, so that with the help of particular insect bringing about pollination, pollinia of one species cannot bring about pollination on the stigma of another species. Such kind of mechanical isolation is also common in the other members of family *Asclepiadaceae* and *Orchidaceae*.

The occurrence of mechanical isolation among animals was recognized quite early. As, soon after the discovery of the manifold structural differences in the genital armatures of different species of insects, it was asserted by Dufour (1844) that these genital armatures act like lock and key, preventing hybridization between individuals of different species. For example, interspecific crosses in *Drosophila*

may cause injury or even death to the participants, and the same is true in *Glossina* (tsetse fly) and in pulmonate snails of subfamily *Polygyrinae*. However, **Karl Jordan** (1905) contradicted Dufour's hypothesis by showing that out of 698 species of *Sphingidae* family of *Insecta*, 48 were not different in their genitalia from other species of the family, while in about 50% of the species with geographic variation in colour, there was geographic variation in the structure of the genitalic armatures. Since that time much additional information has accumulated indicated the slight importance of the genitalic armatures as isolating mechanisms in animals.

According to **Mayr** (1970) the genitalic apparatus of animals is a highly complicated structure and is the pleiotropic by-product of very many genes of the species. Any change in the genetic constitution of the species may result in an incidental change in the structure of genitalia. As long as this does not interfere with the efficiency of fertilization, it will not be selected against.

B. POSTMATING OR POSTZYGOTIC ISOLATING MECHANISMS

Postzygotic isolating mechanisms are those which prevent the growth of hybrid individuals after fertilization has occurred or which reduce the fertility of the F_1 hybrids or the viability of their descendants. They are caused by following methods :

I. GAMETIC MORTALITY

It has been postulated that sperm can tolerate only narrow ranges of physiological conditions (pH, temperature, etc.) and that these conditions are favourably met in female members of the same species. Deviation from the physiological compatibility would prove lethal to sperm, and, few, if any, would survive to fertilize the egg. Therefore, even though members of these two groups could copulate, the mating process would not lead to viable offspring.

Further, the sperms may encounter an antigenic reaction in the genital tract of the female, so that, they become immobilized and killed before they have a chance to reach the eggs. Patterson reported such "insemination reaction" to occur in many *Drosophila* which leads to an enormous swelling of the walls of the vagina and the the subsequent killing of the spermatozoa. In certain sterile human females sperm mortality due to excessive acidic conditions of vagina has been reported by many medical journals. Sperm mortality also may occur due to physiological inability (*i.e.*, fertilizin-antifertilizin reaction) of sperms to penetrate the egg membrane of the alien species.

2. ZYGOTIC MORTALITY

The development of the fertilized hybrid egg is often irregular and development may cease at any stage between fertilization and adulthood, zygotic mortality can be caused due to several cytological, genetical, embryological, molecular biological, biochemical and physiological reasons in most animals. For example, certain hybrid zygotes of *Ambystoma mexicanum* lack nucleolus and in the absence of nucleolus, such zygotes die without undergoing cleavage.

Certain author have included the isolations due to sperm mortality and zygotic mortality under physiological isolation. Such physiological isolation also can occur as an incompatibility between the embryo and the female parent Interspecific hybrids between two species Jimson weed (*Datura*) result in the death of the embryo at the eight-cell stage. By removing the embryos and culturing them artificially, viable seeds can be produced that germinate and grow into healthy offspring.

3. HYBRID INVIABILITY

Many naturally occurring [illegible] hybrid vigour and fertility, bu
the reproductive failure of fully
they are less well adjusted to available ecological niches than individuals of the parental species. Also, species hybrids are usually less successful than individuals of pure species in courtship, when definite behaviour patterns and species-specific stimuli play an important role. Ecological and ethological inferiority, thus, reduces their chances of leaving offspring.

4. DEVELOPMENTAL HYBRID STERILITY

Sometimes postzygotic isolation involves in the production of vigorous but sterile species hybrids. This type of hybrid sterility involves the abnormal development of gonads, or abnormal meiosis or abnormality in gamete formation. It is usually most common in male animals and plants. For instance in viable F_1 hybrids between different species of *Drosophila* and flies, also between various species of mammals such as cattle × yalk, cattle × buffalo, horse × zebra, and between some species of birds such as mallard and muscory ducks. The abnormality occur due to poor growth and a low rate of mitosis in the cells of seminiferous tubules. Further, developmental abnormality also occur at the time of meiosis of spermatocytes due to which either no sperm is produced or if any sperm is produced that remains undifferentiated, to be unable for fertilization. A. Muntzing (1930) have used the terms, **haplontic sterility**, where the sterility is set in haploid parts (gametes) and **diplontic sterility**, where the sterility is brought about due to events before meiosis or after fertilization in diploid tissues.

5. SEGREGATIONAL HYBRID STERILITY

In both plants and animals hybrid sterility may result from abnormal segregation at meiosis of either whole chromosomes or blocks of genes contained in chromosomal segments. If the chromosomes of the parental species are not homologous (*i.e.*, chromosomes of both parents of a hybrid contain individual gene dissimilarities), they cannot pair at all. Further, segregational hybrid sterility or genetic isolation may also be caused due to genetically controlled spindle abnormalities, asynapsis or desynapsis and similar other abnormalities. For instance, in the hybrid (*Raphanobrassica*) between the radish and cabbage, the nine chromosomes derived from the radish may not pair at all with the nine chromosomes derived from the cabbage, so that at meiosis one observes 18 single chromosomes instead of nine pairs found in the parental species. Since these unpaired chromosomes are unable to line up on the meiotic metaphase spindle, they are distributed irregulary to the poles caused daughter cells with unbalanced complements of chromosomes, the genes of which are unable to direct the development of the pollen grains or embryo sacs.

Further, sometimes chromosomal sterility may result when the chromosomes from two parents do not lack homology but differ due to structural changes like translocation or inversions (chromosomal aberrations). In such a case the pairing of chromosomes will be imperfect and results in the segregation to the gametes of abnormal disharmonious combinations of genes (*i.e.*, sterile gametes). For instance, in hybrids in between *Primula verticillata* and *P. floribunda*, the sterility in hybrid is known due to small structural difference between parental chromosomes. Likewise, the functional offspring, mules of horse and donkey are sterile. The horse and donkey each contribute 33 chromosomes to the offspring or (species hybrid) mule. During meiosis of gametogenic cells of the mule, however, the chromosomes fail to pair. This failure is thought to be the result of the degree of genic dissimliarity between the horse and donkey chromosomes, so that they do not "recognize" one another at homologues. Since the orderly separation of chromosomes into gametes is necessary for fertilization to take place, the mule is sterile.

There is evidence that genetic isolating mechanisms are occassi-

absent, and species recognition is rigid. Young cowbirds, for ... leave the company of their foster parents and flock together as soon as they are independent. In the human species, however, conditioning is an important isolating mechanism. The free interbreeding of individuals coexisting in a geographical region is strongly influenced by religious, ecomomic and cultural barriers.

6. F_2 BREAK DOWN

In both animals and plants, there are examples of hybrids which are highly or at least partly fertile, but which give rise to weak, abnormal, or sterile progeny in the second (F_2) generation. This phenomenon is called **hybrid breakdown**. Classical examples of hybrid breakdown are provided by the F_1 hybrids, *Gossypium arboreum* × *G. herbaceum* and *Gossipium barbadense* × *G. hersutum* In both of these cases, F_1 progeny is either missing or is very weak.

From the developmental point of view, F_2 break down is similar to segregational sterility, except that the effects of the segregation of disharmonious gene combinations are delayed until after fertilization.

THE COACTION OF ISOLATING MECHANISMS

The interbreeding of closely related sympatric species of animals is prevented by a whole series of ecological, behavioural, and cytogenetical factors, usually several for each species pair. One factor is often dominant, such as acoustic stimuli in certain frogs, grasshoppers, cicadas and mosquitoes; chemical contact stimuli in some insects; sex stuffs in Lepidoptera and certain marine organisms (*Bonellia*, etc.), and visual stimuli in certain birds, fishes and insects. The sterility barrier may be strong or weak but is rarely tested except [illegible] Thus, the isolating [illegible] one breaks down, [illegible] habitat barrier is broken, individuals of two species may still be separated from each other by behaviour patterns. If these also fail, the mates may be unable to produce viable hybrids, or if hybrids are produced they may be sterile (see Mayr, 1970).

THE GENETICS OF ISOLATING MECHANISMS

From the multiplicity of isolating mechanisms that protect every species, one can conclude that a considerable number of genes are involved. Almost any gene that changes the adaptation of a population may have an incidental effect on the interaction between male and female. Conversely, a mating advantage of a male will become established in a population provided it does not seriously lower the fitness of his offpsring.

Detailed analysis of the genetics of isolating mechanisms other than sterility exist only for the genus *Drosophila*. Many mutations in *D. melanogaster*, such as yellow, white, and bar influence the mating success of the mutant individuals. Further studies have established now that behavioural isolation has a highly complex genetic basis. In addition to genes controlling the specificity and variation of the signals exchanged between males and females, there are others that control mating derive and levels and rhythms of activity.

ROLE OF ISOLATING MECHANISMS

One of the evident functions of isolating mechanisms is to increase the efficiency of mating. Where other closely related species do not occur, courtship signals can "afford" to be general, non-specific and variable. Where other related species coexist, however non-specificity of signals may lead to wasteful courtship and delays, even where no heterospecific hybridization occurs. Under these circumstances there will be a selective premium on precision and distinctiveness of signals. This is examplified by *Parus* bird of Tenerife island, and *Regulus* song bird of Azores.

Moreover, each species is a delicately integrated genetic system that has been selected through many generations to fit into a definite niche in its environment. Hybridization usually leads to a breakdown of this system and results in the production of disharmonious types. It is the function of isolating mechanisms to prevent such a breakdown and to protect the integrity of the genetic system of species. Any attribute of a species that would favour the production of inferior hybrids is selected against, since it results in wastage of gametes. Such selection maintains the efficiency of the isolating mechanisms and indeed help to perfect them. Isolating mechanisms are among the most important biological properties of species.

ORIGIN OF ISOLATION

Following two theories have been proposed about the origin of isolation :

Wallace has proposed the first theory. According to him, isolation mechanisms are selected by natural selection to root out lowly fitted hybrids between species and allow only those genotypes that have developed better isolation mechanism. There are instances where hybrids survive better under vigorous environmental stresses than the pure races. The serious objection to this theory principally comes from Darwin If the back-crossing and introgression by a certain hybrid population would lead to the steady state deterioration of the pre-existing mechanism. Thus, Wallace's argument of improvement of isolation is failed.

Poulton and Muller put forwarded the second idea regarding origin of isolating mechanism. According to their idea, isolation mechanisms originate as a by product of genetic divergence of isolated populations. Genetical studies reveal that hybrids between two new species must be effectively sterile or inviable. Natural selection then picks out such instances, improves accessory isolating mechanisms, such as failure of sterile species survival. Such a selection ultimately renders hybrid production lesser and lesser in nature.

CHAPTER 7

SPECIATION

The entire course of evolution depends upon the origin of new populations (species) that have greater adaptive efficiency than their ancestors. The study of population divergence or speciation is, therefore, crucial to an understanding of evolution. Before the discussion of speciation process, a brief discussion of the term species is desirable :

SPECIES, RACE AND DEME

A species may by defined as an interbreeding population, which is reproductively isolated from other similar but morphologically distinguishable populations. In other words, a species may be envisioned as an isolated pool of genes flowing through space and time, constantly adapting to changes in its environment as well as to the new environments encountered by its extensions into other geographical regions. At various intervals of time and in different environments of this gene pool may become isolated. Such isolates may (1) become extinct, (2) reunite with the parental species, or (3) differentiate during isolation to form new species which in turn pass through space and time. Certain general characteristics of animal species are following :

(i) Each species is an isolated pool of genes possessing regional (racial, populational) characteristics in gene complexes which are inter-connected by gene flow.

(ii) Each species fills an ecological niche not exactly utilized by another species.

(iii) Each species remains in process of continually adjusting to its environment.

(iv) Each species possess a constellation isolating mechanisms that directly or indirectly prevent exchange of genes with related species.

(v) Each species has the capacity to give rise to new species provided some form of geographical or spatial isolation, gives its isolates an opportunity to develop a unique gene pool without being swamped by gene flow from the parental species.

Lastly, a species is a population of individuals with similar structural and functional characteristics, which have a common ancestry and in nature breed only with each other. It is collection of demes, the smallest population units which are the groups of genetically similar individuals bearing an intimate temporal and spatial

relation to one another. Each deme is isolated to some extent from adjacent demes but there is always the possibility of genetic exchange between them. Even distantly located demes may contribute genetic material to one another over a period of time by the gradual passage of certain genotypes from one deme to another. Thus demes are open genetic systems that are affected by gene flow from adjacent populations, that is they are only partially isolated populations.

An intermediate population unit which exists in between species population and local population (deme) is **subspecies**, or **geographical race** or **race**. A subspecies is an aggregate of local populations of a species inhabiting a geographic subdivision of the range of the species and differing taxonomically from other populations of the species.

The nature of species, subspecies and deme now arms us to solve the following questions : How from a single stock of population arise different species ? or in other words, how speciation occur ?

NATURE OF SPECIATION

Speciation means the formation of species (**Mayr, 1970**). The true meaning of the term "origin of species" used by **Darwin** was understood only rather recently. The pre-Darwinian evolutionists did not understand its exact significance, even Darwin himself seems to have considered "origin of species" the same as "evolution". He confused two essentially different problems, subsuming them under the single heading "origin of species". One of these, is evolutionary change, in which Darwin was primarily interested and for which Darwin used the following terms—"phyletic evolution", "modification in time," or "decent with modification" **Phyletic** evolution means evolution in a line or lineage. Species A living in a certain region, in the course of time undergoes change so that the descendants are sufficiently unlike their distant ancestors to be considered a different species. *Mesohippus* evolving into *Miohippus* may be taken as an example of such phyletic evolution in which species A has became species B. Typically, species A will have disappeared in the process, leaving species B in place. Similarly as times goes on species B may evolve into, and be replaced by species C and so on. This phyletic pattern of evolution along a time axis is abundantly evident in the sequences of fossil forms.

According to **Mayr** (1970) such evolutio ary transformation of a species does not necessarily leads to multiplication of species or speciation. Because an isolated population on an island, might change in the course of time from species A through B and C into species D without ever splitting into several species. Consequently, in the end there will be only one species on the island, just as at the beginning.

The essential aspects of phyletic evolution is the continuous genetic and evolutionary change within the populations composing the species, without the development of reproductive isolation between subspecies or demes of a species, and consequently without its breaking into several species.

Speciation in its restricted modern sense, however, means the splitting of a single species into several, that is **multiplication** of species.

The opposite of splitting of a single species into several daughter species is the **complete fusion of two species.** Since the product of such a process would be an entirely new unit, truely a new biological species, it is legitimate to consider the fusion of two species into one as a form of speciation, which is the reverse of a multiplication of species. Since species are defined as reproductively isolated populations, the fusion of two species is on the whole, a logical contradiction. Yet occasionally in nature a previously existing reproductive isolation breaks down and two previously distinct sympatric species merge. This happens most easily in species in which the isolating mechanisms are primarily ecological. Such fusion method of speciation has been reported in two species of birds, towhees *Pipilo erythrophthalmus* and *P. ocai*, both of which fuse because of breakdown of isolation and form a new species.

In this chapter, a detailed discussion will be made only for speciation by multiplication of species as other methods of speciation namely transformation of species (phyletic speciation) and reduction in number of species (fusion of two species), are of little significance.

POTENTIAL MODES OF SPECIATION

According to Mayr (1970), true speciation or multiplication of species may occur by following agencies :

A. Instantaneous speciation (through individuals)
1. Genetically
(a) By single mutation in asexual "species"
(b) By macrogenesis
2. Cytologically, in partially or wholly sexual species.
(a) By chromosomal mutations or aberrations (translocation etc.)
(b) By polyploidy
B. Gradual speciation (through populations)
1. Geographical speciation.
2. Sympatric speciation.

A. INSTANTANEOUS SPECIATION

The process of instantaneous speciation may be defined as the production of a single individual (or the offspring of a single mating) that is reproductively isolated from the species to which the parental

stock belongs and that is reproductively and ecologically capable of establishing a new species population. It can be achieved by following methods :

1. INSTANTANEOUS SPECIATION THROUGH ORDINARY MUTATION

Mutation is a genetic phenomenon of such relatively high frequency that in a higher animal with more than 10,000 gene *loci* almost every individual will be the carrier of a new mutation. Such mutations merely increase the heterozygosity of a population ; they do not lead to the production of new species. Any mutation drastically affecting reproductive behaviour or ecology will be selected against, if it lowers the viability, or will displace the original allele if it is of higher viability. In neither case will there be any origin of discontinuities. It is evident that oridinary mutation cannot produce new species in sexually reproducing species.

Certain phenomena among rotifers, cladocerans and nematodes suggest the occasional occurrence of **asexual speciation by mutation.** For example, in the rotifer order *Bdelloidea*, there occur no male individual in entire order and so it is assumed that the ancestral species of this order was parthenogenetic. This ancestral species evolved into over 200 species, about 20 genera and 4 families only due to asexual speciation by mutation

Vegetative reproduction by growth and splittiug, thus, might offer a favourable condition for instantaneus speciation. This form of reproduction, however, occurs only in some of the lowest groups of animals, such as sponges, coelenterates (hydroids), turbellarians and bryozoans, groups in which the taxonomy is as yet too uncertain for a study of speciation.

2. INSTANTANEOUS SPECIATION THROUGH MACROGENESIS

The sudden origin of new species, new higher taxa, or quite generally of new types by some sort of **saltation** (a change by leap across a discontinuity) has been termed **macrogenesis.** The macrogenesis theory of speciation has been supported by some modern geneticists such as **Goldschimdt** (1940) and **Schindewolf** (1936, 1950). According to this theory, the production of a new type by a complete genetic reconstruction (macrogenesis) or by a major "systemic mutation" is the crucial event in speciation. Such an event will produce a **"hopeful monster"** (as **Goldschmidt**, 1940 called it) which will become the ancestor of a new evolutionary lineage. The believers of macrogenesis theory also proposed that all new types appear in the fossil record suddenly and abruptly. These types are not connected with the ancestral types by intermediates, they claim, and cannot be derived from them by gradual evolution.

All these views of macrogenesis theory have been bitterly criticized by many modern evolutionists. The basic objection to macrogenesis theory is that it can never be proved by any kind of evidences, because it is obviously impossible to witness the occurrence of a major jump, particularly one that achieves simultaneously reproductive isolation and ecological compatibility. Further, Simpson (1953) and Rensch (1960) have criticized this theory by showing palaeontological evidences which clearly suggest that one 'type' can be derived from a previously existing one. Moreover, modern genetic studies have shown that the production of a monster or freak individual due to mutation does not provide the raw material for speciation as such freak individuals have low viability, do not get any mate for sexual reproduction and subsequently fail to establish reproductive isolation from normal members of the parental population, therefore, macrogenesis is a poor source of speciation (Mayr, 1963, 1970).

3. INSTANTANEOUS SPECIATION THROUGH CHROMOSOMAL ABERRATIONS

Closely related species often differ more conspicuously in their karyotype than in their morphology. Among aspects of the karyotype that differ are chromosome number ; the number of metacentric or acrocentric chromosomes ; the presence and kind of paracentric or pericentric inversions ; or of supernumerary chromosomes, and just about every aspect of chromosomal aberrations. Visualizing this fact most cytogeneticists believed that chromosomal mutations have a significant role in the causing of instantaneous speciation. This belief is based on two assumptions—(1) that the degree of difference displayed by two species requires a speciation process of such drastic dimensions that only chromosomal mutation can qualify, and (2) that reproductive isolation between two species cannot be achieved without chromosomal reorganization. Now, it is known that chromosomal rearrangements in most cases does not produce new species, however, only in few cases it produce speciation.

(i) Chromosomal rearrangement without speciation—Except deleterious chromosomal mutations, most kinds of chromosomal aberrations (mutations) lead to **chromosomal polymorphism** rather than to the development of isolating mechanisms. The paracentric inversions of *Drosophila* are a well-known example of such chromosomal polymorphism. Yet, each species has its own species-specific polymorphism, and only very rarely do even the most closely related species share the same chromosomal polymorphism. This fact underlines the drastic nature of the chromosomal aberration during much of speciation. This phenomenon suggests that there is no necessary correlation between chromosomal mutations and speciation, since either can occur without the other.

(ii) Speciation coinciding with a chromosomal mutation—If the chromosomal mutations during speciation would not have some selective (evolutionary) advantage, they would not have occurred so frequently in nature. Consequently, they have following two advantages in chromosomal speciation—(a) chromosomal mutations have the potential to serve as (or contribute to) isolating mechanisms, and (b) the locking up and protection of a particularly favourable gene complement through a chromosomal mutation may create a new supergene as Wallace (1959) first of all recognized it. Both of these components of chromosomal speciation can subsequently be improved by natural selection, either during a period of segregation in a geographic isolate or during subsequent **parapatric speciation** or by both process. The term **parapatric** stands for the population or species which remain geographically in contact but not overlapping and rarely or never interbreeding. (Mayr, 1970).

(a) Chromosomal mutations as potential new isolating mechanisms : Any change in the structure of the chromosomes is called chromosomal mutations or chromosomal aberrations, whether it is an **inversion, translocation, duplication,** or any other change in the linear sequence of the genes or in the mechanics of the chromosome (for instance, spindle attachment). Chromosomal mutations (mostly inversions) are estimated to occur at a rate of 1 in 1000. Most of these are sufficiently deleterious to be eliminated at once, that is, before the mutation's carrier can reach reproductive age. Other kinds of chromosomal mutations are capable of giving rise to a system of balanced polymorphism and they will be retained in the population. In addition, there is a third class of chromosomal mutations which appears to reduce the **fecundity** (a term used for reproductive potential as measured by the quantity of gametes, particulary eggs, produced) of the heterozygotes to some extent. The heterozygotes containing some kind of chromosomal mutation that seems important in speciation, usually encounters following difficulties during meiosis (gametogenesis) : (i) meiotica synapsis (partial failure of chromosome pairing), or (ii) malorientation of multivalents at the first meiotic metaphase, or both. Both of these difficulties lead to the production of gametes carrying chromosomal deletion or duplication or broken or acentric chromosomes and thus lead to a significant reduction in the fecundity of male hybrids.

Unlike **gene or point mutations**, chromosomal mutations usually have no effect on the visible phenotype of the individual, so, they are difficult to detect. Further, unlike the heterozygotes containing point mutations, the heterozygotes of chromosomal mutations are not shielded by dominance and such heterozygotes with their meiotic difficulties are the prime target of selection (John and Lewis, 1969). Finally, chromosomal mutation is not a change in the

DNA programme, but a change in the linear sequence of genes (duplication or a deletion). The most frequent chromosomal change the fusion of two acrocentric chromosomes into a single metacentric chromosome. The result is the reduction in chromosome number. A trend toward such a reduction is wide spread among animal taxa. For instance, the most primitive isopods have 28 haploid chromosomes and this number is independently reduced through fusion to the lower number of 8 in several unconnected lines. It is now clear that most differences in chromosome numbers between closely related species of animals is the result of such fusions.

If only fusions have occurred during evolution, soon all species would have only a single pair of chromosomes. Obviously, there should be some r[illegible] the chromosome number [illegible]uld be the exact reverse of [illegible] of a metacentric chromosome into two acrocentrics. Most cytologists doubted such fission to occur. However, increase in the chromosome number (not due to polyploidy) whatever the mechanism, are frequent in animal evolution and may well play an important role in speciation (see Mayr, 1970).

(b) Chromosomal mutation and the production of new supergenes—It becomes now clear that gene contents rather than mechanical qualities, as discussed in preceding heading, determine in many cases whether or not a new gene arrangement can establish it [illegible] of chromosomes [illegible] A new gene arr[illegible] and, by prote[illegible]

lations, are better adapted for the marginal environment of the species border than are genotypes from centre of the species range. To him, the most important aspect of the chromosomal reorganization is the protection from disruptive recombination that it affords certain new supergenes. Chromosomal mutation, thus, is an instrument of ecotypic adaptation.

It is evident that these two aspects of chromosomal mutation—the production of mechanical incompatibilities and development of new supergenes–reinforce each other. There will be an steady selection for an improvement of the adaptation supergene and this will tend to produce an increase in genic heterozygote inferiority. This, turn, will strengthen the effectiveness of the cyto-mechanic isolating mechanisms.

4. INSTANTANEOUS SPECIATION THROUGH POLYPLOIDY

Polyploidy is a multiplication of the normal chromosome number. It is very wide spread among plants and is an important mechanism of speciation in the plant kingdom (Stebbins, 1950, Grant,

1963). Except conifers, one third of all species of plants have arisen by polyploidy. Among animals the polyploidy is much rarer. Among animals it occurs only in those groups which reproduce parthenogenetically. In few parthenogenetically reproducing animal groups such as lumbricid earthworms, turbellarians and in certain groups of weevils polyploidy is the principal method of speciation.

B. GRADUAL SPECIATION

Gradual speciation is the gradual divergence of populations until they have reached the levels of specific distinctness. Two modes of gradual speciation have been postulated—(i) one involving geographical separation of the diverging populations (**geographic speciation**), and, (2) the other involving divergence without geographic separation (**sympatric speciation**).

1. GEOGRAPHIC OR ALLOPATRIC SPECIATION

Geographic or allopatric speciation states that in sexually reproducing animals a new species develops when a population that is geographically isolated from the other populations of its parental species acquires during this periods of isolation characters that promote or guarantee reproductive isolation after the extenal barriers break down (**Mayr, 1942**). The geographic speciation is the almost exclusive mode of speciation among animals, and most likely the prevailing mode even in plants, is now quite generally accepted.

2. SYMPATRIC SPECIATION

The majority of authors until fairly recently considered sympatric speciation, that is, speciation without geographic isolation to be the prevailing mode of speciation. Such a speciation is based on two postulates : (a) the establishment of new populations of a species in different ecological niches within the normal cruising range of individuals of the parental population, and (b) the reproductive isolation of the founders of the new population from individuals of the parental population. Gene flow between daughter and parental population is postulated to be inhibited by intrinsic rather than extrinsic factors. The concept of sympatric speciation is far older than that of geographic speciation and goes back to pre-Darwinian days.

Definition of sympatric speciation—The method of origin of reproductive isolating mechanisms within the dispersal area of the offspring of a single deme is called sympatric speciation. The size of the dispersal area is determined, for instance, in marine organisms by the dispersal of the larval stages. In most insects it is determined in the adult state by the more mobile sex. Since there are normally a great many ecological niches (= the constellation of environmental factors into which a species (or other taxon, fits) within the dispersal area of a deme, niche specialization is impossible without continued new pollution by immigrants in every generation.

Reasons for postulating sympatric speciation : Numerous biological phenomena suggest, on first sight, the occurrence of sympatric speciation. One is the abundance of finely adapted sympatric species in local fauna. For instance, there are several hundred (perhaps 500) species of bees of the genus *Perdita* in North America. All are oligolectic, confining their visits to the flowers of a single species or of a group of closely allied species. Many species of *Perdita* may be found at a single locality but never together because they occur on different plants or in some cases at different times of year. A similar ecological specificity characterizes the species of many other genera of animals. All such species are perfectly adapted to the particular environment in which they occur. Could such a perfect fitting of species into their ecological niches have evolved as a purely accidental by-product of genetics changes accumulated during geographic isolation ? Recent understanding of genetics of geographic speciation have shown that ecological differences can be acquired in geographic isolation and can be increased (by "character displacement") after the secondary establishment of sympatry.

Biological and host races : A necessary corollary of any theory of gradual speciation is that there should exist in nature some 'forms' or varieties' or 'populations' that are incipient species. The supporters of sympatric speciation have always cited the occurrence of biological races as examples of such incipient species. Most of the early supporters of sympatric speciation adhered to a morphological species definition and assumed that the acquisition of ecological or other non morphological species characters preceded the acquisition of morphological species characters. Any discrete population characterized by non-morphological characters was accordingly interpreted as a biological race and incipient species. Thus, kinds of animals that show no (or only slight) structural differences, although clearly separable by biological characters, are called biological races. This definition of biological race, however, includes following heterogenous assemblage of phenomena in it :

Table : Phenomena listed as biological races.

Occurrence	Population	
	Same	Different
Sympatric	Morphs	Polyploids
	Clones Host "races"	Ecological races Host races Sibling species
Allopatric		Geographical races Semispecies Sibling species

Morphs, clones, geographic races with distinctive physiological or ecological properties, semispecies and polyploids obviously do not qualify as incipient species under the concept of sympatric speciation. Most so-called biological races are now known to be sibling species. The only biological races that, at least in principle might qualify as incipient sympatric species are seasonal races and host races.

Seasonal races—It has been postulated by various authors that a species with a very long breeding season might be sympatrically split into two if the genetic continuity between the earliest and last breeders of year could somehow be interrupted. This occurrence of seasonal races is often cited as evidence for such a process of speciation.

Host races—In many species of animals, particularly, nematodes and insects, temporary strains called **host races** may develop on specific host plants or animals. Host races have following characteristics :

(1) Preference for a given species of host plants may and nearly always does have a double basis-conditioning (including larval conditioning) and a genetic predisposition.

(2) Nearly all species with host races concentrate in a given district on one host ; yet they also have the ability to establish themselves on a variety of other host plants, particulary under crowded conditions, and may have different preferred host plants in different districts.

(3) Local populations (demes) of insects often have considerable genetic variability with respect to host specificity. Any mono- [illegible] bees, beetles (*Acmaeodera*, [illegible] *mys*, leaf-Diptera and [illegible] t will come in contact, [illegible] ant species other than its normal host. If the species has the appropriate genetic consitution it will establish itself on the new host and this according to the Ludwig theorem, leads to an expansion of the food niche of the species.

(4) It is also found that if such a mixing of demes or local populations is prevented artificially and an inbred strain is selected experimentally on a single one of several original hosts, such a strain may become progressively less tolerant ecologically until a stage is reached when it may be difficult to re-establish it on any of the other original host species.

(5) Mortality occurs whenever a strain is established on a new host. The more usual the host, the heavier the initial mortality.

Among the many kinds of so called biological races, it is the host races that has the best claim to the designation of "biological race" (*Mayr*, 1970).

Means of sympatric speciation—Thus we can conclude that the sympatric speciation is caused by following natural situations—sibling species ; monophagous species ; parasites ; species swarms ; and the instantaneous splitting of fossil lineages.

HYPOTHESIS OF SYMPATRIC SPECIATION

Certain evolutionists have forwarded following hypothesis for sympatric speciation, but all of these are not supported by evidences :

1. Homogamy—The concept of homogamy was postulated under the pre-Mendelian theory of blending inheritance because without its random mating would soon have led to complete elimination of all genetic variability. According to this concept the most similar individuals of a population tend to mate with each other. Further, it postulated that monogamy would lead to homogamy. Both of these assumptions are supported by facts (Mayr, 1947) except that in a single case of birds where monogamy is a rule but never leads to homogamy. In case of two kinds of geese, namely snow goose and blue goose, monogamy leads to homogamy and both are found to belong to a single species, (*Anser coerulescens*) and are found to have differences in colour pattern due to a single gene, semidominant for blue (Cooke and Cooch 1968). However, a mild form of homogamy occurs in many animals with highly variable adult size but that is quite insufficient for sympatric speciation.

2. Conditioning—Many workers such as Thorpe (1945) and other have suggested that the establishment of a new sympatric species population (*e.g.*, insects) in a new niche might be achieved through conditioning. But Mayr (1947) contradicted it by showing that complete isolation of the two populations was never achieved by any of the conditioning experiment of Thorpe with insects.

3. Preadaptation and niche selection—Most hypotheses of sympatric ecological speciation postulate that dispersing individuals search actively for that particular niche to which they are best adapted on the basis of their particular genotype. There is some evidence for the validity of this assumption, since a given species usually has well-defined species-specific habitat preferences and these are not necessarily identical for all the various genotypes of a species. Most habitat selection is, however, species-specific only in a very generalized way and is also often affected by non genetic influences (conditioning and so forth). Only in an exceptional case will an individual search out that particular subniche for which it is specifically preadapted by its genetic constitution.

The concept of sympatric speciation by preadptation is quite typological in making the assumption that a single gene preadapts an individual for a new niche. Indeed it would require a verit-

systemic mutation to achieve the simultaneous appearance of a genetic preference for a new niche, a special adaptedness for this niche, and a preference for mates with a similar niche preference. The known facts do not support these assumptions.

SYMPATRIC SPECIATION BY DISRUPTIVE SELECTION

The method of natural selection for phenotypic extremes in a population, until a discontinuity is achieved is called **disrupitve selection (Mather, 1955)** or **diversifying selection (Dobzhansky)**. **Muller (1940)** was first to suggest that the accumulation of different sets of specific modifiers might lead to sympatric speciation. **Thoday and Gibson (1962)** recently have studied different aspects of disruptive selection and they have found that when simultaneous selection for high and low bristle number was carried on in a population of *Drosophila melanogaster*, it responded with a strongly bimodel distribution within 12 generations of selection. However, when these selection experiments were repeated by other workers, similar results were not obtained. When the selection was phenotypically successful, one of the resulting morphs was of lowered viability and could be maintained only with difficulty in competition with the more successful morphs. Further, a selection pressure that would permit the survival of only two opposite extremes is unlikely under the variable conditions of natural environment.

A polymorphism established by diversified selection is unlikely to lead speciation. A species would lose all the advantages of improved utilization of the environment acquired through adaptive polymorphism if it were to split into a series of narrowly specialized species. **K. Jordan** suggested that selection of different physiological varieties within a population can have only two outcomes, either polymorphism or extinction of inferior types. Jordan's coclusion is well illustrated by the North American butterfly *Limenitis arthemis*.

According to **Mayr (1970)**, geographic (allopatric) speciation is the only important mean for gradual multiplication of species because sympatric speciation is not supported by strong evidences.

Differences between allopatric (geographic) and sympatric speciation—The theories of sympatric and allopatric speciation agree in their emphasis on the importance of ecological factors in speciation. They differ in the sequence in which the steps of the speciation process follow each other. The theory of allopatric speciation lets an extrinsic event which separate the single gene pool into several gene pools, with the ecological factors playing their major role after the populations have become geographically separated. According to the theory of sympatric speciation, the splitting of the gene pool is itself caused by ecological factors, and any spatial isolation of the populations formed thereby is a secondary, later phenomenon.

ECOLOGY
(Environmental Biology)

CHAPTER 1

INTRODUCTION

No micro-organism, plant or animal species including man is an isloated organism living in a void. Each of them is surrounded by a host of physical conditions that can be measured in terms of chemical composition, texture, pressure, temperature, and humidity, as well as being surrounded by a host of other living organisms which can be described in such terms as plants, animals, food, parasites, and enemies. Studies of the inter-relationships of organisms with their physical and biotic environments are termed **environmental biology** or **ecology**. These words are very much in the public consciousness today as we become aware of some of past and current ecologic malpractices. It is important for everyone to know and appreciate the principles of this aspect of biology so that he can form an intelligent opinion regarding topics such as insecticides, detergents, mercury pollution, sewage disposal, power dams, urbanization and their effects on mankind, on human civilzation, and on the world we live in.

The term ecology *(oekologie)* is derived from two Greek words-*oikos*-means 'house' or 'place to live' and—*logos* means 'a discussion or study'. Literally, ecology is the study of organisms 'at home', in their native environment. The term first of all introduced by **Reiter** in 1868, but because the German biologist **Ernst Haeckel** first of all fully defined this term and he made an extensive use of this term in his writings, therefore, usually he is falsly credited for the coinage of the term ecology (see **C. B. Knight, 1965**).

DEFINITION OF ECOLOGY

The traditional definition of ecology is *'the study of an organism and its environment'*, however, different ecologists have defined it variously. **Ernst Haeckel** (1869) defined ecology as, *the total relation of the animal to both its organic and its inorganic environment.'* **Charles Elton** (1927) in his pioneering book *Animal Ecology* defined ecology as *'scientific natural history'*. Although this definition does point out the origin of many of our ecological problems, yet it is much broad and vague like Haeckelian definition of ecology. In 1936, **Taylor** defined ecology as *"the science of all the relations*

of all organisms to all their environments.' Allee *et. al.* (1949), in their definition of ecology, clearly emphasizes the all-encompassing character of this field of study. According to them—"*Ecology may be defined broadly as the science of the interrelation between living organisms and their environment, including both the physical and biotic environments, and emphasizing interspecies as well as intra-species relations.*" Though, F. J. Vernberg and W. B. Vernberg (1970) are completely agreed with Allee *et. al's* definition, yet there are certain ecologists which are not satisfied with this definition and have provided their own definitions of ecology. For instance, Andrewartha (1961) defined ecology as '*the scientific study of the distribution and abundance of organisms*'. G. A. Petrides (1968) has defined ecology as '*the study of environmental interactions which control the welfare of living things, regulating their distribution, abundence production and evolution.*" Eugene Odum (1963, 1970) has defined ecology as the "*structure and function of nature.*" C. J. Krebs (1972) has shown his satisfaction over the definition of Odum for its emphasizing the form and function idea that permeates biology, but, he considered it vague. He, however, proposed the modified version of Andrewartha's definition of ecology, according to which "*ecology is the scientific study of the interactions that determine the distribution and abundance of organisms*". According to M. E. Clark (1973) "*Ecology, the science of the environment, is a study of ecosystems, or the totality of the reciprocal interactions between living organisms and their physical surroundings*". Two Indian authors Ananthakrishnan and Viswanathan (1976) have defined ecology as '*the study of the ways by which individual organisms, populations of species and communities of populations respond to diverse environmental pressures, i.e., physical and biotic pressures.*' This definition merely is the modified version of the definition of ecology which has been originally proposed by T. Lewis and L. R. Taylor (1967) in their book, *Introduction to Experimental Ecology*. According to Lewis and Taylor '*Ecology is the study of the way in which individual organisms, populations of same species and communities of populations respond to these changes.*' Lastly to avoid this ecological jargon we can rely upon the simple definition of ecology which has been provided by *Chamber's Twentieth Century Dictionary* (1972). According to it, *ecology is the study of plants or of animals, or of peoples and institutions, in relation to environment.*

HISTORICAL BACKGROUND OF ECOLOGY

The roots of ecology lie in natural history, which is as old as man himself. The vague beginning of ecology may be traced back

to prehistoric man, who utilized environmental information to hunt food. fishing, trap animals, find edible vegetation, and locate shelter to survive the hardships imposed by nature. An increased knowledge of the importance of environmental conditions led quite naturally to religious rituals, worship of weather gods, and rain dances, all of which are quite prevalent in different social groups and tribes of Indian civilization. The establishment of agriculture increased the need to learn about the practical ecology of plants and domestic animals.

1. ECOLOGY IN INDIAN CLASSICS

Early Indian philosophers and thinkers had some knowledge of ecology as has been revealed by the Indian classical writings, in the Vedic, Upanishadic, Pauranic, and Epic literature. **Charaka** considered the important factors of *Vayu* (air and gases), *Jala* (water), *Desha* (topography) and *time* in regulating the life of plants (see **R. Misra, 1962**). The great classical Indian Poet **Kalidasa** has displayed his ecological faculty in his classics—'*Meghdoot*' and '*Abhigyan Sakuntlam*'.

2. EARLY GREEK'S IDEAS OF ECOLOGY

The early Greek philosophers too were well aware of the importance of environmental studies. One of the paper entitled on *Airs, waters and places* of **Hippocrates** stressed on the need of ecological background for medical students. According to him—"whoever wished to investigate medicine properly, should proceed thus : in the first place to consider the seasons of the year, and what effects each of them produces....." In the fourth century B. C. **Aristotle** made references about the habits of animals and environmental conditions prevailing in certain areas, in his writings on natural history. One of the Aristotle's student, **Theoprastus** (370–250 B. C.) who is regarded as the first true ecologist, wrote about the association of plant communities and the relation of plants to each other and the non-living environment.

3. ECOLOGY IN 18TH CENTURY

After a considerable long gap of many centuries, the first naturalist who made a serious attempt to systematise knowledge concerning relations of animals to environment was **Buffon** (1707–1788). Though such works as *Historia animalium* by **Konard Gesner** (1516–1565); 14 volumes of collected works of natural history by **Ulisse Aldrovandi** (1522–1605); the natural histories of fishes and birds by **Pierre Belon** (1517–1564). *The plants* by **Andrea Caesalpino** (1519–1603), the botanical work for **Gaspard Bauhin** (1550–1624); demographical studies about human ecology by **Graunt** (1664): studies

of the sexuality of plants by **Rudolph Camerarius** (1665–1721), the works of English naturalist **John Ray** (1627–1705), studies on natural history of insects by French naturalist **Rene Reaumur** (1683–1757) and the works of many other naturalist doubtlessly laid the foundation upon which **Buffon** built his remarkable work. **Buffon** considered that animals and plants developed adaptations which enabled them to favourably respond to their environment and termed this **environmental induction**. He touched on many of our modern ecological problems and recognised that populations of man, other animals and plants are subjected to the same processes. Buffon also explained how the great fertility of every species was counterbalanced by innumerable agents of destruction. He thus dealt with problems of population regulation.

Malthus published one of the earliest controversial books on demography. In his *Essay on Population* (1798) he calculated that although the numbers of organisms can increase geometrically (1, 2, 4, 8, 16, 32.....), their food supply may never increase faster than arithmetically (1, 2, 3, 4, 5.....). Such a great disproportion between these two powers of increase led **Malthus** to infer that reproduction must eventually be checked by food production. Many workers questioned the ideas of **Malthus**. For example, in 1841 **Doubleday** brought out his true law of population. To contradict the ***Malthus*** idea, he believed that whenever a species was threatened, nature made a corresponding effort to preserve it by increasing the fertility of its members. Human populations that were undernourished had the highest fertility; those that were well fed had the lowest fertility. **Doubleday** explained these effects by the oversupply of mineral nutrients in well-fed populations.

4. ECOLOGY IN 19TH CENTURY

In 1807 **Humbolt** after extensive travels of tropical and temperate South America, discussed the geographical distribution of plants and animals in relation to climate. Saint-Hilaire (1859) used the term "ethology" for the study of relations of organisms to environment and outlined a volume, which he never wrote, on instincts, habits, and other ecological matters. The term ethology, though was not accepted by early ecologists, but this term is now used as an important ecological discipline dealing with animal behaviour. In 1869 **Haeckel** proposed "oecologie" for the relations of organisms to organic and inorganic environments. The modern term *ecology* is derived from the word oecologie. The observations of **Priestley** and **Scheele** that plants produce oxygen led to an understanding of the inter-relationships of plants and animals. The discovery that

green plants and animals use carbon dioxide and water to form organic matter for use by animals and resulting in the release of oxygen and water as wastes, provided new understanding of ecological food chains. **Justus Von Liebig** (1803–1873) initiated the idea of carbon and nitrogen cycles in nature. **Louis Agassiz** (1807–1873) initiated the research in marine ecology by establishing many laboratories for marine ecological research. **C. Mobius** (1877) introduced the word "**biocoenosis**" to designate a group of organisms as an ecological unit. **Charles Darwin** (1809—1882) and **Alfred R. Wallace** (1823—1915) have increased our knowledge of island or insular life with their characteristically different types of environmental conditions. **Hensen** (1887) made excellent quantitative studies of plankton. **S. A. Forbes** (1887), in a classical paper on "*The Lake as a Microcosm*" suggested that the species assemblage in a lake was an organic complex and that by affecting one species we exerted some influence on the whole assemblage. Thus each species maintains a 'community of interest' with the other species, and we cannot limit our studies to a single species. **Forbes** believed that there was a steady balance of nature, which held each species within limits year after year, even though each species was always trying to increase its numbers.

Schroter (1896) introduced the terms "**autecology**" and "**synecology**". **Herdman** (1896) indicated the economic possibilities of scientific agriculture. Danish botanist **Warming** (1895, 1909) raised questions about the structure of plant communities and the associations of species in these communities. In 1899 **H. C. Cowles** described *plant succession* on the sand dumes at the Southern end of Lake Michigan.

5. ECOLOGY IN THE 20TH CENTURY

At the beginning of the twentieth century ecology was a young, but an established science and such eminent ecologists as **Wasmann** (1901), **Dahl** (1901) and **Wheeler** (1902) were discussing whether Saint Hilaire's ethology or Haeckel's ecology should be used to designate the science of relations of organisms to environments. The latter term has gradually come into general usage. **Case** (1905) discussed environmental conditions of past geological periods and has continued to make contributions in that field. Two botanists, **Clements**, in his "*Research Methods in Ecology*" (1905) and subsequent writings, and **Warming** in his "*Oecology of plants*". (1909, 1925) have done much to clarify ideas and contribute terms. **Adams** (1913) gave an excellent review of significant ecological literature, urged the usefulness of ecological surveys, and discussed methods to be used in making them. **Shelford** (1912) demonstrated excellent

examples of ecological succession and has made notable contributions concerning animal communities (1913) and environmental factors (1929). One of the interesting aspects of development of ecology during the first third of this century remains that a tendency toward increased specialization in ecology has occurred as is evidenced by the establishment of such disciplines as **palaeoecology**, a study of environmental conditions and life as it existed in past ages. Pollen analysis, radioactive dating, and palaeontology have aided the palaeoecologists. Other specialized fields of ecology which took their birth during this time, are **zoogeography**, the scientific study of the geographic distribution of animals ; **oceanography**, the study of the biotic and physical conditions existing on oceans, bays, and estuaries, and **limnology**, the study of the living and non-living components of inland waters. With the establishment of specialized disciplines of ecology, the value of application of ecology to such fields as agriculture, forestry, wildlife conservation, fisheries management, and pest control became apparent, and thus a new descipline called **applied ecology** came into its existence.

In the second decade of 20th century great emphasis was laid on statistical studies on populations, sampling techniques, and community studies. During this period several important texts published—**Lotka's** *Elements of Physical Biology* ; **Elton's** *Animal Ecology* ; **Volterra's** *Animal Ecology* ; **Gause's** *The Struggle for Existence*, and **Bodenheimer's** *Problems of Animal Ecology*. Certain distinguished ecologists of earlier half and present decade of this century are **Clements, Cowles, Shelford, Pearse, Oosting, A. M. Woodbury, Hutchinson, Deevey, Lindeman, F. E. Smith, Slobodkin, Hairston, T. Park, O. Park, Emerson, MacArthur, G. L. Clark, C. B. Knight, Odum, R. L. Smith, F. J. Vernberg, C. J. Krebs, R. Misra, M. S. Mani**, etc.

Development of ecology during this century have been greatly stimulated by organization of ecologists. The British Ecological Society was founded in 1913 and this society sponsored two Journals namely *Journal of Ecology* (started in 1913) and *Journal of Animal Ecology* (started in 1932). Following the British, the Ecological Society of America was established in 1915 which also sponsored two publications, *Ecology* in 1920 and *Ecological Monographs* in 1931.

Currently, papers of ecological nature appear in a variety of Journals, reviews, monographs, periodicals and magazines, certain important of which are following—*Science, Nature, Evolution, Bioscience, Endeavour, Journal of Soil and Water Conservation, Advance*

Ecological Research, Archivio di Oceanographia Limnologia, Proceedings of Ecological Society Australia, Biological Bulletin, Journal of Experimental Botany, Journal of Experimental Zoology, Biological Review, Geographical Review, Journal of Wildlife Management, Quaternary Biological Review, Turtox News, Marine Biology, J. Parasitology, and Scientific American. A variety of Indian Journals also publish papers of ecological significance; some of them are following: *Hydrobiologia, Journal of Marine Biol. Ass. India, Andhra University Mems. Oceanography, Journal of Madras University, Bulletin of Madras Government Museum, Indian Journal of Fisheries, Indian Journal of Entomology, Indian Journal of Agriculture Science, Proceedings of Indian Academy of Science, Records of Zoological Survey of India and Tropical Ecology.*

A PREVIEW OF ECOLOGY

The ecology is the study of ecosystems or the totality of the reciprocal interactions between living organisms and their environments. The term **organism** refers to a dynamic biological unit which is greatly influenced by an enveloping and fluctuating environment. For a given organism, the environment consists of all the surrounding physical and biological factors with which it interacts. Maelzer (1965) suggests that **environment** should be defined as the sum total of everything that directly influences the animal's chances to survive and reproduce. The intimately local and immediate surrounding of the organism is called **microenvironment**, while, the **macroenvironment** is the sum total of the physical and the biotic conditions existing external to the organism and its microenvironment. The total life-supporting environment remain restricted to a thin surface zone around our planet earth. This restricted life-supporting zone of earth is called the **biosphere**. The biosphere too is not entirely hospitable to life; in one region called **parabiosphere**, severe environmental conditions permit inhabitation by some organisms only in the resistant stages of their life cycle. The parabiosphere includes broad areas such as the higher altitudes and a greater part of the cold polar regions, as well as numerous localized regions such as volcanos, geysers, and grossly polluted areas. The region of the biosphere in which active metabolic processes of organisms are possible is called the **eubiosphere**. Fear has been expressed by many modern ecologists that the parabiosphere is increasing in size at the expense of the eubiosphere, owing to the destructive activities of man.

Further, the biosphere includes four major environmental categories or **habitats—marine, brackish, fresh water,** and **terrestrial habitats.** Each of these habitats may be further subdivided into

smaller categories. For example, a fresh water environment may be large lake, a pond, a river, or a stream. Likewise, terrestrial environment may include following sub categories, all of which are called **biomes**—tundra, coniferous forest, deciduous forest, broad-leaved ever green subtropical forest, grassland, desert, chaparral and tropical rain forest. Each of the subtype of the four habitats has a typical set of physical and biological features and each forms a **ecological complex ecosystem**. The ecosystem is a natural unit or a subunit of biosphere which composed of **non-living, physical or abiotic** and **living, biological or biotic components** whose interactions result in a stable self-prepetuating system.

Among the main physical (abiotic) components of an ecosytem are such **climatic factors** as solar radiation, temperature, rainfall, and such **physical factors** as light, pressure, geomagnetism, and such **chemical factors** as acidity, salinity and the availability of inorganic nutrients needed by plants. The biological (biotic) factors in an ecosystem are the totality of all living organisms and their organic by-products. This include not only the larger plants and animals that are readily observed, but all the microscopic organisms as well bacteria in the soil or sediments, in the digestive tracts of animals or infecting plants and animals ; plankton that live in water ; viruses that cause disease ; fungi that live in the soil or on decaying organisms ; mites that feed on bacteria, and fungi and so on.

Each kind of living organism found in an ecosystem is called a **species**. A species comprises of all those organisms which are sufficiently alike genetically so as to make them distinguishable from all other types of organisms. Within an ecosystem, there usually exists a wide variety of species, all of which interact directly or indirectly with each other.

All individuals belonging to a particular species in a given ecosystem form a **population**. We thus speak of the population of red-legged frogs in a pond, of the population of herring in the North Sea or of the population of the blue-green alga in a eutrophied lake. The size of a population of any given species is determined by a balance between its **reproductive potential** and the **environmental resistance**. Further, within an ecosystem there is a great number of populations of different species, all interacting with one another as a **community** and with the physical environment as well. A community, thus can be defined as an assemblage of populations inhabiting an area possessing some uniformity in physical feature and vegetation. While a population is formed of a number of animals and plants inhabiting an area, a community is an aggregate of several species of plant and

animal population—the **biotic community** which forms a distinct ecological unit. A biotic community, thus, is a naturally occurring assemblage of plants and animals, living in the same environment, mutually sustaining and interdepending, constantly fixing, utilizing and dissipating energy. The place that an organism occupies in a



which largely reflects the life forms of the plants and which influences the nature and distribution of animal life in the community.

The structure of an ecosystem ultimately depends upon the photosynthetic plants (called **primary producers**) which support it. *These primary producers utilize energy of solar radiation for synthesis* of organic molecules from carbon dioxide, water and nutrient minerals. Part of the energy absorbed by plants is used for their own maintenance, the rest going into the synthesis of plant tissues or **biomass**. Plant biomass serves as food for **herbivorous** animals feeding on plants. Herbivorous are thus the **primary consumers**. They in turn become food for **carnivores**, who are **secondary consumers**. Each of these steps in the transfer of the original plant biomass through an ecosystem is known as **trophic**, or feeding level. This type of feeding sequence forms a **food chain**, or more accurately **food web**, since omnivorous species, such as man, may feed upon anything from plants to top level carnivores. The last category of the food web is formed by **decomposers**. Plant lose leaves or die, animals excrete wastes and, on dying, become carcasses. Eventually all these dead materials, if not eaten by **scavengers**, are decomposed, their organic matter being converted back into organic nutrients. The major decomposers are bacteria and fungi living in sediments or soil where organic debris accumulates.

The study of a food-web reveals the fact that basic elements of living systems, carbon, oxygen, nitrogen, phosphorus, and so forth, are originally obtained in the form of minerals (CO_2, H_2O, NO_3^-, $PO_4^{\equiv}$) by plants. They then pass from one trophic level to the next in the form of organic molecules. Eventually the decomposers return them, as mineral nutrients, to the air, water and soil, to be used again by plants. Mineral nutrients are thus **recycled** over and over again within an ecosystem. Further, the original source of energy for the entire ecosystem is the sun. A fraction of this solar energy, initially captured during photosynthesis, is dissipated at each trophic

level as waste metabolic heat. Thus, in any ecosystem energy is utilized only once.

Lastly, occasionally natural or man-made cataclysms (*e.g.*, fire, floods and avalanches are the natural cataclysms, while, man-made cataclysms include the bulldozing of the landscape, abandoning of unproductive fields or creation of a new lake behind a dam) occur which destroy a stable ecosystem, and a new one gradually takes its place. In such cases one can observe a sequence of biotic stages as a new ecosystem develops on an initially barren site. This is known as **ecological succession**. During chronological ecological succession, early colonists are often replaced, and species diversity increase until eventually a stable community is achieved. It is hypothesized but not yet proved, that species diversity leads to community stability in a self-reinforcing manner.

BRANCHES OF ECOLOGY

Ecological studies may deal with the study of the individual organism or an individual species in relation to their habitat (**autecology**) or the study of groups of organisms which are associated together as unit—the biotic community in relation to a particular area or biotope or habitat (**synecology**). **Demecology** is that branch of ecology which deals with the ecology of populations. Early ecologists have recognized two major subdivisions of ecology in relation to plants and animals—**plant ecology** and **animal ecology**. But when it was found that in the ecosystems plants and animals are very closely associated and interrelated, then, both of these major subdivisions of ecology into plant ecology and animal ecology became vague. Besides these major subdivisions the ecology has been classified in the following branches according to the level of organization, kind of environment or habitats and taxonomic position :

1. **Habitat Ecology** : It deals with the study of different habitats of the biosphere. According to the kind of habitat, ecology is subdivided into **Marine Ecology**, **Freshwater Ecology**, and **Terrestrial Ecology**. The terrestrial ecology too is further subdivided into **Forest Ecology**, **Cropland Ecology**, **Grassland Ecology**, *etc.*, according to the kind of study of its different biomes.

2. **Ecosystem Ecology** : It deals with the analysis of ecosystem for structural and functional point of view including the interrelationship of physical (abiotic) and biological (biotic) components of environment.

3. **Conservation Ecology** : It deals with methods of proper management of natural resources like land, water, forests, sea, mines, etc., for the benefit of human beings.

4. Production Ecology : It is the modern subdivision of ecology which deals with the gross and net production of different ecosystems like freshwater, sea water, agriculture, horticulture, etc., and tries to do proper management of these ecosystems so that maximum yield can be get from them.

5. Radiation Ecology : It deals with the study of gross effects of radiations and radioactive substances over the environment and living organisms.

6. Taxonomic Ecology : It is concerned with the ecology of different taxonomic groups and eventually includes following subdivisions of ecology—**plant ecology, insect ecology, invertebrate ecology, vertebrate ecology, microbial ecology** and so on.

7. Human Ecology : It deals with the study of man with his environment.

8. Space Ecology : It is modern subdivision of ecology which remains concerned with the development of partially or completely regenerating ecosystems for supporting life of man during long space flights or during extended exploration of extra-terrestrial environments.

Thus, the roots of ecology lie in natural history, human demography, biometry, and applied problems of agriculture, horticulture and medicine.

RELATIONSHIP OF ECOLOGY WITH OTHER DISCIPLINES

Modern ecology is a multidisciplinary science which depends on a variety of disciplines like **physics, chemistry, mathematics, statistics, meterology, climatology, geology, geography, economics, sociology, agriculture science, forestry, horticulture, genetics, physiology,** etc. All these disciplines have helped in the better understanding of many ecological principles.

For instance, meterological and climatological data for certain geographic localities allow for a more subtle interpretation of results. A basic knowledge of forestry can be invaluable for a forest-ecologist to understand forest type distribution, floristic composition and prevalent environmental factors. Likewise, statistical data helps in interpreting the reasons for activity, population increases, migrations, probability of ecological events occurring in a particular area, sampling techniques and reliability of results. Palaeontology (geology) provides information about the ancestral organisms and environmental situations prevalent in past. Evolution and genetics are utilized to interpret the reason for organic changes when

with environmental conditions, establishment of new populations and species, environmental effects on genetic populations and so on. Such interdisciplinary approaches to ecology, consequently have given rise to following sub-divisions of ecology.

1. **Ecological genetics** : An ecologist recognised a kind of genetic plasticity in the case of every organism. In any environment only those organisms that are favoured by the environment survive. The branch of ecology dealing with genetics in relation to ecology is called ecological genetics.

2. **Palaeoecology** : It deals with the movements of biotic elements based on palaentological evidence, which provides information about ancestral organisms and environmental conditions existing in the past.

3. **Ecophysiology** : The factors of environment have a direct bearing on the functional aspects of organisms. The ecophysiology deals with the survival of populations as a result of functional adjustments of organisms with different ecological conditions of the ecosystems.

4. **Chemical ecology** : It deals with the adaptations of animals or preferences of particular organisms like insects to particular chemical substances.

5. **Pedology** : It is a branch of terrestrial ecology and it deals with the study of soils, in particular their acidity, alkalinity, humus content, mineral contents, soil type, etc., and their influence on the organisms.

6 . **Ecogeography** : It deals with the study of the role of the environment in animal distribution. It is related with biogeography which is concerned with the structural and functional relations of living organisms in space, which form the immediate environment of the individuals as well as populations. Ecofloras and ecofaunas are the lowest units of which a biogeographhic flora or fauna is made up of.

7. **Ecological energetics** : It deals with energy conservation and its flow in the organisms within the ecosystem. In it thermodynamics has its significant contribution.

Because of its far-flung involvements with so many fields ecology, is often regarded as a generality rather than a speciality. Visualizing this fact, an ecologist, **A. Macfadyen** (1957) wrote in his book *Animial Ecology: Aims and Methods—The ecologist is something of a chartered libertine. He roams at will over the legitimate preserves of the plant and animal biologist, the taxonomist, the physiologist; the behaviourist, the meterologist, the geologist, the physicist, the chemist, and even the sociologist; he poaches from*

all these and from other established and respected disciplines. It is indeed a major problem for the ecologist in his own interest, to set bounds to his divagations.

MATERIALS AND METHODS IN ECOLOGY

Ecology has been attacked on three broad fronts the **mathematical**, the **laboratory**, and the **field**. These three approaches are interrelated, but some problems have arisen when the results of one approach fail to verify those of another. In current ecological investigations several advanced technological methods are employed to deal quantitatively with large complex systems such as the ecosystem. Tracer methodology, mass chemistry (spectrometry, colorimetry, chromatography, etc.), remote sensing, automatic monitoring, mathematical modeling and computer technology are providing the tools for such ecological analysis. However, most ecologist study ecology from either a **descriptive** or **functional** point of view. The descriptive point of view is mainly natural history and proceeds by describing the vegetation groups of the world, such as temperate deciduous forests, tropical rain forests, grassland and tundra, and by describing the animals, plants and micro-organisms and their inter-relationships for each of these ecosystems. The functional point of view on the other hand, is oriented more toward **relationships** and seeks to analyze general problems common to most or all of the different areas.

SIGNIFICANCE OF ECOLOGY FOR MAN

Man is himself an organism within an environment. Like other animals man is influenced by the physical features of his environ-

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proper regulation he often has a destructive effect. For instance, by applying certain ecological principles to such fields as agriculture, biological surveys, game management, pest control, forestry, horticulture, and fishery biology, he has received tremendous economi . gains. Its knowledge is found critically important for intelligent conservation whether in relation to soil, forest, wildlife, water supply or fishery resources. Further, though man has been known to control his environment successfully to meet his needs, but indiscriminate control of different pathogens or pests such as bacteria,

fungi, weeds, insects, rodents, etc., through use of different chemical poisons or pesticides (bacteriocides, fungicides, insecticides, etc.), the release of massive quantities of radioactive debris, discarded chemicals and industrial waste into rivers result in atmospheric and aquatic pollution, which may have short and long term ecological effects. Further, rapid growth of urbanization and fast rate of multiplication of human population have resulted in fatal threat of scarcity of wild life, food, open space, and of survival.

There are certain other ecological problems. Agriculture and now forestry are concentrating upon monoculture—single species ecosystems—in spite of the difficulties and dangers attendant on unnaturally simplified ecosystems that lack a diversity of species. Over much of the world, especially in the grasslands, we continue to disrupt the energy balance through overgrazing and end up with eroded mountain sides, silt-clogged streams and lakes, and a scarcity of water. Therefore, future of human life on earth demands more knowledge about the ecosystems and other ecological problems.

CHAPTER 2

ENVIRONMENT

Each and every living organism has its specific surrounding, medium or environment to which it continuously interacts and remains fully adapted. The environment is the sum total of physical and biotic conditions influencing the responses of the organisms (S.C. Kendeigh, 1974). The life supporting environment of planet earth—the biosphere is composed of following three chief media—air, soil and water, which are the components of three major sub-divisions of the biosphere—atmosphere, lithosphere and hydrosphere, respectively. These media are not completely isolated from each other, however, some of the atmospheric gases are dissolved in all natural waters, and some moisture is persent almost everywhere in the atmosphere. Each of these media and their subsequent major subdivisions of biophere can be discussed separately in following manner :

A. ATMOSPHERE AND AIR

The multilayered gaseous envelope surrounding the planet earth is called atmosphere. The atmosphere remains in contact with all the major types of environment of earth, interacting with them and greatly affecting their ability to support life. It filters sunlight reaching the earth, affect climate, and is a reservoir of several elements essential for life.

PHYSICO-CHEMICAL STRUCTURE OF ATMOSPHERE

According to R. L. Smith (1974) the atmosphere surrounding the earth consists of a series of following layers—exosphere, heterosphere, mesosphere, stratosphere and troposphere.

1. Exosphere—The exosphere is the outer fringe of earth's atmosphere which extends some 20,000 miles from the planet. Its dominant element is hydrogen and its temperature ranges from 32° to 10,000°F. In this region the atoms and particles are so far apart that rarely collide and friction is negligible. Its high temperature is caused by solar energy (radiant energy of sun). The lower part of exosphere contains abundance of helium besides hydrogen, is called helium zone.

2. Heterosphere—The exosphere is followed by ionosphere and thermosphere of heterosphere. In heterosphere

oxide occur in a ionized state and their ionization is caused by ultraviolet radiation. The outer reach of ionosphere (zone of ionized air) is 250 miles from earth. Its temperature is 200°F and its upper limits are characterized by a layer of oxygen largely ionized into ozone byultraviolet radiation. The lower sub region of heterosphere is called thermosphere. It is 125 miles out from the earth and contains mixture of nitrogen and oxygen in the ratio of 1 : 1. In thermosphere molecules of air are so widely spaced that high frequency audible sounds are not carried by the atmosphere. Further, in it the temperature decreases as the distance from the sun increases.

3. Mesosphere—The mesosphere is that layer of air which is within 55 miles of the earth's surface. This is a region of cold temperature (—120°F). The temperature of mesosphere, however, is gradually increases as mesosphere leads to stratosphere.

4. Stratosphere—The stratosphere is 30 miles away from the earth, having a fairly constant temperature at —75°F to —45°F in its outer reaches which increases upto 30°F at its lower boundary. Within the stratosphere, ozone (O_3) accumulates sufficiently to produce a well marked ozone layer called **ozonesphere**, which extends from about 13 to 23 miles above the sea level. In ozonesphere, the sunlight ionizes oxygen to ozone by photochemical dissociation. The ozonosphere completely absorbs solar radiation, ultraviolet radiation from the sun and also a lot of the solar infra-red thus becoming warmer than adjacent layers above or below. The amount of ozone present varies from time to time, probably with season and with solar activity, but because of its extra heat, it acts like a blanket that reduces the cooling rate of the earth and thus adds to the effect of water vapour.

5. **Troposphere** : The lowest region of atmosphere which hangs at 5 miles over the earth at the poles, nearly 10 miles over the earth at the equator, and which subjects to differential heating, temperature inversions and convertion currents is called **troposphere**. In it temperature drops rapidly from 30° to —70°F. Troposphere is the layer of sulphates and is the region of strong air movements and cloud formation, *i.e.*, it is the locus of most weather phenomena. Man along with other living organisms live in troposphere.

THE AIR

The gaseous mixture of troposphere, is utilized by most organisms in respiration to liberate energy from food during oxidation and is called air. The air has a relatively constant composition of gases, as has been shown in following table :

Table : Composition of atmospheric air.

Component	% by volume	%by weight.
Nitrogen (N_2)	78.09	75.54
Oxygen (O_2)	20.93	23.14
Argon (A)	0.93	1.27
Carbon dioxide (CO_2)	0.03	0.03
Miscellaneous	0 02	0 02

Miscellaneous item includes traces of hydrogen, ozone, radon, helium, neon, krypton, xenon, sulphur dioxide, hydrogen sulphide, ammonia, methane, etc. Besides these gases, air may carry suspensions of liquids such as water in clouds and solids such as dust from the ground (soil), smoke from fires or salt from ocean spray.

In general, the composition of these gases in air in other media on earth, such as water or soil, is in equilibrium with the atmosphere. In special ecological habitats such as the anaerobic regions of deep lakes or sand-mud flats, however, the composition of gases of air is altered dramatically. Slight differences occur in the atmosphere at different latitudes and, at places where gases are entering or leaving the atmosphere, such as volcanoes, fires, smelters, cities, metropolitan areas, and vegetation.

Physiologic-ecologic interrelationships of gases and animals—The atmospheric composition is not always the most important factor to organism ; rather, it is the partial pressure of a gas, especially oxygen and CO_2, which influences the existence of life, as illustrated by altitudinal studies on organisms. The partial pressure of a gas is the product of the total barometric pressure times the concentration of gas in dry air. For every 18,000 ft., the barometric pressure is reduced by half, but the composition of air is essentially unchanged. To illustrate this effect on animals, the following critical altitudes have been identified for most humans : above 10,000 ft., oxygen must be added to inspired air ; at 35,000 ft., 100 per cent oxygen must be inhaled; and at 50,000 ft., little oxygen reaches the alveoli even if 100 per cent oxygen is breathed (see Vernberg and Vernberg, 1970).

The solubility characteristics of oxygen and carbon dioxide in water are different, for carbon dioxide is about 200 times more soluble than oxygen. Both temperature and salinity have pronounced effects on the solubility of these two gases ; with either increased

temperature or increased salinity, there is a decrease in solubility in water as has been shown in following table :

Table : Coefficients of saturation of CO_2 in water at different temperatures and salinity (ml/litre in equilibrium with 760mm Hg)

Temperature, °C	Salinity, ‰		
	0	28·91	36·11
0	1715	1489	1438
12	1118	980	947
24	782	695	677

If gases are to be functionally important to an organism, there must be a mechanism whereby gases can enter and leave the body. Apparently the passage of oxygen across a membrane is accomplished *by diffusion*. The rate of diffusion can be calculated by **Fick's law**, which is based on concentration coefficient, amount of surface thickness of the membrane, and time, and is expressed as the diffusion coefficient (Davson, 1959). The diffusion coefficient value is not the same for different animal tissues. For example, some representative values of diffusion coefficient of O_2 are : muscle, 0.000014 ; connective tissue ; 0.0000 11 ; water, 0.000034 ; air, 11.0 ; chitin, 0.000013. Not only is the diffusion rate different in various tissues, but the behaviour of each gas also differs. Carbon dioxide, for example, diffuses through water and animal tissues 25 times faster than oxygen.

Air as a medium for living organism—Air is not a easy and suitable medium to support life (biota) and actually no organism ever originated in air, though, certain aquatic and terrestrial organisms have become secondarily adapted for aerial existence. Air has so much less **buoyancy** than water that organisms emerging from water or land immediately subjected to a stronger pull of **gravity** which hold them to the earth. They are also exposed to **evaporation** of water from their bodies, which threatens loss of water from the protoplasm and death by desiccation. Changes in **temperature** are much more drastic in air and there is great danger of chilling or overheating. Further, periods of light exposure are much longer and much more intense in air than in water or upland. The problem of **mineral supply** becomes acute in the air.

Biota of the air—Only a few micro-organisms, plants and animals have invade the air. The most common examples of aerially adapted animals are—flying fish (*Exocoetus volitans*), flying frog, flying lizard (*Draco volans*), flying phalangers (*Petaurus*), bats (*Eptesicus*), and birds.

B. LITHOSPHERE AND SOIL

The earth is a cooled, spherical, solid planet of solar system, which rotates on its axis and revolve around the sun at a certain

constant distance. The solid component of earth is called **lithosphere.** The lithosphere is multilayered including following main layers—**crust, mantle, and outer and inner core.** The **core** is the central fluid or vapourised sphere having diameter of about 2500 kms from the centre and is possibly composed of nickel-Iron. The **mantle** extends about 2900 kms above the core. This is in molten state. The outermost solid zone of the earth is called **crust** which is about 8 to 40 kms above the mantle. The crust is very complex and its surface is covered with soil supporting rich and varied biological communites, for living organisms find in the soil an environment providing food, shelter, and concealment from predators (**Wallwork, 1970**).

THE SOIL

The word soil is derived from a Latin word '*solum*' meaning earthy material in which plants grow. The science which deals with the study of soil is called **Soil Science, Pedology** (*pedos*—earth) or **Edaphology** (*edaphos*—soil). The study of soil is significant for us due to various reasons. Soil is a natural habitat for micro-organisms, plants and animals. Its knowledge is helpful in practices of agriculture, horticulture, forestry, etc., such as cultivation, irrigation, artificial drainage, and use of fertilizers. Pedology is important also for geology, petrology, minerology, palaeobotany and paleozoology.

The soil is the loose, friable, unconsolidated top layer of earth crust which is the site of decomposition of organic matter and mineral materials and is the crossroad of terrestrial communities. It provides mechanical anchorage to plants, besides serving as a reservoir of food materials and water. It is the site where nutrient elements are brought into biological circulation by mineral weathering. The soil harbours the bacteria which incorporate atmospheric nitrogen into the soil. Plant roots occupy a considerable portion of the soil ; tie the vegetation to the soil ; and pump water and its dissolved minerals to other parts of the plant for photosynthesis and other biochemical processes (see **Smith, 1974**).

Further, a **mature soil** is that state of soil that has assumed the profile features (*i.e.*, succession of natural layers) characteristic of predominant soils on the smooth uplands within the general climatic and botanic regions in which it is found (**Marbut, 1926**).

SOIL FORMATION OR PEDOGENESIS

Soil, is a stratified mixture of inorganic and organic materials, both of which are decomposition products. The mineral constituents of soil are derived from some parent material, the **soil forming rocks** by fragmentation or weathering, while, organic components of soil are formed either by decomposition (or transformation) of dead

remains of plants or animals or through metabolic activities of living organisms present in the soil. Before discussing the soil-forming processes, a brief discussion about the nature of soil forming rocks will be highly desirable :

Soil forming rocks : Basically, there are three kinds of soil forming rocks—**igneous rocks, sedimentary rocks and metamorphic rocks.** The main characteristics can be represented in a summarized form in following table :

Table : Classification, characteristics and mineral composition of the principal soil-forming rocks (Wallwork, 1970).

Class of rock	Mode of formation of rock	Type	Description of rock-types	Principle minerals of soil-forming rocks
A. Igneous rocks	Formed by cooling of molten megma (larva)	1. Granite	Usually light in colour, coarse to medium grain	Quarts, feldspar, mica, amphibole, iron oxides
		2. Diorite	Gray to dark in colour, coarse to medium grain	Feldspar, amphibole iron oxide, biotite
		3. Basalt	Dark to black in colour, dense to fine grain	Feldspar, pyroxene, iron oxide, biotite
B. Sedimentary rocks	Formed by deposition of weathered minerals which derived from igneous rocks	1. Shales	Light to dark in colour, thinly laminated	Clay minerals, quartz
		2. Sandstone	Light to red in colour, granular and porous	Quartz, clay minerals, iron oxides, calcium carbonate
		3. Limestone	Light, grey, red, brown, or black in colour, fine grained and compact	Calcite, dolomite, iron oxides, clay minerals
C. Metamorphic rocks	Formed by change of pre-existing rock (*i e.*, igneous or sedimentary rock) through heat and pressure	1. Gneiss	With light and dark bands	Much as in granite from which it is formed
		2. Schist	Foliated structure	Much as in basalt or shale form which it is formed
		3. Slate	Gry to black in colour, compact and uniform texture	Similar composition to shale form which it is formed

Class of rocks	Mode of formation of rocks	Type	Description of rock-types	Principle minerals of soil-forming rocks
		4. Quartzite	Light to brown in colour, compact and uniform texture.	Similar composition to sandstone from which it is formed.
		5. Marble	Light, red, green or black in colour, compact, fine to coarse texture.	Calcite, dolomite formed from lime stone.

Chemistry of minerals of soil-forming rocks—Rocks are the chemical mixture of numerous kinds of minerals. The chemical nature of certain most common and abundant minerals of soil-forming rocks has been listed in following table :

Table : Chemical composition of some common soil minerals.

Minerals	Chemical Constituents
A. Sand and silt minerals	
1. Quartz or silica	SiO_2
2. Feldspars	
a. Orthoclase	$K_2Al_2Si_6O_{16}$
b. Plagioclase	$NaAlSi_3O_8$
c. Calcium feldespar	$CaAl_2Si_2O_8$
3. Micas	
a. Muscovite	$K(OH)_2Al_2(AlSi_3)O_{10}$
b. Biotite	K, Mg, Fe, Al silicate
4. Pyroxene	$(Mg, Fe)SiO_3$
5. Amphbiole	$(Mg, Fe)_7(Si_4O_{11})_2(OH)_2$
6. Olivine and serpentine	$(Mg\ Fe)_2SiO_4$
7. Calcite ; magnesite ; and dolomite	$CaCO_3$; $MgCO_3$; and $(CaCO_3, MgCO_3)$
8. Iron Oxides	
a. Haematite	Fe_2O_3
b. Magnetite	Fe_3O_4
c. Limonite	$FeO(OH), xH_2O$
B. Clay minerals	
1. Kaolin	$Al_2O_3, 2SiO_2, 2H_2O$
2. Montmorillonite	$(Ca, MgO)Al_2O_3, 5SiO_3, 5H_2O$

Besides the tabulated minerals following minerals occur in low percentage in soil-forming rocks—Toarmalive (Boron silicas or Aluminium with alkali metals and Iron and Magnesium), Rutile (Titanium oxides), Zircon (Zirconium oxide), and Gauconite (hydrated silicon of iron and potassium).

PROCESS OF SOIL-FORMATION

The processes which are involved in the formation of mature soil can be studied under following heads :

A. WEATHERING OF SOIL-FORMING ROCKS

Soil formation is started by disintegration or weathering of parent rocks by some physical, chemical and/or biological agents, because of which the soil-forming rocks are broken down in small particles called regoliths. Regoliths are the basic materials which under the influence of various other pedogenic processes finally develop into mature soil.

(a) Physical weathering : The physical weathering agents are primarily climatic in character, exerting a mechanical effect on the substratum with the result that fragments are comminuted into progressively decreasing particle sizes (*i.e.*, regoliths). Such climatic weathering of rocks does not causes any chemical transformation of rock-minerals and commonly occurs in deserts, in high alttiudes, in high latitudes, and inocalities with marked topographic relief and sparse vegetation cover. The agents which are involved in climatic weat hering of rocks, are **temperature, water, ice, gravity** and **wind** (see **Wallwork, 1970**).

The temperature causes break down of those rocks which have heterogeneous structure, due to the fact of differential expansion and contraction coefficient of materials composing the rocks. Minerals composing the rocks have got different degrees of expansion. These minerals expand in the high temperature of day and contract when the temperature falls. The differential expansion and contraction of different minerals of rocks set up internal tensions and produce cracks in the rocks and consequently, the rocks weather into finer particles.

In its liquid state water causes mechanical weathering of rocks by following methods : (i) **Rain water**—Natural water falling either in the form of rain drops or hail storm on the surface of rock with beating effect bring about abrasion of massive rocks into smaller particles. (ii) **Torrent water**—Rapidly flowing water rolls the heavy rock masses such as rock boulders along the bottom of stream and grinds them into finer particles. (iii) **Wave action**—The wave actions are most effective in sea shores. The rapidly striking water waves dislocate solid particles of varying diameters from sea shore rocks and the debris is then settled at the sea bottom to form marine soil. Water also acts as a mechanical carrier. Nearly 5 billon tons of mineral matter is annually carried away in solution from land to sea (see **Pearse, 1939**).

In its freezing and ice-melting states water causes rock-weathering by **frost action** and **glacier formation.** Water in the form of frost or ice, is an extremely effective physical weathering agent of rocks. It seeps into rock crevices, freezes due to sudden fall of temperature of rocks, expands about nine per cent of its original volume, exerts a pressure (expansion force) of approximately 150 ton/ft^2 and eventually cracks the rock into smaller pieces. Likewise, in summer when ice at mountain tops starts melting and glaciers (huge sliding masses of ice) move downwardly on the slope, then, during the glacier movement, the rock over which they move is gradually worn down to produce fine particles which are deposited as **drift** or **till,** when glacier finally retreats.

Gravitation weathering action is most effectively demonstrated by land slides and rock slippages caused by earth quakes and faulting during which the rock is fragmented by abrasion and the forces of impact. Lastly, the rapid stormy **wind** carrying suspended sand particles causes the abrasion of exposed rock. It acts like a mechanical carrier in moving the particles over the surface of earth as **dunes** or **drifts** and in transporting large quantities of fine suspended particles long distances.

(b) Chemical weathering : The physical disintegration produces a greater surface area of rock exposed to the **chemical weathering,** which occurs simultaneously with physical weathering and continues much beyond that. During chemical weathering, a chemical transformation or decomposition of parental mineral materials into new mineral complexes or secondary products occurs. For example, primary minerals that contain aluminium and silicon, such as feldspar, are converted to secondary mineral such as clay. Because chemical weathering requires the presence of moisture and air as essential factors therefore chemical weathering is not effective in deserts. It includes following reactions :

1. **Solution**—Water is the most potent chemical weathering agent in most kinds of rocks through its solvent action. Solution helps in the removal of water-soluble minerals of weathered rocks. For example, soluble rocks like gypsum, limestone and those with a calcareous content get weathered by the solvent action of water which increases in the presence of CO_2 and organic acids formed by decay of organic remains of plants and animals. The solution of these minerals may become absorbed on the surface of negatively charged colloidal particles or are removed by leaching.

2. **Hydroylsis**—Hydrolysis involves an exchange of constituent parts between water and minerals such as the chemical water with strong bases producing hydroxides of iron,

calcium, aluminium, etc. Hydrolysis mostly act in combination with other reaction such as oxidation/reduction or carbonation as in the production of secondary kaolinitic clay minerals from primary orthoclase—

$$\underset{\text{Orthoclase}}{K_2Al_2Si_6O_{16}} + 2H_2O + CO_2 \rightarrow \underset{\text{Kaolin}}{Al_2O_3.2SiO_2.2H_2O} + 4SiO_2 + K_2CO_3$$

The release of calcium, magnesium, potassium, sodium, and silicates into the soil solution, as a result of hydrolysis, enhances the availability of these ions to plants.

3. **Oxidation**—Oxidation results when oxygen is added to minerals to produce oxides which when dissolves in water weakens the rock and bring about weathering. It occurs in well-aerated, well-drained soil. Because iron is especially reactive with water and oxygen, iron-bearing minerals are prone to rapid decomposition. For example, due to oxidation iron oxidised in the red ferric state.

$$\underset{\text{Ferrous oxide}}{4FeO} + O_2 \rightarrow \underset{\text{Ferric oxide}}{2Fe_2O_3}$$

Further, oxides and sulphides of Iron, Aluminium, Manganese, etc., are easily oxidised and cause into the weathering of rocks.

4. **Reduction**—Reduction occurs in wet, badly drained, poorly aerated sites such as deep zones of earth crust. It means removal of oxygen from minerals. For example, red ferric oxide may reduced to the gray ferrous state as follows :

$$2Fe_2O_3 \rightarrow 4FeO + O_2$$

5. **Carbonation :** In carbonation water and carbon dioxide combine to form carbonic acid which reacts with hydroxides of calcium, magnesium and other minerals of rock to form carbonates and bicarbonates in following manner :

(i) $$CO_2 + H_2O \rightarrow \underset{\text{Carbonic acid}}{H_2CO_3}$$

(ii) $$\underset{\substack{\text{Calcium}\\\text{hydroxide}}}{Ca(OH)_2} + CO_2 \rightarrow \underset{\substack{\text{Calcium}\\\text{carbonate}}}{CaCO_3} + H_2O + CO_2 \rightarrow \underset{\substack{\text{Calcium}\\\text{bicarbonate}}}{Ca(HCO_3)_2}$$

The slightly soluble carbonates of these minerals either accumulate deeper in the rock material or are carried away, depending upon the amount of water passing through. The solubility of these carbonates, leaves gaps in rocks and consequently makes them weak enough for weathering process.

6. **Hydration :** During hydration water molecules become chemically attached to particular rock mineral, due to which the volume of parental material increases and hydrated minerals become soft and more readily weatherable :

$$\underset{\text{Hematite}}{2Fe_2O_3} + 3H_2O \rightarrow \underset{\text{Limonite}}{2Fe_2O_3.3H_2O}$$

(c) **Biological Weathering :** Though the surface of bare rock is unsuitable for many forms of life, even then a number of micro oragnisms (bacteria protozoans fungi, nematodes, etc.), lichens and mosses can gain a foothold. These early colonizers transform the rock into a dynamic system, storing energy and synthesizing organic material (Jacks, 1965). Their activities alter the mineral composition as well as the physical structure of the rock. For example, lichens are present in the initial stages of biological succession and their growth may cause cracking or flaking, exposing greater area of rock to further weathering. The lichens and mosses extract mineral nutrients such as P, S, Ca, Mg, K, Na, Fe, Si, and Al, from the rock. These elements are combined with organic complexes, and eventually return to the developing soil when the vegetation decomposes.

Products of weathering and soil types : The soil which is formed by weathering of soil-forming rocks is called **embryonic or primary soil. The secodary or transported** soils are those which are carried to other places by carriers like gravity, water, glacier and wind. Soil material transported from one area to another is known as **loess or eolian ; that transported by water as alluvial, lacustrine** (lake soil) and **marine deposits ; that transported by glacial ice as glacial drift, till** and **moraines ; that transported by gravity as colluvial soil** and that transported by sand storms as **sand dunes.** In a few places soil material come from accumulated organic matter as **peat.** The soil which develops *in situ* above parent bedrock is called **residual** or **sedentary soil.**

B. MINERALIZATION AND HUMIFICATION

The decomposition or weathering of inorganic soil matter is followed by decomposition of organic matter which is started at an early stage of pedogenesis, (*i.e.*, the biological weathering) and continues upto much later stages. During early sages of pedogenesis, the organic contents of the embryonic soil are not very high, as the vegetation and its associated fauna are not richly developed. Also, the products of organic composition frequently occur in combination with those of inorganic decomposition as complexes, not easily separable into component parts in these initial stages. However, as both of these decomposition processes develop side by side and in concert, the size of soil particles and the spaces between them become smaller with the result that the water holding capacity increases. This together with the increasing amount of plant nutrients and organic material, allows the soil to support higher plant life, such as grasses ; the protective covering effect and binding action of root

systems so provided, promotes a greater stability of soil structure. Thus, soil development begins under some influence of plants. They root, draw nutrients from mineral matter, reproduce and die. Their roots penetrate and further breakdown the regolith. The plants pump up nutrients from its depth and add them to the surface, and in doing so recapture minerals carried deep into the material by weathering processes. By photosynthesis plants capture the sun's energy and add a portion of it as organic carbon to the soil each year. This energy source the plant debris, enables bacteria, fungi (such as *Rhizoctonia solani*, *Armillaria mellea*, etc.), earthworms and other soil organisms to colonize the area.

The breakdown of organic debris into humus is accomplished by decomposition and finally mineralization. Higher organims in the soil—millipedes, centipedes, earthworms, mites, springtails, grasshoppers, and others—consume fresh material and leave partially decomposed products in their excreta. This is further decomposed by micro-oganisms, the bacteria and fungi, into various compounds of carbohydrates, proteins, lignins, fats, waxes, resins and ash. These compounds are broken down into simpler products such as carbon dioxide, water, minerals and salts. This latter process is called **mineralization.** The residual amorphous incompletely decomposed black coloured organic matter which undergoes mineralization is called **humus.** The process of humus formation is called **humification.** Muller (1879, 1884) has recognized two kinds of humus—**mor** and **mull.** Mor **humus** is acidic and supports an abundant fungal growth and low number of soil bacteria. Fungal mycelia may help to bind together particles of humus and decomposition litter into matted layers and in a well developed mor, three such layers can be distinguished, namely a surface litter or **L layer,** comprising undecomposed leaves and twigs, below which is the **fermentation** or **F layer,** in which decomposition has proceeded same way toward the development of humus, and beneath this, the **humus** or **H layer,** in which degraded humus fractions accumulate. Mor has low calcium content and developed on sandy soils under conifers. **Mull humus** is neutral or slightly alkaline and contain rich microflora of bacteria. It lacks distinct layering of the mor, largely because the presence of calcium compound favour the development of a rich earthworm fauna which promotes a greater mixing of organic and mineral materials. The mull humus develops in brown forest soils under tree species having a relatively high calcium content, such as alder, elm, bass wood, aspen and few conifers (red cedar). Intermediate between the two types of humus is the 'moder' which has a richer and varied fauna.

In order to emphasize the part played by living plants and animals in soil formation, Taylor (1930) proposed the following formula :

$$S=M\,(C+V+VA+A)^{t}+D$$

where, A signifies animals; C=Climate ; D=erosion or deposition ; M=parent material ; S=soil : t =Time and V=vegetation.

C. FORMATION OF ORGANO-MINERAL COMPLEXES

During final stage of pedogenesis, colloidal particles which are formed due to weathering, humification and mineralization accumulates and may aggregate into crumbs or concretions. Some colloidal humus particles may become associated with mineral particles to form organo-mineral complexes. According to Wallwork (1970) the crumbs or organo-mineral complexes are formed by following two mechanisms—electro-chemical bonding and cementing. In electro-chemical bonding method of crumb formation, aggregation of negatively charged colloidal clay and/or humus particles of water molecules and metallic ions, particularly calcium, takes place. The cementing mechanism of crumb formation involves the action of substances absorbed on the surface of soil particles which effectively glue them together.

The crumbs increase the total pore space in the soil, allowing good aeration and drainage. Eventually, a characteristic profile develops under the influence of climate, vegetation, parent material and the activities of the soil communities. The mature soil, thus, becomes a complex system of living and non-living materials not inert but active.

SOIL PROFILE

Soil profile is the term used for the vertical section of earth crust generally upto the depth of 6ft or upto the parent material to show different layers or horizons of soil for the study of soil in its undisturbed state. It is made up of a succession of horizontal layers or horizons, each of which varies in thickness, colour, texture, structure, consistency, porosity, acidity and composition.

In general soils have following four horizons an organic or O-horizon and three mineral (A, B, C) horizons. Some workers recognized a D horizon, in which rocks are in active weathering state, in between C and R horizons. R horizon is the consolidated bed rock on which a soil profile rests. A and B horizons form the true soil or solum. Each horizon of soil profile is further subdivided. Horizon subdivisions are indicated by arabic numbers, *e.g.*, O_1, O_2,

A_1, A_2, etc. Different horizons of soil-profile have following characteristics :

(i) O-horizon—The O-horizon, once designated as L, F, H, or Ao and Aoo, is the surface layer forming above the mineral layers and composed of fresh or partially decomposed organic material, as found in temperate forest soils. It is usually absent in cultivated soil. O-horizon contains both kinds of humus (*e.g.*, mull and mor)

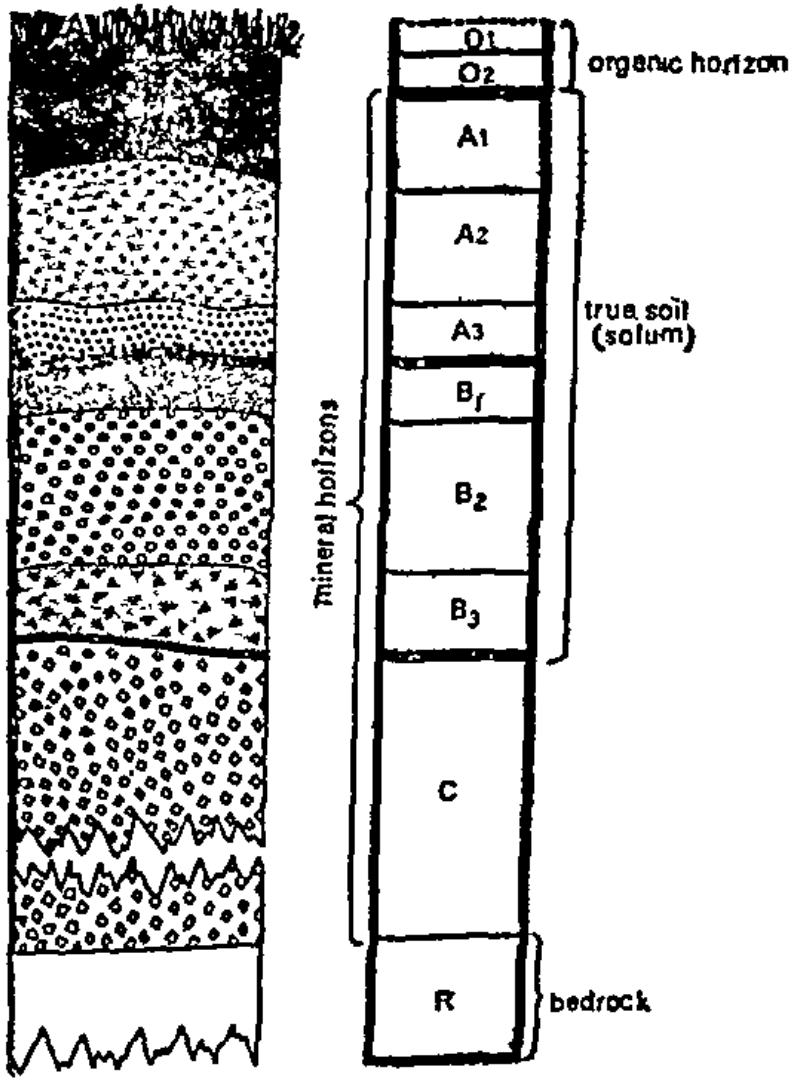


Fig. 2·1. A generalized profile of soil. O_1 : Louse leaves and organic debris : O_2 : organic debris partly decomposed or matted. A_1 : A dark coloured horizon with a high content of organic matter mixed with mineral matter light-coloured

and is subjected to the greatest changes in soil temperatures and moisture conditions and contains most organic carbon. O-horizon and upper part of A horizon is the region where life is most abundant.

(ii) **A-horizon**—It is characterized by major organic matter accumulation, by the loss of clay, iron and aluminium and by the development of organo-mineral complexes, granular crumbs or platy structures.

(iii) **B-horizon**—It lies below A horizon and also called **sub-soil.** It is characterized by an alluvial concentration of all or any of the silicates, clay, iron, aluminium, and humus, alone or in combination and by the development of blocky, prismatic or columnar structure.

(iv) **C-horizon**—Below B-horizon and above the surface of weathered parent rock, is the zone of regolith or C-horizon. It is a light-coloured horizon containing weathered parent material.

(v) **R-horizon**—Below all these horizons may lie the **R-horizon,** which is the region of consolidated rock.

MORPHOLOGY OF SOIL

In the field, differences among soils and among horizons within a soil are primarily reflected by variations in texture, arrangement, structure and colour.

1. Texture of soil—The texture of a soil is determined by the production of different sized soil particles. Mineral fractions or particles of soil are called **"soil fractions"** or **"soil separates".** The soil particles have been classified into **gravel, sand,** and **clay** on the basis of their size differences. Gravel consists of coarse particles larger than 2·0 mm. Sand ranges from 0·02 to 2·0 mm in diameter and is easily seen and feels gritty. Silt consists of particles from 0·002 to 0·02 mm in diameter, which scarcely can be seen by the naked eye, feels and looks like flour. Clay particles range below 0·002 mm in diameter, are too fine to be seen even under the ordinary microscope, and are colloidal in nature. Clay controls the most important properties of soils, including plasticity and exchange of ions be ween soil particles and soil solution.

Most soils are a mixture of these various particles. Based on the proportions of the various particles contained in them, soils can be grouped into 5 textural groups : (1) **Coarse textured** solls are loose, consist mainly of sand and gravel. They retain very little moisture and supply some plant nutrients. (2) **Moderately coarse** soils includes sandy loam to very fine sandy loam. (3) **Medium textured** soils are mixture of sand, silt and clay high enough to water and plant nutrients. (4) **Moderately fine textured**

high in clay. They are moderately sticky and plastic when wet, the may form a crust on the surface if organic matter is low. They hav a high moisture-holding capacity. (5) Fine textured soils contai more than 40 per cent clay, may be sticky and plastic when wet an hold considerable water and plant nutrients, but may have restricte internal drainage. All these 5 textural soil-groups include followin 12 textural classes which have been recognized on the basis of rela tive percentages of soil particles (*i.e.*, sand, silt and clay) :

Table : Textural classes of soils.

Soil classes or Textural names	Range in relative percentage of soil		Separates
	Sand	Silt	Clay
1. Sandy soil	85—100	0—15	0—10
2. Loamy sand	70—90	0—30	0—15
3. Sandy loam	43—80	0—50	0—20
4. Loam	23—52	28—50	7—27
5. Silt loam	0—50	50—88	0—27
6. Silt	0—20	8—10	0—12
7. Sandy clay loam	45—80	0—28	20—35
8. Clay loam	20—45	15—53	27—40
9. Silty clay loam	0—20	40—73	27—40
10. Sandy clay	45—65	0—20	35—45
11. Silt clay	0—20	40—60	40—60
12. Clay	0—45	0—40	40—100

2. Structure of soil—Soil separates (sand, silt and clay) are held together in clusters or shapes of various sizes, called **aggregates** or **peds**. The arrangement of these peds in earth crust is called **soil structure**. Like texture, there are many types of soil structure. Soil peds may be classified as **granular, crumb-like, plate-like** or **platy, blocky, prismatic** and **columnar**. The properties of different soil peds can be tubulated in following table :

Table : Characteristics of peds.

Types of ped	Properties
1. Granular	Small, spherical and non-porous.
2. Crumb-like	Small, porous and spheroidal.
3. Agranular blocky	Block-like with sharp ends, one end may be pointed.
4. Sub-granular blocky	Block-like but bounded by other aggregates.
5. Platy	Plate-like, sometimes plates are overlapped.
6. Prismatic or columnar	Prism-like but without rounded surface.

Structureless soil can be either single-grained or massive soil aggregates (peds) tend to become larger with increasing depth. Structure is influenced by texture, air, moisture, organic nutrients, micro-organisms, root-growth and soil chemical status.

3. **Soil colour**—Soils exhibit a variety of colours. Soil colour may be inherited from the parental material (*i.e.*, **lithochromic**) or sometimes it may be due to soil forming processes (**acquired or genetic colour**). Though colour has little direct influence on the functions of a soil, but is important in the identification of soil type. In temperate regions dark-coloured soils generally are higher in organic matter than light coloured ones. Well-drained soil may range anywhere from very pale brown to dark brown and black, depending upon the organic matter-content. Red and yellow soils are the result of iron oxides, the bright colours indicating good drainage and good aeration. Other red soils obtain their colour from parent material and not from soil-forming processes. Well-drained yellowish sands are white sands containing a small amount of organic matter and such colouring matter as iron oxide. Red and yellow colours increase from the cool regions to the equator. Quartz, kaolin, carbonates of lime and magnesium, gypsum and various compounds of ferrous iron give whitish and grayish colours to the soil. The grayest are permanently saturated soils in which the iron is in the ferrous form.

Soil colour and temperature—Soil colour influences the soil temperature. The dark coloured soils absorb heat more readily than light coloured soils. Ramdas *et. al.*, (1936) showed that black cotton soils of Poona absorbed 86% of the total solar radiations falling on the soil surface as against 40% by the grey alluvial soil.

PHYSICAL PROPERTIES OF SOIL

Soil possesses many characteristic physical properties like density, porosity, permeability, temperature, water and atmosphere, each of which can be studied under following separate headings :

1. **Soil density**—Average density of soil is 2·65 gms. per ml. Density of soil varies greatly depending upon the degree of weathering.

2. Porosity—The space present between soil particles in a given volume of soil are called **pore spaces**. The percentage of soil volume occupied by pore space or by the interestitial spaces is called **porosity** of the soil. Porosity of soil depends upon the texture and structure compactness and organic contents of soil. Porosity of the soil increases with the increase in the percentage of organic matter in the soil.

The pore spaces are of two types—**(1) Micro-pore spaces** (capillary pore spaces) and **(2) Macro-pore spaces** (non-capillary pore spaces). Capillary pore spaces can hold more water and restrict the free movement of water and air to a considerable extent, whereas macro-pore spaces have little water holding capacity and allow free movement of moisture and air in the soil under normal conditions.

3. Permeability of soil—The characteristic of soil that determines the movement of water through pore spaces is known as **soil permeability**. Soil permeability is directly dependent on the pore size, therefore, it is higher for the loose soil with large number of macro-pore spaces than it is for compact soil with numerous micro-pore spaces.

5. Soil temperature—Soil gets its heat energy from different sources such as solar radiation, decomposing organic matter, and heat formed in the interior of earth. The temperature of soil is effected by its colour, texture, water content, slope, altitude of the land and also by climate and vegetational cover of the soil. Evaporation of water from soil makes it cooler. Black soils absorb more heat than white soils. Sandy soils absorb more heat and radiate it out quickly at night than clay or loam soils.

The soil temperature greatly affect the physico-chemical and biological processes in the soil. For example, the germination of seeds, normal growth of roots and biological activity of soil-inhabiting micro-and macro-organism require proper and specific temperature.

6. Soil water—In soil, water is not only important as a solvent and transporting agent but in various ways it maintains soil texture, arrangement and compactness of soil particles and makes soil habitat livable for plants and animals. It comes in soil mainly through infilteration of precipitated water (dew, rain, sleet, snow, and hail) and irrigation. According to improved classification method of **Bonyoucos** (1920), soil water is classified into following types—gravitational and ground water, capillary water, hygroscopic water, combined water and water vapour.

(i) Gravitational water—In a well-saturated soil, the accessory (extra) amount of water displaces air from the pore spaces between soil particles and percolates downwardly under gravitational influence and finally it is accumulated in the pore spaces. This accumulated excess water of large soil spaces is called **gravitational water**. When this gravitational water further percolates down and reaches to the level of parent rock, it is called **ground water**. Both kinds of these soil waters are ecologically important in the leaching of nutrients

(ii) Capillary water—The water which held by capillary forces (*i.e.*, surface tension and attraction forces of water molecules) in smaller soil channels, when the gravitational water and ground water has been drained, is called **capillary water**. Capillary water occurs as a thin-film around soil particles in the capillary spaces and represents the normal available water to the plants. It remains in soil for long periods and carries with it nutrients in solution. Humus has more capillary water than soil minerals.

(iii) Hygroscopic water—Soil particles retain some water so tenaciously that the plants cannot absorb this. Such soil water is called hygroscopic water.

(iv) Combined water—Combined water is the water of chemical compounds held by chemical forces of molecules such as hydroxides of silicon, iron and aluminium. It is of no ecological significance.

(v) Water vapour—Some soil water occurs as moisture or water vapours in the soil atmosphere.

Further, the total amount of water present in the soil is called **holard**. The quantity of water that plant-roots can absorb out of holard is called **chresard** and that amount of soil water which cannot be absorbed by plant-roots is called **echard**.

6. Soil atmosphere—Gases found in pore spaces of soil profiles form the **soil atmosphere**. The soil atmosphere contain three main gases namely O_2, CO_2, and N_2. Soil air differs from atmospheric air in having more of moisture and CO_2 and less of O_2. The soil atmosphere is affected by temperature, atmospheric pressure, wind, rainfall, etc. Loam soils with humus contains a normal proportion of air and water (about 34% air and 66% water) and therefore are good for majority of crops.

CHEMICAL PROPERTIES OF SOIL

Soil is a mixture of various inorganic and organic chemical compounds and exhibits certain significant chemical properties, all of which can be discussed as follows :

(a) Inorganic elements and compounds of soil : The chief in-

organic constituents of soils are the compounds of following elements—Al, Si, Ca, Mg, Fe, K and Na. Soil also contains smaller amounts of compounds of following inorganic elements—B, Mn, Cu, Zn, Mo, Co, I, F, etc.

(b) Organic matter of soil : The cheif organic component of soil is humus which chemically contains—amino acids, proteins, purines, pyrimidines, aromatic compounds, hexose sugars, sugar alcohols, methyl sugars, fats, oils, waxes, resins, tannins, lignin and some pigments. Further humus is black coloured, odourless, homogeneous complex substance.

(c) Colloidal properties : As soil is composed of crystalloids and colloids, therefore, it exhibits all the physico-chemical properties which are related with these two soil particles. Colloids for example exhibit adsorption, electrical properties, coagulation, Tyndal phenomenon, Brownian movement, dialysis, etc.

(d) Soil pH : Many chemical properties of soils centre around soil reaction. As regards their nature, some soils are **neutral**, some are **acidic** and some **basic**. The acidity, alkalinity and neutrality of soils are described in terms of hydrogen-ion concentrations or pH values. A pH value of 7·0 indicates neutrality, a value above this figure (7·1—14·00) indicates alkaline conditions and a value below (0—6·9) indicates acid conditions. Normally, the pH value of soils lies between 2·2 and 9·6. In India, acidic soils (pH below 5·5 to 5·6) occur in the high rainfall areas of Western ghats, Kerala. Eastern Orissa, West Bengal, Tripura, Manipur and Assam. The saline, alkali or basic soils (called '*Usar*', contain pH upto 8·5) of India, occur in U.P., West Bengal, Punjab, Bihar, Orissa, Maharashtra, Madras, M.P., A.P., Gujrat, Delhi and Rajasthan.

Highly acidic and highly saline or alkaline soils often remain injurious for plant growth, micro-organisms, etc. Neutral or slightly acidic soil, however, remain best for the growth of majority of plants.

CLASSIFICATION OF SOILS

Soils are of various types as regards to their physical, chemical and biological properties. Similar to biology, in pedology, soils are classified into orders, suborders, great soil groups and subgroups, etc. All of these can be summarized in following table :

Table : Classification of soils.

Soil order	Soil sub-order	Great soil groups
A. Zonal soils	1. Humid region	(i) Podozolic yellow soil with black humic top soils. (ii) Red yellow podozolic soils.

Soil order	Soil Sub-order	Great soil groups
		(iii) Non-laterised red soils.
		(iv) Laterised red soils.
		(v) Degraded grey and black clay soils.
		(vi) Alluvial soils.
	2. Arid region (Desert soils)	(i) Red desert soils
		(ii) Dark soil
B. Interzonal soils	1. Calcimorphic (calcareous soils)	(i) Red soil (developed over limestone).
		(ii) Black soil (developed over limestone).
		(iii) Red soil (developed over marls)
		(iv) Black soil.
	2. Holomorphic	(i) Saline soil
	3 Hydromorphic	(i) Ground water lateritic soil
		(ii) Planosols.
		(iii) Organic soils.
C. Azonal soils	1. Lethosols	
	2. Regosols	
	3. Alluvial soil.	

MAJOR SOIL TYPES OF INDIA



hills of Deccan, Madhya Pradesh, eastern Ghats, parts of Orissa, Assam, Mysore and Kerala. Besides these there are mountain soils, desert soils, peaty and other organic soils. Peaty soils are mostly found in Kerala. They are black and acidic (pH 3·9).

SOIL AS HABITATS FOR ANIMALS

Soil is used by various animals as a refuge from enemies, to escape desiccation and extremes of temperature; as material for abodes, and as a highway. Clay is cold, dense, poorly aerated and though it holds much water, does not give up water readily to plants and animals. Sand is dry, loose (cave in readily) and has a variable temperature. Rock heats and cools quickly, has little available water and practically no air, and is usually too resistant to allow penetration by burrowing animals. Alkaline soils are "physiologically dry"

because the salts present hold the water so that it is not readily available to organisms. Humus has a great water capacity and gives up water readily, is usully well-aerated and furnishes nitrogen as well as mineral salts and water. It remains rich in biota. Peaty soils are raw humus and are uninhabitable for most animals because they are poorly aerated and have an acid reaction. In general, the character of the vegetation cover serves as a good index of the habitability of soils for animals; conditions that make plants flourish are usually favourable for animals.

SOIL FAUNA AND SOIL FLORA

Soil supports a wide array of organisms of different body-sizes and taxonomic groups. Generally, soil organisms are classified into three major groups namely **micro-fauna** and **micro-flora**, **meso-fauna**, and **macro-fauna**. Mesoflora and macroflora because occur above the surface of soil (land-surface), therefore, are excluded from this discussion :

1. **Microfauna**—It includes animals with body size within the range of 20μ to 200μ. It includes all Protozoa and small-sized mites, nematodes, rotifers, tardigrades and copepode Crustacea. Soil inhabiting **protozoans** like amoeba, ciliates, zoomastigine flagellates occur near the surface soils, while the testate forms like *Thecamoeba*, *Euglypha* and *Difflugia*, have a wider vertical distribution. The common terrestrial **polyclad** is *Bipalium*. The **nematodes** such as *Rhabditis*, *Diplogaster*, *Tylenchus*, *Heterodera*, *Aphelenchoides*, *Mononchus*, *Pratylenchus*, *Xiphinema* and *Criconemoides*, abound by as much as 1–3 million in raw humus soils to 20 million/m_2 in grass land soils.

2. **Microflora**—The microflora of soil includes bacteria, soil fungi, soil actinomycetes, blue green algae and algae. In soil, microflora bacteria form about 90 per cent of the total population. Fungi and algae together represent one per cent and actinomycetes cover only 9 per cent,

Soil bacteria grow fairly well in the neutral soils richly supplied with organic nutrients. Soil inhabitant bacteria fall into two categories namely—**autotrophic bacteria** and **heterotrophic bacteria**. The autotrophic bacteria derive their energy from the oxidation of simple carbon compounds or from inorganic substances and their carbon from the atmospheric CO_2. The common autotrophic bacteria of soil are nitrifying bacteria, hydrogen bacteria, sulfur bacteria, iron bacteria, manganese bacteria, carbon monoxide bacteria and methane bacteria. Most of soil bacteria are heterotrophic bacteria depending upon the organic matter of soil for their energy source and are

primarily concerned with the decomposition of cellulose, and other carbohydrates, proteins, fats and waxes. They bring about mineralization of organic matter of soil and release considerable amount of nitrogen, phosphorus and other nutrients for plants. The common nitrogen-fixing bacteria of soil are *Rhizobium* (occurs in root nodules of leguminous plants); *Azobacter* and *Clostridium pasteurianum* (the latter two are free occurring in soil).

Majority of **soil fungi** are found in acidic soils. They may be parasitic, saprophytic and symbiotic. **Parasitic fungi** of soil infect roots of plants and cause plant diseases such as cotton root rot and many kinds of wilts, rusts, blights and smuts. Certain wilt-forming fungi produce toxins which are harmful, for example, *Fusarium lini*, which causes wilt of flax (*Alsi*) secrets HCN and *Fusarium udum*, a fungus causing wilt of pigeon pea (*Arhar*) secretes fusaric acid in the roots of host plants. However, certain parasitic fungi produce growth stimulating substances for host plant. *Fusarium* sp., for example, have been found to secrete Gibberellin and Gibberellic acid ($C_{19}H_{22}O_6$). **Symbiotic fungi** of soil live on the roots of certain plants and both fungus and plants are benefitted. **Saprophytic fungi** depends on dead organic matter of soil and derive their energy from decomposition of the latter. They break down cellulose, lignin, and gum, sugars, starch, protein, etc., into simple gradients to be utilized by higher plants or nutrients.

Actinomycete fungi prefer saline soils and bring about the decomposition of organic matter such as cellulose. They produce a variety of antibiotics of great economic significance for man. The most important blue green algae of soil are those which fix nitrogen in soil. *Anabaena, Nostoc, Microcystis* are important nitrogen fixing blue green algae of soil. They also make soil aggregates because of having mucilage.

3. **Mesofauna**—Mesofauna include animals with body size within the range of 200μ to 1 cm. The micro-arthropods Acari (mites) and Collembola (spring tails) are important member of this group which also includes the larger nematodes, rotifers, and tardigrades, together with most of the isopods, Araneida (spiders), Chelognethi (pseudoscorpions), Opiliones (harvestmen), Enchytraeidae (pot-worms), insect larvae and smaller millipedes (Diplopoda), isopods and molluscs.

Among **annelids** the microscopic enchytraeids ore represented by *Enchytraeus fridericia* and *Achaeta lumbricellus*, which are more abundant in organic soils and forests than in grass land. *Oniscus, Porcellio,* and *Armadillidum* are the most common isopods (crustacean) of the tropics in the humid zone. Among the soil



arachnids, mites are the most predominant. Mites flourish in moist organic soils and certain mites such as *Galumna, Cepheus, Hemorobates* occur in lichens and mosses. Certain mites, such as, *Schelorbates* and *Brachychthornus* live in humus. The mites are saprophagous, predatory and phytophagous and are involved in the process of organic decomposition and its resultant processes. Certain arachnids like scorpions, *Thelyphonus, Galeodes,* and some spiders are crepuscular, hiding under rocks or in crevices in soil and in loose litter, and has no ecological significance in decomposition like other arachnids. Many opiliones or harvestmen occur in forest litter, frequently preying upon soil organisms.

Besides mites, only the pseudoscorpions or **chelognathae** occur in surface soils and most decaying vegetations. Of the xerophil litter inhabitants are *Stenatemnus indicus, Dhanus indicus, Fealla indica* and the hygrophil inhabitants living in the litter and under stones are—*Comsaditha indica, Tyrannochthonius madrasensis, Tyrannochithonius chelatus* and *Hygrochelifer indicus* (Murthy, 1964), feeding on Collembola, enchytraeids, etc.

The common millipedes or diplopodes of forest soil which are chief decomposers of soils are *Spirostreptus, Thyropygus, Glomeris, Arthrosphaera, Polydesmus, Iulis,* etc. **Tardigrades** or bear animalcules occur in surface layer of moist soils in grassland and being represented by the *Macrobiotus* and *Hypsibius.*

Among insects, apterygote Collembola form numerically the most important groups of soil insects. Others like Dermaptera, psocids, Dictyoptera, Isoptera, Coleoptera, a few Hymenoptera and some Diptera also occur in soil, sometimes as juveniles. The termites such as *Reticulotermes* and *Odontotermes* are important soil-dwellers of tropics and play an important role in the break up of organic materials and their mixing up with mineral soils. Among the Hymenoptera, ants are the most important soil dwelling forms. Among Collembola, Onchuridae, Isotomidae, Poduridae, and Entomobrydae are richly represented in the soil both in number and species composition. Large-sized Collumbola such as *Tomocerus, Entomobrya* and *Orchesella* occur in surface layer, while the smaller *Onychurus, Tullbergia,* etc., occur in deeper layers of soils.

Among Diplura (Mollusca), *Anajapyx, Japyx* and *Campodea* are often found in small numbers in moist soils under stones and in humus. Proturans are more abundant than Diplura and very common in moist forest and grassland soils which abound in species of *Eosentomon, Acerentomon* and *Acerentulus.*

1. A tremandous quantity of water exists on earth in three forms : gaseous, liquid, and solid. Not only is 75 per cent of the earth's surface covered by water but the atmosphere also contains an abundance of aqueous vapour, and the polar region is ice-covered. Moreover, at least 60 per cent of active protoplasm is water.

2. Water is an extremely inert body in relation to most other chemical substances.

3. It has unique thermal properties such as heat capacity, latent heat and higher freezing point.

(a) **Heat capacity :** Water has high heat capacity and it can withhold large amounts of heat. Because of the high heat capacity of water, oceans and lakes tend to maintain a relatively constant temperature, and therefore the temperature of the biosphere is relatively stable. This property of water is functionally important to animal life. For example, a 165lb. man at rest produces sufficient heat to raise his body temperature more than 32°C, but if the heat capacity of his body were to correspond to that of many other substances, this amount of heat production would raise his temperature 100–150°C.

(b) **Latent heat of melting and evaporation :** The latent heat of melting is the number of calories required to convert 1 gram of solid at the freezing point into 1 gram of liquid at the same temperature. This value is about 90 for water, and means that the amount of heat necessary to melt ice is the same as that required to raise the temperature of the resulting ice water to 80°C. The latent heat of evaporation is defined as the number of calories required to change 1 gram of liquid into vapour. For water this value is 536; therefore as much heat is required to boil away 1 gram of water as to raise the temperature of 536 grams through 1°C. These properties of water are important not only because they moderate the temperature of the biosphere, but also because they play a basic role in the evaporation of water and its precipitation as rain and as dew in the **hydrological (water) cycle.**

(c) **Thermal conductivity :** Although water is a poor thermal conductor compared to metals, among the common liquids it is excellent : for example, the conductivity value for silver is 1·10, for water, 0 0125, for alcohol, 0·00048, and for benzene, 0·00033.

(d) **Expansion before freezing :** The relationship between temperature and density, or mass per unit volume, is very unusual. When water is cooled from room temperature, it contracts, becoming denser until it reaches a maximum density at 3·94°C. If cooled further, it begins to expand again. At the freezing point (0°C) it

4. Macrofauna of soil : Macrofauna of soil includes those animals whose body size is greater than 1 cm. Here belong the majority of Lumbricidae, the Mollusca, the largest insects and arachnids and the soil-dwelling vertebrates.

Earthworms usually occur in abundance in alkaline and moist soils and sparse in acid soils. They have been proverbial for their influence on the process of decomposition of organic materials, breaking up litter fragments and mixing them throughly with mineral soils resulting in the formation of organic soils. Some of the common Indian annelidan species of soil are *Megascolex*, *Pheretima* *Octochaetus*, *Drawida* and *Moniligaster*. Among chilopods the carnivorous *Scolopendra* and *Lithobius* are common in moist soils feeding on leaf litter inhabitants.

Among soil vertebrates, following animals are well adopted for burrowing life in soils—*Ichthyophis*, *Cacopus* *systema*, *Breviceps* (Amphibia), *Sphenodon*, *Uromastix*, limbless lizards and snakes (Reptilia), *Talpa*, *Dasyurus*, *Notoryctes* and various insectivores and rodents (Mammalia).

C. HYDROSPHERE AND WATER

About three-fourth of the earth's surface (71%) is covered with **hydrosphere**, the main component of which is water. Water is one of the most unusual natural compounds found on earth, and it is also one of the most important. Life on earth began in the seas, and water in some form or other is absolutely essential to the maintenance of all life. Water is one of the main agent in pedogenesis (soil formation) and is the medium for several different ecosystems. It permeates the atmosphere and the outer layers of lithosphere and has uneven distribution on earth, so that, some of the great ocean depths are approximately six or seven miles (9750 meters). Further, water in its two forms, salt water and fresh water, forms two chief aquatic environments—namely **marine environment** and **fresh water environment** of earth. The oceans holding marine environment are two and one half times more extensive than land and provide over 300 times the living space, since they are habitable throughout their entire depth by certain groups of organisms. Water is obviously heavier than air which imparts a greater buoyancy to the aquatic medium, enabling organisms to float at variable levels. The most unique features of water concern its physical properties.

PHYSICAL PROPERTIES OF WATER

Henderson (1913, 1924) listed in his stimulating book, *The Fitness of the Environment*, the following characteristics of water which are favourable to biological systems :

1. A tremandous quantity of water exists on earth in three forms : gaseous, liquid, and solid. Not only is 75 per cent of the earth's surface covered by water but the atmosphere also contains an abundance of aqueous vapour, and the polar region is ice-covered. Moreover, at least 60 per cent of active protoplasm is water.

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expands markedly, unlike almost all other substances. Thus ice will always float on the top of a lake or stream, and it is very unusual for an aquatic ecosystem ever to freeze solid, unless it is very small.

4. No other compound compares to water as a **solvent**. So many different substances can be dissolved in it that it is known as the **universal solvent**. More things, in fact, can be dissolved in water than in any other liquid. This is especially true of inorganic chemicals which split, or dissociate to form electrically charged entities termed **ions**. Ionization influences most electrical phenomena and many chemical phenomena of solutions. It is probable that all natural elements are soluble in water, at least in trace amounts, and that they are all found in natural water at some place or other on the earth's surface. In addition, many organic chemicals are water-soluble. Thus, water is the main medium by which chemical constituents are transported from one part of an ecosystem to the other. It is the only medium by which these constituents can pass from the abiotic portion of the ecosystem into the living portion. Even in the driest of terrestrial environments, nutrient materials pass into the roots of plants in aqueous solution; when air is breathed by animals, oxygen is dissolved in water at the surface of the lung before it can cross the mucous membrane and be absorbed by the blood.

5. Water has the greatest **surface tension** of all common liquids, except mercury. The role of surface tension is most obvious in the way it allows certain things, such as pollen, dust, and water striders, to remain at the surface of a water body even though they are denser than the water. More important however, the high surface tension of water allows soils to contain a significant amount of water through capillary attraction and to make it avilable to terrestrial plants. In addition, surface tension has profound effect on certain basic physiological and biochemical reactions within living organisms.

Visualizing these unique physical properties of water, **Henderson** (1913, 1914) concluded that no other known substance could be substituted for water as a basic abiotic environmental factor. Now, ecologists have known certain other important physical properties of water which affect life in someway. Some of the additional important physico-chemical properties of water are following :

(i) Viscosity : Water is a fairly viscous liquid. Animals that live and move in water need to be much more streamlined than those that move through air, because the resistance to motion in a viscous medium is high. But at the same time, the viscocity of water allows organisms to swim using relatively simple movements.

Further, high viscosity of water protects the aquatic animals and plants from the mechanical disturbances.

(ii) Buoyancy : Water is a buoyant medium. Organisms can exist in it without specialized supportive structures such as are needed by organisms that inhabit terrestrial environments.

(iii) Transparency : Water is a transparent medium. Its transparency enables the penetration of light to the depths where it is ultimately absorbed. Different wave lenghts are absorbed at different depths. The long heat waves are stopped near the surface. Shorter waves with more energy penetrate successively farther. The ultraviolet rays penetrate beyond 100 meters. The zone upto which light rays penetrate is called as **photic zone** and below this zone there is complete darkness and organisms that require light cannot live. The transparency of water is greatly affected by the presence of suspended particles, phytoplankton, etc., which absorb light and so penetration of light in turbid water is less.

(iv) Pressure : Organisms living at sea level experience a pressure of about 15 psi, which is defined as 1 atm (760 mm of Hg). Pressures increase with increased depth of water at the rate of one atmosphere (1 atm) for every 10 meters of descent. Organisms inhabiting the floor of deep-sea areas at depth of 10,500 meters are exposed to hydrostatic pressure of about one ton per square centimeter. Pressure influences solubility, ionic dissociation, and surface tension, and water is slightly compressible with increased pressures.

(v) Salinity : Salinity has been defined as "the total amount of solid material in grams contained in one kilogram of the water, when all the carbonate has been converted into oxide, bromine and iodine replaced by chlorine and all organic matter completely oxidised." All types of natural waters contain various amounts of differents salts (ions) such as Na, K, Mg, Cl, SO_4, PO_4, CO_3; HCO_3, NO_3, etc. and all these salts are responsible for the **saltiness, salinity** or **salt content** of water. The salinity of marine water is rather constant being about 3·5‰. The salinity of fresh water varies greatly. Some salt lakes may have a salinity of 25‰ to 30‰ which greatly restricts life in them.

Table : Comparison of some of the principal ions found in different kinds of water.

Water	Na	K	Ca	Mg	Cl	SO_4	CO_3	Total per liter
1. Soft water	0·016	—	0 01	0 0005	0 019	0 007	0 012	0·065
2. Hard fresh	0 021	0 016	0 62	0 014	0 041	0·025	0 119	0 301
3. Sea	10 56	0·30	0·40	1·27	18 98	2 65	0·71	34 85
4. Great salt lake	65 54	3·76	0 065	4 47	110 08	13·04		197·51

Salinity of water acts as an important limiting factor for the distribution of a number of species of plants and animals. Certain animals such as, *spider crab*, *Maia*, etc., can tolerate only narrow fluctuations in salinity of water and are known as **stenohaline animals**. While some animals such as *Mytilus*, *Aplysia* etc., can withstand wider ranges of salinity and are called **euryhaline animals** However, there are certain animals, such as, *Anguilla*, *Salmon*, etc., which are both stenohaline and euryhaline.

CHEMICAL PROPERTIES OF WATER

Water consists primarily of a single compound, H_2O. It is a universal solvent and most chemical compounds ionize readily in water and provide many radicals and considerable versatility in the rearrangement of chemical substances. It has following chemical properties :

a. Solubility of gases in water : Most gases dissolve readily in water, most notably those that are essential for life. The concentration of any gas in water generally varies between zero and a theoretical maximum or saturation. The latter is the amount of gas that can be dissolved in water when the atmosphere and the water are in equilibrium with one another. Except for water falls and very-turbulent streams, the water in natural ecosystem is seldom in equilibrium with the atmosphere. A gas may show a deficit, if it is being utilized in the ecosystem faster than it is going into solution across the air water interface, or it may be supersaturated, if it is being produced in the ecosystem faster than it is being released from solution across the interface. The concentration of important gases may vary from widely in any ecosystem, both horizontally and vertically.

The saturation level of any gas in water depends on several variables, most notably temperature, salinity, the concentration of the gas in the atmosphere, and its relative solubility in water. The greater the concentration of a gas in the atmosphere, the greater its concentration in the water will tend to be, depending on its relative solubility in water.

Table : Comparison between equilibrium concentrations of important gases in the atmosphere and in water (Clapham, Jr. 1973)

Gas	Atmospheric concentration	Saturation in water
Oxygen	210 cc./l (21%)	7 cc /l (32·9%)
Nitrogen	780 cc./l (78%)	14 cc /l (65·7%)
Carbon dioxide	0·3 c.c /l (0·03%)	0·3 cc /l (1·4%)

1. Oxygen : One of the most critical factor in an aquatic environment is the amount of oxygen in the water because most living organisms (excepting anaerobic forms) require this gas for respiration. In contrast to atmosphere, the oxygen becomes limitting factor for aquatic animals as the saturation concentration of oxygen in water is governed by temperature and salinity. As is evident in accompanying table, the lower the temperature, the greater the oxygen-retaining capacity of water, wether it is fresh water or sea water.

Table : Comparison of the saturation concentration of oxygen in freshwater and salt water environments with varying temperatures.

Water	Temperature, °c	Saturation point in milliliter per litre
Fresh water	0	10·27
Salt water	0	8·08
Fresh water	30	5·57
Salt water	30	4·52

In fresh water areas of any depth and in the salt water habitat, there are three recognizable zones with regard to oxygen concentration. A surface stratum, where the oxygen tends to be in equilibrium with the atmosphere above. Below this surface stratum of variable depth is an intermediate stratum where oxygen values fluctuate in accordance with existing factors. Respiration, decomposition of organic materials (stagnant ponds) and stream pollution all tend to reduce the amount of available oxygen, while photosynthetic activity will often balance or more than balance oxygen loss. The deepest layers of water will usually have a very low oxygen concentration in the deeper lakes and oceanic areas because the continual decomposition of organic debris, the respiration of organisms inhabiting these deeper waters, and the complete absence of photosynthetic activity in these lower strata will tend to deplete the oxygen concentration. The deep stratum is entirely dependent upon the slow transport of oxygen from the overlying intermediate layer.

Because oxygen is needed for the respiration of all heterotrophs, it tends to be taken out of solution continuously by these organisms. The only means by which it can enter the water are via solution at the air-water interface or through photosynthesis by aquatic plants. Oxygen is transferred to deeper water by diffusion or through circulation of the water. The rate of oxygen solution at the surface is

highly variable, depending mainly on the turbulence of the water and the presence of waves spray and foam.

Some organisms (*e.g.*, aquatic beetles, true bugs, fresh water snails, larvae of mosquitoes, whales, seals, porpoises, alligator, crocodiles, etc.) are independent of the oxygen concentration in water because they move to the surface and obtain atmospheric oxygen. Ingenious devices such as air tubes or the ability to trap air bubbles in surface hairs or under wings cover are utilized by some of these forms to insure submersion for an interval of time. Some bacteria and animals (*e.g. Teredo, Mytilus edulis*, etc.) can live anaerobically by breaking down glucose, glycogen and other carbohydrates and obtain oxygen from this source.

2. Nitrogen : Nitrogen is significantly less soluble in water than oxygen. But because it constitutes 78 per cent of the atmosphere, it still accounts for about 65 per cent of the dissolved gases at equilibrium. It is fairly inert chemically and does not react with water, although some bacteria, fungi, blue-green algae, and so on, can use it to satisfy their nitrogen requirements, and other bacteria can produce it through reduction of nitrate under conditions of very low oxygen concentration.

3. Carbon dioxide ; The decomposition of organic matter and the respiratory activity of aquatic plants and animals produce carbon dioxide. This gas is one of the essential raw materials necessary for photosynthetic activity by green plants. Carbon dioxide combines chemically with water to produce carbonic acid (H_2CO_3), which influences the hydrogen ion concentration (pH) of water. Carbonic acid dissociate to produce hydrogen (H^+) and bicarbonate (HCO_3^-) ions. The bicarbonate radical may undergo further dissociation forming more hydrogen (H^+) and carbonate (CO_3^-), as represented in following reactions :

$$\underset{\text{atmospheric}}{CO_2} + H_2O \underset{\text{air-water interface}}{\rightleftharpoons} \underset{\text{dissolved}}{CO_2} + H_2O \rightleftharpoons \underset{\text{carbonic acid}}{H_2CO_3} \rightleftharpoons H^+ + \underset{\text{bicarbonate}}{HCO_3^-}$$

$$\downarrow\uparrow$$

$$2H^+ + \underset{\text{carbonate}}{CO_3^-}$$

The amount of free or uncombined carbon dioxide in water is of ecological importance : it governs the precipitation of *calcium* in the form of calcium carbonate ($Ca\,CO_3$). Calcium precipitates when temperatures and salinity are high and the amount of uncombined carbon dioxide is low. This means more carbonate (CO_3^-) is present to combine with the calcium cation (Ca^{++}). These conditions exist in shallow tropical waters, where evaporation is high. This raises the salinity and photosynthetic activity of plants and

reduces the quantity of free carbon dioxide in water. The precipitation of calcium carbonate in tropical areas as the Bahamas explains the preponderance of thick calcareous shells of shallow water tropical molluscs, plankton and algae. In deep oceanic waters, temperatures are low and there are no photosynthetic plants, consequently, the carbon dioxide content of the water is high. Deep water fauna (molluscs, crustaceans) possess very fragile skeleton because the precipitation of calcium carbonate is minimum.

4. Hydrogen sulfide : The deeper layers of many bodies of water, including ponds, lakes, and some estuaries, may contain significant amounts of the toxic gas, hydrogen sulfide, which is released by decaying organic matter. If concentrations of the gas build up, all life but anaerobic bacteria excluded from the area (*e g.*, deeper strata of Black sea).

b. Hydrogen ion concentration (pH) : One property of natural waters is their acidity or alkalinity. About one water molecule in every 10,000,000 splits into two, producing a hydrogen ion (H^+) and a hydroxyl ion (OH^-) :

$$H_2O \rightleftharpoons H^+ + OH^-$$

In pure water, there are equal numbers of H^+ and OH^- ions, it therefore has a **neutral reaction.** Some natural waters, however, acquire an excess of H^+ and are **acidic,** while others, with an excess of OH^- are **alkaline.** For example, in fresh water habitats such as acid bogs, swamps and drainage streams carrying water from these areas are acidic, contain pH value as low as 1.4. Despite the high acidity, rich populations of acidophilic flora and fauna thrive under such conditions. On the other hand, certain lake waters may be quite alkaline, particularly in lime stone areas, where the pH may range from 10 to 12. Basophilic plants and animals are found in these areas. In a vast majority of fresh water localities pH value range between 5·5 and 8·5. Oceanic areas exhibit little change in pH value over vast areas because of the effective buffering action of anions such as HCO_3^- and $CO_3^=$. Surface waters of open ocean have pH values of 8·0 to 8·4, but deeper water is closer to neutrality (pH 7·4 to 7·9). There is often a wider range of pH values in shallow marine waters, estuaries and tide pools.

HARD AND FRESH WATERS

Water containing soluble salts of calcium and magnesium such as chlorides, sulphates and bicarbonates, is called **hard water.** The hardness of water may be of two types—**temporary hardness** and **permanent hardness.** Temporary hardness is caused by dissolved calcium bicarbonate or magnesium bicarbonate. Temporary hard

water can be converted into soft water by boiling, as at higher temperatures soluble bicarbonates of calcium and magnesium are converted into insoluble carbonates of calcium and magnesium. Permanent hardness of water is caused by dissolved chlorides or sulphates of calcium and magnesium. This hardness cannot be removed by boiling and requires some specific chemical treatments

THE HYDROLOGIC CYCLE

Water continuously circulates between the atmosphere and the earth's surface (lithosphere) in what is known as the **hydrologic cycle.** The energy for driving this cycle and thus ensuring a constant supply of fresh water on land comes from the sun. Solar heat evaporates water from the ocean which is the great reservoir of water. A lesser amount of water is also evaporated from the surface of the land and from plants, a process known as **evapotranspiration.** All these vaporized water forms clouds which moved by winds, may pass over land where they are cooled enough to precipitate the water as rain or snow. Some of the precipitated water soaks into ground, some runs off the surface into streams and goes directly back to the sea. The ground water is returned to the surface by springs, by pumps and by transpiration—the movement of water in plants from roots to leaves. Water inevitably ends up back in the sea, but it may become incorporated into the bodies of several different organisms, one after another, en route.

EFFECT OF FACTOR OF AQUATIC ENVIRONMENT ON AQUATIC ORGANISMS

The aquatic environment is subject to water movements ranging from small vertical circulations to strong currents. The streams have a unidirectional movement and in seas the movement is reversible. Many aquatic animals have accordingly taken to sedentary or sessile lives depending on water movements. Radial symmetry is a characteristic of such animals. Transformation from a sessile to a locomotive existence favours a bilateral symmetry. The water currents of water often abrade the inhabiting flora and fauna and varied modifications are encountered to withstand this abrasive action. Thick scales, strong shells and many attachment devices like the hold-fasts and suckers all are the results of this environmental stress. The ability to breathe air dissolved in water, at times even resorting to anaerobic existence, the modification of various senses to respond to stimuli characteristic of aquatic environments, the phenomenon of osmoregulation, and above all the phenomenon of external fertilization are other spectacular physiological adaptations to live in an exclusively aquatic medium.

RELATION OF ANIMALS TO MOISTURE

Water makes up a large proportion of the bodies of plants and auimals, whether they live on land or in water. Active cytoplasm holds about 70—90 per cent of water. It has several important phyciological properties. Animal obtain water (*i*) by drinking, (ii) by absorbing it through their skin from contact with some damp ground, (*iii*) directly from their food or (*iv*) from water produced by metabolism. The method of obtaining water and the relation to the supply of liquid water as well as resistance to the drying effects of the surrounding atmosphere are important in determining the distribution of animals. Different animals have evolved following adaptive features to live in drying atmospheres—

1. **Impervious skin :** The drier habitats (deserts, etc.) are invaded by only those animals which contain a thick impermeable body covering. Such integuments occur in many insects, birds and mammals. Some mammals like men, apes and horses lose much water (and salt) through sweat glands in heat regulation. Most rodents and some ruminants like antelope nearly or completely lack sweat glands. Moist skinned forms (most amphibians and earthworms), certain mites and softbodied insects are restricted to swamps, stream margins, moist soils and other similarly damp places.

2. **Internal lungs or tracheal system :** Mode of respiration has some correlation with water. Crustaceans, with their gills covered by a water-retaining carapace, carry with them a liquid environment for their gills. The scaly body covering of a fish may be practically impermeable to water and exchange of gases may be limited to gills and gut. Internal lungs, whether in pulmonate snails, land isopods, spiders or higher vertebrates (amphibians, reptiles, birds and mammals) together with the internal tracheal system of insects are water saving. Much water is lost by in breathing even in animals having internal lungs

3. **Dry excretion :** A further water-saving device is the excretion of concentrated, relatively dry nitrogenous and faecal waste materials. Water-saving insects, reptiles and birds excrete nitrogenous waste as solid uric acid.

4. **Suspended animation :** Certain animals, usually with simpler organization, such as rotifers, nematodes, tardigrades, desert snails, etc., retain their vitality in long dry environment. Other forms (frogs, toads, etc.) **aestivate** during droughts and are active during moist season of the year.

5. Migration : Many birds and mammals of arid zones migrate when water becomes scarce or as a result of drought or for other reasons, the food supply is less.

6. Water from food and from metabolism : Most herbivores and carnivores live on the moisture obtained with food. Many insects utilize the high water contents of plants to meet their water requirements. In fact, most animals make use of water released during metabolism when fats and carbohydrates are broken and water is also produced by the oxidation of hydrogen or carbon in the body of the animal. The camel in the desert can go on for 11 or more days without drinking water since it can use water of metabolism obtained from the oxidation of the fat in its hump as well as water stored in special compartments in the stomach.

AQUATIC FLORA AND FAUNA

With a few exceptions, almost all the taxonomic classes of micro-organisms, plants and animals have their representatives in aquatic environment. Each of them possessing specific morphological and physiological adapatations to exploit its specific environment fully. They will be discussed in the following chapters.

SNOW AS HABITAT

Snow which is the solid state of liquid water, is an integral factor in the physical environment. It is important particularly in the higher latitudes and on the higher parts of mountains where snow cover may last through the year. Its significance is increased by the large amount of land surface in northern Eurasia and North America. Snow covers much of the available food for many animals and is a factor causing migration, both of birds and mammals, toward areas that are somewhat free from its direct influence. Certain mammals (rodents, etc.) tunnel through the snow near the underlying masses and get access of food and often protection from winter cold. In the Tundra, the non-hibernating lemmings (*Dicrostonyx*) breed during winter beneath the snow. Snow prevents the locomotion of mammals and of running birds, and animals with long feet only are well adapted for moving on snow cover.

CHAPTER 3

ABIOTIC ENVIRONMENTAL FACTORS

The distribution, abundance, growth and reproduction of the organisms comparising the individual members of species populations are controlled by certain **environmental factors.** These factors are of two basic sorts : **abiotic environmental factors,** which determine the interactions between the population and the abiotic environment and **biotic environmental factors,** which may refer to interactions between different populations or to instinctive control mechanisms that are internal to the population itself (Clanham,1973). In previous chapter, a discussion has been made of three fundamental media, namely soil, water and air which provide necessary conditions for sustaining life and which act as abiotic factors. In the present chapter, all the remaining abiotic environmental factors will be discussed.

NATURE OF RESPONSE OF ORGANISMS TO ABIOTIC ENVIRONMENTAL FACTORS

Probably the first response of any organism to a change in the environment is physiological (Kendeigh, 1974). Different abiotic environmental factors influence organisms physiologically in various ways (Fry, 1947). These effects may be classified as follows :

1. **Lethal**—Causing death ; for instance, extreme heat or cold, lack of moisture, and so forth.

2. **Masking**—Modifying the effect of some other factor. Low relative humidity increases the rate of evaporation of moisture from body surfaces so that warm-blooded animals are able to survive at otherwise intolerably high air temperatures.

3. **Directive**—Producing an orienting response in relation to some environmental response so that the organism gets itself into favourable conditions.

4. **Controlling**—Influencing the rate at which some process functions, but not entering the reaction. Temperature, pressure, and viscosity, for instance, affect metabolism, secretion, and locomotion.

5. **Deficient :** Some abiotic environmental factors curtail an activity of an organism because some essential ingredient, such as salt, oxygen, or the like, is absent or at unfavourably low concentration.

Further, the same abiotic environmental factor may produce different effects at different times and under different conditions. Temperature may be lethal, if extreme ; masking, as when cold reduces the demand of cold-blooded organisms for food ; directive, by inducing a search for more favourable locations ; or controlling, as a modifier of the rate of metabolism.

ESSENTIAL ELEMENTS AND LIMITING FACTORS

The individual organisms of species population, in order to grow and multiply, must be supplied with certain essential materials. Of the hundred four (*i.e.*, 104) naturally occurring chemical elements on the earth, all living organisms are believed to utilize only 16 different chemical elements for their survival. These are called **essential elements.** Several other elements are needed in small quantities by some species. They are summarized in following table :

Table : Essential elements (Clapham, 1973).

Macronutrients : Elements used in relatively large quantities.	**Micronutrients : Elements generally needed in relatively small quantities.**	***Micronutrients : Element needed by certain species in relatively small quantities.***
Carbon	Iron	Sodium
Hydrogen	Manganese	Vanadium
Oxygen	Boron	Cobalt
Nitrogen	Molybdenum	Iodine
Phosphorus	Copper	Selenium
Calcium	Zinc	Silicon
Magnesium	Chlorine	Fluorine
Sulphur		Barium

Some of these tabulated elements are used in *relatively large* quantities as the fundamental building blocks of organic tissues and are called **macronutrients.** Others, the **micronutrients,** are used in much smaller quantities. The micronutrients are also known as **trace elements.** For instance, a crop such as corn will remove over 45 kg. of nitrogen per acre from the soil but only 10—15 g. of boron. In addition to the nutrients required by all species, some organisms have special nutrient requirements.

THE LIEBIG-BLACKMAN LAW OF LIMITING FACTORS

An organism is seldom, if ever, exposed solely to the effect of a single factor in its environment. On the contrary, an organism is subjected to the simultaneous action of all factors in its immediate surroundings. However, some factors exert more influence than do others, and the attempt to evaluate their relative roles had led to the development of the law of the minimum by the German biochemist Justus Liebig in 1840.

Liebig while investigating the relationship between the available amounts of essential elements and plant growth, discovered that crop yield was frequently limited by elements other than those utilized in the largest quantity. Freely translated, a part of his statement on his experimental results is that "*growth is dependent on the amount of food stuff that is presented in minimum quantity.*" This statement has come to be known as Liebig's law of minimum. It is now usually incorporated with a law of limiting factors developed by a British physiologist F. F. Blackman (1905), who at the beginning of this century investigated the factors affecting the rate of photosynthesis. He listed five factors involved in controlling the rate of photosynthesis : amount of CO_2 available, amount of H_2O available, intensity of solar radiation, amount of chlorophyll present, and temperature of the chloroplast. Blackman discovered that the rate of photosynthesis is governed by the level of the factor that is operating at a limiting intensity. Further work on limiting factors and substances has shown that a high level of one factor will modify the limiting effect of a second, a process described as **factor interaction.** The same principle of limiting factors applies well to animal functions.

Since the rate of a process may be controlled by too great an amount of a substance, such as heat, as well as by too small an amount, and since the presence or abundance of an organism may be limited by a variety of environmental factors, biotic as well as abiotic, and since the limiting effect may be due to two or more interacting factors rather than a single isolated one (Shelford, 1952), the law of minimum has been restated by Taylor (1934) in broad ecological terms, as follows—"*The functioning of an organism is controlled or limited by that essential environmental factor or combination of factors present in the least favourable amount. The factors may not be continuously effective but only at some critical period during the year or perhaps only during some critical year in a climatic cycle.*"

THRESHOLD AND RATE

Every environmental factor varies through a wider range of intensity than any single organism can tolerate characteristically.

there is for each individual organism a lower and an upper limit in the range of an environmental factor between which it functions efficiently. For any one factor, different organisms find optimal conditions for existence at different points along the range, hence their segregation into different habitats.

The **thresold** is the minimum quantity of any factor that produces a precipiteableeffect on the organism. It may be the lowest temperature at which an animal remains active, the least amount of moisture in the soil that permits growth of a plant, the minimum intensity of light at which a photoreceptor is stimulated, and so forth. Above the threshold, the rate of a function increases more or less rapidly as the quantity of heat, moisture, light, or other environmental factor is increased, until a maximum rate is attained. Above the maximum, there is usually a decline in the rate of a process either because of deleteriouseffect produced, the interference of some other factor or exhaustion.

SHELFORD'S LAW OF TOLERATION

Organisms may be limited in their growth and their occurrence not only by too little of an element or too low an intensity of a factor but a[illegible]t or too high intensity of the factor. [illegible]necessary for the growth of all green [illegible]ntration of carbon dioxide in the atmosphere will, under certain circumstances, increase the rate of plant growth, but very considerable increases become toxic. Likewise, small additions of arsenic to the human diet actually have a tonic effect, further increase in the dosage, however, soon proves *fatal*.

The idea that factors could be limiting at their maximum as well as minimum quantities was incorporated in **law of toleration** formulated by **V. E. Shelford** in 1913. This law postulates that each ecological factor to which an organism responds has maximum and minimum limiting effects between which lies a range or gradient that is now known as the **limits of tolerance**.

Between the **lower** and **upper** limits of tolerance lies a broad

[illegible]

optimum and narrow **zones of physiological stresses** in between the range of optimum and lethal zones.

Upper and **lower limits of tolerance** are intensity levels of a factor at which only half of the organisms can survive. These

limits are sometimes difficult to determine, as for example with low temperature, organisms may pass into an inactive, dormant, or hibernating state from which they may again become functional when the temperature rises above a threshold. At high tempertures, there may be similar inactivation or aestivation before the lethal level is attained. Even without dormancy occurring, there are normally zones of physiological stress before the limits of tolerance are reached.

The species as a whole is limited in its activities more by conditions that produce physiological discomforts or stresses than it is by the limit of toleration themselves. Death verges on the limits of toleration, and the existence of the species would be seriously jeopardized if it were frequently exposed to these extreme conditions. Therefore, in retreat before conditions of physiological stress there is a margin of safety, and the species adjusts its activities so that limits of toleration are avoided. There is a variation in hardiness of individuals within a species, so that some hardy individuals find existence possible under conditions that disrupt other individuals. The population level of a species becomes reduced before the limits of its range are actually reached.

Further, species vary in their limits of tolerance to the same factor. For example, the Atlantic salmon spends most of its adult life in the sea, but goes annually into fresh water streams to breed. Most other marine fishes are killed quickly when placed in freshwater, as are freshwater fishes when placed in salt water. The following terms are used to indicate the relative extent to which organisms can tolerate variations in environmental factors. The prefix *steno-* means that the species, population, or individual has a narrow range of tolerance and the prefix *eury-* indicates that it has a wide range.

Table : Terminology for ranges of certain abiotic environmental factors.

Stenothermal — Eurythermal	Pertaining to temperature
Stenohaline — Euryhaline	Pertaining to salinity
Stenohydric — Euryhydric	Pertaining to water
Stenophagic — Euryphagic	Pertaining to food
Stenobathic — Eurybathic	Pertaining to depth
Stenoecious — Euryecious	Pertaining to niche or habitat selection

Moreover, a plant or animal may have a wide range of tolerance for one factor in the environment, but a relatively narrow range of tolerance for another condition. Thus, we find that some species of freshwater fishes are eurythermal but they are stenohaline.

TYPES OF ABIOTIC ENVIRONMENTAL FACTORS

The abiotic environmental factors can be classified into two basic classes namely **physical abiotic factors** including **temperature,** quantity and spectral quality of **light,** depth of water (*i.e.*, **pressure**), **geomagnetism, gravity, wind** and **precipitation** ; and **chemical abiotic factors** which include such factors as **nutrient availability** and **pH.**

TEMPERATURE

Temperature is one of the essential and obvious changeable environmental factors. It penetrates into every region of the biosphere and profoundly influences all forms of life by exerting its action through increasing or decreasing some of the vital activities of organism, like behaviour, metabolism, reproduction, ontogenetic development (*viz.*, embryogenesis and blastogenesis) and death. Temperature is a universal influence and is frequently a limiting factor for the growth or distribution of animals and plants. Normal life activities go on smoothly at a specific temperature or at a specific range of temperatures. This is called the **optimum temperature** or the optimum range of temperature. Organisms react to any rise or fall of the optimum temperature range and biotic communities more often encounter alterations only due to extremes of temperature (*viz.*, **minimum** or **maximum** temperatures). The interaction of temperature with certain other abiotic environmental factors such as humadity, etc., cause into many other climatic changes which influence the living organisms in one way or another.

NATURE OF TEMPERATURE

Temperature is a measure of the intensity of **heat** in terms of a standarized unit, and is commonly expressed as degrees on either the **Fahrenheit** or **Celsius scale.** Heat is a form of energy, and as such is necessary for the very existence of life. Heat may be received or lost by animals as molecular vibrations transmitted from one part of an environment to another by radiation, convection, or conduction. It may be received as radiant energy, of course largely from the sun. Heat may be produced by mechanical work as in Hawk moth, the heat is generated by vibrating the wings before take-off ; or by chemical reactions.

HEAT BUDGET

The temperature at the earth's surface is governed by the brightness of the sun ; the constancy of brighthess of sun has remained virtually unchanged for about 3 billion years. In fact, average terrestrial temperatures now probably do not differ radically from those at the earth's beginning (Schwarzschild, 1967). The total amo-

unt of heat entering the biosphere from the sun must balance the amount lost per unit time if temperatures are to remain unchanged, since the flow of geothermal heat from the interior of the earth is small by comparison and probably has been negligible for at least the past 500 million years. The estimate of this energy flow is referred to as the **heat budget** (**Vernberg** and **Vernberg**, 1970). Heat budget have been projected for the total surface of the earth as well as for special environments.

TEMPERATURE FLUCTUATIONS IN DIFFERENT ENVIRONMENTS

Environmental temperatures fluctuate both daily and seasonally. Different environments such as fresh water, marine and terrestrial environments, are subject to varied reponses to fluctuating temperatures. Temperature fluctuations are comparatively less in the aquatic environment than in the terrestrial environment. The increase in depth of aquatic medium often increases the temperature fluctuations. There exists a distinct difference in the response of living organisms of the freshwater and sea to temperature fluctuations because of the presence of dissolved salts in sea water. The minimum temperature in the sea is —3° C, while in freshwater ponds it never goes below 0°C. The maximum temperature of ocean generally goes upto 36°C, but in shallow pools of fresh water and tide pools of littoral zone, temperature may go higher. In deeper bodies of water, heating and cooling are restricted to the surface strata. But the deeper layers also get a lot of heat as a result of what is usually termed **vertical circulation**, where in, due to circulatory movement of water, surface waters are brought to the deeper regions, and vice versa. Studies on the vertical changes of temperature have led to the hypothetical classification of the freshwater media into three strata. The superficial layer of freshwater is constantly stirred by wind and is called **epilimnion**. It is the layer of warmer

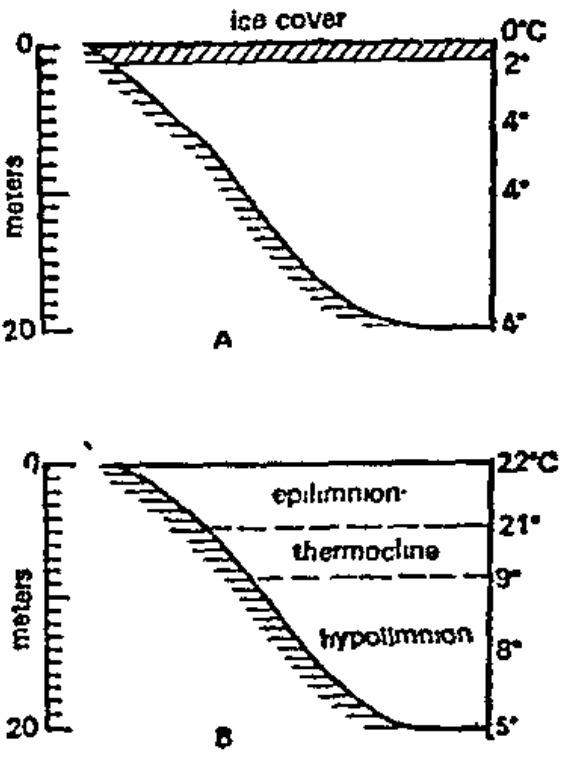


Fig. 3·1. Thermal stratification of a deep lake of the temperate region. A-during winter, B-during summer.

water and its temperature may rise upto 27°C during summers. The stagnant water of the bottom constitutes the **hypolimnion**. The hypolimnion has temperature of 5°C only. In between epilimnion and hypolimnion occurs an itermediate zone called **thermocline** or **metalimnion**, which has rapid vertical temperature changes. During winter, the temperature of epilimnion of a freshwater lake becomes 0°C and the lake becomes ice covered. The process of differentiation of fresh-water habitat into these three strata is called **thermal stratification.**

In the terrestrial environment the seasonal and daily fluctuatations in temperature are varied and marked. The lowest temperature recorded for any land mass is —70°C (Siberia in 1947). Higher temperatures may likewise go often 85°C as in certain deserts at noon. However, the water in hot springs and geysers may approach 100°C, and even higher temperatures occur sporadically in the very special situations presented by volcanic areas. In Rajasthan the highest temperature exceeds 50°C. On land, diurnal variations of temperature are quite staggering. The fluctuation between day and night temperatures may be 17°C as in ordinary land masses or 40°C as in deserts. The Thar Desert of South Rajasthan (India) shows a diurnal range of 20—30°C for all seasons. Difference in latitudes often causes variation in the annual temperature cycles with visible effects on the organisms. Further, with the increased altitude for every 150 meters, the decrease in temperature of 1°C takes place.

The differential responses of land and sea to the temperature, bestow upon the terrestial and aquatic organisms certain special adaptations to with stand the rapid changes of temperature. Because of, narrow range of temperature fluctuations in the aquatic media, the relative stability of the aquatic media has led to the adaptation of aquatic animals to a more or less constant temperature. But this constancy in temperature of aquatic media has its certain own drawbacks as, a slight alteration in temperature often brings about mass killings. For example, torrential insects habitually inhabiting waters with temperature range of 2—4°C at altitudes of 1000—1400 meters are adversely affected by even a slight increase in the temperature of water (Mani, 1968).

RANGE OF TEMPERATURE TOLERANCE

Life in this universe exists within a range of —200 to 100°C. Though normal life persists within narrow temperature limits of about —10 to +50°C. Individual species survive in a smaller range and are active within even narrower limits. Many nematodes, rotifers and tradigrades have shown to withstand cooling to —272°C with-

out ill effects. Larvae of chironomids and certain other Diptera have been found to thrive at temperatures near 55°C. Praying mantis is reported to live in bare grounds at a temperature of 62°C in deserts. Some algae and bacteria are reported to live in hot springs at 88°C. Non-photosynthetic bacteria inhabiting hot springs can actively grow at temperatures greater than 90°C (Bott and Brock, 1969). On the other hand, some arctic algae can complete their life cycles in places where the temperatures barely rise above 0°C. Further, the eggs of the acanthocephalan *Macracanthorhynchus hirudinaceus* have been known to withstand temperatures from —10 to —45°C for about 140 days, and desiccation at temperatures up to 39°C for a period of 265 days.

The organisms (microbes, plants and animals) which can tolerate very large fluctuations in temperature are called **eurythermal organisms**. For example, cyclops, toad, wall lizard, grass snake, man, etc., are the eurythermal animals. The organisms which can tolerate only a small variation in temperature are termed **stenothermal organisms**. The common stenothermal animals are fishes, snails, coral reefs, etc. In organisms all metabolic processes necessary for life start at a certain **minimum temperature** and increase with rise in temperature until they reach the maximum level at a temperature called **optimum temperature**. Further rise in temperature beyond optimum brings about decrease in metabolic rate, until it ceases at a temperature called **maximum temperature**.

Thus the favourable temperature range for any particular species is determined by the prevailing temperature at which normal physiological activities of the animals take place, as in the distribution of the rotifer *Keratella procura* (Nayar, 1970). This rotifer species is known to appear in the ponds of Pilani, Rajasthan (India), when the temperature is below 24°C and to disappear when it rises above 24°C, the frequency of distribution reaching a peak during months of October to March, with the fall in temperature.

POIKILOTHERMS AND HOMOTHERMS

An animals response to wide ranges in temperature is influenced by its physiology. All invertebrates, lower chordates (hemichordates, urochordates and cephalochordates), cyclostomes, fishes, amphibians and [illegible] or poikilothermic organisms. The temperature range that a poikilothermic animal tolerates can be correlated closely with the environmental temperatures normally encountered in nature : tropical

animals can not withstand low temperatures ; polar animals can not withstand high temperature ; temperate-zone animals survive a wide temperature range. Many poikilothermic animals show a rather precise discrimination of temperature. If the temperature decreases or increases appreciably, these animals may tolerate and adjust to change, may avoid the change by seeking a less exposed environment or may become inactive. Some animals respond by becoming dormant during periods of extreme temperatures. Such dormancy is called estivation at high temperature and hibernation at low temperatures. For example, most poikilotherms become inactive when the temperature of their surroundings goes below 8°C or rises to 42°C.

A few exceptional poikilotherms, especially insects, certain amphibians and reptiles, exercise a degree of thermo-regulation by either physiological or behavioural mechanisms. For example, Hawk moths can rise the temperature of their flight muscle to 32°—36°C by vibrating the wings before take-off and gregarious butterfly larvae may raise their temperature 1½—2°C when clustered together. Locust hoppers may increase their temperature 10°C by basking sideways in the sun. Ants move their larvae to warm or cool places within the nest and bees maintain temperatures within their hives between 13° and 25°C by fanning with their wings to evaporate water droplets when it is too hot, or releasing body heat through increased metabolic activity, when too cold.

When temperature drops, lizards bask in the sun to achieve the desired body temperature ; once this thermal level is attained, they will divide their time between sun and shade to maintain it. Poikilotherms such as desert dwellers that live in environments where the temperature is apt to be very high are often nocturnal and thus avoid the highest temperatures of the day. Some poikilotherms, both vertebrates and invertebrates, lower their body temperatures slightly by evaporative cooling. In frogs and reptiles, evaporative cooling can occur through the skin or via the respiratory tract by panting (Warburg, 1967). Among the invertebrates, evaporative cooling has been reported in tropical intertidal-zone animals (Lewis, 1963).

In contrast to poikilotherms, birds and mammals can, within limits, maintain constant body temperatures, regardless of temperature variations of air and water. Such animals are termed warm blooded or homeothermal. Their life processes are adj[illegible] to function at the animal's normal temperature[illegible] a [illegible] 38°C in mammals and 3 to 4° highe[illegible] If its [illegible] control fails, the animal dies. The hom[illegible] mals

the constancy of body temperature by a combination of several factors: (1) a thermo-regulating centre in the brain ; (2) insulation ; (3) a peripheral vascular response to ambient temperature ; and (4) metabolic compensation.

Among these factors, insulation is highly important particularly in enabling large arctic animals to withstand very low temperature. If the temperature is lowered, the oxygen consumption rate of tropical mammals increases, whereas that of arctic mammals remains basal. This response pattern is due to differences in insulation ; some arctic animals are fur-insulated, whereas others are insulated with a layer of blubber. Many small animals, however, are not well insulated and must seek heat retention by other means, often by huddling together.

Homeothermic animals living in very hot environments can not tolerate greatly elevated body temperatures and they utilize methods that facilitate heat transfer to the environment, including an increased peripheral blood flow and surface cooling by sweating or panting. Birds may accomplish this by rearranging their plumage so that more skin is exposed. There is however, no reduction in metabolic rate. Animals subjected to high temperature may exhibit diurnal patterns of behaviour—that is, they may reduce locomotor activity during the heat of the day or may move into the shade to avoid direct sunlight.

Various species shift between homeothermy and poikilothermy and are known as **heterotherms** (**Ananthakrishanan** and **Viswanathan**, 1976). These include animals such as the pigmy mouse and the little pocket mouse, which respond to temperature extremes by estivating or hibernating, and others such as the humming bird, which experience a nocturnal drop in temperature.

Plants too can be divided into the following three categories on the basis of their heat tolerating capacity : **megatherms, microtherms** and **mesotherms**. Megatherm plants occur in warm habitat (*e.g.*, desert vegetation). Microtherm plants occur in cold habitat, (*e.g.*, plants of high altitudes). Mesotherm plants are the plants of the habitat which is neither very hot not very cold (*e.g.*, aquatic plants).

FACTORS OR VARIABLES AFFECTING ORGANISMAL RESPONSE TO TEMPERATURE

The response of an organism to temperature is affected by a number of factors such as **thermal history, genetic differences, diet, size, stage in life cycle, sex, moulting, parasitism, hormones**, etc.

1. Generally, animals found in warmer environments can withstand higher temperatures than animals from colder situations. It is also

temperatures better than those from the warmer climates. These differences between warm-climate and cold-climate populations may reflect basic genetic differences or may be phenotypic expressions resulting from different thermal histories. 2. The thermal resistance of a species may be closely identified with the genetic composition of the parent stock, as illustrated by hybrid development in two anuran species, *Bufo valliceps* and *B. luetkeni* (Ballinger and McKinney, 1966) The lower lethal temperature for development of *B. valliceps* is 18°C ; for *B. luetkeni*, 22°C. In their hybrids, the lower lethal limit is found to be in between that of the two parents at 19·5°C. 3. In some animals a relationship between the total intake of food and resistance to thermal stress has been demonstrated. Some species are more sensitive to elevated temperatures when they are starved for even short periods of time. Goldfish showed increased resistance to high temperature when placed on a high fat diet (Hoar and Cottle, 1952). 4. The size of animal body is found to have some correlation with thermal lethal limits of animals. In some species, the smaller animals are more resistant to higher temperatures than the larger ones ; in some species the reverse is noted, and in other species size is not an apparent variable. Further, some smaller animals die faster at low temperatures than do larger animals of the same species, but at higher temperatures body size is not a factor. 5. Many species of animals have exceedingly complex life cycles during which the larval stages are not only morphologically dissimilar from the adult but also occupy different ecological niches. In the wharf crab *Sesarma cinereum*, thermal requirements of the planktonic zoeal stages are different from those of adults because these larvae are limited a smaller temperature range than are the adults. 6. The female sex is found to be more tolerant than males to the temperature fiuctuations. 7. Among certain invertebrates, such as, in some crabs moulting adversely affected heat resistance, but had no effect on other species. 8. Mud-flat snails, *Nassarius obsoleta*, which are heavily infected with trematode larvae (*i.e.*, parasites) can not withstand high temperatures like the non-parasitized snails. 9. Hormones have been shown to influence cold and warm resistance as well as acclimation (*viz.*, temperature adaptation) to temperature, especially in mammals and a few other invertebrates.

EFFECT OF TEMPERATURE ON PLANTS AND ANIMALS

Temperature has been found to affect the living organisms in various ways, for example, it has significant role on the cells, morphology, physiology, behaviour, growth, ontogenetic development

hopper species—*Melanoplus sanguinipes* and *Camnula pellucida* when reared at 32°C produce 20—30 times as many eggs than those reared at 22°C (see **Ananthakrishnan and Viswanathan,** 1976). On the other hand, the fecundity of certain insects such as cotton stem weevil (*Pempherulus affinis*) was found to decline with an increase in temperature beyond 32·8°C (**Ayyar and Margabandhu,** 1941).

4. **Temperature and sex ratio :** In certain animals the environmental temperature determines the sex ratio of the species. For example, the sex ratio of the copepod *Macrocyclops albidu* is found to be temperature dependent. As the temperature rises there is a significant increase in number of males. Similarly in plague flea, *Xenopsylla cheopis*, males outnumbered females on rats, on days when the mean temperature remains in between 21-25°C. But the position becomes reverse on more cooler days.

5. **Temperature and ontogenetic development :** Temperature influences the speed and success of development of poikilothermic animals. In general complete development of eggs and larvae is more rapid in warm temperatures. Trout eggs, for example, develop four times faster at 15°C than at 5°C. The insect, chironomid fly *Metriocnemus hirticollis,* requires 26 days at 20°C for the development of a full generation, 94 days at 10°C, 153 days at 6·5°C, and 243 days at 20°C, (**Andrewartha and Birch,** 1954). However, the seeds of many plants will not germinate and the eggs and pupae of some insects will not hatch or develop normally until chilled. Brook trout grow best at 13°C to 16°C, but the eggs develop best at 8°C.

6. **Temperature and growth :** The growth rates of different animals and plants is also influenced by temperature. For example, the adult trout do not feed much and do not grow until the water is warmer than 10°C. Likewise, in the oyster *Ostraea virginica,* the length of the body increases from 1·4 mm to 10·8 mm when temperature is increased from 10°C to 20°C. Sea urchin *Echinus esculentus* shows maximum size in warmer waters. Corals flourish well in those waters which contain water below 21°C.

7. **Temperature and colouration :** The size and colouration of animals are subject to influence by temperature. In warm humid climates many animals like insects, birds and mammals bear darker pigmentation than the races of some species found in cool and dry climates. This phenomenon is known as **Gloger's rule.** In the frog *Hyla* and the horned toad *Phrynosoma,* low temperatures have been known to induce darkening. Some prawn (crustacean invertebrates) turn light coloured with increasing temperature. The walking stick

Carausius has been known to became black at 15°C and brown at 25°C.

8. Temperature and morphology : Temperature also affects the absolute size of an animal and the relative properties of various body parts **(Bergman's rule)**. Birds and mammals, for example attain a greater body size when they are in cold regions than in warm regions, and colder regions harbour larger species. But poikilotherms tend to be smaller in colder regions. Body size has played a significant role in adaptation to low temperature because it has influenced the rate of heat loss. According to **Brown** and **Lee** (1969), larger wood rats have a selective advantage in cold climates, apparently because their surface to air ratio and greater insulation permit them to conserve metabolic heat. For opposite reasons small-sized animals are favoured in deserts.

Further, the extremities of organism like tail, ears and legs of mammals often appear to be shorter in colder climate **(Allen's rule)**. Mice reared at 31° to 33·5°C have longer tails than those of the same strain reared at 15·5° to 20°C. Moreover, the races of birds with relatively narrower and more acuminate wings tend to occur in colder regions, while those in warmer climates tend to be broader **(Rensch's rule)**. Temperature also influences on the morphology of certain fishes and is found to have some relation with the number of vertebrae **(Jordon's rule)**. Cod which hatches off New Foundland at a temperature between 4° and 8°C has 58 vertebrae, while that hatches East of Nantucket at a temperature between 10° and 11°C has 54 vertebrae.

9. Temperature and cyclomorphosis : The relation between seasonal changes of temperature and body form is manifested in a remarkable phenomenon termed **cyclomorphosis** exhibited by certain cladocerans like *Daphnia* during the warm months of summer. These crustaceans show a striking variation in the size of their helmet or head projection between the winter and summer months **(Coker, 1971)**. The helmet develops on the *Daphnia* head in spring, it attains its maximum size in summer and disappears altogether in winter to provide usual round shape to the head. Such a kind of cyclomorphosis in the terms of size of the helmet is clearly showing a corelation to the degree of warmth of different seasons. These prolongations of the helmet have been interpreted as an adaptation aiding floatation since the buoyancy of winter becomes reduced as the temperature increases **(the buoyancy hypothesis)**. According to other interpretation **(*viz.*, stability hypothesis)**, the helmet acts like the rudder and gives greater stability to the animal. Besides temperature,

such structural polymorphism can be caused by other environmental factors including the food.

10. Temperature and animal behaviour : Temperature generally influences the behavioural pattern of animals. In temperate waters the influence of temperature on the behaviour of wood borers is profound. For example, in the winter months in general, both *Martesia* and *Teredo* occur in smaller numbers in comparison with *Bankia campanulata* whose intensity of attack is maximum during the winter months. Further, the advantage gained by certain cold blooded animals through thermotaxis or orientation towards a source of heat are quite interesting. Ticks locate their warm blood hosts by a turning reaction to the heat of their bodies. Certain snakes such as rattle snake, copper heads, pit vipers are able to detect mammals and birds by their body heat which remains slightly warmer than the surroundings. Even in the dark these snakes strike on their prey with an unnerving accuracy, due to the heat radiation coming from the prey. The arrival of cold weather in temperate zones causes the snakes to coil up and huddle together.

11. Temperature and animal distribution : Because the optimum temperature for the completion of the several stages of the life cycle of many organisms varies, temperature imposes a restriction on the distribution of species. Generally the range of many species is limited by the lowest critical temperature in the most vulnerable stage of its life cycle, usually, the reproductive stages. Although the Atlantic lobster will live in water with a temperature range of 0° to 17°C, it will breed only in water warmer than 11°C. The lobster may live and grow in colder water but a breeding population never becomes established there. Not only temperature effect on breeding in the geographical distribution but also temperature effects on survivality (*i.e* , lethal effect of temperature), feeding, and other biological activities are responsible in geographic distribution of animals. As noted earlier in this chapter, the animals from colder geographic regions are generally less heat tolerant and more cold tolerant than those animals from warmer regions ; for example, members of *Aurelia*, a jelly fish from Nova Scotia die at a water temperature of 29-30°C, while *Aurelia* from Florida can tolerate temperatures upto 38.5°C. Thus, lethal limit of temperature may regulate the range of distribution of *Aurelia*.

Generally, the distribution of shallow-water marine species can be assigned to four types of zonation. In the first type, northward distribution is dependent on thermal lethal limits during the winter months, and southern distribution is dependent on summer

danger of plants from desiccation during extremes of heat and save them from death.

2. **Removal of water from tissue :** Dried seeds, spores and cysts avoid freezing because there remains no liquid in them that can freeze. Due to removal of water from seeds, the cold resistance of seeds of certain plants increase upto the extent that their exposure for 3 weeks to 190°C, does not diminish their germinating capacity.

3. **Dormancy :** Dormancy includes two already discussed phenomena namely **hibernation** and **aestivation.** During both kinds of dormancies metabolic rate becomes reduced, body temperature becomes low and heart beat rate is also reduced.

4. **Thermal migrations :** Thermal migrations occur only in animals. The journeys taken by animals that enable them to escape from extremely hot or cold situations are referred to as **thermal migrations.** For example, desert animals move to shaded places to avoid burning heat of noon and some animals like desert reptiles and snakes become nocturnal to avoid heat of the day. The frogs, toads, other amphibians, turtles, etc., make short trips into or out of water (or moist places) and this provides desired cooling and warming to the animal.

LIGHT AND RADIATIONS

The radiant energy from the sun is the basic requirement for the existence of life on the earth. This source of energy is of fundamental importance to the photosynthetic production of food by plants and as mentioned previously, the heat budget of the world is dependent on solar radiation. Although we generally, think only in terms of visible light, the sun emits other radiations of different wavelengths-cosmic rays, gamma rays, X-rays, ultraviolet rays, infra-red rays, heat waves, spark discharges, radar waves, radio waves, slow electro-magnetic waves. Biologists have been primarily interested in only three regions near the centre of the electromagnetic spectrum—(1) the infra-red, (2) the visible light and (3) ultraviolet regions. The **infra-red wavelengths,** which are the longest of these three, are not visible to the human eye ; they contribute to the warmth of the earth at the high altitudes in the terrestrial atmosphere. **Visible light,** is only a small fraction of the radiation spectrum and contains the frequency of wavelengths ranges from 390 to 700 millimicrons ($m\mu$). It is made up of a series of colours ranging from violet through indigo, blue, green, yellow, orange and red, all constituting the **visible spectrum.** Light energy, thus, reaches the earth as electro-magnetic waves of solar radiation with tremandous velocity and supplies most of the warmth the earth receives from the sun and also supplies

the main source of energy which is utilized in photosynthesis of plants, oriented and rhythmic behaviour of animals, bioluminescence, periodicities of occurrence and periods of inactivity. Unlike temperature, light is a non-lethal ecological factor and it has a specific direction in its flow. The wavelength of ultraviolet light from the sun is shorter than that of visible light, and it produces the upper levels of the earth known as the ionosphere.

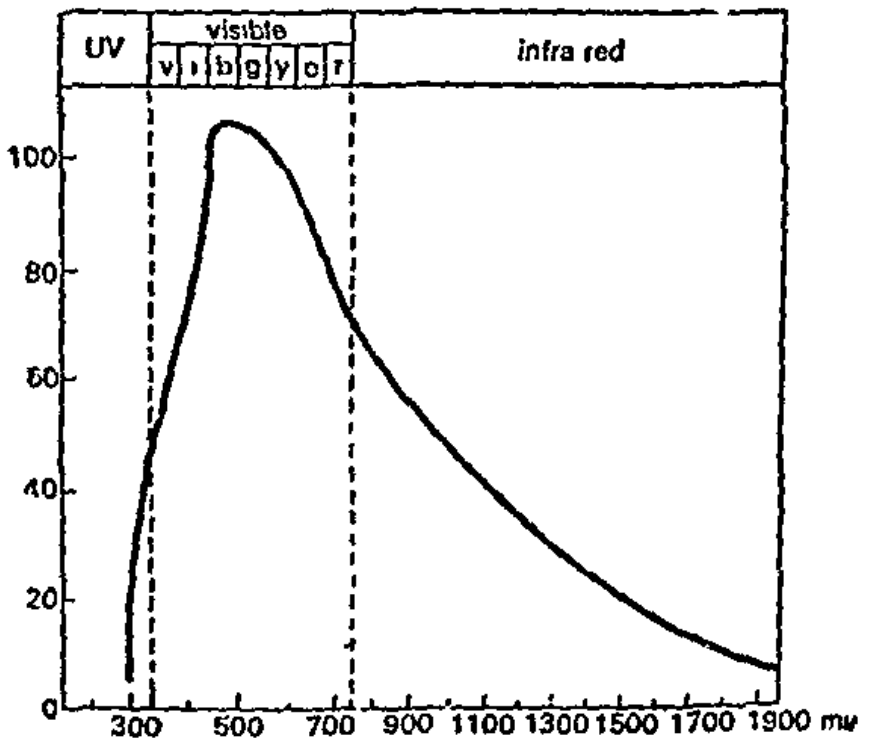


Fig. 32. Spectral distribution of ultraviolet light, visible light and infra-red radiation at the earths surface

LIGHT RECEPTORS OF ANIMALS

Since animals depend on light for orientation, diurnal migrations and synchrony of rhythmic activities light reception probably is the most important sensory modality in the exploration of the environment. It is not surprising, therefore, that receptors for light are common to almost all animals ; in those with better developed photo-receptors, light greatly influence behaviour. Light receptors may be well-defined organs such as the vertebrate eye, the compound eyes of crustacea and insects, the simple eyes or ocelli of other arthropods and invertebrates, and the dermal light receptors.

LIGHT VARIATIONS IN DIFFERENT ENVIRONMENTS

Light energy varies with different media. The transparency of air and water is important in regulating the amount and quantity of light that may be available in particular habitats. For example, the intensity of light reaching the earth's surface varies with the angle of

incidence, degree of latitude and altitude, season, time of day, amount absorbed and dispersed by atmosphere and a number of climatic and topographical factors such as fog, clouds suspended water drops, dust particles, etc., when the angle of incidence is smaller, light rays have to travel by a longer distance through the atmosphere, which resulting into relative reduction in intensity. Likewise, sun's altitude changes due to differences in latitude, changes in the season and in the time of day. When sun remains overhead, the intensity of sunlight over the earth's surface will be greatest. At higher latitudes the intensity of light becomes correspondingly reduced. The illumination or intensity of daylight is greatly diminished by moisture, clouds, and dust in the atmosphere and also by forest vegetation. The direction and slope also affect light intensity. There will be no light on the one side of the slope.

The light which enters in the aquatic media, comes from sun by passing through the atmosphere existing above the water surface and hence, that is subjected to all kinds of atmospheric factors like that of terrestrial media. About 10% of the sunlight which falls over the water surface, is reflected back and rest 90% of that pass downward in the water and is modified in respect to intensity, spectral composition, angular distribution (refraction) and time distribution. The phytoplankton, zooplankton, suspended organic and inorganic particles either reflect or absorb the light rays. Further, in water there is a selective absorption of light at various depths. The longer light rays are absorbed near the surface and in general the shortest light rays penetrate deepest. Thus, long heat and infra-red rays are absorbed in the upper layers of wa[illegible]; red rays are completely absorbed a[illegible]tres

etc. On the basis of light factor certain ecologists have classified plants into **sciophytes, shade loving, or photophobic plants** which have best growth under lower intensities of light, and **heliophytes or photophilous plants** which have best growth in full sunlight. The light factor affects following aspects of plant life :

A. Direct effects of light on plants : Light affects directly following aspects of plant life—1. It is an essential factor in the formation of **chlorophyll** pigment in chlorophyllous plants. 2. It has a very strong influence on the number and position of **chloroplasts.** The upper part of the leaf which receives full sunshine has larger number of chloroplasts which are arranged in line with the direction of light. In leaves of plants which grow under shade, chloroplasts are very few in number and are arranged at right angle to the light rays, thus increasing the surface of light absorption. 3. Light has its most significant role in **photosynthesis.** During photosynthesis, the green plants which are the "primary producers" of an ecosystem, synthesize their carbohydrate food from water and CO_2 in the presence of sunlight. Thus, during photosynthesis, the solar radiant energy is transformed into the chemical or molecular energy which remains stored in chemical bonds of carbohydrates and this chemical energy is utilized by other chlorophyllous and non-chloroyhyllous parts of plants, all animals, bacteria and viruses in their different life activities. The rate of photosynthesis is greater intermittant light than in the continuous light. At high intensity of light a photo-oxidation of chlorophylls and other enzymes takes place, which consequently reduce the rate of synthesis of carbohydrates and proteins. However, high intensity of light results in the formation of **anthocyanin** pigments. It is for this reason alpine plants have beautifully coloured flowers. 4. Li[illegible] of auxins or growth hormones : [illegible] result[illegible] in in[illegible], [illegible] amount of growth hormones, as a result of which they are elongated with weak pale yellow stems with very few branches. 5. Light also influence certain chemical compounds of plants which effect the differentiation of specialized tissues and organs. 6. Leaf structure too is influenced by the intensity of light. The thickness of leaf increases with the increasing light intensity. 7. The development of flowers, frui[illegible]light or r[illegible]ures and causes delicacy. For example, vegetative crops like turnips, carrots, potato and beets give highest yield in regions with high percentage of cloudy days. Intense light favours the development of

violet rays are known to cause mutational changes in the DNA of various organisms.

2. Effect of light on metabolism : The metabolic rate of different animals is greatly influenced by light. The increased intensity of light results in an increase in enzyme activty, general metabolic rate and solubility of salts and minerals in the protoplasm. Solubility of gases however, decreases at high light intensity. Cave dwelling animals are found to be sluggish in their habits and to contain slow rate of metabolism.

3. Effect of light on pigmentation : Light influences pigmentation in animals. Cave animals lack skin pigments. If they are kept out of darkness for a long time, they regain skin pigmentation. The darkly pigmented skins of human inhabitants of the tropics also indicate the effect of sunlight on skin pigmentation. The skin pigments synthesis is dependent on sunlight. Light also determines the characteristic patterns of pigments of different animals which serve the animals in sexual dimorphism and protective colouration. Animals that dwell in the depths of the ocean where the environment is monotone, though pigmented do not show patterns in their colouration.

4. Effect of light on animal movements : The influence of light on the movement of animals is evident in lower animals. Oriented locomotory movements towards and away from a source of light is called **phototaxis** Positively phototactic animals such as *Euglena, Ranatra,* etc., move towards the source of light, while, negatively phototactic animals such as planarians, earthworms, slugs, copepodes, siphonophores, etc., move away from the source of light.

The light directed growth mechanisms are called **phototropisms** which occur in sessile animals. Phototropisms also includes responsive movement of some body part of some active animal to the light stimulus, such as the movement of flagellum of *Euglena,* towards light and movements of polyps of many coelenterates.

The velocity or speed of the movement of certain animals is also regulated by light. It has been observed that animals when responding to light reduce their velocity of movement and these movements which are non-direction are called **photokinesis.** Photokinesis may be a change in linear velocity (**rheokinesis**) or in the direction of turning (**klinokinesis**). During photokinesis when only a part of the body of an animal deviates aways from the source of light, the reaction is termed **photoklinokinesis.** Larvae of *Musca domestica* show such movements. When animals are confronted with two lights of equal brightness, they move towards or away to a position that is

distance between the two lights. This is termed **phototropotaxis.** Attraction of males towards the flash of the female is called **telotaxis.** Movements of animals at a constant angle towards the source of light is to **light compass reaction** or **celestial orientation.**

Celestial orientation : Some organisms, particularly arthropods, birds and fish, utilize their time sense as an aid to find their way from one area to another. To orient themselves, the animals use the sun, moon, or stars as a compass. To do this, they utilize both their biological clock and observations on the azimuthal position of the sun in relation to an established direction. The **azimuth** is the angle between a fixed line on the earth's surface and a projection of the sun's direction on the surface. Using the sun as a reference point involves some problems for animals because the sun moves. The target angle changes throughout the day. But animals which use the sun as a reference, correct their orientation somehow. Such celestial orientation has been observed in fishes, turtles, lizards, most birds, and such invertebrates as ants, bees, wolf spiders and sand hoppers.

5. **Photoperiodism and biological clocks :** Regularly occurring daily cycles of light (day) and darkness (night) have been known to exert a profound influence on the behaviour and metabolism of many organims. Underlying such environmental rhythms of light and darkness are the movements of the earth relative to the sun and the moon. The earth's rotation on its axis results in alternation of night and day. The tilt of the earth's axis, along with the annual revolution around the sun produce the seasons. The response of different organisms to environmental rhythms of light and darkness is termed **photoperiodism.** Each daily cycle inclusive of a period of illumination followed by a period of darkness is called the **photoperiod.** The term **photophase** and **scatophase** are sometimes used to denote the period of light and the period of darkness respectively. Different animals have evolved different morphological, physiological, behavioural and ecological adaptations during the course of their evolution to varying photoperiods, which provide them with environmental information regarding the intensities of natural light.

(a) Daily responses : Circadian rhythms : Life evolved under the influence of daily and seasonal environmental changes, so it is natural that plants and animals would have some rhythm or pattern to their lives that would synchronize them with fluctuations in the environment. For years biologists have been intrigued over the means by which organisms kept their activities in rhythm with the 24 hour day, including such phenomena as the daily pattern of leaf and petal movement in plants, the sleep and wakefulness of animals

and the emergence of insects from pupal cases. At one time biologists thought that these rhythmicities were entirely **exogenous**, that is, the organisms responded only to external stimuli such as light intensity, humidity, temperature and tides. But now it is well investigated that most animals possess **internal** or **endogenous** rhythms in synchrony with the external or exogenous rhythms of the environment, due to which they remain able to measure the length of day. The internal or endogenous rhythms are approximately of 24 hours duration, while the exogenous or environmental rhythms are exactly of 24 hours duration. The term **circadian** (from the Latin *circa*, about, and *dies*, daily) has been used to denote these daily rhythms. The period of circadian rhythm, the number of hours from the beginning of activity one day to the beginning of activity on the next, is called **free running**. Photoperiod plays a role in providing time signals, for adjustments of the animals concerned to these daily rhythms. Circadian rhythms apparently are internally driven or endogenous, are affected little by temperature changes, are insensitive to a great variety of chemical inhibitors, and are innate, not learned from or imprinted upon the organisms by the environment.

The innate character of circadian rhythm is demonstrated by several animals. When *Drosophila* are kept under constant conditions from the larval stage , they will still emerge from pupae with a regular circadian rhythm. Eggs of chicken and lizards kept under constant conditions produce animals that later show regular circadian cycles. The circadian rhythms has been observed in zooplanktons, polychaete annelids, many insects (Lepidoptera, Diptera, Hymenoptera, Neuroptera, Coleoptera, Orthoptera, Odonata, etc.), most birds, and certain mammals.

The possession of a circadian rhythm that can be entertained to environmental rhythms provides plants and animals with a **biological clock**, which is an integral part of cellular structure and is a chemosensory system very receptive to the environmental stimuli. The biological clocks of different animals run or oscillate continuously and environment does not initiate or stop their function. At most certain environmental stimuli may serve to regulate the functions of biological clocks.

(b) **Annual rhythms : Circannual rhythms :** Solary day, lunar day, tidal rhythms, monthly and annual rhythms are also of common occurrence among animals. Endogenous annual cycles or circannual rhythms have been known in many animals like ground squirrels, warblers and other birds, some crayfishes and slugs. The circannual rhythms are of adaptive value for timing seasonal events and specify

MASS MOVEMENTS IN AIR AND WATER

The air and water media are always in a state of flux. Constant currents of water and air (wind) influences the inhabiting organisms. The currents of water besides being an influencing factor of the concentration of gases and salts also directly act as limiting factors. Corresponding differences in structure are met with in stream inhabiting organisms. On land, air currents or wind forms are important limiting factor. Birds confine themselves to shelter on days when the wind is strong. The transportation of microbes, plants and animals by hurricanes to hitherto unoccupied land may change the nature of the whole biotic community.

TIDE

Tides affecting biota comprise a regular rhytmic rise and fall oceanic water twice a day, as a result of which changes occur in the shore line. Tides are regulated by moon. At new moon and full moon the attraction of the sun and moon on tides reinforce each other and cause tides to rise higher and fall down without changing the mean sea level. This is called **spring tide**. During the first and last quarters of the moon the forces oppose each other and the daily fluctuations are much smaller due to neutral effects of the forces and this is the **neap tide**. Covering and uncovering of the tidal zone directly affect the biota by covering them with water and exposing them to air and sunlight, respectively.

CHEMICAL ABIOTIC FACTORS

Oxygen is primary source of life. It becomes a limiting factor in its complete absence, but, due to its abundance it very rarely becomes a limiting factor. High CO_2 concentrations become limiting to animals. pH value often becomes a limiting factor in different habitats. Non-availability of certain micronutrients such as calcium carbonate becomes a limiting factor for land snails (calciphilic species). *Sellana radiata*, the common limpet of Madras has been known to react to the changes of sea water concentrations of various ions. For example, it respond to very small dilutions of the sodium chloride of 0.0001 per cent.

MICROCLIMATE

Uptill now, we have discussed the influence of different ecological media and of different abiotic environmental factors on the living organisms. All these factors form the macroclimate which is measured by the meteorologists But the climate in which animals actually live is very much different from the gross environmental conditions.

the levels of migratory activity that are just sufficient for the birds to reach the vicinity of their species - specific winter quarters. The circannual rhythms also effects gonadial activities, reproductive cycles, metamorphosis, and adaptations to cold (development of fur and feather coats of animals during winter), and so on.

6. Effect of light on reproduction : In many animals (*e.g.*, birds) light is necessary for the activation of gonads and in initiating annual breeding activities. The gonads of birds are found to become active with increased illumination during summer and to regress during shorter periods of illumination in winter.

7. Effect of light on development : Light in some cases (*e g.*, *Salmon* larvae) accelerates development, where as, in other (*e.g.*, *Mytilus* larvae) it retards it.

Further, occasionally the output of sunlight is increased by the development of **sunspots**. As a result of this excess energy is radiated to space and this naturally increases the output of solar energy near the earth A direct consequence of this is the greater evaporation of water which results in cloud formation to prevent more exposure to sunshine and thus to equalize temperature and modifying climate.

PRESSURE

Barometric pressure have little effect on organisms. An amoeba in motion in spite of stopping under slight pressure is insensitive to increased pressure on the whole body. But in an ocean, pressure is an unavoidable ecological factor. In general, an increase of hydrostatic pressure is inversely proportional to the pace of life. While as altitude increases atmospheric pressure decreases. Pressure influences solubility, ionic dissociation and surface tension and water is slightly compressible with increased pressures. Since incompressible bodies are influenced very little by increased pressure, they will sink in the sea, and apparently this factor is important in food settling to the deep-water benthic fauna.

GEOMAGNETISM AND GRAVITY

The earth may be described as a magnetized sphere in which magnetic axis is inclined about 11 degrees relative to the geographical axis. Geomagnetism and gravity are involved in the adaptation of an organism to its environment. Like light, temperature and pressure they are omnipresent ecological factors with which life steadily interacts. The living systems of planarians and snails have been found to be extraordinarily sensitive to magnetic fields of earth.

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This immediate environment of animal is called effective climate or microclimate and relates to such of the habitats of animals as those living in the soil within crevices of barks of trees, inside sheaths of grasses, within burrows, in ants nests, beneath logs of wood or rock, within caves, on vegetation in sand dunes, within plant galls and a variety of habitats which are occupied by endoparasites Woodlice, centipedes and millipedes, for example, remain restricted to dark microhabitats during the daytime to prevent rapid water loss for transpiration. When temperature falls and relative humidity increases at night, these animals emerge of the daytime microhabitats.

CHAPTER 4

BIOTIC ENVIRONMENTAL FACTORS

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upon the coactions or interaction of other members within the species (*intraspecific interactions* or *coactions*) or between members of heterogeneous groups (*interspecific interactions* or *coactions*). These coactions may either be beneficial to the participants—**cooperation**, or harmful—**disoperation**. Interspecific cooperations include mutualism, commensalism, and many of the interrelations that establish the community as a dynamic unit. Coactions that are harmful, to at least one of the participants, include amensalism, parasitism, predation, and competition. Many of these coactions act to regulate density or, along with climate, cause fluctuations or even more drastic changes in number of individuals from time to time, and so constitute **biotic environmental factors**. Other biotic factors are ethics (established behavioural interrelations between individuals characteristic of a species) and other inherent properties of members of a species.

INTERSPECIFIC BIOTIC FACTORS

Table : Basic types of interspecific interactions. (Odum, 1971)

Types of interaction	Effect on population growth and survival of two populations, A and B				General results of interaction
	when not interacting		when interacting		
	A	B	A	B	
1. Neutralism (A and B incependent)	0	0	0	0	Neither population affect the other
2. Competition (A and B competition)	0	0	—	—	Population most affected and eliminated from niche
3. Mutualism (A and B partners or symbionts)	—	—	+	+	Interaction obligatory for both
4. Protocoperation (A and B cooperates)	0	0	+	+	Interaction favourable to both, but not *obligatory*
5 Commensalism (A commensal ; B Host)	—	0	+	0	Obligatory for A ; B not affected
6. Ammensalism (A amensal, B inhibitor or antibjotic)	0	0	—	0	A inhabited ; B not affected
7. Parasitism (A parasite , B host) 8. Predation (A predator ; B prey	—	0	+	—	Obligatory for A ; *B* inhibited

\+ Population growth increased
— Population growth decreased
0 Population growth not affected

1. NEUTRALISM

Neutralism is the most common type of interspecific interaction. Neither population directly affects the other. If there are interactions, they are of a more subtle and indirect type. The mere presence of the two species should not directly affect the population level of either. For example, robins (birds) and squirrels living in a forest present a good example of neutralism, as, neither serves as food for the other, neither has any direct interaction say that both may inhabit the same tree yet both are clearly part of the forest community.

2. COMPETITION

Competition refers to the type of interaction in which two individuals or species vie for a limited amount of food, water,

nesting space, cover, mates or other resources. When these materials are in more than adequate supply for the demands of those organisms seeking them competition does not occur ; When they are inadequate to satisfy the needs of all the organisms seeking them, the weakest, least adapted, or least aggressive individuals are often forced to do without going elsewhere, or become locally extinct. This phenomenon is generally known as the **competitive exclusion principle of Hardin** (1960). Though, competition may result in death for some competitors, but this is from fighting or being deprived of food or space rather than being killed for food as in predation or by disease as in extreme parasitism.

Types of competition : Competition may be either direct or indirect. It is **direct** where there is active antagonism, struggle, or combat between individuals ; **indirect**, when one individual or species monopolizes a resource or renders a habitat unfavourable to the establishment of other organisms having similar requirements. Direct competition or **interference**, is evident in the fighting of bull seals for large harems and of grouse for a better position in the social hierarchy ; in chasing and colour display by fish and birds for defense of territories ; in the singing and calling of frogs, birds and some mammals as invitations for mates ; and in the excretion of chemicals that affect the behaviour or health of other organism (**allelochemistry**) Indirect competition, or **exploitation** is common among plants when certain species monopolize the water and nutrient resources of the soil or available light so that competing species cannot maintain themselves. Once an area is well saturated with established individuals to seek homes elsewhere, even in less favourable situations, than to intrude (see **Kendeigh, 1974**). Trout, for example grow more rapidly to large size when other trout species and forage species are low or absent (**Cooper, 1959**). Further, two species of butterflies, the monarch and the queen monarch of florida, are attracted to the flowers of the same milkweed plant, upon which they both feed and lay eggs and on which the larvae feed. The non-migratory queen monarchs, however, avoid some species of milkweed that the monarch utilize. But after the monarchs have flown northward, the queen monarchs oviposit on this milkweed. Thus the disappearance of the monarchs coincides with a population increase in the queen monarchs.

Degree of competition : Competition is usually keenest between individuals of the same species, **intraspecific competition**, because they have identical requirements for food, mates, and so on, and because they are more nearly equal in their structural

behavioural adaptation. **Interspecific competition** occurs where different species require in common at least some materials or condition. The severity of competition depends on the extent of similarity or overlap in the requirements of different individuals and the shortage of the supply in the habitat. It is generally the case that the more unlike the kinds of competing organisms, the less intense the competition. Yet birds compete with squirrels for acorns, nuts, and seeds; insects and ungulates compete for food in grassland; the bladderwort plant competes with small fish for entomastraca and other planktons.

The immediate test of success in competition is survival; the ultimate test is leaving the largest number of established offspring.

Effects of competition on population : Competition has five important effects in the animal community : 1. Allelochemistry, establishment of social hierarchies. 2. Establishment of territories. 3. Regulation of population size. 4. Segregation of species into different niches, and 5. Speciation. The first three effects are chiefly intraspecific, while, last two effects are interspecific. In any case, competition for the resources of the ecosystem, both interspecific and intraspecific, is a prime means of population regulation. No population can exist at a higher density than can be maintained on the basis of the availability of needed resources as intraspecific and interspecific competition will remove those individuals that are less able to exploit the resources of the ecosystem (**Clapham, Jr., 1973**).

3. MUTUALISM

Mutualism is an ob[illegible] that is strongly beneficial to both species. Th[illegible] to this relationship (*i.e.*, [illegible] to intimate association of [illegible] less of benefits or the lack of them and hence includes mutualism, commensalism and parasitism (see Smith, 1974). Mutualism may be facultative, when the species involved are capable of existence independent of one another, or obligate, when the relationship is imperative to the existence of one or both species.

Mutualism i[illegible] the associations of fungi and algae to [illegible] bacteria with the roots of legumes, a[illegible]e roots of many flowering plants. Further, there are many intimate relations between plants and animals. Mutualism is evident in the presence of photosynthetic algal cells in the protective ectoderm of green hydra, and those associated with turbellarians, molluscs, annelids, bryozoans rotifers, protozoans and the egg capsules of salamanders. The algae give off oxygen, benefiting the animals, which in turn supply CO_2

and N_2 to the plants. Likewise, certain beetles, ants and termites cultivate fungi for food. Bacteria in the caeca and intestine of herbivorous birds and mammals aid in the digestion of cellulose. The cross pollination of flowers by the agency of insects and birds seeking nectar and pollen is of such great importance that many structural adaptations in both plants and animals fit the one to the other to ensure the success of the function. Moreover, birds and mammals are of great importance as agents of plant distribution. Seeds, fruits, even entire plants become attached to feathers or fur or ingested seeds are eaten and eliminated unharmed with the faeces.

Interspecific mutualism is nicely demonstrated by the flagellate protozoan, *Trichonympha* an obligate anaerobe in the gut of several species of wood-eating termites where it digest cellulose. *Trichonympha* also occurs in the alimentary canal of wood-eating roach *Cryptocercus*. The termite and roach reduce the wood to small fragements, passing them through the alimentary canal to hind gut, where the protozoans digest the cellulose, changing it into sugar. The host benefits the protozoa by removing harmful metabolic waste products and maintaining anaerobic conditions in the intestine.

4. PROTOCOOPERATION

Protocooperation is less extreme sort of interaction than mutualism in which the interaction is clearly beneficial to both species, allowing the equilibrium population levels of both to be higher than they otherwise would be. However, it is not obligatory for either species. Examples of protocooperation are many, including the relationship between water moccasins and large birds such as herons and ibises on several islands off the west coast of Florida. The birds nest in the lower branches of relatively unprotected trees, while the snakes congregate around the bases. This protect the bird from tree climbing predators such as racoons. In turn, the snake feed, in part, on fish dropped by the birds and the occasional baby birds that falls out of the nest (Ehrenfeld, 1970).

There is no clear demarcation between protocooperation and mutualism, as in many cases where a mutually beneficial relationship is obligatory for one species but not for the other. For instance, the nitrogen-fixing bacteria in the roots of legumes plant could not survive without the host plant. However, the host plant could probably survive without the bacteria.

5. COMMENSALISM

Commensalism defines the coaction in which two or species are mutually associated in activities centering on one species at least, derives benefit from the

other associates are neither benefited nor harmed. The concept of commensalism has been broadened in recent years, to apply to coactions other than those centering on food ; cover, support, protection, and locomotion are now frequently included (Baer, 1951 ; Kendeigh, 1974).

The epiphytes [illegible] beard' [illegible] roots draw nourishment from the humid air. All these epiphytes depend upon the trees for support only. They manufacture their own food by photosynthesis. Likewise, ectocommensals or epizoite animals are associated with another animal for the purpose of anchorage and protection. Many small animals, for example, become attached to the outside of larger ones, such as the protozoans *Trichodina* and *Kerona* on *Hydra*, vorticellids (*Vorticella*, *Epistylis*) or various other aquatic organisms, branchiobdellid annelids on crayfish the barnacles, which attach themselves to the backs of whales and shells of horse-shoe crabs and so on. The remora fishes attach themselves to the bellies of sharks, swordfish, tunny, barracuda, or sea turtle by means of a dorsal fin highly modified into a suction disk on the top of the head. They are of small size and are not burdensome to the host. The host benefits the remora, however, for when the host feeds, the scraps of food floating back are swept up by the remora.

Commensals may also be internal. For example, many harmless protozoans occur in the intestinal tract of mammals, including man. The pea crab, *Pinnotheres*, lives as a commensal in the mantle cavity of certain sea mussels ; the crap steals food collected by the host mollusc but does little if any other known injury. The hermit crab normally lives inside gastropod shell. The pitcher of the pitcher plant found in bogs furnishes a breeding site or home for certain species of midge flies, mosquitoes, and tree toads. Many kinds of microorganisms, plants and animals (such as small crabs, shrimps, polychaets, ophiuroids, ascidians, etc.) live in the canal system of sponges.

The nest of one species often furnishes shelter and protection for other species as well. Ant nests may contain guest species of various other insects ; some birds place their nests close to wasps, bees or ants for the protection offered by these insects. Woodchuck burrows are used also by rabbits, skunks and racoons, especially in winter.

6. AMENSALISM

Contrasting with commensalism is amensalism, a situation in which one population definitly inhibits the other while remaining

unaffected itself. By so modifying the environment, the organism improves its own chance of survival. Ammensalism commonly involves some type of chemical interaction, by which the organisms of one species affects the well-being and growth of individuals or the population biology of other, is called **allelochemic** (see, Whittaker. 1970, Smith, 1974; Kendeigh, 1974). The inhibitor substance may be inorganic chemicals such as acids or bases which are produced by pioneering organisms (inhibitor species) and which reduce the competition for nutrients, light, and space, between the amensal species and inhibitor species. The production of relatively simple organic toxins is another source of chemical inhibitors. These toxins inhibit seedling growth in the vicinity. This may affect succession of plant species, especially important in the early stages (Muller 1966). A third type of inhibitor chemical is the **antibiotic** potent antimicrobial agent. It is a substance produced by an organism, which, in low concentrations, can inhibit or kill the growth of another organism.

The majority of inhibiting chemical are produced as secondary substances by plants and released into the soil through the roots or leaf wash. The suppression of growth through the release of chemicals by a higher plant is known as **allelopathy**. For example, walnut tree produces a non-toxic substance, juglone (Bode, 1958) which is found in its leaves, fruits and other tissues. When the leaves or fruits fall on the ground, juglone is released to soil, where it is oxidized to a substance that inhibits the growth of certain under story species and garden plants such as heaths and broad-leafed herbes, and favours others such as bluegrass and blackberries.

Atibiotics produced by bacteria, fungi, actinomycetes, and lichens are widespread in nature and may be one of the reasons why bacteria pathogenic to man cannot multiply well in soils. A number of antibiotics such as penicillin, have been used extensively in human medicine.

Allelochemic effects are of great variety in both plants and animals : repellants, escape substances, supprssants, venoms, inductants, counteractants, attractants signals, stimulants, autotoxins, autoinhibitors, and so on. **Pheromones** (ectohormones) are chemical messages between members of a species especially important in reproductive behaviour, social regulation and recognition, alarm and defense, territory or trail marking, food location, and so on. Many of these effects are beneficial to the individual, while others serve for competitive purposes (Whittaker and Feeny, 1971).

7. PARASITISM

Parasitism is a kind of harmful coactions (disoperation) between two species. It is the relation between two individuals wherin one individual called **parasite** receives benefit at the expense of other individual called **host**. Parasitism is mainly a food coaction, but the parasite derives shelter and *protection from the host, as well.* A parasite usually parasitize a host which is larger in body size than it. Further, a parasite does not ordinarily kill its host, at least not until the parasite has completed its reproductive cycle. However, a host may die due to some secondary infection or suffer from stunted growth, emaciation, or sterility. The balance between parasite and host is upset if the host produces antibodies or other substances which hamper normal development of the parasite. In general the parasite derives benefit from the relation while the host suffers harm. But this does not mean that all parasites are harmful. The former category constitutes the **pathogenic parasites**.

Classification of parasites—Parasites exhibit a tremandous diversity in ways and adaptations to exploit their hosts. The parasites may be **viral parasites** (*e.g.*, bacterial, plant and animal viruses), **microbial parasites** (*e.g.*, bacteria, protozoa, fungi, etc.), **phytoparasites** (*e.g , plant parasites*) and ***zooparasites*** (*e.g., animal parasites* such as platyhelminthes, nematodes, arthropodes, etc.) .They may parasitize microorganisms, plants and animals. They may occur *on* [illegible] (ectoparasites) or live within the body of the [illegible] live in the *alimentary* [illegible] or other tissues of host. Ectoparasites may be parasitic only in the immature stages—the hairworm larvae, parasitic in aquatic insects ; only the adult parasitic—fleas, on birds and mammals ; or both larvae and adults may be parasitic—the blood sucking lice and flies, biting lice, mites, and ticks that occur on birds, mammals, and sometimes reptiles, and the monogenetic trematodes on fish. Similar relation occurs in endoparasites but in them usually all the stages of life are parasitic—entozoic amoebae, trichomonad flagellates, opalinid ciliates, sporozoans, pentastomids, nematodes, digenetic trematodes, acanthocephalons, cestodes, and some copepodes.

Further, endoparasites rely on various means of transport from one host to another so that their survival, range of distribution, and life cycle are unaffected. For the endoparasite, the interior of its host [illegible] outside environ- between the microenviron-

ment directly influence the parasite while alterations in the macro-environment influence it directly. Thus, mutual adaptations and tolerance between the host and parasite are important factors facilitating successful transmission of endoparasites, and climatic fluctuations tend to alter the physiology of the parasite, host or vector. Mutual adaptation is the parasite's ability to establish and maintain itself in a favourable location within the host and exist in a proper form for sufficient length of time enabling subsequent transport to another host and continuation of species.

Animals may also be parasitic to plants. Nematodes infest the roots of plants. Wasps or gnats form galls on plants such as oaks, hickories, willows, roses, goldenrods, and asters. Mites stimulate formation of witches brooms in hackberry. A variety of insects the larvae of which are leaf miners, wood borers, cambium feeders, and fruit eaters should be included here.

Lastly, parasites may be full-time (or permanent) parasites or part-time (or temporary) parasites. Mosquitoes and bugs that suck the blood of their hosts are temporary parasites. Some temporary parasites spend only a part of their life cycle as parasites. For example, glochidium larva of *Anodonta* (fresh water mussel) attaches itself to the body of the fish by means of its hooks and penetrates inside the fish integument to remain burried there for several weeks and finally emerges out as the young mussel to lead an independent existence. Permanent parasites, however, spend their life completely on other organisms. The common examples of permanent parasites are—*Plasmodium*, *Entamoeba histolytica*, and other protozoan pathogens, different platyhelminthes, nematodes, arthropods, etc.

Parasitic adaptations—While studying parasitism as a biotic factor influencing the life and activities of parasites and hosts we often encounter manifold adaptations, both offensive and defensive, being developed by the host, as well as by the parasite. A endoparasite entering a host often meets with the antibodies or phagocytic cells produced by the host. The ectoparasites, and endoparasites have following parasitic adaptations :

(1.) In parasitic animals, a reduction of organs of special sense of nervous system, and of locomotory organs occur.

(2.) Most ectoparasites develop some clinging organs such as hooks, suckers, etc., to get attached with the body of their hosts. They also develop special piercing and sucking organs to suck the blood of animals or sap of plants. Certain blood-sucking ectoparasites such as leeches, blood-sucking mosquitoes, etc., contain certain anticoagulent enzymes in their salivary secretions.

3. Most endoparasites exhibit anaerobic respiration, high rate of reproduction, parthenogenesis, hermaphroditism, polyembryony, intermediate hosts and a complicated life-cycle (Lapage, 1951). Some endoparasites, such as tapeworm, have become so adapted to the host that they no longer require a digestive system. They simply absorb their food directly through their body wall. Further, parasites that live within the bodies of plants and animals possess cuticles or develop cysts resistant to the digestive enzymatic action of the host.

4. Many parasites pass their entire existence in a single host, others require one, two, even three intermediate hosts. It is of ecological significance that both primary and intermediate hosts of a parasite occur in the same habitat or community. Even then the hazards to successful passage from one host to another are so great and mortality so high that prodigious quantities of offspring are produced to ensure that at least a few individuals will complete the cycle.

5. Parasites are transferred from one host to another by active locomotion of the parasite itself ; by ingestion, as one animal sucks the blood of or eats another ; by ingestion as an animal takes in eggs, spores, or encysted stages of the parasite along with its food or drinking water ; as a result of bodily contact between hosts ; or by transportation from host by way of vectors. For example, the bacteria that cause tularemia in man are carried from rabbit to rabbit by ticks. Man contacts the disease when he handles infected rabbits but the incidence of infection is greatly reduced in the autum when cold weather forces the ticks (*i.e.*, the vectors of bacteria) to leave the rabbits and go into hibernation.

Host specificity of parasites : Certain parasites, such as copepodes, contain a wide range of hosts and they are ubiquitously present in various invertebrates and fishes. Most parasitic genera, however, are adapted to hosts of one phylum only. For example, each order of birds possesses its own particular species of tapeworms This is true even when several order of birds live in the same habitats as do, for, instance, grebes, loons, herons, ducks, waders, flamingoes and cormorants (Baer, 1951). Species of flagellate protozoans that occur in termite alimentary canals are largely host—specific. Some species of gall wasps attack only one species of oak. Where a single species parasitizes two or more host species, the shape and structure of the gall formed around the egg and larva on both hosts is similar. When several insects are found on the same

of parasite produces its own characteristic gall form. The parasite-specific gall formation is found to be related with parasite-specific enzyme (Kinsey, 1930).

Certain parasites remain restricted to special habitats within the host. The roundworm lives near the duodenum of the digestive tract and soil nematodes live in the rootlets of plants. Biting lice remains restricted to the head or body regions of birds. Some zoo-parasitic nematode species are found throughout the body in connective tissue, but not in the gut ; some occur only in the digestive tract and associated organs ; certain species occur in the glandular crop of birds, but others only in the caecum ; many species occur exclusively in the lungs or in the frontal sinuses Such fine restrictions of parasites to particular microenvironments—hosts or organs is a consequence of precise physiological and morphological adaptations that permit the parasite to survive and complete the life cycle only under very special conditions. Likewise, the life cycle of the parasite is often closely synchronized in time with that of its host (Foster, 1969).

Effects of parasite on the host : disease : Parasites may not cause immediate mortality but they cause damage to body structures, should it become excessive, may cause death. Because of these parasite caused anatomical damages, the finely adjusted balance of different vital processes of host's body become disturbed and host is said to be diseased. There are a number of causes of diseases. Parasites are one ; physiological stress, nutritional deficiency and poisoning are others. Some of common parasitic agents of disease and consequent mortality of animals are following :

1. Viruses are the potent agents of several disastrous diseases of plants and animals including man. For instance, some viruses are the agents of hoof and mouth disease in deer, spotted fever in rabbit, encephalitis and distemper in foxes.

2. Bacteria may produce localized inflamatory changes in tissue, enter the blood stream, or produce powerful poisons known as toxins. They cause a variety of diseases, notably tularemia, para-typhoid, and tuberculosis among birds and mammals, as well as other diseases in lower types of organisms.

3. Fungus spores of *Aspergillus* may be drawn into the lungs of ground-feeding birds, where they germinate and grow, causing aspergillosis disease. Fungus may also develop on the external surface of animals.

4. Protozoan parasites are especially important in the mentary tract and in the blood. A sporozoan species of *Eim* damages the walls of the intestine in upland game birds and ca coccidiosis disease; *Toxoplasma* becomes encysted in the brai rodents; *Leucocytozoon* is a common blood parasite of waterf and game birds.

5. Worm parasites, such as tapeworms; nematodes, acanthocephalans may wander through the hosts body doing me anical injury as well as destroying and consuming tissues. The h may respond by forming a fibrous capsule or cyst around an en dded parasite.

6. External parasites such as ticks, fleas, lice, mites, and do not commonly produce serious mortality by themselves they are often vectors transmitting protozoa, bacteria and from one animal to another. Heavy infestations of external pa however lower the vitality or vigour of an animal and cause of fur or feathers.

7. Nutritional deficiency in vitamins or minerals, or balance among carbohydrates, proteins, and fats may malformations, lack of vigour, even death.

8. Food poisoning, **botulism**, occurs when certain come contaminated with the toxins released by the bacter *ridium botulinum*. Many waterfoul are striken in some lo

9. Physiological stress (Selye, 1955) is a term th to be applied to changes produced in the body non-sp many different agencies which, may accompany any dis of stress include loss of appetite and vigour, ache an loss of weight. Internally, physiological stress cau abnormalities, such as, acute involution of the lym diminution of the blood eosinophils enlargement secretory activity of the adrenal cortex, and a vari in the chemical constitution of the blood and tissues.

10. Accidents, ageing, starvation, and so or included as important causes of mortality.

Organisms that produce disease generally fall categories. They are either present in the body at normally virulent, or they are normally absent from the momment the host is infected by them attack, even a mild one of some diseases often co complete immunity from further attacks of same sites, disease can reduce populations, exterminat restrict the distribution of host. For example, s

ally or unintentionally introduced nearly exterminated some tribes of North American Indians (*viz.*, Human populations). Malaria in southern Asia depressed population growth.

Social parasitism : Social parasitism describes the exploitation of one species by another, for various advantages. It is a kind of parasitism in which the parasite foists the rearing of its young into the host. Social parasitism in various stages of development is found among some higher vertebrates and insects. For example, there [illegible] of birds—old world cuckoos [illegible] orth America, both of which [illegible] ier they deposit their eggs in [illegible] eggs and young to the care [illegible] waylays foraging workers of another species and snatches away the food they are transporting or the robber species may deliberately rob another nest of food. Some species of ants make slaves of the workers of other species. Such kind of dependency of one species on another also occurs in other social insects such as termites, wasps and bees. Social insects are apparently the only animals other than man to have succeeded in domesticating other species, and of cultivating plants particularly fungi for food.

Parasitodism : Some Diptera and Hymenoptera deposit their eggs in the immature stages of other insects ; the larvae on hatching feed on the host until they are fully grown. In such type of parasitism in which host generally dies of the larval depredations before the larva emerges and the parasitoid larva generally lives in spite of the hosts death, is called **parasitodism.** It stands in between parastism and predation. The parasitoids may in turn be infested with **hyperparasitoids.** In theChicago, *Samia cecropia,* a saturniid moth, suffers the destruction of nearly 23% of its cocoon by an ichneumonid parasitoid, *Spilocryptus extrematis,* which deposits an average of 33 eggs on the inside of each coccon or on the surface of larva. The host larva dies in a few hours after the parasitoid hatches and the ichneumonid larva moves about freely, feeding on the cuticle or burrowing into the tissues to drink the body fluids. *S. extrematis* is infested by another hyperparasitoid, *Aenoplex smithii* which causes about 13% destruction of *S. extrematis* cocoons. A chalcidid, *Dibrachys boucheanus*, fed both upon *S. extermatis* and as a tertiary parasitoid, upon *A. smithii.*

8. PREDATION

Predation is a form of disoperation, at least in point of immediate effects, since one animal kills another animal or plant for

food. Like parasitism, predation is important in community dynamics but both differ in the point that a predator tends to be larger than its prey, and it catches its prey from without, while a parasite is smaller than its host and consume it from within (see Clapham, Jr., 1973).

COMPONENTS OF PREDATION

The main components of predation. are predators and prey. A successful **predator** has following characteristics :

1. The hunting ability of a predator remain well developed.

2. By their hunting activities predators can be regarded as specialized or generalized. **Specialized predators** are those adapted to hunt only a few species They are forced to move when the vulnerability of a staple prey item drops to a point where the predator population cannot support itself. For example, Peale's falcon shows a marked preference for ducks and pheasants. Deer exhibit a pronounced preference for certain species of browse (Klein, 1970). **Genaralized predators,** not so restricted in diet, adjust to other food sources. The horned owl and buted hawk have a large range of collective prey available. Foxes can shift to a vegetable and carrion diet, should conditions require it.

3. Hunting ability and success of predator involve the development of a searching image on the part of the predator. Once it has secured a palatable item of prey, the predator finds it progressively easier to find others of the same kind.

4. Though a predator may have a strong preference for a particular prey, it can turn in the time of relative scarcity to an alternate, more abundant species that provides more profitable hunting. For example, if rodents are more abundant than rabbits and quail, foxes and hawks will concentrate on them instead of game animals.

5. Habitat preferences or overlapping territories can bring predator and prey into close contact, increasing prey risks. For example, predatory rainbow trout in Paul Lake, British Columbia, moves into the shoals when their prey, the red side shiner, are most heavily concentrated there.

6. Age, size, and strength of prey influence the direction that predation takes. Predator select food on the basis of size. Mountain lions, for example, avoid attacking large healthy elk, which they cannot successfully handle, and concentrate instead on deer and young or feeble elk (Hornocker, 1970).

7. Predators hunt only when it is necessary for them to procure food. The searching rate of predator is influenced by the

speed of the predator relative to the speed ard escape reactions of the prey, to the distance at which predators first notice and attack the prey and to the proportion of attacks that result in successful capture.

Like the predators, the prey has its certain defensive specializations. The prey risk is determined by density of prey population, availability of food and protective cover (concealment place), movement, activity, habits, size, age, strength and escape reactions of prey.

Regulating effect of predation : It is commonly concluded that the predator—prey interaction causes a reduction in the prey population, it is deterimental in same fashion to the prey. This belief has led to extensive "predator control" efforts conducted in the name of wildlife conservation. But the coevolution of species within natural ecosystems has in fact led to a dynamic balance between the populations in a community, so that the population sizes of predator and prey species are inter-regulated by feed back mechanisms that effectively control the population of both. As the models of **Lotka-Volterra** and **Nicholson-Bailey** stated, a predator that consistently destroyed more prey organisms than the prey population could stand would drive itself, as well as the prey, into extinction, because it would cut off its own food supply. Likewise, if the level of predation is too low, a prey population—whose evolutionary history has, after all, conditioned it to a normal level of losses to predation—may become too large, where upon it can destroy its food supply.

Ricker (1954) has recognized two kinds of predation—A and B. In type A predation, predators of any given abundance take a fixed number of prey species during the time they are in contact, usually enough to satiate themselves. The surplus prey escape. Trout feeding on an evening hatch of mayflies would come under this category. There is a functional response but no numerical response. Type B predation exists if predators of any given abundance take a fixed fraction of a prey species, as though the prey were captured at random encounters. In other words, the amount of prey eaten is proportional to the abundance of predator and the abundance of the prey. There is both a functional and a numerical response. Many vertebrates, however, do not follow the same functional and numerical response in relation to prey and predator. As predators take most or all the individuals of the prey species that are in excess of a certain minimum number, as determined by the carrying capacity of the habitat and social behaviour. The prey species com-

pensates for its losses through increased litter and brood size and greater survival of young. For this reason this type of predation, Ricker's type C, is called **compensatory.** The population level at which predators no longer find it profitable to hunt the prey species has been called the **"threshold of security"** by Errington (1946). As prey numbers increase above this threshold, the surplus animals are no longer tolerated in the area, and become vulnerable to predation. Below the threshold of security, functional response of the predator is very low and numerical response is non-existent. Above the threshold, functional response is marked and numerical response could occur.

INTRASPECIFIC BIOTIC FACTORS

Besides interspecific interactions of biotic factors, there occur intraspecific interactions which operate along with other abiotic and biotic ecological factors in insuring the stability of animal populations. Some of the significant intraspecific biotic factors are following :

1. COLONIZATION

An early manifestation of intraspecific cooperation in the evolution of animals is the grouping of free-living protozoans to form **colonies,** and the further development of such colonies into multi-cellular metazoans that thereafter behave and respond as unit organisms. Whether the first gathering of protozoan cells to form colonies developed for better protection from some predator or environmental condition, improved utilization of food supplies, or more efficient reproduction, it is impossible to say (see **Kendeigh,** 1974). The colonial form, however, must have had survival value to persist.

Outcomes of colonization : *During the course of evolution the* colonization quickly led to **division of labour** between somatic and reproductive cells, as occurs in *Volvox*, and later to division of labour between somatic cells themselves, so that different cells or organs *became specialized to serve the particular functions* of digestion, respiration, circulation, excretion, and so on. Cooperation *among* cells, tissues, and organs gave greater metabolic efficiency to *the* whole individuals and resulted in evolution to the highest types of *animals.*

2. AGGREGATION

Aggregation refers to the tendency among animals to concentrate in numbers larger than found in normal distribution, and which congregate under various circumstances mostly by their own move-

ments. Besides modifying the abiotic environment, more particularly the microclimate, aggregations bring about changes in the morphology, physiology and ethology of the aggregating members, there by acting as a very effective biotic factor. Aggregations of animals have some survival significance—as hundreds, sometimes thousands, of spotted lady beetles hibernate under leaves at the forest-edge. Mayflies, midges, and mosquitoes swarm for mating purposes. Millions of bats roost together in large caves. The migratory locust moves from one locality to another in immense hordes, and birds usually migrate is flocks.

Aggregations may be permanent such as societies of social insects such as termites, ants, bees and wasps; or temporary. The temporary aggregations are formed when certain animals become gregarious by coincidence or for some mutual benefit. A good example is the gragarious habit observed in many species of membracid insects (*Homoptera*) and sometimes the same host plant lodges two different gregarious species, the individuals of both crowding together and mingling with each other very closely. Such close harmony is indicated by *Oxyrhachis tarandus* and *Otinotus oneratus*, both of which live on the plant *Prosopis spicigera*. The egg masses of the two species are very often laid so close to each other that egg slits of one species partially overlap the other. According to Ananthasubramanian and Ananthakrishnan (1975), the gregarious species happen to be highly prolific and they lay egg masses in close proximity, the nymphs as well as adults showing very little tendency towards migration with the result that several generations are passed through on the same plant, often on the same twig. They have recognized following kinds of aggregations in insects—1. Certain species (*e.g.*, *Leptocentrus moringae*, *Telengana nigrodata*, etc.) remain gregarious as adults and solitary as nymphs; 2. Some species (*e.g.*, *Coccosterphus tuberculatus*) remain solitary as adults, and gregarious as nymphs: 3. Some species such as *Leptocentrus rhizophagus* remain gregarious in I instar, and solitary thereafter ; 4. The species of *Oxyrhachis krusadiensis* and *Gargara mixta* remain gregarious as both nymphs and adults but not living with other species; and 5. Certain gregarious species live with other gregarious species as both nymphs and adults (*Oxyrhachis tarandus*, *Oxyrhachis refescens*, *Otinotus oneratus*).

Causes and advantages of aggregations : It is very often the environment that derives animals into collective groups. Scarcity of space, light, utilization of the same food and shelter, collective breeding and various other conditions induce the animals to aggregate

or congregate. These aggregations are capable of tolerating different ecological hazards more efficiently than they have been living solitarily. Insects and vertebrates survive the acute cold of winter by forming groups which help to generate, retain and conserve heat. This is one of the basic advantages of aggregations and is termed as the **group survival value**. Coveys of bobwhite quail roost in close circles, at night. Perhaps this enables detection of predators approaching from any direction but by that behaviour the birds can tolerate lower air temperatures and for a longer time than isolated birds can. Similarly, mice huddle in low air temperatures, a behaviour that reduces heat radiation and consequent need for frequent feeding.

Further, a single muskox or bison may succumb to a pack of wolves. When in a group, the males form a circle facing outward with the females and young inside, whereby they are usually able to ward off the attack. Similarly, a single wolf has difficulty in killing a deer ; a single coyote, in killing a pronghorn antelope. But in packs the wolves can overpower a deer and a pack of coyote can chase a pronghorn to exhaustion.

All these beneficial effects of aggregations, however, lost if the aggregation is either too small or too large. For example, the longevity of *Drosophila* is greatest with a population density of 35 to 55 flies in 28-g culture bottle. Smaller densities are unable to control the growth of the yeasts on which they feed ; greater densities exhaust the food supply and excessive amounts of excreta accumulate.

3. SOCIAL ORGANIZATION

There are many sorts of social organizations that operate in animal populations They range from those of bees, ants, wasps and termites, which rival only urban man in their degree of division of labour and responsibility among individuals in the population, to those of certain fish and birds, which show well-developed social organization during certain periods, such as the mating seasons, but which may show much weaker organization at other times. In highly organized insect societies, specialization occurs both in morphology and behaviour. The primary castes of termites and ants are the winged reproductive males and females, the wingless sterile soldiers that possess large mandibles and irritating glandular secretions, and the smaller, wingless often sterile workers. The soldiers defend the colony against predacious enemies ; this function is assumed by workers in bees and wasps, among which a distinct soldier caste is lacking. In termites, the soldiers may be either males or females ; in ants they are females. The worker caste in ants is usu-

or females. In primitive termites the nymphs of other castes substitute for workers. The workers collect food, cultivate gardens of fungi, take care of domesticated aphids or coccids, feed the other castes, and built shelters.

It has been argued cogently by Wynne-Edwards (1962, 1964) that social organization may be an extremely important mechanism in the regulation of population density for certain animals, especially vertebrates. The precise role of social organization in population regulation is not clear, and the mechanisms by which it has evolved are extremely controversial But the existence of social organization within animal populations can be demonstrated and a population's social structure may affect its density regardless of whatever other effects it may have on the population. The most important types of social organization that can act to control the sizes and distribution of animal populations are territoriality and social hierarchies.

(a) Territoriality: Many animals 'stake out' a specific volume of space for their activities. These activities may include nesting, feeding, and a number of other functions. The territory they have staked out is defended with great intensity against invasion by other individual of the same species. In some cases, this defense will be extremely aggressive, often involving actual bloodshed. In even more instances, the confrontation will be highly ritualized, with the threat of attack being much more important than the attack itself. The type of defense depends largely on the evolutionary level of the behaviour mechanism ; ritual confrontation seems to be much more advanced than genuine fighting. Entry into the territory by an individual of another species will provoke a very different reaction. If the territorial animal perceives no threat from the visitor, there will be essentially no response whatever, but if he does perceive a threat, the response will be extremely aggressive.

Further, the territorial instinct provides a division of the available space into more or less discrete subvolumes There may be some overlap, or there may be none. Each territory represents the amount of space for nesting or feeding by a single individual or nesting pair. The number of territories is thus related to the total amount of energy, nutrients, etc., which are available to the species. If these change in time, the number of territories may also change. Size of any specific territory is a function of both the total number of nesting pairs that can be maintained in the area in question and the relative ability of individuals to defend them. Consequently a very strong individual will be able to maintain a larger tetritory a weak one. Thus, in species with a well-developed territorial insti

all individuals that can maintain a territorial claim have an excellent probability of extracting from it sufficient food for themselves and their offspring. Those that cannot effectively excluded from reproduction and/or feeding. This is an effective mechanism for maintaining population size at a reasonable maximum, both by controlling the amount of reproduction in the population as a whole and by limiting the number of individuals who can feed to a number that the ecosystem can sustain. In addition, it insures that the strongest individuals will tend to reproduce, because they obtain territories first and maintain them most effectively, and that those members of the populations who starve or do not reproduce will be the weakest ones. It further guarantees that the strongest members will always have sufficient food and space, by selective mortality of weak individuals to adjust demand to supply rather than by sharing the burden over the whole population.

Territorial concepts were first developed in relation to birds, among which male establishes a territory from which he derives off all other males of his species, and sometimes females other than his male also, but generally ignores those of different species. But later on many mammals, ranging from bears to deer mice, were found to exhibit territorial behaviour, as do lizards, fishes and some insects.

In the views of **Clapham, Jr.** (1973), territoriality is a factor in the forces of natural selection acting upon the population as well as a mechanism for regulating the population. For example, in sea birds and other animals whose nesting space is very small compared to their feeding area. The feeding areas for these birds cover vast areas of the sea with no subdivision whatever into individual territories, but their nesting space consists of very tiny sites in closely crowded areas. The number of nesting sites, however, is closely regulated by tradition and nesting does not take place outside the traditional areas. Thus, there is intense competition for traditional nesting sites, but no nesting elsewhere. The net result of these taboos appears to be equivalent to that of territoriality. Those individals have attained a nesting site have a high probability of reproducing successfully. Those that have no nesting site are prevented from reproducing just as in territoriality, this mechanism seems to provide a ceiling on reproduction and to assure that the strongest individuals (*i.e.*, those that obtain one of the traditional nesting sites) are the ones that carry on the population.

(b) Social Hierarchy : Implicit in the idea of territoriality is that some individuals are "better" in some fashion than other

individuals in the population. This may be shown in greater capabilities of certain individuals to keep and maintain territories, or it may also be shown in a discrete social hierarchy within a non-territorial species. The most typical social hierarchy in nature is termed the **pecking order** The pecking order is the name which is derived from the social order by which domestic barnyard fowl—that have rather well-developed pecking orders—peck at each other without fear of retaliation. Each individual in the population is known to all other individuals in the population, and each has a social rank. The order in which individuals feed or obtain nesting sites depends on this rank, with individuals of low rank deferring to those of higher rank. When food and nesting sites remain in adequate supply for the entire population, all individuals have access to them. When they are not, only the animals at the top of the hierarchy remain successful in obtaining them. The rest starve, are excluded from nesting sites, or are inhibited in their sexual development. The pecking order, thus, can be an exceedingly effective means of pegging the population size to the current state of the environment, generally the availability of food, water, or nesting space, while insuring that a number of individuals in the population both survive and reproduce.

4. PSYCHOLOGICAL FACTORS

There are certain reactions by populations to unusual conditions, most commonly crowding, which do not come into play under normal conditions, and which seem like breakdowns in the normal psychological balances of the organisms. These include **cannibalism, inability to breed or care for young, increase in rate of spontaneous abortion** and similar problems. In many species, they act to control the population size when the more normal controls have failed.

An example of a relatively simple psychological factor acting to control population size is shown in the fish guppy, *Lebistes reticulatus* (Silliman and Gutsell, 1958). When stable experimental populations are maintained by regularly removing a percentage of the fish, significant numbers of the young survive, although some may be cannibalized by their parents. When no artificial predation is practiced the guppies breed continuously, but they remove the entire breeding surplus through cannibalism. Evidently, the normal control mechanism for guppy populations is predation from other fish higher in the food chain. But if, for any reason this mechanism is lost, the population can regulate its own numbers through cannibalism.

Such psychological regulation of population has also been studied in albino rat (Calhoun, 1962). When rats were raised in densities higher than normal, they showed psychological abnormalties such as hypersexuality, homosexuality, asexuality, cannibalism, inability of a mother to raise her pups successfully to weaning, increased rate of spontaneous abortion, breakdown in the premating rituals and abnormal crowding around certain places such as food hopper and water fountains. Different biotic factors of the environment thus tend to modify the activities of animals like the abiotic factors.

CHAPTER 5

ANIMAL POPULATION

The word population (*L. populus*, people) which originally referred to a group of people occupying a particular space, now includes groups of organisms occupying a particular space. Different ecologists have defined the population variously. **Odum** (1971) has defined population as a collective group of organisms of the same species (or other groups within which individuals may exchange genetic information) occupying a particular space. **Clapham, Jr.,** (1973) defined population as the total assemblage of individuals of a given species found in any ecosystem under study. **Krebs** (1972) **Smith** (1974), and **Ananthakrishnan and Viswanathan** (1976), are basically agreed upon the definition of the population which considers it as a group of organisms of the same species occupying a particular space at a particular time. Anyhow, population is one unit through which energy flows and nutrients are cycled ; it is a self-regulating system that helps in maintaining stability in the ecosystem and its properties include such parameters as abundance and population density, range, genetic variability, adaptations of the species to its environment, demands of the species on the environment, and factors associated with population growth, such as birth rate (natality), death rate (mortality) and age distribution. The study of the numbers for organisms and what determines their abundance and distribution is called **population ecology.**

Further, the ultimate constituents of the population are **individual organisms** that are potentially interfertile. The population may be subdivided into **demes** or **local populations**, which are groups of interbreeding organisms, the smallest collective unit of a plant or animal population. A population has characteristics or "biological attributes" which it shares with its component organisms, and it has characteristics or "group attributes" unique to the group. Among the former, the population has a life-history in that it grows, differentiates, and maintains itself as does the organisms. It has a definite organization and structure that can be described. While, group attributes such as birth rate, death rate, age ratio and genetic fitness, apply only to the population (see **Odum, 1971**).

of fertility is an actual level of performance in the population based on the numbers born. While fecundity is the potential level of performance (or physical capacity) of the population (Krebs, 1972). For example, the fertility rate for an actual human population may be only one birth per 8 years per female in the child-bearing ages, wheras the fecundity rate for humans is one birth per 9 to 11 months per female in the child bearing ages.

Natality rate may be expressed as the number of organisms born per female per unit time. The crude natality rate of a population can be expressed as follows :

$$\text{Birth (Natality) rate (b)} = \frac{\text{number of births per unit time}}{\text{average population}}$$

or

$$b = \frac{dNn}{Ndt}$$

where b = natality rate per unit time, d = entity that is changing, N = initial number of individuals in the population Nn = number of new individuals added to the population by natality, t = time.

The birth rate is the function of the density of the population. As a general rule, if the population density is unusually low, the birth rate is also low, because the probability of males and females finding each other to mate is small. If population density is unusually high, the birth rate may also be relatively low, because of inadequate nutrition or the physiological or psychological aberrations associated with crowding. There is often an optimum density at which the birth rate is maximized. This is generally a relatively low density. Thus the **maximum or absolute natality** is the theoretical maximum production of new individuals under ideal conditions. To attain this maximum a species would exist under ideally optional ecological and genetic conditions. **Ecological or realised natality** refers to increase of population under given set of environmental conditions. It is not constant for a population but may vary with the size, composition and fecundity of a population.

FECUNDITY

Species vary greatly in the characteristic number of generations, broods or litters produced per year, and in the sizes of them. Protozoans often divide so rapidly that they produce a new generation every few hours. Plankton organisms, less fecund, may produce a new generation every few days. Many vertebrates breed but once a year, some large animals only once every two or three years. Several species of small birds and mammals have two or more broods per year. Rodents may continue to breed throughout the

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winter under favourable environmental conditions, so that their reproductive potential is enormous.

The maximum size of a litter is determined by the physiological and morphological characteristics of the species. In mammals, which produce viviparous young, the size of uterus and body cavity as well as the number of mammary glands for suckling the young after birth act as limiting factors for the size of the litter. In birds there is a limit on the number of eggs that one individual can cover and successfully incubate. However in species that do not take care of their eggs after laying, the number produced may be limited only by the energy resources of the parent.

The number of eggs or young produced per litter is correlated inversely with the amount of attention (*viz.*, parental care) that they require When parental care is altogether lacking, invertebrates may lay 1,000 to 500,000,000 eggs at one maturation ; where there is some protection afforded by brood pouches, 100 to 1,000 eggs may be laid ; with a high degree of brood protection, 1 to 10 or more eggs may be laid. Mammals seldom have more than a dozen young in a single litter and, in larger species, usually only one. Characteristic clutch size among birds varies from 1 to 15 ; rarely, 20.

Further, there is a limit on the size of the brood or litter that adult warm-blooded animals can successfully feed and raise to maturity. There is no advantage, for example, for starlings to have broods larger than five. In larger broods, each individual receives less food, and hence has less vigour and weight on leaving the nest. In such weak individuals, mortality increases either before fledging or in subsequent months (**Perrins, 1965**). In those species that feed their young in the nest, the clutch size has evolved through natural selection to the greatest number that can be hatched and raised successfully through efforts of the adults (**Lack, 1967**). In those species whose young leave the nest and feed themselves at hatching, the clutch size depends in large part on the capability of the female to mobilize energy in her body to produce eggs of a particular size (**Ryder, 1970**). The variability in clutch, and litter size for most species allows them to take advantage of temporarily improved conditions.

The size of brood, litter or clutch is also regulated by weather. For example, the clutches laid by birds during periods of hot weather are usually smaller than those laid when temperature is moderate (**Kendeigh, 1941**). Clutches laid by related species in temperate latitudes tend to be larger than those laid in the tropics. Likewise, the fecundity of white-tailed deer is higher with good forage than

with poor forage. The reproduction is generally more successful after periods of high mortality than during years of abundance.

MORTALITY OF POPULATION

Mortality is the population-decline factor and thus, in terms of its effect on the group, is antithetic to natality. As natality can be expressed as a rate of gain or birth of individuals in unit time mortality can be expressed as a loss of individuals in unit time—the death rate. Generally mortality is expressed as specific mortality, that is, the number of members of an original population dying after the lapse of a given time. The crude rate of death of a population can be calculated by this equation :

$$\text{Death rate (d)} = \frac{\text{number of deaths per unit time}}{\text{average population}}$$

The rate of mortality (death) is related with density like natality. Mortality is almost highest at very high densities because the hazards of over crowding and the greater likelihood of concentrated predation or spread of disease, but it is often quite high at very low densities as well, because a number of individuals of a given species are often better able to survive during a period of stress than is a single individual. For instance, heat generated by a cluster of bees is sufficient to allow survival of the cluster in temperatures low enough to kill the bees were they not clumped together.

The death rates vary among species and are correlated with rates of reproduction (natality) The death rate of a species is influenced by a number of factors such as destruction of nests, eggs or young by storms, wind, floods, predators, accidents, and desertation of parents, but of fundamental importance is the number of young that are born in relation to the carrying capacity of the habitat. When more young are born than the habitat can support, the surplus must either die or leave the area. Because, the number of survivours is more important than the number dying, so mortality is better expressed as survival or as life expectancy, the average number of years that members of a population have left to live.

Mortality rate is usually expressed as a percentage of individuals dying within a given time. The mortality rate in many species varies from one age level to another, thus, a mean death rate has only general significance. In birds, however, the death rate is nearly constant once they become adult, and it is then apparent that it varies inversely with adult longevity. In adult penguins, pelicans, shore birds, gulls, and swifts, the annual mortality rate is commonly between 12 and 30 per cent; in herons, hawks, and owls it is about 30 per cent; in ducks, doves, and song birds it is between 40 and 68

per cent, while in gallinaceous birds it is the highest, 60 to 80 per cent. As mortality varies positively with age in the majority of organisms, the specific mortalities at particular ages can be illustrated in the form of life table.

LIFE TABLES

Species differ widely in the number of young produced, each year, in the average age to which they live and in their average rate of mortality. When sufficient facts about a species are known, a **life table** that tabulates the vital statistics of mortality and life expectancy for each age group in the population may be formulated. In such tables, age is usually represented by the subscript index x and is some convenient fraction of a species mean life span, such as year or stage of development. The life table is set up on the basis of an initial cohort of 100, 1000, or 100,000 individuals; and the number living to the beginning of each successive age interval is symbolized as lx. Plotting these data gives a **survivourship** curve for the species, and a number of survivourship curves have been worked out for different species. The number dying within each age interval is designated as dx and gives a mortality rate. The rate of mortality during each age interval is commonly expressed as the percentage of the number at the beginning of the interval 100 (dx/lx) and is indicated as qx. Survival rate is the difference between the mortality rate and 100 per cent ($100-qx$) and is expressed as sx. Life expactancy (ex) is the mean time that elapses between any specified age and the time of death of all animals in the age group. For example we can consider following life table for budworms of Canada :

Table : Life table for the 1952-53 generations of the spruce budworm in New Brunswick, Canada (after Morris and Miller, 1954, source Kendeigh, 1974).

x	lx	Factors responsible for dx	dx	qx
Eggs	1000	Parasites	17	2(−)
		Predators	86	9(−)
		Others	6	1(−)
		Total =	109	12
Instar I	891	Dispersal, etc.	428	48
Hibernacula	463	Winter	79	17
Instar II	384	Dispersal	242	63
Instars III-VI	142	Parasites	51	36
		Disease	3	2
		Birds	20	14
		Others	61	43
		Total =	135	95

x	lx	Factors responsible for dx	dx	qx
Pupae	7	Parasites	0·6	8
		Predators	0·7	10
		Others	1·3	18
		Total =	2 6	36
Moths	4 3	Total for generation =	995·7	99·5

Uses of life tables : Life tables are useful for computing the average longevity of a population, for showing the age composition of a population, for indicating critical stages in the life cycle at which mortality is high, for showing differences between species, for showing the success of the same species in different biotopes, for furnishing information of value in game and fish exploitation (yield) and in control of pests.

Types of survival curve : Because which is really vital for the population is not which members die, but which members survive, specific mortality is expressed by **survivorship curve.** If it could be assumed that all members of an original population had the same capacity for survival (environmental effects for the moment are ignored), plotting the number of surviving individuals against time would produce a survivorship curve in the form of a right angle. The curves of survival plotted from life table may be of three types. In type 1 a cohort finds environmental conditions ideal ; all members, born at the same time, live out the full physiological life span characteristic of the species, and all die at about the same time. The survivorship curve of modern industrialized man approaches type 1. In type 2, the rate of mortality is constant at all age groups, so that an individual's chance of living another year is just as good at one age as another. This approximately true for some adult birds. Type 3 shows extremely heavy mortality early in life, as will such forms as the oyster, where most of the millions of eggs spawned never hatch, but those few individuals that survive have a high life expectation there after. Most survivorship curves observed under natural conditions are intermediate between these three types (e.g., deer, annelid worms, insects, and isopods).

AGE DISTRIBUTION OF POPULATION

It is obvious that populations that differ in the relative numbers of young and old will have different characteristics, different birth and death rates, and different prospects. In other words, age distribution is an important population characteristic which influences both natality and mortality. Mortality usually varies with age, as

chances of death are more towards the earlier and later periods of life span. Likewise, reproduction (natality) is restricted to certain age groups, for example the middle age groups in the higher animals. The distribution of ages in a population may remain fairly constant, or it may change throughout the generation time. The population age distribution is related to the growth rate of the population, and it can be used to estimate whether a population is expanding or contracting. Further, the ratio of the various age groups in a population determines the current reproductive status of the population and indicates what may be expected in the future. Usually a rapidly expanding population will contain a large proportion of young individuals, a stationary population more even distribution of age classes and a declining population a large proportion of old individuals.

METHODS OF MEASURING THE AGE OF POPULATION

There are several different ways of measuring age, and there are two main ways of presenting age distribution in a population. The simplest measurement for age is the raw age itself. In only one species of organism is under investigation, it is probably the best scale to use, in addition to being the simplest. However, it is difficult to compare age distributions of different species using raw ages if the life spans are different, as, for instance, between elephants and mice. For the purpose of comparing age distributions, a useful means of portraying age is as a percentage of total life span. The maximum observed life span of a species is determined, and ages are presented as percentages of that maximum. This presentation format for age data can be applied equally well to any species of organism, regardless of its life span. A related means of presentation is percentage of deviation from mean life span. In this format, the average age at death is computed and ages presented in terms of deviation from this mean age. Both of these formats require that the maximum or mean life span, respectively, be shown for each species, because this information is not presented in data converted to percentage form.

A some what different type of age portrayal is the ecological or functional age, where the life span of a species can be divided up into various functional units, such as prereproductive, reproductive, and postreproductive, or egg, larva, nymph, and adult. The appearance of individuals of the various functional ages may be quite similar, as in man, or quite different, as in animals that undergo metamorphosis. Functional age breakdowns can be extremely useful in comparing certain features of different populations,

such as the relative importance of specific environmental factors on the population as a whole. If we know both the percentage of time spent by an organism in specific functional ages and the environmental factors that have the greatest effect on each age, we have gained considerable insight into the characteristics of the species in question. This knowledge is essential if we are trying to design a procedure to control a best species on an economically important crop, and it is necessary to ascertain which stage in the life cycle of the pest is most vulnerable to attack by which means.

Age pyramids : The first means of portraying age distribution is the **age pyramid.** An age pyramid is a vertical bar graph in which the number or proportion of individuals in various age ranges at any given time is shown from youngest at the bottom of the graph to oldest at the top. It is very useful in monitoring the commericial exploitation of food species by man among other things. Ages may be shown in any of the formats we outlined. An age pyramid represents the age distribution of a living population at a specific moment; thus, as the population age distribution changes over a period of time, the form of the age pyramid does too. From an age pyramid, one can collect the information that whether a population is expanding, contracting, or stable. When compared to the age pyramid of a stable population, if age pyramid shows an increased number of juveniles (*i.e.,* a broader base), it is expanding ; if it shows a decreased number of juveniles (*i.e.,* a narrower base), it is contracting. Consequently, a triangular pyramid will represent a growing population and an urn-shaped pyramid will indicate an increased number of middle aged and old organisms compared to the young. However a bell-shaped pyramid will denote a stationary or stable population having an equal number of young and middle-ages classes.

Biotic potential and environmental resistance : The term **biotic potential** or reproductive potential, refers to the inherent power of a population to increase in numbers when the age ratio is stable and all environmental conditions are optimal. The biotic potential is defined mathematically as the slope of the population growth curve during the logarithmic phase of growth. When environmental condition are less than optimal, the rate of population growth is less. The difference between the potential ability of a population to increase and the actual change in size of the population is a measure of environmental resistance.

The sum of the physical and biologic factors which prevent a species-from reproducing at its maximum rate is termed the **environmental resistance.** Environmental resistance is often low when a

species is first introduced into a new territory so that the species increases in number at a fantastic rate as when the rabbit was introduced into Australia and English sparrow and Japanese beetle were brought into the United States. But as a species increases in number the environmental resistance to it also increases, in the form of organisms that prey upon it or parasitize it, and the competition between the members of the species for food and living space, and consequently, population decreases. An equilibrium will be reached either by decreasing the birth rate (natality) or by increasing the mortality rate.

GROWTH FORM OF POPULATION

The growth is the most fundamental dynamic feature that a species population display. Populations characteristically increase in size in a sigmoid, S-shaped or logistic fashion. When a few organisms are introduced into an unoccupied area, the growth of the population is at first slow **(positive acceleration phase)** then becomes very rapid **(logarithrmic phase)** and finally slows down as the environmental resistance increases **(the negative acceleration phase)** until an equilibrium level is reached around which the population size fluctuates more or less irregularly according to the constancy or variability of given environment. The level beyond which no major increase can occur represents the saturation level or **carrying capacity.** The carrying capacity or equilibrium density is represented by the letter K It is often useful to define the maximum rate of growth of the population. This parameter, generally termed the **intrinsic rate of natural increase,** is symbolized r_0 and represents the growth rate of a population that is infinitely small. Accordingly such type of population growth can be described by following **logistic equation :**

$$dN/dt = r_0 N (K-N)/K$$

If one desires to represent sigmoid growth of the population graphically, he has to plot the time on the X-axis and the number of organisms on the Y-axis, and consequently, will get a characteristic S-shaped sigmoid curve. Human population and growth of yeast, *Drosophila* or rabbit in laboratory conditions, follow such S-shaped population growth.

Another kind of population growth curve, called **J-shaped growth curve** is obtained when the density of the organisms increases rapidly, in compound interest fashion, and then stops abruptly as environmental resistance or other limits become effective more or less suddenly.

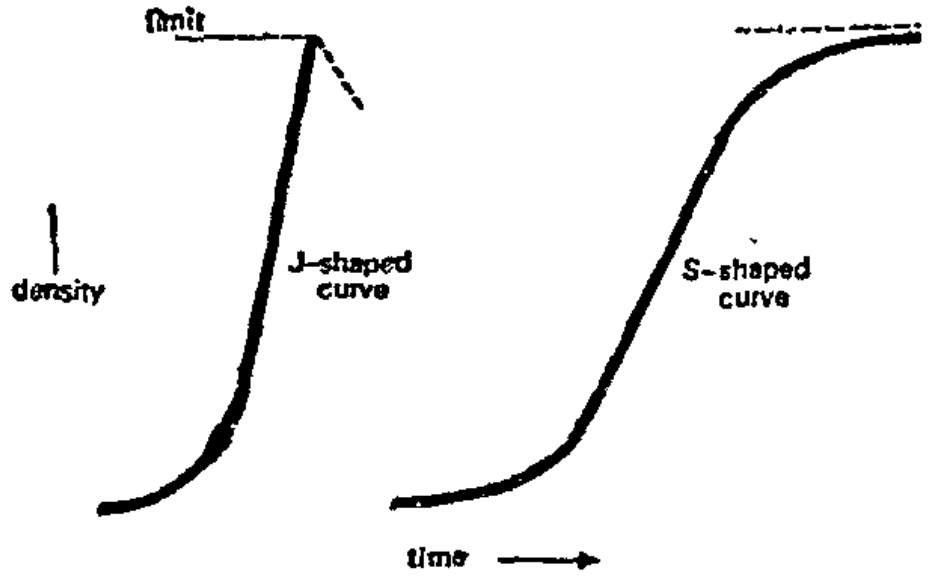


Fig. 5·1. J-shaped and S-shaped population growth curves.

GROWTH RATE OF POPULATION

The rate of growth of a population is expressed as the number of individuals by which the population increases divided by the amount of time that elapses while this population increase is taking place :

$$\text{Growth rate } (r) = \frac{\text{number of births } (b) - \text{number of deaths } (d)}{\text{average population in time interval.}}$$

The actual change in population number ($\triangle N$) over any span of time ($\triangle t$) is equal to rN. This can be written $\triangle N/\triangle t = rN$ or, using the symbology of the calculus, the rate of change of the population at any instant time (dN/dt) can be expressed $dN/dt = rN$. This is equivalent to saying that the number of individuals at any arbitrary time t, or Nt, is related to the number of individuals at the beginning, N_0, by the equation $Nt = N_0e^{rt}$, where $e = 2{\cdot}71828.....$, the base of the natural logarithms.

If r is constant, the growth of population will be exponential. If r is positive ($b>d$), the population shows an exponential increase to indefinite density and if r is negative ($b<d$), it shows an exponential decay to extinction. It is impossible for a population to change at an exponential rate indefinitely. However, there are many cases in which conditions are such that b is substantially larger than d for a period of time, following which conditions change so that d becomes much larger than b. The responses of populations to variations of this sort is an exponential "population explosion" during favourable conditions, followed by a "crash" when conditions change. Diatom populations in Lake Michigan, USA, for example, undergo such exponential increases at different times of years, triggered by variations in abiotic factors within the lake, followed by equally rapid declines.

POPULATION DISPERSION

Populations have a tendency to disperse, or spread out in all directions, until some barrier is reached. Accordingly, population dispersion is the movement of individuals into or out of the population area. It takes three forms : **emigration**—one-way outward movement; **immigration**—one-way inward movement; and **migration**—periodic departure and return. Dispersion supplements natality and mortality in shaping population, growth, form and density, and also it plays a significant role in the distribution of plants and animals even to the areas previously unoccupied by the members of the population. Most types of population dispersion occur due to a number of reasons such as for obtaining food, avoiding predators, preventing overcrowding, result of action of wind and water, environmental factors as light and temperature, breeding behaviour, physiological reasons as secretion of some hormone or for interchange of genetic material between populations.

EMIGRATION

Emigration under natural conditions occur when there is overcrowding in the migratory locust, lemming, grouse, snowy owl, snowshoe rabbit, Arctic fox, gray squirrel and occasionally in other species. This is generally regarded as an adaptive behaviour that regulates the population on a particular site and prevents over-exploitation of the habitat. Further, it leads to occupation of new areas elsewhere. By dispersing into new localities, there is opportunity gained for interbreeding with other populations leading to more genetic heterozygosity and adaptability.

It is, of course, population pressure that is responsible in large part for the dispersal of the young and extension of ranges into new areas. Under normal conditions adult animals, especially among the higher vertebrates, are well established on their territories and the youngs are forced to seek homes elsewhere. Among insects, there is a relation between emigration and inherited behaviour tendencies. Individual tent caterpillars, both larvae and adults, differ innately in the extent to which they show activity even within same colony. In the development of populations of excessive size, spread of infestations of the insect into new regions is largely by the more active individuals. The outbreak finally terminate when the proportion of sluggish individuals comes to predominate in the population (Wellington, 1966). Continuous emigrations are rare and when they occur, result in **depopulation**. Equilibrium of populations is maintained in such circumstances by enhancing the reproductive ability as well as by decreased mortality among the populations.

IMMIGRATION

Immigration leads to a rise in population level, causing an **overpopulation** which may lead to an increase beyond the carrying capacity. These immigrations result in increased mortality among the immigrants or decreased reproductive capacity of the individuals. Both emigration and immigration are initiated by weather and other abiotic and biotic environmental factors.

MIGRATION

Migration is a peculiar kind of population dispersion which involves the mass movement of entire population. This can occur only in mobile organisms and best developed in insects like the butterfly *Danaus plexippus* and in the migratory dragonflies *Libellula quadrimaculata* and *Pantala flavescens* , in fishes like eels, in birds and in certain mammals. Most two-way migratory movements are rhythmic processes of populations and regular periodicity is a common feature. Very often, environmental periodicities control these migratory movements, as for example day and night rhythm, lunar periods, tides and changing seasons. The monarch butterfly-*Danaus plexippus*, travels very long distances and their migrations are found to be pathed every year through their same conventional routes, and their migratory movements is initiated by the oncoming winter and the return trip being influenced by spring.

In most cases, migration of population may occur for food, shelter, or reproduction. Better utilization of uninhabited or hitherto untouched habitats and their resources is the greatest benefit derived from the migratory movements. However, during migration of population mortality of numerous individuals may occur due to different ecological hazards such as temperature fluctuations, scarcity of food, predation, etc. Anyhow, migration has certain benefits for populations—as it enables wider dispersion of populations ; it avoids intraspecific competition for food, shelter or any other means.

At last we can say that population dispersion is brought about by population increase, improvement of habitats or deterioration of habitatats, sparseness of population and by changes in or extremes of weather.

REGULATION OF POPULATION SIZE

The inherent tendency of all animal populations is to increase in numbers. But this increase in number is however, not infinite since the carrying capacity of the environment always imposes a restriction upon it. Thus, after reaching the carrying capacity the population density tends to fluctuate above and below

and such fluctuations in population between upper and lower limits tend to give some stability to the population. The nature of the processes that regulate the numbers of plants and animals is a major problem of population ecology.

Many groups of animals and plants are provided with unique and intrinsic self-regulatory mechanism such as failure of reproduction and self-inflicted mortality for controlling the size of the population. Populations grow when natality exceeds mortality, and they decline when mortality exceeds natality. However, limitation of animal numbers on a population is brought about by the action and interaction of two basic regulatory processes namely **density independent** and **density dependent factors**. Density independent factors are the extrinsic factors which tend to regulate the density of a population under different conditions, appearing to act on the population and inflict loss of individuals irrespective of the population density. Variations in space or cover, favourable weather and food occur independently of population densities and may cause drastic changes in the abundance of animals. Such ecological or environmental factors influcence negatively or positively all the individuals of a population irrespective of density.

The density dependent factors are intrinsic or biotic factors and they depend on coaction between individuals within the same population or between populations of different species. Density-dependent factors may stabilize populations at an asymptote, the level of which is determined by the carrying capacity of the environment. Some of the important density dependent factors are competition, reproductivity, predation, emigration and disease. The combination of factors or any specific factor involved in the density dependent action may vary from species to species. Further these species have been known to display the property of intercompensation. According to intercompensation, if there is a change in the environment to relive the population of pressure from an existing effect, then the population increases till it reaches a level when the second effect takes over. Thus, if the predators that normally keep herbivorous animals down are removed, the population of herbivorous may increase to become overcrowded and result is starvation. A supply of abundant food at this junction would make the individuals susceptible to diseases due to the intensity of crowding (*Wilson and* Bossert, 1971). Generally, the population fluctuations controlled by extrinsic factors tend to be irregular and correlated with the variation in one or more major physical limiting factors such as tempera-

corticosteroids, an excess or imbalance of which produces the alarm response or physiological shock.

In the latter part of the winter of a year of peak abundance, with the stress of cold weather, lack of food and the onset of the new reproductive season putting additional demands on the pituitary to secrete gonadotropins, the adrenal-pituitary system fails, becomes unable to maintain its normal control of carbohydrate metabolism, and low blood sugar (hypoglycemia), convulsion and death ensue.

According to Kendeigh (1974) several instrinsic factors such as disease, predation, food factor and natural selection affect animal cycles in addition to several extrinsic factors such as weather, solar radiation, and so on.

CHAPTER 6

BIOTIC COMMUNITIES

A monospecific assemblage of living organisms is a population. An assemblage of population living in a defined area or habitat is called **biotic community.** A biotic community is thus is a complex assemblage of **microbial community, plant community** and **animal community. Allee** *et al.* (1949) has defined community as *a natural assemblage of organisms which, together with its habitat, has reached a survival level such that it is relatively independent of adjacent assemblages of equal rank ; to this extent given radiant energy, it is self-sustaining.* However, **Ananthakrishnan** and **Viswanathan** (1976) considers community as any assemblage of ecologically related organisms composed of two or more species. The community units may be very large, like the continent-wide coniferous forest, or very small like the community of invertebrates and fungi in a decaying log. A different community occurs in each different habitat and environmental unit of larger size, and in fact the composition and character of the community is an excellent indicator of the type of the environment that is present.

The importance of the community concept does not lie so much in the populations themselves, as in the set of functional interactions between them. In preceding chapter, we have observed how individual populations interact strongly with one another, both as food supplies and as a population regulatory agents. But populations also interact with other members of their communities in much more ingenious fashions, which may have little direct role in the regulation of the specific groups involved, but which are fundamental to the maintenance of the community as a whole and hence to the existence of any species in the community. For instance, there is no direct interaction between deer and nitrogen bacteria in a forest. They belong to different food chains ; both have their own population dynamics, and they are regulated by different aspects of the physical or biotic environment. Yet without nitrogen bacteria, nitrogen could not cycle, and deer could not exist (nor any other form of life, for the matter). Conversely, without members of the grazing food chain such as deer, nitrogen bacteria would have no

nous wastes to feed upon. Thus, in a biotic community there exists a great interdependence among species of different communities (*i.e.*, plant community, microbial community and animal community). The very existence of any population is due to the fact that other populations also exist which make available energy and nutrients, regulate population size, recycle wastes, and buffer the community against the vagaries of the physical environment Population exist, in fact, because they are parts of biotic communities.

Further it has been suggested that the community is analogous to a superorganism in which the various component organisms may represent the structural and functional units in the same manner that different types of cells make up an organism. As such, a community exhibits all those life processes of an individual organisms that were mentioned elsewhere ; that is, metabolism, reproduction, growth, and adaptive responses to environmental fluctuations (see Vernberg and Vernberg, 1970).

TYPES OF COMMUNITIES

According to Kendeigh (1974), the communities may be distinguished as major or minor **Major communities** are those which, together with their habitats, form more or less complete and self-sustaining units or ecosystems, except for the indespensable input of solar energy. **Minor communities**, often called societies, are secondary aggregations within the major community and are not, therefore, completely independent units as far as circulation of energy is concerned.

COMMUNITY STRUCTURE

As communities are dynamic entities of an ecosystem, they include populations that interact constantly with one another, with the patterns of interaction showing continuous variation, both in time and in space. But at all times and in all situations there is a pattern to the organization of the community that is ultimately *determined by* the complex of environmental factors characterizing the community. This characteristic pattern often termed **community structure**, is reflected in the roles played by various populations, their ranges and the types of areas they *inhabit*, the *diversity* of species in the community and the spectrum of interactions between them, and the precise flow patterns of energy and nutrients through the *community* (Clapham, Jr., 1973). Consequently the structure of community includes following aspects :

ECOLOGICAL NICHE

Each species has a specific position within th[illegible] designated as **ecological niche**, and [illegible] oft[illegible] the

functional status or total role of the species within the community. The niche comprises all the bonds between the population and the community and ecosystem in which it is found. These bonds include factors such as the tolerance ranges and optima for all abiotic environmental factors, the sorts of organisms that can be utilized for food by the population, as well as the organisms that feed upon it, the areas in which the species can live, and the population structure of the species. Every population has an ecological niche and this niche is the major determinant of the structural, physical and behavioural adaptations of the population. Recently, the concept of niche has been extended to categories such as **spatial niche** or **habitat niche, trophic niche, climatic niche,** etc.

Different communities in ecosystems characterized by similar environments are often exceedingly similar in their structure, and they may contain one or more niches that are essentially identical. The adaptations of populations inhabiting these niches may also be exceedingly similar, even though they are totally unrelated. For example, the succulent (xerophytic) plants of the southwestern United States are cacti ; in the deserts of southern Africa, there are plants of the spurge family that are virtually identical. In two pairs of birds namely the little auk (a bird of order of gulls) and Magellan diving petrel (a bird of the order of albatross) ; and the eastern meadowlark (an American icterid bird) and yellow throated longclaw (an African Motacillid bird), though each bird is totally unrelated to the other, but they all are virtually identical in appearance and basic adaptations. This phenomenon of similar but unrelated species in different geographical areas is called **ecological equivalence.** It gives much insight into the essence of the niche. The adaptations of an organism to its niche are the result of an extensive period of evolutionary change. In order for two unrelated species to have evolved to a point of ecological equivalence, the niches to which they are adapted must be essentially identical, even though they may be separated by many thousands of miles. It would be impossible for different species to inhabit the same niche in widely separated areas if the niche and the interactions of which it is composed were a property of population alone. Clearly, the structure of communities set in similar environments is so similar that essentially identical niches can develop in them. This means that the principles by which biologial communitie are organized surpass the individual communities, and that the evolution of species may proceed in a fairly predictable manner, controlled by the structure of the community in which it exists, which

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itself is a function of the fundamental abiotic features of the environment.

On the other side, no two species populations can occupy the same niche in a single community (cf. **Gause's principle**). In such a case, one will always be superior and derive the other into local extinction through competition. For different species to survive within a single community, there must be some difference between their niches. In Gause's classic experiment, for example, different species of *Paramecium* were raised together. When *P. aurelia* and *P. caudatum* were the experimental species, only *P. aurelia* was left after sixteen days. The niches of these two species are essentially identical, and the more rapid population growth of *P. aurelia* allowed it to overwhelm *P. caudatum*. However when *P. bursaria* and *P. caudatum* were cultured together, both survived. Even though their food requirements are the same, they live in different parts of the culture chamber, and this simple separation is sufficient to enable each species to have a separate niche-and hence to survive.

There remains a possibility of an overlapping of niches of different species. Of the six species of parasitic Hymenoptera attacking the cocoons of the sawfly *Neodiprion swainei* the four indigenous species overlapped in their distribution although each species occupied one position in the **niche space** ; some even developed a 'zone of dominance' over the other species (Price, 1971). If the amount of overlap is very small then the two populations can exist simultaneously in essentially separate niches. If the overlapping is virtually complete, then the population that is more successful in exploiting the environment will drive the less successful population into extinction through interspecific competition. For example, locusts usually eat grass and wild plants and do not then compete with man, but when large swarms migrate, they eat crops and so compete with man for food. However, abundance of food supply in situations where the same food resource was exploited by different species. averted competitive exclusion. Many closely allied herbivorous fishes coexist in the same habitat utilizing the same food and this is attributed to the sumptuous supply of algae (Freyer, 1959), generally among two populations.

The number of potential niches in the community is a function of the degree to which the development of community under a given environmental regime leads to a divergence or separation of partially overlapping niches. If the tendency for separation is high, then the community will be more complex than if it is low. Stable environments which are characterized by communities having a

large number of separate niches, foster the separation of niches, whereas harsh environments characterized by communities with fewer niches, tend not to foster niche separation. The mechanism for niche separation is natural selection.

SPECIES ABUNDANCE AND DIVERSITY OF THE COMMUNITY

The number of populations in a community varies greatly over a wide range. Some communities of one prominent plant species (single population) may occupy a single layer of a habitat such as the desert salt-grass community or any other community of tundra, while, the communities, such as tropical rainforest or a coral reef, may be multispecies and multilayered system and may contain thousands of populations. The number of species in the community is termed the **richness. Species diversity** closely follows the number of constituent populations, when populations constituting the communities are small there tends to be more diversity, because the coexistence of different species in a community is regulated by the basic resources available within the ecosystem. But species abundance is not the only basis for measuring species diversity in all cases. A gradual increase in species diversity is apparent from the polar regions to the tropics.

Since a community is a natural assemblage of organisms that have evolved together to a high collective persistence level, its structure is based on its relatively distinctive species composition—which may be either sharply different from that of an adjacent community or may show degrees of overlap. Some species are found in only one community (an **exclusive species**), whereas others live in many communities (an **ubiquitous species**). Typically in the aggregation of species that make up a community, only a few species are abundant in number of individuals or biomass (weight) and the majority of species are relatively rare.

COMMUNITY DOMINANCE

Not all organisms in the community are equally important in determining the nature of the whole community. Out of the numerous kinds of organisms (or species) that might be present in a community, a *few species generally exert the major controlling influence* by virtue of their numbers, size or activities. This phenomenon where in various species within the community are dominated by one species or more than one species, is termed **community dominance**, and the dominating species are recognized as **community dominants**. Plants are more frequently dominant in terrestrial communities than are animals. In aquatic communities, animals are

relatively more important in this role, although dominance is often not developed.

According to Clements and Shelford (1939), dominance is most commonly expressed in the reactions of an organism on its habitat. Community dominants shoulder the full impact of the climate or the environment but modify this effect for other organisms within the community by tempering light, moisture, space, and other conditions. Only those other organisms that find these modified physical conditions tolerable can exist within the community. Furthermore, dominants are ordinarily the most prominent species in the community, make up its greatest mass of living material (biomass), and serve as the major source of food, substrate, and shelter for the animals that are present. In a forest community, trees are dominant. They decrease light intensity, increase the relative humidity, intercept precipitation, monopolize most of the moisture and nutrients in the soil, decrease wind velocity, and furnish shelter and food for animals. Grasses play a similar, though less conspicuous, role in prairie communities; sedges, rushes and cattails in marsh communities; sagebrush in the arid habitat of the Great Basin; mussels and barnacles on a rocky seashore; and so forth.

In certain cases, community dominance is demonstrated in coactions, direct effects of organisms on each other. In some fresh water ponds, carp and suckers may consume much of the submerged vegetation. This coaction thus prevent constituents from assuming their usual role in community, and by so much prevents the occurrence of animal species that depend directly upon the plants. These fishes also react upon the habitat by stirring up the bottom, from which they derive organic matter, thereby greatly increasing the turbidity of the water. Penetration of light into the water is reduced, greatly handicapping sunfish, bass, and other species which locate visually.

In ancient days, bison on great plains of U.S.A. fed on the luxuriant taller grasses more extensively than on the short grasses, with the consequence that, over extensive areas, short grass species replaced tall grasses almost entirely. Thus bisons were coactant with and dominant over the composition and character of the community (Larson, 1940). Similarly, overpopulations of the European rabbit alter the character of the forest by feeding upon the seedlings of oak, beech, and hornbeam to the exclusion of other species. When introduced into Australia, the European rabbit converted grassy areas into desert-like tracts. Though, animals are more common coactors than plants, plant pathogens may occasionally exert dominance in

this way. For example, the pathogenic fungus-chestnut blight virtually eliminated the chestnut population from the deciduous forest of eastern North America during the first few decades of the twentieth century.

Further, in a forest community in which trees are dominant, species in the lower stratum of shrubs modify the habitat still further and even the herbs exert some control over the physical conditions on the surface of the ground. The herbs and shrubs are termed as **subdominant species** and they have to tolerate the conditions established for them by dominants, but they in turn are the modifier of the community composition in a secondary manner.

TROPHIC STRUCTURE OF THE COMMUNITY

The diversity and hierarchical arrangements of population (*i.e.*, dominant-subdominant communities) or niches in a community present only one aspect of structure of community. Equally important are those patterns of interpopulation interactions that bind all populations together. The most important of these is the **trophic structure**, which determines the patterns of movement of energy and nutrients through the community. Through the **food web** one can made the graphic depiction of the actual flow of food materials from population to population through the community. Communities, at least major ones, have many trophic levels such as producers (most green plants) consumers (most animals) and decomposers (most microbes). The organisms most consistent in their trophic levels are the green plants ; few of them consume other organisms. (Some autotrophs, however, do gain significant quantities of their nutrients from other plants, or even animals. These include certain epiphytic plants like the mistletoe, whose habitat is the upper branches of larger trees, as well as the insectivorous plants such as venus's flytrap and pitcher plant. with regard to energy, all of these plants are autotrophs ; with regard to nutrients, they are partially "herbivores" or even" carnivores".) The situation is less well marked in herbivores. Some eat only plants (except when they ingest an animal by mistake). But some like the ostracod, may regularly eat animals if they catch them, even though their usual diet consists of plants. Carnivores are still less consistent. Many species are predominantly primary carnivores ; that is, they eat mainly herbivores. Many others are mainly secondary carnivores, with a diet composed largely of primary carnivores and so forth. But few carnivores being highly selective in their diet, will avoid eating any animal they are able to catch, if they are hungry. In addition, many animals, such as raccoons, bears and man are omnivorous and will feed at all trophic

levels present in the community, while others, such as frogs and many insects, occupy different trophic levels at different stages in their life cycles.

The food web of a community often has great amount of flexibility in the pathways through which energy may flow. In the community, though all animals have some choice regarding their food supply, some of them, however, have the opportunity to feed at more than one trophic level. This choice provides a multitude of potential pathways for energy flow, and it has an important role in the stability of the community.

COMMUNITY STABILITY

One of the most important considerations about communities is their stability, or lack of variation in time. There are two discrete senses in which this term is used. The first, **community stability**, refers to the degree of fluctuation in population size of the populations comprising the community. The second, **environmental stability**, refers to the fluctuations in the abiotic factors of the ecosystem. The two types of stability are closely related, and stable communities are generally to be found in stable environments, while unstable environments are more often characterized by unstable communities.

The global range of variation in community stability is extraordinarily broad. Some communities like the tropical rainforest or the coral reef, appear exceedingly stable, and the densities of their populations are constant over long periods of time. Others, such as the tundra, are extremely variable, and they are made up of populations that fluctuate widely in abundance. Much of this fluctuation is due to variations in environmental factors which the community cannot avoid. However, genetically determined adaptations that have arisen through natural selection also play a part in determining the responses of a population to envir[illegible] fluctuatio[illegible]

Further, the structure of c[illegible] which [illegible] is found has a major role in dete[illegible] o[illegible] variability to which the species [illegible] There [illegible] o mechanisms by which communit[illegible] [illegible] of

or an open field adjacent to the forest. The second mechanism by which community structure affects community stability is community diversity. But about the correlation of community stability and community diversity, many ecologists have expressed their doubts (Hairston *et al.*, 1968).

CORRELATION OF ENVIRONMENTAL GRADIENTS AND COMMUNITY GRADIENTS

Environmental units which afford uniform environmental conditions to the inhabiting species are called **biotopes**. The physical features of a biotope maintain the populations within the communities. In drastic environmental conditions the flora and fauna undergo abrupt changes and only those that are adapted to withstand the rapid variations are preferred by the environment. For example, in communities of littoral zone or of deserts, only those organisms that are typically adapted to withstand the environmental hazards can survive. Thus, different environmental factors (*e.g.*, temperature, rainfall, salinity, etc.) have a profound control over the populations so that habitats and communities vary along different environmental gradients or there is intergrading among them. For example, the gradual slope of land into the sea exhibits various grades of environmental factors and animal communities also correspondingly show a gradation from the shore through the intertidal zone to the deep sea region. Characteristic of an environmental gradient is a distinct zonation, each zone harbouring its own assemblage of species adapted to one another and giving way at a sharp boundary to another assemblage of species adapted to one another. For example the elevation gradient includes decreasing mean temperatures, decreasing lengths of growing seasons, increasing rainfall, increasing wind speeds, and so on, toward high elevation. All these factors act together on plants and animals. The assemblage of environmental factors that change together through the space along which a community gradient occurs and that influence its population may be termed as complex-gradient (see Whittaker 1970). The environment gradient (complex gradient) and community gradient or coenocline, go hand in hand and both gradients collectively form the ecocline, the gradient of ecosystem. Gradient analysis of different ecoclines have revealed following trends and patterns :

1. Along a gradient from a "favourable" environment to an "extreme" environment there is normally a decrease in the productivity and massiveness of communities. The decrease in amount of organic matter per unit area is expressed in decrease of height of dominant organisms and percentage of the ground surface covered.

2. Towards increasingly unfavourable environments there is a stepping-down of community structure and a reduction of stratal differentiation, with generally smaller numbers of growth forms, (The different classes or kinds of forms of plants such as trees, lianas, shrubs, epiphytes, herbs and thallophytes are referred to as growth-forms) arranged in fewer and lower strata.

3. Some kinds of species-diversities of certain communities decrease from favourable to extreme environments, whether the latter are extremes of drought, or of cold, or of adverse soil chemistry or (for the sea cost) of tidal exposure.

4. Each growth-form has its characteristic place of maximum importance along the ecoclines—the rosette trees in some tropical forests, semishrubs in desert and adjacent semiarid communities, and so on. Some growth forms, for example, grasses and grass-like plants may have more than one area of importance along the major ecoclines.

5. Some growth-forms may be dominant in similar environments in widely different parts of the world. Thus, similar environments on different continents tend to have communities of similar physiognomy (study of form and structure in biotic or natural communities is called **physiognomy**). This adaptive convergence at the level of the community is one of the major generalizations about the geography of life.

ECOTONE AND EDGE EFFECT

In the ecocline, the line of demarcation (boundary-line) between two communities is often very difficult in view of the chances of overlapping of one community over another. Such demarcation will be conspicuous only when the dominants of the adjacent communities show clear and characteristic differences. The transition zone between two or more diverse communities is called the **ecotone**. The common examples of ecotone are following—the border between forest and grassland, the bank of a stream running through a meadow or between a soft bottom and hard bottom marine community. An ecotone thus, presents conditions that are intermediate to the communities which are on either side of it. The ecotones may be narrow or very wide, extending to large areas. The community on either side of the ecotone may have a typical structure, but the ecotone is strikingly different. The ecotone has a higher diversity than either of the main communities, a diversity that is not directly controlled by the climate or further fundamental environmental factors, but because of the migrations of individuals of different species-populations from both

communities. Further, a number of special populations can become adapted specifically to the ecotone, even when both of the major communities are too simple or are otherwise unsuited for successful colonization by the species. This potential for the ecotone to act as habitat for species found in neither major community is termed the edge effect. A common example of the edge effect in action can be seen in those species of owl that live in or near ecotones between forests and grassland. They depend on forest trees for nesting, and they do their hunting in the grassland, where they depend on field rodents for food. In man-made communities such as agricultural fields, the transition zone (ecotone) between the field and the forest may act as a refuge for animal species formerly found in the plowed area, as well as for other plants such as weeds. Ecotones of this type are also the prime habitat of many species of insect, game bird, and mammal.

COMMUNICATION BETWEEN COMMUNITIES

It is impossible to draw a boundary line across which certain species do not naturally range. The shoreline, which would seem the most definitive of community boundaries, is regularly crossed by adult frogs, some insects, otters, beavers, snakes, shore birds, and muskrats, among other species. The range of several species of plants may include both sides of a shoreline. In addition, certain species, such as toads and many insects, spend their larval period in the water and their adulthood on land. Many large ungulates such as moose and deer have their summer range in one area and their winter range in another. Habitual passage from one community to another is the simplest type of communication between communities.

A second type communication involves two adjacent communities whose members seldom interact and tend to be restricted to one of the communities under normal conditions. But, under conditions of great stress in one ecosystem, such as forest fire, abnormal snowfall, certain kinds of intermittent pollution, and the like, animals can move temporarily into the other community, using as a refuge from which they can repopulate their own habitat after the stress conditions have passed. This type of interaction is characteristic of most communities that are subject to periodic catastrophe.

COMMUNITY STRATIFICATION

From structural point of view biotic communities exhibit vertical differentiation or stratification, horizontal differentiation and temporal differentiation (Whittaker, 1970). Stratification of

communities involve the formation of distinct strata or layers or storeys in vertical and horizontal plane by the organisms.

A. VERTICAL DIFFERENTIATION (STRATIFICATION OF COMMUNITY)

Most communities show vertical structure, differentiation or stratification—different species occur at different heights above the ground, or depths below the water surface. The species have different positions along a vertical gradient of depth in community and decreasing light intensity. Intensity of light necessarily decreases from the surface of the community, which is in full sunlight, downward; light absorption by the organisms themselves is a principal reason for this extinction of light with depth. In a forest one may observe a several-storied physiognomy with a number of growth-forms (in each of which there may be a number of species) occurring one above the other to form the community's vertical structure.

Vertical stratification in plants : The forest trees with their upper foliage in full sunlight, form the canopy or uppermost level. The leaves and branch surfaces of the canopy trees may absorb and scatter more than half of the sunlight energy, but beneath the canopy there is a lower layer of smaller trees utilizing some of the remaining light. This lower tree stratum usually contains both younger individuals of the canopy tree species, and mature trees of other, smaller species that do not normally reach canopy height. Less than 10 per cent light reaching the upper canopy may penetrate through the tree foliage of both levels, and spectral composition of the remaining internal light of the forest is changed from that of sunlight. Species of a third level of vegetation, shrubs, are adapted in utilizing this weaker light within the forest, further reducing the light that reaches herbs beneath the shrub layer. The remaining light (1 to 5 per cent of incident sunlight) in many forests supports the growth of the herb layer. Beneath the herbs, mosses on the ground may form still another vegetation layer. In some dense forests only a fraction of 1 per cent of incident sunlight reaches the forest floor.

By reaching the canopy, the forest trees may gain advantage, where abundant sunlight supports photosynthesis, but the tree must spend much of the energy of photosynthesis in the growth of woody tissue of stem and branches to support the foliage in the canopy. There may be apparent disadvantages in the low light intensities in which the forest herbs must live, but the herbs need not spend its more modest photosynthetic profit on woody supporting tissue. Forest structure, thus, involves a gradient of growth-forms—upper and lower trees, upper and lower shrubs, upper and lower herbs, and soil-

surface mosses—in adaptation to the gradient of light intensity. Along the gradient-growth-form designs change from one extreme (the upper tree with foliage in full sunlight, massive supporting stem and branch structure, and a root system smaller in mass than the above ground structure) to herbs with adaptations at the other extreme (photosynthesis at low levels of light intensity, small investment in above ground supporting structure, and accumulation of reserve food in a root system more massive than the above ground structure).

Vertical stratification in animals : Even as different plant species are adapted to different positions in this vertical gradient, so different animal species also occupy different levels in the forest. Different groups of bird species, for example, may be found feeding and nesting near the ground, in the shrub and small tree foliage beneath the canopy, and in the canopy itself. Different arthropod species occur at different levels from the canopy downward to the herb stratum and to and below the ground surface. A group of animals—mites and spring tails, millepedes and centipedes, ground beetles, and so on—occur primarily in the leaf litter on the soil surface; these animals, which are seldom seen on the surface by daylight are called **cryptozoans**. Other animals occur at different depths in the soil, in which different plant species also have their roots extending to different depths.

Vertical stratification in plankton—In the plankton too there is a degree of vertical differentiation in the adaptation of different species to different levels and light intensities. Vertical movement affect the distribution of these species, however, and vertical differences in the community are less evident than in the forest. Communities of lighted zones below tide levels on the ocean floor show a differentiation determined in part by light intensities. Vertical differentiation is thus a common feature of many biotic communities.

B. HORIZONTAL STRATIFICATION OF COMMUNITY

Communities show horizontal differentiation (stratification), expressed in the patchy or mosaic-like occurrence of species, and in correlations of species that tend to occur separately in relation to the patches. Some plants appear to have even distribution, among them the shrubs in some deserts in which it is reasonable to suppose that it is less probable that one shrub will be close to another shrub of the same species (hence within the area of its root effects) than farther away from it. Singing birds. divide a community into territories, and each male of a species establishes and defends an area in which the pair nests and feeds ; these territories may be of the same

order of size for all pairs of a given species in community. It seems clear that interaction among the birds has brought about a distribution that is more regular than random.

Departures from regular, even or random distribution toward positive contagion (contagious distribution refers to clumped distribution of a species) are very common in many kinds of communities. The plants of the forest floor are concentrated in patches, with few individuals between the patches. There may be following three basic causes of contagion of forest plants :

1. **Dispersal from parent plant :** Seeds from a plant may fall near that plant, producing when the seeds grow a clump of young plants. Plants spreading from a parent plant by runners or rhizomes may form clumps of individuals.

2. **Differences in environment :** The forest floor is a mosaic of patches receiving more light, or less, through the canopy. Light differences among these patches may influence the development of patches of herbs.

3. **Species interrelations :** One species may be dependent on another (for example, an herb parasitic on the roots of a tree species), or an herb population may be denser under trees of a given species because of the effects of the trees on the soil.

Animal populations too show varying degrees of contagion. Marine plankton animals and fish may be strongly clumped into schools. Most of the smaller animals of the forest show contagious distributions, with the degrees of contagion ranging up to the very strong contagion of ants in their colonies. Each species may have its own degree of clumping and spacing of clumps, different from the patterns of other species.

C. TEMPORAL DIFFERENTIATION OF COMMUNITY

Besides the differentiation of communities in space, there occur a differentiation of community in time also. Environments of biotic communities are rhythmic : in most environments light, temperature and other environmental factors go through daily and yearly cycles. In some communities, especially those of ocean shores, there are also complex rhythms set by the rise and fall of tides. In general, there occur rhythms of function in biotic communities in adaptation to rhythms of environment.

Plankton population fluctuate rapidly in many water bodies, with species replacing one another in periods of days and weeks. A number of major species may occur as plankton dominants in the course of the year. Thus in freshwater lake species of diatoms and other yellow-green algae may predominate in the winter plankton.

As water temperatures warm in later spring and early summer, these are replaced by desmids and other green algae. At peak summer temperatures blue-green algae may predominate or share dominance with the green algae ; as temperatures cool dominance shifts back toward the green and yellow-green algae. Each species has an inactive stage in which it survives the season unfavourable for its activity. Each species has its own place in the annual pattern, determined by its own response to the fluctuations of temperature and other environmental factors. The plankton community shows differentiation in time : different groups of species occur at different times during the seasonal cycle. The year-round total number of plankton species is much larger than the number present at a given time.

Seasonal and daily differentiation (stratification) occurs also in forests. One group of insects are active in the day time, another group at night, and a third group may be active in the twilight transitions of morning and evening. In terrestrial communities flycatchers, warblers, and other insectivorous birds are active by day, bat at night, and nighthawks in the dusk. Progress of the seasons is marked by the appearance of different groups of plants in flower and different groups of insects visiting these flowers. In deciduous forests spring beauties, dogtooth violets, and other herbs develop their foliage and flower early, before the trees are in leaf.

ALTERATION OF COMMUNITY PATTERNS IN TIME

Communities are not static ; they change. This change can be understood on several levels The simplest is the growth, interaction and death of individual organisms as they pass through their life cycles, affected by the cycles of seasons and other natural phenomenon. But there are other levels of community change that act over longer time spans and that account for much larger changes in community composition and structure. These include **ecological succession**, the reaction of communities to alterations in fundamental regional abiotic factors, and community evolution.

ECOLOGICAL SUCCESSION

The replacement of one community by other kinds of communities in course of time due to changes in physical conditions is called **ecological succession** or **ecosystem development.** The ecological succession extends over a long time and is a gradual and continuous process which continues until a stable community called **climax community** is reached. The entire sequence of communities characteristic of a given region is termed a **sere** and the individual transitional communities are called **seral stages** or seral communities. Seres

are sometimes classified according to the predominant force that is bringing them about. These forces are biotic, climatic, physiographic, and geologic and their resultant seres are commonly called bioseres, cliseres, eoseres, and geoseres. Further, there exist two major types of successional patterns—when succession starts from a barren area, such as bare rock or open water, it is called primary succession. But if an advanced successional stage retrogresses or is destroyed, the recovering successive stages leading toward a climax community represent secondary succession. The general theory underlying the concept of ecological succession defines a successional series from pioneer stage to the climax as a xerosere, if it starts from bare rock or soil, or as hydrosere, if it begins from open water. Varying numbers of seral stages are formed, and any relatively stable stage below the climax represents a subclimax. Further, in successive seral stages there is not only a change in the species of organisms present but an increase in the number of species and in the total biomass.

Thus, as a lake fills with silt it changes gradually from a deep to a shallow lake or pond, then to marsh, and beyond this, in some cases, to a dryland forest. When in an area of forest a farm field is abandoned, a series of plant communities grow up and replace one another—first annual weeds, then perennial weeds and grasses, then shrubs, and trees—until a forest ends the development. If a land slide exposes a surface of rock in the mountains, the surface may be successively occupied by sparse cover of lichens ; a spreading moss mat ; grasses, which enter and become a meadow ; a shrub thicket, which overtops and suppresses the grasses ; a first forest stage of smaller trees, which seed into the shrub thicket, grow through it and replace it ; and a final stage of larger trees, which take dominance from the first trees and may form a larger and potentially permanent forest community. All these processes of community development represent the simple cases of ecological succession.

According to Whittaker (1970) most ecological successions involves following characteristics :

1. There is usually progressive development of the soil, with increasing organic content, and increasing differentiation of layers or horizons toward the mature soil of the final community.

2. The height, massiveness, and differentiation into strata of the plant community increase.

3. Productivity, the rate of formation of organic matter per unit area in the community, increases with increasing development of the soil and of community, structure and increasing utilization by the community of environmental resources.

4. As height and density of above ground plant cover increase, the microclimate within the community is increasingly determined by characteristics of the community itself.

5. Species-diversity increases from the simple communities of early succession to the richer communities of late succession.

6. Populations rise and fall and replace one another along the time gradient in a manner much like that in stable communities along environmental gradients. The rate of this replacement in many cases slows through the course of succession as smaller and shorter-lived species are replaced by larger and longer-lived ones.

7. Relative stability of the communities consequently increases. Early stages are in some cases of instability, with populations rapidly replacing one another; the final community is usually stable, dominated by longer-lived plants, which maintain their populations with community composition no longer changing directionally.

Further, while primary succession is a long term phenomenon, secondary succession occurs quickly and proceeds rapidly. In the process of succession, the first community or organisms to become established are called **pioneer communities**. The successful species of a seral stage of succession often modify the environment resulting in a situation where in later successional species can stand up to the rigours of the competition. For example, changes in the communities of birds are regulated by the structure of vegetation, availability of perching sites, canopy height and structure, etc. By developing vegetation cover, modification of environment comes into effect and activity pattern of mosquitoes in different types of vegetation have been known considerably (Haddlow, 1961). In forest communities there exist post-sunset and pre-sunset peaks of activity, not met with in those living in banana plantations where the periodicity was much less marked.

Examples of ecological succession : One of the classic studies of ecological succession was made on the shores of Lake Michigan. As the lake has become smaller it has left successively younger sand dunes, and one can study the stages in ecological succession as one goes away from the lake. The youngest dunes nearest the lake, have only grasses and insects; the next older ones have shrubs such as cottonwoods, then evergreens and finally there is a beech-maple climax community, with deep rich soil full of earthworms and snails.

As the lake retreated it also left a series of ponds. The youngest of these ponds contain little rooted vegetation and lots of bass and blue-gills. Later the ponds become choked with vegetation and

smaller in size as the basins fill. Finally the ponds become marshes and the marshes get transformed into a meadow with further addition of soil. Soon grasses grow, shrubs develop and later trees appear. Eventually, an aquatic plant community of the pond becomes transformed into a terrestrial forest community. Further, in this case of ecological succession, the aquatic animal community gets replaced in an orderly manner from purely fresh water forms through marsh inhabitants and meadow grazers to terrestrial dwelling forms.

Another dramatic example of community succession began August 7, 1883, when a volcanic explosion occurred on the Indonesian island Krakatoa, causing part of the island to disappear. The remainder was covered with hot volcanic debris to a depth of 60 meters and all life was obliterated. A year later some grass and a single spider were found. By 1908, 202 species of animals had taken up residence on the island. This increased to 621 species by 1919, and to 880 species by 1934, when there was a young forest on one part of the island.

Ecological succession can be demonstrated in the laboratory. If a few pieces of dry grass are placed in a beaker of pond water, a population of bacteria will appear in a few days. Next, flagellates appear and eat the bacteria, then ciliated protozoa such as paramecia appear and eat the flagellates. Finally predator protozoa such as *Didinium* will appear and eat the paramecia. The protozoa present as spores or cysts attached to the grass, emerge in a definite succession of protozoan communities.

The phenomenon of succession in animal communities has had far reaching implications of an applied nature, in particular in the fouling by sessile marine organisms (many tubicolous polychaetes, hydroids and barnacles) of the bottom of ships and piers. Seasonal progression and succession are two significant factors which affect the change in fouling complexes and concentration of larvae, certainly of attachment growth rate and longevity. According to Daniel (1954) and A. Raja (1959) the duration of breeding seasons govern seasonal progression and succession. Studies on the distribution and succession of sedentary organisms in the Madras harbour indicate that in one of the sites studied *Modiolus striatulus* and *Cynthia* sp. were the first to attach themselves to an unoccupied habitat. Barnacles appeared a month later with *Cynthia* and *Modiolus* until colonial ascidians like *Botryllus* and *Botrylloides* sps. and the bivalves *Mytilus viridis, Ostrea madrasensis* came on the scene. Activities of the barnacles were limited by the settlement of these forms and within a period of three months these fouling organisms

reached the peak of complexity (see **Ananthakrishnan** and **Viswanathan**,1976).

Climax : The community that ends a succession is termed a climax. The nature of the climax has been the subject of considerable debate. At one time it was believed that if an area were given sufficient time, all ecosystems would show succession to a single climax type controlled by the regional climate, regardless of the original surface upon which the ecological succession started or the composition of any of the intermediate stages. Acceptance of the doctrine of regional or climatic climax as the normal end point of succession is due largely to the work of **F. E. Clements** (1916), and a large terminology grew up to describe those stable communities that evidently represented the end points of succession but that were not climatic climaxes. Recent study, however, suggest that the natural variation of the abiotic environment, including local heterogeneity, more broad-scale topographic variations, and soil variation stemming from differences in the rocks from which the soils were formed are significant aspects of the environment, and that they are important enough that community differences related to them can always be expected. A useful review of the climax concept is given by **Whittaker** (1953). However, ecological succession is nondeterministic; it proceeds by the dynamic interaction of natural selection and the progressive changes in available niches without consideration of a "goal". Depending on the current state of the ecosystem, there may be several different paths by which succession can proceed at any time in a given area. Thus the species that can be incorporated into a developing community may be quite varied and may depend on chance as well as the array of environmental factors. It is difficult or impossible to predict the composition of the community in a given ecosystem at some time in the future, even if we can compare it with the pattern of succession in a similar ecosystem. Even slight differences in environmental conditions at some time during succession may lead to the establishment of different communities. If these community differences are reflected in different sorts of community environment interactions, there may be a divergence in succession, so that the climaxes are disimilar even though the regional climate is the same. Soil erosion and fire are the mechanisms by which community differences can result in the establishment of different climaxes under a single climatic regime.

Aceording to the **Whittaker** (1970), central phenomenon of the concept of climax is the community's relative stability. The populations of a climax community show some irregular fluctuations in

time because of environmental change and the character of population process, and some climaxes show more regular or cyclic fluctuation. In the climax, however, there are fluctuations around a stable relatively constant, mean condition. Stability of the climax entails balances maintained in the function of a dynamic, living system.

For a species population in a climax community to be stable, there must be a balance between natality and mortality, between income of new individuals by reproduction and outgo (or spending) of individuals by death. Ideally, such balances of natality and mortality would, over longer periods at least, characterize all the species populations of the climax community. The balance is also applied to the intake and release of matter and energy by the community as a whole. This kind of constancy in the system, based on the balancing of income and outgo and superimposed on an underlying flow through the system, is known as a **dynamic equilibrium** or **steady state**. A pool along a stream examplified such state as in it water flows but with equal rates of inflow and outflow. The pool itself an object or system stays the same—in steady state. Climax, thus, implies a steady-state condition in a natural community.

COMMUNITY RETROGRESSION

It is in some cases possible to drive the successional process backwards by disturbing a community. A grassland under heavy grazing may show first a reduction in coverage of the plants most palatable to the cattle. With continued grazing the total grass coverage is reduced, the more palatable plant species disappear, and weeds that may be almost absent from the undisturbed grassland appear and spread with continued grazing and tampling, most of the grass may be destroyed and, despite the unpalatable weeds that survive, the soil is exposed to erosion. The end result of such community retrogression may be depending on the topographic position and soil, a mud field with erosion channels or a rocky mountain slope stripped of much of its soil. Thus by severe and continued disturbance all the trends of succession—soil development, community stature, productivity, diversity, stability, and extent of modification of environment by community—have been reversed toward zero.

COMMUNITY EVOLUTION

Like the responses of communities to changing abiotic conditions, community evolution involves progressive changes in climax communities. Because the evolution is exceedingly slow, it cannot be

observed in operation, and few instances from the fossil record are sufficiently complete to show the process in action

The example that best demonstrates evolution of the basic structure of the community is that of the development of a terrestrial community of a modern type by early reptiles some 250 million years ago. Between the time when vertebrates (amphibians) first became able to lead a predominant terrestrial existence some 350 million years ago and the establishment of an essentially modern type food web some 100 million years later, the structure of terrestrial community was decidedly different from what it is now Development of the modern type of community structure required not only a complete rearrangement of the niche structure of the community but also the evolution of new species that could fill the new niches (Olson, 1961, 1966).

Attainment of the adaptations needed for terrestrial life by the first amphibians did not in itself establish a land-based vertebrate community. These early amphibians were carnivores, and the only animals inhabiting the land environment were insects. It is inconceivable that the clumsy locomotor system of early amphibians would have allowed them to prey effectively on animals such as insects. Thus the first communities inhabited by terrestrial vertebrates are best regarded as extensions of aquatic communities, with the land habit as an adaptation to improve the capabilities of organisms whose prime food supply was aquatic invertebrates and fish.

By some 300 million years ago, reptiles had evolved that could feed effectively on terrestrial invertebrates. An entirely land-based community was theoretically possible in which all herbivore niches were assumed by invertebrates and some of the carnivore niches by vertebrates. However, the palaeoecological evidences suggest that most contemporary carnivorous vertebrates were unable as yet to realize an entirely terrestrial carnivore niche, so that the great majority of the energy flow through the community continued to pass through the aquatic route. The typical food chain to the highest terrestrial vertebrate carnivore was plant→aquatic invertebrate→aquatic-invertebrate-feeding vertebrate→semi-aquatic predator→terrestrial predator.

By 250 million years ago terrestrial herbivorous vertebrates had evolved and a fully terrestrial vertebrates community could come into being. From this time onward the basic structure of the terrestrial community was of an essentially modern sort, with all consumer trophic levels occupied by a wide range of animals, both vertebrate and invertebrate.

Such evolutionary changes in the structure of communities are caused by a large number of factors. One factor is changes in the regional climate It was becoming progressively drier during the period under consideration, and the development of a land-based community reasonable response to this sort of change. Indeed many evolutionary changes in community structure can be explained on the basis of responses of major changes in the regional abiotic factors of the environment (Axelrod, 1950, 1958). But other chief factors of evolutionary change in community includes reorganization of the community's structure in response to the realization of niches that had not previously existed in the community.

CHAPTER 7

ECOSYSTEMS

The ecosystems are the basic functional ecological units which include both the organisms and the non-living environment, each influencing the properties of the other and both necessary for maintenance of life. They are complex self-sustaining natural systems which include all the interactions that bind their living and non-living components together into the stable systems. These interactions include those between organisms, the relationship between organisms and the physical environment, and the interactions of various phases of the non-living environment which mold its own change.

The word ecosystem was coined by A. G. Tansley (1935). The prefix *eco* means environment. A *system,* according to Webster, is "an aggregation or assemblage of objects joined in a regular interaction or interdependence." Churchman (1968) has defined a system as "a set of parts coordinated to accomplish a set of goals." The goal of a living system such as the tissues and organs that make up the animal body is a coordinated effort to cause a flow of energy through the system and thus maintain life. However, ecosystem is a very broad term and according to many ecologists (*e.g.*, Smith, 1974) it incorporates following aspects in it :

1. The ecosystem is a major ecological unit. It contains both abiotic (*e.g.*, water, soil, air, temperature, light, etc.) and biotic (*i.e.*, biotic communities) components through which nutrients are cycled and energy flows.

2. To accomplish those cycles and flows ecosystems possess a number of structural [illegible] soil, water, nutrients, produce[illegible]

3 [illegible] to the flow of energy and the cycling of materials through the structural components of the ecosystem.

4. The total amount of energy that flows through a natural sys[illegible] ene[illegible] abi[illegible] ma[illegible] [illegible]ch feeding level.

5. Ecosystems tend toward maturity; in so doing they pass from a less complex to a more complex state. This directional change is called succession. Early stages of succession are characterized by an excess of potential energy and a relatively high energy flow per unit of biomass. In mature ecosystems there is less waste and less accumulation of energy because the energy flows through more diverse channels.

6. The major functional unit of the ecosystem is the population. It occupies a certain functional niche that is related to the population's role in energy flow and cycling of nutrients.

7. Both the environment and the amount of energy fixation in any given ecosystem are limited. When a population reaches the limits imposed by the ecosystem, its numbers must stabilize or failing this, decline (often sharply) from disease, strife, starvation, low reproduction and so on.

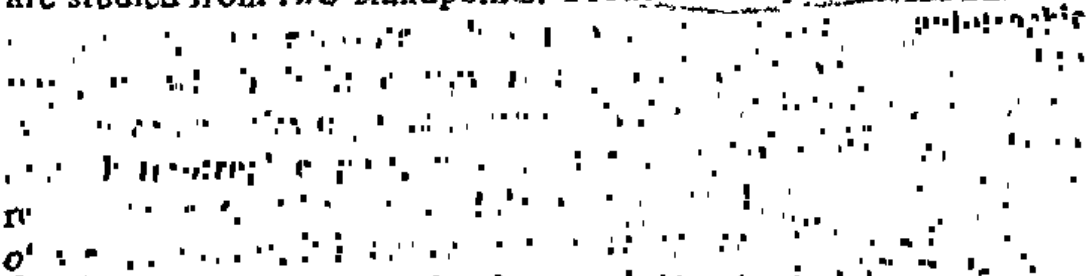
8. The ecosystem has historical aspects : the present is related to the past, and the future is related to the present.

9. The distribution of ecosystems on the earth is remarkably small in geographic extent when compared with the total volume of the earth. What we term the biosphere includes only the surface layer of solid ground or sediment beneath bodies of water, the oceans lakes and streams, and that portion of the atmosphere inhabited by flying or floating organisms such as birds, bats, and bacteria.

In preceding chapters we had a detailed discussions of different abiotic and biotic components of the ecosystems, and to avoid unnecessary repitition in this chapter, we will restrict our detailed discussion only to the flow of energy in ecosystems and also to biogeochemical cycles of the ecosystems.

COMPONENTS OF THE ECOSYSTEMS

Conventionally, the structural component of the ecosystems are studied from two standpoints. From the trophic (nourishment)



units in a pond ecosystem. I. Abiotic substances

inorganic and organic compounds of the environment. The inorganic components of the ecosystems are carbon dioxide, water, nitrogen, calcium, phosphate all of which are involved in material cycles

(geochemical cycles). The organic components of the ecosystems are proteins, carbohydrates, lipids and amino acids, all of which are synthesized in the biotic phase. The climate, temperature, light and other physical factors are also normally included in abiotic components of the ecosystems. 2. Producers—producers of the ecosystems are the autotrophic organisms (mostly green plants) which can manufacture complex organic materials from simple inorganic substances. 3. Macroconsumers or [illegible] organisms (mostly animals) that ir [illegible] of organic matter. The primary macroconsumers (herbivores) feed on the plants and the secondary macroconsumers (carnivores) feed on the primary consumers. 4. Microconsumres, saprotrophs or decomposers—The saprotrophs are the heterotrophic organisms (mostly bacteria and fungi) which break down the complex compounds of dead organ[illegible] osition products [illegible] lable to the produc[illegible] producers,

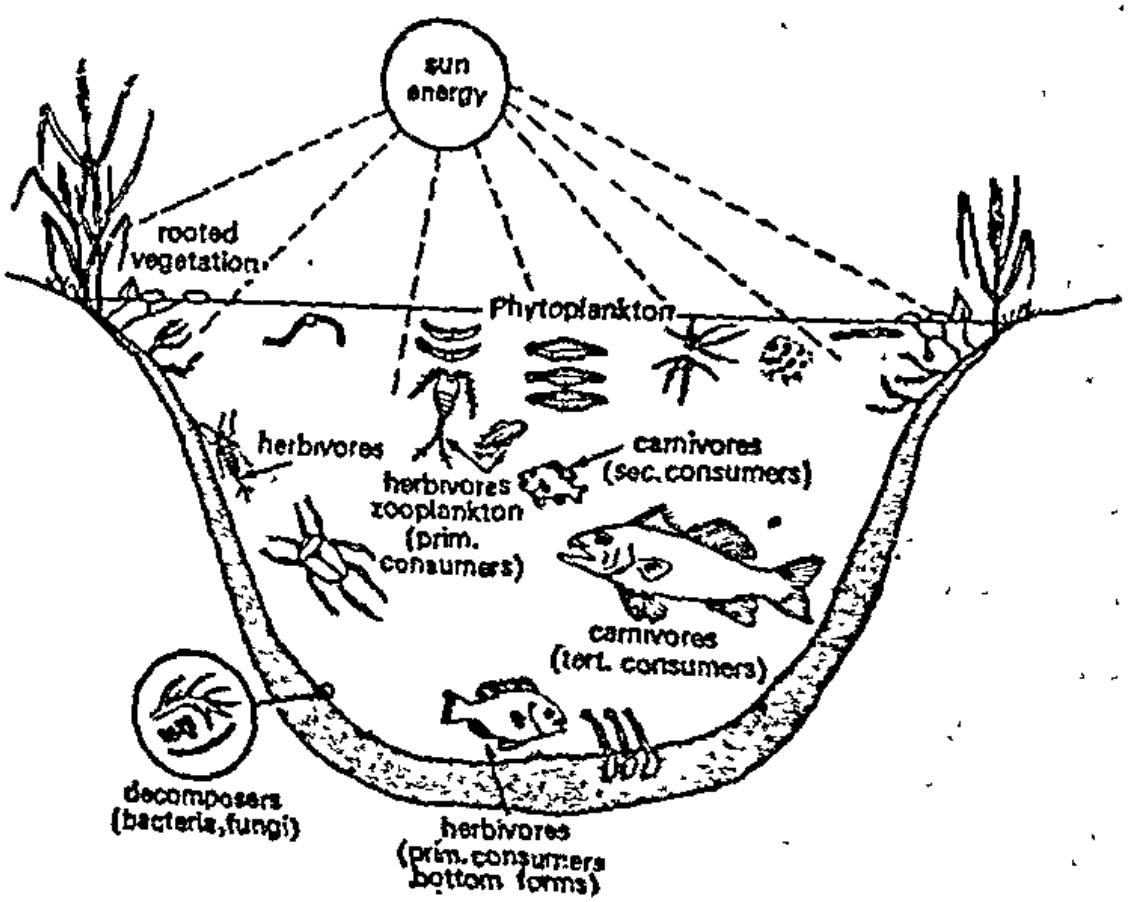


Fig. 7·1. A small fresh water pond as an example of an ecosystem. The component parts—producer, consumer and decomposer or reducer organisms, plus the non-living or abiotic components are indicated.

phagotrophs and saprotrophs, thus, make up the biomass of the ecosystem—the living weight.

EXAMPLE OF ECOSYSTEMS

A classic example of an ecosystem is a small lake or pond. (Fig. 7·1). The abiotic or non-living parts of a fresh water pond include the water, dissolved oxygen, carbon dioxide, inorganic salts such as phosphates, nitrates and chlorides of sodium, potassium, and calcium, and a multitude of organic compounds such as amino acids, humic acids, etc. The living part of the fresh water pond can be subdivided according to the functions of the organisms, *i e.*, what they contribute toward keeping the ecosystem operating as a stable, interacting whole. In a fresh water pond there are two types of producers, the larger plants growing along the shore or floating in shallow water, and the microscopic floating plants, most of which are algae, that are distributed throughout the water as deep as light will penetrate. These tiny plants, collectively referred to as **phytoplankton**, are usually not visible unless they are present in great abundance and give the water a greenish tinge. Phytoplanktons have more significance as food producers for the fresh water pond ecosystem than are the more readily visible plants.

The macroconsumers or phagotrops of pond ecosystem include insects and insect larvae, crustacea, fish, and perhaps some fresh water clams. Primary consumers such as **zooplankton** (animal plankton) found near the surface of water and **benthos** (bottom forms) are the plant eaters (herbivores) and secondary consumers are the carnivores that eat the primary consumers. There might be some tertiary consumers that eat the carnivorous secondary consumers.

The ecosystem is completed by saprotrophs or decomposer ozoans and fungi, which from dead producer and consumer organisms either into small organic molecules, which they utilize themselves, or into inorganic substances that can be used as raw materials by green plants.

FEED-BACK LOOP

Most interactions between living organisms, or between organisms and the abiotic phases of the ecosystem, are mutual; activities of organisms are met by responses. The most common mechanism for these responses is the feed-back loop. Feed-back is extremely important in natural ecosystems, as it is the prime regulatory mechanism for the ecosystem as a whole and may affect both the rate and direction of whatever changes take place within it. A **feed-back loop** can be defined as the relationship in which a change in some original

state of being "feeds back" onto that original state to alter the rate or direction of further change. There are two types of feed-back. In a **positive feed-back** relationship, the feed-back process leads to a self-sustaining change; in a **negative feed-back** relationship, the process maintains a dynamic homeostasis, or steady state. Thus positive feed-back relationships result in continual growth or decline of the entity in question, whereas negative feed-back results in a stabilization of the entity at some dynamic equilibrium.

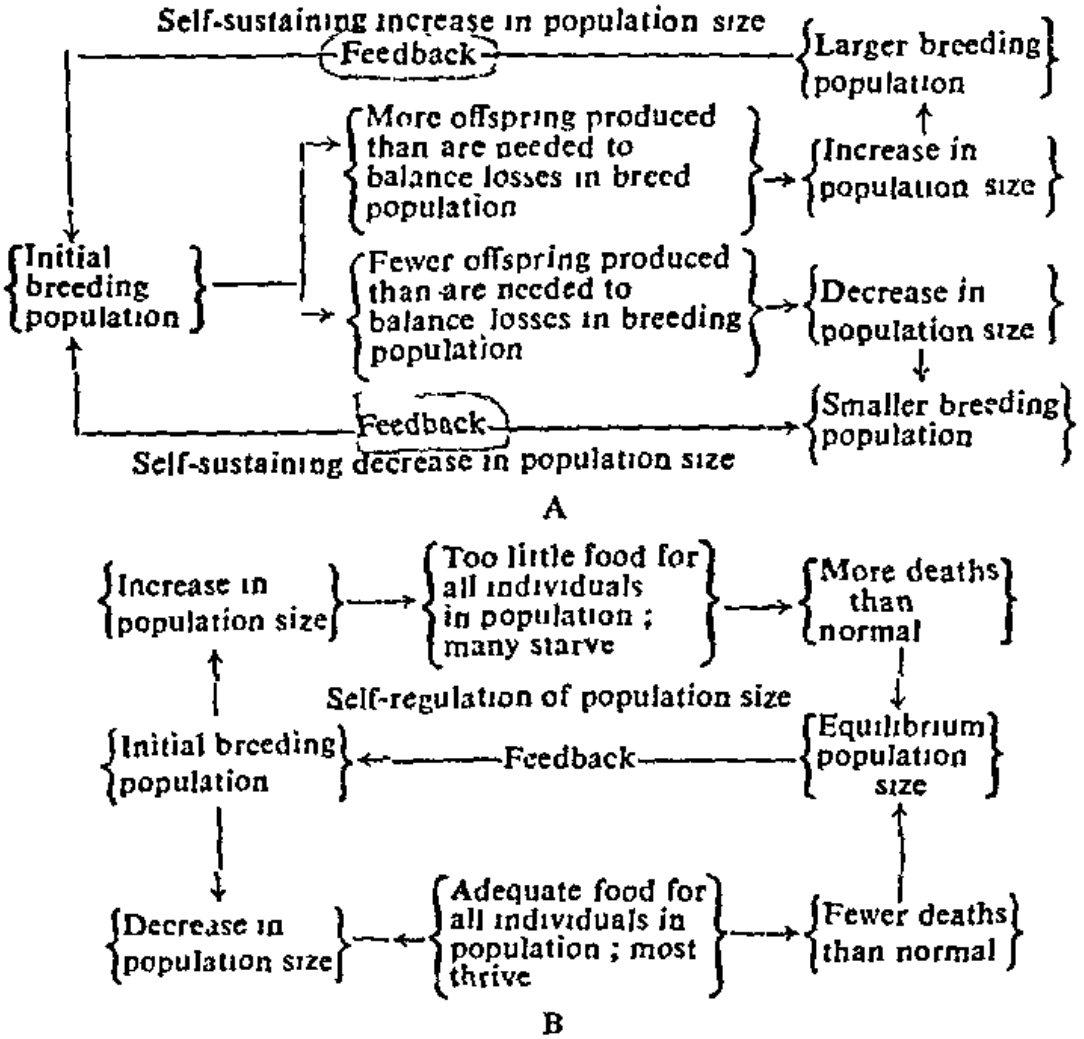


Fig 7·2. Generalized diagram of the operation of feedback mechanisms in natural ecosystems. Both figures refer to the operation of these mechanisms in the regulation of population size in a hypothetical population of animals : positive feedback leading to self-sustaining change (A), and negative feedback leading to self-regulated homeostasis (B)

Fig. 7·2 shows an example of positive and negative feedbacks. In both, we start with a given number of animals. In the positive feedback loop, production of a large number of offspring leads to a larger population than the original. This then becomes the

reproducing population, which can produce even more offspring. The result is a self-sustaining population increase, driven by the offspring feeding back into the breeding population. In the same way, if more breeders die than are replaced each year (which happens in some populations), this loss will be reflected in a smaller breeding population and will result in self-sustaining decline in population number by the same type of positive-feedback loop. In the negative feedback loop, the population is controlled by external factors, such as food supply. If the population increases in size, there will not be sufficient food to go around, and more individuals than usual will die from starvation ; conversely, if the population decreases in size, there will be more than enough food to go around, and the animals will be well-nourished, leading to a smaller than normal die off. In either case, the equilibrium population size is regained. Most interactions between different species of organisms, and many interactions between a given species and its physical environment depend on either positive or negative feedback. All organisms in an ecosystem are part of several different feedback loops at any point in time. Some of these relationships are negative, while others are positive.

BIOGEOCHEMICAL CYCLES

The total mass of all the organisms that have lived on the earth in the past 1 5 billion years is much greater than the mass of carbon and nitrogen atoms present. According to the **Law of Conservation of Matter,** matter is neither created nor destroyed ; obviously the carbon and nitrogen must have been used over and over again in the course of time. The earth neither receives any great amount of matter from other parts of the universe nor does it lose significant amounts of matter to outer space. The atoms of each element, carbon, hydrogen, oxygen, nitrogen, phosphorus, calcium and the rest, are taken from the environment, made a part of some cellular component of an organism and finally, perhaps by a quite circuitous route involving several other organisms, are returned to the environment to be used over again. The cyclic movements of chemical elements of the biosphere between the organism and the environment are referred to as **biogeochemical cycles,** after **Vernadskii** (1934). "Bio" refers to living organisms and 'geo' to the rocks, soil, air and water of the earth.

ORGANIC AND ABIOTIC PHASES OF GEOCHEMICAL CYCLES

Insofar as an element is necessary for the maintenance of life, its movement through biotic communities (organisms) can be viewed

in the terms of food chain. The flow of a chemical element through the food chain can be viewed as the organic phase of the biogeochemical cycle. Further, the biogeochemical cycles also include abiotic phases which are the functions of the chemistry of the elements in question. These abiotic phases are of critical importance to the ecosystem, as the major reservoirs for all nutrient elements are external to the food chains, and flow in the abiotic phases tends to be much slower than in the organic phase. The rapidity and direction of nutrient cycling through the abiotic phases determine not only the distribution of the element in the total environment, but also its availability to living systems. There are two classes of abiotic phases in biogeochemical cycles, a sedimentary phase, which is part of all cycles, and an atmospheric phase, which is possessed by some. In some cycles, such as nitrogen, the atmospheric phase is more important than the sedimentary. In others, such as phosphorus, the atmospheric phase is essentially non-existent. In still others, such as sulphur, both phases are present and their relative importance depends on other environmental factors. Biogeochemical cycles that have dominant atmospheric phases are often called **atmosphere-reservoir cycles**; those whose sedimentary phase is dominant are termed **sediment-reservoir cycles.**

TYPES OF BIOGEOCHEMICAL CYCLES

There are two types of biogeochemical cycles, the gaseous and the sedimentary. In gaseous cycles the main reservoir of nutrients is the atmosphere and the ocean. In sedimentary cycles the main reservoir is the soil and the sedimentary and other rocks of the earth's crust. Both involve biotic and abiotic agents, both are driven by the flow of energy and both are tied to the water cycle.

A. WATER CYCLE

Living organisms, atmosphere and earth maintain between them a circulation of water and moisture, which is referred to as **water cycle** or **hydrologic cycle.** As we have already discussed in chapter 2, water forms a very significant factor of environment and *without the cycling of water, biogeochemical cycles could not exist,* ecosystems could not function, and life could not be maintained. Water is important for an ecosystem for several reasons—it is the medium by which nutrients are introduced into autotrophic plants; it is an important part of living tissue, either as liquid water or as part of essential organic molecules; it serves as a means of thermal regulation for both plants and animals; it is the medium by which sediments—a prime source of mineral nutrients—are removed from or added to local ecosystems; it covers the great majority of the

earth's surface, and is the dominant feature of all aquatic ecosystems.

The hydrologic cycle is driven by solar energy and gravity. More than 80 per cent of the total insolation that is not lost immediately as electromagnetic radiation goes to evaporate water. The atmospheric water vapour produced by this means can then condense around particles of dust in the atmosphere, often called nucleation particles. The atmosphere possesses a limited capacity for holding water vapour, thus, the droplets formed by this means are heavy enough to fall as precipitation under the influence of gravity. Eventually, the hydrologic cycle can be defined as an alternation of evaporation and precipitation, with the energy used to evaporate the water being dissipated as heat in the atmosphere as the water condenses.

Distribution of water in earth's surface : Water is not evenly distributed throughout the earth. Almost 95 per cent of the total water on earth is chemically bound into rocks and does not cycle. Of the remainder, about 97·3 per cent is in the ocean, about 2·1 per cent exists as ice in the polar caps and permanent glaciers, and the rest is fresh water, present in the form of atmospheric water vapour ground water, soil water, or inland surface water (Nace. 1967)

Table : Distribution of water in the earth's crust and surface (Clapham, Jr., 1973).

	A. Chemically bound water of rocks : Does not cycle	
1.	Crystalline rocks	$250{,}000 \times 10^{17}$ Kg.
2.	Sedimentary rocks	$2{,}100 \times 10^{17}$ Kg.
	B. Free water : Moves via hydrologic cycle	
1.	Oceans	$13{,}200 \times 10^{17}$ Kg.
2.	Ice caps and glaciers	292×10^{17} Kg.
3.	Ground water to a depth of 4000 m.	$83{\cdot}5 \times 10^{17}$ Kg.
4.	Fresh-water lakes	$1{\cdot}25 \times 10^{17}$ Kg.
5	Saline lakes and inland seas	$1{\cdot}04 \times 10^{17}$ Kg.
6.	Soil moisture	$0{\cdot}67 \times 10^{17}$ Kg.
7.	Atmospheric water vapour	$0{\cdot}13 \times 10^{17}$ Kg
8.	Rivers	$0{\cdot}013 \times 10^{17}$ Kg.

The rate of cycling of water : The rate of cycling between surface and atmosphere is very rapid. The amount of water vapour in the atmosphere is sufficient, on the average, to cover the entire earth to a depth of 2·55 cm. But the average annual rainfall for the earth is about 81 1 cm (Furon, 1967) and in some places it ranges upto 1,200 cm. This means that the average turnover time for atmospheric water is about 11·4 days, or that the equivalent of all the

water vapour in the entire atmosphere falls as precipitation and is re-evaporated more than 32 times per year.

Further, the distribution of evaporation and rainfall is quite uneven. If we compare the annual evaporation and precipitation over land and sea, we find that relatively more water precipitates on land than evaporates from land. This is fortunate from the point of view of terrestrial organisms. Even so, the amount of rainfall on the open ocean is proportionately greater than that on land, taking into account the relative percentages of the earth's surface covered by land and sea.

Nature of hydrologic cycles : The hydrologic cycles over the oceans is extremely simple—the water is evaporated from the surface of the ocean and water vapours form the clouds which when cool down precipitate the water as rain fall. But several routes are open to precipitation that falls on land—direct evaporation, transpiration, entery of water into ground water system and runoff. Consequently, the routes of hydrologic cycles on land can be divided into following three main categories—the rapidly cycling portion, or **evapotranspiration,** which includes the evaporation and transpiration, the less rapidly cycling water, or surface runoff, and very slowly cycling ground water that seeps into the soil can end up in any one of these three categories.

1. **Evapotranspiration :** Evapotranspiration includes evaporation and transpiration. **Evaporation** refers to water that is evaporated directly from any surface other than a plant, such as a lake, soil surface, or animal skin. In most cases, the main effects of direct evaporation are the moderate the temperature of local area and to allow the hydrologic cycle to continue. In some ecosystems, evaporation also leads to a concentration of salts in the water of soil which may be a critical environmental factor. **Transpiration** is water that evaporates from the surface of leaves of plants. Transpiration acts to move the biogeochemical cycles for all mineral nutrients that enter the food chain via the roots of plants.

2. **Surface runoff :** If transpiration is related to the mechanism of nutrient uptake, the gross movement of soluble and solid particles in the ecosystem is accomplished largely by runoff. Nutrients that have accumulated in sediments or soils can be eroded by streams and removed altogether from a local ecosystem, or soluble nutrients may be carried by soil. Seepage into surface waters, where they are removed from the area. Streams may carry sediment particles which can be chemically altered through additional weathering so that the nutrient elements they contain may be utilized by organisms. Finally

moving water acts as an agent of **erosion** which removes soil and allows weathering of the underlying rock to make their nutrients available to plants.

3. **Ground water :** Ground water is water that saturates either sediment or rock below the water table. In general, it is not tapped by plants for transpiration and it is too deep to be directly evaporated from the soil surface. It is an exceedingly important reservoir for water which moves from one place to another under the influence of gravity. The area where the net water movement is from the surface into the ground water systems is termed a **catchment area ;** areas where ground water reaches the surface and runs off are termed **springs.** A rock body through which ground water flows is called an **aquifer.** A well drilled into an aquifer that has sufficient hydrostatic pressure to force water up into it is called an **artesian well.**

The hydrologic cycle on land thus includes evapotranspiration of water from earth's surface and leaf surface→formation of clouds →precipitation→surface runoff+accumulation of water as ground water→return of water to sea via streams or direct evaporation and cloud formation, and so on.

B. GASEOUS CYCLES

The gaseous geochemical cycles are of following types :

1. **The oxygen cycle :** Oxygen (O_2), the by-product of photosynthesis, is involved in the oxidation of carbohydrates with release of energy, carbondioxide and water. Its primary role in biological oxidation is that of a hydrogen acceptor. The break-down and decomposition of organic molecules proceeds primarily by dehydrogenation. Hydrogen is removed by enzymatic activity from organic molecules in a series of reactions and is finally accepted by the oxygen, forming water. Though oxygen is necessary for life, but being very active chemically, molecular O_2 may be toxing to living body cells. Therefore, for the protection from toxic effects of molecular O_2, cells possess the cellular organelles called **peroxisomes** which mediate oxidative reactions resulting in the production of hydrogen peroxide which in turn is used through the mediation of other enzymes as an acceptor in oxidizing other compounds.

The major supply of free oxygen which supports life occurs in the atmosphere. There are two significant sources of atmospheric oxygen. One is the **photodisassociation** of water vapour in which most of the hydrogen released escapes into outer space. The other source is **photosynthesis,** active only since life began on earth. Because photosynthesis and respiration are cyclic, involving both the release and utilization of oxygen, one would seem to balance the

other, and no significant quantity of oxygen would accumulate in the atmosphere. However, at some time in the earth's history the amount of oxygen introduced into the atmosphere had to exceed the amount used in the decay of organic matter and that tied up in the oxidation of sedimentary rocks. Part of the atmospheric oxygen represents that portion remaining from the unoxidized reserves of photosynthesis—coal, oil, gas, and organic carbon in sedimentary rocks. The amount of stored carbon in the earth suggests that 150×10^{20}g of oxygen has been available to the atmosphere, over 10 times as much as now present, 10×10^{20}g (Johnson, 1970). The main non-living (abiotic) oxygen pool consists of molecular oxygen, water, and carbon dioxide, all intimately linked to each other in photosynthesis and other oxidation-reduction reactions, and all exchanging oxygen with each other. Oxygen is also biologically exchangeable in such compounds as nitrates and sulfates utilized by organisms that reduce them to ammonia and hyrogen sulfide.

The cycling of oxygen is very complex. As a constituent of CO_2, it circulates freely throughout the biosphere. Some carbon dioxide combines with calcium to form carbonates. Oxygen combines with nitrogen compounds to form nitrates, with iron to ferric oxides, and with many other minerals to form various other oxides. In these states oxygen is temporarily withdrawn from circulation. In photosynthesis the oxygen freed is split from the water molecule. This oxygen is then reconstituted into water during plant and animal respiration. Some part of the atmospheric oxygen that reaches the higher levels of the toposphere is reduced to ozone (O_3) by high-energy ultravoilet radiation.

2. The carbon cycle : The carbon being a basic constituent of all organic compounds and a major element involved in the fixation of energy by photosynthesis, is so closely tied to energy flow that the two are inseparable. The source of all the fixed carbon both in living organisms and fossil deposits is carbon dioxide, CO_2, found in the atmosphere and dissolved in the waters of the earth. During photosynthesis, carbon from atmospheric CO_2 is incorporated into the production of the carbohydrate, glucose, $C_6H_{12}O_6$, that subsequently may be converted to other organic compounds such as polysaccharides (sucrose, starch, cellulose, etc.), proteins and lipids. All the polymeric organic compounds containing carbon are stored in different plant-tissues as food and from them the carbon is passed on to the trophic levels of herbivores or phyto-parasites, or retained by the plant until it serves as food for decay organisms (*viz.*, decomposers). Some of the carbon is returned to the atmosphere

(or the enveloping aqueous medium) in the form of CO_2, a by-product of plant respiration, in which, a considerable portion of glucose is oxidized to yield CO_2, H_2O and energy as follows :

$$\frac{1}{6}C_6H_{12}O_6 + O_2 \rightarrow CO_2 + H_2O + Energy$$

The CO_2 which is released as the by-product of plant respiration is again used by plants in photosynthesis. This is the basic carbon cycle which is simple and complete. Decomposing micro-organisms are important in breaking down dead material with the release of carbon back into the carbon cycle.

Similarly, carbon taken up by herbivores, or photoparasites may travel a number of routes. It may be incorporated into protoplasm (assimilation) and stored until the organism dies, where upon it is utilized by decomposers; it may be released through animal respiration; it may serve as live food for other organisms; or finally it may be stored in the environment as CO_2. Similar fates awaits carbon at the carnivore trophic levels. In fact, all the carbon of plants, herbivores, carnivores and decomposers is not respired but some is fermented and some is stored. The carbon compounds that are lost to the food chain after fermentation, such as methane, are readily oxidized to carbon dioxide by inorganic reactions in the atmosphere. As for the storage of carbon in sediments, just as deposition works to store materials, erosion may uncover them, and inorganic chemical weathering of rock can oxidize the carbon contained there. Some carbon is permanently stored in sediments and not uncovered by weathering; it may be replaced by carbon dioxide released from volcanoes and other similar examples of intense geological activity. In modern age man has greatly increased the rate at which carbon is passing from sedimentary form to carbon dioxide. The combustion of fossil fuels is a significant means of recycling sedimentary carbon much faster than natural weathering.

Small portion of carbon, espacially in the sea, is found not as organically fixed carbon, but as carbonate (CO_3^-), especially calcium carbonate ($CaCO_3$). $CaCO_3$ is very commonly used for shell construction by such animals as clams, oysters, some protozoa, and some algae. Carbon dioxide reacts with water to form carbonate in the following three step reaction :

$$\underset{}{CO_2 + H_2O} \rightleftarrows \underset{\text{Carbonic acid}}{H_2CO_3} \rightleftarrows \underset{}{H^+ + HCO_3^-} \rightleftarrows \underset{\text{Bicarbonate}}{2H^+ + CO_3^-} \quad \underset{\text{Carbonate}}{}$$

The precise amount of each of these constituents in the water depends on the pH of the water. Organisms such as clams can combine bicarbonate or carbonate with calcium dissolved in the water to produce calcium carbonate. After the death of the animal, this

calcium carbonate may either dissolve or remain in sedimentary form.

Certain control mechanisms are inherent in the carbon cycle. The rate of carbon utilization is dependent on its availability. If excessive amounts of carbon are taken up in any one phase of the cycle, other phases of activity may be inhibited or slowed down. For example, if the pH of water is alkaline, more carbon is tied up in a carbonate and less is in solution. This removal of carbon in solution would upset the equilibrium established between the atmospheric and the dissolved CO_2 and the net effect would be a movement of CO_2 into solution until equilibrium was reached.

Peculiarities of carbon-cycle : Though carbon-cycle exhibits basic similarity with other biogeochemical cycles, yet it is unusual in that the organic phase is not essentially a complete cycle within itself. The organic (biotic) and atmospheric (abiotic) phases, however, are so closely intertwined that the rapid cycling typical of the organic phase is present. The multiplicity of paths along which carbon can flow is typical of biogeochemical cycles in general, and provides a well-buffered system with adequate feedback mechanisms to insure an adequate supply of the carbon. It is significant that all phases of the cycle yield carbon dioxide at some time, and carbon dioxide is the raw material for them. Thus, despite its relative low concentration in the atmosphere (0·03 per cent), carbon in a form in which it can be used by living organisms is virtually always present.

3. The nitrogen cycle : Nitrogen is an essential constituent of different biologically significant organic molecules such as amino acids and proteins, pigments, nucleic acids and vitamins. It is also the major constituent of the atmosphere, comprising about 79 per cent of it. The paradox is that in its gaseous state, N_2, abundant though it is, nitrogen is unavailable to most life. Before it can be utilized it must be converted to some chemically usable form.

To be used biologically, the free molecular nitrogen has to be fixed and fixation requires an input of energy. In the first step molecular nitrogen, N_2, has to be split into two atoms : $N_2 \rightarrow 2N$. The free nitrogen atoms then must be combined with hydrogen to form ammonia, with the release of some energy :

$$2N + 3H_2 \rightarrow 2NH_3$$

This fixation comes about in two ways. One is by high-energy fixation such as cosmic radiation, meteorite trails, and lightning that provide the high energy needed to combine nitrogen with oxgen and hydrogen of water. The resulting ammonia and nitrates are carried to the earth in rain water. The second method of nitrogen-fixation

which contributes about 90 per cent of fixed nitrogen of earth, is biological. Some bacteria, fungi, and blue-green algae can extract molecular nitrogen from the atmosphere and combine it with hydrogen to form ammonia. Some of this ammonia is excreted by the nitrogen-fixing organism, and thus becomes directly available to other autotrophs. Some of these nitrogen-fixing organism may be free-living, either in the soil (*e.g.*, bacteria-*Azobacter* and *Clostridium*) or in water (*e g* , blue-green algae-*Nostoc*, *Calothrix* and *Anabaena*) and produce vast quantities of fixed nitrogen. In other cases, certain symbiotic bacteria of genus *Rhizobium*, although unable to fix atmospheric nitrogen themselves, can do this when in combination with cells either from the roots of legumes (*e.g.*, peas, beans, clover and alfalfa) and of other angiosperms such as *Alnus*, *Ceanothus*, *Shepherdia*, *Elaeagnus* and *Myrica*, or from the leaves of African genera of *Rubiaceae* and *Pavetta*. The bacteria invade the roots or leaves and stimulate the formation of root-nodules or leaf-nodules, a sort of harmless tumor. The combination of symbiotic bacteria and host cells remains able to fix atmospheric nitrogen and for this reason legumes are often planted to restore soil fertility by increasing the content of fixed nitrogen. Nodule bacteria may fix as much as 50 to 100 kilograms of nitrogen per acre per year, and free soil bacteria as much as 12 kilograms per acre per year. Further both free soil bacteria (*Azobacter*, and *Clostridium*) produce ammonia as the first stable product and like the symbiotic bacteria, they require molybdenum as an activator and are inhibited by an accumulation of nitrates and ammonia in soil.

Recently, certain lichens (*Collema tunaeforme* and *Peltigera rufescens*) were also implicated in nitrogen fixation (Henriksson, 1971). Lichens with nitrogen-fixing abilrty possess nitrogen-fixing blue green species as their algal component.

Nitrogen fixed by symbiotic and non-symbiotic micro-organisms in soil and water is one source of nitrogen. Another source is organic matter. The nitrogenous wastes and carrion of animals are degraded by the detritus organisms, nitrogen is converted to the amino form (*e.g.*, L-Alanine). The amino group ($-NH_2$) is liberated from the organic molecules to form ammonia ; this process is called deamination. Certain specific bacteria, most notably of the genus *Nitrosomonas*, can oxidize ammonia to nitrite (NO_2) by the reaction.

$$2NH_3+3O_2 \rightarrow 2NO_2^-+2H_2O+2H^+$$

This reaction takes place in the soil, in lake or sea water or sediments, and whenever ammonia is being released and oxygen is present. As fast as nitrite is produced, other bacteria, such as

Nitrobacter, can combine nitrite with oxygen to form nitrate (NO_3) by the reaction :

$$2NO_2^- + O_2 \rightarrow 2NO_3^-$$

Both of these reactions which are performed by two nitrifying bacteria—*Nitrosomonas* and *Nitrobacter* are the parts of a single biological process called nitrification. In nitrification process, thus, ammonia is oxidized to nitrate and nitrite yielding energy. This energy is used by the bacteria to make their organic materials directly

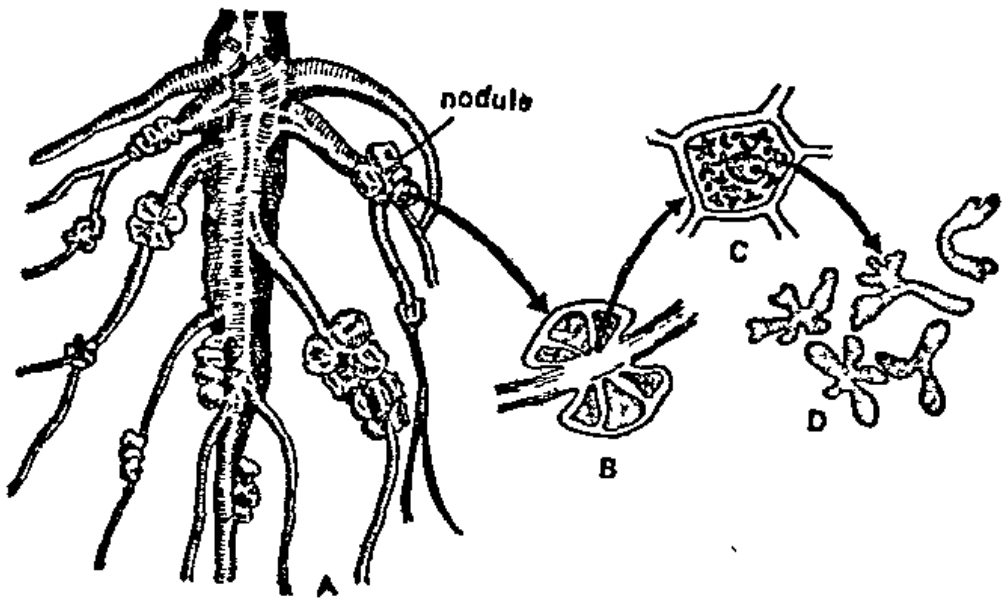


Fig 7.3. Root-nodules of a legume plant. A—Legume root with root nodules B—T.S. of root nodule. C—Single cell of nodule, D—Nitrogen-fixing bacterium, *Rhizobium*.

from carbon dioxide and water. Nitrate can be taken up by autotrophs at the beginning of food chain.

Under certain circumstances, nitrate is either not produced in the nitrogen cycle or it is degraded before it can be utilized by autotrophs. Degradation of nitrate is called denitrification, and may be important when oxygen concentration is low. Denitrifying bacteria such as *Pseudomonas* can use the energy of the nitrate ion to drive their metabolism, and in so doing, they break the nitrate down to nitrite, ammonia, or molecular nitrogen :

$$C_6H_{12}O_6 + 12NO_3^- \rightarrow 12NO_2 + 6CO_2 + 6H_2O$$
$$C_6H_{12}O_6 + 8NO_2 \rightarrow 4N_2 + 2CO_2 + 4CO_3^- + 6H_2O$$
$$C_6H_{12}O_6 + 3NO_2 \rightarrow 3NH_3 + 6CO_2 + 3OH^-$$

If denitrification is significant in an ecosystem nitrite is transitory and is also degraded into either ammonia or molecular nitrogen.

Cycling of nitrogen in the ecosystems : The sources of inputs of nitrogen under natural conditions are the bacterial fixation of

finely tuned circuits which meet demands for the needed elements through several pathways, each controlled by effective self-regulating feedback loop.

C. SEDIMENTARY CYCLES

Mineral elements required by living organisms are obtained initially from inorganic sources. Available forms occur as salts dissolved in soil water or lakes, streams, and seas. The mineral cycle varies from one element to another, but essentially it consists of two phases; the salt-solution phase and the rock phase. Mineral salts come directly from the earth's crust by weathering. The soluble salts then enter the water-cycle. With water they move through the soil to streams and lakes and eventually reach the sea, where they remain indefinitely. Other salts are returned to the earth's crust through sedimentation. They become incorporated into salt beds, silts and limestones; after weathering they again enter the cycle.

Plants and animals, the living components of the ecosystems, fulfil their mineral requirements from mineral solutions in their environments. Other animals aquire the bulk of their minerals from plants and animals they consume. After the death of living organisms the minerals are returned to the soil and water through the action of the organisms and process of decay.

There are different kinds of sedimentary or mineral cycles, depending on the kinds of elements, but following two cycles are very significant for a ecosystem :

(i) **Sulfur cycle :** Sulfur, like nitrogen, is an essential part of protein and amino acids and is characteristic of organic compounds. It exists in a number of states—elemental sulfur, S, sulfides, sulfur monoxide, sulfite, sulfates. Of these the three are important in nature are elemental sulfur, sulfides and sulfates.

The sulfur cycle is both sedimentary and gaseous (*i.e.*, it includes gaseous phase and sedimentary phase). The sedimentary phase of sulfur cycle is long-termed and in it sulfur is tied up in organic and inorganic deposits. From these deposits, it is released by weathering and decomposition, and is carried to terrestrial and aquatic ecosystems in a salt solution. Atmospheric (gaseous) phase of sulfur-cycle is less pronounced and it permits circulation on a global scale.

Sulfur enters the atmosphere from several sources—the combustion of fossil fuels, volcanic eruption, the surface of the oceans and gases released by decomposition. Intially sulfur enters the atmosphere as hydrogen sulfide, H_2S, which quickly oxidizes into another volatile form, sulfur dioxide, SO_2. Atmospheric sulfur dioxide,

atmospheric nitrogen, addition of inorganic nitrogen in rain from such sources as lightning fixation and fixed "juvenile" nitrogen from volcanic activities, ammonia absorption from the atmosphere by plants and soil, and nitrogen accretion from windblown aerosols, which contain both organic and inorganic forms of nitrogen. In terrestrial ecosystems, nitrogen, largely in the form of ammonia and nitrates is taken up by plants, which convert it into amino acids and proteins. Animals (primary macro-consumers) may eat the plants and utilize the amino acids from the plant proteins in the synthesis of their own proteins and other cellular constituents. When animals and plants die, the decay bacteria convert the nitrogen of their proteins and other compounds into ammonia. Animals excrete several kinds of nitrogen—containing wastes-urea, uric acid, creatinine, and ammonia-and the decay bacteria converts these wastes to ammonia. Ammonia may be lost as gas to the atmosphere, may be acted upon by nitrifying bacteria, or may be taken up directly by plants. The nitrates may be utilized by plants, immobilized by microbes, stored in decomposing humus, or leached away. This material is carried to streams, lakes, and eventually the sea, where it is available for use in aquatic ecosystems. There nitrogen is cycled in a similar manner, except that the large reserves contained in the soil humus are largely lacking. Life in the water contributes organic matter and dead organisms that undergo deposition and subsequent release of ammonia and ultimately nitrates. In aquatic ecosystem atmospheric nitrogen is fixed by numerous blue-green algae.

Under natural conditions nitrogen lost from ecosystems by denitrification, volatilization, leaching, erosion, wind blown aerosols, and transportation out of the system is balanced by biological fixation and other sources. Both chemically and biologically, terrestrial and aquatic ecosystems constitute a dynamic equilibrium system in which a change in one phase affects the other.

Comparison of nitrogen-cycle and carbon-cycle : A comparison of the nitrogen and carbon cycles points out some obvious differences between them. Although the atmospheric phase clearly constitutes the main reservoir for both elements in the total environment, atmospheric nitrogen exists in a very different form from that in which it is taken up by autotrophs. But it constitutes a source from which losses sustained by the organic phase can be made up. Also, there are more inorganic chemical reactions external to the food chain in the nitrogen cycle than in the carbon cycle. Despite these differences, both the nitrogen and carbon cycles are examples of

finely tuned circuits which meet demands for the needed elements through several pathways, each controlled by effective self-regulating feedback loop.

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soluble in water, is carried back to earth in rainwater as weak sulfuric acid, H_2SO_4. Whatever its source, sulfur in a soluble form, mostly as sulfate (SO_4^-) is absorbed through plant roots, where it is incorporated into certain organic molecules, such as some amino acids (*e.g.*, cystine) and proteins. From the producers the sulfur in amino acids is transferred to the consumer animals, with excess being excreted in the feces.

Excretion and death carry sulfur in living material back to the soil and to the bottoms of ponds, lakes, and seas where the organic material is acted upon by bacteria of detritus food. Chain within the detritus food chain, the sulfhydryl group (—SH) of amino acids (*e.g.*, L-cysteine) is separated from the rest of the molecule as hydrogen sulfide (H_2S) by most decomposing bacteria as a normal part of the degradation of proteins. In an aerobic environment, the hydrogen sulfide is oxidized to sulfate by bacteria specially adapted to perform this conversion :

$$H_2S + 2O_2 \rightarrow SO_4^- + 2H^+$$

The sulfate produced then can be reused by the autotrophs. In anaerobic environments, such as bottom of certain lakes, it is impossible to oxidize sulfide by this means, because the process of oxidation requires oxygen. But if infrared radiation is present in these environments, there are photosynthetic bacteria that can use it to manufacture carbohydrates and oxidize sulfide either to elemental sulfur or sulfate :

$$6CO_2 + 12H_2S + h\nu \rightarrow C_6H_{12}O_6 + 6H_2O + 12S$$

$$6CO_2 + 12H_2O + 3H_2S + h\nu \rightarrow C_6H_{12}O_6 + 6H_2O + 3SO_4^- + 6H^+$$

Elemental sulfur can also be utilized by other bacteria to form sulfate. If oxygen is present, the reaction is quite rapid.

$$2S + 3O_2 + 2H_2O \rightarrow 2SO_4^- + 4H^+$$

Under anerobic conditions, elemental sulfur can still be oxidized to sulfate by certain bacteria if nitrate is present :

$$6NO_3^- + 5S + 2CaCO_3 \rightarrow 3SO_4^- + 2CaSO_4 + 2CO_2 + 3N_2$$

None of these bacterial reactions is unidirectional; under certain conditions, sulfate can also be reduced either to sulfide or to elemental sulfur by bacteria. This series of reactions operating within the organic phase of the sulfur cycle provides a rather finely tuned mechanism for regulating the availability of sulfur to autotrophs.

The sulfur is removed from the organic phase in the form of elemental sulfur which is unsoluble and accumulates in sediments. If iron is present in the sediment, it can combine with sulfide to form iron sulfides, all of which are highly insoluble :

$$Fe^{++} + S^- \rightarrow FeS$$

$$Fe(ionic)+2S(ionic) \rightarrow FeS_2 \text{ (Ferrous sulfide or pyrite)}$$

FeS_2 is highly insoluble under neutral and alkaline conditions and is firmly held in mud and wet soil. Some ferrous sulfide is contained in sedimentary rocks overlying coal deposits. Exposed to the air in deep and surface mining, the ferrous sulfide oxidizes and in the presence of water produces ferrous sulfate and sulfuric acid :

$$2FeS_2+7O_2+2H_2O \rightarrow 2FeSO_4+2H_2SO_4$$

$$12FeSO_4+3O_2+6H_2O \rightarrow 4Fe_2(SO_4)_2+4Fe(OH_3)\downarrow$$

In this manner sulfur in pyritic rocks, suddenly exposed to weathering by man, discharges heavy slugs of sulfur, sulfuric acid, ferric sulfate and ferrous hydroxide into aquatic ecosystems. These compounds destroy aquatic life and cause acidic water.

(ii) **Phosphorus cycle :** Phosphorus cycle has no atmospheric phase. It occurs naturally in environment as phosphate (PO_4^-, or one of its analogues, HPO_4^- or $H_2PO_4^-$), either as soluble inorganic phosphate ions, as soluble organic phosphate (*i.e.*, as a part of a soluble organic molecule), as particulate phosphate (*i.e.*, as part of an insoluble organic or inorganic molecules) or as mineral phosphate (*i.e.*, as part of a mineral grain as found in a rock or sediment). The ultimate source of phosphate in the ecosystem is crystalline rocks. As these are eroded and weathered, phosphate is made available to living organisms, generally as ionic phosphate This is introduced into autotrophic plants through their roots, where it is incorporated into living tissues. From autotrophs, it is passed along the grazing food chain in the same fashion as nitrogen and sulfur, with excess phosphate being excreted in the faeces An extreme example of faecal phosphate is the tremandous guano deposits built up by birds on the desert west coast of South America. Phosphates can also be released as particulate matter from forest and grassland fires.

In the detritus food chain, as large organic molecules containing phosphates are degraded, the phosphate is liberated as inorganic ionic phosphate. In this form it can be immediately be taken up by autotrophs, or it can be incorporated into a sediment particle, either in the soil of a terrestrial ecosystem or in a sediment of an aquatic ecosystem. The sedimentary phase of phosphorous cycle remains comparatively slow than the organic phase.

Besides phosphorus, there are biogeochemical cycles for all the other nutrients (minerals) used by living organisms, as well as some that are not. Most of them has complete cycles in sedimentary phase. Their availability depends on their solubility in water and availability of water as solvent.

Thus, the geochemical cycles of different chemical substances are closed : the atoms are used over and over again. To keep the cycles going does not require new matter but it does require energy, for the energy cycle is not a closed one. Further, the patterns of flow, both of energy and of chemical substances, are of great significance. The simpler patterns involves energy, as the sources of energy are external to the ecosystem, and flow is unidirectional through it. Chemical substances, on the other hand, are finite and have their origin inside the ecosystem; thus they must continnously cycle within the system.

ENERGY FLOW IN ECOSYSTEMS

Energy can be defined as the capacity to do work, whether that work be on a gross scale as raising mountains and moving air masses over continents, or on a small scale such as transmitting a nerve impulse from one cell to another. There are two kinds of energy, potential and kinetic. Potential energy is energy at rest. It is capable of and available for work. Kinetic energy is due to motion, and results in work. Work that results from the expenditure of energy can both store energy (as potential energy) and arrange or order matter without storing energy.

The expenditure and storage of energy is described by two laws of thermodynamics [illegible] called law of conservation of er[illegible] *destroyed.* It may [illegible] or act upon matter [illegible] and transformations take place, no gain or loss in total energy occurs. Energy is simply transferred from one form or place to another. When wood is burned the potential energy present in the molecules of wood equals the kinetic energy released, and heat is evolved to the surroundings. This is an **exothermic reaction.** In **endothermic reaction,** energy from the surrounding may be paid into a reaction For example, in photosynthesis, the molecules of the products store more energy then the reactants. The extra energy is acquired from the sunlight, but even than there is no gain or loss in total energy. Further though the total amount of energy involved in any chemical reaction, such as burning wood, does not increase or decrease, much of the potential energy stored in the substance undergoing reaction is degraded during the reaction into a form incapable of doing any further work. This energy ends up as heat serving to disorganize or randomly disperse the molecules involved, thus making them useless for further work. The measure of this relative disorder is named entropy.

The second law of thermodynamics states that whenever energy is transformed from one kind to another, there is an increase in entropy and a decrease in the amount of useful energy. Thus, when coal is burned in a boiler to produce steam, some of the energy creates steam that performs work, but part of the energy is dispersed as heat to the surrounding air. The same thing happens to energy in the ecosystem As energy is transformed from one organism to another in the form of food, a large part of that energy is degraded as heat and as a net increase in the disorder of energy. The remainder is stored in living tissue

Three sources of energy account for virtually all the work of the ecosystem : gravitation, internal forces within the earth and solar radiation. The last is very significant for ecosystem. The solar radiations which originate from sun is the source of energy for life and life is what sets the ecosystem apart from other natural systems.

ENERGY IN AN ECOSYSTEM

Living organisms can use energy in several forms, but all can be grouped under one of two headings : radiant and fixed. Radiant energy is in the form of electromagnetic waves such as light. Fixed energy is potential chemical energy bound up in various organic substances which can be broken down or reacted with something else in order to release their energy content.

Only a small fraction of the light energy reaching the earth is trapped ; considerable areas of the earth have no plants, and plants can utilize in photosynthesis only about 3 per cent of the incident energy. This radiant energy of sun is converted into chemical or potential energy by photosynthesis in plants. The photosynthetic autotrophs :

maintenance of body tissues and functions of the plants. All required energy which is provided by oxidation of some of the sugar produced by photosynthesis to give CO_2, H_2O and usable chemical energy :

$$C_6H_{12}O_6+6O_2\rightarrow 6CO_2+6H_2O+\text{ usable energy}$$

Oxidation of sugar or any other organic molecule to get usable energy by organism is called **respiration.** The energy released by respiration is lost permanently to the ecosystem.

Primary production : All the energy that the plant actually fixes results in the formation of sugar. Conversely, all the sugar produced in the leaves of green plants is derived from carbon dioxide and water which have been combined by the solar energy. Thus, one can speak of the energy incorporated into living tissue either in terms of the light energy utilized or in terms of the sugar produced. Because all the energy used by the plant is converted into chemical energy, it should theoretically be possible to determine the entire energy uptake of plants by measuring the total amount of sugar produced. This quantity is termed **gross primary production or gross productivity.** Because, it is not easy to measure gross primary production in most ecosystems because some of the sugar produced by photosynthesis is lost immediately through respiration. So, if one measures the total organic material actually present in the plant (*i.e.*, biomass), then indirectly he will measure the gross primary production less the respiration, or **net primary production.** In addition, there are direct measurement techniques involving the rate of uptake of radioactive carbon ^{14}C. Productivity is generally expressed in terms of grams or kilocalories per sq metre per day or per year. It can also be expressed as $P_g=P_n+R$, where P_g is the gross productivity or energy used to do work, P_n is net productivity, and R is respiration or energy to do work. Net production is an increase of stored energy with time, while biomass is total stored energy at any time. If gross production equals respiration ($P_g=R$) no change in energy content results. But when P_g is less than R biomass decreases, and when P_g is greater than R there is an accumulation of biomass.

The gross productivity is dependent on many things, including climatic conditions such as temperature, rainfall, and total solar radiation, and such other features of the abiotic environment as the availability of nutrient materials essential for life, such as nitrogen, phosphorus and sulfur. Following table lists the gross primary production or gross productivity over one year, for several different environments :

Table : Estimated annual gross primary production of a range of ecosystem (Odum, 1971; Clapham, Jr., 1973).

Ecosystems	Area (10^6 Km.^{2})	Gross primary productivity (ly./Yr.)
Marine		
1. Open ocean	326.0	100
2. Coastal zones	34·0	200
3. *Up welling zones*	*0 4*	*600*
4. Estuaries and reefs	2 0	2,000
Terrestrial		
1 Deserts and Tundras	40 0	20
2. Grasslands	42·0	250
3. Dry forests	9 4	250
4. Boreal coniferous forests	10 0	300
5. Cultivated land	10 0	300
6 Moist Temperate Forests	4 9	800
7. Mechanized Agriculture	4 0	1,200
8. Wet tropical, subtropical Forests	14 7	2,000

Secondary production : The potential energy resulting from primary production furnishes the energy required by other trophic levels in an ecosystem. Some energy in the form of food is consumed

per cent of the ingested food, where as the assimilation coefficiency of carnivores is typically higher. For example, various species of fish absorbed between 86 and 96 per cent of the ingested food, but the efficiency absorption of different classes of food substances varied : about 92—98 per cent of the ingested protein was absorbed, while only 80 per cent of the fat and about 63 –79 per cent of carbohydrates was absorbed by the fishes (**Pandian**, 1967). The unassimilated material leaves the animal's body as waste material to serve as an energy source for other organism (detritus feeders and saprotrophs). Assimilated energy is used by these consumers species for various metabolic processes, such as respiration, excretion, and secretion. The resultant amount of energy stored in the tissues of heterotrophs is termed **net secondary production.** The **gross secondary production** is equivalent to the total plant material ingested by the herbivore, less the materials lost as faeces, That is, it is the food that actually passes through the walls of the gut. *Unlike*

gross primary production, gross secondary production can be measured directly by determining the amount of material defaecated.

Respiration of the population : In previous sections it has been discussed that gross production and net production and the total energy involve respiration or the cost of maintenance of the population. This is expressed in terms of oxygen utilized for vital processes or of carbon dioxide eliminated and so termed **respiration of the population.**

Patterns of flow of energy through the ecosystems : Thus, in an ecosystem one can observe the transfer or flow of energy from one trophic level to other in succession. Primary producers trap radiant energy of sun and transfer that to chemical or potential energy of orga[illegible] fats. When [illegible] decompose it) liberated is just equal to the amount of energy used in synthesizing the substances (first law of [illegible] energy is heat and not useful [illegible] mics). If this animal in turn [illegible] transfer of energy from a herb[illegible] in useful energy occurs as the [illegible]

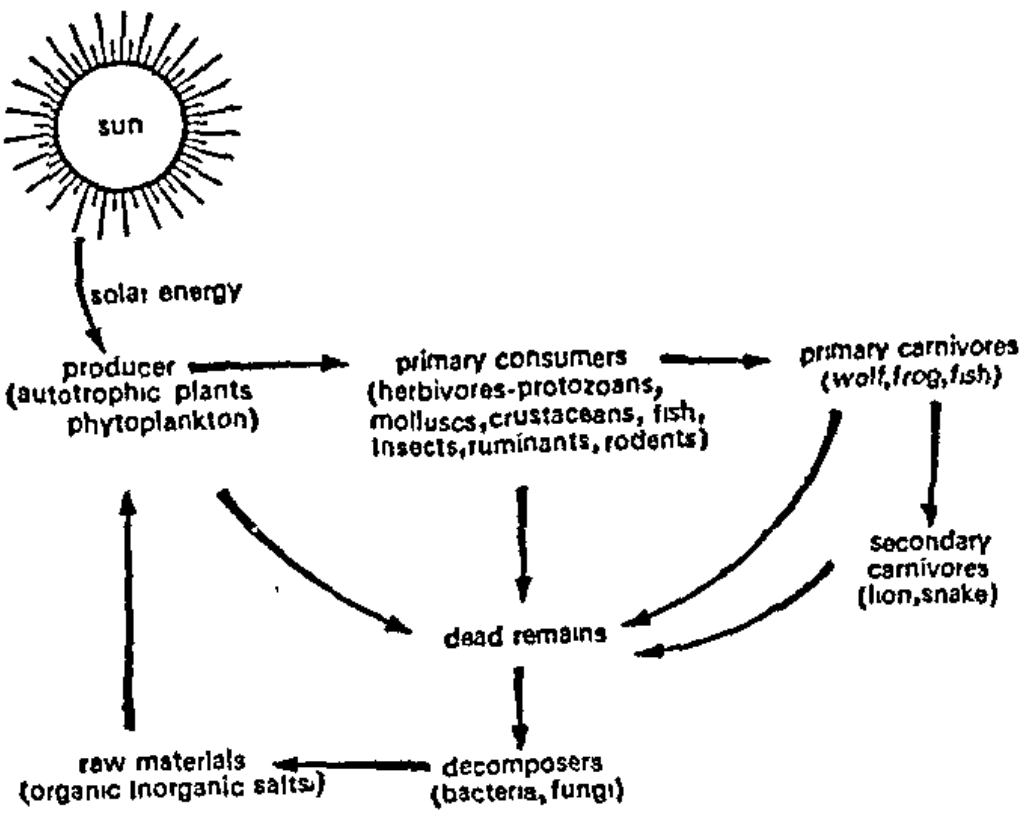


Fig. 7 4. A generalized food chain of the ecosystems

the organic substances of the first (herbivore or omnivore) to liberate energy to synthesize its own cellular constituents. Such transfer

restricted to four or five, since the energy available decreases with each step. In other words, the energy fixed in organisms such as in aquatic plants in a pond passes through the ecosystem in the form of plant→herbivore→carnivore, where only three steps are encountered in the chain. But very often, the chains are very complicated with several steps being involved. For example, the food-chain of shore ecosystem includes plant→herbivore→carnivore 1→ carnivore 2→carnivore n. Basically two types of food chains are recognized—grazing food chain and detritus food chain.

1. Grazing food chain : The grazing food chain starts from green plants and ends to carnivores by passing through herbivores. Thus, gross production of a plant in an ecosystem may meet three fates—it may be oxidized in respiration, it may die and decay and it may be eaten by plant eating animals or herbivores. In herbivores the assimilated food can be stored as carbohydrates, protein or fat, transformed into relatively simple substances or rebuilt by the animal into much more complex organic molecules. The energy to perform these transformations is supplied by respiration. Like autotrophs, the ultimate disposition of energy in herbivores occurs by three routes : respiration, decay of organic matter by bacteria and other decomposer organisms and consumption by carnivores.

The **primary carnivores** or **secondary consumers** eat herbivores or primary consumers of the ecosystems. And likewise, **secondary carnivores** or **tertiary consumers** eat primary carnivores. The total energy assimilated by primary carnivores, or **gross tertiary production**, is derived entirely from the tissues of herbivores, and its disposition into respiration, decay and further consumption by other carnivores is entirely analogous with that of the herbivores. It seems clear that much of the energy flow through grazing food chain can be described in terms of the categories or **trophic levels**, outlined as follows :

{Autotroph primary producers} → {Herbivore primary consumer} → {Primary carnivore secondary consumer} → {Secondary carnivore tertiary consumer} → etc.

As a general model for the direction and extent of energy flow within communities, the food chain is very useful. It demonstrates the amount of energy found at any trophic level, the transfer of energy

from one trophic level to the next, and the amount of energy lost from the grazing food chain, either through respiration or decay. Further the producer→herbivore→carnivore chain is a **predator chain. Parasitic chains** also exist wherein smaller organisms consume larger ones without outright killing as in the case of the predators.

2. Detritus food chain : The organic wastes, exudates and dead matter derived from the grazing food chain are generally termed **detritus.** The energy contained in this detritus is not lost to the ecosystem as a whole ; rather it serves as the source of energy for a group of organisms (detritivores) that are separate from the grazing food chain, and generally termed the **detritus food chain.** The detritus food chain represents an exceedingly important component in the energy flow of an ecosystem. Indeed in some ecosystems, considerably more energy flows through the detritus food chain than through the grazing food chain. In the detritus food chain the energy flow remains as a continuous passage rather than as a stepwise flow between discrete entities. The organisms of the detritus food chain are many and include algae, fungi, Protozoa, insects, rotifers, annelid worms, species are highly specific in eat almost anything. Many Protozoa, for instance, need certain specific organic acids, vitamins, and other nutrients before they can thrive ; on the other hand, the guts of small Collembola (a group of tiny soil insects) have been reported to contain decaying plant material, fungal fragments, spores, fly pupae, other Collembola, parts of decaying earthworms, and cuticle from their own faecal casting (Hale, 1967). In contrast to the grazing food chain, in which energy storage is entirely within the tissues of living organisms, energy storage for the detritus food chain may be largely external to the organisms, and is the detritus itself.

Detritus organisms ingest pieces of partially decomposed organic matter, digest them partially, after extracting some of the chemical energy in the food to run their metabolism, excrete the remainder in the form of slightly simpler organic molecules. The wastes from one organism can immediately be utilized by a second which repeats the process. Gradually, the complex organic molecules present in the original wastes or dead tissue are broken down to much simpler compounds, sometimes all the way to carbon dioxide and water. In most instances, however, the organic material is broken down until all the easily biodegradable material has been degraded, and all that is left are some refractory organic substances

termed **humic acids**, or simply, humus. In a normal environment the humus is quite stable and will form an essential part of the soil.

Links between grazing and detritus food chains : Just as energy enters the detritus food chain from the grazing food chain as leaf litter, or dead organisms, energy can re-enter the grazing food chain from the detritus food chain if organisms from the latter are consumed by grazers. Considering the diversity of the detritus community, it is not surprising that many of these serve as prey for carnivores in the grazing food chain. A common example is robins eating earthworms. In addition, many insects, among them beetles and flies, spend their larval period in the detritus food chain and their adulthood in the grazing food chain. But through such links through which energy passes from detritus food chain basic into grazing food chain, very little amount of energy is passed in comparison to the amount of energy which flow from grazing food chain to detritus food chain.

ECOLOGICAL PYRAMIDS

In the successive steps of grazing food chain—photosynthetic autotroph, herbivorous heterotroph, carnivores heterotroph, decay bacteria—the number and mass of the organisms in each step is limited by the amount of energy available. Since some energy is lost

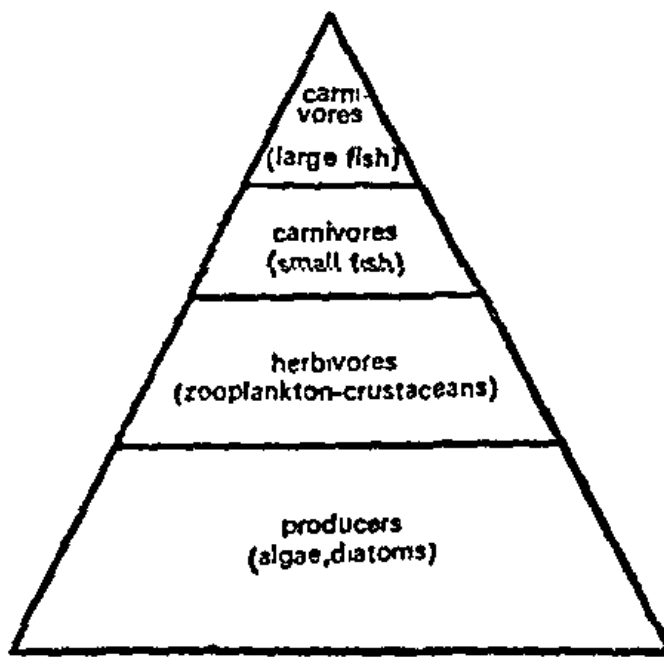


Fig. 7·5. A pyramid of numbers in a lake ecosystem.

also trophic function of the ecosystem. In many ecological pyramid, the producer form the base and the successive trophic levels make up the apex.

Thus, communities of terrestrial ecosystems and shallow water ecosystems contain gradually sloping ecological pyramids because these producers remain large and characterized by an accumulation of organic matter. This trend, however, does not hold for all ecosystems. In such aquatic ecosystems as lakes and open sea, primary production is concentrated in the microscopic algae. These algae have a short life-cycle, multiply rapidly, accumulate little organic matter and are heavily exploited by herbivorous zoo-plankton. At any one point in time the standing crop is low. As a result, the pyramid of biomass for these aquatic ecosystems is inverted : the base is much smaller than the structure it supports.

TYPES OF ECOLOGICAL PYRAMIDS

The ecological pyramids may be of following three kinds :

1. **Pyramids of number :** It depicts the number of individual organisms at different trophic levels of food chain. This pyramid was advanced by **Charles Elton** (1927), who pointed out the great difference in the numbers of the organisms involved in each step of the food chain. The animals at the lower end (base of pyramid) of the chain are the most abundant. Successive links of carnivores decrease rapidly in number until there are very few carnivores at the top. The pyramid of number ignores the biomass of organisms and it also does

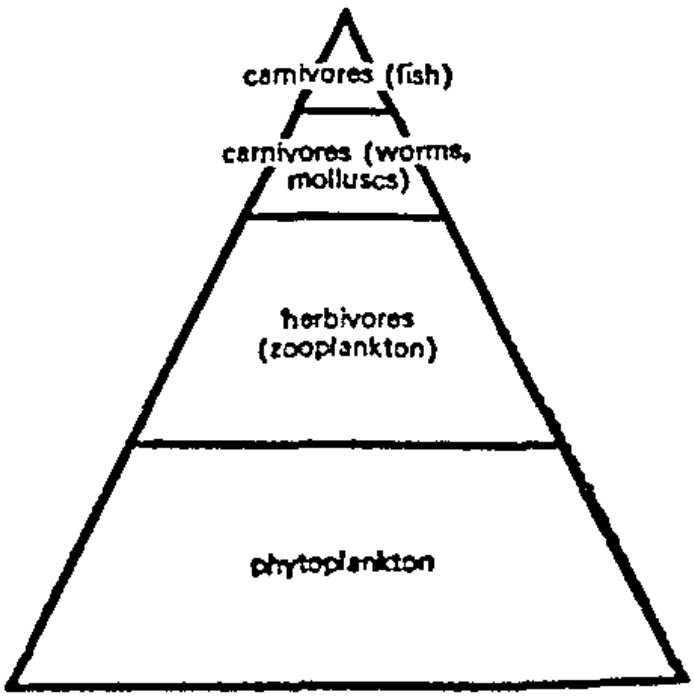


Fig. 7·6. Pyramid of biomass for a marine ecosystem.

not indicate the energy transferred or the use of energy by the groups involved. The lake ecosystem provides a typical example for pyramid of number (Fig. 7·5).

2. Pyramid of biomass : The biomass of the members of the food chain present at any one time forms the pyramid of the biomass Pyramid of biomass indicates decrease of biomass in each trophical level from base to apex. For example, the total biomass of the producers ingested by herbivores is more than the total biomass of the herbivores in an ecosystem. Likewise, the total biomass of the primary carnivores (or secondary consumer) will be less than the herbivores and so on.

3. Pyramid of energy : When production is considered in terms of energy, the pyramid indicates not only the amount of energy flow at each level, but more important, the actual role the various organisms play in the transfer of energy. The base upon which the pyramid of energy is constructed is the quantity of organisms produced per unit time, or in other words, the rate at which food material passes through the food chain. Some organisms may have a small biomass,

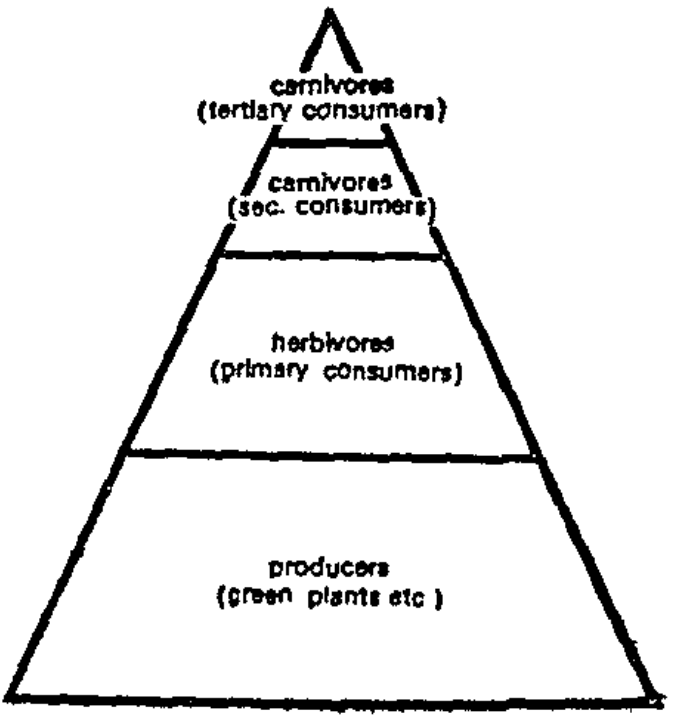


Fig. 7·7. Energy pyramid of an ecosystem.

but the total energy [illegible] and pass on may be considerably [illegible] a much larger biomass. Energy [illegible] se less energy is transferred from [illegible]. In cases such as in open water [illegible]ss bulk than consumers but the [illegible] be greater than that of the next producers support could not be greater than that of the producers themselves. This high energy flow is maintained by a rapid turn over of individual plankton, rather than an increase of total mass.

CHAPTER 8

AQUATIC ECOSYSTEMS : FRESH-WATER COMMUNITIES

The subject of ecology can be approached through discussions of the principles and concepts of the science as they apply to different levels of organization, the individual, population, community and ecosystem. Another general approach, the **habitat approach**, describes the distinctive features of the major habitats and their subdivisions, how they are organized, the organisms present in each and the ecologic role of these organisms in that region (*i.e.*, the identity of the major producers, consumers and decomposers). According to Elton (949) habitat is definable as an area possessing uniformity of physiography, vegetation, climate or any other quality the investigator assumes important. Four major habitats can be distinguished : **marine, estuarine, fresh water** and **terrestrial.** No plant or animal is found in all four major habitats and, indeed, no animal or plant is found everywhere within any one of these. According to recent ecological trend only two major habitats or more specifically ecosystems namely **aquatic ecosystems** and **terrestrial ecosystems,** are recognized.

AQUATIC ECOSYSTEMS

Liquid water covers about three quarters of the earth's surface, either as oceans or as freshwater. Virtually all these waters contain life in one form or other ; hence aquatic ecosystems would be important for their sheer volume, if for nothing else. But aquatic ecosystems are historically the source of life on earth. Even now, tiny single-celled marine plants are the main source of the earths' oxygen supply, because most photosynthetic oxygen is derived from these plants (**Clapham. Jr.,** *1973). In addition, aquatic ecosystems* are simpler in many ways than terrestrial ecosystems. The reason for this is that the omnipresent environmental factor which sets the tone for all aquatic systems, regardless of their *biotic complexity,* is water. It is the medium within which all aspects of the ecosystem coexist, both living and non-living ; it is the source of all nutrients for aquatic life, including the gaseous nutrients such as oxygen and

carbon dioxide ; it is the medium by which organic and inorganic wastes and sediments are distributed throughout the ecosystem. The amount of light energy reaching the community is determined by the way in which light is absorbed by water ; the heat properties of water determine much of the circulation patterns within the ecosystem and are a major control over the structure of the aquatic community that exists in any one place.

SUBDIVISIONS OF AQUATIC ECOSYSTEMS

Global aquatic systems fall into broad classes definable by salinity, or amount of material dissolved in water—the **fresh-water ecosystem** and **saltwater ecosystems.** The latter may include inland brackish water, as well as marine and estuarine habitats. In fact, estuary represents a transitional zone between a river and the sea and it contains dissolved solid content intermediate between those of fresh and marine waters. Fresh-water ecosystems, the study of which is known as **limnology**, are conveniently divided into two groups—**lentic, standing or still water habitats** and **lotic or running water habitats.** Both can be considered on an environmental gradient. The lotic follows a gradient from springs to mountain brooks to streams to rivers. The lentic involves a gradient from lakes to ponds to bogs, swamps and marshes.

FRESH WATER ECOSYSTEMS : PHYSICO-CHEMICAL NATURE OF FRESHWATER

The fresh water of both kinds—lentic and lotic, has low percentage of dissolved salts and is subjected to the influence of an array of physical and chemical factors. The rise and fall of these factors very frequently affect the fauna, altering their number and diversity. Some of the important factors of fresh water environment are following :

1. PRESSURE, DENSITY AND BUOYANCY

The pressure imposed on a lake-dwelling organism is the weight of the column of water above it plus the weight of the atmosphere. *In all fresh water environments* maximum pressure is much less than in the ocean, and organisms appear to adjust to them readily. The absence of animal life from deep water is ordinarily a consequence of low oxygen supply, or low temperature, rather than pressure.

The density of water varies inversely with temperature and directly with the concentration of dissolved substances. Water is most dense at approximately 4°C and becomes progressively less dense as it cooled below +4°C. Ice also expands markedly the

colder it gets It is because the coldest water is at the surface in winter that ice forms there, rather than at the bottom of a lake. In summer, the coldest waters of deep lakes are at the bottom. Dissolved salts increase the density of water; the density of most inland water-bodies is much less than that of the ocean. However, when great evaporation occurs in a lake having no outlet, the lake may come to contain a higher precentage of salts(*i.e.*, hypersalinity) than the ocean. The few species capable of living in these very salty lakes (*e.g.*, Great Basin of USA) include some algae and Protozoa, the brine shrimp *Artemia gracilis* and the immature stages of two brine flies, *Ephydra gracilis* and *E. hians*.

According to the law of Archimedes, the buoyancy of an object is equal to the weight of the water it displaces. Buoyancy varies with the density of water, and is influenced by the factors that affect density. Viscosity, the measure of the internal friction of water, varies inversely with temperature and also influence buoyancy. Most aquatic organisms keep stations by swimming movements or have special adaptations to decrease the specific gravity of the body and take advantage of any turbulance in the water. For this purpose fresh water aquatic organisms have some swimming adaptations, clinging organs (in case of animals of lotic habitat) or following adaptations—absorption of large amounts of water to form jelly-like tissues; storage of gas or air bubbles within the body; formation of light-weight fat deposits within the body or oil droplets within the cell; increase of surface area in proportion to body mass, which increase frictional resistance (Davis, 1955). When an organism so equipped cease to function, and it sinks to

2. TEMPERATURE

Further, the lentic water of lakes and ponds undergo thermal stratification phenomenon according to the seasons. Thermal stratification have been reported most frequently in the lakes of tropical countries like Java, Sumatra, etc. In fact, according to their temperature relations, lakes have been classified into three types : (1) **Tropical lakes** in which surface temperatures are always maintained above 4°C ; (2) **Temperate lakes** in which surface temperature vary above and below 4°C and (3) **Polar lakes** in which surface temperatures never go above 4°C. The seasonaly regulated thermal stratification of lentic habitats has a significant influence on their inhabitant biotic communities. Decreasing temperatures often cause a fall in metabolism, resulting in a lower rate of food consumption. The extremes of lower and higher temperature have lethal effects on the aquatic organisms. So fluctuations in temperature of aquatic media regulate the breeding periods, initiate hibernation, gonadial activitation and a number of other biological phenomena such as thermally oriented migration, etc , of fresh water biota. On the ba[illegible] or[illegible] ra[illegible] ra[illegible] **mothermal forms** (narrow range of temperature, *e.g*, *Aeolosoma, Megascolex mauritii)*, **stenomaximothermal** (*e.g., Dero limosa*) or **stene-optimothermal forms** (*e.g., Branchiodrilus semperi and B. menoni*) (Sitaramiah, 1966).

3. LIGHT

Light influences fresh water ecosystems greatly. The freshwaters often have a lot of suspended material. While affording protection to the light sensitive species, these substances more often obstruct the light that normally reaches the water. The degree of such obstruction of light influence the productivity of the fresh water ecosystems. A shallow lake receives light to its very bottom resulting in an abundant growth of vegetation both phytoplankton and rooted vescular plants, These plants in living or dead states form nice food for consumers of grazing food chain or organisms of detritus food chain, respectively. The running water contain little plant or animal plankton not due to the lack of sunlight but because of the action of the currents in washing it away. Further, light controls the orientation and changes in position of attached species and their nature of growth and it also caused into diurnal migrations of planktonic species of fresh water.

4. OXYGEN

Chemically pure water is biologically uninhabitable and all fresh water contains an array of chemical substances. The oxygen, which is a most essential chemical component of life processes, remain dissolved in fresh waters. The aquatic environments which remain in close proximity with atmosphere contain an abundance of oxygen, that reaches the water either by direct diffusion or by movements of water like wave action or water circulation. Lotic (moving) water of streams and rivers often have a high percentage of oxygen. Aquatic plants supply water with oxygen that is formed as a product of photosynthesis. Rooted vegetation of shallow water zones and floating phytoplankton of open waters also produce oxygen. The amount of photosynthetically produced oxygen remain high at warm temperatures and greater light intensities.

The oxygen level in a tropical pond exhibits diurnal variation—it remains at peak between 14·00 and 17·00 hours of day (Sreenivasan, 1964). Oxygen contents of a fresh water body are depleted in numerous ways. Primarily oxygen is utilised in the respiration of organisns and decomposition of dead orgnisms in the aquatic environment. While photosynthesis remains restricted to the surface layers of water containing phytoplankton and exposed regions of rooted vascular plants, respiration and decomposition occur at all levels. In stagnant pools with a lot of decaying vegetation oxygen content often reaches a stage of complete depletion. The reduction in dissolved oxvgen is magnified by the release of many gases as end products of decomposition or by the mixing up of waters of low oxygen content reaching the habitat as an inflow. Aquatic animals with very few exceptions, *i.e.*, those that breathe air, utilize the oxygen dissolved in water. Certain fresh water inhabitants such as many anaerobic bacteria and insect larvae of chironomids perform anaerobiosis and requires no oxygen.

5. CARBON DIOXIDE

Aquatic vegetation and phytoplankton require carbon dioxide for photosynthetic activity. The carbon dioxide of fresh water environments is produced as the end product of respiration and of decomposition. Carbon dioxide also diffuses directly from the atmosphere and is readily dissolved in water to result in carbonic acid (H_2CO_3) which affects the pH of water. It is also present in the fresh water as carbonates and bicarbonate of calcium, magnesium and other minerals. The growing plants and lime depositing bacteria and other animals may cause a depletion in carbon dioxide resources. Photosynthesis is the major cause for its drain. The high saturation levels

of O_2 and CO_2 have been found to have toxic effects on aquatic biota.

6. OTHER GASES

Streams and lakes contaminated by sewage and stagnant pools with decaying vegetation show an abundance of the gas hydrogen sulphide which is a decomposition product. This gas is highly toxic to living organisms and results into complete denudation of bottom fauna. Methane and carbon monoxide are other toxic gases which are the products of decomposition. Nitrogen, hydrogen, sulphur dioxide and ammonia are some of the other gases which are found dissolved in fresh waters.

7. DISSOLVED SALTS AND SALINITY

Fresh water being efficient solvent contains many solutes in solution, but even then its salt contents remain under $\frac{1}{5}$ °/$_{oo}$ than marine water which contains about thirty-five parts per thousand (°/$_{oo}$) dissolved salts (see **Clapham, Jr.**, 1973). Different dissolved salts reach the water by erosion, inflow and decay of aquatic forms. Dissolved substances have peculiar significance for floating aquatic vegetation and phytoplankton, since these organisms do not depend on the substratum for the supply of nutrients. Compounds of nitrogen, phosphorus and silicon are most important substances found dissolved in fresh water. Nitrate, nitrites and ammonium salts are essential for the food of aquatic vegetation such as algae and water weeds. Nitrate always remain available due to nitrogen cycle occurring between nitrogen fixing bacteria and nitrogen consuming plants. Ammonium salts in excess have a lethal effect on the fauna. Dissolved silicates of freshwater are readily utilized by diatoms and sponges in constructing their body structures such as shell in case of diatoms and spicules in case of sponges.

All fresh water environmants also contain small amounts of phosphorus which more often acts as a limiting factor. Utilization of phosphorus by plankton during the periods of abundance may result in a total elimination of other plants that require the element (phosphorus). Many other elements like calcium magnesium. manganese, iron, sodium, potassium, sulphur, and zinc are found dissolved in water and influence the fauna variously. Iron being a growth promoting element for plants exists as the compound of oxygen (ferrous oxide) or sulphur (ferrous sulphide) in different freshwater bodies. Its influence is often modified by the pH of water. Calcium is an essential element for plants. The abundance and scarcity of carbonate of calcium determine the faunal composition. Deposition of calcium carbonate in water called marl is produced

by the activity of plants. External coverings of arthropods and the shell of molluscs and tubes of some worms need calcium carbonate, snails are found to develop a heavy shell if the waters in which they lived contained excess of calcium. Bryozoans, sponges, and cladocerans prefer an increased calcium content.

Due to low salinity of fresh water animals face the problem of *osmoregulation. Because the salt concentration of body fluids of* animals remain higher than the fresh water hence the water continuously tend to enter the body which should be readily removed. Most aquatic animals (*e. g.*, Protozoa and fishes) have the means to excrete extra amount of water of body by osmoregulation. For this purpose Protozoa employ contractile vacuoles and other multicellular invertebrates and chordates use excretory organs, such as nephridia, kidney, etc.

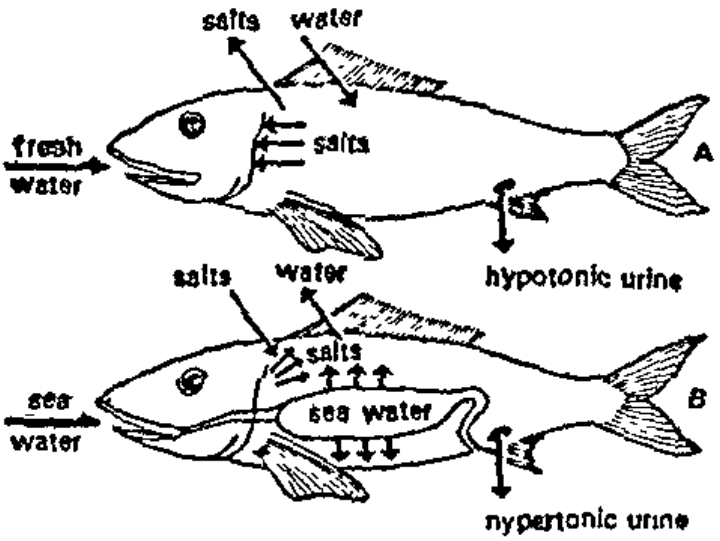


Fig. 8·1. Osmoregulation in (A) fresh water fish and (B) marine fish.

8. PH OR HYDROGEN ION CONCENTRATION

In freshwater environments pH is a determining factor for the biota by becoming a limiting factor. The pH value of different freshwater bodies may fluctuate seasonally and annually. The pH of surface waters and deeper waters exhibit marked differences. Sreenivasan (1968) has reported a marked pH variation of 2·2 units between surface waters and deeper waters in the Sandynulla reservoir in the Nilgiris (India). Though pH range species specific, yet lower aquatic forms in general showed little reaction to alterations in pH, while higher aquatic organisms (*e.g.*, fishes) responded quickly to little pH variations.

LENTIC ECOSYSTEMS

... standing water (freshwater) ... wamps, bogs, meadows, etc. ... ems or wetlands have been tabulated in following table :

Table : Classification of wetland or lentic ecosystems (excluding lakes and ponds) (Smith, 1974).

A.	**Inland freshwater areas**	
1.	Seasonally flooded	Soil covered with water or waterlogged during variable periods but well-drained during much of the growing season. In upland depressions and bottomlands. Bottomland hardwoods to herbaceous growth.
2	Fresh water meadows	Without standing water during growing season ; water logged to within few inches of surface Grasses, rushes, sedges broad-leaf plants.
3.	Shallow freshwater marshes	Soil waterlogged during growing season ; often covered with 6 inches or more of water. Grasses, bulrushes, spike rushes, cattails, arrowhead, smartweed, pickerelweed. A major water-fowl production area.
4.	Deep freshwater marshes	Soil covered with 6 inches to 3 feet of water. Cattails, reeds, bulrushes, spikes rushes, wild rice. Principal duck-breeding area.
5.	Open fresewater marshes	Water less than 10 feet deep Bordered by emergent vegetation : pondweed, naiads, wild celery, water lily. Brooding, feeding, nesting areas of ducks.
6	Shrub swamps	Soil waterlogged; often covered with 6 inches or more of water. Alder, willow, button-bush, dogwoods. Ducks nesting and feeding to limited extent
7.	Wooded swamps	Soilwaterlogged; often covered with 1 foot of water. Along sluggish streams, flat uplands, shallow lake basins. North of USA : tamarack, arborvitae, spruce, red maple, silver maple, south of USA : water oak, overcup oak, tupelo, swamp black gum cypress.
8.	Bogs	Soil waterlogged; spongy covering of mosses. Heath shrubs, sphagnum, sedges.
B.	**Coastal freshwater areas**	
9.	Shallow freshwater marsh	Soil waterlogged during growing season ; at high tides as much as 6 inches of water on landward side, deep marshes along tidal rivers, sounds, deltas Grasses and sedges. Important water-fowl areas
10.	Deep freshwater marshes	At high tide covered with 6 inches to 6 feet of water. Along tidal rivers and bays. Cattails, wild rice, giant cutgrass.
11.	Open freshwater	Shallow portions of open water along fresh tidal rivers and sounds. vegetation scarce or absent. Important water-fowl areas.

I. LAKES AND PONDS

Lakes are inland depressions containing standing water. They may vary in size from small ponds of less than acre tc large seas covering thousands of square miles. They may range in depth from a few feet to over 5,000 feet Ponds, however, are considered as small bodies of standing water so shallow that rooted plants can grow over most of the bottom. Most ponds and lakes have outlet streams and both are more or less temporary features on the landscape because filling no matter how slow, is inevitable.

The aquatic habitats of lake and pond remain vertically stratified in relation to light intensity, wave length absorption, hydrostatic pressure, temperature, etc. In a lake, for example, there are three well recognized horizontal strata namely : (i) Shallow water near the shore forms the **littoral zone.** It contains upper warm and oxygen rich circulating water layer which is called **epilimniou.** The littoral zone includes rooted vegetation. **(ii) Sublittoral zone** extends from rooted vegetation to the non-circulating cold water with poor oxygen zone, *i.e.*, **hypolimnion. (iii) Limnetic zone** is the

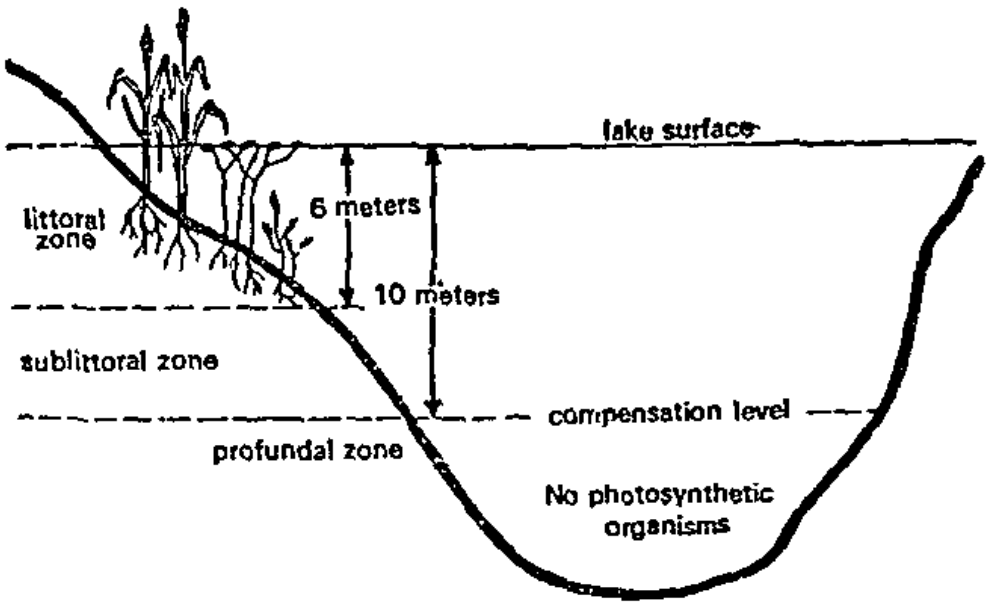


Fig 8·2. Different zones of a deep freshwater lake.

open water zone away from the shore. It is the zone upto the depth of effective light penetration where rate of photosynthesis is equal to the rate of respiration. **(iv) Profundal zone** is the deep-water area beneath limnetic zone and beyond the depth of effective light penetration. **(v) Abyssal zone** is found only in deep lakes, since it begins at about 2,000 meters from the surface. Ponds have little vertical stratification. In them littoral zone is larger than the limnetic zone and profundal zone. In a small pond the limnetic and

profundal zone are not found. Lakes thus differ ponds in having relatively larger limnetic zone and profundal zone than littoral zone.

Further, lentic water of a lake or pond is also classified on the basis of the depth of light penetration enabling photosynthesis into **trophogenic zone** (includes littoral plus sublittoral zones), and a **tropholytic zone** (upper part of profundal zone). The former is often distinguished by abundant plant growth and dependent fauna, while the latter denotes a general absence of vegetation and harbours mostly saprobes. In between the two zones is the **compensation level** which forms a boundary between two zones. It exhibits perfect equilibrium between respiration and photosynthesis.

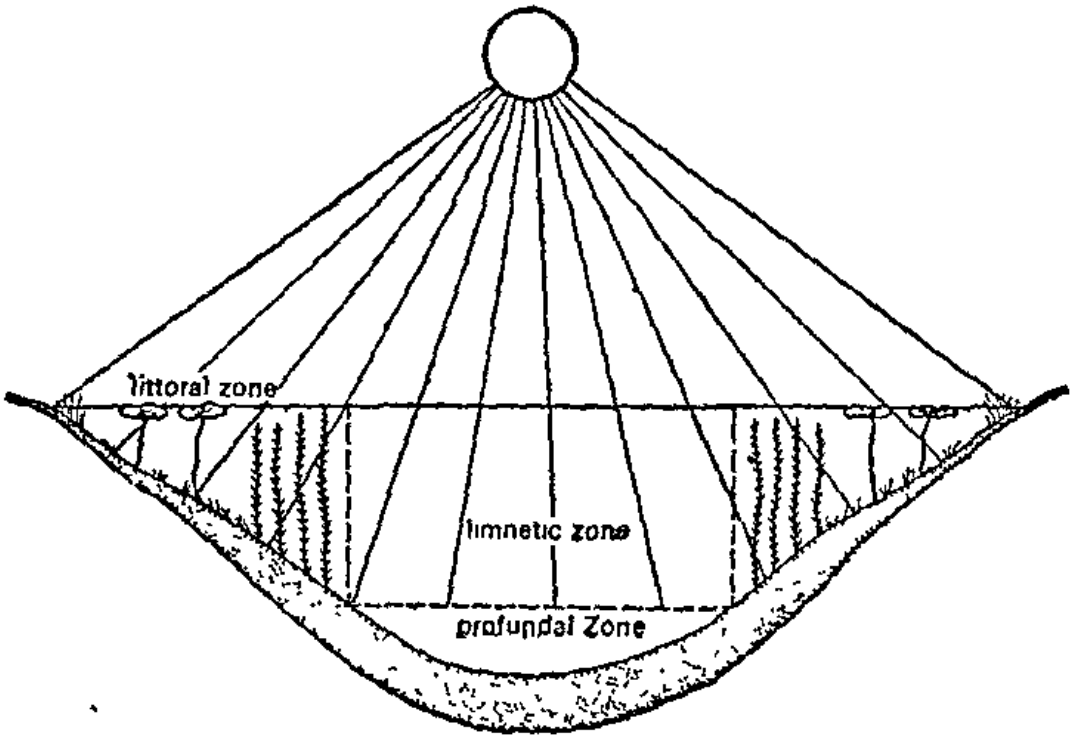


Fig. 8·3. Different zones of a freshwater pond.

Physico-chemical properties of lakes and ponds : Lakes have the tendency to become thermally stratified during summer and winter to undergo definite seasonal periodicity in depth distribution of heat and oxygen (For details see chapter 3, page 59—60). Light too penetrates only to a certain depth, depending upon turbidity. These gradations of oxygen, light and temperature profoundly influence life in the lake, its distribution and adaptation.

Different classifications of lakes on the basis of physical factors productivity, etc., exist. Based on temperature **Hutchinson** (1957) classifies lakes into **dimictic, monomictic** and **polymictic lakes**. The dimictic lakes exhibit two overturns every year, while monomictic lakes present only a single overturn per year. The monomictic lakes

may be **cold monomictic** and **warm monomictic**, the former being characterized by a circulation only during summer, while the latter has a complete circulation in winter as well. Polymictic lakes present circulations throughout the year. Based on the humic acid contents the lakes of world have been classified into **clear water lakes** and **brown water lakes**, brown water contains high humus content. Clear water lakes may be divided into two types—the **oligotrophic type**, whose water is poor in nutritive plant material and shows nearly equal distribution of oxygen during summer and winter months and whose mud bottom contains little organic material; and the **eutrophic type**, which is rich in nutrients. At greater depth below the thermocline in summer euthrophic lakes show a considerable reduction in oxygen content and their mud bottom is composed of typical muck. In ponds strasification is of minor significance.

Origin of lakes and ponds : Lakes and ponds arise in many ways. For example, North American lakes were formed by glacial erosions and deposition and a combination of the two. Glacial abrasions of slopes in high mountain valleys engraved basins, which filled with water from rain and melting snow to produce tarns. Retreating valley glaciers left behind crescent-shaped ridges of rock debris, which dammed up water behind them. Lakes are also formed by the deposition of silt, driftwood, and other debris in the beds of slow-flowing streams. Craters of extinct volcanos may fill with water and landslides can block off streams and valleys to form new lakes and ponds. Futher man intentionally creates artificial lakes by damming rivers and streams for power, irrigation, and water storage, or by constructing small ponds and marshes for water, fishing and wild life. Man made lakes are often called **impoundments**. They may be a 'close' type or 'open' type depending on the discharge of water into it. Sandynulla is a closed impoundment, while Pykara is an open impoundment (Sreenivasan, 1957). Impoundments differ from natural lakes in thermal and oxygen variations and are often characterised by a low percentage of bottom dwellers or benthos.

Ponds occur in most regions of adequate rainfall. Temporary ponds, which are often shallow and quick drying contain a characteristic lentic environment. Permanent ponds contain water throughout the year, while flood-plain ponds are formed when a stream shifts its position, leaving the formed bed isolated as a body of standing water.

BIOTIC COMMUNITIES OF LAKES AND PONDS

Different organisms of the lentic environment can be ecologically classified based on whether they are dependent on the sub-

stratum or free from it. Organisms depending on the substratum are called pedonic forms and those that are free from it are the limnetic forms. Further, the aquatic organisms may also be classified into following groups depending upon their sizes and habits :

1. **Neuston :** These are unattached organisms that live at the air-water interface. They may include floating plants such as duckweed, as well as many types of animals. Animals that spend their lives on top of the air-water interface, such as water striders, are termed epineuston, while others, including insects such as diving beetles and back swimmers, which spend most of their time on the underside of the air-water interface and obtain much of their food from within water, are termed **hyponeuston.**

2. **Plankton :** These are forms which are found in all aquatic ecosystems except for fast-moving rivers. They are small plants and animals whose powers of self-locomotion are so limited that they cannot overcome currents. Thus their distribution is controlled largely by the currents in their ecosystems. Most planktons (phytoplanktons and zooplanktons) can move a bit, however, either, to control their vertical distribution or to seize prey. Certain animal planktons or zooplanktons are exceedingly active and move relatively great distances considering their small size, but they are so small that their range is still controlled largely by currents and such planktons are also known as **nektoplankton.**

3. **Nekton :** Nektonic animals are swimmers and are found in all aquatic systems-except for the fastest-moving rivers. In order to overcome currents, these animals are relatively large and powerful ; they range in size from the swimming insects of quiet water, which may be only about 2 mm long, to the largest animal that has ever lived on earth, the blue whale.

4. **Benthos :** The benthos includes the organisms living at the bottom of the water mass. They occur virtually in all aquatic ecosystems. The benthos organisms living above the sediment-water interface are termed **benthic epifauna** and those living in the sediment itself are termed **infauna.**

Biota of littoral zone : Lentic aquatic life is most prolific in the littoral zone. The littoral zone of a lake remains rich in pedonic flora especially upto the depth to which effective light penetration is possible facilitating the growth of rooted vegetation. At the shore proper are the **emergent vegetation** which remain firmly rooted in the shore substratum but their tops with thin chlorophyll bearing regions are exposed. Interspersed with these plants are the cattails (*Typha*), bulrushes (*Scirpus*), arrowheads (*Sagitaria*) and

pickerelweeds. Out slightly deeper are the rooted plants with floating leaves such as the water lilies—*Nymphaea, Potamageton*, etc. Still deeper in the littoral zone of lake are the fragile thin-stemmed water weeds, rooted but totally submerged. Such a submerged vegetation includes plants like *Elodea, Vallisneria, Myriophyllum*, etc.

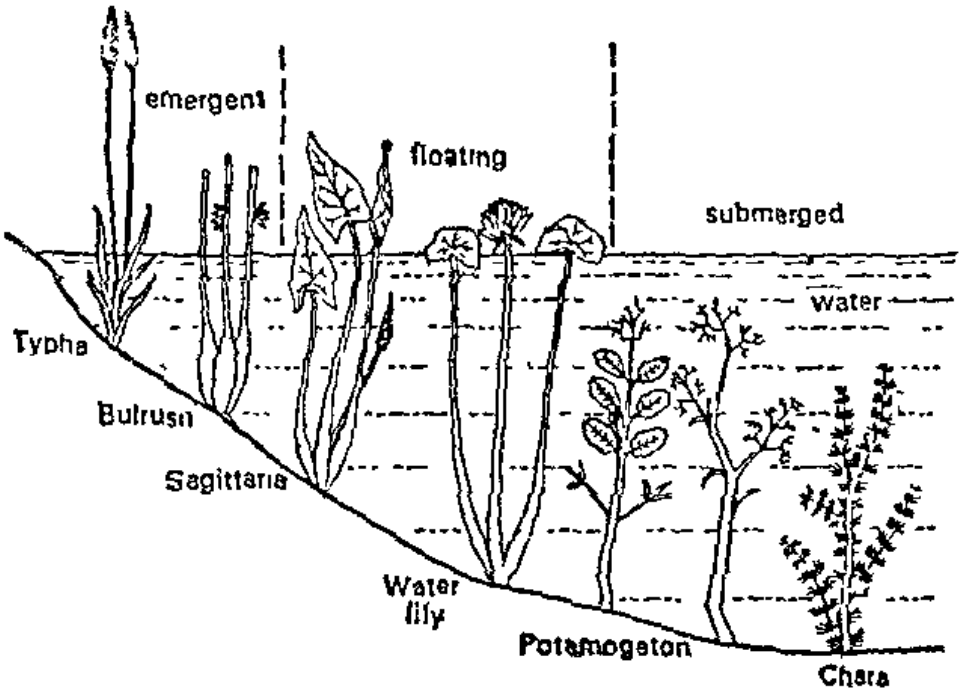


Fig. 8 4. Flora of the lentic habitat.

The phytoplankton of littoral zone of lake is chiefly composed of diatoms *(e.g., Navicula, Cyclotella)*, blue green algae *(e.g., Microcystis, Oscillatoria)*, green algae (*e g , Cosmarium, Staurastrum)* and holophytic flagellates. Sreenivasan (1970) has observed the abundance of marine dinophycean flagellate *Ceratium hirudinella* in some impoundment of India.

The littoral zone also contains great concentration of animals, which remain distributed in recognizable communities. In or on the bottom are various dragonfly nymphs, crayfish, isopods, worms, snails and clams. Other animals live in or on plants and other objects projecting up from the bottom. These include protozoans like *Vorticella, Stentor* ; larvae of *Dytiscus, Laccotrophes*, leech-like *Glossophonia*, climbing dragonfly, damsel fly nymphs, rotifers (*Brachionus*), flat worms, Bryozoa, hydra, snails (*Lymnaea*) and other. With the exception of a few rotifers like *Keratella, Brachionus, Diurella*, and *Trichocerca* and crustaceans like *Cyclops, Daphnia* and *Moina*, most freshwater animals of lentic habitat depend in one way or other on the aquatic vegetation. Larvae of *Chironomus* are found

underneath the leaves of floating plants. The zooplankton of littora zone consists of water fleas such as *Daphnia*, rotifers and ostracod The free swimming fauna (Nekton) includes *Paramecium*, *Euglena Ranatra*, *Corixa*, *Dytiscus*, larvae of *Culex* and of *Chaoborus*, *Gerris Gyrinus*, etc. Among the vertebrates are frogs, salamanders, snake

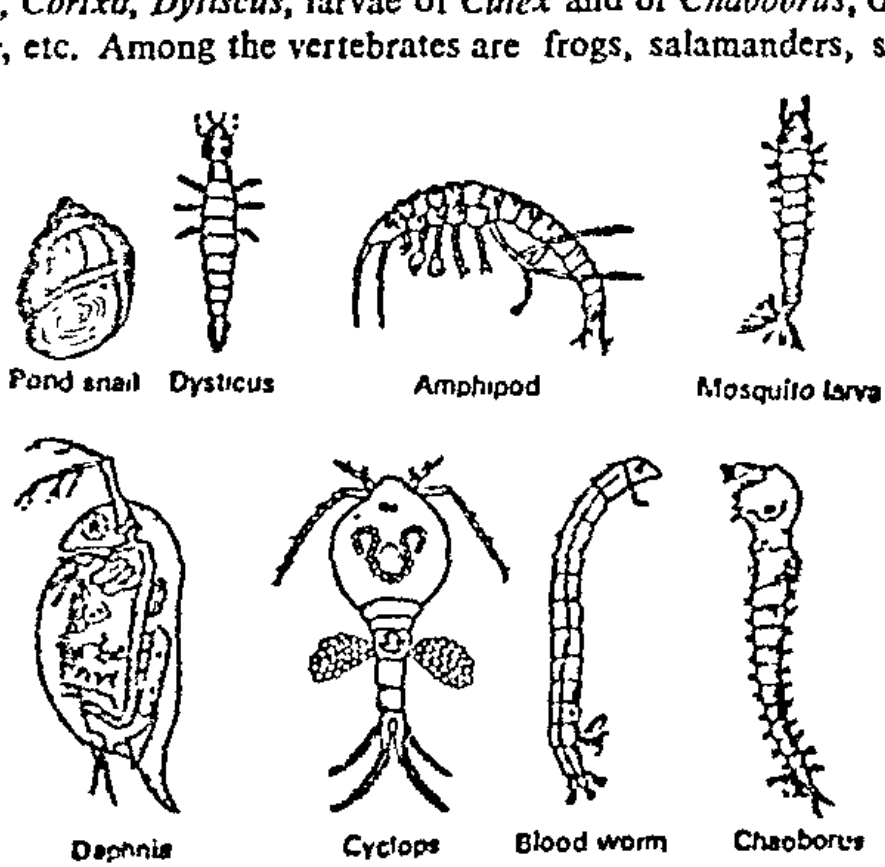


Fig. 8·5. Fauna of littoral zone of lentic habitat.

and turtles are the nektons of littoral zone. Floating members of the community (neuston) are whirliging beetles, water spiders and numerous Protozoa. Many pond fishes such as sun-fish, top minnows, bass, pike and gar spend much of their time in the littoral zone.

Biota of limnetic zone : Limnetic zone is the region of rapid variation, with the water level, temperature and oxygen content varying from time to time. In certain Himalayan lakes which have a glacial origin, change in water level is a conspicuous phenomenon, the onset of summer bringing forth a rapid and sudden rise in water level. Various protozoans which are capable of encystment during adverse ecological conditions, tardigrades like *Macrobiotus*, rotifers like *Rotaria*, *Philodina*, copepods, snails and frogs occupy the limnetic zone. Sedentary and slow moving forms are excluded from this zone because of predators and lacking of permanent substratum for attachment. Many microscopic plants (*Volvox*, *Euglena*) and fishes also occupy this zone. The limnetic zone has autotrophs in abundance.

Biota of profundal zone : The deep (profundal) life consists of bacteria, fungi, clams, blood worms (larvae of midges), annelids and other small animals capable of surviving in a region of little light and low oxygen. In the profundal zone, autotrophs cannot produce food, and the main source of energy is detritus that rains out of the limnetic zone. All the organisms of this zone are heterotrophs, either as detritus feeder or as carnivores. Most of our largest lake fishes inhabit the dark waters of hypolimnion for most of their lives. In addition to detritus from the limnetic zone, food washed into lakes by rivers can settle out and serve as food for profoundal animals.

Benthos of lake bottom : The lake bottom in young lakes may be of the original rock ; in older lakes, it will have been covered with sediment to form a uniform substrate of mud or sand. The benthos community includes several species of insect larvae, including those of small mosquito like midges, burrowing mayflies, clams, snails, and tubeworms.

DISTRIBUTION OF OXYGEN AND DISSOLVED NUTRIENTS IN LAKES

The productivity of a lake depends on the amounts of oxygen and available nutrients dissolved in the water. As a general rule, the littoral zone behaves as a normal ecosystem, with oxygen being produced by photosynthesis within the community, as well as being constantly mixed into the water at the surface. Productivity within the littoral is directly controlled by the nutrients cycling within the littoral zone ecosystem and biogeochemical cycles apparently operate as do in terrestrial ecosystems, with sediments serving as a storage place for inorganic nutrients such as phosphate, and some rooted plants being able to draw nutrients out of the sediments through their roots (McRoy and Barsdate, 1970) But in the pelagic zone (limnetic and profundal zones), the distribution of oxygen and nutrients is exceedingly uneven, and this has a profound effect on the behavior of the lake as a whole, especially in those whose pelagic zone is much larger than the littoral.

The epilimnion of a lake is typically well oxygenated, both because of the production of photosynthetic oxygen in the epilimnion and because of the oxygen uptake at the surface. The amount of nutrients dissolved in the water is highly variable, mainly because most of the nutrients in the epilimnion have been incorporated into living tissue. Thus, production of epilimnion is controlled by amount of available nutrients, which, in turn is controlled primarily by the rate of cycling within the epilimnion, the rate of loss to the hypo-

limnion of nutrient-rich detritus, and the rate of return of soluble nutrients from the hypolimnion during overturn. The fact that only heterotrophic metabolism proceeds in the hypolimnion means that the level of production is determined by the amount of detritus raining into it. As the decomposition of detritus release soluble nutrients, therefore, there is an inverse relationship between oxygen concentration and concentration of vital nutrients. It is paradoxical that the greater the productivity of a lake the lower the oxygen concentration in its hypolimnion. For example, oligotrophic lake which is virtually sterile biologically has low production but its water remain well saturated with oxygen from top to bottom (orthograde oxygen curve). Likewise, a eutrophic lake has high productivity, but there is substantial oxygen depletion from top of the hypolimnion to the bottom, with depletion most extreme in the bottom layers (clinograde oxygen curve). However, in most lakes, oxygen is thoroughly mixed and is essentially at saturation during overturn.

LOTIC ECOSYSTEMS

Moving-water or lotic ecosystems includes rivers, streams, and related environments. They are remarkably variable, ranging in size from Ganga, Yamuna, Hindon, Gomti, etc., to the trickle of a small spring. They vary from raging torrents and water-falls to rivers whose flow is so smooth as to be almost unnoticeable. A given river varies considerably over its length, as it changes from a mountain brook to a large river. This is most noticeable in the abiotic factors of the environment, but all features of the ecosystem vary in response.

CHARACTERISTIC OF LOTIC ENVIRONMENT

Moving waters differ in three major aspects from lakes and ponds : current is a controlling and limiting factor; land-water interchange is great because of the small size and depth of moving water systems as compared with lakes; oxygen is almost always in abundant supply except when there is pollution. Temperature extremes tend to be greater than in standing water. Besides these, the most distinctive features of moving water ecosystems are those related to their motion, *i.e.*, the rate of flow and the stream velocity. The rate of flow or discharge refers to the volume of water passing a given observation point during a specific unit of time ; it is measured in units such as m^3/sec , $ft.^3$/sec., or acre-feet/sec., (one acre-foot is equivalent to a volume of water 1 acre in area by 1 foot deep). It tends to increase steadily going down stream, as tributaries join with the main river. The velocity of flow is the speed at which the water

and is measured in m./sec., ft./sec., or mi./hr. Velocity is variable, but it also tends to increase downstream with increasing discharge. A factor that may be even more important to the biotic community is the **turbulence**, or the irregularity of the motion of the water particles. Perfectly even flow, in which water particles move parallel to one another, is called **laminar flow**. It is contrasted with **turbulent flow**, in which the movement of water particles is highly irregular In highly turbulent water the erosive power is great, the sheer forces at the water-sediment interface at the bottom of the river are powerful, and the amount of oxygen incorporated into the water is very high. In streams with laminar flow the erosive power is lower, the shear forces at the bottom of the stream are lower, and relatively less oxygen is incorporated into the water than turbulent waters.

The moving-water ecosystems can be divided into following two ecosystems depending up on the velocity and rate of flow of water body .

1. Rapidly flowing water and 2. Slowly flowing water.

1. RAPIDLY FLOWING WATER

Rapidly flowing water (of fast moving torrential streams, etc.) can be defined as the portion of the stream in which the flow is both rapid and turbulent. Everything that is not attached or weighted is swept away by the current; this includes organisms and sediment particles alike. The substrate tends to be rock or gravel, and the fragments are rounded and smoothed by the water. The habitat itself is an extremely diverse one. Physical parameters such as sheer force and rate of water movement tend to be quite different on top of a rock fragment, between rock fragments, or beneath rock fragments, and different species can exploit these differences in microhabitat. Aquatic plants, in addition, provide microhabitat for some torrential animals. The rarely occurring phytoplankton of rapid or riffle zone [illegible] algae (*e.g.*, *Clado-*

[illegible] animals of rapidly flowing water streams live among the mosses and flowering plants like *Eriocaulon miserum*, *Hydrolyum lichenoides*, and *Duroea wallichii*. Animals inhabiting these plants have a torpedo-like body which enables them to offer minimum resistance to the current. Some animals such as funnel mouthed tadpoles of *Megalophrys* and tipulid larvae live entangled in the roots of these plants. They possess devices for fixation and have hydrostatic organs.

Some animals such as *Cephalopteryx, Helodes, Phalacrocera, Gammarus*, etc. live among leaves and stems of aquatic plants. All these animals possess hook-like structures on their body helping them in anchoring on leaves and stems. The larvae of simulids and chironomids, however, live on the exposed surfaces of plants.

2. Rock inhabitant forms : The animals that live on the tops of exposed rocks have an efficient mechanism for staying in one place, otherwise, they will be swept away. The organisms in this microhabitat are universally flattened. Some, like the fresh water limpet, *Ferrissia*, are virtually flat, offering little resistance to the current, and holding themselves in place with a very large powerful foot that extends over almost the entire area of the shell. Others, such as water penny, the larvae of the riffle beetle, and *Baetis* larvae, are not only almost flat, but each of their legs possesses hooked claws that enable them to hold onto the substrate more firmly. Larvae of *Simulium* and chironomids cling to rock top by grapling hooks at the posterior end of the body. Caddishfly worms contain both of these structures (claws on legs and hooks at posterior end of body). The mayfly nymphs *Iron* and *Psephenus* attach themselves to the rocks by means of functional pads. Some animals, including fresh-water sponges, actually cement themselves to the surface of the rocks. Others, such as caddish flies, build "houses" out of sediment or wood fragements, which are then cemented firmly to the rocks. Exposed surfaces of rocks also contains few sessile algae and few plants. A significant amount of organic detritus is washed into rapid-water ecosystems from up stream and adjacent terrestrial areas, however. This is the source of the most of energy in fast water ecosystems, and is much more important as an energy source than primary production within the stream itself. In consequence, the majority of primary consumers of such a microhabitat are detritus eaters.

3. *Inhabitant forms of spaces between rocks :* Numerous different kinds of animals live in the spaces between the rocks. Many of them, such as mayfly and stonefly naiads, are flattened and have certain behavioral adaptations to hold them in place. These include **thigmotaxis**, by which they cling instinctively to any hard surface such as rock or another insect larva. **Rheotaxis**, by which the animals orient themselves to face into current and move upstream is another adaptation of this sort. In many of these naiads, the combination of the organisms shape and behaviour is such that the current presses the insect tightly against the rock, increasing the friction between the animal and its substrate. Other insect

burrow into the sediment, such as tubeworms, naiads of the burrowing mayflies, *Sialis* (alderfly), midge *Chironomus*, and several other insect larvae, as well as, clams, nematodes, snails and rotifers.

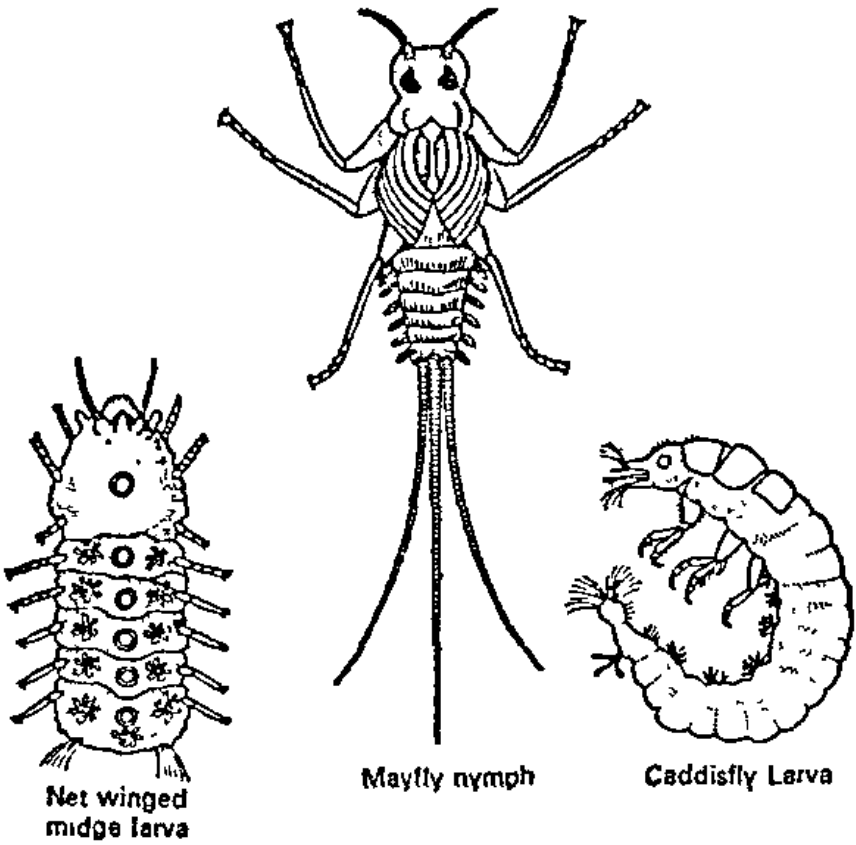


Fig. 8·6. Some inhabitants of lotic environment.

Swimming organisms are also abundant, including not only fishes. Such as carps, cat fishes, suckers, stingers, spoonbills, etc., which tend to be different species from those of fast-water areas, but also larger Crustacea such as amphipods (fresh-water shrimps). Finally, several insects spend most of their time at the surface of the stream. These includes forms such as water striders, water boatmen, backswimmers, and predaceous diving beetles. Zooplankton is abundant, including a rich assemblage of Protozoa and smaller Crustacea such as Cladocera (water fleas) and copepods. The richness of food afforded by slow streams attract a large number of reptiles (water snakes, crocodiles, turtles) and amphibians.

Plant life is also abundant in a slow-water ecosystem. It includes rooted vascular plants such as pond weeds and grasses, firmly attached aquatic mosses and large multicellular filamentous algae. Motile algae such as diatoms and flagellates may abound in the open water. In all, the primary productivity of slow-water ecosystem is higher than that of the rapid-water ecosystem, and the community is relatively less depandent on food materials from

outside. Further, the detritus food chain consisting of organisms such as bacteria and fungi is much better developed in this community as the partially decomposed organic debris that comprises the main food source for these organisms accumulates in the mud bottom. In some slow-moving streams, in fact, the bottom muds contain more organic material than mineral fragments.

In slow-water streams oxygen concentration remains main limiting factor. The high level of animal activity, coupled with an active detritus food chain, can withdraw a large amount of oxygen from water of a slowly moving stream. In addition, the low level of turbulence means that less oxygen is incorporated into the water at the surface. Thus the dissolved oxygen content of a slowly moving stream is likely to be much lower than that of a fast- moving stream. This is commonly reflected in the fauna of the stream. Fishes tolerant of low oxygen levels such as carps and catfish are the most common fishes in slow water, while species with high oxygen demands, such as trout are found in fast water,

CHAPTER 9

AQUATIC ECOSYSTEMS : ESTUARIES AND MARINE COMMUNITIES

Waters of all streams and rivers ultimately drain into the sea ; the place where this fresh water joins the salt water is called an estuary. Estuaries are the transitional zones between the sea and rivers and are the sites of unique ecological properties possessing a characteristic biological make up. They are semienclosed coastal bodies of water that have a free connection with the open sea and within which sea water is measurably diluted with fresh water from rivers. Not all rivers open into estuaries : some simply discharge their runoff into the ocean. Estuaries differ in size, shape and volume of water flow, all influenced by the geology of the region in which they occur. As the river reaches the encroaching sea, the stream-carried sediments are dropped in the quite water. These accumulates to form **deltas** in the upper reaches of the mouth and shorten the estuary. When silt and mud accumulations become high enough to be exposed at low tide, **tidal flats** develop, which divide and braid the original channel of the estuary. At the same time, ocean currents and tides erode the coast line and deposit material on the seaward side of the estuary, also shortening the mouth. If more material is deposited than is carried away, then **barrier beaches, islands,** and **brackish lagoons** appear.

PHYSICO-CHEMICAL ASPECTS OF ESTUARIES

Current and **salinity,** both very complex and variable, shape life in the estuary, where the environment is neither fresh-water nor salt. Estuarine currents result from the interaction of a one-direction stream flow, which varies with the season and rain fall, with oscilating ocean tides, and with the wind. The salinity of estuaries varies vertically and horizontally and fluctuate amazingly between 0·5 to 35 per cent. Due to low salinlty fresh water has a lowet density than sea water (±1·00, as opposed to I 03). Were there no tides in an estuary to mix fresh and salt water, the lighter fresh water would simply flow over the heavier sea water and dissi-

pate in the ocean. However, the tidal action acts as a plunger to thoroughly flush the estuary and mix the fresh and salt water. This has several results. The water level in the estuary fluctuates regularly, unlike that of river. For this reason the habitat that is covered at high tide and uncovered at low tide is a prominent one in the estuary, and has no analogue in any purely fresh water ecosystem. Further, the the salinity is exceedingly variable and may change by a factor of ten over the course of a day at any location. At low tide, most of water passing through the estuary is fresh-river water, and the salinity is correspondingly low. At high tide, most of the water may be of marine origin, and the salinity correspondingly high. Flow may be somewhat stratified, or mixing may be incomplete, and the salinity at any time may vary considerably from one location to another. The intensity of current, and hence of the degree of mixing, is a function of the intensity of the tides and of the rivers flow rate. In other words, an estuary is an exceedingly variable environment. Further, salinity of estuaries remain highest during the summer and during periods of drought, when less fresh water flows into the estuary. It is lowest during the winter and spring, when rivers and streams are discharging their peak loads. No stenohaline organism (such as Echinodermata, Cephalopoda, and other molluscs) could hope to survive in the estuaries. Likewise, no organism that could not tolerate strong currents and the turbid water that results from strong currents could live there.

The temperatures in estuaries fluctuate considerably diurnally and seasonally. Waters are heated by solar radiation and inflowing and tidal currents. High tide on the mud flats may heat or cool the water, depending on the season. The upper layer of estuarine water may be cooler in winter and warmer in summer than the bottom a condition that, as in a lake, will result in a spring and autum overturn.

Anyhow, all estuaries have high productivity. As estuary's high productivity is exceeded among aquatic communities only by coral reefs. The reason is that although an estuary may be a harsh environment in some ways, it is not in others. Because the typical estuary is shallow and turbulant, the amount of dissolved oxygen tends to be fairly high although it may be low in bottom layers of those estuaries where water does not mix from top to bottom. More important, the tidal action acts to accumulate (concentrate) the nutrient and energy materials that wash in from upstream or, in some cases, that enter from the nutrient rich bottom waters of the sea. The mechanism of nutrient concentration is very simple—parti-

culate nutrient material enters the estuary at its upper end, is carried seaward by the falling tide and is brought back through the estuary by the rising tide, and so on for several cycles. The length of time it takes from a nutrient particle to traverse the estuary is substantially greater than it would take for it to traverse a similar length of even the most slowly flowing river. Thus, the estuary acts as a nutrient trap, with an average nutrient level significantly higher than either the river or the sea that it connects. Likewise, the concentration of energy-rich organic materials remain high in estuaries.

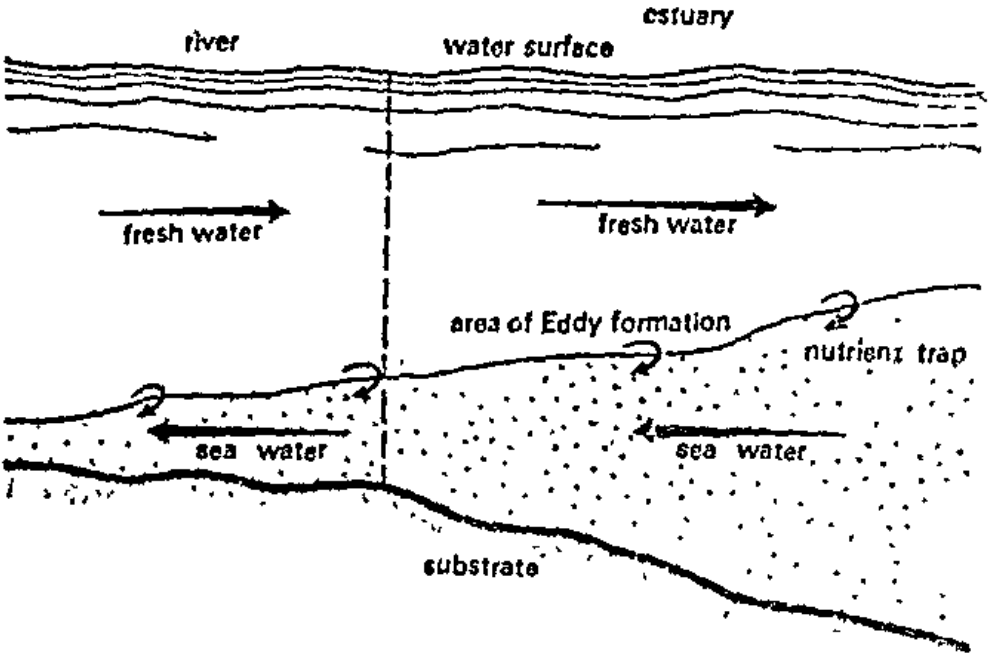


Fig. 9·1. Diagram showing formation of nutrient traps and horizontal turbulance or eddy.

The primary result of the concentration of nutrients and fixed carbon is a very high level of production within the detritus food chain. This has two aspects. First the nutrient material is broken down bacterially at a very high rate, and recycled back into soluble form. This allows a very high rate of gross primary production for estuarine plants. More important, perhaps, the amount of nutrient-rich organic detritus allows a level of productivity for detritus-eating animals much higher than could be maintained on the basis of the primary production by estuarine plants

BIOTIC COMMUNITIES OF ESTUARIES

Carikker (1967) has classified the regions of estuaries into the **head**, where freshwater enters the estuary, **upper, middle** and **lower reaches** with increasing range of salinities and the mouth with salinity nearly equal to the sea. He has also classified the animals inhabiting the estuarine region into 'oligohaline (0·5 to 5 per cent),

mesohaline (5 to 18 per cent) in the upper reaches **polyhaline** (18 to 25 per cent) in the lower reaches, 25 to 30 per cent in the middle and **euhaline** (30 to 50 per cent) in the mouth of the estuary. **Krishnamoorthy** (1963) in fact reported that the extent of penetration of the polychaets *Marphysa gravelyi*, *Diopatra uariabilis*, *Clymene insecta*, *Loimia medusa*, *Glycera embranchiata*, *Onuphis eremita* into the Adyar estuary in Madras from the Bay of Bengal vary with varying salinities at constant temperatures.

Furthermore a estuary being a transitional zone between the freshwaters and seas is an ecotone and, therefore, typical estuarine forms are unique in their habitat. Thus, the estuarine community is a mixture of three components : the marine, the fresh water and the brackish water. However, the diversity of both fresh-water and marine components reaches a maximum. Total diversity of the estuarine community is lower than that of either of the more normal environments. The number of interactions between species is not high and some estuarine populations may fluctuate greatly in size. The plants of the estuary are of four basic sorts : phytoplankton, marginal and marsh vegetation, mud-flat algae, and epiphytic plants-growing on the marginal marsh vegetation. Because of the turbid water found in estuaries, phytoplankton are normally uncommon. However, in Hooghly-Matla estuarine system in west Bengal, **Gopalakrishnan** (1971) has reported an abundance of phytoplanktonic forms—several species of diatoms, *Synedra*, *Navicula*, *Rhizostoma*, *Fragilaria*, *Asterionella*, *Biddulphia*, *Planktoniella*, *Hemidiscus*, *Chaetoceros*, *Cyclotella*, *Stephanodiscus*, *Triceratium*; several species of green algae like *Pediastrum*, *Spirogyra*, *Eudorina*, *Tribonema*, *Closterium*, *Zygonema*, *Pandorina*, *Volvox*, *Chlorella*; and blue-green algae like *Microcystis*, *Oscillatoria*, *Anabaena*, and *Trichodesmus*. Most estuarine algae are of marine origin. Further, the most significant estuarine plants are marsh grasses such as *Spartina*, *Salicornia*, and *Scirpus*, as well as some submerged filamentous algae such as *Cladophora*, *Chara* and *Enteromorpha*. Very few animals feed on these plants directly, but a very large amount is consumed as detritus.

The estuarine animal communities include zooplanktons and other animals For example, the planktonic animal forms of Hoogly-Matla estuary of India include flagellate protozoans such as *Euglena*, [illegible] such as *Difflugia*, and *Paracalanus*; cladocerans of the genus *Bosmina*, *Bosmi[illegible]*

Ceriodaphnia, *Moina*, besides a number of isopods (Gopalakrishnan, 1971). All these forms remain confined to water and lead a pelagic existence. The best known eustarine animals are detritus feeders like oysters (*Ostrea* sp. of Lake Chilka), clams, lobsters, and crabs. Several insect larvae, annelid worms, molluscs, enter the estuary from the fresh-water, while, most marine phytoplankton, Crustacea, annelid worms, anemones and Bryozoa enter a estuary from marine ecosystems. For example, out of 130 species of fishes and 30 species of prawns of Hoogly-Matla estuary, several fishes such as *Hilsa*, *Harpodon*, *Mugil*, *Trachyurus*, etc., migrate from sea in to the mouth of the estuary, to form important fish catches, while some like palaemonid prawns are fresh-water inhabitants and come to live in estuaries.

Adyar estuary of Madras coast is found rich in invertebrate and vertebrate fauna (Aiyar and Panikkar, 1937). The common invertebrates of this estuary are sea anemones (*e.g.*, *Phytocoetes gangeticus*, *Phytocoeteopsis ramunni*, *Stephensonaetis ornata*, *Pelocoetes exul*, *Bolocractus gopalai*, etc.,) ; hermaphrodite nereids (*e.g.*, *Lycastis indica*) : the tubicolous polychaet *Diopatra variabilis* ; the burrowing polychaet *Marphysa gravelyi*, the oligochaet *Pontodrilus bermudensis* ; the mysids *Rhopalophthalmus egregius* and *Mesopodopsis orientalis* ; the crabs *Uca annulipes*, *Uca triangularis*, *Neptunus pelagicus*, *Scylla serrata*, *Varuna litterata*, *Sesarma tetragonum* ; the hermit crabs *Clibonarius olivaceus* and *Clibanarius padavensis*, and several molluscs (*e.g.* *Ostraea arkanensis*, *Modiolus undulatus*, *Meretrix casta*, *Cuspidaria cochinensis*, *Stenothyra blanfordiana*, *Amnicola stenothyroides*, *Potamides cingulatus*, *Stylger gopalai* and *Bursatella leachii*) The vertebrate fauna of Adyar estuary includes the fishes like *Etroplus maculatus*, *Etroplus suratensis*, *Acentrogobius viridipunctatus*, *Acentrogobius neilli*, *Panchax parvus*, *Aplocheilus melastigma*, and mud skipper *Periophthalmus koelreuteri* and snakes such as *Natrix piscator* and *Cerberus rhyncops*

In short, the estuarine ecosystem is a complex and interesting one. It is also very vulnerable environment, because it has served as conduits for shipping and as sites for cities throughout the history of man. The organisms that inhabit an estuary are adapted to a vastly changing environment, but because estuaries are strategically located, man-made alterations to their ecosystems are substantially greater than in almost any other ecosystem.

MARINE ECOSYSTEMS

The marine environment of seas and oceans is large, ing 70 per cent of the earth's surface. The volume of

of marine environment lighted by the sun is small in comparison to the total volume of water involved. This and the dilute solutions of nutrients limits production. It is deep, in places nearly 4 miles. All the seas are interconnected by currents, dominated by waves, influenced by tides and characterized by saline waters. Not only the seashore and banks which are the homes of many organisms but the open ocean, many hundreds of miles away from land, supports plant and animal communities of great diversity and complexity.

PHYSICO-CHEMICAL ASPECTS OF MARINE ENVIRONMENT

In the marine environment, the most important physical factors which influence marine life are light, temperature, pressure, salinity, tides and currents.

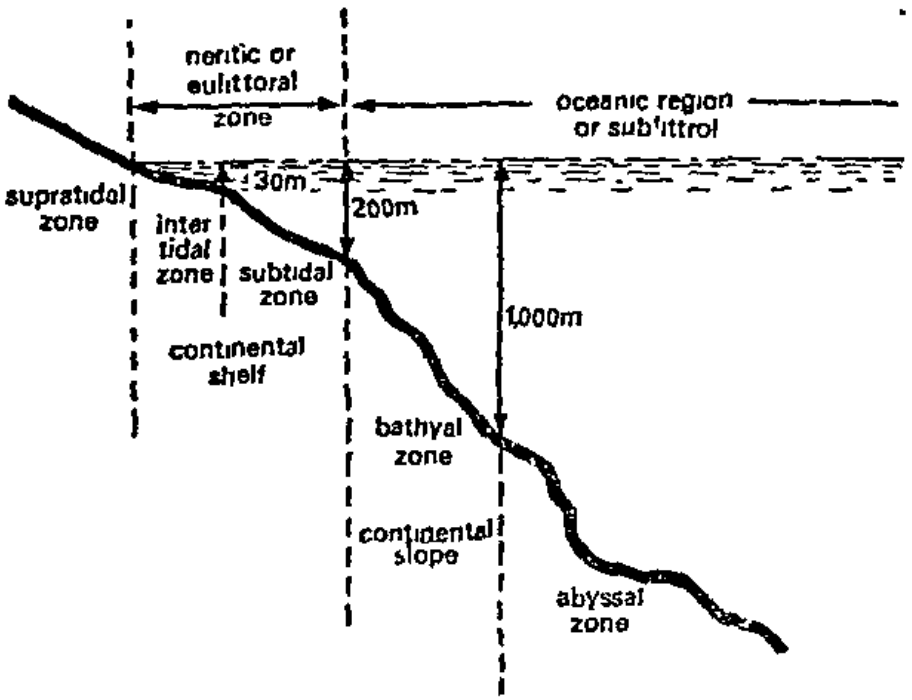
1. Light : Light is a very significant factor in regulating the pattern of distribution of marine animals and it contributes significantly to organic production. The autotrophic primary producers exploit the light energy in the photosynthetic production of food for primary macroconsumers of marine ecosystem. The amount of light exploited in photosynthesis depends on intensity of light and turbidity of water. For example, a ten meters deep turbid coastal zone receives an equal amount of light energy as a hundred meters deep clear oceanic zone, but the maximum intensity at a ten meters coastal water is greater. Light determines diurnal migrations of marine organisms, and it also regulate colour pattern of marine animals. The deep sea fauna which lives in total darkness exhibit either colourlessness or uniform colouration. It is also somehow related with the development of visual sense organs as shown by absence of functional eye in deep-sea animals.

2 Temperature : The range of temperature in sea is far less than that on land. Arctic waters at 27°F are much colder than tropical waters at 81°F, and currents are warmer or colder than the waters through which they flow. Seasonal and daily temperature changes are larger in coastal waters than on the open sea. The surface of coastal waters is the coolest at dawn and the warmest at dusk. In general, sea water is never more than 2° to3° below the freezing point of fresh water or higher than 81°F. At any given place the temperature of deep water is almost constant cold, below the freezing point of fresh-water. Unlike fresh-water, sea water does not have a density maximum at 4°C ; rather it becomes continuously denser as it gets colder.

3 Pressure : Pressure in the ocean varies from 1 atm at the surface to 1,000 atm at the greatest depth. Pressure changes are

many times greater in the sea than in terrestrial environments and have a pronounced effect on the distribution of life. Certain organisms are restricted to surface waters when the pressure is not so great, where as other organism are adapted to life at great depths. Some marine organisms, such as the sperm-whale and certain seals, can dive to great depths and return to the surface without difficulty.

4. Zonation of marine environment : Just as lake exhibit stratification and zonation, so do the seas. The ocean itself is divided into two main divisions, the **pelagic**, or whole body of water, and the **benthic**, or bottom region. The pelagic region is further divided into two provinces ; the **neritic**, water that overlies the continental shelf, and the **oceanic provinces**. The **continental shelf** is the underwater extension of the continent and it generally extends to a depth of roughly 125–200 m. The edge of the continental shelf may be within a few kilometers of the shore, or it may be seve-



Fig, 9·2. Different zones of sea.

ral hundred kilometers from shore. From the edge of the continental shelf there is a more rapid descent, the **continental slope**; to the broad flat **abyssal plain** that underlies most of the ocean at a depth of 4,000—5,000 m. On the abyssal plain, there are extensive mountain ranges, or **midoceanic ridges**, some of which have tips projecting above sea level. In addition, there are very deep troughs, which drop down below 11,000 m. below sea level. Life exists from the very top of sea to the bottom in virtually all areas, althe abundance is exceedingly variable.

The benthic life zones are defined in terms of these physical subdivisions. The **littoral**, or **intertidal** zone, is the zone between high tide and low tide levels. The **sublittoral** extends from the low tide mark to the edge of the continental shelf, with the **bathyal zone** comprising the continental slope. The **abyssal zone** includes the abyssal plains, and the **hadal zone** includes any life in the deep trenches below 5,000 m.

The pelagic zone is divided into three vertical layers or zones : from the surface to about 200 m is the **photic zone**, in which there is sharp gradients of illumination, temperature, and salinity. The region below this zone is called **aphotic zone**. From 200 to 1,000 m where very little light penetrates and where the temperature gradient is more even and gradual and without much seasonal variation, is the **mesopelagic zone** of aphotic zone. It contains an oxygen-minimum layer and often maximum concentrations of nitrate and phosphate. Below mesopelagic is the **bathypelagic zones**, where darkness is virtually complete except for bioluminescence, and where temperature is low and pressure is great,

5. **Stratification of marine environment :** The upper layers of ocean water exhibit a stratification of temperature and salinity. Depths below 300 m are usually thermally stable. In high and low latitudes temperatures remain fairly constant throughout the year. In middle latitudes temperatures vary with the season, associated with climatic changes. In summer the surface waters become warmer and lighter, forming a temporary seasonal thermocline. In subtropical regions the surface waters are constantly heated, developing a marked permanent thermocline. Between 500 and 1,500 m a permanent but relatively slight thermocline exists

Associated with a temperature gradient is a salinity gradient or **halocline**, especially at the higher latitudes. There the abundant precipitation reduce surface salinity and causes a marked change in salinity with depth. Thus in the middle latitudes in particular, the two produce a marked gradient in density. Water masses form density layers with increasing depth. Because density of seawater does increase in depth and does not reach its greatest density at 4°C as with fresh-water, there is no seasonal overturn. This results in a normally stable stratification of density known as the **pycnocline**. Because there are marked changes in temperatures with depth in the open ocean and thus with density, the pycnocline often coincides with the thermocline. Below the pycnocline is the deep zone which is oxygen rich, cold and comprises 80 per cent of the ocean.

6. **Salinity :** The marine animal life has specific osmoregulatory adaptation for high saline sea waters. The absence of many animal species in marine environment has been related to their inability to tolerate the high salt contents of sea water. Except few insects such a *Halobates* and shore collembolans like *Isotoma* and *Sminthurus*, most insects do not occur in marine ecosystems. The salinity of marine water fluctuates from place to place and it is caused due to the dissolved salts such as chlorides of sodium, potassium, calcium and magnesium, and also the sulphates of calcium and magnesium.

7. **Currents and tides :** Sea water is never static and waves, currents, and tides are the regular features of sea water. All these phenomena are controlled by winds, cosmic forces and varying water densities. Most world-wide water currents occur at the surface and at great depths of sea water. These currents determine the interchange between the surficial and deep water masses, as well as horizontal movements. Both the horizontal and vertical movements of ocean water are significant, but for different reason. Within a 'given current, such as warm Gulf stream, for instance, the water mass retains its identity for great distances, and the warm-sea community ranges far north of what have been anticipated as its northernmost limits. In addition, the climate of terrestrial ecosystems is strongly affected by the nature of adjacent water masses. The prevailing winds blow across the ocean and are heated or cooled by the waters they traverse. In marine ecosystems, however, the oceanic current system controls the distribution of productivity of marine communities through vertical mixing of water masses. Because most of the ocean is permanently stratified, any essential nutrient that settles out of the upper layers, either as an inorganic mineral or as organic detritus, is not returned to the upper layers at the same location. This had led to such an improverishment of the surficial waters of the oceans that productivity in most parts of the open oceans is about the same as that of desert, because of the very low concentrations of nutrients, especially phosphorus. At the same time, the lowest waters of the ocean are nutrient-rich. There are certain regions in the world, notably at the west coasts of continents and in subpolar latitudes in both hemispheres, where deep currents rise to the surface. These are called zones of upwelling, and even though their waters are very cold, they are sites of intense production because of their relatively high concentration of nutrients. Nutrients are then cycled back to the rest of the ocean via surface currents.

forms are the Forminifera and Radiolaria, arrow worms (*Sagitta*), certain annelid worms, swimming snails, jelly fish and most abundant of all the crustaceans such as shrimps, copepods and cladocerans. Among the temporary zooplankton are the larva of larger animals from all marine environments and even some fresh-water environments. For example, in the zooplanktons of inshore waters of Mandapam (South India) Prasad (1956) reported following animal species–

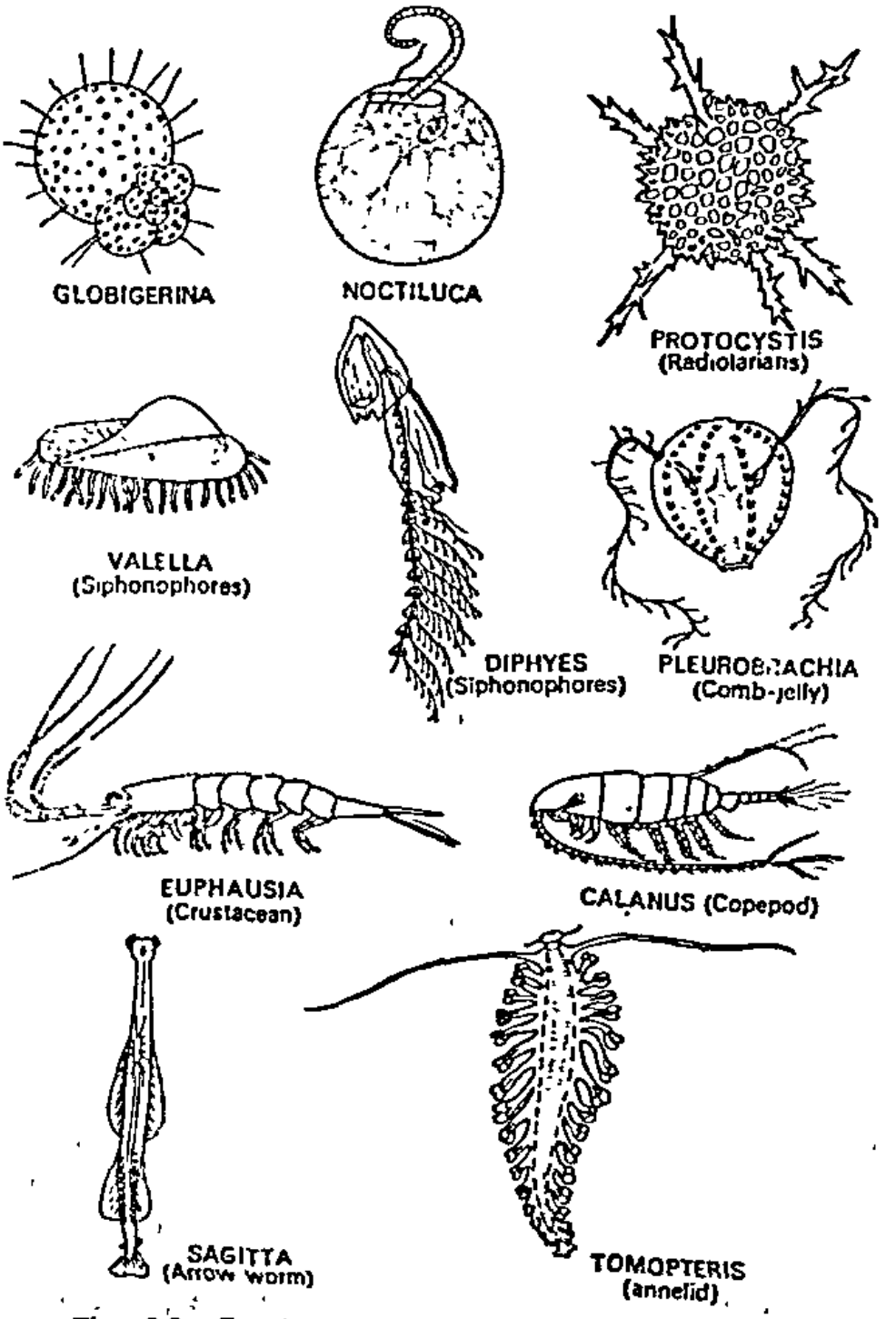


Fig 9-3. Certain holoplanktons of pelagic zooplanktons.

Tides are water movements which are caused by some astronomical factors. They represent a rhytmic rise and fall of water and often waves of long wave lengths characterize the process. The chief astronomic force behind the tidal rhytm is the attraction of the moon and sun depends on the movement of earth in relation to the moon and the sun. Tides influence the sea-shore fauna variously, as low tides expose the shore and high tides flood the substratum.

MARINE COMMUNITIES

The sea biota is not abundant but contains well marked diversity. Every major group of algae and almost every major group of animals can be found some where in the oceans. The only striking omissions are the vascular plants and insects, which are abundant in estuaries but which have few or no marine representatives. The marine biotic commuities can be studied separately for different life zones of the ocean as follows :

A. BIOTIC COMMUNITIES OF OCEANIC REGION

The oceanic region or pelagic zone is less rich in species and numbers than the coastal areas, but it has its characteristic species. Many of these are transparent or bluish and since the sediment-free water of the open sea is marvelously transparent, these animals are nearly invisible. Animals that are too thick to be transparent frequently have smooth shiny and silvery bodies which make them invisible by mirroring the water in which they swim. Further, the animals of the pelagic zone of ocean encounter stable and uniform environmental conditions due to the continunity of the waters of the sea. This continuity of sea waters causes the individuals of the open ocean to stay away temporarily or permanently from any solid objects, and they are provided with different locomoter organs. However, the planktonic forms lack a locomoter organ and float freely in the vast expanse of the sea.

1. Biotic communities of pelagic zone : The pelagic plankton also called **epiplankton** are exceedingly diverse. **Phytoplankton** includes diatoms and dinoflagellates, which together produce most of the organic carbon in the sea (and most of the oxygen in the atmosphere), as well as other forms of golden-brown algae and flagellated green algae. Some seaweeds, such as the large brown algae *Sargassum*, may have a floating stage. The **zooplankton** of pelagic zone include representatives of every major phylum and most minor phyla, either as permanent members of the plankton community **(holoplanktonic forms)** or as transients during their larval stages **(meroplanktonic forms)**. Common among permanent planktonic

forms are the Forminifera and Radiolaria, arrow worms (*Sagitta*), certain annelid worms, swimming snails, jelly fish and most abundant of all the crustaceans such as shrimps, copepods and cladocerans. Among the temporary zooplankton are the larva of larger animals from all marine environments and even some fresh-water environments. For example, in the zooplanktons of inshore waters of Mandapam (South India) Prasad (1956) reported following animal species–

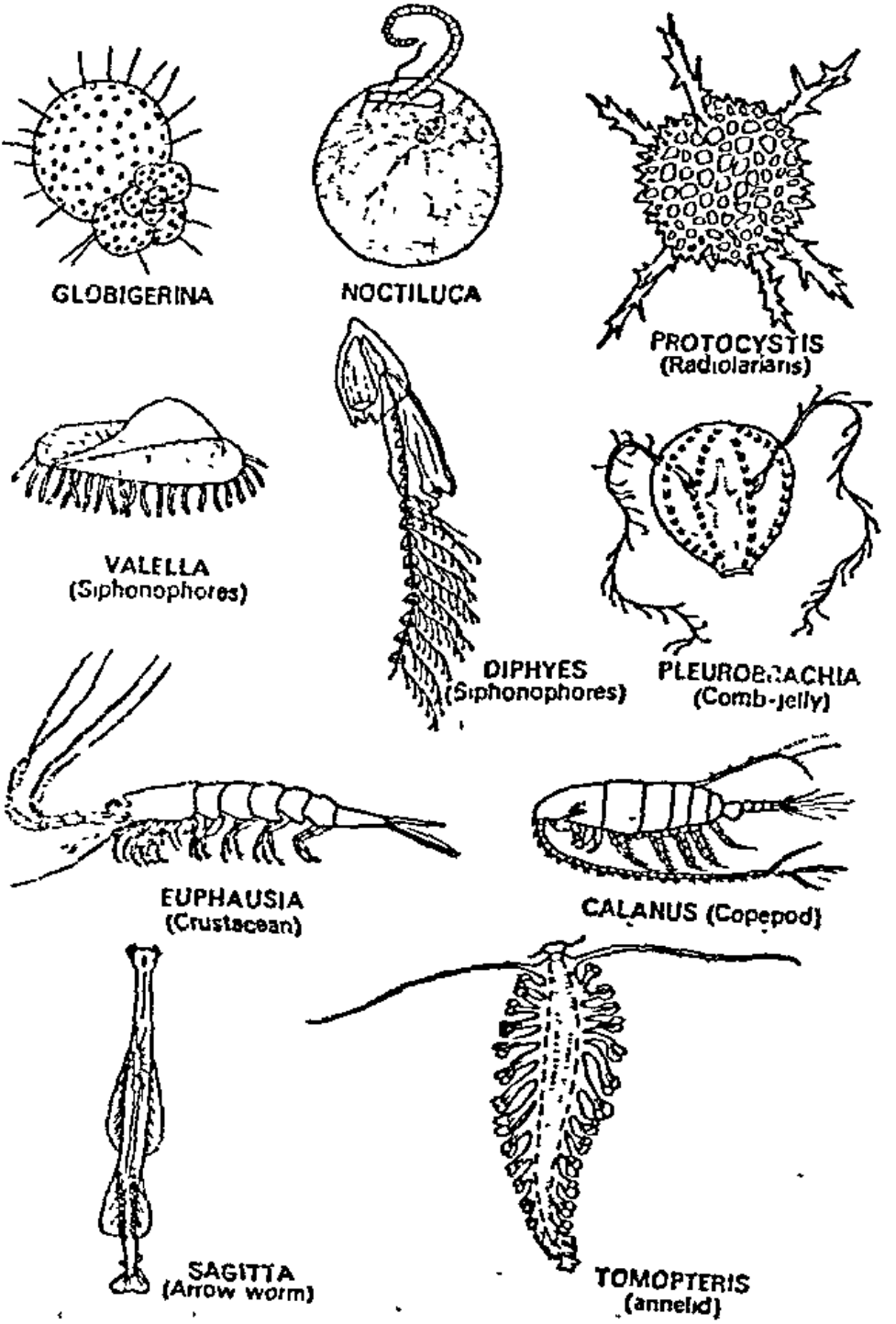


Fig 9·3. Certain holoplanktons of pelagic zooplanktons.

flagellate protozoan *Noctiluca miliaris* ; larval forms of coelenterates like *Planula, Ephyra, Semper's* larva, and *Cerianthus* larva ; medusae of certain coelenterates such as *Bougainvillia, Obelia, Charybdea, Rhizostoma* and *Rhopalina* ; ctenopheres like *Pleurobrachia* and *Beroe* ; larvae of many polychaet annelids and annelids such as *Tomopteris, Alciopa* and *Autolytus*, several species of chaetognaths like *Sagitta* and *Spadella* ; heteropod and pteropod molluses like *Carinaria, Pterotrachea, Cliona, Creseis*; larvae of echinoderms such as bipinnaria, ophiopluteus, echinopluteus, auricularia; tornaria of *Balanoglossus*; species of copepods like *Acartia, Calanopia, Labidocera, Centropages, Paracalanus, Canthocalanus, Acrocalanus, Eucalanus* and *Microsetella* ; larvae of decapod *Lucifer* ; protochordate species of Larvacea. Thaliaceae and Ascidiaceae such as *Thalia democratica, Jasis zonaria, Salpa cylindrica* ; appendicularian-*Oikopleura diocia, O. para* and *O. cephalocera* and also cephalochordate *Amphioxus.*

The largest animals in the pelagic region are the nekton. The include cephalopods such as saquid and nautili among the inverte rates, as well as many marine vertebrates, such as bony fishes, shar sea turtles and whales. In addition, sea birds also feed on many the same food types as these large carnivores. Air-breathing nekt such as the turtles, and whales, are found mainly in the photic zo but fishes extend from the sea surface to the bottom. Certain fi such as tuna, shark, sardine, mackerel, herring, bonito, and anch live near the surface. Those fishes which occur at greater de are often grotesque and unlike any fish found at the surface. T tend to be small—15 to 20 m is large for the ocean deeps they are exceedingly dispersed. Many have luminescent appen with which to lure prey, or mouth that look several sizes too for the rest of the body. Food is not plentiful in the deep w of the sea, so these fishes must go for long periods of time w food, and then consume as much as they can when they h chance.

(ii) Biotic communities of abyssal benthic zone : The benthic zone or deep sea of oceanic region is pitch dark and sal absence of light in this environment excludes the possi any growth of vegetation or other photosynthetic organism sequently, most of the deep sea dwellers depend upon the (dead body and excreta of surface forms) which sink to bottom while a large number of species are active predators ever the deep ocean benthos is surprisingly diverse. Food i more plentiful on the bottom than it is in the deep wate ocean, because the bottom sediments are the ultimate rest

for all detritus raining out of the upper layers (oceanic surface and pelagic zones). The bottom of the sea is a soft ooze, made of the organic remains and shells of Foraminifera, Radiolaria, and other animals and plants. Sea cucumbers, brittle stars, crinoids (sea lilies), sea urchins, certain benthic fishes, and several types of crustaceans, as well as sea anemones, clams, and similar animals are all found on

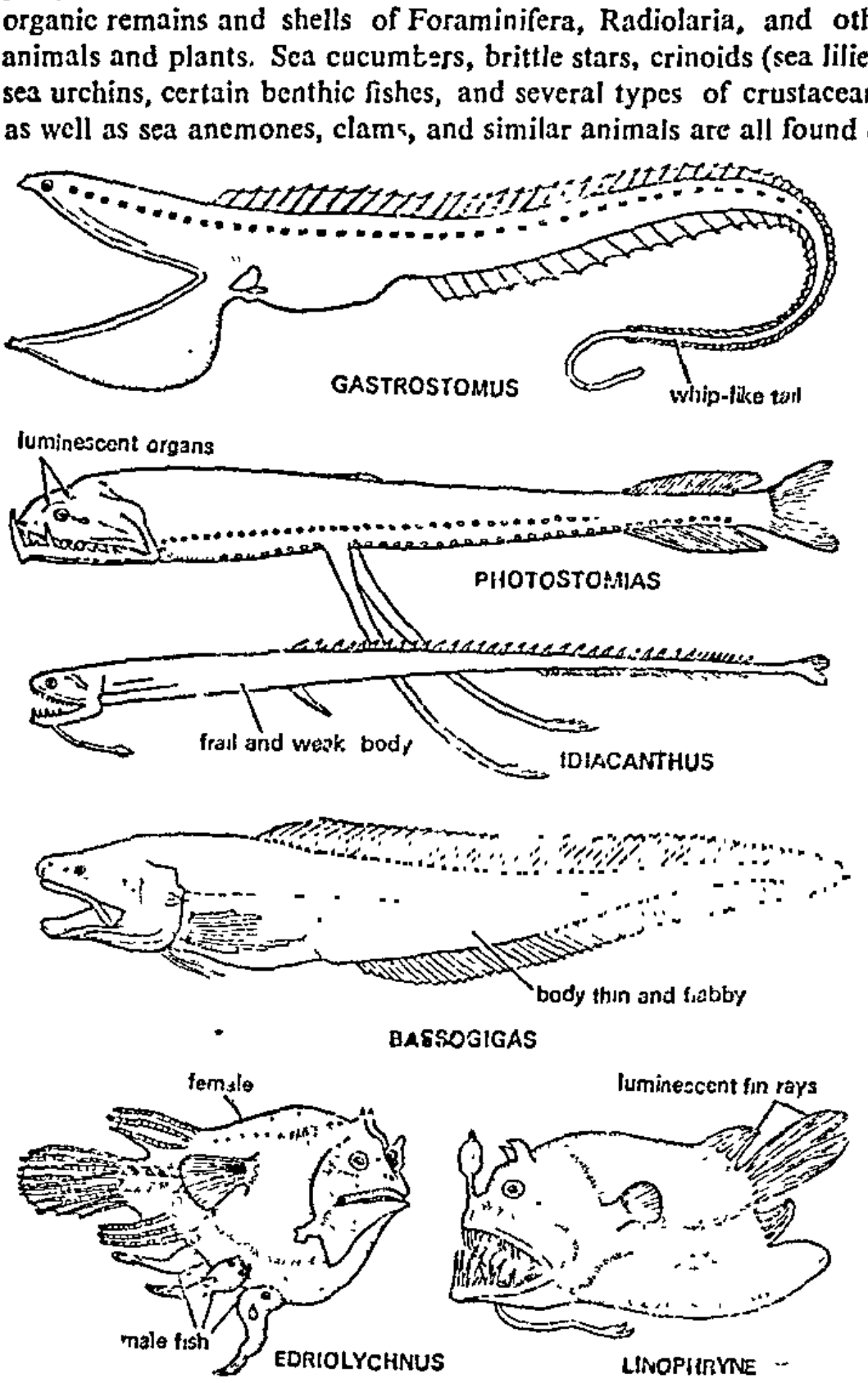


Fig 9·4. Fishes of the deep waters of the ocean.

the bottom, not only on the abyssal plains, but some even at the bottoms of the deepest ocean trenches. Many of them are detritus feeders but a large number of them are carnivores. In fact, the biomass of carnivorous brittle stars is often higher than that of the detritus feeders that serve as their food source ; however, their metabolic rate is much lower. The great diversity of sea bottom fauna has been related with great constancy in the physical environment of the deep sea.

B. BIOTIC COMMUNITIES OF CONTINENTAL SHELF

The communities of continental shelf are both richer and more diverse than those of open ocean. Diatoms and dinoflagellates are still the most productive phytoplankton, but in the shallower regions green, brown, and red algae anchored to the bottom may be of great importance. Several kinds of these seaweeds are harvested from rocky shores as human food or for some commercial purpose in eastern countries, USA and India. The zooplankton of continental shelf are generally the same as in the pelagic region, but some purely pelagic species have been replaced by neritic species, and the overall diversity is some what higher. The temporary zooplankton are much more abundant over the shelf region than in the open ocean.

The neckton of the neritic sea are both diverse and well known. The significant neckton species of the region are large squid, whales, seals, sea otters, and sea snakes. The most numerous necktonic forms, of course, are the fishes, which include many species of shark as well as herring-like species such as menhaden, herring, sardine and anchovy ; cods and their relatives, such as haddock and pollack; salmon and sea trout ; flounder and other flatfish such as sole, plaice and halibut ; and mackerels, including tuna and bonito.

Communities of sea shore or intertidal zone : The littoral, intertidal or eulittoral zone, is the region of sea shore which exists between the high and low tide lines. The region of the high tide mark is called **supratidal** or **supralittoral zone.** The region of low tide is called **subtidal zone. Davenport** (1903) has divided sea shore or intertidal zone into a **submerged zone** which is the portion of the shore below the low tide exposed by very low tides, a **lower beach** occupying the area between the mean low tide and mean high tide and an **upper beach** which is reached by the highest of high tides. The intertidal zone is the most variable zone in the entire sea. It is completely covered at high tide, and is completely uncovered at low tide except for tide pools. Any organism that is to survive in the intertidal must be either resistant to periodic dessication or able

to burrow to water level. For example intertidal polychaets escape desiccation by resorting to a tubicolous mode of life whereby they can resist desiccation by retaining sufficient moisture till the advent of the next wetting. Barnacles can survive desiccation by remaining in a state of suspended activity during dry periods. Many plants of sea shore contain jelly-like substances such as agar, which absorb large quantities of water and retain it while the tide is out. Further, the majority of shore animals utilize oxygen dissolved in water and when the low tide exposes them periods of drought, they utilize the oxygen stored in the blood plasma, *e.g.*, nereid like, *Arenicola*. Barnacles achieve this by enclosing on air bubble between the shells.

One of the outstanding characteristics of this region is the ever present action of the waves, and the organisms living on a sandy or rocky beach have lad to evolve ways of resisting wave action. The many seaweeds have tough pliable bodies, able to blend with the waves without breaking, while the animals either are encased in hard calcareous shells, such as those of mollusks, Bryozoa, starfish, barnacles and crabs, or are covered by a strong lethery skin that can bend without breaking, like that of the sea anemone and octopus. Many sedentary shore animals (*e.g.*, sponges, tunicates, etc.) have special modes of adhesion which keep them firmly attached to the substratum. However, animals of sandy beaches escape wave action by taking burrowing mode of life, because, these lack a hard substratum for the attachment of the animals.

The biota of intertidal zone also have to over come the wide fluctuations in salinity and temperature. Thus, like the estuary, this variable environment is a zone in which an organism must be adapted to a broad range of environmental conditions. Like the estuary also, it is an area of very high productivity with a simple community, many of whose members may be exceedingly abundent. The diversity of biota of intertidal zone is determined by nature of substratum whether loose sand or mud or rocky coast. Ecologists have recognized three major types of intertidal zones or seashores, namely, **rocky, sandy and muddy. Eltringham** (1971) has included a fourth type of sea shore, the **pabble shore** in this classification. Each type of sea shore possess specific biota each of which remain specificially adapted for its peculiar habitats. Here we will discuss only rocky shore and sandy beach habitats.

1. Biotic communities of rocky shore : The rocky shore presents solid substratum for the attachment of many sessile animals which often remain abundant here. The animals which are sedentary and inhabit rocky shore are limpets like *Patella, Haliotis, Fissurella,*

and oysters, barnacles, tunicates and bryozoans. Sessile organisms like the sponges, the colonial hydrozoans, anthozoans like *Gammaria* and *Zoanthus* attached to rocks. Certain animals such as some sponges, annelids and molluscs either bore the soft rocks like limestone or seek protection in the cervices of hard rocks. All these sedentary sessile animals are adopted for filter feeding.

Further, all these animals of rocky shore occurs in successive zones. At the uppermost end is a zone of bare rock marking the transition between land and sea. Next is a spray zone with dark patches of algae on which the periwinkles (*Littoria*) graze. Below this is the zone called barnacle zone regularly covered by the high tide ; rocks in this zone are encrusted with acorn barnacles, limpets and mussels (*e.g.*, *Mytilus*). This zone is a vulnerable zone being exposed to the brunt of wave action and active predation by starfishes and gastropods. The mussels act like hard substratum for tunicates, sponges and small muscles. The next one is the zone of oysesters is less vulnerable and includes green algae (*Enteromorpha* and *Ulva*) and barnacles. The next zone includes mussels, *i.e.*, boring bivalves, which remain within cervices with exposed siphons only to be protected from waves and predation, The chief species of borer bivalves are *Pholas, Martesia, Hiatella, Tridacna,* etc. Besides bivalves a variety of filter feeder bryozoans, and brachiopods and tubucolous polychaets like *serpulids, terebellids, sabellids* and *cirratulids* which live in tubes are the chief inhabitants of this zone. The subtidal zone includes red algae, brown algae (Laminarians and fucoids), sea anemone, sea urchin, corals, etc.

For example, the Okha coast of Gujrat in India is a typical rocky sea coast which has well defined fauna and flora (**Gopalakrishnan,** 1970). Dominant weeds of subtidal zone of Okha coast are *Sargassum, Dictyopteris, Gracilliaria, Padina, Ulva, Polysiphonia,* etc. This subtidal zone also contains sea urchins, serpulid tubes of species *Pomatoleius crosslandi*, and mollusc species like *Trochus radiatus, Cypraea arabica, Thais alveolata.* The upper limit of the dense weed growth of subtidal zone marks the lower end of eulittoral or intertidal zone, where occur a dense clusters of *Crassostrea cucullata* and *Littorina* sp. The intertidal zones includes three subzones, *i.e.,* the subzone of patchy weed growth ; 2. the subzone of limpets like *Sellana radiata* and barnacles ; and 3. the subzones of oysters and barnacles. The supratidal zone is inhabited by green algae and molluscs. Chlorophyceae like *Enteromorpha, Ulva, Bryopsis, Codium, Halimeda, Caulerpa, Dictyosphaeria* and *Cladophora, Phaeophyceae* like *Padina, Sargassum, Colpomenia, Iyengaria, Spathoglo-*

ssum, *Dictyopteris* and *Turbinaria ;* Rhodophyceae like *Gracilaria*, *Hypnea*, *Acanthophora*, *Polysiphonia*, *Cryptonemia* are the chief algae of the area. The common animals of this area are coelenterates species *Stoichactis*, *Ivalactis*, and *Gyrostoma* ; Nereid like *Eunice*, *Tubifex* ; molluscs like *Chiton*, *Nerita rumphii*, *Cypraea integerrimus*, *Pilumnus vespertilio*, seaweed grazer (nudibranch) molluscs, *Aplysia benedicti*, *Onchidium*, *Elysia grandifolia*, *Eolis*, *Doris* and floating mollusc *Janthina*, etc. The midlittoral reef community of Okha coast consists of fishes like *Epinephelus*, *Petrachus*; the crab *Pilumnus vespertilio*, the annelid *Eurythoe complanata* and molluscs like *Murex*, *Bursa*, etc. Rock cervices of intertidal zone harbour within them crabs like *Atergatis*, *Grapsus*, *Pilumnus*, *Matuta*, *Charybdea*, *Gelasimus* , squillids like *Sesarma* and *Gonodactylus* ; coelenterates like *Stoichactis*, *Ivalactis*, *Zoanthus*, *Lobophytum* ; the hydroid species like *Lytocarpus*, *Sertularia* and *Plumularia* ; and annelids like *Sepula*, *Polynoe*, *Eurythoe*, *Sabella*, etc.

(ii) Biotic communities of sandy shore : The sandy shore may be even more harsh than the rocky shore. It is subjected to all the extremes of the latter (temperature, salinity, turbidity, wave action, etc.) plus inconvenience of a constantly shifting substratum. The last makes life on the surface almost impossible ; life has retreated below the surface. Generally sandy beaches are characterized by gentler wave action. Because of the prolonged time taken for drying up, these beaches are suitable for animal life Decaying seaweeds and dead remains of animals result in the addition of organic matter to the sand. The coastal subsoil water is the environment of a special interstitial brackish water fauna. Most of the interstitial organisms are small, elongate, vermiform with transparent bodies. They have no eye but possess well developed adhesive organs and sense organs. They are negatively phototactic and gregarious in habit. They may be herbivore, carnivore, omnivore or detritus feeder. Large animal forms which are few in number prefer coarse sand grains and smaller one which very abundant, prefer finer sand. The principal animal groups of interstitial water are the Nematoda, Turbellaria, Annelida, Gastrotricha and Acarina Certain animal groups such as Protozoa, occur sporadically. The top 20 cm of sand between low and mid-tide levels of the beach is occupied by the inhabitants such as Hydrozoa, Turbellaria, Nemertina, Rotifera, Archiannelida, Polychaeta, Ostracoda, Halacaridae and Nudibranchiata. Species of Gastrotricha, Kinorhyncha, and Isopoda occur at mid-tide levels preferably in deep layers of sand. Oligochaets are sporadic and are found at different levels. Harpacteoid copepods remain restricted to mid tide level.

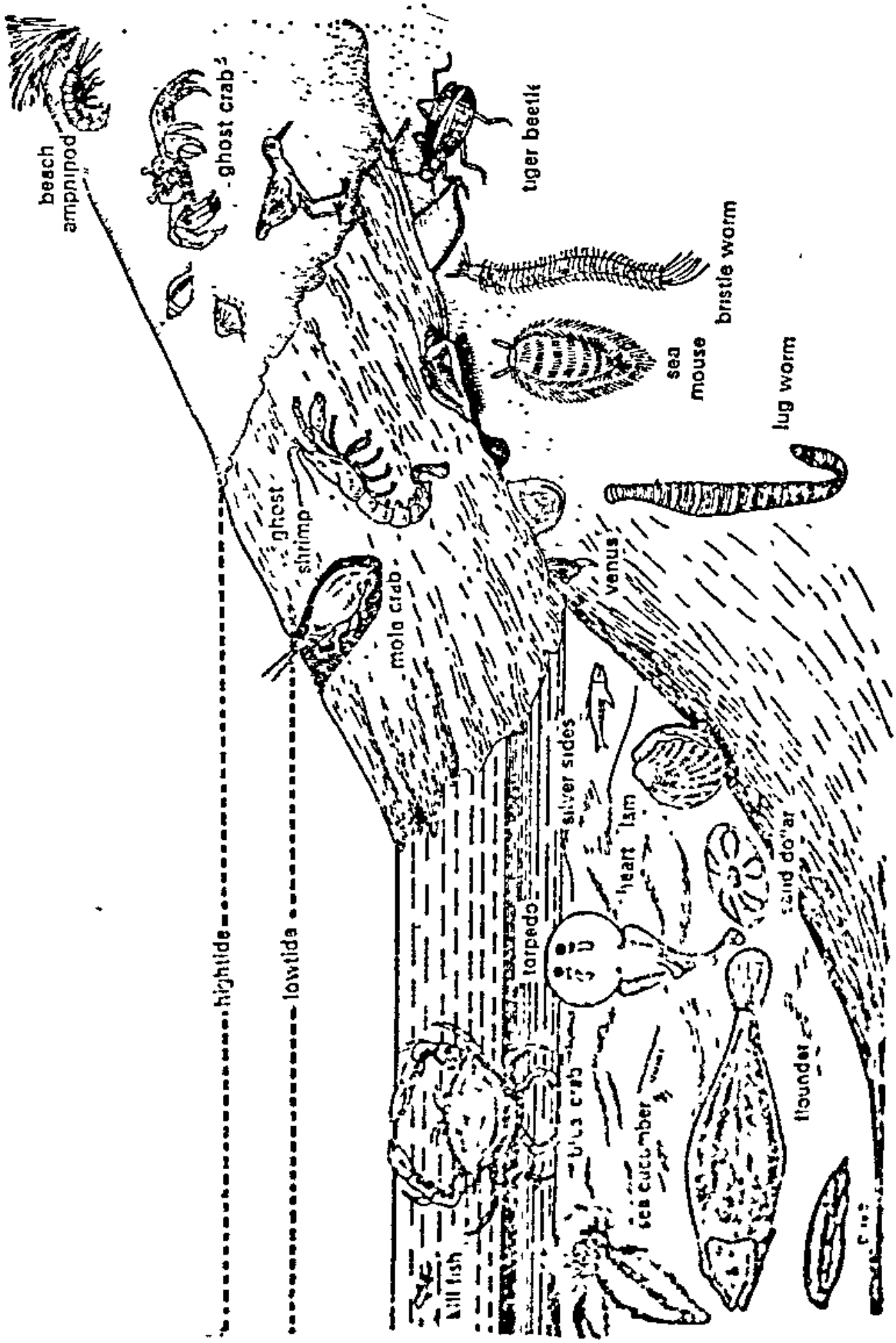


Fig. 9-5. Life on a sandy ocean beach along the Atlantic coast.

Tardigrades occupy the region between mid-tide and high-tide. Thus, sandy beaches also possess characteristic successive zones or zonations of animals.

For example, according to studies of Ganapathy and Rao (1959) sandy beach fauna of Visakhapatnam coast though lacks or contains small number of tubicolous polychaets and Crustacea due to very unstable substratum, but include following animal species—spionid *Prionospio krusadiensis*, *Aricia* and *Lumbriconeries* all of which possess the capacity to secrete mucus and formation of cover of sand grain over the body for protection, *Glycera lancadivae*, *G. alba*, *Nerina bonnieri*, *Pisionidens indica*, *Pistone complexa* and archiannelids *Saccocirrus minor* and *Protodrilus* also occur. The crustacean fauna of this zone includes *Emerita asiatica*, *Albunea symnista* (burrowing), amphipod species *Harpinia* sp., isopod *Sphaeroma walkeri*, mysid shrimp *Gastrosaccus spinifer*, hermit crabs *Clibanarius aretheusus* living within the shells of *Thais* and *Cerithium*, the eight-oared swimming crab *Matuta victor*, the spider crab, *Philyra scabriuscula*, *Ocypoda platytarsis* and *O. macrocera*. Lamellibranchs like *Donax cuneatus* and *Pamphia textile*, burrowing snails like *Simum neritoideum*, common gastropods like *Oliva gibbosa* and *Terebra* sp., *Bullia vittata* are also found along the coast of Visakhapatnam. This beach also contains eel *Ophichtys orientalis* and siphonophore coelenterates like *Physalia*, *Velella* and *Porpita*; and medusae like *Aequoria* and *Chiropsalmus*.

CORAL REEF AS A SPECIALIZED OCEANIC ECOSYSTEM

Coral reefs are the specialized ecosystems of ocean which are among the most productive of all ecosystems anywhere, with a diversity equalled only by tropical rainforests. The reef environment is very rugged one because it is constantly subject to the pounding of the waves, and parts of the reef are often above water at low tide. But reef is also a very rich environment for those species that can withstand the physical pounding of water. The amount of oxygen is very high. During the day, it may reach 250 per cent of saturation because of the production of oxygen by algae in the reef structure. Likewise, the tremandous amount of CO_2 produced by the metabolism can be carried off before it becomes too high.

Reefs can be found wherever a suitable substrate exists in the lighted waters of the tropics, away from areas of continental sediments which would tend to bury the reef in mud or cold upwellings which would tend to lower the temperature too much. They may form **barrier reefs** (*e.g.*, barrier reef in Australia which contains

canal or lagoon between them and coast) adjacent to continents; atolls or ring-shaped or horse-shoe-shaped islands on the top of submarine heights, or fringing reefs, as in Mandapam, Appa Islands and Hare Islands. In all modern reefs, the main reef structure is formed by madreporarian corals of following families-Poritidae (*Porites minicoiensis, Porites mannarensis*), Acroporitidae (*Acropora intermedia*), Astraeidae and Milliporidae. Secretions of calcareous algae go into the building up of reefs. Equally important are calcareous Bryozoa, lamellibranchs and gastropod molluscs and Foraminifera, which become significant elements in the total reef structure. Although the name 'coral reef' implies that corals should be the most abundant organism, algae of many sorts comprise over three times the biomass of the corals. In addition, the calcareous algae are not only producers ; their skeletons act to cement the entire reef to make it more resistant to the pounding surf.

Coral reef normally exist in water with mean temperature 20·5°C, but they can withstand very high temperatures. The growth of coral reef is influenced by salinity and reduced tides. Moderate water movement around the coral reefs help in the circulation of food.

BIOTIC COMMUNITIES OF CORAL REEF

Coral reef ecosystems harbour a abundant and well diverse biota. The well known Krusadi Island is the coral island of gulf of Manaar. It's dominant corals are *Porites, Montipora, Poecilopora, Acropora, Madrepora, Favia, Symphylia, Psammocora, Platygyra, Merulina* and *Leptastrea.* In the Krusadi area a luxuriant growth of sea weeds such as *Sargassum, Caulerpa, Turbinaria, Spatoglossum, Gracilaria, Ulva, Enteromorpha, Chaetomorpha* and *Polysiphonia.* The abundant sponges of this reef are *Spirastrella incorstans* and *Callyspongia fibrosa.* The other inhabitants of this reef are following —hydroid coelenterates like *Syncoryne, Sertularia, Eudendrium, Pennaria, Tubularia, Thyroscypus, Plumularia;* alcyonarians like *Clavularia, Alcyonium, Lobophytum, Sclerophytum, Teleste;* zooantharians like *Zoanthus, Gemmaria;* polychaet annelids like *Nereis, Polynoe, Eunice, Lepidonotus, Iphion, Eurythoe, Hesione;* sipunculid species like *Dendrostomum, Aspidosiphon* and *Phascolosoma;* molluscs like *Doris, Dendradoris, Acanthochiton, Ischnochiton, Haliotis, Emarginula, Trochus, Turbo, Nerita, Natica, Cypraea, Thais;* and echinoderms like *Holothuria atra, H. Scabra, H. edulis* and starfish *Proteaster lincki.*

Beautifully pigmented fishes like *Holocentrus, Lutianus* sp., *Pomacanthoides, Chaetodontopsis, Chaetodon, Linophora, Thallasoma*

and *Zanclus* and the eels *Gymnothorax undulatus* and *G. punctatus* are commonly frequent in the coral reefs. These inhabitants of coral reef community, when exposed to low tides are subjected to predation by large number of birds like the crested tern *Sterna bergii*, golden plover *Pluvialis dominica fulva*, etc.

According to Clapham, Jr. (1973) the great productivity of reefs is obviously caused by something more than the abundance of light and oxygen. He considers nutrient cycles which runs very quickly and efficiently between the components of reef ecosystem to be main cause of great productivity of reef ecosystems. In fact, no organic matter is incorporated permanently into the sedimentary system portion of reef. The conditions of rapid nutrient cycling allow a mechanism by which the natural paucity of nutrients in the ocean can be effectively circumvented It may also allow a gradual increase in nutrient availability by incorporating the nutrients of the non-reef organisms that wash into the reef, while still continuing to prevent the reef's own nutrients from being lost to the deeper seas.

CHAPTER 10

TERRESTRIAL ECOSYSTEMS

Only about a quarter of the earth's surface is dry land, yet the complexity and variegation of terrestrial ecosystems are much greater than those of aquatic ecosystems. There is no unifying theme in terrestrial ecosystems analogous to the physical properties of water. Rather, the variety of climates, the diversity of the lithosphere, and heterogeneity of terrestrial biotic communities all conspire to give rise to such a variety of themes that they are woven together in nature to give an incalculable diversity of biotic communities and terrestrial ecosystems.

PHYSICO-CHEMICAL NATURE OF TERRESTRIAL ECOSYSTEMS AND THEIR COMPARISON WITH AQUATIC ECOSYSTEMS

An aquatic system is essentially a **single-phase system**, where water sets the tone for entire habitat. A terrestrial ecosystem, on the contrary, is a **three-phase system**, where the characteristics of the habitat are a function of the atmosphere and climate, the soil, and the biotic community itself. The atmosphere is the source of oxygen for animals and carbon dioxide for plants. Both are sufficiently common and well-mixed in the atmosphere that they are essentially never limiting (For details see chapter 2, page 17–20). Air as a medium of support is much less buoyant than water. Thus, an organism need relatively little support to in it

by soil, rock, and vegetation, whose specific heat are much lower. A given quantity of heat energy absorbed by solid material can change its temperature two to five times as much as the same energy absorbed by water. Thus heat is gained and lost rapidly by terrestrial ecosystems, with a resultingly wide fluctuations of temperature, both diurnally and seasonally.

Table : Specific heats of several common substances. (Clapham, Jr., 1973).

1. Fresh water	1·00
2 Sea water	0·93
3. Wet mud	0·60
4. Moist sandy clay	0 33
5. Sol d rock	0 20
6. Copper	0 09

In terrestrial ecosystems, the soil serves two major functions—it provide support for living organisms, and it is the source for all essential nutrients except for carbon, oxygen, and hydrogen (For detail see chapter 2, Page 20–37). Even its supportive role is different from the way in which a lake sediment support rooted vegetation. Because the entire weight of all living organisms is borne by the soil, there is no partition of support between the soil and the air. The role of soil as a source of nutrients is unique to trrestrial environments. It is the site of the entire detritus food chain (see chapter 2, Page 38–42) and thus is central to the biogeochemical cycling of nutrient materials. Different types of soils have different properties that affect the availability of nutrients to plants, so the productivity of the terrestrial ecosystems is very closely tied to the chemistry of of the soil. Further, in terrestrial biotic communities interactions between species are real but they are simple, involving relationships like predation, mutualims, parasitism and the like. In contrast to aquatic ecosystems, living organisms of terrestrial ecosystems leave a permanent mark on the system. For example, the role of plants in breaking down and weathering rock and building soil is much more pronounced than it is in almost all aquatic ecosystems. In addition, the succession that takes place in a terrestrial community is almost entirely a function of the organisms within the ecosystem, unlike aquatic successions such as lake eutrophication, which are dependent in large part on materials being washed in from out side.

Lastly, in each of these two ecosystems, namely aquatic and terrestrial ecosystems, several types of major subdivisions c

recognized : thus one can distinguish freshwater, estuárine and marine aquatic ecosystems, and several major types of terrestrial ecosystems such as prairies, forests, tundra, etc. The former are distinguished on the basis of a major chemical difference (*i.e.*, salt content), the latter generally on the basis of the predominant type of vegetation (grass, tree, etc.) (see **Kormondy, 1976**).

CLASSIFICATION OF TERRESTRIAL ECOSYSTEMS

The earth surface—the continental land masses have been classified by biogeographers and ecologists into following regions :

(i) Biogeographic realms.

(ii) Biomes.

(I) BIOGEOGRAPHIC REALMS OR REGIONS

Careful studies of the distribution of plants and animals over the earth have revealed the existence of six major biogeographic realms, namely **Palaearctic realm, Nearctic realm, Neotropical realm, Ethiopian realm, Oriental realm** and **Australian realm,** each characterized by the presence of certain unique organisms. Each of these realms embraces a major continental land mass and each remains separated by oceans, mountain ranges, or desert.

(II) BIOMES

Within these biogeographic realms and established by a complex interaction of climate, other physical factors and biotic factors, are large community units called biomes. A biome is a large community unit characterized by the kinds of plants and animals present This may be contrasted to the ecosystem, which is a natural unit of living and non-living components that interact to form a stable system in which the exchange of materials follows a circular path. Thus, an ecosystem might be a small pond or a large area coextensive with a biome, but it includes the physical environment as well as populations of animals, plants and micro-organisms.

In each biome the kind of climax vegetation is uniform—grasses, conifers, deciduous trees—but the particular species of plant may vary in different parts of the biome. The kind of climax vegetation depends upon the physical environment and the two together determine the kind of animals present. The definition of biome includes not only the actual climax community of a region, but also the several intermediate communities that precede the climax community. Further, there is usually no sharp line of demarcation between adjacent biomes ; instead each blends with the next through a fairly broad transition region termed an ecotone. There is, for

example, an extensive region in Northern Canada where the tundra and coniferous forests blend in the tundra-coniferous ecotone. Some of the biomes recognized by ecologists are **Tundra biome, alpine biome, forest biome, grassland biome, desert biome.**

1. TUNDRA BIOME

Tundra presents the most common example of "fragile ecosystem" (**Clapham, Jr., 1973**). Tundra which means "marshy plain", lies largely north of latitude 60°N (*i.e., between the Arctic ocean and* polar ice-caps and the forests to the south) and is characterized by the absence of trees, the presence of dwarfed plants, and an upper ground surface that is wet, spongy and uneven, or hummocky, as a result of freezing and thawing of this poorly drained land (**Kormondy, 1976**). Some five million acres of tundra stretch across Northern America, Northern Europe and Siberia. Although there is variation from place to place within the biome, temperature, precipitation, and evaporation are characteristically low, the warmest months averaging below 10°C and the wettest with about 25mm of precipitation. Despite the small amount of precipitation, water is usually not a limiting factor because the rate of evaporation is also very low.

The ground usually remains frozen except for the uppermost 10 to 20 cm, which thaw during the brief summer seasons. The *permanently frozen deeper soil layer* is called **permafrost.** The permafrost line which may exist at a depth of a few centimeters to several meters, is the ultimate limit of plant root growth, but the immediate control is the depth to which soil is thawed in summer. The rather thin carpet of vegetation of tundra biome includes few species : grasses and sedges are characteristic of numerous marshes and poorly drained areas, but large areas consist of ericaceous heat plants (bilberries and dwarf huckleberries), low flowering herbs, and lichens. Perhaps the most characteristic arctic tundra plant is the lichen known as "reindeer moss" (*Cladonia*). The animals that have adapted to survive in the tundra are caribou or reindeer, the arctic hare, arctic fox, polar bear, wolves, lemmings, snowy owls, ptarmigans and during the summer, swarms of flies, mosquitoes and a host of migratory birds.

Further, certain animals such as caribou and reindeer are highly migratory because there is not enough vegetation produced in any one local area to support them. Though tundras are barren areas yet a surprisingly large number of organisms have become adapted to survive the cold. During the long daylight hours of the brief

summer, the rate of primary production is quite high. The production from the vegetation on the land, from the plants in the many shallow ponds that dot the landscape and from the phytoplankton in the adjacent Arctic Ocean provides enough food to support a variety of residant mammals and many kinds of migratory birds and insects. Diversity is very low, and despite the extreme hardiness of the tundra organisms, their growth rate tend to be very low, and the community as a structural unit is exceedingly vulnerable

Moreover, Alpine tundra is quite similar to some of Arctic tundra, but in the absence of permafrost and in the growing season, mosses and lichens are less prominent, flowering plants more so.

2. HIGH ALTITUDE OR THE ALPINE BIOME

The region of mountain above the timber line contains a distinct flora and fauna and is referred as the **alpine zone.** Alpine zone remains conspicuous in those mountains whose peaks reach up to the niveal or snow zone, as in the Himalayas. The alpine zone (zone which lies between timber line and snow zone) includes in the descending order, a **sub-snow zone** immediately below the snow zone, a **meadow zone** in the centre and a **shrub zone** which gradually merges into the timber zone. According to Mani (1968) snow zone of Himalayas lies over 5100m above mean sea level and alpine zone exists at a height of 3600m. From an ecological view point, the zone above the limits of tree growth (timber line) exhibits extreme environmental conditions which greatly influence the biota of this region.

The characteristic features of high altitude environment are following—a low air density, low oxygen and carbon dioxide contents and water vapour, high ozone content, high atmospheric transparency affording greater penetration of light, cold, snow cover, increased rate of desiccation, high wind velocity, insolation of high intensity during the day ard rapid radiation during the night, the high glare from the sky and snow, high intensity of ionizing radiation and the exclusion of trees. Alpine zone of Himalayas is characterized by a sparseness of animals groups, the scarcity being relatively important as far as tropical Indian, South Chinese, Indo-Chinese and Malayan derivatives are concerned. Important constituents of the fauna of this zone are cold-adapted palaearctic forms (*Mani, 1968*).

Many invertebrates of alpine zone are predatory and occur in lakes, streams and ponds Among vertebrates fishes and amphibians are totally lacking and reptilian fauna is greatly impoverished.

However, pelobatid frog, *Aleurophryne mammata* is found to occur at 4500 meters of height in Himalayas and viviparous *Mabuya varia* has been reported to live at 4000 meters of height in Mount Kilimanjaro. Some of the representative vertebrates of Himalayan alpine zone are—crow *Corvus corax* ; snow partridge *Lerwa nivicola* ; snow leopard *Felis unica* and *Felis lynx* ; Eropean leech marten *Mustela foina* ; the vole *Lagomys* ; Tibetan yak, *Bos grunnius* ; Tibetan sheep *Ovis hodgsoni* ; pamir sheep *Ovis poli* ; ibex *Capra sibirica*; markhor *Capra falconeri* ; and Persian wild goat *Capra aegagrus*.

A large number of insects and arachnids remain best adapted to Himalayan alpine zone and at 6900 meters height. The torrential streams which are the products of melting snow contain many mayflies, stoneflies and caddisflies. Some of common insects of this zone are—stonefly *Rhabdiopteryx lunata* ; grassophers like *Bryodema* and *Gomphomastax* ; apterous species *Conophyma* ; *Dicranophyma* ; *Spingonotus* ; tettigonid *Hypsinomus fasciata* ; earwigs like *Anechura* ; bugs like *Dolmacoris, Tibetocoris*, and *Nysius ericae* ; aquatic beetle *Amphizoa* ; carabids like *Bembidion, Carabus Calosoma, Harpalus, Trechus, Calathus, Bradytus, Broscus*, and *Dyschirius* ; staphylinids like *Atheta, Aleochara, Geodromicus, Oxypoda, Philonthus* and *Tachinus* ; tenebrionids like *Bioramix* and *Chianalus* ; ants like *Formica picea* ; bumble bees like *Lapidariobombus separandus* ; butterflies like *Colias, Argynnis* and *Parnassius* ; the fly *Deuterophlebia* ; collembolans like *Entomobrya, Isotoma, Proistoma, Hypogastrura, Isotomurus, Tomocerus* and *Onychiurus* ; and numerous simulids blepharocerids, syrphids, anthomyids tachinids and sarcophagids, (Ananthakrishnan and Visvanathan, 1976).

3. FOREST BIOMES

The word forest is derived from Latin *'foris'* meaning outside, the reference being to village boundary or fence, and must have included all uncultivated and unihabited land. Today a forest is any land managed for the diverse purposes of forestry whether covered with trees ,shrubs, climbers, etc., or not (see S.P. Sagreiya, 1967). The forest biomes include a complex assemblage of different kinds of biotic communities. Optimum conditions of temperature and ground moisture responsible for the growth of trees contribute greatly to the establishment of forest communities. In addition, 50 mm rainfall is a pre-requisite for the growth of trees. The nature of soil, wind and air currents determines the distribution (abundance or sparseness) of forest vegetation. Normally ecologists recognise among forest communities such features as their evergreen nature,

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of Himalayas lies over 5100m above mean sea level and alpi
exists at a height of 3600m. From an ecological view point, t
above the limits of tree growth (timber line) exhibits e
environmental conditions which greatly influence the biota
region.

The characteristic features of high altitude environm
following—a low air density, low oxygen and carbon
contents and water vapour, high ozone content, high atmo
transparency affording greater penetration of light, cold, snow
increased rate of desiccation, high wind velocity, insolation
intensity during the day and rapid radiation during the ni
high glare from the sky and snow, high intensity of ionizing ra
and the exclusion of trees. Alpine zone of Himalayas is charac
by a sparseness of animals groups, the scarcity being re
important as far as tropical Indian, South Chinese, Indo-C
and Malayan derivatives are concerned. Important constitu
the fauna of this zone are cold-adapted palaearctic forms
1968).

Many invertebrates of alpine zone are predatory and o
lakes, streams and ponds Among vertebrates fishes and amp
are totally lacking and reptilian fauna is greatly impove

(ii) Temperate deciduous forest : The temperate forest biomes are characterized by a moderate climate and broad-leaved deciduous trees, which shed their leaves in fall, are bare over winter and grow new foliage in the spring. These forests are the characteristics of north America, Europe, Eastern Asia, Chile, part of Australia and Japan, with a cold winter and a annual rainfall of 75—150 cm and a temperature of 10—20°C. In these biomes the precipitation may be fairly uniform throughout the year. In India, at elevations of 9000—12,000' in Himalayas occur temperate vegetation including pines, fir, yew and juniper trees with an undergrowth of scrubby rhododendrons.

Soils of temperate forests are podozolic and fairly deep. Trees are quite tall—about 40—50m in height—and their leaves are thin and broad. The predominant genera of this biome are maple (*Acer*), beech (*Fagus)*, oak *(Quercus)*, hickory (*Carya*), basswood *(Tilia)*, Chestnut *(Castnea)*, cottonwood *(Populas)*, sycamore *(Platanus)*, elm *(Ulnus)*and willow *(Salix)*. In some locations, coniferous vegetation may be quite predominant and that includes white pine (*Pinus strubos)*, hemlock *(Tsuga canadensis)* and red cedar *(Juniperus virginianus*). There are not many epiphytes or lianas save for some species of mosses, algae and lichens growing on tree trunks,and a few vines, notably *Vitis*, the grape. The understory of shrubs and herbs in the deciduous forest is typically well-developed and richly diversified, with a coniderable portion of the photosynthesis and flowering attuned to the short days of the spring season, prior to the leafing out of and consequent shading by the tree canopy. Accordingly, there are often two separate herb assemblages. One consists of spring flowers, which bloom before the trees have expanded their leaves and are gone by summer, and the other is adapted to the low light levels of the forest floor and lasts into the fall.

The animals orginally present in temprate forests were deer, bears, squirrels, gray foxes, bobcats, wild turkey and wood peckers. Other common animals of this region are invertebrates like earthworms, snails, millipedes, Coleoptera and Orthoptera and vertebrates like amphibians such as newts, salamanders, toads, and cricket frogs; reptiles such as turtles, lizards and snakes ; mammals such as racoon opossum, pigs, mountain lion, etc., and birds like horned owl, hawks, etc. All these animals and plants show a profound seasonality; some may even hibernate throughout the winter. The range of animal size and adaptations is wide ; the largest animals include such forms as the deer and black bear. The dominant carnivores are large, including the wolf and mountain lion, although smaller carnivores such as fox and skunk are also common. Diversity of fauna is lower than in

whether deciduous or indeciduous, as well as their shape, whether broad- leaved as in temperate forests or more needle-like as in the conifers. On the basis of these features the forest biomes of the world have been classified into following biomes—**coniferous forest, tropical forest, and temperate forest.** All these forest biomes are generally arranged on a gradient from north to south or from high altitude to lower altitude.

(i) Coniferous forest : Cold regions with high rainfall and strongly seasonal climates with long winters and fairly short summers are characterized by boreal conifer forest which is transcontinental. For example, adjacent to the tundra region either at latitude or high altitude is the **northern coniferous forest,** which stretches across both North America and Eurasia just south of the tundra (*i.e.*, Canada, Sweden, Finland, Siberia and Mussoorie). The term **Taiga** is applied to the northern range of coniferous forests. This is characterized by evergreen plant species such as spruce (*Picea*, etc.), spruce (*Picea, glauca*), fir (*Abies balsamea*) and pine trees (*Pinus resinosa, Pinus strobus*) and by animals such as snow shoe hare, the lynx, the wolf, bears, red fox, porcupines, squirrels, amphibians like *Hyla* and *Rana*, etc. Large size is common, both in the trees, which range up to some 40 m in height, and in vertebrates, which include the giants of several groups of animals, such as moose, caribou, elk, grizzly bear, wolverine, beaver, and several large species of birds. Species diversity is low, and pure stands of trees and shrubs are common, as are outbreaks of defoliating insects of several sorts (*e.g.*, bark beetles, saw flies, geometrid moths, etc.). Understory trees are uncommon as a result of the continual low light penetration. Among common understory associates are orchids and ericaceous shrubs like the blue berry. The thalloid mosses and lichens being very rich understory vegetation.

Boreal forest soils are thin podozols, and are rather poor both because the weathering of rock proceeds slowly in the cold environments, and because the litter derived from conifer needle is broken down very slowly and is not particulary rich in nutrients. These soils are acidic and mineral deficient, the result of the movement of a large amount of water through the soil; in the absence of a significant counter upward movement of evaporation, soluble essential nutrients like calcium, nitrogen, and potassium are leached sometimes beyond the reach of roots thereby leaving no alkaline-oriented cations to encounter the organic acids of the accumulating litter (Kormondy, 1976). The productivity and community stability of a boreal conferous forest are lower than those of any other biome.

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any of the rain forests and a few species seems clearly to be dominant.

(iii) **Temperate evergreen woodland :** Many parts of the world have a Mediterranean-type climate with warm, dry summers and cool, moist winters. These are commonly inhabited by low evergreen trees with small hard needles or slightly broader leaves. The most important area of tropical evergreen woodland in North America is the **'Chaparral'** of the Pacific Coast, the Mediterranean **'maquis'**, Spanish **'encinar'** and **'melle scrab'** on Australia's South Coast are the same type of community. In such a woodland, trees are essentially lacking, although shrubs may range up to 3—4 m in height. Species diversity is roughly intermediate between that of a temperate deciduous forest and a drier grassland. Fire is an important factor in this ecosystem, and the adaptations of the plants enable them to regenerate quickly after being burned. The characreristic animals of temperate evergreen woodland or chaparral are mule deer, brush rabbits, wood rats, chipmunks, lizards, wem-tits and brown towhees. Small-hooved cursorial ungulates are the dominant herbivores. Saltatorial (jumping) animals and many fast moving ungulates are also common in this fauna.

(iv) **Temperate rainforests :** The temperate rainforest is a colder ecosystem than any other rainforest. Such a forest has a definite seasonality, with both temperatures and rainfall varying throughout the year. Rainfall is high, but fog may be very heavy and actually more important as a source of water than rainfall. The diversity is much lower, both in plants and animals, in comparison to warmer rainforests, yet it remain still higher than other temperate forest types. The dominant trees (canopies) are coast redwood (*Sequoia sempervirens*) of the Pacific coast of North America and the alpine ash (*Eucalyptus regnans*) of Australia and Tasmania, both of which reach more than 100 m in height Epiphytes and lianas are common but are not abundant like those of other rainforests. The animals of temperate rainforests are similar to those of deciduous forests, but show a somewhat higher diversity.

(v) **Tropical rainforests :** Tropical rainforests occur near the equator in Central and South America, Central and Western Africa (Congo, Zambesi river), Southeast Asia (parts of India and Malaysia), Malaya, Borneo, New Guinea and Northwest Australia. Tropical rainforests are among the most diverse communities on earth. Both temperature and humidity are high and constant. The annual rainfall which exceeds 200 to 225 cm is generally evenly distributed throughout the year.

The flora is highly diversified : a square mile may contain 300 different species of trees, a diversity unparalleled in any other biome. The extremely dense vegetation of the tropical rainforests remain vertically stratified with tall trees often covered with vines, creepers, lianas, epiphytic orchids and bromeliads Under the tall trees is a continuous evergreen carpet, the canopy layer, some 25 to 35 meters tall. The lowest layer is an understory of trees, shrubs, herbs like ferns and palms, all of which become dense where there is a break in the canopy. Nearly all plants are evergreen, and those that do lose their leaves entirely do so at irregular intervals with no apparent regard to the climatic regime. The leaves of most plant are of moderate size, leathery and dark green in colour. Their roots are often shallow and have swollen bases or flying buttresses.

Soils of tropical rainforests are red latosols, and they may be exceedingly thick The high rate of leaching makes these soils virtually useless for agriculture purposes, but if they are left undisturbed the extremely rapid cycling of nutrients within the litter layer (due to decomposition) can compensate for the natural poverty of the soil. It is the nature of the soil, both its potential for high leaching as well as its chemical composition that promotes a rock-like quality when exposed to air, that largely has prevented western style agriculture from being applied to the tropical forests (Kormondy, 1976).

Invertebrate density and abundance are very high in tropical rainforests, but while vertebrates are diverse, they are not as abundant as in many other communities. The common invertebrates of these forests are worms, snails, millipedes, centipedes, scorpions, isopods, spiders, insects, planarians and leeches. Among insects, heteropterans, orthopterans, blattids, mantids, phasmids, bees, termites and ants are most common. The common vertebrates of tropical rainforests are the arboreal amphibian *Rhacophorus malabaricus*; aquatic reptiles, chameleons, agamids, geckoes, and many species of snakes, many species of birds, social birds being predominant; and a variety of mammals. Nocturnal and arboreal habits are most common in many mammals such as insectivores, leopards, jungle cats, ant-eaters, giant flying squirrels, monkeys and sloths. But in New Guinea and Northern Queensland, where monkeys are absent, there are arboreal kangaroos (*Dendrolagus* sp.), despite the fact that the basic anatomy of the kangaroo is not particularly well-suited to arboreal life. Further, in the foot hills of the forest zone of peninsular India covered with dense tropical vegetation, we have the tiger (*Panthera tigris*), the elephant (*Elephas maximus*), samber deer (*Rusa unicolor*), muntjac (*Muntiacus muntjak*), the gaur (*Bibos gaurus*), the chital or

spotted deer (*Axis axis*), and the swamp deer (*Rucervus duraucelli*) as the major ground dwelling mammals (see **Ananthakrishnan and Viswanathan, 1976**).

(vi) Tropical seasonal forests : Tropical seasonal forests occur in regions whose total annual rainfall is very high, but segregated into pronounced wet and dry periods. Tropical seasonal forests are found in Southeast Asia, Central and South America, Northern Australia, Western Africa and the tropical islands of the pacific as well as India and Southeast Asia. In exceedingly wet tropical seasonal forests, commonly known as **monsoon forests**, the annual precipitation may be several times that of the tropical rainforest. Trees may reach heights over 40m, but are more commonly 20–30m high. Stratification is of a relatively simple type with a single understory tree layer, canopy is deciduous and understory is evergreen. Teak is often a major large tree in the best-known tropical seasonal forests, those of India (Central India) and Southeast Asia. Bamboo is also an important climax shrub in these areas, although in other areas it is important only in earlier stages of the succession.

(vii) Subtropical rainforests : In regions of fairly high rainfall but where temperature differences between winter and summer are less marked, as in Florida(USA), the broad-leaved evergreen subtropical biome is found. The vegetation includes mahogany, gumbo limbo, bays, palms, oaks, magnolias, tamarinds, all laden with epiphytes (of pineapple and orchid families), ferns, vines and strangler fig (*Ficus aureus*). This, stratification is simpler, with only one understory tree horizon. All these plants tend to be evergreen, but may lose their leaves during the dry season. Animal life of subtropical forest is very similar to that of tropical rainforests.

4. TROPICAL SAVANNA BIOMES

Savannas are tropical grasslands with scattered, drought-resistant trees which generally do not exceed about ten meters in height and do not forming a canopy. Thus, a savanna is an intermediate between a forest and a grassland. Savannas constitute extensive areas in Eastern Africa, which support the richest diversity of grazing mammals in the world, and also occur in Australia, South America and Asia. The climate is generally characterized by a rainy (May through October) and dry (November through April) season ; in the llanos of Venezuela, for example, nearly 90 per cent of the annual rainfall of 130 cm falls during the rainy season. The latosol soils of savannas are nutrient poor owing a heavy leaching, quite widely distributed in savannas are soils called laterite which when dried harden to rock-like consistency thereby precluding their use in wes-

termstyle agriculture (Kormondy, 1976). Although climate and soil are significant regulating factors in this biome, the controlling factor appears to be fire, which gives grasses and certain species of trees a powerful advantage over other tree species. As a result of this latter factor, species diversity is quite low in comparison to adjacent tropical forests ; in some situations a single species of both grass and tree may be dominant over large areas.

The trees of savannas are resistant to desiccation, and may be either deciduous or evergreen. Their leaves are often hard and drought-resistant. Grasses are the most conspicuous plants, and may reach heights of 1½—2 m. Gigantism of certain animal groups in these tropical savannas is as pronounced as it is in the boreal forest including such giants as many antelopes, giraffes, elephants, buffalo and lions (Clapham Jr., 1973). In addition, a rich insectan fauna especially those with strong mandibles capables of mastication are conspicuous in this biome. Grasshoppers and termites are encountered in large numbers.

5. GRASSLAND BIOMES

The grassland biome is found where rainfall is about 25 to 75 cm per year, not enough to support a forest, yet more than of a true desert. The seasonality of grasslands is pronounced, both with respect to rainfall, which is concentrated in the summer and to temperature. Grasslands typically occur in the interiors of continents and include the tall grass prairies, short grass prairie or great plains and arid grassland of North America as well as the steppes of Eurasia (Southern Russia, Siberia and Asia), the veldt of Africa and the pampas of South America (Argentina). The grassland [illegible] ties are open land co [illegible] irregular rainfall, shar [illegible] high radiation. Since [illegible] cluded from these co [illegible] These winds carry the [illegible] provide natural pasture for grazing animals (herbivore) which are excluded from predation by predators which hide in thick vegetation and prey upon them.

In grasslands, the stratification is reduced essentially to a single story, but within that level, species diversity may be as high as in a deciduous forest-especially for the tall grass prairies. Only along the streams are trees to be found, but the "gallery forests" within a few meters of stream bank are is characteristic of grasslands. The grasses comprising the grasslands can be divided into two basic groups, the tall grasses more than 1 m high, which are found in moist portions

of the grassland, and the short grasses less than 1 m high, which are found in the drier regions. For example, prior to its conversion to agriculture and urban development, the tall grass prairie toward east of North America was dominated by species of bluestern (*Andropogon*) forming dense covers four to six feet tall. Westward, Buffalo grass (*Buchloe dactyloides*) and other grasses but a few centimeters high dominated the landscape. Flowering herbs including many kinds of composites are common, but much less important than grasses.

dering it dark ; this upper portion remains neutral to slightly alkaline because of the continued replenishment of cations like calcium and potassium through the upward movement associated with evaporation.

Africa. The large herbivores are no where near as diverse as they in savanna areas. Likewise, carnivores are relatively small, such as coyotes, weasels, badgers, foxes, ferrets, owls and rattle snakes. Rodents such as prairie dogs, rabbits, and ground squirrels are common. Most herbivores characteristically aggregate into herds or colonies ; this aggregation provide some protection against predators. The characteristic birds of grasslands are prairie chickens, meadow larks, and rodent hawks. Further, saltatorial motion is widespread, not only in mammals such as rabbits and kangaroo rats, but also in insects, such as grasshoppers and crickets. This probably has to do with increasing rate at which animal can move through or over the tall grasses, as well as making it harder for a predator to catch the animal. Visibility in the grasslands is exceedingly good and the premium placed on efficiency including predators is very high.

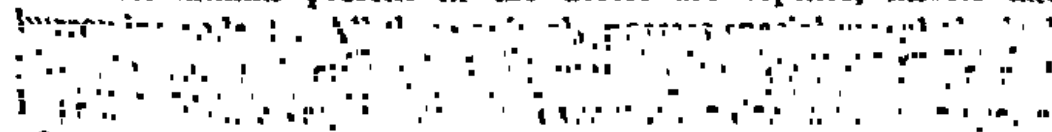
The common insects of steppes are termites, locusts (*Locusta migratoria, Schistocerca gregaria* and *Melanoplus* sp.) bees, burrowing wasps mutilid wasps, bumble bees and blister bees. The steppes also harbour an undisturbed reptilian fauna. Lizards and snakes are met with in large numbers and possess remarkable diversity. Most of them are fossorial, insectivorous and carnivorous.

6. DESERT BIOMES

Deserts are the biome formed in the driest of environments, Temperature may range from very hot as in hot deserts to very cold as in cold deserts. Major hot d
the tropics of cancer and ca
10 mm. The most important hot deserts of world is the Sahara-Arabia-Gobi desert complex extending from Africa to Central Asia and contains highly irregular and very insignificant rainfall, and low humidity due to excessive evaporation. Fairly extensive hot deserts also occur in India (Sind-Rajasthan deserts), South America (Chile), North America and Australia. The cold deserts occur at high elevations where the temperatures are low and rainfall scanty as the air losses all its moisture content as it ascends higher and higher. Cold deserts occur in Ladakh regions of Himalayas, Tibet, and Bolivia Arctic. The hot and cold deserts may also be distinguished by differences in plant population which are mostly succulent type (e.g., cactus, palo verde trees, creosote bush, etc.). Most cold deserts have sage brush.

Low erratic precipitation coupled with soil and air temperatures that are extremely high by day and drop abruptly by night, low humidity, and high insolation are the major desiccating environmental factors to which desert vegetation and animals have adapted. Desert plants which tend to be shrubs are adapted to drought conditions through reduced leaf size and the dropping of leaves in dry conditions, both reducing water loss via evapotranspiration. The roots of most desert plants remain well developed and occur in the top meter of the soil to take maximum possible advantage of any rainfall. Further, the root hairs on many desert plants are ephemeral, drying back under drought conditions and thereby reducing potential water loss by osmosis. Yet other species are short lived annuals that complete their life cycles during the short-moist period. In most hot deserts, there occur plants such as cacti, water-storing succulents such as acacias, euphorbias, cacti, prickly pears, etc., which are adapted by their protoplasmic colloids, which enable the accumulation of substantial water reserves, as well as by a reduced leaf surface, which obviates water loss via evapotranspiration.

The animals present in the desert are reptiles, insects and



of gazelle and all animals have cursorial, fossorial and/or salt-

atorial adaptations. Some desert animals are nicely adapted for high extremes of temperature. For example, the lethal temperature of different species of insects found to be following—for the camel spider *Galeodes granti* it is 50°C, for the *Gryllus domesticus* it is 40°C and for the forficulid *Labidura riparia* it is 38°C (Cloudsley—Thompson, 1962). Diurnal rhythms are perhaps the best method of avoiding the heat [illegible] many blossom [illegible] *Akis spinosa* remain active during the day, and the centipede *Scolopendra clavipes* restricts its activities to night time.

Further, certain reptiles and certain insects are well adapted for survival in deserts because of their thick, impervious integuments and the fact they excrete dry waste matter. A few species of mammals have become secondarily adapted to the desert by excreting very concentrated urine. They avoid the sun by remaining in their burrows during the day. Kangaroo rat and pocket mouse, both are able to live without drinking water by extracting the moisture from the seeds and succulent cactus they eat. The camel and the desert birds (Ostrich, etc.) must have an occasional drink of water but can go for long periods of time using the water stored in the body. Most insects of deserts are herbivores and as a correlation the number of small insectivorous lizards found in the desert is usually high.

Fauna of Indian deserts : Prakash (1974) have recognized four habitat types in the Indian desert based on land forms namely **aquatic, sandy, and rocky ruderal habitats.** The aquatic habitat of Indian deserts which remains confined to the perennial lakes, are inhabited by various species of fishes like *Labeo nigripinnis, Oxygaster clupeoides, Tor khurdee, Puntius amphibia, Noemacheilus denisonii.* Among amphibians of the Indian desert are a species of toad (*Bufo andersonii*) and five species of frogs (Indian bull frog *Ramna tigrina*, cricket frog *Rana limnocharis* and burrowing frog *Rana breviceps* being dominant species). Among reptiles, there occur two species of testudines (Loricata), 18 species of lizards and 18 species of snakes. Of the lizards, some species like *Acanthodactylus cantoris, Calotes versicolor, Uromastix hardwicki, Ophiomorus tridactylus* are predatory on the desert locust inhabiting localized areas in the Thar desert. The avian fauna of the Indian desert includes species like painted partridge *Francolinus pictus pallidus*, grey partridge *Francolimus pondiceriamus*, common quail *Coturnix coturnix*, black breasted quail *Coturnix coromandelicus*, rock bush quail, the little bustard

quail *Lurnix sylvatica*, button quail *Turnix tanki*, great Indian bustard *Choriotes nigriceps*, florican *Sypheotides indica*, sandgrouses like *Petrocles exustus erlangeri*, *Petrocles alchata*, *P. senegallus*, and *P. indicus indicus*. During winter numerous aquatic birds become abundant in Indian deserts. Such birds are white fronted goose *Anser albifrons*, wigeon *Anas penelope*, garganey *Anas querguedrila*, red crested pochard *Netta fufina* and tufted duck *Aythya futigola*. Among the predominant predatory birds are two species of the vultures namely *Gyps bengalensis* and the white vulture *Neophron percnopterus*.

The mammalian fauna of Indian deserts includes many species, some of which have been tabulated in following table :

Table : Certain common mammals of Indian deserts (Prakash, 1974).

Order	Species	Popular Name
1. Chiroptera	*Rosettus arabicus*	Fruit bat
	Pteropus giganteus giganteus	Fruit bat
	Rhinopoma kinneari	Rat-tailed bat
	Rhinopoma hardwicki	The lesser rat-tailed bat
	Taphozour perforatus	Tomb bat.
	Megaderma lyra	Indian false vampire
	Rhinolophus lepidus lepidus	Horse-shoe-bat
2. Insectivora	*Hemiechinus auritus collaris*	Long ear hedgehog
	Paraechinus micropus	Long ear hedgehog
	Suncus muinus sindensis	House Shrew
3. Pholidota	*Manus crassicaudata*	Scaly ant eater
4. Lagomorpha	*Lepus nigricollis caudatus*	Hare
	Lepus nigricollis dayanus	Hare
5. Rodentia	*Funambulus pennanti*	Northern palm squirrel
	Hystrix indica indica	Indian crested porcupine
	Rattus rattus	House rat
	Mus musculus rufescens	House mouse
	M. musculus bactrianis	House mouse
	Gerbillus gleadovi	Indian hairy footed gerbil
	Tatera indica	Gerbil
	Merriones hurrianae	Gerbil
6. Artiodactyla	*Sus scrofa cristata*	Wild boar
	Boselaphus tragocamelus	Blue bull
	Antilope cervicapra rajputani	Black back
	Gazella gazella bennetti	Gazel
7. Carnivora	*Canis lupus*	Wolf
	Canis aureus	Asiatic jackal
	Vulpes vulpes	Red fox

Order	Species	Popular Name
	Vulpes bengalensis	Bengal fox
	Lutra perspicillata	Indian otter
	Viverricula indica	Small Indian Civet
	Herpestes auropunctatus	Small Indian mongoose
	Felis chaus prateri	Jungle cats
	Felis libyes ornata	Jungle cats
	Panthera pardus sindica	Panther

All these mammals have well specialized adaptations for survival in thermal extremes and low humidity. Many of them possess diurnal rhythmic activities.

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