Fertilization

Fertilization is a complex process which involves the fusion of male and female gametes followed by the fusion of their cytoplasm. Fertilization begins with the sperm's approach to the egg and ends with the fusion of egg and sperm pronuclei.

Mechanism of fertilization:
The process of fertilization completes in the following five stages :

(1) Encounter of spermatozoa and ova
(2) Approach of the sperm to the egg.
(3) Acrosome reaction and penetration.
(4) Activation of ovum.
(5) Migration of pronuclei and Amphimixis

1. Encounter of Spermatozoa and Ova:

A major problem in sexual reproduction is how to bring together the spermatozoa and ova in the same locality in a fluid medium, so that individual sperms may reach the surface of ova at the right time.

The primary needs for the encounter of spermatozoa and ova are fluid medium for the act of fertilization and delivery of large quantities of spermatozoa close to the number of ripe eggs at the right time.
According to the place and nature of fluid medium following two kinds of fertilization have been reported :
(1) External fertilization, (2) Internal fertilization.

1. External fertilization. This type of fertilization occurs in liquid medium outside the bodies of parent animals. Among freshwater animals (fishes, amphibians and freshwater invertebrates), the timing of spawning of egg by the female and shedding of sperm by the male parent are very specific, As their spermatozoa remain active usually for a few minutes, the sperms are delivered directly to the eggs of an individual female immediately after egg laying. But marine forms shed eggs and sperms freely into the surrounding water. The time interval between the laying of eggs and the shedding of sperms may even be weeks or months, because the saltish sea water serves as an important physiological medium for gametes.

During external fertilization, the movement of spermatozoa in a liquid medium is entirely at random and the spermatozoa collide with the eggs as a matter of chance which occurs regularly in nature, partly due to enormous number of spermatozoa produced by the male and partly because the eggs being relatively larger targets, they can be hit by sperms fairly well.

2. Internal fertilization. This type of fertilization occurs inside the body of the female. In oviparous forms such as reptiles and birds, the eggs are completely enclosed in impermeable egg membranes. In ovoviviparous and viviparous animals the eggs are retained within the metarnal body throughout development. In all such cases the spermatozoa are delivered internally in the body of the female by some type of copulatory mechanism or by intromittant organ of the male. In such forms the fertilization may occur in the lower part the oviduct (e.g., Urodela), in the upper portion of oviduct (e.g., salamanders, reptiles, birds and mammals) or in the ovarian follicles in viviparous fishes (e.g., *Gambusia affinis*) and eutherian mammals (e.g., *Ericulus*).
In case of internal fertilization, the movement of the spermatozoa from the site of deposition to the site of fertilization usually depends on the active swimming of the spermatozoa themselves or transported passively by muscular contractions of the female tract and also by the counter currents in the cilia - propelled, backward flowing liquid content of the tract

2. Approach of the sperm to the egg:
The sperm reaches near the ovam by adopting one of the following methods:

A. Chemotaxis: Chemotaxis is the ability of the sperm to detect difference in concentration of some substance released into the water by the egg and to move from an area of lower to one of greater concentration of that substance. Apparently, such a chemical attraction of male gamete towards the ovum operates only in the plant, mosses and ferns. However, chemotaxis is also suspected to be operative in some animals such as coelenterates and fishes. In them spermatozoa appear to be guided to the egg by chemical substances. For example, in the hydroid, *Campanularia*, the eggs are produced by reduced female gonangia, which are enclosed in a theca with an opening at the distal end. During fertilization spermatozoa have been observed to converge toward the opening of theca. The attractant chemical substance extracted from female gonangia by Miller (1966) was found to be a small molecule having molecular weight less than 5000 daltons.

B. Fertilizin and anti-fertilizin interactions: We have known for many years that the eggs and sperms of some animals including mammals are chemically attracted to one another. These eggs release into the surrounding medium substances that bring about agglutination (clumping of the spermatozoa onto the surface of the egg) of the sperm. The cause of agglutination of spermatozoa was studied by Lillie (1919). He observed that fertilizin and anti fertilizin occur in the eggs and sperms of the animals respectivily. They are directly involved in the reaction between the egg and the sperm.

The main source of fertilizin is the egg itself and it is located in the plasma membrane. However, in the eggs of sea urchin and other echinoderms, it is produced by the layer of jelly surrounding the egg, and becomes accumulated in the external gelatinous coat.

The fertilizin is a gel formed of glycoprotein or mucopolysaccharide. As a protein, it contains a number of amino acids and as a polysaccharide it includes molecules of one or more monosaccharides.

Both the amino acids and polysaccharides vary from one species to another, that is why each species possesses its specific type of fertilizin.

The molecules of fertilizins are quite large - the molecular weight is about 300,000-and each molecule may have more than one 'active group', so that one fertilizin particle may become attached to two or more spermatozoa thus binding them together. The surface layer of cytoplasm of spermatozoa (i.e., sperm plasma membrane) contains another protein species specific acid known as antifertilizin.

The remarkable peculiarity of the fertilizin and antifertilizin is that they combine in a specific manner; that is, the egg fertilizin of a species reacts best with the sperm antifertilizin of the same species. Reactions between the species which are nearly related to each other are much weaker. The reaction between fertilizin and antifertilizin is very much similar to the reaction between antigen and antibody. In both cases, a 'chemical lock' is formed between two complementary substances. Thus during capacitation and contact stage of fertilization when spermatozoa and eggs of same species come in physical contact of each other, a chemical lock is established between the antifertilizin molecules of spermatozoa and fertilizin molecules of
unfertilized egg and due to the fact many spermatozoa adhere to the surface of an unfertilized egg.

C. Capacitation. In mammals the normally ejaculated or epididymal sperm do not show acrosome reaction unless they have been for some time in the female genital duct (vagina and Fallopian tube). This time span varies with the species and physiological condition of the female. It is more than 1 hour in the mouse, at least 1.5 hours in the ewe, 2-4 hours in the rat, and 6 hours in the rabbit and man. During this time probably, the coating substances on the sperm surface particularly those on the acrosome, are gradually removed. The receptor sites on the acrosome are, thus, exposed to enable the sperm to recognize signals arising from the ovulated egg. This is called capacitation. The factors that induce capacitation in the female genital tract do not appear to be strictly species-specific.

Significance of capacitation: According to Guyton (1986) the process of capacitation of human beings and other mammals results from the following effects and has the following significance : The acrosome of the human sperm contain extremely powerful hydrolytic and proteolytic enzymes that could literally destroy the male genital tract if all the sperms would release these enzymes prematurely. Fortunately, the fluids in seminiferous tubules, in the epididymis and in the vas deferens, all contain minute floating vesicles filled with large amounts of cholesterol. This cholesterol is continually supplied to the cellular membrane covering the acrosome, toughening this membrane and preventing release of the enzymes. After ejaculation, the sperms swim away from the vesicles and gradually lose their excess cholesterol during the next few hours. In doing so they become "capacitated so that the acrosomes can now release the enzymes that allow a sperm to enter or penetrate the ovum.

3. Acrosome Reaction and Penetration:

The surface of the ripe egg in most animal eggs are enveloped by one or more egg membranes or gelatinous layers or follicle cells or both, outside the plasma membrane. These layers constitute barriers for the penetration by spermatozoa and serve in preventing fertilization by more than one spermatozoa or by sperm of other species. When the sperm is attached to the surface of the eggs, it becomes motionless. Its penetration through egg membranes and also through the plasma membranes of the egg is achieved by some physico-chemical activity of the sperm acrosome.
Certain enzymatic proteins called sperm lysins are produced presumably by the sperm acrosome. The sperm lysins differ from one animal group to another. In some cases the dissolution of the egg envelopes may be brought about by simpler means. Thus, it is believed that the jelly coat of echinoderm eggs may be dissolved as a result of acidification of seawater by carbon dioxide produced by the spermatozoa in the course of their respiration. In the case of eggs with very thick and resistant envelopes, such as the egg envelopes of fishes and insects, the sperm cannot reach the egg at all points but must penetrate through a special canal, the micropyle, left in the egg envelope, the chorion.

In mammals, when the eggs are released from the ovary, they are commonly encased in a layer of follicular cells, called corona radiata. These cells are held together by an adhesive cementing substance called hyaluronic acid, a mucopolysaccharide. The corona radiata, thus, acts as a barrier through which the sperm must first penetrate to reach the plasma membrane of the egg. For this purpose, the sperm's acrosome produces an enzyme, hyaluronidase, which serves to dissolve the adhesive substance and disperses the cells of corona radiata. The breaking of the membranous barriers is not only mediated by lytic agents provided by the acrosome of the sperm, but the acrosome itself undergoes morphological changes and forms acrosomal filament which helps the sperm penetration into the egg interior. The entire process has been well illustrated in echinoderms, annelids and *Saccoglossus* (hemichordata).

Acrosome reaction and penetration in *Saccoglossus*: Penetration and acrosomal reaction of spermatozoa of *Saccoglossus* has been best described by Colwin and Colwin (1967). A sperm of *Saccoglossus* has spherical nucleus, a flat tail and an acrosomal vesicle at the forwarding end of the sperm head.

The acrosomal vesicle is bound by an acrosomal membrane and contains a large, dense acrosomal granule. The granule is surrounded in large part by fine, grainy material except at the apex where an apical space lies between the granule and membrane. The space between the acrosomal membrane and sperm plasma membrane and also, the space between acrosome nuclear membrane, are filled by some material called periacrosomal material.

As the sperm of *Saccoglossus* makes its initial contact with the egg envelope, the following events occur (Figs. 2,3).

(a) **Bursting of acrosome**: Apex of the acrosome bursts so that the membrane of sperm and acrosome open apically and consequently expose the interior of the acrosomal vesicle to the outside.The two membranes join around the margin of the opening.

(b) **Release of lytic enzymes**: Acrosomal granule is released and comes in contact with the egg envelope. It contains lytic enzymes which make passage through the egg envelopes. Therefore, shortly after its release, the acrosomal granule disintegrates and disappears.

(c) **Formation of acrosomal tubule**: Shallow depression of the acrosomal membrane close to the nucleus now deepens and soon lengthens into a long slender acrosomal tubule. The tubule becomes twice as long as the sperm nucleus.

(d) **Eversion of the acrosomal membrane**: Rest of the acrosomal membrane everts and is added to the acrosomal tubule at its base. This is simply an unfolding of the already continuous membrane.

(e) **Fusion of acrosomal tubule with egg membrane**: Acrosomal tubule gradually enters through the passage of egg envelope, which has been made previously by acrosomal lytic enzymes and ultimately touches and fuses with the egg plasma membrane.

(f) **Passage of sperm contents**: Fertilization cone protrudes from the egg and engulfs acrosomal tubule. The nucleus of sperm is drawn out towards the fertilization cone. The acrosomal tubule dissolves; the elongated nucleus along with the middle piece of sperm is engulfed into the egg cytoplasm.

In other animals similar types of events have been observed during the penetration of sperm into the egg envelope, i.e., before penetrating the egg contents and before activating the egg for further developmental events, the spermatozoa of many animals themselves get activated like the spermatozoa of *Saccoglossus*. In them sperm activation includes rupturing of acrosome and formation of acrosomal filament or acrosomal tubule. However the number and size of acrosomal tubules may vary from species to species, as some annelids (e.g., *Hydroides hexagonus*) have several acrosomal tubules.

The mammalian spermatozoa though possess acrosome do not develop acrosome filaments. The acrosomal membrane and the egg membrane are dissolved at the point of contact and the sperm is drawn into the interior of the egg.

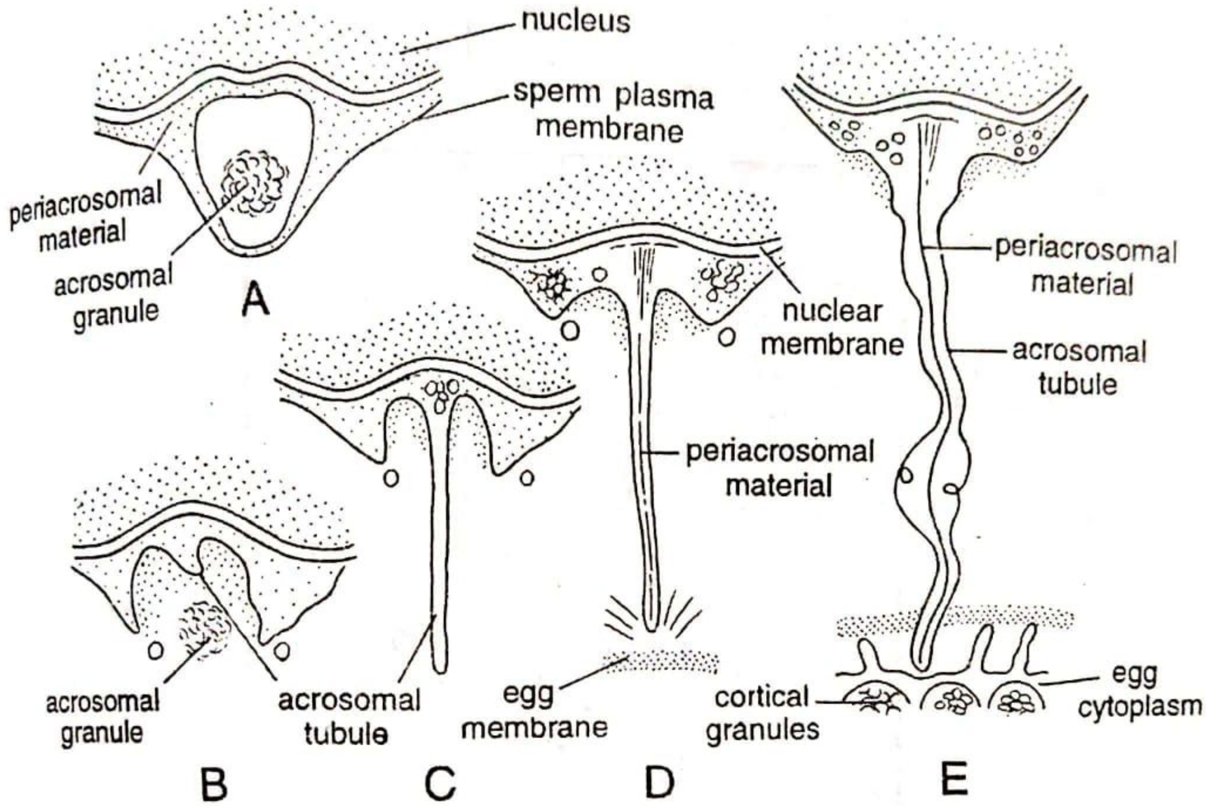

Fig. 2. Changes in the spermatozoon during fertilization in *Saccoglossus* **A.** Acrosome of inactivated spermatozoon. **B.** Extrusion of acrosomal granules. **C.** Formation of acrosomal tubule. **D.** Acrosomal tubule reaches egg membrane. **E.** Acrosomal filament reaches the surface of egg cytoplasm.

Penetration

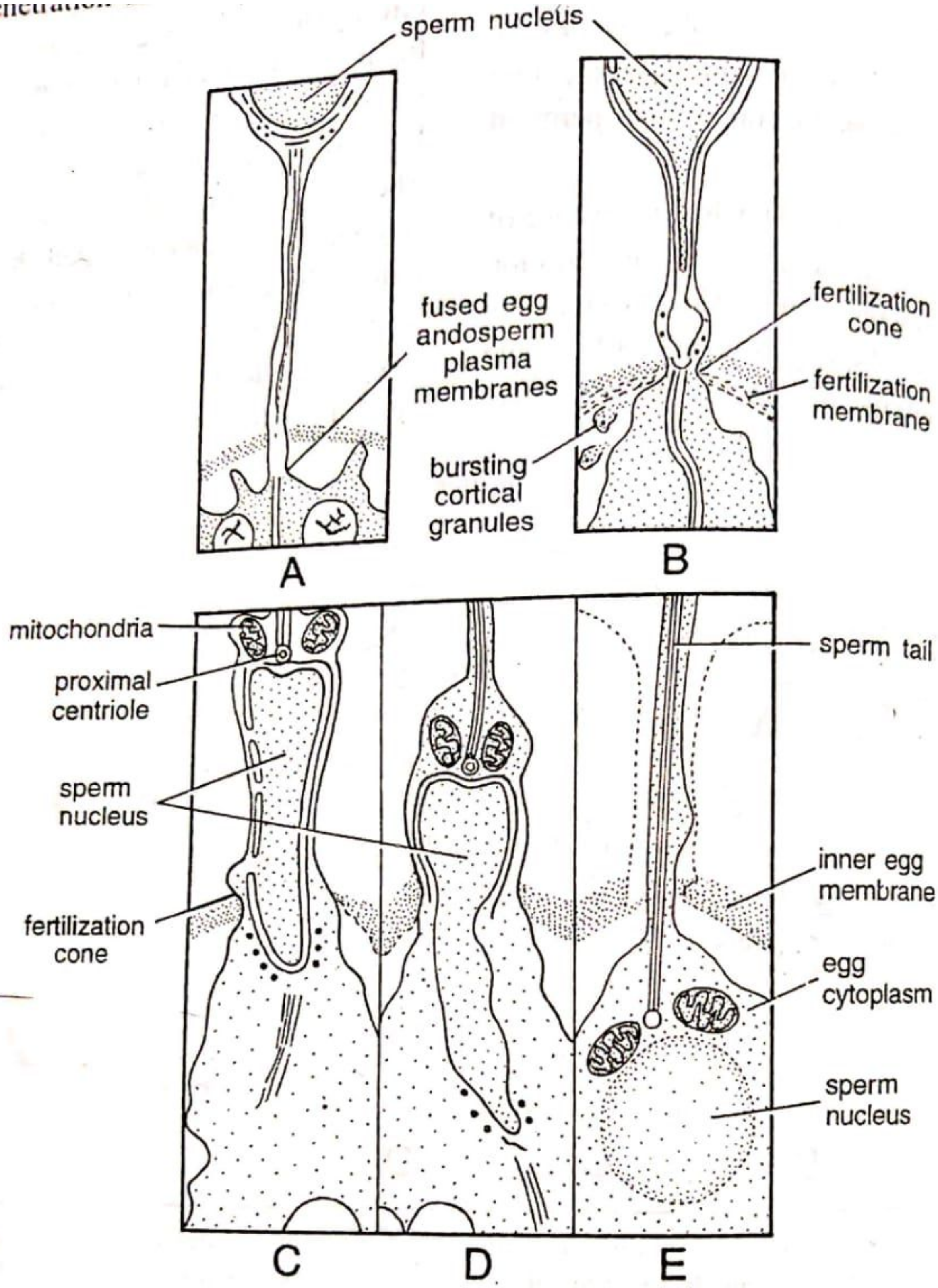

Fig. 3. Spermatozoon of *Saccoglossus* penetrating into the egg cytoplasm.

4. Activation of Ovum

Activation of ovum is that aspect of fertilization by which an egg is released from its inactive state and begins to develop. As soon as the apex of acrosomal tubule of a sperm touches the surface of egg plasma membrane fusion of both membranes (i.e., plasma membrane of sperm and egg) over this limited area of contact takes place and a single continuous mosaic membrane is formed. Thus, the plasma membrane of both gametes (sperm and ovum) becomes continuous and forms a single cell, called zygote. At this very time, certain very important changes occur in the cytoplasm of egg:

(A) Fertilization cone formation.

(B) Cortical reactions and formation of fertilization membrane.

(C) Metabolic activation.

A. Fertilization Cone Formation :

Immediately after the acrosomal filament of sperm touches the surface of the egg, the cytoplasm of the egg bulges forward at the point of contact, producing a process of hyaline cytoplasm called the fertilization cone.

Fertilization cone appears in many forms. It may be in the form of a more or less simple conical protrusion or it may consist of several irregular pseudopodium-like processes, or in some cases it may take the form of a cytoplasmic cylinder stretching forward along the acrosomal filament or tubule. Whatever its shape, the gradually engulfs the sperm and then begins to retract.

B. Cortical Reactions and Formation of Fertilization Membrane

Even before the fertilization cone is formed and the sperm penetrates into the interior of egg, a chain of physico-chemical reactions is set in the cortex. All these reactions are collectively called cortical reaction. These reactions may differ from one group of animals to another, but in most groups, the cortical reactions lead to the formation of fertilization membrane outside the egg plasma membrane. This membrane blocks the entry of the late arriving spermatozoa in the egg interior and thus avoids polyspermy. The process of cortical and fertilization membrane formation in different geoups of animals is as under:

1. **Sea Urchins :** In sea urchins, as soon as the apical end of acrosomal tubule touches the surface of egg from the site of contact, a wave like colour change from yellow to white travels rapidly arounf=d the egg cortex and is immediately followed by the elevation of fertilization cone from the egg surface and the formation of fertilization membrane around the egg plasma membrane. Electron micrographs of Sea Urchins' unfertilized eggs show that the egg cortex is bound by two membranes : an outer vitelline membrane and an inner plasma membrane.

Beneath the plasma membrane occurs a layer of cortical granules. A fertilization membrane is formed in the following stages:

The outer vitelline membrane separated from the plasma membrane undergoes expansion and becomes the outer layer of the fertilization membrane. The cortical granules explode and release the following three components:

1. **Dark, denser, lamellar and folded bodies** (i.e., electron opaque material) of the cortical granules unfold and fuse with the inner side of the already elevated membrane, the vitelline membrane.

2. **Globules** (hemisphere bodies), fuse together and build up a new surface of the viscous hyaline layer, just at the outer side of the egg plasma membrane. The hyaline layer adhered closely to the surface of the egg and during cleavage, it helps to keep the blastomeres together.

3. **The liquefied component** of the cortical granules fills the perivitelline space between the new egg surface and now the completed and elevated fertilization membrane. It contains mucopolysaccharides and abundant water.

All these structures, namely vitelline membrane and contents of cortical granules, thus form the fertilization membrane, which is much thicker (up to 900Å) and stronger.

In vertebrates, the changes which occur in the cortex are similar to sea urchins with some minor exceptions. e.g., the unfertilized eggs of some mammals (man, rabbit, etc.) have cortical granules. In them, the sperm penetration is not followed by the formation of fertilization membrane but, the cortical granules burst open and release their contents into the perivitelline space, i.e. the space between the egg plasma membrane zona pellucida.

In urodel amphibians and some mammals, which lack cortical granules, neither any cortical reaction nor fertilization membrane formation occurs.

C. Metabolic Activation:

After the sperm penetrates the unfertilized egg, a series of cytoplasmic reaction is initiated. Following metabolic changes occur in the egg during fertilization:

1. **Changes in plasma membrane**: The permeability of plasma membrane increases for the molecules of water and certain other chemicals like ethylene glycol, phosphate, K^+, etc. During fertilization, the electrical potential of plasma membrane becomes more positive and gradually becomes more negative. The change in the electrical potential of the membrane is governed by the unequal distribution of chloride ions (Cl^-).

Besides this, a plasma membrane enzyme adenyl cyclase becomes activated at the time of fertilization and it starts the formation of a chemical molecule 3' 5' cyclic AMP, which is supposed to activate most of the metabolic reactions in a fertilized egg.

2. **Ionic changes:** Certain intracellular changes occur in the concentration of cations, especially those of sodium, potassium and calcium. The change in calcium ion concentration in a fertilized egg has great significance in the metabolic activation of the egg.

3. **Changes in the rate of respiration**: In a fertilized egg, the rate of respiration either increases (e.g., sea urchins) or decreases (e.g., *Chaetopterus*) or remains static (e.g., *Bufo*). There appears to be a relation between the post-fertilization oxygen consumption and the stage of maturation of the egg at fertilization. Because at the time of fertilization the sea urchin egg has completed maturation, the egg of *Bufo* is at the second maturation division stage and the egg of *Chaetopterus* is at the first maturation division stage. The increased oxygen consumption is related with the oxidation of glycogen and other food stuff of the egg and synthesis of numerous ATP molecules.

4. **Co-enzyme changes:** In a fertilized egg, inter-conversion of coenzyme, NAD into another co-enzyme NADP and also NADPH due to phosphorylation of the NAD in the presence of an enzyme NAD Kinase takes place.

NAD Kinase

NAD + ATP ----------------› NADP +ADP

There is ample evidence that NAD kinase enzyme although present in the unfertilized egg, is in inactive state. It is activated only at the time of fertilization. The increased NADP and NADPH contents may initiate many synthetic pathways of fertilized eggs.

5. Changes in the rate of protein synthesis: The cytoplasm of a mature unfertilized egg contains complete machinery for protein synthesis, such as DNA molecules, tRNA, MRNA, ribosomes and proteolytic enzymes required during protein synthesis, but none or very little protein synthesis occurs because the mRNA of unfertilized egg remains 'masked'. There is evidence that during later phases of oogenesis some inhibitor or repressor proteins are manufactured in sea urchin’s egg which inactivate chromosomal genes, mRNA molecules, ribosomes etc. During fertilization, there is an increase in proteolytic activity of the egg immediately following the penetration of sperm, which removes these inhibitor proteins from them and unmasks the mRNA and active protein synthesis is started. In the egg of frog, however, the rate of protein synthesis is increased quite early at the stage of ovulation itself.

6. Initiation of mitosis: The initiation of mitosis for cleavage is the most significant aspect of egg activation. The initiation of mitosis occurs because (i) the rate of DNA synthesis increases with great pace immediately after fertilization, (ii) the unfertilized egg cytoplasm although possesses a centriole, yet this centriole is incapable of division and also to form a mitotic spindle. Thus, sperm stimulates the first mitotic division (cleavage) of fertilized egg by contributing its centriole to the egg.

In some animals after fertilization, a considerable amount of acid is formed during the first several months.

Theories of Activation:

The process of activation of egg by sperm during fertilization is still not very clear. However, various theories have been put forward by different embryologists to explain the mode of activation of egg, but the most accepted theory is the Repressor theory of activation.

Repressor theory of activation:

Very recently, Monroy (1965) has given some convincing arguments that the egg is activated by sperm in molecular terms. According to him, during maturation of egg, the energy yielding systems are blocked, so, all the reactions such as metabolic activities inside the egg cytoplasm, etc., requiring a large amount of energy are also inhibited. The accumulation of inhibitory substances during maturation of egg, seems to be the most likely explanation and indeed, he recognized one such inhibitor.

Monroy's repressor theory has been supported by many modern embryologists, such as Tomkins, et al., (1969), Metafora, et al., (1971), Berrill (1971) and D. Epel (1973), etc. Now, there are large amount of evidence that the repressor substances manufactured by the egg in the later phases of maturation, inhibit both the metabolic activities inside the cytoplasm and the genetic activities of the nucleus. The key reaction of fertilization is, thus, the removal of the repressors, releasing at the same time, the cytoplasmic metabolic activities and the activity of the nuclear genetic system.

Recent studies have shed some light on the initial phenomenon that activates the egg. It appears that after acrosome reaction there is an elevation of Ca++ and an increase in pH inside the sperm and that localized increase in cytoplasmic Ca++ (at the place of sperm and egg contact), is sufficient to trigger the exocytosis of the cortical granules and elevation of the fertilization membrane. This is followed by a propagating wave of Ca^{++} released from intracellular stores and by an increase in cytoplasmic pH. These two components (Ca++ and pH) mainly appear to be the regulators that trigger the various cytoskeletal changes occurring in fertilization.

Components of Sperm in the Egg Interior:

Many variations have been observed in different groups of animals, as to how much part of the sperm is engulfed into the interior of egg during fertilization. In most cases, the sperm nucleus, peri acrosomal material, proximal centriole and mitochondria make their entry as a rule. The plasma membrane of the sperm becomes one of the entities of plasma membrane of the egg. In mammals, complete structure of sperm (i.e., head, middlepiece and tail) penetrates into the egg cytoplasm. In echinoderms the sperm tail remains exterior to the vitelline membrane, while in Nereis, only sperm nucleus and proximal centriole enter the egg cytoplasm. There is no definite proof that any constituent of the sperm except for the nucleus and centrosome play any active part in subsequent development. The mitochondria have been observed in some cases to scatter in the cytoplasm of egg, but it is not known how long they maintain their existence there.

In vertebrates, as a rule the egg completes its first meiotic division in the ovary and reaches the metaphase stage of the second meiotic division. At this stage all further progress is arrested, ovulation takes place and the egg may become fertilized. The second polar body gets extruded only if the egg is fertilized by a sperm.
In ascidians, the egg reaches only the metaphase of the first meiotic division when it becomes ripe and if fertilized, only then the egg completes the first reduction division and carries out the second meiotic division.

Migration of Pronuclei and Amphimixis

At the time of penetration of sperm inside the egg cytoplasm, the sperm nucleus remains compact and its mitochondria and centriole remain situated behind it. To perform the act of amphimixis, the sperm nucleus has to undergo two activities (i) it has to become pronucleus, and (ii) it has to migrate to the site of amphimixis. As the sperm nucleus moves inwards from the site of fertilization cone, it soon rotates through an angle of 180°C, so that its mitochondria and centriole assume the leading position. Besides this rotation, the sperm nucleus starts swelling and its chromatin, which is very closely packed, becomes finely granular. It ultimately becomes vesicular and has an appearance like the interphase nucleus and is called male pronucleus.

At the same time, inside the egg cytoplasm, the sperm aster forms around the proximal centriole of the sperm. As the male pronucleus develops and migrates towards the site of amphimixis, the sperm aster seems to lead it. The site of amphimixis lies either near the centre, i.e., in case of microlecithal and mesolecithal eggs or in the centre of the active cytoplasm at the animal pole of microlecithal and telolecithal eggs. As the sperm pronucleus and centriole move inward, it may be accompanied by some cortical (outer cytoplasm) and subcortical cytoplasm. If the latter is heavily pigmented, as in amphibian eggs, the trajectory (the path) of the sperm pronucleus may be marked by pigmented granules trailing along its path. This is called **penetration path**. This movement of the sperm appears to be directed and some investigators feel that it is due to a chemotaxic effect of chemicals liberated by the female pronucleus. During this movement toward the female pronucleus, the sperm may have to deviate from its penetration path. If it does, the new pathway taken is referred to as **copulation path**.

In some cases the sperm need not alter its direction. In these cases the penetration and copulation paths would be identical.

Before amphimixis the nucleus of the egg also undergoes certain changes like the sperm nucleus. After the completion of the second meiotic division, the haploid nucleus of the egg is located near the surface of the egg in the form of several vesicles, known as karyomeres. In a fertilizing egg, these karyomeres fuse together to form a female pronucleus which swells, increase in volume and becomes vesicular. It also migrates towards the site of amphimixis.

Amphimixis: The fusion of male and female pronuclei is called as amphimixis. In vertebrates, the nuclear membranes of both pronuclei (male and female) are broken down at the point of contact and their contents unite in one mass surrounded by a common nuclear membrane. At the approach of first cleavage of fertilized egg, the nuclear membrane dissolves, chromosomes of maternal and paternal origin become arranged on the equator of the achromatic spindle.

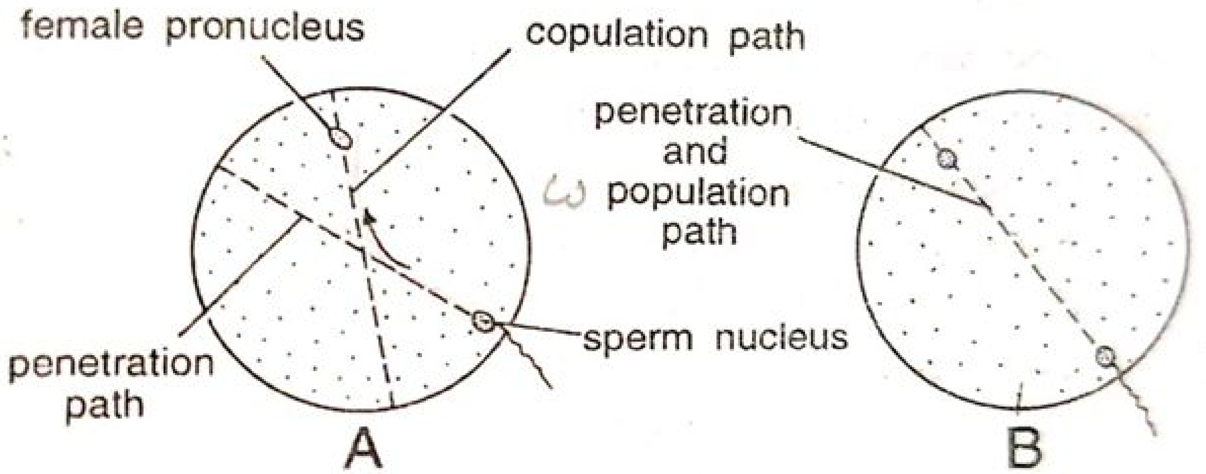

Fig. 8. Possible sperm paths during fertilization.

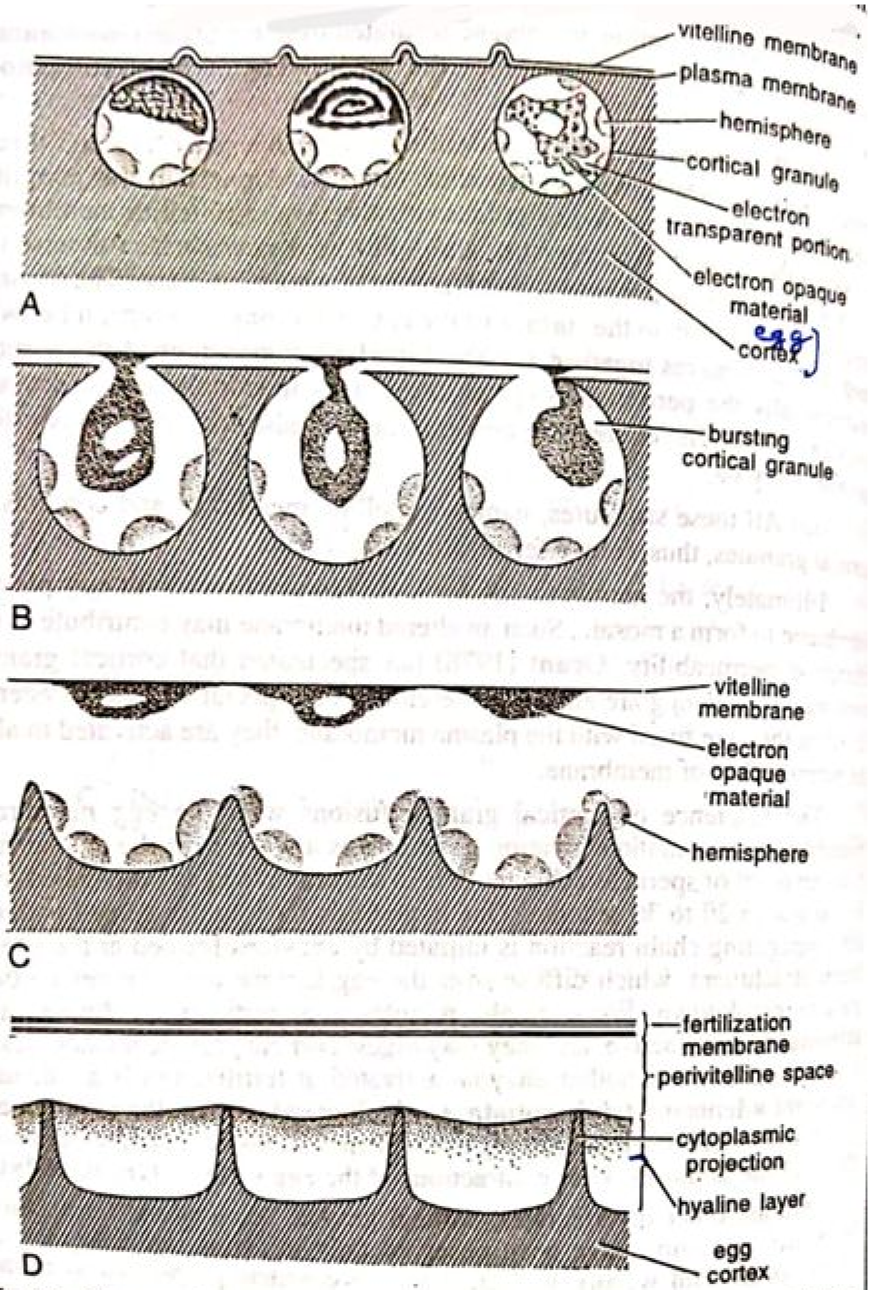

Fig. 8.9. Changes of the egg cortex of the sea urchin *Clypeaster japonicus* following fertilization. A—Unfertilized egg; B—The explosion of the cortical granules; C—Adhesion of electron-opaque material to the vitelline membrane now lifted up and formation of fertilization membrane. The hemispheric bodies remain at the surface of the egg and give rise to the hyaline layer; D—The egg surface upon completion of these changes (After Berrill, 1971).