The Cell

Structure & Function

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INTRODUCTION

Cell biology or cytology, may be defined as the science which is concerned with the structural and functional organization of protoplasm, and with the relation of this organization to the phenomena of metabolism, growth, differentiation, heredity and evolution. In other words, cell biology or cytology is a branch of biology which deals with the morphological, chemical and functional studies of cells. The cell is the essential morphological and physiological unit in the structure of living organisms. In ancient times, the biologists considered plants and animals to be composed of few elements repeated in each of them. They were taking into consideration only the macroscopic structures of the organism such as roots, leaves and flowers in case of plants, and the segments or organs familiar in animals.

After the invention of the magnifying lenses, it was found that there occurs a highly complicated system of microscopic elements known as the cells which were considered as the units of living matter. The discovery of cells is of utmost importance since we are living now in an analytical period of science. Thus, it is essential to analyze the biological processes, that is, to separate them into their essential elements in order to realize or investigate the chemicla and energy transformations which are collectively known as the phenomenon of life.

Now it became realized that the cells are the basic structures of all living organisms including plants and animals. This is the general scope of the rather newly evolved science, namely "Cytology" or "Cell Biology".

In fact, many text books are available on their modern science, each with its own trend and personal interests. But in writing this books, we have intended to adopt a certain trend, which we believe – in view of our long experience in teaching of this subject and doing vast research work in it - to be quite convenient as a basic building stone for any advanced and post- graduate studies. In brief, the general trend was to include as much as possible of information in this important field. within a special frame comprised of "The Cell Structure and Cell Function". Nonetheless, this frame is an essential point, or even the sole of Biology since Biology - in general - is basically concerned with the inter - relations between structure and function.

The second point of concern in editing this book was to decide on one of the two obtions which are of considerable importance in a such newly and fast advancing science. One of those two options was to abandon the classical knowledge in the subject and concentrate on the modern data which became widely spread and gaining more and more interest after the invention and construction of the recent tools, and equipments used in studies and researches on the cell like the phase contrast microscope, electron microscope, ultraviolet – and fluorescent microscopes, etc... The authors have reached a general decision in this regard: not to abandon or neglect the basic cytology and in the mean time to have a satisfactory and good grasp of modern cell biology. For example, as much as possible, a basic cell structure of cell organelle was to be described as it was observed for the first time by the old equipments, and then turned to be examined and described by the recent tools and equipments. This was of course accompanied by disclosing all the secrets which became known after such modern investigations, in a simple, direct and easy manner.

The authors also believe in the essential importance of instrumentations and diagrammatic representations of several points in the subject. These points were also well covered in this text.

The authors hope that this book will be another building brick in this field and fulfil the purposes aimed to from it.

At the end, the authors take this chance to offer their thanks to "Dar Al-Maaref" Publishing Institution in Cairo, and especially to the section of "University Publications" headed by Mr. Mohamed Abdel Rahman and his assistant, Mr. Aly Galal for the interest, help and all burdens taken during publishing this book.

The authors
El – Banhawy & Khattab
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CHAPTER 1

GENERAL CONCEPTS OF CELL BIOLOGY

After the invention of magnigying lenses, it was found that there occurs a highly complicated system of microscopic elements known as the cells which were considered as the units of living matter. The discovery of cells is of utmost importance since we are living now in an analytical period of science. Thus, it is essential to analyze the biological processes, that is, to separate them into their essential elements in order to collectively known as "the phenomenon of life".

The advent of the phase contrast microscope provides a profound knowledge of the cellular organization, not only as it appears in the dead cell after fixation, but also as it is seen in the living condition.

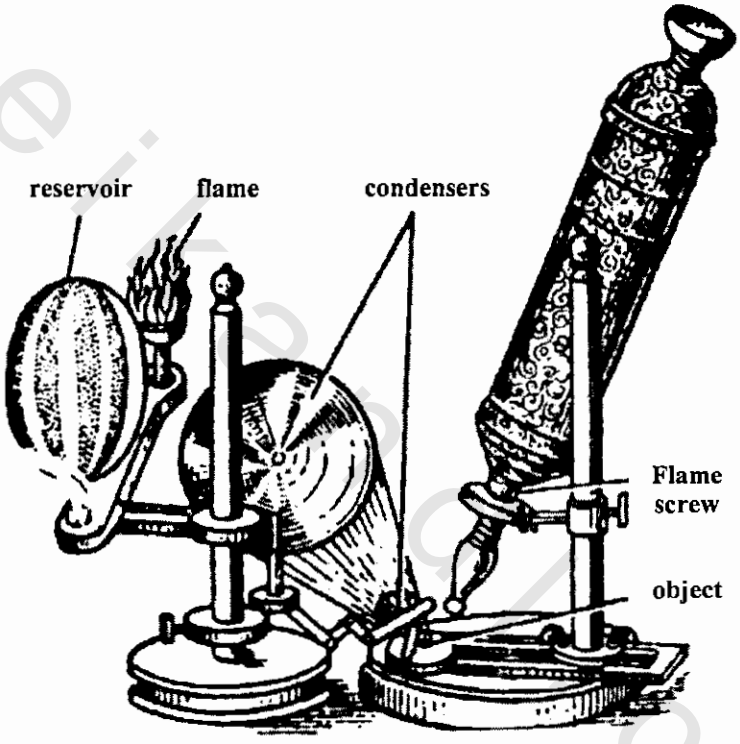
In recent years, the improvement cytological techniques and the application of modern methods (e.g., X-ray, polarized light, fluorescent and electron microscopy) has greatly changed our knowledge about the cell structure and organization. The biochemical investigation have also demonstrated the products of living matter and even the elements (e.g. DNA, RNA, proteins, etc.) of the living cells. A number of more detailed structures at the macromolecular level have, therefore, been recognized. This has resulted in a new branch of science known as **"molecular biology"**; the scope of which includes the investigation and orientation of the molecules and of the intermolecular structure of the essential constituents of the cells. This important field of science has flourished only in the last few years.

Inspite of the vast knowledge of cellular structure and activity, a question arises and up till now is unsolved: the question is whether the vital processes in the cell are of a purely physicochemical (mechanical) nature, or does there exist in the organism a special force which regulates these different biological processes? The tools accessible for investigating the different biological activities include physical, chemical as well as different experimental procedures. We agree with Goddard (1958) that "When we truly understand the cells, we will understand life itself".

HISTORY OF CYTOLOGY

Discovery of the cell "Cell Theory" :

The term cell, as first used by Robert Hooke in 1665, meant a hollow chamber surrounded by a definite wall.



(Fig. 1)

Hook's compound microscope

Durig the seventeenth and eighteenth centuries the cell wall was considered to be the most important part of the plant cell. It was later realized that the cells are not always empty spaces, and in the nineteenth century the cell content, which was described by different authors as gelatinous juice, attracted the attention of a great number of investigators. In this juice, the nucleus was discovered by Robert Brown in 1831. Although the discovery of the nucleus is usually attributed to Robert

Brown, it was first seen in the red blood corpuscles of the salmon by Leeuwenhöek in 1800, then in tissue cells by Fontana in 1781.

In 1833, the German botanist Schleiden formulated the couception that cells are the structural units of plants; in other words, Schieiden discovered that all tissues of plants are composed of cells. His results were confirmed and extended to animals in 1839 by the German Zoologist Schwann, who used for the first time the term "**Cell Theory**" for the concept that 'The cells are organisms and animals as well as plants are aggregates of these organisms, arranged in accordance with difinite laws".

Although the theory that all plants and animals are built up of cells is associated with the names of Schleiden (1838) and Schwann (1839), many workers as Mirbel (1898-09), Lamarck (1809), Turpin (1826), Meyer (1830) and von Mohl (1831) had previously dealt with the theory in a form more or less complete.

In lower organisms, such as the Rhizopoda and Foraminifera, Dujardin (1835), a French protozoologist, described the content of the cell, which he called "Sarcode" as a gelatinous, homogeneous and transparent substance which is elastic, contractile and insoluble in water.

The name protoplasm was first given to the cell conternts of animals and plants by Purkinje (1840) and von Mohl (1846) respectively.

The essential similarity which exists between the sarcode and the protoplasm of animal and plant cells was established by Max Schultze (1861) who offered a theory which later was called by Hertwig (1892) the "Protoplasm Theory".

It was then realized that protoplasm (Gr. Protos = first, and Plasma = formation) is the principal constituent of the cells of animals as well as plants, and that walls are only found in plant cells but are lacking in animal cells. The cell was then defined as a mass of protoplasm (cytoplasm) containing a nucleus and bounded by a delicate cell membrane. Such a definition is better than the older one which regards the cell as "a unit of living matter", but is still far from being satisfactory for the following. In the first place, in many protozoa the protoplasm contains two or more nuclei. Likewise, in metazoa. Cells may contain two nuclei as in the anterior mesenteric ganglion neurones of the rabbit, or many nuclei as in the striated muscle fibres. Secondly, some cells may either apparently lack a nucleus as the mature mammalian red blood corpuscles or may contain nuclear matter scattered through them without forming a definite nucleus as in some protozoa. And lastly, it has been claimed that connections may

be found between cells, and therefore, it is a false conception to consider the organism as composed of discrete cells which co-operate physiologically but are fundamentally independent.

The modern opinion considers the organism as the individual with a common life running through it all, and the cells, not as units of which it is built up, but rather as parts into which it is divided in order to proceede for the necessary division of labour involoved in such complex process of life. The word cell thus conveniently remains as a useful descriptive term to denote that "the cell is a mass of prtoplasm surrounded by a delicate membrane and containing one or more nuclei at least through a stage in its differentiation".

Special attention was focused upon the extraordinary modifications that take place within the nucleus at each cell division. Thus, there was discovered the phenomenon of amitosis or direct division (Remak, 1841) and that of indirect division or mitosis or karyokinesis (Schneider, Strasburger, Schleicher, Flemming). Another important discovery, was that of the entry of the sperm into the frog's egg which was first described by Newport in 1854, then in 1875 Hertwig showed that the sperm pronucleus fuses with that of the egg.

In the cytoplasm, notable advances were made in our understanding of its components. The discovery of the mitochondria by Altmann (1890) and the Golgi apparatus by Camillo Golgi (1898) led to much valuable work. Both the mitochnndria and the Golgi bodies are present in practically all kinds of cells throughout the animal kingdom.

Improvements in the methods of demonstrating the cell components in fixed material and the use of vital dyes have opened a vast field of research. The contributions to the subject are too numberous to be mentioned here. However, the main contributions will be dealt with later.

In recent years, not only has there been a marked improvement in the methods of cytological technique, but some entirely new methods of approach such as the use of the phase contrast microscope, the electron microscope, the polarized light, the ultra-centrifuge, the microdissection apparatus, X-ray, ultra violet light and the administration of certain chemicals have been evolved. Thus fertile and fresh fields of research have been opened.

RELATIONSHIP BETWEEN CYTOLOGY AND OTHER BRANCHES OF BIOLOGY

It is obvious. From the different investigations, that there is a close relationship between cytology and other branches of biology. For example, it is strongly allied to genetics which deals mainly with the hereditary factors carried on the chromosomes which are the important constituents of the nucleus. That is why both branches are given together a common name "cytogenetics".

A close relationship also exists between cytology and taxonomy since recent studies on the latter are mainly based on differences in the number and form of chromosomes. This also aided so much the study of evolution. Cytology is also closely related to embryology, physiology and ecology. Not only this, but since disease is primarily an abnormal activity in cells, the close relation between cytology on one hand and pathology and medicine on the other hand should be obvious. In this respect the growth of cancer cells, and the effects of viruses and bacteria on cell structure and function should be mentioned. Cytology as has been stated by Sharp appears to occupy a key position in the science of biology since everything the organism does has a part of its cause in protoplasmic activity. This means that all biological problems have a cytological element in them. Cytology is, therefore, an integral part of biology, and the future progress of the science will depend very largely upon how well such integration is maintained, Cytology is entering upon a new period when a clearer understanding will be obtained of the various cell components and their rôle in cellular activities, a period when cytology will solve some of the problems of medicine, agriculture and of biology as a whole.

CHAPTER 2

INSTRUMENTS AND METHODS IN THE FIELD OF CELL BIOLOGY

The last quarter of the nineteenth century contributed to the twentieth century many of the fundamental discoveries and suggestive theories.

The present century is fortunate in having several new and extremely valuable tools and techniques. Observation of the detailed structures of cells and tissues have been carried out by using different types of microscopy. These instruments are designed to increase the resolving power and contrast.

Light microscopy :

There are many kinds of the ordinary light microscopes ranging from simple to compound ones. The type of microscope depends on glass lenses and on the visible light. The light microscopes are widely used by students in all biological laboratories (Figs. 2 & 6)

Phase contrast microscopy :

The phase contrast microscope is mainly used for the study of unstained living cells. It acts mainly to increase the differences between the refractive indices of the different cellular components, and hence they become more clearly detectable. The phase contrast microscope allows the examination of living cells without the use of the usual technical procedures as fixation, dehydration and staining which may cause some morphological and chemical changes. It is also applied for the investigation of the effects of different chemical and physical agents on the living cell and for the examination of the artifacts produced as the result of fixation and staining (Figs. 3 – 5 & 7)

Interference microscopy :

Both the phase contrast microscope and the interference microscope are based on the fact that although biological structures are highly transparent to visible light, they cause phase changes in transmitted radiations. These phase differences in the refractive index and thickness of different parts of the object are highly detectable. With the interference microscope it is easy to determine the optical phase difference for the various cellular

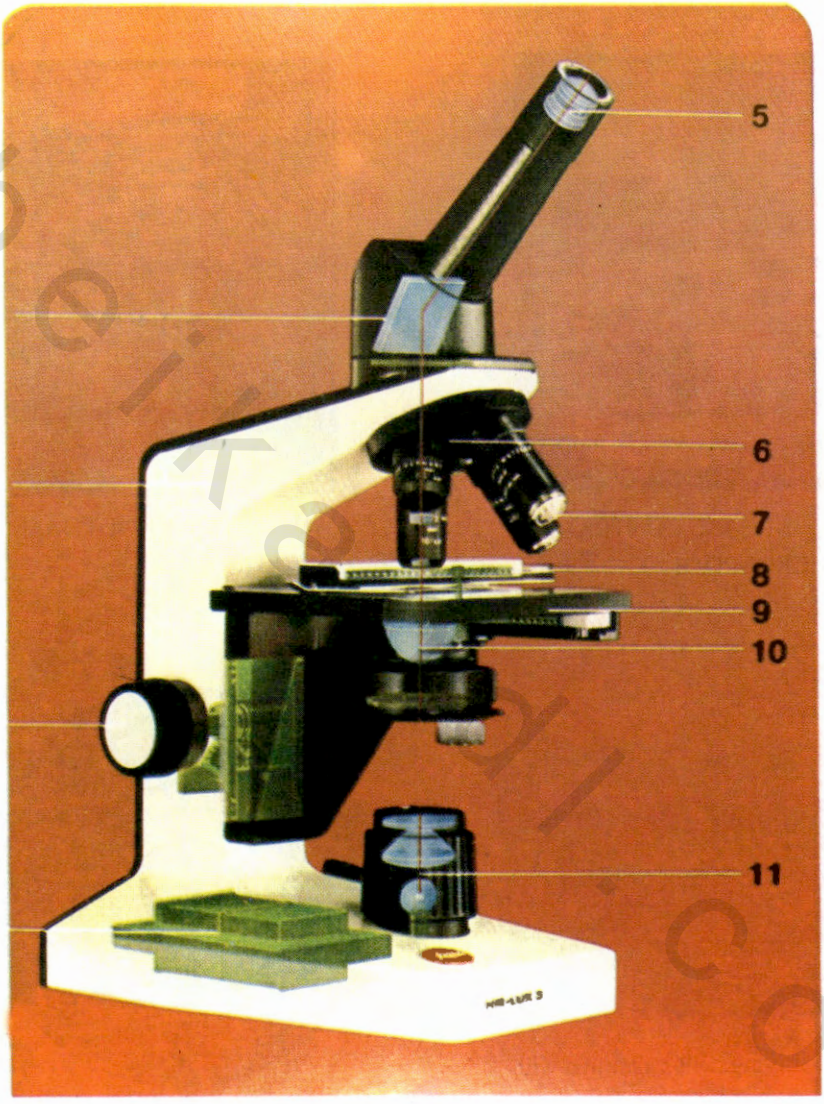


Fig.2 : A MODERN LIGHT MICROSCOPE

1- Foot 2- Adjustment Screw 3- Arm 4-Body Tube 5- Ocular lens
6- Nose piece 7- Objective 8- Clips 9- Stage 10- Diaphragm
11- Light Source

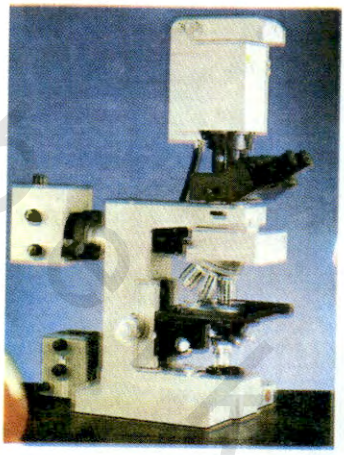
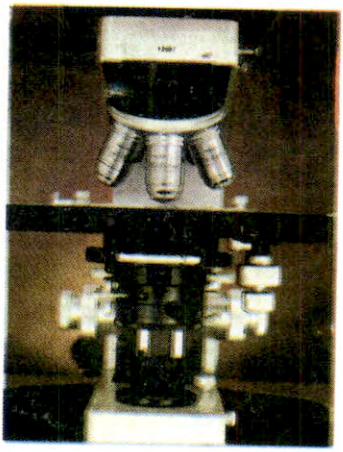


Fig .3 PHASE CONTRAST MICROSCOPE



ADJUSTED SET OF PHASE MICROSCOPY

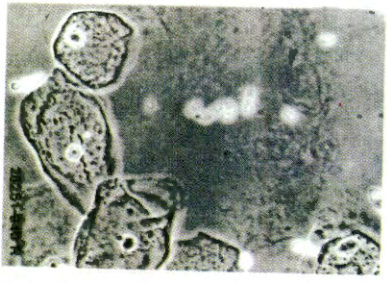


Fig. 4: Living cells examined with phase contrast microscopy

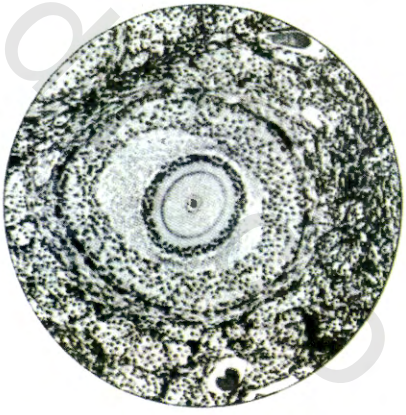


Fig. 5 : Graafian follicle examined with phase contrast microscopy

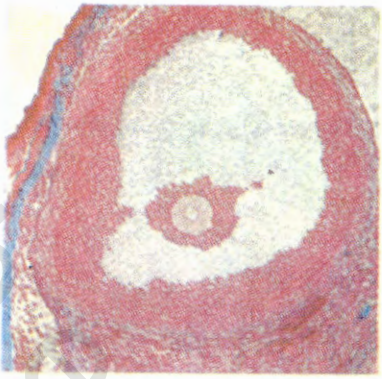


fig.6 : Light Microscopy (Graafian follicle)

Fig. 7: phase contrast microscopy (Urinary sediment)

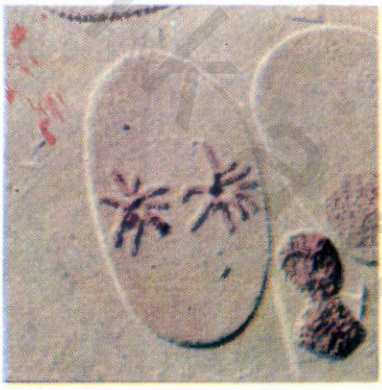


fig. 8: Interference Microscopy (cell division)

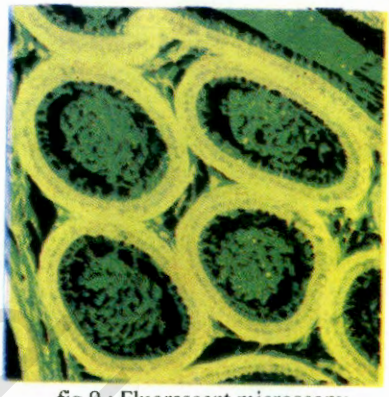


fig.9 : Fluorescent microscopy (Seminiferous tubules)

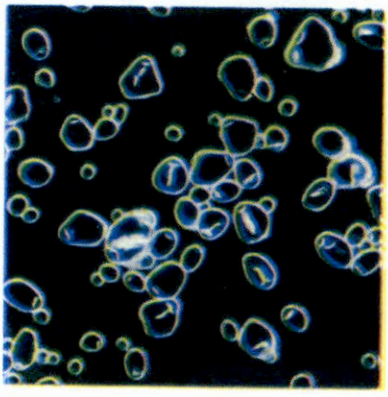


fig.10: Dark field Microscopy (Bacterial forms)

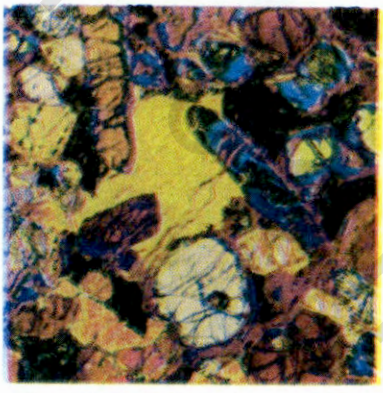


fig.11: Polarization microscopy (Crystal varieties) Different specimens examined by different microscopes.

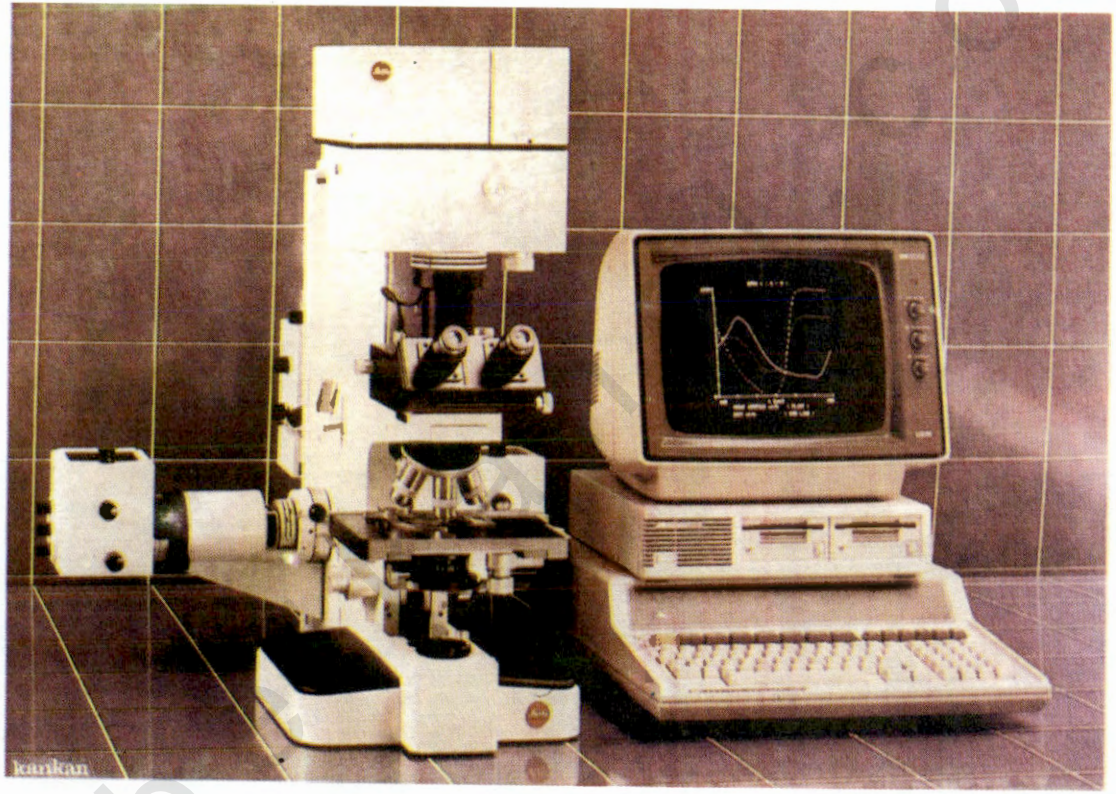


fig 12: Cytophotometer for quantitative cytochemistry

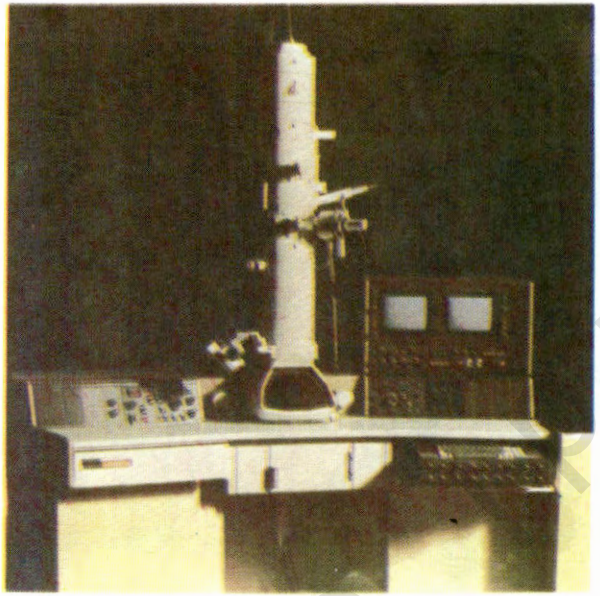


fig .13 : Electron microscope

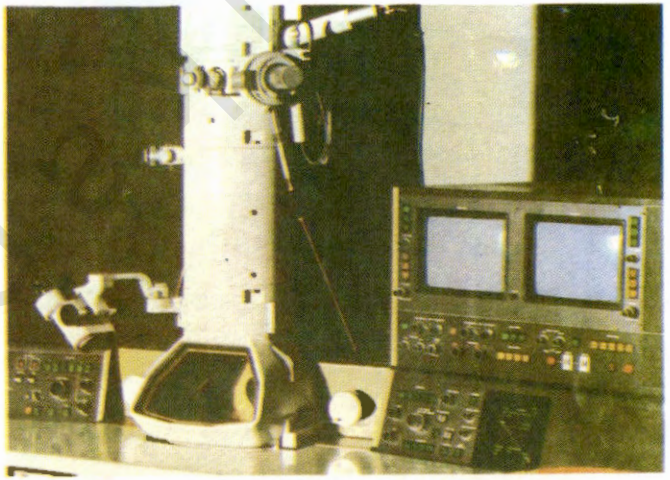


fig.14 : Enlarged basal part of hte electron microscope

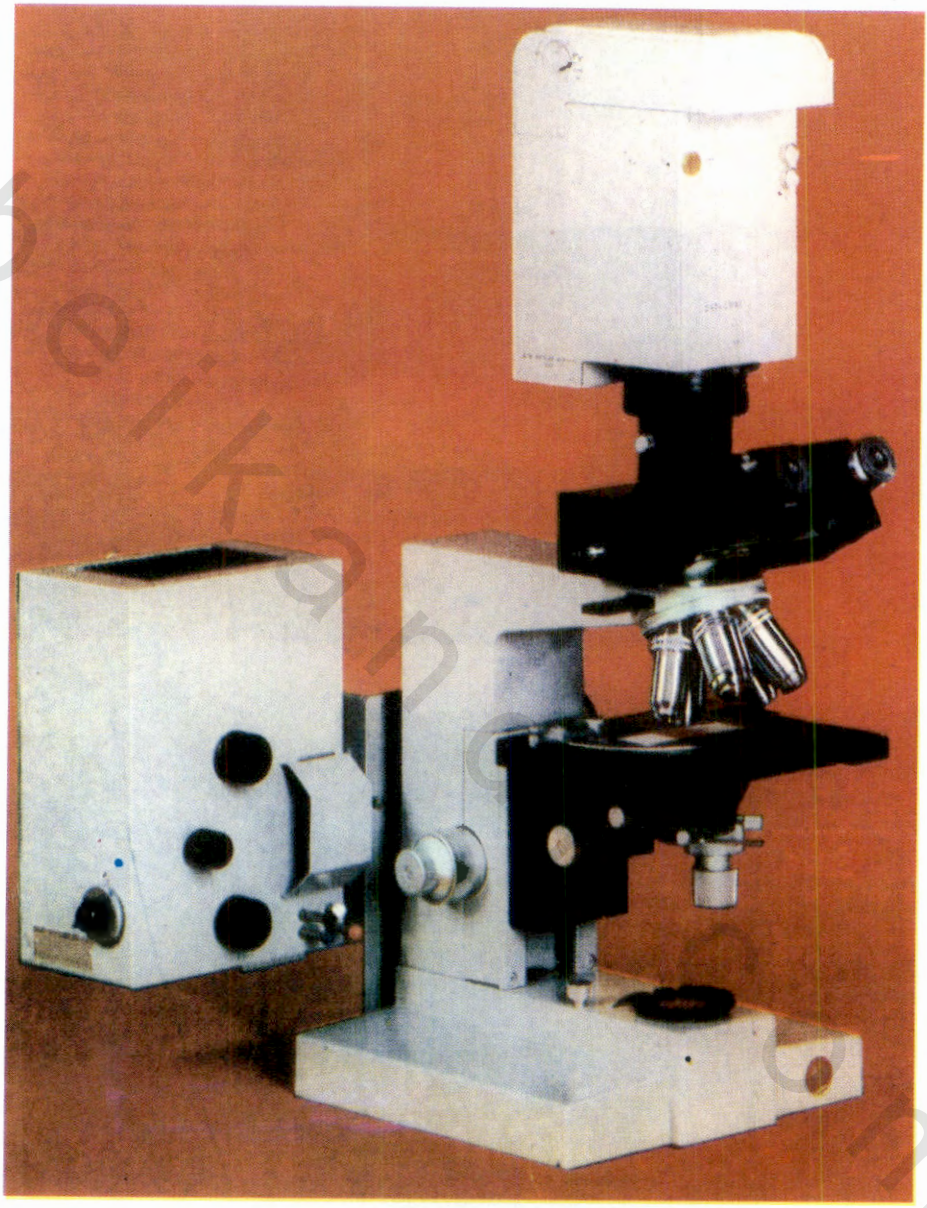


fig. 8B Ultraviolet Microscope

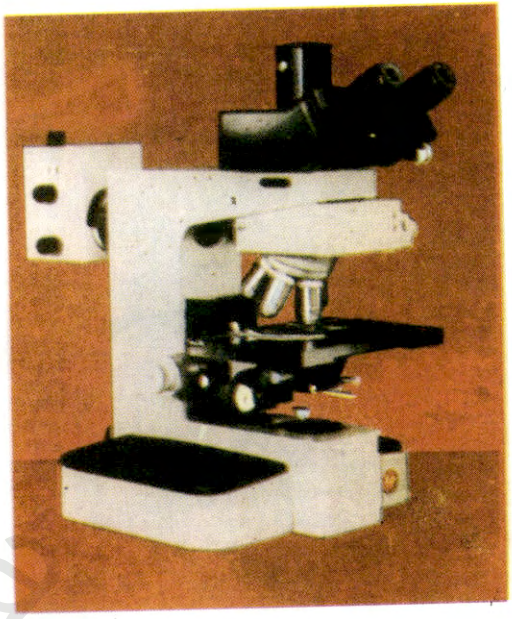


fig. 10 A
Dark field Microscope

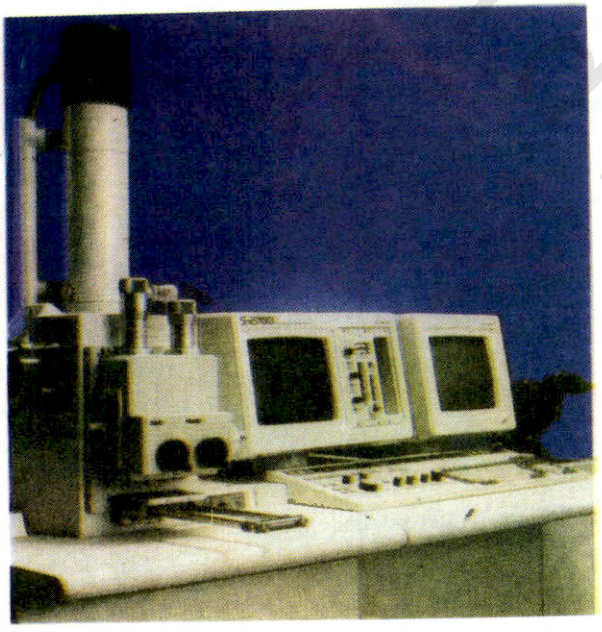


fig. 16 A
Ultramodern Scanning
Electron Microscope

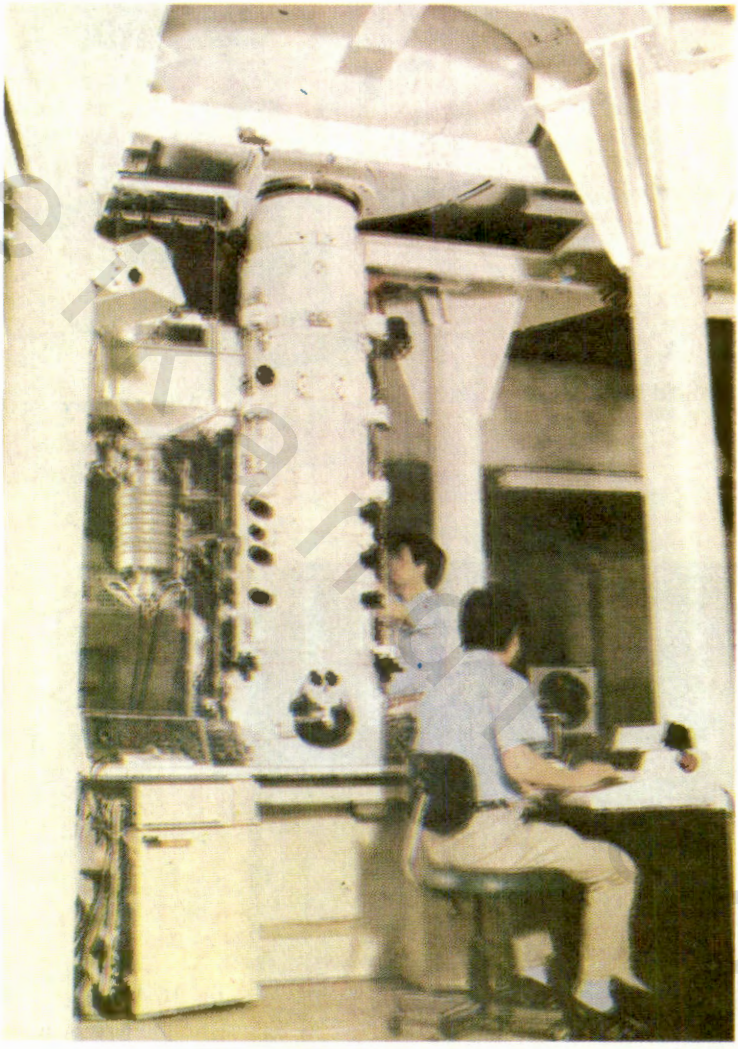


Fig.16 : the most recent Hitachi electron microscope (exhibited 1987 - 1988).

structures and consequently to measure their dry weight. Moreover, it is possible to detect small and continuous changes in refractive index. So, the interference microscope has an advantage of giving quantitative analysis of living cells and the effect of different chemicals and agents on them. (Fig. 8).

Ultraviolet microscopy :

The visible region of the light spectrum lies between 6500 A° (red) and 4500 A° (violet). Wave lengths shorter then 4000 A° are called ultraviolet. In this region some components of the cell such as nucleic acids absorb certain wave lengths. So, such microscopy is used to measure the nucleic acid content of cell nuclei.

Fluorescence microscopy :

Some chemical substances – when irradiated with ultraviolet light-they absorb the radiation and emit visible light. Cellular contents which absorb these chemicals in living cells can therefore be observed as fluorescent areas when illuminated with ultraviolet light. This method is very sensitive and is especially used for studying how molecules enter or are absorbed on to cells. (Fig. 9).

Darkfield microscopy :

Darkfield microscope is used for the study of living cells. This type of microscopy is based on the fact that light is scattered at the boundaries of particles having different refractive indices. In the darkfield microscope there is a special condenser which illuminates the object obliquely, and hence the direct light cannot enter the objective; consequently the object appears bright owing to the scattered light, and the dark background. For example, if a living cell in tissue culture is examined under the darkfield microscope the nucleolus, nuclear membrane and some of the cytoplasmic organoids (e.g. the mitochondria) and inclusions (e.g. lipid droplets) appear bright, whereas the background of cytoplasm appears dark. (Figs. 10 & 11).

Cytophotometer :

Cytophotometry is applied now- on a large scale – for the accurate determination of the amounts of materials which have the ability to absorb ultraviolet rays at certain wave lengths, particularly nucleic acids and specifically DNA. For this purpose, a cytophotometer was constructed

embodying an ultraviolet microscope, a broad screen and other accessories. (Fig. 12).

Electron microscopy :

The electron microscope is used for studying the fine or ultrastructure of the cell. In the light microscope the magnification is largely determined by the objective and ocular lenses, and a maximum magnification of 500 to 1500 x can be obtained, On the other hand, in the electron microscope, in which a beam of high speed electrons, properly controlled by electromagnetic lenses, could produce an immense increase in resolution. The resolving power of electron microscopes may reach 5 A°.

In recent microscopes, a wide range of magnifications can be reached by introducing an intermediate lens. Direct magnifications as high as 160,000 X can be obtained; the micrographs can be enlarged 1,000,000 X or more. Thus, The electron microscope has brought into vision the most detailed structures which were never observed before in the cells.

The electron miscroscope consists of a vacuum chamber, the electron microscope column in which an electron source (the electron gun) generates a beam of electrons and an optical system which projects an image onto a fluorescent screen or a photographic plate or film (Figs.13-15).

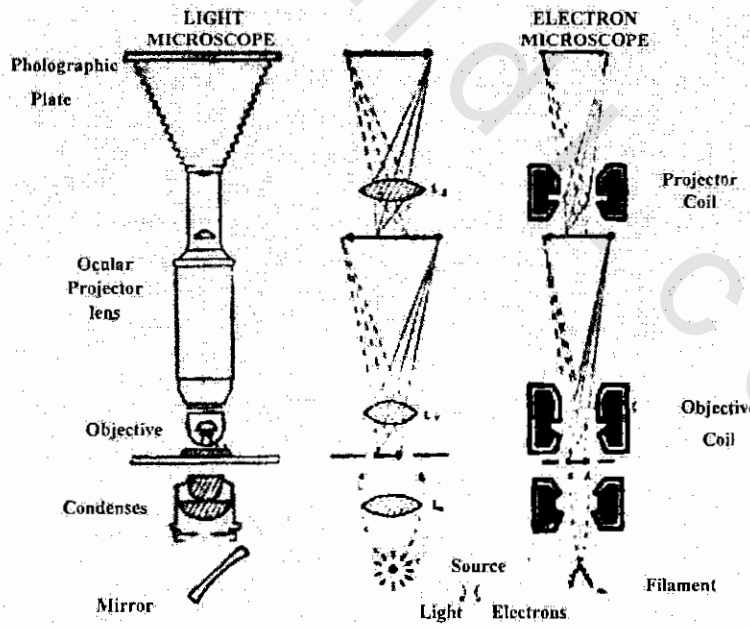


Fig. 15: Light pathways in light and electron microscopy.

The electron beam is obtained by heating of, for example, a tungsten wire – the cathode filament. The electrons are accelerated by the accelerating high voltage applied between the cathode and the anode. The anode is kept at ground potential and the cathode filament assembly at a high negative potential. The electric insulation between the cathode and the rest of the microscope column is ceramic or glass.

The optical system consists of a magnetic condenser lens system which concentrates the electron beam onto the specimen and the magnetic objective lens and the projector lens system which together produce a magnified image on a· fluorescent screen or a photographic plate or film. The electron beam in the electron microscope must pass through a well evacuated space to prevent scattering of electrons.

Generally speaking, the electron microscope uses a beam of electrons instead of light as its source of illumination. The electrtons pass through the specimen and fall upon a photographic plate where they produce an image of the specimen. The magnification of the electron microscope is very high and thus it allows the study of the ultrastructure of the cell constituents.

Recently, a new electron microscope was constructed which provides a total magnification of about 2,000,000 times, thus enabling the visualization of atoms and molecular surfaces. (Fig. 16).

Micromanipulator :

Micromanipulator is widely used in the cytological studies. This aparatus enables the dissection or injection of normal living cells under the very high power objectives.

Methods for studying cells :

Certain and specific methods are used for studying the cell. Its constituents and its chemical organization. Two main procedures are widely applied.

1 - Vital examination (t.e., examination in the living condition).

2 - Fixed cells by using different fixatives.

Examination of living cells :

Vital and supravital examination (staining takes place inside and outside the body respectively) can be carried out on living cells which may be stained with specific dyes such as Janus green and neutral red.

Methods of tissue culture are also very valiable in studying the cell structure. These methods are well progressing that a culture of living fibroblasts continued to live for more than 30 years.

Fixatives used in cell biology :

Using a certain chemical to preserve cells tissues in a state very close to that of the living condition with the least amount of artifacts is a process called **fixation** and the chemical used is the fixative. The choice of the fixative depends on the nature, type and aim of the study. For example, for studying the nucleus and chromosomes acid fixatives are usually employed; for studying the activity of enzymes acetone, formaldehyde etc. which preserve some enzyme systems are used. Fixatives used in studying the cells and their constituents are very selective and one should seek the proper fixative that precipitates protein in its finest form so that he appearance of the cell is not modified.

The best protein fixatives are the following :

Formalin (formaldehyde) :

The reactions of formalin with tissue proteins are numerous and complex since it can combine with a number of different group of substances which become precipitated in the cells and tissues. But many of these combinations are reversible by the simple process of washing, whereas others are irreversible.

The most important reaction of formalin is the transformation of a compound containing a reactive hydrogen atom into a hydroxyl compound:

RH + CH O ------- R. CH. (OH).

The hydroxyl compound is also reactive it may condense with a further H atom to form a methylene bridge (– CH –)

R. CH_2 (OH) + HR ------- R – CH2 – R + H_2O

These methylene bridges are readily ruptured by hydrolysis. Methylene bridges may be formed between two similar group such as NH_2, or between NH_2, and NH.

Between pH6 and 8 formalin reacts with keratin (the essential protein constituent of hair and skin) without affecting the S – S links of cystine. In more alkaline solutions it is considered to reduce S – S to two SH groups, and subsequently to react with these forming, in some cases, a methylene bridge (S – CH_2 – S) in place of the original disulphide link.

The groups particularly involved in the fixation of proteins are the peptides, hydroxyl, carboxyl as well as the sulphur-containing proteins.

As regards the formalin remaining bound to protein after fixation, it as been found that a considerable amount of it could be removed by washing in running water up to 24 hours. But inspite of this, there still remains certain traces of formalin in the tissues. Such formalin residues may prevent the proper treatment of the fixed tissues, and hence in formalin-fixed material sections must be carefully washed with distilled water to get rid of the formalin residues in the tissues.

The knowledge of the action of formalin on tissue compounds was primarily obtained from tanning and wool industries; collagen and reticulin are the two known substances in this connection. A great deal of this work has been done with formalin under quite histological conditions (fro example, at pH ranging from 4 to 10 at various temperatures); and it was found by some authors that the amount of formalin bound by various proteins dropped sharply in most cases when the pH rose above 1.0. In histological and cytochemical work. Formalin is nearly always used in buffered solutions at or above the neutral point. This intensifies theaction of formalin. This is regarded as being due to the fact that the polymerized form of HCHO exists in water in a hydrated form as methylene glycol (CH_2 (OH_2)) which is a very effective fixative.

The significance of these observations in cytological and histochemical practice is that treatment of proteins with formalin, followed by adequate washing with distilled water is likely to leave the majority of active substances in a condition which enables them to react readily with any dye reagent.

Alcohol and Acetone :

Alcohol and acetone are two protein precipitants which have been widely used as fixative in enzyme cytochemistry. Inspite of the morphological disturbances which they produce in the tissues they don not produce a marked alteration in the reactive groups of enzymes.

It must be pointed out that the effects of alcohol and acetone on proteins are to some extent reversible, and many of the original properties of the proteins are sometimes regained when the effecting agent (alcohol or acetone) is removed.

Metallic ions and complexes :

As regards the application of metallic ions in fixation, histologists and cytologists are mainly concerned with chromlum, mercury and osmium.

Chromium :

Chromium fixatives have the property of forming certain complexes with water, and these complexes combine with protein substances forming other complexes similar to those produced under the effect of formalin. The reaction of chromium depends, to a large extent, on the pH of the medium. For example, at pH ranging from 1 to 4 it was found that the amount of chromium bound by the collagen substance is increased. It was also found that the amount of chromium bound by egg albumen was much decreased as the pH was changed from 4 to 7.

Generally, it has been established that cytological and histochemical fixatives containing chromium must be adjusted at a pH lower than 2.9.

However, chromium fixatives give good results in case of glycogen, lipids, nucleic acids and mitochondria fixation of preservation.

Mercury :

Mercury salts are much more commonly employed in routine histological and cytological work. The complexes formed as the result of reaction of mercury with the tissue proteins are much more stable than those produced by the other metallic substances.

Following fixation with mercury fixatives, coarse mercury precipitates usually removed by the application of iodine solution. This is followed by thorough washing with sodium thiosulphate which acts to remove the iodine crystals. Sodium thiosulphate is then eliminated from the tissue by careful washing in distilled water.

As regards the röle of the this metal on the tissues and particularly on their proteins, it might be mentioned that Hg ++ behaves like other metallic ions in combining with the acid groups of proteins especially carboxyl and hydroxyl, and the posphoric acid of nucleoproteins. It differs from chromium in not forming complexes capable of binding together the adjacent protein chains and also from the majority of metals in its selective affinity for SH groups. If a small reactive SH groups it will react readily with these, and the quantity of a mercuric salt is added to a protein containing stability of the mercury – sulphur bond is greater than that between mercury and any other grouping. Some workers were able to prepare a fraction of serum albumen which contained one SH group per

molecule. When this was allowed to react with a mercury salt the resulting protein substance was found to contain – an atom of mercury per albumin molecule. The reaction which takes place can be illustrated as follows:

$RSH + HgCl_2$ ------- $RSHgCl + HCl$

(protein mercaptide)

$RSHgCl + R.SH \rightleftharpoons (RS)2\ Hg + HCl$

The second reaction is a slow one, and both reactions are reversible, but while the second can be reversed by any reaction which forms an undissociated mercury complex, the first is only reversed by reagents (e.., cysteine) which form equally stable mercury derivatives. But it must be pointed out that in preparatins including proteins not all the SH groups present are available for the reaction. However, the important point in cytochemistry is whether the reaction of mercury with the various protein groups is reversible by the ordinary processes of embedding, washing and removal of the coarse mercury precipitates by means of iodine and thiosulphate. no specifice investigations on this point have been made but it is certain that some mercury remains bound by the acid group in nucleoproteins and other proteins and it is probable that some remains bound to SH groups also. It is therefore advisable to avoid the use of mercurial fixatives in cytochemical work when critical work is carried out on nucleic acids or sulphydryl groups. Other protein reactions as Millon, Saguchi and tetrazolium salt can be employed.

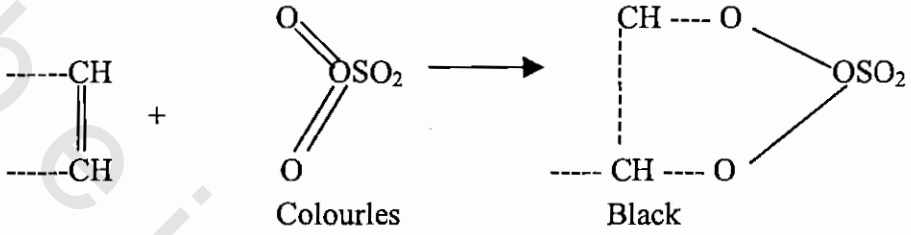
Osmium :

Osmic acid (osmium tetroxide, OSO_4) is used as a general cytological fixative particularly for the demonstration of the Golgi apparatus and mitochondria. Its use in histology is limited because of its poor penetrating properties, but it retained great interest due to its wide application in the preparation of tissues for electron microscopy.

As regards its rôle it has been assumed that nusaturated fats reduce OSO_4 with the formation of black compounds containing osmium or its hydroxide; and this has been suggested as being due to oxidation of the double bonds between adjacent carbon atoms.

Porter (1953) found that 2 percent OSO_4 forms gels with albumen, globulin and fibrinogen. The clear gel which forms relatively slowly with albumin is regarded as an indication of fine micellar or even unimolecular

binding. Wolman (1955) believes that the longer time of fixation produces certain damage due to over-oxidation. Also, it is well-known that alcohol extracts myelin sheathes of the nerve fibres completely, but this does not occur after treatment with OSO_4 which is explained as being due to complete binding of lipid and protein.



Colourles Black

CHAPTER 3

THE PROTOPLASM

Protoplasm is the living matter of which animals and plants are essentially composed. Huxley, in his famous essay of 1808, defined it as "the physical basis of life" since the activities of animals and plants are ultimately due to chemical and physical changes associated with protoplasm. The word protoplasm is commonly used for the various substances of which both nucleus and cell-body consist. The protoplasm of the nucleus is known as **karyoplasm**, while the extranuclear protoplasm is known as **cytoplasm**.

Inclusions of various sorts such as the yolk granules in eggs, the secretions in gland cells, the fat globules in adipose cells, etc. may be found in the cytoplasm due to the activity of either the cytoplasm or the nucleus or both. These inclusions produced by the protoplasm, but not actually part of it, are usually known as "deutoplasm" or "metaplasm". Protoplasms vary from one cell type to another and are specific for organs and for species.

Chemical coposition of protoplasm :

The structure of portoplasm has given rise to much controversy. Its exact composition is unknown since it is impossible to analyse protoplasm without killing and coagulating it by reagents and thereby bringing about in it some chemical changes. Besides, protoplasm is always associated with some of its products. Various types of cells also show chemical differences comparable to their morphological variations; thus a nerve cell differs from that of a liver cell, and so on. However, there are certain substances generally found in all tissues. These may be divided into organic such as proteins, carbohydrates and fats, and inorganic such as water and inorganic salts (or mineral ions).

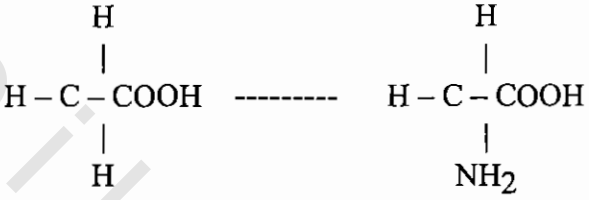
ORGANIC COMPONENTS OF THE CELL :

(A) PROTEINS :

Proteins are the most abundant organic compounds in animal protoplasm. They are charateristic of living substance and of substances derived from it, and they play a great part in tissue building.

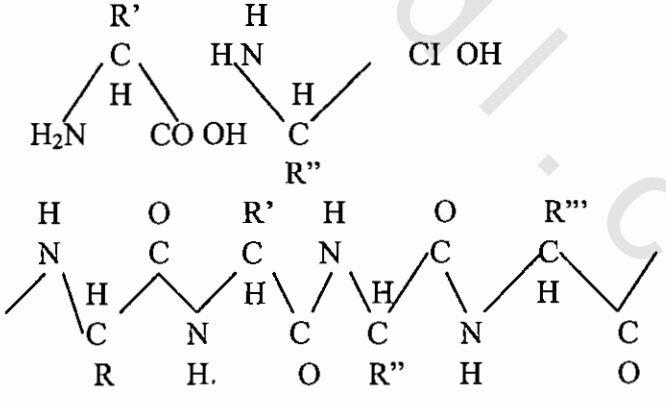
Proteins contain C. H, O and N usually with small amounts of S, P or other elements. Protein molecules are very complex and built up of amino acids. Since there is a great number of amino acids which are capable of great variety of combinations among proteins and therefore, it is impossible to have any chemical formula for protoplasm.

The **amino acids** are organic containing an amino group (-NH,2); in other words, amino acids are derived from aliphatic acids, such as acetic acid (CH3COOH), by replacement of an alpha hydrogen by the amino group.



Acetic acid Aminoacetic (glycine)

Amino acids can combine with each other to form long chains; this property is due to the presence of a carboxyl group (- COOH) and a basic amino group (-NH_2) in each molecule. Such substances, which contain at the same time acid and basic group, are called **amphoteric**. The condensation of amino acids to from a protein nolecule takes place in such a way that the acid group of one molecule combines with the basic group of another nolecule with the loss of H_2O.



R, R`,R", etc. represent radicals of different amino acids).

The importance of proteins in protoplasmic activity is partly due to their amphoteric properties, they may act either as acids or bases under proper conditions. In general most of them act during life as acids.

The amino acids are substances of very great physiological importance, since proteins contained in the food are broken down into amino acids during the process of digestion. These amino acids pass into the blood stream, then into the cells which require them, and finally they are built up by the action of intracellular enzymes into the proteins of the animal's body. The amino acids can be obtained from the proteins by hydrolysis with acids, alkalies, or digestive enzymes. Free amino acids constitute the so-called amino-acid pool from which the cell draws its building units for the synthesis of new proteins.

Types of Proteins :

Proteins may be divided into 3 main categories :

1 - Simple proteins which, on hydrolysis, yield exclusively amino acids. Of the simple proteins the most important are the albuminus, histones and protamines.

(a) **Albumins** are soluble in water and coagulable by heat.

(b) **Globulins** are soluble in acids, alkaline and salt solutions, but not in water.

(c) **Histones** are soluble in water but insoluble in dilute ammonia. Histones may combine with nucleic acid to form nucleohistones which are present in many cells as those of pancreas and thymus.

(d) **Protamines** which are soluble in water and incoagulable by heat. They may combine with nucleic acid to from nucleoproteins.

2 - Conjugated Proteins are those in which a simple protein is combined with another substance called prosthetic group. Th the conjuagated proteins belong the following.

(a) **Nucleoproteins** which are combinations of nucleic acid with proteins (i.e., in which the prosthetic group is formed of nucleic acids) and which play an important part in the cell. They are the chief constituents of the chromosomes. Different organisms vary from each other in respect to the character of their nucleoproteins.

(b) **Glycoproteins** (mucoproteins) in which proteins are combined with carbohydrates, e.g. nucin.

(c) **Lipoproteins** in which proteins are combined with higher fatty acids.

(d) Phosphoproteins, such as casein, in which proteins are combined with phosporus.

(e) Chromoproteins include a series of substances of great biological importance which are characterized by their particular colours. To these compounds belong the haemoglobin, haemocyanin and a series of respiratory enzymes such as cytochromes and flavproteins.

3 – Derived Proteins include coagulated proteins, as well as partly hydrolysed proteins. This group includes proteoses, peptones and polypeptides.

Rôle of proteins as mechanical supports :

Proteins are very important consituents of most of the skeletal structures which are very important in the mechanical support of the body such as the white and yellow fibres, cartilage, muscles, scales and fin rays.

In addition, the polarization microscopy reveals the presence of submicroscopic fibrillae in many structures such as cilia, flagella, myonemes of the protozoa, tails of spermatozoa, fibres of astars and spindles. The vibratile cilia and their extraellular roots contain protein particles oriented in the axial direction.

Many cytologists suggest the existence of a certain structure inside the cell which is refereed to as the "**cytoskeleton**"; this consists of a network of fine subicroscopic fibrillae distributed throughout the whole cytoplasmic matrix. These reticular fibrils are responsible for supporting the protoplasm and maintaining its mechanical properties. Besides, they regarded to be responsible for the thixotropic changes, namely reversible gelation and solation.

Reticular proteins were clearly demonstrated in some organisms as plasmodium. When this organism is pressed whith a microneedle there appear elastic and resistant fibres which offer certain resistance to the force exerted. Moreover, according to some workers, a certain structural proteinic cmponent known as ellipsin persists after all the soluble proteins have been extracted. This substance forms the basis for the permanent structures such as the cellular and nuclear proteins.

(B) CARBOHYDRATES :

The carbohydrates are compounds of C, H and O with the latter two in the ration of 2: 1, i.e., in the same ratio by which they are present in water. The most important carbohydrates in the animal protoplasm are glucose, galactose and glycogen. Carbohydrates taken into the body are transformed into simple sugars before being absorbed. These sugars are oxidized in the tissues and are the most important source of energy which can be used for different body functions.

Carbohydrates may be classified as monosaccharides, disaccharides and polysaccharides. The first two are known as sugars because of their sweet taste. They are soluble in water and alcohol and easily pass through semipermeable membranes. The polysaccharides, on the contrary, form

colloidal solutions with water, do not crystalize and do not pass across living membranes.

The monosaccharides are simple sugars with an empirical formula Cn (H_2O)n. The most important of the monosaccharides in the cells are pentoses and hexoses. Those are found usually combined with proteins and lipids. The pentoses are one of the main components of nuclear chromation. Among the pentoses, ribose and deoxyribose intervene in the constitution of nucleic acids. Glucose ($C_6H_{12}O_6$) is the hexose mainly involved in the energetic changes of the cell.

The disaccharides are the result of condensation of two molecules of monosaccharides with the loss of one molecule of water:

$$2\,C_6H_{12}O_6 \xrightarrow{-H_2O} C_{12}H_{12}O_{11}$$

or the disaccharides, the most important are sucrose (cane suger) and maltose (malt sugar) in plants, and lactose (milk sugar) in animals Maltose is built up of glucose; lactose is built up of glucose and galactose, and sucrose is formed of glucose and fructose.

Maltose = glucose + glucose
Lactose = glucose + galactose
Sucrose = glucose + fructose

The polysaccharides are formed by the condensation of many molecules of monosaccharides with a corresponding loss of water molecules :

$$n\,C_6H_{12}O_6 \xrightarrow{-nH_2O} (C_6H_{10}O_5)n$$

of the polysaccharides, the most important are starch and cellulose in plants and glycogen in animals.

Starch forms the reserve substance in plant cells and is synthesized from CO2 and H20 by means of chlorophyll.

Cellulose is the main constituent of most plant cell-walls and also enters in the formation of series of structures which from part of the supporting skeleton of plants.

Glycogen may be considered as the starch of the animal cells, and it is a substance of great importance in animals. It exists free in many tissues and serves as a source of energy in the body. Although glycogen is found in many tissues and organs, the largest proportion is present in the liver and in the muscle.

The amount of glycogen varies according to the diet, but normally it is about 3% of the total wight of the liver,. It is continually broken down and

synthesized in the organism from glucose molecules in the liver and from lactic acid in muscle (Pasteur- Meyerhof Cycle). It can also be synthesized from proteins and amino acids.

Glycogen is fairly soluble in water (15-20%) and may by dissolved in the protoplasm, It is difficult to demonstrate it in the living cell, but it can be demonstrated histochemically by the iodine reaction which gives a reddish brown colour with glycogen. In addition, glycogen gives a deep violet colour with periodic acid Schiffs reaction (PAS) and exhibits a dark red colour with best carmine staining.

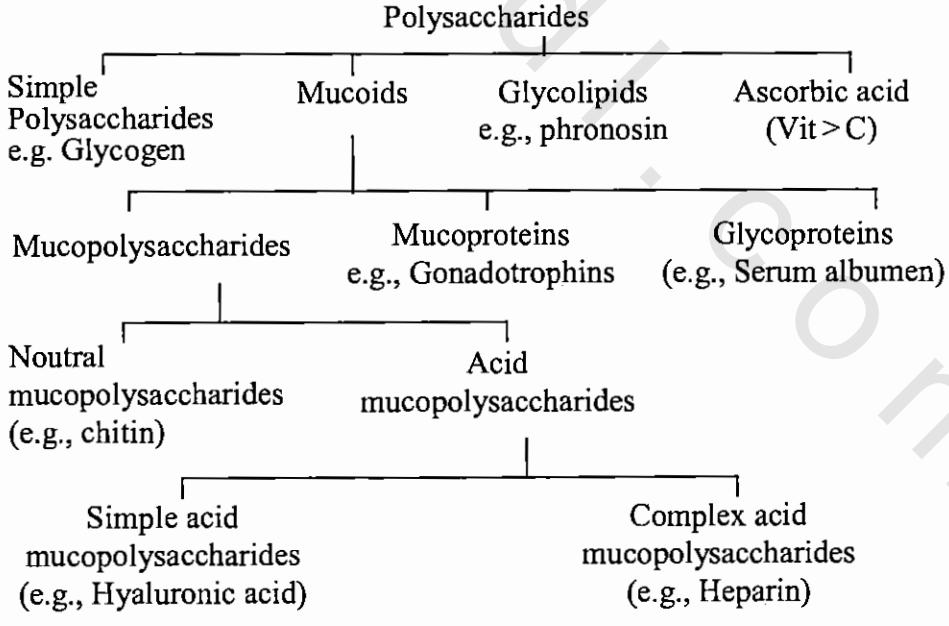
Mucopolysaccharides, Mucoproteins and Glycoproteins :

These different names include a vast number of compounds which are very important in the molecular organization of the cell and particularly of the intercellular substances.

Acidic mucoploysaccharides, and particularly hyaluronic acid, chondriotin – suphuric acid and mucoitin sulphuric acid are important in cytology. All the three substances are found in the ground substance of connective tissue where they act as binding and protective agents. The three substances also occur in the umbilical cord.

Hyaluronic acid is present in the synovial fluid, vitreous humour and aqueous humour. It is a binder of cells and it can be easily hydrolysed by the action of hyaluronidases.

Polysaccharides can be summarized as follwos:



NUCLEIC ACIDS :

As has been already mentioned the carbohydrates form compounds with other substandes such as proteins, amino acids and others. One group of compounds of particular interest for the cytologist is the nucleic acid group.

The nucleic acids are chemical compounds of great biological importance. All living organisms contain nucleic acids in the from of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Some viruses may contain only RNA, e.g., tobacco mosaic and poliomylitis viruses, and others contain only DNA, e.g., bacteriophages, vaccinia and adenoviruses. In bacteria and higher cells both types of nucleic acids are found.

DNA is mainly present in the nucleus and forms the major part of the chromosomes (about 90-95%) when the cell is dividing. During interphase, DNA is in the chromatin. In the mucleus, DNA is combined with proteins (histones or protamines) forming nucleoproteins.

RNA is found both in the nucleus and in the cytoplasm. It is found in small amounts in the nucleolus, chromosomes and chromatin. In the cytoplasm it forms a large part of the ribosomes.

(B) Components of nucleic acids :

Nucleic acids have a complex chemical structure. They result from the linkage of many units called **nuclctides**. Each nucleotide is composed of 3 molecules; a pentose sugar (ribose or deoxyribose) to which a molecule of phosphoric acid is attached on one side, and a molecule of nitrogenous base (purine or pyrimidine) on the other.

Within the nucleotide the combination of a pentose with a base constitutes a nucleodside.

Phosphoric acid links the nucleotides by joining the pentose of two consecutive nucleosides with an ester phosphate bond.

Pentoses include two types, one for each kind of nucleic acid: ribose in RNA and deoxyribose in DNA. Both ribose and deoxyribose have a bentagonal ring with five carbons.

Pyrimidine bases comprise mainly cystosine, thymine and uracil Cystosine is found in both RNA and DNA, while thymine is characteristic of DNA and uracil of RNA. Thus, DNA and RNA differ, not only in the structure of the sugar, but also in one of the pyrimidine bases.

Purine bases comprise mainly adenine and guanine which are common to both DNA and RNA.

Structurally, DNA molecule is composed of two polynuclcotide chains running in opposite directions and helically coiled about each other around a single axis. The chains are united by hudrogen bonding of their bases, in such a manner that adenine is only linked with thymine, and cytosine with guanine.

It should be noted that the varying sequence of the four bases along the DNA chain forms the basis of genetic information.

RNA plays an essential rôle in protein synthesis in the living cells of the body.

(C) LIPIDS :

The fatty constituents of protoplasm comprise the true fats and a number of more complex derivatives containing nitrogen and phosphorus such as the pospholipids. Lipids are relatively insoluble in H_2O, but soluble in organic solvents such as benzene, petroleum ether and chloroform.

Lipids can be classified as :

1. **Simple lipids** which are alcohol esters of fatty acids. Of the simple lipids the most important are **neutral fats** (glycerides), usually called **triglycerides** which are triesters of fatty acids and glycerol. Glycerol is a trihydric alcohol; its formula is:

$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array}$$

It can react with 3 molecules of an acid to from a triglyceride, e.g., it can react with butyric acid (C2H7COOH) to give a tributyrin which is the simplest fat occuring in butter.

$$\begin{array}{lcccl} CH_2OH & & HOCC_2H_7 & & CH_2.O.CO.C_3H_7 \\ | & & & & | \\ CHOH & + & HOCC_2H_7 & \text{-------} & CH.O.CO.C_3H_7 \quad + 3H_{20} \\ | & & & & | \\ CH_2OH & & OOCC_2H_7 & & CH_2.O.CO.C_3H_7 \\ \text{Glycerol} & & \text{Butyric acid} & & \text{Tributyrin} \end{array}$$

Hence, the general formula of a fat is :

CH_2. O. CO. R

|

CH. O. CO. R

|

CH_2. O. CO. R

where R varies according to the fatty acid present.

The triglycerides serve as stores of energy and are divided into fats and oils; the former are solid at $20C^o$ whereas the latter are liquid at this temperature. On hydrolysis with an alkali, the fat or oil yields fatty acids.

2. **Steroids,** include such substances as the sex and adrenal cortical hormones, vitamin D and the bile acids.

   The bile serve as protein denaturants and emulsifiers to aid digestion.

   Each steroid consists of an aliphatic ring system which may have one or more aliphatic unsaturated double bonds as well as various side chains.

   Steroids possessing an OH group are called sterols, **Cholesterol** is a widely distributed sterol and is found in bile, adrenal glands, etc.; and is also the principal constituent of the wool fat.

   Ergosterol is a sterol found in plants. It exists also under the skin and changes into the antirachitic vitamin D upon irradiation with ultra-violet light.

3. **Compound or conjugate lipids** contain PN or P and N, in addition to the C.H and O present in fats. Hence the complex lipids yield, on hydrolysis, other compounds in addition to the alcohol and fatty acids. Among these are:

(a) **Phospholipids,** these are fats containg phosphate and nitrogen such as lecithin, cephalin, sphingomyelin and the acetal phospholipids.

(b) Glycolipids or cerebrosides which are fatty acids combined with nitrogen containing carbohydrates. Cerebrosides and phosholipids are found principally in nervous tissue as essential consituents of myelin.

4. **Carotenoids** (lipochromes) which are red and orange cell pigments. They are insoluble in water but soluble in organic solvents. Among the carotenoids are carotenes found in carrots and grass, xanthophyll in leaves, vitamin A and egg yolk pigment.

5. **Other lipoidal substances** such as xanthocyanins in plants and certain melanin-like phenolic polymers which are soluble in organic solvents.

Visible and masked lipids :

It is of great importance from the cytological point of view to differentiate between "visible" lipids and invisible or "masked" lipids. The former generally are visible directly in the form of refractile droplets which give readily the typical reactions of lipids as staining with sudan dyes and blackening with osmium tetroxide. Masked lipids, on the other hand, do not give the usual lipid reactions unless they are set free by using unmasking procedures which sometimes consist of proteolytic enzymes.

Significance of lipids in body tissues :

The importance of fats in animal tissues and their rôle vary according to their location and disposition. Glycosides serve as stores of energy and may provide a protection against cold and injury. Lecithin is believed to play are an important role in the metabolic activities in the liver. Phospholipids and cerebrosides are found principally in the nervous tissue as constituents of the myelin. Of the steroids, the bile acids act to emulsify the fats, thus aiding in their digestion. Cholesterol is important in regulating the mechanical properties of epidermis and hairs.

Fat is present in most tissues as well as in the depöts. Depöt fat is mainly neutral fat (triglycerides), whereas the lipids of the tissues consist both of neutral fat and of phospholipids, the former is mainly stored fat but the latter are present as essential constituents of the structure of the protoplasm.

After plogonged fasting, the neutral fat content of tissues becomes greatly depleted, whereas the phospholipid content is much less affected. For example, the phospholipid contens of the brain remains high during starvation since the cerebral pospholipids are not stored materials but are substances essential for the biological activities of the tissues.

It is, therefore, clear that fats are grouped into two categories: (a) **constant fats:** these are mainly phospholipids ad do not disappear during fasting; (b) **variable fats**: these represent the depôt ones which are utilized by the fasted animals. Recent work indicates that, especially in the liver, phospholipid may be stored in the form of phospholipid-nucleoprotein complexes which play a central rôle in cellular netabolism and may be used during fasting.

There is a considerable increase in the fat content of the liver in certain cases, as in poisoning with arsenic, phosphorus, chloroform, carbon tetrachloride and some drugs as well as many infective processes. Generally, the liver plays an important rôle in fat metabolism. The normal liver contains about 4% of lipids of which 25% is essential fat and 75%

are phospolipids. The amount of neutral fat arises in the liver during the pesiods of starvation as fat passes from the depots to the liver to be oxidized. Later, as the fat stores become exhausted, the fat content of the liver falls gradually.

Inorganic Components of the Cells:

The inorganic constituents occur in the protoplasm in the form of salts or in combination with proteins, carbohydrates and lipids. They may be combined with amino acids to from hormones (thyroxine), or with proteins to form compounds such as haemoglobin (Fe), cytochromes (Fe), haemoeyanin (Cu), etc., or with purines or pyrimidines and a pentose in nucleotides.

Salts are dissociated into anions (e.g., CI) and cations such as Na+ and K+ which are important in regulating the osmotic pressure and maintaining the acid-base equilibrium of the cell.

The concentration of various ions in the intracellular fluid differs from that of the interstitial fluid, for example, the cell has a high concentration of K+ and Mg ++ while Na + and CI are mainly found in the exracelular fluid. The dominant anion of cells is the phosphate; and some bicarbonate is also present.

Calcium is found in the circulating blood and in cells as free ions, and combined with phosphate and carbonate ions in bone. Phosphate, also, occurs in the blood and tissue fluids; much of the phosphate is bound in the from of phospholipids, nucleotides and phosphoproteins. Other inos present in tissues are sulphate, carbonate, bicarbonate magnesium and amino acids. Ion is found in non-ionized from (metal carbon linkage) in haemoglobin, cytochromes and some enzymes such as catalse and cytochrome oxidase.

For the mentainance of the normal cellular activities, it is necessary that a well balanced equilibrium of different ions should exist in the medium.

Water:

Water plays a very important rde in the life of the cell. No single substance is of greater significance in the life of the organism than water. It acts as a natural solvent for mineral ions and other substances. It is a medium for reaction, and participates in reactions through hydrolysis and dehydration. It also acts as a medium for dispersion which is very important for the colloidal structure of the protoplasm. Water is also important in the exchange of substances between the cell and its environment as for example; the exchanges between the cell and the

lymph. It is used to absorb heat by virtue of its high specific heat coefficient and thus prevents drastic temperature changes in the cell.

Water constitutes about 75-85% of the protoplasm. The amount of water in the cell varies greatly under different conditions and also varies from one tissue to another. The quantity of water in the cells varies also with age; in older individuals there is less water in tissues than in younger.

Water exists in free and bound form within the organism. Free water represents 95% of the total cellular water with the exception of bone cells; it is the chief solvent in the cell and it is a medium for metabolic processes.

Bound water (5% of the total cellular water) is mainly tied to the polar groups of proteins; in other words, there is a considerable portion of the water in the cell physically "bound" in the colloidal structure of the protoplasm, and must be considered as integral part of the living system. The bound water resists the effects of very low temperatures, remaining unfrozen after the free the water has crystalized. It is probably that crystalization of free water that kills the protoplasm at low temperatures.

PHYSICAL CHARACTERISTICS OF PROTOPLASM :

Protoplasm is a complex colloidal system since it possesses the properties of colloids. **A colloid** consists of extremely fine particles called **dispersed phase** suspended in another substance called **cotinuous phase** or **dispersion medium**. Dissolved substnces behave differently as regards their capacity for passing trough organic membranes such as parchment paper. Some substances like salts and sugars pass through such a membrane, whereas others such as starch in solution, egg-albumen or gelatin do not traverse such membranes. The first class of substances is known as **crystalloids** and the second as **colloids.** Colloids give shapeless masses of material when evaporated, whereas crystalloids on evaporation yield crystals of diffinite shapes.

There are two types of colloids; one is known as **suspensoid,** e.g., Indian ink in which small particles of carbon forming the dispersed phase are held in suspension in a fluid. The second type of colloids is known as **emulsoid** in which both phases are liquids as in milk which consists of small particles of fat (cream) suspended in water. Although both phases in emulsoids are liquids they do not mix with each other.

In most emulsoids the two phases can change places, e.g., a solution of gelatin in water below certain temperature, the continuous phase is gelatin

and the colloid is more or less solid. In this condition, it is known as gel, but when temperature rises the water becomes the continuous phase and the colloid becomes more or less fluid and is a now known as sol. Protoplasm shows the same behaviour, that is to say, under the influence of internal and external stimuli protoplasm is capable of conversion from gel to sol and vice versa; and thus the protoplasm is a **reversible emulsoid.** In other words, the consistency of protoplasm may vary in different cells and from moment to moment in the same cell from that of a freely flowing fluid to a rather firm gel. This change is reversible and is often spoken of solation and gelation as a **reversible gelation** (sol --- gel). Solution and gelation are the basis of amoeboid movement.

The most important character of protoplasm as a colloidal system lies in the fact that it possesses numerous very small particles dispersed in the continuous phase. This condition affords a very large surface between the particles of the dispersed phase and the continuous phase, and since most of the reactions take place at such surface the colloidal nature of protoplasm is of great importance.

As regards the structure of protoplasm, there have been many theories:

The firs **(fibrillar theory)** regarded the protoplasm as consisting of fibres which are continuous, and form a reticulum through out the cell. This reticulum is enclosed in a fluid, like a sponge in water. Some investigators believed that the fibres are discontinuous.

The fibrillar theory was followed by the **alveolar theory** which regarded the more solid portion of the protoplasm as forming a foam which encloses the fluid in cavities or alveoli.

The third theory, the **granular theory,** claimed that the protoplasm consists of graules (bioblasts of Altmann) suspended in a fluid.

It ha been shown, however, that the apparent distribution of protoplasm in threads, bubbles or granules is due primarily to the coagulation of protein by different agents. Thus, any study on the structure of protoplasm must be carried out on the living cell.

CHAPTER 4

GENERAL STRUCTURE OF THE ANIMAL CELL

The animal cell may be defined as a mass of protoplasm surrounded by a delicate membrane and containing one or more nuclei at least through a stage of its development. The protoplasm of the cell- body is usually known as **cytoplasm** or **cytosome**, while that of the nucleus is referred to as **karyoplasm.**

It has been found that in any species there is a relationship between the size of the nucleus and the size of the cytoplasm, this is known as the **"Karyoplasmic ratio" or 'Nucleo – cytoplasmic ratio"**. This has been first originated in protozoa and then extended to other animals. The relation usually holds in cells which are homologous (i.e., of similar origin and function) as shown in sea-urchin larvae either from fertilized or unfertilized eggs. However, under different conditions this ratio may vary. The karyoplasmic ratio has considerable importance in the life of the cell, and is probably one of the factors which brings about cell- division. (Fig. 17)

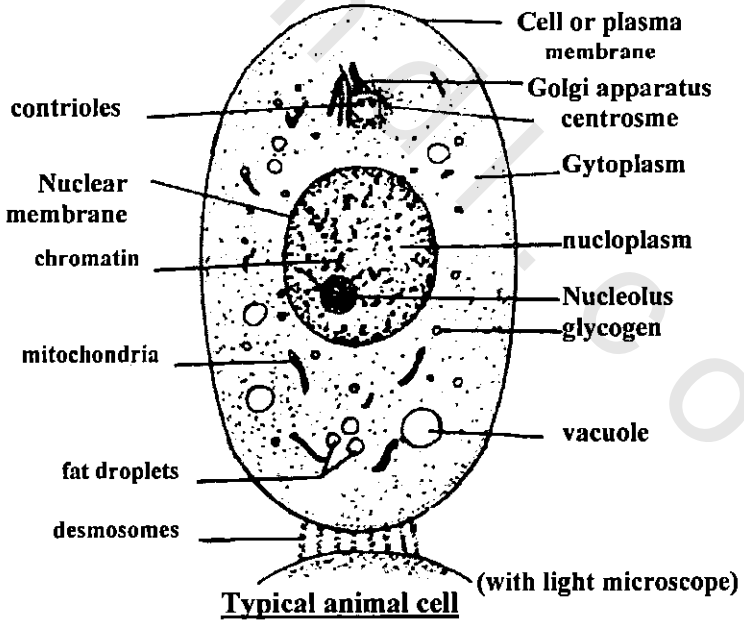


Fig. 17: As seen by light microscopy.

The animal cells vary in from and structure according to their location and functional activities. Some of them are of variable forms as the white blood cells, and others are of stable from as the epithelial cells and nerve cells. The shape of the cell is dependent upon many factors such as functional activities, viscosity of the protoplasm, surface tension and the mechanical pressure due to adjoining cells.

Animal cells show, marked variation in size. The diameter of the red blood cell, for example, is nearly 7.5μ. whereas other cells of human body range in diameter of human body range in diameter from 2ooμ – 15,000 μ. The eggs of birds are seen by the naked eye; and in some large animals the nerve cells may reach several feet in length. Function and age are the two major factors which influence the size of both cell and nucleus.

The cytoplasm :

The term cytoplasm or cytosome is applied to all the protoplasm lying outside the nucleus. It is limited externally by the **plasma membrane** or **plasmalemma** which is the outer living part of the protoplasm and is usually invisible. The plasma membrane is a thin membrane which plays an important role in the regulation of the exchange of materials which occur between the cell and its surrounding. Outside the plasma membrane of some cells occurs a non- living substance which is a secretion product; this is known as **extraneous** coat in animal cells and cell-wall in plant cells.

The **fundamental** or **ground cytoplasm** (also named hyaloplasm) appears, in living cells, as homogeneous, structureless substance in which certain structures such as the **Golgi bodies,** the **mitochondria** and the **cell centre** can be distinguished. These structures are specialized parts of the living material and are usually known as **organoids** or **organelles.** Besides, the cytoplasm contains a number of **inclusions** which are formed as the result of the activities of the protoplasm, i.e. they are non protoplasmic and temporary and usually known as **metaplasm, deutoplasm, paraplasm** or **inclusions.** The inclusions comprise accumulations of material either elaborated by the cell or resulting from catabolism or ingested substances. Such inclusions are fat droplets, secretion granules, yolk globules glycogen, pigment fat granules, crystals, etc. Some cells, especially the old, may contain vacuoles of different sizes.

The ground cytoplasm or hyalopasm, in electrom micrographs, contains a very delicate structure known at **the endoplasmic reticulum**. This is a system of tubular and vacuolar elements surrounded by very thin membranes. The endoplasmic reticulum, thus divides the ground cytoplasm into an inner phase separated by a membrane from an outer phase which is the **hyaloplasmic** or cytoplasmic matrix. This matrix is therefore, the continuous phase of the ground cytoplasm that surrounds all other components present in it such as the Golgi apparatus, the mitochondria, the endoplasmic retuculum, etc.

The nucleus:

The mucleus in the interphase (the phase between two successive divisions) contains the **chromatin** substance; some of the larger flakes of chromatikn are called **chromoscentres, karyosomes** or flase mucleoli because they are morphologically similar to some nucleoli. The chromatin substance is distributed through out the nuclear sap (nucleoplasm). In addition, there are one or more spherical bodies, **the nucleoli** which differ from the karyosomes in some staining properties and in chemical composition. The nucleus in surrounded by **the nuclear envelope** (nuclear membrane).

The cell which we have already described is a typical cell in which the nucleus and the cytoplasm with its organoids are found. Such cell which contains a true nucleus is referred to as **eukaryotic cell** (Gr.: Karyon = nucleus), but if the nuclear envelope is lacking, and thus the nuclear substance is in direct contact with the rest of the protoplasm, the cell is called **prokaryotic cell** (e.g. bacteria, some algae and most viruses).

However, the electron microscope has shown many cellular details as shown in figures (18 & 19).

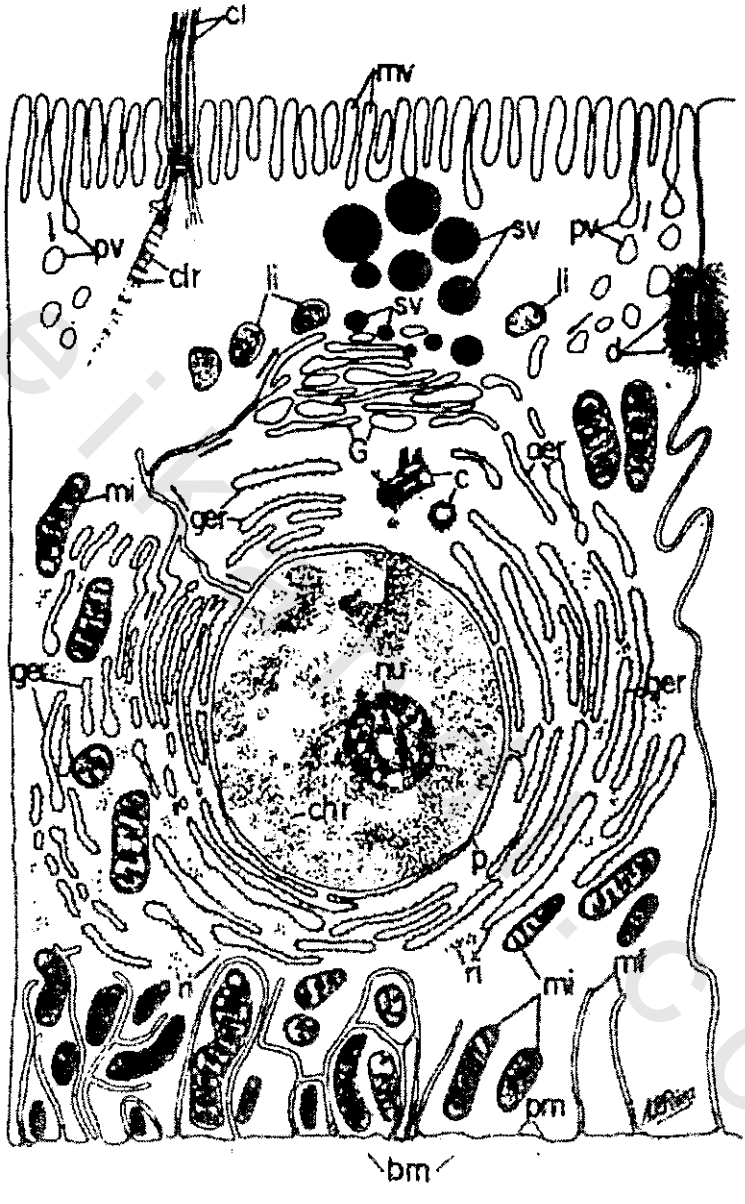


Fig. 18:

A hypothetical cell illustrated by The Electron Microscope Ci, Cilia; Secretory vesicles; desmosome; G, Golgi elements; C, centriole; mi, mitochondria; ger, granular E.R, nu, nucleus; chr, chromatin, p, pore.

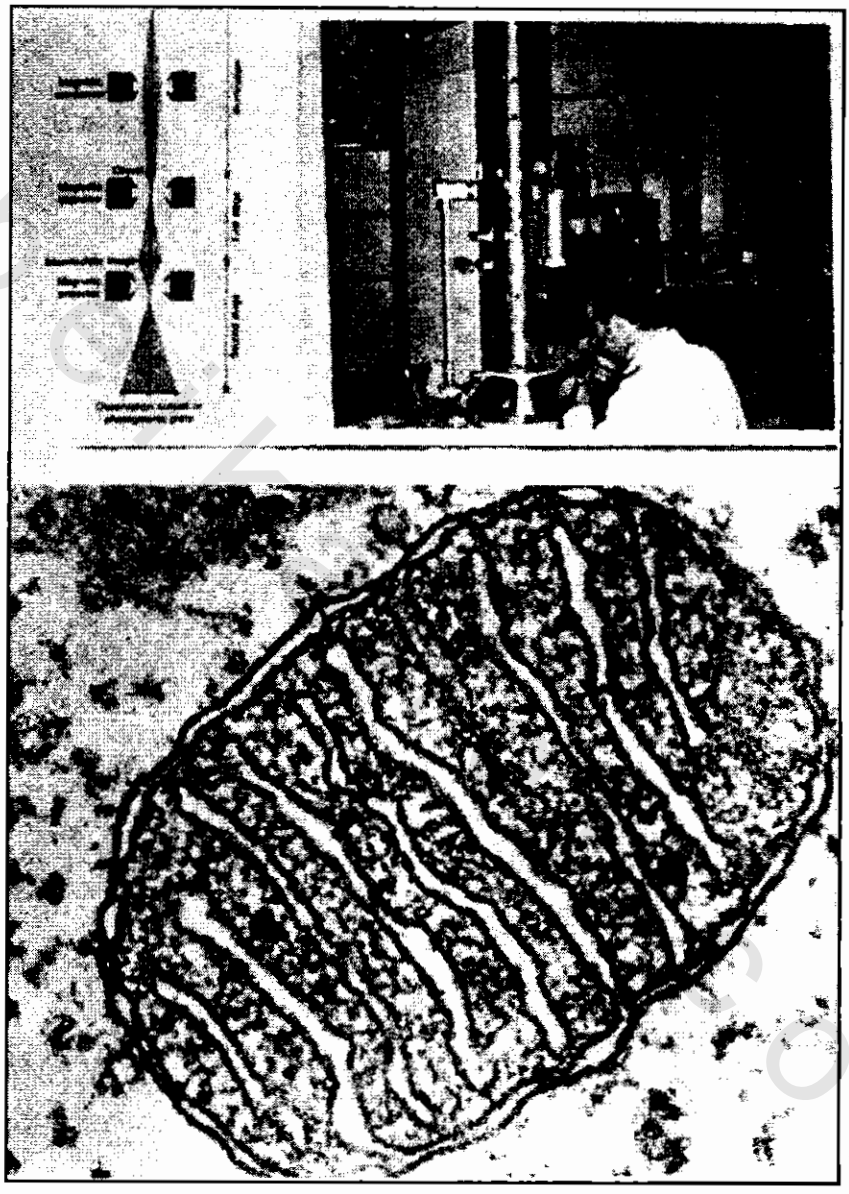


Fig. 19 : A minute granular mitochondrion seen by electron microscopy.

CHAPTER 5

THE PLASMA MEMBRANE

As early as 1855, it was recognized that the outer limiting membrane of the cell or plasma membrane (sometimes known as the cell membrane) plays a unique role in the life of the cell. This membrane is essentially a premeability barrier that controls the passage of molecules and ions between the cytoplasm and the surrounding medium.

Although the plasma membrane is so thin it cannot be observed by the light microscope, yet its presence was revealed by microsurgical experiments. For example, if a cell is punctured by a microneedle the cytoplasm flows outside the cell; this indicates the presence of a limiting surface membrane. Also, if a dye is injected into a cell by a micropipette this coloured material remains inside the cytoplasm and does not diffuse to the exterior.

In some cases, the plasma membrane may become clearly identified as in the case of the eggs of sea-urchins in which the penetration of the sperm into the egg is followed by the separation of a thin membrane which is presumably the plasma membrane becomes thickened forming the fertilization membrane (Fig. 20) which prevents penetration of other sperms into the fertilized egg. The space, left between this membrane and the surface of the egg is known as the perviteline space.

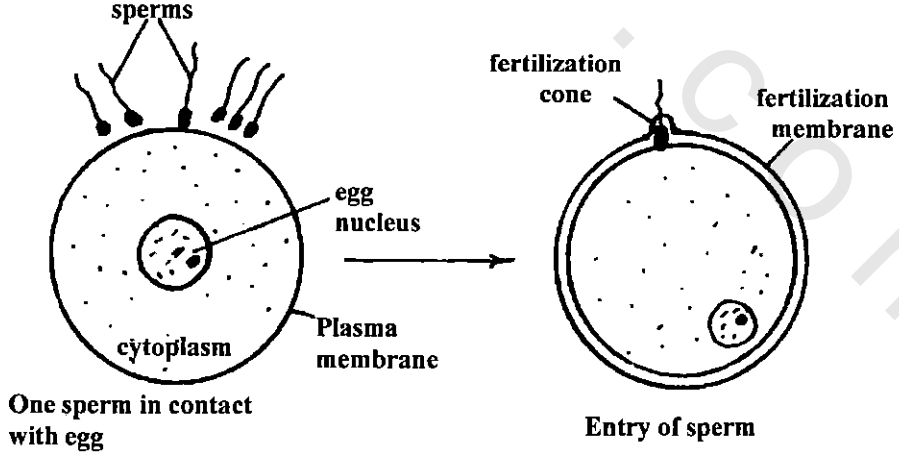


Fig. 20: Diagram showing the fertilization membrane.

Besides the cell membrane, there exixts in many plant cells an outer visible thick wall which covers or adheres to the plasma membrane of plasmalemma. In animal tissues, what is seen separating the cells, and thought to be the surface membrane, is nothing but the cementing substance adhering the cells together.

Structure of the plasma membrane:

Early investigators showed that the plasma membrane is composed of a thin layer of lipid. This was obtained form the studies of cellular permeability of sea-urchin eggs, red blood cells and muscle fibres. These studies showed that the penetration of molecules into the cells depends, to a great extent on their solibility in lipid substance. The more these substances are soluble in such solvents and the more rapildy they penetrate into the cell. For example, urea and ethers were found to penetrate too rapidly; glycerol shows moderate penetration; however, other substances; such as galactose never penetrate. Further biochemical investigations showed the presence of a protein subtance in the plasm membrane. The presence of fibrous proteins in the plasma membrane was indicated from its physical properties, such as its elasticity, mechanical ability to expand and contract and its surface tension.

As regards the molecular organzation of lipids and proteins in the plasma membrane, some investigations have introduced the view that protein molecules are oriented in the plane of the envelope surface, whereas lipid molecules are oriented radially.

Danielli (1952) proposed that lipids are arranged in the form of a bimolecular layer, that is, two layers of lipid molecules, these layers are sandwiched between two protein layers. The protein layers consist of polypeptide chains; each chain being about 50 m U and are attached to similar chains by hydrogen bonds which actotie the various parts of the membrane together. The polypeptide chains are regarded to be largely responsible for the elasticity and mechanical strength of the plasma membrane.

This view was later modified by the same author (Danielli, 1954) who declared that the plasma membrane consists of a double layer of lipid molecules separating the two proteing sheets. The lipid layer is not continuous, but is interrupted by certain pores (7 A^{o} in diameter) which permit the penetration of non-lipoid substances into the cells (Fig. 21).

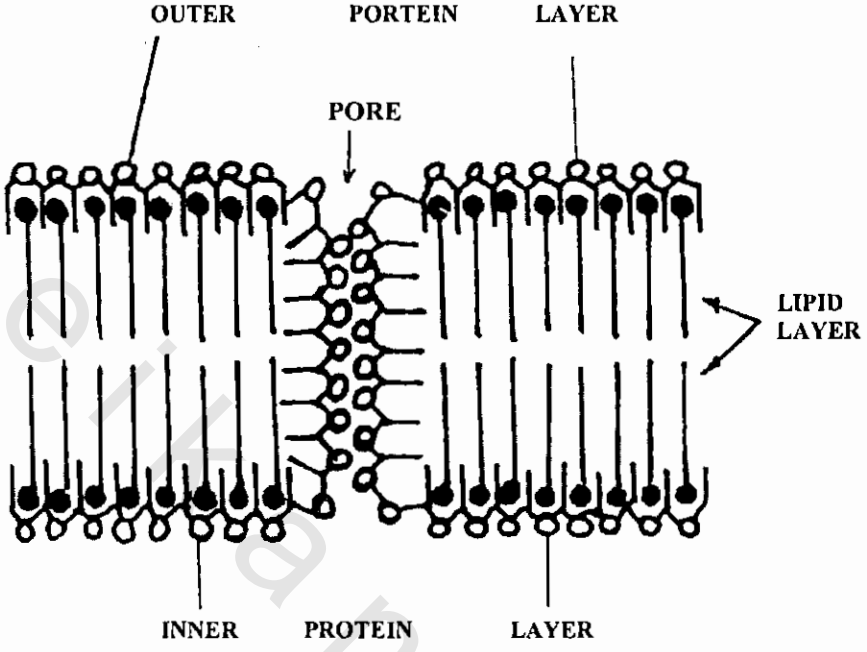


Fig. 21 Diagram of molecular structure of membrane (after Damielli, 1954)

The presence of pores in the plasma membrane was later clearly demonstrated by the electron microscope.

Robertson (1959) established that the plasma membrane appears in electron micrographs as a triple-layered structure consisting of two dense bands separated by a central clear zone of lipid (Figs. 21 and 22). He suggested the well known nomenclature "the unit membrane" or the "tripartite structure" for the plasma membrane. The tripartite structure shown in electron micrographs coincides with the hypothetical model of the molecular configuration of the plasma membrane put forward by Danieli. The total thickness of the unit membrane is in the range of 75-100 A^{o}. The lipid layer is 25-30 A^{o} thick A^{o} the outer protein layer is 25 A^{o} thick and the inner one is 25-35 A^{o} thick, depending on the procedures used. The middle layer is a bimolecular layer of lipids, with its non-polar hydrophobic groups facing inwards and its polar hydrophilic group(-COOH) facing outwards. The outer layer of the tripartite membrane has a surface coating of mucopolysaccarides called the glycocalyx.

The plasma membrane in electron micrographs possesses a morphology which depends, to a large extent, on the fixatives, embedding medium, stain used and the thickness of the section.

The fluid mosaic theory:

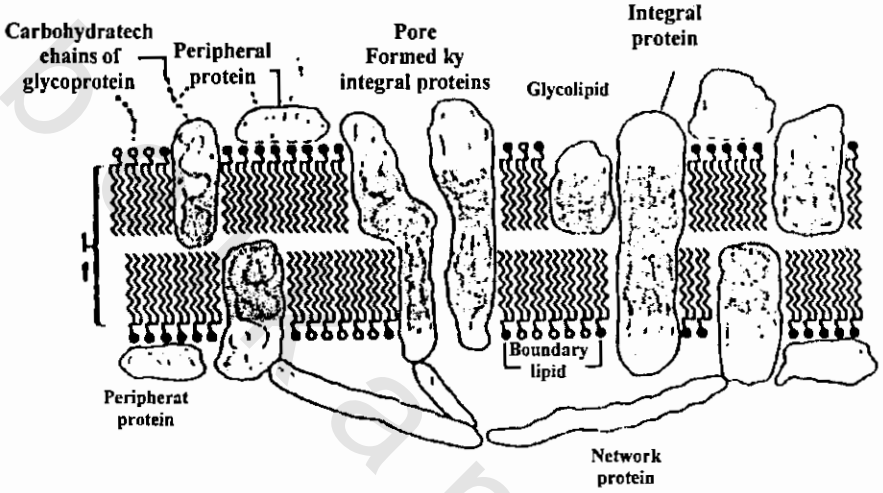
The fluid mosaic model presented by Singer and Nicholson (1974) concerning the membrane organization is explained as follows:

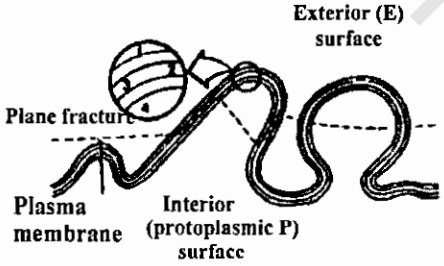
- Membrane lipids are arranged predominently in the form of a bilayer (double layer) which is frequently interrupted by the presence of embedded proteins.

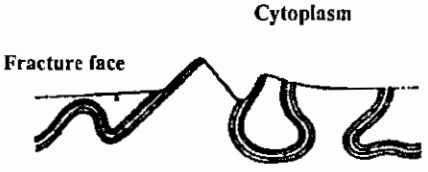
- Membrane proteins, of two kinds: integral proteins embedded in the lipid bilayer in the form of mosaic arrangement, and peripheral proteins bound to the surface of that bilayer.

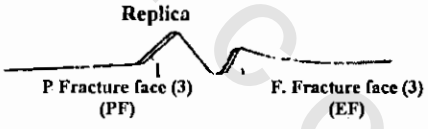
- The lipid bilayer is essentially a fluid substance permetting lateral mobility of both the lipid protein molecules, and hence they are capable of transitional movements within the whole bilayer. (Fig. 22).

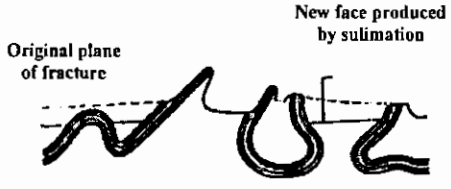
The fluid mosale model of membrane structure.











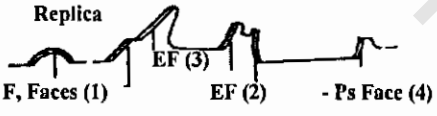


Fig. 22: Membrane faces.

Chemical compositions of the palsma membrane

Lipids :

The universal presence of lipid bilayer in biological membranes is well known inspite of the widespread differences in the particular kinds of lipids involved. However, there is a wide range of variation in the lipid-protein ratio in the different cell membranes. In most of the membranes, the majority of lipids is formed of phospholipids although glycolipids predominate in myelin membranes. Also, cholesterol is a significant component of animal plasma membranes.

The majority of phospholipids present in the biological membranes consits of phosphatidyl choline, phosphatidyl ethanolamine and sphingomyelin.

It has been proved that the distribution of the phospholipids is highly asymetrical. It is assumed that this asymetry is rather stable and there is no exchange of lipids across the lipid bilayer.

Carbohydrates :

Hexose, hexosamine and sialic acid are bound to the outer surface of the plasma membrane of some cells such as the hepatic cells. A small amount of sialic acid – in the form of gangliosides – aslo exist in the plasma membrane of the liver cells. However, gangliosides form the essential constituents of the plasma membrane surface of nerve cell.

Proteins :

Membrane proteins are usually classified into:

Integral (intrinsic) and peripheral (extrinsic) proteins. Intergral proteins are embedded in the lipid bilayer and could be removed or extracted by certain specific treatments, where they were estimated to constitute about 70% of the two protein types.

Integral proteins are bound to the surface membrane by certain hydrophobic interactions with the ends or tails towards the lipid bilayer and may either extend along the bilayer completely or only embedded in one of the two sides of this layer. Such integral proteins must be in the form of amphipathic molecules whose hydrophobic regions are exposed at the membrane surfaces. Such arrangement is thermodynamically stable and accounts for the major membrane proteins including most of the membrane – associated enzymes, receptors and antigens.

Peripheral proteins are bound to the membrane - by relatively weak interactions with the hydrophilic regions of the lipid bilayer. Such proteins are easily removed from the membrane surfaces. These peripheral proteins do not cover the entire surface of the lipid bilayer, but leave many vacant or empty spaces where the lipid head regions are exposed at the membrane surface.

Relation of lipid and protein in some cell membranes:

Tissue	**Protein %**	**Lipid %**
Human central nervous system (CNS)	20	79
Bovine CNS myelin	23	76
Rat skeletal muscle	65	45
Rat liver	60	40
Human erythrocytes	60	40
Rat liver mitochondria	70	29

Membrane fluidity :

The first indication that the cell membrane may be a fluid rather than a rigid structure came from studies using electron – spin resonance (ESR) spectroscopy to monitor the mobility of the phospholipid molecules within the lipid bilayer.

The fluidity of the lipid bilayer has also been investigated by a certain differential scanning calorimentry technique. This technique applies the fact that the transition between different physical states, e,g., from solid to liquid or liquid to gas is accompanied by the uptake of heat. The transition temperature from solid to liquid for most biological membranes – is below the physiological tempreatures at which they normally function. This indicates that under physiological conditions, it is in the liquid state and 70-90% of the total lipid is in the liquid state. The remaining lipid is insoluble as it is tightly associated with the membrane proteins.

Modifications of the plasma membrane :

Light microscope observations have revealed that regions of the cell surface of certain cells are involved in many physiological process such as absorption, secretion and fluid transport.

Electron microscopy has permitted a much better analysis and interpretation of these cell differentiations. Topographically they are described as:

I - Specializations of the apical cell surface:

The so-called striated border of the covoluted tubules of the kindney seen by the light microscope are but slender processes called **microvilli** (0.6,0.8 ų long and 100 m ų in diameter). The electron microscope shows that the apical plasma membrane of these cells project into these slender processes or microvilli. This microvilli increase the surface of absorption of the cell, and the space between them form a kind of sieve.

II - Specializations of contact surfaces between cells :

In epithelial rissues there four types of contracts at the sides of these cells. These are:

(a) Desmosomes or Macula Adherens :

They are found in a number of epithelial cells and appear, under the light microscope, as darkly stained bodies. Thes were considered as intercellular bridges. Fine tonofibrils converge upon the desmosome and were believed to pass through the bridge. The electron microseope shows that there is no continuity between the cells. The desmosomes (Figs: 25 – 29 – 30) are local thickenings of the opposing membranes. Tonofilamnets radiate from these thickened regions into the cytoplasm. The intercellular space contains a central disc or line. Usually there are regions of looser contact between the desmosomes and even real intercellular spaces for free circulation of fluids.

Fig. 23:

Electron micrograph of two adjacent plasma membranes of interstial cells, Note the triple – layered structure of each membrane. Arrows point to some pores. (From Kurts: Electron Microscopic Anatomy). Magnification: X 360,000.

Fig. 24: Electron micrograph of the human red blood cell plasma membrane, The arrows indicate the three – layerd (dense – light – dense) unit membrane structure of the plasma membrane. (Fom Robertson) Magnification: X 187,000.

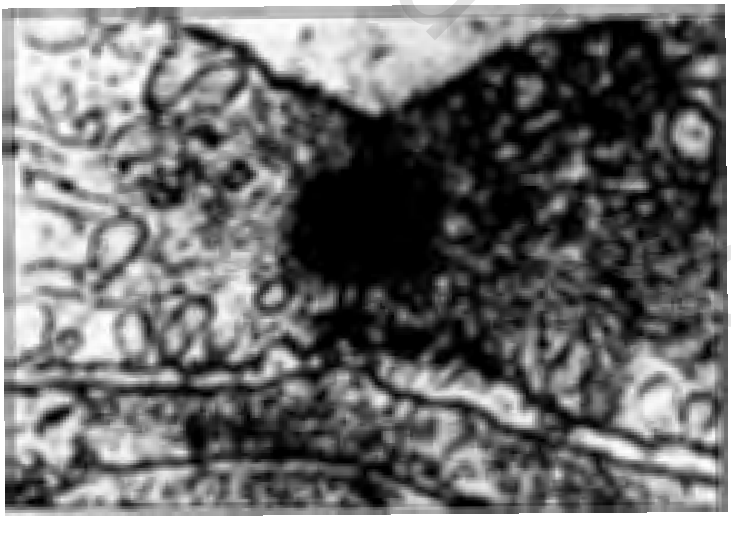


Fig. 25: Desmosomes.

Fig. 26: Intestinal epithelium showing microvilli, (From Fawcet: the Cell: Maginification: X 14,000.

(b) Terminal bars or Zonula Adherens :

These are similar to the desmosomes, but the tonofibrils are lacking.

(c) Tight junctions or Zonula Occludens :

Here the adjacent cell membranes have fused and, therefore, there is no intercellular space.

(d) Synaptic junctions :

Synapses represent the physiological continuity between neurones.

(e) Plasmodesmata :

Also representing a certain type of junctions.

III - Specializations of the cell base :

At the cell base of certain cells involved in rapid water transport, numerous infoldings of the palsma membrane penetrate deeply into the cytoplasm. These folds form septa which branch and anastomose to divide the cytopasm into compartments which frequenlty contain mitochondria. (Fig: 27).

Coats of the plasma membrane :

A layer of material may or may not loosely cover the surface of the cell and lies outside the plasma membrane. Electron microscopy reveals that there is generally a gap of about 100 A° between the plasma membranes of two apposed cells. This gap may contian glycoproteins and other polysaccharides in the form of hyaluronic acid. This covering is known as glycocalyx.

Functional significance of plasma membrane :

The plasma membrane acts to contorl the penetration of the dissolved substance to the cell and prevents the diffusion of protoplasm outside the cell. Such phenomena are known collectively as permeability which can be defined as "the rate of movement of a substance through a permeable layer under a certain driving force".

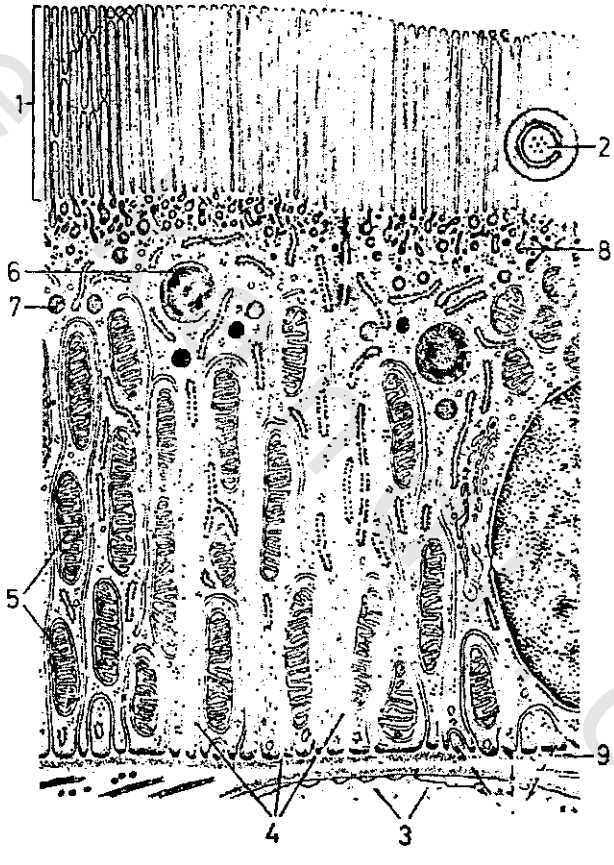


Fig: 27: Microvilli (Brush border of a proximal tubule epithelial cells of the rat kidney:

1 – Microvilli
2 – Tranverse section of microvilli
3 – Blood capillaries
4 – Connective tissue
5 – Mitochondria
6 – lysosomes
7 – Perixosomes
8 – Pinocytotic vesicles
9 – Basement lamina

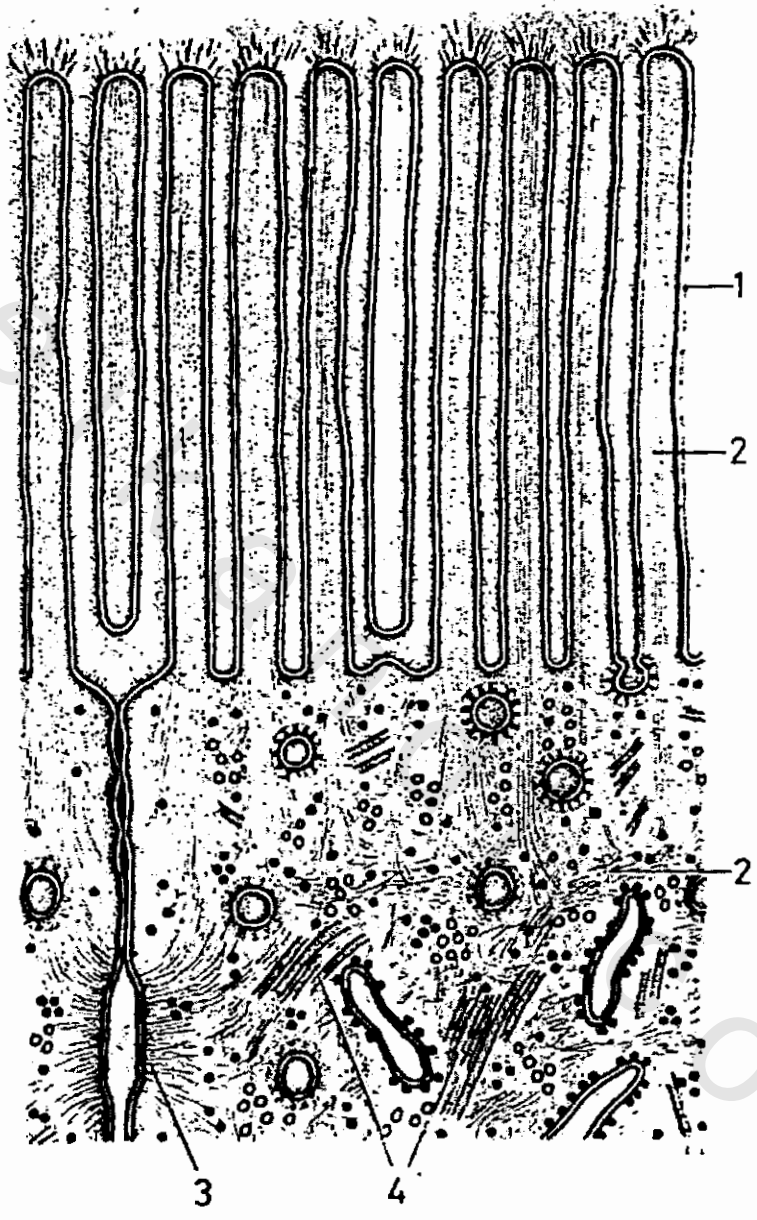


Fig: 28 : Microvilli (Brush border)

1 – Microvillus projections. **2 – Microfilaments.**

3 – Zonula adherens. **4 – Microtubules.**

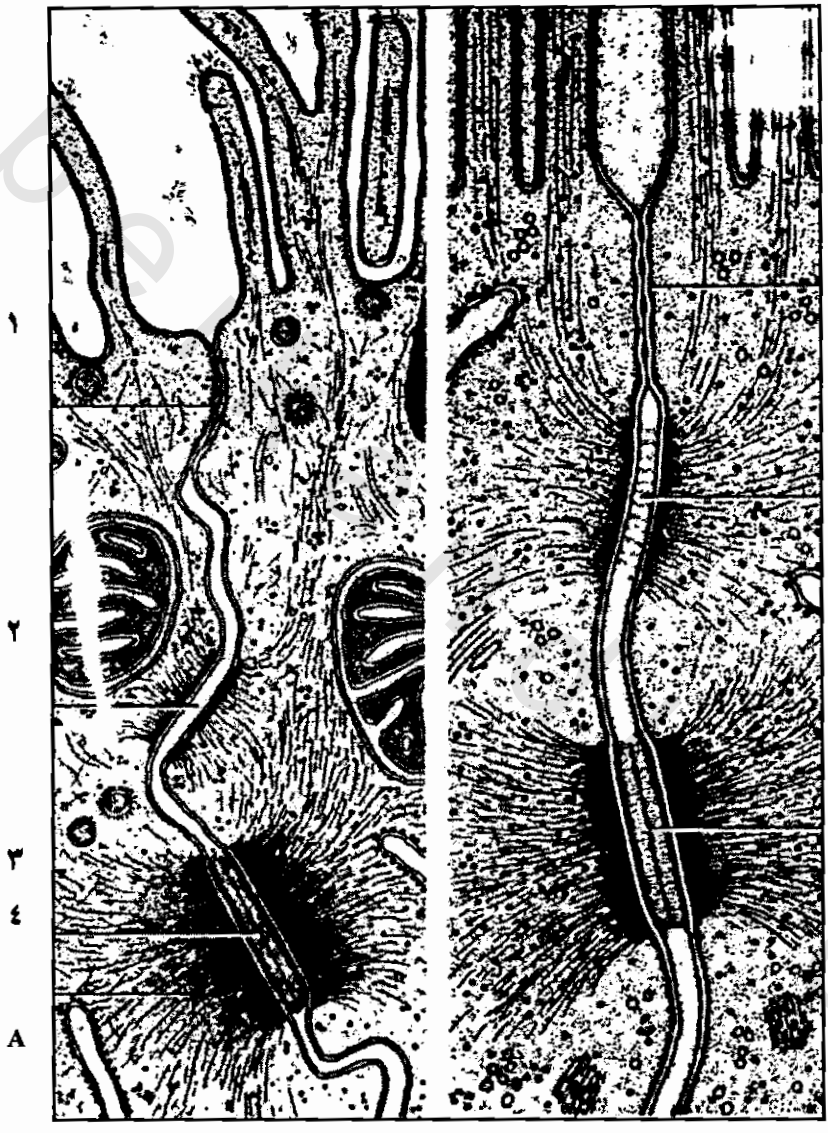


Fig: 29 : Intercellular junctions (Tracheal cells of rat).

1 – Zonule occludens. **2 – Zonule adherens.**

3 – Maculae adherens. **4 – Tonofilaments.**

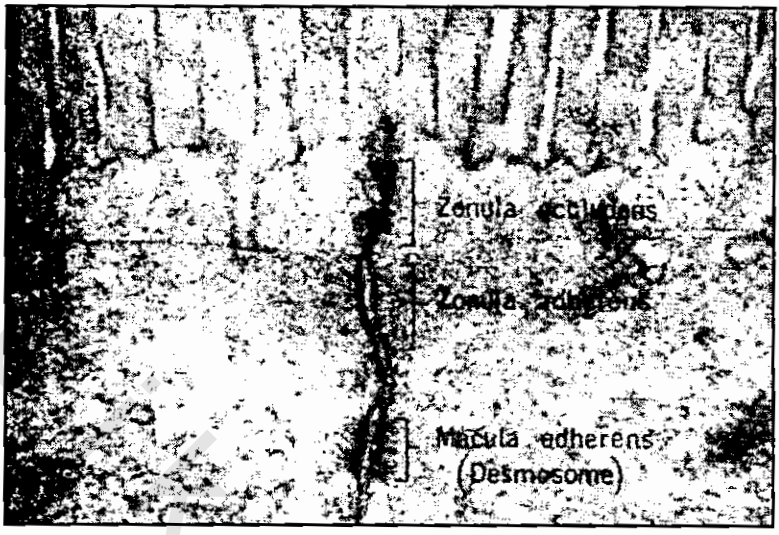


Fig: 30

Intestinal epithelium showing junctional complexs of epithelia. Magnification: X 47,000.

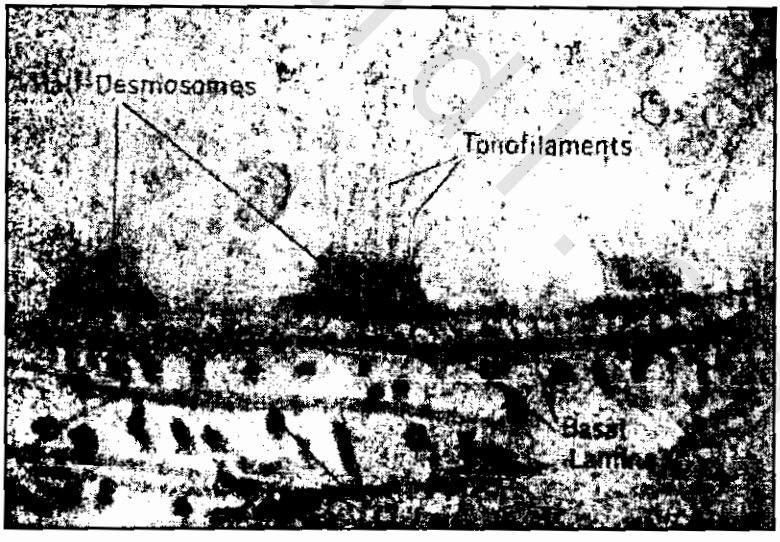


Fig: 31

Basal surface of a cell in the epidermis showing the tonofilaments. (From Fawcett; after Hay). Magnification: X 42,000.

Permeability is of fundamental importance as it is the mechanism which regulates the entrance of certain substances which are essential for the synthesis of the living materials. It also regulates the out flow of excretory materials and water which are eliminated from the cells.

Cellular permeability depends on many factors such as the physiological state of the cell as well as the various external conditions, e.g., the tonicity of the medium, temperature, and others.

As the palsma membrane is the immediate contact between the cell and its surroundings, so, the major part of the exchanges between the cell and its environment is through active or passive transport of ioins and molecules. The active ingestion of larger particles and of solutes by the cell is carried out through phagocytosis or pinocytosis.

The plasma membrane is involved not only in the uptake of solids and solutes, but also in the passage of cellualr products (i.e., secretion and excretion) to the extracellular fluid.

Merocrine secretion which involves the fusion of the bounded membrane of the secretory ganule with the plasma membrane, the content of the secretory granules there by becomes extracellular. The process takes place during the release of zymogen granules from the pancreas and in many other exocrine and endocrine glands (Fig. 32).

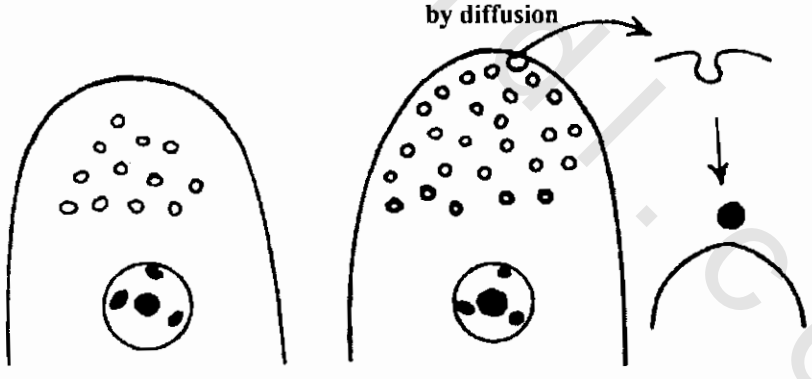


Fig: 32 : Cells showing merocrine section.

APORINE secretion is a completely different mechanism found in the submandibular sweat gland of rabbit. The luminal surface of the cell bulges outwards to form a secretory projections. This is a spherical body attached to the cell by a narrow stalk. The secretion body is then set free (Fig. 33)

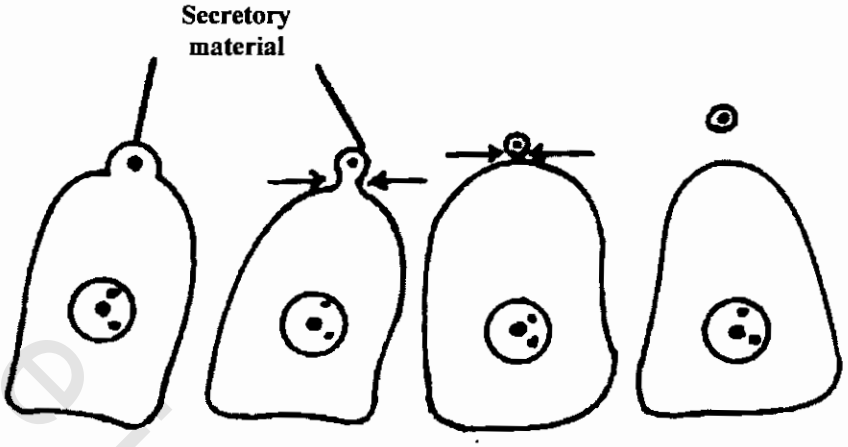


Fig: 33 : Cells showing apocrine secretion.

Mechanism of permeability :

Several ideas have been introduced to explain the cell permeability. These can be summarized in the following:

1 - According to Danielli (1954), certain substances enter the cell through the membrane pores, and others pass by simple diffusion. The latter are supposed to be those soluble in lipoidal material. Of the non-lipid-soluble substances, those with smaller molecular size pass through the pores more rapidly than those of the larger size.

2 - Some investigators declared that there is a strong relationship between the permeability and the metabolism of the cell. In other words, penetration of a substance is facilitated when this substance is needed for cellular metabolism. For example, an amino acid penetrates easier when it is to be incorporated into the cellular proteins. Also, in case of sugars, molecules of glucose diffuse with great ease while other sugars do so with difficulty. This is due to the fact that glucose is strongly needed for the general metabolism of the body and it is mostly utilized as soon as it enters the cell. On the other hand, sugars as sucrose which are metabolized very slowly also penetrate very slowly into the cell.

3 - Another view regards that penetration of ions is often linked to the respiration of cells. Hence, respiratory enzymes are of special importance in this respect. However, enzymes in general were found to be highly important in penetration of phosphates and sugars. Rothstein (1954) found that the enzyme phophates is localized in the membrane of yeast, and

thisenzyme is markedly invoved in the permeability of phosphates and glucose.

Besides, Gomori's reaction for phosphatases shows strong activities for these enzymes in the cell membranes especially those which are capable of considerable glucose reabsorption as in the kidney and small intestine.

According to Demis (1954), the enzyme invertase occurs in the plasma membrane of yeast cell which is known to be strongly concerned with the penetration of sugars inside the cells.

4 – Another idea was introduced which regardes that RNA is strogly related to permeability. This is based on the observation that cell membranes give a strong positive cytochermical test for RNA. Thus, when ribonuclease was added from outside to amphibian eggs it strogly affected the permeability of ions.

5 – Biophysical studies indicate that ionic interchange takes place through electrically charged pores. This was explained by the fact that the plasma membrane contains non-charged pores as well as others with a positive charge and others with a negative charge. The positively charged pores attract the anions (e.g., CI) and repel the cations (e.g., Na +). An opposite activity is carried out by the negatively charged pores. These pores are considered to be located principally in the proteinic layer of the plasma membrane.

Role of cell permeability in the life of the organism :

Permeability is very important in regulation the various metabolic activities of the cells and consequently of the whole organism. The plasma membrane has a special property of selectivity which allows the rapid diffusion of certain substances which are continuosuly needed by the body as glucose and certain amino acids that are to be incoporated into cellular proteins. The less needed substances as sucrose, for example, is less admitted into the cell.

Also, oxygen which is required in large amounts by the cells has a great power of permeability.

On the other hand, the first products of glucose, utilized for example by the contracting muscles, such as glycerol derivatives which are valuable to the cells, are not allowed to escape easily.

During muscle work lactic acid is produced. This would be toxic if allowed to accumulate inside the cells. In this case the plasma membrane allows this substance to escape easily to the surrounding blood vessels.

Asregands the valuable substances to which the plasma membrane is [ermeable, it was found that these are stored in the cells by being changed from one form to another, thus amino acids are stored as proteins, fatty acids as fats, and sugars as glycogen. In each case, such polymenzation acts to prevent the penetratin of these substaces through the plasma membrane.

Cellular permeability also plays an important role in the removal of the toxic subtances which are harmful to the body. This process takes place mainly by the detoxication of these subtances. In this case, the toxic subtances are conjugated with amino acids or with sulphuric acid. Thus, bromobenzene of menthol, which are toxic substances produced by some cells, are converted into new molecules. These molecules cannot penetrate into the cells, and once reaching the blood stream they are filtrated off by the glomeruli of the kidney, and are not reabsorbed from urine in the tubules of the kidney. Thus, these toxic substances are prevented from going into the cells and their excretion in the urine is secured.

CHAPTER 6

THE GROUND CYTOPLASM CYTOPLASMIC MATRIX ENDOPLASMIC RETICULUM ERGASTOPLASM RIBOSOMES MICROSOMES

The **ground cytoplasm** or the hyalopalsm appears, under the light microscope, as an amorphous structurless region in which certain structures such as the Golgi apparatus and mitochondria are embedded. In some cells, it was discovered that some regions of the ground cytoplasm were stained with basic byes; these were identified **as basophilic or chromidial** cytoplasm. The basophilic cytoplasm was called by Garnier (1887) **ERGASTOPLASM**. The ergastoplasm included the basophilic regions of the ground cytoplasm such as the Niss bodies in nerve cells and the basophilic clumps in liver cells.

The ergastoplasm consists of osmiophilic granules which contain a very high concentraion of ribonucleoprotein (RNP). The granules are either scattered freely in the cytoplasm or attached to a system of membranes which form canaliculi (i.e., the endoplasmic reticulum). In the presence of the RNP granules and the endoplasmic reticulum it is **called "organized ergastoplasm".** If only the granules are visible, as in case of embryonic cells, it is known as **"unorganized ergstoplasm"** or we may simply speak of RNP granules. (Fig. 34).

ENDOPLASMIC RETICULUM
And Cytoplasmic Matrix

Studies carried out by the electorn microscope showed that the ground cytoplasm or **hyaloplasm** contains a very delicate structure which has been called by Porter (1954) the **endoplasmic reticulum**. This structure occurs in all types of animal cells except the mature red blood cells.

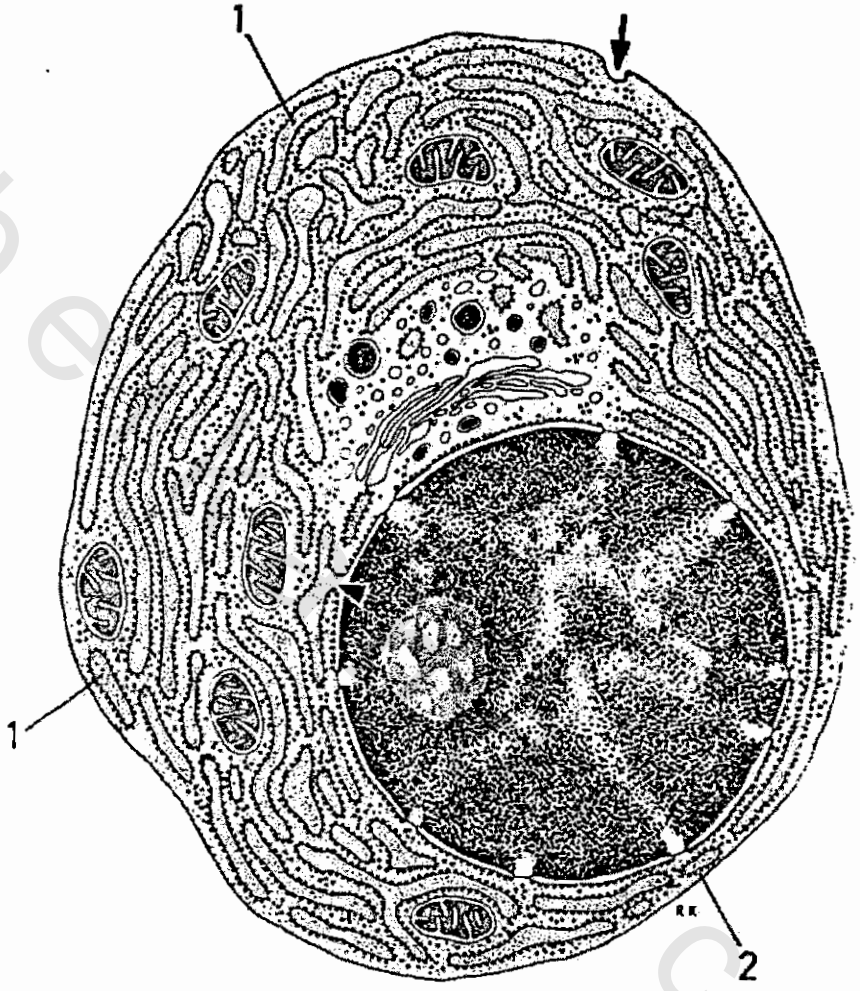


Fig. 34: Rough endoplasmic reticulum (Plastocyte of rat).

1 – Cisternae of Rough E.R.

2 – Hyaloplasm.

The endoplasmic reticulum (ER) is a system a tubular and/or vacuolar elements surrounded by very thin membranes. In some types of cells, such as those of the liver and pancreas, these membranous structures are very mumerous, whereas in other cells such as the muscle cells, they are very few. The membrances of the ER enclose internal spaces, and therefore create in the cytoplasm an internal part or inner phase separated from the outer part or continous phase which is the cytoplasmic or hyaloplasmic matrix. These two phases, in electron micrographs, differ in density and structure from each other.

In the cytoplasmic matrix, ctyoplasmic organoids and inclusions can be distinguished. The matrix is thus the fundamental and most important part of the cell. Its components carry out the biosynthetic functions of the cell. It contains the enzymes necessary for energy production, mainly by anaerobic glycolsis. Furthermore, the specialized matrix is the site of many fibrillar differentiations found in specialized cells such as keratin fibres, myofibrils, neurofibrils and neurotubules. Some mechanical properties of the cytoplasm such as elasticity, contractility, rigidity and intracellular movements (e.g., cyclosis) are mainly related to the cytoplasmic matrix.

The network arrangement of the endoplasmic reticulum was first observed in tissue cultures. In such case, the different elements were found to form a continuous system. However, this system is not fixed for rigid, and under the conditions of cytolysis it breaks up into separate vesicles.

The profiles or sections of the elements of the endoplasmic reticulum are variable, they may be rounded or elongated according to the direction of sectioning.

Types of endoplasmic reticlum:

1 – Granular or Rough Endoplasmic Reticulum:

This type of endoplasmic reticulum is characterized by the presence of numerous minute particles arranged on the outer surfaces of the membranes of the reticulum (Fig. 18). These particles are rich in RNA and proteins and hence they are known as the ribonucleoprotein particles (RNP) or **ribosomes**. Similar particles are also found in the cytoplasmic matrix. (Figs. 35 & 36).

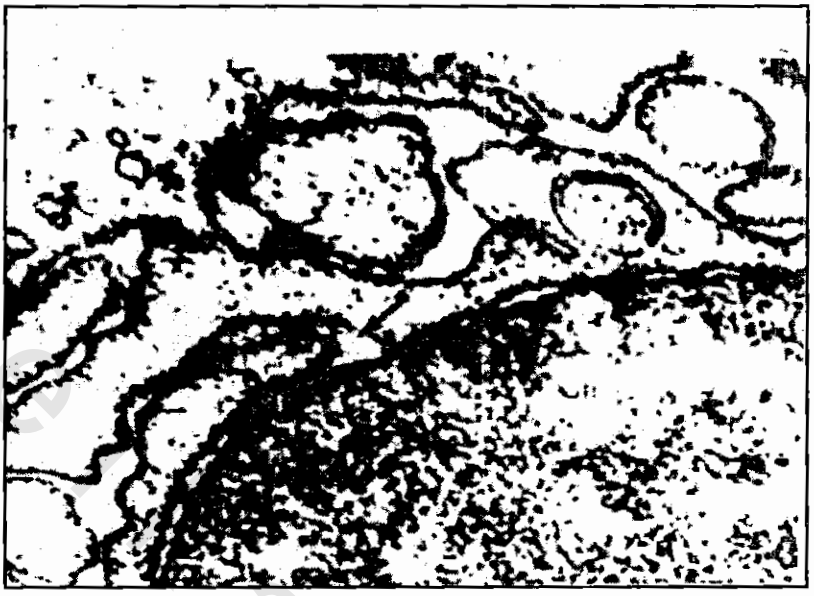


Fig. 35: Rndoplasmic Reticulum (Rough or Granular type originating from the outer layer of the nuclear membrane.

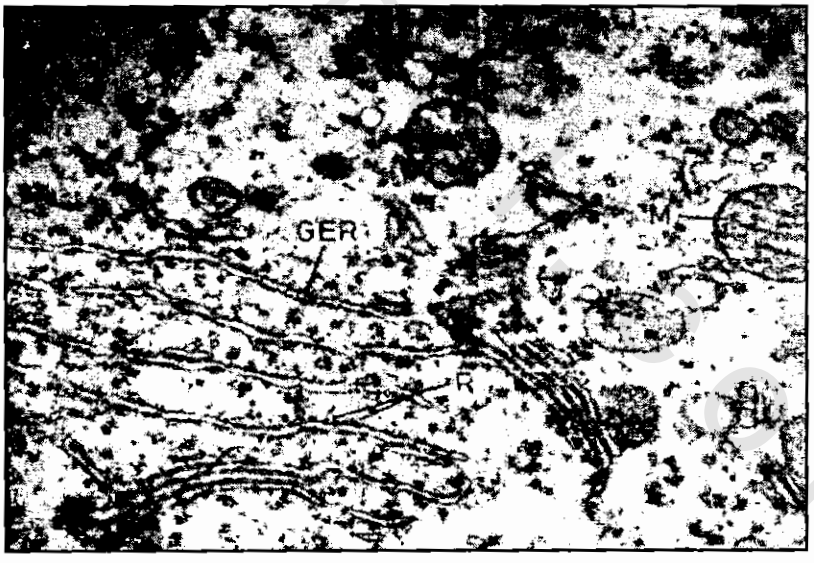


Fig. 36: Electorn micrograph of cat motoneurone showing granular endoplasmic reticulum (GER) ribosomes (R) and mitochondria (M).

The granular endoplasmic reticulum is well developd in the basophilic regions of the cytoplasm (i.e., ergastoplasm) and is mainly concentrated in the basal regions of the cells especially the secretory ones as the exocrine pancreatic cell.

The majority of the elements of the endoplasmic reticulum is on this case exist in the form of lamellar structures or flattened cisternal vesicles. These elements usually contain certain accumulation of material known as the **intracisternal inclusions**.

The rough ER is widely distributed in the growing cells and in those engaged with protein synthesis.

2 – Agranular or Smooth Endoplasmic Reticulum :

This type is characterised by the lack of ribosomes (Fig. 37). Its elements are usually tubular. This type found in the pigment epithelial cells of the retina of the eye and in the striated muscle cells.

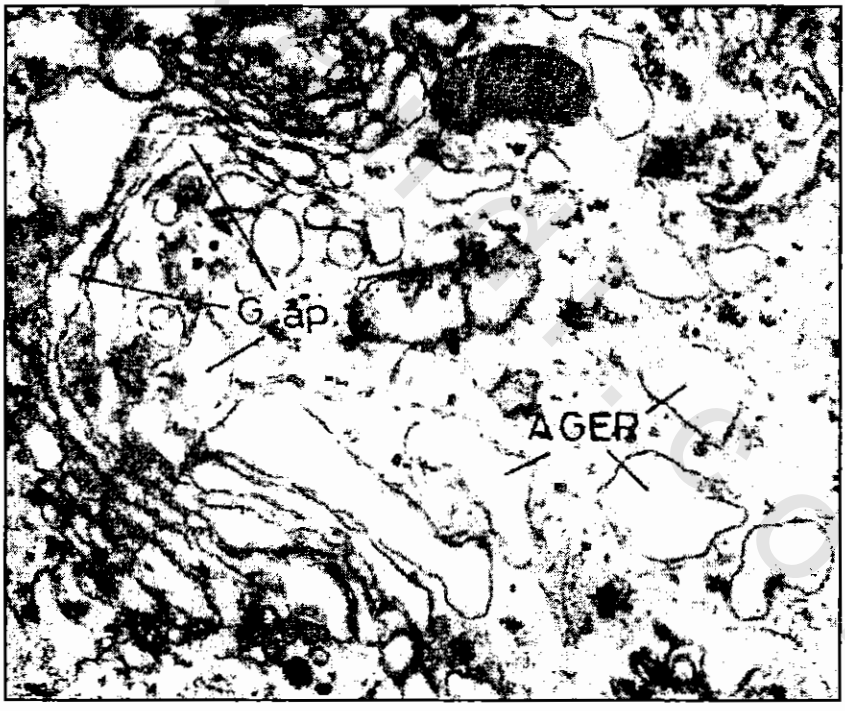


Fig. 37:

Electorn micrograph showing agranular endoplasmic reticulum (AgER) and Golgi apparatus (G. ap.).

However, both the rough and the somooth types may occur in the same cell as in the case of liver cells where the rough type is found mainly in the central region of the cell, whdreas the smooth type exists along the cell margins.

Relationship between the ER and Nuclear Envelope :

This endoplasmic reticulum is closely associated with the nuclear envelope. The envlope consists of two membrames, the outer one being continous with the adjacent elements of the endoplasmic reticulum. Hence, it was regarded that the nuclear envelope is a part of the endoplasmic reticulum.

According to Porter (1960), the nuclear envelope is the constant part of this system and the cytoplasmic parts are derivatives or extensions of this envelope. This explains the observation that the endoplasmic reticulum is present in the newly-formed red blood cells, and is absent in the mature ones.

Significance of the Endoplasim Reticulum :

It has been found that the endoplasmic reticulum plays an important role in cellular activities, particularly in **protein** synthesis and cell **secretion.**

According to some authors, the rough type is capable of incorporating amino acids into proteins. This was indicated by the presence of large zymogen granules in the swollen sacs of the endoplasmic reticlum of the exocrine pancreatic cells. Also, in the hen's oviduct the albumen substance was found to be concentrated in the cavities of the endoplasmic reticulum. Furthermore, it was found that the wide cisternae of the endoplasmic reticulum in thyroid gland cells were filled with colloidal substance (Wissig, 1960).

It is regarded that the newly synthesized proteins or enzymes make their way across the cisternal membrances from the ribosomes, and then condense out as large intracisternal granules. From this initial site of condensation, the secretory material in transported as granules or in a dissolved form to the Golgi apparatus, where they are again condensed into more compact granules which are then emitted into the cytoplasm and are later released form the cells. (Fig. 38).

The rough endoplasmic reticulum is also strogly related to cell growth and differentiation. For example, in Hydra it was found that the interstitial cells from which the cnidoblasis arise are very rich in RNP granules, but

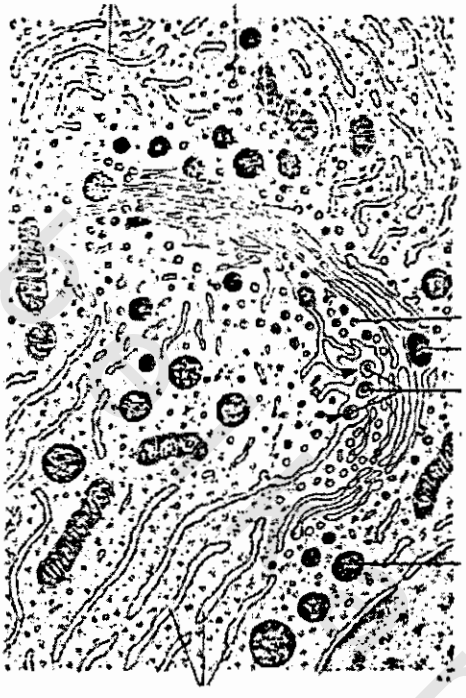


Fig. 38: Rough endoplasmic reticulum and neurosecretory material in a neurcosecretory cell in rate.
1 – Cisternae of E.R. 2 – Small vesicles.
3 – Smooth tubules. 4 – Granular material.
5 – Secretory granules.

nearly devoid of endoplasmic reticulum. Later, after a series of divisions when the cell begins its differentiation the ER becomes prominent. In addition, during the differnetiation of nerve cells the endoplasmic reticulum was increased 3-4 times that of the young undifferentiated cells.

Generally, the smooth endoplasmic reticulum is regarded to be related to the transmission of excitatory impulses in the cells (see saroplasmic reticullum). It is most evident in cells which are mainly concerred with lipid production, carbohydrate metabolism, ion transport and electrolyte excretion; this may indicate that the endoplasmic reticulum is concerned with these functions.

Pathologically, the endoplasmic reticulum is broken down in some cases into separate narrow and wide vacuolar structures without any clear connection with the nuclear membrane.

RIBOSOMES

Traditionally the ribosomes are generally studied in relation to the endoplasmic reticulum because in higher cells ribosomes are frequently attached to some intracellular membranes of the endoplasmic reticulum. De Robertis considers the ribosomes as a constant part of the cellular matrix.

The ribosome is a definite submicoscopic particle composed of ribonucleic acid and protein. Ribosomes have an essential role in **protein synthesis**, the process by which amino acids are assembled in a definite sequence to produce the polypeptide chain.

The ribosome are found in all cells except mature **red blood cells** of mammals. They occur in the protoplasm of bacteria (there is no ER). Most

of the ribosomes are free in the sytoplasmic matrix of yeast cells, reticulocytes, meristematic plant cells and embryonic neurones.

In cells which are involved in protein synthesis, e.g., enzyme secreting cells, most ribosomes are attached to the membranes of certain parts of the emdoplasmic reticulum. It has been presumed that the lipoprotein membrane assist in removing the newly synthesized protein from the ribosomes and helps in transporting and excreting it.

The major constituents of ribosomes are proteins and ribonucleic acids in approximately equal proportions with little or no lipid. In other words, the ribosome is a ribonucleoprotein particle, with ribosomal RNA and protein present in roughly equivalent weights. Ribosomal RNA forms and accumulates in the nucleolus under DNA control.

Ribosomes are generally considered as cytoplasmic components of the cells. Howvere, similar particles were discovered in the nucleus. In fact, the nuclear ribosomes are more heterogeneous in size and also in RNA protein content than cytoplasmic RNA.

The ribosomes are either free or bound to the membranes of the ER. The bound ribosomes are found in great amounts in animal cells that secrete proteins such as hormones or disgestive enzymes.

In the electron micrigraphs, the ribosome is spherical in the majority of cells, but may be rod or polygonal in shape. The ribosomes consist of two distinct subunits which can be reversibly separated by various treatment, e.g., by lowering the Mg2 = concentration of the medium.

Ribosomes

At low concentrations of Mg^{++} eukaryotic ribosomes sediment in sucrose gradients with a sedimentation coefficient of 80S. Prokaryote ribosomes are smaller and sediment at 70S. The following table shows the sizes of various ribosomes.

Ribosomes	Size	Subunits		RNAs		
Eukaryotes	80S	60S	40S	28S^{+} 5S	5.8S	18S
Prokaryotes (Bacteria)	70S	50S	30S	23S 5S		16S
Mitochondria (mammals)	55S	35S	25S	21S 3S		12S

The 80S ribosomes (eukaryotes) have subunits of 60S and 405 subunits while 70S ribosomes (prokaryotes) have subunits 50S and 30S subunits.

Ribosomes vary from 150-250 A^0 in diameter (300 A^0 in striated muscle). Nissl's substance is formed of packed masses of ribosomes.

Another type of ribosomes is polyribosome or polysome. Polysomes appears as 4-7 ribosomes united by a fine filament (10-20 A^0 in diameter). The filament uniting the ribosomes is considered to be messenger RNA. Polysomes are regarded as the functional units in the synthesis of protein.

MICROSOMES

The microsomes are not specific components of the cell. They constitute a heterogeneous group of cellular structures that can be isolated by centrifugation.

By using the ultracentrifuge, a fraction of submicroscopic particles could be obtained; this is the "microsome fraction: or microsomomes". Detalied observation of the microsome fraction shows the three main components of the endoplasmic retculum, i.e., the membrane of the endoplasmic reticulum, the ribosomes attached to the outer surface of the membrane and the content of the fragments may be found as in the liver microsomes which were found to consists of parts of the endoplasmic reticulum in the form of isolated vesicles, tubules and some cistern with ribosomes attached. However there is in addition to the ER, the microsomal fraction contains Golgi membranes and other cell fragments. In the liver, glycogen, ferritin and some lipid droplets were also found.

CHAPTER 7

MITOCHONDRIA

The mitochondria or chondriosomes were described by Altmann (1890) in nerve cells, then by Benda (1879), and after that they have been discovered in all cells of both animals and plants.

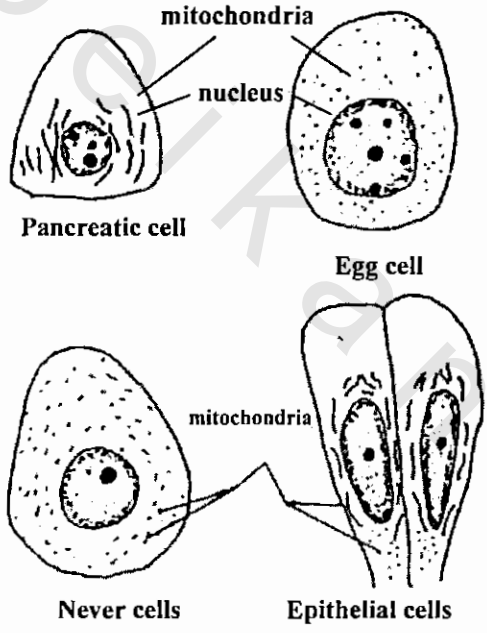


Fig. 39: Different forms of mitochondria.

The mitochondria are living organiods in the form of small rods, threads, granule or spheres. The rods and threads are often referred to as chondrioconts, the granules as chondriomites and the spheres as chondriospheres. (Fig. 39).

According to Cowdry, the mitochondria have been given about 50 names. The term mitochondria which has remained in common use comes from two Greek words: mitos = filament and chondros = granule.

Mitochondria are considered as the "Power palnts" of the cells or biological machines in which the transformation of the chemical energy of food stuffs into a sort of energy used by the cells takes place.

Demonstration :

The mitochondria have been seen in various living cells by different authors especially with the phase contrast microscope. They are not easily observed with the normal light microscope owing to their low refractive index. They are supravitally stained with diluted solutions of Janus dyes (green and black). A dilute solution of Janus green stains mitochondria vitally greenish blue. This staining is due to the action of the cytochrome oxidase system present in the mitochondria, which maintains the dye in its

oxidized (coloured) form. In the surrounding cytoplasm the dye is reduced to a colourless leukobase.

In fixed materials, the mitochondria are stained with Altmam's acid fuchsin, Regaud's iron-alum haematoxylin, Bend's crystal violet, alizarin, Campy's fluid, etc. The mitochondria are labile structures that are readily disintegrated by the action of fixatives. For this reason, mitochondria are fixed by methods that stabilize the lipoprotein structure by the prolonged action of oxidizing agents, such as osmium tetroxide, chromic acid and potassium dichromate.

Morphology of mitochondria :

The shape of the mitochondria often appears to be characteristic for one particular organ. For example, in the pancreatic cells they are in the form of long threads; in sperms and eggs they are granular, in the nerve-cells they take the form of short rods and granules; in the intestinal epithelial cells they are filamentous in the apical part and at the sides of the nucleus, but granular in the basal cytoplasm. They may change in form during certain functional states as in the hepatic and pancreatic cells.

Although the shape of the mitochondria is usually uniform in the different cells of the same organ, yet in some cases different kinds of mitochondria are found in the same organ as in case of mammalian liver. This phenomenon is referred to as **"mitochondrial heterogencity"** and is considered to be related to the activity of the component cells. In the peripheral cells of the hepatic lobules numerous filamentous mitochondria occur, in the cells of the control labules there are few granular ones, whereas a mixture of rod-like, granular and filamentous mitochondria exists in the intermediate regions of the hepatic lobules. Some authors consider the outer lobular cells as the zone of **"maximum activity"**, the inner area as the zone of "maximum repose", whereas the region in between as the zone of **"intermediate activity"**. This was explained as being due to the fact that the portal blood circulation carrying the products of digestion to the liver reaches the peripheral regions of the lobules first

The size of mitochondria is variable. In the majority of cells, the width is relatively constant (about 0.5 u) and the length is variable, reaching a maximum of 7 u. Depending on the functional state of the cell, it is possible to find very thin (0.2 u) or thick rods (2 u). The size and shape of

the fixed mitochondria depend also on the osmotic pressure and pH of the fixative. (Fig. 40).

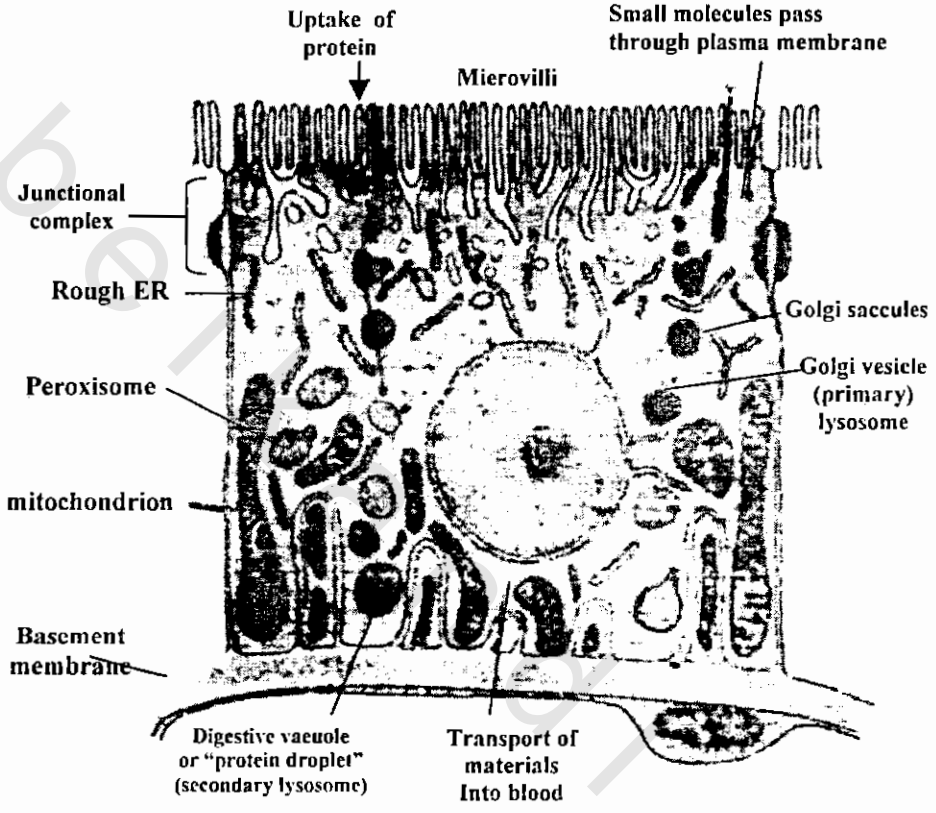


Fig. 40: Kidney cell showing mitochondria.

The number of the mitochondria varies with the cell type and function: it is nearly constant in each cell type. The mitochondria are more numerous in the specialized organs (e.g., liver and kidney) than in the others of less activity (as thymus and adrenals).

The number ranges from a few per cell to thousands in some cells, e.g., the average number of mitochondria in mamalian liver cells is 2.500; this number drops very greatly in hepatoma cells (about 220-800).

The mitochondria are usually **distributed** throughout the cytoplasm, but in rare cases they are restricted to certain regions of the cytoplasm, e.g., in kidney cells they are concentrated in the basal region next to the blood

capillaries. Sometimes, they accumulate around the nucleus or in the peripheral region of the cytoplasm; such distributions are more frequent in pathological conditions, Similar locations of mitochondria are also brought about by overloading the cells with inclusions (e.g., glycogen and fat) which displace these organoids.

The distribution of mitochondria within the cytoplasm should be considered in relation to their function as energy suppliers. In some cells they can move freely, carrying adenosine trophosphate (ATP) where needed, but in others they are located permanently near the region of the cell where presumably more energy is needed. In the rod-and cone-cells all mitochondria are located in a portion of the inner segment, while in motor neurones they are concentrated towards the periphery.

Ultratructure of mitochondria :

The mitochondrion, in electron micrographs, appears to be surrounded by an outer smooth limiting membrane (of about 60 A^{o} thick) which is probably related to the permeability properties of the organoid. Within this membrane, and separated by a very small space (60-80 A^{o}), there is an inner membrane (60 A^{o} thick) extending into the mitochondrial cavity in the form of a number of septa or partitions known as the internal ridges or "cristae-mitochondriales" which divide the internal cavity incompletely into a number of small chambers. The inner membrane divides the mitochondrion into tow chambers: (a) the outer chamber lies between the two membranes and in the core of the ridges, (b) the inner chamber bounded by the inner membrane and is occupied by a relatively dense material which is usually known as the mitochondrial matrix.

The mitochondrial matrix is generally homogeneous but in some cases it may show a finely filamentous material or small granules of high density. These granules are considred as sites for binding bivalent cations particularly Mg Ca.

The structure of the outer mitochondrial membrane is nearly the same in the different cell types, but that of the inner membrane and the intrnal ridges show some differences in the fifferent cells. Even in the same imtochondrion, the two membranes are different from each other.

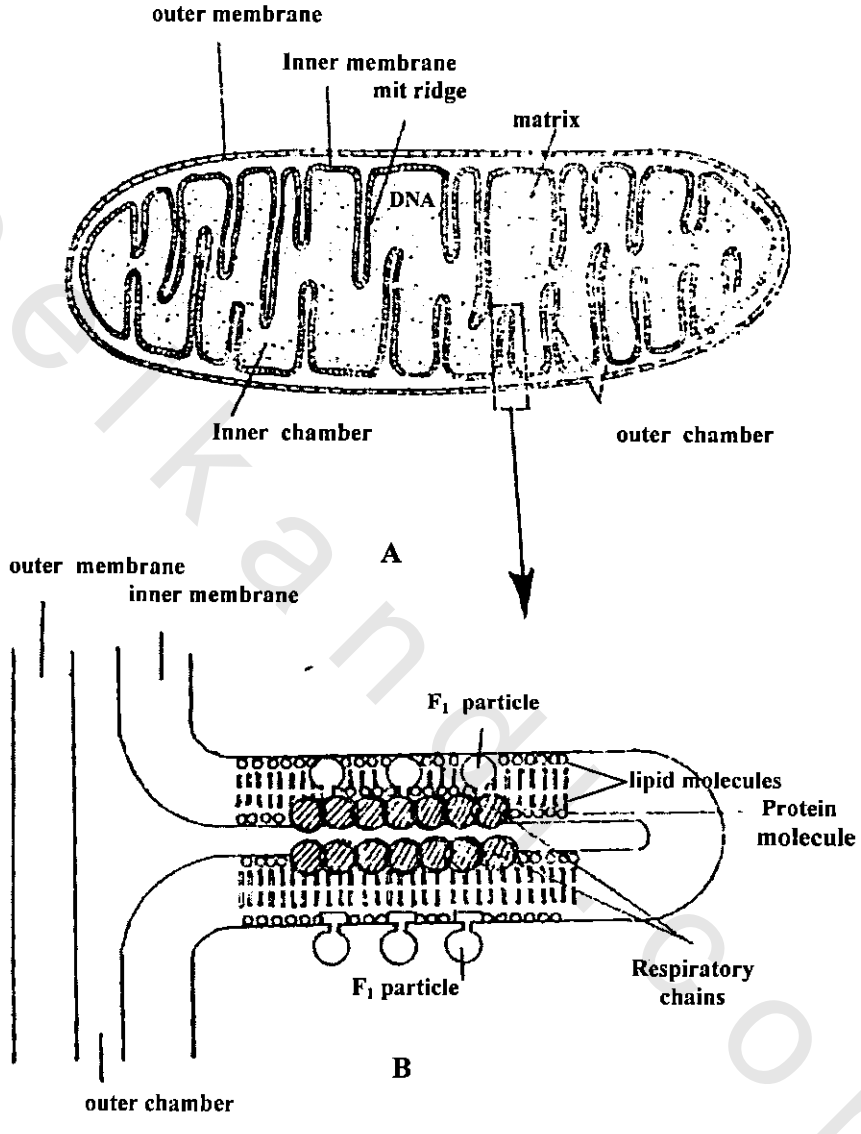


Fig. 41

A Diagram of the ultrastructure of a mitochondrion. B Diagramm showing the molecular organization of a mitochondrial ridge (crest). After Lehninger.

The mitochondrial ridges divide the inner chamber incompletely into a unmber of small chambers. The ridges usually run transversely across the mitochondrion. In some cases they are arranged parallel to the long axis of the mitochondrion, in others they are arranged concentrically with in the mitochondrion. Sometimes, the ridges branch forming complex network. The presence and appearance of these ridges are regarded to be modifications for obtainining a surface film of maximum extent; thus, a large area is provided for the intramitochndrial activities. This is clear from the observation that the motochondria of the muscle cells (including cardiac and flight muscles) possess large numbers of mitochondrial ridges (Fig. 41 – 44).

Fig. 42: Ultrastructure of a mitochondrion.

The mitochondria also, possess, certain utlramicroscopic particulates which are arranged regularly on the mitochondrial ridges, and are assumed to represent certain assemblies of respiratory enzymes. In liver cells the mitochondrion contains about 15.000 of these assemblies, whereas in the flight insect muscle there may be as much as 100.000 assemblies in each mitochondrion and hence the motochondria are regarded as the "enzyme batteries".

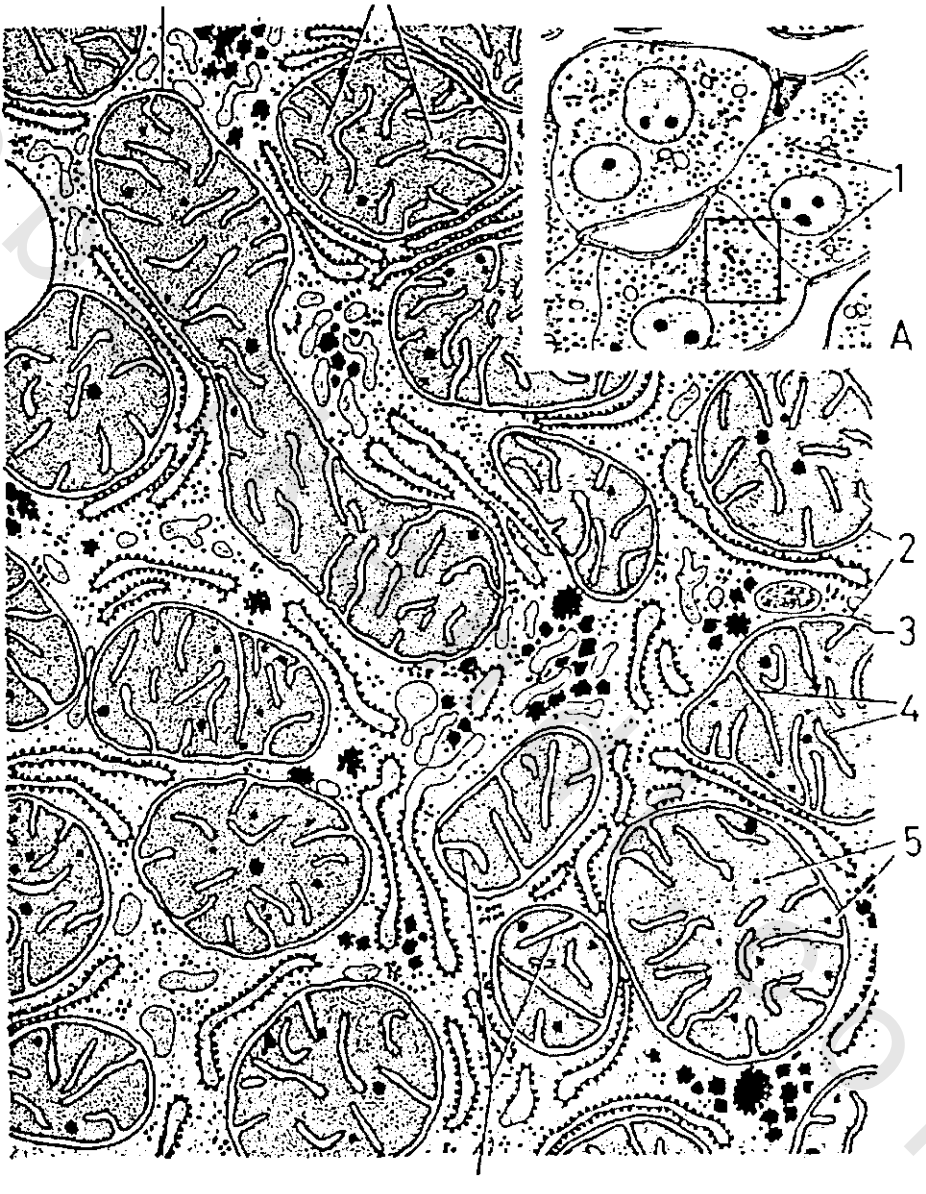


Fig. 43 : Mitchondria (Rat liver cell)

1 – Mitochondrion.
2 – Outer mitochondrial membrane.
3 – Inner mitochondrial membrane.
4 – Mitochondrial ridges.
5 – Internal chambers.

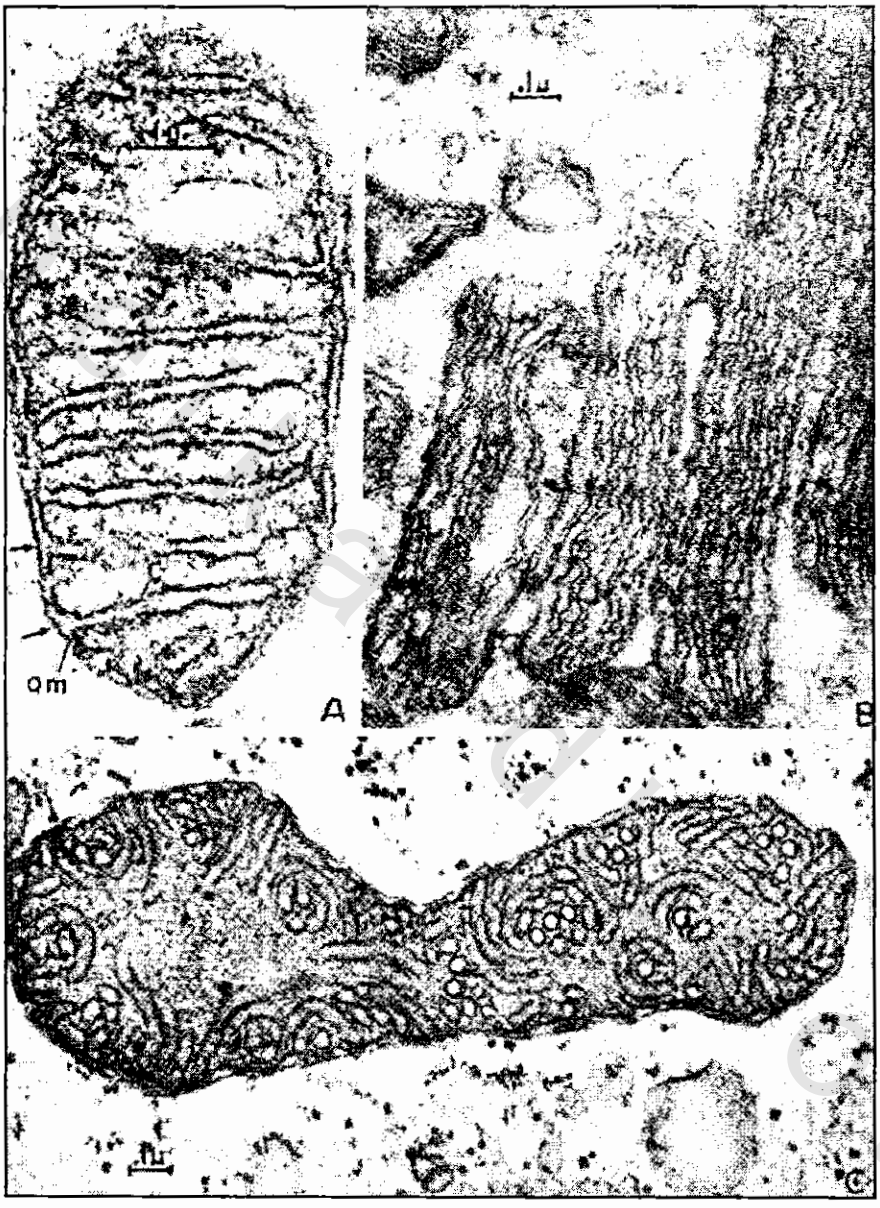


Fig. 44 : Electron micrographs showing modifications of itochondrial ridges which appear as transvers ridges (A), longtudinal ridges (E) and tubular ridges (C), (After Andro).

Behaviour :

The mitochondria respond to changes in the osmotic pressure of the medium. They have been seen in a state of constant movement. The mitochondria show two main types of motion which are agitation and displacement from one part of the cell to another.

Some authors claimed that the mitochondria are sensitive to heat and appear to melt at about 50°C,: this has been denied by other workers.

Some variations in mitochondria structure are related to the special functional state of the cell. For example, after an animal has starved for several days, **liver mitochondria** are swollen, with clarification of the matrix and changes in their internal structure. After refeeding they turn back to the normal condition (Fig. 45).

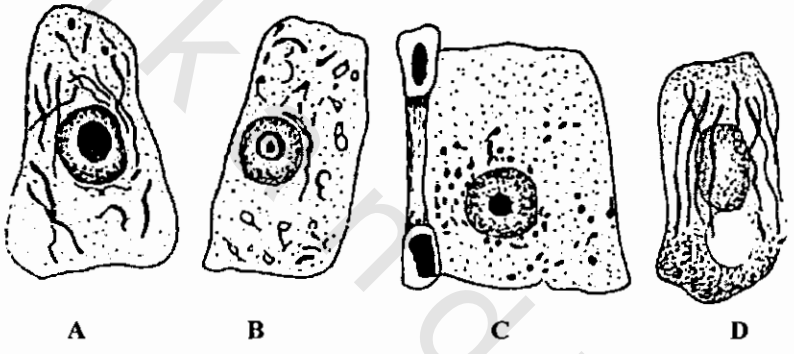


Fig. 45 :

Hepatic cells of a fish A, at of time eating; B, 9 hours later; C, 24 hours after eating; and D, 48 hours after eating. (From De Robertis et al., Cell Biology).

The mitochondria are very sensitive indicators of cellular injury. Thus, pinching of tissues with the forceps before fixation is liabel to cause them to brteak up into granules. It has been also found that the mitochondria break up and coalesce during scurvy. In cyanide poisoning although mitochondria maintain their shape yet their movement is inhibited.

In ultracentrifuged material the mitochondria are shifted towards the centrifugal end of the cell, wherwas the Golgi apparatus is shifted towards the centripetal end, i.e., the mitochondria are heavier than the Golgi bodies. In addition, the mitochondria were isolated from the liver cells by the prosess of centrifugation.

During cell division, the mitochondria tend to aggregate around the spindle, and upon division of the cell they are shared out between the resulant cells.

Chemistry and the functions of mitochodria :

The chemical composition of mitochondria seems to vary in different tissue under different conditions and affected by pathological changes in the cells. The mitochondria contain both lipid (about 30%) and protein (about 70%). DNA & RNA are components of mitochondria.

Bourne (1942) suggested that mitochondria may be surrounded by an adsorbed layer of protein which prevents them from being stained by fat dyes. Millon's reaction specific for proteins gives positive results with the mitochondria. If proteins are digested by pepsin, the mitochondria are no longer detected with Millon's reaction.

The use of fluorescence microscopy shows that the mitochondria contain **Vitamin A**. Mitochondria give, also, positive reaction with antimony trichoride which is characteristic for carotenoids in general. Mitochondria were demonstrated by some investigators in acetic silver nitrate preparations; this denotes the presence of **Vitamin C**. It is also claimed that members of **Vitamin B-complex** are found in the mitochondria.

Iron, which is an important constituent of the cytochrome enzymes, is found in mitochondria.

The mitochondria contain, according to some authors, **proteolytic enzymes**. This was illustrated by the following example: in Amoebs, the food particles were brought into intimate contact with mitochondria then a food vacuole was formed which encloses both mitochondria and food particles. Digestion of food in this vacuole was accompanied with the disappearance of mitochondria which are presumably used up during this digestive process.

Respiratory enxymes such as cytochrome oxidase and succinic dehydrogenase are found in the mitochondria. These are regarded to be exclusively the sites of oxidative processes in the cells. Green (1951) reported that even when mitochondria were broken into small fragments, they were still capable of carrying on their oxidative processes. Long-chained fatty acids were found to be oxidative into carbon dioxide and water under the effect of mitochondrial fragments.

Localization of respiratory enzymes in the mitochondria was shown by the fact that cyanide, which is known to inhibit the respiratory processes, was also found to prevent the mitochondria from carrying on the respiratory functions.

As an indications for the role mitochondria in respiration, it was found (Moussa and Banhawy, 1962) that when insects were treated with insecticides, the mitochondria coalesced together forming larger bodies. This was reflected externally by deep and violent respiratory movement of insects. This indicates that the coalescence of mitochondria resulted in marked reduction in the respiratory surface (mitochondrial surfaces). Consequently, a small amount of oxygen is consumed in such cells, and this forces the inscet to perform a strong effort in respiration to accelerate the respiratory activities. With prolonged effect of the insecticide, most of the mitochondria disappeared from the cells. At this stage, the respiratory movements were gradually inhibited and then the insect died.

In a mitochondrion, many enzymes and conezymes work in an orderly fashion, in addition to numerous cofactors, vitamins and metals essential for mitochondrial function. The only fuel that a mitochondrion needs is phosphate and adenosine diphosphate (ADP), the final product is: ATP + CO_2 + H_2O.

The three major food stuffs of the cell (carbohydrates, proteins and fats) are ultimately degraded in the cytoplasm to give acetyle coenzyme A. When this penetrates the mitochondrion, the acetate group enters the Kreb's tricarboxylic acid cycle (citric acid cycle) in which, after a complex series of steps involving several enzymes, it is decarboxylated, losing CO_2, at several points in the cycles. Pairs of electrons (or their equivalent hydroger atoms) are removed by dehydrogenases and enter into the respiratory chain, at the end of which they combine with molecular oxygen to form water.

The respiratory chain (also called electron transport pathway) is the main energy transforming system of mitochondria. The main components of the respiratory chain are two flavo-protein enzymes (succinic and DPN dehydrogenases), four cytochromes and also non-heme iron, copper and coenzyme Q.

At three points along this chain transforming mechanisms use the energy lost by a pair of electrons to form ATP from ADP and phosphate (see also Chapter 22 for more details).

Localization of the mitochondrial enzyme system

Matrix	Membranes
Kreb's cycle :	Respiratory chain enzymes:
Aconitase	ADP dehydrogenase
Malic dehydrognase	succinic degydrogenase
Fumarase	cytochrome oxidases
Isocitric dehydorgenase	
Condensing enzyme	
Pyruvic and ketoglutaric	
Dehydogenase .. . ect.	
Fatty acid cycle ..	
Crotonase	
Acyl dehydrogenase	
DNA polymerase	
RNA polymerase	

If a suspension of mitochondria is submitted to sonic vibrations, the soluble proteins contained in the matrix are released leaving the membranous parts in the sedimental fraction.

These soluble proteins comprise most of the enzymes invloved in the Kreb's and fatty acid cycles. The matrix also contains different nucleotides as well as nucleotide coenzymes and inorganic electrolytes such as K+, HPO4 =, Mg++, Cl – and SO4 =, The insoluble fraction contains all the respiratory chain together with the energy coupling enxymes of oxidative phosphorylation. It has been suggested that the inner membrane and the crests of the mitochondria contain most, if not all, the enzymes involved in this chain.

The enzymes of the inner membrane are organized in compact "asslemblies" that are regularly spaced in the mitochondrial crest. The respiratory enzymes are in equimolecular quantities in these assemblies. i.e., there is a molecule of succinic cehydrogenase or DPN dehydorgenasw for each cytochrme and cytochrome oxidase in the chain.

The Functions of Mitochondria can be summarized in the following:

1 - The mitochondria are the respiratory centres of the cell; this is due to their rich supply of respiratory enzymes.

2 - The mitochondrai, according to some authors, **possess hydrolytic and synthesizing enzyme activities** in lower animals. This has been demonstrated by Horning in Amoeba. Horning believes that the mitochondria in proteozoa are used for the digestion of food. It was, also, found that during the feeding process of Opalina, the vegatative naterial collected on the surface of mitochondria. After a time, certain granules were separated from this material and were distributed in the cytoplasm. These were found to be protein particles synthesized from the vegetative food under the effect of mitochondria.

3 - It is claimed that the mitochondria are responsible for the **production of zymogen** granules of pancreas. If this is so, the mitochodria, therefore, produce enzymes to be used for the extracellular digestion and so their functions in the higher and lower animals are the same.

4 - They are concerned with **fat metabolism** in the cell. Since Altmann, it has been noted that there is a relationship between mitochondria and lipid. In the liver or pancreas, after a short period of starvation, the mitochondria come into contact with lipid droplets. Electron microscope images suggest that an active process of fat netabolism takes place under 'the action of the fatty acid oxidases present in the mitochondria. It is known that after a short period of starvation, the metabolism of the cell changes and instead of carbohydrates, fatty acids are actively oxidized and degraded to be utilized in the common pathway of the Kreb's cycle found in the mitochondria.

5 - They play part in the formation of **albuminous yolk** in eggs.

6 - They form **the sheath of the axial filament of sperm** middle-piece.

Origin of Mitochondria :

There is no general agreement as regards the exact origin of mitochondria. Three possible mechanisms of mitochondrial formation have been suggested.

1 - **Division of pre-existing mitochondria**: The majority of workers consider mitochondria to arise from the pre-eexisting ones through different processes of fission and multiplication. This finds support in the behaviour of the mitchondria during division and development of cells.

2 - De Novo Synthesis: This view resulted from centrifugation experiments of sea-urchin eggs. It was found that certain fraction (centripetal) that did not contain mitochondria, as judged by staining method develop a full set of these organoids in later stages of

development. These findings were interpreted as an indication that mitochondria originated without pre-existent elements. However, the possibility of the existence of submicroscopic mitochondria in the centripetal fraction cannot be discarded.

There is a biochemical evidence that the half life of liver mitochondria is about 5 to 100 days which indicate that the cellular synthesis of mitochondria may be a continuous process.

3 – The third assumption is based on electron microscopy. Continuities of the mitochondrial membrane with the plasma membrane or the endoplasmic reticulum and unclear envelope have been observed. This has led to the suggestion that mitochondria are formed by these membranes.

Pathological changes :

Mitochondria are affected by pathological changes in the cells.

This can be illustrated by the following :

1 - In malignant cells, the mitochondria undergo fatty changes. They usually swell up, lose most of their internal ridges (Fig. 46) and become transformed into fat droplets.

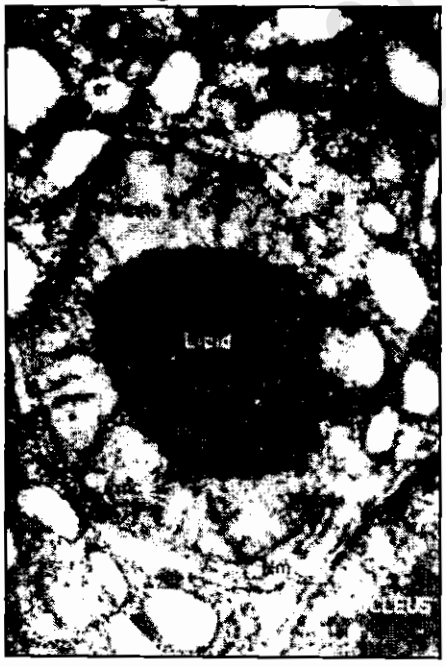


Fig. 46: Intimate relation of mitochondria to lipid droplets, ec-endoplasmic reticulum; mc, mitochondrial crests; mitochondria; Nm, nuclear membrane.

2 - It has also been reported that mitochondria of malignant cells react rapidly to ultraviolet radiations.

3 - During scurvy, the filamentous mitochondria are collected in the form of a dense mass around the uncleus instead of their homogeneous distribution in the cytoplasm. Then these filaments become fragmented into small granules which swell upn and become vesicular. In advanced condition the mitochondria disappear gradually from the cells.

4 - In nerve cells treated with insecticides the mitochondria are often grouped together forming few large bodies which move gradually to the cell periphery and finally most of them disappear completely.

5 - In morphine poisoning the mitochondrial rods of mamalian neurones break up into granules and the staining ability of the mitochondria decreases.

6 - It has been stated that in sulphonal poisoning the mitochondria of liver cells become granular and migrate towards the cell periphery.

7 - In phosphorous poisoning the filamentous mitochondria of the pancreas are broken down into small granules which fuse together and give rise to fat droplets.

8 - Asphyxia produced marked changes in mitochondria (Fig. 47 A & B).

9 - The mitochondrial movement is inhibited by narcotics.

10 - During fasting and ageing, the filamentous mitochondria are transformed gradually into granular elements which disappear progressively from the cells. In the ageing liver cells, the mitochondrial remnants are changed into red-coloured particles which show marked accumulation in the sinusoidal poles of the cell.

Fig. 47 : A

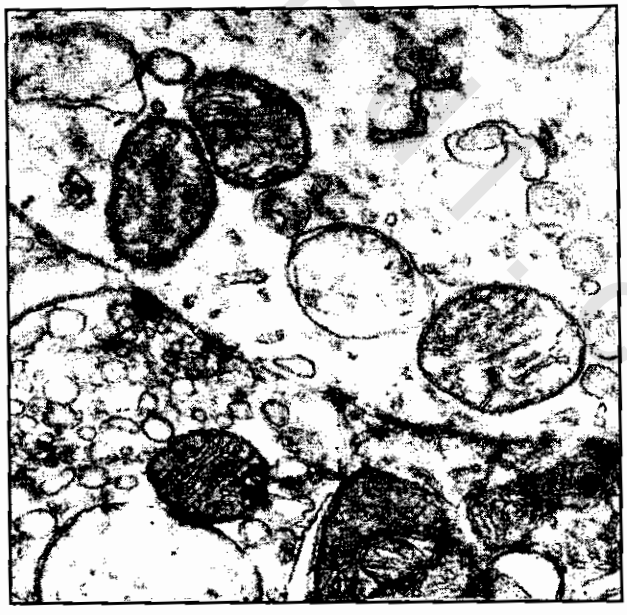


Fig. 47 : B

Mitochondria of asphyxiated neurones.
(After khattab 1970).

CHAPTER 8

THE GOLGI APPARATUS

This cellular organelle was discovered for the first time by the Italian neurologist Camillo Golgi in 1898. He noticed that when the nerve cells of cats and birds were treated with either silver nitrate or osmic acid (osmium tetroxide O5O4), there appeared in those cells a dense net-like structure surrounding the nucleus (perinuclear). He gave the name "Reticulo interno" to this structure, and described it as being highly argentophilic owing to its high affinity for staining and impregnation with silver nitrate, and it is also strongly osmiophilic due to its high affinity to osmium tetroxide which gives this structure an intense black colour.

Fig. 48 : Golgi apparatus (A : Intestinal epithelial cell, B : Nerve cell).

Since that time extensive studies have been carried out on this structure, which later became known as the "Golgi apparatus" after the name of its first discoverer, such studies exceded those carried on any other known cellular structure. (Fig. 48 & 49).

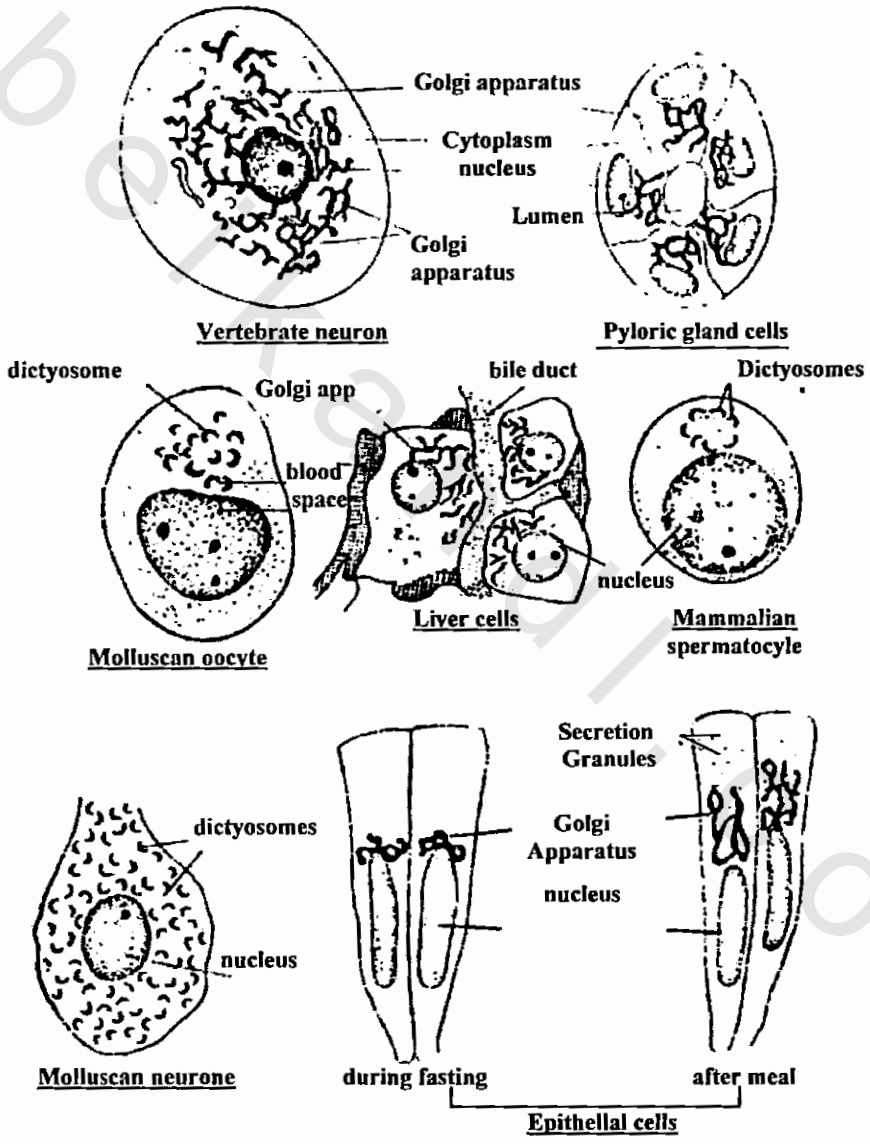


Fig. 49 : Forms and localization of the Golgi apparatus.

The Golgi apparatus controversies :

No cellular components described in animal cells have consituted such a constant challenge to cytologists than the Golgi apparatus. Generally speaking, it can be said that there have been two schools of thoughts concerning the apparatus of Golgi. The first (Getenby, Beams, Moussa, etc.) believes that the structure is a definite and independent cytoplasmic organoid which impregnates with silver (i.e., argentgophil) or osmium (i.e., osmiophil) after appropriate fixation. The second (Benzeley, Parat, Baker, etc.) claimed that the classical Golgi apparatus seen after fixation and impregnation with silver or osmium does not exist as such in the living cell but is produced under the effects of fixatives or the reduction of silver nitrate or osmium tetroxide in, around, and between lipid bodies or on the mitochondria or the Nissl bodies.

The majority of investigations now support the first school. This is clear from the following :

I. The lipid-Golgi apparatus or the "Vacuome hypothesis"

The "vacuome hypothesis" is upheld by some workers (Parat, Covell, Scott, Baker, Thomas, and others) who claimed that the lipid granules and vacuoles (stainable with neutral red and sudan black) form the basis for an artificial deposition of silver or osmium to produce the classical Golgi apparatus network of vertebrate nerve cells.

This view has not been accepted by several authors because of the following:

(A) Using the phase contrast microscope. Moussa in 1952 was able to see the Golgi apparatus network in the living unstained sympathetic neurones of the mouse. This network resembles in shape, size and distribution the Golgi apparatus given by the classical techniques of silver or osmium.

(B) When the sympathetic neurones were stained vitally with neutral red, or with sudan black after fixation, and there examined under the phase contrast microscope they showed unstained network (negative image of the Golgi apparatus) surrounding the nucleus and leaving a cortical area of the cytoplasm free form the Golgi apparatus but filled with lipid ganules which were stained with neutral red and sudan black. This indicates that the lipid granules and the argentophil Golgi apparatus can be demonstrated simultaneously in the same cell.

(C) In the young berve cell, the Golgi apparatus was demonstrated in the form of an argentophil and osmoiphil network lying in the axon-

hillock. This organoid was not stained with sudan black in these young stages which show the absence of lipid bodies. In other words, the lipid bodies are lacking in young nurones in which the Golgi apparatus is well developed.

(D) According to the different specific gravities of the Glogi apparatus and the lipid bodies they can be separated by means of the ultra-cntrifuge (Moussa, 1950 and 1952). Ultra-centrifugation shows that the Golgi apparatus is a definite structure of specific gravity more than that of the lipid.

(E) Gatenby and Noussa (1949, 1950) have pointed out that the lipid vesicles and granules of Baker and others are the secretion products of the Golgi apparatus particularly in old animals, and that the Golgi apparatus of vertebrate neuones is a canalicular system; the wall of the canal (i.e, the Golgi substance) is argentophil and osmiophil.

(F) By using the electron microscope the Golgi apparatus (Similar to that described by Gatenby and Moussa) has also been described by several authors (Beams, Dalton, Felix, etc.).

That the Golgi apparatus and lipid bodies are two different structures has been also established by the work of several authors (Moussa, 1950, 1952; Beams et al., 1952; Dalton et al., 1953; Gatenby, 1953; Lacy. 1954; Moussa and Banhway, 1953; Moussa, 1956; Moussa and Khattab. 1963 and Moussa and El-Beih, 1970). It is worth mentioning that Baker (1963) admits the existence of a definite argentophil and osmiophil Golgi apparatus reticulum in vertebrate nerve cells and does not still believe in the lipid Golgi apparatus.

II. Golgi apparatus and Mitochondria :

In 1915, Monti claimed that the Golgi apparatus of vertebrate and inverebrate neurones resembles the mitochondria in position, quantity and orientation; so she believed that the mitochondria represent in life the Golgi apparatus.

Monti's claim seems to reappear from time to time in one form or another. In 1948 Thomas suggested that the classical Golgi apparatus of mammalian neurones is produced by running together of the lipid bodics and "filamentous" mitochondria on impregnation with osmium. The mitochondria Golgi apparatus of Thomas was adopted by Baker (1949) and Chopra (1960).

According to Moussa and his colleagues (1948-1970) no connection could be traced between the mitochondria and the Golgi apparatus. Although the mitochondria occur as granules and short rods distributed throughout the cytoplasm in the various stages of development, the Golgi apparatus is in the form of a canlicular network which lies as the axon-hillock of embryonic neurones. Then with the increase in age, this network extends around the nucleus and finally spreads in the cytoplasm, leaving always the peripheral part of the cytoplasm free from it but occupied with mitochondria.

Furthermore, Moussa (1952) was able to demonstrate both the Golgi apparatus and the mitochondria in the same cell.

That the Golgi apparatus and the mitochondria of vertebrate and invertebrate cells are two different and independent structures has been also established by using the electron microscope (Beams et al., 1952, etc..), utracentrifuge (Brown, 1936; Moussa, 1950, 1952), and by cutting the sciatic nerve (Moussa, 1956). Moussa found that, on cutting the sciatic nerve of the cat, the Golgi apparaus of the neurones corresponding to the cut nerve fibres was shifted to the cell periphery leaving a zone around the nucleus which is free from the Golgi apparatus but still contains its mitochondria.

In addition, the chemical composition of the mitochondria differs completely form that of the Golgi apparatus.

III. Golgi apparatus and Nissl substance: See Chapter II.

IV. Golgi apparatus and Holmgren camals :

During the early stages of the investigation of the Golgi apparatus the subject was confused by the claim of Holmgren that it was identical with a system of clear canals (opening at the cell surface) which he claimed to have discovered in many cells and which he called the "trophospongium". Cajal added to the confusion by referring to the Golgi nets as Golgi-Holmgren canals.

The evidence obtained from ultracentrifugation of nerve cells, shows that the Holmgren canals become stratified in a position different from that of the Golgi apparatus. The position and the structure of both systems are also different; and the general opinion of cytologists now is that there is no connection between the two systems.

Form, size and distribution :

The Golgi apparantus varies in **size** from one type of cells to another. It is large and active in secretory, nerve and gential cells; relatively smaller in cells in which no obvious secretory activity can morphologically by identified.

In general, the **form** and **size** of the Golgi apparatus are characteristic for each cell type; nevertheless, they vary in the same cell according to its functional activity and age. For example, in starved rabbits the Golgi apparatus of epithelial cells occurs as a compact reticulum lying between the nucleus and the luminal surface of the cell. After feeding, the Golgi apparatus hypertophies and secrection granules arise in close association with the Golgi substance (Moussa and Khattab, 1957a). As regards the effect of age, conspicuous changes in the morphology and chemistry of mammalian, amphibian and avian neurones have been described and illustrated by us (1952, 1955, 1963, 1970). As a general rule, ageing is accompanied by fragmentation of the network into small rods and granules.

In mitosis, the Golgi apparatus usually breaks up into small particles or granules which are distributed more or less even in the cytoplasm. Cytoplasmic division causes an approximate halving of the Golgi substance. The arrangement of Golgi bodies during the stages of cell division varies in different animals, but in all cases they are shared out between the resultant cell.

The position of the Golgi apparatus is relatively fixed for each type of cells. In the nerve cells it completely surrounds the nucleus, but in the exocrine gland cells (e.g., epithelial cells of intestine and epididymis) it lies between the nucleus and the excretory pole. This polarity is variable in endocrine glands except in the thyroid gland where the apparatus is present towards the centre of the follicle.

Chemical Compostion :

The Golgi apparatus is a combination of proteins and lipids; in other words, the apparatus is composed basically of a lipoprotein in which the lipid is usually masked, i.e., the lipid is combined with protein in such a way that it does not give the typical lipid reactions.

Through a series of investigations including different types of somatic cells (e.g., cells of nervous system, stomach, intestine, liver, pancreas, uterus and others) of vertebrates and invertebrates the present authors (1949-1975) have established that the Golgi apparatus is not sudanophil;

and that the proteinic part in neurones is composed of tyrosine and glutathione. In addition, polysaccharides have been found in the Golgi substance of intestinal epithelial cell. The Golgi canals, on the other hand, contain thiamine pyrophosphatase and some glycosyl transferases which transfer oligoscaccharides to glycoproteins.

Sometimes the lipid portion is not masked as in the germ cells of mollusca and annelida (Gatenby and Moussa, 1949). In addition, unmasking of the lipid part of the Golgi bodies has been reported by us in the senile condition of cells of both vertebrates and invertebrates.

Demonstration:

The Golgi apparatuscan be demonstrated by the silver impregnation methods of Aoyama, Cajal and Da Fano, and by the osmication methods of Kolatchev-Nassonov and Mann-Kopsch. The apparatus is also demonstrated by the thiamine pyrophosphatase technique, this is a staining procedure which is based on the presence of specific phosphatases in the Golgi saccules.

In fixed or supravitally stained cells the Golgi substance of somatic cells does not stain with the lipid dyes (e.g., neutral red methylene blue and sudan dyes).

In living unstained cells of both invertebrates and vertebrates Golgi bodies – comparable to those demonstrated by the classical techniques – have been seen by different authors especially with the phase contrast microscope.

In ultracentrifuged material the Golgi apparatus takes up position well below fat, but above the heavier mitochondria.

Development of the Golgi apparatus :

(A) Development in vertebrates :

The Golgi apparatus has been studied in the various development stages of the nerve cells of mammals (Moussa, 1952; Moussa and El-Beih, 1970), birds (Moussa and Khattab, 1963) and amphibians (Moussan and Banhawy, 1955) in both living and fixed cells with the help of the phase contrast microscope. (Fig. 50).

In embryonic stages the Golgi apparatus appears as a tiny reticulum lying adjacent to the nucleus at the axon hillock of the cell. The Golgi apparatus retjculum spreads gradually on both sides of the nucleus until it becomes perinuclear.

In the full-grown animals the Golgi apparatus spreads in the cytoplasm but does not reach the cell periphery.

In the old stage the Golgi apparatus reticulum fragments into short filaments and rods. Fragmention goes on until, in the senile condition, the Golgi apparatus is completely transformed into small irregular bodies.

It should be noted that the canalicular nature of the Golgi apparatus is clear even in the very early embryonic stages. The wall of the canal is argentophil and osmiophil; but does not stain with either sudan dyes or neutral red. The lumen of the canal is argentophobic and osmiophobic; it contains a fluid which is very probaly secreted by the Golgi apparatus.

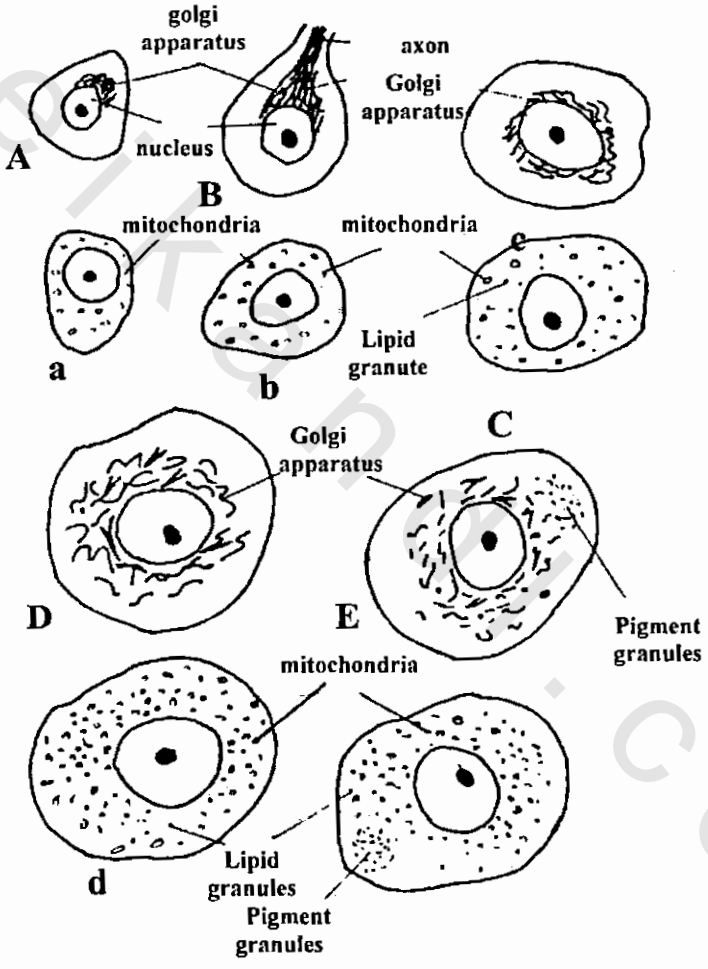


Fig. 50 :

Developmental stages of vertebrate neurones showing Golgi apparatus mitochondria and lipid granules, A and B, neurones of an embryo. C,D and E, neurones of young, adult and old individuals. a,b,c, d and e same ages as above.

(B) Development in invertebrates :

The development of the Golgi apparatus was followed in the various stages of development of the nerve cells of mollusca (Moussa, 1950) and insecta (Moussa and Banhawy, 1960), and it has been found that there are differences in the details, but as a general rule the stages of development in both are alike. (Fig. 51).

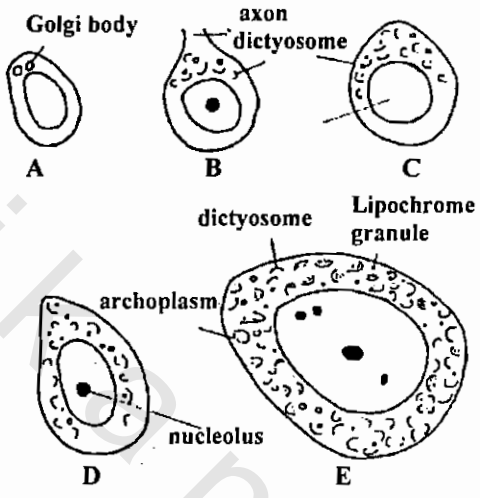


Fig. 51 :
Development of the Golgi apparatus (dictyosomes) of limnaea neurones (After Moussa).

Ultrastructure :

According to some workers the Golgi apparatus of vertebrates seen by the electron microscope (Fig. 52) consists of: (a) a unmber of elongated flattened sacs or cisternace running parallel to each other, (b) a group of large vacuoles lying near the margin of the flattened sacs, and it is assumed that such vacuoles are due to the dilatation of some of the tubular cisternate; (c) clusters of small vesicles.

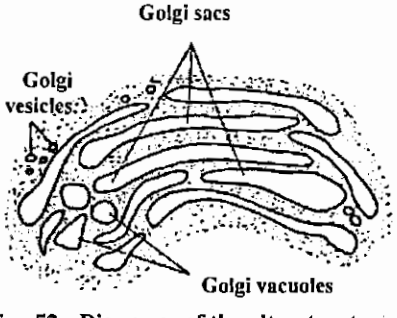


Fig. 52: Diagram of the ultrastructure of the Golgi apparatus.

In other words, the electorn microscope revealed a system of elongated membrane-bound sacs and assoicated vesicles in the places where the classical cytologists had located the Golgi apparatus.

The Golgi **dictyosomes** appear in the electron micrographs as flattenend curved sacs together with clusters of small vesicular bodies at their edges.

Functions of the Golgi apparatus :

Studies with the light and phase contrast microscopes indicate clearly that the Golgi apparatus is closely associated with **the formation of specific secretions** in different types of cells. Most of these studies have been confirmed by using the recent techniques and the electron microscope. The following examples demonstrate the role of the Golgi apparatus in the production of secretions:

(A) The manner of formation of acrosome in spermatozoa (Fig. 53 B) is the classic evidence that the Golgi appsratus is the centre of secretory synthesis. This has been confirmed by the use of the electron mictoscope (Bugos and Faweett, 1955). In the young spermatid the Golgi apparatus elements surround the centrosphere. As development proceeds large vacuoles are formed by the dialations of the Golgi elemnts (the flattened sacs). Each Vacuole encloses a granule which is formed by the Golgi apparants. Then the granules run rogether forming 2 or 3 larger granules, which finally fuse together forming a single strcuture called the proacrosome which is enclosed in a vacuole. The proacrosome then comes in contact with the anterior pole of the nucleus and is now known as the acrosome. The spermatid elongates and the acrosome becomes flattened over the anterior part of the nucleus and assumes its final form. At the same time the acrosomal vacuole forms the head cap of the spermatid and the Golgi apparatus migrates to the opposite pole of the nucleus.

(B) The investigations of several authors (Bowen, CJl, Nassonov, Moussa, Banhawy, Khattab, and others) indicate clearly the association of the Golgi apparatus with **the production of secretions in various types of externally secreting glands** (e.g., secrection of pepsin by peptic cells of stomach, bile by the liver cells and zymogen granules by the exocrine cells of pancreas). In one of these investigations it has been found that in rabbits starved for 48 an hours, then killed, the Golgi apparatus is very compact and lies close to the luminal pole of the nucleus (Fig. 56). When starved rabbits were fed, and then killed after half and hour, the Golgi apparatus begins to extend with few argentophil granules. Most of these granules are in close association with the Golgi substance. The apparatus extends more in rabbits which were killed one hour after feeding, in addition more secretory granules were observed). Two hours after feeding the Golgi apparatus becomes very extensive, more heavily impregnated and occupies the luminal part of the cytoplasm. In these cells the secretory argentophilic granules are transformed into larger non-agentopilic spheres which run together forming larger bodies, of which many were seen to be accumulated at the luminal pole of the cell.

The argentophil granules which appear after feeding, are the young or prozymogen (propepsinogen) granules; these are transformed into argentophobic nature zymogen spheres (pepsinogen) by the Golgi apparatus (Moussa and Khattab, 1957b).

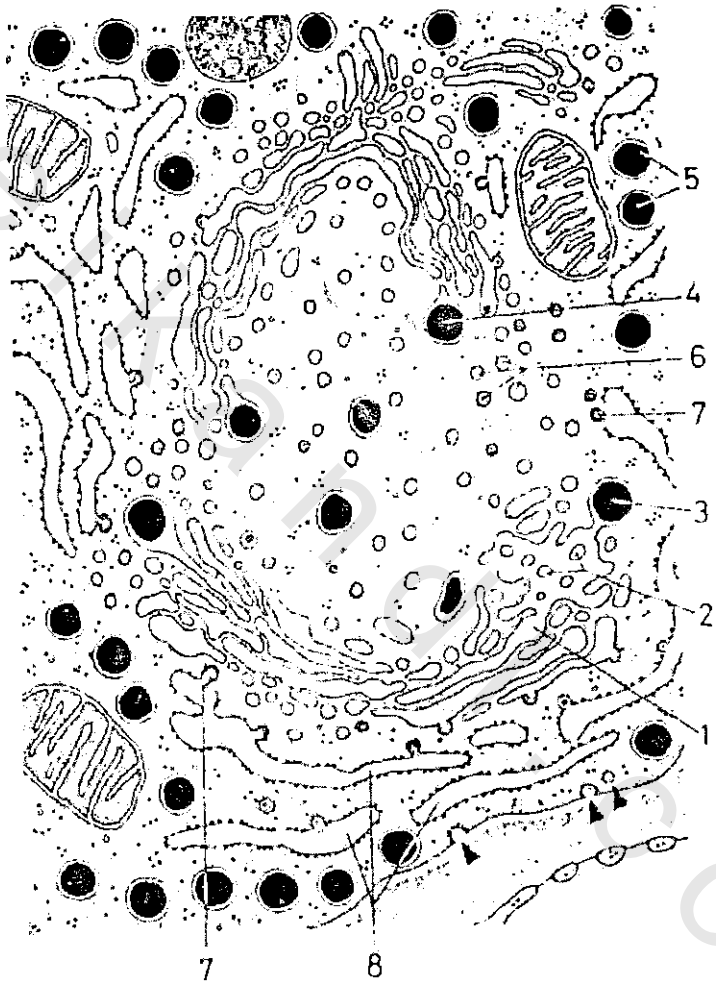
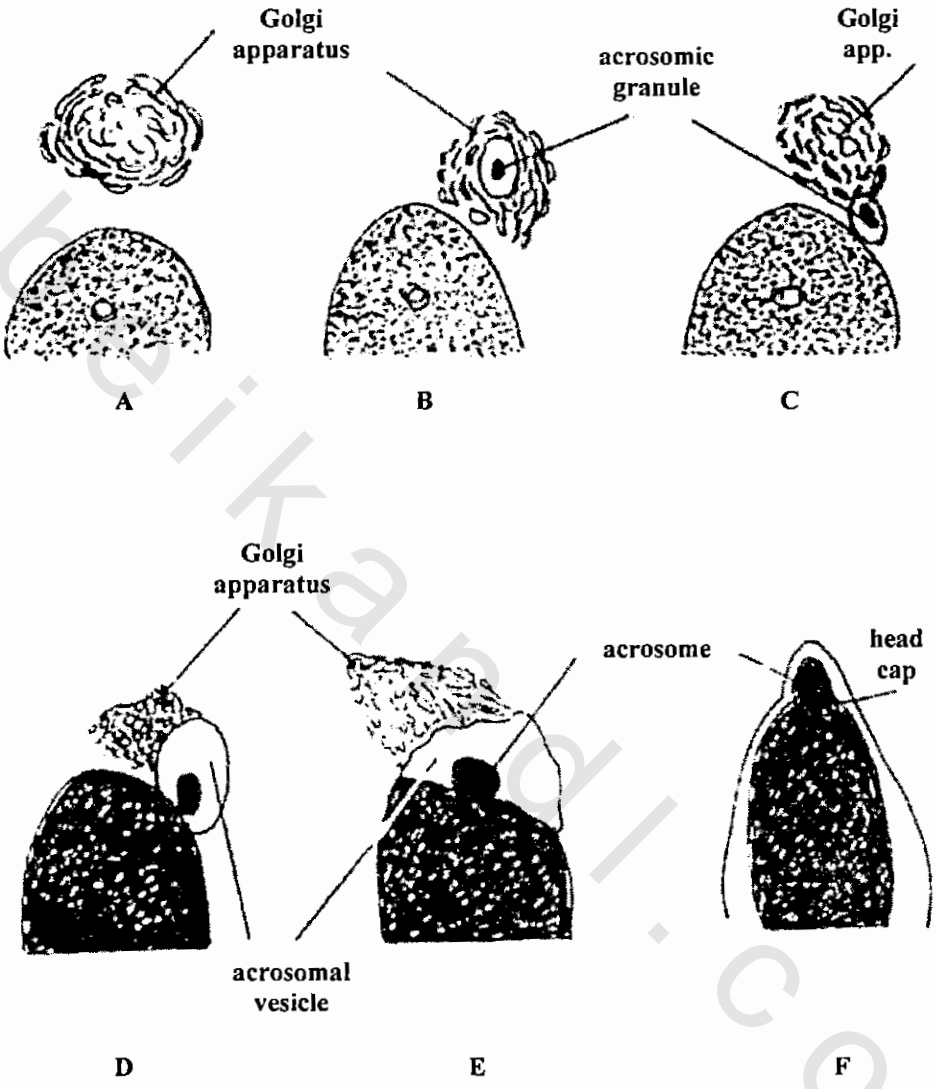


Fig. 53 A: Golgi apparatus

1 – Golgi cisternal. 2 – Internal inclusions. 3 – Osmiophilic.
4 – Golgi vacuoles. 5 – Secretory granules. 6 – Golgi vesides.
7 – Advanced vesicles.



(Fig: 53: B): Diagram (from electron micrograph) to illustrate the formation of the acrosome (From Burgos and Fawcett).

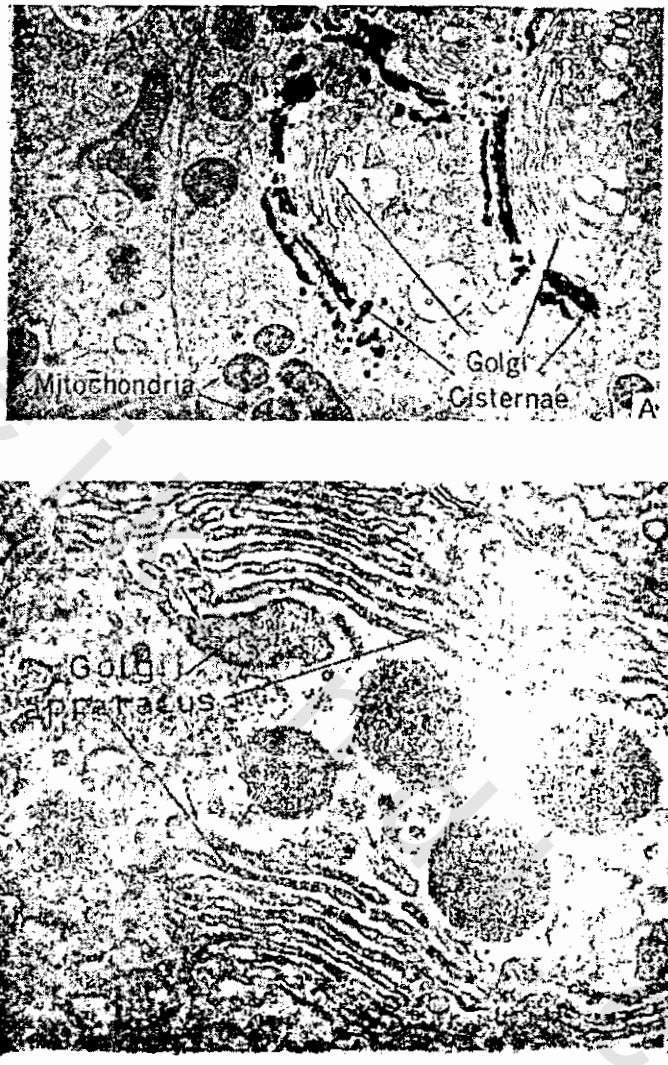


Fig. 54:
Electron micrographs showing the Golgi apparatu (From Fawcet, after Daniel Friend).
Upper: showing selective deposition of osmium on or in the outer Golgi cisternae (Sacs) but not in the inner ones.
Lower: showing that the distended Golgi sacs contain a flocculent material (see at arrows) resembling the early secretion granules.

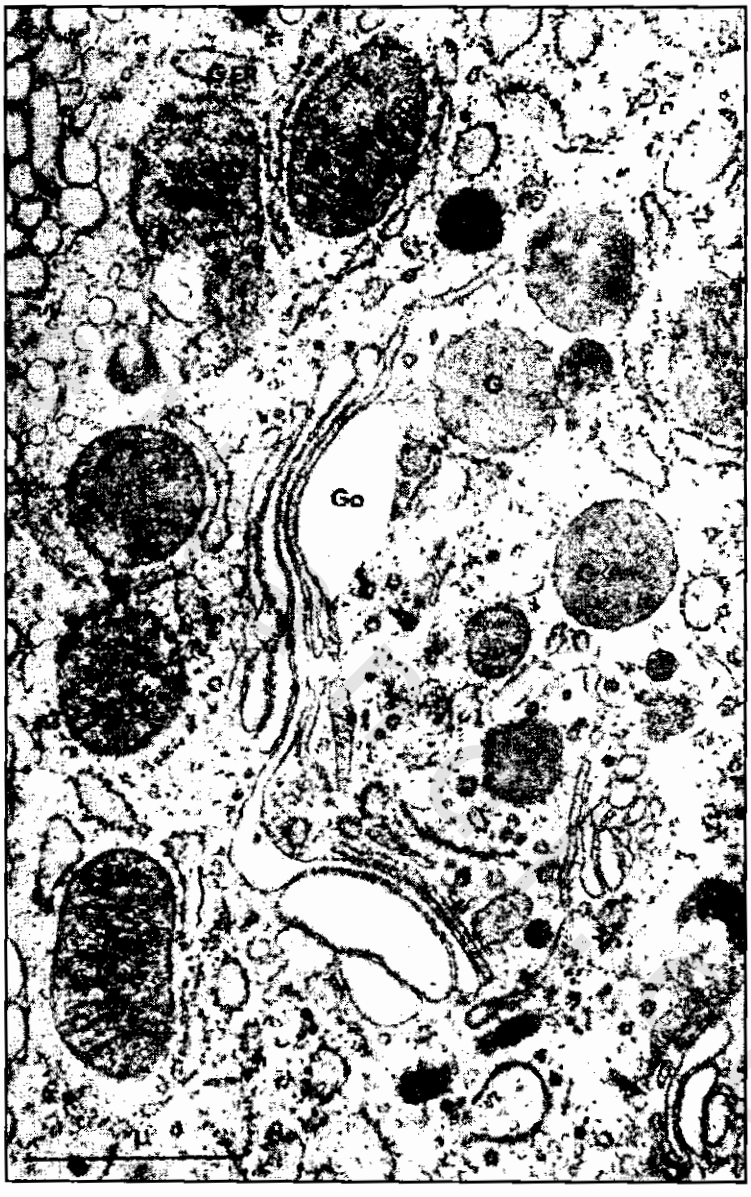


Fig. 55:
Electron micrograph showing the Golgi apparatus (Go), Notice also the granular endoplasmic reticulum (ER), zymogen granules (G.) mitochondria (M.) and ribosomes (R) Magnification: X 30,000 (From Toner and Carr).

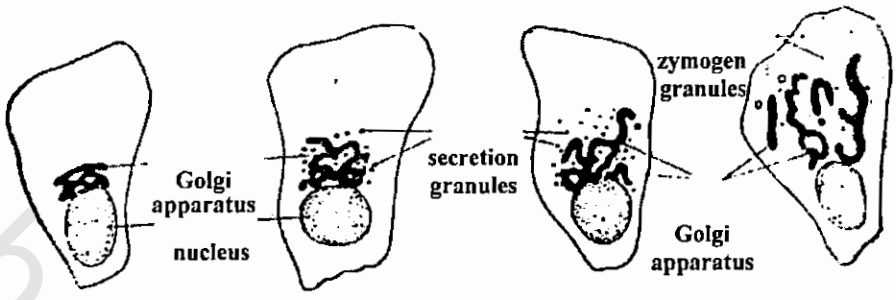


Fig. 56:
Golgi apparatus and secretion of enzymes in peptic cells of rabbit. A.D. showing the morphological changes of the Golgi apparatus during the different phases of activiy. A. cell showing compact Golgi reticulum after 48 hours fast; B. Golgi reticulum enlarged and secretory granules appeared in its field (half an hour after feeding); C, cell in an advanced stage of secretion (one hour after feeding); D, cell showing the transformation of some of the argentophil secretory granules (propesinogen) into non-argentophil pepsinogen spheres (After Moussa and Khattab).

(C) In goblet cells, the Golgi apparatus is the site of formation of mucus (Cajal, 1914). This has been later confirmed by using the electron microscope.

(D) The Golgi apparatus region in epithelial cells, nerve cells and others has been shown to contain alkaline and acid phosphatases; this suggests the exitence of a certain relationship between the Golgi apparatus and these enzymatic particles. This has been confirmed in the Golgi elements isolated by centrifugation. Recently, the electron microscope reveals the formation of primary lysosomes (sites of acid phosphatase activity) by the Golgi apparatus (Novikoff, 1964). Remarkably enough, the Golgi apparatus shows acid phosphatase activity (Fig. 68), a phenomenon which was also stressed by Weizel and his associates (1967). (E) Gatenby and Moussa believe that the senility pigment and lipid granules in vertebrate neurous are secreted by the Golgi apparatus. In addition, the liopochrome bodies of molluscan neurones, as pointed out by Moussa (1950), are secreted by the Golgi dictyosomes. This has been confirmed by Moussa and Banhawy (1960) and Banhway and Anwar (1970) who demonstrated the stages of neurosecretion in insects. In the locucst, the fifth instar nymph and the adult represent the active or secretory phase in which the Golgi dictyosomes accumulate in groups forming irregular or ring-shaped structures each enclosing a chromophobic sphere secreted by the

dictyosomes. This is transformed under the effect of the dictyosomes, into a number of small osimiophilic granules which fuse together forming one or two large osmiophilic granules that become emitted into the cytoplasm (Fig. 57).

(F) The investigations of Bourne, Moussa and others indicate that vitamin C is associated with the Golgi apparatus. Bourne indicated that the Golgi apparatus of kidney tubule cells segregates vitamin C; and Moussa suggested that the Golgi apparatus of mammalian sympathetic neurones may secrete or condense vitamin C.

(G) According to Cramer and Ludford (1925) the Golgi apparatus of intestinal cell is concerned with the **synthesis of fat** from absorbed fatty acids and glycerol.

(H) It is also believed that the Golgi apparatus is concerned with the secretion of synovial fluid in the joints, in the formation of the enamal of teeth, the production of pigment by the cells of the iris epithelium and the formation of fatty yolk in eggs.

(I) In their study on the morphological and chemical changes in ageing of the Golgi apparatus of amphibian neurones. Moussa and Banhawy (1955) pointed out that the Golgi apparatus of old and senile neurones fragments into filaments which are continous with the lumens of the Golgi elements, then the vesicles separate from their mother elements and lie free in the cytoplasm. According to these authors these vesicles are the transformations of the Golgi apparatus in ageing neurones.

According to some authors the Golgi apparatus acts as a condensation membrane for the concentration of the diffused cytoplasmic products into certain granules or droplets which are later expelled form the cell. In this respect, the Golgi apparatus is regarded to withdraw water from the maturing secretory material, which becomes packed into compact granular elements. This view is also adopted to explain the role played by the Golgi apparatus in the segregation of lipid droplets in the duodenal epithelial cells (Weiss, 1955)

Related to the function of concentrating secretion products is the homology suggested between the Golgi apparatus of higher animals and the contractile vacuole (which expels water from the cell to the outside) present in protozoa and lower animals (Gatenby, Dalton and Felix, 1955).

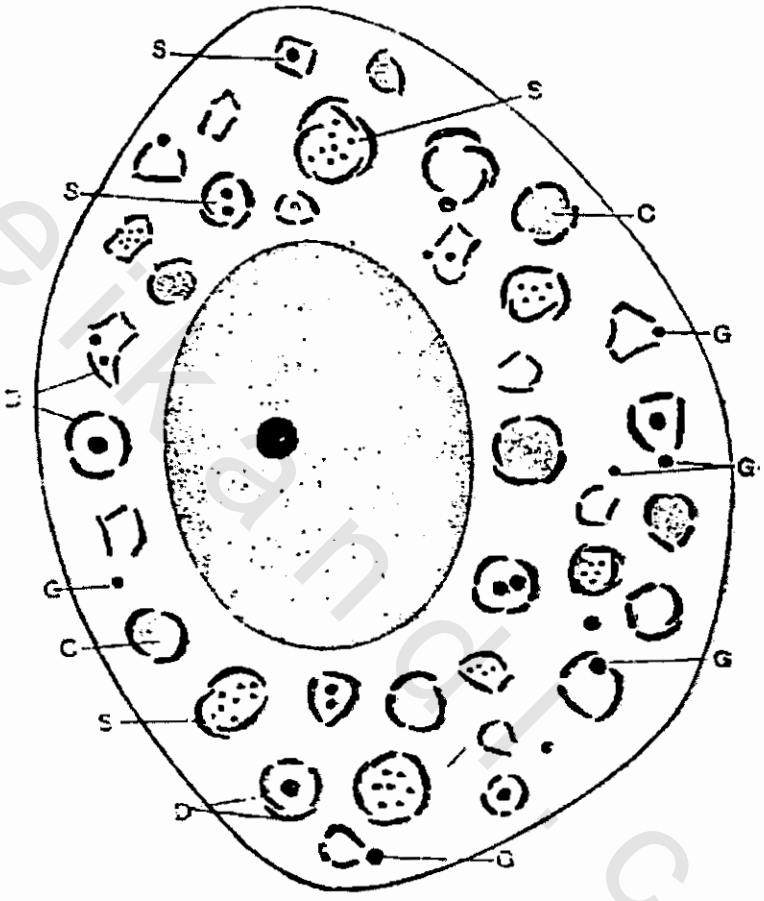


Fig. 57:

Large motorneurone of adult locust showing the arrangement of the Golgi dictyosomes D and the various stages or secretion (after Moussan and Banhawy). The successive stages of secretion are illustrated as follows:

(1) Secrection of the chromophob sphere (C).

(2) (2) Formation of minute secretion granules (s_1).

(3) Fusion of the minute secretion granules into one or two large granules (S).

(4) Emission of the secreted granules (G), G represents various stages of the emission of the secretion granules.

The use of tracers and electron microscope shows that proteins are primarily synthesized on the endoplasmic reticulum (by ribosomes), then the secretions pass from the endoplasmic reticulum to the Golgi apparatus where they undergo maturation, and finaly they are liberated into the cytoplasm.

However, by using the same technique as above, it has been found that the Golgi apparatus is the only site of synthesis of the complex polysaccharides, none has been detected in the ribosomes or the endoplasmic reticulum (Young, 1973). It has also been suggested that the glycoproteins formed in the Golgi apparatus migrate to the surface of the cell to form the cell coat.

From all the previous examples it is clearly established that the Golgi apparatus is concerned with the formation of specific **secretions** in different types of cell, but the part which it plays in the process has been interpreted in different ways. Some cytologists are of the opinion that the apparatus synthesises **secretory substances**.

According to others, the apparatus is transformed into secretory products, whereas others believe that the Golgi apparatus acts as a **condensation membrane** for the concentration, in drops or granules, of products eleborated elsewhere in the cytoplasm. Sometimes the apparatus represents the site of major steps in the completion of synthesis initiated in the endoplasmic reticulum or other of major steps in the structures in the cell. It is obvious, from the accumulated data and the fore – mentioned examples, that all these accumulated data and the fore – mentioned examples, that all these views are correct and that the relationship between the Golgi apparatus and secretion is dependent upon the nature of secretory product.

Pathological changes of the Golgi apparatus :

The Golgi apparatus is a dynamic system which responds to altered levels of activity. Under physiological and pathological conditions the apparatus shows changes particularly in size and position. The morophological alterations (and sometimes the chemical) have been described (Moussa and collaborators 1956-1975, under a variet of pathological cases as shown in the following: (Figs. 58-61).

(1) Section of the Sciatic nerve leads to the migration of the Golgi apparatus network of the corresponding neurones from the perinuclear region to the cell periphery.

(2) The Newcastle disease virus is associated with swelling accumulation and movement of the Golgi apparatus towards the cortical part of the cytoplasm.

(3) Vitamin B- complex deficiency causes fragmentation of the network of the Golgi apparatus of mammalian neurones into spherical-shaped bodies and short fragments which are mainly concentrated around the mucleus. Fragmentation proceeds and Golgi material becomes hardly visible.

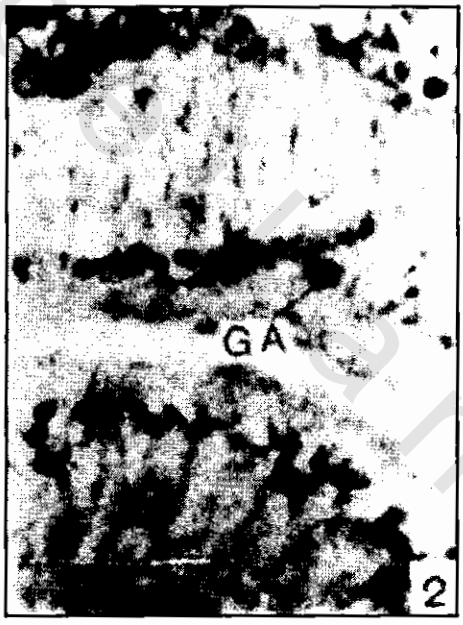


Fig. 58: Golgi app. In epithelial cell of fasted animal.

(4) Treatment with insecticides produces marked alterations in the morphology and topography of the Golgi elements. In general, they are fragmented into small particles, which are thinnedoutandgradually disappeared in advanced stages of injury. The secretion granules accompanying the normal Golgi bodies also disappear from such cells.

(5) In morphine poisning the Golgi apparatus network if mammalian neurones swells and loosens, then breaks up into fragments. As morphine injection goes on for a long period gradual fragmentation of the Golgi apparatus occurs and finally disappears completely.

(6) Electrical stimulation, irradiation, cold, phosphorous poisoning and others have also marked effects on the morphology, chemistry and behaviour of the Golgi apparatus of both vertebrates and invrtebrates.

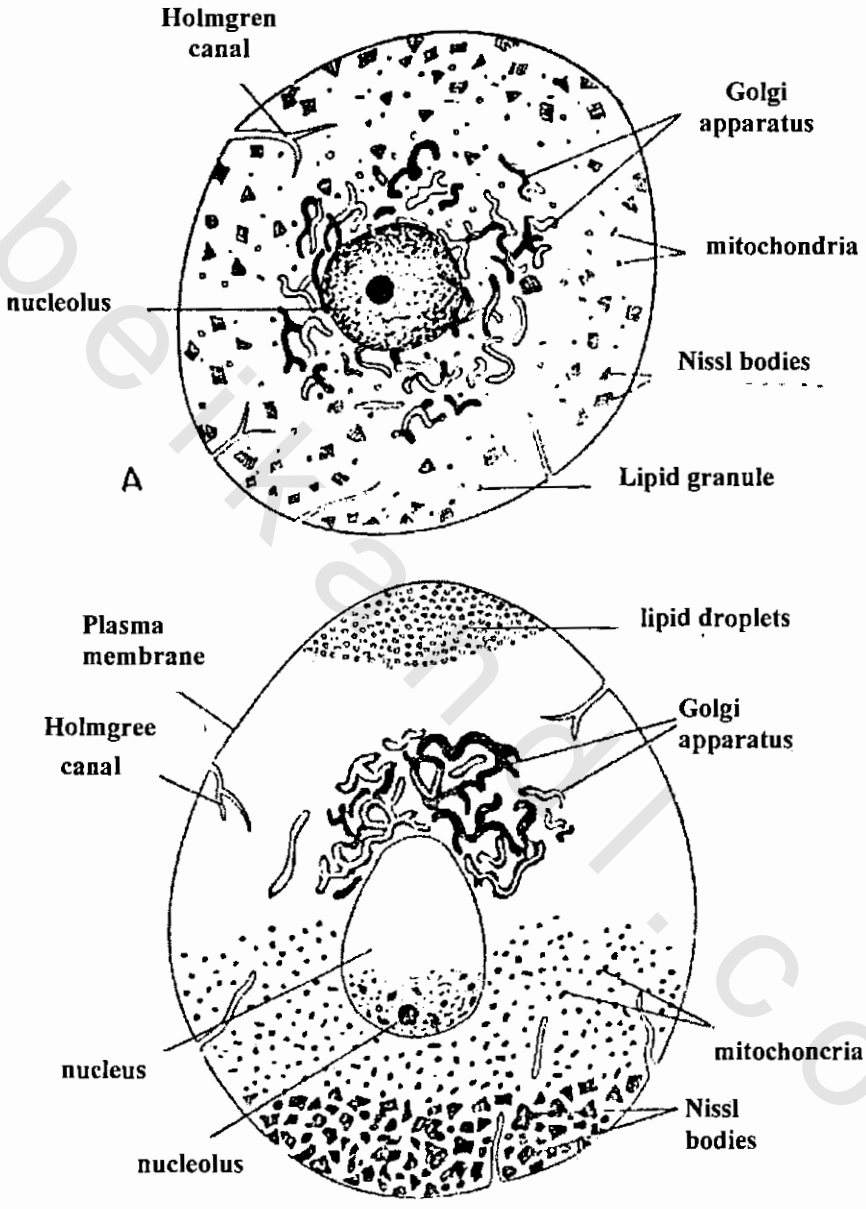


Fig. 59:
Effect of nltracentrifugation on vertebrate neurones (after Moussa).
A : Normal newurone, B: Ultracentriguged neurone.

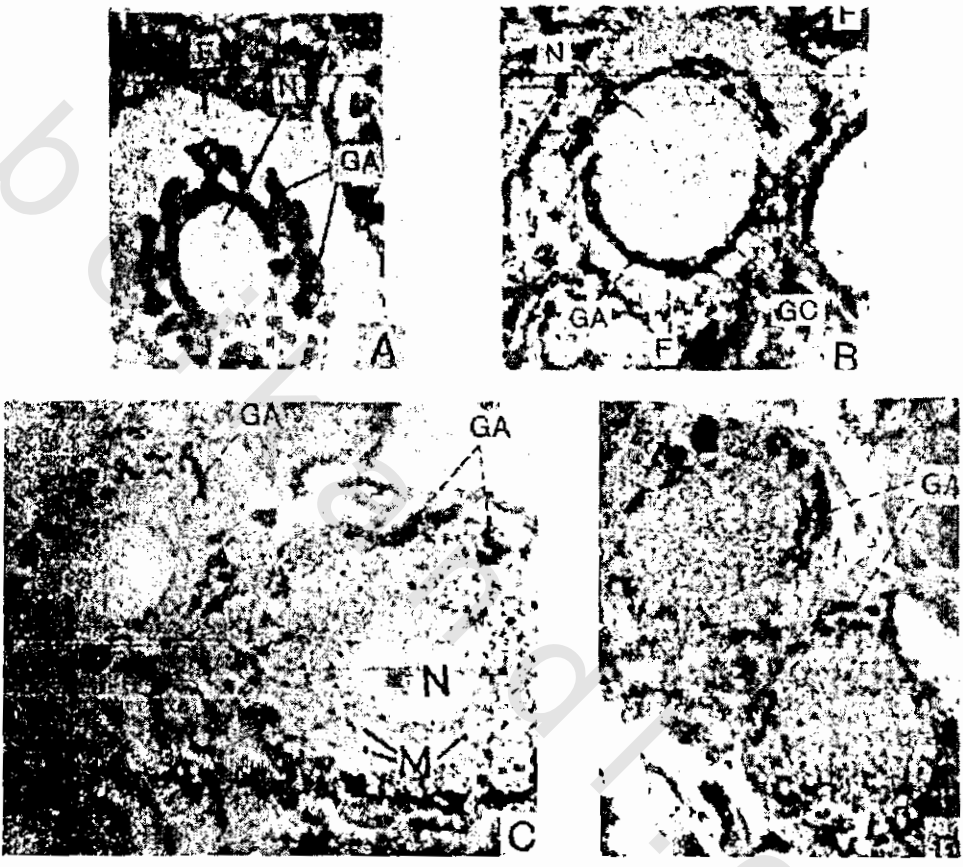


Fig. 60:

Spinal ganglion cells of the cat (C.D) showing the movement of the Golgi apparatus (GA) from the perinuclear region to the cell periphery after cutting the corresponding sciatic nerve. The small cell in C shows partial retispersion, e.e., the Golgi apparatus has not yet reached the cell periphery. Retispersion in larger cells is earlier than in the smaller. Figure C also indicates that retispersion takes place before chromatolysis. The mitochondria (M) are scattered throughout the cytoplasm (F). Compare the Golgi apparatus in these cells with that in the normal spinal ganglion cell of cat (A) and sympathetic neurone of mouse (B.) (After Moussa).

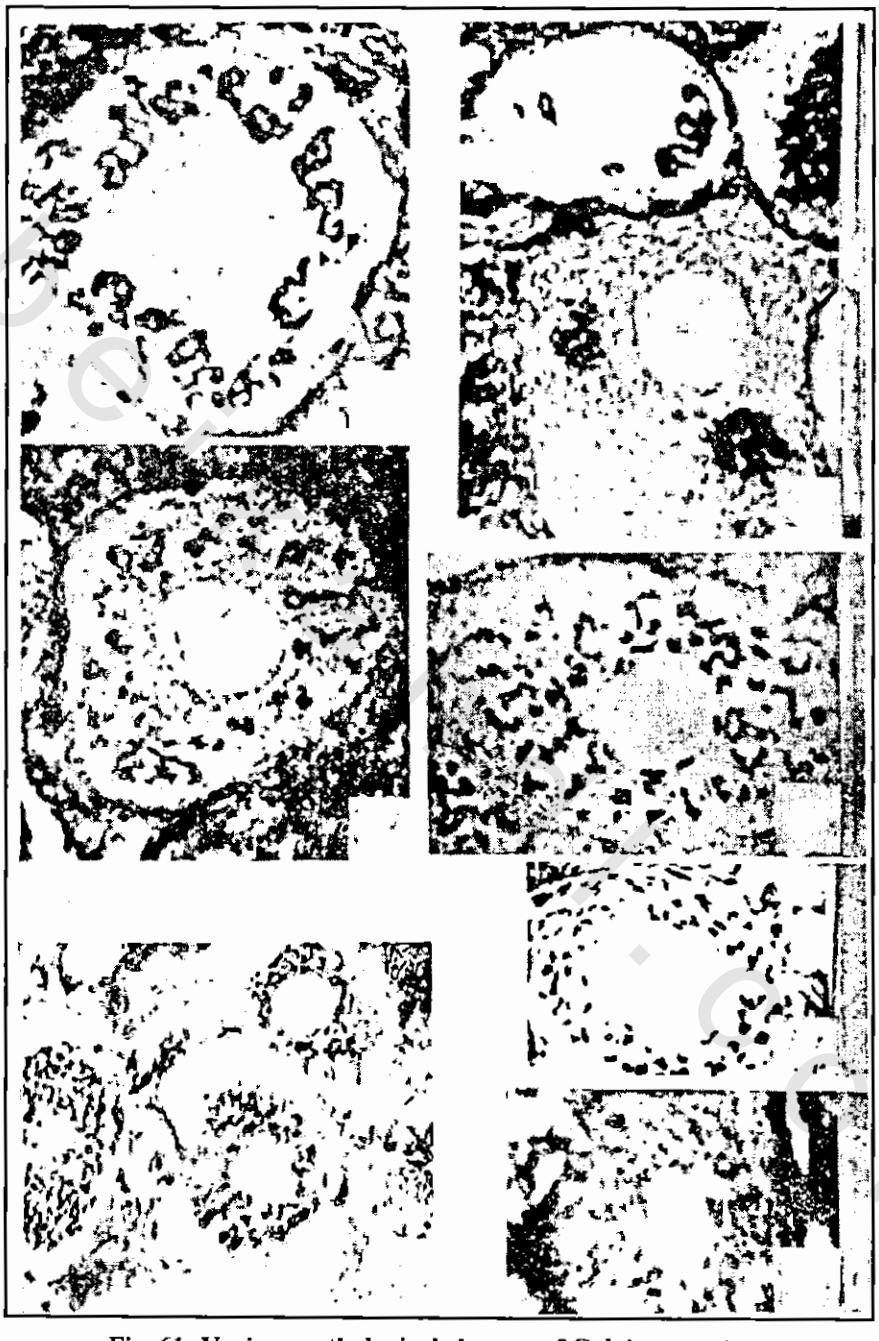


Fig. 61: Various pathological changes of Golgi apparatus.
(After khattab)

CHAPTER 9

LYSOSOMES AND PEROXISOMES

LYSOSOMES

The lysosomes were first described by the Duve and his collagues in 1955 in the liver cells of rate as a distinct group of cytoplasmic particles. Later they were demonstrated by many authors in the majority of animal cells. Furthermore, there is now considerable evidence for the presence of lysosome-like structures in plant cells.

Lysosomes appear under the light microscope as small vesicles or granules (Fig. 62 - 63) which are slightly smaller than the mitochondria, in the electron micrographs (Fig. 64 - 65) they are seen as sac-like structures, each being surrounded by a single thin lipoprotein membrane.

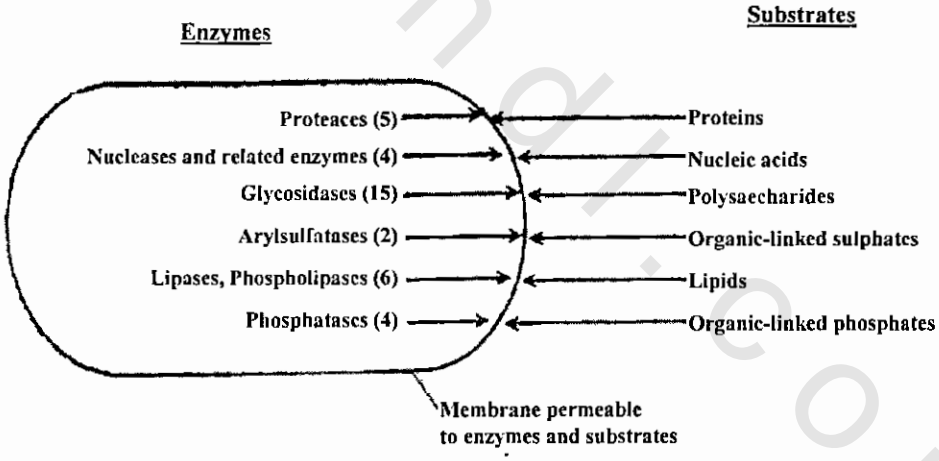
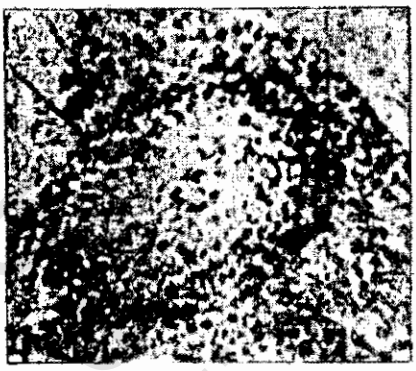
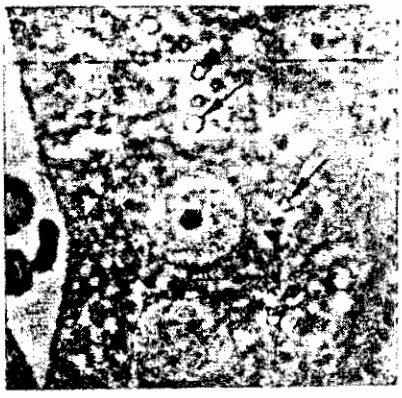


Fig. 62:

Diagram showing enzymes (36 hydrolases) in lysosomes. The number indicates how many enzymes hydrolyze each class of substrates shown to the right. (After de Duve et al.).





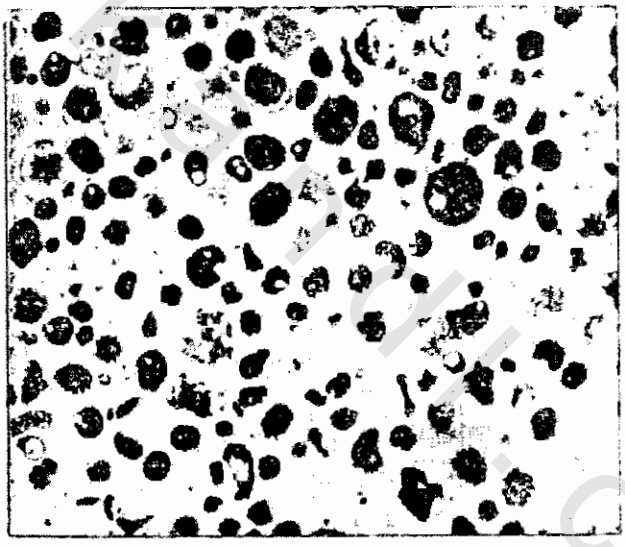


Fig. 63:

Left, Lysosomes (indicated by arrows) in the cells of the mouse kidney viewed under the phase contrast microscope, Magnification: X 500. (From Threadgold The ultrastructure of the animal cell).

Fig. 64:

Right, Lysosomes in a nerve cell seen under the light microscope some lysosomes are in the form of rounded vesicles.

Fig. 65:

Lower, Electron micrograph of isolated lysosomes from normal rat liver, (Form Dingie: Lysosmes) X 11,000.

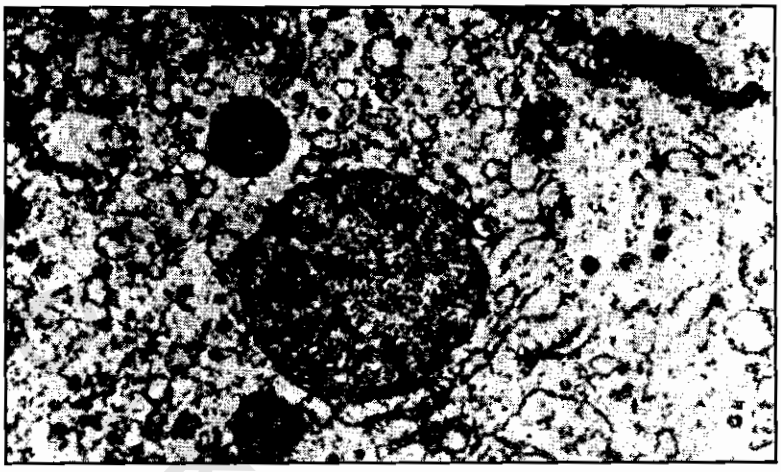


Fig. 66:

Hepatic parenchymal cell showing typical dense body type of lysosome (L) with a peripheral clear "halo" surrounded by unit membrane, Note also a multivesicular lysosome and a mitochondrion (M). (From Dingel: Lysosomes).

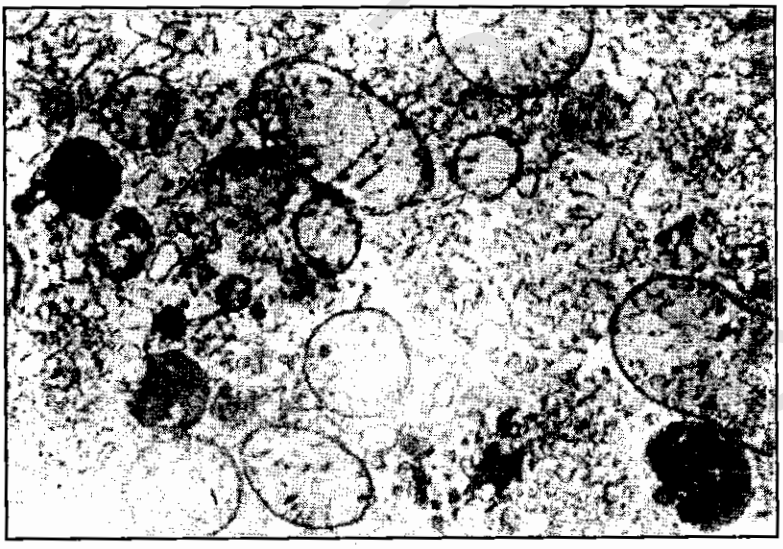


Fig. 67:

Electron micrograph of liver phenobarbital treated hamster showing helerogenecity of lysosomes and deformed mitochondria.

Lysosomes are rich in acid hydrolases such as acid phosphatase, cathepsin, ribonuclease and deoxyribonuclease (Fig. 66). All these hydrolytic enzymes work in a mild acid medium. They break bown cellular material such as protein, nucleic acids, and polysaccarides. The particles are, therefor, known as lysosomes-that is, bodies which can digest or lyse substances. It has been clearly shown that when the limiting membrane of these particles is ruptured, the contained enzymes are related causing destruction of the cellular constituents and complete dissolution of the cell. For this reason the lysosomes are also known as the "suicide bags".

Demonstration:

The identification of lysosomes is proved by the demonstration of acid phosphatase activity in the cells. This enzyme is the most important of all the hydrolytic enzymes enclosed in the lysosomes.

For the demonstration of the lysosomes, formol-calcium frozen sections are incubated in a medium containing lead nitrate and the enzyme substrate, sodium B-glycerophosphate. This medium is adjusted at pH 5.0 and kept for sometime at 37° C. This is followed by a short treatment with yellow ammonium sulphide. Lysosomes (sites of acid phosphatase activity) are distinguished as dark brown vesicles. According to this procedure the B-glycerophosphate is hydrolysed by acid phosphatase, and the phosphate ions liberated are captured by lead ions producing insoluble lead phosphate. This colourless precipitate is then converted into brownish black lead sulphide by reaction with ammonium sulphide. As a control, some sections are incubated in a medium lacking the enzyme substrate sodium B-glycerophosphate where negative rsults are obtained.

Distribution and size :

In the intestinal epithelial cells the lysosomes are mainly located in the apical cytoplasmic regions in which the Golgi apparatus is found. In the liver cells they are also, acccumulated in the Golgi apparatus region. This suggests the existence of a close relationship between the lysosomes and the Golgi apparatus, as it also indicates that lysosomes play and important role in the metabolic activities of these cell. Figure 68 demonstrates acid phosphatase activity in the Golgi apparatus sacs.

The size of lysosomes varies according to form, origin and function. In most lysosomes as those of liver cells the diameter is about 05u. However, the largest are several micra in diameter as in the kidney cells of mammals. The smallest lysosomes are the Golgi vesicles in which the diameter is 25-50 m u.

Types of lysosomes :

There are four types of lysosomes: (Figs. 67-71)

1 - **The original or primary lysosomes** (or storage granules) are small bodies in which enzymatic content is manufactured (sythesized) by the ribosomes and accumulated in the endoplasmic reticulum. The histochemical results pointed towards a relatioship of the endoplasmic reticulum and the Golgi bodies. From the reticulum the enzymatic content penetrates into the Golgi region with the formation of vesicles giving a strong acid phosphatase reaction; in other words, primary lysosomes appear to be partially produced by the Golgi apparatus.

2 - **The heterophagosomes or digestive** vacuoles (or secondary lysosomes) result form the phagacytosis or pinocytosis of foreign material by the cell. The phagosome gives a positive reaction for acid phosphatase (this is the indication of the lysosome) which may be due to the association with á storage granule (Golgi vesicle or primary lysosome). Under the action of the hydrolytic enzymes, the engulfed material is progressively digested; the end products pass through the lysosomal membrane into the cytoplasm.

3 - **The residual bodies** are the final particles containing the undigested material. In Amoeba and other protozoa, these residual bodies are elininated by defecation. In the cells of higher animals they may remain for a long time and may be important in the ageing process as in case of the pigment granules in neurones of old animals. However, Gatenby and Moussa (1951) pointed out that the pigment granules of old neurones are secrected by the Golgi apparatus.

4 - **The autophagic vacuoles** in which the lysosomes contain a part of the cell in a process of digestion (e.g., a mitochondrion or portion of the endoplasmic reticulum. A large number of these vascuoles have been obsereved in some physiological and phthological processes. For example during starvation the liver cell contains many vacuoles in which mitochondrial remnants may be detected. This is a mechnism by which the cell can feed upon its own substance without irreparable damage

All these types of lysosomes are very stable in the living cells.

Functional significane of lysosomes:

Lysosomes play an importan role in a variety of cellular activities such as **intracellular digestion**, lipofuscin formation (i.e., formation of coloured lipid granules) and **carbohydrate metabolism**. Hence, they are makedly abundant in the cells engaged in carbohydrate metabolism such as those of the liver, kidney and small intestine. (Fig. 72).

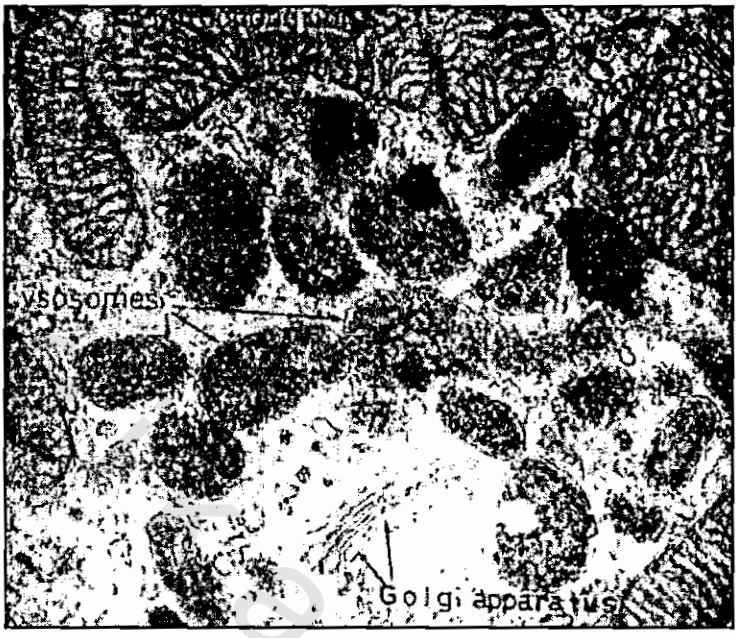


Fig. 68:

Electron micrograph of suprarental cortex showing the limiting membrane of the lysosome.

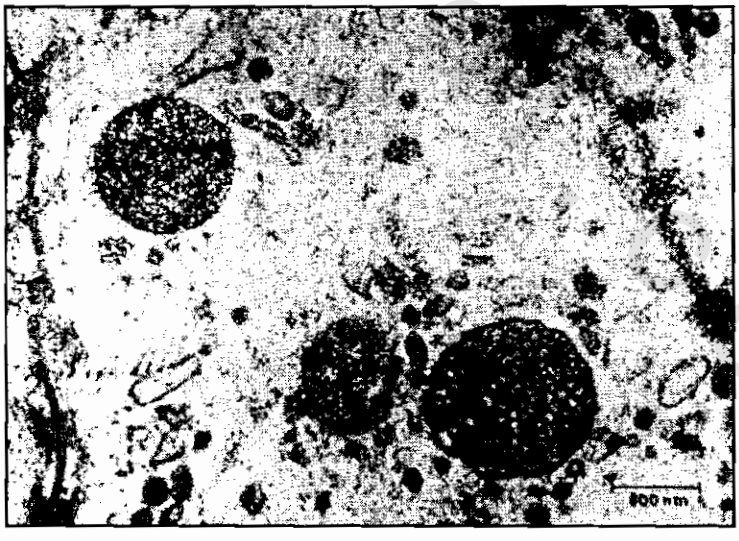


Fig. 69:

Cytochemical preparation showing two multivesicular bodies (heterolysosomes) from rat ileum. (After Johnson).

Fig. 70:

Motoneurones of a rat administered with high dose of gusathioa insecticide showing a high inrease in the number of lysosomes. (After Banhawy and Gansuri).

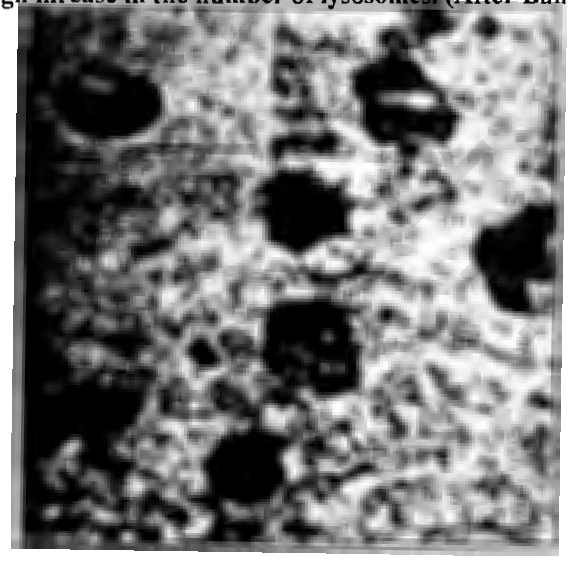


Fig. 71:

Alterations in lysosomes in cat motoneurones after asphyxiation of the spinal cord. In (A) the unit membrane of the lysosome is shown by the arrow. In (B) the number and size of lysosomes are highly increased. The unit membranes are lost and the phosphatase activiy seems to diffuse to the surrounding cytoplasmic constituents as shown by the arrows. Glycogen granules (GL) lie close to the lysosomes. (After Khattab).

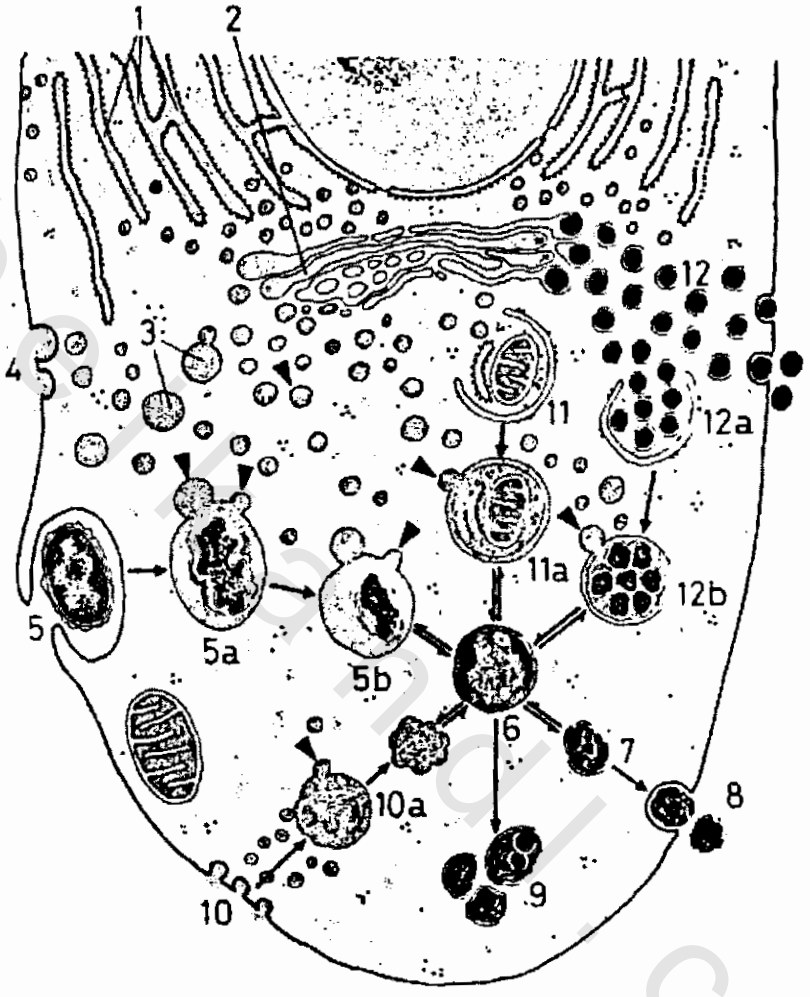


Fig. 72: Lysosomal activities:

1 - Rough E.R. 2 - Golgi apparatus. 3 - Primary lysosome.

4 - Excreted enzymes. 5 - Baderkium or other foreign substance

5 a - Phagosome 5 b - Break down of the bacteria.

6 - Secondary lysosome. 7 - Residual body.

8 - Exocytosis (Extrusion from the cell). 9 - Residual bodies.

10.a - Pinocytosis. 11 - Autophagocytic vacuole.

11.a - Autophagosome. 12 - Secretory granules.

12.a - Autophagocytic vacuole.

12.b - Secretory granules degeneration resulting in a secondary lysosome once again.

Under certain conditions lysosomes take all and, and active part in the **removal of certain cellular and tissue contents** by engulfing and digesting them. For example, during metamorphosis in amphibia, there is occasional destruction and degeneration of considerable numbers of cells as happens when the tadpole tail degenerates. It has been shown that the tail due to the presence of a high concentration of hydrolytic enzymes (e.g., cathepsin) which are contained in the lysosomes present in the tail cells, and which are assumed to be discharged outside the cell to exert their lytic actions.

It is very likely that lysosomes play an important role in the fertilization of ova. The sperm releases lysosomal enzymes which facilitate its penetration at a certain point on the surface of the ovum.

It is believed that lysosomes play an yessential role **in morphogenesis, ageing** and in some diseases. They may share in the process of **carcinogenesis** (i.e., the changing of normla cells to cancer cells).

Physiological and Pathological changes:

Lysosomes are markedly affected by many physiological and pathological conditions as shown in the following:

1 - Fasting of animals results in a marked diminution of these particles which almost disappear completely if fasting is prolonged.

2 - Ageing is also accompanied by a gradual loss of lysosomal particles specially in the liver cell.

3 - In hepatoma cells and in the liver cells of tumour-bearing animals lysosomes were also markedly decreased as compared to the normal healthy liver cells (Banhawy, 1964).

4 - Treatment with X-ray irradiation results in either the accumulation of lysosomal particles in some kinds of nerve cells, or in their disintegration and ultimate disappearance as in liver cells. However, it is believed that long treatment with X-rays causes the disruption of lysosomal membranes and as a result the enclosed enzymes are liberated. Being free in the cytoplasm, the enzymes act to dissolve most of the cellular constituents and this will lead finally to the degeneration and lysis of the cells.

5 - Necrosis and autolysis are also accompanied by considerable alterations in the number and size of lysosomes.

6 - Treatment with insecticides causes conspicuous effects on the lysosomal contents of mammalian liver cells and nerve cells. In the

Purkinje and spinal cord cells, the lysosmes are considerably increased in size and number, but in the spinal ganglion neurones they are less affected.

7 - The lack of oxygen affects lysosomes. In asphyxia, the lysosomes rupture and the releasing enzymes result in the lysis of the cell (Khattab, 1967).

PEROXISOMES

Peroxisomes are particles (of about 0.3-1.5 u in diameter) similar to the lysosomal particles. These particles are rich in the enzymes peroxdase, catalase, D amino oxidase, and a lesser amount if urate oxidase.

Peroxisomes occur not only in the liver and kidney of vertebrates but also in protozoa, yeast and many cell types of higher plants. Those present in plant cells contain different enzymes including the enzymes of glyoxylate cycle, and hence are called glyoxysomes.

The peroxisomes are ovoid granules limited by a membrane. They contain a fine granular substance which may condense in the centre formimg an opaque core. The number of peroxisomes per cell of the rat liver ranges from 70-100, whereas the number is 15 to 20 for the lysosomes.

Origin of the peroxisomes :

Peroxisomes are initmately related to the endoplasmic reticulum. They are frequently observed to be continuous with the membranes of the endoplasmic reticulum. They appear in both plant and animal cells as dilatations of the endoplasmic reticulipum. The enzymes of peroxisomes are synthesized by the ribosomes.

Peroxisomes are usually found in close contact with chlorophlasts and mitochondria in green plant cells. This may reflect a close metabolic relation among these organoids.

Functional activities of Peroxisomes:

The liver peroxisomes contain four enzymes which are involed in the metabolism of hydrogen peroxide H_2O_2. The enzymes urate oxidase. D-amino oxidase and u-hydroxylic acid oxidase synthesize hydrogen peroxide which catalase destroys it. Since H_2O_2 is a toxic substance to the cell it is probable that catalase plays a protective rôle.

CHAPTER 10

NISSL BODIES

The Nissl or tigroid bodies were first described by Nissl in 1889. They are also known as the chromophil or basophil elements owing to their strong affinity for basic dyes. These bodies are characteristic for the nerve cells and they have not been noticed in any other cell type.

They occur as granules in the invertebrate nerve sells, but in the vertebrate nerve cells they are in the form of granules and flake-like elements of various shapes and sizes (Figs. 73 – 79). Their presence is mainly confined to the cytoplasm, though they extend along the dendrites. The axons are usually devoid of these bodies.

Chemical composition:

The Nissl bodies consist mainly of nucleoprotein. The nucleic acid in Nissl substance is the ribonucleic acid (RNA), and the protein fraction consists mainly of arginine and hsitidine.

Demonstration :

The bodies of Nissl are easily demonstrated following appropriate fixation and staining. They are strongly coloured with basic stains such as toluidine blue, Borret's methylene blue and Giemsa stain. They have been seen in living unstained neurones by using the phase constrast microscope.

Mode of occurrence:

As to the mode of occurence of Nissl bodies in the living condition some authors believe that the Nissle substance occurs in the living cells in a diffused form and that the fixatives are responsible for their coagulation. This view is supported by Cowdry, Young and others. However, the majority of cytologists as Beams, King, Catenby, Mouusa, Banhway, Khattab and others are against this view and consider the flakes and gtanules as real structures and not artifacts of fixation.

The claim that Nissl bodies appear only as a product of fixation of post-mortem changes cannot be admitted because of the following:

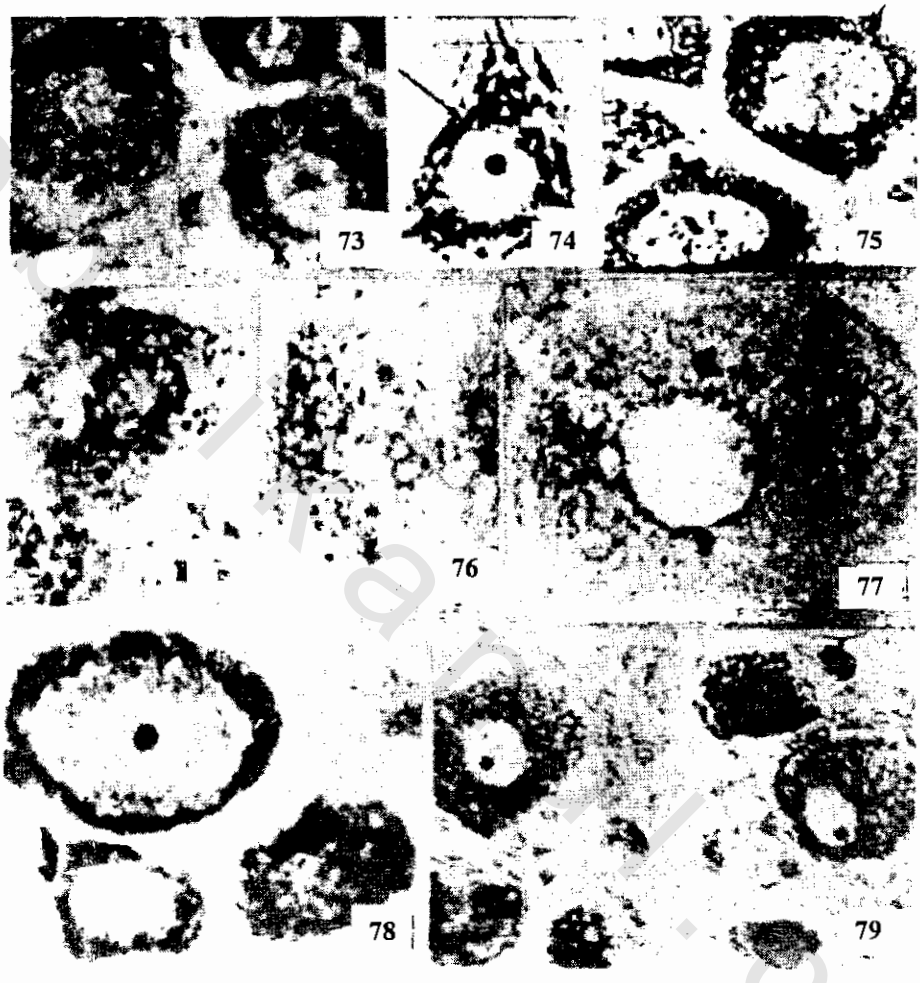


Fig. 73: Chick spinal ganglion neurones showing Nissl bodies in the form of irregular flakes and granules. (After Mousa and Khattab).

Fig. 74: Rat spinal cord motoneurone showing large flakes of Nissl substance. (After Banhawy, Khattab and Gansuri).

Fig. 75: Nissl bodies in locust neurones. (After Mousa and Banhawy).

Fig. 76, 77: Effect of insecticides on the Nissl bodies of locust. In BHC treated locusts the Nissl bodies decrease in number and show a considerable accumulation at the axon hillock. In locust poisoned with sodium arsenate, the Nissl bodies are greatly reduced in number, (After Moussa and Banhawy).

Fig. 78, 79: Spinal ganglion neurones of rat poisoned with tamaron and glutathion, Notice tigrolysed Nissl bodies into dusty particles in the latter. (After Banhawy and Ganzuri).

1 - Using the ultraviolet microscope the similarity of Nissl bodies in both living and fixed neurones was noted.

2 - The phase-contrast microscope provides strong evidence that the Nissl bodies are not artifacts of technique bu are real cytoplsmic constituents.

3 - Using the ultracentrifuge the Nissl bodies are readily moved towards the centrifugal pole of the cell without any alteration of their well-defined form seen in the living condition.

4 - In tissue culture it was found that the Nissl bodies in living chick neurones do not undergo any marked change after a good fixation.

Physiological significance and behaviour:

1 - The Nissl bodies undergo marked diminution during fatigue or hard exercis, then are reformed during the rest of the animal. Hence, it is concluded that these bodies are closely associated with the functional activities of the nerve cells. However, some authors believe that they are stores of oxygen in the nerve cells.

2 - These bodies were found in mollusca to disappear hours following the removal of ganglia provided that they were kept in a current of sea-water. If the ganglia were felt inside the animal after its death the Nissle substance accumulate (after ten hours) at the axon-hillock, then began to travel along the axon leaving the cell-body empty. Such movement were more prominent in cells kept under anaerobic conditions than in those left in normal sea-water. Hence, it was concluded that it was the lack of oxygen which brought about the migration of Nissl bodies along the axon of the cell after its death.

3 - Hyden (1948) followed the changes which took place in the Nissl bodies during development, trauma and degeneration and came to the conclusion that the Nissl substance changes considerably according to the physiological state of the cell. This author also investigated the cytochemical changes in the neurones after cutting off a neurite and found that after a few days the Nissl substance disappears and at the same time the quantity of nucleic acid drops completely in two weeks. Thus, he concluted that Nissl bodies are linked up with the nucleoprotein metabolism on one hand, and on the other hand with the motor and sensory functions of the neurons.

4 - Pathological effects were also reported following axon-sectioning. In such case, the substance of Nissl began to disappear from the central part of the cell. Tis continued until few granules together with some

small flakes were left at the peripheral region of the cytoplasm. After fifteen days, the Nissl bodies were reformed.

5 - Nissl bodies of insects (Mouusa and Banhawy, 1959) and mammals (Banhawy et al, 1972) are markedly affected by various insecticides. In insect neurones the Nissl bodies accumulate into small masses in the cytoplasm of small neurones in large ones they became lesser and most of them migrated towards the cell periphery, and in some cells they completely disappeared.

6 - Nissl nodies are also highly affected by virus infection (Moussa and Khattab, 1961). In yound chicks infected with Newcastle disease virus the Nissl bodies became smaller in size and their stainability decreased. In the adult they were fragmented into small bodies and granules which were faintly stained.

Nissl bodies and the Golgi apparatus:

Some authors claimed that the Nissl bodies are responsible for the appearance of the Golgi apparatus reticulum due to the deposition of reduced silver and osmium on them. This claim cannot be accepted because of the following:

(1) The topography of both Golgi apparatus and Nissl bodis were studied in insects, amphibia, birds and mammals by Moussa and his collaprators who found that the position of the Golgi apparatus does not coincide which that of the Nissl bodies in young neurones in which the Golgi apparatus is juxtanuclear, whereas the bodies of Nissl lie at the cell periphery. The Golgi apparatus appears in embryonic stages as a reticuum (in case of vertebrates) or crescents (in invertebrates) lying at the axon-hillock of the cell; then the apparatus spreads gradually around the nucleus until it surrounds it completely. In the full-grown animals the Golgi apparatus occupies a considerable part of the cytoplasm, but does not reach the cell periphery. The Nissl bodies, on the contrary, occur in embryonic stages as a diffused substance in the cytoplasm; then the substance is differentiated into irregular flakes and granules which is at the cell periphery. In the adult and old stages both the Golgi apparatus and Nissl bodies become scattered in the cytoplasm.

(2) Ultracentrifugation of vertebrate and invertibrate nerve cells showed that the Golgi apparatus and Nissl bodies differ markedly in their specific gravities. The Nissl bodies appeared to be the heaviest cytoplasmic components and hence thay were easily displaced towards

the centrifugal pole of the cell. The golgi apparatus, being lighter was shifted and became concentrated in the centripetal half of the cell.

(3) The Golgi apparatus and Nissl bodies were demonstrated side by side in unstained neurons fixed in alcohol-acetic acid and viewed under the phase-contrast microscope.

(4) The electron microscope shows that the Golgi apparatus and Nissl bodies are two different structures, and that Nissl bodies represent a picture comparable to that illustrated by the normal light microscope.

(5) As regards the chemical composition, Moussa and his associates (Banhawy, Khattab and El-Beih) found that the Golgi appeartus is highly proteinic containg tyrosine and glutathione whith a lillte amount of masked lipid. The Nissl bodies, on the contrary, consis of ribonucleic acid, arginine and histidine.

CHPATER 11

THE CELL CENTRE

The division centre or the cell centre is a cytoplasmic organoid which is present in the vast majority of animal cells and in certain lower plant cells It plays an important role in the mechanism of cell division.

Localization:

In the interphase condition it usually lies just near the nucleus towards the larger area of the cytoplasm. Sometimes it occupies the geometrical centre as in the macrocytes (Leucocytes with kidney-or hose-shoe-shaped nucleus) or when the nucleus is small and is displaced. Generally, the position of the centre is fixed for each type of cells.

Structure :

With the light microscope, it usually appears in fixed and stained preparations as a single or double (diplosome) deeply stained granule called the centriole.

The centrioles are not generally visible in the living condition by the light microscope; however, Cleveland (1953) was able to see the centriole in the living cells and followed it during mitosis of fibroblasts in its division and migration towards the opposite poles of the cell. In addition, the characteristic position of the centrioles, their constancy, their behaviour during mitosis, their colouration, with acid stains and their affinity for iron haematogxylin leave no doubt that they are real structures in the cytoplasm. The centriole, therefore, is permanent component of the cytoplasm, but in some instances it seems to arise de novo.

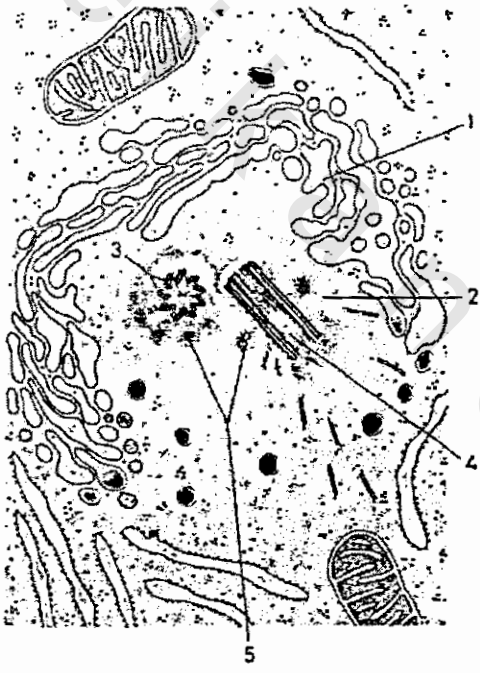
As regards the zones that surround the centriole, it was assumed that in fixed and stained preparations of cells in mitosis the centriole is frequently surrounded by a clear zone called microcentrum or centrosme and then by a denser zone known as archoplasm or centrosphere, from which the aster or astrosphere radiates, and or the surface of which the Golgi bodies, especially of the germ-cells, usually lie.

During the mitotic prophase, as the centrioles separate and migrate towards the poles the microcentrum seems to form an elongated body or

bridge which is called **centrodesmosis** from which the mitotic spindle seems to arise.

Ultrastructure of the centriole:

In electron micrographs, the centriole appears as a small cylinder of 0.15 u in diameter and 0.3-0.5 u long. Its interior is of low density, but the wall of the cylinder is rather dense and contains nine sets of small rods or tubules of 150-200 A^{o} in diameter oriented parallel to the axis. No tubules are present in the centre of the cylinder, thus the pattern of the centriole is "9 + O" as shown in figures 80-81.



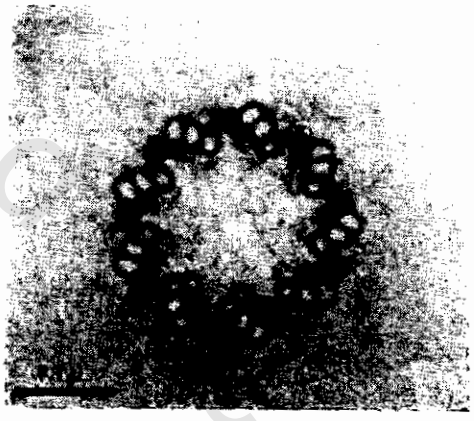


Fig. 80:

Ultrastructure of the centriole.

1 – Golgi apparatus.
2 – Centrosome (Centrosphere).
3 – Triplets.
4 – Cylinderial structure.
5 – Satellite.

Fig. 81: Cross section of a centriole from chick pancreas.

According to some authors, there are also certain pericentriolar structures kown as satellites observed as dense masses of babout 700A° around the centriole and are sometimes attached to its wall.

Other authors described certain structures that can be interpreted as small daughter centrioles that seem to arise at right angles from the other centriole. This disposition may be related to the mechanism of reduplication of the centriole and to the fact that the two centrioles in the diplosome are generally disposed at righ angles (Fig. 82). It has also been suggested that the pericentriolar components are not constant, but are transient structures related to the phase of activity of the centriole.

Fig. 82: Ultrastructure of a centriole (Ce) and chromosome (Chr).

Centrioles and other cell components:

The relationship of the centriole and the other cell components has been clarified. The. Position of the centriole is usually fixed for each type of cells. Generally speaking, the position of the centiole is relatively **fixed and axial**; if one draws a line between the centre of the nucleus and the centriole it will coincide with the axis of the cell as in some cylindrical epithelial cell.

The relation between the centriole and the Golgi apparatus has been observed with the light and electron mocroscopes where the Golgi bodies form a crown around the centriole in the early stages. Golgi membranes may be seen in contact with the cell centre.

The existence of the centrosomes (macrocentrum) and the centrodesmosis is demonstrated by the light microscope if not confirmed by the electron microscope. It is at present difficult to decide whether these structures are real structures or artifiacts of technique.

Centrioles, cilia and flagella:

Studies with the celctron microscope show that the structures of the **basal bodies** of **cilia,** flagella and of the proximal centriole of seprmatids are similar to those recognized in other centrioles (each basal body has the same 9 + 0 structure as a centriole). The formation of cilia has been followed in some developing cells and it has been found that the pairs of filaments (or tubules) that constitute the typical structure of cilia and sperm tails are formed in direct continuity with the tubules of one centriole whereas the other centriole remains inactive.

In addition, it has been found that the outer segment of the retinal rods and cones develops from a primitive cilium and is also related to the activity of one centriole.

Cilia and flagella resemble centrioles in having nine sets of tubules arranged in a cylinder 0.15- 0.2 u in diameter.

They differ form centrioles in the following:

(a) They have an additional par of tubules in the centre of the cylinder, thus, their pattern is "9 + 2" instead of "9 + 0" in case of centrioles (Figs. 83 -84).

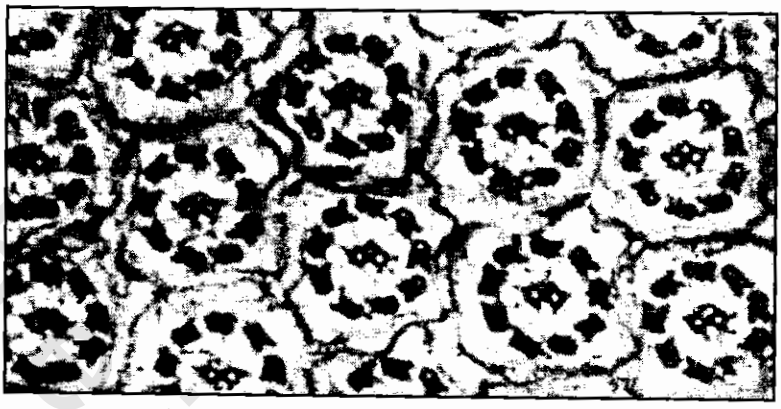


Fig. 83:

T.S. Showing the internal structure of epithelial cilia. Magnification: X 37,000.

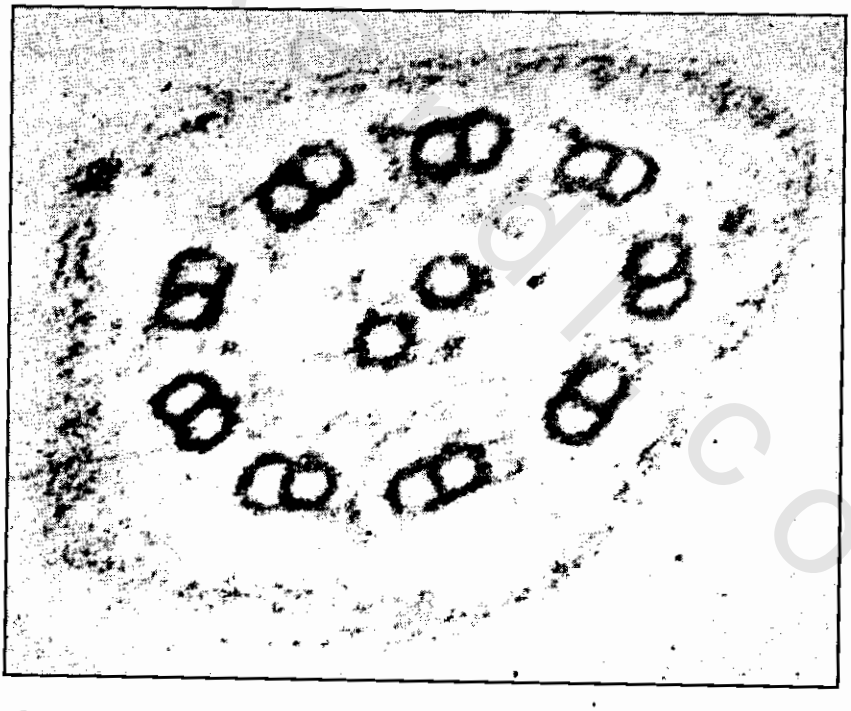


Fig. 84:

Electron micrograph of a T.S. of a flagellum. Maginification: X 259.000 (From Ambrose and Dorethy: Cell Biology).

(b) The peripheral sets of cilia and flagella are "doublets" of two tubular elements each, while in centrioles they are triplets; "arms" usually extend from the doublets.

(e) Mature cilia and flagella are bounded by membrane which is an extension of the plasma membrane, whereas the centrioles lie within the cytoplasm and have no surrounding membrane.

It is worth mentioning that when cilia and flagella are detached and isolated from the cell they can beat if adenosine triphosphate (ATP) is added to the medium; thus, it is probable that ATP provides the energy used in motion. This also indicates that cilia and flagella are not passive organoids moved by the action of the rest of the cell.

Centrioles and mitotic apparatus:

It has been mentioned before that the cell centre plays an important part in the mechanism of cell division. This is because intimately related to the function of the cell centre is the formation of **mitotic apparatus** during cell division (Fig. 85 – 87). This term is applied to the ensemble of structures which constitute the **achromatic figure** (aster and spindle) in the classical description of mitosis.

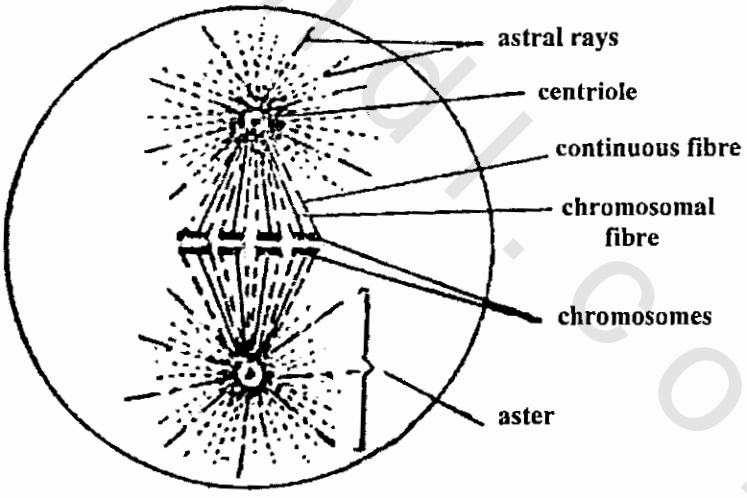


Fig. 85: Diagram of mitotic apparatus at metaphase.

Prior to nuclear division the centriole is divided into two, this is followed by the division of the centrosome. The two new centrosomes separate and move apart, and each finally takes up a position at the opposite pole of the nucleus. From each centrosome, the cytoplasm begins

to radiate into delicate fibrils (non-staining) and the **astral rays**, which between the centrosomes form a spindle. The astral rays together with the spindle form the **amphiaster.**

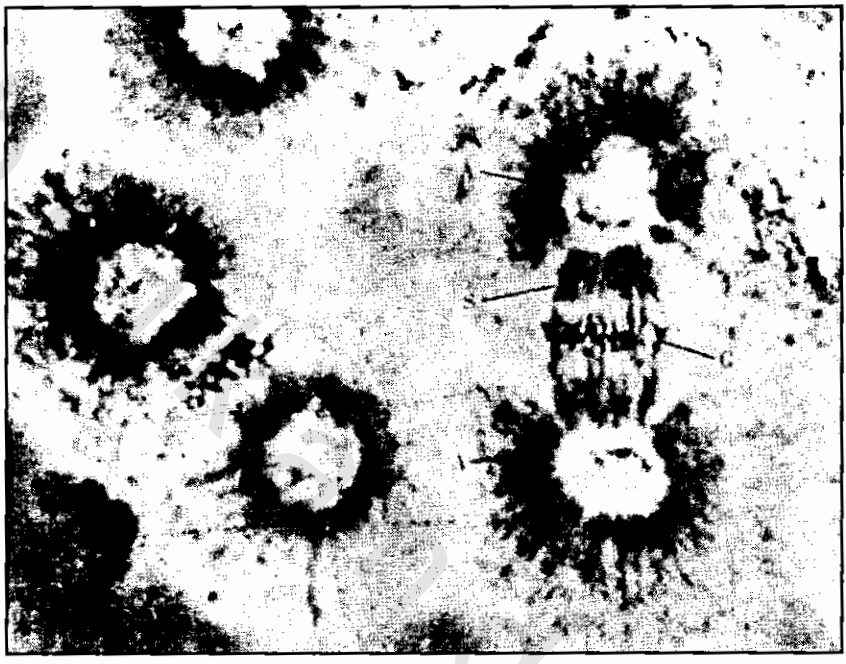


Fig. 86:

Phase contrast micrograph of the mitotic apparatus isolated from sea urchin eggs Notice the spindle (S), aster (A) and chromosomes (C).

In fixed and stained cells the spindle fibres appear to be made up of continuous fibres running from pole to pole and of **chromosomal or half-spindle** fibres which are short fibres, each of which connects each half-chromosomes with one or other of the spindle poles. In other words, the **half-spindle** fibres extend from the spindle poles to the chromosomes, and so they correspond in number to that of the chromosomes. It is claimed that **interzonal connections** stretch between the anaphase chromosomes of some animals.

It was generally believed that the spindle fibres are not apparent in the living cell and that they are produced by adding acid to the medium, but Schrader (1944) believes in the reality of the fibres and was able to see

them in the living dividing cells of a mite and in some flagellates. In addition studies with polarization optics and with phase contrast and electron microscopes have demonstrated the reality of both the spindle and the astral fibres.

Fig. 87:

Electron micrograph of a spermatocyte from cock testis showing two pairs of centrioles after replication. In this metaphase view can also be seen the spindle fibres and chromosomes.

By the microdissection needle or the centrifugal force the spindle can be moved. The asters, also, can be moved through the cytoplasm and their individual rays can be bent and twisted by means of the microdissection needle.

Part of the spindle originates from the cytoplasm, but the half-spindle fibres - which connect the chromosomes with the poles of spindle - probably arise from nuclear material.

CHAPTER 12

FIBRILIAR STRUCTURES

In some kinds of cells having certain speialized functional activities, the cytoplasm is modified in various ways giving rise to specific fibrillar structures such as neurofibrils and myofibrils.

NEUROFIBRILS

The neurofibrils are only found in nerve cells. They appear in the form of fine filaments in the cytoplasm. They run in all directions and continue into the axon and dendrites (Fig. 88-90).

Since the discovery of the neurofibirls by Remak (1843), there has been much controversy as regards their real existence. Some authors were unable to see the neurofibrils in the living conditions and hence consider them as artifacts of technique. However, many investigators were able to see them in living vertebrate and invertbrate nerve cells especially with the phase contrast microscope. Furthermore the living axoplasm of the giant nerve fibres of some animal examined with the polarization microscope shows a weak positive birefrengence. This indicates the extistence of submicroscopic elongated material oriented along the axis. Later, the axoplasm of the myelinated nerve fibres has been extruded and separated from the myelin sheath thus the examination of these structures with the electron microscope has become possible. In fixed preparations the neurofibrils are demonstrated following certain procedures as Cajal's or Willis sivler nitrate technique.

Ultrastructure of neurofibrils:

In electron micrograph the neurofibrils of the spinal ganglion neurones of the albino rat appear as a net-like structure of intermingled threads. In the extruded axoplasm, the electron microscope revealed a fibrillar material formed by fibrils of indefinite length, smooth contour and a diameter of 100 to 490 A° .

In the non-myelinated nerve fibres of the neurohypophysis the fibrillar material of the axon shows thin filaments (100 A° or less in diameter) and thicker fibrils (200 to 300 A°). The latter are of a definite length and correspond to the fibrils described previously in the extruded axoplasm. Such structures are termed "neuroprotofibrils". They have a definite tubular appearance with edges of markedly higher density. Two types of axoplasmic fibrils, namely, thin neurofilaments and thicker tubules or neuroprotofibrils had been also described. These structures are closely related to the endoplasmic reticulum. Hence, it is concluded that both

neurofilaments and neuroprotofibrils correspond to the neurofibrils seen in the classical histological and Cytological preparations.

Figs. 88-89 :

Spinal cord neurones of chick showing neurohibrillae extending also along the cell process. (After Moussa and Khattab).

Fig. 90 : Neurofibrils in the spinal cord cells.

Development of neurofibrils:

In the early embryonic stages, the authors reported that the neurofibrils exist in the spinal cord and spinal ganglion neurones as thin intermingled filaments lying at the axon – hillock. These filaments become more distinct in the cells of late embryonic stages and start to extend around the nucleus. However, in the majority of these neurones, they are still accumulated at the axonal pole of the cell and extend as fine threads along the axon.

In the advanced developmental stages, the neurofibrils increase in amount and appear to enclose the uncleus. The fibrils are intermingled throughout the cytoplasm; they collect into bundles that pass into the cell processes. In old and senile ages, some of the neurofibrils are fragmented transversely and accordingly, they were mistaken by some investigators for the mitochondria.

Significance of neurofibrils :

The exact role of neurofibrils is still debatable. Some neurologists consider that the neurofibrils play a certain role in nerve conduction. Otheres, being against this view, believe that nerve conduction depends essentially on the surface membrane of the axon and degeneration after its cutting indicate that the neurofibrillar material is very senssitive and that it is destroyed prior to the disappearance of nerve conduction.

According to some authors, neurofibrils are trophic (nutritive) elements for the axon, and thus are concerned with growth and synthesis of materials essential for the axon.

At present, there is considerable evidence that the axoplasm is continually produced by the perikaryon of the nerve cell.

Furthermore, some cytologists suggest that this fibrillar (or tubular) material may carry essential enzyme systems or other components which are used at the nerve ending for the transmission of the nerve impulse.

Relation between neurofibrils and other cytoplasmic organelles:

Some authors, who were unable to demonstrate the neurofibrils in the living condition, denied the presence of these structures and claimed that the linear arrangement of the mitochondria has been erroneously interpreted as neurofibrils. Others believe in the existence of these structures and the mitochondria in the same cells, and that both constituents differ in morphology and distribution.

The neurofibrils are in the form of therads surrounding the nucleus and passing into the cell processes, bu the mitochondria exist as granules and short rods scattered in the cytoplasm. Besides, in the early embryonic stages the mitochondria are scattered in the cytoplasm, whereas the neurofibrils appear as a group of threads lying at the axonal pole of the cell and extending along the axon.

Using the ultracentrifuge, its was possible to displace the neurofibrillae of the mammalian spinal ganglion neurones to the centrifugal pole of the cell, wherease the mitochondria were shifted towards the centripetal pole.

Pathological changes of neurofibrils :

Neurofibrils give a clear indication of the activity of the cell, their thickness is said to vary according to the different physiological conditions. They respond in various ways to the effect of different chemical substances. A severe action us brought about by insecticides on the neurofibrils in the neurones of the medulla of rat. They are markedly fragmented and show progressive disappearance from the treated cells. In some of these cells, the presence of neurofibrils becomes only restricted to the axons; other cells become almost completely devoid of neurofibrils.

However, the neurofibrils of Purkinje cells are more resistant to the action of insecticides.

MYOFIBRILS

The cytoplasmic matrix is highly differentiated in the muscle fibres. Most of this substance is modified to form a special type of fibrils known as myofibrills which are characterized by a great ability of contraction.

Structure :

The myofibrils are homogeneous in the smooth (involuntary) muscles, but in the skeletal (voluntary) and cardiac muscles they are striated showing the presence of dark zones alternating with light ones. In these cells a small part of the cytoplasm (called sarcoplasm) remains as it was in the embryonic conditions. This portion of undifferentitated cytoplasm lies between the myofibrils specially in the region surrounding the nucleus.

Skeletal muscles are made up of bundles of large multinucleated cylindrical fibres ranging from **10 to 100** u in diameter and serveral millimetres or centimetres in length. The whole muscle fibre is surrouded by a sarcolemma (plasma membrane); this is an electrically organized membrane which can be activated by the nerve impulses. Consequently, the whole fibre is activated.

The myofibris constitute the contractile machinery of the muscle fibre. Each myofibril is identified as a fine thread-like structre of about 1u in diameter. Under the light microscope, the myofibrils show the usual striations which are characteristic for the whole muscle fibre.

Each myofibril is composed of repeating contractile units or sarcomeres (Fig. 91 – 92), each of which is limited by a band known as the telephragma or Z-line. In other words, the scrcomere is the regions between two successive Z-lines along a myofibril. The Z-line is situated in the middle of the less dense region referred to as the l-band which corresponds to the relatively clear zone. Another band, the A-band is of greater density than that of the 1-band. Under certain conditions, a less dense region (called Hansens disk or H-disk) is seen in the middle of the A-band dividing it into two dark semi-disks. In a relaxed mammalian muscle the A-band is about 1.5 u long, while the 1-band is about 0.8 u. The zones of thin filaments (1-bands) can easily by disinguished from those of thick filaments (A-bands) by the light microscope.

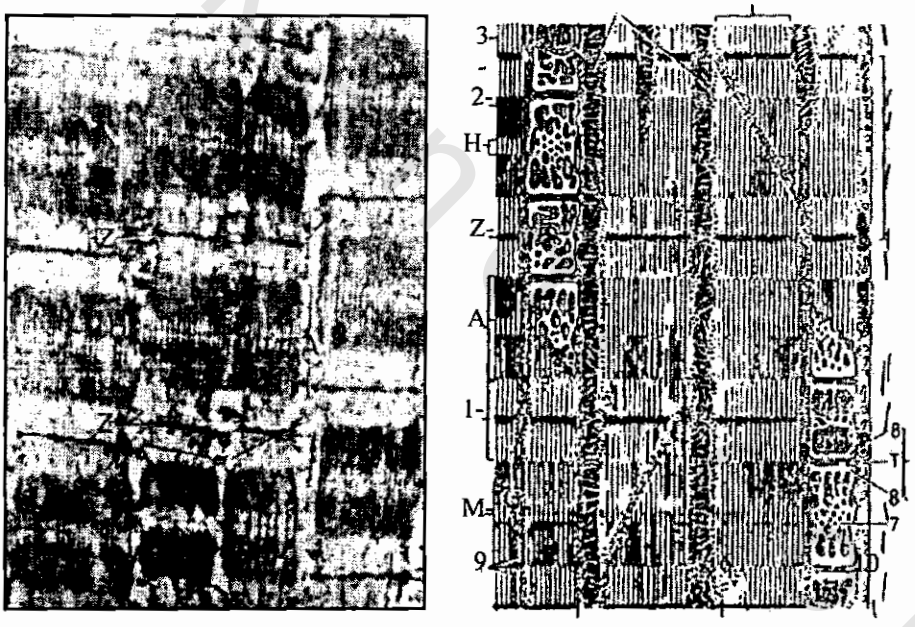


Fig. 91:
Electron micrograph of striated muscle of rabbit. Magnification: X 14,000. (From Ambrose and Dorothy: Cell Biology). Each myofibril is traversed by a regular banded structure of A-band Z-line and H-disk or band.

Fig. 92: Myofilaments (Ultra structure)

1 – Myofibrils. **2 – Myosin filaments forming the A-bands.**

3 – Actin filaments 1-1-band. **Z- line and H-zone.**

(A-bands) by the light microscope.

Ultrastructure of myofibrils :

Electron microsope shows that the myofibrils are composed of regularly arranged fine structures called myofilaments.

There are two main kinds of myofibrils in vertebrates and insects, one kind is 100 A° in diameter and about 1.5 micron long and the other is 60 A° diameter and 21 microns long.

In a relaxed condition, the I-band consists of thin filaments, while the H-band or disk consists of thick filaments. Both thick and thin filaments are found in the A-band. The regular arrangement of the two types of filaments is well seen in a cross section through the A-band. In vertebrate muscles, each thick filament is surrounded by **6 thin** filaments, each of which lies symmetrically among three thick ones. Thus, it is clear that the thin filament of one sacromere goes across the Z-line to the next sarcomere.

The two types of filaments (thick and thin) are linked together by a system of cross bridges which plays an important part in muscle contraction. Each thick filament joins the six adjacent thin ones every 400 A° in a helical manner which has certain significance in the process of contraction and relaxation (Fig. 93).

Fig. 93:

Striated muscle showing two nyofibrils, one of which is tangentially cut and shows the disposition of the sarcoplasmic reticulum. The transverse component of this system is represented by the triad (notice the relationship of the triad to the Z-line). The longitudinal componnet of the sacroplasmic reticulum forms anastomosing tubules (st) on the surface of the sarcomere. (After Porter).

Macromolecular mechanism of contraction :

The changes taking place in the submicroscopic structure of muscle fibres show clearly that contraction occurs at the level of the myofilaments. This mechanism consists mainly of a reversal of banding during contraction. These changes can be studied in the living condition by using the phase contrast microscopy. It should be noted that the A-band remains constant in a wide range of muscle lengths, but the 1-band changes in accordance with contraction. At the same time, the length of the H-band varies with contraction in such a way that the distance between the end of the H-band of one sarcomere and the other remains constant. These findings lead to the so-called **sliding mechanism theory of contraction**. According to this concept, the two types of filaments maintain their length but slide with respect to each other (Fig. 94). In other words, the muscle contracts by a sliding of the fine filaments within the larger filaments. The coarse filaments are myosin, while the fine filaments are actin. When the contraction is strong enough, the ends of the thin filaments will meet and then the thick ones. New bands may be seen (inversion of the banding, suggesting a certain degree of crumpling and overlapping of the filaments.

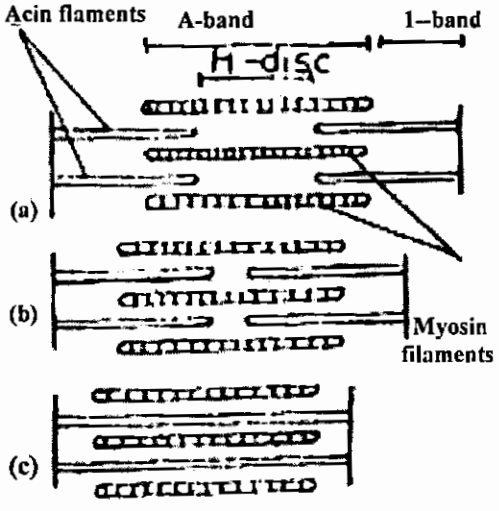


Fig. 94: (a), (b) and (c), progressive shortening of the muscle takes place by sliding of the actin filaments between the myosin filaments.

Structural proteins of myofibrils:

Recent studies indicate that the contractile structure of the muscle fibres consists almost exclusively of proteins. Of these, about 90% are myosin (fibrous protein) and **actin** (globular protein); both of them can be separated and purified. The myosin fraction forms about one-half of the total protein; it can be extracted from the myofibril with appropriate salt solutions leaving the actin intact. It is known that myosin split ATP, and that actin has ATP or derivatives bound to it.

When the two isolated proteins are placed together in as test tube they form the complex known as **actomyosin** which can contract in the

presence of ATP. This experiment shows that the interaction between actin and myosin is essential for the contraction to take place. It has been found that the thick filaments (100 A^{o}) are built up of myosin and the thin (60 A^{o}) ones of actin (a second protein called tropomyosin also occurs in the thin filaments); and that the interaction between actin and myosin observed in the test tube occurs in nature between the actin (thin) and myosin (thick) filaments; in other words, the interaction of the two sets of filaments is responsible for the force of contraction.

It is believed that the interaction takes place by way of the cross bridges and that each of these bridges is probably related to one myosin molecule. Hence, it was stated that the cross bridges could oscillate and hook up to the specific sites of the actin molecule. In this way, a pull of about 100 A^{o} in length could be produced before the bridge retuns to the original position. For each of these cycles a phosphate group of the ATP is libreated.

These data show clearly that the submicroscopic structure of the myofibrils provides an excellent example of the coupling of the energetic processes with the actual machinery involved in function (muscular contraction). In this case, it is clear that **form and function are so intimately related** in the realm of molecular organization that one connnot be separated from the other.

Sarcoplasmic reticulum :

The sarcoplasmic reticulum occurs within the sarcoplasm of the muscle fibre. This sacroplasm contains also mitochondria. Golgi apparatus and few RNP granules. In addition, glycogen particles (providing a storage supply of carbohydrates) accumulate in different regions of the muscle fibre being found in variable amounts depending on the fibre type. Thus, in the so-called red muscles (which contract more slowly) the sarcoplasm is more abundant than in the pale muscles.

The sarcoplasmic reticlum can be considered as the smooth endoplasmic reticulum of the striated muscle. It is a continuous membrane-limited reticulum which consists of vesicles and tubules. Special terminal cisternae occur at the level of the 1-band; between these a row of small vesicles is found. The tubules lying between the terminal cisternae are arranged longitudinally on the surface of the A-band of the sarcomere. This structure is repeated between all myofibrils and is also continuous across the muscle fibre, making connections with the surface membrane at the level of the Z-lines.

The role of the sarcoplasmic reticulum :

The ppssible role of the sarcoplasmic reticulum in the physiology of the muscle fibre is that it serves to transmit the excitatory impulse intracellularly. It has been claimed that the membrane of the sarcoplasmic reticulum, separating two different compartments within the cell, is electrically polarized in the same way as the surface membrane of muscle; and that this membrane is capable of conducting impulses inside the muscle fibre in order to activate the contractile elements. It has been also found that the sarcoplasmic reticulum contains the relaxing factor which inhibits the activity of ATP of the myofibrils giving rise to relaxation after contraction. The membranes of the reticulum are capable of fixing calcium ions.

CHAPTER 13

THE PLANT CELL

Cells in general have the same architecture and structure, whether they are plants or animals, whether we deal with unicellular or multicellular organisms. Even bacteria and certain blue algae which were thought to be non-nucleated cells, have certain equivalents to a mucleus. The unicellular organisms have basically the same structure as all the other cells.

The main differences between animal cells and plant cells are that the latter contain **plastids** (the most important of which are **the chloroplasts), a cell wall covering the plasma membrane and a large** vacuole or vacuoles in many types.

GENERAL MORPHOLOGY OF PLANT CELL

In a living cell the chloroplasts are the most prominent organelles. They are embedded in a clear hyaline Cytoplasm which is confined to the periphery of the cell or to strands crossing the **central vacuole** (Fig. 95).

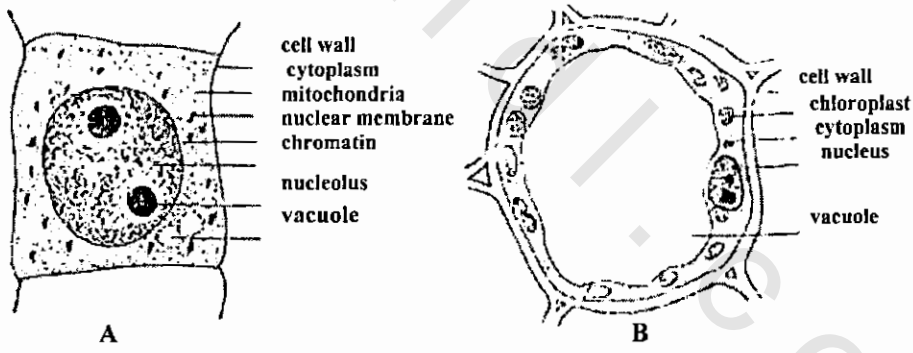


Fig. 95 : Plant cells. A, a meristematic cell from the root of onion. B, parenchyma cell from a leaf.

The major component of vacuoles is water with dissolved substances including atmospheric gases, salts, sugars, organic acids, soluble proteins and certain pigments. The red colour of many flowers is a result of pigments being concentrated in the vacuoles of the cells in the flower petals. The most familiar pigments are anthocyanins. Anthocyanin pigments dissolved in the sap are also reponsible for the red, blue and

purple colours of autumn leaves, flowers, fruits and stems.The interfaces of the cytoplasm-vacuole and the cytoplasm-wall are characterized by special cytoplasmic membranes that control the passage of substances into and out of the cytoplasm. These membranes are not visible with the light microscope, but because of their activities their existemce has long been recognized. The membrane separating the cytoplasm and the vacuole is known as the **tonoplast**, whereas the membrane limiting the outer surface of the cytoplasm is called the **plasma membrane** or the **plasmalemma**.

When living plant cells are examined under the microscope, the cytoplasm appears to circulate at a rapid rate. The mitochondria, chloroplasts and other cell components can be observed moving about in the cell. With careful examination small completely spherical particles can be also observed moving rapidly through the cell; these are the spherosomes which are poorly preserved in fixed preparations. It is believed that the spherosomes are formed by the endoplasmic reticulum. Small immature spherosomes are pinched off form the tube-like endoplasmic reticulum. These immature microbodies enlarge and give the type of spherosomes found in many cells. Frequently endargement continues and a fat body develops; this development may indicate that the spherosome forms a special cell structure involved in fat production and storage, but the exact function of these spheromomes is uncertain. These spherosomes are bounded by a single unit-membrane.

A single **nucleus** is embedded in the cytoplasm, and it may be seen flattened against the cell wall. The nucleoli can also be sen in the living cell.

In fixed and stained cells, cell walls, chlorophasts, mitochondria, microbodies and Golgi bodies can be easily demonstrated. Within the nucleus there are generally clumps of chromatin material. In a plant cell, the plasmalemma is covered by a thicker cell wall which is traversed by tunnels known as plasmo-desmata which communicate with the neighbouring cells.

ULTRASTRUCTURE OF PLANT CELL

It is natural in syudying a plant cell to subdivide this basic unit into: nucleus, chloroplast, mitochondria, endoplasmic reticulum. Golgi bodies, cytoplasmic matrix and tonoplast,. These entities have different functions, thus there exists within the cell a number of what might be considered as microenvironments. Each microenvironment is surrounded by a system of

semipermeable lipoprotein membranes which insulate it form other environments. These limiting membranes while maintaining the activities within, allow for the communication and the exchange of metabolites with the surrounding medium. (Fig. 96-97).

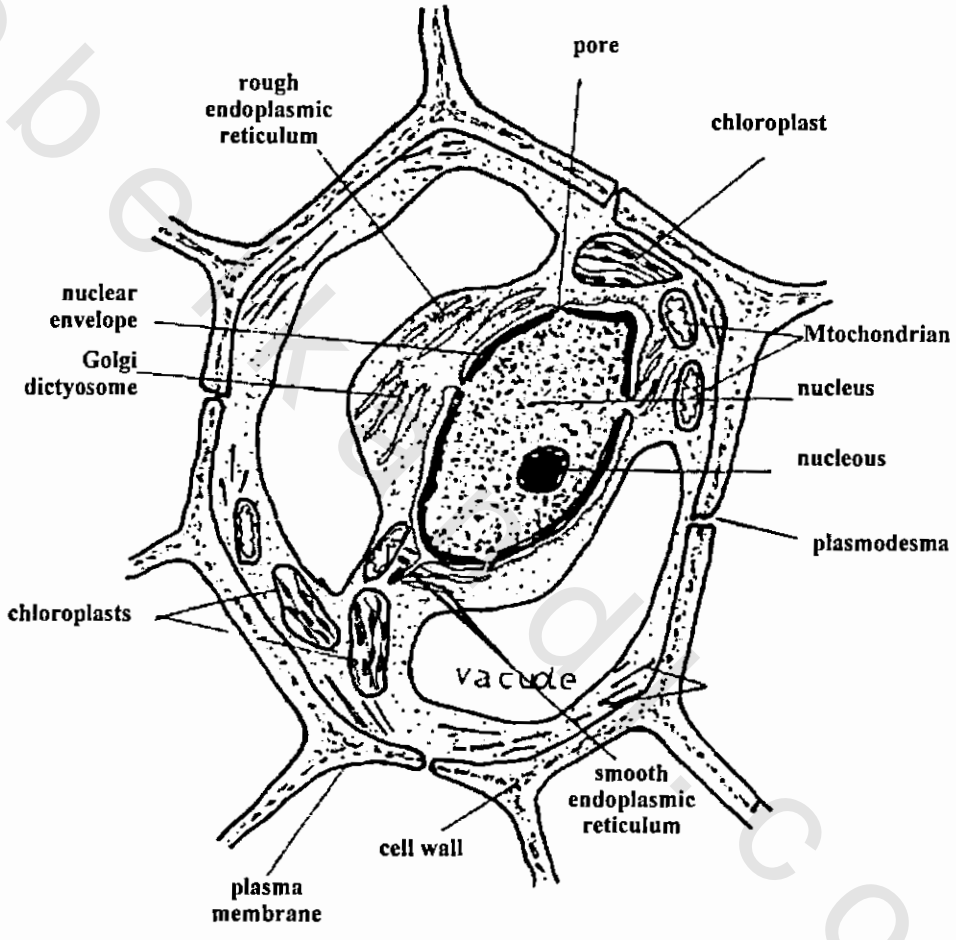


Fig. 96 : General ultrastructure of plant cell (form De Robertis et al, cell Biology).

All cellular membranes are composed of two principal classes of compounds: lipids and proteins. A large number of evidences supports the hypothesis that these two structures are assembled in more or less the same way throughout nature. Whether the membrane occurs in plant or in an animal, whether it envelopes the entire cell or subcellular particle, the basic structural pattern and dimensions are similar within very narrow

limits. The membranes constitute a much smaller percentage of plant cells than of the animal cells. This is related to the formation of huge vacuoles during cell growth and differentiation, this results in the compression of the cytoplasm which becomes restricted to the periphery of the cell.

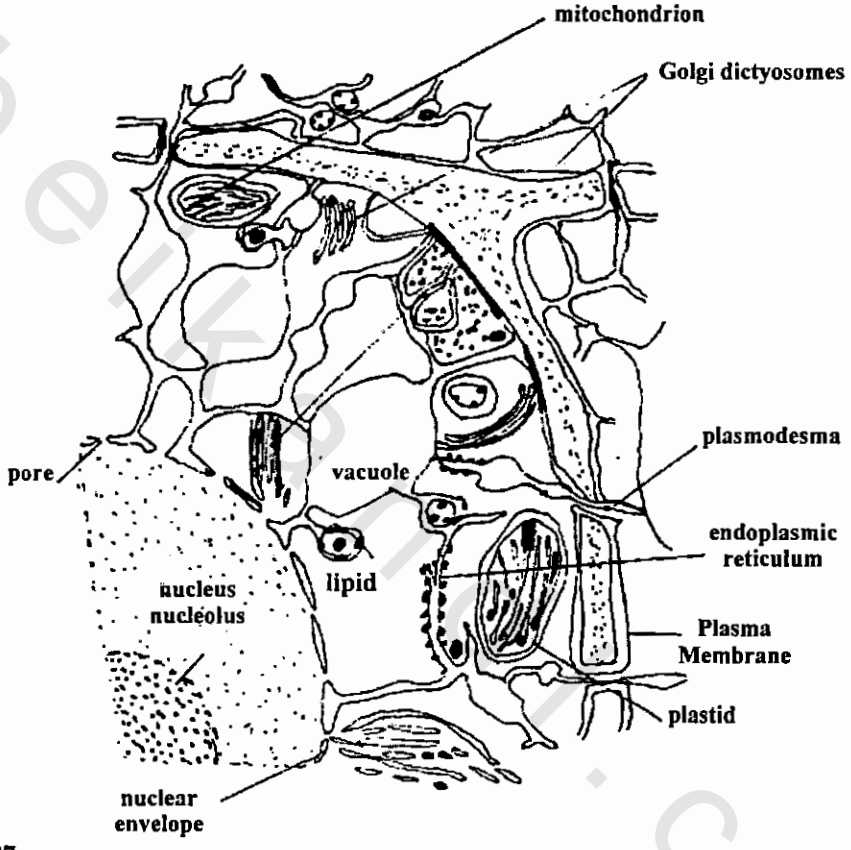


Fig. 97:

Plant cell ultrastructure and its intercellular relationships (From De rebertis et al, after Buvat).

The plasma membrane:

The plasma membrane or the plasmalemma separates and encloses the entire living portion of the cell (protoplast) from the external cell wall. Its major role in plant cells is to contorl the difference in intracellular and extracellular concentrations of inorganic salts and organic substances involved in metabolism.

As regalrds the chemical coposition of the plasma membrane (and in general, all the cellular membranes of plant cells) it is different from most animal membranes. The lipids of most animal membranes consist, mainly of cholesterol and phospholipids. However, that lipids of plant membranes are: phospholipids (mainly lecithin), glycolipids (formed of galactose and lipid), sulpholipids (sulphur containing lipids) and sterols (formed of sterol-B-sistosterol and spinosterol). The protein component of plant cellular membrane system is made up of many protein units.

Electron micrographs of plant cells show that the cellular membranes follow the typical architecture of the living membranes. Each of these membranes consists of a light line between two dark lines. These membrances are thought to be composed of central bimolecular lipid layer sandwitched between two monomolecular layer of protein.

Cell walls :

The outer boundary of the plant cell, produced by the living protoplast, consists of a non-livig rigid srtuctre called the cell wall. With relatively few exceptions, all plant cells possess walls which may be thick, thin or sculptured. It is assumed that these walls have protective and supportive functions and, in addition, determine the shape and the texture of the cells. The cells walls are built up essentially of **cellulose** (a polysaccharide made of glucose subunits) which is produced by the cell. In some plants especially multicellular ones, secondary walls are characterized by the presence of cavities or depressions known as pits. Cells with only primary walls contain somewhat similar depressions called **primary-pit fields.** Pits and primary pit-fields originate when the cell wall is formed unevenly, leaving depressions. These pits facilitate the movement of materials from one cell to another. Pits are usually found in non-living cells that are concerned with conduction and support as fibres and tracheids. Sometimes, thickened over changes are formed around the pits; these are referred ro as **bordered pits.** In other cases, the over-hanging borders are absent and the pits are known as **simple** pits. With special staining techniques many primary pit-fields are shown to have **pores** in the plasmalemma of the living cells through which fine, delicate strands of cytoplasm connect the **adjacent** cells. These cytoplasmic projections are called **plasmodesmata** (Fig. 97) within the thin palsma membrane of a cell is continuous with that of the adjacent one. Within the plasmodesmata are tubules which are continuous with the cisternae of vacuoles of the endoplasmic reticulum of the two adjoining cells.

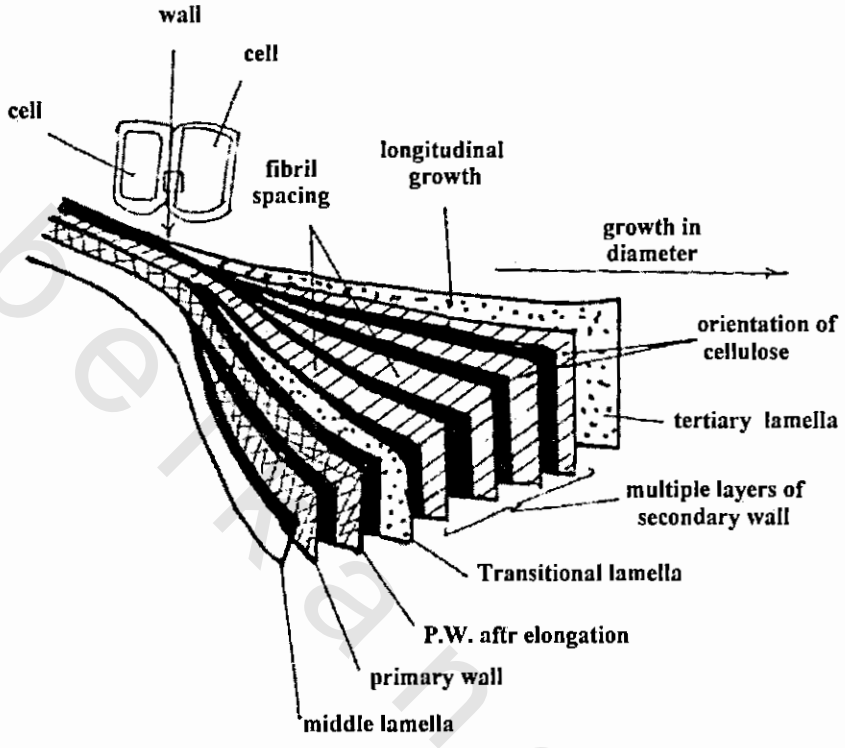


Fig. 98 : Diagram showing the various layers of the cell wall of a higher plant (after Muhlethaler).

On the basis of its development and structure, the cell wall (Fig. 98) consists of three distinguishable well defined regions; and intercellular substance called middle lamella, a primary cell wall and a secondary cell wall.

(a) The **middle lamella** is a relatively thin layer of intercellular material formed between two adjacent cell walls during division of the plant cells. This lamella is composed of pectin, calcium, cellulose and other prolymers. Owing to the consistency of pectin, the middle lamella is a viscous jelly-like substance. However, in woody tissues it is heavily lignified. In plant tissues, the middle lamella serves as an intercellular cementing material.

(b) **The primary cell wall**, found in all plant cells, is the first deposition product of the protoplasm during the early stages of growth and development. It is relatively thin and quite plastic and capable of considerable extension as the cell increses in size. It is composed of pectin

but contains hemicellulose, cellulose and non-cellulose polysaccharides. The primary wall is well demonstrated in meristematic cell. It is free of pits or irregular thickenings except where the plasmodesmata are found. Because of their some what similar mak-up, the middle lamella and the primary wall are difficult to be distinguished and are usually refered to collectively as the **primary wall.**

(c) **Secondary cell wall:** Ageing of the cell may bring about the deposition by the protoplast of more material on the primary wall to form a secondary wall. In many cell types after the secondary wall formation is completed, the living components of the cell die and disappear. The secondary cell wall is formed of cellulose, hemicellulose, pectic compounds and additional depositions and thickenings of other compounds such as lignin (a complex polymer that imparts stength and rigidity to the cell wall), subrin, cutin and cutin waxes. In many fugi and in yeasts the cell wall is formed of **chitin**.

The oreintation of cellulose in the cell walls is represented in Figure 98. As cellulose is a polymer of glucose molecules together with other cellulose molecules, it forms a structure called micelle (or elementary fibril). Micelles are in turn organized into larger bundles called microfibrils which can be seen with the light microscope. The microfibrils are then arranged into layers forming the cell wall.

The primary cell wall is composed of cellulose microfibrils running many directions, whereas the secondary wall is formed of parallel microfibrils which are more densely packed than those of the primary wall (Muhlethaler, 1961). In other words, the secondary wall may contain distinct layers; in this case, the fibrils in each layer are parallel, but the fibrils of different layers are oriented at angles to one another.

(d) A thin **tertiary cell wall** may also be found.

Cytoplasmic matrix and endoplasmic reticulum:

In the undiffierentiated plant cells, represented by the meristematic cell, the cytoplasmic matrix contains a very scanty amount of membranes. These membranes are hardly demonstrated as they are hidden by overwhelming ribosomes which lie free in the cytoplasmic matrix and are not attached to any membrane. Using glutaraldehyde as a fixative for meristematic cells, it has been possible to show a system of **microtubules**

below the plasma membrane. These microtubules are unbranched and 250A° in diameter and extend for several microns (Porter, 1966). These tubules are, probably, involved in the formation of the cell wall.

Granular and agranular endoplasmic reticulum are observed in differentiated cell (Fig. 97). **The agranular endoplasmic reticulum** seems to be common in cells which are mainly concerned in steriod production and carbohydrate metabolism, with transport of electrolytes as in the sieve elements and companion cells of ducurbit phloem. This reticulum is well demonstrated in the plasmodesmata. The **granular endoplasmic reticulum** is highly developed in cells active in protein synthesis as in the cells of glandular hairs of Drosera leaves. In leaf primordia, granular and agranular endoplasmic reticulum have been demonstrated. However, in the more differentiated cells the ribosomes are fewer and the agranular reticulum containing large vacuoles filled with fluid occupies a vast area of the cytoplasmic matrix. The functions of the endoplasmic reticulum in both animals and plants are similar.

Golgi apparatus :

It is the electron microscope which revealed the occurrence of the Golgi dictyosomes in all the living plant cells. These dictyosomes are similar to those of the invertebrate cell, but they differ from those of the somatic cells of vertebrates. The Golgi dictyosomes or Golgiosomes are scattered throghout the cytoplasm without definite localization. The dictyosomes are formed of stacks of flattened cisternae which are dilated at the edges as those of the invertebrate dictyosomes. Vesicles are found attached to the cisternae which are pinched off releasing the excretory product. These vesicles are aggregated at the telophase stage at the equatorial plate and fuse to form the cell plate. There are numerous evidences that the Golgi dictyosomes are concerned with secrection and formation of cell plate and membranes of the cell. The dictyosomes contain certain specific enzymes such as thiamine pyrophosphatase and inosinic diphosphatase.

Mitochondria :

The structure of the mitochondria in both animal and plant cells is nearly the same. The main difference is that plant mitochondria contain fewer crests and a larger matrix than those of the animal cells. However, cells engaged in photosynthesis contain more mitochondrial crests than those of other plant cells.

For other details on the cytoplasmic organoids see the previous chapters on these organoids in animal cells.

PLASTIDS

Plastids represent a variety of cytoplasmic organoids which are related to the metabolic processes of plant cells. These, together with cell walls, are truly distinctive plant structures. They are present in all plants, with the exception of bacteria, certain algae, myxomycetes and fungi. The plastids of plant cells are of various forms. The forms exhibited by plastids are plates, discs and spirals. They contain several pigments (e.g., chlorophyll and carotenoids) and components (e.g., starch). (Figs. 99 – 102).

Origin and development of plastids :

Plastids are believed to originate from minute, defined precursor structures called proplastids. The proplastids, typically found in young cells, are transmitted from one generation to another during cell division. Proplastids are self-perpetuatig organoids. They are surrounded by a double membrane.

In the presence of light, the inner membrane of the proplastid grows and gives off vesciles which are arranged to form larger discs. In the dark, tubular structures are formed which are arranged in a lattice. When these proplastids are placed in light the lattice becomes arranged in the form of the well known lamellar structure of the chloroplast. This process of development is strongly affected by the lack of light. When plants are grown under low light intensity, the vesicles formed in the proplastids aggregate forming one or more prolamellar bodies. Some times the vesicles form a crystalline pattern consisting of regularly connected tubules. When these plants are exposed to light again, the vesicles may fuse into layers and develop again into the lamellar structure (grana). The entire development of the chloroplast is controlled by a number of genes which regulate the formation of the lamellar structure and its moelcular organization.

Types of Plastids:

1. Leucoplasts:

These are colourless plastids which are generally found in variegated leaves, stems, roots, embyronic cells, sexual cells and in the regions of the plant not receiving light. Functionally, leucoplasts serve as starch-forming and storage centres of protein and fats. Some leucoplasts produce essential oils.

Fig. : 99:

A chloroplast from a leaf cell corn. At D notice the two delimiting membranes. The grana (G) consists of stacks of sac-like thylakoids. Stroma membranes (S) run between the grana. Magnification: X 64,000. (From Novikoff and Holtzman, after Shumway).

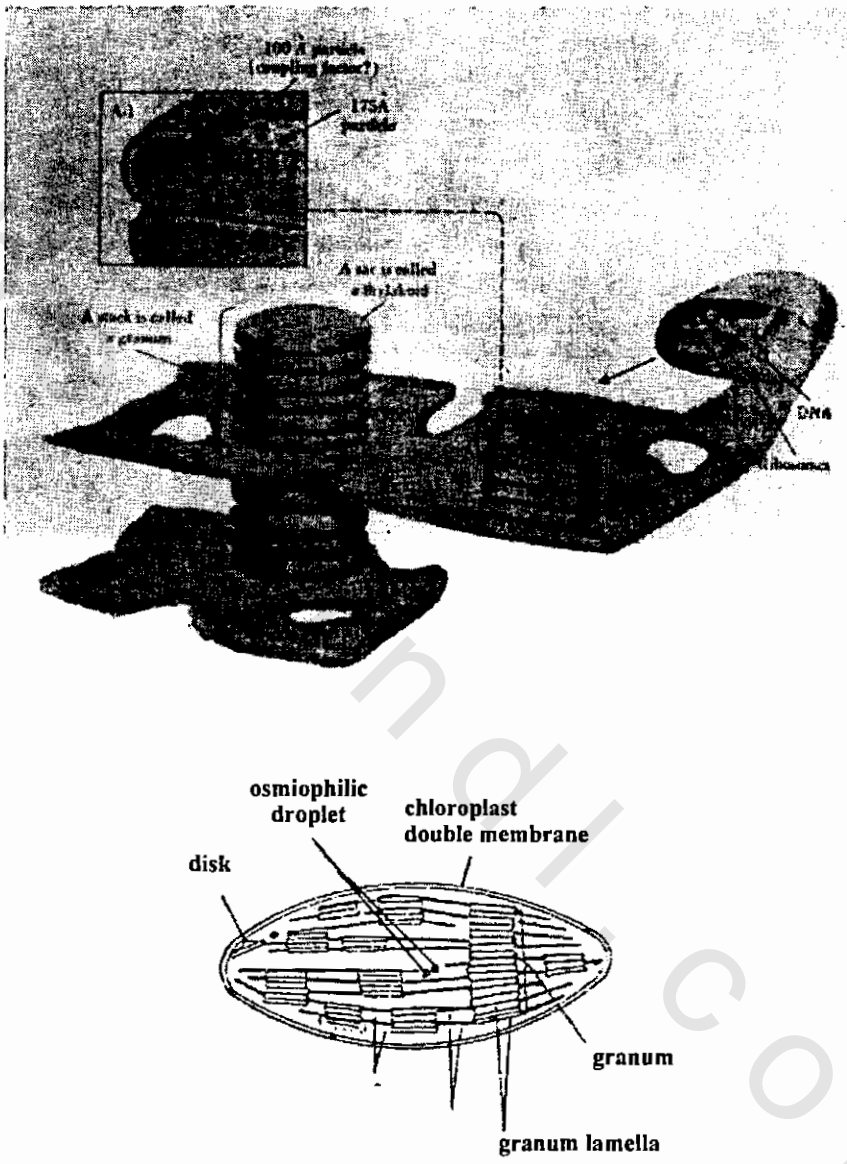


Fig. : 100:

Upper: Structure of a chloroplast of a higher plant. A-1 shows the particle distribution in the grana membranes. Lower: structure of a barley chloroplast (after von Wettstein).

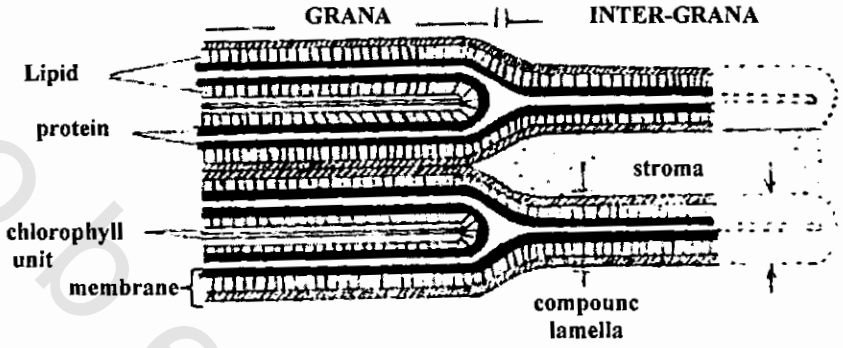


Fig. : 101:
Upper: Digram of the macromolecular structure of a chloroplast (After Hodge).

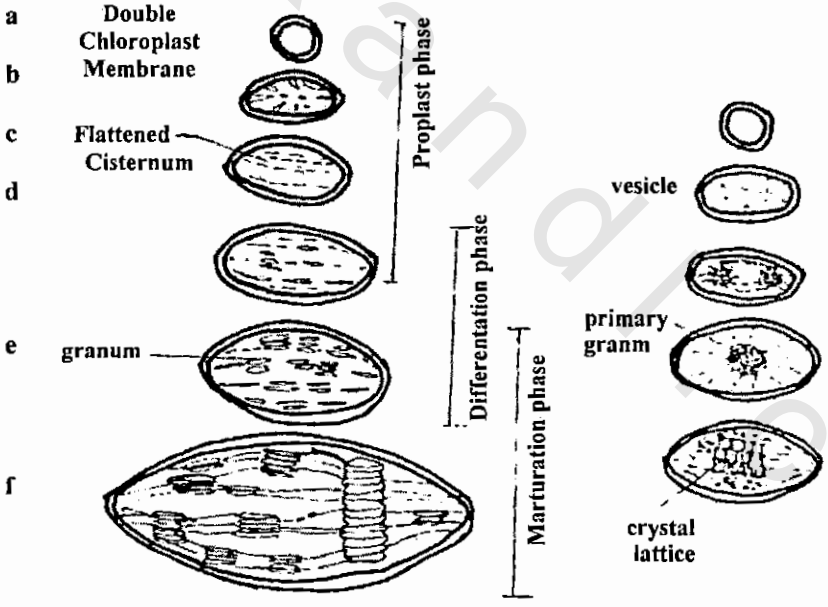


Fig. : 102:

A, phases in the development of a proplastid into a chloroplast in the presence of light. B, Same, but in the dark. (From Brachet and Mirisky: The cell).

2. Chromoplasts:

These are plastids that owe their charcteristic colour to the presence of various carotenoids; they are typically yellow, orange or red. Although they are disc-shaped, some of them appear as spindle, angular, spherical or rod-bodies.

Chromoplasts may be plastids in which chlorophyll disappears and becomes less dominant as in the autumn leaves in which carotenoids, originally present in leaves, become the dominant pigments as chlorophll is broken down in autumn.

In other cases the chromoplasts originate directly from the proplastides. In chromoplasts there are several pigments such as lycopene in red tomatoes, and phycoerythrin as well as phycocyanin in algae.

3. Cholorplasts :

These are green plastids which owe their colour to the presence of large quantities of the green pigment **chlorophyll**. Chloroplasts vary in **shape and size** from one species to another. In leaves of the higher plants they are spherical, oval or disc-shaped. Algae usually have one or two huge chloroplasts which are in the form of cups or elongate spirals. About 20% of the volume of leaf cells is occupied by the chloroplasts. There are 20 to 40 chloroplasts in each cell of the higher plants. Chloroplasts are relatively large organoids which range in size drom 4 to 6 Ų in diameter and 0.5 to 1μ in thickness. However, chloroplasts of plants grown in shade are larger in size and contain more chlorophyll than those grown in sunlight. The size of the chloroplast is countrolled by certain genetic and sexual characters, e.g., chlorophlasts in polyploid cells are larger than that of those of the corresponding diploid cell.

Chloroplasts are sometimes **distributed** evenly throughout the cytoplasm, but are usually packed near the nucleus or at the periphery of the cell. Their distribution usually depends upon the light intensity.

Chloroplasts are self-perpetuating organoids that propagate by division. This takes place by the elongation of the plastid; this is followed by a constriction in the central region separating it into two equal portions.

Chloroplasts are characterized by their high resistance to osmotic changes and fixatives and by their strong reducing action. Also, they swell when kept in distilled water.

Structure of chloroplast :

By the light microscope the chloroplast appears to contain minute granules called "grana" which are embedded in a clear matrix called the **"stroma".**

Electron micrographs of chloroplasts (Figs. 99-102) show an elaborate and highly organized internal strcuture. Each chloroplast is bounded by two membranes. The outer membrane appears similar to the plasma membrane of the cell. The inner membrane is similar to the membrane system of the chloroplast itself.

In addition to the external double membrane, there are internal membrane systems which consist of closed flattened sacs called **lamellae** or **thylakoids**. These membranes contain the chlorophyll and the light conversion apparatus in eukaryotic cells. The thylakoids olso represent the sites of oxygen production and photophosphorylation processes. Thylakoids when stacked one on top of the other produce a structure called granum (Menke, 1962). Membranes extending between the stacks of grana are referred to astroma lamellae (intergranal lamellae). In other words, the grana consits of stacked sacs resembling a pile of coins, and they are connected to each other by membranes (intergranal lamellae) running in the stroma.

Fine granules in the grana membranes have been demonstrated by using very high magnifications (up to 3000,000). These granules are referred to as **quantasomes**, and were considered to be the basic units of photosynthesis. This concept was recently disputed (Howell et al., 1967). Chlorophyll and other pigments are localized in a single layer between the lamellae of a granum. Reserchers have calculated that each quantsome contains about 230 chlorophll molecules.

Internally, the chloroplast contains a non-membranous region known as the stroma. The stroma contains the enzymes necessary for the fixation of carbon dioxide into sugar, and the ribosomes are involved in protein synthesis. The stroma contains, also, lipid globules starch granules, pyrenoid bodies. RNA and DNA. Moreover the chloroplasts contain some cytochromes, vitamin K and E and some metallic atoms such as Fe, Cu, Mn and Zn. In addition, magenesium is found in the chlorophyll moelcule.

Function of chloroplasts :

Chloroplasts in sunlight are capabel of transforming CO_2 and H_2O into carbohydrates and O_2 by a process called **photosynthesis**. This process, by which the solar energy is transformed into chemical energy, is the most

important chemical process in the world. The food stuffs produced are utilized by all plants and all animals for their metabolic processes. This means that chlorophyll transforms and stores the light energy coming from the sun into chemical energy in the food stuffs. This stored energy is released during the oxidation processes through the enzymes located in the mitochondria. So, chlorophyll can carry the process of photosynthesis in the presence of light, while mitochondria carry on the process of oxidative phosphorylation independent of light. During the process of photosynthesis O2 is liberated, whereas the process of oxidation (oxidative phosphorylation) needs O2, thus the former process in endergonic (i.e., it consumes energy) but the latter is exergonic (i.e., it liberates energy).

As regards the nucleus, the chromosomes, the mitosis and the meiosis they are very similar to those of animal, These will be dealt with in some of the following chapters, and the illustrations will include material from both animals and plants.

CHAPTER 14

THE INTERPHASE NUCLEUS

The nucleus was discovered as a constant part of the cell by Robert Brown in 1831. Since then many studies have been carried out on this cellular component. It is well-known that the nucleus passes, in the course of its life, through two periods; and interphase (the phase between two successive divisions) or metabolic period and a mitotic period (period of division).It was usual for a time to describe the interphasic period as a "resting stage", but this is not accurate because the nucleus is physiologically active at all times; this means that the nucleus does not pass by any period of rest during its life.

The nucleus is a permanent structure in all living cells with the exception of the mature red blood cells of mammals. In certain lower animals a typical nucleus is not found, but is represented by a number of granules of nuclear substance (chromatin granules) scattered in the cell as in certain flagellates. In bacteria no nuclear structure is demonstrated by ordinary methods, but certain authors consider the scattered granules having microchemical characteristics of the nuclei as representing the true unclei. With the aid of the electron microscope, it has been possible to demonstrate certain nucleoid bodies in bacteria with which characteristics of nuclei. This nucleoid substance appears as a region which is less opaque than the cytoplasm. There is no definite nuclear envelope or membrane in bacteria.

In viruses, nucleoproteins are present and regarded to represent the nucleoid substance.

Shape of the nucleus :

The shape of the nucleus is usually related to that of the cell; however, it may be completely irregular. As a rule, most nuclei are spherical or oval in shape; sometimes they may be elongate or lobed as in vertebrate leucocytes, and in some cases they may be irregularly branched as in the secretory cells of many insects.

Size of the nucleus :

The nuclear size is usually much variable but in general there is a relationship between the volume of the nucleus and that of the cytoplasm. This may be expressed numerically in the so-called nucleocytoplasmic index "NP".

$$NP = \frac{\text{Nuclear volume (Vn)}}{\text{Cell volume (Vc) - Vn}}$$

This means that when the volume of the cytoplasm increases, the volume of the nucleus also should increase. The lack of maintenance of the "NP" ratio is probably one of the factors which brings about cell division.

As regards the number of nuclei in the cell, almost all cells are mononucleate but binucleate cells are found as in case of some hepatic, nerve and cartilage cells. Polynucleate cells are also found such as the osteoclasts of bone marrow. Syncytia are, also, examples of polynucleate ells. A syncytium is a mass of protoplasm containing many unclei as in the striated muscle fibres.

Nuclear localization:

The position of the nucleus varies from one type of cells to another, but is generally characteristic for each cell type. In embryonic cells it usually lies in the central area, but it commonly becomes displaced as differentiation advances and in accordance, with the formation of reserved substance in the cytoplasm. For example, in adipose cells or in eggs rich in yolk the nucleus is forced towards the periphery by the accumulation of metaplasm. In glandular cells it lies in the basal portion, whereas the apical region is occupied by the secretory granules. Generally speaking, the nucleus is usually situated near the centre of cytoplasm, and in some cases they lie at one side of the cell.

Strncture of nuclei :

(A) Living nuclei :

In living stained or unstained cells the nucleus appears as a mere refractive sphere lying in the middle of the cytoplasm and separated from it by the nuclear membrane. Its interior is, in general, homogeneous excpet for the presence of one or more refractile sperical boldies called **the nucleoli** (signular; nucleolus). In some cases, the nucleus is not homogeneous, but has a granular structure.

In the electron micrographs the nucleus appears empty, but the nucleoli may be distinguished as luminous bodies.

With the micromanipulation it can be demonstrated that the nuclei are usually denser than the cytoplasm and some unclei can be extracted intact with the microneedle. In other cases, when the nuclear membrane is punctured, a liquid material which is the nuclear sap or karyolymph flows out of the nucleus. The nuclear membrane behaves like a true morphologic membrane and not as simple interface for it exerts certain resistance to external pressure and can even become folded or wrinkled.

When the microneedle break through the membrane it can be moved in the interior of the nucleus without encountering resistance, and the nuclolus can be displaced easily.

(B) Fixed nuclei :

In fixed and stained material the structure of the nucleus is distinguished by its great complexity. The following structures are indentified in such nuclei :

1 - Nuclear membrane.

2 - Karyolymph (nuclear sap).

3 - Nucleoli and Karyosomes.

4 - Chromatin.

1 - **Nuclear membrane** or **Karyotheca** appears as a clear outline in opitcal sections. The nuclear membrane possesses mechanical strength as can be detected with mictomanipulator. It divides the cell into two main regions; the nucleus and the cytoplasm. Both having a different physical and chemical structure. This membrance controls the flow of certain substance from the nucleus to the cytoplasm and vice versa.

By the aid of the electron microscope it was found that the nuclear membrane or the **nuclear envelope** consists of two layers; an outer porous layer and an inner one which is apparently continuous (Fig. 103 – 105). The porous layer is about twice as thick as the inner one. The pores are about 400 A^{o} in diameter and have a regular arrangement; the distance between the pore centres being about 1000 A^{o}. At the edge of the pores, the two membranes of the unclear envelope are in continuity. It has been suggested that large molecules may pass through the pores and the small molecules and the ions may pass by diffusion.

The outer layer of the nuclear envelope is connected with the membranes of the endoplasmic reticulum.

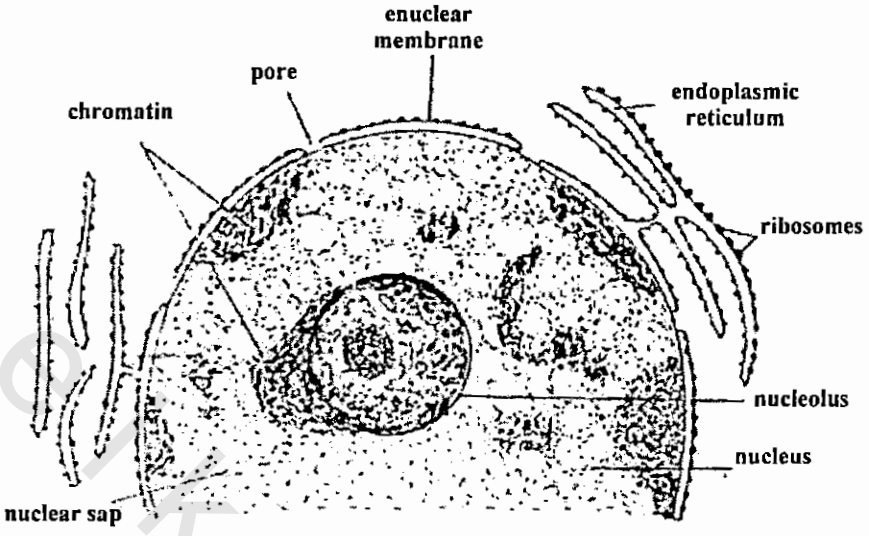
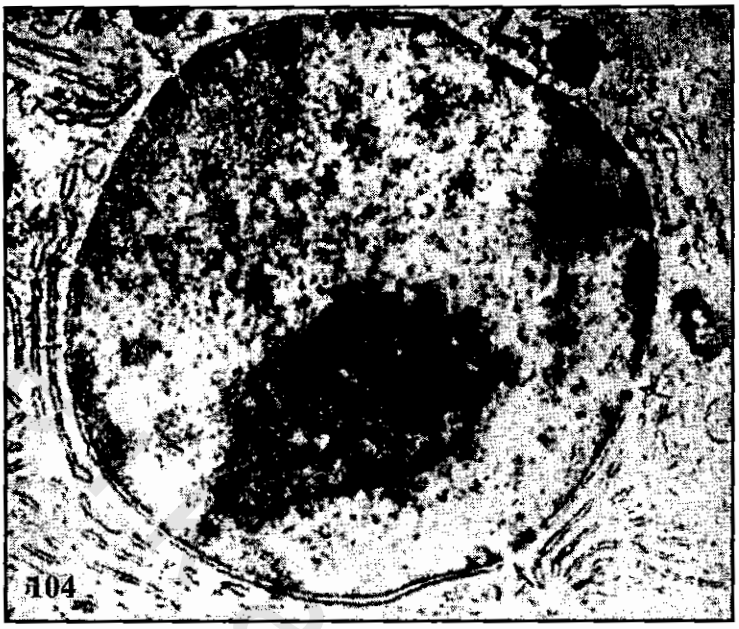


Fig. 103 :

Diagram of the nucleus. Notice the nuclear membrane and its connection with the endoplasmic reticulum.

The nuclear envelope is considered as a part of the vacuolar system (consisting of endoplasmic reticulum and Golgi apparatus). It has long been known that the nuclear boundary either disappears or undergoes major organization during cell division, and that a new boundary is formed around each of the two daughter unclei. During mitotic prophase the nuclear envelope breaks up into a number of vesicles which become disperesed in the cytoplasm and are indistinguishable from the membrane elements of the endoplasmic reticulum. The new envelope is formed at the end of the mitotic process around each of the daughter nuclei. This involves migration of membrane elements of the endoplasmic reticulum onto the surface of the chromosomes where they eventually coalesce and become reorganized into a double layered membrane structure.

The permeability of the nuclear membrane has been studied in isolated nuclei. The nuclei of amphibian oocytes are permeable to salts, sugars, polypeptides and even proteins as haemoglobin. Also in intact cells, it was shown that pyronin staining of the nucleoli disappears 15 minutes following treatment of cells with the enzyme ribonuclease. It was explained that this enzyme - which has a molecular weight of about 13.000-enters the nuclei through the nuclear pores.



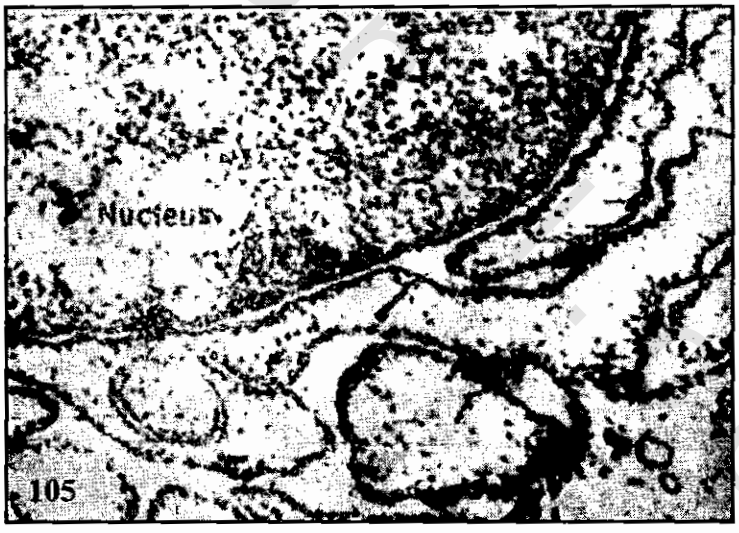


Fig. 104 :

Nucleus showing pores. The two membranes of the nuclear envelop are clearly visible.

Fig. 105 :

Electron micrograph showing the continuity of the nuclear membrane with the endoplasmic reticulum.

2 - **Nucleoli :** Each interphase nucleus contains one or more **nucleoli** which are more less spherical in shape. The nucleoli are often of considerable size (particularly in nerve cells and oocytes), being either single or multiple. The nucleolus appers to be formed of two different parts, an amorphous part and a filamentous one.

The electron microscope revealed the existence of a definite organization within the nucleolus. In some cells an irregular fibrillar structure can be observed, but in others it appears compact and relatively homogeneous.

During mitosis, the nucleoli undergo cyclic changes; they disappear at the beginning of one mitosis and reappear at the end of mitosis. They are formed during the telophase of mitosis in association with specific regions of specific chrmomosomes of the complement, such chromosomes are referred to as **nucleolar chromosomes** to distinguish them from other members of the complement. The nucleolus has a high protein content, RNA and some lipid.

The nucleoli **(plasmosomes** or **true nucleoli)** are, like the cytoplasm, readily stainable with acidic dyes. Other bodies are found in the nuclei which stain with basic dyes and are known as the **karyosomes** or the **chromatin nucleolei** (or **false nucleoli**). The karyosome is a mass of chromatin which appears to act as reservoir of chromation from which the chromosomes may draw part at least of their supply when nuclear division is approaching. In certain cases the karyosome represents a single chromosome which remains compact during the interphase of the nucleus. The plasmosome, on the other hand, is often regarded as a reservoir of nutritive material.

3 - **Karyolymph :** Inside the nuclear membrane is the **nuclear sap (nucleoplasm)** or **haryolymph** which is usually a clear acidophilic fluid of low viscosity. Sometimes the nuclear sap is in the gel condition. The nuclear sap fills the nuclear space between the other nuclear components.

The nucleoplasm appears in electron micrographs as consisting of a pattern of irregular-shaped particles or granules. Little is known regarding its chemical constitution. It is proteinic in nature, containing some RNA and giving positive cytochemical reactions of glycoproteins. A number of hydrolytic enzymes (e,g,, ribonuclease, alkaline phosphatase, dipiptidase) occur in the nucleus and may be specific constituents of the nucleoplasm.

4 - **Chromatin:** In the fixed interphase nucleus one usually sees a network of highly stained material upon or in which are carried granules or flocculent masses of highly stainable substance called chromatin. The chromatin is the physical basis of inheritance, containing the genes or factors which constitute the particulate chemical entities upon which all characters of the organism have been constructed during development. The nuclear network and granules are not visible in the living cell, and they are regarded as artifiacts of technique.

As noticed in the living condition, when a cell passes from the interphase into the prophase of cell division the chromatin granules, characterstic of the interphase nucleus, are no longer seen and their place is taken by the deep blue-staining structures called the **chromosomes**. These structures were given this name because they are deeply coloured bodies (chroma = colour soma = body).

The Chromosomes are usually invisible in the living interphase nucleus due to their having nearly the same refractive index as the nuclear sap. In few cases, however, this thread-like chromosomes can be seen.

It was formerly believed that the chromosomes disappear at the end of each nuclear division and re-form at the beginning of the next mitosis. In other words, it was believed that after each division the chromosomes break up into chromatin granules which at the beginning of next mitosis reassemble up into chromosomes. But, it is now generally accepted that the chromosomes persist throughout the interphase stage of the nucleus; in other words, the chromosomes maintain their individuality form division to division that the same chromosomes which became invisible at the end of one division reappear at the beginning of the next. This is based on the following :

(1) An optically identical set of chromosomes is handed on from cell to cell.

(2) Genetical evidence regarding the distribution of the hereditary factor, or genes which are arranged in a definite linear manner.

(3) In some cases, particularly with the phase contrast microscope, the chromosomes become visible at the beginning of one mitosis in the same position which they occupied at the end of the preceding division, for example, during the eleavage stages of Ascaris the ends of the telophase chromosomes lie in lobe-like projections of the nucleus. The lobes persist

theoughout the interphase stage, and at the prophase the ends of the chromosomes become visible in the lobes which they occupied at the end of the preceding telophase.

That the chromosomes do actually persist through the interphase stage is also supported by the isolation of chromatin threads from the non-dividing nuclei of leukemic cells. So, the nuclear network of linen thread with granules of chromation at the points of intersection is an artifact of technique and is not visible in living condition.

In the interphase nucleus the chromosomes, probably due to their high water content, are unfixable, this only applies to the interphase nuclei but there is evidence that fixed preparations of nuclei in mitosis present a very accurate picture of what is taking place in the living cell.

CHAPTER 15

THE CHROMOSOMES

Although chromosomes were named for the first time by Waldeyer in 1888 yet they were described previously by Hofmeister forty years rarlier.

A chromosome may be defined as a nuclear component with a special organization, individuality and function. It is capable of autoduplication (self-duplication) and of maintaining its morphological and physiological characteristics throughout successive cell divisions.

Chromosomes have received marked attention because of their fundeamental role in heredity, variation, mutation and evolution and in their control of morphogenesis, multiplication, and the equilibrium of vital processes.

Chromosomes are generally visible only during cell division, when they appear as intensely stained rods. Their appearance depends on the physiological state of the celll. In certain cases they appear as delicate slender filaments, and in other cases as compact bodies.

Chromosome continuity :

It is believed that the chromosomes persits throughout the interphase, but are invisible in the majority of the nuclei. It has been suggested that the invisibility of the chromosomes is due to their high water content; according to this view as the chromosomes pass into the interphase stage they take up water and become unfixable. At the beginning of prophase they undergo partial dehydration and become "fixable" and visible as lightly stained threads. They undergo further dehydration, the result is that fixability increases throughout prophase until it reaches its maximum at the metaphase. It follows that chromosomes become gradually more deeply stained and compact as the prophase proceeds.

According to Gresson (1984) the invisibility of the chromosomes during the interphase is not only due to the high water content, but may in part be due to the changes following the transference of uncleic acid. It should be noted that the history of the chromosomes during the interphase is not known with certainity; also, in all cases the chromosomes at the very earliest prophase are separate, and therefore there is no "continuous spireme" as described by some of the earlier workers.

Morphology of Chromosomes :

It has been established that each prophase chromosome is composed of two longitudinal halves called Chromatids. In some cases the longitudinal division is clearly visible during the early prophase and in others the two chromatids lie very close together so that the split is not apparent. There is still some dispute as to the stage at which the chromosomes become double. Some authors believe that the division of the chromosome in preparation for the next division takes place during the interphase; others clain that doubling occurs during the preceding telophase. The chromatids are made up of **chromomeres** connected by non-staining intervals (called interchromomers), and the division of the chromosome is brought about by the doubling of the chromomeres.

The morphology of the chromosomes (Fig. 106) is best studied during the metaphase and anaphase of cell division during which they appear as cylindrical bodies with marked affinity towards the basic dyes. They can be seen in living condition by the phase contrast microscope, and they show an intense absorption in the ultraviolet spectrum at 2600 A^{o}.

Four types of chromomsomes are distinguished in animal and plant cells (Fig. 107). These are:

1 - **Metacentric** or V-shaped chromosomes in which the two arms are equal or nearly equal.

2 - **Submetacentric** or hook-shaped chromosomes, i.e., chromosomes having two unequal arms.

3 - **Acrocentric** or rod-like chromosomes, each being formed of a single straight rod (two arms, one of which is very small).

4 - **Telocentric** or rod-like chromsomes in which the centromere lies at the proximal end.

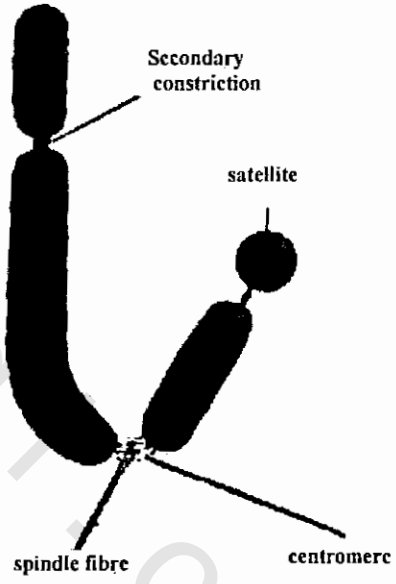


Fig. 106 :
External view of a chromosome.

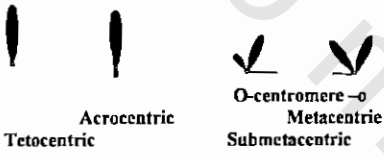


Fig. 107 :
Types of chromosomes according to the position of the centromere.

The centromere :

There is a special region of the chromosome by which the latter is attached to the spindle during cell division. This is known as the **Centromere** or **kinetochore** or **primary constriction** (Fig. 108). It is less stainable than the other regions of the chromosome. In certain large chromosomes two slender filamets can be seen traversing the centromere. These filaments represent the chromonemata. In some organisms a small granule or spherule (kinosome) is found at the middle of each filament. While (1935) believes that the non-staining region is the ture attachment organ, whereas Darlington (1936) regards the granule as the actual organ of attachment. It is now believed that the term centromere designates the whole region of attachment to the spindle. Very frequently the metaphase and anaphase chromosomes are bent at the centomere so that they appear to consist of two parts or limbs with a non staining gap in between.

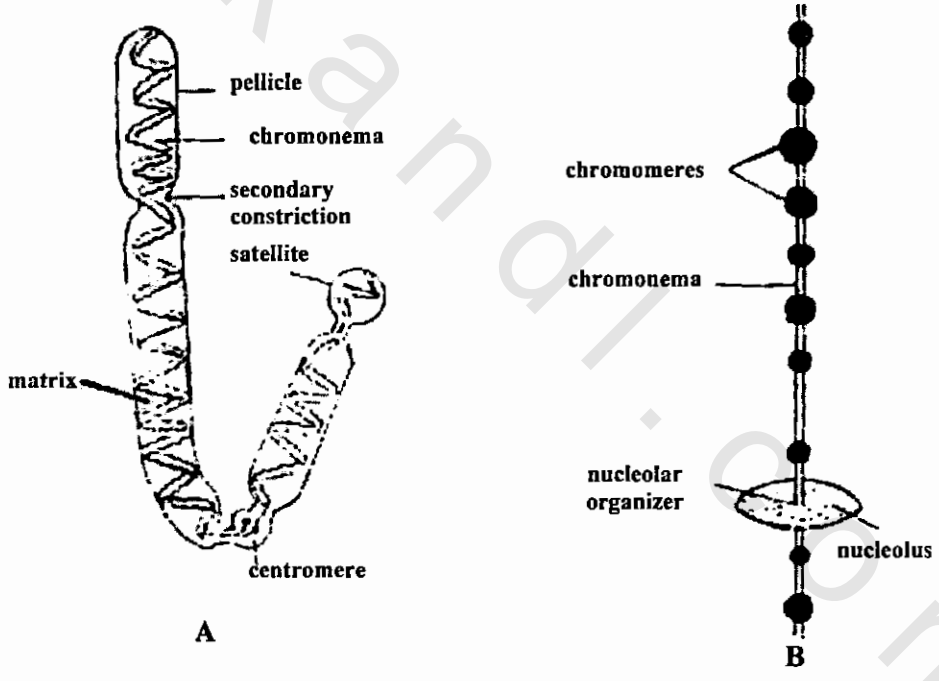


Fig. 108 :

A, Diagram of a chromosome showing the inner structure with the two chromonemata and major and minor spirals.

B. Diagrammatic representation of the interphase chromosome.

The portions of the chromosome on either side of the centromere are known as the arms. These are equal or unequal in length depending upon the position of the centromere which is constant for a given chromsosme. According to the position of the centromere the chromosomes appear in different forms which are V-shaped or metacentric, hook-shaped or submetacentric and straight rod (acrocentic and telocentric). The shape of the chromosome may be changed due to the effect of certain drugs, e.g., a submetacentric chromosome becomes straight under the influence of colchicine.

Usually, there is only one centromere in each chromosome (monocentric). However, there may be two centeromeres (dicenteric) or more (polycentric).

It is very likely that the centromere has a relation with the movement of chromosomes during cell division.

Secondary constrication :

Chromosomes may possess secondary constrictions on one or both of their arms; the position of which is also constant. The secondary constricition is distinguished from the primary constriction (where the centromere lies) by the absence of marked angular deviation of the segments of the chromosome on either side.

Telomeres :

These are the extremities of a chromosome that persent specific genetic properties. If chromosomes are fractured by X-rays, the resultign segments may fuse again except with the telomeres. Thus, it appears that the telomeres that possess a certain polarity which prevents other segments from joining with them.

Nucleolar zone :

There is also another constrictin which certain chromosomes present this is not easily distinguished from the secondary constriction this is known as the **nucleolar zone** or the **nucleolar organizer of nucleolus-forming region**. This is closely related to the formation of the nucleolus, and usually each cell contains two chromosomes known a **nucleolar chromosomes** which possess this special characteristic.

In the anaphase the exact extent of the nucleolar organizer in not evident since the chromosome is very compact and the nucleolus is absent. During the telophase, where the matrix loses its stainability and disappears, the nucleolus makes its appearance in connection with the

chromonenmata at or near the constriction. It should be noted that the chromatic substance of the nucleolus is derived from all the chromosomes found in the nucleus, but in some way it is collected or organized as nucleolus only at the nucleolar zone. It is apparent, therefore, that the nucleolar constituent passes into the chromosomes.

Satellite :

The small segment of the chromosome distal to the nucleolar zone is known as the satellite or trabant. The satellite is a rounded or elongated prominence separated from the body of the chromosome by a delicate chromatic thread. The size of the satellite is variable and the thread may be short or long, but for each particular chromosome they are always constant. In nucleolar chromosomes the formative regions may occur in the part where a satellite is joined with the chromosome.

It is believed that the region which produces the nucleolus is made up of:

(a) **Nucleolar organizer**: This is the region which lies at the end the chromosome where the filament joining the satellite arises.

(b) **Nucleolar body:** This is the part of the nucleolus where its development begins before the appearance of the nucleolus itself.

(c) **Filament of the satellite :** This is a continuation of the chromonema without the matrix. It is a permanent formation of the chromosome.

Karyotype :

The most important characteristics identifying individual chromosomes in mitosis are their number, relative size, structure, behaviour and internal organization. There are other characteristics, such as linear contraction, degree of spiralization and volume, which are subject to physiological variations.

The **number** of chromosomes is constant for all the somatic cells throughoust the individuals of a species. Not only is the number constant but each somatic cell has a similar set of chromosomes. Since chromosomes differ in size and form then in majority of animals the chromosomes are found in pairs; the members of a pair are of similar size and shape and are referred to as **homologous chromosomes**. Therefore every **somatic or diploid** set consists of two **haploid** sets; one is the paternal set contributed by the spermatozoon and the other is the maternal

set contributed by the ovum. Each haploid set of chromosomes is designated by N and the diploid by 2N.

The **shape** of the chromosomomes is characteristic for each species. The shape may be changed by chemical agents or radiation. Spontaneous alterations may occur in nature. The criteria used for morphological identification are mainly based upon the position of the centromere, on the secondary constriction, and on the existence and localization of satellite.

The **size** of the chromosomes is relatively constant and is important in the individualization of each one of the members of a complex. The relative dimensions of the chromosomes usually differ from each other. The length of the chromosome may vary fro 0.2 u to 50 u; the diameter varies between 0.2 u and 2 u.

The name **Karyotype** is given to the group of constants or characteristics (including the number, the form and the size, and other characteristics such as primary and secondary constrictions) which should be taken itno consideratin when identifying a particular chromosmal set. This Karyotype (Fig. 109) is characteristic of an individual, race, genus, or larger grouping. The Karyotype is usually represented by a diagram called an idiogram in which the pairs of homologous chromosomes are arranged in series of decreasing size.

Internal Structure of Chromosomes:

The internal structure of chromosomes (Fig. 108) can be studied by using special methods such as X-ray diffraction, polarization microscopy, electron microscopy, ultraviolet microspectrophotometry, protein chemistry, enzymology, experimental genetics and cytogentics. By these methods one can see a slender thread known as **chromonema** which is coiled into a spiral along the length of the chromosome. The chromonoma is embedded in a **matrix,** which is covered externally by the **pellicle**. The matrix (also called the sheath, hyalolemma or calymma) acts as filling substance that covers the chromonema (p.: chromonemata), and hides its spiral form.

The chromonema is present throughout the entire nuclear cycle, and in which presumably the genes are carried; the matrix, on the other habd, is conspicous only during certain stages of mitosis. It should be remembered that along the chromonema are the **chromomeres** which are clearly visible during the prophase when the threads have less chromatic substance, but during anaphase they are rarely visible. Some cytologists deny the existence of the matrix. But the invisibility of the spiral in many metaphase chromosomes appears to be an evidence in favour of the presence of a matrix.

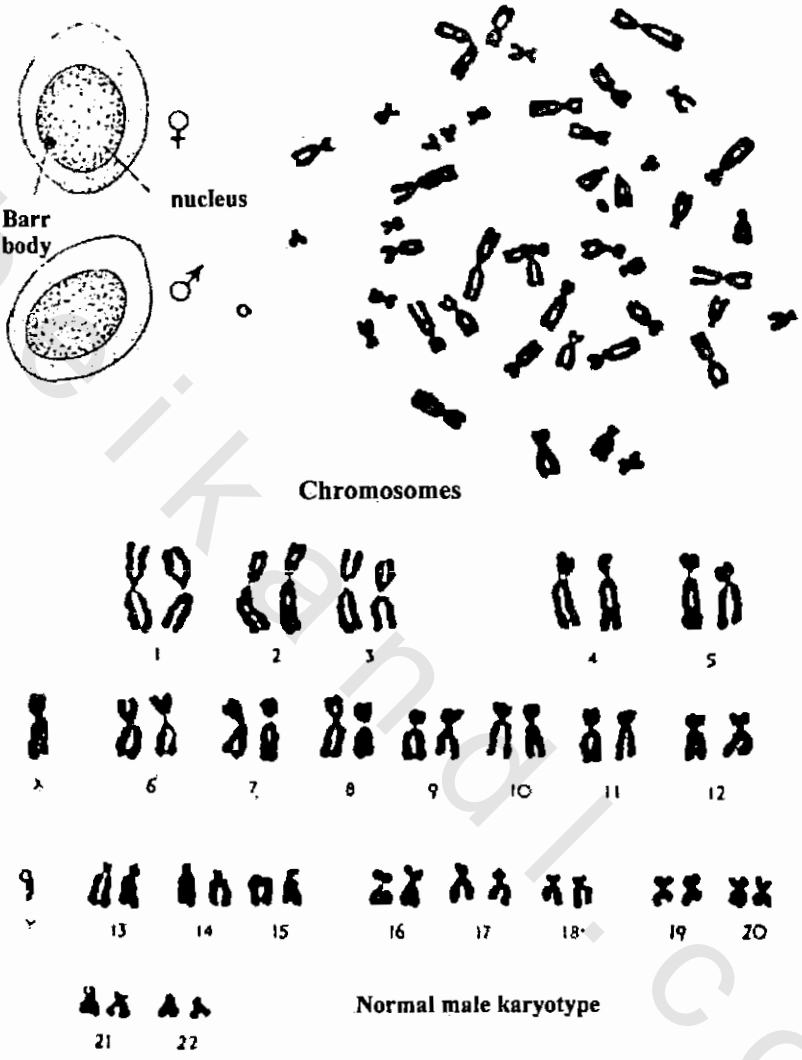


Fig. 109 : Chromosomes.

The chromosome is composed of two coils; a major coil which has from 10-30 gyres, and a minor coil lying perpendicular to the major coil and is composed of many small gyres. The number of major coils depends upon the length of the chromosome, Very long chromosomes may possess 20-30 gyres, whereas very short chromosomes do not form even one complete gyre.

The contraction of the chromosomes during the prophase is due to the coiling of the chromonema so that it assumes a spiral structure, except at the regions of the spindle attachment, which is therefore seen as a constriction.

Ordinary methods of fixation show the metaphase chromosomes as homogeneous cylindrical rods, but special methods of fixation (fixing in boiling water, treatment with ammonia fumes or strong acids or squeezing the chromosomes under a cover glass) reveal that each metaphase chromatid is coiled to form a spiral. White believes that this is the true structure in the living condition, and that the special methods of fixation only slighly separate the gyres and thus reveal the spiral. In other words, the spirals are not usually visible either in living condition or in preparations fixed by ordinary techniques, due to the presence of the gyres of the spring in contact with one another. In addition, it is found that a certain amount of nucleic acid covers the whole structure. In plants with large chromosomes such as Lillacae the spiral structure is clearly seen.

The coiling is usually characteristic for a given chromsome. In some cases, the direction of coiling is uniform being clockwise or anti-clockwise, and in other cases, this direction varies in the different parts of the chromosome, being clockwise in certain regions and anti-clockwise in the other ones. The direction of coilling may change at the spindle attachment, but does not necessarily do so. It should be also mentioned that the two chromatics of a pair may be coiled separately or together, in the former the chromosome is 8-shaped when seen in T.S., but in the latter it has a circular or an oval corss-section.

During the telophase the chromosomes undergo a process of de-condensation and de-spiralization, and if the interphase is long the spiral structure may disappear before the next prophase. But if the interphase is short the early prophase chromosomes are coiled into a loose spiral which Darlington (1935) calls the relic spiral, or remains of the metaphase spiral of the previous division. The relic spiral disappears by the middle of the prophase and the new spiral begins to develop at the end of this period and is completed by metaphase. There for, we have two kinds of spiral: those of the early prophase and those which develop at the end of prophase and are completed by metaphase. The metaphase spirals are, therefore, not a continuation of the early prophase ones.

Heteropyknosis :

In some cases it has been shown that certain chromosomes or parts of chromosomes (chromosomal regions) stain differently, from the rest of the

chromosomes in the same nucleus during a certain stage, or stages of nuclear division. This phenomenon is refered to as heteropyknosis (Greek differential staining: Hetoros = different; Pyknos = dense). If a chromosome or chromosomal region stains more deeply than the others in the nucleus it is spoken of as **positively heteropyknotic**, but if it stains more lightly it is said to be **negatively heteropyknotic**.

Heteropycnosis is very common among the sex chromosomes, but it has been also found among ordinary chromosomes (autosomes) as in the toad. where an autosome with a negative heteropycnosis during the metaphase of the first meiotic division has been found (Saez, Rojas and De Robertis, 1936).

Only one type of heteropycnosis is usually present in the nuclei of a given organism, but in certain locus both positive and negative heteropycnosis appear clearly at different stages in the development of the male germ-cells. Chromosomal regions which become heteropycnotic at some stages of nuclear division are said to be **heterochromatic**, whereas those which do not undergo heteropycnosis are known as euchromatic. The heterochromatins are the chromosomal parts which remain condensed during interphase, forming the chromocentres or false nucleoli. Heterochormation is regarded as a state of chromosome and not a special substance.

Sex chromatin (Barr body):

Barr and Bertram (1949) found a little more or less darkly stained body of chromatin in the nerve cells of female cats that could not be seen in similar cells of male cats. This body usually lies against the inner surface of the nuclear membrane. The same authors extended their studies to other kinds of cells and to other species where they were always able to demonstrate this body in the nuclei. This body became referred to as **Barr body.**

Later, it became known that the appearance of Barr body in cells of females is caused by one X-chromosome being coiled tightly along most or all of its length during the interphase. As a result of its tight coiling, it would be dense enough when stained to constitute a visible Barr body. In addition, it also become known that in case of the presence of two X-chromosomes, only one is active (euchromatic) and the other is at rest (in a heterochromatic state). It is in this state that it appears as a Barr body. This explains the non-existence of a Barr body in cells of males in which a single X-chromosome is found, and in such case, it must exist in an active state.

Chemical Composition of Chromosomes :

The chromosomes are chemically made up of nucleoproteins, i.e., a combination of simple proteins with nucleic acids.

The protein part is essentially composed of histones, these are present in all cells. Proteamine appears and exists only in the sperms of some fishes. However, in 1943 Stedman and Stedman isolated besides the histone and the deoxyribose nucleic acid a new type of protein called **chromosomin from** the nuclei of the fish spermatozoa.

The nucleic acids are aggregates of units called nucleotides. A nucleotide is composed of phosphoric acid, a pentose-sugar and a purine or pyrimidine base. The affinity of chromosomes towards the nuclear stains such as haematoxylin is due to the prsence of basic groups.

According to some authors the prophase chromosome consists of a protein fibre called the **Chromonema**, in certain regions of which deoxyribose nucleic acid accumulates to produce small bead-like structres known as **chromomers**; however, other workers are of the opinion that the chromomeres are regions of superposed coils. The **internodes** (interchromomeres) in between the chromomeres contain little or no nuclei acid so that they are almost colourless in stained preparations. This moniliform appearamce of the chromosome is characteristic of the meiotic prophase, but also occurs in most of mitotic divisions. When the nuclear membrane vanishes the amount of nucleic acid in the chromosomes increases. Since the nucleic acid is present in cytoplasm of rapidly dividing cells but is absent or scanty in cells which have ceased to divide, therefore, the nucleic acid is probably removed form the cytoplasm to the chromosomes at the beginning of mitosis and is transferred from the chromosomes to the cytoplasm after each nuclear division.

Nucleoli disappear in the prophase and become visible in the telophase; they usually arise in connection with a definite region of a chromosome called the **nucleolar organizer**. They contain ribose nucelic acid, which is characteristic of the cytoplasm, but it is probable that the two types of nucleic acid are mutually convertible, and that the nucleoli play a part in the transference of nucleic acid to and from the chromsosomes.

Giant chromosomes:

At certain stages of the cycle of some cells special types of giant chromosomes are found. These are characterized by their very large size and the increase in the nucleus and cell volumes. To the giant chromosomes belong the polytene chromosomes found in larvae of Diptera (e.g., in salivary gland), and the Lampbursh chromosomes occuring in the oocytes of animals. (Figs. 110-112).

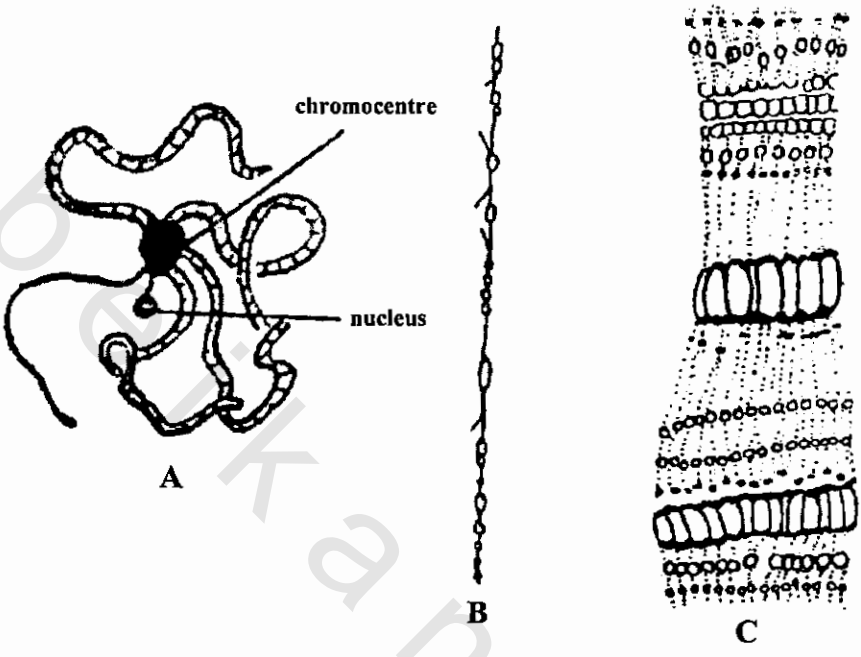


Fig. 110 : Diagram showing the structure of the polytene chromosomes.

A : Diagram of salivary gland chromosomes of Drosophila. The paternal chromosome (in white) and the maternal (in black) are paired.

B : Showing chromomena of a polytene chromosome. Notice the different chromomeres.

C : Polytene chromosome showing the organization of the chromonemata and chromomers.

Fig. 111 : Giant chromosomes from the salivary glands of the Fruit fly.

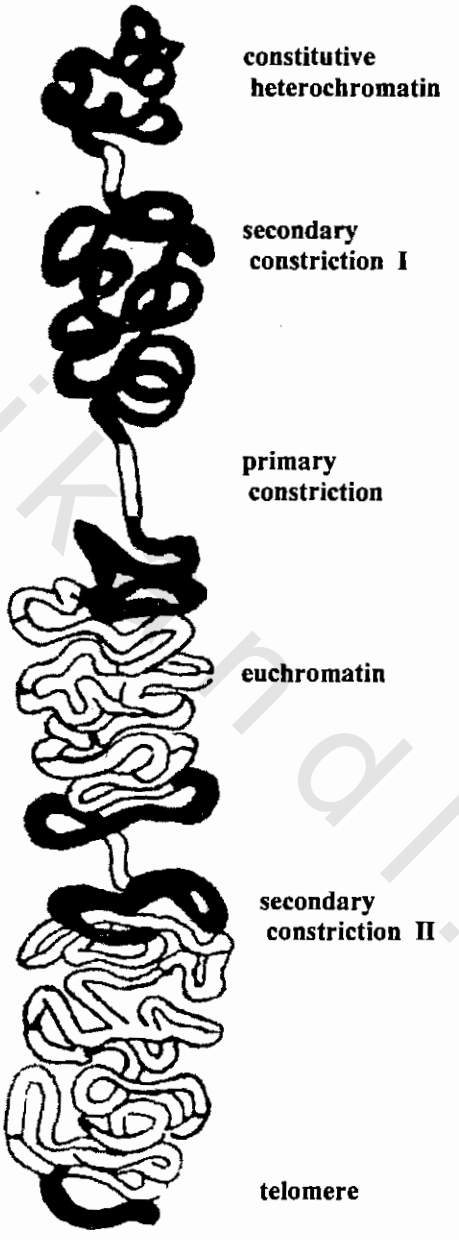


Fig. 112 :
Diagram of constitutive heterochromatin in a mammalian metaphase chromosome.

Polytene chromosomes :

The polytene chromososmes were first demonstrated by Ballbini in 1881 in the salivary glands of the Larva of Chironomus. Then these chromosomes were studied by a number of cytologists (Heitz and Bauer, 1933; Oainter, 1934 and Koller, 1935) who pointed out their importance in cytogenetics.

Since the salivary chromosomes are usually paired throughout their length and show a chromomeric structure they are analogous in several respects to pachytene bivalents. But some authors but referred to as polytenic chromosomes – a term which is convenient since chromosomes of this type are not confined to salivary gland nuclei but are found in other tissues of Diptera as in the gut epithelium, Malpighian tubules and nerve cells.

Each chromosome is closely paired with its homologue, i.e., each two homologous chromosomes are completely in contact throughout their length. Thus, the number of the chromosomes corresponds to the haploid and not to completely the diploid number. The chromosomes are spirally wound around one another as in a piece of two-stranded rope, but it should be noted that they are not wound into a tight spiral and therefore they are about 50 times the length of the ordinary metaphase chromosomes.

The structure of these chromosomes was explained as being due to the fact that certain successive longitudinal divisions take place in the normal chromosomes until they constitute a massive and coarse element similar to a rope. Hence, they were given the name "giant chromosomes".

If the salivary chromosomes are crushed under a cover glass and suitably stained (e.g. in acetocarmine which is a fixing and staining fluid) each will be seen to be mad up of a series of **dark bands** separated by non-staining zones known as **internodes** or **interbands**.

In other words, the polytene chromosomes are markedly striated transversely having the appearance of striated muscle fibres. Along the length of each chromosome, there is a series of dark bands alternating with other clear zoned called interbands.

The dark bands are rich in nucleic acid and are stained intensely with ordinary nuclear dyes (Feulgen-positive); the internodes, on the other hand, contain little of nucleic acid. The interbands are of fibrillar appearance, more elastic than the bands and do not stain with basic dyes.

The bands are of varying thickness and result from the accumulation of chromomeres. Some large bands particularly in inert regions of chromosomes

consist of vesicular chromomeres which are sometimes referred to as **heterochromomeres**. Certain of the thicker bands are made up of finer bands with several extermely short internodes.

The bands of a polytene chromosome vary in size and they correspond exactly in position in the two homologous chromosomes. The position, size and number are always constant for a particular chromosome in any individual of the same species. Thus, "chromosome maps" have been made from salivary shromosomes showing the number and disposition of the bands, and it is easy to verify any disarrangement of alteration in the order of their linear structure. Since some of the thicker bands are compound, i.e., composed of several thinner ones lying very close together it is difficult to know the exact number of bands in a chrmosome. The total number of bands in the X-chromosome of **Drosophila melanogaster** is about 4,000. The bands are mostly in pairs, i.e., each two adjacent bands are of the same thickness.

Each band is considered as a disk since they extend through the whole diameter of the chromosome. Furthermore, each band consists of several granules (about 256 in Chironomus) which are more or less completely fused to form a transverse plate. The granules in one band are connected with those in the next band on either side by means of fine threads.

In Drosophila the heterochromatic regions around the centromeres are as a result of their homology, fused together to form a single mass known as Chromocentre to which the nucleolus is attached by means of a thread. In other Diptera such as Chirononmus the salivary gland chromosomes are separate and unconnected and therefore the chromocentre is absent. The lack of the chromocentre in such Diptera is probably due to the lack of the hololegous regions immediately adjacent to the centromeres. The study of the salivary gland chromosomes is of great importance as regards the chromosome structure.

Chromatin nature of polytene chromosomes :

Some of the bands of the polytene chromosomes consist of euchromatin and others consist of heterochromatin.

The euchromatic regions were regarded by Casperson (1950) to consist of DNA associated with histones. They also give a strong Gomori reaction for alkaline phosphatase. The euchromatin regions are regarded to carry the major genes.

The **heterochromatic** regions also contain DNA, but the amount of DNA is liable to many changes according to the different physiological

and pathological conditions. For example, cold treatment was found to induce DNA deficiency. Thus, these segments stain with different grades according to the NDA content. This is the phenomenon which has been referred to as **Heteropyknosis**. The more intensely stained region is known to be **positively heteropyknotic** and the more lighly stained region is known to be **negatively heteropyknotic**.

The clear discs (interbands) are Feulgen negative which indicates that the DNA is absent.

Lampbrush chromosomes :

These chromosomes are much longer (about 3 times) than the polytene chromosomes. They are seen in occytes during the first meiotic division. This phase corresponds to a period of maximum synthesis where formation of yolk takes place. Each chromosome consists of a central axis carrying many small leteral branches (Fig. 113), thus acquiring the shape of a test tube brush or lampbrush. The growth of the type of chromosomes results from the increase of the size of chromonemata.

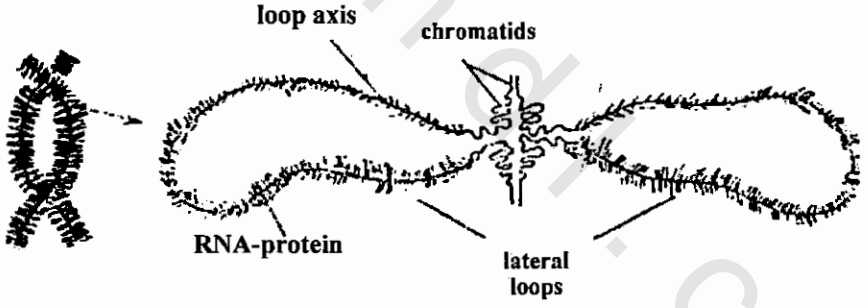


Fig. 113 :

Diagram of the structure of a lampbrush chromosome. (after Miller).

CHAPTER 16

CHEMICAL AND MACROMOLECULAR ORGANIZATION OF THE NUCLUES

The physiology of the nucleus from the angle of its chemical and macromolecular organization is the basis of a new field referred to as **"molecular genetics"**.

In these new fields – nuclear physiology and molecular genetics-control and regulation of cellular functions are investigated; the investigations are mainly concentrated on the part played by the nucleic acids in genetic function.

Cytochemical study of the nucleus ;

In studying the chemical organization of the nucleus two approaches are usually followed.

(1) The biochemical approach which depends on isolating a large number of nuclei to allow analysis by using the biochemical techniques.

(2) The cytological approach in which cytophotometric, cytochemical and autoradiographic techniques are employed.

From these studies it has been found that the nucleus is of a complex chemical organization in which the **nucleoproteins** are the most important components. Nucleoproteins result from the combination of nucleic acids (DNA and RNA) and proteins. The protein part has several components; the well known of which are the basic protamines and histones. In addition, several acidic proteins (the so- called non histone proteins) are found.

The chemical composition of the nucleus, therefore, includes the following:-

1- Deoxyribonucleic acid (DNA).

2- Ribonucleic acid (RNA).

3- Basic proteins : Protamines and histones.

4- Non-histone or acidic proteins (residual protein, chromosomin and enzymes).

5- Other nuclear components.

1 – DEOXYRIBONUCLEIC ACID (DNA)

Deoxyribonucleic acid has a complex chemical structure. The molecule of two stands coiled together in a double helix (Fig. 114). Each strand is a chain of nucleotides. The nucleotide (Fig. 115) consists of a pentose sugar (deoxyribose) with a phosphate group attached on one side and a nitrogenous base (purine or pyrimidine) on the other. The nucleotides are linked together by joining the pentose sugar of two consecutive nucleosides (combination of a pentose with a base) with phosphate bond. Four different bases are found in different nucleotides: adenine (A) and guanine (G) belong to purines; cytosine (C) and thymine (T) belong to pyrimidines (see Fig, 115).

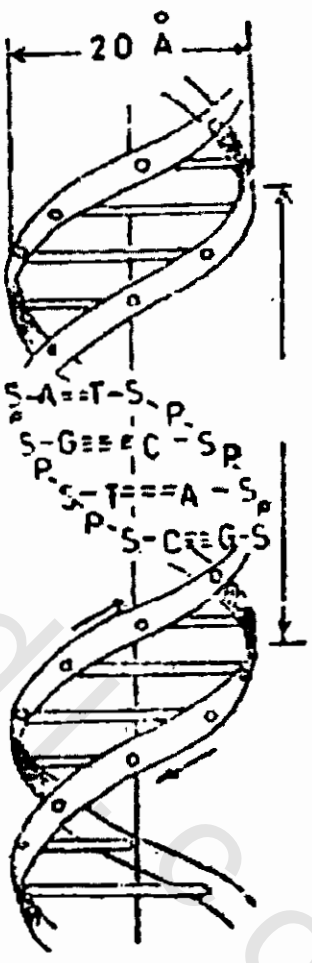


Fig. 114 :
The double helix of DNA (Watson and Crick model). The two chains are held together by hydrogen bonds between the bases.
A. addenne; T, theymine: G. guanine; P. phosphate; S, deoxyribose sugar; C, cytosine.

Pyrimidines

Are monocyclic, each consisting of a six membered ring, whereas purines are dicyclic. The ratios of purines and pyrimidines differ in different DNA molecules, but the amount of purines equals that of pyrimidines, i.e., A + G = T + C. Furthermore, in the DNA molecule the amount of adenine equals that of thymine (i.e.,A = T); also the amounts of guanine and cytosine are equal., i.e., G = C.

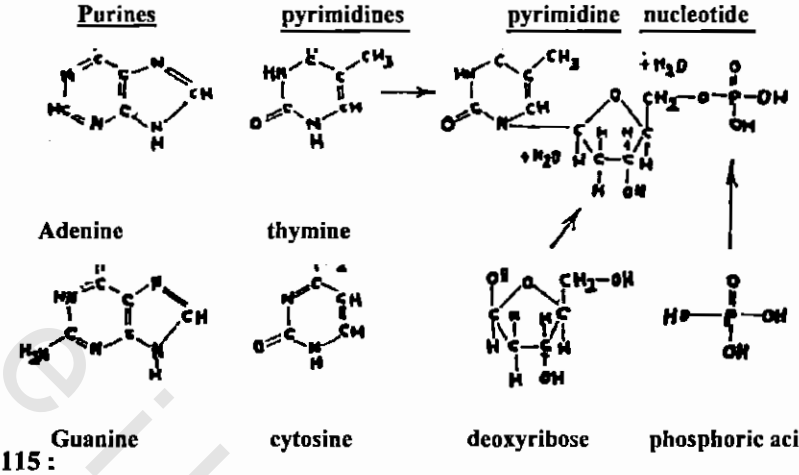


Fig. 115 :

The basic building units of DNA. Left. The four bases: adenine, guanine thymine and cytosine. Right a pyrimidine nucleotide formed from thymine deoxyribose and phsophoric acid.

The two strands of DNA molecule are united together by the hydrogen bonding of their bases in such a way that every A on one strand is linked with a T on the other, while every G is linked with a C (Fig. 116). Thus, the two strands of a DNA molecule are complementary to each other.

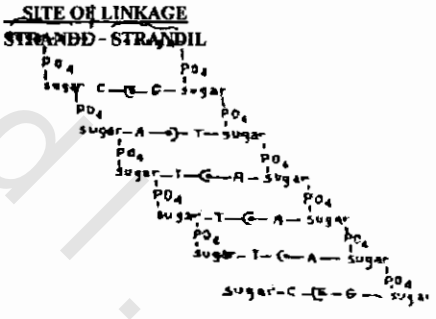


Fig. 116 : Portion of a double-stranded DNA molecule showing how deoxyribonucleotids of one stand are joined to those of the other strand through their bases; adenine is joined to thymine, and cytosine to guanine.

The high degree of polymerization of DNA molecule and the varying sequence of the four bases along the DNA chain gives rise to a very large number of structurally different DNA molecules, and thus an extraordinary amount of genetic information can be recorded. DNA is usually referred to as the genetic code since it is mainly responsible for the determination and transmission of hereditary characters. One should remember that since phosphoric acid and deoxyribose (two of the components of DNA) are constant, the information is coded only by the sequence of the four bases (adenine,

guanine, cytosine and thymine). Thus, the genetic "dictionary" has only a four unit "language". Under the electron microscope the DNA molecule appears as long, unbrancehed microfibrils with a diameter of 20 A°. The DNA molecule may reach several millimeters in length.

DNA DUPLICATION (replication) :

The duplication of DNA molecules takes place mainly accompanying cell division. In mitosis the amount of DNA in each of the resulting nuclei is the same; this is due to the fact the DNA molecules are arranged lengthwise in the chromosomes and that they split longitudinally accompanying the splitting of the chromosomes. It is also evident that in meiosis, each of the resulting haploid muclei contains only half the amount of DNA found in the diploid nucleus.

At the beginning of mitosis, condensation of DNA into visible coiled chromatin threads (the chromosomes) takes place. It is clear that the main step involved in this process is the doubling of the DNA content of the nucleus. It is thought that this takes place before the visible condensation of the chromatin material into the chromosomes. However, it is certain that the molecules of DNA are duplicated inside the chromosomes prior to the splitting of the chromosomes.

As has been previously mentioned, the molecule of DNA is composed of two strands coiled togther in a double helix. These helices are held together by hydrogen bonds between the nucleotides, in such a way that the guanine (G) of one chain is hydrogen bonded to the cytosine (C) of the other and the adenine (A) of one to the thmine (T) of the other.

Duplication (Fig. 117) starts by the unwinding of the two complementary strands of the DNA molecule. Each strand then, acts as a template for the production of another complementary strand. During the formation of the new strand A will come opposite to T found in the old strand, C will come opposite to G and vice versa. At the end of this process, two DNA molecules are formed; each of which consists of an old strand and a newly - synthesized complementary strand. Each of these molecules now forms a chromatid. The two chromatids separate from each other forming two daughter chromosomes. DNA duplication is not a prerequisite to cell division, because in certain cases the duplication takes place without division of the cell, but the reverse is not true, i.e. cells

division does not take place without a previous duplication of the DNA content. (Figs. 118-119).

Chromosome duplication and DNA:

In the prophase stage there is seen for the first time the condensation of the chromatin into coiling, spiraling threads which are the chromosomes. It is generally agreed that the chromosome which is seen with the light microscope is itself a duplex structure, i.e. it contains two perhaps more equivalent strands called chromatids. The chromatids are the structures which will be separated from each other as mentioned before. In some cases, it has been suggested that each of these chromatids is itself duplex being made up of "half chromatids", each of which is replicated, so that a chromatid coil contains a new DNA strand and an old DNA strand.

The exact mechanism of replication is still not clear. According to some authors, the complementary strands, which are usually interlocked together, must rotate at their free ends in order to separate prior to the replication process. Others claim that the complementary strands fragment at each gyre, separate and then replicate. The first view was rather modified recently by some authors who declared that the duplication of a DNA molecule could occur in a continuous manner without requiring the prior separation of strands. The duplication could begin at one end, this opening up the strand and providing the energy for the rotation of the two lengthening daughter strands.

DNA and genetic information (Genetic code):

As to the rôle of DNA in storing and transmitting information to be used in protein synthesis it would appear that the specific sequence of the four bases among the linear structure provides the code necessary for the determination of the protein structure. Thus, the amount of 20X 10-12g of DNA in the human fertilized egg is sufficient to determine all the hereditary characteristics of the mature person.

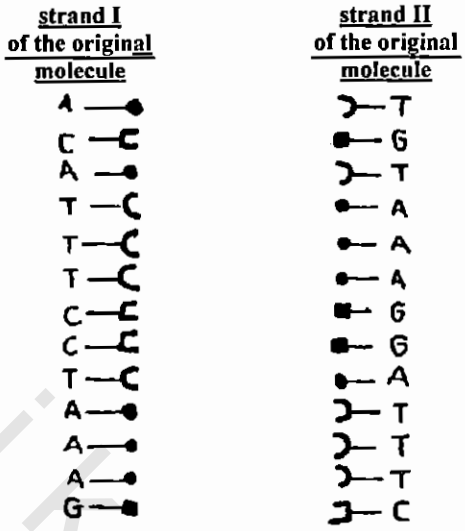


Fig. 117:

The first step in the duplication of DNA molecule is the separation of its two strands.

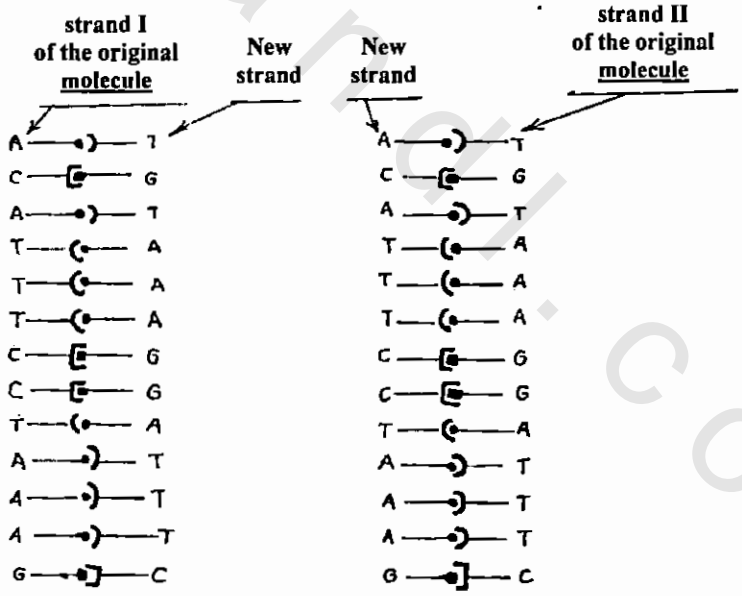


Fig. 118: The second step in duplication in which a new strand is synthesized beside each of the two original strands. An A always forms beside a T, and vice versa, and a C, besides a G and vice versa. Each of the resulting double – stranded molecules is identical with the one in Fig. 120.

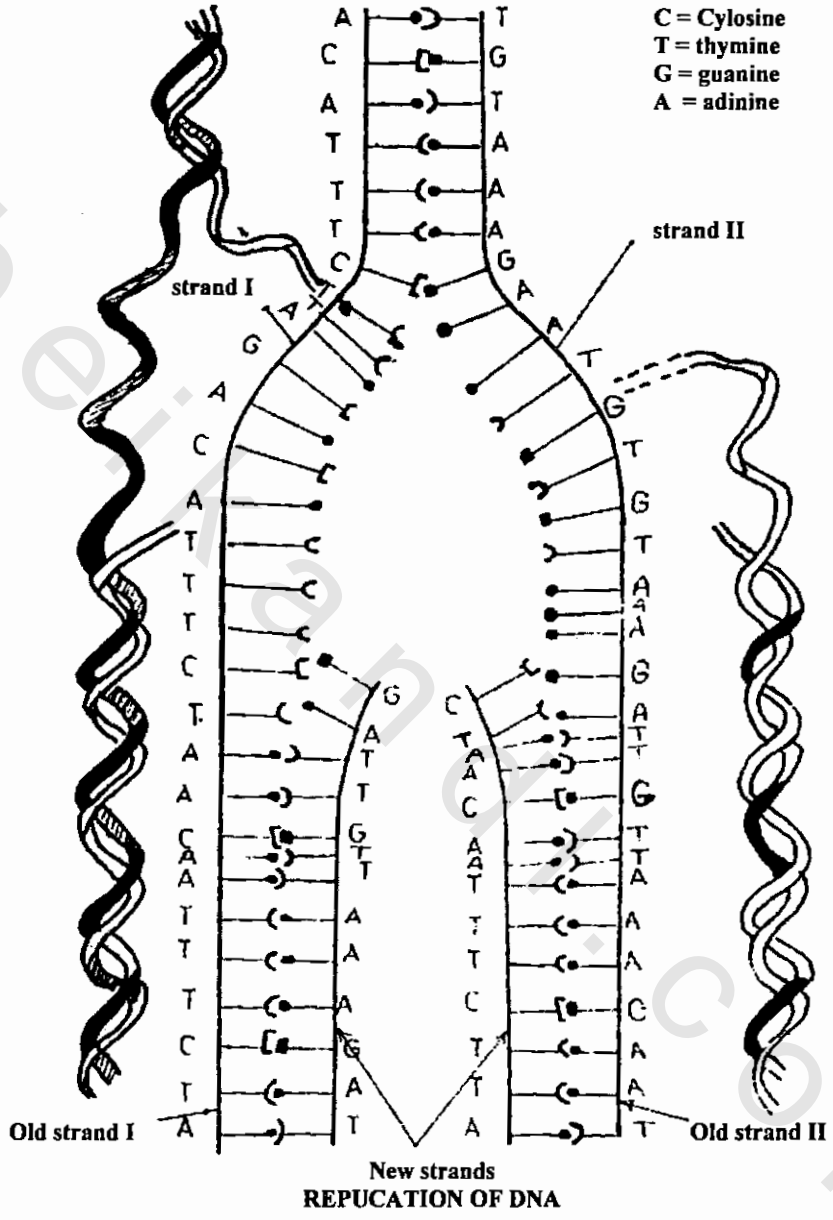


Fig. 119:

Diagram showing the two steps (mentioned in Fig. 121 A and B) in the duplication of a DNA molecule.

The question now is how information is given by the DNA molecule or in other words, **what is the nature of the genetic code?**

It is well-known that information is given by words, and words are composed of letters. Words give different information not only because they are composed of different letters, but also because the same letters can by arranged in different sequences to give different words of different meanings. For example, the letters A, T and R can be used as ART, TAR, RAT, etc., i.e., the 3 letters can be arranged to give different words of different meanings. When the DNA molecule is considered it will be found that it has an alphabet of only 4 chemical letters adenine (A), guanine (G), cytosine (C) and thymine (T). These letters form certain words that convey different information, and can be written and arranged into long sentences. The possibility of this was clearly demonstrated with early invention of the telegraph when it was found possible to convey any known language by a 2 – letter code; the 2 letters being a "dot" and a "dash" (-). Combination of dots and dashes which from the **Morse code** can convey just as much, varied information as can the whole letters of any language. Since genetic information is finally expressed in the form of proteins and since there are about 20 amino acids, only a code of no more than 20 words is needed, each of which specifies a certain amino acid. In this connection, it must be pointed out that the extensive work carried out on the bacteria, Neurospora showed tat each gene (segment of DNA) dictated the specificity of an enzyme so that a direct retationship between a gene and a protein was suggested. It was found that Neurospora are built up of proteins from available precursors. Mutations (produced by X-ray treatment) were unable to build up proteins unless certain enzymes were added to the medium. This shows that a certain gene disappeared which was necessary for the activation of the specific enzyme.

2 - RIBONUCLEIC ACID (RNA)

The structure of RNA resembles that of DNA with the exception that ribose and uracil are found in RNA instead of dexyribose and thymine found in DNA. Thus, RNA and DNA differ not only in the structure of the pentose sugar (ribose in RNA: and deoxyribose in DNA), but also in one of the pyrimidine bases (uracil in RNA; and thymine in DNA). A third difference is that RNA is single-stranded (except in certain cases as in viruses), whereas DNA is double- stranded. The RNA is synthesized within the nucleus by using only one strand of DNA as template.

RNA plays an essentilal rôle in protein synthesis in the living cells of the body. The protein synthesis is controlled indirectly by DNA; this takes place as follows; The DNA molecule carries certain genetic information which is transcribed into the RNA molecule (which carries it to the cytoplasm); then the genetic information is translated into proteins (i.e., protein synthesis).

$$\text{.. DNA} \xrightarrow{\text{transcription}} \text{m RNA} \xrightarrow{\text{translation}} \text{Protein}$$

Three main types of RNA are now recognized:

1 - **Ribosomal** RNA (rRNA) is contained mainly in the ribosomes which are scattered in the cytoplasm. Ribosomal RNA is formed in the nucleus and probably accumulated in the nucleolus which is the site of rRNA synthesis. Ribosomal RNA is present in two sizes and constitutes the largest fraction of the RNA in the cell (75 to 85% of the total RNA).

2 - **Messenger RNA** (m RNA) is synthesized in the nucleus as part of the heterogeneous nuclear RNA. It is built up of molecules of different sizes. The name "messenger" comes from its function of transcribing the genetic information from the DNA molecule in the nucleus and carrying it to ribosome to be tanslated into protein. The longer the m RNA molecule, the longer is the message carries it and the longer is the protein it can from.

Transfer RNA (t RNA) is of low molecular weight and is formed by about 67 nucleotides.

The name "transfer" is based on the rôle of this type of RNA in protein synthesis. Each t RNA molecule carries one amino acid molecule to a ribosome (the site of protein synthesis) in the cytoplasm.

PROTEIN SYNTHESIS :

The various experiments carried out in the filed of molecular biology have indicated that DNA contains the information necessary of the synthesis of proteins in the living material. This process is mediated by RNA which is synthesized from DNA in the nucleus and then becomes emitted into the cytoplasm where the main bulk of protein synthesis takes place (Fig. 120), From histochemical and autoradiographic evidences it is clear that DNA duplication and RNA synthesis in the nucleus take place at different times during the nuclear division cycle. At one time the DNA acts as a template for the synthesis of a duplicate DNA molecule, and at another time it acts as a template for synthesis of and RNA molecule.

The messenger RNA (m RNA) which is synthesized along the course of only one molecule of the two strands of a DNA molecule carries the information from this DNA to the site of protein synthesis. In other words, the m RNA carries an inscription of the sequence of nitrogenous bases arranged along the DNA molecule with the 3-letter words of (A,G,C) and U. The sequence of nitrogenous bases in DNA is usually refered to as **genetic information.**

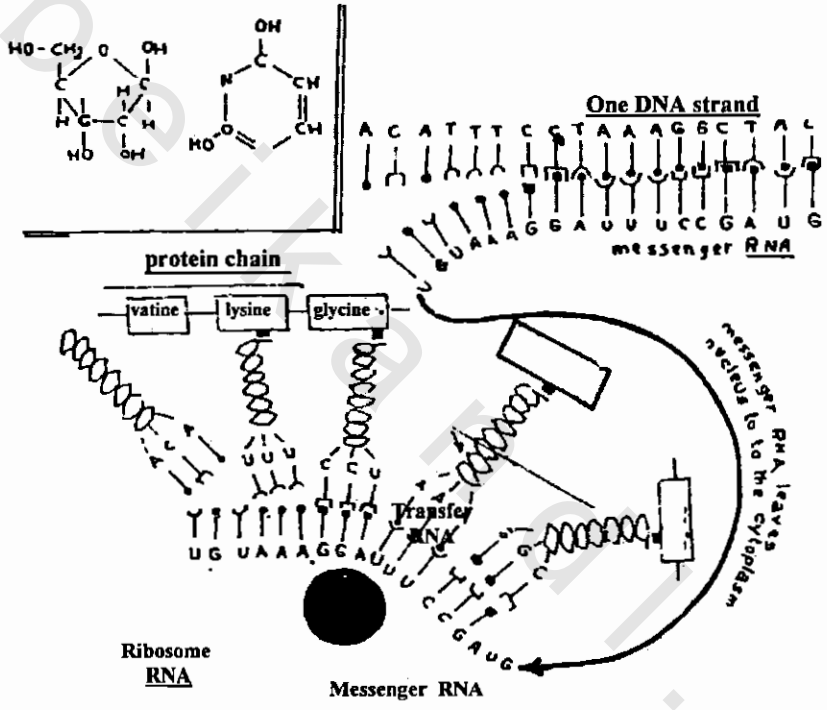


Fig. 120 Diagram showing protein synthesis.

The other variety of RNA, namely the transfer RNA (t RNA), acts to define the types of amino acids selected for protein synthesis. **The RNA** is also formed from DNA in the nucleus and passes out to the sites of protein formation in the cytoplasm, i.e., the ribosomes. Ribosomes are submicroscopic particles found in all cells that synthesize protein from bacteria to cells in the ograns of mammals. They are either attached to the membranes of the endoplasmic reticulum or lie free in the cytoplasm. Their size ranges from 150-200 A^0 in diameter. They contain 40% RNA

which is referred to as rebsosomal RNA (rRNA); the rest being protein. Ribosomal RNA is similarly produced from RNA in the nucleus and then reaches the cytoplasm.

Several types of t RNA exist in the living material, for each of the 20 amino acids found in nature. The different kinds of t RNA act in the same way, i.e., each becomes attached to its respective amino acid which has either come to the cell by the way of the blood stream, or has been synthesize inside the cell. Each type of t RNA identifies its specifice amino acid by a 3-letter word (3 nitorgenous bases at its end) and becomes attached to it. Thus a t RNA amino acid (AA) is produced. There are 20 different 3-letter words suficient to identify the 20 amino acids. The tRNA-AA complex reaches the ribosome where the 3-letter code word of the t RNA fit with 3 corresponding letter words along the molecule of messenger RNA which, in turn, becomes attached to the respective amino acid.

In other words, the whole process can be summarized as follows: A long molecule of m RNA is formed from DNA molecule in the nucleus and moves to the ribosomes in the cytoplasm. There it lies in the midst of a pool of 20 amino acids, each of which carries a distinguishing tabe of t RNA (3- letter word) at its end. The m RNA calls out the 3-letter word of t RNA which is attached to a specific amino acid. This particular t RNA fits the corresponding 3-letter word on the m RNA. For example, a t RNA with the 3-letter word ACA- specific for the amino acid valine-fits the 3-letter word UGU – specific for the amino acid valine- fits the 3-letter word UGU on the messenger RNA; and tRNA with the 3-letter word UUU-specific for the amino acid lysine- fits the 3-letter word AAA on mRNA; etc... (Fig. 120). Thus amino acids become arranged according to the sequence of nitrogenous bases along the mRNA which is originally transcribed from the DNA in the nucleus. After this arrangement is made and the macromolecule of protein is completed, the messenger and transfer RNA are probably broken down. Hence, it is clear that different types of protein molecules can be formed dependent on the number and arrangement of amino acids involved in the process. Worthy of mention that all these processes are initiated and activated by several series of synthesizing enzymes. Homard (1962) declared that the amino acids forming one molecule of haemoglobin can arrange themselves side by side in about 90 seconds.

3 - BASIC PROTEINS

PROTAMINES AND HISTONES

Protamines :

They are simple proteins which are rich in arginene (basic amino acid). They occur mainly in fish spermatozoa. They have a very low molecular weight (of about 4000 daltons).

Histones :

They have a molecular weight of 10,000 to 18,000, and are found in all nuclei of higher plants and animals. They contain a high proportion of lysine and arginine. They also contain histidine and other basic residues.

Histones and protamines are tightly bound to DNA by salt linkages. Several histone fractions have been isolated. Many investigators have expressed the concept that hisone, by its close association with DNA at certain stages of the cell cycle, amy regulate gene activity. The histones also act as a chromosomal "glue" that binds together the genetic units of DNA. Furthermore, histones in nucleoprotein complex can partially protect DNA from radiation damage.

4 - NON-HISTONE PROTEINS

The non-histone proteins a re acidic. In the interphase nuclei, as well as in the chromatin, the amount of acidic proteins is very great. Some of the non-histone proteins may be linked to DNA; others are not linked. Among those which are not linked to DNA are the **residual proteins.** The non-histone proteins are very heterogeneous. They are characteristic for tissues and species. Unlike histones they are synthesised throughout the cell cycle. The synthesis takes place in the cytoplasm, and after that the proteins are caried out into the nucleus.

The nuclear phosphoproteins (an importan component of the acidic proteins) undergo rapid phosphorylation and dephosphorylation and are present in the euchromatin.

It is very probable that acidic proteins play an important rôle in gene regulation.

Nuclear Enzymes :

The cell nucleus is devoid of the essential respiratory enzymes, such as cytochrome oxidase and succinic dehydrogenase. On the other hand, some glycolytic enzymes such as aldolase, endolase and 3- phosphoglyceraldehyde dehydrogenase have been found in the nucleus. These findings suggest that

the cell nucleus has a predominantly anaerobic metabolism using glycolysis as a main source of energy. However, isolated nuclei can synthesize ATP by the aerobic process.

The most important nuclear enzymes are involved in the synthesis of nucleic acids. Thus, DNA polymerase is able to synthesize DNA unsing a primer (i.e., a short chain of DNA) and the triphosphates of the four **deoxyribonueleotides** (Komberg, 1957). RNA polymerase can from messenger RNA using DNA as a template (Stevens, 1961). In the nucleus, there are some enzymes (such as, adenosine diaminase musleoside posphorylase and guanase) which are concerned with nucleoside metabolism. Some special enzymes (e.g., catalase and arginase) appear to be concentrated in certain nuclei but absent in others.

5 - OTHER NUCLEAR COMPONENTS

Nuclear lipids have been studied in isolated nuclei, and it has been found that the lipid content of the nucleus is very low. In cancer cells the amount of nuclear lipids is increased, e.g., the quantity of free cholestrol may be as much as 45 times the normal.

The nucleus contains some minerals, the ash of the nucleus is composed of phosphrous, potassium, sodium, calcium and magnesium.

THE NUCLEOLUS

Structure and Cytochemistry

Under the light microscope the nucleolus generally appears to be structurally homogeneous, although small corpuscles of vacuoles are sometimes observed. The vacuoles may move towards the periphery of the nucleolus forming a clear area. The nucleolus is freqhently attached to the nuclear membrance and some of these vacuoles and material of the dense part of the nucleolus seem to pass into the cytoplasm., After fixation, the nucleolus is Feulgen negative which indicates that it does not contain DNA, but it cotains RNA. The nucleolus may be surrounded by a ring of Foulgen- positive chromatin, which represents heterochromatic regions of one or more chromosomes associated with he nucleolus.

Ultrastructure :

Studies with electron microscope have revealed the existence, within the nucleolus of a definite submicroscopic orgnization. In some cells, the nucleoli have an irregular fibrillar strucure and in some others the structure appears to be compact and relatively homogeneous. In the

majority of the nucleoli, the following components can be distinguished: (a) a granular protein made up of closely aggregated round particles of 150-200 A° in diameter, and often placed at the periphery; (b) a fibrillar portion built up of fibrils (50-80 A in length); (c) an amorphous zone of low electron density; and (d) ncleolus associated chromatin found around the nucleolus.

The nucleolar material has been observed to pass into the cytoplasm of the living cell.

Cycle of the nucleolus during cell division :

Early studies have revealed that the nucleoli seem to disappear at the beginning of cell division (prophase) (and reappear at the end of the division (telophase). The nucleoli are intimately related to the chromosomes. Each nucleolus lies in contact with a chromosome, the point of union is a special region called the nucleolar organizer.

There are at least two chromosomes (nucleolar chromosomes) each of which produces a nucleolus which origiates during the telophase at the nucleolar organizer as a small granule which is Feulgen-negative. Each nucleolus increases in volume, then the corresponding nucleoli fuse into a single mass. They remain united until the beginning of the next prophase where the nucleolus takes up its globular from, When the nuclear membrane starts to disintegrate the nucleolus is liberated from the nucleolar chromosomes and then disappears. The nucleolar zone of the chromosome is important in the organigtion of the proteins and of the RNA which are found in the nucleolus,. According to Chouinard (1969) the only permanent component of the nucleolu is the chromatin loop which coils inside the nucleolar zone of the correstonding chromosome. This loop contains the genetic information for synthesis of the nucleolar material.

Isolation of nucleolus :

The nucleolus has been isolated from oocytes of marine animals. Isolated nucleoli were found to conatin 3 to 5% RNA. The protein content of the nucleolus is high. The naked protein component is phosphoproteins. Histones are lacking. There is a high concentration of **orthophosphate** in the nucleolus, which may serve as a precursor of the RNA phosphorous (Tandler et al., 1962). The enzymes of the nucleolus are the acid phosphatase, nucleoside phosphorylase and DPN synthesizing enzymes.

Nucleolar functions :

A possible relationship of the nucleolus with protein synthesis was first suggested by Caspersson (1939). The following are the postulated sites of origin of the different types of RNA,: mRNA and tRNA are synthesized in the chromosomes; and rRNA is also derived from the chromosomes; and rRNA is also derived from the chromosomes (DNA) but in a special region related to the nucleolus in which numerous copies of rRNA are formed. According to this theory rRNA represents a homologous transcription of a restricted region of DNA which is accumulated in the nucleolus prior to its penetration into cytoplasm. The nucleolar organizer of chromosomes would be the sites primarily devoted to the large scale production of cytoplasmic ribosomes. Thus, the presence of large nucleoli in all rapidly synthesizing cells would be explained on the basis of the large number of copies of rRNA needed for protein synthesis.

CHAPTER 17

CELL DIVISION

In the nineteenth century the division of cells was observed by zoologists and botanists. Cell division includes both the division of the mucleus and the division of the cytoplasm; the former precedes the latter.

Biologists have long distinguished two kinds of division in the somatic cells which are named mitosis and amitosis according to the behaviour of the nucleus.

MITOSIS

Mitosis or karyokinesis is the division of the nucleus to form daughter nculei. It takes place before the division of the cytoplasm. Mitosis (sometimes referred to as indirect nuclear division) is the common and regular method in all the higher animals and plants, It is a continuous dynamic process but for purposes of description and generalization is subdivided into 4 phases or stages which are prophase, metaphase, anaphase and telophase (Figs. 121 & 122). For convenience in descriprion it seems best to insert a stage which can be called prometaphase between prophase and metaphase, and to subdivide each of prophase, anaphase and telophase into two parts.

1. Prophase :

In the majority of interphase nuclei the "fixability" is zero but at the beginning of prophase the chromosomes become "fixable" – thus the first sign of prophase is the appreance within the nucleus of invisible long thread-like chromosomes which are usually coiled and stain lightly with the nuclear dyes. Under the high power of the mictoscope each chromosome is seen to be made up of linear series of particles of different sizes, the chromomeres, connected by a more lightly stained thread. The linear arrangement of the chromomeres in each chromosome is constant; and adjacent chromomeres have a tendency to coalesce auring fixation and thus a single large visible granule may really be clump of several chromommeres.

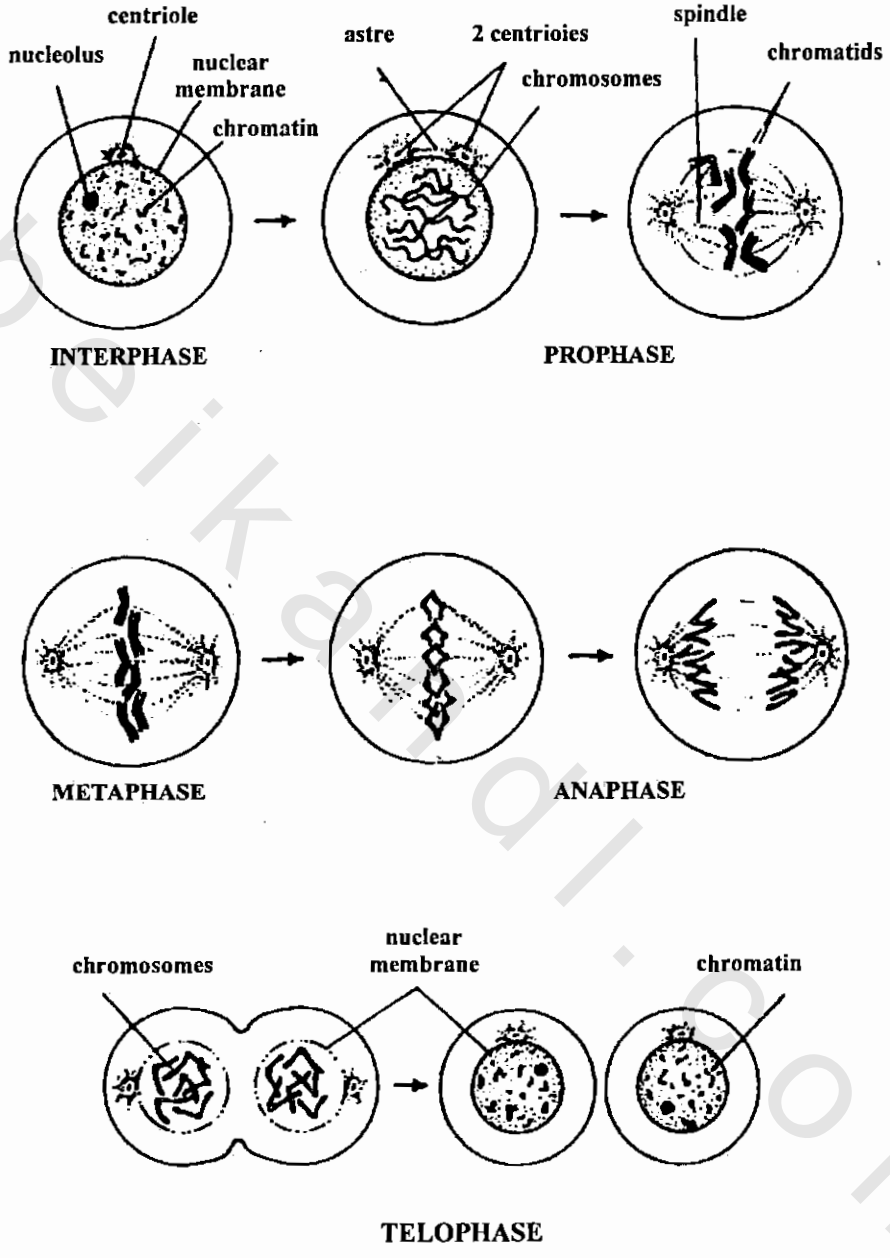


Fig. 121 : Diagram showing the various stages of mitosis.

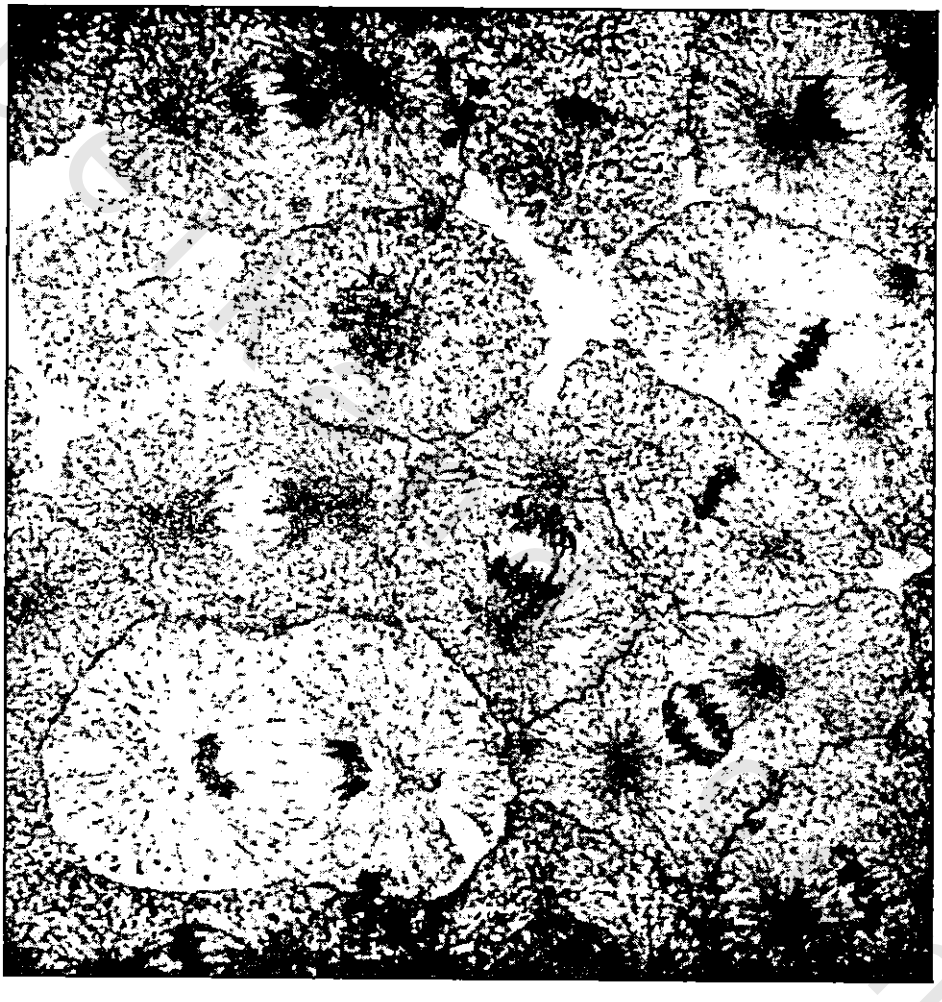


Fig. 122 : Micrograph of animal cells showing meiosis.

As prophase proceeds the chromomsomes increase considerably in volume and at the same time they gradually shorten and thicken. Thus, three processes are involved in the development of prophase: **dehydration, growth** and **condensation or contraction.**

It has been established that each prophase chromosomes is lonigitudinally split. i.e., divided into two halves called **chromatids** (or **daughter chromosomes**). In other words, the chromsosomes are always double from the very beginning of prophase, with the two chromatids of which they are composed closely approximated throughout their length. Each double thread is provided with a single unstainable body known as the centromere. Apparently the **Centromere** remains undivided during prophase and only divides at metaphase.

It should be noted that the chromosomes are always separate at the very earliest prophase; thus there is **no "continuous spireme"** as described by some earlier cytologists.

During the prophase, the **nucleoli** commonly disappear; the disappearance takes place mostly in the late prophase and sometimes in the early prophase.

As regards the **centriole** we find that when the prophase begins the centriole divides into two if it has not already divided. The two centrioles migrate towards the opposite poles of the cell. As they do so, the cytoplasm around them becomes arranged in delicate fibrils known as **astral rays** which radiate around each centriole forming a star-shaped figure known as the **aster.** The rays (these are microtubules of 100-230 A^{o} in diameter) between the centrioles form a spindle which passes across the centre of the cell where the chromosomes are lying irregularly.

Another type of spindle formation has been found. This is referred to as **metaphase** spindle and in which the centrioles are polarized before the beginning of division, and the spinlde is formed at the metaphase. Mitiosis in which the centrioles and asters are found is known as **astral or amphiastral mitosis**, but if the centrioles are absent in it called **anastral mitosis**. The former is common in animals and in some lower plants, whereas the latter is common in higher plants and in certain invertebrates.

At the end of the prophase the nucleolus and the nuclear membrane suddenly disintegrate. The period between the disappearance (or disintegration) of the nuclear membrance and the moment when the spindle is fully formed is called **prometaphase.**

2. Metaphase :

In the metaphase the chromosomes are arranged at the equator of the spindle forming the **equatorial plate** which is chracteristic of metaphase. The chromosomer may either lie within the spiblde of form a ring around the equator. It should be noted that he chromosomes are double and lie with their centromeres in the equatorial plane, so that each chromatid is connected by a half-spindle fibre (called chromosomal fibres) with the opposite pole of the spindle. The chromosomes are connected to the spindle fires by means of the centromeres.

3. Anaphase :

During the anaphase the chromtids, which must be now called chromosomes, separate and each moves towards the corresponding pole of the spindle. In the early anaphase the **centromeres** begin to move apart towards opposite poles while the arms of chromosomes remain in contact. Finally the arms separate as if pulled apart, and in the late anaphase the central region of the spindle elongates so that the two groups of the chromosomes may be carried beyond the original position of the spindle poles. Since the movement of chromosomes begins at the contromere, i.e., at the attachment region so the movement, according to some workers, seems to be due to a force of repulsion which develop in the points of attachment, or, according to other authors, it may be due to the contraction of the spindle fibres (chromosmal fibres) and the elongation of continous fibers (fibers running from one pole to the other).

4. Telophase :

This is the final phase of cell division which begins when each set of chromosomes arrives at its corresponding pole. The chromosomes of each set are reconstituted into a new daughter nucleus, usually by passing through stages more or less comparable with reverse process of prophase. So, in this stage a nuclear membrane and mucleoli reappear, and each mucleus assumes the character of the interphase mucleus. It should be remembered that the nucleoli reappear at the nucleolar organizers (nucleolus-forming regions). The fibres of the spindle amd asters begin to disappear. It is now believed that the chromosomes persist throughout the interphase stage of the nucleus, in other words, the chromosomes maintain their individuality from division to division; meanwhile a constriction appears around the equatorial region of the cell. The constriction deepens until it divides the cell into two daughter cells, each of which is a replica of the original cell except in size. The division of the original cell except

in size. The division of the cytoplasm is known as cytokinesis and it takes place along a plane which passes through the equator of the spindle and at right angles to the spindl's length.

The duration of the mitotic cycle varies from 10 minutes to several hours. This depends upon the species, its physiclogical conditions, as well upon certain external factors.

Comment on mitosis :

As a result of mitosis, each chromosome is divided into two equal and exactly similar halves. Since the chromosome is the carrier of genetic information, then each of the two resulting copies will carry the same genes.

Mitosis is also dependent on the state of the cells, being more active during embryonic development, repair of injury, tumours, etc..

The duration of mitosis is nearly fixed for each cell type. For example, in the cells of fruit fly mitosis takes place in 9 minutes whereas this period 100-200 minutes in the cells of chick.

Body cells of fully mature indiviuals differ form each other as regards their capacity of cell division. In this respect, they are divided into 3 main categories:

1 - Cells which divide continounsly throughout the whole life span of the individual, e.g., Malpighian layer cells of the skin.

2 - Cells which usually stop division at maturity such as the livers cells, but under some conditions (e.g., hepatectomy, liver injury, etc.) they regain their capacity of division.

3 - Cells which appear to have lost completely their capacity of cell division, and this capacity is not regained under any condition, as in case of nerve cells.

AMITOSIS

Amitosis or direct nuclear division is frequent among protozoa but is very rare in the metazoa. Sometimes it occurs in pathological conditions. In such division the nucleus elongates and becomes constricted in the middle and finally the two halves separate and move apart. The division of the nucleus takes place without disappearance of nuclear membrane and without any complicated rearrangement of the chromatin. The cytoplasm then, becomes constricted in its middle and eventually divides between the

two nuclei giving rise to two daughter cells which resemble the original one except in size.

MEIOSIS

The somatic cells of an animal contain a definite number of chromosomes known as the diploid number, while the germ cells of the same animal contain only half the normal number, i.e., the haploid number so that when a spermatozoon and an ovum unite in fertilization the normal number is restored.

The type of cell division by which the somatic or diploid number of chromosomes (say 2 N) is reduced to the haploid number (i.e., N) is known as meiosis or reduction division. Meiosis occurs only in the germ cells, and may be looked upon as two modified mitosis in the course of which the chromosomes only divide once. The two divisions are called the first and second meiotic divisions (Fig. 123); and they are usually separated from one another by a very short interphase. In some organisms the interphase is missing.

First Meiotic Division :

As the prophase of the first meiotic division is long and complicated it is convenient to sub-divide it into several stages which in the order of their occurrence are known as: preleptotene, leptotene=, zygotene pachytene, diplotene and diakinasis. The prophase is then followed by metaphase, anaphase and telophase. Then comes the second meiotic division.

Preleptotene stage :

Preleptotene stage is the earliest part of the prophse of the first meiotic division. It is of short duration and corresponds to the very early prophase of an ordinary mitosis. In this stage the chromosomes are very thin and difficult to be demonstrated.

Leptotene stage :

In this stage the chromosomes become fore visible as slender, long threads, whose number is equal to the somatic number according to some workers the chromosomes are not longitudinally divided, i.e., each chromosome is made up of a single chromatid and not of two chromatids lying very close to each, if this is so, this will be an important difference between the chromosomes of the leptotene stage and those of the early prophase of an ordinary mitosis. According to other authors the chromosomes are longitudinally divided and that the division of the chromosomes into two chromomatids takes place before the loeptotene stage. Another point of difference is that the chromomers are usually more distinct than during mitosis.

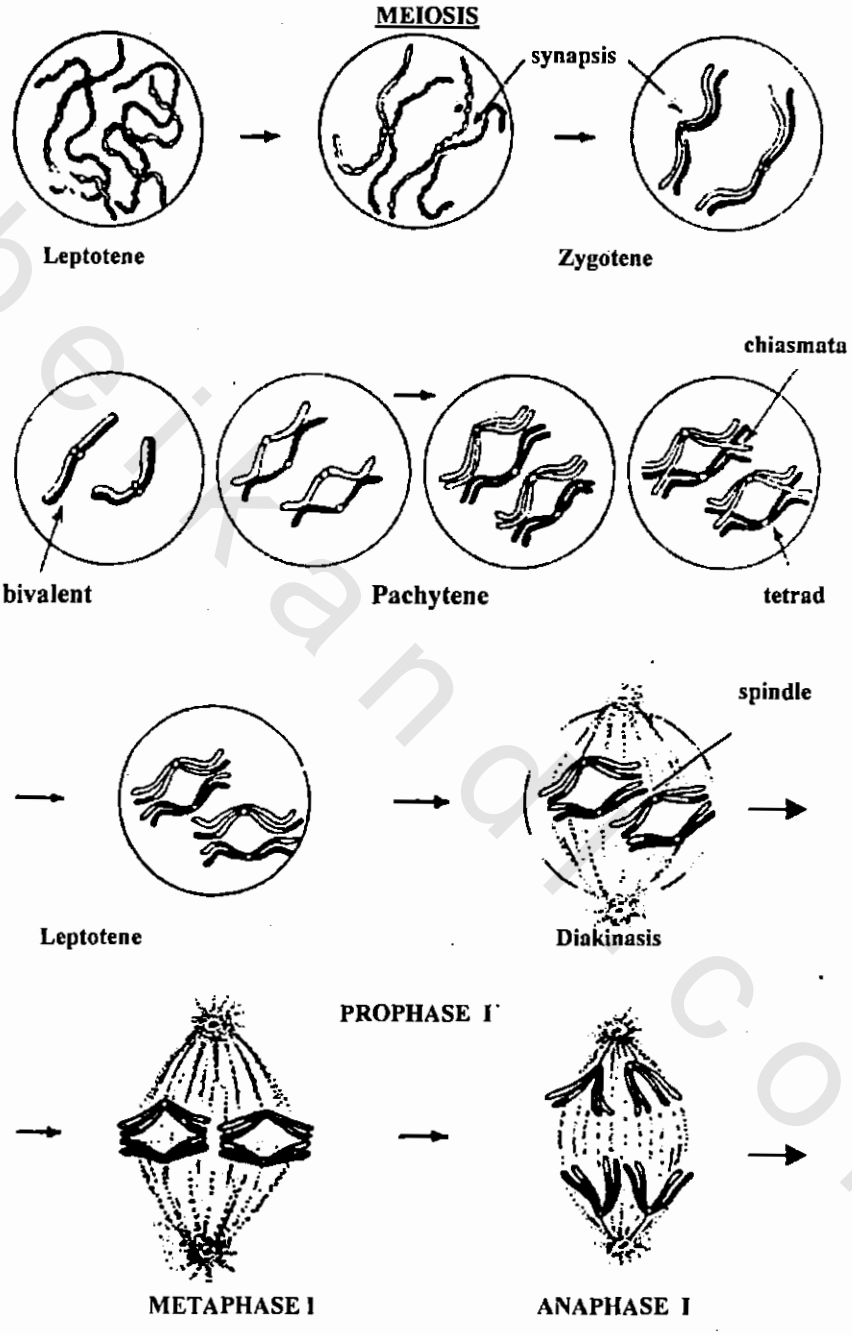
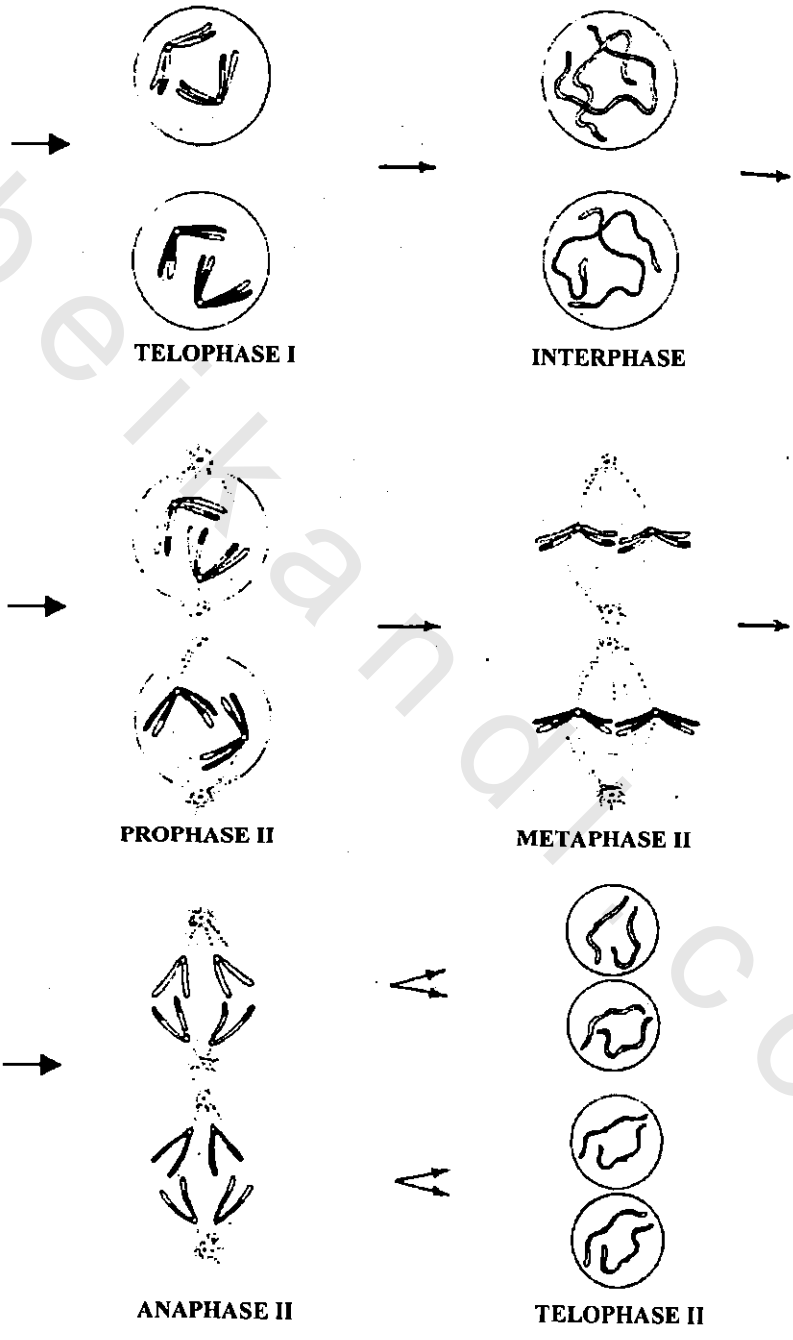


Fig. 123 : Diagram showing the various stages of meiosis.



The chromosomes may lie without any regular orientation in the nucleus, or may be polarized directing one or both ends towards one side, forming what has been celled a "bouquet". In the polarized type the chromosomes preserve the arrangement of the preceding telophase, i.e., with all the centromeres lying at one side of the nucleus and the chromosomes arranged as in a bunch of flowers.

Zygotene Stage :

Leptotene is followed by another stage known as zygotene, during which pairing of the homologous chromosmes takes place (i.e., each two homologous chromosomes become arranged side by side, and thus they tend to be arranged in paris). The zygotene pairing is usually referred to as synapsis although this term is falling into disuse. The arrangement during pairing is variable. When chromosomes are polarized, pairing starts at the centromeres and then extends along the whole length of the chromosomes, but if not pairing may begin at any point. As pairing takes place the chromosomes begin to shorten and thicken.

It should be noted that the pairing is not merely between hololog0us chromosomes, but always between strictly homologous chromomeres, this can be demonstrated in chromosomes in which the chromomers are distinct since the sizes of the chromomers are slightly different. Pairing can be also demonstrated by the behaviour of inverted regions where a part of a chromosome becomes reversed. If we call the chromomers in one homologous chromosome a, b, c, d, e, f,.. and those in the other a', b', c' d' e', f',.. then a will pair with a', and b with b' and so on. If a short region has become inverted in one homologous chromosome but not in the other then the inverted region will remain unpaired and forms a loop in the middle as in figure 124 A, but if a longer portion is inverted the loop will twist round so that the region is completely lost from one chromosome then, the corresponding region in the homologous chromosome will form and unpaired loop as represented in figure 124 C. The process of pairing seems to result from a force of attraction which operates between homologous chromomeres (genes). This attraction is specific and seems to act through considerable distances since some of the pairing chromosomes at zygotene appear at opposite poles during lepototene stage. It is likely that pairing force is identical with the force which keeps the two chromatids of a chromosome together throughout their length in the prophase of mitosis.

Pchytene Stage :

When the pairing of the chromosomes is completed the nucleus is said to be in the pachytene stage. The chromosomes become shorter and thicker, and as a result of pairing the apparent chromosome number is

reduced to half. This reduction as we said is only apparent since each thread is double or bivalent. Each bivalent corresponds to and ordinary mitotic chromosome at mid-prophase, but it should be noted that the bivalent arises by pairing of two entirely distinct chromosomes instead of by splitting of a single one in case of mitosis. Another important difference is that pachytene bivalent has two centromeres lying very close together, whereas the mitotic prophase chromosome possesses only one.

About the middle of the pachytene stage a longitudinal splitting in each homologue takes place in plane perpendicular to that of the pairing. The result is that each bivalcnt chromosome is composed of 4 chromatids, and hence was formerly called a tetrad. The chromatids of each homologue are referred to as the daughter chromatids.

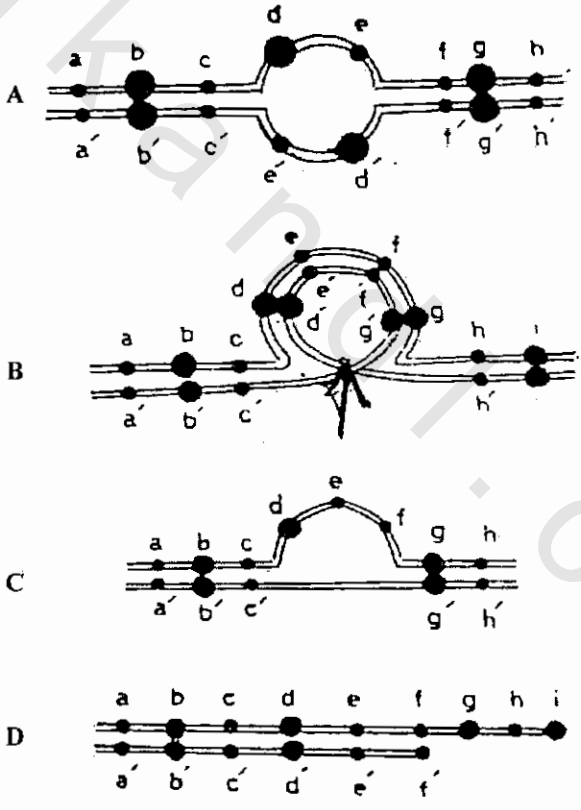


Fig. 124 :

Chromosome pairing in which one of the pairing homololgues has an inverted (A and B) or deleted (C and D) segment. C, an intercalary deletion; D, a terminal deletion. In C and D three chromomeres are lacking.

Pachytene may be subdivided into a two strand stage (before the chromosomes have split) and a 4-strand stage (after they have split). At the beginning of pachytene the two threads lie parallel to one another but they soon begin to wind round one another, thus when splitting occurs it results in 4 threads two of which are wound round the other two.

When longitudinal splitting of each chromosome takes place transverse breaks may occur at the same level in the two internal homologous chromatids. Then, both segments of the chromatids interchange and fuse together with those of the homologues. The interchange does not take place between daughter chromatids, but only between homologous chromatids. Therefore, two chromatids remain intact while portions of the other two exchange by crossing over (Fig. 124 D).

Diplotene Stage :

As soon as the homologous chromosomes have split longitudinally they start to repel each other and therefore to separate. In other words, the attraction force between homologous chromosomes before they have split becomes converted into a repulsion after splitting. The moment at which the two homologous chromosomes start to separate marks the transition from pachytene to diplotene. The separation is not complete, since the homologues remain held together at certain points known as chiasmata. These are the points of exchange between the partner chromatids and they are considered as the physical basis of genetical crossing-over. Chiasmata are present in all animals and plants except in very few cases. At least one chaisma is found in each bivalent and there may be several. Chiasmata are generally interstitial, that is to say, they are formed in between the ends of the chromosomes. The chiasmata become reduced gradually being displaced outwards along the length of the chromosome. This displacement is called **terminalization.**

Diakinesis :

This stage corresponds to the late prophase of mitosis. It is characterised by the contraction of chromosomes and continuation of terminalization until the chiasmata disappear completely. The transition form diplotene to diakinesis takes place gradually. In diakinesis the bivalents continue to contract by spiralization. Special techniques reveal that each chromatid forms a double spiral in which each gyre of the major spiral consists of

several gyres of the minor spiral. Because of the thickening of the chromatids the split between them, which was clear during the middle and final phases of the diplotene stage, becomes more difficult to see.

The **nucleoli** behave much as in mitosis. Rotation is usually completed by the beginning of diakinesis but the process of terminalization may continue until the metaphase of the first meiotic division; and the number of interstitial chiasmata decreases. When terminalization is completed, the members of the pair of homologues remain in contract only at their distal extremities where the centromeres are located.

Prometaphase :

As in mitosis, the period between the disappearance of the muclear membrane and the moment when the spindle is fully formed is called prometaphase. During this stage spiralization reaches its maximum and the bivalents become arranged at the equator of the spindle to begin the metaphase.

Metaphase 1:

This differs from the metaphase of somatic mitosis in the fact that each bivalent has two independent centromeres which do not divide as in mitosis. The centromeres lie at equal distances above and below the equatorial plane. It should be remembered that at mitosis, on the other hand, each chromosome has a single centromere and so all the centromees lie in the equatorial plane.

Anaphase 1 :

Due to a force of repulsion, each centromere travels towards the nearest pole of the spindle, dragging after it the attached chromatids. As they do so the chiasmata which have not already been terminalised move along to the ends of the bivalent. In the late anaphase the central region of the spindle elongates and completes the separation of each bivalent into 2 dyads (1/2 bivalent).

Telophase 1 :

When each group of the anaphasic chromosomes reaches the respective pole the telophase begins. This is similar to that of an ordinary somatic mitosis except that each group of chromosomes may remain in condensed state, in the case the two daughter chromatids diverge from one another so

that an acrocentrie chromosome looks like X. The firsy meiotic division results in the formation of secondary spermatocytes in the ,ale and secondary oocytes in the female.

Following the the telophase there is a short period called **interkinesis or interphase**.

The chromosomes may become unfixable and pass into the condition of the interphase nucleus or they may persist in a condensed state and undergo no changes between the anaphase of the first division and the metaphase of the second.

Second meiotic division :

This process takes place in each of the above resulting nuclei. It also includes the following stages:

Prophase II :

Each centriole divides into two, and these travel to the opposite poles of the cell. The spindle is formed, the nuclear membrance disappears, and the chromosomes become associated with the spindle fibres. Each chromosome is still formed of two chromatids.

Metaphase II :

Chromosome become arranged on the equator of the spindle, each chromosome is now a dyad consisting of two chromatids held together at the centromere.

Anaphase II :

The centromere uniting each two chromatids divides, and thus the daughter chromatids separate from each other. They travel away towards the corresponding poles, and each being now an independent chromosome.

Telophase II :

In each set the chromosomes become grouped together near the corresponding pole of the spindle. They become thinned and elongated. A nuclear membrane is formed round each group of chromosomes, and thus two nuclei are produced, each containing the haploid number of chromosomes.

Comment on meiosis :

Meiosis consists of two successive divisions. In the first division half of the chromosome pass into each daughter nucleus. In the second divisions each chromosome divides into two chromatids. At the end four moclei are produced from the original cell, each contains the haploid (N) number of chromosomes. Thus, four gametes are formed, each containing N. chromosomes.

When two gametes fuse together the zygote contains 2 N. If reduction division does not take place, then the chromosomes are duplicated at each fusion.

In addition, by the process of crossing-over exchange of genes takes place, and thus the zygote formed receives a variable supply of genetic factors from both the male the female parents.

CHAPTER 18

CHROMOSOMES AND GENETICS "CTYOGENEYICS"

Genetics is the science of heredity. Heredity means the transmission of anatomical, physiological and mental characteristics from one generation of the next (excluding transmission through tradition and education).

The hereditary material consists of minute structures known as genes which are arranged in liniar series in the chromosomes. The gene is a hereditary factor which determines the development of a particular character.

Cytogenetics is based on the inter-relations between cytology and genetics. This is clear from the fact that chromosomes are the carriers of genetic factors. Any change which takes place in the chromosomes is reflected in the genetic constitution of the resulting offspring.

Cytogenetics is mainly concerned with the cytological and molecular bases of heredity, variation, mutation and evolution of organisms. In this chapter we are going to deal with these aspects except the molecular which has been dealt with in another chapter.

Mendelian Heredity

We owe to Gregor Mendel (1865) the discovery of two of the fundamental lwas of heredity on which the modern theory of heredity is based.

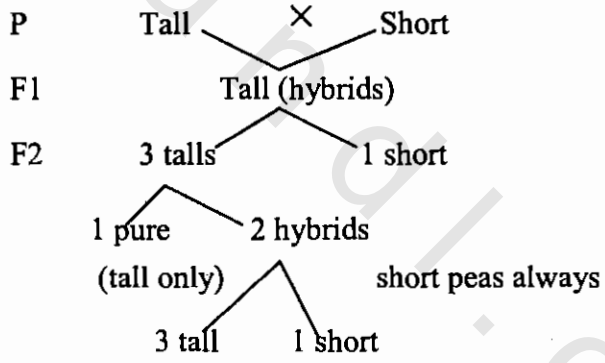
The rediscovery in 1900 of Mendel's work on heredity and the recognition that the history of the chromosomes during gametogensis and fertilization gives a cytological explanation of his works opened up new field in nuclear cytology and led to the establishment of the science of cytogentics.

The result of Mendel's experiments with garden peas was published in 1866 in a small local natural hisoty journal where it was securly buried for 34 years until rediscovered independently by Correns. Tschermak and De Vires 16 years after Mendel was dead. Mendel obtained his results by observing single characters instead of looking to the whole appearance; and his work showed that the characters concerned were tansmitted from the parents to the offspring according to certain laws. Sutten in 1902 and

1903, and De Vries in 1903 were the first to give a clear and ad equate cytological explanation of Mendel's work.

Mendel's First Law
"The Law of Segregation of Genes"

Mendel found that in a tall variety of pea is crossed with a short variety the offspring (hybrids) of the first generation (F1) are all tall. If these are allowed to self-fertilized their offering (second filial generation, or F2) are tall and short in the ration of 3 talls to 1 short. The short peas of the second filial generation when self fertilized give short individuals only, and so they are pure for the character of shortness. When the tall pears of the F2 generation were allowed to self-fertilized, one-third produced tall individuals only, and thus is pure for the character of tallness, while the other two-thirds produced tall and short peas in the ration of 3:1, and therefore carry the factors for both tallness and shortness.

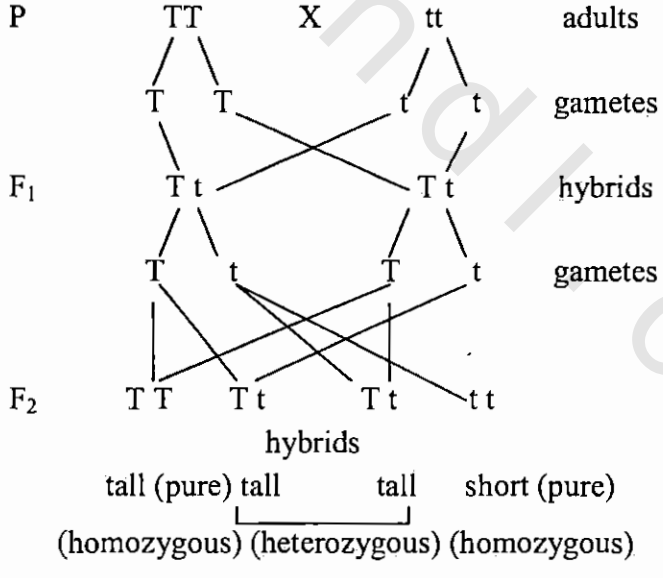


The above results can be explained by assuming that the tall variety contains in its germ-cells a factor for tallness (something that makes the plants tall; this hereditary factore is called the gene) and that the short variety carries a factor (gene) for shortness. The individuals of the F1 generation contain both genes; the gene for tallness is contributed by one parent and the gene for shortness by the other parent; and since the F1 is tall it is evident that the gene of tallness is **dominant** and that of shortness is **recessive**; and when both genes are brought together the domainant prevents the expression of the recessive.

It is usual to denote a dominant factor on gene in any cross between alleles by a capital letter and its recessive allele by the corresponding small letter; thus in our case T stands for tall and t for short.

The 3: 1 ratio that appears in the second (F2) generation can be explained as follows: the young germ-cells of the F1 generation ceontain a gene for tallness and a gene for shortness, and during meiotic division the two genes separate, so half the eggs will contain the gene for tallness and half the gene for shortness. The same is true for th pollen grains. The Chance of fertilization of any egg by any pollen grain will give on the average 3 talls to 1 short; this as regards the phenotypes (i.e., outward appearance). But as regards the genotypes (i.e.,. genetic constitution) there are 3 kinds of offspring: 25% contain genes for tallness, 25% contain 2 genes for shortness and are pure for tallness and shortness respectively; 50% contain a gene for tallness and another for shortness as in the F1 generation.

This inheritance with dominance can be illustrated in the following diagram[1]:



(1) Genes occur in pairs known as allelomorphs (alleles) whose members are derived from the father and the other from the mother. Alleles occupy corresponding loci on the chromosomes which are homologous. The two genes composing an allelomorphic pair may be similar (i.e. homologous) or dissimilar (i.e., hetorozygous)

So, as **phenotypes** are concerned we have two kinds: 3 talls : 1 short:

; but as genotypes are concerned we have three kinds :

1 1 1
-- TT : -- Tt : -- tt.
4 2 4

Therefore, we can say that if hybrids in any generation resemble each other in all somatic characters, they are **phenotypes**. In other words, the sum of the characters exhibited by an individual is called its phenotype. The term is also applied to a group of individuals all of their characters are alike.

The total gene-content of an organism is called its **genotype.** Genotype, therefore, is the genetic constitution of an individual. The term is also applied to a group of individuals which are identical in their genetic constitution.

A factor (or gene) that is expressed when another factor (or gene) is concealed is called the dominant. The concealed factor (gene) is the recessive. It must be doubled to be expressed.

Breeding experiments which deal with single or unit characters in animals yield similar results. For example, if a black guinea pig is mated to a white one, all the offsprings are black. Black is, therefore, dominant over the white. In the F2 generations the guinea pigs occur in the ratio of 3 blacks to 1 white.

The same relation can be illustrated by the inheritance of eye colour in man. If a blue mates with a pure brown the children are brown. If two of the these children marry, their children (F2) will be brown and blue-eyed in the ratio of 3 : 1.

Mendel's Second Law

"The Law of Independent Assortment"

Mendel's first law deals with the results of a mononhybrid cross. According to this law the F1 of a monohybrid cross is genetically uniform, but in the next generation the two unit characters (genes) segregate in difinite numerical proportions depending on the type of cross. The cytological basis of Mendel's first law is the separation, at meiosis of the two members of a pair of homologous chromosomes into different gametes.

Mendel's second law deals with the results of dihybrid or polyhybrid crosses, i.e., crosses between individuals which differ genetically in respect of two or more pairs of allelomorphs. It states that each pair of allelomorphs segregates as though none of the others were present, so that the segregation ratio in respects of all character painrs concerned can be calculated by combination of the individual segregation ratios. The cytological basis for this law is provided by the fact that at the reduction division, when the members of each pair of chromosomes separate and shared by different gametes, the movements of any chromosome pair are independent of those of all the others. The principle of independent assortment thus applies only to genes which are situated on different pairs of chromosomes. These genes are said to recombine freely with one another.

Black coat in the mouse is dominant over albino, and straight hair is dominant over wavy. When an albino wavy mouse is crossed to a mouse-form a strain which is homozygous; for black and straight hair, the F1 is heterozygous for both genes and phenotypically black with straight hair.

Let the gene for black hair be represented by B and that for albino by b; and the gene for straight hair by S and for wavy by s. Therefore, F_1 would be represented by BBSS (for black straight hair) and bbss (for albino wavy hair). The former parent produces gametes all of which carry BS; and the latter produces gametes all of which carry bs; and the resulting F_1 hybrid offspring arising from a union of two of these gametes will have the genotype BbSs.

At gamete formation in an F_1 individual the chromosome carrying B separates from the one carrying b, and the chromosome carrying S from; that carrying s. The movements of these two chromosome pairs are independent of each other, so that four type of gametes are produced in equal numbers in respect of these two pairs of alleles. These gametes are BS, Bs,bS,bs.

When F_1 individuals mated together, each of the four kinds of sperms produced by the males has equal chance of fertilizing each of the four kinds of eggs produced by the females. Thus 16 equally possible combinations are obtained. i.e., 16 possible types of fertilized eggs are produced.

The simplest way to determine the expected combinations produced by random union among gametes is to arrange gametes from the 2 sexes on 2 sides of a checkerboard[1] as follows:

The segregation ratios of genotypes and phenotypes can be read from this scheme. They will always appear in the same squares of the checkerboard, and can be referred to them quickly if the squares in each row are numbered from left to right. For the phenotypes the ratio is:

9 black straight (showing both dominate): 3 black wavy (showing the first dominant and the second recessive): 3 albino straight (showing the second dominant and the first recessive): 1 albino wavy (showing both recessives).

The same ratio is obtained much more easily by multiplying the two segregation ratios for the two pairs of unit characters, thus:

Bb X Bb gives 3 black: 1 albino

Ss X Ss gives 3 straight: 1 wavy

BbSs X BbSs gives (3 black + albino) (3 straight + wavy)

= 9 black straight + 3 black wavy + 3 albino straight + 1 albino wavy.

Linkage

Genes which are situated on the same chromosome are called linked genes. Mendel's second law does not apply to linked genes. Linkage occurs when genes located on the same chromosome remain linked together in passing from one generation to another. It is natural that if two genes in one chromosome enter across together from one parent they stay together in the offspring, and if they enter from separate parents they remain swparate in the offspring.

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(1) It is usual to present croses in the form of a checkeroad, with the factors of genes of the male gamete written above the top squares and those of female by the side squares. The latter, within each square represent the combination of one male and one female gamete to form a zygote. The number of squares in the checkerboard represents the number of possible reults. The letter combination in any squar can be interpreted to find the phenotypes and genotypes of the individuals.

P BBSS × bbss

black straight albino wavy

BS BS gametes bs bs

BbSa

F_1 black straight

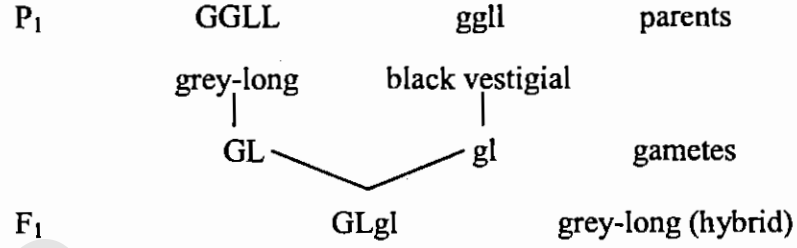
BbSs × BbSs

♂ gametes	BS	Bs	bS	bs
♀ gametes BS	BBSS Blck straight	BBSs black straight	BbSS black straight	BbSs black straight
Bs F2	BBSs black straight	BBss black wavy	BbSs black straight	Bbss black wavy
bS	BbSS black straight	BbSs black straight	bbSS albino straight	bbSs albino straight
bs	BbSs black straight	Bbss black Wavy	bbSs albino straight	bbss albino Wavy

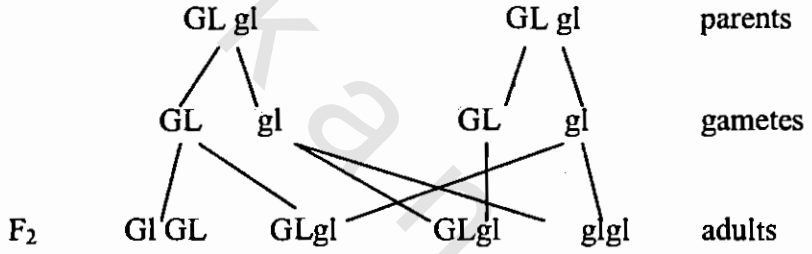
There are various degress of linkage ranging from complete linkage, where genes keeps always together in the same chromosome in successive generation, to the other extreme in which genes show only a very slight tendency of holding together than of assorting freely.

The following example shows a typical complete linkage in Drosophila. If a wild-type fly with grey body (G) and long wings (L) is crosed with a fly showing the two recessive mutations of black body (g) and vestigial

wings wings (1), the F_1 offispring will be all long-winged, grey-bodied; i.e., phenotypically like the wild-type parent.

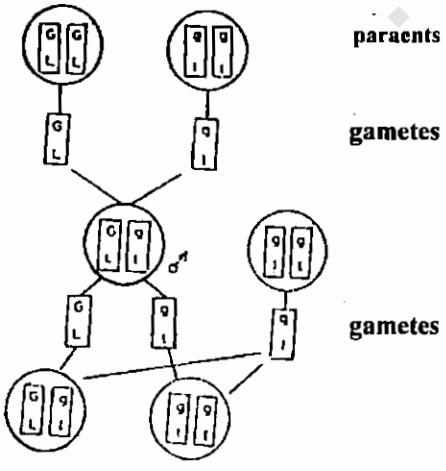


In the F_1 generation there is not the usual free assortment of the characters, but long-winged, grey-bodied and vestigial-winged, black-bodied flies appear in the ratio of 3:1 according to the following:



This shows that long-winged and grey-bodied are linked together, the same applying to the vestigial-winged and black-bodied.

This typical case of complete linkage can be worked out on the chromosome theory as shown in the following diagrm:.



The phenomenon of inkage is common among both animals and plants and its discovery has led to further advances in our knowledge of the mechanism of inheritance. By a long series of genetical experiments it was possible to arrange the allelomorphic characters of Drosophila into groups in accordance with their linkage relationships. Such groups are called linkage groups and it was found that the number of such groups was the same as the haploid number of the chromosomes. This fact gives additional support to the view that it is the **chromosomes which carry the genes.**

Crossing-over

Linkage is very rarely complete. In both sexes of animals and plants there occurs a stage, immediately preceding meiosis, when the two members of each pair of chromosomes come in close contact with one another and by a process of breakage and reunion exchange segements. This process is called crossing-over, because it enables mutated genes to cross over from their member of the chromosome pair to the opposite member and there to become recombined with a different set of genes.

Evidence shows that crossing-over is not haphazard, but gives definite numerical results as seen from the following examples:

When grey female Drosophila with long wings is crossed to a recessive black-vestigial male, the offspring in F_1 are all grey body with long wings.

If one of the F_1 females, i.e., grey-long dihybrid fly, is back-corssed to a male showing the two recessive characters, there are produced four kinds of offspring, namely grey-long and black-vestigial like the grandparents, and two new combinations, grey-vestigial and black-long. The two latter classes are spoken of as the cross-over classes or shortly crossovers. In this experiment there 83 per cent (41.5 + 41.5) of instances that represent non-crossovers, and 17 percent (8.5 + 8.5) of crossovers, or new combinations. The percentage of crossovers is definite for a given stock of given age and under given environmental conditions.

It should be also noticed that independent assortment involves whole chromosomes, but crossing-over has to do with **parts of chromosomes only** (Fig. 125).

The above mentioned example can be shown diagrammatically as follows:

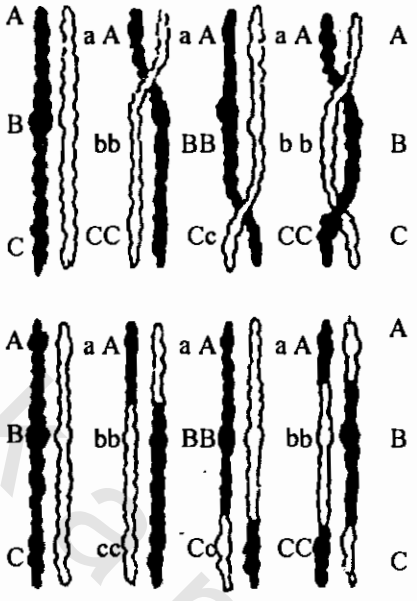


Fig. 125 :

Diagram showing the mechanism (upper row) and the results (lower row) of crossing-over in a pair of chromatids. I, no crossing over, 2 crossing over between A and B; 3 crossing over between A and B and between B and C. The lower row shows the resulting chromatids which pass into the gametes.

It is clear from this scheme that in two chromosomes the two characters grey and long that entered a dihybrid cross together from one parent came out together, i.e., stay linked together. The same applying to the other characters black and vestigial. But in the other two chromosomes crossing over had taken place. The gene for black goes over into the other chromosome and the gene for grey goes over into the chromosome that gave up its black gene.

CHIASMATA :

It has been already mentioned that, during the liptotene stage, the homologous chromosomes tend to separate but remain united together at few points where two out of the four chromatids cross-over from one chromosome to the other forming an X; this is known as **Chiasmata** (Fig. 126).

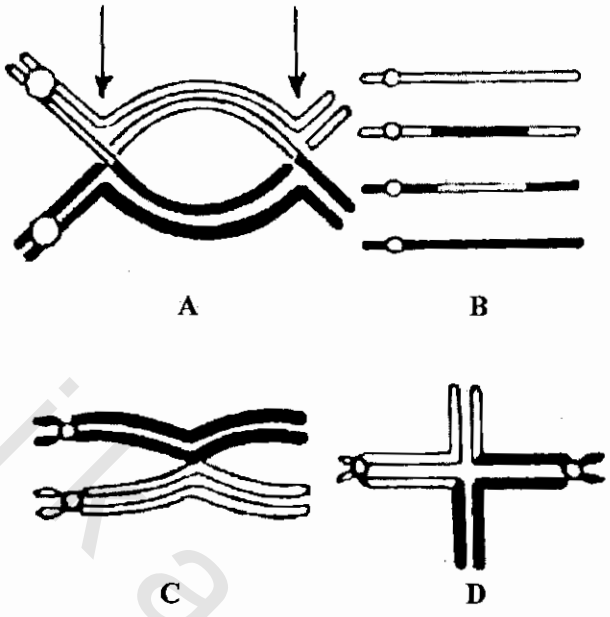


Fig. 126 :

Diagram of the relationship between teo successive chiasmata (A) and the products of the double exchange (B) in the same bivalent. The arrows point to the two chiasmata. C, an equal bivalen with a single chiasmata. D, the same bivalent after rotation.

In other words, we can say that a **Chiasma** is formed by 2 of the 4 chromatids of a bivalent breaking at the same level at the end of the pachytene stage and then joining diagonally so that there is an exchange of parts (crossing-over) between two chromatids one of which is paternal and the other is maternal. Each chiasma is, therefore, a visible sign that a single genetical cross-over has occurred.

·With very few exceptions, the chaismata are universal feature of all plant and animals; and usually they are interstitial (i.e., they occur between the ends of the chromosomes).

Due to the repulsion force which operates between the homologous chromosomes of a bivalent during the diplotene stage they form loops between the chiasmata and therefore the diplotene stage usually has a characteristic appearance. A bivalent with a single chiasma about half-way along its length forms a four armed structure while a bivalent with several chiasmata appears as a series of loops with an incomplete half loop at each end. In bivalents with single chiasma two of the arms rotate through an

angle of 180° (relative to the other two arms); the result is that a bivalent with a single chiasma which looks like figure 126 C at early diplotene comes to look like figure 126 D at late diplotene. In case of bivalents with many chiasmata the rotation is usually through an angle 90°, so that successive loops between the chiasmata come to lie in planes at right angles to one another.

It is clear that the chromosomes which separate at the anaphase of the first meiotic division are not identical with those which paired during the zygotene stage as a result of the break which takes place during the pachytere stage, at the same level in 2 of the 4 chromatids of a bivalent, an interchange of parts occurs between the paternal and maternal chromosomes. The new chromatids, thus, interchange will be mixed.

It is probable that the break in a chromatid is due to a localized strain set up in the spirally twisted chromosomes. As the the result of the break the strain is reduced in the adjacent region of the chromosome and consequently crossing over does not take place for some distance on either side of a chiasma. This is known as **interference**.

The number of chiasmata is variable. There is at least one chiasma in each bivalent and there may be as many as 13. The average number of chiasmata which are found in a bivalent or in all the bivalents of a nucleus is spoken of as the **Chiasma frequency**. If on the average there is one chiasma in a bivalent, then this bivalent is said to have a chiasma frequency of 1.0. In some species crossing over is restricted to certain regions, but frequently it may take place in any regions of the chromosome.

Terminalization :

One of the changes taking place in the bivalents consists of an actual moving of the chiasmata towards the ends of the chromosomes; the movement may be slight or all the chiasmata may be shifted to the exterme ends of the bivalent. In the latter case the 4 chromatids remain in contact at their ends. It should be noted that the visible chiasma moves, but the points where the paternal and maternal portions of the chromatid have fused together (the point of genetical crossing over) do not move.

Terminalization (Fig. 127a) is due to the repulsion force which is obviously greater inside a closed loop than in terminal half-loop; it may continue into the **metaphase**.

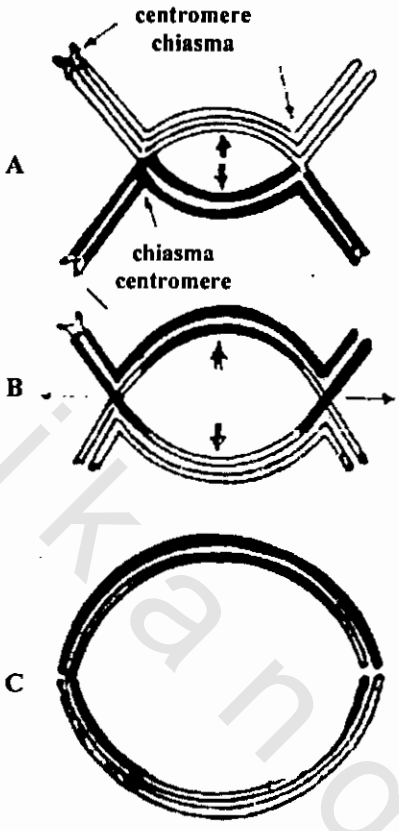


Fig. 127 :

Diagrams to show terminalization of two chiasmata in a bivalent with terminal spindle attachments (centromeres). A, the bivalent at early diplotene when the chiasmata correspond in position to the points of crossing-over, B, at late diplotene showing partial terminalization; C, at diakinesis when terminalization is completed. The thick arrows represent the force of repulsion inside the loop, the thin arrows the direction in which the chiasmata are moving (After white).

Structural rearrangements of the Chromosomes

Some rearrangements such as inversion and deletion have already been mentioned during the study of meiosis.

Sometimes a part of a chromosome is transferred to another chromosome which may not be homologous. This process is known as translocation (Fig. 127b). Translocation may or may not involve a reciprocal exchange of parts. One of the resulting chromosomes may be without a centromere, while the other possesses two; consequently they do not behave normally during

mitosis. Reciprocal translocations are present in nature in some animals and plants.

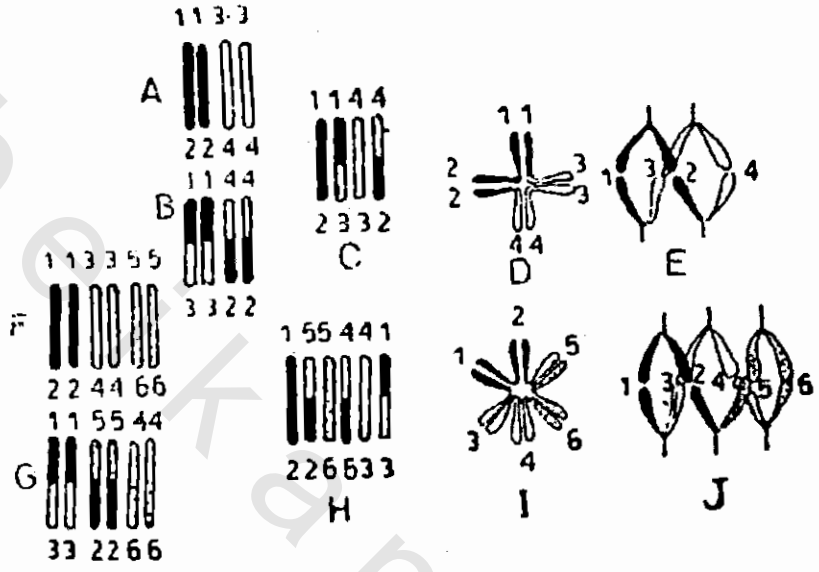


Fig. 127 : Translocation between tow (B-E) and between three (C-3).

We should distinguish between translocation and crossing-over. In translocation a fragment of one chromosome becomes attached to a non-homologous chromosome which remain unbroken; but in crossing-over there is a reciprocal exchange of homologous parts of homologous chromosomes.

When a piece of chromosome breaks off and becomes stuck on to its chromosome, or when a part of a chromosome for some reason is dropped out there is left behind a deficient chromosome which has lost the whole block of genes that was located in the missing part.

This loss of a part of chromosome due to the chromosome breaking at one or two points is known as deletion. Deletions, as well as inversions, are usually intercalary. On the other hand, the occurrence of a portion or portions of a chromosome more than once is spoken of as **duplication**.

These portions may be situated next to one another or may be separate; they may be inverted or occupy their normal positions in the chromosome.

Changes in Chromosome Numbers

These include the following cases:

Haploidy: Some plants and animals have a monoploid set of chromosomes, i.e., a complete haploid set of genes. This means that the homolgous chromosomes and genes are absent. Examples of this case are seen in insects. e.g., drones (males) of ants, wasps and bees.

Polyploidy : This means the presence of more than two haploid sets of chromosomes. Such cases may be triploids, tetraploids, pentaploids, and so forth, Cells showing reduplicated chromosomes are frequently met with in certain pathological conditions and can be brought about by certain subtances as colchicine, and by the effect of heat or cold. Such agents act to prevent the formation of the spindle and thus cell division does not come to its end. After certain time, cells start the process again, but now they possess the double number of chromosomes.

Aneuploidy : This case is seen when one or more chromosomes reduplicate and the organism is said to be polysomic. This is caused by the failure of the chromosomes to separate during meiosis. One of the chromosomes together with its homologue pass to the same pole and are contained in the same gamete. This phenomenon is also called non-disjunctions. When such gamete unites with a normal gamete, an individual called trisomic individual is produced. This produces certain genetic characters as mangolism in humans.

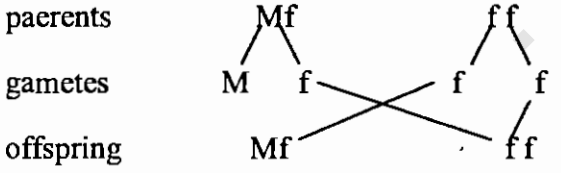
Alloploidy : This type of chromosome variation is produced in crosses between two species that have different sets of chromosomes. The resulting hybrid has a different number of chromosomes than the parents.

CHAPTER 19

CHROMOSOMES AND SEX DETERMINATION

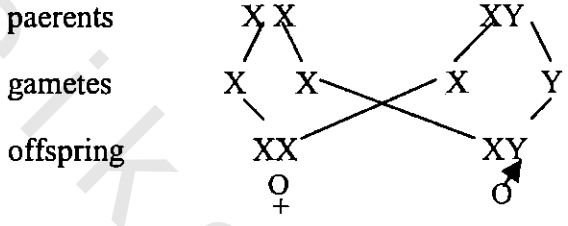
While the mechaism of sex determination is fairly well understood, no way of cotrollingy the sex of offspring is at present known. Various hypotheses, mainly of historical interest, have been put forward. Dawson thought that the left and right ovaries gives eggs alternately male and female; but this is wrong, since a woman or animal with one ovary removed can produce offspring of either sex from the one that remains. Another theory is that of Schenck; that by feeding the mother on a special diet the sex of the foetus could be influenced. We know, how, however, that the young male or female foetus cannot be turned into the opposite sex by feeding. Its sex was established at the time of ferutilization. Genetic studies indicated that maleness and femaleness, are genetic characters which are transmitted from parents to offspring in the same way as the other hereditary characters. In other words, sexual genes occur in the chromosomes, and thus qualitative and quantitative differences between the sexes are found.

It was Correns (1907) who showed, for the first time, that sex may be determined according to the law of Mendelian segregation. He was studying the plant Bryonia in which the male is heterozygous for sex and the female is a homozygous recessive.



Modern research has shown that, except in birds and butter flies, the male is heterogametic - meaning that the male produces gametes of two sorts: male - producing and female - producing: the eggs all being homogametic (of one sort as regards sex - determinatin). Thus the problem of controlling sex – determination would be solved if we could separate and use the male-producing and the female – producing sperms.

In all animals, there are autosomes, or ordinary chromosomes, as well as sex chromosomes. There are 2 sex chromosomes in man, an X and a Y, the former is large than the latter. In the second maturation division the X and Y are separated and divides between the daughter cells, so that half of the sperms have 22 autosomes and an X, and the other half 22 and a Y. The ripe eggs all have an X chromosome. If the sperm with the fertilizes an egg the developing organism will be female but if the sperm with a Y fertilizes an egg, the offispring will be a male. This is called the XY – XX type of sex determination.



From the fact that the human sperms are divided into two equal groups of an X and a Y chromosomes, one could assume that the sexes would be equal in number in a population, at an-early age at least. The sex ratio is usually expressed in number of males to 100 females.

TYPES OF SEX DETERMINATION

There are the following main types of sex determination:

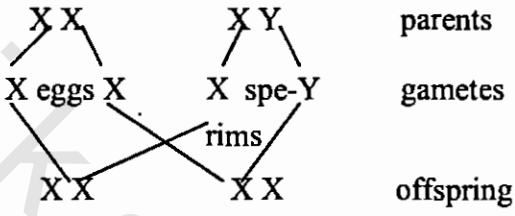
(1) Sex determination by the genic equilibrium between the X chromosomes and the autosomes (sex determiantion in Drosophila or "Bridges Balance Theory of Sex") :

In Drosophila, the female has the sex-chromsome constitution which is symbolized as XX. The male is YY.

One might suspect that the Y chromosome contains the male determiners and that any animal possessing the Y becomes a male. But it has been found by Bridges (1913-16) that sex chromosomes in Drosophila are not always segregated regularly since ova containing XX instead of an X are found. When an ovum of this type is fertilized by a Y-bearing spermatozoon, an **exceptional female** XXY is produced.

X X
FEMALE

X Y
MALE

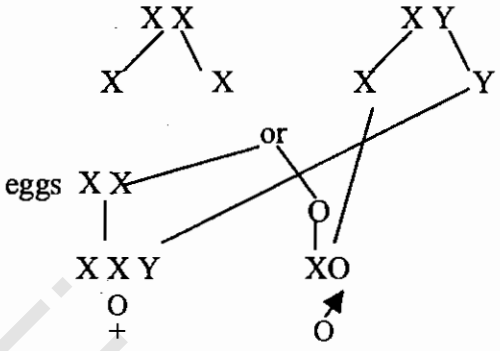


Individuals of the constitution XO can be obtained if an ovum lacking the X chromosomes is fertilized by a spermatozoon bearing the X chromosome, such individuals are **sterile males** though they have complete normal morphology. It is natural therefore, to conclude that the Y chromosome in Drosophila has nothing to do with sex determination though it is necessary for male fertility.

In considering the XY - XX type it has been stated that femaleness is brought about by the double dose of X, and maleness by the single dose of X. There is some truth in this conclusion, but the problem of sex determination become further complicated by the finding of individuals with an intermediate sexuality as well as supersexed individuals.

If X represents an X-chromosome, and A represents one haploid set of autosomes, therefore a female Drosophila will be XYAA and the male X AA. But since the Y chromosome is inert the male can be considered as XAA. If we suppose that the factors of feamleness (F) are located in the X chromosomes, the factors of maleness (M) would be located in the autosomes. This means that sex depends upon several genes which must be distributed among the sex chromosomes and the autosomes. And that sexuality is conditioned by the ratio between the number of X chrononosomes and that of the autosomes. Bridges has been able to show that a triploid fermale Drosophila (XXXAAA) crossed with a normal male

(XAA) gives rsie to four types of eggs, which upon fertilization with the two types of sperms produce the following:



Eggs	Sperms	Zygotes	Relation $\frac{X}{A}$	Individuals
2 X 2A	XA	3 X 3 A	1.00	triploid female
1 X 1A	XA	2 X 2 A	1.00	diploid female
2 X 1A	XA	3 X 2 A	1.50	super female
1 X 2A	XA	3 X 3 A	0.67	intersex
2 X 2A	A	2 X 3 A	0.67	"
1 X 1A	A	1 X 2 A	0,50	male
2 X 1A	A	2 X 2 A	1.00	diploid female
1 X 2A	A	2 X 3 A	0.33	super male

From the above table, it is clear that sex is not determined by the absolute number of X chromosomes but by the relation between the chromosomes and the autosomes. If this relation is 1 the animal should be a female, if it is ½ it is a male, but if X/A is between 1 and 0.5, that is 0.67 or 0.75 it is intersex (showing a mixture of male and female characters); if the ratio is above 1 a super famale is produced, and if below ½ a super male is given. Although these organisms are supresexed they are sterile, and this shows that there is particular grade of sexuality allows reproduction.

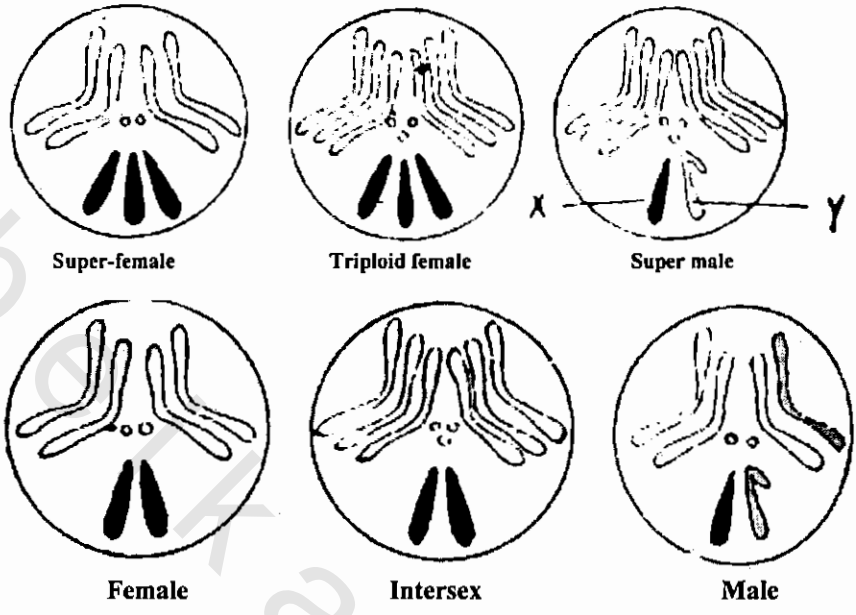


Fig. 128 : Sexual types in Drosophila melanogaster.

Polygenic nature of X-chromosomes in Drosophila :

That the X chromosome contains several female determinating genes distributed along the length of the chromosome is established by using various fragments of X chromosome obtained by X-rays treatment. It has been found that the longer the fragment introduced into the intersex X2X3A, the greater is the feminizing influence on the intersexes.

As regards the autosomes it has been shown that both of the long autosomes contain male determiners in certain regions, whereas the smalles pair of autosomes seem not to contain male genes. According to White (1945), the sex in Drosophila is probably determined by the equilibrium between two systems of polygenes in the X and in the large autosomes.

(2) Sex determination by the mutual action of the X and Y chromosomes or Sex determination by the Y chromosomes: ("Melandrium type") :

In the plant Melandrium the female sex chromosome constitution is XX and the male is XY. In this plant sex is determined by sex chromosomes. Unlike Drpsophila the Y chromosome contain the male determiners. These are effective so that even a tetraploid plant of a consitution XXXY is a

male. This means that the male deteminers on the Y can counteract a triple dose of female determiners.

Plants that have X and 4 sets autosomes (instead of the normal two) are females although they have only one X to 2 sets of autosomes. I.e., the same proportion as in a male. Thus, sex is not determined by the proportions of X and autosomes, as in Drosophila, but by the interaction of X and Y.

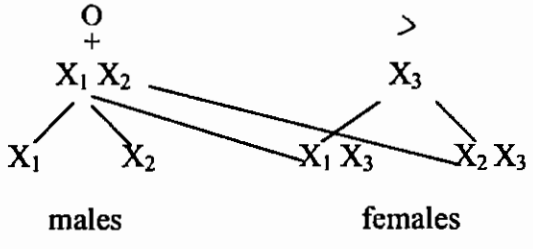
In the amphibian Axolot females YY were obtained, the sex depends upon the presence or absence of the Y chromosome.

(3) Sex determination by the action of a series of multiple homozygotic and heterozgotic allels situated at corresponding locations (Sex determination in Parthenogenetic of Haploid organisms):

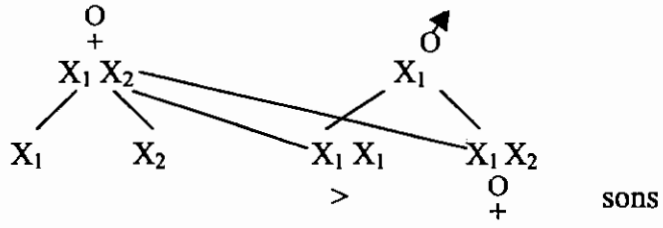
In many social Hymenoptera such as honey-bees and ants there are diploid females resulting from fertilized eggs and haploid males resulting from unfertilized eggs by **Parthenogenesis**.

In habrobracon (a wasp) it has been found that the eggs may develop either with or without fertilization. The unfertilized eggs develop into males, the fertilized eggs as a rule develop into females, but if the parents are closely related then some of the fertilized eggs develop into males. Accordingly, the males are either haploid or diploid (biparents). There fore, according to white sex is determined by a series of multiple homozygotic and heterozygotic alleles (allelomorphs) situated at corresponding locations. Any heterozygotic individual (X1,X2; X2, X3; X1, X2; etc.) is a female, but a homozygotic individual (X1, X1; X2; X2; X3; X3; etc.) is a diploid male, and the hemizygotic individuals (X1; X2; X3; etc.) are haploid males.

If a female X1 X2 is mated to unrelated male X3 all the fertilized eggs will be heterozygous X1X3 and X2X3 (daughters) as follows:

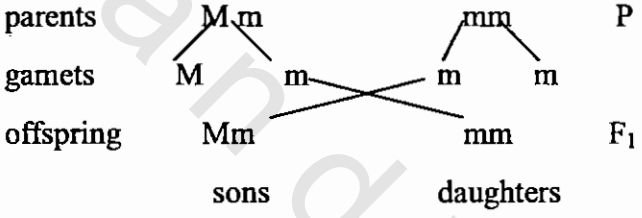


On the other hand, if a female X1X2 is mated to a related male say X1 the result will be X1X2 females (heterozygous) and X1X1 males (homozyguos), in addition there will be X1 and X2 males.



(4) Sex determination by the different action of a single pair of genes:

It has been found that a single pair of allelomorphic* genes is responsible for sex determination in certain organisms as in Culex molestus where it has been proved by linkage experiments involving the white eye gene that sex is determined by a single pair of genes. Malenes is dominant to femaleness. Sex inheritence was explained on the assumption that the male was heterozygous with the two genes Mm, and that the female was homozygous with the two genes mm. The result of the mating will be:



Sex is determined also by single pair of genes in the case of the fish Lebistes. It is probable that similar conditions are found in many Amphibians and Teleosts in which there is genotypic control of sex but no sex chromosomes were detected.

(5) Sex determination by the action of the environment :

Sex chromosomes are not the only factors responsible for the determination of sex, and there is evidence that in exceptional cases the sex is influenced by environmental conditions. As an example of this type is the marine annelid called **Bonellia viridis** in which the female is one metre in length while the male is only a few millimetres (about $\frac{1}{500}$ of the female). The male lives at first attached to the proboscis of the female, and

* Allelomorph is applied to those genes which occupy one and the same locus upon a particular chromosome. In other words, when two genes are located on the same locus in two hmologous chromosomes, they are allelomorphs.

later within its oviduct or intestine. The very young larvae are free living and are neither males nor females. If the larva finds its way to the oviduct or intestine of the female it becomes a male. But if lives indepedent it grows to a female. The oviduct of this worm secretes a substance which is able to change the female into a male. Experiment carried out by Nowinski in 1934 show that the oviduct extract can change the sex of the larvae. Later on, it has been found that putting the larvae in an acid medium changes them into males. It seems, therefore, that sex determination in this case depends on a chemical reaction imposed on the larva from the outside and is not produced by the larva itself. This method of sex determination is very exceptional. Usually sex is determined by chromosomes.

HERMAPHRODITISM AND SEX REVERSAL

The **hermaphrodite** is an animal possessing a testis and an ovary. Animals may be **simultaneous** (e.g., earthorm, and snail), **protogynous** (female first, male later) or **protandrous** (male first) hemaphrodites.

The word **gynandromorphism** refers to the secondary sexual characters, e.g., butterfly with one side female and the other males, and the organism need not necessarily be a true hermaphrodite. Gynandromorph (Gyne = woman; and = man; morphe = form) is an individual exhibiting a combination of male and female characters. This chromosomes of both sexes in different parts of the body.

Most of the so-called human hermaphrodites are merely gynandromorphs, usually females lacking the factor or factors which in women normally supress the development of such male secondary characters as the beard.

Change of sex from female to male is comparatively common in poultry, many cases in such egg laying birds as the White Leghorn being recorded. In these, after the exhaustion of the oocytes in the ovary, the birds is still comparatively young and vigorous, and by some means a new crop of germ cells emerges, but along male lines. These birds may develop testes, cock hackles, combs and even spurs, and will fight the other cocks. This is protogynous hermaphroditism.

HYBRID STERILITY AND MEIOSIS

A typical sterile hybrid is the mule, which is a hybrid between the mare and the jackass. The male mule is definitely infertile, but there is some doubt about the infertility of the female mule. The horse egg has the

haploid number of 19, the ass sperm has 32, so that the diploid offspring has 51. At meiosis in the mule, normal synapsis (i.e., zygotene pairing) obviously cannot occur, because there are no proper synaptic mates (i.e., homologous chromosomes), and from that period the rest of meiosis is out of gear and no functional sperms or eggs are produced. The male is more vigorous, hardier, free from disease than either parents.

CHROMOSOMES AND EVOLUTION

The hereditary material carried in the chromosomes has a constant arrangement. Howevere, changes may take place in the chromosomes, brought about by accidents which disturb their regularity and produce disarrangements in the structure of their parts. Such changes have been artificially produced, however, they are sometimes found in nature. Treatment with abnormal temperatures, with ionizing radiations and with certain chemicals will increase the rate of gene mutations and structural alterations in the chromosomes.

The changes in the chromosomes can be summarised as follows:

1. Genic mutations (intragenic); these are the most important class of mutations in evolution.

2. Changes in the chromosome number due to:

(a) Increase or decrease in the number of chromosoome sets; this results in **polyploidy or haploidy**.

It is known that in the ´somatic nuclei of a diploid organism there are two chromosomes of each kind (the homologous chromosomes); however, organisms with nuclei containing more than two haploid sets of chromosomes are sometimes found in nature. An organism containing more than two haploid sets of chromosomes in its somatic cells is called a **polyploid**, If the orgamism contains 3 chromosomes of every kind (or 3 sets) in its nuclei it is referred to as **triploid**; those with 4 sets (or four chromosomes of every kind) are called **tetraploid,** and so on.

(b) Increase or diminution of one or more chromosomes (i.e., of less than a whole set of chromosomes); this produces polysomy.

3. Structural alterations (Fig. 129. 1 – 7) in the segmenth of the chromosomes due to:

Intrachromosomic rearrangements (intergenic): inversion.

(b) Interchromosomic rearrangements: translocations.

(c) Loss or increase of chromosomic segments: deficiences or **deletions and duplications**.

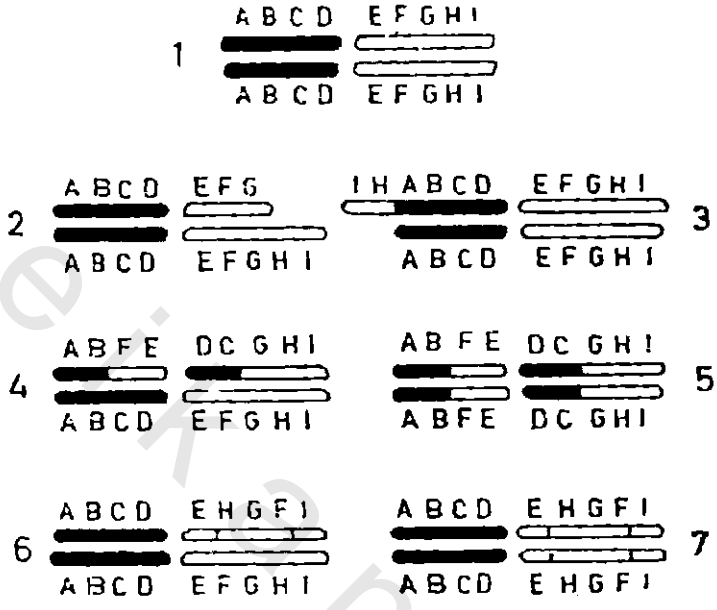


Fig. 129: Diagram showing chromosomal changes (see also Figs. 125 and 129).

The term mutation, when used in its broader sense, means any relatively premanent change in the germ plasm other than one that results from Mendelian segregation and recombination. Mutations in this sense can be classified as:

(1) Changes in genes, i.e., gene mutations or mutations.

(2) Changes in chromosome number (resulting in polyploidy haploidy or heteroploidy).

(3) Changes in the arrangement of the chromatin (i.e., chromosome segments) due to translocations, inversions, deletions and duplications. The term mutation as ordinarily used means a change in gene; in other words, when we speak of mutations we usually have gene mutations in mind.

Mutations might cause changes of various degrees ranging from very small to very large. In Drosophila, for example, a mutation might cause the eyes (normally red) to become only slightly lighter than normal or it might cause them to lose all their pigment and become white, i.e., gene mutations can result in new kinds of genes. Evolution is possible because

a gene can change and reproduce itself in its changed form. Sometimes, a mutant gene mutates back to its normal allele; this is spoken of **as reverse mutation.**

Polyploidy is due to multiplication of one or both of the haploid sets of chromosomes brought about by the failure of cells to divide when the chromosomes of the germ-cells split in the process of cell division or by the omission of the reduction division during the formation of the gametes. In some instances an organism may develop in the **haploid** contdition, as in male bees, with only a single set of chromosomes present.

Polyploidy can be artificially produced by treament with abnormal temperatures and with the drug colchicine. It sometimes produces morphological or physiological changes in plant which are of commercial value as increasing vitamin A in maize and vitamin C in tomato or producing bigger flowers and seeds or increasing fertility and so on.

Changes in the characteristics of an individual which lead to species formation originate as changes in the chromosomes. The appearance of a new character is sometimes due to alteration in the number of structure of the chromosomes, but is often brought about by gene mutations. In plants, new species have arisen through polyploidy, but in animals structural rearrangements of the chromosomes and gene mutations are apparently the most important factors.

Mutations affect a single gene at a time, and since species usually vary in several genes, it is very likely that new species usually appear as the result of several mutations. Each mutation affects one or more characters and further mutations may result in the appearance of a new species.

As the effect of a gene is determined not only by the nature of the gene itself, but also by the genes located in the adjacent regions of the chromosome, it is probable that structural rearrangements may alter the functions of several genes.

If a new variety or species is to become established it must be isolated geographically, or prevented in some other way from breeding with the parent stock or related groups; otherwise intermediate types might result and prevent specific distinctness.

CHAPTER 20

CELLULAR MOVEMENTS

The energy procuced by the cell is stored in the form of ATP (adenosine triphosphate) and other energy rich phosphates. This energy is used in **chemical transformations** (e.g., protein synthesis) and can also be consumed in the **mechanical activity.** Several forms of energy can be included in this type, but the most important form of energy is clearly seen in **the cell motion.**

In certain cases cell movement takes place within the protoplasm without any change in the shape of the cell; such motion is known as cytoplasmic **streaming or cyclosis.** In other cases, the movement occurs by the emission of pseudopodia which cause a true displacement of the cell (**amoeboid movement**). Sometimes movement may take place in cilia and flagella; this type of motion is spoken of as **ciliary and flagellar motion.** In addition, movement may occur in specific cytoplasmic fibrils; such motion is celled **muscular motion.**

Amoeboid, ciliary and flagellar types of motion are common in unicellular organisms. Embryonic animal cells may move during organogenesis (development of organs) and histogenesis (development of tissues). In tissue cultures, healing wounds and cancers, the cells are capable of free movement, in adult or mature animals, visible movement is carried out only by gametes, ciliated epithelia, wandering amoeboid cells and muscle cells.

1 - CYTOPLASMIC STREAMING or CYCLOSIS

Cytoplasmic streaming can be easily observed in many plant cells. In ciliated protozoa (e.g., Paramecium) similar but slower movements are visible. In higher animals, intracellular movements can be detected in some cells (Fig. 130). Mitotic division (including the displacement of the cell centre, the chromosomes and other cell organoids) also belongs to the group of intracellular movements.

In studying scyclosis plant cells have been used especially the cylindroid cells of the alga Nitella in which the protoplasmic layer is thin (about 15 μ surrounding a central vacuole of 0.5 mm by 10 cm). It has been found that motion occurs in the more liquid inner part of protoplasmic layer.

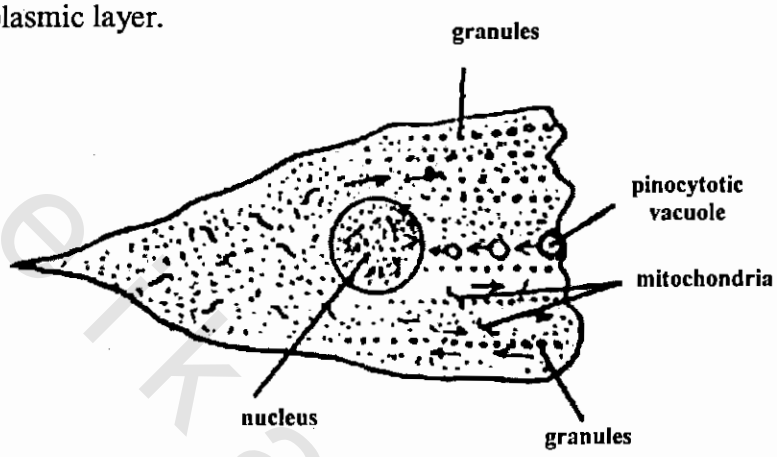


Fig. 130:
Diagram showing the cytoplasmic movements in a mammalian fibroblast cell.

Cyclosis may be started in some plant cells by chemicals (chemodynesis) or by visible light (photodynesis); and is controlled by temperature, by the action of ions, or by changes of pH.

Cyclosis can be increased by some auxins (plant growth hormones) and stopped by mechanical injuries, electrical shocks, or some anesthetics, As a general rule, all the factors which decrease cell viscosity increase the speed of cytoplasmic streaming and vice versa.

Viscosity is one of the most important factors in the mechanism of cyclosis; this is because the protoplasmic colloid may pass from the liquid state to an almost solid one (by means of the reversible sol-gel changes).

2 - AMOEBOID MOVEMENT

In cyclosis the protoplasmic components are simply displaced without any change in the shape of the cell, whereas in **amoeboid movement** (or motion) the cell is deformed. The shape of the cell is changed, cytoplasmic projections (pseudopodia) are emitted, and into these pseudopodia the protoplasm flows. This type of movement is called **amoeboid movement** because it can be seen easily in Amoeba. It takes place also, in other types of cells as in case of leucocytes in which pseudopodia are given and movement occurs. In tissue cultures cells may free themselves from the

rest of the tissue and move out actively. This happens also in epithelia in which the desmosomes connecting the cells disappear. Furthermore, the cells - in epithelial repair-free themselves and slide actively towards the depth of the wound.

Generally, the amoeboid movement takes place when the cells are attached to some solid substratum., In its simplest form there is an axial cytoplasmic streaming which continuously displace a tubular body. Some amoebae are monopodial (one pseudopodium); others may be polypodial. The pseudopodia have many shapes: they may be cylindrical lobopodia, fine filamentous or branching filopodia; and sometimes the filaments anastomose forming reticulopodia as in foraminifera.

The Amoeba is the ideal cell in which one can observe this kind of movement. It has a clear ectoplasm, which expands considerably towards the end of the pseudopodium (hyaline cap), and an endoplasm which constitutes the greater part of its mass. The recent studies distinguish in Amoeba the axial endoplasm, which is surrounded by a "shear zone" in which the particles move more freely (Fig. 131). At the advancing end occurs the hyaline cap, and just posterior to it lies at the "fountain zone" where the axid endoplasm appears to contract actively and flows below the ectoplasmic tube. The tail process (uroid) lies at the opposite end, and near it occurs the "recruitment zone" where the endoplasm is recruited from the walls of the ectoplasm in the posterior third of the ogranism. In different amoebae the rate of progerssion varies from 0.5 to 4.6 μ per second. This rate is modifid by temperature ad other environ mental factors. In sufficient 02 slows the movement but the absence of calcium ions stops it.

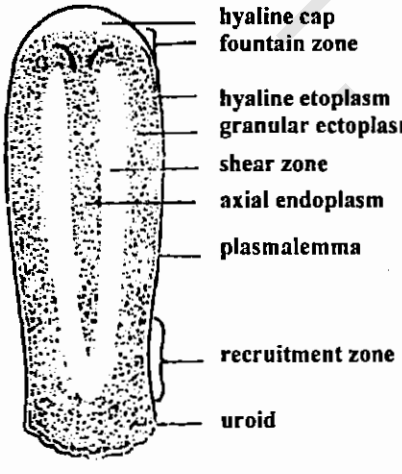


Fig. 131: Amoeblod structure and movement (From Allen).

The mechanism of amoeboid movement is based on the structure of the cell matrix, and especially on the hypothesls that it contains a network of protein molecules held together by different kinds of cross linkages. Changes in such forces and in the degree of folding or length of the protien chains may cause sol gel transformations and protoplasmic contraction in a certain region of the protoplasm.

3 - CILIARY MOTION

In contrast to amoeboid motion which takes place on a solid substrate and involves cellular deformation, **ciliary movement** is adopted to a liquid medium and is carried out by minute appendices. These are contractile filaments which vary in number and size. If they are few and long they are called **flagella,** but if they are short and numerous they are spoken of as **cilia.** These motile processes are found in protozoa and in many animal cells. Various cilia may fuse forning larger conical appendices called the **cirri**, or membranes known as the **undulating membranes.**

The class flagellata is characterized by the presence of thin flagella. Among the metazoa, the spermatozoa have flagella and the epithelial cells have cilia.

Ultra structures of cilia and flagella :

The ciliary apparatus is formed of :

(1) The **cilium :** this is a slender cylindroid process which projects from the free surface of the cell.

(2) The **basal body** or **granule** from which it originates.

(3) The **ciliary rootlets :** These are fine fibrils which arise from the basal granule in some cells, and converge into conical bundles. They end towards or near the nucleus.

Both cilia and flagella are extremely delicate filaments whose thickness is often at the limit of the resolving power of the light microscope and so they do not show any internal structure.

Some epithelial cells have appendices which resemble the cilia in shape, but are immobile; these are called **stereocilia.**

Ultrastructure of cilia and flagella :

It was found that each cilium of flagellum contains 11 fibrils. The spermatozoon tail built upon the same plan of cilia and flagella. In a cross section of cilium, the total diameter of which is about 2000 A^{o}, an outer ciliary membrane surrounds the ciliary matrix and is continuous with the plasma membrane. Embedded in this matrix are nine pairs of filaments, whereas two single filaments are at the centre (Figs. 83&84). Cilia can be defined as long cylindrical processes tapered at the tip and composed of an axial filament complex embedded in a matrix and enclosed in a ciliary membrane which is continuous at the base with the cell membrane.

Mechanism of ciliary motion:

The current hypothesis for the mechanism of ciliary motion is based on knowledge of the fine structure of cilia. One hypothesis is that: (1) the matrix of the cilium is stiff; (2) The nine peripheral pairs can contract and propagate waves of contraction from the base to the tip; The centreal pair is not contractile but specialized for rapid condition; (4) The impulse that initiates the beat arises rhythmically from the basal body and spreads sequentially to the different fibrils.

The shortening of the fibrils 1,2,9,3 and 8 causes the cilium to bend forwards. In the recovery phase, these fibrils relax and a contraction is initiated in fibrils 4,7,5 and 6 which produces the recovery stroke. In this mechanism the effective or intial stroke would be helped by the rapid propagation along the contracting fibres 4,7,5 and 6. Several findings show the importance of ATP in cilia, flagella ad spermatozoa tails, ATP stimulates filamentous movement (Fig. 132).

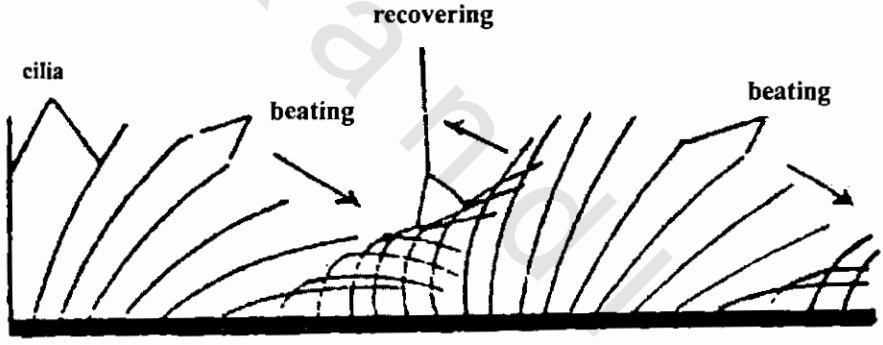


Fig. 132:

The cilia on the surface of a cell beat in coordinated waves such that at any given moment some are in the effective (down stroke) portion of the cycle and some are recovering.

4 - MUSCULAR MOVEMENT

This is the most highly developed type of cell contractility. The muscle cells are adapted to mechanical work by unidirectional contraction; thus most muscles are elongate and spindle-shaped. The majority of the cytoplasm of the muscle cell is occupied by contractile fibrils (the **myofibrils**) which are the functioanal units. The structure and function of myofibrils are present in chapter 12.

CHAPTER 21

PERMEABILITY

Permeability includes the passage of materials through membranes. This process is of utmost importance for the life of the organism since it is the mechanism which regulates the entrance of certain substances which are essential for synthesis of living materials, and it also controls the outflow of excretory substances and water which are to be eliminated from the body cells.

Permeability depends on two main factors:

1. Characteristics of the cell and nuclear membranes for the passage of molecules.
2. Driving forces of the molecules.

In addition, permeability is highly influenced by the surrounding conditions such as temperature and tonicity of the medium (osmotic pressure).

Osmotic pressure and permeability:

Osmotic pressure is essential for the life of the organism. It plays a fundamental rôle in the formation of certain body fluids such as lymph and interstitial fluid.

Osmotic pressure is well demonstrated in plant cells. When cells are put in a solution having the same osmotic pressure as that of the intracellular fluid, the cytoplasm remains sticking to the cell wall. If the solution of the medium is more concentrated than the intracellular fluid, the cells lose water and the cytoplasm retracts from the rigid cellulose wall. On the contrary if the solution of the surrounding medium is less concentrated the cells swell up even to the point of bursting.

In animal cells, the plasma membrane is permeable to water and to certain solutes; and the osmotic pressure is preserved by means of mechanism which regulates the concentration of the dissolved substances in the interior of the cells. The passage of different solutes through the membranes of animal cells does not take place with the same facility.

The cell membrane maintains a balance between the osmotic pressure of the intracellular and the interstitial fluid.

Solutions may be isotonic, hypertonic or hypotonic with respect to the intracellular fluid.

1 - **Isotonic solutions** in which the osmotic pressure is similar to that of the cells. For example, 0.95% NaCl solution is isotonic in relation to mammalian cells.

2 - **Hypotonic solutions** in which the osmotic pressure is less than that of the cells, e.g., a 0.66% NaCl solution is hypotonic for mammalian cells, but isotonic for amphibian ones.

3 - **Hypertonic solutions**, with osmotic pressure greater than that of the cells.

Regulation of osmotic pressure :

Regulation of the osmotic pressure is very essential in the different cellular activities. In higher animals the kidneys are the main organs for regulating the osmotic pressure. In these organs the hydrostatic pressure of the blood brings about the outward passage of water from the glomerulus in the form of urine.

In many unicellular animals the contractile vacuole maintains the osmotic equilibrium. This organoid, which is an osmoregulator structure, extracts the excess of water from the protoplasm and expels it into the surrounding medium; this is why the contractile vacuoles occur in Amoebae which live in fresh water and not in those living is salt water. In the first case, Amoeba is surrounded by hypotonic solution, thus water continuously diffuses inside the body. In the other case, Amoeba in salt weter is surrounded by a solution which is nearly isotonic for the internal fluid. If small traces of water diffuse inside they will be eliminated by simple diffusion through the cell membrane.

Experiments with red blood corpuscles showed that these cells belong to the group of objects known as "osmometres" there are objects which are capable of changing their volumes in media of different tonicities by the exchange of water alone.

Methods of determination of cell permeability :

1 - **Plasmolysis** : This method depends upon the observaion of plasmolysis under the microscope. In animal cells the excellent material for the study of permeability especially of water are the eggs of some marine animals such as **chaetopterus.** The eggs are spherical in shape and have a constant size under normal conditions. They swell up in hypotonic solutions and shrink in hypertonic ones without losing their spherical

shape. The swelling and shrinkage can be determined by measuring the diameter of the egg and then calculating the volume.

2 - **Haemolysis :** In this method the permeability is calculated by measuring the degree of haemolysis of erythrocytes by various optical devices. This is carried out by changing the intensity of colour of the solution in which the red blood corpuscles are suspended; this measurement (related to the time factor) gives a fairly accurate idea of the penetrability of the substance.

3 - **Radioactive isotopes** : Permeability to electrolytes is better studied with the application of radioactive isotopes. In this case, the tracer elements are used instead of the normal compounds, and then their premeability is measured by the Geiger counter. This method is valuable in determining the amount of substance which penetrate inside the cell during a definite period of time.

4 - **Diffusion and Active Transport** : The minute pores in the palsma membrane serve for the free diffusion of molecules up to a size of 7 A^{0}. It is through these pores that lipid-insoluble substances of very small sizes such as water molecules pass with relative ease between the interior and the exterior of the cell.

Substances are transported through the plasma membrane by two major processes: (1) diffusion, and (2) active transport.

(1) Diffusion :

This means the movement of substances in a random fashion by the normal motion of the matter. The dissolved substance diffuses from one compartment to another when the concentration is higher in the first.

The diffusion rate =

$$\frac{\text{Concentration gradient X cross section X temperature}}{\text{Moleuclar weight X distance}}$$

As we have mentioned the cell membrance is a sheet of lipid covered on each surface by a layer of protein.

The lipoprotein membrane acts as a limiting barrier between the exterior and the interior. Unless the substances is a lipid-soluble it cannot pass through the membrane. Few substances are soluble in the lipid of the cell membrane as well as in water, these include O2, CO2, alcohol and few other less important ones. When one of these substances comes in contact

with the membrane it immediately becomes dissolved in the lipid, so it diffuses with ease as well as in the watery dedium on either side of the membrane. Thus, the random motion of the molecule may take it in through the membrane, or it may take it back out of the membrane to the side from which it came.

If the substances dissolves very poorly or does not dissolve at all in lipids, its diffusion will be greatly retarded, e.g., H20 which is almost completely insoluble in lipids does not pass through the lipid material at all; but instead H2O passes through the minute pores (8 A^{o}). The same is true for the very small water-soluble ions and molecules; only the size of the particles should be smaller than the size of the pore.

The following table shows the relationship of effective diameters of different substances to pore diameter :

Substance	Diameter in A^{o}	Ratio to pore diameter
Water molecule	3	0.38
Urea molecule	3.6	0.45
Cl^{-}	3.86	0.84
K^{+}	3.96	0.49
Na^{+}	5.12	0.64
glycerol mol.	6.2	0.77
glucose mol.	8.6	1.04
sucrose mol.	10.4	1.30
lactose mol.	10.8	1.35

Still there are some substances which are insoluble in lipids and have larger diameters than the pore diameter like glucose which can cross the membrane by what is called **facilitated diffusion**. The mechanism by which this type of diffusion takes place is a follows:

(a) Glucose combines with a carrier substance C at point (1) to form the compound Cgl. This combination is soluble in the lipid so that it can diffuse to the other side of membrane (Fig. 133).

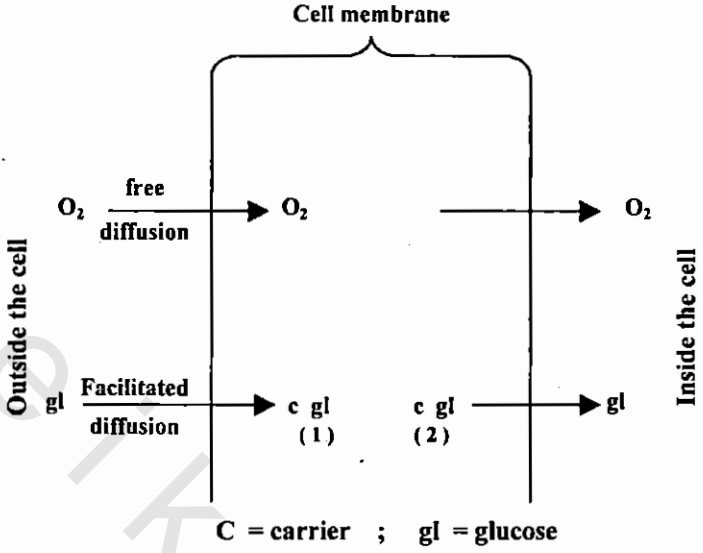


Fig. 133 : Diagram showing facilitated diffusion.

(b) At point (2), at the other side of the membrane glucose breaks away from the carrier and passes in the inside of the cell, while the carrier diffuses back to the outside surface of the membrane to pick up still more glucose and transport it also to the inside. Thus, the effect of the carrier is to make the glucose soluble in the membrane; and without the carrier the glucose cannot pass through the cell membrane. It is possible that sepcific enzymes exist to catalyize these chemical reactions. In some instances the process takes place without any requirement for extra energy.

(2) Active Transport:

It is evident that all subtances diffuse from a higher to a lower concentration, and no substance can diffuse against a concentration ghradient (from lower to higher or as is called uphill). The process of moving molecules up hill agaisnt a concentration gradient is called active transport. Among the substance that are actively transported through the plasm memtrane in at least some parts of the body are Na, K,Cl Fe, I, urea, several sugars and amino acids.

The mechanism of active transport is similar for all subsances, being dependent on transport by carriers (Fig. 134).

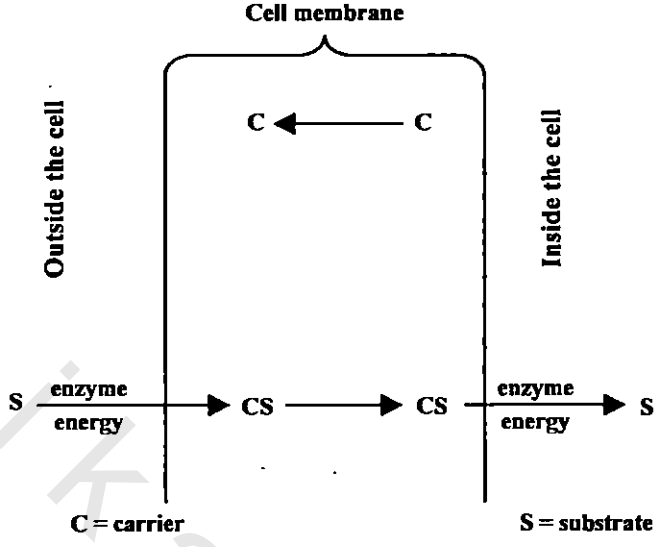


Fig. 134 : Diagram showing active transport.

1 - The substances (S) combines with carrier (C) when it enters the membrane.

2 - At the inside surface of the membrane (S) separates from the carrier and is released to the inside of the cell.

3 - (C) then moves back to the outside to pick up more (S).

This shows the similarity between this mechanism and facilitated diffusion. The difference is that energy is imparted through ATP to the system in the course of active transport so that transport can occur against a concentration gradient.

The carrier is believed to be a phsospholipid or a protein. Several carrier system exist in all membranes each of which transports only certain specific substances; for example, one carrier system transport Na+ to the outside of the cell membrane and probably transports K+ to the inside at the same time. It appears that the Na+ - K+ transport system is linked to ATPase which is found associated with the cell membrane. It is believed now that Na+ - K+ ATP ase is related to the active transport of Na+ and K+.

4 - **Vant Hoff-Mariotte Law :** This procedure, is especially concerned with permeability of water. According to this law, the volume of an object that can be described as an "osmometre" is reversibly proportional to the

tonicity of the surrounding medium. Thus, as the volume increases the tonicity decreases and vice versa.

To explain this law, it is assumed that the mammalian red blood cell is represented by a model in which a thin, non-rigid, non-elastic surface membrane surrounds a quanity of haemoglobin and salts in solution. This membrane is permeable to water and anions but not to cation. Such a model would shrink or swell by the transfer of water alone when placed in solutions of higher or lower osmotic pressure than that in its interior. Thus, it behaves as a "perfect osmometre" in accordance with the vant Hoff-Mariotte law.

If V represents the cell volume, T tonicity of the medium, and W the fraction of the cell volume occupied by water, then the above law is represented as follows:

$$V = w\,(1/T - 1) + 1$$

As above mentioned, an isotonic solution is that in which the tonicity is the same as that of the cell. Thus, the cell never swells or shrinks in such a medium. The animals own plasma is usually taken as the ideal isotonic solution of the tonicity T = 1.0 and the same value (1,0) is that of electrolytes, sugars, etc. in which the red cell volume remains unchanged.

As the tonicity decreases, the volume of the cell increases until a critical volume Vn is reached with a critical tonicity Tn. Any volume increase beyond this, results in haemolysis. In this condition An is the surface area of the cell which is now of spherical shape, thus:

$$Tn = \frac{W}{(Vn/Vo - 1) + W}$$

The critical volume of the human red cells is 1.6 Vo.

From this law it has been found that red blood cells are typical osmometres. This is clear from the following evidences:

1 - There is a certain critical volume for the red blood cell which depends on the tonicity of the medium and consequently on the amount of penetrating water. Any increase above this critical volume was found to cause the lysis and destruction of the cell.

2 - Red blood cells do not haemolyze in the same manner in the same tonicity. This is due to the differences in their water contents and their original shapes and volumes.

3 - In hypertonic solutions there are certain limits to the extent of the shrinkage of cells due to the loss of H2O. It was generallly found that in a medium as hypertonic as 20% NaCl only about half of the cell water is removed. The remaining water is not osmotically transferable under these conditions. Removal of half the cell water raises the concentration of haemoglobin to about 60%, haemoglobin becomes cycstalline and water is very difficult to be removed from such crystals.

Generally, it has been found that the permeability of water varies from 0.1 to 3.0 and is expressed by the number of cubic microns of water which pass through 1-2 u of cell surface per ninute at a temperature of 20°C.

Permeability to lons (Donnan's Law of Eqilibrium) :

The moleucles of non-electrolytes (which do not dissociate in water into charged ions) pass through membranes nore readily than the electrically charged particles (ions). However, ions are very important in the maintenance of the biological activities and their passage into or our from the cell is of special importance due to the electrical charge they carry. This type of permeability is illustrated by **Donnan equilibrium** (the relation existing between two solutions of electrolytes separated by a membrane through which one of the ions cannot pass) in the following manner:

If a solution of sodium chloride (Na+ Cl-) is separated by a membrane from another diffusible solution as potassium chloride (K+ Cl-), the ions will be distributed between the two compartments until an equilibrium is established.

But, if a solution of sodium chloride is separated from a solution of Congo red (Na+ and a radical R-) by a membrane which is permeable to the Na+ and Cl- ions but impermeable to the R ions, the initial distribution of substances is represented in the following manner provided that the number of ions on each side of the membrane is represented by a and b.

I	II
a. Na+	b. Na+
a. Cl-	b. R-

The ions of Na+ and Cl-pass through the membrane in the direction 1-11 and in the opposite direction until an equilibrium is reached when the

speed of diffusion in the two directions is the same. If the number of ions traversing the membrane from compartment I is represented by X, then in case of equilibrium in side II there will be the original number of ions b + and X number of ions (coming from the side 1). On side I, on the other hand, there will be a lesser X number of ions as shown in the following:

I	II
a ___ X. Na^+	b + x, Na^+
a.___ X. Cl^-	b. R^-
	x. Cl^-

Since the velocity of diffusion from I --- II is proportional to the concentration of Na+ and Cl- inside I, this can be expressed as (a-x)2. At the same time, the speed of diffusion from the opposite side, i.e., from I --- II is proportional to the product of concentration of a+ and Cl- in side II which may be represented by (b+x) x. The ionic equilibrium is reached when the speeds of diffusion are the same in both directions; this can be expressed as follows:

$$\overset{\text{I}}{(a-x)^2} = = = \overset{\text{II}}{(b+x)\ x}$$

From this equation which is known as **Donnan's fundamnetal** equation, the following results are deduced:

1 - The concentration of ions of Na+ and Cl- in side I is the same.

2 - The number of Na+ ions is greater in compartment II than in I.

3 - The concentration of Cl- is greater in I than II.

4 - The concentration of Na+ in the compartment II is greater than that of Cl-.

Significance of Donnan's equilibrium in cellular activites :

Donnan equilibrium is very important in the cellular and biological activities. It explains the ionic equilibria established between the cells and the surrounding medium where there are diffusible and non-diffusible ions in the system. Proteins are examples of such non-diffusible ions. On the basis of this equilibrium, the difference in the content of bicarbonate and chloride between the erythocytes and the serum (taking haemoglobin as the non-diffusible ions) was explained. Haemoglobin, as well kown, is present in high concentrations in the erythrocytes.

Furthermore, the metallic ions (both anions and cations) enter and leave the cells. It is probable that this interchange is active in cells and tissues during growth, since the concentration of salts remains constant whereas the mass of protoplasm is increasing. It also very intense in many secretory cells (such as those of the salivary ad gastric glands) which lose a great quantity of salts that are replaced from the blood cross the vascular membrane. Likewise, muscular and nervous tissues show an active interchange of ions during their physiological activities.

Moreover, Donnan's equilibrium shows that the penetrability of the different metallic ions is variable. (Penetrability means the relative speed with which a substrate crosses the plasm membrane under standard conditions. Penetrability is a property of the cell membrane). Anions penetrate more rapidly than cations (Cl- > Na+). The order of penetration of anions is as follows:

Nitrate > Chloride > Acetate > oxalate > Sulphate

In case of cations, it is as follows:

Potassium > Sodium > lithium > Magnesium > Calcium.

In case of sugars, a large number of molecules of glucose were found to diffuse into the cell to maintain the equilibrium, whereas in other sugars (as sac charose) small amounts can maintain this equilirium. This is due to the fact that glucose is highly needed by the cells and is continous in metabolic activities, whereas saccharose is metabolized very slowly.

Penetration of solids and fluids :

Related to the activity of the plasma membrane are the processes of phagocytosis and pinocytosis. By these processes solids and fluids enter the cell. Both processes are similar and thus can be included under the term endocytosis. **Ekxocytosis** is the reverse process by which membrane-lined products are discharged at the cell membrane.

Phagocytosis :

This term is usually applied to the ingestion of solid particles sufficient to be seen with the ordinary light microscope such as bacteria (Phagocytosis means cell eating). In phagocytosis the particle is engulfed by the formation of pseudsopodia, and the cytoplasmic membranes eventually fuse to give a membrane-limited vacuole (Fig. 135).

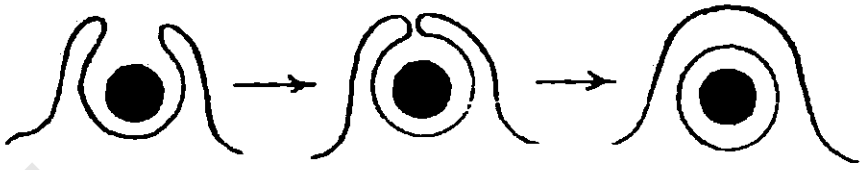


Fig. 135 :

Serial drawings to illustrate phagocytosis. The opposing evaginated pseudopodia eventually fuse and trap the particle.

This phenomenon is observed in a large number of protozoa and among certain metazoa as the granular leucocytes and histocytes of connective tissue. The intake of paticles of vital dyes by the living cells is also regarded as a kind of phagocytosis.

Cells differ according to their power of phagocystosis. For example, some cells can take in virus pritcles and others cannot. In some of these phagocytic cells, the viruses undergo multiplication and become much increased in number. Also, malignant cells differ in their ability to phagocytose; some types are phagocytic and others are not. According to some authors phagocytosis is more common by malignant fibrolblasts in tissue cultures than by normal fibroblasts. In addition, some cells have certain power of selectivity with regards to phagocytosis. Thus, likewise, cells can phagocytose neutral red particles but cannot phagocytose tubercle bacilli or carbon particles. Similarly, cells which are derived from monocytes are thus modified in their power of phagocytosis from the normal cells which are able to phagocytose bacteria and carbon particles.

In all such cases, it is not necessarily that such objects must penetrate through the plasma memtranes, but it is most likely that these objects come into contact with the cell membrane and sink into the substance of the cell so that the substance of the cell flows about them. In either case, the plasma membrane might be carried by these objects into the cytoplasm of the receiving cell and then becomes dissolved or digested.

PINOCYTOSIS :

Pinocytosis is a term which indicates drinking by cells. This is in contrast to phagocytosis which means eating by cells. Pinocytosis was observed for the first time by Lewis (1931). By this process, in tissue culture the complex fluids of culture media containing proteins and other substances which canot diffuse into the cells are engulfed by the wavy pseudopodia of these cells. When the globules of fluid are first taken in they are entirely enclosed by the surface membrane of the cells which invest them. This later disappears as the fluid becomes a part of the cytoplasm. In other words, pinocytosis is often used to describe the formation of any small vacuole (Fig. 136) through the formation of very active membranous fringes which fall back on to the cell surface, thus trapping a droplet and incorporating it into the cytoplasm. In such cells, the peripheral region is usually wavy instead of being smooth and even. The production of wavy pseudopodia by these cells was followed by motion pictures by many investigators.

Fig. 136 : Serial drawings to illustrate pinocytosis.

Macrophages, as seen in tissue cultures, exhibit the property of pinocytosis better than any other variey of cells. Malignant sarcoma cells also show active pincocytosis in tissue cultures. The process then is similar to that in normal macrophages. Lewis (1947) regards that the cells of carcinoma might not possess the property of pinocytosis since they are epithelial and sessile and they cannot produce pseudopodia.

Micropinocytosis and rhopheocytosis (cell aspiring):

They do not involve the formation of pseudopodia, in both cases the plasma membrane invaginates into the cytoplasm forming small vesicles (Fig. 137).

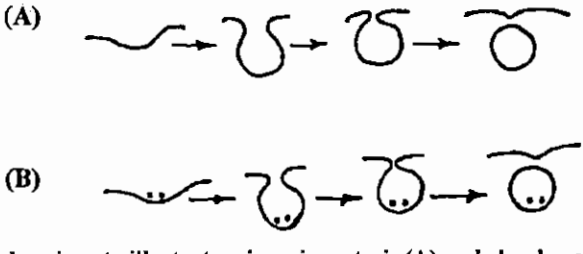


Fig. 137: Serial drawings to illustrate micropinocytosis (A) and rhopheocytosis (B).

The difference between micropinocytosis and rhopheocytosis is that the attachment of macromolecules to the plasma membrane is an essential feature of the latter process. In micropinocytosis liquids or solids are incorporated into vacuoles without prior attachment to the plasma membrane.

CLASMATOSIS :

This process takes place in a manner opposite to that of pinocytosis. In this case, the cell extends certain processes outward surrounded with plasma membrane. These processes later become pinched off and separated. The investing plasma membrane is lost with the result that materials included in these processes leave the cells without passing through the plasma membranes.

Three examples of clasmatosis by normal cells are known. The first is well known in megakaryocytosis of the bone marrow in which fragments of their pseudopodia extending through the delicate vascular walls break off and give rise to platelets. The second is that observed in living pancreatic cells vitally stained with neutral red. These cells produce certain extensions of the plasma membranes which contain cytoplasm as well as secretion substances. They separate off into the lumen of the pancreatic duct where secretion passes out. The third example is found in the thyroid gland. In this case, it was observed that in the living cells certain colloidal material extends into the lumen of the cellular follicles being contained in certain extensions of the cytoplasm. Then, similarly, they become separated from the cells with the result of liberation of colloid from the cells (without passing through the plasma membrane).

CHAPTER 22

CELLULAR ENZYMES AND CELL RESPIRATION

The cell carries on a variety of functions such as synthesis and breakdown of various substances at normal body temperature. These different chemical reactions are carried out with the intervention of enzymes which are capable of speeding up or slowing down the different reactions.

Enzymes are complex proteins which form colloidal solutions when dissolved in water. Their activity is influenced by different factors such as the concentration of the substrate and of the enzyme, temperature (it increases the rate of reaction up to a certain limit). The pH (each enzyme has optional hydrogen-ion concentration) and metal activators. e.g., Na+, K+, Mg++, Zn++, Ca++, Co,++ and Fe++.

Enzymes are usually divided into two categories:

1 – Hydrolytic enzymes: These are concerned with hydrolysis of the different types of food substances.

2 – Respiratory enzymes: These are mainly responsible for the respiratory activities of cells and tissues.

Hydrolytic enzymes are secreted in an inactive from, known as **zymogen** granules. These pass to the cavities of the body where they become activated by certain substances called **kinases**, e.g., trypinogen is activated by enterokinase into active trypsin.

In respiratory enzymes certain compounds may influence the activity of these enzymes. Some of these cmpounds are known as coenzymes which unite with the inactive enzyme (called apoenzyme) thus forming an active complex or holoenzyme. Glutathione and riboflavin belong to this group.

Enzymes are also classified into :

A = Intercellualr enzymes which are secreted by the cells into intercellular tissue spaces as, for example, hyaluronidase which is secreted into the interstitial tissue of the testis.

B = Intracellular enzymes : these are located entirely within the cell, and serve to maintain all the cellular activities,

From the cytological pint of view, the latter group is the more important one. Some of these enzymes can also act outside the cells such as ascorbic acid ad ribonulease. On the other hand, other enzymes lose their activities outside the cells as in the case cytochromes.

In this respect, 3 classes of enzymes are distinguished:

(1) Lyoenzymes : These are dissolved directly in the protoplasm and are easily extracted.

(2) Desmoenzymes: These are bound to the protoplasm and therefore cannot be extracted by the normal methods.

(3) Endoenzymes: These are apparently adsorbed or bound to membranes. The extraction of these enzymes is possible only when the membrane is destroyed by certain chemical or mechanical procedures.

At regards the exact **localization of enzymes** in the cells, some are localized in the cytoplasmic matrix such as glycolytic enzymes; some hydolytic enzymes occur in separate compartments. e.g., lysosomes; other enzymes are attached to the cellular membranes, whereas all the respiratory enzymes are localized in the mitochondria.

Respiratory or oxidative enzymes : These are exclusively localized in the mitochondria, and are regarded to carry out all the oxidative recesses. These oxidative enzymes are localized in the stalked particles of the mitochondria. They are arranged in a definite sequence. The stalked particles have been partially broken down by some electron microscopists into their constituents. The stalked particles are separated form the broken mitochondria as spherical particles having a diameter of 150A°. This corresponds to a molecular weight of 1.4. million which would be expected from an assemble of the enzymes which catalyze the reactions. These isolated particles can carry out respiration and electron transfer.

The most important respiratory enzymes are the cytochromes – the iron containing enzymes. There are at least five types of cytochromes in animal mitochondria. Known as cytochromes b, C1, C2, a and a2 (cytochrome a2 is probable identical to the enzyme cytochrome oxidase 0. They differ slightly by their different absoroption bands spectrosopically.

Another type of respiratory enzymes is that which is capabel of separating hydrogen from various substances. This group of enzymes is known as **dehydrogenases**. One of them, succinic dehydrogenase, is well-known and acts to activate phosphates from succinic acid.

The oxidation processes take place inside the cell and very important in liberating the energy necessary for the cellular activities. These oxidative processes are collectively known as **intracellular respiration.**

For a long time, it was thought that oxidation and its opposite process, reduction, were based only on the direct chemical combination with O_2 or the loss of this element. It is now known that under anaerobic conditions within the cell, although no O_2 is available yet a series of oxidations and reductions take place.

Modern view of oxidation and reduction :

According to this view, oxidation is explained as the withdrawal of electrons from a molecule or atom, and reduction as the addition of electrons.

Therefore, besides the direct combination with oxygen, the following chemical changes are considered as oxidations:

(a) The loss of hydrogen, e.g., ascorbic acid is oxidized to dehydro-ascrobic acid by the loss of 2 atoms of H.

(b) Addition of H2O with the simultaneous loss of H as in case of the formation of acetic acid ($C_2H_4O_2$) from ethyl alcohol.

(c) The loss of electrons without simultaneous addition of O2 or loss of H. For example, bivalent iron Fe++ (ferrous) is oxidized into trivalent iron Fe+++ (ferric); in other words, a ferrous ion is convereted to a ferric ion by loss of an electron (Fe++ -- Fe+++ + e).

However, in all such chemical processes the fundamental fact of oxidation is the loss of electrons from the molecule or atom oxidized. In a similar way the reverse process, reduction takes place by the addition of electrons.

CELL RESPIRATION :

The exergonic processes produced in the living cells through which organic substances are oxidized releasing chemical energy indicate what we call **cell respiration.**

There are two types of respriation:

(a) Aerobic respiration where molecular O2 is utilized.

(b) Anaerobic respiration where molecular H2 is separated from substances.

Anaerobic respiration :

The exergonic processes are carried out in the absence of molecular oxygen in the cytoplasm of all living cells. The best example is anaerobic **glycolysis**, i.e., the break down of glucose. In muscle cells

$C_6H_{12}O_6$ ---$2C_3H_6O_3$ + 68,000 calories.

glucose lactic acid

The most important product of anaerobiosis is pyruvic acid.

In glycolysis the glucose molecule is split to form two molecules of pyruvic acid. In glycolysis, oxygen is not required and energy is released, that is why it is called **anaerobic oxidation**.

The degradation of glucose to pyruvic acid (anaerobic glycolysis) takes place as follows (Fig. 138):

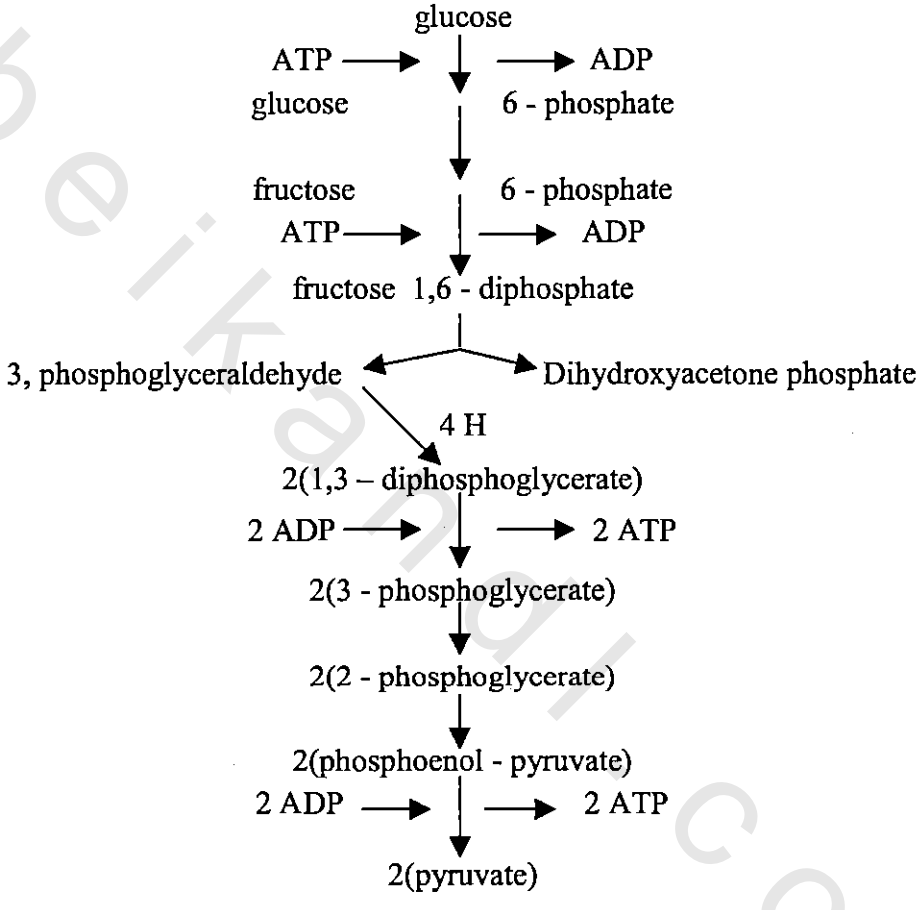


Fig. 138 : Anacerobic respiration.

The net reaction :

Glucose + 2 ADP + 2 PO4 ----- 2 pyruvic acid + 2 ATP + 4H. The net result of the degradation of one molecule of glucose is 2 molecules of ATP.

Enzymes and coenzymes of glycolysis :

Enzymes	Coenzymes or activators
Hexokinase	Mg++
Glucose 6-phosphatase	Mg++
Phosphohexose isomerase	Mg++
Phosphofructokinase	Mg++
Diphosphofructose phosphatase	Mg++
Aldolase	Zn++, Co+, Fe++, Cu++
Phosphglyceraldehyde dehydrogenase	DPN
Phosphoglyceric acid kinase	Mg++
Phosphoglyceromutase	Mg++
Endolase	Mg++ Mn++
Pyruvic acid kinase	Mg++ K+
Lactic acid dehydrogenase	DPN, Mg++

The oxygen is not required in glycolysis, for this reason this process is called anaerobic glycolysis. The net result of this process is 2 molecules of ATP form one molecule of glucose used. This is very inefficient compared with 38 molecules of ATP produced when pyruvic acid is used for aerobic respiration (oxidation of carbon to Co2) in the mitochondria. However, anaerobic glycolysis is very important when a rapid supply of energy is needed since it represents the main exergonic source as in embryonic and cancer cells. Anearobic respiration also takes place in differentiated normal cells such as muscles.

Aerobic Respiration :

In aerobic respiration molecular oxygen is used for combustion of organic substances:

$C_6H_{12}O_6 + 60_2$ -- 6 CO_2 + energy (686, 000 calories), glucose.

This process takes place in the mitochondria which are scattered throughout the cytoplasm of all the eukaryotic cells. Thus, the mitochondria are knosn as "plants of energy". The number, localization and distribution of mitochondria

differ in the different types of cells depending on the metabolic activity of each cell type.

Aerobic respiration is intimately related to anaecrobic respiration. The degradation of glucose (in anaerobic respiration) in the cytoplasmic matrix gives rise to pyruvate which enters the mitochondria after its conversion into acetyl coenzyme A. Acetyl coenzyme A enters into a series of reactions called **tricarboxylic acid or Krebs cycle** and degraded to CO2 and hydrogen atoms. In fact, all the food stuffs (fats, proteins and carbohydrates) are degraded in the cytoplasmic matrix to acetyl coenzyme A which is derived from two major sources which are the glucose and the fatty acids.

The enzymes responsible for the Krebs cycle are contained in the mitochondria.

The steps of the kreb's cycle (Fig. 139) are as follows :

1 - Pyruvate is first converted to acetyl – coenzyme A.

2 - Oxaloacetate combines with acetyl coenzyme A to form citrate which is the first substrate in Krebs cycle.

3 - Citrate loses one molecule of water to form aconitate.

4 - On addition of H_2O the ace onitate is convered to isocitrate.

5 - The oxidation of isocitrate to oxalosuccinate reduces nicotinamide-adenin dinucleotide (NADP+) to NADPH.

6 - Oxlalosuccinate loses CO_2 and forms a-oxogultarate.

7 - A-oxoglutarate is decarboxylated in the presence of coenzyme A forming succinyl-Co-A which gives rise to succinate.

8 - Part of the succinate is used in later stages for the formation of ATP. The remainder of succinate undergoes a dehydrogenation to fumarate.

9 - Fumarate by addition of H_2O gives malate.

10 - Malate is oxidized to oxaloacetate with the formation of one more molecule of NADH from NAD+.

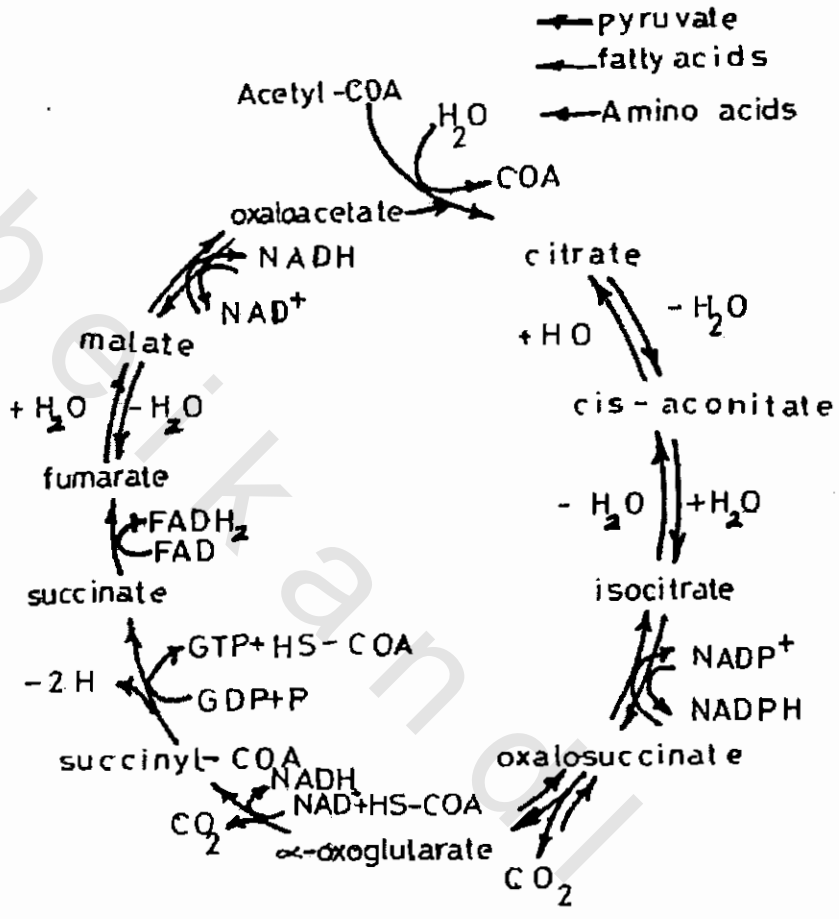


Fig. 139 : The Krebs cycle.

Then the krebs cycle (or citric acid cycle) is repeated, the oxalosacetate again combines with acetyl-coenzyme A to give citrate.

Respiratory chain :

The hydrogen atoms are subsequently oxidized releasing still more energy to form ATP. An oxidized molecule from citric acid cycle (krebs cycle) is subjected to a series of oxidation reduction reactions as shown in figure 140 within the mitochondria. Figure 140 is a simplified version of the electron transport chain in which reduced nicotinamide adenine nucleotides (NADPH, NADH), reduced flavins (FADH2),cytochromes and molecular O2 are indicated as components.

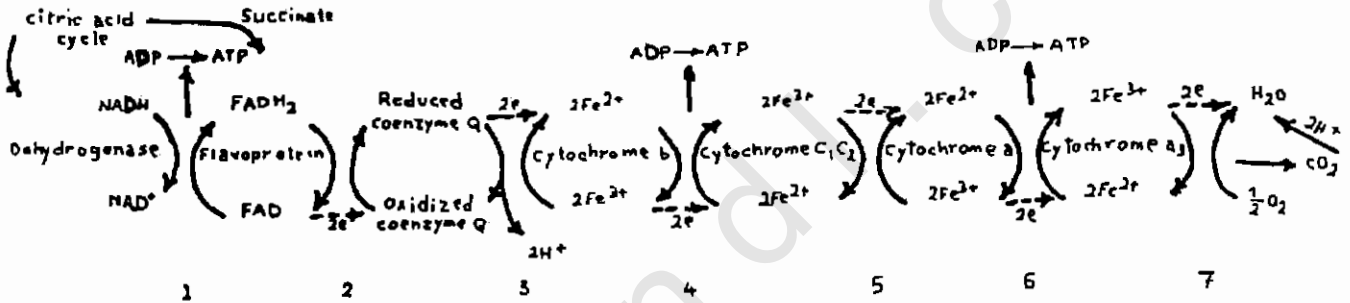


Fig. 140 : Successive oxidation-reduction reactions in the respiratory chain which occur in the particles attached to the membranes of the mitochondria.

The Cytochromes - iron containing enzymes are involved in the oxidation-reduction reactions and depend on the change Fe+++ + e----- Fe++.

Cytochrome a3 or cytochrome oxidase earries out the final stage of transfering electrons to oxygen and combining it with the hydrogen ions to form water. This is the only stage in aerobic respiration at which oxygen is needed.

The oxidation reduction reactions are shown in figure 140.

Oxidative phosphorylation :

As has been already mentioned the energy released at various stages along respiratory chain is utilized to produce ATP from ADP. This means that this energy released is stored in ATP. The process of ATP formation is called oxidative **phosphorylation**, because phosphate is added to ADP energy from oxidation.

$ADP + P_I$ + energy ----- ATP (1 = inorganic)

The net gain of ATP molecules from the complete oxidation of one molecule of glucose in the cell is 38 molecules. Figure 141 is a diagrmmatic representation of aerobic respiration showing the **krebs cycle, the respiratory chain**, and its coupling with **oxidative phosphorylation**.

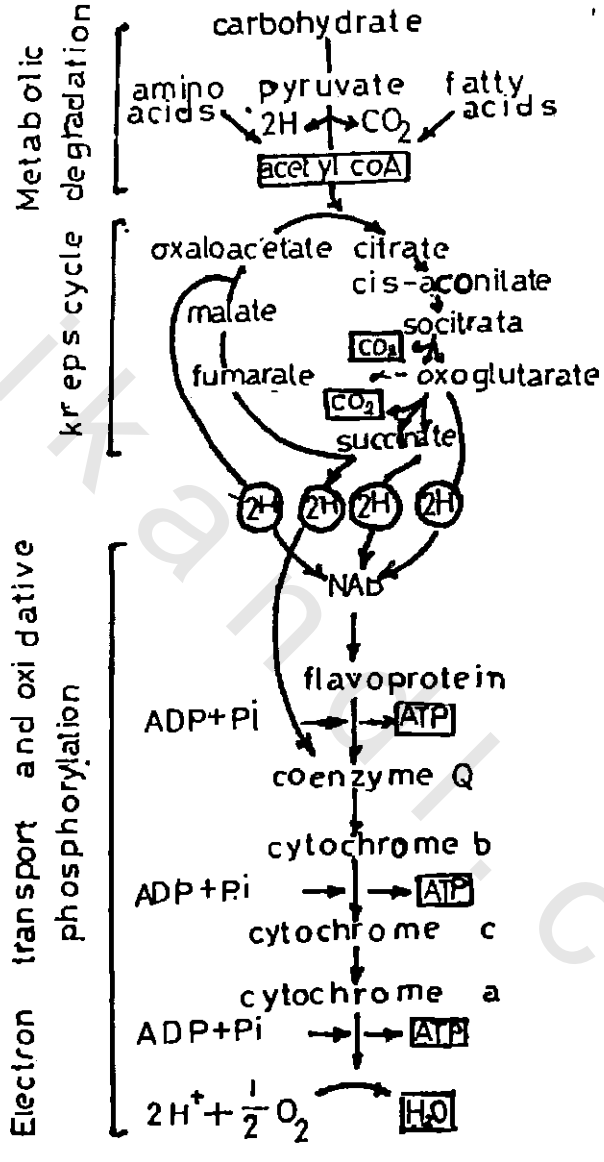


Fig. 141 :

Diagrmmatic representation of aerobic respiration showing the krebs cycle, the respiratory chain and its coupling with oxidative phosphorylation.

CHAPTER 23

CELL SECRETION

The secretory process is usually defined as the result of a cellular activity which takes place place in three stages: (1) absorption by the cell of certain substance (this passage of materials through the plasma membrane in known as ingestion); (2) formation inside the cell of the products (this is called synthesis); (3) elimination of the secretory products (this stage of the cell activity is called extrusion). The products of secretion may be used by other cells, many stimulate or inhibit other cells, or may act chemically on other substances, or may be totally eliminated from the animal. Generally speaking, secretion may be defined as the process by which cells synthesize substances that will be used by other cells or eliminated from the body.

Secretion is one of the most complex cellular functions. Most of the cellular components (the Golgi apparatus, endoplasmic reticulum, ribosomes, nucleolus and nucleus) participate directly or indidrctly in the secretion process. The roles of these components in the process of secretion have been discussed in previous chapters.

Secretory Cycle

Nature of secretory products :

Secretory substances are either clearly visible in the cells and are thus easily illustrated by cytological techniques, or are not visible in the cells and are not demonstrated by cytological methods. Hence, two kinds of secretions are recognized.

1 – In one case, the products of secretion are easily visible with the light microscope. These products are accumulated in the cell and then are eliminated outside the cell. These substances may be dense and refractile granules, vacuoles, droplets and so forth. They occupy a difinite position in the cell and usually possess specific histochemical reactions. This case is clearly observed in certain types of cells as in the pancreas. Cells of the pnacreas belong to the category of cells of zymogenic secretion, namely, protein-rich proenzymes. In the bottom of the cell, the mucleus, ribonucleoprotein substances and elongated mitochondria are found. During active secretion, the apical part of cell is occupied by certain

refractive granules rich in protein, these are the zymogen granules. Among the zymogen granules the Golgi apparatus reticulum is found. Latter, the zymogen granules become liquified and pass out through the rupture of the apical part of the cell membrane. During secretion the Golgi apparatus is enlarged and its stainability is highly increased.

2 – In the second category of secretions, it is proved physiologically that cells carry on the process of secretion, but the products of secretion cannot be illustrated cytochemically. A good example is the parathyroid gland (Fig. 142) which secretes a powerful hormone that regulates calcium metabolism in this case, the existence of the secretory cycle may be demonstrated by studying the modifications which are produced in the cell components during the activity of the gland. Thus, when the gland was injected with a single large dose of parathyroid extract, certain cytological and cytochemical changes were observed. First of all, the cells acquired a homogeneous aspect. Osmic acid showed the Golgi apparatus in the form a simple reticular apparatus. After some time, the apparatus of Golgi became fragmented into small elements which were scattered in the cytoplasm. Besides, a large number of vacuoles and vesicular mitochondria were observed in the cells. In this case, the secretion products are not visible, but the different changes in the cytoplasmic organoids and inclusions reflect the phases of secretion in the cells.

Fig. 142 : Parathyroid cells showing secretory activity. A, cell in repose with a simple Golgi apparatus. B, hypertrophy and fragmentation of the Golge apparatus, showing accumulation of secretion. D, dark cell in stage of excretion. (From De Robertis et al).

Methods for the study of the secretory cycle

1 - Cytological study of the secretory cycle :

(a) By using classical techniques :

The secretory cell may be compared to a complex machine which produces secretory substances and expels them. The secretion process is continuous and thus the different cells of the same secretory gland represent different stages of activity. Therefore, the histological or histochemical preparations obtained from such a gland represent different morphological features according to their different activities. It is clear that when a cell is fixed and stained, the image obtained shows only one step of function at which the cell is fixed. In the study of secretion the time factor important and must be taken into consideration in order to interpret the results of cytomorphological analysis. The best method for studying the secretion process is, therefore, vital observation for a sufficient time.

In order to get a nearly uniform picture, certain stimuli are used which modify the activity of cells and arrange these activities in a uniform manner. Thus, if it is fasted in order to bring about a state of repose of the gland. Then the cells are stimulated by a stimulating substance (as pilocarpine) which causes the rapid excretion of the products of secretion. In this way, all cells represent nearly a similar picture of activity in the whole gland.

Fig. 143 : Process of secretion in the thyroid gland, studied by freezing drying technique. Thyroid cells (1.,2,3,) 30 to 60 minutes after the injection of the hormone ec, excreted droplet; cd colloid droplets; bc, basal colloid. 4 and 5 thyroid cells 3 and 22 hours after injection. (From De Robertis et al.).

(b) By using the freezing drying method :

This method is valuable in the study of the secretory cycle, since it acts mainly to use the different cellular activities very rapidly. Therefore, no further changes are allowed to take place in the cells during fixation. Besides, the method shows the different soluble products and protein secretions even when they are in great dilution. For example, in the thyroid gland (Fig. 143) this method revealed an intracellular colloid which was not detected by classical techniques. By the freezing drying method, it was also possible to follow the different stages of formation and excretion of this secretion colloid. In these experiments, when the animal was injected with thyrotropic hormone, there appeared after few minutes – at the apical pole of the cell numerous droplets of colloid which are eventually excreted into the interior of the thyroid follicle. The outward passage of the secretory substances takes place by the rupture of the cell membrane at the apical part.

II - Biochemical studies of secretory cycle :

By using biomicroscopic and biometric methods some interesting results concerning secretion have been obtained. The number of pancreatic zymogen granules was measured at different intervales after stimulation with pilocarpine, Curves expressing the ratio if synthesis and extrusion of these granules were then obtained. The number of granules was compared with the concentration of carboxypolypeptidase (a digestiv enzyme excreted into the panreatic juice) and dipeptidase (an enzyme found only in the tissue). It was found that carboxypolypeptidase reached a minimum concentration after 3 hours of pilocarpine injection and was restored after 9 hours, the concentration of dipeptidase was changed in the opposite direction.

It was also found that the rates of resynthesis of amylase, protease, lipase and carboxypeptidase are very rapid after the expulsion of the granules, and as early as 30 minutes following stimulation, the synthesis of characteristic proteins of the enzymes takes place and reaches the resting level after 6 hours.

III - Studies with Isotopes :

Isotopes have been introduced in the study of cell secretion. After injection of glycine-C14 or methionine-S35 stimulation of the gland shown that the radio activity of the pancreatic juice increases rapidly and reaches a maximum between the second and fifth hours. The anino acid penetrates the cell very rapidly, reaching a maximum in about 10 minutes, and the expulsion of the secretory products is also very rapid.

These different results indicate that the problem of secretion is ralated to the enzymyatic machinery necessary for the synthesis of proteins. In other words, it is suggested that in secretory cells there is a biochemical organization of the various enzyme systems which is concerned with the submicroscopic structure, particularly with the RNP granules. In the pancreas, the RNP granules which are attached to the membrane of the endoplasmic reticulum are incroporated more rapidly. On the other hand, free RNP granules show less and slower incroporation. According to these data, the synthesis of the specific enzyme proteins of the pancreas occurs, first in the RNP granules (ribosomes) attached to the membrances of the endoplasmic reticulum. The newly synthesized proteins are then transported into the cisternae of the endoplasmic reticulum (intracisternal granules), and by this route reach the Golgi apparatus where they are formed and stored as zymogen grnules; the granules are then released into the lumen of the acinus (Fig. 144).

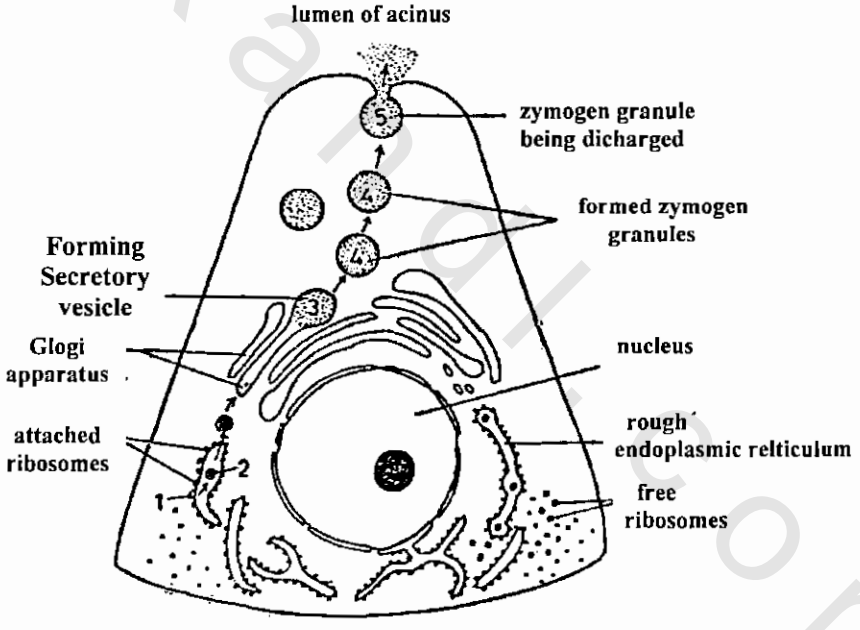


Fig. 144 :

Secretory process in the pancreatic acinus. 1, ribosomal stage; 2, endoplasmic reticulum stage; 3, Golgi apparatus stage; 4, zymogen stage; 5, release of zymogen into the lumen of the acinus.

Origin of secretion material :

Several theories were introduced to explain the origin of secretion material. These are summarized as follows:

1 - Nuclear theory :

According to this theory, the nucleus is responsible for the formation of the secretion granules. This is one of the old theories. However, nucleolar extrusion has been described and illustrated by several authors (Figs. 145-147). The fundamental functions of the nucleus and the nucleolus should also be mentioned here, e.g., the production of the different RNA molecules by genes present in the DNA of the chromosomes; the function of the nucleolus in the biogenesis of ribosomes; and the polyribosomes as the sites of synthesis of different proteins.

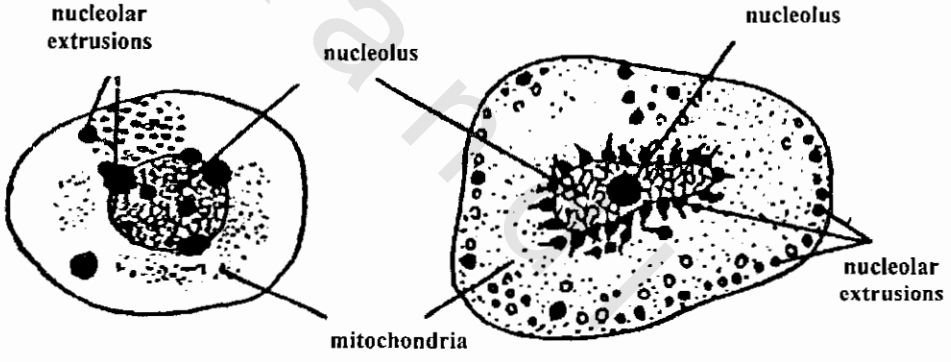


Fig. 145 :

Spermatocyte (A) and oocyte (B) of Saccocirus showing nuclear extrusions. (After Gatenby).

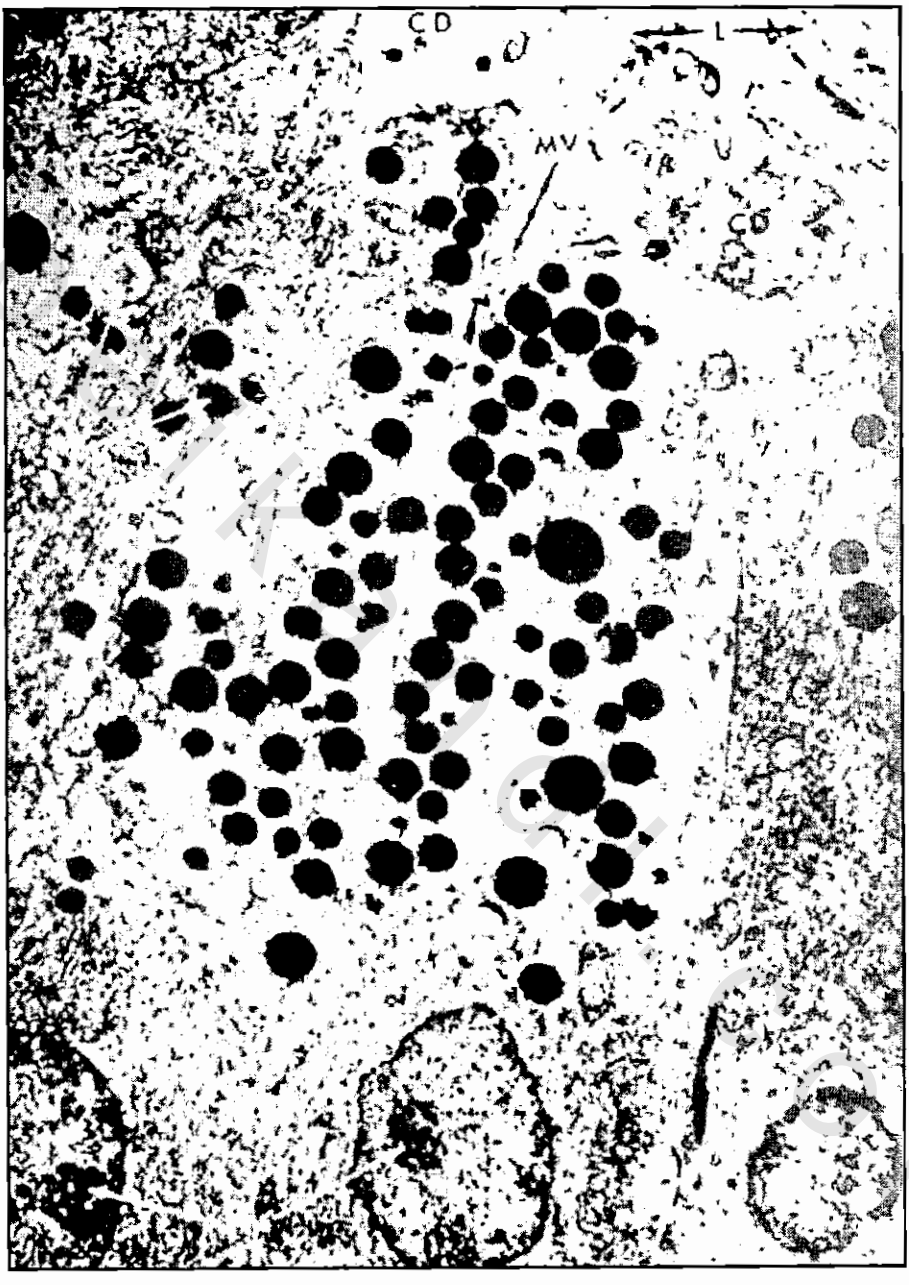


Fig. 146 : Electron micrograph showing secretory (black) or zymogen granules.

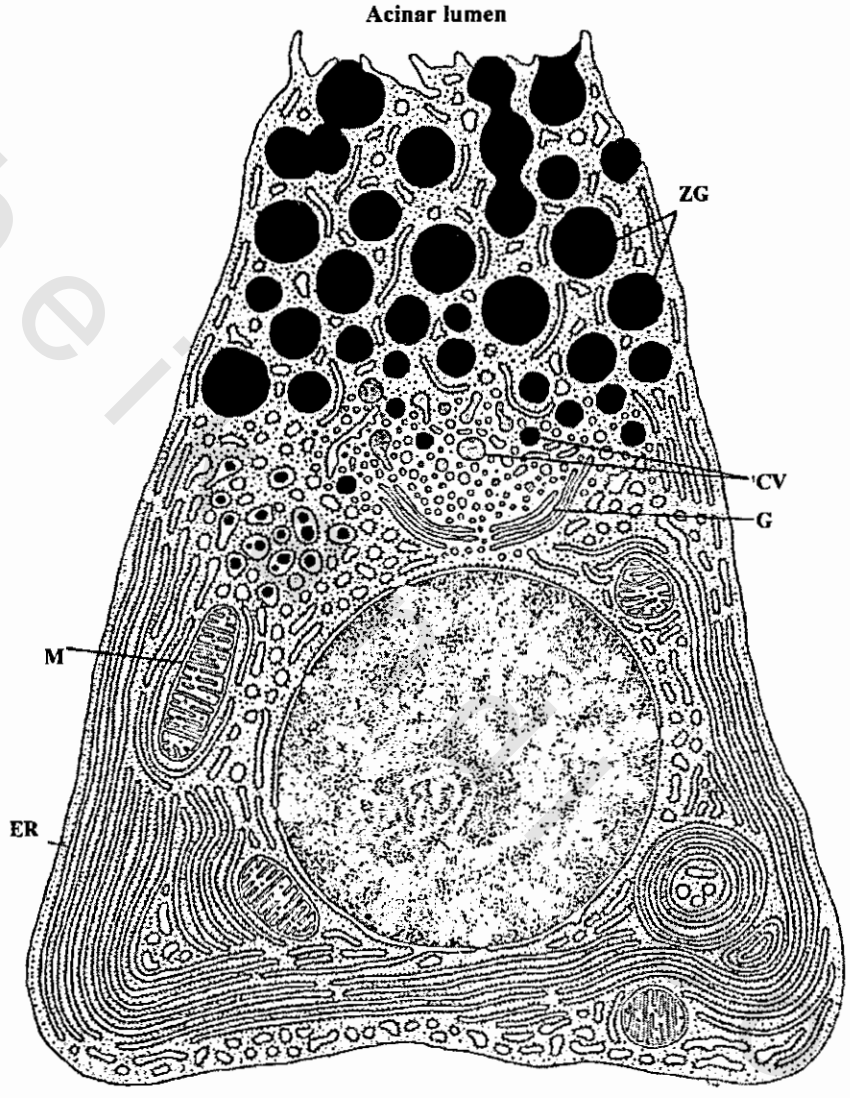


Fig. 147 :

Idealized pancreatic acinar cell showing stacked endoplasmic reticulum (ER), mitochondria (M), the Golgi complex (G), condensing vacuoles (CV), and zymogen granules (ZG). The apical end of the cell is at top, beyond which lies the acinar lumen.

2 - Chromidial theory :

Toward the end of the last century certain authors described the secretory granules and the morphological changes which the cells undergo during the secretory cycle. Then they suggested that the Nissl substance comes out form the nucleus, and that this substance is responsible for the formation of the secretion granules. But recently, it has been confirmend that the Nissl bodies do not originate from the nucleus and that they are not responsible for the elaboration of secretion granules.

3 - Chondriosome theory :

Later, with the development of cytological techniques the mitochondria were demonstrated in the cells. Some authors claimed that secretion granules are derived from the subsance of the chondriosomes.

4 - Golgi apparatus theory :

A large number of authors believe that the Golgi apparatus plays a fundamental role in the secretory activity of the cells. This has been shown by the following examples:

(A) In many secretory cells as the goblet cells, salivary gland cells and exocrine cells of the pancrease the Golgi apparatus is much hypertrophied during the process of secretion. This apparatus is enlaraged and spread in the apical region which becomes occupied by the secretion (or zymogen) granules. Similar changes were also recorded in the parathyroid gland, hepatic cells and enamel-secreting cells during the secretory activity.

(B) A good example of the relationship of the Golgi apparatus to the secretory activity is shown in the uterine gland cell (Fig. 148). In initmate association with the Golgi apparatus there appears a secretory substance in the form of round and oval corpuscles.

(C) In the secreoty epithelial and peptic cells of mammals the Golgi apparatus is demonstrated in the fasted animals in the form of a dense and compact reticulum close to the apical pole of the nucleus. After feeding, the Golgi network begins to spread and its meshes are much extended. At the same time, certain granules appear in the Golgi apparatus region. There are the secretions of zymogen granules. These granules are gradually increased until they occupy most of the secretory pole of the cell. Later, these granules are extruded from the cells.

(D) Applying the acetic-silver nitrate method of Bourne, it was posible to demonstrate ascorbic acid granules in the region of the Golgi apparatus. This led to the conclusion that the Golgi apparatus is closely related to the production of ascorbic acid in the cells.

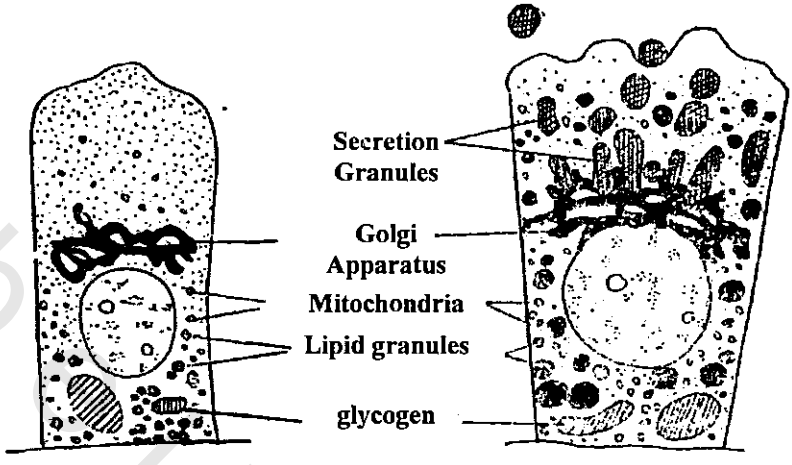


Fig. 148 : Secretory changes in the uterine gland cells. Notice secretion granules appearing in the meshes of the Golgi apparatus (see Gravid cell) and some are being extruded from the cell.

(E) In the nerve cells of mollusca, the senior author was able to prove that the coloured lipoidal pigments (lipofuchsin granules) were secreted as a result of activity of the Golgi dictyosomes. At first, these elements are lying very close to the Golgi dictyosome, then they move away and become scatterd in the cytoplasm (Fig. 149).

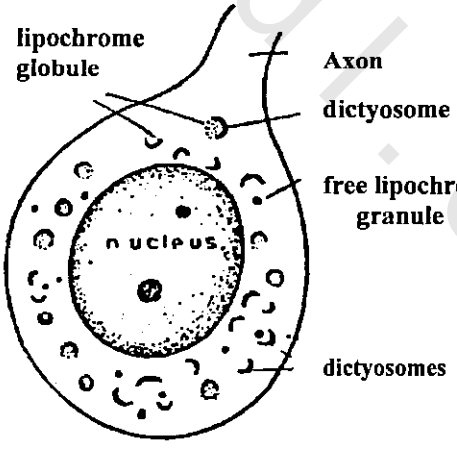


Fig. 149 :

Neurone of middle-aged Limnaea unstained and viewed under the phase contrast microscope showing the secretion of lipochrome granules by the Golgi dictyosomes. (After Moussa).

Significance of the secretion granules :

It is established that the granules demonstrated in the majority of gland cells represent secretion products. Thus, for example, it is believed that the zymogen granules of the exocrine pancreatic cells contain the various enzymes (in the form of proenzymes) found in the pancreatic juice. Also the granules in the peptic cells of the stomach contain pepsin. It has also been proved by accurate methods that some products of secretion are really found in the granules or droplets occurring in the cells. For example, when the content of the granules in the pancreatic cells was determined quantitatively at various times following stimulation with pilocarpine, and at the same time the concentration of carboxy polypeptidase was analyzed, a correlation was found between both of them. This result indicates that carboxypolypeptidase is really found in the zymogen granules.

From the submicroscopic point of view, several studies have been carried out to clarify the relationship between the fine structure of the cytoplasm and the secretion products.

In other words, studies by using the electron microscope and the ctyochemical techniques have been made in order to find out the correlation between structure and function. It is now generally believed that the ribosomes take major part in the process of protein synthesis, and that the Golgi apparatus is the site in which maturation of the secretion products takes place. Palay (1958) made extensive electron microscopic studies on some apocrine, merocrine and holocrine glands. The most significant work of this author in this respect is that carried out on the cells of the adrenal gland which are known to produce catecholamines (epinephrine and norepinephrine). The catecholamine material reduces osmium tetroxide very greatly and hence it can be detected even if it is found in a very small amount. At first, very small secretion droplets are visible in the cytoplasm just near the nuclear membrane. Small vesicles belonging to the Golgi apparatus become filled with very dense material of catecholamine. These vesicles migrate towards the cell periphery while increasing in density and size. Thus, the cytoplasm becomes filled with catechol-containing droplets (about 160 m u in diameter)., in this gland excretion of the secretory material is mediated through the splanchnic nerves which innervate the cell by the terminal endings which have a number of snyaptic vesicles (Fig. 150). These findings are cholinergic, which means that stimulation of the nerve releases acetyle choline thus activates the execration of catecholamine. The electrical srimulation which produces the activity in the nerve endings as shown by the highly increased synaptic vesicles, and the amount of acetylcholine is also much more released.

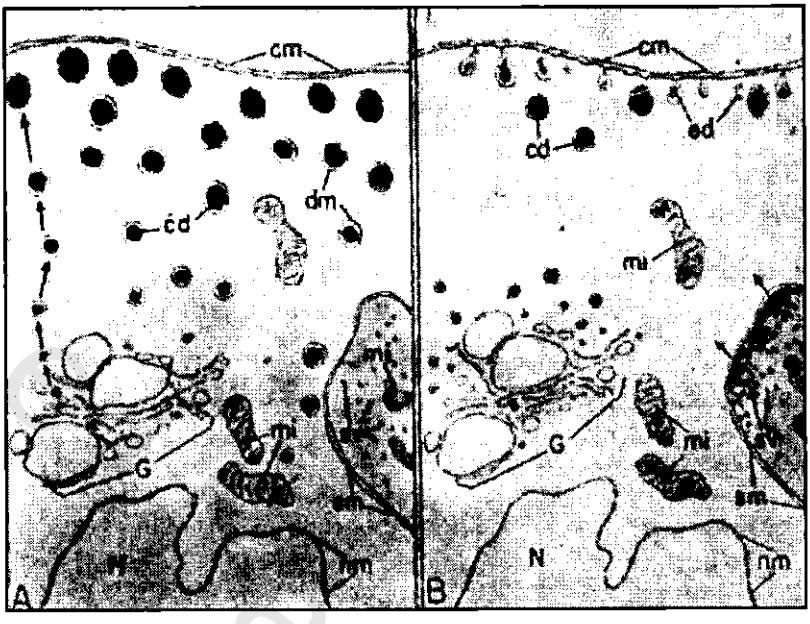


Fig. 150 :

Diagram showing the mechanism of secretion in the chromaffin cell from the adrenal gland of hamster.

A: Cell in the resting stage showing the storage of mature catchol droplets (cd) concentrated near the cell membrane (cm.). Within the Golgi apparatus (G.) new secretion is bieng formed at a slow rate. SV synapyic vesicles; ml, mitochondria; N, nucleus; nm, nuclear membrane; sm, surface membrane.

B: Cell after strong electrical stimulation by way of the splanchnic nerve. Most of the cd have disappeared. The nerve ending shows an increase of synaptic vesicles. (After De Robertis and Sabatini).

Concerning the actual expulsion of the secretory product into the intercellular spaces, it was found that catechol-containing droplets become attached to the surface membrane. At the beginning, they increase in size and become less dense (swelling); then the dense material is evacuated leaving empty spaces which probably disappear within the surface membrane. Simultaneously, new croplet are formed in the Golgi apparatus. By the mechanism, acetylcholine, epinephrine and norepinephrine of other action agents may be synthesized stared and then discharged at the surface membrane under the influence of a stimulus.

In has been found that the release of catecholamines takes place in the presence of Ca++.

CHAPTER 24

SENILITY AND DEATH OF CELLS

The cells usually pass by period of differentiation by which they are adapted to perform their specific role which ends with death.

In adult individuals certain cells remain undifferentiated and keep their reproductive capacity. But, most of the body tissues undergo a process of differentiation followed by senility changes. In this respect tissues usually fall in on the following categories:

1 - **Labile cells:** whose life is short and senility takes place immediately before reaching an advanced degree of cellular differentiation. Examples are the erythrocytes and granulocytes of blood which are continually destroyed and replaced by the multiplication of the undifferentiated cells of the bone marrow. In addition, the epithelial cells of the epidermis (Fig. 151), corneal and certain glands and mucous membranes are continually renewed. The life time of such cells is usually dependent upon many factors, some of which are mechanical and others are chemical.

2 - **Stable cells:** In these cells the multiplication capacity is maintained until the end of the growth period of the organism. Examples are the parenchymatous cells of the liver, pancreas, salivary glands, kidney, thyroid, parathyroid and smooth muscles.

3 - **Perennial cells:** These undergo differentiation very early during embryonic development. Multiplication of undifferentiated cells gradually decreases and stops within the embryonic period. When this stage has been reached the number of differentiated cells can no longer increase; but increase in the cell volume occurs. Examples are the striated muscle fibers and nerve cells which undergo senility changes but persist until the death of the animal.

According to Leblond and Walker (1956), there is certain renewal which takes place in the cells of the organism. The rate of renewal of the cells and their life span are now studied by the techniques involving labeling of the cells with radio-isotopes and tracing them with autoradiography. This labeling technique is based on the fact that the synthesis of RNA generally takes place prior to cell division.

SENILITY CHANGES

Cellular changes during senility :

Cell senility is usually accompanied by certain cytological and cytochemical changes. The most marked feature is the accumulation of the pigments of exhaustion, (also called the **"wear and tear"** pigments) which occur particularly in the nerve cells and in lesser degree in the cells of the liver, kidney, testis, ovary and thyroid. These senility pigments are considered by Casselman (1951) to be derived from the oxidation of unsaturated lipids. This has not been accepted by Moussa (1952) and Moussa and Banhawy (1955) who indicated that the senility pigment granules are secreted by the Golgi apparatus of old and senile neurones, and that the granules after being fully formed leave the Golgi element and lie free in the cytoplasm then travel towards the axon-pole of the cell forming a dark mass in senile nerones (Fig. 152). A similar accumulation of pigment granules in human sympathetic neurones was described by Murray and stout !1947) and regarded by them as an indication of the coming death of the nerve cell. These senility pigments are not soluble in fat solvents and are stained with sudan black.

However, there is certain discussion as to the significance of pigmentation as a sign of ageing of the nerve cells. The genral opinion is that the accumulation of pigment in senile cells is due to the progressive difficulty of the cells in exctreting the poorely soluble products, and this is regarded as an important factor in ageing of cells.

Other changes observed in this connection are: the accumulation of small droplets of fat, a decrease and fragmentation of the Nissl substance and a decrease of the cell volume.

Several authors managed to study senility changes in tissue cultures. Cultured cells, like the whole organism, pass through a series of stages, but at a much faster rate. These stages include: increase in mass, differentiation and organization, equilibrium, senility, death and dissolution. These changes are generally characterized by the appearance of vacuoles and fat droplets in the cytoplasm; then the cells are disintegrated.

During senility the nucleus is shrunken and its staining ability is highly increased; at the same time its structural details are progressively lost. This

process is spoken of as **nuclear pyknosis** (pyknos = dense) and is followed by the cell death.

Furtheremore, the normal senility, especially in nerve cells is accompanied by a retraction of the cell boundaries, loss of transparaeny of the nuclear sap, a great decrease in the **Nissl substance** (Fig. 153) and ascorbic acid content (Figs. 154 and 155), fragmentation of the Golgi apparatus and the formation of senility pigments. Degeneration of neurofibrils (Fig. 156) was also described. In addition, the **glycogen** found in the spinal ganglion neurons of some young and adult animals is replaced by a mucoprotein in senile condition.

Whereas fibrosis is a common feature of senility in some organs such as the thyroid, it does not appear in other ones such as the liver, in this latter case, giant nuclie and intranuclear inclusions are often seen.

It must be noted that besides these cellular changes, some other alterations are observed in the whole body which could not be separated form these cellular changes. Calcium shows marked increase in the blood and also in many tissues (except bone). The increase of the calcium content has been also reported in the cytoplasm of plant cell, in sea – urchin eggs and in some other tissues. These changes are probably related to decrease in cell permeability. However , it has been found that the experimental reduction of calcium increases longivity. The suggestion has been made that calcium is bound to ribonucleoprotein at all surfaces, and that age changes are reflected in calcium binding.

It has also been reported that there is a decrease in magnesium and an increase in iron and potassium.

As regards the organic compounds it has been found that cholesterol and insoluble proteins are increased. On the otherother hand, acid and alkaline phosphates and estrases decrease with advance of age. A decrease in tissue respiration and an inhibition of protein synthesis have been also reported.

Causes of senility :

Several views were introduced to explain ageing or senility; these can be summarized as follows:

1 - According to one view senility is due to an **alteration of the colloidal state of the protoplasm**. In this case, the degree of dispersion decrease, with loss of water and of electrical charge. In the mean time, protein substances which are resistant to the action of enzymes increase, but the other content diminishes. Example is the cornification process that occurs in some stratified epithelia.

2 - Senility may be due to the **accumulation of residues**, which together with condensation of colloids and decrease of cell permeability disturb the metabolism of the cell. But there is certain argument hwether these residues are the cause or the result of senility.

3 - The medium in which the cells survive are considered to be of great importance. Changes in its composition may affect the development and may cause senility. However, Cowdry believes that senility is due to the internal medium, where the regulatory memchanisms which maintain the uniformity of the blood are disturbed with ageing and the tissue fluids are also altered.

4 - Some authors consider that the **hormonal control** of the body "tissues is of utmost importance". According to this concept senility is due to the decrease in the synthesis of the governing homones such as those of the pituitary gland (e.g., growth and gonadotrophic hormones). This is because the formation and function of many vitamins, hormones and coenzymes are dependent upon these governing hormones.

5 - **External factors** (e.g., accumulation of metabolic products lack of food, and some factors in the plasma of senile animals) are considered to be the cause of senility of cultured cells. However, some authors suggest that the internal media contain some factors which affect the life history of cultured cells.

6 - Some authors are of the opinion that senility is due to factors which are found in the cell itself. This means that some cells have not only a fixed determination of their morphological and functional differentiation but also a predetermined limit of their potential life.

From the above-mentioned views, ageing of cells is due to factors which are either internal, external or lying in the cell and capable to limit the duration of its life.

DEATH OF CELLS

Cell senility leads ultimately to **catabiosis** (Gr.: Kata = down; bios = live) and to death. Howevere, clear knowledge about the cytoplysiological changes occuring in the cell is still lacking. Cell death is generally defined as the irreversible cessation of the vital phenomena, in some cases, however, death is produced rapidly by agents that cause an instantaneous coaglation or precipitation of the protoplasm (as in case of fixation, death by heat or by various agents), otherwise, cessation of cellular activities take place gradually. A cell many undergo irrepairable injury but some of its functions may continue for a certain time. If a tissue is ground some of

the metabolic phenomena such as oxygen consumption, fermentation and glycolysis continue for sometimes, but the processes decline rapidly with time until they disappear completely.

Other features of cell death are the lack of reproduction, growth and movement. Acidosis is usually found in the dead cells indicating certain oxidation-reduction variations (or pH), ultraviolet light is absorbed more intensely by the dead cells.

Both the cytoplasm and the mucleus of dead cells are diffusely stained by vital dyes such as neutral red, methylene blue and Janus dyes.

In cultured cells, the following changes are considered as signs of death:

- Retraction of pseudopodia and rounding up of the cell.
- Dissolution of the mitochondria.
- Diffuse vital staining of the cytoplasm and the nucleus.
- Coagulation and shrinkage of the nucleus.

Postmortem Changes :

It must be born in mind that the rapid death of the cell which is produced by such agents as fixatives that stop at the same time all enzyme activities-does not produce any postmortem alteration. The changes which occur during the slow and gradual death of the cell are usually grouped under the name **necrobiosis** (Gr.: nekros = death, bios = life). These changes are not postmortem.

In the different types of cells, some of the necrobiotic changes are variable, but, as a general rule, they include the diminution of the cell viscosity and liquefaction of the cytoplasm (necrobiosis by liquefaction). Sometimes, the viscosity is increased and the protoplasm is coagulated (necrobiosis by coagulation).

Death is usually preceded by a period characterized by an alteration of the normal metabolism and by degenerative changes of different types (fatty, waxy, pigmentary, etc.).

The **cloudy swelling** (appearance of protein granules in the cytoplasm) appears. This is preceded by the accumulation of acids and by the lowering of the pH. The cloudy swelling is generally followed by a progressive imbibiton of water and finally by the disintegration and dissolution of the cell. The mitochondria are fragmented into granules which undergo swelling.

The nucleus also undergoes different postmortem changes. In general, the nuclear structure and stainability resist the autolytic phenomena for a longer

time, and in many cases, the stainability increase. Pyknosis is generally accompanied by a shrinkage of the nucleus and the disappearance of its structural details.

Cytophotometric methods show that the increase in basophilia is not real because it does not correspond to an increase of absorbing material, but to the diminution of the nuclear volume. The protein content is greatly decreased, the DNA is gradually lost; this indicates that proteolysis occurs first. After that, the nucleases begin their action and break down the nucleic acid molecules. Then the nucleus loses its stainability and dissolves **(Karyolysis)**, either with or without previous nuclear fragmentation **(Karyorrhexis).**

The postmortem alterations of the cell are generally due to the activity of intracellular enzymes which start their action after the death of the cell. This hydrolytic enzymes break down the large molecules especially protein molecules (proteolysis). Simultaneously, the lack of oxygen favours anaerobic fermentation and the production of many acids particularly lactic acid. The accumulation of small molecules and ions resulting from autolysis increases the imbibition of water and thus caused the swelling of the cell.

It is established that irreversible coagulation of the protoplasm is one of the postmortem phenomena. This is often followed by the digestion, swelling and liquification of the cell.

Fig. 151 : Section of human skin. A, germinal zone; B, zone of differentiation; C, granular zone; D, zone of cell death (with pyknotic nuclei); E, keratinized zone (From Strehler: Time, Cells, and ageing).

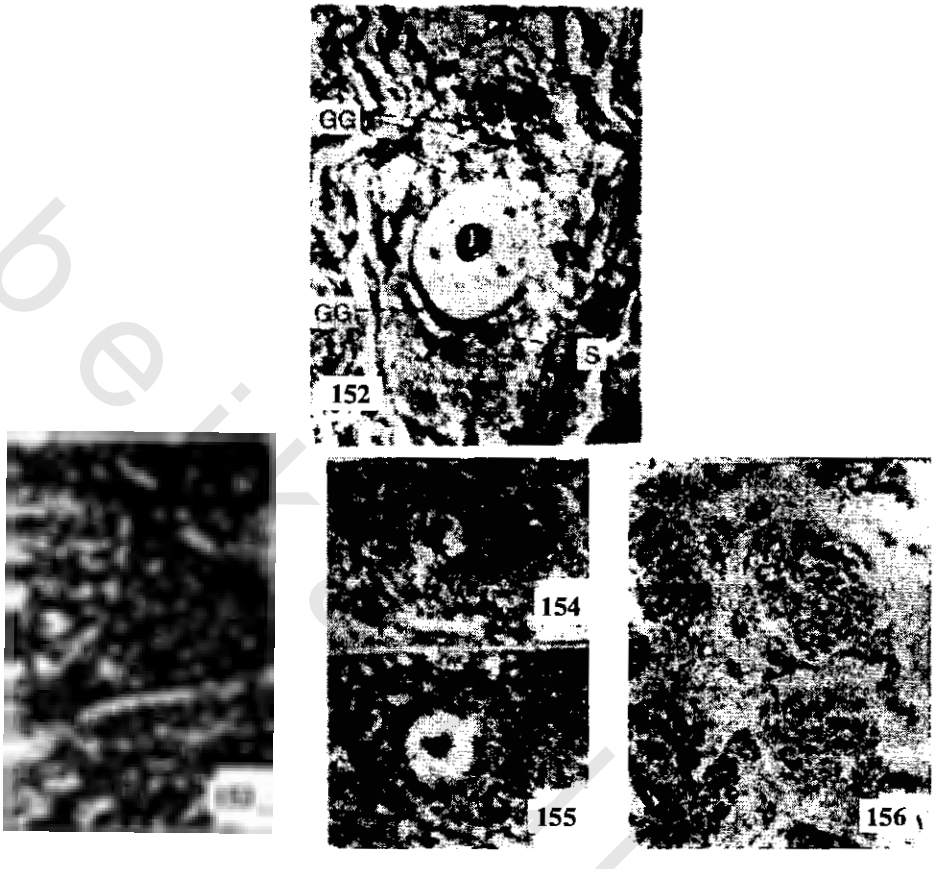


Fig. 152 : Mouse neurone viewd under the phase contrast microscope showing senility changes (After Moussa) GG., negative of Golgi apparatus. P, pigment or senility granules collected in the axon pole, S, sudanophile granules still in the golgi apparatus region.

Fig. 153 : Senile spinal ganglion neurones of guinea pig showing the decrease in Nissl bodies which become smaller in size and most of them are in the form of granules. (After Moussa and El-Beth).

Fig. 154, 155 : Spinal ganglion neurones of young (Fig. 154) and old (Fig. 155(chicks showing vitamin C. (After Moussa and Khattab). Notice the decrease in number and staining of vitamin C granules in the old neurone.

Fig. 156 : Spinal cord neurone of old chick showing neurofibrillae. Compare with Fig. 93 of young chick.

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