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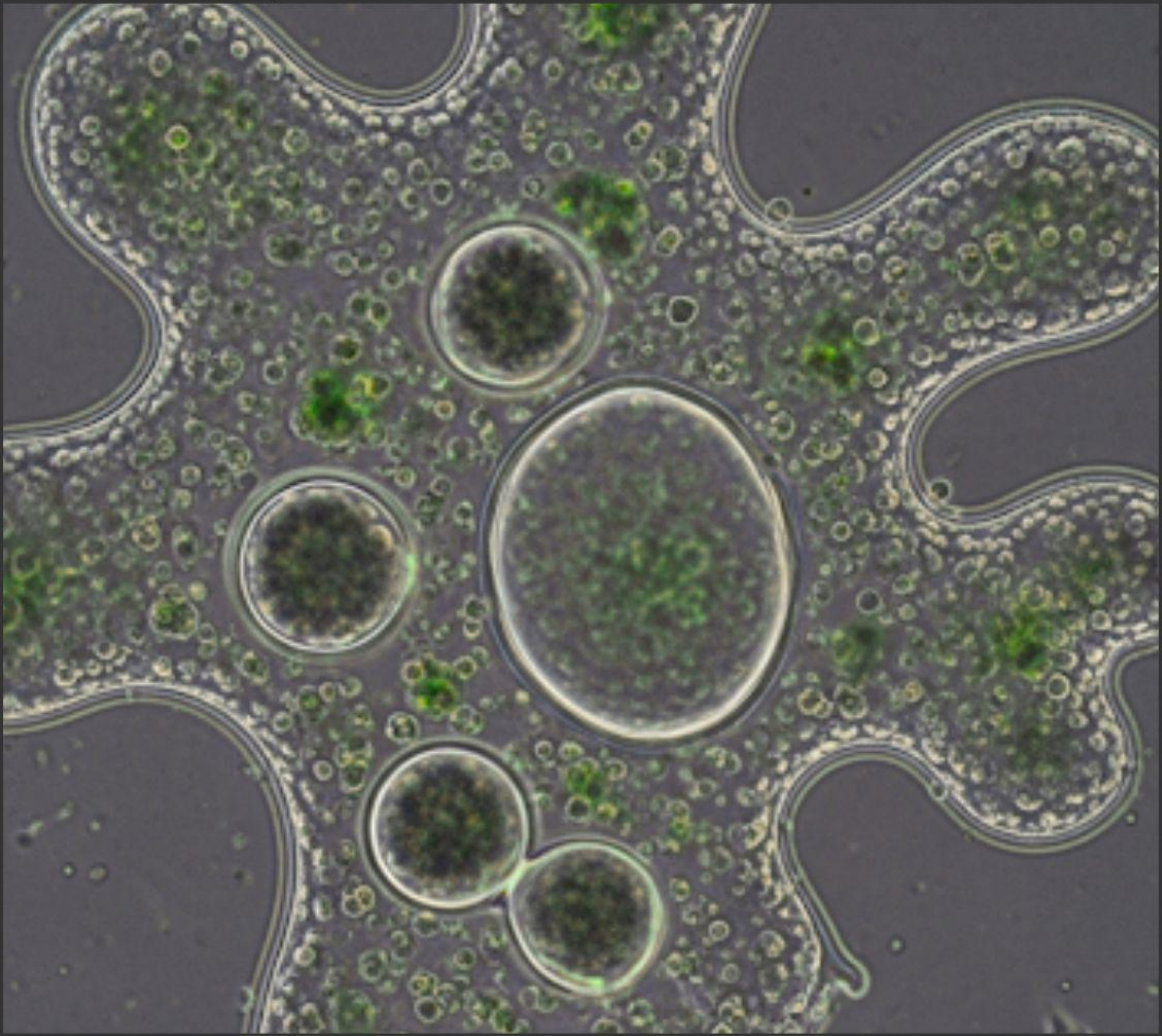
Chapter 1

INVERTEBRATES

CHAPTER SERIES

Amoeba proteus



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Amoeba proteus

(Proteus Animalcule)

CLASSIFICATION
(SYSTEMATIC POSITION)

Kingdom: Protista
Phylum: Protozoa
Superclass: Sarcodina
Class: Rhizopoda
Sub-Class: Lobosia
Order: Amoebodia
Genus: *Amoeba*
Species: *proteus*

INTRODUCTION

You must have heard of microorganisms in standing waters. Amoeba is one of the protozoans found in stagnant waters with organic matter and can be observed under a light microscope. Russel von Rosenhof discovered *Amoeba* in 1755. Because *Amoeba* can change its shape, it was named "Little Proteus" after the mythical Greek sea-god Proteus who had the power to change its shape. There are several species of *Amoeba.* A. *proteus* is the common and best-known species. Other common species are A. *radiosa*, A. *discoides*, and A. *verrucosa*. In this chapter you will go through the type study of A. *proteus*. *Amoeba* is a free-living, solitary animalcule. It is found in fresh-water ponds, pools, ditches and streams having plenty of decaying organic matter. It is generally found in the bottom mud or on the underside of aquatic vegetation in shallow waters. It is rarely found in free water as it requires substratum to glide on from place to place. It moves and feeds with the help of pseudopodia (False feet). It is omnivorous in nutrition. It responds to stimuli and reproduces by asexual methods only. *Amoeba* has a great power of regeneration. It forms cysts during unfavourable conditions. Amoebas are considered to be immortal, as no individual dies whenever it divides into two daughter amoebas.

CULTURE OF AMOEBA

Cultures of *Amoeba* can be easily prepared in the laboratory by "hay-infusion method". Some hay, dry leaves, grass, twigs, seeds or banana peels are boiled in distilled water for about 15 minutes. To prepare culture medium, tap water should not be used because it is usually chlorinated. After boiling, the water is filtered and allowed to stand open for 2-3 days. This allows abundant bacterial growth. Now, some half-rotten leaves are taken from a pond, washed and put into the filtrate. Within a week, the filtrate is full of large numbers of amoebae. A culture can also be maintained in the laboratory by keeping amoebae in small covered petri dishes containing a few boiled wheat grains. A drop should be taken from the bottom of the container onto the slide, which will show several amoebae when observed under the microscope.

MORPHOLOGY
(EXTERNAL FEATURES)

A. Size and shape

Amoeba proteus is a unicellular microscopic animalcule which measures about 250 to 600 um (0.25 to 0.6 mm) in diameter. An amoeba may be just visible to the naked eye, as a minute white speck. It is slightly greyish in colour. Under the microscope, it appears like an irregular 'Jelly-like' tiny mass of protoplasm, which changes its shape by pushing out and withdrawing a finger-like process, called a pseudopodium (Plural: pseudopodia). When it withdraws all its pseudopodia, it becomes spherical in shape.

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B. *Plasmalemma (Plasma membrane)*

Plasmalemma is a semipermeable, thin and elastic outer membrane of *Amoeba*. It possesses a good power of regeneration and quickly repairs itself, if injured. As revealed by electron microscope, it is the usual "trilaminar" unit membrane of fluid mosaic architecture, consisting of a lipid bilayer and many peripheral proteins. A "glycocalyx" of adhesive glycolipids and glycoproteins occurs at the outer surface of the plasmalemma. Also a fringe of extremely fine, finger-like outgrowths, called microvilli, projects at the outer surface. The plasmalemma prevents the animal sticking to the substratum, keeps the protoplasm intact and regulates the exchange of materials between the protoplasm and the surrounding water. Plasmalemma is dynamic in structure due to which Amoeba can change its shape.

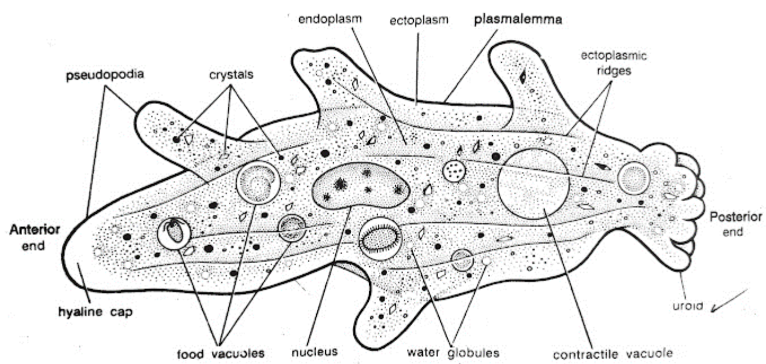

Figure 1. Ultrastructure of *Amoeba proteus*

C. *Pseudopodia (False feet)*

Pseudopodia or "false feet" (Gr. pseudo=false; podium=foot) are irregular projections from the body surface (Figure 2). These can be of variable sizes. Mostly they are broad and finger-like with blunt ends. Blunt pseudopodia are also known as lobopodia. These are formed as a result of liquefaction and flowing forward of the cytoplasm. In an actively moving *Amoeba*, new pseudopodia can be seen forming towards the direction of movement, while the older ones are in the process of withdrawal at the trailing hind end (Uroid). The older pseudopodia usually appear as wrinkles on the hind end of the body. The pseudopodia move by "pressure flow" mechanism and assist the animal in locomotion and food ingestion. Various theories have been put forward by different researchers for amoeboid movements.

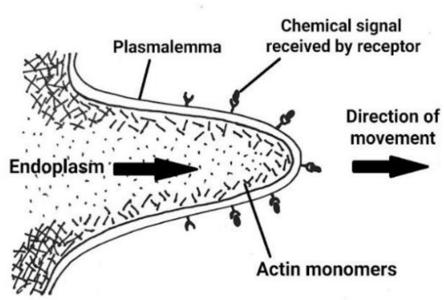

Figure 2. A single pseudopodium

D. *Cytoplasm*

Cytoplasm is the fluid substance present inside the plasmalemma. Cytoplasm is differentiated into two distinct regions: the outer ectoplasm and the inner endoplasm.

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(i) **Ectoplasm**. Ectoplasm is the outer layer of the cytoplasm. It is a thin, clear and transparent layer lying just within the plasmalemma. It is somewhat rigid, contractile and under tension (gel state). Ectoplasm is clearly visible at the tip of a pseudopodium, where it forms a distinct "hyaline cap". Hyaline cap is responsible for maintaining the shape of the pseudopodia. As pseudopodia are formed due to endoplasmic pressure, the hyaline cap prevents bursting of pseudopodia from the anterior end.

(ii) **Endoplasm**. Endoplasm is the inner layer of the cytoplasm and forms the bulk of the body of Amoeba. It is fluid like granular and semi-transparent. Endoplasm keeps on moving regularly. Streaming movement of endoplasm is termed as "cyclosis" which helps in the distribution of materials inside the body of Amoeba. Previously, it was stated that the endoplasm was differentiated into two parts: an outer "Gel" (Plasmagel) layer and an inner "Sol" (Plasmasol) mass (Mast, 1926). But, modern studies have proved that ectoplasm is the 'Gel' state and endoplasm is the 'Sol' state of colloid cytoplasm. Whenever a pseudopodium is formed, plasmagel forms an outgrowth into which plasmasol flows and exerts a forward pressure. This results in the formation of a pseudopodium. The two colloidal states, gel and sol, are interchangeable also. Sol can convert into gel (Gelation) and gel can convert into sol (Solation).

E. *Cytoplasmic inclusions*

Organelles in *Amoeba* include a nucleus, contractile vacuole, food vacuoles and water vacuoles, endoplasmic reticulum with ribosomes, mitochondria, Golgi apparatus, lysosomes, minute crystals etc.

(i) Nucleus. Nucleus is the controlling centre of any cell. *Amoeba* has a single large flattened, circular, disc-like or concavo-convex nucleus. Generally it is situated in the central part of endoplasm, the plasmasol. It may be difficult to see the nucleus in living *Amoeba* but can be seen under microscope after staining. As Amoeba is a eukaryote, its nucleus (DNA) is bounded by a thin double nuclear envelope, intercepted by pores. Nucleoplasm is highly granular and refractive to light. The nucleoplasm contains several nucleoli and a large number of scattered chromatin granules (Chromidia). The nucleus controls the vital activities of the body of Amoeba by making different proteins through transcription and translation processes.

(ii) Food vacuoles. Food vacuoles are the membraned spaces enclosing food particles or droplets. Several spherical bodies, varied in size, containing water and food in various phases of digestion, are seen in plasmasol moving inside the body along with cyclosis (streaming movements of cytoplasm). These food vacuoles are temporary structures and are formed when *Amoeba* engulfs food along with water. They vary in shape and size according to the size of ingested food. Digestion takes place within these food vacuoles with the help of lysosomal enzymes. They disappear with the egestion of residual material from the body. Thus they are not permanent structures.

(iii) Contractile vacuole. Contractile vacuole is generally seen in endoplasm at the posterior end of the body under light microscope. It appears like a clear bubble filled with watery fluid, enclosed by a unit membrane. Surrounding this membrane is a region containing many tiny feeder or accessory vacuoles. Contractile vacuoles are meant for osmoregulation. As the name suggests, it is a pulsatory organelle, rhythmically dilating (Diastole) and contracting (Systole) after short intervals as per the need. It is surrounded with an envelope of mitochondria which provide sufficient energy for active water movement from the endoplasm into the vacuole in diastolic phase.

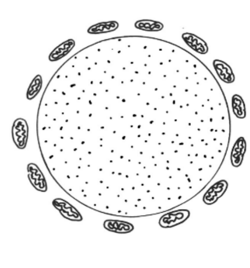

Figure 3. A single contractile vacuole with mitochondrial envelope.

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(iv) Water vacuoles. These are several small and spherical, non-contractile vacuoles appearing in the endoplasm as droplets of colourless fluid. Their significance is still unknown. They may be functioning to pour their contents into the contractile vacuoles as a part of osmoregulation.

(v) Other organelles. A number of eukaryotic organelles are revealed in *Amoeba* by the electron microscope. These include endoplasmic reticulum with ribosomes, mitochondria, Golgi apparatus, lysosomes; numerous minute, regularly shaped crystals, called biuret (carbamylurea) and triuret (carbonyldiurea) are also found in the endoplasm. These organelles are responsible for all respective functions as they do in a normal eukaryotic cell.

PHYSIOLOGY OF *AMOEBA*

A. LOCOMOTION (Amoeboid movement)

Amoeboid movements refer to the movements of an organism lacking any particular shape like *Amoeba*. *Amoeba* moves from one place to another by means of temporary finger-like processes of the body, which are termed "Pseudopodia" (False feet). They are referred to as 'false' because they are temporary and disappear after sometime. The characteristic irregular movements of *Amoeba* are termed as amoeboid movements. Its pseudopodia, which are broad and have rounded tips, are called "Lobopodia" (Blunt pseudopodia). Pseudopodia are formed as a result of flowing endoplasm in the forward direction (Figure 2). Sometimes, several pseudopodia are formed at the same time but usually one survives which is in the right direction and becomes larger, while others disappear. The speed of movement in *Amoeba* is about 0.02 to 0.03 mm per minute. The speed is attained by the pressure exerted by the plasmasol onto the plasmagel in a definite direction.

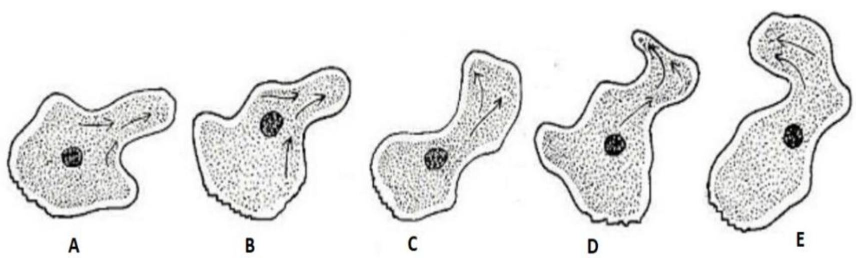

Figure 4. Movement in *Amoeba proteus*

THEORIES OF AMOEBOID MOVEMENTS

Amoeba moves by making pseudopodia. Several theories have been put forth to explain the mechanism of the formation of a pseudopodium. The important theories have been described below:

1. Adhesion theory. According to the theory of adhesion, a pseudopodium is formed by a sort of a pulling out of the cytoplasm at a place where the force of adhesion increases. This theory is purely an imaginative speculation. This theory lacks distinctive and firm evidence.

2. Contraction-hydraulic theory. This theory was put forth by Schultze in 1875. He was of the view that plasmagel (ectoplasm) undergoes contraction at the posterior end and causes protoplasmic current to flow forward, pushing the more fluid-like plasmasol (endoplasm) forward. This results in the formation of pseudopodium and propelling the body forward.

3. Surface-tension theory. Amoeboid movements were initially explained through this theory by Berthold (1886), supported by Rhumbler and Butschi (1898). According to this theory, protoplasm is a fluid that exerts a tension of the protoplasmic mass at its surface in all directions, making the mass spherical. When the surface tension changes due to external or internal factors, an outflow occurs that forms a pseudopodium. In a pseudopodium, fluid flows from the centre and moves backwards along the sides. The theory assumes that the body contents are liquid, while the outer surface remains rigid and gelatinised. This type of movement is also known as "Fountaining streaming movement."

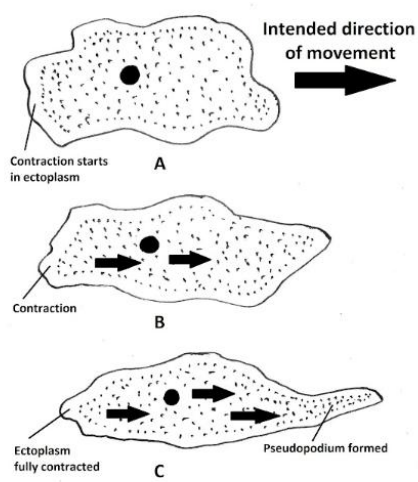

Figure 5. Contraction-hydraulic theory of pseudopodia formation

4. Rolling movement theory. This theory was put forward by Jennings in 1904. He observed it in A. *verrucosa*. The basic principle behind this theory was that Amoeba is a ball-like organism and it rolls on the surface. To demonstrate this fact, he marked the surface of an *Amoeba* by administering a particle of lamp black, which adhered firmly over there. He noticed that as the Amoeba moved, the lamp black particle also rolled over along with the amoeba. First the particle reached the top and then in the anterior position. Then the particle went below the Amoeba. After some time the particle again attained the original position where it was administered. Such a movement is possible only in A. *verrucosa*, which is devoid of pseudopodia but not in A. *proteus* which moves with pseudopodia.

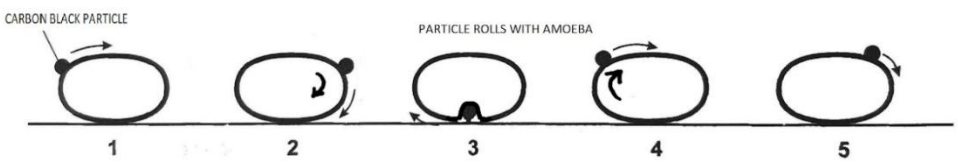

Figure 6. *Amoeba verrucosa* showing rolling movements

5. Walking movement theory. In A. *proteus*, Dellinger (1906) has described what he called 'walking'. According to him, the tip of the elongated pseudopodium touches the substratum and remains attached to it till another pseudopodium is formed and touches the substratum. Similarly, the second pseudopodium will remain attached till the third one is not formed and the process goes on in the direction of movement (Figure 7).

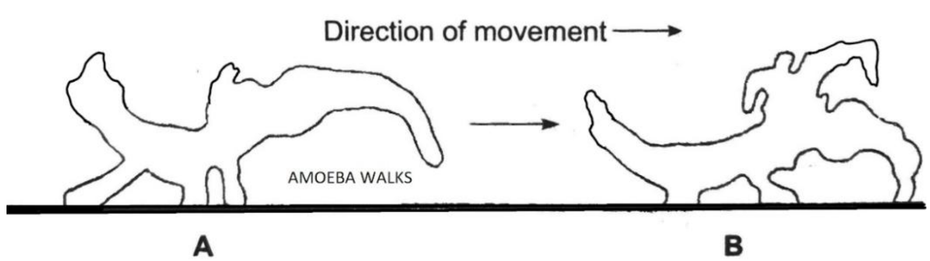

Figure 7. *Amoeba* walking on pseudopodial tips

6. Sol-gel theory. This theory was first advocated by Hyman (1917) and later supported by Pantin (1923-26), Mast (1925); Rinaldi and John (1963). This theory is widely accepted by the scientific community. According to this theory,

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the pseudopodia are formed and are withdrawn back due to change in the viscosity of the cytoplasm from 'gel to sol' and 'sol to gel'. This theory reveals that the cytoplasm of *Amoeba* consists of an outer layer of jelly-like consistency (Plasmagel) which surrounds a fluid mass (Plasmasol). Plasmasol has two types of contractile proteins in it:

a. Actin
b. Myosin

Actin protein subunits are freely moving in the plasmasol. These actin monomers can bind together with a process of polymerisation to form a mesh-like form (Figure 8). This change from sol (monomer state) into gel (mesh stage) and is called "gelation". However this process is reversible. Depolymerization results in the breakage of mesh-like form into a fluid-like sol form and is called "solation".

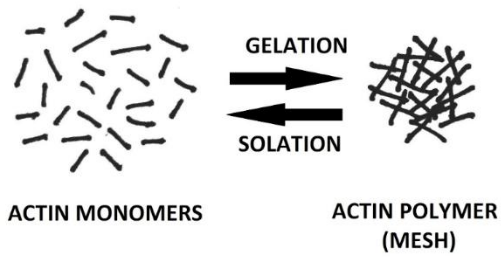

Figure 8. Actin polymerization and vice versa

Locomotion by the transformation of states can be explained by five main steps:

(a) Attachment to substratum: *Amoeba* attaches itself to the substratum.

(b) Pseudopodium formation: The plasmalemma of the *Amoeba* contains chemical signal receptors (9A). Whenever the Amoeba receives some chemical signals (e.g. food), it tries to move in that direction. At the anterior end, local partial liquifaction of the plasmagel occurs. Actin mesh loses its polymeric form and the general surface becomes soft (9B). This causes the central plasmasol (endoplasm) which is under tension to flow forward towards this point. This movement of the plasmasol exerts pressure and forces the plasmagel to produce a bulge towards the direction of the movement. This point is the starting point of pseudopodium formation.

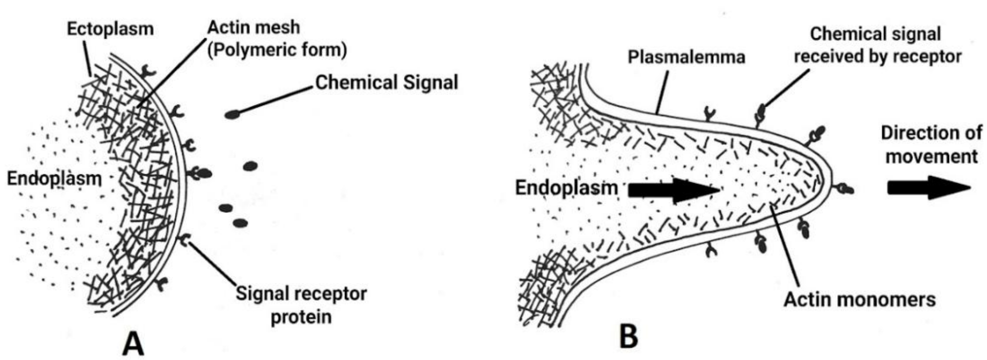

Figure 9. Formation of a pseudopodium

(c) Plasmasol converts into plasmagel (Gelation): At the anterior end, as plasmasol enters the newly formed pseudopodium, it rapidly changes into plasmagel on the periphery thus forming a gelatinized tube. In this tube, plasmasol continues to enter.

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(d) Plasmagel converts into plasmasol (Solation): At the posterior end, the plasmagel undergoes solation and forms plasmasol. This plasmasol produced at the posterior end may go to the centre and to the anterior end. Thus, a constant flow of plasmasol is maintained from behind in the direction of movement.

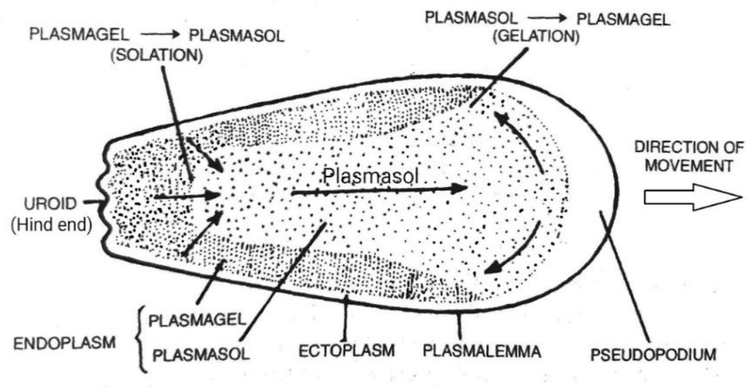

Figure 10. Sol-Gel transformation theory

(e) Withdrawal of pseudopodium: The tube of elastic plasmagel (as described in step c) contracts and is withdrawn back. This action is aided by the protein myosin. Though the pseudopodium is withdrawn, the main bulk of the body travels forwards. Plasmagel comes back from the sides towards the posterior end as the pseudopodium is withdrawn. This further makes space for more of the plasmasol to move anteriorly.

This cycle of steps (b) to (e) is repeated so as to get a movement in the required direction by the *Amoeba*.

7. Folding and unfolding theory. Folding and unfolding of the protein chain results in contraction and relaxation of protein molecules (Goldacre and Lorch,1950). They reported that the long-chain protein molecules contract and become folded at the posterior end representing 'solation'. The protein molecules unfold at the anterior end representing 'gelation'. The unfolded straight protein molecules come back from the anterior side to the hind end from sides as shown in the diagram. The process of folding and unfolding requires energy which is delivered by the breakage of ATPs.

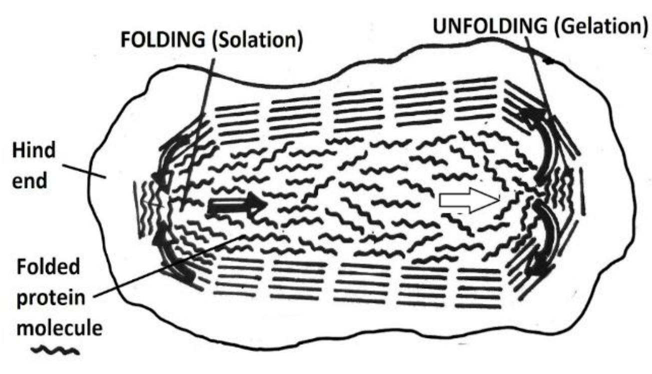

Figure 11. Folding and unfolding theory

8. Fountain-zone contraction theory. This theory suggested that the region near the tip of the forming pseudopodium is under pressure (Allen, 1961). Here, the everting plasmasol changes into plasmagel to form a wall or fountain zone, and this anterior region tension is developed which is transmitted to the hind end. At the posterior end, the protein chains unfold by which the plasmagel is converted into plasmasol. Thus, the animal is pulled forward by the contraction or tension at the anterior end.

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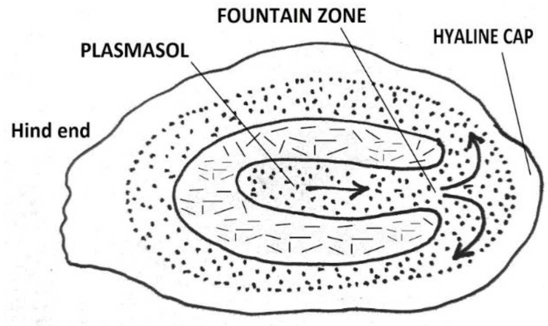

Figure 12. Fountain-zone contraction theory

9. Contraction-hydraulic theory. This theory was proposed by Rinaldi and John (1963). According to this theory, contraction of the plasmagel layer at the hind end (uroid) exerts a hydraulic pressure upon the plasmasol. Resistance to this pressure is minimum at the front end. Thus, more of the plasmasol tends to flow forward into the pseudopodium. At the anterior end, the plasmasol is converted into gel (Gelation zone). This converted gel comes back from the periphery to the hind end and is back converted into sol (Solation zone) and the cycle is repeated. This is how the *Amoeba* travels forward in the direction of movement.

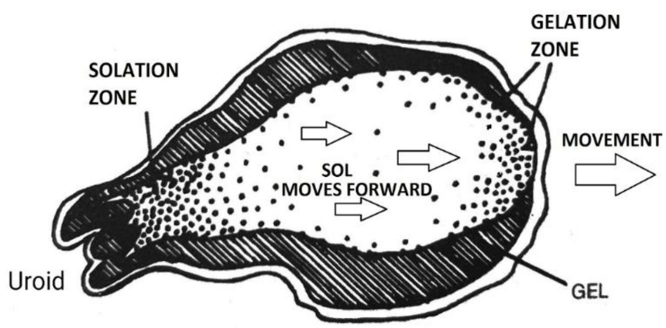

Figure 13. Contraction-hydraulic theory

B. RESPIRATION

Respiration refers to the intake of oxygen and giving out of carbon dioxide by the *Amoeba*. *Amoeba* has no special respiratory organelles. The exchange of gases (O and CO_2), takes place through the general body surface (Plasmalemma) by a process known as "Diffusion". Diffusion literally means the transportation of particles or gas from a region of higher concentration towards the region of lower concentration. Now, we have to understand the process of diffusion to understand the process of respiration in *Amoeba*.

Oxygen status: *Amoeba* lives in the freshwater. The concentration of oxygen in the surrounding water is much higher than inside of the *Amoeba*. *Amoeba* tends to use its oxygen in the oxidation process. Thus, oxygen levels further decrease inside. So, there is a concentration difference of oxygen in between the outside water and cytoplasm of Amoeba. As a result of this concentration gradient (difference), oxygen from the outside water enters inside the *Amoeba* simply through the plasma membrane. This transportation of oxygen is carried until the concentration gradient becomes zero. The oxygen brings about enzymatically assisted oxidation of carbohydrates, fats and proteins and breaks them into end products. The energy liberated in the oxidation reaction results in the formation of ATP molecules within mitochondria. Further, in the cytoplasm, ATP molecules are broken down into ADP and inorganic phosphate to release energy.

Carbon Dioxide status: On the other hand, as *Amoeba* utilizes oxygen for food respiration, more and more carbon dioxide is formed inside the cytoplasm. This creates a gradient in the concentration of carbon dioxide in between the outside water and the inside of the *Amoeba*. Here, the concentration of carbon dioxide is less towards the

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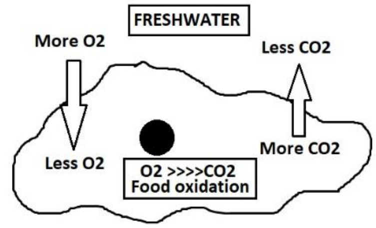
outside water as compared to the cytoplasm. Hance, due to this concentration difference carbon dioxide tends to move out of *Amoeba* into the surrounding water.

Figure 14. Respiration by diffusion



Whole of this process of oxygen intake and carbon dioxide expulsion due to the concentration difference is known as diffusion. Thus, Amoeba requires no special organs or structures for respiration.

C. NUTRITION

The process of nutrition in Amoeba can be understood by sub-dividing it into different phases like intake of food (ingestion), breakage of food into simpler components (digestion), taking off of the required digested matter (absorption), using the absorbed components for different metabolic activities (assimilation) and removal of waste products (egestion).

Amoeba is holozoic and omnivorous. It eats small organisms, such as diatoms, bacteria, rotifers, flagellates, ciliates, algae and other animal and plant matter. *Amoeba* is generally seen engulfing Paramecia. *Amoeba* shows a distinct sense of food selection. It generally avoids unwanted objects, such as inorganic particles. Amoeba tends to move in the direction of food particles.

(i) Ingestion of food (intake of food). *Amoeba* has a plasma membrane and has no mouth for food ingestion. Ingestion may occur at any point of the body, but usually this is done at the advancing end. Rumbler (1930) described the ingestion in *Amoeba*. It can occur in several ways.

(a) Import. This is passive food-ingestion for which *Amoeba* has to make less effort. The food passively sinks into the body by rupturing the plasmalemma and ectoplasm at the point of contact. The ruptured site soon heals up..

(b) Invagination. In this process, the part of plasmalemma and ectoplasm that comes in contact with a food particle invaginate, forming a tube-like structure. Food is taken inside this tube and the tube is elongated. When the food completely reaches inside the body of Amoeba, the tube detaches and a food vacuole is formed. The tube itself takes the form of a food vacuole (gastriole).

The process of taking in solid food is termed "Phagocytosis" or "Cell Eating" and the food vacuole is termed 'Phagosome'. The process of taking in liquid food is termed "Pinocytosis" or "Cell Drinking" and the food vacuole is termed 'Pinosome'. The intake of food is termed as Endocytosis whereas expelling the food out is termed as Exocytosis, Egestion or 'Cell Vomiting'.

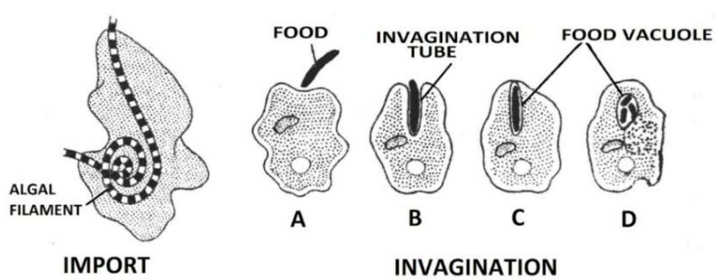


Figure 15. Import and Invagination (A to D) processes in *Amoeba*.

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(c) Circumvallation (For active preys). If the prey is "active", just like a *Paramecium*, Amoeba eats by a method called Circumvallation. In this method, *Amoeba* extends pseudopodia around the prey forming a cup-like structure known as a food cup. Here, the food cup is quite big in size and does not touch the prey. So, there is no intimate contact between *Amoeba* and the *Paramecium*. The opened face of the food cup constricts gradually and finally closes. Thus, a food vacuole is formed and the *Paramecium* is now inside the body of *Amoeba*. Water is also taken along. The vacuole's shape and size may vary with the enclosed prey. If prey is large, larger food cups and food vacuoles may be formed. Several food vacuoles may also be present in the endoplasm of an actively feeding *Amoeba*, moving about with endoplasmic cyclosis. Now the live *Paramecium* inside the food vacuole will be killed and digested.

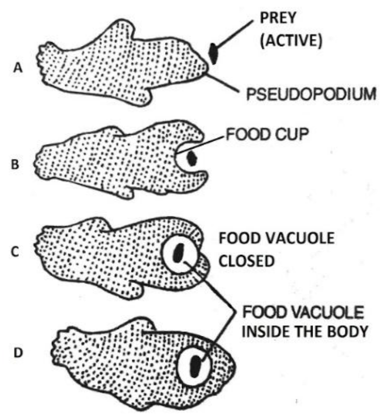

Figure 16. Amoeba ingesting the active prey by circumvallation.

(d) Circumfluence (For inactive prey). If prey is very less active or inactive, *Amoeba* eats by the process termed Circumfluence. For ingesting less active food particles like algae and bacteria, *Amoeba*, extends pseudopodia around the prey and encloses it completely. Here, the food cup is in intimate contact with the food. Ultimately, the food cup closes and a food vacuole is formed in the cytoplasm.

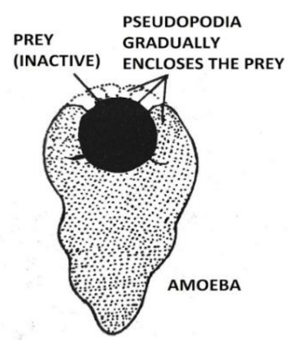

Figure 17. Amoeba ingesting the inactive prey by circumfluence.

(e) Pinocytosis. Pinocytosis is also referred to as "cell drinking" because here the food is in liquid form. *Amoeba* can ingest liquid food in colloidal or droplet form out of the surrounding medium by pinocytosis. First, a pseudopodium is formed towards the site of the food droplet. On the tip of that pseudopodium, pinocytic channels start developing as invaginations in which the liquid food is taken. These channels deepen and ultimately small pinocytotic vesicles (Pinosomes) are pinched off from these channels enclosing the food droplets. Thus the liquid food reaches the cytoplasm of Amoeba. Further, lysosomes attach to these vesicles for digestion of the liquid food.

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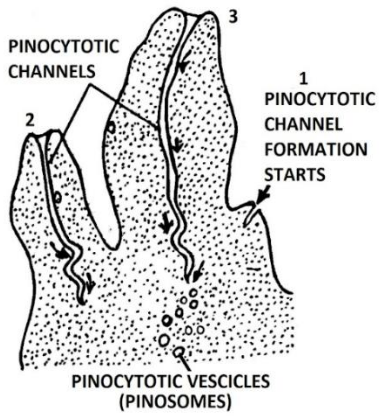

Figure 18. Amoeba ingesting colloidal food through pinocytosis.

(iii) Digestion (Breaking food into simpler forms). Digestion is the breakdown of food components into simpler molecular forms that can be absorbed and assimilated for making energy. Whenever food is ingested by any of the methods discussed above, food is present inside a food vacuole {Gastriole or phagosome (solid food) or pinosome (liquid food)}. The food present within the food vacuole is digested with the help of lysosomes. Lysosomes contain lysosomal enzymes (Amylolytic, proteolytic and lipolytic) to digest all types of food materials including carbohydrates, proteins and lipids respectively. Lysosomes fuse with food vacuoles to form "phagolysosomes" and release their enzymes into them. But before the actual enzymatic digestion, an acidic phase is attained by the food vacuoles.

(a) ***Acidic phase (Killing the prey):*** In the early phase of digestion, when the food vacuoles are formed, the medium of the vacuole is changed to acidic (pH between 4.0 and 6.5). This acidic state is attained by active 'influx of H^+' into the vacuole. Generally it contains inorganic acids (perhaps HCl). As more and more H^+ enter the vacuole, pH decreases and the medium becomes acidic. This acidic medium kills the prey. If the prey is alive, active and big in size, acidity inside the food vacuole is decreased to a large extent so as to kill that prey.

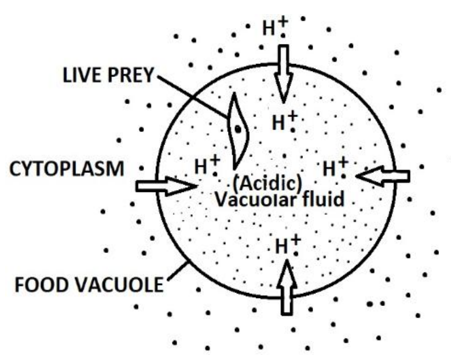

Figure 19. Acidic phase of digestion

(b) ***Basic phase (Enzymatic phase):*** After the acidic phase and killing of the prey, the second phase of digestion starts. In this phase, the medium of food vacuole becomes distinctly alkaline. To attain the alkalinity, excessive H^+ are pumped out of the food vacuole. When the medium becomes slightly basic, digestive processes take place. Now the lysosomal enzymes are poured over the prey and the digestion process starts because the pH required for their action has been attained by the food vacuole. Digestion of starch, proteins and fats takes place by the enzymes amylase, protease and lipase respectively.

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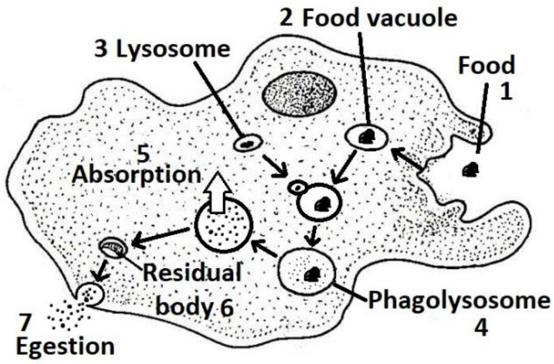

Figure 20. Process of digestion, absorption and egestion

(iv) Absorption and assimilation. After the digestion, the soluble molecular forms resulting from digestion diffuse through the membrane of food-vacuoles into the surrounding cytoplasm. This is called absorption of food. Each vacuole remains in the endoplasm for about 15 to 30 hours. During this period, the vacuole continuously circulates with the cyclosis of plasmasol. As digestion goes on, more and more digested food is absorbed and the food vacuoles gradually decrease in size. Absorbed materials are stored as reserve food in the form of glycogen and fat. These molecular forms ultimately become part of the cytoplasm.

After these digested materials are absorbed in the cytoplasm, they are used for making energy with the oxidation process. Their released energy is used to carry out various reactions in the body of Amoeba. Thus, using these products in the metabolic processes by the Amoeba is known as assimilation.

(v) Egestion (Removing the wastes). After the process of absorption from the food vacuole, it becomes smaller in size. As all the necessary materials have been absorbed from the food vacuole, now it contains the residual waste materials only. Now this food vacuole is known as 'Residual body'. The residual waste products are removed from the body of *Amoeba* through the process of egestion (Cell vomiting). Egestion of indigestible food may occur at any point on the surface of the body of *Amoeba* as there is no anus. Residual bodies generally move to the hind part of the body and are egested. Plasmalemma immediately closes up to prevent any loss of cytoplasm in this process.

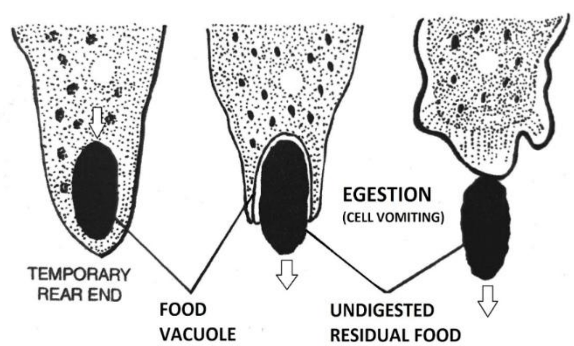

Figure 21. Process of egestion or cell vomiting

D. EXCRETION

(Removal of nitrogenous wastes)

Excretions refers to the elimination of nitrogenous byproducts of metabolism from the body. Animals may produce different nitrogenous waste products on the basis of availability of water such as ammonia, urea and uric acid. Amoeba is an ammonotelic animal because it excretes nitrogenous wastes in the form of ammonia. Here, in Amoeba, most of the nitrogenous waste (ammonia) diffuses out of the body through the general body surface in the surrounding water in the form of ammonium ions. There are no specialized organelles or structures for this process. Some amount of ammonia is dissolved in water which is discharged through the contractile vacuole. Ammonia reacts with water to form ammonium ions which go out of the Amoeba by the process of diffusion. This happens because of the concentration gradient of ammonium ions in between the outside water and cytoplasm of *Amoeba*.

$$NH_3 + H_2O \leftrightarrows NH_4^+ + OH^-$$

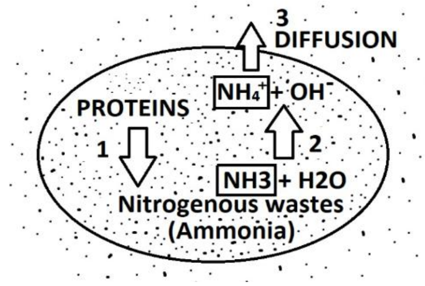

Figure 22. Process of excretion in *Amoeba*

E. OSMOREGULATION
(Regulation of salt and water)

Amoeba lives in freshwater which has a low concentration of salts. The cytoplasm has a higher concentration of salts as compared to outer water. As plasmalemma is semipermeable in nature, the phenomenon of osmosis occurs. As osmosis is the movement of solvent from a low concentration solution to a higher concentration solution, water from outside tends to move inside the Amoeba. This process is termed 'endosmosis'. Water is also taken in along with food-particles. Some water is formed within the cytoplasm as a result of metabolic activity. If all this water accumulates in the body of *Amoeba*, it will soon swell and burst. Hence the excess water must be removed from the body of *Amoeba*. The process of removal of excess water is called osmoregulation. It takes place by "contractile vacuoles". Amoeba has only one contractile vacuole. A contractile vacuole is about 20-30 µm in diameter and contains accumulated fluid which is less dense than the surrounding cytoplasm. It contracts and relaxes at regular intervals and the phases are termed systole and diastole respectively. It is surrounded with an envelope of mitochondria which provide sufficient energy for active water movement from the endoplasm into the vacuole under diastolic phase.

(a) Diastole (Relaxation): In diastole, the contractile vacuole grows in size. It gathers more and more water from the endoplasm of Amoeba. Mitochondria give the required energy for the active uptake of the water into the vacuole. It also fuses with small accessory vacuoles which collect water from the cytoplasm and appear in its close vicinity. After fusion it becomes bigger and attains its optimum size. Then, it becomes stationary and reaches the temporary hind end.

(b) Systole (Contraction): At the hind end, it fuses with the plasmalemma. At the point of fusion, plasmalemma breaks off and the vacuole releases the contents to outside of the *Amoeba*. This is its systole phase. Schneider (1960) observed that the wall of a contractile vacuole is provided with contractile myofibrils which bring about the con-traction of the vacuole. Due to the force of contraction, the contents of vacuole which contain water, CO_2, ammonia, etc. are discharged to the outside. As soon as the old vacuole disappears, its place is taken up by a new vacuole. The rate of contraction of a vacuole varies from a few seconds to several minutes.

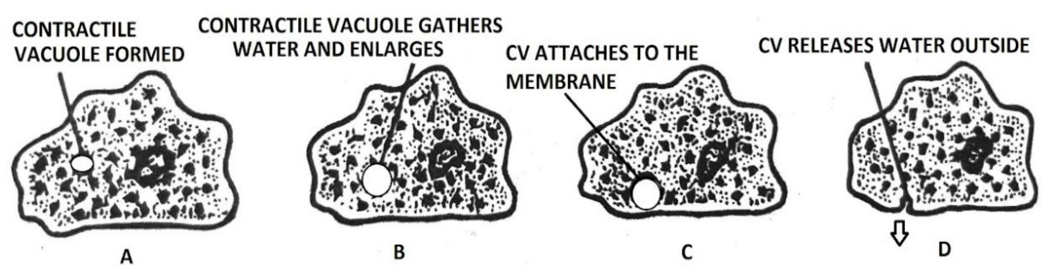

Figure 23. Process of osmoregulation in *Amoeba*

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F. Behaviour
(Responses to stimuli)

Behaviour is the overall response of an animal. Behaviour involves the manner in which an animal responds to the environmental conditions. *Amoeba* does not have a nervous system and special sense organs for perceiving the stimuli. Still it behaves differently to different stimuli. The responses to stimuli are called taxes (singular, taxis). A taxis may be either positive or negative. Positive taxis is that in which the organism moves towards the stimulus. Negative taxis is that in which the organism moves away from the stimulus. *Amoeba proteus* shows both types of taxes, positive as well as negative, to different stimuli. Taxes are named according to the nature of stimulus. With respect to the kinds of stimuli, taxes are classified as follows:

1. Phototaxis (Response to light). *Amoeba* searches for a dim or weak light. It avoids both direct sunlight and total darkness. So we can say that Amoeba is negatively phototactic to sunlight and positively phototactic to dim light.

DIM LIGHT + STRONG LIGHT -

2. Chemotaxis (Response to chemicals). It avoids chemicals. *Amoeba* is negatively chemotactic to solutions of alkalies, salts and sugars. It responds positively to the food particles or live prey like *Paramecia*.

ACID / ALKALI - FOOD / PREY +

3. Thermotaxis (Response to heat). *Amoeba* responds negatively to both low and high temperatures. Optimum temperature lies between 20°C and 25°C. So we can say that Amoeba is negatively thermotactic to a medium at 15°C.

Below 15°C - 20°C and 25°C + ABOVE 30°C -

4. Thigmotaxis (Response to touch). *Amoeba* is positively thigmotactic to food particles. It responds positively to those objects upon which it glides or rests. It avoids any mechanical contact.

FOOD / RESTING OBJECTS + MECHANICAL CONTACT -

5. Rheotaxis (Response to water current). Amoeba lives in freshwater and it prefers to move along the flowing water. Thus, it shows positive rheotaxis.

WATER CURRENT +

6. Galvanotaxis (Response to electric current). When an electric current is passed through a medium containing Amoebae, these stop moving. They withdraw their pseudopodia and become round. But if the applied electric current is weak, it prefers to move towards the negative pole (cathode).

LARGE CURRENT - WEAK CATHODE +

7. Geotaxis (Response to gravity). Amoeba locomotes on the substratum inside the water medium. The response of *Amoeba* to gravity is positive as it remains at the bottom of a petri dish having water.

GRAVITY +

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G. REPRODUCTION
(Making next generations)

Reproduction is essentially asexual and takes place by various methods such as binary fission, multiple fission and sporulation. *Amoeba proteus* does not reproduce sexually. Though some observers have described a temporary fusion (conjugation) between two amoebae which seems to be sexual reproduction. But it's not considered to be a true sexual process. The various reproductive methods have been described here.

1. Binary fission (Breaking into two). Binary means 'two' and fission means 'break'. It results in the division of the parent *Amoeba* into two daughter amoebae. It is the most common mode of reproduction when the Amoeba is reproducing under normal conditions. Division involves the nuclear division (karyokinesis) followed by cytoplasmic division (cytokinesis) as in a normal cell. The nucleus divides by mitosis and involves the normal phases of mitosis including prophase, metaphase, anaphase and telophase.

(A) KARYOKINESIS: It involves the following phases:

(a) Prophase. In this phase, *Amoeba* withdraws its pseudopodia and becomes rounded. Contractile vacuole disappears and the cytoplasm loses its transparency. The nucleoli are disintegrated. After this, a very large number (about 500) of very small chromosomes appear in the central nucleoplasm. The chromosomes are ready to be arranged at the metaphasic plate.

(b) Metaphase. The metaphasic stage is marked by the arrangement of chromosomes at the metaphasic plate or equator. Each chromosome splits longitudinally and attains a paired form. Daughter chromosomes of each side become attached to the spindle fibres arising from multiple poles.

(c) Anaphase. In this stage, daughter chromosomes start to move towards opposite poles. Here the constriction of the nuclear membrane also begins. The major change is that the multipolar metaphasic spindle becomes unipolar.

(d) Telophase. During this stage, the constriction of the nuclear membrane is completed and the nucleus is finally divided into two daughter nuclei. The nucleoli begin to reappear.

(B) CYTOKINESIS:

After the division of the nucleus (karyokinesis), cytokinesis follows. *Amoeba* stretches and constricts in the middle. Large pseudopodia are formed at opposite poles, drawing both the daughter amoebae in opposite directions. It ultimately divides parent *Amoeba* into two daughter amoebae. These grow in size and in turn, repeat the same cycle of binary fission to make further daughter amoebae.

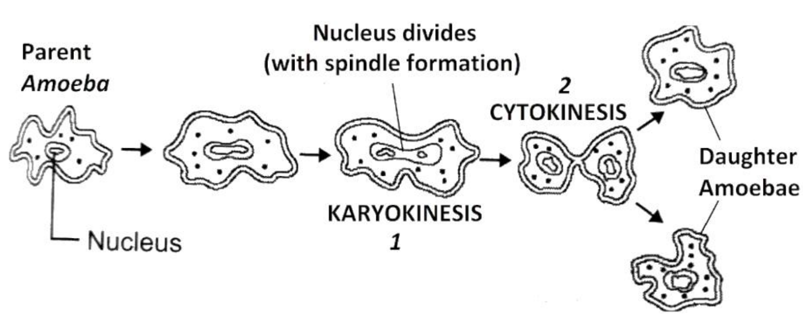

Figure 24. Binary fission in *Amoeba proteus*

Immortality: *Amoeba proteus* reproduce by binary fission in normal living conditions. In binary fission, the parent *Amoeba* divides into two and becomes a part of the offspring. Nobody dies in this process. Thus, there exists a

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continuity of life. So, Amoeba is referred to as potentially immortal. However, death may occur under conditions like starvation or accident.

2. Multiple fission (Breaking into many). Under normal conditions of living, Amoeba reproduces by binary fission. But under abnormal, adverse or stressful conditions, Amoeba chooses to reproduce by multiple fission. Because under unfavourable conditions, survival is difficult, Amoeba tries to make many daughter Amoebae so as to ensure the next generation.

Whenever Amoeba faces unfavorable conditions, it forms a "cyst wall" around itself to cope up with the conditions. This is a three-layered, protective, chitinous wall under which it is safe. After secreting the cyst wall it becomes round and inactive. It stops feeding. Inside the cyst, the nucleus repeatedly divides to form several daughter nuclei. Then, these nuclei arrange themselves near the periphery, close to the plasma membrane. Each daughter nucleus becomes enveloped by a small amount of cytoplasm and plasma membrane. These structures formed are called pseudopodiospores. Whenever conditions become favourable, the cyst breaks off liberating the young pseudopodiospores. They feed and grow rapidly to become adults.

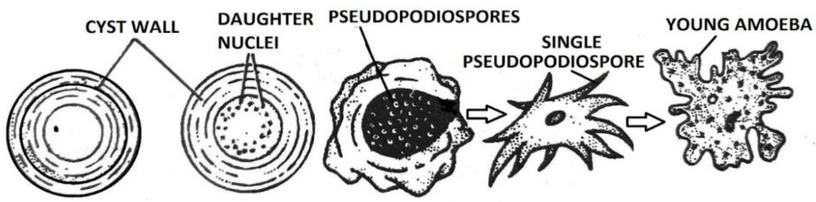

Figure 25. The process of multiple fission

3. Sporulation (Making many spores). According to Taylor, during unfavourable conditions, A. *proteus* multiplies by sporulation. This process is similar to the multiple fission but without the formation of cyst wall. As in the multiple fission, the parent nucleus divides into several nuclei. Each nucleus gathers some cytoplasm and develops a spore membrane. Under favourable conditions, the parent body disintegrates. About 200 spores are liberated. Each spore hatches into a small "amoebula". These amoebulae grow in size and become adult Amoebae to lead an independent life.

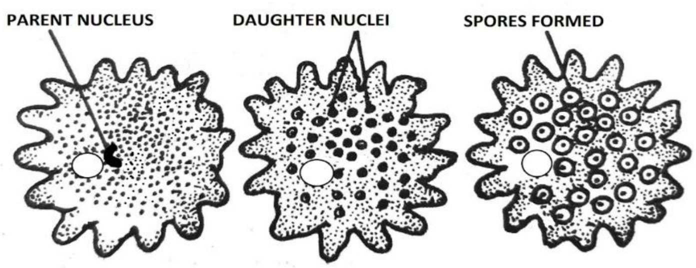

Figure 26. Sporulation in *Amoeba proteus*

4. Conjugation (Temporary fusion): Observers have described a temporary fusion between two amoebae. In this process, two old amoebae come close and join by their plasma membranes. After some time, they separate. The separated individuals are now the young ones. It is observed that this temporary union enables the two amoebae to lead a more active and vigorous life. This phenomenon is also termed 'rejuvenation'. The molecular mechanisms behind this phenomenon are still unknown.

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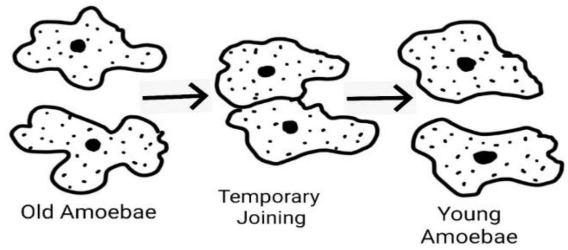

Figure 27. Conjugation in *Amoeba*

Biological significance of Amoeba

(1) Amoeba serves as a classic example of a unicellular organism, illustrating the basic structure and functions of a single cell.

(2) The process of binary fission in *Amoeba* provides a clear and simple model for understanding mitotic cell division.

(3) Despite being a single-celled organism, *Amoeba* exhibits various responses to stimuli, reflecting the earliest form of sensitivity seen in the animal kingdom.

(4) The specialized organelles within *Amoeba* demonstrate the initial stages of cellular division of labour, each contributing to essential life processes.

(5) The large number of chromosomes in *Amoeba* (approximately 500) indicates the presence of numerous isolated genes, similar to those found in the chromosomes of more complex animals.

(6) *Amoeba* offers a rudimentary analogy to the anatomical systems of higher animals; for instance, its food cup resembles the oral cavity, the food vacuole functions like the digestive tract, pseudopodia act as limbs, and the contractile vacuole performs excretory roles similar to the kidney or bladder.

In summary, *Amoeba* functions as a complete, self-sustaining organism, carrying out all fundamental life processes such as feeding, digestion, absorption, waste elimination, respiration, excretion, and reproduction.