



**BSCZO- 203**

**B. Sc. II YEAR  
DEVELOPMENTAL BIOLOGY &  
APPLIED ZOOLOGY**



**DEPARTMENT OF ZOOLOGY  
SCHOOL OF SCIENCES  
UTTARAKHAND OPEN UNIVERSITY**

**BSCZO-203**

# **DEVELOPMENTAL BIOLOGY & APPLIED ZOOLOGY**



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## UNIT: 1 GAMETOGENESIS

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## ***1.1 OBJECTIVES***

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- 1 To understand the Basic concept of gametogenesis.
- 2 To study their types of eggs, Spermatogenesis and Spermatogenesis.
- 3 To describe Chemical and metabolic events during gamete formation.

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## ***1.2 INTRODUCTION***

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**Gametogenesis** is a biological process by which diploid or haploid precursor cells undergo cell division and differentiation to form mature haploid gametes. Depending on the biological life cycle of the organism, gametogenesis occurs by meiotic division of diploid gametocytes into various gametes, or by mitotic division of haploid gametogenous cells. For example, plants produce gametes through mitosis in gametophytes. The gametophytes grow from haploid spores after sporic meiosis. The existence of a multicellular, haploid phase in the life cycle between meiosis and gametogenesis is also referred to as alternation of generations.

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## ***1.3 BASIC CONCEPT OF GAMETOGENESIS***

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Gametogenesis is the process by which male and female sex cells or gametes, i.e., sperms and ova are formed respectively in the male and female gonads (testes and ovaries). The gametes differ from all other cells (= somatic cells) of the body in that their nuclei contain only half the number of chromosomes found in the nuclei of somatic cells. Meiosis forms the most significant part of process of gametogenesis. Gametogenesis for the formation of sperms is termed spermatogenesis, while that of ova is called oogenesis. Both spermatogenesis and oogenesis comprise similar phases of sequential changes.



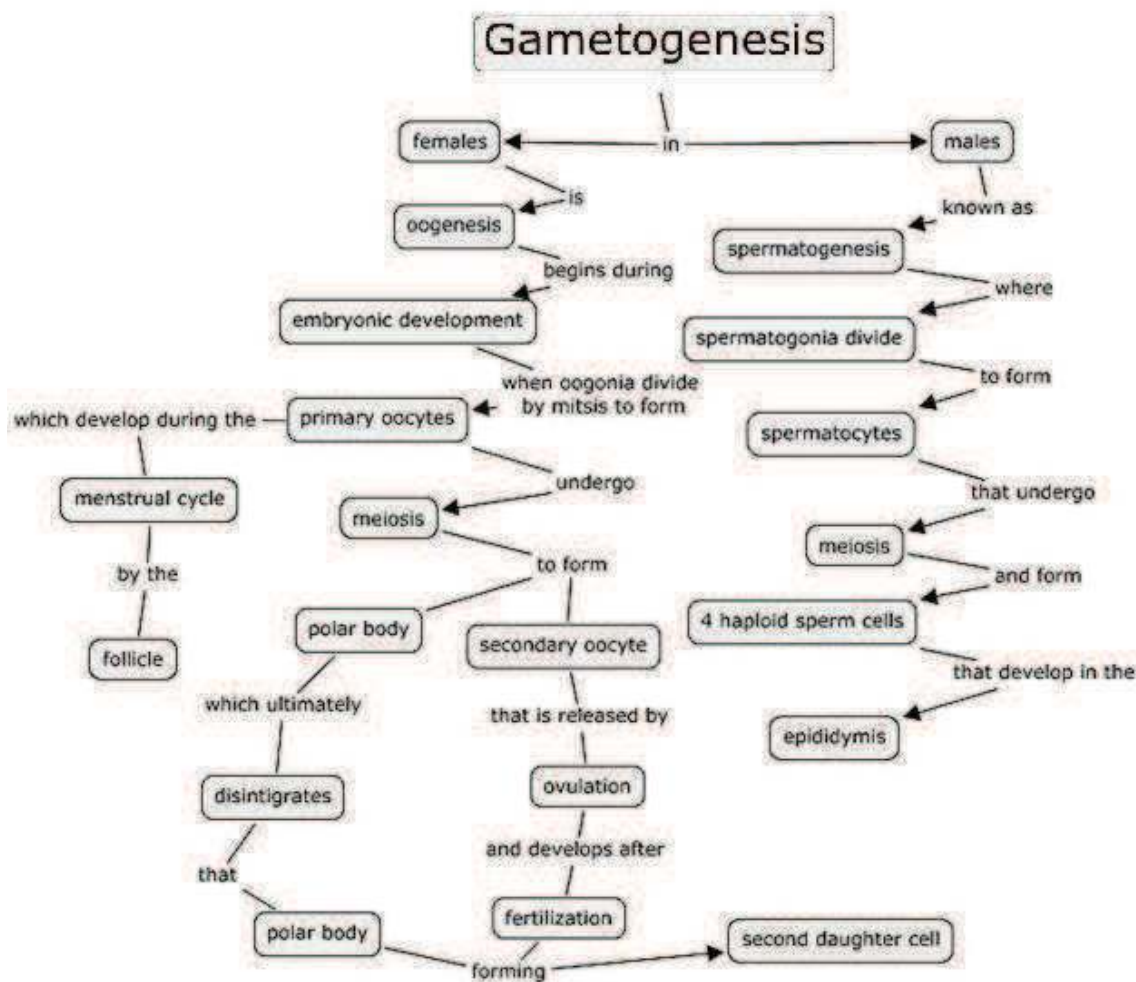


Fig. 1.1 Gametogenesis

### 1.3.1 TYPES OF EGGS

**A. Based on the quantity of yolk, the eggs are of the following types:**

**(1) Alecithal eggs:** The ova or eggs with no yolk are called alecithal. But this is idealistic feature because even in man and other eutherian mammals where egg size is smallest, the egg cytoplasm is not completely free from yolk.

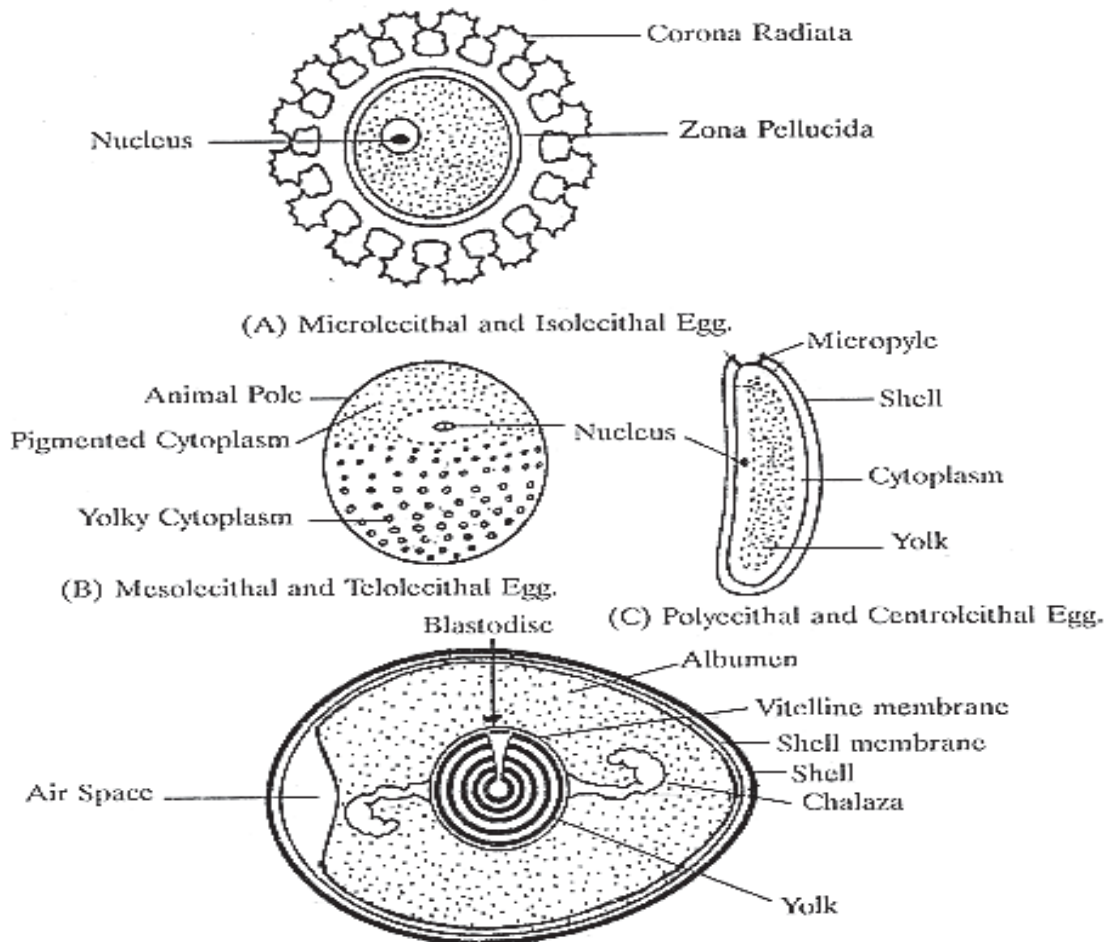
**(2) Microlecithal eggs:** They contain very small amount of yolk, e.g., eggs of sea urchin, tunicates, and amphioxus. In marsupials (kangaroo) and eutherian mammals (man) eggs contain very little amount of yolk and hence these eggs are called alecithal (almost free of yolk).

**(3) Mesolecithal eggs:**

These eggs contain moderate amount of yolk, e.g., eggs of *Petromyzon* (lamprey), lung fish, frogs and toads.

**(4) Macrolecithal eggs:**

They contain large amount of yolk, e.g., eggs of insects, sharks, bony fishes, reptiles, birds and prototherian mammals (*Fig.1.2*).



*Fig. 1.2 Macrolecithal and Telolccithal Egg.*

**B.** Based on the distribution of yolk in the cytoplasm eggs are of the following types:

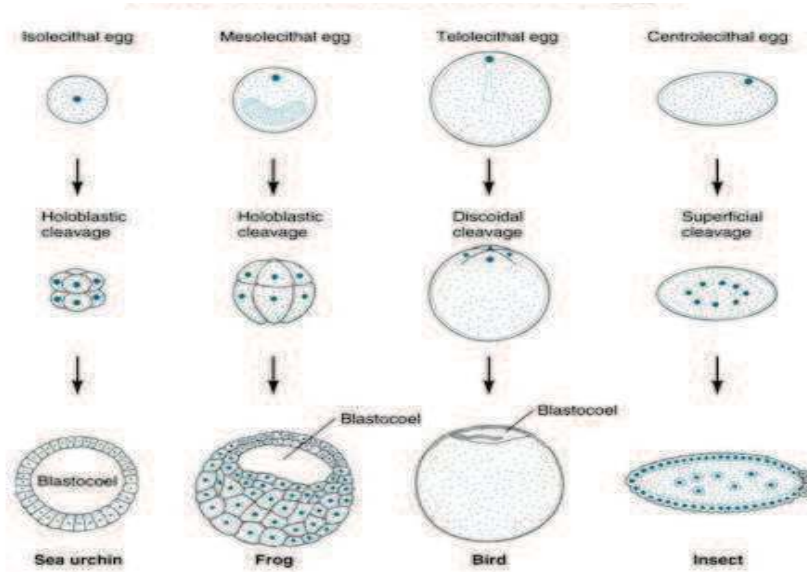


Fig. 1.3 typical cleavage patterns of Isolecithal, Mesolecithal, Telolecithal and Centrolecithal eggs.

**(i) Homolecithal eggs:**

The yolk is uniformly distributed all over the ooplasm (cytoplasm of the egg) e.g., eggs of echinoderms and potochordates.

**(ii) Telolecithal eggs:**

The yolk is concentrated in the vegetal half e.g., eggs of amphibians.

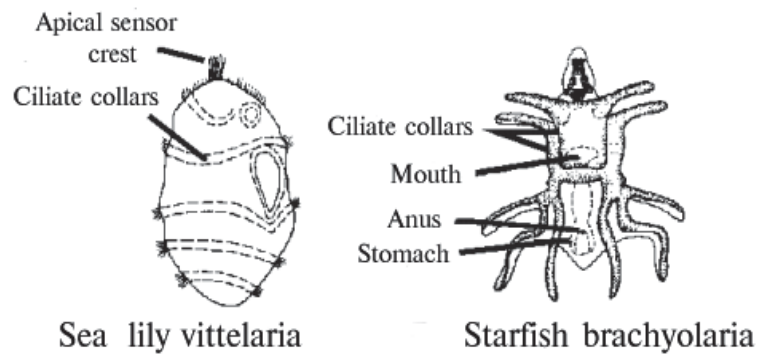


Fig 1.4

**(iii) Meiolecithal eggs:**

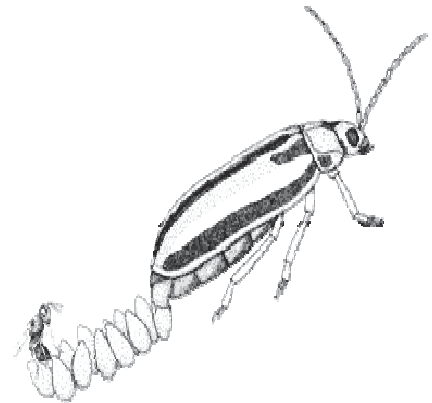
The yolk is very large which occupies nearly the entire ooplasm, leaving free only a small disc-like area of cytoplasm for the nucleus e.g., eggs of reptiles, birds and egg laying mammals.



*Fig 1.5 Meiolecithal eggs*

**(iv) Centrolecithal eggs:**

The yolk is localized at the centre e.g., eggs of insects.



*Fig 1.6 Centrolecithal eggs*

### 1.3.2 SPERMATOGENESIS

(1)

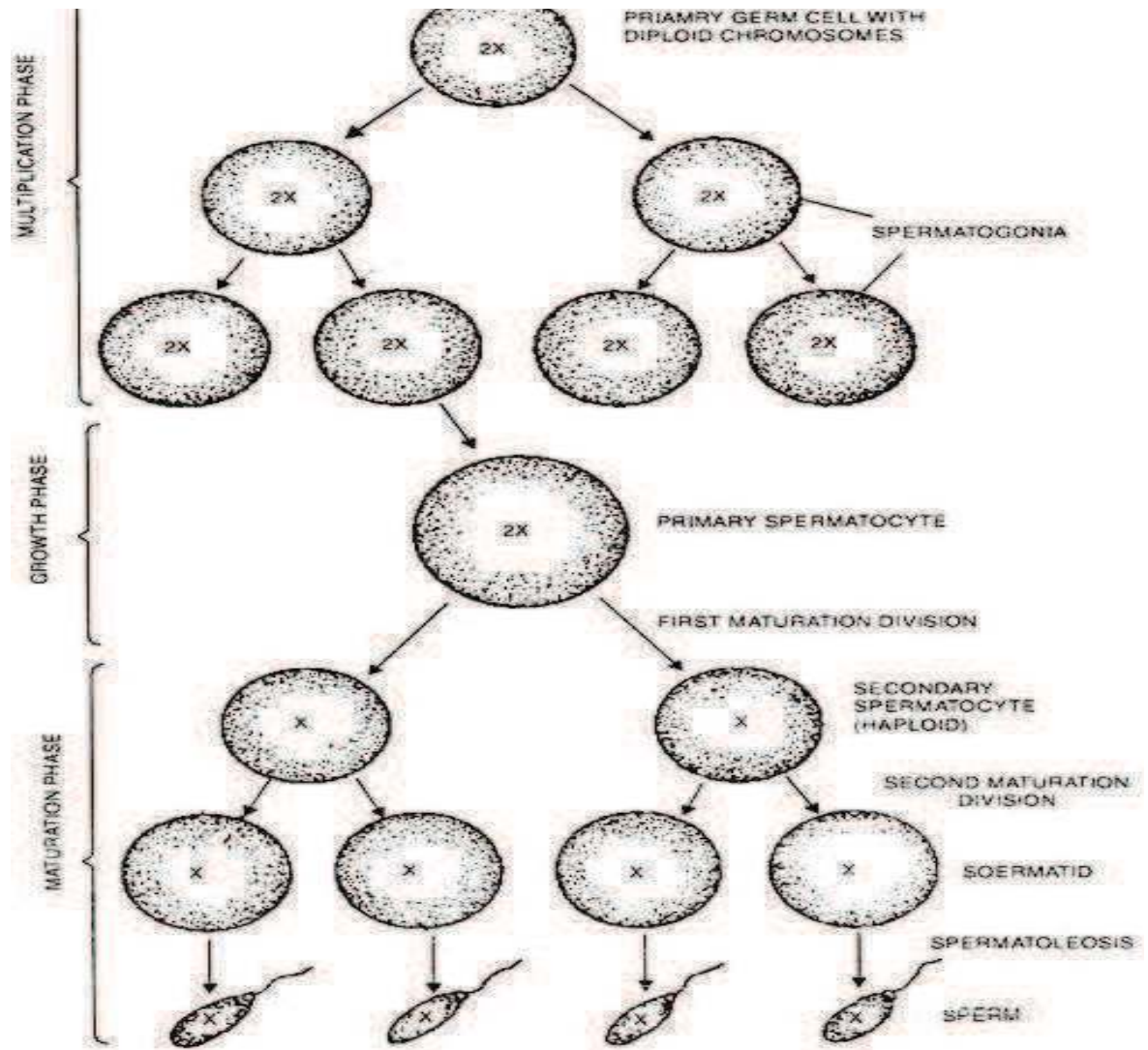


Fig. 1.7 Spermatogenesis

Spermatogenesis process in which spermatozoa are produced from spermatogonial stem cells by way of mitosis and meiosis division. The initial cells in this pathway are called spermatogonia, which yield primary spermatocytes by mitosis. The primary spermatocyte divides meiotically (Meiosis I) into two secondary spermatocytes; each secondary spermatocyte divides into two spermatids by Meiosis II. These develop into mature spermatozoa, also known as sperm cells. Thus, the primary spermatocyte gives rise to two cells, the secondary spermatocytes, and the two secondary spermatocytes by their subdivision produce four spermatozoa.

Spermatozoa are the mature male gametes in many sexually reproducing organisms. Thus, spermatogenesis is the male version of gametogenesis, of which the female equivalent is oogenesis. In mammals it occurs in the seminiferous tubules of the male testes in a stepwise fashion. Spermatogenesis is highly dependent upon optimal conditions for the process to occur correctly, and is essential for sexual reproduction. DNA methylation and histone modification have been implicated in the regulation of this process. It starts at puberty and usually continues uninterrupted until death, although a slight decrease can be discerned in the quantity of produced sperm with increase in age (see Male infertility).

## **(2) Purpose:**

Spermatogenesis produces mature male gametes, commonly called sperm but specifically known as spermatozoa, which are able to fertilize the counterpart female gamete, the oocyte, during conception to produce a single-celled individual known as a zygote. This is the cornerstone of sexual reproduction and involves the two gametes both contributing half the normal set of chromosomes (haploid) to result in a chromosomally normal (diploid) zygote.

To preserve the number of chromosomes in the offspring – which differs between species – each gamete must have half the usual number of chromosomes present in other body cells. Otherwise, the offspring will have twice the normal number of chromosomes, and serious abnormalities may result. In humans, chromosomal abnormalities arising from incorrect spermatogenesis results in congenital defects and abnormal birth defects (Down Syndrome, Klinefelter's Syndrome) and in most cases, spontaneous abortion of the developing fetus.



**(3) Location:**

Spermatogenesis takes place within several structures of the male reproductive system. The initial stages occur within the testes and progress to the epididymis where the developing gametes mature and are stored until ejaculation. The seminiferous tubules of the testes are the starting point for the process, where spermatogonial stem cells adjacent to the inner tubule wall divide in a centripetal direction—beginning at the walls and proceeding into the innermost part, or lumen—to produce immature sperm. Maturation occurs in the epididymis. The location [Testes/Scrotum] is specifically important as the process of spermatogenesis requires a lower temperature to produce viable sperm, specifically 1°-8 °C lower than normal body temperature of 37 °C (98.6 °F). Clinically, small fluctuations in temperature such as from an athletic support strap, causes no impairment in sperm viability or count.

**(4) Duration:**

For humans, the entire process of spermatogenesis is variously estimated as taking 74 days (according to tritium-labelled biopsies) and approximately 120 days (according to DNA clock measurements). Including the transport on ductal system, it takes 3 months. Testes produce 200 to 300 million spermatozoa daily. However, only about half or 100 million of these become viable sperm.

**(5) Stages:**

The entire process of spermatogenesis can be broken up into several distinct stages, each corresponding to a particular type of cell in human. In the following table, ploidy, copy number and chromosome/chromatid counts are for one cell, generally prior to DNA synthesis and division (in G<sub>1</sub> if applicable). The primary spermatocyte is arrested after DNA synthesis and prior to division.

Cell type	ploidy/chromosomes in human	DNA copy number/chromatids in human	Process entered by cell
spermatogonium (types Ad, Ap and B)	diploid (2N) / 46	2C / 46	spermatocytogenesis (mitosis)
primary spermatocyte	diploid (2N) / 46	4C / 2x46	spermatidogenesis (meiosis I)
two secondary spermatocytes	haploid (N) / 23	2C / 2x23	spermatidogenesis (meiosis II)
four spermatids	haploid (N) / 23	C / 23	spermiogenesis
four functional spermatozooids	haploid (N) / 23	C / 23	spermiation

### (6) Spermatocytogenesis

The process of spermatogenesis as the cells progress from primary spermatocytes, to secondary spermatocytes, to spermatids, to SpermSchematic diagram of Spermatocytogenesis

Spermatocytogenesis is the male form of gametocytogenesis and results in the formation of spermatocytes possessing half the normal complement of genetic material. In spermatocytogenesis, a diploid spermatogonium, which resides in the basal compartment of the seminiferous tubules, divides mitotically, producing two diploid intermediate cells called primary spermatocytes. Each primary spermatocyte then moves into the adluminal compartment of the seminiferous tubules and duplicates its DNA and subsequently undergoes meiosis I to produce two haploid secondary spermatocytes, which will later divide once more into haploid spermatids. This division implicates sources of genetic variation, such as random inclusion of either parental chromosomes or chromosomal crossover, to increase the genetic variability of the gamete.

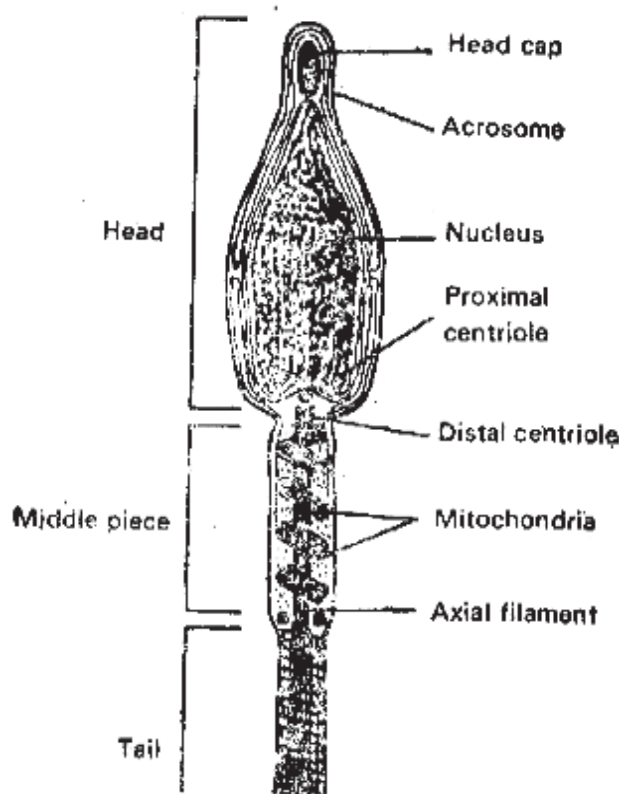
Each cell division from a spermatogonium to a spermatid is incomplete; the cells remain connected to one another by bridges of cytoplasm to allow synchronous development. It



should also be noted that not all spermatogonia divide to produce spermatocytes; otherwise, the supply of spermatogonia would run out. Instead, spermatogonial stem cells divide mitotically to produce copies of them, ensuring a constant supply of spermatogonia to fuel spermatogenesis.

### (7) Structure of sperm:

During spermiogenesis, the spermatids begin to form a tail by growing microtubules on one of the centrioles, which turns into basal body. These microtubules form an axoneme. The anterior part of the tail (called midpiece) thickens because mitochondria are arranged around the axoneme to ensure energy supply. Spermatid DNA also undergoes packaging, becoming highly condensed. The DNA is packaged firstly with specific nuclear basic proteins, which are subsequently replaced with protamines during spermatid elongation. The resultant tightly packed chromatin is transcriptionally inactive.



*Fig 1.8 Structure of sperm*

The Golgi apparatus surrounds the now condensed nucleus, becoming the acrosome. Maturation then takes place under the influence of testosterone, which removes the remaining unnecessary cytoplasm and organelles. The excess cytoplasm, known as residual bodies, is phagocytosed by surrounding Sertoli cells in the testes. The resulting spermatozoa are now mature but lack motility, rendering them sterile. The mature spermatozoa are released from the protective Sertoli cells into the lumen of the seminiferous tubule in a process called spermiation.

The non-motile spermatozoa are transported to the epididymis in testicular fluid secreted by the Sertoli cells with the aid of peristaltic contraction. While in the epididymis the spermatozoa gain motility and become capable of fertilization. However, transport of the mature spermatozoa through the remainder of the male reproductive system is achieved via muscle contraction rather than the spermatozoon's recently acquired motility.

### (8) Role of Sertoli cellss

At all stages of differentiation, the spermatogenic cells are in close contact with Sertoli cells which are thought to provide structural and metabolic support to the developing sperm cells. A single Sertoli cell extends from the basement membrane to the lumen of the seminiferous tubule, although the cytoplasmic processes are difficult to distinguish at the light microscopic level.

Sertoli cells serve a number of functions during spermatogenesis; they support the developing gametes in the following ways:

- a) Maintain the environment necessary for development and maturation, via the blood-testis barrier
- b) Secrete substances initiating meiosis
- c) Secrete supporting testicular fluid

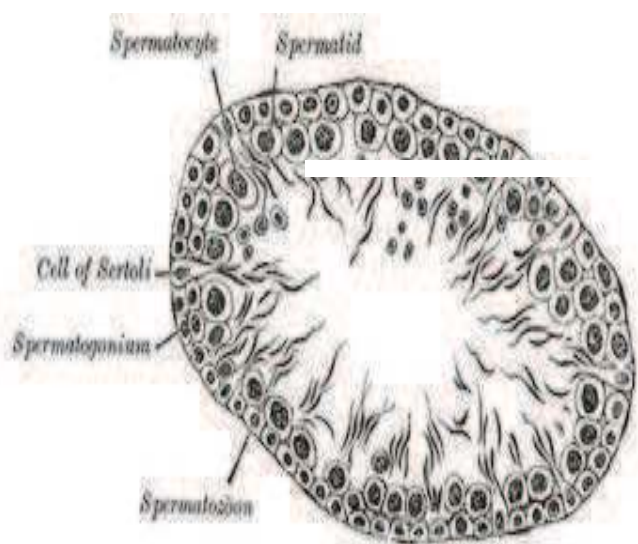


Fig 1.9 Sertoli cell

d) Secrete androgen-binding protein (ABP), which concentrates testosterone in close proximity to the developing gametes.

e) Testosterone is needed in very high quantities for maintenance of the reproductive tract, and ABP allows a much higher level of fertility

d) Secrete hormones affecting pituitary gland control of spermatogenesis, particularly the polypeptide hormone, inhibin.

e) Phagocytose residual cytoplasm left over from spermiogenesis.

- f) Secretion of anti-Müllerian hormone causes deterioration of the Müllerian duct.
- g) Protect spermatids from the immune system of the male, via the blood-testis barrier.
- h) Contribute to the spermatogonial stem cell niche.

The intercellular adhesion molecules ICAM-1 and soluble ICAM-1 have antagonistic effects on the tight junctions forming the blood-testis barrier. ICAM-2 molecules regulate spermatid adhesion on the apical side of the barrier (towards the lumen).

### **(9) Influencing factors**

The process of spermatogenesis is highly sensitive to fluctuations in the environment, particularly hormones and temperature. Testosterone is required in large local concentrations to maintain the process, which is achieved via the binding of testosterone by androgen binding protein present in the seminiferous tubules. Testosterone is produced by interstitial cells, also known as Leydig cells, which reside adjacent to the seminiferous tubules.

Seminiferous epithelium is sensitive to elevated temperature in humans and some other species, and will be adversely affected by temperatures as high as normal body temperature. Consequently, the testes are located outside the body in a sack of skin called the scrotum. The optimal temperature is maintained at 2 °C (man)–8 °C (mouse) below body temperature. This is achieved by regulation of blood flow and positioning towards and away from the heat of the body by the cremasteric muscle and the dartos smooth muscle in the scrotum.

Dietary deficiencies (such as vitamins B, E and A), anabolic steroids, metals (cadmium and lead), x-ray exposure, dioxin, alcohol, and infectious diseases will also adversely affect the rate of spermatogenesis. In addition, the male germ line is susceptible to DNA damage caused by oxidative stress, and this damage likely has a significant impact on fertilization and pregnancy. Exposure to pesticides also affects spermatogenesis.

### **(10) Hormonal control**

Hormonal control of spermatogenesis varies among species. In humans the mechanism is not completely understood; however it is known that initiation of spermatogenesis occurs at puberty due to the interaction of the hypothalamus, pituitary gland and Leydig cells. If the pituitary gland is removed, spermatogenesis can still be initiated by follicle stimulating hormone (FSH) and testosterone. In contrast to FSH, LH appears to have little role in spermatogenesis outside of inducing gonadal testosterone production.

FSH stimulates both the production of androgen binding protein (ABP) by Sertoli cells, and the formation of the blood-testis barrier. ABP is essential to concentrating testosterone in levels high enough to initiate and maintain spermatogenesis. Intratesticular testosterone levels are 20–100 or 50–200 times higher than the concentration found in blood, although there is variation over a 5- to 10-fold range amongst healthy men. FSH may initiate the sequestering of testosterone in the testes, but once developed only testosterone is required to maintain spermatogenesis. However, increasing the levels of FSH will increase the production of spermatozoa by preventing the apoptosis of type A spermatogonia. The hormone inhibin acts to decrease the levels of FSH. Studies from rodent models suggest that gonadotropins (both LH and FSH) support the process of spermatogenesis by suppressing the proapoptotic signals and therefore promote spermatogenic cell survival.

The Sertoli cells themselves mediate parts of spermatogenesis through hormone production. They are capable of producing the hormones estradiol and inhibin. The Leydig cells are also capable of producing estradiol in addition to their main product testosterone. Estrogen has been found to be essential for spermatogenesis in animals. However, a man with estrogen insensitivity syndrome (a defective  $ER\alpha$ ) was found produce sperm with a normal sperm count, albeit abnormally low sperm viability; whether he was sterile or not is unclear. Levels of estrogen that are too high can be detrimental to spermatogenesis due to suppression of gonadotropin secretion and by extension intratesticular testosterone production. Prolactin also appears to be important for spermatogenesis.

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### 1.3.3 OOGENESIS

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- (1) **Oogenesis:** **Ovogenesis** or **oögenesis** is the creation of an ovum (egg cell). It is the female form of gametogenesis; the male equivalent is spermatogenesis. It involves the development of the various stages of the immature ovum.
- (2) **Oogenesis in mammals-** Diagram showing the reduction in number of the chromosomes in the process of maturation of the ovum. (In mammals, the first polar body normally disintegrates before dividing, so only two polar bodies are produced.)

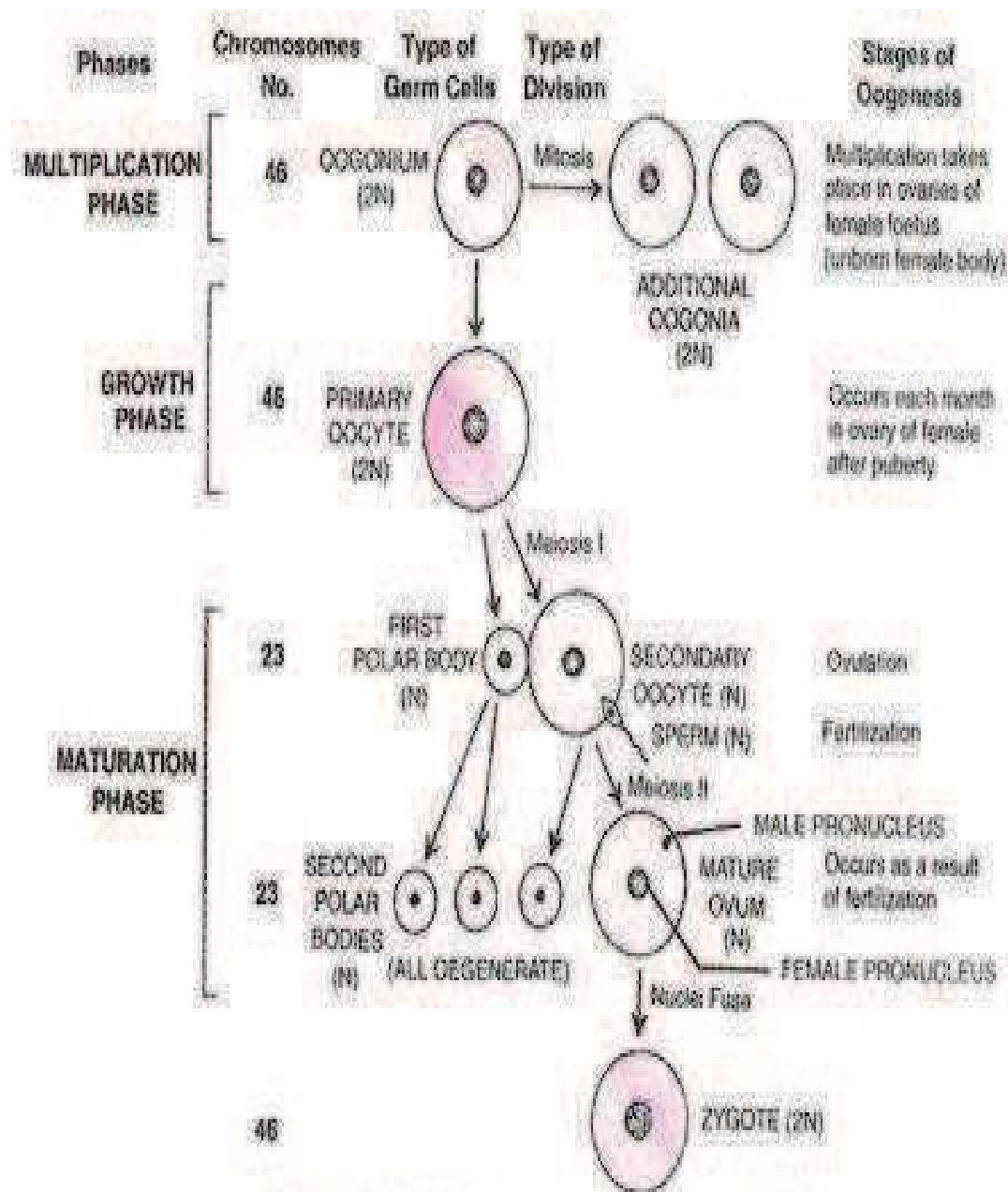


Fig 1.10 Stage in oogenesis (diagrammatic)

In mammals, the first part of oogenesis starts in the germinal epithelium, which gives rise to the development of ovarian follicles, the functional unit of the ovary.

Oogenesis consists of several sub-processes: oocytogenesis, ootidogenesis, and finally maturation to form an ovum (oogenesis proper). Folliculogenesis is a separate sub-process that accompanies and supports all three oogenetic sub-processes.

Oogonium —(Oocytogenesis)—> Primary Oocyte —(Meiosis I)—> First Polar Body (Discarded afterward) + Secondary oocyte —(Meiosis II)—> Second Polar Body (Discarded afterward) + Ovum

It should be noted that oocyte meiosis, important to all animal life cycles yet unlike all other instances of animal cell division, occurs completely without the aid of spindle-coordinating centrosomes.

### (3) The creation of oögonia

The creation of oögonia traditionally doesn't belong to oögenesis proper, but, instead, to the common process of gametogenesis, which, in the female human, begins with the processes of folliculogenesis, oocytogenesis, and ootidogenesis.

### (4) Maturation of the oocyte in amphibians

The egg is responsible for initiating and directing development, and in some species (as seen above), fertilization is not even necessary. The accumulated material in the oocyte cytoplasm includes energy sources and energy-producing organelles (the yolk and mitochondria); the enzymes and precursors for DNA, RNA, and protein syntheses; stored messenger RNAs; structural proteins; and morphogenetic regulatory factors that control early embryogenesis. A partial catalogue of the materials stored in the oocyte cytoplasm, while a partial list of stored mRNAs. Most of this accumulation takes place during meiotic prophase I, and this stage is often subdivided into two phases, **previtellogenesis** (Greek, “before yolk formation”) and **vitellogenesis**. Cellular components stored in the mature oocyte of *Xenopus laevis*.

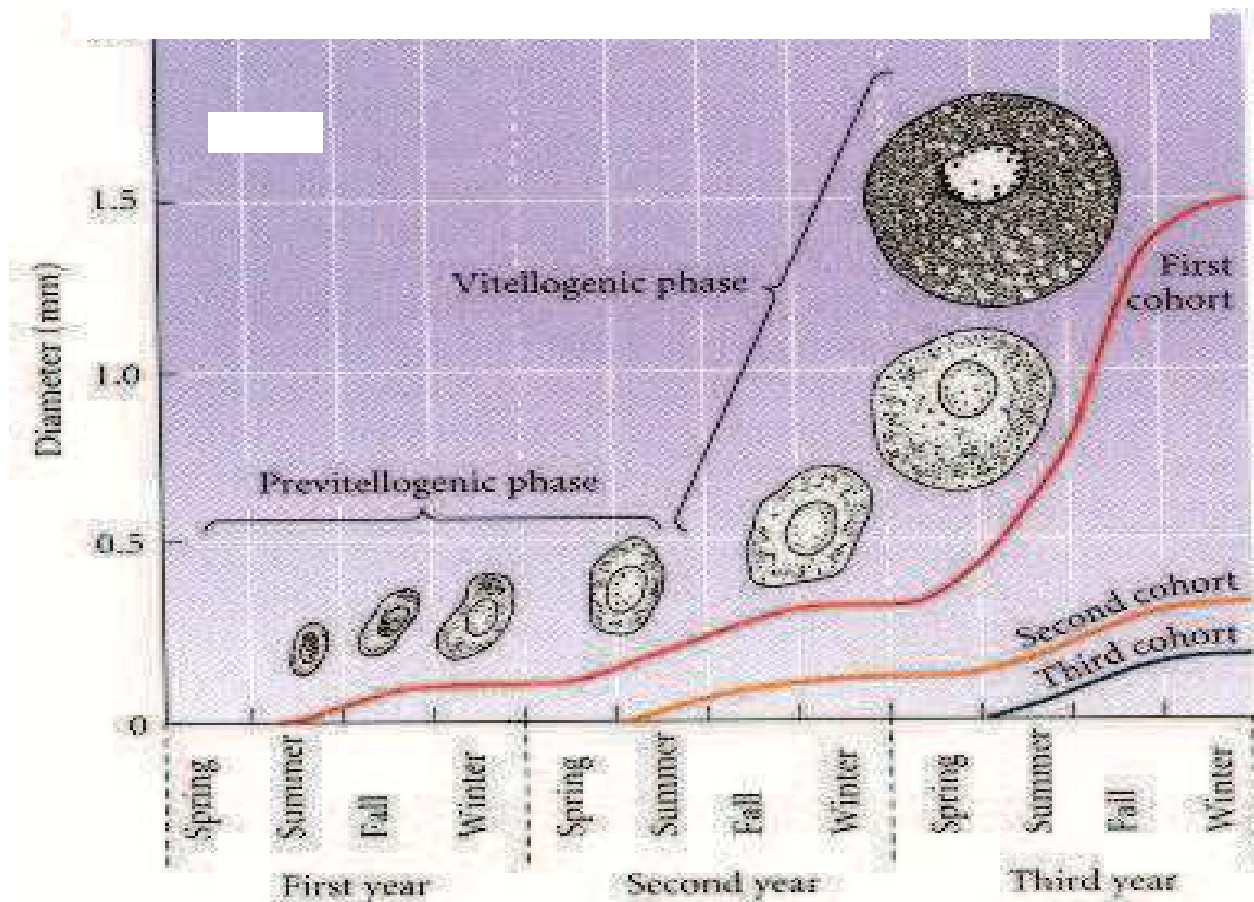


Fig 1.11 Growth of amphibian oocyte

The eggs of fishes and amphibians are derived from an oogonial stem cell population that can generate a new cohort of oocytes each year. In the frog *Rana pipiens*, oogenesis takes 3 years. During the first 2 years, the oocyte increases its size very gradually. During the third year, however, the rapid accumulation of yolk in the oocyte causes the egg to swell to its characteristically large size). Eggs mature in yearly batches, with the first cohort maturing shortly after metamorphosis; the next group matures a year later.

Growth of oocytes in the frog. During the first 3 years of life, three cohorts of oocytes are produced. The drawings follow the growth of the first-generation oocytes. (After Grant 1953.)

Vitellogenesis occurs when the oocyte reaches the diplotene stage of meiotic prophase. Yolk is not a single substance, but a mixture of materials used for embryonic nutrition. The major yolk component in frog eggs is a 470-kDa protein called **vitellogenin** is synthesized in the liver and carried by the bloodstream to the ovary (Flickinger and Rounds 1956). This large



protein passes between the follicle cells of the ovary, and is incorporated into the oocyte by **micropinocytosis**, the pinching off of membrane-bounded vesicles at the bases of microvilli (Dumont 1978). In the mature oocyte, vitellogenin is split into two smaller proteins: the heavily phosphorylated **phosvitin** and the lipoprotein **lipovitellin**. These two proteins are packaged together into membrane-bounded **yolk platelets**). Glycogen granules and lipochondrial inclusions store the carbohydrate and lipid components of the yolk, respectively.

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### 1.3.4 CHEMICAL AND METABOLIC EVENTS DURING GAMETE FORMATION

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(1) **Metabolism** is the set of life-sustaining chemical transformations within the cells of living organisms. The three main purposes of metabolism are the conversion of food/fuel to energy to run cellular processes, the conversion of food/fuel to building blocks for proteins, lipids, nucleic acids, and some carbohydrates, and the elimination of nitrogenous wastes. These enzyme-catalyzed reactions allow organisms to grow and reproduce, maintain their structures, and respond to their environments. The word metabolism can also refer to the sum of all chemical reactions that occur in living organisms, including digestion and the transport of substances into and between different cells, in which case the set of reactions within the cells is called intermediary metabolism or intermediate metabolism.

(2) Metabolism is usually divided into two categories: catabolism, the *breaking down* of organic matter, for example, by cellular respiration, and anabolism, the *building up* of components of cells such as proteins and nucleic acids. Usually, breaking down releases energy and building up consumes energy.

(3) The chemical reactions of metabolism are organized into metabolic pathways, in which one chemical is transformed through a series of steps into another chemical, by a sequence of enzymes. Enzymes are crucial to metabolism because they allow organisms to drive desirable reactions that require energy that will not occur by themselves, by coupling them to spontaneous reactions that release energy. Enzymes act as catalysts that allow the reactions to proceed more rapidly. Enzymes also allow the regulation of metabolic pathways in response to changes in the cell's environment or to signals from other cells.

(4) The metabolic system of a particular organism determines which substances it will find nutritious and which poisonous. For example, some prokaryotes use hydrogen sulfide as



a nutrient, yet this gas is poisonous to animals. The speed of metabolism, the metabolic rate, influences how much food an organism will require, and also affects how it is able to obtain that food.

(5) A striking feature of metabolism is the similarity of the basic metabolic pathways and components between even vastly different species. For example, the set of carboxylic acids that are best known as the intermediates in the citric acid cycle are present in all known organisms, being found in species as diverse as the unicellular bacterium *Escherichia coli* and huge multicellular organisms like elephants. These striking similarities in metabolic pathways are likely due to their early appearance in evolutionary history, and their retention because of their efficacy.

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## 1.4. SUMMARY

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(1) **Gametogenesis**, by definition, is the development of mature haploid gametes from either haploid or diploid precursor cells. The precursor cells undergo cell division in order to become **gametes**. This may sound like a very technical definition, but by the end of this lesson you'll understand it.

(2) Organisms can be either **diploid** or **haploid**. Those that are diploid, like you and me, have two copies of their DNA per cell. Those that are haploid have one copy of their DNA per cell. As mentioned in the definition, gametes are all haploid. So, if you are already a haploid cell, you undergo regular cell division (**mitosis**). However, if you are diploid you have to make haploid gametes. That is, you have to create cells with only one copy of DNA each. This is also done by a special type of cell division called **meiosis**.

During the process of **mitotic cell division** a cell makes a complete copy of its DNA. Then, when the cell divides, the DNA is split between the two daughter cells. Thus, each daughter cell gets a complete, exact copy of the parent cell's genetic information (DNA). This type of cell division is a one-step process.

(3) **Meiotic cell division** is a two-step process. Meiosis begins with a diploid cell that has two copies of DNA. One comes from the father and one from the mother. The cell divides twice producing four haploid cells. The first division is called **Meiosis I**. It involves replication of chromosomes but allows 'gene shuffling' between the maternal and paternal chromosomes. The second division is called **Meiosis II**. It results in haploid cells.

(4) What is gene shuffling? Imagine you have a red blanket from your dad and a light blue one from your mom. Mitotic cell division keeps the blankets (or chromosomes) separate. So, all cells have red blankets and light blue blankets. Meiosis allows the cells to make a patchwork quilt out of mom and dad's blankets. This creates genetic diversity which is an advantage to sexual reproduction. The general steps for meiosis are described in the first figure.

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## 1.5 GLOSSARY

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<b>Allele</b>	-An alternative form of a gene.
<b>Anther</b>	-The terminal pollen sac of a stamen, inside which pollen grains with male gametes form in the flower of an angiosperm
<b>Binary fission</b>	-the type of cell divisions by which prokaryotes reproduce; each dividing daughter cell receives a copy of the single parental chromosome
<b>Budding</b>	-A method of asexual reproduction common in some lower animal groups in which part of the body wall bulges outward and eventually forms a new individual, which becomes detached from the parent. Budding can also occur in single-celled organisms such as yeast
<b>Carbohydrate</b>	-A family of organic molecules with the general formula $(CH_2O)_x$ , ranging from simple sugars, such as glucose and fructose, to complex molecules, such as starch and cellulose.
<b>Cell membrane</b>	-the outer boundary of cells, the structure of which is visible only under the electron microscope.
<b>Cellulose</b>	-A type of unbranched polysaccharide carbohydrate that is composed of glucose sugars.
<b>Cellular respiration</b>	-the most prevalent and efficient catabolic pathway for the production of ATP, in which oxygen is consumed as a reactant along with the organic fuel
<b>Centromere</b>	-The centralized region joining two sister chromatids.
<b>Chromatid</b>	-One of a pair of duplicated chromosomes produced during the S phase of the cell cycle, which are joined together at the centromere
<b>Chromatin</b>	-the aggregate mass of dispersed genetic material formed of DNA and protein and observed between periods of cell division in eukaryotic

	cells.
<b>Chromosome</b>	-a long, threadlike association of genes in the nucleus of all eukaryotic cells and most visible during mitosis and meiosis. Chromosomes consist of DNA and protein.
<b>Cilium</b> (plural, <b>cilia</b> )	-A short cellular appendage specialized for locomotion.
<b>Clone</b>	-A lineage of genetically identical individuals.
<b>Coarse adjustment knob</b>	-knob located on the arm of a microscope used to obtain an approximate focus.
<b>Condenser</b>	-concentrates light from the illuminator below.
<b>Contractile vacuole</b>	An organelle that pumps excess water out of many freshwater protist cells.
<b>Crossing over</b>	-the reciprocal exchange of genetic material between non-sister chromatids during synapsis of meiosis I.
<b>Cytokinesis</b>	-the division of the cytoplasm to form two separate daughter cells immediately after mitosis.
<b>Cytoplasm</b>	-the entire contents of the cell, exclusive of the nucleus, and bounded by the plasma membrane.
<b>Diffusion</b>	-The natural effect of a solute moving from an area of higher concentration to an area of lower concentration
<b>Ectotherm</b>	-An animal, such as a reptile, fish, or amphibian, that must use environmental energy and behavioral adaptations to regulate its body temperature.
<b>Double stranded</b>	-two adjacent strands. For example DNA has two adjacent polynucleotide strands wound into a spiral shape
<b>Endoplasmic reticulum</b>	-a series of interconnected, flattened cavities lined with a membrane about 4 nm thick, which is continuous with the nuclear membrane.
<b>Endotherm</b>	-An animal that uses metabolic energy to maintain a constant body temperature, such as a bird or mammal.
<b>Energy of activation</b>	-The energy required to initiate a (bio) chemical reaction.
<b>Fat (triacylglycerol)</b>	-A biological compound consisting of three fatty acids linked to one glycerol molecule.
<b>Fine adjustment knob</b>	sed for minor adjustments in the focal length of a slide at high magnifications.

<b>Focal length</b>	-the distance from the object at which the objective lens is in focus
<b>Fructose</b>	-A simple carbohydrate (monosaccharide) that is a structural isomer of glucose and considered to be an atypical ketose
<b>Gametes</b>	-Haploid egg or sperm cells that unite during sexual reproduction to produce a diploid zygote.
<b>Gametogenesis</b>	The process where haploid gametes are produced from diploid cells via meiosis. In animals the two processes are spermatogenesis and oogenesis.
<b>Gene</b>	-One of many discrete units of hereditary information located on the chromosomes and consisting of DNA.
<b>Glucose</b>	- ( $C_6H_{12}O_6$ ) An important monosaccharide (simple carbohydrate) that acts as a primary energy supply for both plant and animal cells.
<b>Glycogen</b>	-An extensively branched glucose storage polysaccharide found in the liver and muscle of animals; the animal equivalent of starch
<b>Gonads</b>	-The male and female sex organs; the gamete-producing organs in most animals.
<b>Haploid</b>	-Referring to a cell nucleus it contains one of each type of chromosome.
<b>Illuminator</b>	-The light source on a microscope
<b>Isoosmotic (isotonic)</b>	-Refers to the concentration of solutes on either side of a semi-permeable membrane being equal, resulting in no net movement of water molecules across the membrane.
<b>Karyokinesis</b>	-The division of the cell nucleus.
<b>Leaf</b>	-A thin organ arising from the node on the stem of a plant. The main site of photosynthesis.
<b>Lipid</b>	-One of a family of compounds, including fats, phospholipids, and steroids, that are insoluble in water.
<b>Locus (pl. Loci)</b>	-A particular place along the length of a certain chromosome where a given gene is located.
<b>Lysosome</b>	-A membrane-enclosed bag of hydrolytic enzymes found in the cytoplasm of eukaryotic cells.
<b>Meiosis</b>	-A type of nuclear division associated with sexual reproduction, producing four haploid cells from a single diploid cell, the process

	involving two cycles of division.
<b>Metabolism</b>	-The totality of an organism's chemical processes, consisting of catabolic and anabolic pathways.
<b>Microsporangium</b>	-The sporangium from which the microspores are formed, which in higher plants is the pollen sac.
<b>Microspore</b>	-The smaller of the two types of spore produced by ferns and higher plants, giving rise to the male gametophyte. In Tracheophytes the microspore is the pollen grain
<b>Microsporocytes</b>	-Thousands of cells (pollen mother cells) found within a young microsporangium.
<b>Msds (material safety data sheets)</b>	One of the three elements of WHMIS, consisting of a technical bulletin which provides more detailed information about a hazardous product.
<b>Nucleolus (plural, nucleoli)</b>	-A specialized structure in the nucleus, formed from various chromosomes and active in the synthesis of ribosomes.
<b>Objective</b>	-A magnifying element found on the revolving nosepiece of a microscope.
<b>Ocular</b>	-The eye-piece of a microscope which serves to magnify the object.
<b>Oogenesis</b>	-The process in the ovary that results in the production of female gametes.
<b>Origin of life</b>	-The process by which biomolecules, subcellular structures, and living cells have come into existence.
<b>Osmosis</b>	-The net movement of water molecule across the cell membrane towards areas of higher solute concentration
<b>Ovary</b>	-In flowers, the portion of a carpel in which the egg-containing ovules develop. In animals, the structure that produces female gametes and reproductive hormones.
<b>Oviduct</b>	-A tube passing from the ovary to the vagina in invertebrates or to the uterus in vertebrates
<b>Oxygen</b>	-A colourless, tasteless gas forming about 21% of Earth's atmosphere and capable of combining with all other elements except the inert gases
<b>Oxygenated blood</b>	-Blood that has become enriched with oxygen as it exchanges with the

	lungs
<b>Phagocytosis</b>	-A type of endocytosis involving large, particulate substances.
<b>Plasma membrane</b>	-The outer boundary of cells which is only visible with an electron microscope.
<b>Plasmalemma</b>	-The cell membrane that also lines the connecting plasmodesmata between living cells.
<b>Poikilothermic</b> (ectotherm)	-Any animal whose body temperature follows that of the surrounding environment.
<b>Protein</b>	-A three-dimensional biological polymer constructed from a set of 20 different monomers called amino acids.
<b>Pyruvic acid</b>	-An important 3-carbon molecule formed from glucose and glycerol in glycolysis.
<b>Reducing agent</b>	-Any substance capable of removing oxygen from a molecule or of adding hydrogen, that is, it is capable of contributing electrons to a process.
<b>Respiration</b>	-A process by which gaseous exchange -oxygen and carbon dioxide-takes place between an organism and the surrounding medium.
<b>Ribosome</b>	-A cell organelle constructed in the nucleolus, consisting of two subunits and functioning as the site of protein synthesis in the cytoplasm
<b>Rough endoplasmic reticulum</b>	-Endoplasmic reticulum when it is covered with ribosomes is referred to as rough E
<b>Septum</b> (plural, <b>septa</b> )	-Any dividing wall or partition that occurs between structures or in a cavity.
<b>Sex cells</b>	-Gametes. In the male it is the sperm and in the female it is the egg.
<b>Sexual reproduction</b>	-A type of reproduction in which two parents give rise to offspring that have unique combinations of genes inherited from the gametes of the two parents.
<b>Smooth endoplasmic reticulum</b>	-Endoplasmic reticulum that is not covered with ribosomes and gives rise to the Golgi Apparatus.
<b>Sperm</b>	Spermatozoon; a small, usually motile male gamete

<b>Somatic cell</b>	-Any of the cells of a plant or animal except the reproductive cells.
<b>Starch</b>	-A polysaccharide carbohydrate consisting of two forms of glucose units, amylose and amylopectin.
<b>Storage material</b>	-Any compound that accumulates naturally within a cell, for example, the starch grains of potato tubers and glycogen in liver cells.
<b>Stroma</b>	-The fluid of the chloroplast surrounding the thylakoid membrane; involved in the synthesis of organic molecules from carbon dioxide and water
<b>Testis (plural, testes)</b>	-The male reproductive organ, or gonad, in which sperm and reproductive hormones are produced.
<b>Thylakoid</b>	-A flattened membrane sac inside the chloroplast, used to convert light energy to chemical energy
<b>Tissue</b>	-A large group of cells of similar structure in plants or animals that performs a specific function. (ex. muscle, phloem, etc.)
<b>Unicellular</b>	-Made up of one cell.
<b>Uterus</b>	-The enlarged posterior portion of the oviduct in which the embryo implants and develops in viviparous species. It is also called the womb of female humans.
<b>Vital stains</b>	-The staining of cells while alive, which has been used particularly for studying the movements of parts of embryos
<b>Zygote</b>	-The diploid product of the union of haploid gametes in conception; a fertilized egg.

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## ***1.6 SELF-ASSESSMENT QUESTION***

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- Q1 Give an account of the process of spermatogenesis?
- Q2 What is spermatogenesis? Describe the various steps of this process.
- Q3 Describe the metamorphosis of spermatozoon?
- Q4 What is gametogenesis? Describe the process of spermatogenesis and its significance in a Mammal.
- Q5 What is oogenesis? Give an account of oogenesis. State the difference between oogenesis and spermatogenesis?
- Q6 Classify with example the various types of eggs?

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## ***1.7 TERMINAL QUESTIONS/ANSWER***

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### **SHORT QUESTION**

- Q.1** Why there is unequal division of egg cytoplasm during oogenesis?
- Q.2** Why sperm are motile?
- Q.3** Why Lamp brush chromosome are formed in large yolk egg?
- Q.4** Draw the diagram of sperm?
- Q.5** Difference between Macrolecithal eggs a Microlecithal eggs.

### **Multiple Choice Questions:**

- Q.1** Which of the following cells is normally diploid?
- (a) First polar body
  - (b) Spermatid
  - (c) Spermatozoa
  - (d) Primary spermatocytes
- Q.2** Spermatozoa are nourished during their development by
- (a) Sertoli cell
  - (b) Interstitial cell
  - (c) Nurse cells
  - (d) Germinal epithelial cells
- Q.3** Maturation of spermatozoa occurs in
- (a) Epididymis
  - (b) Vas deferens
  - (c) Prostate
  - (d) None of the above
- Q.4** Partially phosphorylated phosphovitin protein is
- (a) Insoluble in water
  - (b) Soluble in water
  - (c) Indigestible
  - (d) None of these
- Q.5** Enzyme protein kinase is present in



- (a) Acrosome of sperm
  - (b) Mitochondria of all ova
  - (c) Mitochondria of yolky eggs
  - (d) Lysosomes
- Q.6 The follicle cells surrounding the mammalian oocyte form
- (a) Zona radiata
  - (b) Corona radiate
  - (c) Zona pellucid
  - (d) Jelly coat
- Q.7 The release of eggs from ovary is called
- (a) Oviparity
  - (b) Ovulation
  - (c) Oviposition
  - (d) Parturition
- Q.8 In large yolky eggs, the yolky component is called
- (a) Ooplasm
  - (b) Deutoplasm
  - (c) Cytoplasm
  - (d) None of these
- Q.9 Vertebrates, the protein yolk is synthesized in
- (a) Yolk nucleus of oocytes
  - (b) Follicle cells
  - (c) Nurse cells
  - (d) Liver cells
- Q.10 Acrosomal vesicle of sperm is derived from
- (a) Golgi apparatus
  - (b) Endoplasmic reticulum
  - (c) lysosome
  - (d) Peroxisome

**Answers**

1 (d) 2 (a) 3 (a) 4 (b) 5 (c) 6 (b) 7 (b) 8 (b) 9 (d) 10 (a)

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## ***1.7 REFERENCES***

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1. **Varma P.S**, A Text Book of Embryology.
2. **Agrwal V.K**, A text Book of Zoology.
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## UNIT: 2 FERTILIZATION

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### ***CONTENTS***

2.1 Objectives

2.2 Introduction

2.3 Basic concept of fertilization

2.3.1- Approximation of gametes.

2.3.2- Acrosome reaction

2.3.3- Formation of fertilization membrane

2.3.4- Egg activation

2.3.5- Prevention of polyspermy

2.4 Summary

2.5 Glossary

2.6 Self-assessment question

2.7 Terminal questions/answer

2.8 References

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## 2.1 OBJECTIVES

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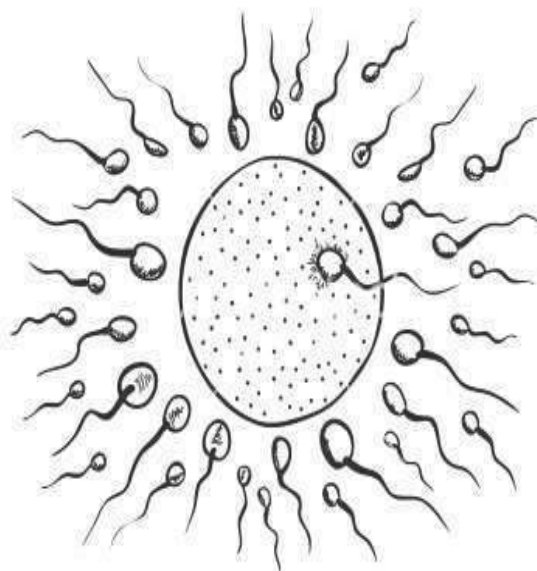
- 4 To understand the Basic concept of Fertilization.
- 5 To study of approximation of gametes, Acrosome reaction, formation of fertilization membrane and egg activation.
- 6 To describe prevention of polyspermy

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## 2.2 INTRODUCTION

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Fertilization also known as generative fertilization, conception, fecundation, syngamy and impregnation, is the fusion of gametes to initiate the development of a new individual organism. In animals, the process involves the fusion of an ovum with a sperm, which first creates a zygote and then leads to the development of an embryo. Depending on the animal species, the process can occur within the body of the female (internal fertilization), or outside (external fertilization). The cycle of fertilization and development of new individuals is



*Fig. 2.1 fertilization*

called sexual reproduction. During double fertilization in angiosperms the haploid male gamete combines with two haploid polar nuclei to form a triploid primary endosperm nucleus by the process of vegetative fertilization.

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## 2.3 BASIC CONCEPT OF FERTILIZATION

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Human fertilization is a complicated process that results in a fertilized egg. The fertilized egg will mature in the womb of its mother until birth. This lesson will go over the process, basic definition, and some symptoms of human fertilization.

### 2.3.1 APPROXIMATION OF GAMETES

The name gamete was introduced by the Austrian biologist Gregor Mendel. A **gamete** (from Ancient Greek gamete from gamein "to marry") is a cell that fuses with another cell during fertilization (conception) in organisms that sexually reproduce. In species that produce

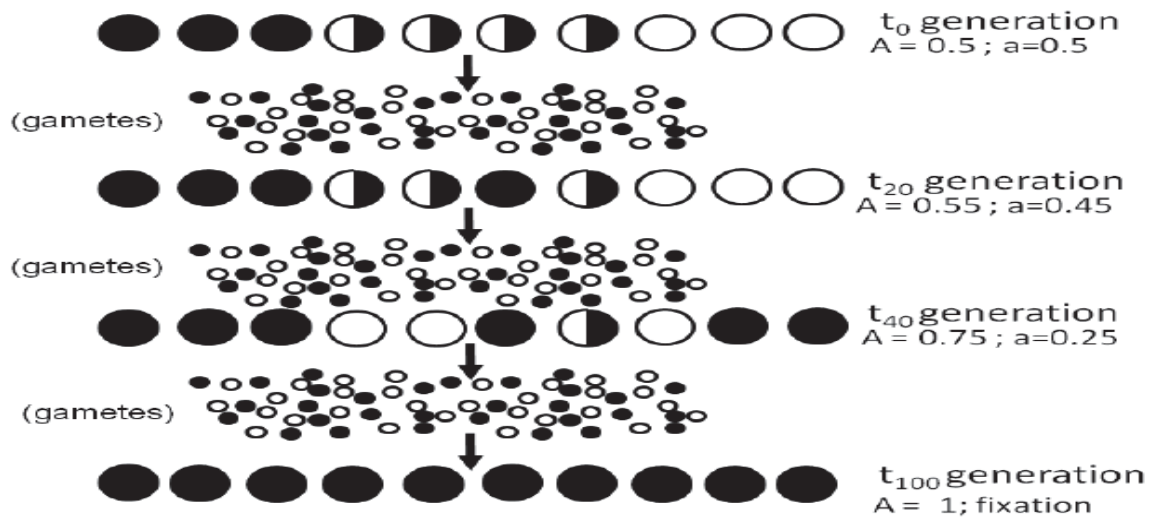


Fig. 2.2 Approximation of gametes

two morphologically distinct types of gametes, and in which each individual produces only one type, a female that produces the larger type of gamete—called an ovum (or egg)—and a male produces the smaller tadpole-like type—called a sperm. This is an example of anisogamy or heterogamy, the condition in which females and males produce gametes of different sizes (this is the case in humans; the human ovum has approximately 100,000 times the volume of a single human sperm cell). In contrast, isogamy is the state of gametes from both sexes being the same size and shape, and given arbitrary designators for mating type. Gametes carry half the genetic information of an individual, one ploidy of each type, and are created through meiosis.

#### (a) Dissimilarity

In contrast to a gamete, the diploid somatic cells of an individual contain one copy of the chromosome set from the sperm and one copy of the chromosome set from the egg cell; that

is, the cells of the offspring have genes expressing characteristics of both the father and the mother. A gamete's chromosomes are not exact duplicates of either of the sets of chromosomes carried in the diploid chromosomes, and often undergo random mutations resulting in modified DNA (and subsequently, new proteins and phenotypes).

### (b) Gender determination in humans and birds

In humans, a normal ovum can carry only an X chromosome (of the X and Y chromosomes), whereas a sperm may carry either an X or a Y (a non-normal ovum can end up carrying two or no X chromosomes, as a result of a mistake at either of the two stages of meiosis, while a non-normal sperm cell can end up

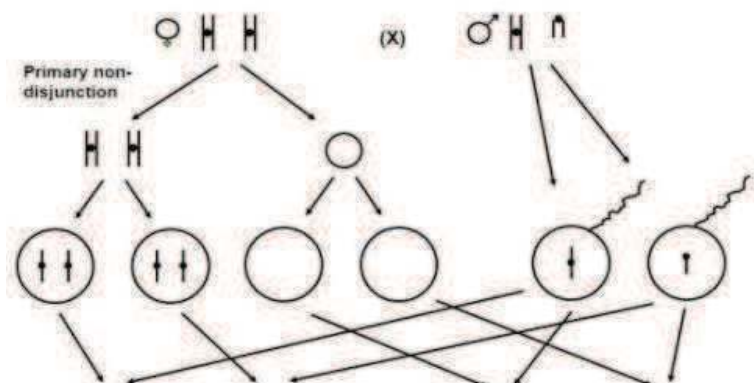


Figure 2.3 Sex Determination in Humans

carrying either no sex-defining chromosomes, an XY pair, an XX pair as a result of the aforementioned reason); ergo the male sperm can play a role in determining the gender of any resulting zygote, if the zygote has two X chromosomes it may develop into a female, if it has an X and a Y chromosome, it may develop into a male. For birds, the female ovum determines the sex of the offspring, through the ZW sex-determination system.

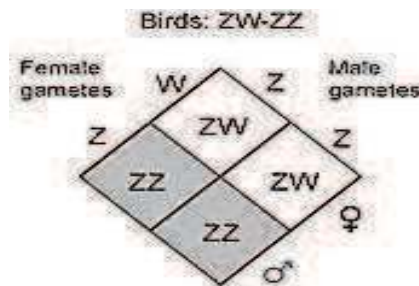


Figure no 2.4

### (c) Artificial gametes

Artificial gametes, also known as In vitro derived gametes (IVD), stem cell-derived gametes (SCDGs), and In vitro generated gametes (IVG), are gametes derived from stem cells. Research shows that artificial gametes may be a reproductive technique for same-sex male couples, although a surrogate mother would still be required for the gestation period.<sup>[5]</sup> Women who have

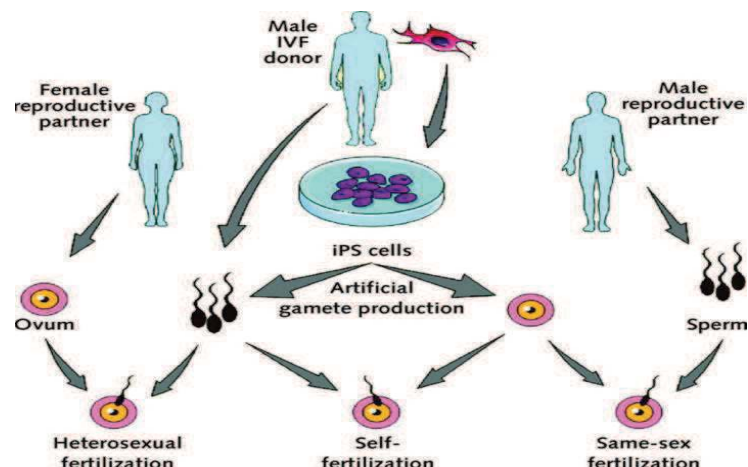


Figure 2.5 Artificial gametes

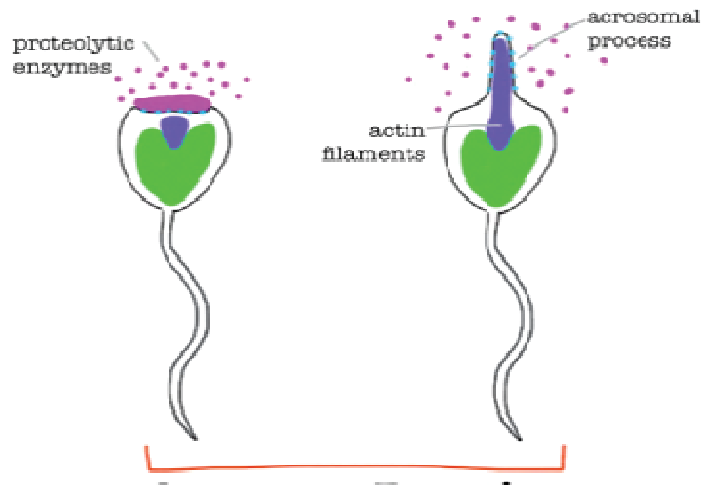
passed menopause may be able to produce eggs and bear genetically related children with artificial gametes. Robert Sparrow wrote, in the *Journal of Medical Ethics*, that embryos derived from artificial gametes could be used to derive new gametes and this process could be repeated to create multiple human generations in the laboratory. This technique could be used to create cell lines for medical applications and for studying the heredity of genetic disorders. Additionally, this technique could be used for human enhancement by selectively breeding for a desired genome or by using recombinant DNA technology to create enhancements that have not arisen in nature.

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### 2.3.2 ACROSOME REACTION

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During fertilization, a sperm first fuses with the plasma membrane and then penetrates the female egg in order to fertilize it. Fusing to the egg



*Fig. 2.6 Acrosome Reaction*

usually causes little problem, whereas penetrating through the egg's hard shell can present more of a problem to the sperm. Therefore, sperm cells go through a process known as the **acrosome reaction** which is the reaction that occurs in the acrosome of the sperm as it approaches the egg. The acrosome is a cap-like structure over the anterior half of the sperm's head.

As the sperm approaches the zona pellucida of the egg, which is necessary for initiating the acrosome reaction, the membrane surrounding the acrosome fuses with the plasma membrane of the oocyte, exposing the contents of the acrosome. The contents include surface antigens and numerous enzymes which are responsible for breaking through the egg's tough coating and allowing fertilization to occur.



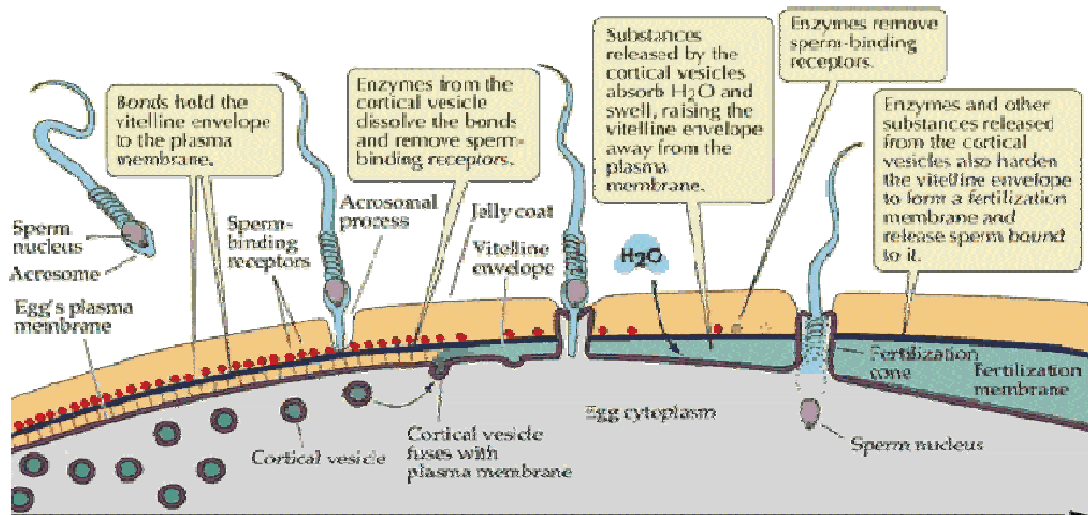


Fig. 2.7 Acrosomal Reaction

#### (a) Variations among species

There are considerable species variations in the morphology and consequences of the acrosome reaction. In several species the trigger for the acrosome reaction has been identified in a layer that surrounds the egg.

#### (b) Echinoderms

In some lower animal species a protuberance (the acrosomal process) forms at the apex of the sperm head, supported by a core of actin microfilaments. The membrane at the tip of the acrosomal process fuses with the egg's plasma membrane.

In some echinoderms, including starfish and sea urchins, a major portion of the exposed acrosomal content contains a protein that temporarily holds the sperm on the egg's surface.

#### (c) Mammals

In mammals the acrosome reaction releases hyaluronidase and acrosin; their role in fertilization is not yet clear. The acrosomal reaction does not begin until the sperm comes into contact with the oocyte's zona pellucida. Upon coming into contact with the zona pellucida, the acrosomal enzymes begin to dissolve and the actin filament comes into contact with the zona pellucida. Once the two meet, a calcium influx occurs, causing a signaling cascade. The cortical granules inside the oocyte then fuse to the outer membrane and a transient fast block reaction occurs.

It also alters a patch of pre-existing sperm plasma membrane so that it can fuse with the egg plasma membrane.

A sperm penetration assay includes an acrosome reaction test that assesses how well a sperm is able to perform during the fertilization process. Sperm that are unable to properly go through the acrosome reaction will not be able to fertilize an egg. However, this problem only occurs in about 5% of men that have the test done. This test is rather expensive and provides limited information on a man's fertility.

In other cases, such as in the wood mouse *Apodemus sylvaticus*, premature acrosome reactions have been found to cause increased motility in aggregates of spermatozoa promoting fertilization

#### (d) The process

The acrosomal reaction normally takes place in the ampulla of

the fallopian tube (site of fertilization) when the sperm penetrates the

secondary oocyte. The sperm cell acquires a

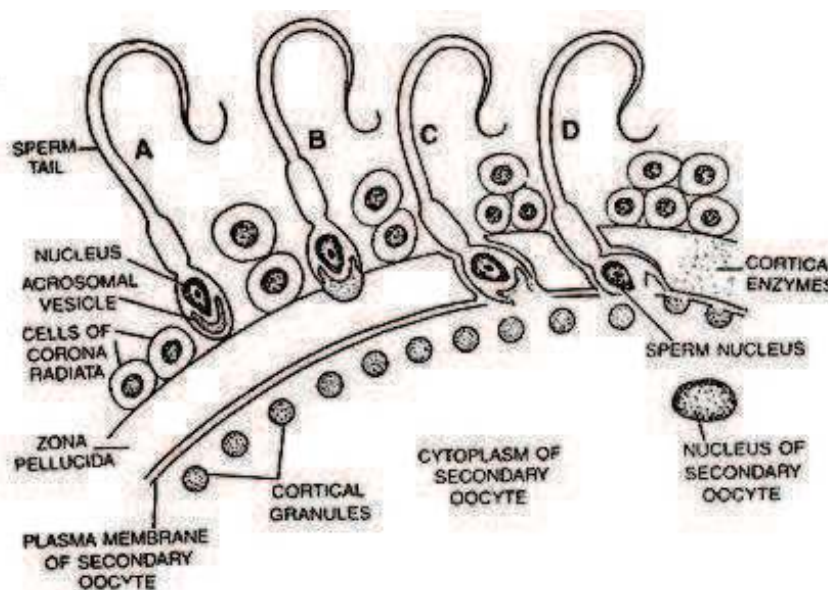
"hyperactive motility pattern" by which its

flagellum produces vigorous whip-like

movements that propel

the sperm through the cervical canal and uterine cavity, until it reaches the isthmus of the fallopian tube. The sperm approaches the ovum in the ampulla of the fallopian tube with the help of various mechanisms, including chemotaxis. Glycoproteins on the outer surface of the sperm then bind with glycoproteins on the zona pellucida of the ovum.

The first stage is the penetration of corona radiata, by releasing hyaluronidase from the acrosome to digest cumulus cells surrounding the oocyte and exposing acrosin attached to the inner membrane of the sperm. The cumulus cells are embedded in a gel-like substance



**Fig. 2.8 Stages of sperm entry into the ovum during fertilization**

made primarily of hyaluronic acid, and developed in the ovary with the egg and support it as it grows. After reaching the zona pellucida the actual acrosome reaction begins.

Acrosin digests the zona pellucida and membrane of the oocyte. Part of the sperm's cell membrane then fuses with the egg cell's membrane, and the contents of the head sink into the egg. In the mouse it has been demonstrated that ZP3, one of the proteins that make up the zona pellucida, binds to a partner molecule on the sperm. This lock-and-key type mechanism is species-specific and prevents the sperm and egg of different species from fusing. The zona pellucida also releases Ca granules to prevent additional sperm binding. There is some evidence that this binding triggers the acrosome to release the enzymes that allow the sperm to fuse with the egg. It is likely that a similar mechanism occurs in other mammals, but the diversity of zona proteins across species means that the relevant protein and receptor may differ.

Upon penetration, if all is occurring normally, the process of egg-activation occurs and the oocyte is said to have become activated. This is thought to be induced by a specific protein phospholipase c zeta. It undergoes its secondary meiotic division, and the two haploid nuclei (paternal and maternal) fuse to form a zygote. In order to prevent polyspermy and minimise the possibility of producing a triploid zygote, several changes to the egg's cell membranes renders them impenetrable shortly after the first sperm enters the egg. The aforementioned process describes the physiologically relevant events. One should however bear in mind that a certain percentage of sperm cells will undergo a spontaneous acrosome reaction without the presence of the ovum. Those cells are not able to fertilise the egg, even if they do reach it later. Other cells will spontaneously shed their acrosome during the process of apoptosis/necrosis.

#### **(e) In vitro fertilization**

When using intracytoplasmic sperm injection (ICSI) for IVF, the implantation rate is higher in oocytes injected with spermatozoa that have undergone acrosome reaction (~40%) vs. those injected with nonreacted spermatozoa (~10%). The implantation rate is ~25% in when injected with both reacted and nonreacted spermatozoa. The delivery rate per cycle follows the same trend.

The acrosome reaction can be stimulated in vitro by substances a sperm cell may encounter naturally such as progesterone or follicular fluid, as well as the more commonly used calcium ionophore.

**(f) Assessment**

Birefringence microscopy, flow cytometry or fluorescence microscopy can be used for assessing the shedding of the acrosome or "acrosome reaction" of a sperm sample. Flow cytometry and fluorescence microscopy are usually done after staining with a fluoresceinated lectin such as FITC-PNA, FITC-PSA, FITC-ConA, or fluoresceinated antibody such as FITC-CD46. The antibodies/lectins have a high specificity for different parts of the acrosomal region, and will only bind to a specific site (acrosomal content/inner/outer membrane). If bound to a fluorescent molecule, regions where these probes have bound can be visualised. Sperm cells with artificially induced acrosome reactions may serve as positive controls.

For fluorescence microscopy a smear of washed sperm cells are made, airdried, permeabilized and then stained. Such a slide is then viewed under light of a wavelength that will cause the probe to fluoresce if it is bound to the acrosomal region. At least 200 cells are viewed in an arbitrary fashion and classified as either acrosome intact (fluorescing bright green) or acrosome reacted (no probe present, or only on the equatorial region). It is then expressed as a percentage of the counted cells.

For assessment with flow cytometry the washed cells are incubated with the chosen probe, (possibly washed again) and then sampled in a flow cytometer. After gating the cell population according to forward- and side-scatter the resulting data can be analysed (E.g. mean fluorescences compared). With this technique a probe for viability, like propidium iodide (PI) could also be included in order to exclude dead cells from the acrosome assessment, since many sperm cells will spontaneously lose their acrosome when they die.

### 2.3.3 FORMATION OF FERTILIZATION MEMBRANE

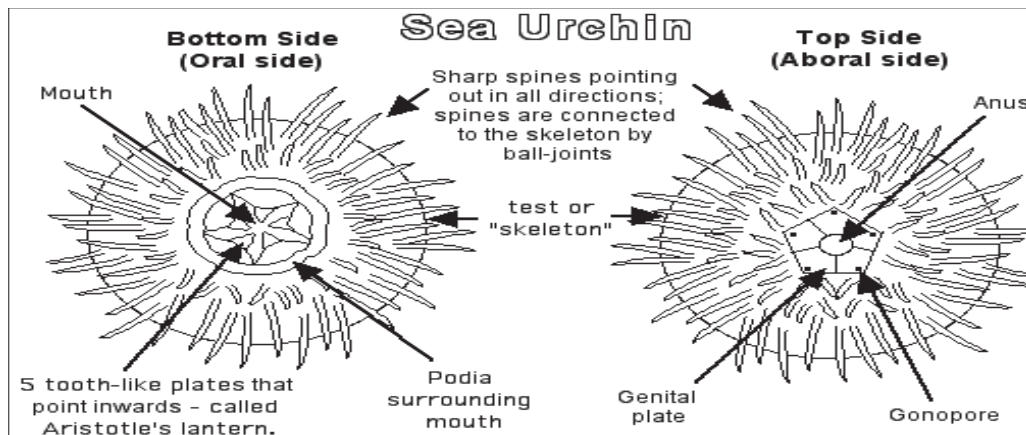


Figure 2.8 Sea Urchin

The most spectacular changes that follow fertilization occur at the egg surface. The best known example, that of the sea urchin egg, is described below. An immediate response to fertilization is the raising of a membrane, called a vitelline membrane, from the egg surface. In the beginning the membrane is very thin; soon, however, it thickens, develops a well-organized molecular structure, and is called the fertilization membrane. At the same time an extensive rearrangement of the molecular structure of the egg surface occurs. The events leading to formation of the fertilization membrane require about one minute.

At the point on the outer surface of the sea urchin egg at which a spermatozoan attaches, the thin vitelline membrane becomes detached. As a result the membranes of the cortical granules come into contact with the inner aspect of the egg's plasma membrane and fuse with it, the granules open, and their contents are extruded into the perivitelline space; *i.e.*, the space between the egg surface and the raised vitelline membrane. Part of the contents of the granules merge with the vitelline membrane to form the fertilization membrane; if fusion of the contents of the cortical granules with the vitelline membrane is prevented; the membrane remains thin and soft. Another material that also derives from the cortical granules covers the surface of the egg to form a transparent layer, called the hyaline layer, which plays an important role in holding together the cells (blastomeres) formed during division, or cleavage, of the egg. The plasma membrane surrounding a fertilized egg, therefore, is a mosaic structure containing patches of the original plasma membrane of the unfertilized egg and areas derived from membranes of the cortical granules. The events leading to the formation

of the fertilization membrane are accompanied by a change of the electric charge across the plasma membrane, referred to as the fertilization potential, and a concurrent outflow of potassium ions (charged particles); both of these phenomena are similar to those that occur in a stimulated nerve fibre. Another effect of fertilization on the plasma membrane of the egg is a several-fold increase in its permeability to various molecules; this change may be the result of the activation of some surface-located membrane transport mechanism.

**(a) Formation of the zygote nucleus**

After its entry into the egg cytoplasm, the spermatozoal nucleus, now called the male pronucleus, begins to swell, and its chromosomal material disperses and becomes similar in appearance to that of the female pronucleus. Although the membranous envelope surrounding the male pronucleus rapidly disintegrates in the egg, a new envelope promptly forms around it. The male pronucleus, which rotates 180° and moves towards the egg nucleus, initially is accompanied by two structures (centrioles) that function in cell division. After the male and female pronuclei have come into contact, the spermatozoal centrioles give rise to the first cleavage spindle, which precedes division of the fertilized egg. In some cases fusion of the two pronuclei may occur by a process of membrane fusion; in this process, two adjoining membranes fuse at the point of contact to give rise to the continuous nuclear envelope that surrounds the zygote nucleus.

**(b) Biochemical analysis of fertilization**

Many of the early studies on biochemical changes occurring during fertilization were concerned with the respiratory metabolism of the egg. The results, however, were deceiving; the sea urchin egg, for example, showed an increased rate of oxygen consumption as an immediate response to either fertilization or parthenogenetic activation, in apparent support of the idea that the essence of fertilization is the removal of a respiratory or metabolic block in the unfertilized egg. Extensive comparative studies have shown that the increased rate of oxygen consumption in fertilized sea urchin eggs is not a general rule; indeed, the rate of oxygen consumption of most animal eggs does not change at the time of fertilization and may even temporarily decrease.

At the time of fertilization the egg contains the components required to carry out protein synthesis, and enhance development, through an early embryonic stage called the blastula. Most immediate post-fertilization protein synthesis is directed by molecules of ribonucleic acid, known as messenger RNA, that were formed during oogenesis and stored in

the egg. In addition, protein synthesis up to the blastula stage (up to a much earlier stage in the mammalian embryo) is directed by the cell components called ribosomes, which are present in the unfertilized egg; new ribosomes, as well as molecules of another type of RNA involved in protein synthesis, called transfer RNA, are synthesized at later stage in embryonic development (gastrulation). Eggs fertilized and allowed to develop in the presence of the antibiotic actinomycin, which suppresses RNA synthesis, not only reach the blastula stage but their rate of protein synthesis is the same as that in untreated embryos.

Unfertilized sea urchin eggs, as well as those of other marine animals studied thus far, have a very low rate of protein synthesis, suggesting that something in the unfertilized egg inhibits its protein synthesizing machinery. Since the rate of protein synthesis increases immediately following fertilization, it may depend on some change in, or removal of, an inhibitor. In the sea urchin egg, for example, the low efficiency of the protein synthesizing apparatus apparently depends on certain properties of the ribosomes. Most of the ribosomes found in an unfertilized sea urchin egg are single ribosomes (so-called monosomes); soon after fertilization, however, the single ribosomes interact with messenger RNA molecules thus giving rise to the polyribosomes, which are the active units in protein synthesis. This process also occurs in eggs of a few other marine animals that have been studied. The protein-synthesizing inefficiency of unfertilized sea-urchin-egg ribosomes is caused by an inhibitor that is associated with them and interferes with the binding of messenger RNA molecules to the ribosomes; the inhibitor is removed almost immediately following fertilization, perhaps by enzymatic breakdown.

It thus appears that at least in the sea urchin egg the overall rate of protein synthesis is controlled at the ribosome level and that the first step in the activation of protein synthesis following fertilization is the “turning on” of the ribosomes. In vertebrates such as amphibians, activation of protein synthesis takes place at the onset of egg maturation, apparently initiated by the action of a hormone, progesterone. The effect of progesterone is not mediated by the nucleus but is a direct effect on the cytoplasm.

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### 2.3.4 EGG ACTIVATION

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**Egg** (or **ovum/ Oocyte**) **activation** is a series of processes that occur in the oocyte during fertilization.



Sperm entry causes calcium release into the oocyte. In mammals, this has been proposed to be caused by the introduction of phospholipase C isoform zeta (PLC $\zeta$ ) from the sperm cytoplasm, although this remains to be established definitively. Activation of the ovum includes the following events:

1. Cortical reaction to block against other sperm cells
2. Activation of egg metabolism
3. Reactivation of meiosis
4. DNA synthesis

#### (a) Sperm trigger of egg activation

The sperm may trigger egg activation via the interaction between a sperm protein and an egg surface receptor. Izumo is the sperm cell signal, that will trigger the egg receptor Juno.<sup>[1]</sup> This receptor is activated by the sperm binding and a possible signaling pathway could be the activation of a tyrosine kinase

which then activates phospholipase C (PLC). The inositol signaling system has been implicated as the pathway involved with egg activation. IP<sub>3</sub> and DAG are produced from the cleavage of PIP<sub>2</sub> by phospholipase C.

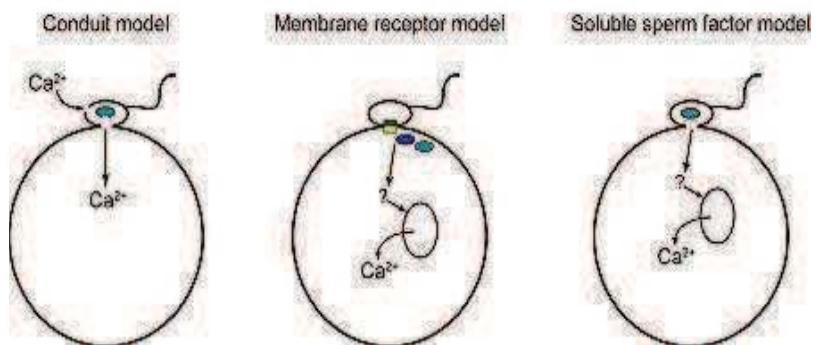


Fig. 2.9 Egg activation

However, another hypothesis is that a soluble 'sperm factor' diffuses from the sperm into the egg cytosol upon sperm-oocyte fusion. The results of this interaction could activate a signal transduction pathway that uses second messengers. A novel PLC isoform, PLC $\zeta$ , may be the equivalent of the mammalian sperm factor. A 2002 study demonstrated that mammalian sperm contain PLC zeta which can start the signaling cascade.

#### (b) Fast and slow block to polyspermy

Polyspermy is the condition when multiple sperm fuse with a single egg. This results in duplications of genetic material. In sea urchins, the block to polyspermy comes from two mechanisms: the fast block and the slow block. The fast block is an electrical block to



polyspermy. The resting potential of an egg is  $-70\text{mV}$ . After contact with sperm, an influx of sodium ions increases the potential up to  $+20\text{mV}$ . The slow block is through a biochemical mechanism triggered by a wave of calcium increase. The rise of calcium is both necessary and sufficient to trigger the slow block. In the cortical reaction, cortical granules directly beneath the plasma membrane are released into the space between the plasma membrane and the vitelline membrane (the perivitelline space). An increase in calcium triggers this release. The contents of the granules contain proteases, mucopolysaccharides, hyalin, and peroxidases. The proteases cleave the bridges connecting the plasma membrane and the vitelline membrane and cleave the bindin to release the sperm. The mucopolysaccharides attract water to raise the vitelline membrane. The hyalin forms a layer adjacent to the plasma membrane and the peroxidases cross-link the protein in the vitelline membrane to harden it and make it impenetrable to sperm. Through these molecules the vitelline membrane is transformed into the fertilization membrane or fertilization envelope. In mice, the zona reaction is the equivalent to the cortical reaction in sea urchins. The terminal sugars from ZP3 are cleaved to release the sperm and prevent new binding.

#### (c) Reactivation of meiosis

The meiotic cycle of the oocyte was suspended in metaphase of the second meiotic division. Once PLC $\zeta$  is introduced into the oocyte by the sperm cell, it cleaves phospholipid phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) into diacyl glycerol (DAG) and inositol 1,4,5-trisphosphate (IP<sub>3</sub>). In most cells, this occurs at the cell membrane however, evidence suggests that the PIP<sub>2</sub> required for oocyte activation is potentially stored in intracellular vesicles dispersed throughout the cytoplasm. The IP<sub>3</sub> produced then triggers calcium oscillations which reactivate the meiotic cycle. This results in the production and extrusion of the second polar body.

#### (d) DNA synthesis

4 hours after fusion of sperm and ovum, DNA synthesis begins. Male and female pronuclei move to the centre of the egg and membranes break down. Male protamines are replaced with histones and the male DNA is demethylated. Chromosomes then orientate on the metaphase spindle for mitosis. This combination of the two genomes is called **syngamy**.

The sperm contributes a pronucleus and a centriole to the egg. Most other components and organelles are rapidly degraded. Mitochondria are rapidly ubiquitinated and destroyed. **Oxidative stress theory** is a hypothesis that it is evolutionarily favourable for

mitochondria from the father to be destroyed, as there is a greater possibility that the mitochondrial DNA has become mutated or damaged. This is because mtDNA is not protected by histones and has poor repair mechanisms. Due to the increased metabolic activity of the sperm compared to the egg, due to its motility, there is greater production of reactive oxygen species and therefore greater chance of mutation. Furthermore, sperm are exposed to reactive oxygen species from leukocytes in the epididymis during transit. Additionally, quality control of spermatozoa is much worse than for the ovum, as many sperm are released whereas only one dominant follicle is released per cycle. This competitive selection helps to ensure the most 'fit' ova are selected for fertilisation.

**(e) Artificial oocyte activation**

Oocyte activation may be artificially facilitated by calcium ionophores, something that is speculated to be useful in case of fertilization failure, such as still occurs in 1–5% of intracytoplasmic sperm injection (ICSI) cycles. Another method is by using the drug Roscovitine, this reduces the activity of M-phase promoting factor activity in mice.

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### **2.3.5 PREVENTION OF POLYSPERMY**

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As soon as one sperm has entered the egg, the fusibility of the egg membrane, which was so necessary to get the sperm inside the egg, becomes a dangerous liability. In sea urchins, as in most animals studied, any sperm that enters the egg can provide a haploid nucleus and a centriole to the egg. In normal **monospermy**, in which only one sperm enters the egg, a haploid sperm nucleus and a haploid egg nucleus combine to form the diploid nucleus of the fertilized egg (zygote), thus restoring the chromosome number appropriate for the species. The centriole, which is provided by the sperm, will divide to form the two poles of the mitotic spindle during cleavage.

The entrance of multiple sperm—**polyspermy**—leads to disastrous consequences in most organisms. In the sea urchin, fertilization by two sperm results in a triploid nucleus, in which each chromosome is represented three times rather than twice. Worse, since each sperm's centriole divides to form the two poles of a mitotic apparatus, instead of a bipolar mitotic spindle separating the chromosomes into two cells, the triploid chromosomes may be divided into as many as four cells. Because there is no mechanism to ensure that each of the four cells receives the proper number and type of chromosomes, the chromosomes would be apportioned unequally. Some cells receive extra copies of certain chromosomes and other

cells lack them. Theodor Boveri demonstrated in 1902 that such cells either die or develop abnormally.

Aberrant development in a dispermic sea urchin egg. (A) Fusion of three haploid nuclei, each containing 18 chromosomes, and the division of the two sperm centrioles to form four mitotic poles. (B, C) The 54 chromosomes randomly assort on the four spindles.

Species have evolved ways to prevent the union of more than two haploid nuclei. The most common way is to prevent the entry of more than one sperm into the egg. The sea urchin egg has two mechanisms to avoid polyspermy: a fast reaction, accomplished by an electric change in the egg plasma membrane, and a slower reaction, caused by the exocytosis of the cortical granules.

#### **(a) The fast block to polyspermy**

The **fast block to poly-spermy** is achieved by changing the electric potential of the egg plasma membrane. This membrane provides a selective barrier between the egg cytoplasm and the outside environment, and the ionic concentration of the egg differs greatly from that of its surroundings. This concentration difference is especially significant for sodium and potassium ions. Seawater has a particularly high sodium ion concentration, whereas the egg cytoplasm contains relatively little sodium. The reverse is the case with potassium ions. This condition is maintained by the plasma membrane, which steadfastly inhibits the entry of sodium ions into the oocyte and prevents potassium ions from leaking out into the environment. If we insert an electrode into an egg and place a second electrode outside it, we can measure the constant difference in charge across the egg plasma membrane. This **resting membrane potential** is generally about 70 mV, usually expressed as  $-70$  mV because the inside of the cell is negatively charged with respect to the exterior.

Within 1–3 seconds after the binding of the first sperm, the membrane potential shifts to a positive level, about  $+20$  mV. This change is caused by a small influx of sodium ions into the eggs. Although sperm can fuse with membranes having a resting potential of  $-70$  mV, they cannot fuse with membranes having a positive resting potential, so no more sperm can fuse to the egg. It is not known whether the increased sodium permeability is due to the binding of the first sperm or to the fusion of the first sperm with the egg.

Membrane potential of sea urchin eggs before and after fertilization. (A) Before the addition of sperm, the potential difference across the egg plasma membrane is about  $-70$  mV. Within 1–3 seconds after the fertilizing sperm contacts the

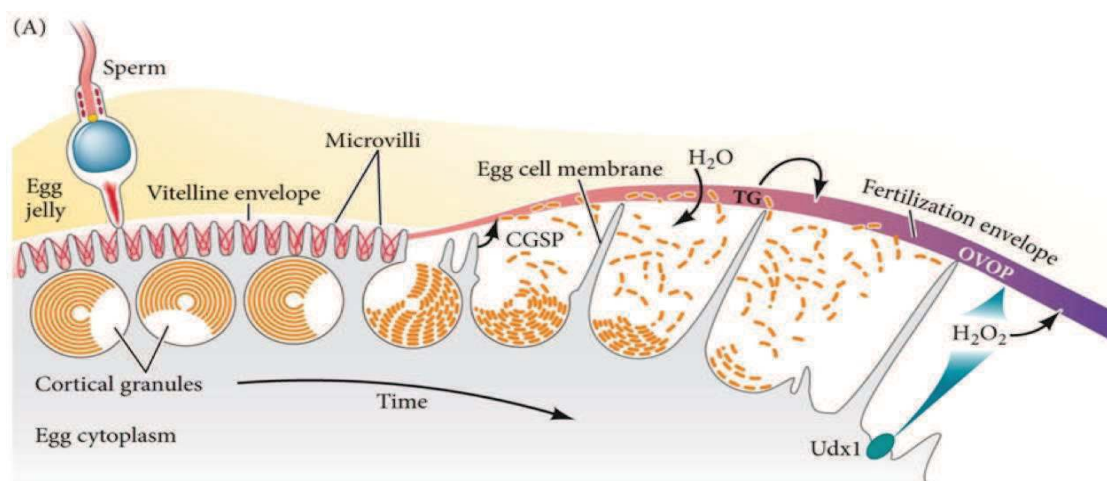
The importance of sodium ions and the change in resting potential was demonstrated by Laurinda Jaffe and colleagues. They found that polyspermy can be induced if sea urchin eggs are artificially supplied with an electric current that keeps their membrane potential negative. Conversely, fertilization can be prevented entirely by artificially keeping the membrane potential of eggs positive. The fast block to polyspermy can also be circumvented by lowering the concentration of sodium ions in the water. If the supply of sodium ions is not sufficient to cause the positive shift in membrane potential, polyspermy occurs. It is not known how the change in membrane potential acts on the sperm to block secondary fertilization. Most likely, the sperm carry a voltage-sensitive component (possibly a positively charged fusogenic protein), and the insertion of this component into the egg plasma membrane could be regulated by the electric charge across the membrane. An electric block to polyspermy also occurs in frogs, but probably not in most mammals.

**(b) The slow block to polyspermy**

The eggs of sea urchins (and many other animals) have a second mechanism to ensure that multiple sperm do not enter the egg cytoplasm. The fast block to polyspermy is transient, since the membrane potential of the sea urchin egg remains positive for only about a minute. This brief potential shift is not sufficient to prevent polyspermy, which can still occur if the sperm bound to the vitelline envelope are not somehow removed. This removal is accomplished by the **cortical granule reaction**, a slower, mechanical block to polyspermy that becomes active about a minute after the first successful sperm-egg attachment.

Directly beneath the sea urchin egg plasma membrane are about 15,000 cortical granules, each about  $1\text{ }\mu\text{m}$  in diameter. Upon sperm entry, these cortical granules fuse with the egg plasma membrane and release their contents into the space between the plasma membrane and the fibrous mat of vitelline envelope proteins. Several proteins are released by this cortical granule exocytosis. The first are proteases. These enzymes dissolve the protein posts that connect the vitelline envelope proteins to the cell membrane, and they clip off the binding receptor and any sperm attached to it. Mucopolysaccharides released by the cortical granules produce an osmotic gradient that causes water to rush into the space between the plasma membrane and the vitelline envelope, causing the envelope to expand and become

the **fertilization envelope**. A third protein released by the cortical granules, a peroxidase enzyme, hardens the fertilization envelope by crosslinking tyrosine residues on adjacent proteins. The fertilization envelope starts to form at the site of sperm entry and continues its expansion around the egg. As it forms, bound sperm are released from the envelope. This process starts about 20 seconds after sperm attachment and is complete by the end of the first minute of fertilization. Finally, a fourth cortical granule protein, hyalin, forms a coating around the egg. The egg extends elongated microvilli whose tips attach to this **hyaline layer**. This layer provides support for the blastomeres during cleavage.



*Fig. 2.10*  
Cortical granule exocytosis. Schematic diagram showing the events leading to the formation of the fertilization envelope and the hyaline layer.

As cortical granules undergo exocytosis, they release serine proteases (CGSP) which cleaves the proteins linking the vitelline envelope to the cell membrane. Mucopolysaccharides released by the cortical granules form an osmotic gradient, thereby causing water to enter and swell the space between the vitelline envelope and the cell membrane peroxidases (OVOP and Udx1) and transglutaminases (TG) then harden the vitelline envelope, now called the fertilization envelope.

In mammals, the cortical granule reaction does not create a fertilization envelope, but its ultimate effect is the same. Released enzymes modify the zona pellucida sperm receptors such that they can no longer bind sperm. During this process, called the **zona reaction**, both ZP3 and ZP2 are modified. The cortical granules of mouse eggs contain an enzyme that clips off the terminal sugar residues of ZP3, thereby releasing bound sperm from the zona and preventing the attachment of other sperm. Cortical granules of mouse eggs have been found

to contain N-acetylglucosaminidase enzymes capable of cleaving N-acetylglucosamine from ZP3 carbohydrate chains. N-acetylglucosamine is one of the carbohydrate groups that sperm can bind to, and Miller and co-workers (1992, 1993) have demonstrated that when the N-acetylglucosamine residues are removed at fertilization, ZP3 will no longer serve as a substrate for the binding of other sperm. ZP2 is clipped by the cortical granule proteases and loses its ability to bind sperm as well. Thus, once a sperm has entered the egg, other sperm can no longer initiate or maintain their binding to the zona pellucida and are rapidly shed.

### **(c) Calcium as the initiator of the cortical granule reaction**

The mechanism of the cortical granule reaction is similar to that of the acrosomal reaction. Upon fertilization, the intracellular calcium ion concentration of the egg increases greatly. In this high-calcium environment, the cortical granule membranes fuse with the egg plasma membrane, releasing their contents. Once the fusion of the cortical granules begins near the point of sperm entry, a wave of cortical granule exocytosis propagates around the cortex to the opposite side of the egg.

In sea urchins and mammals, the rise in calcium concentration responsible for the cortical granule reaction is not due to an influx of calcium into the egg, but rather comes from within the egg itself. The release of calcium from intracellular storage can be monitored visually using calcium-activated luminescent dyes such as aequorin (isolated from luminescent jellyfish) or fluorescent dyes such as fura-2. These dyes emit light when they bind free calcium ions. When a sea urchin egg is injected with dye and then fertilized, a striking wave of calcium release propagates across the egg. Starting at the point of sperm entry, a band of light traverses the cell. The calcium ions do not merely diffuse across the egg from the point of sperm entry. Rather, the release of calcium ions starts at one end of the cell and proceeds actively to the other end. The entire release of calcium ions is complete in roughly 30 seconds in sea urchin eggs, and the free calcium ions are resequestered shortly after they are released. If two sperm enter the egg cytoplasm, calcium ion release can be seen starting at the two separate points of entry on the cell

Wave of calcium release across sea urchin eggs during fertilization. A sea urchin egg is preloaded with a dye that fluoresces when it binds calcium. When the sperm fuses with the egg, a wave of calcium release can be seen, beginning at the site of sperm.

Several experiments have demonstrated that calcium ions are directly responsible for propagating the cortical granule reaction, and that these calcium ions are stored within the egg itself. The drug is a calcium ionophore (a compound that transports free calcium ions across lipid membranes), allowing these cations to traverse otherwise impermeable barriers. Placing unfertilized sea urchin eggs into seawater containing this compound causes the cortical granule reaction and the elevation of the fertilization envelope. Moreover, this reaction occurs in the absence of any calcium ions in the surrounding water. Therefore, must be causing the release of calcium ions already sequestered in organelles within the egg.

The calcium ions responsible for the cortical granule reaction are stored in the endoplasmic reticulum of the egg. In sea urchins and frogs, this reticulum is pronounced in the cortex and surrounds the cortical granules. In *Xenopus*, the cortical endoplasmic reticulum becomes ten times more abundant during the maturation of the egg and disappears locally within a minute after the wave of cortical granule exocytosis occurs in any region of the cortex. Once initiated, the release of calcium is self-propagating. Free calcium is able to release sequestered calcium from its storage sites, thus causing a wave of calcium ion release and cortical granule exocytosis.

Endoplasmic reticulum surrounding cortical granules in sea urchin eggs. (A) The endoplasmic reticulum has been stained with osmium-zinc iodide to allow visualization by transmission electron microscopy.

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## ***2.4 SUMMARY***

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Fertilization is a cell-cell recognition process that occurs between two distinct cells: a small asymmetric and motile sperm cell and a large and nonmotile egg. The stages of fertilization can be divided into four processes: 1) sperm preparation, 2) sperm-egg recognition and binding, 3) sperm-egg fusion and 4) fusion of sperm and egg pronuclei and activation of the zygote. The specific structures of the sperm and egg that are important for fertilization will be discussed and experiments that led to the identification of the egg receptor for the sperm and the sperm receptor for the egg will be described. Membrane fusion of sperm and eggs is an incompletely understood process, but the discovery of proteins known as ADAM proteins on the sperm surface has suggested new mechanisms to explain sperm-egg fusion. Finally, we will consider how fertilized eggs prevent additional sperm from fusing (a condition known as polyspermy) and how the fertilized egg is activated to begin development.



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## 2.5 GLOSSARY

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**Abdomen:** The belly, that part of the body that contains all of the structures.

**Anesthesia:** Loss of feeling or awareness, as when an anesthetic is administered.

**Birth control:** Birth control is the use of any practices, methods, or devices.

**Birth rate:** The birth rate is usually given as the number of live births divided. .

**Bowel:** The small and large intestine.

**Catheter:** A thin, flexible tube.

**Cell:** The basic structural and functional unit of any living thing.

**Cervix:** The cervix is the lower, narrow part of the uterus (womb)

**Chemotherapy: 1.** In the original sense, a chemical that binds to.

**Contrast:** Short for "contrast media." Contrast media are X-ray dyes used to provide contra...

**Cryopreservation:** The process of cooling and storing cells, tissues, or organs.

**Donor:** The giver of a tissue or an organ, such as a blood donor or kidney donor.

**Ectopic:** In the wrong place, out of place. For example, an ectopic kidney.

**Ectopic pregnancy:** A pregnancy that is not in the uterus.

**Egg donor:** A woman who provides her own eggs for another woman.

**Embryo:** An organism in the early stages of growth and differentiation.

**Endometriosis:** The presence of tissue that normally grows inside the uterus (womb).

**Estrogen:** A female steroid hormone that is produced by the ovaries and, in lesser amounts,...

**Fallopian tube:** One of the two Fallopian tubes that transport the egg.

**Fertilization:** The process of combining the male gamete, or sperm.

**Fetus:** An unborn offspring, from the embryo stage.

**Gamete:** Germ cell.



**Genetic:** Having to do with genes and genetic information.

**Genetic disease:** A disease caused by an abnormality in an individual's genome.

**HCG:** Human chorionic gonadotropin.

**Hormone:** A chemical substance produced in the body that controls.

**Hysteroscopy:** A procedure to see inside the uterus (the womb) using a viewing

**ICSI:** Intracytoplasmic sperm injection.

**Implant: 1.** To embed; to set in firmly. In embryology, the fertilized egg implants.

**Implantation:** The act of setting in firmly.

**In vitro:** In glass, as in a test tube. An in vitro test is one that is done in glass.

**In vitro fertilization:** A laboratory procedure in which sperm are put in a special dish.

**Infection:** The invasion and multiplication of microorganisms such as bacteria, viruses.

**Infertile:** Not able to conceive after a year of regular intercourse without contraception.

**Infertility:** Diminished or absent ability to conceive and bear offspring.

**Insemination:** The deposition of semen in the female reproductive tract.

**IVF:** In vitro fertilization.

**Laboratory:** A place for doing tests and research procedures, and for preparing chemicals a...

**Louise Brown:** See: Brown, Louise.

**Maternal: 1.** Pertaining to the mother as, for example, the maternal mortality rate.

**Maternal age:** The age of the mother at the time of delivery. Advanced maternal age is usual

**Oral contraceptive:** A birth control pill taken by mouth. Most oral contraceptives.

**Outpatient:** A patient who is not hospitalized, but instead comes to a physician?

**Ovarian:** Of or pertaining to the ovary.

**Ovary:** The female gonad, one of a pair of reproductive glands in women.

**Pain:** An unpleasant sensation that can range from mild, localized discomfort to agony.

**Pelvic:** Having to do with the pelvis, the lower part of the abdomen

**Pelvic exam:** An examination of the organs of the female reproductive system.

**Perinatal:** Pertaining to the period immediately before and after birth.

**Pregnancy:** The state of carrying a developing embryo or fetus within the female body.

**Pregnant:** The state of carrying a developing fetus within the body.

**Progesterone:** A female hormone, the principal hormone that prepares the uterus to receive .

**Recipient:** In medicine, someone who is given something, such as a blood transfusion

**Reproduction:** The production of offspring. Reproduction need not be sexual

**Semen:** The fluid that is released through the penis during orgasm.

**Shortness of breath:** Difficulty in breathing. Medically referred to as dyspnea.

**Speculum:** An instrument that is used to widen the opening of the vagina so that the cervix

**Sperm:** A sperm is the male "gamete" or sex cell. It combines with the female

**Surgeon:** A physician who treats disease, injury, or deformity via operative

**Surgery:** The branch of medicine that employs operations in the treatment of disease

**Syndrome:** A combination of symptoms and signs that together represent a disease process.

**Tubes:** The "tubes" are medically known as the Fallopian tubes.

**Ultrasound:** High-frequency sound waves. Ultra-sound waves can be bounced off tissues by us...

**Uterus:** A hollow, pear-shaped organ that is located in a woman's lower abdomen

**Vagina:** The muscular canal that extends from the cervix to the outside of the body.

**Viable:** Capable of life. For example, a viable premature baby is one who is able to survive

**Womb:** The womb (uterus) is a hollow, pear-shaped organ located in a woman's lower abdomen.

**ZIFT:** Stands for zygote intrafallopian transfer, a method used to treat infertility

**Zygote:** The cell formed by the union of a male sex cell (a sperm) and a female sex cell

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## ***2.6 SELF ASSESSMENT QUESTIONS***

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Q1 Describe in detail the various events in fertilization?

Q2 Write a note on an acrosome reaction during fertilization?

Q3 Write an essay on fertilization?

Q4 Describe the mechanism of fertilization in details?

Q5 Give an account of changes occurring in the ovum during fertilization?

### **Short answer question**

Q1 What is the effect of fusion of gametes or fertilization?

Q2 Give the definition of fertilization?

Q3 What brings about adhesion of sperm with egg?

Q4 What is an acrosomal filament?

Q4 Draw the diagram of sperm with labels?

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## ***2.7 TERMINAL QUESTIONS/ANSWER***

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### **1. Fusion of male and female gametes are called**

- (a) Syngamy
- (b) Karyogamy
- (c) Fertilization
- (d) Isogamy

### **2. The membrane formed around the egg after the attachment of sperm to egg surface is**

- (a) Vitelline membrane
- (b) Fertilization membrane
- (c) Perivitelline space
- (d) Egg membrane

### **3. The region where sperm enters the ovum is called.**

- (a) Reception cone

- (b) Animal pole
- (c) Vegetal pole
- (d) Grey crescent

**4. Androgamones are hormones secreted by**

- (a) Sperm head
- (b) Leydig cells
- (c) Follicle cells
- (d) Pituitary gland of males

**5 After a sperm has penetrated the surface of ovum during fertilization, entry of additional \_\_\_\_\_ is prevented by**

- (a) Formation of vitelline membrane
- (b) Formation of fertilization membrane
- (c) Condensation of yolk
- (d) Reorientation of pigments

**6. Chemical nature of fertilizing is:**

- (a) Protein
- (b) Glycoprotein
- (c) Carbohydrate
- (d) lipid

**7. The process of fusion of male and female pronuclei is called**

- (a) Syngamy
- (b) Plasmogamy
- (c) Karyogamy
- (d) None of these

**8. Entry of sperm pronucleus in the ovum initiates**

- (a) First maturation division in egg nucleus
- (b) Second maturation division of the egg nucleus
- (c) Separation of first polar body
- (d) First cleavage

**Answers**

**1 (a) 2 (b) 3 (a) 4 (a) 5 (b) 6 (a) 7 (c) 8 (b)**

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## **2.7 REFERENCES**

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3. **S.Gilbert** The eighth edition of Scott *Gilbert's Developmental Biology*

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## UNIT: 3 CLEAVAGES AND EMBRYONIC INDUCTION

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### ***CONTENTS***

- 3.1 Objectives
- 3.2 Introduction
- 3.3 Cleavage
  - 3.3.1- Patterns of cleavage
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### **3.1 OBJECTIVES**

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- To understand the Patterns of cleavage.
- To study control of cleavage patterns
- To describe chemical changes during cleavage
- To impart knowledge of significance of cleavage
- To understand embryonic induction and concept of organizer

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### **3.2 INTRODUCTION**

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Fertilization results into the formation of zygote. The process of segmentation (cleavage) immediately follows fertilization or any other process which activates the egg. Cleavage consists of division of the zygote into a large number of cellular entities. The cells which are produced during segmentation are called blastomeres. The process of segmentation prepares the groundwork for the future design of the embryo by producing adequate number of cells. The cleavage also establishes the fundamental conditions for the initiation of next developmental stage Gastrulation.

During embryonic development morphogenetic communication between cells and cell populations bring the exchanges of chemical and contact signals between groups of cells, which in turn alters the fate of cells. Such cellular interaction is called embryonic induction.

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### **3.3 CLEAVAGE**

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Cleavage or segmentation is a series of cell division of the fertilized egg through which it is converted into multicellular structure.

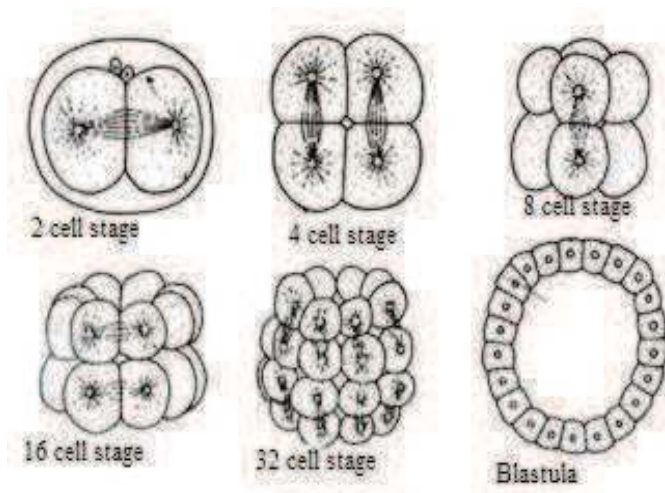
Cleavage can be characterized as that period of development in which no growth occur and chemical conversion of reserve food (yolk, glycogen) into active cytoplasm and the active cytoplasmic substances into nuclear substances like DNA, RNA and proteins.

### 3.3.1 PATTERNS OF CLEAVAGE

The Pattern of cleavage due to Organization of egg may be of the following types

#### (1) Radial cleavage:

When the cleavage planes cut the zygote in such a manner that there appears a radial symmetry in the resulting blastomeres, the cleavage is called a radial cleavage (Fig. 3.1).



*Fig.3.1 Radial changes in sea cucumber*

For example, the frog's zygote divides by a vertical furrow into two equal blastomeres. The second cleavage furrow is also vertical but appears at right angles to the first. Thus four blastomeres are produced. These four blastomeres remain sticking together. A horizontal cleavage then appears above the equatorial region to cut the four blastomeres into eight blastomeres with four smaller "upper" blastomeres and four bigger "lower" blastomeres. At this stage each bigger blastomere has a smaller blastomere sitting on its "head" and the blastomeres are arranged in four radial planes. A blastula produced by radial cleavage can be cut along any meridian to get into two identical halves. Radial cleavage is found in echinoderms.

#### (2) Biradial cleavage:

When the first three division planes do not stand at right angles to each other, the cleavage is termed as biradial. Examples are found in polychaetes and ctenophores.



### (3) Spiral cleavage:

The spiral cleavage is found in those forms in which there is a rotational movement of cell parts around the North Pole to South Pole axis of egg, leading to a displacement or inclination of the mitotic spindles with respect to the symmetrically disposed radii.

Here, the cleavage planes are neither vertical nor horizontal but are slanting in relation to this axis. Moreover, each blastomere divides to form one bigger cell (macromere) and a smaller cell (micromere). In such a cleavage the blastomeres of upper tier (micromeres) sit over the junction between each two of the vegetal blastomeres (macromeres).

This is due to oblique position of the mitotic spindles (Fig 2). Therefore it is also called oblique cleavage.

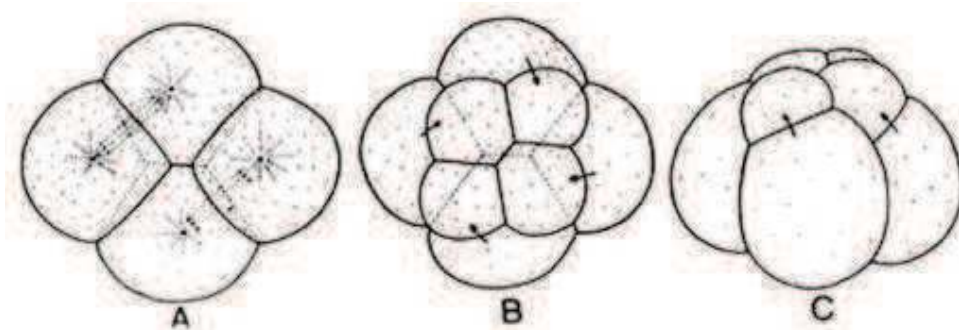
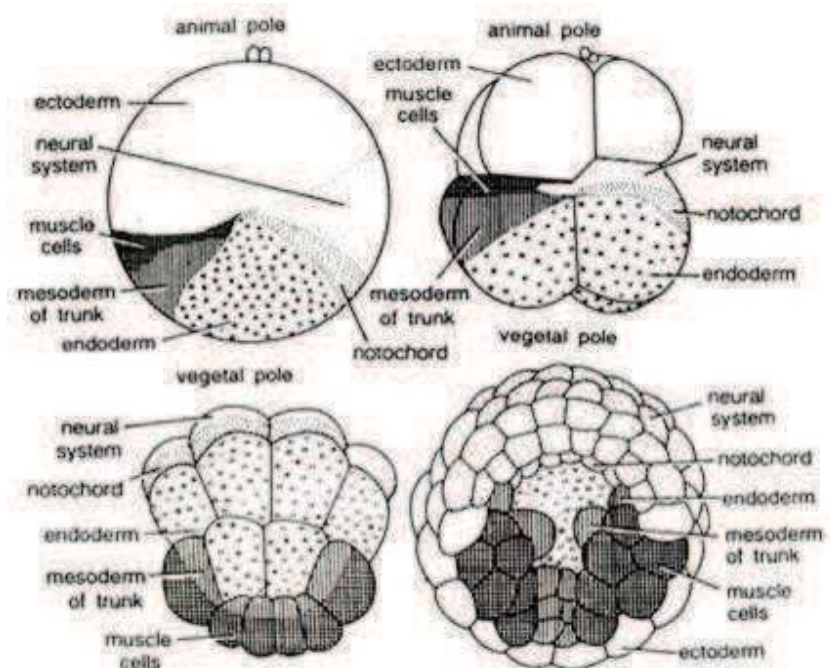


Fig.3.2 Spiral cleavage in multi Trochus (a) Four cell stage, cell preparing for division (b) Eight cell stage, Animal pole view (c) Eight cell stage, Lateral view

In successive cleavages the mitotic spindles are arranged in a sort of spiral. The turn of spiral may lie in a clockwise direction (dextral cleavage—right handed) or anticlockwise direction (left handed—sinistral cleavage). Examples are found in Turbellaria, nematoda, rotifera, annelides and all the molluscs except urophalopods.

### (4) Bilateral cleavage:

In bilateral cleavage, the blastula can be cut vertically only along one plane to get two identical halves, the right and the left. Cleavage activity on one side is mirrored by Activity on the other side. In most cases, the plane of bilateral symmetry is established by the plane of first cleavage furrow, which is bilaterally symmetrical (Fig. 3.3). Examples are found in tunicates, Amphioxus, amphibians, and higher mammals.



*Fig.3.3 Cleavage of Ascidian egg showing bilateral cleavage*

According to the concept of potency, which refers to the total range of developmental possibilities, that an egg or a blastomere is capable of realizing under any imposed conditions either natural or experimental, the following two types of cleavage have been recognized:

**(a) Determinate:**

The fertilized egg forms all the parts of the embryo by repeated division. Some eggs or ova have, even before cleavage, different regions earmarked to form different parts of the embryo. For example, in the Ascidian eggs the region, which will form the endoderm, is fixed. If this region is dissected out from a fertilized egg, the embryo formed later will have no endoderm. Such eggs with predetermined regions are called mosaic (or determinate) eggs.

Cleavages in mosaic eggs follow a precise pattern and each blastomere has its characteristic position and unalterable fate. Here cleavage separate different organ forming regions and are called determinate or mosaic cleavage. Examples are of nematodes, annelids, molluses and ascidians, which show determinate type of cleavage.

**(b) Indeterminate:**

In vertebrates the plan of cleavage is less rigid. Here the fertilized eggs do not have predetermined region. If the region which normally forms the endoderm removed from a fertilized sea urchin egg, the embryo formed later will still have the endoderm. Such eggs are called regulative or indeterminate eggs.

In these eggs, as there are no predetermined regions and the cleavages cannot separate such regions, they simply cut the eggs into segments which have the potential of forming any organ. This type of cleavage is called indeterminate or regulative cleavage. Eggs of some groups of invertebrates and of all vertebrates show indeterminate cleavage.

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**3.3.2 CONTROL OF CLEAVAGE PATTERNS**

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(1) A simple way to regulate cell divisions differentially during development would be to express all but one of the required factors constitutively, and to then modulate expression of the single remaining, rate-limiting factor. Our findings are consistent with this type of regulation, and suggest that *stg* mRNA is the rate-limiting factor whose expression governs mitotic patterns after interphase 14. The most suggestive of these findings are first, that *stg* mRNA is expressed in a spatio-temporal pattern that anticipates the mitotic pattern, and second, that the *stg* gene is required zygotically for initiation of the first patterned mitosis. Since cell-cycle arrest occurs earlier in *stg* mutants than in any other characterized zygotic mutant, it seems likely that maternal supplies of other cell-cycle factors persist past the time when *stg* becomes required. The work of Lehner and O'Farrell on *Drosophila* cyclin A provides a clear example of a product that is required for mitosis yet seems not to be rate-limiting during the first zygotically controlled divisions. In contrast to *stg*, cyclin A derived from maternal mRNA is sufficient to support divisions through mitosis 15, and the level of cyclin A protein has little influence on the timing of mitoses.

(2) We have demonstrated directly only that *stg* is required for mitosis 14, but our observations of mitotic patterns in hypomorphic and temperature-sensitive *stg* mutants suggest that *stg* is required for subsequent embryonic mitoses (Edgar, unpublished data). In addition, clonal analysis has indicated that *stg* is required for cell divisions in the imaginal discs during metamorphosis (Terle and Saint, personal communication). Although such observations do not address whether *stg* continues to be rate-limiting during later mitoses, the

continued correlation of *stg* expression with mitotic patterns suggests that *stg* expression could determine the timing of all the postblastoderm divisions.

(3) Perhaps our most significant finding is that the pattern of embryonic cell divisions may be predicted from the expression pattern of *stg* messenger RNA. This implies that the mitotic pattern may be controlled through differential rates of *stg* transcription and/or RNA degradation. While we know little about RNA degradation in *Drosophila*, recent studies have defined a plethora of factors involved in generating complex spatio-temporal patterns of transcription during embryogenesis these factors are encoded by the “selector” genes that set up patterns and determine cell identities in the embryo. To date, most of the selector genes studied at the molecular level encodes DNA binding proteins of the zinc finger or homeodomain types that reside in the nucleus, and are widely believed to be transcription factors. This is fitting, since it has long been thought that selector genes determined cell fates by modulating the expression of “cytodifferentiation” genes encoding products directly responsible for cell structure, movement, and division. Our understanding of *stg*’s function clearly classifies it as a cytodifferentiation gene, and thus as a likely target of selector-gene regulation.

(4) There are more compelling reasons than theory, however, to believe that *stg* is a selector-gene target. One is that the selector-gene expression patterns exhibit uncanny similarities to the *stg* expression patterns. Preceding mitosis 14, *stg* expression along the dorsoventral axis of the embryo is broken up into at least six distinct patterns that fall into the six dorsoventral domains defined by selector genes such as *zerknüllt*, *twist*, and those of the *spitz* group. Along the anteroposterior axis, *stg* expression is divided into different patterns in the head, thorax, abdomen, and tail, regions that are determined by differential expression of selector genes of the gap and homeotic classes (Gaul and Jäckle, 1987). Within these regions, *stg* expression occurs in reiterated patterns with double- and single-segment periodicity that resembles pair-rule and segment-polarity gene expression patterns.

(5) In theory, the various selector-gene expression patterns subdivide the embryo into enough uniquely specified domains to account for virtually all aspects of *stg* expression during cycle 14. Moreover, the patterns of mitosis 14 are altered in predictable ways in many selector-gene mutants (Foe, personal communication). We expect that the altered patterns of mitosis in these mutants will be correlated with altered patterns of *stg* expression. Accordingly, we would like to propose that *stg* activity, and thus the mitotic pattern, is

regulated at the transcriptional level by a variety of selector-gene products. Much of this regulation may be indirect, but the relative timing of selector-gene and *stg* expression suggests that direct interactions, perhaps using combinations of selector-gene encoded transcription factors, are involved.

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### 3.3.3 CHEMICAL CHANGES DURING CLEAVAGE

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Significant chemical changes go on in the fertilized egg during cleavage. They are:

- (1) **Increase of nuclear material:** During cleavage a steady increase in nuclear material (predominantly DNA) is observed. Cytoplasm of the egg is the source of such nuclear material. Cytoplasmic DNA contained in mitochondria and yolk platelets are available.
- (2) **RNA synthesis:** During cleavage messenger RNA (mRNA) and transfer RNA (RNA) are synthesized during cleavage, especially in late stages.
- (3) **Synthesis of proteins:** Throughout the period of cleavage there is steady and spectacular increase in protein synthesis.

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### 3.3.4 SIGNIFICANCE OF CLEAVAGE

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- (i) It converts a unicellular zygote into a multicellular embryo.
- (ii) It maintains the cell size and nucleo-cytoplasmic ratio of the species.
- (iii) Cleavage produces large number of cells or blastomeres required for the building of offspring's body.
- (iv) During cleavage quick mitotic division of blastomeres occurs following which there is no growth of blastomeres.
- (v) Cleavage brings about the distribution of cytoplasm among the blastomeres.

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### 3.4 *EMBRYONIC INDUCTION*

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Embryonic induction defines as the process of communication between cells required for their differentiation, morphogenesis and maintenance.

(1) In amphibian embryos, the dorsal ectodermal cells in a mid-longitudinal region differentiate to form a neural plate, only when the chorda-mesoderm is below it. Chorda-mesoderm is the layer formed by cells invaginated from the region of the dorsal blastoporal lip, which forms the roof of archenteron.

(2) Mangold (1927) selected a small part of dorsal blastoporal lip from an early gastrula of *Triturus cristatus* and grafted it at a place near the lateral lip of the blastopore of the host gastrula of *T. taeniatus*. The graft cells grew in number and spread inside the host gastrula to form an additional chorda-mesoderm at this place. This chorda-mesoderm subsequently induced the ectoderm of the host gastrula to form an additional neural tube.

(3) The graft cells themselves formed an additional notochord. As the host gastrula developed further, it grew into a double embryo joined together. One of the embryos was the regular one, while the second was the induced one. The latter did not develop a complete head. This experiment clearly showed that the dorsal blastoporal lip of the blastula had the ability to induce the formation of the neural plate in the ectoderm of the host. This phenomenon is called neural induction. Other parts of an embryo can similarly induce the formation of other structures. This influence of one structure in the formation of another structure is called embryonic induction.

(4) In fact, the entire development of an organism is due to a series of inductions. The structure, which induces the formation of another structure, is called the inductor or organizer. The chemical substance that is emitted by an inductor is called an evacuator. The tissue on which an evacuator or inductor acts is called the responsive tissue.

(a) **Historical Background of Embryonic Induction:** For the discovery of neural induction, the German embryologist, Hans Spemann and his student, Hilde Mangold (1924) worked a lot and for his work Spemann received Nobel Prize in 1935.

(b) These two scientists performed certain heteroblastic transplantations between two species of newt, i.e., *Triturus cristatus* and *Triturus taeniatus* and reported that the dorsal lip

of their early gastrula has the capacity of induction and organization of presumptive neural ectoderm to form a neural tube and also the capacity of evocation and organization of ectoderm, mesoderm and endoderm to form a complete secondary embryo.

(c) They called the dorsal lip of the blastopore the primary organizer since it was first in the sequence of inductions and as it had the capacity to organize the development of a second embryo. Later on, the primary organizer was reported to exist in many animals, e.g. in frogs (Daloq and Pasteels, 1937); in cyclostomes (Yamada, 1938); in bony fishes (Oppenheimer, 1936); in birds (Waddington, 1933) and in rabbit (Waddington, 1934).

(d) Primary organizer and neural induction have been reported in certain pre-vertebrate chordates, such as ascidians and *Amphioxus* (Tung, Wu and Tung, 1932). In 1960 and 1963 Curtis investigated and reported that the organizer of gastrula of *Xenopus laevis* can be distinguished in the cortex of gray crescent of a fertilized egg.

(e) Holtfreter (1945) gave an account of how an enormous variety of entirely unspecific substances-organic acids, steroids, kaolin, methylene blue, sulphhydryl compounds, which had nothing in common except the property of being toxic to sub-ectodermal cells-produced neurulation in explants. Barth and Barth (1968, 69) provided further information about the chemical nature of embryonic induction.

### **(5) Types of embryonic induction:**

Lovtrup (1974) classified different types of embryonic induction into two basic categories-endogenous and exogenous inductions.

#### **(a) Endogenous induction:**

Certain embryonic cells gradually assume new diversification pattern through the inductors that are produced by them endogenously. Due to these inductors, these cells undergo either self-transformation or self-differentiation. Examples of such induction were reported in Mesenchymal cells of ventral pole of Echinoid and in small sized, yolk-laden cells of dorsal lip of amphibian blastopore.

#### **(b) Exogenous induction:**

When some external agent or a cell or a tissue is introduced into an embryo, they exert their influence by a process of diversification pattern upon neighbouring cells through contact



induction. This phenomenon is called exogenous induction. It may be homotypic or heterotypic depending on the fact that whether the inductor provokes the formation of same or different kind of tissues respectively (Grobstein, 1964). In homotypic induction, a differentiated cell produces an inductor. The inductor not only serves to maintain the state of the cell proper, but also induces adjacent cells to differentiate according to it, after crossing the cell boundaries. Best example of the heterotypic exogenous induction is the formation of a secondary embryonic axis by an implanted presumptive notochord in amphibians.

**(6) Experimental evidences to induction:**

Spemann and Mangold (1924) transplanted heteroplastically a piece of the dorsal lip of the blastopore of an early gastrula of pigmented newt, *Triturus cristatus* and grafted it near the ventral or lateral lip of the blastopore of the early gastrula of pigmented newt *T. taeniatatus*. Most of the graft invaginated into the interior and developed into notochord and somite's and induced the host ectoderm to form a neural tube, leaving a narrow strip of tissue on the surface.

With the development of host embryo, an additional whole system of organs was induced at the graft – placement area. Except for the anterior part of the head, almost a complete secondary embryo comprising of the additional organs was formed. Posterior part of the head was present as indicated by a pair of ear rudiments. Since in this experiment the type of transplantation involved was heteroplastic, it was found that notochord of secondary embryo consisted exclusively of graft cells; the somites consisted partly of graft and partly of host cells (Fig. 3.5). Few cells, which did not invaginate during gastrulation, were left in the neural tube. The bulk of the neural tube, part of the somites, kidney tubules and the ear rudiments of the secondary embryo consisted of host cells. The graft becomes self-differentiated and at the same time induces the adjoining host tissue to form spinal cord and other structures including somites and kidney tubules. Spemann (1938) described dorsal lip of the early gastrula as a “primary organizer” of the gastrulative process. However, organization of the secondary embryo results from a series of both inductive interactions and self-differentiate changes in the host and donor tissues. Hence, now a days the term “embryonic induction” or “inductive interactions” is preferred. The part, which is the source of induction, is called “inductor”.



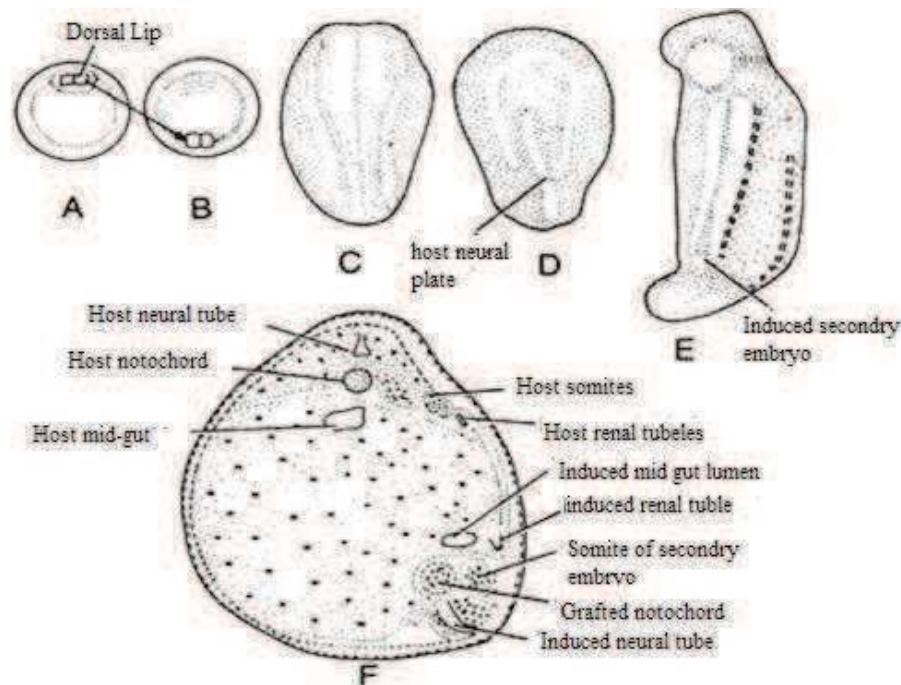


Fig.3.5 Induction of secondary embryo in triturus by transplanting a piece of dorsal lip to the future belly region of another gastrula(A-B) and C-E are the stage of resulting primary embryo with a secondary embryo attached to it, where F is the T.S of same embryo.

#### (7) Characteristics of the organizer:

Organizer has the ability for self-differentiation and organization. It also has the power to induce changes within the cell and to organize surrounding cells, including the induction and early organization of neural tube. Primary organizer determines the main features of axiation and organization of the vertebrate embryo.

Induction is a tool-like process, utilized by this center of activity through which it affects changes in surrounding cells and as such influences organization and differentiation. These surrounding cells, changed by the process of induction, may in turn act as secondary inductor centers with abilities to organize specific sub-areas.

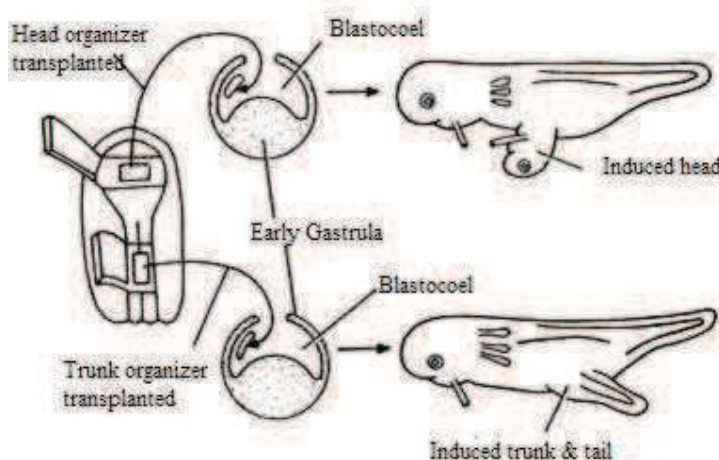
Thus, the transformation of the late blastula into an organized condition of the late gastrula appears to be dependent upon a number of separate inductions, all integrated into one coordinated whole by the “formative stimulus” of the primary organizer located in the pre-chordal plate area of the endodermal -mesodermal cells and adjacent chorda-mesodermal material of the early gastrula.

**(8) *Regional specificity of the organizer:***

Vital-staining experiments of Vogt with newt eggs have shown that the material successively forming the dorsal blastoporal lip moves forward as the archenteron roof. Transplants taken from this region are also able to induce a secondary embryo or the belly of a new host i.e. the archenteron roof acts as a primary inductor in essentially the same way as does the dorsal lip tissue proper. The inductions of neural inductor are found to be regionally specific and the regional specificity is imposed on the induced organ by the inductor.

Therefore, the inductive capacity of the blastoporal lip varies both regionally and temporally. Most of the dorsal and dorso-lateral blastoporal material is necessary for a graft to induce a more or less complete secondary embryo. Spemann (1931) demonstrated that during gastrulation anterior part of the archenteric roof invaginates over the dorsal lip of the blastopore earlier.

Dorsal blastopore lip of the early gastrula contains the archenteric and deuteroccephalic organizer and the dorsal blastopore lip of the late gastrula contains the spinocaudal organizer. Inductions produced by the dorsal lip of the blastopore taken from the early and the late gastrula differ in accordance with exception; the first tends to produce head organs and the second tends to produce trunk and tail organs (Fig. 3.6).



*Fig 3.6 The separation of neural inductor into head & trunk organizer*

(9) As invagination continues and the dorsal lip no longer consists of prospective head endo-mesoderm but progressively becomes prospective trunk mesoderm; it acts as a trunk-

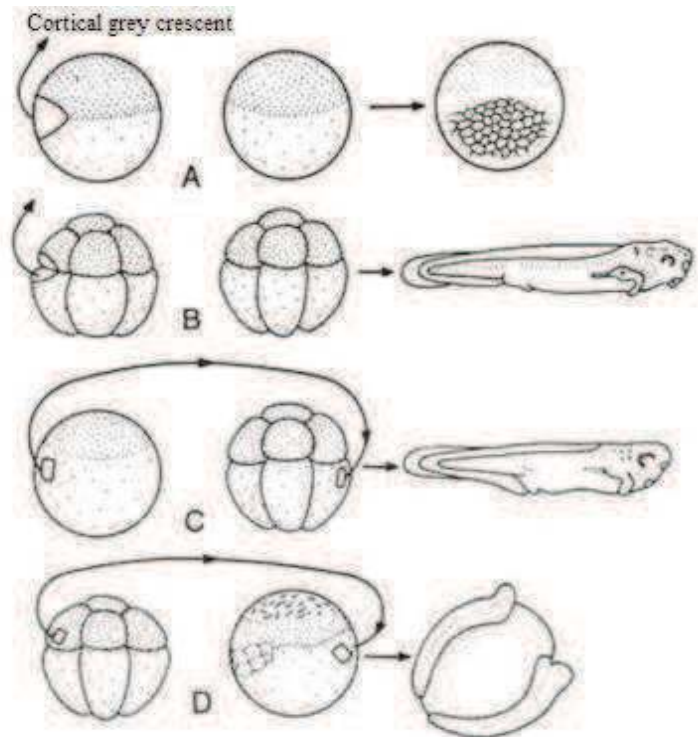
tail inductor. The most caudal region of the archenteron roof, in fact, specifically induces tail somites and probably other mesodermal tissues. The archenteron roof induces entirely different class of tissues; various neural and meso-ectodermal tissues by its anterior region and various mesodermal tissues by its most posterior region.

Therefore, differences in specific induction capacities exist between head and trunk level of archenteron roof and are related to the regional differentiation of the neural tissue into archencephalic (including fore-brain, eye, nasal pit), deuterencephalic (including hind-brain, ear vesicle) and spinocaudal components. Thus, archenteron roof consists of an anterior head inductor including an archencephalic inductor and a deuterencephalic inductor and a trunk or spinocaudal inductor.

#### **(11) Primary induction and gray crescent:**

The dorsal lip region of the blastopore at the onset of gastrulation can be traced back to the gray-crescent of the undivided fertilized amphibian egg. It was conceived by some developmental biologists that the crescent material of egg cortex initiated gastrulation and has the capacity of neural induction. A.S.G.Curtis (1963) performed a series of experiments of transplanting parts of the cortex of the fertilized egg of the clawed toad, *Xenopus* is at the beginning of cleavage

.In one experiment, the gray-crescent cortex was excised from the fertilized egg and it was observed that the cell division though proceeded undisturbed, the gastrulation failed to take place (Fig. 3A). In another experiment, the gray crescent cortex of uncleaved fertilized egg was excised and transplanted into a ventral position of a second egg, so that the egg receiving the graft had two gray crescents on opposite sides.



*Fig.3.7 Experiment of Curtis on Xenopus*

As a result, egg cleaved to form a blastula, which underwent two separate gastrulation movements to produce two separate primary nervous systems, notochord and associated somites. Similar experiments conducted on the eight-cell stage showed that something had happened during the short – interval represented by the first three cleavages.

Gray crescent cortex of the eight-cell stage still retained its inductive capacity when grafted to younger stages. Removal of the gray crescent at this stage no longer inhibits subsequent gastrulation and normal development, the missing crescent properties being replaced from adjacent cortical regions.

According to Curtis, a change in cortical organization spreads across the surface of the egg during the second and third cleavages, starting from the gray crescent; when this change is completed, interactions, probably of a biophysical nature, can take place among various parts of the cortex.

**(12) Mechanism of neural induction:**

Development of the ectoderm overlying the roof of the archenteron into neural tissue suggests a direct action upon the ectodermal cells, either by surface interaction or by chemical mediation.

(a) One of the broad possibilities is surface interaction of the cells at the inductive interface. The contact of the two cellular layers may provide a device whereby the structural pattern or geometry or behaviour of the ectodermal cell membranes is altered directly by the underlying chorda mesodermal cells.

Thus, the spatial configuration of the latter membranes might induce a change in the spatial configuration of the ectodermal cell membranes, this in turn producing in the interior of the cell changes that determine its development into neural plate. A morphological arrangement of this kind could account for quick and effective transmission of the inductive effect.

(b) Another broad possibility is a chemical mediation of the inductive effect. Therefore, a chemical substance or substances produced and released by inducing chorda mesoderm cells at the archenteron -ectoderm interface may act upon or enter the ectodermal cells to initiate cellular activities leading to neural development. A great deal of evidence favours the idea of an exchange of material between cells and also suggests that a diffusible substance may act as effective inductive stimulus.

**(13) Chemical basis of neural induction:**

The results of numerous studies to elucidate the mechanism of induction and to identify the chemical substance or substances presumed to be involved have not yielded good results. It was found that many different tissues, embryonic or adult, from a great variety of different species, were capable of inducing nervous tissue in amphibian embryos. Moreover, some foreign tissues were found to be much more potent inductors after they had been killed by heat or alcohol treatment.

This fact remains against the concept of a universally present 'masked organizer's, released in the primary inductor region. Few inorganic agents as iodine and kaolin, local injury, exposure to saline solutions of excessively high or low pH, cause neural differentiation in ectoderm. These findings establish the early grand concept of master-

chemical embryonic organizer of Holtfreter's sublethal cytolysis. It has the concept of reversible cell injury liberating neural inductor.

Different chemical substances of either gray crescent or dorsal lip or chord mesoderm are separated by different biochemical methods to find out the molecule which causes the neural induction and then the inductive capacity of each molecule was tested separately. Few experiments show that inducing substance is a protein.

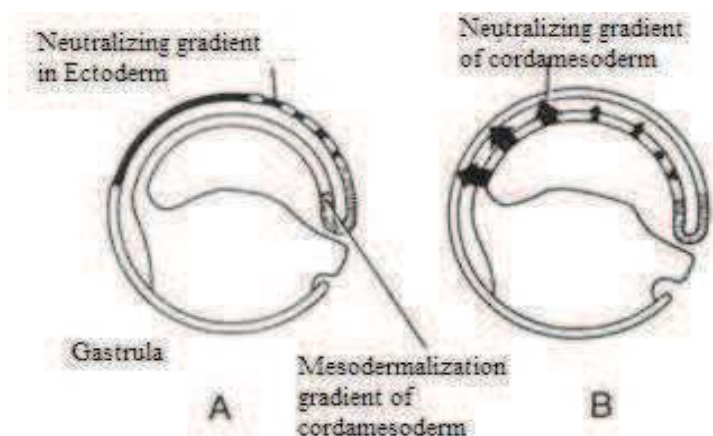
Exhaustive attempts were made by different embryologists to understand the real mechanism of neural induction. Some theories have been put forward to understand the mechanism of neural induction, out of which the most important are as follows:

**(a) Protein denaturation theory of neural induction:**

According to Ranzi (1963) neural induction and notochord formation are related to protein denaturation. Site of notochord formation is amphibian gray crescent, which is a center of high metabolic activity. Such centers of greater metabolic activity correspond to sites of protein denaturation.

**(b) Gradient theory of neural induction:**

Toivonen (1968) and Yamada (1961) stated that two chemically distinct factors are involved in the action of the primary inductor. Out of these two factors, one is neutralizing agent and the other is mesodermalizing agent. These experiments were conducted with denatured bone marrow and liver as the inductors.



*Fig.3.8 (A) The neutralizing gradient in ectoderm over lying in the mesodermalization gradient of chordamesoderm, (B) Neutralizing gradient of chordamesoderm*

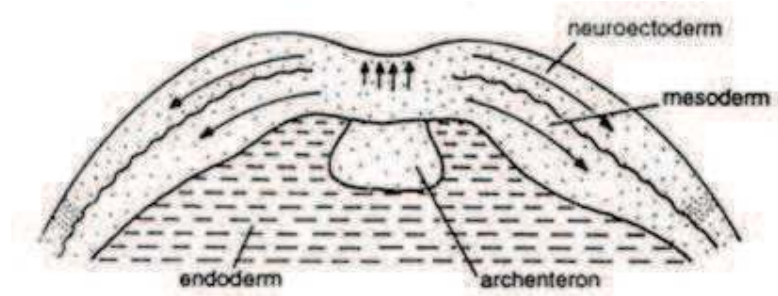


Regional specificity of the embryonic axis arises from the interaction between two gradients: neutralizing principle has its highest concentration in the dorsal side of the embryo and diminishes laterally, while the mesodermalizing principle is present as an antero-posterior gradient with its peak in the posterior region.

Anteriorly the neutralizing principle acts alone to induce forebrain structures, more posterior the mesodermalizing principle acts along with the neutralizing one to induce mid-brain and hind-brain structures, while even more posteriorly the high concentration level of the mesoderm gradient produces spino-caudal structures (Fig.3.8).

**(c) One factor hypothesis of neural induction:**

Nieuwkoop (1966) using living notochord as the inductor, postulated that only one factor which first evokes ectoderm to form neural tissue and later causes ectoderm to transform into more posterior and mesodermal structure (Fig.3.9) is involved.



*Fig.3.9 Medio lateral spreading of an inductive action within the mesoderm & a similar spreading of the neutralizing action in the overlying ectoderm.*

In one experiment, consisting of combining isolated gastrular ectoderm with a piece of notochord and then removing the notochord tissue after varying lengths of time, it was found that only 5 minutes exposure to inductor caused a part of the ectoderm to transform into brain and eye structures.

**(d) Ionic theory of neural induction:**

According to Barth and Barth (1969), the actual process of induction may be initiated by release of ions from bound form, representing a change in the ratio between bound to free ions within the cell of the early gastrula. Induction of nerve and pigment cells in small

aggregates of prospective epidermis of the frog gastrula were found to be dependent on the concentration of the sodium ions.

Normal induction of nerve and pigment cells by mesoderm in small explants from the dorsal lip and lateral marginal zones of the early gastrula is dependent on the external concentration of sodium. Thus, normal embryonic induction depends on an endogenous source of ions and that an intracellular release of such ions occurs during late gastrulation.

**(14) Genetic basis of neural induction:**

There are evidences that the component tissues of neural inductor become differentiated prior to ectodermal cells. During this process, the rate of transcription of mRNA and differential activation of genes becomes many fold, while the differentiation of ectodermal cells is set in only after mid-gastrulation. According to experiments conducted by Tiedemann (1968), after 2 to 7 days of cultivation of dorsal blastopore lip of young *Triturus* gastrula with adjacent ectoderm in a medium containing sufficient quantities of Actinomycin-D to inhibit RNA synthesis, induction could not take place, but some differentiation of muscle and notochord occurred. It shows that mRNA by transcription from the DNA was required, which also requires the presence of Actinomycin-D. Therefore, no neural induction could be detected in this experiment.

**(15) Time of neural induction:**

Neural induction occurs at the time when the material of chordamesoderm moves from the dorsal lip of blastopore inward and forward (Saxen and Toivonen 1962). The inductive stimuli exhibit a time gradient, which may be crucial with regard to action and reaction events.

**(16) Embryonic induction in different chordates:**

Although neural induction was first discovered in urodele amphibians, it was found that the dorsal lip of the blastopore and the roof of the archenteron of other vertebrates have the same function. The chordamesoderm in all vertebrates induces the nervous system and sense organs. Neural inductor has been investigated in the following chordates:

(i) In Cyclostomes, especially in lampreys, the property of neural induction lays in the presumptive chorda mesodermal cells of dorsal lip of the blastopore. Prior to cyclostomes, in Ascidians different blastomeres of eight cell stage have the following presumptive fates-(i)

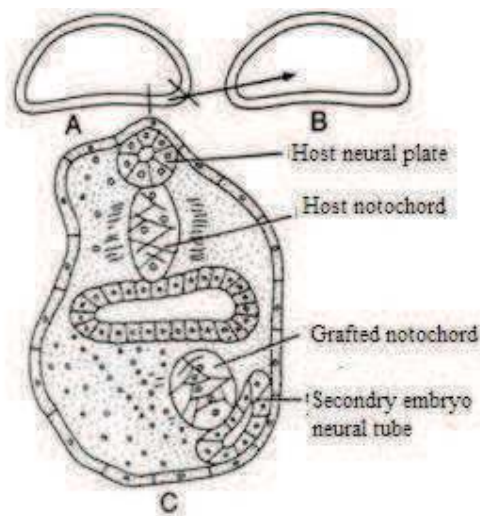


the two anterior animal pole blastomeres produce head epidermis, pulps and the brain with its two pigmented sensory structures, (ii) two posterior animal pole blastomeres produce epidermis, (iii) two anterior vegetal blastomeres produce notochord, spinal cord and part of the intestine (iv) two posterior vegetal cells produce mesenchyme, muscles and part of the intestine.

From these experiments, Raverberi (1960) concluded that the formation and differentiation of brain by two anterior animal blastomeres is dependent on the induction of two anterior vegetal blastomeres, which act as neural inducers. It was further concluded that the two anterior vegetal blastomeres gave rise to diverse tissues, namely, endoderm, notochord and spinal cord.

(ii) Wu and Tung (1962) proved the existence of the primary organizer and neural induction in *Amphioxus*. They transplanted pieces of tissues from the inner surface of the dorsal blastopore lip of an early gastrula of *Amphioxus* into the blastocoel of another embryo in the same stage (Fig.3.10) and observed that secondary embryo developed in the ventral region of the host with a notochord and mesoderm produced by the graft and the neural tube from host tissue.

Thus, the chordal tissue of *Amphioxus* gastrula possesses the power of neural induction, while mesodermal and endodermal tissues have little such inductive power.



*Fig.3.10 Neural induction in Amphioxus*

(iii) In bony fishes, inductions of secondary well developed embryos were produced by transplanting the posterior edge of the blastodisc which corresponds to the dorsal lip of the blastopore, into the blastocoel of another embryo (Fig.3.11) or by transplanting the chordamesoderm and ectoderm. Neural inductions were also obtained by transplanting the dorsal lip of the blastopore in the sturgeon.

(iv) In frogs, the induction of secondary embryo can be produced by the dorsal lip of the blastopore transplanted into the blastocoel of a young gastrula, in very much the same way as in newts and salamanders.

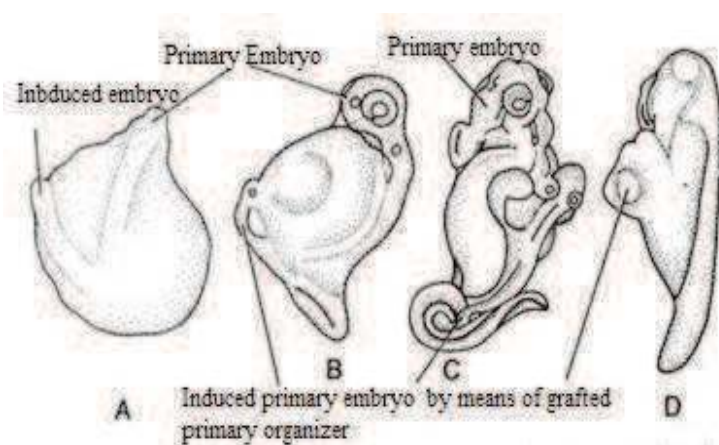


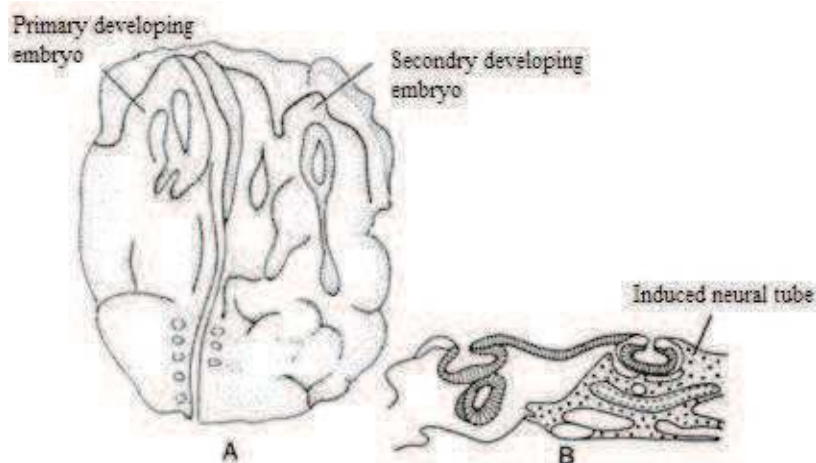
Fig 3.11 Induction of secondary embryo by means of grafted primary organizer in (a) Lamprey, Pench & Frog.

(v) In reptiles archenteron has the same inducing activity as in other vertebrates but there is no experimental proof of occurrence of neural inductor.

(vi) In birds the existence of primary organizer was established by Waddington and co-workers. Anterior half of the primitive streak was the inducing part similar to the lips of the blastopore in amphibians. In the experiment whole blastoderms were removed from the egg in early gastrulation and cultivated in vitro on the blood plasma clot.

From another embryo, parts of the primitive streak were then inserted between epiblast and hypoblast, inductions of secondary embryos obtained. Primitive streak was found dependent on the underlying hypoblast for its formation (Fig.3.12).

(vii) A successful neural induction was performed in a rabbit embryo by cultivating the early blastodisc on a plasma clot and implanting the primitive streak of the chick as inductor. Tissues of the mammalian gastrula were found having competence for neural induction. Anterior end of a rabbit embryo, with two pairs of somites, induced a neural plate in a chick embryo when placed under a chick blastoderm.



*Fig 3.12 Induction of secondary embryo by means of grafted primitive streak in a birds*

#### **(17) Other types of embryonic inductions:**

Along with gastrulation growth, various organ systems of the embryo begin to differentiate and acquire the power of inducing the differentiation of later formed structures or organs such as eyes, ears, limbs and lungs, etc. These organs develop organizing property and become the source of induction.

Therefore, this series of organizers can be called as secondary, tertiary and quaternary organizers. Progressive development of embryonic organs is dependent on sequential induction. One embryonic tissue interacts with the adjacent one and induces it to develop and this process continues in sequence.

#### **(a) Development of eye in chick:**

The first sign of the development of the eyes is a bulging at the lateral sides of the prosencephalon. These are the rudiments of the optic vesicles which lie beneath the head ectoderm. Meanwhile, the distal part of each optic vesicle (the future sensory layer)

invaginates and presses against the proximal part (the future pigment layer of the retina, iris and ciliary body). This results in the formation of the optic cup, the elimination of the original lumen of the optic vesicle and the formation of a new lumen, the future vitreous chamber.

The lens is formed from the lens placode, a thickening of the ectoderm formed in response to an inductive signal from the optic cup. The lens sinks beneath the surface of the ectoderm, the latter becoming the cornea.

As the lens continues to grow, the cells in the thickened region lose their ability to divide and become converted into fibres that will become the core of the adult lens. New fibres are formed from the cells at the periphery of the lens which divide rapidly and become arranged in concentric circles around the original core. By the time of hatching there are three concentric layers of fibres, the core, the intermediate layer of irregularly arranged fibres, and the radial layers which continue to grow after hatching. The lens capsule, which is an extracellular material with a high collagenous component, starts to form about day 7. The ciliary body develops close to the lens, its role being to secrete the fluid of the vitreous chamber.

As the lens loses contact with the ectoderm a space is formed, the anterior chamber of the eye. The corneal epithelium develops from the ectoderm covering the anterior chamber, whilst the corneal stroma forms from the mesenchyme and becomes visible on day 4 as a thin layer beneath the epithelium. It becomes thicker as mesenchyme cells migrate into it during day 7.

The iris arises from cells at the margin of the anterior chamber at about day 7. Removal of the lens results in disorganization of the components of the anterior chamber. The retina is formed from the optic cup. Its inner layer becomes the neural retina and its outer layer the pigmented retina.

The choroid and sclera differentiate from the mesenchyme around the optic cup, forming the inner pigmented vascular layer, and the outer, fibrous layer, respectively. The melanophores of the choroids are derived from cells of the neural crest that reach the eye during day 2 and develop pigment on day 7. Cartilage starts to form in the sclera on day 8.

The eyelids start to form at about 7 days from a circular fold of skin surrounding the eye. The choroid fissure usually begins to close in the region near the lens about day 4. At this time a ridge of mesoderm, carrying with it a blood vessel, migrates along the choroid fissure into the

posterior chamber of the eye and enlarges during day 5 to form the pecten. The pigment cells of the pecten are derived from the pigmented retina. The pecten is a structure characteristic of birds, and it is thought that it acts not only by bringing oxygen and nutritive materials to the eye but that it may also play a role in vision. The vitreous humour is secreted by the cells of the optic cup.

**(18) Types of inductors:**

On the basis of various experimental evidences Lehmon (1945) said that specific regionality of induction effects present in the dorsal lip of the blastopore. He further said that the roof of the archenteron definitely possess specific induction activities for the differentiation of head and trunk regions. On the basis of the regional specificity he classified the inductors into three groups. They are:

- 1) **Archenocephalic inductor:** Due to induction effect of this inductor partial head, fore-brain, eye, nasal cavities are formed.
- 2) **Deuterencephalic inductor:** By its induction effect posterior portion of the head, ear cavities etc. are formed. As archenocephalic and deuterencephalic inductors induce the formation of different parts in the head region so they together are known as cephalic or head inductors.
- 3) **Spino-caudal inductor:** Their inductive influence leads to the formation of spinal cord and different structures of the tail region.
- 4) **Development of Eye in Chick**

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### **3.5 CONCEPT OF ORGANIZER**

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The effect of embryonic interaction or organizer is a morphogenetic effect by which one organic tissue transmits a chemical substance that influences other embryonic part to produce a structure that otherwise could not come into existence. The embryonic tissue which exerts such an influence is called an inductor and the chemical substance secreted by an inductor is known as evocators. The tissue on which evocator works and the tissue responses is known as responsive tissue. The action of the indicator through evocator is known as induction action or organizer action. This process of induction influences greatly the protein synthesis mechanism of responsive tissues as a result of which definite structure forming cells become very active.

Spemann's experiment (1924): A German embryologist Hans Spemann and his student Hilde Mangold (1924) performed transplantation experiment on a newt *Triturus cristatus*, an Urodela of class Amphibia. Spemann grafted a piece taken from the dorsal lip of early gastrula of *Rana* sp. to the lateral lip region of the early gastrula of *Triturus cristatus*. The embryo of *Rana* sp. is donor and the embryo of *Triturus* is the host. They observed that the cells of the grafted piece enter into the gastrula and form notochord and somites. In this embryo its own dorsal lip of blastopore forms neural groove, notochord, mesoderm etc. Similarly the grafted tissue influences to form notochord, neural groove and mesoderm. That is in the same embryo double set of notochord, nerve cord and mesoderm are produced. In this case donor tissue has secreted some chemical substances which has induced to form neural groove, notochord etc. in the host embryo. The donor tissue had pigments and the induced neural groove has also coloured pigments. After the completion of the gastrulation they observed that a larva has developed with two heads. One head is due to normal development and the other head production has been induced by donor tissue.

They examined the larva under the microscope and found that notochord, renal tubules, gut etc. have been formed by the tissue of the host embryo as a secondary set. If the donor tissue would not have been grafted such secondary structures would not develop. From this experiment they concluded that dorsal lip of the donor had influenced greatly the tissue and thus has brought about change in the host tissue development. If it is not the fact then how a head had developed in the abdomen of the host. This secondary head formation is due to induction effect of donor tissue. This process of influencing other tissue was termed as induction by Spemann and the tissue that induced the tissue was known as the inductor or organizer.

### 1. Primary organizer:

Spemann continued his grafting experiments taking tissues from different zones of the gastrula and observed that except dorsal lip of the early gastrula other zone of tissue can not create any induction effect but when dorsal lip is grafted a complete embryo is formed. He named the dorsal lip as organizer as this dorsal lip organizes the developmental process of the embryo. According to him this dorsal lip induces to form neural tube and the neural tube then induces to form the eyes. The dorsal lip is composed of chorda-mesoderm and as it primarily acts as inducer so he named the dorsal lip or chordamesoderm as primary organizers.

## 2. Secondary, tertiary and quaternary organizers:

As the gastrulation proceeds due to primary organizer's induction primary organs begin to form and the early stages of organ development are known as organ rudiments. These organ rudiments themselves may act as organizer and then they are known as secondary organizer. Tissues formed by the action of secondary organizer may in turn induce further development. Then they are known as tertiary organizer. These successive stages of organizer activities start from the primary organizer.

How these organizers act in succession can clearly be understood from the examples of the development of eye in amphibian, chick etc. First of all due to induction effect of the primary organizer forebrain and within the forebrain eye forming cells are produced. These cells push out as a vesicle outside the forebrain. These vesicles are known as optic vesicle. This vesicle grows through the lateral mesenchyme and reaches the epidermis.

As soon as the vesicle comes in contact with the epidermis the outer layer of the vesicle invaginates to form a double layered optic cup. The inner layer of the optic cup is formed of sensory cells and the outer layer is formed of pigmented cells. They two together form the retina. The chemical substances secreted by the optic cup induce to form the lens between the optic cup and the epidermis. The peculiar thing is that if the optic vesicle is prevented from coming in contact with the epidermis there will be no lens formation. So the optic cup acts as secondary organizer. Similarly lens and retina together induce to form cornea so lens and retina together act as tertiary organizer and so on.

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## 3.6 SUMMARY

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In embryology, **cleavage** is the division of cells in the early embryo. The zygotes of many species undergo rapid cell cycles with no significant growth, producing a cluster of cells the same size as the original zygote. The different cells derived from cleavage are called blastomeres and form a compact mass called the morula. Cleavage ends with the formation of the blastula.

Depending mostly on the amount of yolk in the egg, the cleavage can be **holoblastic** (total or entire cleavage) or **meroblastic** (partial cleavage). The pole of the egg with the highest concentration of yolk is referred to as the vegetal pole while the opposite is referred to as the animal pole.



Cleavage differs from other forms of cell division in that it increases the number of cells without increasing the mass. This means that with each successive subdivision, the ratio of nuclear to cytoplasmic material increases.

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### 3.7 GLOSSARY

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<b>Agar-</b>	A polysaccharide complex extracted from seaweed (Rhodophyceae) and used as an inert support for the growth of cells, particularly bacteria and some cancer cell lines.
<b>Ambystoma mexicanum-</b>	Mexican axolotl (amphibian). A salamander that shows neoteny.  the adult may retain the larval form, but can reproduce. The neotenuous, aquatic axolotl will metamorphose into the terrestrial form if injected with thyroid or pituitary gland extract.
<b>Animal cap-</b>	Pigmented animal hemisphere of the amphibian blastula.
<b>Animal Pole-</b>	In most animal oocytes the nucleus is not centrally placed and its position can be used to define two poles. That nearest to the nucleus is the animal pole, and the other is the vegetal pole, with the animal-vegetal axis between the poles passing through the nucleus.
<b>Anterior-posterior axis -</b>	Body axis extending from the anterior to the posterior pole of a bilaterally symmetric embryo (or animal).
<b>Archenteron-</b>	Cavity formed by the endoderm during gastrulation; will later become the gut lumen.
<b>Axial mesoderm -</b>	the mesodermal tissue that gives rise to the notochord and somites.



<b>Blastocoel-</b>	Fluid-filled cavity that forms in the embryo after the morula stage.
<b>Blastomeres -</b> cleavage, in	One of the cells produced as the result of cell division, the fertilized egg.
<b>Blastoporal Groove-</b> The	The groove formed as result of the formation of bottle cells. The groove presumably results from the invagination produced by apical constriction, as well as the basal expansion of the basal ends of the bottle cells, which forces nearby tissue to roll.
<b>Blastoporal Pigment Line-</b>	The first visible sign on the surface of the amphibian embryo that gastrulation is underway. The blastoporal pigment line forms as a result of the apical constriction of bottle cells, thereby concentrating the pigment granules near the apex of each of the bottle cells, and marks the initial phase of formation of the blastopore lip.
<b>Blastula-</b>	Stage of embryonic development of animals near the end of cleavage but before gastrulation. In animals where cleavage (cell division) involves the whole egg, the blastula usually consists of a hollow ball of cells.
<b>Bottle cells -</b>	Epithelial cells that temporarily become bottle-shaped, owing to the contraction of their apical margins and the expansion of their basal margins; found at the site of initiation of gastrulation in amphibian embryos.
<b>Calcium Wave-</b>	Chain reaction of intracellular $\text{Ca}^{2+}$ release and uptake that accompanies the cortical reaction.

**Cell Cycle-** Period between the formations of a cell by the division of its parent

Cell and the formation of two new cells by cell division.

**Convergence and extension-** Convergence of an epithelial sheet toward a central site, followed

by its extension along a single axis through forceful intercalation of the cells of the epithelium

**Cortex-** Gel-like cytoplasmic layer just below the egg plasma membrane.

Thickened coelomic epithelium of developing gonads.

**Cortical Reaction-** Wave of exocytosis that occurs as the cortical granules fuse with

the egg plasma membrane and release their contents after sperm-egg fusion.

**Cytokinesis-** Division of the cytoplasm during mitosis.

**Deep Cells -** Generally, non-epithelial cells in a vertebrate embryo. In amphibians, deep cells underlie the superficial epithelial cells of the animal cap and marginal zone.

**Diploid Genome** diploid Cells have its chromosomes in homologous pairs, and thus having 2 copies of each autosomal genetic locus. The diploid number (2n) equals twice the haploid number and is the characteristic number for most cells other than gametes.

**Dorsal lip of the blastopore-** Site of initiation of gastrulation in the amphibian embryo. The

dorsal lip, which forms at the site of the gray crescent, forms the dorsal margin of the blastopore.

<b>Ectoderm-</b>	Germ layer that gives rise to the epidermis and nervous tissue.
<b>Endoderm-</b>	Germ layer that gives rise to the respiratory organs, gut, and the gut accessory glands.
<b>Endoplasmic Reticulum - (ER)</b>	Membrane system that ramifies through the cytoplasm. The membranes of the ER are separated by 50-200 nm and the cisternal space thus enclosed constitutes a separate compartment.
<b>Epidermis-</b> layer	Outer epithelial layer of a plant or animal. May be a single layer  that produces an extracellular material (as for example the cuticle of arthropods), or a complex stratified squamous epithelium, as in the case of many vertebrate species.
<b>Extracellular Matrix -</b>	ECM) any material produced by cells and secreted into the Surrounding medium, but usually applied to the non-cellular portion of animal tissues. The ecm of connective tissue is particularly extensive and the properties of the ecm determine the properties of the tissue
<b>Fibronectin -</b> kD	Glycoprotein of high molecular weight (2 chains each of 250  linked by disulphide bonds) that occurs in insoluble fibrillar form in extracellular matrix of animal tissues, and soluble in plasma, the latter previously known as cold-insoluble globulin.
<b>Fluorescent dextran-</b> weight	A chemically modified form of dextran, a high molecular

polysaccharide, which carries one of several different fluorescent moieties ("tags") that fluoresce upon excitation with the appropriate wavelength of near ultraviolet or visible light.

**G1-** Phase in the cell cycle between the completion of cell division and

the initiation of DNA synthesis.

**G2-** Phase in the cell cycle between the completion of DNA synthesis and the next cell division.

**Gap Phases-** The phases of the cell cycle known as G1 and G2, during which relatively less obvious cellular activity is visible

**Gastrula-** Stage of embryonic developments in animals when gastrulation occurs; follows the blastula stage.

**Gastrulation -** Process by which cells of the blastoderm are translocated to new

positions in the embryo, producing the three primary germ layers.

Return to Cleavage-

**Germ layer-** The main divisions of tissue types in multicellular organisms. Diploblastic organisms (eg. coelenterates) have two layers, ectoderm and endoderm; triploblastic organisms (all higher animal groups) have mesoderm between these two layers. Germ layers become distinguishable during late blastula/early gastrula stages of embryogenesis, and each gives rise to a characteristic set of tissues, the ectoderm to external epithelia and to the nervous system for example, although some tissues contain elements derived from two layers.

**Germ Plasm -** Region of the egg containing the determinants of the germ cell

line.

**Gray crescent -**  
the

Region of intermediate pigmentation in the marginal zone of the amphibian egg caused by a shift in the pigmented egg cortex toward the site of sperm entry; marks the future site of the dorsal lip of the blastopore.

**Induction-**  
neighboring

Alteration of cell fate as a result of interactions with cells.

**Integrin -**  
binding to

Super family of cell surface proteins that are involved in extracellular matrix components in some cases.

**Intercalation -**  
contact

Expansion process whereby cells from different layers lose contact with their neighbors and rearrange into a single layer, which consequently spreads laterally, owing to an increase in surface area. 2. Generation of missing positional values during regeneration when cells of disparate positional values are brought together after amputation.

**Involuting marginal zone--** Vegetal portion of the marginal zone of the *Xenopus* embryo that

turns inside the embryo during involution.

**Involution-**  
and

Process by which an expanding epithelium turns over on itself and continues to spread in the opposite direction along its basal margin.

- Keller sandwiches-animal** One of several types of explants of tissue lying immediately to the blastopore lip of an early *Xenopus* gastrula, and including involuting marginal zone, non-involuting marginal zone, and animal cap cells, named for Ray Keller, who devised the technique.
- Leading edge mesoderm - zone** The mesoderm at the extreme vegetal edge of the marginal zone which forms a free edge after the marginal zone is turned inside the embryo. The dorsal leading edge cells form head mesoderm; ventral leading edge cells form heart tissue. Leading edge cells possess lamellipodia at their free margin.
- Marginal zone -** Region of intermediate pigmentation between the pigmented animal hemisphere and the unpigmented vegetal hemisphere of the amphibian egg.
- Maternal Mrna-** Messenger RNA found in oocytes and early embryos that is derived from the maternal genome during oogenesis.
- Mediolateral interdigitation** -An intercalation movement in which cells move between one another towards the midline of the forming anterior-posterior (A-P) axis, resulting in the elongation of the entire array of rearranging cells along the A-P axis.
- Meiosis-** A specialised form of nuclear division in which there two successive nuclear divisions (meiosis I and II) without any chromosome replication between them. Each division can be divided into 4 phases similar to those of mitosis (pro-, meta-, ana- and telophase). Meiosis reduces the starting number of  $4n$  chromosomes in the parent cell to  $n$  in each of the 4 daughter cells. Each cell receives only one of each homologous

chromosome pair, with the maternal and paternal chromosomes being distributed randomly between the cells

**Mesoderm-**

connective

Middle of the three [germ layers]; gives rise to the musculo-skeletal, blood vascular, and urinogenital systems, to tissue (including that of dermis) and contributes to some glands.

**Metazoans -**

Animals whose bodies consist of many cells, as distinct from Protozoa, which are unicellular; all animals commonly recognized as animals. Sponges (Parazoa, q.v.) though also multicellular, differ so much from other multicellular animals that they are not usually included in the metazoa.

**Microfilaments-**

Contractile cytoskeletal actin filaments of 6-nm diameter.

**Microtubules-**

Components of the cytoskeleton composed of hollow cylindrical rods, 25 nm in diameter, formed of 13 rows of solid tubulin protofilaments that run parallel to the microtubule long axis.

**Mitochondrial -**  
varying

Microscopic bodies occurring in cytoplasm of every cell in numbers except in bacteria and blue-green algae (actually the mitochondria are derived from common ancestors as the bacteria and blue green algae). Contain DNA, ribosomes, and many enzyme systems; comprise power plant of cell, producing energy (in form of ATP) for many cell functions

**Mitosis -**

The usual process of nuclear division in the somatic cells of eukaryotes. Mitosis is classically divided into four stages. The chromosomes are actually replicated prior to mitosis during the S phase of the cell cycle. During the first stage, prophase, the

chromosomes condense and become visible as double strands (each strand being termed a chromatid) and the [nuclear envelope] breaks down. At the same time the mitotic spindle forms by the polymerisation of [microtubules] and the chromosomes are attached to spindle fibres at their kinetochores.

**Mrna -** (messenger RNA) RNA species that contains the information to specify the amino acid sequence of proteins and that is translated on the ribosome.

**Neoteny-** The persistence in the reproductively-mature adult of characters usually associated with the immature organism.

**Neural Fold-** bilaterally symmetric infoldings of the neural plate that then seal dorsally to form the neural tube.

**Neural induction-** In vertebrates the formation of the nervous system from the [ectoderm] of the early embryo as a result of a signal from the underlying [mesoderm] of the archenteron roof; also known as primary neural induction. The mechanism of neural induction is not yet clear

**Neural plate-** A region of embryonic ectodermal cells, called neuroectoderm, that lie directly above the notochord. During neurulation, they change shape, so as to produce an infolding of the neural plate (the neural fold) that then seals to form the neural tube.

**Neural tube-** The progenitor of the central nervous system. See neural plate, neurulation.

**Neurulation-** The embryonic formation of the neural tube by closure of the neural plate, directed by the underlying notochord.



**Non-involuting marginal zone-** Animal portion of the marginal zone of the *Xenopus* embryo

that spreads in front of the animal cap but does not involute during gastrulation.

**Notochord-**

An axial mesodermal tissue found in embryonic stages of all chordates and protochordates, often regressing as maturity is approached. Typically a rod-shaped mass of vacuolated cells. It lies immediately below the nerve cord and may provide mechanical strength to the embryo.

**Notoplate-**  
notochord.

Ventral portion of the neural plate in contact with the

**Nucleic Acid-**

Linear polymers of nucleotides, linked by 3',5' phosphodiester linkages. In DNA, deoxyribonucleic acid, the sugar group is deoxyribose, and the bases of the nucleotides adenine, guanine, thymine and cytosine. RNA, ribonucleic acid, has ribose as the sugar, and uracil replaces thymine. DNA functions as a stable repository of genetic information in the form of base sequence.

**Oogenesis-**

The process of egg formation.

**Organogenesis -**  
animal

The process of formation of specific organs in a plant or animal involving morphogenesis and differentiation.

**Protists-**  
in

Group of animals differing from the rest (Metazoa and Parazoa)

consisting of one cell only, i.e. one continuous mass of cytoplasm, but resembling them and plants, and differing from bacteria, in

having at least one well-defined nucleus, of eucaryotic type.

<b>Reductive division-</b> does	Cell division in which the volume of the two daughter cells not increase. The result is a progressive increase in cell number, without a corresponding increase in the size of the tissue. Reductive divisions are characteristic of cleavage in early embryos.
<b>S phase -</b> with	cell cycle phase during which the quantity of DNA doubles, replication of the chromosomes.
<b>Signal transduction -</b> receptor	The cascade of processes by which an extracellular signal (typically a hormone or neurotransmitter) interacts with a receptor at the cell surface, causing a change in the level of a second messenger (for example calcium or cyclic AMP) and ultimately effects a change in the cell's functioning (for example, triggering glucose uptake, or initiating cell division). Can also be applied to sensory signal transduction, eg. of light at photoreceptors.
<b>Somites-</b>	Blocks of tissue in the trunk derived from the originally unsegmented paraxial mesoderm.
<b>Spiral Cleavage-</b>	Pattern of early cleavage found in molluscs and annelids (both mosaic eggs). The animal pole blastomeres are rotated with respect to those of the vegetal pole. In some molluscs, the handedness of the spiral twist is maternally inherited.
<b>Substratum -</b>	The solid surface over which a cell moves, or upon which a cell grows: should be used in this sense in preference to substrate, to avoid confusion.
<b>Superficial cells-</b>	epithelial cells on the surface of an amphibian embryo.

<b>Taxol-</b>	Drug isolated from yew ( <i>Taxus brevifolis</i> ) that stabilizes microtubules: analogous in this respect to [phalloidin] that stabilises microfilaments.
<b>Trypan Blue -</b>	Biological stain used to determine cell viability. Trypan blue is unable to cross intact plasma membranes, and so only labels dead cells.
<b>Vegetal pole-</b>	The surface of the egg opposite to the animal pole. Often the cytoplasm in this region is incorporated into future endoderm cells.
<b>Vital Dyes-</b>	a substance that imparts a color or fluorescence to living cells (hence the word "vital") without causing perturbation of normal cellular functions. Vital dyes are used to mark specific groups of cells that are to be followed during subsequent phases of development. Examples include Nile blue, Neutral red, and the fluorescent marker rhodamine isothiocyanate.
<b>Walter Vogt-</b>	Embryologist most famous for his use of vital dyes to construct fate maps of amphibian embryos. Vogt placed small vital dye marks on the surface of amphibian embryos at various stages of development to study the movements and fates of various regions of the embryo. His fate mapping studies at the gastrula stage as an important prelude to more modern investigations of amphibian gastrulation.
<b>Xenopus-</b>	The genus of African clawed toads, <i>X. laevis</i> is widely used in developmental biology and was formerly used in pregnancy diagnosis. Ovulates easily under influence of luteinising hormone.

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### **3.8    *TERMINAL QUESTIONS/ANSWER***

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1. Define the cleavage and Give the process of cleavage patterns?
2. What do you understand by the embryonic Induction?
3. Write a short note on the embryonic development in chick?
4. Explain the concept of organizer?
5. Write a short note on the significance of Cleavage?

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## **UNIT: 4 BLASTULATION AND GASTRULATION IN FROG AND CHICK**

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### ***CONTENTS***

- 4.1 Objectives
- 4.2 Introduction
- 4.3 Blastulation and Gastrulation process
  - 4.3.1- Blastulation in Frog
  - 4.3.2- Gastrulation in frog.
  - 4.3.3- Blastulation in chick
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- 4.4 Summary
- 4.5 Glossary
- 4.6 Self-assessment/Terminal question & Answer
- 4.7 References
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## 4.1 OBJECTIVES

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1. To understand the Basic concept Blastulation and Gastrulation process
2. To study of Blastulation & Gastrulation in Frog and chick
3. To describe foetal membrane and their formation and significance

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## 4.2 INTRODUCTION

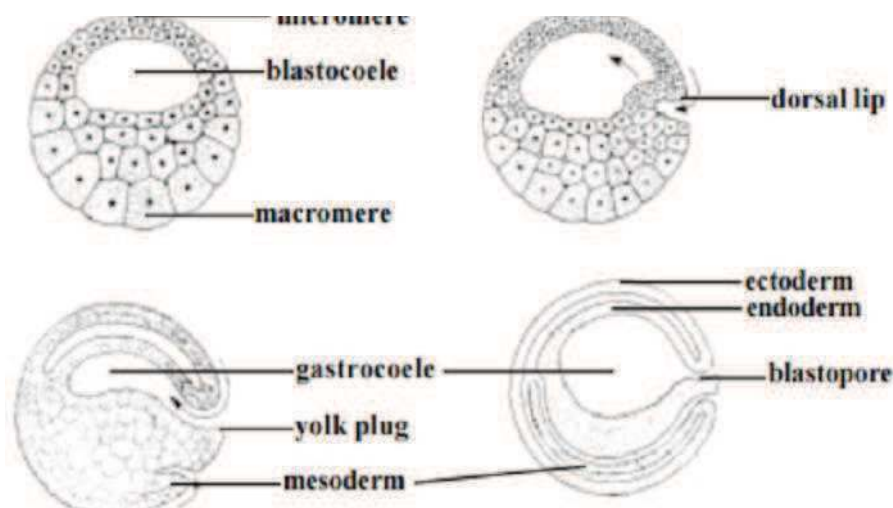
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### (1) Blastulation

As a result of repeated cleavage, a solid ball of blastomers is produced. It is Known as **morula**. Later on blastomers rearrange themselves on the periphery of the egg to form single layer blastoderm leading to the formation of a fluid filled cavity called the **blastocoels**. This Structure is known as **blastula** and the process of formation of blastula known as Blastulation.

### (2) Gastrulation

Gastrulation is a process involving large scale movement of blastula cells resulting in the formation of three germ layers. The three layered structure is known as gastrula and the process involved is called gastrulation.



*Fig. 4.1 Gastrulation of Frog*

Gastrulation in frogs resembles closely the gastrulation of many other animals. We see the three germ layers forming just as they would in humans. In the end, we'll see the process look

very similar, if not the same, as the gastrulation process of human beings. So for the most part, once you get down the concept of gastrulation, you essentially understand it for most animals out there.

One of the main differences in frogs over humans is that there is a larger amount of yolk-laden cells that are due to become the endoderm. These cells also end up forming a yolk plug. Also, in frogs, the **blastocoel**, hollow space inside of the blastula, is slightly off-center, but this doesn't make much of a difference in the gastrulation process.

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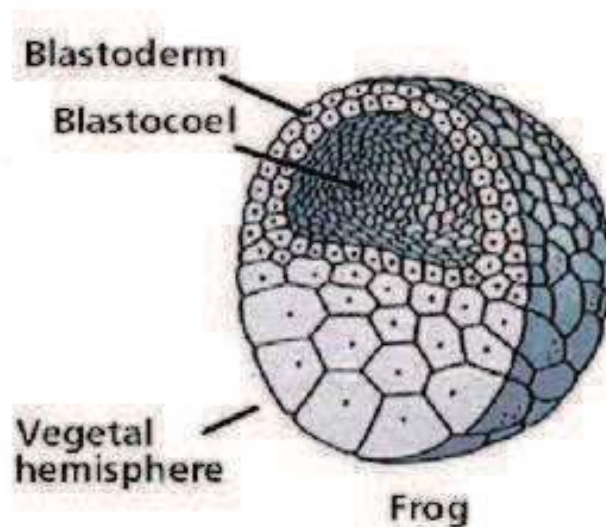
### ***4.3 BLASTULATION AND GASTRULATION PROCESS***

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#### **4.3.1 BLASTULATION IN FROG**

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The first division of the zygote is said to be the cleavage or segmentation. This division is mitotic. The cleavage is said to be of the holo blastic type (the entire zygote divides).



*Fig 4.2 Blastula of Frog*

The first cleavage results in the formation of two cells of unequal size. Hence the cleavage is said to be of the unequal type. The pigmented half contains cells that are smaller in size than the half containing the yolk.

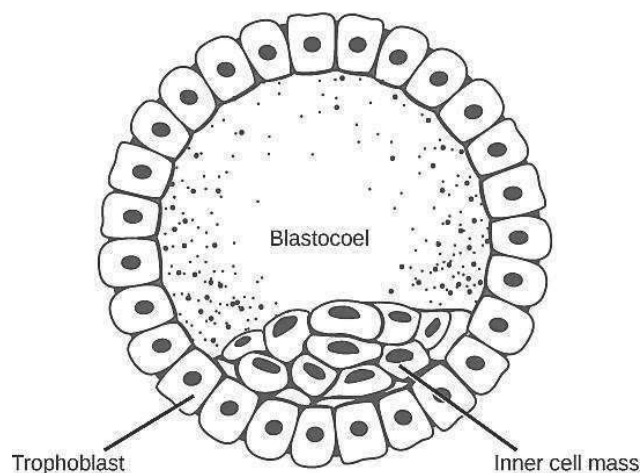
- (a) The first cleavage is meridional, that is it passes through both the animal and vegetal poles. The cleavage begins near the animal pole and extends downwards to the vegetal poles. It appears like a shallow groove on the zygote and bisects the grey crescent area. The two cells that are formed as a result of the first cleavage are called blastomeres. The formation of the first two blastomeres is completed in about 3 to 3.5 hours after fertilization.
- (b) The second cleavage takes place about sixty to seventy minutes after the first cleavage. This cleavage is also meridional but it takes place at right angles to the first plane of division. As a result of this division four cells or blastomeres are formed. All the four blastomeres are not identical.
- (c) Two of them contain parts of the grey crescent while the other two are without it. The third cleavage which begins about eighty minutes after the second cleavage is latitudinal (horizontal) and is at right angles to the second cleavage and passes slightly above the equator. The third cleavage results in the formation of eight cells. Of these eight cells the four cells towards the vegetal pole are larger in size and have yolk content. These are called megameres or macromeres.
- (d) The four upper cells towards the animal pole are smaller in size, pigmented and are called micromeres. The eight cell stage is completed approximately about 5.5 hours after fertilization. The fourth cleavage is meridional and it consists of two cleavage planes passing between the first and the second cleavage. This takes place in about 20 minute's time after the third cleavage. As a result of this cleavage, sixteen cells are formed of which eight are pigmented micromeres (towards the animal pole) and the remaining eight are yolk filled megameres (towards the vegetal pole).
- (e) The four cleavages is completed approximately about six and half hours after fertilization
- (f) The fifth cleavage is double and horizontal. It consists of two latitudinal cleavage furrows one of these cleavages is above and the other below the third cleavage furrow. As a result of this thirty two cells are formed of which sixteen are pigmented micromeres and the other sixteen yolk megameres. These thirty two cells are arranged in forties of eight each. The thirty two cell stage is formed approximately about seven and half of hours after fertilization. From this stage onwards the division becomes rather irregular (in fact the



unequal division begins from the third cleavage itself due to the unequal distribution of yolk which seems to determine the cleavage pattern.

The rate of division also varies between the micromeres and megameres. It has been seen that the micromeres divide at a faster rate than the megameres. Initially the continued division of blastomeres forms a solid ball like structure. It is called the morula stage, as this has superficial resemblance to a mulberry fruit. Morula stage gives rise to a stage called the blastula which is a hollow ball like structure.

(i) **Blastula:** At the end of cleavage the solid ball of cells give rise to blastula which consists of number blastomeres. The characteristic features of the blastula stage are the presence of a well-defined cavity called the blastocoel. This is the beginning of the primary body cavity. The process of the formation of blastula is called blastulation. The blastula of frog is called amphiblastian as the cavity is confined to only the animal pole. The vegetal pole however is composed of a solid mass of non-pigmented yolky cells.

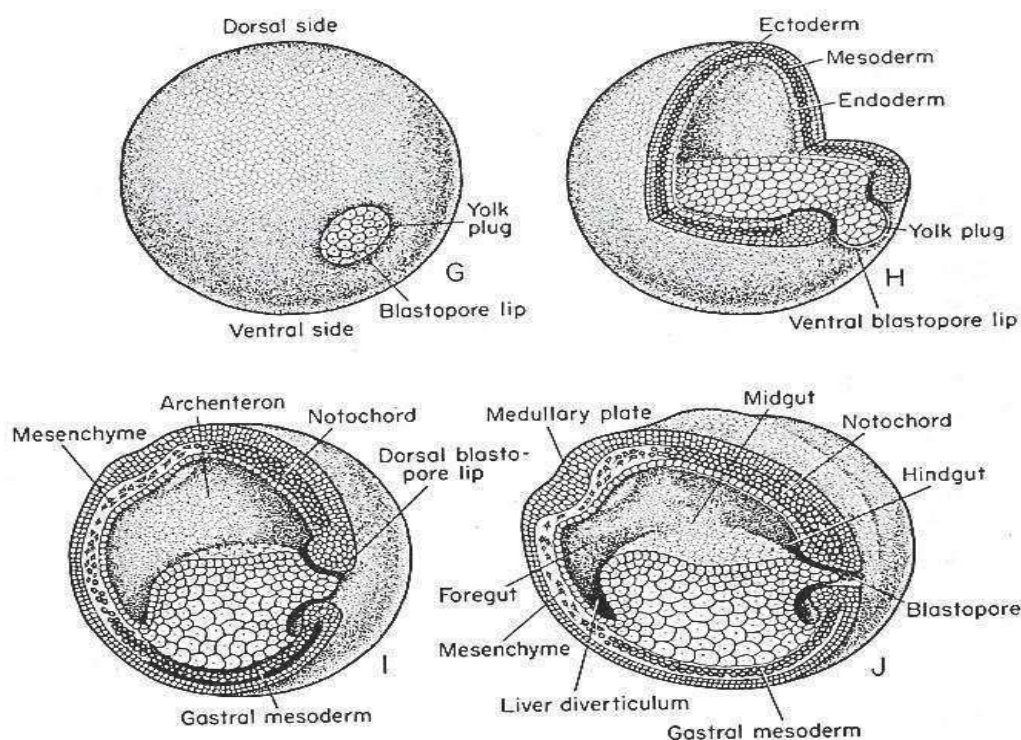


*Fig.4.3 Blastula*

In the thirty two cell stage, the blastula consists of a single layer of cells and is called the early blastula. The pigmented cells (micromeres) are found in the anterior half while the yolky megameres are present in the posterior half. As has been already pointed out, the blastocoel lies entirely in the anterior half. The blastula of frog is hollow and has a very well developed blastocoel. It is said to be a coeloblastula.

As segmentation proceeds, the number of cells in the blastula increase; so also the blastocoel. The floor of the blastocoel is flat while its top portion is arched. The roof (top) is made up of three to four layers of pigmented micromeres while the floor is formed by yolky megameres. Between the micromeres and the megameres and along the equator is found a group of cells which are intermediate in size (between megameres and micromeres). These cells constitute the germ ring. The germ ring is formed in the region of the grey crescent.

### 4.3.2 GASTRULATION IN FROG

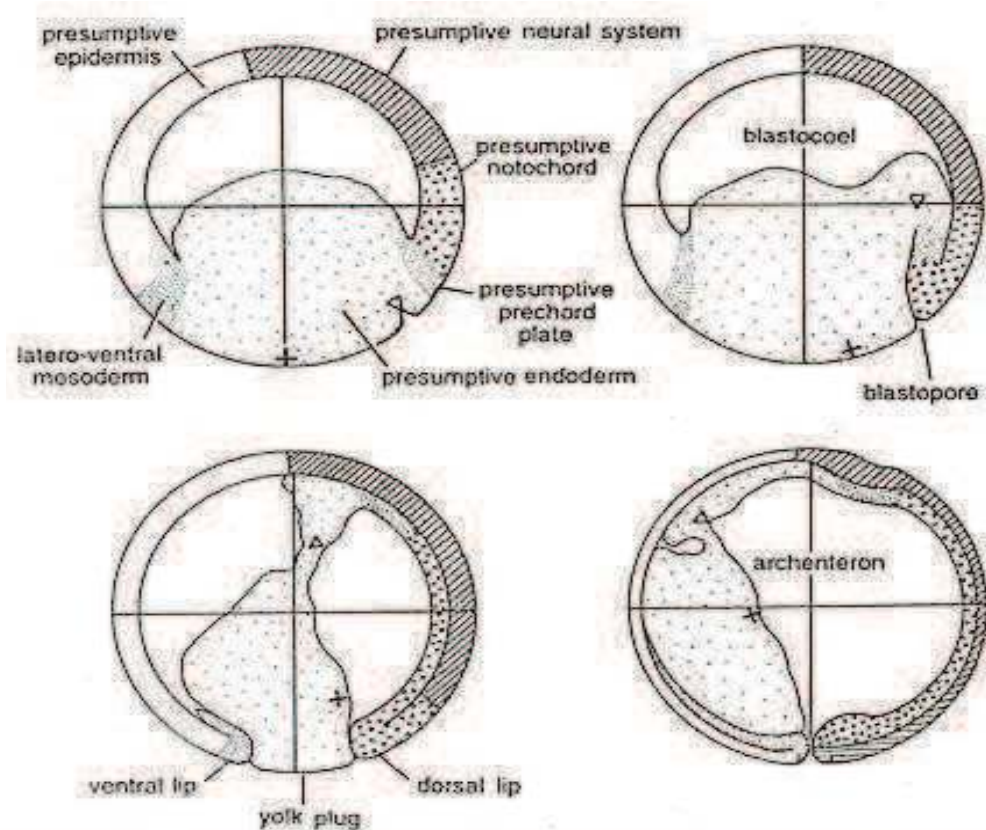


*Fig. 4.2 Gastrulation in frog*

Gastrulation is the process of highly integrated cell and tissue migrations of prospective endodermal and mesodermal areas to their definite positions into the interior of the embryo. These movements are self-determined and interdependent and are termed morphogenetic movements, creating new relationships and ultimately a triploblastic embryo. The cellular preparations for these movements take place during cleavage. The amphibian embryo undergoes a midblastula transition during which the cell cycle slows down (as a result of acquisition  $G_1$  and  $G_2$  phases of the cell cycle), cell division becomes a synchronous, the cells gain the ability to move from their original positions, and the transcription of new

mRNA is seen from the nucleus for the first time in the animal's life. In *Xenopus*, this transition occurs immediately after the twelfth cleavage (Newport and Kirschner, 1982). There occur three types of morphogenetic movements in amphibian gastrulation.

**(a) Invagination:** In frog embryos, gastrulation is initiated at the future dorsal side of the.



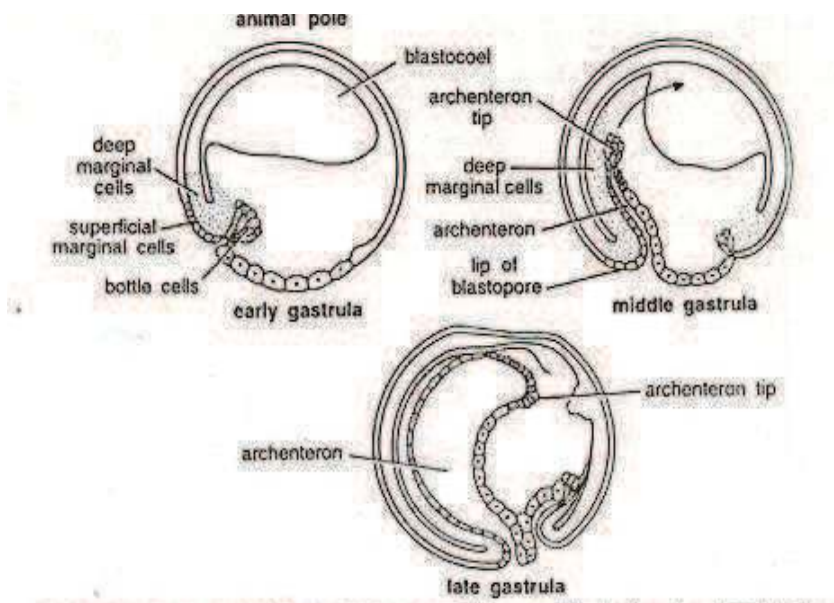
*Fig. 4.4 Migration of the presumptive organ forming areas of the blastula during the gastrulation in the Frog. The triangle marks the endodermal cells which start invagination and form the foregut. The cross marks the position of endodermal cells which were at the vegetal pole when gastrulation began and which are covered by epibolic growth of ectoderm.*

embryo, just below the equator in the region of the grey crescent. (Fig.4.4). Here the marginal endodermal cell sinks into the embryo thus forming a slit like blastopore. These cells now change their shape and become flask shaped. These are called as bottle cells. The bottle cells maintain the contact with the outer surface with the help of cytoplasmic strands whereas their main body is displaced towards the inside of the embryo. Therefore in frog, gastrulation begins in the marginal zone near the equator of the blastula. Here the endodermal cells are not so large or so yolky as the most vegetal blastomere.

Thus although the bottle cells may be responsible for creating the initial groove, the motivating force, this appears to come from the deep layers of marginal cells. Furthermore,

this deep layer of cells appears to be responsible for the continued migration of cells into the embryo.

**(b) Involution:** The next phase of gastrulation involves the involution of the marginal zone cells, while the animal cells undergo epiboly and converge at the blastopore. On reaching the tip of the blastopore, the marginal cells turn inward and travel along the inner surface of the outer cells sheets (Fig. 4.5). Thus, the cells constituting the lip of blastopore are constantly changing. The first cells to form the dorsal lip are endodermal cells that invaginated to form the leading edge of the archenteron.



*Fig. 4.5 Invagination of endodermal cells in an amphibian egg. The bottle cells originate from superficial marginal cells and become the archenteron tip. The involuting cells that form the mesoderm are derived from the deep marginal cells.*

These cells later become the pharyngeal cells of foregut. As these first cells pass into the interior of the embryo, the blastopore lip becomes composed of involuting cells that are precursors of the head mesoderm. The next cells involuting over the dorsal lip of the blastopore are called the chorda mesoderm cells. These cells will form the Notochord, a transient mesodermal “back bone” that is essential for initiating the differentiation of nervous system.



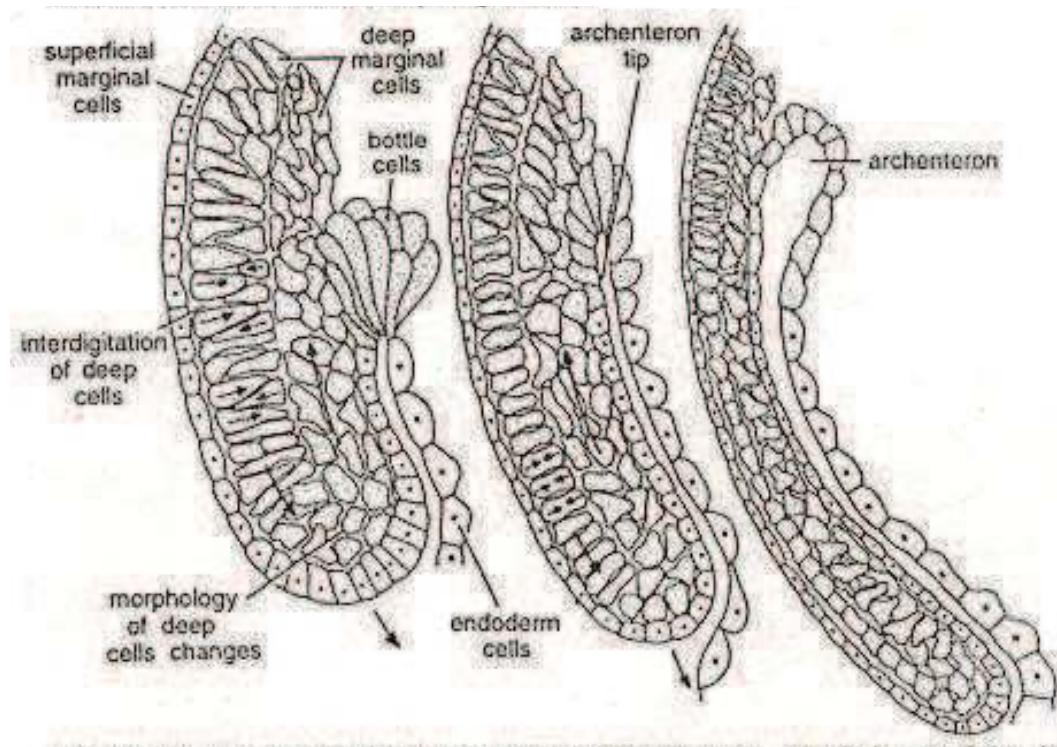
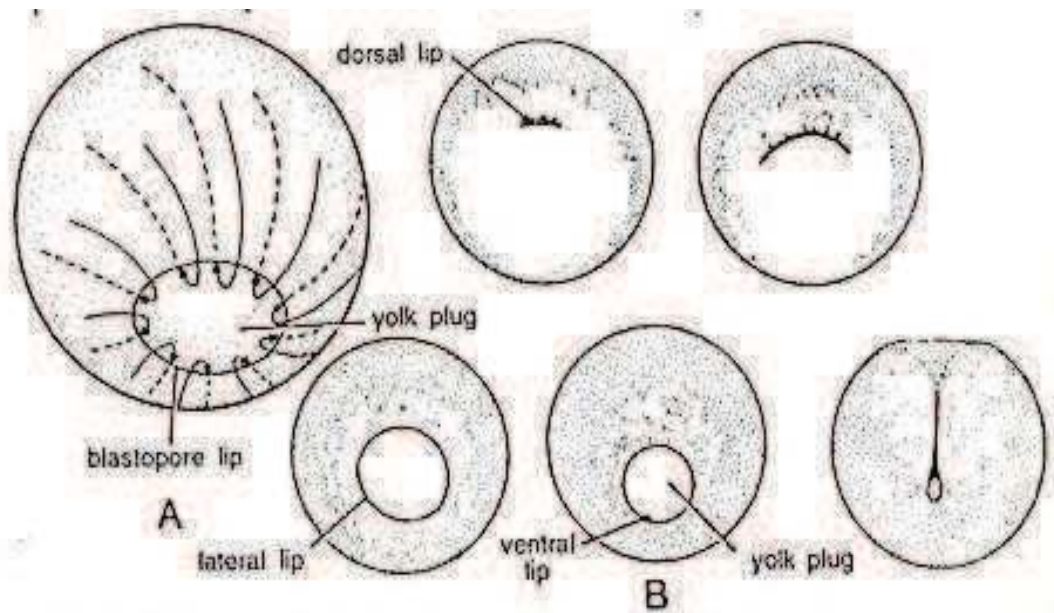


Fig. 4.6 Integrative model of cell movements during gastrulation. (A) Early gastrulation is characterized by the interdigitation of the marginal deep layers and by involution (B, C) in later gastrulae, the deep marginal cells flatten and the formerly superficial cells from the wall of the archenteron. Bottle cells are darkly stippled.

### (c) Epiboly:

As the new cells enter the embryo, the blastocoels are displaced to the side opposite the dorsal blastopore lip. Meanwhile, the blastopore is displaced vegetal and widens as more animal hemisphere cells converge at the blastopore lip. The widening blastopore develops lateral lips and finally a ventral lip over which the additional mesodermal and endodermal precursor cells pass. With the formation of the ventral lip, the blastopore has formed a ring around the large endodermal cells that remain exposed on the surface (Fig. 4.7).

The remaining patch of the endoderm is called the yolk plug and it too, is eventually internalized. At this point, all the endodermal precursors have been brought into the interior of the embryo, the ectoderm has encircled the surface and the mesoderm has been brought between them.



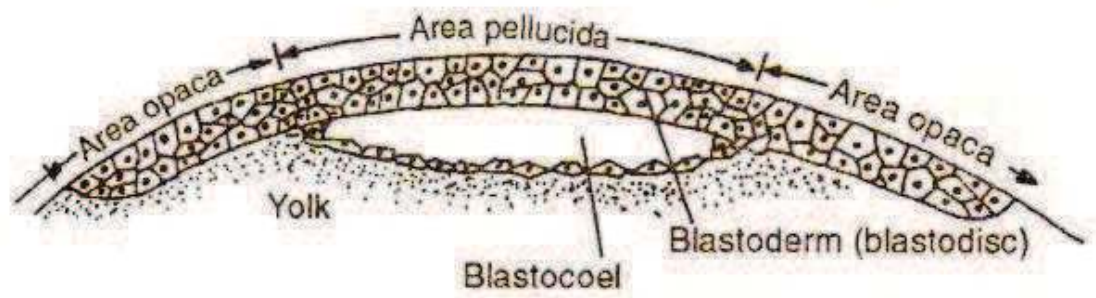
*Fig. 4.7 Epiboly of ectoderm (A) Morphogenetic movements of the cells migrating into the blastopore and then under surface (B) changes in the region around the blastopore, as the dorsal, lateral and ventral lips are formed in succession when the ventral lip completes the circle, the endoderm becomes progressively internalized (After Gilbert, 1988).*

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### 4.3.3 *BLASTULATION IN CHICK*

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The morula condition is of short duration. Almost as soon as it is established there begins a rearrangement of the cells preceding the formation of the blastula. A cavity is formed beneath the blastoderm by the detachment of its central cells from the underlying yolk while the peripheral cells remain attached. The space thus established between the blastoderm and the yolk is termed the segmentation cavity (blastocoele). The central part appears more distinct



and transparent due to the presence of the segmentation cavity called area pellucida the marginal area of the blastoderm in which the cells remain undetached from the yolk and closely adherent to it, is called the zone of junction. This zone looks opaque and white and is known as area opaca. With the establishment of the blastocoels the embryo is said to have progressed from the morula to the blastula stage.

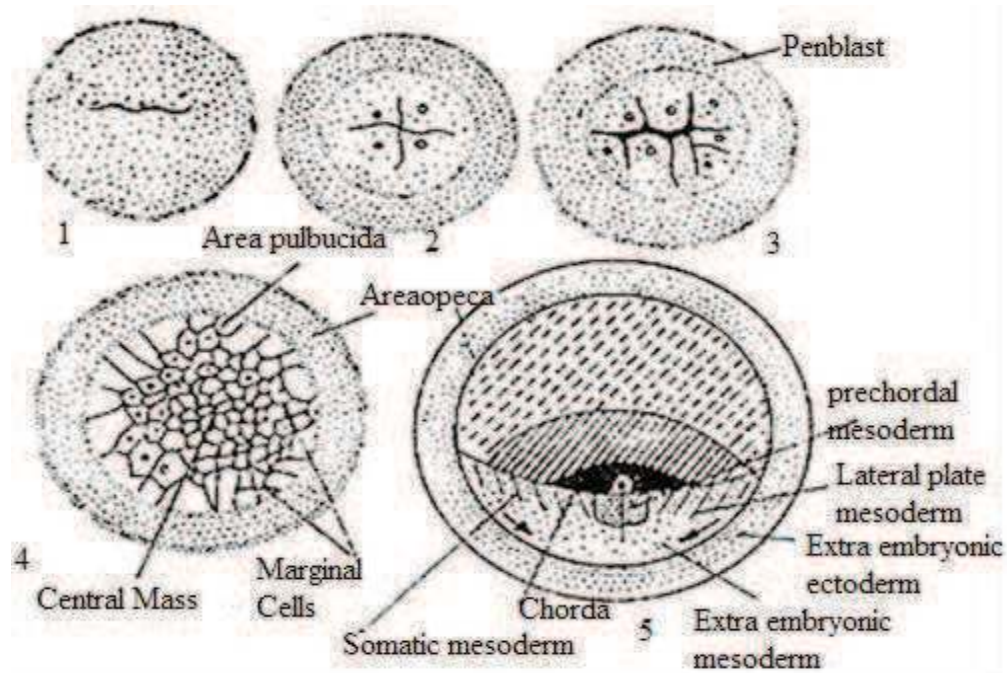


Fig. 4.8 Cleavage in egg, e-4 early stages, 4-blastula; 5. Prospective map and later formative movements

At this magnification the complete yolk must be imagined as about three feet in diameter. The structure of the bird embryo in these stages may be brought in line with the morula and blastula stages of forms having little yolk if the full significance of the great yolk mass is appreciated. Instead of being free to aggregate first into a solid sphere of cells (morula) and then into a hollow sphere of cells (blastula), as takes place in forms with little yolk, the blastomeres in the bird embryo are forced to grow on the surface of a large yolk sphere. Under such mechanical conditions the blastomeres are forced to become arranged in a disc-shaped mass on the surface of the yolk. If one imagines the yolk of the bird morula removed, and the disc of cells left free to assume the spherical shape dictated by surface tension its comparability with the morula in a form having little yolk becomes apparent.

The process of blastulation also is modified by the presence of a large amount of yolk. There can be no simple hollow sphere formation by rearrangement of the cells if the great bulk of the morula is inert yolk. But the cells of the central region of the blastoderm are nevertheless separated from the yolk to form a small blastocoele. The yolk constitutes the floor of the blastocoele and at the same time by reason of its great mass nearly obliterates it. If we



imagine the yolk removed from the blastula and the edges of the blastoderm pulled together the chick blastula approaches the form of the blastula in embryos with little yolk.

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#### 4.3.4 GASTRULATION IN CHICK

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In the chick, the process of gastrulation is prolonged and highly modified than that of frog and *Amphioxus*. It is already started when the egg of chick is laid and completes well into the second day of incubation. The main characteristic of avian gastrulation is the formation of primitive streak.

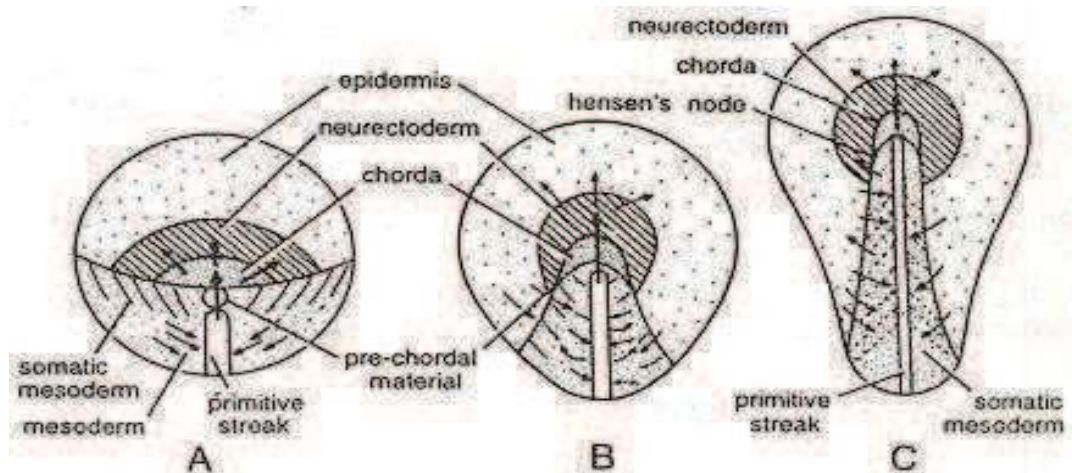
The streak is first visible as a thickening of the cell sheet at the central posterior end of the area pellucida. This thickening is caused by the migration of cells from the lateral region of the posterior epiblast towards the centre. As the thickening narrows, it moves anteriorly and constricts to form the definitive primitive streak. This streak elongates 60-75 percent of the length of the area pellucida and marks the anterior posterior axis of the embryo.

As the cells converge to form the primitive streak, a depression forms within the streak—the primitive groove, through this primitive groove the migrating cells pass into the blastocoel.

At the anterior end of the primitive streak is a regional thickening of cells called the primitive knot or Hensen's node. There is a funnel shaped depression in the centre of the node through which cells can pass into the blastocoel. As soon as the primitive streak is formed, blastoderm cells begin to migrate over the lips of the primitive streak and into the blastocoel.

The cells which migrate through the Hensen's node pass down into the blastocoel and migrate anteriorly to form head mesoderm and notochord, and those cells which pass through the lateral portion of the primitive streak form the majority of endodermal and mesodermal tissues. The cells entering the inside of the avian embryo form a loosely connected mesenchyme. Moreover, no true archenteron is formed in avian gastrula. As the cells enter the primitive streak, the streak elongates toward the future head region. At the same time, the secondary hypoblastic cells continue to migrate anteriorly from the posterior margin of the blastoderm. The first cells to migrate through the primitive streak are those destined to become the foregut.

Inside the blastocoel, these cells migrate anteriorly and eventually displace the hypoblast cells in the anterior portion of the embryo. The next cells entering the blastocoel through Hensen's node also move anteriorly, but they do not move as far ventrally as the presumptive



endodermal cells.

*Fig. 4.9 Formation of the primitive streak and mesoderm during gastrulation in heavily telolecithal egg of chick (dotted arrows indicate movements below the surface)*

These cells remain between the endoderm and the epiblast to form the head mesoderm and the chorda mesoderm (notochordal) cells. These early ingressing cells have all moved anteriorly, pushing up the anterior midline region of the epiblast to form the head process. Meanwhile, cells continue migrating inward through the primitive streak. As they enter the blastocoel, these cells separate into two streams. One stream moves deeper and joins the hypoblast along its mid-line, displacing the hypoblast cells to the sides.

These deep-moving cells give rise to all the endodermal organs of the embryo as well as to most of the extra embryonic membranes. The second migrating stream spreads throughout the blastocoel as a loose sheet, roughly mid-way between the hypoblast and the epiblast.

This sheet gives rise to mesodermal portions of the embryo and extra embryonic membranes. By 22 hours of incubation, most of the presumptive endodermal cells are in the interior of the embryo, although presumptive mesodermal cells continue to migrate inward for a longer time.

Now the second phase of gastrulation begins. While the mesodermal ingression continues, the primitive streak starts to regress (disappearance of primitive streak) moving Hensen's node from near the centre of the area pellucida to a more posterior position.

As the node moves further posteriorly, the remaining (posterior) portion of the notochord is laid down. Finally the node regresses to its most posterior position, eventually forming the anal region in true deuterostome fashion. By this time, the epiblast is formed entirely of presumptive ectodermal cells. As a consequence of this two step gastrulation process; avian (and mammalian) embryos exhibit a distinct antero-posterior gradient of developmental maturity. While the posterior portions of the embryo are undergoing gastrulation, cells at the anterior end are already starting to form organs. For the next several days the anterior end of the embryo is seen to be more advanced in its development than the posterior end.

While the presumptive mesodermal and endodermal cells are moving inward, the ectodermal precursors surround the yolk by epiboly. The enclosure of the yolk by the ectoderm takes greater part of 4 days to complete and involves the continuous production of new cellular material at the expense of the yolk and the migration of the- presumptive ectodermal cells along the undersides of the vitelline envelope.

Thus, as avian gastrulation draws to a close, the ectoderm has surrounded the yolk, the endoderm has replaced the hypoblast, and the mesoderm has positioned itself between these two regions. Thus the fully formed chick gastrula consists of these three germ layers- ectoderm, chorda-mesoderm and the endoderm.

**(f) Significance of the primitive streak:**

The primitive streak with its Hensen's node is analogous to the blastopore and its dorsal lips of amphibian gastrula. The only difference is that the avian blastopore is elongated whereas amphibian blastopore is circular. Some homologies are as follows:

- (1) The primitive pit represents the dorsal opening of the blastopore (neurenteric canal).
- (2) The primitive node corresponds to the dorsal lip of blastopore (future tail bud).

(3) The primitive groove and folds are comparable to the opposed lateral lips of the blastopore. (4) The posterior end of primitive streak may be compared with the ventral region of the blastopore (future anal opening).

(5) The first cells which migrate through the primitive streak are those destined to become foregut. This situation is again similar to amphibians.

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### 4.3.5 FATE MAPS

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In developmental biology, fate mapping is a method of understanding the embryonic origin of various tissues in the adult organism by establishing the correspondence between individual cells (or groups of cells) at one stage of development, and their progeny at later stages of development. When carried out at single-cell resolution, this process is termed cell lineage tracing.

The first attempts at fate mapping were made by inferences based on the examination of embryos that had been fixed, sectioned, and stained at different developmental time points. The disadvantage of this technique was that observation of single points in developmental time provides only snapshots of what cell movements are actually occurring and what fates are being assigned. Early embryologists thus had to infer which cells became what tissues at later stages.

Early embryologists used "vital dyes" (which would stain but not harm the cells) to follow movements of individual cells or groups of cells over time in *Xenopus* frog embryos. The tissue(s) to which the cells contribute would thus be labeled and visible in the adult organism. The first person to develop and use this technique to study cell fate was embryologist Walter Vogt in 1929. Vogt used small chips of agar impregnated with a vital dye, (such as Nile Blue or Nile Red) which he placed on a particular cell or population of cells in *Xenopus* embryos until the dye absorbed into the yolk platelets within the desired cell(s). Once the cells were effectively labeled, the agar chip could be removed and the embryo was allowed to develop normally. With this method, Vogt was able to discern movements of particular cell populations and the ultimate organ or tissue into which they integrated. Although innovative for the time, this technique is limiting in that the size of a chip of agar may not accommodate single-cell resolution studies at later stages of development, since successive cell divisions will yield smaller cells (until the embryo

develops into a larval form that can eat, and thereby grow larger). Additionally, the cell or cell population of interest must be superficial, since the agar chip with the dye must be placed on the surface of the embryo.

The information Vogt gathered from his tracing experiments of distinct cells and populations of cells in *Xenopus* was then pooled to construct a fate map. The map was a representation of an early-stage embryo (such as a blastula) that has particular regions highlighted which are known to give rise to specific tissues in the adult organism. For instance, in Figure 1, Nile blue staining of a 32-cell blastula at the dorsal side of the animal pole yields a blue-stained brain and (depending on the size of the agar chip) may also stain the anterior portion of the notochord.

In 1978, David Weisblat and colleagues in Gunther Stent's lab at Berkeley improved the technique of single-cell resolution fate mapping by injection of horseradish peroxidase (HRP) enzyme, and later fluorescent peptides (1980), into individual cells in *Helobdella triserialis* (leech) embryos during early development. All progeny of the injected cells could later be discerned by staining for HRP using benzidine substrate or visualized by fluorescence microscopy. This technique allowed the experimenter greater control and selectivity over what cell was labeled and traced. However, the opaque character of the HRP stain prevented use of vital dye nuclear counter-stains such as Hoechst 33258 (blue) to observe the mitotic state of the injected cell's progeny. Also, embryos had to be fixed in order to stain for the HRP, thus allowing only a single time point view of each individual leech embryo injected. The use of fluorescent peptides such as Rhodamine-D-protein (red, RDP) and Fluorescein-D-protein (yellow/green, FDP) conjugated to large carrier molecules to prevent diffusion through cell gap junctions, alleviated several of the shortcomings of HRP injection. Leech embryos injected with the fluorescent tracers could be visualized, and images collected of the same specimen at multiple time points, without fixation. The fluorescent tracers could also be combined with nuclear Hoechst staining to visualize the mitotic status of the progeny of injected cells. Seth Blair, also in Stent's lab, introduced a novel ablation technique that could be used in tandem with lineage tracing to pursue the questions relating to developmental potential changes in cell fate in experimentally perturbed embryos that were first raised by Roux and Driesch (1980). For this purpose, specific cells were ablated by microinjection with Pronase (an enzyme that digests proteins) to ablate the cell; later modifications of this technique employed DNase or the ricin A chain. The single-cell injection technique is now also in use by researchers studying other model

organisms such as *Xenopus* (frogs), *Danio rerio* (zebrafish), and *Caenorhabditis elegans* (worms).

#### 4.3.6 FOETAL MEMBRANES: THEIR FORMATION AND SIGNIFICANCE

In amniotes certain tissues of the developing embryo do not enter into the formation of embryo proper, but helps in care and maintenance of developing embryo. These part termed as Foetal membranes or extra embryonic membranes. Developed foetal membrane serve for nutrition, respiration, excretion and protection of the embryo. The extra embryonic membranes are chorion, amnion, yolk sac, and allantois.

##### Foetal membranes of chick:

In the development of chick these membranes develop from original blastoderm. The central part of blastoderm forms embryo proper, the marginal blastoderm gives extra embryonic membranes. Amnion and chorion develop from somatopleurae, yolk sac and allantois, develop from splanchnopleurae.

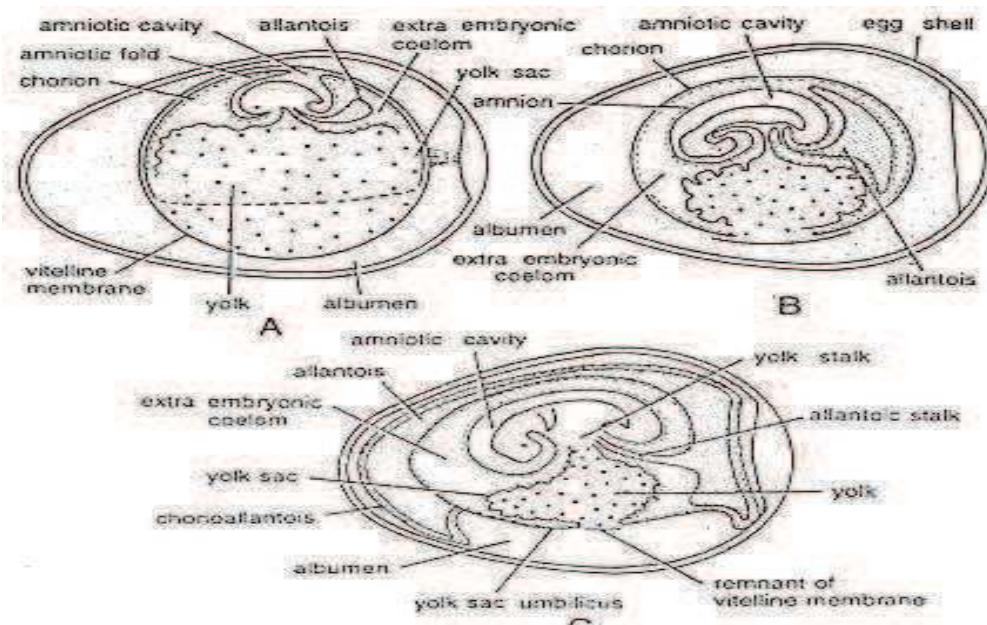


Fig. 4.10 Stages in the development of foetal membranes in chick



**(a) Amnion & Chorion :** In the development of embryo amnion and chorion are closely associated, Amnion is bag like covering over the embryo, it separates the embryo from internal environment, Amnion is developed from somatopleuric amniotic folds. These folds are head fold, lateral folds and tail folds.

- i. At about 30 hours of incubation, in front of the head of embryo a head fold is developed, it is called amniotic head fold.
- ii. At about third day of incubation amniotic tail fold is developed. It grows opposite to head fold.
- iii. Mean while lateral folds will develop, they grow dorsomedially.
- iv. After some time head fold, lateral folds, and tail fold will fuse near posterior end of a embryo.
- v. At 72 hrs. of incubation they are still not fused. They show an opening called amniotic umbilicus, afterwards they unite.
- vi. After their union at the point of union "sero-amniotic raphae" is present. It is a fold.
- vii. Because of this union outer chorion inner amnion will form, because it is developed from somatopleure. In chorion ectoderm is present outside and mesoderm is present inside. In amnion ectoderm is inside, mesoderm is outside. Hence the space between amnion and chorion is called exocoel or extraembryonic coelome.

**(b) Functions of chorion:-**

- i. The extra embryonic coelome is filled with a fluid. It gives protection to the developing embryo.
- ii. This coelome gives space, for developing allantois.

Chorion combines with allantois and acts as a respiratory organ.

**(c) Functions of Amnion:**

- i. Amnion is sac like structure around embryo. It contains amniotic fluid. It will protect embryo from mechanical shocks and dessications.
- ii. It protects the embryo when the egg is laid. It gives artificial aquatic environment for growth of embryo.

**(d) Yolk sac:**

- i. At 16 hours of incubation, yolk sac makes its appearance.

- ii. It develops from Splanchnopleurae contains endoderm and mesoderm layers.
- iii. The Splanchnopleurae instead of forming a close gut, it will grow over yolk, and becomes yolk sac. The primitive gut is present above the yolk. This yolk region is in contact with midgut. Finally the yolk sac is communicated with midgut through an opening.

**(e) Functions of Yolk sac :**

It digests the yolk, and the digested food will be circulated through blood to the developing embryo. Hence yolk sac is considered as a nutritive organ of the embryo.

**(f) Allantois :**

It develops from the ventral part of caudal end of the hindgut at third day of incubation. It develops from Splanchnopleurae This Splanchnopleurae contains endoderm and mesoderm. The allantois grows rapidly, and occupies the entire exocoel. The mesoderm of the chorion and mesoderm of allantois will unite. It forms chorio allantoic membrane. Allantois is connected to the hindgut, and is called as allantoic stalk. As the embryo is growing the allantoic and yolk stalk are brought together. Their mesodermal layers will unite. It is called umbilical stalk. It is covered by somatic umbilicus.

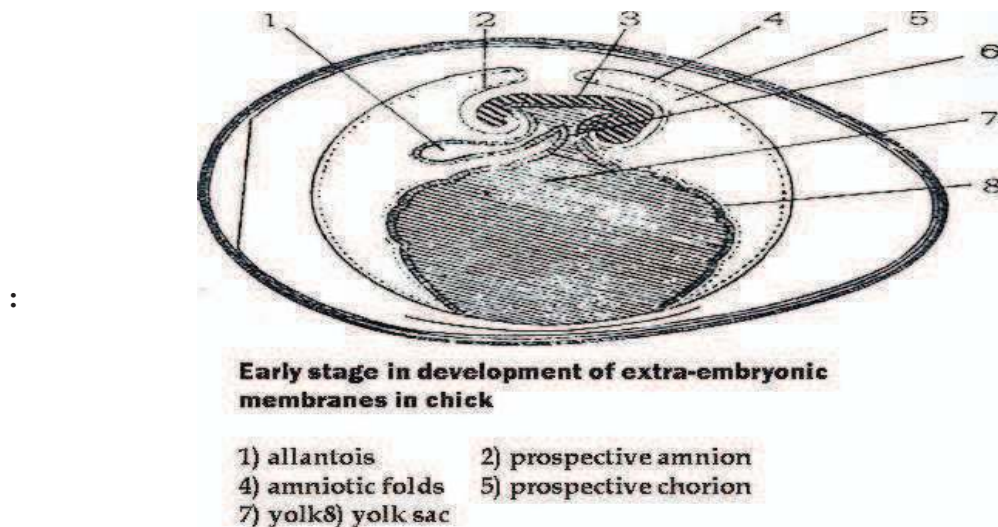
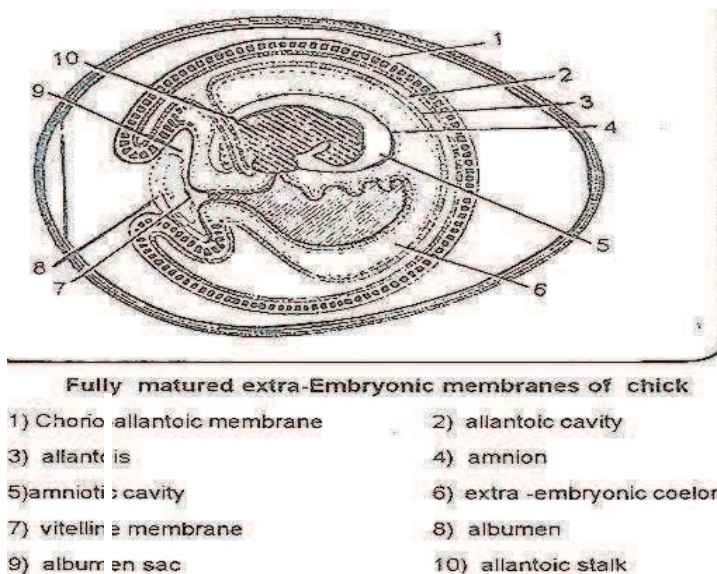


Fig 4 .11 Allantois



**(g) Function of Allantois:**

- i. Allantois is richly vascularised. Hence it works as respiratory organ.
- ii. It stores nitrogenous waste material of the embryo.



*Fig 4.12 fully matured extra embryonic membrane of chick*

In later development the allantoic circulation will absorb calcium from the shell. This calcium is used in construction of bones in young ones. Allantois absorbs calcium from shell. Hence the shell becomes thin. It helps in rupturing the shell during hatching. These are the membranes that develop outside the embryo but in close association with it and they carry out certain specific functions. In human beings foetal membranes are amnion, chorion, yolk sac and allantois.

**Extra embryonic membranes of mammals:**

**(i) Amnion:** This is formed above the embryo. This consists of a cavity (amniotic cavity) and encloses a fluid called amniotic fluid. The embryo is suspended into the amniotic cavity by the umbilical cord. The amniotic fluid provides a shock absorbing effect to the embryo against bumps infections etc.

The watery fluid around the embryo helps in maintaining constant temperature and pressure and protects the embryo in case the mother has a fall. The amniotic fluid is derived from the mother's blood and contains foetal cells. This is made use of in the prenatal sex test

for the foetus- known as aminocentesis. In aminocentesis the amniotic fluid is drawn out with a syringe and the cells are tested for the presence of the sex chromosomes.

(ii) **Chorion:** The chorion completely surrounds the embryo and has small projections all around it during early stages of development. The chorion is composed of trophoblast on the outside and mesoderm on the inside. Chorion protects the embryo and forms placenta for metabolic exchange between the mother and the foetus.

(iii) **Yolk sac:** This is formed below the embryo. In human beings this contains a fluid but no yolk. It is vestigial organ. Its wall is made up of trophoblast and endoderm. The yolk sac functions as the region of formation of blood cells upto about 6th week of development when the liver of the foetus takes up this function.

(iv) **Allantois:** This is a small bag like structure that develops from the gut of the embryo and near the yolk sac. This membrane develops around the third week of development. Gradually the allantois shrinks in size and gets enclosed in the umbilical cord. Allantois helps in the formation of umbilical arteries and veins. The allantois also forms blood cells.

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#### 4.4 SUMMARY

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Embryo development begins with a sperm fertilizing an egg to become a zygote which undergoes many cleavages to develop into a ball of cells called a morula. Only when the blastocoele is formed does the early embryo become a blastula.

The **blastula** (from Greek *βλαστός* (blastos), meaning "sprout") is a hollow sphere of cells, referred to as blastomeres, surrounding an inner fluid-filled cavity called the blastocoele formed during an early stage of embryonic development in animals

The blastula precedes the formation of the gastrula in which the germ layers of the embryo form. A common feature of a vertebrate blastula is that it consists of a layer of blastomeres, known as the blastoderm, which surrounds the blastocoele

**Gastrulation** is a phase early in the embryonic development of most animals, during which the single-layered blastula is reorganized into a multilayered structure known as the **gastrula**. Before gastrulation, the embryo has a continuous epithelial sheet of cells; by the end of gastrulation, the embryo has begun differentiation to establish distinct cell lineages, set

up the basic axes of the body (e.g. dorsal-ventral, anterior-posterior), and internalized one or more cell types including the prospective gut. In triploblastic organisms the gastrula is trilaminar ("three-layered"). These three *germ layers* are known as the ectoderm, mesoderm, and endoderm. In diploblastic organisms, such as Cnidaria and Ctenophora, the gastrula has only ectoderm and endoderm. Gastrulation takes place after cleavage and the formation of the blastula. Gastrulation is followed by organogenesis, when individual organs develop within the newly formed germ layers. Each layer gives rise to specific tissues and organs in the developing embryo. The **ectoderm** gives rise to epidermis, the nervous system, and to the neural crest in vertebrates. The **endoderm** gives rise to the epithelium of the digestive system and respiratory system, and organs associated with the digestive system, such as the liver and pancreas. The **mesoderm** gives rise to many cell types such as muscle, bone, and connective tissue. In vertebrates, mesoderm derivatives include the notochord, the heart, blood and blood vessels, the cartilage of the ribs and vertebrae, and the dermis.

In mammals the blastula is referred to as a blastocyst. The blastocyst contains an embryoblast (or inner cell mass) that will eventually give rise to the definitive structures of the fetus, and the trophoblast, which goes on to form the extra-embryonic tissues.

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## 4.5 GLOSSARY

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**Albumen** - The "white" of a bird's egg which provides both protein and water for the growing embryo.

**Allometric growth** or **allometry** - phenomenon whereby parts of the same organism grow at different rates. Contrast with **isometric growth**.

**Amniocentesis** - prenatal diagnostic procedure in which amniotic fluid is withdrawn from amniotic sac in order to obtain fluid and fetal cells which are analyzed for metabolic and/or genetic disorders, and to test the maturity of the fetus' lungs.

**Amnion** - the innermost membranous sac enclosing the embryo of an **amniote**; it becomes filled with amniotic fluid. One of the four amniote extraembryonic membranes; derived from the **somatopleure** (combination of ectoderm and somatic mesoderm)

**Amniote** - higher vertebrate capable of terrestrial reproduction, and having an **amnion** during its development. Includes reptiles, birds and mammals, which share a common ancestor.

**Animal hemisphere** - half of an egg or embryo that contains less yolk and/or which divides more rapidly in comparison to the **vegetal hemisphere**. In eggs or embryos with considerable yolk, the animal hemisphere will be the upper half when the embryo is allowed to settle by gravity - yolk cytoplasm being denser than yolk-free cytoplasm.

**Axoneme** - motor section of flagellum constructed of microtubules emanating from the centriole at the base of the flagellum.

**Blastocoel** - fluid-filled cavity found in the interior of a **blastula** or **blastocyst**.

**Blastocyst** - cleavage stage mammalian embryo; a hollow ball of cells made of outer **trophoblast** cells and an inner cell mass.

**Blastoderm** - cell layer formed during cleavage of telolecithal and centrolecithal eggs.

**Blastomere** - any embryonic cell formed during cleavage.

**Blastopore** - site of gastrulation initiation and later the opening of the archenteron at the vegetal region of certain embryos (e.g., echinoderm and amphibian); in **deuterostome** embryos it is the future anus of the organism.

**Blastula** - a cleavage stage embryo, typically a hollow ball of cells surrounding a cavity called the **blastocoel**; this term is used for (among others) echinoderm and amphibian embryos.

**Blood islands** - also known as angiogenetic clusters; masses of splanchnic mesodermal cells found in the yolk sac of amniotes. The first blood forming tissue of the embryo, responsible for red blood cells and vitelline blood vessels.

**Bottle cells** - epithelial cells found at the initial site of gastrulation, lining the initial archenteron, that temporarily become bottle-shaped; they maintain contact with the outer surface of the embryo, but the majority of the cell is inside the embryo. Also known as flask cells.

**Centriole** - microtubule-based structure that divides prior to mitosis; with the pericentriolar material constitutes the **centrosome** it is associated with the poles of the spindle apparatus during **karyokinesis**.

**Chordate** - organism having a **notochord** at some stage of development - a rigid cartilaginous rod in the back extending from anterior to posterior; this group includes the vertebrates.

**Chorionic somatomammotropin** - aka placental lactogen, a hormone that promotes maternal breast development during pregnancy.

**Chromosomal puff** - expanded region of a polytene chromosome indicative of active messenger RNA synthesis.

**Cytoplasmic segregation** - restriction of factor(s) into one daughter cell but not the other to specify cell fate, a mechanism associated with **autonomous specification**.

**Cytotrophoblast** - inner cellular layer of the trophoctoderm (**trophoblast**), between the **syncytiotrophoblast**, and chorionic villus capillaries; part of the mammalian placenta. In contrast to the **syncytiotrophoblast**, made of individual mononucleate cells.

**Dalton** - measure of molecular weight or mass. One hydrogen atom has mass of 1 Da. Proteins and other macromolecule molecular weights are usually measured in kDa or kD (kilodaltons) - 1000 Da.

**Dauerblastula** - permanent "blastula" of ciliated epidermal cells formed by experimental isolation of animal pole cells of the sea urchin embryo. "dauer" is from the German dauern - "to endure"

**Delamination** - splitting of one cellular sheet or layer into two parallel layers.

**Deoxynucleotides** - components of DNA, containing the phosphate, sugar and organic base; when in the triphosphate form, they are the precursors required by DNA polymerase for DNA synthesis (i.e., ATP, CTP, GTP, TTP).

**Deuterostomes** - broad classification of **triploblastic animals** including echinoderms and chordates that tend to share certain embryological traits; among these the formation of the "mouth second" (hence the name) during gastrulation, after the future anus, which is comes from the **blastopore**, the site of gastrulation initiation. (Contrast with **protostomes**)

**Discoidal cleavage** - incomplete division of the blastodisc, a region of yolk-free, active cytoplasm; characteristic of birds, fishes and reptiles.

**Ectoderm** - (1) the outer cellular membrane of a diploblastic animal. (2) a: the outermost of the three primary germ layers of a triploblastic embryo. b: a tissue (as neural tissue) derived from this germ layer.

**Endoderm** - One of the three primary germ layers formed in the embryo, moved into interior by cell movements during gastrulation. In vertebrates, this innermost layer of cells goes on to form the linings of the gut (esophagus, stomach, intestines, rectum, colon), pharyngeal pouch derivatives (tonsils, thyroid, thymus, parathyroid glands), lungs, liver, gall bladder, pancreas. In amniotes, extraembryonic endoderm participates in the formation of the **allantois** and **yolk sac**.

**Epiboly** - literally, "over the ball," usually the growth of epidermal ectoderm to cover the surface of the embryo during gastrulation.

**Epigenesis** - theory holding that development is a gradual process of increasing complexity. (This contrasts with **preformationism**, which holds that the organism is already present in the gamete(s), merely growing and unfolding during development.) For example, organs are formed de novo in the embryo rather than increasing in size from pre-existing structures.

**Epithelial** (adj.) - belonging to a sheet of tightly joined, polarized cells.

**Exogenous** (adj.) - arising from a source outside the organism or cell.

**Fate map** - diagram that takes the larval or adult structure of an organism and "maps" it onto the region of the embryo from which it arises

**Gastrulation** - stage in animal development following cleavage characterized by extensive cell movement and rearrangement to form a "three-layered" embryo of ectoderm, mesoderm and endoderm.

**Hensen's node** - regional thickening of cells at the top (anterior) of the primitive groove through which gastrulating cells migrate anteriorly to form tissues in the future head and neck. The functional equivalent of the dorsal lip of the blastopore ('organizer') in amphibians,

the region is found in birds, reptiles and mammals (strictly, the mammalian equivalent is called simply the 'node'). Also known as the primitive knot.

**Macromere** - large **blastomere**; in the sea urchin embryo, the four relatively large cells that result from the fourth cleavage of the vegetal tier are macromeres. Contrast with **micromere** and **mesomere**.

**Marginal zone** - region near the equator of the amphibian blastula, where the animal and vegetal hemispheres meet; gastrulation begins among these cells.

**Meroblastic cleavage** - incomplete cleavage, characteristic of zygotes with large accumulations of yolk.

**Merogones** - egg fragments (in sea urchins) that can divide and develop, even if they have only a haploid nucleus.

**Mesenchyme** - mesodermal cells in a developing embryo with the ability to move freely and individually.

**Mesoderm** - primary embryonic germ layer of **triploblastic** animals found between the outer **ectoderm** and the inner **endoderm**, which (in chordates) gives rise to notochord, bone, cartilage, muscle, other connective tissues, somatic gonad, urogenital tracts, kidneys, heart and circulatory system, blood, and portions of extraembryonic membranes (in amniotes).

**Neural tube** - hollow cylindrical structure of neuroepithelial cells (in chordate embryos) that will give rise to the brain and spinal cord; an ectodermal derivative.

**Neuroblast** - dividing neuronal precursor cell

**Nucleic acid hybridization** - coming together (annealing) of single-stranded nucleic acid sequences by hydrogen bonding of complementary bases to form double-stranded molecules; this process is the basis for molecular biological techniques in which a labeled probe sequence is used to detect another identical or similar sequence (e.g., Southern hybridization, Northern hybridization).

**Nucleosome** - unit of chromatin consisting of a short length of DNA (about 140 bp) wrapped twice around a core of eight histone proteins (two each of H2A, H2B, H3 and H4).

**Primitive streak** - thickening of the epiblast cell layer caused by movement of mesodermal cells into the blastocoel; this structure is characteristic of avian, reptilian and mammalian gastrulation.

**Somite** - block of dorsal mesodermal cells adjacent to the notochord during vertebrate organogenesis. These transient structures define the segmental pattern of the embryo, and subsequently give rise to vertebrae and ribs, dermis of the back, and skeletal muscles of the back, body wall and limbs.

**Triploblastic** (adj.) - having three embryonic germ layers (**ectoderm**, **mesoderm**, and **endoderm**); characterizes all animals except cnidarians, ctenophores and sponges which are considered diploblasts, lacking true mesoderm.

**Vegetal hemisphere** - typically, the yolky half of an egg or embryo, opposite the **animal hemisphere**. In many embryos, cells in the vegetal hemisphere divide more slowly than those in the animal hemisphere.

**Visceral arches** - see **pharyngeal arches**.

**Vitelline envelope/membrane**- membrane outside the plasma membrane forming a fibrous mat over the sea urchin egg; becomes the fertilization membrane via the cortical reaction.

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## 4.6 SELF-ASSESSMENT QUESTION

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Q 1 Describe the process of blastulation in chordates?

Q 2 Describe various types of blastula found in chordate?

Q3 what do you understand by gastrulation? Describe the processes of gastrulation in A mesolecithal and polylecithal egg?

Q4 what is gastrulation? Compare the gastrulation in frog and chick.

Q5 what is blastulation? Difference between blastulation and gastrulation.

Q6 what do you understand by fate maps? Explain in brief.

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## 4.7 TERMINAL QUESTIONS/ANSWER

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**Q. 1** What do you mean by morphogenetic movement?

**Q. 2** Differentiate between invagination and evagination?



- Q. 3** Describe significance of gastrulation?
- Q. 4** What is primitive streak?
- Q. 5** What do you mean by gastrula movement?
- Q. 6** What is blastocyst?
- Q. 7** What is hypoblast?
- Q. 8** What do you mean by foetal membrane?

### **Terminal questions/answer**

**Q 1 As a result of gastrulation,**

- (a) The rounded blastula becomes oval
- (b) The single layered blastula changes to double layerd gastrula
- (c) Three germinal layers are differentiated
- (d) Blastocoel is formed

**Q.2 The cavity formed during gastrulation by the process of invagination in blastula**

**Is called**

- (a) Blastocoel
- (b) Segmantation cavity
- (c ) Subgerminal cavity
- (d) Archenteron

**Q.3 Primitive streak is formed in the embryo of**

- (a) Ampioxus
- (b) Frog
- (c ) Birds
- (d) Mammals

**Q.4 The nucleocytoplasmic ratio in the early blastomere is**

- (a) Lower than in the somatic cells
- (b) More than in the somatic cells
- (c ) The same as in the somatic cells
- (d) None of these

**Q.5 Eggs of insects are**

- (a) Mesolcithal and centrolcithal

- (b) Telolecithal and oligolecithal
- (C) Meggalecithal and centrolecithal
- (d) Megalecithal and centrollecithal

**Q.6 In highly yolky eggs of birds ,gastrulation occur by**

- (a) Invagination and involution
- (b) Infiltration and delamination
- ( c ) Ingression or infiltration
- (d) Epiboly and invagination

**Q. 7 In mammalian eggs, gastrulation occur by the process of**

- (a) Delamination
- (b) Involution
- (c ) Ingression
- (d) Invagination

**Q.8 in frog, gastrulation is completed by**

- ( a ) Epiboly
- (b) Emboly
- (c) Both epiboly and emboly
- (d) Delamination

**Q.9 The embryonal area in chick embryo is located in**

- (a) Area opaca
- (b) Area pellucid
- ( c ) Area vasculosa
- (d) Area vitelline

**Q.10 Morphogenetic movement the occur during embryogenesis are**

- (a) Irreversible
- (b) Reversible
- (c ) Both a and b
- (d) Temporary

#### **Answers**

1 ( c ) 2 (d) 3 (c) 4 (a) 5(d) 6 (c) 7 (a) 8 (c) 9 (b) 10 (a)

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## **4.9    *REFERENCES***

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## UNIT- 5 AQUACULTURE

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### ***CONTENTS***

5.1- Objectives

5.2-Introduction

5.3 General principles of aquaculture

5.3.1 Induced breeding

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And the by-product of fishing Industry

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5.5 Pearl Culture

5.6-Terminal question

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## ***5.1 OBJECTIVES***

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The main objectives of aquaculture are:

- To boost economy of the nation by way of increasing per production.
- To generate employment opportunities for the unemployed persons.
- To utilize fully the natural water resources available to the maximum.
- To uplift the socio-economic status of the people of Indian sub-continent.
- To earn foreign exchange revenue by transport of fish to foreign countries.
- To culture ornamental fishes for beautifying aquariums.
- To culture larvicidal fishes for control of mosquito larvae.
- To increase production of food, in the form of fish, and decreasing the pressure on other food items.

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## ***5.2 INTRODUCTION***

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The term ‘Aquaculture’ means culture of all aquatic forms like fish, prawns, molluscs and sea weeds in fresh, brackish as well as marine waters. The ‘Aquaculture’ includes the type of culture systems utilized, e.g., pond culture, cage culture, pen culture, etc. The type of organisms cultured e.g., fish, oyster, shrimp or prawns etc. The origin of aquaculture dates back to at least three thousand years ago, but unlike agriculture, which has been the most important way of obtaining food on land, aquaculture has until recently contributed very less to mankind due to age old methods in use coupled with lack of proper knowledge. But now the picture is changing rapidly as aquaculture is gaining more importance in the today’s modern world, due to increase in population and shortage of food.

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## ***5.3 GENERAL PRINCIPLES OF AQUACULTURE***

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The evidence about fish culture practised in India years ago came from the ‘kautilya’s ‘Arthshastra’ in which he mentioned about the secret means of keeping fish in reservoirs. ‘King Someshwara’ of Chanakya Dynasty described the methods of fattening the fish in ponds.

There is a long period regarding fish culture in India until, collection and transportation of carp spawn, from rivers and stocking the ponds, was developed traditionally in Bengals,

Bihar and Orissa by the end of 19<sup>th</sup> century. This technique spread in other states also and a notable advance in fish culture in Bengal was the construction of 'Bundhs' for carp breeding. Warm water fish culture got a boost when under the guidance of 'H.C. Wilson' the first big fish farm with facilities of carp breeding came into existence in Tamil Nadu (TN).

The Indian Council of Agriculture Research (ICAR) recommended sponsoring fisheries research schemes by State Governments and Universities on different aspects of fish culture. For extending fish culture activities to all parts of the country Govt. Of India established the Central inland fisheries Research Institute (CIFR) at Barrackpore (west Bengal). The pond culture substation of CIFRI was started at Cuttack (Orissa) in 1949 for finding solutions to problems of fish culture in ponds and thereafter a considerable thrust has been laid on research programmes in inland fisheries.

Development after 1970 has led to the use of 'Second generation Techniques' including mammalian hormones, steroids, prostaglandin, and its analogues to make the cultivated species spawn for seed production.

The central institute of Fisheries Education (CIFE) Versova, Mumbai, Aquaculture Research and Training Centre, Kakinana, Central Marine Fisheries Research Institute (CMFRI), Cochin; Central Institute of Freshwater Aquaculture (CIFA), Kausalyganganga, Orissa, Central Institute of Brackishwater Aquaculture, Chennai, have been established for the development of aquaculture in India.

#### **(a) Trout Propagation: History**

The trout originated in France and the monk Don Pinchot of 14<sup>th</sup> century discovered the artificial propagation of trout eggs. Being a sport fish the, trout culture spread to almost all continents thereafter. Commercial trout culture developed in countries like France, Denmark, and Japan and recently in Norway and Italy. With the development of 'Cage farming' of Trout in Norwegian 'firods' salmonoid culture achieved a remarkable place in production and public attention.

The fish culture in North America was cantered earlier towards propagation of salmon and trout. In 18<sup>th</sup> century trout hatcheries were established. Gradually the trout propagation spread to the temperature and semi temperate areas of central and South America. The

British introduced trout in Asia and Africa, mainly to develop sport fisheries. The first attempt to transplant trout in India was made at Nilgiris in Western Ghats of Tamil Nadu.

**(b) Exotic Fish culture in India and other countries**

Besides trouts there are other species which from their place of origin have been introduced in other countries for culture purpose, e.g. *Cyprinus carpio* is the most extensively cultivated species worldwide. In India, the common carp (*Cyprinus carpio*) is cultured in combination with Indian major carps.

The silver carp (*Hypophthalmichthys molitrix*,) has been introduced in many countries and cultured in China, Taiwan, Thailand, Malaysia, Japan, Sri Lanka, India, Pakistan, Nepal, Philippines, Russia, Myanmar, Hong Kong, Singapore and Israel. In India for the first time fingerlings of silver carp were brought from Japan in 1959. Likewise, the Grass Carp (from China) and *Tilapia mossambica* (from Africa) have been transplanted throughout the world for cultural practices. There was resistance to its introduction in many countries as it was considered a pest by some countries. The larvivorous fishes were also introduced in different parts of the world.

The catfish family Clariidae enjoys the widest range of geographical distribution and species *Clarias gariepinus* (Nile catfish) from Africa was introduced in South Vietnam in 1974. From Vietnam it reached Cambodia, Laos and Thailand and it gets entry in India, in 1993.

**(c) Coastal Aquaculture**

The oldest form of 'Coastal Aquaculture' is the 'Oyster Farming' practised by the early Romans, Greeks and Japanese. In Japan around 2000 years ago coastal aquaculture was practised. Aristotle mentioned the cultivation of Oysters in Greece. The culture of mussels and clams developed at a quite later stage. The terms 'mariculture' and 'sea Farming' are frequently used for raising organisms in the marine environment until recently, and from the worldwide interest, that the sea farming has received in recent years, it appears that sea will be cultivated on a large-scale. The animals now cultured include shrimps, lobsters, oysters and clams.

**(d) Sea weed Culture:** Sea weed culture is relatively of recent origin. The earliest reference about 'sea weed' culture appears to have published in Japan in 1952. It was after 2<sup>nd</sup> world war the culture of edible sea weeds expanded and intensified considerably and practised in Korea, Taiwan and China. In Philippines and Hawaii several species of algae are regularly eaten. Most of the species of sea weeds contain 'Gelatin', used for the preparation of Jelly

and Jams. Dried sea weeds are regularly used in domestic cookery for making soups, pudding etc. The active ingredient in the sea weeds is 'Agar' (Sodium Alginate) which is used as a gelling substance.

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### Aquaculture in India

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India is the second largest producer with about 9% share of the world's total aquaculture. The top producer is China (57%).

#### Freshwater Aquaculture in India:-

Inland aquaculture emerged today, as a major fish producing system in India (Fig 5.1) with production around 1.7 million ton/yr. Carp accounts for over 80% of cultured fish. Major carps cultured are Rohu (*Labeo rohita*), Catla (*Catla catla*) Mrigal (*Cirrhinus mrigala*), Grass carp (*Ctenopharyngodon idellus*), Common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), Magur (*Clarias batrachus*), singhi (*Heteropneustes fossilis*) Rainbow trout (*Salmo gairdneri*) and giant prawn (*Macrobrachium rosenbergii*).



Fig.5.1 catching of fish

West Bengal is the largest producer of fish in India. The state is also the largest supplier of fish seed and supplies nearly 80% of the carp seed demand of the country.

The Central Institute of Freshwater Aquaculture (CIFA) Kausalyaganga (Orissa) established in 1986 is the top level institute in the country with the motto of conducting research on different aspects of freshwater aquaculture and undertaking transfer of technology to the field. The institute serves as the lead centre on carp farming.



**Brackish water Aquaculture in India:-**

The area of brackish water available in India for aquaculture is 1.19 million ha. Traditional shrimp farming practices are popular in Kerala, West Bengal, and Goa. The yields vary from 300 to 1000 kg/ha/year. Because of its high commercial value, Giant tiger prawn (*Penaeus monodon*) is the dominant species in commercial production; although, Indian white prawn (*Penaeus indicus*) is also farmed in several places. Shrimp production by farming reached a record value in 1994-95. Subsequently production suffered a setback due to a ban imposed by the Supreme Court of India in response to petition filed by environmentalists pleading that shrimp farming had created severe environmental damages. Subsequently many shrimp farms in coastal areas were closed. Intensive shrimp farming is banned and only modified improved farming is permitted with a productivity of around 2 to 2.5 ton/ha/yr. West Bengal is the highest producer of shrimps in India. Its estuarine area in the Sunderbans is ideally suited for the extensive culture of prawns and shrimps.

**Marineculture (Sea Farming) in India:-**

Indian 'Sea farming' progress has been very slow. Of the 1.2 million ha. of potential land identified for shrimp farming only about 100,000 ha. is utilized. The slow progress in this area is due to the collapse of the shrimp farming industry because of environmental concerns and disease problems. Although the present attention is towards diversification of fish species other than shrimp the commercial ventures are constrained due to unreliable seed supply and lack of technology for commercial marine finfish hatchery seed production. The culture of sea bass (*Lateolabrax niloticus* sp.), milkfish (*Chanos chanos* sp.) grey mullets (*Mugil sp.*), pearl spot (*Etroplus sp.*) etc. has been practiced in India since 1960's.

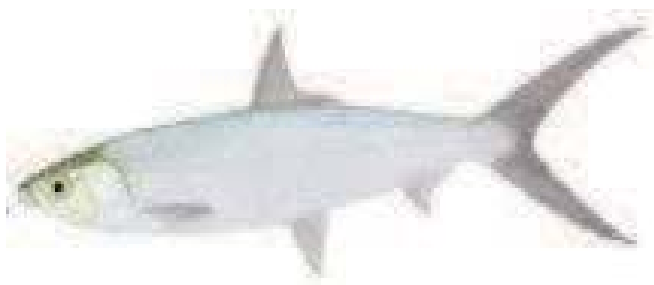


Fig.5.2 *Chanos chanos* (Milk fish)

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### 5.3.1 INDUCED BREEDING

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The artificial process by means of which the extract of the pituitary is introduced inside the body of both the matured male and female fishes, then the carps after being excited lay eggs in the pond water and subsequently fertilization takes place and the process is called **induced breeding** of fishes. This process of breeding is also known as **hypophysation**.

#### 1. Collection of pituitary extract:

From the matured fishes of both sexes either belonging the same species as the recipient or a closely related the pituitary glands are collected. It is preferred to collect the pituitary gland from freshly killed fishes. But it has been observed that the pituitary glands taken from five to eight days old ice-preserved fishes have also given successful results. In the fish markets, where the head of the cut fishes are available the pituitary glands can be taken out from the posterior end of the cranium through the foramen magnum after cleaning the brain tissue.

#### 2. Preparation of pituitary extract:

For the induced breeding of fishes the preparation of pituitary extract is very important. It is very easy to prepare. Immediate after the collection the pituitary glands are kept in absolute alcohol for dehydration. After 24 hours, the alcohol is changed for further dehydration and defatting. The glands are then weighed and preserve in fresh alcohol in dark colored phials. It may be stored at room temperature or in a refrigerator. The weight of each pituitary gland varies from 7 to 19 mg in Rohu weighing 1kg to 3.8 kg and 3 to 23 mg in Mrigal weighing 0.3 to 3.6 kg. At the time of injection to carps for the induced breeding, the required quantity of pituitary glands are taken out of the phials and the alcohol is allowed to evaporate. The glands are then macerated with a tissue homogenizer either in distilled water or 0.3 percent of saline water. The gland suspension is then centrifuged and the supernatant fluid is drawn into a hypodermic syringe for the injection.

#### 3. Method of injection and spawning:

During the rainy season, the extract of the pituitary gland of the same species which is prepared on the above said scientific process is injected in the muscle of the matured carps, 1.5 kg the 5.5 kg weight in general. Just before evening, per one female with two males of the approximate same body weight are to be injected the pituitary extract by hypodermic syringe. Injection of the carps is to be done outside of the water lying on a piece of sponge which is

used only to avoid the injury of the carps. In case of male carps the pituitary extracts are introduced once and in case of female carps it is introduced twice.

Then the carps, i.e., one female and two male are placed in a breeding hapa for spawning. Inside of the breeding hapa both the female and male carps are excited. After the excitation the female carps lays eggs. The eggs are externally fertilized by the spermatozoa that are discharged by the males. After that all the fishes are removed from the breeding hapa and then the eggs are collected by a net and are transferred to the inner part of the hatching hapa. After 14 to 18 hours, the spawns enter into the outer hapa and the induced breeding process completed. Then the spawns are collected from the outer hapa and transferred to the pond for nursery.

#### **Advantages of induced breeding:-**

- i) Eggs and spawns of carps collected from the river bed, there are possibility of mixture of other fish eggs and spawns. Whereas, in the induced breeding there is no possibility of mixture and as a result the pure form of fish seeds are obtained.
- ii) Desired species of carps can be cultured through the induced breeding.
- iii) Large numbers of eggs are available from a fish through induced breeding.
- iv) In the same season, a carp can be induced to breed more than once.
- v) Transportation cost becomes very low as the carps can be breed in any desired pond.
- vi) Between the different species of fishes hybridization can be done and it is possible to get hybrid variety of fishes.

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### **5.3.2 COMPOSITE FISH CULTURE**

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Fish is the cheapest and most easily digestible animal protein and was obtained from natural sources from time immemorial for consumption by human beings. However, due to over exploitation and pollution, the availability of fish in natural waters have declined considerably forcing scientists to adopt various methods to increase its production. Fish farming in controlled or under artificial conditions has become the easier way of increasing the fish production and its availability for consumption. Farmers can easily take up fish culture in village ponds, tanks or any new water body and can improve their financial position substantially. It also creates gainful employment for skilled and unskilled youths. The technology developed for fish culture in which more than one type of compatible fishes are cultured is the most advanced technique and popular in the country. This technology is

known as Composite Fish Culture. This technology enables to get maximum fish production from a pond or a tank through utilization of available fish food organisms in all the natural niches, supplemented by artificial feeding. Any perennial fresh water pond/tank retaining water depth of 2 metres can be used for fish culture purpose. However, the minimum level should not fall below one metre. Even seasonal ponds can also be utilised for short duration fish culture.

Here, fingerlings of fast growing compatible species of fishes with different feeding habits are employed. Indian major carps such as *catla catla* (catla,) *Labeo rohita* (rohu), and *Cirrhina mrigala* (mrigal) and exotic carps such as silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio*) grass carp (*Ctenopharyngodon idella*) are stocked together.

### 1. Fish species involved in composite fish culture:-

Depending on the compatibility and type of feeding habits of the fishes, the following types of fishes of Indian as well as Exotic varieties have been identified and recommended for culture in the composite fish culture technology:

#### Indian Major Carp (IMC)

Species	Feeding habit	Feeding zone
Catla	Zoo plankton feeder	Surface feeder
Rohu	Omnivorous	Column feeder
Mrigal	Detritivorous	Bottom feeder

#### Exotic carps

Silver carp	Phytoplankton feeder	Surface feeder
Grass carp	Herbivorous	Surface, column and marginal areas
Common carp	Detritivorous/Omnivorous	Bottom feeder

**2. Potential:** The area under tanks and ponds available for warm fresh water aquaculture is estimated to be 2.85 million ha. In addition 0.78 million ha of swamps, beels, etc. and low lying water logged area not good for agriculture as also any agriculture land can be converted for fish farming. Out of the total inland fish production around 60% is contributed by the culture sector. The average productivity from ponds at present is to the tune of 2160 kg/ha/year. This shows the tremendous scope for fish culture in the country. The area of 4.56 lakh ha brought under scientific fish culture by 1997-98 is only 16% of the potential area of tanks and ponds available for development showing immense possibilities for horizontal expansion of composite fish culture.

### **Technical parameters that needs to be considered for Composite Fish Culture**

#### **1. Selection of Pond:**

The main criteria to be kept in mind while selecting the pond is that the soil should be water retentive, adequate supply of water is assured and that the pond is not in a flood prone area. Derelict, semiderelict or swampy ponds can be renovated for fish culture by dewatering, desilting, repair of the embankments and provision of inlet and outlet. The pond may be owned by the individual or taken on lease in which case the lease period should be more or coterminous with the repayment period.

#### **2. Pond Management:**

Pond Management plays a very important role in fish farming before and after the stocking of fish seed.

##### **A) Prestocking:**

In case of new ponds, prestocking operations starts with liming and filling of the pond with water. However, the first step for existing pond requiring development deals with clearing the pond of unwanted weeds and fishes either by manual, mechanical or chemical means. Different methods are employed for this.

- Removal of weeds by Manual/Mechanical or through Chemical means.
- Removal of unwanted and predatory fishes and other animals by repeated netting or using mahua oil cake @ 2500 kg/ha meters or by sun drying the pond bed.

- Liming:-The tanks which are acidic in nature are less productive than alkaline ponds. Lime is used to bring the pH to the desired level. In addition lime also has the following effects -

- a) Increases the pH.
- b) Acts as buffer and avoids fluctuations of pH.
- c) It increases the resistance of soil to parasites.
- d) Its toxic effect kills the parasites.

**B) Fertilization:**

Fertilization of the pond is an important means of intensifying fish culture by increasing the natural productivity of the pond. The fertilization schedule has to be prepared after studying the quality of the pond soil. A combination of both Organic and Inorganic fertilizers may be used for best results. The fertilizer programme has to be suitably modified depending on the growth of the fish, available food reserve in the pond, physico-chemical conditions of the pond and climatic conditions.

**C) Manuring:**

- i) Organic manuring may be done in monthly installments @ 1000 kg/ha.
- ii) Inorganic fertilization may be done at monthly intervals alternating with organic manuring. However, the monthly rate of fertilization will depend on pond productivity and the growth of the fishes. It should be ensured that excess fertilization does not take place which may result in eutrophication.

**D) Harvesting:**

Harvesting is generally done at the end of first year, when the fishes attain average weight of 750 gm to 1.25 kg. A production of 4 to 5 tons/ha can be obtained in a year. However, for the purpose of working out economics' a production level of 3 tons/ha/year may be considered. Harvesting is done by partial dewatering and repeated netting. In some cases complete dewatering of ponds is resorted to.

### 3) Vertical expansion of fish culture:

A number of measures are now being employed by the entrepreneurs to increase the per hectare production of fish. Important measures adopted are stocking of Yearlings by stunning the growth of fish seed during first year, heavy stocking and multiple harvesting after the fishes attain a size of 500 gm. multiple stocking and multiple harvesting, use of aerators, integrated fish farming with animal husbandry activities like dairy, poultry, piggery or duckery to get daily organic manuring to the pond thus increasing its fertility. It is possible to increase the per hectare production of fish to 7 to 10 tons per ha per year by employing different methods as indicated above.

### 5.3.3 LAYOUT OF FISH FARM AND ITS MANAGEMENT & THE BY-PRODUCT OF FISHING INDUSTRY

Fish farming or pisciculture involves raising fish commercially in tanks or enclosures such as (fish ponds), usually for food. It is the principal form of aquaculture, while other methods may fall under mariculture. Farming carnivorous fish, such as salmon, does not always reduce pressure on wild fisheries. A facility that releases juvenile fish into the wild for recreational fishing or to supplement a species' natural numbers is generally referred to as a fish hatchery. Worldwide, the most important fish species produced in fish farming are carp, tilapia, salmon, and catfish.

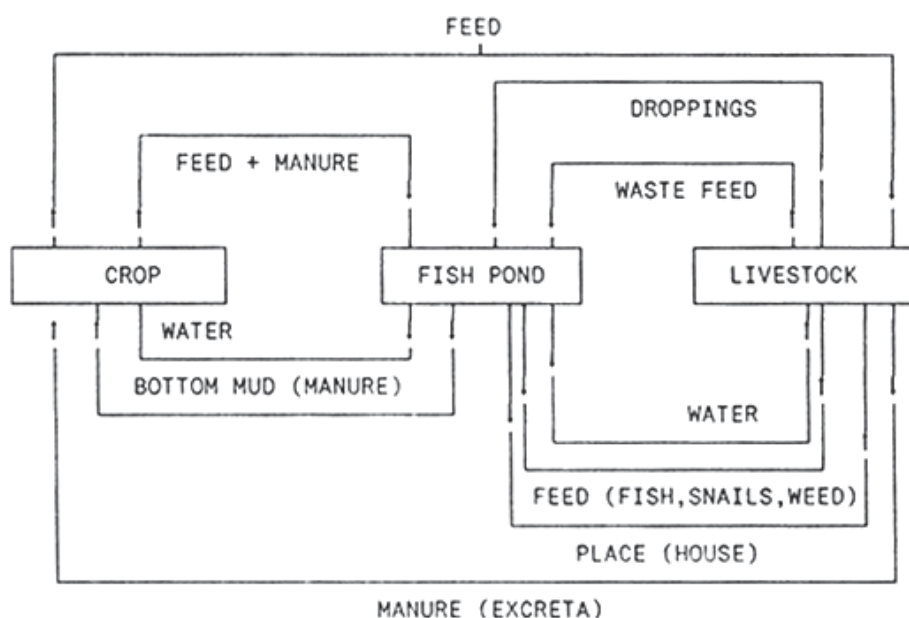


Fig.5.3 Diagrammatic representation of Layout of fish pond

Within intensive and extensive aquaculture methods, numerous specific types of fish farms are used, each has benefits and applications unique to its design. The traditional fishery by-products are fishmeal, fish body and liver oils, fish maw, etc. Fish protein concentrate, fish albumin, glue, gelatine, pearl essence, peptones, amino acids, proteins, fish skin leather etc. are some other by-products generally processed out of fish and fish waste. Fish and other aquatic organisms are also processed into various food and non-food products.

**By-Product of fishes:-**

Fishes are consumed as food in fresh condition. Some of them are also utilized after the preservation. During preservation and processing, some materials of fish and prawn are discarded as waste. Similarly some trash and distasteful fishes are unsuitable for human consumption. These waste material and above fishes become an important source of fish by-products, which in turn are used to produce different useful fish by-products. A shimmery substance found on fish scales, most usually obtained from herring and one of many by-products of commercial fish processing, can also be used for pearlescent effects, primarily in nail polish, but is now rarely used due to its high cost, bismuth oxy-chloride flakes being used as a substitute instead.

- Fish protein concentrate (FPC) is a stable protein concentrate prepared from whole fish or other aquatic animals or parts thereof. Protein concentration is increased by removal of water, oil, bones and other materials. Traditionally dried or otherwise preserved products do not fall within this definition. Development of FPC has paved the way for converting a wide range of whole fish into protein concentrate, which has no resemblance to the original raw material, for human nutrition.
- Fish Protein Concentrate is a gritty, colorless, odorless and tasteless powder. It is stable up to 3-4 years at room temperature without any significant change in flavour. Proximate composition of a representative sample of FPC is given below. The greater quantity of highly digestible protein, available lysine and minerals makes FPC a highly nutritious product.



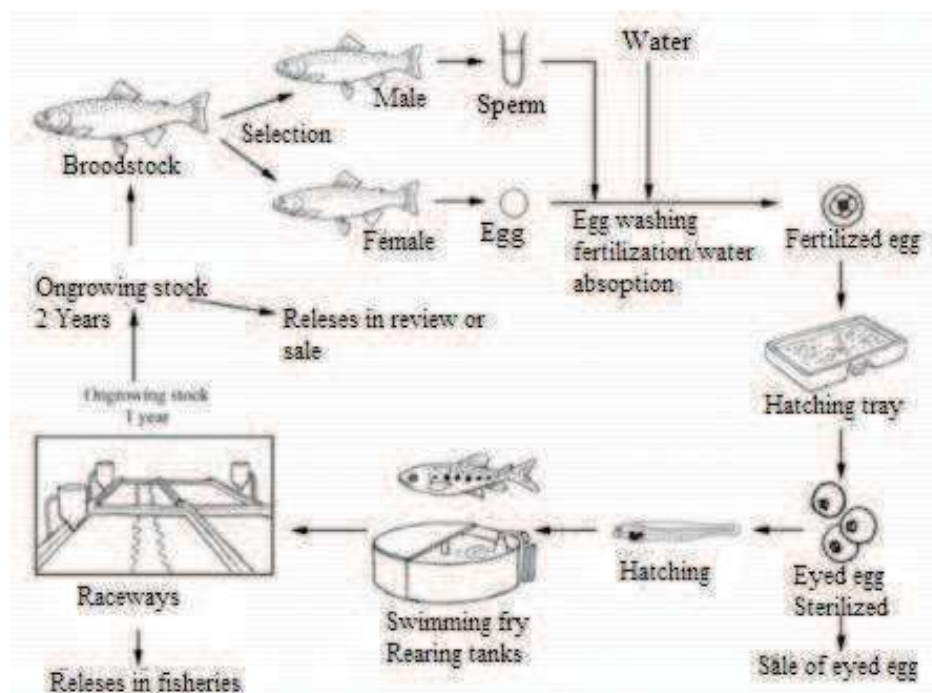


Fig 5.4 Production cycle of fish

#### Uses of By-product:-

- Though FPC is intended for human consumption it is not relished for consumption as such. It is therefore incorporated as a protein supplement in human diet. 5-10 per cent level FPC in bread and biscuit is considered the acceptable limit. 35 g per person per day is a recommended level of use of FPC.
- Fish undoubtedly is one of the most nutritious foods available for human consumption. Fish flesh on an average contains 15-20 per cent protein. Some species of fish contain very high amounts of body oil. Few species of fish like shark, cod etc. are good sources of liver oil. Fish processing and filleting industries turn out large quantities of fishery waste. All these are good sources of high quality protein, fat, minerals etc.
- The traditional fishery byproducts are fishmeal, fish body and liver oils, fish maw, isinglass etc. Fish protein concentrate, fish albumin, glue, gelatin, pearl essence, peptones, amino acids, protamines, fish skin leather etc. are some other byproducts generally processed out of fish and fish waste. Chitin and chitosan processed out of shrimp, crab and other crustacean waste are byproducts of high economic value.

Biochemical and pharmaceutical products like bile salts, insulin, glucosamine etc. are some other fishery byproducts of great significance.

## 5.4 PRAWN CULTURE

Prawn is a common name for small aquatic crustaceans with an exoskeleton and ten legs (i.e. a member of the order decapods), some of which can be eaten. The term "prawn" is used particularly in the United Kingdom, Ireland, and Commonwealth nations, for large swimming crustaceans or shrimp, especially those with commercial significance in the fishing industry. Shrimp that fall in this category often belong to the suborder Dendrobranchiata. A freshwater prawn farm is an aquaculture business designed to raise and produce freshwater prawns or shrimp<sup>1</sup> for human consumption. Freshwater prawn farming shares many characteristics with and many of the same problems as, marine shrimp farming. Unique problems are introduced by the developmental life cycle of the main species (the giant river prawn, (*Macrobrachium rosenbergii*)).

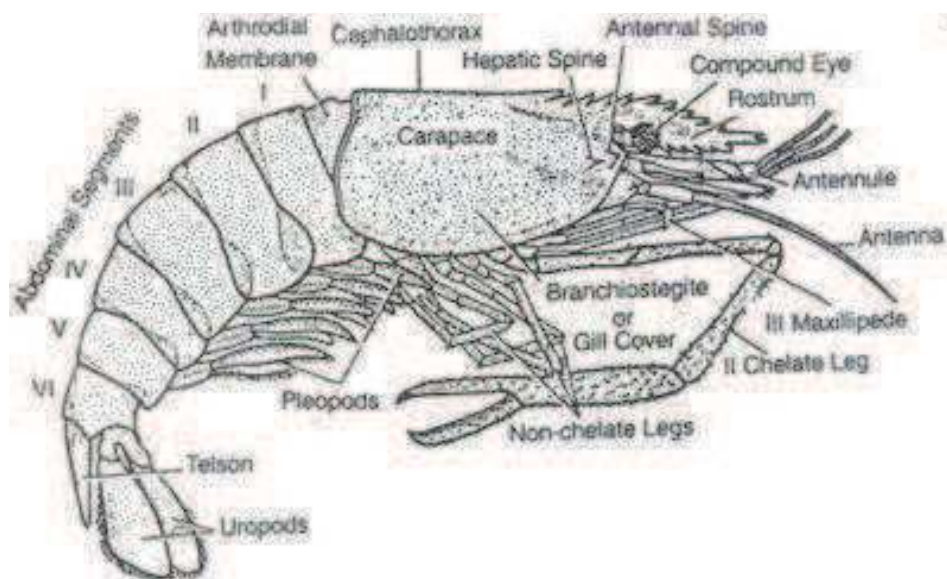


Fig.5.5 External feature of Palaemon

### Habit, habitat and food of Prawns:-

Before going for the prawn culture we should have some knowledge about the habit, habitat and food of prawns. Prawn inhabits all sorts of water, much as in sea-water, estuaries and fresh-water. They are generally living at the bottom of water and are avoiding sun-rays. The

marine and brackish water species spawn in sea. The hatchlings are incapable to swim, so they are drifted along with the current to the coastal waters or estuaries where they undergo development till they reach the juvenile stage. The post larvae feed upon the dead organic matter of plants and animals and upon small benthonic organisms. The juvenile prawn has to enter the sea. The fresh-water species like *Macrobrachium* sp. spawn in fresh-water, and then they are drifted to estuaries and after attaining the juvenile stage swim back to fresh-water. Prawns are consuming the organic substances, microscopic animals and plants as their food material. Among the animals, minute insect, snail, larvae of mollusca and echinodermata as well as aquatic weeds, algae, moss etc., are taken as their food material.

Giant river prawns live in turbid freshwater, but their larval stages require brackish water to survive. Males can reach a body size of 32 cm and females grow to 25 cm around. In mating, the male deposits spermatophores on the underside of the female's thorax, between the walking legs. The female then extrudes eggs, which pass through the spermatophores. The female carries the fertilized eggs with her until they hatch; the time may vary, but is generally less than three weeks. A large female may lay up to 100,000 eggs. From these eggs hatch zoeae, the first larval stage of crustaceans. They go through several larval stages before metamorphosing into post larvae, at which stage they are about 8 mm long and have all the characteristics of adults. This metamorphosis usually takes place about 32 to 35 days after hatching. These post larvae then migrate back into freshwater.



Fig.5.5 Freshwater prawn farming

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## **Prawn Culture in Fresh water**

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The culture of prawn is done in three ways, extensive, semi intensive and intensive.

In extensive culture the juvenile prawns are trapped from their natural habitat and are allowed to develop in some artificial water bodies e.g. rice fields. Here the prawns feed and grow till they are harvested. In the semi-intensive culture the juveniles are trapped from the conditions in the water bodies. Required size prawns are then collected for marketing.

Intensive culture requires much time and infrastructure to reap good results. Here the culture proactive starts from very beginning i.e., from spawning and culminate into the harvesting. All the factors required for the development and growth of prawns are carefully regulated and monitored here.

The economically important culturable varieties of freshwater prawns in India are giant fresh water prawn (*Macrobrachium rosenbergii*) and monsoon river prawn (*macrobrachium malcolmsonii*). The giant fresh water prawn is of prime importance due to its large size, fast growth, and excellent food value and consumer preference.

### **Distribution:**

The prawns inhabit wide range of environmental conditions in tropical and sub-tropical zones. In nature, they migrate to estuarine zone for breeding and the juveniles (15-25mm) migrate back to the freshwater.

### **Food habits:**

The prawns are omnivorous and eat frequently. They commonly feed on aquatic worms, insects and their larvae, small sized molluscs and crustacean, flesh and offal fish and other animals, grain, seeds algae and tender leaves and stems of aquatic plants.

### **Life cycle**

The gaint fresh water prawn and the monsoon river prawns attain sexual maturity in 5 to 7 months in fresh water. They breed throughout the year with a peak at beginning of rainy season. The copulation takes place between the hard shelled sexually matured male and fresh moulted female (Premating moult). The male deposits its sperms on the ventral surface of the

female's cephalothorax close to the genital orifice so that, the eggs are fertilized as they are liberated through the female genital pores. Then the fertilized eggs are transferred to the brood chamber which is formed by the downward prolongation of the abdominal shell. The berried females descend to the brackish water of the rivers (estuary) for spawning.

Each female lays 5000 to 30000 eggs. Smaller females (8-10 g) lay 500-600 eggs/g and large females (60g) lay 300-400 eggs/g. The eggs appear bright orange in colour in the beginning, becoming progressively brown turning grey and finally becoming dark grey before hatching. The eggs are carried by the female in its brood chamber for 15 to 25 days depending on the water temperature. The larvae are liberated in water after hatching. The larvae pass through 11 developmental stages before changing into post-larvae. The larvae are planktonic and develop in brackish water for 3 to 6 weeks depending on the water temperature. The post larvae resemble the miniature adult prawns and develop the habit of crawling rather than free swimming and benthic in behaviour. These post larvae begin to migrate upstream into freshwaters within one or two weeks after metamorphosis and are able to swim against water current.

**Seed:**

The seed is obtained from two sources. The seed collection from natural water is carried out from rivers. The other method is to induce breed the prawns under controlled conditions in the hatchery.

**Nursery Phase:**

Nursery phase is an intermediate step between hatchery and culture ponds. In this, the post larvae (1.0cm) produced in hatchery or collected from natural resources are reared in specially prepared nurseries so as to grow them into juveniles (2.0 to 3.0 cm.) Nurseries may be earthen ponds, cement cisterns, or plastic pools enclosing a space of 50 to 200m<sup>2</sup>.

Before stocking in nurseries, the post-larvae are thoroughly acclimatised to freshwater conditions by subjecting them to low level of salinity. Nurseries are stocked @ 2000 to 3000 post-larvae per m<sup>2</sup>. Post-larvae at this stage tend to cling to any available surface inside the nurseries. In order to provide more surface for clinging, bunches of polythene strips are placed in nurseries with the help of sinkers.

**Pond:**

Ponds of 0.1 to 0.2 ha area is ideal for the culture of freshwater prawns. The pond bottom should be slightly sloping with a sump made at the deeper end to facilitate harvesting. Rectangular ponds having a length with ratio of 2.5:1 are desirable for easy netting during harvesting. The pond depth should be within a range of 0.75 to 1.2 m. To prevent erosion proper bundhs should be constructed. The internal and external slopes of the bundhs should be 2:1 to 3:1 and 1.5:1 to 2:1 respectively. Proper inlet and outlet should be provided in opposite direction at shallow and deeper ends of the pond respectively.

**Water:**

Water supply and its quality are of vital importance for prawn's culture. Criteria for water suppliers for prawn farming are as follows:-

**pH:** 7.0-8.5, Total hardness ( $\text{CaCO}_3$ ) 50 to 100 ppm, Temperature  $18^{\circ}$ - $34^{\circ}$  C (optimum  $29^{\circ}$ - $31^{\circ}$ C), Dissolved oxygen more than 4.00 ppm, Salinity 0.25 to 0.75 ppm, Calcium Less-than 100 ppm, Phosphorus Less than 1 ppm, Nitrates Less than 1 ppm. The water intake through the inlet pipe should be screened, in order to supply sediment free water and prevent entry of small fish, eggs, larvae etc. into the pond.

**Pond Management**

Preparation of pond: Ponds are prepared in the same manner as it is done for carps involving steps like removal of predators, clearance of weds, liming and fertilization with organic manures. The pond is lime @ 250 to 500kg of chicken droppings per hectare). This organic base on the bottom increases the fertility of the pond and enhances the production of benthic organisms which is the prime natural food of the prawns.

**Stocking:**

Ten to fifteen days after liming and fertilization, prawn seed is stocked. Generally, 3 to 4 weeks old post-larvae to the prevailing water temperature in the pond by floating the polythene bags containing seed in the pond for 15 to 20 minutes before emptying then into the pond. Seed should not be stocked when water temperature is below  $20^{\circ}$ C. The optimum temperature for stocking is  $22^{\circ}$  to  $24^{\circ}$ C.



**Stocking rate:**

The recommended rate of stocking for monoculture operations is 7,000 to 10,000 per hectare and for polyculture with carps, just half of this number (15,000 to 25,000 per hectare.) In India, polyculture with carps is more preferred as it gives better scope for utilisation and conversion of natural and supplementary food and thus works out more economical. Compatible fish fingerlings viz. Catla, rohu, silver carp and grass carp are stocked @15, 00 to 2,000 per hectare before stocking the prawn seed.

**Feeds:**

The prawns feed actively at night and rest during the day. Natural feeds in culture ponds serve as a good source of food for the stocked prawns. However, in commercial operations, the natural productivity is not sufficient where the prawns are stocked at higher densities. Therefore, it is essential to provide supplementary feed for achieving higher growth.

**Feeding rate:**

The quantity of feed is adjusted through trial and error. To avoid cannibalism, the prawns are not kept hungry. Good practice for the farm operator is to feed the animals according to their demand. Feeds are placed in wide plastic trays or earthen troughs kept at several points along the marginal shallow area. In monoculture, feed conversion ratio is 1:7-9 when raw (wet) feeds are used. Feeding with compound dry feeds ration of 1:2-3 has been obtained. Growth and survival: Growth and survival depends on a number of factors like food, stocking density, water quality, climate, pond size and productivity of pond.

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## ***5.5 PEARL CULTURE***

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Pearls are made by molluscs, like oysters. Pearls are small and often white but sometimes in pale colours or even black. They are often rounding, but sometimes half-round, oval, or in different shapes. Pearls are often used for jewellery. A cultured pearl is a pearl created by an oyster farmer under controlled conditions. Cultured pearls can be farmed using two very different groups of bivalve mollusk, the freshwater river mussels, and the saltwater pearl oysters. A pearl is formed when the mantle tissue is injured by a parasite, an attack of a fish or another event that damages the external fragile rim of the shell of a bivalve or gastropod mollusk. In response, the mantle tissue of the mollusk secretes nacre into the pearl sac, a cyst that forms during the healing process. Chemically



*Fig.5.6 various pearl*

speaking, this is calcium carbonate and a fibrous protein called conchiolin. As the nacre builds up in layers of minute aragonite tablets, it fills the growing pearl sac and eventually forms a pearl.

#### **Physical properties of pearl:-**

The unique luster of pearls depends upon the reflection, refraction, and diffraction of light from the translucent layers. The thinner and more numerous the layers in the pearl, the finer the luster. The iridescence that pearls display is caused by the overlapping of successive layers, which breaks up light falling on the surface. In addition, pearls (especially cultured freshwater pearls) can be dyed yellow, green, blue, brown, pink, purple, or black. The very best pearls have a metallic mirror-like luster. Because pearls are made primarily of calcium carbonate, they can be dissolved in vinegar. Calcium carbonate is susceptible to even a weak acid solution because the crystals of calcium carbonate react with the acetic acid in the vinegar to form calcium acetate and carbon dioxide.

#### **Natural Pearl**

Natural pearls are nearly 100% calcium carbonate and **Conchiolin**. It is thought that natural pearls form under a set of accidental conditions when a microscopic intruder or parasite enters a bivalve mollusk and settles inside the shell. The mollusk, irritated by the intruder, forms a pearl sac of external mantle tissue cells and secretes the calcium carbonate and conchiolin to cover the irritant. This secretion process is repeated many times, thus producing



a pearl. Natural pearls come in many shapes, with perfectly round ones being comparatively rare.

Typically, the build-up of a natural pearl consists of a brown central zone formed by columnar calcium carbonate (usually calcite, sometimes columnar aragonite) and a yellowish to white outer zone consisting of nacre (tabular aragonite). In a pearl cross-section, these two different materials can be seen. The presence of columnar calcium carbonate rich in organic material indicates juvenile mantle tissue that formed during the early stage of pearl development. Displaced living cells with a well-defined task may continue to perform their function in their new location, often resulting in a cyst.

### **Cultured Pearls**

Cultured pearls are the response of the shell to a tissue implant. A tiny piece of mantle tissue (called a graft) from a donor shell is transplanted into a recipient shell, causing a pearl sac to form into which the tissue precipitates calcium carbonate. There are a number of methods for producing cultured pearls: using freshwater or seawater shells, transplanting the graft into the mantle or into the gonad, and adding a spherical bead as a nucleus. Most saltwater cultured pearls are grown with beads. Trade names of cultured pearls are Akoya, white or golden South Sea, and black Tahitian. Most beadless cultured pearls are mantle-grown in freshwater shells in China, and are known as freshwater cultured pearls.

Cultured pearls can be distinguished from natural pearls by X-ray examination. Nucleated cultured pearls are often 'preformed' as they tend to follow the shape of the implanted shell bead nucleus. After a bead is inserted into the oyster, it secretes a few layers of nacre around the bead; the resulting cultured pearl can then be harvested in as few as six months.

When a cultured pearl with a bead nucleus is X-rayed, it reveals a different structure to that of a natural pearl. A beaded cultured pearl shows a solid center with no concentric growth rings, whereas a natural pearl shows a series of concentric growth rings. A beadless cultured pearl (whether of freshwater or saltwater origin) may show growth rings, but also a complex central cavity, witness of the first precipitation of the young pearl sac.

### Pearl Formation

Natural pearls are formed by nature, more or less by chance. On the other hand, cultured pearls are human creations formed by inserting a tissue graft from a donor oyster, upon which a pearl sac forms, and the inner side precipitates calcium carbonate, in the form of nacre or "mother-of-pearl". The most popular and effective method for creating cultured pearls are made from the shells of freshwater river mussels harvested in the midwestern states of the U.S., from Canada to the Gulf of Mexico. Shells with the common names, "Washboard" "Maple Leaf" "Ebony" "Pimple back" and "Three Ridge" are popular for use in pearl culture due to their compatibility with the host animal, and the nacre they are to be covered by. These high-quality and sought-after shells are first sliced into strips and then into cubes. The edges and corners are ground down until they are a roughly spherical and then milled to become perfectly round, and brought to a highly polished finish.

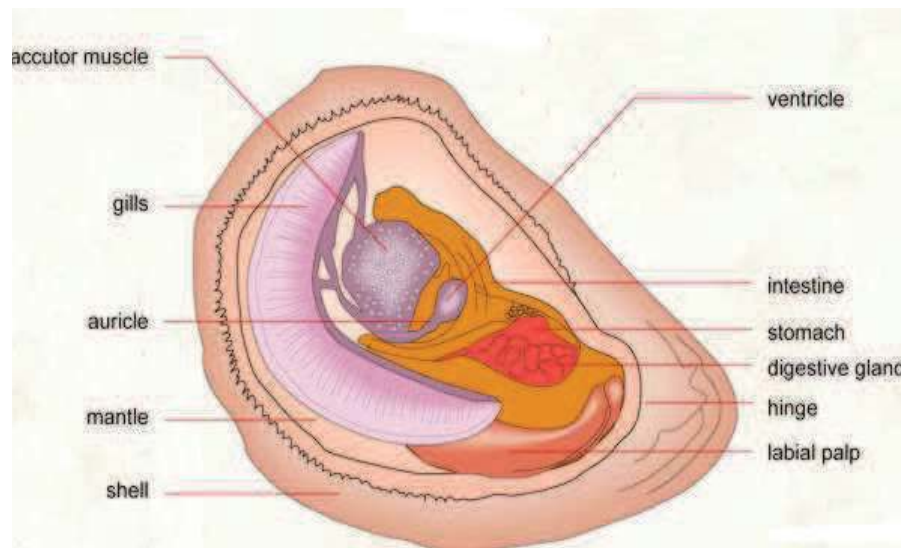
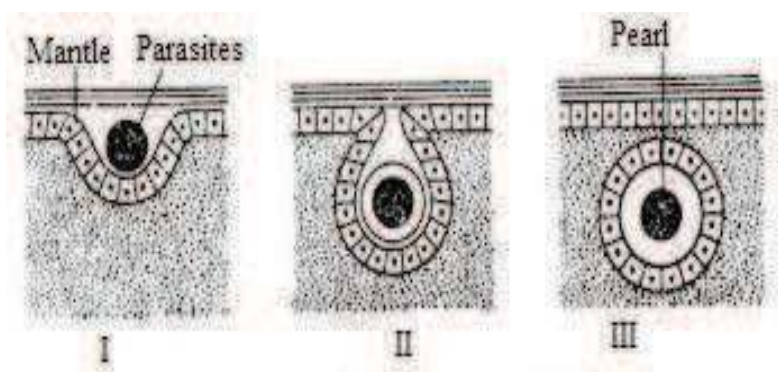


Fig.5.7 Oyster

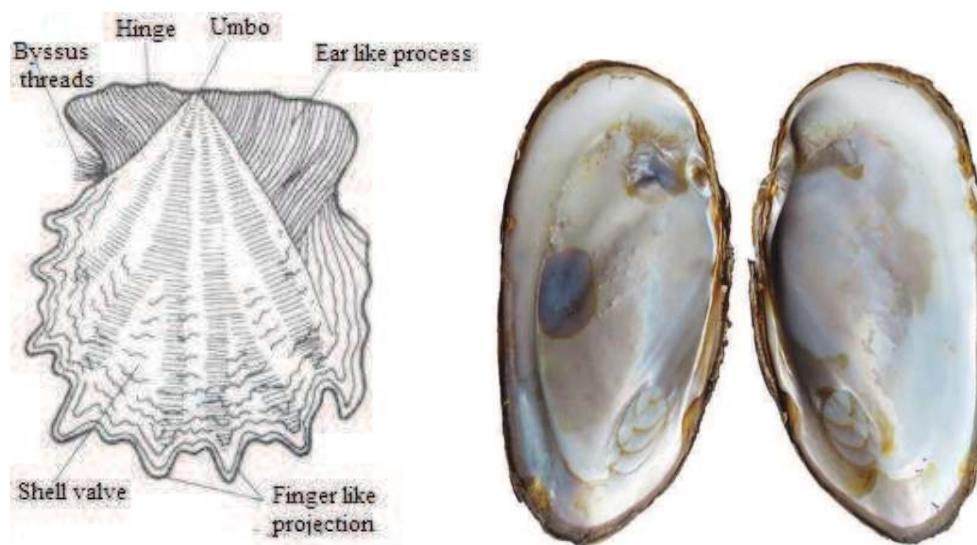


*Fig.5.8 Stage in pearl formation*

After the nucleus is ready, the next step is obtaining the mantle tissue. The mantle tissue is harvested from one oyster and cut into small pieces. After obtaining the mantle tissue from the first oyster it is time to operate on the second animal. The oyster is placed in warm water to relax the animal. Then it is gently pried open and mounted in a stand to be operated on. A small incision is made and the nucleus is inserted along with a small piece of mantle gland. The oyster is then placed back in the water and allowed over several years to coat the nucleus with nacre. The nucleus is coated in many layers of this nacre, so that when pearls are cut in half, visible layers can be seen.

#### **Pearl Producing Molluscs:-**

Although a number of bivalves have the ability to produce pearl under suitable climatic condition, high quality pearl obtained only from pearl oysters of genus *pinctada* roding belonging to the class Bivalvia and family pteriidae. A number of species of this genus like *P.vulgaris*, *P.chemnitzii*, *P.margaritifera*, *P.anomioides* etc. are found in India water. *P.vulgaris* is a common oyster distributed in the gulf of kutch, gulf of mannar and the palk bay.



*Fig.5.9 External feature of pearl oyster*

Apart from the true pearl pearl oyster belonging to the genus *pinctada* a large number of the other marine and a few fresh water mollusks are also found to produced pearls. Mostly the pearl oyster inhabits on the ridges of rocks dead corals forming extensive pearl banks at a depth of 18 to 22 meters.

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## **5.6 TERMINAL QUESTION**

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- Give detail account of the Aquaculture?
- Give detail account of the composite fish culture?
- Write a short note on the By-product of fishing Industries?
- Explain the layout of fish farm with suitable diagram?
- Write a short note o the pond management?
- Give principles of Aquaculture?
- Give detail account of the culture of fresh water prawn?
- Give an account of the method of prawn fishing
- Give detail account of the pearl culture?
- Give detail account of the management of the fish culture programme?
- Give detail account of the induced breeding?

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## UNIT- 6 SERICULTURE

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### ***CONTENTS***

- 6.1- Objectives
- 6.2-Introduction
- 6.3 Different kind of silk producing insect
- 6.4 Host plant of Insect
- 6.5 Rearing & disease of silkworm
- 6.6 Reeling and fiber technology
- 6.7-Terminal question
- 6.8-References

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## 6.1 OBJECTIVES

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Study of Different kind of silk producing insect and the Host plant of Insect and motivating the students to plant high yielding mulberry varieties to increase income and productivity. Imparting training in mulberry cultivation, silkworm rearing and silk reeling. Enhance skill of students for increased productivity and to prevent silkworm diseases.

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## 6.2 INTRODUCTION

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Sericulture is the art and technology of raising silkworms for the production of raw silk. The word Sericulture is derived from the Greek word *Sericos* (meaning silk) and the English word Culture (meaning Rearing). There are several species of silkworms but the most extensively studied silkworm is *Bombyx mori*. It is an agro based cottage industry, but each person involved in it must have some technical knowledge about it. It requires enough patience, ability to adapt to new scientific development, etc. Sericulture, or silk farming, is the cultivation of silkworms to produce silk. Although there are several commercial species of silkworms, *Bombyx mori* (the caterpillar of the domesticated silk moth) is the most widely used and intensively studied silkworm.

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## 6.3 DIFFERENT KIND OF SILK PRODUCING INSECT

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### Mulberry Silk worm (*Bombyx Mori*)

Mulberry silk is the most common among the many kinds of silk. It makes up 90% of the silk supply in the world. This popular kind is produced by the ***bombyx mori* silkworms** which are fed from the mulberry bush (thus the name). Since it is a common kind of silk, acquiring it is easy. Countries such as China, Japan and Korea have an abundance of it in particular. One of the disadvantages of using this kind of silk is that it needs special care to maintain its smooth texture. It also is often obtained in an unethical way, by killing the silkworms in their cocoons to extract the long fibres. For more information on this, have a look at our article The Pros and Cons of Silk Production, which also includes information on vegetarian silks, or ‘**Peace Silks**’.

**Tussar Silk worm**

Tussar silk worm *Antheraea paphia* belongs to the family Saturniidae. Tussar silk is produced by **tussar silk worms**. Unlike other silks, this one has a distinct light golden to dark brown colour. This is a result of the tannin-rich leaves that tussar silkworms consume.

**Muga Silk worm:-**

Muga silk worm ***Antheraea assamensis*** is the semi-domesticated silk worm, **another member of the** same family Saturniidae. Muga originates in India, and the silk it is known for its golden brown and glossy texture. Along with the Eri (below) and Pat silk, Muga silk is sometimes referred to as Assam silk, as fabrics produced from these silkworms were reserved for the exclusive use of royal families in Assam. Muga's silk porosity can sometimes become a disadvantage as it limits the use of bleach (which for us more eco-conscious types is an advantage!) but also means that it can't be dyed. Silk is said to be one of the strongest fibres around – and it's certainly the case with these thicker tussars and makes it an ideal material for couches, jackets and sweaters.

**Eri Silk worm:-**

Eri silk is derived from the domesticated silkworm ***Philosamia rinini***. It is a fine silk that is almost as white in colour as the *Bombyx mori* (Mulberry Silk we talked about above). Even though Eri is spun from the cocoons of domesticated silk worms, it is a “peace silk” because the silk caterpillars are not destroyed in the cocoon but are allowed to emerge as moths and live a full lifecycle. Because the cocoons are damaged when the moth emerges, Eri silk is spun rather than reeled. It usually has the matt appearance of wool or cotton, but the sheen and softness of silk and is commonly cultivated in India, Japan and China. Aside from the *Bombyx mori*, this is the only other silkworm that is completely domesticated, so it relies on human intervention to be developed. And just like the *Bombyx Mori*, the name “Eri” is said to derive from the Assamese word “Era”, which means castor plant – the food that the worms feed on. This is a very durable silk and makes a great material for clothing, and surprisingly, soft furnishings such as curtains and couches. The only drawback of using such silk is the fact that it is heavy to wash. It may also harbor micro organisms as it is easier for them to stick on its thicker layers.



**Mussel Silk:-**

As the name implies, this silk is produced by mussels – yes, the same ones that can be found on seabeds. It is also sometimes called **Sea Silk**. This differs from the other silk types we've mentioned so far as it is not produced by silkworms. It is difficult to source this kind of silk nowadays due to the effects of pollution on its source. Since its production is rare, it counts as one of the most expensive silks to date. The most common type of mussel, or more accurately mollusk used in the production is the Byssus, and this fabric tends to be called **Byssus Cloth**. There is an amazing explanation of the fabrics and the differences between them here.

**Spider Silk:-**

Like Mussel silk, you may be surprised to learn that spider silk has long been used by ancient cultures. But unlike other types of silk, this is the most difficult one to produce as spiders cannot just be bred like silkworms. Spiders cannot produce as much yarn as silkworms either. But though the production of this type of silk may seem difficult, its output is certainly worth the effort. It is regarded as one of the most durable types of silk as it is now being utilized in the production of telescopes, bulletproof vests and wear-resistant clothing etc.

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## **6.4 HOST PLANT OF INSECT**

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**Host plants of tasar silkworm:-**

The important food plants are Arjun (*Termanalia Arjuna* Roxb. Fr. De Wright & Am.), Asan (*Termanalia tomentosa*, W& A) and Sal (*Shorea robusta* Gaertn). Besides these, the silkworm feeds on Phutuka (*Melastoma melabathricum* Linn.), Bogori (*Zizyphus jujuba* Mill) etc, are some of the endemic species of the tropical region of India. The temperate species of this silkworm, namely as, *Antheraea proylei* Jolly, feeds on different species of Quercus which are widely distributed in this region.

**Host plant of Mulberry silkworm: -** Mulberry silkworm is a monophagous insect which reared on the leaves of mulberry only; the morin present in the leaves helps to attract the silkworm.

**Host plants of Muga silkworm:-** Muga group comprises of *Antheraea assama* Westwood, *A. knyvetty*, *A. compta* and *A. helferi* are endemic polyphagous insect and feeds on different

*host plant species*, formerly named as *Machilus bombycina* (King ex Hook. f.) and *Soalu* (*Litsea monopetala*) and few other food plants, likewise *Digloti* (*Litsaea salicifolia* Hook), *Mejankari* (*Litsaea cubeba* Lour.), *Bogori* or *ber* (*Zizyphus jujuba* Mill), *Champa* (*Michelia champaca* Linn.), *Bhomloti* (*Symplocos grandifolia* Wall), *Patihonda* (*Actinodaphne obovata* Blume), *Gamari* (*Gmelina arborea* Linn.) *Panchapa* (*Magnolia sphenocarpa* Roxb.), *Katholua* (*Cyclocodaphne nitida* Roxb.), *Gansarai* (*Cinnamomum glanduliferum* Meissn), *Bojramoni* (*Xanthoxylum rhesa* DC.).

**Host Plants of Eri Silkworm:** - *Eri group comprises of Eri* (*Samia ricini* Donovan and *Samia Cynthia*) is widely available. Besides this wild silkworms like *Attacus atlas* and *Cricula* species are distributed in this region. *Castor* (*Ricinus communis* Linn.) is primary food plants of Eri silkworm and commonly distributed in this region. However, *Kesseru* (*Heteropanax fragrans* Roxb.) is also considered as another primary perennial food plants. Besides these two, eri silkworm being polyphagous feeds on several alternative food plants viz. *Borkesseru* (*Ailanthus excels* Roxb.), *Barpat* (*Ailanthus grandis*), *Topioca* (*Manihot esculanta* Crantz), *Gulanha* (*Plumeria acutifolia*), *Gamari* (*Gmelina arborea*), *Payam* (*Evodia flaxinifolia*), are endemic to this region.

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## 6.5 REARING & DISEASE OF SILKWORM

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The word rearing does not mean only the feeding of caterpillars as often understood but a continuous care from egg laying through aestivation, hibernation, incubation, early stage larval care, late stage larval care, late stage larval to the production of cocoon. So, for proper and step-wise study of rearing one should proceed from Grainage Technology.

### Grainage Management

The aim of establishment of grainage is to provide good quality of seed to rearers and maintenance of original quality of races. For this purpose due care should be taken of the 'crop of silkworm' for seed production from the very beginning' i.e., the caterpillar stage, by providing them with proper nutrition and protection from the attack of diseases. Keeping these points in view initial selection is made on the basis of percentage of dead pupae during normal development. If it is above the limit seed should not be purchased for seed production. First selection is made by separating out dead cocoons and next selection in the grainage.

After grainage management the next step is the supply of seed of caterpillars to the farmers. The supply is of two types depending on the knowledge of rearers i.e., supply of eggs and 2<sup>nd</sup> instar larvae.

The old rearers who are well versed with the rearing technique may purchase eggs for the rearing but new and untrained rearers knowing nothing about the rearing should always be given 2<sup>nd</sup> instar caterpillars for this purpose. Much care should be taken for the rearing of 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instar caterpillars and 4<sup>th</sup> and 5<sup>th</sup> instar caterpillars are mostly reared either on hanging trays often with nylon nets or on the floor.

### **Spinning of Cocoons**

This is the period when the caterpillar stops feeding and starts to secrete a pasty substance from the silk gland. In this condition worms should be picked up and transferred to the spinning trays and kept in a position of slope (slanting) to the sun for a short period. Within three days spinning is over and the cocoon is formed and this is the last phase of the rearing of silkworm.

**Quality of cocoon:** The quality of cocoon is dependent on the raw silk yield, filament length, and real ability and splitting.

**Marketing of cocoon:** The price of cocoon is fixed during every season of the rearing. This price is, however, watched by the Government and cocoons are purchased by the rearers.

### **Post-cocoon processing**

The method of obtaining silk thread from cocoon is known as post-cocoon processing. This includes Stifling and Reeling.

1. **Stifling:** The process of killing the cocoons is termed as stifling. Seri culturists should be very much careful that before the emergence of silkworm (The cocoons which do not have cut holes.) good sized cocoons of 8 to 10 days old are selected for further processing and dropped into hot water or subjected to steam or dry heat, sun exposure for 3 days or fumigation. In this way pupae or cocoons are killed. The killing of the cocoon in boiling water helps in softening the adhesion of the outer threads to separate freely, facilitating the unbinding of silk threads.

2. **Reeling and spinning:** The process of removing the threads from the killed cocoon is called as reeling. Four or five free ends of the threads of these cocoons are passed through

eyelets and guides to twist into one thread and wound round a large wheel from which it is transferred to spools. Thus, the silk obtained on the spool is called as raw silk or reeled silk. The waste outer layer or damaged cocoons and threads are separated, teased and then the filaments are spun. This spun silk is called as 'Spun silk'.

**1. Emergence of moth and fertilization:** When kept for emergence at room temperature, mass emergence of adults takes place. As per their nature, just after emergence, male moth starts moving around the female. Males are very much active whereas the females which are loaded with eggs are incapable of flying. If not separated at once in cages males start copulating with the females but the eggs obtained from this female mated from the male of the same stock is useless for the seed. So the males and the females just after emergence have to be separated into separate cages without their mating. Now one female of one lot is kept with the male of the other lot and at once they form pair and copulate for about 3 hours. After completion of mating, males should be separated and may be used for the fertilization of other females. But one male cannot fertilize more than two females. Now fertilized females are subjected to egg laying.

**2. Egg laying:** Just after fertilization, female starts egg laying and in duration of 24 hours it completes egg laying process. The eggs laid by one female are about 400 to 500 varying according to the different races. Female dies after egg laying. These eggs are called as SEED. These eggs are kept in sterilized trays and stored at 4°C under laboratory conditions or sometimes kept at hill stations in diapause conditions.

**3. Hatching:** This is an important phase of sericulture industry because as soon as the larvae are hatched they start feeding voraciously. So only those Sericulturists who would be able to supply sufficient amount of fresh mulberry leaves to the young hatched larvae, could perform successful sericulture programme otherwise young ones will die resulting great loss to sericulture industry. This is why the hatching has to be controlled, accelerated or postponed by artificial treatments under refrigerated conditions. For proper hatching of seeds(eggs) advanced techniques have been developed in which eggs are collected and kept with mulberry leaves, working as stimulant for hatching in shady places on white sheet of paper in insect proof trays on a stool. For this purpose the legs of stool must be kept in water so that insects may not crawl and damage the hatching eggs. It is also notable that if the eggs are placed in the same position in which they are laid, hatching will not be 100%. So it is advisable that the eggs kept in trays should be moved with the help of feather. The group of

caterpillars hatched at various stages should be kept separately. Thus, one should be careful that hatching must be coincided with the best season of the mulberry.

#### 4. Experimental data of egg laying and hatching:

If after 120 minutes of oviposition eggs are kept at 10°C for 24 hours then transferred to the oven at 16°C for 4 days and further soaked in hydrochloric acid (15% at 46°C) for 5 minutes.

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#### Diseases of Silkworm

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The Sericulture Industry suffers from a number of diseases in Tropical regions of South East Asia. The maggot disease, pebrine, polyhedrosis and flacherie are the diseases which cause severe damage to this industry. The poisoning by tobacco and Muscardines is also reported to be harmful but is not very common.

##### A. Maggot Disease

This disease is caused by *Tricholyga sorbillans*, a fly belonging to the order-Diptera and Family-Techinidae. It is distributed throughout India, Japan, Korea, China, Vietnam and Thailand. The presence of milky white cylindrical eggs on the skin of silkworm larvae is a symptom of this disease. The number of eggs laid by the fly on a silkworm larva varies from several to more than fifty but usually they are two to three. After 30-40 hours of egg laying, maggot makes a hole on the ventral side of egg shell and on the skin of the silkworm. Thus, maggot penetrates into the body of silkworm larvae and starts eating the tissues of larvae. When the maggot penetrates into the larval body, the big black mole is formed on that part of the skin. The segments in which maggot exists, swell up and bend as a result the attacked larva becomes inactive and loses appetite. Usually fourth and fifth stadia are attacked by the maggots. The larvae, attacked up to the fourth stadia die before making cocoon but usually do not attain the pupal stage.

##### B. Pebrine

This is one of the worst diseases of silkworm. Sericulture was once damaged by this disease in all the countries involved in this industry but some countries have overcome this disease and succeeded in getting pebrine-free-silkworm-eggs for reeling cocoons. *Nosema bombycis* Nageli, belonging to Microsporida, is the casual micro-organism of this disease. The infection takes place through the mouth of larvae at the time of feeding or through the mother's ovary. When new spores are formed in the tissues of alimentary canal of silkworm, they are discharged with the faeces and make a source of infection. When new spores are

formed in the tissues of alimentary canal of silkworm, they are discharged with the feces and make a source of infection. When the spore enters into the digestive tract of silkworm, two nuclei, contained in the sporoplasm, are divided into four nuclei and at the same time the polar filament projects and penetrates into the cells of the alimentary canal. Further, they enter into the blood and swim in it. They are distributed throughout the host body, attacking various tissues, specially the fat bodies and organs, excluding chitinous tissues and nucleus of cells. When the hypoderm is attacked and its cells die, the affected part becomes black due to the formation of melanin. Inside the body, the milky white spots or marks are observed on the silk gland or on the surface of the alimentary canal. In case of severe infection of the eggs the whole of yolk nearly gets filled with the micro-organisms resulting in their (eggs) death. Whereas, in case of slight infection the eggs hatch but the larvae carrying infection die at the third moult without making cocoon.

1. **Examination of mother moths:** All the mother moths producing eggs should be carefully examined one by one and eggs laid by healthy moths only be taken for further use. The unqualified eggs should be burnt away.

2. **Forecasting and correcting examination:** In order to make the pebrine examination more reliable, the forecasting and correcting examinations are carried out for the eggs of the seed cocoons.

The materials employed for forecasting examinations are the excrement of mature larvae, late moulting larvae, dead larvae, cocoons or pupae and moths accelerated to emerge out of cocoons. For the correcting examination a few eggs of each reproductive egg-batch are taken and incubated and thus, emerged larvae are used as the material for examination of pebrine germs.

3. **Removal and disinfection of pebrine germs:**

The spores of pebrine may survive for a number of years in an ordinary type of rearing room if the environmental condition is humid. Therefore, rearing rooms, tools and other utensils should previously be cleaned and washed to remove the pebrine diseased eggs, carcasses of infected larvae, pupae, moths and dead cocoons, faeces of diseased larvae and so on. The pebrine spores can be destroyed by treatment with 2% formalin for 30 minutes, 0.5% sublimate for 5 minutes, 5% chlorinated lime for 30 minutes, current steam for 30 minutes and sun shine in the summer for 7 hours.

4. **Care on rearing silkworms:** The tendency of the infestation by the pebrine spores has been observed to occur more when silkworms are reared in dry and cool conditions than in the hot and wet conditions. If the larval period becomes longer, the infection of pebrine is severe, so care should be taken on these points during the course of selection of the seed cocoons by the rearers.

### C. **Polyhedrosis in Silkworms**

Three types of polyhedrosis are found in silkworms:

- (1) Nuclear polyhedrosis.
- (2) Intestinal cytoplasmic polyhedrosis.
- (3) Intestinal nuclear polyhedrosis.

#### 1. **Nuclear polyhedrosis (Grasserie Jaundice):**

It is caused by a kind of virus which forms polyhedral in the nuclei of the cells of fatty tissues, dermal tissues, muscles, tracheal membranes, basement membrane, epithelial cells of midgut and blood corpuscles. The polyhedral are commonly hexagonal and rarely tetragonal in shape, containing large number of virus in them. The polyhedral enter the body fluid. The virus present inside the polyhedral maintains its pathogenic power for a number of years in the rearing room but the isolated virus loses its pathogenic power in a period of short time.

2. **Intestinal cytoplasmic polyhedrosis:** This type of polyhedral are formed in the cytoplasm of midgut cells but in a few cases they are formed in the goblet, too. The polyhedron of this disease contains plenty of viruses. The infected cells of midgut rupture and polyhedral are released into the gut. Thus, the faeces, excreted become whitish, containing plenty of polyhedra.
3. **Intestinal nuclear polyhedrosis:** In this disease the virus makes polyhedra inside the cytoplasm and nucleus of the midgut cylindrical cells. The polyhedral formed in this disease are large sized. The translucent cephalothorax, shrinkage of body and diarrhea are the symptoms of this disease also. The mode of infection and countermeasures are similar to those of nuclear polyhedrosis.

**D. Flacherie**

Flacherie is the genetic name of some kinds of silkworm diseases, carcasses of which rot due to the attack of bacteria. Flacherie may be divided as follows:

1. **Infectious flacherie caused by a kind of virus:** The various symptoms of flacherie are loss of appetite, translucent cephalothorax, vomiting and diarrhea but the real diagnosis can be made after the microscopic observation of the virus.

The pathogen of this disease is a spherical virus which does not form polyhedral in the body of silkworm larvae. The virus multiplies in the tissues of midgut and is released into the gastric juice and is excreted along with faeces which are the source of infection. The virus infects the larvae of silkworm orally.

**Countermeasures:**

- (1) Resistant silk worm races should be selected.
- (2) The rearing room, tools and utensils should be well disinfected.
- (3) In order to maintain the good health of silkworm, high quality mulberry leaves should be provided.
- (4) Favourable conditions like temperature and humidity should be maintained in rearing rooms.
- (5) The faeces, diseased larvae and dead bodies should be piled in the compost.
- (6) Some of the chemicals like hydrochloric acid, formalin, chlorinated lime may be used as disinfectants for this virus.

**2. Gastric injury caused by physiological disturbance of silkworms followed by the multiplication of bacteria:**

Due to the supply of bad quality of mulberry leaves the digestive physiology of the mulberry leaves the digestive physiology of the silkworm is disturbed and multiplication of bacteria in the gastric cavity takes place. Thus, the combined action of physiological disturbances and bacterial activity in the gut are major causes of this disease. In unfavorable climatic conditions, the bacteria like Streptococci sp. Coli aerogenous bacilli or proteus group bacilli attack the weakened silkworms.



The control measures against this disease are to keep healthy conditions of rearing silkworms.

**3. Bacterial intoxication:** This disease is caused by a toxin of some bacilli, *Bacillusthuringiensis* Var. The larvae attacked by this toxin become unconscious, later soften, become darkish and finally rot off. The infection occurs orally and can retain the toxicity as long as for seven years in some cases.

The countermeasures are the disinfection of the rearing room and instruments.

**4. Septicaemia:** This disease is caused by infection of some bacteria as *Bacillus megatherium*, *B. proteus*, *B. prodigiosus*, *B pyocyones* in the blood of silkworms. This disease is rear in the larval stage but it causes severe damage to the pupae and the moths during the period of egg production. The infection is caused through the wounds on the skin.

#### **E. Green Muscardine**

It is a fungal disease of silkworms. There are a number of muscardine in silkworm but only green muscardine (*Spicaria prasina*) has been noticed to affect the larvae of silkworm in Vietnam.

The infection may be observed at the third and fourth stadia of silkworm. In the beginning stage a big black spot is observed on the ventral side.

The green-tubes of fungus develop into mycelia in the blood and bear cylindrical spores which are separated from mycelia and further form mycelia which bear cylindrical spores again. Thus, all the organs of silkworm are attacked by this fungus disturbing their normal functioning. As a result the diseased larvae do not moult and finally die.

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## **6.6 REELING AND FIBER TECHNOLOGY**

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The process of removing the threads from the killed cocoon is called as reeling. Four or five free ends of the threads of these cocoons are passed through eyelets and guides to twist into one thread and wound round a large wheel from which it is transferred to spools. Thus, the silk obtained on the spool is called as raw silk or reeled silk. The waste outer layer or damaged cocoons and threads are separated, teased and then the filaments are spun. This spun silk is called as 'Spun silk'. The raw silk is further boiled, stretched and purified by acid or by fermentation and then carefully washed over again and again to brings about the well known luster on the thread.

The modernization of the reeling and spinning process by automation and various labour saving processes has opened a new way to this cottage industry in the world.

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**Life History of Mulberry Silkworm, *Bombyx mori***

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The adult of *Bombyx mori* is about 2.5 cm in length and pale creamy white in colour. Due to heavy body and feeble wings, flight is not possible by the female moth. This moth is unisexual in nature and does not feed during its very short life period of 2-3 days.

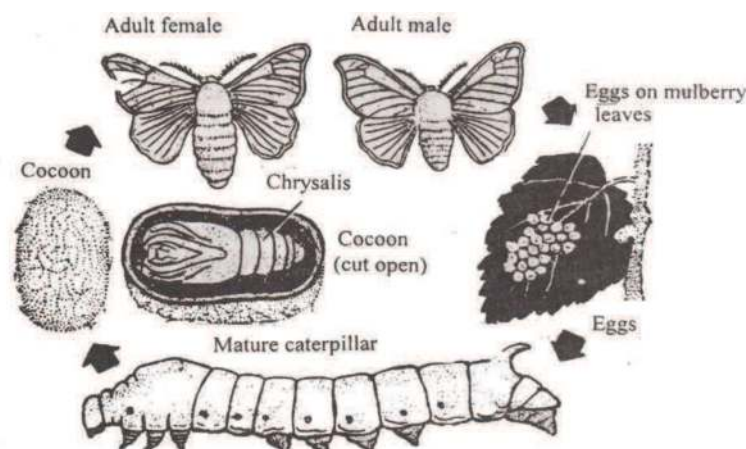
**Fertilization:** Fertilization is internal preceded by copulation. Just after emergence, male moth copulates with female for about 2-3 hours and if not separated they may die after few hours of copulating with female.

**Egg laying:** Just after copulation, female start egg laying which is completed in 1-24 hours. One moth lays 400 to 500 eggs depending upon the climatic condition and the supply of food material to the caterpillar from which the female moth is obtained. The egg laying is always in form of clusters and covered with gelatinous secretion of the female moth which helps them in proper attachment.

**Hatching:** The egg after ten days of incubation hatch into a larva called as caterpillar. Hatching is the most important phase of silk moth's life. After hatching, caterpillars need continuous supply of food because they are voracious feeders. If proper supply of mulberry leaf is not possible the development of caterpillar would not be in proper course. Sometimes, due to lack of food material, young caterpillars die causing great loss to the sericulture industry. It is recorded that in uni-voltine race hatching of eggs takes one month after laying.

**Caterpillar:** The newly hatched caterpillar is about 0.3 cm in length and is pale, yellowish-white in colour. The caterpillars are provided with well developed mandibulate type of mouth-parts adapted to feed easily on the mulberry leaves. The caterpillar is twelve segmented and the abdominal region has ten segments having five pairs of pseudo-legs. It is also provided with a small dorsal horn on the anal segment. Because of its being very much tender, the 1<sup>st</sup> instar larva can feed only on very soft leaves of mulberry plants. As they are voracious feeders, they grow rapidly which is marked by four moultings caterpillars get changed into 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> instars respectively. It takes about 21 and 25 days after hatching. The full grown caterpillar is 7.5 cm in length. It develops salivary glands, stops feeding and undergoes pupation. The time taken for the full growth of the caterpillar from

young to the well grown stage varies with regard to the temperature, humidity, food supply and type of race. Weight of the full grown caterpillar varies from 4 to 6gm.



*Fig 6.1 Life cycle of Silk worm*

**Pupa:** The caterpillars stop feeding and move towards corner among the leaves and secrete a sticky fluid through silk gland. The secreted fluid comes out through spinneret (a narrow pore situated on the hypopharynx) and takes the form of long fine thread of silk which hardens around the body of the caterpillar in the form of a covering called as Cocoon.

**Cocoon:** Cocoon is the white colored bed of the pupa whose outer threads are irregular while the inner threads are regular. The length of continuous thread secreted by a caterpillar for the formation of cocoon is about 1000-1200 meters' which requires 3 days to complete. The threads are wound around the cocoon in concentric manner. The binding of threads round the cocoon is very interesting and quick going phenomenon achieved by the constant round motion of the head of the caterpillar from one side to the other at the rate of 65 times per minute. Now the silkworm pupa is covered within a thick, oval white or yellow silken cocoon. It is estimated from the data obtained by practical application that one pound of silk can be obtained from 2500 cocoons.

## SILK

Silk is a pasty secretion of the silkworm produced by the silk gland. The silk gland is actually modified salivary glands which are long and sac like. As this pasty secretion comes in contact with air, it becomes hard and forms strong and pliable silk stands. This secretion forms two cores of fibroin: (i) a tough elastic insoluble protein consisting of 75% of the fiber's weight

and cemented together with sericin from the middle region of the silk gland at the time of secretion, and (ii) a gelatinous protein which is easily soluble in warm water. Some quantity of wax and carotenoid pigments are also detected. The diameter of the silk fibers is 0.0045 to 0.0082cm. Its elasticity is found to be 20%.

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### **Economic Importance of Silk**

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The raw silk is used in the manufacture of woven materials and the knitted fabrics for the preparation of garments, parachutes, parachute cords, fishing lines, sieve for flour mills, insulation coil for telephones and wireless receivers and tyres of racing cars. Fabrics for garments in various weaves, plain, twill, stain, crepe, georgette and velvet, knitted goods such as vests, gloves, socks, stockings, dyed and printed ornamented fabrics for saris, jackets, shawls and wrappers are made out of this material.

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### **Silk-Research Institutes**

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- (1) Central Sericultural Research and Training Institute (CSR and TI) Mysore (Karnataka).
- (2) Central Sericultural Research and Training Institute (CSR and TI) Berhampore (West Bengal).
- (3) Central Tasar Research and Training Institute (CSR and TI) Ranchi (Jharkhand).
- (4) Central Silk Technological Research Institute (CSTRI) Bangalore (Karnataka).

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### **Regional Silk-Research Stations**

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A number of Research Centers have been established in different parts of the country for carrying out research in mulberry, oak tasar, muga and eri silk:

#### **Mulberry Silk-Research Station**

1. Bangalore (Karnataka)
2. Chamaraj Nagar (Karnataka)
3. Salem (Tamilnadu)
4. Coonoor (Tamilnadu)
5. Kalimpong (West Bengal)
6. Dhule (Maharashtra)
7. Pampore (Kashmir)
8. Anantpur (Andhra Pradesh)

9. Jorhat (Assam)
10. Ranchi (Jharkhand)
11. Koraput (Orissa)
12. Mothabari (West Bengal)
13. Majra (Uttar Pradesh)

**Oak Tasar Silk Research Station**

- (1) Batote (Jammu and Kashmir)
- (2) Bhimtal (Uttar Pradesh)
- (3) Imphal (Manipur)

**Muga Silk Research Station**

- (1) Bako (Assam)

**Eri Silk Research Station**

- (1) Mendipather (Meghalaya).

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**6.7 TERMINAL QUESTION**

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Question 1. Give an account of the species of the Silkworm and describe the life history of *Bombyx mori*.

Question 2. Give a detail account of the of silkworm disease.

Question 3. Describe the rearing of mulberry silkworm.

Question 4. Write a short note on the cocoon processing in sericulture.

Question 5. Write a short note on the maggot disease of silkworm.

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## UNIT: 7 APICULTURE

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### ***CONTENTS***

7.1- Objectives

7.2- Introduction

7.3- Honey bees of India

7.4- Management of Bees colonies

7.5- Bees enemies & their control

7.6-Extraction & processing of honey

7.7-Role of Honey bees in pollination.

7.5- Summary

7.6- Self assessment question

7.7-References

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## **7.1 OBJECTIVES**

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- To provide the knowledge to the learners about Apiculture
- To promote the formation of Bee Improvement groups.
- To liaise with bee-keepers with a view to establishing bee improvement groups.
- To advise and encourage bee-keepers to promote our aims and objectives.

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## **7.2 INTRODUCTION**

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Beekeeping or apiculture is the maintenance of honey bee colonies, commonly in man-made hives, by humans. A beekeeper (or apiarist) keeps bees in order to collect their honey and other products that the hive produces (including bees wax, propolis, pollen, and royal jelly), to pollinate crops, or to produce bees for sale to other beekeepers. A location where bees are kept is called an apiary or "bee yard." Apiculture or beekeeping is the practice of maintaining honeybee colonies, usually in hives. This could be for collecting honey and beeswax, or for pollinating crops, or for the purpose of selling bees to other beekeepers. About 85 per cent crops plants are cross-pollinated, and colonies of honeybees, placed in the field when the crop is in flowering stage, can set about the needed pollination; abundance of pollinators helps in early setting of seeds, resulting in early and a more uniform crop yield.

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## **7.3 HONEY BEES OF INDIA**

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Honey bee belongs to the class **Insecta**, order **hymenoptera** and family **apidae**. There are five well recognized types of bees found in the world.

- (i) *Apis dorsata* (Rock bee)
- (ii) *Apis florea* (Little bee)
- (iii) *Apis indica* (Indian bee)
- (iv) *Apis mellifica* (European bee)
- (v) *Apis adamsoni* (African bee)

Out of these five types, three are common in India. They are

1. *Apis dorsata* (Rock bee)
2. *Apis florea* (Little bee)

### 3. *Apis indica*(Indian bee)

***Apis dorsata***: - which is commonly called as rock bee, is the largest Indian variety with an average size of about 20 mm. It builds large comb (0.90 x 15 metres) on tree branches, under caves, or under roofs of high buildings. They are migratory species as during June and July they swarm to the hills, but in winter come back to the plains. They have been found up to the height of 3,500 feet above sea level.

This variety is yet to be successfully hived. Researchers are going on, on the behaviour of this variety in order to domesticate them. This variety has the highest honey yield (average 15 kg. per colony per year) amongst Indian bees. Sometimes, the yield exceeds 30 kg. Per colony per year. This bee is notorious for its ferocity and tendency to make unprovoked, sometimes fatal, mass attack on persons who approach its hive.

***Apis floralae***: - which is commonly called as little bee, is a miniature of the rock bee. It is a plain species and rarely occurs above 1000 feet of sea level. It builds small comb (about 15.24 cm. across) on the branches of trees, or in bushes, or under the wall of the buildings. The yield of honey from this type is very little (few ounces per colony per year), and the production does not compensate the labour undergone on it.

***Apis Indica***: - Popularly known as Indian bee, is of commonest occurrence on the plains and forests of India. There are several regional strains of it, of which plain, transitional and hill varieties are three recognized types. Picea strain is found in hills at an altitude up to 7,000 ft, Pironi is distributed along the transition between altitudes of 3,000 to 4,000 ft. whereas, and Lighter indica is a plain strain found up to an altitude of 1,500 ft. It builds several parallel combs (about 30 cm across) in protected places like hollow of trees, caves, in rocks and in other such cavities. Due to their mild nature and average output of honey between 3 kg. to 5 kg. Per colony per year, they are amongst the best of the Indian variety to be hired in artificial conditions. *Apis mellifica* or European bee is very common all over the Europe. This bee is similar to *Apis indica* in its habitates. There are several varieties and strains of this bee amongst them the Italian variety is the best. It yields an average of 100 to 400 lbs. of honey per year per colony. Attempts to domesticate this been in India on large scale has yet not been proved to be a success.



The yield of honey from Indian bees is quite poor compared to their Italian or South African counter-parts. The South African yield of 100 kg. Per hive is about twelve times more than the Indian average of 4.5 kg. Per hive per year. Cross breeds have developed and experiments are going on at Palampur campus of the Punjab Agricultural University. The crosses between Indo- Italian swarms have yielded 51 kg. of honey per hive. But all these are in experimental conditions and at present we can only hope for a brighter future in the field of apiculture.

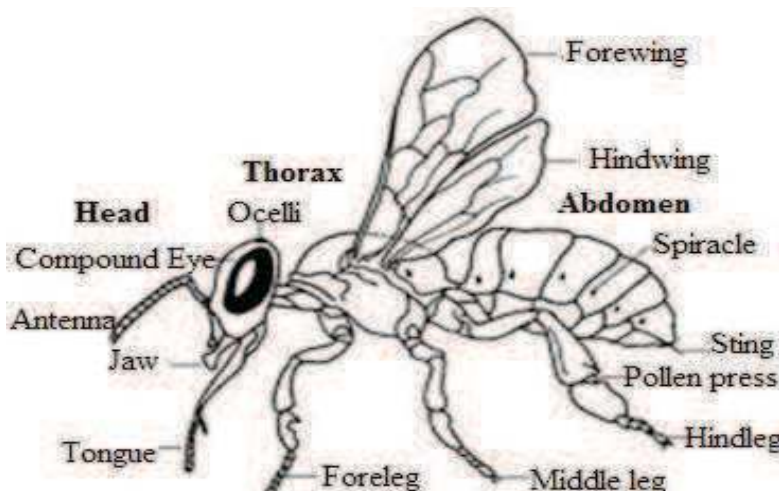


Fig.7.1 Anatomy of Honey bee

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### Habit and Habitat

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Honey bees are highly organized social insects reported from all over the world. Although they are active throughout the year but in winter season they do little work and do not rear the brood. In spring seasons i.e. at the time of flowering they prepare a strong colony with honey rich combs. They exhibit polymorphism and good division of labor. The bee hives with thousands of individuals are observed hanging down from the branches of the trees and ceilings of houses. The workers communicate information's for the location of the food sources through the 'Waggle Dance', a phenomenon called as 'Language of the bees', by the eminent biologist Kari Von Frish. He has mentioned that the rate of dance is directly proportional to the distance of the food.

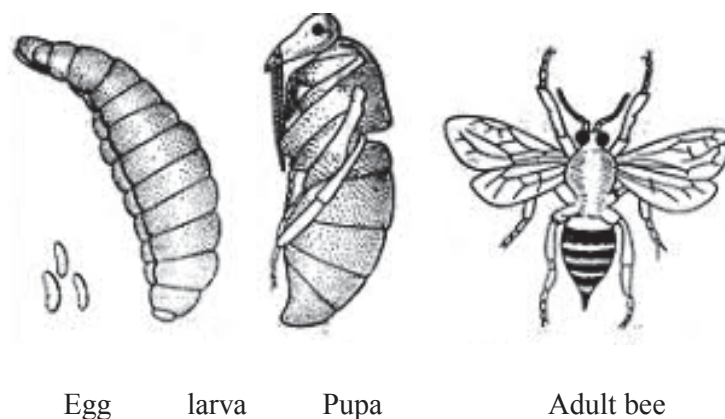
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### Life History

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After mating the queen generally lays one egg in one brood cell. The eggs are pinkish colored, elongated with cylindrical body generally attached to the bottom of the cell. Larvae

emerge out from both the fertilized as well as unfertilized eggs. Thus, the larvae from the unfertilized eggs form the drones while the workers are developed from the larvae of the fertilized eggs. Amongst the larvae of the workers one is fed on the royal jelly, a special diet secreted by the young workers in the colony and becomes the queen of the colony. The royal jelly consists of digested honey and pollen, mixed with a glandular secretion into the mouth of the workers. After 5 days of feeding the cell is sealed and the larvae undergo pupation. It spins a thin silken cocoon and pupates completely. Emergence of the young ones takes place after three weeks and they get busy in the indoor duties for about 2 to 3 weeks. Later on they are sent for the outdoor duties. All the bees pass through a complete metamorphosis with the various changes in the life-cycle taking place within the comb.



*Fig 7.2Life history of Apis Indica*

**Swarming:** The process of leaving off the colony by the queen is termed as swarming. It happens towards the end of spring or early summer but the real cause of swarming is still not well known. In summers when plenty of food is available and the hive is overcrowded by the bees, the queen leaves the hive on a fine fore-noon with some old drones and workers and establishes a new colony at some other place. Now in the old hive a worker is given Royal Jelly and is converted into a new queen of the colony. This new empress of the colony never tolerates her successor, as a natural law in the hive, so she orders to kill the other sisters, if any, in the hive.

**Supersedure:** When the egg laying capacity of the old queen is lost or it suddenly dies, a new young and vigorous queen takes the position of the old queen and is called as supersedure.

**Absconding:** The migration of the complete colony from one place to another takes place due to some unfavorable conditions of life, such as destruction of the comb by termites or wax-moths and scarcity of nectar producing flowers around the hive. This phenomenon is quite different from that of swarming.

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## ***7.4 MANAGEMENT OF BEES COLONIES***

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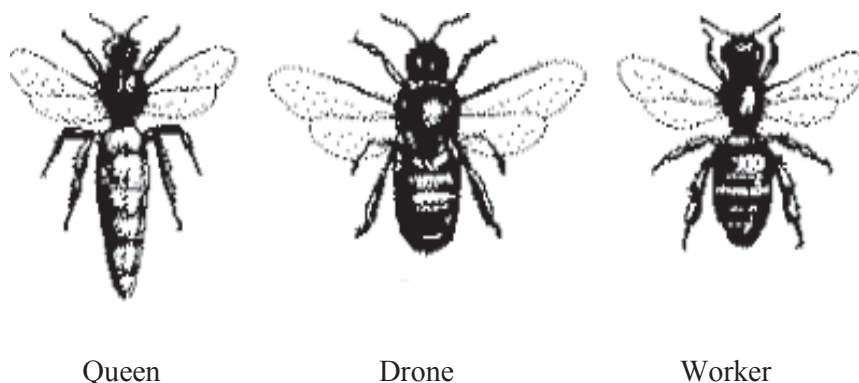
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### **Social Organization of Honey Bee**

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A highly organized division of labour is found in the colony of honey bees. A good and well developed colony of bees has 40 to 50 thousand individuals consisting of 3 castes viz., Queen, drones and worker. The queen lays fertilized and unfertilized eggs both. From unfertilized eggs male bees emerge which are known as Drones whereas from the fertilized eggs worker bee is produced. The workers when fed on Royal jelly, develop into Queen.

**Queen:** It is a well-developed fertile female provided with immensely developed ovaries. Commonly one queen is found to be present in each hive and feeds on Royal Jelly. She is the queen in real sense as the Mother of the Colony, guarded by a number of attendants and never allotted any duty except egg laying. Egg laying is the sole function of the queen throughout her active life span. The queen is 15 to 20mm in length and can be easily distinguished by her long tapering abdomen, short legs and wings. Structurally she is unable to produce wax or honey or gather pollen nectar. By the combination of ovipositor-cum sting, a structure is developed which aids in egg laying. It is said that the queen gets mated only once in her life but in a single chance of mating, drone releases 2 crore sperms which are sufficient for the fertilization of the eggs at the time of egg laying by the female throughout her life span. In recent researches in U.S.A it has been reported that out of 110 queens only 55 mated twice before egg laying. It is also a fact that queen lays fertile and unfertile eggs both in accordance to her will but the factors governing such selective activity are still not known. One queen lays about 1,500 and 2,000 eggs in a day depending upon the seasonal variation and other ecological factors.



*Fig. 7.3 Queen, Drone and Worker*

**Workers:** Although the workers are the smallest of the three castes but they function as the main spring of the complicated machinery like honey bee colony. Like the queen, they are also produced from the fertile eggs laid by the queen and live in a chamber called as 'WORKER CELL'. It takes 21 days in the development from the egg to the adult and the total life span of a worker is about 6 weeks. The workers are atrophid female which sacrifice themselves for the well-being of the colony. The total indoor and outdoor duties of the colony are performed by the workers only. That is why they are provided with some special structures for particular work.

- (1) Long proboscis for sucking the nectar.
- (2) Strong wings for fanning.
- (3) Pollen baskets for the collection of pollen.
- (4) Powerful sting to defend the colony against any attack.
- (5) Wax gland for wax secretion.

The workers which are engaged in outdoor duties collect the nectar, pollen, gum and water which are received and stored properly by the house bees. The Indoor workers are further sub-grouped for specific duties. Some of them which are very sincere attend the queen while some others look after the nursery called as NURSERY BEE. Some produce wax for the formation of the new hive and are known as BUILDERS. The repairing of the comb is done by the REPAIRERS. The dead body and other impurities are removed from the hive by the CLEANERS. The fanning in the hive is performed by the wings of the FANNERS. Several other functions like honey storage and ripening are also done by the workers. The guard bee

always watches at the gateway. It is said that up to half of their life period workers perform indoor duties and later on become engaged in outdoor duties.

**Drone:** The drone is the male member of the honey bee colony which fertilizes the queen so called as King of the colony. They take 24 days to develop from the egg to the adult stage. The sting and the wax glands are absent but in the males the reproductive organs are very well developed. They are reared from an unfertile egg in large DRONE CELLS. Drones are totally dependent on the workers and have been seen begging for honey from the workers. The sole duty of the drone is to fertilize the virgin queen. At the time of swarming the drone follows the queen, copulates and dies after copulation.

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## 7.5 BEES ENEMIES & THEIR CONTROL

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Enemies of the bees harm the colony in different ways so they have attracted considerable attention in the different region of the country. The wax moth *G.mellonella* & *A.grisella*, wasp (*vespa spp.& palarus sp.*) black ants *componotus compressus* and bees eaters etc, are the common enemies of the honey bees comb and honey.

Enemies of Honeybees:-

### 1. Wax Moth (*Galleria mellonella*):

It is one of the most important enemies of the bee colony causing serious damage particularly to weak colonies. The caterpillars live in the silken tunnels made by the bees, feed on the propolis, pollen, and wax in the combs. The presence of loose dislodged particles in the hive is the first symptom of attacks. When the infestation is serious, the comb is seen covered with silken webs with the numerous black faecal particles of the caterpillars. The insect can be controlled by frequent examination of hive, cleaning all the crevices and removing all debris.

### 2. Ants:

The black ants and red ants are dangerous enemies of the bee. They attack weak colonies and carry away the honey, pollen and the brood. By providing ant pans around the bases of the stand or oil bands over the stands ants can keep away.

### 3. Wasp:

It waits near the entrance of the hive; catches bees as they come out, macerate them for feeding the juice to its young. By reducing the width of the alighting board of the hive, the wasps can be prevented from sitting near the entrance. The wax beetle, birds and deaths head moth are also other enemies of honeybees.

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## 7.6 EXTRACTION & PROCESSING OF HONEY

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### Selection of Bees for Apiculture:-

For running a good apiary, selection of honey bee is of much importance, so the following should be kept in mind at the time of selecting honey bees for apiculture:

- (1) Honey bees should be of gentle temperament.
- (2) Honey bees should have capability to construct strong colony.
- (3) It should have ability to protect from enemies.
- (4) Honey bee should have energetic and industrious workers.
- (5) Workers can suck juice from numerous varieties of plants.
- (6) Bees on the whole can produce more and more honey from its comb.
- (7) Bees can form their comb easily at any place.

The apiculture scientists engaged in genetics are trying to find out such cross races which would not be of ferocious nature but be a good honey producer in India; *Apis Indica* is the best bee for apiculture industries due to its gentle nature and having efficient and prolific workers.

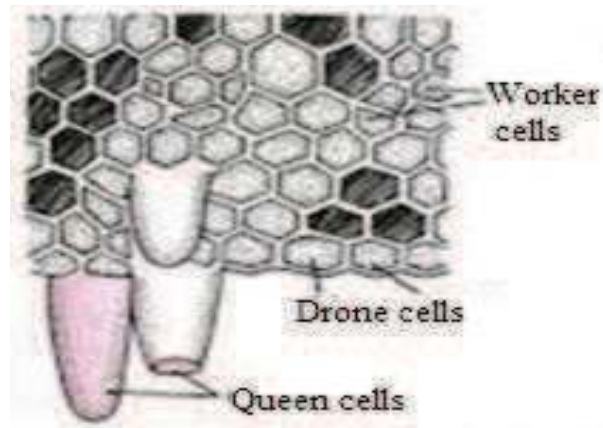
### METHODS OF BEE KEEPING:-

The ultimate aim of bee keeping is to get more and more honey in pure form. The old method commonly used by old apiculturists is very crude, cruel and of unplanned type. This old method is called as indigenous method.

### Indigenous Method:-

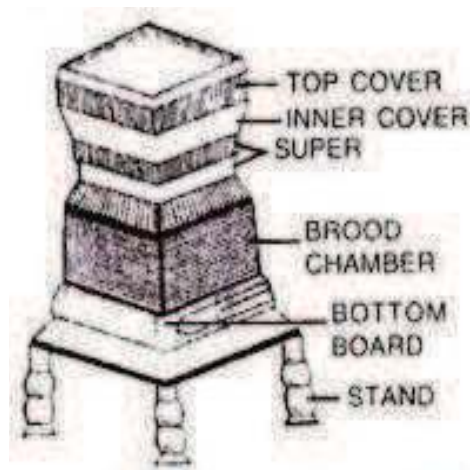
1. **Hive:** Two types of hives are used in indigenous method of bee keeping e.g., wall or fixed hive and movable hive.

- (a) **Wall or fixed hive:** It is purely natural type of comb because the bees themselves prepare the hive at any space on the wall or trees. There is an opening on one side through which bees come out of the hive.



*Fig.7.4 Hive of Apis Indica showing various cells*

- (b) **Movable hive:** It comprises of hollow wood logs, empty boxes and earthen pots etc. placed in verandas of houses. There exist two holes; one is for entrance and the other for exit of the bees. The swarmed bees usually come to the box on their own accord. Some bee keepers use to take the clusters of the swarms from a tree and keep them in the hive.



*Fig.7.5 A Movable hive*



**2. Extractive of honey:** For honey extraction, burning fire is brought near the bee hive at the night as a result of which bees are either killed or they escape off. Further the hive full of honey is being removed, cut into pieces and squeezed to get honey. Sometimes smoking is done so that the bees may escape from their hives.

**3. Drawbacks of indigenous method:** The indigenous method of bee keeping suffers from a number of drawbacks due to which it is not recommended by present day panel. These drawbacks are: (i) Honey become impure because at the time of squeezing, the brood cells, pollen cells, honey cell and larvae are also extractes. (ii) The colony becomes weak due to killing of the eggs and the larvae at the time of squeezing. (iii) Formation of new hive by the escaped bees requires extra energy which affects the yield. (iv) The activities of the bees can be controlled. (v) The hivation of bees on the same place is only matter of chance. (vi) The honey robbers, like, rat, ant, wasp and monkeys may affect the hive easily. (vii) The race improvement programme may not be applied, so no possibility for the selection of the best is there. (viii) The hazards created by climatic factors cannot be controlled.

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### **Modern Method of Apiculture**

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To overcome the drawbacks of indigenous method an advanced method based on scientific facts has been developed. It has opened a new era for the cottage industry in India and has also given an opportunity for lakhs of unemployed persons to keep them busy in this business. From this cottage industry programme the routine agricultural work may not suffer. First of all care was taken to improve the texture of the hives and during this race hive patterns were introduced in India. The Newton model with 7 to 10 frames ( $21 \times 14.5$  cm) in the brood chamber with a shallow super ( $21 \times 6.5$  cm sized frames) has been most popular in south, east and central India. Longstroth hive containing 10 frames ( $44.8 \times 23$  cm) has been used as a standard hive in Himachal Pradesh, Jammu and Kashmir and Punjab. In Uttar Pradesh another type of hive has been in use which was evolved at Jyolikote (Nainital) apiary and contained 8 frames ( $30 \times 18$  cm). After gaining experience from the above mentioned hives, Indian Standard Institute has standardized the hives of small and big sizes accommodating frames  $21 \times 14.5$  cm and  $31 \times 20.4$  cm respectively.

Now-a-days a typical type of movable hive is constructed which is capable of expansion or contraction according to the requirement of the place, season and climatic conditions.



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**Advantages of Modern Method**

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In the modern method of bee keeping there are several advantages which encourage the well planned bee keeping.

- (1) A proper watch on the activities of the bees can be had.
- (2) A strong colony can be developed by providing sugar, syrup, and pollen substances to honey bees.
- (3) Swarming of bees is checked by modern hive.
- (4) The same hive is used again and again so the workers pay their attention more for the honey and not for the hive formation.
- (5) Under adverse climatic conditions the hive can be transferred from one place to the other for the protection of the bees.
- (6) Comb can be protected from the enemies.
- (7) Pure honey in large quantity can be obtained.

**Precautions:** For the proper management of bee keeping programme following precautions should be taken.

- (1) The hive should not be kept more than half a mile away from the place from where the bees have to collect the nectar and the pollen.
- (2) People must know about the bee keeper for proper contact.
- (3) The boxes must be kept under shade at cool places.
- (4) Industry should be near the road for proper transport facilities.
- (5) Fresh water reservoir should be near the hive.
- (6) Good flora for the collection of pollen and nectar should be there.

**Products of Bee Keeping**

The chief products of bee keeping industry are

1. Honey and
2. Bee wax.

**Honey**

It is truly an insect product of high nutritive value. The food value of honey may be estimated by the presence of about 80% sugar in it.

**Production of honey:** One should not be confused that honey is a direct plant product because the nectar, pollen and cane-sugar bearing secretions of flowers are ingested by honey bees, get mixed with the saliva and undergo certain chemical changes due to enzyme action. At this stage cane-sugar (sucrose) is converted into invert sugars i.e., dextrose and levulose. At this very time some ingredients of bees are also added to the mixture and reduce the water content. The whole mixture is then collected in the honey sac (crop) until the honey reaches the hive. As the honey bee reaches the hive this compound is regurgitated in the hive cell and is known as the honey. Now honey is concentrated by a strong current of air produced by the rapid beating of worker's wings, crawling over the cells.

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### ***7.7 ROLE OF HONEY BEES IN POLLINATION***

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Since the **honey bee** is the most important insect that transfers pollen between flowers and between plants, the word "**pollination**" is often used to describe the service of providing **bees** to **pollinate** crop plants. Pollination is the transfer of pollen grains, the male sex cells of a flower, from the anther where they are produced to the receptive surface, or stigma, of the female organ of a flower. Since the honey bee is the most important insect that transfers pollen between flowers and between plants, the word "pollination" is often used to describe the service of providing bees to pollinate crop plants. This service is now more important than ever in the Midwest because the acreage of insect pollinated crops is large as compared with the number of all kinds of bees (honey bees, humble bees, and solitary bees) that are available to provide pollination. In many states the estimated number of colonies (hives) of bees has dropped drastically in recent years. For example, in Illinois the estimated number of hives dropped from 101,000 in 1964 to 46,000 in 1984. These two figures are probably much more accurate than some of the older, larger estimates that may have reflected state pride more than reality. Because of the reduction in numbers of bees, growers in any state can no longer assume that there are sufficient numbers of bees nearby to produce the best possible crop from insect pollinated plants.



*Fig.7.6 Honey bee hive placed in Apple Orchard*

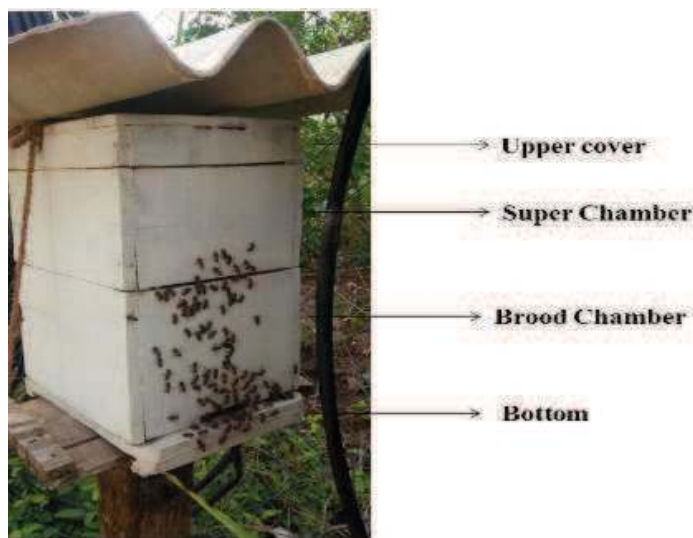
Honey bees are good pollinators for many reasons. Their hairy bodies trap pollen and carry it between flowers. The bees require large quantities of nectar and pollen to rear their young, and they visit flowers regularly in large numbers to obtain these foods. In doing so, they concentrate on one species of plant at a time and serve as good pollinators for this reason. Their body size enables them to pollinate flowers of many different shapes and sizes. The pollination potential of the bees is increased because they can be managed to develop high populations. The number of colonies can also be increased as needed and the colonies can be moved to the most desirable location for pollination purposes.

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## Hive

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The house of honey bees is termed as hive or comb. It consists of hexagonal cells made up of wax secreted by the worker's abdomen. These hives are hanging vertically from rock, building or branches of trees. Each hive has thousands of hexagonal thin walled fragile cells arranged in two opposite rows on a common base. The resins and gums secreted from the plants are used for the repairing of the hives. The young stages are generally occupying the lower and central cells in the hive which are the 'BROOD CELLS'. In *A. dorsata* brood cells are similar in shape and size but in other species brood cells are of three types viz., WORKER CELL for workers, DRONE CELL for drones and QUEEN CELL for the queen. Queen cell cannot be used again while the rest are used a number of times. There are no special cells for lodging the adults which generally keep clustering or moving about on the surface of the comb. The cells are mainly intended for



*Fig.7.7 Hive of bee*

the storage of honey and pollen especially in the upper portion of the comb while those in lower part are for brood rearing.

### **Flora for apiculture**

Although honey bees can collect nectar and pollen from quite a long distance but the flora for apiculture is also important. The flora may be of wild or cultivated type. The more nectar yielding plants are neem, jamun, soapnutect.. The other plants like maize, rose and sorghum are good sources of pollen. Some plants like plum, cherry, apple, sheesham, coconut, guava, mustard etc., are good sources for nectar and pollen both.

### **Chemical composition of honey:**

Honey is sugar rich compound having the following constituents:

(1) Levulose	-38.9%
(2) Dextrose	-21.28%
(3) Maltose & other sugars	-8.81%
(4) Enzymes & pigments	-221%
(5) Ash	-1.0%
(6) Water	-17.20%

**Storage of honey:** After long duration in the stored condition, the honey may be granulated and fermented.

- (a) *Granulation of honey:* The stored honey becomes granular after long duration. Such type of granulation property is the best evidence of pure honey. It is considered that 10 parts of dextrose combine with one part of water, hence forms crystals. Due to less solubility levulose is not crystallized and gives cloudy appearance. Crystallization is mainly accelerated by the presence of minute air bubbles, colloids and pollens.
- (b) *Fermentation of honey:* After crystallization honey is subjected to fermentation. Due to crystallization of dextrose 9% moisture is released, which dilutes the remaining levulose of the honey and the action of yeasts present in air, flowers and soils takes place on levulose and dextrose resulting in the fermentation of honey.



**Economic Importance of honey:** Honey is used by human beings in different ways of which the most important is as food and medicine.

- (a) *Food value:* It is estimated that 200gm of honey provides as much nourishment as 11.5 liter of milk of 1.6kg cream or 330gm meat. 2.1gm of honey provide as much as 67K. cal of energy. Its sugars, minerals, vitamins and other vital elements are readily absorbed by the systems. Honey may be taken by healthy men as well as those who are ill. It can be taken at any time any season and by persons of all ages even those just born. It is used in the preparation of candles, cakes and bread. In illness it is preferred over milk because more than half of the body energy is provided burning of dextrose.
- (b) *Medicinal value:* Honey is mildly laxative, antiseptic and sedative, generally used in Ayurvedic and Unani systems of medicine. It is quite helpful in building up of the hemoglobin of the blood and also used as preventive against cough, cold and fever, as blood purifier and as a curative for ulcers on tongue and alimentary canal. Its regular use is recommended after severe cases of heart attack for malnutrition, indigestion and diabetes. It is also found that typhoid germs are killed by honey within 48 hours, those of branchio-pneumonia in 4 days and of dysentery in 50 hours.
- (c) *Other uses:* Other than food and medicine, honey is used in numerous ways. It is used in the preparation of bread, cake and biscuits. It enhances their preserving quality. Much amount of honey goes in making alcoholic drinks. In poultry and fishing

industries honey is widely used. In laboratory, honey is used to stimulate the growth of plants, the bacterial culture, and inoculation of seeds of cloves, in insect diet and in the preparation of poison baits for fruit flies.

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## **7.5 SUMMARY**

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Honey bees are highly organized social insects reported from all over the world. Although they are active throughout the year but in winter season they do little work and do not rear the brood. In spring seasons i.e. at the time of flowering they prepare a strong colony with honey rich combs. They exhibit polymorphism and good division of labor. Beeswax is a very useful by-product of bee keeping industry. It is yellowish to grayish brown in colour and insoluble in water but completely soluble in ether. Commonly it is a wrong impression to suppose that honey bees convert the pollen into beeswax because beeswax is also a natural secretion of the worker bees and is poured out in thin delicate scales of flakes. The various beeswaxes differ only due to change in the proportions of these constituents. Large quantities of beeswax produced and exported, come from *Apis dorsata* bees, Indian Standard Institutions have fixed standards for pure beeswax in order to facilitate its export. Beeswax is used in the manufacture of cosmetics, for Catholic churches, face cream, paints, ointments, insulators, plastic works, polishes, carbon paper and many other lubricants. It is also used in the laboratory for microtomy with the common wax for block preparation of tissues.

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## **7.7 SELF ASSESSMENT QUESTION**

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- Give an account of the social organization and life history of the honey bees?
- Describe the method of Bees Keeping?
- Explain the bee's enemies?
- Give the short account of the species of honey bees?
- What is beeswax?
- Give the economic importance of honey bees?

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## ***7.8 REFERENCES***

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## UNIT-8 LAC CULTURE

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### CONTENT

8.1- Objectives

8.2-Introduction

8.3 Different kinds of Lac producing Insect

8.3. 1 Host Plant

8.3.2 Life cycle of Lac insect

8.6-Terminal question

8.7-References



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## 8.1 OBJECTIVES

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The study of Different kinds of Lac producing Insect and Host Plant and discuss the Life cycle of Lac insect and Disease of Lac insect.

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## 8.2 INTRODUCTION

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Lac is the scarlet resinous secretion of a number of species of lac insects, of which the most commonly cultivated species is *Kerria lacca*. Cultivation begins when a farmer gets a stick (broodlac) that contains eggs ready to hatch and ties it to the tree to be infested. Thousands of lac insects colonize the branches of the host trees and secrete the resinous pigment. The coated branches of the host trees are cut and harvested as sticklac. The harvested sticklac is crushed and sieved to remove impurities. The sieved material is then repeatedly washed to remove insect parts and other soluble material. The resulting product is known as seedlac. The prefix seed refers to its pellet shape. Seedlac which still contains 3–5% impurities is processed into shellac by heat treatment or solvent extraction.

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## 8.3 DIFFERENT KINDS OF LAC PRODUCING INSECT

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Lac insect (*Tachardialacca*) previously known as *Laci ferlacca* is a minute, resinous, crawling scale-insect which inserts its beak into plant tissues, sucks juices, grows and secretes lac from the hind end of the body. Its own body ultimately gets covered with lac in the 'Cell'. lac is actually secreted for its protection and not for the food of the insect. The commercial lac is produced in large quantities by female as a protective covering of its body which is injurious to the host plants.

### Classification

Lac----- Insect (lakh ka-kira)

Phylum -----Arthropoda

Class -----Insecta

Order-----Hemiptera

Genus -----Tachardia

Species -----lacca

**Male:** male is red in colour and 1.2 to 1.5 mm in length. It secretes bright creamy lac. It has reduced eyes and ten segmented antennae. The mouth-parts are of piercing and sucking type.

Thorax bears three pairs of legs and one pair of hyaline wings. The abdomen is eight segmented and terminates into a short, chitinous prominent genital sheath containing penis. On either side of this genital sheath a white elongated caudal seta is found.

**Female:** Female is larger than males and measures about 4 to 5 mm in length. The pyriform body of the female is enclosed in a resinous cell. The head, thorax and abdomen are not clearly distinct. The mouth-parts are of piecing and sucking type. The antennae are clearly visible and degenerated. The posterior end of the body has a median and two lateral processes. The legs are in degenerated form.

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### 8.3.1 HOST PLANT

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The lac insects have more than one host plant. The selection of suitable host plant for the cultivation of lac is of much importance. To establish the lac industry one should know well about the topographic and climatic conditions for the growth of host plants suitable for the particular region. Brun (1958) has mentioned that 113 varieties of host plants are found in the geographical Indian regions including Pakistan and Myanmar. Out of these 113 host plants only 14 are very common in India which is as follows:

- Kusum – *Schleichera oleosa*
- Babul – *Acacia nilotica*
- Ber – *Zizphus mauritanias*
- Palas – *Butea monosperma*
- Ghont- *Zizphus xylopyra*
- Khair – *Acacia catechu*
- Peepal – *Ficus religiosa*
- Gular – *F. glomeratu*
- Pakapi – *F. virens*
- Putkal- *F. globella*
- Mango- *Mangifera indica*
- Sal – *Shorea robusta*
- Shisham- *Dalbergia sisso*
- Fig- *Ficus carica*

The quality of lac is directly related with the quality of host plant. So far, no artificial product has been able to replace the lac. Khair, Kusum and Babul give better quality of lac when

sown directly in the field. But Palas, Ber and Ghont give good crop when they are first sown in nursery and then transplanted to the lac growing field. Palas and Ber produce a particular type of lac which is called as 'Kusumi lac'.

### **CULTIVATION OF LAC:-**

Lac cultivation is a complicated process, so the cultivators should know well about the inoculation, swarming period and harvesting of lac.

#### **Inoculation:-**

The first procedure in the lac cultivation is the inoculation of lac insect. Inoculation is the process by which young one gets associated properly with the host plant. Inoculation is of two types:

1. **Natural inoculation:** The inoculation taking place in natural way is very simple and common process during which the swarmed nymphs infect the same host plant again and start to suck the juices from the twigs. The natural incubation of swarmed nymphs has some drawbacks which are as follows:
  - (a) **Incomplete nutrition:** Lac insects with their piercing and sucking mouth-parts, pierce into succulent twigs and suck the cell sap of the same host plant for nutrition. If the cell sap of the same host plant is further sucked out by the swarmed nymphs of the second crop continuously, the growth of the host plant would be retarded. In this way lac insect may not be able to get enough nutrients from the same host plant. The lac insects due to lack of sufficient nutrients lose their proper development, thereby affecting the production of lac also.
  - (b) **Irregular inoculation:** During the natural inoculation it is not sure that uniform sequence of inoculation takes place. If inoculation is not of continuous fashion, a regular crop of lac may not be obtained.
  - (c) **Unfavorable climatic conditions:** At the time of swarming a number of factors like high intensity of sunlight, heavy rainfall, flow of wind etc. affect the proper inoculation of nymphs. These natural environmental factors may also affect the host plant at the same time and may cause a gap of inoculation resulting in irregularity of the lac crop.
2. **Artificial inoculation:** The main idea behind the artificial method of inoculation is to check all possible drawbacks of natural inoculation.

In this method first of all host plant should be pruned in January or June. The twigs bearing insect nymphs which are about to swarm or just before swarming are cut in sizes ranging between 20 to 30cm in length. Then the cut pieces of these twigs are tied to fresh trees in such a way that each stick touches the tender branch of the tree at several places which form bridges for the migration of the nymphs. After swarming, these twigs should be removed and separated from the host plant. The following precautions should be taken in artificial inoculation:

- (1) One must ensure that the twigs, which are going to be tied on fresh host plant, are having good number of nymphs or eggs. It is also possible that from many of the twigs nymphs have swarmed out, thus inoculation would prove unsuccessful.
- (2) The twigs provided with eggs or nymphs should be without any parasite and predator.
- (3) The eggs or nymphs present on the twigs should be healthy and about to swarm so that one has not to wait for longer period and thus save time.
- (4) For the uniformity of inoculation, 3 to 4 twigs should be utilized.
- (5) Host plants should be changed from time to time for the proper nutrition of the nymphs.

These insects are very small and if they move to a long distance there are chances of mortality of the nymphs. Due to maximum contact of twigs, swarming nymphs have not to move for long distance and find suitable places to establish on the host plant.

### **Inoculation Period**

In India two types of crops viz., Rangini and Kusumi are grown in a year. The Rangini crop is of two types called as Kartiki and Baisakhi crop which produce Kartiki and Baisakhi lac respectively. The Kusumi crop is also of two types viz., Agahani and Jethi which produce Agahani and Jethi lac respectively.

Thus, the inoculation periods of all the four types of crops are different. The inoculations of Kartiki, Baisakhi, Agahani and Jethi crops are recommended in months of June to July, October to November, July and January to February respectively. But if continuously four crops are taken, the plant would not get any rest which may cause less production of lac.

### **Swarming**

It is very important phase in the life history of lac insect. We should have accurate knowledge about the actual date of the swarming. At the time of swarming, the upper surface has yellow spot on the anal region. At this stage muscle contracts and insect gets detached from the place of attachment. Thus, it leaves a hollow cavity which later on gets covered with wax also. When these eggs are to be hatched out they become orange colored. It is an indication that swarming has taken place. Thus, by trials and learning methods i.e., by practice one could know about the exact date of swarming by looking at the colour of the eggs.

### **Harvesting of Lac**

The process of collection of ready lac from host tree is known as harvesting. In common practice the harvesting is of two types.

1. **Immature harvesting:** The harvesting of the lac before swarming is called as immature type of harvesting and the lac thus obtained is known as 'ARI LAC'.
2. **Mature harvesting:** The collection of crop after the swarming is called as mature harvesting and the lac obtained is known as 'MATURE LAC'. The harvesting of lac before the swarming has some drawbacks because the lac insects may be damaged at the time of harvesting which would affect the population of lac insects and ultimately result in great economic loss to the cultivators. But in case of palas lac (Rangini lac) it is found that Ari lac gives better production. Therefore, Ari lac harvesting is recommended in case of palas only. In all other cases immature harvesting should be discouraged. It is also found that in cold areas mature crop yields quality of lac.

### **Harvesting period:**

The harvesting periods of different crops are quite different in accordance with the inoculation of crops. kartiki crop is harvested in October to November whereas, Baisakhi crop in May and June. The other crops like Agahani and jethi are harvested in January to February and June to July respectively.

### **Composition of Lac**

Lac is a complex substance having large amount of resins, together with sugar, water and other alkaline substance. The percentage of various constituents is as given below.

(1) Resin	- 68 to 90%
(2) Dye	-2 to 10%

- |                       |              |
|-----------------------|--------------|
| (3) Wax               | -6%          |
| (4) Albuminous matter | -5 to 10%    |
| (5) Mineral matter    | -3 to 7% and |
| (6) Water             | -3%          |

### Properties of Lac

- (1) Lac is not soluble in water but easily soluble in alcohol. This property of lac has great value for insulation of electrical connections.
- (2) Lac is easily fusible on heating.
- (3) Lac has adhesive quality.
- (4) It has binding property when mixed with alcohol.
- (5) lac is also soluble in weak alkali like ammonia.
- (6) Lac is a bad conductor of heat.

### ENEMIES OF LAC CULTIVATION

Lac cultivation is destroyed by biotic and abiotic factors:

1. **A biotic enemies:** These are high intensity of light, high temperature, high humidity, heavy rainfall and flow of wind.
2. **Biotic enemies:** The main biotic enemies of lac cultivation are mammals and insects, Krishnaswami et al. (1957,59), and Gepulpure et al.(1963), have reported that squirrel, rats and monkeys cause great damage to the lac crop.  
The insects are very powerful enemies of lac crop. Annual loss due to the insect enemies is to the tune of about four lakh maunds. The insects damage the crops in different ways.
3. **Parasites:** The lac insects are parasitized by eight species of chalcidoid parasites like, *Parenthrodryinusclavicornis*, *Erencyrtusdewitzil*, *Tachardi-aephagustachardiae*, *Tachardiaephagustachardiae* var. *somervilli*, *Eupelmustachardiae*, *Coccophagustschirchil*, *Marietta javensis* and *Tetrastichuspurpureus*. These parasites lay their eggs into lac insects and parasitised 4.8 to 9.9 % of lac insects per year and 1/3 of the parasitised cells are males. Thus, it may be concluded that parasitization is not a major cause of the damage to the lac cultivation.
4. **Predators:** Predators cause very severe damage to lac cultivation and 35% of the lac cells are damaged by two predators viz., *Eublemaamabilis* Moore (Lepidoptera:

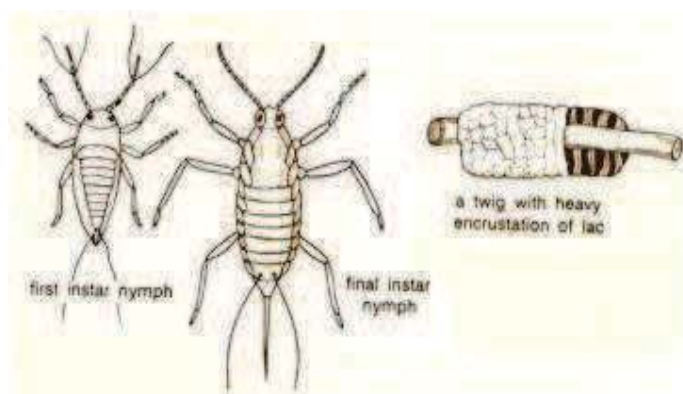
Noctuidac) and *Holocerapulverea*Meyr (Lepidoptera: Blastobasidae). Female lays eggs near encrusted twings from larva emerge and feed on lac insects.

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### 8.3.2 LIFE CYCLE OF LAC INSECT

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Each mature female just after fertilization lays about 200 to 500 eggs in a cell in which she is enclosed. The oviposition takes place into the incubating chamber which is formed by the contraction of the body of the female in forward direction inside the lac cell. The eggs are laid in the months of October and November. After six weeks of lying, the eggs are hatched into first instar aymphs in the month of November and December. When nymphs emerge they are in quite large number. This mass emergence of the nymphs is known as 'SWARMING'



*Fig.8.1 Life cycle of Lac Insect*

**Nymph:** At the time of emergence the nymphs are about 0.5 mm in length, red coloured and boat-shaped. The head bears paired antennae, ocelli and ventrally situated piercing and sucking type of mouth-parts. The mouth-parts are provided with proboscis. The tree segmented well developed thorax contains two pairs of spiracles and only one pair of walking legs. The abdomen contains two pairs of legs and terminates into a pair of long caudal setae. The active nymphs can crawl to a considerable distance so, just after emergence they start moving in search of food and reach their host plants, preferably on young and succulent shoots because the young nymphs are unable to settle very close to each other on the twig of the host plant which further collapses completely and forms a continuous covering even on the lower surface of the twing. The number of nymphs that settle per square inch area is about 150 to 200. Settled nymphs suck the sap form the twing of the host plant and start to secrete

the resinous substance by special dermal glands which are located all over the body. As the resinous secretion comes in contact with air, it soon becomes hard and forms a coating over the body of nymph and is called as 'CELL'. Within this cell various life processes like growth of the nymph, morphological changes and lac secretion take place.

The male 'CELL' is elongated and cigar-shaped having two holes i.e., anterior and posterior. From the posterior hole which is covered by a flap or operculum, the male insect comes out by pushing open the operculum. After six to eight weeks of stationary life the nymphs are metamorphosed as a result of which some (30%) active winged males and maximum (70%) emerge in the form of females which are wingless. The females get fixed on the host plant in resinous mass.

Due to short life period males do not take major part in the secretion of lac but female secretes lac throughout her life and its life span is longer than males. Major quantity of lac is secreted from females. The life cycle period depends mainly on ecological factors of the region.

### **Economic Importance**

In 19<sup>th</sup> century lac dye was in more use than lac resin. Presently due to availability of a better and cheaper annaline dyes the use of lac as a dye has been discarded. The manifest uses of lac is one of the Nature's standing gifts. The various used to which it is put are:

- (1) It is utilized in the preparation of gramophone records. Previously this industry utilized major part of the lac produced annually. But now days to a great extent plastic is being used in this trade.
- (2) It is of utility to Jewellers and Goldsmiths who use lac a filling material in the hollows in gold ornaments like bracelets, armlets, bangles and necklaces etc.
- (3) It is an essential gradient used extensively for making polishes, paints and varnishes for finishing wooden as well as metal furnitures and doors etc.
- (4) It is utilized for the preparation of toys, buttons, in pottery and artificial leather.
- (5) It is used in the manufacture of photographic material, lithographic ink and for stiffening felt and hat materials.
- (6) It is used as an insulating material for electrical goods.

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## ***8.6 TERMINAL QUESTION***

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1. Discuss on the host plant of Lac Insect?
2. Describe the natural inoculation of Lac Insect
3. Give a detail account of the life history of Lac Insect?



4. Explain the swarming of the Lac Insect?
5. Explain the Cultivation of the Lac Insect

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## ***8.7 REFERENCES***

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## UNIT: 9 POULTRY

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### ***CONTENTS***

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## **9.1 OBJECTIVES**

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1. The objective of this chapter is to understand the poultry farming.
2. To understand their food & feeding habits.
3. A detailed study of breeds of fowl.
4. To understand the disease caused to them.
5. Understanding the economic importance of poultry & poultry products.

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## **9.2 INTRODUCTION**

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Fowls are widely distributed as domesticated animal since times immemorial. In twentieth century poultry keeping has become an important small scale industry due to modern need for palatable and nutritive food which it provides in the form of eggs as well as adult animals. The storage and transport facilities also have helped to a great extent in its becoming popular as a trade. This naturally has attracted the attention of the people at large and the scientists in particulars necessitating new researches on breeding, hatching and rearing of fowls. India is the native place of the wild jungle fowl but little attention has been paid for developing poultry industry in comparison to other countries. In country like India, the increased egg consumption is essential for the proper nutrition of human beings. The researches carried out at the Imperial Veterinary Research Institute, Izatnagar have demonstrated the high biological value of eggs and recommended the consumption of eggs in supplementing the defective human diets. For the successful management of poultry industry one should have detailed idea about the poultry ground, habitat of fowl breeds, breeding and rearing of chickens. Though there are several Government Poultry Farms in India but the poultry is almost entirely in the hands of poor persons, thus eggs available are of small size.

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## **9.3 HABITAT OF FOWL**

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1. **Soil.** For fowls, soil should be sandy, gravelly and abounding in kunkar alongwith good amount of lime. The stony soil is not suitable for fowls because stones cause damage to feet of the fowl. The heavy soil with high percentage of moisture is harmful. The marshy, dirty and drained grounds are fatal to the animals.
2. **Shelter.** The poultry should be protected from the cold winds and heavy rains. They should not be allowed to walk in water.

**3. Shade.** The poultry should be well protected from hot sun and hot wind in summer season, so the site for the poultry ground should be such as to have a number of shrubs and trees. In summer season the west side of the poultry house should be closed in the day time otherwise they will die due to westerly wind. The places where trees are not found, a shed of 10 to 15 square feet should be made with bamboo and straw. The shed should be kept 3 feet above the ground surface with the help of bamboo posts. The ground where such shades are not possible, it may be constructed with metal and raised 2 feet from the ground on stone pillars. The ground beneath the shed should be raised about 3 inches from general surface for good drainage.

### **Fowl-House**

Fowls can be reared in the hills of India without houses but in plains the specially constructed houses are essential for fresh and cool air in the early morning and evening. One adult fowl needs twenty five cubic feet of space. A house of 6 x 5 x 5 feet has sufficient accommodation for six fowls. An open shed or verandah must be attached to this house as a run way to the fowls for exercise. It is recommended that for six fowls a run of 40 x 30 feet is sufficient.

The fowl house may be either of wood or brick or metalled but the house of 'all metal' is best. The outdoor of houses must be towards south. It is advisable to construct a number of small houses rather than a single large one. The roof of poultry houses should be of either corrugated iron sheets, thatch, or wood. For the proper ventilation, the south side of the house should be enclosed with half inch mesh wire netting and other 3 sides have openings (12" x 6") and covered with wire-netting. The door of the house should be made up of an angle iron frame covered with mesh wire netting. The floor of the house should be pucca cemented and covered with coarse sand or dry earth sprayed with kerosene oil or phenyl. The sand layer must be changed after one month. The tick-proof perches of strong wood (3" in width) should be placed inside the house above 1.5 feet from the ground running parallel at 1.5 feet from the wall. The laying nets made up of 9 inch earthen gumlas (1.5 feet diameter, 9 inch depth), provided with dry ashes, sand or earth, should be kept in the corners of the poultry house for egg laying. A small gumla filled with old lime and mortar and the other gumla filled with coarse shell grits should be placed in the shed to supply the necessary grit and lime essential for the formation of egg shell. Light is also essential for the well-being of poultry, so these houses should always be constructed where natural light is possible without

which fowls will die due to cold. Near the shed door or in the yard, a vessel filled with clean and fresh water should also be placed. Care should be taken against sun-warmed water which causes cholera in the fowls. The house and shed should be cleaned daily and water should be changed twice a day. All wood work must be cleaned and painted with a mixture of tar and kerosene oil. The fowl house must be rat-proof because they destroy the eggs of chickens, and carry diseases into the fowl house. The broken glass should be spread on the floor under the bricks to make a pucca floor because rats can not burrow due to pieces of glass. Other enemy is snake which enters into the fowl-houses through the holes made by rats. Fowls also attack small snakes, kill and eat them but if snakes are cobra, krait or russel's viper the fowls are killed due to snake biting. One way to check the rats and snakes is to fill up the holes of poultry house regularly.

### **Food and Feeding of Fowls**

The quality and balanced quantity of food material are the back-bones of poultry. The oats, peas, grams and beans are good food for fowls. The rice and Indian-corn (makka) are not proper food for the birds. The skimmed-milk, butter-milk and curds, mixed with ground wheat and barley, are of good nutritive value for them. The skimmed-milk is highly nutritive for young chicks' and should be given in clean vessels. The boiled potato, mixed with equal part of wheatbran, should be given for the fattening of fowls. Some animal foods like ground fresh bones and meat are also good for the health of fowls. The green food as fresh tender grass, garlic, lettuce, onions are essential part of food and should be given uncooked. The mustard seed, hemp seed and linseed should be given after some interval during cold season, rain and at the time of moulting.

Poultry keeper should have good idea about the amount of food allowed to the fowls at different stages of the life span, type of breeds and area of yards at poultry farms. The best diet recommended by Mr. A.J. Macdonald, ex-Poultry Research Officer, Indian Veterinary Research Institute, Izatnagar, U.P. is given below.

Maize meal	20 parts
Ground oat	20 parts
Wheat bran	40 parts
Earthnut meal	20 parts
Common salt	1 part

A good amount of green food and calcium should also be given with the above food. Too much grain feeding to fowls is very much harmful to them.

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## ***9.4 BREEDS OF FOWLS***

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The whole poultry industry is centred round the fowls so the selection of good breed of birds for particular area is essential. The origin and evolution of various breeds and their varieties are due to selective breeding for producing the fowl of different shape, size, colour, comb structure, ear lobe's etc. Domestic fowls may *be* categorised into two groups like Desi or the indigenous and the exotic or the improved.

### **Desi Breeds (Indigenous Breed)**

The term desi is used to include all indigenous fowls exhibiting great variations in their shape, size and colour which, even if they have local names or pronounced characteristics e.g., Tenis, Naked-neck, Chittagong, Punjab brown, Aseel, Ghagus, Lolab, Titre, Kashmir faverolla, Bursa, Danki, Tellicherry, Kala hasti, Karaknath etc., are pure breed of fowl. The desi hen is, however, the best mother, as a rule, and makes an ideal sitter. She is also known to be very vigilant and a good forager. The pure desi breeds are Assel, Chittagong, and Ghagus.

### **Aseel**

Aseel, means real or true, is the name given to an indigenous breed, because of its endurance, power and fighting qualities. The word Aseel is probably misnomer of the word 'Asli'. The original Aseel is a medium-sized aggressive bird commonly known as the Reza or the Tikra. The remarkable endurance of an Aseel even during the most critical stages of the fight is proverbial, because it fights to the last and prefers going down fighting to withdrawing. Pure specimens of this breed are now rare and are available with some fanciers in parts of Andhra Pradesh, Karnataka and Uttar Pradesh. Several varieties, mostly crosses between Aseel and local fowls are known in other parts *viz.*, Nhurie (white), Hyderabad peela (red), Yakhud (Black and red), Dhummar (blue dust), Teekar (Brown black), Zava (laced), Patteda (single combed) etc. Aseel is one of the best table birds as the meat is, delicious and flavoured. Because of its poor growth and low fertility, this breed cannot be bred on commercial scales as table bird.

**Chittagong or Malay.**

It is a native of Malay Peninsula and bred in Chittagong (Bangladesh) and is found in some parts of eastern India. The colour of this bird is white with splash of golden markings on the wing. This bird grows faster and is ideal for table purposes as its flesh is excellent.

**Ghagus.**

It is a peculiar Indian breed resembling the Faverolle in appearance but having feathers on the legs. It is a big and hardy breed found in Andhra Pradesh and Karnataka. Ghagus is a good table bird. The hen is a fair layer, good sitter and real mother.

**Bustra**

It is a minor breed of desi class found in small numbers in Gujarat and Maharashtra particularly around Mumbai. The birds have body conformation typical of layers, and are majestic, alert, deep bodied and light-feathered. The hen of this breed is poor layer.

**Exotic Breeds**

The term 'Improved' is applied generally to the exotic and comparatively modern breeds of Chickens imported into this country, where they are bred and acclimatised to the local conditions. The exotic breeds of poultry were introduced in India by early European residents such as missionaries, planters and civil servants. The exotic breeds have become quite popular here. According to the standard classification 'Chickens' are grouped into many classes which are further subdivided into about fifty breeds. Each breed has several varieties which differ from one another.

**American Class****Polymouth Rock.**

It is one of the most popular breeds of USA as it can attain good size with excellent flesh. It has fair egg laying capability. Six varieties of this breed have been reported of which Barred Polymouth Rock Cocks like those of the Rhode Island-Red, are good for upgrading the ordinary village or desi hens. Other varieties of polymouth Rock breed are the white, Buff. Silver-pencilled, Partridge, Columbian and Blue. The white variety of this breed has been proved to be profitable as it has good capacity for egg and broiler production. The standard weight of this breed is cock-5.5 Kg, hen-4.5 Kg, cockered-5 Kg and pullet-4 Kg.

**Wyandotte**

It has a rounded body set close to the ground. Like Polynouth Rock, it is good breed for general purposes. It is very well adapted for flesh production and can lay good number of eggs of standard quality. The different varieties of this breed are white, silver-laced, Buff, Partridge, Golden-laced, Silver-pencilled, Columbian and Black. The standard weight of this breed is cock-5 Kg, hen-4 Kg, cockered-4.5 Kg and pullet-3.5 Kg.

**Rhode Island Red**

It is one of the most popular breed of fowl in India with good quality of flesh, and the hens are very good layers. This breed is hardiest of all breeds which can survive well in varying climatic conditions. The flocks of this breeds are kept in government poultry farms and by commercial poultry keepers. The chickens grow quickly, are easy to rear and well suited for wet and heavy rainfall regions. There are two varieties of the Rhode Island Reds : the single comb and the Rose-comb which are otherwise identical. The single comb variety is more, popular of the two. The standard weight of this breed is cock-5 Kg, hen-3 Kg, cockered-4 Kg and pullet-2.5 Kg.

**New Hampshire.**

The New Hampshire is a relatively recent Island Red stock. Because of its unusual hardiness, it has gained great popularity in India. Its popularity is also due to its rapid growth, early maturity, fertility, hatchability and good laying capacity. The standard weights are the same as those of the Rhode Island Red.

**Asiatic Class**

The important members of this class are Brahma, Cochin and Langshan. They are not of any special value for their flesh but these breeds together with those of the Mediterranean class have formed the centre for the progressive development of new breeds and varieties. Fowls of this class mature slowly, are poor foragers and persistent sitters.

**Brahma**

The earliest Brahma is said to have come from India in the Brahmaputra area, where the fowls of original type, commonly known as 'Grey Chittagongs' are still to be found. This breed is massive in appearance and is one of the largest domesticated chickens. The standard weights of this breed is cock-6.5 Kg, hen-4.5 Kg, cockered-5 Kg and pullet-3.5 Kg.



**Cochin.**

This breed hails originally from the Shanghai district in China and was called as the Shanghai fowl. The breed may be Buff, White, Black or Partridge. Its outstanding characteristics are a massive appearance, and well feathered shanks. The feather is long and profuse, which makes the bird appear larger than it really is.

**Langshan**

This has its origin in the Langshan district of China. As compared with Brahma and Cochin, the Langshan has a shorter but deeper body. It is graceful bird with well proportioned body and with a single comb. The standard weight of this breed is : Cock-5 Kg, hen-4 Kg, cockerel-4.5 Kg and pullet-3 Kg. This class comprises of six breeds like, Sussex Oprington, Australorp, Cornis, Dorking and Red cap.

**I. Sussex.**

The breeds of this class are provided with prominent breast, and are well developed with excellent fleshing qualities. Fowls of this breed have single comb and horn, coloured beaks, shanks and toes. There are three varieties 'among the sussex as the light sussex, the Red Sussex, and the speckled sussex. The Light Sussex is the most popular among them, and although originally a meat breed, good laying strains have since been' developed which make it a good all-round utility bird. The standard weight of this breed is cock-5 Kg, hen-3.5 Kg, cockered-4 Kg and pullet-3 Kg.

**II. Orpington.**

It is generally a good table bird, but good egg laying strains have also been developed by selective breeding and management. It has single comb. The bird may be Buff, Black, White or Blue. Of these, the buff variety is the most popular. The black is also important as it has led to the development of an increasingly popular breed-the Australorp of this class. The standard weight is cock-5.5 Kg, hen-4 Kg, cockered-4.5 Kg and pullet-3.5 Kg.

**III. Australorp.**

This breed was developed in Australia from the blackorpington. It is developed as good egg layer and considerable amount of flesh is obtained from this breed which make it a good dual purpose breed. This breed is gaining more and more popularity in India, and like the Rhode Island Red is well suited for small-sized flocks in the 'backyard' type of poultry keeping,

especially in the wet and heavy rain fall regions. The standard weight of the bird is : cock-4.5 Kg, hen-3.5 Kg, cockered-4 Kg and pullet-3 Kg.

#### **IV. Cornish.**

It is originally known as Cornish Indian, breed which is said to have been developed in England from the crossing of Indian Aseel and the Malay, and English Game breeds. It is reputed for its close feathering and compact well-flashed body which has a distinctive shape. The standard weight is cock- 4 Kg, hen-3 Kg, cockered-3.5 Kg and pullet-2.5 Kg.

#### **V. Dorking.**

The dorking, like the Sussex, are characterised by long, broad, deep and low-set bodies. Dorking has a rose-comb and the other two varieties have a single comb. All Dorkings have five toes. The standard weight is cock- 5 Kg, hen-3.5 Kg, cockered-4 Kg and pullet-3 Kg.

#### **VI. Red Cap.**

The name 'Red Cap' is derived from the large rose-comb which is the characteristic of this breed. It is medium-sized bird possessing a fairly long body and a rather prominent breast. The standard-weight is cock-4Kg, hen;-3 Kg, cockered-3.5 Kg and pullet-2.5 Kg.

### **Mediterranean Class**

There are six breeds classified as Mediterranean because of their origin from the Mediterranean region. These breeds are Leghorn, Minorca, Ancona, Spanish, Andalusian and Buttercup. The fowl of this class are small sized, mature early and good foragers of active temperament but they are non sitters. The maintenance of the fowl is economical as their food requirements are comparatively less. They are good layers.

#### **Leghorn.**

Leghorn is small, active and known for the harmony of its different parts. It has a relatively long back, prominent breast and comparatively long shanks. The common varieties of Leghorn are white, brown, black and buff. Some less common varieties are silver-red, black-tailed Red and the Columbian. The white variety has gained popularity all over the world. Next in order of popularity are brown and black. The white, buff and brown varieties are subdivided further on the basis of the character of comb *i.e.* whether it is rose or single comb.

### **I. White leghorn**

It was first introduced into India by foreign missionaries and tea planters about 80 years ago. This variety is not ideal for meat purposes. In India, white leg horn has given good results especially in drier regions, while it does not seem to thrive and produce well on heavy soils and in wet areas. It is most popular as egg producing breed. It matures within 5.5 to 6 months, when pullet starts laying.

### **II. Brown leghorn**

This breed seems to be hardier than the white leg horn and its colours, especially in the chicks, serves as a natural camouflage against predators. This breed is also equally popular because of its excellent productive capacity. It is used for upgrading desi-fowls in areas where birds of white colours are not preferred.

### **III. Black leghorn**

It is not as good layer as the white or brown varieties and is usually mistaken for black Minorca, but can be easily recognised by their head and body conformation of Leghorn. This variety has no special advantage over the other variety of the breed.

### **Minorca.**

It is noticed for its length of body, large comb and long wattles. In fact it is largest of the Mediterranean breeds. There are black, white and buff varieties. The black and white varieties are further sub-divided into the single and rose comb varieties. The most popular is the single comb black Minorca which has an attractive metallic black plumage and an unusually large comb. They are good layers and produce large-size white eggs. The chickens grow fast and are good for table purpose. The standard weight is cock-2 Kg, hen-1.5 Kg, cockered-1.5 Kg and pullet-1 Kg.

### **Ancona.**

The Ancona is of the same general type as the Leghorn. The breed has both the single-comb and rose-comb varieties of which the former is relatively more popular, although the breed as a whole is not so much favoured as the white leghorn. The standard weight of this breed is cock-3 Kg, hen-2 Kg, cockered-2.5 Kg and pullet-1.5 Kg.

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## ***9.5 BREEDING IN FOWLS***

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For successful poultry keeping, systematic breeding of fowls must be practised. The following care should be taken and kept in mind:

- (1) The largest and best farm fowls should be selected.
- (2) The weak, sick and stunt birds should not be taken for breeding.
- (3) Hens should always be best layers.
- (4) Two years old hens should be selected to breed with the cocks of one year age.
- (5) The male fowl should be of different family from the hen with which mated.
- (6) The hen must be mated with a cock superior to her.
- (7) For successful breeding nutritive food and intelligent management is essential.

For the breeding purposes the cock, selected, should have good size, bone, flesh, broad chest and colour. He should be active, healthy, energetic, young and of one year in age. The hen chosen for breeding should be equally good having almost the same qualities as the cock. If she is active and young she can lay a good number of large eggs. The number of hens allowed to mate with One Cock should be limited and in no case be more than four.

### **Cross Breeding**

For cross breeding purposes a good knowledge of the characteristics of different breeds is essential because all crosses are not good. The first cross of both, the pure hen and pure cock, is the best egg laying but should never be used for further breeding. For cottage industry of the rapid production of eggs and fowls for human consumption, the breeding of cross-breed fowls can be made.

### **Selection of Best Layer**

It is very difficult to select the best layers among the hens. An active intelligent-looking bird, with a bright comb, would always be the better layer than the dull and lazy hen. The thin, sickly, sluggish and poorly feathered hens should always be avoided for breeding. It is said that the greater the capacity the better the layer. The hen in which pelvic bones are fine and wide apart will be good layer. One has to be careful that a very fat hen will not at all be a good layer. Hence the hens should neither be very fat nor too thin.

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## **9.6 EGGS AND HATCHING**

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The hens normally start egg laying from February and continue till August with some intervals. The hens undergo moulting from July to August so should not be forced for egg laying. Few hens lay from October to January. The best time for egg laying depends upon the climate of a particular region. The rainy season is the best time to raise chickens, because:

- (1) There is availability of green food for birds.
- (2) Plenty of animal-foods are also found available.
- (3) The trees and shrubs are in full foliage to provide protection and shed to the fowls.
- (4) The chickens raised in this season grow fast and attain large size which can easily survive in cold season.
- (5) The eggs of rainy season are very fertile and hatch out well.

But the chickens should be Well protected from frequent showers and heavy rains. The chickens hatched from October to the end of January, develop very well throughout India except the hills. The most favourable period in the plains of India is from January to April.

### **Selection of Eggs**

The selection of eggs is very important so following care should be taken:

- (1) Eggs of best hens must be set.
- (2) The fresh eggs (3-5 days old in hot weather, and 7-10 days old in cold weather) must be set.
- (3) The eggs of too small and too large size must be rejected. The eggs should be of ordinary size and of smooth surface which should only be allowed to hatch.

### **Treatment of Eggs**

After placing the eggs under the hen, one should see every day that they are all right because sometimes eggs get broken in the nest. If any egg is broken the remaining eggs must be removed, washed with water, dried and the ashes and earth of the nest should also be changed. The water used for washing must be at 102°F. The cleaned and dried eggs should be placed under the washed hen in new nest. The infertile and addled eggs must be removed from the nest. The fertility of eggs can be tested by taking a cardboard having hole of an egg shape. The eggs for testing should be held on the hole against strong light. If the egg is found

transparent it is infertile but if small body is observed floating in the centre of the egg, it has an embryo. 11 test should be performed after 14 days of egg setting. Another method for testing eggs is to place the egg after 20th day of setting in a large bowl having warm water (102°F). After one minute the eggs, with live chicken will start wriggling in water whereas infertile and addled eggs will float the water surface. On the 20th or 21st day of egg setting the chickens usually come out of the shell. The chickens after emergence do not take food up 30 to 36 hours, so they must be with mother undisturbed for the minimum period of 24 hours.

### **Precautions for Hatching**

At the time of hatching following care should be taken

- (1) Hen should be set at night in a well ventilated and semi dark place.
- (2) Seven or eight eggs should be placed under one hen.
- (3) Before, setting, hens should be examined that they are free from insects. If they are carrying some insects they should be dusted with suitable insect powder.
- (4) Care should be taken of that the hens come out of the nest daily for food. The maize should be given to hen daily. The fresh water and a dry dust bath should also be given daily.
- (5) Hen should be left alone at the time of hatching of the eggs.

### **Rearing of Chickens**

The chickens during their growing stage must be kept under proper management. Different breeds need separate type of nourishment, shelter and exercise. After 36 hours of hatching chickens should be taken out of the old nests with the mother, placed in a clean box or on clean floor under a basket at a dry and warm place. The mother should be given good nutritive food and should be placed with her chickens in the box. After every interval of 2 hours, the food must be given to the chickens in very small quantities. The best food for chickens up to the age of 3 days is stale bread-cumbs moistened with milk and oat meal and broken wheat. Upto the age of 45 days chickens must be fed six times daily. After 45 days to six month's age they must be fed 4 times daily. First feed of the day should be given just after sunrise and last at sunset. Feeding board should always be clean. A very small quantity of 'Poultry powder' given in the soft food to chickens is found to be very much beneficial. A little finely-chopped onions and garlic should be given twice a week. After the age of 45 days, half-cooked meat and raw onion with wheat-bran should be given at an interval of one "day. The earth-worms and white ants are very nutritive for chickens and they feed on them

voraciously. After 3 months of age, oil-cake should be given to the chickens once a day. The perfectly clean water should always be kept before the chickens. Very small quantity of potassium permanganate should also be added to the drinking water to protect the chickens from sickness and other troubles. The overfeeding of chickens causes greatest danger to their liver and heart.

The standard ratio of chicken food of 100 Kg is given here as under:

Finely cracked wheat	40 Kg
Finely cracked maize	40 Kg
Cracked rice	10 Kg
Bajra	5 Kg
Cracked charcoal	2.5 Kg
Cracked granulated bone	2.5 Kg

When chickens attain the age of six months, they should be fed three times a day on grain, green food and moist mash at morning, noon and at evening respectively.

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## ***9.7 DISEASES OF POULTRY***

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The poultry keeper should always be careful against the diseases. The most common disease amongst fowls is **RANIKHET DISEASE** which is caused by a filter-passing virus. The Disease affects the fowls of all ages. In this disease bird opens the beak, becomes thirsty, suffers from fever and yellowish white diarrhoea occurs. The crop contains undigested food and the beak is filled with mucus. It is followed by nervous symptoms like twisting of the head, circular walking and wings get paralysed. The birds become very weak and die within 2 to 3 days. Mortality is very high, about 98 to 100 per cent. If a bird recovers it has immunity for life. This disease is transmitted from one fowl to another due to contact with the mucus discharged from the beak. The Indian Veterinary Research Institute Mukteswar (U.P.) has prepared a vaccine which is a boon for poultry industry. The dead birds should be properly disposed of by burying or burning. The chickens should be vaccinated as they attain the age of 40 days. The other common diseases are loss of feather, feather-eating and egg-eating which can be cured by giving large run and some of the poultry powder or tonic mixture. Some diseases are serious but not infectious *viz*; Apoplexy, caused by over feeding; Bumble

food due to very large size; Cramps due to exposure in wet or cold; Crop bound, due to over feeding, Diarrhoea, due to over-feeding and dirty water, Dysentery, Liver disease etc. Some diseases are contagious like Chicken-pox, Cholera, Cold etc. The birds should be destroyed and burnt or buried. The disease caused by a tick is known as Spirochaetosis (tick fever). The parasites feed on the blood of fowls in night and leave them during day time. For the prevention of this parasite poultry houses should be made up to 'All metal'. It is advisable for poultry keepers that 'prevention is better than cure' so they must be careful that the fowls should not be attacked by their enemies.

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## ***9.8 POULTRY PRODUCTS***

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The main products of poultry farming are eggs and meat in addition to some by-products like manure, feather etc. In India, the primary aim of poultry farming is to obtain egg. The old, surplus and weak stocks are used for the table. The eggs and poultry meat are among the richest sources of protein and vitamins.

### **Egg**

Eggs contribute to the palatability of a variety of dishes and constitute a rich source of easily digestible animal protein. Eggs are regarded as one of the good sources of vitamin A, riboflavin, phosphorus and iron. As per weight for weight data, an egg contains same quantity of animal protein as poultry meat and pork, about two-thirds that of whole milk cheese, and three-quarters that of beef. Eggs are coagulated on cooking. They provide appetizing foods when fried, boiled and poached. They have a leavening (fermenting) action in some foods such as angel food cake and sponge cake, a thickening action in custard, a combining action in noodles and doughnuts. The eggs are used as binding and coating agent during the preparation of soft breads. The formation of large crystals in ice-cream and candy can be reduced or checked by adding the eggs. The egg albumen acts as a clarifying medium for coffee or soups. It is very much useful in many non-food industries due to the adhesive and coagulating nature of egg white. The number of eggs used for other commercial purposes ego glazing, book binding, medicinal or pharmaceutical preparations, tanning etc. is comparatively very small.

As regard the structure of egg, it is one of the most wonderful things in nature because of its ability to develop and produce independent life under favourable conditions. The general shape of egg is irregularly ovoid in that one end is broader and some what flatter than the



other. This difference in the shape between the two ends is of considerable significance in the development and hatching of embryo. The egg consists of shell and shell membranes 12%, albumen and chalazae 56% and yolk 32%.

**Shell:**

The shell of an egg composed, mainly, of inorganic salts (chiefly calcium carbonate) forms the hard, rigid and porous protective covering for the soft contents within. The pores allow interchange of moisture and gases like oxygen and carbon dioxide between the egg contents and the outside atmosphere. The number of pores per unit area of the broad end is must larger than that found anywhere else on the surface of the egg. The outer surface of the shell is covered by a thin cuticular structure. Of the two principal layer of shell, the outer is thin relatively dense and compact, and protects against the entry of bacteria and other unwanted micro-organisms. The inner layer of the shell is granular and besides assisting in maintaining the strength and rigidity of the shell, serves as a source of calcium for the growing embryo. Inside the shell, there are two rough and fibrous membranes, one attached to the shell and the other to the thick white. As the shell contents shrink with cooling and evaporation of moisture, the membranes separate to form the air-cell, usually found at the broader end of egg. The outer membrane can only be separated from the shell with greatest difficulty.

**Albumen**

(Egg white). The albumen of the egg consists of four different layers. The outer layer of the thin white is just inside the inner shell membrane and forms about 20% of the total white. This layer is directly in the contact of shell membrane except at both the ends of the egg where the thick white comes in contact with the shell membranes. Inside the outer thin white, the layer of the thick white is found which constitutes about 50% of the total white of an egg. Enclosed within thick white is the inner layer of thin white. The inner most very thin layer of thick white surrounds the vitelline membrane that encloses the yolk. The main difference between the thick and the thin is due to the presence of the 'mucin' in the thick white. The chalazae are the coiled cords of thick white, twisted in opposite direction, arising from the thick white layer at each end and terminating in the chalaziferous layer (inner thick white). The chalazae keep the egg yolk nearly in the centre of the egg and give protection to the egg against rough handling. It also helps the yolk to turn easily within the white. The chalazae significantly are of more value as they ensure uniform temperature for the developing embryo

during incubation. The white acts as a shock-absorber, protecting the embryo and yolk from damage, and as a source of food for the growing embryo.

### **Yolk**

Beside the blastodisc, the yolk (yellow portion) is the only part of the egg that has its origin in the ovary. The other parts of egg like shell membranes; albumen and shell are added one after another during the passage of the egg through the oviduct. In a fresh egg the yolk rests slightly above the centre because it is lighter than the albumen of egg. The shape of a fresh egg is usually spherical but if egg is kept for some time, the yolk increases in size by taking water from the white and shows a relatively flattened shape than of the fresh egg. The blastodisc or female germ cell is essentially found in the egg but further development of the egg starts after fertilization by the sperm. Therefore, for incubation only fertile eggs should be taken because the infertile eggs are incapable of development so, they should be disposed off soon.

### **Composition of egg**

The actual composition of egg is based upon the breed of the bird, their habitat and the ecological conditions of the region where poultry farm is located. Whole liquid egg (excluding shell) consists of 36% yolk and 64% white. Protein is the major constituent of white and rest of the white is formed of small amount of sugars, minerals and fat. The two third of solid yolk is fat and one third is protein. The nature of yolk protein is different to that of white. The other constituents of yolk are lactic acid, creatine, creatinine choline and alcohol.

#### ***(a) Carbohydrate***

The sugar (glucose) constitutes the major carbohydrate part in the egg. The glucose is more in egg white in comparison to the yolk. An average of glucose content in the hen's egg is as healthy egg-0.45%, egg white-0.47% and egg yolk-0.14%. The egg contains very small amount of carbohydrate which yields a reducing sugar on hydrolysis. It is notable that sugar in free form causes heavy deterioration, if present in dry egg products. So, free sugar is eliminated from eggs before drying either by fermentation or by enzymatic oxidation to gluconic acid.

***(b) Proteins.*** Protein constitutes 12% of an egg, of which 64% is from egg white and the rest by yolk. Ovalbumin constitutes about 70% of the total protein in egg white and identified into AI' A2 and A3 proteins. During the storage of eggs this protein is converted into a more

stable form 'S-ovalbumin' which is highly resistant to denaturation. Another protein in egg white is canalbumin which is about 17% and reported to be found in two forms like ovomucoid and lysozyme. The egg yolk proteins include vitellenin, livetins, phosvitin, phosphoproteins, lipoproteins, lipovitellin and lipovitellenin. The egg proteins contain all of the essential amino acids required for the development and growth. The egg proteins have been found to be of higher biological value in comparison to the proteins of meat, soya bean, milk, wheat, ground nut etc. The protein of egg white is comparatively highly nutritive than that of egg yolk. The egg proteins possess the functional properties of coagulation, foaming and emulsification which are also significant for the consumers of eggs.

**Non-protein nitrogenous substances.** The non-protein nitrogenous substances like lecithin, free cholin, ovine, and other bases are also identified in the eggs.

**(a) Lipids.** The ether-soluble lipids amount to 30-35% of the fresh egg yolk (60-70% on dry basis), and the phosphatids 4 to 12%. Component fatty acids of the glyceride and- the phosphatid fractions of the egg yolk are palmitic, myristic, stearic, oleic, hexadecenoic, linoleic and unsaturated C<sub>22</sub> acid. The yolk of hen contains 1.8% of cholesterol.

**(b) Vitamins.** Eggs are very good source of riboflavin, vitamin A and vitamin D. The different vitamins present in egg are vitamin-A, vitamin-B12 vitamin-D, vitamin-E, vitamin-K, riboflavin, folic acid, niacin, thiamine, pantothenic acid, biotin, choline chloride, pyridoxine and inositol. The losses in vitamin contents during storage of egg are very low.

**(c) Enzyme.** Some of the enzymes like amylase, diastase, peptidase, phosphatidase, oxidase, various proteolytic enzymes, mono and tributyrases, catalase, tryptic proteinase, lipase, erepsin and salicylase are reported to be present in the egg.

**(d) Minerals.** Eggs are rich in mineral contents. The minerals like-calcium, iron, phosphorus, sodium, potassium magnesium, sulphur, zinc, chloride, manganese, iodine, copper,fluorine, selenium are reported to be present in the egg. The trace elements present in the egg include lead, chromium, aluminium, molybdenum, strontium, vanadium, titanium and barium.

**(e) Pigments.** The carotenoids of egg yolk are lutein and zeaxanthin. Ovoflavin, a nitrogenous pigment is also found to be present in the egg. The brown pigment of egg shell is Oorodein, which is identical with hematoporphyrin. The blue-green colour of egg shells, named oocyan, is considered to consist, in part, of the bile pigment biliverdin.

**Clearing of egg:-**

After laying eggs may become dirty due to so many factors. The dirty eggs should never be washed with ordinary type of cold water. The shell of egg should not be rubbed with the wet cloth or sand paper. For clearing the dirty eggs, sanitizer and detergent (1 % sodium hydroxide) solutions are used. Further it should be washed for 5 minutes in warm water (40 to 43°C) containing a detergent. The egg washing equipment has been developed by the central Food Technological Research Institute, Mysore. With this equipment 1000 to 1500 eggs are properly washed within one hour. This can be used advantageously for clearing eggs on a commercial scale.

**Preservation and processing of eggs**

The production of clean and wholesome eggs has received considerable attention in the developed and developing countries of the world. Eggs are used for household purposes, for confectionery and for other industrial purposes. About 95% of the eggs are used for table and cooking purposes and remaining 5% are used in confectionery. The urban demand of egg is of prime importance from marketing point of view. In India 20% of the eggs produced do not reach the consumers in a good condition and deteriorate during transport from the place of production to the place of consumption. According to the survey made by the Directorate of Marketing, Ministry of Food and Agriculture, Government of India, the deterioration in the quality of eggs is due to the various factors *viz.*, hot weather condition, storage in warm dry places, dirty eggs, fertile eggs, ungraded eggs and defective packing and handling. It is recorded that 5% of entire spoilage of egg is due to the bacterial contamination. The uncared eggs deteriorate quickly. So, eggs should be collected within a few hours after being laid. The preservation of the quality of egg is of great importance in marketing. The problem is however, beset with great practical difficulties, especially under conditions of village production. For the proper care freshly laid eggs are taken to the egg room, maintained at 16°C and 75% RH, and cooled as soon as possible. Even in cool weather, eggs deteriorate when left in the nest in which hens are laying. Eggs should be collected in suitable wire baskets atleast two or three times daily. The following measures are found to be useful in preserving the eggs.

**Production of infertile eggs**

The fertile eggs deteriorate rapidly than the infertile eggs at suitable higher ranges of temperature so, the production of infertile eggs instead of fertile ones helps in preserving egg quality considerably.

Infertile eggs can be obtained by separating the cocks from the hens except during the breeding seasons. It is common impression that the presence of a male bird is necessary for the hens to lay eggs. Hens are capable of laying without the presence of cock bird and the eggs thus obtained are infertile. But this is, however, not always possible under the existing conditions of poultry rearing in rural areas, therefore, defertilization of eggs has to be recommended. Defertilization. For the defertilization, eggs are kept in hot water maintained at a temperature range of 135F to 145F for about 15 minutes to destroy the germ. The defertilized egg is equally good as infertile egg and can be kept without deterioration for longer period. In villages, an ordinary long, wide mouthed tin vessel is used for maintaining temperature between 135F to 145F. Eggs to be defertilized are put in an open basket of wire and dipped into the hot water for 15 minutes after which they are removed and stored in some cool places. In well organised poultry centres, somewhat more elaborated defertilization plants are installed at little cost which consists of water tanks with mechanical stirrers electrical heating arrangements, some wire baskets and egg cooler.

**Egg cooling.**

It is well known that temperature above 68°F is favourable for the development of embryo and consequently makes for rapid deterioration of egg quality. A temperature below 68°F is suitable for maintaining the quality and freshness of the egg. In winter season, cooling devices is not needed when temperature is normally below 68°F but when temperature is higher than 68°F, a cooling device is necessary. The ideal of course, is refrigerated coolers. Some of the cooling systems which can be adopted by poultry farmers are given below.

**(a) Cool room.** Egg room can be cooled by providing all the outlets with khus tattis kept constantly moist by sprinkling water on the floor. If possible, a ceiling fan will be very much helpful for cooling the room.

**(b) Underground cellar.** If a cool room is not possible an underground pit should be made which can provide desirable low temperature for the proper cooling of eggs.

**(c) Earthen pot.** A large earthen pot can be maintained at lower ranges of temperature by keeping it partially buried in a sand heap on which water is sprinkled frequently. In the

bottom of pot a layer of dry straw or hay should be kept to avoid the percolating dampness. The pot should be kept in well ventilated place and the mouth of the pot should be covered with thin muslin so that air and moisture can pass freely inside the pot.

**(d) Cold storage.** The egg quality is fairly well maintained for about nine months in cold storage at 0°C and 85% RH. In India the cold storage of egg is now under practice in well organised poultry farms but in rural areas where refrigeration facility is not common, the cold storage of eggs has been taken upon a large scale.

### **Freezing eggs**

It is One of the best means of conserving the quality of eggs. In this method deterioration is arrested, and the frozen eggs can be held in cold storage for an extended period until needed. In this method, shell eggs are placed in cold storage to preserve the quality. After thorough chilling, eggs are taken to candling room. After the eggs are candled, they are transferred to the breaking tables, where the shell is broken against a blunt knife located above a small tray which is supplied with cups to hold the contents of the egg. Separated yolk, and white, or mixed whites and yolks, can be preserved through freezing which checks the growth of bacteria. Frozen whole eggs are used for preparing cakes, pastries, ice-cream etc.

### **Drying eggs**

The drying of eggs is more convenient way of preserving the eggs than even freezing. The drying process reduces eggs to about one-fourth of their original weight, so that about 70 normal-sized eggs make 1 kg of the dried product. The dry egg products like dried whole egg, dried yolk and dried whites, are prepared in farms. In this method the egg pulp is forced under pressure into a drying chamber and sprayed through a nozzle, The incoming air is held at higher ranges of temperature while the exhaust air has a temperature of lower ranges. The spray dried product is usually a fine powder while the pan dried product is made up of flakes or scales which can be ground into powder.

### **Lime sealing of egg**

The lime sealing of eggs prevent the evaporation of moisture and escape of carbon dioxide through shell pores. For lime sealing, shell eggs are dipped for about 18 hours in lime water, containing powdered salt.

**Oil coating of egg**

The oil coating of shell egg for conserving the quality of egg is an economical and convenient method for common poultry farmers. The oils used for this purpose are carnation oil, a white mineral oil refined from paraffin and coconut oil. The Central Food Technological Research Institute, Mysore has developed a perfect method in which eggs are coated with oil- based on a petroleum product to which some fungistatic and bacteriostatic agents are added. The eggs, kept in wire baskets are dipped for 5 to 10 seconds in a vessel containing the coating oil. Further, baskets having eggs are taken out from the vessel and hung on a hanger for about one hour. During this period fan can be used for proper drying of oil coated eggs. The dried eggs are ready for storage. The coating oil in the vessel can be used for many times with proper filtration and sterilization so that oil may not get dirty. The oil coated eggs can be kept for about 30 days at room temperature (25-27°C) and 80 days at 13°C. Oil coating should be done as soon as possible after the eggs are laid. The oil should be colourless, tasteless and odourless.

**Water-glass method**

It is a good method for preserving the quality of eggs. For this purpose, commercial water-glass (sodium silicate) is mixed with cooled boiled water in a definite ratio and is kept in an earthen pot and both are mixed thoroughly with an wooden piece. Eggs are dipped in the above solution and covered pots are kept in a cool place.

**Products of egg**

A number of useful products like albumen flakes, frozen egg yolk and egg powder are prepared from eggs.

**1. Albumen flakes:** For the preparation of albumen flakes, the thick albumen of egg is broken by the microbial fermentation and glucose is removed. Now this content is acidified and dried in the form of albumen flakes. The albumen flakes are used for the preparation of sensitive mixtures for coating zinc or aluminium foils for offset printing. The flakes are also used for the tanning of costly leather.

**2. Frozen yolk:** The yolk obtained as by product during the processing for albumen flakes are used as such or frozen for use in various purposes. The major products of frozen yolk are plain yolk, sugared yolk, salted yolk and yolk emulsions. Sugared yolk and salted yolk is mixed with 10 per cent sugar and salt respectively which acts as anticoagulant and minimise the chemical changes in yolk during freezing but they affect physical properties of yolk.



Another method for the preservation of frozen yolk is by the addition of 6 per cent sodium chloride and 1 per cent of sodium benzoate to it. The frozen yolk after treatment with 0.04 per cent pepsin can be kept undamaged for about four months.

**3. Egg powder:** For the preparation of egg powder, eggs are cleaned in running water, dipped into 2% solution of bleaching powder. The cleaned eggs are broken and liquid thus obtained is churned and filtered to separate pieces of shell and chalazae. For the removal of sugar, 0.5% yeast is added and kept at 36°C for 1.5 hours. Now fermented liquid is pasteurized at 60-61°C for about 30 minutes, cooled and 1 N HCl is added to bring the pH to 5.5. This solution is spray dried at an inlet temperature of 160°C and an outlet temperature of 60°C, keeping the automizer at 20,000 r.p.m. Thus the egg powder is obtained which is further kept in vacuum self-drier at 60°C for about 3 hours. In this powder 1.2% sodium carbonate is added and is canned in sealed containers. The egg powder thus obtained consists of moisture 13-15%, protein 45%, lecithin and fat 38-40%. The dried egg powder can be kept for long period even at higher ranges of temperature. It is very easy to transport the egg powder and the proteins are with the same amino acids as in shell egg. For the preparation of custards, pies etc. the egg powder is commonly used.

### **Poultry Meat**

The use of poultry birds for table is gaining more and more importance in our country which is managed either as a separate enterprise or in conjunction with commercial egg production. The birds for table should be well-fleshed on thighs and breast, fast growing and should have ability for high ratio of gain in live weight to the feed consumed. The weight of mature chicken may vary from 1 Kg to 5 Kg' according to the species and feed of birds. The leg represents more of total weight in male chicken in comparison to the females. For the purpose of human consumption, poultry meat should be soft, sound, clean and healthy. It contains less fat than other meats. The tenderness and flavour of meat is influenced by the age and sex of the bird. Chicken upto 85 days of age have a very tender meat and between 85 to 115 days of age have tender meat which can be cooked by roasting. The meat of mature hens is not soft for table purpose.

### **Composition of poultry meat**

The use of table birds is gaining more and more success as meat is a good source of protein, vitamins B, phosphorus and iron. The losses of these constituents are least during the course of cooking particularly with respect to vitamins B.



**Nitrogenous substances** The protein of poultry muscle consists of extracellular protein which includes actinomyosin, globulin X, myogen and myoglobin. In addition, other nitrogenous substances like creatine, carnosine, anserine, adenosine triphosphate, urea, ammonia, uric acid and amino acids are found *to be present in* crude poultry muscle. The bones are made up of *calcium phosphate* and collagen. The collagen of skin is changed to gelatin at the time of the poultry cooking which gives a desirable flavour to *the* soup. The poultry proteins are provided with highly digestible constituents and essential amino acids.

**Fat** The distribution of fat constituent varies in accordance with the age, sex, nutrition of the bird and tissue. The abdominal tissue contains about 80% fat, while the breast tissue contains very low (0.3%) fat percentage. The fat may be either neutral fat or phospholipids which contain tetraenoic, pentaenoic and hexaenoic acids. The acidity of the fat is taken as an index of freshness. The increased acidity of fat indicates the deterioration in the poultry meat.

**Enzyme** Many enzymes like lipase, catalase, oxidase, peroxidase, reductase, amylase, invertase, glycogenase, maltase, proteinase and antitrypsin are found to be present in the poultry fat and muscles.

**Minerals** A large number of minerals like calcium, potassium, sodium, iron, phosphorus, magnesium, chlorine and sulphur are reported to be present in the poultry muscles. Phosphorus and iron are found in good quantity. The trace elements like copper, iodine and manganese are also present in the poultry muscle.

**Vitamins** The poultry meat is very good source of riboflavin and nicotinic acid which is directly related with the nutrients given to birds. The other vitamins like vitamin A and vitamin D are present in the liver. The dark muscles of chicken are good source of thiamin and riboflavin.

### **Processing the Bird for Meat**

The killing of bird for table purpose, dressing and evisceration (to disembowel) come under the poultry processing. Some consumers prefer to purchase living birds while some wish to buy dressed bird as per their requirements.

**Dressing** Before killing, birds should be housed and should be given sufficient food and water. Before 3 hours of slaughter, feed should be taken away from the birds but water should be there for all the time. The carotid arteries are cut for complete bleeding because incomplete bleeding results in a dark, unacceptable product. Feathers are removed by immersing the bled bird in water at particular temperature for 3-5 minutes which is termed as,

scalding. Further, feathers are removed by hands or by some simple mechanical measures. The hairs from carcasses are removed by passing them through a hot flame after drying.

**Evisceration** Commonly birds for table are purchased in eviscerated or ready-to-cook form so proper care must be paid to save from bacterial infection, rancidification of fat and much moisture losses.

**Chilling and freezing** Just after dressing, the carcass should be first kept below 4-5°C and further at 0°C. The process of chilling is performed by slush ice for sudden fall in the body temperature of carcass so that excessive desiccation of the carcass may be checked. Thus, the carcass can be stored in good condition for 20 to 30 days. One should take care that faeces of the bird itself may not come in contact with the carcass to avoid the contamination during this process. During the course of chilling, the free amino acids and basic nitrogen content of meat slightly increases but at the expense of proteins. If the poultry meat is kept below -9°C temperature, the losses due to microbiological activity ceases as a result the souring and sliming of meat do not affect the quality of meat. Now-a-days, with the advancement of poultry industry in India, large poultry processing system have been established at various centres which have very good capacity for the poultry processing.

**Canning** For facilitating the distribution and storage of surplus poultry meat canning is very useful method for maintaining the quality of meat. In canning, the meat is sterilized and protected by a pack container particularly where refrigeration facility is not available.

### **Meat Products**

**Canned chicken** The old hens after the age of 20 months become uneconomical for egg laying so they are canned for regular supply of poultry meat. The central Food Technological Research Institute, Mysore has developed methods for canning solid-packed processed chicken in the form of whole chicken, whole boneless chicken, cut-up chicken without bone, cut-up chicken with bones and selected parts such as breast, thigh etc. In the process of canning chicken broth and chicken jelly are obtained which are used in the preparation of tonics.

**Sausages** The small pieces of meat obtained from old hens, roosters and culled birds are mixed with vegetables and after seasoning with spices they are converted to sausages. The product consists of moisture 62-65%, protein 15-17%, fat 15-17% and carbohydrates 3-4%.

**Chicken essence** The pieces of meat of healthy young chicken are partially hydrolysed with boiled water and extract is concentrated under vacuum. Further, this concentrated extract is

sterilized and fat is removed and diluted to required standard. Now suitable preservatives are added and the contents are sealed packed.

**Baby food** Some of the baby foods, as a good source of protein, iron and nicotinic acid, are prepared by the meat and broth. The skin and bones are used for the preparation of skin paste or dehydrated product. The baby foods are devoid of any fibre.

**Feathers** The feathers of poultry birds are of good commercial purpose. They constitute 4-9% of live weight of bird. They are washed, dried and packed for commercial uses. Feathers are utilized for making pillows and quilts. Due to their light weight, softness and high insulating power the down feathers are of great demand in foreign countries. Since long feathers are under use for making shuttle cocks.

**Poultry manure** The poultry manure, obtained from the dropping of poultry birds, and constitutes nitrogen 2%, phosphoric acid 1.25% and potash 0.75% so it is highly valuable for the standing crops in the field. Forty birds can give nearly a ton of deep litter per year which is sufficient for fertilizer needs of a hectare of maize or paddy.

**By-product feed.** A number of poultry by-products like blood-meal, feather-meal, poultry by-product meal and hatchery by-product meal are used as good sources of nutrients for meat producing animals and poultry. These by-products supply protein, essential amino acids, fat, vitamins and good amount of minerals. The feathers obtained from poultry slaughter house are treated under high pressure and feather-meal or hydrolysed feather is formed which contains, 80 per cent protein of which 70 percent is highly digestible. To obtain the poultry by-product meal the dryrendered parts of the carcasses of slaughtered poultry like head, feet, undeveloped eggs, gizzard and intestine are grounded. A mixture of egg shell, infertile and unhatched eggs and culled chicken are cooked, dried and grounded to obtain poultry hatchery by-product meal which contains calcium 18.1 %, 413 mg/ 100 g of phosphorus and essential amino acids.

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## 9.9 SUMMARY

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Poultry farming is the practice of raising domestic birds like chickens, turkeys, ducks and geese, as a subcategory of animal husbandry for meat or eggs for food agriculture. A hen begins to lay eggs when it is six months old and the egg laying bird is called 'broody hen. The eggs collected are called laying hens while the chickens bred for meat are called broilers. Most poultry are raised in intensive farming techniques. Seventy four percent of poultry meat in the world and 68 percent of eggs are produced in this way. Poultry farming involves

breeding and raising chicks for various purposes, in egg producing farms, birds are typically housed in rows of battery cages. High yielding varieties of chicken include Aseel, Ghugas, Basara and Brahma found in different states of India. High yielding foreign breeds or exotic breeds are classified into four classes namely, American, Asiatic, English and Mediterranean based on the geographical area they have evolved in. These classes comprise breeds that are reared for eggs, meat, or both.

A good crop of eggs is obtained by keeping poultry healthy. It is important to ensure nutritious feeding and proper housing. Chicken on a poultry farm are fed with grains, groundnut cake, rice husk, wet mixtures and green food to eat that are rich in carbohydrates, proteins fats and minerals. Poultry is routinely medicated, by antibiotics, in feed or drinking water, to treat disease or to prevent disease outbreaks arising from over- crowded or unsanitary conditions.

Poultry farming is a booming business, which can provide employment opportunities to small farmers and give them supplementary income along with nutritional support. Poultry contributes to improved human nutrition and food security by being a leading source of high quality protein in form of eggs and meat. It acts as a key supplement to revenue from crops and other livestock enterprises, thus avoiding over dependency on traditional commodities. For smallholder farmers in developing countries poultry represents one of the few opportunities for saving, investment and security against risk. In some of the countries, family poultry accounts for approximately 90 percent of the total poultry production. Poultry is the smallest livestock investment.

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### ***9.10 SELF ASSESSMENT QUESTION***

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#### **Long Answers Type Questions**

1. Explain the food & feeding habits of the poultry?
2. What are indigenous breeds, explain with suitable examples?
3. Explain the economic importance of poultry & its products?
4. Write a note on Diseases of poultry?
5. Describe exotic breeds & their economic significance with examples?

**Short Answer Type Questions**

1. Which exotic breed is best for egg production?

Ans. White Leghorn s

2. What is the most important ingredient in a good poultry feeding ration?

Ans. A constant supply of clean, fresh water

3. How long is the incubation period for chicken eggs?

Ans. 21 days

4. What is a female chicken less than 1 year of age called?

Ans. Pullet

5. What is the collective term for the feather covering of a bird?

Ans. Plumage

**Fill in the blanks:**

1. \_\_\_\_\_ acts as bird's teeth by grinding the food.

2. \_\_\_\_\_ type chickens are more prone to malfunction.

3. Indian indigenous breed are \_\_\_\_\_, \_\_\_\_\_ & \_\_\_\_\_.

4. The most common disease amongst fowls is \_\_\_\_\_.

5. A poussin is a type of \_\_\_\_\_.

**Multiple Choice Questions**

1. The hatchery should be situated at least how much distance away from other poultry houses.

a) 100ft b) 500ft c) 1000ft d) none of the above

2. For maximum egg production, the photoperiod is

a) 8 hours b) 14 hours c) 18hours d) 22hours

3. Fresh poultry excreta contains.....% water

a) 50-60% b) 60-70% c) 70-80% d) 80-90%

4. During incubation testing of egg should be done at

a) 10th day b) 15th day c) 18th day d) 21th day

5. A guinea fowl is a

(a) Young pigeon

(b) Castrated chicken

(c) Rock Cornish game hen

(d) Relative of the pheasant

**Answer:**

**Fill in the blanks:**

1. Gizzard
2. Meat
3. Aseel, Malay & Ghagus
4. Ranikhet disease
5. chicken

**Multiple Choice Questions:**

1. c
2. b
3. c
4. c
5. d

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## ***9.11 GLOSSARY***

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**Anthelmintic:** medication given to treat a bird with internal parasites

**As hatched:** description of a group of chicks that have not been sorted

**Avian:** pertaining to birds

**Axial feather:** the short wing feather located between the primary and secondary flight feathers

**Banding:** putting a tag or band with identification on it to the wing or leg of a bird

**Bantam:** a chicken breed that is one third to one half the size of a standard breed.

- Beak:** the hard protruding mouth part of a bird consisting of an upper and a lower part
- Bedding:** material scattered on the floor of a poultry house to absorb moisture and manure (also called litter)
- Billing out:** the act of chickens using their beaks to scoop feed out of a feeder and onto the floor
- Broiler:** a meat type chicken
- Broody:** a hen that is sitting on eggs with the intent of hatching them
- Chick:** young (baby) chicken
- Cluck:** sound a hen makes after laying an egg
- Coccidiosis:** a parasitic infection (coccidia) in the intestinal tract of poultry
- Comb:** the fleshy red outgrowth on the top of a chicken's head
- Contract grower:** a farmer that grows chickens, under contract, for a broiler company
- Coop:** the house or cage in which poultry are housed
- Crossbred:** the offspring of parents from different varieties or breeds
- Crumbles:** a poultry feed that has been pelleted and then the pellets broken up
- Cull:** to remove a bird from the flock because of productivity, age, health or personality issues
- Depopulate:** to destroy an entire flock
- Dressed:** cleaned in preparation for eating (feathers and guts removed)
- Forage:** to scratch the ground in search of food
- Fowl:** domesticated birds raised for food or other similar purpose; also refers to a hen at the end of its productive life
- Fryer:** a young meat type chicken
- Hatchery:** a place where eggs are incubated and chicks hatched
- Hen:** adult female poultry including chicken, turkey, duck, pigeon, pheasant, etc.
- Mite:** a type of external parasite
- Molt (Moult):** a part of the hen's reproductive cycle when she stops laying and loses her body feathers
- Mounting:** when the rooster mates with a hen
- Nest egg:** artificial egg placed in a nest to encourage hens to lay there
- Pecking order:** the social rank of individuals within a flock
- Perch:** the area above the ground where birds will sit, primarily for sleeping at night
- Plumage:** the total set of feathers covering a bird
- Poultry:** a term for domestic fowl raised for meat, eggs, feathers, work or entertainment

**Roaster:** a meat type chicken raised to a size that makes them suitable for roasting

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## **UNIT 10      ECONOMIC IMPORTANCE OF**

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# MAMMALS

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## *CONTENTS*

- 10.1 Objectives
- 10.2 Introduction
- 10.3 Economic Importance of Mammals in Agriculture
- 10.4 Dairy
- 10.5 Leather
- 10.6 Wool and Fur Industries
- 10.7 Terminal question & Answer
- 10.8 Glossary
- 10.9 References

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## *10.1 OBJECTIVES*

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After reading these units we will know about the Economic Importance of Mammals in Agriculture, Horticulture, Dairy product, Leather product and leather industries and Wool and Fur Industries.

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## ***10.2 INTRODUCTION***

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Mammals have attracted the attention of human beings since time immemorial because *Homo sapiens* have been always gazing for their food, shelter, protection and comfort. The status of mammals may be considered from the point of view of their value in nature and also to their importance in raising the economy of mankind. Mammals have always dominated the regions they inhabit and thus influence the direction of evolution and maintain a balance of complex ecological conditions.

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## ***10.3 ECONOMIC IMPORTANCE OF MAMMALS IN AGRICULTURE***

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Mammals have attracted the attention of human beings since time immemorial because *Homo sapiens* have been always gazing for their food, shelter, protection and comfort. The status of mammals may be considered from the point of view of their value in nature and also to their importance in raising the economy of mankind. Mammals have always dominated the regions they inhabit and thus influence the direction of evolution and maintain a balance of complex ecological conditions. But man has treated only those mammals with respect who may be of direct value to him. The comprehensive account about the importance of mammals may be assessed into two sub-divisions:

- (1) Indirect value of mammals,
- (2) Direct value of mammals.

### **Indirect Value of Mammals**

The phytophagous mammals greatly influence the flora of an area, for example the short-grass prairies were certainly affected by the millions of Bison, Prairie dogs and other herbivores that inhabited them. In nature a large number of lagomorphs, rodents and ungulates damage the trees by feeding on bark but others, help to reseed the vegetation by burying nuts and seeds. Seeds of many plants are spread when they pass out through the digestive tract of mammals or by being attached to their hairs. Pollen eating and nectar feeding bats cause pollination in a number of plants. In grass lands, where there are large number of ungulates, the soil enriching effect of mammal's droppings is also of great value.

The activities of carnivorous mammals like foxes, cats etc. are also of much importance in controlling the number of mice, rats, and other rodents and rabbits. The detailed ecological study of carnivores suggests that even sick and weak from the population should not be removed. So carnivores are the necessity of natural community to control the number of herbivores, and the population of carnivores are, in turn, regulated by the abundance of prey species. Literature regarding this reveals that the relationship of herbivorous and carnivorous mammals has not been well studied purely from ecological point of view.

### **Direct Value of Mammals**

For the comprehensive study of economic importance of mammals two categories can be made *vir.* Usefulness of mammals and harmfulness of mammals.

### **Usefulness of Mammals**

Mammals which promote welfare of human-being are known as useful mammals. For proper study, the useful aspects can be grouped into 4 categories:

- (1) Direct use of mammals,
- (2) Use of mammal products,
- (3) Other commercial products,
- (4) As zoo and laboratory animals,

### **[I] Direct use of mammals**

Some mammals are helpful to human beings with regard to their activities. They are used for agricultural, and transport purpose, as scavengers, in hunting and sports, as pets and predator of pest mammals.

**1. Agriculture.** Even in the era of advanced science and technology a large number of mammals are employed in agricultural work. Mammals like ox, buffalo, camel etc. are commonly found to be used for the 'tilling of the soil. Due to tilling, the soil becomes highly

fertile because of its increased water absorbing capacity; aeration of soil and reaching of organic materials to the lower levels. Buffalo, ox and camels are also used for irrigation of crops specially in the areas where other irrigation facilities are not available. The dung and urine of cow, buffalo, sheep, goat etc. are used in the form of highly nitrogenous natural manure. This manure is also used in gardening for good sized flowers and fruits. Manure is also prepared from the remains of the whaling products and the guano of bats dug from the floor of caves. It is also a rich and valuable fertilizer.

**2. Transport** A large number of transport facilities have been evolved and are being used in various fields but many mammals are also used for the purposes of transport at different places. Man has expert and trained horses, ox, camel, buffalo and elephants for the transport either in cart or as such. Camel, ass, donkey, horse, and elephant are used as beasts of burden. Camels are widely used in desert for transport purposes. Certain mammals are used for transport in specific regions e.g., Reindeers and Tundra dogs in Tundra Pradesh, Yak in Tibet and Bison in America.

**3. Scavengers** The importance of mammals as scavenger can not be ignored. Without this valuable aid of the mammals the whole of the world would be an open sewer. Hyaenas, jackals and pigs always function as natural scavengers due to their feeding habit. Sometimes it is found that dogs are also feeding on the excrement of human-beings.

**4. Hunting and sports** From time immemorial hunting has been of interest to human-beings for recreation and men in all parts of the world engage themselves in this activity. Sport hunting is commonly considered to be of two types *i.e.*, 'big game' and 'small game'. The big game may provide protection from a number of larger carnivore's *viz.* lion, wild boar, tiger etc. and also may be used as food. For this game, hunter usually has to travel far from his home. The fur hides and skins are also obtained in big games which are a good source of money. The small game is generally restricted to the smaller mammals such as rabbits, squirrel etc. and is played locally. In small game the use of weapons is not essential. Some persons are professional hunters. A number of larger mammals are becoming extinct, so their hunting is strictly prohibited now-a-days. Mammals like dogs and horses are quite helpful at the time of hunting.

**5. Pets** The dependency of human-beings on mammals led to domestication of some mammals for proper care and continuous supply of some useful materials in daily life. Before

pre-historic period domesticated mammals had started to act under the direction of man. Dog was probably one of the mammals domesticated even before the written history. During-the course of hunting dog provides its superior and sincere help about the presence of small and dangerous predators. It also follows the foot steps of the prey, harasses them and even may kill finally. At some places the dogs are used for transportation, guarding, herding and protection of live stock, food, skin etc. The sheep and goats have been domesticated since 9,000 years. Cattle, water buffalos and horses have been domesticated since 6,000 4,000 and 4,000 years respectively. Apart from these mammals, human-beings use many other mammals either as domestic or as domesticated lots. Thus, it is concluded that man is greatly dependent upon pet mammals for a number of functions, so pet mammals like dogs, cats, cattle; sheep, goats etc. are generally found in association with civilized man. Bernett (1948) discovered that policedogs assist the police department by tracking down culprits by their distinctive smell.

**6. Predator of pests** The role of pest predating mammals is of much importance. They reduce the population of some pests which are damaging the economy of the nation. So, there are certain restrictions on the hunting of those mammals who prey on pests. Next aim behind the restriction of hunting of those mammals is to preserve the species in nature. Cat is one of the most important mammalian predators which prey upon rats which is very serious pest of food grains in houses and crops in the field. In a number of government offices cats are used predating on rats and thus help in reducing the number of rats. The Humburg Government has fixed a special budget to provide milk and meat for 10,000 domesticated cats. Humburg is a very big port of the world where food grains are loaded and unloaded in huge quantities. Mongoose is the other mammal predating on rats. Foxes also cause great harm to the population of rats. Bhatia and Singh in 1959 have mentioned that squirrels entering in the cages of locusts prey on the hoppers and adults both.

#### **[IT] Use of mammalian products**

The mammalian products are being used in various ways by human-beings in the form of food, shelter, medicine, oil, musk and also as some other commercial products.

**1. Food** Food is the first necessity of life. The food value of mammals is in the form of meat, milk and their products. With the increasing number of domestic mammals and good transport facilities, man has increased his dependence upon non-vegetarian diet, obtained from mammals. Eskimos use essentially the marine mammals for their food. Kangaroos and

wallabies are very important for the food of Australian aborigines. Although hunting is known to be a sport but a number of mammals after hunting are used as food e.g., rabbit, squirrel, deer, whales, seals etc. A number of ungulates as cattle, goats, sheep etc. provide very nutritive food to human-beings. The pork and beef are taken as major food materials throughout the world. Many tribes of the Andaman and Nicobar Islands (Jawa, Holchus, and Oongees) prefer to feed on the flesh of pigs. Thus, mammals are used as highly proteinous non-vegetarian diet for human-being throughout the world.

The use of mammalian milk has proved to be a boon for mankind since time immemorial. Milk has provided life for the man. Mammals like cow, buffalo and goat provide milk for human consumption to take as such or in the form of butter, curd and ghee or for the preparation of other edible materials. Dairying has been a good business for the earning of money in cities but in villages the milk, curd and ghee are small sources of income especially to the poor people.

Denmark, Holland and Belgium have developed advanced techniques in dairying industry and thus they can earn good money. In India adequate efforts have not been made to establish improved dairy. Once upon a time India was greatest source of milk in the world and thereby cow was regarded as 'mother' by the 'Hindus' but now-a-days due to lack of uncultivated land and other facilities mammalian milk is being less consumed by man day-by-day. The other reason is its high cost which is beyond the reach of common man. Although Government of India has planned some schemes to improve the dairying industry but the results are not very satisfactory due to lack of proper management and its being restricted only up to cities.

**2. Shelter** The mammalian skins, hairs and furs are used for the protection and covering of the human body. Now-a-days mammals are probably being used more for shelter as compared to that of food. Wild boars, peccaries, kangaroos, seals, sea lions and capybaras are hunted for their hides. So called fashionable and rich people use furs of soft texture for their coats, particularly their ladies' must have fur atleast around their neck. Thus, fur trade is subjected not to the actual needs of human-beings but to social whims. In India alone out of 1/5th of the world's out-put of hide and skin 1/3rd is obtained from goats. But in the world market of hide, India has not been able to provide good quality material. Soni (1969) calculated from some data that India produces hide and skin worth 50 crores rupees annually.

The most common fur yielding domesticated mammals are beaver, mink, otter, shrew, fox, sheep, goats, cats and rabbits. The hair and wool of sheep and goats are spun into thread and woven into fabrics.

**3. Medicine** The mammals are also of great medicinal value. The cow and goat milk is given to diseased persons because of its easily digestible property. The whale liver provides a good quantity of vitamin A which is highly recommended for eye diseases and proper maintenance. Some mammalian organs like kidney, pancreas, liver, and some hormones, secreted by endocrine glands *viz.*, adrenal, thyroid, pituitaries and thalamus are used directly or in the form of their extracts to cure the disease of human-beings.

**4. Oil** Before the knowledge of petroleum, the oil obtained from mammals was in much greater demand. Whales are still hunted for their oil which is used primarily in the manufacture of soap and margarine in paints and for softening the leather. The renowned 'Sperm oil' a waxy inedible fat was used for candle manufacture, but today it is being used in cosmetics and ointment, as a lubricant for fine machines and in the manufacture of detergents and shoe polishes.

**5. Dung** Cow dung is a good source of natural nitrogenous fertilizer. Some people in villages use dried dung as fuel for cooking the food materials. In recent years cow dung is being used in small scale industry of Gobar Gas Plant. The gas obtained from this plant is now widely used in villages for light and cooking purposes.

**6. Musk** Musk is known since pre-historic period and has been mentioned in several old literatures. It is produced by Asian and African civet, American skunks and musk deer. Musk is widely used as a base in perfume industry. Ambergris, a waxy substance found in the intestine of some sperm whales and formed by certain abnormalities in the function of the intestine is also used in perfume industry as fixative.

**7. Ivory** It is also well known since very old time and is obtained from elephants and walrus. It is very much valuable and is used for piano keys, billiard balls, in knives etc. The hippopotamus teeth, narwhal tusks and the teeth of sperm whales are also sold for ivory purposes.

### **[III] Other commercial products**

**1. Hides and skins** The hides and skins are tanned and used for the preparation of number of articles like purses, cases, belts, shoes, sandals, suit cases etc.

**2. Carcass** From the carcass of mammal's fat is obtained and is used widely in the preparation of soaps and lubricants in leather and textile industry. From the carcass of a healthy mammal 5 to 50 pounds of fat can be obtained according to the size and health of the animal. Tennis and badminton rackets are prepared from the iritestine of some mammals. Now-a-days, there are 250 flaying carcass and 250 marketing depots in the small scale industry sector.

**3. Horns and hoofs** The horns and hoofs are consumed in the preparation of fancy toys, combs, frames, handles of knives, buttons etc.

**4. Hairs** The hairs obtained from pigs, camels and horses are used for the preparation of brushes of hard nature viz., for polishing the shoes, painting, coat brush etc.

**5. Skeleton** Bones of the dead mammals are of great economic value and are used for the preparation of weapons for defence and offence both. Bone-charcoals are used in sugar industry for clearing mollases. The bones of elephant and camel are of high cost in the markets. Gum and gelatins are obtained from the skeleton of whales and are used in the manufacture of candles and photographic films etc. The bones of mammals are widely used for the manufacture of phosphate fertilizers like super-phosphate which now-a-days is commonly used by farmers in their cultivated land for good growth and production of crops.

#### **[IV] As zoo and laboratory animals**

A large number of mammals like lions, tigers, deer, bears, monkeys, rabbits, foxes, elephants, camels, blue bulls, hyaenas, wolves, etc. are well maintained in a number of national and international zoological gardens and parks. A lot of visitors see them from a close range for their entertainment whiles some from academic point of view also. So, it is a good source of income. Some mammals like rats, jackals, rabbits, squirrels, monkeys are used widely in research laboratories for investigations in various fields. Thus, apart from economic benefits mammals are quite useful from academic points also.



**Dairy Industry**

The dairy industry, covering the production, processing and distribution of milk and milk products is unique in its importance as it is concerned with valuable food stuffs universally consumed by man. Milk is the fresh lacteal secretion of milch animals naturally intended for the nourishment of the offspring, but exploited as an article of food by human-beings. The young ones of other mammals survive only upon their own mother's milk but man uses milk of other mammals as additional source of nutrients for his offspring as well as for himself. Man also uses mammalian milk for a variety of preparations like curd, butter, chees, sweet etc. For the proper and regular supply of milk, man has domesticated a number of mammals. The only mammals which have received attention worth the name are cows, goats and buffalos. The other animals like sheep, cammels, asses and mares are milked in certain confined localities but as producers of milk they are of little importance.

The dairy cattle thrive best in areas where pasturage and other green forage are grown in abundance. Extremely cold climates are not suitable because of the lack of green forage and much expense for protecting the animals from the weather. The modern dairy industry is somewhat recent in origin. In earlier phase cows were kept to furnish milk for the farm family. The marketing of milk began when customers called at the farm or farmers to distribute their milk directly to users. Within the past 100 years advances along five different lines have caused milk and milk products to become important articles of commerce:

1. The processing of milk by factory system was started in the middle of 19th century, resulting in greater uniformity of product.
2. A number of technological advances like concentrating milk and sealing it in container in sterile conditions, distribution of milk in bottles and other advanced processing of milk began.
3. The first milk sold in towns and cities came from nearby farms. The knowledge of refrigeration of milk not only aided in keeping milk fresh for a longer period but also made possible the shipping of dairy products to all parts of the world.
4. In earlier days the method of transporting milk to large cities was to ship it on railways. The motor vehicles and paved roads made possible the supplying of fresh milk to markets hundreds of kilometres distant from farms where the milk is produced.
5. The adoption of pasteurization and the enforcement of laws requiring proper food value in dairy products greatly benefited the entire dairy industry.

**Breeds of Dairy Animals****Cow**

The number of well recognised cow breeds in India is about fifty. In addition, a large number of other types which do not confirm to any definite breed characteristics exist, and are treated as non descripts. The nondescripts are very poor producer of milk. The principal breeds of cows are Haryana, Kankrej, Ongole, Rath, Deoni, Gir and Kangayam. Deoni is found in the locality of North-western and western parts of Hyderabad and are good milkers in the region. Gaolaois common in Ward ha and Chindwara districts. Gir is good milker and found in Kathiawar, Rajputana and Baroda regions. Haryana is very good milker found in the vicinity of Rohtak, Hissar, Karnal, Delhi and Uttar Pradesh. Kankrej is common in South east of Rann of Kutch and Ahmedabad region and is fair milker. Ongole is also a good milker and found in Guntur districts. Rath is fairly good milker which is found from Rajasthan to North-western part. Shahiwal and Sindhi are very good milker found in Punjab, Haryana and Uttar Pradesh.

**Buffalo**

The buffalo is generally regarded as a very good dairy animal in India. The breeds of buffalo are lesser than cow. Murrah is very good milker which is distributed throughout Punjab, Haryana and Uttar Pradesh is a good milker and found in forest regions of Kathiawar. The breeds of Nili and Ravi are common near Ferozepur district of Punjab and are very good milk yielding varieties. The Surti are economical milkers and common in Gujarat.

**Goat**

The goats are used all over India for supplementing deficiencies in milk production. About 20 per cent of the goat population is used for milk production. Though there are many breeds of goats, some are used commonly in different regions of India. The various breeds of goat which are good milkers, are Jamunapari in Ganges-Jamuna riverine tracts. Beetal in Punjab, Bar-bari in Uttar Pradesh but not giving good results in Tarai belt; Outchi in Kathiawar and Kutch, Osmanabad in Hyderabad region, North Gujarat in Kathiawar, Kutch and North Gujarat, Marwari in Jodhpur, Surti near Surat, Sirohi in Sirohi and Palampur and Malabari in North Malabar region.

**Breeding**

Near about the middle of the 18th century, dairy farmers began the improvement of cattle and other farm livestock. The chief practices followed were the mating of related animals and close culling. The rearing and maintenance of proved and standard breed for distribution upto village level is undertaken in Government farms. The bulls are used for selective breeding in areas of well-defined breeds. Attempts are being made to upgrade non-descripts in several areas by repeated forward crossing. These attempts have given encouraging results but the number of bulls available for breeding purposes is inadequate. The progeny of Shahiwal, Sindhi, Haryana or other well defined bulls, yield atleast twice as much milk as the non-descript dam. The second cross shows a further increase in the production of milk.

Considerable amount of cross-breeding using imported bulls, has been carried out during the last 50 years. In the earlier years, the breeds favoured in India were the Ayreshier. A large number of Friesians were later imported which are reported to have given best results. The Indian Friesian yields on an average 4000 Kg of milk per lactation as compared to 5000 Kg given by Friesian cows. The Friesians are regular calvers, their productivity, however, is dependent on the care and attention given to them. These are maintained in favourable areas and moved to the hills in summer. The yield goes down as the percentage of Friesian blood is increased or decreased. It has been found that an increase in the production of Friesian blood increases the capacity to produce but the constitution of animal is not equal to the strain imposed in producing the milk to capacity. Although cross-breeding with foreign breeds improves the milk production, it is not widely adopted as the animals need expert care and management, not usually given to cattle in India. Experience shows that the cow produced by repeatedly back-crossing the half-bred is superior to the cow started with. For more effective utilization of limited number of breeding bulls now available, artificial insemination has been introduced throughout the country. In the very beginning the artificial insemination was followed at regional research centers in Calcutta, Patna, Montgomery, Bangalore and Izatnagar.

For establishing a good goat farm, a beginning can be made by taking a few good milch-yielding mother breeds with their male and female kids. After making a beginning with small number, in about a couple of years, it would be possible to sort out best milk yielding mothers and those not needed for participating in the breeding programme, may be castrated at the age of 3 to 4 months and may be disposed off at the appropriate time.

### Feeding Stuffs

The feeding stuff for dairy cattle may be broadly classified into roughages and concentrates. The roughages consist of succulent feeds (natural grazing, cultivated grasses, cultivated fodders and root crops) and dry fodders (hay, straw, chaff). The concentrates consist of carbohydrates-rich materials (oil seed, oil seed cake and meals). In addition to roughages and concentrates, dairy animals also require a certain amount of common salt to keep them in good condition.

Investigations in this area show that a number of materials hitherto considered as wastes, can be utilized as cattle feed eg.mango seed, jaman seed, and mahuaflowers (concentrates) and ground nut husk, bajra and coffee husk (roughages). In addition to distillary products (malt sprout, millets and molasses) feeds of animal origin eg.fish meal, blood meal and bone meal may be employed as cattle feed. The table below gives information about the feeding stuff, commonly fed to dairy cattle in India.

Dry rough ages	Green fodder	Concentrates
Wheat straw	Maize	Gram
Oat hay	Lucerne	Bran
Rice straw	Berseem	Cotton seed
Maize stalks	Oat	Cotton seed cake
J u war stalks	Juwar	Mustard cake
Legume straw	Bajra	Linseed cake
Grass hay	Elephant grass	Til.cake
Bajra stalks		Ground nut cake
Berseem hay		

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## 10.4 DAIRY

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Milk is produced by the mammary glands which are specialised skin glands. The actual secretion of milk by the mother is stimulated at birth by a lactogenic hormone (galactogen or prolactin) which comes from pituitary' gland located at the bottom of brain, adrenal hormones also are essential for lactation. During gestation, the production of lactogenic hormone from pituitary is inhibited by the presence of another hormone, estrogen which disappears at birth. In some mammals the stimulus of sucking offspring serves, through the nervous system, to stimulate the secretion of lactogen.

### **Marketing and distribution**

In urban areas 60 to 70 percent of the total milk requirements is produced within the municipal limits, the rest is obtained from adjoining rural areas.

Only 6 to 8 per cent of the total milk produced in the country is transported from rural to urban centres for consumption as milk and milk products. Nearly 2/3rd of milk received from outside the municipal limits comes from within 8 to 15 kilometres of the towns, and the remaining 1/3rd from beyond this distance. A part of the milk consumed in large cities, like Calcutta, Mumbai, Chennai, Delhi and others, it is obtained from localities situated at a distance of even 75 kilometres. Some successful efforts have been made to organize the production and marketing of milk on a cooperative basis.

### **Prices**

The price of milk varies to a great extent from place to place. In rural areas there is practically no good market facility for fluid milk. The milk leftover after meeting the demands of the producer's family is converted into butter, ghee or khoa. These products are sold at weekly markets at prices largely determined by the distance of the market from the village. As a rule, cow milk is cheaper than buffalo milk but it may not be true for all the time and for all the places.

### **Milk Products**

A variety of milk products is. Known in India and some of them figure in interstate trade. Dairy products such as cheese, butter, condensed milk, milk powder, curd etc. make the dairy farming a highly attractive industry.

### **Dahi (Curd)**

It is prepared by souring milk with a lactic acid starter which is usually curd of the previous day. For the preparation of curd, milk is first boiled and cooled from 60 to 70°F. Further, starter is added and is kept untouched. Thus, curd becomes ready for consumption in 10 to 12 hours. Curd contains 0.6 to 1.0 per cent titratable acid expressed as lactic acid. It has a lactic flavour and a compact smooth texture. Its composition varies with the quality of milk used, the types of organisms present in the starter and the time allowed for souring. The organisms present in the curd are mainly streptococci (*Streptococcus thermophilus*) and *Lactobacillus casei*. Curd is normally consumed at the place of production. Curd contains 84.79% water, 7.7% fat, 3.4% protein, 4.6% lactose, 0.7 to 0.8% lactic acid, 0.7% minerals, 0.12% calcium and 0.95% phosphorus.

### **Cream**

Cream is a fat containing fraction separated from milk by centrifuging the liquid milk. The separator, commonly employed, consists of bowl with a large number of conical discs arranged one above the other with intervening spaces. Milk enters through an opening in the centre and as the bowl is rotated, 3,000 to 20,000 rpm, the lighter fraction which is cream, is driven towards the centre and the heavier fraction or skimmed milk is drawn towards the periphery and drawn out.

The second method to obtain the cream is gravitational method in which milk is kept in a container at 50°F. After 24 hours cream automatically comes on the surface of the milk which is taken out by spoon. The yield and quality of cream vary according to the quality of milk and to the speed of separator. Skimmed milk is obtained as a by product which contains 0.04 to 0.5% fat and is used in the manufacture of condensed milk, milk powder, butter milk and cheese. The yield of cream is about 10% from a buffalo milk, 6% from cow milk and 7.5% from mixed milk. The composition of cream is 56% fat, 1.6% protein, 3% lactose, 0.4% minerals and 39% water.

### **Butter**

Butter is a mixture of milk fat, butter milk and water. Salt and colouring materials are often added. It is good source of vitamin A and fair source of vitamin D. Butter is characterized by spread ability, a characteristic not found in butter substitutes. This probably is due to the glyceride structure of butter fat and to the presence of lower saturated fatty acids.

Desibutter or makhkhan is prepared by churning curd after dilution with water, in earthen or tinned metal pots by a wooden pole to one end of which beaters are attached which is known as "mathani". The butter usually contains 18 to 25% water and varying quantities of curd. The composition of butter is 14% water, 83.5% fat, 1.5% lactose, 0.3% minerals and 0.8% aluminium.

### **Creamy butter**

The production of creamy butter in India is confined to few dairies. For the preparation of creamy butter, cream is aerated and ripened at 75 to 95°F to develop the desired smell and to facilitate churning. The ripening is effected usually by the addition of natural starter such as curd, fermented cream and butter milk or commercial starters containing pure cultures of lactic acid bacteria. The ripened cream, after dilution with cold water is mixed with a small quantity of dye and then churning is started at temperature 50 to 60°F. Churning is usually carried out in the mornings, when temperature is low otherwise ice is added to maintain the desired temperature. The whole operation takes 30 to 40 minutes. Butter milk is drained off and the butter is washed repeatedly with cold water. It is then salted, up to 2.5%, by adding salt solution either in the churn or after taking out the butter. Excess of moisture is pressed out to give a product with a firm and compact texture.

### **Ghee**

Ghee or clarified butter is obtained from butter by eliminating water. It is next to milk in importance as a dairy product. It can be stored over long periods and for this reason it is preferred in comparison to butter in most of the tropical countries for common people. Ghee is produced in India according to the traditional process involving sour curdling of milk, recovering butter and heating the butter to remove water. Desi butter is preferred to creamy butter as ghee obtained from the former is melted into ghee at once or after storage up to 10 days. It may be partially dehydrated and later converted into ghee according to the demand of market. The temperature employed for clarifying butter varies from 80 to 125°C. Short exposure to 120°C does not interfere with the formation of grain nor does it diminish the carotene and vitamin A contents of the ghee. To ensure proper grain formation, the tins to

which ghee is transferred should be kept undisturbed and cooling should be allowed to take place gradually. The appearance of colour and grain structure influences its market value. The ghee of cow is yellow and that of buffalo is whitish. The grain in buffalo ghee is bigger than that of cow ghee. The composition of ghee varies according to the composition of the milk from which it is derived. Ghee is mainly used for cooking, frying and taking directly with the food materials. Ghee is subjected to extensive adulteration in the trade.

### **Malai**

When milk is heated, a layer of fat and coagulated proteins, malai, is formed on the surface. Slow heating helps to increase the thickness of the layer. The volume of malai can be increased by boiling the milk until a voluminous froth is formed and cooling slowly over a dying fire. Malai is either consumed directly, or used in the preparation of sweets. Its composition is moisture 60 to 70%, fat 25 to 30%, proteins 3 to 3.5%, lactose 3.3 to 3.8% and ash 0.4 to 0.5%.

**Condensed milk** It is obtained by evaporating milk at 130 to 135°C in a vacuum p:m to the required concentration. The concentrate is homogenized to prevent the separation of fat, cooled and fortified if necessary. Stabilizers such as disodium hydrogen phosphate or calcium chloride, are added to prevent coagulation during the sterilization. The condensed product is cooled rapidly from 80-86°F and held at that temperature for 15 to 20 minutes. The cooling is so controlled that the crystals are of small dimensions and remain in suspension in the viscous liquid.

### **Khoa**

It is prepared by the rapid evaporation of water from the milk. It is usually prepared from buffalo milk by heating with brisk stirring in flat-bottomed shallow steel pots until the volume is reduced to about one-fifth. The product is gathered in a compact mass, cooled, and packed for markets.

Alum is sometimes added to the milk during the boiling to give a smooth texture to the product. Khoa is consumed directly or used as an ingredient of sweets. It can be kept for 3 to 4 days without deterioration. The common adulterants of khoa are cereal flours.

### **Cheese**



Cheese is the product made from the curd, obtained from whole or skimmed milk with or without added cream by coagulating the casein, and then further the separated curd is treated by ripening ferments. Soft cheese, known as 'Paneer' is prepared by using coagulants as the source of coagulating enzyme for clotting milk. Milk is warmed to about 100°F and the crushed coagulants are tied in cloth and dipped in it. The milk curdless in 30 to 40 minutes. The coagulum is placed on a muslin cloth and the whey is drained out. The process of cheese manufacture varies very much but not to such an extent as may be with different characters of the final product.

### **Future prospects**

The present retail prices of milk in urban areas in India are higher than those in the other countries. This is due, principally, to inadequate supply of milk. With a view to increase the per capita consumption of milk, the Planning Commission has suggested the adoption of measures for raising milk production in suburban areas. Emphasis has been laid on the need for maintaining hygienic conditions during the collection, transportation and distribution of milk and for enforcing measures of quality control.

The setting up of a statutory milk board for each urban area, consisting of representatives of producers, distributors, consumers, Municipal Corporation. Health authorities and the state Governments is a good initiative from Planning Commission. The matters related to the handling, distribution, quality control, imports and price of milk, and milk products come under the jurisdiction of the board. The financial assistance needed will be provided by government, municipalities and cooperative banks. Village schemes have been formulated for improving the breeds of milk cattle and for ensuring adequate fodder supplied for dairy cattle.

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## ***10.5 LEATHER INDUSTRY***

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Since the dawn of civilization man had started the use of animal skin to wrap his body before the invention and knowledge of clothes. Today, when a number of synthetic fibres and other materials are under use, the position of hides and skins in the world market is increasing day-by-day, of course, for different purposes. The skin of a number of mammals is being used for the preparation of clothings, attaches, purses, shoes, belts, etc. after proper tanning. Out of the total production of leather in India, 86-7% is obtained from the hides of dead animals and a very little quantity *i.e.*, 13.3% from the slaughtered animals. The livestock population in

India is highest in comparison to that of any other country in the world as such the production and status of skin and hide industries in India is on the top in the world. Yet due to the lack of proper technology and low grade tanning process it has so far never been possible to compete in the world market particularly regarding the quality of leather. Thus there is an urgent need for proper tanning of skin in this industry.

### **Animals of Leather Industry:**

The principal animals in this industry are goat, sheep, cow, buffalo, lamb, snakes, lizards, tigers, crocodiles, varanus, etc. The skin of cow is called as 'kips' and that of the buffaloes as 'buffs'. The cow hides are available from Tamilnadu, Uttar Pradesh, West Bengal, Kerala, Madhya Pradesh, Bihar, Maharashtra and Orissa since long but now-a-days only Kerala and Bengal are producing cow skins. The goat skins are common from Uttar Pradesh, Bihar, Rajasthan, West Bengal, Madhya Pradesh, Mumbai, Chennai and Hyderabad.

### **Processing of Skin Industry :**

To obtain the leather from skin and hides is a long and complicated process. The states where the cow slaughter is banned by State Government, only fallen animals are used to get hides, whereas, in other States like West Bengal and Kerala leather is obtained from the skin of slaughtered and fallen animals both.

### **Flaying**

The traditional job of flaying has been taken by the hereditary flayers in villages. During the process of flaying some amount of flesh and fat tissues remain attached to the hide due to which the weight of hide increases. To minimize the knife cut on the hide it is essential to have flesh and tissues with it. The carcass is hanged with a pole with the hind limb upward and flayer pulls down the hide over the back of dead animals with his left hand. Now the hide undergoes further processing or curing.

### **Curing**

Due to the atmospheric temperature and presence of bacteria and humidity, the hides containing flesh and other tissues start decaying just after flaying. Therefore, the swelling of skin occurs within 12 hours after flaying. The bacterial action causes the liquification of the

flesh and fat tissues but this bacterial action must be prevented by the use of antibacterial agents.

### **Salting**

The cured skin is now placed for salt treatment. The common salts used for this purpose are sodium sulphate, sodium chloride (common salt), potassium nitrate etc. The salting is performed by spraying the powdered salts on the fleshy surface of the hide. Now the salt treated skin is ready for being sent to factories for tanning.

### **Tanning**

The tanning of skin is an ancient art in India, and was being practised before Christian Era. Before the war of independence of 1857 tanning was mostly confined to the villages but after that in 1867, British Government established 'The Government Harness and Saddley Factory' at Kanpur. Only vegetable tanned leather was produced based on the technology taken from England. Another factory 'Cooper, Allen and Company' was set at Agra and Kanpur to produce the same leather. The chrome tanning was started in the Government Schools of Chennai, Calcutta and Kanpur as a subject. The first chrome tannery was established in Chennai in 1903.

#### **2. Pre tanning.**

The pre tanning process consists of a number of steps viz, soaking, liming, unhearing, fleshing and scudding, deliming, pickling. The pickled skins and hides are treated with organic, inorganic and synthetic' tanning agents like basic chromium sulphate; basic salts of aluminium, zirconium, and iron; formaldehyde; quinone; aliphatic sulphonylchloride, fish oil and synthetic polymerized materials (syntants).

#### **3. Post tanning**

After proper tanning the skin is subjected to a number of mechanical, physical as well as chemical processes viz., removal of water and tan liquor, splitting and shaving, neutralizing, bleaching and dyeing, setting out, samming, drying, staking, buffing and finishing to provide the fancy touch to leather for commercial grace.

#### **Number of public and private sector producing leather and leather goods are:**

1. Cooper, Allen and Company, Kanpur.
2. Cawnpore Tannery Limited, Kanpur.

3. Shewan Tanner, Kanpur.
4. United Provinces Tannery Company Limited, Kanpur.
5. DayalbaghTaj Tanneries Limited, Agra.
6. Bata Shoe Company Limited, Batanagar andMokama.
7. National Tannery Company Limited, Calcutta.
8. Chrome Leather Company Limited, ChromePET, Chennai.
9. Government Tannery Phulbani, Orissa.
10. Gordon, Woodroffe Leather Manufacturing Company Limited, Pallavaram, Chennai.
11. Mysore Chrome Tanneries, Bangalore.
12. Western India Tannery Limited, Mumbai.
13. Gold Filled Leather Works, Mumbai.

### **Enemies of skin industry:**

The skin industry also suffers from its enemies like warble-fly and tick. Warble fly makes a number of holes on the hides and skins due to which its value decreases from commercial point of view. If the attack is severe, the products are made useless for any commercial purpose. In previous decade 25,000 goat skins, exported to U.S.A. from Kanpur, were severely attacked by warblefliesresulting in the loss of about 100,000 rupees. Ticks are also found damaging the skins and hides. The ticks cause about 20 to 30 per cent damage of the whole stock of skins and hides in Punjab, Uttar Pradesh and Rajasthan.

### **Recent efforts**

The Government of India established "All India Khadi and Village Industries Bored in 1953 and entrusted the job of looking after the proper processing and utilization of hides. and skins. This board manages the production of a number of skin articles in which about 2 million workers are actively engaged in India, The small scale industries produce about 70 per cent of the skin products. In the Central Leather Research Institute (CLRI), Chennai, scientists are actively engaged to improve the quality of leather by modern techniques of leather processing. In U.P. 'The Uttar Pradesh Leather Development Corporation' (UPLDC)

has been recently established for encouraging the leather industry. Recently UPLDC has sanctioned for starting a factory in Basti district at a cost of about fifty lacs.

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## ***10.6 WOOL & FUR INDUSTRY***

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The wool yielding sheeps are inhabiting the arid regions of Northern India specially in plains as well as in the hills. The important places *viz.*, Saurashtra, North Gujarat, Kutch, Kashmir and the foot hill districts of Himachal Pradesh and Garhwal are most favourable for providing the natural conditions suitable for raising fine woollen types of sheep. The largest sheep population has been recorded at plateau of Deccan and Vindhya Mountains. The wool obtained from the sheep of Kashmir is finer in comparison to that of other places. The Magra and Chokla is the best breeds from Bikaner, and Kutchi from Joria (Rajasthan) which are famous for the superior type of carpet wools.

### **Types of Wool in India**

The proper and systematic classification of wool with regard to its quality is not yet properly known but it is classified according to the territorial nomenclature as given here under:

Wool type	Colour
Joria	Superior white
Harmal	White grey
Bikaneri	Super white
Rajputana	Yellow grey
Bibrik	White grey
Marwar	Yellow grey
Vicanere	Skin wool/Common black

### **Physical Properties**

The colour of the wool varies from species to species of the sheep and also the climatic conditions of the area. The real wool fibre is hygroscopic, elastic, durable, bad conductor of heat and is not easily inflammable. Due to its nature (bad conductor of heat) more heat is produced when the wool is wet. The microscopic study of the wool fiber seems to have cellular structure. In the transverse section of the wool fiber two regions are distinguishable *i.e.*, a central core of hard cells in the periphery and a medulla somewhat softer, situated in

the centre. The affinity of wool to dye absorption and the easy twist are the characteristic features of pure wool. The diameter of the wool fiber has been noticed to be 12 to 80  $\mu$ . The wool produced in India has a remarkable property of regaining the original shape when pulled and has abradable resistance.

### **Chemical Properties**

The wool fiber is made up of keratins which are actually, the polymers of the proteins and have higher sulphur contents. It consists of a number of polypeptide chains of amino acids. The various amino acids which constitute the wool protein are: arginine, histidine, lysine, alanine, methionine, threonine, tyrosine, cystine, leucine, iso-leucine and valine.

### **Removal and Wool from Sheep**

The shearing of wool is essential to promote the health of sheep. It also provides protection from natural enemies viz., ticks and mites which infect the sheep and cause various diseases. The removal of hairs from the sheep should be done carefully in the mild weather. The recommended periods for shearing of wool are winter (February to March) and rainy (August to September) seasons when rich grazing ground is available. Before the removal of hairs the sheep should be washed properly. The sharpened shears must be used for shearing purposes.

### **Processing of Wool Manufacture**

The manufacture of wool from sheep hairs is a complicated process consisting of cleaning, drying, bleaching, dyeing and twining.

**Cleaning** First of all wool obtained from sheep should be washed with cold water in specially constructed water tanks. After washing, the wool should be pulled out from the tank for further processing.

**Drying** The properly washed wool should be kept in open sun for drying for two to three days.

**Bleaching** The well dried wool would always be of faint and rough colour, therefore, it is essential to bleach it properly. The bleaching should be done by a number of physical processes because the chemicals destroy the wool in this condition.

**Dyeing** The bleached wool absorbs any dye rapidly so care should be taken that the amount of dye and the dyeing period is accurate.

**Spinning and twisting** Now the coloured wool is sent to mills for spinning and twisting. The spinning and twisting of wool is performed by a number of devices which varies from region to region. The shepherds use the spinning wheels to make the thread.

### **Recent Efforts**

The management of wool production in India has been unplanned since long with the result that the persons who are engaged in wool production are not getting proper price. The Indian Council of Agricultural Research, New Delhi has taken over the management of wool production and trading since 1937 and has appointed an authority, 'Sheep and Wool Development Officer' to control the whole production as well as the sale of the wool. The annual budget regarding the wool production is prepared by the officer and sent to the Govt. of India. The annual production of wool by different states has been recorded to be maximum from Rajasthan. The other states come on the position (production-wise) as : Rajasthan > Uttar Pradesh > Punjab > Gujarat > Andhra Pradesh > Tamil Nadu > Maharashtra > Karnataka > Jammu and Kashmir > Himachal Pradesh > Bihar.

### **Fur and Fur Industry**

The word fur is derived from old French word *forre* and *juene* meaning a 'sheath' or 'covering'. Fur is what may be described as the soft 'down' dense growth of fibres (small hairs) covering the skin of certain animals (mammals). Fur bearing animals also have a covering of longer hairs called as 'guard' hairs or over hairs which protect the underlying fur from injury and prevent it from matting or falting. Thus there is difference between 'fur' and 'hair' although both protect the animal from cold and storm.

The chief needs of the primitive man in the colder regions of the earth were food and warmth and it is quite reasonable to assume that the use of animal flesh for food from that of animals' fur for warmth was not long separated. Thus the practice of wearing fur is as old as humanity and obviously it outdates the art of spinning and weaving. Necessity came first but luxury soon followed, and the utilitarian and the beautiful marched side by side from remote antiquity into the present day. It is no more an article of clothing but now of luxury and decoration. This is why furs have tremendous fascination for woman kind and she assumes her 'furs' when she wishes to impress, not merely when she is desirous of keeping warm.

Assyrians, Romans and Greeks all, made lavish use of furs. It is recorded that Herodotus, and queen Samitamias (2182 B.C.) brought 8000 tiger skins from an Indian campaign. Some of these skins were put as uniforms for the royal household troops. In the time of Homer (850 B.C.), Armenia was the centre of a large fur trade and skins were coming from Siberia, Russia, Persia, Bokhara and even from far away places for distribution in Europe and elsewhere. The use of fur gradually became popular mainly with the royal family particularly the ladies in the various countries. The man did the hunting and fighting and the ladies adorned themselves, with the furs. Prof. Blach has mentioned: "Man must ever slave and toil, that vanity may wear the spoil". The woman has achieved equality rather superiority with regard to wearing of fur. There was a gorgeous display of furs at the coronation of Queen Victoria. The wearing of furs today is practically universal among the fair sex in all the civilized countries where the temperature warrants it and frequently when it does not.

Valuable furs are obtained from those regions where the winter temperatures is low and the growth of the fur on animals is thick and luxuriant. It is a fact that 'the colder the climate, the better the fur'. Canada, North America, Northern Europe and Siberia are among the important countries which supply fur. Himalayan districts also produce a certain number of large fur bearers, such as leopard, snow leopard, snow lynx, and tiger.

An immense organisation is necessary in this trade which is a most hazardous game, and many fortunes have been lost especially owing to sudden changes in fashion. There is an old saying, 'Furs when wanted are diamonds, when not wanted, charcoal'. The retailers at least live and prosper or fail under civilized conditions, but the man at the other end of the trade, in some cases thousands of kilometres away, who actually gets the skin *i.e.* the hunter is usually a combination of both. The hunter has to face death from cold, starvation, fatigue or even at the teeth and claws of the denizens of the wild. They have to face the sand storms of the desert or the blizzards of the Arctic. At the end of the chain is the hunter, striving against the most terrific hardships that can be conceived and at the other, the designer.

Hudson bay to all intents and purposes is the true centre of the hunting grounds. It is ice-locked for nearly eight months every year. Skilled men are needed to get the precious skins from regions, difficult in transport facilities and where even rivers are frozen. In the more northern regions, extending up to and beyond the Arctic circle, the fur trade depends to a large extent on Eskimos. After trapping, the animals are slaughtered and cut there at the collection



base and then the skins and the blubber conveyed to the nearest camp and thence dog-siege or taken by other means to a fur company outpost.

### **Dressing**

Technical and artistic skill of high order is required to get the raw skin manufactured. The first process through which they go is that of 'dressing' (curing). To dress a skin implies changing it from somewhat raw state to a condition of softness, indescence and beauty. The function of the fur skin dresser is to make the skin suitable for the use in the later stages of the trade.

The art of dressing skins has been known in some form or the other from very early days. Probably Chinese discovered alum as a best preservative and they claim to have made use of fur for 3000 years. Thus their mode of dressing skins continued for centuries. The procedure varies widely somewhat according to the nature and condition of the skin treated but in every instance there are at least four distinct stages.

1. The preliminary cleaning and softening of the pelt.
2. The fleshing: The removal of fleshy matter and blubber from the skin.
3. The leathering: Formation of leather on the skin which is actually a form of tanning.
4. Lastly the final cleaning.

It would be worthwhile to describe a simple and almost common process for dressing the skin.

1. The skins are first soaked in salt water for twelve hours if the weather is very hot and for twenty four hours if cool. Then it is conveyed to a *centrifugal* machine which *rotating* at a terrific speed clears away *the superfluous* moisture.
2. The fur is neat cleaned with benzine and then sent to the unhairer.
3. This craftsman (unhairer) after thoroughly warming the skin stretches it over rounded wooden block and with a blunt, two handled, sickle-shaped knife presses strongly with a dragging action, on the fur for pulling out the rough hair by the root, and leaving the soft fur or down untouched. This process may have to be repeated thrice or four times for removing all the top hairs.
4. The skins next go to the drums (a gigantic wooden cylinder) filled with dry saw dust or other suitable material. The drum is rotated for an hour or more, the saw dust by this means being forced into the fur and further cleaning takes places.

5. The skins from the drum are put into tanks containing ordinary fresh water and soak there overnight.
6. It then goes to the flesher for shaving off all the remains of the fat or grease. In this process the flesher runs the skin with a drawing motion over the edge of a very large knife tied in a vertical position. In some cases the skins are fixed and the knives are run.
7. After this the skins are hung up in a hot room preferably between 120° and 130°F. In this chamber the skins get thoroughly dried and at the same time, get hard once more.
8. Again the skin is put on the drum but this time for the purpose of being broken down *i.e.* made flexible, and when the desired flexibility is reached, this process is stopped.
9. The leather side is then thickly covered with grease which is spread evenly.
10. The skins are then folded up, greased inside and put in a huge tub where a person (or persons) treads them steadily for a varying period. It is this process which is known as leathering. The treader is a skilled worker and he should know when to stop this process. If in treading insufficient time is given the skin will not leather properly but in patches. On the otherhand if it has been done for a longer period the hair will mat and the skin will be spoilt.
11. However coarser skins are 'trampled' in a machine which is double wooden pistons working in troughs.
12. Lastly the skins are turned to the fur side and are thoroughly rubbed with saw dust, then to the drum where the saw dust may be changed six times before they are finally cleaned.

After each drumming the saw dust is removed completely from the skin either by beating with hand or other kind of drums, sometimes termed as 'cage' whilst the fur comb is constantly used.

Although the process of dressing originated in a primitive and haphazard manner, the modern fur skin dressing is a highly developed scientific process requiring, incidentally, considerable mechanical equipment.

The skins thus dressed are known as furs and ready for grading which is done by the assorter who is a skilled and highly paid person.

**Dyeing**

In early days the dyeing of the furs was mainly carried out with the negotable or mineral colouring matters. The modern development in dyeing took place from the latter part of the 19th century. Many chemical compounds known as fur bases have been developed. The whole process was revolutionized by the discovery of aniline dyes by Sir W.H. Perkin, an Englishman. The use of synthetic compounds as dyes has opened a new era for the production of many new colours on furs.

On the technical side of dyeing great secrecy is maintained. Each dyer jealously guards his methods from competition as thousands of experiments are made before really satisfactory dye process is evolved. Huge amount of literature is available on the dyeing industry. The importance of fur dyeing increased in the 1950s and new colour became an important selling point among fur retailers. From traditional browns and blacks of natural colours different shades depending on the fashion of the day and the liking of the people were developed. Different countries excel in different dyes. The chief dyeing centres of Europe are France, Belgium and London. At one time Leipzig was supreme in this field but later on Frankfurt, London and New York became important. U.S.A. has developed resin as a formidable competitor and New York has the largest fur processing industry.

Dyeing by dipping and brushing; cleaning the leather by repeatedly passing over a revolving emery wheel, and removing any top hairs left after unhairing is a highly ingenious process. Topping is a favoured process among the furriers. It means artistically and lightly brushing the top hairs with the dye, so that a badly marked skin be provided with rich colouring as is found on a good quality specimen. Topping is also done to fresher up partly worn skins which assume perfectly new appearance after going through this process. In U.S.A. a chemical process for straightening the curly fleece of yearling lamb skins has been developed. After undergoing this process the product is known as 'Mouton' which is used for aviators coats, bed room slippers and coat linings. It is durable and the fibers stay straight. They are resistant to moisture and can be easily cleaned.

**Fur Manufacturing**

The making of fur into garments technically designated as 'Furriery' has progressed to a great extent. From the primitive stage of sewing it has now become a highly skilled and intricate

occupation because of the advancement in fashions. Paris is famous for originating fur fashions, then Italian designers are also well known. At present New York is the largest manufacturing centre. The persons involved in the trade are, designers, cutters and sewers which are all highly technical jobs. Formerly sewing was done by hand but today highly efficient fur sewing machines do the work. Liners and finishers then complete the job.

There are two main types of fur manufacture. The first one is known as the letting out technique in which every skin is sliced into diagonal strips of .5 to 1.5 cm in width and then sewing these strips together, thus making longer and narrower strips without showing seams on the fur side. The other skin-on-skin method is less costly. In this case one full skin is sewed adjacent to another in an uniform alignment. Some firms get the left overs of full skins and specialize in sewing them in the form of blanket like plates.

### **Caring for Furs**

Great care should be taken in keeping and maintainance of the fur and fur garments. It should be remembered that even a little care taken of these beautiful and usually costly articles of wearing is well repaid thousand fold. Some of the suggestions in short are :

1. Keeps the fur hanging upward in darkness.
2. Before putting them on and also when taking them out, give them a shake. Shake them with the floke of the fur *i.e.* from the head.
3. Beat them occassionally with a light care gently.
4. Comb them gently but frequently with a fur comb.
5. If the fur gets wet never dry them in front of a fire or strong sun but hang them up in a room in a drought preferably. In this way it will dry gradually and when the moisture is gone, shake them well and comb.
6. The worst enemy of the furrier as well as the keeper of the fur garment is the 'Moth' which causes havoc. It is the grub of the moth which is responsible for the damage. The insect shelters in the fur and lays its eggs at the roots of the hairs. When eggs hatch, the grubs attack the roots and thus the hairs fall and large bare patches appear. (a) The whole stock, if possible, should be kept in cold storage, (b) Beating with light cane is most effective in this case also. (c) Pepper, campher and nephthaline can be used.

**By-products**

Fur cuttings and guard hairs are by-products of the fur industry. They are used in the manufacture of fabrics. Inferior grades are used as fertilizers and for glue stock.

**Fur bearing animals**

The principal fur bearing animals of the World belong to the order Carnivora of the class mammalia. The other fur bearing groups are Rodentia, Ungulate and Marsupialia. Some insectivora like moles and others also provide fur but they are not important from commercial point of view.

In India the number of fur bearing animals is small and restricted almost entirely to the Himalayan region. The animals in other parts of the country possess relatively coarse hair. During the British period in India, animals such as leopard, snow leopard, snow lynx and tiger were hunted by Gurkhas and the skin purchased by Indian merchants operating from Almora whilst the bulk of the central Asiatic fur were despatched by Camel, donkey and bullock transport to Peshawar and then by rail to Mumbai from where they were shipped to London and New York.

In a limited space of this book it would not be possible to give a description of all the fur bearing animals, but a few facts on the more important species should serve the necessary purpose.

**1. Badger (Lat. *meles*, Fr. *Blaireau*,)** This animal is to be found in both the old and the New World. In Russia, the hairs were used- for shaving brushes. All badgers have a peculiar white marking on the head and back. It is nocturnal and carnivorous. The American badger produces the best fur, the Chinese and the Japanese are coarse and inferior in texture.

**2. Bear (Lat. *Ursus*, Fr. *Ours*)** four kinds of bear are in common use in the fur trade *i.e.* Black, Brown, Grizzly and White. The largest is the polar bear the skin of which has even touched 3 metre in length and weighing about 500 to 800 lb. The Eskimos are very much fond of bear meat and are stated to kill the animal in an interesting but shrewd manner. The 'grizzly' bear (Lat. *Ursus horribilis*- grisly bear) 'known to hunters as 'uncle Eph' is the most formidable to the tribe. It is 2.5 metre long and reputed to be very cunning and armed with claws 10-15 cm in length. The name 'grizzly' denotes the colour of the fur which varies from pale grey to brown grey. To wear the collar of the grizzly claws denotes that the person has killed the bear single handed. This fur 'is mostly employed in the manufacture of motor and

floor rugs. The brown bear is a synonym of the Russian bear which in addition to Russia is found all over Europe. The dancing bears are of the brown and black variety.

**3. Beaver** The animal is from 30 to 90 cm in length and has a tail which resembles a paddle. The fur is of chestnut shade. It is found in Canada, Russia, Norway and also U.S.A.

**4. Chinchilla** A native of South America, is a small squirrel like land rodent. The fur is extremely beautiful slate grey with delicate dark markings and the leather is almost as thin, as tissue paper. The animal varies from 20 to 25 cm in length and is a Vegetarian.

**5. Ermine:** It is probably the most celebrated fur in the world. Ermine fur is really the winter dress of the Stoat which is an animal of the weasel tribe belonging to Carnivora. It is 25 cm in length and most ferocious blood thirsty, savage and cruel. A peculiarity in this animal is the great change that takes place in their natural covering on the approach of winter. It is light brown in summer which becomes white on the arrival of winter with the exception of the tip of the tail which always remains black. It is good and hard wearing fur, but prone to be yellow with the passage of time. It is used as a garment for children and well to do persons and also as a trimming to furs of different animals.

**6. Fisher** It is a mammal and it does not fish and is a type of large weasel which is about 60 to 90 cm long. It is very swift in its movements and is supposed to attack and eat the percupines also. Fur is rich dark brown and the long, hairs are practically black.

This creature inhabits Canada, certain parts of United States and parts of Northern India.

**7. Fox** There are numerous varieties of fox in common use in the fur trade. A passing mention may be made here of the principal ones. They are found all over the world. They range in colour from pure white to almost equally pure black with numerous grey, red and blue varieties. Pure black fox is both rare and expensive. The usually seen black fox skin is a red or white dyed to black. The different varieties are:

**(a) Blue fox** This is not blue but rather of a slate colour. It is small not very plentiful, and hence costly.

**(b) Cross fox** This is handsome rather flamboyant skin and a variety of American red fox. It is generally of a yellow or orange tone.

(c) **Grey fox** It is also known as virginia fox and is handsome, about 75 cm in length.

(d) **Patagonian fox** It is from South America and resembles a jackal rather than a fox.

(e) **Redfox.** This animal inhabits both the old and the new World (Europe, U.S.A., Canada and Asia). The American variety is most popular in fur trade. In colour it is reddish brown. Feeds chiefly on rabbits and is an enemy of poultry farmer. The colouring and quality of the fur vary to a great extent.

(f) **Australian fox.** The fur coming from the sub-tropical climate is of poor quality.

(g) **Silver fox.** It is the king of foxes from the point of view of beauty. The skins are by far most expensive of the fox species and vary in colour from all black to all silver. This fox will further be described under 'fur farming'.

(h) **White fox.** It is found in the Arctic region and usually on the sea shore. It is a small animal of about 60 cm and gregarious rather or migratory disposition. It turns snow white in winter only. The skin is of singular beauty and quite devoid of smell.

**8. Lamb.** They are found almost all over the World. The fur is extremely popular, being both handsome and hard wearing. It is used for every purpose to which the fur is put. The best lamb skins, silky with tight curl and fine glass, undoubtedly come from Bokhara, whilst others of inferior quality are obtained from Afghanistan, India, Persia, Asia and Rumania.

**9. Leopard.** This animal and the panther are considered to be of the same species and found distributed freely over the whole of Asia and Africa. The skins make handsome rugs. The bass drummer uses the skin as an apron.

**10. Marten.** There are four martens in common use in the fur trade viz .thebaum, stone, Japanese and American or Canadian. It varies from 45 to 60 cm in length. It is arboreal, greedy and extremely active animal. Its fur is silky, the best specimens, being of a rich dark brown colour and the inferior skins being lighter and yellower. The darker the fur, the more valuable it becomes. Hence the paler martens are frequently dyed or 'topped'.

**11. Mink.** This is another savage animal of the weasel tribe. It inhabits Canada, U.S.A., Russia, China and Japan. In Canada it is one of the main fur bearing animals. It is about 45 cm long and spends as much time in the water as on land. The fur is close, dense and thick. The leather or the pelt is also thick and comparatively heavy. The colour varies from yellow to red but as a rule it is oak like brown. The hair is short and close and the under fur is usually dark brown. The fur is used as garments for both the sexes and is rather more popular in Canada and U.S.A. than in Europe.

**12. Musquash** This animal is found in a belt extending from Alaska to Virginia in addition to Southern States. It is one of the most important animals used in the fur trade. The skin varies from 15 to 30 cm in length. The fur is a chestnut brown, the belly part being white and the under fur bluish or bluish white. It is warm and durable, expensive and in great demand for coats and linings.

**13. Opossum** There are two distinct kinds, one American and the other Australian. In colour it is grey, the top hair being light grey and the under fur is a dirty white. The fur is of coarse quality.

**14. Sable** It is found in D.S.A., Canada, Japan and Russia. It was once ambition of every woman to possess a set of sables as it was available in large quantities, but the supply today is all too small.

**15. Seals** Broadly speaking seals are either hair seals or fur seals. The fur seals really a sea-lion is now sold very sparingly owing to the regulations framed to prevent its extinction. It is highly gregarious in nature. The fur is in its best condition in June and July. The price of the seal garments is prohibitive.

**16. Tiger** The best tigers come from Central Asia. The well known Bengal tiger, is richly market but very poorly furred whilst the Manchurian is often 5 to 8 cm deep in the fur and many times more expensive. They are used as rugs. The other animals which are used in the fur trade are Pitch-having pungent odour; Lynx, Mermot, mole, dog, monkey, rabbit, squirrel and wolf.

### **Fur Farming**

Fur farming is one of the oldest of the occupations. The Chinese have fanned dogs, sheep and goats for countless centuries, utilizing the flesh for food and the skins for trade. If we term the sheep a fur bearing animal, it becomes the oldest form of farming in the world.

A number of animal-husbandry practices of live stock raising were adapted in fur farming also but some of the fur farm problems were specific to these wild species because of their being in confinement. This is why specific research became necessary for handling some of the animals.

Germany has fanned Corsican sheep in Harymountains and also extensive farming in silver fox for musquash and. beaver has been done. Soviets fur Syndicate is farming black and blue fox, deer and other animals. The farm on the Komandorsky Island is one of the most ancient in the World. The Canadian Govt. in collaboration with the Canadian National Silver Fox



breeders association established a research station as early as 1920 at Sumarside and the U.S.A. Govt. Started 'The United States fur animal experiment station' "at Saratoga Springs, New York in 1923. The United States Govt, by 1940 was conducting research on fur animals in cooperation with the colleges and universities in the States of Wisconsin, New York, Pennsylvania, Washington and Alaska. In Canada several provinces are also doing fur animal research in addition to 'Prince Edward Island' the 'home of the industry'.

In Great Britain there are farms for silver fox, skunk, marten, mink, fisher, musquash and nutria. Mole farms are also very popular and lucrative. Similarly rabbit farming is popular. The Fur Board Ltd. is a controlling body of rabbit breeders. Chinchillas are farmed in Andes blue and silver foxes in Norway and rabbit, nutria, marten, fisher, Opossum and skunk in France, Sweden and Denmark who also began similar research because of increasing importance of fur farming in their economy.

Some suggestions for establishing a fur farm are given here. There are many others which may not be possible to describe all in detail.

- (1) The farm should be kept at a secluded and "quiet locality. Any thing that tends to irritate the animal be avoided.
- (2) Only accustomed persons should visit the animals and that too when absolutely necessary. Strange faces have extremely bad effect.
- (3) Proper food to their living be provided at fixed time. They must be well fed but never over fed.

Silver fox farming Out of all the animals fox farming is most popular. The breeding of silver foxes in captivity was started in 1894 on Prince Edward Island. At first it was carried with secrecy till a beautiful silver fox skin was sold at London at a very high price. The British Crown knighted Charles Dalton for this work. Charles and R.T. Oulton both Canadians were at one time considered to be pioneers in this field.

A silver fox is mutation of red fox in which the red colour is replaced by black. The white band on the guard hairs gives the silver effect. The early captured foxes were nearly black but by selective mating over a period of 20 years, the bright silver fox was produced in which the white bands were extremely wide and black was free from rusty brown, Pair mating was first deemed necessary, but finally polygamous mating became the practice.

**Blue fox fanning**

It is done principally in Norway. It is interesting to note that in Alaska, the entire island were based for this work and the foxes ran wild. Due to lack of controlled breeding, the whole business came to a stop by 1940s.

**Mink ranching**

These animals were produced in Captivity for their fur as early as 1866 but by 1930 they were produced in quantity. After the middle of twentieth, there were three times as many mutations as the dark, natural coloured mink. The mutation mink breeders association controlled the market strictly. The sexes are kept separately and the polygamous mating is the common practice.

**Chinchilla rising**

As mentioned early it was a native of South America. In early 1900s Chinchilla furs were obtained from trapped animals. Due to trapping there was scarcity hence an American M.P. Chapman started their farming. By mid 1950s more than 5 lacs of these animals were being kept in captivity in U.S.A. and Canada. By 1954, it became one of the biggest trades in fur.

**Martens**

It is an attractive and interesting forest fur animal. Attempts have been made to raise them in Captivity but have failed because of only about 15% to 20% of the females produce young ones. They breed in July and August. Twenty litters were obtained over several years at the United States Fur Experiment Station.

Rabbit skins from animals raised primarily for food have been extremely used in the fur trade from time to time. Very few rabbits were raised primarily for their fur.

The other fur animals such as Karakul and other breeds of sheep have been raised in Russia and other countries for hundred or more years. South-West Africa by 1950s was producing several million Persian lamb skins after many years of selective mating.

The raising of several other animals in Captivity was initiated and developed for selling on a promotional basis by 1950s, but it had not materialized commercially, because of the availability of skins from wild variety. The animals that have been described or mentioned earlier under 'Fur animals' have almost all been raised at sometimes or the other. It all depended on fashions and the finding of new striking colours. There is no doubt that even with the natural coloured animals, improvement can be made by selective mating that can give them greater fashion appeal. It is only the scarcity of the wild types that will be a contributing factor towards successful farming of those animals.

### **Fur Farming in India**

In India the main commercial types of fur skins produced are lamb skins and kid skins. But the trade in the skins of wild animals is very limited.

#### **Lamb skins**

Various Karakul sheep were the first fur bearing animals to be put under farmirig. The lambs of these sheep have black or grey wool with tight curls. The quality of lamb skin depends on the variety of sheep from which it is obtained, the age of the lamb when slaughtered and also the nature of the curls. Lambs have to be slaughtered within a few days of their birth for obtaining good skin with very tight curl of fur. In India even the pregnant sheep are aborted to take out the lamb and skinned for getting high price. Lamb skins from South India are of inferior quality and major portion of them is used for preparing 'Astrakhan' caps. .

There are four grades of lamb skins-Moire, Nazakkcha, Guldar and Plain depending on the degree of curliness of the wool and the waves which the curls form. Moire is obtained from the premature lambs and is of best quality. Nazakkcha is obtained from the lambs killed within twenty four hours of their birth and the last two variety-Guldar and Plain from those animals which are killed within six to twelve day of birth. India imports lamb skins from Tibet.

#### **Kid skins**

Rajasthan and other dry areas in India provide skins of fairly good quality. Their prices are fixed according to the uniformity in the arrangement and alignment of skin hair, the quality of the hair and the size and colour of the skins. In this case also there are graded and the first quality is Moire, the second Surface Patterns and the third is designated as Plain. Moires are obtained from kids killed within two days and Surface Patterns within seven days of their birth. The rest come under the third quality. These skins are exported to France, U.K. and U.S.A. which is the chief buyer. They are cheap in comparison to the lamb skin.

**Skin of other animals**

The skins of many wild animals like marten (*Martes foina* Enx1.) of Kashmir and eastern Himalayas 'snow leopard' (*Panthera pardus* Linn) of Kashmir, Tibet and Sikkim, and Himalayan marmot (*Marmota bobak* Muller) and long tailed marmot (*Marmota caudata*) of the Himalayas are reported, to be exported to some extent from Calcutta port. Tiger and deer skins are mostly used locally. Tibet and Nepal supply small quantities of skins to Kashmir, U.P. and West Bengal depending upon the requirement. The skins of tiger's panthers, lynxes, foxes, jackals, bears, mongooses, otters, squirrels, hares and deer are also collected but their demand in the foreign market is irregular.

**Markets and Marketing**

Profits made in the fur trade are not dependent on the occasional specimen skins collected but on the quantities coming a few at a time, gradually pile up in huge quantities and sold by auction or negotiation.

In the past, say thirties, London was the chief fur market but later on St. Louis and New York became the chief centre. Under American stimulus the fur trade went mad and prices rose so high that at once time it looked that the fur could only be used by very rich persons. Presently the other countries involved in the trade are Russia, Germany and other European countries.

In India fur skins are obtained mainly from Punjab, Himachal Pradesh, Uttar Pradesh, Rajasthan, Delhi and Gujarat.

Major portion of the fur skin produced in India is exported to foreign countries; Delhi is the main market for the distribution of fur skins, handling even more than 75% of the total Indian supplies. The skins collected at Agra and Jaipur are also brought to Delhi. During the period of mid-August to mid-April more than 80% of the skin is collected and the rest during other months. As regards the local demands of lamb and kid skins, it is confined to furs of inferior quality. Fur skins of all types cured and tanned in India are used for gloves, ladies coats, caps and other fancy articles.

It may be pointed out that increasing demand for fur skins all over the world and the lure for high prices for them have led to the destruction of rather more or less extinction of many fur bearing animals. At the same time the decrease in the supply of furs of high quality has made it costly and also created a demand for inferior furs. These days even artificial and synthetic

furs are being produced on a factory scale. One fact is also to be remembered that the effect of the destruction of some of these animals for fur is boon for automatic control of the Agriculture crops and other products which otherwise are destroyed by these creatures.

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## ***10.7 SELF ASSESSMENT QUESTION***

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### **Long Answers Type Questions**

1. Describe briefly about Dairy industry?
2. Explain all the processes involved in Fur industry?
3. Describe economic importance of animals to humans with suitable examples?
4. Define breed & explain all the cattle breeds found in India?
5. Write about wool industry & process in it briefly?

### **Short Answer Type Questions**

1. What hormone is important in the milking process?

A. Oxytocin

2. What is Flaying?

Ans. To strip the skin off

3. Name the animals which are reared for fur farming?

Ans. Mink, Chinchilla, Blue fox, Rabbits, Martens, Sheep, Goat etc

4. Name the types of wool found in India?

Ans. Joria, Harmal, Bikaneri, Rajputana, Birbik & Marwar

5. Name well recognised cow breeds in India?

Ans. Sahiwal, Gir, Deoni, Ongole, Kankrej, Rath & Kangayam.

### **Fill in the blanks:**

1. \_\_\_\_\_ is the major enemy of skin industry.
2. \_\_\_\_\_ breed of buffalo is the best producer of milk.
3. Musk is obtained from \_\_\_\_\_, \_\_\_\_\_ & \_\_\_\_\_
4. A group of animals related by descent and similar characters like appearance, size and configuration are known as \_\_\_\_\_
5. The management of animal for milk and its product for human consumption is called \_\_\_\_\_

**Multiple Choice Questions**

1. The process of removing the fleece of sheep along with a thin Layer of skin is called :-
  - a. Rearing
  - b. shearing
  - c. Sorting
  - d. Scouring
2. What is the name of process which involves pulling & twisting of strands of a fibre?
  - a. Ginning
  - b. Weaving
  - c. Spinning
  - d. None of these
3. The yellow colour in the creamy layer of milk is caused by
  - a. *Pseudomonas synxantha*
  - b. *Peudomonassyncyanea*
  - c. both a & b
  - d. *S. marcescens*
4. Pashmina is obtained from :-
  - a. Changthangi goat
  - b. Sannen goat
  - c. Jamunapari goat
  - d. Merino goat
5. The highest milk producing country in the world is :-
  - a. U.K.

- b. Germany
- c. U.S.A
- d. India

**Answer:****Fill in the blanks:**

1. Warble fly
2. Murrah
3. Civet, Musk deer & Skunk
4. Breed
5. Dairying

**Multiple Choice Questions:**

1. Shearing
2. Spinning
3. *Pseudomonas synxantha*
4. Changthangi goat
5. India

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## ***10.8 GLOSSARY***

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**Breeding:** the mating and production of offspring by animals

**Buffalo:** a heavily built wild ox with backward-curving horns, found mainly in the Old World tropics:

**Chinchilla:** a small South American rodent with soft grey fur and a long bushy tail

**Condensed milk:** milk that has been thickened by evaporation and sweetened, sold in tins

**Dung:** the excrement of animals; manure

**Dyeing:** A process in which the color of a fur is changed

**Fur:** the short, fine, soft hair of certain animals.

**Ivory:** a hard creamy-white substance composing the main part of the tusks of an elephant, walrus, or narwhal, often used to make ornaments and other articles

**Lagomorphs:** a mammal of the order *Lagomorpha*, which comprises the hares, rabbits, and pikas.

**Mammals:** a warm-blooded vertebrate animal of a class that is distinguished by the possession of hair or fur, females that secrete milk for the nourishment of the young, and the birth of live young

**Marten:** a semi-arboreal weasel-like mammal found in Eurasia and North America, hunted for its fur

**Mink:** a small semiaquatic stoat-like carnivore native to North America and Eurasia

**Musk:** a strong-smelling reddish-brown substance which is secreted by the male musk deer for scent-marking and is an important ingredient in perfumery

**Pasteurization:** The process of heating milk or cream (and other beverages) to a specific temperature for a specified time to destroy any potential harmful microorganisms and increase its keeping qualities

**Phytophagous:** feeding on plants.

**Ranching:** A large farm on which a particular crop or kind of animal is raised

**Salting:** spraying the powdered salts on the fleshy surface of the skin

**Skin:** the thin layer of tissue forming the natural outer covering of the body of a person or animal

**Tanning:** A process in which the raw pelts are skinned, fleshed, soaked, and washed in special solutions to prepare them for use in garments.

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## ***10.9 REFERENCES***

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## UNIT 11 STORE GRAIN PESTS

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### CONTENT

- 11.1 Objective
- 11.2 Introduction
- 11.3 Pulse beetle (*callosobruchus maculatus*)
- 11.4 Rice weevil (*sitophilus oryzae*)
- 11.5 Wheat weevil (*trogoderma granarium*)
- 11.6 Rust red flour beetle (*tribolium castaneum*)
- 11.7 Lesser grain borer (*rhizopertha dominica*)
- 11.8 Summary
- 11.9 Terminal question & Answer
- 11.10 Glossary
- 11.12 References

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## ***11.1 OBJECTIVE***

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1. The objective of this chapter is to understand the pests of stored grain food.
2. To understand their habits & identification.
3. A detailed study of their life-cycles.
4. To understand the nature of damage caused by them.
5. Understanding the precautions & control measures to be taken against them.

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## ***11.2 INTRODUCTION***

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Since the cradle of human race, man has utilised animals for various purposes like food, clothing, weapons etc. If some animals proved to be useful while other caused great damage to the economy of human beings. These not so useful animals are a competition to man for food & natural resources. These are termed as pests, which affect the human beings directly or indirectly. A pest is any animal which becomes a source of nuisance & causes loss to humans. It has been estimated that between one quarter & one third of the world grain crop is lost each year during storage; much of this is due to insect attack. In addition, grain which is not lost is severely reduced in quality by insect damage. Many grain pests preferentially eat out grain embryos, thereby reducing the protein content of food grain & lowering the percentage of seeds which germinate. Direct feeding by stored grain pest reduces grain weight, nutritional value & quality of stored grains. Infestation also causes contamination, odour, mould & heat damage problems which reduces the worth of the grain & result in making it unfit for processing into food for human beings & animals.

## SOME COMMON STORED GRAIN PEST'S:-

### 11.3 PULSE BEETLE (*Callosobruchus maculatus*)

1. **Pulse beetle (*Callosobruchus maculatus*):** It is a species of beetle commonly known as cowpea weevil or cowpea reed beetle. It is a member of the leaf beetles family Chrysomelidae hence not a true weevil.

#### Systematic Position:

**Kingdom** – Animalia

**Phylum** - Arthropoda

**Class** – Insecta

**Order** – Coleoptera

**Family**- Chrysomelidae

**Genus** – *Callosobruchus*

**Species** – *maculatus*

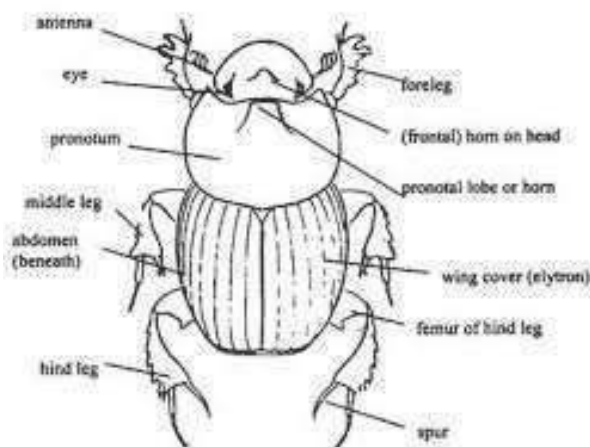
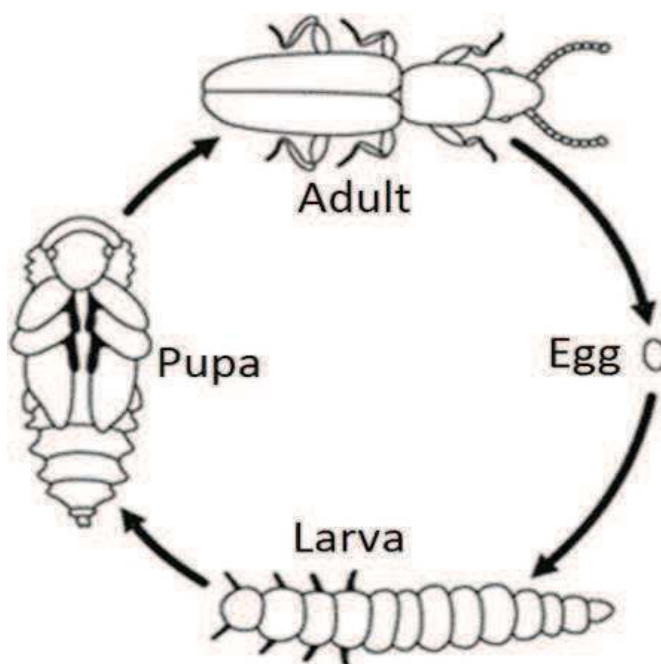


Fig11.1 Pulse beetle

**Habits:** It is a common pest of stored legumes which is cosmopolitan in distribution except Antarctica. This beetle originated in West Africa & moved around the world with the trade of legumes & other crops. It is a major pest of cowpea, green gram & lentils. It lacks the 'snout' of a true weevil; it is elongated in shape, reddish brown in colour with grey & black sheath elytra having two central black spots. The last segment of the abdomen extends out from elytra, which also have black spots. There are two morphological forms of the species i.e. a flightless form & a flying form. The flying form is more common in the beetle that develops in high larval density & high temperature. Flying form has longer life span but lower ability to produce eggs. The beetle is sexually dimorphic as males can be easily distinguished from females as they are brown while females are overall darker. Female beetles are larger than male beetles in size. Elytra are larger in females as compared to males. It is medically harmless to humans.

**Life-cycle:** A female beetle after copulation lays up to hundred eggs & out of which all hatches. Eggs are layed on the surface of the bean which are small, translucent, shiny, oval, inconspicuous & doomed structures. The duration of egg stage is 5-6 days, upon hatching the larva bite through the base of egg: through the testa of the seed & into the cotyledons. The developing larva feeds entirely within a single seed, excavating a chamber as it grows eating the tissue just under surface leaving a very thin layer through which it exits when get matures. It emerges after a larval period of 3 to 7 weeks depending on conditions as gestation period is shorter in summer while longer in winter. Larval crowding i.e. 9-10 larvae feeding within one bean limits the resources leading to mortality. The larvae takes 24-36 hours to mature completely into an adult after emerging out, life span of the beetle is 10-14 days. The adult does not require food or water as larval stage is the feeding stage. There are two growth stages namely fruiting stage & post-harvest stage. The adult beetles that emerges out mate with the others that develop on the same host bean.



*Fig11.1 Life cycle of Pulse beetle*

**Nature of Damage:** *Callosobruchus maculatus* is a serious store pest, causing enormous damage to almost all kind of pulse grains. It prefers cowpea (*Vigna catjang*) but it also infest the seeds of different pulses such as red gram, arhar, lentil, pea, small pea, mung, urid, moth,

soyabean, khesari etc. It also causes damage to seeds in pods of red gram in the field. Damage to the pulse grain is mainly caused by the developing larvae. Just after hatching young larvae bores into the grain, feed upon the contents of the grain making them almost hollow and empty, clustering of grains, decay or powdering of pulses which produces foul smell. As development occurs entirely within the seed, the immature stages are not normally seen. The optimum development conditions are 32<sup>0</sup> C temperature & 90 % relative humidity. As the pods become dry the ability to infest them decreases where as the thrashed seeds are more susceptible to pest attack throughout storage.

**Control Measures:** For the control & eradication of the beetle following methods should be adopted which are enlisted below.

1. **Chemical Control:** It is the use of chemicals such as fumigants, contact insecticide & pesticides to control storage insects. Chemical measures provide immediate disinfestations of the commodity & the space enclosing it.
2. **Physical & Mechanical Control:**
3. **Biological Control:** Animals used as predators of Pulse beetle are parasitoid wasps (*Dinarmus basalis*) which target small larvae. The organism used as parasite of the beetle is a mite (*Uscana mukerji*) which feeds upon egg & prevents them from hatching.

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### 11.4 RICE WEEVIL (*Sitophilus oryzae*)

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**Rice-Weevil (*Sitophilus oryzae*):** The rice weevil is a stored product pest of not only rice but almost of all cereals & their products. It is called as rice weevil because it's breeding habits & life-cycle was first of all studied in rice

**Systematic Position:**

**Kingdom** – Animalia

**Phylum** - Arthropoda

**Class** – Insecta

**Order** – Coleoptera

**Family**- Curculionidae

**Genus** – *Sitophilus*

**Species** – *oryzae*

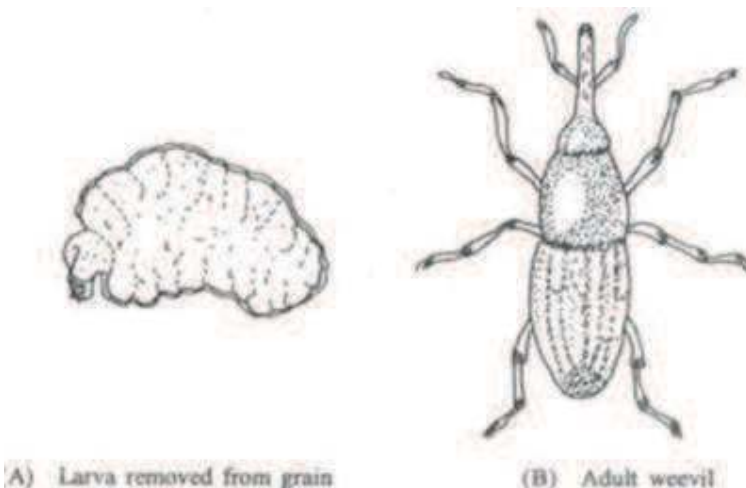


Fig 11.3 *Sitophilus oryzae*

**Habits:** It is the commonest pest that one encounters in all kinds of stores. It is believed that it originated in temperate countries. But now days it is the most widely distributed stored product pest of the world through shipments of rice. It commonly occurs in temperate & warm countries, rice weevil does not only attacks rice but several crops like wheat, maize, barley etc. Rice weevil causes the most substantial loss to stored grains in world amounting 18.30%. The beetle has a relatively short developmental period & high populations can easily be built up, adult weevil is reddish brown in colour measuring 3-4 mm in length. Head is projected forward into a long snout like rostrum with a pair of stout mandibular jaws at the extremity of the rostrum. Four light red spots on elytra are arranged in a cross on the wing covers due to this it is easily confused with the similar looking maize weevil. Females are larger than males & sexual dimorphism occurs as rostrum of males is shorter, broader as compared to the females. Adult weevils are able to fly.

**Life-cycle:** After copulation, adult female bores a hole in the grain with the help of its powerful jaws & deposit a single egg in the grain cavity. In search of suitable site in the grain

a mother beetle may bore at several parts of a grain but only one egg is laid down in a single grain, female can lay up to 300-550 eggs in 4-5 months i.e. 2-6 eggs per day. Eggs are deposited within the hole sealing it with the gelatinous fluid secreted from her ovipositor, which are white, elastic, tiny & oval in structure. The egg hatches within 4-5 days under optimum conditions, the larva develops within the grain hollowing it out while feeding leaving the shell intact. The tiny, white, fleshy legless grubs with yellowish brown head & biting jaws bores down into the grain, feeding on its starchy content. The grub stage lasts for 19-34 days & a fully matured grub makes a pupal cell inside the grain called as eclosion & pupates. The pupal stage lasts for 3-6 days under optimum conditions but in unfavourable conditions it may extend up to 20 days. The adults formed after pupation bores its way out of the grains, immediately after emergence the adult weevil are ready for breeding. The duration of life cycle of rice-weevil & number of generations completed in a year depends upon the weather conditions like temperature & humidity; 5-7 generations are completed in a year. The size of the newly bored adult weevil is directly proportional to the size of the grain in which the larval period has been spent, larger & healthier grains produces larger & healthier weevils.

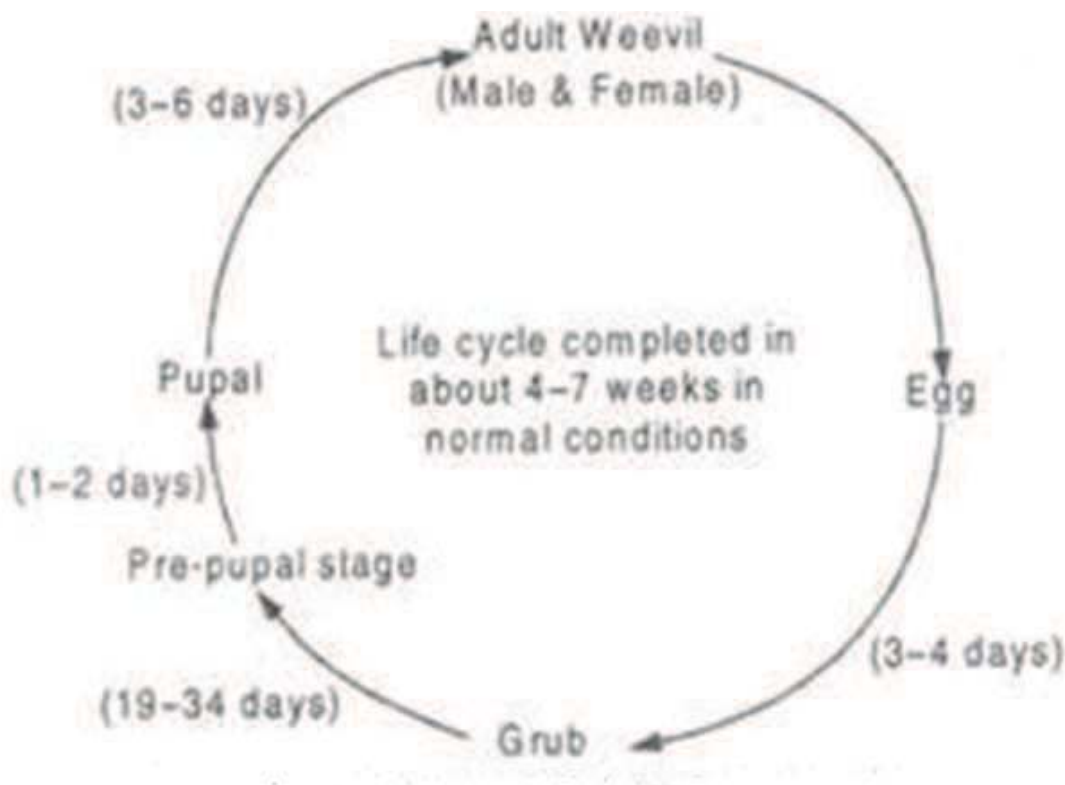


Fig 11.4 Life cycle of the *Sitophilus oryzae*

**Nature of Damage:** As adult rice weevil can fly, they fly down to nearby fields where it infests the ripe grains, both adults & larva feed upon the grains making them unconsumable. The damage caused by them extent up to 50% of the total grain stored at a particular place. Larvae are the more destructive as they feed voraciously on the content of the grain but leave the shell of the grain intact. Adults also feed upon flour i.e. milled cereals but the larvae cannot develop in it unless the material is caked. Feeding causes clustering of grains producing foul smell.

**Control-Measures:** For the control of rice weevil following methods should be adopted-

**1. Physical & Mechanical Control:** The weevil is unable to breed at a grain moisture content of 9%, hence dry storage of grains can avoid infestation by the pest. Previously infested grain & its debris should be removed out from trucks beds, transport wagons, grain dumps & elevator buckets to avoid re-infestation during new storage. Ceilings, walls, ledger, braces & handling equipments should be thoroughly cleaned or removed which are ideal places of hiding. Thermal disinfestation techniques should be implanted by drying grains on a concrete platform by the increasing the temperature up to 60<sup>0</sup>C for 10 minutes. Mechanical disinfestation includes refinement of grains from simple turning of grains through bulk handling systems to the use of sophisticated percussion machines in flour mills etc.

**2. Chemical Methods:** This method includes use of various chemical techniques like fumigation, insecticide, aerosol sprays, traps etc to eradicate rice weevil.

**(i) Fumigation:** It is the use of toxic gas to disinfest a commodity in an enclosure, the purpose of fumigation is to obtain immediate relief from pest. Phosphine is mainly used for fumigation against rice weevil.

**(ii) Insecticides:** These chemicals are used as surface layering for eradication of insects; these are available as dust formulations admixed with cereals or as liquid treatments.

**E.g.** Malathion, Lindane, Permethrin etc

**(iii) Aerosol Spray:** After vacuuming of the storage areas, it is disinfested by spraying FS MP Aerosol which is odourless & fast acting. It can be used in those areas which are left unaffected by other means such as sink, pantries, cabinets, cervices, cracks etc where adults & larvae of the pest likes to hide. Another spray used is PT-PHANTOM.



(iii) **Rice-weevil Traps:** These traps act on the pheromone, it is placed where weevil suspect activity is observed. This trap uses strong pheromones or attractants to lure adults, once they crawl or fly into the holding tray, the thick catching oil will hold them.

(2) **Biological Control:** The organism used for the predation of rice weevil is parasitoids *Anisopteromalus calandrace* which attacks the larvae & adults & stop their development.

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### 11.5 WHEAT WEEVIL (*Trogoderma granarium*)

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**Wheat Weevil (*Trogoderma granarium*):** The pest is cosmopolitan in distribution; it is also called as cabinet beetle. It initially originated in South Asia, wheat weevil is regarded as the most destructive pest of the grain products & seeds.

#### Systematic Position:

**Kingdom** – Animalia  
**Phylum** – Arthropoda  
**Class** – Insecta  
**Order** – Coleoptera  
**Family** – Dermestidae  
**Genus** – *Trogoderma*  
**Species** – *granarium*

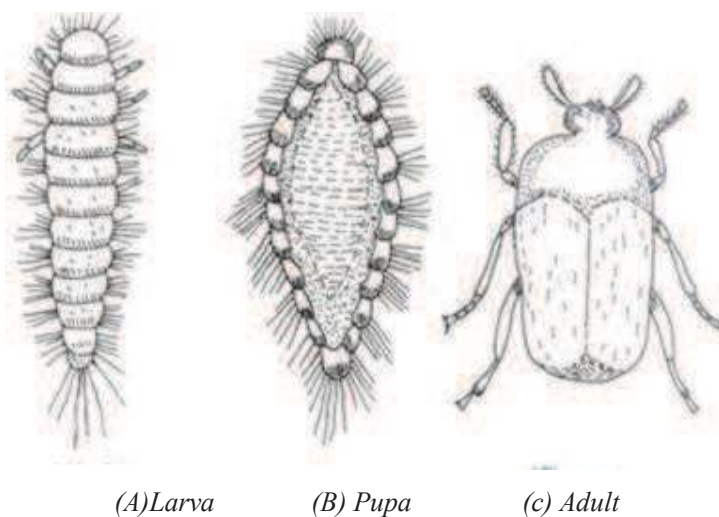


Fig 11.5 *Trogoderma granarium*

**Habits:** *Trogoderma granarium* is considered as the worst invasive species worldwide, it is a major pest of wheat in the Indian subcontinent, U.S.A, U.K & Germany. It is more commonly found in the warmer & dry regions with low moisture content food, beside wheat it also infest barley, oats, rye, maize, rice, flour, malt & noodles also. It is omnivorous in nature as it feeds upon dried blood, dried milk, fish meal, wool, goat skins, dead mice & dried insects. The adults are characterized by oblong-oval, light to dark brown coloured body bearing markings; body is covered with fine hairs which give a velvety appearance. Elytra contains indistinct red brown markings, rostrum is well developed especially in males. Eyes are emarginated; head is small & usually deflected with 11 segmented antennae. Males are

smaller than females, adult weevil have wings but usually don't fly & feed very little. Adults are short lived as life span of males is 7-12 days where as that of females is 20-30 days but mated females live up to 4-7 days only.

**Life-Cycle:** After 2-3 days of emergence, copulation takes place between adult beetles, the Female start laying eggs after 5 days of mating. The eggs are generally laid in crevices in godown or in grain heaps, average number of eggs laid by a single female per day is 25 which last for 5-7 days. The eggs are laid 40°C which are initially milky white, later pale yellowish, typically cylindrical with one end rounded & the other more pointed bearing a number of spines like projections, broader at the base & tapering distally. Eggs are laid loosely & singly in the host material, which hatch in 8-14 days at optimum conditions. There are five moults in the development of larvae & cast skin is shed following each moult, larval duration lasts for 30-50 days. During unfavourable conditions the number of moults increase up to 8-10 level & the larval duration prolong for a period of 200 days to 4 years. In winters or in scarcity of food the larvae enter diapause & development ceases, it remains inactive & lives in crevices, cracks or other concealed places. Young larvae are unable to feed upon whole grains & depend on damaged grains. Larvae are uniformly yellowish white, except head & body hairs are brown, as the larvae increases in size their body colour changes to a golden or reddish brown & more body hairs develop. Head is barbed with segment like constrictions & tail is shorter. At the last ecdysis i.e. moulting of cuticle, the larval skin splits but the pupa remains within the skin for the whole of its life. After 6-16 days of pupal period adult weevils emerge out which become sexually mature immediately. The development takes 4-6 weeks for completion at 95°F optimal temperature.

**Nature of Damage:** It is considered to be a serious pest of stored wheat grains along with it also attacks rice, maize, oat, jaw pulses, oilseeds & other products like copra, dry fruits etc. The damage to the grains is caused by the larval stages while the adults are harmless as they do not feed. This pest is mostly active during July-October period in which they are capable of causing heaviest damage to stored grains. The infestation occurs mainly at superficial layers of grain as the pest is not able to penetrate deep into the grain. The destruction of the embryo of the grain is the major damage caused by this pest but during heavy infestations complete grain is damaged. Reproduction of the beetle is so rapid that larvae are found in large numbers on the surface of grain, they spread generally by movement of infested goods & container during diapause condition. The most favourable conditions for multiplication & damage is in bulk grain under extended storage, apart from the destruction of grain products

by beetle, infested products contaminated with body parts, setae & cast larval skins result in gastro-intestinal irritation, asthmatics & allergens to sensitised individuals.

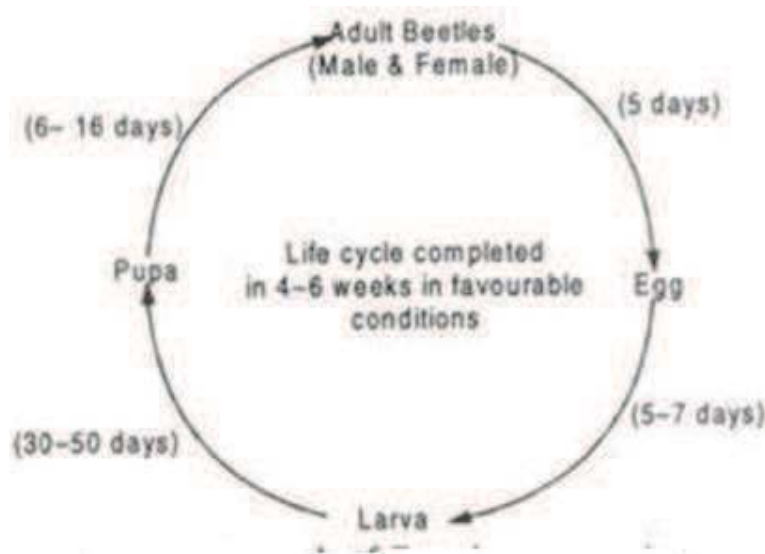


Fig 11.6 Life cycle of the *Trogoderma granarium*

**Control-Measures:** The obvious signs of kharpa beetle infestations are the larvae & cast skins, for its eradication following control measures should be adopted-

1. **Chemical Control:** This method includes use of various chemicals means such as fumigation, insecticide, irradiation, spray etc to eliminate the pest.

(i) **Fumigation:** The fumigants which are used to control kharpa beetle work at higher dosage as this beetle is resistant to fumigants which are generally used for other stored grain pests. High concentration of fumigants is to be maintained over the fumigation period to allow penetration into all cracks & crevices. The most effective fumigant is the Methyl Bromide which is used in enclosed conditions, other fumigants used is Phosphine at 10<sup>0</sup>C which helps in destroying of pest & its larvae.

(ii) **Irradiation:** Irradiation is the use of ultra-violet rays to sterilise the adult beetles & disrupt their life cycle, which keep a check at the population of weevil.

(iii) **Neem powder:** It is an effective & cheap method to control the pest in stored conditions where it repels the kharpa beetle due to its strong odor & also antipest ability.

2. **Physical & Mechanical control:** This type of control methods includes disinfestation of the commodity by heating, using trapping nets, cleaning of grains by machines etc.

(i) **Heating:** This step involves drying of wheat grains prior to storage by solar heat on a cemented surface or metal sheets, the grain temperature is increased up to 60°C & maintained for 10-20 minutes, which kills the live pests of grains i.e. larvae & adult weevils.

(ii) **Trapping nets:** These are used in warehouses & other storage facilities; it combines a feeding attractant for larvae while a pheromone for adult males. The method is based on using sticky traps placed on a suspended position; these specialised traps used for the species *granarium* are wall mountable, known as the Biolure box trap.

3. **Biological Control:** Biological control is an efficient & healthier alternative method of chemical use in eradication of pests, the predator used to control the kharpa beetle is a parasitoid *Laelius padatus* (wasp) which stings the developing larvae & releases its egg inside the body. The developing wasp inside pest larvae feeds upon it & emerges out when it is dead.

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### 11.6 RUST RED FLOUR BEETLE (*Tribolium castaneum*)

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**Rust red flour beetle (*Tribolium castaneum*):** The weevil is commonly called as rust red flour beetle, it is cosmopolitan in nature. It initially originated in the Indian Subcontinent but now found throughout all tropical, subtropical & warm temperate regions of the world.

#### Systematic Position:

**Kingdom** – Animalia

**Phylum** - Arthropoda

**Class** – Insecta

**Order** – Coleoptera

**Family** – Tenebrionidae

**Genus** - *Tribolium*

**Species** – *castaneum*



Table 1. The mean developmental time days for *T. confusum* at 29°C.

Life stage	<i>T. confusum</i>
egg	5.5
larvae	22.4
pupae	7.0
Total	34.9

Fig 11.7

*Tribolium castaneum*

**Habits:** It is a worldwide serious pest of stored products particularly food grains like flour, cereals, pasta, biscuits, nuts, oil cakes, dried fruits, meal beans, dried pet food, dried flowers, chocolate seeds & even dried museum specimens. This beetle is the most important pest of stored products inside the home, food industry & grocery stores. It is different from the confused floor beetle (*Tribolium confusum*) by having different antennae shape & its ability to fly under stressed conditions. The body of the adult beetle is flattened reddish brown in colour, thorax & abdomen are distinct. Antennae are well developed, eyes are reddish black in colour, chewing mouth parts present & grooved wing covers with last few segments being abruptly much larger. Female beetles are polyandrous in mating behaviour i.e. during a single copulation period; a single female will mate with multiple different males. This polyandrous mating behaviour by female beetles is shown in order to increase their fertility assurance, by mating with an increased number of males, female beetles obtain greater amount of sperm. The adults are long lived as females can live up to 3-4 years.

**Life-Cycle:** After copulation a female beetle lays about 400-500 eggs, which are laid singly in flour & dust of the grains, they soon get covered with small particles of dust & flour as they are moist & sticky when freshly laid. Eggs are minute, slender & cylindrical in shape with rounded at both ends & of whitish colour, they hatch in 5-12 days under favourable conditions. The freshly hatched grub is small, worm like, cylindrical & wiry in appearance, body segments have a number of fine hairs and the terminal segment is furnished with a pair of spine like appendages. The grub is pale yellowish in colour, larvae go through 5-12 instars and the larval period ranges from 27-29 days under favourable conditions. Pupation takes place on the surface of food; pupa is naked in appearance, initially white but gradually becomes yellowish, pupal stage lasts for 5-7 days. The total life cycle from egg to emergence of adult takes about 6 weeks, there are about 4-7 generations in one year.

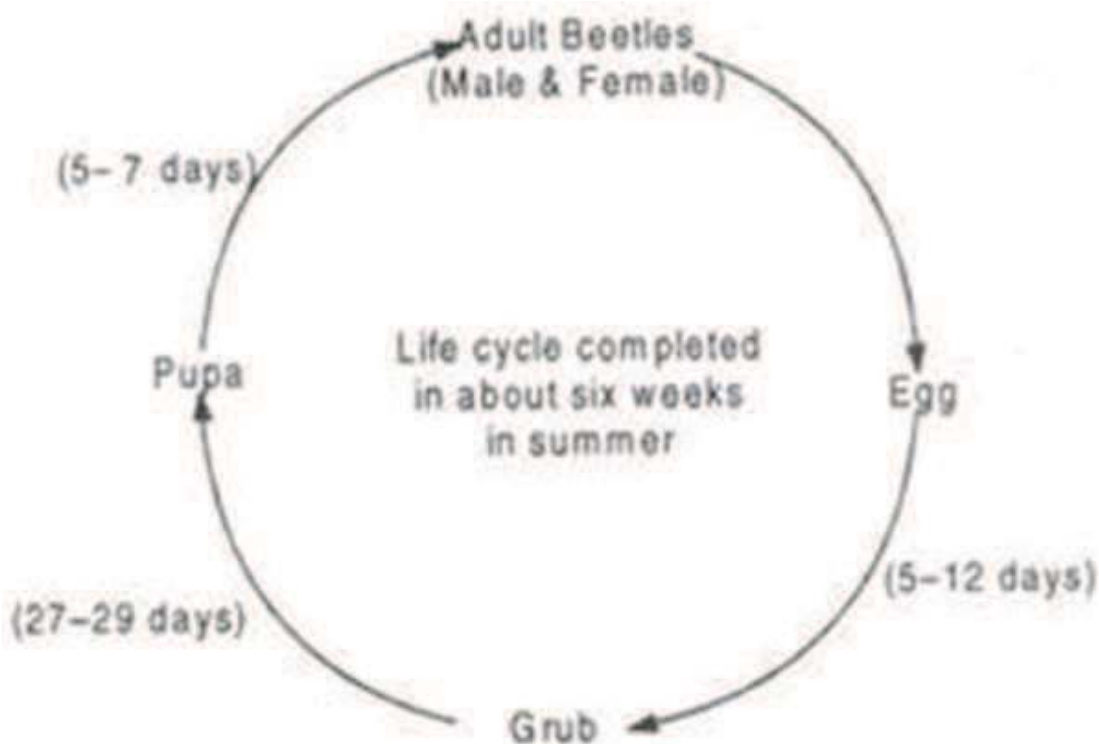


Fig 11.8 Life cycle of the *Tribolium castaneum*

**Nature of Damage:** This pest is found infecting all stored products, both the larvae & adult cannot damage sound grains, but they feed on those grains which already have been damaged by other pests. The main strength of rust red flour beetle is its high reproductive rate, quick maturing to adult stage with easy dispersal & migration which leads to a new colony with help of few individuals. It is a serious pest of prepared cereal products such as atta, suji & maida found in abundance in flour mills, in case of heavy infestations flour & maida turns greyish-yellow & develop red taints which become mouldy & emits pungent foul smell. It also damages the seeds & grain by feeding internally, contaminating with its larval casts & faeces.

**Control Measures:** *Tribolium castaneum* can be controlled by the implantation of the following measures which are as follows –

1. **Chemical measures:** Applications of chemicals sometimes become essential for complete mortality & prevention of the weevil.

(i) **Fumigation:** It is a procedure by which stored grain pests are removed by means of poisonous gas called as fumigant. The fumigant is produced & concentrated as a gas is lethal for the specific living pests, the gas penetrates into the interior of largely invisible, incipient

eggs & larvae. Generally Methyl bromide, Magnesium & Aluminium phosphide are used as fumigants.

(ii) **Insecticides:** These are used as surface treatments by making a cover or layer above the infested commodity with the pests. These insecticides come in various forms like powder & liquid. Examples are Tetrachlorovinphus, Melathion, Deltamethrin, Diazinon etc.

2. **Physical & Mechanical methods:** It involves following methos-

(i) **Reducing intergranular space:** As adult beetles being very soft & weak at the beginning cannot move freely in grains & are restricted to top layer hence to stop their movement deep into the grain mass is done by placing 7-10 cm layer of dry sand at the top of grains. To prevent mixing of sand with the food, a paper or polythene sheet is placed on the top surface of commodity.

(ii) **Coating with Oil:** This technique is only for the small quantity of grains in which non-drying oils is mixed uniformly with whole grains are kept in closed container. The layer of oil prevents laying of eggs or larvae hatching on grain surface. The oils preferred for this purpose are Neem oil, Vegetable oil, Castor oil, Niger oil, Sesame oil etc.

(iii) **Drying heating:** In this method solar drying of infested grain is done on a cemented platform & the temperature is raised upto 60<sup>0</sup>C & maintained for 20 minutes, which kills the pests if any present. Solar absorbance surfaces are best used for this technique.

3. **Biological control:** The rust red flour beetle can be controlled by parasites, parasitoids & predators that affect its different stages of life cycle.

(i) **Entomopathogens:** A fungus *Brauveria baesiana* is used as an entomopathogen of the beetle which causes white muscardine disease, leading to formation of microscopic spores which when come in contact with the body of pest germinates, penetrates cuticle & grow inside killing the pest within few days. It is an epidemic disease which travels from one individual to another leading to massacre of beetles.

(ii) **Parasites:** The mite *Acarophenax lacunatus* which is parasitic in nature is used to feed upon the larvae of the beetle & stops its growth.



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### 11.7 LESSER GRAIN BORER (*Rhizopertha dominica*)

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#### Lesser Grain Borer (*Rhizopertha dominica*)

It is commonly called as lesser grain borer & Australian wheat weevil, it is considered as second destroyer of stored grains after rice weevil. It initially originated in the Indian subcontinent, but now is cosmopolitan in distribution.

#### Systematic Position:

**Kingdom** – Animalia

**Phylum** - Arthropoda

**Class** – Insecta

**Order** – Coleoptera

**Family** – Bostrichidae

**Genus** - *Rhizopertha*

**Species** – *dominica*

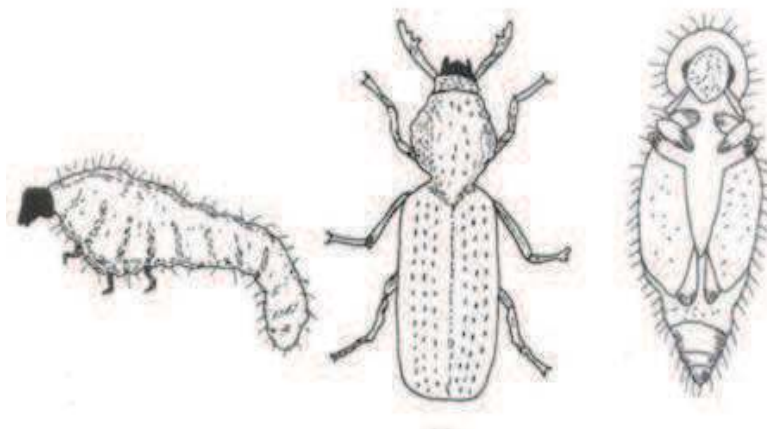


Fig 11.8 *Rhizopertha dominica*

**Habits:** It is the serious pest of stored products throughout the tropics, Australia & USA. It is also found in temperate countries due to its ability for prolonged flight & international trade of food products. It is found mainly in cereal & food stores also in animal feed processing facilities, it largely feeds on stored cereal seed including wheat, maize, rice, oats, barley, sorghum & millets beside these it is also found on a wide variety of foodstuffs including beans, dried chillies, turmeric, coriander, ginger, carrava chips, biscuits & wheat flour. It can be distinguished from other store pests by its cylindrical shape & small size, it measures about 3mm in length. Adult beetle is polished dark brown or black in colour with a roughened surface. Head is inserted into a hood-like triangular structure lying under the thorax; head possesses powerful jaws with which it causes serious damage to grain & also any part of the wooden structure in the store to hide over unfavourable conditions. The elytra are parallel sided; head is not visible from above with propotum having rasp like teet at front.



**Life-Cycle:** After copulation the mother beetle lays 300-500 eggs singly or in clusters near the embryo end of the grains, eggs can also be simply dropped down between grains & are also laid in powdery starchy materials lying outside. Eggs are pear shaped; glistening white when freshly laid, but become pinkish opaque as the larvae start developing inside the egg shell. The duration of egg stage is 5-6 days during summer, while 7-11 days during winter, the newly hatched larva is quite active & is campo-deiform in shape i.e. C shaped grub creamy white in colour. It burrows at once into the grain or crawls' actively feeding on the loose starchy material; grub completes its development either within grains or in the flour where it undergoes larval mounds. There are three larval instars; the first two instars are not recurved while the third & fourth instars have head & thorax recurved towards abdomen. A fully grown larva is dirty white in colour with brown head, which is retracted into thorax, three pairs of legs with swollen anterior end. The whole body is covered with tiny hairs, average larval period is about 40 days, pre-pupal & pupal period's lasts for a week & it changes into adult form. The adults eat their way out of the grain, which are winged & may fly, the total life cycle from egg up to emergence of the adult is about 6-8 weeks. There are generally 5 generations in a years, adults live for 4-8 months, life cycle may be completed outside the grain as it is found breeding on flour.

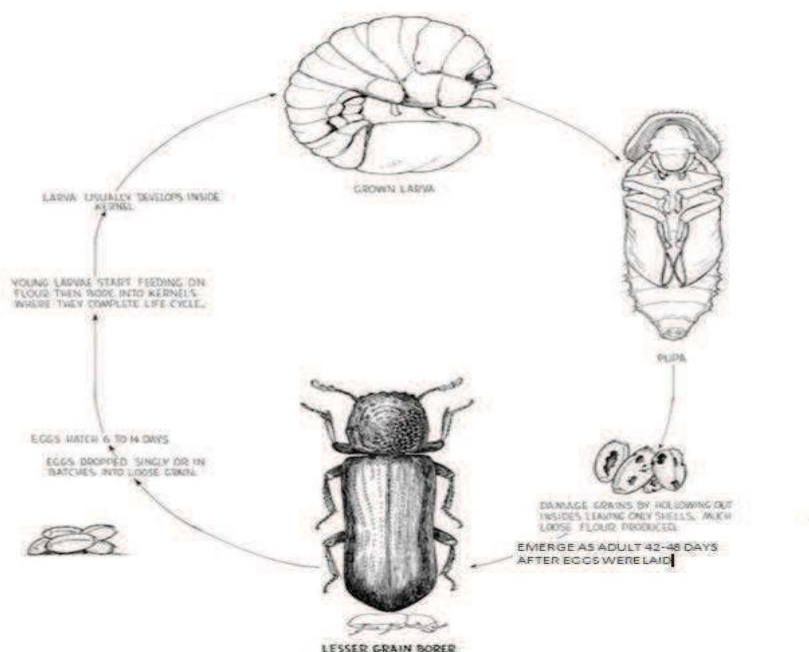


Fig 11.9 Life cycle of the *Rhizopertha dominica*

**Nature of Damage:** Both the larvae & adults are destructive in nature i.e. primary pests; it bore irregularly shaped holes into whole grains causing weight loss of grains. It reduces rice to dust & also produces more faeces i.e. excreta than any other species, it can tolerate the moisture content as low as 7 percent. Grains when heavily attacked by this pest are hallowed out, until a thin shell remains, the larger grains such as maize up to 4 beetles can be found at a time in a single grain. If the female lays eggs away from grains only the first stage larvae are able to enter the grain because of their straight body while the second stage larva due to its curved shape cannot penetrate the grain. It causes weight loss of grain up to 40% besides any other species of stored grain pest, it also reduces germination rates & vigour of the grains letting it easy prey for secondary pests & fungi. The larvae of beetle emerging out from eggs laid near the grain straight way enters into the grain.

**Control-Measures:** Prevention is always the most economic & efficient method of controlling these pests, following methods can be adopted to eradicate the pest.

#### 1. Chemical control

(i) **Fumigation:** Once the pest is distributed within the grain mass, fumigation is the only method of relieving the problem. Phosphine is the commonly used fumigant to control insect infestations in stored conditions; other fumigants used are Magnesium phosphide, phosphine mixed with CO<sub>2</sub>.

(ii) **Insecticides:** These are generally sprayed on surface as they degrade faster, the period of protection depends upon commodity treated temperature, grain moisture content & insecticide used. These remove pests populations from buildings, store house & godowns. The commonly used insecticides for eradication of the weevil are Wheat-ENT47, Soyabean-ENT13, Corn-ENT16, Neonicotinoids, Methoprene & diflubenzuron etc.

(iii) **Insect growth regulators (IGR):** These are sprayed or dusted directly onto the grain & protect grain from infestation from two weeks to one year. It controls the adults by suppressing progeny production & also causes mortality, these IGR have low toxicity to mammals but takes longer to control the pest & are more expensive than other insecticides, fumigants & chemicals. Commonly used IGR are Methoprene, Pyriproxyfen, Lufenuron & Fenoxycarb.

2. **Biological control:** By the use of natural enemies like predators, parasites, parasitoids & entomopathogens.

(i) **Predators:** *Ferretiosoma nigrescans* i.e. clown beetle is able to feed on the larvae. *Xylocoris flavipes* i.e. reutes feeds upon the instar larval stages of the pest.

*Cheyletus eruditus* known as hunting mite feeds upon eggs of the beetle.

(ii) **Parasites & Parasitoids:** Parasitic egg mite *Acaroph enax lacunatus* is used as a parasite of the beetle. Generally hymenoptera parasitoid used against the beetle are *Anisopteromalus calandrea* & *Choetospila elegans* are effective in reducing beetle as they are very small, do not feed on grain & can be easily removed from grains as it attacks the larva of beetle that are feeding inside the grain.

(iii) **Entomopathogens:** These are bacteria, fungi, protozoans or viruses which act as endoparasite of insects that enters into host through natural body openings & causes virulence. The commonly used entomopathogens for the beetle are Bacteria(*Bacillus thuringiensis*), Nematode(*Steinernema felitae* & *Steinernema carpocapsae*), Fungi(*Beauveria bassiana* & *Metarhizium anisopiliae*).

3. **Physical & Mechanical control:** The measures to control insect infestation generally used are cleaning & drying, mechanical disinfestations includes refinement of the grain through simple handling system to the use of sophisticated machines like entomoleters etc. Physical control includes thermal disinfestations by heating of grains through simple exposure to the heat of sun. In this traditional procedure disinfestation is achieved by exposing grain in thin layers to the sun light on a cemented platform that will drive off any adult or larvae. Hot air grain drying systems are also used for this purpose in the reconditioning of infested grain.

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## 11.8 SUMMARY

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Insects are of great importance to the mankind from centuries, some are beneficial while others are harmful in different ways. Insects cover more than  $\frac{3}{4}$  of the entire world fauna, insects attack a large number of crops in the field & causes great loss to human health & economy. Of all demands of man, food is the prime importance & because of population pressures the task of increased food production has become all the more important. Human struggle against pests of stored grains is very old, as it loses one third of the food produced to pests around the globe. The numbers of known insect species causing damage to crops are about 1000 out of which 70 species are responsible for maximum loss.

Most of the pests of store grains belong to order Coleoptera which contains mostly Weevils & beetles. The word "coleoptera" is from the Greek, *koleos*, meaning "sheath"; and, *pteron*, meaning "wing", thus "sheathed wing", because most beetles & weevils have two pairs of wings, the front pair, the "elytra", being hardened and thickened into a shell-like protection

for the rear pair and the beetle's abdomen. The order contains more species than any other order, constituting almost 25% of all known animal life-forms. The diversity of beetles & weevils is very wide-ranging. They are found in almost all types of habitats. The major insects damaging food products are Pulse beetle, Rice weevil, Wheat weevil, Rust red flour beetle and lesser grain borer.

Pulse beetle (*Callosobruchus maculatus*) is a serious pest of stored gram & cowpea, grub is the infective stage for stored food grains. The holes on grains are the first evidence of infestation; infested grains become useless for human consumption. Rice weevil (*Sitophilus oryzae*) is the most common & widely spread stored food pest around the globe, its larva are more destructive causing damage up to 50%. It not only attacks rice but many other cereals & food grains.

Wheat weevil (*Trogoderma granarium*) is also called as cabinet beetle, the worst invasive species worldwide. There are five moults, omnivorous in nature, rapid reproduction causing heavy infestations to the food grains. Rust red flour beetle (*Tribolium castaneum*) is called as rust red flour beetle, cosmopolitan & polyandrous mating nature. A serious pest of prepared cereal products, infecting internally & producing foul smell in food products, flies under stressed conditions.

Lesser grain borer (*Rhizopertha dominica*) it is the Australian wheat weevil, possess ability for prolonged flight. 5 generations in a year, both the larvae & adults are destructive as causing weight loss of grains. It reduces rice to dust & produces fraes in larger amounts. Control measures of food grain pests are necessary as they causes heavy economic loss, which includes chemical, mechanical, physical & biological.

Chemical control produces immediate effect as it removes heavy infestations by the help of fumigation, insecticides, insect growth regulators & aerosol sprays but they are harmful to humans also as produces delitrous effects when chemically treated food grain consumed. Physical method involves thermal drying & mechanical method includes cleaning, drying, refinement & use of sophisticated machines. Biological control is the safest method for pest control as it has no side effects upon human health; it uses natural enemies like parasites, predators, parasitiods, entomopathogens & hormonal traps.

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## ***11.9 TERMINAL QUESTION & ANSWER***

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### **Self assessment Questions**

#### **Long answer type questions**

1. Describe the habit and life cycle of Pulse beetle in detail?
2. Explain the nature of damage caused and control measures of Rice weevil?
3. Explain the life cycle of Wheat weevil with help of diagrams?
4. What are the control measures of store grain pests explain in detail?

#### **Short answer type questions**

1. Name some storage pests belonging to Tenebrionidae?

**Ans.** Red flour beetle & Long headed flour beetle.

2. Name a dermestid storage pest?

**Ans.** Khapra beetle is a dermestid storage pest.

3. Name the pests that occur both in the field and storage?

**Ans.** Rice weevil & pulse beetle are the pests that occur both in field & storage.

4. Pulse beetle prefers which type of pulse?

**Ans.** Pulse beetle prefers split pulses.

#### **Fill in the blanks**

1. Presence of irregular holes of 1.5 mm diameter on grains of rice, sorghum, wheat, barley, maize in storage is due to attack by \_\_\_\_\_
2. Merely placing the gunny bags on the heap of grains helps in the collection of \_\_\_\_\_
3. Adults of \_\_\_\_\_ are powerful fliers and can move across godowns
4. Long headed flour beetle: *Latheticus oryzae* resembles \_\_\_\_\_

5. Gaseous quinones released to the medium produces a readily identifiable acid odour in heavy infestations of \_\_\_\_\_

**Multiple choice questions**

1. Adult beetles infesting stored products are characterized by:  
**a.** rather hard shell-like bodies **b.** soft, very fragile bodies **c.** transparent body walls **d.** body covered with scales and hairs
2. In general, stored grain mass temperatures above allow insects to survive in lower moisture content:  
**a.** 30°F **b.** 40°F **c.** 50°F **d.** 60°F
3. The stored grain infesting weevil does not fly.  
**a.** Maize weevil **b.** Granary weevil **c.** Rice weevil **d.** Lesser grain weevil
4. Pheromone and food attractant traps may include:  
**a.** Perforated plastic probes **b.** Multi-layered corrugated paper **c.** Wing, delta, and diamond traps **d.** All the above
5. To be effective fumigants must:  
**a.** Be applied in enclosed areas **b.** Reach a lethal concentration in all areas **c.** Be held at a lethal concentration for a minimum amount of time **d.** All the above
6. The pheromones used in warehouse storage of processed commodities attract both sexes of the species:  
**a.** Repelling **b.** Sex **c.** Aggregation **d.** Reproduction

**Answers**

**Fill in the blank:**

1. Rice weevil: *Sitophilus oryzae*
2. Khapra beetle: *Trogoderma granarium*
3. Lesser grain borer: *Rhizopertha dominica*
4. Tribolium castaneum
5. Red flour beetle: *Tribolium castaneum*

**Multiple choice questions**

1. a
2. d
3. b
4. d
5. d
6. c

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**11.10 GLOSSARY**

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**Antennae:**paired sensory organs originating on the insect head

**Apex:**the portion of a body part farthest from the base or point of attachment. The apex of the elytra is the portion at the rear end of the elytra.

***Bacillus thuringensis:*** A bacteria used as biopesticide.

***Coleoptera:****the order comprised of the beetles; sheath winged*

**Cowpea:** A food-legume crop belonging to the Vigna family, resistant to dry climate conditions.

**Diapause:**a condition of suspended animation; no activity or development occurs

**Elytra:**first pair of wings that are modified to form a hard shell

**Flour beetle:** A pest which feeds with grains and flour, resistant to pesticides

**Grain borer:** A cosmopolitan beetle living in and that eats stored grain

**Grub:** an insect larva in the Order Coleoptera

**Head:**the first or anterior division of the insect body where the eyes and antennae are found

**Integument:** outer covering or cuticle of the insect body

**Instar:** The growth period between molts. (The term is usually applied to arthropods.)

**Khapra beetle:** Pest of stored grains

**Larval:** pertaining to immature stage of juvenile insects

**Mandibles:** first pair of jaws in insects

**Oil cake:** Small residues of oil seeds after oil extraction, used as fodder

**Organochlorine insecticide:** A kind of insecticide which is not easily cleared, as it is passed along the whole alimentary chain, and has a strong impact on the environment

**Snout:** lengthening of the head to give the appearance of a nose or snout

**Ventral:** The under surface of the abdomen; from below

**Weevils:** Any beetle from the Curculionoidea superfamily. Weevils are less than 6mm (0.24in), and herbivorous

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## 11.11 REFERENCES

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## **UNIT 12 IPM [INTEGRATED PEST MANAGEMENT]**

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12.1 Objective

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12.3- Basic concept of Pest Management

12.4 Biological and Chemical control

12.5 Elementary knowledge of pesticides and integrated pest management

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12.8 Terminal Question & Answer

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## 12.1 OBJECTIVE

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- The objective of this chapter is to keep the pest number below ETL (economic threshold level) instead of their eradication.
- To understand the various types of pesticides.
- To understand the conservation of environment including bio-diversity.
- To understand how crop protection be made feasible, safe & economical.
- Understanding various preventive measures & their health effects.

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## 12.2 INTRODUCTION

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**Integrated pest management (IPM)**, also known as **integrated pest control (IPC)** is a broad-based approach that integrates practices for economic control of pests.

The concept of Integrated Pest Management is nothing new and is widely implemented on field crops and orchards throughout the world. Implementation in the urban environment, in home gardens, landscapes, golf courses and structural settings, presents special challenges. Urban IPM or pest control programs that incorporate reduced use of pesticides in homes, private and commercial landscapes, golf courses and structural settings, is an expanding field with increased support from university and industry research.



After World War II, the research scientist and chemical industries started to produce pesticides of various types. Plant protection measures have included legal, physical, mechanical, cultural, biological, chemical etc. singly or in combination, though with different degree of success. The physical measures involve mainly cold storage and exposure to dry or steam heat. Irradiation of grains and use of electric shocks are yet on a pilot scale. Mechanical measures are used locally for specific cases. The cultural methods of clean cultivation, destruction of infested plants and crop rotation etc. are widely adopted biological control measures has been successful in case of some insect pests but chemosterilization has not yet made any major impact in the field of plant protection.

### **Introduction to IPM**

IPM is based on taking preventive measures, monitoring the crop or site for the level of the pest(s), assessing the potential for pest damage, and choosing appropriate actions. Many different tactics may be available, including cultural practices, biological control agents, pesticides, pest-resistant varieties, mechanical methods and physical barriers. In IPM, these tactics may be combined into a plan that best suits the particular situation. It is a comprehensive approach dedicated to removing causes rather than just treating symptoms.

Almost all farmers do at least some IPM through normal crop production practices. Integrated pest management is a balanced, tactical approach to pest control. It involves taking action to anticipate pest outbreaks and to prevent potential damage. IPM utilizes a wide range of pest control strategies or tactics. The goal of this strategy is to prevent pests from reaching economically or aesthetically damaging levels with the least risk to the environment.

IPM programs are very site-specific. IPM is based on the identification of pests, accurate measurement of pest populations, assessment of damage levels, and knowledge of available pest management strategies or tactics that enable the specialist to make intelligent decisions about control. IPM offers the possibility of improving the effectiveness of pest control programs while reducing some of the negative effects. Many successful IPM programs have reduced pesticide use and increased protection of the environment.

Pesticide use is and will continue to be significant in food and fiber production, forestry, turf and landscape maintenance, and public health. Pest management has shifted from relying

heavily on pesticides to using an integrated approach based on pest assessment, decision making, and evaluation.

### Defining IPM

Since the 1930's, over 60 definitions of IPM have been published. Here is a basic definition which will be used.

**Integrated Pest Management (IPM)** is the coordinated use of pest and environmental information along with available pest control methods, including cultural, biological, genetic and chemical methods, to prevent unacceptable levels of pest damage by the most economical means and with the least possible hazard to people, property, and the environment".

**Integrated** means that all feasible types of control strategies are considered and combined as appropriate to solve a pest problem.

**Pests** are unwanted organisms that are a nuisance to man or domestic animals, and can cause injury to humans, animals, plants, and property. Pests reduce yield and/or quality in plants ranging from field crops, fruits and vegetables, to lawns, trees, and golf courses.

**Management** is the process of making decisions in a systematic way to keep pests from reaching intolerable levels. Small populations of pests can often be tolerated; total eradication is often not necessary, or feasible.

### The Basics of IPM

All of the components of an IPM approach can be grouped into three activities. The first is **monitoring**; the second is **assessing the pest situation**; and the third is **taking action**.

- (i) **Monitoring:** Supervising activities in progress to ensure that they are on course and on schedule, in meeting the objectives and performance target.
- (ii) **Assessing the pest's situation:** It is a frame work of macro environmental factors used in the environmental scanning component of strategic management.
- (iii) **Taking action:** Researches on attractants, repellants, pheromones, chemosterilants, hormones, insect pathology, insect nutrition, host resistance and biological control has great importance. For success through integrated pest management a well balanced training programmes with high competence, dedication with cooperativeness is essential among the entomologist of the field.

**IPM is information intensive** and relies on scouting and monitoring programs for the collection of field data about key factors such as:

- Pest population identification
- Disease pressure
- Weather conditions and degree-days
- Pest date of first occurrence of biological events in their annual cycle
- Crop growth stage
- Presence, reliance and preservation of beneficial organisms

**IPM uses decision support systems** for determining if control measures are necessary and what measures are most appropriate. Such as:

- Economic thresholds - the pest population level that inflicts crop damage greater than the cost of control
- Availability of selective pesticides
- Action levels - pest level when action should be applied to prevent pest from reaching injurious levels
- Environmental risk measurements (i.e. impacts on pollinators)
- Disease forecasting systems

**IPM programmes seek to avoid pest damage** through practices such as:

- Use of field sanitation and reduction of pest habitat
- Crop rotations
- Selection of pest/disease tolerant or resistant seeds and varieties
- Judicious use of pesticides that prevent pest infestations
- Resistance management

### **Why Practice IPM?**

You might be wondering why you should even consider IPM when pesticides so often succeed at controlling pests. Here are some reasons for using a broader approach to pest management than just the use of pesticides.

- **Many IPM practices** are used before a pest problem develops to prevent or hinder the buildup of pests.
- **Keep a Balanced Ecosystem.** Every ecosystem, made up of living things and their non-living environment, has a balance; the actions of one creature in the ecosystem

usually affect other, different organisms. Many of our actions in an ecosystem can change this balance, destroying certain species and allowing other species (sometimes pests themselves) to dominate. Beneficial insects, such as the ladybird beetle and lacewing larvae, both of which consume pests, can be killed by pesticides, leaving fewer natural mechanisms of pest control.

- **Reliance on Pesticides can be Problematic.** Pesticides are not always effective when used as a singular control tactic. Pests can become resistant to pesticides. In fact, some 600 cases of pests developing pesticide resistance have been documented to date, including populations of common lamb-quarters, house flies, Colorado potato beetle, Indian meal moth, Norway rats, and greenhouse whitefly.
- **IPM Is Not Difficult.** You will have done much of the “work” for an IPM approach if you’ve figured out the problem (the pest), determined the extent of the pest population, and decided on the best combination of actions to take.
- **Maximize Effectiveness of Control Tactics.** Pest control practitioners, following traditional programs, sometimes apply pesticide treatments on a calendar based schedule regardless of the stage of development of the target pest and the number of pests present. Using an IPM approach will ensure that all control tactics, including pesticides, are used at the proper time and only to reduce pest damage to acceptable levels. This will reduce costs from unnecessary pesticide applications and insure that control tactics are used when they will be most effective.
- **Promote a Healthy Environment.** The definition of IPM promotes a careful consideration of all pest control options with protection of the environment a key goal.
- **Natural Enemies Conserved.** Parasites and predators are part of the natural control mechanism for some pest populations. These natural controls are considered and protected in an IPM program
- **Maintain a Good Public Image.** A thoughtful approach to pest control, which protects the environment and provides an abundant, affordable crop and safe living conditions, is a basic goal of IPM.

### How do IPM programmes work?

IPM is not a single pest control method but, rather, a series of pest management evaluations, decisions and controls. In practicing IPM, growers who are aware of the potential for pest infestation follow a four-tiered approach. The four steps include:

- **Set Action Thresholds**

Before taking any pest control action, IPM first sets an action threshold, a point at which pest populations or environmental conditions indicate that pest control action must be taken. Sighting a single pest does not always mean control is needed. The level at which pests will either become an economic threat is critical to guide future pest control decisions.

- **Monitor and Identify Pests**

Not all insects, weeds, and other living organisms require control. Many organisms are innocuous, and some are even beneficial. IPM programs work to monitor for pests and identify them accurately, so that appropriate control decisions can be made in conjunction with action thresholds. This monitoring and identification removes the possibility that pesticides will be used when they are not really needed or that the wrong kind of pesticide will be used.

- **Prevention**

As a first line of pest control, IPM programs work to manage the crop, lawn, or indoor space to prevent pests from becoming a threat. In an agricultural crop, this may mean using cultural methods, such as rotating between different crops, selecting pest-resistant varieties, and planting pest-free rootstock. These control methods can be very effective and cost-efficient and present little to no risk to people or the environment.

- **Control**

Once monitoring, identification, and action thresholds indicate that pest control is required, and preventive methods are no longer effective or available, IPM programs then evaluate the proper control method both for effectiveness and risk. Effective, less risky pest controls are chosen first, including highly targeted chemicals, such as pheromones to disrupt pest mating, or mechanical control, such as trapping or weeding. If further monitoring, identifications and action thresholds indicate that less risky controls are not working, then additional pest control methods would be employed, such as targeted spraying of pesticides. Broadcast spraying of non-specific pesticides is a last resort.

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## ***12.3 BASIC CONCEPT OF PEST MANAGEMENT***

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### History of Pest Management

- (i) 2500 BC First records of insecticides; Sumerians used sulfur compounds to control insects and mites.

- (ii) 200 BC Romans advocated oil sprays for pest control.
- (iii) 300 AD First records of biological controls; Chinese used predatory ants in citrus orchards to control caterpillar and beetle pests.
- (iv) 1880 First commercial spraying machine.
- (v) 1930 Introduction of synthetic organic compounds for plant pathogen control.
- (vi) 1940 First successful use of an entomopathogen; Milky Spore (*Bacillus popilliae*) used to control Japanese beetle.

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## ***12.4 BIOLOGICAL AND CHEMICAL CONTROL***

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### **Biological Control of Insect Pests:-**

Most pests have natural enemies that control or suppress them effectively in some situations. Natural enemies, including pathogens and insects, are being used successfully as biological control agents to manage certain directed against pests that are not native to a geographical area. Introduced pests often cause problems in their new locations because they lack natural enemies to help control them. Laws have been enacted that strictly control the importation of all organisms, including biological control agents, into the United States, to prevent these organisms from also becoming pests. Biological control also involves the mass release of large numbers of natural enemies into fields, orchards, greenhouses, or other locations to control specific pests. This method usually does not have long-term results, so these natural enemies must be released periodically. Several natural enemies are reared or cultured commercially. Predatory mites are used to control plant-feeding spider mites. Parasitic wasps and lacewings are used to control various insect pests. Nematodes and fungi are being studied as biological control agents for certain weeds and some insects. General predators, such as praying mantids and lady beetles, are sold with claims made for biological control. In many cases, however, their effectiveness has not been established.

Biological pest control is the use of a living organism to control another living organism. The importance of using biological control agents to control insect and disease pests is often overlooked. Biological agents of landscape pests include.

### **Predators:**



Common arthropod predators of insects include lacewings, predatory mites, minute pirate bugs, lady bird beetles and spiders. Either the adult or immature stage may prey on insect pests, so it is important to properly identify all the life stages of predator arthropods. Some predatory arthropods have greater impacts on pest populations than others. Vertebrate pest management should include the use of natural enemies. Examples include predators, such as hawks, owls and coyotes that prey on rodents. Natural enemies can be found in all habitats including landscapes, aquatic sites, and crop land and surrounding areas.

**Parasites:**

The life cycle of insect parasites develops in or on an insect host. The parasite feeds on body fluids or organs, usually killing the host. Common parasites include wasps, flies and nematodes. Most parasites are specialized in their choice of a host.

**Weed Feeders:**

Insects, grazing animals, and some fish, such as grass carp, consume plant leaves, stems, seeds, flowers and fruits. Insects are often specific to a single species of weed, while grazing animals and fish feed on a broader array of vegetation. Weed feeders seldom eradicate an infestation. However, they are useful in slowing the spread of weeds.

**Pathogens:**

Weeds, arthropods and vertebrate pests can be infected by pathogens, including viruses, bacteria and fungi. When environmental conditions are favourable for the pathogen, a disease outbreak can occur which may decimate the pest population. This same principle applies to disease outbreaks in all species, including humans. Most pathogens are specific to certain groups of plants or animals. A pathogen commonly found in soil is *Bacillus thuringiensis*, or “Bt”. Bt is a bacterial that is effective at controlling insects in their larval stage. It is used commercially to control mosquitoes, black flies and other insects. It is considered safe to humans and other non-target organisms. Biological control may be accomplished in one or a combination of several ways:

**Conservation:**

This is the process of using, protecting and encouraging existing populations of natural enemies. Examples of conservation include avoiding the use of insecticides when beneficial insect populations are high or providing nesting or roosting sites for birds of prey. Conservation is the most cost-effective form of biological control.

**Augmentation:**

This occurs when more individuals are added to an already existing population of biocontrols at a site. For instance, many species of predator and weed-feeding insects can be collected in the field or raised commercially, and may be released to increase existing populations to a level where they are effective against the pest.

**Importation:**

This method relies on introducing a population of beneficial organisms not currently present to a given site. This is often done to manage non-native pest species, such as the noxious weeds saltcedar and leafy spurge, or insect pests like the Russian wheat aphid.

The Nevada Department of Agriculture, in cooperation with USDA – Animal Plant Health Inspection Service (APHIS) and Plant Protection and Quarantine (PPQ) is using biological controls to manage a number of pests in Nevada.

Russian wheat aphid (*Diuraphis noxia*), a recently introduced insect, is a serious pest of barley, wheat and other small grains. Parasitic wasps, syrphid flies and different species of lady bird beetles have been released experimentally with the hope that they will contribute to the control of this damaging aphid. Attempts to control the noxious weed leafy spurge.

(*Euphorbia esula*) have included beneficial insects. Three species of flea beetle and a midge species have been released in Nevada in an attempt to decrease the population of this weed to manageable levels.

**Chemical Control of Insect Pests:**

Chemical controls are pesticides that are either naturally derived or synthesized. Pesticides often play a key role in pest management programmes and frequently may be the only control method available. Major benefits associated with the use of pesticides are their effectiveness, the speed and ease of controlling pests, and, in many instances, their reasonable cost compared with other control options. Usually pest damage stops or pests are destroyed within a few hours (for insects) to a few days (for weeds) after application of a pesticide. Using a fungicide may provide immediate, short term protection against microorganisms.

A pesticide is defined as any material that is applied to plants, the soil, water, harvested crops, structures, clothing and furnishings, or animals to kill, attract, repel, regulate or interrupt the growth and mating of pests, or to regulate plant growth. Pesticides include a

wide assortment of chemicals with specialized names and functions. They are commonly grouped according to the type of pest they control.

- **Avicides** control pest birds.
- **Bactericides** control bacteria.
- **Disinfectants (antimicrobials)** control microorganisms.
- **Fungicides** control fungi.
- **Herbicides** control weeds and other undesirable plants.
- **Insecticides** control insects and related arthropods.
- **Miticides (acaricides)** control mites.
- **Molluscicides** control snails and slugs.
- **Nematicides** control nematodes (roundworms).
- **Predicides** control predatory vertebrates.
- **Piscicides** control pest fish.
- **Repellents** repel insects, related invertebrates, birds, and mammals.
- **Rodenticides** control rodents.
- **Defoliants** cause leaves or foliage to drop from plants.
- **Desiccants** promote drying or loss of moisture from plant tissues.
- **Growth regulators** are substances (other than fertilizers or food) that alter the growth or development of a plant or animal.

Each group of pesticide includes several classes or families. For example, the classes of insecticides include, among others, the organophosphates, organochlorines, carbamates, pyrethroids, botanicals, insecticidal soaps, and microbials. The pesticides within a particular class have similar chemical structures or properties or share a common mode of action. The mode of action of a pesticide is how the pesticide works. In other words, it is what specific system in the pest is affected by the pesticide. The various classes of chemicals work in different ways and present different risks and problems.

Pesticides also vary in their selectivity. Fumigants, for example, are nonselective, controlling a wide variety of pests—fungi, insects, weeds, nematodes, etc. Some non-selective herbicides control any plant given a sufficient dose. In contrast, **selective pesticides** control only certain species of pests or affect only a certain stage of pest development. For example, certain herbicides control broadleaf weeds while not harming grasses and ovicides kill only the eggs of certain insects, mites, and related pests.

Pesticides may move in various ways after they come in contact with a host. **Systemic pesticides** are absorbed through leaves or roots and then transported within the treated plant. Similarly, systemic insecticides can be eaten by or injected into livestock to control certain pests. By contrast, **contact pesticides** are not absorbed by treated plants or animals. These pesticides must directly touch the pest or a site the pest frequents to be effective.

The use of pesticides in a proper manner and in accordance with the label. Examples of a pesticide application would include applying a dust pesticide into a wall void to control ants or using baits in a crack to control cockroaches.

Chemical controls include pesticides applied to manage pests. Pesticides should be viewed as a last-resort treatment to prevent significant damage to plants in the landscape, or as available and possibly necessary treatment for agricultural commodities or to protect human health. Pesticides are important tools, but they should be used only when necessary and in conjunction with other management tools. The development of a pest problem often signals poor management practices, so a review of the management protocols and cultural practices for a given landscape, field or property should be made prior to applying pesticides.

In the urban environment, the tendency is to use pesticides as preventative measures to ensure “perfect” landscapes. Pesticide use for this purpose is based on perceived threats from pests, but many times no actual pest has been identified and no damage is visible. Not only is this pesticide application philosophy expensive and unnecessary, it may also have significant environmental consequences. For example, over application of weed-and-feed-type products on lawns can have serious effects on adjacent ornamental plants, particularly trees planted in or adjacent to turf. The use of pesticides for structural and institutional pest control must first take into account the potential exposure to the residents of the building as well as potential health effects. When inside a structure, pesticides tend to break down more slowly than when in the outdoor environment, so residual effects must be considered. This limits the number and type of pesticides that are available for such applications. These products are highly regulated.

## ***12.5 ELEMENTARY KNOWLEDGE OF PESTICIDES AND INTEGRATED PEST MANAGEMENT***

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Pesticides are designed to kill bugs that are harmful to plants. Pesticides kill specific pests on plants such as slugs, beetles and flying insects. The chemicals used in most pesticides can kill more than just garden pests; they can kill the helpful organisms that live in the soil. Some of these chemicals can remain in the soil for years, effectively keeping necessary micro-organisms from working the soil.

Common chemical pesticides that are used in gardens and by large-scale crop producers include the following:

- Basic Copper Sulfate
- Silica Gel
- Sodium Fluoride
- Carbon Disulfide
- Hydrogen Cyanide
- Methylchloroform
- Fenthion
- Boric Acid

There are literally hundreds of pesticides that have been manufactured and applied to soil in the past. We are beginning to understand the ramifications of using these toxic chemicals on the soil. In places where the chemicals are used extensively, plants will no longer grow at all, or will fail to thrive.

### **Types of pesticides**

Many pesticides can be grouped into chemical families. Prominent insecticide families include organochlorines, organophosphates, and carbamates.

Type of Pesticide	Target Pest Group
Algicides or Algaecides	Algae
Avicides	Birds

Bactericides	Bacteria
Fungicides	Fungi and Oomycetes
Insecticides	Insects
Miticides or Acaricides	Mites
Molluscicides	Snails
Nematicides	Nematodes
Rodenticides	Rodents
Virucides	Viruses

## INTEGRATED PEST MANAGEMENT

Integrated pest management, or IPM, is a system of controlling pests by combining biological, mechanical, cultural, physical and chemical control methods in a way that minimizes economic, health and environmental risks. Pests are monitored by regular and careful inspections. The inspections identify pests and the conditions contributing to the pest problems. Based on the inspection the technician then decides what actions are necessary. The knowledge of the pests biology and habits will help in determining what methods or techniques would best control the pests at the lowest potential exposure possible.

IPM as "the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment.

### Integrated Pest Management Stands as:-

**Integrated:-** A focus on interactions of pests, crops, the environment and various control methods. This approach considers all available tactics and how these tactics fit with other agricultural practices used.

**Pest:** - An organism that conflicts with our profit, health or convenience. If a species does not exist in numbers that seriously affect these factors, it is not considered a pest.

**Management:** - A way to keep the pests below the levels where they can cause economic damage. Management does not mean eradicating pests. It means finding tactics that are effective and economical, and that keep environmental damage to a minimum.

### **Necessity of Integrated Pest Management**

For the safe and successful operation against pest population the integrated approach for pest control is required due to following reasons:

- (a) **Side effect-** The indiscriminate use of any control measure may cause harmful side effects in the environment.
- (b) **Environmental pollution-** Continuous use of any biological agent may cause unwanted change in the environment.
- (c) **Pest resurgence-** Continuous use of pesticides causes disruption of the environment and target species may recover from pesticide action.
- (d) **Pesticide resistance-** Pest may develop tolerance against pesticide being use.
- (e) **Secondary pest outbreak-** Due to application of pesticide, other non-target insects may attain the level of pest.
- (f) **Residual effect-** Pesticide residue may cause hazard to man or his pets.
- (g) **Non target species-** Pesticide being used continuously may damage useful species such as pollinators, wildlife etc.

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## ***12.6 SUMMARY***

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Integrated Pest Management (IPM) is an approach that emphasizes nonchemical pest prevention, focusing on facility maintenance and sanitation before considering chemical options for pest management. IPM is a multidisciplinary endeavour; it takes from branches of crop science and then assembles information from the disciplines like Agronomy, Entomology, Plant pathology, Agricultural Engineering & Climatology. The history of pest management gives us a perspective of how IPM came into being and how we have learned about effective ways to control pests. In 1967, the term “Integrated Pest Management (IPM)” was first used. Rapidly, the concept became popular to describe making informed

management decisions based on a thorough understanding of the factors that affect the pest or pests involved.

Integrated Pest Management (IPM) is the coordinated use of pest and environmental information to design and implement pest control methods that are economically, environmentally and socially sound. IPM promotes prevention over remediation and advocates integration of multiple control strategies to achieve long-term pest management solutions. IPM consists of gathering information, interpreting data, creating a flexible management plan, making timely decisions and taking the proper action. Information gathering and decision-making techniques include: accurate pest identification, learning about the weak link in a pest's life-cycle or biology, scouting and monitoring crops in fields and greenhouses, using action thresholds to minimize spraying, and keeping records of findings to assess the effectiveness of management decisions.

Along with information gathering and decision-making techniques, a variety of preventative and curative control methods are used to construct a complete IPM management plan for each pest, crop and farm. Cultural, mechanical, physical, genetic, and biological controls help prevent severe pest problems, while pesticides are used when additional control measures are required. **Cultural controls** are modifications of the crop production systems that suppress pest populations and occurrence which includes the use of better site selection, crop rotation, modifying planting times or plant spacing, improved water and nutrient management for better crop health.

**Mechanical and physical controls** consist of using supplies, equipment, or some factor, such as temperature, humidity or light, to disrupt pest life cycles and suppress populations by cutting, crushing, burying or excluding pests with implements and barriers, or by heating, cooling, drying, wetting, or regulating light. **Biological control** is the use of naturally occurring or introduced beneficial organisms to control or suppress pest populations. Biological control agents come in all shapes and forms including: beneficial insects, mites, spiders, nematodes, fungi, bacteria, viruses, protozoa and plants. **Pesticides** should be used in conjunction with the control measures previously mentioned and only when pest population densities will cause economic damage, or when environmental conditions favour disease. Selective insecticides are products that primarily target the pest you wish to control, with few or no detrimental effects on most beneficial. Broad-spectrum insecticides should only be used if no other viable options exist to manage the pest.



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## 12.7 GLOSSARY

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**Adult Stage:** The stage that is sexually mature and in which procreation occurs.

**Abdomen:** The posterior body division of an arthropod.

**Antenna (plural: antennae):** The paired segmented sensory organs, borne one on each side of the head, commonly termed horns or feelers.

**Biological control:** The action of parasites, predators, or pathogens in maintaining another organism's population density at a lower average level than would occur in their absence. Biological control may occur naturally in the field or result from manipulation or introduction of biological control agents by people.

**Broad-spectrum pesticide:** A pesticide that kills a large number of unrelated species

**Diapause:** A period of physiologically controlled dormancy in insects.

**Economic threshold:** A level of pest population or damage at which the cost of control action equals the crop value gained from control action.

**Exclusion:** Keeping a pest and crop separate from one another.

**Frass:** Solid fecal material produced by insects.

**Fumigation:** Treatment with a pesticide active ingredient that is a gas under treatment conditions.

**Infestation:** The presence of a large number of pest organisms in an area or field, on the surface of a host or anything that might contact a host, or in the soil

**Instar:** The larval or nymph stage of an immature insect between successive molts.

**IPM:** A pest management strategy that focuses on long-term prevention or suppression of pest problems through a combination of techniques.

**Juvenile:** Immature form of a nematode that hatches from an egg and molts several times before becoming an adult.

**Larva (plural: larvae):** The immature form of insects that develop through the process of complete metamorphosis including egg, several larval stages, pupa, and adult. In mites, the first-stage immature is also called a larva.

**Mandibles:** Jaws; the forward-most pair of mouthparts of an insect.

**Meconium:** Fecal pellet excreted by a larva before pupation.

**Metamorphosis:** The change in form that takes place as insects grow from immatures to adults.

**Molt:** In insects and other arthropods, the shedding of skin before entering another stage of growth.

**Monitoring:** Carefully watching and recording information on the activities, growth, development, and abundance of organisms or other factors on a regular basis over a period of time, often utilizing very specific procedures

**Nymph:** The immature stage of insects such as grasshoppers and aphids, that hatch from eggs and gradually acquire adult form through a series of molts without passing through a pupal stage.

**Oviposit:** To lay or deposit eggs.

**Pest resurgence:** The rapid rebound of a pest population after it has been controlled.

**Pheromone:** A substance secreted by an organism to affect the behavior or development of other members of the same species; sex pheromones that attract the opposite sex for mating are used in monitoring certain insects.

**Pupa:** The nonfeeding, inactive stage between larva and adult in insects with complete metamorphosis.

**Quarantine:** A period of enforced isolation that is required to prevent movement of undesirable organisms.

**Target pest:** A pest species that a control action is intended to destroy.

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## ***12.8 TERMINAL QUESTION & ANSWER***

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### **Long Answer Type Questions**

1. Explain IPM & its components?
2. What is Biological control & its advantages?
3. Describe Chemical control along with its health effects?
4. What do you mean by pesticide resistant. Explain with examples?
5. Describe mechanical & physical control methods with suitable examples?

### **Short Answer Type Questions**

**1. Adjusting the planting date for a crop to avoid a pest outbreak is an example of what type of IPM practice?**

Ans. Cultural control

**2. What is a LD-50?**

Ans. The dose of a substance that will kill 50% of a population.

**3. What is the best approach for successful management of pest populations in IPM?**

Ans. Monitoring, Identifying, Evaluating & Choosing

**4. Why are sticky traps for insects often not a good outdoor monitoring method?**

Ans. Because they attract other non target species at outdoor which produces hindrance.

**5. Give realistic objective when starting an IPM program for plant disease organisms?**

Ans. To plan an IPM program identify the pathogen and know its life cycle and biology.

**Multiple Choice Questions**

1. The three basic elements that most fertilizers contain and are displayed on the bag are:
  - a. nitrogen, phosphorous, potassium
  - b. iron, sulfur, zinc
  - c. magnesium, calcium, iron
  - d. copper, manganese, boron
2. Most pesticide poisonings occur from
  - a. inhalation
  - b. getting it on skin
  - c. swallowing
  - d. None
3. The three signal words that may be found on a pesticide label in the order of most toxic to least toxic are:
  - a. danger>caution>warning
  - b. danger>warning>caution
  - c. toxic>irritant>safe
  - d. None
4. Which of the following are practices which can be used to reduce or prevent insect pest problems?
  - a. sanitation
  - b. insecticides
  - c. crop rotation
  - d. All of the above
5. All \_\_\_\_\_ are pesticides
  - a. insecticides
  - b. fungicides
  - c. herbicides
  - d. all of the above
6. What term is used in IPM to indicate the level at which pests must be controlled?
  - a. Insect level
  - b. Disease severity

c. Action Threshold

d. Pest number

**Fill in the blanks**

1. IPM stands for \_\_\_\_\_
2. The 3 types of cides are \_\_\_\_\_
3. \_\_\_\_\_ attract beneficial insects to plants attacked by pests.
4. Pest control measures are best implemented before the \_\_\_\_\_ stage of the pest.
5. \_\_\_\_\_ are typically a fly or tiny wasp that develops inside another insect.

**Answers**

**MCQ**

1. a
2. b
3. b
4. d
5. d

**Fill in the blanks**

1. Integrated Pest Management
2. Pesticide, herbicide & insecticide
3. Insect growth regulators
4. destructive
5. Parasitoids

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***12.9 REFERENCES***

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## UNIT 13 PARASITOLOGY

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### ***CONTENTS***

13.1 Objective

13.2 Introduction

13.3 General character of Parasites

13.4 Disease Caused and control measure of E. Histolytica

13.5 Trypanosoma

13.6 Leishmania

13.7 Giardia

13.8 Ascaris

13.9 Ancylostoma

13.10 Enterobius

13.11 Wucheria

13.12 Wucheria & Schistosoma

13.13 Summary

13.14 Terminal Question & Answer

13.15 Reference

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### ***13.1 OBJECTIVE***

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The objective of this chapter is to understand the general classification of Parasites and Understand the life-cycles of the organism and their pathogenesis and transmission patterns. we will also discuss the treatment & control measures of parasitic infections.

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### ***13.2 INTRODUCTION***

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A parasite is a living organism, which takes /draws its nourishment & other needs from a host; the host is an organism which supports the parasite by providing food & shelter. The study of parasites is termed as parasitology. The father of parasitology is an Italian Francesco Redi (1632-1723). Parasites are organisms which adapt themselves to live in or on other organisms termed host. A larger number of diseases are caused by several living organisms like Bacteria, Viruses, Fungi, Protozoa, and Arthropods & Helminthes. Almost all the multicellular animals are harbouring at least one protozoan parasite or the other. Parasitology is the area of biology concerned with the phenomenon of dependence of one living organism on another. A misconception about parasitic infections is that they occur only in tropical areas. Although most parasitic infections are more prevalent in the tropics, many people in temperate and subtropical areas also become infected, and visitors to tropical countries may return with a parasite infection. The parasites are antigenically and biochemically complex, as are their life histories and the pathogenesis of the diseases they cause. During their life, parasitic organisms typically go through several developmental stages that involve changes not only in structure but also in biochemical and antigenic composition. Humans are hosts to nearly 300 species of parasitic worms and over 70 species of protozoa, some derived from our primate ancestors and some acquired from the animals we have domesticated or come in contact with during our relatively short history on Earth. Disease transmitted from animals to man are known as zoonotic diseases.

Parasites can be of different kinds according to places where they live, such as –

1. **Ectoparasite:** A parasitic organism that lives on the outer surface of its hosts.  
e.g. lice, ticks, mites, etc.
2. **Endoparasite:** A parasite that lives inside the body of their host.  
e.g. *Entamoeba histolytica*
3. **Facultative parasite:** An organism that exhibits both parasitic & non-parasitic modes of living & hence does not absolutely depend on the parasitic way of life, but is capable of adapting to it if placed on a host.  
e.g. *Naegleria fowleri*
4. **Accidental parasite:** When a parasite attacks an unnatural host & survive.  
e.g. *Hymenolepis diminuta* (rat tapeworm)
5. **Erratic parasite:** When a parasite wanders into an organ in which it is not usually found.  
e.g. *Entamoeba* in the liver & lungs of humans.
6. **Monogenetic parasite:** The parasite which needs only one primary host to complete its life cycle.  
e.g. *Giardia*
7. **Digenetic parasite:** The parasite which needs two hosts i.e. one primary host & one secondary host to complete its life cycle.  
e.g. *Leishmania*

**Similarly hosts are also of different kinds:**

1. **Definitive host/ Primary host:** A host where the parasite undergoes a sexual method of reproduction.
2. **Intermediate/ Secondary host:** A host where an asexual cycle of development takes place.
3. **Reservoir host:** A host that makes the parasite available for the transmission to another host & is usually not affected by the infection.
4. **Natural host:** A host that is naturally infected with certain species of parasites.
5. **Accidental host:** The host that is under normal circumstances not affected with the parasite.
6. **Symbiosis:** Parasites may be pathogenic & non-pathogenic, there is a dynamic equilibrium which exists between the host & parasite of different species and is termed as symbiont and this relationship is called as symbiosis.

There are three common symbiotic relationships between two organisms-

- (i) **Mutualism:** Is an association in which both partners are metabolically dependent upon each other & one cannot live without the help of the other, however, none of the partners suffer any harm from the association.  
e.g. Certain species of flagellated protozoa living in the gut of termites. These protozoans depend entirely on carbohydrate diet, acquire it from termites & in return they synthesize cellulases which is utilized by termites in digestion.
- (ii) **Commensalism:** Is an association in which the commensal takes the benefit without causing any harm to the host.  
e.g. Most of the normal floras (bacteria) of the human body are commensals
- (iii) **Parasitism:** Is an association where one of the partners is harmed & other lives at the expense of the other.  
e.g. Schistosoma as endoparasite of man

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### ***13.3 GENERAL CHARACTER OF PARASITES***

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1. Parasites are found mostly in moist habitats, some free living species inhabit freshwater & marine environment where as terrestrial species inhabit decaying organic matter.
2. Parasites vary substantially in size & shape. Smaller species may be microscopic where as larger species are visible to the naked eye. Protozoan parasites have no cell wall therefore can assume any infinite variety of shapes.
3. Locomotion is done by many modes in different species i.e. by pseudopodia, cilia & flagellum in protozoans while in metazoans locomotion is done by muscular contraction & relaxation.
4. Parasites derive pre-digested nutrition directly from the host body, hence there digestive system is poorly developed or absent.
5. Nervous system of parasites is of primitive type.
6. Respiration in parasites takes place through diffusion or general body surface as they reside inside the host where oxygen availability is less.
7. Excretion in protozoans takes place through vacuoles where as in metazoans through specialised organs.



8. Parasites can be both monoecious & diecious. Protozoans reproduce through asexual method by binary fission whereas metazoans reproduce by sexual method.
9. Reproduction rate is very high in parasites as they produce high no of eggs in very short time.
10. Fertilization is external.
11. Development is indirect with immature larval stages which are motile, feeding & independent.
12. Cleavage is spiral.
13. Presence of protective covering to withstand harsh environmental conditions such as envelope or cyst in protozoans whereas cuticle or tegument in metazoans.
14. Presence of adhesive structures in parasites to remain at the specific site of infection such as suckers, hooks, rostellum.

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### 13.4 DISEASE CAUSED AND CONTROL MEASURE OF *E.HISTOLYTICA*

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#### ENTAMOEBIA

##### Systematic Position

**Phylum-** Protozoa

**Subphylum-** Sarcomastigophora

**Superclass-** Sarcodina

**Class-** Rhizopodea

**Subclass-** Lobosia

**Order-** Amoebida

**Genus-** *Entamoeba*

**Species-** *histolytica*

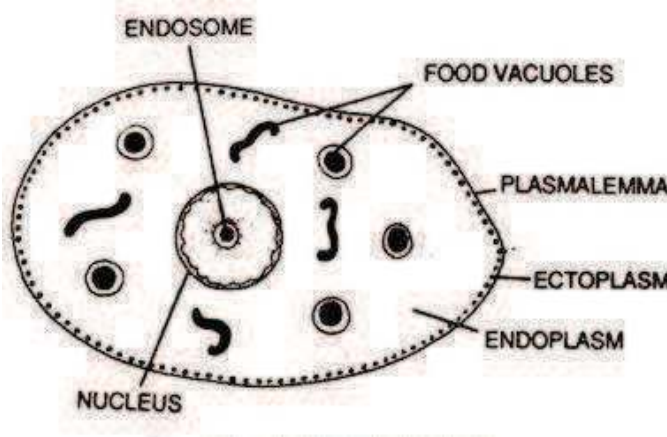


Fig13.1 *Entamoeba histolytica*

*Entamoeba histolytica* is a protozoan parasite responsible for a disease called amoebiasis. It occurs usually in the large intestine and causes internal inflammation as its name suggests (histo = tissue, lytic = destroying). 50 million people are infected worldwide, mostly in

tropical countries in areas of poor sanitation. In industrialized countries most of the infected patients are immigrants, institutionalized people and those who have recently visited developing countries.

### General Characters

1. It is found mainly in tropics & subtropics in addition to that higher in rural and densely populated urban areas.
2. It is a monogenetic parasite which affects mainly children's and young adults.
3. It is a microscopic endoparasite of man that is commonly found in the upper part of the large intestine.
4. It occurs in three distinct forms i.e. Trophozoite/magna form, precystic /minuta form and cystic form.
5. It is holozoic in nature and feed by phagocytosis.
6. Encystation i.e. formation of a cyst wall during unfavourable environmental conditions is an adaptation by this parasite to thrive in harsh conditions.
7. It reproduces asexually by binary fission.
8. Metacyst is the infective stage of parasite.
9. It causes Amoebic dysentery disease.

Inside humans *Entamoeba histolytica* lives and multiplies as a trophozoite. Trophozoites are oblong and about 15–20  $\mu\text{m}$  in length. In order to infect other humans they encyst and exit the body. The **life cycle** of *Entamoeba histolytica* does not require any intermediate host. Mature cysts (spherical, 12–15  $\mu\text{m}$  in diameter) are passed in the feces of an infected human. Another human can get infected by ingesting them in fecally contaminated water, food or hands. If the cysts survive the acidic stomach, they transform back into trophozoites in the small intestine. Trophozoites migrate to the large intestine where they live and multiply by binary fission. Both cysts and trophozoites are sometimes present in the feces. Cysts are usually found in firm stool, whereas trophozoites are found in loose stool. Only cysts can survive longer periods (up to many weeks outside the host) and infect other humans. If trophozoites are ingested, they are killed by the gastric acid of the stomach. Occasionally trophozoites might be transmitted during sexual intercourse.

### LIFE CYCLE

It is a monogenetic parasite. Its life cycle concludes within a primary host i.e. man. Inside humans *Entamoeba histolytica* lives and multiplies as a trophozoite. Trophozoites are oblong

and about 15–20  $\mu\text{m}$  in length. Trophozoites multiply asexually by binary fission inside the wall of large intestine, which grow rapidly in size feeding upon bacteria and host tissue. In order to infect other humans they encyst and exit the body. Mature cysts (spherical, 12–15  $\mu\text{m}$  in diameter) are passed in the feces of an infected human. Transmission of the parasite takes place by the intake of contaminated food & water with faecal matter containing tetra nucleate cyst. The cystic or minuta form is excreted out with faeces which infect new host when ingested and pass down the alimentary canal & reach small intestine. After 5-6 hours excystation takes place thereby releasing tetra nucleate amoeba, called the excystic amoeba or metacyst. These metacyst immediately start dividing to produce 8 small uninucleate amoebulae or metacystic trophozoites, which invade intestine mucous lining & grow into mature trophozoites. Both cysts and trophozoites are sometimes present in the feces. Cysts are usually found in firm stool, whereas trophozoites are found in loose stool. Only cysts can survive longer periods (up to many weeks outside the host) and infect other humans. If trophozoites are ingested, they are killed by the gastric acid of the stomach. Occasionally trophozoites might be transmitted during sexual intercourse.

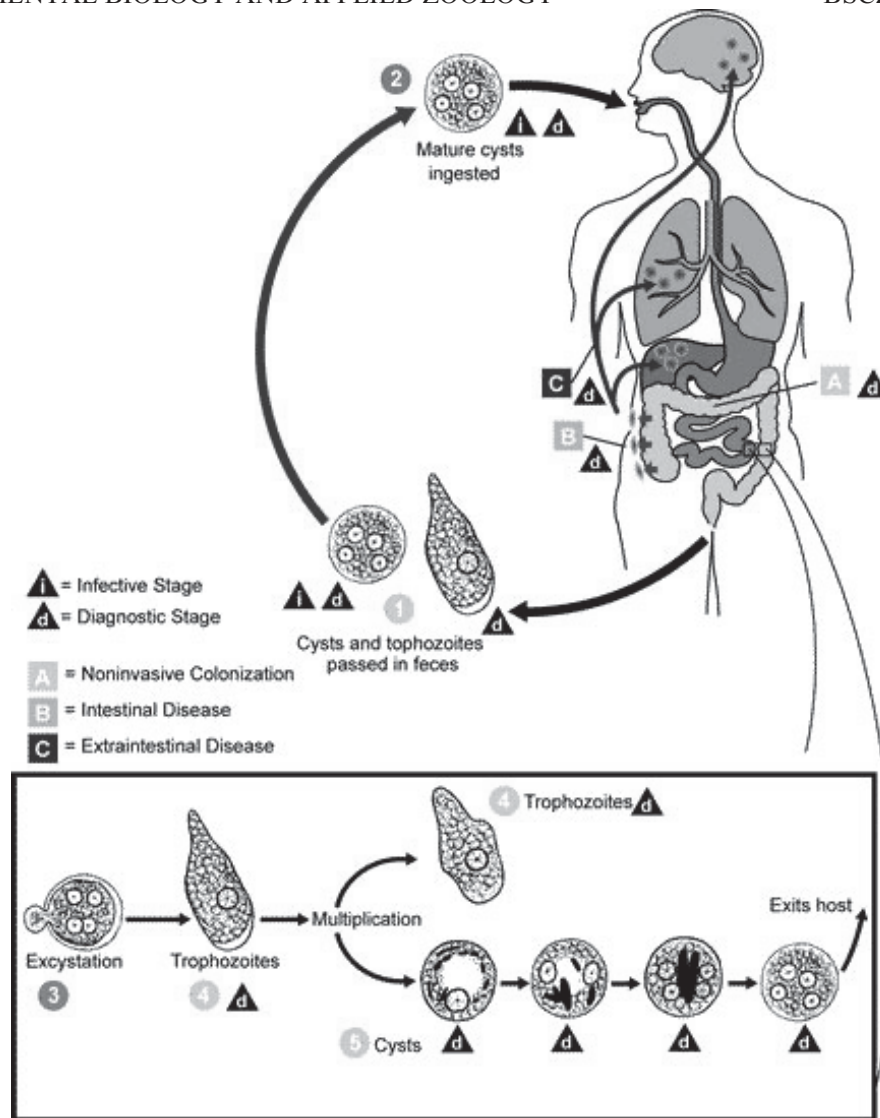


Fig13.2. Life Cycle

### PATHOGENECITY

Several protozoan species in the genus *Entamoeba* colonize humans, but not all of them are associated with disease. *Entamoeba histolytica* is well recognized as a pathogenic amoeba, associated with intestinal and extraintestinal infections. The other species are important because they may be confused with *E. histolytica* in diagnostic investigations.

*Entamoeba histolytica* causes Amoebiasis or Amoebic dysentery. It causes ulcers which are flask shaped containing lymphocytes, blood corpuscles, cellular debris and bacteria which lead to formation of cavity filled with pus. The blood and ulcer contents pass outside with the stool. Infected person's stool is usually acidic and

consists of swarms of entamoeba which causes the new infection. Sometimes trophozoites make their way through blood circulation into brain, liver, spleen, lungs and gonads where they destroy tissues.

**Symptoms** of these more **severe infections** include:

- anemia
- appendicitis (inflammation of the appendix)
- bloody diarrhea
- fatigue
- fever
- gas (flatulence)
- genital and skin lesions
- intermittent constipation
- liver abscesses (can lead to death, if not treated)
- malnutrition
- painful defecation (passage of the stool)
- peritonitis (inflammation of the peritoneum which is the thin membrane that lines the abdominal wall)
- pleuropulmonary abscesses
- stomach ache
- stomach cramping
- toxic megacolon (dilated colon)
- weight loss.

### **Prevention**

To **prevent** spreading the infection to others, one should take care of personal hygiene.

Always wash your hands with soap and water after using the toilet and before eating or preparing food. Amoebiasis is common in developing countries. Some good practices, when visiting areas of poor sanitation:

- Wash your hands often.
- Avoid eating raw food.
- Avoid eating raw vegetables or fruit that you did not wash and peel yourself.
- Avoid consuming milk or other dairy products that have not been pasteurized.
- Drink only bottled or boiled water or carbonated (bubbly) drinks in cans or bottles.

Natural water can be made safe by filtering it through an "absolute 1 micron or less" filter and dissolving iodine tablets in the filtered water. "Absolute 1 micron" filters are found in outdoor/camping supply stores.

**Diagnosis**

Amoebiasis is diagnosed by your health care provider under a microscope by finding cysts and (rarely trophozoites) from a stool sample. The results are usually said to be negative, if *Entamoeba histolytica* is not found in three different stool samples. But it still does not necessarily mean that you are not infected because the microscopic parasite is hard to find and it might not be present the particular samples. A blood test might also be available but is only recommended, if your health care provider believes that the infection could have spread to other parts of the body. Trophozoites can be identified under a microscope from biopsy samples taken during colonoscopy or surgery.

*Entamoeba histolytica* should be differentiated from the non-pathogenic *Entamoeba dispar*. The two are morphologically identical and differentiation must be based on immunologic or isoenzymatic analysis or molecular methods. They can be distinguished under a microscope, if *Entamoeba histolytica* has ingested red blood cells. *Entamoeba dispar* is about 10 times more common. If either one is found, then you are usually treated.

**Treatment**

If you are experiencing amoebiasis symptoms, you are treated with two antibiotics. The preferred drugs are metronidazole or tinidazole immediately followed with paromomycin, diloxanide furoate or iodoquinol. Asymptomatic intestinal amoebiasis is treated with paromomycin, diloxanide furoate or iodoquinol.

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### ***13.5 TRYPANOSOMA***

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**Systematic Position****Phylum-** Protozoa**Subphylum-** Sarcomastigophora**Supesclass-** Mastigophora**Class-** Zoomastigophorea**Order-** Kinetoplastida**Genus-** *Trypanosoma***Species-** *gambiense***GENERAL CHARACTERS**

- It is found mainly in South East Asia, Central & West Africa.
- Trypanosomes are essentially found near moist places.
- Trypanosoma is a monophyletic group of unicellular parasitic flagellate protozoa characterized by possession of a flagellum near posterior end of body.
- Trypanosomes infect a variety of hosts & cause various diseases.
- Polymorphism is shown by trypanosomes on the basis of position of kinetoplast & blepharoplast and the course taken by the flagellum.
- Trypanosomes are heteroxenous i.e. requiring more than one obligatory host to complete its life cycle.
- They are mostly transmitted via a vector.
- Majority of trypanosomes are blood feeding invertebrates.
- Inside an invertebrate host they are generally found in intestine where as in a mammalian host they occupy blood stream.
- It is microscopic, elongated, leaf like tapering at both ends.
- The anterior end contains the free flagellum where as posterior end is blunt.
- Whole body is externally surrounded by thin, elastic covering called pellicle.
- Undulating membrane is an adaptation for locomotion inside the host body. It is a membranous fold which is attached to the pellicle originating from the flagellum.
- Cytoplasm is enclosed within the pellicle.

- Nucleus is large, oval & vesicular within the cytoplasm.
- It reproduces asexually by longitudinal binary fission.
- The long & slender trypanosomes show adaptations to antibodies produced by host body & continue to survive and multiply.

Trypanosomiasis, also known as "sleeping sickness," is caused by microscopic parasites of the species *Trypanosoma brucei*. It is transmitted by the tsetse fly (*Glossina* species), which is found only in rural Africa. Although the infection is not found in the United States, historically, it has been a serious public health problem in some regions of sub-Saharan Africa. Currently, about 10,000 new cases each year are reported to the World Health organization; however, it is believed that many cases go undiagnosed and unreported. Sleeping sickness is curable with medication, but is fatal if left untreated.

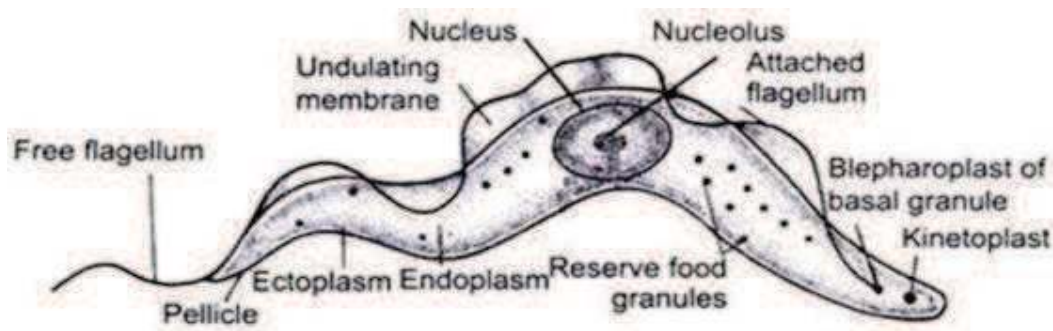


Fig 13.3 *Trypanosoma*

### LIFE-CYCLE

Trypanosoma exhibits polymorphism *i.e.* occur in four morphological forms which are-

**Leishmanial (Amastigote) form:** It is round or oval with a nucleus, blepharoplast & kinetoplast. Flagellum reduced and fibril like embedded in cytoplasm.

**Leptomonad (Promastigote) form:** Body elongated nucleus large & anteriorly located blepharoplast and kinetoplast. Flagellum short & unattached.

**Crithidial (epimastigote) form:** Body elongate, blepharoplast & kinetoplast placed immediately anterior to nucleus. Undulating membrane inconspicuous. Crithidial form is represented only in salivary glands of tse-tse fly.

**Trypanosomid (Trypomastigote) form:** Elongate body with blepharoplast & kinetoplast situated near the posterior end. Undulating membrane conspicuous.



Trypanosoma is digenetic that completes its life cycle in two hosts. The primary host is man and the secondary host is a blood sucking insect called tsetse fly (*Glossina palpalis*). The infection to humans by trypanosomes is transmitted by the bite of tsetse fly which contains infective metacyclic forms in salivary glands. The tsetse fly feeding upon mammalian blood releases the contaminated trypanosomes into his blood stream. All stages of trypanosome in human are extracellular i.e. present in the blood plasma. The vector takes in short stumpy forms along with the blood which continue to develop inside it into long slender forms & multiply by asexual method. After transformation inside vector they move into its mouth region & metamorphose into crithidial forms having shortened body, reduced flagellum. The crithidial form further transform into slender metacyclic non-motile form which is the infective stage.

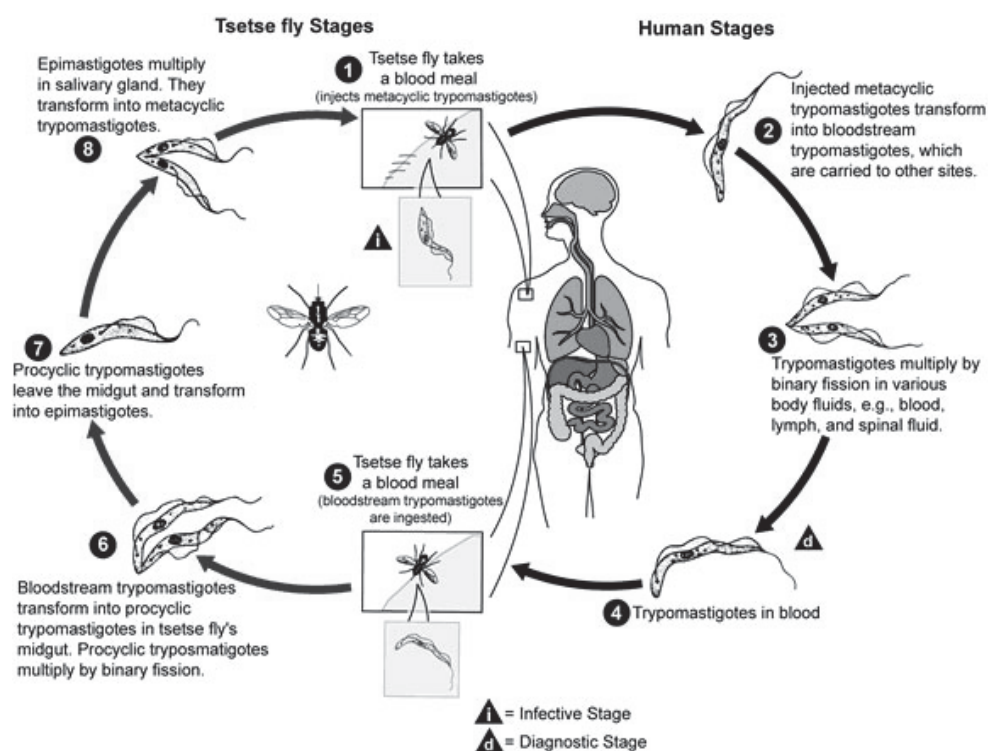


Fig: 13.4 Life Cycle of Trypanosoma

During a blood meal on the mammalian host, an infected tsetse fly (genus *Glossina*) injects metacyclic trypomastigotes into skin tissue. The parasites enter the lymphatic system and pass into the bloodstream. Inside the host, they transform into bloodstream trypomastigotes, are carried to other sites throughout the body, reach other blood fluids (e.g., lymph, spinal fluid), and continue the replication by binary fission. The entire life cycle of African Trypanosomes is represented by extracellular stages. The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host. In

the fly's midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission, leave the midgut, and transform into epimastigotes. The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission. The cycle in the fly takes approximately 3 weeks. Humans are the main reservoir for *Trypanosoma brucei gambiense*, but this species can also be found in animals. Wild game animals are the main reservoir of *T. b. rhodesiense*.

## **PATHOGENECITY**

Protozoan hemo flagellates belonging to the complex *Trypanosoma brucei*. Two subspecies that are morphologically indistinguishable cause distinct disease patterns in humans: *T. b. gambiense* causes West African sleeping sickness and *T. b. rhodesiense* causes East African sleeping sickness. (A third member of the complex, *T. b. brucei*, under normal conditions does not infect humans.)

Trypanosomes cause Gambian trypanosomiasis /Sleeping sickness in man. The metacyclic promastigote are introduced subcutaneously by the bite of tsetse fly & multiply in 2-3 days. There is itching, swelling, pain & redness developed at the site where vector bites. The earliest sign of generalized infection is loss of weight, fever followed by headache & pain in the joints. 5-12 days after infection the parasite is found in the blood stream swimming freely. Special character of disease is enlargement of cervical lymph nodes called as winterbottom's sign. The disease sleeping sickness is caused when parasites invade cerebrospinal fluid of central nervous system.

The clinical course of human African trypanosomiasis has two stages. In the first stage, the parasite is found in the peripheral circulation, but it has not yet invaded the central nervous system. Once the parasite crosses the blood-brain barrier and infects the central nervous system, the disease enters the second stage. The subspecies that cause African trypanosomiasis have different rates of disease progression, and the clinical features depend on which form of the parasite (*T. b. rhodesiense* or *T. b. gambiense*) is causing the infection. However, infection with either form will eventually lead to coma and death if not treated.

*T. b. rhodesiense* infection (East African sleeping sickness) progresses rapidly. In some patients, a large sore (a chancre) will develop at the site of the tsetse bite. Most patients develop fever, headache, muscle and joint aches, and enlarged lymph nodes within 1-2 weeks of the infective bite. Some people develop a rash. After a few weeks of infection, the parasite

invades the central nervous system and eventually causes mental deterioration and other neurologic problems. Death ensues usually within months.

*T. b. gambiense* infection (West African sleeping sickness) progresses more slowly. At first, there may be only mild symptoms. Infected persons may have intermittent fevers, headaches, muscle and joint aches, and malaise. Itching of the skin, swollen lymph nodes, and weight loss can occur. Usually, after 1-2 years, there is evidence of central nervous system involvement, with personality changes, daytime sleepiness with nighttime sleep disturbance, and progressive confusion. Other neurologic signs, such as partial paralysis or problems with balance or walking may occur, as well as hormonal imbalances. The course of untreated infection rarely lasts longer than 6-7 years and more often kills in about 3 years.

## DIAGNOSIS

The diagnosis of African Trypanosomiasis is made through laboratory methods, because the clinical features of infection are not sufficiently specific. The diagnosis rests on finding the parasite in body fluid or tissue by microscopy. The parasite load in *T. b. rhodesiense* infection is substantially higher than the level in *T. b. gambiense* infection.

*T. b. rhodesiense* parasites can easily be found in blood. They can also be found in lymph node fluid or in fluid or biopsy of a chancre. Serologic testing is not widely available and is not used in the diagnosis, since microscopic detection of the parasite is straightforward.

The classic method for diagnosing *T. b. gambiense* infection is by microscopic examination of lymph node aspirate, usually from a posterior cervical node. It is often difficult to detect *T. b. gambiense* in blood. Concentration techniques and serial examinations are frequently needed. Serologic testing is available outside the U.S. for *T. b. gambiense*; however, it normally is used for screening purposes only and the definitive diagnosis rests on microscopic observation of the parasite.

All patients diagnosed with African trypanosomiasis must have their cerebrospinal fluid examined to determine whether there is involvement of the central nervous system, since the choice of treatment drug(s) will depend on the disease stage. The World Health Organization criteria for central nervous system involvement include increased protein in cerebrospinal fluid and a white cell count of more than 5. Trypanosomes can often be observed in cerebrospinal fluid in persons with second stage infection.

## TREATMENT

All persons diagnosed with African Trypanosomiasis should receive treatment. The specific drug and treatment course will depend on the type of infection (*T. b. gambiense* or *T. b. rhodesiense*) and the disease stage (i.e. whether the central nervous system has been invaded by the parasite). Pentamidine, which is the recommended drug for first stage *T. b. gambiense* infection, is widely available in the U.S. The other drugs (suramin, melarsoprol, eflornithine, and nifurtimox) used to treat African trypanosomiasis are available in the U.S. only from the CDC. Physicians can consult with CDC staff for advice on diagnosis and management and to obtain otherwise unavailable treatment drug.

There is no test of cure for African trypanosomiasis. After treatment patients need to have serial examinations of their cerebrospinal fluid for 2 years, so that relapse can be detected if it occurs.

## PREVENTION & CONTROL

There is no vaccine or drug for prophylaxis against African trypanosomiasis. Preventive measures are aimed at minimizing contact with tsetse flies. Local residents are usually aware of the areas that are heavily infested and they can provide advice about places to avoid. Other helpful measures include:

- Wear long-sleeved shirts and pants of medium-weight material in neutral colors that blend with the background environment. Tsetse flies are attracted to bright or dark colors, and they can bite through lightweight clothing.
- Inspect vehicles before entering. The flies are attracted to the motion and dust from moving vehicles.
- Avoid bushes. The tsetse fly is less active during the hottest part of the day but will bite if disturbed.
- Use insect repellent. Permethrin-impregnated clothing and insect repellent have not been proved to be particularly effective against tsetse flies, but they will prevent other insect bites that can cause illness.

Control of African trypanosomiasis rests on two strategies: reducing the disease reservoir and controlling the tsetse fly vector. Because humans are the significant disease reservoir for *T. b. gambiense*, the main control strategy for this subspecies is active case-finding through population screening, followed by treatment of the infected persons that are identified. Tsetse fly traps are sometimes used as an adjunct. Reducing the reservoir of infection is more difficult for *T. b. rhodesiense*, since there are a variety of animal hosts. Vector control is the

primary strategy in use. This is usually done with traps or screens, in combination with insecticides and odors that attract the flies.

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## ***13.6 LEISHMANIA***

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### **Systematic Position**

**Phylum-** Protozoa

**Subphylum-** Sarcomastigophora

**Supesclass-** Mastigophora

**Class-** Zoomastigophorea

**Order-** Kinetoplastida

**Genus-** *Leishmania*

**Species-** *donovani*

### **GENERAL CHARACTERS**

- It is a digenetic protozoan parasite which needs two hosts to complete its life cycle.
- It occurs mainly in temperate zones of the world.
- Its primary host is mammal/man where as secondary host is blood sucking sand fly.
- It lives inside the intestine & visceral organs like spleen, bone marrow, liver & lymph glands.
- Body of the parasite is covered externally by protective covering called pellicle.
- Undulating membrane is absent.
- It shows dimorphism i.e. occurs in two forms in its life cycle which are –
- Leishmanial /Amastigote form: This form is non-motile, smaller, round & oval which lives inside the primary host man in its blood cells.
- Leptomonad/ Promastigote form: This form is motile, long, elongated, slender & spindle shaped with a flagellum.
- It reproduces asexually by longitudinal binary fission.
- In order to avoid destruction by the immune system & thrive, it hides inside the hosts cells.
- It resides inside man in the reticuloendothelial system of the viscera, where amastigote form multiplies until the host cell is destroyed.
- It causes the disease black fever / kala azar.

Two developmental stages are formed: amastigotes and promastigotes. The amastigotes are small spherical non-flagellated cells ranging from 2-4 $\mu$ m in diameter. The nucleus and kinetoplast are surrounded by small ring of vacuolated cytoplasm and the cells are among the smallest nucleated cells known. Promastigotes are thin elongate cells with an anterior kinetoplast and an emergent free flagellum. They are generally lance-like in shape and range in size from 5-14 $\mu$ m in length by 1.5-3.5 $\mu$ m in width. Different parasite species are generally not differentiated by morphological differences, but rather on the basis of geographical, biological and clinical features.

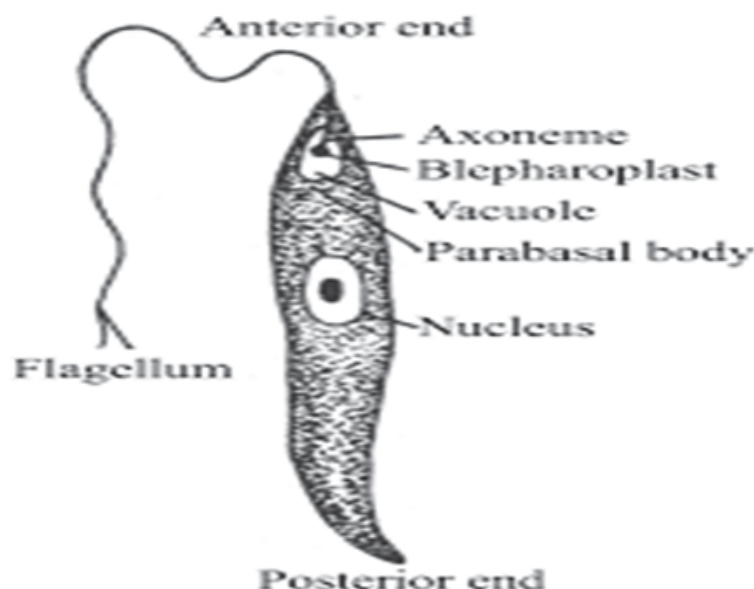


Fig. 13.5 *Leishmania donovani*

Leishmaniasis is a parasitic disease that is found in parts of the tropics, subtropics, and southern Europe. It is classified as a Neglected Tropical Disease (NTD). Leishmaniasis is caused by infection with *Leishmania* parasites, which are spread by the bite of phlebotomine sand flies. There are several different forms of leishmaniasis in people. The most common forms are **cutaneous leishmaniasis**, which causes skin sores, and **visceral leishmaniasis**, which affects several internal organs (usually spleen, liver, and bone marrow).

### LIFE –CYCLE

Since the parasite is digenetic therefore it needs two hosts for completion of its life cycle i.e. primary host is man where as secondary host is blood sucking invertebrate sand fly (*Phlebotomus*).



- (i) In Human: It is transmitted by the bite of vector, firstly before entrance they are in the form of promastigote/ leptomonad but after infection changes into amastigote/ leishmanial form & undergo slow multiplication after which they reside in liver, spleen, bone marrow & lymph nodes where their number increases from few to several hundred inside the host cell and ruptures liberating parasites which infect new cells.
- (ii) In Invertebrate/Vector: The parasite enters the invertebrate in amastigote form. It feeds upon an infected person blood. This form further develops inside vector's midgut and get elongated to form a flagellum, where they increases in number & migrate to pharynx & now are ready to infect new person when vector finds new host.

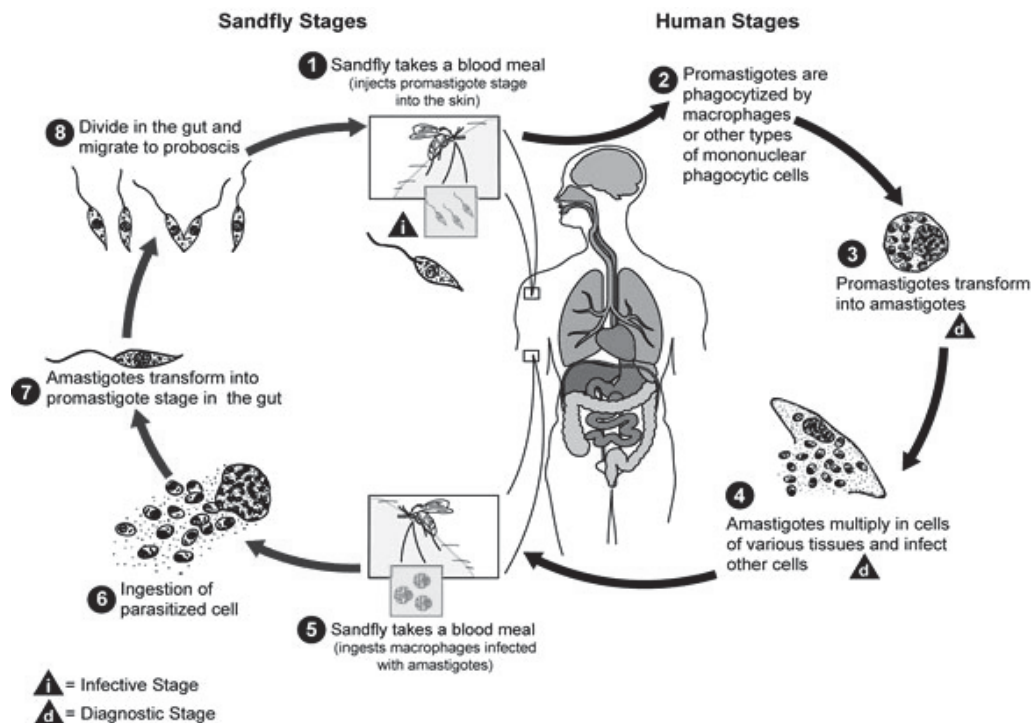


Fig 13.6 Life Cycles

*Leishmania* is transmitted by the bite of infected female phlebotomine sandflies. The sandflies inject the infective stage (*i.e.*, promastigotes) from their proboscis during blood meals. Promastigotes that reach the puncture wound are phagocytized by macrophages and other types of mononuclear phagocytic cells. Promastigotes transform in these cells into the tissue stage of the parasite (*i.e.*, amastigotes), which multiply by simple division and proceed to infect other mononuclear phagocytic cells. Parasite, host, and other factors affect whether the infection becomes symptomatic and whether cutaneous or visceral leishmaniasis results. Sandflies become infected by ingesting infected cells during blood meals. In sandflies, amastigotes transform into promastigotes, develop in the gut (in the hindgut for leishmanial

organisms in the *Viannia* subgenus; in the midgut for organisms in the *Leishmania* subgenus), and migrate to the proboscis.

### **PATHOGENECITY**

Leishmaniasis is a vector-borne disease that is transmitted by sandflies and caused by obligate intracellular protozoa of the genus *Leishmania*. Human infection is caused by about 21 of 30 species that infect mammals. These include the *L. donovani* complex with 2 species (*L. donovani*, *L. infantum* [also known as *L. chagasi* in the New World]); the *L. mexicana* complex with 3 main species (*L. mexicana*, *L. amazonensis*, and *L. venezuelensis*); *L. tropica*; *L. major*; *L. aethiopica*; and the subgenus *Viannia* with 4 main species (*L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) panamensis*, and *L. (V.) peruviana*). The different species are morphologically indistinguishable, but they can be differentiated by isoenzyme analysis, molecular methods, or monoclonal antibodies.

This parasite cause's disease black fever also known as Kala-azar as its name suggests during the disease the skin of patient becomes dark, rough which gives black appearance. Symptoms begin with intermittent fever, weakness, and diarrhea; chills and sweating that may resemble malaria symptoms are also common early in the infection. As organisms proliferate & invade cells of the liver and spleen, marked enlargement of the organs, weight loss, anemia, and emaciation occurs. With persistence of the disease, deeply pigmented, granulomatous lesion of skin, referred to as post-kala-azar dermal leishmaniasis, occurs. The organs of the reticuloendothelial system (liver, spleen and bone marrow) are the most severely affected organs. Reduced bone marrow activity, coupled with cellular distraction in the spleen, results in anaemia, leukopenia and thrombocytopenia. This leads to secondary infections and a tendency to bleed.

There are several different forms of leishmaniasis in people. The most common form is **Cutaneous leishmaniasis**: This causes skin sores. The sores typically develop within a few weeks or months of the sand fly bite. The sores can change in size and appearance over time. The sores may start out as papules (bumps) or nodules (lumps) and may end up as ulcers (like a volcano, with a raised edge and central crater); skin ulcers might be covered by scab or crust. The sores usually are painless but can be painful. Some people have swollen glands near the sores (for example, under the arm, if the sores are on the arm or hand).



**Visceral leishmaniasis:** which affects several internal organs (usually spleen, liver, and bone marrow) and can be life threatening. The illness typically develops within months (sometimes as long as years) of the sand fly bite. Affected people usually have fever, weight loss, enlargement (swelling) of the spleen and liver, and low blood counts—a low red blood cell count (anemia), a low white blood cell count (leukopenia), and a low platelet count (thrombocytopenia).

**Mucosal leishmaniasis** is an example of one of the less common forms of leishmaniasis. This form can be a sequela (consequence) of infection with some of the species (types) of the parasite that cause cutaneous leishmaniasis in parts of Latin America: certain types of the parasite might spread from the skin and cause sores in the mucous membranes of the nose (most common location), mouth, or throat. The best way to prevent mucosal leishmaniasis is to ensure adequate treatment of the original cutaneous (skin) infection.

## DIAGNOSIS

Various laboratory methods can be used to diagnose leishmaniasis—to detect the parasite as well as to identify the *Leishmania* species (type). Some of the methods are available only in reference laboratories. In the United States, CDC staff can assist with the testing for leishmaniasis.

Tissue specimens—such as from skin sores (for cutaneous leishmaniasis) or from bone marrow (for visceral leishmaniasis)—can be examined for the parasite under a microscope, in special cultures, and in other ways. Blood tests that detect antibody (an immune response) to the parasite can be helpful for cases of visceral leishmaniasis; tests to look for the parasite itself usually also are done.

## TREATMENT

Before considering treatment, the first step is to make sure the diagnosis is correct. Treatment decisions should be individualized. Health care providers may consult CDC staff about the relative merits of various approaches. Examples of factors to consider include the form of leishmaniasis, the *Leishmania* species that caused it, the potential severity of the case, and the patient's underlying health.

The skin sores of **cutaneous leishmaniasis** usually heal on their own, even without treatment. But this can take months or even years, and the sores can leave ugly scars. Another potential concern applies to some (not all) types of the parasite found in parts of Latin

America: certain types might spread from the skin and cause sores in the mucous membranes of the nose (most common location), mouth, or throat (**mucosal leishmaniasis**). Mucosal leishmaniasis might not be noticed until years after the original sores healed. The best way to prevent mucosal leishmaniasis is to ensure adequate treatment of the cutaneous infection.

If not treated, severe (advanced) cases of **visceral leishmaniasis** typically are fatal.

## **PREVENTION & CONTROL**

No vaccines or drugs to prevent infection are available. The best way for travelers to prevent infection is to protect themselves from sand fly bites. To decrease the risk of being bitten, follow these preventive measures:

Avoid outdoor activities, especially from dusk to dawn, when sand flies generally are the most active.

### **When outdoors (or in unprotected quarters):**

- Minimize the amount of exposed (uncovered) skin. To the extent that is tolerable in the climate, wear long-sleeved shirts, long pants, and socks; and tuck your shirt into your pants. (See below about wearing insecticide-treated clothing).
- Apply insect repellent to exposed skin and under the ends of sleeves and pant legs. Follow the instructions on the label of the repellent. The most effective repellents generally are those that contain the chemical DEET (N, N-diethylmetatoluamide).

### **Note:-**

Bed nets, repellents, and insecticides should be purchased before traveling and can be found in hardware, camping, and military surplus stores. Bed nets and clothing that already have been treated with a pyrethroid-containing insecticide also are commercially available.

### **When Indoors:**

- Stay in well-screened or air-conditioned areas.
- Keep in mind that sand flies are much smaller than mosquitoes and therefore can get through smaller holes.
- Spray living/sleeping areas with an insecticide to kill insects.
- If you are not sleeping in a well-screened or air-conditioned area, use a bed net and tuck it under your mattress. If possible, use a bed net that has been soaked in or sprayed with a pyrethroid-containing insecticide. The same treatment can be applied to screens, curtains, sheets, and clothing (clothing should be retreated after five washings).

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## 13.7 *GIARDIA*

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### Systematic Position

**Phylum-** Protozoa

**Subphylum-** Sarcomastigophora

**Supesclass-** Mastigophora

**Class-** Zoomastigophorea

**Order-** Diplomonadida

**Genus-** *Giardia*

**Species-** *lamblia*

*Giardia intestinalis* is a protozoan flagellate (Diplomonadida). This protozoan was initially named *Cercomonas intestinalis* by Lambl in 1859. It was renamed *Giardia lamblia* by Stiles in 1915 in honor of Professor A. Giard of Paris and Dr. F. Lambl of Prague. However, many consider the name, *Giardia intestinalis*, to be the correct name for this protozoan.

### General characters

- *Giardia* is commonly called as ‘Grand Old Man of the Intestine’.
- It is found mainly in tropics & subtropics.
- It is a monogenetic diplomonad flagellate protozoan parasite.
- It is cosmopolitan in nature.
- It is found as a parasite in digestive tract of man.
- It is similar to shape in *Entamoeba*.
- It bears four flagella on each side.
- It is bilaterally symmetrical, pear-shaped with two nuclei.
- Transmission is by ingestion of the infective cyst.
- It exhibits polymorphism .i.e. two distinct forms.
- The life cycle consists of two stages, the trophozoite and cyst.
- It reproduces by binary fission
- It causes disease Giardiasis.

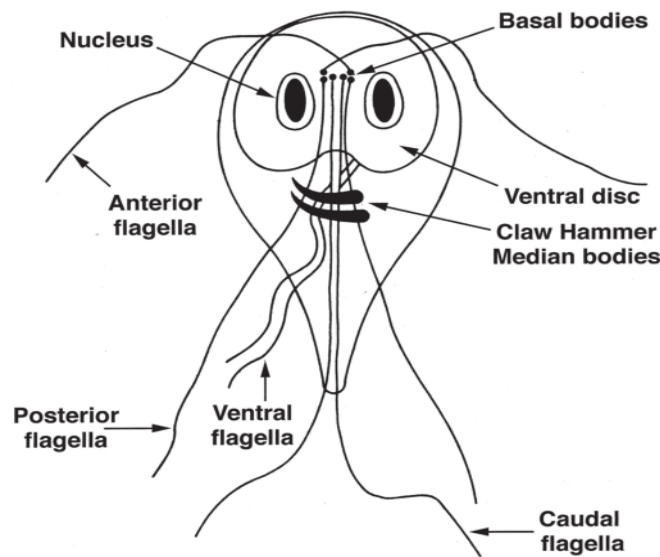


Fig 13.7 *Giardia*

### LIFE –CYCLE

Infection with *Giardia* is initiated by ingestion of cysts. Each cyst gives rise to two trophozoites during excystation in the intestinal tract. Gastric acid stimulates excystation, with the release of trophozoites in duodenum and jejunum. The trophozoite is the feeding form motile & pathogenic, it is somewhat pear shaped which get attach to the intestinal villi by the ventral sucking discs and start feeding upon mucous secretions till they attain maturity. After maturation they multiply by longitudinal binary fission and transform into cystic form. It is the infective stage which is round & oval in shape; get passed out with the faeces. Ready to be ingested along with contaminated water & food by a new host to cause a new infection.

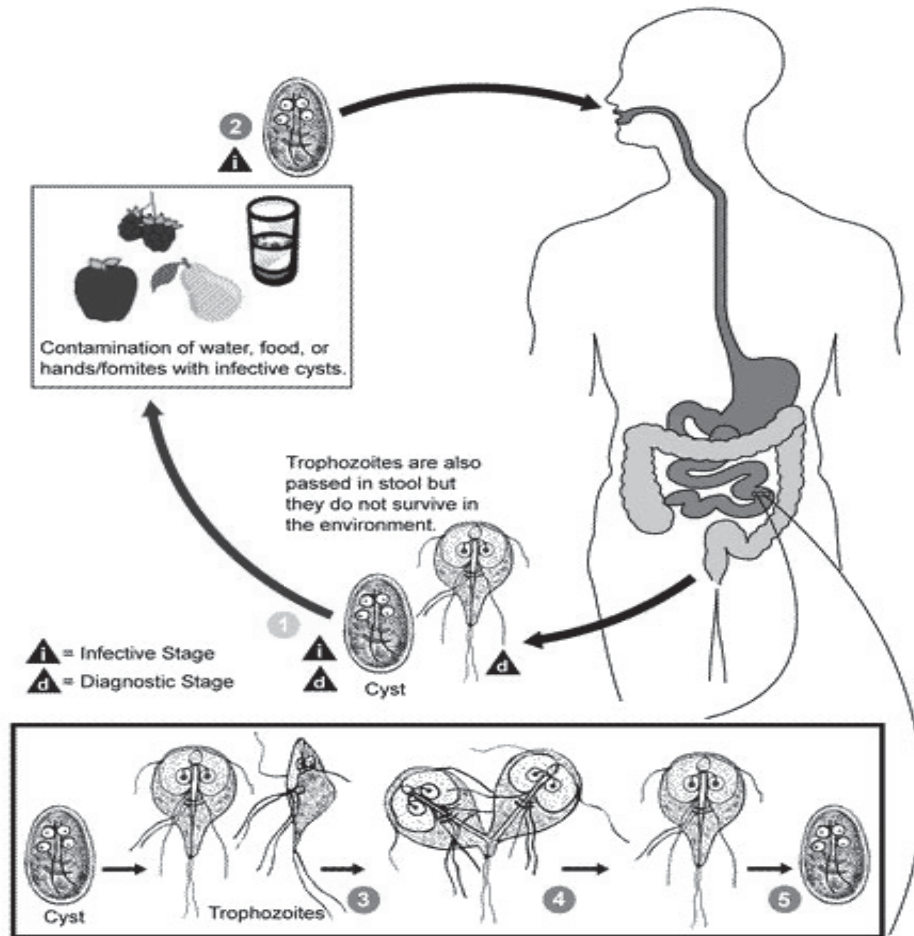


Fig 13.8 Life Cycle of *Giardia*

Cysts are resistant forms and are responsible for transmission of giardiasis. Both cysts and trophozoites can be found in the feces (diagnostic stages) (1). The cysts are hardy and can survive several months in cold water. Infection occurs by the ingestion of cysts in contaminated water, food, or by the fecal-oral route (hands or fomites) (2). In the small intestine, excystation releases trophozoites (each cyst produces two trophozoites) (3). Trophozoites multiply by longitudinal binary fission, remaining in the lumen of the proximal small bowel where they can be free or attached to the mucosa by a ventral sucking disk (4). Encystation occurs as the parasites transit toward the colon. The cyst is the stage found most commonly in nondiarrheal feces (5). Because the cysts are infectious when passed in the stool

or shortly afterward, person-to-person transmission is possible. While animals are infected with *Giardia*, their importance as a reservoir is unclear.

### Diagnosis & Detection

Because *Giardia* cysts can be excreted intermittently, multiple stool collections (i.e., three stool specimens collected on separate days) increase test sensitivity. The use of concentration methods and trichrome staining might not be sufficient to identify *Giardia* because variability in the concentration of organisms in the stool can make this infection difficult to diagnose. For this reason, fecal immunoassays that are more sensitive and specific should be used.

Rapid immune-chromatographic cartridge assays also are available but should not take the place of routine ova and parasite examination. Only molecular testing (e.g., polymerase chain reaction) can be used to identify the sub types of *Giardia*.

### Illness & Symptoms

Giardiasis is the most frequently diagnosed intestinal parasitic disease in the United States and among travelers with chronic diarrhea. Signs and symptoms may vary and can last for 1 to 2 weeks or longer. In some cases, people infected with *Giardia* have no symptoms

Acute symptoms include:

- Diarrhea
- Gas
- Greasy stools that tend to float
- Stomach or abdominal cramps
- Upset stomach or nausea/vomiting
- Dehydration (loss of fluids)

Other, less common symptoms include itchy skin, hives, and swelling of the eye and joints. Sometimes, the symptoms of giardiasis might seem to resolve, only to come back again after several days or weeks. Giardiasis can cause weight loss and failure to absorb fat, lactose, vitamin A and vitamin B12

In children, severe giardiasis might delay physical and mental growth, slow development, and cause malnutrition

*Giardia* causes giardiasis which includes violent diarrhoea, excess gas, stomach cramps, nausea, blood & pus in stools resulting in dehydration. It also causes the villi of small intestine to atrophy & flatten resulting in malabsorption.

### **Sources of Infection & Risk Factors**

#### **General Epidemiology**

Giardiasis is a diarrheal illness caused by the parasite *Giardia intestinalis* (also known as *Giardia lamblia* or *Giardia duodenalis*).

Giardiasis is a global disease. It infects nearly 2% of adults and 6% to 8% of children in developed countries worldwide. Nearly 33% of people in developing countries have had giardiasis. In the United States, *Giardia* infection is the most common intestinal parasitic disease affecting humans.

People become infected with *Giardia* by swallowing *Giardia* cysts (hard shells containing *Giardia*) found in contaminated food or water. Cysts are instantly infectious once they leave the host through feces (poop). An infected person might shed 1-10 billion cysts daily in their feces (poop) and this might last for several months. However, swallowing as few as 10 cysts might cause someone to become ill. *Giardia* may be passed from person-to-person or even from animal-to-person. Also, oral-anal contact during sex has been known to cause infection. Symptoms of giardiasis normally begin 1 to 3 weeks after a person has been infected.

*Giardia* infection rates have been known to go up in late summer. Between 2006-2008 in the United States, known cases of giardiasis were twice as high between June-October as they were between January-March.

Anyone may become infected with *Giardia*. However, those at greatest risk are

- Travelers to countries where giardiasis is common
- People in childcare settings
- Those who are in close contact with someone who has the disease
- People who swallow contaminated drinking water
- Backpackers or campers who drink untreated water from lakes or rivers
- People who have contact with animals who have the disease
- Men who have sex with men

#### **Molecular Characterization**

*Giardia intestinalis* (aka: *G. duodenalis*, *G. lamblia*) can be subdivided based on molecular analysis into what are known as different genetic assemblages (A,B,C,D,E,F, and G). Some

of these assemblages can be classified even further into subtypes like for example A-I, A-II, A-III, A-IV. Each assemblage is capable of infecting certain species, and some assemblages are more commonly seen than others

<b>Molecular Characterization</b>	
<b>Assemblages</b>	<b>Some Species Commonly Infected</b>
A-I	Humans and animals (cats, dogs, livestock, deer, muskrats, beavers, voles, guinea pigs, ferrets)
A-II	Humans (more common than A-I)
A-III and A-IV	Exclusively animals
B	Humans and animals (livestock, chinchillas, beavers, marmosets, rodents)
C and D	Dogs, coyotes
E	Alpacas, cattle, goats, pigs, sheep
F	Cats

## Prevention & Control

### ❖ Practice good hygiene.

- *Everywhere*
  - Wash hands with soap and clean, running water for at least 20 seconds; rub your hands together to make lather and be sure to scrub the backs of your hands, between your fingers, and under your nails.
    - Before, during, and after preparing food
    - Before eating food
    - Before and after caring for someone who is sick
    - Before and after treating a cut or wound
    - After using the toilet
    - After changing diapers or cleaning up a child who has used the toilet
    - After blowing your nose, coughing, or sneezing
    - After touching an animal or animal waste
    - After handling pet food or pet treats
    - After touching garbage
  - Help young children and other people you are caring for with handwashing as needed.



Thoroughly washing your hands after gardening can help prevent exposure to parasitic diseases.

- *At childcare facilities*
  - To reduce the risk of spreading the disease, children with diarrhea should be removed from child care settings until the diarrhea has stopped.
- *At recreational water venues (for example, pools, beaches, fountains)*
  - Protect others by not swimming if you have diarrhea (this is most important for children in diapers).
    - If diagnosed with giardiasis, do not swim for at least 1 week after diarrhea stops.
  - Shower before entering the water.
  - Wash children thoroughly (especially their bottoms) with soap and water after they use the bathroom or after their diapers are changed and before they enter the water.
  - Take children on frequent bathroom breaks and check their diapers often.
  - Change diapers in the bathroom, not by the water.
- *Around animals*
  - Minimize contact with the feces (poop) of all animals, especially young animals.
    - When cleaning up animal feces (poop), wear disposable gloves and always wash hands when finished.
  - Wash hands after any contact with animals or their living areas.
- *Outside*
  - Wash hands after gardening, even if wearing gloves.

❖ **Avoid water (drinking and recreational) that may be contaminated.**

- Do not swallow water while swimming in pools, hot tubs, interactive fountains, lakes, rivers, springs, ponds, streams or the ocean.
- Do not drink untreated water from lakes, rivers, springs, ponds, streams, or shallow wells.
- Do not drink poorly treated water or ice made from water during community outbreaks caused by contaminated drinking water.
- Do not use or drink poorly treated water or use ice when traveling in countries where the water supply might be unsafe.
- If the safety of drinking water is in doubt (for example, during or after an outbreak, in a place with poor sanitation or lack of water treatment systems), do one of the following:
  - Drink bottled water.
  - Disinfect tap water by heating it to a rolling boil for 1 minute.

- Use a filter that has been tested and rated by National Safety Foundation (NSF) Standard 53 or NSF Standard 58 for cyst and oocyst reduction; filtered tap water will need additional treatment to kill or weaken bacteria and viruses.

❖ **Avoid eating food that may be contaminated.**

- Use safe, uncontaminated water to wash all food that is to be eaten raw.
- After washing vegetables and fruit in safe, uncontaminated water, peel them if you plan to eat them raw.
- Avoid eating raw or uncooked foods when traveling in countries with poor food and water treatment.

❖ **Prevent contact and contamination with feces (poop) during sex.**

- Use a barrier during oral-anal sex.
- Wash hands right after handling a condom used during anal sex and after touching the anus or rectal area.

❖ **Clean up after ill pets and people.**

*Giardia* is hard to completely eliminate from the environment, but you can decrease the risk of human infection or of your dog's or cat's reinfection if it has been ill. The risk of acquiring *Giardia* infection from your dog or cat is small, but there are some steps you can take to minimize your exposure.

Clean and disinfect your home in this way:

- **Hard surfaces** (for example: cement and tile floors, pet crates, tables, trash cans, etc.)
  - **Cleaning**
    - Wear gloves.
    - Remove feces and discard in a plastic bag.
    - Clean and scrub surfaces using soap. Rinse surface thoroughly until no obvious visible contamination is present.
  - **Disinfection**
    - Wear gloves.
    - Disinfect according to manufacturer guidelines using **one** of the following:
      - Quaternary ammonium compound products (QATS) <sup>1</sup>, which are found in some household cleaning products; the active ingredient may be listed as alkyl dimethyl ammonium chloride.
      - Bleach mixed with water (3/4 cup of bleach to 1 gallon of water) <sup>2</sup>.

- Follow product instructions, ensuring the product stays in contact with the surface for the recommended amount of time.
- Rinse with clean water.
- **Carpet / Upholstered Furniture**
  - **Cleaning**
    - Wear gloves.
    - If feces are on a carpet or upholstered furniture, remove them with absorbent material (for example, double layered paper towels).
    - Place and discard the feces in a plastic bag.
    - Clean the contaminated area with regular detergent or carpet cleaning agent.
    - Allow carpet or upholstered furniture to fully dry.
  - **Disinfection**
    - Wear gloves.
    - Steam cleans the area at 158°F for 5 minutes or 212°F for 1 minute.
    - QATS are found in some carpet cleaning products and can also be used after cleaning to disinfect. Read the product labels for specifications, and follow all instructions.
- **Other items (toys, clothing, pet bed, etc.)**
  - Household items should be cleaned and disinfected daily while a dog or cat is being treated for *Giardia* infection.
  - **Dishwasher**
    - Dishwasher-safe toys and water and food bowls can be disinfected in a dishwasher that has a dry cycle or a final rinse that exceeds **one** of the following:
      - 113°F for 20 minutes
      - 122°F for 5 minutes
      - 162°F for 1 minute
    - If a dishwasher is not available, submerge dishwasher-safe items in boiling water for at least 1 minute (at elevations above 6,500 feet, boil for 3 minutes).
  - **Washer and Dryer**
    - Clothing, some pet items (for example, bedding and cloth toys) and linens (sheets and towels) can be washed in the washing machine and then heat-dried on the highest heat setting for 30 minutes.
    - If a clothes dryer is not available, allow clothes to thoroughly air dry under direct sunlight.

## Treatment

- *Giardia* trophozoites under scanning electron microscope. Credit: Waterborne Disease Prevention Branch, CDC
- Several drugs can be used to treat *Giardia* infection. Effective treatments include metronidazole, tinidazole, and nitazoxanide <sup>1</sup>. Alternatives to these medications include paromomycin, quinacrine, and furazolidone. Some of these drugs may not be routinely available in the United States.
- Different factors may shape how effective a drug regimen will be, including medical history, nutritional status, and condition of the immune system. Therefore, it is important to discuss treatment options with a healthcare provider.

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## 13.8 ASCARIS

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### Systematic Position

**Phylum-** Nematoda

**Class-** Phasmodia

**Order-** Ascaroidea

**Suborder-** Ascarinae

**Family-** Ascaridae

**Genus-** *Ascaris*

**Species-** *lumbricoides*

### General characters

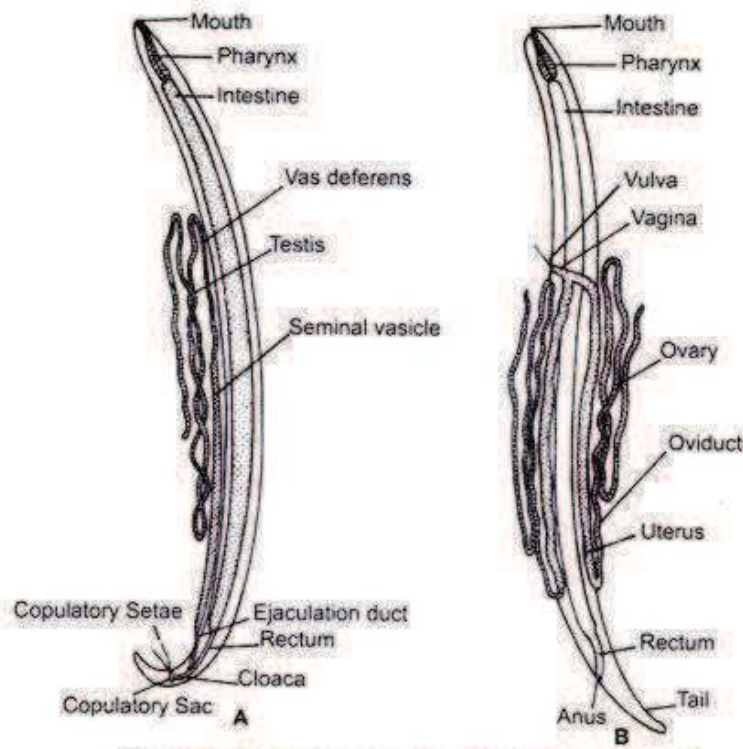
- It is an endoparasite of man found in small intestine.
- It is cosmopolitan in distribution.
- It is commonly called as round worm.
- It is monogenetic nematode.
- Body is un-segmented, elongated, cylindrical in shape, white pinkish in colour tapering at both ends.
- Female ascaris is larger than male ascaris.
- It is dioecious in nature.
- It respire anaerobically i.e. in the body of host where it lives, the amount of oxygen is relatively less inside intestine to which it adapts by carrying anerobic respiration.
- Locomotory structures are absent as they need not move in search of food.

- To protect from the harmful effects of digestive enzymes of the host, body is covered by tough protective cuticle.
- Digestive system is not well developed as it feeds upon partially digested food of host which is also an adaptation.
- Excretory system is primitive with absence of flame cells.
- Nervous system is primitive.
- To remain at the specific site of infection they attach to the surrounding tissues provided with adhesive structures like hooks.
- Low metabolic rate.
- The reproductive potential is very high by laying 2,00,000 eggs day which ensures the contamination of new host.
- Eggs are enclosed inside a protective covering called cyst to thrive in harsh environmental conditions.
- Fertilization is external.

An estimated 807-1,221 million people in the world are infected with *Ascaris lumbricoides* (sometimes called just "*Ascaris*"). *Ascaris*, hookworm, and whipworm are known as soil-transmitted helminths (parasitic worms). Together, they account for a major burden of disease worldwide. Ascariasis is now uncommon in the United States.

*Ascaris* lives in the intestine and *Ascaris* eggs are passed in the feces of infected persons. If the infected person defecates outside (near bushes, in a garden, or field) or if the feces of an infected person are used as fertilizer, eggs are deposited on soil. They can then mature into a form that is infective. Ascariasis is caused by ingesting eggs. This can happen when hands or fingers that have contaminated dirt on them are put in the mouth or by consuming vegetables or fruits that have not been carefully cooked, washed or peeled.

People infected with *Ascaris* often show no symptoms. If symptoms do occur they can be light and include abdominal discomfort. Heavy infections can cause intestinal blockage and impair growth in children. Other symptoms such as cough are due to migration of the worms through the body. Ascariasis is treatable with medication prescribed by your health care provider.



*Fig 13.9 (A) Adult Male (B) Adult Female*

## LIFE –CYCLE

The egg containing larva when ingested with contaminated raw vegetables causes ascariasis. Ingested eggs hatch in the duodenum. The larvae penetrate the intestinal wall and circulate in the blood. From the heart they migrate to the lungs, ascend to the trachea, descend to the oesophagus and finally reach the small intestine to become adult. The female pass immature eggs which pass to the soil and mature in 2 weeks.

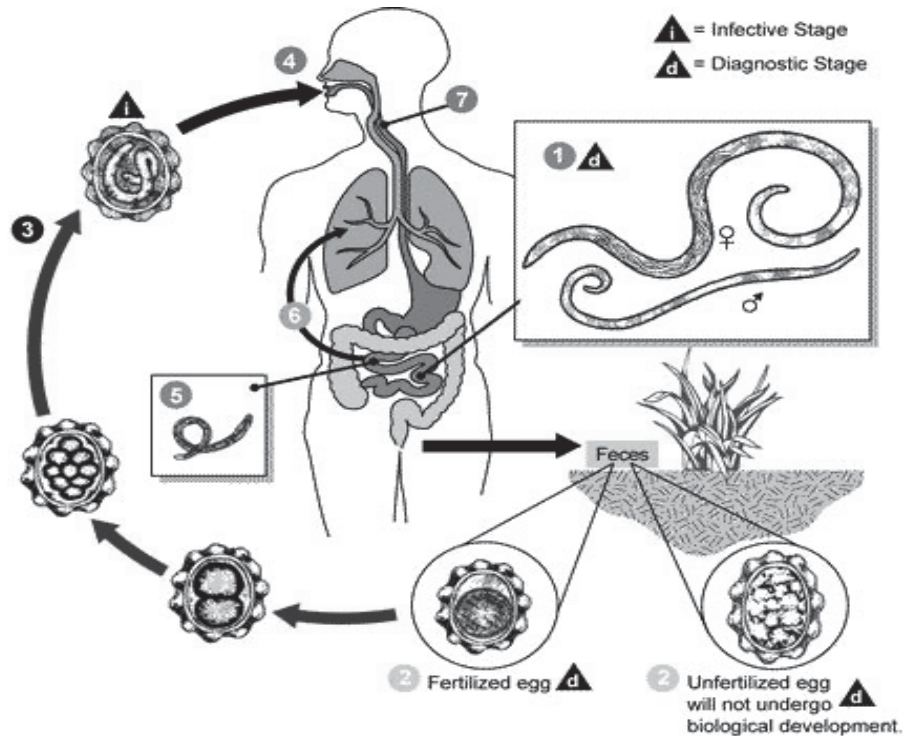


Fig. 13.10 Life Cycle

Adult worms live in the lumen of the small intestine. A female may produce approximately 200,000 eggs per day, which are passed with the feces. Unfertilized eggs may be ingested but are not infective. Fertile eggs embryonate and become infective after 18 days to several weeks, depending on the environmental conditions (optimum: moist, warm, shaded soil). After infective eggs are swallowed, the larvae hatch, invade the intestinal mucosa, and are carried via the portal, then systemic circulation to the lungs. The larvae mature further in the lungs (10 to 14 days), penetrate the alveolar walls, ascend the bronchial tree to the throat, and are swallowed. Upon reaching the small intestine, they develop into adult worms. Between 2 and 3 months are required from ingestion of the infective eggs to oviposition by the adult female. Adult worms can live 1 to 2 years.

### **PATHOGENECITY**

*Ascaris lumbricoides* is the largest nematode (roundworm) parasitizing the human intestine. (Adult females: 20 to 35 cm; adult male: 15 to 30 cm.)

It causes ascariasis which results in inflammation of alveoli tissue, injuries to vital organs while Adult worms in the intestine cause abdominal pain and may cause intestinal obstruction especially in children. Larvae in the lungs may cause inflammation of the pneumonia-like symptoms.

People infected with *Ascaris* often show no symptoms. If symptoms do occur they can be light and include abdominal discomfort. Heavy infections can cause intestinal blockage and impair growth in children. Other symptoms such as cough are due to migration of the worms through the body. Ascariasis is treatable with medication prescribed by your health care provider.

**Diagnosis**

The standard method for diagnosing ascariasis is by identifying *Ascaris* eggs in a stool sample using a microscope. Because eggs may be difficult to find in light infections, a concentration procedure is recommended.

**Treatment**

Anthelmintic medications (drugs that rid the body of parasitic worms), such as albendazole and mebendazole, are the drugs of choice for treatment of *Ascaris* infections. Infections are generally treated for 1-3 days. The drugs are effective and appear to have few side effects.

The best way to prevent ascariasis is to always:

- Avoid ingesting soil that may be contaminated with human feces, including where human fecal matter ("night soil") or wastewater is used to fertilize crops.
- Wash your hands with soap and warm water before handling food.
- Teach children the importance of washing hands to prevent infection.
- Wash, peel, or cook all raw vegetables and fruits before eating, particularly those that have been grown in soil that has been fertilized with manure.

Transmission of infection to others can be prevented by

- Not defecating outdoors.
- Effective sewage disposal systems.



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### ***13.9 ANCYLOSTOMA***

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**Systematic Position****Phylum-** Nematoda**Class-** Phasmodia**Order-** Strongyloidea**Family-** Ancylostomidae**Genus-** *Ancylostoma***Species-** *dudoenale*

*Ancylostoma Duodenale* was discovered by an Italian physician, Angelo Ducini Looss in 1898. It is found in the small intestine of millions of people chiefly in Europe, Africa, India, China, Japan, Srilanka and Pacific Islands.

**General characters**

- Generally known as hook worm.
- It is a monogenetic parasite of humans.
- Found in tropical & sub-tropical, temperate regions of the world.
- It is greyish white in colour.
- Body is ventrally curved.
- Females are larger than males.
- Presence of cup shaped cavity armed with cutting plates bearing teeth/lancets for adhesion to the host.
- Secretes anti-coagulant.
- Respiration anaerobic as live inside the intestine where supply of oxygen is less.
- Body covered with cuticle to protect from hosts digestive enzymes.
- Production of enormous number of eggs i.e. high fertility rate.
- Minute size & resistant nature of eggs helps in their wide dispersal.
- Sexes are separate.

The hookworm, *Ancylostoma duodenale*, is a nematode that mainly parasitizes humans. However, it can also be found in a range of paratenic hosts, including dogs, cats, pigs, and

even coyotes. These tiny, s-shaped worms only grow to be roughly 8-13 mm in length. Although it is very small, it still contains a very vicious “bite”.

Each hookworm contains two very powerful ventral teeth, along with small pairs of teeth located deeper in its capsule to help it bite and attach itself to its victims. Once inside, the parasite will hook itself onto the intestines and continually drain blood from its host, up to 1 mL blood per individual per day. Sexual dimorphism occurs. The male worm is 8 to 11 mm x 0.4 mm in size. The posterior end of the body forms a bursa made of three lobes, out of which one is dorsal and two are lateral. A pair of long spicules passes from the genital canal to the outside through cloaca. A gubernaculum is also used during copulation to help guide the spicules.

The female averages 10 to 13 mm x 0.6 mm in size. The posterior end of the body tapers to a rather blunt point. The vulva is located at a point about two-thirds the length of the body from the anterior end. Eggs are ovoidal, thin-shelled and measure 56 to 60  $\mu\text{m}$  x 34 – 40  $\mu\text{m}$ .

*A. duodenale* infects humans mainly through direct contact, which usually occurs through the foot. However, it can also be transmitted through the consumption of under-cooked meats such as lamb, beef, and pork. The parasites are released back into the soil through human feces.

Infants are more vulnerable than adults, and infections in children can lead to permanent growth deficiencies.

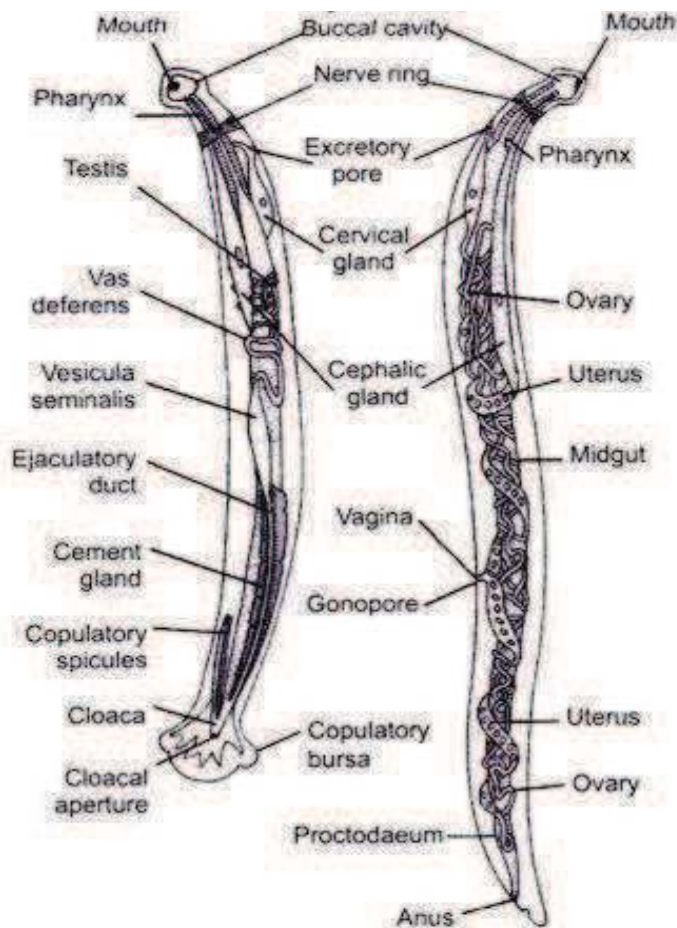


Fig.13.11 Male and Female *A. duodenale*

### LIFE-CYCLE

It is monogenetic in nature & involve only one host in its life cycle i.e. man. Adult male and female worms live in the small intestine. The female lays eggs, which contain immature embryo in the 4 cell stage. When the eggs pass in the stool to the soil and under favourable conditions of temperature, moisture and oxygen, they hatch into larvae, which molt twice and become infective. When the filariform larvae penetrate the skin, they circulate in the blood, reach the lungs, ascend to the trachea, descend to oesophagus to reach the small intestine and become adults, where they get attached to mucous lining & feed on blood of host. The adult copulate to produce eggs which are fertilized externally. Fertilized eggs are passed out with host faeces which under favourable environmental conditions hatch into rhabditi form larva & wait to infect a new host through skin contact.

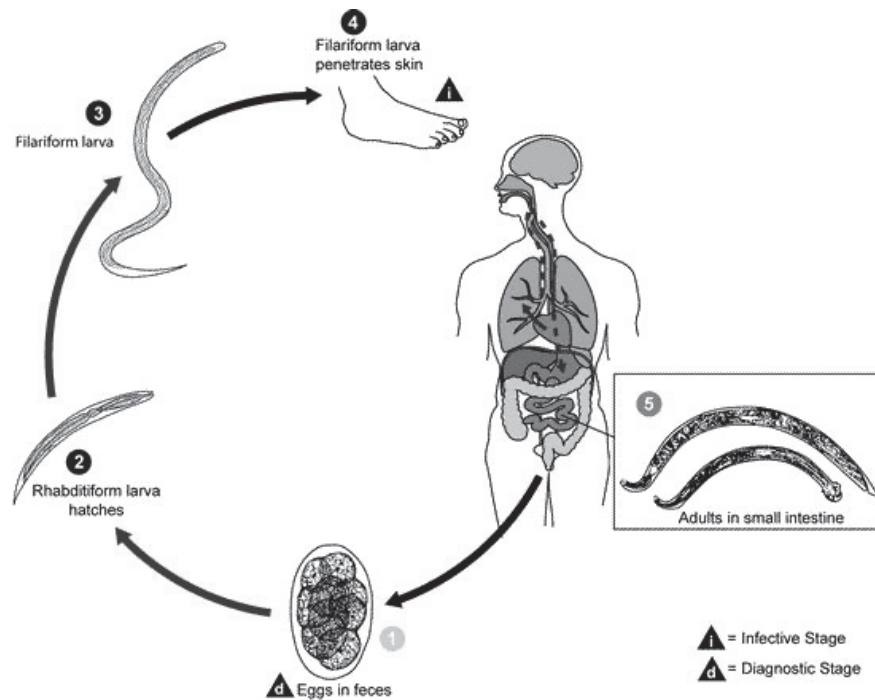


Fig 13.12 Life cycle

Eggs are passed in the stool, and under favorable conditions (moisture, warmth, shade), larvae hatch in 1 to 2 days. The released rhabditiform larvae grow in the feces and/or the soil, and after 5 to 10 days (and two molts) they become filariform (third-stage) larvae that are infective. These infective larvae can survive 3 to 4 weeks in favorable environmental conditions. On contact with the human host, the larvae penetrate the skin and are carried through the blood vessels to the heart and then to the lungs. They penetrate into the pulmonary alveoli, ascend the bronchial tree to the pharynx, and are swallowed. The larvae reach the small intestine, where they reside and mature into adults. Adult worms live in the lumen of the small intestine, where they attach to the intestinal wall with resultant blood loss by the host. Most adult worms are eliminated in 1 to 2 years, but the longevity may reach several years.

Some *A. duodenale* larvae, following penetration of the host skin, can become dormant (in the intestine or muscle). In addition, infection by *A. duodenale* may probably also occur by the oral and transmammary route. *N. americanus*, however, requires a transpulmonary migration phase.

**PATHOGENECITY**

Ancylostoma causes ancylostomiasis in which itching of skin, severe inflammation, diarrhoea, constipation with bloody stools. Adult worms in the intestine feed on blood causing iron deficiency anaemia. The larvae cause inflammation of the lungs. In severe conditions mental retardation in children is caused.

Anemia of iron deficiency is the principal host reaction to the intestinal infection by adult worms. Other symptoms are fever, abdominal pain, diarrhoea, food fermentation, constipation, myocarditis, eosinophilia, loss of health and collapse. Children are more susceptible than adults. Mental and physical growth is retarded in children and growing youth.

To check the epigastric pain, the patient may start eating even dirt, so called “dirt eaters”. If the infection is not controlled, it may lead to fatty degeneration of heart, liver and kidneys, ending in the death of the patient.

**Prevention and Control**

Many drugs are available to treat ancylostomiasis. The most commonly used drug is tetrachloroethylene or blephenium hydroxynaphthoate, because of its high efficiency and low toxicity. Other antihelmintic drugs used are Hexylresorcinol, thymol, oil of chenopodium, dithiazanine iodide, piperazine salt, pyrvinium pamoate. Thioabendazole can be given, but only under strict supervision of a physician. Food is usually supplemented by iron to compensate haemoglobin deficiency.

**Control:**

1. Proper sewage disposal in affected areas.
2. Keep soil free from contamination of larvae.
3. Educate the people in endemic area concerning the source of infection.
4. Wear shoes regularly.

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### ***13.10 ENTEROBIUS***

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**Systematic Position****Phylum-** Nematoda**Class-** Secernentea**Subclass-** Spiruria**Order-** Oxyurida**Family-** Oxyuridae**Genus-** *Enterobius***Species-** *vermicularis***General characters**

- Commonly called as Pin worm.
- It is a monogenetic parasitic nematode.
- It is white in color.
- Females are larger in size than males.
- They give thread like appearance.
- Found in upper part of the colon.
- Sexual dimorphism occurs.
- Tail of female is long & pointed where as that of male is blunt & curved.
- Male is monarchic while female is didelphic i.e. possessing two wombs.

Pinworm infection is caused by a small, thin, white roundworm called *Enterobius vermicularis*. Although pinworm infection can affect all people, it most commonly occurs among children, institutionalized persons, and household members of persons with pinworm infection.

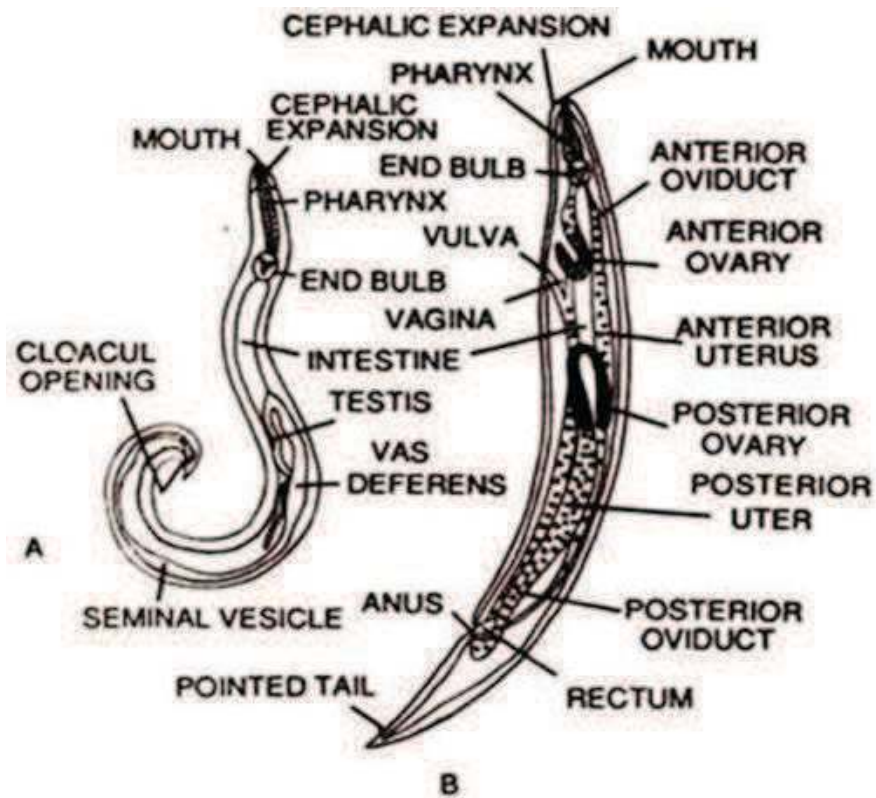
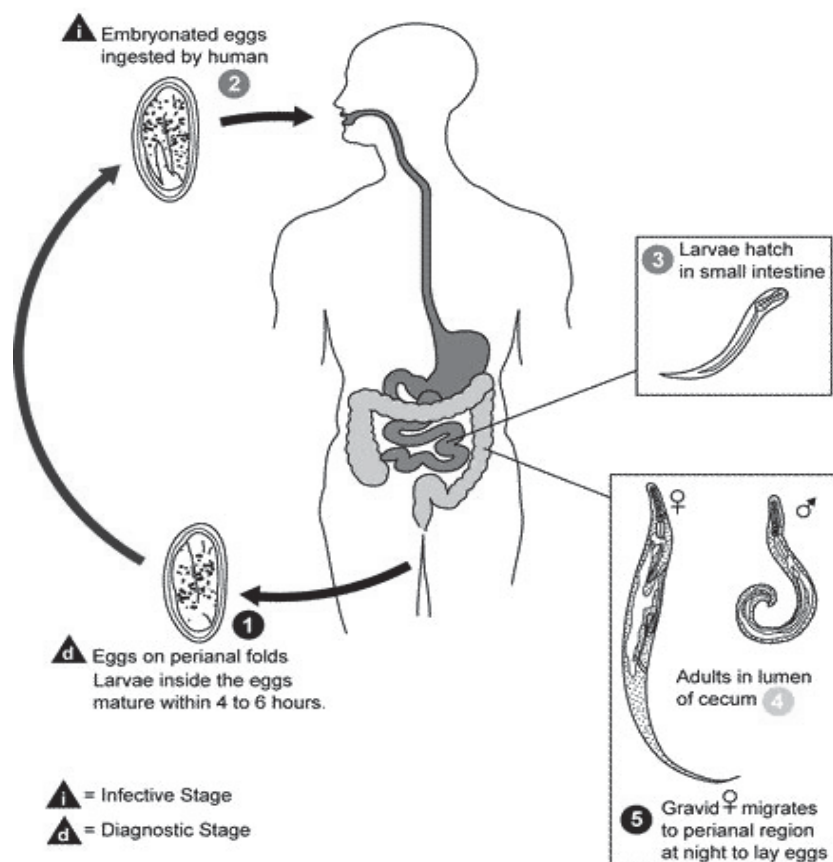


Fig 13.13 *Enterobius vermicularis*

### LIFE-CYCLE

As it is monogenetic in nature life cycle is simple with man being the only host. Adult worm lives in the large intestine. After fertilization, the male dies and the Female moves out through at night to the anus to glue its eggs on the peri-anal skin. The eggs are plano-convex and contains larva. When the eggs are swallowed, they hatch in the small intestine and the larvae migrate to the large intestine to become adult.



*Fig.13.14 Life Cycle*

Eggs are deposited on perianal folds. Self-infection occurs by transferring infective eggs to the mouth with hands that have scratched the perianal area. Person-to-person transmission can also occur through handling of contaminated clothes or bed linens. Enterobiasis may also be acquired through surfaces in the environment that are contaminated with pinworm eggs (e.g., curtains, carpeting). Some small number of eggs may become airborne and inhaled. These would be swallowed and follow the same development as ingested eggs. Following ingestion of infective eggs, the larvae hatch in the small intestine and the adults establish themselves in the colon. The time interval from ingestion of infective eggs to oviposition by the adult females is about one month. The life span of the adults is about two months. Gravid females migrate nocturnally outside the anus and oviposit while crawling on the skin of the perianal area. The larvae contained inside the eggs develop (the eggs become infective) in 4 to 6 hours under optimal conditions. Retroinfection, or the migration of newly hatched larvae from the anal skin back into the rectum, may occur but the frequency with which this happens is unknown.



**PATHOGENECITY**

Pinworm infections are more common within families with school-aged children, in primary caregivers of infected children, and in institutionalized children.

A person is infected with pinworms by ingesting pinworm eggs either directly or indirectly. These eggs are deposited around the anus by the worm and can be carried to common surfaces such as hands, toys, bedding, clothing, and toilet seats. By putting anyone's contaminated hands (including one's own) around the mouth area or putting one's mouth on common contaminated surfaces, a person can ingest pinworm eggs and become infected with the pinworm parasite. Since pinworm eggs are so small, it is possible to ingest them while breathing.

Once someone has ingested pinworm eggs, there is an incubation period of 1 to 2 months or longer for the adult gravid female to mature in the small intestine. Once mature, the adult female worm migrates to the colon and lays eggs around the anus at night, when many of their hosts are asleep. People who are infected with pinworm can transfer the parasite to others for as long as there is a female pinworm depositing eggs on the perianal skin. A person can also re-infect themselves, or be re-infected by eggs from another person.

The migration of the worms causes allergic reactions around the anus and during night it causes nocturnal itching and enuresis. The worms obstruct the appendix causing appendicitis. Parasite also causes loss of appetite, insomnia, hysteria, restlessness and inflammation to the patient.

The most common clinical manifestation of a pinworm infection is an itchy anal region. When the infection is heavy, there can be a secondary bacterial infection due to the irritation and scratching of the anal area. Often the patient will complain of teeth grinding, and insomnia due to disturbed sleep, or even abdominal pain or appendicitis. Infection of the female genital tract has been well reported.

**Diagnosis & Treatment**

A person infected with pinworm is often asymptomatic, but itching around the anus is a common symptom. Diagnosis of pinworm can be reached from three simple techniques. The first option is to look for the worms in the perianal region 2 to 3 hours after the infected person is asleep. The second option is to touch the perianal skin with transparent tape to

collect possible pinworm eggs around the anus first thing in the morning. If a person is infected, the eggs on the tape will be visible under a microscope. The tape method should be conducted on 3 consecutive morning's right after the infected person wakes up and before he/she does any washing. Since anal itching is a common symptom of pinworm, the third option for diagnosis is analyzing samples from under fingernails under a microscope. An infected person who has scratched the anal area may have picked up some pinworm eggs under the nails that could be used for diagnosis.

Since pinworm eggs and worms are often sparse in stool, examining stool samples is not recommended. Serologic tests are not available for diagnosing pinworm infections.

The medications used for the treatment of pinworm are mebendazole, pyrantel pamoate, or albendazole. Any of these drugs are given in one dose initially, and then another single dose of the same drug two weeks later. Pyrantel pamoate is available without prescription. The medication does not reliably kill pinworm eggs. Therefore, the second dose is to prevent re-infection by adult worms that hatch from any eggs not killed by the first treatment. Health practitioners and parents should weigh the health risks and benefits of these drugs for patients under 2 years of age.

Repeated infections should be treated by the same method as the first infection. In households where more than one member is infected or where repeated, symptomatic infections occur, it is recommended that all household members be treated at the same time. In institutions, mass and simultaneous treatment, repeated in 2 weeks, can be effective.

### **Prevention and Control**

Washing your hands with soap and warm water after using the toilet, changing diapers, and before handling food is the most successful way to prevent pinworm infection. In order to stop the spread of pinworm and possible re-infection, people who are infected should bathe every morning to help remove a large amount of the eggs on the skin. Showering is a better method than taking a bath, because showering avoids potentially contaminating the bath water with pinworm eggs. Infected people should not co-bathe with others during their time of infection.

Also, infected people should comply with good hygiene practices such as washing their hands with soap and warm water after using the toilet, changing diapers, and before handling food.

They should also cut fingernails regularly, and avoid biting the nails and scratching around the anus. Frequent changing of underclothes and bed linens first thing in the morning is a great way to prevent possible transmission of eggs in the environment and risk of reinfection. These items should not be shaken and carefully placed into a washer and laundered in hot water followed by a hot dryer to kill any eggs that may be there.

In institutions, day care centers, and schools, control of pinworm can be difficult, but mass drug administration during an outbreak can be successful. Teach children the importance of washing hands to prevent infection,

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### ***13.11 WUCHERERIA***

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**Systematic Position****Phylum-** Nematoda**Class-** Phasmodia**Order-** Filarioidea**Family-** Filariidae**Genus-** *Wuchereria***Species-** *bancrofti***General characters**

- It is a digenetic nematode parasite.
- It is confined to tropical & sub tropical countries.
- It is an endoparasite of human blood & lymphatic system.
- Its primary host is man where as secondary host is invertebrate i.e. mosquito.
- It is filiform & cylindrical in shape with terminal blunt ends.
- These are creamy white in colour.
- Sexes are separate.
- Females are longer than males.
- Its larvae are termed as microfilaria which shows diurnal rhythm.
- Sheath covering on microfilaria for protection from host immune system.
- Adhesive structures are present to remain attached to the host.
- Polyembryony occurs i.e. presence of many larval stages.

There are three different filarial species that can cause lymphatic filariasis in humans. Most of the infections worldwide are caused by *Wuchereria bancrofti*. In Asia, the disease can also be caused by *Brugia malayi* and *Brugia timori*.

Lymphatic filariasis, considered globally as a neglected tropical disease, is a parasitic disease caused by microscopic, thread-like worms. The adult worms only live in the human lymph system. The lymph system maintains the body's fluid balance and fights infections. Lymphatic filariasis is spread from person to person by mosquitoes. The adult worm lives in the human lymph vessels, mates, and produces millions of microscopic worms, also known as microfilariae. Microfilariae circulate in the person's blood and infect the mosquito when it

bites a person who is infected. Microfilariae grow and develop in the mosquito. When the mosquito bites another person, the larval worms pass from the mosquito into the human skin, and travel to the lymph vessels. They grow into adult worms, a process that takes 6 months or more. An adult worm lives for about 5–7 years. The adult worms mate and release millions of microfilariae into the blood. People with microfilariae in their blood can serve as a source of infection to others.

People with the disease can suffer from lymphedema and elephantiasis and in men, swelling of the scrotum, called hydrocele. Lymphatic filariasis is a leading cause of permanent disability worldwide. Communities frequently shun and reject women and men disfigured by the disease. Affected people frequently are unable to work because of their disability, and this harms their families and their communities.

Programs to eliminate lymphatic filariasis are under way in more than 50 countries. These programs are reducing transmission of the filarial parasites and decreasing the risk of infection for people living in or visiting these communities.

The infection spreads from person to person by mosquito bites.

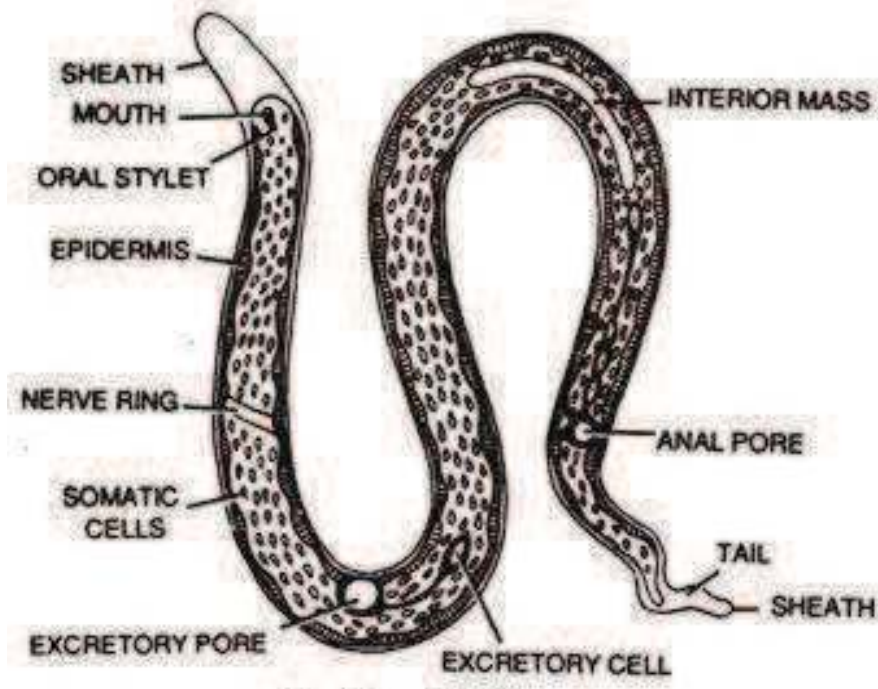
A wide range of mosquitoes can transmit the parasite, depending on the geographic area. In Africa, the most common vector is *Anopheles* and in the Americas, it is *Culex quinquefasciatus*. *Aedes* and *Mansonia* can transmit the infection in the Pacific and in Asia.

Many mosquito bites over several months to years are needed to get lymphatic filariasis. People living for a long time in tropical or sub-tropical areas where the disease is common are at the greatest risk for infection. Short-term tourists have a very low risk.

Lymphatic filariasis affects over 120 million people in 73 countries throughout the tropics and sub-tropics of Asia, Africa, the Western Pacific, and parts of the Caribbean and South America.

Only four countries are currently known to be endemic: Haiti, the Dominican Republic, Guyana and Brazil.

In the United States, Charleston, South Carolina, was the last known place with lymphatic filariasis. The infection disappeared early in the 20th century. Currently, you cannot get infected in the U.S.

Fig 13.15 *Wucheria bancrofti*

### LIFE-CYCLE

The filariform larvae are introduced through the skin by the bite of the arthropod intermediate host. The larvae invade the lymphatics, usually the lower limb, where they develop into adult worms. The microfilariae are liberated into the blood stream. They remain in the pulmonary circulation during day, emerging into the peripheral circulation only during night, to coincide with the biting habit of the vector.

The parasite when sucked up by vector moves into its stomach where they metamorphosis from long slender form to plump sausage shaped organism ready to cause infection to a new host when bitten by vector. Infective larvae are transmitted by infected biting mosquitoes during a blood meal. The larvae migrate to lymphatic vessels and lymph nodes, where they develop into microfilariae-producing adults. The adults dwell in lymphatic vessels and lymph nodes where they can live for several years. The female worms produce microfilariae which circulate in the blood. The microfilariae infect biting mosquitoes. Inside the mosquito, the microfilariae develop in 1 to 2 weeks into infective filariform (third-stage) larvae. During a subsequent blood meal by the mosquito, the larvae infect the human host. They migrate to the lymphatic vessels and lymph nodes of the human host, where they develop into adults.

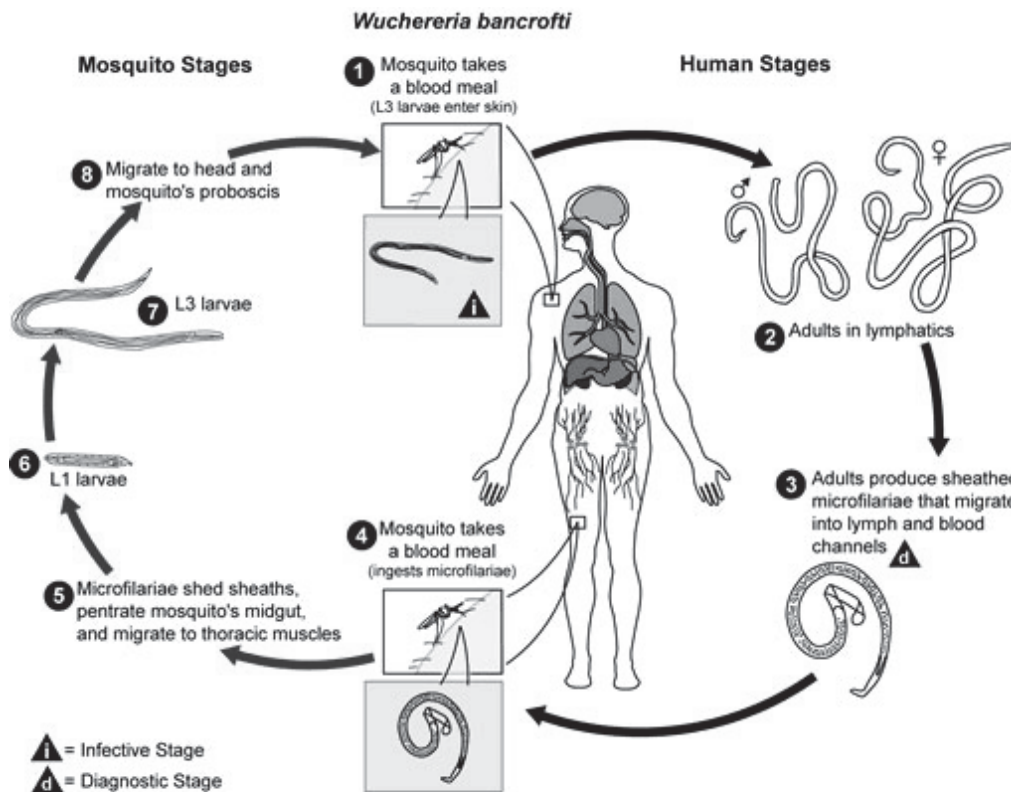


Fig.13.16 Life Cycle

During a blood meal, an infected mosquito introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound. They develop in adults that commonly reside in the lymphatics. The female worms measure 80 to 100 mm in length and 0.24 to 0.30 mm in diameter, while the males measure about 40 mm by .1 mm. Adults produce microfilariae measuring 244 to 296  $\mu\text{m}$  by 7.5 to 10  $\mu\text{m}$ , which are sheathed and have nocturnal periodicity, except the South Pacific microfilariae which have the absence of marked periodicity. The microfilariae migrate into lymph and blood channels moving actively through lymph and blood. A mosquito ingests the microfilariae during a blood meal. After ingestion, the microfilariae lose their sheaths and some of them work their way through the wall of the proventriculus and cardiac portion of the mosquito's midgut and reach the thoracic muscles. There the microfilariae develop into first-stage larvae and subsequently into third-stage infective larvae. The third-stage infective larvae migrate through the



hemocoel to the mosquito's proboscis and can infect another human when the mosquito takes a blood meal.

Different species of the following genera of mosquitoes are vectors of *W. bancrofti* filariasis depending on geographical distribution. Among them are: *Culex* (*C. annulirostris*, *C. bitaeniorhynchus*, *C. quinquefasciatus*, and *C. pipiens*); *Anopheles* (*A. arabinensis*, *A. bancroftii*, *A. farauti*, *A. funestus*, *A. gambiae*, *A. koliensis*, *A. melas*, *A. merus*, *A. punctulatus* and *A. wellcomei*); *Aedes* (*A. aegypti*, *A. aquasalis*, *A. bellator*, *A. cooki*, *A. darlingi*, *A. kochi*, *A. polynesiensis*, *A. pseudoscutellaris*, *A. rotumae*, *A. scapularis*, and *A. vigilax*); *Mansonia* (*M. pseudotitillans*, *M. uniformis*); *Coquillettidia* (*C. juxtamansonia*).

### **PATHOGENECITY**

Lymphatic filariasis is caused by nematodes (roundworms) that inhabit the lymphatic vessels and lymph nodes of a human host. *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* cause lymphatic filariasis.

*Wuchereria* causes disease termed as Filariasis. The adult worm obstructs the flow of lymph in the lymph nodes and the lymphatic vessels draining the lower limbs and the external genitalia become swollen. The skin becomes thick and fissured. The disease is also called elephantiasis in which the body parts grow in enormous size. The major symptoms include lymphangitis, lymphedema, fever, headache, myalgia, hydrocele and chyluria.

Although the parasite damages the lymph system, most infected people have no symptoms and will never develop clinical symptoms. These people do not know they have lymphatic filariasis unless tested. A small percentage of persons will develop lymphedema. This is caused by fluid collection because of improper functioning of the lymph system resulting in swelling. This mostly affects the legs, but can also occur in the arms, breasts, and genitalia. Most people develop these symptoms years after being infected.

The swelling and the decreased function of the lymph system make it difficult for the body to fight germs and infections. These people will have more bacterial infections in the skin and lymph system. This causes hardening and thickening of the skin, which is called elephantiasis. Many of these bacterial infections can be prevented with appropriate skin hygiene and exercise.

Men can develop hydrocele or swelling of the scrotum due to infection with one of the parasites that causes LF specifically *W. bancrofti*.

Filarial infection can also cause tropical pulmonary eosinophilia syndrome, although this syndrome is typically found in persons living with the disease in Asia. Symptoms of tropical



pulmonary eosinophilia syndrome include cough, shortness of breath, and wheezing. The eosinophilia is often accompanied by high levels of IgE (Immunoglobulin E) and antifilarial antibodies.

### **Diagnosis and Treatment**

The standard method for diagnosing active infection is the identification of microfilariae in a blood smear by microscopic examination. The microfilariae that cause lymphatic filariasis circulate in the blood at night (called nocturnal periodicity). Blood collection should be done at night to coincide with the appearance of the microfilariae, and a thick smear should be made and stained with Giemsa or hematoxylin and eosin. For increased sensitivity, concentration techniques can be used.

Serologic techniques provide an alternative to microscopic detection of microfilariae for the diagnosis of lymphatic filariasis. Patients with active filarial infection typically have elevated levels of antifilarial IgG4 in the blood and these can be detected using routine assays.

Because lymphedema may develop many years after infection, lab tests are most likely to be negative with these patients.

#### ***Patients currently infected with the parasite***

Diethylcarbamazine (DEC) is the drug of choice in the United States. The drug kills the microfilaria and some of the adult worms. DEC has been used world-wide for more than 50 years. Because this infection is rare in the U.S., the drug is no longer approved by the Food and Drug Administration (FDA) and cannot be sold in the U.S. Physicians can obtain the medication from CDC after confirmed positive lab results. CDC gives the physicians the choice between 1 or 12-day treatment of DEC (6 mg/kg/day). One day treatment is generally as effective as the 12-day regimen. DEC is generally well tolerated. Side effects are in general limited and depend on the number of microfilariae in the blood. The most common side effects are dizziness, nausea, fever, headache, or pain in muscles or joints.

DEC should not be administered to patients who may also have onchocerciasis as DEC can worsen onchocercal eye disease. In patients with loiasis, DEC can cause serious adverse reactions, including encephalopathy and death. The risk and severity of the adverse reactions are related to *Loa loa* microfilarial density.

The drug ivermectin kills only the microfilariae, but not the adult worm; the adult worm is responsible for the pathology of lymphedema and hydrocele.

Some studies have shown adult worm killing with treatment with doxycycline (200mg/day for 4–6 weeks).

***Patients with clinical symptoms***

Lymphedema and elephantiasis are not indications for DEC treatment because most people with lymphedema are not actively infected with the filarial parasite.

To prevent the lymphedema from getting worse, patients should ask their physician for a referral to a lymphedema therapist so they can be informed about some basic principles of care such as hygiene, exercise and treatment of wounds.

Patients with hydrocele may have evidence of active infection, but typically do not improve clinically following treatment with DEC. The treatment for hydrocele is surgery.

**Prevention and Control**

The best way to prevent lymphatic filariasis is to avoid mosquito bites. The mosquitoes that carry the microscopic worms usually bite between the hours of dusk and dawn. If you live in an area with lymphatic filariasis:

- at night
  - sleep in an air-conditioned room or
  - sleep under a mosquito net
- between dusk and dawn
  - wear long sleeves and trousers and
  - Use mosquito repellent on exposed skin.

Another approach to prevention includes giving entire communities medicine that kills the microscopic worms -- and controlling mosquitoes. Annual mass treatment reduces the level of microfilariae in the blood and thus, diminishes transmission of infection. This is the basis of the global campaign to eliminate lymphatic filariasis.

Experts consider that lymphatic filariasis, a neglected tropical disease (NTD), can be eradicated and a global campaign to eliminate lymphatic filariasis as a public health problem is under way. The elimination strategy is based on annual treatment of whole communities with combinations of drugs that kill the microfilariae. As a result of the generous contributions of these drugs by the companies that make them, tens of millions of people are being treated each year. Since these drugs also reduce levels of infection with intestinal worms, benefits of treatment extend beyond lymphatic filariasis. Successful campaigns to eliminate lymphatic filariasis have taken place in China and other countries.

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## ***13.12 SCHISTOSOMA***

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### **Systematic Position**

**Phylum-** Platyhelminthes

**Class-** Trematoda

**Subclass-** Digenea

**Order-** Strigeidida

**Family-** Schistosmatidae

**Genus-** *Schistosoma*

**Species-** *mansoni*

### **General characters**

- It is a digenetic blood fluke parasitic trematode.
- It is found near the moist places.
- This parasite resides inside the blood vessels of primary host.
- Adult worms reside in pairs.
- Its secondary host is snail.
- Sexes are separate.
- The female lives inside the gynecophoral canal of male.
- Female lay enormous eggs.
- Many larval forms are found in its life cycle like miracidium & cercaria.
- They secrete hepatic enzyme which helps in feeding.

Schistosomiasis, also known as bilharzia, is a disease caused by parasitic worms. Although the worms that cause schistosomiasis are not found in the United States, more than 200 million people are infected worldwide. In terms of impact this disease is second only to malaria as the most devastating parasitic disease. Schistosomiasis is considered one of the Neglected Tropical Diseases (NTDs).

The parasites that cause schistosomiasis live in certain types of freshwater snails. The infectious form of the parasite, known as cercariae, emerges from the snail, hence contaminating water.

You can become infected when your skin comes in contact with contaminated freshwater. Most human infections are caused by *Schistosoma mansoni*, *S. haematobium*, or *S. japonicum*.

Schistosomiasis occurs in places with poor sanitation. School-age children who live in these areas are often most at risk because they tend to spend time swimming or bathing in water containing infectious cercariae.

If you live in, or travel to, areas where schistosomiasis is found and are exposed to contaminated freshwater, you are at risk.

Areas where human schistosomiasis is found include:

- *Schistosoma mansoni*
  - Distributed throughout Africa: There is risk of infection in freshwater in southern and sub-Saharan Africa—including the great lakes and rivers as well as smaller bodies of water. Transmission also occurs in the Nile River valley in Sudan and Egypt
  - South America: including Brazil, Suriname, Venezuela
  - Caribbean (risk is low): Dominican Republic, Guadeloupe, Martinique, and Saint Lucia.
- *S. haematobium*
  - Distributed throughout Africa: There is risk of infection in freshwater in southern and sub-Saharan Africa—including the great lakes and rivers as well as smaller bodies of water. Transmission also occurs in the Nile River valley in Egypt and the Mahgreb region of North Africa.
  - found in areas of the Middle East
- *S. japonicum*
  - found in Indonesia and parts of China and Southeast Asia
- *S. mekongi*
  - found in Cambodia and Laos
- *S. intercalatum*
  - found in parts of Central and West Africa.

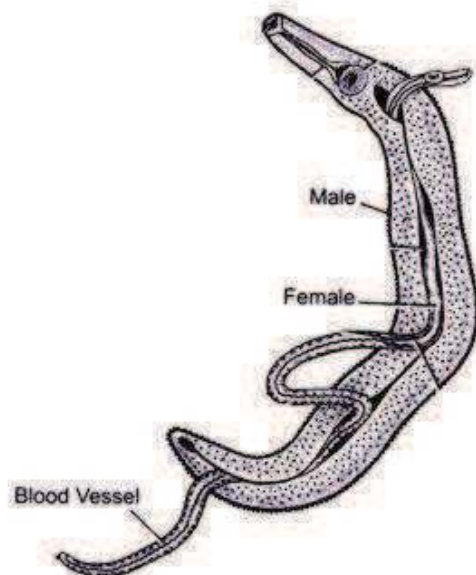


Fig 13.17 *Schistosoma mansoni*

## LIFE-CYCLE

Adult worms reside in pairs: the female lying in the gynecophoral canal of the male. After fertilization, eggs are passed into the venules. A larval form – the miracidium - develops within the egg. Its lytic enzymes and the contraction of the venule rupture the wall of the venule liberating the egg into the perivascular tissues of the intestine or urinary bladder. The eggs pass into the lumens and organs and are evacuated in the feces or the urine. On contact with fresh water the miracidia hatch from the eggs and swim about until they find the appropriate intermediate host, snail, which they penetrate. After two generations of sporocyst development and multiplication within the snail, the fork-tailed cercariae emerge. Infection to man takes place during bathing or swimming. The cercariae penetrate the skin and are carried into the systemic circulation and pass through to the portal vessels grow & mature Within the intra hepatic portion of the portal system, the worms feed and grow to maturity.

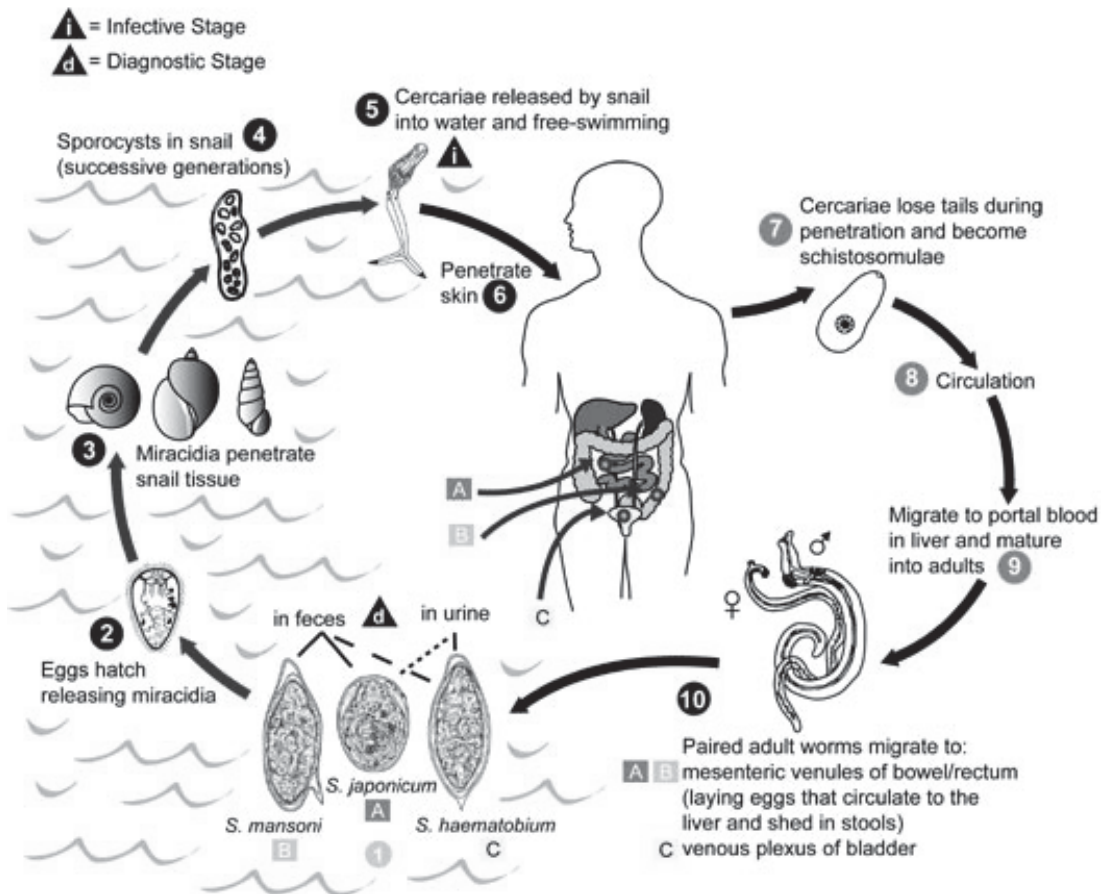


Fig 13.18 Life cycle *Schistosoma mansoni*

Eggs are eliminated with feces or urine. Under optimal conditions the eggs hatch and release miracidia, which swim and penetrate specific snail intermediate hosts. The stages in the snail include 2 generations of sporocysts and the production of cercariae. Upon release from the snail, the infective cercariae swim, penetrate the skin of the human host, and shed their forked tail, becoming schistosomulae. The schistosomulae migrate through several tissues and stages to their residence in the veins. Adult worms in humans reside in the mesenteric venules in various locations, which at times seem to be specific for each species. For instance, *S. japonicum* is more frequently found in the superior mesenteric veins draining the small intestine, and *S. mansoni* occurs more often in the superior mesenteric veins draining the large intestine. However, both species can occupy either location, and they are capable of moving between sites, so it is not possible to state unequivocally that one species only occurs in one location. *S. haematobium* most often occurs in the venous plexus of bladder, but it can also be found in the

rectal venules. The females (size 7 to 20 mm; males slightly smaller) deposit eggs in the small venules of the portal and perivesical systems. The eggs are moved progressively toward the lumen of the intestine (*S. mansoni* and *S. japonicum*) and of the bladder and ureters (*S. haematobium*), and are eliminated with feces or urine, respectively. Pathology of *S. mansoni* and *S. japonicum* schistosomiasis includes: Katayama fever, hepatic perisinusoidal egg granulomas, Symmers' pipe stem periportal fibrosis, portal hypertension, and occasional embolic egg granulomas in brain or spinal cord. Pathology of *S. haematobium* schistosomiasis includes: hematuria, scarring, calcification, squamous cell carcinoma, and occasional embolic egg granulomas in brain or spinal cord.

Human contact with water is thus necessary for infection by schistosomes. Various animals, such as dogs, cats, rodents, pigs, horse and goats, serve as reservoirs for *S. japonicum*, and dogs for *S. mekongi*.

### **PATHOGENECITY**

Schistosomiasis is caused by digenetic blood trematodes. The three main species infecting humans are *Schistosoma haematobium*, *S. japonicum*, and *S. mansoni*. Two other species, more localized geographically, are *S. mekongi* and *S. intercalatum*. In addition, other species of schistosomes, which parasitize birds and mammals, can cause cercarial dermatitis in humans.

*Schistosoma* causes schistosomiasis. Patients infected with it suffer from terminal haematuria and painful micturition. There is inflammation of the urinary bladder (cystitis), and enlargement of spleen and liver. Cercarial dermatitis (swimmers itch) and dysentery (mucus and blood in stool with tenesmus) as well as enlargements of the spleen and liver.

Infection occurs when skin comes in contact with contaminated freshwater in which certain types of snails that carry the parasite are living. Freshwater becomes contaminated by *Schistosoma* eggs when infected people urinate or defecate in the water. The eggs hatch, and if the appropriate species of snails are present in the water, the parasites infect, develop and multiply inside the snails. The parasite leaves the snail and enters the water where it can survive for about 48 hours. *Schistosoma* parasites can penetrate the skin of persons who come in contact with contaminated freshwater, typically when wading, swimming, bathing, or washing. Over

several weeks, the parasites migrate through host tissue and develop into adult worms inside the blood vessels of the body. Once mature, the worms mate and females produce eggs. Some of these eggs travel to the bladder or intestine and are passed into the urine or stool.

Symptoms of schistosomiasis are caused not by the worms themselves but by the body's reaction to the eggs. Eggs shed by the adult worms that do not pass out of the body can become lodged in the intestine or bladder, causing inflammation or scarring. Children who are repeatedly infected can develop anemia, malnutrition, and learning difficulties. After years of infection, the parasite can also damage the liver, intestine, spleen, lungs, and bladder.

### *Common Symptoms*

Most people have no symptoms when they are first infected. However, within days after becoming infected, they may develop a rash or itchy skin. Within 1-2 months of infection, symptoms may develop including fever, chills, cough, and muscle aches.

### *Chronic schistosomiasis*

Without treatment, schistosomiasis can persist for years. Signs and symptoms of chronic schistosomiasis include: abdominal pain, enlarged liver, blood in the stool or blood in the urine, and problems passing urine. Chronic infection can also lead to increased risk of bladder cancer.

Rarely, eggs are found in the brain or spinal cord and can cause seizures, paralysis, or spinal cord inflammation.

## **Diagnosis and Treatment**

Stool or urine samples can be examined microscopically for parasite eggs (stool for *S. mansoni* or *S. japonicum* eggs and urine for *S. haematobium* eggs). The eggs tend to be passed intermittently and in small amounts and may not be detected, so it may be necessary to perform a blood (serologic) test.

Safe and effective medication is available for treatment of both urinary and intestinal schistosomiasis. Praziquantel, a prescription medication, is taken for 1-2 days to treat infections caused by all *Schistosoma* species.



## Prevention and Control

### *Prevention*

No vaccine is available.

The best way to prevent schistosomiasis is to take the following steps if you are visiting or live in an area where schistosomiasis is transmitted:

- Avoid swimming or wading in freshwater when you are in countries in which schistosomiasis occurs. Swimming in the ocean and in chlorinated swimming pools is safe.
- Drink safe water. Although schistosomiasis is not transmitted by swallowing contaminated water, if your mouth or lips come in contact with water containing the parasites, you could become infected. Because water coming directly from canals, lakes, rivers, streams, or springs may be contaminated with a variety of infectious organisms, you should either bring your water to a rolling boil for 1 minute or filter water before drinking it. Bring your water to a rolling boil for at least 1 minute will kill any harmful parasites, bacteria, or viruses present. Iodine treatment alone will not guarantee that water is safe and free of all parasites.
- Water used for bathing should be brought to a rolling boil for 1 minute to kill any cercariae, and then cooled before bathing to avoid scalding. Water held in a storage tank for at least 1 - 2 days should be safe for bathing.
- Vigorous towel drying after an accidental, very brief water exposure may help to prevent the *Schistosoma* parasite from penetrating the skin. However, do not rely on vigorous towel drying alone to prevent schistosomiasis.

Those who have had contact with potentially contaminated water overseas should see their health care provider after returning from travel to discuss testing.

### *Control*

In countries where schistosomiasis causes significant disease, control efforts usually focus on:

1. reducing the number of infections in people and/or
2. eliminating the snails that are required to maintain the parasite's life cycle.

For all species that cause schistosomiasis, improved sanitation could reduce or eliminate transmission of this disease. In some areas with lower transmission levels, elimination of schistosomiasis is considered a "winnable battle" by public health officials.

Control measures can include mass drug treatment of entire communities and targeted treatment of school-age children. Some of the problems with control of schistosomiasis include:

1. Chemicals used to eliminate snails in freshwater sources may harm other species of animals in the water and, if treatment is not sustained, the snails may return to those sites afterwards.
2. For certain species of the parasite, such as *S. japonicum*, animals such as cows or water buffalo can also be infected. Runoff from pastures (if the cows are infected) can contaminate freshwater sources.

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### ***13.13 SUMMARY***

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Parasites include one of the most important interactions in the living world which has wide variations in degree & in its impact on the host. A truly successful parasite obtains nutrients & shelter from host. The parasites which produce injury to their hosts are pathogens. While parasites are adapted to living in or on their hosts, they can only survive by producing offspring capable of finding new hosts. The key to understanding their dispersal through the world is through knowledge of their life-cycles or modes of transmission.

About two dozen species of parasitic protozoans are found to be getting their nutrition on human-beings, Protozoans which are parasitic on human-beings cause serious disease to man, these are characterized by obligatory nature, host specificity, secretion of toxic substance & having more than one host.

Some common human protozoan parasites are Entamoeba, Trypanosoma, Leishmania, Giardia etc. Entamoeba is a monogenetic parasite of humans causing amoebic dysentery, transmitted through infected food & water. Trypanosoma is a digenetic parasite of human blood causing lymphadenitis; it is transmitted by Tse-tse fly a vector.

Leishmania is the causative agent of kala-azar or visceral leishmaniasis, it is a digenetic parasite spread by vector sand fly. Giardia is a flagellated protozoan causing diarrhoea in human-beings; transmission takes place with contaminated food & water.

Nematodes are generally termed as round worms, they inhabit fresh water, salt water, animal & plant tissues. They are pseudocoelomate, worm like, cylindrical with unsegmented body & unisexual in nature. *Ascaris* is commonly called as round worm & causes ascariasis which is most common in children; it is transmitted through unhygienic food, water & sanitary habits.

*Ancylostoma* is commonly known as hook worm, it causes ancylostomiasis & infective stage is filarial larva which penetrates the skin of the feet & reaches the lungs.

*Enterobius* is commonly called as Pin worm & the most common parasite of humans. It causes appendicitis & anal region infections, good sanitary habits help in its prevention. *Wucheria* is commonly called as Filaria worm & causes disease filariasis. It is spread by the vector a mosquito (*Culex fatigans*), eradication of its vector is the best prevention of the parasite.

Helminthes are generally leaf like with body covered by cuticle, they have organs of attachment. *Schistosoma* is commonly known as Blood fluke & found mainly in the blood vessels of human beings. It causes disease schistosomiasis, for completion of its life cycle it needs water & intermediate host snail. It has three larval forms miracidium, cercaria & metacercaria respectively. Metacercaria is the infective stage of the parasite.

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### ***13.14 SELF ASSESSMENT QUESTION***

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#### **Long Answers Type Questions**

1. Describe the pathogenesis of *Entamoeba*?
2. What is the flagellated responsible for causing kala azar?
3. What are the common characteristics of leishmania?
4. With the help of labelled diagram explain the life-cycle of *Trypanosoma*?
5. Write a detailed note on parasitic adaptations in *Ascaris*?
6. What disease is caused by *Ancylostoma*?
7. Describe the life history of *Wucheria bancrofti*?

**Short Answer Type Questions**

1. Name the primary & intermediate host of Trypanosoma?

**Ans:** Human being's are the primary host where as Tsetse fly (Glossina) is the intermediate host.

2. Name the polymorphic forms of Schistosoma?

**Ans:** Miracidium & Cercaria larvae.

3. What is the causative organism of Kala-azar?

**Ans:** Leishmania donovani is the causative organism of kala-azar.

4. Name the disease caused by Entamoeba?

**Ans:** The disease caused by entamoeba is amoebic dysentery or amoebiasis.

5. Maximum number of eggs released by single female Ascaris in a day?

**Ans:** A single female Ascaris can release upto 2,00,000 eggs in a day.

**Fill in the blanks:**

1. Giardia is commonly called as.....

2. Schistosoma is..... in nature.

3. Enterobius is.....

4. .... is commonly used as a worm drug.

5. Zoological name of sand-fly is.....

**Multiple Choice Questions**

1. Microfilaria is larva of:

- (a) Tapeworm
- (b) Pin worm
- (c) Mud worm
- (d) Filarial worm

2. Enterobius is generally known as:

- (a) Pin worm
  - (b) Whip worm
  - (c) Eye worm
  - (d) Gut worm
3. Ascaris is:
- (a) Host
  - (b) Bisexual
  - (c) Dioecious
  - (d) Digenetic
4. Pre-cystic stage of Entamoeba is called:
- (a) Magna form
  - (b) Minuta form
  - (c) Feeding form
  - (d) Growing trophozoite
5. Leptomonad form of leishmania is found in:
- (a) Man
  - (b) Sand fly
  - (c) Tsetse fly
  - (d) Bed fly
6. Trypanosoma lives inside the human body in
- (a) lymph
  - (b) blood
  - (c) fluid
  - (d) none

**Answer:****Fill in the blanks:**

1. Grand Old Man of the Intestine
2. Sanguivorous
3. Monogenetic
4. Piperazine
5. Phebotomus

**Multiple Choice Questions:**

1. d
2. a
3. c
4. b
5. b
6. b

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### ***13.15 GLOSSARY***

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**Autoinfection:** self infection

**Blepharoplast:** A small granule-like body, usually appearing in the cytoplasm, from which an axoneme arises. Axonemes may form rod-like structures in the cytoplasm, cilia, or flagella.

**Cercaria :** The free-swimming larva of a trematode (usually possessing a tail) which escapes from a sporocyst or redia generation within the intermediate, molluscan host and constitutes the transfer stage to the next host

**.Chyluria:** lymphatic fluid

**Commensal:** An association, usually an obligate one, in which an organism lives on or in another, usually larger organism (the host) and derives its nourishment from the host organism without causing damage to the latter

**Contamination:** The general meaning of this term is clear in that it refers to feces in the context of parasitic disease, however, it should be remembered that a contaminated object is not necessarily infective in as much as the parasite may not be in its infective stage.

**Cyst:** An organism together with the enveloping membrane or wall secreted by that organism; the stage of a protozoan in which the organism is encased in a "cyst wall "; an encysted organism; a protected or more resistant stage that may be involved in transmission to a new host.

**Definitive host:** An animal that harbors a parasite where it reaches sexual maturity in or on it.

**Digenetic:** Three or more generations (literally "two", adult and larval) required for completion of one life cycle (or generation), as in digenetic trematodes. In parasitology, application of this term is virtually limited to those trematodes requiring one or more intermediate hosts

**Filariform:** (juvenile) A post-feeding-stage, of a nematode characterized by its delicate, elongate structure and its slim, capillary esophagus. Also, the infective stage of hookworm, filarial worms, and some other nematodes.

**Gynecophoral canal:** This is a canal in the male schistosome where the adult female worm is carried.

**Haematuria:** Presence of blood in the urine

**Hermaphrodite:** having both sexes in one

**Infective Stage:** That stage in the life cycle of a parasite during which it is capable of producing infection

**Intermediate host:** Hosts normally infected with certain parasites, which are also capable of infecting humans.

**Molluscicide:** Chemical used to kill snails

**Proglottid:** a unit of tapeworm body

**Trophozoite:** The active, vegetative stage of a protozoan.

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