BIO 231
BIOLOGY OF CYTOLOGY, GENETICS AND PHYSIOLOGY

NATIONAL OPEN UNIVERSITY OF NIGERIA

BIO 231: CYTOLOGY, GENETICS AND PHYSIOLOGY

COURSE GUIDE

NATIONAL OPEN UNIVERSITY OF NIGERIA

This study is designed to give you an insight on how to study this unit. Each unit starts with an Introduction which leads you into what to expect within the unit. This is followed by the objectives which sets out what you are supposed to know at the end of each unit. Keep the objectives which sets out what you are supposed to know at the end of each unit. Keep the objectives in mind as you read the text and you can repeatedly ask yourself if you have achieved the objectives by understanding what the text contains.

The objectives are followed by the main contents of the text. Diagrams are put in place to encourage you to view what you are reading about. The diagrams are in two-dimensionals but you can put them in 3 dimentionals by obtaining biological samples where possible. Try to draw the diagrams and label them correctly, mind the spellings on the diagrams and of the names mentioned; this is very important.

The conclusion gives you highlight of what you have read in the text. The summary links the unit you have just read to other units in this course.

There are Self Assessment Question (SAQ) interposed in the units. These are to guide you to realize the objectives set out at the beginning of the units. You should sit down, time yourself and attempt these SAQ's. Some of the answers can be found in the text. If you can not answer the SAQ's satisfactorily, then re-read the appropriate section in the text until you are able to fulfill the set objectives.

Tutor-Marked Assignments (TMA) come last. These require deductive reasoning on your part. Some of the answers may be found in the text. You may need to consult other textbooks to answer these questions.

The department will set a time for the submission of the TMA for each unit. You will have to meet the deadline as the marks will be part of your final grade in the course. This system is to encourage you to read steadily and not leave everything to the end of the final examination.

The section that may tend to scare you in this course are those on Genetics. There is no magic about Genetics. It is part of life and living and should be of interest to you as a biologist. Follow the examples given and understand the general principles behind them so that you can use them to solve other problems.

I wish happy and fruitful reading.

COURSE CONTENTS

UNITS	TITLE
1	The Microscope
2.	Preparation and function of the Cell
b)	Structure and Function of the Cell
4	Membranes: General Structure and Function
5.	Cell Types
6.	Tissues, Organs and Systems
7.	Cellular Mechanics
8.	Transcription
9.	Translation
10.	Proteins and Enzymes: Structure and Function
11.	Theory of Germplasm: Cell Division
12.	Gametogenesis in Plants and Animals
13.	Introduction to Genetics
14.	Chromosome Theory of Inheritance
15.	Principles of Segregation
16.	Principles of Independent Assortment (Mendel's Second Law of Inheritance)
17.	Probability

18. Quantitative/Polygenic Inheritance

19. Sex Determination and Sex Linkage

20. Sex Linkages.

BIO 231: BIOLOGY OF CYTOLOGY, GENETICS AND PHYSIOLOGY

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NATIONAL OPEN UNIVERSITY OF NIGERIA

MODULE 1

UNIT 1: THE MICROSCOPE

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Overview

Cytology is the study of cells. Since the cell is the basic structural and functional unit of an organization, it is important that we do a study of the cell to be able to know how each cell functions and how the function affects the whole organism. As you already know, an organism can be made up of one or more cells. An organism consisting of only one cell is called a ***unicellular*** organism. One with more than one cell is called a ***multicellular*** organism.

In a unicellular organism, the single cell is able to carry out all the processes that characterizes life i.e. feeding, respiration, excretion, reproduction, growth, response to stimulus (irritability) etc. In multicellular organism, each cell is able to carry out these functions but the cells relate to one another. A

group of cells structurally alike and performing the same functions form a ***tissue***. Similarly, tissues performing the same functions group to form an ***organ***, organs form ***systems*** and many systems form an organism.

e.g epithelial cells → epithelial tissue (e.g. in skin)

epithelial tissue + muscle tissue → organ (e.g. inner lining of gut)

lungs + trachea → Respiratory system

Respiratory + Reproductive etc → animal (an organism)

A thorough understanding of the cell and functions of its various parts will lead us to a clear understanding of genetics. Genetics is the study of inheritance. This can not be fully understood unless you know the genetic materials held in the nucleus perform.

The nature and behaviour of the genetic material is very essential for the study of genetics.

The instrument used in studying the cell is the ***microscope.*** This has gone through a lot of developmental advances. A lot of advances are still being made on the microscope. We shall thus begin with the study of the history of development of the microscope. We shall then study the structure of the cell and the functions of its parts. This will enable us to cope with the learning of the rules and laws of genetics. Genetics tell us how and why a child resembles his parents or even fore-parents. Studies in genetics are being applied to improve quality and quantity of food crops. And as you might have read, there is cloning and genetic engineering going on in laboratories of research centres and these affect our lines either now or in the future.

1.0 Introduction

The study of Cytology is of the utmost importance to the understanding of the particulate nature of cell organization, cell function and the basis of life as we know it. Our present knowledge of cellular organization is a result of many years of intensive scientific research, and the information now available I the field have prove very useful in other fields such as taxonomy, agriculture, medicine, biochemistry etc.

The tools of cytology are very diverse and sophisticated depending on the level of study. At this level, it is essential to emphasize on the principles of

microscope action and cellular chemistry. Thus we should be able to appreciate the basic fundamentals of cytology.

This unit concentrates on the basic principles of microscope action. Cells are very minute substances and thus need to be enlarged or magnified before they can be studied. In this unit, aspects such as ***resolution, magnification*** and microscope optics are included

The development of cell science (cytology) as a discipline would not have reached the present stage without the discovery of the microscope. Except for some rather large unicellular cells most cells are very small and require a microscope for study. Even for those large cells that can be seen with the naked eye, the use of the microscope is essential in order to study the internal components of the cells.

Various methods of fixation and staining now enable specific parts of cells to be selectively revealed for study under the microscope. The staining procedures also provide information about the chemical and physiological properties of cells. These processes require adequate understanding of the use of the microscope. The microscope is therefore one of the most useful tools in cytology.

2.0 Objectives

At the end of this unit, you should

1. be familiar with the historical development of the microscope and the different kinds in existence.

2. know the different types of microscopes and how they function.

3. be familiar with the use and care of the microscope.

3.1 Brief History

The word "Microscope" was coined by Grovani Faber in 1625 A.D. The discovery of the microscope however, (dating back to 1000 A.D) followed the observation that water in a glass bowl magnified objects therein, and that a tiny drop of water acted as a magnifier. Thereafter, the present optical microscope emerged from the work of Anthony von Leevwenhoek, and others, between 1673 and 1683 A.D. These discoveries consisted of careful observations of minute plano-convex and bi-convex lenses capable of magnifying objects 30 to 200 times their normal sizes. With these

arrangements, bacteria, red blood cells, spermatozoa etc became visible to the naked eye for the first time.

The careful manipulation of two simple lenses to give the compound microscope was achieved by Galileo in 1610 A.D. Robert Hook, in 1665 A.D, made a microscope that was capable of observing opaque objects. Since then, the microscope had undergone modifications to evolve the present-day Compound Microscope, which is highly refined and versatile, and could be used to view many kinds of object. The phase-contrast microscope was developed in 1932 by F. Zernike, while the first electron microscope was constructed, also in 1932, by M. Knoll and E. Reiska.

The above historical review shows that the present laboratory microscope is the product of many years of devoted hard work by many scientist and should be treated with care and respect.

3.2 Microscopy

Several types of microscopes are available for the study of biological materials. Basically, they can be classified by the types of light source they use. The optical microscope is most general in use and will be discussed more extensively in this unit.

The light or optical microscope, of course, uses visible light, this could be from the sun or from an electric lamp. There are certain modifications of this, namely the polarization, phase contrast, interference , and dark-field microscopes. Microscopes which utilize invisible radiation, e.g. the ultra-violet, X-ray, and electron microscope are more recent developments.

The usefulness of any type of microscope depends not only on its ability to magnify, but more importantly its ability to resolve details. Beyond certain limits, magnification adds no new details. An ordinary light microscope can magnify about 1500 times the original size.

The resolution power of a microscope is a measure of its capacity to separate or distinguish quite clearly two points lying close together. Beyond the resolving power of any microscope, two points will appear as one. The resolution with lens systems is limited by the wave-length of light and by the numerical aperture (or light gathering capacity) of the objective lens. The resolving power of a well-constructed light microscope is about 0.2 μ.; any two points lying closer in less than this will be seen as one point.

3.2.1 The Light (or Optical) Microscope

Basically the light microscope is a system containing two magnifying glasses or lenses. An ***objective*** lens provides the initial magnification, and an ***ocular*** lens is placed symmetrically so as to magnify the primary image a second time (Fig.1.1). An additional condensing lens is normally employed beneath the stage of the microscope (Fig. !.1) to concentrate the light from its source into a very bright beam illuminating the object, thus providing sufficient light for the inspection of the magnified image.

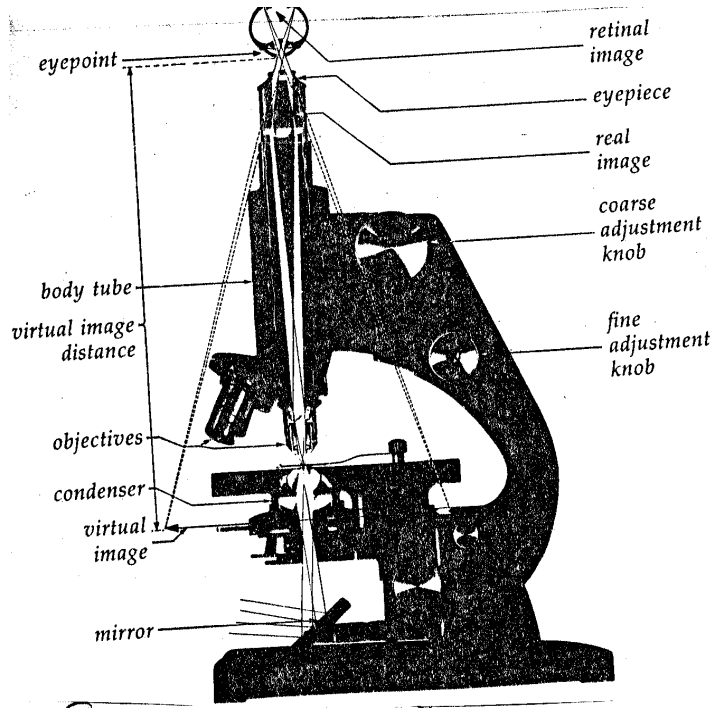


Fig 1.1: Construction of a typical compound microscope. Objective lenses of different power can be selected by rotating the knob which is focused on the specimen by the condenser located just below the stage. Courtesy of Optical Company.

3.2.2 The Polarizing Microscope

This instrument was developed by mineralogists who employ it in their study of crystalline materials. Many natural objects, including crystals and fibres, exhibit an optical property known as double refraction or birefringence. In biological materials, birefringence is caused by the orientation of particles too small to be resolved even by the best lenses. Thus, an examination of birefringence permits

deductions to be made concerning the organization of structure not demonstrable by regular methods of microscopy.

In its simplest form, the polarizing microscope is a conventional microscope in which a Nicol prism (or Polaroid sheet) is interposed in the light path below the condenser. This "Polarizer" converts al light passing through the instrument into plane polarized light, or light which vibrates in one optical plane only. A similar second prism, termed the "analyzer" is placed within the barrel of the microscope above the objective lens. When the analyzer is orientated so that its polarizing direction is parallel to that of the polarizer below, one sees the image. However, if the analyzer is rotated until its axis is perpendicular to that of the polarizer, no light ca pass through the ocular lens and the field is black. The field remains black if an ***isotropic***, or singly refractive, object is placed on the stage. A birefringent object, however, will appear light upon a dark background when examined in this manner. Birefringence, or ***anisotropy***, is exhibited by many biological structures, for example, muscle fibres, certain connective tissue fibres, lipid droplets within the adrenal cortex, and the rods and cones of the retina.

3.2.3 The Phase Contrast Microscope

Lack of contrast has always been a problem in biological work because the refractive indices of cytoplasm and its inclusions are similar. In normal microscopy, one overcomes this problem by staining differentially, but this is subject to numerous limitations, among which is that the stained biological object being viewed is in a dead state. Phase microscopy provides a method whereby contrast is created by purely optical means.

The refractive index is a measure of the optical density of an object, or the speed at which a light wave travels through it. Air, for instance has a refractive index of approximately 1.0, water about 1.3, and glass about 1.5. What this means is that light travels fastest, in air, water, and glasses will not emerge at the same time; they will emerge out of phase with each other.

The phase contrast microscope consists of optical plates placed within the condenser and objective lenses which converts the phase differences into amplitude differences. Briefly, therefore, differences in refractive index are rendered directly visible. Objects ordinarily transparent become visible through contrast differences. The phase contrast microscope is of no particular assistance in the study fixed

and stained biological preparation in which transparency differences are not important. The instrument finds its application chiefly in the study of living cells and of unstained tissues.

3.2.4 The Interference Microscope

The interference microscope, like the phase contrast microscope, depends upon the ability of an object to retard light. Unlike the phase contrast microscope which depends upon the specimen diffracting light, the interference microscope sends through the specimen two separate beams of light which then are combined in the image plane. After recombination, difference in retardation of light results in interference which can be used to measure the thickness or refractive index of the object under investigation.

3.2.5 The Dark-Field Microscope

This microscope utilizes a strong oblique light that does not enter the objective lens. A special dark-field condenser, in which no light passes through the centre of the lens, is employed. Light thus reaches the object to be viewed at an angle. So oblique that none of it can enter the objective lens. Small particles present in the field will reflect some light into the objective lens and appear as glistering spots. Thus it is possible to visualize particles far below the limits of bright light resolution. The effect is similar to the phenomenon of dust particles "seen" in a beam of sunlight entering a darkened room.

Dark-field examination is also se din the examination of small transparent objects such as chylomicrons, which are invisible in the glare of bright field illumination, and of microincineration specimens

The microscopes just discussed all utilize visible light. However, images can be formed by rays other than visible light; and in this instance, since the image cannot be viewed directly, they are made visible by means of a suitably sensitized photographic film. In general, the rays used in these special microscopes all have shorter wave lengths than that of visible light and thus permit higher resolution.

3.2.6 The Ultraviolet Microscope

Ultraviolet light cannot pass through ordinary optical lenses; the lenses are opaque to ultraviolet (UV) light. Hence quartz lenses are used in UV microscopes. In principle, this system allows an improvement in resolution about twice that of ordinary microscope (0.1μ).

Ultraviolet light is employed in ***fluorescence microscopy***. Many substances have the property of emitting visible light when irradiated by invisible rays. UV light is focused upon the specimen which glows and can be observed but is emitted fluorescence. Fluorescence may be naturally occurring in the specimen or may result from the introduction of fluorescent dyes bound to certain specific components of the specimen.

3.2.7 The X-Ray Microscope

X-rays have a shorter wavelength than visible or ultraviolet light and therefore has a greater penetration and, theoretically, a higher resolution power. By using preparation techniques similar to those used in light microscopy, the specimen can be placed upon a photographic emulsion and exposed to soft X-irradiation. The small X-ray, picture obtained is subsequently magnified optically. This process is known as contact microradiography. In ***projection x-ray microscopy***, a point source of x-rays casts a enlarged image of a nearby object upon a distant fluorescent screen or photographic plate. It is the contrast obtained as a result of differences in x-ray absorption that is utilized in these procedures. Resolution is either case is not particularly high.

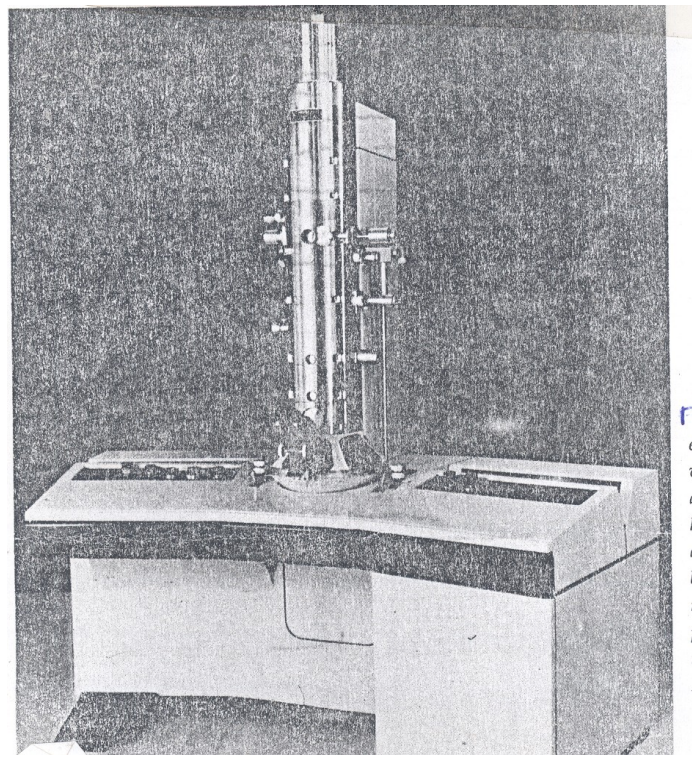


Fig. 1.2: a high-performance electron microscope. The electron lenses are housed in the cenral column, which is maintained under a high vacuum. To the right and left of the column are banks of controls by which lens current (focus) can be regulated. The image can directly viewed on a fluorescent screen through the binocular microscope; for permanent high resolution records, photographs are made. Specimens and photographic plates can be introduced without breaking the vacuum in the main column. Courtesy of the Perkins-Elmer Corporation.

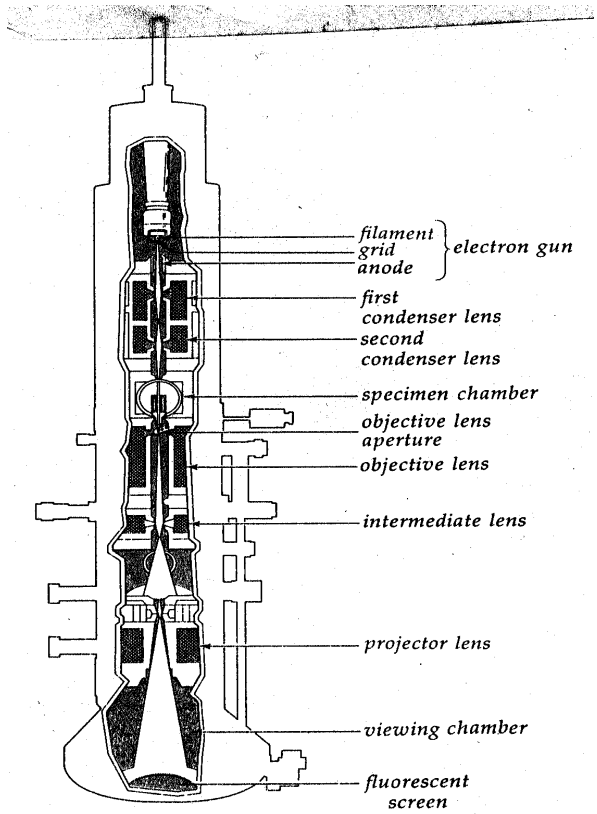


Fig. 1.3: Cutaway view of the lens column of the instrument shown in Fig. 3-14. Modified from the original diagrams supplied by the Perkins-Elmer Corporation.

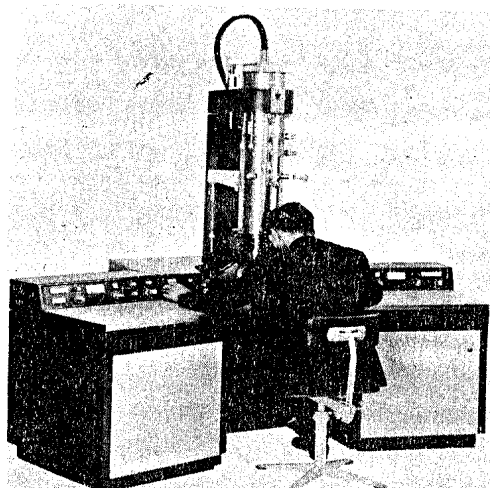
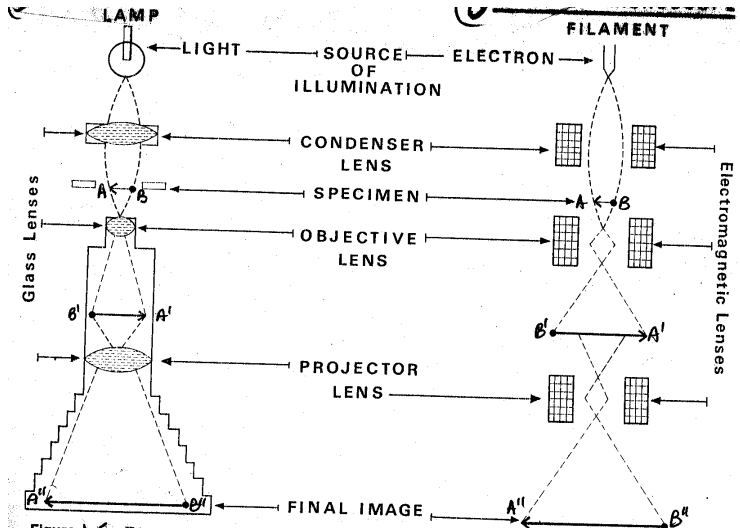


Fig 1.4: a modern electron microscope (Courtesy of Philips Electronic Instrumentss)

(a) LIGHT MICROSCOPE (b) ELECTRON MICROSCOPE



3.2.8 The Electron Microscope (EM)

The development of this type of microscope has revolutionized our entire concept of the cell. The EM (Fig. 1.2, 1.3 and 1.4) uses an electron beam instead of light and electromagnets instead of glass lenses. There are two types of electron microscopes:

a. The Transmission Electron Microscope (TEM)

b. The Scanning Electron Microscope (SEM)

Freed from the limitations imposed by the wavelength of light, the electron microscope has a resolving power five hundred times as great as the optical microscope. A good optical microscope can only magnify an object without loss of details about 1,500 times. The electron microscope can achieve magnification of over 500,000 times. To appreciate the magnifying power of the electron microscope, you can imagine an object the size of a pin head being enlarged, without loss of details, to over one kilometer. The impart on the EM on biology has been tremendous. Materials which had hitherto been described as structureless have been shown to have elaborate fluids organization and so called homogenous fluids are now known to contain a variety of complex structures.

In the electron microscope, the illuminating source is a beam of high velocity electrons accelerated in a vacuum (Fig. 1.3) (Since the EM

operated in a vacuum, the specimen to be observed must be thoroughly dried, so as not to contain any trace of water or moisture; hence live specimens cannot be viewed in an EM). The beam is passed through the specimen and focused upon a fluorescent screen or photographic plat by a series of electromagnetic or electrostatic fields (Fig. 1.5b). The wavelength of the electrons depends upon the acceleration voltage used. At voltages used routinely, the wavelength of the electrons are of the order of 0.05Å (Å, an ongstrom unit = 1 x $10^{-4}\mu$ or 1 x 10^{-7} mm). The electric or magnetic fields usd as lenses are imperfect and do not have the numerical aperture of optical lenses. The practical limit of resolution of the E.M is about 2Å, and the usual limit for biological preparation is about 3.5 Å.

The EM permits the observation of cells and tissue structure beyond that seen with the light microscope. Structures smaller than individual macromolecules can not be visualized. Structures seen under the EM are commonly described as ***fine structure or ultrastructure.*** Just as special techniques for preparing specimen for examination

3.3 The Compound Microscope

The compound microscope is a combination of two simple microscopes (a) the objective and (b) the ocular (or eyepiece) separated by a long tube known as he TUBE or BARREL. In the tube the objective is the lens closer to the object, while the ocular or eyepiece is the lens closer to the eye. In more recent times, improvements on the microscope have been mainly on mechanical rather than optical properties. The features listed below are clearly noticeable on the microscope you will be using in the laboratory. The features should be examined closely and you should make effort s to understand the function of each component so as not to cause any serious damage when using the microscope.

3.3.1 Features of Improvement on the Microscope

(a) The limb of the microscope is attached to the body tube and to the stage which carries the slide, in such a way that all optical parts above and below the stage are in correct alignment for the life of the instrument. This construction also provides a convenient carrying handle, with no distruption on the optical alignment of the component parts. (Fig. 1.1)

(b) The microscope is more or less streamlined with, the limb deeply covered and sharp edges or corners reduced to a minimum.

(c) A large distance between the optic axis and the limb is provided. This allows the fitting of a large square or circular mechanical stage with easy complete rotation. Thus large objects or spherical stage attachment are readily accommodated.

(d) The inclined binocular body can be quickly interchanged with vertical or inclined monocular tubes, with the alignment of the eye piece and objective lenses remaining undisturbed.

(e) The rapid interchange of substage condensers for light and dark fields is another improvement on the microscope.

3.4 Optical Principles of the Microscope

The construction of the optical compound microscope is based on the principle of the effect of light rays passing media of different reflective indices, and of their passing through different thickness of the same medium, which in this instance, is glass. Objects are distinguished from each other because they reflect light of varying intensity and colour. In an unstained tissue the different parts can only be distinguished if they have different refractive indices. This is because the light reflected by the different parts will vary in intensity and colour.

When two rays of light from the same source pass through media of different refractive indices, the ray passing through the medium of higher refractive index is retarded relative to that passing through the medium of lower reflective index. Thus the two rays emerging from the two media, though still of the same amphitude (i.e. intensity) will be "out of step", and will thus ***interfere*** with each other when recombined. It is this interference that enables the observer to discriminate between different parts of the object. This INTERFERENCE EFFECT (the underlying effect in microscope viewing) depends on the ***wavelength*** (λ) of the light used for illumination of the object under examination.

From two objects to be discriminated in the microscope field of view, the distance between the objects must not be less than the wavelength (λ) of the light used; otherwise the two images will merge and remain as one (i.e unresolved). This minimum distance (approximately 0.3 μm) is called the ***resolving power*** of the microscope. In fact, the minimum size of objects that

can be seen with the average "student microscope" (with maximum magnification of x 400) is about 0.5 μm. With good quality microscope, it is about 0.3 μm, while with a "research type" microscope (using oil immersion objective) magnification x 1200) it is about 0.1 μm.

Another optical constant of great importance is microscopy is the ***numerical aperture*** (N.A.) of the objective. The ability of he objective lens to resolve or differentiate structures depends on the numerical aperture of the objective lens; the higher the numerical aperture of the objective the greater the resolution. The relationship between the N.A. and the resolving power of the microscope can be represented by the following equation.

$$R.P = \frac{\lambda}{2 \times NA}$$

where λ = wavelength of light employed
N.A = numerical aperture
R.P = resolving power

The NA is a function of the refractive index of the medium between the objective and the object, and the angular aperture of the object.

$$NA = n\sin\mu$$

Where n = refractive index of the medium between the object and the objective lens

μ = half the angular aperture of the objective

The N.A. will usually be found stamped on the objective mount either with or without the letter "NA". For general work, sufficient resolution will be obtained from a 4 mm objective having NA of 0.70.

The ocular is a biconvex lens combination of focal length (f2) greater than that of the objective. The ocular acts as an ordinary magnifying glass and produces a virtual inverted, but still larger image, B"A" (Fig 1.5a). Since the eye cannot satisfactorily focus onto any object within 25.5 cm distance of it, it focuses further into the final, much enlarged image B"A"(see Fig 1.2b) at the optimum distance of distinct vision (the near point) or at a point still farther from the eye.

The size of image B'A' relating to that of the object, AB, is directly proportional to the relative distance between the objects, the objective and the image, B'A'. Thus the size of the image B"A" can be controlled, altered or changed by altering the tube length of the microscope; the greater the tube length, the larger the image. For construction purpose the tube length is limited, for convenience, to a standard value of 160 mm, while some manufacturers use a standard tube length of 170 mm.

3.5 More Information on the Microscope

A. The Body Tube

Carries the objective or the resolving nose piece at the lower end. The microscope may carry a straight monocular body tube; this is essential for certain works requiring high powers where the introduction of prisms between the objective and the eye piece causes too much lose of light or which may be considered to adversely affect the clarity of the fine details of the image. In addition, such straight tube is necessary for measuring, drawing, microprojection and photomicrography.

The microscope may also carry an inclined monocular body tube. The angular eye piece somehow extends the mechanical tube length (i.e. more than the conventially 160 mm), thereby leading to increase in magnification. This extension over the standard is usually corrected by aligning the objective lens with a reflecting prism. The tube inclination usually varies between 30 and 45^0 from the vertical. The inclined monocular tube is very useful for prolonged observations.

B. The Coarse Adjustment

This is used for rapid and precise focusing of the objective. In most microscope models turning the pinion heads in a clockwise direction moves the body tube down, while moving it in a counterclocking direction moves the body up. In some other microscope models, the pinion moves the stage and substage as a unit, the body tube and objectives remaining in a fixed position. This later type of movement limits the range of possible movement, thereby restricting the lower limit of the objective power. An adjustment displacing the body tube has a long range so that he movement of the coarse adjustment knob is smooth and precise, with no loss of motion or play in the slides. The knob should not be stiff, nor too loose, so that the body tube sinks with its own weight. With careful handling and understanding, the

coarse adjustment should focus up to x 40 without recourse to the fine adjustments.

C. The Fine Adjustment

In nearly all compound microscopes, the fine adjustment moves a long-bearing slide, carrying the coarse adjustment, body tube, and objectives very slowly and precisely for a range of 2-3 mm. The operational direction is similar to that of the coarse adjustment. The fine adjustment is of paramount importance, and unless it operates very smoothly and with no trace of backlash, prolonged visual work will be tedious, and vertical high power photomicrography cannot be accomplished. In some microscope models, the fine adjustment moves to the stage or that part of the stage carrying the object slide. This type of movement is not very desirable, unless the substance carrying the condenser is moved at the same time.

D. The Substage

The substage is attached directly to the lower end of the limb. The main purpose of all adjustments below the stage is to allow for accurate focusing of the condenser. It also provides precise means of quickly centering any light or dark field condenser to the opic axis of the microscope. The simple substage contains a sleeve for the condenser, a clamping screw which has a swing-out ring to hold the dark ground stops, or glass colour filters. In the compound substage, the ring or fork carrying the condenser is filled with centering screws in order that all types of condensers can be quickly and easily centred to the objectives.

E. The Stage

The stage is attached to the limb by means of a broad, rigid fork, or bracelet. The mechanical arrangements for moving or orientating the slide vary from very simple to complex. In the modern microscopes, the two methods are:

i. the plain stage with spring clips

ii. the plain stage with a built-in mechanical device

The built-in mechanical system is activated by micrometer screws situated at right angles to the two directions of stage movement.

3.6 Some Hints on Focusing

I. The coarse adjustment is capable of sharply focusing all objectives up to the high dry 40X, and sometimes nearly the 90X oil immersion objective. Thus, only a fraction of movement should be needed for the final critical focusing with he fine adjustment. The haphazard turning of the fine adjustments for more than one resolution should be avoided.

II. use the correct methods for illumination. The image of the lamp diaphragm, or that of the light source may serve as a guide.

III. Bring the prominent part of the object to the centre of the field of view under the 10X objective before going to a higher power.

IV. Focusing downwards under high power with the coarse adjustment should be avoided in order to minimize damage to the slide and the front lens of the objective.

3.7 Magnification

An object viewed through a light microscope becomes enlarged as a result of the production of a magnified virtual image through the ocular (Fig. 1.2B). the degree of magnification is expressed in diameters.

The magnification by the objective lens is known as the primary magnification. This is equal to

$\frac{\lambda}{f}$ where λ = length of the microscope tube (usually 160 mm) and

f = focal length of the objective (usually marked on it).

The total magnificient (objective and eye piece lenses) is found by multiplying the primary magnification of the objective by the magnification of the eye piece.

i.e Total Magnification = $\frac{\lambda}{f} \times e$

where e = magnification of the eye piece

The appropriate total magnification for various lens combinations used in routine laboratory work is shown in Table 1.1

Table 1.1 Approximate Total Magnification for various lens combinations

OBJECTIVE			EYE PIECE						
FOCAL	Primary	Numerical	MAGNIFICATION						
LENGTHS	Magnification	Apperture	No. 0	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
(f)	(λ/f)	(N/A)	X4	X5	X6	X8	X10	X12	X16
2" or 50 mm	x 3.2	0.1	13	16	19	26	32	38	48
1" or 25 mm	x 6.4	0.17	26	32	38	51	64	76	96
2/3" or 16 mm	x 10	0.25	40	50	60	80	100	120	150
1/3" or 8 mm	x 20	0.60	80	100	120	160	200	240	300
1/6" or 4 mm	x 40	0.65	160	200	240	320	400	480	600
1/12" or 2.0 mm	x 80	-	320	400	480	640	800	960	1200
1/15" or 1.7 mm	x 94	1.25	376	470	564	752	840	1128	1410

3.8 Care of the Microscope

The microscope is a combination of carefully selected and balanced parts. Despite the attempts to make the instrument rigid and capable of withstanding much strains, the careful arrangements will quite often get damaged. The following faults may be quickly discovered:

1. The stage or inclinable limb showing signs of wobble.

2. Slack mirror allowing the mirror to move out of position, or making centering of the mirror difficult.

3. Difficulty in aligning the objective or condenser.

4. Sloppiness in the coarse adjustment resulting in objects slipping out of focus when left for a few minutes.

These faults are best corrected by trained experts in such work.

A microscope should be stored in the case provided, with the lid shut to keep out dust. Any dust which settle on the back lens of the objective during storage should b e carefully removed with a carmel hair brush. Dirty dusty or scratched lens must never be cleaned with a duster or handkerchief. These should be cleaned with lens tissues, applying a rotary motion, after first breathing on the lens.

If the lens has acquired some greasy deposits or if the dirt is difficult to remove, this should be wiped off with a lens tissue bearing a trace of benzene or xylene. Excess benzene or xylene should be avoided as this will dissolve the lens cement. Polish off immediately, but gently, using dry lens tissue. The microscope stage should be kept clean always, but not by wiping with a lens tissue; a clean linen can be used or this purpose.

Self Assessment Questions

1. Name at lease three types of microscopes used in modern laboratories.

2. Explain the relationship between the resolution of a microscope and the numerical aperture of its objectives.

3. What is the significance of the numerical aperture.

4.0 Conclusion

1. Cytology is the study of the cell which is the structural and functional unit of a living organism.

2. Genetics is the study of the principles of inheritance.

3. The microscope has come a long way in its building and modification.

4. Types of microscope are:

    a. The light (optical microscope)

    b. The Phase contrast microscope

    c. The electron microscope (E.M.)
        i. Transmission electron microscope (TEM)

        ii. Scanning electron microscope (SEM)

5. The microscope is a series of magnifying lenses aligned with each other to bring about the enlargement of an object.

6. The compound microscope is a combination of two simple microscope (a) the objective and (b) the ocular (or eye piece) separated by a barrel.

7. The compound microscope is designed for easy carriage.

5.0 Summary

The invention of the microscope has enhanced the study of the cell. The electron microscope can enlarge an object up to 500,000 times and has a higher resolution than the optical microscope. The impact of the electron microscope on the study of organism has been tremendous and has helped biologists to better understand the cell and the phenomena of genetics.

6.0 Tutor Marked Assignment (TMA)

1. List three differences between the optical and electron microscopes.

2. Make a detailed diagram of a light microscope showing details of its parts.

7.0 Reference

Roberts, M.B.U. 1975 Biology: A Functional Approach. E.L.B.S and Nelson, Lagos

Answers to Self Assessment Questions

1. (a) Light (optical) microscope

   (b) Phase contrast microscope

   (c) Electron microscope (EM)
   (i) Transmission and (ii) Scanning electron microscope

(2) $\frac{\lambda}{2NA}$

Where λ is the lights wavelength and NA is the numerical apertive.

The resolving power of the microscope is the power to distinguish between two points closely side-by-side, NA is the ability of the objective lens to resolve or differentiate structure.

(3) The NA is the ability of the objective lens to resolve or differentiate structures and is a function of the refractive index of the medium between the objective and the object and the angular aperture of the objective.

UNIT 2: PREPARATION OF BIOLOGICAL MATERIALS FOR MICROSCOPY

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1.0 Introduction

Having gone through the historical development and the different types of microscopes, it is suitable, at this point, to discuss how materials can be prepared for viewing under the microscopes. It will be obvious to you that cells, tissues and organs cannot be studied to advantage unless they are suitably prepared for microscopic examination. The methods of preparation fall logically into two groups:

1. Methods involving the direct observation of living cells and
2. Methods employed with dead cells (fixed or preserved)

Living tissues are usually more difficult to handle and are valuable for a short period only. Nevertheless, it is important that you become aware of the methods by which living cells may be observed and understand the ways in which they differ from fixed cells. In the living cell, structure and function may be studied simultaneously. Living cells may be seen to move to ingest

foreign material, occasionally to divide and to carry out other functions. Dead or fixed cells cannot do these things.

2.0 Objectives

At the end of this unit you would be expected to:

1. Describe the methods of preparing for viewing living specimen under the microscopy.

2. Describe the preparation of dead tissue for light microscopy.

3. Describe the preparation of dead tissue for electron microscopy.

4. Enumerate stains used in preparrng specimens for microscopy.

5. Explain the phenomenon of histochemistry.

3.1 Observing Living Tissue

Unicellular organisms and, occasionally, free cells may be studied directly under the microscopy while they are still alive. Free cells are colourless and the structures within them lack contrast. This difficulty may be overcome by using a phase contrast microscope e.g. the web of the frog foot, the wing of the bat and the buccal pouch of the hamster. Thin sections of relatively thick organs such as liver and kidney may be viewed by ***transillumination*** with quartz rods, which produce a cold light and avoid coagulation of protoplasm. Small pieces of tissue for microscopic examination may be excised, placed in some relatively harmless liquid such as serum or an 0.85 per cent aqueous solution of sodium chloride, and teased apart gently with needles of fine steel or glass.

Prolonged preservation of living cells outside the body can be achieved through ***tissue culture***: fragments of tissue are removed aseptically, transferred to a physiological medium and kept at a temperature normal for the animal from which the tissue was taken. The cultures are placed in thin glass vessels or in hanging drops on a cover glass mounted over a hollow slide. In this way they are available for observation under the microscope. In such cultures, growth, multiplication, and in some cases differentiation of cells into other cell types can be observed directly. Tissue culture is a valuable method for the study of cancer and of many viruses.

Microdissection involves the use of an instrument which moves very fine glass needles with precision under the microscope. In this way small portions of a cell, such as a nucleus, can be removed and the effect observed.

Two staining methods have been applied successfully to living animals or surviving cells. In vital staining, dyes are injected into the living animal. The activity of certain cells will result in the selective absorption of the colouring material by these cells. An example of this procedure is the staining by trypan blue of macrophages on the basis of their ability to phagocytose foreign particles. ***Supravital staining*** involves the addition of dye stuff to a medium of cells already removed from the organism. Examples of this techniques are the staining of mitochondria in living cells by Janus green, of lysosomes by neutral red, and of nerve fibres and cells by methylene blue.

Finally, motion picture records aid in the understanding of cellular activities. Lapsed time films made of individual living cells or of tissue cultures help to analyse processes such as mitosis, phagocytosis and amoeboid movement. Slow-motion films of such rapid processes as the beating of cilia permit analysis of the action.

3.2 Preparation of Dead Tissue

3.2.1 Light Microscopy

Use is made of sections, which are thin slices cut from a piece of fixed tissue which is then stained, mounted in a medium of suitable refractive index upon a slide, and finally covered with a covership. The procedure for producing a histological section involves the following steps:

(a) Removal of the Specimen

For cytological purposes and for the best histological preparations, the material should be removed from an anesthetized animal. In the case of human material, this is scarcely ever possible except in an autopsy of the dead. Surgical material represents the source of human material since, frequently, some normal tissue is removed together with abnormal tissue or diseased tissue.

b) Fixation

The primary objective of fixation is to preserve the protoplasm with the least alteration form the living state. Fixing fluids act as preservative, inhibiting autolytic changes and bacterial growth. They coagulate the protoplasm thus rendering it insoluble and harden the tissue so that sectioning is facilitated. They may or may not preserve carbohydrates and lipids. Many fixatives also increase the affinity of protoplasm for certain stains.

Examples of fixing agents are formation, alcohol, mercuric bichloride, potassium bichromate, and certain acids (picric acetic, osmic). No single fixative possesses all the desirable qualities, and many reagents are used in mixtures, such as Bouin's fluid, Zenker's, and Susa's fluid. The choice of a fixative is usually determined by the particular tissue or component that is to be studied.

c) Embedding

Prior to embedding, the fixed specimen is washed to remove excess fixative and then dehydrated by passing it through increasing strengths of alcohol or some other dehydrating agent. The tissue/specimen is then ***"cleaned"***; this involves the removal of the dehydrating agent and its replacement by some fluid which is miscible both with the dehydrating agent and the embedding medium. Clearing agents include Xylol, chloroform, benzene and ceda-wood oil

After clearing, the tissue is infiltrated with the embedding agent, usually paraffin or celloidin. After infiltration, the embedding agent is made to solidify so that a firm homogenous mass containing the embedded tissue/specimen is obtained.

For special studies, tissues can be embedded in paraffin without subjecting it to preliminary treatment with fixatives, dehydrating solutions or clearing agents. The method of ***freeze-drying*** is used, and in this, the fresh tissue is frozen rapidly and dehydrated, while still frozen, in vacuum at a low temperature. The dried tissue is then embedded. In the ***freeze-substitution*** modification of this method, the ice within

the frozen tissues replaced by alcohol at a very low temperature prior to embedding.

d) Sectioning

Tissue embedded in paraffin may be sliced very thin to between 3 and 10 microns (μ) thick using a ***micrometer***. Each section is transferred to a clean glass microscope slide on which little egg albumen has been smeared. Water is run under the section and the slide is placed on a warming plate. The water evaporates and the section settles down on the glass surface, to which it becomes attached. The mounted section is now ready for staining.

e) Staining

The purpose of staining is to enhance natural contrast and to make more evident various cell and tissue components and extrinsic materials. Most stains are dissolved in aqueous solution which will not mix with paraffin; and thus to stain a paraffin section it is necessary to remove the paraffin by placing the section in a paraffin solvent or ***decerating agent***, usually xylol or tolud. This step is omitted in the case of a section which has been embedded in celloidin. The section is hen passed through descending strengths of alcohol prior to staining; this is so that it can easily mix with the aqueous dye.

f) Mounting

After staining, excess dye is removed by washing with water or alcohol, depending on the solvent of the dye, and the section is dehydrated through ascending grades of alcohol. After removal from the clearing agent, a drop of mounting medium eg. Canadian balsam, which has a similar refractive index to that of glass, is placed on the section. The preparation is covered with a cover slip and allowed to dry.

3.2.2 Electron Microscopy

In general, the method of preparing biological materials for electron microscopy is similar to that stated for light microscopy with some important points of difference. Much smaller pieces of tissue are used since preservation and fixation of cell fine structure is more critical and requires rapid interaction with the fixative. Blocks are commonly

1 cu. Mm or less in size. Tissue must be obtained fresh since past mortem changes are more obvious at the higher resolution of the electron microscope. The procedures of fixation, dehydration and embedding, though similar to those employed in light microscopy, are affected rapidly because of the small pieces of tissue involved. Since paraffin is not suitable for very thin sectioning required for electron microscopy. It is replaced as an embedding medium by some agents, usually a plastic material such as Epon or Araldite, which produces a firm block minute sections about 0.25 mm square ad about 300 to 500 Å thick are cut from the embedded block using a special, precision – built microtones and glass or diamond knives. The minute sections are mounted on perforated copper grids for viewing in the electron microscope. Thick sections, about 0.2 to 1.0 micron thick, of such plastic embedded material can be mounted on a glass slide, stained and examined by light microscope.

3.3 Freeze - Etching

This is another method of preparing materials for electron microscopy. This method involves a purely physical preparation which may allow examination of specimens virtually free of artifacts. The specimen is frozen, cut into small pieces, briefly warmed to etch the cut surface by vacuum – sublimation and a replica of the surface made by heavy metal shadowing. The frozen specimen is thawed and the replica can then be placed on a specimen grid and viewed in a conventional electron microscope or in ***scanning electron microscope*** which produces three-dimensional images. The method helps to distinguish natural from artificial structures and allows examination of the surface of single cells or of such structures as cytoplasmic membrane systems.

Self Assessment Questions 1

1. List the procedure for the preparation of a specimen for light microscopy.

2. list at lease 3 advantages of viewing life specimens.

3. List the differences between preparation of materials for light microscopy and electron microscopy.

3.4 Autoradiography

This is a special technique which employs the microscope, either light or electron, only as a visual aid. The technique involves introducing tracer

isotopes into the organism either by feeding or by injection; the tracers follow the same metabolic pathways as do the naturally occurring elements. Their presence in an organ can be detected by autoradiography.

After the administration of a tracer isotope, the organ or tissue under investigation is removed and processed in the normal manner for light or electron microscopy. The section is placed in close contact with a photographic emulsion and allowed to remain in the dark for a certain period of time. After subsequent photographic development of the sensitive emulsion, the radioactive tracer elements will appear as dark areas lying over the cells or components of cells in which the radioactivity is located. This method has been used to achieve some excellent results: for instance, the localization of radioiodine in the thyroid gland and of phosphorous (using radiostrontium) as a substitute in bone.

3.5 Examination and Interpretation of sections

The ability to interprete histological section is a skill which you have to develop. In gross anatomy, structure is studied in three dimensions. However, sections are viewed in a two-dimensional frame. It thus takes a good frame of mind for the student to relate the two dimensional (3-d) form.

It is important to reconstruct a 3-d mental picture of cells, tissues and organs from the two-dimensional (2-d) sections. In this wise, you must bear in mind the plane of sectioning applied. A single section of an organ may give a very false impression of the cell/tissue/organ architecture. It is thus important to use several sections taken in different planes in order to make a reasonable interpretation of structure of complex organs.

Also, the mere identification and notation of structures are not sufficient. You must try to interprete the functional significance of what you observe. Dead structures are examined for the purpose of throwing light upon their condition in life. Conditions which were hitherto dynamic in life have been converted to a static form in the permanent histological section.

You should also bear in mind that not all sections are perfect. Owing to the techniques used in preparation, sections may not be accurate representations. Alterations appearing in section but which do not occur in life specimen are called artifacts and you should try and identify them as such in your sections. Artifacts may be due to different chemicals used in the histological technique, resulting in shrinkage, or due to sectioning, leading to folding or wrinkling of the section, or to defects caused by a imperfect knife.

When viewing histological sections, you may often be confused by the varying appearance of different constituents of cells and tissues due to the use of a number of different staining techniques. Your interpretation of any microscopic preparation should involve an appreciation or consideration of the staining technique used. Although it is not necessary for you to be conversant with details of the various staining tecnniques used, you should understand the general principles, uses, and results of the common staining procedures.

3.6 Histological Stains

In general dyes used for staining are complex organic chemicals which often show some variability in performance. They may be classified based upon their use with regards to tissue or cell components. Dyes may be of general use staining either the nucleus or the cytoplasm, or they may be specific with regard to particular components. e.g some may stain for fats, protein, carbonhydrates, chloroplasts Golgi body etc.

Stains in general use are considered to be either bases or acids, but in fact they are neural salts having both acidic and basic radicals. A ***basophil*** is a stain whose colouring properties are in the basic radical of the neutral salt, and in most cases are attracted to substances with acidic tendencies eg. ribonucleic acid (RNA). Similarly when the dye exhibit its basic radical, it is known as an ***acidophil*** (an acid dye) and stains basic substances (for instance, the general cytoplasm)

Examples of Stains

1. **Hematoxylin** – nuclear stain in most common use; its property is due to the presence in solution of its oxidation product, hematein. (Thus, a freshly prepared solution of hematoxylin must be allowed to "ripen" or "age" or oxidation to occur prior to use). When stained with such a dye, nuclei appear blue. Iron hematoxylin which stains nuclei blue or black has a wide application. In most methods employing iron hematoxylin one overstains with the dye and regressively differentiates in a weak acid or in ferric salt solution. By careful ***differentiation***, which may be viewed directly under the microscope, such organelles as chromosomes, mitochondria, Golgi apparatus and the contractile elements of muscle may be visualized.

2. **Carmine –** a red to purple nuclear stain was formerly very popular but is little used today.

3 **Basic Aniline Dyes –** are a group of stairs used extensively. The group includes:

i) Azure A

ii) Toluidine blue

ii) methylene blue

These stains are employed also in the identification of mucopolysaccharides which stain ***metackromatically*** (meta, beyond; chroma, colour). This means that mucopolysaccharides, when stained with one of these dyes will take on a colour different from that of the dye employed. It is thought that substances which demonstrate metachromasia do so because they are capable of concentrating the dye or of altering its molecular state. Mucin, matrix or cartilage, and granules of mast cells are demonstrated readily by their metachromatic staining.

Other basic aniline stains in common use are: brilliant cresyl blue, neutral red and Janus green, all of which are non-toxic and may be used also as vital or supravital stains.

Acidic dyes commonly employed to stain the general cytoplasm, include i.) eosin ii) picric acid iii) azo dyes (e.g. chromotrope) and (iv) the acid diazo dyes – trypan blue and trypan red. The latter two are used as vital stains.

Most histological sections are stained with both a basic stain and an acid stain. The commonest combination is hemtoxylin are stained dark purple or blue, and practically all cytoplasmic structures and intercellular substances are stained pink.

3.7 Histochemistry

This is a field of research which is expanding rapidly and is base on the fact that deposition of specific stain in certain regions is a result of chemical or physical properties of the structure. Histo chemical methods are even now available for many inorganic and organic substances. Eg. Prussian blue stain is used for the detection of ferric iron; in sections treated with potassium ferrocyanide deposits are coloured blue.

The Feulgen reaction for the identification of deoxyribonucleic acid (DNA) is an example of a histochemical test. Enzymes may be detected by histochemical method to localize the site of a specific enzyme. This is done by incubating the section under examination at body temperature in the

presence of a suitable substrate; the products of the resultant chemical (enzymatic) reaction is converted into a chemical substance of a definite colour.

Glycogen can be stained by Best's carmine stain or by the periodic acid-Schoff reagent. In either case, glycogen can be differentiated from other polysaccharides by the fact that the staining property of the latter substances is resistant to digestion by salvary amyglass.

Not all reactions in histochemisry rely upon chemical affinities. Fat can be detected in section which have not been exposed to fat solvents by stain such as Sudan III, Sudan IV and Sudan black B. These stains have a physical affinity for lipids and are absorbed by the fat.

Immunocytochemistry is one branch of histochemistry that is gaining grounds. At the light microscopic level, the fluorescent antibody technique is a sensitive method for the localization of specific polysaccharides or protein. The basis for immnocyto-chemistry is the fact that the body reacts to foreign substances, ***antigens***, by producing specific substances, ***antibodies*** which combine with and inactivate the antigens. Antibody molecules are chemically aged with fluorescent dye molecules and their reactions with antigens can be visualized in the ultraviolet (UV) microscope. The method has been used to identify the cells of origin of protein hormones, the intracellular localization of various enzymes and the sites of protein such as myosin. The method has been adapted for use with the electron microscope by conjugating an antibody with a metalloprotein such as ***ferritin,*** which naturally possesses a distinct appearance in the electron microscope. This method enables the investigator to localize precisely the site of the antigen-antibody reaction.

4.0 Conclusion

In this unit, we have discussed how biological specimens are treated for histological observations.

1. Living organisms, particularly unicellular ones can be observed using the phase contrast microscope.

2. Tissue culture presents a means of observing cells for prolonged periods.

3. Two staining methods are used with living or surviving cells. In ***vital staining*** dyes are injected into the living animal or plant; due to selective absorption certain cells (target cells) will pick up the stains.

In supravital staining dyestoy is added to the medium of cells already removed from the organism.

4. Stages involved in the preparation of specimen for viewing with the light microscope include:

   a) removal of the specimen (b) fixation (c) embedding (d) sectioning (e) staining and (f) mounting.

5. Similar preparations are made for electron microscopy with some important points of differences:

   a much smaller pieces of tissue are used for the EM.

   b. fixation, dehydration and embedding are effected more rapidly.

   c. paraffin is substituted with plastic materials (eg. Epon or Araldite) in EM

   d. copper grids are used for mounting prepared EM specimens rather than glass

6. Autoradiography is achieved by injecting tracers to fellow metabolic pathways and locate sites of action.

7. Staining techniques must be considered in viewing histological materials under the microscope. This is because artifacts may appear due to the different chemicals used or a defective knife used in sectioning etc.

8. Different sections should be made to obtain a composite 3-d structure of the specimen.

9. Stains may be acidophilic (attracting basic structures) or basophilic (attracting acidic structures). A combination of stains may be used eg. hematoxylin – eosin (H.E.)

10. Histochemistry I premised on the fact that some chemicals react specifically with certain structures within a cell or tissue.
11. Immunocytochemistry is based o the property of the body to produce antibodies in response to antigen (foreign object) entering it. Fluorescent dyes are linked to the antibody and its sites of reaction are monitored.

5.0 Summary

We have looked at histological/cytological methods so that we can understand what efforts might have gone into any slide preparation or photomicrograph we come across. As a biologist you should be able to observe both living specimens and stained ones. You should also be able to do the staining yourself and preserve it for future references of used in teaching your students in biology practicals.

6.0 Tutor-marked Assignments

1. Describe in details the various steps involved in preparing a fixes slide of a rats kidney.

2. Name three acidophilic dyes and three basophilic dyes and what structure they stain.

7.0 References

Leeson, T.S. and C.R. Leeson . 1970, Histology 2nd Edition, W.B Saunders Company. Philadelphia

Answers to Self Assessment Questions

(1) (i) Removal of specimen, (ii) Fixation, (iii) Embedding (iv) Sectioning (v) Staining (vi) Mounting

(2) (i) Microdization can be done (ii) Little experiments like osmotic response can be performed (iii) Motion picture records can be made (iv) Lapsed time filsm of individual living cells or tissue cultures can reveal such processes as mitosis, phagocytosis and ameboid movement (v) Slow-motion films can permit analysis of such actions as the beating of cilia.

(3) For light microscopy paraffin or wax can be used for embedding but this is not strong enough for preparation for the EM where a plastic material such as Epon or Araldite is used. (ii) A much smaller piece of tissue is uded in EM (iii) Less time is spent on fixation, dehydration and embedding for the EM preparation because of the small specimen size.

UNIT 3: STRUCTURE AND FUNCTION OF THE CELL

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1.0 Introduction

In another course you have learnt about the Cell Theory which states that "the cell is the basic structural and functional unit of all living organisms". This means that all living organisms are made up of cells; or that cells are the building blocks of all living organisms.

You have also learnt that some organisms are made up of only one cell which performs all the characteristic functions of life. These organisms are known as ***unicellular*** organisms. Some other organisms are made up of several cells

each performing a particular function to sustain the organism. These are known as ***multicellular*** organisms.

In unit 1 you leant abut the usefulness of the microscope n biological observations. This unit is concerned with the structure and function of the cell.

2.0 Objectives

At the end of this unit you would be able to:

1. Distinguish between a prokaryotic and eukaryotic cell.

2. Describe the structure of a generalized animal cell as seen under the electron microscope..

3. Describe the structure of a generalized plant cell as seen under the electron microscope.

4. list the differences between prokaryotic and eukaryotic cells

3.1. Different Types of Cells

The cell theory states that all living organisms comprise of cells as the structural and functional units. Some organisms consist of only one cell and are called ***Unicellular*** organisms. Others are made up of many cells and are called ***Multicellular*** organisms.

When it comes to the levels of cells, these can be classified according to the complexity of the cell involved. Organisms that show the simples form of complexity in not having any internals membranes are called ***prokaryotes (or prokaryotes)*** (fig. 3.1) These organisms eg. the bacteria ***Escherichia coli*** , blue-green algae and mycoplasma lack a membrane-bound nucleus. Organisms with discrete nuclei in their cells are called ***eukaryotes (or eukaryotes)*** (Fig. 3.2.) e.g. amoeba and clhlamydomonas.

An aberration in the cell Theory exists in the viruses. These are biological particles which are made up of an outer protein coat (the capsid) which encloses a genetic material (DNA or RNA). The virus on its own has no metabolic capabilities until it gets within a suitable host, takes over the host's metabolic processes to generate its own kind. Such structures are described as ***acellular*** e.g. Tobacco Mosaic virus (TMV).

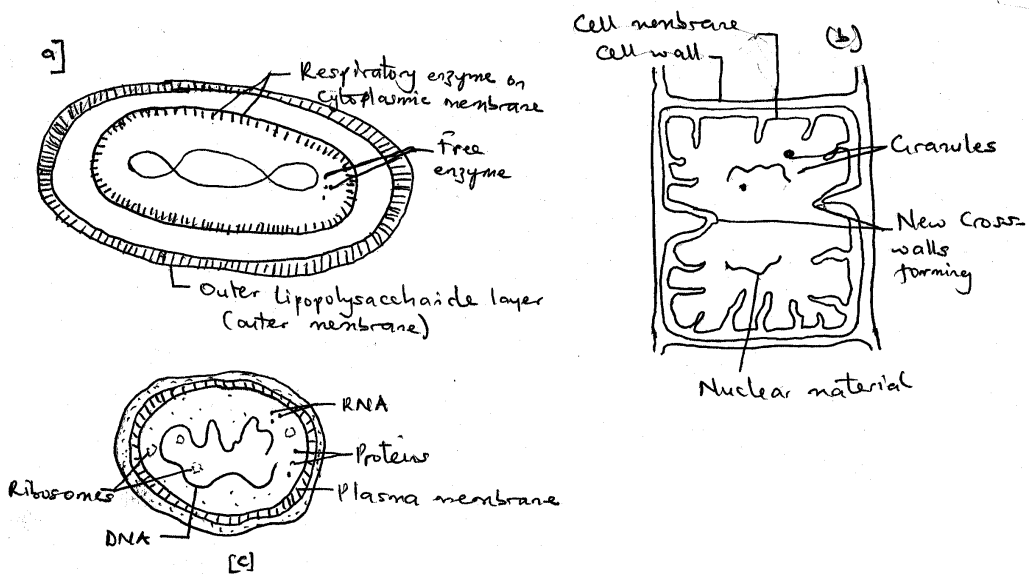


Fig. 3.1 Examples of Unicellular prokaryotic cells sowing (a) bacterium (e.g. E. coli) (b) blue-green alga (c) mycoplasma. Notice the absence of any internal membrane; the projections within the mycoplasma all arise from the cell membrane to increase surface area for respiration.

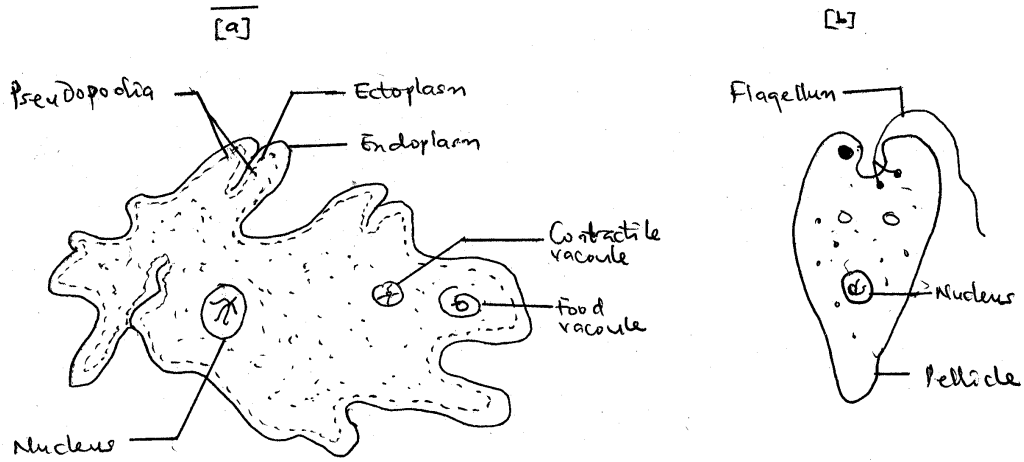


Fig. 3.2 Unicellular Eukaryotic organisms (a) Amoeba proteus and (b) euglena viridis. Notice a distinct,membrane-bound nucleus.

3.2 Coarse Structure of a Cell

The structure of a cell as seen with a good optical (light) microscope. As previously stated n unit 1, the best light microscope can magnify up to 1,500

time the original size of the object (specimen). However, most laboratory microscopes you will come across at this level do not have that capacity.

What is seen of a coarse structure of a typical animal cell is a structure that is bounded on the outside by a limiting membrane, called the ***cell (or plasma) membrane***. The cell membrane is the cell's contact point with its environment and through it materials enter or leave the cell. In general, plasma membranes are ***semi-permeable*** allowing certain molecules and particles to enter the cell while disallowing others.

Within the eukaryotic cell there is a ***nucleus*** which is bounded by a membrane the ***nuclear membrane***. The nucleus is the centre of control of all cellular activities. An enucleated eukaryotic cell (ie one whose nucleus has been removed) can only function for a limited period; an example is the human erythrocyte which has a half life of 60 days (maximum life span is 120 days)

The rest of the cell is made up of the ***cytoplasm***, in which are contained various particulate organelles (membrane systems) and soluble substances. These carry out the various chemical reaction that are typical for the cell. However, under the light microscope, the cytoplasm may look like an amorphous (formless)body and these organelles may not be too distinct, or clearly visible (Fig. 3.3a)

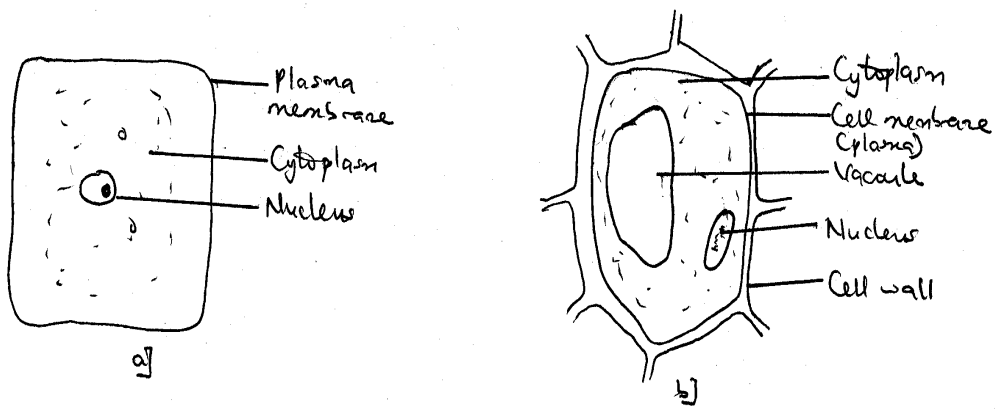


Fig. 3.3 Coarse structures of (a) a typical animal cell and (b) a typical plant cell.

T typical plant cell when viewed with the light microscope will show a ***cell wall*** enclosing the ***cell (plasma) membrane***. Inside can be seen the ***nucleus*** delineated by a membrane. Within the ***cytoplasm*** one may be able to see a ***cell vacuole*** which contains cell sap. (Fig. 3.3b)

3.3 Fine Structure of a Cell

The structure of the cell as seen under the electron microscope (E.M.)is called the ***fine structure (or ultra structure)*** of the cell. As you already know from Unit 1, the EM has the capacity to magnify an object over 500,000 times and has a resolution of 0.05Å. The E.M. thus can magnify and resolve far better than the light microscope (L.M.).

Under the EM various structures will be seen as membrane –bound entities. These include the ***plasma membrane, endoplasmic reticulum, Golgi Body (of Dictyosome), mitochondria, lysosomes, Ribosomes, Centrosome***, the nucleus and also in plant cells, ***plastids***, ***and vacuoles***. Fig. 3.4 shows a schematic representation of an animal cell as can be seen under the E.M.

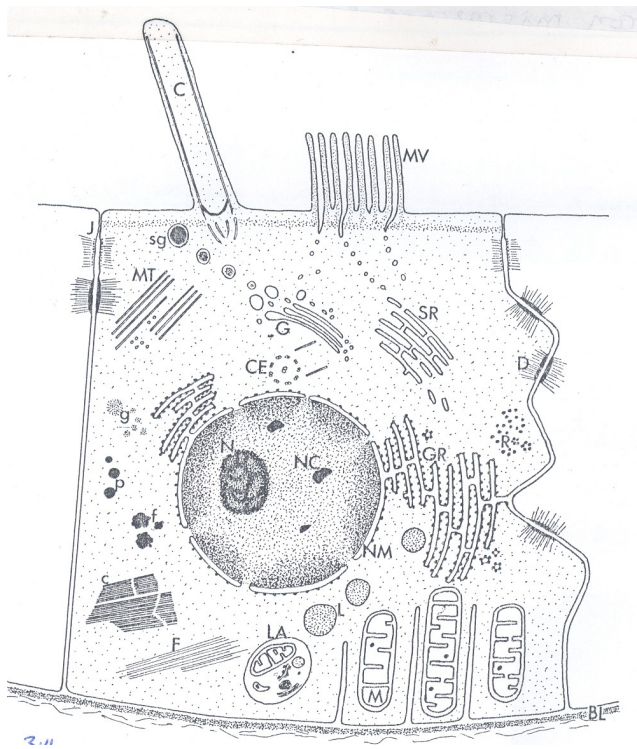


Fig. 3.4: Schematic diagram of the cell. Nuclear components and cytoplasmic organelles are indicated with capital letters, inclusions with small letters and BL indicates the basal lamina, supported by reticular fibers beneath. C = cilium, CE = c entrosome, D = desmosome (macula adherens). F = filaments, G = Golgi apparatus, GR = granular endoplasmic reticulum, J = junctional complex, L = lysosome, LA = autolysosome (secondary lysosome), M = mitochondrion, MT = microtubules, MV = microvillus, N = nucleolus, NC = nuclear chromatin, NM = nuclear envelope, R = ribosomes,

SR = smooth (agranular) emdoplasmic reticulum; c = crystal, f = fat, g = glycogen, p = pigment, and sg = secretion granule.

3.3.1 The Plasma Membrane

Under the light microscope the plasma membrane is seen as a thin line. But under the EM it is seen as being made up of three layers; two dark layers separated by a light one. Chemical analysis and other advanced studies have revealed that the membrane has a lipoprotein (fat, plus protein) composition.

The two dark regions of the membrane are made up mainly of protein while the light one in-between consists of lipids. The plasma membrane, therefore, consists of a thin sandwich of lipids between two layers of proteins. This structure of the membrane is known as the ***unit membrane*** and it is characteristic of all cell membranes. The membrane will be discussed more fully in the next unit.

The protein constituent of the membrane gives the cell its wettability and flexibility. There are pores which perforate the membrane at regular intervals (Fig. 3.5. While small molecules eg. molecules of water, can pass through these pores, larger molecule cannot. This is one way in which the membrane controls what goes in an out of the cell.

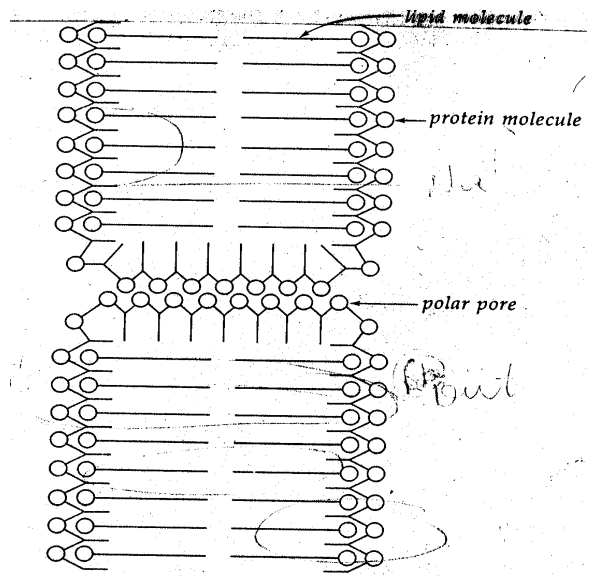


Fig. 3.5: The Danielli-Davson model for membrane structure. A lipid bilayer is stabilized by layers of protein on both outer surfaces. At intervals, pores, presumably coated with protein extend through

the membrane. These "pores" are extremely minute, and give the membrane in these regions polar properties, with the result that water, but not larger polar molecules, penetrates the membrane readily. Redrawn, original courtesy of J.F. Danielli.

The protein molecules are long and complex and so can fold or unfold. In this way the membrane can expand or contract. This could also provide a possible means of controlling or selecting which molecules may enter or leave he cell. Such a membrane is said to be ***selectively permeable (or semi-permeable)***. Although all membranes have the same basic unit structure described above, the various membranes in the cell are of different thickness and have different biochemical properties, since they contain different proteins.

3.3.2 The Endoplasmic Reticulum (ER)

This is a system of membranes found within the cytoplasm. It consists of cavities of tubes lined with a thin membrane. The cavities are interconnected and the membrane is continuous with the nuclear membrane and the plasma membrane. In portions of the endoplasmic reticulum, a number of granules are attached to the membranes on the matrix side (the side in contact with the cytoplasm). Such is known as ***rough endplasmic reticulum (RER) or granular ER***. These granules are rich in ribonucleic acids and are called ***ribosome***. They are the sites where proteins e.g hormones and digestive enzymes are synthesized in the cell. The general function of the RER is to isolate and transport the proteins which have been synthesized by the ribosomes. The ER is therefore a sort of intracellular transport system which makes the transport of materials from one part of the cell to another easy. In this connection it is pertinent to note the connection between the ER and the nucleus (see Fig. 3.4) This provides a route by which materials might move from the cell to the cytoplasm and vice versa. The RER is particularly abundant in cells engaged in protein synthesis.

Where the ER is not associated with ribosomes, it is called a ***smooth endoplasmic reticulum (SER).*** This is not usually directly continuous with the RER and its function is believed to be the synthesis and transport of lipids.

3.3.3 The Golgi Body (Dictyosome)

This is another characteristic system of the cytoplasmic membrane. It resembles the SER but is smaller, more compact and discontinuous.

It consists of stacks of flattened cavities which are linked with the SER.

The Golgi Body is believed to be involved in the secretion of glycoproteins; these are proteins conjugated with carbohydrate. The proteins are synthesized in the channels of the rough ER. From here, they are transported in vesicles to fuse to form the Golgi Body. In the Golgi Body carbohydrate is added on to the proteins and the resulting glycoproteins are discharged in vesicles.

The structures we have considered so far is the plasma membrane, the ER (RER and SER) and the Golgi Body form the membrane systems of the cell. And as mentioned ealier, all membranes, wherever they occur, have the unit membrane which we have also referred to is the nuclear membrane, which is continuous with the ER and the plasma membrane. Next we shall consider structures that are more particulate ad which constitute the cytoplasmic particles.

3.3.4 Cytoplasmic Particles

(i) **Mitochondria (sing mitochondrion)** are found in virtually every type of cell except bacteria, blue-green algae and the red blood cells of mammals.

Each mitochondrion is surrounded by a double lipoprotein membrane similar to the plasma and nuclear membranes. The inner membrane is variously folded into projections called ***cristae***. This provides an increased internal surface area for chemical activities (see fig. 3.7 and 3.8)

The outer membrane is elastic, thus the mitochondrion is able to swell or contract with relative ease. The degree of stretching of the membrane controls the size of the substances entering the mitochondrion. The outer and inner membranes constitute the membrane phase while the rest of the mitochondrion is the liquid phase or the ***matrix.***

The Mitochondria are the respiratory centres of the cell. They are concerned with the energy problems of the cell. The matrix contains many soluble enzymes which act on metabolic fuels such as carbohydrates, breaking them down to carbon dioxide (CO_2) and water (H_2O) with the release of energy. The energy thus produced is in form of a special complex molecule called ***adenosine triphosphate (ATP)*** which is the

energy "currency" of the cell. ATP production occurs on the inner membrane but the enzymes come from the matrix; so both the inner membrane and matrix are involved in ATP production.

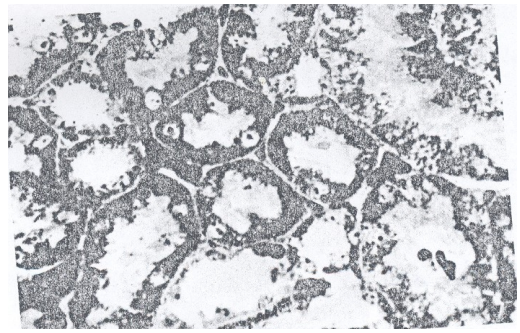


Figure 3.6: Photomicrograph to show mitochondria which appear as dark striations beneath and around nuclei. Kidney tubules, iron hematoxylin stain, x 350.

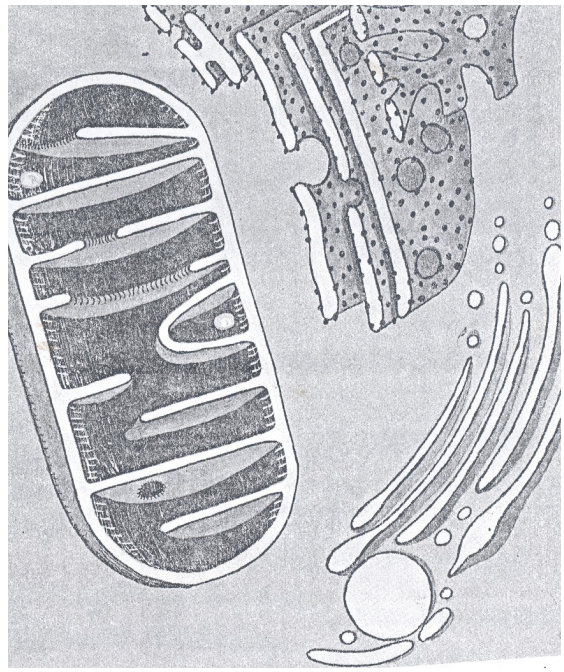


Fig. 3.7: Diagram to illustrate the electron microscopic appearance of a mitochondrion (left), elements of the granular endoplasmic reticulum (top right), and the Golgi apparatus (bottom right)

Cells which by their function expend a lot of energy contain a large number of mitochondria and they are to be seen highly packed in that part of the cell where the energy is required e.g. the tail of animal spermatozoa is densely packed with mitochondria. They are also found in great number alongside the contractible fibrils in muscles particularly in the flight muscles of insects and at the surface of cells where active transport occurs.

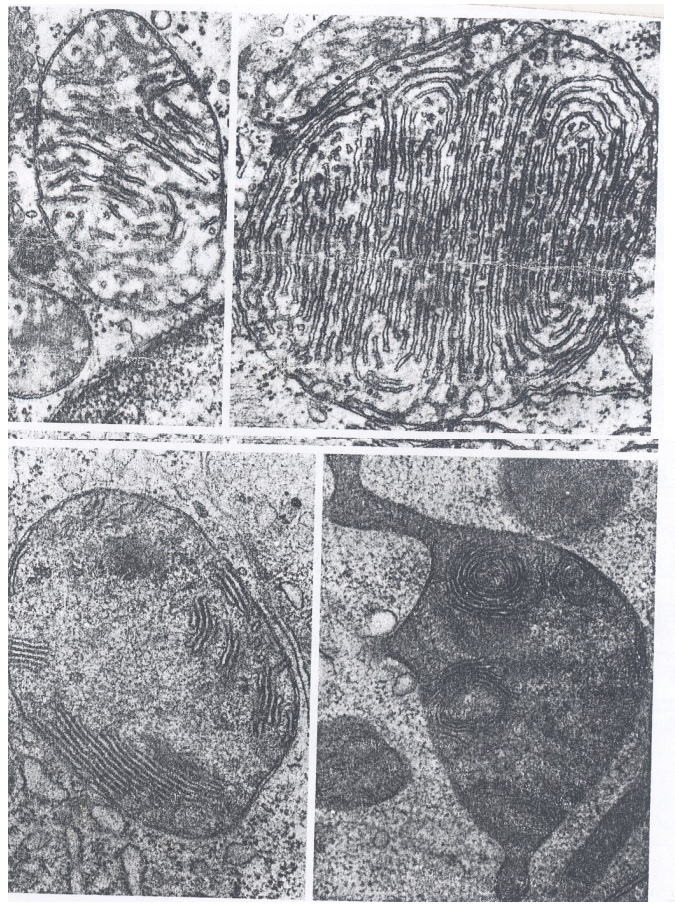


Fig. 3.8: Electron micrographs of mitochondria showing different morphological types. Top left: from striated muscle (note, also the presence of a centrole in cross section). Top right: from cardiac muscle. Bottom left and right: from interstitial cells of the human test's. All x 40,000

(ii) Lysosomes: These resemble mitochondria externally but are distinguishable by their lack of internal cristae. The enzymes contained in them are also different from those of the

mitochondria. Lysosomal enzymes are mainly hdroxylases, and their main function is the degradation of large molecule by the addition of water (hydro = water, lysis = break). In this way large molecules are broken down into smaller ones like when starch is broken down into sugars. The lysosomes also have an important role to play in the destruction of worn-out organelles in the cell. Such old organelles are enclosed in a sac-like structure formed by a membrane and the lysosomes pour their content of enzymes into the sac (see fig 3.9). After digestion the products are absorbed into the surrounding cytoplasm and used for the synthesis of new organelles.

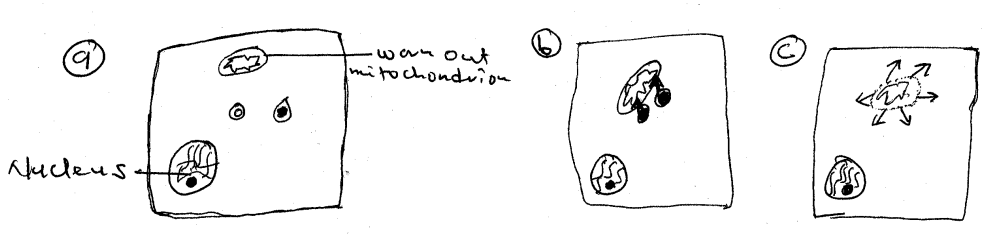


Fig. 3.9.: Destruction of a worn-out organelle (a) (Mitochondrion) by lysosomes (a) worn-out mitochondrion (b) fusion of organelle with lysosomes which digest the organelle (c) dispersion of digested organelle.

(iii) Ribosomes: We have already come across these in association with the rough endoplasmic reticulum (RER) and at that time we noted that they function as sites for protein synthesis. Ribosomes may also lie free in the cytoplasm; it is for this reason they must be included in a discussion of cytoplasmic particles. The function, however, remains are associated with ER or they lie free in the cytoplasm.

Each ribosome consists of two sub-units. The smaller sub-unit is surmounted on the larger one. They are designated as the 30S (in bacterial) and 40S (in animals) for the smaller units and 70S and 80S respectively for the large sub-units. The S is called a Sverdberg unit) and is a function of their sedimentation rates).

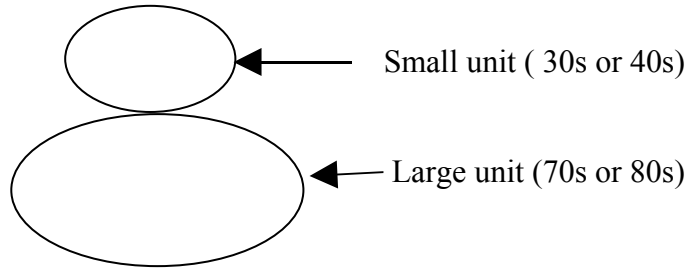


Fig. 3.10 Subunits of the ribosome

(iv) **Plastids:** Plastids are peculiar features of the cytoplasm of plant cells. They are classifeied as leucoplasts depending on whether they are coloured or not. Leucoplasts are colourless plastids in which starch is deposited. They occur in cells not usually exposed to light eg. in deep-seated cells, but they may develop green coloured plastids and are of two types: those that contain red orange or yellow pigments. Plastids that contain chlorophyll are called ***chloroplasts*** while those that contain the other colours are termed ***chromoplasts***. In the present discussion, we shall concentrate on chloroplasts to which green plants owe their colour, and without which photosynthesis (the process by which green plants manufacture carbonhydrates from water and carbon dioxide) cannot take place. The energy needed for this very important process is trapped from the sun by thechlorophyll which is contained in chloroplasts.

Electron microscopic studied have revealed the chloroplasts as complex structures. On the outside it is bounded by a double membrane and internally it is organized into a series of lamella areas (***grana)*** and non-lamella areas ***(stroma),*** see fig. 3.11 below:

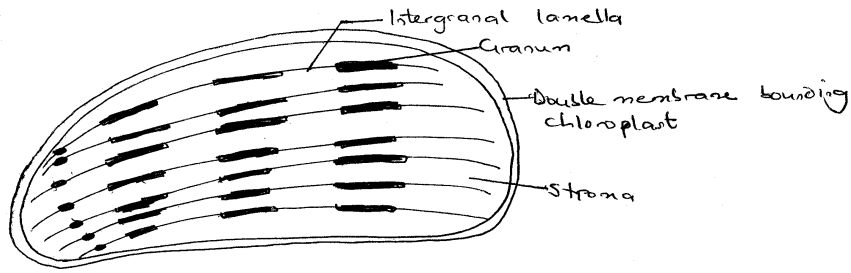


Fig. 3.11 Diagram of a chloroplast (notice that the inner membrane is not thrown into folds, but there are stacks of membranes called grana (sing granum and intergranal lamella)

Within the grana the chlorophyll molecules are precisely arranged. The chlorophyll is in a single layer and is sandwiched in-between layers of proteins, and closely associated with lipids and carolenoids. This arrangement make for efficiency in photosynthesis. It has been found that anything that leads to a disturbance in the lamellar structure of a granum leads to a reduction in the efficiency of photosynthesis.

The stroma can be visualized as the aqueous part of the chloroplast which contains dissolved salts and enzymes.. Enzymes are also part of the lamellar structure of the granum. The other plastids mentioned earlier differ from chloroplasts in that they lack the lamellar structure of chloroplasts.

(v) The Centrosome (Centriole)

This is a cytoplasmic particle which is more characteristic of animal than plant cells. It lies just outside the nucleus and functions in cell division. It possesses a high degree of internal organization. It is a cylindrical structure with a detailed structure that is similar to that of the cilia and flagella; in fact it is involved in the formation of cilia and flagella in certain cells. The centriole has the same 2 + 9 tubular structure found in cilia and flagella.

Fig. 3.12 Diagram of a Centriosome (centriole) (a) longitudinal (b) cross-section.

(vi) **The Nucleus:** This is the most prominent feature of the cell under the microscope. It is the controlling centre of the cell, in that it provides information to the cytoplasm to kept it (the cytoplasm) functioning for an indefinite period. A cell in which the nucleus has been removed eventually dies. For instance, the mature red blood corpuscles (erythrocytes) of man which lack nuclei are short-lived.

The nucleus is bounded externally by a double-layered membrane – the ***nuclear membrane*** (fig 3.1.4) the nuclear membrane has the characteristic unit membrane structure of all cellular membranes. The outer layer of this nuclear is continuous with the membranes of the ER. This nuclear membrane is punctuated by a series of pores, the ***necleopores***, (fig. 3.15). which provide a route through which materials move from the nucleus to the cytoplasm and vice versa.

When the nucleus is appropriately stained the central portion shows a network of fine thread with coarser lumps of heavier substances. This is the ***chromatin***. The chromatin is the ***chromosomes*** in a diffuse state. This diffuse state of the chromosomes permits maximum surface contact with the nuclear sap. We shall discuss the chromosomes later during cell division. Also present in the nucleus is a dense rounded body – the ***nucleolus*** which is a proetein – rich particle; it is also very rich in RNA

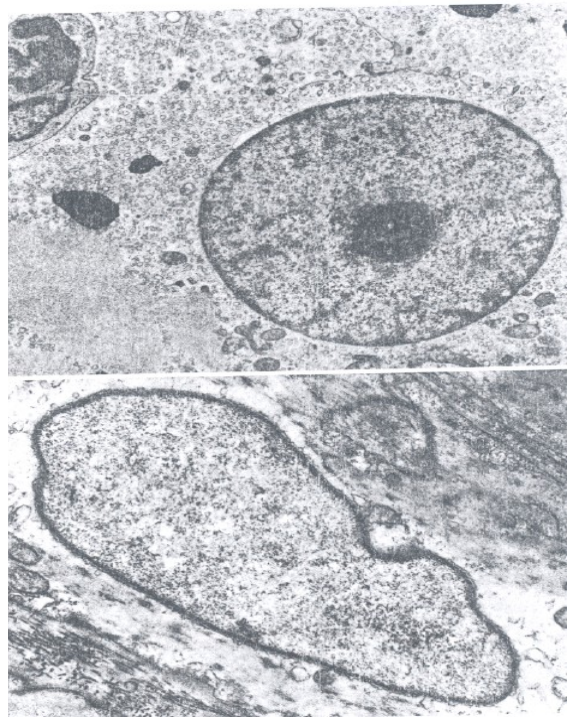


Fig. 3.13: Electron micrographs demonstrating interphase nuclei. Top: the large nucleus (right) is of an interstitial (Leydig) cell of the human testis and shows a spherical profile with nuclear envelope, central nucleolus, chromatin granules and karyoplasms. The nucleus (top left) of a connective tissue cell is of highly irregular outline x 12,500. bottom: the elongated nucleus of a smooth mjuscle cell shows nuclear envelope an fibrous lamina internal to it. No nucleolus or chromatin granules are seen x 17,000

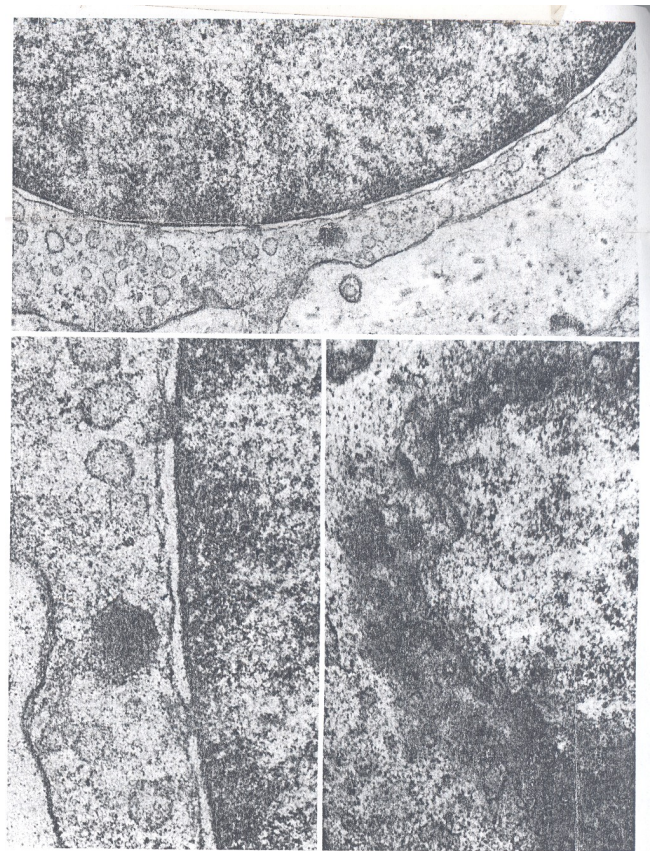


Fig. 3.14: Electron micrographs demonstrating the nuclear envelope and nuclear pores. To: the nucleus lies above and four pores are shown. X 2,000. Bottom left: higher magnification showing two pores. X 68,000. Bottom right: tangential section of nuclear envelope showing nuclear pores as circular profiles, nucleus on the right. X 18,000

3.3.5 Vacoules

A vacuole is a fluid-filled space within a cell. The fluid (or cell sap) is a solution of various salts in water. Animal cells usually contain numerous minute vacuoles while in many plant cells a single large vacuole which takes up most of the volume of the cell is present.

Vacuoles are formed at cell surfaces by a process of ***pinocytosis*** by which the cell takes in substance in liquid form. At points on the surface of the cell, invaginations or infoldings of the plasma membrane occurs. These develop into flask-like structures which are then called ***micropinocytic vesicles***. Later the neck of theflask closes up and the vesicle is sealed off from the exterior. It becomes entirely

enclosed within he cell and moves freely in the cytoplasm; in this condition it is known as a ***vacuole.*** The single large vacuoles which are characteristic of mature plant cells are believed to be formed by a fusion of many small vacuoles.

The means by which larger particles are taken into the cell is called ***phagocytosis***. This is clearly demonstrated during the formation of good vacuoles by amoeba. It is also the means by which white blood cells engulf bacteria and other harmful foreign particles.

3.3.6 The Cytoplasm

This is a solution of many substances, mainly protein in water. It is a transparent slightly viscuous fluid which fills the cell, and in which the various structures (organelles) discussed above are suspended.

3.4 Cell Size

Cells are very diverse structures not only in size and shape, but also in structure and function. Hence, it is pertinent to always remember that there is really o "typical cell".

Most cell sizes range from about 1/10μm to 1mm. Bacterial cells are among the smallest and their sizes fall in this range of 1/10μm to 1mm. Most of the cells that make up the bodies of plants and animals fall in this group. Large cells eg. eggs and unicellular organisms such as Amoeba and paramecium measure up to 1mm while the really large cells such as ostrich eggs could measure several inches. From the above it can be seen that the range of size of cells is indeed very wide. The large ones being over 10,000 times as big as the small ones.

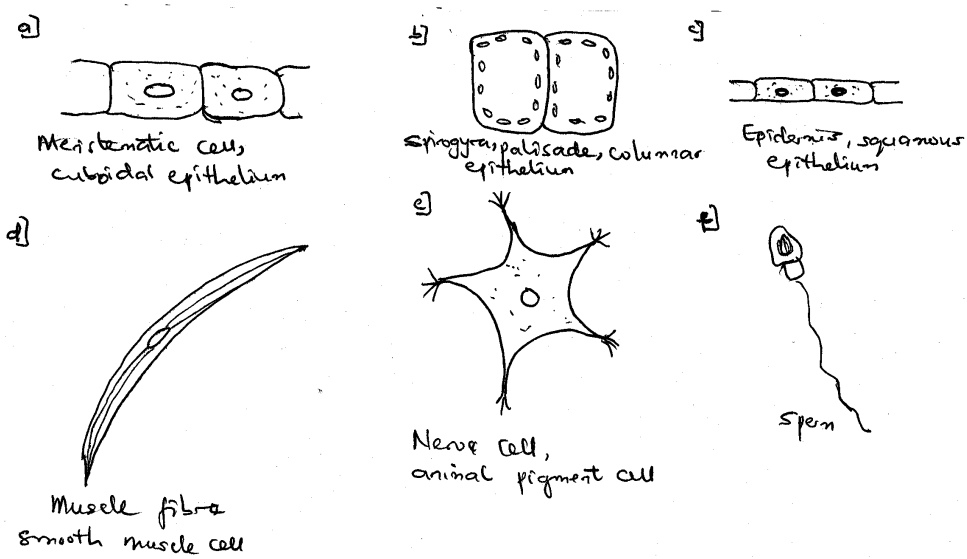
The chart below gives some indication of the distribution of cells on the basis of size. The chart also shows the limit of resolution of the human eye, the light microscope and the electron microscope.

	1mm	giant cells, protozoans e.g. Amoeba
	1/10mm	large cells, small cells
Human eye	1/100mm	most cells
	1/1000mm	= 1μm

Light microscope	1/10μm	smallest cells eg. bacteria
	1/100	many cells organelles, viruses
Electron microscope	1/1000μm	= 1mm molecules
	1/10 mm	Atoms
	1/100mm	
	1/1000mm	= 1μm

3.5 Cell Shapes

Cells are as variable in shape as in size. They could be spherical e.g. cells of the coccus bacteria, or cubical e.g. cells of the cuboidal epitheha. Other shapes of cells are: cylindrical e.g. columnar epithelia, rod bacteria, parenchyma of palisade mesophyll of the leaf; linear e.g. fibres of various types – and a host of others. Fig. 3.15 below illustrates different cell shapes..



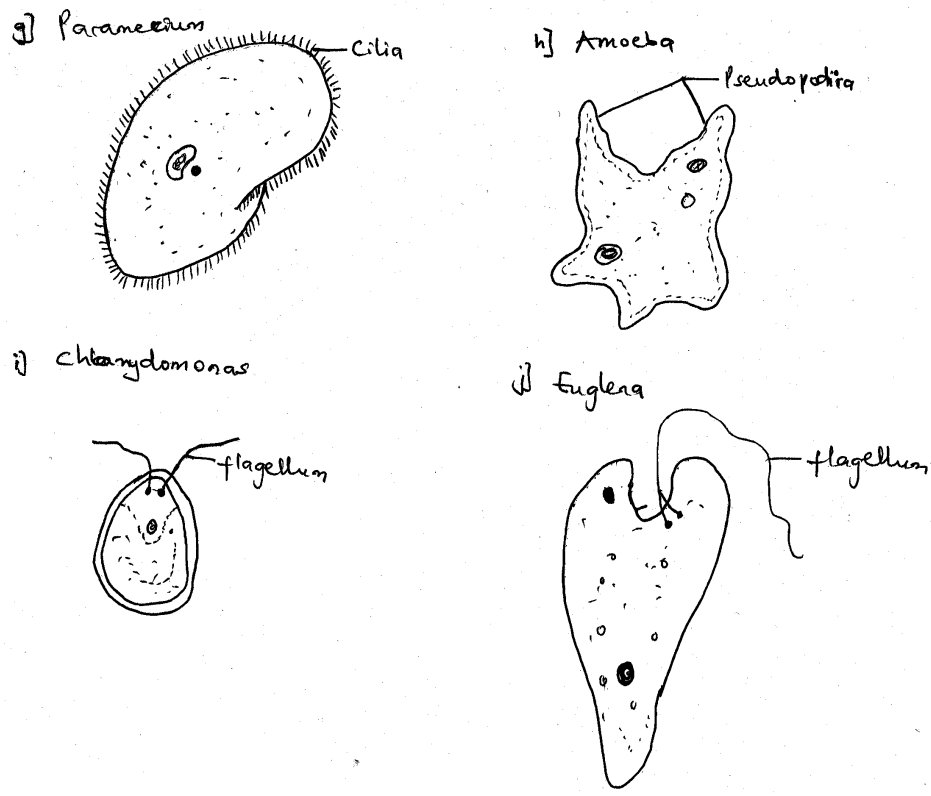


Fig. 3.15: Different cell shapes

The range of structure and functions of both plant and animal cells is discussed more fully in unit 5 of this course. You will therefore have a more complete picture of cells and tissues at that stage.

4.0 Conclusion

We have, in this unit considered most of the important structures of a typical cell. As stated earlier there is really no typical cell size each cell is unique. However, the structures we have high-lighted in this discussion are those to be found in most cells. In some cases where there has been a need to point out limited occurrence, this has been done.

A typical cell can then be said to consist of:

(a) **Cell Membrane:** highly structural, lipoprotein, tri-layered structure; marks boundary of the cell and controls entry and exist of substances into and out of the cell respectively.

(b) **Cytoplasm**: A viscous liquid of many substances in solution. In it are suspended numerous important structures:

(i) mitochondria – energy work house of the cell, produces ATP

(ii) lysosome – membrane bound, contain lytic enzymes

(iii) ribosome – rich in RNA, for protein systhesis

(iv) Golgi Body – flattened membrane sacs, secretion of glycoprotein

(v) Endoplamic reticulum – membrane channels, RER contains ribosome for protein synthesis, SER, no ribosomes for lipid synthesis.

(vi) Vacoules – fluid filled membrane bond, contain water plus various salts.

(vii) Plastids (in plant cells) – leucoplasts – colourless, chromoplasts – contain coloured pigments (green, red, orange, purple); green coloured plastids are called chloroplasts contain chlorophyll used by plants in absorbing light energy to carry out photosynthesis for synthesis or carbohydrates.

(viii) Centromsome (centrioles in animal cells) functions for formation of spindle fibres for movement of chromosomes in cell division.

(c) **Nucleus:** also suspended in the cytoplasm. Bounded by a double membrane (nuclear membrane) with pores (nucleopores); contain chromosomes on which are situated genes the hereditary materials. It is the controlling centre of the cell.

5.0 Summary

Since the cell is the most basic structural and functional unit of a living organism, knowing about its structure and function wil enable us know more about the whole organism. Sometimes the shape of and structure of a cell may dictate the function of the cell. A common structure among cells may indicate common functions.

Since a group of similar cells form a tissue, the function of each cell in the tissue will determine the function of the tissues. Likewise, as tissues group

together to form an organ the functions of the tissues will determine the function of the organ. Similarly, organs form systems and a group of systems form the organism. Thus, we can see how a cell influences the whole organism.

Self Assessment Questions 1

1. Where in plant or animal cells will you find the following?

   (i) Cell Wall
   (ii) Centrosome
   (iii) Chloroplasts
   (iv) Vacuoles
   (v) Mitochondria

2. Define the following terms.

   (i) Procaryotes
   (ii) Eukaryotes
   (iii) Unicellular
   (iv) Acellular
   (v) Multicellular

3. The basis structure of all membranes is the same. What term is used to describe his unit structure of membranes?

6.0 Tutor-marked Assignment

1. Draw and label fully and correctly a typical animal cell as seen under the electron microscope.

2. Draw and label the fine structure of each of the following:

   (i) Plasma membrane
   (ii) Mitochondria
   (iii) chloroplast

3. Discuss the functions of the following:
   (i) Plasma membrane
   (ii) Endoplasmic Reticulum
   (iii) Mitochondria
   (iv) Plastids
   (v) nucleus

7.0 References

Leeson, T.S. and C.R. Leeson . 1970, Histology 2nd Edition, W.B Saunders Company. Philadelphia

Wolfe, S.L. 1972, Biology of the Cell. Wadswork Publishing Company Inc., Belmont California

Answers to Self Assesssment Questions

1. (i) Cell Wall – outer covering of a plant cell.

   (ii) Centrosome – animal cell, near the nucleus

   (iii) Chloroplasts – cytoplasm of platn cells

   (iv) Vacoules – cytoplasm of plant cells

   (v) Mitochondria – cytoplasm of plant and animal cells.

2. Ref. 3.1 for definitions

3. Trilamellar structuture of the unit membrane.

UNIT 4: MEMBRANE: GENERAL STRUCTURE AND FUNCTION

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1.0 Introduction
2.0 Objectives
3.1 Structure of Membrane
3.2 Functions of Membrane
4.0 Conclusions
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1.0 Introduction

Membranes are very important to living organisms. An organism contains at least one type of membrane; the plasma membrane is very common to all living organisms. In animal cells it makes contact with the outside environment of the cell; nothing goes in or come out except through the plasma membrane. In plants, the cell has a rigid cell wall covering the plasma membrane. By its nature, the cell wall is freely permeable to many substances. It is when they get to the plasma membrane that selection takes place. In this wise the plasma membrane is said to be a semi-permeable structure. And, although the plasma membrane is the only membrane structure I prokaryotic cells, it could be extensive through infolding to increase its surface area; respiration takes place on the infolded plasma membrane of the prokaryotic cell.

Another unique feature of cellular membranes is that regardless of where they occur, they all share the same structural plan. The general structure of a membrane is known as the “unit membrane”. Since the organelles are the functional components of cells, and since they are all membrane-bound, a look into the structure of the membrane will enhance our understanding of cell structure and function.

2.0 Objectives

At the end of this unit you would be expected to:

1. Know and be able to describe the structure of the "unit membrane"

2. Outline the functions of membranes in the cell.

3.1 General Structure of Membrane

Membranes are lipo-protein complexes the molecular structure of which have been the subject of many speculative models. The important concepts of the various models have been embodied in the so-called "Fluid mosaic model" which states (1) that lipids and integral proteins are disposed in a kind of mosaic arrangement and (2) that biological membranes are quasi –fluid structures in which both the lipids and the integral protein are able to perform transitional movement within the overall bilayer.

As recent as the 1940's and 1950's the cell was regarded simply as an unorganized "bag of enzymes". This concept did not seem unreasonable at the time, because most of the known cellular reactions could be duplicated, in vitro, with only the enzymes and substrates present.

It is such as in the case of lysosomes which contain lytic enzymes rich in acid phosphatases. Lysosamal fractions were worked on by a chemist De Duve who obtained it as a fraction had centrifugal properties intermediate between those of mitochondria and microsome. These enzymes were later found to be enclosed by membrane and are not readily available to substrate unless the membrane is ruptured.

The existence of the plasma membrane was discovered by such an indirect way. When cells were in isotonic solutions they remained intact (fig. 4.1b). When placed in hypotonic solutions they imbibe water until they expand beyond the point of no return and then burst (fig. 4.1a). In a hypertonic media the cells shrink (fig. 4.1c). As a result of these findings it was proposed that the cell is enclosed within a semi-permeable membrane. Further elaboration with the electron microscope has revealed more about the plasma membrane.

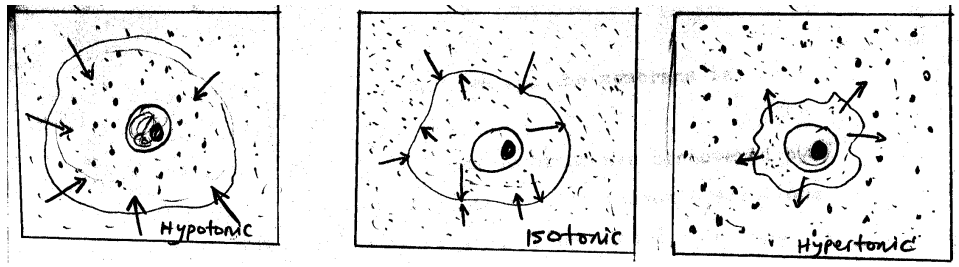

Fig. 4.1 Animal cells in solutions of different concentrations. In (a), the cells cytoplasm is more concentrated than the surrounding solution (hypotonic); this results in water entering the cell till it bursts. In (c) the surrounding solution is more concentrated (hypertonic) than the cell's cytoplasm; hence water leaves the cytoplasm and the cell shrinks. In (b) the cytoplasm has the same (isotonic) concentrates as the external medium; hence the same amount of water that enters the cell leaves it; thus the cell remains the same.

Hints of the importance of membrane structure within a cell began to emerge with continuing investigations in biochemistry, particularly I mitochondira and chloroplasts in which particular reaction sequences could be studied only in intact organelles, or at least subfractions of the organelle, were retained. These subfractions were identified in many cases as a system of membranes. The organizations of enzymes on or within membranes was found to greatly modify their properties; often dissolution of membrane structure completely inhibited enzymatic activity.

Classification of a cell into either as prokaryotes or eukaryote is based on the abundance and placement of membranes within the cell. Prokaryotes, in general are usually unicellular cells which lack an internal membrane system called organelles. Electron microscopic studies have revealed the abundance of membranes in eukaryotes and has shown in effect that the eukaryotic cell is highly ordered rather than unorganized, and that much of the order is based on a frame work of membranes (or organisms).

The membranes framework in eukaryotes include the plasma membranes, the cytopasmic and vacuolar system (including SER, RER, microsomal fraction, golgi complex, lysosomes), mitochondria, chloroplasts and the nuclear envelope. The only membrane system in prokaryote is the plasma membrane; but it is effective in such a way that it serves the functions of organelleds n eukaryotes.

The realization of the importance of membranes has spurred a tremendous burst of research into membrane ultra-strucure. The membrane became to the 1970's and 1980's what nucleic acid was to the 50's and 60's. However, the burst of enthusiasm has not yet led to a complete solution of the problem of

membrane structure but has yielded several tentative models that extend speculation to the molecular level of membrane structure. (Fig 4.2)

A number of hypothesis have been proposed and some are currently favoured. They are bases heavily on evidence derived from electron microscopy and other physical and chemical techniques. While the models differ in detail, they all agree on the membrane as consisting of globular subunits of protein and lipid complex which can be arranged either in a lamella or particulate substructure.

The lamellar mode states that the membrane is constructed fro laminations of sheet-like layers of protein and lipids. The most widely favoured lamella mode considers the membrane as consisting of a double layer of lipids between two layers of proteins, in a sort of sandwich in which the lipid forms the meat and the protein the bread.

The particulate hypothesis, in contrast, considers that the membrane is made up of a single layer f globular subunits like a layer of marbles. The lipid is considered to form the core of the subunit particle, with the protein bound to he surface of the lipid, forming an exterior coat.

Neither model can be eliminated by evidence at hand. Despite the obvious differences between the two models, both are bases on the known properties of membrane lipids and proteins. It is even possible that both models are correct and that membranes may occur in either the lamellar or particulate form, depending on their location and function in the cell.

In support of the lamella model Gorter and Grendel in 1925 extracted lipids from isolated erythrocyte membranes and showed that enough lipid was present to provide a coating two molecules in thickness over the entire surface of the cell. Danielli and Dawson proposed that the cell is covered by a bimolecular layer of lipids with the polar hyper……parts of the molecules extending into the surrounding and the non-polar hydrophobic chains associated together in the centre of the bilayer. (fig 4.3). They noted that the surface tension of living cell is much lower than the values obtained for oil droplets, they proposed that the bilipid layer is coated on both the internal and external surfaces by a layer of protein, which acts to reduce the surface tension (Fig. 4.4).

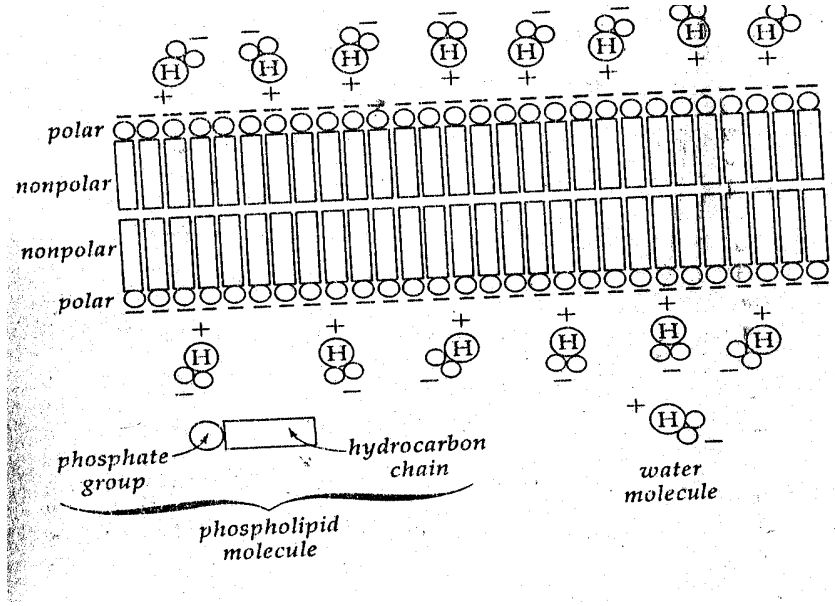

Fig 4.3: The presumed arrangement of phospholipids molecules in a bilayer.

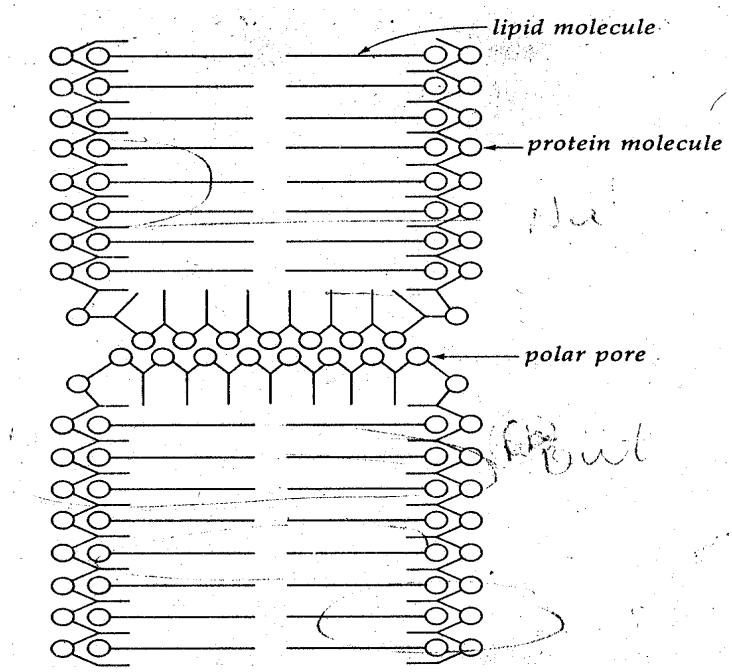

Fig 4.4: The Danielli-Davson model for membrane structure. A lipid bilayer is stabilized by layers of protein on both outer surfaces. At intervals, pores, presumably coated with protein, extend through the membrane. These "pores" are extremely minute, and give the membrane in these regions polar properties, with the result that water, but not larger polar molecules penetrates the membrane readily. Redrawn original courtesy of J.F. Danielli.

The hydrophilic portions of the membrane lipids are proposed to face outwards towards the surface of the bimolecular layer of lipids, at these surfaces the lipids are associated with proteins. The protein layers on the inside surface of the membrane (the surface facing the cytoplasm) probably differ, in specific molecular composition, from the protein layer that faces the external environment.

This model is attractive since it agrees with the usual electron microscope image which sows a trilarmellar membrane structure, which looks like a railroad tract withy two dark parallel lines each (in the plasma membrane) about 30Å thick, and an inner lighter zone also about 30Å thick. Presumably, the central layer of the unit membrane would correspond to the hydrophobic portions of the lipids while the two dense lines would represent the protein and the hydrophilic portions of the lipids. This structure is called the "unit membrane".

Differences in thickness and asymmetry of the layers have been observed in various membrane types. The thickness of the unit membrane has been found to be greater in the plasma membrane (10m) than in the intracellular membranes of the ER or Golgi complex (5 to 7mm).

The important concepts of the various models of membranes have been embodied in the so-called "fluid mosaic model" of membrane structure. This states, (1) that the lipids and integral proteins are disposed in a kind of mosaic arrangement (Fig 4.5 and (2) that biological membranes are quasi-fluid structures in which both the lipids and the integral proteins are able to perform translational movement within the overall bilayer. This model regards both the lipid and integral protein to be "amphipathic" is exhibit hydrophilic and hydrophobic group within the same molecules. The hydrophobic chains of lipids are enclosed in the iner space of the bilipid layer; the protein too have their polar regions protruding from the surface and non-polar regions embedded in the hydrophobic interior of the membranes. (Fig. 4.5)

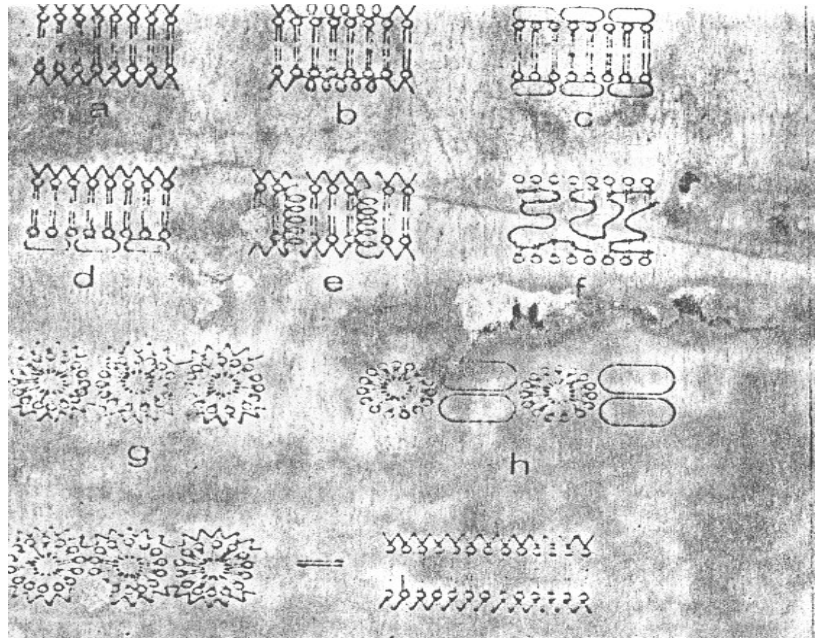

Fig. 4.2.

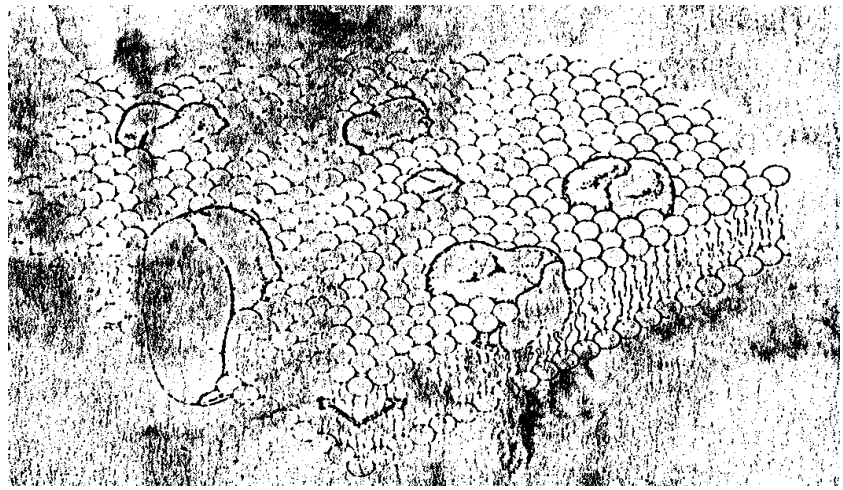

Fig. 4.5

The fluidity of membranes is supported by experiments which have demonstrated the rapid lateral diffusion of the lipids and the fact that the integral proteins can also undergo translational displacements within the bilayers.

As a result of these diffusibilities it has been possible to fuse two different cells together, for example fusing of mouse L cell and human transformed cells influenced by Sendai viruses. At the beginning both cells surfaces could be recognized by their different labels but after 40 minutes considerable intermixing of the antigens had occurred, so that the two labels could no longer be recognized.

Also observations of living cells have revealed that membranes are continually being assembled and disassembled. Sometimes with great rapidity. This indicates that membranes are always in a dynamic state, not static.

The fluidity of membranes depends upon the state of unsaturation of the fatty acid components of the lipids. The more unsaturation that exists within the fatty acids the more fluid the membrane is. Membranes undergo a physical phase transition from a flexible fluid-like liquid crystalline state to a solid gel structure as a function of temperature. The temperatures at which the phase transition occur are dependent on the composition of the amphipathic lipids. The lipids with more unsaturated fatty acids have lower transitions than those with more saturated fatty acids; longer chain lengths have higher transition temperatures than shorter chain lengths; Cis fatty acids have a lower transition temperature than trans-unsaturated fatty acids.

The significance of the heat transition is clearly apparent when membranes in homeothernic and poikilothermic animals are compared. By controlling their internal environment homeotherms do not expose their membrane systems to marked temperature changes. Cold blooded poikilotherms on the other hand are exposed to marked shifts in temperature. Undoubtedly, all membrane-bound enzymes, transport processes, receptor sites etc. are embedded within lipids, and therefore their activities would be markedly altered by the state of the lipids within membranes which in turn is a reflection of the surrounding temperatures. Thus it can be shown that lipids of the mitochondria from homeotherms have a higher proportion of saturated fatty acids them those from poikilotherms.

The same phenomenon exists in the membranes of cells on reindeer legs. The temperature in the deer's legs is higher towards the body than towards the hooves. The deer compensates for this by having more unsaturated fatty acids in the membranes of the cells near the hooves.

Membranes lipids comprise the matrix that give form and structure to membranes in which membrane proteins are imbedded. All members contain amphipathic lipids that include phospholipids and glycolipids. (Table 4.1 & 4.2) Phospholipids are composed of fatty acids linked to a glycerol backbone. Generally two fatty acids are attached to the glycerol with the third position available for one of the specific compounds encountered in phospholipids, suchas, choline or ethanolamine.

Threee major types of lipids found in nature are: fats, phospholipids and steroids. Fats consists of fatty acids, a series of long hydcrocarbon, chains, linked to a glycerol backbone. A triglyceride results when all 3 carbons of glycerols are attached to a fatty acid. Most phospholipids have a structure similar to triglycerides, except that in place of one of the fatty acids they have a more complex chain phosphate and nitrogen-containing groups. Steroids have a skeleton based on the structure of cholesterol. Glycolipids are also found in cellular membranes.

The phospholipids and steroids are polar molecule i.e. amphipathic with hydrophobic and hydrophitic ends. Plasma membranes and other cellular membranes are rich in polar lipids.

Table 4-1 Lipid Composition of Animal and Bacterial Membranes (in per cent)								
				Microsomal Fractions				
	Myelin	Erythrocyte	Mitochondria	1	2	Escherichiacoli	Bacilus megateriu	Chroloplast
Cholestrol	25	25	5	6	*	0	0	*
Phosphatidyl ethanolamine	14	20	28	17	8	100	45	0.6
Phosphatidyl serine	7	11	0	0	9	0	0	*
Phosphatidyl choline	11	23	48	64	48	0	0	4.1
Phosphatidyl inositol	0	2	8	11	6	0	0	1.4
Phosphatidyl glycerol	0	0	1	2	0	0	45	5.3
Cardiolipin	0	0	11	0	2	0	0	0.6
Sphingomyelin	6	18	0	0	9	0	0	*
Cerebroside	21	0	0	0	0	0	0	*
Cerebroside sulfate	4	0	0	0	0	0	0	*
Ceramide	1	0	0	0	0	0	0	*
Lysyl phosphatidyl glycerol	0	0	0	0	0	0	10	*
Galactosyl diglyceride								14.7
Digalactosy diglyceride								35.3
Sulfoquinovosyl diglyceride								4.9

* Not analysed

Adapted from E.D. Korn, Structure of biological membranes. Science 153:1491 (1966). Chloroplast data from J.S. O'Brien, J. Theoret, Biol. 15:307 (1967)

Table 4-1

	Total Lipid		choles-	Total	other	Phospho-
	mg/ml		terol	ganglioside	glyco-	lipid
	packed	gm/cell			lipids	
			(%)	(%)	(%)	(%)
Cat	6.04	3.45×10^{-13}	26.8	8.8	3.1	61.3
Cow	4.44	2.58×10^{-13}	27.5	5.5	2.2	64.8
Dog	5.76	4.84×10^{-13}	24.7	11.8	10.9	52.6
Goat	6.14	1.23×10^{-13}	26.2	5.7	17.9	50.2
Guinea Pig	5.72	4.41×10^{-13}	27.0	2.2	15.2	55.6
Horse	5.37	2.58×10^{-13}	24.5	15.5	8.0	52.0
Pig	4.33	2.52×10^{-13}	26.8	3.3	10.1	59.8
Rabbit	4.57	4.15×10^{-13}	28.9	4.5	0.8	65.8
Rat	5.08	3.15×10^{-13}	24.7	6.3	2.0	67.0
Sheep	4.91	1.62×10^{-13}	26.5	7.8	2.5	63.2

Adapted from G. Rouser et al., Lipid composition of animal cell membranes, organelles, and organs, D. Chapman, ed. Biological Membrane. New York: Academic Press, 1968, p.5. see this paper for original references.

Table 4-2

Membrane proteins fall into two general classes: peripheral and integral protein. The peripheral proteins are loosely bound and can be displaced by hypotonic exposures, strong salts, mild detergents or sonication. Examples of peripheral proteins include cytochrome “o” which is loosely associated with the outer face of the inner membrane of mitochomdria, spectrin of erythocytes and β-lactalbumin which is loosely associated with the plasma membrane of mammary glands cells. In addition, the periplasmic binding proteins of the plasma membrane of bacteria are also classified as peripheral proteins.

The integral proteins are tightly bound to membrane lipids bilayers and may include a large number of functional proteins that participate as transport carriers, drug and hormone receptor sites, antigens and a large number of membranes bound enzymes (e.g. ATPase). Examples include NAD-cytochrome b5 reductase which is tightly coupled to the homoprotein, andcytochrome oxidase which is imbedded in the endplasmic reticulum of eukaryotic cells. They are usually attached to lipids to form lipo-proteins (i.e proteolipids) or may be attached to digosaccharrides to form glycoproteins. They require drastic procedures for isolation.

3.2 Functions of Membranes

Membranes are involved in:

1. Pinocytosis - intake of small molecules-plasma membrane

2. Phagocytosis – ingestion of large particles-plasma membrane

3. Exocytosis – expulsion of particles from cell-Golgi, plasma membrane.

Bioenergetics

4. Photosynthesis in plant chloroplasts.

5. Oxidatve phosphorylation-production of ATP in mitochondria

6. Storage – eg. lysosomes are stored I membrane (b) protection, were it not for the membrane autolysis by lysozomal enzymes will take place.

7. Cell recognition – contact inhibition where it fails cancer results.

8. Anrtigen – antibody reaction (Ag – Ab) – plasma membrane

9. Shape and size – plasma membrane.

10. Increase in surface area eg. microvilli, infoldings of mitochordria and chloroplast membranes, ER and Golgi apparatus.

11. Transmision of nerve impulse-nerve cell membranes (b) protection of nerves-myeling sheath.

12. Maintenance of heat-saturated vs unsaturated membranes.

Self Assessment Questions

1. Name five membrane-bound structures (organelles) within an eukaryotic animal cell.

2. Name one organelle found in plans but not in animal cell.

3. Name one organelle generally found in animal but not in plant cell.

4. What macromolecules make up the structure of a membrane?

5. Show diagrammatically how lipids will react in aqueous solution to form layers.

4.0 Conclusion

1. Membranes consist of lipids and proteins (Lipo-proteins).

2. The lipids are arranged in a bi-layer with the hydropholic ends tucked in on the inside and the hydrophilic ends on the outside.

3. Proteins now sandwich this bi-layer of liquids.

b. the lipids appear as one layer with two layers of proteins, one on each side.

c. this gives the membrane its tralamella outlook.

4. The trilamella structure of the membrane is known as its "unit structures".

5. The unit structure is the basic structure of al cellular membranes.

6. Membranes exhibit fluidity which is demonstrated by the rapid lateral diffusion of the lipids, making it possible to fuse two different cells together.

7. Membranes are continually being assembled and disassembled, sometimes with great rapidity. Hence membranes are always in a dynamic state, not static.

8. Membranes with more unsaturated fatty acids are more fluid than those with saturated fatty acids.

9. Membrane proteins are of two types (a) peripheral which are loosely bound and (b) integral proteins which are tightly bound to membrane lipid bilayers.

10. Two major types of lipids are fond in membranes: Phospholipids and glycolipids. Membrane lipids give form and structure to membranes.

5.0 Summary

Membranes are important structures within the cell and this warrants a unit to discuss the structure and functions of membranes. Membrane structures in the cell include plasma membrane, SER, RER, Golgi Body, mitochondria, chloroplasts, lysosomes, centrioles and ribosomes. Within their various organelles the membranes carry out different functions which gives the particular cell its function and contribute to the general functioning of the organism itself.

6.0 Tutor marked Assignment (TMA)

1. Make a diagrammatic drawing of the unit structure of the membrane.

2. List nine (9) functions of membranes within a cell.

7.0 References

1. Roberts, M.B.V. 1975. Biology: A Functional Approach, E./L.B.S. and Nelson. Lagos

2. Stephens, G.C. and Barbara B. North. 1974. Biology. John Wiley & Sons, Inc. New York

3. Wolfe, S.L. 1972. Biology of the Cell. Wedsworth Publishing Co. Inc., Belmont, California.

Answers to Self Assessment Questions

1. Plasma membrane, SER, RER, mitochondria, lysosome, nucleus, ribosome.

2. Chloroplast

3. Centriole (centrosome)

4. Lipids and proteins (lipo-protein)

5. See fig. 4.3

UNIT 5: CELL TYPES

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1.0 Introduction

In Unit 3 we dealt with structures which are common to most cells, and we pointed out then that while cells have a lot of features in common, a lot of variations also occur among them. We shall now study some examples of cell types, but before we so this, we shall first consider the differences between plant and animal cells. You will find in some subsequent units that

some of the differences I the activities of plants and animals are due to these differences in the basic structure of their cells.

2.0 Objectives

This unit is devoted to the study of different types of plant and animal cells. We have noted in an earlier unit 3 that there are various types of cells on the basis of size, shape, structure and function. In this unit, we shall consider some examples of these various types of cells and note the aspects in which one type of cell differs from another.

At the end of this unit, therefore, you should be familiar with the range of cell types that make up the bodies of plants and animals. You should be able to name different cell types found in animals and plants.

3.1 Differences between Plant and Animals Cells

Fig. 5.1 (a) and (b) below illustrate the main features of a plant and an animla cell. The diagrams show the prominent differences between the two types of cells.

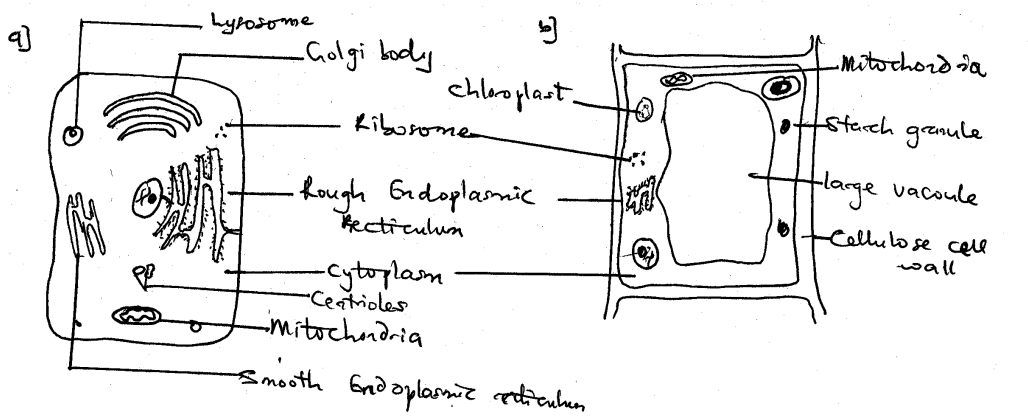


Figure 5.1 diagrams illustrating differences between (a) animal cell and (b) plant cell.

3.1.1 Cell Wall

The first major difference is the existence of a surrounding rigid wall, exterior to the plasma membrane in the plant cell. The absence of this cell wall, in animal cells underlies some of the functional differences between plant and animals. The cell wall, which contains cellulose,

confers a certain amount of rigidity on the cell but also ensures constancy of shape. Thus, plant cells have to a large extent a constant shape as opposed to some animal cells whose shape is constantly changing e.g an ***amoeba***, a human leucocyte (WBC).

3.1.2 Plastids

Another major difference is that plastids (e.g. chloroplasts) are present in plant cells but absent in animal cells. Plastids, particularly chloroplasts, enable plants to manufacture carbonhydretes from simple inorganic substances (water and carbondioxide) using sunlight as a source of energy. This process is known as photosynthesis. Animals are incapable of carrying out this process, because they lack plastids, and so have to obtain their food in complex forms. Thus the basic differences in the nutrition of plants and animals are related to the presence or absence of chloroplasts respectively. Plants are holophytic, animals are heterophytic.

Some other behavioural differences between plants and animals are associated with this difference in nutrition. One such difference is that animals usually move about searching for food while plants usually do not move. Water and carbon dioxide are the raw materials for photosynthesis are usually readily available from the soil and the atmosphere. There are exceptions to this difference between plants and animals. Some plants, especially aquatic ones are motile; and some e.g insectivorous plants and mucor, take in insects and organic foods.

3.1.3 Centrosome (Centriole)

Most animal cells have centrosomes (centrioles), which are involved in the movement of chromosomes during cell division. Plants cells lack centrioles, although spindle fibres are still formed and chromosomes still move during cell divison.

3.1.4 Starch and Glycogen Granules

While plant cells have starch granules, these are absent in animal cells. Glycogen is the form of storage of carbonhydrates in animal cells but not in plant cells.

3.2 The Plant Cell Wall

Fig 5.2 illustrates the structure of a typical plant cell wall. It consists of a number of layers which are different in origin and chemical composition.

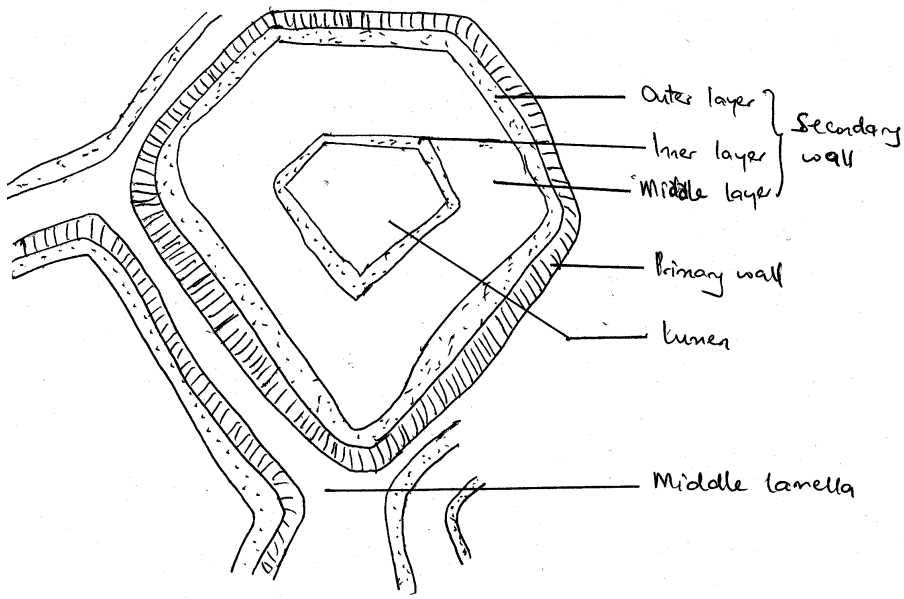


Fig. 5.2: Diagram of plant cell wall.

3.2.1 Middle Lamella

The layer which forms the first partition between two cells as they are formed during cell division is called the middle lamella or intercellular substance. It is shared by two adjacent cells together. The main chemical constituent of the middle lamella is pectin.

3.2.2 The Primary Wall

The next layer is the primary wall. It lies between the middle lamella and the plasma membrane. While the cell is still growing and enlarging the primary wall is thin and elastic, but when the cell stops growing the wall may thicken. Chemically this wall is primarily made up of cellulose, various kinds of sugars and proteins. (Cellulose is a complex polysaccharide which is formed by a consideration of many glucose molecules).

3.2.3 Secondary Wall

The next layer of the cell wall is the secondary wall which forms between the primary wall and the plasma membrane. The secondary wall may be thin but it is usually thickened to varying degrees. When thickened it may consists only of cellulose but usually other chemicals may become deposited on the cellulose layer. Some of these other chemicals are ***lignin*** and ***suberin***. When such chemicals are present the wall is said to be lignified or suberised. These chemicals are impervious to water and air. The cells in which they occur are therefore usually non-living. The thickening material is usually not uniformly laid down but takes a number of different patterns. It may be arranged in rings, spiral bands or patches of thick and thin areas. Some of these are illustrated below in Fig. 5.3. These arrangements allow for flexibility:

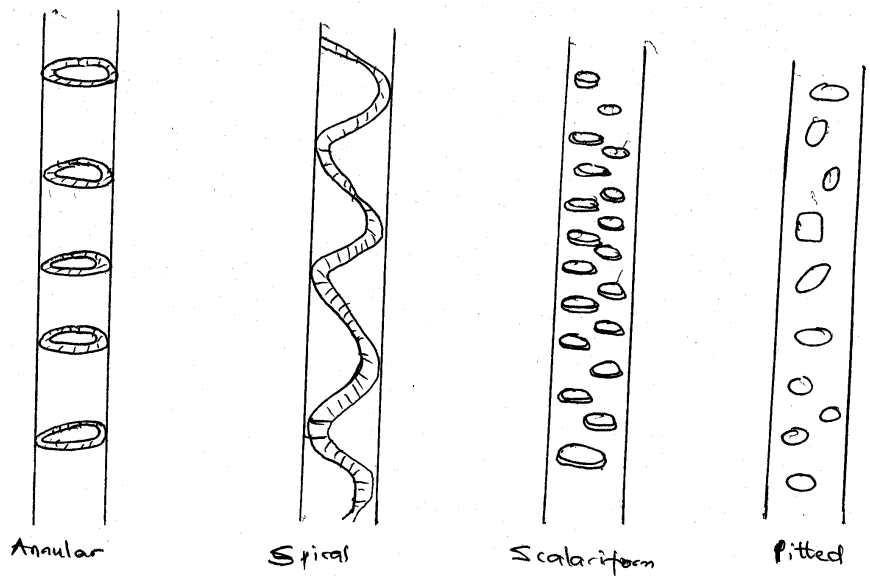


Fig. 5.3: Various patterns of cell wall thickening

3.2.4 Pits

The secondary wall is not usually laid over the entire primary wall. It is absent altogether in some places, which remain thin. Such areas that remain thin in the walls of plants (while the rest of the wall is thickened) are called **pits.** When pits of two adjacent cells coincide, a pit pair is formed. This is illustrated in Fig. 5.4

The structure of a pit may be simple, when its side walls are plain and parallel or more complex when various modifications are present. An extreme situation is seen in ***bordered pits*** in which the pit cavity is over-arched by an extension of portion of the cell wall (fig. 5.4. b).

The cytoplasm of the adjoining cells are continuous in the form of slender strands, called plasmodesmate, across pits. This ensures continuity of cytoplasm in organisms and facilitates the movement of molecules between adjacent cells.

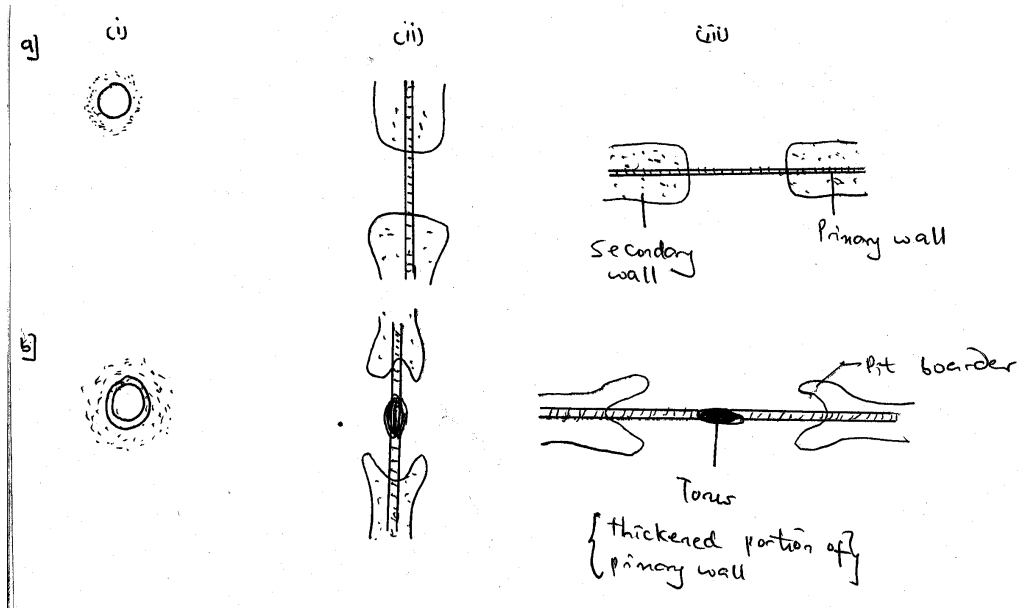


Fig. 5.4: Structure of pits (a) simple pit (b) boarded pit
(i) surface view (ii) longitudinal section (iii) cross –section.

3.3 Types of Plant Cells

If you examine critically any plant in your environment you will notice that is it made up of a number of parts which are markedly different and distinguishable. The leaves can be readily distinguished from the branches and from the flowers and fruits. These external differences could be a manifestation of the different types of cells that make up the entire plant. The cells are different in structure as well as in function and this enables the plant

to carry out its various functions. We shall now consider some of these different types of cells found in plants.

3.3.1 Meristematic Cells

Meristematic cells are the least differential of all the cells found in the plant body. They are found at growing points of root an shoot apices where cell division results in the production of new young cells. Other types of cell can be imagined to be derived from meristematic cells by various modifications arising as a result of growth of the cell. The structure of a meristematic cell is illustrated below in Fig. 5.5 (a). Its main characteristic feature is its small size and cubical shape. It has a large prominent nucleus compared with the size of the cell. It has a dense cytoplasmic content.

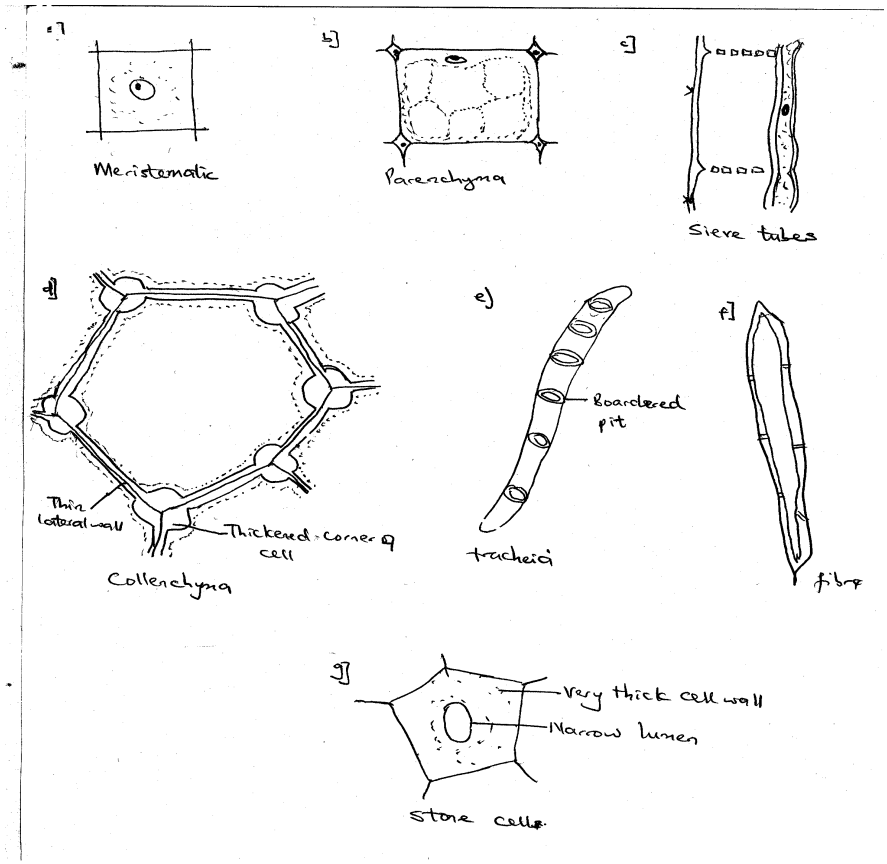


Fig. 5.5 various types of plant cells.

If vacuoles are present, they are usually small in size and numerous in quantity. The wall of a meristematic cell is thin and cellulotic. With

all these characteristics you can determine that the meristematic cell is a living cell.

3.3.2 Parenchymatous Cells

These are large and thin walled. The wall is cellulose, hence it is a living cell. The cytoplasm usually contains a large vacuole which may be central, restricting the cytoplasm to a living of the wall and displacing the nucleus to one side of the wall. (Fig. 5.5b). Parenchymatous cells form the bulk of the tissue of the plant. They may contain chloroplasts as in the leaf.

3.3.3 Collenchyma Cells

Collenchyman cells resemble parenchyma cells but they are thickened a the corners (fig. 5.5d). The thickening is of cellulose. As a result of the thickened corners, collenchyma cells give some mechanical strength to the plant.

3.3.4 Sclerenchyma Cells

Sclerenchyma cels have greatly thickened walls and the thickening materials include chemicals other than cellulose. These chemicals include ***lignin*** and ***suberin*** which are impervious to water and air. At maturity these cells are non-living. They lack nuclei and cytoplasm. This group of cells includes a number of different types: tracheids (Fig. 5.5e) vessels and stone cells (Fig. 5.5g).

(a) **Tracheids**: are short and irregularly shaped. They have greatly thickened walls with only a very narrow lumen.

(b) **Fibres**: are long and narrow with tapering ends. Its lumen is also narrow.

(c) **Vessels**: are greatly elongated cells. They are made up of a system of elongated cells joined end to end to form continuous pipes. They have larger lumina (sing lumen) through which water is concducted. It is in the walls of vesselsthat different methods of thickening are conspicuous. Young vessels hve the ring type of lignined thickening called annular thickening; slightly older ones are spirally thickened which may be of the sclariform, reticulate and pitted types. These sclerenchyma cells are suitably adapted for mechanical strengthening. The

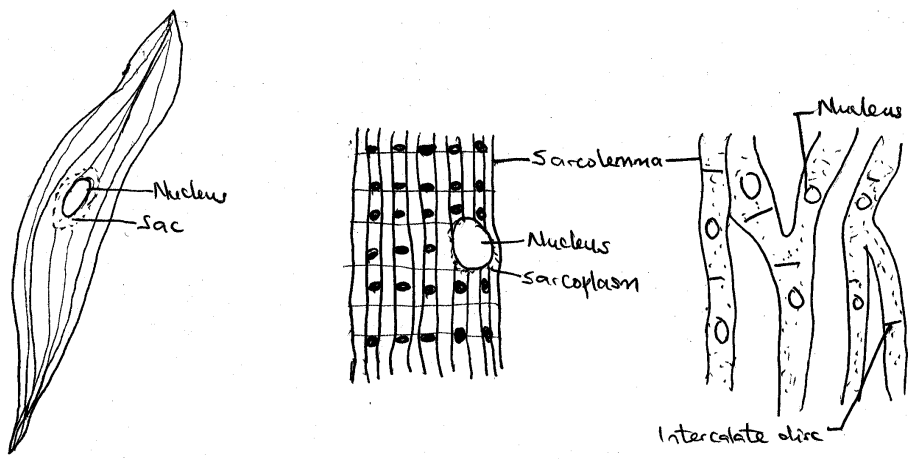
presence of thickened lignified secondary walls confer on these cell types great tensile strength for this purpose.

3.4 Types of Animal Cells

You will recall that, when discussing plant cell types, we noted that an important difference between plant cells is in their shapes, but a more important difference is related to the structure of the walls. In studying animal cells we found there is no cell wall, and so differences between animal cells are based mainly on shape as well as function. We will now discuss some common types of animal cells.

3.4.1 Muscle Cells

The muscle is made up of different types of cells whose common characteristics is their high contractility (ability to move by contracting). The different muscle cells or fibres: ***smooth*** or ***unstriated*** muscle, ***striped*** or ***striated*** muscle and cardiac muscle. These are illustrated below in Fig.5.6 (a) (b) and (c).



a) Smooth muscle b) Striated muscle c) Cardiac mucle

Fig . 5.6: Types of muscle cells (a) smooth (b) striated (c) cardiac muscle.

(a) Smooth (Unstriated) Muscle Cells

Each unstriated muscle cell is elongate, and tapers gradually at each end (ie. Spindle shaped). It consists of a centrally placed nucleus which is surrounded by a mass of cytoplasm, the ***sacroplasm***. The rest of the cell consists of very delicate

myofibillae (myofibrils) which run the length of the cell. These are not surrounded by a well defined membrane.

Smooth muscle is mainly visceral in distribution, forming the contractile portion of the wall of the digestive tract from the midpoint of the oesophagus to the anus, including the ducts of glands associated with the digestive system. It is found in the respiratory, urinary and genital systems and in arteries, veins, capillaries, and larger lymphatic ducts. In addition, it is present in the dermis and in the iris and ciliary body of the eye.

(b) Striated Muscle Fibres

The individual striated muscle fibre or cell is long, cylindrical and multinucleate, the ends tapering to a point or being somewhat rounded or notched at the junction of muscle and tendon. They differ from the cells of unstriated muscle in that they are enclosed in a definite membrane, the ***sarcolemma***. Below the sarcolemma re embedded many nuclei each in layer of sarcoplasm. The rest of the cell is composed of microfibrillae which run through the length of the cell. Alternating bands of light and dark stripes cross the cell giving it a striated appearance.

Striated or skeletal muscle is that which the layman recognizes as muscle and comprises the flesh or meat of animals. They are attached to the long bones of the hands and legs and enable movement. Our backside, which we sit on, is made up of striated muscle fibres. Striated muscle fibres is what is built up by lifting weights in the gymnasium.

(c) Cardiac Muscle Fibres

Cardiac muscle, which is involuntary, contract rythymically and automatically. It is found only in the myocardium (muscle layer of the heart) and in the wall of the large vessels joining the heart. They contract throughout the life of the animal; when they cease death occurs.

Cardiac muscle fibres have a structure which is intermediate between that of unstriated and striated fibres. A cardiac muscle fibre by light microsopy is a muscle cell joined end to end at specialized junctional zones called ***interclated discs***.

The fibres in any region run mainly in parallel fashion but "cross beams" are numerous and this gives the false impressions of a synetical network. Between the fibres is fine connective tissue, the endomysium containing small blood vessels and lymphatics.

The cardiac muscle fibre is enveloped by a thin sarcolemman similar to that of skeletal muscle, and sarcoplasm is abundant with numerous mitochondria. Myofibrils are separate by mitochondria arranged in rows between them, with consequent obvious longitudinal striation.

3.4.2 Blood Cells

Blood contains two main types of cells or corpuscles, the red blood corpuscles (RBC) or erythrocytes and white blood corpuscles (WBC) or leucocytes.

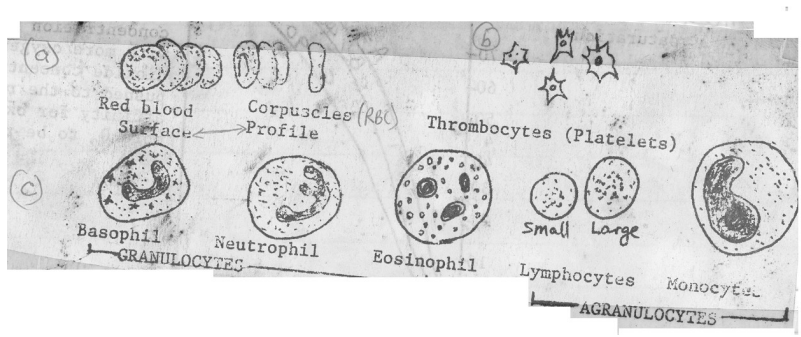


Fig. 5.7: Formed elements of blood (i.e. solid corpuscles)

(a) Erythrocytes

The erythrocytes are biconcave circular discs. They are bounded by a thn elastic envelope and thus are easily distorted by pressure. When the pressure is removed, the original bi-concave disc shape is restored (fig. 5.7a). The shape allows blood to flow through small capillaries. The cytoplasm of the erythrocyte contains the respiratory pigment haemoglobin which gives blood is red colour. There is no nucleus in the mature RBC but they are present in the RBCs of other vertebrates; this is a distinctive faeture of human RBC. Because it lacks a nucleus the RBC is short-lived, with a half-line of 60 days and a maximum life span of 120 days.

Erythrocytes function in the transportation of oxygen (O_2) from the lungs, releasing it in the tissues and carrying carbon dioxide from the tissues to the lungs. Haemoglobin is the pigment that carry the gases.

(b) leucocytes (white blood cells, WBC)

leucocytes are of two types (i) granular (granulocytes) and (ii) non-granular of agranular. The granulocytes have granules within their cytoplasm and these react to certain stains. Cells whose granules stain with acid stains are called acidophils, while those that react with basid stains are basophils. Granulocytes are the ***basophilis***, ***neutrophilis*** and ***eosinophils*** while agranulocytes are the ***lyniphocytes*** and ***monocytes***. The nucleus of the different leucocytes are variable in shape but the commonest condition is that of an irregularly lobed for which is said to be polymorphous (Fig. 5.7c).

3.4.3 Nerve Cells

A nerve cell consists of a cell body which comprises a system of branching fibres. The cell body consists of cytoplasm and a nucleus. The cytoplasm contains granules (Nissl granules) which stain readily with methylene blue. In the cytoplasm is also a system of neurofibrillae. Out of the fibres that arise from the cell body, one is especially long. The shorter ones are called ***dendrites*** and through them the nerve cell (neurone) receives impulses. The specially long fibre is the axon or ***axis cylinder*** and through it impulses are sent out. In most cases the axis cylinder is surrounded by a fatty myelin sheath outside which is a thin membrane called the ***neurolemma***. Just below the neurolemma is a very thin layer of cytoplasm in which nuclei is embedded at intervals. The structure of a nerve cell (neurone) is illustrated in Fig. 5.8

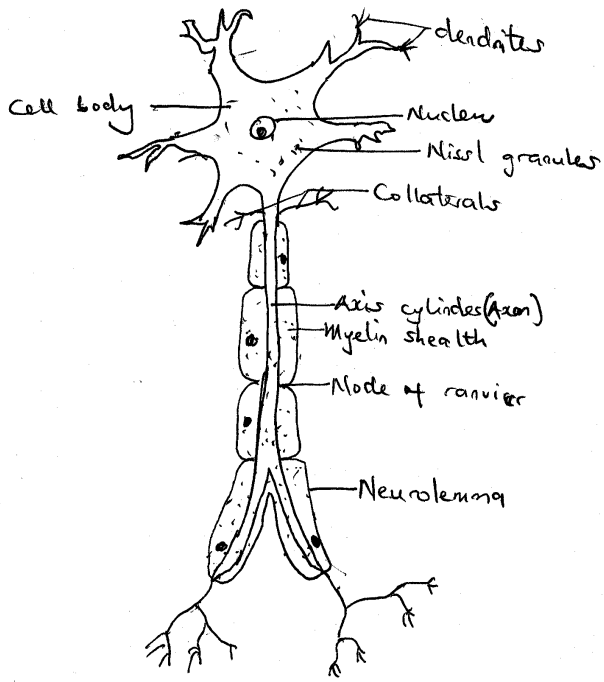


Fig. 5.8: Structure of a nerve cell (neuron)

4.0 Conclusion

In this unit we have discussed a number of cell types found in plants and animals. The examples are by no means exhaustive. Many other types of cells exists. We did consider the following types of plant and animal cells.:

(a) Plant Cells

(i) Meristematic Cells – small, isodiameric, dense cytoplasm, prominent , centrally placed nucleus; thin cellulose wall, therefore a living cell; numerous, small vacuoles.

(ii) Parenchyma Cells - Irregularly shaped, vacuolated thin cellulose wall, therefore living cell; they contain chloroplasts.

(iii) Collenchyma Cells - like parenchyma cell, but with cellulose – thickened corners; also a living cell;gives mechanical strength to the plant.

(iv) **Sclerenchyma Cells** – Thick secondary walls impregnated with chemicals e.g. lignin suberin, which are impervious to water and gases; therefore at maturity not a living cell. This group include different cell types e.g. tracheids and fibre cells.

(b) Plant Cells

(i) **Muscle Cells –** (a) smooth, (b) striated (c) cardiac. Fibrows in structure and highly contractible.

(ii) **Blood Cells -** (a) Red blood corpuscles (RBC) or erythrocytes biconcave discs; contain haemoglobin which gives blood its red colour; anucleate in humans; carry O_2 and CO_2. (b) white blood corpuscles (WBC) or leucocytes. Granulocytes – basophils, neutropluils and acidopluils, contain granules in cytoplasm. Agranulocytes – lymphocytes and monocytes; smooth cytoplasm – no granules.

Nucleus of many shapes (polymorphic) fight against germs (defence of body)

(iii) **Nerve Cells –** Cell body contains nucleus and branches called dendrites through which impulses are received, granules or Nissl bodies within cytoplasm; longest dendrite called axon or axis cylinder also ends in dendrites through which impulses leave the neuron as myelin sheath which has notches called Nodes of Ranvier.

5.0 Summary

In an earlier unit we discussed what a "typical" eukaryotic cell looks like under the microscope (light and electron microscopes). We also mentioned that there is really nothing like a "typical" cell but that most cells contain what had been shown in the "typical" cell. We have now seen that there are many types of cells, each one specially designed for its functions.

Self -Assessment Questions

1. Illustrate the structure of a typical plant cell wall.

2. What do you understand by primary wall and secondary wall. Explain each term.

3. Make a detailed diagram of a neurone.

4. Draw and label the following cell types: unstriated muscle cell, striated muscle cell, erythrocyte and leucocyte

6.0 Tutor-Marked Assignment

1. Name and describe the formed elements of blood.

2. Name and describe four different types of plant cells.

7.0 References

Hickman, C.P. 1970, Integrated Principles of Zoology 4th Edition. The C.V. Mesby co., St. Louis.

Leeson, T.S. and C.R. Leeson . 1970, Histology 2nd Edition, W.B Saunders Company. Philadelphia

Answers to Self Assessment Questions

1. Ref. Fig. 5.2

2. See sections 3.2.2 and 3.2.3

3. See fig. 5.8

4. See fig. 5.6 and 5.7

MODULE 2

UNIT 6: TISSUES, ORGANS AND SYSTEMS

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1.0 Introduction

Many large organisms, animals or plants are composed of hundreds of cells. In some big plants, such as trees there are many millions of cells; also in animals such as man, there are millions of cells. Such organisms are said to be ***multicellular***. On the other hand, there are organisms which are composed of one cell only e.g. bacteria, chlamydomonas, Amoeba' such organisms are said to be ***unicellular***.

Let us compare the cells of multicellular organisms with human beings. If a person lives alone, absolutely cut off from contact with any other human being (e.g. a hermit), he will have to do everything for himself. A unicellular organism is like that. It performs all the processes of life within itself.

Now within a community of people nobody does everything for himself. People split themselves up into groups (consciously or not) and each group does one or perhaps several kinds of work ***only.*** Some grow food, such as farmers; others manufacture it, such as bakers; others make clothes, and others are responsible for the health of the rest; and so forth. This splitting up of all necessary work and allocating it to different people or group of people is called ***division of labour.***

In this respect each multicellular organism represents the community, and each cell represents one human being within the community. Human beings modified to their special work; farmers become proficient in filling the soil, sowing and reaping; doctors in treating the sick, and so on so do cells become modified in order to carry to their special work successfully.

How do cells go about becoming "specialists" in a field? Remember that you and I came from one single cell after fertilization – the ***zygote***. This cell divides through mitosin to form a ball. The cells are at first all alike. But as the number of cells increase they begin to group themselves into clusters and start to look and act differently from other cells. thus a group of cells become the head, another the arm, another the liver etc. This is called ***differentiation.*** Thus, in all living organisms especially the more advanced, there is a ***morphological differentiation of structure with a physiological division of labour.*** Fig. 6.1 shows different forms of animal cell elements, which have become modified to perform various functions.

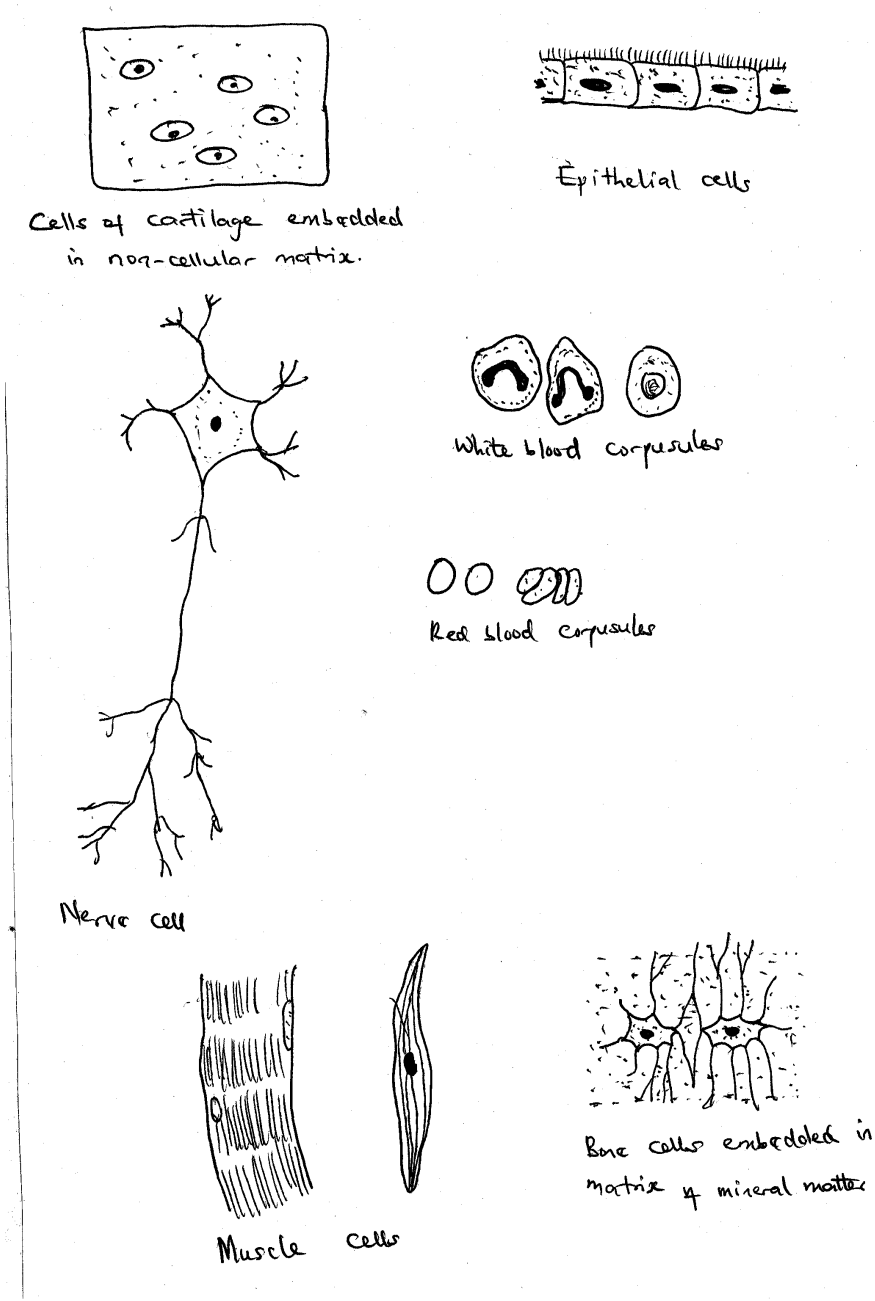


Fig. 6.1: various forms of animal cells

Division of labour has another important characteristic. Men having the same kind of work to do often group themselves together: coal miners, for example, congregate around the coal mines, and farmers relegate themselves

to the land. Thus, within a community of people, so far as is possible, there is a segregation according to work (or function). So also in living organisms; many cells which have the same kind of work to perform group themselves together. Such groups are called ***tissues*** e.g. epithelium. When different tissues group together to perform a singular function they are called an ***organ*** e.g. kidney. Many organs group or work together to form a ***system*** e.g. reproductive system. A group of systems come together to form the **organism.**

2.0 Objectives

At the end of this unt you should be able to:

1. name different tissues within animals and plants and discuss their functions.

2. name and describe different organs in plants and animals and specify their functions.

3. discuss various systems in plants and animals with their functions.

3.1 Plant Tissues

A tissue is a group of similar cells which perform the same function. Usually, the cells that make up a tissue are contiguous. We shall now consider a few plant tissues.

3.1.1 Meristematic Tissue

A meristematic tissue is made up of meristematic cells, whose structure we have already described in unit 5. (see Fig. 5.5a). In the tissue the cells are closely fitted together. There are no air spaces between adjacent cells (see Fig. 6.2). the meristematic cells are capable of cell division and so a meristematic tissue is a growing tissue. It is found at growing points at the apices of stems and roots.

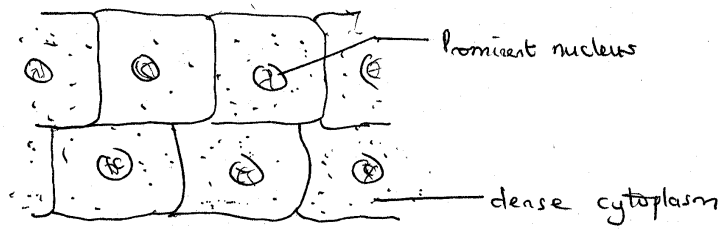
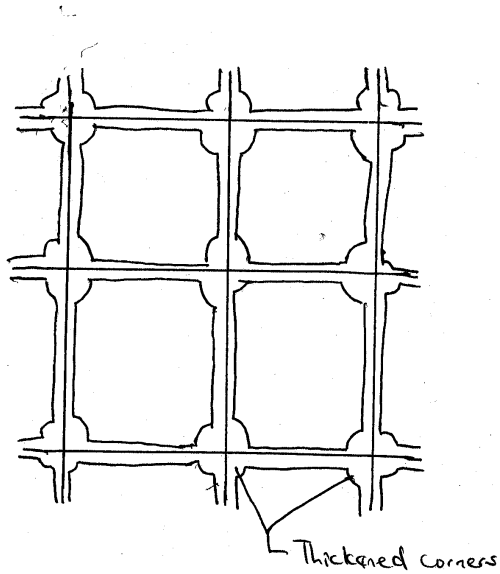


Fig. 6.2 Meristematic Tissue

3.1.2 Parenchymatous Tissue

This is made up of parenchyma cells whose structure we discussed in Unit 5. In the tissue the cells are not closely fitted. Air spaces occur among adjacent cells (Fig. 6.3). Parenchymatous tissue is a filling tissue. It forms the ground tissue and may also serve as storage tissues, string water or food.



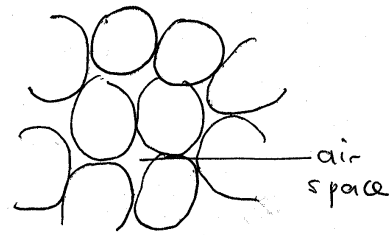


Fig. 6.4: Collenchyma Tissue

Fig. 6.3: Parenchyma Tissue

3.1.3 Collenchyma Tissue

This is familiar to parenchyma tissue but the cells are thickened at the corners (fig. 6.4) and their distribution is more restricted. The presence of thickenings at the corners of the cells give some mechanical strength to organs where the tissue occurs. Collenchyma tissue is usually found at the periphery organs.

The tissues we have considered so far are made up of cells that are all of the same type. For this reason the tissues are said to be ***simple.*** Other tissues are made up of different types of cells. Each cell type has a different function. These are called **complex** tissues and examples are considered below:

3.1.4 Xylem

As a complex tissue the xylem is made up of different cell types among which are the following; parenchyma fibre, sclerieds and vessels (see unit 5: 3.3.2 – 4). Fig 6.5 illustrates the features of xylem tissue. The proportions in which the different cell types are present varies in different xylem tissues. In spite of the number of

different cells, each with its characteristic function, the xylem tissue as a whole, functions in the conduction of water and mineral salts up the plant.

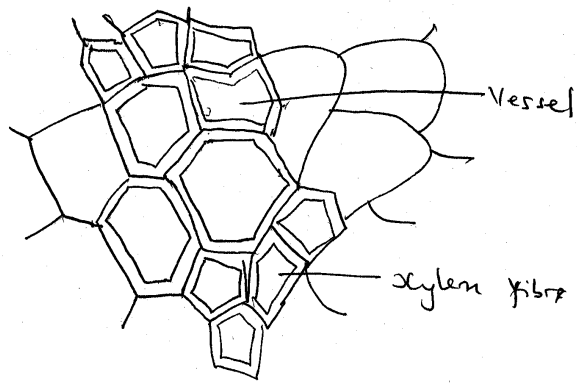


Fig. 6.5: Xylem Tissue

3.1.5 Phloem

Like the xylem, the phloem is a complex tissue and the following cell types are present: parenchyma, fibre, sieve tubes and companion cells. The parenchyma and fibre cells have the characteristic features earlier described. The sieve tubes are formed from a row of elongated cells joined end to end. The cross walls between adjacent cells are perforated and are called sieve plates. While the sieve tube is maturing a small portion of it is cut off by a longitudinal wall. This small portion is densely filled with cytoplasm and contains the original nucleus of the parent cell. A mature sieve tube cell therefore lacks a nucleus. The smaller cell called companion cell remains closely associated with the sieve tube cell and this arrangement usually helps in identifying phloem in a plant section. The structure of phloem is illustrated below in Fig. 6.6

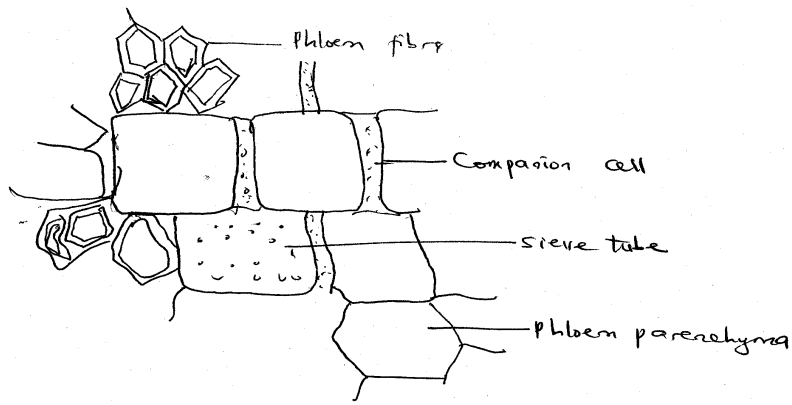


Fig. 6.6: Phloem Tissue

3.2 Animal Tissues

In this section, we shall consider a few examples of animal tissues just as we have done for plants in the preceding section. After this we shall then proceed to examine selected plant and animal organs in order to demonstrate the organization of tissues into organs.

3.2.1 Epithelium

The epithelium is a sheet or tube of closely fitted cells, i.e with minimal intersticial material between the cells. It covers the exposed surfaces of the body and lines cavities and tubes. One surface of the epithelium is therefore free while the other rests, usually, on a connective tissue.

Epithelium may be classified into different groups using a number of different criteria. One of these classifications is based on the height of the cell relative to its breath. (The height of the cell is measured at right angles to the extension of the sheet). On this bases we have: ***columnar, cubical*** and ***squamous*** epithelia (in order of decreasing height)

Epithelia may also be classified on the basis of whether the sheet is one cell thick (***simple epithelium***) or many cells thick (***stratified epithelium***).. In some cases some cells of the epithelium may be ***secretory***, and it is then called ***glandular epithelium***. Fig 6.7 below illustrates different epithelia.

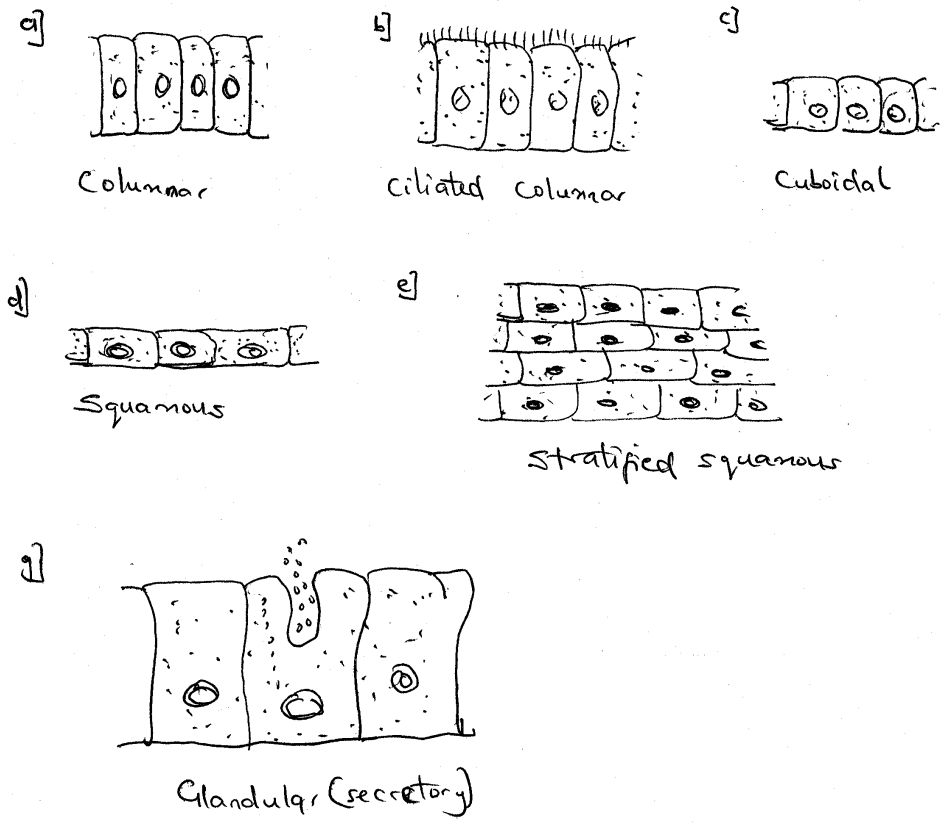


Fig. 6.7: a – f. Different types of animal epithelial cells.

3.2.2 Connective Tissue

This is the tissue that supports and binds together the various organs and tissues of the animal body. It is extensive in distribution and strong in texture. Essentially, it consists of a ground substance or matrix in which various structures are embedded. The matrix of areola tissue consists of a gelatinous glycoprotein matrix and four types of cells and two types of fibres are present. The cell types of fibroblasts which synthesize the fibres mast cells (which are phagocytic) The fibres present are collgen fibres (white fibres) and elastic fibres (yellow fibres). It is the fibres that give the connective tissue its strength and toughness. The particular type and proportion of fibres present depends on the stresses to which the tissue is usually subjected. Fig. 6.8 below illustrates the structure of a connective tissue.

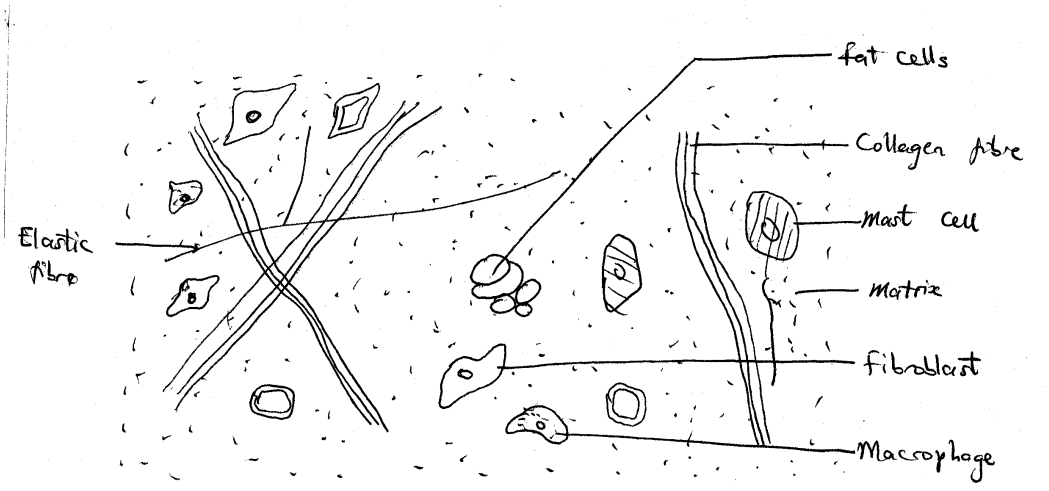


Fig. 6.8

3.2.3 Blood – a Liquid Tissue

Blood is a liquid and consists of a fluid ***plasma*** in which many different types of cells are suspended, the suspended cells are called the ***formed elements.*** Plasma is a complex mixture of solution of inorganic salts and blood proteins. The salts present include sodium clhloride, sodium bicarbonate, potasssium sulphate and potassium phosphate. The presence of these salts make the blood slightly alkaline. The proteins in the blood are of three types: ***albumins, globumins*** and fibrinogen. Other substances present in blood plasma include glucose, fats, amino acids, hormones and urea.

The cells (corpuscles) found in the blood are the **red blood corpuscles (RB erythrocytes), white blood cells (WBC or leukocytes) and Platelets (thrombocytes).** You should note that RBC does ***not*** stand from red blood ***cells*** because mature erythrocytes are ***not*** cells in that they contain no nuclei. Also thrombocytes too are not cells but fragments of cells broken off from a type of blood cells known as megakaryocytes; the platelets function in clothing of blood and where they are inadequate. A person can bleed to death (haemophilia). Fig. 6.9 shows the formed elements of blood which make up 45% of blood while plasma constitutes 55%.

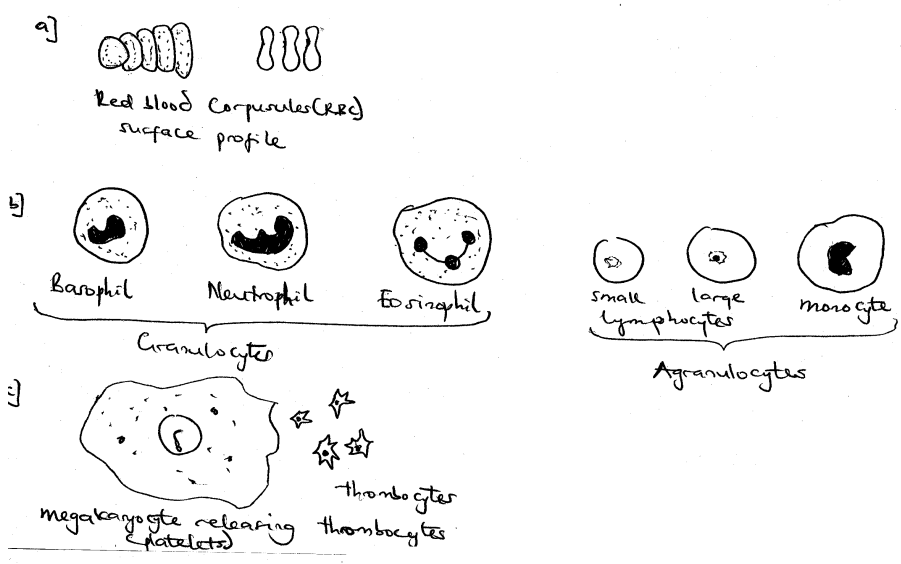


Fig. 6.9 Blood Cells (a) Red Blood Corpuscles (RBC – erythrocytes) (b) White blood cells (c) thrombocytes (platelets) being released from giant megakaryocyte.

3.3 Organs

An organ is a portion of a plant or an animal which forms a structural as well as a functional unit. It is made up of a number of tissues. For example, the leaf is a plant organ. It forms part of the plant and performs the function of photosynthesis. Similarly, the kidney is an organ of excretion in some animals. Other examples of plant organs include the stem, the root and the flower, while those of animals include the eye, the ear, the skin and the nose. In this section we will not treat all the known plant and animal organs. We will describe only a few to illustrate the organization of tissues into organs.

3.3.1 The Leaf

Fig 6.10 illustrates the structure of a leaf. It is evident from this that it is made up of a number of different tissues. On the upper surface there is upper epidermis which is a tissue made up of epidermal cells. Each of the cells is tabular in shape, it is slightly broader that it is high. The cells are in most part closely fitted together, thus forming a

continuous covering over the surface of the organ. There is a layer of curtain over the epidermis. This is the cuticle and it is impervious to water and gases. It is a protective tissue. Beneath the epidermis is a layer of cells which are elongated at right angles to the surface of the leaf. These are specialisd parenchymatous cells. These cells are richly supplied with chloroplasts which line the walls of the cells. The layer is called palisade mesophyll. It may be one or more cell layers deep. Beneath this is the spongy mesophyll which is made up of irregularly shaped and loosely packed parechymatous cells. The cells of the palisade mesophyll. The mesophyll layer (palisade and spongy) is the site of photosynthesis in the lead. This is possible here because of the occurrence of chloroplasts in the cells.

Within the spongy mesophyll layer are the ends of the vascular tissue. This is made up of phloem and xylem tissues. The xylem brings water and mineral salts to the leaves while the phloem carries manufactured food from leave to other parts of the plant.

The lower surface of the leaf is bounded by the lower epidermis which has a structure similar to that of the upper epidermis. Both the upper and the lower epidermis are perforated by special pores called stomata (sing stoma). It is through these that gases are exchanged between the interior of the leaf and the surrounding atmosphere. Each stoma is made up of a stomatal pore surrounded by two special epidermal cells called guard cells. Unlike the ordinary epidermal cells, they contain chloroplasts. In dicotyledoneous leaf, guard cells are kidney (or bean) shaped.

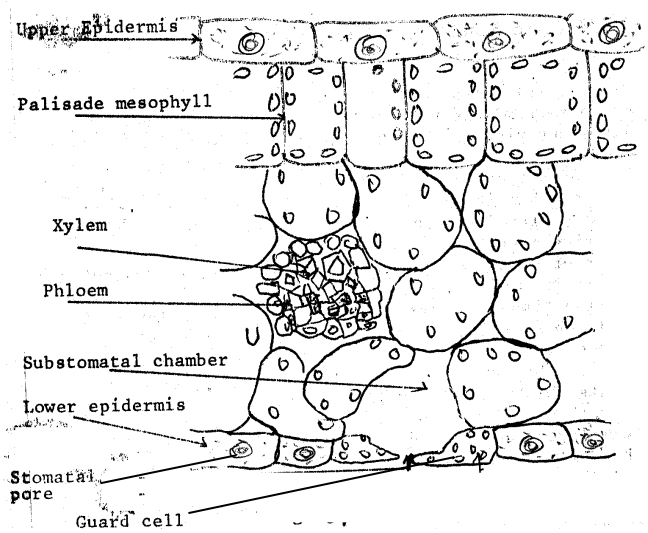


Fig. 6.10 Structure of a dicot leaf

3.3.2 The Root

The root is a plant organ which usually attaches the plant firmly to the ground and absorbs water and mineral salts from the soil. Fig. 6.11 illustrates the structure of a young dicotyledonous root. It is bounded on the outside by a layer which is made up of tabular closely fitted cells. This is called the piliferous layer and it is protective in function. The young root is perforated at intervals by stomata for gaseous exchange. At intervals some epidermal cells elongated at right angles to the axis of the root. These extensions of epidermal cells are called root hairs. Root airs are important in the absorption of water and mineral salts from the soil. You will come to this later in the course.

Within the epidermis is the cortex which is made up of parenchymatous cells. The cortex is usually many cell layers deep. Food or water may be stored in the cortex. It is bounded on the inside by a single layer of special cells. The layer is the pericycle and its cells are distinctive in that they are thickened on the inner and lateral walls only. The tissues that occupy the centre of the root constitute the cascular or conducting tissues. These are the food-conducting phloem tissue and xylem tissue which are concerned with the conduction of water and mineral salts within the plant. The xylem occurs as a stellate (star-shaped) structure, i.e. it has radiating arms. The tip of the arms are occupied by young xylem on the inside is called exarch. You will come across a different arrangement later.

The xylem is said to be diarch, tetrarch, or polyarch depending on whether there are 2, 4 or many xylem arms i.e. protoxylem groups.

The phloem occurs in groups which alternate with the protoxylem groups. There are usually the same number of phloem groups as are protoxylem ones.

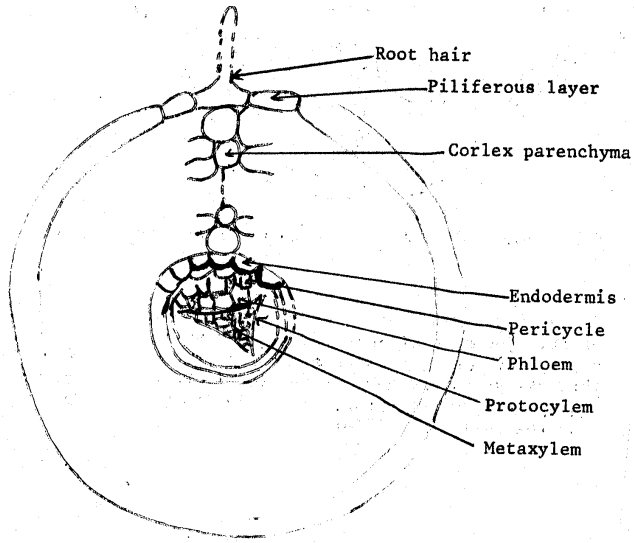


Fig. 6.11: Structures of a young dicut root

3.3.3 The Stem

The structure of a young dicotyledonous stem is illustrated in Fig. 6.12.

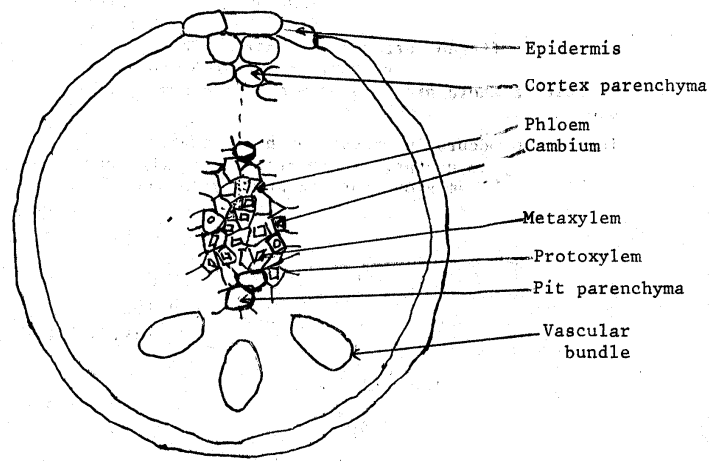


Fig. 6.12: Structure of a young dicot stem

It is self-explanatory. Write up a description of it. How many tissues can you recognize? What are these? Can you observe some differences between this structure and that of the root. When you have studied the two structures properly you will recognise the following differences.

Table 6.1: Differences between root and stem structures

Root	Stem
The centre is occupied by vascular tissue.	Pith occupies the centre of the stem
Wider Cortex	Narrow Cortex
Xylem and phloem on different radii.	Xylem and phloem on same radius-radial arrangement
Protoxylem vessels exterior - exarch	Protoxylem vessels interior – endarch.

3.3.4 The Skin

The skin is an organ whose main functrion (s) include protection, sensitivity to touch and excretion of waste substances e.g. salts. Its structure is illustrated in fig. 6.13. Note the different tissues which are associated in the formation and function of this organ. How many of these can you identity? Name them. You should have recognized the following: tissues, epidermal, muscle, blood, nervous tissues.

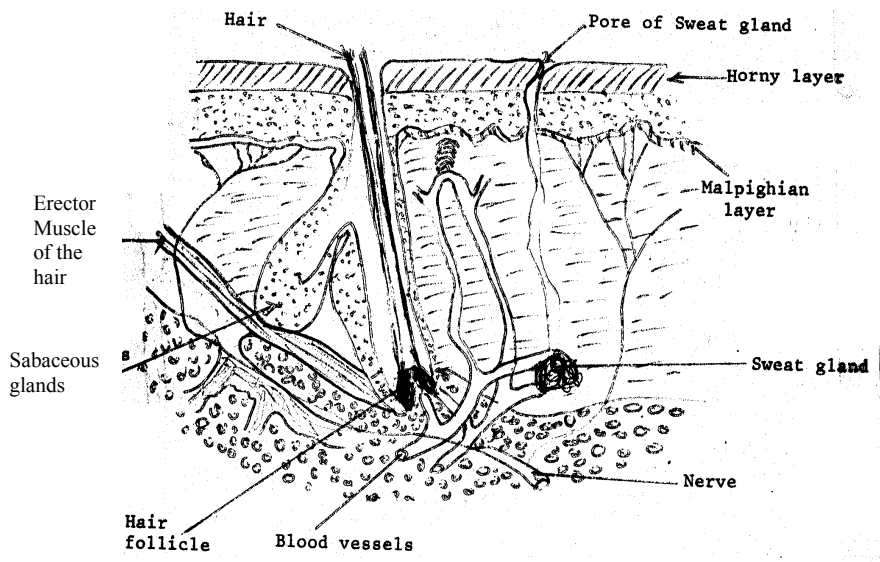


Fig. 6.13 Structure of a Skin.

3.3.5 The Kidney

The kidney is mainly responsible for the excretion of urea from the body. Its gross structure is illustrated in Fig. 6.14 (a) and this is seen to consist of an outer cortex and an inner medulla. A tube of the ureter which enlarges into a head, the pelvis, is embedded in the medulla. Waste substances which are extracted from the blood are poured into the pelvis from where they are directed by the ureter to the bladder for temporary storage. Fig. 6.14 (b) is that of a nephron which is the structural unit of the Kidney. Each kidney contains about one to two million nephrons. These are loosely embedded in connective tissue and are richly supplied with blood vessels. The Bowman's capsule, the proximal tubule and distal tubule are contained in the cortex while the U-shaped limb lies in the medulla. The collecting duct leads into the pelvis. You will learn more about the functioning of this organ later in this course. For now make sure that you are familiar with its structure.

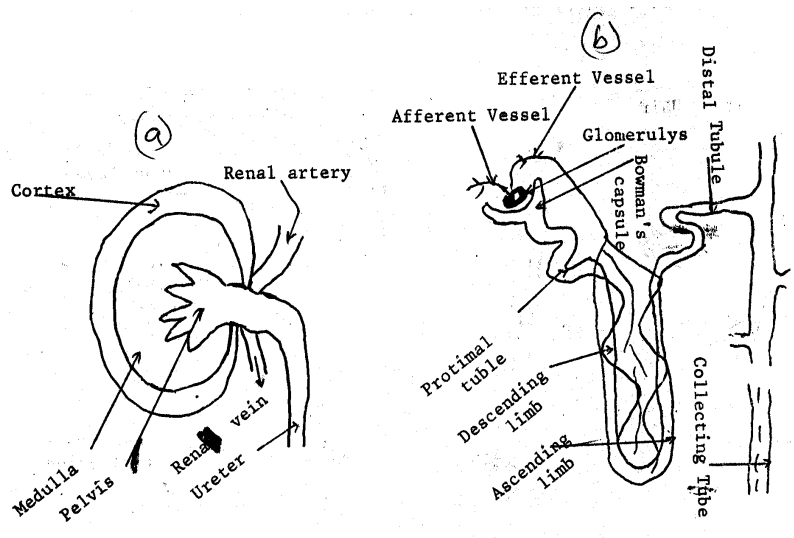


Fig. 6.14: Structure of the kidney (a) Gross structure (b) structure of a single nephron.

3.3.6 The Liver

The liver performs many functions among which are:

(i) regulation of sugar lipids and amino acids

(ii) production of heat and bile

(iii) the formation of red blood cells

(iv) elimination of haemoglobin from used red blood cells.

(v) Storage of blood and vitamins.

Later you will consider in detail how these functions of the liver are performed. Its structure is illustrated in Fig. 6.15

It consists of numerous cylindrical lobules each of which is filled with numerous liver cells. The liver cells are arranged in rows which radiate from the centre towards the periphery. Branches of the hepatic arlery, hepatic portal vein and bile duct run side by side with the liver lobules.

As stated previously organs are grouped together into systems for the performance of a major function in both plants and animals. Systems are more numerous and conspicuous in animals than in plants, as animals are more complex in organization. Among the systems in animals are the digestive ; the nervous, the circulatory, and reproductive systems. The circulatory, and the reproductive systems. The vascular system is the most outstanding one in plants

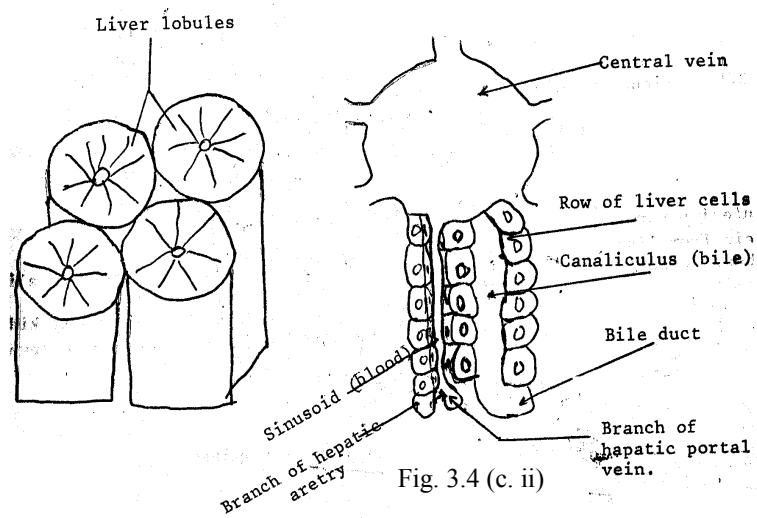


Fig. 3.4 (c. ii)

Fig. 6.15: Structure of the liver (a) Gross structure

(b) Details structure

in later courses in this programme you will deal with these systems in details. At the moment we will consider the structure of a selected few to illustrate the essential features.

3.4 Systems

3.4.1 Circulatory System

This is the system by which blood carrying food and other dissolved substances are carried round the body. During the circulation of the blood substances diffuse from the blood into organs in which they are required. For example as the liver, glucose and dissolve foods diffuse out of the blood into the liver, and at various tissues oxygen diffuse out of the blood into the tissue cells. At the same time substances also enter the blood from various organs. For example, glucose, amino acids, fatty acids diffuse into the blood from the alimentary canal; oxygen diffuse into the blood from the lungs; carbon dioxide diffuse into the blood from the tissues. The structure of the circulatory system in man is illustrated in Fig. 6.16

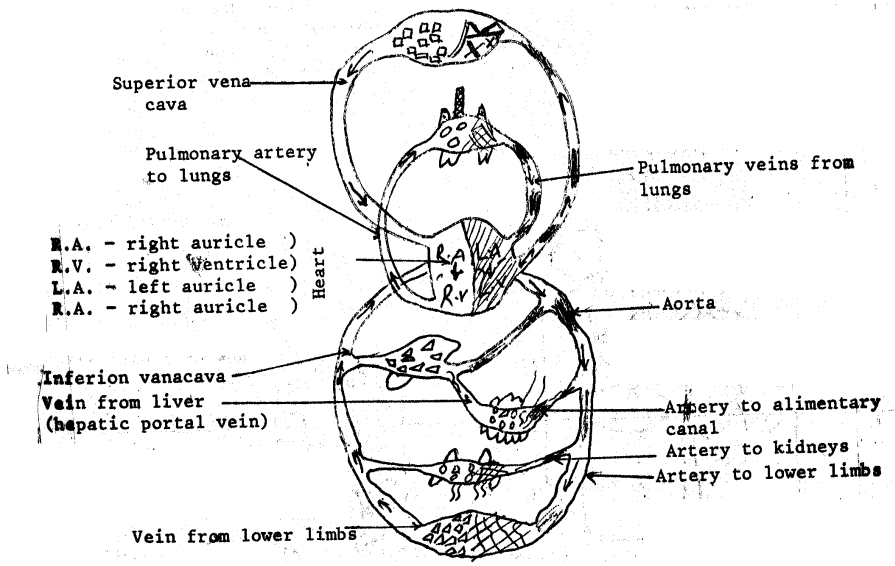


Fig. 6.16 Mammalian circulatory system

3.4.2 The Digestive System

As the name suggests this system deals with the break down of foods into the component parts. Starch is broken down into glucose units,

protein into amino acid units and fats into fatty acids. The break down is by means of enzymes which are produced by various organs and glands and poured into the digestive canal where the actual breakdown takes place. Thus the digestive system consists of a tract, the alimentary canal which runs from the mouth and opens out at the anus. Associated with this tract are various glands and organs as stated above. These features are illustrated in Fig. 6.17

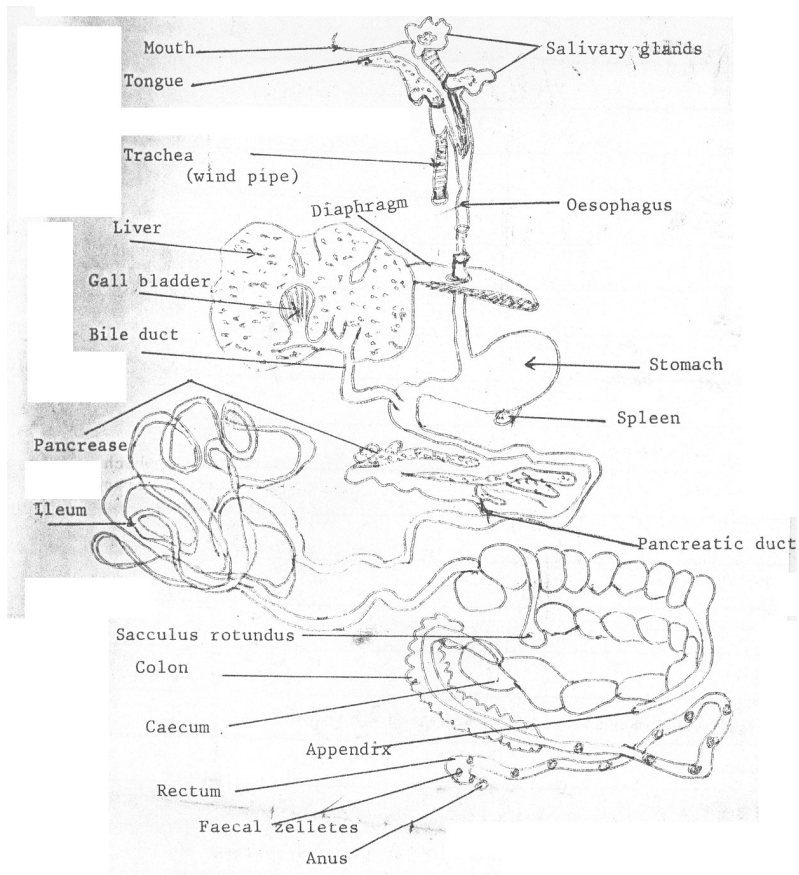


Fig. 6.17: Mammalian Digestive Systems

3.4.2 The Nervous System

The nervous system is concerned with the perception of and reaction to stimuli. It consists of the central nervous system which is made up of the brain and spinal cord. Associated with the central nervous system is a system of peripeheral nerves which end in various organs and tissues. An outline of the nervous system is illustrated in Fig. 6.18

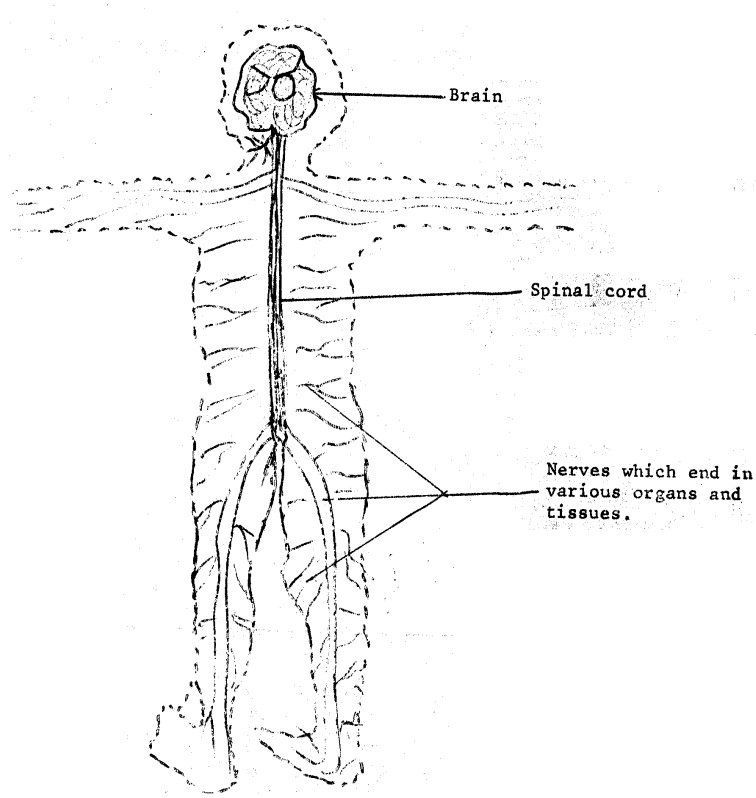


Fig. 6.18: Diagramatic representation of human nervous system.

4.0 Conclusion

In this unit we have considered the structure of tissues and organs in plants and animals, and selected examples of systems in animal. As was stated in the introduction, the main objective of this lecture is to illustrate the organization of tissues into organs and organs into systems.

In later lectures you will learn more about what had been introduced in this lecture and also about other structures found in plants. You will also learn in details how the organs and systems function. For the time being form up of numerous cells which are however organized in a definite manner. There are different types of cells which are grouped into organs each of which is associated with a definite major function.. At still a higher level, organs are grouped together into systems.

Table 6.2 From cell to the Organism

Cells	**→ Tissues**	**→ Organs**	**→ Systems**	**→ Organism**
	Meritems	lead	digestive	plant
	Parenchyma	stem	circulatory	animal
	Collenchyma	root		
	Sclerenchyma	skin		
	Xylem	kidney		
	Phloem	liver		
	Epithelium			
	Blood			
	connective			

Self Assessment Questions

Self Assessment Questions

1. What do you understand by the terms: tissue, organ and systems?

2. Mention five examples of plant and animal organs.

3. Give five examples of plant and animal organs.

4. Enumerate the systems you have studied in this lectures. Do you know of others? Name them.

5. Give an account of the structure of the following: epithelium, blood, meristematic tissue, collenchyma, xylem and phloem.

6. Illustrate the structure of the following with large clearly labeled diagram; root, stem, leaf, skin, kidney and liver.

7. Draw and label mammalian circulatory system, digestive system and nervous system.

5.0 Summary

We have studied the different types of cells found in both plants and animals. When cells of the same type group together to perform he same function, they form a tissue. Different tissues that are placed contiguously and perform the same function are called an organ. A set of ***organs*** that work together for a purpose make up a system. Two or more systems work together to form an ***organism.*** Hence you will again realize the cell as the basic structural and functional unit of an organism.

6.0 Tutor-marked Assignment

1. Write short illustrated notes on the following:

   a) a cell

   b) a tissue

   c) an organ

   d) a system

In all cases treat at least one example each from plants and animals.

2. Write brief annotated brief on the male human reproductive system.

7.0 References

1. Brimble. L.J.F., 1960. (Intermediate Botany, Macmillan and co. Ltd. New York.

2. Lesson, T.S. and C.R. Leson, 1966. Histology, W.B. Saunders co., Philadelphia

3. Uduebo, a. 1980. SEPP Biology 002. COSIT, University of Lagos.

Answers to Self Assessment Questions

1. See summary 5.0

2. See Table 6.2.

3. See table 6.2

4. Digestive, circulatory, nervous; name others you know e.g. excretory, respiratory, reproductive.

5. See appropriate sections of the unit

6. See appropriate sections of the unit.

7. fig. 6.16 and 6.17 should act as guide.

UNIT 7: CELLULAR MECHANICS

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1.0 Introduction

In a previous unit, we examined the structure of cells as seen under both the light and electron microscopes. Many more structures could be seen with the electron microscope. Since we are now familiar with the organelles; such as the ***nucleus, endoplasmic recticulum, Golgi complex, ribosomes, chloroplasts and mitochondria***, we should go further to see how each of these organelles works and how each's functions relate to other organelles and the cell in particular and the whole organism in general.

2.0 Objectives

In this module we shall examine what each organelle does within the cell and how its functions affect or relate to the other organelles, the cell as a unit and the whole organism.

3.0 Cellular Mechanics

The Nucleus

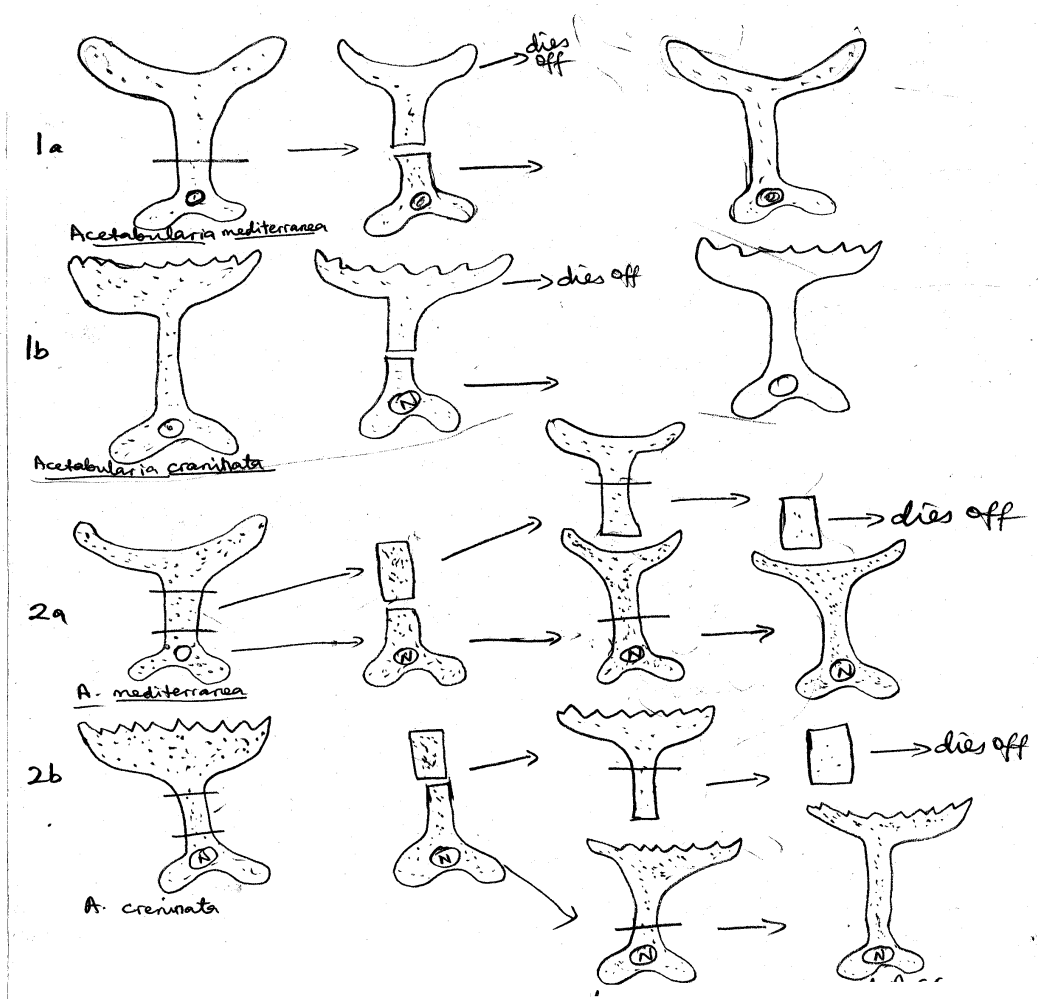
As stated in unit 3, the nucleus is the nerve or control centre of the cell. It is the functional genetic apparatus in the eukaryotic cell and is responsible for the reproduction of the cell in producing genetically similar daughter cells.

The role of the nucleus as the functional genetic apparatus of the cell was demonstrated by Hammering, a German Scientist. In a classic experiment using the large single-celled mushroom-shaped alga, ***Acetabularia***. The body consists of "head" a stalk about 40mm in length, and a base of root like rhizoids. The nucleus is situated at the base of the stalk. Hammering worked with two different types namely A. ***mediterranea***, whose head is cup-shaped, and A. ***creninata*** whose head is tentacled-like. (Fig. 7.1 a & b).

In experiment 1 Hammering cut off the stalk and head region of the acetabularia. The part bearing the nucleus i.e the rhizoid regenerates while the stalked head dies. Further in Experiment 2, he separated the stalk form the head and root and put the stalk into sea water; the stalk regenerates a new head. When he then cut off the regenerated head, no new head grew from from the stalk. He postitulated that the nucleus produced some substances which, while still present in the just separated stalk induced the growth of a new head; but at the time of the second cut (Fig. 7.1 a & b) the "nucleic substance" had been depleted and a new head could not be regenerated.

To buttress his point that the nucleus was influential in the regeneration and type of head performed experiments 3 and 4. the head that grew was that of the specie that congtributed the nucleus. This clearly demonstrates the importance of the nucleus in the control of cell development.

Fig. 7.1 Hammelings experiments with Acetabularia mediterranea and A. creninata



In Fig. 7.1 a & b he cut off the head and root,the head dies off while the root (rhizoid) regrows another head. In 2a & b he separated the stalks and roots; the stalk and roots; the stalk grew a new head, but when this is cut off again, it dies off; the root grew a new head and when that was cut off, it grew another one again.

3.2 The Central Dogma of Molecular Biology

The way in which the nucleus controls cell development and activities can be summarized by the following annotation which denotes the central dogma of molecular biology.

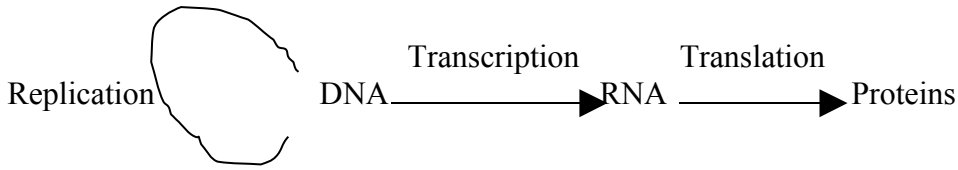


Fig. 7.2: The Central Dogma of Molecular Biology

We should remember that the nucleus contains the genetic material which is DNA, with some associated proteins; This is in exclusion of some few viruses in whch RNA is the genetic material. Evidences, both direct and indirect, have shown the DNA as the genetic material in eukaryotic cells;

Some of these are:

(1) That DNA is metabolically very stable, with its amount per cell in a given species of higher organisms being fairly constant and unaffected by changes in the environment or nutrition; no other cellular constituent (RNA, Proteins, Carbonhydrates or lipids) satisfies this need.

(2) In higher organisms, experiments have shown that the DNA of the cell is very largely found in the chromosomes.

(3) The amount and composition of bases in DNA is specie specific, with the ration of the four purines and pyrimidine bases for all tissues of the same organism remaining constant but being different from those of others.

(4) The rate of mutation in a cell is highest at the wavelength of maximum absorption of ultraviolet (UV) light by DNA (ie. 260nm). This suggests that DNA is the hereditary material and when irradiated undergoes chemical changes which cause mutation in the cell.

Briefly, let us examine what is being expressed in the central dogma of molecular biology, and then go on to see the mechanics of it. Firstly, DNA

can reproduce (i.e replicate) or copy itself. This is an essential property of a genetic material which must be able to reproduce its own exact copy. Again, DNA can be read and copied (i.e transcribed) into RNA. The message on the RNA can, in turn, be translated into proteins.

Within all living systems, the many basic structures of the organism are formed by proteins, and their basic activities are regulate with precision by specific catalytic proteins – the enzymes. It is now clear that the production of proteins in a cell is ultimately, if directly, controlled by deoxyribonucleic acid (DNA) which is principally contained in the nucleus. Since proteins control the metabolism of the cell, the nucleus can thus be seen to be the control centre of the cell.

3.3 DNA Replication

During the so-callled "resting" stage which a cell undergoes prior to cell division, the DNA of the chromosomes must be duplicated to ensure that each daughter cell receives the full complement of genetic information into its nucleus. According to the Watson-Crick model, DNA occurs as a helical structure containing two strands which are complementary to one another. The repeating monomer in a nucleic acid is called a NUCLEOTIDE, each and one **base.** The base is is a ring-shaped molecule that contains nitrogen and has basic properties. There are four different types of bases I DNA – tow purines, adenine and guanine, and two pyrimidines, thymine and cytosine. One the double helical strands one purine is bounded to a pyrimidine through weak hydrogen bonds. It has been established the guarnine binds to cytosine with three (3) hydrogen bonds. (G=C) while adenine binds thymine with two (2) hydrogen bonds. The bonds can be represented thus G = C T = A.

Guanine never binds to thymine, nor adenine to cytosine. The bases are arranged in random sequence, one above the other with the sugar phosphate as the backbone. The bases are bonded towards the centre of the helix. As the first step in replication, the relatively weak hydrogen bonds between the base pairs of the two DNA stands are broken by an enzyme, DNA polymerase. Previously, synthesized nucleotide triphosphate units then move in, and form hydrogen bonds with exposed bases on both of the old strands which act a templates; a pyrophosphate is lost in the process. Once the nucleolides are in position; the enzyme DNA – polymerase directs the formation of phosphate bonds between them.

As a result, there are ultimately two identical molecules where one existed before, each molecules containing one old and one new strand. This is known as semi-conservative replication (fig. 7.3).

Other possible forms of replication are conservative and dispersive replications. In the conservative type after the new strands have grown on the old strands they separate from the templates and join together to form a double helix while the old stands too re-group.

Thus, we would have two old strands together and the two new strands together in their respective helixes. In the disruptive type, the new strands are formed on the old strands which serve as templates. Then, there are crossing ever between the old and the new strands. Anyone resulting strand would thus be part of and part new (Fig. 7.3).

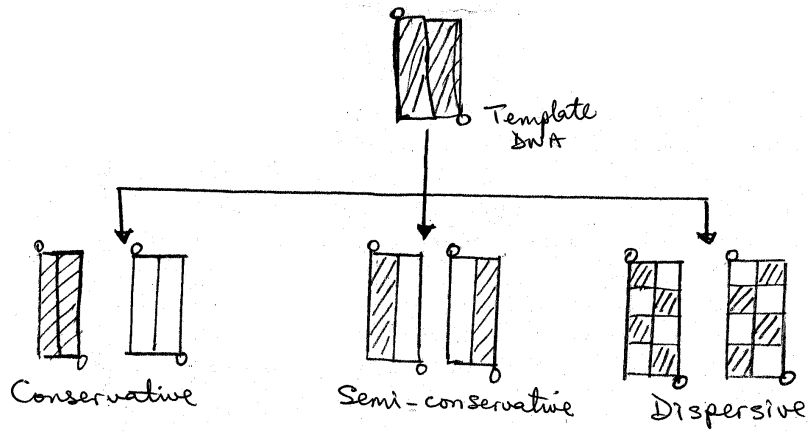


Fig. 7.3: Different types (theories)of DNA replication.

(a) In conservative replication the old strands reunite and the second double helix consists of two new strands.

(b) in the semi-conservative replication, the double helix are made up of one old and one new strands.

(c) In dispersive replication the parental (old) strand is randomly scattered among the components of the new (daughter) strand. The DNA strands are drawn as blocks, rather than as double-helix, for simplicity.

3.3.1 Replication is Semi-Conservative

To test the correct mode of replication, meselson and stahl in 1958 grew a culture of ***E coli*** in a medium in which the sole source of nitrogen was 15NH4CL. After several generations of growth the cells were washed clean of 15NH4CL and 14NH4CL was added. At short

intervals cells were removed, the DNA was carefully extracted and analysed for relative 14N and 15N content by equilibrium density gradient centrifugation. The results depicted in Fig. 7.4 strongly gave support to the semi-conservative mechanism of replication over the other two types.

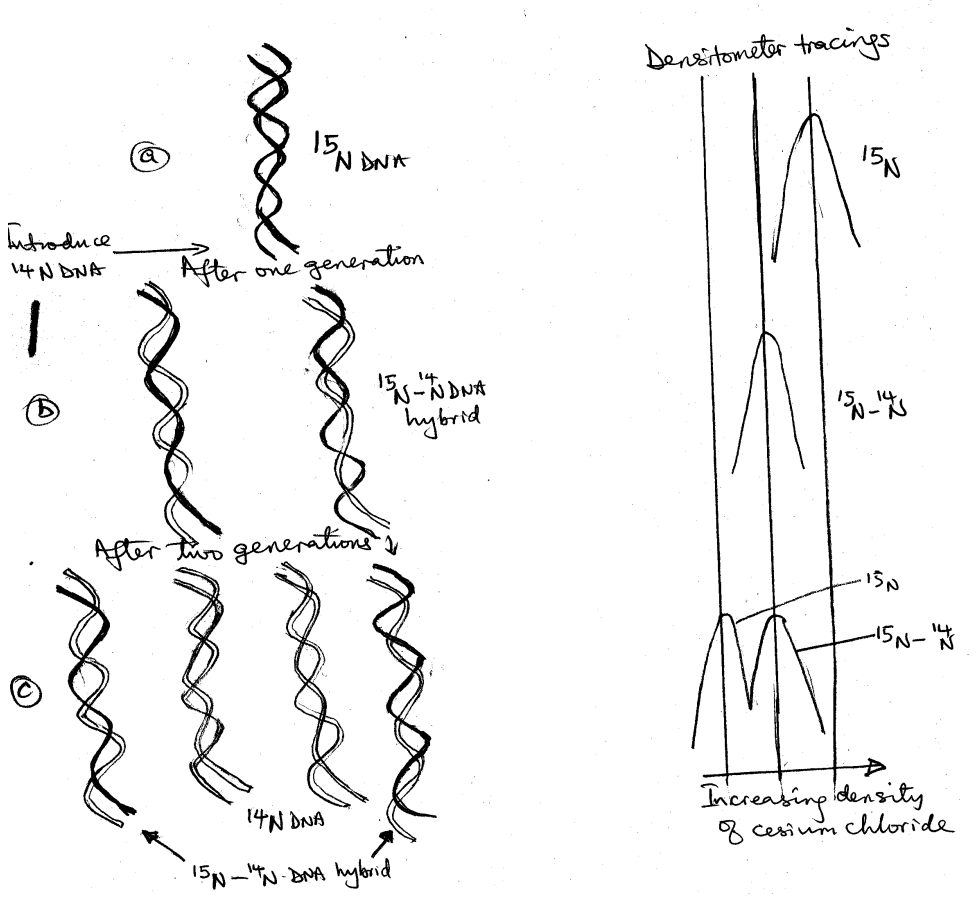


Fig. 7.4: Meselson-stahl experiment to demonstrate semi-conservative replication of DNA. Left: sketch of DNA structure (15N DNA stands shown in black, 14N DNA strands in white) right: corresponding cesium chloride densitometer tracings.

(a) ***E coli*** cells with 15N DNA

(b) One generation after 14 nucleotides were added shows 15N – 14N DNA hybrid

(c) After two generations pure 14N and 15N – 14N hybrid DNA exist.

3.3.2 Mechanism of Replication

Interphase chromosomes are visible in the light microscope when appropriately stained. Under these conditions, chromosomes show regional differentiation into two distinct kinds of organization, termed ***heterochromatin*** and ***euchormatin***. Heterochromatic regions are condensed and stain darkly; euchromatic regions are more dispersed and stain lightly. It has been observed that the heterochromatin replicate late in the cell cycle and are thought to represent genetically inert chromatin. The euchromatic regions replicate early in the cell and have the potential for genetic expression.

From the above, it is clear that DNA replication is fragmentary. In-vitro and in-vivo works have confirmed that replication is discontinuous and in a 5' ⟶ 3' direction on both sided of a duplex DNA; hence, it means that replication has to be anti parallel (in both directions) on the two strands.

The process begins at several initiation points along the duplex strands (Fig 7.5). The strands must separate before initiation can begin; this is accomplished by unique proteins called ***Unwinding proteins*** which are of low mws (35,000) and are found in both prokaryotic and eucauyotic organisms; the protein binds to one of the two strands. Initiation begin usually in regions which in A – T base pairings, since the A – T base pairs have lower energy bonds than G – C pairs.

Okazaki, a Japanese biochemist, in 1968 proposed the discontinuous DNA replication mechanism to explain semiconservative replication. According to his explanation, segments of DNA are unwound at certain replication starting points. A protein complex containing RNA and DNA polymerases first synthesizes a short segment of RNA (50 – 100 residies) co-joined to the growing DNA strand. The RNA – DNA primer is known as Okazi fragment named after its discoverer (Fig. 7.6). The RNA primer has a triphosphate residue for its 5' position and a free 3' OH terminus to which the new DNA attaches; hence growth of the new DNA strand is in the 5' ⟶ 3' direction. Elongation continues 500 1000 until deoxyribonucleotide residues have been added; at this time a termination point would have been reached.

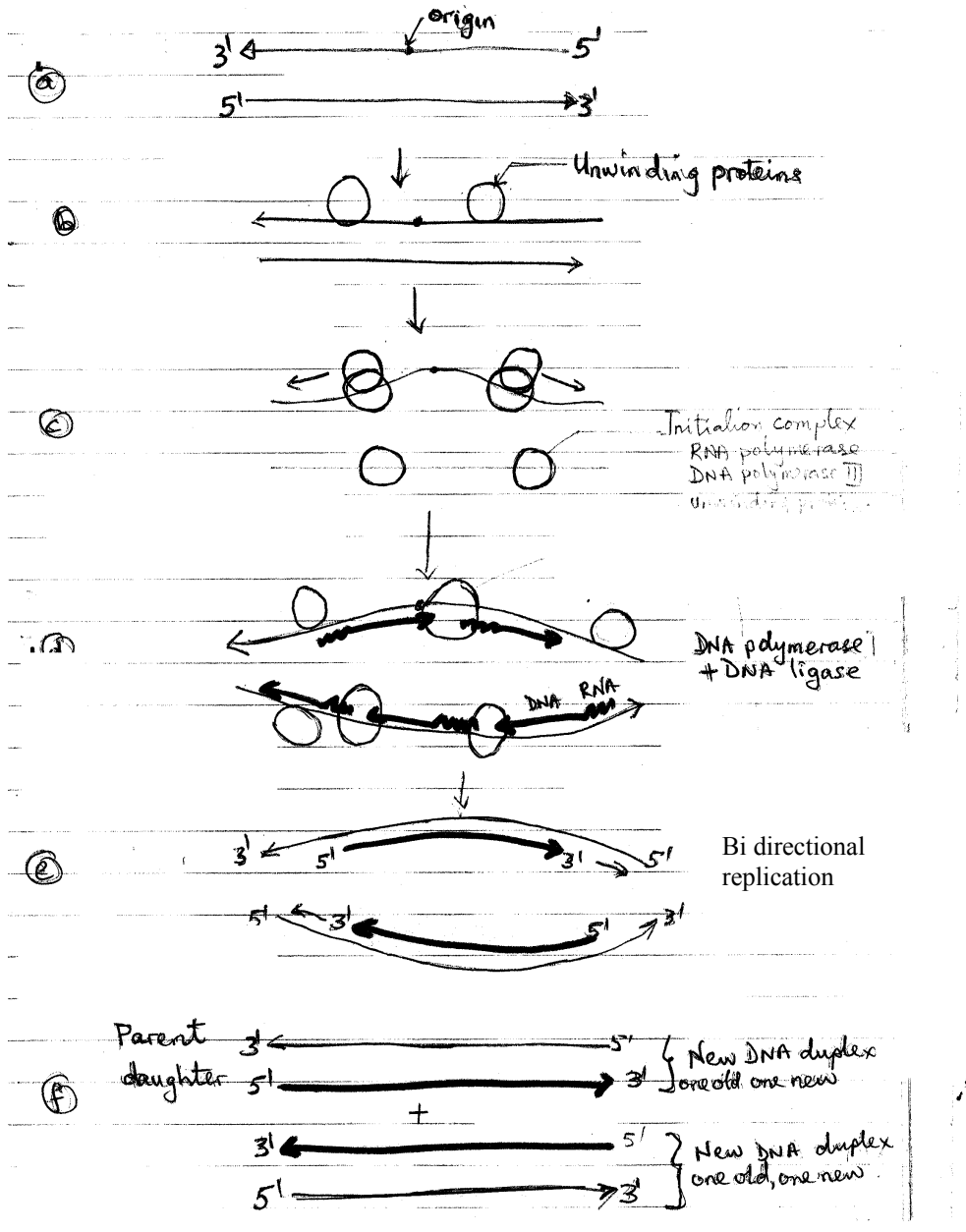


Fig. 7.5: Okazaki explanation for discontinous DNA……

(a) DNA duplex showing origin point of replication

(b) Unwinding proteins bind to origin point, usually rich in A – T nucleotides.

(c) DNA duplex separated into two separate strands by unwinding protein, allowing initiation complex to bind to freed DNA strands.

(d) Short segments of RNA primer (50 – 100 residues) are first made and later co-joined to short DNA strands. The DNA – RNA duplex is known as "Okazaki fragments".

(e) Later the RNA - primers are excised and the DNA fragments are joined together by DNA ligase; replication if bi-directional on the DNA duplex.

(f) Two DNA duplexes result containing a parent and a new strand each.

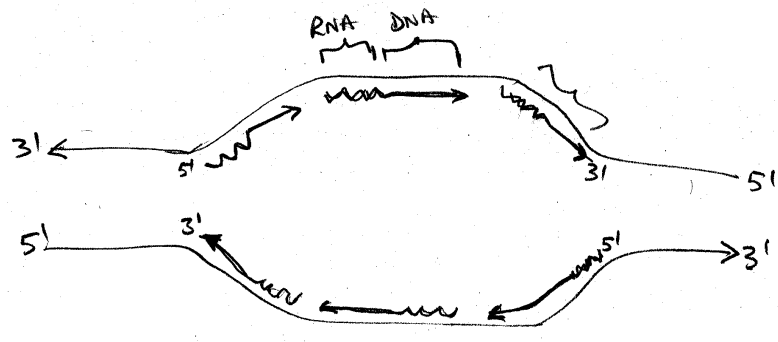


Fig. 7.6: Okazaki's explanation for discontinuous DNA replication.

As the DNA chains grow and approach each other i.e as the 3'OH terminus approaches the 5' ppp terminus (A) (see fig. 7.7) three events must occur:

(a) Excision of the RNA primer fragment

(b) Filling in of the remaining gaps with deoxyribonucleotide residues,

(c) Fusion of the DNA fragments by a phosphate diester bond to form a continuous DNA daughter strand. DNA polymerse 1, the enzyme discovered in E coli by kornberg in 1955 is the enzyme that fulfils requirements (a) and (b) DNA ligase now accomplishes the joining of the DNA fragrments.

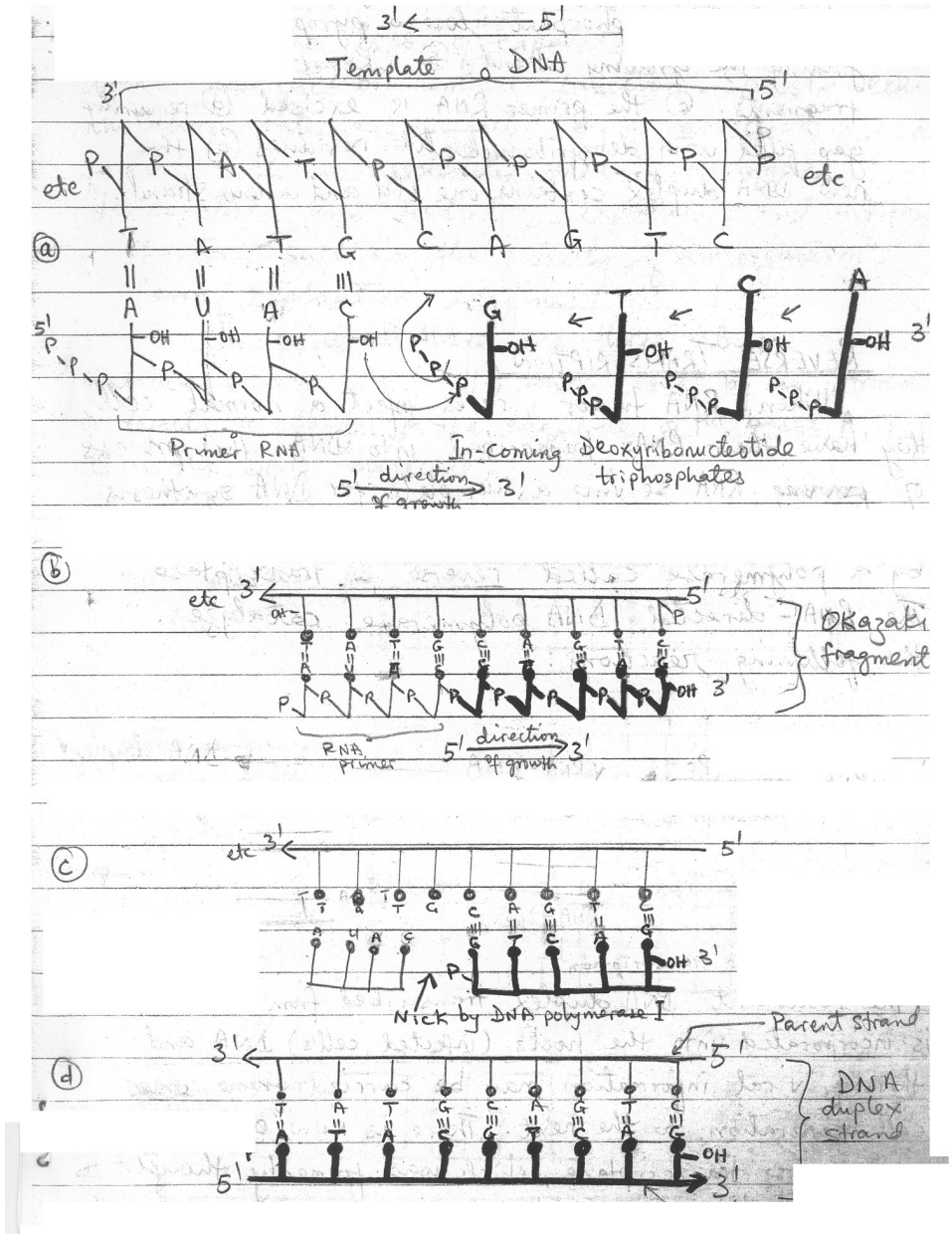


Fig. 7.7: Further diagrammatic explanation on DNA replication.

The diagram shows only a segment of the DNA replicating fragment

(a) DNA replication is preceded by an RNA primer with a 5' ppp lead and a 3' OH tail end to which deoxyribonucleotides join. The deoxyrebonucleotide triphosphates lose a pyrophosphate in joining the growing strand.

(b) Okazaki's RNA – DNA fragments

(c) The primer RNA is excised

(d) Remaining gap filled with deoxyribonucleotide residues

(e) The new DNA duplex contains one old and a new strand

3.3.3 Reverse Transcription

When RNA tumor viruses infect a normal cell, they have their RNAs transcribed into DNA. This process of RNA serving as templates for DNA synthesis is known as reverse transcription and is mediated by a polymerase called reverse transcriptase. The RNA – directed DNA polymerase catalyzes the following reactions; (Fig. 7.8)

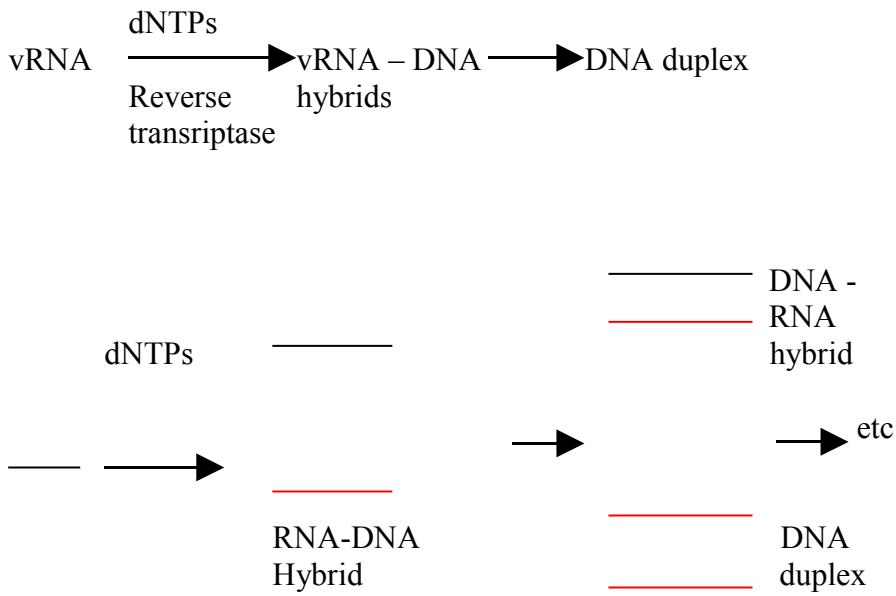


Fig. 7.8: Reverse transcription

The result DNA duplex transcribed from the viral RNA is incorporated into the host's (infected cell's) DNA and thereby viral information may be carried from one cell generations to the next. There is now also information that

reverse transcriptase, which was formerly thought to be limited only to virus particles, also occurs in normal cells.

3.4 Mutation

Mutation can be defined as an abrupt, stable change of a gene which is expressed in some unusual phenotypic (visible traits) character, frequently as a biochemical modification. In mutation there may be a loss of the capacity to carry out some specific biochemical function. Spontaneous mutation rates are affected by a variety of external factors such as dietary mutagens, temperatures and radiation exposure, and internal factors such as , the fidelity of an organism's own replication enzymes.

In general, there are three classes of mutation (shown in fig. 7.9) which result by an introduction of defects or changes in the sequence of the bases, A, T, G & C in the DNA molecule.

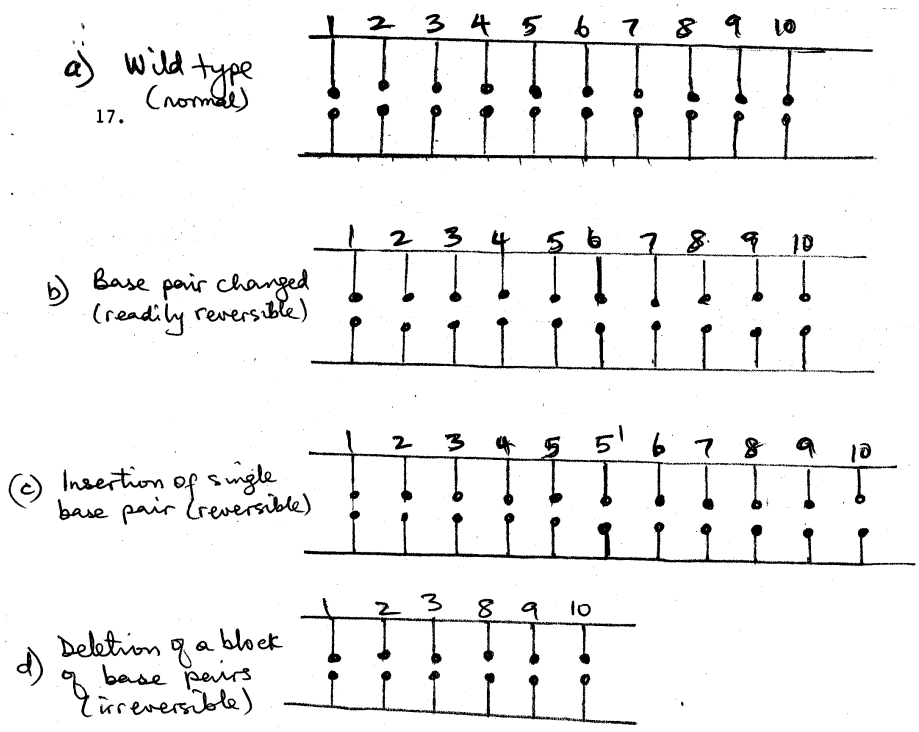


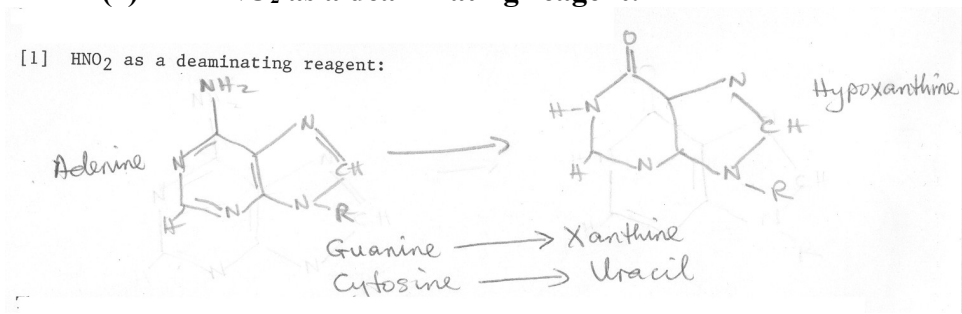
Fig. 7.9: Classes of mutation

3.4.1 Physical and Chemical Mutagenesis

In nature, mutations may occur by accident either by physical or chemical changes of bases in DNA or by shifting the condon reading frame by deletion, addition or modification of DNA base.

Examples of chemical mutagenesis include the following:

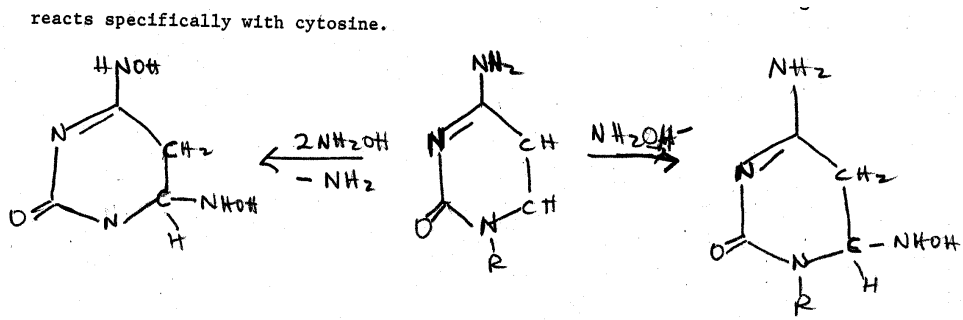
(1) HNO_2 as a deaminating reagent:



The conversion of adenine to hypoxanthine will result in the incorrect pairing with cytosine; change of cytosine to uracil leads to adenine base pairing, while guanine to xanthine results in cytosine pairing which is normal.

(2) Hydroxylamine

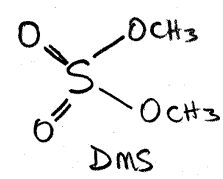
This is a very powerful mutagen but only with isolated systems, since the normal components of a cell would readily scavenger the reagent. The reagent reacts specifically with cytosine.



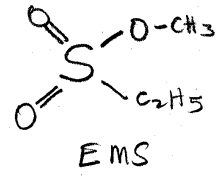
The new derivatives pair with adenine.

(3) Alkylating Reagents

Alkylating reagents – dimethyl sulfate (DMS) and ethyl methane sulfonate (EMS) are specific for guanine.

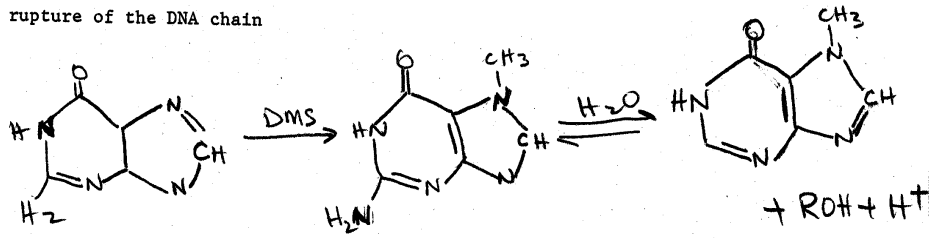


DMS



EMS

The reaction which follows methlation leads to the formation of a quaternary nitrogen which destabilizes the deoxyribosis link and releases deoxyriboside. The loss of a base can lead to replacement by any of four bases or even rupture of the DNA chain.



These compounds as well as another alkylating reagent, B-chloroethylamine, a nitrogen mustad, are very toxic.

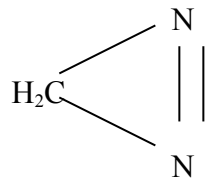
(4) Methylating Reagents

These are very extremely mutagenic compounds and thus are very dangerous to use unless very careful precautions are taken; they include

N – methyl – N' – Nitro – N- nitrosoguanidine

$$0_2N - NH - C - \underset{\displaystyle CH_3}{\overset{|}{N}} - N = 0$$

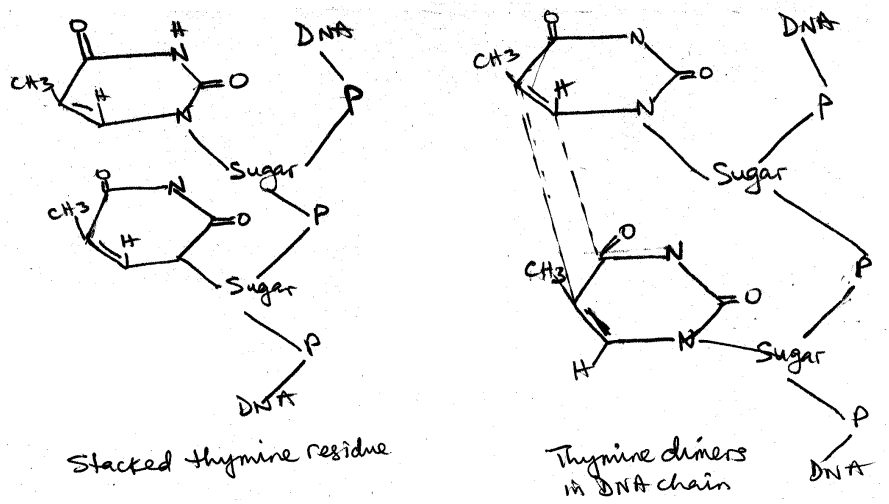
This reagent readily converts to diazomethane



An extremely effective methylating reagent. This compound used commonly for the methylation of carboxylic acid and amino groups, must be treated with great respect and care. The reagent methlates nucleic acids.

(5) Un and X-Ray Radiation

Mutagenic action by ultraviolet (UV) and X-=ray irradiation is very effective in inducing mutagenesis. X-rays probably react with DNA by a free radical mechanism to cause single-stranded chain breaks in the DNA chain. Ultraviolet irradiation with a strong absorption at 260nm leads frequently to a photochemical dimerization of two adjacent thymines, thymine-cytosine or two cytosines to dimmers. Thymine residues are particularly susceptible to the following reaction.



Formation of thymine-dimers caused by ultraviolet or X-ray irradiation.

3.5 DNA Repair Mechanisms

X-ray and ultraviolet irradiations cause damage to cell DNA. X-radiation causes dimerization (fig. 7.10) All cells possess the machinery by which damage to the DNA can be eliminated, rapidly repaired and the original form of the DNA double helix restored.

Both DNA polymerase I (Kornberg's enzyme) and DNA ligase are involved in DNA repair mechanisms. After UVradiation, repair commences by the nicking of the damaged. DNA strand on the 5' side of the thymine dimmer by an endonuclease. Oligonucleotides (ie.,short strips of nucleotides) containing thymine dimmers are removed by the 5' →3'.

Exonuclease activity of DNA polymerase I (Fig. 7.10)

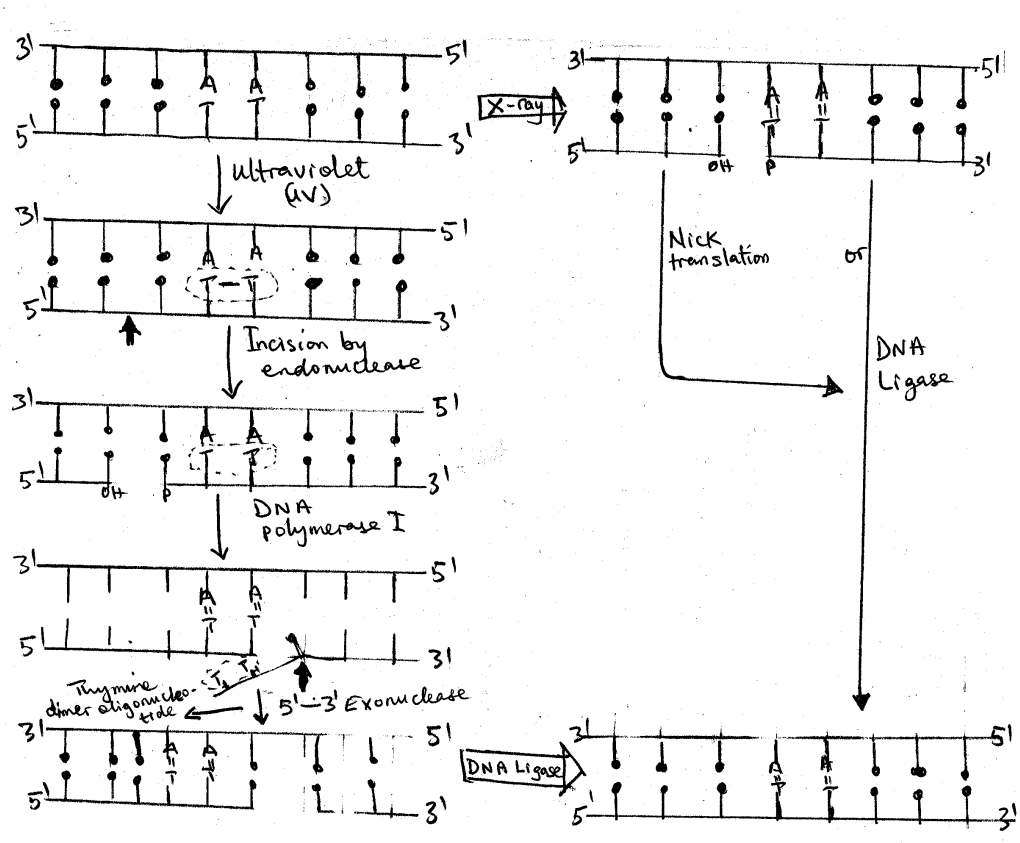


Fig. 7.10 Repair mechanism for DNA

This enzyme also ensures that nucleotide triphosphate, while losing a pyrophosphate each, are fixed into the gap, with the complementary DNA strand serving a template. The final gap is linked up by exactly the same

mechanism as described in DNA replication, namely by DNA ligase. X-ray radiation damage is readily repaired by the use of DNA polymerase I to remove the damaged strand, filling of the gap, and then fusing the gap by the DNA ligase.

4.0 Conclusion

1. In this unit, we have examined an experimental proof that showed the nucleus as the control centre of all cellular activities.

2. The properties of the genetic material, DNA has been stated.

3. The central Dogma of cell/molecular Biology also shows that the DNA contained on chromosomes within the nucleus dictates the RNA type to be made.

4. A triplet set of nucleotide dictates the sequence of amino acids to be arranged in the protein chain; proteins are "translated" form the triplet sets on RNA.

5. Replication of DNA is semiconservative; distruptive and dispersal replication do not take place.

6. Replication is discontinuous and in a 5' $\longrightarrow$ 3' direction on both sides of a duplex DNA.

7. Mutation can occur in many ways

   i) through dietary mutagens
   ii) temperature exposure
   iii) exposure to radiation e.g. UV, X-ray
   iv) chemical mutagens

8. Cells have repair mechanisms to DNA damage.

9. Viral RNA causes reverse transcription by serving as template within its host and being transcribed into DNA.

5.0 Summary

This unit has exposed the DNA as being the genetic material. Its sequence of nucleotides dictated the genetic messages (genes) carried from generation to generation. For a cell to divide and multiply, DNA copies have to be made; this is called replication. Mistakes can occur in replication and this results in mutation. Mutation changes the genetic message to be relayed. Mutation can lead to some genetic diseases e.g. cancer.

Self-assessment Questions

1. Discuss Hammerling's experiment by which he showed that the nucleus controls cellular activities.

2. What are nucleotides and where can they be found?

3. State and explain the Central Dogma of Cell Biology.

4. What is reverse transcription?

6.0 Tutor-marked Assignment

1. Discuss the three types of possible replication methods and explain why one is chosen over the others.

2. Describe the mechanisms of DNA replication.

3. Name three agents of mutation.

4. Describe the mechanism of DNA repairs.

7.0 References

1. Wolfe, S.L. 1972. Biology of the Cell. Wedsworth Publishing Co. Inc., Belmont, California.

2. Roberts, M.B.V. 1975. Biology: A Functional Approach, E..L.B.S. and Nelson. Lagos

Answers to Self Assessment Questions

1. Ref. Section 3.1

2. Nucleotides are repeating units of DNA and RNA, each containing a base, sugar and phostphate molecules. The bases of DNA are Adenosine (A), Thymine (T), Guanine (G) and cytosine (C); in RNA uracil (U) replaces thymine.

   The sugar of DNA is a deoxyribose sugar while ribose sugar exists on RNA

3. 

4. Reverse transcription is when a viral RNA enters a cell and takes over the cellular machineries to use its RNA as template for making DNA

UNIT 8: TRANSCRIPTION

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3.4 Post-Transcriptional Processing of RNA
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4.0 Conclusions
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1.0 Introduction

The central Dogma of Molecular Biology states that DNA can replicate itself ie. Make a carbon-copy of itself. It went further to say that RNA can be copied off from the DNA sequence on the double helix. However, unlike in replication only one strand is copied; this strand that is copied is called the sense strand. This implies that a single strand of RNA is copied from the double helix of DNA.

The synthesis of RNA takes place in the nucleus. However, since RNA function in the cytoplasm it has to be transported thence, probably through the nucleopores in the nuclear membrane.

Three types of RNA are made. These are the ***messenger*** (mRNA), ***transfer*** (tRNA) and the ***ribosomal*** (rRNA).

2.0 Objectives

At the end of this unit you should be able to:

(1) Describe how RNA is synthesized from DNA

(2) Know the different sugars in DNA and RNA.

(3) Know the differences in the bases in DNA and RNA

(4) Tabulate the differences between DNA and RNA synthesis

(5) Describe the structure of the three types of RNA

3.1 Biosynthesis of RNA - Transcription

With the exception of the biosythesis of RNAs in such organelles as mitochondria and chloroplasts, in eukaryotic cells, the site of DNA-dependent. RNA biosynthesis (transcription) is the nucleus, while the nucleus appears to contain the enzymes and genes for ribosomal RNA (rRNA) biosynthesis, the enzymes responsible for the synthesis of transfer RNA (rRNA) and messenger RNA (mRNA) are localized in he nucleoplasm. In prokaryotic organisms, RNA polymerase occurs in the cytoplasm.

The biosynthesis of ribonucleic acid requires the presence of the enzyme RNA polymerase, magnesium ions and triphosphate ribonucleotides. Another very essential requirement is the presence of a nucleic acid (DNA or RNA) template. Unless there is a nucleic acid template present, the polymerase will form RNA with a random arrangement of nuclieotide bases. It is the chromosomal DNA which normally provides a template for RNA formation in most cells. The DNA must become single strained to serve as a template for RNA formation (Fig.8.1)

3.2 DNA as Template for RNA Transcription

Although the nucleotide sequence in DNA helix was said to be random, it must be empphasised that for each specie of organism, the nucleotide sequence on DNA is very specific for all cells within the organism; by "random" is only meant that there is no rule as to which of the bases occurs in whatever sequence. But the sequence in which they occur must be repeated for every generation except where mutation occurs.

Certain sequences of the DNA on chromosomes are arranged to form particular information called ***genes*** or ***cistrons.*** These genes are on the length of the DNA.

DNA acts as template for the making, i.e, transcription of RNA. For this to happen, the DNA first unzips, with the two strands separating due to breakage of the hydrogen bonds between them. However, it is only one of the strands that serve as template for transcription and is known as the sense strand while the other is known as the ***Non-sense*** or ***anti-sense*** strand.

Thus in transcription, a complementry strand of RNA is formed to the sense strand of the DNA double helix (Fig. 14). The process of transcription is mediated by RNA-polymerase, a DNA-dependent enzyme which attaches to the DNA molecule and catalyzes the formation of phosphate between the ribonucleotides. When the end of the gene or cistron is reached RNA-polymerase terminates the synthesis.

The DNA sense strand serves as template for the three types of RNAs i.e. mRNA, rRNA and tRNA (Fig. 8.2).

Table 8.1: components of **E Coli** RNA polymerase

Sub Unit	Molecular Wt.	Number	Function
β^1	165,000	1	DNA binding
β	155,000	1	Initiation and catalytic site
σ	95,000	1	Initiation
α	39,000	2	Not known
ω	9,000	1	Not known
ℓ	200,000		Terminator Factor

is employed for the copying of one strand and symmetric transcription for the copying of both template DNA strands. The core enzyme will transcribe symmetrically but the reaction if slow and non-specific. With the G factor, the holoenzyme transcribes DNA asymmetrically, initiating RNA chain at specific promoter sites.

3.2.1 Association with DNA Template

RNA biosynthesis does not require a template prime as in DNA replication. Transcriptioon begins at specific promoter sites in the DNA template and terminates at the end of a defined sequence (Fig. 8.3) presumably the RNA core polymerase repeatedly associates and dissociates with the DNA template until a promoter site is found; promoter sites must have specific sequence that are recognized by the holo – RNA polymerase as suitable binding site. The sigma factor is an essential component for binding at the promoter site. With the G factor, only the sense strand will be recognized and read correctly.

Since the 5' end of many RNAs has either PPP A or PPPG, either ATP or GTP is probably bound initially by the RNA polymerase at the promoter site. The sigma (σ) factor is needed for initiation of transcription but once started it dissociates and the core RNA polymerase completes the transcription. (fig. 8.4). The rate of transcription is apparetently controlled by the rate of initialtion and not be the rate of elongation. As soon as the polymerase moves away from the initiation site to continue and complete transcription, a second RNA polymerase molecule can bind at the same initiation site to begin a second transcript.

3.2.2 Termination

At the end of a gene, a sequence of bases must signal the completion of transcription. A release factor, rho (p), an oligomeric protein, with a molecular weight of 200,00, presumably attaches to the RNA polymerases blocking further transcription and the RNA product is released.

3.3 Transcription in Eucaryotes

Eucaryotic cells contain at least three different nuclear RNA polymerases. RNA polymerase I, an Oligomeric protein found in the nucleous, synthesizes ribosomal RNA (rRNA); it has a molecular weight of 500,000 – 700,000 and requires either Mg^{2+}or Mn2+. RNA polymerase II is an oligomery protein found in the necleoplasm; it requires Mn^{2+} for the synthesis of messenger RNA (mRNA). RNA polymerase III, also found in the necleoplasm is responsible for the synthesis of transfer RNA (tRNA).

Polymerase IV is localized in the inner mitochondrio membrane and is concerned with the asymmetric transcription of mitochondrial ribosomes. In higher plants, which contain chloroplasts, a DNA-dependent RNA polymerase has been characterized. All these polymerases in eukaryotic cells have G like initiation factors associated with them.

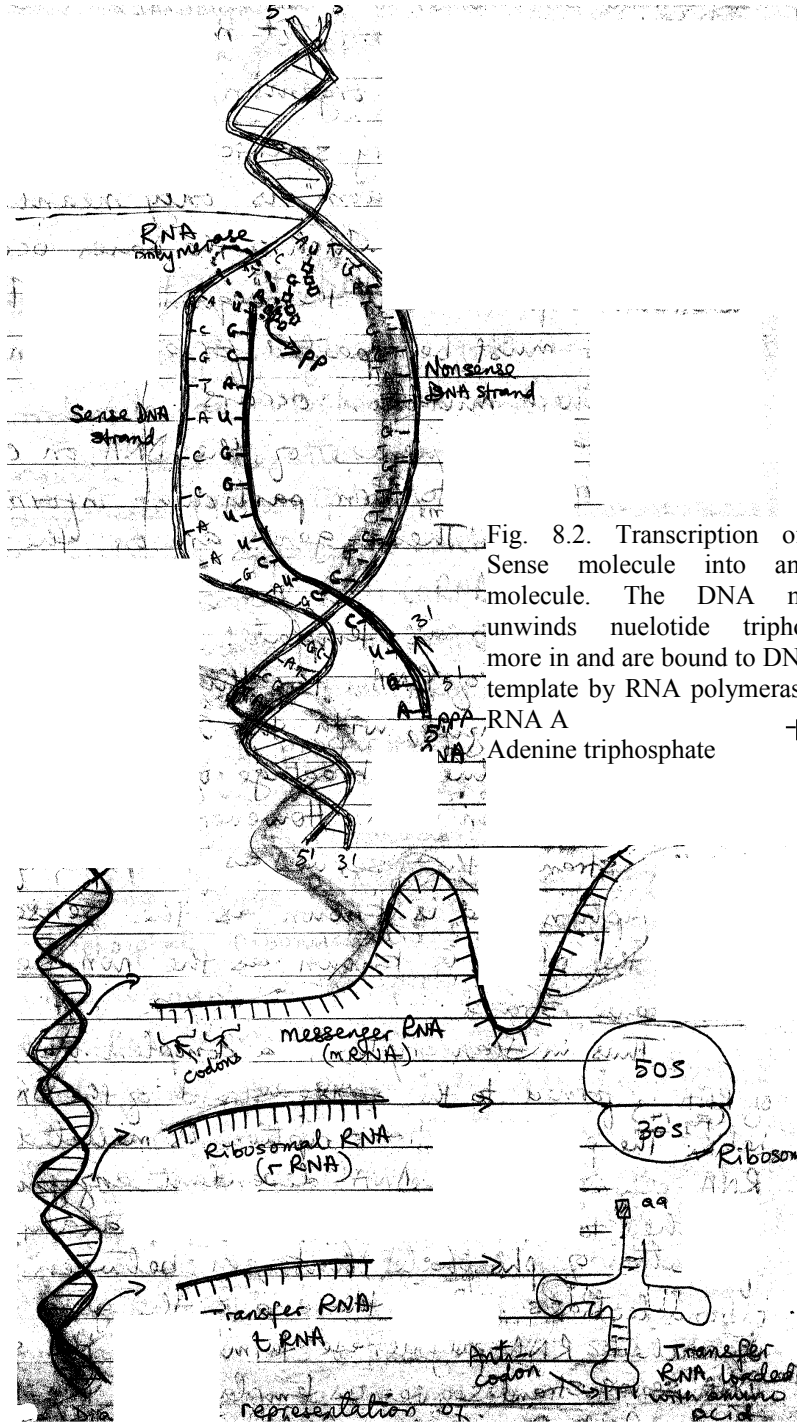


Fig. 8.2. Transcription of DNA Sense molecule into an RNA molecule. The DNA molecule unwinds nuelotide triphosphates more in and are bound to DNA sense template by RNA polymerase to for RNA A

Adenine triphosphate

Fig. 8.2: Transcription of DNA sense molecule into an RNA molecule. The DNA molecule unwinds, nucleotide triphosphates move in and are bound to DNA sense template by RNA polymerase to form RNA.

Fig. 8.3: Diagrammtic representation of the production of the three, kinds of RNA that

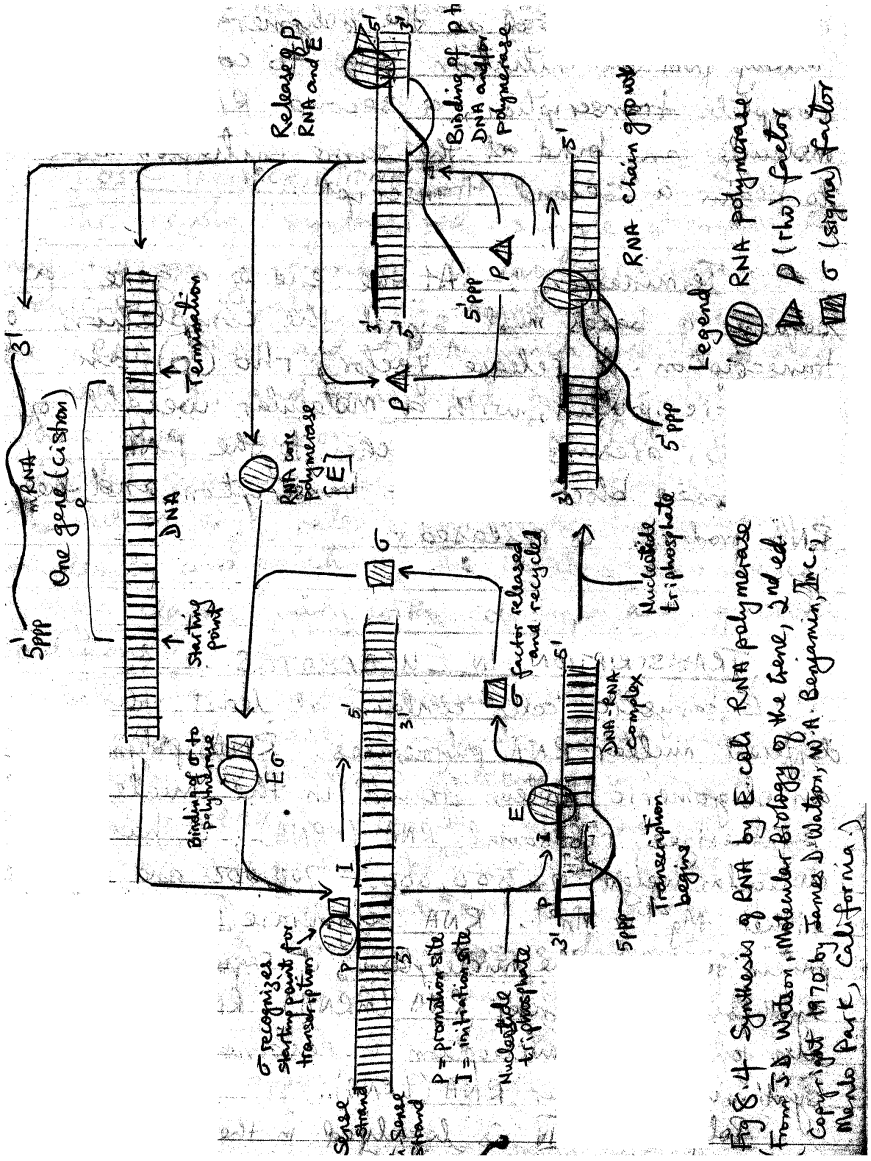


Fig. 8.4: Synthesis of RNA by E.Coli RNA polymerase

3.4 Post – Transcriptional Processing of RNA

The newly synthesized single-stranded RNAmay undergo a number of modifications. Transcription results in the synthesis of precursors for mRNA, rRNA and tRNA which are modified if necessary, and transported to the cytoplasm where they function. The nuclear RNA's can be divided somewhat arbitrarily into four classes, based on size, rate of turn-over, sequence complexity and function. The relative abundance of these classes vary with cell type and growth conditions. The class termed heterogenous nuclear RNA (hnRNA) is generally the most abundant. These molecules range from about 1,000 to 50,000 nucleotides in length. Processing of mRNA by cleavage also has been suggested on the basis of differences between bulk hnRNA and cytoplasmic RNA. These two RNA populations show different, though overlapping size distributions in higher eukaryotes. mRNA chain lengths range from about 1,000 to 10,000 nucleotides, whereas hnRNA chain lengths appear to range from 1,000 to 50,000 nucleotides.

Thus, in eukaryotic cells a prescursor, heterogenous nuclear RNA (hnRNA) is first synthesisezed in the nucleoplasm by DNA-dependent RNA polymerase. It is then degraded by a nuclear nuclease to mRNA that is then translocated to the cytoplasm. Where it becomes associated with a string of ribosomes. Most eukaryotic mRNAs are monocistronic, that is, code for only one polypeptide.

The precursor tRNA also passed out from the nucleus into the cytoplasm through the nucleoplasses. In the cytoplasm it then undergoes secondary folding into its typical clover-shape form (Fig. 8.2). This structure is aided by hydrogen bondings among the bases which do overlap; the anticodon is located in the central petal of the clover leaf (fig. 8.5). Ribosomes are made up of two subunits which enter the cytoplasm through the pores in the nuclear envelope and are in essentially nascent (complete) forms. On analysis, the ribosomes of bacteria contain about 60% RNA and 40% protein by weight. No lipids or polysaccharides are found in the bacteria ribosomes. Eucaryotic ribosomes contain a smaller proportion of RNA with the ration of RNA; protein approaching a value of 1 i.e. RNA protein = 1 : 1.

The protein complements of ribosomes are exceedingly complex. Bacteria ribosomes may contain 50 to 60 different proteins; eukaryotic ribosomes show greater complexity and may contain as many as 150 different polypeptide chains. Three classes of ribosomal proteins exist as defined by their strength of bonding to ribosomes. In prokaryotes the holding together of ribosomal units depends on the concentration or Mg^{2+}. About 30% of the protein is only very loosely attached and is easily removed; the removal of this protein does not interfere with protein synthesis in cell – free systems.

Another factor which is about 50% of the proteins can be reversibly removed by adjusting the concentration of Mg^{2+}. The remaining 20% called "core" protein is very tightly bound, its removal results in the irreversible destruction of the ribosome.

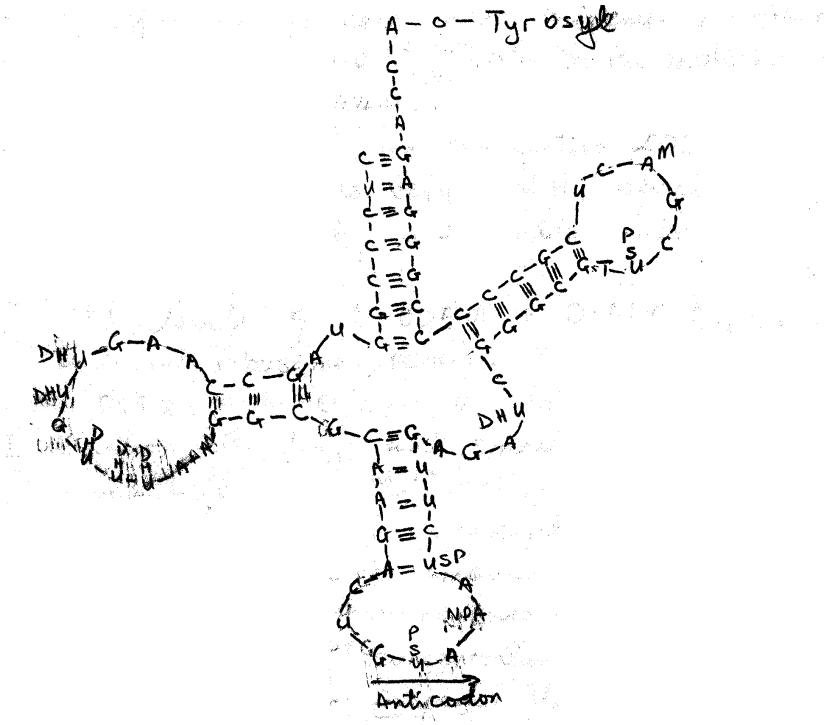


Fig. 8.5: Tyrosine tRNA as an example of tRNA showing its clover leaf structure,with an indication of the suggested base-pairing. The anticodon the sequence of three bases first over the arrow at the bottom of the molecules.

At levels of 0.005m to 0.01m Mg^{2+}, about 30% of the protein is only very loosely attached and is easily removed;the removal of this protein does not interfere with protein synthesis in cell-free systems. Another fraction which is about 50% of the proteins can be reversibly removed by adjusting the concentration of Mg^{2+}. The remaining 20%, called "core" protein is very tightly bound, its removal results in the irreversible destruction of the ribosome. At levels of 0.005m to 0.01m Mg^{2+}, bacterial ribosome remain intact. At levels below 1,000 mg^{2+} ions/ribosome, the ribosomal sub units are irreversibly denatured if the "core" proteins are removed. Eucaryotic ribosomes are not denatured by just low Mg^{2+} concentration but you have to raise the PH or increase the concentration of monovalent cations or increase the concentration of PO_4 or CO_3 which have high affinity for Mg^{2+} . These

observations point to the possible role of Mg^{2+} ions in binding the subunits of ribosomes together.

The ribosomes in both prokaryotic and eukaryotic cells are made up of two sub-units, one smaller than the other. Fig. 8.5, shows that the eukaryotes have larger ribosomes (80s) which dissociates less easily into component sub units of 40s and 60s.. however, mitochondrial ribosomes in eukaryotic cells resemble procryotic ribosomes in being 70s and 50s sub unit; hence the mitochondrial ribosomes from mammalian cells are smaller than the cytoplasmic ones, and they closely resemble bacteria ribosomes. It has also been shown that protein synthesis in mitochondria and chloroplasts is inhibited by chloroamphinicol, an inhibitor which is effective against bacterial protein synthesis in eukaryotic cells.

Chloroamphenicol has no effect on protein synthesis in the cytoplasm (i.e. on ribosomes) in eukaryotic cells. This points to an evolutionary relationship between mitochondria, chloroplasts and bacteria. It will be noted that the sub uits do not add up to the whole units (i.e. 30s + 50s ≠ 70s) and 40s + 60s ≠ 80s). This is because the "S" s not a weight unit, but a notation called Svedberg unit (names after its originator) which denotes the sedimentation rate rather than weight.

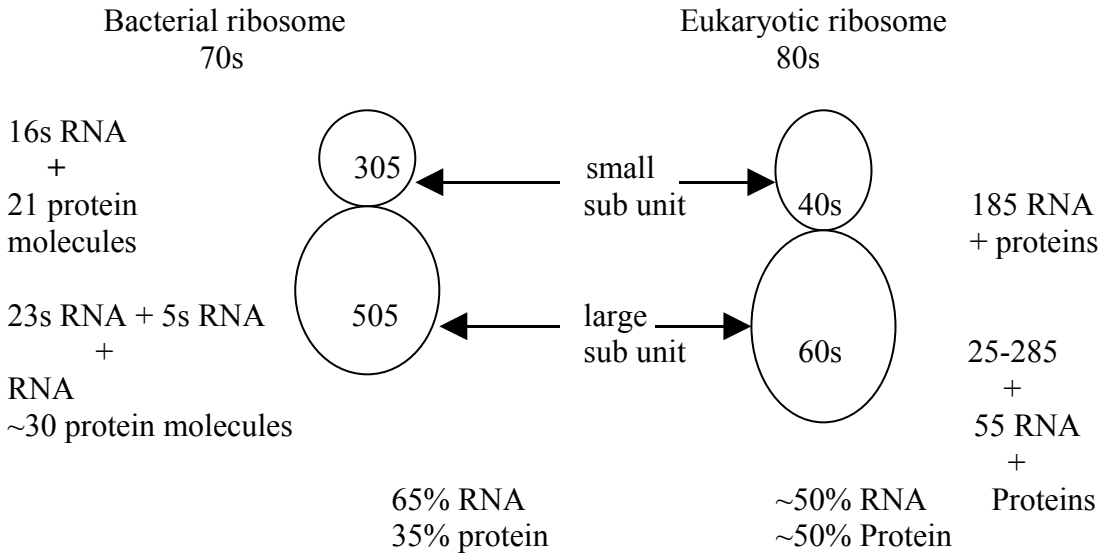


Fig. 8.6: Schematic drawing of a bacterial ribosome and a ribosome from a eukaryotic cell.

3.5 Differences between RNA, DNA and their Syntheses

There are four bases in both RNA and DNA. Adenine (A), cytosine (C) and guanine (G) which occur in the DNA molecule are also present in RNA, but the thymine (T) of DNA is replaced by ***Uracil*** (U) in RNA. Thus the compatible pairs of nucleotides in the transcription of RNA are as follows:

DNA Molecule			RNA Molecule	
Adenine	(A)	=	Uracil	(U)
Thymine	(T)	=	Adenine	(A)
Guanine	(G)	=	Cytosine	(C)
Cytosine	(C)	=	Guanine	(G)

Besides the substitution of thymine by uracil RNA differs from DNA in two more ways. Whereas the sugars in the DNA nucleotide is a deoxyribose pentose sugar, that in RNA is a ribose pentose sugar (Fig. 8.6). DNA occurs as a double strand whereas RNA exists as a single – stranded molecule.

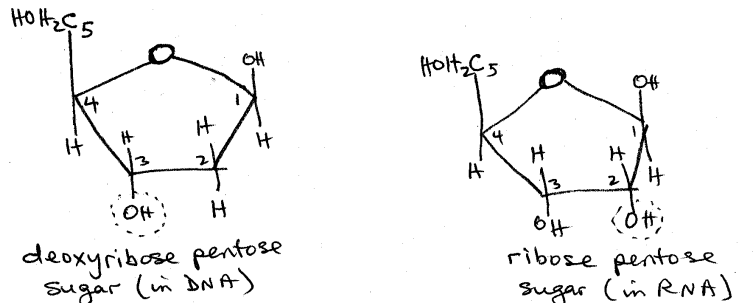


Fig. 8.6: Structures of Deoxyribose (DNA) and ribose (RNA) pentose sugars.

In replication, the two strands of DNA are read and copied with four strands resulting thereof. In transcription only one of the DNA strand is read resulting in three strands – 2DNAs and IRNA.

An RNA primer preceeds DNA synthesis whereas no primer is needed for RNA synthesis. It should be noted that the raw materials needed for transcription of RNA and the replication of DNA, i.e nucleotide triphosphates, come from the cytoplasm, passing through the pores in the nuclear membrane to get to the nucleus. RNA though synthesized in the nucleus functions in the cytoplasm. 90% of DNA are contained and function in the nucleus; remaining extranuclear DNA occur in the mitochondria and chloroplasts.

Table 8.2: Comparison between DNA synthesis (replicate and RNA synthesis transcription)

	DNA Synthesis		RNA Synthesis
1.	Double stranded helix	1	Single stranded
2	Both strands are read and copies	2.	Only one strand (serve strand) is read and copy.
3.	4 strands result thereof in two double but contained helix	3.	Three strands (2DNA + 1RNA)
4.	A short strand of RNA primer preceeds DNA replication	4.	No primer is needed
5.	Whole length of the chromosome ie, DNA is copied, albeit in discrete pockets which later are joined together	5.	Only shoet lengths of the DNA (i.e, region of a gene or cistron are copied to make RNA.
6.	The DNA is read in the 3' - 5' direction hence synthesis of the new DNA strand is from its 5' PPP -> 3' 04 direction	6.	Same here
7.	ATP, GTP, CTP and TTP are the nucleotide raw materials.	7.	ATP, GTP, CTP and UTP are the nucleotide raw materials.
8.	Deoxyribase sugar in incorporated it the sugar-phosphate backbone	8.	The sugar here is a ribose pentose
9.	Newly synthesized DNA remains in the nucleus	9.	Newly synthesized RNA move into the cytoplasm through the nucleopore.

4.0 Conclusion

1. Synthesis of RNA takes place mainly in the cytoplasm.

2. Three types of RNA are transcribed from DNA (a) messenger (mRNA) (b) transfer (tRNA) and ribosome (rRNA).

3. mRNA and t-RNA are synthesized in the nucleoplasm, while r-RNA is synthesized in the nucloplasm.

4. mRNA is a straight chain; t-RNA is clover-leaf shaped while r-RNA is made up of two units, a smaller and a larger unit.

5. The smaller unit is designated 30s (in prokaryotes e.g. bacteria) and 40s (in eukaryotic cells). The larger unit of r-RNA is designated 70s and 80s I prokaryotes and eukaryotes respectively.

6. Bases found in DNA are A, T, G and C. Bases found in RNA are A, U, G and C.

7. Differences exists between DNA and RNA synthesis.

5.0 Summary

We have gone a step further in describing the Contra Dogma. We have examined the synthesis (transcription) of RNA from DNA. It is most essential that the messages (codons) on DNA are correctly copied (transcribed) into RNA to result in the right protein being eventually made. This process (translation) is treated in the next unit.

Self-assessment Questions

1. (a) Name the three types of RNA transcribed in the cell.

   (b) Make diagrammatic drawing of each.

   (c) State where they are synthesized in the cell and their sites of action.

2. What different base pairs exists between DNA and RNA?

3. What are a “sense” and “non-sense” strands?

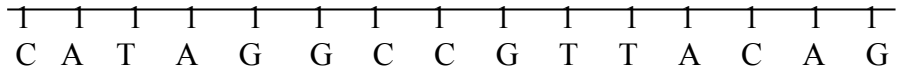
4. Draw the diagram of a deoxyribose and a ribose sugar. Where will you find either one of them?

6.0 Tutor marked Assignment

1. Below is the sequence of a “non-sense” DAN strand.

(a) Compute the sequence of its “sense” counterpart DNA strand.

(b) What is the sequence of RNA to be formed from the DNA double helix?



2. list five differences between DNA and RNA synthesis

3. Describe the synthesis of RNA from DNA double helix template.

7.0 References

1. Wolfe, S.L. 1972. Biology of the Cell. Wedsworth Publishing Co. Inc., Belmont, California.

2. Roberts, M.B.V. 1975. Biology: A Functional Approach, E..L.B.S. and Nelson. Lagos

UNIT 9: TRANSLATION (SYNTHESIS) OF PROTEINS FROM RNA

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1.0 Introduction

The third stage of the Central Dogma of Molecular Biology involves the synthesis of proteins from the message coded in triplet nucleotide sets on the mRNA. Earlier we have seen how RNA is formed from the reading of a gene on the chromosome (DNA strand). The three types of RNA formed (m-RNA, t-RNA, rRNA) migrate into the cytoplasm where they all work in unison to source for appropriate amino acids which are then linked up into a chain to form proteins.

2.0 Objectives

At the end of this unit, you should be able to:

1. Describe the mechanism of protein synthesis.

2. Name the codons that represent each amino acid

3. Name the anticodon that will attach to the right codons

4. Know the functions of each type of RNA

5. Name some of the twenty naturally occurring amino acids.

6. Describe with the aid of diagrams

   (i) Primary Structure

   (ii) Secondary Structure

   (iii) Tertiary Structure

   (iv) Quartenary structure of a protein.

3.1 Translation: Protein synthesis from RNA

The specific directions for the synthesis of protein are transcribed from the sense DNA template into mRNA. Each molecule of mRNA contains information necessary to assemble a specific sequence of amino acids into a complete polypeptide chain; this chain may be either a complete protein molecule or a polypeptide submit a protein molecule.

The information transformed to, and contained on the mRNA are in form of three linear nucleotides forming a codeword or "codon". Using the four bases in all different 3-base combinations, 64 different codewords can be formed. (Table 9.1). This is more than sufficient for the 20 known amino acids (Table 2.2) that make up protein molecules. Hence we can have more than one codon coding for each amino acid, but no one codon can code for more than one. For example, one codon, ACC codes for the amino acid threonine; other codons that code for this same amino acid are ACU, ACA and ACG (Table 9.1).

After release for its DNA template, the newly-synthesised mRNA passes through the nuclear envelope into the cytoplasm and attaches to series of ribosomes (fig. 9.1). The ribosomes, of course are RNA particles coupledwith proteins. Surrounding the ribosomes is a pool of various tRNA's with attached amino acids. Each tRNA (Fig. 9.2) contains a recognition site (called an ***anti-codon***) for the code designating these amino acids in the mRNA. For example the tRNA carrier of threonine would contain the anti-codon UGG (or UGA, UGU or UGC).

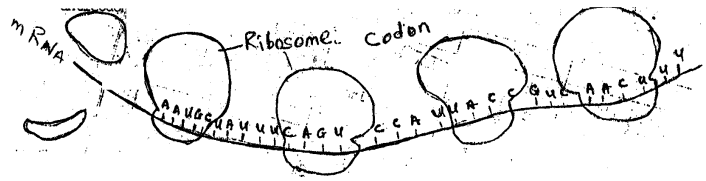


Fig. 9.1 several ribosomes become attached to a mRNA strand during protein synthesis. Each ribosomes is made up of two units which become dissocited when detached from RNA.

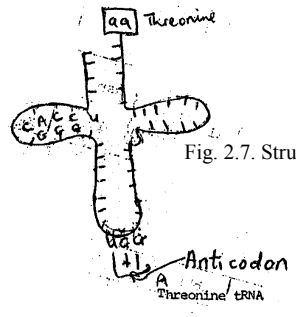


Fig. 2.7. Structure of t, RNA

Fig. 9.2

When the right anti-codon on tRNA finds the right codon on the mRNA temporary bondings will be formed. Then the next t-RNA comes around and binds to the next set of codons (Fig. 9.2). The two amino acids are now united in a chain and the first t-RNA dissociates from its codon to search for another amino acid to hook up with. The ribosome move along the m-RNA and the subsequent codons dictate the amino acid to be attached to the growing chain. A set of triplets on the mRNA dictate the end for that particular protein synthesis; to this triplet set, called ***terminator***, no t-RNA binds. At the sight of the terminator protein synthesis ends, and the polypeptide chain detaches; it can on its own fold up into its tertiary structure to form a protein, or join up with other polypeptide chains to form protein.

Table 9.1: The genetic code, codons, consisting of 64-triplet combination and their corresponding amino acids.

	U	C	A	G	
U	UUU) Phe) UUC)) UUA) Leu	UCU)) UCC) Ser) UCA))	UUA)) Tyr UAC) UAA Termina tor	UGU)) Cys UGC) UGA Termina tor	U C A

	UUG	UCG)	UAG " "	UGG Tryp	G
C	CUU)) CUC) Leu) CUA)) CUG	CCU)) CCC) Pro) CCA)) CCG)	UAU)) His CAC)) CAA) GIUN) CAG)	CGU)) CGC)) Arg CGA)) CGG)	U C A G
A	AUU)) AUC) Ileu) AUA) Met Initi AUG alisation	ACU)) ACC) Thr) ACA)) ACG)	AAU)) asPN AAC)) AAA)) Lys AAG)	AGU)) Ser AGC)) Arg AGA)) AGG)	U C A G
G	GUU)) GUC) Val) GUA)) GUG)	GCU)) GCC) Ala) GCA)) GCG)	GAU)) Asp GAC)) GAA)) Giu GAG)	GGU)) GGC)) Gly GGA)) GGG)	U C A G

Table 9.2: The twenty amino acids found in all proteins.

	Amino acid	Abbreviation		Amino acid	Abbreviation
1.	Alanine	Ala	13	Methionine	Met
2.	Argine	Arg	14	Phenylalanine	Phe
3.	Asparagine	AspN	15	Proline	Pro
4.	Aspartic acid	Asp	16	Serine	Ser
5.	Cysteine	Cys	17	Threonine	Thr
6.	Glutamic acid	Glu	18	Tryptophan	Tryp
7.	Glutamine	G1uN	19	Tryosine	Tyr
8.	Glycine	Gly	20	Valine	Val.
9.	Histidine	His			
10.	Isoleucine	Ileu			
11.	Leucine	Leu			
12.	lysine	lys			

3.1.1 Initiation of Protein Synthesis

The first step in the initiation of protein synthesis is the binding of mRNA to the ribosome. The mRNA combines with the smaller 30s unit of the ribosome. The first amino acid to be laid down in the synthesis of any bacterial protein is N-formyl-L-methionine (fmet0 which is coded for by the triplet codon AUG; this codon thus a series as an ***initiation codon.***

The initiation of protein synthesis depends on the interactions between mRNA, fmet – tRNA, the initiation codon AUG and the 30s ribosomal subunit to form the initiation complex, this process requires GTP and mg^{2+}. Once formed the whole complex then combines with the 50s subunit to make up during the formation of the initiation complex, it is hydrolysed when the two subunits (30s – 50s) combine.

These ribosomal proteins simply called the F3 or the ***initiation factors*** have been shown to be required for the process of chain initiation. Two of these seem to be essential for the binding of the fmet – tRNA and the normal mRNA to the 30s subunit; the third protein is concerned with linking the two ribosomal subunits (fig. 9.3). Thus it seems that the bacterial ribosomes are diassociate until the 30s subuits form the initiation complex.

What this initiation phenomenon means is that, every bacteria protein must have Fmet as its N-terminal amino acid residue. However, either the formyl grou or the fmet itself seems to be hydrolized off the protein before it is released from the ribosome. It is only the first AUG triplet in the mRNA which specifies for fmet; all subsequent AUG triplets code for methione.

The GUG triplet, which normally codes for valine, can also code for fmet – tRNA and thus services as an initiation codon. Investigations have shown that the region between the chain termination of one protein and the initiation codon of another one is not translated.

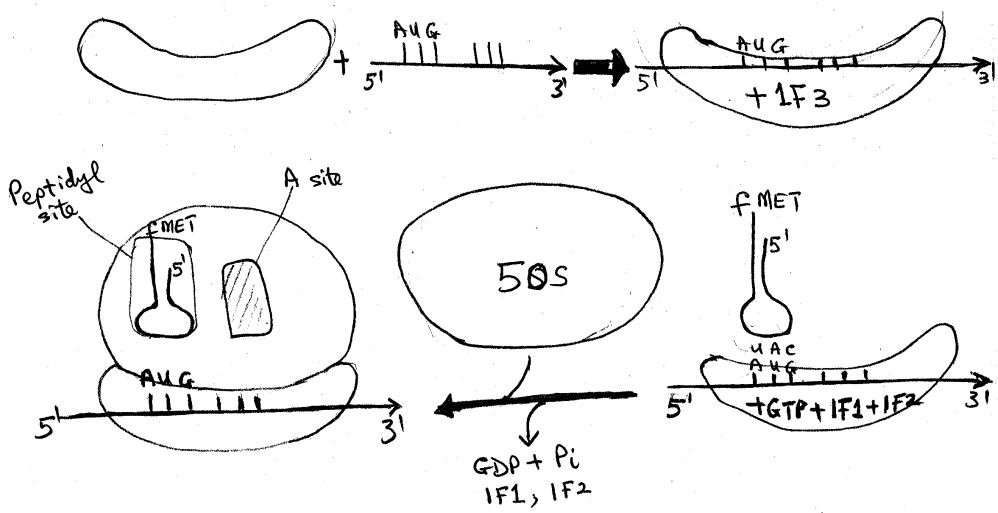


Fig. 9.3 steps by which the 70s initiation complex is formed in **E coli** (1F1, IF2, 1F3 are the protein initiation factors).

3.1.2 Chain Elongation

Once the initiation complex has combined with the 50s subunit of the ribosome the actual polypeptide synthesis then begins. The next codon is then engaged by the second amino acyl – tRNA complex bering the appropriate anticodon; this occurs at the A site (Fig. 9.3) GTP, mg^{2+} and two elongation factors EFTU and EFTs, are involved. Peptide bond is then formed between the fmet's carbonyl group and the amino group of the newly-attached amino-acyl tRNA.

The reaction is catalysed by an enzyme called peptidyl transferase, which is a constituent of the 50s ribosomal subunit; as a result of the transfer, the +-RNA which was originally attached to the f-met is freed, and a dipeptide is left attached to the second tRNA. The next codon on the mRNA is filled, in similar manner by an acyt-tRNA with the correct anticodon; peptide bond will be formed with the preceeding amino acid with the subsequent hydrolisis and release of its tRNA (fig. 9.4) The sequence continues until the end of the chain is reached.

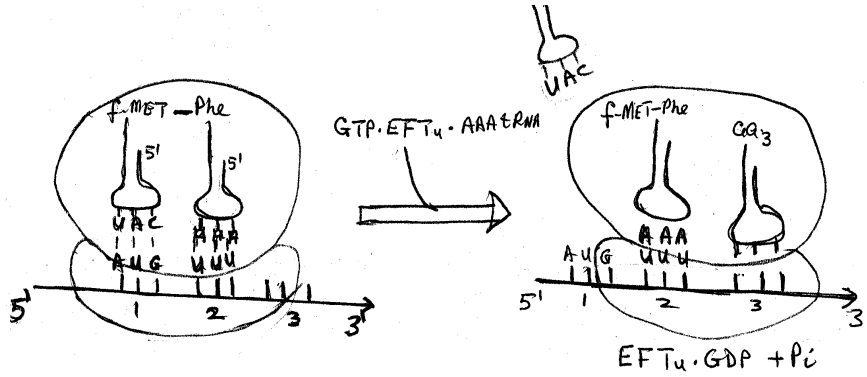


Fig. 9.4: The elongation process.

3.1.3 Chain Termination

The peptide chain continues to grow until a set of triplets on the mRNA dictates the end of that particular protein synthesis. This triplet is called a termination codon or a terminator and does not bind any tRNA. Three of such terminators exist – UAA, UAG and UGA. Upon reaching the termination codon, protein synthesis stops and the polypeptide chain detaches. Release of the ribosomal proteins, the releasing factors, R_1 and R_2.

3.2 The Peptide Bond

In a protein or polypeptide the amino acid are joined together through the carboxyl and amino groups by an amide linkage (Fig. 9.5). This linkage is known as a peptide bond. For alanine and glycine, this give alanylglycine which is a dipeptide.

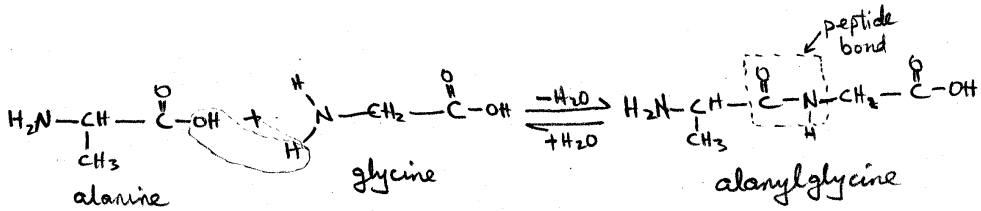


Fig. 9.5: The peptide Bond (between alamine & glycerine)

The peptide bond itself is the -CO-NH- link, and it can be split by hydrolysis in acid solution or in the presence of a suitable enzyme. If a third amino acid is joined to the alanylglycine dipeptide, it will become a tripeptide (Fig. 9.6) a peptide chain containing three or more amino acids is known as a ***polypeptide.*** If the third amino acid is leucine, we obtain alanylglycylleucine.

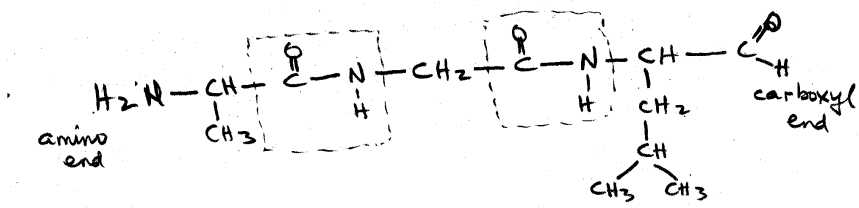


Fig. 9.6: Elongation of Peptide chain (alanylglycyl-leucine chain)

It should be clear by now that any polypeptide will have an amino group at one end and a carboxyl group at the other. Thus, we can write the general structure of a polypeptide chain where R stands for the side chain of any one of the 20 different amino acids.

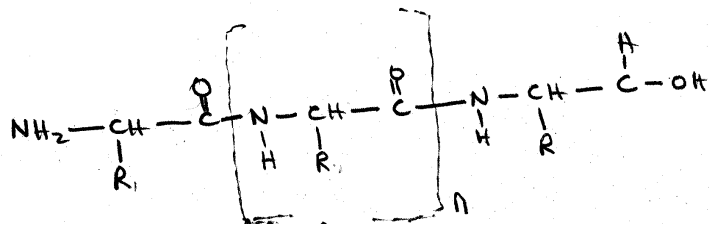


Fig. 9.7: The polypeptide chain

3.3 Protein Structure and Conformation

3.3.1 Primary Structure

The sequential arrangement of amino acids as synthesized from the mRNA on polysomes is known as the ***primary structure*** of a protein. The released polypeptide chain can undergo structural modifications; these modification will result in the polypeptide assuming secondary, tertiary and quaternary structures. (Fig. 9.8)

3.3.2 Secondary Structure

The secondary structure of a protein involves the formation of hydrogen – bond interactions between amino acid residues fairly close to one another in the primary structure. (Fig. 9.9)

3.3.3 Tertiary Structure

This involves extensive coiling or folding to produce a complex, somewhat rigid structures (fig. 9.10). This folding normally occurs

from interactions between amino acid residues relatively far apart in the sequence (Fig. 9.10)

3.3.4 Quartenary Structure

This results from interaction between separate polypeptide units of a protein containing more than one subunit (Fig. 9.11) the & and B chains of haemoglobin. Where the subunits are the same the protein has a homogeneous quartenary structure (or promoter); where dissimilar the protein has a heterogenous quartenary structure (=oligomeric protein e.g. haemoglobin).

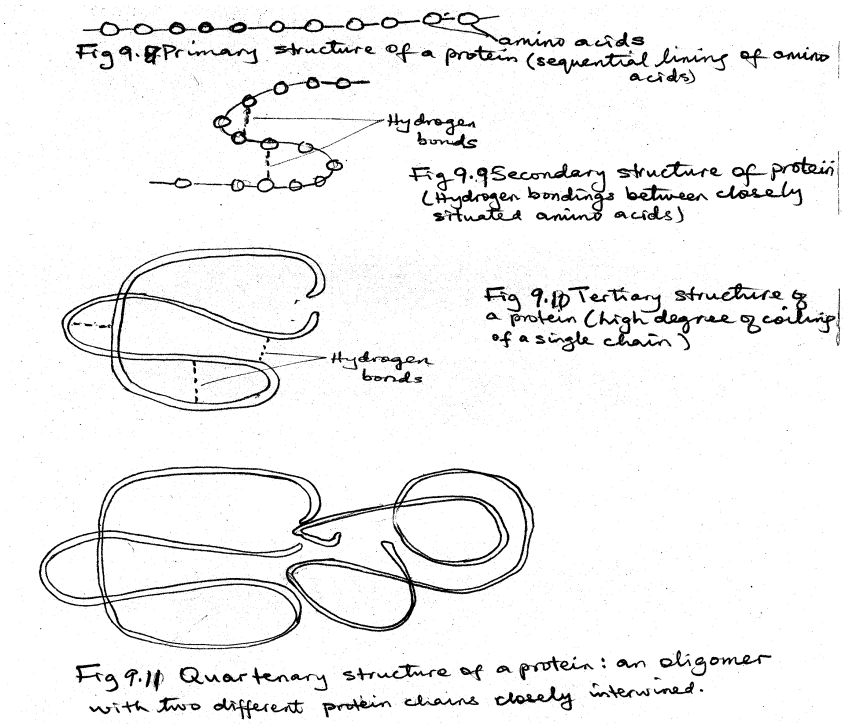


Fig 9.8 - 9.11:

4.0 Conclusion

We have now got to the third phase of the Central Dogma of Molecualar Biology – Translation or protein synthesis from RNA. You will remember that the DNA contained in the nucleus holds the message as to what kind of RNA is formed. The three types of RNA formed (m-RNA, tRNA and rRNA) all function in the cytoplasm. So they have to migrate into the cytoplasm carrying their messages written off from the DNA. The mRNA, which is s

straight chain bears the message of the sequencing of amino acids in the protein chain to be formed. It attaches to the ribosomes (rRNA). The tRNA hooks on to an appropriate amino acid and through its anticodon end attaches to the codon on the mRNA. It remains there till the next tRNA brings the second amino acid; there is peptide bond formed between the amino end of and the carboxyl ends of the two amino acid. A di-peptide is formed, linked by a peptide bond. Chain elongation continues with the addition of a third amino acid, and so on until a terminator codon is reached which terminated the sequence of protein formation.

The protein chain thus formed goes from its primary straight-chain structure, into the secondary structure, formed by hydrogen bonds between closely-linked amino acids. The protein chain may go into a tertiary structure by much infolding. When associated with another protein chain, the polypeptides may form a quartenary structure e.g the alpha (α) and beta (β) chains of haemoglobin.

5.0 Summary

With the study on protein synthesis ie. Translation, we have gone the whole route of the Central Dogma of Molecular Biology. Now you know that the DNA contained within the nucleus bears the genetic codes which can be duplicated (replicated) in mitosin to continue the same messages throughout generations of the cell. Genes on the DNA can be read of (transcribed) into RNA which go into the cytoplasm to dictate the type of proteins to be synthesized. The proteins activate metabolic reactions in the form of enzymes. It is the type of enzymes that determine the reactions and functions of the cell. Thus, we see that the nucleus is the control centre of the cell.

Self-assessment Questions

1. Where will you find a codon? and an anticodon.

2. Name the three types of RNA transcribed from DNA (b) identify where each is synthesized in the cell (c) what work does each RNA do in the cell.

3. Describe the (a) primary (b) secondary (c) tertiary and (d) quartenary structure of a protein.

6.0 Tutor-marked Assignments

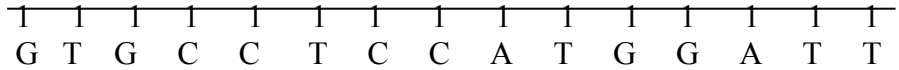
1. Below is the sequence of a "non-sense" DNA strand.

   (a) Compute the sequence of its counterpart "sense") DNA strand.

   (b) What is the sequence of the mRNA to be synthesized from this DNA helix.

   (c) Which amino acids will be contained in the protein synthesized from this RNA.

Nonsense DNA Strand



2. List five differences between DNA and RNA

3. What amino acids will these codons code for?

   (i) GGG (ii) UUU (ii) AAG (iv) CGG (v) UAA (vi) UUG (vii) GAU (viii) CCU (ix) CUA (x) UAU.

7.0 References

1. Wolfe, S.L. 1972. Biology of the Cell. Wedsworth Publishing Co. Inc., Belmont, California.

2. Roberts, M.B.V. 1975. Biology: A Functional Approach, E..L.B.S. and Nelson. Lagos

Answers to Self Assessment Questions

1. Codons are a set of three contiguous nucleotides onmRNA that determine which amino acid will fall in place. (b) An anticodon is a triplet set of nucleotiede on a tRNA that attaches to the codon and deposits the appropriate amino acid in the protein sequence.

2. (i) mRNA (ii) tRNA (iii) rRNA

b. both mRNA and tRNA's are synthesized in the nucleoplasm of the nucleus rRNA is made in the nucleolus.

c. (i) mRNA caries the codons that stipulate the sequence of amino acids in a protein.
(ii) tRNA carries the appropriate amino acid to the site of protein synthesis
(iii) rRNA is the actual site where protein synthesis takes place.

3. Ref Figs. 9.8 to 9.11

UNIT 10: PROTEINS AND ENZYMES: STRUCTURE AND FUNCTION

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4.0 Conclusion
5.0 Summary
6.0 Tutor-marked Assignment
7.0 References

1.0 Introduction

It was stated earlier in this course that the nucleus is the nerve centre of the cell in that it controls the activities of the cell. It was also stated that genetic materials which carry information from one generation to another are contained within the nucleus; specifically they are contained on the chromosomes which are basically made of DNA. DNA is capable of making copies of itself (replication) before cell division and this ensures equal distribution into daughter cells. From DNA, RNA can be copied (transcription). The RNAs function in the cytoplasm for the synthesis of proteins.

Three types of RNA are made from DNA. The messenger RNA (mRNA) is a straight chain that bears the codons which determine the sequence of amino acids in a protein. The transfer RNA (tRNA) carry amino acids at one end and bear the anticodon on the other end. The anticodon links with the codon and amino acids that are side-by-side join through peptide bonds to form chains of protein synthesis take place in the cytoplasm.

The protein thus made goes into folds to maintain its characteristic shape – every protein, to function properly, must assume its characteristic shape. A protein can be structural, making up part of the cell, especially the cell membranes where it forms the characteristic unit structure. Proteins can also be enzymes which catalyze the physiologically reactions that take place in the cell and for which the cell is well known. Without enzymes reactions go on at painstakingly slow pace. But the pace is quickened in the presence of the right enzyme which combine with the ***substrate*** to form the product. The product may also serve as a ***substrate*** for other enzymes to work upon.

Thus in this unit, we come to see how the nucleus controls the activities of a cell. The messages carried on the DNA within the nucleus carries the message which determine what kinds of proteins are synthesized. The type of proteins (or enzymes) within a cell determines what kind of biochemical reactions take place in the cell. The type of biochemical reactions within a cell characterizes the cell.

2.0 Objectives

At the end of this unit, you would be able to:

1. Describe the structure of proteins

2. List the classes of proteins

3. Describe how enzymes work

4. Explain the factors that affect enzyme activity

5. State the effects of activators and inhibitors on enzyme action.
6. Relate enzyme structures and functions to their evolutionary trends.

3.1 Proteins

3.1.1 Structure of Proteins

1. Primary Structure

The primary structure of a protein is the linear sequential order of amino acid residues making up its polypeptide chain. The focal point is the peptide linkage between each of the amino acids; no other forces or bonds are involved in this term.

aa_1 —— aa_2 — aa_3 — aa_4 — aa_5 —— aa_6 —— aa_7……aa_n

2. Secondary Structure

After the amino acids have been linked sequentially by peptide bonds, the protein chain undergoes coiling into a helix (a spiral of fixed diameter; ie. a cock-screw shape. This secondary characteristics are enabled by hydrogen bonding, between O_2 and H_2 atoms near the peptide linkage (Fig. 10.2)

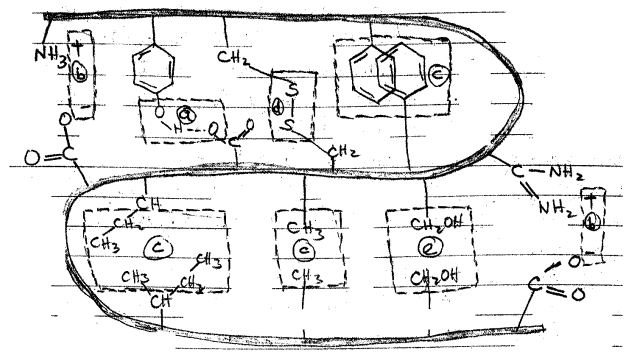


Fig. 10.2: Some types of non-covalent bonds which stabilizes protein structure: (a) hydrogen bonding (b) electrostatic interaction (c) hydrophobic interaction of non-polar side chains caused by the mutual repulsion of solvent (d) disulfide linkage, a covalent bond (e) dipole-dipole interaction (from C.B. Anifinsen, The Molecular Basis of Evolution, John Wiley & Sons, NY 1959)

3. Tertiary Structure

This is the folding of the helix or sheet at characteristic places to produce a complex, somewhat rigid interaction (fig. 10.3). The folding normally occurs from interactions between amino acid residues relatively far apart in the sequence. The term ***conformation*** refers to the participation of the secondary and tertiary structures of the polypeptide chains in molding the structure of the protein and is of great importance in determining the fine structure and unique catalytic properties of biologically active proteins (ie. enzymes and carriers); a change from the correct conformation may lead to loss of activity.

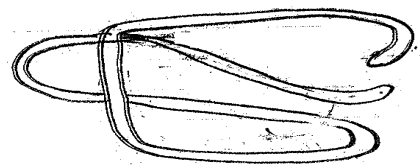


Fig. 10.3: Sketch illustrating the complicated folding of a globular protein stabilized by non-covalent bonds illustrated in fig 10.2

4. Quartenary Structure

This is formed when two or more polypeptide chains come together to form one complex active protein molecule. .Each subunit has its own independent three-dimensional conformation (Fig. 10.4). the quarternary structure is stabilized by weak interactions rather than by covalent bonds. An example of protein with quartenary structure is haeme of haemoglobin which has a pair each of an α and a β chain. A protein with identical subunits has a homogeneous quartenary structure; if the subunits are dissimilar a heterogenous quartenary structure is obtained. The haeme protein is thus a heterogenous protein. Subunits can also be called ***protomers*** and a protein made up of more than one protomer isan oligomeric protein.

Protein coiling in proteins is not static; only a fraction of the peptide chain may be coiled at any one time. The tertiary folding is stabilized by bonds between side chains e.g. (S-S bonds) or "R" groups; but these bonds are usually weak and easy to break.

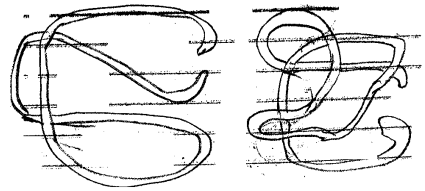


Fig. 10.4 Quartenary structure of s globular protein: an oligomer with two different protomers.

Protein structure is extremely sensitive to changes in the environment such as an increase in temperature, increases in the energy of the molecular vibration or random motion which may disrupt some of the weak bonds that maintain protein structure. If the hydrogen ion concentration changes, so does the charge on the exposed "R" groups and the whole folding pattern may shift. Inorganic salts have similar effect.

Not only do the different sequences of amino acid make different kinds of proteins, but a particular protein itself may exist in many different forms because of changes in the forces that underlie its coiling and foldings.

3.1.2 Classification of Proteins

Proteins are classified based on their conformation or three-dimensional structure and on their solubility properties. Within these criteria there are two categories of proteins namely ***FIBROUS*** and ***GLOBULAR*** proteins.

(a) **Fibrous Proteins:** are composed of individual elongated filamentous polypeptide chains arranged in parallel rows, and joined laterally by several type of cross-linkages to form sheets of fibres which are fairly stable, tough and insoluble in water and dilute salt solutions. Examples of fibrous proteins include structural proteins such as keratin (found in skin and feathers) collagen (found in skin, cartilage and bone) and silk.

(b) **Globular Proteins:** in contrast to fibrous proteins, contains polypeptide chains which are extensively folded and compact, with little, if any room for molecules of water in its interior. They are folded to produce roughly spherical shapes, with all polar R groups of the amino acids on the outside and are hydrated; on the inside are hydrophobic R groups which thus repel water from the interior. They are usually soluble in

water and perform a multitude of functions in living systems. These include the enzymes, some animal hormones (e.g oxytocin, vasopresin, adrenocorticotrophic hormone (ACTH), and insulin), transport proteins (e.g haemoglobin) cytochrome C (involved in aerobic respiration), blood proteins (serum albumin, antibodies, glycoproteins) and some seed storage proteins.

(c) Another method of classification groups proteins into ***simple*** **and** ***conjugated*** proteins. Simple proteins yield only amino acids upon hydrolysis. Conjugated proteins consist of a protein molecule which is bound to a non-protein molecule known as a prothetic group. Simple protein include both fibrous and globular proteins. Examples of conjugated proteins are NEUCLEOPROTEINS (nucleic acid + proteins) GLYCO OR MUCOPROTEINS (carbonhydrates + proteins), LIPO-PROTEINS (lipids and proteins), CHROMOPROTEINS (metallo-pigments) and PHOSPHOPROTEINS (which contain phosphate groups other than those found in phospholipids or nucleic acids).

3.2 Enzymes

In this unit we shall define what an enzyme is. We shall consider the basic properties of the enzyme and its importance in the life and activities of a cell. We shall learn such new definitions as competitive, non-competitive inhibition, allosteric enzyme, regulatory enzymes, oligomeric enzyme, active centres etc.

An enzyme is a protein that is synthesized in a living cell and catalyses or speeds up a thermodynamically possible reaction so that the rate of the reaction is compatible with the biochemical process essential for the maintenance of the cell. The essential thing here is that the enzyme does not itself get altered at the end of the reaction; it remains unchanged and is capable of carrying out further catalysis on more substrates. The enzyme in no way modifies the equilibrium constant (km) or the ΔG (free energy change) of a reaction. Enzymes, being proteins can be denatured by (a) heat (b) strong acids and bases (c) organic solvents (d) temperatures or other materials that denature proteins.

3.2.1 Mechanism of enzyme Action

The molecules of any compound are always in constant motion, the speed depending on the nature of the compound i.e. gas, liquid or solids (gas molecules are much freer than liquid molecules and far much freer than solid molecules). Only molecules that can teach a certain level of energy can undergo specific reaction. Enzymes function by increasing the proportion of molecules having sufficient energy to react, thus speeding the rate of the reaction. This they do by decreasing the energy required for the reaction, not by decreasing the energy required for the reaction, not by increasing the amount of energy in a molecule.

For a substrate to be converted to a product an energy barrier, ΔG, must be

S + E ⇌ P + E Where S = Substrate
E = enzyme P = product

Overcome. This barrier is called the ENERGY OF ACTIVATION (ΔG). This is like having to climb a hill before reaching a valley (fig. 10.5 & 10.6). The enzyme greatly reduces this "hill", barrier, or energy of activation for the hjydrolysis of sucrose to glucose and fructose subunits is about 32,000 calories per mole, but the presence of the enzyme invertase lowers it to about 9.400 calories thus an enzyme catalyzed reaction proceeds at a tremendously faster rate than would the same non-catalyzed reaction.

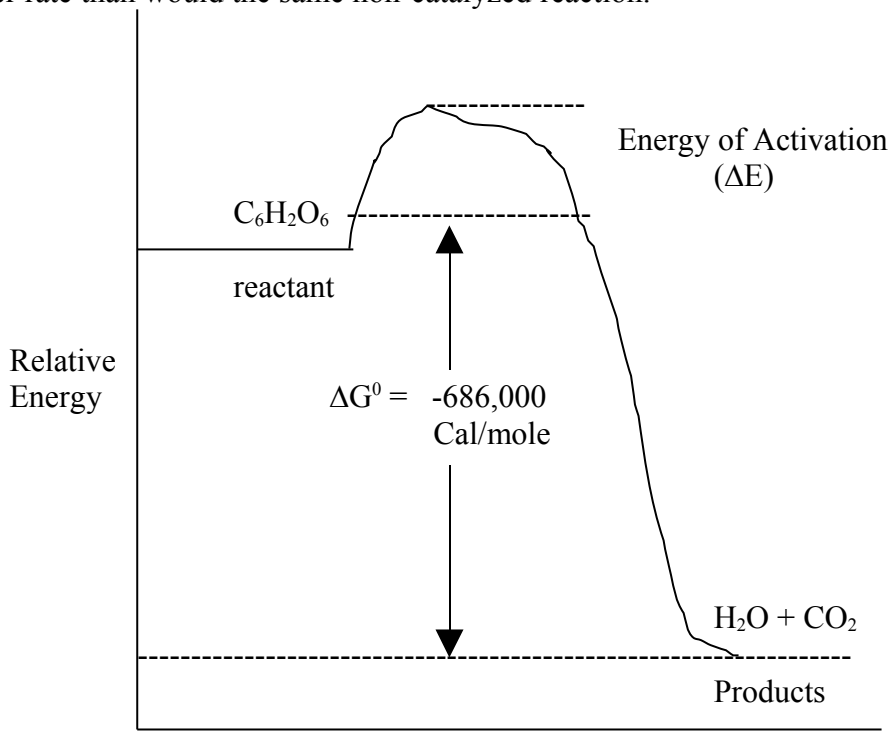


Fig. 10.5: Diagram illustrating the effect of a spontaneous reaction of the requirement of energy of activation. Even though the conversion of glucose to the product (CO_2 & H_2O) is a "down hill" reaction and proceeds with the release of a large amount of free energy, spontaneous oxidation of glucose at room temperature (without an enzyme) proceeds at an immeasurably slow rate. This is because an increments of energy must first be added to raise the molecule to a state at which spontaneous reaction may occur. The increment is shown as the energy of activation in the diagram.

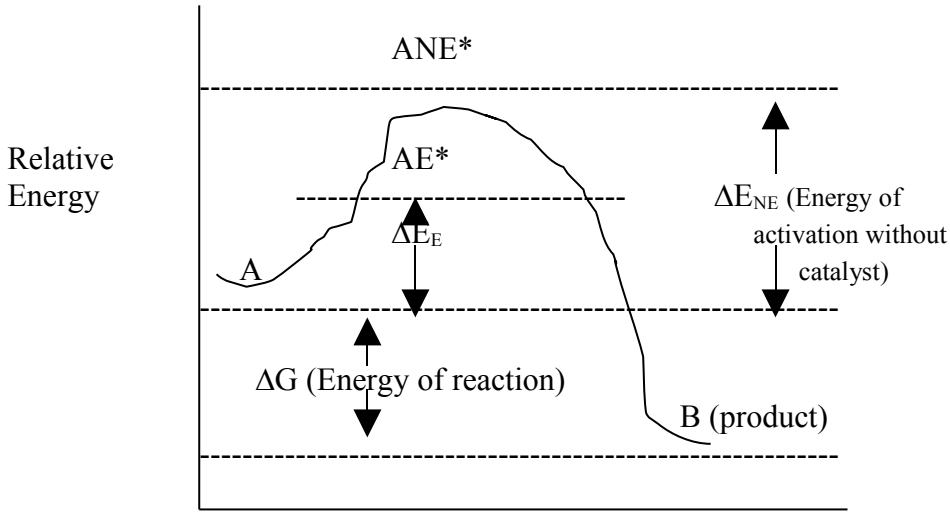


Fig. 10.6

Fig. 10.6: Diagram showing energy barriers of a reaction with and without and enzyme. A is the substrate energy level; B product energy level; ΔA^*_{NE} indicates the activated complex in a non-enzymic reaction; ΔA^*_E shows the energy level of activated complex in an enzymic reaction; ; ΔE_{NE} is the energy of activation for no-enzymic reaction; ΔE_E is the energy of activation in an enzyme catalysed reaction and ΔG is the difference in the free energy in A $\longrightarrow$ B.

3.2.2 Effect of Enzyme Concentration and Substrate Concentration

An enzyme-catalysed reaction at varying substrate concentration is ***diphasic***. With fixed enzyme concentration, an increase of substrate will result at first in a very rapid rise in velocity or reaction rate. As the substrate concentration continues to increase, however, the rate of the reaction begins to slow down until, with a large substrate concentration, no further change in velocity is observed. (Fig. 10.7)

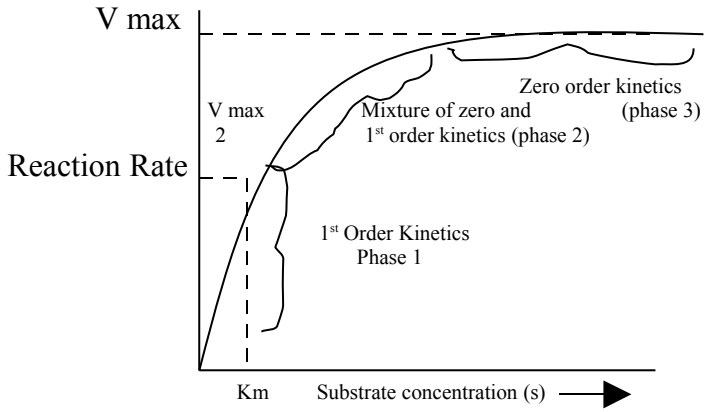
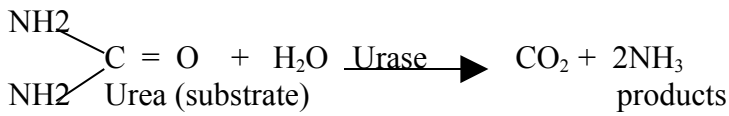


Fig. 10.7: Effect of substrate concentration on rate of reaction, assuming that enzyme concentration if constant.

As shown in fig. 10.7 above, at low substrate concentrations, the active sites on the enzyme molecules are not saturated by the substrate (Fig. 10.8 (i)). Thus, the substrates molecules are quickly acted upon and the rate is fast (phase 1). As the amount of substrate molecules increases, the active sites on the enzyme molecules are covered to a greater degree (phase 2) until at saturation, no more sites are available. The enzyme is working at full capacity and now the rate is independent of substrate concentration (phase 3). In excess substrate concentration, the rate remains constant until more enzymes are added.



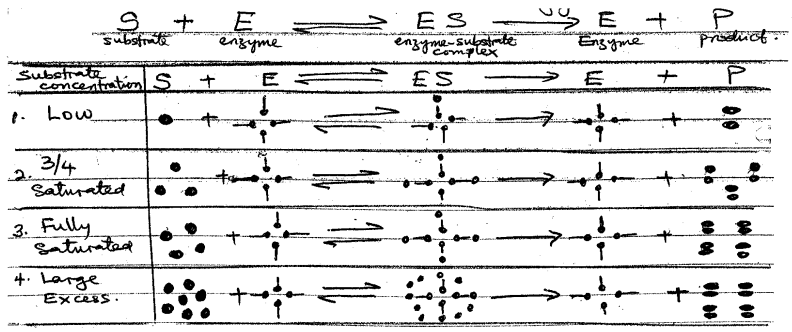


Fig. 10.8: Diagramatic illustration of the effect of substrate concentration on saturation of active sites of enzyme molecules. Note that for a unit time interval, cases 3 and 4 give the same amount of product (p) despite the large excess of substrate in case 4.

3.2.3 Important Terminologies in Enzymology

1. **Enzymology:** Study of the structure and functions of enzymes.

2. **Enzyme:** A protein synthesized in a living cell which speeds up a thermodynamically possible reaction.

3. **Active Sites:** That region of the protein which participates in the conversion of substrate to product.

4. **Enzyme Unit:** amount of enzyme which will catalyze the transformation of 1μ mole of substrate per minute under defined conditions.

5. **Specific Activity:** Unit of enzyme per milligram of protein.

6. **Catalytic Centre Activity:** number of molecules of substrate transformed per minute per catalytic center.

7. **Optimum Temperature:** The highest temperature at which an enzyme works best, above and below this the reaction rates are lower.

8. **Optimum pH:** The highest pH at which an enzyme functions best, above and below this pH, enzymic activities are lower

9. **A Subunit:** is the material that is converted or worked upon by the enzyme and is converted to the product.

3.2.4 Factors that affect Enzyme Activity

Enzymes, being proteins, are affected by the same factor that affect proteins. Enzymes are affected by such factors as temperature, pH, salt concentration etc.

3.2.5 Effect of Temperature

a non-catalyzed reaction can be greatly increased by raising the temperature of the environment. However, such a condition can be highly unfavourable in a living cell. In fact, enzymes being proteins, are sensitive to elevated temperatures which results in the denaturation of the enzyme protein, by decreasing the effective concentration of the enzyme and thus decreasing the reaction rate.

Enzyme activity is strongly influenced by temperature. As the temperature increase so does the reaction. The random motion of the molecules increases with temperature making collision between reactive molecules more possible. However, as temperature continues to increase, the rate of enzyme – catalyzed reactions decreases again (Fig. 10.9) because the secondary and tertiary structures of the enzyme is distrupted.

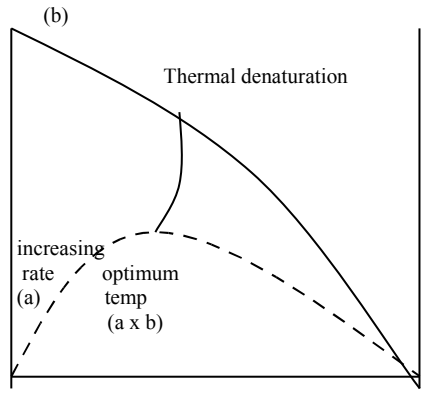


Fig. 10.9: Effect of temperature on the reaction rate of an enzyme – catalysed reaction (a) represents the increasing rate of a reaction as a function of temperature (b) represents the decreasing rate as a function of thermal denaturation of the enzyme. The dasked line curve represent the combination of (a & b) showing optimum temperature.

The reactivity of an enzyme is affected by both extremes of temperature. At low temperatures the enzyme is inactive; but there is increase in reaction rate as the temperature is raised-up to about 450C. above 45)C, thermal denaturation sets in, and at about 550C, rapid denaturation destroys the catalytic function of the enzymes protein (fig. 10.9). The increase in temperatures increases the energy of molecular vibrfation or random motion which may distrupt some of the weak bonds that maintain protein structures (i.e secondary and tertiary)

3.2.6 Effect of pH

Since enzymes are proteins, changes in hydrogen ion concentrations or pH, profoundly affect the ionic character of the amino (NH_2) and carboxylic (COOH) groups of the protein; so also will it affect the changes on the exposed "R" groups; it will thus affect the whole charges carried on the protein. The whole folding pattern will shift and this will therefore markedly affect the catalytic site and conformation of the enzyme; inorganic salts have similar effect.

Low or high pH values can cause considerable denaturation and hence inactivation of the enzyme protein (Fig. 10.10). Each enzyme protein has an optimum pH at which it best functions, above and below this results in reduced reaction rate or complete inactivation.

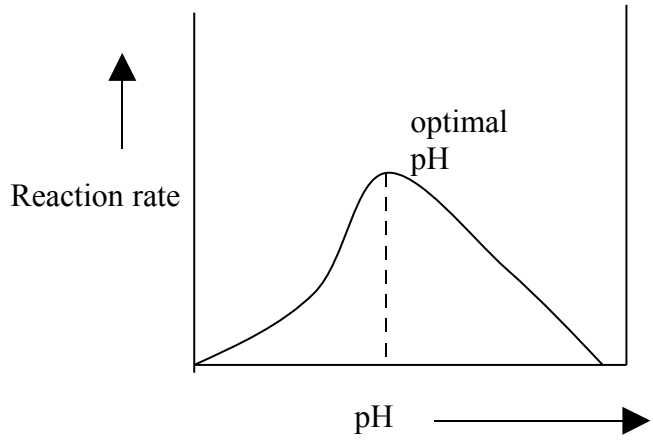


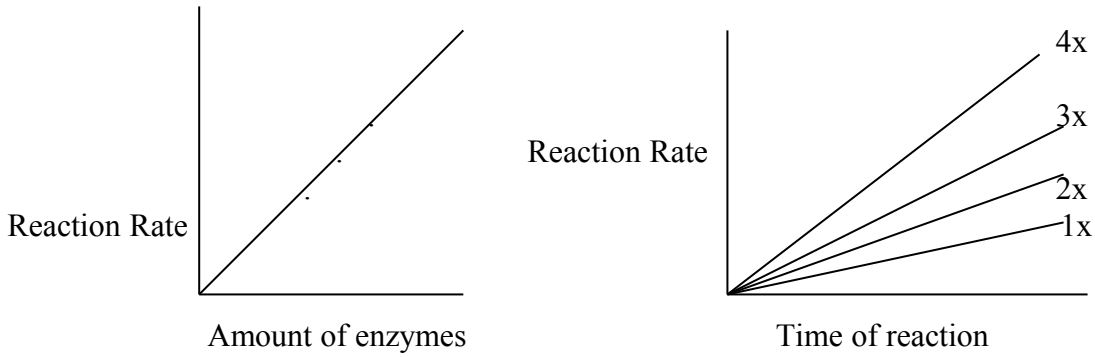
Fig. 10.10 Effect of pH on an enzyme-catalyzed reaction

Different enzymes have different pH optimum at which they work best. For example, the salivary amylase (ptyalin) works best at

neutral or slightly alkaline pH (7.4); the gastric enzymes, pepsin and rennin, work best in acidic environment aptly provided for in the stomach. The other digestive enzymes in the intestine work in alkaline medium and that is why the acid from the stomach has to be neutralized by bile salts, failure of which results in ulcers.

3.2.7 Effect of Enzyme Concentration

As is true of any catalyst, the rate of an enzyme-catalyzed reaction depends directly on the concentration of the enzyme. Fig. 10.11 depicts the relationship between the reaction rate and increasing enzyme concentration in the presence of excess substrate. As can be seen in the diagrams the reaction rate increases with increasing enzyme concentrations.



3.2.8 Specificity

One important characteristic of an enzyme is its substrate specificity. This is because of the conformation of the complex protein molecule, the uniqueness of its active site and the structural configuration of the substrate molecule which make an enzyme and its substrate fit in like lock and key. Hence and enzyme will select only specific compounds for attack.

An enzyme will also exhibit ***group specificity*** by which a general group of compounds may serve as substrates. Thus, a series of aldohexoses may be phosphorylated by a kinase and ATP. If the enzyme will only attack one single substrate e.g glucose and no other monosaccharide, it is said to have ***absolute group specificity***; if it attacks a homologous series of aldohexoses then it has a ***relative group specificity.***

An enzyme may also exhibit ***stereospecificity*** towards substrate. Thus an enzyme may have optical specificity for a D- or L- optical

isomer. For example L- amino acid oxidase attacks only the L-amino acids, whereas D-amino acid oxidases only react with the D-amino acid isomers.

$$\text{L – amino acids} \xrightarrow[\text{L – amino acid oxidase}]{O_2} \alpha\text{-keto acids} + NH_3 + H_2O_2$$

$$\text{D – amino acids} \xrightarrow[\text{D – amino acid oxidase}]{O_2} \alpha\text{-keto acids} + NH_3 + H_2O_2$$

There are, however, a small group of enzymes which catalyses an equilibrium between the L and D isomers through an intermediate complex with pyridoxal phosphate. Thus, alanine racemase catalyzes the reaction

$$\text{L – alamine} \underset{\text{racemase}}{\overset{\text{alamine}}{\rightleftharpoons}} \text{D- alamine}$$

Still other enzymes exhibit specificity towards geometric or cis-trons isomers. Fumerase will readily add water across the double bond system of the ***trans*** isomer of fumaric acid but is completely inactive towards the ***cis*** isomer, maleic acid.

3.2.9 Evolutionary Trends in Enzymes

Through many different proteins could be constructed from random arrangements of amino acids, the enzyme proteins in different organisms are surprisingly similar. This similarity results partly from evolutionary relationships. For example, man is more closely related to monkeys than to horse and this closeness is reflected in their respective protein structure; sytochrome C (a respiratory pigment) in man is very identical to the cytochrome C in monkeys except for one amino acid. However, horse cytochrome C differs from the human protein by twelve (12) amino acids. The more distant the relationship, the larger the difference between analogous proteins.

A protein must have a particular shape in order to catalyse a specific reaction. Many reactions in diverse organisms are identical. Therefore enzymes that perform the same function in different plants and animals must have analogous structures. The fact that variation in structure occurs demonstrates only that other portions of the

enzyme molecules are not needed for catalytic activity and are thus free to change.

Thus an enzyme protein molecules has specific and non-specific portions. This may be demonstrated by removing small portions of the molecule and testing the remainder for activity. The enzyme may remain functional until the active surface site is affected.

Proteins are like words spelt out with amino acid "letters", the spelling (ie. amino acid sequence) at the active site must be accurate while other letters do not carry a message and can be changed at random. The type of proteins or enzymes made in an organism or cell is established by its genetic material, the nuclei acids. Differing genes on the nuclei acid (DNA) being expressed indifferent cells contribute to differentiation of cells.

3.2.10 Action of Enzyme

Many enzymes have their catalytic activity stimulated by non-substrate molecules which are known collectively as ACTIVATORS. Examples of activators are ***cofactors*** and ***coenzymes***. Other substances retard or inhibit enzyme activity. These are known as INHIBITORS; of these there are those that compete with the substrates for the enzyme as well as those that do not compete ie. competitive and non-competitive inhibitors respectively.

3.2.11 Activators

(a) Cofactors

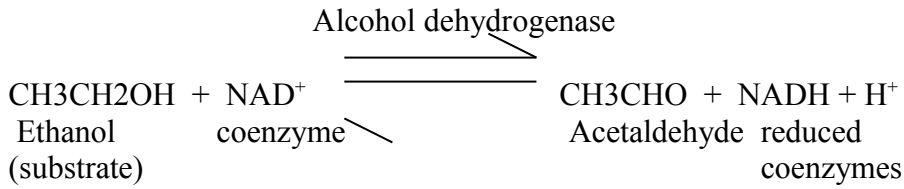
Many enzymes require small heat-stable molecules, known as cofactors, for their activity. Often such cofactors are metal ions such as magnesium and sodium ions (mg^2 and Na^+). The cofactor may from a weak association with the enzyme such that they can be removed by dialysis – (if the enzyme – cofactor complex is put in a dialysis bag (eg. cellophane sac)) which is suspended in a large volume of buffered solution of the same osmotic strength, the cofactors diffuse out of the dialysis leaving the enzyme which is not able to pass through. The enzyme will be inactive until the appropriate ion (cofactor) is added back.

A true cofactor differs from a metal ion which is tightly bound to the enzyme by a special prosthetic group, such as the hene ion of cytochrome C or heaemoglobin cofactors possible combine loosely

with the enzyme and it tertiary structure to an active configuration. Some cofactors have been found to combine with the substrate other than the enzyme.

(b) Coenzymes

A group of relatively small non-protein organic molecules which are essential for the activities of some enzymes. Coenzymes differ from the simpler (metal ions) cofactors in that they are fairly complex molecules. Some coenzymes (eg NAD^+ or NADPT, FAD^+) are involved in transferring electrons, protons or groups of atoms form one reaction to another and these actually take part in the reaction of one of the substrates.

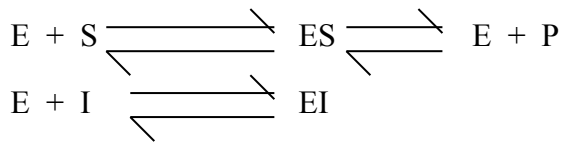


3.2.12 Inhibitors

These are substances that hinder the performance of an enzyme. Their action can be reversible or irreversible, competitive or non-competitive.

(a) Irreversible Inhibitors

An irreversible inhibitor forms a covalent bond with a specific function, usually an amino acid residue, which may, in some manner, be associated with the catalytic activity of the enzyme. The inhibitor cannot be released by dilution or dialysis. The concentration, and hence the velocity of the active enzyme is lowered in proportion to the concentration of the inhibitor and thus the effect is that of a non-competitive inhibition.

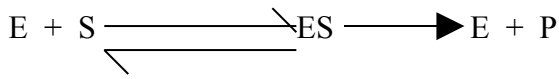


(b) Reversible Inhibition

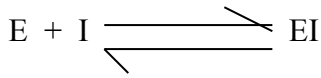
This involves equilibrium between enzyme and the inhibitor. There are three different types of reversible inhibition.

(c) Competitive Inhibition

Since an enzyme must physically combine with its substance in order to form an enzyme – substrate complex (Fig. 10.12) a molecule other than the substrate will inhibit the action of the enzyme competitively if it combines reversibly with the same site on the enzyme as the substrate. Thus, in the presence of both substrate and inhibitor, the enzyme can take part in two distinct reaction, either



or



The inhibitor may not be structurally related to the natural substrate, but combines with the enzyme at or near the active site (Fig. 10.12 & 10.13). Hence the inhibitor and substrate therefore compete for the same active site on the enzyme. The extent of inhibition will depend on the relative amount of inhibitors and substrates and also on the relative affinity of the enzyme for these two molecules. A high concentration of substrate will, however, overcome the inhibition by causing the enzyme to combine more with substrate than inhibitors.

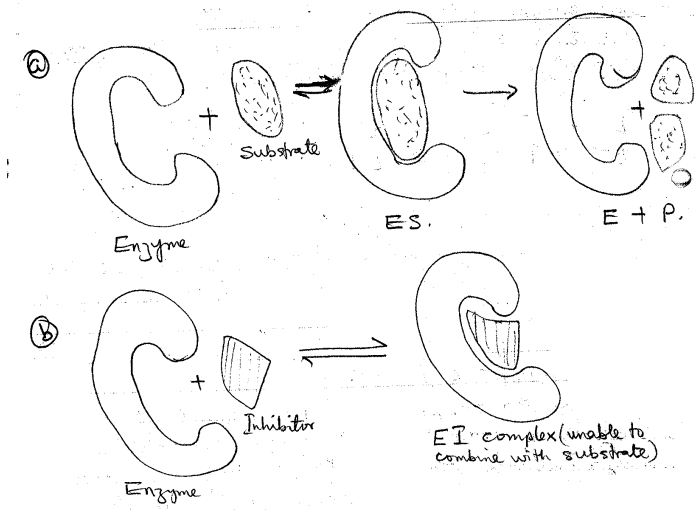


Fig. 10.12: Lock-and-key model for the binding of substrates and inhibitors by enzymes (a) Formation of enzyme – substrate complex; this may either dissociate or result in the conversion of the substrate to the products. (b) A competitive inhibitor may also bind with the enzyme and prevents it from reaching with the substrate.

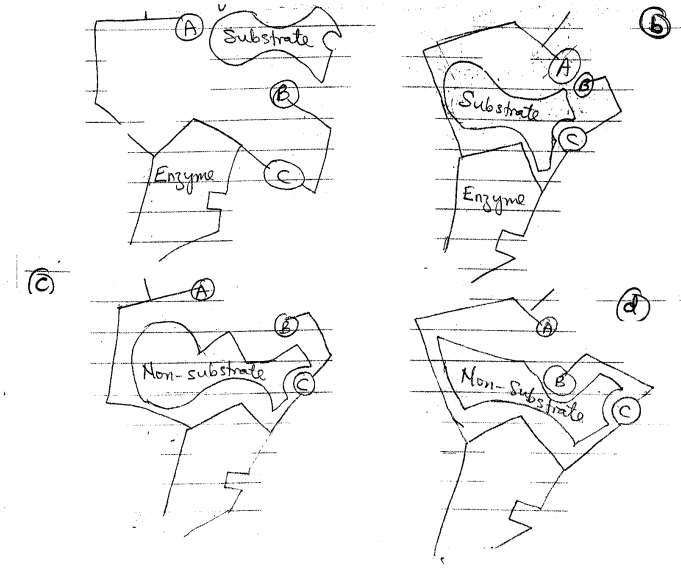
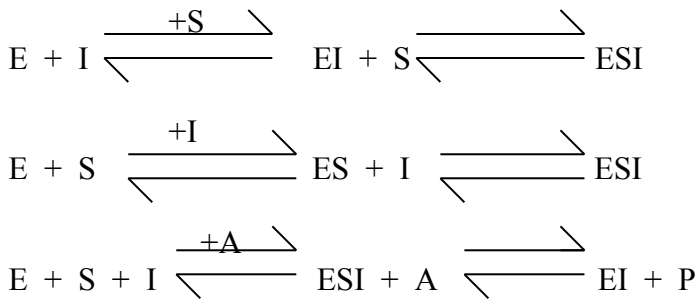


Fig. 10.13: The induced-fit model for the combination of enzyme and substrate. In (a) the catalytically important groups (A & B) of the enzyme are spread apart but are brought together by the substrate (b) this alignment allows the reaction to occur. In c and d the non-substrate molecules are capable of attaching to the binding site c, but they do not have the correct shapes to give the proper alignment of A & B and so they act as competitive inhibitors.

If the shape of the competitive inhibitor is sufficiently close to that of the substrate position at the active center (Fig. 10.12) however, since it is not identical to the substrate, the inhibitor cannot induce the correct arrangement of the catalytic groups to enable the enzyme to catalyze a reaction. A non-competitive inhibitor cannot act in this way, since it does not occupy the same position on the enzyme as does the substrate (Fig. 10.14)

3.2.13 Non-Competitive Inhibition

A non-competitive inhibitor is generally thought to combine with the enzyme in some way that may not prevent the additional binding of the substrate. Many non-competitive inhibitors act by causing conformational changes in the enzymes they inhibit in such a way that the catalytic site is distrupted; although the substrate binds, the catalytic activity of the enzyme is greatly reduced. An enzyme–substrate-inhibitor complex is formed (fig. 10.14); but this is incapable of carrying out the reaction to yield the end products. The extent of the inhibition will depend on the relative amount of enzyme and inhibitor. The effects of non-competitive inhibitors cannot be overcome by increasing the substrate concentration. However, an ***activator*** could overcome the effects of the inhibitor. Heavy metals such as Hg^{2+} or Pb^{2+} can combine irreversibly with some enzymes to cause this sort of inhibition.



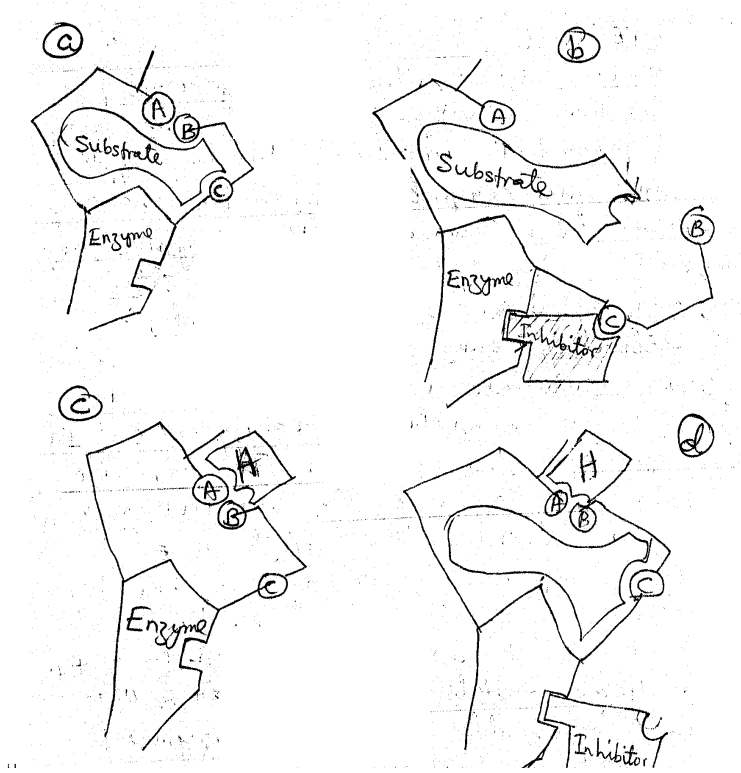


Fig. 10.14: Extension of the induced –fit model for enzyme –substrate combination to explain the effects of an inhibitor and activator (H). (a) The catalytically important groups A & B are brought close together in the correct alignment for enzyme activity. (b) When inhibitor binds group C, the enzyme cannot achieve the active conformation. The inhibitor will be non-competitive if its presence does not alter the binding of the substrate, but a competitive one if the chain containing A & B were significantly involved in substrate binding and it does not allow substrate to bind. (c) the activator (H) stabilizes the active site. (d) the activator (H) overcomes the effects of the inhibitor.

3.2.14 Relationship between Enzyme Structure and Activity

1. At the level of the primary structure, a modification of amino acid sequence in the region of the active center will distort the active site and may prevent the enzyme from combining with the substrate or from carrying out the reaction.

2. Also, any amino acid substitution which causes a charge in the reactive amino acid side chains (serine, histidine, glutamic, acid etc) at the active center will interfere with catalytic activity.

3. If the secondary (2^0) or tertiary (3^0) structure of an enzyme is altered by an inhibitor or activator, or by some changes in the conditions, this can influence the activity of the enzyme by altering the accessibility of the substrate to the active center or by changing the orientation of the catalytic groups.

Many enzymes are known to be composed of several protein subunits. Such an enzyme may have several catalytic centers, each one on a different subunit. The more closely associated the subunits are, the less the enzyme can bind the substrate. The binding of the substrate to the catalytic sites stimulates the conversion of the less active, tightly aggregated form into a more active, and substantially dissociated form. As soon as a substrate molecule has become bound to one catalytic site, the remaining sites will have a greater tendency to bind the substrate. This type of behaviour can be illustrated by the binding of oxygen to haemoglobin; although haemoglobin is not an enzyme, it is composed of four subunits which show co-operative interaction.

3.2.15 Isoenzymes

Even within a single cell, some enzymes exist in more than one molecular forms. These multiple forms of the same enzyme are called ***isoenzymes***. Thee consist of different polypeptide chains. For example, lactic dehydrogenase has four subunits, each of which may be either of two polypeptide chains with different amino acid sequences. Five lactic dehydrogenase isoenzymes have been separated by starch gel electrophoresis. All these isoenzymes have the same molecular weights and catalyze the same reaction, although they have different Km values for lactic acid.

3.2.16 Classification of Enzymes

Enzymes are normally classified into six major groups depending on the type of reactions they are involved in.

1. **Oxidoreductases:** are involved in biological oxidation and reduction reactions – and so are closely related to respiratory processes in the cell i.e. respiration and fermentation. This class include (a) ***dehydrogenases*** which bring about oxidation in conjunction with coenzymes such as NAD^+ and $NADP^+$ which act as hydrogen acceptors. (b) ***peroxidases*** which are hydrogen peroxide (H2O2) as oxidant (c) ***hydroxylases*** which introduce hydroxyl groups (d) ***oxygenases*** which introduce molecular oxygen in place of double bonds in the substrate.

2. **Transferases:** catalyze the transfer of one carbon group (methyl, formyl, carboxyl, aldehydyl or ketonic groups, alkyl groups, nitrogenous, phosphate and sulfide containing groups from substrate to acceptor molecules.

3. **Hydrolases:** include esterases, phosphatases, glycosidases, peptidases etc which hydrolyze esters, phosphatase, glycosidic and peptide bonds by the introduction of water.

4. **Lyases:** remove groups from their substrates (not by hydrolysis) leaving double bonds. This class also include ***decarboxylases*** (that release CO^2 from various substrates) ***aldolases*** (that catalyze aldel consensations or the reverse and have an important role in carbohydrate metabolism including the breakdown of hexoses into 3-carbon units)

5. **Isomerases:** include racemoces, epimerases, cis-trans isomerases, intramolecular oxidoredectases and intra molecular transferaces which catalyse the interconversion of stereoisoers of amino acids and sugars respectively.

6 **Ligases:** catalyze the joining together of two molecules couples with the breakdown of a pyrophosphate bond in ATP or a similar triphosphate also known as synthetases, these enzymes are involved in such important reactions as the linkage of amino acids to transfer RNA (tRNA) in the first state of protein synthesis.

Table 10.1: major classes of enzymes and their actions

S/No	Enzyme Class	Type of Protein Catalyzed
1	Oxidoreductases	***Removal of addition or electrons with the associated loss or gain of protons.***
2.	Transferases	Transfer of a group from one molecule to another.
3.	Hydrolases	Splitting of a bond by the addition of water.
4.	Lyases	Removal of a group to leave a double bond, or the addition of a group to a double bond.

5.	Isomerases	Conversion of a molecule into a different structural isomer through a re-arrangement.
6.	Ligases	Synthesis of a new molecule from two precurors by a process coupled to a reaction with a large negative free energy charge.

4.0 Conclusion

The synthesis of proteins concludes the message sent from the nucleus (DNA) which were read into RNA and interpreted into proteins. There are many genes present on each chromosome but some are closed and cannot be read, while others are opened and are read into proteins. The closing and opening of particular genes on chromosome determine the level of ***differentiation*** a cell has undergone. Essentially, differentiation means that different cells produce different proteins.

The proteins thus produced can be classified as either fibrous or globular, simple or conjugated. Proteins may also be structural or functional. Structural proteins from part of the cell structure ie. Membrane while functional ones serve as enzymes, catalyzing biochemical reactions.

Enzymes act to reduce the energy necessary to get a reaction started and going. The workings of an enzyme may be affected by its own concentration, concentration of the substrate, temperature and pH. Many enzymes require small heat-stable molecules known as cofactors for their activity. Also a group of relatively small non-protein organic molecules called co-enzymes for their activities. Enzymes can be inhibited by inhibitors which may be competitive or non-competitive and their inhibition may be reversible or non-reversible.

Enzymes are specific in action. They can be classified as oxidoreductases, transferases, hydrolases, lyased, isomerases or ligases.

5.0 Summary

Enzymes are essential in the functioning of a cell. The type of enzymes produced in a cell determines the type of biochemical reaction the cell in involved in. Some cells produce proteins for their internal us while others produce them for use outside the cell.

In this unit we have now seen how the nucleus controls the activities that go on in a cell. In the ensuing units we shall further see how the chromosomes within the nucleus determine the genetic characteristics of cells and organisms. This is the basis of Genetics and the genetic make-up of a cell determines the types of proteins synthesized in it which also determines the way the organism looks – phenotype ie yellow, tall wrinkled etc.

Self Assessment Questions

1. Enumerate the physical factors that affect the functioning of an enzyme.

2. Describe the following structures of a protein (a) Primary (b) secondary (c) tertiary (d) quartenary

3. In what ways do you classify proteins?

4. Discuss evolutionary trends in proteins.

5. What are (a) co-enzymes (b) cofactors?

6.0 Tutor-marked Assignment

1. Describe with the aid of diagrams how a competitive enzymes works.

2. List classes of enzymes and what they do; give examples.

7.0 References

1. Roberts, M.B.V. 1975. Biology: A Functional Approach, E./L.B.S. and Nelson. Lagos

2. Wolfe, S.L. 1972. Biology of the Cell. Wedsworth Publishing Co. Inc., Belmont, California.

Answers to Self Assessment Questions

1. Ref. section 3.22, 3.25, 3.26, 3.2.7

2. Ref section 3.1

3. Ref section 3.1.2

4. Section 3.2.9

5. Section 32.11

MODULE 3

UNIT 11: THEORY OF THE GERMPLASM: CELL DIVISION

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6.0 Tutor Marked Assignment

7.0 Reference

1.0 Introduction

This story was postulated by Weismann in 1895. Weismann divided the organisms into two types of tissues, the SOMATOPLASM and the GERMPLASM. The sometoplasm or somatic tissues are responsible for the vegetative aspects of the life of the organism. The germplasm on the other hand is tissue set aside for gamete production only. Since sexual reproduction involves the effusion of two gametes to form the zygote which in turn develops into the offspring, it is only materials of the germplasm, which is transmitted to the next generation.

Prior to the formulation of Weismann's theory, evidence had been produced to show that Chromosomes re constant in number in any given species; that each parent contributes the same number of chromosomes and finally that it is the nucleus and not the cytoplasm, which is important in determining the type of offspring. Weismann as part of his theory said that there must be a reduction in half of the number of hereditary units during the formation of gametes. Since fertilization involves the fusion of two gametes, one from each parent, non-reduction of the number of hereditary units would result in a doubling in each generation. Although Weismann did not categorically say so, his theory did imply that the hereditary factors are contained in the chromosomes. Accumulated evidence has since shown that the genes are on the chromosomes. Therefore, if there were no reduction in the number of chromosomes, there would be a doubling of the number. However, the evidence, as mentioned above is that gametes contain only half the number of chromosomes found in non-gametic cells of the same organism. The reduction is accomplished by the cell division process of meiosis. Meiosis is therefore restricted to the germplasm. The other cell division process, mitosis, occurs in both the somatoplasm and the germplasm.

2.0 Objectives

At the end of this unit, you should be able:

1) To describe what is involved in each of the two cell division processes.

2) To discuss the consequences of the various steps leading to cell division.

3) To appreciate and discuss:

   a) the identical aspects of these processes in both plants and animals; and

   b) the fact that the above account for why some laws of inheritance apply to both plans and animals.

4) Know why the cell undergoes these elaborate divisions; you should be able to discuss the significance of each type of division

3.1 Introduction to Cell Division

In this unit, we shall consider two types of cell division – *MITOSIS AND MEIOSIS.* Both types of cell division occur in eukaryotic acellular (unicellular) and multicellular organisms.

Multicellular organisms, as mentioned earlier, possess two broad classes of tissues – somatic and germ tissues. In sexual reproduction, the offspring is produced as a result of the fusion of two cells called GAMETES. In animals, they would be the sperm from the male and egg from the female. In plants the pollen contains the male gamete while the ovule contains the female equivalent. The germ tissues consist of cells, which divide to give rise to gametes. Of the two types of cell division, meiosis occurs only in the germ tissues leading to the production of gametes.

In a cell the nucleus contains all the information for the activities of the cell, and, in the final analysis, information regarding the whole organism. Mitosis and meiosis are the cell division processes by which the nuclear information of a cell is apportioned to the daughter cells.

3.2 Mitosis (See Figures 11.1 & 11. 2)

We shall be looking at the mitotic process as being divided into a number of stages for convenience of description. It is however, important to note that although there are distinguishing features for each stage; mitosis is a continuous process with one stage blending into another. The five stages of mitosis are Interphase, Prophase, Metaphase, anaphase and Telophase.

3.2.1 Interphase

This stage is sometimes described as the "resting stage". Such a characterization, however, only refers to the fact that the cell is not dividing. There would be normal metabolic processes going on in the cell. Also, in the cell preparing to divide the duplication of the nuclear appears simply as a jumble of thread-like fibres called CHROMATIC fibres. It is impossible to trace any particular fibre within the mass. The interphase nucleus also contains a nucleolus of nucleoli. Duplication of nuclear material is usually completed in the interphase stage but it may extend into the next phase.

All cells, which are going to divide, are involved in the necessary preparations including growth of the cell. Because there is so much activity in the cell we shall avoid the term "resting stage". The preparations for mitosis carried out during interphase include the replication involved in the formation of the mitotic spindle. No chromosomes are visible in the interphase nucleus. Instead, the nucleus merely appears as a jumble of find threads called chromatin fibres (Figure 11A)

3.2.2 Prophase

All syntheses, which had not been completed in interphase are completed during this stage. The chromatic fibres characteristic of the previous stage gradually assume another form. They progressively become shorter as a result of the phenomenon known as condensation – coiling of the chromatia fibres. In their condensed form they are rod-like structures which take up stain and are therefore called chromosomes (or coloured bodies).

Each chromosome is a duplex structure made up of two strands called sister chromatids. The sister chromatics which are identical copies are held together at a point along their length by a single structure called the centromere or kintochore.

The location of the centromere is characteristic of a given chromosome type. Condensation proceeds in such a way that each chromatid is coiled and then the sister chromatids are coiled in relation to each – when they are intertwined the coiling is said to be plectonemic and when they are called parallel to each other, being therefore easily separable, the coiling is ***paranemic.***

As the chromosomes become visible the nucleolus gradually disappears. The mitotic spindle i.e. the structure on which the division of the chromosomes will occur is also assembled. In animal cells, the centrosome divides giving rise to two structures called ***asters.*** As the asters move apart

the mitotic spindle appears between them (Figure I1.b). Each aster has fibres radiating from it, in addition to the fibres connecting the two asters.

At the end of prophase condensation is completed i.e. the chromosomes have attained their shortest length. Also formation of the mitotic spindle is completed while the nucleolus disappears completely and the nuclear membrane breaks down. No more synthesis occurs.

3.2.3 Metaphase

With the breakdown of the nuclear membrane, the chromosomes move on to the mitotic spindle. They arrange themselves in a single plane at the equator of the spindle. In other words if one looked at the equator form one of the poles of the spindle, all the chromosomes would be seen, none lying directly on top of the other. The centromere of each chromosome is connected to the asters constitute the poles. One can still talk of poles in plant cells even though there are no asters. The poles are the ends of the spindle.

3.2.4 Anaphase

Recall that in prophase each chromosome appeared as a double stranded unit made up of two sister chromatids held together by a single centromere. The prophase and metaphase chromosomes can therefore be each defined as a structure made up of two sister chromatids and a centromere. In the light of what happens during anaphase it may be useful to qualify the centromere by describing it as a "single functional centromere". Since all syntheses occurred in interphase, resulting in a duplication of the chromosome, it is logical to assume that the centromere was also duplicated, but continues to behave and appears as a single unit.

At the beginning of anaphase, the centromere splits and the sister chromatids now separate. An important aspect of the definition of a chromosome is the number of centromeres rather than the number of strands. Besides a chromatid is essentially half of a chromosome. The implication of all this therefore is that with the splitting of the centromere we now have single – stranded chromosomes.

There is evidence to show that the centromere is a vital structure for the movement of a chromosome during anaphase, hence the alternative name Kintechore. *The Greek root "Kine-" signifies motion). As anaphase progresses the daughter chromosomes move to opposite poles with the centromere leading and the arms of the chromosome trailing. As a result of this the configuration assumed by a chromosome during anaphase may be an inverted V-shape or J-shape or an I-shape (Figure 11.1D). This shape is

determined by the location of the centromere along the length of the chromosome. If the centromere is located in the middle, that is, the arms of the chromosomes are equal in length, the chromosome is described as ***metacentric,*** if the centromere is located such that one arm is longer than the other, the chromosome assumes a J-shape during anaphase. Such a chromosome is ***sub-metacentric.*** The two other types of chromosomes are often classified together, ***acrocentric,*** and ***telocentric.*** These chromosomes assume an I-shape.

The forces responsible for the movement of the chromosome are complex and are best discussed in a course on cytology.

3.2.5 Telophase

In this stage, the two identical sets of chromosomes arrive at the opposite poles (Figure I1.1E). (Recall that each chromosome) was duplicated during prophase and what were once sister chromatids, but now daughter chromosomes, move apart in anaphase). The chromosomes are clustered at the pole and a nuclear membrane begins to form around each cluster of chromosomes. As a result of the tight clustering individual chromosomes are not recognizable. As formation of the nuclear membrane progresses, decondensation i.e. uncoiling of the chromosomes occurs. The nucleolus is also reformed. Notice that the decondensation restores the appearance of chromatin fibres found in interphase. With the formation of the nuclear membrane, division of the nucleus – called Karyokinesis – is completed and two daughter nuclei are retained in one cell. In others, however, the cytoplasm is divided to give two daughter cells. This division is described as ***Cytokinesis***. It occurs simultaneously with the re-formation of the nuclei and therefore it is one of the events during telophase.

In animal cells a furrow, i.e. an in-pushing of the cell membrane, occurs around the cell at a level corresponding to the equator of the spindle, usually also the middle of the cell. As telophase progresses, the furrow deepen, constricting the cytoplasm, and eventually separating it into two daughter cells. In plant cells, a cell plate – an aggregation of material – is formed in the equatorial region in the spindle. The cell plate then grows outwards to the periphery of the cell, eventually separating the cell into two daughter cells. The cell plate becomes the middle lamella normally found between two plant cells.

3.2.6 Significance of Mitosis

In chemical terms, the nucleus contains DNA, and the duplication mentioned earlier also refers to the DNA. The evidence is that the DNA is the

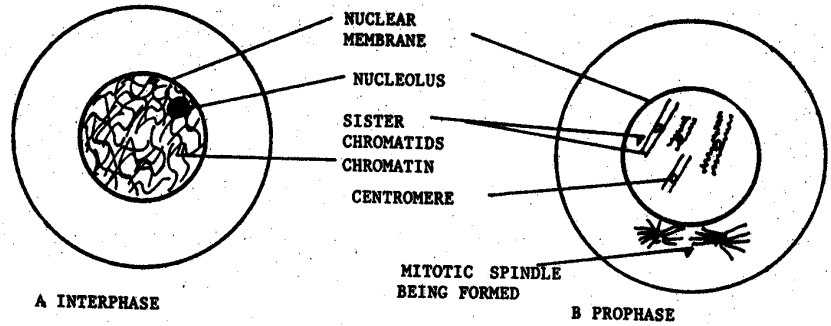
chromosomes. If the amount of DNA is the non-dividing cell is designed as X that at the end of interphase or the cell in prophase, the cell would have ZX units of DNA. By the same token the daughter calls at the end of the mitosis would be X units.

In terms of chromosomes mitosis ensures that the daughter cells have identical numbers as well as types of chromosomes. There are also identical numbers as well as types of chromosomes. They are also identical in the same ways with the mother cell.

It seems obvious therefore, that the elaborate process of mitosis is designed to ensure numerical and chemical identity between the mother cell and the daughter cells. Mere constriction of the interphase nucleus would not have ensured the desired goal. There would have been a random distribution of the chromosomes and therefore their chemical content.

The process of mitosis therefore underscores the point that the nucleus is the repository of all information for the normal functioning of the cell. Therefore by ensuring that the daughter cells, the mother cells and indeed the original zygotic cell (the product of fertilization) are quantitatively and qualitatively identical, mitosis virtually guarantees normal development and functioning of the organism. Notice that the outcome of mitosis automatically means that in vegetative (asexual) reproduction the offspring are identical with each other and with their parent.

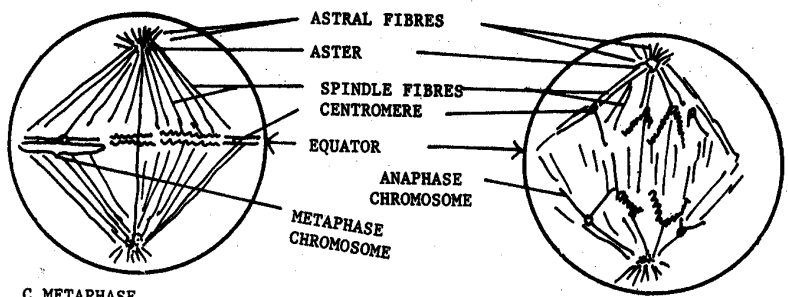
FIGURE 1.1: MITOSIS IN AN ANIMAL CELL



A INTERPHASE

B PROPHASE

Each chromosome at this stage is made up of two sister chromatids and a centromere. Straight chromosomes from one parent and "zig-zag" from the other parent.



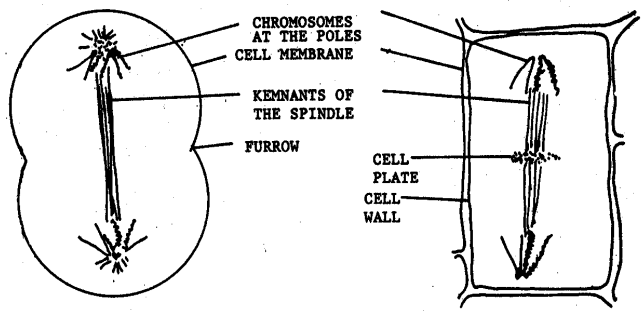
C METAPHASE

All the chromosomes are on a single plane at the equator of the cell.

D ANAPHASE

The centromere leads the arms of the chromosome toward the pole. The anaphase chromosome is single-stranded.

FIGURE 1: (cont'd)



E. TELOPHASE
The chromosomes become more tightly packed later, and a nuclear membrane is formed around each group of chromosomes.

F. Telophase in a plant cell.

NOTE: Homologous chromosomes from each parent cannot be distinguished under the microscope. They have been represented with straight and zig-zag lines merely to emphasize:

(i) that there is equal contribution from each parent.

(ii) that each daughter cell receives an exact copy of each chromosome.

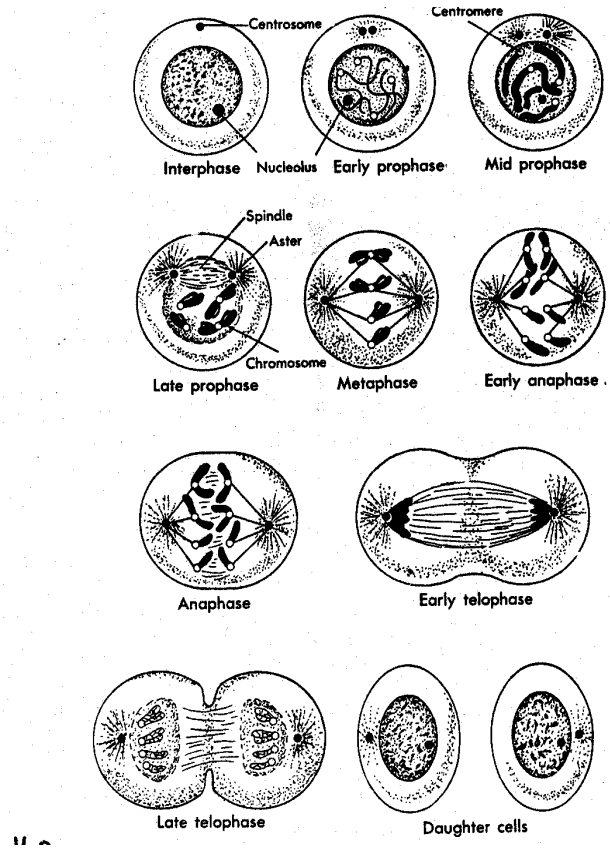


Fig. 11.2: Diagram of mitosis in an animal cell with 4 dimensions

3.2.7 Summary of Mitosis

Mitosis is a cell division process, which occurs primarily in the somatic tissues (somatoplasm). Wherever it occurs the process is the same. At the onset there is duplication of the cellular material, most importantly of the nuclear material. The nuclear content then assumes the form of short rod-like structure called chromosomes. Although behaving as single units, the chromosomes are double in structure. On the mitotic spindle, the centromere, holding together the two strands of the chromosome, the sister chromatids, splits giving rise to two identical daughters' chromosomes which move to opposite sides. Two daughter nuclei in tow daughter cells are the end products. They are identical with each other and with the mother cell with respect to their nuclear content.

3.3 Meiosis

3.3.1 Introduction to Meiosis

In many organism examination of the chromosomes shows that there are two of each type of chromosomes present in the vast majority of cells. Put differently each cell has two sets chromosomes. Such a cell or an organism is described as ***diploid.*** The two members of each chromosome type as described as ***homologues*** chromosomes.

In contrast to diploid cells, there are in the diploid organism cells, which have only one set of chromosomes. These cells are described as ***haploid*** or monoploid. They are the ***gametes.*** The diploid number of chromosomes is designated as 2n while the haploid number is represented as n, reflecting the fact the former has two of n different types of chromosomes while the latter has only one set of n different types of chromosomes.

Even though n is constant for the species of organisms, it may vary from species to species.

Meiosis results in the reduction in half of the chromosome number in a cell form 2n to n (two sets to one set) in the daughter cells. It eventually leads to the formation of gametes which are also haploid and will at fertilization restore the diploid number of chromosomes. It is not surprising therefore that meiosis in diploid organisms is restricted to the germplasm i.e. the testes and ovaries in animals and the anthers and ovaries in plants.

A convenient term for a cell, which is going to undergo meiosis is meiocyte. The process of meiosis consists of two successive cell divisions, designated meiosis – I and meiosis – II. The meiocyte, which undergoes meiosis therefore, produces for haploid cells. The phases of meiosis are essentially the same as in mitosis and are identified by the suffix I or II.

Note: If the suffix is left out it means that the stage referred to is a mitotic one.

3.3.2 Meiosis - I

3.3.2.1 Interphase I

This stage is no different from the mitotic interphase. There is duplication of chromosomal material. Therefore at the end of interphase-I the nucleus of the meiocyte contains twice as much DNA as a diploid non-dividing cell. Only chromatin fibres and the nucleolus are visible in the nucleus.

3.3.2.2 Prophase – I

This phase is similar to the mitotic prophase in some respects but it is more complex. One indication of complexity is the fact that there are five sub-stages namely: Leptotene, Zygotene, Pachytene, Diplotene and Diakinesis. (There may be another suffix used for the first four names, in some books, but we shall use those stated in this text). As pointed out during our consideration of mitosis, the various stages and sub-stages are merely important sign-posts for consideration of the cell division process. The whole process is continuous and cannot in reality be put into neat little packets.

3.3.2.2a Leptotene

The chromosomes become visible in the nucleus but at this time they appear as long as slander threads. Because of the length of these threads, it is usually not possible to trace any chromosome from tip to tip within the entwined lot. Remember that even though the chromosomes in this stage are very thin, they are visible because of some degree of condensation of the chromatin fibres.

One striking feature of leptotene is the fact that under the right microscope the "threads" appear to be single. The implication of this compared to what we saw in mitosis, is that duplication of the chromosomes has not occurred. The chemical evidence, however, indicates that there is twice as much DNA as in a non-dividing diploid cell. In spite of this evidence, by convention, it is accepted that there are not chromatids at this stage. Because the common tool used for investigation, the light microscope, does not show double-stranded chromosomes.

3.3.2.2b Zygotene

At this stage, the chromosomes still appear as single threads, but now, homologous chromosomes attract each other and undergo pairing which proceeds from one end similar to the way in which a zipper is pulled shut (figure 11.3C). This pairing process is called synapsis. Synapsis is very specific in that only homologous i.e. corresponding regions of the chromosomes pair. Note that because each parent contributes one set of chromosomes to the offspring each synapsing homologous pair is made of one paternal a done maternal chromosome.

3..3.2.2c Pachytene

At this stage, the condensation of the pronounced, making the chromosome appear thicker. Each chromosome is now visibly made up of two chromatids. Therefore each synapse unit of a pair of chromosome is made up of four chromatids. This unit of four chromatids is called ***tetrad,*** indicating the four chromatids or a ***bivalent,*** (Figure 11.3d) referring to the pair of homologous chromosomes. As in the mitotic prophase, the centromeres still appear and behave as single structures. Parts are also most invariably exchanged in a reciprocal manner between synapsed homologues. We shall consider this phenomenon known as ***crossing-over*** in more detail later.

3.3.2.2d Diplotene

This stage is characterized by repulsion between homologues. The once closely paired and entwined homologues pull apart from each other except at a few points where they are still attached. These points of continued attachment are called chiasmata (singular: chiasma) (Figure 11.3e). The centromeres do not participate in chiasmata formation. The chiasmata are though to represent the breakage points at which the reciprocal exchange in crossing-over occurred. Only two non-sister chromatids are involved in any chiasma (Figure 11.3e). A chiasma can occur at any point along the length of the chromosome (with the exception mentioned above). The number and position of chiasmata are variable. Condensation continues.

3.3.2.2e Diakinesis

This is the last sub-stage of prophase I. Condensation reaches its peak at this stage making the tetrads appear as short, thick and heavily-straining bodies. As a result of the condensation, the chiasmata appear to move towards the tips of the tetrads. The chiasmata which were originally close to the tip of the tetrad may "slip" off at this stage. This phenomenon of apparent movement of the chiasmata towards the tips of the paired chromosomes is called ***terminalization.*** The tetrads gradually move to the periphery of the nucleus. The nucleolus disappears and the nuclear membrane breaks down.

3.3.2.3 Metaphase – I

Following the disintegration of the nuclear membrane, the tetrads move on to the equatorial plate of the division spindle which was formed during prophase – I. They are attached by their centromeres, which still appear and behave as single structures. The tetrads are oriented on the spindle such that each centromere is biased towards one of the two poles of the spindle (Figure 11.3a). In other words, if the spindle is oriented vertically, one chromosome

in the tetrad is on top of the other. As a result, the chromosomes are in effect arranged in to planes (cf. the single plane in mitosis).

Recall that every diploid individual ha one set of paternal and maternal chromosomes. Hence the same is true of the composition of the tetrad /bivalent. If we distinguished a north pole and a south pole for the spindle, the orientation of the members of the bivalent with respect to the poles is random.. In other words, on the average, in half of the cells undergoing meiosis, the paternal chromosome (centomere) will be biased toward the north and it will be reserved in the other half. More importantly, however, is the fact that the orientation of the one bivalent is independent of another bivalent's. If bivalent-I is oriented with the paternal chromosome biased toward the north pole and maintained that way, the orientation of bivalent-II is such that in appropriately fifty per cent of the dividing cells the paternal chromosome would be biased toward the north pole as for I; in the other fifty per cent the paternal chromosome of bivalent-II will be biased toward the opposite pole i.e. the south pole. In other words, when there are two pairs of chromosomes there are two possible metaphase-I orientations of the bivalents. When there are three pairs of chromosomes, four different metaphase orientations are possible. (Try to draw them after looking at one metaphase-I in Figure 11.3g). A formula for deriving the total number of possible metaphase orientations is 2^{n-1} where n is the number of bivalents i.e. the haploid number. Thus if n = 4 the number of possible orientations is 2^{4-1} = 23 = 8.

Note: For n = 1 the number of possible orientations is $2^{1-1} = 2^0 = 1$. This is because there is no point of reference i.e. north or south pole in he cell. A point of preference is obtained when one bivalent is kept constant. The use of north and south pole was for ease of description.

3.3.2.4 Anaphase – I

In anaphase-I there is no division of the centromeres (c.f. mitotic anaphase). The homologous chromosomes of the bivalent separate and begin to move to opposite poles. The centromere leads towards the pole dragging along the two sisters' chromatids attached to it. There is also repulsion between the sister chromatids. Each unit of one centromere and two sister chromatids is called a ***dyad.*** We have said that each tetrad is made up of homologous chromosomes from maternal and paternal sources. Therefore, on this basis alone, we find that for each metaphase-I orientation there are two different groups of chromosomes, and so two types of cells will be produced. The formula for metaphase-I orientations is 2^{n-1} . The corresponding formula for anaphase-I will therefore, by 2 x 2^{n-1}, which is the same as 2^n. Thus a cell with two pairs of chromosomes in anaphase-I and ultimately, four different

types of cells. It is necessary to underscore the point that we are considering maternal and paternal homologous chromosomes as different merely because of their origin. This is an assumption which simplifies the discussion.

3.3.2.5 Telophase - I

Whether this phase occurs or not varies form one species to another. In some organisms the chromosomes pass directly into prophase-II and in some others, they pass directly into metaphase-II and in some others, they pass directly into metaphase-II. Whatever the case, the arrival of the dyads at the poles marks the end of anaphase-I. We shall for purposes of simplicity take the rather, a typical case in telophase-I: on arrival at the poles, the chromosomes undergo some measure of decondensation while nuclear membranes are formed around the two groups of chromosomes. The nucleolus is re-formed and cytolcinesis occurs.

The consequence of such a meiosis-I division is production of to daughter cells each of which has a reduced number of chromosomes, although each chromosome is made up of two sister chromatids. This structure of the chromosome (a dyad) is reflected in the amount of DNA present in each of the two cells. Recall that there is a doubling of the normal amount of DNA as a result of synthesis during interphase-I such that if the normal amount were 2X, there would be 4X. This is reflected in the resulting chromosomes which are two stranded. The separation of synapsed homologous chromosomes into two daughter nuclei results in nuclei which have 2X amount of DNA. This is the same amount of DNA that is found in the non-dividing diploid cell.

The double-stranded chromosomes (dyads) and the amount of DNA present in this products of meiosis-I constitute the rationale for meiosis-II. In other words, in spite of the reduction of the number of chromosomes (centromeres), not all necessary aspects have been reduced in order that fertilization might restore the normal situation with respect to chromosome number and amount of DNA.

3.3.3 Meiosis – II

3.3.3.1 <u>Interphase-II</u>

As mentioned above, this phase may or may not occur. If it occurs, t is often of short duration and there is only partial condensation. More important, however, is the fact that there is not more DNA synthesis. The chromosomes

were duplicated in Interphase –I. This phase is sometimes described as ***interkinesis,*** which is a "resting stage" between divisions.

3.3.3.2 Prophase-II

This phase, if it occurs, is also short. The repulsion between sister chromatide first evident in anaphase-I persists. The spindle for the second meiotic division is often oriented at right angles to that of meiosis – I. This is most readily visible in plant cells.

3.3.3.3 Metaphase-II

The dyads arrange themselves in the equatorial plate and are held in the spindle by fibres attached to their centromeres (c.f. mitotic metaphase). Note that the same events are occurring simultaneously in the two products of meiosis-I. (see Figure 11.30I).

3.3.3.4 Anaphase-II

The centromeres divide and the daughter chromosomes (monads – single – stranded) move to opposite poles. This event, by reducing the number of strands in the chromosomes also reduces the amount of DNA (see Figure 11.3J).

3.3.3.5 Telophase – II

On arrival at the poles, the chromosomes are enclosed in a nuclear membrane with concomitant formation of the nucleolus. Cytokinesis also occurs. If thee was no cytokinesis after meiosis – I, this one would yield four cells at one. The end products in all cases are four haploid cells (Figure 11.3K).

Assuming that there was no exchange of material between homologous chromosomes (i.e. no crossing-over), two of the four cells would carry the paternal homologue of a chromosome while the other two would carry the material homologue. If crossing-over occurred between two non-sister chromatids in a tetrad, those two chromatids would be mixtures of maternal and paternal parts. The consequence would be four different types of cells but such that two contain non-crossover chromosomes while the other two would contain cross-over (recombined) chromosomes. As we shall se later, the genes are on the chromosomes; therefore, if the constitution of the paternal and maternal homologues were different, the crossover chromosomes would be different from each other and would both be different from the non-crossover homologues.

3.3.4 Significance of Meiosis

The reductional nature of meiosis ensures that the number and types of chromosomes (hence the amount of DNA and number and types of genes) in a species is kept constant from generation to generation. This is a critical condition for normal development.

As we shall see later, various genetic combinations are possible even within the guideline just mentioned. However, the combinations are not all equally viable or equally fit to endure changes in the environment. Meiosis by its nature constantly reshuffles the genetic combinations, thus providing the material for evolution as well as ensuring the survival of the species. The reshuffling is brought about by the independent orientation of different bivalents and by crossing-over.

Fig. 11.3 Meiosis

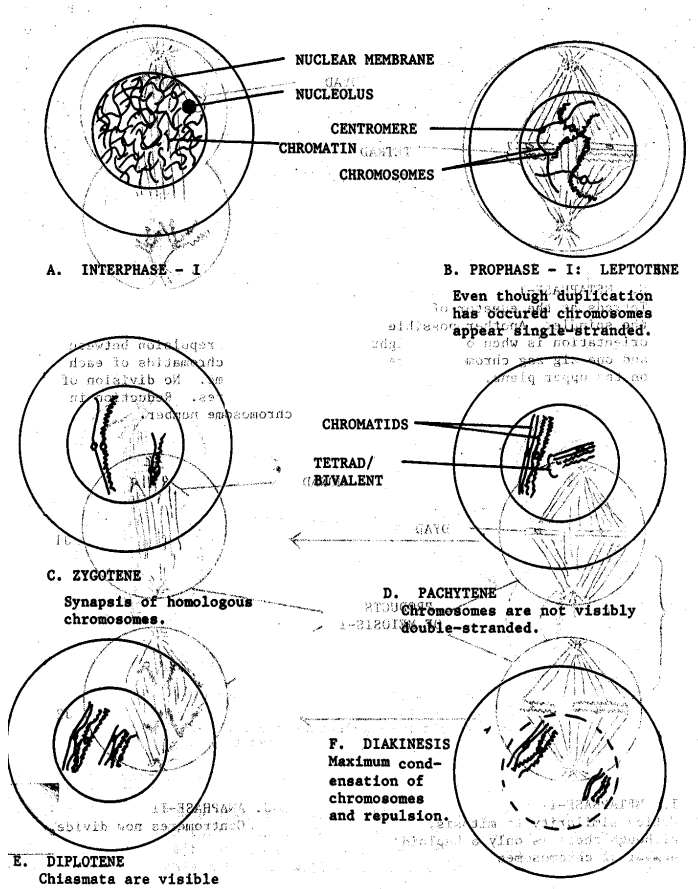
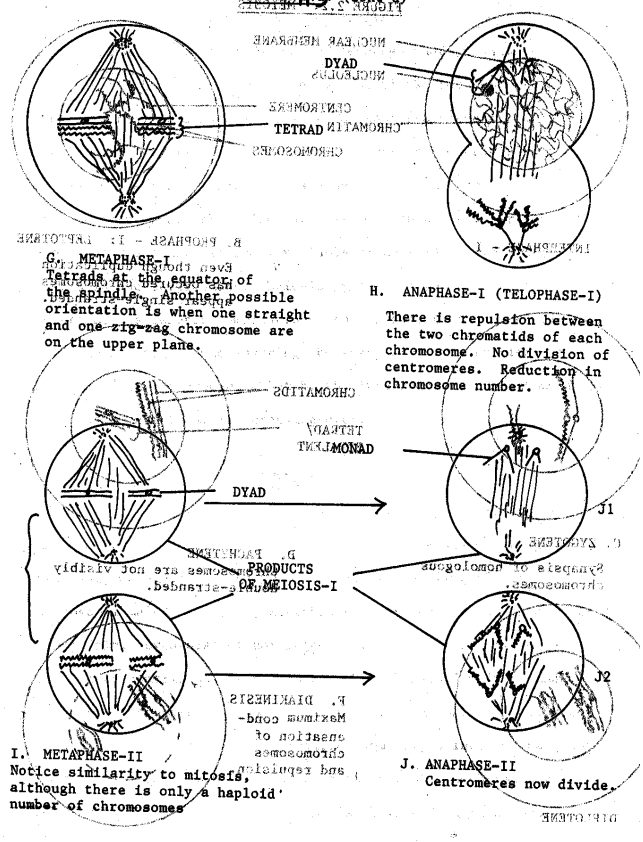


FIGURE 43 (cont'd)



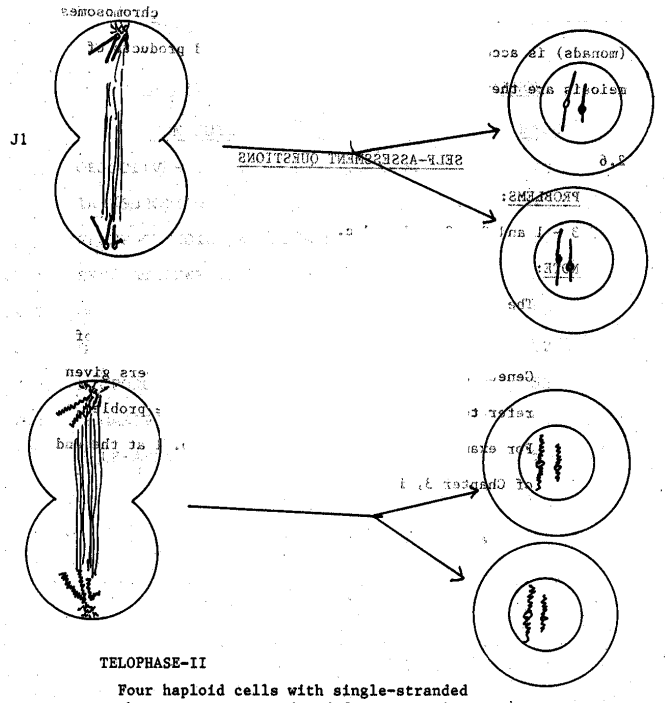
G. METAPHASE-I
Terrads at the equator of the spindle. Another possible orientation is when one straight and one zig-zag chromosome are on the upper plane.

H. ANAPHASE-I (TELOPHASE-I)
There is repulsion between the two chromatids of each chromosome. No division of centromeres. Reduction in chromosome number.

I. METAPHASE-II
Notice similarity to mitosis, although there is only a haploid number of chromosomes

J. ANAPHASE-II
Centromeres now divide.

FIGURE 1.3: (cont'd)



TELOPHASE-II

Four haploid cells with single-stranded chromosomes are produced from one meiocyte. There is a corresponding reduction in the amount of DNA.

NOTE:-

These diagrams merely illustrate the points emphasized in the Unit. One major feature of meiosis has been left out, that is, crossing-over which occurs at the chiasmata. It will be discussed under linkage.

4.0 Conclusion

Meiosis is made up of tow cell division events – meiosis – I and meiosis – II. Each division is made up of interphase, prophase, metaphase, anaphase and telephase. There is doubling of the chromosomal material during interphase-I. Prophase-I is characterized by a pairing (synapsis) of homologous chromosomes i.e. similar chromosomes contributed by each parent. Each paired unit is made up of two centromeres and two pairs of sister chromatids. Meiosis – I results in the separation of the paired homologues into two daughter cells. Although there is a reduction in half o the chromosome (two chromatids). The production of single-stranded chromosomes (monads) is accomplished in meiosis-Ii. The end products of meiosis are therefore four haploid cells.

5.0 Summary

At this point of fertilization, the genetic materials of both the sperm and the egg fuse to form the zygote. The sex cells (sperm and egg) contain half of

the number of the genetic materials (chromosomes) contained in the body of the adult parents. The zygote now divides into multiples of cells, million in the case of humans, all containing the "full load" of chromosomes of the parental body cells.

It can be seen that fertilization restores the diploid number of chromosomes into the zygote. Each sex cell thus contain half or diploid number of chromosomes. Can you imagine that would happen if somatic cells containing diploid number of chromosomes acted as the sex cells?

Students Assessment Question

1 Explain with the aid of diagrams, the following:

a) metacentric b) sub-metacentric c) acrocentric

d) telocentric

2 What can you say of the following conditions of an organism whose ovum contains 14 chromosomes?

a) haploid b) condition and number of chromosomes in the zygote

3 Explain the following terms and their conditions

a) somatic cells b) germ cells in the human body

4 In what ways is mitosis different in plant and animal cells?

5 List the stages of

a) mitosis b) meiosis

Answers

1) See Section 3.1.2.4

2) a) haploid – number of chromosomes in the gametes i.e. ovum and sperms, in this case 14 (i.e. n).

b) the zygote contains twice the number of chromosomes in the ovum i.e. 2n = 28.

3) a) somatic cells are the body cells and contain diploid number of chromosomes i.e. 2n = 46; 23 pairs.

b) the human germ cells are the spermatozoa and the ovum and each contains haploid number of chromosomes i.e. 23.

4)

	Animals	**Plants**
a)	Presence of centriole	No centriole
b)	Cytokinesis by pinching of cytoplasm	Because of rigid cell wall cytokinesis by lying down of cell, plate across daughter cells.

5

Mitosis	**Meiosis**
Interphase	Interphase I
Prophase	Prophase I
Metaphase	Leptotene
Anaphase	Zygotene
Telophase	Pachytene
	Diplotene
	Diakinesis
	Metaphase I
	Anaphase I
	Telophase I
	Interphase II
	Prophase II
	Metaphase II
	Anaphase II
	Telophase II

6.0 Tutor-Marked Assignment

1) With the aid of diagrams, detail the sequences of meiosis in an animal cell with 4 chromosomes.

2) Discuss the reasons for the elaborate processes of

a) mitosis and b) meiosis

3) List the differences between mitosis and meiosis

7.0 Reference

Williams, G. O. (2001). Biology 302 – Genetics-I, Lecture 1 – 3. Distance Learning Institute, University of Lagos, Akoka, Lagos

UNIT 12: GAMETOGENESIS IN PLANTS AND ANIMALS

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1.0 Introduction

Gametogenesis, as the name implies, is the process by which an organism produces its gametes. It was mentioned earlier in this series of lectures that gametes are normally haploid. We have also seen how the reduction of chromosomes form the diploid to the haploid number is accomplished in the process of meiosis. However, meiosis is but one step in the process of gametogenesis. We shall consider briefly the situation in animals and plants.

2.0 Objectives

The steps and consequences of meiosis are essentially the same in both sexes of an organism and in all diploid organisms. But we know that the end products of meiosis are not necessarily the gametes. On completion of this topic, you should be able to:

1) to describe how male and female gametes are formed in animals and flowering plants.

2) to highlight the similarities as well as the differences between sexes as well as organisms.

3) most importantly, you should be able to recognize the essential similarity of the chromosomal constitutions of all gametes. This fact accounts for:

a) why chromosomes number is constant from generation to generation.

b) why the same basic laws of heredity apply to all diploid organisms.

3.1 Gametogenesis in Animals

3.1.1 Spermatogenesis

In male animals the processes leading to the formation of spermatozoa are collectively called the process of spermatogenesis. The diploid cells destined to give rise to sperms are the primordial germ cells, call the SPERMATOGONIA (sing. - sper-maogonium). These cells are present in the semi-niferous tubules of the testes. Spermatogonia undergo mitotic divisions, giving rise to the meiocytes called the PRIMARY SPERMATOCYTES. Since each spermatogonium is diploid the primary spermatocytes are also diploid (Figure 12.1).

The primary spermatocytes undergo the first meiotic division, each giving rise to two haploid SECONDARY SPERMATOCYTES. Each secondary spermatocyte in turn undergoes the second meiotic division giving rise to two haploid SPERMATIDS. The spermatid then undergo a metamorphosis consisting of morphological and intra-cellular changes culminating in the formation of the sperm. This process of metamorphosis from spermatid to sperm is called SPERMIOGENESIS. In summary then one primary spermatocyte gives rise to four functional spermatozoa.

3.1.2 Oogenesis

Oogenesis is the formation of the female gamete, and it occurs in the ovary. The diploid primordial germ cell in the female is called the OOGONIUM. It undergoes mitosis to produce more oogoria. Each oogonium grows in size and matures into a PRIMARY OOCYTE. Like the primary spermatocyte, he primary oocyte undergoes meiosis-I giving rise to two haploid cells. But in this case, the two cells are not equal in size, unlike the situation in spermatogenesis. The larger of the two cells is the SECONDARY OOCYTE and the smaller one is the FIRST POLAR BODY. (Figure 12.1).

The secondary oocyte undergoes meiosis-II, giving rise to a large cell called the OOTID, and a small cell called the SECOND POLAR BODY. The first polar body may undergo meiosis-II, giving rise to two polar bodies. The production of the unequal-sized cells in meioses-I and I of the oocytes is due

to the fact that the spindle in the oocyte is displaced toward the periphery of the cell, rather than being in the centre. You will recall from our discussion of mitosis, that cytokinesis occurs at the equatorial region of the spindle. The same is true of meilosis, except that in the oocyte the equator of the spindle does not correspond to the middle of the cell. The consequence of this is the production of two unequal-sized cells.

The ootid undergoes a maturation process and becomes the OVUM. In many animals the process of oogenesis is not completed until the sperm has penetrated the egg. For instance, the secondary oocyte may remain in metaphase-II or anaphase-II until it is penetrated by a sperm. In some insects, annelids, the frog and the mouse, it is the penetration by the sperm which triggers the completion of meiosis. In others such as the sea urchin meiosis is completed before sperm penetration. Regardless of the pattern of oogenesis, however, the final act in the process of fertilization, i.e. the fusion of the male and female nuclei does not occur until meiosis-II has been completed.

In summary, oogenesis in animals results in the production of one ovum and two or three polar bodies. The polar bodies are non-functional and eventually degenerate.

3.2 Gametogenesis in Plants

Gametogenesis is somewhat different in plants. In plants there are sporophyte and gametophyte generatins. In anagiosperms (flowering plants), the sporophyte is dominant and the gametophyte is greatly reduced, and is dependent on the sporophyte. The male gametophytes, called the MICROGAMETOPHYTES are in the anthers while the female equivalent, the MEGAMETOPHYTES are in the ovary.

3.2.1 Microsporogenesis

This is the process by which the male gametes of plants, the MICROSPORES, are produced.
The anther contains the pollen mother cells, MICROSPOROCYTES, which are diploid. Each microsporocyte undergoes meiosis to produce four microspores, which are haploid. The microspores mark the beginning of the gametophyte generation.

Within each spore the nucheus undergoes a mitotic division without cytokinesis. The spore thus becomes a cell with two haploid nuclei, one of which is the TUBE NUCLEUS and the other the GENERATIVE NUCLEUS.

This cell with two nuclei develops into the pollen grain which is the MICROGAMETOPHYTE.

On the stigma the pollen grain germinates, producing the pollen tube with two haploid nuclei. Within the tube the generative nucleus undergoes a mitotic division, giving rise to two functional SPERM NUCLIE. Although three are two sperm nuclei both as we shall see later participate in only one fertilization event. Therefore, microsporogenesis is similar to spermatogenesis in that each pollen mother cell (meiocyte) give rise to four microspores, each of which in turn develops into a microgametophyte (pollen grain) which is the gamete. (Figure 12.2A)

3.2.2 Megasporogenesis

Formation of the female gamete varies among different plants. The ovary may contain one or more ovules. Each ovule however, contains a single diploid mega megaspore mother cell, the MEGASPOROCYTE.

In maize, the megasporocyte undergoes meiosis and gives rise to four haploid MEGASPORES. Three of these cells then degenerate. The fourth megaspor then develops and enlarges to become the megagametophyte, also called the EMBRAYO SAC. (Note the similarity with the situation in animals)..

The nucleus (which is haploid) of the meganmetophyte then undergoes three successive mitotic divisions without cytokinesis, producing eight haploid nuclei in the embryo sac (Figure 12.2). The middle one of the three mnuclie becomes the EGG NUCLEUS and the other two are the SYNERGIDS. Each of these nuclei is surrounded by cell walls. Two nuclei remain in the middle of the mbryo sac and are called the POLAR Nuclei. The remaining three nuclei migrate to the chalazai end of the embryo sac (Figure 12.2C). These three are each surrounded by cell wall and are described as the ANTIPODALS. The synergids and the antipodals eventually degenerate. The antipodals form nutritive tissue in maize.

At fertilization, one of the sperm nuclei of the pollen grain fuses with the egg nucleus. The product of this fusion will develop into the diploid sporophyte generation. The other sperm nucleus fuses with the two polar nuclei, giving rise to a triploid (3n) nucleus, which will develop into the endosperm.

The situation in ***Lilium*** is slightly different, as shown in the summary below. However, in spite of the differences, the basis processes underlying the transfer of chromosomes (and genes) from one generation to the next via the gametes are essentially the same in plants and animals. In both groups, the

four cells produced from a meiocyte in the male develop into functional gametes. On the other hand, in females only one becomes the egg, even though in plants as many as eight nuclei may be present in the embryo sac. Most importantly, the gametic nuclei are haploid, thus giving rise to a diploid zygote. The endosperm is a nutritive tissue which is not a part of the next generation. This fact is reflected in the chromosomal constituion of the endosperm; in maize it is triploid (3n) but in ***Lilium*** it is pentaploid (Sn).

It is the isimilarity of the process of gametogenesis in the plants and animals which accounts for the equal applicability of the basic laws of inheritance to both. It is therefore, of paramount important that you understand the consequences of mitosis and meiosis

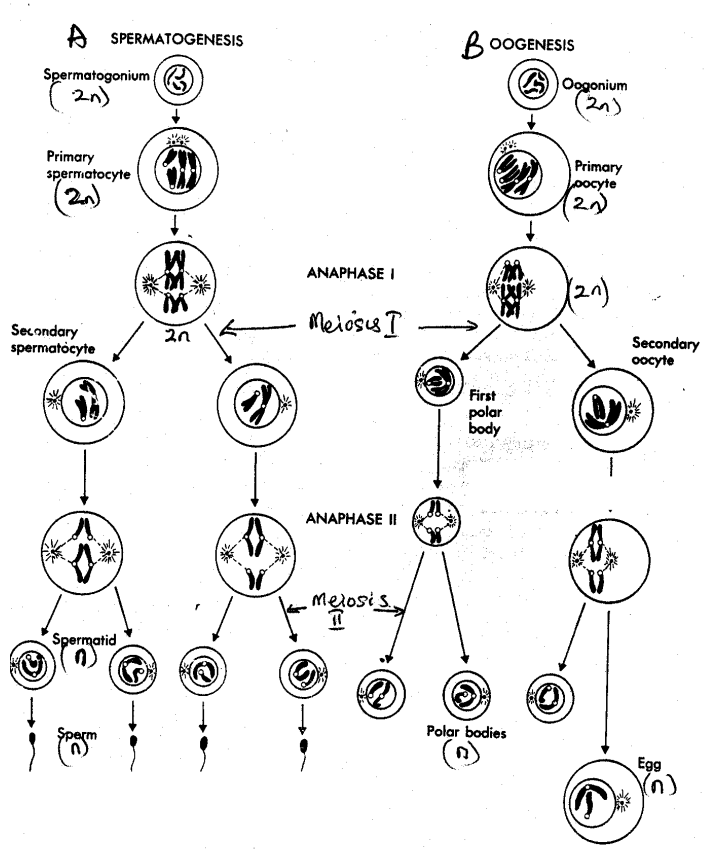

Fig. 12.1: Diagram representing the meiotic sequence in the male and in the female animal. Left: Process of spermatogenesis, resulting in the formation of four sperm. Right: Oogenesis, resulting in the formation of one egg and three polar bodies.

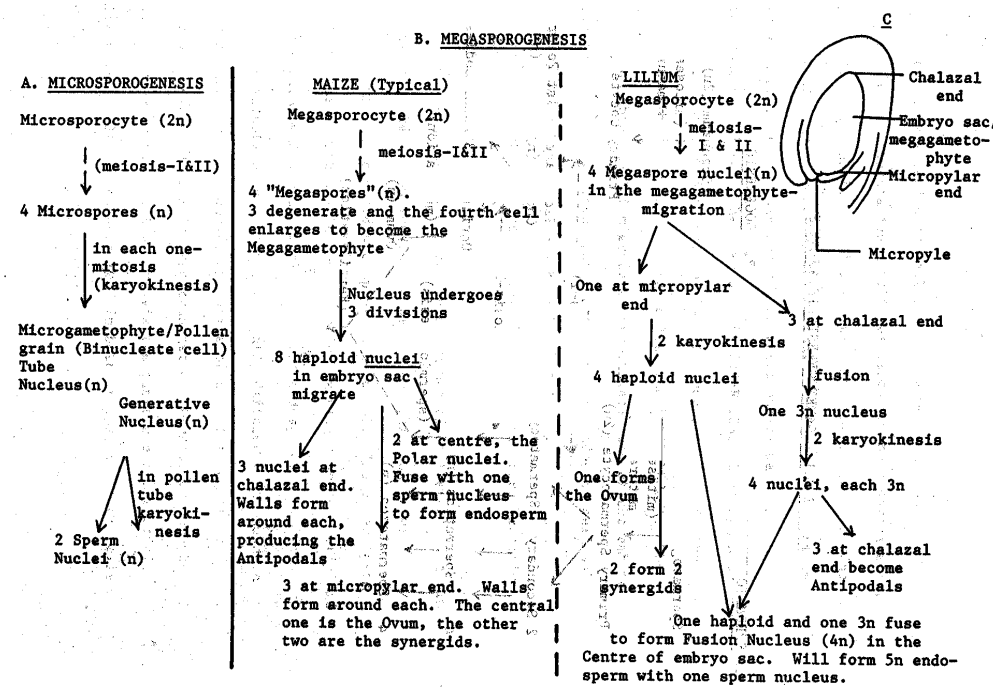

Fig. 12.2

4.0 Conclusion

Gametogenesis is the process by which an organism produces its gametes.

Gametes are normally ***haploid,*** this cndition is by reduction division (meiosis) of chromosones from the *diploid* germ cells.

In male animals the production of spermatozoa is through the process of ***spermatogenesis;*** the spermatozoa are haploid. Testis is where perms are formed. Four spermatoa are formed from one spertogeonium.

Oogenis is the process for the formation of the female gamete and it occurs in the ovary. The diploid primordial germ is called the *oogonium.* It undergoes mitosis to produce more ***oogonia. Each oogonium grows in size and matures into a*** primary oocyte. The primary oocyte divides by meiosis to produce one large cell, the *secondary oocyte* and the smaller one, the *first polar body.*

The secondary oocyte undergoes meiosis II to givew rise to a large cell called the second polar body. The ootid undergoes matuartion and becomes the ovum.

Hence, for each oogenesis, one ovum is produced with large cytoplasm and three smaller polar bodies with little or no cytoplasm. The cutoplasm is distributed in favour of the ovum sot that it will have enough food reserve for the zygote (fetilised egg) to grow upon before implantation, in the case of mammals or during incubation in the case of reptiles and birds.

Gametogenesis in plants involves sporaphute and gametophyte generations. In angiosperms (flowering plants) the sporophyte is dominant while the gametophyte is greatly reduced and is dependent on the sporophyte.

The male gametophytes, the **microgametophuphytes,**are in the anthers while the females equivalents, the ***megagametophytes*** are in the ovary. Both are haploid in chromosome number.

5.0 Summary

Spermtogenesis and oogenesis are the basis for the generationof new life, because when the sperm fertilizes the ovum a new beginning – the zygote – ensues. It is the zygote which later develops into the full organism. Gametogenesis is very important in genesis because this is the process whereby genetic characteristics are distributed into the sex cells. When the chromosomes of the sex cells pair up and fuse, the genes begin to express themselves as their dominant or recessive in the pairs. The expressions of the genes into visible characteristics give the phenotype of the genes. The genotype of the characteristics is based on whether the paired genes are the same (homologous) or different (hetegorous). All these will be learnt in genetics in the units that follow.

Self Assessment Questions

1) Define the terms:

   a) Gametogenesis
   b) Spermatogenesis
   c) Oogenesis
   d) Microsporogenesis
   e) Megasporogenesis

2) What are the conditions of the chromosomes in

   a) Spermatozoa
   b) Ovum
   c) Microgametophytes
   d) Megagametophytes

Answers

1. a) **Gametogenesis:** The formation of sex cells from promordal germ cell, through the process of meiosis.

b) **Spermatogenesis:** Meiotic reductions of the chromosomes in the diploid spermatogonia to produce haploid spermatozoa.

c) **Oogenesis:** Meiotic reduction of the chromosomes in the diploid oogonium to produce haploid ovum and three smaller polar bodies.

d) **Microsporogenesis:** Formation of haploid sperms in plants

e) **Megasporogenesis:** Formation of haploid ovum in plants

2 a) Spermatozoa b) Ovum

c) Microgametophytes – haploid (n)

d) Megagametophytes – haploid (n)

6.0 Tutor Marked Assignment

1) With the aid of diagrams, explain the process of oogenesis in humans; explain the number of chromosomes at each stage.

2) With the aid of well-labelled diagrams, describe microsporogenesis in plants and explain the number of chromosomes at each stage.

7.0 References

Williams, G. O. (2001). BIY 302 – Genetics – I. Distance Learning Institute, University of Lagos.

UNIT 13: INTRODUCTION TO GENETICS

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1.0 Introduction

A feature of students' attitude to Genetics is that, Genetics is a very difficult course, which one takes only because it is compulsory. Some students reinforce this negative attitude by the rationalization that they do not intend to do post-graduate work in Genetics! These attitudes are not deceptively correct because they make you neglect some of the more important aims of education. If you avoided the challenge posed by some courses, you would be denying your intellect the stimulation it needs to spur it to greater heights.

Besides, for a long time, Nigerian secondary school students have been denied adequate exposure to Genetics because some people avoided the challenge. Genetics is a vital aspect of everyday life and of Biology, and no biologist, regardless of his level or interest, should avoid a meaningful exposure to it.

Dobzhansky, aptly summarizes the need for a broad exposure as follows:

> "The advancement of science is, in the main, the business of specialists. And as science expands, the specialists tend to become narrow specialists. Some specialists have become disgustingly narrow. Narrow specialists are ENDANGERED and DANGEROUS (emphais mine) – endangered because their own inner lives are impoverished; dangerous because they are liable to be easy pray for exploitation by those with power or with money, for purposes inimical to both science and to the interests of mankind as a whole … There should exist, however, scientists able and willing now and then to abandon the protective shells of their specialties, and to engage in surveying broad vistas … people at large will have their inner life enriched if they gain an appreciation of what

> science and scientific attitude really are. Some aspects and achievements of science are everyone's business" (Dobzhansky, 1964).

It is hoped that at the end of the course, you would have gained an understanding of the principles governing the transmission of hereditary traits. All societies are interested in understanding how certain traits are inherited in living things, including man.

The concern, or better still, the puzzle about genetic inheritance in man is perhaps most succinctly expressed in this portion of a poem by Aldous Huxley's "Fifth philosopher":

A million million spermatozoa
All of them alive;
Out of their cataclysm but one poor Noah
Dare hope to survive

And among that billion minus one
Might have chanced to be
Shakespeare, another Newton, a new Donne
But the One was Me

Why was that one me? Why did normal parents produce an albino of short parents a tall child, or tall parents a short child? It is important for our well-being that we should be able to answer simple questions about heredity without resorting to "old wives tales". But Genetics is not solely concerned with man, it is of great importance in agriculture.

It is further hoped that at the end of this course, you will be able to appreciate the fact that:

"Increased knowledge of heredity means increased power of control over the living things, and as we come to understand more and more the architecture of the plant or animal we realize what can and what cannot be done towards modification or improvement …

It is not, however, in the economic field, important as this may be, that Mendel's discovery is likely to have most meaning for us: rather it is in the new light in which man will come to view himself and his fellow creatures …, if it is shown that the qualities of man, his body and his intellect, his immunities and his disease, even his very virtues and vices, are dependent upon the ascertainable presence or absence of definite unit-characters (genes) whose mode of transmission follows fixed laws, and if also man decides that

his life shall be ordered in the light of this knowledge, it is obvious that the social system will have to undergo considerable changes" (Punnett, 1910).

This course deals in the main, with the basic principles governing heredity. Examples are chosen merely to illustrate these principles. To that extent therefore, you will not be expected to memorize examples, which may be new to you. This approach is dictated not only by the fact that the basic laws of heredity are applicable to most organizations, but also by the belief that with a good understanding of the principles one can make extrapolations to explain particular situations.

Much of the difficulty, which students have with Genetics stems from the fact that they had been used to purely descriptive aspects of biology. Genetics on the other hand largely entails logical reasoning based on a number of interdependent principles often involving some calculations. These calculations are within the scope of anyone who has studied elementary mathematics.

Genetics is a course, which demands alertness and consistent work in the forms of reading and practice.

A note of warning should be sounded here: You would be deceiving yourself and also doing yourself a disservice, if you merely read genetics as literature. It indeed entails practicing on questions that boarder on the principles and laws of genetics. You will have to work examples typifying these principles and laws to have the concepts of genetics running in your blood.

2.0 Objectives

At the end of this unit, you would be expected to:

1) Develop an appreciation of the growth of Genetics

2) Know some of the important names in the development of Genetics

3) Know some of the theories in the evolution of Genetics, as well as the merits and demerits of such theories.

3.1 History of Genetics

Genetics is primarily and originally a science dealing with heredity i.e. the transmission of characteristics from parents to offspring. From such considerations, laws are derived concerning the relationships. In addition

genetics also involves a study of the factors, which show the relationship between parents and offspring and which also account for the many characteristics which organisms possess. You are familiar with the observations that "Like begets like", that children tend to resemble their parents as well as their siblings (or sibs i.e. their brothers and sisters), but they also tend to vary or look different from one another in many ways.

Genetics is the science, which tries to account for similarities and variations between related individuals. The science studies the transmission of hereditary factors from parents to offspring. Put differently, it is a study of biological "communication" between generations using the hereditary factors. Another facet of the science is the study of the expression or effect of the factors during development.

If one were to put the above "descriptive definition" of Genetics in a capsule form, Bateson, who coined the term Genetics in 1906 aptly defines it as follows:

Genetics is the science dealing with heredity and variation, seeking to discover laws governing similarities and differences in individuals related to descent. The factors which are transmitted were called "Genes" by Johannsen in 1909.

As mentioned above, Genetics weeks to provide explanations to the phenomenon of heredity and variation. It is therefore not surprising that the beginnings of genetics date back to the centuries before Christ. Around 400 BC Hippocrates theorized that small representative elements of all parts of the parental body are concentrated in the semen. It is these elements, which provide the building blocks for the corresponding parts of the embryo. According to this theory characteristics acquired by parents can be transmitted to offspring.

Aristotle, one century later disproved the theory postulated by Hippocrates, pointing out the facts that crippled and mutilated parents do not always produce abnormal offspring. Aristotle in turn advanced the theory that the father's semen provides the plans according to which the amorphous blood of the mother is to be shaped into the offspring. Put differently the semen supplied the FORM while the mother's blood supplied the SUBSTANCES. It is important at this point to note that Aristotle recognized that biological inheritance consists of a transmission of information for embryonic development, and not simply a transmission of samples of body parts. Because the information in the seminal fluid could not be seen, it was regarded as a mystical influence. Early in the 17th century, Harvey called this influence the AURA SEMINALIS.

In the 17th and 18th centuries, new theories of inheritance were propounded, following the discoveries of the egg and the sperm. One theory was the PREFORMATION THEORY, which depending on the school of thought, stated that either the egg or the sperm contains the entire organism in a miniaturized but perfect form. In the case of men, the theory postulated a miniature human being, called a homunculus, present in the sperm. This theory was postulated by Swammerdam. Not too surprisingly there were scientists who claimed that they saw homunculi in spermatozoa. They even drew diagrams to illustrate what they saw. One person who made an elaborate drawing of homunculus was Hartsoeker. The major drawback with the pre-formation theory is the fact that is implies that one homunculus contained another, which in turn contained yet another ad infinitum.

Another theory of development was the THEORY OF EPIGENESIS. In the 18th century Wolff discovers that adult structures in plants and animals arise form embryonic tissues, which do not resemble the corresponding adult structures. In other words, there is no pre-formation. But Wolff thought that mysterious vital forces were responsible for what he thought was a ***de novo*** origin of adult parts. Wolff's view modified in the 19th century by Von Baer who stated that adult parts arise as a result of a gradual transformation or differentiation of embryonic tissues into increasingly specialized tissues. Although the modified epigenetic theory is correct. It did not account for the form in which the materials to be transformed existed in the original embryonic cell, zygote.

Early in the 19th century, Maupertuis postulated that minute particles from each part of the body of the parents are united in sexual reproduction such that during development particles from the male dominate in some cases; in other cases those form the female parent dominate. In one important aspect, this theory recognized the fact that an offspring receives two of each type of particle, one from each parent, but exhibits only one. However, by suggesting that the body parts contribute particles, this theory leads to the theory of evolution advanced by Lamarck. According to Lamarck's interpretation characteristics such as well-developed muscles acquired by parents in the course of their lift can be transmitted to their offspring. This idea was formalized by Charles Darwin as the "Provisional Hypothesis of Pangenesis." According to Darwin,, exact miniature replicas, called ***gemules***, of the body parts and organs are carried in the blood stream, to be assembled in the gametes. In the zygote, the gemmules from both sexes come together and are parceled out to form the appropriate structures during development. Since a gemmule is an exact replica of a parental part it means that acquired characteristics should be inherited by the offspring. If that were so it would be easy to understand evolution. Recall that the theory of

pangenesis is essentially the same theory advanced by Hippocrates in the 5th century B. C. and disproved by Aristotle.

The theory of pangenesis lends itself readily to testing, and it was tested by August Weismann toward the end of the 19th century. He cut off the tails of mice for 22 generations, yet the offspring of such mince continued to show tails of normal length in every generation. The experiment can be represented schematically as follows:

Generation I: Cut off tails of the mice and mate them.

Generation II: Offspring with tails; repeat operation

Generation III:Offspring with tails; repeat operation

Generation IV: Offspring with tails; repeat operation

: :

: :

Generation XXI: Offspring with tails; repeat operation

Generation XXII: Offspring with tails.

The result therefore showed that it cannot be true that acquired characteristics can be inherited.

In spite of this proof there are people who still accept the inheritance of acquired characteristics. Perhaps the most prominent adherent in recent times was prominent adherent in recent times was the Russian, Trofim Lysenko. He coerced many Russian geneticists to accept the theory, because he wielded political power. Lysenko dominated Russian agriculture and biology from 1984 to 1964. They were years of successive failures of soviet agriculture.

To replace the theory of pangenesis Weismann proposed the GERMPLASM THEORY in 1885. According to this theory, multicellular organisms are made up of two types of tissues, viz the somatoplasm and the germplasm. The somatoplasm is made up of tissues which are essential for the functioning of the organism, but they do not determine what is transmitted to the offspring. In other words changes in the somatic tissues are not transmitted. The tail of a mouse is a type of somatic tissue. On the other hand the germplasm is tissue whose sole function is the formation of

gametes. Since the gametes give rise to the offspring, changes in the germplasm may lead to changes in the offspring. Notice, however, that the theory does not indicate what the germplasm transmits.

Many biologists including Kolreuter compared the similarities and differences between plant hybrids and their parents. A hybrid is an offspring from two different parental types. Kolrenter found that although hybrids from two parental stocks are usually similar, such hybrids if fertile usually produce offspring which show considerable diversity. The results of such hybridization studies were recorded simply as qualitative observations.

Kolreuter and many others after him did not record the ratios in which the original parental characters occurred among the progeny. As we shall see later, it is therefore not surprising that the early hybridizers did not discover any underlying principles of inheritance. Thus even though they made many important observations, the hybridizers pre-date the origin of genetics.

In many ways Genetics is a precise and somewhat mathematical science dealing with specific offspring ratios which are predictable on the basis of the known genetic constitutions of the parents. In the reverse process, the genetic constitution of the different types of offspring they produced.

Gregor Mendel, an Austrian monk, is regarded as the father of Genetics. It is generally agreed that Mendel's success can be attributed to the fact that he was lucky in choosing the garden pea, Upisum ***Sativum,*** for his studies. This plant, although, normally self-pollinating can be easily cross-pollinated. Mendel was also successful because he studies the inheritance of single contrasting characters (i.e. smooth versus wrinked), unlike his predecessors who studied several characters simultaneously. Equally important was the fact that Mendel counted and carefully recorded the numbers of each type of offspring from each of his crosses.

Mendel published his results in 1866 after he had reported them at a Natural science meeting in 1865. He clearly stated the laws of inheritance which can be derived from his results. The law constitute the foundation stones of Genetics. In spite of the fundamental nature of Mendel's discoveries and the clarity with which he stated his results and conclusions, his papers had no immediate impact on the scientific world. However, one Russian botanist, I. F. Schmalhansen stressed the importance of Mendel's findings soon after they were published. Mendel's discovery did not have an immediate effect because the related information required for understanding his deductions were not available at the time. Thus it may be said that Mendel was "ahead of his time".

After publication of Mendel's results other relevant information about development were provided by various workers. In 1875, O. Hertwig and later, Fol, and Strasburger described the process of fertiliztion including the fusion of the egg and the sperm nuclei. Between 1880 and 1885 Fleming, ran Beneden and Strasburger described chromosomes and their division in mitosis as well as their constancy in number. Later Hertwig and Stransburger developed the theory that the nucleus contains hereditary material. These discoveries were reflected in Weismann's theory of the Germplasm. Weismann postulated that in the process of gametogenesis, i.e. the formation of gametes there must be a reduction in half of the number of chromosomes. If that were not so there would be a doubling of the chromosome number at each fertilization. However, as mentioned earlier the chromosome number is constant from generation to generation. The postulate by Weismann of reduction in chromosome number was later observed by Boveri and other investigators. The process involved is ***meiosis.***

Three investigators unaware of Mendel's work and results independently carried out similar plant breeding experiments. During the process of writing their findings for publication, they each came across Mendel's paper and they referred to it in their rediscovery of the Mendelian laws of inheritance. Although the three people, Correns, do Vries and Tschermak are generally regarded as the rediscoverers, Stern and Stern (1978) do not think that Tschermak's work on its own could have yielded the laws of inheritance. Hence, there should be only two rediscoverers.

Although the laws of inheritance were first demonstrated with plants, Cue'not and also Bateson in 1902 showed that the laws apply equally to animals.

From this brief history of Genetics one would hope that you would derive and appreciation of the tortuous steps leading to the establishment of various laws in science.

4.0 Conclusion

Genetics is a vital aspect of everyday life and of Biology and biologists, and even non-biologists, should be fully exposed to it. Every father wants to be sure that the baby brought from the hospital is his own, and farmers want improved farm products – both animals and plants.

These aspects are being further improved by genetic engineering which result in better agricultural products. Increased knowledge of heredity through genetics means increased power of control over living things.

Genetics is the science dealing with heredity and variation and is governed by laws. The history of genetics dates even before Christ. Hippocrates, Aristotle Maupertuis, Lamark Mendel and Charles Darwin are some of the eminent scientists who have contributed to the knowledge of genetics.

Genetics is a precise and somewhat mathematical science dealing with specific offspring ratios which are predictable on the asis of the known genetic constitutions of the parents. It should not be studied like literature but examples should be worked out so as to be acquainted with the mathematical rules guiding heredity.

5.0 Summary

The history of genetics has been discussed in the Unit so as to let you appreciate the toils and labour of those who had worked in the field before. You could also develop your own ideas in genetics and be reckoned with as one of the greats.

Self Assessment Questions

1) Discuss the beliefs of Hippocrates with regards to inheritance

2) What was Aristotle's theory that shattered Hippocrate's theory on heredity

Answers to Self Assessment Questions

1) Refer to Section 3.1

2) Refer to Section 3.1

6.0 Tutor Marked Assignment

1) Discuss the current theories regarding heredity.

7.0 References

Williams, G. O. (2001). BIY 302 – Genetics – I. Distance Learning Institute, University of Lagos.

UNIT 14: CHROMOSOME THEORY OF INHERITANCE

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1.0 Introduction

Some of the material covered under this topic is repetition of what we covered as part of the "History of Genetics." However, this is necessary in order to reduce the problem of recall at such an early stage.

2.0 Objectives

The nucleus controls the activity of the cell. It is therefore, reasonable to assume that the genes which constitute the ultimate basis of what a cell must also be in the nucleus, specifically, on the chromosomes. You should be able to explain:

1) The parallels between the behaviour of genes and chromosomes, which led to the formation of the chromosome theory of inheritance;

2) The essential features of the chromosome theory;

3) The evidence in support of the theory.

↔↘↔ Deduction of Chromosome Theory of Inheritance

Hertivig working with sea urchins and some other investigators working with other organisms, discovered that two equal-sized nuclei, one from the sperm and the other from the egg fuse at fertilization. This is in spite of the fact that the egg is much larger than the sperm. In other words the difference in the amount of cytoplasm not the nuclear content. Based partly on this fact and the results of crossing (mating) different types Hertvig, and Stransburger also in 1885 advanced the theory that the cell nucleus must contain the hereditary material.

Earlier in 1883, Eduoard Beneden had discovered in Parascaris Equorum (formerly Ascaris megalocephala – these names seems to be still preferred) that the fertilized egg of this nematode contains only four chromosomes. Furthermore, at the time of fertilization, the sperm and the egg nuclei contain two chromosomes each. In the light of this fact one could be more specific about the equal nuclear contribution by both the male and female parent to the zygote. The components of the nucleus that are visibly distributed during cell division are the chromosomes. It is therefore, quite logical to conclude that because the parents contribute equal numbers of chromosomes, the chromosomes must be the carriers of hereditary material.

Reasoning without the benefit of knowledge of van Beneden's discovery, Roux, also in 1883, in a purely hypothetical discussion of the significance of the mitotic process strongly implied (did not say so categorically) that the chromosomes are the bearers of hereditary material. Roux's approach was teleological i.e. he started from the standpoint that there must be a reason fro the elaborate mitotic process. (For example, it is teleological to say that we developed eyes because we needed to see.) The question in essence was "why should the division of a simple structure like nucleus be so complicated?'

According to Roux, if one assumed that there are in the nucleus, very many submicroscopic units which control the life processes of cell, then it would be understandable that great care should be taken in dividing the nuclear content.

On the other hand, mere constriction of the cell would be sufficient for dividing the cytoplasm. Roux reasoned that a suitable method for ensuring an identical distribution of the very many submicroscopic units into each daughter cell would be for each unit to be divided first, and then the sister units would be separated. The tasks of division and separation would however be greatly facilitated if the units were arranged like beads on a string. There would be several such assemblies, carrying different units, in the cell. During cell division each "string of beads" would then split longitudinally, and the halves would move into separate daughter cells. Roux then went on to say that because the mitotic process is so elaborate it must serve a purpose in the organism. The purpose is the equal distribution of the nuclear material important for the physiological and development processes of the cell. We know today that Roux's "units" are the ***genes,*** the hereditary material, and they are carried on the chromosomes.

In formulating his theory of the Germplasm in 1885, Weismann specifically said that the chromosomes function as the carriers of hereditory units, but the chromosome theory was still to be clearly stated.

After the rediscovery of the Medilian Laws in 1900, it did not take long before the parallel becharious of the Mendelian foctors (the genes) and the chromosomes were identified. The fact that the observable type of transmission of chromosomes (i.e. the cytological evidence) correspoonds to the deduced type of transmission of genes (the Mendelian Laws of inheritance) was pointed out independently by Sutton and by Boveri in 1903. Their conclusions constitute the Chromosomes Theory of Inheritance. The main points of the theory are:

1. That genes are located on chromosomes such that one member of a pair of genes is on one chromosome and the other member is on a partner chromosome, i.e. the homologous chromosome with which it synapses in meiosis.

2. Different pairs of genes are located on different chromosomes. This is not to say that there is only one gene on each chromosomes. This is not to say that there is only one gene on each chromosome. Rather, the point is that non-homologous chromosomes carry different genes. There is more than one gene on each chromosome.

The parallels between the genetic and cytological facts which form the basis for the theory are:

i) In diploid organisms, genes occur in pairs and so do chromosomes.

ii) Members of a gene pair separate at the time of gamete formation so that each gamete receives only one member of the pair. The same is true for chromosomes (cf. Anaphase-I).

iii) The members of different gene pairs recombine at random at the time of segregation during gamete formation.

Sutton and Boveri did not have corresponding evidence for chromosomes but they also did not have evidence to the contrary. Recall the fact that the metaphase-I orientation of one bivalent did not influence the orientation of another vivalent. This piece of evidence was provided later and it confirmed the assumption that No. (iii) was also applicable to chromosomes.

The most convincing proof of the theory that genes are on chromosomes was provided by Boveri in his experiments with the sea urchin. Boveri worked

with a species in which 2n = 36. In other words at fertilization each gamete contributes a haploid number of chromosomes of n = 18. Normally, only one sperm fertolizes an egg but there are rare exceptions in which more than one sperm fertilizes the egg. This condition is called polyspermy. It is called dispermy when only two sperm are involved. Polyspermic embryos die early in development. We shall consider the simplest case, i.e. the dispermic embryos. Boveri found that there was great viariability in the time of death and also in the type of organ whose abnormal development led to death.

The sea urchin embryo can be divided into four guardrants, each of which arose from one of the first four cleavage blastomeres are cells. Boveri observed that the four quadrants often develop differently, thus one guadrant may be normal and the other three abnormal but in different ways and to different degrees. This variability in development of different parts of the same embryo was a very important observation by Boveri. How does one acccount for it?

At fertilization in the sea urchin the sperm contributes a centriole which divides to form the two poles i.e. the asters of the itotic spindle which is formed as the asters move apart. Each of the 18 chromosomes contributed by each gamete in normal fetilization becomes duplicated and comes to lie at the metaphase plate (equatorial plate). This is normal mitosis. The zygote contains 36 chromosomes and to blastomeres are formed as a result of the first cleavage. Following the seocnd cleavage a total of for blastomeres gives rise to cells which will form one quadrnat of the embryo.

When there is dispermy, two centriole are introudced into the egg. Each divides giving rise to two asters. The effect of dispermy is the production of four asters in the zygote. The four asters are arranged like the corners of a square. Since there is a spindles are fund in the zygote such that they are correspond to the sides of the square. When such a zygote divides, for blastomerses are formed at one in the first division. As mentioned earlier, each blastomere gives rise to a quandrant in the embryo.

In order to answer the question we posed earlier, we have to try to answer another question, manely, “How do the chromosomes behave in a quadripolar division?’ The zygote in question is made up of contributions from two sperm and the egg. The nucleus of each of these gametes contains 18 chromosomes, therefore, there will be 54 chromosomes. This is a 3n number of chromosomes and it is said to be a triploid number. The chromosomes are duplicated as in normal mitosis. However, when they move on to the equatorial plates of the spindle, they are distributed at random on the spindles. The consequence of this random distributionis that each of the four

resulting blastomeres may contain different types of numbers of chromosomes.

Boveri was able to show that the abnormal development of a dispermic embryo was the result of the erratic chromosme distribution rather than dispermy per se. In other words, dispermy does not invariably lead to abnormal development. Bovery analyzed his results as follows: He found that the size of a nucleus is dependent on the number of chromosomes present in it. Therefore, he compared the sizes of the nuclei with the degree of developmental success (i.e. the degree of normal development) in each quadrant of an embryo as well as with degree of developmental success in quadrants having similar-sized nuclei in other embryo.

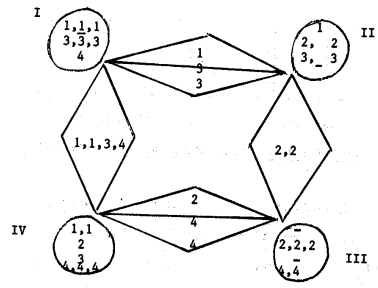
	EMBRYO A				**EMBRYO B**			
Nuclear Size	**QUADRANTS**				**QUADRANTS**			
	I	II	III	IV	I	II	III	IV
1		+					111	
2	1111		+			11		
3								+
4				11	+			

1111 = Highest degree of developmental success.

Table 14.1: Comparison of Development in Two Dispermic Embryos

From Table 14.1, one can see that similar-sized nuclei may result in different abnormalities, hence the different degrees of developmental success. Boveri therefore concluded that the variability in development is a reflection of qualitative rather than quantitative differences between nuclei in different quadrants. For instance if development were dependent on nuclear size only, quadrants I and III having similar-sized nuclei should have had similar degrees of developmental success.

Let us now look at a hypothetical example using only four instead 18 types of chromosomes. In this example we shall also assume that in order to have normal development, each type of chromosome must be represented at least once. Since n = 4, the dispermic zygote would contain 12 chromosomes. Recall that the distribution of the chromosomes on the spindles is a random process. The diagram below is therefore only one of many possible ways in which the 12 chromosomes might be distributed on the four spindles. In this arrangement, only one quadrant develops normally.



Note: 1 - 4 = Chromosome types

1 - IV = Blastomeres that will form quadrants

I & IV = Have equal-sized nuclei. Some for II and III.

Only IV is normal since all 4 types of chromosomes are present.

Since Boveri was aware that the chromosomes vary in shape and size he concluded that there are qualitative differences between chromosomes. Specific abnormalities would therefore, arise when particular chromosomes were missing. This would be the case only if different chromosomes carried different genes.

As a further test of his hypothesis about qualitative differences between chromosomes, Boveri found the expected frequency with which any quadrant might lack all three of any one of the 18 types of chromosomes. He found that the expected frequency compared favourably with the observed frequency of abnormally developing quadrants.
One of the main points of the chromosome theory is that different chromosomes carry different genes. It is pertinent under the circumstances to ask weather the chromosomes are asked whether the chromosomes are stable structures or whether they disintegrate during interphase and are reassembled during prophase. If that were so it would also be probable that genes would "move" from one chromosome to possibly a non-homologous chromosome. There would also be the possibiolity that the genes are not normally carried on chromosomes. The fact that chromosomes are stable structures which maintain their integrity even during interphase, was established by Boveri using the fertilized eggs of Parascaris equorum. In this mematode the arms of the chromosomes are not completely retracted at the end of telophase to give a spherical nucleus. Boveri found that at the end of telophase, the two daughter nuclei are mirror images of each other as shown in Figure 14.1

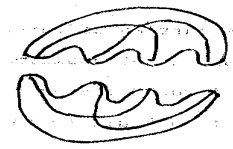


Figure 14.1: Mitotic Daughter Nuclei of P. Equorum (2n = 2)

These nuclei retain their shape until the next prophase when the chromosomes reappear. The chromosomes reqppear with their tips still in the projectins from the nucleus. It is therefore, reasonable to conclude that the chromosomes did not lose their identity from generation to generation.

↔↘↕ Other Evidence in Support of the chromosome Theory

In our consideration of cell division, we found that the chromosomes in a cell could be considered as sets, such that a diploid cell would have two sets of chromosmes. The general terms used to describe the number of whole sets of chromosomes is "“ploidy”. Continuing on the same theme, there are ***euploid*** aneuploidy conditions. The term euploidy is used to describe variations in the numbers of whole sets of chromosomes haploid = n; diploid = 2n; traploid = 3n. These variations which involve whole sets of chromosomes generally result in normal development. Aneuploidy on the other hand refers to variations in the numbers of individual chromosomes. Such variations give unbalanced sets of chromosomes.

From the discussion of Boveri’s sea urchin experiments above, it is obvious that aneuploidy provides a lot of information in support of the theory that genes are located on chromosomes. The same is true for the assertion that different chromosomes carry different genes. In this section then we shall be considering mainly evidence from aneuploid conditions.

In discussions of chromosomes one often talks of karyoty and idiogram. A karyotype is an individual’s chromosomes complement in terms of number and size of chromosomes as well as the location of the centromere in the different chromosomes. The idiogram on the other hand is a diagrammatic representation of an individual’s karyotype with the different chromosomes arranged in order of decreasing size.

In the plant, ***Datura,*** the haploid number is 12. Blakeslie and Bell found that unusual plants occasionally arise. These unusual plants contain 25 instead of the normal 24 chromosomes. These plants look different from the normal diploid plant.

Twelve different types each having 25 chromosomes can be identified in terms of the seed capsule. It was found that each of the twelve variants possessed a different one of the twelve types of chromosomes. In other words, in each variant, a given chromosome was present in triplicate. This aneuploid condition in which three instead of two of a given chromosome are present is described as a trisomy. Thus, if the different chromosomes are numbered 1 – 12, an individual with Trisomy – 1 (or Triplo – 1) has three of chromosomes – 1 present. Note that as we said earlier, these trisomic plants have only one chromosomes extra, hence the total number is 25 or 24 + 1 which can be stated as 2n + 1; with the exception of the particular chromosome under consideration all the other chrommosomes are in pairs. With respect to the example of Datura under consideration, the aneuplooid effect due to Trisomy – 2 and so on. Because the effect of each trisomy is distinguishable from all the others, it is logical to conclude that different chromosomes carry different genes.

Normally in mitosis, the two daughter chromosomes move to opposite poles during anaphase. Very rarely, however, mistakes do occur and both daughter chromosomes migrate to the same pole. This situation is described as non-disjunction. Non-disjunction can also occur in both meiosis –I and meiosis-II. In the former case, homologous chromosomes would be involved while the latter would be similar to mitotic non-disjunction. Non-disjunction will give rise to aneuploid conditions.

Trisomic conditions also occur in man. One example is Trisomy – 21. This chromosome imbalance produces a condition known as Down's syndrome. The term syndrom is used when a number of symptons characterise an ailment. This particular case was first described by Down. In man, the diploid number is 46 but those affected with Down's syndrome have 47 chromosomes, the extra being chromosome – 21. Amongst other symptoms, affected invidiuals are mentally retarded.

Where it has been studied (e.g. U.S.A.) the occurrence of Trisomy – 21 (production of an egg with 24 chromosomes) has been found to be associated with the age of the mother. The proof of the effect of maternal age is that in general population, the occurrence of Trisomy – 21 is one in 600 live births.

However, when different age groups are considered separately, the frequency for mothers about 20 years old is one in 3,000, but for mothers around 45 years, the frequency of occurrence rises to one in 40 live births. The rise in frequency starts when the woman is about 35 years. A corresponding study keeping the female age fairly constant but varying the father's age does not show any difference between age groups. The reason for the association with the age of the mother is not known.

Non-disjunction is not the only cause of Trisomy – 21. Although it was said ealier that every chromosome maintain its integrity (with the exception of reciprocal exchange between homologues during crossing-over) it sometimes happens that a portion of one chromoosme is transferred to another chromosome, usually or non-homologue. This phenomenon is known as trans-location. Chromosome – 21 is a very samll chromosome while 14 is fairly large. In some very rare individuals the bulk of 21 has been translocated to 14 to give a chromosome designated 14.21.

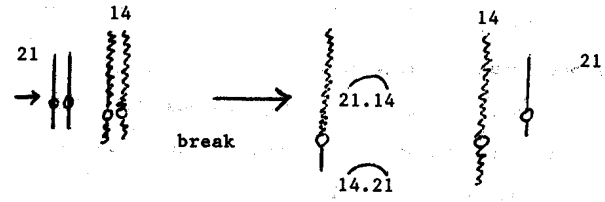


Figure 14.2: Translocations Involving Human Chromosomes 14 and 21

The translocation occurs as shown above in a diploid individual. The samll chromosome 21.14 is lost without any adverse effect and so the person has 45 vhromosomes, but is normal because virtually all of 14 and 21 are combined in the 14.21 chromosome. If the egg produced carries both the 14.21 and the free 21, it would have two doses instead of one of 21. Fertilisation by a normal sperm would therefore, produce an individual with 46 chromosomes but with threee effective doeses of chromosome – 21. Notice that the fact that particular effects are associated with specific trisomic conditions and also, the fact that the translocated 14.21 can be transmitted unchanged are proof that chromosomes retain their integrity.

If non-disjunction can produce a gamete containing two of one type of chromosome, the reverse situation is also possible. There are cases known in which as organism carries only one instead of two of a given chromosome; such individuals are said to be monosomic for that chromosome. Monosomy – 21 is not known in man, so the condition is assumed to be non-viable. The same is true for monosomy – 14. These cases illustrate the point that in some organisms, unlike the sea urchin studied by Boveri, the mere presence of some genes Is not a sufficient condition for normal development, rather the genes must be present in a balanced dose. In Droiophila melanogaster, a fruit fly, the haplo – IV (monosomy – IV) condition survives although the fines have reduced viability and fertility.

Some other aneuploid conditions are:

Tetrasomy = 2n + 2 i..e. two extra of a given chromosome.

Double Trisomy = 2n + 1 + 1 i.e. one extra of each of two different chromosomes.

Nullisomy = 2n – 2 i.e. a given chromosome has both members absent.

The significance of chromosomes as well as dosage of chromosomes with respect to characteristics exhibited by organisms extends to sex determination as we shall see later. It is sufficient to mention one extreme example here, namely, the honey –bee in which male are haploid while females are diploid.

When an organism has more than two whole sets of chromosomes i.e. 3n or more such as individual is described as being polyploid. The 3n individual is a triploid individual; tetraploid = 4n and pentaploid = 5n. Polyploidy is rather common in plants but it is rare and often easily recognizable because with certain limits they are larger than their diploid counterparts.

4.0 Conclusion

Rather than try to summarize the examples considered, it is sufficient to say that the chromosome theory of inheritance states that the genes are an integral part of the chromosomes. The basis for this generalization is the fact that particular deviations from say the normal diploid chromosome number, whether euploid or aneuploid have specific detectable effects. These specific effects are an indication that chromosomes carry genes and more specifically that different chromosomes carry different groups of genes.

6.0 Summary

We have learnt that genes are bourne on chromosomes, and occur in pairs in diploid organisms. The gene pairs separate at the time of gamete formation so that each gamete receives only one member of the pair. Pairing is restored when members of different gene pairs recombine at random. The randomness of recombination is the basis of genetics.

Self Assessment Questions

1) Explain the following terms:

   a) Haploid b) Diploid c) Polyploidy

2) Explain what is meant by non-disjunctions

3) Explain trisomy in man

Answers to self Assessment Questions

1. a) Haploid: Set of chromosomes (impaired) that occurs in sex cells; denoted by (n)

   c) Diploid: Set of Chromosomes (paired) that are found in somatic
   cells; denoted by (2n)

   d) Polyploidy: Set of chromosomes more than 2n i.e. 3n or more; more common in plants than in animals. Polyploid individuals are often easily recognizable because with certain limits they are larger than their diploid counterparts.

2 Non-disjunction is when in mitosis the two daughter chromosomes move to some poles during anaphase.

3 Trisomy in man usually involves chromosome 21, which instead of being in pairs occurs in triplet. This chromosome imbalance produces a condition known as Down's Syndrome.

6.0 Tutor Marked Assignment

1) Explain the conditions that give out the chromosomes (DNA) as the genetic materials

7.0 References

Williams, G. o. 2001: biy 302 – Gnetics – 1 Module. Distance Learning Institute, University of Lagos.

UNIT 15: Principles of Segregation

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1.0. Introduction

You will recall that Mendel succeeded where many before and after him had failed. Mendel succeeded for two major reasons.

1. He analyzed his results both in a qualitative as well as in a quantitative way.

2. In making his initial crosses, he chose pairs of clearly contrasting characters for which each of the plants he started with words true/pure breeding. The term true breeding is used to describe cases in which a cross between two individuals possessing the same character yields only progeny which are identical with one another and with the parents with respect to that character. It is also applicable to cases of self-fertilization yielding the same results.

2.0 Objectives

In this and subsequent units you are not expected to memorise specific examples, instead, you should understand how the principles involved can be derived from the examples used. This unit is supposed to inculcate an understanding of:

1. Some of the terms used in Genetics. In this and other units new terms are used. They are an essential part of the vocabulary of Genetics. You have to learn them whenever they occur.

2. What is involved when a cross is written.

3. The steps, types of evidence and types of deductions which led to Mendel's formulation of the first law of inheritance.

4. To recognize the evidence which indicates monohybrid inheritance.

5. To explain the bases for the various phenotypic and genotypic ratios.

6. To make the necessary deductions of phenotype from genotype and vice-versa, as well as derive offspring from parents and vice-versa.

7. To state and explain the first law in your own words.

3.1 Mendel's first Law of Inheritance

Mendel made many crosses, and for each cross he used a pair of characters which were such that a plant can only exhibit one but not both characters. A cross was made by transferring pollen grains from the anthers of one plant to the stigma of another plant or of the same plant for cross-pollination and self-pollination respectively.

The plants used for the initial cross constitute the parental or P-generation. Their progeny constitute the First Filial generation, abbreviated as F_1 – generation. The progeny of the F_1 as a result of either crossing two F_1 or of self pollinating an F_1 constitute the F_2 or, second filial generation.

In one experiment, Mendel crossed parents which were true breeding for yellow seeds with parents which were true breeding for green seeds. This cross was done in two ways:

1. Yellow (ovum) x green (pollen) $\longrightarrow$ Yellow F_1

2. Green (ovum) x yellow (pollen) $\longrightarrow$ Yellow F_1

In cross-1, the yellow parent was the female parent and in cross-2 the role were reversed. Cross-2 is referred to as a reciprocal cross of cross-1 or vice-versa. In other words the characters used in a reciprocal cross are exactly the same at the initial cross; the difference is merely a reversal of male and female roles. The F_1 progeny of the two crosses are indistinguishable from each other and from the yellow parent. In both crosses also all the F_1 were yellow.

The reciprocal cross provides a very important piece of information. The fact that the progeny of the two crosses are identical indicates that the male and female contributions to the progeny are equal. This is in spite of the fact that the pollen grain contributes virtually no cytoplasm to the offspring. Mendel deduced that fact of equal hereditary contribution from his results and as we saw earlier, it was only much later that Hertwig and others provided cytological evidence that the nuclear contributions are indeed equal. Mendel's conclusions for reciprocal crosses are also applicable to animals. The result obtained from the reciprocal cross is therefore, evidence in support of the chromosomes theory of inheritance.

In the next step of the experiment, Mendel planted the yellow F_1 seeds and self-pollinated (selfed) them when they flowered. This step of the experiment is the same as crossing two F_1 yellow. The yellow F_1 seeds gave different results from crosses between two parental yellow types. While the parental yellow were pure breeding the F_1 yellow were not. Yellow F_1 progeny from reciprocal crosses gave identical F_2, confirming the initial conclusion. The F_2 progeny consisted of yellow and green seeds. When Mendel pooled the results of the F_1 crosses he got 6,022 yellow and 2,001 green F2. Further analysis gave a ratio of 3.01 yellow: 1 green among the F_2.

Using the same scheme Mendel tested a number of characters. His results for some crosses are shown below:

Note that it is no longer necessary to specify the sex of each parental type. You are not expected to memorize this table. It is simply to give credence to the conclusions drawn.

Table 15.1: Some results of Mendel's experiments on Sweet Pea

S/No	Parental Characters	F_1	F_2	Ratios
1	Yellow x Green Seed	All yellow	6022 yellow; 2001 green	3.01;1
2	Round x wrinkled	All round	5474 round; 1850 wrinkled	2.96:1
3.	Green x yellow Pods	All green	428, green; 152 yellow	2.82:1
4.	Axial x terminal flowers	All axial	651 axial: 207 terminal	3.14:1

Although only four crosses are shown in the table, it is obvious that even though a particular character is not visible in the F_1 it is not lost nor is it modified i.e. it does not blend with the other character. The fact that it remains unchanged can be shown by comparing the F_2 green with the parental green; they are indistinguishable in other words the hereditary unit responsible for the green colour was merely latent in the F_1. Mendel called the hereditary units "factors". Johannsen called them "genes" later.

Also in the table we find that in each cross all the F_1 resemble one parent and there is a constant ratio of approximately 3:1 of the two parental characters. In order to account for these results Mendel made assumptions and explained his results along the following lines.

He assumed that each of the true breeding parents carries two identical hereditary factors which are responsible for their particular character. For instance, in the first cross the yellow parent would carry two identical factors making for yellowness, and the same would be true for the green parent. These factors can be represented with symbols. We can therefore represent the two factors in the yellow parent as YY. The two factors in the green parent can be represented as YY. When each parent produces gametes, the pairs of factors separate so that only one factor enters a gamete (compare Mendel's assumption which the separation of homologous chromosomes in anaphase-I and also with Welsmann's theory of reduction). As a result of the separation, the gametes from the yellow parent contain only Y factor and those from the green parent contains only one y factor also. Each parent produces only one type of gamete but there is no way to distinguish between the two Ys or the two ys in the green parent.

When the gametes from the two parents fuse at fertilization, a zygote i.e. the F_1 is formed containing two factors, one Y and one y. Hence the F_1 may be designated Yy. From the table, the observed character exhibited by the F_1 is yellow, which corresponds to the Y-factor inherited from the yellow parent. Since a y-factor was also inherited from the green parent but not exhibited, the y-factor is latent in the F_1.

The yellow character is said to be ***dominant*** over the green character because when the two types of factors responsible for both characters are present in the same individual only the yellow character is exhibited. In the same way the Y-factor is said to be dominant over the y-factor. The green character is said to be ***recessive*** to the yellow character. The same terminology is used to describe the relationship of the y-factor to the Y-factor.

The factor for the yellow trait is designated Y because yellow is dominant and the factor for green is designated y because green is recessive. The same letters used as the symbol for both the yellow and green characters because they are alternate forms of the same character. In other words a seed is either yellow or green but not both. Although we have been using gene (hereditary factor) and character interchangeably, the character is the effect produced by the gene. The symbols Y and y are therefore alternate forms of the same gene. They are called ***alleles***. Alleles are modifications of the same gene, hence variations of the same symbol are used to designate them.

We assumed earlier that each parent carried a pair of alleles for the characters in question, hence we would use symbols to represent the genetic constitution of each parent and also of the offspring. The term for the genetic constitution is ***genotype***. For example the genotype of the yellow parent is YY. The effect produced by the genotype (which we had called character) is called the ***phenotype***. Before continuing with our discussion of Mendel's experiment, it is important to draw your attention to the fact that identical phenotypes do not necessarily indicate identical genotypes. In the example under consideration the phenotype of the F_1 are indistinguishable from that of the yellow parent yet according to our explanation so far the yellow parent is YY while the yellow F_1 are Yy.

According to Mendel's assumption, given the parental genotype and the types of gametes produced, the F_1 are Yy. What type of gametes would the F1 produce? We had concluded that because the F2 green was not different from the green in the P-generation, the contribution of the green parent to the F1 must have retained its integrity and merely remained latent. In effect therefore, we also have to assume that the y allele remained unchanged in the F1. In spite of the difference in genotype there is no reason to assume that the processes leading to gamete formation in the F1 would be different. Again the two alleles must separate so that only one, Y or y, enters each gamete. It is most important that you recognize the fact that only one allele would be in any given gamete. When both alleles were identical as in the parental generation, each parent produced only one type of gamete. But you will recall that at the end of meiosis-I each daughter cell contains one member of a homologous pair of chromosomes. Genes are on chromosomes so the same situation applies. More specifically then, 50% of the gamete formed by each F1 would contain the Y-allele and the other 50% would contain the y-allele.

Fertilization i.e. gametic fusion according to Mendel is a random process, i.e. the Y-bearing pollen does not preferentially fertilized either the Y-bearing or y-bearing ovule. Both types of fusion are equally frequent because there are equal amounts of the two types of gametes. We can easily represent random

fertilization by using the Punnett squares (designed by Punnett). All the four boxes are equally possible in this case, and together constitute a unit.

	POLLEN		
		Y	Y
EGG	Y	YY	Yy
	y	Yy Yellow	Yy Green

Fig. 15.1 Punnett Square

The genotype in each box is produced by the fusion of the corresponding gametes. The contents of the boxes represent the F_2 and they are equally visible. Mendel's actual results given earlier in Table I show that the ration of yellow: green in the F_2 was 3:1. The Punnett squares show the same type of ratio, and in addition, how the ratio was arrived at. It shows the genotypes contained in the two phenotypic groups.

The results produced by Mendel's assumptions and shown in the Punnett square allow the following predictions to be made:

1. The green F_2 will be pure breeding if they are either self fed or crossed to the pure-breeding green of the P-generation because they have the same genotype. (yy).

2. One-third of the yellow F_2 i.e ¼ of all the F_2 will also be pure breeding for the yellow phenotype since they are YY in genotype.

3. Two-third of the yellow F_2 i.e. 2/4 of all the F_2 will yield the same results as the F_1 if they are self fed. They will give yellow and green F_3 in a ratio of 3:1.

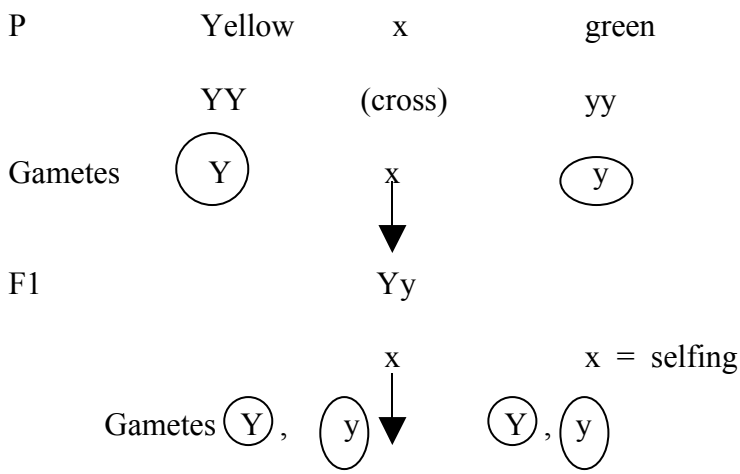
You can convince yourself with respect to the fractions which are expressed in quarters by indicating the fractions of the gametic types i.e. ½ Y and ½ y. A fusion event in the Punnett square is "like" an algebraic multiplication such that Y x Y ⟶ YY (***NOT*** Y^2, that is why an ***arrow*** is used instead of =). If therefore you now include the fraction of the gametic type we shall have ½ Y x ½ Y ⟶ ¼ YY.

Mendel tested these predictions and obtained the expected results, thus confirming the correctness of this assumptions – there are a pair of factors (alleles); there is segregation and there are dominant and recessive alleles.

We can re-summarise these and other facts as follows:

A diploid organism contains pairs of homologous chromosomes such that the members of each homologous pair separate into two cells during meiosis. A gene may occur as different forms of the same functional unit; the different forms are called alleles. A diploid organism contains only two alleles for any give phenotype, and the alleles may be identical as in YY or different as in Yy. Because there are only two of any alleles and because there is only a pair of any give chromosome type, we can say that one allele is on one chromosome and the other alleles is on its homologous partner. Recall the parallel behaviour of the genes and the chromosomes.

We can summarise Mendel's experiments with seed colour as shown below:



F2 ¼ Y Y; 2/4 : ¼ yy

¾ yellow ¼ green

3 yellow : 1 green

Mendel derived the First law of inheritance, also called the Principle of segregtion from these results. Mendel's First law of inheritance states that:

"In the formation of gametes, the members of a gene pair i.e. a pair of alleles, segretate from each other so that only one or the other member is contained in each gamete."

The law states that: In the formation of gametes, the members of a gene pair i.e a pair for alleles, segregate from each other so that only one or the other member is contained in each gamete.

Although the law has been formally stated, it is not intended that you should memorise it. Rather, you should understand it and be able to apply it. As you can see, it deals only with gamete formation. If you can not correctly derive the gametes then the offspring you derive would not be viable!

3.2 Some Definitions

3.2.1 Locus

This is the specific point on a chromosomes, occupied by a gene. Thus alleles occupy the same locus on homologous chromosomes. We had said earlier, that genes do not normally move from chromosome to chromosome. The locus of a gene is constant. The only aspect that varies is the allele that may be at that locus on a particular chromosomes.

3.2.2 Homozygous/Heterozygous

A genotype is said to be homozygous when both alleles are identical e.g. YY or yy, and it is heterozygous when the alleles are different e.g. Yy. Homozygous organisms are called homozygotes. By the same token heterozygotes are heterozygous individuals. From the definitions and the discussions above homozygotes are pure breeding types if self fed or crossed to similar homozygotes.

3.2.3 Backcross

This is a cross between an offspring and one of its parents an individual that is genotypically identical with one parental type.

3.2.4 Testcross

This is a cross between an individual whose genotype is not known and another individual who is known to be homozygous resessive for the trait in question. The testcross by its design makes it possible to determine the unknown genotype. For example we know that in the garden pea, axial flowers is dominant over terminal flowers. Suppose a plant had axial flowers and we had to determine the genotype of the plant. We would make a testcross.

i.e. Axial Flowers x terminal

7 x aa

The genotype which we give the plant with axial flowers will be determined by the types of progeny we get. The critical as pect of the test-cross however lies in the fact that the homozygous recessive parent (terminal flowers in our example) produces only one type of gamete and the gamete contains only a recessive alleles. Because the alleles is recessive, any allele from the other parent which it fuses with can be easily determined. Suppose our test-cross yielded two types of offspring as shown below:

Test cross: Anxial flowers x terminal

Genotype: 7 x aa

Gamete: (a)

Test cross: Anxial flowers x terminal

Genotype: 7 x aa

Gamete: (a)

F_1 Phenotype: Axial : terminal

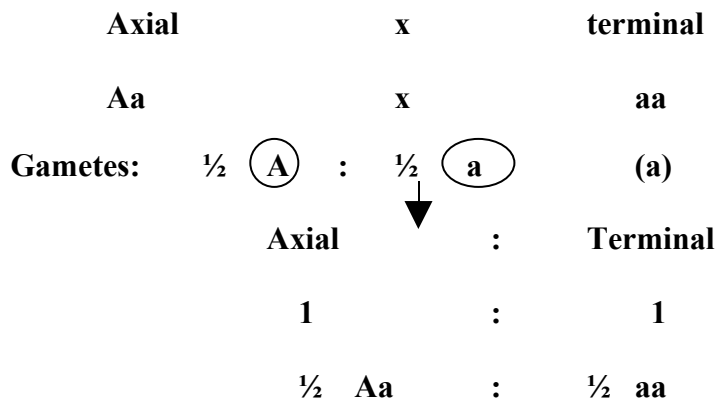
Ratio: 1 : 1

Partial genotype -a -a

Since the recessive parent produces only one type of gamete half of the F_1 genotype is known, as indicated. In order to have a terminal phenotype, a recessive trait, there must be homozygosity for the recessive allele. Hence that genotype is aa, and the axial parent must

have contained "a" as part of its genotype. The axial F_1 also has "a" as part of its genotype but the phenotype is a dominant one, thus requiring that at least a dominant "A" allele be present. Such an allele could only have been contributed by the parent with unknown genotype which also has an axial phenotype.

Therefore the genotype of the axial parent is Aa and the cross is



3.2.5 Phenotypic Ratio

This is the ratio of the different phenotypes in the progeny of a cross, based on the fraction of the different phenotypes. For instance in the testcross above, the phenotypic ratio is 1 : 1, but among the F_2 in Mendel's experiment the ratio was 3 yellow: 1 green.

3.2.6 Genotypic Ratio

This is the ratio of the different genotypes among the progeny of a cross. The genotypic ratio may or may not be identical with the phenotypic ratio. It depends on the parental genotypes.

3.2.7 Monohybrid Cross

This is a cross in which the parents differ with respect to only one trait which is controlled by only one gene (and its alleles). The example of Mendel's cross is a monohybrid cross. One pure breeding parent was yellow and the other green, but the trait was seed colour controlled by the one gene with the alleles Y and y. The F_1 combining the traits and alleles from both parents is a monohybrid. It is a hybrid with respect to one locus.

3.2.8 Genetic Symbols

As we found earlier symbols are used to designate the game responsible of a given trait. The same basic symbol may be modified to designate the alleles of that gene. We therefore use symbols to represent the genotypes of an individual.

The choice of symbols is somewhat arbitrary so you will sometimes find different symbols for the same gene in different books. There are however some common patterns which we shall adopt, except when convention demands something different.

Usually a single letter chosen from one of the phenotypes is used and the capital form represents the dominant allele while the lower case represents the recessive. It is often best to state which phenotype corresponds to a symbol, e.g. yellow = Y and green = y. Equally important is the need to ensure that the same letter is used for alleles since that is the only way of making it unambiguous that the phenotypes belong to the same gene. You would be correct if you use yellow = G and green = g, but I would mark you wrong if for the ***alternate*** phenotypes of yellow and green you wrote the allelic symbols as "Y" and "g" respectively. I would take it that these are alleles to two different genes occupying two different loci, so that a genotype such as Yg would not be taken as heterogenous. It would be taken as incomplete, since as we shall see later it represents a gamete carrying alleles from two loci.

One deviation from the above pattern is found in ***Drosohilia*** genetics. By convention the wild type alleles (i.e. the most common type found in the wild) are written with a "+" as superscript e.g. "w^+." The less common allele is written as "w". The symbol implies neither dominance nor recessiveness. This aspect has to be stated.

4.0 Conclusion

We have covered very specific information as well as principles which apply equally to plants and animals. By specific information I mean the type contained in Table 6.1, for instance. I do not expect you to memorise that table nor do I expect you to know whether a particular trait is dominant or recessive. On the basis of the facts you can easily determine that if you know the principles. In the example, yellow is said to be dominant because in the F_1 from a cross between pure breeding yellow and green was also passed on to the $_{F1}$.

I expect you to be able to give the genotypic and phenotypic ration from a cross and also to be able to derive the types of offspring a cross between two

parental types will produce as well as the converse i.e to be able to derive the probable parental genotype given sufficient information about the offspring.

You would almost certainly have a lot of difficulty if you did not try to understand how results are obtained, you will never be able to memorise all the different situations. Yet you can quite easily master the principles for deriving gametes, hence offspring and parental genotypes. "F_1" or "F_2" do not designate any specific genotypes or phenotypes, nor does "backcross" imply a specific genotype. Yet a testcross must definitely include a homogenous recessive parent. You should memorise definitions but you should equally know how and when to apply them.

5. Summary

Nuclear contribution by both gametes that form the zygote is equal, although the ovum has more cytoplasm than the sperm. Genes are the hereditary factors found on the chromosome and occur in pairs in diploid organisms. The predominant gene of the pair, where they are different i.e heterozygous, is said to be dominant while the latent one is said to be recessive. Both the dominant and recessive genes are called alleles with the dominant being represented with capital letter (e.g Y) and the recessive by small letter (e.g. y)

Self Assessment Question

1. Define the terms
   a) alleles b) genotype c) phenotype d) locus

2. Define the following terms with examples
   a) dominant b) recessive c) heterozygous d) homozygous

6.0 Tutor-marked Assignment

Using the Punnett square show the offspring of a cross between Yy and Yy; explain the genotypes and phenotype of the offspring.

7.0 Reference

Williams, G.O 2001, BIY 302 – Genetics –1, Module 5, Distance Learning Institute, University of Lagos.

Answers to Self Assessment Questions

1. **a.** alleles are modifications of the same genes, hence variations of the same symbols are used to designate them e.g Y for yellow and y for green.

b. Genotype stands for the genetic constitution of an organism e.g. an organism may be yellow but still bear the green allele; hence the genotype of this particular type would be Yy.

c. Phenotype is the visible characteristics produced by the genes (genotype) present within an organism. Eg. Yellow seeds may have the genes Yy or pure YY; green seeds will be yy.

2. Dominant – is an allele that shows and overshadow the other counterpart in a pair. Eg yellow (Y) overshadows green (y).

b. Recessive is the latent allele of a gene that is overshadowed, but remains unchanged, only to resurface in future generations.

c. Heterozygous is when the two alleles of a gene are different e.g. Yy.

d. Homozygous is when the two alleles of a gene are the same e.g Yy or yy.

MODULE 4

UNIT 16

UNIT 16: PRINCIPLES OF INDEPENDENTASSORTMENT (MENDEL'S SECOND LAW OF INHERITANCE)

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1.0 Introduction

The Chromosome is a long t twining chain of genetic materials. It has been established as the site where genes will be found. There are many genes to be found on one chromosome. For example in the human being the gene for tallness, and fair skin may be found on one chromosome. The father of a child may be tall and dark while the mother is short and fair-skinned. A child may take the father's height and the mother's fairness. This means that the two genes present on the same chromosomes have been assorted independently. This phenomenon could have been assorted independently. The phenomenon could have happened at the time of cross-over in metaphase stages of meiosis. Independent assortment, the thrust of Mendel's Second Law of Inheritance, may involve more than two genes on a chromosome.

2.0 Objectives

In spite of its importance as a basic laws, the Principles of Segregation deals with the transmission of only one locus, in isolation from other loci. However, there are cases in which it is necessary to consider more than one locus at a time. The second law governs such situations. On completion of this topic, you should be able:

1. to state and explain the second law

2. to explain the fact that in spite of the apparent differences, the first law is implied and therefore, obeyed in the second law by each of the loci being considered together.
3. to determine the phenotypic and genotypic ratios among the progeny when two or more loci are involved in a cross.

4. to determine the gametic and offspring genotypes from given parents and parental genotypes from offspring genotypes or phenotypes and ratios.

3.1 Mendel's Second Law of Inheritance

Although Mendel's first law is a very important basic law, it deals with the pattern of inheritance of alleles at only one locus. However, it is very rarely the case that two individuals differ at only one locus. Mendel recognized this fact and so performed experiments in which he studied the simultaneous inheritance of more than one phenotype. The simplest example of such a case is one in which there are two phenotypes, each controlled by one locus.

Instead of using Mendel's example, we shall consider an example from the fruit fly, ***Drosophila melanogaster***. This will give us a chance to use another series of genetic symbols. The term wild type is used to describe any phenotype which is most common in flies in the wild; such a phenotype may be either dominant or recessive. The wild type fly has red eyes and long bristles on various parts of the body. A mutuant gene, purple, changes the eye colour to purple. This gene is represented by the symbol, pr, and its wild type allele for red eyes is symbolized as pr + the superscript "+" indicating that it is a wild type allele. Another gene, spineless (ss), drastically reduces the length of the bristles. Its wild type allele is represented as SS^+. Notice that the symbol is derived from the mutant and not the wild type. The reason for this is that there may be other non-allelic mutant genes which alter the same wild type feature. For instance, the red eye colour may be changed to white by the gene, w, whose wild type allele is w^+.

Coming back to the example we are considering we can make the following dihybrid cross i.e. a cross in which the parents differ with regard to two characters. Our initial cross is with two pure breeding parents as follows:

P: purple, spineless x wild type eyes and bristles

F_1: (Dihybrid) wild type eyes and bristles

The F_1 is described as a dihybrid because it is the product of parent who differ with regard to two characters. In future crosses, the wild type eye colour and bristles will be referred to as red and normal respectively. The wild type phenotype of the F_1 indicates that both purple and spineless are recessive, since they are not expressed when present in combination with their respective wild type alleles.

At this stage of our experiment, the result of our cross is not different from that of a monohybrid cross; all the F_1 have the same phenotype. (Review your monohybrid cross.) We therefore, do not know whether we are dealing with one or to loci. In order to answer the question we can cross the F_1 inter as i.e. cross to F_1. The result from such a cross using hypothetical numbers is shown below:

F_1 red, normal x red, normal

↓

F_2 red, normal; red, spineless; purple, normal; purple, spineless

290 : 108 : 101 : 32

Approx. ratio 9 : 3 : 3 : 1

As shown above four phenotypic classes of F_2 are obtained. The numbers immediately below the classes show the number of each class of progeny. These numbers work out to a ratio (use the smallest number, 32, to divide all the numbers) of 9 : 3 : 3 : 1. Using the sum of the ratios as the denominator, 9/16 of the F_2 are red, normal, 3/16 are red, spineless etc. This result looks very different from the F_2 of the monohybrid cross in which there were only two phenotypic classes in a ratio of 3:1.

A close examination of the distribution of the phenotypes among the F_2 of the cross above shows that there are 398 red and 133 purple, giving a ratio approximately 3:1. There are also 391 normal and 140 spineless and again the ratio is 3:1. Thus when we look at each trait separately, we find that it is behaving according to the first law of inheritance. We can therefore, re-write our experiment so far as two separate monohybrid experiments, shown below:

P (1)	red	x	purple	(2)	normal	x	spineless
F1		red				normal	
F2	red	:	purple		normal	:	spineless
	398	:	133		319	:	140
	3		1		3		1

The fact that the dihybrid cross can be broken into two monohybrid crosses raises the question: "why does the F_2 ratio change when we look at both traits simultaneously?" The change, however, is only an apparent one since the 9 : 3 : 3 : 1 ratio is the same as the expression (3 red: 1 purple) (3 normal : 1 spineless). When that expression is expanded i.e. multiplied, it gives 9 red, normal : 3 red, spineless : 3 purple, normal : 1 purple, spineless. In other words the situation is such that what is inherited at on locus does not prevent or determine what is inherited at another locus. There is a ***random combination*** of both loci in the progeny.

This conclusion is confirmed by examining a testcross. Recall that one of the parents in a testcross is homozygous recessive; in this case two lock are involved, as one parent is purple, spineless in phenotype. Notice that this testcross is also a backbone since the homozygous recessive parent is identical with one of the parents in the F generation of our cross. The testcross is as shown below:

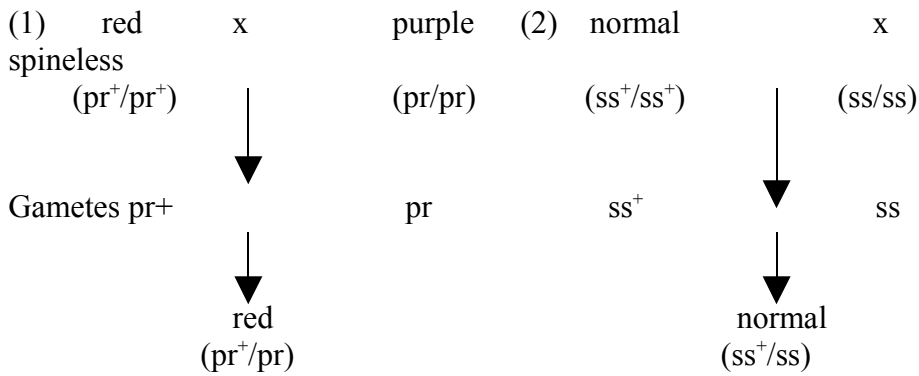
Red, normal (F_1) x purple, spineless

Red, normal: red, spineless; purple normal; purple, spineless

Ratio: 1 : 1 : 1 : 1

Here again as in the F_2 of our original cross, there are four phenotypic classes, but the ratio is 1 : 1 : 1 : 1. Recall that in the monohybrid testcross the ratio was 1 : 1. In order to account for the result of the testcross we must go back to determine the genotype of the F_1 which we used in the testcross. We found that purple was recessive to red and spineless recessive to normal bristle. We also found that the cross could be broken into

two crosses and then recombines. Since the P generation parents were pure breeding our crosses and progeny are:



The genotypes are listed under the corresponding phenotypes. When combined, the genotypes of the pure breeding parents are pr^+/pr^+ , ss^+/ss^+, and pr/pr, ss/ss. The red, normal F_1 are pr^+/pr, ss^+/ss . Although we have derived the genotype of the F_1, one could still ask: what type of gametes did the F parents produce to give the F_1? At this stage it is pertinent to recall some of the relevant principles in answering the question.

1. According to the Chromosomes Theory, every type of chromosome must be represented in the gamete. Since the genes are on the chromosome, it follows that every gene/locus (two loci in that case) must be represented in the gamete.

2. According to Mendel's first law, there is a segregation of alleles, so only one member of a pair enters the gamete. Therefore there must be segregation at every locus present.

In each P – parent, the alleles at each locus are identical, so each parent produces only one type of gamete. The red, normal parent will produce ***pr^+, ss^+*** gamete and the purple, spineless will produce ***pr, ss*** gametes. Fertilization will produce the doubly hererozygous F_1 genotype (i.e. there is heterozygosity at each locus) derived above. The genotype conforms with the red, normal F_1 phenotype. On the basis of the preceeding, the genotype of the F_1 testcross parent is pr^+/pr, ss^+/s and that of the purple, spineless parent is pr/pr, ss/ss.

The next step is to determine the genotype of each of the four classes of the testcross progeny. We can easily determine one half of the genotype of each class of progeny because the purple, spineless testcross parent is (it has to be) homozygous for each of the two recessive genes. This parent will therefore produce only one type of gamete, having a genotype of pr, as we found with

one of the P – parents. Since these two alleles which are contributed to all the testcross progeny are both recessive, they will not obscure the effects of the alleles contributed by the F_1 parent. Put differently we can say that the phenotypes of the testcross progeny will be determined by the genotypes of only the gametes produced by the F_1 parent. The testcross can therefore be represented as shown in the table below (Table 16.1)

Table 16:1 Testcross showing genotypes and phenotypes

(i)	Phenotype:	Red, normal (F1) x Purple spineless
(ii)	Genotype:	pr^+/pr ss^+/ss pr/pr ss/ss
(iii)	Phenotype:	Red, normal; red, spineless; purple, normal, purple, spineless
(iv)	F_1 Gamete:	pr^+, ss^+; pr^+, ss pr , ss^+ pr , ss
(v)	Other Gamete:	Pr, ss ; pr , ss ; pr , ss ; pr , ss
(vi)	Progeny Genotype	pr^+/pr, ss^+/ss; pr^+/pr, ss/ss; pr/pr, ss^+/ss; pr/pr, ss/ss
(vii)	Ratio	1 : 1 : 1 : 1

Note: gametes on lines (IV) and (V) are from the parental genotype in (ii).

We can derive the testcross progeny genotype shown above as follows: We already determined all the other lines except lines (iv) and (vi). Let us first determine the genotype of the red, normal class or progeny. From line (v) one now know that the other parent contributed pr, as which are both recessive. The phenotype, however, is dominant. Therefore, the F_1 parent must have contributed two dominant alleles in order to produce the observed phenotype. This contribution is represented in line (iv) under that phenotype. When combined, line (iv) and (v) give us the appropriate genotype for that class of progeny.

In the case of the red, spineless class, in order to have the red phenotype the F_1 parent must contribute the dominant alleles, pr^+ and for spineless, there must be homozygosit for the recessive alleles. Therefore in this case the gamete from the F_1 parent is pr^+, as in line (iv). You can go through the steps of determining the gametic contributions of the F_1 parent in the last two classes.

From the table above, it is obvious that the F_1 parent produced four types of gametes. The four types are possible because, as we derived, the F_1 are heteroxygous at both loci. We can also see that with respect to each locus two of the four types of gametes contain one allele, e.g. pr^+ while the other

two contain the other allele, pr, using the same example. Line (vii) shows that the four different (phenotypes) genotypes among the progeny are present in a ratio of 1 : 1 : 1 : 1. In other words each class represents ¼ of the testcross progeny. Since the phenotypes (and genotypes) which we obtained among the testcross progeny were in the final analysis determined by the gametic contributions of the F_1 parent, we have to conclude that (1) four different types of gametes are produced by the F_1 parent in a ratio of 1 : 1 : 1 : 1; (2) fertilization is a random process, determined only by the proportions of the different types of gametes present. In other words all the different types of gametes from the F_1 parent are equally likely to fuse with the gamete from the other parent.

We examined the test-cross in order to facilitated our understanding of the results obtained when two F_1 were crossed, and we obtained F_2 progeny in a ratio of 9 red, normal: 3 red, spineless: 3 purple, normal: 1 purple, spineless. In our examination of the testcross we found that fertilization by the gametes from the F_1 is a random process, we can therefore, use the Punnett squares to diagram the gametic fusions when two F_1 are crossed. The gametes are as we derived earlier.

Gametes

	pr^+, ss^+	pr^+, ss	pr, ss^+	pr, ss
pr^+, $1ss^+$	pr^+/pr^+ , ss^+/ss^+ 1	pr^+/pr^+ ss^+/ss 2	3 pr^+/pr ss^+/ss^+	pr^+/pr ss^+/ss 4
pr^+, ss	pr^+/pr^+ , ss^+/ss	pr^+/pr^+ , ss /ss ////////////////////	pr^+/pr , ss^+/ss^+	pr^+/pr^+ , ss^+/ss ////////////////////
pr, ss^+	3 pr^+/pr $ss^{+/ss+}$	pr^+/pr ss^+/ss 4	//////////////////// pr/ pr , ss^+/ss^+ 7	3 pr/ pr $ss^{/ss+}$ /////////////////
pr, ss	4 pr^+/pr , ss^+/ss	6 pr^+/pr , ss /ss ////////////////////	pr/ pr , ss^+/ss 8	9 pr/ pr , ss/ss ////////////////////

Since there are four types of gametes from each parent, there would be sixteen possible fusions (boxes). In addition since the gametes are present in equal proportions each box represents 1/16 of the F_2. Therefore by counting the number of boxes corresponding to each phenotype we can determine the frequency of that class of progeny. The fourth phenotype classes are identified by different types of shading. For instance in order to have a dominant phenotype for each locus, there must be at least one dominant allele at each locus. This class of progeny is represented by the unshaded boxes which are nine in number. Therefore 9/16 of the F_2 are red, normal. Thus we

find that the ratios obtained from the Punnett squares correspond to the observed results of the same cross split into two monohybrid crosses.

The genotypic ratio in the F_2 is different from the phenotypic ratio. The different genotypes among the progeny have been numbered in the Punnett squares. There are nine different genotypes in a ratio of 1: 2 :2 :4 :1 : 2 : 1 : 2 : 1. Make sure you understand how the genotypes correspond to the phenotypes. The fractions are in sixteenths like the phenotypic fractions and they add up to one.

From the above results we can conclude that the inheritance pattern (both in the formation or gametes and in the fusion of gametes) does not influence the pattern at another locus. The apparent discrepancy we found due to the independent behaviour of each locus. It is pertinent to emphasis that regardless of the method used to derive the F_2 frequencies, the sum of the frequencies must be equal to 1 since together they constitute the F_2 progeny from a specific kind of cross.

Mendel derived his second law of inheritance (the Law of Independent Assortment) governing the simultaneous inheritance of genes at two or more loci, after analyzing the results of crosses similar to the one we have discussed. The law may be stated as follows:

> "In the formation of gametes, the two alleles of a given gene assort independently of the pairs of alleles of other genes on non-homologous chromosomes".

You should not try to memorise the law as just stated. You should instead try to understand the meaning of the law so that you can explain it in your own words. The law as stated above is not in Mendel's words since Mendel did not know of chromosomes nor did he use the term alleles and genes.

Given an individual whose genotype is AaBb, we can diagram the production of gametes using the second law as follows:-

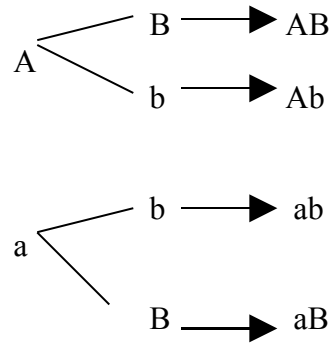
	B	AB
A	b	Ab
	b	ab
a		
	B	aB

According to the first law and each pair of alleles will segregate. But recall that meiosis-I leads to the production of only two cells. Therefore, the A allele has to go into the same cell with one of the alleles at the other locus.

However, since the two loci assort independently, one locus does not influence the other. Hence, there are two equal possibilities of which allele will go into the same cell with A then b must go with a and vice versa. As shown in the diagram there are four equally possible combinations. (Diagram meiosis in a cell with two pairs of chromosome inserting one pair of alleles on one pair of homologous chromosomes – only one member of the pair of alleles on each chromosome:).

4.0 Conclusion

We have seen so far that an individual heterozygous for one locus produces two types of gametes in equal frequencies. When two such individuals are crossed, four combinations (2 x 2 as seen in the Punnett squares) are expected among the progeny. The four types of gametic fusions really amount to three different genotypes in a ratio of 1:2:1. However, because of dominance only two phenotypes are observed, in a ratio of 3:1. We have also seen the situation when there is heterogygosity for two lock – there are four types of gametes, sixteen (4 x 4) types of gametic fusions, nine different gemetypes and four phenotypes. What happens when there is triple heterozygosity e.g. Aa Bd Dd? Gamete formation is as follows:



There are eight gametic genotypes, leading to 64 (8 x8) possible types of gametic fusions. In this case there will be 27 different genotype and eight different phenotypes if there is dominance. Below is a table showing the number combinations possible when two parents heterozygous for the same number of loci are crossed. Note the last column of the table applies only when there is complete dominance between a pair of alleles.

Number of pairs Of heterozygous Loci	Number of gamete genotypes from each parent	Number of gametic fusions	Number of different zygotic Genotypes	Number of different kinds phenotypes

1	2 or 2^1	4 or 4^1	3 or 3^1	2 or 2^1
2	4 or 2^2	16 or 4^2	9 or 3^2	4 or 2^2
3	8 or 2^3	64 or 4^3	27 or 3^3	8 or 2^3
4	16 or 2^4	256 or 4^3	81 or 3^4	16 or 2^4
n	2^n	4^n	3^n	2^n

As shown in the last line, the power in the expressions is the same as the number of heterozygous loci. Instead of merely memorizing the formula in the last line, you should learn to derive the expressions for one and two loci by understanding what they mean and why they are so.

5.0 Summary

The first and second laws of Mendel are the guiding principles of Genetics. It is very important that you familiarize yourself with these laws and know their applicabilities. This will greatly ease your understanding of Genetics.

Self Assessment questions

1. State and explain Mendel's second law.

2. Define (a) genotype (b) phenotype

3. Explain why phenotypes are different from genotypes.

6.0 Tutor-marked Assignment

Certain Parameia are thin because of a homozygous nuclear gene. What is the phenotypic expectation for the clones derived from ex-conjugants of a single mating of th^+ th^+ by th^+ th? How would cytoplasmic mixing affect your expectation? Why?

7.0 References

Williams, G.O 2001, BIY 302 – Genetics –1, Module 5, Distance Learning Institute, University of Lagos.

Answers to Self Assessment Questions

1. Mendel's second law states that in the formation of gametes, the two alleles of a given gene assort independently of the pairs of alleles of other ones on non-homologous chromosomes. This means that two loci assort independently and one locus does non influence the other. Eg consider two heterozygous alleles Aa and Bb. If (A) gamete goes with b to form (Ab) zygote, then ((a) must go with ((B) to form (aB).

2. **a.** Genotype is the type of alleles of genes carried within a cell e.g. AB, Ab, aB etc.

   b. Phenotype is the visible characteristics exhibited as a result of genes contained in an organism.

3. Phenotypic ratios are different from genotypic ratios because of the incidence of dominance. For example if

UNIT 17: PROBABILITY

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1.0 Introduction

We saw when discussing meiosis that, with regards to any pair of homologous chromosomes, an organism can produce two types of gametes. Thus when there are n-pairs of chromosomes, 2^n types of gametes can be produced. For instance, in man there are 23 pairs of chromosomes, therefore, every human being is capable of producing 2^{23} i.e 8,388,608 different types of gametes, assuming that the two chromosomes which make a pair are different.. If every human being has the potential to produce such a large variety of gametes, it follows that even if the constitution of say the egg is kept constant, it would still be a matter of chance which one of the large variety of sperms would fertilize the egg. Thus in genetics there is a chance factor or probability to be considered with regard to whether a particular type of offspring will be produced by a given mated pair.

2.0 Objectives

From meiosis and the laws of inheritance it should be obvious that, depending on the genotype, a variety of gametes can be produced by any organism. Since fertilization is a random process it follows that it would be a matter of chance which types of offspring would be produced and in what proportions by any pair of parents. You should be able, on completion of this topic:

1. to explain and use the basic probability principles summarized at the end of this topic;

2. to appreciate the fact that genotypic and phenotypic rations correspond to probabilities when expressed as fractions;

3. to use the fact contained in No. 2 above to determine phenotypic and genotypic ratios from complex genetic crosses. In such cases the Punnett Squares are an ineffective tool because of their size.

4. to apply probability to simple human conditions.

3.1 General Principles

Before looking at probability as applied to genetics, let us consider an everyday example. A coin has two sides, a head and a tail. When the coin is tossed it will come up head or tail usually. If the coin is not loaded so that it is biased in favour of one side, we would expect that in a large number of trials, ***approximately*** 50% of the results would be head and 50% would be tails. The result of one toss does not influence the result of another toss. In other words the ratio of heads or tails would be ***approximately*** 1 : 1. Notice that I am not saying that exactly 50% of the result would be heads and 50% tails. The results could deviate from the ideal 1 :1 ratio for any given set of trials. However, statistical tests would show that the deviations from expectation is indignificant i.e. we would be correct if we said that the ratio was approximately 1 : 1

The fact that we can draw the above conclusion means that we can also make the generalization that, when an unbiased coin is tossed the probability that it will come up heads is ½. By the same token the probability that it will not come up heads is ½, which is the same as saying that the probability it will come up tails is 1/2,. State as a formula, we can say that the

$$\text{Probability of a Given event} = \frac{\text{Number of favourable occurences}}{\text{Number of possible occurences}}$$

Going back to our example, we would ask for the probability of a head. A coin has only two sides so there are only two possible occurrences and only one of them is favourable i.e. what we want. Therefore, the probability is ½. We could expand the numbers, as in the large experiment, in which case we would get a probability that is acceptable as ½.

3.1.1 First Law of Probability

In considering the probability of heads or the probability of tails we are considering the probability of single events. However, we could

ask a different question; what is the probability that if we toss a coin, we shall get either a head or a tail? Since we have only two possible occurrences when a coin is tossed and both are favourable according to the question, the answer to the question is 1. It is ***certain*** that we shall get head or tail. Thus when it is certain that an event will occur the numerical probability is 1. Instead of using the formula above which would be 1+ ½ = 1, we could have arrive t the same answer by adding the probabilities of each of the two events i.e. ½ + ½ = 1. We can summarise this operation by saying that the probability of occurrence in one trial, of either of two mutually exclusive events is the sum of the probabilities of individual occurrence. The events must be mutually exclusive i.e. both cannot occur in one trial, it has to be one or the other as in the coin toss.

In the coin example we found that the sum of the probabilities of the different types of occurrences is 1. This principle applied even if the number of type of occurrences is greater than two. The reason is that in the formula given above "the number of possible occurrences" constitute both the numerator and the denominator. Put differently, for a given set of events e.g throwing dice, it is certain that one possibility will occur. As we said earlier when an event is certain, the probability is one. It is important that you remember that the sum of all the probabilities for a given set of events is never greater than 1. Using this principle we could answer the question: "what is the probability that a coin will not come up head?" as follows:

Probability of not head = 1 – Probability of head i.e. 1 – ½ which is ½.

A dice has six sided which are equally possible therefore the probability that it will not show 6 when thrown is 1 – 1/6 which is 5/6. Notice that this is the probability that either 1, 2, 3, 4 or 5 will show. The converse of when an event is certain to occur is when it is impossible i.e it can not occur. The probability in such a case is 0. For instance we can not come up with 7 when we toss a dice. Therefore if the numerator in our formular is 0 the fraction has to be zero. This in effect means that the probability of an event occurring can never be less than 0 i.e it can not be written with a minus sign. Therefore, the probability of an occurrence is greater than or equal to 0, and less than or equal to 1 but never greater than 1.

In the examples of the coin toss or the dice, the probability of obtaining a head is equal to that for a tail and the same is rue for all the six sides of the die. However, such equality is not always true for

all the possibilities of an event. Notice that the formula given above does not require such equality.

3.1.2. Genetic Considerations

The probabilities of the different possibilities depend on the type of event under consideration. For instance when two monhybrids are crossed, ¾ of the progeny have the dominant phenotypes while ¼ have the recessive phenotype. In simple terms then we can say that 3 out of 4 progeny would have the recessive. The reason as we saw from the Punnett squares is that there are 4 possible types of fusion between the parental gametes to give the progeny. Thus we could ask the question; what is the probability that the first offspring of two heterozygotes (monohybrids, Aa x Aa) will have the dominant phenotype?" The answer is ¾ since 3 out of the 4 possible gametic fusions will produce dominant phenotypes.

One important aspect of a consideration of probabilities, is the fact that the answer is highly dependent on the phrasing of the question. Compare the last question with this one: "What is the probability that two monohybrid parents will produce an offspring having the dominant phenotype?" The answer is 1 because these parents are potentially capable of producing ***an*** (at least one) offspring with dominant phenotype. In the first question we were considering a particular offspring, the ***first***. In the same way the answer to the question, what is the probability that the first child of Aa x aa parents will have the recessive phenotype, is ½. The reasons are:

1. a particular child is indicated, and

2. there are only two possible genotypes and phenotypes, Aa and aa.

Earlier, we found that the sum of all the probabilities for a given series of events is always 1. We also know that from a cross of two monohybrid parents, ¾ of other progeny have the dominant phenotype. However, such progeny can be either AA or Aa in genotype. Among the progeny with the dominant phenotype, the two genotypes are present in a ratio of 1 : 2 respectively.

QUESTION: "what is the probability that if a farmer put his hand ***in a bowl containing the yellow seeds*** from a cross of Yy x Yy,he would pick up a seed which is YY in genotype?"

The phrasing of the question eliminates the green seeds from the number of possible occurrences to be considered. The relevant seeds occur in a ratio of 1 : 2, therefore, the answer is 1/3. The probability of Yy would be 2/3; so again the sum of the probabilities for this series of events involving only yellow progeny is (1/3 + 2/3) equal to 1. (The answer to the question would have been ¼ if the question had been, what is the probability that the farmer would pick a YY seed from among the progeny of a Xy x Xy cross?) It is important for you to remember that the sum of all the probabilities is one even if you have re-adjusted the total number of possibilities.

The probability of occurrences of either of two mutually exclusive events in one trial is the sum of the probabilities of their individual occurrences.

QUESTION: What is the probability of picking in one attempt, a seed with the dominant phenotype from a bowl containing the seeds from a cross of Yy x Yy?"

The question is the same as asking for the probability of either a YY or Yy genotype. The probabilities are ¼ and ½ respectively but both genotypes would give a dominant phenotype. Therefore the answer to the original question is ¼ + ½ i.e ¾. If the question had asked for homozygous genotype, the answer would be for either YY or yy i.e. ¼ + ¼. Which would be 1/2 .

3.1.3 Second Law of Probability

If two coins are tossed simultaneously, the appearance of a head or tail on one coin does not in any way influence what appears on the other coin. Thus we have the following possible combinations: HH, HT, TH and TT. All of these combinations are equally probable, assuming the coins were not biased. Using the probability formula, the probability that both coins would appear heads in a single toss is ¼. The same would be true for both coins appearing tails. However, the answer with respect to the situation where the coins show up differently will depend on the question asked. For example, "what is the probability that one coin will appear head and the other tail?" According to the possible combinations listed earlier, there are two favourable occurrences (combinations) which satisfy the condition HT and TH, therefore the answer is 2/4 i.e. ½ . A different question is: "what is the probability that the ***first*** coin will be head and the ***second*** tail?" In this case the answer is ¼ because we have specified

what we expect of each coin, making only the HT combination the favourable occurrence.

We can derive the probabilities for different combinations without listing all the possible occurrences. The probability of a head for each coins are tossed or one coin is true for tail. When two coins are tossed or one coin is tossed twice, the probability of an HH combination is ½ x ½ which is ¼ as we found earlier. The same is true for the other combinations. Note that there again, the sum of the probabilities for all the possible combinations is 1. The principle which we have discussed in this and the proceeding paragraph may be summarized as follows:

4.0 Conclusion

The probability of the simultaneous occurrence of two or more independent events is equal to the product of the probabilities of their individual occurrences. This principle applies only when one is dealing with independent events i.e the outcome of one trial does not influence the outcome of the other trials. In the example we considered, one coin does not influence the outcome of the next toss of the same coin.

5.0 Summary

Probability appears, at first hand, difficult but with careful reasoning you will be able to follow the principles involved. It is very applicable to Genetics, especially when considering Mendel's 2nd Law of Inheritance which states that two or more alleles segregate independently of one another. The same is true when throwing two dices or coins, each one of the coins can turn heads up regardless of what the other turns up.

6.0 Tutor-marked Assignment

1. An albino (aa) man of blood type MN marries a heterozygote for albinism (Aa) also of MN blood type. They plan to have 4 children. If you assume independent segregation, what is the exact probability they will have

   a) no albinos

   b) 2 non-albino children with MN blood type

   c) 3 children with M blood type?

2. After meiosis of the genetype Aa Bb in Neurospora you obtain 100 asci. If you assume independent segregation how many ascospores do you expect to have the following genetic constitution: AB? Ab plus aB?

7.0 References

Williams, G.O 2001, BIY 302 – Genetics –1, Module 5, Distance Learning Institute, University of Lagos.

Herskowitz I. H. 1973, Principles of Genetics. The Macmillan Co. New York.

UNIT 18: QUANTITATIVE/POLYGENIC INHERITANCE

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2.0 Objectives
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1.0 Introduction

In our considerations so far we have dealt with phenotypes which can be cleary distinguished from one another regardless of:

1. the type of relationship that exists among the alleles at one locus – complete or incomplete dominance or codominance.

2. the complexity of the genotype, in terms of the number of lock involved and the type of interaction among the alleles at the different loci e.g. epistatic interactions.

Put differently every genotype or class of genotype hs a distinctive phenotype. For instance, in the ABO blood group the blood types can be clearly distinctive classes of phenotypes are described as QUALITATIVE traits. The genes are said to show DISCONTINUOUS variation is their phenotype.

2.0 Objectives

1. In this unit as in the earlier ones you should be able to recognize the fact that the basic principles of inheritance are still operative.

2. The difference in this case lies in how the genotype determines the phenotype.

3. In order to be able to appreciate No. 2, you have to be able to state and explain the assumptions which form the basis for all the discussion in this module.

   You should be able to:

   (i) to account for the shades of difference between the variety of phenotypes in a given polygenic trait.

   (ii) to determine the number of genes controlling a trait

   (iii) to determine the genotypes for various phenotypes and vice-versa, as well as the frequencies of various phenotypic and genotypic classes.

   (iv) to use the probability method in the determination in (iii) above.

   (v) to explain the fact that in some instances polygenic traits mayhave only phenotypes, produced by a threshold effect.

3.1 Continuous Variation

There are traits which flow – CONTINUOUS variation. The different phenotypic classes are small so that the classes are not sharply distinguishable or immediately obvious. For example, in a population not everybody is the same height; there seems to be somebody in every possible position from the shortest to the tallest. Weight and skin colour are other traits which also show continouos variation. The differences between the various classes of such genetically determined traits is therefore, described as ***quantitative*** inheritances.

Evidence from a number of experiments on quantitative inheritance show that more than one gene is involved in the phenotype that is produced. One can therefore talk of a trait controlled by ***multiple genes*** or ***polygenes***. The latter term is used more widely. The roles of the alleles at the different loci in the production of the phenotype issuch that , on a simplified basis, one can recognise two types of alleles at each locus – CONTRIBUTING and NON-CONTRIBUTING alleles. These are merely terms adopted for convenience of description because it is highly unlikely that an allele has no effect. Thus a genotype with omly non-controlling alleles still has a “basis” phenotype.

In order to facilitate the study of quantitative inheritance some simplifying assumptions have to be made. There are those who argue, with good reasons

against some of the assumptions, but, be that as it may, we shall use them because they reduce the complexity of the problem. Also, in many cases valid predictions can be made using them. The assumptions are:

1. The effect of each contributing alleles on the phenotype is equal to the effect of any other contributing allele. Moreover, the effect is in addition to a base or minimum value. Thus, we may say that each allele contributing to a height adds 2.5 centimeters to the minimum height of one hundred and fifty centimetres.

2. The effects of the contributing alleles are additive. Therefore, if we continued with the example in No. 1, we could consider a genotype with six contributing alleles. In this case the total effect of the contributing alleles would be 15 centietres (i.e. 6 x 2.5) which would be added to the minimum height. Thus the total height would be 165 centimetres (i.e. 150 + 15).

2. There is no dominance at each locus; instead we recognise contributing and non-contributing alleles as mentioned earlier. In other words at any locus one alleles does not obscure the effect of the other alleles and the effect of homozygosity for two contributing alleles is greater than that of heterozygosity for one contributing and one non-contributing allele. Yet when there is dominance, homozygotes and heterozygotes have identical phenotypes. The assumption of contributing and non-contributing alleles is also different from intermediate and codominance because in both of these cases as well as in dominance the other alleles has a distinctive phenotype. The non-contributing allele is not ascribed a specific phenotype.

   One important consequence of the first three assumptions is that different genotypes will have the same phenotype is the total number of contributing alleles is the same in all the genotypes.

4. There is no epistasis. It is necessary to assume this condition in which the genotype at one locus does not mask the effect of the genotype at another locus because if that were not the case it would be difficult to assign a value for the effect of each allele. In the absence of epistasis there is a direct correspondence between the genotype and the phenotype.

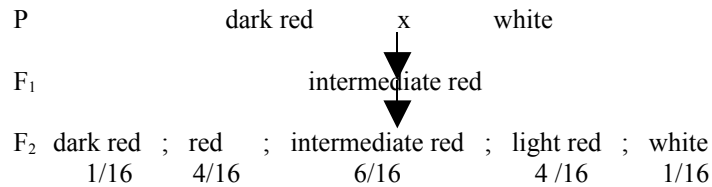
5. There is no linkage among the loci controlling the trait. Linkag, you will recall, is one of the factors which modifies the genotypic and phenotypic ratios and the modified ratios are difficult to calculate. As

we shall see later there may be as many as four or more loci involved in some traits and that would of course increase the complexity of the linkage calculations.

3. The environment has no effect on the genotype. In other words the phenotype is entirely attributable to the genotype. Certainly this assumption is a great simplification of the true situation. You know from personal experience that most people will not attach their potential maximum height and weight if they are under-nurished. In the same way various factors in the environment influence various traits in an organisms. In spite of this obvious fact the environmental effect is not assigned any value because its true or even approximate value is variable and difficult to quantify consistently, ignoring it therefore, greately facilitates quantification of the effect of the alleles involved.

I should emphasise the point that although the above assumptions are acknowledged as being merely for the purposes of simplification, conclusion based on them have been found to be very useful even in applied genetics.

The first significant breakthrough on the problem of quantitative inhetitance was by Nilsson-Ehle in 1909. He worked on the colour of wheat kernel. The F_1 from a cross between pure-breeding dark red-kerneled and white-kneneled parents were all of an intermediate red colour. This type of F_1 from such a cross is consistently with incomplete dominance. The F_2 generation, however, forces a rejection of that hypothesis. In the F_2 generation, one-sixteenthe (1/16) of the progeny has the same dark red colour of the P-generation parents. The same was true for white progeny in the F_2. The fact tht the fractions of the different classes of progeny are in sixteenths, mean that the trait is controlled by two loci. But unlike what we found when considering the second law of inheritance with complete dominance, Nilsson-Ehle obtained Five phenotype classes among the F_2, instead of four. The presence of five phenotypic classes was also different from what is obtained when other types of genetic interactions are operative. The phenotypes in the crosses and among the progeny are shown below:



As mentioned earlier the fractions are indicative of two loci. The two types of alleles at each locus can be distinguished by a superscript. Thus we can assume that the A^1 and B^1 alleles contribute to red pigment production while

A^2 and B^2 are non-contributing. The superscripts "1" and "2" as in "A^1" and "A^2" rather than capital and small letters such as "a" nd "A" were chosen to distinguish the contributing and non-contributing alleles because capital and small letters have already been used to represent dominant and recessive alleles. We assumed that there is no dominance so it would be best to avoid any confusion in that direction. Much as you are advised to use the same method of representation used in these modules, you would not be penalized as long as you clearly define whatever method you opt for.

Since the parents were pure breeding the dark red parent would be $A^1A^1B^1B^1$ and the white would be $A^2A^2B^2B^2$. The rationale for characterizing the dark red parent as being homzygos for only contributing alleles is that, that colour indicates the presence of the maximum amount of red pigment while white is due to the total absence of red pigment. Put differently the maximum number of contributing alleles any offspring can have is four if there are only two loci involved and four of such alleles would produce the maximum amount of red pigment.

Given the above parental genotypes, the F_1 would inherit a maximum of two contributing alleles, both from the drdk red parent. Since the contributing alleles have equal effects which are also additive, the F_1 with only two contributing alleles would have half as much red pigment as the parent. Hence its phenotype would be an intermediate red. The F_1 is heterozygous at both loci (doubly heterozygous) and independent assortment during gamete production would yield gaments with 2, 1 or 0 contributing alleles Random Fertilization would in turn yield F_2 progeny with 4,3,2, 1 or 0 contributing alleles. The phenotypes corresponding, would be different. Therefore, there would be five classes of F_2 as Nilsson-Ehle observed. The whole cross can be written as:

A^1 and B^1 = contributing alleles A^2 and B^2 – non-contributing alleles.

P :- dark red x white

$A^1A^1B^1B^1$ ↓ $A^2A^2B^2B^2$

F1 :- intermediate red

$A^1A^2B^1B^2$

Gametes: A^1B^1 : A^1B^2 : A^2B^1 : A^2B^2

Contributing alleles in gametes	2	1	1	0

F2 :- dark red	red	intermediate red	light red	white
$A^1A^1B^1B^1$	$A^1A^1B^1B^2$ $A^1A^2B^1B^1$	$A^1A^1B^2B^2$ $A^2A^2B^1B^1$ $A^1A^2B^1B^2$	$A^1A^2B^2B^2$ $A^2A^2B^2B^1$	$A^2A^2B^2B^2$
Fraction:- $\frac{1}{16}$	$\frac{2}{16} + \frac{2}{16} = \frac{4}{16}$	$\frac{1}{16} + \frac{1}{16} + \frac{4}{16} = \frac{6}{16}$	$\frac{2}{16} + \frac{2}{16} = \frac{4}{16}$	$\frac{1}{16}$
Contributing Alleles:- 4	3	2	1	0

On the basis of the above and other experiment using larger numbers of loci it is possible to draw up the table below:

Table 3.1
Expectations in the F2 – Generation due to Polygenic differences between two homogenous Parents.

P-generation: Number of loci (pairs of polygenes) in which two parents differ	Fraction of F2 like either pure-breeding parents	Number of genotypic classes in the F2 - generation	Number of phenotypic classes in the F2 - generation
1	¼	$3 = 3^1$	$3 = 2 \times 1 + 1$
		$9 = 3^2$	
2	$1/16 = (¼)^2$	$27 = 3^3$	$5 = 2 \times 2 + 1$
		59,049	
3	$1/64 = (¼)^3$		$7 = 2 \times 3 + 1$
10	$\frac{1}{1,048,576} = (¼)^{10}$	3486784401	21
20	$\frac{1}{1,099,511,627,776} = (¼)^{20}$	3^n	41
n	$(¼)^n$		$2n + 1$

From the table it is obvious that increases in the number of genotypic classes is not proportional to increase in the number of polygenes determining a trait. Rathe, the increase in the number of genotypes greatly outsripts the increase in the numer of genotypes greatly outstripts the increase in the number of polygenes. The increase in the number of genotypes also results in an increase in the number of phenotypic classes, making it more difficult to distinguish between the phenotypic classes.

If two heterozygotes are crossed, the table shows that the number of genotypic classes is larger than the number of phenotypic classes wherever the number of oci at which they difer isgreater than one. This means that there would be more than one genotype in a number of the phenotypic classes. In such cases it is necessary to know the number of genotypes in a given phenotypic class in order to be able to calculate the fraction of such progeny accurately. It is important that you bear in mind the fact that we are referring to number of genotypes not number of different genotypes. The distinction is a fine one but nonetheless a very important one. The former category includes similar gentypes which occur moe than onec s well as different genotypes. Thus in Nilsson-hele's experiment which we considered earlier, the class of F_2 progeny with intermediate red phenotype contains three different genotypes. Yet according to the distinction which we are tryin to make, the number of genotypes in that phenotypic class is Six, because the $A^1A^2B^1B^2$
genotype can occur in four different ways, making up 4/16 of the total F_2 progeny.

The above explanation makes it necessary to repeat what we are trying to accomplish: How to calculate the fraction of a given phenotypic class of progeny from a cros between two parents heterozygous for the polygenes under consideration. Suppose there are n loci involved. In any cross between two heterozygotes for one locus all genotypes occur in a fraction of ¼ i.e. Aa x Aa $\longrightarrow$ AA, Aa, aA, and aa. For n loci the fraction will be $(1/4)^n$. Recall that for two loci, there are 16 boxes in the Punnett squares, making the fraction of each genotype in the box, 1/16 which is the same as $(1/4)^2$. In other words n = 2. However , in order to get the correct fraction b elonging to that class, $(1/4)^n$ must be multiplied by a coefficient which is the number of genotypes in that class. The coefficient may be calculated by either the binomial method in your textbook (pp. 188 and 189 in the 4th edition) or by the factorial method. I shall discuss only the factorial method. The formula for the number of genotypes in any phenotypic class is:

$$\frac{(2n)!}{X!\,(2n - X)!}$$

Where n is the number of loci (i.e 2n is the number of alleles in the genotype).

X is the number of contributing alleles in that phenotypic class and 2n – X is therefore, the number of non-contributing alleles.

Therefore the fraction of a phenotypic class among the progeny is

$$\left[\frac{(2n)!}{X!\,(2n - X)!} \right] = (1/4)^n$$

3.2 Worked Example

Question

In human population the shortest height is one metre and the tallest is two metres. Five loci quantitatively determine height.

(a) What is the effect of each contributing alleles?

(b) If people of intermediate height marry

(i) What fraction of their children would be expected to be like their parents genotypically?

(ii) What fraction of their children would be expected tohave the intermediate phenotype?

(iii) What fraction of their progeny would be expected to have intermediate height and be homogous for contributing alleles, A^1 and B_1 in their genotype.
(Assume the loci are A, B, C. D, and E)

Answer:

(a) Assume that the shortest height is homozygous for only non-contributing alleles and also that the tallest are homozygous for contributing alleles. Therefore the additive effects of the contributing alleles will account for the difference in height between the tallest and the shortest. Therefore the contributing alleles will account for one metre in height. There are five loci and the tallest are homozygous for only contributing alleles. Hence 10 equally additive alleles produce some metre or 100 cm.

. . The effect of each contributing alleles is

$$\frac{100}{10} = 10 \text{ cm}$$

(b) People of intermediate height would be 1.5 metres in height. The additional 50cm. will be produced by five contributing alleles in addition to five non-contributing alleles in the genotype.

(i) Let us assume that the genotype of both parents is $A^1A^2B^1B^2$ $C^1C^2D^1D^2$ E^1E^2 (a number of other genotypes will produce the same effect).

If the parents are as sucy assumed the fraction of A^1A^2 from A^1A^2 x A^1A^2 is ½.

. . The fraction that is genotypically like the parents is:

$$\underline{\quad\quad} \; (½)^5$$

(if you do not understand how this answer was arrived at, go back and review the lectures on Probability)

(ii) As mentioned above people of intermediate height have 5 contributing and 5 non-contributing alleles. It was also stated earlier that the fraction (probability) of any given genotype from such a cross is ¼ at each locus. For the five loci, that would be $(¼)^5$. but there are a number of genotypes possible with five contributing and five non-contributing alleles. The formula for calculating this number or coefficient has been given. When applied, the expression to be solved is

$$\frac{10!}{5!\,(10-5)!} \times (¼)^5 \quad \frac{10!}{5!5!} \times (¼)^5$$

$$\frac{10 \times 9 \times 8 \times 7 \times 6}{5 \times 4 \times 3 \times 2 \times 1} \times (¼)^5$$

$$= 252 \times (¼)^5$$

(iii) Since the relevant progeny are both intermediate in height and homozygous for contributing alleles at loci A and B, four of the five contributing alleles have been determined. The fifth contributing allele will be due to heterozygosity at either locus C, D or E i.e three different possibilities. The fraction of $A^1A^1B^1B^1$ from a cross of two

$A^1A^2B^1B^2$ is $(¼)^2$ For the three other loci if there is heterozygosity for a contributing allele at the C locus, the two other loci must be homozygous for non-contributing alleles, giving 2/4 x ¼ x ¼ and the fraction for the full genotype would be

¼ x ¼ x 2/4 x ¼ x ¼

sicne there are three possibilities for geneotypes at loci C, D and E, the fraction of this relevant type of progeny is:

$$3 \times 2/4 \times (¼)^4$$

3.3 Other Considerations

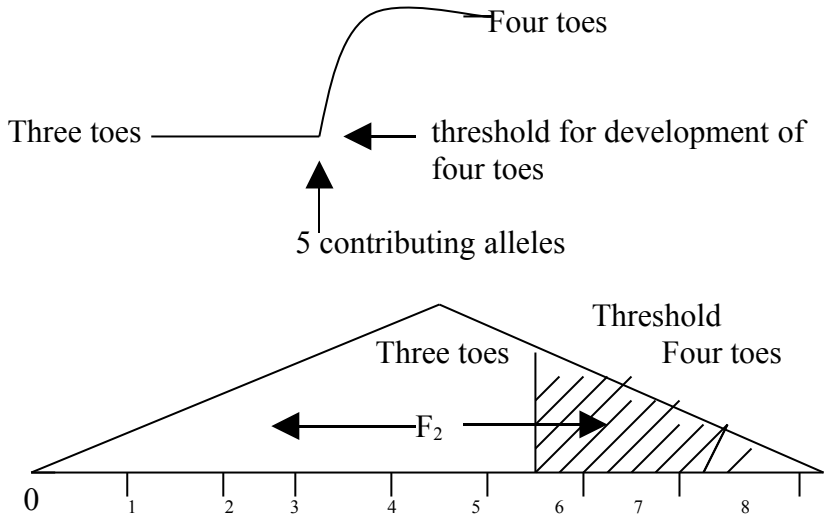
It is estimated that skin colour in man is determined by a minimum of three and a maximum of six additive loci. If we assumed that four loci are involved with the A^1, B^1, C^1 and D^1 alleles contributing to pigment production while A^2, B^2, C^2 and D^2 are non-contributing, a marriage between pure black and pure white would produce mulatto children with intermediate skin colour. Their genotuype in this particular cross would be $A^1A^2B^1B^2C^1C^2D^1D^2$. On the other hand, a marriage between two mulattoes would be cpabel of producing the whle spectrum of skin colours. This is because both parents can produce gametes which contain only contributing (i.e. $A^1B^1C^1D^1$) or non-contributing (i.e. $A^2B^2C^2D^2$) alleles in addition to other gametic genotypes. The point being made here is that because of the way in which skin colour is determined it is possible for a couple to have children whoa are either much lighter or darker in complexion than either of them. Infidelity on the woman's part therefore may not be the correct explanation when a child with a markedly different skin colour from the rest of the family is born.

Although the discussion of polygenic inheritance so far has characterized it as a condition in which there is a continuum of phenotypes, that is not always the case. There are polygenic traits for which only two phenotypes are produced. For example, in the guinea pig the normal hind foot has only three toes, but there are some strains with four toes. Crosses between the pure breeding three-toed and four-toed strains produced F_1 which are nearly all three-toed. The F_2 from this type of cross consists of three-toed and four-toed individuals in a ratio of approximately 3 three—toed : 1 four-toed. This ratio suggest that we are dealing with a pair of alleles in one locus exhibiting complete dominance by the allele for three toes.

Pure breeding	P	three-toed	x	four-toed
	F_1	nearly all are three-toed		
	F_2	three-toed	:	1 four-toed

Crosess with the F2 however do not support the one locus hpothesis. Instead it is more reasonable to assume that there are approximately four additive loci involved, but that is not all. In order to have fur toes there must be at least about five contributing alleles present. If there are less than five contributing alleles then only three toes develop, but if there are five or more, four toes develop. In other words, there is a threshold or level which must be reached before there would be development of four toes. Any number of contributing alleles below the threshold produce only three toes.

In discussing this particular example of threshold effect on the phenotype, I have not gone into the ramification. The important point to bear in mind is that the alleles are additive. The effects of the different alleles must be added in order to reach the threshold at which the effect, in this case development of four toes, becomes visible. Equally important is the fact that once thr threshld is re3ached the effect (phenotype) is the same regardless of the number of contributing alleles present. What we have discussed with reference to number of toes may be summarized by the following diagrams.



According to the hypothesis to account or the three-and four-toed phenotypes if A^1, B^1, C^1 and D^1 are contributing and A^2, B^2, C^2 and D^2 are not, then parents who are $A^1A^1B^1B^1C^1C^1D^2D^2$ would be pure-breeding for four-toes. The same would be true of $A^1A^1B^1B^1C^1C^1D^1D^2$ parents but of $A^1A^1B^1B^1C^1C^2D^1D^2$ parents. Try to explain why the statements are correct.

4.0 Conclusion

1. A trait is controlled byh a ***multiple genes*** or **polygenes**

2. Alleles appear in pairs and two types can be recognized – contributing and non-contributing alleles.

3. Certain simplifying assumptions have been made to facilitate the study of quantitative inheritance. Among these are

   (a) at any locus one allele does not obscure the effect of the other allele.

   (b) the effect of each contributing allele on the phenotype is equal to the effect of any other contributing allele.

   (c) the effect of the contributing alleles are additive.

   (d) there is no epistasin.

   (e) there is no linkage among the loci controlling the trait.

   (f) the environment exerts no effect on the genotype.

UNIT 19: SEX DETERMINATION AND SEX LINKAGES

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1.0 Introduction

Many men blame their wives for having only female children; some couple have only male children. Is it the fault of the woman for having only daughters? How exactly does a baby get to be a boy or a girl? This unit, among other things, examine this phenomenon.

2.0 Objectives

In many sexually reproducing organisms, especially in animals, the two sexes are distinct. There are a numbe of factors which determine the sex of an individual. The materials discussed should enable you to:

1. distinguish between autosomes and sex chromosomes;

2. account for the sexes produced by the chromosomal constitutions discussed;

3. explain the pattern of inheritance and expression of genes on the sec chromosomes;

4. explain the terms sex-linked, sex imited and sex influenced traits.

3.1 Sex Determination

One of the characteristics of living things is the ability to reproduce their own kind. The method of reproduction; however, may be either of two kinds, although some organisms are capable of using both kinds. The two kinds of reproduction are asexual or vegetative reproduction and sexual reproduction. One major feature of sexual reproduction is the fusion of two types of

gametes. In many organisms – all higher organisms the two gametes are morphologically different. There is no fusion of gametes in asexual reproduction.

Sexually reproducing organisma may be either ***monoecious*** or ***dioecious***. In the former case one individual produces both kind of gametes. In other words both male and female parts are present in the same individual. Dioecius organisms however, are characterized by the fact the sexes are separate; any normal individual is either male or femal and therefore produces only one type of gamete. Put differently, among dioecious organisms, the primary sex difference is in the types of gametes produced. In addition, there are secondary sexual characters which distinguish the sexes. However, we will not be concerned with the secondary differences per se. Rather, our time will be spend in condidering the underlying geneticf mechanisms responsible for the differences. What is the genetic basis for the fact that one zygote develops into a male while another develops into a female?

In 1891, a German biologist, Henking, observed that in certain insects, the nuclei of half of the sperm contain an extra structure. He called the structure the "X-body" (similar usage of x in algebra to designate unknown quantity) because its role is the nucleus was not known. In 1902 McClung, an American biologist, found that the somatic cells of female grasshoppers contain 24 chromosomes while male cells contain 23 chromosomes. In 1905, Wilson and Stevens, also Americans, identified Henking's X-body as a chromosome. They arrived at this conclusion after studying gametogenesis (oogenesis and spermatogenesis) in a number of insects. The X-body therefore, became known as the X-chromosome. In addition to the X-chromosome, another chromosome showing some similarities in behavior with the X was discovered. It was named the Y chromosome to indicate the closeness in behaviour.

In general therefore, three types of chromosome can be identified, although not all the three are necessarily present in all sexually reproducing organisms. The three types of chromosomes are the AUTOSOMES, the X – and the Y – chromosomes. The number of autosomes is the same in both sexes of all but a few organisms. The X – and the Y – chromosomes are designaed the SEX CHROMOSOMES because, although their numbe is constant (same as autosomes), for any member of a species, the number and types of sex chromosomes present depend on the sex of the individual (Table 19.1)

Table 19.1: The Chromosomes Number and Types in a Few Animals

	AUTOSOMES	S E X CHROMOSOMES X	Y	O M E S 2n
Fruitfly ♀	3 pairs	1 pair	-	8
(Drosophila melanogaster) ♂	3 pairs	1	1	8
Grasshopper ♀	11 pairs	1 pr.	-	24
(Zonocerus Variegatus) ♂	11 pairs	1	-	25
Man ♀	22 pr.	1 pr.	-	46
(Homo sapiens) ♂	22 pr.	1	1	46
Chimpanzee ♀	23 pr.	1 pr.	-	48
(Fantroglodytes ♂	23 pr.	1	1	48
Chicken ♀	38 pr.	1 z*	1W	78
(Gallus domesticus) ♂	38 pr.	1 pr. of z	-	78

* The choice of nomenclature is merely to underscore the fact that in some organisms it is the male which has two homologous sex chromosomes. This obviously is a different pattern. Some geneticists prefer the x-y nomenclature.

It is obvious that when the sec chromosomes are the same type they would behave like any pair of homologous chromosomes during meiosis-I, segregating to opposite poles. However, in spite of the differences in their nomenclature, the x (z) and the Y(W) behave like a pair of homlogues in meiosis-I. Although we shall not discuss that aspect, there is in fact partial homology between the two chromosomes. Be that as it mayy, the net result of the meiotic behaviour of the sex chromosomes is that the individual with one pair of X- or z- chromosomes produces only X- or z- bearing gametes. On the other hand an XY or ZW individual will produce two types of gametes – X bearing and Y-bearing or Z- and W- bearing. The grasshopper male also produces two types of sperms X-bearing and O-bearing (pronounced zero-bearing i.e. no ex chromosome). The sex which produces only one type of gamete is described as the ***homogametic sex*** while the sex which produces two tyhpes of gamestes is called the ***heterogametic sex***. Thus in man the male is the heterogametic sex but in the chicken it is the female.

Frm the table above, we can distinguish three different patterns of sex chromosomes distribution, the XX – XY, XX – XO and ZZ – ZW. Since these sex chromosal constitutions are the basic differences between the chromosomal complements or the nuclei of the two sexes, it is reasonable to assume that the sex chromosomes are associated with sex determination. The assumption is not far fetched. Genes determine much of what an organism is. According to the Chromosome Theory, the genes are on the chromosomes and different chromosomes carry different genes. Differences in chromosomal constitutions should therefore, result in different phenotypes.

In the light of the information so far presented the assumption regarding the significance of the sex chromosomes is a logical one. However, there are a number of questions which also require answers that would specify the role of the sex chromosomes. For instance, is the ***Drosophila*** female, a female because it carries two X-chromosomes, or is it a female because it carries two Y-chromosomes? We can ask similar questions about the male also. Is it a male because it carries one X-chromosomes or because it carries a Y-chromosomes? Are the autosomes involved in sex determination? Man and Drosophila are very different organisms yet they have the same pattern of sex chromosome distribution. Are the two systems of sex determination similar?

3.2 Sex Determination in Drosophila

Normally in meiosis-I homologous chromosomes go to opposite poles so that every gamete gets one of each pair of homologous pair. Deviations do occur however, such that both members of a pair go to the same pole. The condition where a pair of chromosome fall to separate durng cell division (meiosis an mitosis) is described as NON-DISJUNCTION. Note that as defined non-disjunction can occur between a pair of homologues in meiosis-I or between sister chromatids (chromosomes) in meiosis-II and mitosis.

If non-disjuntion of the X-chromosomes occurred during meiosis-I in the female Drosophila two type of eggs can be produced. In one case the egg will be XX, containing 5 instead of 4 chromosomes. The other type of egg will not contain any sex chromosome, there would be only three autosomes. If these eggs are fertilized by normal sperm, we would get the following sex chromosomal constitutions: XXX, XXY, XO, OY. The OY condition is very rear and the zygote dies in the egg, so the condition is lethal. The XXX and XXY conditions give rise to females. XXXY females are fertile and are phenotypically indistinguishable from XX females. On the other hand, the XXX female is frail and sterile, and dies quite early, sometimes in the pupil stage. The XXX female is known as a metafemale although some authors still use the original nomenclature, superfemale. Given the characteristics

just described, the term superfemale is a misnomer. The XO fly is phenotypically indistinguishable from normal males but it is also sterile.

From the results just considered the mechanism of sex determination in the fly may be stated as follows: In the fly with a ***diploid number of autosome***, sec is determined by the number of x-chromosomes present, such that the fly is a female if there is more than one x-chromosome present. It is , however, a male if there is only one x-chromosome. The Y-chromosome does not play a role in sex determination, but it carries the genes which determine femaleness, the fact that the OY condition is lethal means that the x-chromosomes carries some genes which are necessary for viability.

The preceeding account of sex determination is ***Drosophila*** is only a part of the picture, hence the emphasis on the "diploid number of autosomes" in the paragraph above. The complete theory of sex determination in ***Drosophila*** as proposed by Bridges in 1925 involves an interaction between the autosome and the x-chromosomes, with great significance given to the ratio of x-chromosomes to complete sets of autosomes. The reason is that there is some evidence indicating that genes for maleness are distributed among the autosomes. I have opted not to discuss this aspect of the theory because we would almost invariably be considering only diploid conditions. If you are interested in the complete theory, there is a good account in your recommended textbook. But to reiterate the point made searlier, for the purposes of this course, it is sufficient to say that in conjunction with a diploid number of autosomes the sex of the fly is female when there is more than one x-chromosomes and male when there is only one x-chromosomes present.

The validity of the above conclusion is evidenced by flies known as gynandromorphs or gynanders. Such flies are made up of male and female parts. Themale sections of the fly are samller than the female parts. The male sections of the fly are samller than the female parts since male flies are smaller than female. The male parts of the gynandromorphy are XO and the female part are XX. The proof that the chromosomal conditions are as stated comes from the expression of recessive genes on the x-chromosome. The theory is that male parts have only one x-chromosome. Therefore, any recessive gene onthat x-chromosome will be expressed. On the other hand female parts would not express such traits if they were heterozygous for the genes. Indeed, it is found when the the appropriate experiment is done, tht the parts which have the recessive phenotype are only the male parts. All the female parts have the dominant phenotype expectee for a heterozygous genotype. A heterozygous genotype would be possible only if there are two homologous chromosomes, in this case two x-chromosomes, present in the same cell.

The evidence and the ensuing theory of sex determination in Drosophila indicated that it is the x-chromosome tht is involved in sex determination. On the strength of this it is reasonable to conclude that a number of loci (genes) is involved in the determination of sex. All things being equal, that is the normal situation. However, there is evidence that single identifiable loci may play significant role in sex determination. For example, in ***Drosophila melanogaster***. There is a recessive autosomal gene (i.e. a gene on an autosome as opposed to one on a sex chromosome) which when homozygous transforms XX-zygote i.e. female zygote into males which are sterile. The effect is only XX zygotes. The gene, transformed I symbolized as ***tra.*** From what I have said so far, there are two possible types of males which are also homozygous for tar; they are ZY, tra/tra which are sterile males. The tra/tra genotype nullifies (i.e. it is epistatic) the effects of the female determining genes on the X-chromosomes present. I should emphasise the point that normally the recessive tra allele is not present, rather it is the dominant wild type allele, tra^{+}, which is present, and this allele has no epistatic effect on the pattern of sex determination.

3.3 Sex Determination in Man

Human males and females, as shown in the table earlier, are XY and XX in the sex chromosomal constitution. This is the same constitution found in ***Drosphila***. A logical question that arises about this is whether the mechanism of sex determination in man is the same in ***Drosphila***. Relevant places of evidence indicate that the mechanism is different.

As in Drosphila, evidence that help in the elucidation of the pattern of sex determination in man came from cases of abnormal sex chromosomal constitution. Available data from studies in the U.S. show that one in 500 – 800 (1/500 – 1/800) male births, i.e. babies recognizable as males, results in individuals affected with a condition known as Klinefelter's Syndrome. The conditions was first described by Klinefelter's and the term syndrome indicates the fact that the condition is characterized by a member of specific abnormalities. In this case, some of the abnormalities are that although the external genitals appear normal, the testes are small and there is little or no sperm production; these males are therefore, sterile. The arms and legs are longer than normal. There is usually some enlargement of the breasts (a condition known as gynecomastia) and intelligence is often below average. Chromosomal studies show that Klinefelter males have 47 chromosomes instead of 46. The chromosomal constitution is made up of a normal complement of autosomes, i.e 22 pairs, their sex chromosomal constitution is XXY.

Another major sex abnormally in man is Turner's Syndrome, occurring with a frequency of between one in 5,000 and one in 3,000 female births. Those affected with this syndrone, first described by Turner, are recognizable as females but they are poorly developed; the same is true of the secondary sexual characteristics – breasts etc. Affected females are shorter than average and have folds of skin on both sides of the neck. The condition is described as "webbing" of the neck because the folds are similar to those between the toes of a duck. Although there is often a degree of mental retardation there are a few Turner females who are anything but mentally retarded. Affected females have a normal complement of autosomes but only one X-chromosome making a total of 45 chromosomes. The sex chromosomal constitution is therefore XO as opposed to XX for the normal female.

The triple-X condition XXX is also known in man. These individuals have a normal complement of autosomes plus three X-chromosomes, making a total 47 chromosomes. They are female. Some are quite normal and most are fertile but others exhibit varying degrees of abnormality. Some have below average intelligence, some have poor development of the external and internal genitalia.

The OY condition has not been found in man, but it is known to be lethal in mice embryo. Therefore, it can be assumed to be also lethal in man.

The phenotype of the sex chromosomal constitutions descried so far indicate that in man as in ***Drosophila***, the X-chromosome carries viability genes. Hence, at least one must be present if the embryo is to survive. However, the mechanism of sex determination in man is such that the Y-chromosome carries the male-determining genes but the X-chromosomes also carries some genes for femaleness. These constitutions are based on the fact that the XY and XXY constitutions are male and XO, XX and XXX i.e no Y present are female. There is also the fact that there is development of some female secondary characteristic e.g. gynecomasty, in XXY males.

Further evidence in support of the male-determining effect of the human Y-chrosome is the observation that XXXY, XXYY, XXXXY are Klinefelter males. The logical conclusion is therefore, that the presence of a Y-chromosome, leads to a male regardless of the number of X-chromosomes present.

There is information regarding the distribution of the male-determining genes along the length of the Y-chromosome as well as the distribution of genes involved in sex-differentiation on both the X and Y-chromosomes. I shall not consider these aspects in this course.

One of the major points in the Chromosomes Theory of Inheritance is that a normal chromosomes complement is necessary for normal development. The cases of sex chromosomal imbalance which we have considered, underscore the point. Although there is some measure of normality in XXX females, XXXX and XXXXX females show increasing degrees of mental retardation.

XYY individuals are males as expected and are very rare in the population. They are generally above average height and in most cases are below normal in intelligence. There are no consistent major abnormalities associated with this constitution. However, the XYY condition is of social interest because there is some evidence that it might predispose such men to criminality. The emphasis is on predisposition because the evidence for a direct association with criminality is not conclusive. There are many XYY men who do not run into conflict with the law. However, it is pertinent to note that in some countries the XYY condition is considered in much the same way as the insane criminal.

Although in the normal pattern of sex determination in man, no specific gene is identified as having a specific role, there is evidence that might in fact be the case. In man there is a condition known as testicular feminization or male pseudohermaphroditism. Examination of those affected with this trait show them to be chromosomally XY but possessing female characteristics externally. Internally, however, they possess testes and lack ovaries and fallopian tubes. They behave as females and many are known to be married but are of course sterile. The only known effect of the gene for this trait on XX females is the absence of axillary and pubic hair in some carrier women The gene also transforms XXY zygotes into sterile females. It has not been possible so far in man to determine whether the gene responsible is on an autosome or on the X-chromosome. If it is an autosomal trait, then it would have to be a dominant trait. Thus both carrier females and affected males would be heterozygous for the gene. On the other hand the gene could be either a recessive or dominant gene on the X-chromosome. The recessive gene would be expressed in all XY zygotes inheriting it since there is no other X-chromosome which could carry a dominant gene. Whatever the case, the gene is transmitted through only females, and it overrides the male-determining effect of the Y-chromosome.

The pattern of sex determination in man is the same in most mammals. However, in mice the XO females are fertile although not as fertile as normal XX females. Also in mice the gene for testicular feminization is known to be located in the X-chromosome and it is dominant.

The XX/XO system of sex determination is similar to that in ***Drosophila*** in that male have only one X-chromosome while females have two. Note,

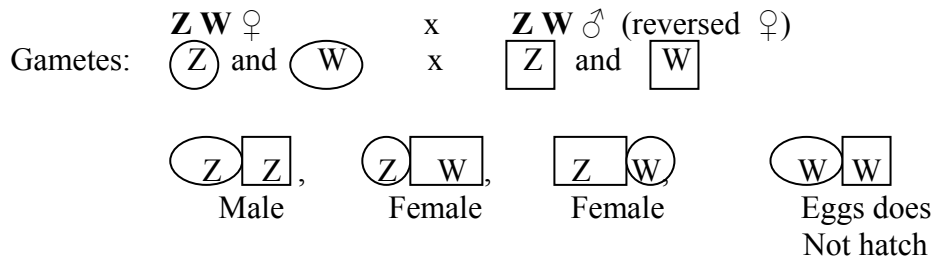
however, that the ***Drosophila*** male is different with respect to the fertility gene, which are located in the Y-chromosome.

Using the term which I introduced early in this series of lectures, in the system of sex determination which we have considered so far, the female is the homogametic sex (all eggs contain an X-chromosome) and the male is the heterogametic sex, producing two types of sperm one of which contains an X-chromosome while the other contain either a Y-chromosome or no sex chromosome.

3.4 Other Organisms

The next system which I will consider is much that the male is homogametic and the female heterogametic. For this reason, the sex chromosomes are often designated differently; they are represented as Z (the equivalent of the X) and W (= Y). In this system the male is ZZ and the female is ZW or ZO in some animal. This system is found in the chicken; males are ZZ and females are ZW. One interesting aspect of sex determination in the chicken is the fact that sex reversal without chromosomal change is possible in females. Normally only one, the left, of the two presumptive gonads develops into an ovary. The other is essentially rudimentary or better still dormant. If the left gonad i.e. the ovary is destroyed, the chicken even as an adult is transformed into a fertile ZW male.

Such a reversed female would be a heterogametic male. When this male is mated to a normal female, the cross would be:



The observed ratio of two females to one male instead of the expected 1:1 ratio among the progeny of this cross is proof that the male was a revered female with a ZW sex chromosomal constitution. (Diagram a normal ZW x ZZ cross to convince yourself that the expected sex ratio is 1 female : 1 male).

The chromosomal systems of sex determination is not restricted to animals. For instance, in the dioecious plant, ***Lychnis*** XX plants are female and bear only pistillate flowers. There is, however, evidence to show that the type of

flower produced is dependent on theratio of X to Y chromosomes. When the ratio is 4X : 1Y the plant produces mainly perfect flowers (i.e. hermaphroditic flowers with stamen and pistil) but occasionally there are some staminate flowers. Therefore, it is possible to suppress the male determining effect of the Y-chromosome by increasing the number of X-chromosomes.

Sex chromosomes also play a role in sex determination in some haploid organisms. For instance, in liverworts meiosis in the sporophyte, the diploid asexual generation, results in the production of two types of gametophytes (gamete producing plants). The one bearing the X-chromosomes develops into the archegonium which produces the egg cell. The one bearing the Y-chromosome develops into the antheridium and will produce the sperm. Fertilization will restore the XY constitution of the diploid asexual sporophyte.

In the hymenoptera – base and wasps – the system of sex determination does not depend on sex chromosomes. In the honey bee, for example, workers and the queen bees are females. Chromosomal analysis shows that they are diploid (2n = 32). Male bees (drones), develop by parthenogenesis i.e from unfertilized eggs and are haploid, with 16 chromosome. Thus on a generalized basis one can say that the 2n condition leads to femaleness while the haploid condition leads to maleness. Careful studies have however, shown that a large number of genes are involved and more specifically it is the extensive heterozygosity for the large number of genes which leads to a female bee. By the same token homozygosity for a large number of the genes leads to a diploid male. For our purposes however, it would be sufficient to generalize the mechanism in terms of chromosomes number.

The patterns of chromosomal sex determination which have been discussed can be summarized as shown in the Table 19.2 below:

FEMALE	MALE	ORGANISMS
*AAXX	AAXY	Man and other mammals; some dioecious anglosperms (plants): ***Drosophilia****
AAXX	AAXO	Grasshopper, Cockroach and many orthoptera and hemiptera.
AAZW/ZO	AAZZ	Birds, reptiles, some amphibia, some fishes and lepidoptera.

AX	AY	Liverworts (plants)
AA	A	Hymenoptera.

*A = one haploid set of autosomes.

** Drosophila technically does not belong in this group since the Y is not sex determining.

Self Assessment Questions

1. What is the diploid number of chromosomes in humans?

2. Explain how (a) a boy (b) a girl is formed at fertilization.
3. What is (a) heterogametic sex
   (b) homogametic sex

4. What are (a) autosomes (b) sex chromosomes?

5. What are the sex chromosomes in man?

4.0 Conclusion

Reproduction is one of the general characteristics of all living organisms. Sexual reproduction is a more advanced form of reproduction than asexual reproduction and involves the fusion of sex cells or gametes which are quite distinct from one another.

A sexually reproducing organism may be ***monoecious*** in which case an individual produces both types of sex cells or gametes, or ***dioecious*** in which two distinctly different individuals in dioecious organisms are designated male or female. The chromosomes that determine the sex of an organism are known as ***sex chromosomes***; others within the cell are known as autosome. In man, there are 22 pairs of ***autosomes*** (i.e. 44 autosomal chromosomes) and one pair (i.e 2) sex chromomoses. The sex chromosomes are designated X and Y. the Y chromosomes determines maleness.

5.0 Summary

Sex chromosomes are important in the formation of distinct sexes in living organisms. They are also responsible for some sex-linked diseases in organisms e.g. haemophilia and colour blindness in man. Some of these will be discussed in the next unit.

6.0 Tutor-marked Assignment

1. Discuss Klinfelter's Syndrome.

2. Discuss Down's Syndrome.

7.0 References

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Answers to Self Assessment Questions

1. There are 23 pairs or 46 chromosomes in the human cell.

2. (a) A boy is formed when a Y chromosome sperm fuses with the ovum to form XY.
   (b) A girl is formed when an X sperm from the father fuses with the ovum to form XX.

3. (a) An heterogametic sex is one that contains differing sex chromosomes e.g. XY as in a boy.
   (b) A homogenetic sex is one where the sex chromosomes are the same.

4. (a) Autosomes chromosomes within a cell other than those that can determine the sex of the individual.
   (b) Sex chromosomes are those that can determine the sex of the organism.

5. The sex chromosomes in man are the X and Y chromosomes.

UNIT 20: SEX LINKAGES

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2.0. Introduction

The sex of an organism is determined by the sex-chromosomes. These are designated by the letters X or Z for females and Y or W for males. The remaining chromosomes are called autosomes. The sex chromosomes carry some genes which are responsible for certain traits. Some genes are predominant on the X-chromosomes while some are linked to the Y-chromosomes. Such traits as night-blindness, colour blindness, haemophilia, deep/soprano voice and hairy chests are all linked to the sex chromosomes.

2.0 Objectives

At the end of this unit you would be expected to:

1. Know about the discovery of the Barr Body.
2. Understand the term holandric genes linked with the Y-chromosomes.
3. Give examples of sex-linked traits
4. Give examples of sex-influenced genes.

3.1 Sex Linkage

From the preceding series of units we have found that the basis of chromosomal sex determination is the fact that one chromosome or a pair of non-homologous chromosomes does not occur in the same numbers in both sexes. These chromosomes are therefore, named the sex chromosomes and symbolized as X and Y in some organisms or Z and W in others. In all oujr

considerations of the transmission of genes hitherto, no attempt hasd been made to specify the sex of each parent. This was because the genes being considered are on the autosomes which you will recall are present in equal numbers in both sexes. That is not the case with the sex chromosomes. Hence, it is pertinent to inquire into the pattern of transmission and expression of genes on the sex chromosomes. The term linkage you should recall is used to describe the occurrence of genes on the same chromosome. By convention however, sex "linkage" refers to genes on the X- or Z-chromosome, even though the Y- and the W- chromosomes are also sex chromomoses. The reason for such a usage of the term is the fact that in most organisms no specific loci on the Y- or W- chromosomes have been identified as being responsible for particular traits. With regard to the Y or W, one almost invariably refers to a group of genes rather than single genes as being responsible for say, fertility or determination of sex.

One of the most clear-cut pieces of evidence illustrating sex-linked inheritance was reported by Morgan in 1910 from crosses with ***Drosophila melanogaster***. The crosses are shown below:

P red-eyed ♀ x white-eyed ♂

↓

F_1 all ♀♀ red; all ♂♂ red

½ ½ i.e 1:1 ratio of ♀♀:♂♂

↓

F_2 all ♀♀ red; red ♂♂ ; white ♂♂

½ : ¼ ; ¼ i.e 2:1:1

also 3 red : 1 white

Backcross:

P red ♀ (F1) x white ♂

↓

F1 **red** ♀♀ ; white ♀♀ ; red ♂♂ ; white ♂♂

¼ ¼ ¼ ¼

From the F1 of the original cross, one would conclude that the white-eye phenotype is recessive to the wild type i.e. red eye. The evidence that the eye-colour trait is probably controlled by one locus is the fact that there is only one pair of alternative phenotypes – red or white. The next question is whether the trait is due to an autosomal gene. If trait were an autosomal trait

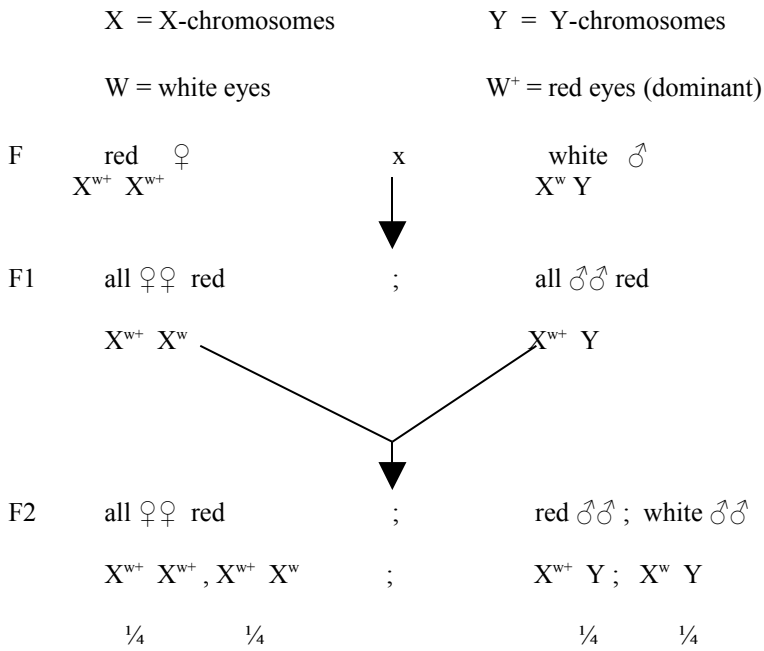
we would have expected both the male and female F_1 in the original cross to be heterozygous for the pair of alleles, since both sexes have the same numbers of each autosome. If that were so we would then have expected in the F_2 a ratio of 3 red to 1 white. ***BUT*** with the sexes evenly distributed between the two phenotypic classes. Instead, we find that one-half of the males are white and the other half red but all the females are red-eyed.

In the backbone, one half of the F1 are white and the other half red. Furthermore, one half of the white are females and the other half males. The same is true for the red-eyed class. These ratios of 1 red : 1 white and equal numbers of males and females in the two classes are similar to what one would expect with an autosomal trait. However, the fact that the results of the original crosses were so different from what would be expected for an autosomal trait made Morgan seek other explanations. It is necessary to note here that my attempt to explain sex linkage based on the results of Morgan's crosses is not strictly along the same steps. For example, Morgan based his hypothesis on the results of the backbone using his hypothesis. Instead I have chosen to discuss all the crosses and in the process explain sex linkage.

The result of the P-generation cross indicates that the white eye phenotype is recessive. If this were an autosomal trait white-eye flies would have to be homozygous. On the other hand if the locus of the gene were on the X-chromosome only white-eyed female need be homozygous for the recessive allele because they have two X-chromosomes. By the same token red-eyed females would be either homozygous dominant or heterozygous.

The heterozygous female would produce two types of eggs – one containing the X-chromosome with the dominant allele and the other containing the X-chromosome with the recessive allele. Both types of eggs can be fertilized by a Y-bearing sperm. If the egg with the dominant allele is fertilized the eye colour of the resulting male offspring is of course red because the dominant allele is present. However, one can only deduce the basis of the phenotype in the case of the egg with the recessive allele fertilized by a Y-sperm. The phenotype has to be white because there is not other X-chromosome present which could possibly carry a dominant allele. Even though the X and the Y segregate like a pair of homologues they are not homologous chromosomes. In other words there is no locus corresponding to the white locus of the X and Y. Therefore, the male fly has one set of sex-linked genes. The sex which has only one X or Z chromosomes and therefore, only one set of sex-linked genes is described as being HEMIZYGOUS for that set of genes. To underscore the point, one can say that when an individual is hemizygous for any gene, that gene will be expressed regardless of whether it is recessive or dominant.

Based on Morgan's deductions one can assign genotype to the parents and progeny in the crosses. In writing the genotypes, each allele will be written as a superscript on a capital X denoting the X-chromosomes. This is merely to remind you of the sex chromosomal constitution of the individuals. You need not include the X if you can keep track of male and female genotypes.



Ratio of red white is 3 : 1

Notice, however, that all the females are red!

The genotypes assigned to the parents and progeny in the crosses adequately account for their phenotypes. The genotypes are of course a consequence of the hypothesis that the eye colour gene is located on the X-chromosome with no corresponding locus, and therefore no alleles, on the Y-chromosome. In the crosses above the white-eyed P male is hemizygous and his X-chromosome (designated by the heavy type) is transmitted to his daughters only. Thus some inherit their X-chromosome exclusively from their mother. Mothers can transmit either of their X-chromosomes to both sons and daughters. Since a father transmits his X-chromosomes to daughters only, only some of his grandsons by his daughters will inherit his X-chromosome. Thus under normal circumstances, an XY male never transmits his X-chromosomes to his sons. This transmission of the P-generation's male X-chromosome to F_2 sons via the F_1 daughters is sometimes referred to as "crisscross" inheritance.

(Assign genotypes to the backcross parents and progeny as was done for the original cross).

Morgan's postulate has the following implications:

1. A cross of white-eyed females with white-eyed males will yield only white-eyed progeny.

2. Heterozygous red females will always produce and expected proportion of 50% red-eyed and 50% white-eyed male progeny regardless of the type of males to which they are mated. However, only matings of these females to white males will produce white daughters in a proportion of 50% red-eyed and 50% white-eyed. (Recall the backcross).

3. F1 males from white ♂ x red ♀ (pure breeding) will produce only red-eyed females; but all sons from the cross will be white-eyed.

4. F2 females from a P cross of pure-breeding red ♀ x white ♂ will all be red but of two genetypes : ½ W^+/W

5. Reciprocal crosses for sex-linked genes will yield different results, contrary to what is found with autosomal genes:

red ♀ x white ♂	white ♀ x red ♂
red ♂♂ x	all ♂♂ white, all ♀♀ red

The reason for the differences is the fact that the male is hemizygous for sex-linked genes.

Results from different crosses prove that these implications are correct and that they also apply to other sex-linked genes. Hence Morgan's sex-linkage hypothesis is a valid one.

Another demonstration of sex-linked inheritance in ***Drosophila*** is as follows. There is an X-chromosome which is ring-shaped instead of rod-shaped. During the early cleavage stages in development there is a tendency for the chromosome not to be included in some nuclei. The loss of the ring X-chromosome from some nuclei in an XX embryo results in a condition in which some cells retain their original X condition and other cells would be XO. The latter types of cells would be hemizygous for the genes on the remaining rod X-chromosomes.

Since the XO constitution is male determining, the parts of the fly containing the XO cells would give rise to male structures whil the XX cells would develop into female structures. The extent of the mosaicism of male and female parts would depend on the distribution of the two types of cells and the relative numbers of the two type of cells. We have already characterized this type of fly as a gynandromorph.

With respect to sex-linkage, there is recessive allele which makes for yellow body chitin and bristles. Another recessive allele makes for twisted bristles. The embryo could be made heterozygous for the two loci such that the normal X-chromosomes carries the two recessive alleles while the ring X-chromosomes carries the dominant alleles for normal colour and straight bristles. Under such circumstances the male parts of the gynandromorph would be recognized by the fact that in addition to being smaller than the female parts, they would be yellow and would have twisted bristles. Such phenotypic differences between the XO male parts and the XX female parts bear testimony to the facts that the genes are on the X-chromosome and that the male is hemizygous for X-linked genes. The XO phenotypes for the genes are no different from the phenotypes in XY moles. Note that the phenotype in the gynandromorphy are further proof that the XO constitution in Drosophilia leads to male development.

Inheritance of the X-chromosomes of man follow the same pattern as in Drosophila. Therefore, with respect to X-linked traits sons are never like their fathers, instead they are more like their mothers! It is only daughters won inherit their fathers's X-chromosome. It is equally interesting to note here, that the sex of the child is determined by which of the father's two types of sperm fertilizes the egg. All things being normal it is a matter of chance, 50%, which type of sperm fertilizes the egg. Thus a family of six daughters is not highly improbable – $(½)^6$ i.e one out of sixty-four families of six children.

A number of sex-linked traits are known in man and they follow the same patterns of inheritance as sex-linked traits in ***Drosophila***. Most of the known sex-linked traits in man are recessive. These tratits are rare in the population but males are more frequently affected than females. The reason in that the male is hemizygous, therefore any male who inherits the recessive allele from his mother will be affected. On the other hand female who is affected must have an affected father and a mother who is either heterozygous (called a "carrier") or affected. Put differently, if she inherited the abnormal recessive allele from one parent she might inherit the dominant normal allele from the other parent. In mathematical terms if the frequency of affected males in the population is q, the frequency of females would be q^2 because they have to be

homozygous. q^2 is less than q because q is often in the range of 1/1000 or even much less than that. A few examples of recessive x-linked traits are night blindness, hemophilia in which the affected cannot distinguish between red and green, deficiency for the enzyme glucose-6-phosphate dehydrogenase and ocular albinism.

The inheritance of sex-linked genes in XX/XO organisms follows the same pattern as in the XX/XY types which we have discussed using the ***Drosophila*** example. In ZZ/ZW animals the only difference is in the fact that it is the female which is heterogametic, therefore daughters never inherit Z-linked genes from their mothers.

3.2 Barr Body

One interesting aspect of sex-linked inheritance in mammals is connected with the presence of a dark staining body – the Barr body (named after the discoverer) – found in the interphase nucleus of most female somatic cells. The Barr body is one of the two x-chromosomes in the female. Furthermore it is not the same X-chromosomes which occurs as the Barr body in all cells. Mary Lyon and others proposed the Lyon or inactive-X hypothesis about the Barr body. Very simply the hypothesis states that the genes in the X-chromosome forming the Barr body are inactive. Therefore, every female somatic cell is technically hemizygous. Since it is not the same X-chromosome which is inactive over the entire body, a female who is heterozygous for a trait would have the two phenotypes of her body. She would be a mosaic. For instance, in man the condition known as anhidrotic ectodermal dysplasis has as one of is characteristics, the absence of sweat glands in the skin. A woman wh is heterozygous for this trait two types of patches on her body – no sweat glands and sweat glands. Although the Lyon hypothesis is clearly applicable to many x-linked traits, there are some which deviate from the hypothesis.

3.3 Y-Linkage

The y-chromosome does not occur in mammalian females or occurs only under special circumstances in ***Drosophila*** females. Therefore, any traits present on the Y-chromosome will be transmitted from father to son only. In other words the genes and their corresponding traits will occur in only one sex. Such genes therefore, would be described as ***holandric genes***. The occurrence of an unusual amount of hair on the ear rims of some men was once thought to be holandric but the absence of the expected hairs in some males has cast some strong doubts on a Y-linked explanation. In man only two traits – a testis – determining factor and Y-histocompatibility – are known definitely to be Y-linked.

3.4 Sex – Limited Traits

Sex-limited traits are also referred to as sex-limited genes. However, I prefer the former term because as we shall see, the genes occur in both sexes although it is not expressed in one. One example which is of agricultural importance is the production of milk in cattle. The bull chosen to sire the dairy cattle is just as important as the cows because the genes controlling high milk production also occur in males but males do not produce milk because they are not equipped (differentiated) to do so. Other examples are the size and shape of the penis in males, breast development in women, heavy beard development in men and testicular feminization.

3.5 Sex – Influenced Genes

Sex-influenced genes are also described as sex-controlled or sex-modified genes/traits. These are genes which are expressed in both sexes, unlike the genes for sex-limited traits. However, in this case the type of expression is different in the sexes given the same genotype. One common example is "pattern" baldness, which has a genetic basis. In the population there are many more males than female affected with this trait. The reason is that affected males are either homozygous or heterozygous i.e. BB or Bb but only homozygous BB females are bald. In other words although the trait is dominant men, it is recessive in females. Another sex-influenced trait in man is singing voice in which low bass males and high soprano females seem to have the same genotype.

4.0 Conclusion

Sex chromosomes are designated by X and Y in some organisms or by Z or W in others. Genes on the autosomal chromosomes are present in equal numbers in both sexes. However, the sex chromosomes do not carry equal numbers of genes.

By convention sex-linkage refers to genes on the X or Z sex chromosomes and not the Y and W chromosomes. This is because for most organisms the genes on the Y and W – chromosomes no specific genes have yet been identified; rather, one refers to a group of genes as being responsible for, say, fertility or determination of sex.

The Barr body is a dark staining body found in the interphase nucleus of most female somatic cells. It is one of the two sex (x) chromosomes in the female. The Y chromosomes determines maleness in mammals and is not found in

the females. The Y chromosome bears such genes (holandric genes) responsible for at testic-determining factor and Y-histocompatibility.

Sex-limited traits or genes are responsible e.g for high production of milk in cattle, size and shape of the penis in males, breast development in women, heavy beard development in men and testicular feminization.

Example of sex-influenced genes/traits is pattern baldness which has a genetic factor. Affected males are genetypically BB r Bb but only homozygous BB females are bald; hence the trait is dominant in men that another sex-influenced trait in man is singing voice in which low bass males and high soprano females seem to have the same genotype.

5.0 Summary

We started with the knowledge that the nucleus controls the activities of the cell. We narrowed the control center to the chromosome which carry information on themselves. The set of information is called genes. Genes are responsible for the characteristics/traits that are shown by the organisms. Genes occur on all sets of chromosomes – both autosomes and sex chromosomes. The genes on autosomal chromosomes are common in all cells but those on the sex chromosomes are not; this gives rise t5o sex-linked, sex-limited and sex-influenced genes/traits.

6.0 Tutor-marked Assignment

7.0 Reference

Williams, G.O 2001, BIY 302 – Genetics –1, Module 5, Distance Learing Institute, University of Lagos.

Herskowitz, I. H.. 1973, Principles of Genetics. The Macmillan Co. New York.

Self Assessment Questions

1. What letters are used to sybolise the sex chromosomes?

2. In man which of the sex-chromosomes is responsible for maleness?

3. What is a Barr body

Answers to Self Assessment Questions

1. The letters X or Z and Y or W

2. The Y sex chromosome is responsible for maleness in man (i.e XY)

3. The Barr body is a dark staining body found in the interphase nucleus of most female cells and it is one of the two X-chromosomes.