

PROCEDURE

- (i). With a sterile wire loop, place a small drop of both culture on a clean slide. If the culture is taken from a solid medium, first place a drop of sterile water on the slide, and thoroughly mix it in a circular form
- (ii). Gently stir the loop in the film on the slide to obtain an even distribution of cells over the area.
- (iii). Flame neck of bottle from which bacterial culture has been taken and replace the cap.
- (iv). Air dry the slide, or hold it high above the Bunsen flame.
- (v). Heat fix the smear when it is dry by passing it, several times over the Bunsen flame, Note that too much heat will distort the shape of the cells. The slide should merely feel warm to the back of the hand.)
- (vi). Flood the smear with stain and allow the dye to act (allow 30 seconds) in crystal violet and
- (vii). Decolourize with 75% ethanol or acetone
- (viii). Wash the slide gently in running water over the sink to remove excess stain.
- (ix). Counter stain with safranin for 30 seconds
- (x). Wash with water.
- (xi). Blot dry and observe slide under oil immersion

EXERCISE

- (i). Draw the cell morphology (shape) you have observed).
- (ii). Name the type of shape you have observed.

ACTIVITY 4: Gram Staining of Bacteria

AIM: To identify Gram positive and Gram negative bacteria.

MATERIALS

- (i) Bacterial culture,
- (ii) Glass slide,
- (iii) Wire loop Bunsen Burner,
- (iv) Crystal violet,
- (v) Lugols iodine,
- (vi) 95% ethyl alcohol,
- (vii) Safranin distilled water.

PROCEDURE

- (i) Prepare a heat fixed smear as shown in Activity 3 (procedure 1 to 5).
- (ii) Flood the smear with crystal violet and allow it for 30 seconds.
- (iii) Wash it gently in running water.
- (iv) Flood smear with Grams iodine solution allow it to act for about one minute.
- (v) Drain off excess iodine and rinse with gently running water.
- (vi) Decolorize by using 95% ethyl alcohol for 30 seconds.
- (vii) Wash in gently running water.
- (viii) Counter stain with safranin for 30 seconds.
- (ix) Wash in gently running water and blot dry.
- (x) Examine slide under oil immersion.

EXERCISE

- (i) Draw the shape of the cells you have observed
- (ii) What is the colour of the cells?
- (iii) What Grams reaction are the cells?

ACTIVITY 5: Bacterial Smear Preparation

AIM: To Demonstrate how to prepare bacterial smear

MATERIALS

- (i) Bunsen burner,
- (ii) Bacterial culture plate,
- (iii) A clean glass wire loop.

PROCEDURE

- (i) Wash slide gently but thoroughly with vim or detergent.
- (ii) Rinse properly in clean flowing tap water
- (iii) Wipe slide with a clean dry cotton cloth or tissue paper.
- (iv) Hold slide with forceps and flame by passing it 6 to 10 times through a blue Bunsen flame and allow to cool before laying down to avoid cracking.
- (v) In case of fluid material (e.g both culture) using a sterilized wire loop take a loopful of the culture and spread thinly on the slide.
- (iv) With solid material (e.g culture on agar plate), a loopful of distilled water is placed in a clean grease free slide.
- (v) The loop is then sterilized and a small quantity of bacterial sample obtained by picking the colony with the sterile loop and the charged loop transferred to distilled water on the slide and thoroughly emulsify the mixture to spread evenly on the slide.
- (vi) Allow the smear to air dry completely
- (vii) Pass the slide with the side containing the smear uppermost three times through the bunsen flame to heat kill and fix the organisms to the slide
- (viii) Allow smear to cool

DISCUSSION

- (a) List two ways in which a bacterium cell differs from a typical animal cell.
- (b) Give three ways by which a scientist can tell the difference between different kinds of bacteria.
- (c) Why is it necessary to wash hands before a meal and after using the lavatory?

3.16 TUTOR MARKED ASSESSMENT QUESTIONS

HAVING READ THROUGH **CHAPTER THREE**, ANSWER THE FOLLOWING QUESTIONS IN THE SPACES PROVIDED.

1. (a) Define the Term Prokaryotes?

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2 × $\frac{1}{2}$ = 1 Marks

(b) What are Bacteria?

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$2 \times \frac{1}{2} = 1$ Marks

c) Write briefly on the following:

(i) Flagella

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(ii) Nucleoid

$2 \times \frac{1}{2} = 1$ Marks

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(iii) Capsule

$2 \times \frac{1}{2} = 1$ Marks

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(d) List **Eight** types of Bacteria in Nature.

$2 \times \frac{1}{2} = 1$ Marks

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$8 \times \frac{1}{2} = 4$ Marks

2(a). Explain briefly the term Archaea

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$4 \times \frac{1}{2} = 2$ Marks

(b) Distinguish Between Aerobic and Anaerobic Respiration in Bacteria.

(i) Aerobic

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$2 \times \frac{1}{2} = 1$ Marks

(ii) Anaerobic

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$2 \times \frac{1}{2} = 1$ Marks

(c) What is Binary Fission in Bacteria?

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$2 \times \frac{1}{2} = 1$ Marks

(d) In a Tabular Form Distinguish between Asexual and Sexual Reproduction in Bacteria.

Asexual Reproduction	Sexual Reproduction

$6 \times \frac{1}{2} = 3$ Marks

3(a) List **Eight** Factors affecting the Growth of Bacteria:

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(b) Write briefly on:

$8 \times \frac{1}{2} = 4$ Marks

(i) Gram positive Bacteria.

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(ii) Gram negative bacteria.

$2 \times \frac{1}{2} = 1$ Marks

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(c) Mention the Diseases caused by the following Bacteria:

$2 \times \frac{1}{2} = 1$ Marks

(i) *Vibrio cholerae*

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(ii) *Clostridium tetani*

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(iii) *Mycobacterium tuberculosis*

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(iv) *Staphylococcus aureus*

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(d) State the Benefits of the following Bacteria:

$2 \times \frac{1}{2} = 1$ Marks

(i) *Azotobacter*

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$2 \times \frac{1}{2} = 1$ Marks

(ii) *Streptomyces*

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$2 \times \frac{1}{2} = 1$ Marks

(iii) *Methanogen*

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$2 \times \frac{1}{2} = 1$ Marks

4.(a) List **Six** uses of Bacteria you have studied.

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$6 \times \frac{1}{2} = 3$ Marks

(b) In a tabular form state **Three** differences between Bacteria and Cyanobacteria.

Bacteria	Cyanobacteria.

$4 \times \frac{1}{2} = 2$ Marks

c) State how Prokaryotic Cells differ from Eukaryotic Cells structurally.

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$4 \times \frac{1}{2} = 2$ Marks

(d) What do you understand by the term Eukaryote?

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$4 \times \frac{1}{2} = 2$ Marks

Chapter Four

PROTOZOA, ALGAE AND FUNGI CELLS

Emem Okon Mbong [PhD]

4.1 INTRODUCTION

Protozoan is eukaryotic microorganism. Although they are often studied in *zoology* courses, they are sometimes considered as part of the microbial world because they are unicellular and microscopic. *Protozoa* are notable for their ability to move independently, a characteristic found in majority of species. They usually lack the capability for *photosynthesis*, although the genus *Euglena* is renowned for *motility* as well as *photosynthesis* (and is therefore considered both as an algae and a protozoan). Although most *protozoa* reproduce by asexual methods, sexual reproduction has been observed in several species. Most protozoan species are aerobic, but some anaerobic species have been found in the human intestine and animal rumen.

Protozoa are located in the moistest habitats. Free-living species in freshwater and marine environments and terrestrial species inhabit decaying organic matter. Some species are parasites of plants and animals. They play an important role as zooplankton, free-floating aquatic organisms of the oceans. Here, they are found at the bases of many food chains, and they participate in many food webs. *Algae* are *eukaryotic organisms* that have no roots, stems, or leaves but do have chlorophyll and other pigments for carrying out *photosynthesis*. *Algae* can be multicellular or unicellular. Unicellular *algae* occur most frequently in water, especially as planktons. Phytoplankton is the population of free-floating microorganisms composed primarily of unicellular algae. In addition, *algae* may occur in moist soil or on the surfaces of moist rocks and woods. *Algae* live symbiotically with *fungi* as lichens. According to the Whittaker scheme, *algae* are classified into seven divisions, of which five are considered to be in the Protista kingdom and two in the plantae kingdom. The cell of an alga has eukaryotic properties and some species have flagella with the "9-plus -2" pattern of *microtubules*. A nucleus is present, and multiple chromosomes are observed in mitosis. The chlorophyll and other pigments occur in chloroplasts, which contain membranes known as *thylakoids*. Most *algae* are *photoautotrophic* and carry out *photosynthesis*. Some forms, however, are *chemoheterotrophic* and obtain energy from chemical reactions and nutrients from organic matter. Most species are saprobes, and some are parasites. Reproduction in algae occurs in both asexual and sexual forms. Asexual reproduction occurs through *fragmentation* of colonial and *filamentous algae* or by spore formation (as in *fungi*). Spore formation takes place by mitosis. Binary fission also takes place (as in bacteria). During sexual reproduction, *algae* form differentiated sex cells that fuse to produce a diploid *zygote* with two sets of chromosomes. The *zygote* develops into a sexual spore, which germinates when conditions are favorable to reproduce and reform the haploid organism with a single set of chromosomes. This pattern of reproduction is called the alternation of generations. *Fungi* are eukaryotic and have membrane-bound cellular organelles and nuclei. They have no plastids of any kind (and no chlorophyll). The hyphae of the *fungi* are of two general kinds: some are *septate* and are divided by septa (walls) that separate the cylindrical hypha into cells; in the non-septate *fungi*, the hypha is one long tube. The septa are perforated, however, permitting the cytoplasm to flow throughout the length of the filament.

Mitosis occurs in the non-septate *hyphae*, but there is no accompanying cytokinesis (division of the cytoplasm), so the *hyphae* are *multinucleate* (with many nuclei). The special name for this condition – an organism or part of an organism with many nuclei not separated by walls or membranes – is *coenocyte*, and the organism is coenocytic. A few *fungi* - called by the general name yeasts - are single-celled and nonfilamentous most of the time. The only *flagellated* cells in the kingdom are the *flagellated* gametes of the *chytrids*. The *fungi* are all *heterotrophic* but some are parasitic while the *saprobies* are saprotrophic and digest, recycle materials from dead organisms.

4.2 LEARNING OBJECTIVES

After reading this chapter, you should be able to:

- (i) Explain the meaning of protozoa.
- (ii) State the characteristics of protozoans.
- (iii) Compare cells in protozoan.
- (iv) Examine some forms of protozoans (flagellates, sarcodines, ciliates and sporozoans).
- (v) Define the term algae.
- (vi) Discuss the characteristics of algae.
- (vii) Explain some forms of algae: (euglenophytes, diatoms, dinoflagellates, green algae, brown algae, red algae, slime molds and water molds).
- (viii) Give the meaning of fungi.
- (ix) Describe the traits of fungi cell: (filamentous fungi cell, the growth of Mushroom, specialized hyphae).
- (x) Highlight some forms of fungi cells: (zycomycetes, ascomycetes, basidiomycetes, deuteromycetes (yeast, the life cycle of yeast)).
- (xi) State the activities of fungi (fungi as decomposers, fungi as mycorrhizae and fungi as lichens);
- (xii) Discuss the functions of fungi to humans.

4.3 PROTOZOA

4.3.1 Meaning of Protozoans

Protozoa are single-celled eukaryotes (organisms whose cells have nuclei that commonly show characteristics usually associated with animals, most notably mobility and *heterotrophy*). They are often grouped in the kingdom Protista together with the plant-like algae and fungus-like *water molds* and *slime molds*.

Many protozoans do have some animal like traits, such as the ability to move. But because of protozoans' structure and development, most scientists now think that they are most closely related to other protists.





4.3.2 General Characteristics of Protozoa

(a) **Size and shape:** *Protozoa* vary substantially in size and shape. Smaller species may be the size of *fungus cells*; larger species may be visible to the unaided eye. Protozoan cells have no cell walls, and therefore can assume an infinite variety of shapes. Some genera have cells surrounded by hard shells, while the cells of other genera are enclosed only in a cell membrane.

Many *protozoa* alternate between a free-living vegetative form known as *atrophozoite* and a resting form called a *cyst*. The *protozoa* cyst is somewhat *analogous* to the bacterial spore, since it resists harsh conditions in the environment. Many *protozoa* parasites are taken into the body in the form of cyst. Most protozoa have a single nucleus, but some have both a *macronucleus* and one or more *micronuclei*. Contractile vacuoles may be present in *protozoa* to remove excess water and food vacuoles are often observed.

- (a) **Nutrition and Locomotion:** *Protozoa* are *heterotrophic microorganism*, and most species obtain large food particles by *phagocytosis*. The food particle is ingested into a food vacuole. *Lysosomal enzymes* then digest the nutrients in the particle, and the products of digestion are distributed throughout the cell. Some species have specialized structures called *cytosomes*, through which particles pass in *phagocytosis*. Many *protozoa* species move independently by one of three types of locomotor organelles; flagella, cilia or pseudopodia. Flagella and cilia are structurally similar having a “9-plus -2” system of *microtubules*, the same type of structure found in the tail of animal sperm cells and certain cells of unicellular algae.
- (b) **Reproduction:** sexual and asexual modes of reproduction have been observed in many species of *protozoa*.
- (c) **Respiration:** Aerobic and anaerobic means of respiration have been seen in some species of *protozoa*.

Table 4.1: Comparison of Cells in Protozoan

	Flagellates	Sarcodines	Ciliates	Sporozoans
				
Example	<i>Trypanosoma</i> , <i>Trichonympha</i> etc.	<i>Amoeba</i> , <i>Entamoeba</i> , <i>Foraminiferans</i> , Radiolarians etc.	<i>Paramecium</i> , Didinium, Stentor, Vorticella etc.	<i>Plasmodium</i>
Motion	Long undulipodia	By pseudopods	Short undulipodia	No special organs
Feeding	Absorbs	Engulfs	Engulfs	Absorbs
Parasitic	A few	A few	A few	All
Shell	None	Some calcium carbonate some silica	None	None

Source: (Slesnick *et al*, 1985)

4.3.3 Some forms of Protozoans:

(a) Flagellates

Members of this phylum are considered to be the most primitive of the protozoans. Most biologists think that ancient *flagellates* are the ancestors of present day plants and animals.

Flagellates or mastigophores (mas'te ge forz) - vary greatly in size. Each flagellate bears at least one **undulipodium**, while some have thousands. Many flagellates live independently in fresh water or salt water, but some live inside tissues of plants and animals. The picture in Table 4.1 shows a **flagellate** of the genus *Trichonympha* that lives inside the intestines of termites, where it digests particles of wood. Because termite cannot digest wood themselves, they cannot live without the **flagellates** that perform this function. Within the termite's intestines, the **flagellates** receive food and protection. Other **flagellates** are parasitic and cause diseases. A flagellate called *Trypanosoma* causes African sleeping sickness. This **flagellate** lives in the bodies of tsetse (tset'se) flies. When these flies bite humans or other mammals, they transmit the disease causing organism.

(b) **Sarcodines**

Amoebas are some of the most familiar of all protists. Grouped with their relatives and **radiolarians**, they make up the **sarcodine** phylum.

A living *Amoeba*, such as the one in Figure 4.1, shows constant changes of shape. As this organism moves, it slowly pushes out pseudopods. Cytoplasm that flows into the pseudopods moves *Amoeba* along a surface. *Amoeba* moves in this way in response to stimuli, such as light, chemicals, and physical contact. *Amoeba* also uses pseudopods to engulf food, such as bacteria or other protists. After surrounding the other organism, *Amoeba* forms a vacuole around the organism by **phagocytosis**.

Various *Amoebas* live in fresh water, saltwater, or soil, while others are parasites that live in the bodies of animals. *Entamoebahistolytica* is a parasite of humans. It causes amoebic dysentery, an intestinal disease.

Not all **sarcodines** are shapeless blobs like *Amoeba*. Many **sarcodines**, including the **foraminiferans** and **radiolarians** shown below, form calcium carbonate. Long, thin pseudopods poke through openings in the shells to form netlike traps for prey. **Foraminiferans** digest food while it is still outside of the shells.

Radiolarians secrete intricate, internal shells made of silica, a hard compound. Like **foraminiferans**, they capture prey with pseudopods extended through holes in their shells. **Radiolarians** draw their prey inside their shells for digestion.

The shells of both of these types of sarcondines make up a large part of the sediment on ocean floors. Accumulations of **foraminiferans** shells help form various types of limestone, such as chalk. The white cliffs of Dover on the English seacoast are composed largely of **foraminiferans** shells. Layers formed from **radiolarian** shells have produced some of the earth's silica-containing rock.

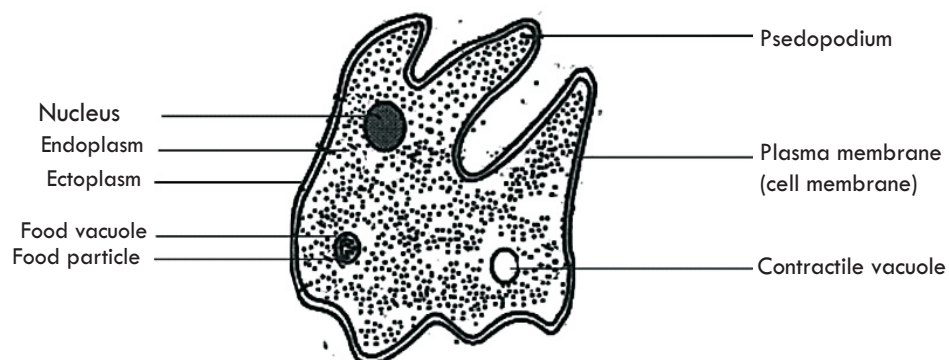
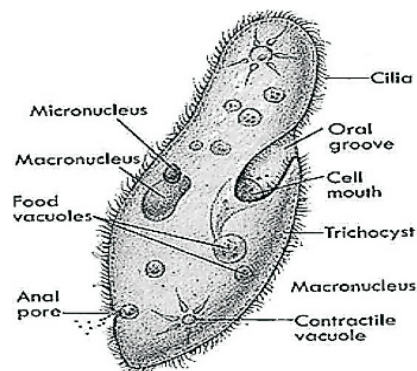


Figure 4.1: Microscopic appearance of *Amoeba Proteus*.

Source: Iddo-Umeh (1996).

(c) **Ciliates**

Ciliates are possibly the most complex and highly organized protozoans. Their name is derived from their undulipodia, the short, bristle like organs of locomotion present in all ciliate species. These organs propel a cell by beating in a coordinated, wave like fashion. **Ciliates** vary greatly in size and shape. But almost all ciliates are free-living species found in freshwater or saltwater. One common ciliate is *Paramecium*, shown in Figure 4.2. Unlike *Amoeba* with its changing shape, *Paramecium* has a fixed, slipper like shape. *Paramecium* swims rapidly using the short undulipodia that cover its body. These organs operate like paddles moving *Paramecium* backward as well as forward. Undulipodia are also important to *Paramecium* for taking in food. They move food into the gullet, pocket-like opening. From the gullet, food enters a food vacuole where enzymes digest the food. Digested wastes are expelled from the food vacuole through an anal pore. Excess water is expelled by the contractile vacuoles. *Paramecium* has a small (micro) and a large (macro) nucleus. The small micro-nucleus controls reproduction and the large macronucleus control respiration and other cell activities. *Paramecium* generally reproduces asexually. Occasionally, it can reproduce sexually by **conjugation**, in which two paramecia join and exchange the genetic material of their micronuclei. *Paramecium* has a defense mechanism that it can use against **predators**. When threatened, *Paramecium* shoots dart-like projectiles at its enemy.



**Osmoregulation in paramecium is carried out by contractile vacuoles, which actively expel water from the cell to compensate for fluid absorbed by osmosis from the surroundings. The number of contractile vacuoles varies from one, to many depending on species.*

Figure 4.2: Microscopic appearance of *Paramecium aurelia*.

Source: Campbell (1993).

Note: *Paramecium*, an example of ciliate complexity, is covered by thousands of individual cilia (LM). Associated with each cilium are trichocysts, bubble-like organelles that discharge sticky, **proteinaceous threads**. Although these threads are released in the presence of predators, they have little effect and are thought to function mainly in stabilizing the cell during feeding. Other genera have toxic trichocysts. *Paramecium* feeds mainly on bacteria. Rows of cilia along a funnel-shaped oral groove move food down into the cell mouth where the food is engulfed by phagocytosis. The food vacuoles combine with lysosomes and, as the food is digested; the vacuoles fuse with a specialized region of the plasma membrane that functions as an anal pore. *Paramecium*, like other freshwater protists, constantly takes in water by osmosis from the hypotonic environment. Bladderlike contractile vacuoles accumulate the excess water from radial canals and periodically expel it through the plasma membrane by contractions of the surrounding cytoplasm.

(a) **Sporozoans**

All *sporozoans* are parasitic protozoans that lack undulipodia as adults. Like many parasites, *sporozoans* have complicated life cycles with several different stages. *Sporozoans* feed by absorbing dissolved nutrients from their hosts. Various *sporozoans* species cause serious diseases in humans and in both wild and domesticated animals. For example *sporozoans* in the genus *Plasmodium* cause malaria.

Biologists have long studied the complex life cycles of various species of *Plasmodium* to find a way to control malaria. The diagram in Figure 4.3 outlines the stages of the life cycle of *Plasmodium* species. The parasite lives alternately within the bodies of mosquitoes and humans. In the female *Anopheles* mosquito, *Plasmodium* reproduces sexually. *Gametes* combine to form *zygote*, which divide and produce large numbers of spore-like cells. These cells are transmitted to human blood in the saliva of a mosquito that bites humans. In the human, *Plasmodium* reproduces asexually. It completes several divisions in the human's liver and red blood cells. The chills and fever of malaria, which occur at regular intervals, coincide with the release of newly produced *Plasmodium* cells from red blood cells. When an uninfected mosquito bites an infected human, the mosquito picks up many of these *sporozoans*, and the cycle begins again. One way to control the disease is to destroy the breeding grounds of mosquitoes.

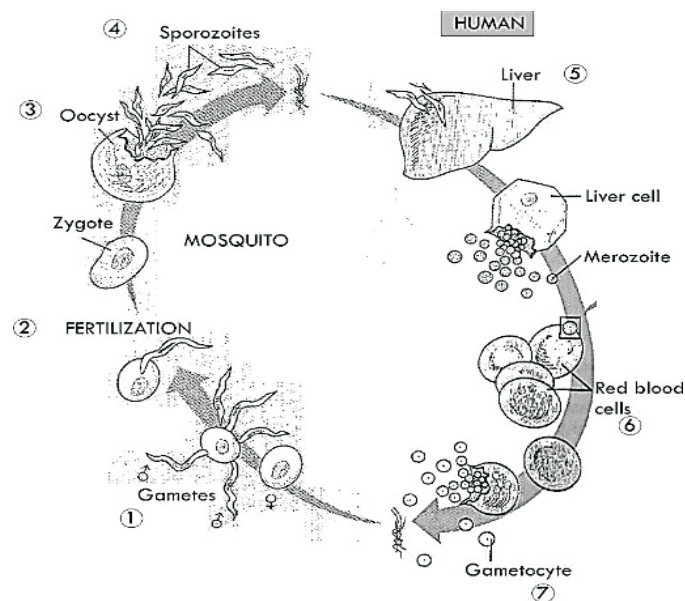


Figure 4.3: The Life Cycle of Plasmodium.

Source: Campbell (1993).

Note: (1) Female *Anopheles* mosquito bites a person infected with malaria and picks up plasmodium gametocytes along with blood. (2) Gametes form, fertilization occurs in mosquito's digestive tract, and a zygote forms. The zygote is the only diploid stage in the life cycle. (3) An oocyst develops from the zygote in the wall of the mosquito's gut. Thousands of sporozoites develop in the oocyst, and then migrate to the salivary gland. (4) The mosquito bites another person, infecting the victim with the plasmodium sporozoites. (5) The sporozoites enter the victim's liver cells. After several days they undergo multiple divisions to become merozoites, which then infect the victim's red blood cells. (6) The merozoites grow and divide asexually into great numbers of new merozoites, repeatedly breaking out of the blood cells at intervals of 48 or 72 hours (depending on the species). This causes periodic chills and fever. Some of the merozoites infect new red cells. (7) Some merozoites divide to form gametocytes, which complete the life cycle in a new female mosquito.

(b) **Slime molds**

Slime molds are unusual organisms with complicated life cycles as shown in Figure 4.4. They are amoeba-like at one stage and fungus-like at another stage. These strange creatures grow on damp soil, rotting logs, and other decaying vegetation in forests. They are mostly unicellular, plasmodial or colonial, phagotrophic, wall-less organisms. The non-reproductive or vegetative stage of the common slime mold is a large, amoeboid mass of cytoplasm surrounding numerous nuclei. This sticky mass is called a *plasmodium* and can be white, yellow, or red. (This stage bears no relation to *Plasmodium*, the sporozoan genus). A slime mold *plasmodium* flows along slowly, using an amoeboid motion, while engulfing and absorbing organic nutrients. Like *Amoeba*, the *plasmodium* can respond to stimuli. For example, it can avoid obstacles and dry areas.

Dry or cold conditions are unfavourable to *plasmodium* growth. Under these conditions, a *plasmodium* stays in one place, thickens, and begins to grow small stalks or fruiting bodies. You can follow these stages in the diagram above. Meiosis occurs in cells at the tips of the fruiting bodies, and cell walls cover individual **monoploid** nuclei, forming spores. Slime mold spores are released to the environment where they can remain dormant for long periods. When conditions are favourable, these spores germinate, forming **gametes** equipped with flagella. These **gametes** swarm together and fuse in pairs to form **zygotes**. Each **zygote** can produce a new *plasmodium*.

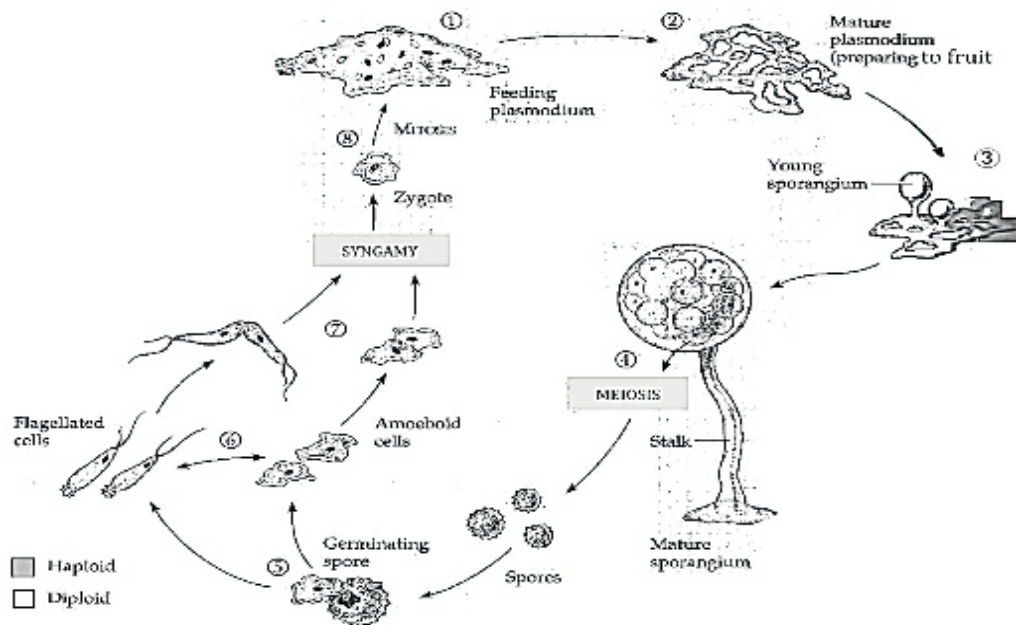


Figure 4.4: Life cycle of a Plasmodial slime mold.

Source: Campbell (1993).

Note: (1) The feeding stage is a multinucleate (coenocytic) plasmodium that lives on organic refuse. (2) The plasmodium often takes a weblike form, an adaptation that increases the surface area contacting food, water, and oxygen. (3) The plasmodium rounds into a mound and erects stalked fruiting bodies called sporangia. (4) When conditions become harsh within the bulbous tips of the sporangia, meiosis produces haploid spores. (5) The resistant spores germinate to become active haploid cells when conditions are again favourable. (6) These cells are either amoeboid or flagellated, the two forms readily reverting from one to the other. (7) These cells unite in like pairs (flagellated with flagellated and amoeboid with amoeboid) to form diploid zygotes. (8) Repeated division of the nucleus of the zygote by mitosis, without cytoplasmic division, forms a feeding plasmodium and completes the life cycle.

4.4 ALGAE

4.4.1 Meaning of Algae

Algae are numerous groups of chlorophyll-containing, mainly aquatic eukaryotic organisms ranging from microscopic single-celled forms to multicellular forms 30 meters or more long. Distinguished from plants by the absence of true roots, stems and leaves and by a lack of non-reproductive cells in the reproductive structures. Classified into the six phyla: Euglenophyta, Crysophyta, Pyrrophyta, Chlorophyta, Phaeophyta and Rhodophyta. The study of algae is known as **phycology** and one who studies algae is called phycologist.

4.4.2 General Characteristics of algae

- (a) Occurrence of algae: On the basis of habitat, algae are classified into three groups;
- Aquatic forms e.g. fresh water form (e.g. *Spirogyra*) and marine water form (e.g. *Polysiphonia* and *Sargassum*)
 - Terrestrial algae e.g. *Vaucheria* and *fritschiella*
 - Algae of unusual habitats include:
 - (i) **Halophyticalgae:** Algae present in highly saline water e.g. *Dunaliella*.
 - (ii) **Epiphyticalgae:** Algae grown on the surface of other plants/algae e.g. *Oedogonium*.
 - (iii) **Epizoicalgae:** Algae grown on animals such as snails and fishes e.g. *Cladophora* grows on the shells of snails.
 - (iv) **Endozoicalgae:** Algae growing inside animals e.g. *Zoochlorella* grows inside hydra.
 - (v) **Symbiotic algae:** symbiotic association with fungi in lichen, in Bryophytes (*Anthoceros*), in Pteridophytes (*Azolla*), gymnosperms (corolloid roots of cycas) and in angiosperms.
 - (vi) **Parasitic algae** e.g. *Cephaleuros*.
 - (vii) **Thermophyte algae:** growing in hot spring e.g. *Heterohormogonium*.
 - (viii) **Fluviatile algae:** Algae found in rapidly running water such as water falls e.g. *Ulothrix*.
- (b) **Plastids in algae:** Except in cyanophyceae (blue green algae), pigments in algae are found in membrane bound organelles called plastids. In blue green algae, plastids are absent, pigments located at peripheral cytoplasm called chromoplasm. Plastids are Leucoplast and chromoplast.
- (c) **Reserved food materials in algae:** it is also called food reserve. It is the stored form of food in the cells for energy.
- (i) **Cyanophyceae:** cyanophycean starch
 - (ii) **Chlorophyceae:** Starch
 - (iii) **Rhodophyceae:** floridean starch
 - (iv) **Phacophyceae:** Laminan, manitol and oil
- (d) **Reproduction in algae**
- (i) **Vegetative reproduction:** cell division, fission, fragmentation, Hormogonia, Formation of adventitious branches, tubers, buddings etc., are the important vegetative reproduction methods in algae.
 - (ii) **Asexual reproduction:** By a variety of motile or non – motile spores, zoospore, aplanospore, hypnospore, tetraspore, autospore, a kinetes etc, are the important spore types in algae.
 - (iii) **Sexual reproduction:** here the unions of gametes are involved: Autogamy, hologamy, isogamy, anisogamy and Oogamy are all types of sexual reproduction in algae.

- (a) Alternation of generation: it is also called alternation of phase, it is a term primarily used to describe the life cycle of plants. Most algae have an alternation of many celled haploid **gametophytic generation** with many celled diploid **sporophytic generation**, which alternate regularly.
- (b) Major classes of algae (algal systematics)
- (i) **Cyanophyta:** Blue green algae, prokaryotes.
- (ii) **Euglenophyta:** motile, protozoan-like algae lack true cell wall.
- (iii) **Chrysophyta:** Golden-brown algae = *diatoms*
- (iv) **Pyrrophyta:** Dinoflagellates
- (v) **Chlorophyta:** *Green algae*
- (vi) **Rhodophyta:** Red algae
- (vii) **Pacophyta:** Brown algae

4.4.3 Some Forms of Algae

(i) Euglenophytes

Euglenophytes are unicellular organisms that have one or two undulipodia and contain chloroplasts. They divide asexually by mitosis. Also, a *euglenophyte* has a protein layer called a pellicle beneath its cell membrane, giving it a fairly rigid shape while allowing some movement.

This group is named *Euglena*, which is shown in Figure 4.5. This organism has an eyespot- a spot of red pigment covering a light-sensitive area-enabling the organism to detect light. *Euglena* uses light to make its own food through *photosynthesis*. In the absence of light, *Euglena* can absorb organic materials to use as food. The organism has an opening called a **reservoir** into which contractile vacuoles discharge excess water.

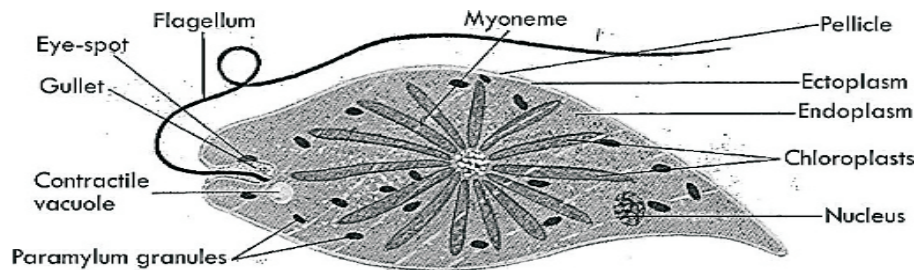


Figure 4.5: Microscopic appearance of *Euglena viridis*

Source: Campbell (1993).

Note: *Euglena*. A motile protist with chloroplasts, this unicellular alga uses its long flagellum for propulsion. Near the base of the flagellum is an eyespot that functions as a pigment shield. Depending on the position of the organism, it allows light from only a certain direction to strike a light detector, the swelling at the base of the long flagellum. These structures seem to function in phototaxis, which is important for these photosynthetic algae. *Euglena* lacks a cell wall but has a strong, flexible covering made of protein beneath its plasma membrane.

(ii). Diatoms

Diatoms, such as the one below, are golden **brown algae**-or chrysophytes. They are important producers of food and oxygen in the oceans. Most species contain yellow-brown pigments and store food in the form of oils rather than starch.

The cell wall of a diatom has two halves. The rim of one half tightly overlaps the rim of the other half, like a pillbox. Silica in the cell wall forms a hard shell that remains long after the rest of the diatom has decomposed. Diatom shells pile up on lake and ocean bottoms over millions of years, forming deposits of a crumbly substance called diatomaceous earth (see Figure 4.6). People use this material as an abrasive in silver polish and toothpaste, as well as in swimming pool filters.

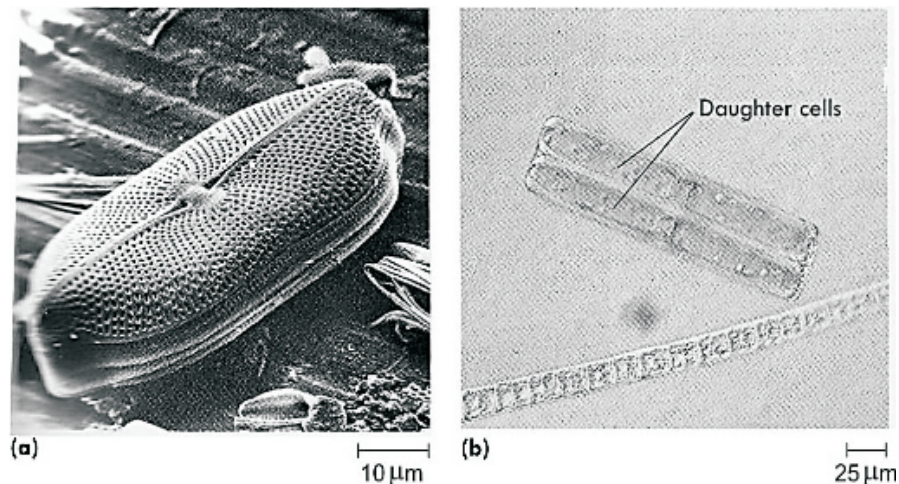


Figure 4.6: Diatoms.

Source: Campbell (1993).

Note: (a) The glasslike shells consist of two halves that fit like the bottom and lid of a shoe box. Tiny pores in the ornate shells allow for the exchange of gases and other substances between the cell and its environment. The shape of the shell and its pattern of pores are used for classifying diatoms. This species is *Navicula monilifera* (SEM). (b) In this side view of a species of *Pinnularia*, the cell has just divided by mitosis (LM). Each daughter cell keeps half of the parent cell's wall and builds a new complementary half.

(iii) **Dinoflagellates (pyrrophytes)**

Dinoflagellates are single-celled organisms that are also called pyrophytes. These organisms spin like tops as they swim in ocean water. Most **dinoflagellates** have two undulipodia that each beat within a groove. One groove extends the length of the cell while the other encircles the mid section. Except for the grooves, the surface of such **dinoflagellates** is covered by armorlike, cellulose plates.

Among protists, dinoflagellates are second only to **diatoms** in abundance and importance as producers of food and oxygen. Many **dinoflagellates** are bioluminescent, which means they can change chemical energy into light. This bioluminescence can be seen at night from a ship passing through waters inhabited by dinoflagellates and other bioluminescent organisms. The disturbed water, as in ship's flashes of light. For this reason, these organisms are also called fire algae.

(iv). Green Algae (chlorophytes)

Green algae or chlorophytes display great diversity both in size and shape. The phylum includes both single-celled and multicellular forms. Multicellular species that live in sea water, such as *Ulva* shown in Figure 4.7, are often larger than their freshwater relatives. Various freshwater **green algae** are single cells, colonies or filaments. *Chlamydomonas*, shown in Figure 4.8 is one example of freshwater **green algae**. Some **green algae** live on damp surfaces of soils and trees, and many live in symbiotic associations with protozoans, fungi, and animals. Some species of green algae form true multicellular tissues, in which different cells are specialized to perform different functions. These organisms differ from some protists and monerans which clump to form multicellular colonies. Because the cell in a colony can still function independently. A long colony is not considered to be a multicellular organism.

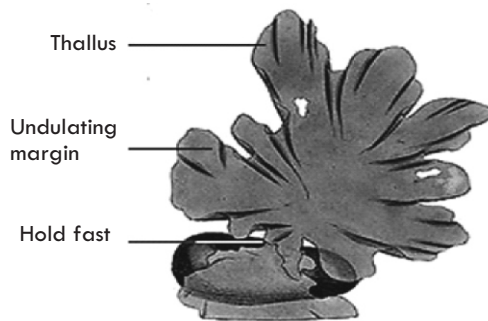


Figure 4.7: Microscopic appearance of *Ulva lactuca* (Sea lettuce)
Source: en.Wikipedia.org

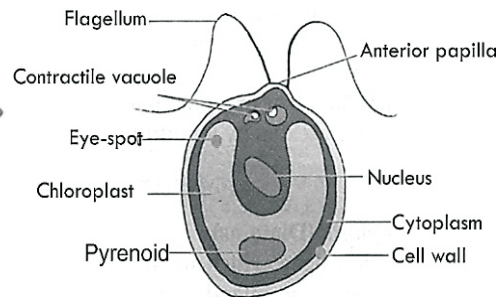


Figure 4.8: Microscopic appearance of *Chlamydomonas reinhardtii*
Source: Ndu, Asun and Aina (2013).

(v). Brown Algae (phaeophytes)

Brown algae or **phaeophytes** - are multicellular seaweeds that superficially resemble plants. They have root like, stem-like, and leaf-like parts, which differ from true roots, stems and leaves in their internal organization. Almost all species of **brown algae** live in oceans, and most species are large. **Brown algae**, the giant kelp, usually grow in lengths up to 60 meters and form large marine “forests” offshore. Huge masses of one species, *Sargassum nitans*, float in the open waters of the Sargasso Sea part of the Atlantic Ocean. Smaller species dominate the rocky shores of the earth's coastal regions. Cells of **brown algae** contain numerous small vacuoles and store food in a compound known as laminarin. Their numerous chloroplasts are usually disk-shaped but also occur in other shapes, such as stars, spirals, and ribbons.

(vi). Red Algae (Rhodophytes)

Rhodophytes are multicellular seaweeds commonly known as **red algae**. Their red colour is due to two pigments which mask chlorophyll. Compared to **brown algae**, they are small, ranging in length from a few centimeters to a few meters. Most species of **Red algae** usually have delicate lace-like shapes. Most **red algae** are marine seaweeds that grow attached to rocks at greater depths than other types of algae can grow. Some **red algae** grow 175 meters below the ocean surface. They can grow at great depths because their red pigments absorb blue light which can penetrate more deeply into water than other colours of light can. Another distinguishing trait of red algae is that, unlike most organisms, they have no cells with undulipodia. Their cells also lack **centrioles**. People eat some kinds of **red algae** which are a good source of iodine. Red algae are important as a source of agar, the substance on which scientists grow bacteria and fungi.

4.5 FUNGI

4.5.1 Meaning of Fungi

A fungus is any member of the group of eukaryotic organism that includes micro-organisms such as *yeast* and molds, as well as the more familiar *mushrooms*. These organisms are classified as a kingdom, which is separate from the other eukaryotic life kingdoms of plants and animals.

4.5.2 Traits of Fungi Cell

The cells of fungi, like those of protists, are eukaryotic. In addition, most fungal cells are tubular in shape. As in monerans and plants, a rigid cell wall encloses fungal cells. The cell walls of fungi are made of chitin, the same tough substance found in the body covering of insects. Fungal cells have large central vacuoles, as do plant cells. Unlike plants, fungi never contain chloroplasts and thus, do not carry out *photosynthesis*.

How fungi get food most clearly distinguishes them from other eukaryotic organisms. Fungi secrete digestive enzymes into a food source. These enzymes break down molecules of the food source in the soil, a rotting log, the carpet of needles on a forest floor, or perhaps a piece of bread. Then the fungi absorb the already digested materials through their cell walls and membranes.

The life of a fungus begins when the fungus germinates from a reproductive cell called a spore, as shown in Figure 4.9. Like the resting cell of monerans, a fungal spore is often covered by a thick coat. A few fungi continue life as single cells, but most fungi, such as molds and *mushrooms*, develop into multicellular organisms.

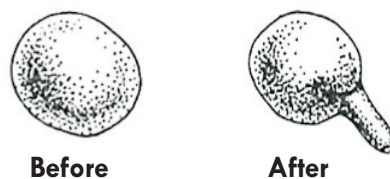


Figure 4.9: Germinating spore of a fungus.

Source: (Slesnick et al, 1985).

4.5.3 Filamentous Fungi Cell

In a multicellular or filamentous-fungus, a tiny, tubular filament grows out from the spore. This filament is called a hypha. In many species of fungi, the *hyphae* contain cross wall or septa-that divide the filaments into cell-like units. Usually, the septa have large perforations that enable cytoplasm and even nuclei to move freely from one unit to the next. In other fungal species, the *hyphae* do not have septa.

As a hypha grows, it branches out and eventually forms a tangled mat called *mycelium*. Usually you cannot see the spreading mycelium because it grows embedded in its food source.

4.5.4 Water molds

Water molds make up another protist phylum whose members cannot produce their own food. Most *water molds* live in water or moist soil and obtain their nutrients from dead organic material. A few *water molds* are parasites that live on the tissues of plants or animals. *Water molds* produce long thread-like filaments that can absorb nutrients (see Figure 4.10). Like

many other protists, **water molds** have undulipodia during their life cycles. The spores of **water molds** each bear two flagella. One parasitic species, **phytophthora** infestans or potato blight plays an important role in history. The filaments of this parasite extend into moist leaves, as shown to the right. Filaments invade the plant cells and absorb nutrients, eventually killing the plant. **Phytophthora**, which means “plant destroyer”, ruined the entire potato harvest in Ireland in 1845 and 1846, resulting in mass migrations and more than one million deaths from starvation.

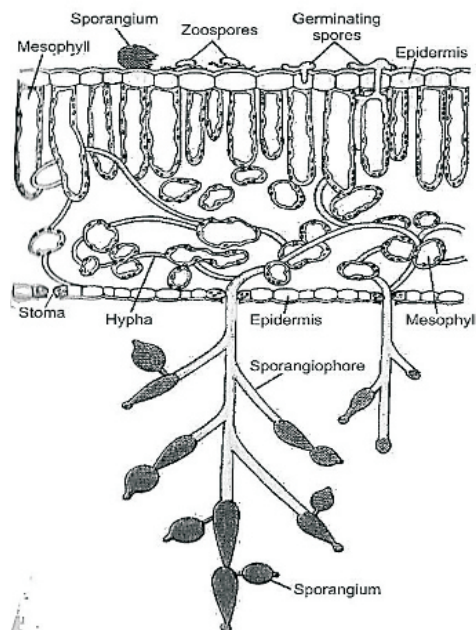


Figure 4.10: Water mold invading Potato.

Source: Raven, Evert and Eichhorn (1999).

Note: *Phytophthora infestans*, the cause of the late blight of potatoes. The cells of potato leaf are shown in Figure 4.9 as water mold invades potato leaf. In the presence of water and low temperatures, either of two events can occur. Zoospores can be released from the sporangia and swim to the germination site (as shown here), or the sporangia can germinate directly through a gem tube.

4.5.5 The Growth of Mushroom

The only part of a **mushroom** that can be seen above the surface is the reproductive structure, or fruiting body. These reproductive structures of **mushrooms** appear only when conditions are favorable for spore production.

A **mushroom** begins to develop as a knot of **hyphae** covered by a thin membrane. As growth continues, the membrane breaks, revealing a cap which covers a series of paper-thin tissue layers called gills that radiate like spokes of a wheel around the stalk. Countless spore-producing **hyphae** cover the surface of the gills. These **hyphae** can produce half a million spores a minute for several days!

As the hidden mycelium of a *mushroom* uses up its food supply, growth occurs at the tips of the *hyphae*. The mycelium grows outward in an ever widening circle, much like ripples on a pond. The fruiting bodies of *mushrooms* develop above the most recent growth of the mycelium. Thus, a mycelium can produce a circle of *mushrooms* at its outer edge called a fairy ring, as shown in Figure 4.11.

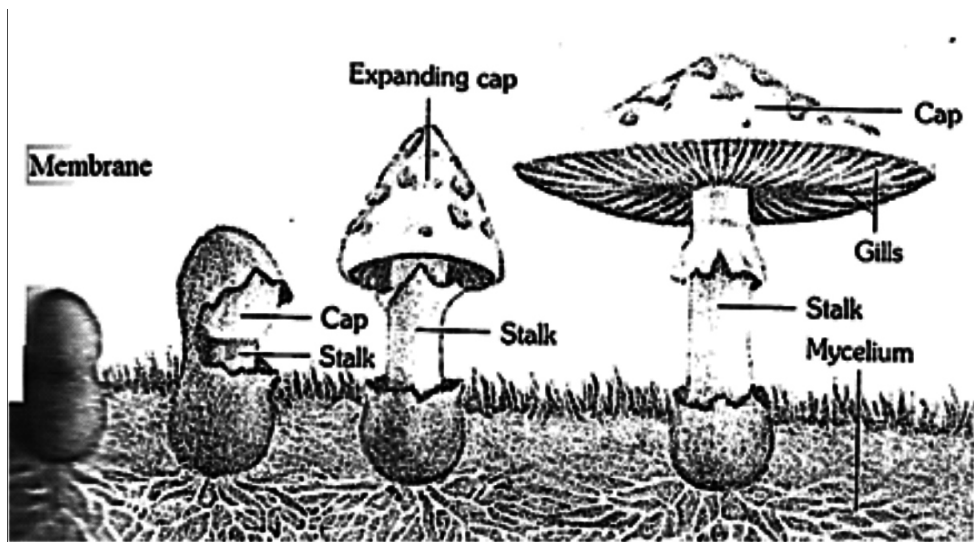


Figure 4.11: Growth of a mushroom fruiting body.

Source: (Slesnick et al,1985).

4.5.6 Specialized Hyphae

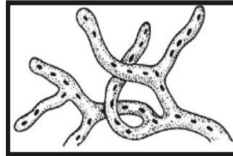
In addition to specialized reproductive *hyphae*, some fungi have specialized non-reproductive or vegetative structures. For example, certain parasitic fungi have *hyphae* that can penetrate the cells of their hosts. These specialized *hyphae* are called *haustoria*. A few fungi can actually capture living animals. Specialized *hyphae* have developed for a variety of purposes in some fungal groups. *Hhaustoria* are enlarged, convoluted hyphal cells adapted for absorption that contact the plasmalemma of host cells after wall penetration. They are present in the rusts, powdery mildews, and some *Mycorrhizae* fungi.

4.5.7: Some Forms of Fungal Cells:

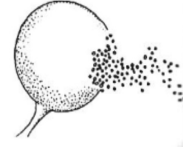
(a) Zycomycetes

Name of Group	Vegetative Structures	Asexual Reproductive System
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Zygomycetes
Conjugation Fungi
Example: Rhizopus, bread mold

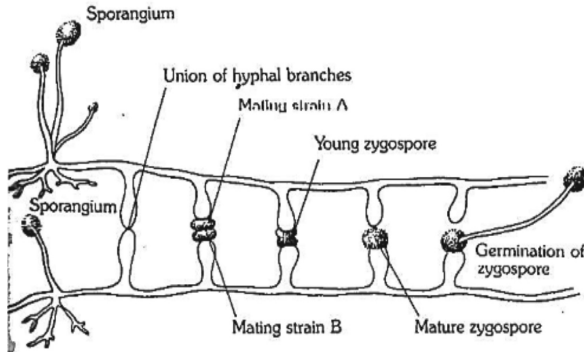


In the conjugation fungi, the vegetative hyphae do not have septa, so cytoplasm can move freely through the filaments. However, septa separate the vegetative hyphae from the reproductive hyphae.



Asexual spores of a conjugation fungus, such as a bread mold, develop within a case -or sporangium-found at the tip of a spore -producing hypha. When the sporangium burst, it releases hundreds of spores that air currents carry to new environments.

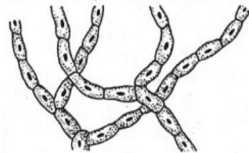
Zygomycetes	Vegetative Structures	Sexual Reproductive System
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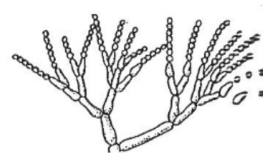
In bread mold, sexual reproduction requires two mating types, or strains. The two strains, called “+” and “-” look identical. When cells of the se two strains come together, a diploid zygote called a *zygospore* is formed. When conditions are favorable, the spore cell’s nucleus undergoes meiosis, producing monoploid spores.

(b) Ascomycetes

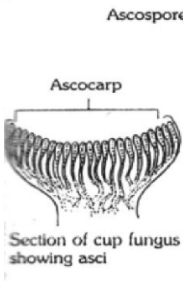
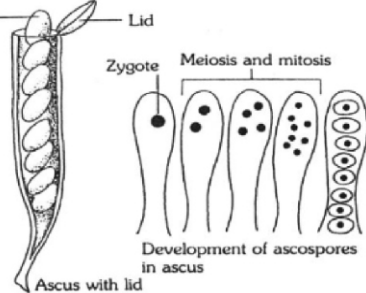
Sac Fungi
Example: Peziza, a cup fungus



In the sac fungi, a large central pore perforates the septa of the vegetative hyphae. Cytoplasm and cell organelles can migrate freely from one compartment to another.

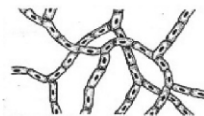


A few sac fungi, including the morels, do not have asexual spores most do not develop within cases. The specialized hypha that produces asexual spores is the conidiophore, and the spores are conidia.

Ascomycetes	Vegetative Structures	Sexual Reproductive System
 <p>Section of cup fungus showing asci</p>	 <p>Ascospore Lid Zygote Meiosis and mitosis Development of ascospores in ascus Ascus with lid</p>	<p>In sac fungi, the visible fruiting body is called an ascocarp. Two nuclei from different organisms of the same species fuse, forming a diploid zygote. Division by meiosis and then mitosis results in eight monoploid ascospores. A case called an ascus contains these spores. Numerous asci line ascocarp.</p>

(c) Basidiomycetes

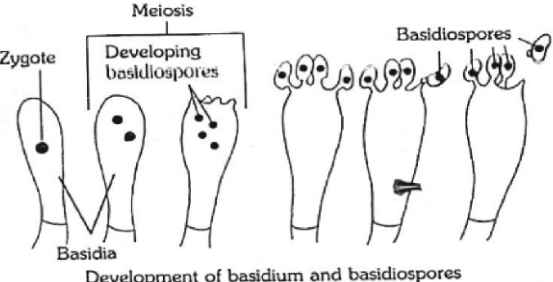
Club Fungi
Example: Agaricus, an edible mushroom



In the club fungi, the vegetative hyphae have septa much like those of the sac fungi.



Many club fungi, including the mushrooms, do not produce asexual spores. When asexual spores do occur, they form as buds, fragments, or conidia.

Basidiomycetes	Vegetative Structures	Sexual Reproductive System
 <p>Zygote Meiosis Developing basidiospores Basidia Basidiospores Development of basidium and basidiospores</p>		<p>In club fungi, the visible reproductive structure is called a basidiocarp. Within the basidiocarp, monoploid nuclei of different strains fuse to form a zygote. These club-shaped zygotes are called basidia. When the diploid zygote undergoes meiosis, four monoploid basidiospores are formed.</p>

Source: (Slesnick *et al*, 1985).

(d) Deuteromycetes

About 10,000 species of fungi are placed in a “catchall” category called “imperfect” fungi, or Deuteromycetes. Members of this group are imperfect in the sense that they have no known means of sexual reproduction. Scientists sometimes discover a sexual stage in what they thought was an imperfect fungus. Then, the fungus is usually classified into one of the other three groups of fungi.

Hyphae of imperfect fungi have septa. Conidiophores, similar to those in the sac fungi, produce asexual spores. The well-known mold *penicillium* is an imperfect fungus, always found growing on spoiled/leftover foods in the kitchen.

(a) The Yeast

This is a group of reduced or degenerate Ascomycetes. They are non-mycelial ascomycetes that do not form fruiting bodies, but have dominant growth form as unicell which may be glucose, ellipsoid or short cylinders. Some fungi occur as *yeast* under certain environmental conditions but as multicellular filaments under other conditions. For example, a species of *Mucor* develops as a filamentous form in the presence of oxygen. In the absence of oxygen it exists as *yeast*. *Yeasts* occur abundantly both in soil and in association with living plants and animals. They are particularly abundant in places that have a high content of sugar, such as in the nectar of flowers or on the surfaces of ripening fruits.

Unlike most fungi, a *yeast* cell obtains energy and raw materials by absorbing sugar and then breaking down within the cell. In the presence of oxygen, *yeasts* break down sugar by aerobic respiration. This process releases carbon dioxide and water. In the absence of oxygen, *yeasts* break down sugar by fermentation. The final products of this process are carbon dioxide and alcohol.

The Life Cycle of Yeast

Yeasts can reproduce either asexually or sexually. In asexual reproduction, a small pouch of *bud* develops on the surface of the yeast cell. The nucleus of the *yeast* migrates to the vicinity of the *bud* and divides by mitosis. Then, one of the new nuclei and some cytoplasm are pinched off from the yeast cell.

In sexual reproduction, *monoploid* yeast cells fuse to form a diploid zygote. The diploid cell can reproduce by budding, as shown in Figure 4.12. Or the diploid can undergo meiosis. Then, four new *monoploid* cells are formed. The *monoploid* cells generally reproduce asexually by budding for a while. Then, cells of the two different mating types fuse. The diploid zygotes that result from this fusion complete the life cycle (See Figure 4.13).

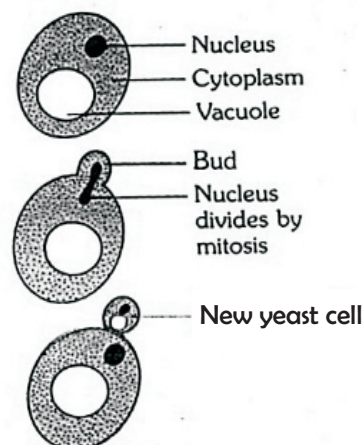


Figure 4.12: Budding of yeast cell.
Source: (Slesnick et al, 1985).

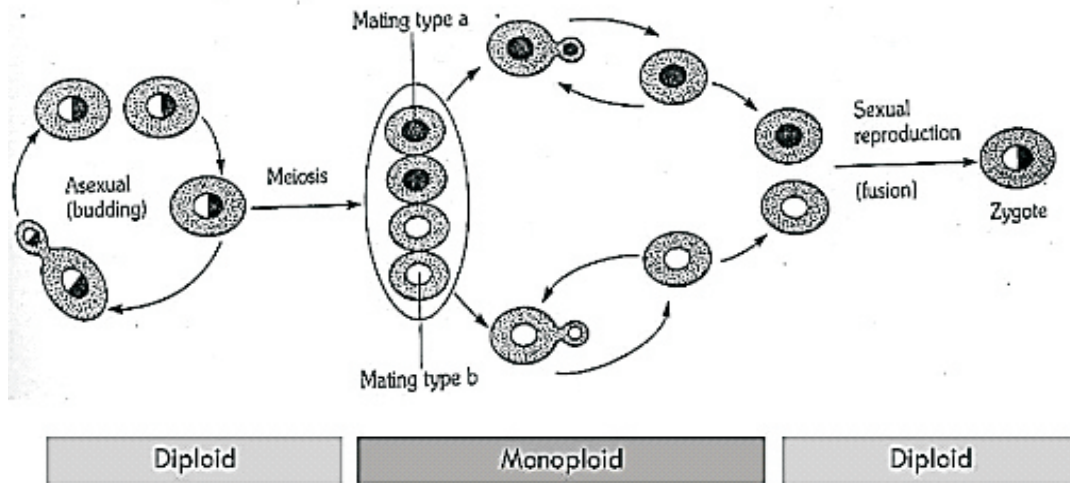


Figure 4.13: Life cycle of yeast cell.

Source: (Slesnick et al, 1985).

4.5 ACTIVITIES OF FUNGI

The activities of fungi affect other organisms in important ways. For example, fungi can change a dead tree into simple compounds that other living things can use for growth and metabolism. Also, fungi are affected by close associations with other kinds of organisms. As you read down you will discover how fungi affect and are affected by other organisms.

(a) Fungi as decomposers

Like monerans and protists, fungi are important *decomposers* of organic materials. Fungi break down these materials into simple, inorganic compounds. Some of these compounds are absorbed by the fungi, but some remain in the environment. Other organisms can use these compounds as raw materials. In this way, a process of natural recycling makes raw materials available continuously for the growth of new organisms.

Fungi are responsible for the decomposition of a wide variety of substances, since almost any organic material can provide food for fungi. In soil, fungi decompose the remains of other organisms. Fungi also break down fabric, leather, fur, wood, paint, and even some plastics.

(b) Fungi as *Mycorrhizae*

The *mutualistic relationship* between a plant root and a soil fungus is called a mycorrhiza. The soil fungus surrounds the root of a plant, and the fungal hyphae penetrate the root tissues, as shown in Figure 4.14. This arrangement provides the plant with a greater surface area for absorption of water and minerals from the soil. In turn, the plant provides the soil fungus with organic nutrients. Because *mycorrhizae* occur in a hidden world beneath the soil surface, many people are not aware of their existence. However, scientists think this relationship is very common, occurring in almost every kind of plant. Fossils estimated to be over 300 million years old show evidence of *mycorrhizae*.

In some cases, a plant will grow in the absence of soil fungi, but the growth will be poor. For example, orchids, cranberries, and pine trees do not thrive in the absence of soil fungi. Some plants require a particular kind of soil fungus, while others form *mycorrhizae* with variety of soil fungi.

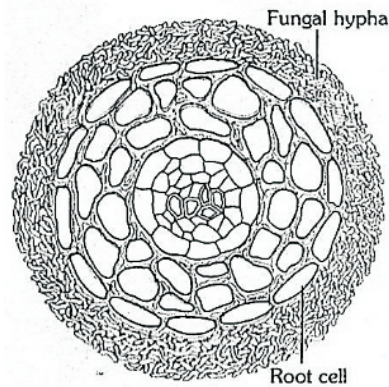


Figure 4.14: Section of a Mycorrhiza.
Source: Adapted from Dutta (2004).

(c) **Fungi as Lichens**

A fungus living in close association with either an algae or a cyanobacterium is called lichen. The algal or bacterial cells are dispersed among the fungal hyphae, as in Figure 4.15. The fungus is the more bulky and conspicuous part of the *lichen*. Most biologists have considered *lichens* to be a *mutualistic relationship*. The photosynthetic algae or bacteria provide nutrients for the fungus, while the fungus seems to protect its partner from drying out. However, a Swiss botanist, Simon Schwendener in 1868, revealed that *lichens* are composite organisms, consisting of fungi that live in partnership with microscopic algae. Other scientists still suggests that the algae and *cyanobacteria* can exist quite well on their own and have developed different degrees of resistance to their fungal partners.

Forms in which *lichens* grows are crust-like lichen, a leaf-like lichen and shrub-like lichen. Larger *lichens*, such as reindeer “moss”, are often abundant enough in arctic regions to provide food for grazing caribou herds. *Lichens* can grow on bare surfaces, such as rock, and in other places that do not permit the survival of other kinds of organisms. *Lichens* can survive extreme heat, extreme cold, and dryness. However, because they absorb substances quickly from the air and from rainwater, *lichens* cannot survive where the air is polluted. *Lichens* are pioneer organisms because they can pave the way for other living things to exist in an environment. Remember that *lichens* can exist on bare rock. In time, acids secreted by the *lichens* begin to break down the rock. This chemical action, combined with a buildup of organic materials from many generations of *lichens*, contributes to the formation of soil.

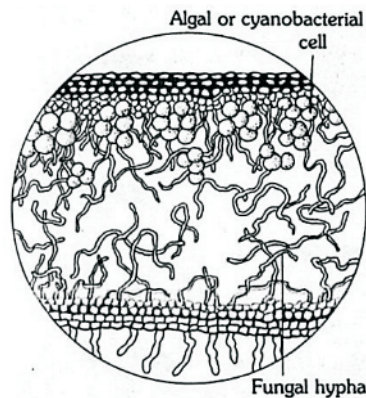


Figure 4.15: Cross section of lichen.
Source: Adapted from Dutta (2004).

4.6 FUNGI AND HUMANS

Humans use fungi in many ways. Gourmets prize edible fungi for their unique flavors and textures are used for food. In addition, the metabolic activities of fungi play an important role in the making of many foods, beverages, medicines, and chemicals useful to humans. On the other hand, some fungi are parasites on human, other animals and plants.

(a) Fungi as Food

Among the edible fungi are yeasts, truffles, morels, puffballs, and mushrooms. Some people eat dried yeast as a vitamin and protein supplement. Other edible fungi are eaten primarily for the unique flavors, although they do contain protein and vitamin D. One of the most highly prized of the edible fungi is the truffle, which grows underground near tree roots as a mycorrhiza. One way to locate truffles is to look for yellow flies that lay their eggs in the ground above these fungi. The fly larvae burrow down and feed on the truffles. Some mushrooms are good to eat, but many are not. If a **poisonous mushroom** is eaten, it can produce severe nausea and even death. Although more than 50 species of edible wild mushrooms grow in Nigeria only a few are easy to identify. Many different mushrooms look alike, and even experienced mushroom farmers and gatherers hunters can be fooled. So when it comes to eating wild mushrooms, the rule of thumb is –don't. Certain species of *Amanita mushrooms*, all similar in appearance, are poisonous. Amanitas cause most deaths from mushroom poisoning. The **fly amanita** is shown in Figure 4.16.



Figure 4.16: Poisonous fly amanita.
Source: (Slesnick et al, 1985).

(b) **Fungi in Industry: Yeast**

A poet once describes happiness as “a loaf of bread, a jug of wine, and thou.” Such happiness would be impossible without the metabolic activities of the *Saccharomyces cerevisiae*. Bread rises with a light, fluffy texture as yeasts ferment carbohydrates and release carbon dioxide gas in bread dough. Similarly, wine gets its alcoholic content when yeasts ferment the carbohydrates in grapes. The same *Saccharomyces* yeasts used in bread-making and wine making are also used in brewing beer.

(c) **Fungi in Industry: Blue-green molds**

The most important **blue-green molds** in industry belong to the genus *Penicillium* and the genus *Aspergillus*. Some species of *Penicillium* produce the widely used **antibiotic**, penicillin. Antibiotic is a substance made by one microorganism, usually a fungus to attack some bacteria. Because it has a similar effect on bacterial infections in humans, penicillin is important as a treatment for these infections.

Other species of *Penicillium* are important in the production of blue cheeses. The metabolic activity of *Penicillium roquefortii* grown on milk in the caves of Roquefort, France, produces Roquefort cheese. *Aspergillus*, another mold, produces citric acid. Many lemon flavored beverages and some medicines owe their taste to the citric acid produced by *Aspergillus*. This fungus also ferments soybean meal, resulting in soy sauce. A partial list of common foods manufactured with fungi includes: cheese, bread, chocolate, coffee, tea, pickles, olives, salami, soy sauce, tempeh, miso etc.

(d) **Fungi as Human Parasites**

Although plants are far more commonly attacked by fungi, humans are not immune to fungal diseases. Fungus infections in humans are often difficult to treat. Fungal spores are able to survive inside the human body for long periods. For this reason, a fungus disease that appears to be cured can reappear at a later time.

Fungi cause several diseases of the human skin, such as athlete's foot and ringworm. Athlete's foot is common during warm weather. Ringworm becomes an epidemic in schools occasionally. No worm is involved in ringworm. The fungus mycelium grows outward in all directions forming a ring usually on the scalp. Even when antibiotics and other medicines are used to treat ringworm, the symptoms can last for months.

Inhaling certain yeasts can cause cryptococcosis. This disease can occur in almost any organ of the body, but it frequently infects the lungs. The yeasts that cause the disease are carried by pigeons, so this cryptococcosis is often found in cities than in rural areas. In the past, cryptococcosis was often fatal. Now, however, treatment with antibiotics can control this disease.

(e) **Fungi as Plant Parasites**

Parasitic fungi cause some of the most destructive plant diseases. These fungi often infect plants that humans use for food. Both rusts and smuts are fungal plant parasites that can reproduce rapidly, affecting large areas in short time.

Nearly half of the world's farmland is used for growing wheat, and wheat rust ruins a tenth of that crop. Serious wheat rust infestations spread throughout United States farmlands in 1916 and again during the 1930s. Farmers stood by and watched as green fields turned red-brown with rust. This disease can be controlled by removing barberry, another host in the life cycle of wheat rust.

Smut attacks such cereal grains as corn, barley, and wheat. After the smut enters a seedling, the hyphae grow up the stem as the plant grows. Eventually, the kernels of grain are replaced by foul smelling smut balls.

4.7 CHAPTER SUMMARY

- Protozoa are unicellular aerobic eukaryotes.
- They have a nucleus, complex organelles, and obtain nourishment by absorption or ingestion via specialized structures.
- Protozoa have been traditionally divided based on their mode of locomotion.
- Flagellates produce their own food and use their whip-like structure to propel forward, ciliates have tiny hairs that beat to produce movement, amoeboid have false feet or pseudopodia used for feeding and locomotion, and sporozoans are non-motile.
- They also have different means of nutrition, which groups them as autotrophs or heterotrophs.
- Cyanobacteria or blue-green algae, are unicellular or multicellular eukaryotes that obtain nourishment by photosynthesis.
- They live in water, damp soil, and rocks and produce oxygen and carbohydrates used by other organisms.
- Cyanobacteria are believed to be the origin of green land plants.
- Fungi (mushrooms, molds, and yeasts) are eukaryotic cells (with a true nucleus).
- Most fungi are multicellular, and their cell walls are composed of chitin.
- They obtain nutrients by absorbing organic material from their environment (decomposers), through symbiotic relationships with plants (symbionts), or harmful relationships with their hosts (parasites).
- They form characteristic filamentous tubes called hyphae that help absorb material.
- The collection of hyphae is called mycelium. Fungi reproduce by releasing spores.
- Activities of fungi include: fungi as decomposers, fungi as mycorrhizae and fungi as Lichens.
- Fungi are also used as food, in the industry for the production of bread and beer, and also in the production of antibiotics.

4.8 STUDENTS' PRACTICAL ACTIVITIES

ACTIVITY 1: Exploring Pond Water

AIM: To observe, identify and recognize movement of different organisms in Fresh Pond Water

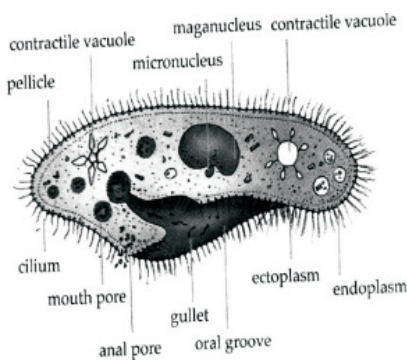
MATERIALS

- (i) Water sample from pond or aquarium
- (ii) Eye dropper
- (iii) Microscope slide
- (iv) Cover slip
- (v) Compound microscope
- (vi) Light source
- (vii) Drawing materials

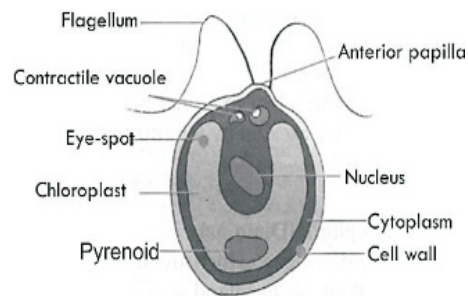
PROCEDURE

- (i) Add one or two drops of pond water to the center of a microscope slide. Try to include in the drops tiny amounts of mud or leaves (organisms often collect around these things).

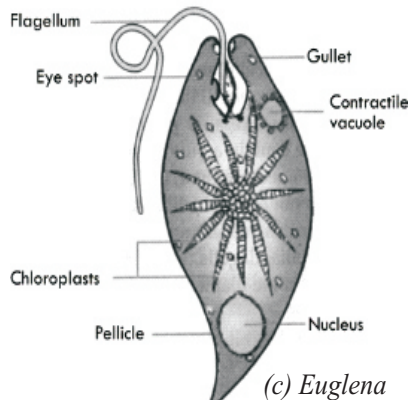
- (ii) Add one or two drops of pond water to the center of a microscope slide. Try to include in the drops tiny amounts of mud or leaves (organisms often collect around these things).
- (iii) Gently place a cover slip over the drop. Look at the drop using the low power of your microscope.
- (iv) Try to find some one-celled organisms in the drop. You may see organisms that resemble those shown in the drawings below. Try to find places where these organisms occur in the largest numbers.
- (v) Center some of the smallest organisms in the field of view and then switch to high power to examine them. Also examine details of the largest organisms.
- (vi) Make drawings of the organisms you find most interesting in Fig. 4.17.



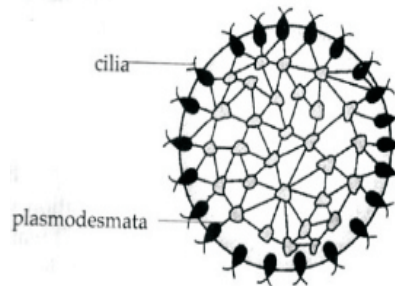
(a) *Paramecium*



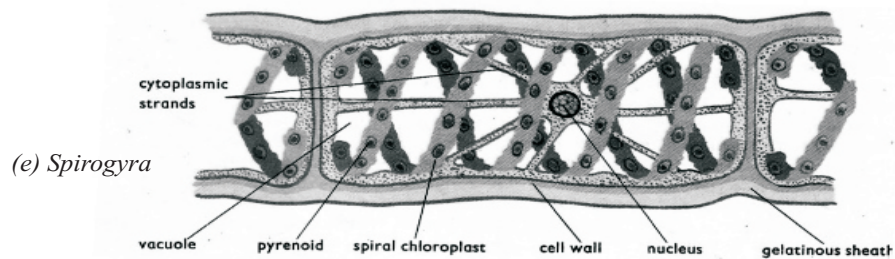
(b) *Chlamydomonas*



(c) *Euglena*



(d) *Volvox*



(e) *Spirogyra*

Figure 4.17: Organisms in Pond water (a-e).

Source: Ndu, Asun and Aina (2013).

ANALYSIS

1. How many different kinds of organisms were you able to recognize?
2. Which kinds of organisms were the most common? How did they move?
3. Which of the organisms probably make their own food? How can you tell?
4. Which organisms, if any, chased and then “swallowed” their food? In what other ways did you observe organisms obtaining food?
How can you explain the greater concentration of organisms around dead leaves and other debris than in open water?

ACTIVITY 2: Response of *Euglena* to Light and Temperature

AIM: To observe the response of *Euglena* to light and temperature

MATERIALS

- (i) Culture of *Euglena*
- (ii) Compound microscope
- (iii) Medicine dropper
- (iv) Microscope slides
- (v) Coverslips
- (vi) Black construction paper
- (vii) Scissors
- (viii) Methyl cellulose
- (ix) Ice
- (x) Beaker
- (xi) Aluminium foil
- (xii) Candle
- (xiii) Matches

PROCEDURE

- (i) Put a drop from the *Euglena* culture on a glass slide and cover it with a coverslip.
- (ii) Make a second slide the same way. Put black construction paper over half of the cover slips. Then place the entire slide in bright light for 20-30 minutes. Make sure that the light does not cause the culture to dry out.
- (iii) Use the low power lens on a compound microscope to examine the *Euglena* culture on the first slide. Look for the nucleus, chloroplasts, eyespot and other organelles. Make a labeled drawing of *Euglena*.
- (iv) Adjust the light with the mirror to observe the long undulipodium of a single *Euglena*. If the *Euglena* moves too fast, slow it down by placing a drop of methyl cellulose on the drop of culture. Notice whether *Euglena* is pushed or pulled by its flagella.
- (v) After 20-30 minutes, remove the second slide from bright light. Compare and record your observations about the distribution of the *Euglena* on the uncovered and covered parts of the slide.
- (vi) Cut a strip of aluminium foil about 30 centimeters long and 2 centimeters wide. Cut a hole in the middle of the strip the size of the hole in the microscopic stage. Place the strip and the slide on the stage, as shown;
- (vii) Heat one end of the strip by placing a candle under it, as shown, cool the other end by placing it in ice. Do not allow the culture to dry out. Observe the distribution of *Euglena* on the slide. Record your observations.
- (viii) If the slide should start to dry out, *Euglena* would not be able to swim. Instead, it would use a creeping motion. Before adding water to the slide, look for this motion.

ANALYSIS

- (i) Of the traits you observed, which of *Euglena*'s traits are plant-like and which are Animal-like?
- (ii) What is the advantage of being able to respond to changes in light and temperature?
- (iii) Do you think *Euglena* would prefer a cool, bright place; a cool, dark place; a warm, bright place; or a warm dark place? Design an experiment to test your hypothesis.

ACTIVITY 3: Isolation of Fungi from Soil

AIM: To isolate and examine fungi from soil

MATERIALS

- (i) Sabouraud Dextrose agar, potato dextrose agar or corn meal agar
- (ii) Distilled water.
- (iii) Soil sample,
- (iv) Petri dishes,
- (v) Test tube.

PROCEDURE

- (i). Prepare mycological agar according to the manufacturers instruction
- (ii). Pour the molten agar in petri dishes and allow to solidify
- (iii). Take a drop or 2 of soil suspension and spread over the surface of the medium.
- (iv). Cover the petri dishes and incubate invertedly at room temperature 3 to 4 days
- (v). Observe the petri dishes for colonies of the fungi

EXERCISE

Observe and describe the shape and colour of the fungal colonies in the petri dishes.

ACTIVITY 4: Demonstration of Budding in Yeast (1)

AIM: To demonstrate and observe yeast in budding stage

MATERIALS

- (i) Slides,
- (ii) Cover slips,
- (iii) Lactophenol cotton blue,
- (iv) Agar culture of *sacharomyces cerevisiae*(yeast),
- (v) Palm wine.

PROCEDURE

- (i). Place a drop of palm wine on a clean slide and cover with cover slip.
- (ii). Examine under microscope.
- (iii). Make drawings of the yeast cells noting the different stages of budding yeast cells. Also note the presence of bacteria and compare the signs of both organisms.
- (iv). Make wet preparations of the pure yeast culture in lactophenol cotton Blue and cover with cover slip.
- (v). Examine under x40 objective nothing budding.

ACTIVITY 5: Budding in yeast (2)

AIM: To examine budding in yeast.

PROCEDURE

- (i) Prepare a yeast culture by adding a few dry yeast pellets to 20 cm³ glucose solution (10%) in a conical flask.
- (ii) Plug the flask with cotton wool and keep it in a dark warm place (Bubbling should occur as the yeast starts to break down the glucose).
- (iii) About 2 to 3 days later, prepare a wet-mount of the yeast culture.
- (iv) Stain the yeast organisms with iodine or methylene blue. Examine the slide under a microscope.
- (v) Draw a single cell showing the cell wall, cytoplasm, nucleus and vacuole as shown in Figure 4.18 .

OBSERVATION

- (i) Look for yeast cells that have reproduced by budding.
- (ii) How many buds are formed by a single organism? Make sketches of yeast showing the buds.

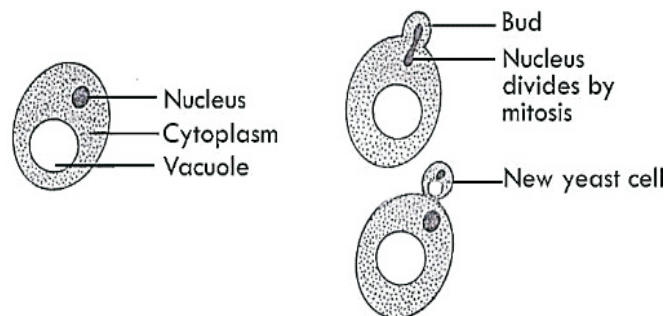


Figure 4.18: Budding in yeast.

Source: (Slesnick et al,1985).

ACTIVITY 6: Spores in Bread Mould

AIM: To examine spores in Bread Mould (Figure 4.19)

PROCEDURE

- (i) To grow bread mould, sprinkle water on a slice of bread, put it in a cupboard in a dark and warm place.
- (ii) Use a hand lens and observe the bread daily.
- (iii) Prepare a wet-mount of the mould and examine the slide under a microscope.
- (iv) Make a sketch showing the rhizoids, stolons, sporangiophores and sporangia as in Figure 4.19.
- (v) Crush a sporangium and examine the pores under high-power objective.

OBSERVATION

- (i) What is the shape of the spores?
- (ii) Does each spore contain a single nucleus or many nuclei?

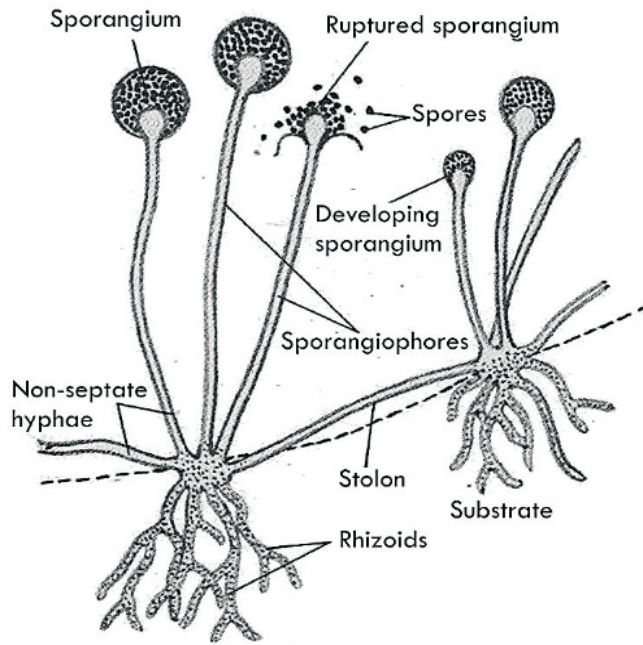


Figure 4.19: *Rhizopus nigricans*.
Source: Ramalingam (2005).

4.8 TUTOR MARKED ASSESSMENT QUESTIONS

HAVING READ THROUGH **CHAPTER FOUR**, ANSWER THE FOLLOWING QUESTIONS IN THE SPACES PROVIDED.

1. (a) Give the Meaning of Protozoa.

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4 × ½ = 2 Marks

(b) Write down **Four** General Characteristics of Protozoa.

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.....

4 × ½ = 2 Marks

- (d) Make a large labeled diagram of a Paramecium to show its external features.

Drawing $6 \times \frac{1}{2} = 3$ Marks

Labeling $4 \times \frac{1}{2} = 2$ Marks

- 2.(a) Make an annotated drawing showing the Plasmodium.

$8 \times \frac{1}{2} = 4$ Marks

(b) Give **Two** examples of the Following Protozoa:

Sacordines

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Flagellates

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Ciliates.

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.....
.....

$3 \times \frac{1}{2} = 1\frac{1}{2}$ Marks

(c) Discuss briefly on:

(i) *Flagellates*

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$2 \times \frac{1}{2} = 1$ Marks

Ciliates.

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$4 \times \frac{1}{2} = 2$ Marks

(b) What is Algae?

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.....
.....

$3 \times \frac{1}{2} = 1\frac{1}{2}$ Marks

(c) Write briefly on:

(i) Halophytic Algae

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$4 \times \frac{1}{2} = 2$ Marks

(ii) Epiphytic Algae.

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.....

$4 \times \frac{1}{2} = 2$ Marks

(iii) Epizoic Algae.

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$4 \times \frac{1}{2} = 2$ Marks

(iv) Endozoic Algae.

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$4 \times \frac{1}{2} = 2$ Marks

(d) Write down the **Seven** major classes of Algae.

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$7 \times \frac{1}{2} = 3\frac{1}{2}$ Marks

3. Make a large label drawing of *Euglena viridis* to show Its external Features.

Labeling $6 \times \frac{1}{2} = 3$ Marks

Drawing $4 \times \frac{1}{2} = 2$ Marks

(b) Distinguish between the following types of Algae with a short note:

(i) Chlorophytes

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$4 \times \frac{1}{2} = 2$ Marks

(ii) Phaeocophytes

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$4 \times \frac{1}{2} = 2$ Marks

(c) Distinguish between the terms:

(i) SlimeMolds

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$4 \times \frac{1}{2} = 2$ Marks

(ii) Watermolds

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$4 \times \frac{1}{2} = 2$ Marks

(d) Explain briefly the term Fungi:

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$4 \times \frac{1}{2} = 2$ Marks

4. Explain briefly how Mushroom grow into fruiting body.

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Drawing $6 \times \frac{1}{2} = 3$ Marks

Labeling $4 \times \frac{1}{2} = 2$ Marks

(b) List **Four** forms of Fungi and give **Two** examples of each group.

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$4 \times \frac{1}{2} = 2$ Marks

(c) With the aid of a life cycle explain how Yeast Cell reproduce.

Drawing $6 \times \frac{1}{2} = 3$ Marks

Labeling $4 \times \frac{1}{2} = 2$ Marks

(d) Discuss Fungi under the following headings of Fungi as Decomposers.

(i) Fungi as Decomposers

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.....
.....

$4 \times \frac{1}{2} = 2$ Marks

(ii) Fungi as Lichen

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$4 \times \frac{1}{2} = 2$ Marks

5. (a) Write short notes on Fungi as Human Parasites.

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$4 \times \frac{1}{2} = 2$ Marks

(b) Briefly explain Fungi as Plant Parasites.

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$4 \times \frac{1}{2} = 2$ Marks

Chapter Five

VIRUSES: DISCOVERY, STRUCTURE AND GENERAL PROPERTIES

Dr. Inemesit N. Bassey & Dr. Amara G. Nwaogu

5.1 INTRODUCTION

A virus is a non-cellular infectious agent consisting only of a nucleic acid core and a protein coat that is sometimes enclosed in a lipid envelop. It can replicate only after its genetic material enters a host cell and directs the cellular **machinery** into synthesizing the materials necessary to produce new virus particles.

Viruses are smaller than bacteria, ranging in size from about 20 nm to 300 nm. They cannot be seen through a light microscope, and can pass through filters that trap bacteria. Many can be crystallized and can only multiply inside living cells. They do, however, contain nucleic acids like DNA or RNA and must therefore be considered as being on the border between living and non-living. Their inert particles are known as **virions**. Most viruses found in animal cells and those attacking bacteria (**bacteriophages**) have DNA. Other animal viruses and plant viruses contain RNA. Electron microscope and x-ray diffraction have shown viruses to be in a variety of shapes, such as spherical (e.g. poliomyelitis), straight rods (e.g. tobacco mosaic virus (TMC) or flexible rods (e.g. potato virus), **bacteriophages** have a distinct "head and tail", while covid-19 is spherical with tiny projections on the surface. Viruses cause many diseases, both in plants and animals. The virus is spread mainly by contact between healthy and infected organisms. Contact may be indirect or direct, e.g., insects such as leaf-sucking aphids can carry viruses from plant to plant, spreading diseases. Virus diseases in plants include leaf curl in tobacco and cotton plants, swollen shoots in cocoa and bunchy tops in bananas. In man, viruses cause diseases such as HIV-AIDS, COVID-19, yellow fever, poliomyelitis, small pox, the EBOLA virus disease (EVD), common cold, influenza, measles, and mumps are also caused by Viruses. The influenza virus is spread in tiny water droplets from the breath of an infected person. Mammals are able to develop **natural immunity** to some of the virus diseases by producing substances in their blood-stream that make the viruses harmless. These substances are called **antibodies**. Artificial protection can be provided by **vaccination**, in which a less harmful form of the virus is injected into the patient.

5.2 LEARNING OBJECTIVES

After reading this chapter, you should be able to:

- (i) Explain the history and discovery of virus.
- (ii) Define virus, viroids and prions.
- (iii) List the types of bacterial viruses for genetic and biochemical research.
- (iv) Describe viruses as a bridge between living and non-living.
- (v) Explain generalized characteristics of viruses.

- (vi) Discuss viral replication/reproduction.
- (vii) State the major classes of viruses (bacteriophages, animal viruses and plant viruses).
- (viii) Enumerate the diseases of humans caused by viruses.
- (ix) Explain the mode of transmission of viral disease in (vii).
- (x) Examine the pattern of occurrence of viral diseases.
- (xi) Discuss on virulent nature of viruses.

5.3 HISTORY AND DISCOVERY OF VIRUS

In 1796, Edward Jenner conducted a classic experiment in which he purposely introduced materials from cowpox lesion on the hand of a milkmaid named Sarah Nelmes, to a cut on the arm of James Phipps. After six weeks, the boy James was exposed to pus from a smallpox patient and Phipps failed to develop smallpox disease. This boy became protected and immune against smallpox due to his initial exposure to a less virulent cowpox agent. This process, **variolation**, though practiced in China and Middle East more than a thousand years earlier was only understood after Jenner's experiment.

In 1885, Louis Pasteur, while experimenting using rabies and fowl cholera agents came up with the word, **vaccination** (from the latin Vacca for “cow”) to discriminate between viruses and other infectious agents, he originated the term “virus” and developed scientific basis for Jenner's experimental approach to **vaccination**.

In 1886, he demonstrated that the sap of mosaic leaves tobacco plant developed **mosaic disease** when injected into healthy plant leaves and the **infectivity** of the sap destroyed when boiled. He thought he was dealing with bacterial agent. However, in 1892, a Russian botanist Dimitri Iwanowsky using porcelain filter developed by Charles Chamberland (1884) (filter that allows the passing of fluid but not bacteria) to filter the sap of diseased tobacco plant observed that the infectious agents filtered through the pores of the porcelain. When he injected the filtered sap into healthy plants, there was the development of mosaic symptoms.

He concluded that the infectious agent was smaller than bacterium. He called this **filterable virus** i.e. poisonous fluid. In 1898, Martinus W. Beijerinck defined the infectious agent as a Contagium vivum fluidum and put forward the concept that virus readily passed through a porcelain filter, with pore size small enough to retain bacteria, suggesting that they were smaller than bacteria. He further observed that the “agent” could diffuse through agar gel that retained bacteria and could not be cultured except in a living plant. This agent was the Tobacco Mosaic Virus (TMV). Fedrick W. Twort (1915) in England and Felix d'Herelle (1916) in Paris independently observed that bacterial colonies were lysed in culture by some agents and the effect transmitted from colony to colony.

They also discovered that, highly diluted material from lysed colonies, even after being filtered through a bacterial filter, still transmitted **lytic** effect which was destroyed after heating. Twort concluded that the **lysis** have been due to a virus. In 1917, d'Herelle, confirmed the above phenomenon and coined the term bacteriophage (bacteria eater). In 1935, an American chemist, Wendell M. Stanley, crystallized the virus causing mosaic disease in tobacco plant and found that the crystals alone were also infectious when inoculated into healthy tobacco plants. This led him to conclude that viruses were not like typical cells of living organism.

In 1938, F.C. Bawden and N.W. Pirie, analysed the crystallized particles and demonstrated that they were made up of protein and ribonucleic acid (RNA). Hershey and Chase (1952) demonstrated that *bacteriophage* DNA carries the genetic information and that infection occurs upon penetration of bacteriophage DNA into living cells. Gierrer and Schwamm (1956) provided proof that the nucleic acid was the infectious agent and *genetic materials*. In that same year 1956, Fraenkel- Conrat confirmed that the genetic material of TMV was the RNA.

TMV is the first virus to be purified in pure crystal form, first pathogen to pass through filter candles and first virus to be identified as consisting of an infectious nucleic acid. Other developments: Mycovirus discovered by Hollings (1982); cynophages by safferman and Marvis (1963); *viroid* by Diener and Raymer (1967); prion by Prusiner (1982) and HIV by LuC Niontagnier (1983).

5.3.1 Origin of Virus

Viruses are better understood, using their evolutionary origins. It was presumed that *viruses* are pieces of genetic material that have escaped from prokaryotic and eukaryote cells and have the potentials to replicate when they get into a susceptible host cell environment. There are three main theories of the origin of *viruses*:

(i) Cellular Origin/Vagrancy Theory

This theory states that viruses evolved from pieces of DNA or RNA that had escaped from genes of larger organism. The escaped DNA may have been from a plasmids or transposons. These DNA molecules replicate and move around to other locations within the genes. These jumping genes may have given rise to viruses.

(ii) Regressive/ Degeneracy Theory

According to this theory, viruses come from small cells that parasitized larger cells. In time, genes not required by their *parasitism* become lost to serve as origin of the present day viruses. Rickettsia and chlamydia are living cells that can only reproduce inside host cells like viruses.

(iii) Co-evolution theory

The theory holds that viruses evolved from complex molecules of protein and nucleic acid at the same time as cells first appear on planet earth.

5.4 VIRUS, VIROIDS AND PRIONS

(i) Virus: A virus is a submicroscopic infectious agent that replicates only inside the living cells of an organism. Viruses consist of a core of nucleic acid surrounded by a protein coat. They are classified on the basis of either DNA or RNA in their core, their size and shape, the number of identical structural units in their cores, and the nature of their protein coats. Viruses infect all types of life forms, from animals and plants to microorganisms, including bacteria and archaea. It takes the metabolic machinery of a living host cell to assemble the viral nucleic acids and proteins necessary to construct new virus particles.

Viruses, which have no cellular structure, are about the size of large molecules. Some can be isolated, purified, and *crystalized*, yet remain virulent. They cannot grow or increase in size and cannot be reproduced outside of a living cell, upon whose DNA they depend for duplication. *Viruses* can be found in every ecosystem on earth and are ever-present in the living world, infecting, affecting and interacting with all organisms, from the miniscule to the gigantic. The study of viruses is known as virology, a subspecialty of microbiology.

(I) **Viroids**

Many plants, including potatoes, tomatoes, apples, and citrus fruits, can be infected by *viroids*. *Viroids* are single stranded RNA molecules that have no surrounding capsids. It is believed that *viroids* are plant pathogens that enter an infected cell and direct the synthesis of new viroids. The viroids then disrupt the *metabolism* of the plant cell and stunt the growth of the entire plant.

(ii) **Prions**

In 1972, American Stanley Prusiner became interested in scrapie, an infectious disease in sheep for which the exact cause was unknown. Although he first suspected a virus, experiments suggested the disease might actually be caused by tiny particles found in the brains of infected sheep. Unlike viruses, these particles contained no DNA or RNA, only protein. Prusiner called these particles *prions*, short for “protein infectious particles”. Although *prions* were first discovered in sheep, many animals, including humans, can become infected with *prions*.

There are some evidences that *prions* cause disease by forming protein clumps. These clumps induce normal protein molecules to become prions. Eventually, there are so many prions in the nervous tissue that cells become damaged. There is strong evidence that mad cow disease (Spongiform encephalopathies) and Creutzfeldt-Jakob disease, a similar disease in humans, may be caused by *prions*.

5.4.1 Definition of Virus

Lwoff (1957) defined virus as an infectious, potentially pathogenic nucleoprotein with only one type of nucleic acid which reproduces from their genetic material and is unable to grow, multiply and lack enzyme system.

Lwoff and Tournier (1962) characterized viruses thus;

- (i) They are potentially infectious;
- (ii) Possess a single nucleic acid;
- (iii) Are not capable of producing their genetic materials on their own;
- (iv) Lack enzyme for energy metabolism;
- (v) Lack cellular organelles;
- (vi) Can replicate only by using host cell materials.

Laria and Darnell (1968) defined viruses as entities, whose genomes are elements of nucleic acid that replicate inside living cells using the cellular synthetic machinery and causing the synthos of specialized elements that can transfer the viral genome to other cells.

Viruses are acelular, lacking cell inclusions and occupying the twilight zone separating the living from the non-living.

Outside the living cell, viruses are inactive or inert and thus cannot be said to be living, however, when considering the diseases caused by them, they act as pathogens against bacteria, fungi, protozoa, plant, and animals.

5.4.2 Occurrence of Virus

Viruses are found in a wide range of hosts including plants, algae, fungi, bacteria, animals, etc. they cause disease in crop plants, forest trees and ornamentals. Important viral diseases of human include influenza (flu), measles (Rubeola), mumps, Rubella (german measles), chicken pox, poliomyelitis, viral hepatitis, Rabies, AIDS, COVID – 19, etc.

5.5 STRUCTURE OF VIRUS

The complete assemblage of an infectious particle is known as viron (see Figure 5.1). A virion consists of a nucleic acid core surrounded by a protein coat called the capsid. The capsid with the enclosed nucleic acid is referred to as the nucleocapsid. The capsid is made up of a number of subunits known as the *capsomer*.

The morphological types of virus or symmetry observed under electron microscope and crystallography have been grouped into three, Helical (cylindrical), *Polyhedral*, and complete viruses as highlighted below.

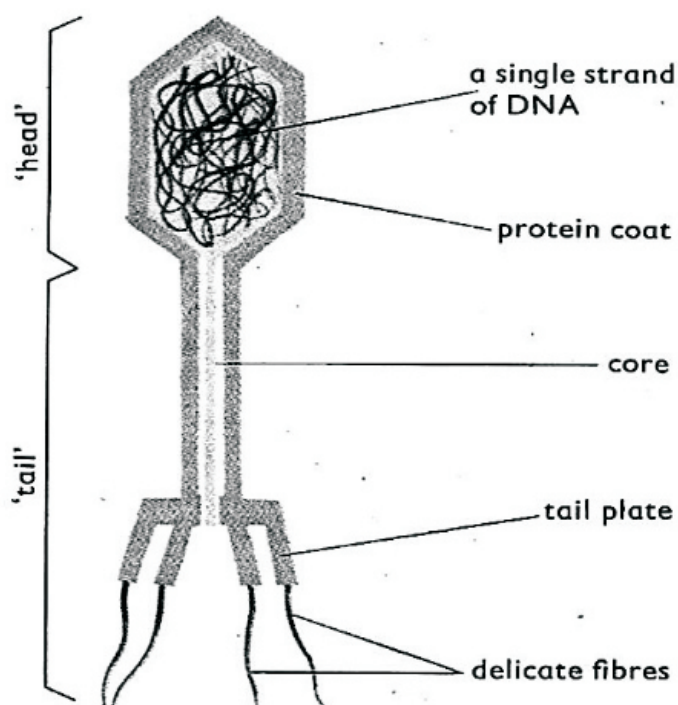


Figure 5.1: A structure of a typical virus [T₂ virus].

Source: Ramalingam (2005).

- (i). Helical (cylindrical) viruses
 - (ii). Polyhedral (icosahedral) viruses
 - (iii). Complete viruses
- (i). **Helical viruses** have elongated, rod-shaped, rigid or flexible helical structure and their capsids around the hollow cylinder. The capsid consists of monomers arranged helically in a *rotational axis*.
- (a) **Naked helical e.g. the TMV** (tobacco mosaic virus)
 This virus is rod shaped, measuring about 280 x 150m, consisting of a protein coat with a hollow of 20A° which encloses a single stranded (ss) helix or coiled RNA. The genetic material (ssRNA) has molecular weight of about 2.06 x 10⁶ Dalton and consists of 6,500 nucleotides.

(b) Enveloped helical e.g. influenza virus

When the helical virus is enclosed within an envelope they are known as *enveloped helical virus*. The envelope is composed of a viral protein and the host cell components *polyhedral* (icosahedral) viruses.

(ii). **Polyhedral structure** has three possible symmetries including tetrahedral, octahedral and icosahedral. An icosahedron is a regular polyhedron with 20 triangular faces and 12 corners. They have either naked or enveloped icosahedral shape.

(a) Naked icosahedral viruses

These include poliovirus, adenovirus and bacteriophage $\times 174$.

(b) Enveloped icosahedral viruses

An example is the herpes virus whose capsid is enclosed inside an envelope of 30nm glycoprotein-lipid complex.

(iii). **Complex viruses**

These are viruses which have unidentifiable capsids or capsids with additional structures. Examples are vaccinia virus and T-even bacteriophage. Enveloped viruses are susceptible to the action of lipid solvent like ether, alcohol, chloroform and bile salts.

5.5.1 Sizes and Shapes of Viruses

Sizes of Viruses: Each virus has a definite size. In general, a virus is far tinier than the smallest known cellular organism. A virus can pass through an opening small enough to hold back a bacterium. In fact, a virus is too small to be seen with an ordinary light microscope. To be seen, viruses must be magnified tens or even hundreds of thousands of times. Such magnification is possible only with an electron microscope. The diagram in Figure 5.2 shows a wide variety of sizes and shapes of a typical virus composed of a core of either DNA or RNA, which is surrounded by a core protein coat.

Shapes of Viruses: Each Virus has its own definite composition and shape. The core of every virus is a small piece of RNA or DNA. This nucleic acid can be single-stranded or double-stranded. A protective coat of protein surrounds the viral core. The shape of the virus depends on the arrangements of protein molecules that make up the protective coat. A virus can be a combination of spiral-shape and many-sided.

The three basic shapes of viruses are shown in the examples below (see Figure 5.2). The *adenovirus*, which causes respiratory infections in some animals, has a crystal-like shape. Each of its 20 sides or faces is triangular. The spiral-shaped virus shown below causes mosaic disease in tobacco plants. Notice how this virus looks like a long, slender cylinder. But when examined closely, the molecular units of the mosaic virus appear to be wound in a spiral. The spaceship-like T_4 virus also shown at the beginning of this chapter has a many-sided “head” and a spiral “tail”. A virus that invades bacteria, such as the T_4 virus is called a bacteriophage.

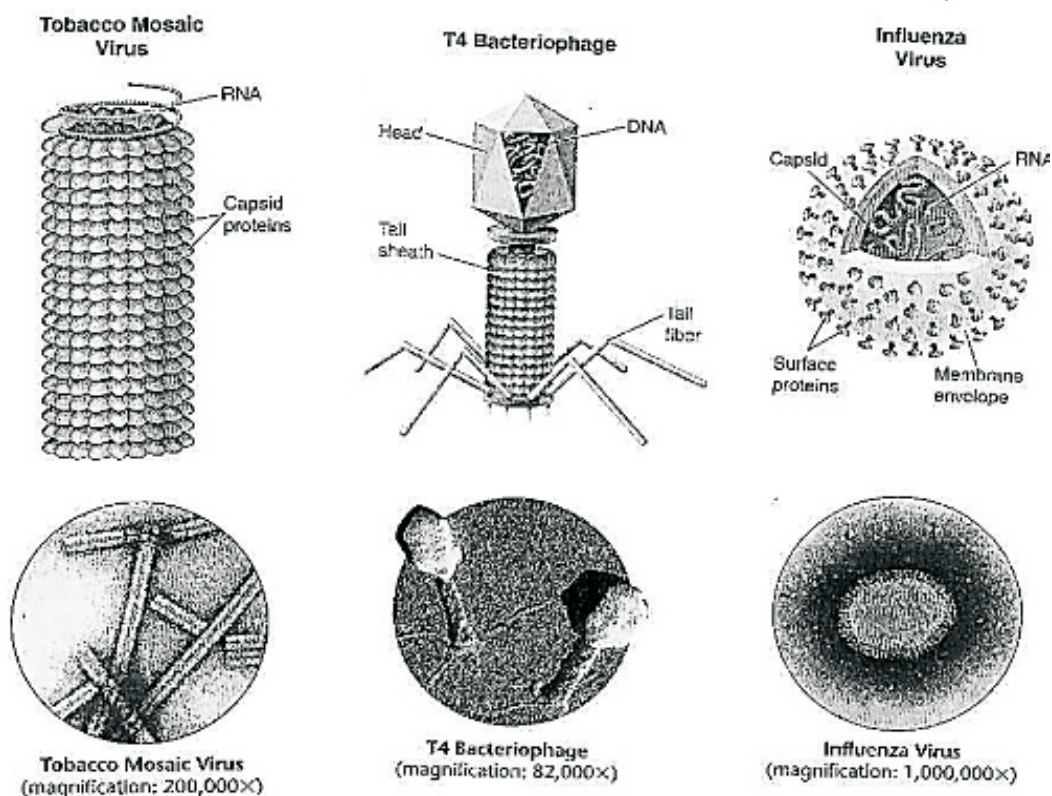


Figure 5.2: Variety of viral structures.

Source: Miller and Levine (2006).

Note: A typical virus is composed of a core of either DNA or RNA, which is surrounded by a protein coat, or capsid. Viruses come in a wide variety of sizes and shapes as shown in the figure above.

Viruses, especially animal viruses, vary in shape and size.

Table 5.1 below shows the approximate shape of some animal and plant viruses.

Table 5.1: Shapes and Sizes of Some Virus Particles

Name of Virus	Shape	Size
Tobacco mosaic virus	Rod- shape	4,500m μ
Potato	Filamentous	Diameter 16.0m μ
Coli-phage	Tadpole-shape	8,670m μ
Polio-virus	Spherical	18m μ
Vaccinia	Brick-shape	290230m μ

Source: Bos (1992)

5.6 PROPERTIES OF VIRUSES AND CLASSIFICATION

(a) Properties

- (i) Viruses are obligate intracellular parasites.
- (ii) They lack enzymes necessary for protein and nucleic acid synthesis and depend on the synthetic machinery of the host cell for replication i.e cannot grow in cell free culture media.
- (iii) They do not have cellular organization.
- (iv) They are unaffected by antibiotics.
- (v) They contain only one type of nucleic acid, either RNA or DNA and never both.
- (vi) They multiply by a complex process and not through binary fission.
- (vii) With few exceptions, viruses are heat labile.
- (viii) The extracellular infectious virus particle is called virion (see Figure 5.3).

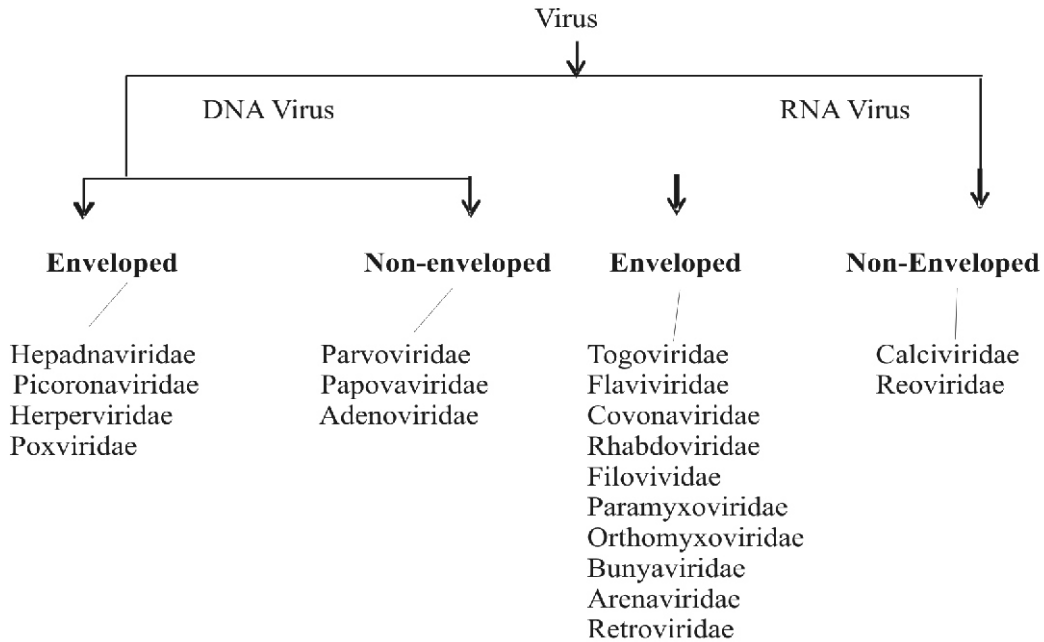


Figure 5.3: Chart showing classification of Virus.

Source: NIOS (2017).

Mnemonics

Non-enveloped DNA Viruses **PAP** *Papova, Adeno, Parvo*

Non-enveloped RNA viruses **PCR** *Picorna, Calci, Reo*

DNA viruses – HHAPPY – Hepadna, Herpes, Adeno, Parvo, Papova, Pox

Note: all DNA viruses have double stranded nucleic acid except for parvovirus, which has single stranded DNA

All RNA viruses have single stranded nucleic acid except for Reoviridae (Rotavirus, Reovirus) which has double stranded RNA

(a) Viral Classification Scheme

- (i) Lwoff-Horme-Tourier system of classification
In 1962, A. Lwoff, R. Horne and P. Tourier proposed a system of classification of viruses known as the LHT system. The LHT system of classification is based on (a) nature of nucleic acid, (DNA or RNA) (b) Symmetry or viral particle (helical, icosahedral, cubic etc) (c) presence or absence of envelope (d) diameter of capsial and (e) number of *capsomer* forming the capsid.
- (ii) Casjens and King's classification
Casjens and King in 1975 classified viruses on the basis of nucleic acid types, symmetry, presence or absence of envelope and location of assembly of envelope.
- (iii) International Committee on *Taxonomy* of Viruses (ICTV) (1990)
The committee developed a universal taxonomic scheme for viruses aimed at describing all viruses classified in more than 2,000 species and in more than 400 higher taxa.
- (iv) Dimmock classification
Dimmock in 2001 classified viruses into six groups based on host preference.
- (v) Baltimore Classification
David Baltimore classified viruses in 2008 based on the method of viral mRNA synthesis. Viruses must generate mRNA from their genome to produce proteins and replicate themselves but different mechanisms are used to achieve this in each viral family.

5.7 TYPES OF BACTERIAL VIRUSES USED IN BIOCHEMICAL AND GENETIC RESEARCH

Bacterial viruses have played a vital role in the development of molecular or cell biology. Thousands of different *bacteriophages* have been isolated; many of these are particularly well suited for studies of specific biochemical or genetic events. Here, we briefly describe four types of *bacteriophages*, all of which infect *Escherichia coli* that have been especially useful in molecular biology research.

(I) DNA Phages of the T series

The T phages of *Escherichia coli* are large *lytic* phages that contain a single molecule of double-stranded DNA. This molecule is about 2×10^5 base pairs long in T₂ T₄ T₅ and T₆ viruses and about 4×10^4 base pairs long in T₁, T₃, T₅ and T₇ viruses. The phage virions consist of a helical protein “tail” attached to an icosahedral “head” filled with the viral DNA. After the tip of a T-phase tail absorbs to receptors on the surface of an *Escherichia coli* cell, the DNA in the head enters the cell through the tail. The phage DNA then directs a program of events that produces approximately 100 new phage particles in about 20 minutes, at which time the infected cell lyses and releases the new phages. The initial discovery of the role of messenger RNA in protein synthesis was based on studies of *Escherichia coli* cells infected with *bacteriophage* T₂. By 20 minutes after infection, infected cells synthesize T₂ proteins only. The finding that the RNA synthesized at this time had the same base composition as T₂ DNA (not *Escherichia coli* DNA) implied that mRNA copies of T₂ and DNA were synthesized and used to direct cellular ribosomes to synthesize T₂ protein.

(ii) Temperate Phages

Bacteriophage, which infects *Escherichia coli*, typifies the temperate phages. This phage has one of the most studied genomes and is used widely in DNA cloning. On entering an *Escherichia coli* cell, the double-stranded DNA assumes a circular form, which can enter either the *lytic* cycle (as T phage do) the *lysogenic cycle*. In the latter case, proteins expressed from viral DNA bind a specific sequence on the circular viral DNA to a similar specific sequence on the circular bacterial DNA.

The viral proteins then break both circular molecules of DNA and the broken ends, so that the viral genes maintains DNA as part of the host chromosome by representing the *lytic* functions of the phage. Under appropriate stimulation, the prophage is activated and undergoes *lytic* replication.

(iii) Small DNA Phages

The genome of some bacteriophages encoded only 10-12 proteins, roughly 5-10 percent of the number encoded by T phages. These small DNA phages are typified by the ϕ X 174 and the filamentous M13 phages. These were the first organisms in which the entire DNA sequence of a genome was determined, allowing extensive understanding of the viral life cycle. The viruses in this group are so simple that they do not encode most of the proteins required for replication of their DNA but depend on cellular proteins for this purpose. For this reason, they have been particularly useful in identifying and analyzing the cellular proteins involved in DNA replication.

(iv) RNA Phages

Some *Escherichia coli* bacteriophages contain a *genome* composed of RNA instead of DNA. Because they are easy to grow in large amounts and because their RNA genomes also serve as their mRNA, these phages are a ready source of a pure species of mRNA. In one of the earliest demonstrations that cell-free protein synthesis can be mediated by mRNA, RNA from these phages shown to direct the synthesis of viral coat protein when added to an extract of *Escherichia coli* cells contain all the other components needed for protein synthesis. Also, the first long mRNA molecule to be sequenced was the *genome* of a RNA phage. These viruses, among the smallest known encode only four proteins; an RNA polymerase for replication of the viral RNA, two capsid proteins, and an enzyme that dissolves the bacterial cell wall and allows release of the *intracellularvirus* particles into the medium (see Figure 5.4).

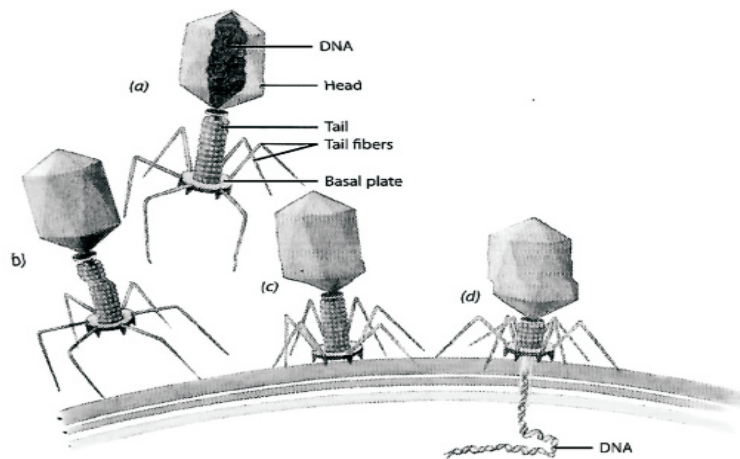


Figure 5.4: Diagram of Viruses infecting Bacteria cell (bacteriophages).

Source: Raven, Evert Eichhorn (1999).

Note: (a) Diagram of a T-even *bacteriophage* showing its attachment (b), (c) to the cell wall of *E. coli* and the injection of DNA into the bacterium (d). The T-even phages, which contain linear, double-stranded DNA, were the first viruses to be studied in detail. The virions are structurally complex. Each consists of an icosahedral head containing the viral DNA and a hollow tail through which the DNA is injected. The tail, which consists of a helical tube, ends with a basal plate to which are attached long, jointed tail fibres.

5.8 VIRUSES AS A BRIDGE BETWEEN LIVING AND NON-LIVING

Viruses are microscopic agents that replicate only inside the living cells of other organism (host) structurally they possess:-

- A genetic material made from either DNA or RNA.
- A protein coat.
- An envelope of lipids.

Unlike bacteria, viruses are acellular particles (meaning they are not made up of living cells, have no energy *metabolism*, they do not grow (they only replicate in host), they produce no waste products and do not respond to stimuli.

Because viruses lack the essential characteristic of living things they are generally considered to be non-living. Viruses must infect a living cell in order to grow and reproduce. They also take advantage of the host's respiration, nutrition, and all the other functions that occur in living things. Therefore, viruses can be considered to be parasites. A parasite depends entirely upon another living organism for its existence, harming that organism in the process.

Table 5.2: Virus and Cells

Characteristics	Virus	Cell
Structure	DNA or RNA core, capsid	Cell membrane, cytoplasm; eukaryotes also contain nucleus and organelles.
Reproduction	Only within a host cell	Independent cell division either asexually or sexually
Genetic Code	DNA or RNA	DNA
Growth and Development	No	Yes; in multicellular organisms, cells increase in number and differentiate
Obtain and Use Energy	No	Yes
Response to Environment	No	Yes
Change Over Time	Yes	Yes

Source: BROCK (1995)

Table 5.2 shows the comparison between viruses and cells. Are viruses alive? If we require that living things be made up of cells and able to live independently, then viruses are not alive. Yet, viruses have many characteristics of living things. After infecting living cells, viruses can reproduce, regulate gene expression, and even evolve. Viruses are at the borderline of living and non-living things.

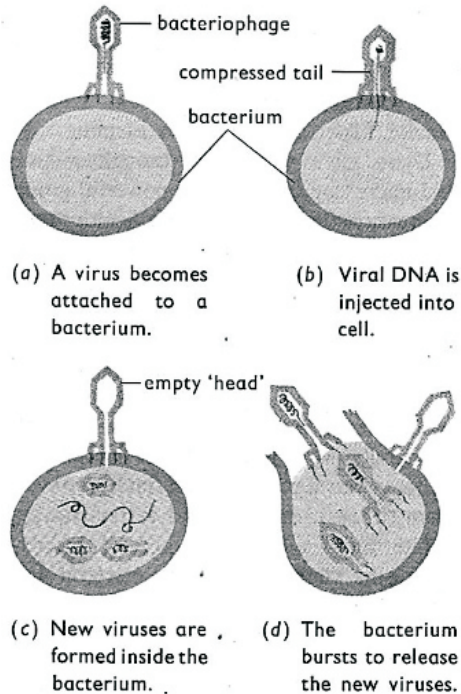
Although viruses are smaller and simpler than the smallest cells, it is not likely that they could have been the first living things, because viruses are completely dependent upon living things, it seems more likely that viruses developed after living cells. In fact, the first viruses may have evolved, along with the cells they infect, over billions of years.

5.8.1 Viral Replication/Reproduction

Viruses replicate in a variety of ways. In all cases, however, the viral DNA or RNA is copied repeatedly, many viral proteins are synthesized, and many new viral particles are assembled inside a suitable host cell. By definition, a cell is a potential host if a virus can chemically recognize and lock onto specific molecular groups at its surface.

Regardless of the variations, **viral replication** always proceeds through the following stages:

- (i) The virus chemically recognizes and becomes attached to a host cell.
- (ii) The whole virus or its genetic material alone (DNA or RNA) enters the cell's cytoplasm.
- (iii) In an act of molecular piracy, information contained in the viral DNA or RNA directs the host cell into replicating viral nucleic acids and synthesizing viral enzymes, capsid proteins, and sometimes other viral proteins (which become incorporated into the host plasma membrane).
- (iv) The viral nucleic acids, enzymes, and capsid proteins are assembled into new virus particles.
- (v) The newly formed virus particles are released from the infected cell.



Viruses usually replicate by lytic or temperate pathways. In a lytic pathway, stages a through d (see Figure 5.5) proceed quickly, and viral particles are released as the cell undergoes lysis. In this context, lysis means the host cell ruptures and then dies after its contents are lost.

In temperate pathways, the virus does not kill the host cell outright. Instead, the infection enters a period of latency, in which viral genes remain inactive inside the host cell and any of its descendants. In some cases of latency, the viral generally become integrated into the host DNA, are replicated along with it, and so are passed along to the daughter cells. In time, damage to the DNA or some other event may activate **transcription** of the viral genes. Ultimately, new viral particles can be produced and the infected cells destroyed.

Figure 5.5: Reproduction in virus.
Source: Ramalingam (2005).

5.8.2 Isolation and Cultivation of Viruses

Viruses cannot multiply and grow outside a susceptible living host cell; therefore, their isolation, enumeration and identification become a difficult task. Methods used for cultivation and isolation of viruses are:

(i) **Animal Inoculation**

In 1909, Landsteiner and Popper pioneered the isolation of polio virus using monkey. Infact mice have also been used for isolating viruses such as coxsackie virus and arbovirus, causative agents of dengue and chikugunya. Inoculating routes might be intracerebral, *subcutaneous*, intraperitoneal or *subcutaneous*. Other animals used include guinea pigs, rabbits and ferrets.

(ii) **Cell Culture**

(Steinhardt *et al*, 1913) introduced tissue culture for isolating viruses: They were able to maintain a culture of vaccine virus in rabbit cornea. In 1949, Ender, Weller and Robbins succeeded in cultivating polio virus in tissue culture.

(iii) **Embryonated Eggs**

In 1931, Good Pasture used embryonated hen's egg to cultivate virus. Different parts of the egg are used for the cultivation of different viruses. The viral agent is inoculated through a hole in certain regions of the egg for example; myxo virus and herpes simplex virus on chorio-allantoic membrane; mumps virus in *allantoic cavity*; influenza virus into the amniotic sac and rabies virus in the yolk sac.

The drilled hole is sealed using gelatin and the egg incubated. Infection produces a visible lesion called pocks.

Viruses in cell cultures can be detected by such methods as:

- (a) **Cytopathic Effect (CPE)** which is the morphological change in the cultured cells produced by the virus growing in those cells. These are seen by microscopic examination.
- (b) **Staining technique**
- (c) Detection of viral nucleic acid by molecular technique such as polymerase chain reaction (PCR).
- (d) Characteristics produced by these methods can help in the presumptive identification of virus isolates.

5.9 VIRAL DISEASES

(a) Viral Disease in Human

Viruses Like bacteria produce disease by disrupting the body's normal equilibrium. In many viral infections, virus attacks and destroys certain cells in the body, causing the symptoms of the disease. Poliovirus infects and kills cells of the nervous system, producing *paralysis*. Other viruses cause infected cells to change their patterns of growth and development. Some common diseases caused by viruses are listed in Table 5.3. Unlike bacterial diseases, viral diseases cannot be treated with *antibiotics*. The best way to protect against most viral diseases lies in prevention, often by the use of vaccines. Vaccinations against smallpox have virtually eliminated this disease. Most vaccines provide protection only if they are used before an infection begins.

Once a viral disease has been contacted, it may be too late to control the infection. However, sometimes the symptoms of the infection can be treated.

Table 5.3: Diseases of Humans Caused by Viruses

Disease	Effect on Body	Transmission
Common cold	Sneezing, sore throat, fever, headache, muscle aches	Contact with contaminated objects; droplet inhalation
Influenza	Body aches, fever, sore throat, nasal congestion, headache, dry cough, fatigue	Contact with contaminated objects; droplet inhalation
Smallpox	High fever, fatigue, head and back aches, rash	Contact with contaminated objects; droplet inhalation
AIDS	Helper T cells, which are needed for normal immune system function, are destroyed	Contact with contaminated blood or bodily fluids; pregnant women to babies during delivery or during breastfeeding
Chicken pox	Fever and weakness, red itchy rash	Contact with rash, droplet inhalation
Measles	High fever, sore throat, cough, rash, sneezing, swollen eyelids, white spots on cheek lining	Droplet inhalation
Hepatitis A	Jaundice, fatigue, abdominal pain, appetite loss, nausea, diarrhea, fever	Human waters, contaminated water and food
Hepatitis B, Hepatitis C	Jaundice, fatigue, abdominal pain, appetite loss, nausea, diarrhea, joint pain	Contact with contaminated blood or bodily fluids
West Nile	Fever, headache, body ache	Bite from an infected mosquito

Source: Cann (1997).

(b) Prion Diseases of Humans

These include:

- **Human Immunodeficiency Virus (HIV) /Acquired Immune Deficiency Syndrome (AIDS)**

It is a pandemic disease. The word “immune deficiency” signifies that the immune system becomes very weak. It is a disorder of cell-mediated immune system of the body. Lymphocytes are the main cells of the immune system i.e. T-lymphocytes and B-lymphocytes. 'Helper T' lymphocytes play a great role in regulating the immune system. Damages to or destruction of 'Helper' lymphocytes lead to the development of a cellular immune deficiency which makes the patient susceptible to wide variety of other infections.

- **Coronavirus Diseases**

Most common symptoms:

- (i) Fever
- (ii) Dry cough
- (iii) Tiredness

Less common symptoms:

- (i) Aches and pains
- (ii) Sore throat
- (iii) Diarrhoea

- (iv) Conjunctivitis
 - (v) Loss of taste or smell
 - (vi) A rash skin, or discolouration of fingers or toes
- Serious symptoms:
- (i) Difficulty breathing or shortness of breath
 - (ii) Chest pain or pressure
 - (iii) Loss of speech or movement
- Creutzfeldt Jacob Diseases (CJD)
 - Gerstmann – Strausster – scheinker syndrome (GSS) Syndrome
 - Fatal familial insomnia

(c) Viral Diseases in Animals

Viruses produce serious animal diseases as well. An epidemic of foot-and-mouth disease, caused by a *virus* that infects livestock, swept through parts of Europe in the late 1990s. Thousands of cattle were destroyed in efforts to control the disease. American authorities took special precautions to guard against the spread of the foot-and-mouth *virus* to North America.

Some animal viruses can even cause *cancer*. An example of these *oncogenic*, or tumor-causing, viruses is the Rous sarcoma *virus*, which infects chickens. Scientists have learned a great deal about *cancer* by studying the genes of these *oncogenic* viruses, which disrupt normal controls over cell growth and division.

(d) Viral Diseases in Plants

Many viruses, including tobacco mosaic virus etc, infect plants. These viruses pose serious threats to many agricultural crops. Farmers in many countries, including the United States, struggle to control them. Like other viruses, plant viruses contain a core of nucleic acid and a protein coat.

Unlike animal viruses, most plant viruses have difficult time entering the cells they infect. This is partly because plant cells are surrounded by tough cell walls that viruses alone cannot break through. As a result, most plant viruses are adapted to take advantage of breaks in the cell wall caused by tears in leaf tissue, breaks in stems or roots, or simply through microscopic cell wall damage caused by human or animal contact with the plant. Many plant viruses are spread by insects. The feeding action of an insect pest often provides a perfect opportunity for viral infections to spread. Potato yellow dwarf *virus* is spread by an insect known as the leafhoppers. Leafhoppers feed on potato leaves, and they also carry the *virus* in their tissues. As leafhoppers move from plant to plant, they spread the infection, threatening an entire crop if they are not controlled.

Once inside the plant, many viruses spread rapidly, causing severe tissue damage, mottled leaves, and wilting, and sometimes killing the infected plant. Plant viruses infect many valuable fruit trees, including apples and peaches, and have caused serious losses in the potato crop.

5.9.1 Major Classes of Viruses

(i) **Bacteriophage:** The *bacteriophages* are a class of viruses that infect bacterial cells. Although *bacteriophages* may have adverse effects on a host cell, they have been utilized in genetics research. *Bacteriophages* are used as research tools in early experiments that were designed to reveal whether DNA or proteins are the molecules of inheritance. *Bacteriophages* are now being used as research tools in *genetic engineering*.

- (ii) **Animal Viruses:** Table 5.4 lists a few types of animal viruses that are responsible for diseases as varied as warts, chicken pox, common cold, and several forms of *cancer*. Among them are the influenza viruses responsible for recurring pandemics. Many animal viruses infect animal cells through *endocytosis* and depart from them either by *exocytosis* or by lysis. Briefly, the plasma membrane of a host cell is stimulated into dimpling inward after virus particles have become attached to it. The resulting endocytic

vesicle transports the virus particles into the cytoplasm.

Animals have defenses against the initial attack of different animal viruses. Even so, some virus particles may escape detection and *latency*, only to be reactivated at some further time. For example, the Herpes simplex virus is latent in the vast majority of people and it can cause cold sores each time it is reactivated. Similarly, humans who have had chicken pox might still harbour the virus, which may cause a skin disease (shingles) if it becomes reactivated later in life.

Retroviruses are RNA viruses that infect animal cells and that follow temperate pathways of replication. You may wonder how the genetic material of an RNA virus becomes integrated into host cell DNA. After the viral RNA molecule enters the cytoplasm, a viral enzyme (reverse transcriptase) uses it as a template and synthesizes a DNA “transcript” on it. The transcript, not the RNA itself, becomes integrated into the host DNA. This is what happens in people infected by the human immune deficiency virus, or HIV, the causative agent of AIDS. All Retroviruses have reverse transcriptase they cause *transcription* to occur in reverse order i.e. synthesis of DNA from RNA, in normal *transcription* RNA is synthesized from DNA. The DNA is transcribed into RNA.

Table 5.4: Classification of Animal Viruses

DNA Viruses	Some Diseases
Adenoviruses Herpesviruses: H. simple type I H. simple type II Varicella-zoster Epstein-Barr	Respiratory infections Oral herpes, cold sores Genital herpes Chickenpox, shingles Infectious mononucleosis, implicated in some cancers
Papovaviruses Parvoviruses Poxviruses	Benign and malignant warts Roseola (fever, rash) in small children, aggravates sickle-cell anaemia. Smallpox, cowpox
RNA Viruses	Some Diseases
Picornaviruses: Enteroviruses Rhinoviruses Togaviruses Paramyxoviruses Rhabdoviruses Coronaviruses Orthomyxoviruses Arenaviruses	Polio, hemorrhagic eye disease, hepatitis A (infectious hepatitis) Common cold Encephalitis, yellow fever, dengue fever Measles, mumps Rabies Respiratory Infections Influenza Hemorrhagic fevers
Reoviruses Retroviruses: HTLV I, II HIV	Respiratory, intestinal infections Associated with cancer AIDS, ARC

Source: NIOS (2017).

(iii) **Plant Viruses:** Known plant viruses cause infectious diseases only after they have successfully penetrated the protective wall of plant cells. Typically, aphids and other insects that feed on plants assist in the infection. Virus particles may be clinging to their piercing or sucking devices, and when those devices penetrate plant cells, the virus enters with them.

Viruses are known to cause more than a thousand diseases in plants. Viral diseases can greatly reduce yields of a variety of crops, including potatoes, tomatoes, cauliflowers, cucumbers, turnips and barley. Outward symptoms of infection include mottled and blistered leaves, misshapen or unusually small fruit, tumors on roots, and colour changes in flowers.

Certain viruses affect the outward appearance of tulips and some other plants. The tulip blossom has colourless streaks because some of its pigment-containing cells are attacked by the colour breaking virus. Because tulip fanciers sometimes admire the variegated blossoms, commercial growers keep viruses infected bulbs in their inventory, but they keep them isolated from non-infected bulbs. Table 5.5 shows some common plant viruses.

Table 5.5: Some common Plant Viruses

Types of Virus	Target Plant
RNA Viruses: Closterovirus Comovirus Cucumovirus Hordeivirus Potaxvirus Tobamovirus (tobacco mosaic virus)	Beets Cowpeas Cucumbers Barley Potatoes Tobacco
DNA Viruses: Caulimovirus Geminivirus	Cauliflower Maize

Source: NIOS (2017).

5.9.2 Modes of Transmission

(a) Modes of Viral Disease Transmission

- (i) **Direct contact:** by touching open sores or other lesions on an infected person. (This is where “contagious disease” comes from; the Latin, contagion means touch or contact). Infected people also can transfer pathogens from such lesions to their own hands or mouth and so contaminate someone else through a handshake or a kiss. COVID– 19 and HIV are spread primarily by direct contact.
- (ii) **Indirect contact:** by touching doorknobs, food, diapers, hypodermic needles (as used by drug abusers), or other objects that were previously in contact with an infected person. Food that is moist, not refrigerated, and not too acidic can be contaminated by an assortment of viral particles, including the ones responsible for typhoid fever.

- (i) **Biological vectors:** These include mosquitoes, flies, fleas, ticks and other arthropods that transport pathogens from infected people or contaminated material to new hosts. Many pathogens only use vectors like taxis, or vehicles. Many depend on vectors as *intermediate hosts*. This means that a portion of the pathogen's life cycle must be carried out inside the vector. Aedes mosquitoes, for instance, are *intermediate hosts* for the causative agents of dengue.
- (ii) Inhaling pathogens that have been ejected into the air, example by coughs and sneezes.

(b) Transmission of Human Immunodeficiency Virus (HIV)

- (i) Sexual transmission
 - Most common mode of infection is heterosexual transmission
 - Anal intercourse > Vaginal intercourse > Oral sex
 - Male to female transmission greater than female to male
- (ii) Blood and blood products
 - Whole blood, packed red blood cells, platelets and leucocytes can transmit the Infection.
 - Fresh frozen plasma or clothing factors can also transmit infection.
 - Artificial insemination (by semen).
 - Organ transplant
 - Intravenous drug abuse
- (iii) Occupational transmission
 - Exposure to needles and other sharp medical instruments.
- (iv) Maternal- fetal/infant transmission
 - Probability of transmission of HIV from mother to infant/foetus ranges from 15 to 25% in industrialized countries and from 25 – 35% in developing countries

Mode of transmission from mother to foetus

 - (a) In uterus (During pregnancy)
 - (b) During delivery (perinatal) – most common
 - (c) After birth by breast feeding – least common.

5.10 PATTERN OF OCCURRENCE AND VIRULENCE

(a) Occurrence

Infectious diseases often are described in terms of their patterns of occurrence.

As the name suggests, sporadic disease break out irregularly and affect only a few people. *Endemic diseases* occur more or less continuously but are localized to a relatively small portion of the population e.g. Yellow fever and Lassa. During an *epidemic*, a disease abruptly spreads through large portions of the population for a limited period. When influenza breaks out along the East Coast of North America, this is an epidemic. When epidemics break out in several countries around the world in a given time span, they collectively are called a pandemic. AIDS is pandemic as more than 300 million have been infected so far worldwide. Covid-19 is another viral infection that has assumed pandemic proportion. As of 24 May, 2022 Nigeria recorded 255,972 confirmed cases of Covid-19.

(a) Virulence

Virulence is the relative ability of a pathogen to overcome body defenses and cause disease, and this depends on how easily it can avoid detection and destruction in the tissues of a particular individual. Every pathogen has its own *virulence* factors.

For example, a certain virus may cause a mild disease within 24-hours whereas toxins produced by the *Clostridium* may kill a person within a few hours.

There can be great individual differences in the responses to many pathogens. Most important is the overall state of the body's immune system. Immune systems can be weakened by chronic fatigue or stress while in some cases and they can be essentially shut down (e.g during AIDS).

5.11 CHAPTER SUMMARY

- In 1796, several scientists demonstrated that viruses readily passed through a porcelain filter with a pore size small enough to retain bacteria, suggesting they were smaller than bacteria.
- It was also observed that this filterable virus could not be cultured except in a living host and that its cells were not like typical cells of a living organism.
- In the 1950's, proof was provided that nucleic acid was the infectious agent.
- There are several theories about the origin of the virus.
- The difference between viruses, viroids, and prions is based on the arrangement of their nucleic acids and the nature of their protein coats.
- Viruses are obligated and acellular, with a single nucleic acid that can only replicate using host material; they are classified morphologically based on symmetry observed under an electron microscope.
- They are actually bridges between living and non-living organisms.
- There are various viral classification scheme and regardless of the variation, virus replicate by attaching to host cell, introduction of genetic material (DNA) or (RNA) into the host cytoplasm, replication and assembly of viral nucleic acids, release of newly formed viral particles.
- Bacteriophages are viruses that attack bacteria. In replicating, they become attached to a susceptible cell, which they penetrate, with their DNA or RNA directing the synthesis of new virus molecules from host material; the assembled new viruses are released when the host cell dies.
- They cannot grow outside of a susceptible living host cell, but some scientists have discovered some techniques for the isolation and cultivation of viruses.
- Viruses attack humans, animals, and plants, causing diseases and various effects on the host.
- Generally, they can be broken into major classes based on the types of hosts.
- Their mode of transmission or spread can be direct, indirect, biological vectors, or by inhalation. Their patterns of occurrence can be endemic, epidemic, or pandemic.
- Viral diseases such as chicken pox, measles, mumps, and yellow fever, have declined since immunizations against the diseases have become widespread.

5.12 STUDENTS' PRACTICAL ACTIVITIES

ACTIVITY 1: Activity 1 & 2 in this practical should be carried out with the supervision of a professional. Your teacher will help you to focus at a **bacterium under a light microscope**. Take a very good look at the slide of a bacterium and compare with that of a virus. You will therefore notice that a virus is 50 times smaller than bacterium. Can you now see how small a virus is?

ACTIVITY 2: Spread of Viruses through a population by sharing body fluid

AIM: To determine how viruses are spread through a population by sharing body fluid

MATERIALS

- (i) Plastic pipet
- (ii) Body fluid
- (iii) Test reagent
- (iv) Numbered vial solution

PROCEDURE

- (i) Obtain a numbered vial of solution and a plastic pipet from your instructor.
- (ii) Record your student name and vial number on the class data sheet.
- (iii) Share bodily fluids with another person in lab. Use the plastic pipet to withdraw solution from your vial and place 5 drops of your solution in another classmate's vial. Your classmate will also share fluids with you in the same way. Return the cap to the vial and invert to mix.
- (iv) Write the name of the person you shared bodily fluids with in the record book.
- (v) Exchange bodily fluids with another person following the directions above. Record the name of the person with whom you exchanged fluids.
- (vi) Exchange fluids with another student (different than the first two) and record his/her name below. You should complete three total fluid exchanges.

DISCUSSION

The laboratory instructor will add a drop of the test reagent to determine when someone is infected with the disease. During testing the sample tested turns pink when one is infected. If it turns yellow after testing then one is not is infected. If a person is positive for a disease, that means the person must have contracted the disease from the laboratory when sharing bodily fluids.

CONCLUSION

A virus is not considered a living organism as it only contains DNA surrounded by a protein coat. However, viruses aserious infectious agents cause condition such as AIDS, chicken pox, and herpes. Viruses must have a living host cell to reproduce. The virus takes over the protein building machinery of the cell to create new viruses to spread the infection. Viruses can infect many different types of organisms, both prokaryotic and eukaryotic.

One way a virus can be spread through a population is by sharing bodily fluids such as saliva, blood, or semen. We will demonstrate how quickly a virus can spread through today's simulation activity.

5.13 TUTOR MARKED ASSESSMENT QUESTIONS

HAVING READ THROUGH **CHAPTER FIVE**, ANSWER THE FOLLOWING QUESTIONS IN THE SPACES PROVIDED.

1.(a) Define briefly the following terms:

Virus:

.....
.....
.....
.....
.....
.....
.....

2×½ =1 Marks

Viroids

.....
.....
.....
.....

$2 \times \frac{1}{2} = 1$ Marks

Prions

.....
.....
.....
.....

$2 \times \frac{1}{2} = 1$ Marks

(b) According to Lwoff and Tournier in (1962), virus was characterized as:

.....
.....
.....
.....
.....

$4 \times \frac{1}{2} = 2$ Marks

c) Make a large labeled drawing of a T₂ Virus to show its essential features.

Drawing $6 \times \frac{1}{2} = 3$ Marks
Labeling $4 \times \frac{1}{2} = 2$ Marks

(d) Write down the shapes and sizes of the following Virus as listed in the tabulations Below.

	Shape	Size
Tobacco Mosaic Virus		
Polio Virus		
Potato Virus		

4 × $\frac{1}{2}$ = 2 Marks

1.(a) Enumerate **Six** properties of Viruses.

6 × $\frac{1}{2}$ = 3 Marks

(b) List **Four** types of Bacteriophages which affect *Echerichia coli* that have been useful in Molecular Research.

.....

4 × $\frac{1}{2}$ = 2 Marks

(c) Virus Replication proceeds through **Four** phases, namely:

.....

4 × $\frac{1}{2}$ = 2 Marks

(d) State **Four** methods in which Viruses can be detected in Cell Culture.

.....
.....
.....
.....
.....

4 × ½ = 2 Marks

3. (a) In a Tabular form State the effect and transmission of the following Viral diseases in human body.

Disease	Effect on body	Transmission
Chicken Pox		
Small pox		
Common Cold		

4 × ½ = 2 Marks

(b) Write Down three most common and three less common symptoms of Coronavirus

(i) Three most common symptoms are:

.....
.....
.....
.....
.....

3 × ½ = 1½ Marks

(ii) Three less common symptoms are:

.....
.....
.....
.....
.....

3 × ½ = 1½ Marks

(c) In a tabular form place the following common Plant Virus and their target plants.

Types of virus	Target Plant
Germini Virus	
Clotero Virus	
Potax Virus	
Como Virus	
Cumuno Virus	
Hordel Virus	

$6 \times \frac{1}{2} = 3$ Marks

(d) Write briefly on the term **Virulence**.

.....

.....

.....

.....

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.....

$4 \times \frac{1}{2} = 2$ Marks

Chapter Six

ULTRASTRUCTURE IN EUKARYOTIC PLANT AND ANIMAL CELLS

Dr. Reggan B. Agbor & Dr. Innocent C. Anidu

6.1 INTRODUCTION

All organisms are composed of structural and functional units of life called "cells". The bodies of some organisms, like bacteria, protozoans, and some algae, are made up of a single cell (unicellular), while bodies of fungi, plants, and animals are composed of many cells (multicellular). The human body contains about one trillion cells. Cells vary in size and structure as they are specialized to perform different functions, but the basic components of a cell are common to all cells. This chapter deals with the structure common to all types of cells. Cells are minute, varying in diameter between 10 and 100 micrometers. They number in billions in larger organisms, such as trees. Plant cells are bound by *cell walls*. The living part of the cell, within the *cell walls*, is the *cytoplasm*, and it consists of fluid *cytosol* and various organelles, the most important of which is the *nucleus*. Cells are the smallest units that still retain the characteristics of life, including complex organization, metabolic activity, and reproductive behavior. All cells have a plasma membrane that surrounds an inner region of *cytoplasm*. The plasma membrane keeps events within the cell separate from the surrounding environment so that the events proceed in organized, controlled ways. *Eukaryotic cells* have a *nucleus* and other organelles (membrane-bound compartments) within the cytoplasm. The membranes of these organelles separate different chemical reactions in the cytoplasm and so allow the reactions to proceed in an orderly fashion. Prokaryotic cells (**bacteria**) do not have comparable organelles.

6.2 LEARNING OBJECTIVES

After reading this chapter, you should be able to;

- (i) Define eukaryotic cell and state its characteristics.
- (ii) Discuss cell ultrastructure in eukaryotic plant and animal.
- (iii) State the physical and chemical nature of cytoplasmic matrices.
- (iv) Differentiate the cell ultrastructure between membranous and non-membranous organelles.
- (v) Identify the cell organelles and state their functions.
- (vi) State the similarities between mitochondria and chloroplast.
- (vii) Highlight the major differences between plant and animal cells.
- (viii) State the similarities between both plant and animal cells.

6.3 MEANING AND CHARACTERISTICS OF EUKARYOTIC CELL

Eukaryotic cells are cells that contain a *nucleus* and organelles, and are enclosed by a plasma membrane. Organisms that have eukaryotic cells include protozoa, fungi, plants and animals. These organisms are grouped into the biological domain Eukaryota.

Eukaryotic cells are larger and more complex than prokaryotic cells which are found in Archaea and *Bacteria*, the other two domains of life. Characteristically, eukaryotic cells contain a variety of structures called organelles, which perform various functions within the cell. Examples of organelles are ribosome, which make proteins, the *endoplasmic reticulum*, which sorts and packages the protein, and mitochondria, which produce the energy molecule adenosine triphosphate (ATP). They also have true *nucleus*, which contains the *genetic material* DNA and is surrounded by a nuclear envelope. All the organelles are stabilized and given physical support through the *cytoskeleton*, which is also involved in sending signals from one part of the cell to the other. In eukaryotic cells, the *cytoskeleton* is composed mainly of three types of filaments: microtubules, microfilaments and intermediate filaments. The gel-like substance that surrounds all the organelles in the cell is called *cytosol*.

6.4 CELL ULTRASTRUCTURE IN EUKARYOTIC PLANT AND ANIMAL

Structurally, there is no "typical cell", since no particular cell anywhere possesses all the features of a cell. However, all cells share certain attributes in common (Figure 6.1a). A typical cell as seen under the light microscope consists of two main parts, namely:

- (i) The outer covering coat or envelope called *cell wall* (in plants) or cell membrane, also called plasma membrane (in animals), as shown below in Figure 6.1b.
- (ii) The *protoplasm*, which is differentiated into two distinct regions-the cytoplasm and the *nucleus*, is bound with a membrane that contains chromosomes.

Protoplasm is essentially the living part of the cell. In its living state, the protoplasm is **transparent** and **colloidal**. The principal component of the protoplasm is water, which accounts for between 75% and 90% of inorganic and organic substances, including food materials.

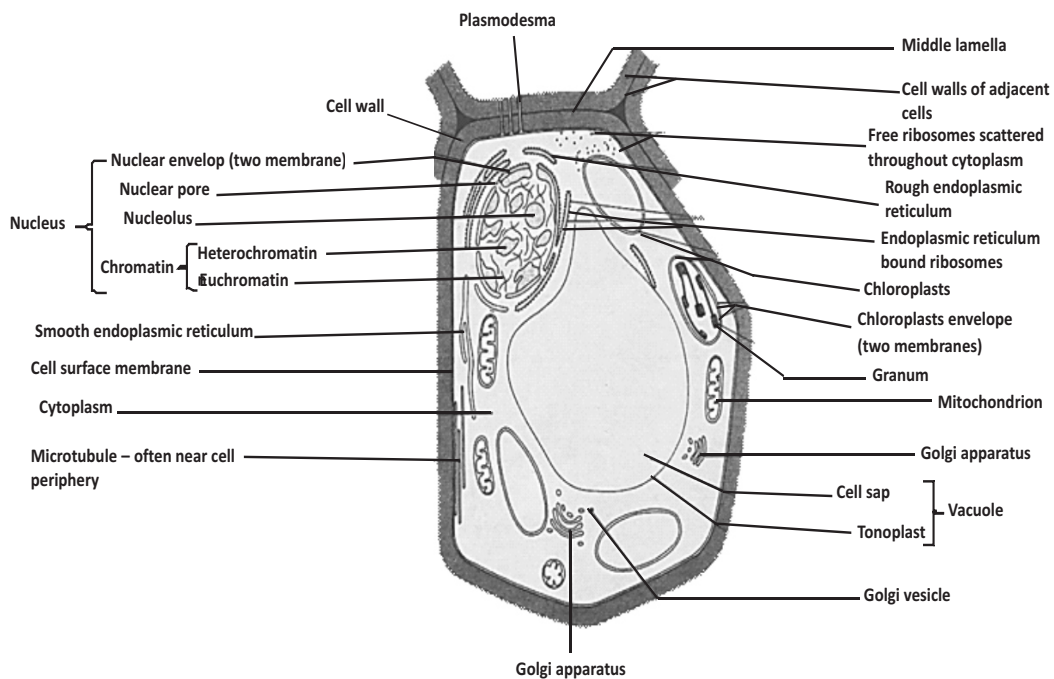


Figure 6.1: (a) Generalized Plant Cell.
Source: (Taylor et al, 1997).

Note: The cell is surrounded by a plasma membrane and contains a nucleus, ribosome, Endoplasmic Reticulum, Golgi apparatus, mitochondria, peroxisomes, and microfilaments and microtubules. However, plant cells also contain organelles called plastids. Here, chloroplast is present as an important plastid which carries out photosynthesis, converting sunlight to chemical energy stored in sugar and other organic molecules. Another important feature is the presence of large central vacuole. It stores chemicals, breaks down macromolecules, which helps in the process of plant growth. The membrane of the vacuole is called the Tonoplast. Outside a plant cell's plasma membrane is a thick cell wall, which helps maintain the cell's shape and protects the cell from mechanical damage.

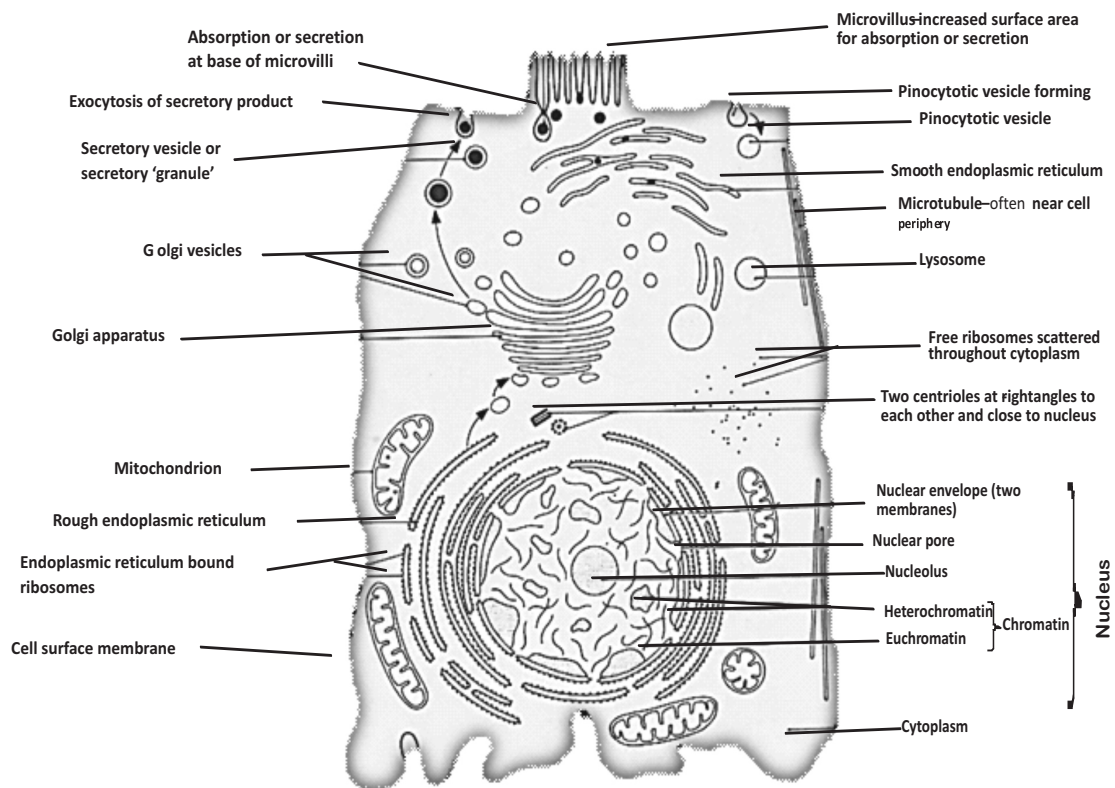


Figure 6.1: (b) Generalized Animal Cell.

Source: (Taylor et al,1997).

Note: The generalized drawing of an animal cell here carries the most common features found in animal cell. The Nucleus is bordered by an envelope consisting of two membranes. The cytoplasm is full of specialized organelles suspended in a semi-fluid medium called the cytosol. Within the cell are some components called organelles. Nucleus is found here in which inherited genes reside in the form of DNA Nucleolus is also found which assembled particles called ribosomes, which function in the synthesis of proteins.

6.5 CYTOPLASMIC MATRIX (Chemical Organization of the Cell)

Cytoplasmic matrix or cytosol is the fluid and soluble portion of the cytoplasm that exists outside the organelles.

6.5.1 Physical Nature of Cytosol (Cytoplasmic Matrix)

The *cytoplasmic matrix* is a colourless or greyish, translucent, viscid, gelatinous or jelly-like colloidal substance. It is heavier than water but capable of flowing. In the past, there has been a lot of controversy about the physical nature of the matrix. Different workers advanced different theories which are represented as follows:

- i. Reticular theory suggests that the matrix is composed of reticulum of fibres or particles in the ground substances.
- ii. Alveolar theory was proposed by Butschili in 1892 and according to him, the matrix consists of many suspended droplets or alveoli or minute bubbles resembling the foams of emulsion.
- iii. Granular theory was propounded by Altmann in 1893. This theory supports the views that the matrix contains many granules of smaller and larger size arranged differently. These granules were known as bioplasts.
- iv. Fibrillar theory was proposed by Fleming and it holds that the matrix is fibrillar in nature.
- v. Colloidal theory has been forwarded very recently after the electron microscopical observations of the matrix. According to the recent concept, the matrix is partly a true solution, partly a colloidal system.

Cytoplasmic matrix like many colloidal systems, shows the property of phase reversal. For example, gelatin particles are dispersed through water in a thin consistency that is freely shakable. Such a condition is called a sol. When the solution cools, gelatin now becomes the continuous phase and the discontinuous phase. Moreover, now the solution has stiffened and becomes semisolid and is called a gel.

6.5.2 Chemical Organization of Cytoplasmic Matrix

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

- (i) **Chemical elements:** Of the 92 naturally occurring elements, 46 elements are found in the cytosol. Twenty four (24) of these are considered essential for life, while others are present in cytosol only because they exist in the environment which the organism interacts. Out of the 24 essential elements, six play important roles in living systems. These major elements are Carbon, Hydrogen, Nitrogen, Oxygen, Phosphorus and Sulphur. Most organic molecules are built with these six elements. Another five essential elements found in less abundance in living systems are calcium, potassium, sodium, chlorine and magnesium. Several other elements, called trace elements, are also found in minute amounts in animals and plants, but are nevertheless essential for life. These are iron, iodine, molybdenum, manganese, cobalt, selenium, copper, chromium, vanadium, silicon, Nickel, fluorine and boron.
- (ii) **Ions:** The cellular function of certain ions in cytoplasmic matrix is highlighted in Table 6.1. An ion is a charged atom or group of atoms in which the number of electrons is different from the number of protons. If the number of electrons is less than the number of protons this is referred to as a positive ion, also called a cation, and if the number of electrons are more than the number of proton. It is a negative ion and referred to as anion. Examples of ions are: Sodium ion (Na^+), Chloride ion (Cl^-), and oxide ion O^{2-} ferric ion, Fe^{3+} .

- (iii) **Electrolytes and Non-Electrolytes**
The matrix also consist of both *electrolytes* and non-electrolytes
- (iv) **Electrolytes:** The *electrolytes* play a vital role in the maintenance of osmotic pressure and acid-base balance in the matrix. Magnesium ion (Mg^{2+}) ions, and phosphate, are good examples of *electrolytes*.
- (v) **Non-electrolytes:** Some minerals occur in matrix in non-ionizing state. The non-electrolytes of the matrix are Na, K, Ca, Mg, Cu, I, Fe, Mn, Fl, Mo, Cl, Zn, Co and Ni. The iron (Fe) occurs in *haemoglobin*, *ferritin*, *cytochrome* oxidase. Calcium occurs in the blood matrix and the bones. The Cu, Mn, Mo and Zn are useful as *cofactors* for enzymatic actions. The iodine and fluorine are essential for the thyroid and the enamel *metabolism* respectively.

Table 6.1: Cellular functions of certain ions

S/N	Elements	Ionic present	functions
i.	Molybdenum	MoO_4^{2-}	Co-factor or activator of certain enzymes (e.g. nitrogen fixation, nucleic acid metabolism, aldehyde oxidation)
ii.	Cobalt	Co^{2+}	Constituent of vitamin B ₁₂
iii.	Copper	Cu^+ , Cu^{2+}	Constituent of plastocyanin and co-factor of respiratory enzymes.
iv.	Iodine	I ⁻	Constituent of thyroxine, triiodothyronine and other thyroid hormones.
v.	Boron	BO_3^{3-} , $B_4O_7^{2-}$	Activates arabinose isomerase
vi.	Zinc	Zn^{2+}	Co-factor of certain enzymes (e.g., carbonic anhydrase, carboxypeptidase)
vii.	Manganese	Mn^{2+}	Co-factor of certain enzymes (e.g., several <u>kinases</u> , isocitric decarboxylase).
viii.	Iron	Fe^{2+}	Constituent of haemoglobin, myoglobin and cytochromes.
ix.	Magnesium	Mg^{2+}	Constituent of chlorophyll; activates ATPase enzymes.
x.	Sulphate	SO_4^{2-}	Constituent of coenzyme A, biotin, thiamine, proteins.
xi.	Phosphate	PO_4^{3-} , $H_2PO_4^-$	Constituent of lipids, proteins, nucleic acids, sugar phosphates, nucleoside phosphates.
xii.	Calcium	Ca^{2+}	Constituent of plant cell walls; matrix component of bone tissue; co-factor of coagulation enzymes.
xiii.	Potassium	K^+	Co-factor for pyruvate kinase and K^+ stimulated ATPase.

Source: After Raven and Johnson (1976).

6.5.3 Types of Compounds of Cytosol: The term *cytosol* is used to refer to the liquid phase of the cytoplasm in an intact cell.

Chemical compounds are conventionally divided into two groups: organic and inorganic. **Organic compounds** form 30% of a typical cell, the rest are the **inorganic substances** such as water and other substances as shown in Table 6.2.

Table 6.2: The approximate percentage composition of the Mammalian cytosol

Substances	Percentage
Water	65
Protein	18
Fat	10
Carbohydrate	5
Other organic	1
Inorganic	1

6.6 CELL MEMBRANE

The cell membrane's main function is to serve as a boundary between the cell and its environment. It is not, however, inert but a functional organelle. It may permanently exclude certain substances from the cell while permanently retaining others. Some substances may pass freely in and out through the membrane. Yet others may be excluded at one moment only to pass freely across the membrane on another occasion. On account of the membrane's ability to permit different substances to pass across it at different rates, it is said to be partially or semi-permeable.

There is little dispute that the *cell membrane* is made up almost entirely of two chemical groups – proteins phospholipids. In 1972, Singer and Nicholson suggested a structure for the *cell membrane*, that it is a bimolecular *phospholipid* layer with inwardly directed *hydrophobic tails* and a variety of protein molecules with an irregular arrangement. Some proteins occur on the surface of the *phospholipid* layer (peripheral or extrinsic proteins) while others extend into it (integral or intrinsic proteins) and some even extend completely across (transmembrane protein). Viewed from the surface, the proteins are dotted throughout the *phospholipid* layer in a mosaic arrangement. Other research suggests that the *phospholipid* layer is capable of much movement, i.e. as fluid. It was this fact which gave rise to its name, the *fluid-mosaic model*. Also present in the membrane is *cholesterol* which interacts with the *phospholipids* to make the membrane less fluid. The proteins in the membrane have a number of functions. Apart from giving structural support they are very specific, varying from cell to cell. It is this specificity which allows cells to be recognized by other agents in the body, e.g. enzymes, hormones and antibodies. In the *fluid-mosaic model*, it is probable that the proteins also assist the active transport of materials across the membrane.

6.7 MEMBRANOUS ORGANELLES

6.7.1 The Nucleus

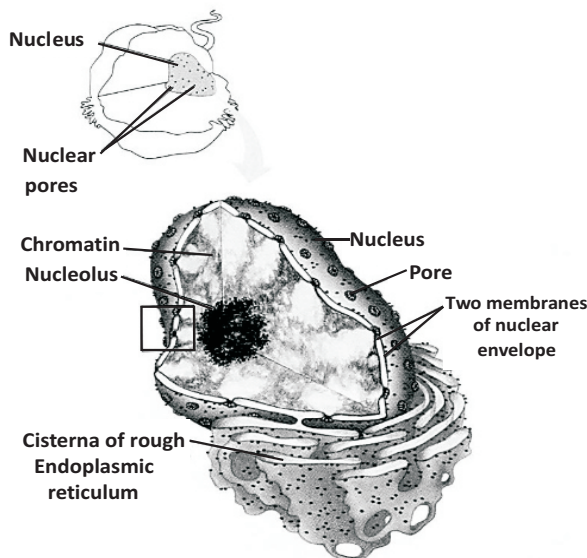
When viewed under a microscope, the most prominent feature of a cell is the *nucleus*, while its shape, size, position and chemical composition vary from cell to cell, its functions are always the same, namely, to control the cell's activity and to retain the organism's hereditary material, the chromosomes. It is bounded by a double membrane, the nuclear envelope, the outer membrane being continuous with the *endoplasmic reticulum* and often having ribosomes on its surface.

The inner membrane has three proteins on its surface which act as anchoring points for chromosomes during interphase. It possesses many large pores (typically 3000 per nucleus) 40-100nm in diameter, which permit the passage of large molecules, such as RNA, between it and the cytoplasm. The cytoplasm-like material within the nucleus is called **nucleoplasm**. It contains **chromatin** which is made up of coiled DNA bound to proteins. During cell division, the **chromatin** condenses to form the chromosomes but these are rarely, ever visible in a non-dividing cell. The denser, more darkly stained areas of **chromatin** are called heterochromatin.

Within the nucleus are one or two small spherical bodies, called nucleolus (Figure 6.2). They are not distinct organelles as they are not bounded by a membrane. They manufacture ribosomal RNA, a substance in which are especially rich, and assemble ribosomes. Nucleus is an organelle found in both plant and animal cells.

The functions of a nucleus are:

- (i) It contains the genetic material of a cell in the form of chromosomes;
- (ii) It acts as a control centre for the activities of a cell;
- (iii) It carries the instructions for the synthesis of proteins in the nuclear DNAs;
- (iv) It is involved in the production of ribosomes and RNAs;
- (v) It is involved in cell divisions.



Note: The nucleus. Within the nucleus, the genetic material is in the dispersed form known as chromatin; a darker -staining nucleolus is also visible. The nucleolus is the site of ribosome synthesis. The nuclear envelope which consists of two membranes separated by a narrow space is perforated with pores.

Figure 6.2: The nucleus and its envelope.

Source: Campbell (1993).

6.7.2 The Plastids

Plastids occur only in plant cells. They contain pigments and may synthesize and accumulate various substances.

Plastids are of the following types and functions

- (i) **Leucoplasts** are colourless **plastids** of embryonic and germ cells lacking **thylakoid** and ribosomes.

- (iiv) **Amyloplasts** produce starch.
- (iv) **Proteinoplasts** accumulate protein
- (v) **Oleosomes or elaioplasts** store fats and essential oils.
- (vi) Chromoplasts contain pigment molecules and are coloured organelles. Chromoplasts impart a variety of colours to plant cells, such as red colour in tomatoes, red chillies and carrots, various colours to petals of flowers and green colour to many plant cells. The green coloured chromoplasts are called **chloroplasts**. They have chlorophyll pigment and are involved in the photosynthesis of food and so act like kitchens of the cell.

Chloroplasts have diverse shapes in green algae but are round, oval or discoid in shape in higher plants (see Figure 6.3). Like mitochondria, each chloroplast is bounded by two membranous envelopes, both of which have no chlorophyll pigment. However, unlike mitochondria, there occurs a third system of membranous layer within the boundary of the inner membrane, called grana and are stacks of membrane-bounded flattened discoid sacs, arranged like neat piles of coins. The grana form the main functional units of chloroplast and are bathed in the homogeneous matrix, called the stroma. The stroma contains a variety of photosynthetic enzymes and starch grains. A chloroplast contains many such interconnected grana on which are located various photosynthetic enzymes and the molecules of green pigment chlorophyll and other photosynthetic pigments to trap the light energy. They contain DNA, ribosomes and complete protein synthetic machinery.

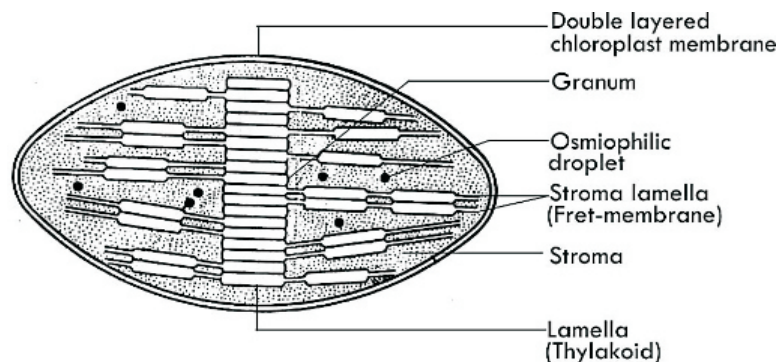


Figure 6.3: The Structure of a Chloroplast.

Source: Jain (2007).

Note: Chloroplast, a specialized member of a family of plant organelles called plastids, contains chlorophyll and other pigments, which function in photosynthesis. Chloroplasts are enclosed by two membranes surrounding the fluid called stroma, in which are embedded the thylakoids. These flattened sacs may be stacked to form grana.

6.7.3 The Mitochondrion

Mitochondria are found within the cytoplasm of all eukaryotic cells, although in highly specialized cells such as mature red blood cells, they may be absent (Figure 6.4). They range in shape from spherical to highly elongated and are typically $5\mu\text{m}$ in length and $0.2\mu\text{m}$ across. They are bounded by a double membrane, the outer one controls the entry and exit of chemicals. The inner membrane is folded inwards, giving rise to extensions called cristae, some of which extends across the entire organelle. They function to increase the surface area on which respiratory processes take place. The surface of these cristae has stalked granules along its length.

of cristae increases in metabolically active cells, giving weight to the proposition that respiratory enzymes are located on them.

Functions of Mitochondrion

1. Mitochondrion is the site of ATP synthesis in the cell.
2. It has a role to help maintain the intracellular environment. It stores capases responsible for triggering *apoptosis* and is able to transiently store calcium contributing to calcium homeostasis.
3. In brown adipose tissue, mitochondria have an alternative function of heat production using the electron transport chain.
4. Mitochondria replicate their DNA by a process called binary fission and can use this to make multiple copies in one mitochondrion. Their DNA has maternal lineage which means their DNA is passed from mother to child with little change.

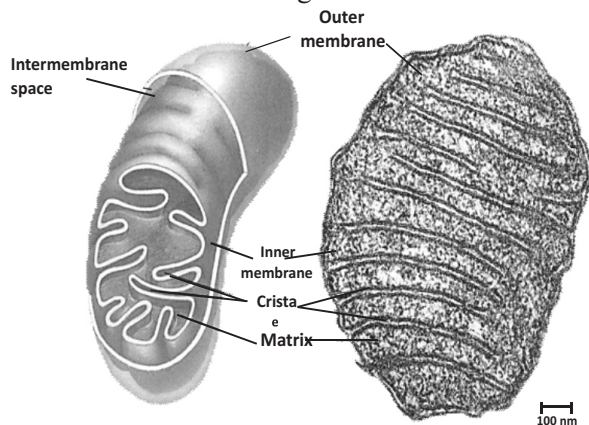


Figure 6.4: The mitochondrion.
Source: Campbell (1993).

Note: The mitochondrion.

The double membranes of the mitochondrion are evident in the drawing and the micrograph (TEM). The cristae are infoldings of the inner membrane. The three dimensional drawing emphasizes the relationships between the two membranes and the compartments they bound: the intermembrane space and the mitochondrial matrix.

6.7.4 Endoplasmic Reticulum

The *endoplasmic reticulum* (ER) is an elaborate system of membranes found throughout the cell, forming a cytoplasmic skeleton. It is an extension of the outer nuclear membrane with which it is continuous. The membranes form a series of sheets which enclose flattened sacs called *cisternae*. The membranes of the ER may be loosely organized or tightly packed. Where the membranes are lined with ribosomes they are called rough endoplasmic reticulum. The rough ER is concerned with protein synthesis and is consequently most abundant in those cells which are rapidly growing or secrete enzymes (see Figure 6.5). In the same way, damage of a cell often results in increased formation of ER in order to produce the proteins necessary for the cells' repair. Where the membranes lack ribosomes they are called smooth endoplasmic reticulum. The smooth ER is concerned with lipid synthesis and is consequently most abundant in those cells producing lipid-related secretions e.g. the sebaceous glands of mammalian skin and cells secreting *steroids*.

The functions of the Endoplasmic Reticulum (ER) are:

- (i) It provides a large surface area for chemical reactions;
- (ii) It provides a pathway for the transport of materials through the cell;
- (iii) It produces proteins, especially enzymes (rough ER);
- (iv) It produces lipids and *steroids* (smooth ER);

- (v) It is used in collecting and storing synthesized materials;
- (vi) It provides a structural skeleton to maintain cellular shape (e.g. the smooth ER of a rod cell from the retina of the eye).



[Magnification: 40,000 x]

Figure 6.5: The rough endoplasmic reticulum (RER).

Source: Miller and Levine (2006).

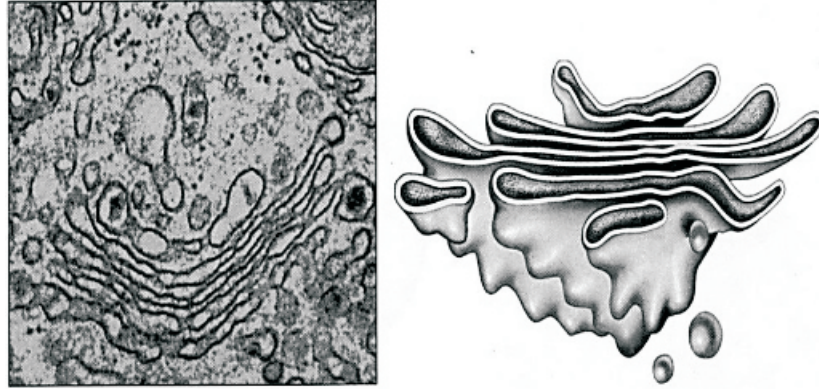
Note: The endoplasmic reticulum consists of a series of parallel membranes, encrusted with ribosomes, enclosing a system of interconnected flattened cavities. The membrane of the ER encloses a compartment called the cisternal space.

6.7.5 Golgi Apparatus (dictyosome)

The *Golgi apparatus*, named after its discoverer, Camillo Golgi, has a similar structure as the smooth endoplasmic reticulum but is more compact (Figure 6.6). It is composed of stacks of flattened sacs made of membranes. The sacs are fluid-filled and pinch off smaller membranous sacs, called vesicles, at their ends. There is normally only one *Golgi apparatus* in each animal cell but in plant cells there may be a large number of stacks known as *dictyosomes*. Its position and size varies from cell to cell, but is well developed in secretory cells and neurons, and is small in muscle cell. All proteins produced by the endoplasmic reticulum are passed through the Golgi apparatus in a strict sequence. They pass first through the cis *Golgi network* which returns to the ER any protein wrongly exported by it. They then pass through the stack of cisternae which modify the proteins and *lipids* undergoing transport and add labels which allow them to be identified and sorted at the next stage, the trans Golgi network. Here the proteins and *lipids* are sorted and sent to their final destinations. In general the Golgi acts as the cell's post office, receiving, sorting and delivering proteins and *lipids*.

Functions of Golgi apparatus

- (i) It Produces *glycol-proteins* such as mucin required in secretions, by adding the carbohydrate part to the protein;
- (ii) It produces secretory enzymes, e.g. the digestive enzymes of the *pancreas*;
- (iii) It secretes carbohydrates such as those involved in the production of new cell walls;
- (iv) It transports and storing *lipids*;
- (v) It forms lysosomes.



[Magnification: 45,700 x]

Figure 6.6 Three -Dimensional drawing of a Golgi apparatus; drawn from Electron Micrographs of a Secretary Animal Cell.

Source : Adapted from Jensen and Park (1967).

Note: The Golgi apparatus consists of stacks of membranous sacs that synthesize various macromolecules and also modify store, sort, and export products of the ER. One side of a Golgi stack, the *cis* face, receives secretory proteins from the transitional ER through ER transport vesicles. Once inside, these proteins can be chemically modified and sorted before release from the *trans* face of the Golgi stack in vesicles.

6.7.6 Lysosomes

The cytoplasm of animal cells contains many tiny, spheroid or irregular-shaped, membrane-bounded vesicles known as **lysosomes**. The **lysosomes** are originated from Golgi apparatus and contain numerous (about 50) **hydrolytic enzymes** (e.g. acid phosphatase that is **cytochemically** identified) for intracellular and extracellular digestion. They digest the material taken in by **endocytosis** (such as **phagocytosis**, **endocytosis** and **pinocytosis**), parts of the cell (by autophagy) and extracellular substances. Lysosomes have a high acidic medium (pH5) and this acidification depends on ATP- dependent proton pumps which are present in the membrane of lysosomes and which accumulate protons (H^+) inside the lysosomes (Figure 6.7). Lysosomes exhibit great polymorphism i.e. there are four types of lysosomes;

- (i) Primary lysosomes (**storage granules**)
- (ii) **Secondary lysosomes** (digestive vacuoles)
- (iii) **Residual bodies**
- (iv) **Autophagic vacuoles.**

The lysosomes of plant cells are membrane-bounded **storage granules** containing hydrolytic digestive enzymes e.g. large vacuoles of **parenchyma cells** of corn seedlings, protein or aleurone bodies and starch granules of cereal and other seeds.

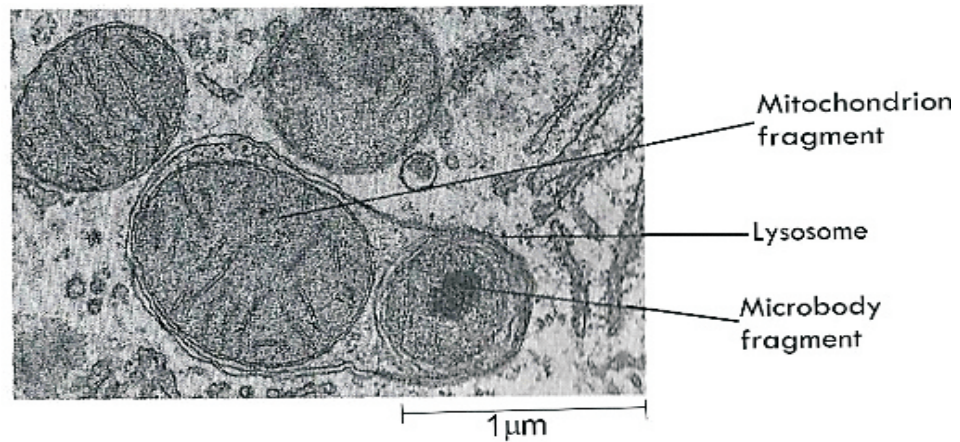


Figure 6.7: Lysosome of white blood cells in a rat.
Source: Campbell (1993).

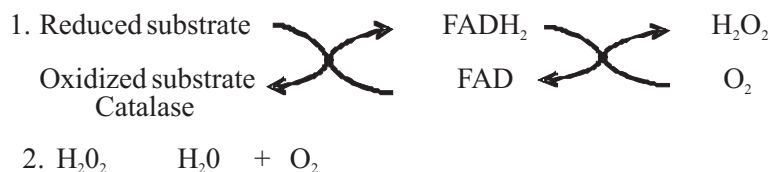
Note: A lysosome is a membrane enclosed bag of hydrolytic enzymes originating in the rough ER and processed and released from the Golgi apparatus. Its acidic microenvironment is optimal for the functioning of its enzymes in recycling monomers from cell macromolecules and in digesting substances ingested by phagocytosis.

The functions of lysosomes are:

- (i) To digest materials which the cell consumes from the environment. In the case of white blood cells, this may be bacteria or other harmful material. In protozoa, it is the food which has been consumed by **phagocytosis**. In case the material is broken down within the lysosome, useful chemical are absorbed into the cytoplasm and any debris is egested by the cell by **exocytosis**.
 - (ii) To digest parts of the cell, such as worn-out organelles, in a similar way to that described above. This is known as autophagy. After the death of a cell they are responsible for its complete breakdown, a process called **autolysis**;
 - (iii) To release enzymes outside the cell (**exocytosis**) in order to break down other cells, e.g. in the reabsorption of tadpole tail during **metamorphosis**;
- In view of their functions, it is hardly surprising that lysosomes are especially abundant in secretory cells and in phagocytic white blood cells.

6.7.7 Microbodies: Peroxisomes and Glyoxysomes

Microbodies are spherical or oblate in form. They are bounded by a single membrane and have an interior or matrix which is amorphous or granular. **Microbodies** are most easily distinguished from other cell organelles by their content, catalase. Catalase can be visualized with the electron microscope when cells are treated with the stain DAB (i.e., 3, 3 – diamino benzidine). The product is electron opaque and appears as dark regions in the cell where catalase is present. By applying this technique **microbodies** have been observed by electron microscopy and subsequently isolated from various mammalian tissues such as liver, kidney, intestine and brain. Recent biochemical studies have distinguished two types of **microbodies**, namely **peroxisomes** and **glyoxysomes**. These two organelles differ both in their enzyme complement and the types of tissue in which they are found. **Peroxisomes** are found in animal cells and the leaves of the higher plants. They contain catalases and oxidases (e.g. Damino – oxidase and urate oxidase). In both, they participate in the oxidation of substrates, producing hydrogen peroxide which is subsequently destroyed by catalase activity:



6.7.7.1 Types of Microbodies

(a) Peroxisomes

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

Functions of Peroxisomes

- (i) **Hydrogen Peroxide Metabolism:** **Peroxisomes** are so called, because they usually contain one or more enzymes (i.e. **D – amino acid oxidase** and urate oxidase) that uses molecular oxygen to remove hydrogen atom from specific organic substances (R) in an oxidative reaction that produces hydrogen peroxide (H_2O_2):

$$\text{R}_\text{H} + \text{O}_2 \longrightarrow \text{R} + \text{H}_2\text{O}_2$$
- (ii) **Glycolate Cycle:** The glycolate cycle brings about the formation of the amino acids – glycine and serine – from the non-phosphorylated intermediates of photosynthetic carbon reduction cycle. The **glycolate pathway** also generates C_1 compounds and serves as the generator of precursors for nucleic acid biosynthesis.
- (iii) In green leaves, there are **peroxisomes** that carry out a process called **photorespiration** which is a light stimulated production of CO_2 that is different from the generation of CO_2 by mitochondria in the dark.
- (iv) β -oxidation: **Peroxisomes** of rat liver cells contain enzymes of β -oxidation for the metabolism of fatty acids.
- (v) Mammalian cells do not contain D-amino acids, but the **peroxisomes** of mammalian liver and kidney contain D-amino acid oxidase. The presumed role of this enzyme arises from breakdown and absorption of peptidoglycan material of intestinal bacteria.

(a) Glyoxysomes

Glyoxysomes are found in the cells of yeast, Neurospora and oil rich seeds of many higher plants. They resemble peroxisomes in morphological details, except that, their crystalloid core consists of dense rods of 6.0m diameter. They have enzymes for fatty acid metabolism and **gluconeogenesis** i.e. conversion of stored lipid molecules of **spherosomes** of germinating seeds into the molecules of carbohydrates.

Functions of Glyoxysomes

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

- (ii) **Glyoxylate cycle:** The *glyoxylate pathway* occurs in glyoxysomes and it involves some of the reactions of the Krebs cycle in which citrate is formed from oxaloacetate and acetyl – CoA under the action of citrate synthetase enzyme.

6.7.8 Vacuoles

A fluid-filled sac bounded by a single membrane may be termed a *vacuole*. Within mature plant cells there is usually one large central *vacuole* (see Figure 6.8). The single membrane around it is called the tonoplast. A plant *vacuole* contains a solution of mineral salts, sugars, amino acids, wastes (e.g. *tannins*) and sometimes also pigments such as anthocyanins.

Functions of Plant Vacuoles

- (i) The sugars and amino acids may act as a temporary food store;
- (ii) The anthocyanins are of various colours and so in many coloured petals to attract pollinating insects, or fruits to attracts animals for dispersal;
- (iii) They act as temporary stores for organic wastes, such as *tannins*. These may accumulate in the *vacuoles* of leaf cells and are removed when the leaves fall;
- (iv) They occasionally contain hydrolytic enzymes and perform functions similar to those of lysosomes.
- (v) They support herbaceous plants of woody plants by providing an osmotic system which creates a pressure potential.

In animal cells, *vacuoles* are much smaller but may occur in large numbers. Common types include food vacuoles, *phagocytic vacuoles* and *contractile vacuoles*. The latter are important in the *osmoregulation* of certain protozoans.

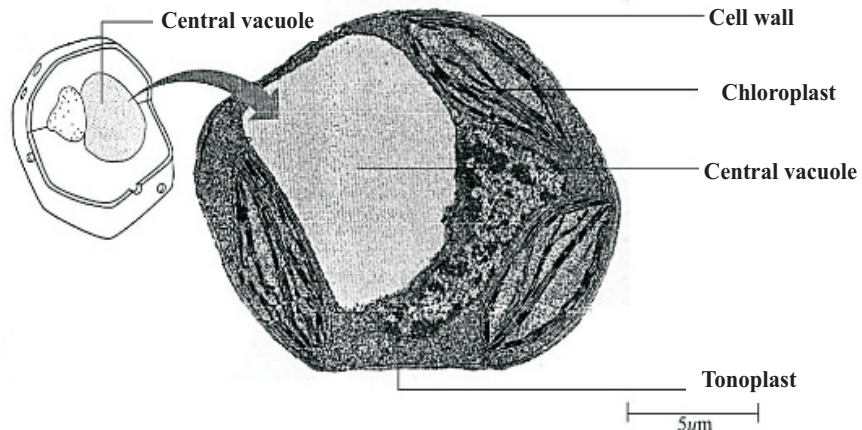


Figure 6.8: Plant cell showing a vacuole.

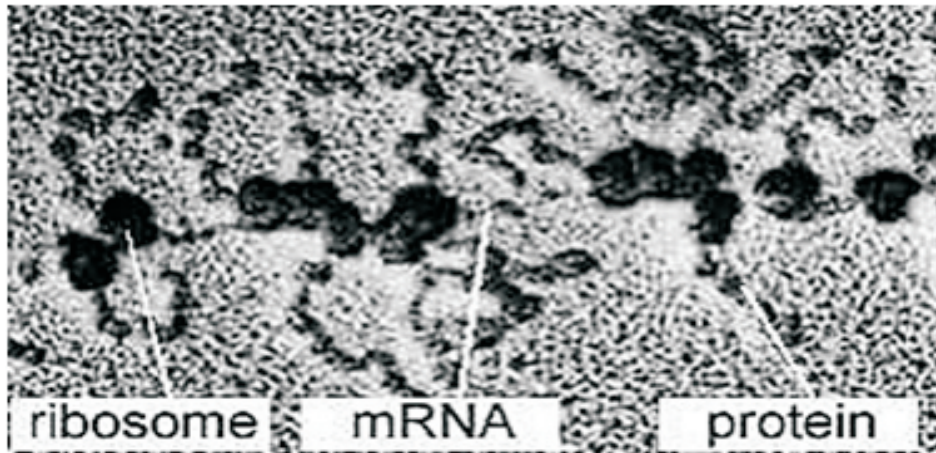
Source: Campbell (1993).

Note: The central vacuole is usually the largest compartment in a plant cell, comprising 80% or more of a mature cell. The cytoplasm is generally confined to a narrow zone between the vacuole and the plasma membrane. The membrane bounding the vacuole, the tonoplast, separates the cytosol from the solution inside the vacuole, which is called cell sap. Like all cellular membranes, the tonoplast is selective in transporting solutes; therefore, cell sap differs in composition from the cytosol. The vacuole functions in storage, waste disposal, hydrolysis, protection, and growth (TEM).

6.8 NON-MEMBRANOUS STRUCTURES

6.8.1 Ribosomes.

Ribosomes are small cytoplasmic granules found in all cells. They are approximately 20 nm in diameter in eukaryotic cells (80s type) but slightly smaller in prokaryotic ones (70s type). They may occur in groups called *polysomes* and may be associated with endoplasmic reticulum or occur freely within the cytoplasm. Despite their small size and their enormous number, accounts for up to 20% of the mass of the cell. **Ribosomes** are made up of one large and one small sub-unit, and comprise ribosomal RNA and protein (Figure 6.9).



6.8.2 Storage Granules

Every cell contains a limited store of food energy. This store may be in the form of soluble material such as the sugar found in the vacuoles of plant cells. It may also occur in insoluble form, as grains or granules, within cells or organelles.

Starch grains occur within chloroplasts and the *cytoplasm* of plant cells. Starch may also be stored in specialized leucoplasts called amyloplasts. Glycogen granules occur throughout the cytoplasm of animal cells. The stored animal starch or glycogen, oil or fat droplets found within the cytoplasm of both plant and animal cells.

6.8.3 Microtubules

Microtubules occur widely throughout eukaryotic cells but are not found in prokaryotic ones. They are slender, unbranched tubes 24nm in diameter and up to several microns in length. They are made of two similar proteins alpha-and beta-tubulin, each of which comprises 450 amino acids.

The Functions of Microtubule are:

- (i) It provides an internal skeleton (cytoskeleton) for cells and help determine their shape;
- (ii) It aids transport within cells by providing routes along which materials move;
- (iii) It forms a framework along which the cellulose cell wall of plants is laid down;
- (iv) As major components of cilia and flagella where they are grouped in a very precise way and contribute to their movement;

- (v) Found in the spindle during cell division and within the centrioles from which the spindle is formed. Here it help to draw chromosomes or chromatids to opposite poles.

6.8.4 Cilia and Flagella

Cilia and **flagella** are almost identical, except that cilia are usually shorter and more numerous. Cilia are up to 25µm long whereas **flagella** may be 1000µm long. Both are approximately 0.2µm in diameter. They are found in a limited number of cells but are nevertheless of great importance. They function to either move an entire organism, e.g. cilia on the protozoan *Paramecium*, or to move materials within an organism e.g. the cilia lining the respiratory tract move mucus towards the throat. In the human respiratory tract there are about 200 cilia (each 7µm long) on each epithelial cell, giving a density of 10^9 cilia per cm^2 .

(a) Distinguishing Features between Cilia and Flagella

- (i) The **flagella** are less (1 or 2) in number than the cilia which may be numerous (3000 - 14000 or more) in number.
- (ii) The **flagella** occur at one end of the cell, while the cilia may occur throughout the surface of the cell.
- (iii) The **flagella** are longer (up to 150µm), while the cilia are short (5 - 10µm) appendages of the cytoplasm
- (iv) The **flagella** usually beat independently, while the cilia tend to beat in a coordinated rhythm.
- (v) The **flagella** exhibit undulatory motion, while the cilia move in a sweeping or pendular stroke.

(b) Functions of the Cilia and Flagella

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

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

6.8.5 Centrioles and basal bodies

Centrioles occur in most algal cells (notable exception being red algae), moss cells, some fern cells and most animal cells. They are absent in prokaryotes, red algae, yeast, cone-bearing and flowering plants (conifers and angiosperms) and some non-flagellated or non-ciliated protozoans (such as *amoeba*). Some species of *amoebae* have a flagellated stage as well as an amoeboid stage; a centriole develops during the flagellated stage but disappears during the amoeboid stage.

Centrioles and **basal bodies** are cylindrical structures which are 0.15 to 0.25µm in diameter usually 0.3 to 0.7µm in length, though some are short as 0.16µm and others are as long as 8µm. Both have the following ultrastructural components: cylindrical wall, Triplets, lickers, cartwheel, ciliary rootlets, basal feet and satellites.

The **microtubules** of **centrioles** and **basal bodies** contain the structural protein, tubulin, along with lipid molecules. The **centrioles** and basal bodies contain a high concentration of ATPase enzyme. There exists a controversy whether **centrioles** and **basal bodies** have DNA and RNA.

Functions of Centrioles and Basal bodies

- (i) Formation of **basal bodies** and ultimately the cilia is the specialized function of the **centrioles** in the cell.
- (ii) The critical function of a pair of **centrioles** in most animal cells is to act as a focal point for the centromere. The centromere (also called the cell centre) organizes the array of cytoplasmic microtubules during interphase and duplicates at mitosis to nucleate the two poles of the mitotic spindle.
- (iii) Sometimes centrioles can serve several functions in turns: for example, prior to each division in *chlamydomonas*, the two flagella resorb and the basal bodies leave their position to act as mitotic poles.
- (iv) In spermatozoon, one centriole gives rise to the tail fibre or flagellum.
- (v) **Centrioles** and basal bodies are also involved in ciliary and flagellar beat.
- (vi) **Centrioles** and basal bodies have a role in the reception of optical, acoustic and olfactory signals.

6.8.6 Microfilaments

Microfilaments are very thin strands about 6nm in diameter. They are usually made up of the protein actin with a small proportion of myosin. As these are the two proteins involved in muscle contraction, it seems probable that **microfilaments** play a role in movement within cells and possibly of the cells as a whole in some cases.

6.8.7 Microvilli

Microvilli are tiny finger-like projections about 0.6µm in length on the membranes of certain cells, such as those of the intestine epithelium and the kidney tubule. They should not be confused with larger villi which are multicellular structures. **Microvilli** massed together appear similar to the bristles of a brush, hence the term brush border given to the edge of cells bearing **microvilli**. Actin filaments within the **microvilli** allow them to contract, which, along with their large surface area and facilitate absorption.

6.8.8 Cellulose Cell Wall

A **cell wall** is a characteristic feature of plant cells. It consists of cellulose **microfibrils** embedded in an amorphous polysaccharide matrix. The matrix is usually composed of polysaccharides e.g. pectin or lignin. The **microfibrils** may be regular or irregular in arrangement. The cell wall determines the structure of the cell, the texture of plant tissues, and many important characteristics that distinguish plant as organisms (see Figure 6.10). All plant cells have a primary wall. In addition, many also have a secondary wall, which occurs interior to the primary wall. The region between the primary walls of adjacent cells is the **middle lamella**, a pectin-rich layer cementing together the primary walls of adjacent cells. Cellulose is the principal component of primary and secondary walls. The cellulose microfibrils of primary walls occur in a cross-linked matrix of noncellulosic molecules, including **hemicelluloses**, **pectins** and glycoproteins. Due to the presence of pectins, primary walls are highly hydrated, making them more plastic.

Actively dividing and elongating cells commonly have only primary walls. Secondary wall contains hemicelluloses but apparently lack *pectins* and glycoproteins. **Lignin** may also be present in primary walls but is especially characteristic of cells with secondary walls. Lignin adds comprehensive strength and rigidity to the wall. The chemical nature and staining reactions of various component of plant cell wall are highlighted in Table 6.3.

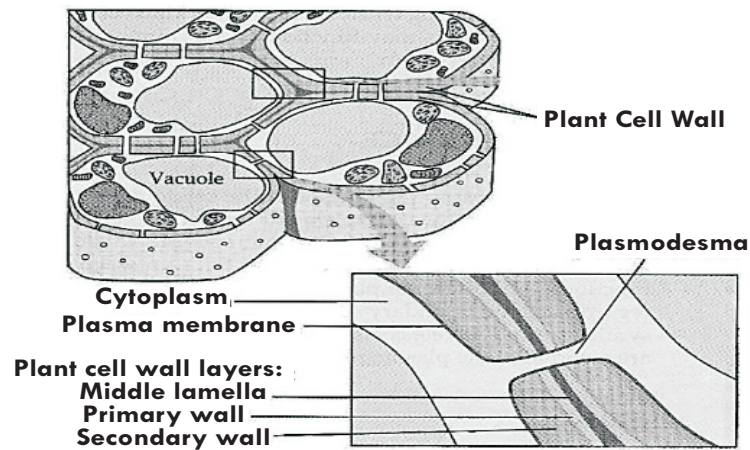


Figure 6.10: Plant cell walls.

Source: Adapted from Jensen and Park (1967).

Note: Young cells first construct thin primary walls, often adding stronger secondary walls to the inside of the primary wall when growth ceases. A sticky middle lamella cements adjacent cells together. Thus, the multilayered partition between these cells consists of adjoining walls individually secreted by the cells. The walls do not isolate the cells: The cytoplasm of one cell is continuous with the cytoplasm of its neighbors via plasmodesmata, channels through the walls.

Table 6.3: Chemical Nature and Staining Reactions of various components of Plant Cell Wall

Substance	Chemical unit	Staining reaction
(i) Cellulose	Glucose	Chlorzinic iodine (stains violet)
(ii) Hemicellulose	Arabinose, Xylose, mannose, glucose and galactose	No specific stain
(iii) Pectin	Glucuronic and galacturonic acid	Ruthenium red
(iv) Lignin	Coniferyl alcohol (e.g, hydroxyphenyl prophase)	Phloroglucinol hydrochloride (stain rose) and Chlorine iodine (stains yellow)
(v) Cuticular substances	Fatty acids	Sudan III (stains orange)
(vi) Mineral deposits	Calcium and magnesium as carbonates and silicates	

Source: After Raven and Evert (1981)

Functions of the Cell Wall:

- (i) It provides support in herbaceous plants. As water enters the cell osmotically the cell wall resists expansion, and an internal pressure is created which provides *turgidity* for the plant;
- (ii) It gives direct support to the cell and the plant as a whole by providing mechanical strength. The strength may be increased by the presence of lignin in matrix between the cellulose fibril;
- (iii) It permits the movement of water through and along it, and contributes to the movement of water in the plant as a whole, in particular in the cortex of the root;
- (iv) In some cell walls, the presence of cutin, suberin or lignin in the matrix makes the cells less permeable to substances. Lignin helps to keep the water within the xylem and cutin in the epidermis of leaves prevents water being lost from the plant. Suberin in root endodermal cells prevents movement of water across them, thus concentrating its movement through special passage cells;
- (v) The arrangement of the cellulose fibrils in the cell wall can determine the pattern of growth and hence the overall shape of cell;
- (vi) Occasionally, cell walls act as food reserves.

6.9 SIMILARITIES BETWEEN MITOCHONDRIA AND CHLOROPLASTS

- (i) Both contain their own DNA (the genetic material) as well as their own RNA (for protein synthesis). Thus, they can self-duplicate to produce more of their own kind without the help of nucleus.
- (ii) Since *chloroplasts* and mitochondria contain their own DNA the hereditary molecule and also their own ribosomes, they are termed semi-autonomous only because they are incapable of independent existence though they have ribosomes and DNA.

6.10 SIMILARITIES BETWEEN PLANT AND ANIMAL CELLS

- (i) Both have a nucleus.
- (ii) Both cells possess mitochondria which are the power house of all cells.
- (iii) Both cells possess chromosomes.
- (iv) Both cells possess nucleolus.
- (v) Both cells possess lysosome.
- (vi) Both cells possess golgi bodies.
- (vii) Both cells possess cytoplasm or cytosol.
- (viii) Both cells possess endoplasmic reticulum (ER).
- (ix) Both cells possess ribosome.
- (x) Both cells possess microtubules and chromatin.
- (xi) Both cells possess peroxisomes.

6.11 DIFFERENCES BETWEEN PLANT AND ANIMAL CELL Even though all living organisms are made up of cells that contain similar structures, there are differences between the structures of the cells of plants and animals as shown in Table 6.4.

Table 6.4 Major differences between plant and animal cell

S/N	Plant Cells	Animal Cells
(i).	Tough, slightly elastic cellulose cell wall present (in addition to the cell membrane)	Cell wall absent only a membrane surrounds the cell
(ii).	Pits and plasmodesmata present in the cell wall	There are no pits and plasmodesmata because of absence of cell wall.
(iii).	Middle lamella join cell walls of adjacent cells	Middle lamella absent, cells are joined by intercellular cement
(iv).	Plastids, e.g. chloroplast and leucoplasts, present in large numbers	Plastids absent.
(v).	Mature cells normally have a large single, central vacuole filled with cell sap.	Vacuoles, e.g. contractile vacuoles, if present, are small and scattered throughout the cell.
(vi).	Tonoplast present around the vacuole	Tonoplast absent
(vii).	Cytoplasm normally confined to a thin layer at the edge of the cell	Cytoplasm present throughout the cell
(viii)	Nucleus present at edge of the cell	Nucleus anywhere in the cell but often centrally located
(ix)	Lysosomes not normally present	Lysosomes almost always present
(x)	Centrioles are absent in higher plants	Centrioles are present
(xi)	Cilia and flagella absent in higher plants	Cilia or flagella often present
(xii)	Starch grains used for storage	Glycogen granules used for storage
(xiii)	Only some cells are capable of division	Almost all cells are capable of division
(xiv)	Few secretions are produced	A wide variety of secretions are produced

Source: DeDuve (1996)

6.12 CHAPTER SUMMARY

- Structurally, plant and animal cells are very similar because they are both eukaryotic cells.
- Only living things have cells. Plant cells can be distinguished from animal cells by three characteristics.
- Plant cells are bounded by a cell membrane and a rigid cell wall whereas animal cells have only a cell membrane to protect their inside from the outside environment.
- Plant cells also have mitochondria and chloroplasts (organelles that are used in energy generation), while animal cells only have mitochondria.
- Plant cells contain vacuoles, storage units that are absent in many animal cells.
- The similarities between plant and animal cells are that they are both eukaryotic cells (cells that contain nucleus-bound organelles).

- They share many kinds of cell parts or organelles, such as the nucleus, mitochondria, endoplasmic reticulum, golgi apparatus, lysosomes, cytosol, etc.
- The two eukaryotic cells have similar reproduction processes of mitosis and meiosis, using their DNA that is housed in the cell nucleus.
- Mitochondrion, which is an organelle common to both plant and animal cells, is unique because it is the power house of the cells, which is responsible for energy production.
- Several plastids are present in plant cells. These include Leucoplasts, Amyloplasts, Proteinoplasts, Oleosomes or Elaioplasts, Chloroplasts, and Chromoplasts.

6.13 STUDENTS' PRACTICAL ACTIVITIES

ACTIVITY 1: Observation of animal cell using light microscope

AIM: Toprepare and observe animal cell (unstained and stained) using light microscope

MATERIALS

- (i) Microscope
- (ii) Disinfectant
- (iii) 2 Microscope slides
- (iv) Labels
- (v) 2 cover slips
- (vi) Wash bottle
- (vii) Disposal jar for slides
- (viii) Filter paper/absorbent paper
- (ix) Disposal jar for inoculating lops/swabs
- (x) 2 Disposable inoculating loops/mouth swabs
- (xi) Methylene blue stain (1%)
- (xii) Timer
- (xiii) Seeker/mounted needle
- (xiv) Disposable gloves

PROCEDURE 1: (*Unstained Animal Cell*)

- (i) Familiarize yourself with all procedures before starting.
- (ii) Set up the microscope
- (iii) Scrape inside cheek surface with a disposable inoculating loop and transfer the sample to the slide. Put the loop into the disposal jar.
- (iv) Cover the sample with one drop of water.
- (v) Apply the cover slip to this preparation as follows:
- (vi) Place the cover slip at the edge of the water at an angle of 45 to the slide.

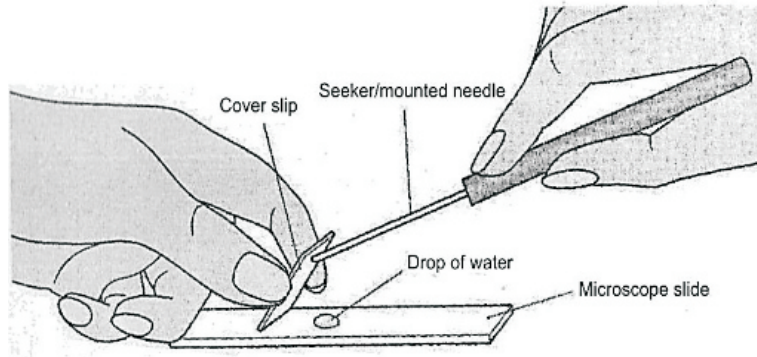


Figure 6.11: Application of cover slip.

Source: Adapted from Laboratory Handbook for Teachers (2015) (https://www.education.ie->Ic_biology_sup).

- (a) Slowly lower the cover slip onto the water, supporting it with the seeker/mounted needle, until it is in place. This helps to avoid trapping air bubbles (Figure 6.11).
- (i) Dry the slide if necessary and label it.
- (ii) Examine under the microscope following the usual procedure.
- (iii) Draw labeled diagrams of what you see at $\times 100$ at $\times 400$.

PROCEDURE 2: (*Stained Animal Cell*)

- (i) Scrape inside cheek surface again using a disposable inoculating loop and transfer sample onto the second slide. Put the loop into the disposal jar.
- (ii) Air dry the slide.
- (iii) Cover sample with one drop of methylene blue solution.
- (iv) Allow to stand for one minute.
- (v) Gently, using a wash bottle, wash excess stain from the slide.
- (vi) Apply a cover slip to this preparation as in Figure 6.11.
- (vii) Dry the slide carefully with filter paper/absorbent paper and label it.
- (viii) Examine under the microscope following the usual procedure.
- (ix) Draw labeled diagrams of what you see and $\times 100$ at $\times 400$.

ACTIVITY 2: Observing Plant Cell using Light Microscope

AIM: To Prepare and observe plant cell (unstained and stained) using light microscope

MATERIALS

- (i) Microscope
- (ii) 2 Microscope slides
- (iii) 2 Cover slips
- (iv) Beaker for used slides
- (v) Iodine stain
- (vi) Petri dish
- (vii) Onion

- (viii) Chopping board
- (ix) Small scissors
- (x) Small paintbrush
- (xi) Sharp knife
- (xii) Labels
- (xiii) Seeker/mounted needle
- (xiv) Filter paper/absorbent paper
- (xv) Dropper
- (xvi) Disposable gloves

PROCEDURE 1: (*Unstained Plant Cell*)

- (i) Familiarize yourself with all procedures before starting.
- (ii) Set up the microscope.
- (iii) Place a drop of water on the slide.
- (iv) Cut the onion in half.
- (v) Separate two fleshy leaves and locate the epidermis between them.
- (vi) Peel off the epidermis and cut it into small pieces.
- (vii) Put these pieces into water in a petri dish.
- (viii) Transfer one piece into the drop of water on the slide, using the small paintbrush.
- (ix) Apply the cover slip.
- (x) Dry the slide if necessary and label it.
- (xi) Examine under the microscope following the usual procedure.
- (xii) Draw labeled diagrams of what you see at $\times 100$ and $\times 400$.

PROCEDURE 2: (*Stained Plant Cell*)

- (I) Prepare another slide as above and stain as follows.
- (ii) Place a drop of iodine solution at one side of the cover slip and draw it across the plant tissue by placing the edge of a piece of filter paper at the opposite side of the cover slip (Figure 6.12).

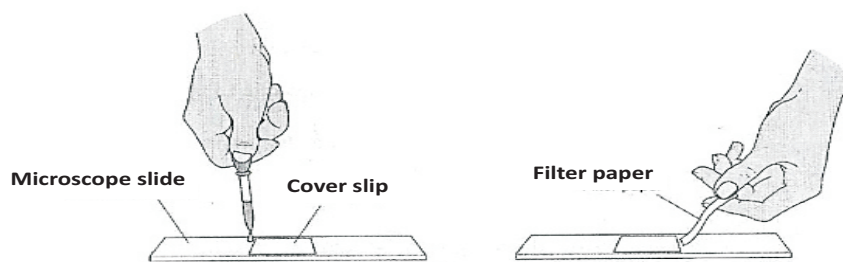


Figure 6.12: Withdrawing stains under cover slip.

Source: Adapted from Laboratory Handbook for Teachers (2015)
(https://www.education.ie->Ic_biology_sup).

- (iii) Dry the slide carefully with filter paper/absorbent paper and label it.
- (iv) Examine under the microscope following the usual procedure.
- (v) Draw labeled diagrams of what you see at $\times 100$ and $\times 400$.

6.14 TUTOR MARKED ASSESSMENT QUESTIONS

HAVING READ THROUGH **CHAPTER SIX**, ANSWER THE FOLLOWING QUESTIONS IN THE SPACES PROVIDED.

1.(a) Explain briefly the meaning of Eukaryotic cell.

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3 × ½ = 1½ Marks

(b). Make a large labeled drawing of a generalized Plant cell to show its essential features.

Drawing 4 × ½ = 2 Marks

Labeling 6 × ½ = 3 Marks

(c) State the **Four** function of each of the following Two Organelles in:

(i)Nucleus

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.....

4 × ½ = 2 Marks

(ii) Mitochondrion

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$4 \times \frac{1}{2} = 2$ Marks

(d) State the Cellular functions of the following Elements.

Sulphur

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Molybdenum

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Manganese

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Zinc

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$4 \times \frac{1}{2} = 2$ Marks

2. (a) What is Cytosol

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$4 \times \frac{1}{2} = 2$ Marks

(b) In a Tabular Form State the Approximate % Composition of the Mammalian Cytosol.

Substance	Preference

6 × $\frac{1}{2}$ = 3 Marks

(c) Write short note on Chloroplast.

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4 × $\frac{1}{2}$ = 2 Marks

(d) What is Lysosome?

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2 × $\frac{1}{2}$ = 1 Marks

3. (a) Highlight the **Four** Types of Lysosome you have Read.

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4 × $\frac{1}{2}$ = 2 Marks

(b) State **Four** functions of Plant Cell Vacuoles?

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4× $\frac{1}{2}$ =2 Marks

(c) List **Three** types of Vacuole?

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4× $\frac{1}{2}$ =2 Marks

(d) State **Four** similarities between Mitochondria and Chloroplast?

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4× $\frac{1}{2}$ =2 Marks

4. (a) In a tabular form, state **Six** major differences between Plant and Animal Cell.

Plant cell	Animal cell

6× $\frac{1}{2}$ =3 Marks

(b) State **Six** similarities between Plant and Animal cell?

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6 × $\frac{1}{2}$ = 3 Marks

(c) State **Four** distinguishing features of Cilia and Flagella?

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4 × $\frac{1}{2}$ = 2 Marks

(d) Enumerate the **Four** types of Plastids found in Plant Cell.

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4 × $\frac{1}{2}$ = 2 Marks

Chapter Seven

ENZYME ACTIVITIES AND REACTIONS IN CELLS

Dr. Godwin E. Udofia & Dr. Dantani D. Odonye

7.1 INTRODUCTION

An enzyme is a substance that acts as a *catalyst* in living organisms; it regulates the rate at which chemical reactions proceed without it being altered in the process. The *biological processes* that occur within all living organisms are chemical reactions, and most are regulated by *enzymes*. Without *enzymes*, many of these reactions would not take place at a perceptible rate. *Enzymes* catalyze all aspects of cell *metabolism*. This includes the digestion of food, in which large nutrient molecules (such as protein, carbohydrates, and fats) are broken down into smaller molecules; the **conservation** and *transformation* of chemical energy; and the construction or synthesis of cellular *macromolecules* from smaller **precursors**. *Enzymes* also have valuable industrial and medical applications. The formatting of wine, the leavening of bread, curdling of cheese, and brewing of beer have been done since the earliest times, but not until the 19th century when these reactions were understood to be the result of the catalytic activity of enzymes. Since then, *enzymes* have assumed increasing importance in industrial processes that involve organic chemical reactions. The uses of *enzymes* in medicine include killing disease-causing microorganisms, promoting wound healing, and diagnosing certain diseases. There are different types of enzymes, each acts only on a particular type of *substrate*. Some, however, do act on groups of *substrates*.

7.2 LEARNING OBJECTIVES

After reading this chapter, you should be able to:

- (i) Define the term enzyme and list the classes of enzymes.
- (ii) List and explain types of plant enzymes.
- (iii) Highlight the properties of enzymes and state the chemical nature of enzymes.
- (iv) Enumerate the functions of enzymes.
- (v) Illustrate the structure of enzyme.
- (vi) State how enzymes are named.
- (vii) Describe the mechanism of enzyme action.
- (viii) Enumerate factors affecting the rate of enzyme activity.
- (ix) Highlight the important groups of proteins.
- (x) Explain the lock and key model which shows how enzymes work.
- (xi) Discuss the following: co-enzymes, co-factors and inhibitors.
- (xii) Define anabolism and catabolism and state their metabolic pathways.

7.3 MEANING OF ENZYMES

Enzymes can be defined as biological *catalysts* that alter the rate of a biochemical reaction in a living organism. An *enzyme* acts as *catalyst* for specific chemical reactions, converting specific set of reactants (called *substrates*) into specific products. **Enzymes** are biological *catalysts* because they are protein molecules made by living cells. **Enzymes** are critically important because in their absence, reactions in the cell would be too slow to sustain life.

The chemical (or chemicals) which enzymes work on is called its *substrate*. An enzyme combines with its *substrate* to form a short-lived enzyme – *substrate* in the complex and greatly increases the chances of a reaction occurring. Once a reaction has occurred, the complex breaks up into products and enzyme. The enzyme remains unchanged at the end of the reaction and is free to interact again with more *substrates*.

Substrate + enzyme = enzyme/substrate complex = enzyme/product complex = enzyme + product(s)

Or $E + S = ES = EP = E + P$

7.4 CLASSIFICATION OF ENZYMES

Enzymes were initially assigned names based on the scientist who discovered them. With further researches, classification became more comprehensive.

Today, according to the International Union of Biochemists (IUB), enzymes are divided into six functional classes and are classified based on the type of reaction in which they are used to catalyze.

The six kinds of enzymes are listed below:

- (a) Oxidoreductases;
The enzyme *oxidoreductase* catalyzes the oxidation reaction where the electrons tend to travel from one form of a molecule to the other.
- (b) Transferases;
The transferases enzymes help in the transportation of the functional group among acceptors and donor molecules.
- (c) Hydrolases;
Hydrolases are *hydrolytic enzymes*, which catalyzes the hydrolysis reaction by adding water to cleave the bond and hydrolyze it.
- (d) Lyases;
It adds water, CO₂ or ammonia across double bonds or eliminates them to create double bonds.
- (e) Isomerases;
The *isomerases* enzymes catalyze the structural shifts present in a molecule, thus causing the change in the shape of the molecule.
- (f) Ligases;
The ligases enzymes are known to charge the catalysis of a *ligation process*.

7.5 TYPES OF ENZYMES

The four enzyme groups are;

- (a) **Proteases:** It breaks long protein chains into smaller amino acid chains and eventually into single amino acids.
- (b) **Amylases:** It reduces polysaccharides to disaccharide – Lactose, maltose and sucrose.

- (c) **Lipases:** It breaks *triglycerides* into individual fatty acids and glycerol.
- (d) **Cellulases:** it digests specific carbohydrate bonds found in fiber.

7.6 PROPERTIES OF ENZYMES

Enzymes are always proteins, and their characteristics therefore reflect the properties of proteins. Their main properties are as follows:

- (i) **They generally work very rapidly:** The speed of actions of an enzyme is expressed as its turnover number. This is the number of substrate molecules which one molecule of the enzymes turns into products per minute. The turn-over number of different enzymes varies from 100 to several millions; for the majority it is about several thousands, the fastest known enzyme is *catalase*, found in tissues which speed up the *decomposition* of hydrogen peroxide into water and oxygen. *Catalase* has a turnover number of 6 million. Its action can be demonstrated by dropping a small piece of a liver into a beaker of hydrogen peroxide: the fizzing that ensues as oxygen is given off is a dramatic demonstration of an enzyme in action. In their speed of action enzymes are much more efficient than inorganic catalysts. The reason is that the enzyme achieves a greater lowering of the activation energy than can be brought about by inorganic catalysts.
- (ii) **Enzymes are not destroyed by the reactions they catalyse:** This is not to say that a given molecule of enzyme can be used indefinitely, but enzymes are very unstable and are readily inactivated by heat, acids, etc. In this respect they differ from inorganic catalysts which are completely stable and can be used over and over again, indefinitely.
- (iii) **An enzyme can work in either direction:** *Metabolic reactions* are reversible and the direction in which they proceed depends on the relative amounts of substrate and products present. The reaction will proceed from left to right until equilibrium between substrate and products is reached.
 $A + B = C$. If for some reasons a large amount of C happens to be the present, the reverse reaction occurs, C being split into A and B until again equilibrium is reestablished. The *enzyme* responsible for accelerating this reaction will catalyze it in either direction depending on conditions. The *enzyme* has no effect on the equilibrium point. It merely speeds up the reaction until equilibrium is reached.
- (iv) **Enzymes are inactivated by excessive heat:** This property of enzymes relates to the fact that they are proteins. Figure 7.1 shows the effect of temperature on the rate of an *enzyme*-controlled reaction. Up to about 40°C the rate increases smoothly, a ten degree rise in temperature being accompanied by an approximate doubling in the rate of the reaction. Above this temperature the rate begins to decline, and about 60°C the reaction ceases altogether. This is because proteins (and in this case *enzymes*) at high temperatures are denatured. For this reason few cells can tolerate temperatures higher than approximately 45°C. Organisms living in situations where the temperature exceeds 45°C either have heat-resistant enzymes or are capable of regulating their body temperature. A striking example of the former is provided by certain blue-green algae that live in hot springs at temperatures of 100°C (see Figure 7.1).

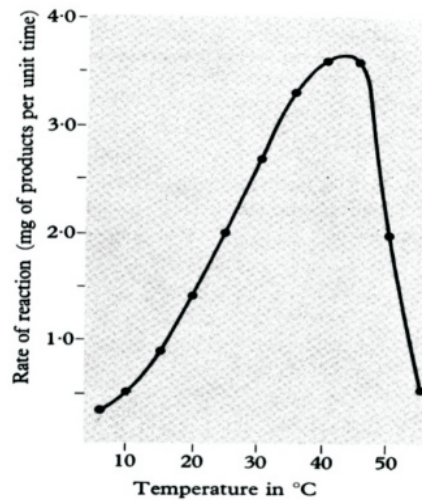


Figure 7.1: The effect of temperature on the rate of enzyme-controlled reactions.

Source: Adapted from Esenowo (2011).

Note: The concentration of enzyme and substrate were kept constant at all the temperatures investigated.

- (v) **Enzymes are sensitive to pH:** Every enzyme has its own range of pH in which it functions most efficiently. Most *intracellular enzymes* function best at or around neutral. Excessive acidity or alkalinity renders them inactive. On the other hand certain digestive enzymes prefer a distinctly acidic or alkaline environment. Thus the protein-splitting enzyme pepsin only functions in an acid medium at a pH of about 2.0 and accordingly is found in the stomach where conditions are markedly acidic. Trypsin, on the other hand, only functions in an *alkaline medium* at about pH 8.5 and is found in the duodenum where conditions are alkaline.
- (vi) **Enzymes are specific:** Normally, a given enzyme will catalyze only one reaction, or a type of reaction. However, the degree of *specificity* varies from one enzyme to another. Most *intracellular enzymes* only work on one particular substrate, but certain digestive enzymes work on a comparatively wide range of related substrates. Thus *catalase* will only split hydrogen peroxide and is ineffective on any other natural substrate, but pancreatic lipase is less specific and will digest a variety of different fats.

7.7 CHEMICAL NATURE OF ENZYMES

All enzymes were once thought to be proteins, but since in the 1980s, the catalytic ability of certain nucleic acids, called *ribozymes* (or catalytic RNAs) has been demonstrated, refuting this *axiom*. Because so little is yet known about the enzymatic functioning of RNA, this discussion will focus primarily on protein enzymes.

A large protein enzyme molecule is composed of one or more amino acid chains called *polypeptide chains*. The amino acid sequence determines the characteristic folding patterns of the protein's structure, which is essential to *enzyme specificity*. If the enzyme is subjected to changes, such as fluctuations in temperature or pH, the protein structure may lose its integrity (denature) and its *enzymatic ability*. *Denaturation* is sometimes, but not always, reversible.

Bound to some enzymes is an additional chemical component called a **Cofactor**, which is a direct participant in the catalytic event and thus is required for enzymatic activity. A **Cofactor** may be either a Coenzyme (an organic molecule, such as a vitamin) or an inorganic metal ion, some enzymes require both. A **Cofactor** may be either tightly or loosely bound to the enzyme. If tightly connected, the **Cofactor** is referred to as a **prosthetic group**.

7.8 FUNCTIONS OF ENZYMES

- (i) Enzymes help in signal **transduction**. The most commonly used enzyme in this process includes protein kinase that catalyzes the **phosphorylation** of protein.
- (ii) They break down large molecules into smaller and absorbable forms for easy absorption.
- (iii) They help in generating energy. ATP synthase is the enzymes involved in the production of energy.
- (iv) Enzymes are responsible for the movement of ions across the plasma membrane.
- (v) Enzymes perform a number of biochemical reactions, including oxidation, reduction, hydrolysis etc, to eliminate the non-nutritive substances from the body.
- (vi) They function to reorganize the internal structure of the cell to regulate cellular activities.

7.9 ENZYME STRUCTURE

Enzymes are linear chain of amino acids, which give rise to a three-dimensional structure. The sequence of amino acids specifies the structure, which in turn identifies the catalytic activity of the **enzyme** (see Figure 7.2). Upon heating, **enzyme's** structure denatures, resulting in a loss of **enzyme** activity, which typically is associated with temperature.

Compared to its substrates, enzymes are typically large with varying sizes, ranging from 62 amino acid residues to an average of 2500 residues found in fatty acid synthase. Only a small section of the structure is involved in catalysis and is situated next to the binding sites. The catalytic site and binding site together constitute the enzyme's active site. A small number of **ribozymes** exist which serve as an RNA-based biological catalyst. It reacts in complex with proteins.

The model below (Figure 7.2) shows an enzyme called **Ribonuclease S**, which breaks up RNA molecules. It is a typical enzyme, being a globular protein and composed of up to several hundred atoms. The darkly shaded areas are called active sites and make up the cleft-where the substrate molecule(s) are drawn to. The correct positioning of these sites is critical for the catalytic reaction to occur. The substrate (RNA in this case) is drawn into the cleft by the active sites. By doing so, it puts the **substrate molecule** under stress, causing the reaction to proceed more readily.

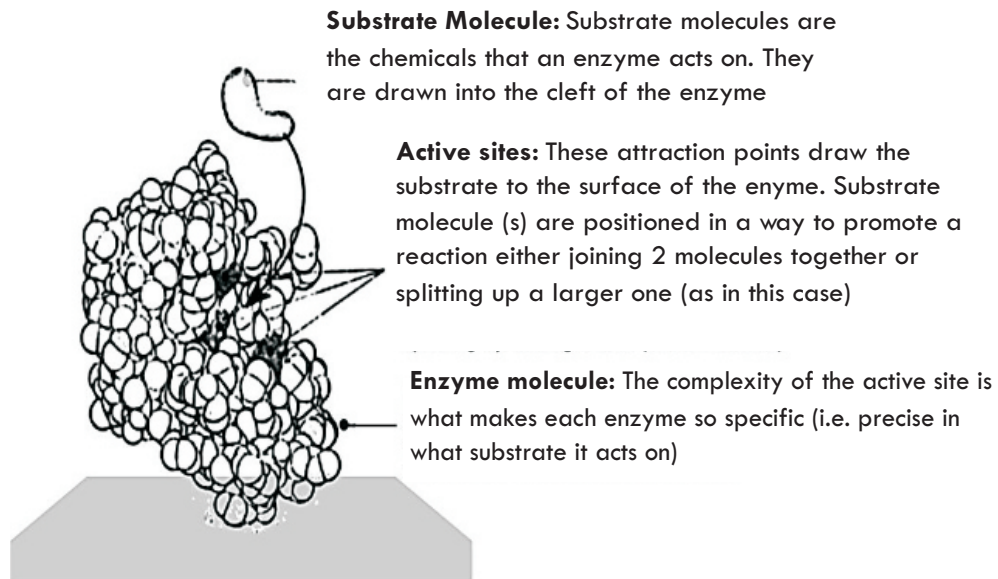


Figure 7.2: Structure of Enzyme.
Source: Lubert Stryer (1981)

7.10 NOMENCLATURE OF ENZYMES

An enzyme will interact with only one type of substance or group of substances, called the substrate to catalyze a certain kind of reaction. Because of this specificity, enzymes are often named by adding the suffix “ase” to the substrate’s name (as in urease, which catalyzes the breakdown of urea). Not all enzymes have been named in this manner. However, to ease the confusion surrounding enzyme nomenclature, a classification system has been developed based on the type of reaction the enzyme catalyzes. There are six (6) principal categories and their reactions.

- (i) **Oxidoreductases**, which are involved in electron transfer.
- (ii) **Transferases**, which transfer a chemical group from one substance to another.
- (iii) **Hydrolases**, which cleave the substrate by uptake of a water molecule (hydrolysis)
- (iv) **Lyases**, which form double bonds by adding or removing a chemical group.
- (v) **Isomerases**, which transfer a group within a molecule to form an isomer.
- (vi) **Ligases, or synthetases**, which couple the formation of various chemical bonds to the breakdown of a pyrophosphate bond in adenosine triphosphate or a similar nucleotide.

7.11 MECHANISM OF ENZYME ACTION

In most chemical reactions, an energy barrier exists that must be overcome for the reaction to occur. This barrier prevents complex molecules such as proteins and nucleic acids from *spontaneously degrading*, and so is necessary for the preservation of life. When metabolic changes are required in a cell, however, certain of these complex molecules must be broken down, and this energy barrier must be surmounted. Heat could provide the additional needed energy called activation energy, but the rise in temperature would kill the cell. The alternative is to lower the activation energy level through the use of a catalyst.

This is the role that enzymes play. They react with the substrate to form an intermediate complex – a “transition state” – that requires less energy for the reaction to proceed. The unstable intermediate compound quickly breaks down to form reaction products, and the unchanged enzyme is free to react with other substrate molecules.

Only a certain region of the enzyme, called the active site, binds to the substrate. The active site is a groove or pocket formed by the folding pattern of the protein. This three-dimensional structure, together with the chemical and electrical properties of the amino acids and Cofactors within the active site, permits only a particular substrate to bind the site, thus determining the *enzyme's specificity*.

Enzyme synthesis and activity also are influenced by genetic control and distribution in a cell. Some enzymes are not produced by certain cells, and others are formed only when required. Enzymes are not always found uniformly within a cell; often they are compartmentalized in the nucleus, on the cell membrane, or in subcellular structures. The rates of enzyme synthesis and activity are further influenced by hormones, *neurosecretions*, and other chemicals that affect the cell's internal environment.

Some experiments conducted, together with other lines of evidence, suggest that in an enzyme-controlled reaction, the substrate molecules combine with the enzyme to form an enzyme-substrate complex (see Figure 7.3). With their various bonds held in relation to each other by the enzyme, the substrate molecules react together to form an *enzyme-product complex*. This splits into the enzyme and product. The enzyme, unchanged by the reaction, can then be used again.

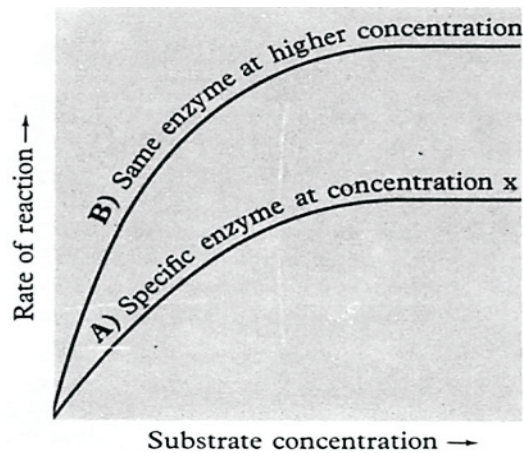
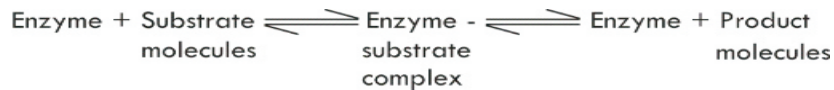


Figure 7.3 : The effect of substrate concentration on the rate of an enzyme-controlled reaction.

Source: Adapted from Esenowo (2013).

Note: Assuming an optimum temperature, when a plateau is reached the only way of increasing the rate of the reaction is to increase the amount of enzyme present.

7.12 FACTORS AFFECTING THE RATE OF ENZYME ACTIVITY

- (i) **Temperature:** Heating increases molecular motion, thus the molecules of the substrate and enzyme move more quickly and of their bumping into rate each other are increased. As a result, there is a greater probability of reaction occurring. The temperature that promotes maximum activity is referred to as the optimum temperature. If the temperature is increased above this level, a decrease in the rate of the reaction occurs despite the increasing frequency of collisions. This is because the secondary and tertiary structures of the enzyme have been disrupted, and the enzyme is said to be denatured. In effect, the enzyme unfolds and the precise structure of the active site is gradually lost (see Figure 7.4). The bonds which are most sensitive to temperature change are hydrogen bonds and *hydrophobic interactions*. For example, the enzymes of bacteria living in hot springs may have an optimum temperature of 70°C or higher. Such enzymes have been used in biological washing powders for high temperature washes. If temperature is reduced to near or below freezing point, enzymes are inactivated, not denatured. They will regain their catalytic influence when higher temperatures are restored. Today, techniques of quick-freezing food are widely used as a means of preserving food for long periods. This does not only prevents growth and multiplication of microorganisms, but also deactivates their digestive enzymes, thus, making it impossible for them to decompose food. The natural enzymes in the food itself are also inactivated. However, once frozen, it is necessary to keep the food at subzero temperatures until it is ready for consumption.

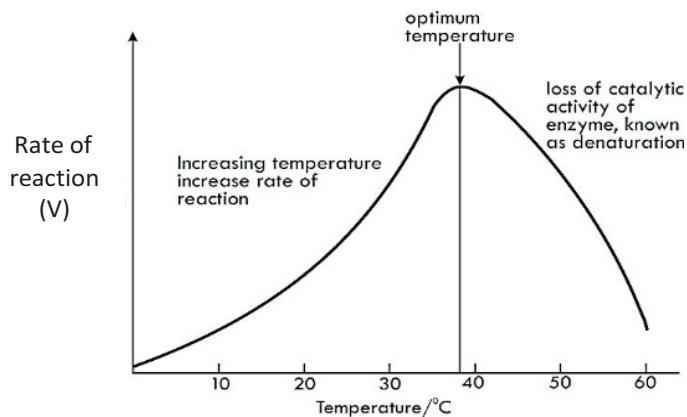


Figure 7.4: The effect of Temperature on the Rate of reaction on Enzyme activity.

Source: (Slesnick et al, 1985).

- (ii) **pH:** Under constant temperature, every enzyme functions most efficiently over a particular pH range (see Figure 7.5). Often this is a narrow range. The optimum pH is that at which the maximum rate of reaction occurs. When the pH is altered above or below this value, the rate of enzyme activity diminishes. As pH decreases, acidity increases and the concentration of H^+ ions increases. This increases the number of positive charges in the medium. Changes in pH alter the ionic charge of the acidic and basic groups and therefore disrupt the ionic bonding that helps to maintain the specific shape of the enzyme. Thus the pH change leads to an alteration of enzyme shape, including its active site. If extremes of pH are encountered by an enzyme, then it will be denatured.

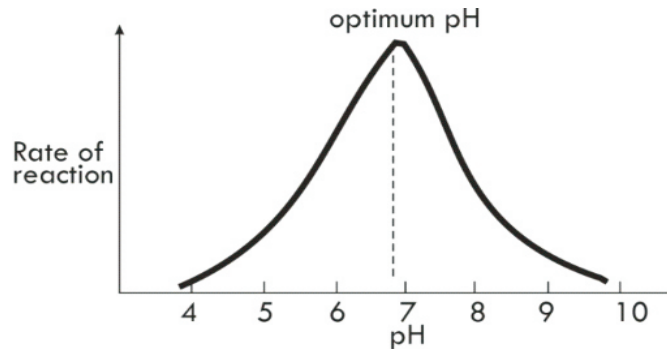


Figure 7.5: The effect of pH on the Rate of reaction on Enzyme activity.

Source: (Slesnick *et al.*,1985).

- (iii) **Substrate Concentration:** The rate of an enzyme reaction increases with increasing substrate concentration for a given enzyme concentration (see Figure 7.6). The theoretical maximum rate (V_{\max}) is never obtained, but there is a point which any further increases in substrate concentration produces no significant change in reaction rate. This is because at high substrate concentrations the active sites of the *enzyme molecules* at any given moment are virtually saturated with substrate. Thus any extra substrate has to wait until the enzyme/substrate complex has released the products before it may itself enter the active site of the enzyme.

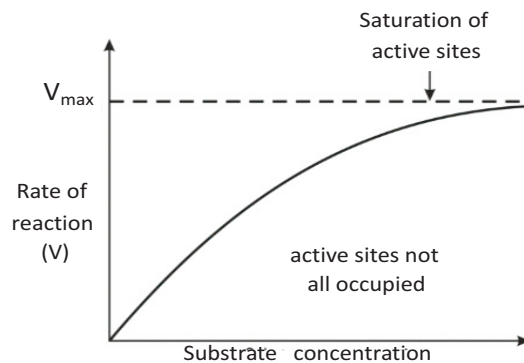


Figure 7.6: The effect of Substrate concentration on the Rate of reaction on Enzyme activity.

Source: (Slesnick *et al.*,1985).

- (iv) **Enzyme Concentration:** The rate of reaction is proportional to the enzyme concentration, provided that the substrate concentration is maintained at a high level, and other conditions such as pH and temperature are kept constant. Normally, reactions are catalyzed by enzyme concentrations. Thus as the enzyme concentration is increased, so will be the rate of the enzyme reaction (see Figure 7.7).

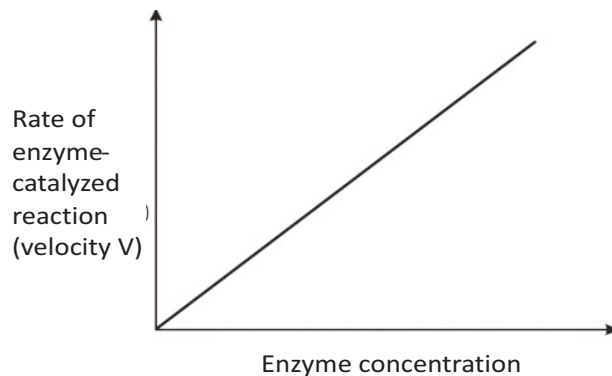


Figure 7.7: The effect of Enzyme concentration on the Rate of enzyme catalyzed reaction on Enzyme activity.
Source: (Slesnick et al ,1985).

7.13 ENZYMES: AS AN IMPORTANT GROUP OF PROTEINS

Many chemical reactions take place inside a cell. The speeds at which cell reactions take place are controlled by catalysts called *enzymes*.

Enzymes are proteins. There are thousands of *enzymes* in living things. We say that *enzymes* are specific; this means that each *enzyme* catalyses a certain chemical reaction or a type of chemical reaction. The substance which the enzyme helps to react is called substrate. The enzyme amylase increase the speed at which starch reacts with water to form sugars (see Figure 7.8).

The enzyme has a group of atoms called the active site. Part of the substrate molecule fits into the active sites. This fit has been described as “a lock and key”



The starch molecules attacked by a water molecule. Weakened by its bonds to the enzyme, the starch molecule is hydrolysed (‘split by water’) to form two molecules of sugar

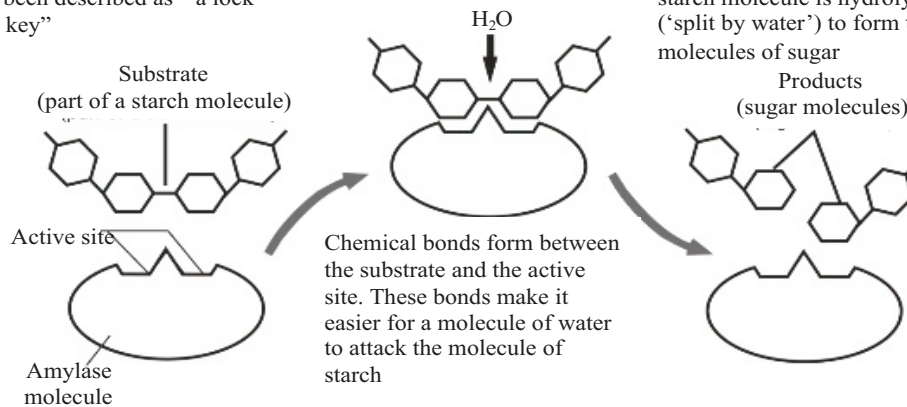


Figure 7.8: How the enzyme anylase catalyses the breakdown of starch
Source: David (1997)

Note: that the enzyme is unchanged at the end of reaction and able to catalyse the breakdown for more starch

7.14 THE LOCK AND KEY MODEL SHOWING HOW ENZYMES WORK

The lock and key model proposed earlier by the German Scientist Emil Fisher (1894) suggested that the substrate was simply drawn into a closely matching cleft on the enzyme molecule. More recent studies have revealed that the process more likely involves an induced fit. See Figure 7.9, where the enzyme and/or the reactants change their shape slightly. The reactants become bound to enzymes by weak chemical bonds. This binding can weaken bonds within the reactants themselves, allowing the reaction to proceed more readily.

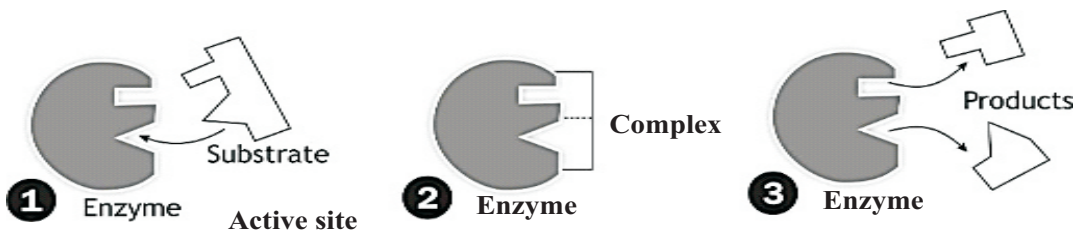


Figure 7.9: How enzymes work.
Source: Biozone International (2000).

The presence of an enzyme simply makes it easier for a reaction to take place. All catalysts speed up reactions by influencing the stability of bonds in the reactants. They may also provide an alternative reaction pathway, thus lowering the activation energy needed for a reaction to take place (see Figure 7.10). Just as one specific key can open only a specific lock. In the same manner the specific enzyme can transform only specific substrate into products. The enzyme must have the correct geometric shape to fit the substrate.

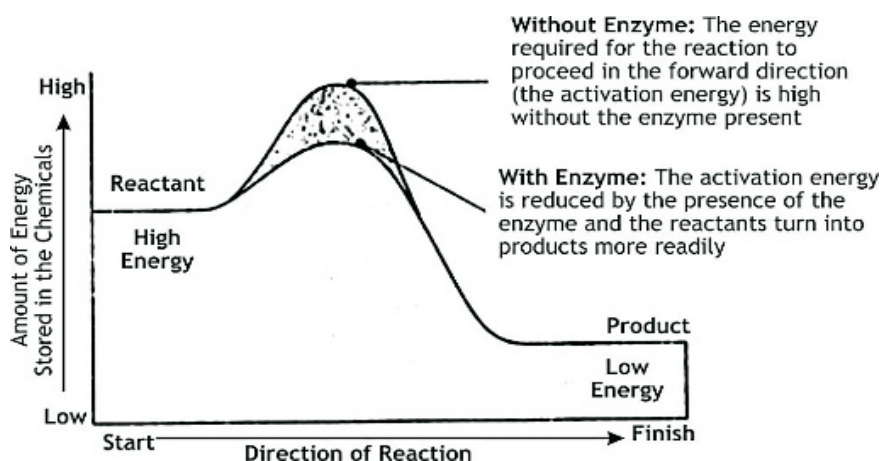


Figure 7.10: Graphical representation of how enzymes work.
Source: Biozone International (2000).



The activation energy 86kj/mole is required without catalyst whereas only 1kj/mole energy is needed in the presence of enzyme.

Note:

Induced fit model of enzyme was first proposed by Koshland in 1954 to explain the protein conformational changes in the binding process. The model suggests that an enzyme, when binding with its surface, optimizes the interface through physical interactions to form the final complex structure. As shown in Figure 7.11 the **lock and key model** states that the active site of an enzyme precisely fits a specific substrate. The induced fit model states that the active site of an enzyme will undergo a conformational change when binding a substrate, to improve the fit.

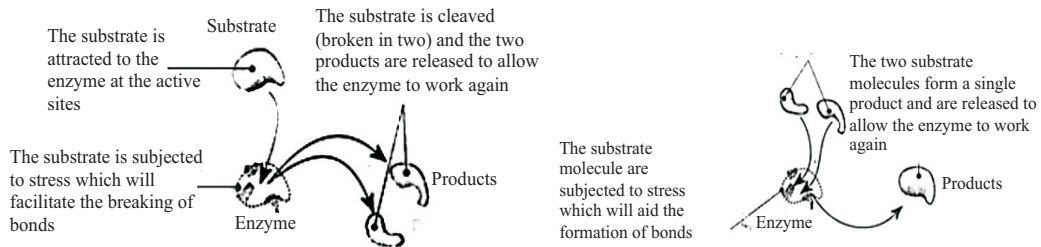
Note:

Metabolism

The sum of all biochemical reactions occurring within a living cell is called metabolism. These are sets of biochemical reactions that occur in living cells in order for the organism to maintain life. There are two major types of metabolism: catabolism and anabolisms.

Catabolic and Anabolic reactions

Anabolic reactions use energy to build complex molecules from simpler **organic compounds** (e.g., proteins from amino acids, carbohydrates from sugars, fats from fatty acids and glycerol); **catabolic reactions** break down **complex molecules** into simpler ones, releasing chemical energy. The key difference between anabolic and catabolic enzymes is that anabolic enzymes catalyze the biochemical reactions that synthesize larger complex molecules from smaller units while catabolic enzymes catalyze the biochemical reactions that break down larger complex molecules into smaller units in Figure 7.12.



Catabolic Reactions

Some enzymes can cause a single substrate molecule to be drawn into the active site. Chemical bonds are broken, causing the substrate molecule to break apart to become two separate molecules. Examples: digestion, cellular respiration

Some enzyme can cause substrate molecules to be drawn into the active site. Chemical bonds are formed, causing the substrate molecules to form bonds to become a single molecule. Examples: protein synthesis, photosynthesis

Figure 7.12: Catabolic and Anabolic Reactions in the cell.

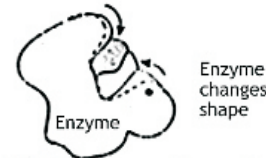
Source:Biozone International (2000).

Induced Fit Model

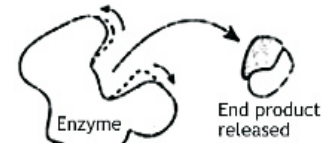
An enzyme and its substrate act like a lock and key. The shape of the enzyme changes when the substrate fits into the cleft:



1 Two substrate molecules are drawn into the cleft of the enzyme



2 The enzyme changes shape, forcing the 2 substrate molecules to combine



3 The resulting end product is released by the enzyme which returns to its normal shape, ready to receive more.

Figure 7.11: Induced Fit Model.

Source: Lubert Stryer (1981).

The 2 substrate molecule are attracted to the enzyme at the active sites

7.15 CO-FACTOR

Most enzymes can only work in the presence of appropriate cofactor. This is a non proteinous organic substance or metallic ion that associates with enzyme for it to function effectively as a catalyst. An enzyme without a cofactor is called apoenzyme, while enzyme and its cofactor together constitute *holoenzyme*. Few Cofactors can be made inside the body of organisms while others must be consumed in food. Cofactors generally serve to supply chemicals groups or properties that are not available in other chemical groups.

Categories of cofactors

(i) Metallic ion Cofactor

Metal ions such as iron, copper, selenium etc. may bind enzyme and substrate together or it may serve as the catalytic center of the enzyme itself. Iron (Fe^{2+}), for example, is a cofactor responsible for the catalytic action of catalase. Metal ions are required at the active sites to form coordinate bonds. These constitute the minerals and are inorganic substances that occur in nature.

(ii) Coenzyme

When the Cofactor is a complex non-protein organic molecule, that binds an enzyme only during catalysis, but remains detached at all times, it is called *Coenzyme*, for example, NAD^+ . The later function as a carrier for transferring chemical groups or atoms from one enzyme to another. The NAD (Nicotinamide adenine dinucleotide) molecules working in conjunction with dehydrogenase enzyme catalyse the transfer of hydrogen atoms from a substrate to NAD.

(iii) Prosthetic group

These are non-proteins, organic or inorganic molecules that are tightly bound to an enzyme for proper functioning of the enzyme. Flavin adenine dinucleotide (FAD) is an example of a *prosthetic group*.

(iv) ATP Cofactor

Adenosine triphosphate ATP is a cofactor with the special ability for property of transferring energy that drives chemical processes including enzymes activities and protein transport.

Type of Cofactors

- i. Vitamins: (Retinol [Vit. A], Thiamine [Vit. B₁], Riboflavin [Vit. B₂], Folic Acid [Vit. B₁₁], Ascorbic acid [Vit. C], Calciferol [Vit. D], Tocopherol [Vit. E] and Phylloquinone [Vit. K]).
- ii. Minerals: (Calcium, Chlorine, Magnesium, Iron, Phosphorus, Zinc, Selenium).
- iii. Organic Non-vitamin

7.16 Inhibitors

Certain substances inhibit or interfere with enzymes, thereby slowing down or stopping enzyme-controlled reactions. These *inhibitors* have excited the interest of biologists partly because of their medical and agricultural applications, but also because they go some way towards confirming the *lock-and-key hypothesis* (see Figure 7.13).

One of the steps in the series of reactions which release energy in cells involves the oxidation of *succinic acid*. The enzyme catalyzing this particular reaction is succinic *dehydrogenase*. Now malonic acid has a molecular configuration similar to that of *succinic acid* and when added to the system reduces the rate of the reaction. What seems to happen is that the malonic acid molecule is so similar to the *succinic acid* that it fits into the active site of the enzyme. It therefore competes with the normal substrate for the active site. While it is attached to the enzyme molecule, it prevents the normal substrate from doing so.

This is known as competitive inhibition. The degree of inhibition depends on the relative concentrations of substrate and *inhibitors*. If the *inhibitor* is sufficiently concentrated a high proportion of the enzyme molecules will combine with it in the reaction with *succinic acid* will reduce. A quite different type of *inhibition* is that in which the *inhibitor* attaches itself permanently to the active site of the enzyme thereby excluding any possibility of the normal substrate taking up its rightful place. In this case the extent of the *inhibition* depends entirely on the concentration of *inhibitor* and cannot be varied by changing the amount of substrate present. As substrate and *inhibitor* are not competing with each other for the active site, this kind of *inhibition* is known as non-competitive (see Figure 7.14).

Arsenic and other metals such as mercury and silver, owe their toxic effects to the fact that they are enzyme inhibitors, in this case, the non-competitive type. Other well-known poisons such as the sulfonamide drugs are competitive enzyme *inhibitors*. The sulfonamide drugs are used against *pathogenic bacteria* and exert their effect by releasing compounds that combine with the bacteria's enzymes. Other enzyme inhibitors include the phosphate insecticides and the nerve gases developed during the Second World War. Both acts in the same way: They attack the enzyme *cholinesterase* which plays a vital role in the transmission of nerve impulses. Cyanide is another potent inhibitor. It combines with the *cytochrome* enzymes responsible for the transfer of hydrogen atoms during cellular respiration and thus blocks the production of energy in cells.

The terms competitive and non-competitive, though useful in understanding the general nature of enzyme *inhibition*, can be somewhat misleading. Nowadays biochemists prefer to use the terms reversible and irreversible for these two kinds of *inhibition*. Nor is *inhibition* confined to substances which combine with the active site. Some inhibitors combined with other parts of the enzymes molecule, altering its shape in such a way that the normal substrate no longer fits the active site. In general all that is required for a substance to qualify as an enzyme-inhibitor is that it should be able to prevent the substrate molecules from combining with the enzyme. In other words the binding of *inhibitor* and substrate with the enzyme must be mutually exclusive.

The fact that poisons are often enzyme inhibitors should not give the impression that enzyme-inhibition is invariably unnatural or disastrous. To a great extent moment-to-moment *variations* in the rate of cellular metabolism are caused by the control of enzyme action by *inhibitor* substances occurring naturally in the body. It has been shown that in some *metabolic pathways* the end-product itself acts as an *inhibitor*. This happens when the end-product is in excess. For instance, when there is a surplus of a particular compound (or the raw materials required for its synthesis) in the diet. In these circumstances the end-product combines with one of the enzymes responsible for its own production so that the formation of further end-product is temporarily slowed down or stopped. This is an example of negative feedback and is important in the normal control of biochemical reactions.

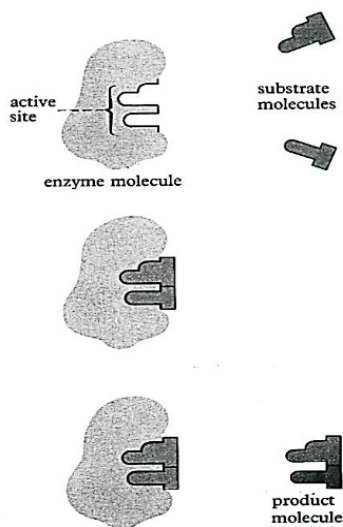


Figure 7.13: Hypothesis explaining enzyme action.
Source: Biozone International (2000).

Note: The substrate molecules fit into the active site where they react together to form the product. The product then leaves the enzyme molecule.

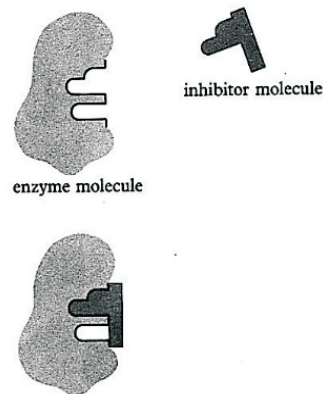


Figure 7.14: In non-competitive inhibition of an enzyme, an inhibitor molecule, similar in structure to one of the normal substrate molecules, fits into the active site and stays there, thereby excluding the normal substrate molecule from the rightful place.

Source: Biozone International (2000).

7.17 CHAPTER SUMMARY

- Enzymes form a specialized class of proteins responsible for catalyzing chemical reactions within the cell and thus are ideal drug targets.
- They are characterized by remarkable efficiency and specificity.
- Enzymes are named by adding the suffix-ase to the name of the substrate that they modify (i.e., urease and tyrosinase).
- They are classified into categories according to the type of reaction catalyzed: oxidoreductase, transferases, hydrolases, lyases, ligases, and isomerases.
- Structurally, the vast majority of enzymes are proteins.
- Also, RNA molecules have catalytic activity (ribozymes).
- Enzyme activity is determined by measuring the amount of product formed or substrate consumed in a reaction at a given time.
- Enzymatic reactions have the advantage of being specific.
- Coenzymes are used as a carrier for transporting chemical groups or atoms from one enzyme to another.
- Cofactors are non-proteinous substances that are associated with enzymes for proper function of the cell.
- The inhibitors interfere with enzymes, thereby stopping enzyme-controlled reactions.
- Metabolism is the sum total of anabolism and catabolism.
- Metabolism takes place only in the living cells and is one of the characteristics of life.
- Commonly, a number of enzymes are used in sequence to convert one substance into another or several products via a series of intermediate compounds.

- Enzymes are important for catalyzing all types of biological reactions, those that require energy as well as those that release energy.
- The chain of reactions is referred to as a metabolic pathway.
- Many such pathways are going on at the same time in the cell.
- The reactions proceed in a controlled manner due to the specific nature of enzymes.
- A single enzyme generally will catalyze only a single reaction.
- Thus enzymes serve to control the chemical reactions that occur within cells and ensure that they proceed at an efficient rate.
- Note that anabolic pathways are those that require energy to synthesize larger molecules.
- While, catabolic pathways are those that generate energy by breaking down larger molecules.
- Both types of pathways are required for maintaining the cell's energy balance.
- However, anabolic pathways assemble large molecules from smaller ones.
- Whereas, catabolic pathways break large molecules into small pieces.

7.18 STUDENTS' PRACTICAL ACTIVITIES

ACTIVITY 1: Determination of enzyme concentration during hydrolysis of sucrose by sucrase (invertase)

AIM: To determine the effect of enzyme concentration on the hydrolysis of sucrose by sucrase (invertase)

MATERIALS

- 2% sucrose solution
- 1%, 0.75%, 0.5% sucrose (invertase) solutions
- Benedict's reagent
- test-tubes and rack
- Water baths at 38°C and 100°C
- Glass rods
- Stop clock
- Distilled water
- Labels
- Bunsen burner

PROCEDURE

- Add 2 cm³ of clear blue Benedict's reagent to 2 cm³ of clear colourless 1% sucrose solution. Heat the mixture in the water bath maintained at 100°C for 5 min (Benedict's test).
- Repeat (i) using 2 cm³ of clear colourless 2% sucrose solution and then 2cm³ of distilled water.
- Boil 5 cm³ 1% sucrose solution.
- Take 8 clean, dry test-tubes, label 1-8, and add 1 cm³ Benedict's reagent to each.
- Add 5 cm³ of 2% sucrose solution to a test-tube labeled S and place in the water bath maintained at 38°C throughout the experiment.
- Add 5 cm³ of 1% sucrose solution to a test-tube labeled E and place in the water bath at 38°C
- Leave both test-tubes and contents in the water bath for 5 min to allow them to equilibrate with their surroundings

- (viii) Add the enzyme solution to the sucrose solution, invert the test-tube to thoroughly mix the two solutions.
- (ix) Immediately start the stop clock and replace the tube containing the reaction mixture in the water bath.
- (x) Throughout the experiment agitate the mixture continuously to ensure thorough mixing.
- (xi) After 30s of incubation remove 1 cm³ of mixture and place in test-tube i.
- (xii) Repeat this procedure every 30s placing the samples in tubes 2-8 in turn.
- (xiii) Heat tubes 1-8 in the water bath at 100°C for 5 min. Note the time when the first positive reducing sugar test is obtained indicated by a brick-red precipitate.
- (xiv) Repeat the experiment using the boiled enzyme from (iii).
- (xv) Repeat the entire sequence/experiment twice using the 0.75% and 0.5% sucrose solution.
- (xvi) Record your observation and comment on your results.

ACTIVITY 2: Investigate the effect of pH on the rate of catalase activity

AIM: To investigate the effect of pH on the rate of catalase activity

MATERIALS

Enzyme source e.g. radishes	Thermometer
Hydrogen peroxide (20% or less)	Knife
Range of buffer solutions – acidic, neutral and alkaline	Chopping board
pH paper	Electronic balance
Washing-up liquid	Weigh boats
Large beaker of water at 25°C	Disposal gloves
Graduated cylinders (100 cm ³)	Labels
Syringe	Timer
Boiling tubes	Dropper

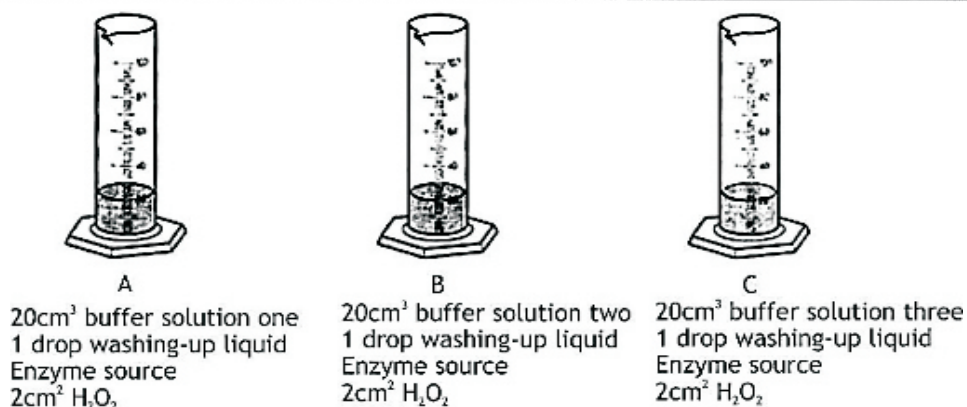


Figure 7.15 Investigate the effect of pH on the rate of catalase activity

Source: Adapted from Laboratory Handbook for Teachers (2015)

(<https://www.education.ie> > Ic_biology_sup).

PROCEDURE

- (i) Familiarize yourself with all procedures before starting as in Figure 7.15.
- (ii) Add 20cm³ of one of selected buffers to a graduated cylinder.
- (iii) Using the dropper, add one drop of washing-up liquid.
- (iv) Add 5g of finely chopped radish to the cylinder.
- (v) Add 2cm³ of hydrogen peroxide to a boiling tube.
- (vi) Stand the cylinder and the boiling tube in the beaker of water at 25⁰C.
- (vii) Pour the hydrogen peroxide into the cylinder.
- (viii) Note the volume in the cylinder immediately and record.
- (ix) Read the volume again after a measured amount of time e.g. 2 minutes, and record.
- (x) Subtract the initial volume from the final volume to get the volume of foam and record.
- (xi) Repeat the procedure from step 3 for each of the other buffer solutions.
- (xii) A graph should be drawn of enzyme activity (volume of foam) against pH. Put pH on the horizontal axis.

Result

pH of buffer	Initial volume (cm ³)	Final volume (cm ³)	Volume of foam produced (cm ³)

Conclusion/Comment**ACTIVITY 3: Investigate the effect of Heat (Denaturation) on catalase activity**

AIM: To investigate the effect of heat (denaturation) on catalase activity

MATERIALS

Enzyme source e.g. radishes	Knife
Hydrogen peroxide (20% or less)	Chopping board
Buffer solution (pH 9)	Electronic balance
Boiling tubes	Weigh boats
Washing-up liquid	Dropper
Syringe	Thermometer
2 water baths (25 ⁰ C, 100 ⁰ C)	Disposal gloves
Graduated cylinders (100 cm ³)	Timer
Test-tube holder	Test-tube rack

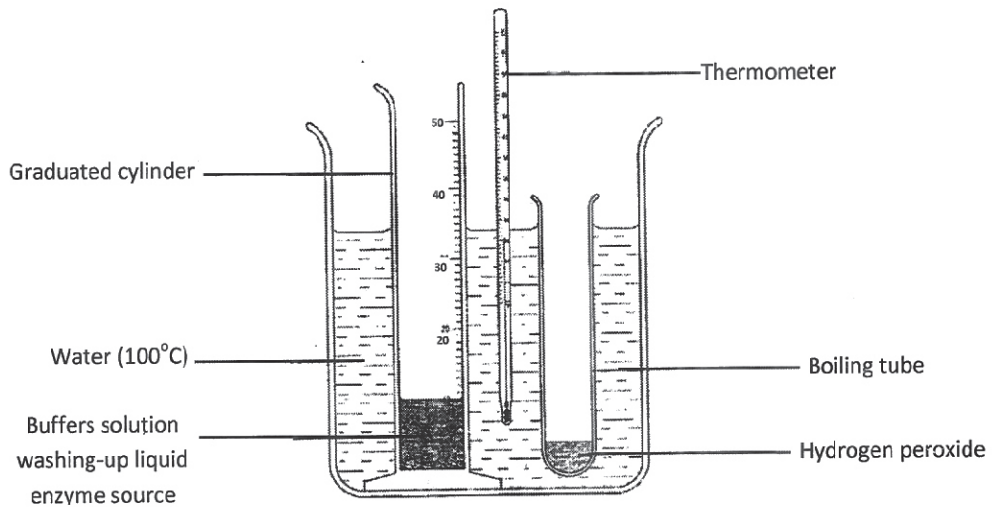


Figure 7.16: Denaturing catalase Enzyme.

Source: Adapted from Laboratory Handbook for Teachers (2015)

(https://www.education.ie->lc_biology_sup).

PROCEDURE

- (i) Familiarize yourself with all procedures before starting as in Figure 7.16.
- (ii) Place 5g of finely chopped radish into a boiling tube (without water) and place the boiling tube into the water bath at 100°C for ten minutes.
- (iii) Remove the boiling tube and allow it to cool.
- (iv) Add 20 cm³ of the buffer to the graduated cylinder.
- (v) Using the dropper, add one drop of washing-up liquid.
- (vi) Add 5g of the heated radish to the cylinder.
- (vii) Add 2cm³ of hydrogen peroxide to the boiling tube.
- (viii) Stand the cylinder and the boiling tube in the water bath until the desired temperature (25°C) is reached.
- (ix) Pour the hydrogen peroxide into the cylinder.
- (x) Note the presence or absence of foam formation and record.
- (xi) Repeat the procedure from step 5 using an unheated radish sample.

Result

Foam formation	Unheated enzyme	Heated enzyme

Conclusion/Comment

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ACTIVITY 4: Catalase Test

AIM: To demonstrate the action of catalase enzymes on hydrogen peroxide to produce water and oxygen

MATERIALS

- (i) Glass slide 3% hydrogen peroxide,
- (ii) Pasteurs pipette 18 to 20 hr old bacterial culture.

PROCEDURE

- (I). Make two drops of hydrogen peroxide on a clean grease free glass A and B
- (ii). Using a clean glass rod transfer the test organism to drop A; Use Drop B as control.
- (iii).Observe immediately for gas bubbling or effervescence which indicates the breaking down of Hydrogen peroxide to water and oxygen catalase enzyme.

7.19 TUTOR MARKED ASSESSMENT QUESTIONS

HAVING READ THROUGH **CHAPTER SEVEN**, ANSWER THE FOLLOWING QUESTIONS IN THE SPACES PROVIDED.

1.(a) Explain briefly the meaning of Enzyme.
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4×½ =2 Marks

(b) State **Six** classification of Enzyme?
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6×½ =3 Marks

(c) Highlight **Four** types of Enzymes you have studied.
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4×½ =2 Marks

(d) List the major **Six** properties of Enzymes.

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6 × $\frac{1}{2}$ = 3 Marks

(e) State briefly the **Six** functions of Enzymes.

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6 × $\frac{1}{2}$ = 3 Marks

2.(a) Describe briefly the structure of Enzyme.

Drawing 4 × $\frac{1}{2}$ = 2 Marks
Labeling 2 × $\frac{1}{2}$ = 1 Marks

(b) State the **Six** Principal Categories/Classification System Based on Enzymes Reactions.

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(ii)

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(iii)

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(v)

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(vi)

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6 × $\frac{1}{2}$ 3 Marks

(c) Represent graphically the effect of temperature on the rate of reactions of Enzyme Activity.

Drawing $3 \times \frac{1}{2} = 1\frac{1}{2}$ Marks
Labeling $4 \times \frac{1}{2} = 2$ Marks

(d) List the **Six** factors affecting the rate of Enzyme Activity.

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$6 \times \frac{1}{2} = 3$ Marks

3.(a) Enzymes as important group of Proteins: explain

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$4 \times \frac{1}{2} = 2$ Marks

(b) Explain the lock and Key Model showing how Enzymes work.

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$4 \times \frac{1}{2} = 2$ Marks

(c) Define the term Co-factor.

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$2 \times \frac{1}{2} = 1$ Marks

4. (a) Explain the term Co-enzyme

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$3 \times \frac{1}{2} = 1\frac{1}{2}$ Marks

(b) Explain the term Metabolic Pathways in relations to Enzymes when Catalizing Reactions.

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$4 \times \frac{1}{2} = 2$ Marks

Chapter Eight

THE NUCLEIC ACID, PROTEINS AND STORAGE OF GENETIC INFORMATIONS IN CELLS

Dr. Anthony A. Adegoke & Dr. Nsima J. Ekong

8.1 INTRODUCTION

Every multicellular organism begins life as a single cell. As the organism develops, the single cell divides and forms new cells. Then, those new cells divide and form more cells. The process continues, eventually forming the entire organism. Some cells will be muscle cells, some will be nerve cells; still others will be bone or blood cells. Each cell will have many jobs to do. The instructions that dictate the functions of each cell are contained within the DNA molecules inside the cell. When James Watson and Francis Crick **elucidated** the structure of DNA, there was great excitement in the scientific world and this won for them the Nobel prizes. Why is DNA so important? What is so special about the structure? **Nucleic acids**, like proteins, are essential for life. They form the genetic material of all living organisms, including the simplest viruses. The term "**nucleic acid**" comes from the fact that it is found mainly in the nucleus. Stains for **nucleic acids** show up nuclei very clearly under the light microscope. The discovery of the structure of DNA (deoxyribonucleic acids), one of the two types of **nucleic acids**, represents one of the outstanding milestones in biology because it finally solved the problem of how living organisms store information needed to control their activities and pass this information on to subsequent generations. Proteins are compounds which contain carbon, hydrogen, oxygen, nitrogen and sometimes sulphur. Proteins are very important because: they are the materials from which new tissues are made. If organisms are to grow and if they are to repair damaged tissues, they need proteins. Basically protein can be classified into simple and **conjugated proteins**. The complete structure of a protein can be described at four different levels of complexity namely Primary, Secondary, Tertiary and Quaternary Structure.

8.2 LEARNING OBJECTIVES

After reading this chapter, you should be able to:

- (i) Explain nucleic acid.
- (ii) Highlight types of nucleic acid.
- (iii) Explain the meaning of biosynthesis of nucleotide.
- (iv) Discuss information storage in cells.
- (v) Highlight the importance of DNA.
- (vi) Draw the structure of DNA.
- (vii) States component of a DNA molecule.
- (viii) Explain how DNA replicates.
- (ix) Describe the structure of RNA.
- (x) Discuss the synthesis of messenger RNA; and RNA codons.
- (xi) Explain the meaning of Protein, Polypeptides and amino acids.
- (xii) Classify Protein into Primary, Secondary, Tertiary and Quaternary structure.
- (xiii) Distinguish between simple protein and conjugated protein.
- (xiv) Describe the messenger RNA in protein synthesis.
- (xv) Distinguish between DNA and RNA.
- (xvi) State the similarities between DNA and RNA.
- (xvii) Define the term mutations.

8.3 MEANING OF NUCLEIC ACID

Nucleic acids are naturally occurring chemical compounds that are capable of being broken down to yield phosphoric acids, sugars and a mixture of organic bases (**purines** and **pyrimidines**). They are the main information-carrying molecules of the cell, and by directing the process of protein synthesis, thereby determining the inherited characteristics of every living thing.

The two main classes of **nucleic acids** are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is the master blueprint for life and constitutes the genetic material in all free-living organisms and most viruses. RNA is the genetic material of certain viruses, but it is also found in all living cells and involved in certain processes such as the making of proteins.

Nucleic acids consist of **nucleotide**, a phosphate, a pentose sugar (ribose or deoxyribose) and a nitrogen base containing **purine** or **pyrimidine**. Usually, phosphate and sugars, alternate as links in the chain while the nitrogen base, projects inward from sugar links. A unit is composed of one molecule of each sugar, phosphate and nitrogen base. A large number of **nucleotides** combine to form a molecule of **nucleic acids**. **Nucleic acids** are universally present in the nucleus and in the cytoplasm of all living cells and are now clearly shown to form the chemical basis of life.

8.4 TYPES OF NUCLEIC ACIDS: RNA and DNA

Nucleic acids are of two types; ribonucleic acid (RNA) and deoxyribose nucleic acid (DNA). RNA occurs in nuclei and cytoplasm. It transfers the information contained in DNA to places in the cell where proteins are made, DNA occurs in cell nuclei. The **genes** that living things inherit from their parents are lengths of DNA. The information that a cell needs to assemble molecules of amino acids in the correct order to make protein molecules is present in its DNA.

RNA and DNA have large complex molecules made up from smaller molecules of compounds called **nucleotides** (Figure 8.1).

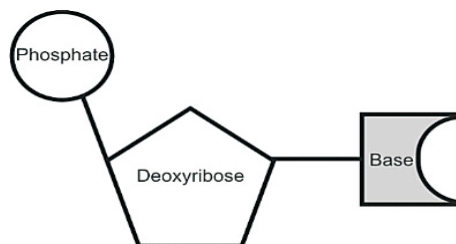
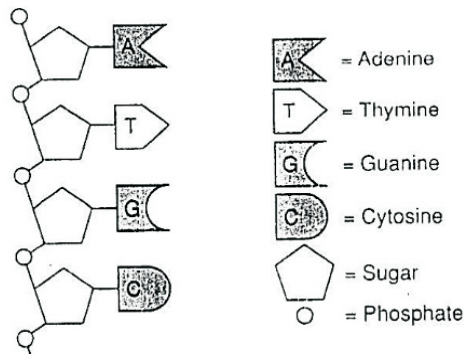


Figure 8.1: A nucleotide in DNA.

Source: David (1997).

Note: The five -carbon sugar deoxyribose occurs in DNA. The base is one or four: adenine, cytosine, guanine or thymine. (In RNA the sugar ribose occurs, and the base uracil is present instead of thymine). There are two sorts of nucleic acid: ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Each sort of nucleic acid is made up of (synthesised) from nucleotides.

The **phosphate group** of one nucleotide molecules combines with the sugar group of another. A strand of alternate sugar groups and phosphate groups is formed. The bases attached to the sugar groups stick out from the strand (see Figure 8.2).



Erwin Chargaff, a biochemist, discovered that the amounts of adenine (A) and thymine (T) in DNA are equal and the amounts of guanine (G) and cytosine (C) are equal. This led to the idea of the one-to-one pairings of adenine-thymine and cytosine-guanine. Chargaff's rule, as it came to be known, helped Francis Crick and James Watson to construct their model of DNA.

Figure 8.2: Part of a strand of DNA.
Source: David (1997).

Deoxyribonucleic acid is a polymer composed of two polynucleotide chains that coil around each other to form a **double helix** carrying genetic instruction for the development, functioning, growth and reproduction of all known organisms and many viruses. There are two nucleic acids (DNA and RNA) associated primarily with cell nuclei. DNA and RNA molecules consist of chains of nucleotides. Four kinds of nucleotides, each with a unique nitrogenous base, occur in DNA. Helical coils of DNA contain coded information determining the nature and proportions of substances in cells and the ultimate form and structure of the organism. RNA has a different sugar and nucleotide. Deoxyribonucleic acid is the information molecule which stores instructions for making other large molecules called protein. These instructions are stored inside each of the cells of a human being, distributed among 46 long structures called chromosomes.

These chromosomes are made up of thousands of shorter segments of DNA, called **genes**. A DNA molecule consists of two such strands bonded together. The bases sticking out from one sugar-phosphate of one strand bond to bases sticking out from one sugar-phosphate from another strand. In the Figure 8.3 you will notice that C bonds to G and A bonds to T. This is always the case in DNA. Remember, in RNA, the base uracil is present instead of thymine).

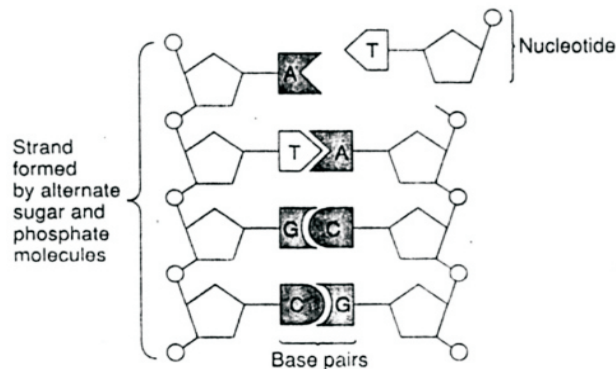


Figure 8.3: Part of a molecule of DNA.
Source : David (1997).

Note: Notice how two sugar-phosphate strands are joined along their lengths as their bases combine. Remember that the bases in a strand of DNA are adenine (A), thymine (T), guanine (G) and cytosine (C). The combination of two strands of DNA to make a double helix molecule depends on A combining with T (or T with A) and G combining with C (or C with G). The combinations are called "base pairs".

As two strands of sugar-phosphate groups combine to form a molecule of DNA, the result resembles a ladder: the sugar-phosphate strands form the sides and base pairs form the rungs. The ladder is in fact twisted into a spiral shape. The shape is called a double helix—two intertwined spiral strands. A molecule of DNA consists of two strands that form a **double helix** structure as seen in figure 8.4. Whereas, each strand has a back bone made of alternating sugar (deoxyribose) and phosphate groups. Attached to each sugar is one of four bases—adenine (A), cytosine (C), guanine (G), and thymine (T). The sequences of nitrogenous bases on the two strands of a DNA molecule are complementary. In addition, the specific sequences of nitrogenous bases that code for particular proteins or regulatory RNA molecules are called **genes**. Nucleic acids are **polynucleotides**, that is, long chain molecules composed of series of nearly identical building blocks called nucleotides (see Figure 8.5). Each nucleotide consists of a nitrogen-containing aromatic base attached to a pentose (five-carbon) sugar, which is in turn attached to a phosphate group. Each nucleic acid contains four or five possible nitrogen-containing bases: **Adenine** (A), **Guanine** (G), **Cytosine** (C), **Thymine** (T), and **Uracil** (U). A and G are categorized as **purines**, and C, T, and U are collectively called **pyrimidines**.

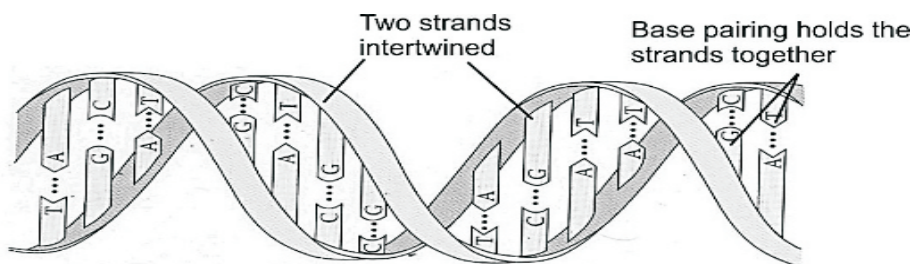


Figure 8.4: The double helix: two connected spiral strands.
Source: David (1997).

The double helix The DNA molecule is usually double-stranded, with the sugar phosphate backbone of the polynucleotides (shown here by blue ribbons) on the outside of the helix. In the interior are pairs of nitrogenous bases, holding the two strands together by hydrogen bonds. Hydrogen bonding between the bases is specific. As illustrated here with symbolic shapes for the bases, adenine (A) can pair only with thymine (T), and guanine (G) can pair only with cytosine (C).

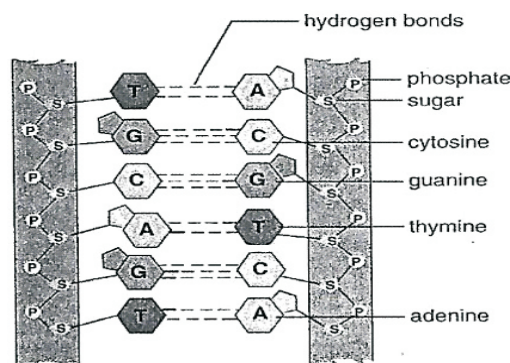


Figure 8.5: The pairing of nucleotides in a tiny portion of a strand of DNA. The variations in sequences of pairs are virtually unlimited.
Source: Stern (2000).

8.5 CHEMICAL STRUCTURE OF DNA

DNA is a polynucleotide, a *macromolecule* (macro = large) made of units called *nucleotides*. Each nucleotide consists of three subunits.

- (i) A pentose (5 carbon) sugar called *deoxyribose*.
- (ii) 4 nitrogenous bases *Adenine* (A), *Guanine* (G) (purine bases) and *Thymine* (T) and *Cytosine* (C) (*pyrimidine bases*).
- (iii) A phosphate group (PO_4) positioned on the sugar (see Figure 8.6).

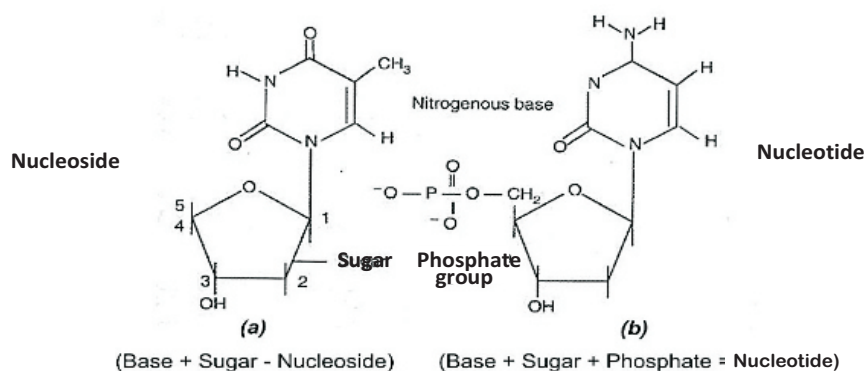


Figure 8.6: Chemical Components of nucleoside and nucleotide.

Source: NIOS (2017).

A base and a sugar combine to form a nucleoside, while it becomes a nucleotide when a phosphate group gets attached.

Base + sugar = nucleoside
 Base + sugar + Phosphate = nucleotide

So there are four *nucleotides* in DNA formed of sugar and nitrogenous base and phosphate.

The four *nucleotides* are not present in equal amounts in a DNA molecule. But the amount of purines (A + G) and that of *pyrimidines* (T + C) is always equal. In other words, A = T and G = C. This is called Chargaff's rule.

8.6 BIOSYNTHESIS OF NUCLEOTIDES

Nucleotides are synthesized from readily available precursors in the cell. The ribose phosphate portion of both purine and pyrimidine *nucleotides* is synthesized from glucose via the pentose phosphate pathway (see Figure 8.7). The six-atom pyrimidine ring is synthesized first and subsequently attached to the *ribose phosphate*. The two rings in purines are synthesized while attached to the ribose phosphate during the assembly of adenine or guanine *nucleosides*. In both cases the end product is a nucleotide carrying a phosphate attached to the 5 carbon on the sugar. Finally a specialized enzyme called a kinase adds more phosphate groups using adenosine triphosphate (ATP) as the phosphate donor to form ribonucleoside triphosphate, the immediate precursor of RNA. For DNA, the 2 hydroxyl group is removed from the *ribonucleosidediphosphate* to give *deoxyribonucleoside diphosphate*. An additional phosphate group from ATP is then added by another kinase to form a *deoxyribonucleosidetriphosphate*, the immediate precursor of DNA.

During normal cell *metabolism*, RNA is constantly being made and broken down. The purine and pyrimidine residues are reused by several salvage pathways to make more genetic material. Purine is salvaged in the form of the corresponding nucleotide, whereas pyrimidine is salvaged as the *nucleoside*.

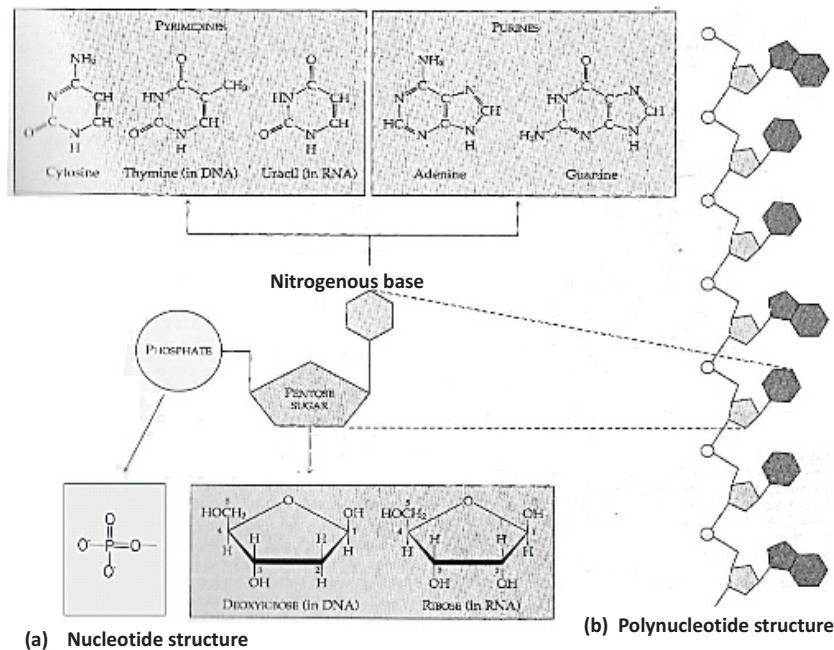


Figure 8.7: The structures of nucleotides and polynucleotides.

Source: Campbell (1993).

(a) Nucleotides, the monomers of nucleic acids, are themselves composed of three smaller molecular building blocks: a nitrogenous base, either a purine or a pyrimidine; a pentose sugar; and a phosphate group. **(b)** In polynucleotides, each nucleotide monomer has its phosphate group bonded to the sugar of the next nucleotide. The polymer has a regular sugar-phosphate backbone with variable appendages, the four kinds of nitrogenous bases.

8.7 INFORMATION STORAGE IN CELLS

For a long time scientists knew that every cell stores information, but the way cells store this information was one of the greatest puzzles in the history of biology. The solution to the puzzle came from combined research in chemistry, physics, and biology. This research showed how the arrangement of atoms in DNA acts as a code for the cells. This code contains the instructions a cell needs to perform all its function.

DNA is important because it holds all of the genetic information that makes an organism. This information is needed for development and survival and is able to be passed along to the next generation. It also influences traits, ranging from appearance to the types of food utilized.

Given how different organisms look, it might seem like organism' DNA should be very different from one another. Amazingly this is not the case. On average, humans for example share around 99.5% of their DNA with someone they are not related to.

A big part of what makes one unique is found in that 0.5% of DNA. And even though we're overall more alike than different, everyone's DNA tells a different story about whom their relatives are and where they are from.

