PARASITOLOGY AND IMMUNOLOGY

(DZOO23)

(MSC - ZOOGOLOGY)

ACHARYA NAGARJUNA UNIVERSITY

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UNIT – I

1.1 PARASITISM : SYMBIOTIC RELATIONSHIPS, KINDS OF PARASITES, ADAPTATIONS FOR PARASITIC LIFE AND ECOLOGY OF PARASITISM

1.1.1 OBJECTIVES

The purpose of this lesson is to:

* study parasitism
* lead to a fuller understanding of host-parasite relationships and
* know the means of transmission of all infective stages

1.1.2 INTRODUCTION

Parasitology is the study of parasitism. In animal parasitism, one species, the parasite, living in or on another species, the host, gains its livelihood at the expense of the host. The host provides both the habitat and the food for the parasites. The parasite always does damage in some degree to its host.

1.1.3 SYMBIOTIC RELATIONSHIPS

The relationship between individuals of different species is termed interspecific relationship. Symbiosis refers to the close physical interrelationship between two different species. Three types of symbiosis are generally recognized. They are mutualism, commensalism and parasitism.

1.1.3.a MUTUALISM

Mutualsim is that relationship in which the host and the symbiont are depended on each other physiologically and the relationship is mutually beneficial. Mutualism is of two types:

A. Mutualism with continuous contact
B. Mutualism without continuous contact

A. Mutualism with continuous contact:

This type of relationship is permanent. The two symbionts are in close contact and physiologically interdependent on each other. Termites and their intestinal protozoa are an example. The termites provide the habitat and food in the form of wood (cellulose) to the protozoa in the intestine. The wood can not be digested by the termites. However, the intestinal protozoa are capable of hydrolyzing the wood for their own and termites use.

An interesting example is furnished by various Sea-anemones (Fig. 1-1) which live on Gastropod shells inhabited by Hermit-crabs. The Sea-anemone is carried from place to place by the Hermit-crab, and in this way secures a more varied and abundant food-supply. On the other hand, the Hermit-crab is protected from the attack of predaceous fishes by retreating into its shell and having exposed the Sea-anemone. The Sea-anemone owing to its toughness, and to the pain caused by its poisonous stinging capsules, is usually avoided as an article of food.

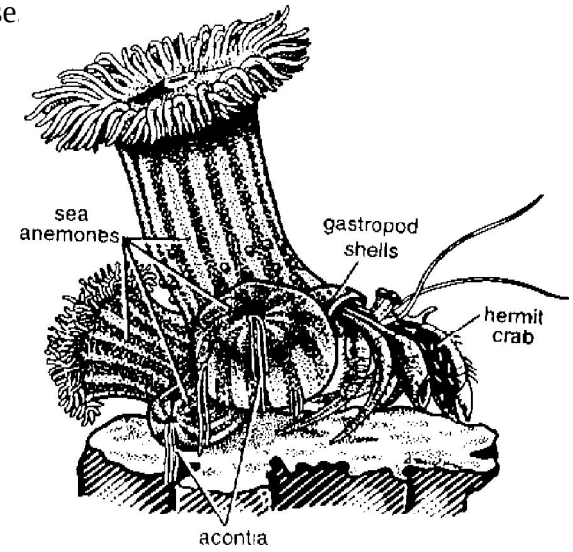

Fig. 1-1. Sea-anemones on Gastropod shells inhabited by Hermit-crabs.

B. Mutualism without continuous contact:

In this case the partners or symbionts are in contact for only a short period. An example is furnished by the cowbird which sits on the back of grazing animals. The cowbirds often sit on the back of grazing animals and feed on the ectoparasites such as ticks and mites. In this way the birds get ready supply of food and the host animal may get rid of its pest. Also the hosts are frequently warned of approaching danger by the activity of birds as watchmen.

Other birds –such as Egyptian plover are found associated temporarily with crocodiles. In this case the crocodile allows the bird to enter its mouth for the search of parasites and leech. The bird removes parasites on the body and leeches from the teeth of the crocodile, and in this way it gets abundant food. On the other hand, the crocodile is relieved from leeches and ectoparasites.

1.1.3.b COMMENSALISM

Commensalism constitutes one type of relationship in which the host provides the habitat and food for its symbionts. The symbionts live without causing benefit or harm to the host. The symbionts, however, are physiologically dependent on the host for their existance. The host, on the other hand, is not dependent on them. Commensalism is divided into two types:

A. Commensalism with continuous contact
B. Commensalism without continuous contact

A. Commensalism with continuous contact:

In this type of commensalism, the commensals remain more or less in permanent contact with their hosts. The familiar examples include ectocommensals and endocommensals. Ectocommensals (epizoites) are found in association with other animals for the purpose of anchorage and protection.

Some examples of ectocommensals are:

i) Ciliates (protozoans) living on the bodies of frogs, fishes, mollusks, arthropods etc.

ii) Hydroids and Sea anemones growing on the bivalve shells.

iii) Sea anemones and crabs are other examples of ectocommensalism.

Endocommensals (microorganisms and protozoans) live within the cavities or tissues of higher plants and animals. Certain of the protozoa (*Entamoeba coli*) living in the alimentary canal of man and *Opalina* in frog intestine are examples of endocommensals.

B. Commensalism without continuous contact:

In this type of commensalism, the partners are in contact for a short period. Examples include:
i) Sharks or whales and sucker fish

Echenies possesses a dorsal sucker; hence it is called sucker fish. It attaches by the dorsal sucker to the underside of sharks or whales or sea turtles. By attaching to sharks or whales or sea turtles, sucker fish are carried to other places and search for food.

ii) Sea cucumber and Pearl fish

An interesting commensal relationship exists between the slender tropical pearl fish and sea cucumbers. These little fish, which are about 15cm. long make their home in the trunk of one of the respiratory trees of certain sea cucumbers. The fish feed on crustaceans. The fish leaves the host at night while it searches for food. After such excursions, the fish forces its way into the cloaca and comes back to the respiratory tree.

1.1.3.c PARASITISM

Parasitism is the condition in which the symbiont is physiologically dependent on the host for its habitat and living. At the same time it is harmful to the host. All of trematodes, cestodes, acanthocephalans, and many of the protozoa and nematodes are examples of true animal parasites. The parasitism can be classified into the following categories:

a. Accidental – when a free living animal accidentally reaches inside the body of host and leads a parasitic life for sometime.

b. Facultative – when the animal is able to live both as parasite and/or free living animal.

c. Obligatory – when the animal needs a suitable host for living. The obligatory parasitism is of two types:

i) Ectoparasitism – the animal lives on external surface of the host.
ii) Endoparasitism – the animal lives inside the body of the host.

1.1.4 KINDS OF PARASITES

Several kinds of parasites are reported, depending on their relationship to the host. The parasitic animals are divided into two groups basing on the location on or in the body of the host.
Ectoparasites: Parasites which live on the external surface of the body of the host or cavities that open directly on to the surface. Monogenetic trematodes, fleas, lice, mites and ticks are some examples.

Endoparasites: Parasites which live inside the body of the host. Usually they live in the alimentary canal, lungs, liver and other organs, tissues, cells, and body cavities. Digenetic trematodes, nematodes, tapeworms and sporozoans are some examples.
Depending on their location within the body, endoparasites are recognized into three types:

Coelozoic parasites: These are parasites living in cavities or alimentary canal of the host.
Example: *Entamoeba histolytica* in human intestine

Cytozoic parasites or intracellular parasites: The parasites which live inside the cells of host tissues.
Examples: *Plasmodium* lives in red blood cells and parechymatous liver cells of man. *Leishmania donovani* lives in white blood corpuscles of blood and cells of liver, spleen, bone marrow, lymph glands, etc.

Histozoic parasites or intercellular or extracellular parasites: These are parasites which live in between the cells of tissues or organs.
Example: *Trypanosoma gambiense* lives in the blood, lymph and cerebrospinal fluid of human beings.

Depending on the amount spent on or in the host, parasites are divided into temporary and permanent parasites.

Temporary or partial parasites: These are parasites which spend only a part of their life cycle on or in the host.

Examples: i) Glochidium larva: The larva of bivalve molluscs, Fresh water mussel. Glochidium larva leads ectoparasitic life on fishes for a short period whereas its adults are free-living.

ii) Mosquitoes: The larvae of blood sucking mosquitoes re free-living whereas the adults are parasitic.

Permanent parasites: These are parasites which spend their entire life in the body of the host.

Examples: *Plasmodium*, *Trichinella*, *Entamoeba*, tapeworms, round worms, filarial worms, etc.

Obligate parasites: These are parasites that must lead a parasitic life wither partly or completely, otherwise, they are unable to survive. Most of the endoparasites are obligate parasites.

Examples: *Taenia solium, Fasciola hepatica, Ascaris, Entamoeba,* etc.

Facultative parasites: These organisms may be leading their life as parasite or free-living form at its will.

Examples: Tuberculosis causing bacterium, *Microbacterium tuberculosis* in man.

Some free-living nematodes such as *Rhabditis* and *Turbotrix.*

Intermittent parasites: These parasites visit and leave their hosts at intervals.
Examples: Mosquitoes, lice, leeches, etc.

Accidental parasites: Nemathelminthese which become parasites when accidentally introduced into other animals.
Examples: Soil and fresh water nematodes.

Incidental parasites: These parasites appear incidentally in unusual hosts under natural conditions.
Examples: *Echinococcus,* the dwarf tapeworm of dogs is sometimes found in children.
The common liver fluke of sheep occurs sometimes in dogs or cats.

Erratic or aberrant parasites: Parasites that wander into unusual places in the normal host.
Example: Ascarids of swine and man may wander from the intestine into the nostrils, liver, or body cavity.

Pathogenic parasites: The parasites which cause injury or disease to their primary hosts are called pathogenic parasites. Most of the endoparasites are pathogenic.
Examples: *Plasmodium, Trypanosoma, Entamoeba histolytica, Ancylostoma, Ascaris, Taenia,* etc.

Non-pathogenic parasites: The parasites which do not cause any harm to their primary hosts are called non-pathogenic parasites.
Examples: *Entamoeba coli, Trichomonas hominis, Enteromonas,* etc.

Hyperparasitism: Sometimes, parasites themselves are parasitized by other organisms. Such parasites are known as hyper parasites.
Examples: *Nosema notabilis* is an hyperparasite on myxosporidian.
Several small amoebae live as hyerparasites on *Opalina,* a common parasite in frog's intestine.

Udonella caligorum, a small monogenetic trematode occurs often in clumps on egg cases of caligoid copepods which are themselves parasitic on fish.

Sexual parasitism: In this type of parasitism either the male or the female becomes parasite on the other sex.

Examples:

In case of *Schistosomes* (blood flukes), (dioecious forms)- the male carries the female permanently in the gynecophoric canal. The female carries its life as parasite on male.

Species of *Bonellia* exhibit remarkable sexual dimorphism. In *Bonellia viridis*, the female trunk may be 8 cm. long, whereas the minute males are only 1 to 3 mm. in length and live within the female's uterus or coelom. The male is entirely dependent on the female for food and shelter (Fig. 1-2).

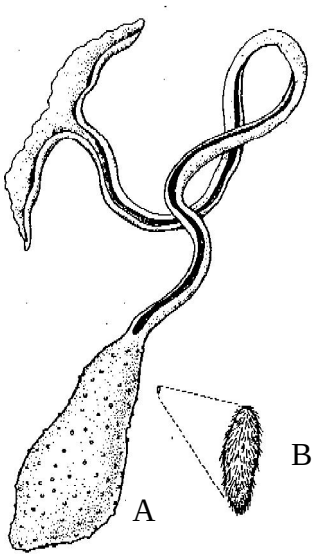

Fig. 1-2. *Bonellia viridis*. A. Female. B. Dwarf male.

1.1.5 ADAPTATIONS FOR PARASITIC LIFE

The basic requirements of a parasites are similar to those of free living animals. They are habitat, food and reproduction. To achieve these requirements, special adaptations have evolved in parasites.

In order to live on or in a host, the parasite must evolve structures for adherence. Monogenetic trematodes have rigid hooks for attaching to the host. Trematodes and cestodes have highly developed suckers for the same purpose.

Parasites of the alimentary canal, lungs, liver and reproductive systems use the natural outlets of these organ systems as avenues of exit for eggs and cysts. The parasites living in the blood stream and tissues generally utilize other animals or means to leave their hosts. Malarial parasites, protozoans and the microfilariae of nematodes use blood sucking arthropods to escape from the hosts and develop further.

Transmission of the infective stage of the parasite to the next host is accomplished by one of three methods. They are passive, active or inoculative. Passive transmission occurs when the infective stages of the parasites contaminate or infect the food or water of the host and are swallowed with them. Examples are the eggs of ascarids cysts of *Enta*

stages of trematodes, cestodes and nematodes also contaminate the food. Active transfer occurs in the hook-worms and the miracidia and cercaria of trematodes. These parasites actively penetrate the bodies of their hosts upon coming in contact with them. Inoculative transmission occurs when the infective stage of the parasite has developed in the body of blood sucking arthropod. For example, *Plasmodium* which develops in female anopheles mosquitoes, transfer back to vertebrate host. Transmission occurs when the mosquito inoculates the parasites into the host during feeding.

The survival of the parasites within the host depends on the ability of the parasite to withstand the destructive action of the digestive juices and the immunological reactions of the host. The tremendous production of eggs as in the ascarids and cestodes, the duplication of sex organs in segments as in cestodes or

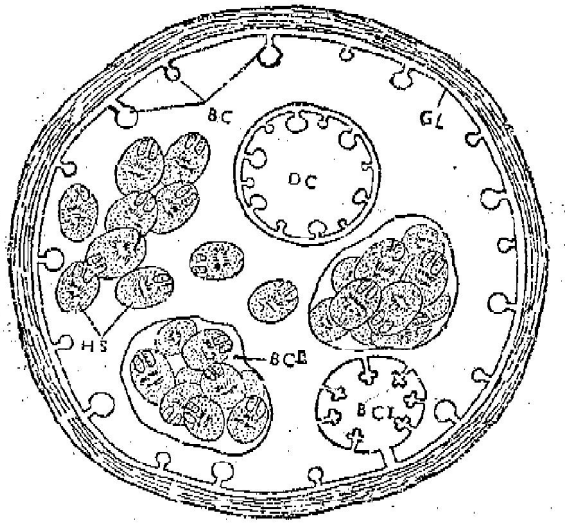

Fig. 1-3. DIAGRAM SHOWING THE DEVELOPMENT OF HYDATID CYST

BC, brood capsule in formation; BC I, brood capsule with scolex; BC II, fully developed brood capsule with scoleces, detached from the side wall; DC, Daughter-cyst; FL, fibrous layer; GL, germ layer; HS, hydatid sands.

vegetative reproduction extending for long periods in sporocysts or rediae of trematodes and scolices in cestodes (*Echinococcus* and *Multiceps*) are some of the biotic potentialities of the parasite for the successful completion of their life cycles (Fig. 1-3).

1.1.6 ECOLOGY OF PARASITISM

Ecology refers to the relationship of the parasite to its habit and habitat for essentials required for the parasitic life. The requirements for development and survival in the physical environment outside their hosts include favourable temperature, adequate moisture, sufficient oxygen and nutrients. In addition, there must be protection from adverse environmental conditions like freezing, extreme temperature and desication. In some parasites, the physical factors of environment provide stimuli for: hacthing of the eggs of many trematodes and cestodes, movement of swimming cercariae to go to light or away from light, the diurnal periodicity of microfilariae, the exit of cercariae from snail host (in day light)and molting of first and second stage larvae of strongyles and trichostrongyles.

The biological environment of the host also provides stimulus for hatching of eggs and growth of larval stages, nourishment, location finding and a favorable site for reproduction. The host may react with the parasite by producing antibodies which prevent the establishment of the parasite.

Only when the physical and biological environmental factors are favorable, the parasite can survive, mature, reproduce and be transmitted to other hosts.

Parasites of terrestrial hosts show some ecological adaptations to meet the adverse factors of the environment. They include thick shelled eggs, ensheathed larval stages or intermediate hosts that provide protection during the growth and transmission period of the host.

1.1.6.a TRANSMISSION

The fertilized eggs of the parasites or the infective stages contaminate the food or water. When the contaminated food or water is swallowed accidentally, or when the infected stages encyst on forage or when the body of an intermediate host lodging the infective stages is eaten by the definitive host, transmission of the parasite occurs. Transmission is involved with ecology and linked in some manner with the food chain. For example, blood sucking intermediate hosts such as mosquitoes inject infective stages of plasmodia or some fialrioids. Cercariae of blood flukes in water directly penetrate the skin of humans. Infective larvae of hookworms and strongyloidids from the soil actively penetrate the skin.

1.1.6.b ESCAPE FROM HOSTS

Cysts, eggs or larval parasites from the digestive, urinary, reproductive and respiratory tracts escape through the natural openings and/or in the discharges of the respective systems of the host. Certain ascarids of the digestive tract are transmitted by passing eggs through feces.

1.1.6.c DIRECT LIFE CYCLES

The Nemathelminthes have both a direct and an indirect type of life cycle. When the life cycle of the parasite is completed without the involvement of an intermediate host, it is called direct life cycle. In case of parasites with a direct life cycle, cysts, oocysts and eggs are passed in the

feces of the host. The infective stage has a limited life span to the adverse facets of the environment. Transmission of the parasite is dependent upon the frequency of the visits of the host in that area where the infective stages were found.

Domestic ruminents confined to a particular area have a high degree of exposure; they frequently have heavy infection. Wild ruminants, on the other hand, may not visit the same spot for sometime. During this interval of time infective stages may have developed and died due to the non entry into the definitive (suitable) host. Feeding habits of definitive hosts also play an important role in transmission of infection. As an example, sheep which feed on grass close to the ground, acquire extremely heavy infection of trichostrongyles, whereas cattle which feed on higher grass are less heavily parasitized by these worms. Humans, particularly children, spread ascarids by contaminating their intermediate and limited habitat with feces containing eggs that are subsequently swallowed with soil. Contamination of the water with feces and supply of such contaminated water to communities or families may be the way of spreading infection. Water contaminated with feces containing cysts of *Entamoeba histolytica* and *Giardia lamblia* is another mode of transmission.

The presence of parasites with direct life cycles is dependent primarily on the occurrence of the definitive host.

1.1.6.d INDIRECT LIFE CYCLES

When the life cycle of the parasite is completed with the involvement of one or two intermediate hosts, it is called indirect life cycle. Trematode and cestode life cycles are indirect, involving one or two hosts (except in primitive caryophyllaeids which are direct).

Parasites with an indirect life cycle need more restrictive requirements. Digenetic trematodes utilize aquatic mollusks, mostly snails as the first intermediate host. The snails may be a single or atmost a few closely related species. Eggs of these flukes are able to develop only if they are in a suitable aquatic habitat with the intermediate host. The host must be present at the right time, in the right place, must be readily infected and must survive until the parasite develops. Also, the cercariae must be released in a location when they can encyst on vegetation or penetrate the next host, whether it is a second intermediate or a definitive host.

The common liver fluke *Fasciola hepatica* is a classical example of a trematode with a single intermediate host. The cercariae can act only in water when they encyst on objects or vegetation to transform into infective metacercariae. However, the longevity of the metacercariae is influenced by physical factors such as moisture, oxygenation, sun light and temperature. The cercariae of blood flukes become active when the water is agitated; with the aid of histolytic enzymes secreted by penetration glands, they penetrate the epidermis and enter the circulatory system.

The Chinese liver fluke, *Clonorchis sinensis* is an example of a trematode with two intermediate hosts. An aquatic snail and fish serve as intermediate hosts for this trematode. In this model, a new set of ecological conditions is required for the parasitic stages. Fully embryonated eggs enclosed in thick shells are passed in the feces of definitive hosts. Hatching occurs in the digestive gland of the snail, that swallows the eggs. Cercariae are discharged into the water. The cercariae encyst on the muscles of fish. The second intermediate host contains the infective metacercariae and is often food of the definitive host.

Cestode life cycles are indirect, involving one or two intermediate hosts. The pork tapeworm of man, *Taenia solium* is a classical example of a cestode with a single intermediate host. The cestodes with a second intermediate host in the life cycle have a more complicated situation. Undeveloped eggs of pseudophyllidean tapeworms deposited in water embryonate and hatch into motile, ciliated hexacanths which, when eaten by cyclops, develop into procercoids. In the digestive tract of fish, they escape from the cyclops and migrate to the muscles and transform into pleurocercoids that are infective to mammals and birds.

The life cycles of nematodes are both direct and indirect. In either case, there is a common pattern consisting of an egg and five developmental stages. These include the first, second, third and fourth larval stages followed by the adult stage. Each stage except the first is preceded by a molt. In general, third stage larvae are infective to the definitive host, whether it is in the soil or in an intermediate host. Eggs of the strongyles, trichostrongyles, oxyurids, ascarids and some trichurids are undeveloped when laid. They depend on favorable environmental conditions for embryonation. Ascarid larvae develop to the second stage in the egg, where they remain without further changes. Hatching and further development occurs in the intestine when they are swallowed by the host. These eggs, both in the unembryonated and embryonated stages are extremely resistant to unfavorable conditions.

1.1.6.e ENTRANCE OF PARASITES INTO HOSTS

Infective stages of parasites enter into their hosts either positively or actively.

Cysts, oocysts, embryonated eggs, metacercariae, cysticerci, cysticercoids, plurocercoids and many hatched larvae such as strongyles, trichostrongyles, dracunculids and filaroids enter the host passively. They are dependent upon the host swallowing them while feeding and drinking. Miracidia, cercariae and certain larval nematodes such as hookworms, strongyloidids and filarioids actively penetrate the epithelial covering of their hosts as a result of stimuli provided by them.

1.1.6.f HOST PARASITE SPECIFICITY AS AN ECOLOGICAL FACTOR

The development and survival of the free living stages depend on the favorable conditions in the external environment. In the same way, the parasite/parasitic stages are able to develop and survive in the compatible circumstances (in the intestinal habitat) of the host. The parasites are able to tolerate the specific aspects of the physiological environment provided by that host. This type of relationship is referred to as host-parasite specificity. Some species of parasites are able to live and reproduce only in a single species of vertebrate host. The malarial parasite and beef and pork tapeworms of man are examples.

1.1.6.g RESISTANCE OF THE HOST AS AN ECOLOGICAL FACTOR

The presence of parasite and their metabolites stimulate the host to react by producing antigens. The phenomenon of self cure of stomach worms (*Haemonchus contortus)* in sheep is an example. When sheep already harboring a population of adult worms receive a new infection of larvae an immune reaction develops in the host's intestine. This reaction creates unfavorable environment by raising the pH of the intestine to a level which is untolerable to the adult worms. They detach the gastric mucosa and are expelled. Also, this altered environment remains unsuitable for the larval stages.

1.1.6.h ECOLOGICAL NICHE

Different species of intestinal parasites can live in the intestine of a single animal. *Entamoeba, Ascaris, Ancylostoma, Hymenolepis* and *Taenia* may all occur simultaneously in the small intestine of man. Each species has its own specific requirements and, therefore, may be regarded as occupying its own niche. The specific requirements (for all the life activities) for all the species can be supplied by the host.

1.1.6.i AGE AND DIET OF THE HOST

The age of the host may alter the bioenvironment. To some extent establishing the infection is difficult. For example, in many species of snails, only young individuals are susceptible to infection. *Toxocara canis* develops readily in the intestine of puppies but not in adult dogs.

Diet of the host may alter the intestinal environment and essential nutrients for the development of the intestinal parasites. A milk diet appears to produce an unfavorable environment for ascarids. Animals/humans on a low level of nutrition are more susceptible to infections. The bioenvironment may be altered because of malnutrition.

1.1.7 SUMMARY

An organism can not exist in nature without any relationship with other animals for getting shelter and nourishment.

The relationship may be intraspecific occurring among individuals of the same species or interspecific existing between different species.

When one member benefits and the other is neither benefited nor harmed, the relationship is termed commensalism.

In a close relationship of two members, when both benefit, it is termed as mutualism.

When one member benefits and the other is harmed, the relationship is termed parasitism.

The parasitism can be classified into accidental, facultative and obligatory types.

The basic requirements of a parasite are habitat, food and reproduction.

Monogenetic trematodes have rigid hooks for attaching to the host.

Digenetic trematodes and cestodes have highly developed suckers for attaching to the host.

Transmission of the infective stages of the parasite to the next host is accomplished by passive, active and inoculative methods.

Passive transmission of the infective stages occurs by swallowing the eggs/cysts through contaminated food.

Active transmission of infective stages occurs by direct penetration of the host.

Inoculative transmission occurs when the infective stages develop in the vector host and inoculated into the final host by vector bites.

Only under the influence of suitable physical and biological environmental factors the parasite is able to survive, mature, reproduce and be transmitted to suitable host.

The transmission of the infective stages is linked with the ecological food chain.

Cysts, eggs or larval parasites from the different systems of the host escape through natural openings of the host.

The nemathelminthes have both a direct and an indirect type of life cycle.

Parasites with direct life cycle are depended on the occurrence of definitive host.

Parasites with indirect life cycle are depended on suitable vector hosts, definitive host and restricted environment.

1.1.8 KEY TERMINOLOGY

Commensalism: A type of symbiotic relationship in which one species benefits from the relationship and the other species (host) is neither benefited nor harmed.

Ectocommensal: An organism living on the surface of other for anchorage and protection.

Ectoparasite: A parasite which lives on the external surface of the host.

Encystment : Forming resistant cysts in response to unfavorable conditions such as lack of food or dessication.

Endocommensal: An organism living within the tissues or cavities of higher animals and plants without causing harm to the host.

Endoparasite: A parasite which lives inside the body of the host.

Epizoite: A commensal animal which lives in association with other animal for anchorage and protection.

Facultative parasite: A free living organism which leads parasitic life for short period in the body of other organism.

Host: An organism which provides shelter and food for other organism.

Mutualism: An association between two organisms, where both organisms are benefited.

Niche: The habitat that favors the living and reproduction of species.

Obligate parasite: A parasite which needs suitable environment for living.

Parasite: An organism which lives on or in the body of other organism (host) and lives at the expense of the host.

Symbiosis: Relationship between two different species wherein one or both the species are benefited and neither is harmed.

1.1.9 SELF ASSESSMENT QUESTIONS

1. Write an account of host-parasite adaptations
2. "Mutualism constitutes one type of relationship" – Justify.
3. Give an account of the ecology of parasitism
4. Transmission of parasitic stages is involved with ecology and linked with food chain – Substantiate.
5. Write short notes on:

a. Symbiosis

b. Kinds of parasites

c. Direct life cycles in parasites.

d. Resistance of the host as an ecological factor – comment

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Dr. P. PADMAVATHI

Unit - I

1.2. *TRYPANOSOMA GAMBIENSE*

1.2.1. OBJECTIVES

The purpose of this lesson is to :

* describe the structure, biology and life cycle of blood parasite, *Trypanosoma gambiense.*
* explain the mode of infection of *T. gsambiense* and
* know the pathogenicity, diagnosis, therapy and prevention of trypanosomiasis.

1.2.2. INTRODUCTION

The genus *Trypanosoma* is an important protomonad haemoflagellate which includes several species. These are endoparasitic in the blood of various vertebrates. Many species of the genus *Trypanosoma* are pathogenic, while some are non-pathogenic. The disease caused by *Trypanosoma* is called *trypanosomiasis*. Of all the species of *Trypanosoma*, only three species are pathogenic in man, viz., *Trypanosoma gambiense*, *T. rhodesiense* and *T. cruzi*. The former two cause a deadly disease known as *sleeping sickeness* in Africa, while *T. cruzi* causes the *chagas' disease* in children in South America. Blood-sucking insects act as vectors for the transmission of disease from one host (vertebrate) to other.

The African trypanosomes belong to a group of closely related polymorphic forms. One of these, *Trypanosoma brucei*, is found in many African wild animals, is highly virulent for domestic animals, especially horses and camels. It is infective for almost every kind of mammal except baboons and man. It is believed that *T. brucei* is the parent form from which two species or strains capable of infecting man have arisen, namely, *T. gambienese* and *T. rhodesiense*. Some authorities consider both

of them distinct species. Some consider that *gambiense* (but not *rhodesiense*) is distinct from *brucei*, and some argue that all of them are mere strains of a single species.

Among trypanosomes, *T. gambiense* is the most prevalent and dreadful species. It inhabits the blood, lymph and cerebrospinal fluid of human beings and in the intestine of blood sucking tsetse fly, *Glossina palpalis*. It causes *'Gambia fever'* or *African sleeping sickness'* which is fatal to man. It is more prevalent in African countries especially in Congo and Nigeria. The trypanosome causing disease in man was first discovered in Gambia by Forde and Dutton in 1902.

1.2.3. MORPHOLOGY:

T. gambiense is a microscopic uniflagellate, polymorphic and digenetic parasite. It has a slender, elongated, colourless, sickle-shaped and flattened body which is tapering at both the ends. The anterior end has a flagellum and is more pointed than the posterior end which is blunt. The body varies in length from 15 to 30 microns and width from 1 to 3

The body is covered by a thin, elastic and firm *pellicle* (Fig.1-4). It is supported by a number of elastic fibrils called microtubules useful to maintain the shape of the animal. A small outgrowth of the pellicle or periplast projects on one side of the body as a thin flat spiral ridge, called *undulating membrane*. This is bounded by the flagellum on the other side. The undulating membrane is supposed to be an adaptive structure for locomotion in a viscous environment (blood, lymph) where it lives.

A small *basal granule* or *blepharoplast* is located near the posterior end. It is accompanid by another granule in front of it which is variously called *kinetoplast*, *kinetonucleus* or *parabasal body*. It contains extra nuclear DNA and hence, it is a self-duplicating body. These two granules are connected by a slender fibril called *rhizoplast.*

The single flagellum takes its origin from the basal granule and runs inside the body towards the opposite end to a certain extent, comes out and forms the outer boundary of the undulating membrane. After reaching the anterior end of the body, the flagellum becomes free and hangs freely as *free flagellum*. The basal granule corresponds to the centriole of a metazoan cell and functions as the controlling organ of the locomotory apparatus.

A large single vesicular *nucleus* lies in the center of the body with a clear *endosome*. The nuclear membrane is double

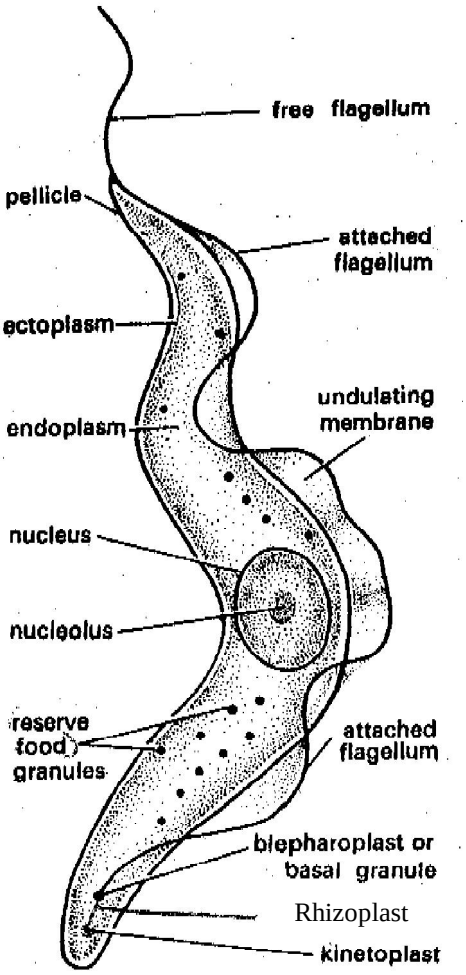

Fig. 1-4. *Trypanosoma gambiense.*

walled and perforated. It is a *trophonucleus* concerned with the vegetative activities of the animal.

The cytoplasm is differentiated into ectoplasm and endoplasm. Contractile and food vacuoles are absent. The cyloplasm contains numerous scattered greenish refractile deep staining granules called *volutin granules*. These granules are metabolic food reserves consisting of glycogen and phosphates. In addition, cytoplasm also contains some small vacuoles having hydrolytic enzymes and all other cellular components like golgi body, mitochondria, endoplasmic reticulum, ribosomes and nucleus.

A. POLYMORPHIC FORMS:

Trypanosoma is a polymorphic form. Hoare (1966) has noticed six morphologic stages in the life cycle of different species of *Trypanosoma*. Of these, four stages are common and the other two stages (Opisthomastigote and Choanomastigote) are absent in flagellates which infect human beings.

These forms have been named mostly on the basis of the arrangement of flagellum, its place of origin and its course through the body. However, two or more such forms occur either in one or both the hosts in the life cycle of the various species of *Trypanosoma*. Four polymorphic forms are as follows (Fig. 1-5).

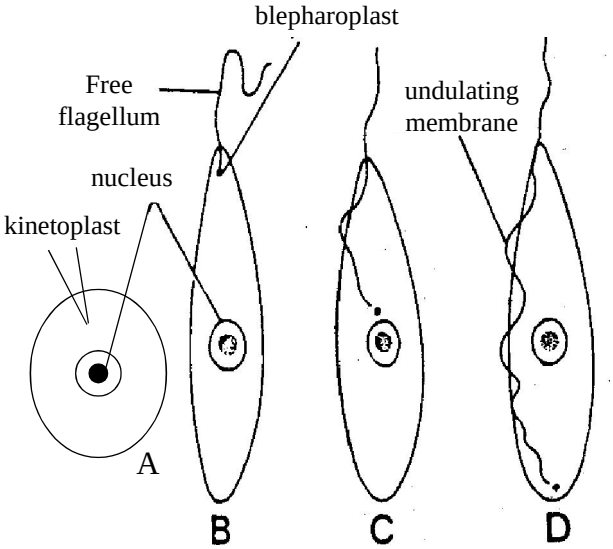

Fig. 1-5. *Trypanosoma,* Polymorphic forms.
A-Leishmanial. B-Leptomonad;
C-Crithidial; D-Trypanosome.

1. Leishamanial (Amastigote): It ha small, oval or rounded body with a nucleus. Basal granule and kinetoplast in the form of reduced dots placed in front of nucleus. Flagellum reduced, fibre-like, embedded in the cytoplasm; external flagellum is not found.

2. Leptomonad (Promastigote): It has an elongated body with nucleus in its center. The basal granule and kinetoplast are situated at the anterior end. A free flagellum originated from the basal granule and no undulating membrane is formed.

3. Crithidial (Epimastigote): Its body is short or elongated but stumpy. The basal granule and kinetoplast are situated in front of nucleus which is central. A long flagellum arises from basal granule and becomes free anteriorly. Undulating membrane ill-developed.

4. Trypanosome (Trypomastigote): Its body is elongated and slender. The basal granule and kinetoplast are situated at the posterior end of the body. Flagellum is large and becomes free anteriorly. The undulating membrane is well developed.

1.2.4. BIOLOGY:

T. gambiense performs its *locomotion* by the wavy movements of the undulating membrane and flagellum. They swim in blood and lymph in the direction of the pointed end of the body. **Nutrition** is of *saprozoic* type by absorbing mainly the sugars from the host blood by diffusion through the body surface. **Respiration** is basically *anaerobic* because it lives in an environment without oxygen. The absorbed glucose undergoes glycolysis to release energy necessary for metabolic activities. **Excretion** of metabolic waste products is carried by diffusion through general body surface into the external environment i.e. blood or lymph of the host. The osmoregulatory mechanism is altogether wanting due to its parasitic mode of habit.

Reproduction: *T. gambiense* reproduces asexually by longitudinal binary fission. Sexual reproduction is completely absent. The division is initiated by basal granule (blepharoplast) and followed by the kinetoplast (Fig. 1-6). The original flagellum remains undivided and goes to one of

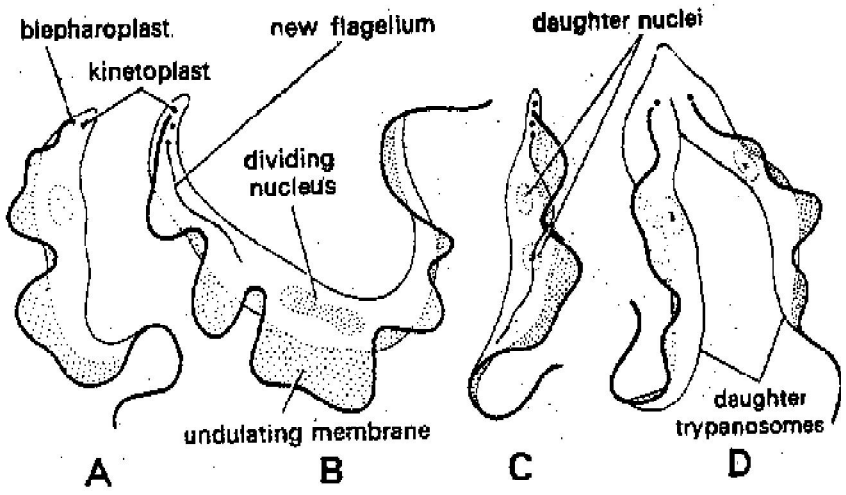

Fig. 1-6. *Trypanosoma*, Stages in binary fission.

the daughters whereas another offspring develops a new flagellum from the margin of undulating membrane. The division starts at the anterior end, extends backwards and finally dividing the organism into two individuals. By repeated division, the parasites increase in the blood of the host until the blood is swarmed with them.

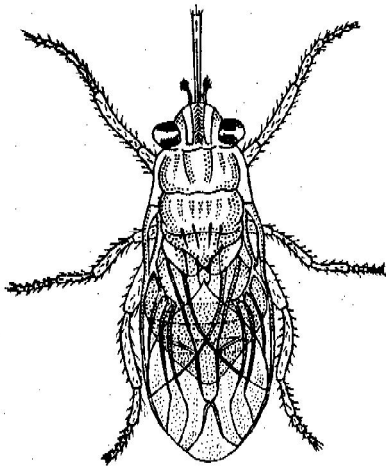

Fig. 1-7. Tsetse fly.

1.2.5. LIFE CYCLE:

The life cycle of *T.gambiense* is completed within two hosts, i.e. *digenetic*. The primary host is a vertebrate i.e. man, and the secondary host or vector is an invertebrate, i.e. blood sucking tsetse fly, *Glossina palpalis* (Fig. 1-7). Sometimes other vertebrates like Antelops, pigs, buffaloes, cattle, etc. offer lodging to *T. gambiense* and serve as *reservoirs*, in which the parasite lies unchanged without

causing any damage to the host tissue. It may remain like this for any number of days waiting for the chances to infect the proper host i.e. human being.

A. Life cycle in Man:

When an infected fly bites a man, the metacyclic forms of the parasites are inoculated into the blood of man (Fig. 1-8). Immediately after entering the blood, they transform into long and slender

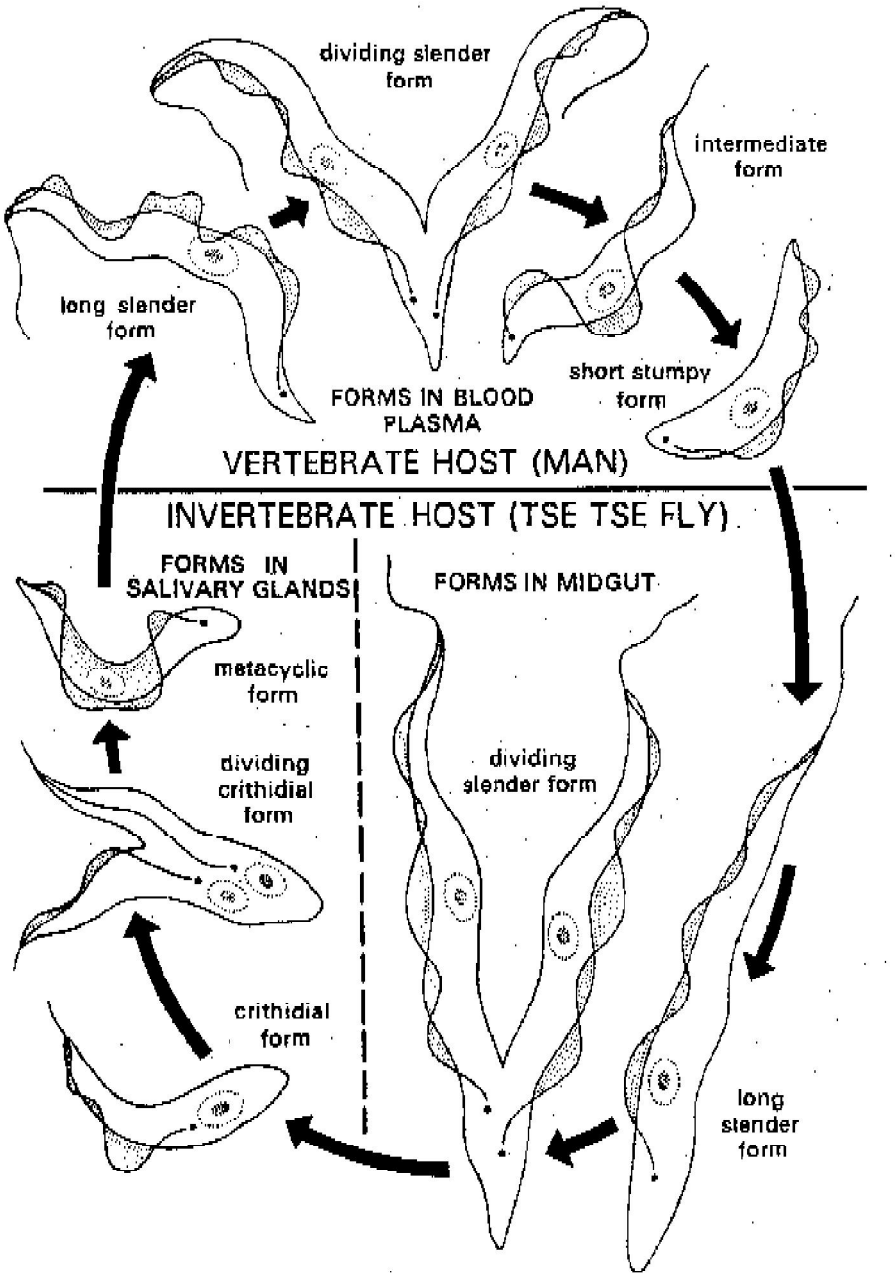

Fig. 1-8. *Trypanosoma gambiense.* Life cycle.

forms with long flagellum. The parasities first live in the blood of the man, but later find their way into the cerebrospinal fluid. While the parasites are in the blood, the infected man develops a kind of fever termed *Gambia fever*, but when they reach the cerebrospinal fluid, various nervous symptoms are produced in the patient leading to a lethargic condition, which has given the name *sleeping sickness* to the disease. The parasites multiply by longitudinal binary fission in the blood and produce three forms *viz*., i) long and thin forms with a free flagellum ii) short and stumpy forms with a reduced flagellum and iii) intermediate forms (Fig.1-9). It has been observed that the parasites periodically increase and decrease in number in the blood of man. The short forms have more resistance against the antibodies produced by the host. They survive the period of depression and the rest die. Sometimes they even migrate to spleen and lymph glands, and live as intercellular parasites. Now they may enter into the brain and cerebrospinal fluids. The short and stumpy forms are capable of developing in the intermediate host, tsetse fly.

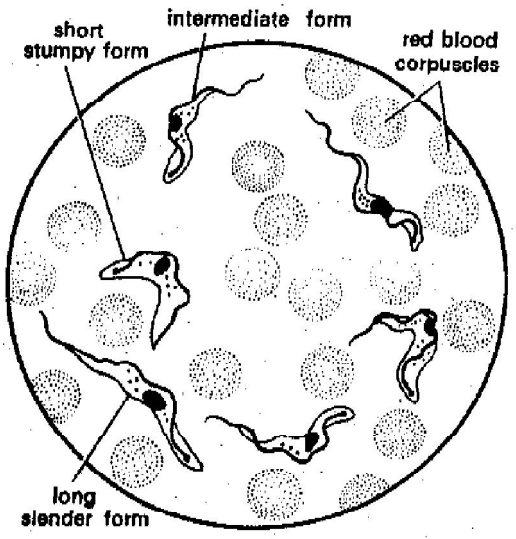

Fig. 1-9. *Trypanosoma gambiense*, Human blood smear to show its various forms.

B. Life cycle in Tsetse fly:

When a tsetse fly sucks the blood of an infected man, a number of parasites enter into the *midgut* of the fly along with the blood (Fig. 1-8). The short and broad forms can only survive, the rest being degenerated. They multiply in the gut producing long and slender forms. After tenth to fifteenth day, long slender forms appear in great numbers which move forward to the *proventriculus* and finally to the *salivary glands.* In the salivary glands they become attached to the walls. Now they multiply and modify into short and broad forms with short flagellum. The nuclear arrangement of these resembles that of 'crithidia', so they are called *crithidial forms*. Again they undergo multiplication, producing *metacyclic forms* (*Trypanosome forms)* which are now infective. These metacylic forms pass down through the ducts and hypopharynx.

The cycle in tsetse fly complets within 20-30 days time and it needs a temperature between 75-85^{0}F. When the fly bites a man, the metacyclic forms enter the blood of man along with the saliva of the fly and the cycle repeats again.

1.2.6. MODE OF INFECTION:

It is by inoculative method, by the bite of the infective tsetse fly, *Glossina palpalis* (both male and female suck blood and can transmit the infection). They bite by daylight, usually in the early morning and evening. The transmission occurs in two ways.

1. Mechanical or direct transmission: When an infective tsetse fly bites a man, some trypanosomes stick to the probosies of the fly and when the fly bites another man, the trypanosomes are introduced into his blood, provided the time between two successive bites does not exceed 24 hours. Such a transmission is termed mechanical or direct as the fly acts merely as a mechanical carrier and parasites do not undergo any change in it.

2. Cyclical transmission: When the fly sucks the blood of an infected man, the parasites along with the blood enter the midgut of the fly, remain there for two days and start multiplying. Parasites can be inoculated in the blood of another man only after undergoing through a set of stages. This type of transmission is known as cyclical transmission.

1.2.7. PATHOGENICITY AND SYMPTOMS:

The bite of an infected fly is usually followed by itching and irritation near the wound, and frequently a local dark red, button like lesion develops. In blood, the parasite multiplies and absorbs nutrients from it. When they are in large numbers, the host's blood gets poisoned with their excretory wastes causing high fever, along with the headache, anemia and aedema. Usually, the parasites succeed in penetrating the lymphatic glands and cause them to swell. Then the parasites enter the cerebrospinal fluid and brain causing a sleeping sickness like condition leading to coma stage and finally resulting in the death of the patient. Development of lethargic condition and recurrence of fever are the symptoms of its infection.

1.2.8. DISEASE DIAGNOSIS:

The disease is confirmed by examining fresh or stained peripheral blood, or by examining the cerebrospinal fluid obtained by lumbar puncture, or by examining the extract of enlarged lymphatic glands.

1.2.9. THERAPY (TREATMENT):

Arsenic and antimony compounds were used earlier for the treatment of trypanosomiasis. Now they are rarely used except for late stages when the parasites have invaded the central nervous system. Two drugs, *Bayer 205* (also called *Antrypol*, *Germanin* or *Suramin***)**, and *Pentamidine* or *Lomidine* are now widely used for both treatment and prophylaxis of human infections. These drugs are low in toxicity, effective in treatment, and prevent reinfection for several months.

1.2.10. PREVENTION (PROPHYLAXIS):

For preventing the infection of this parasite, the following measures are suggested.

1. The infection of this parasite can be prevented by eradicating the vectors or secondary hosts. This can be accomplished by keeping the endemic areas clean and regularly sprayed with insecticides.

2. Care should be taken to keep the reservoir hosts free from its infection.

3. Preventive medicines should be taken frequently and periodically which help to a great extent from its infection.

1.2.11. LIST OF SOME PATHOGENIC TRYPANOSOMES:

Species	Primary host	Site in primary host	Secondary host or vector	Name of disease	Distribution
Trypanosoma gambiense	Man	Blood	*Glossina palpalis* (Tsetse fly)	African (Gambian) sleeping sickness	Central Africa
T.rhodesiense	Man	Blood	*Glossina morsitans* (Tsetse fly)	Rhodesia sleeping sickness	South-eastern coastal areas of Africa
T. cruzi	Man (Children)	Blood	*Triatoma* (Bug)	Chagas disease	South and Central America
T. brucei	Horses, mules, donkeys, camels cattle, swines and dogs	Blood	*Glossina morsitans* (Tsetse fly)	Nagana	Africa
T. evansi	Horses, mules, donkeys, cattle, camels, elephants	Blood	*Tabanus* or *Stomoxys*	Surra in horses	Widely distributed
T. equinum	Horses	Blood	Tabanid fly	Mal de Caderas	South America
T. equiperdum	Horses and donkeys	Blood	No intermediate host. Transmission takes place from host to host during sexual act.	Dourine	Widely distributed
T. hippicum	Horses and mules	Blood	Flies	Murrina or Derren gadera	Panama
T. vivax	Ruminants and horses	Blood	*Glossina* spp.	Virulent	Central and East Africa
T. simiae	Pigs, monkeys, sheep, goats	Blood	*Glossina* spp.	Virulent	Africa

1.2.12. SUMMARY:

1. The genus *Trypanosoma* is parasitic in the blood of most of the vertebrates. It consists of several species, of which some are pathogenic and some are non-pathogenic. The pathogenic forms cause a disease known as *trypanosomiasis,* most commonly referred to as *sleeping sickness.*

2. Of all the species of *Trypanosoma,* only three species are pathogenic in man, namely *T. gambiense*, *T. rhodesiense* and *T. cruzi*. The former two cause sleeping sickness in Africa and the latter causes chagas' disease in children in South America. Blood-sucking insects act as *vectors* for the transmission of disease and many vertebrates act as reservoirs.

3. Among trypanosomes, the most common and dreadful species is *T. gambiense* which inhabits the blood, lymph and cerebrospinal fluid of man and the gut of tsetse fly, *Glossina palpalis*. It causes *''Gambia fever*' or '*African sleeping sickness'.*

4. The parasite has a slender, colorless and slightly flattened body which is tapering at both the ends. The anterior end has a flagellum. The entire body is covered by pellicle. On one side of the body, undulating membrane bounded by flagellum is present. A small basal granule and kinetoplast, connected by rhizoplast, are present at the posterior end of the body. The flagellum takes its origin from the basal granule and runs inside the body and emerges out at the anterior end as free flagellum. Nucleus is a trophonucleus and lies in the center of the body. Reserve food granules called volutin granules are found scattered in the cytoplasm. Contractile and food vacuoles are absent.

5. *Trypanosoma* is a polymorphic form with six morphologic stages. However in human parasites, four forms are common. They are Leishmanial, Leptomonad, Crithidial and Trypanosome forms.

6. *T. gambiense* swim in the blood and lymph of the host with the help of undulating membrane and flagellum. Nutrition is saprozoic. Respiration is basically anaerobic. Excretion of wastes by diffusion through general body surface. It reproduces asexually by longitudinal binary fission.

7. *T. gambiense* completes its life cycle in two hosts, the primary host being the man and the secondary host or vector is the tsetse fly, *Glossina palpalis*. When an infected fly bites a man, the parasites enter the blood of man. The parasites live for sometime in the blood but later find their way into the cerebrospinal fluid. While the parasites are in the blood, the infected man develops Gambia fever, but when they enter into the brain and cerebrospinal fluids, the host enter into a lethargic state of sleeping sickness. In the blood the parasites multiply and produce three polymorphic forms. Of which crithidial form is resistant and able to survive and develop in the intermediate host. When these forms enter the midgut of the fly, they multiply and produce long slender forms. They move forward to the proventriculus and finally to the salivary glands. There they multiply and modified into crithidial forms. Again they unergo multiplication, producing *metacyclic* forms which are infective. When the fly bites a man, the cycle repeats again.

8. Mode of infection is by inoculative method, by the bite of the infective tsetse fly, *Glossina palpalis*. The transmission occurs in two ways namely mechanical or direct, and cyclical transmission.

9. When an infected fly bites, itching and irritation near the wound and a dark red lesion develops. After a few days when the parasites are in blood, fever and headache develop, recurring at regular intervals accompanied by weakness, loss of weight and anaemia. From blood the parasites enter the lymph glands. Finally they invade brain and cerebrospinal fluid that cause unconsciousness and sleeping sickness which ultimately leads to the death of the patient.

10. The disease diagnosis includes the examination of peripheral blood or cerebrospinal fluid or the extract of enlarged lymphatic glands.

11. Various arsenic and antimony compounds were used for effective treatment of this disease earlier. Now Bayer 205 and pentamidine are widely used for both treatment and prophylaxis of human infections.

12. The disease can be prevented by eradicating the vectors; by keeping the reservoir hosts free of infection and by using preventive medicines.

1.2.13. KEY TERMINOLOGY:

Basal body or granule: An organelle equivalent to a centriole at the base of flagellum or a cilium. Also called Blepharoplast.

Contractile vacuole: Large spherical vesicle responsible for osmoregulation in protozoans and some sponge cells.

Diffusion: Movement of molecules from a region of high concentration to one of lower concentration brought about as a consequence of their kinetic energy.

Endoparasite: Parasite that lives inside of its host.

Fission: Asexual division of an organism into two or more progeny.

Flagellum: A long whip-like cytoplasmic organelle. A characteristic of many protozoan and metazoan cells; it is typically long and its motion is a complex whip-like undulation.

Food Vacuole: Cellular vesicle containing ingested food.

Intermediate Host: The host for the larval or developmental stage of a parasite.

Midgut: In arthropods, middle portion of digestive tract, not lined with cuticle.

Pellicle: Protozoan body wall composed of cell membrane, cytoskeleton and other organelles.

Polymorphic: Occurrence of several forms in a single species.

Primary host or Definitive host: The host for the adult stage of a parasite.

1.2.14. SELF ASSESSMENT QUESTIONS:

1. Give an account of the morphology, biology and life cycle of *Trypanosoma gambiense.*
2. Write an account on the mode of infection, pathogenicity, treatment and prevention of trypanosomiasis.
3. Write notes on:
   a. Polymorphic forms of trypanosomes

b. Life cycle of *Trypanosoma gambiense*
c. Therapy and prophylaxis of Trypanosomiasis
d. Pathogenicity and symptoms of African sleeping sickness.

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Dr. P. PADMAVATHI

Unit - I

1.3. *ENTAMOEBA HISTOLYTICA*

1.3.1. OBJECTIVES:

The purpose of this lesson is to :

* describe the morphology, biology and life cycle of intestinal parasite, *Entamoeba histolylica,*
* explain the mode of infection of *E. histolylica*, and
* exemplify the pathogenicity, diagnosis, therapy and prevention of amoebiasis.

1.3.2. INTRODUCTION:

The genus *Entamoeba* belongs to super class Sarcodina, class Rhizopodea and order Amoebida. It is characterized by the presence of a lobe-like pseudopodium. They live as endoparasites in the intestine of higher animals like frog and man. Three species of the genus occur in man. *E. histolylica* is a pathogenic form and causes 'amoebiasis' and 'amoebic dysentery' in man. The other two species are *E. gingivalis* and *E. coli*, which are non-pathogenic. Lambl (1859) first discovered *E. histolytica*, Losch (1875) proved its pathogenic nature, while Schaudinn (1903) differentiated pathogenic and non-pathogenic types of amoebae.

E. histolytica has a world wide (cosmopolitan) distribution. But it is commonly found in epidemic form in tropical and sub-tropical regions than in the temperate region. Its incidence is relatively higher in densely populated areas where the sanitary conditions are poor.

E. histolytica is microscopic and lives as an endoparasite in the upper part of the large intestine i.e. colon of man. It inhabits the mucus and submucous layers of the large intestine. *E. histolytica* has a histolytic power and feed on the juices of tissue cells. It feeds mainly on the tissues of the intestinal wall and often produces severe ulcers and abscesses. In chronic cases it may enter the blood circulation to reach the liver, lungs, brain and other organs. It causes serious and often fatal disease known as amoebic dysentery.

Hora (1952) recognized that there are two races or strains of *E. histolytica* which differ in the size of cysts and pathogenicity. They are 1) a smaller, common, non-pathogenic form – *the small-cyst race* (cyst below 10 μ) 2) a much larger, virulent, pathogenic in man, invading the tissues – *the large-cyst race* (cysts above 10 μ).

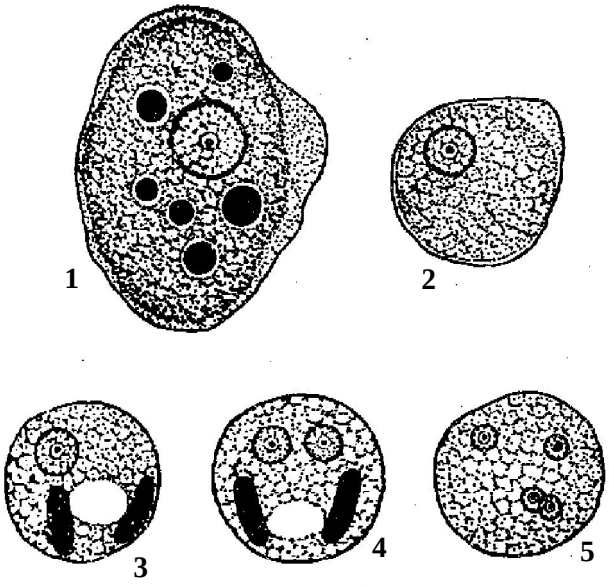

Fig. 1-10. Three stages of *Entamoeba histolytica.*
1. Trophozoite; 2. Pre-cystic stage; 3, 4, 5, Uninucleate, binucleate and quadrinucleate cysts.

1.3.3. MORPHOLOGY:

The morphology of the three stages (Fig. 1-10) in the life cycle of *E. histolytica* namely, the ***magna* or *trophozoite*** stage, the **minuta** or **precystic** stage, and **cystic** stage is described as follows:

i) **Trophozoite stage** (The growing or feeding stage): The trophozoites of *E. histolytica* are large, hence called magna, usually grows to a size of 20 to 30 μ in diameter. It is the most active form, moving with the help of a single pseudopodium. Hence, it is monopodial. It is the feeding form hence pathogenic to man. It resembles amoeba in all structural details.

The entire body is covered by a transparent semipermeable plasmalemma (Fig.1-11). The cytoplasm is differentiated into an outer clear ectoplasm and an inner granular endoplasm. The hyaline ectoplasm gives out a large pseudopodium from any part of the body. The endoplasm contains the nucleus and food vacuoles.

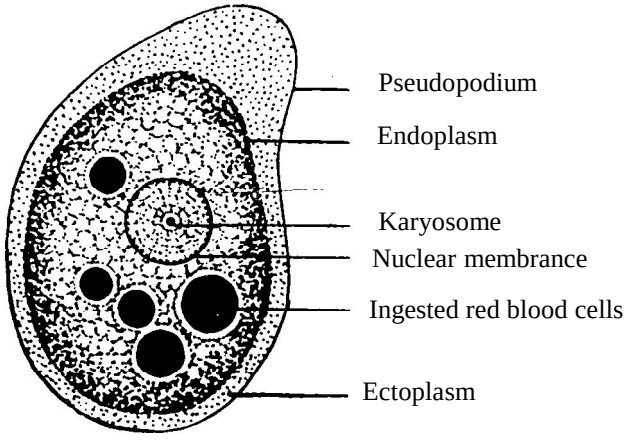

Fig. 1-11. Trophozoite of *Entamoeba histolytica.*

Nucleus is rounded (4-6 μ) and vesicular (Fig. 1-12). It is covered by nuclear membrane, which is encrusted with uniform granules of *chromatin.* There is a small single granular *endosome* or *nucleolus* or *karyosome* which is surrounded by a *halo* ring. Between the endosome and the nuclear membrane radiate numerous spoke-like lines.

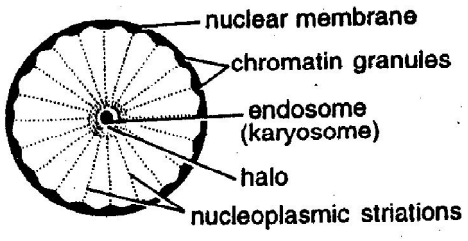

Fig. 1-12. *Entomoeba histolytica*. Vesicular nucleus.

One or more *food vacuoles* are present in the endoplasm which contain red blood corpuscles (RBC) in various stages of digestion. Presence of RBC in food vacuoles is an important characteristic feature of this parasite. Contractile vacuole is absent because *E. histolytica* lives in an environment which is *isotonic*.

The trophozoite form enters into the mucosa and submucosa layers of the intestinal wall by dissolving its tissues. Thus, it makes small wounds in the intestinal lining which later develop into ulcers. After reaching into the intestinal tissues, it ingests RBCs and grows in size.

ii) **Pre-cystic stage:** It is smaller (10 – 20 μ), spherical or slightly ovoid, non-feeding, non-motile and non-pathogenic. It resemble trophozoite form in its structure except that it is smaller in size having small blunt pseudopodium. Endoplasm is free of RBC and other ingested food particles. The relatively larger nuclear structure retains the characteristics of the trophozoite. It lives only in the lumen of the intestine and rarely found in the tissues. It undergoes encystations (cystic stage) and helps in the transmission of the parasite from one host to another.

iii) **Cystic stage:** During encystment the parasite becomes rounded and is surrounded by a highly refractile membrane, called the *cyst wall* (Fig. 1-13). A mature cyst is a quadrinucleate spherical body, its cystoplasm is clear and hyaline, and the nuclear structure retaining the characters of the trophozoite. The cyst varies greatly in size-the "small race" being 6 to 9 μ and the "large race" 12 to 15 μ.

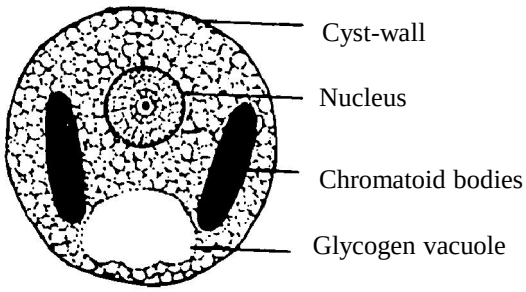

Fig. 1-13. Cyst of *E. histolytica*

The cyst begins as a uninucleate body but soon divides by binary fission and develops into binucleate and quadrinucleate body. During the process of division, the nuclei undergo gradual reduction in size, becoming 2 μ in diameter.

The cytoplasm of the cyst (Fig. 1-13) shows chromatoid bars and a glycogen mass. The number of chromatoid bodies vary from 1 to 4 and the size vary from one-half to two thirds the diameter of the cyst. As the cyst matures from uninucleate to quadrinucleate stage, both the glycogen mass and the chromatoid bodies gradually disappear. Immature cysts passed in the feces may however complete their development outside.

1.3.4. BIOLOGY:

In *E. histolytica* the nutrition is *holozoic.* It feeds mainly upon the blood corpuscles, other host elements, bacteria and yeasts. It also absorbs substances *saprozoically* from the surrounding medium.

Methods of Reproduction: Excystation, Encystation and Multiplication (Fig. 1-14).

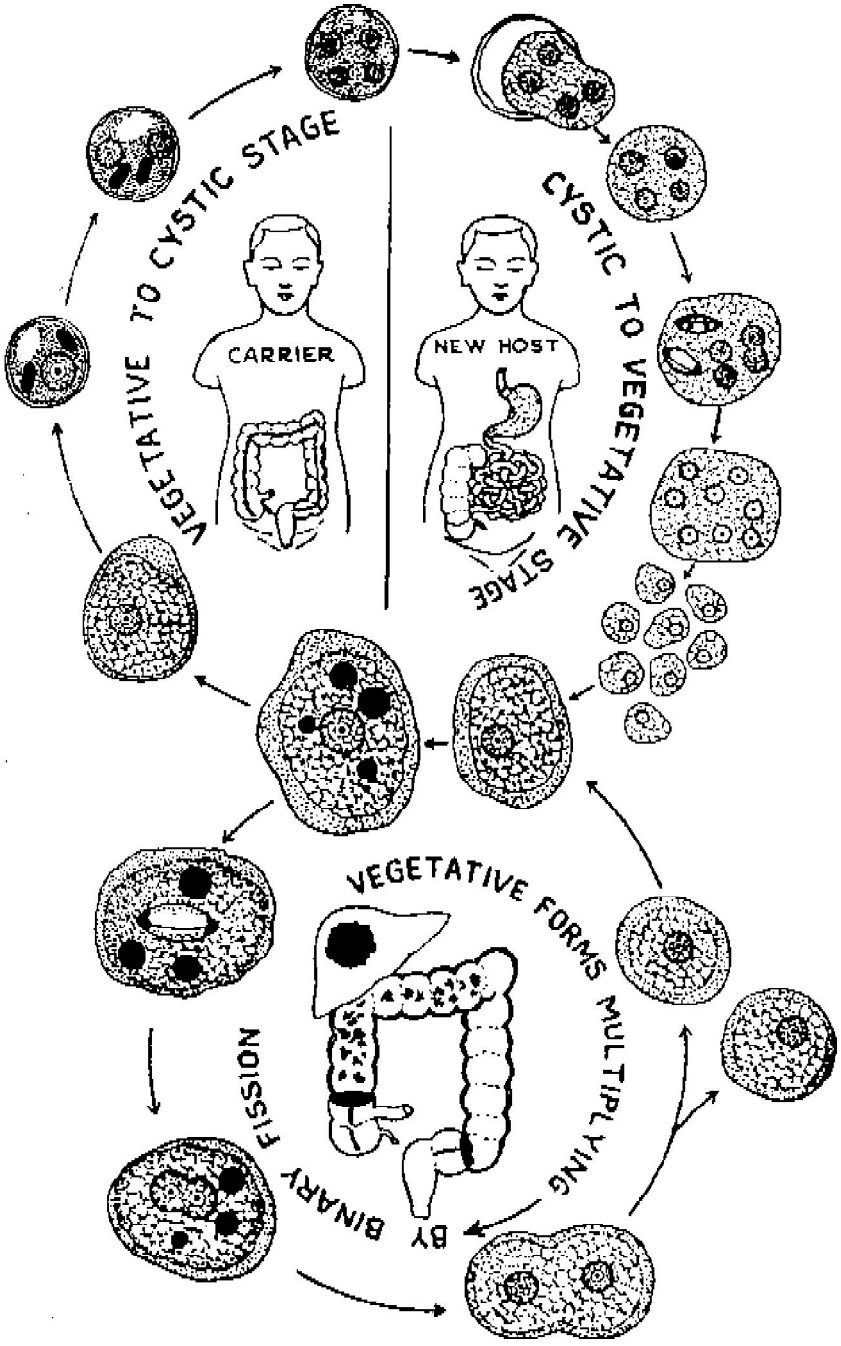

Fig. 1-14. Methods of reproduction of *Entamoeba histolytica.*
Showing excystation and encystation at the top and multiplication of trophoziotes below.

Excystation: This is the process of transformation of cysts to trophozoites and occurs only when the cysts enter into the alimentary canal of man (a susceptible host). During excystation, a quadrinucleate cyst gives rise to eight amoebulae, each one of which is being capable of developing into a trophozoite.

Encystation: This is the process of transformation of trophozoites to cysts and occurs inside the lumen of the intestine of an infected individual. The whole process of encystation takes place within a few hours and the life span of a mature cyst inside the lumen of the bowel of the original host is only two days. It is to be noted that the cysts are not developed inside the tissues of man, neither in the intestinal wall nor in the areas of metastatic invasion (liver, lungs or other organs). The mature cyst is a quadrinucleate body, because during encystment the nucleus has undergone multiplication and given rise to four daughter nuclei.

Both the processes of excystation and encystation may occur in one and same host but after the formation of cysts a transference to another new host is required for the continuation of species.

Multiplication: This occurs only in the trophozoite phase. The trophic forms of *E. histolytica* are exclusively parasitic in their habit, growing at the expense of living tissues and multiplying in large numbers. Reproduction of trophozoites occur by simple binary fission, first of the nucleus which divides by a modified type of mitosis and then, of the cytoplasmic body of the organism.

Note: Generally encystment is not a reproductive process but a means of protection of species from extinction. In *E. histolytica*, however, the nucleus of the cyst multiplies into 4 daughter nuclei from which ultimately 8 amoebulae are developed.

1.3.5. LIFE CYCLE:

E. histolytica is monogenetic which completes its life cycle in a single host, i.e. man. There are mainly two phases of development, *trophozoite* and *cyst* with a transitory stage of *pre-cystic form*.

The mature quadrinucleate cysts are the infective forms of the parasite. When these cysts are swallowed along with the contaminated food and drink by a susceptible man, they are capable of further development inside his gut. The fully developed cysts, thus enter the alimentally canal, pass unaltered through the stomach. The cyst-wall is resistant to the action of the gastric juice but is digested by the action of trypsin in the intestine. The excystation occurs when the cyst reaches the caecum or the lower part of the ileum (natural or slightly alkaline medium). Each cyst liberates a single amoeba with four nuclei, a *tetranucleate* amoeba which eventually forms eight amoebulae *(metacystic trophozoites)* by the division of nuclei with successive fission of cytoplasm. The young amoebulae being actively motile, invade the tissues and ultimately lodge in the submucous tissue of the large gut, their normal habitat. Here they grow and multiply by binary fission. This trophozoite phase of the parasite is responsible for producing the characteristic lesson of amoebiasis. While invading the tissues, sometimes the trophozoites find their way into the liver where their further progress may be arrested. In the liver the trophozoites may grow and multiply for sometime but encystation does not occur. Those parasites that remain in the intestinal wall may cause dysentery (ulcerative colitis).

Under unfavourable conditions, a certain number of these trophozoites are discharged into the lumen of the bowel and are transformed into small pre-cystic forms from which the cysts are developed. But the cysts produced in an infected individual are unable to develop in the host in which they are produced and therefore necessitate a transference to another susceptible host where they can grow and continue their life cycle as stated above.

1.3.6. MODE OF INFECTION:

Transmission of *E. histolytica* from man to man is effected through its encysted stage. Infection occurs through the ingestion of these cysts. In favourable conditions of moisture and temperature the cysts will live for a number of weeks outside the body. Infection to new host takes place through the ingestion of contaminated food and water. Contamination generally occurs through house-flies, cockroaches and food-handlers which carry the cysts on to the human food.

1.3.7. PATHOGENICITY:

E. histolylica causes amoebic dysentery, non-dysenteric infections, and abscesses in liver, lungs and brain.

1. **Amoebic dysentery:** *E. histolytica* secretes tissue dissolving enzyme (histolysin nature) that destroys the epithelial lining of the colon and causes its necrosis and forms the abscesses (small wounds) which later become flask-shaped bleeding ulcers. The cavity of these ulcers is generally filled with mucus, bacteria, amoeba and cell debris which are then liberated into the lumen of the intestine. In severe cases almost the entire colon is undermined. The ulceration of colon may produce severe dysentery.

2. **Non-dysenteric infections:** *Entamoeba* sometimes produces chronic gastrointestinal symptoms but not dysentery. As they pass the infective stages in their stools, they are the carriers of the disease, even though they do not show the symptoms of dysentery. The symptoms associated with chronic amoebiasis are abdominal pain, nausea, headache and nervousness.

3. **Abscesses in liver, lungs and brain:** The parasites are often carried to organs like liver, lungs, spleen and even brain through blood or lymph, where they form local abscesses. In case of liver abscesses, the patient has pain in liver region, fever and high leucocyte number, a condition referred to as *amoebic hepatitis*. When the abscesses are infected by bacteria, they become more harmful.

1.3.8. DISEASE DIAGNOSIS:

The microscopic examination of the stool of an infected man shows the presence of trophozoites and cysts in it. The presence of diamond-shaped, clear and refractile *charcot-Laden crystals* in saline preparations suggests the infection of *E. histolytica*.

1.3.9. THERAPY (TREATMENT):

Amoebiasis if left untreated tends to become chronic and to persist indefinitely. Different drugs are needed i) to stop acute dysentery promptly ii) to eliminate acute or chronic infections from the intestine and iii) to cure hepatic, pulmonary or other extra-intestinal infections. For prompt relief of acute or subacute dysentery, *Emetin* injections are given. Oral administration of certain antibiotics like *Fumagillin*, *Terramycin*, *Erythromycin* and *Aureomycin* are more effective. For eradication of intestinal infections after the dysentery is controlled, or in chronic cases, certain arsenic compounds (*Carbarsone, Thiocarbarsone* and *Milibis*) and a number of iodine compounds (*Yatren, Diodoquin* and *Vioform)* are effective when given over a period of 7 to 10 days. For amoebiasis of the liver or lungs or other extra-intestinal infections *Chloroquine* is quite effective. *Camoform* is useful for both intestinal and extra-intestinal amoebiasis. The most significant advancement in the treatment of amoebiasis is the use of *Metronidazole* and *Tinidazole* as both luminal and tissue amoebicide. *Diodoquin* is used as a prophylactic for travellers in places where there is danger of infection.

1.3.10. PREVENTION (PROPHYLAXIS):

For Community Prophylaxis: i) effective sanitary disposal of faces, ii) protection of water supplies from faucal pollution, iii) avoidance of the use of human excrement as fertilizer, iv) detection and isolation of carriers.

For Personal Prophylaxis: i) use of boiled drinking water, ii) protection of all food and drink from contamination by flies, cockroaches and rats, iii) avoidance of use of raw vegetables iv) personal cleanliness and elementary hygienic conditions are to be observed while taking meals.

1.3.11. SUMMARY:

1. The genus *Entamoeba* lives as endoparasite in the intestine of higher animals. Three species of the genus namely *E. histolylica, E. gingivalis* and *E. coli* occur in man. Of these *E. histolytica* is pathogenic whereas the other two are non-pathogenic.

2. *E. histotylica* causes amoebic dysentery in man. It is more prevalent in densely-populated area where the sanitary conditions are poor especially in tropical and subtropical regions than in the temperate region.

3. *E. histolytica* lives as an endoparasite in the upper part of the large intestine i.e. colon of man. It inhabits the mucus and submucus layers of the large intestine. It feeds on intestinal tissues and produces severe ulcers and abscesses. In chronic cases it may enter the liver, lungs, brain and other organs.

4. In the life cycle of *E. histolytica* three stages namely trophozoite, pre-cystic and cystic stages are found. **i)** *Trophozoite* is large and the most active form, moving with the help of a single pseudopodium i.e. monopodial. It resembles amoeba in all structural details. It lives in the tissues of intestine, feeding on them and causes ulcers, and hence pathogenic to man. Food vacuoles contain

RBC, an important characteristic feature of this parasite. **ii)** *Pre-cystic stage* is small, ovoid, non-feeding, non-motile and non-pathogenic. It resembles trophozoite in structure. It lives only in the lumen of the intestine and rarely in the tissues. It undergoes encystation and helps in transmission from one host to another. **iii)** The encysted parasite is said to be in *cystic stage* which is surrounded by the cyst wall. The cyst begins as a uninualeate body which soon develops into binucleate and quadrinucleate body. The cytoplasm shows chromatoid bodies and a glycogen body.

5. In *E. histolytica* nutrition is holozoic. It feeds mainly on blood corpsuscles. It also absorbs other substances saprozoically. It reproduces by Excystation (cysts to trophozoites), Encystation (trophozoites to cysts) and Multiplication. Multiplication occurs only in the trophozoite phase. They multiply in large numbers by simple binary fission in intestinal tissues.

6. *E. histolytica* is monogenetic which completes its life cycle in a single host i.e. man. The mature quadrinucleate cysts are the infective forms of the parasite. When these cysts are ingested, they enter the alimentary canal. When they reach the caecum or the lower part of the ileum, excystation occurs which results in amoebulae. Young amoebulae (trophozoites) are active, invade the tissues and finally lodge in the sub mucus tissue of the large intestine where they grow and multiply by binary fission. These trophozoites are responsible for producing characteristic lesion of amoebiasis. Sometimes they invade the liver, lungs and brain. Under unfavourable conditions, the trophozoites are transformed into small pre-cystic forms from which the cysts are developed. The cysts enter the new host and the life cycle is repeated as stated above.

7. Infection occurs through the ingestion of these cysts. Infection to new host takes place through the ingestion contaminated food and water.

8. *E. histolytica* causes amoebic dysentery, non-dysenteric infections and abscesses in liver, lungs and brain.

9. The disease caused by *E. histolytica* is diagnosed by the examination of stool. The presence of Charcot-Leyden crystals in saline preparations suggest the infection of this parasite.

10. Acute and subacute dysentery are treated effectively with Emetin injections or with oral administration of antibiotics. For eradication of intestinal infections arsenic and iodine compounds are found effective. For amoebiasis of liver, lung and other extra-intestinal infections, chloroquine is quite effective. Use of Metronidazole and Tinidazole is the significant advancement in the treatment of amoebiasis.

11. The disease can be prevented by following certain personal and community prophylactic measures like the use of boiled drinking water, protecting food from contamination, following personal cleanliness and hygiene, sanitary disposal of faecal matter, etc.

1.3.12. KEY TERMINOLOGY:

Amoebiasis: The term is used clinically to denote all those conditions which are produced in the human host by infection with *E. histolytica* at different areas of its invasion.

Amoebic dysentery: The term signifies a condition in which the infection is confined to the intestinal canal and is characterized by the passage of blood and mucus in the stool. It is to be noted that "amoebic dysentery" is not a synonym of 'amoebiasis'.

Cyst: The stage of an organism where it is enclosed in a resistant wall.

Diffuse amoebic hepatitis: The syndrome of slightly enlarged and tender liver, right upper quadrant pain, intermittent fever and leucocytosis in patients with amoebic dysentery.

1.3.13. SELF ASSESSMENT QUESTIONS:

1. Describe the structure, biology and life cycle of *Entamoema histolytica*.
2. Give an account of the life cycle and pathogenicity of the parasite causing amoebic dysentery in man.
3. Write notes on
    a. Encystation
    b. Excystation
    c. Treatment of amoebiasis
    d. Prophylaxis for amoebiasis.

1.3.14. REFERENCE BOOKS:

Chandler, A.C. and C.P. Read, 1961. *Introduction to Parasitology,* W.B. Saunders Company, Philadelphia and London.

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Dr. P. PADMAVATHI

Unit - I

1.4 *GIARDIA INTESTINALIS* AND *BALANTIDIUM COLI*

1.4.1. OBJECTIVES:

The purpose of this lesson is to :

* describe the morphology, biology and life cycle of intestinal parasites, *Giardia intestinalis* and *Balantidium coli,*
* explain the mode of infection of *G. intestinalis* and *B. coli*, and
* discuss the pathogenicity and treatment of giardiasis and balantidiasis.

1.4.2. *GIARDIA INTESTINALIS:*

A. INTRODUCTION:

Giardia intestinalis, once known as *Giardia lamblia* and *Lamblia intestinalis,* is an intestinal flagellate. It is an odd-looking creature having nuclei and other organelles reduplicated like closely bound Siamese twins (Fig. 1-15 A). They inhabit the upper part of the small intestine instead of the large intestine favoured by all the other intestinal protozoa. *G. intestinalis* inhabit the duodenum and the upper part of jejunum of man. They attach themselves to the surface of the mucosal cells where they absorb nourishment directly from the host. *Giardia* species inhabit the intestines of all kinds of vertebrates from fish to man. However, *G. intestinalis* is found in man and also reported from both old and new world monkeys.

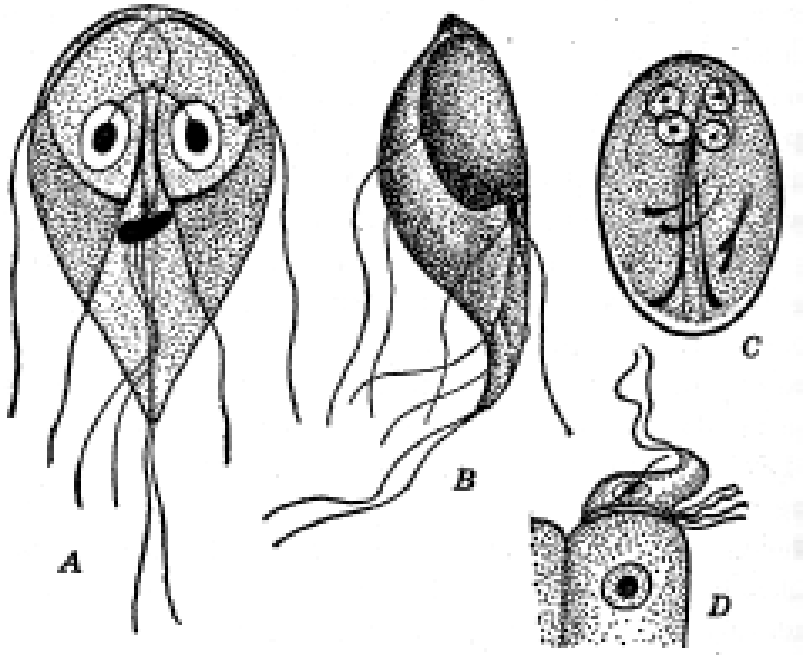

Fig. 1-15. *Giardia intestinalis.* A) face view of trophozoite; B) semiprofile view. C) cyst; D) position of trophozoite resting on epithelial cell.

B. MORPHOLOGY:

G. intestinalis exists in two phases - trophozoite and cyst (Fig. 1-15 A&C).

Trophozoite: In face view, the outline of the body is strikingly like that of a tennis or badminton racket without the handle. The size of the trophozoite is 14 μ long by 7 μ broad. In side view, it resembles a longitudinally split pear. The dorsal surface is convex and the ventral surface is concave with a sucking disc. The anterior end is broad and rounded and the posterior end tapers to a sharp point. The finely tapering posterior end is usually turned up over the convex back. It is bilaterally symmetrical and all the organs of the body are paired. Thus there are two nuclei, two axostyles (a central supporting rod) and four pairs of flagella. The two nuclei have large central endosomes. Between them are two slender rods, the axostyles, to which the nuclei are anchored by slender fibrils.

Cyst: The fully formed cyst is oval in shape and measures 12 μ long by 7 μ broad. The axostyles lie more or less diagonally, forming a sort of dividing line within the cyst-wall. There are four nuclei which may remain clustered at one end or lie in pairs at opposite poles. The remains of the flagella and the margins of the sucking disc may be seen inside the cytoplasm. An acid environment often causes the parasite to encyst.

C. BIOLOGY:

Giardia makes its home in the small intestine, especially in the duodenum, occasionally invading bile ducts. Although *Giardia* infections are found in people of all ages, they are common in children. Although most of the infections were very light, and all of them disappeared spontaneously, usually within 1 to 6 weeks.

The parasites fasten themselves by their hollow faces to the convex surfaces of epithelial cells in the small intestine, their flagella streaming like the barbels of a catfish (Fig. 1-15 D). Sometimes large areas of epithelium are practically covered with them, each one perched on a separate cell. It was estimated that in one instance, the number of cysts in a single stool exceed 14,000,000,000. The number of cysts in an average stool in a case of moderate infection was estimated at over 300,000,000. The motile forms are not normally found in the stools, but in cases of diarrhea dead ones may be present in considerable numbers. They do not ingest solid food nor do they appear to dissolve tissue cells. They feed on the abundant secretion of mucus, and on a variety of aminoacids, vitamins, and other substances which are constantly passing in and out of the intestinal mucosal cells.

Multiplication occurs by binary fission and occasionally multiple fission occurs as in other intestinal flagellates. The cysts are formed intermittently, enormous numbers may be found on one day and then none for several days, when a shower of them again appears. The cysts remain alive in feces for 10 days or more and survive many days in the gut of roaches. The parasite is a very persistent one, so that infections sometimes last for many years, possibly in some cases for life.

D. LIFE CYCLE AND MODE OF INFECTION:

In the trophozoite stage, the parasite multiplies in the intestine of man by binary fission. When conditions in the duodenum are unfavourable, encystment occurs, usually in the large intestine. During encystment, a thick resistant wall is secreted by the parasite and the cell then divides into two within the cyst.

Infection of man is brought about by ingestion of cysts. Within 30 minutes of ingestion, the cyst hatches out two trophozoites which then multiply in enormous numbers and colonise in the duodenum. To avoid the high acidity of duodenum *Glardia* often localizes in the biliary tract (gall bladder).

E. PATHOGENICITY:

As the parasites attach themselves to the convex surfaces of the epithelial cells of the intestine, mechanical interference with absorption, particularly of fats, occurs i.e. malabsorption of fats. Thus it often leads to vitamin dificiencies, particularly of the fat-soluble ones. The presence of large amounts of unabsorbed fats in the stools caused a persistent or recurring diarrhea, often with large amounts of yellow mucus. The symptoms may resemble those of celiac disease, sprue or chronic gall bladder disease. Epigastric pains, vague abdominal discomfort, loss of appetite, apathy, headache, and diarrhea alternating with constipation are common. An allergic dermatitis sometimes occurs. On the other hand, in many cases, there are no evident symptoms. When the parasites invade the bile ducts, they may cause some irritation in the bile ducts, and predispose them to chronic infection (chronic cholecystopathy).

F. DISEASE DIAGNOSIS:

This includes the following:

A. A microscopical examination of a freshly passed stool for the demonstration of *Giardia* trophozoites and cysts; the former are found in a diarrhoeic stool or after a purgative.

B. *Giardia* trophozoites may be recovered both in the bile A (aspirated from duodenum) & B (removed from bile duct) drawn by duodenal intubation.

G. THERAPY:

Giardia infections are very susceptible to the anti-malarial drugs, atebrin, chloroquine and camoquin, given at the rate of 0.1 to 0.2 gram three times a day for about 5 days. Cysts cease to be passed after the second or third day. Atebrin and acranil have been found to be specific for giardiasis. A derivative of Imidazole (Metronidazole) also showed good results. Chloriquine in doses of 0.3 gram base once daily for 5 days is also effective. Patients tend to benefit from the administration of fat soluble vitamins, particularly vitamin A.

1.4.3. *BALANTIDIUM COLI*:

A. INTRODUCTION:

Balantidium is an intestinal ciliate parasite. All the intestinal ciliates of warm – blooded animals belong to the sub-class Euciliata (with macronucleus and micronucleus, and sexual reproduction by conjugation). *Balantidium coli* is a parasite of the large intestine of man, monkeys, and pigs. A parasite in rats identical with *B. coli* was reported from Moscow; and rats and guinea pigs can be experimentally infected.

B. MORPHOLOGY AND BIOLOGY:

B.coli (Fig. 1-16) as found in man is much larger than any of the other protozoan inhabitants of the human intestine and usually measures 50 to 80 μ in length, with a breadth between two-thirds and

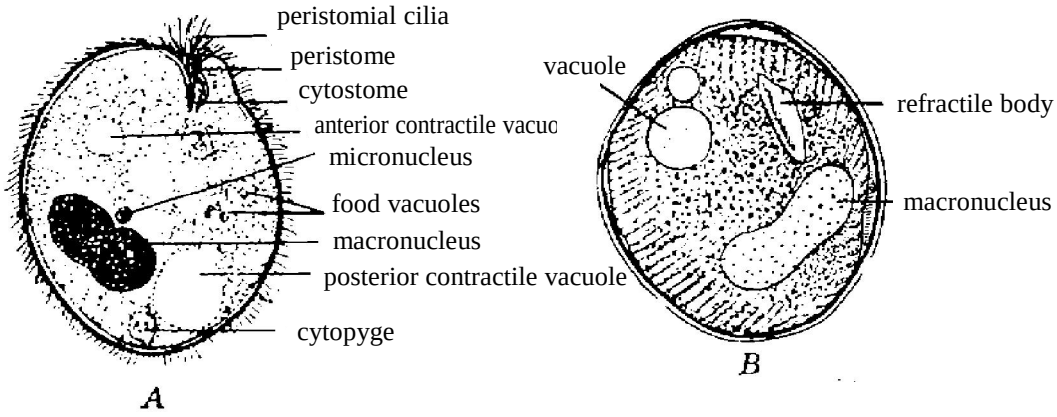

Fig. 1-16. *Balantidium coli.* A. Trophozoite, B. cyst.

three-fourths as great. In pigs it sometimes reaches a length of 200 μ. It is shaped like an egg or pear. At the anterior end an obliquely arranged depression, the peristome is present. It may appear wide open or slit like, and in the bottom of which is the cytostome. The whole body is covered with fine cilia arranged in rows, with special row of longer "adoral" cilia surrounding the peristome. The macronucleus is only very slightly curved, usually with a slight concavity on either side. It usually lies obliquely near the middle of the body and is about two – fifths the length of the body. The micronucleus is very small and inconspicuous. There are two contractile vacuoles, and food vacuoles circulate in the endoplasm.

Like other ciliates, *Balantiduim* divides by transverse fission, a new cytostome being formed by the posterior daughter. A process of conjugation occurs, similar in its general features to that of *Paramecium*. Thick-walled cysts are formed in which single individual is usually enclosed. Slow-moving cilia are at first visible on encysted ciliates, but later all structures except the nudei and sometimes one or more refractile bodies disappear. No multiplication takes place in the cysts.

C. MODE OF INFECTION:

Pigs are usually regarded as important sources of human infection. such infections are rather infrequently reported, but they may be locally common. Of the 20 cases in New York, all in children, 18 had a history of contact with pigs. Apparently large or repeated doses of cysts or special susceptibility is necessary for human infection.

D. PATHOGENICITY:

In man, *B. coli* is known to be a pathogenic parasite though in pigs it appears to be harmless. In man it may cause ulceration of the large intestine and invade the tissues of the walls; the colon is sometimes ulcerated from end to end. Nevertheless, the majority of cases suffer only from diarrhea and may show no symptoms at all; only a small number develop severe or fatal dysentery.

E. TREATMENT:

Balantidium infections have been treated with varying success with some anti-amebic drugs, particularly carbarsone, but there is now evidence that Aureomycin and Terramycin are very effective. These antibiotics are given for 10 to 15 days, the total dosage being from 7 to 28 grams. The ciliates disappear within 2 to 4 days, and no relapses occur for atleast 2 weeks or so after treatment.

1.4.4. SUMMARY:

1. *Giardia intestinalis* is an intestinal flagellate parasite. They inhabit the duodenum and the upper part of jejunum of man.

2. *G. intestinalis* exists in two phases – trophozoite and cyst. *Trophozoite* resembles that of a tennis racket without hand in its face view and a longitudinally split pear in its side view. Dorsal surface is convex and the ventral surface is concave with a sucking disc. All organs of the body are paired and possesses eight flagella. The *cyst* is oval in shape with four nuclei.

3. *Giardia* infections are more common in children. The parasites do not ingest solid food nor dissolve tissue cells. They feed on mucus, aminoacids, vitamins and other substances which are passing in and out of the intestinal mucosal cells.

4. Multiplication occurs mainly by binary fission. The cysts are formed intermittently; enormous numbers may be found on one day and then none for several days and again appears. The cysts remain alive in feces for 10 days or more. The parasite is a very persistent one so that infections last for many years.

5. In the intestine, the trophozoite multiplies by binary fission. When conditions are unfavourable, encystment occurs, usually in the large intestine. During encystment, the cell divides into the within the cyst.

6. Infection of man is through the ingestion of cysts and within 30 minutes, the cyst hatches out two trophozoites. They again multiply in enormous numbers and colonize in the duomenum or in the biliary tract.

7. With the help of sucking disc, the parasite attaches itself on to the convex surfaces of the epithelial cells in the intestine and cause a disturbance of intestinal function, leading to malabsorption of fat. It often leads to the deficiency of fat-soluble vitamins. The presence of fats in the stools cause diarrhea, often with large amounts of yellow mucus. Epigastric pains, vague abdominal discomfort, headache, diarrhea and allergic dermatitis are some of the common symptoms.

8. Disease diagnosis includes the microscopic examination of stools for trophozoites and cysts and by examining trophozoites from bile A and B aspirated from duodenum and bile duct respectively.

9. Atebrin or chloroquine or camoquin, given at the rate of 0.1 to 0.2 g three time a day for 5 days control the *Giardia* infection. a derivative of Imidazole (Metronidazole) is also effective. In addition, the administration of fat soluble vitamins benefit the patients.

10. *Balantidium* is an intestinal ciliate parasite. *B. coli* is a parasite of the large intestine of man, monkeys and pigs.

11. *B. coli* of man is much larger (50-80 μ in length) than any of the other protozoan parasites of human intestine. It is an egg or pear shaped with an obliquely arranged peristome at the anterior end. The body is covered with cilia in rows with adoral cilia in peristome. Macronucleus, micronucleus, two contractile vacuoles and food vacuoles are present in the endoplasm.

12. *Balantidium* divides by binary fission. Conjugation also occurs. Cysts are formed by single individual. No multiplication take place in the cysts.

13. Pigs are the important sources of human infection. In man, *B. coli* is pathogenic whereas in pigs it is non-pathogenic. In man, it causes ulceration of large intestine and colon. The patients suffer from only diarrhea and may show no symptoms at all.

14. Treatment with anti-amebic drug like carbarsone and antibiotics like Aureomycin and Terramycin are very effective in controlling the *Balantidium* infection.

1.4.5. KEY TERMINOLOGY:

Adoral cilia : Cilia within the buccal cavity of certain ciliates.

Bilateral symmetry: The arrangement of the body parts, so that the right and left halves are mirror images of each other.

Binary fission: Asexual reproduction that produces two similar individuals

Conjugation: A method of sexual reproduction in which two unicellular animals unite, exchange nuclear material and then divide as in the *Paramecium*.

Cyst: The stage of an organism where it is enclosed in a resistant wall.

Cytostome: Mouth of a ciliate.

Encystment: Forming resistant cysts in response to unfavourable conditions such as lack of food or desiccation.

Trophozoite: A phase in life cycle of an organism where its principal activity is nutrition and growth.

1.4.6. SELF ASSESSMENT QUESTIONS:

1. Give an account of the morphology, biology and life cycle of *Giardia intestinalis* and *Balantidium coli*.

2. Write an account on the mode of infection, pathogenicity and treatment of intestinal parasites, *Giardia intestinalis* and *Balantidium coli*.

1.4.7. REFERENCE BOOKS:

Chandler, A.C. and C.P. Read, 1961. *Introduction to Parasitilogy,* W.B. Saunders Company, Philadelphia and London.

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Dr. P. PADMAVATHI

UNIT - II

2.1. BIOLOGY, LIFE CYCLE, AND PORTALS OF ENTRY OF *OPISTHORCHIS (= CLONORCHIS) SINENSIS.*

2.1.1. OBJECTIVES:

The purpose of the lesson is to:

- ⇑ know the habit, habitat, and the structure of the *Opisthorchis*.
- ⇑ study its structure and physiology
- ⇑ understand its life cycle and infective stages
- ⇑ learn the symptoms, treatment and control measures of opisthorchiasis

2.1.2. INTRODUCTION:

It is a monoecious, digenetic, liver fluke with two or more asexual generations and an alternation of hosts. A lanceolate distome distinguished with a flat semitransparent body, small suckers, well

formed alimentary canal, an excretory bladder, a pair of posterior lobed testes, an ovary in front of testes, and a gonopore in front of the acetabulum. The development includes a number of larval stages like miracidium, sporocyst, redia, cercaria, and metacercaria.

Systematic Position:

Phylum: Platyhelminthes,
Class: Trematoda,
Order: Digenea,
Family: Opisthorchidae.

2.1.3. HABIT AND HABITAT:

The adults inhabit the small biliary ducts and large bile ducts, often in hundreds or even thousands, of fish-eating reptiles, birds and mammals. The cats, dogs, tigers, foxes, badgers, and minks serve as the common reservoir hosts. In addition, the fluke infests the snails of genera *Parafossarulus, Melanoides, Bulinus or Bythinia* as the first intermediate host and the freshwater fish, primarily of family Cyprinidae, as a second intermediate host before passing to the final host.

It is an important internal human parasite, with heavy infections in local areas, and is widely distributed in the Far East from Korea, Vietnam, Japan through China to Indo-China, and in many parts of India where raw fish is considered as a delicacy among people. It is popularly known as the Chinese liver fluke because of its prevalence in China.

2.1.4. EXTERNAL CHARACTERS:

The adults are small worms with an elongate, flat, leaf-like body. The size varies from 10 to 25 mm in length and 3 to 5 mm in breadth (averages about 18 x 4 mm). The mouth is typically located at the bottom of a muscular oral sucker. A second sucker occurs on the ventral surface in the anterior portion of the body.(Fig. 2-1)

2.1.5. TEGUMENT:

A thin, resistant, non-ciliated, spiny, syncytial cuticle called the tegument clothes the body. The epidermis is lacking. Mesoderm cells secrete the tegument. It protects the parasite from the action of host enzymes and also serves as site of nitrogenous waste excretion, gaseous exchange, and amino acid absorption. Beneath the cuticle are layers of circular, longitudinal, and diagonal muscles. Inside the muscles is a loose mesh of parenchyma (Fig.2 - 2)

2.1.6. DIGESTIVE SYSTEM

The digestive tract is branched due to the absence of blood and circulatory system. It helps in carrying the ingested food to all parts of the body. The mouth leads into a muscular pharynx, which runs into a short esophagus. It branches into two blind pouches, the intestinal caeca, which extend up to the posterior part of the body. The alimentary canal is internally lined with the cuticle.

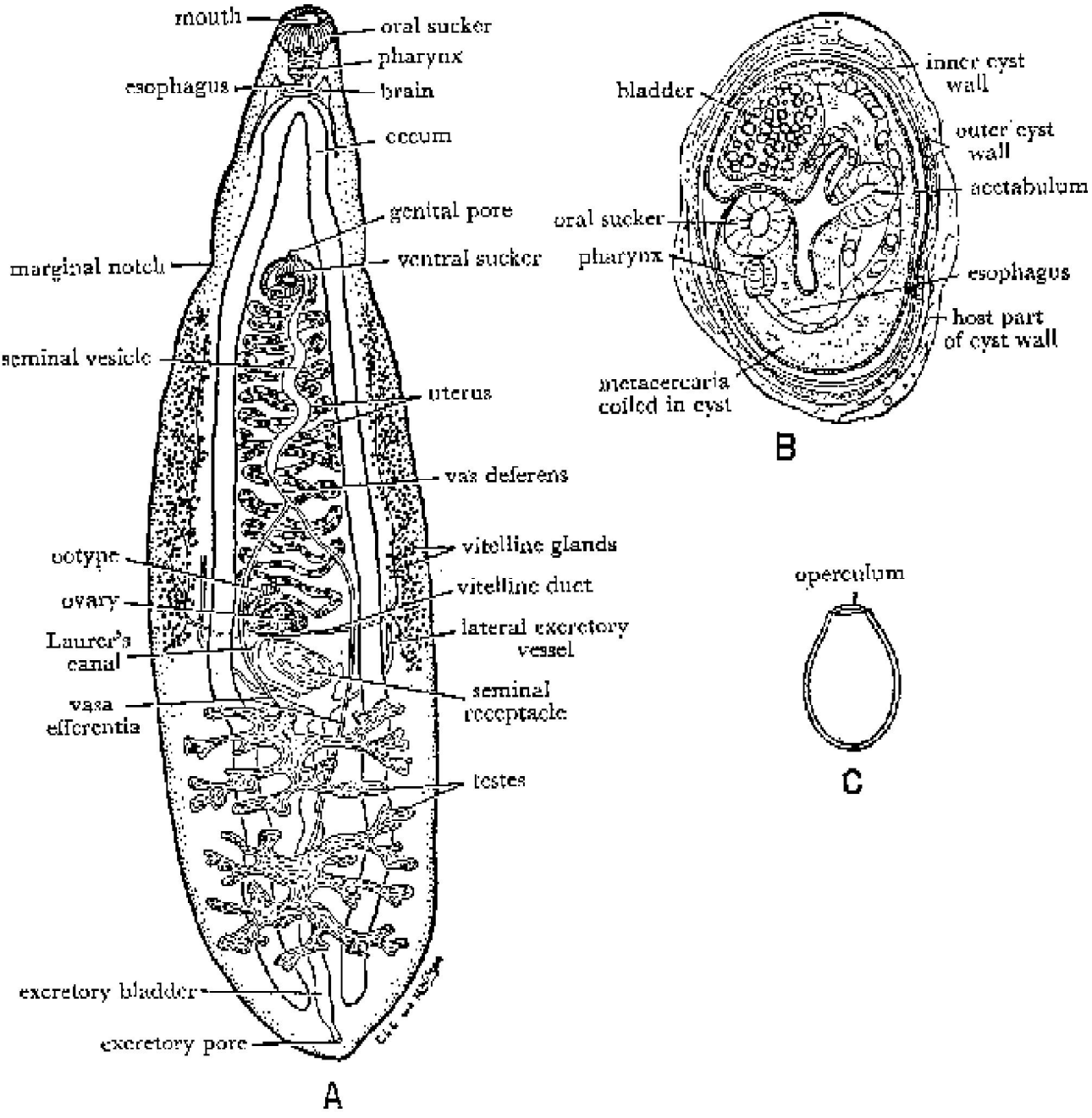

Fig. 2-1. *Opisthorchis sinensis*.
A) Dorsal view of adult. B) Metacercaria. C) Capsule.

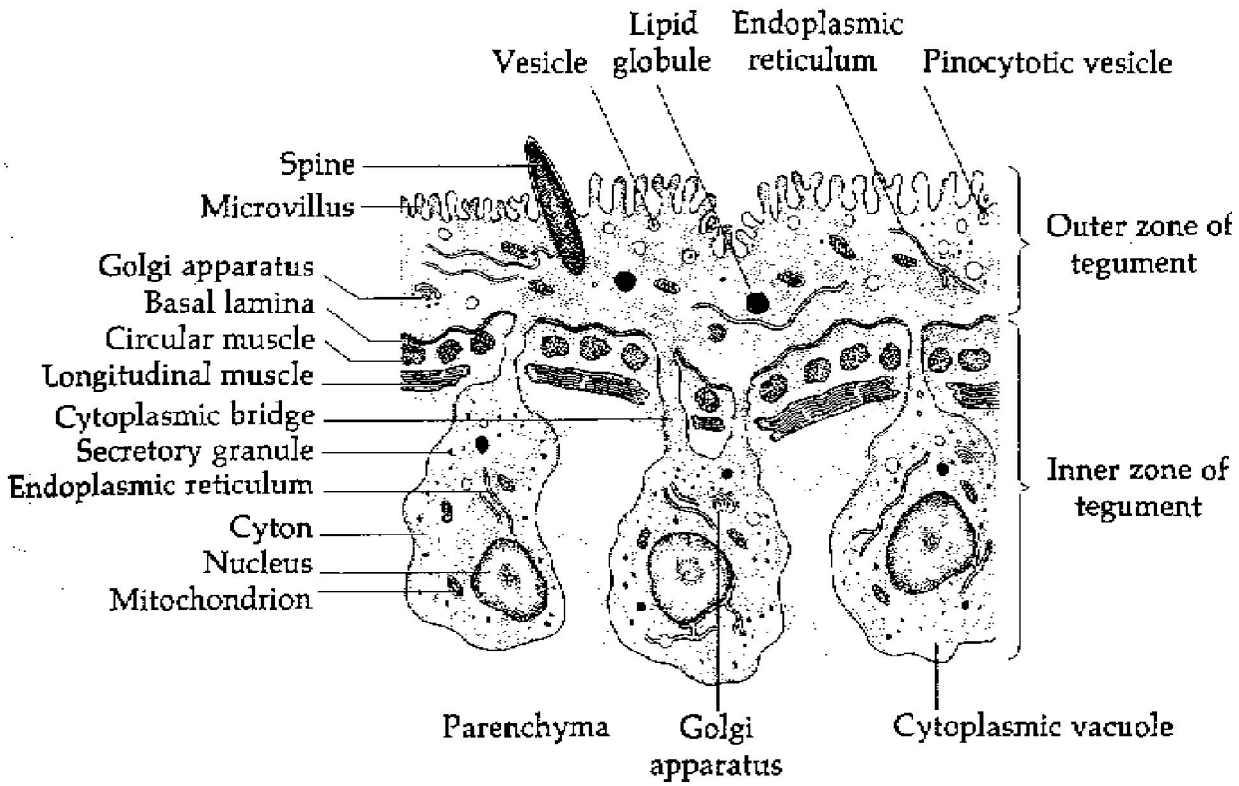

Fig. 2-2. Drawing showing fine structure of the tegument.

2.1.7. NERVOUS SYSTEM AND SENSE ORGANS:

It is of low grade in nature and consists of a pair of small cerebral ganglia at the anterior end of the body, which give off a few longitudinal nerve cords that extend posteriorly. The suckers receive a good nerve supply. Sense organs are almost lacking.

2.1.8. EXCRETORY SYSTEM:

In the absence of the circulatory system, the excretory system has a complicated arrangement of protonephridial tubules to carry waste products out from all parts of the body. The chief excretory and osmoregulatory structures are the flame cells, which keep up a flow of fluid towards the excretory pore. They give off fine branches, which unite to form a pair of main collecting tubules, one on each side. The tubules open posteriorly into a Y- shaped excretory bladder, which opens to the exterior by a single excretory pore. There are two groups of flame bulbs on each side, located on both anterior and posterior tubules.

2.1.9. REPRODUCTIVE SYSTEM:

The reproductive system is more intricate and highly specialized. Adults are hermaphroditic and possess both male and female reproductive organs. It occupies a largest portion of the body.

Male organs

There are two branched testes lying one behind the other in the posterior region. A vas efferens arises from each testis. The two vas efferentia unite in the middle of the body to form a vas deferens, which joins a seminal vesicle. A narrow ejaculatory duct arises from the seminal vesicle to open into a genital atrium, which open s by a gonopore on the ventral surface just in front of the acetabulum. The penis, prostrate glands and cirrus are lacking.

Female organs

There is small lobed ovary lying in front of the testes. It gives out a short oviduct. Behind the ovary lies a sac-like seminal receptacle. Before it joins the oviduct it receives a Laurerís canal, which curves behind seminal receptacle and opens dorsally by a small pore in the middle of the body. There are also separate glands for the production of the yolk and shell material and fluid in which the eggs are carried. The small follicles of vitelline glands lie in the middle one-third of the body on each side. These glands supply yolk and shell material for the eggs. The transverse vitelline ducts that arise from the vitelline glands unite to form a small common duct and this joins the oviduct and then the ootype. Small cells of Mehliís gland surround the ootype. The function of this gland is uncertain and its secretions may provide lubrication for the egg movement through the uterus. The ootype opens into a long coiled uterus, which, in turn, opens into the genital atrium.

In the ootype the fertilized eggs get enclosed with yolk in shells to form capsules. The capsule resembles an electric bulb, yellow-brown in color, and measures 27 x 16 microns in size. It has an operculum and a comma-shaped appendage. After formation the capsules enter the uterus.

2.1.10. LIFE CYCLE

The life cycle is marvelously complicated and includes three hosts. The fish-eating vertebrate, which lodges the adult worms, is the primary host while the snail that eats the eggs is the first intermediate host and the freshwater fish that lodges the final larval stages is the second intermediate host (Fig. 2 - 3)

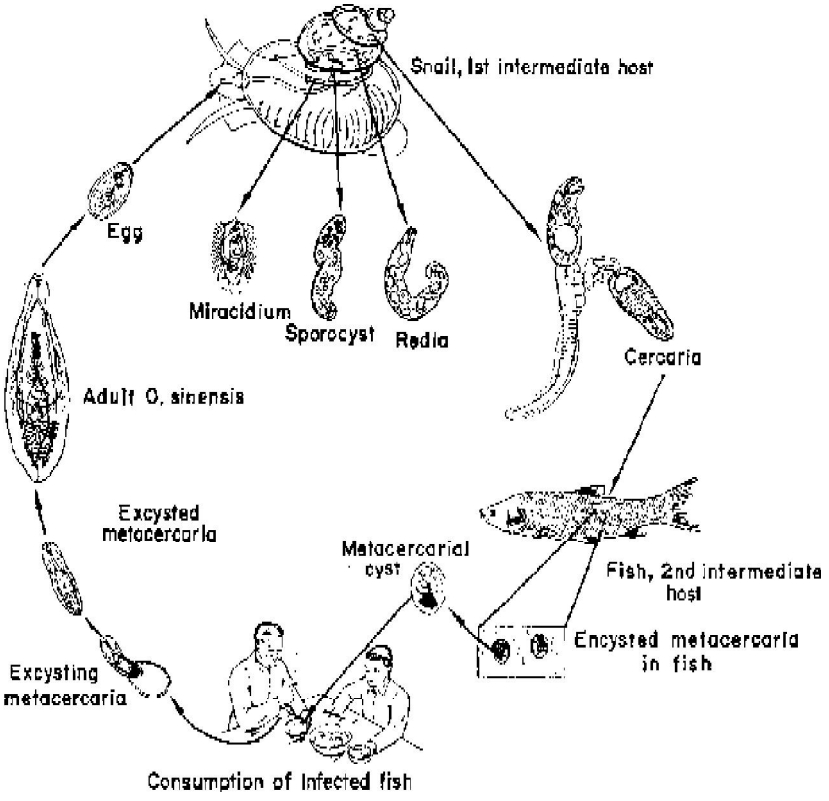

Fig. 2-3. Life Cycle of *Opisthorchis sinensis.*

⑦Mature Adult⑦Eggs⑦Ingested by mollusk⑦Miracidium hatches from egg⑦In mollusk tissues⑦Sporocyst⑦Redia⑦Cercaria⑦Emerge out from snail and enter a freshwater fish⑦Encysts in fish host as metacercaria⑦Ingestion by definite host ⑦Adult.

2.1.10.A. PASSAGE OF EGGS FROM THE HOST IN ITS FECES:

The capsules stored in the wormís uterus pass out through the gonopore into the bile ducts of the man and then reach the intestine and eventually move out along with the feces.

2.1.10.B. DEVELOPMENT OF EGGS:

By the time the capsules escape from the primary (definitive) host, they contain completely formed ciliated embryos. When the ciliated embryos are eaten by the snail (the first Intermediate host), the capsules hatch out in their intestine to produce the miracidia.

2.1.10.C. DEVELOPMENT OF THE PARASITE IN SNAIL (FIRST INTERMEDIATE HOST, AN INVERTEBRATE)

The miracidia are covered by a ciliated epithelium of relatively few large, flat cells. They have short sac-like gut, one or more pairs of penetration glands, one to many pairs of flame cells and excretory tubules, and a cluster of germ cells. They do not feed and usually die within 24 hours. They bore into the tissues of the snail and become round hollow sporocysts. The sporocyst contains numerous germ cells, each of which develops into an embryonic mass. In the snail the mother sporocyst gives rise to a generation of redia, which are devoid of birth pore and projections. The redia has a chambered form with many germ cells, which develop into the infective cercariae.

2.1.10.D. EMERGENCE OF INFECTIVE LARVAE (CERCARIAE) FROM SNAIL

The cercaria is the fourth developmental stage and is the infective stage. The cercaria is of pleurolophocercous type and is characterized by the presence of long tail with fluted lateral fins. It has a finely spined cuticle, seven pairs of penetration glands, fourteen cystogenous glands, several eyespots, and masses of brownish pigment. The cercariae emerge out of the snail and infest a suitable freshwater fish (Fig. 2 - 4)

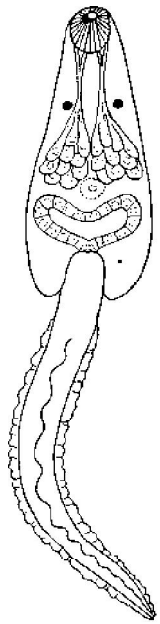

Fig. 2-4. *Opisthorohis senensis*- Pleurolophocercous cercaria

2.1.10.E. SUCCESSFUL ENCYSTMENT OF CERCARIAE IN FISH(THE SECOND INTERMEDIATE HOST, THE VERTEBRATE)

The cercariae escape from the snail and swim to be swallowed by the freshwater fish where the larval stages penetrate the skin and encyst in flesh as metacercariae. About eighty species of fish are found

to prey for the infection. The encystment may rarely occur under the scales or in gills. The metacercariae lose the eyespots, but have the sac-like excretory bladder filled with coarse, refractive granules.

2.1.10.F. ENTRY OF PARASITE (METACERCARIAE) INTO FINAL HOST

Eating uncooked or insufficiently cooked fish containing the metacercariae infects the definitive host. The incidence of infection in man reaches 15 - 70 per cent in local areas and 100 per cent in reservoir hosts. Raw as well as smoked, salted, or dried fish may constitute a source of human infection.

2.1.11. NUTRITION

The physiology of nutrition is not fully known. The digestion is probably chiefly extracellular. The worm ingests food by mouth. The food consists of blood, tissue cells, or tissue exudates. Food is stored mainly in the form of glycogen in mesenchyme, musculature, and ovary. Fine fat droplets may also present in the walls and lumen of the excretory system. The respiratory metabolism of the fluke is of the anoxybiotic type, where the glycogen is utilized and carbon dioxide and fatty acids are given off into the medium. The fluke disturbs the carbohydrate and lipid metabolism of the host. There is a decrease of calcium and potassium and an increase of sodium in hostís blood, and a raise in blood cholesterol and a fall in liver polysaccharides. Hyper or hypoglycemia may occur.

2.1.12. EPIDEMIOLOGY, SYMPTOMS AND PATHOGENECITY

The contamination of pond water with infected human feces or night soil makes the eggs to reach the suitable snails, which feed on the fecal material, and later the cercariae to attack the fishes. Infection occurs whenever the people eat contaminated raw fish. Sometimes the fish may be half-cooked where the heat is not enough to kill cysts in the flesh. For its endemical establishment, these flukes need the presence of suitable snail to serve as an intermediate host and the habit of eating raw fish.

The adult worms cause a chronic widespread disease in humans through blocking the bile passages called clonorchiasis. It is estimated that 19 million human cases exist in the Far East. Although a few dozen worms constitute an average infection, a case having 21,000 worms was reported. The fluke extracts large amounts of blood form the liver, causing anemia and eosinophilia. Early in the infection, there may be signs of indigestion. They injure the epithelium of biliary ducts, and if numerous they may seriously clog them. The walls of the ducts become thickened and in severe cases it may cause marked jaundice, liver cirrhosis and ultimately death. The frequent light infections may show no symptoms at all and the heavy infection may be accompanied by diarrhea, edema, nausea, epigastric pain, vertigo, fluid in body tissues, enlargement and tenderness of liver, abdominal discomfort, and signs of general body toxicity. Diagnosis is confirmed by finding eggs in fecal samples. The eggs are readily killed by desiccation, but can live up to six months at 0 C if kept moist.

2.1.13. MEDICATION (TREATMENT AND PREVENTION):

No completely effective treatment is known. Initially good results were obtained with injections of antimony compounds, but complete cure is not found except in early cases where gentian violet and related dyes are given in the form of coated pills. More recently, fair success has been obtained with chloroquine when used at the rate of 0.5-gm. daily for several weeks.

The prevention would be possible by storing night soil undiluted or adding 10% of ammonium sulfate to kill the eggs before they reach the snail. The prophylaxis rests mainly on avoiding infection through cooking of all freshwater fish that are used as food in endemic areas. The best measure is to prohibit the sale of raw fish in public places and to educate people about the dangers of eating raw fish.

2.1.14. SUMMARY:

- ⇑ A hepatic fluke of fish-eating vertebrates, including man.
- ⇑ More prevalent in China, Korea, Japan, and other Southeast Asian countries.
- ⇑ Adult lives in the bile passages of humans while the larvae infest snails as first intermediate host and fish as a second intermediate host.
- ⇑ Worms are small, elongate and flat distomes having an oral and a ventral sucker.
- ⇑ Body is covered by a protective tegument, which has some physiological significance.
- ⇑ Digestive system consists of mouth, pharynx, and a branched intestine. The digestion is extracellular. The fluke ingests blood and tissue exudes.
- ⇑ Blood and circulatory system are lacking.
- ⇑ Respiration is anaerobic. Lactic acid is end product of glycolysis.
- ⇑ Excretory system consists of a pair of collecting tubules, two groups of flame bulbs, an Y-shaped bladder and a single excretory pore.
- ⇑ Nervous system consists of a pair of cerebral ganglia and a few longitudinal nerve cords.
- ⇑ Sense organs are lacking.
- ⇑ Adults are hermaphroditic and contain both male and female reproductive systems.
- ⇑ Male reproductive system, located in the posterior part of the body, consists of a pair of deeply branched testes, a pair of vas deferentia, a seminal vesicle, and an ejaculatory duct.
- ⇑ Female reproductive system consists of a single lobed ovary, an oviduct, a seminal receptacle, an ootype, a uterus and a laurerís canal.
- ⇑ Separate vitelline and Mehliís glands occur to incorporate yolk and shell material into capsules.
- ⇑ Male and female gonopores are separate.
- ⇑ Copulation is mutual and cross fertilization results.
- ⇑ Life cycle is often complex and involves three hosts, a definitive vertebrate host and two intermediate hosts, a snail and a fish.

- ⇑ Development is indirect involving a series of larval stages like miracidium, sporocyst, redia, cercaria and metacercaria.
- ⇑ Fertilized embryonated eggs pass out of the main host via feces.
- ⇑ When eaten by snail, the eggs hatch out to give miracidia, which penetrate the liver glands to become sporocysts.
- ⇑ Sporocyst produces the redia and the redia produces the infective cercaria.
- ⇑ Fish swallows the cercaria, which encysts in its flesh as metacercaria.
- ⇑ Metacercaria reaches the humans when they eat raw fish.
- ⇑ Fluke is responsible for the occurrence of a serious disease in humans called clonorchiasis.
- ⇑ Severe infection is symptomatic.
- ⇑ Though the treatment is uncertain, the chloroquine gives a good result.
- ⇑ Prevention rests mainly on the consumption of thoroughly cooked freshwater fish and proper storage of night soil.

2.1.15. KEY TERMINOLOGY:

Digenetic Life cycle is completed in two or more hosts with many larval stages.
Distome Having two suckers, an oral and a ventral.
Capsule Encapsulated embryonated egg in ootype.
Cercaria Infective larval stage of digenic trematodes.
Definitive host Principal or main host lodging the adult worms.
Flame cells Excretory cells whose ciliary movement simulates the flickering of a flame.
Genital atrium A chamber receiving both male and female gonopres.
Gonoduct Main duct involved in the transport of the gametes produced by the gonads.
Gonopore External opening of male or female reproductive organs.
Intermediate host Host lodging the larval stages of the parasites.
Laurerís canal A copulatory canal extending from seminal receptacle to the exterior.
Mehliís gland Conspicuous unicellular gland surrounding the ootype.
Metacercaria Encysted cercaria and the final larval stage infective to the primary host.
Miracidium First ciliated larva that emerges out of the capsule.
Monoecious Having both male and female reproductive organs.
Pleurolophocercous Cercaria with a tail having fluted lateral fins.
Polyembryony Sequential development of a number of larvae from germ cells of a zygote.
Protonephridia Collecting excretory tubules equipped with flame cells.
Seminal receptacle A chamber of female reproductive system to receive and store sperms.
Seminal vesicle A chamber of male reproductive system to collect and store the sperms.
Tegument Tough body covering of trematodes and cestodes.

Uterus An organ of female reproductive system to store the fertilized eggs.

Vitelline glands Special glands secreting yolk and shell material during capsule formation.

2.1.16. SELF ASSESSMENT QUESTIONS:

1. Describe the habit, structure and organization in *Opisthorchis sinensis.*
2. Discuss the life cycle and pathogenecity in *Clonorchis.*
3. Write short notes on:
   a. Excretion in China fluke.
   b. Reproductive system in *Clonorchis.*
   c. Clonorchiasis.
   d. Life cycle of *Opisthorchis*.
   e. Labelled diagram of *Clonorchis.*

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Shri **B.V. Krishna Rao**

Unit - II

2.2. BIOLOGY, LIFE CYCLE AND PORTALS OF ENTRY OF *SCHISTOSOMA (=BILHARZIA) MANSONI.*

2.2.1. OBJECTIVES:

The purpose of the lesson is to:

→ know the habit, habitat, and the structure of the blood fluke.
→ study its structure and physiology
→ understand its life cycle and infective stages.
→ learn the symptoms, treatment and control measures of schistosomiasis.

2.2.2. INTRODUCTION:

Schistosoma (=Bilharzia) mansoni is a dioecious, digenetic, human blood fluke, which is notable for its marked sexual dimorphism. The union is of more compassionate nature, as both male and female remain permanently wedded, and always found together in pairs in human hepatic portal system. The uncoupled females live as spinsters while the males are able to develop independently of females. Copulatory organs are absent. It is the smallest and the best-known fluke responsible for one

of the world's most important sufferings, the schistosomiasis or bilharziasis. It can live for 20 years in the primary host. A male miracidium gives rise to a male adult and female one results in a female adult

Systematic Position:

Phylum: Platyhelminthes,
Class: Trematoda,
Order: Digenea,
Family: Schistosomatidae.

2.2.3. HABIT AND HABITAT:

The paired males and females are found in host's portal blood, usually in smaller branches of the inferior mesenteric veins, which drain blood from large intestine and cecal region (Ileocaecal junction). Occasionally they may get into the urinary system and have the eggs voided with the urine.

These flukes are found in Africa, Arabia, Madagascar, Brazil, Puerto Rico, Venezuela, Surinam, Dominican Republic, West Indies, and northern South America. Their presence in Western Hemisphere is believed to be a by-product of the African slave trade.

2.2.4. EXTERNAL CHARACTERS:

The body is thin and cylindrically elongated. The male is comparatively broader and shorter and measures 6.4 to 9.9 mm in length and 1 to 1.2 mm in breadth. The female is longer, slender, delicate and thread-like, and measures 7.2 to 14 mm in length and 0.16 mm in breadth. The male in its ventral deeply incurved body wall, the gynaecophoric canal, permanently carries the female. The color is grayish or pinkish. Both sexes have a small oral sucker around the mouth and a larger ventral sucker in the anterior part of the body. In the male, ventral sucker is large and powerful, and is borne on a slight elevation. The mouth opens at the anterior end. A single gonopore lies ventrally just behind the acetabulum. An excretory pore occurs ventrally at the posterior end (Fig. 2 – 5 and 2 – 6).

2.2.5. TEGUMENT:

The body is clothed with a resistant, rough, spiny cuticle with minute sensory papillae. It is grossly tuberculated with tufts of cilia-like projections in males. It is a mesenchymal secretion and a protective device for the parasite related to the immune reaction of the host. The body is solid and filled with a spongy mesenchymal connective tissue that surrounds all the internal organs.

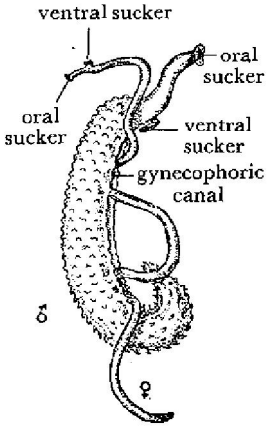


Fig. 2-5. *Schistosoma mansoni*

2.2.6. DIGESTIVE SYSTEM:

The funnel-like mouth leads into a short, narrow esophagus, which is surrounded by clusters of esophageal glands.

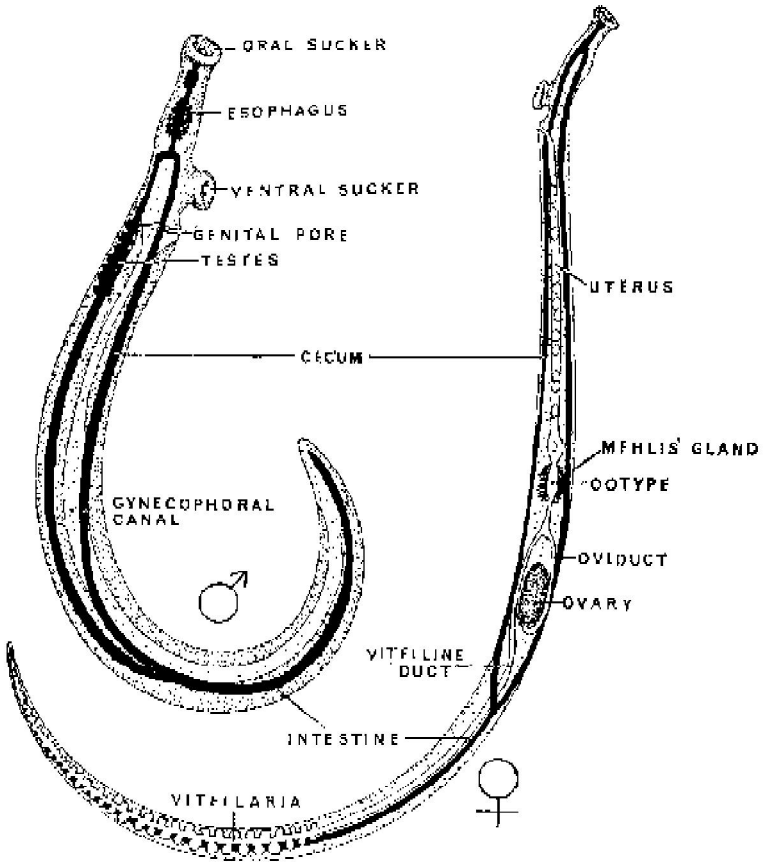


Fig. 2-6. *Schistosoma*, illustrating the main differences between the adult male and female.

The pharynx is lacking. The esophagus opens into an intestine, which bifurcates into two branches that reunite in the middle of the body to form a long and blind-ending caecum. The digestive system up to the intestine is lined by cuticle. The circulatory system is absent.

2.2.7. EXCRETORY SYSTEM:

It consists of flame cells, a pair of protonephridial collecting tubules, a Y-shaped bladder, and a single excretory pore at the posterior end of the body. The flame cells serve both in excretion of nitrogenous wastes and maintenance of osmoregulation.

2.2.8. NERVOUS SYSTEM AND SENSE ORGANS:

It consists of pair cerebral ganglia, two lateral longitudinal nerve cords and transverse connectives. The two ganglia are connected by a broad commissure which lies dorsally, between the oral sucker and esophagus. From this, three pairs of nerves proceed anteriorly and three pairs of cords – dorsal, lateral, and ventral proceed posteriorly. The ventral cords are the best developed. Adhesive organs receive rich nerve supply.

2.2.9. REPRODUCTIVE SYSTEM:

The sexes are separate. The males and females are held together in pairs permanently.

Male reproductive system:

It is simple and multitesticular consisting of 8 – 9 small testes situated anteriodorsally. The minute vas efferentia that arise from the testes unite to form a vas deferens. It joins a seminal vesicle and then enters a penis and finally opens to the exterior by a gonopore.

Female reproductive system:

A long, tubular ovary occurs above the point where the intestinal caeca rejoin. A long oviduct arises from the posterior part of the ovary. It bends upwards and runs forwards to join the ootype that is surrounded by Mehli's gland. The vitelline glands are voluminous and occupy the posterior part of the body. A vitelline duct joins these glands with the oviduct. The ootype gives out anteriorly a thin, straight uterus that contains relatively few capsules. The uterus opens to exterior by a gonopore just behind the acetabulum(Fig. 2 – 7).

2.2.10. LIFE CYCLE:

The life cycle is completed in two hosts. The humans serve as the principal hosts while the snail, primarily of the family Planorbidae (e.g., *Planorbis, Biomphalaria, Australorbis* etc.), acts as the only intermediate host. Wild rodents may act as the reservoir hosts. The paired worms move against the blood stream in a state of permanent copulation. The fertilization occurs in the oviduct when spermatozoa from the male are introduced into the female. The fertilized eggs become encapsulated in ootype by the vitelline secretions. Always there is only one egg in the uterus. A healthy, mated female deposits a single egg at a time and about 300 - 350 a day (Fig. 2 – 8 and 2 – 9).

∅Mature Adult∅Eggs∅Ingested by mollusk∅Miracidium hatches out from egg∅in molluscan tissue∅Sporocyst I∅Sporocyst II∅Cercaria∅Escapes from mollusk∅Penetrate definite host (man) ∅Adult.

2.2.10.A. DEVELOPMENT OF EGGS:

After copulation the female leaves the male to lay eggs, which find its way into the smallest possible blood vessels. The eggs penetrate the gut wall and move into the lumen of intestine and then escape with the feces of the host. The eggs are non-operculate, elongate or oval in

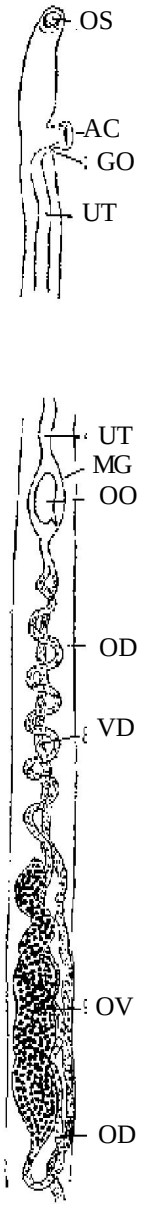


Fig. 2-7 Female system of *S. mansoni* AC, Acetabulum; GO, Gonopore; MG, Mehlis gland; OD, oviduct; OO, ootype; OS, oral Sucker; OV, Ovary; UT, Uterus; OD, Vitelline duct.

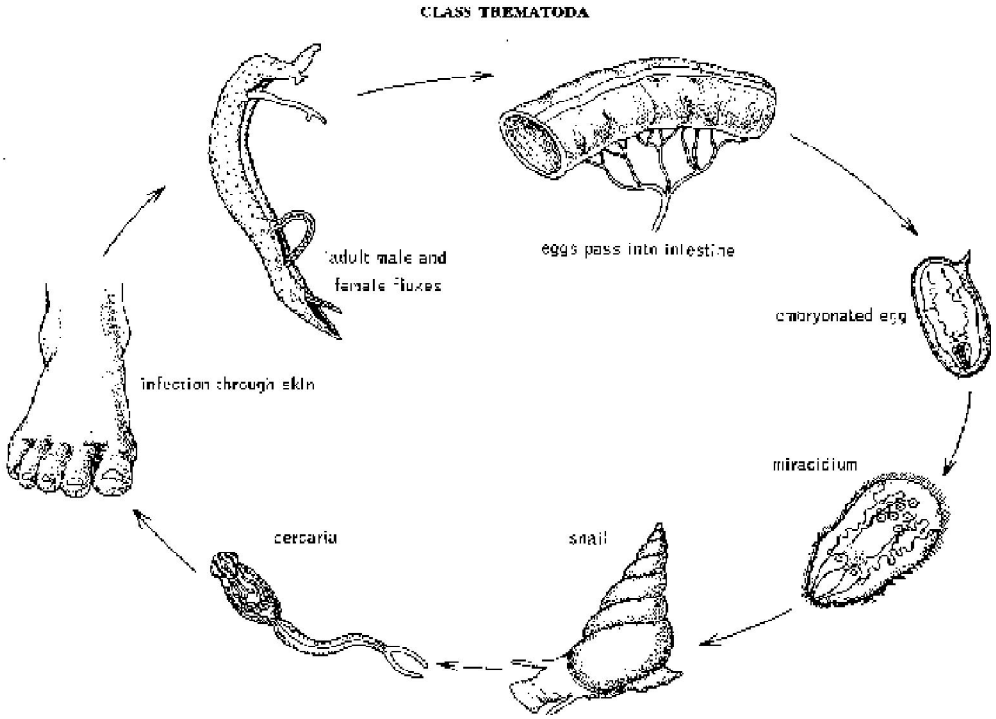


Fig. 2-8. Life Cycle of *Schistosoma mansoni*

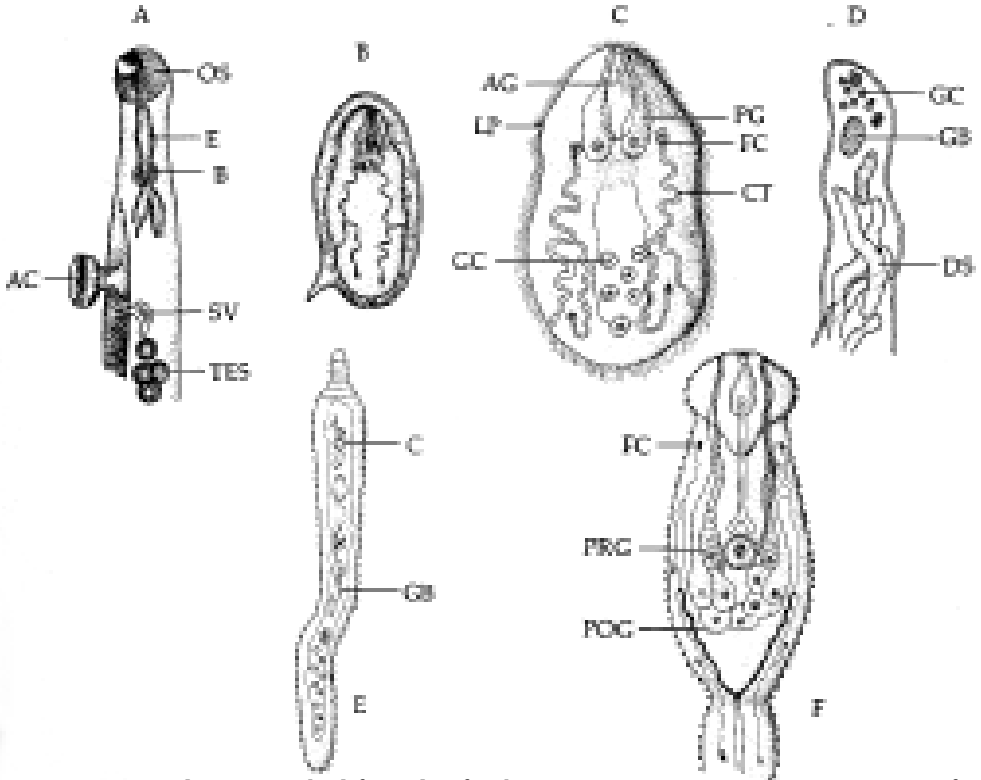


Fig. 2-9. Larval stages in the life cycle of *Schistosoma mansoni.* A. Anterior portion of adult male worm. B. Egg enclosing miracidium. D. Portion of mother sporocyst. E. Daughter sporocyst. F. Body of cercaria. AC, acetabulum; AG, apical gland; B. brain; C, cercaria; CT, collecting tubule; DS, daughter sporocyst; E, esophagus; FC, flame cell; GB, germ ball; GC, germinal cell; LP, lateral papilla; OS, oral sucker; PG, penetration gland, POG, postacetabular gland; PRG, preacetabular gland; SV, seminal vesicle; TES, testis.

shape, and measure 114 -175 microns in length and 45 – 68 microns in diameter. They are of same size having a postero-lateral spine. Each egg contains a motile miracidium larva. On contact with freshwater, the eggs hatch out to release miracidia, which swim about until they find a suitable snail host.

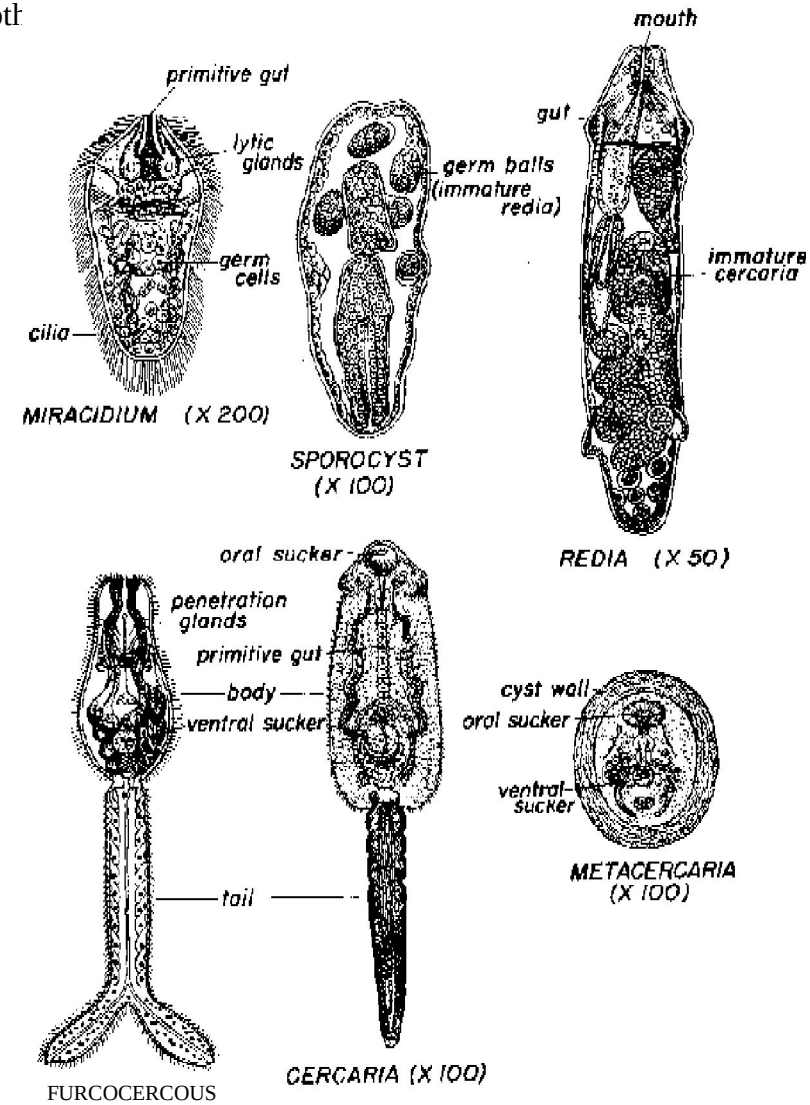
2.2.10.B. DEVELOPMENT OF THE MIRACIDIUM IN SNAIL:

The miracidium has a ciliated epithelial covering, a pair of large anterior penetration glands, a pair of internally separated lateral penetration glands, a large gut, a nerve center, and two pairs of flame cells. It measures 130 x 60 microns in size. Groups of germ cells are present. The miracidium is pressed against the snail by action of its cilia. The adhesive glands secrete a mucoid substance to make it to adhere to the snail. An anterior flagellum elongates and works its way through the snail's epithelium like a drill. The secretions of the penetration glands apparently digest the tissues of the snail, thereby aiding the papilla in enlar
snail's liver and transforms into a moth

Sporocyst reaches a length of I mm in about two weeks. The first generation of sporocysts may again produce miracidia larvae, which in turn produce a second generation of sporocysts. which reach a length of 1.5 mm and produce the cercariae from germ masses at their posterior end. Within 4 – 6 weeks of infection, the mature cercariae emerge out from a birth pore near the anterior end of the sporocyst. The sporocysts survive longest in snail tissues of the head-foot, tentacles, pseudobranchs, mantle collar, and in sinuses and veins. The redia stage is absent (Fig. 2 –10).

2.2.10.C. EMERGENCE OF CERCARIA (INFECTIVE LARVA) FROM SNAIL:

Either first or second generation of sporocysts can produce a very large number of infective cercariae larvae, which are apharyngeal and furcocercous type. It has a short, oval body of about 200 microns length with a slightly longer



FURCOCERCOUS CERCARIA (x 150) OF *S. MANSONI*

Fig. 2-10. Larval types of human flukes.

and forked tail. The entire surface is covered with minute spines. Two suckers, oral and ventral, and excretory and nerve elements are present. The mouth leads into an esophagus and then into a trifid intestine. There are two pairs of pre-acetabular oxyphilic glands and three pairs of post-acetabular basophilic glands to secrete the protective mucoid film. They escape frequently from snail into water in "puffs", a number at a time. For 2 – 3 days they alternately swim and rest in water. If they fail to reach a final host in this time they die. Ruminants can be infected when they drink contaminated water (Fig. 2 - 11)

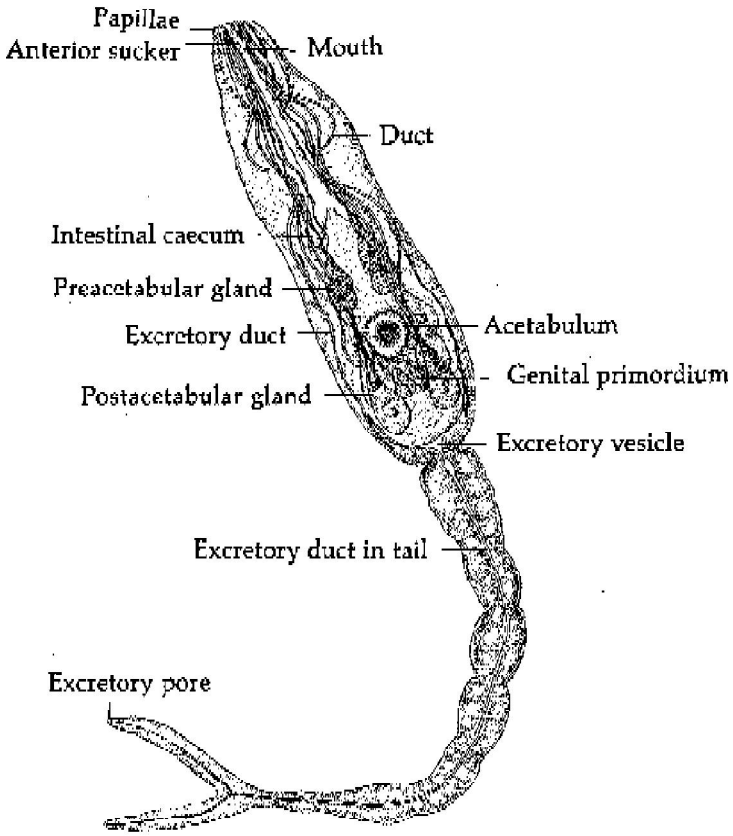


Fig. 2-11. *Schistosoma mansoni* - Furcocercous cercaria

2.2.10.D. ENTRY OF PARASITE INTO FINAL HOST:

Without encystment the cercariae penetrate the human skin rapidly during bathing or swimming in contaminated water or they may be swallowed into mucous membrane of mouth or throat by drinking infected water. They do not survive in the stomach of the host. The penetration requires several minutes when they lose their tails and remain in skin for about eighteen hours. They enter the blood circulation and make their way through the heart and lungs to reach the liver where they mature and finally settle in the portal system. In a week or more they gradually transform into adults and move to the inferior mesenteric veins and capillaries of the sigmoidorectal area.

The adults feed on blood. All the cercariae produced from one egg develop into flukes of same sex. The presence of male is necessary for the sexual maturation in female. Whenever a male finds a female it encloses her in its gynaecophoric canal. Young adults can be found in the lungs by the second or third day after penetration, accumulated in the liver by sixth day and grow rapidly by feeding on portal blood and establish well by fifteenth day. By the 23rd day they migrate to the mesenteric veins where sexual maturation and mating take place. The eggs are produced by the 40th day.

2.2.11. NUTRITION:

Although some absorption of nutrients may take place through the body surface, it ingests blood through its mouth. It feeds almost entirely on blood. Digestion is probably extracellular. Food is

stored chiefly in the form of glycogen and fine fat droplets. The respiratory metabolism is primarily anaerobic type.

2.2.12. EPIDEMIOLOGY, SYMPTOMS AND PATHOLOGY:

It causes the Manson's or Intestinal Schistosomiasis. The human infection occurs with the contact of water containing the infective cercariae. The farmers, washerwomen and rural communities are more prone to the infection. Infected snails show stunted growth and physiological castration. WHO has estimated some 200 million cases of human schistosomiasis exist in the world. China alone account for 20 million cases where as Egypt for about 17 million cases. In Lower Egypt, an incidence of 60% - 90% infection has been observed due to the development of perennial irrigation, which provided good breeding places for the Planorbid snails. Next to malaria, it is probably humanity's most serious parasitic infection.

The clinical course of the disease usually begins with an allergic reaction to the parasites and their by-products. Most commonly the eggs trapped in various tissues cause inflammation. The symptoms include cough, late-afternoon fever, skin eruptions, swelling and tenderness of liver, eosinophilia, anemia, abdominal pain, tiredness, severe dysentery and diarrhea. The chronic stage of the disease is characterized by the gradual impactment of eggs into the walls of the body organs, leading to fibrous thickening and loss of elasticity. There may be a serious liver damage. Occasionally, eggs may also cause lesions of such organs as the brain and lungs. During autopsy, eggs have been found in almost all body structures. The penetration of skin by cercariae causes dermatitis with prickling sensations and intense itching, a condition called "Swimmer's itch".

2.2.13. MEDICATION (TREATMENT AND PREVENTION):

Early diagnosis and persistent treatment usually ensure recovery. Chemotherapy is aimed at killing the adult worms by using anthelmintics orally. Several of the drugs used to treat worm infections affect the nervous system of the parasite and result in muscle paralysis. Other drugs affect the uptake of glucose and consequent production of energy. Generally, compounds of Antimony (Sodium antimony tartarate) are cheap, effective, but toxic, and are given intravenously over a period of four weeks to cure the disease. Recently, low toxic compounds with a high rate of cure, Antimony dimercaptosuccinate, Praziquantel and Oxamniquine have been developed. For dermatitis, Copper oleate ointment or Benzyl benzoate is effective.

Schistosomiasis is ordinarily contacted by working, bathing, or swimming in water populated with snails that carry the worm. Hence prevention is practiced indirectly by eliminating the snails along with cercariae by using molluscicides like sodium pentachlorophenate, dinitro-o-cyclohexyl phenol, copper sulfate etc., but with uneven success. When there are no snails the newly developed miracidia cannot find a suitable host and perish. Educating public and sanitary control of infected water through chlorinating can prevent human infection.

2.2.14. SUMMARY:

→ A dioecious, digenetic, distome with marked sexual dimorphism.
→ An important human blood fluke inhabiting inferior mesenteric veins of hepatic portal system.
→ Found in Africa, Arabia and South America.
→ Male is broader and shorter than the female.
→ Female is permanently lodged in male's gynaecophoric canal.
→ Both sexes have two suckers, a ventral gonopore and a posterior excretory pore.
→ Tegument rough and spiny and is protective in nature.
→ Mouth, esophagus and a bifurcated intestine represent digestive system.
→ Excretory system is protonephridia type consisting of flame bulbs, collecting tubules, a bladder and an excretory pore.
→ Nervous system is of diffused type with a pair of cerebral ganglia and two lateral longitudinal nerve cords.
→ Sexes are separate. Males are multitesticular with a sperm duct, a seminal vesicle, a penis and a gonopore. Females are with a single elongated ovary, an oviduct, an ootype, a uterus, and a gonopore and are associated with vitelline and Mehli's glands.
→ Life cycle is completed in two hosts. Man is the primary host while snail is the intermediate host.
→ A healthy mated female can deposit 300 to 350 eggs per day.
→ Embryonated eggs penetrate the gut wall of human and move into the intestine and escape with feces.
→ On contact with water, the eggs hatch out to produce ciliated miracidia, which penetrate snail's body to reach its liver and transform into sporocysts.
→ Sporocyst produces a large number of infective cercariae with forked tails.
→ Cercaria emerges out from the snail and swims in water to penetrate human skin.
→ Cercaria enters human blood circulation, lose its tail and finally settle in portal veins to attain maturity.
→ Fluke feeds almost entirely on blood.
→ Causes one of the most important human sufferings called "Intestinal Schistosomiais". Also responsible for a skin infection called "Swimmer's itch".
→ Antimony compounds are used in treatment.
→ Prevention is achieved by snail control and by sanitary control of water.

2.2.15. KEY TERMINOLOGY:

Acetabulum A larger ventral sucker in the anterior portion of the body.
Apharyngeal With out pharynx.

Bilharziasis	Other name for Scistosomiasis, a disease caused by the blood fluke, *Schistosoma*.
Digenetic	Life cycle is completed in two or more hosts with a number of larval stages.
Distome	Having two suckers, an oral and a ventral.
Dimorphic	Distinct and separate males and females.
Capsule	Encapsulated embryonated egg in ootype.
Cercaria	Infective larval stage of digenetic trematodes.
Definitive host	Principal or main host where the adult worms attain sexual maturity.
Flame cells	Excretory cells whose ciliary movement simulates the flickering of a flame.
Furcocercous	Cercaria with a long furcated tail.
Genital atrium	A chamber receiving both male and female gonophores.
Gonoduct	Main duct involved in the transport of the gametes produced by the gonads.
Gonopore	External opening of male or female reproductive organs.
Gynaecophoric canal	Ventral folding of the male worm in which the female lodges.
Intermediate host	Host lodging the larval stages of the parasites.
Mehli's gland	Conspicuous unicellular gland surrounding the ootype.
Metacercaria	Encysted cercaria and the final larval stage infective to the main host.
Miracidium	First ciliated larva that emerges out of the capsule.
Ootype	An organ of female system where the fertilized eggs receive yolk and shell material.
Polyembryony	Sequential development of a number of larvae from germ cells of a zygote.
Protonephridia	Collecting excretory tubules equipped with flame cells.
Reservoir host	A host that acts as vector and helps in storing and spreading of the parasite.
Seminal receptacle	A chamber of female reproductive system to receive and store sperms.
Seminal vesicle	A chamber of male reproductive system to collect and store the sperms.
Sperm duct	Main duct arises from the testes leading to gonopore.
Sporocyst	A cyst like larval stage of trematodes that give birth to redia or cercaria.
Tegument	Tough and resistant body covering of trematodes and cestodes.
Uterus	An organ of female reproductive system to store the fertilized eggs.
Vitelline glands	Special glands secreting yolk and shell material during capsule formation.

2.2.16. SELF ASSESSMENT QUESTIONS:

1. What is schistosomiasis? Discuss its epdemiology, symptoms and treatment.
2. Describe the life cycle of *Schistosoma mansoni*. Add a note on its pathogenecity.
3. Give an account of habit, structure and organization of *Schistosoma mansoni*.
4. Write short notes on:
   a. Intestinal schistosomiasis.
   b. Swimmer's itch.

c. Sexual dimorphism in *Schistosoma.*
d. Larval stages of *S. mansoni*.
e. Labelled diagram of *S. mansoni.*
f. Life cycle of human blood fluke.

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Shri B.V. Krishna Rao

Unit- II

2.3. BIOLOGY, LIFE CYLCE, AND PORTALS OF ENTRY OF *DIPHYLLOBOTHRIUM (=DIBOTHRIOCEPHALUS) LATUM.*

2.3.1. OBJECTIVES:

The purpose of the lesson is to:

- ⇑ Know the habit, habitat, and the structure of the broad fish tapeworm.
- ⇑ Study its structure and physiology.
- ⇑ Understand its life cycle and infective stages.
- ⇑ Identify the symptoms, treatment and control measures.

2.3.2. INTRODUCTION:

Diphyllobothrium latum (also known as *Dibothriocephalus latus*) is an extremely largest, polyzoic, pathogenic, human cestode of Eurasian fish-eating countries. It is commonly known as the "Broad fish tapeworm". It is characterized by an elongated scolex with two "bothria", which are short vertical slit-like sucking grooves. It has a long slender neck; a majority segments in similar stage of development; a rosette-shaped uterus; many scattered vitelline glands as dorsal and ventral sheets,

and a common genital mid-ventral opening for cirrus and vagina. The eggs are with pointed ends and are shed through a uterine pore. A plerocercoid larval stage occurs usually in frogs, snakes, birds, or rodents. It can live in human flesh for 5 - 35 years in plerocercoid stage and cause a disease called Dibothriocphaliasis or Sparganosis.

Systematic Position:

Phylum: Platyhelminthes
Class: Cestoda
Order: Pseudophyllidea
Family: Diphyllobothridae

2.3.3. HABIT AND HABITAT:

Adult worms are found living in the small intestine of man and many domestic and wild mammals like cats, dogs, bears, pigs, seals and sea lions. Some may have both birds and mammals as definitive hosts. The intermediate hosts are freshwater copepods and fishes.

D. latum has been well known in the Northern Hemisphere in many parts of the Central Europe (Finland, Ireland etc.), Siberia, Palestine, Japan, Central Africa, Chile and U.S.A.

2.3.4. EXTERNAL CHARACTERS:

It is a large, slender, weakly muscular, veritable monster reaching up to a length of more than 10 meters and a width 10 to 20 mm, with a total of 3000 - 4000 proglottides. The segments for most part are mature and much wider than long. The body (Fig. 2 -12) is distinguished into three regions.

Scolex: It is an elongate, compressed, almond-shaped, anchoring structure. It has two narrow deep longitudinal slit-like grooves called Bothria. Scolex measures 2 -3 x 0.7 - 1 mm in size. Hooks are absent.

Neck: It is a long, slender, narrow part located just below the scolex. It is a zone of segmental proliferation.

Strobila: A continuous ribbon of self-fertilizing proglottides that regularly shrink, die and detach from the posterior end. The anterior 20% of the body is composed of small immature segments and the rest constitutes mature as well as gravid. The mature proglottid is broader than long and has a single set of reproductive organs. The ripe proglottid is longer than wide and shows permanently a central rosette- shaped uterus.

2.3.5. TEGUMEBNT:

The tegument is largely proteinaceous containing certain polysaccharides, glycoproteins, mitochondria, vacuoles and membranes. Its cuticular surface projects into numerous brush-like processes. It is enucleate syncytium specialized for absorption. Beneath it lies a thin circular muscular layer and one or more longitudinal muscular layers containing secretory cells. Within the mesenchyme

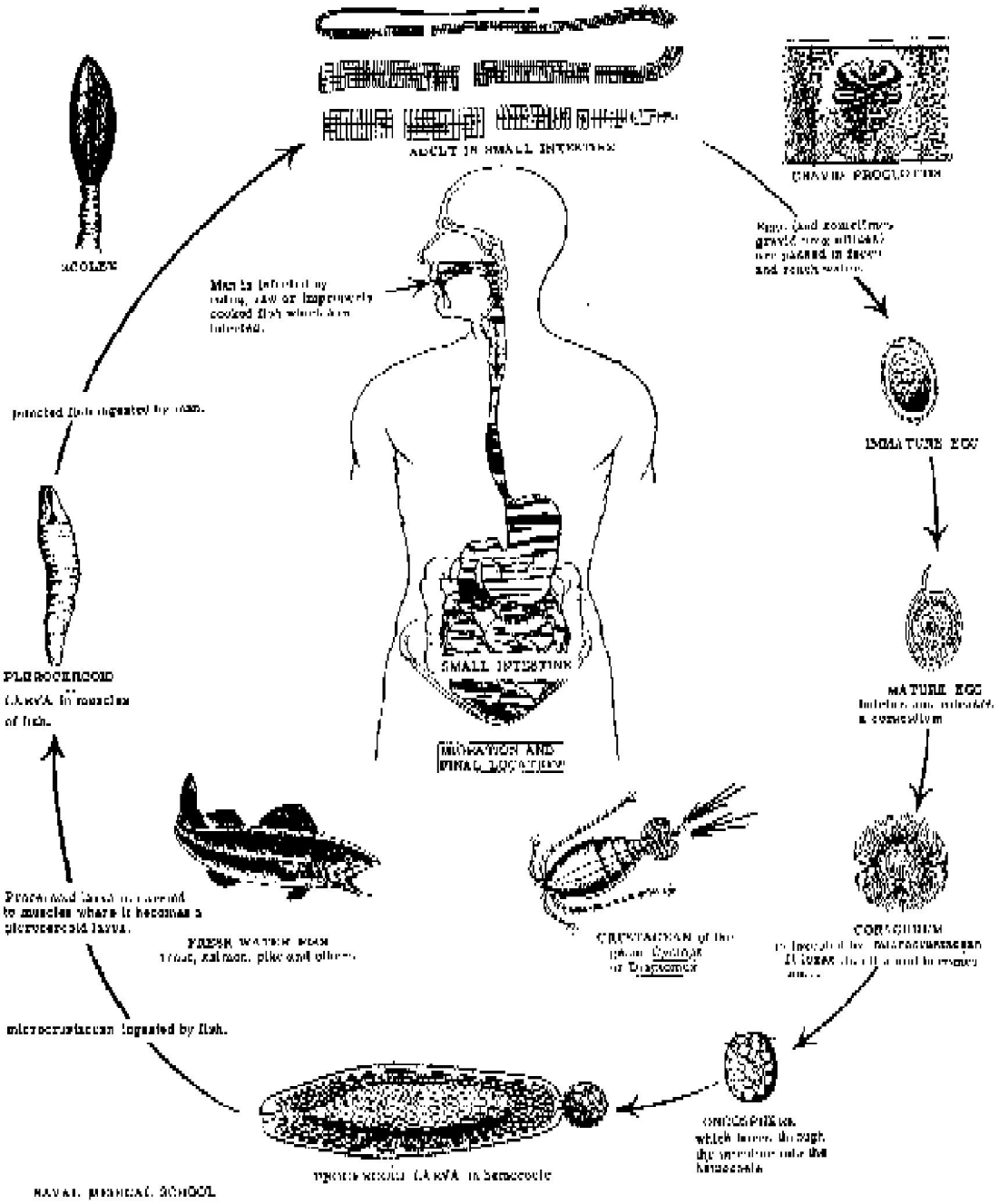

Fig. 2-12. Life cycle of *Diphyllobothrium latum.*

is well-developed transverse, longitudinal, dorsoventtal, and occasionally diagonal muscles. Bothria of scolex are well endowed with muscles.

2.3.6. DIGESTIVE SYSTEM:

As tegument takes the function of absorbing nutrient requirements directly from the intestine of the host, the digestive system is lacking.

2.3.7. NERVOUS SYSTEM AND SENSE ORGANS:

The nervous system consists of a pair of cerebral ganglia at the anterior end, from which extend two main lateral trunks that span the length of the body. There are several other longitudinal nerves that are connected by many branching nerves extending to all parts of the body. A heavier concentration of nerves and neurons occur in such organs as suckers and excretory vesicles. There are no special sensory organs.

2.3.8. EXCRETORY SYSTEM:

The excretory system is of Protonephridia type consisting of flame cells that connect with transverse and longitudinal collecting tubules. The longitudinal vessels are located along each side of the body. Fluid flows anteriorly in the smaller dorsal vessels and posteriorly in the larger ventral vessels. These vessels open directly to the surface or through a caudal excretory vesicle and a terminal excretory pore. In addition to the elimination of metabolic wastes, the tubules also help in maintenance of hydrostatic pressure.

2.3.9. REPRODUCTIVE SYSTEM:

Adults are hermaphroditic with each mature proglottid contains one set of female organs and one set of male organs.

Male Reproductive system:

Testes are numerous, small and round and are scattered in the lateral fields on dorsal side of the proglottid. The vas efferentia of the testes unite to form a coiled sperm duct (vas deferens), which proceeds anteriorly and enlarges into a seminal vesicle and then ends in a median cirrus.

Female Reproductive system:

A symmetrically bilobed ovary is located in the posterior third of the proglottid. Vitellaria containing shell globules and yolk material are dispersed along the lateral areas but ventral to the testes. The vagina is a narrow coiled tube opening on the mid-ventral surface in the anterior part of the segment and running almost straight posteriorly through seminal receptacle into an ootype surrounded by Mehliís gland. Uterus is much twisted and occupies the middle field of the proglottid. It has an inner series of delicate coils as it leaves ootype and then followed by an outer series of large coarse ones. The uterine opening is on the ventral surface, but is inconspicuous (Fig. 2 -13)

It has been estimated that a single female can produce 36,000 eggs daily. Self-fertilizations involving the sexual structures of a single proglottid or reciprocal fertilization between proglottides of the same worm occur.

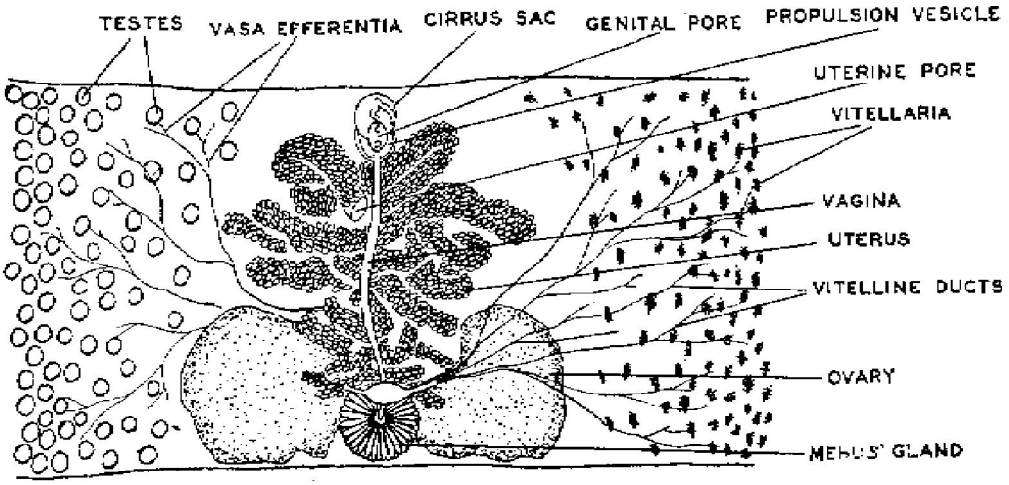

Fig. 2-13. *Diphyllobothrium latum.*- Mature proglottid (Reproductive system)

2.3.10. LIFE CYCLE:

The life cycle is completed in three hosts, man or a domestic mammal as a definitive host and a copepod as a first intermediate host and a fish as a second intermediate host. It includes a free-swimming Coracidium stage, a Procercoid stage in the copepod, a Plerocecoid stage in the fish and a mature Adult stage in the final host (Fig. 2 - 14).

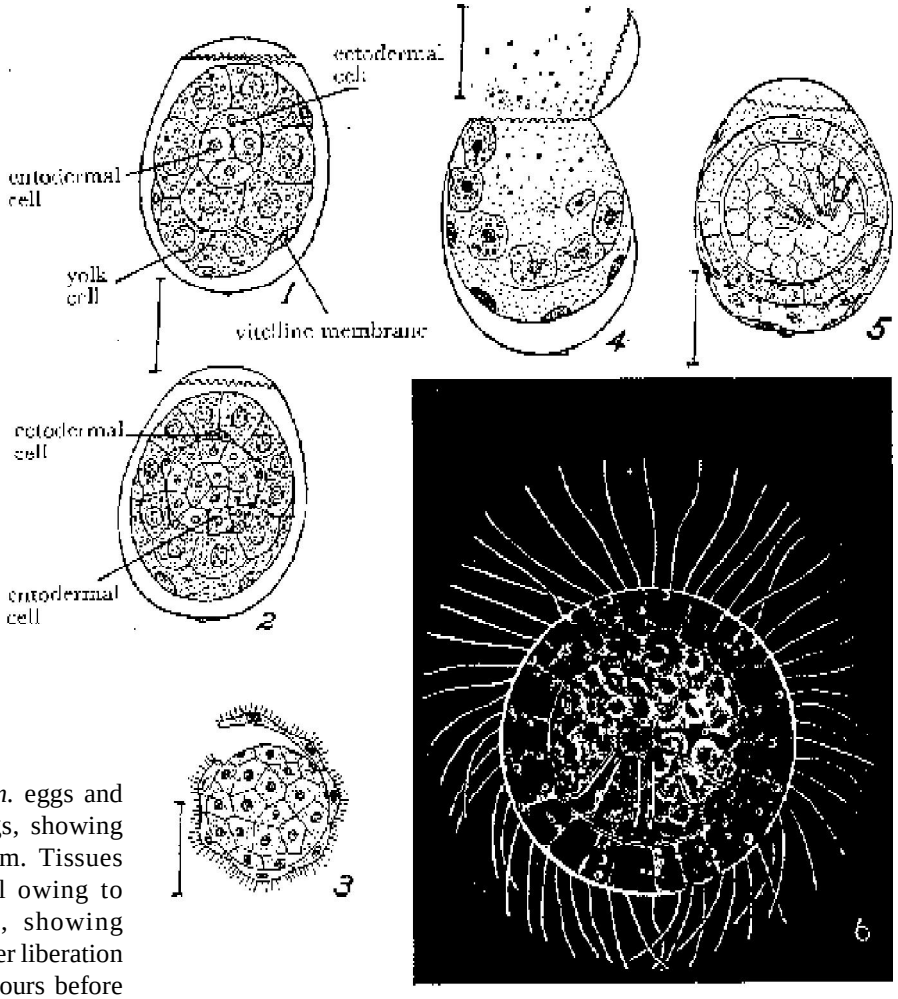

Fig. 2-14. *Diphyllobothrium latum.* eggs and coracidium. (1,2) Segmented eggs, showing origin of ectoderm and endoderm. Tissues shrunk away from the egg shell owing to fixation. 3) Immature embryo, showing development of cilia. 4) Egg just after liberation of the coracidium. 5) Egg a few hours before hatching. 6) Vogel's figure of the coracidium by dark field illumination.
The line near the figures represent 0.02 mm.

⑦Polyzoic Adult⑦Eggs⑦Oncosphere (liberated after ingestion by copepod) ⑦Procercoid (in the body cavity of copepod) ⑦Ingested by fish⑦ Plerocercoid or Sparganum (develop in fish flesh) ⑦Ingested by the definitive host⑦Adult worms.

2.3.10. A. PASSAGE OF EGGS FROM THE PRIMARY HOST:

Eggs are thick-walled, oval and operculate. They shed from the ventral uterine pores of the strobila and are deposited in water in a dormant, non-embryonated state along with feces. They are yellowish to golden brown in color and measure 55 - 76 x 41 - 56 microns in size. Eventually the spent proglottides cease to produce eggs.

2.3.10.B. DEVELOPMENT OF THE EGGS:

Eggs require light and oxygen for hatching. In water medium, either at laying or after development outside, the egg hatches out in a period of about 8 to 12 days to produce an early embryo with three pairs of hooks known as the Hexacanth embryo or Onchosphere. Upon hatching, the Onchosphere that is covered with a ciliated embryophore becomes a free- swimming or free-crawling Coracidium. The Coracidium measures about 50 - 55 microns in diameter. It must be eaten by some copepod within 24 hours to continue its development and to fulfil its destiny or else it perishes. Coracidia are quite host specific and are killed when ingested by a wrong host. When ingested by a Cyclops or Diaptamus, they lose their ciliated coats in the hostís stomach and become naked Onchospheres.

2.3.10.C . DEVELOPMENT OF PARASITE IN INTERMEDIATE HOST:

In copepod host, the Coracidia invade and perforate stomach wall by bringing six hooks together to form a point and then enlarging the opening by spreading the hooks apart. They bore through the body cavity and in 14 to 18 days transform into second larval stage called the Procercoid. The Procercoid is an elongate solid creature with a thick cuticle and bears six hooks at its posterior end and measures about 500 microns in length. It can resist acid, alkali, and a temperature ranging ñ8 to 55 C. The embryonic hooks persist in a bulb-like appendage, the cercomer, which is partially pinched off at the posterior end.

2.3.10.D. EMERGENCE OF PROCERCOIDS FROM COPEPOD HOST AND ENTRY INTO FISH HOST:

The Procercoid undergoes further development only in a carnivorous fish (trout, salmon, pike and perch) that ingest the infected copepods. Its passage through the intestine and body cavity of the fish is very slow. It penetrates the intestine wall by its peristaltic movements and frontal gland secretions and makes its way into various organs like liver, coelom, muscles etc., where it elongates into a third stage, the Plerocercoid larva.

2.3.10.E. DEVELOPMENT OF PARASITE IN THE SECOND INTERMEDIATE (FISH) HOST:

The Plerocercoid has a coiled form and looks like a tapeworm without strobila. Either unencysted in viscera or encysted in muscles or elsewhere, it multiplies asexually. As it resembles another tapeworm, it was mistakenly called as Sparganum and so its infection of a fish or other animal is called as Sparganosis. Plerocercoid is a minute and straight form and measures about 5 - 10 cm in length. As it grows, it becomes increasingly bent and twisted. The anterior end has a depression, which is the withdrawn and inverted scolex. The remainder of the body is white, flat and marked by irregular wrinkles, but without segmentation. It is peculiar that the Plerocercoids are able to re-invade and become re-established in host after host until the normal final host is reached.

2.3.10. F. ENTRY OF PLEROCERCOID INTO FINAL HOST:

All fish-eating mammals including humans are the final hosts, which are infected upon eating inadequately cooked or raw infected fish containing Plerocercoids.

2.3.11. DEVELOPMENT OF PLEROCERCOID TO ADULT IN FINAL HOST:

When ingested by a fish-eating mammal, the Plercocecoid larva attaches itself to the hostís intestine and develops into a mature adult worm. In dogs, there was a little growth for six days after ingestion, then a rapid growth for about ten days when sexual maturity is attained. In about three weeks, it grows to a length of about three feet and begins producing eggs.

2.3.12. NUTRITION:

Adult worms use the waste metabolic products efficiently from the host intestine and absorb the required nutrients through direct contact with the mucosa. They depend on the breakdown of glycogen for the energy release. Tegument may excrete some enzymes, which may digest proteins that are in close contact to the wormís body, and the resulting amino acids are absorbed by pinocytosis. The tissues are rich in vitamin B 12.

2.3.13. EPIDEMIOLOGY, SYMPTOMS AND PATHOGENECITY:

The infection that is acquired by eating undercooked beef, pork, or fish contaminated with Plerocercoids is called Dibothriocephaliasis or Sparganosis. In Finland, about 20% of population are infected while in some communities in the Baltic nearly 100% infection exists.

The symptoms of human infection are often absent. Sometimes, the symptoms like digestive discomfort, loss of weight, progressive weakness, abdominal pain, nervous disorder, erythropenia, hemorrhage, or enteritis may occur. The mature worms located in the proximal part of the intestine usually compete for vitamin B 12 with host, and thus the host may suffer rarely from a unique type of pernicious anemia.

2.3.14. MEDICATION (TREATMENT & CONTROL):

Some anthelmintic drugs containing a mixture of metallic tin, tin oxide and zinc chloride are given before meals for the expelling of the adult worms from human intestine. Atebrin is found to be successful when given on an empty stomach in the morning, followed in 2 to 4 hours by strong saline purge. Chloroquine seems to be little less efficient than the Atebrin.

The best preventive measure is the exclusive use of thoroughly cooked fish, beef, or pork for eating, or quick-freezing of the same at ñ 10 C for 24 hours for the destruction of the cysts. Other measures include the elimination of the reservoir hosts, prevention of flow of untreated sewage into freshwaters, prevention of dogs from eating raw refuse, and educating the general public.

2.3.15. SUMMERY:

- ⇑ A large, slender, polyzoic, fish tapeworm with its scolex bearing two slit-like suctorial bothria.
- ⇑ Adults inhabiting primarily in small intestine of humans and fish-eating domestic mammals of Northen Hemisphere.
- ⇑ Intermediate hosts are a freshwater copepod and a carnivorous fish.
- ⇑ Body is differentiated into scolex, neck and a very long strobila of 3000 ñ 4000 proglottides.
- ⇑ Body is sheathed in a tegument with a brush-like surface for the absorption of nutrients.
- ⇑ Digestive and circulatory systems are lacking.
- ⇑ Nervous system consists of a pair of cerebral ganglia and a pair of lateral longitudinal nerve cords.
- ⇑ Excretory system is protonephridia type with flame cells connected to transverse and longitudinal collecting tubules that open through a caudal excretory vesicle.
- ⇑ Adults are hermaphroditic.
- ⇑ Male system has numerous small testes, a coiled sperm duct, a seminal vesicle, and a cirrus sac ending in a genital atrium.
- ⇑ Female system has a bilobed ovary, an oviduct, an ootype, a twisted uterus, a seminal receptacle and a straight vagina opening into a common genital atrium.
- ⇑ Copulation occurs through self-fertilization.
- ⇑ Life cycle involves three hosts and larval stages like Coracidium (egg), Procercoid (copepod) and Plerocercoid (fish), which is infective to the final fish-eating definitive host.
- ⇑ Capsules are liberated through uterine pore into the hostís intestine and are deposited in water with feces.
- ⇑ Eggs hatch out to produce ciliated, free-swimming, six-hooked embryos called Coracidia.
- ⇑ In copepod, Coracidia bore through body cavity and transform into Procercoids.
- ⇑ When ingested by a fish, Procercoids penetrate the gut wall to reach muscles to become Plerocercoids.
- ⇑ Unencysted Plerocercoids multiply asexually and infect man and other mammals when they eat raw or undercooked fish and cause a disease called Sparganosis.

⇑ Plerocercoids develop into mature worms in the intestine of the definitive host and eggs appear in a few weeks.

⇑ Adults absorb required nutrients directly from the intestinal mucosa, especially vitamin B 12.

⇑ Atebrin, Chloroquine and Tin compounds are found to be effective in the treatment.

⇑ Prevention is obtained by thoroughly cooking fish before eating, educating public and treating sewage, and preventing pet animals from consuming raw refuse.

2.3.16. KEY TERMINOLOGY:

Capsule	Encapsulated embryonated egg in ootype.
Cirrus	A terminal muscular male reproductive organ used in the transfer of sperms into vagina.
Coracidium	Free-swimming or free crawling, ciliated, oncosphere of D. latum.
Cysticercus	Infective stage (bladderworm) of tapeworm in the intestine of intermediate host.
Definitive host	Principal or main host where the adult worms attain sexual maturity.
Digenetic	Life cycle is completed in two or more hosts with a number of larval stages.
Flame cells	Excretory cells whose ciliary movement simulates the flickering of a flame.
Genital atrium	A chamber receiving both male and female gonophores.
Gonoduct	Main duct involved in the transport of the gametes produced by the gonads.
Gonopore	External opening of male or female reproductive organs.
Gravid	Terminal ripe segments with a branched uterus filled with capsules.
Hermaphrodite	Having both male and female organs in the same segment or worm.
Intermediate host	Host lodging the larval stages of the parasites.
Oncosphere	Hexacanth or six-hooked, the first larval stage that hatches out from the egg.
Mehliís gland	Conspicuous unicellular gland surrounding the ootype.
Ootype	An organ of female system where the fertilized eggs receive yolk and shell material.
Plerocercous	Third larval stage of *D. latum* found in fish flesh. It is infective to final host.
Procercoid	Second larval stage of *D. latum* found in copepod.
Protonephridia	Collecting excretory tubules equipped with flame cells.
Reservoir host	A host that acts as vector and helps in storing and spreading of the parasite.
Seminal receptacle	A chamber of female reproductive system to receive and store sperms.
Seminal vesicle	A chamber of male reproductive system to collect and store the sperms.
Sparganos or **Dibothriocephaliasis**	A disease caused by *D. latum* in mammalian hosts.
Sperm duct	Main duct arises from the testes leading to gonophores.
Tegument	Tough and resistant body covering of trematodes and cestodes.
Uterus	An organ of female reproductive system to store the fertilized eggs.
Vitelline glands	Special glands secreting yolk and shell material during capsule formation.

2.3.17. SELF ASSESSMENT QUESTIONS:

1.What is Sparganosis? Discuss life history of *D. latum* and its pathogenecity.

2. Describe the life cycle of *Diphyllobthrium latum* and significance of its larval stages.

3. Give an account of structure and organization of *Dibothriocephalus latus.*

4. Write short notes on:

   a. Structure of *D. latum.*

b. Swimmerís itch.

c. Larval stages of Fish tapeworm.

d. Life cycle of Broad fish tapeworm.

e. Plercecoid larva.

f. Coracidium.

g. Onchosphere stage.

h. Labelled diagram of reproductive organs of *D. latum.*

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Unit - II

2.4. BIOLOGY, LIFE CYLCE, AND PORTALS OF ENTRY OF *ECHINOCOCCUS GRANULOSUS* (= *TAENIA ECINOCOCCOSUS).*

2.4.1. OBJECTIVES:

The purpose of the lesson is to:

- Know the habit, habitat, and the structure of the dog's tapeworm or hydatid worm.
- Study its structure and physiology
- Understand its life cycle and infective stages.
- Identify the symptoms, treatment and control measures of echinococosis

2.4.2. INTRODUCTION:

It is a minute intestinal parasite in the members of canine and feline families. It is characterized by the highest powers of asexual multiplication; lack of epidermis, mouth and digestive tract; limitations of organs of attachment to the anterior end; possession of an elongated jointed body; and occurrence of a complicated life cycle including an intermediate host. It is noted for causing the most serious infection as a hydatid cyst in various organs in man and domestic mammals. Hartmann (1965) first

discovered it in the intestine of the dog, a normal host containing the worms in hundreds to thousands, apparently with little harm.

Systematic Position:

Phylum: Platyhelminthes,
Class: Cestoda,
Order: Cyclophyllidea,
Family: Taenidae.

2.4.3. HABIT AND HABITAT:

The adults inhabit the small intestine of primary hosts like dogs, cats, wolves, foxes and other carnivores while the larval cysts infest the vital organs of secondary hosts like man, monkeys, cattle, sheep, camels, rabbits, horses, pigs, hogs, caribou and other herbivorous mammals.

Human infection with hydatid larvae have been reported from Australia, New Zealand, Tasmania, Southern America, Africa, Southern and Eastern Europe, Middle East, Siberia, China, Mongolia, Japan, India (Punjab), and U.S.A.

2.4.4. EXTERNAL CHARACTERS:

It is a tiny worm measuring 2 – 8 mm in length. The body is flat and opaque white or yellow in color. The body is divided into three regions(Fig. 2 – 14)

Scolex: It is a highly muscular, pyriform organ of attachment, which is about 30 microns in diameter. It is equipped with a retractile apical snout, the rostellum, usually armed with a double circlet of hooks at its base (28 – 50) and four moderately sized adhesive suckers. It contains nephridial canals and central part of the nervous system. It is normally embedded in the host's intestinal mucosa.

Neck: It is a narrow, unsegmented, slender region just behind the scolex. Proliferation of new proglottides occurs in the neck region.

Strobila: It consists of 3 – 4 successively larger proglottides, one immature, one or two mature, and one gravid or nearly ripe. The immature proglottid is indistinct and has a distinct organization except for rudimentary reproductive organs. The mature ones are much larger with well-developed male and female reproductive organs. The gravid segment is much broader and longer than the mature one. Only a single ripe segment occurs at any time with a branched uterus containing 500 – 800 eggs (Fig. 2 –15)

2.4.5. TEGUMENT:

The body is clothed in a transparent cuticle provided with minute spines and sensory endings. As the epidermis is lacking the cuticle rests directly on the mesenchyme. It appears to be secreted by the long-necked subcuticular cells that occur in abundance beneath the subcuticular muscle

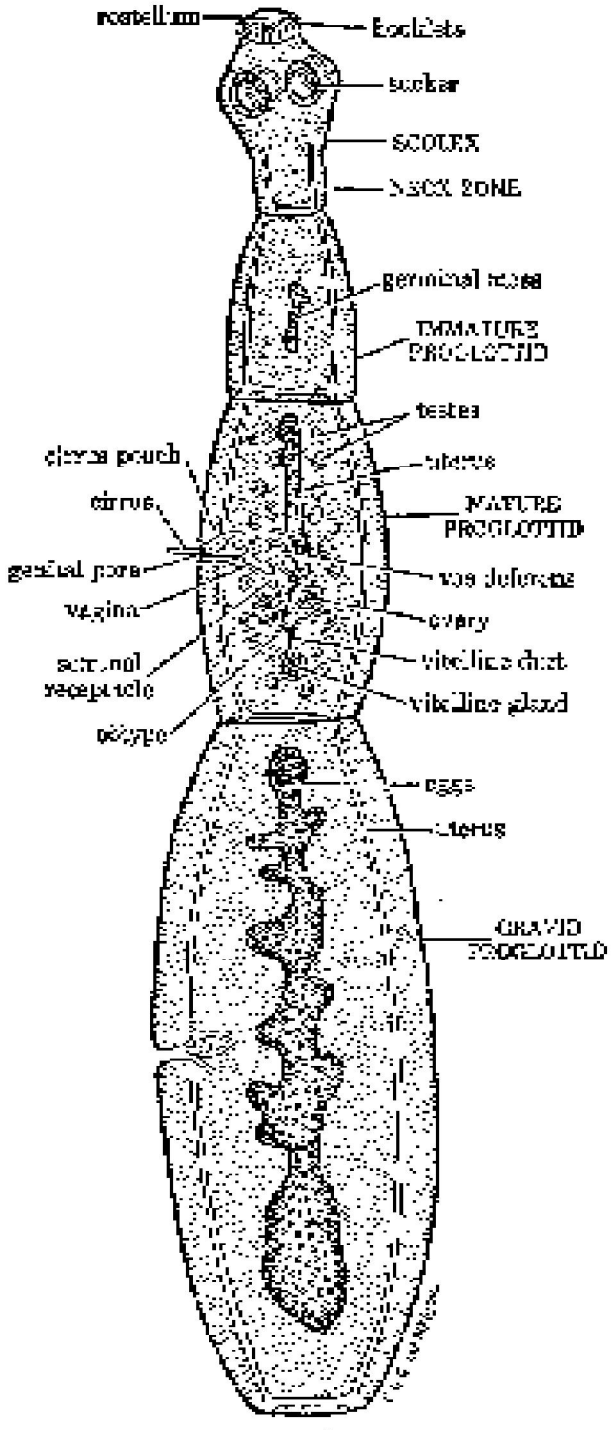

Fig. 2-15. *Echinococcus granulosus*, surface view of whole worm.

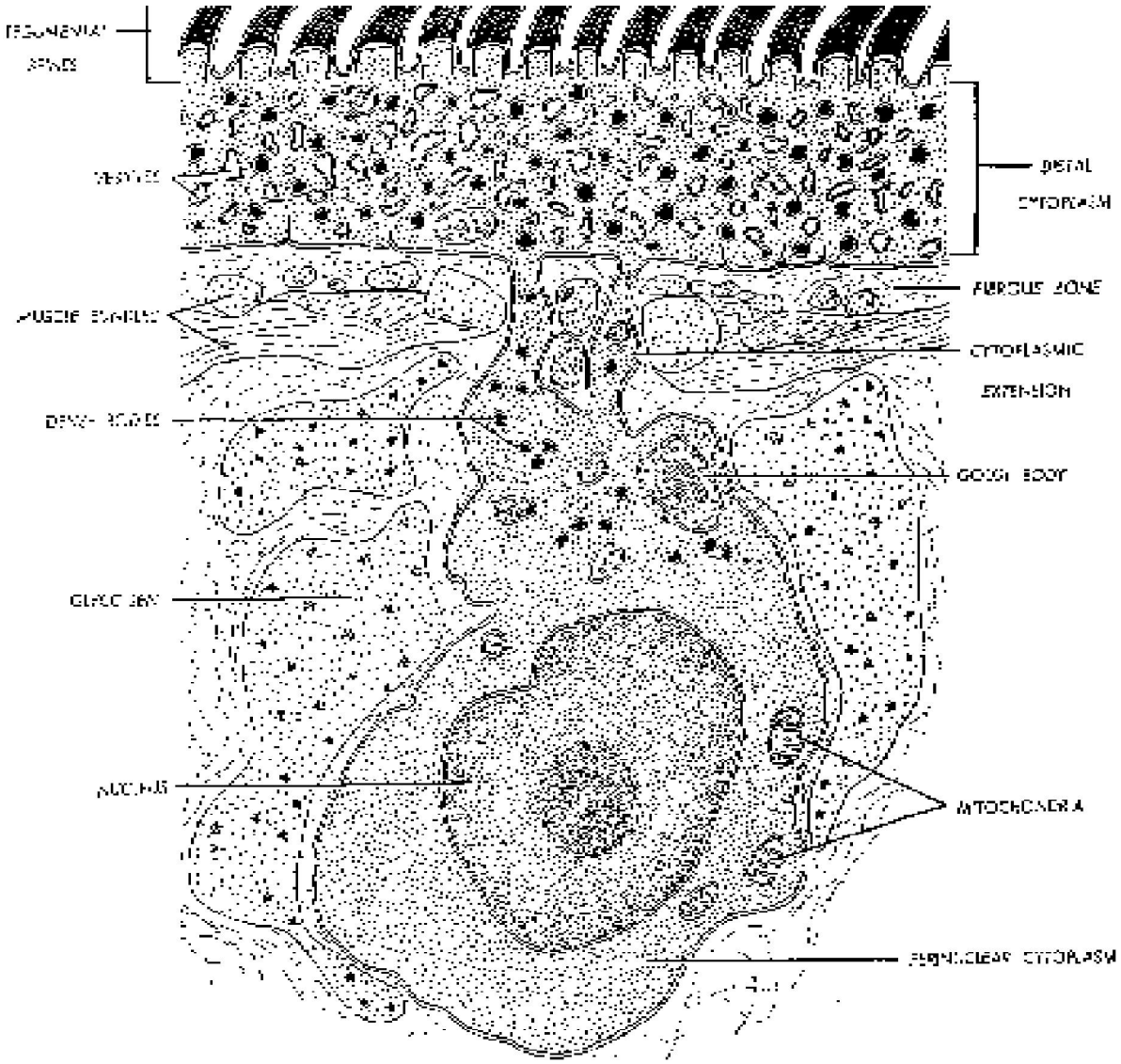

Fig. 2-16. Cellular arrangement of the tegument of *Echinococcus granulosus.*

layer (outer circular and inner longitudinal). Beneath the muscles lie strongly developed mesenchymal musculature, which comprises of dorsovertral, transverse, and longitudinal, seldom diagonal fibers. All the space between internal parts is filled with mesenchyme. (Fig. 2 – 16)

2.4.6. DIGESTIVE SYSTEM:

The digestive system is totally lacking as it absorbs the required nutrients directly from the host intestine through its tegument. The tegument also secretes proteolytic enzymes that act on host proteins and the resulting amino acids are absorbed by the tegument. This type of digestion is known as "membrane digestion". Tegument can take up dipeptides, polypeptides or even entire proteins by pinocytosis.

2.4.7. NERVOUS SYSTEM AND SENSE ORGANS:

There is a pair of lateral longitudinal nerves situated near the excretory canals and run through out the length of the strobila. In each proglottid the longitudinal cords are connected by atleast by one ring commissure situated posteriorly near the transverse excretory canal. The longitudinal cords are ganglionated nerves. The cords and commissures give out numerous branches into the proglottides. In the scolex the two main lateral cords swell into a pair of cerebral ganglia that are connected by a thick transverse commissure. From this brain complex nerves pass anteriorly and laterally to the parts of the scolex.

There are no special sense organs. The body surface and organs of scolex are richly supplied with free sensory nerve endings. There are also numerous tangoreceptors with bulbous or bristle endings situated in a subcuticular nerve plexus.

2.4.8. EXCRETORY SYSTEM:

The organs of excretion and osmoregulation are the protonephridia. They are provided with typical terminal flame bulbs strewn through out the mesenchyme. There are two collecting tubules on each side, a dorsal and a ventral. The ventral canals are larger and are connected in the posterior part of each proglottid by a transverse canal. The ventrals run through the entire strobila and terminate in the last proglottid in a median excretory bladder that opens by a pore. In each proglottid they may open to the exterior by many secondary pores. The longitudinal canals continue into the scolex where they are connected by cross branches or break up into a plexus. The flame bulbs are single cells with a conical flame.

2.4.9. REPRODUCTIVE SYSTEM:

The adults are hermaphroditic. The mature proglottid contains the fully developed bilaterally arranged reproductive systems of both the sexes. The gonads originate from the mesenchyme cells. The gonoducts open together into a common genital atrium located near the center of lateral margin.

Male organs:

The testes are small and numerous round bodies (20 - 30) strewn in the mesenchyme close to the dorsal surface and anterior to the ovary. Small fine ductules (vas efferintia) arise from testes join to form a large, coiled sperm duct (vas deferens), which terminates in an elongated highly muscular body, called cirrus sac that opens into genital atrium. Inside the cirrus sac, there is a non-eversible proximal portion consisting of the ejaculatory duct and seminal vesicle and an eversible distinct portion containing a finger-like copulatory organ lined with bristles or spines, the cirrus.

Female Organs:

A single ovary lies ventrally and posteriorly in the median line. It has two lobes connected by a narrow bridge. The yolk glands occur as numerous small follicles, the ductules of which unite to

form a vitelline duct. It runs transversely, and joins its fellow duct in the median line to form a common vitelline duct, which immediately opens into the oviduct. The oviduct also receives a straight sinuous vagina and then enlarges to form an ootype surrounded by Mehlis gland. The proximal part of the vagina enlarges to form a fusiform seminal receptacle. It harbors sperms received during copulation. Beyond ootype, the ovo-vitelline duct enlarges to form the uterus. The uterus begins to develop only after the gonads and their ducts have attained maturity. As it fills with ripe capsules, it expands into a sac with lateral diverticula. As it lacks an aperture the embryos are freed by the disintegration of the proglottid.

In ootype, the fertilized eggs become enclosed with yolk cells in a shell to form capsules. The eggs develop into embryos within the uterus while still inside the main host. The shed proglottides contain fully developed embryos. Ripe uterus has a broader central stem with lobe-like out-pocketing. Copulation by self-fertilization by eversion of the cirrus into the vagina of the same proglottid is the common method of impregnation.

2.4.10. LIFE CYCLE:

The life cycle is completed in two hosts. The larval hydatid cyst occurs in humans and in sheep, camels, deers, moose, pigs, rabbits and other herbivorous mammals, which serve as secondary hosts. The capsules develop into the onchospheres while they are in the uterus of the gravid proglottid. (Fig. 2-17).

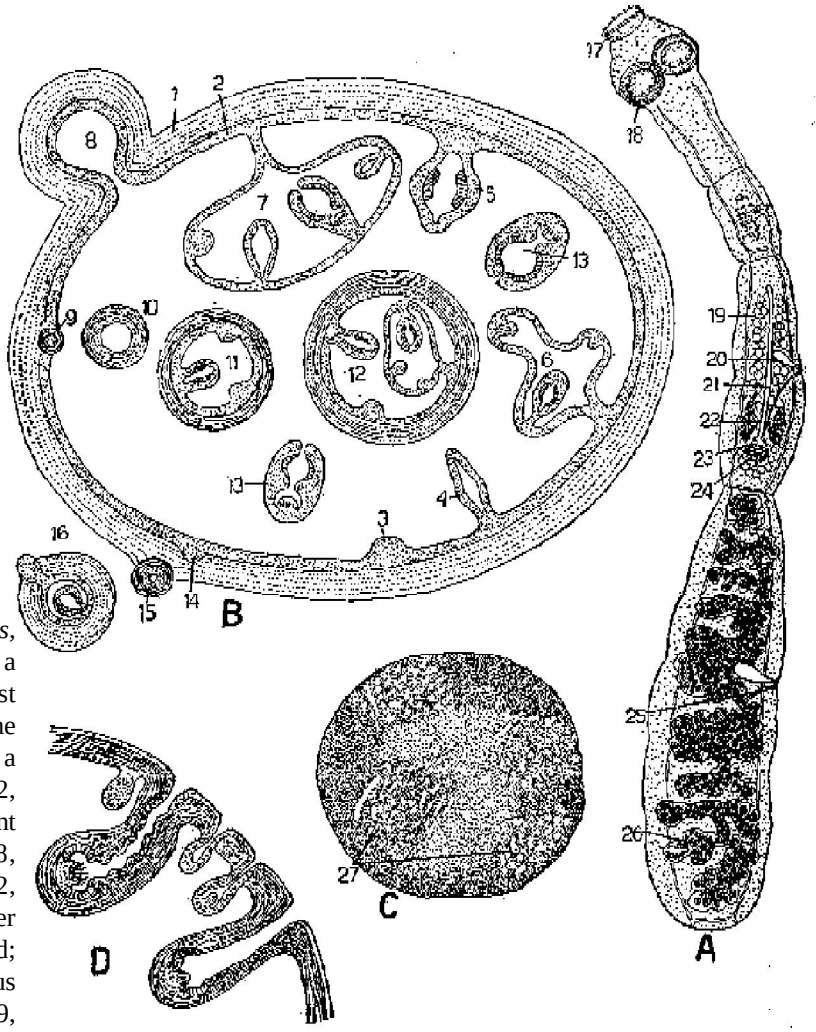

Fig. 2-17. *Echinococcus granulosus*, entire worm, from the dog B. Scheme of a hydatid cyst, original. C. A coenurus cyst showing four groups of scolices. D. Scheme of a section through a scolex group of a coenurus cyst. 1, lamellated cyst wall; 2, germinative layer; 3-7, stages in development of scolices from germinative layer; 8, constriction to form daughter cyst; 9-12, stages in budding of endogenous daughter cyst; 13, isolated scolices free in cyst fluid; 14-16, stages in budding of an exogenous daughter cyst; 17, rostellum; 18, suckers; 19, testes; 20, cirrus sac; 21, young uterus; 22, ovary; 23 shell gland; 24, yolk gland; 25, vagina; 26, ripe uterus with hexacanths; 27, group of scolices.

∅Adult∅Eggs∅Ingested by the Intermediate host (man or mammal) ∅Onchospheres hatch in intestine∅Penetrate intestine∅Hydatid cysts in various parts of body∅Ingested by definitive host (dog) ∅Adult.

2.4.10.A. PASSAGE OF EGGS FROM THE PRIMARY HOST:

As the uterus of ripe proglottid bursts, the capsules are liberated into the intestine of main host (dog) and pass out along with feces, and then gain access to an intermediate host..

2.4.10.B. DEVELOPMENT OF EGGS:

The capsule has a thin delicate shell enclosing one egg and one yolk cell. The egg cleaves totally and unequally into a mass composed of two to three macromeres, three or more mesomeres and a number of micromeres. The macromeres contain yolk. They fuse to form a syncytium that encircles the other blastomeres as the outer embryonic membrane of nutritive nature. The blastomere mass continues to cleave. The mesomeres fuse to form an inner embryonic membrane, which hardens to a thick cuticularized shell. The inner mass of micromeres becomes an Onchosphere.

2.4.10.C. DEVELOPMENT OF PARASITE IN THE INTERMEDIATE HOST:

The shell of the capsule is dissolved in the intestine of the intermediate host and releases a six-hooked larval stage, the Onchosphere. The capsule and the outer embryonic membrane are lost and the Onchosphere hatches from its membranes only after being eaten by an appropriate intermediate host. Infection to a mammalian host occurs through water or in forage. Human infection develops because of intimate association with dogs and also from contaminated fingers. Once swallowed, the eggs pass to the duodenum where they hatch out to release Onchospheres. They penetrate the intestinal wall to enter the mesenteric veins and within three hours they make their way to final place of development in various organs like lungs, liver, spleen, kidneys, heart, brain, eye, muscles or even bones. Here the Oncosphere enlarges and elongates and transforms into a fluid-filled spherical Cysticercus or Bladder stage. The host immediately forms a fibrous envelope (pericyst) around this bladder to make it a Hydatid cyst. The cyst wall consists of an outer cuticle (ectocyst) and an inner mesenchymal layer (endocyst). The hydatid grows very slowly over a period of 5 – 20 years and reaches a size of an orange (10cm) or more. In humans, about 60 % – 75% of the cysts develop in the liver and about 20% in the lungs and rest in other organs.

2.4.10.D. EMERGENCE OF INFECTIVE STAGE (HYDATID CYST):

The hydatid cyst is the infective stage to the main host (Fig. 2 – 18). At the end of one month the cyst measures about 1 mm and in five months it grows to about 10 mm. The cavity is filled with a sterile fluid known as hydatid fluid. It has a complex structure and produces many daughter and granddaughter cysts either inside by endogenous budding or outside by exogenous budding. The internal cysts fell into the fluid cavity of the mother cyst while the external cysts detach from the mother cyst and migrate to other parts of the body to settle as secondaries. The daughter cysts originate

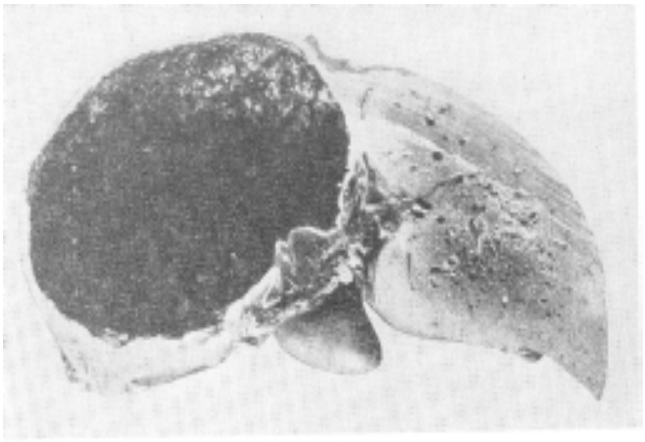

Fig. 2-18. Hydatid cyst. Space formed in human liver resulting from development of an unilocular hydatid cyst.

as invaginations on the wall of the mother cyst and the granddaughter cysts as invaginations on the walls of the daughters. The daughter cysts have only two layers, an inner germinative and an outer fibrous.

The inner germinative layer of the cyst proliferates continuously to produce numerous hollow spherical vesicles called the brood capsules. With age, more and more brood capsules are formed. In the older brood capsules, the inner wall may give rise to a large number of scolices that protrude into the cystic spaces. A fully developed cyst may contain about 15 liters of fluid and millions of scolices, each of which develops into a worm when a definite host ingests the intermediate host. The multiple scolices present a spiny and berry-like appearance, hence the generic name (Gr., echinos, spiny; coccus, berry) of the parasite. The inverted scolices develop and turn right side out, and some fall into brood chamber.

In older cysts, minute daughter and grand daughter cysts and free scolices are found as "hydatid sand" in the fluid. The spherical fluid-filled cysts are called unilocular cysts, which cause great damage to the host. Sometimes, the cyst turns into an irregular, spongy mass devoid of internal fluid, which spreads and grows like a tumor, called the alveolar cyst. It is sterile and undergoes degeneration and calcification. A cyst that is found in the bones is called the osseous cyst. The initial exposure to the parasite normally produces a slowly growing unilocular cyst in liver or lungs while a subsequent infection produces a rapidly growing tumor-like cyst. However, the occurrence of cysts in brain or eye is invariably fatal. In man, the formation of cyst represents the end of lifeline for the parasite. In case of herbivorous mammals, the predatory main hosts may feed up on the viscera of the infected herbivores and the cysts may be ingested.

2.4.10.E. ENTRY OF PARASITE INTO PRIMARY HOST:

The final host gets infected when it eats the carcasses of intermediate host containing hydatid cysts. The scolices in the cyst can differentiate in two directions. If the cyst is ruptured in intermediate host, they leak out into adjacent tissues and each may finally differentiates into another vesicular cyst (secondary hydatidosis) that gives rise to additional scolices. If the final host ingests the

cyst, the scolices emerge out, evaginate, and become attached to the host's intestinal wall to proliferate proglottides.

2.4.10.F. DEVELOPMENT TO ADULT IN FINAL HOST:

Upon reaching the intestine of a new host, each scolex develops an adult strobilated worm. During development, the following stages of maturation have been established .

⇑ Stage 0 - Undeveloped Metacestode: A stage represented on 0^{th} day by an evaginated, but an underdeveloped proscolex.

⇑ Stage 1 – Proglottidization: This stage is represented on 14^{th} day by the appearance of the first proglottid.

⇑ Stage 2 – Appearance of Second Proglottid: This stage appears on 18^{th} day and is defined by the appearance of second Inter-segmental partition.

⇑ Stage 3 - Early Gametogony: This stage appears on 22^{nd} day and is characterized by the appearance of testes in the posterior most proglottid.

⇑ Stage 4 – Genital Pore Formation: This stage occurs on 24^{th} day and is characterized by the appearance of the lateral genital pore.

⇑ Stage 5 – Later Gametogony: This stage occurs on 26^{th} day and is characterized by the maturation of both male and female reproductive systems.

⇑ Stage 6 – Uterus Dilation: This stage appears on 28^{th} day and is characterized by the appearance of the uterus as a hollow thin-walled sac in the center of the maturing proglottid.

⇑ Stage 7 – Oncosphere Formation: This stage occurs on 40^{th} day and is characterized by the appearance of embryonated eggs in the uterus, carrying fully developed hexacanth larvae.

2.4.11. NUTRITION:

Worm obtains its nutrition by direct absorption of the split products of host digestion through its body surface. Though provided with proteolytic enzymes, it does not emit any enzyme into the surroundings. The scolices that are deeply embedded in the intestine wall may absorb some of the host tissue fluids. Lack of carbohydrates in host body was very deleterious and may result in stunted growth in worms and they may also fail to establish in the gut. The main respiratory metabolism is anoxybiotic and operates regardless of the presence or absence of oxygen in the medium. The end products are carbon dioxide and organic acids. Deficiency of vitamin B 12 affects adversely the growth, maintenance and production of hexacanth. Food reserves are chiefly of glycogen and to a lesser extent of various lipoid substances.

2.4.12. EPIDEMIOLOGY, SYMPTOMS AND PATHOGENESIS:

The parasite has both sylvatic and a pastoral epidemiology. The pastoral form of infestation is best known in area where it is passed back and forth between dogs and sheep, cattle, or man. The sylvatic form occurs in circumpolar areas where wolves or foxes and moose or caribou are principally involved. Blowflies can carry eggs from excreta to food. The infection of humans results from too great familiarity with dogs and is common in certain parts of the world, particularly Iceland, Australia, parts of Africa, and southern parts of South America.

The disease produced in the humans by presence of the cysts is called Echinococcosis, Hydatidosis or Hydatid disease. The eggs and proglottides often remain attached to lips of the dogs because of their unsanitary habit of licking their posterior ends. Infection may also occur via contaminated food and drink. The scolices are resistant to gastric juice but are digested by the intestinal juice of various mammals except their natural hosts. Although the adult worms do not live long, the cysts may remain alive for many years. The cysts are harmful when they develop rapidly. Large and migrating cysts can be more serious. Inflammatory reactions in the surrounding tissues of the host result in the development of fibrous tissue.

The osseous tissue is weakened and the liver may be enlarged. The normal functioning of organs may be impaired. Through pressure on or disturbance in vital organs like kidney, spleen, brain or eye, the cyst may produce grave symptoms terminating in death. In 25% of human cases more than one cyst is present and it may be due to original multiple infection or due to the development of detached daughter cysts. In natural wild hosts the cysts may be practically harmless. They do serious harm only when grow to outrageous size in the liver and press on other organs. The rupture of cysts causes allergic symptoms as the bleeding Hydatid fluid contains toxins that may result in vomiting, toxemia, diarrhea, abdominal pain, eosinophilia, capillary obstructions, and sometimes collapse, due to anaphylactic shock. The cysts are diagnosed only by autopsy.

2.4.13. MEDICATION (TREATMENT AND CONTROL):

Surgical excision of the cyst is the only effective treatment. But in many cases even this procedure is often unsuccessful or impracticable as the disease recurs because of the contents of the cyst may escape during operation. It is customary to withdraw part of the fluid from the cyst and replace it with formalin solution, which kills scolices, brood capsules and secondary cysts. Oral application of Arecolin hydrobromide eliminates 95% of worms from dog's intestine. Three doses usually terminate all.

The disease can be prevented by:

- ⇑ Keeping the dogs free from infection by avoiding the accidental ingestion of eggs.
- ⇑ Avoiding too much human intimacy (kissing, licking or playing) with them.
- ⇑ Careful washing of hands after handling them.

- ⇑ Cleaning of the dishes from which they have eaten.
- ⇑ Avoiding food or water that may have been contaminated by them.
- ⇑ Preventing them from eating raw meat, viscera or carcasses of herbivorous mammals.

2.4.14. SUMMARY:

- ⇑ Dwarf tapeworm of dog, which has the highest power of asexual multiplication.
- ⇑ Primarily parasitizes the members of canine and feline families.
- ⇑ Noted for its occurrence as hydatid cyst in man and domestic herbivorous mammals.
- ⇑ The body of *E. granulosis* is distinguished into scolex, neck and strobila.
- ⇑ Scolex has four suckers and a retractile rostellum with a double row of hooks at its base.
- ⇑ Neck is a slender and unsegmented region of proliferation.
- ⇑ Strobila consists of 3 to 4 proglottides: 1 immature, 1 or 2 mature and a terminal ripe proglottid.
- ⇑ Tegument is a syncytial cuticular surface with minute spines and sensory papillae. It helps in "membrane digestion" and nutrient absorption from the host-intestine.
- ⇑ Digestive and circulatory systems are totally absent.
- ⇑ Nervous system has a pair of lateral longitudinal nerves and additional pairs of lateral, dorsal and ventral longitudinal cords connected to a pair of cerebral ganglia and transverse commissure in scolex.
- ⇑ Sense organs act as tangoreceptors.
- ⇑ Excretory system consists of a pair of protonephredial collecting tubules on each side equipped with flame bulbs. At one end they continue into scolex and on the other end open into a median bladder in the terminal segment.
- ⇑ Hermaphroditic with both male and female organs developing in each mature proglottid.
- ⇑ Male consists of a large number of testes, vas efferentia, and a coiled sperm duct leading to a genital chamber through a cirrus sac.
- ⇑ Female consists of two compact ovaries, vitelline glands, oviduct, ootype, vagina, seminal receptacle and genital atrium. Ovo-vitelline duct enlarges to form uterus.
- ⇑ Life cycle is completed in two hosts.
- ⇑ Embryonated eggs liberate into the intestine of main host and pass out along with feces and gain access to intermediate host.
- ⇑ Shells dissolve to release oncospheres, which enter host's blood circulation and make their way to various organs to develop into hydatid cysts.
- ⇑ Cyst produces asexually numerous daughter cysts, grand daughter cysts, brood capsules and scolices.
- ⇑ When a final host ingests infected brood capsule, each scolex evaginates and develops into an adult worm.
- ⇑ Upon infection, humans develop a disease called Echinococcosis or Hydatid cyst disease.
- ⇑ Infection occurs either through physical contact with dogs or food contaminated by their excreta.
- ⇑ Cyst may grow in liver or other organ for years together without being detected.

⇑ Liberation of hydatid fluid can cause severe allergic symptoms.
⇑ Treatment is purely surgical. Injection of formaline can also kill cysts.
⇑ Oral application of Arecolin hydrobromide eliminates worms in dog's intestine.
⇑ Prevention rests mainly in keeping pet dogs free from infection and in avoiding too much human intimacy with them.

2.4.15. KEY TERMINOLOGY:

Alveolar cyst An irregular, spongy, sterile, tumor-like cyst devoid of internal fluid.
Capsule Encapsulated embryonated egg formed in ootype.
Cyst A parasitic stage surrounded by a resistant wall.
Cysticercus or Bladder worm Infective stage of tapeworms in the intermediate host.
Definitive host Principal or main host where the adult worms attain sexual maturity.
Echinococcosis Hydatidosis disease caused in humans by *Echinococcus granulosus*.
Endoparasite A parasite that lives within its host.
Epidemic A disease that affects a large number of people and spreads rapidly.
Epidemiology Study of epidemics.
Flame cells Excretory cells whose ciliary movement simulates the flickering of a flame.
Genital atrium A chamber receiving both male and female gonopores.
Gonoduct Main duct involved in the transport of the gametes produced by the gonads.
Gonopore External opening of male or female reproductive organs.
Habit Specific place where a parasite lives.
Host An organism that harbors a parasite.
Hydatid cyst An infective cystic stage of Echinococcus in vital organs of humans.
Infection Entrance and establishment of a parasite within a host.
Intermediate host Host lodging the larval stages of the parasites.
Mehli's gland Conspicuous unicellular gland surrounding the ootype.
Onchosphere A 6 – hooked larval stage that hatches out from the eggs of tapeworms.
Ootype An organ of female system where the fertilized eggs receive yolk and shell material.
Parasite An organism that depends on its host for some essential metabolite.
Pathogenic The microbe or protozoan or metazoan organism which causes a disease.
Protonephridia Collecting excretory tubules equipped with flame cells.
Reservoir host A host that acts as vector and helps in storing and spreading of the parasite.
Seminal receptacle A chamber of female reproductive system to receive and store sperms.
Seminal vesicle A chamber of male reproductive system to collect and store the sperms.
Sperm duct Main duct arises from the testes leading to gonophores.
Tegument Tough and resistant body covering of trematodes and cestodes.
Unilocular cyst A hollow, spherical infective cyst in the organs of that contains cystic fluid.
Uterus An organ of female reproductive system to store the fertilized eggs.
Vitelline glands Special glands secreting yolk and shell material during capsule formation

2.4.16. SELF ASSESSMENT QUESTIONS:

1. Describe the structure and life cycle of *Echinococcus granulosus.*
2. Discuss the life history of dog's tapeworm and add a note on its economic importance.
3. Give an account of structure and pathogenecity of hydatid cyst.
4. Write short notes on:
   a. Hydatid cyst
   b. Echinococcosis
   c. Dog's tapeworm
   d. Unilocular cyst
   e. Cysticercus larva
   f. Oncosphere
   g. Labelled diagram of unilocular cyst
   h. Labelled diagram of *Echinococcus*

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UNIT – III

3.1 BIOLOGY, LIFE CYCLE AND NATURE OF INFECTION OF *WUCHERERIA BANCROFTI*

3.1.1 OBJECTIVES

The purpose of this lesson is for:

- studying the life cycle of *Wuchereria bancrofti*
- knowing the source of infection
- understanding the pathogenecity of the disease
- knowing prevention and the therapeutic measures of *Wuchereria* infection

3.1.2 INTRODUCTION

Wuchereria bancrofti is commonly called as human filarial worm. It is world wide in distribution. It is very common in tropical countries. In Asian countries, it is reported in coastal areas of India,

China, Japan, Burma, Malaya, Arabia etc.

Wuchereria bancrofti Systematic position

Phylum - Nemathelminthes

Class - Phasmida

Order - Filaroidea

Genus - *Wuchereria*

Species - *bancrofti*

3.1.3 FILARIASIS

Wuchereria bancrofti is also called as *Filer bancrofti*. It causes elephantiasis or wuchereriasis or Bancroftis filariasis. Filarial worms are long, thin tapering worms. Lips are absent around the mouth. The life cycle involves a blood-sucking insect. Adult parasites are Filarial worms. The female Filarial worms produce microfilariae.

Adult Filarial worm lives in the lymph glands or vessels of man. Its larvae live in blood. Hence it is an intercellular endoparasite of man. The Filarial worm is a dreadful human nematode parasite.

3.1.4 DESCRIPTION OF THE PARASITE

Sexes are separate. It exhibits sexual dimorphism. Both the male and female worms live together in highly coiled state. Both of the worms have a long, slender thread like body (Fig. 3-1).

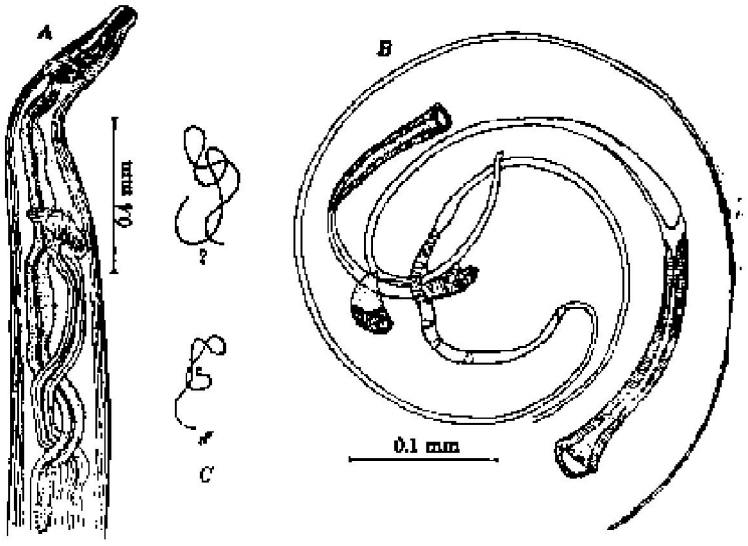

Fig. 3-1. *Wuchereria bancrofti* A. anterior end of female B. Posterior end of Male C. Adult female and male natural size

3.1.5 EXTERNAL AND INTERNAL CHARACTERS

The male and female worms live coiled in the lymph glands and lymph passages of man. The male is smaller than female. The anterior end of the worm is swollen and the posterior end is pointed. Lips are absent around the mouth.

3.1.6 DIGESTIVE SYSTEM

In both male and female the head end is slightly enlarged. The mouth is a simple opening at the head end. The mouth leads into a buccal cavity and then to oesophagus. The oesophagus is simple without bulges and constrictions. The oesophagus is non-glandular and non-muscular.

3.1.7 REPRODUCTIVE SYSTEM

Male worm is 40mm long and 0.1mm in diameter. The posterior end of the male is coiled like a tendril with a pair of copulatory spicules at the cloacal opening. The spicules are unequal in length. A gubernaculum is present.

Female worm is 65 to 100mm long and 0.25mm in diameter. The vulva is situated behind the mouth ventrally. Tail is narrow and pointed. The number of females is more than males.

3.1.8 ADULT WORMS IN MAN

Usually males and females are found in lymphatic vessels/ducts. After copulation, the adult female produces sheathed larvae called microfilariae. These are found in peripheral blood. The Filarial worms are ovoviviparous.

3.1.9 MICROFILARIAE

These are found in the peripheral blood vessels. They gain entry to the peripheral blood vessels by passing from the lymphatic system to the vascular system. Each microfilaria is 200m to 300m in length and 5 to 7m in diameter. It has a long slender body enveloped by a hyaline sheath extending beyond the anterior and posterior ends (Figure 3 –2). Internally there is a stylet, nerve ring and rudiments of adult organs. The fully developed microfilariae stay in the blood vascular system. These microfilariae cannot undergo further development in the human body. They develop further only when they are sucked with blood by the intermediate host (mosquito). If the mosquito does not suck them they die. The life span of microfilaria is about 70 days.

Microfilariae live in deep seated blood vessels during day time but migrate into the peripheral blood vessels at night (between 10p.m and 4a.m) to be sucked by the mosquito. This peculiar behaviour of larvae associated with the nocturnal feeding habit of mosquitoes is called as nocturnal periodicity.

Microfilariae occur as two biologically different forms. In oriental countries like India and China, microfilariae show nocturnal periodicity. Night-biting mosquitoes, Culex fatigans, transmit them.

In Pacific islands, microfilariae show diurnal periodicity and they are transmitted by day biting mosquitoes, *Aedes polynesiensis.*

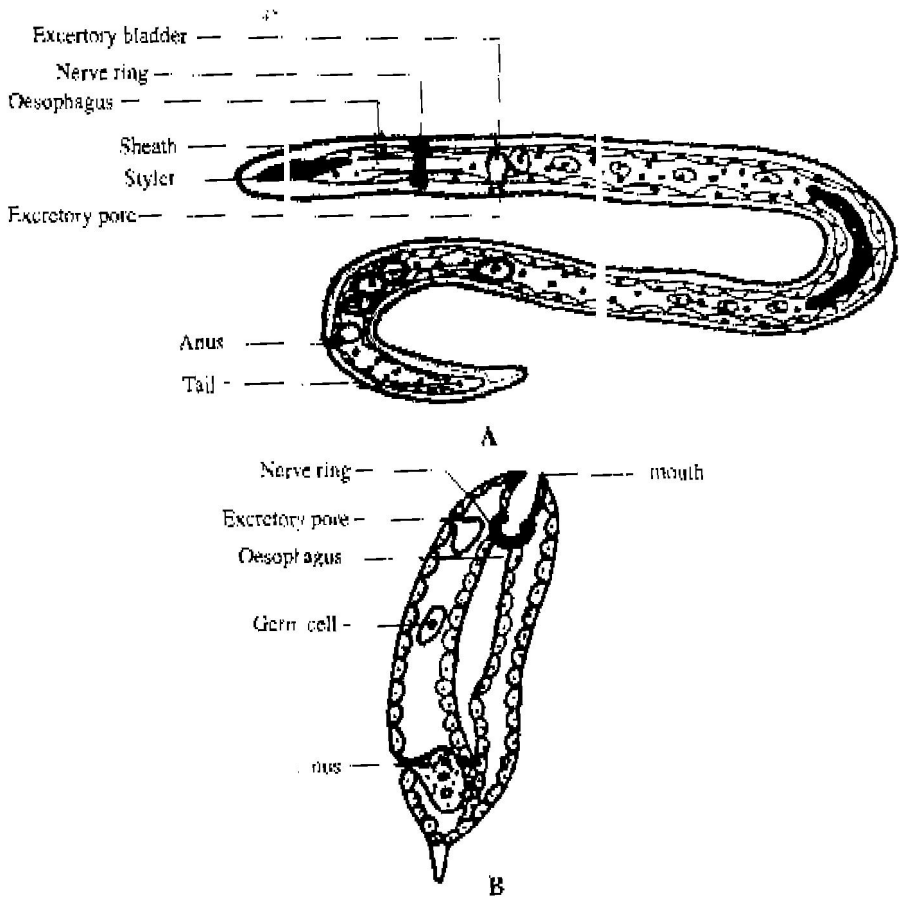

Fig. 3-2. *W. bancrofti* : Larvae A) Micro filarial larva; B) Sausage shaped larva.

3.1.10 LIFE CYCLE

W. bancrofti completes its life cycle within two hosts. Man is the definitive host harboring the adult worms. The intermediate host is a mosquito – *Culex fatigans*, or *Aedes egyptii* or even some anopheles mosquitoes.

3.1.10.A IN FEMALE CULEX

When the female culex mosquito sucks the blood of an infected person, microfilariae enter into the stomach with blood. After entering the stomach, a cycle of maturation commences, and finally, infective microfilariae are found near the mouth parts of the insect (proboscis). During this maturation process, the larvae shed their envelope, penetrate the intestinal wall and enter the thoracic muscles.

The microfilariae transform into short, thick sausage shaped larvae. These are first stage larvae. Soon they undergo one molt, develop gut and begin to grow in length and width. These second stage larvae again undergo one molt to transform into long, slender infective larvae. Infective larva measures 1.5mm in length and 20m in diameter.

The infective microfilariae leave the thoracic muscles, move to the head to reach proboscis.

3.1.10.B IN MAN

When an infected mosquito bites a man, the infective microfilariae are usually deposited on the skin. They stick to the skin and by penetrating the skin, they enter the blood vascular system. The microfilariae are not directly injected into the blood stream as in malaria.

From the skin surface, they burrow into the lymphatic tissue, reaching the lymph nodes. Here, they undergo 2 more molts to transform into adults.

3.1.11 CLINICAL FEATURES

The disease caused by adult Filarial worm is called as filariasis or elephantiasis. The adult worms excite a marked inflammatory response and produce several deleterious effects. These abnormal effects are lymphangitis and lymphatic obstruction.

Lymphangitis is an inflammation of the lymphatics and is produced by mechanical irritation of the worms and other products of the worm. Adults, which live in lymphatic tissue, cause obstruction for the free flow of lymph. Hence the lymphatic vessels become swollen leading to the thickening of lymph glands and consequent fibrosis. Lymphatic obstruction is eventually produced by fibrosis of the lymphatics. This has serious consequences since the lymph flow from the affected part is stopped. This leads to a swelling part and is known as elephantiasis. Lymphatic obstruction occurs in any part of the body producing symptoms. Microfilariae produce no symptoms. Usually scrotal sacs, breasts, legs and arms are affected by filariasis. Obstruction of the lymphatics draining the sac of testis produces hydrocele. Similarly rupture of distended lymphoid varices into the urinary tract produces chyluria.

In the early stage of infection, the obstructed lymph vessels burst releasing toxins and lymph into tissue spaces, scrotal sacs, kidneys, peritoneum etc. As a result the effected organs show swellings. The presence of parasite in blood and early stages of infection cause fever in man.

Obstruction of lymph in scrotal sacs, limbs, breast etc. cause swelling. Fibrous tissue in them grows enormously and the skin becomes hard due to the degeneration of sweat glands. This enormous swelling leads to form a tumor-like solidity. Inflammation may be due to irritation or allergen or toxin liberated by the living or dead worms.

3.1.12 LABORATORY DIAGNOSIS

Microfilariae can be seen as actively motile organisms in “wet” coverslip preparations of patient’s night blood.

Microfilariae can also be seen in stained blood smears.

Biopsy of lymph node is a suspected case may reveal the presence of adult worms.

The peripheral blood also shows abnormal number of eosinophils (eosinophilia).

3.1.13 PREVENTION

The infection can be prevented:

By avoiding mosquito bites

Control of breeding mosquitoes

By killing vectors, their eggs, larvae and pupae.

By using mosquito nets or mosquito repellents during sleeping.
Treatment of carriers by using heterozoan and cyanine.

3.1.14 TREATMENT

No proper medicine is available to expel/destroy the Filarial worms. However, using antifilarial drugs can treat filariasis.

1. Heterazan compound (Diethyl carbamide) can be used to destroy microfilariae
2. MSb (Paramelaminyl phenyl stibonate) is effective on microfilariae and adult worms.

3.1.15 SUMMARY

W. bancrofti is the human Filarial worm.

This Filarial worm is elongated, slender and likes a tendril. Adults live in the lymphatic vessels of man.

Sexes are separate.

Both the male and female worms live together in coiled state.

Females are ovoviviparous.

The larvae produced by the females are called as microfilariae.

Each microfilaria is enclosed in a protective sheath.

Microfilaria is the infective stage to the intermediate host, *Culex pipiens, C. fatigens* and day biting mosquitoes, *Aedes polynesiensis.*

Microfilariae *live* in deep-seated blood vessels during daytime and migrate to peripheral blood vessels during night. This phenomenon is called as nocturnal periodicity.

The disease caused by adults is called as filariasis or elephantiasis. The thickening of lymph glands and the swelling of lymphatic vessels lead to elephantiasis.

Elephantiasis is usually found in scrotum, legs and hands.
Microfilaria can be treated with Heterazan.

3.1.16 KEY TERMINOLOGY

Indirect development: Having a larval stage in the course of development

Oviparous: Egg laying.

Ovoviviparous: Having development occurs within female reproductive tract but nutrition of embryo is derived from yolk or egg. Young are released at birth.

Viviparous: Having development occurs within female reproductive tract but nutrition of embryo is provided directly by mother rather than by yolk of egg. Young are released at birth.

3.1.17 SELF ASSESSMENT QUESTIONS

1. Describe the life cycle of *Wuchereria bancrofti.*
2. Give an account of the life cycle, pathogenicity and control of *Wuchereria bancrofti* infection.
3. Write short notes on:
   i) Bancroftiasis ii) Microfilariae

3.1.18 REFERENCE BOOKS

Barnes R.D. 1980: ***Invertebrate Zoology*.** III Edition, W.B. Saunders Co. Philadelphia

Chandler, A.C. and Read, C.P. 1960: ***Introduction to Parasitology*.** John Wiley and Sons. Inc. U.S.A.

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Dr. P. Padmavathi

UNIT – III

3.2 BIOLOGY, LIFE CYCLE AND NATURE OF INFECTION OF *ANCYLOSTOMA DUODENALE*

3.2.1 OBJECTIVES

The purpose of this lesson is to:

know about the distribution of the parasite, *A. duodenale*
understand the life cycle of hookworm
know about the mode of infection
know about the control methods of ancylostomiasis

3.2.2 INTRODUCTION

The two important hookworms of man are *Ancylostoma duodenale* and *Necator americanus. A. duodenale* is popularly known as the oriental species (Old World hookworm) and *N. americanus* as American hookworm. *A. duodenale* belongs to the super family Ancylostomatoidea. A bucal capsule having ventral teeth or cutting plates characterizes both the male and female worms. The anterior end of the male and female worms is bent dorsally (backward) in the form of a hook, hence the name "hookworm". The males have copulatory bursa. These hookworms are found in the small intestine of millions of people. The female worms are longer than the males.

Ancylostoma duodenale systematic position

Phylum - Nemathelminthes
Class - Phasmida
Order - Strongyloidea

Genus - *Ancylostoma*
Species - *duodenale*

A. duodenale is cosmopolitan in distribution. It is predominant in Europe, North America, West Asia, North China and Japan. In India it is found in some parts of South India and Maharashtra.

3.2.3 HABIT AND HABITAT

Adult hookworms live in the small intestine of man. The hookworm feeds on blood and tissue fluids of host. It is a voracious blood sucker. It is an intestinal nematode of man. It is predominant in humans. Rarely, it is reported in pigs.

3.2.4 EXTERNAL STRUCTURE

It exhibits sexual dimorphism. Mature worms are cylindrical in shape. In both males and females the narrow anterior end is slightly bent or curved dorsally. It is due to the curved anterior end-appearing like a hook, the name 'hookworm' is derived. The body of the worm is slightly curved; in this worm the hook is in the same direction of the body curvature. In both males and females mouth is present at the anterior end. Mouth possesses a large cup shaped bucal capsule having a pair of chitinous plates. These plates have 2 pairs of ventral and one pair of dorsal teeth. The teeth are useful for piercing the tissues of the host (Figure 3-3).

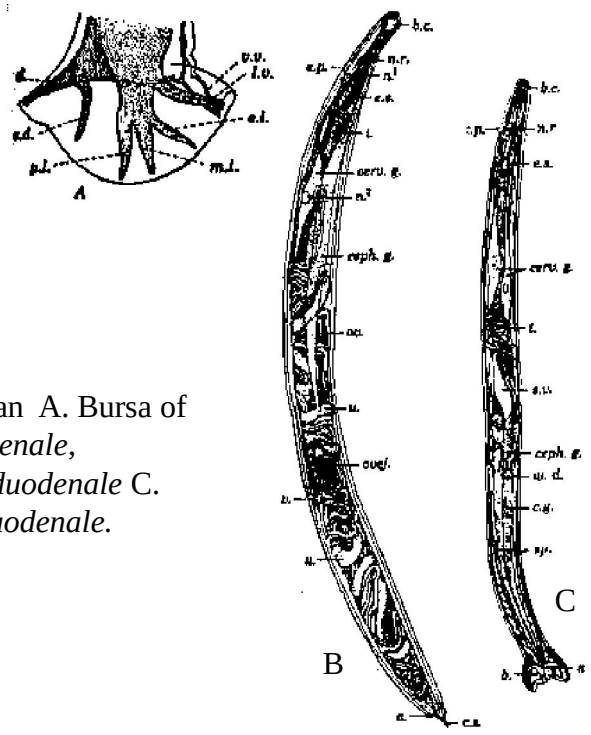

Fig. 3-3. Hookworms of man A. Bursa of *Ancylostoma duodenale*, B. Female *Ancylostoma duodenale* C. Male *Ancylostoma duodenale.*

a. anus; b. bursa; b.c. buccal capsule; ceph.g cephalic gland; cere.g cervical gland; c.g. cement glands; c.p. cervical papilla; c.s. caudal spine; e.p. excretory pore; ej.d. ejaculatory duct; es. esophagus; i. intestine; n^1. nucleus of cephalic gland; n^2. nucleus of cervical gland; n.r. nerve ring; ov. ovary; ovej. ovejector; sp. spicules; s.v. seminal vesicle; t. testis; u. uteres; v. vulva.

Male worm is 8 to 11mm in length and 0.4 to 0.5mm in diameter. The posterior end of the male possesses an umbrella like cuticular expansion called copulatory bursa. As the name suggests, it is used in copulation. The bursa is supported by muscular rays, which are of taxonomic importance for identification of different species of hookworms. The dorsal wall of the cloaca thickens to form a chitinous gubernaculum. It guides the penial or copulatory spicules in copulation.

Females are longer than females. Female worm is 10 – 13mm in length and 0.6mm in diameter. The tail of female is terminating in a minute, straight, blunt caudal point. Vulva is situated ventrally. Eggs are ovoid and thin shelled.

3.2.5 LIFE CYCLE

Man is the only definitive host. There is no intermediate host in the life cycle of *A. duodenale*. The life cycle starts with a fertilized egg. Copulation occurs between male and female worms in intestine. The female worm releases about 10,000 fertilized eggs per day into the intestine. Then they are sent out along with feces of the host. The eggs are 4 celled, which is an identifying character for a hookworm egg in clinical tests.

3.2.5.A THE FREE LIVING PHASE IN DEVELOPMENT

Under favorable conditions, the eggs hatch (within 24 hours) into 1st stage rhabditiform larvae. These are active, feeding larvae with well developed oesophageal bulb. The rhabditiform (L_1) larva feed on fecal matter and organic debris of the soil and undergoes I molt and transforms into 2nd stage rhabditiform larva (L_2). Again, this larva feeds, molts and transforms into 3rd stage, infective, filariform larva (L_3). It is active and nonfeeding larva. The filariform larva remains viable and infective for several weeks under favourable conditions. It shows a long oesophagus without bulb and a small genital primordia.

3.2.5.B ROUTE OF INFECTION INTO THE HUMAN HOST

Normally, the infective, filariform larva actively penetrates the human skin. When human's sole or palm comes in contact with infected soil, these larvae penetrate the skin. Infection also occurs through contaminated food or water by swallowing them. In such cases, larvae develop into adults in the intestine itself.

The larvae, which penetrate the skin, reach the heart and lungs via blood circulation. Finally, they enter the small intestine, undergo molting twice and develop into adults. They transform into adults within 6 weeks after infection. Adult worms live in the intestine for more than 5 years.

3.2.6 PATHOGENECITY

The disease caused by *Ancylostoma* is called as ancylostomiasis or hookworm disease. The penetrating infective larvae cause itching and inflammation of the skin, which is referred as ground, itch.

During migration, infective larvae cause hemorrhages and bronchial pneumonia.

Adult worms of the intestine cause anemia, diarrhea, eosinophilia, loss of appetite, fever, abdominal pain, constipation and loss of health.

Adults cause acute anaemia due to the sucking of blood. Hence, hookworms are also referred as voracious blood suckers.

3.2.7 TREATMENT

Many drugs are available to treat ancylostomiasis. Tetrachloroethylene can be used to treat this disease. This drug is commonly used because of its low toxicity and high efficiency. Other antihelminthic drugs, which can be used to cure ancylostomiasis, are Hexylresorcinol, thymol, dithioazamine iodide, and piperazine salt, Thioabendazole. But these drugs can be used only under strict supervision of a physician. Giving the food supplemented with iron can compensate the hemoglobin deficiency of a patient.

Personal hygiene should be maintained.
Contamination of soil or water by infective eggs or larvae should be prevented.
Pollution of soil by infected feces should be eliminated.
By educating the people not to walk in soil with bare foot.

3.2.8 SUMMARY

A. duodenale is the Old World hookworm.

It is one of the voracious blood sucking nematode parasite of man.

It exhibits sexual dimorphism. Both the males and females possess an anterior curved bucal capsule.

The posterior end of the male terminates into an umbrella shaped copulatory bursa, which is an identifying character of male worm.

The posterior end of the female ends into a straight, caudal spine.

It is digenetic as it has more than one development stage.

Fertilized, 4 celled eggs come out through feces.

It has a feeding, free living, 1st larval stage, and the rhabditiform larva. It shows well developed oesophageal bulb.

Active, non feeding 3rd stage larva is the filariform larva. It is the infective stage. It shows oesophagus without bulb and little genital primordia.

Hookworm infection occurs through contaminated soil, food or water; or by larval penetration of skin. This usually takes place in the skin of the foot, while walking on infected soil. The danger of hookworm infection is enhanced if most people walk around bare foot.

The larval forms may produce ancylostoma dermatitis while penetrating through the skin.

The larvae produce bronchitis or bronchopneumonia while passing through the lung.

The adult worms produce chronic blood loss and thus cause anaemia.

The disease caused by *Ancylostoma* is called as ancylostomiasis

Patients suffer from acute anaemia due to the sucking of blood by adults in intestine.

3.2.9 KEY TERMINOLOGY

Anaemia: A reduction in the quantity of the oxygen-carrying pigment hemoglobin in the blood.

Bucal cavity: Cavity within the mouth opening.

Copulatory Bursa (pl-Bursae): A pouch like structure found in male *Strongyloides* which is used in copulation.

Iron deficiency anaemia: It results from lack of iron, which is necessary for the production of hemoglobin.

3.2.10 SELF ASSESSMENT QUESTIONS

1. Describe the life cycle of *Ancylostoma duodenale*
2. Describe the symptoms and treatment of ancylostomiasis.
3. Write short notes on:

a) External structure of male and female *Ancylostoma caninum*

b) Ancylostomiasis

c) Mode of entry of filariform larvae of *A. duodenale* into the host.

3.2.11 REFERENCE BOOKS

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UNIT – III

3.3 BIOLOGY, LIFE CYCLE AND NATURE OF INFECTION OF *TRICHURIS TRICHIURA*

3.3.1 OBJECTIVES

The purpose of this lesson is to:

know about the habit and habitat of the worm

present the life cycle of the worm

understand the clinical symptoms of trichuriasis

know the control measures of parasitic infection.

3.3.2 INTRODUCTION

The Phylum Nemathelminthes includes the Nematodes or round worms. These are of medical importance. Nematodes have elongated, cylindrical, unsegmented bodies. The sexes are separate. The males are usually smaller than females. The alimentary canal has a mouth and an anus. The reproductive system in the male can be differentiated into testis, vas deferens, seminal vesicle and ejaculatory duct, which has a common opening with an anus. The female has ovaries, oviduct, uterus, vagina and vulva. The female genital pore is separate and opens either on the side of the body or near the anterior end.

Trichuris trichuria systematic position

Phylum - Nemathelminthes

Class - Phasmida

Order - Trichuroidea

Genus - *Trichuris*

Species - *trichura*

3.3.3 HABIT AND HABITAT

It is an endoparasite. The worm lives in the caecum, large intestine and appendix of man (a parasite of alimentary canal). It has world wide distribution but common in tropical countries. These are dioecious. Oviparous.

3.3.4 MORPHOLOGY

The members of the family Trichuridae are characterized by females that are oviparous and by males that have one spicule. The worm resembles a whip; the anterior three fifth is very thin, the posterior two fifths is thick and stout like the handle of a whip. Hence, it is also called whip worm. In both males and females the anterior end of the worm is longer and slender than the posterior end. A long oesophagus is present in the anterior end. The intestine and sex organs are present in the thick posterior end.

Male: Males have one spicule. Posterior end of body rolled dorsally in a spiral; spicule surrounded by a prepuce-like sheath, which evaginates when the spicule is protruded. External surface of sheath is smooth or covered with spines.

Female: Female is slightly larger than male. The worm measures 3 to 4 cm in length. Posterior extremity slightly curved, but not spirally coiled. The female has a single ovary. Vulva nears the junction of the anterior and posterior portions of the body. Oviparous (Figure 3 – 4).

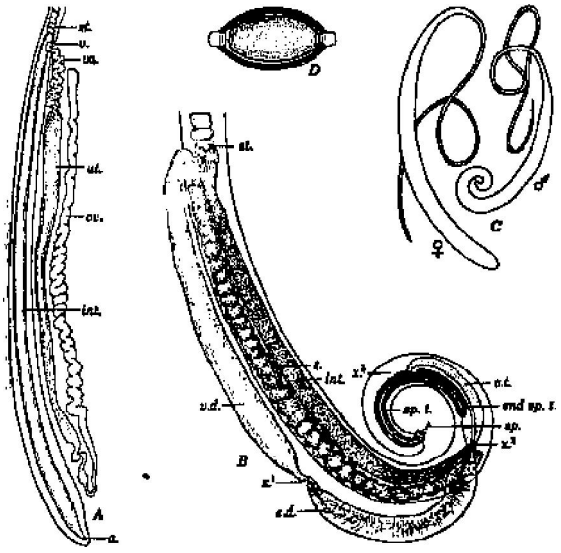

Fig. 3-4. Human whipworm *Trichuris trichiura*
A. Female and B. Male; C. Body shape of male and female; D. egg.

a. anus; c.t. cloacal tube; e.d. ejaculatory duct; int. intestine; ov. ovary; sp. spicules; sp.t. spicular tube; st. begining of stichosome of esophagus; t. testis; ut. uterus; v. vulva; va. vagina; v.d. vas deferens; x^1. junction of vas deferens and ejaculatory duct; x^2. junction of ejaculatory duct and intestine to form cloacal tube; x^3. junction of cloacal tube and spicular tube;

3.3.5 EGGS

The eggs are barrel shaped with thick brown shells, with plugs at both ends. The egg measures about 50X52 microns. The unembryonated eggs have a clear mucoid plug at each end.

3.3.6 LIFE CYCLE

Man is the only definitive host. No intermediate host is required. The *Trichurus* feed on blood or liquefied mucosa, by inserting its greatly elongated anterior end into the mucosa. Coincidentally with this, the posterior end has become enlarged and hangs free in the lumen. This genus is widespread in mammals but is reasonably host specific. The eggs of this whip worm develop without any intermediate host. Non-embryonated ova reach the environment (outside man) through feces. Infection to man occurs by ingestion of the eggs. They develop to adult in intestine. The eggs do not hatch until they are swallowed and, although the larvae do not leave the intestine, they burrow into the mucosa and remain there for a while.

The life cycle of *T. trichuria* is incompletely known. When infective eggs are swallowed with contaminated food and water, the digestive juices of the duodenum, allowing the larvae to escape within 30 to 60 minutes after entering the host promptly dissolve the mucoid plugs. The larvae are carried directly to the caecum. Three molts, all in the caecum are reported. A fourth molt has not been reported but should be expected. They undergo sexual maturity in the caecum.

3.3.7 PATHOLOGY

Normally the worms produce no pathogenic effect. Penetrated tissue prone to bacteria and infectious agents. Petechial hemorrhages, superficially eroded and hyperemic mucosa, extreme irritation of the lower colon prone to prolapse of rectum.

3.3.8 CLINICAL FEATURES

The clinical features of trichuriasis depend on the worm load- Discomfort, flatulence, loss of appetite and weight, nausea, vomiting, mucous diarrhoea or dysentery. Symptomless to chronic debilitates diarrhoea and anemia, with damage to physical and mental development. Lower right Quadrants pain is quite common complaint. Chronic diarrhoea and bloody stools in heavier infections. Weight loss, wasting, rectal prolapse in massive trichuriasis especially in children. Worms visible attached to mucosa under sigmoidoscopy in heavy infections.

In acute infections the whip worms produce acute appendicitis. Mucosa becomes hyperemic, friable and odematous. Stools are mucoid and sticky with streaks of blood, eosinophils and eggs.

3.3.9 LABORATORY DIAGNOSIS

Eggs may be passed in the feces. The eggs should be looked for in the stool specimen. The most important character is that the eggs float in saturated salt solution. This is perhaps the best method of differentiation of the eggs, which are very characteristic.

3.3.10 TREATMENT

Mebendazole is highly active against whip worms. It is found that the ideal dose would be 100 mg (each tablet contains 100 mg of Mebendazole) twice daily for 3 days for whip worm. Dewormis –

50 (Levamisole 50 mg) or Dewormis –150 (Levamisole 150 mg) is also effective against whip worm. The dosage is one tablet 150 mg for adults and one tablet 50 mg for children; the drug is preferably taken after the last meal at night. Special dieting and laxatives are not necessary. The obvious effect on the worm is muscular paralysis. The paralyzed worms are expelled with feces.

Trichuriasis can also be treated with Wormin (Chewable tablet of Mebendazole 100 mg); the dosage would be 100 mg single dose for 3 days.

3.3.11 SUMMARY

It is commonly called as whip worm.

It is an endoparasite.

It inhibits the caecum and large intestine of man.

It has world wide distribution.

It is found commonly in tropical countries.

Sexes are separate.

Females are slightly larger than males.

In both males and females, the anterior portion of the body having pharynx is usually more slender. Usually this slender portion is burried in the mucosa of caecum and/or large intestine.

Mouth is simple. Lips are absent.

The parasite feeds on blood and epithelial cells.

Life cycle is simple.

No intermediate host is involved.

Fertilized eggs of the parasite come out through feces of humans.

Development of eggs takes place in moist soil.

Embryos develop within three weeks.

Man gets the infection through contaminated food and water.

Contaminated food and water contains the eggs with juveniles.

The juvenile larvae undergo metamorphosis and develop into adults.

Heavy infection results in diarrhoea, anemia, constipation, abdominal pain and appendicitis.

3.3.12 KEY TERMINOLOGY

Abdomen: The part of the body cavity below the chest from which it is separated by the diaphragm. The abdomen contains the organs of digestion and excretion (in women it also contains the ovaries and womb).

Appendicitis: Inflammation of the vermiform appendix. Acute appendicitis usually affects young people. The chief symptom is abdominal pain.

Constipation: A condition in which bowel evacuations occur frequently or in which the feces are hard and small or where passage of feces causes difficulty or pain.

Diarrhoea: Frequent bowel evacuation or the passage of abnormally soft or liquid feces. It may be caused by intestinal inflammation.

Dioecious: Having separate sexes i.e. some individuals contain the male reproductive system and other individuals contain the female system.

Dysentery: An infection of the intestinal tract causing severe diarrhoea with blood and mucus.

Flatulence: The expulsion of air or gas from the stomach through the mouth.

Hemorrhage: The escape of blood from a ruptured blood vessel, externally or internally.

Nausea: The feeling that one is about to vomit. Actual vomiting often occurs subsequently.

Petechial hemorrhage: Small round flat dark red spot caused by bleeding into the skin or beneath the mucous membrane.

Prolapse: Downward displacement of organ or a part from its normal position. This may happen if the supporting tissue is weak.

3.3.13 SELF ASSESSMENT QUESTIONS

1. Describe the life cycle of *Trichuris trichura.*
2. Describe the morphology and life cycle of *Trichuris trichura.*
3. Explain the pathology, clinical features and treatment of trichuriasis.
4. Write short notes on:
   (a) Trichuriasis.
   (b) Laboratory diagnosis of trichuriasis.

3.3.14 REFERENCE BOOKS

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UNIT – III

3.4 BIOLOGY, LIFE CYCLE AND NATURE OF INFECTION OF *DRACUNCULUS MEDINENSIS*

3.4.1 OBJECTIVES

The purpose of this lesson is for:

studying the life cycle of *D. medinensis*

knowing the source of infection

understanding the clinical symptoms of the disease

learning the prevention and treatment of dracontiasis

3.4.2 INTRODUCTION

Dracunculus medinensis is popularly known as the guinea worm. The adult nematode parasite (worm) lives in the subcutaneous tissues of man, especially in the leg, ankle, foot and back. Its life

cycle is well understood when compared to any of the other species of the genus occurring in others.

***Dracunculus medinensis* systematic position**

Phylum - Nemathelminthes
Class - Phasmida
Order - Dracunculoidea
Family - Dracunculidea
Genus - *Dracunculus*
Species - *medinensis*

3.4.3 DRACONTIASIS

Dracunculus medinensis is a parasite of the subcutaneous connective tissues of man in many parts of the tropical sections of the world, particularly in Africa and Asia. The parasitic infection is primarily found in rural populations of dry areas. People who obtain water from step wells and ponds become the victims of this parasitic infection. The crustacean intermediate hosts living in these habitats become infected with the larvae of the parasites. When the infected crustaceans are swallowed with the drinking water man gets the infection of guinea worm. The disease caused by its infection is generally referred to as dracontiasis (subcutaneous abscess).

3.4.4 DESCRIPTION OF THE PARASITE

Adult worms are found in the subcutaneous connective tissues of man. Sexes are separate. Males have been recorded n very few instances. Males measure about 40 mm long and females from 60 to 80 mm, with a diameter of 1.7 to 2 mm. The white thread like female lives in the connective tissues beneath the skin. Females are enormously larger than males. Only the female worm is commonly found. It is a very long slender worm. The female may measure upto 1 meter in length. It is found in the subcutaneous tissue of that part of the body, which is likely to come in contact with water – e.g. the backs of water carriers. It is also commonly found in the legs and over the hands of washerwomen. On reaching the skin surface, the worm secretes an irritant substance, which produces an ulcer, through which the worm can protrude. The embryos are small measuring about 50 mm in length. The embryos are coiled structures.

3.4.5 EXTERNAL AND INTERNAL CHARACTERS

In both males and females the mouth is triangular aperture. A quadrangular cuticular plate (external circle) surrounds it. There is an internal circle of four cephalic papillae. These are arranged as a dorsal and ventral doublet. Four double papillae are present on the external circle. Well developed and conspicuous amphids are present. In case of males the posterior end bears 4 pairs of pre anal and 6 pairs of post anal papillae. A pair of large lateral phasmids is located at the posterior end near to the anus. The posterior end of the male is also characterized by the presence of spicules (Figure 3-5).

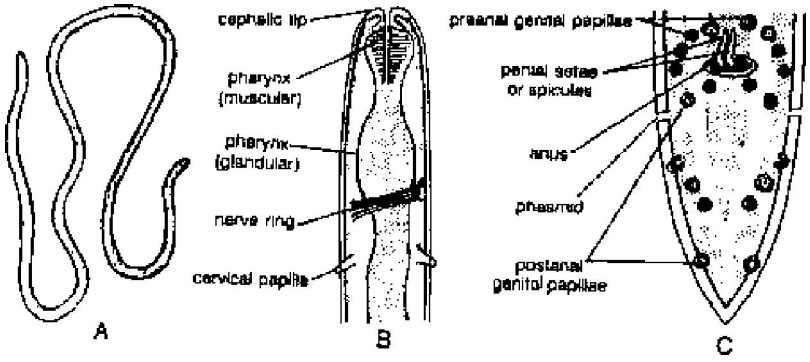

Fig. 3-5. *Dracunculus medinenis* A. Entire; B. Anterior end of male ; C. Posterior end of male in ventral view.

3.4.6 DIGESTIVE SYSTEM

The digestive system is simple and straight in both males and females. Mouth lies at the anterior end of the body. Mouth opens into a slender, muscular portion called oesophagus. It is followed by the larger and longer glandular part called intestine. A median constriction appears in the intestine at the level of the nerve ring and cervical papillae. The intestine is a flattened and nonfunctional in adult. They feed on the subcutaneous connective tissue. The anus lies in the female a little in front of the posterior extremity.

3.4.7 REPRODUCTIVE SYSTEMS

In males ten pairs of genital papillae are present.

In the female, the genital opening lies anteriorly as in other nematodes. In the adult female, the whole body is filled with embryos and vagina is indistinct.

The worms attain sexual maturity in the connective tissues. Copulation takes place between male and female. After fertilization, the male appears to die. Then the gravid female migrates to such body surfaces (ankle, foot and arm) which are likely to be exposed to water.

3.4.8 LIFE CYCLE

D. medinensis completes its life cycle within two hosts. The primary host is man (vertebrate) and the intermediate host is an invertebrate (cyclops). The female, which lives in the connective tissues beneath the skin, releases its larvae into a large blister on the legs or arms. When the limbs are immersed in water, the larvae escape and are subsequently eaten by tiny water fleas (cyclops), in which their development continues. Dracontiasis results from drinking water contaminated with cyclops. The detailed life cycle is given below:

The embryos are liberated directly from the uterus of the gravid female. They have to be discharged from an ulcer on the skin, through which the female protrudes during the act of discharging embryos. This occurs only when the sore is immersed in water.

3.4.8.A RELEASE OF EMBRYOS IN WATER

The liberation of embryos occurs in this way – gravid females lie in the subcutaneous tissue. The anterior end of the female worm pressing outward against the under surface of the skin. A papula is produced due to the pressure of the worm. This papula develops into a blister and finally into an ulcer like sore. When the sore is wetted with water, the female is stimulated and thrusts her anterior end through the sore. Water enters the pseudocoel of the worm through the membrane and produces internal pressure. The internal pressure causes the body wall to rupture and the gravid uterus to prolapse. A mass of first stage larvae, upto a half million, escape into the water.

They have a flattened body, a slender tail, a round anterior end and a well developed alimentary canal with a bulbous oesophagus. For further development, these larvae have to be ingested by the intermediate host, a fresh water crustacean known as cyclops. After releasing the larvae, the female withdraws into the subcutaneous tissue. The female repeatedly releases larvae in water upon the subsequent wettings of the area. In this way the female worm may release her entire brood of millions of larvae. Following the release of entire larvae, the female dies.

Viability of the larvae is only 7 days. They remain active in the water up to 7 days but their ability to infect decreases after day 3. They become non-infective by day 6.

3.4.8.B INGESTION OF LARVAE BY THE INTERMEDIATE HOST

In the water, the intermediate host must eat the larvae (embryos), which is a Cyclops (a fresh water crustacean). Larvae wriggling in the water are ingested by a number of species of Cyclops. The common intermediate hosts are *Cyclops leukarti, C. hyalinus, Eucyclops, Mesocyclops* and *Macrocyclops.* The larvae in the intestine burrow through the wall (with the aid of dorsal tooth) and reach the hemocoel within 1 to 4 hours.

Naturally infected Cyclops generally contain a single larva. The infected Cyclops become relatively inactive and sink to the bottom.

3.4.8.C LARVAL DEVELOPMENT IN CYCLOPS

Larval development proceeds and two molts occur in crustaceans in temperature about 19°C. Larvae are unable to tolerate –78°C for over 6 months and retain their infectivity for intermediate host. During molting the larva looses its tail, becomes cylindrical in shape. Larvae are 240 to 600 μ long. The tail of the larva is of 2 or 3 or 4 microns. Transparent globules are present in the intestine of larva. Also, the larva contains genital primordium consisting of 6 to 8 cells. Lytic substances are also present in larvae of 21 days old or older. After two molts the larva reaches to third stage and then develops into an adult. Then a cuticular sheath is formed around the worm. In this sheath the worm lies Quiescent. These are the infective stages to man. These Quiescent worms must reach the man for further development.

3.4.8.D INFECTION OF THE MAN

Infection of the man occurs when copepods containing Quiescent worms are swallowed with water. The digestive juices of the stomach digest the copepods. The Quiescent worms are liberated in the duodenum. They are rendered active by the hydrochloric acid of the gastric juice. They migrate to

the duodenal wall within 13 hours. From the duodenal wall they migrate to the thoracic and abdominal muscles by day 15. During this time, the worms did not grow in size. A final molt occurs between 15 to 22 man swallows days after the worms.

After the final molt (between 15 and 22 days after they are swallowed), both the male and female worms migrate into the subcutaneous connective tissues. In the subcutaneous connective tissues they become sexually mature within 10 to 12 months. Here they reach adult size. The male fertilizes the female and dies. The females have mucus plug in the vagina indicating the occurrence of insemination in them. The gravid female then migrates to a favorable site, which is likely to come in contact with water.

3.4.9 CLINICAL FEATURES

Usually reddish papular lesion appears on legs. Until the appearance of this reddish papule, the disease remains asymptomatic. Lesion forms blister bearing head of female worm and numerous larvae. The initial symptoms, which appear a year after infection, include itching, giddiness, difficulty in breathing, vomiting and diarrhoea. Later a large blister forms on the skin and may ulcerate and become infected. The disease symptoms also include urticaria, pruritus, allergic symptoms and eosinophilia. When the worm is ruptured accidentally, it may produce intense inflammatory reaction. At the site of inflammation secondary infection may be established or the ulcer can become secondarily infected, and produce a large cystic mass.

If the affected part bearing a fresh ulcer is immersed in water, the worm can be seen emerging to release a milky fluid, which contains embryos.

Adult females in humans extending the anterior end of the body are often clamped in a split stick and slowly withdrawn from the tissues by winding the worms on the stick.

The danger is serious hazards to the diseased persons in case the worm breaks and withdrawn. If this occurs a severe inflammatory reaction occurs at the site. This may result in secondary bacterial infection. The worms can be removed safely from people under aseptic conditions in hospitals.

3.4.10 LABORATORY DIAGNOSIS

Adult worm can be detected by immersing the blister in water.

Detection of embryos in fluid expelled by the worm.

Local lesion shows the head of the worm. Blister shows the accumulation of larvae.

X– ray studies reveal calcified nodules (worm).

Old and degenerated worms tend to become calcified.

Reflected light shows worm under skin.

Eosinophilia in peripheral blood.

Intradermal test can be done for the presence of worm/antigens. If dracunculus antigen is available, skin testing may be positive.

3.4.11 PREVENTION

Eradication of copepods in water wells.

Drinking water after boiling.

Well water should be treated with insecticides to reduce breeding of copepods.

Treatment of carriers by anthelminthic drugs.

Prohibiting the use of water from wells of infection.

3.4.12 TREATMENT

No proper medicine is available to treat dracontiasis. However, the following measures can be employed.

Phenolthioazine is effective on infective larvae and adult worms.

Blisters may be treated with antiseptics and dressings.

Treatment involves killing the adult worms with injection of phenolthiazine.

Adult females extending from the subcutaneous tissues can be clamped in a split stick and removed by winding the worms on the stick. This procedure of removal is hazardous causing much pain to the patient. Also, if the worm breaks and withdraws, this may result in secondary bacterial infection. They can be removed under aseptic conditions or patients can be treated with drugs.

3.4.13 SUMMARY

Dracunculus medinensis is commonly called as guinea worm.

Dracontiasis is common in India, West Africa but also occurs in Arabia, Iran, East Africa and Afghanistan.

The disease caused by *D. medinensis* is called dracontiasis.

Man is the primary host.

Cyclops (water flea) is the intermediate host.

The disease is transmitted to man via contaminated drinking water.

The worm comes out of digested Cyclops and migrates into tissues.

Mature female passes to skin.

Adult female in skin causes host to form blister near head of worm.

The blister filled with larvae.

When the skin is immersed in water, the blister bursts discharging larvae.

When the female moves to new site, a new blister is formed.

In the intermediate host (cyclops), the larvae penetrate the gut and develop in haemocoel (18 – 21 days).

Man is the reservoir host for dracontiasis.

Dracontiasis remains asymptomatic until reddish papular lesion appears on legs.

The initial symptoms, which appear a year after infection, result from the migration of the worm to the skin surface.

The clinical symptoms include urticaria, pruritus, allergic asthma-like response, vomiting, diarrhoea and eosinophilia.

Later a large blister forms on the skin usually on the legs or arms, which eventually burst and may ulcerate and become infected.

Disease can be diagnosed under laboratory conditions by X-ray studies (calcified worm can be identified) and intra dermal test.

Local lesions and blisters also reveal the infection.

Adult females can be removed safely under aseptic conditions in hospitals.

3.4.14 KEY TERMINOLOGY

Abscess: A localized collection of pus anywhere in the body, surrounded by damaged and inflamed tissues.

Blister: A swelling containing watery fluid (serum) and sometimes also blood (blood blister) or pus, within or just beneath the skin. Blisters develop on the hands or feet or at the site of burn.

Connective tissue: Body layer between epithelia. Composed of a fluid or gel extracellular matrix, with or without cells.

Dracontiasis: A tropical disease caused by the parasitic nematode *Dracunculus medinensis* in the tissues beneath the skin.

Eosinophilia: An increase in the number of eosinophils in the blood. Eosinophilia occurs in a variety of diseases, including parasitic infections.

Papulae: A small superficial raised spot or abnormality on the skin.

Phasmid: Paired unicellular glands in the tail region. Chemoreceptive in function. Well developed in parasitic nematodes.

Prolapse: Downward displacement of an organ or a part from its normal position. This may happen if the supporting tissues are weak.

Pruritus: Itching caused by local irritation of the skin.

Ulcer: A break in the skin that fails to heal and is often accompanied by inflammation.

3.4.15 SELF ASSESSMENT QUESTIONS

1. Describe the life cycle of *Dracunculus medinensis*

2. Write about the causative agent and clinical symptoms of dracontiasis.
3. Write short notes on:
    a. Dracontiasis
    b. Guinea worm
    c. Subcutaneous abscess

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UNIT – IV

4.1 INTRODUCTION AND OVERVIEW OF IMMUNOLOGY

4.1.1 OBJECTIVES

The purpose of this lesson is to:

Know about the essential steps for the occurrence of disease.

Provide framework for understanding the immune mechanism.

4.1.2 INFECTION

The infection is a process whereby the microorganisms gain entry into the body of animals and human beings and produce the disease. Disease occurs due to:

(1) the successful entrance of the disease causing pathogen into the host (2) the establishment of the parasite in the host and (3) the growth or development or multiplication of the pathogen within the host. After gaining entrance into the body of the host, the pathogens reach their habitat and induce the disease (causing harmful effects).

4.1.3 SOURCES OF INFECTION

The two major sources of infection are:

(1) Human beings (2) invertebrates and vertebrates

4.1.4 MODE OF TRANSMISSION

Infection may be transferred in three ways – contact infection (direct infection), indirect infection and vector hosts.

4.1.4.A CONTACT INFECTION

When infection is transferred more or less directly from person to person, it is called contact infection or direct infection. Pathogenic bacteria may be transferred by direct contact or by carriers.

The examples for direct infection are gonorrhea and syphilis.

Sometimes, air acts as a vehicle to transmit microorganisms. The pathogens may leave the body through body discharges like saliva and sputum. The examples include tuberculosis (partly from cattle), rabies (from dogs) and common cold (from human beings).

4.1.4.B INDIRECT INFECTION

When disease-causing organisms are transferred indirectly from person to person through water, food and soil, it is called indirect infection. The examples for indirect infection are typhoid fever, cholera and dysentery. Houseflies also contaminate the food.

4.1.4.C INFECTION THROUGH VECTOR HOST

Invertebrates or vertebrates act as vector hosts to transfer the infection.

The examples are:

A blood sucking female anopheles mosquito transfers the organism from man to man (e.g. malarial fever), a blood sucking insect transfers the pathogens from individual to individual (e.g. plague by rat flea).

4.1.5 PORTALS OF ENTRY

Disease causing organisms may utilize various ways to gain entrance into the host. Following are the portals of entry of pathogens:

1) Through skin: When the blood-sucking insects punch the skin, the pathogens may be released on the skin or introduced into the blood.
2) Digestive tract: Typhoid, dysentery and cholera causing organisms may enter through the digestive tract by contaminated food and water.
3) Respiratory tract: Tuberculosis and other respiratory diseases may spread through airways by contaminated air.
4) Urino-genital tract: Sexual diseases like syphilis and gonorrhea may spread by sexual intercourse.

4.1.6 RESISTANCE TO INFECTION

Human beings are exposed to a continuous stream of microorganisms from the childhood. The potential, pathogenic microorganisms cause havoc on the body physiology. Various plants and

microorganisms may produce harmful foreign substances (e.g. toxins); these substances and/or the disease causing microorganisms or the parasites cause disease in animals and human beings.

The effective, protective immune mechanism defends the body against the disease causing pathogens. In the battle with the microbial invaders, the complex defense mechanisms of humans and vertebrate animals prevent the invasion and protect the body. Such complex array of defensive mechanisms is collectively called the immune system.

4.1.7 GENERAL PROPERTIES OF IMMUNITY

In all vertebrates and human beings, the defense system protects the body from invading pathogenic microbes and cancer. The immune system is able to produce an enormous number of immune cells and biomolecules. The immune cells are of different varieties. The biomolecules and the immune cells are able to recognize the harmful invaders; they act together in destroying or expelling the foreign invaders.

The activity of an immune system can be divided into two categories – recognition of the foreign invaders and the immune response.

The immune system is able to recognize variety of foreign invaders (pathogens) and is able to distinguish one another basing on their chemical differences. Also, the immune system is able to discriminate between the foreign molecules and the body's own cells and cellular proteins.

When a foreign organism is recognized by the immune system, it elicits a message to a variety of cells and molecules to mount an immune response called effector response. The pathogens may be neutralized or expelled from the body by the effector mechanism of immune response.

When the body is exposed to the same foreign organism, the animal or the human immune system is able to memorize and induces an immune response. This response is called immune response. The memory response is characterized by a more rapid immune response, which serves to get rid of the infectious organisms. This is also termed as immunological memory of the defense system of the body.

When humans are first exposed to an invading pathogen, their body normally experiences some of the pathogenic effects of the microorganism/parasite. Infected humans may suffer due to some of the early effects of infection. This is because the immune system needs sometime to respond and to destroy the invaded organism (pathogen). However, once the body has been exposed to a particular pathogen, its "immune system" remembers the pathogen and can respond much more quickly to eliminate or destroy the encounter.

The immune system in its normal function protects the body against pathogens; sometimes the immune system turns against the host, causing symptoms that range from discomfort to the onset of disease or even death. This is called as "immune dysfunction". The causes of immune dysfunction include genetic defects and the destruction of immune cells or tissues by chemical agents or by radiation.

4.1.8 HISTORICAL KNOWLEDGE

The observation that individuals who had recovered from certain infectious diseases were thereafter protected from the disease led to the growth of the discipline of immunology. "Immunitis" is the Latin term. The meaning of immunitis is "exempt". This Latin term immunitis is the source of English word immunity, meaning the state of protection from infectious diseases. This is term, which usually means the power of the animal body to resist infection.

The earliest reference to the phenomenon of immunity can be traced by the written reference of Thucydides, the great historian of the Peloponnesian War. In 430 B.C, in describing a plague in Athens, he wrote that only those who had recovered from the plague could nurse the sick when there was an outbreak of plague. Those who had recovered from the plague would not contract the disease a second time. Perhaps, almost 2000 years passed to develop a concept of the "phenomenon of immunity" and to understand/implement in medical practice.

Protection against small pox was obtained by inoculating live organisms from disease pustule. Jenner (1878) used non-virulent cowpox vaccine against small pox infection. Later Pasteur tried successfully vaccine using attenuated organisms against anthrax.

Metchnikoff (1883) suggested the role of phagocytes in immunity. Von Behring (1883) recognized antibodies in serum against diphtheria toxin. Denys and Leclef (1895) suggested that phagocytosis is enhanced by immunization. Ehrlich (1897) put forward side chain receptor theory of antibody synthesis. Bordet (1899) found that lysis of cells by antibody require cooperation of serum factor now collectively known as complement.

Landsteiner (1900) declared human ABO groups and natural isohemagglutinins. Richet and Portier (1902) proposed the term anaphylaxis which is opposite of prophylaxis. Wright (1903) put forward opsonic activity to phagocytosis. Von Pirquet and Schick (1905) described serum sickness after injection of foreign serum. Von Pirquet (1906) correlated immunity and hypersensitivity. Fleming (1922) found lysozyme. Zinsser (1925) suggested the contrast between immediate and delayed type of hypersensitivity. Heidelberger and Kendall (1930-35) put forward quantitative precipitation studies on antigen and antibody reaction.

Coons (1942) introduced fluorescence antibody techniques. Medwar (1958) discovered acquired immunologic tolerance. Portar and Edelman (1959) determined structure of immunoglobulin.

4.1.9 DISCOVERY OF HUMORAL AND CELLULAR IMMUNITY

Elvin Kabat (1930) suggested a fraction of serum to be responsible for protective immunity in animals/humans. He first called this fraction of serum as gamma-globulin (now immunoglobulin). The active molecules in the immunoglobulin fraction are called antibodies. Because antibodies mediated immunity contained in body fluids, the antibodies in body fluids are called as humors. It was called humoral immunity.

Elie Metchnikoff (1883) demonstrated that certain blood cells, which he termed phagocytes, were able to ingest microorganisms and other foreign materials. Noting that the phagocytes were more active in immunized animals than in non-immunized animals, he hypothesized that phagocytes were the major effector of immunity. In the 1950s, the lymphocyte was identified as the cell responsible

for both cellular and humoral immunity. In the key experiments with chickens, Bruce Glick explained that there were two types of lymphocytes: T lymphocytes derived from the thymus mediate cellular immunity and B lymphocytes from the Bursa fabricus mediate humoral immunity, Both systems were shown to be necessary for the immune response (Fig. 4-1).

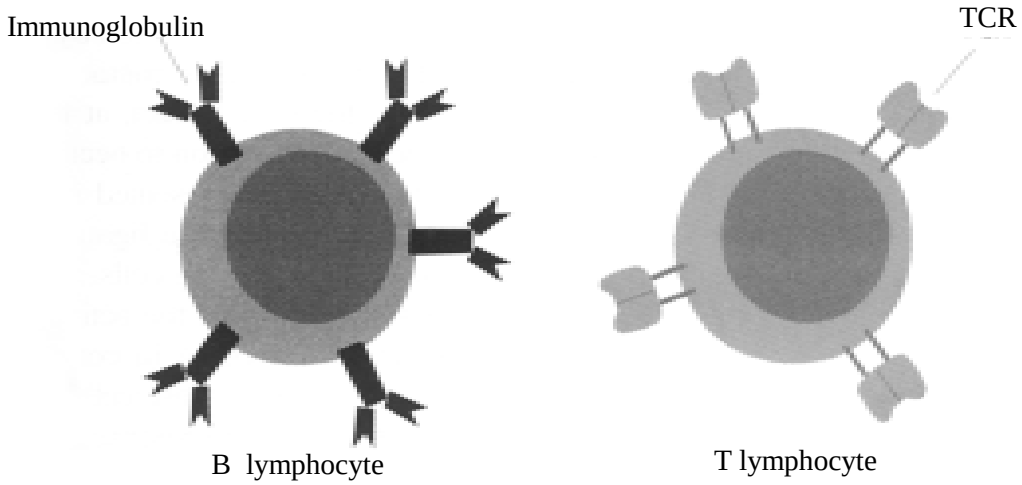

Fig. 4.1. Antigen receptors expressed as transmembrane molecules on B and T lymphocytes.

4.1.10 EARLY CONCEPTS OF IMMUNITY

One of the early theories of immunity explained about the protective antibody molecule – its specificity for foreign material, or antigen (a general term used for a substance that binds with a specific antibody).

In the 1950s, Niels Jarne, David Talmadge and F. Macfarlane Burnet formulated clonal-selection theory. According to this theory, an individual lymphocyte expresses membrane receptors that are specific for a distinct antigen. Binding of antigen to its specific receptor activates the cell, causing it to proliferate into a clone of cells. These cells have the immunological specificity as the parent cell.

4.1.11 THE NON-SPECIFIC COMPONENT OF IMMUNITY

Immunity is the state of protection from infectious diseases. It has both nonspecific and specific components of immunity.

The nonspecific component of immunity, innate immunity is a set of disease resistance mechanisms. These mechanisms are not specific to a particular pathogen.

In general most of the microorganisms encountered by a healthy individual are readily cleared within few days by nonspecific defense mechanisms.

4.1.12 THE SPECIFIC COMPONENT OF IMMUNITY

The specific component, adaptive immunity displays a high degree of specificity as well as the remarkable property of "memory". The adaptive immune response acts against an antigen within

five or six days after the initial exposure to that antigen; this is called as primary immune response. Exposure to the same antigen sometime in the future may result in a memory response. The secondary immune response occurs more quickly than the first; it is stronger and often is more effective in clearing the pathogen. The major components of adaptive immune response are lymphocytes and the antibodies and other molecules they generate.

4.1.13 NOBEL PRIZES FOR IMMUNOLOGICAL RESEARCH

Year	Recipient	Country	Research
1901	Emil von Behring	Germany	Serum antitoxins
1905	Robert Koch	Germany	Cellular immunity to tuberculosis
1908	Elie Metchnikoff	Russia	Role of phagocytosis (Metchnikoff)
	Paul Ehrlich	Germany	and antitoxins (Ehrlich) in immunity
1913	Charles Richet	France	Anaphylaxis
1919	Jules Bordet	Belgium	Complement-mediated bacteriolysis
1930	Karl Landsteiner	Unites States	Discovery of human blood groups
1951	Max Theiler	South Africa	Development of yellow fever vaccine
1957	Daniel Bovet	Switzerland	Antihistamines
1960	F.Macfarlane Burnet	Australia	Discovery of acquired immunological tolerance
	Peter Medawar	Great Britain	
1972	Rofdney R. Porter	Great Britain	Chemical structure of antibodies
	Gerald M. Edelman	United States	
1977	Rosalyn R. Yalow	United States	Development of radioimmunoassay
1980	George Snell	United States	Major histocompatibility complex
	Jean Dausset	France	
	Baruj Benacerraf	United States	
1984	Cesar Milsteing	Great Britain	Monoclonal antibody
	Georges F. Kohler	Germany	
	Niels K. Jerne	Denmark	Immune regulatory theories
1987	Susumu Tonegawa	Japan	Gene rearrangement in antibody production
1991	E. Donnall Thomas	United States	Transplantation immunology
	Joseph Murray	United States	
1996	Peter C. Doherty	Australia	The specificity of the cell-mediated immune
	Rolf M. Zinkernagel	Switzerland	response

4.1.14 SUMMARY

Infectious disease is a condition, which occurs when the adverse changes such as to interfere with the comfort of the host; it is the clinical manifestation of the host reaction to the parasite.

Parasitic diseases depend on the density of distribution of the host population, including ‘reservoirs’.

The parasitic existence of a parasite is dependent on its finding a suitable environment in which it can mature and reproduce.

Parasitic organisms may be carried from host to host by contact, fecal contamination, arthropod vectors and other vectors.

Immunity is the state of protection against foreign organisms or substances (antigens).

Vertebrates have two types of immunity, the nonspecific immunity and specific immunity.

The components of nonspecific immune mechanism are not specific to any one pathogen.

The components of specific immune mechanism display a high degree of specificity – specific to particular pathogen.

The nonspecific immune responses begin much more quickly than specific immune responses.

The specific and nonspecific immune responses do not operate independently of each other.

4.1.15 KEY TERMINOLOGY

Antibody: A globulin produced in the body in response to the presence of an antigen.

Antigen: Any foreign substance, which can cause the production of antibodies

Clone: A race of cells derived from a single parent cell. These cells have the immunological specificity as the parent cell.

Host: An organism, which provides shelter and nourishment to the parasite.

Immunity: This is a term, which usually means the power of the animal body to resist infection by pathogenic microorganisms, parasites or toxins.

Immunoglobulin: Globulins which act as antibodies.

Infection: It is a process whereby the microorganisms enter the body of animals and humans and produce disease.

Memory cell: Clonally expanded progeny of lymphocytes. Memory cells (lymphocytes) are more easily activated than unexposed (naïve) lymphocytes.

Memory response: The immune response, which is triggered due to the exposure to an antigen.

Phagocyte: A cell, which ingests microorganisms.

4.1.16 SELF ASSESSMENT QUESTIONS

1. How the infectious diseases occur? Name the sources and mode of transmission of infection.
2. Name four features of portals of entry of pathogens.
3. Explain the general properties of immunity.
4. Describe the early concepts of immunity. Explain the specific and nonspecific components of immunity.

5. Write short notes on:
   a. Indirect infection
   b. Contact infection
   c. Immune system
   d. Adaptive immune response

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UNIT – IV

4.2 NATURAL IMMUNITY

4.2.1 OBJECTIVES

The purpose of this lesson is to:
Know the types of immunity
Understand the natural immune mechanisms
Describe the importance of natural immunity

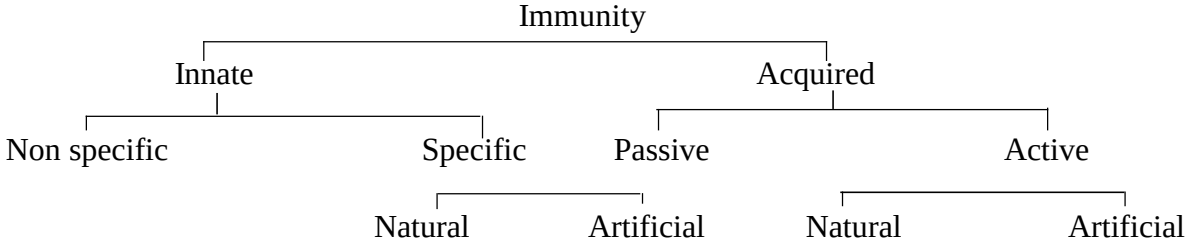
4.2.2 INTRODUCTION

Every organism during its lifetime may be exposed to disease causing agents. The pathogenic, disease causing microbes or their toxic products may cause many diseases in humans. In response to the microbes, the body of any organism and/or humans has evolved a strategy to deal with the microbes and prevents the establishment of infection and onset of disease. The mechanism, which overcomes disease, is collectively called as immune system. The word "immune" (Latin: *immunitis* meaning "*exempt*") implies freedom from a heavy burden. Resistance to infection is termed immunity. An organism, which develops immunity (resistance) against an infectious agent, will remain free of that particular agent throughout its lifetime.

Immunology is the study of the immune system. Immunology is the science, which deals with the body's response to antigenic exposure or challenge. It is an important scientific discipline whose application to the field of medicine has become apparent in recent years. Immunological mechanisms play an important role in the protection of the body against infectious agents but periodically these immune mechanisms also cause damage to the host. Immunological tests are now routinely used in diagnosis of disease and subsequent treatment of patients. Few years ago a new technology called hybridoma technology developed and is revolutionizing immunology. It is through this technique, the production of antibodies against single antigenic determinants (epitopes) is possible. By this technique it is now possible to get huge amounts of homogeneous and specific antibodies. Such antibodies can be used for disease diagnosis and possibly they will be used to treat the patient. The immunological reactions may be harmful or protective and in some cases they may be both at the same time. Immunology has broad biological role involving concept of antigen recognition, specificity and immunlogical memory. In this lesson, discussion is confined to most relevant topics in the field of medicine.

4.2.3 TYPES OF IMMUNITY

The resistance offered by the host to the harmful effect of pathogenic microbial infection is called immunity. Immunity against infectious diseases is of different types.



4.2.4 INNATE IMMUNITY

The natural immunity is also called as innate immunity. This is present in all the vertebrates. Innate immunity may be genetically passed on from one generation to other generation. It does not depend on previous exposure with microorganisms and/or metazoan parasites. This is non specific immunity; it indicates a degree of resistance against to all infectious, pathogenic organisms. Immunity is called as specific immunity when it shows resistance to particular pathogens. Innate immunity can be divided into following types:

4.2.4.A SPECIES IMMUNITY

Individuals of same species show uniform pattern of susceptibility to different bacterial infections. The mechanism of species immunity is conferred by physiological and biochemical differences between tissue of host species. These factors determine whether or not pathogen multiply in them e.g. poliomyelitis, measles, syphilis, leprosy, gonorrhea occur only in man. For example, *Salmonella typhi* produces typhoid fever in man where as mice are resistant to it.

4.2.4.B RACIAL IMMUNITY

Within a species different races show differences in susceptibility to infection e.g. Negroes are resistant to yellow fever and malaria. Algerian sheep show high resistance to anthrax. Such racial

differences are known to be genetic in origin principally induced by persistent environmental stimulus.

4.2.4.C INDIVIDUAL IMMUNITY

An individual in a population shows variation in the immunological response to microbial infections. For example homozygous twins exhibit individual immunity or susceptibility to lepromatous leprosy and tuberculosis. But such type of correlation is not seen in heterozygous twins.

4.2.5 ACQUIRED IMMUNITY

This is a nonspecific immunologic response that occurs following exposure to particular infectious agents; it may be either deliberate attempt called as vaccination or accidental exposure called as infection. The resistance against these particular infectious agents is due to the production of antibodies or sensitized lymphocytes specific to these infectious agents. Specific immune responses are mediated by two mechanisms.

4.2.5.A HUMORAL IMMUNITY

Bursa or bone marrow derived (B) lymphocytes or B cells confer humoral immunity. These cells possess specific immunoglobulins on their cell surface. When B cell interacts with its homologous antigen, the B cell is activated and differentiated into a plasma cell. These cells excrete enormous quantities of immunoglobulins of the same specificity.

4.2.5.B CELL MEDIATED IMMUNITY

Thymus derived (T) lymphocytes or T cells confer cell mediated immunity. These cells express a receptor molecule, which is similar to an immunoglobulin. Upon stimulation they undergo proliferation. T cells are found in peripheral blood and lymphoid tissues.

4.2.6 NON-SPECIFIC IMMUNITY

The natural immune mechanisms do not exhibit specificity. Non specific immunity is innate immunity. Examples include:

Mucous membranes
Enzymes in secretions
Interferons and
Phagocytic cells

4.2.7 SPECIFIC IMMUNITY

The specific immunologic response that occurs to the exposure of pathogens. Examples of Natural specific resistance are:

Passive placental transfer of antibodies
Active recovery of the body from disease
Examples of Artificial specific resistance are:
Passive administration of antitoxin
Active reaction (by the production of antibodies) of the body to vaccination.

4.2.8 CONTRIBUTION OF MECHANICAL BARRIERS

The defense mechanisms of body are related to mechanical barriers as under:

a. The intact skin confers considerable protection against bacteria. With few exceptions undamaged skin is virtually impenetrable to bacteria.

b. Mucous membrane covering (in the areas that are not covered by the skin) of the body protects the body against microbes. The mucous coating of mucosal cells trap the microorganisms. These are removed by other defense mechanisms.

c. The bactericidal activity of sweat and sebaceous secretions contain lactic acid, saturated and unsaturated fatty acids. Saliva, tears, urine and perspiration remove the cells carrying microbes.

d. The mucous membranes of gastrointestinal tract provide protection by secreting bactericidal enzymes released in lumen.

e. Coughing expels the mucous blanket

f. Sneezing, diarrhea and vomiting eliminate pathogenic organisms.

4.2.9 ROLE OF PHYSIOLOGIC FACTORS

The physiologic factors influencing the level of innate immunity in an individual include:

A. Age: Persons who are very young (aged 3 years or younger) or very old (aged 75 years) are much more susceptible to infection. Two extremes of life carry high susceptibility to infectious diseases. The susceptibility of fetus to infection is related to immaturity of its immune apparatus e.g. Coxsackie viruses cause fatal infection in sucking mice but not in adults.

Old persons are highly susceptible to infection due to gradual warning of their immune responses.

Many age differences in specific infections can be related to physiologic factors. Thus coliform bacteria often cause bacterial meningitis during first month of life because bacterial antibodies to these bacteria are IgM and these antibodies fail to cross the placenta. Other such examples are gonococcal vaginitis in small girls, rickettsial infection more severe in advancing age, rubella infection damage the fetus severely but otherwise produce only mild disease.

B. Hormonal influence: The host immune response is affected by hormonal balance. Endocrine disorders e.g. diabetes mellitus is related to susceptibility to infection because of increased carbohydrate levels in tissue. Cortiosteroids depress host's resistance by anti-inflammatory antiphagocytic effect and by suppression of antibody formation and hypersensitivity. The elevated steroid levels during pregnancy may have relation to heightened susceptibility or pregnant women to many infections.

C. Nutrition: Imbalanced diet depresses all types of immune response and thus increasing the risk of infection.

D. Body temperature: Many microorganisms are unable to survive at 37°C

E. Oxygen tension: Anaerobic microorganisms cannot survive or their growth is inhibited in lungs where there is high oxygen tension.

4.2.10 ROLE OF CHEMICAL AND BIOCHEMICAL INHIBITORS

Saliva is bactericidal. High acidity of stomach inhibits bacterial multiplication. The normal body flora plays indirect role in defense of body e.g. intestinal flora by producing bactericides, which are destructive to other bacteria. The acid pH prevents colonization of microbes.

Inflammation: Tissue injury or irritation initiated by entry of bacteria or of other irritant leads to inflammation. It is an important non-specific mechanism of defense. Initially constriction and then dilation of blood vessels of affected site is followed by escape of polymorphonuclear cells into tissue. Microorganisms are phagocytosed and destroyed. Out pouring of plasma helps to dilute the toxic products. A fibrin barrier is laid, serving to wall off the sit of infection.

Fever: It is a natural defense mechanism. It may actually destroy the infecting organism. Fever stimulates the production of interferon and helps in recovery from virus infection.

4.2.11 PHAGOCYTOSIS

Natural defense against invasion of blood and tissue by bacteria or other foreign particles is mediated by phagocytic cells, which ingest and destroy them.

Phagocytic cells may be:

Microphages e.g., polymorphonuclear leukocytes and Macrophages.

Macrophages include:

1) Histiocytes (wandering amoeboid cells in tissue)
2) Fixed reticuloendothelial cells
3) Monocytes.

Phagocytic cells reach sites of inflammation in large numbers and ingest particulate material. Bacteria are phagocytosed into vacuole, which fuses with lysosomes to form phagolysosome. Bacteria are subjected to the action of lytic enzymes in phagolysosome and are destroyed. In chronic granulomatous diseases and in agranulocytosis susceptibility to infection is there because of lack of phagocytosis (Fig. 4-2).

4.2.12 OPSONIZATION

Phagocytosis occurs even in simple systems. Phagocytosis can be enhanced in the presence of blood serum or plasma due to opsonization. The process of enhancing phagocytosis via opsonins is called opsonization. Opsonins found in serum are:

Antibodies,

Fibronictin (glycoprotein),

Leucotrienes (derivatives of arachidonic acid),

Tuftsin (a tetra peptide split product of IgG)

4.2.13 HUMORAL FACTORS WITH ANTIMICROBIAL ACTIVITY

(a) **Lysozyme:** It is a bactericidal enzyme found in nasal, intestinal secretion, seminal fluid and lachrymal secretion.

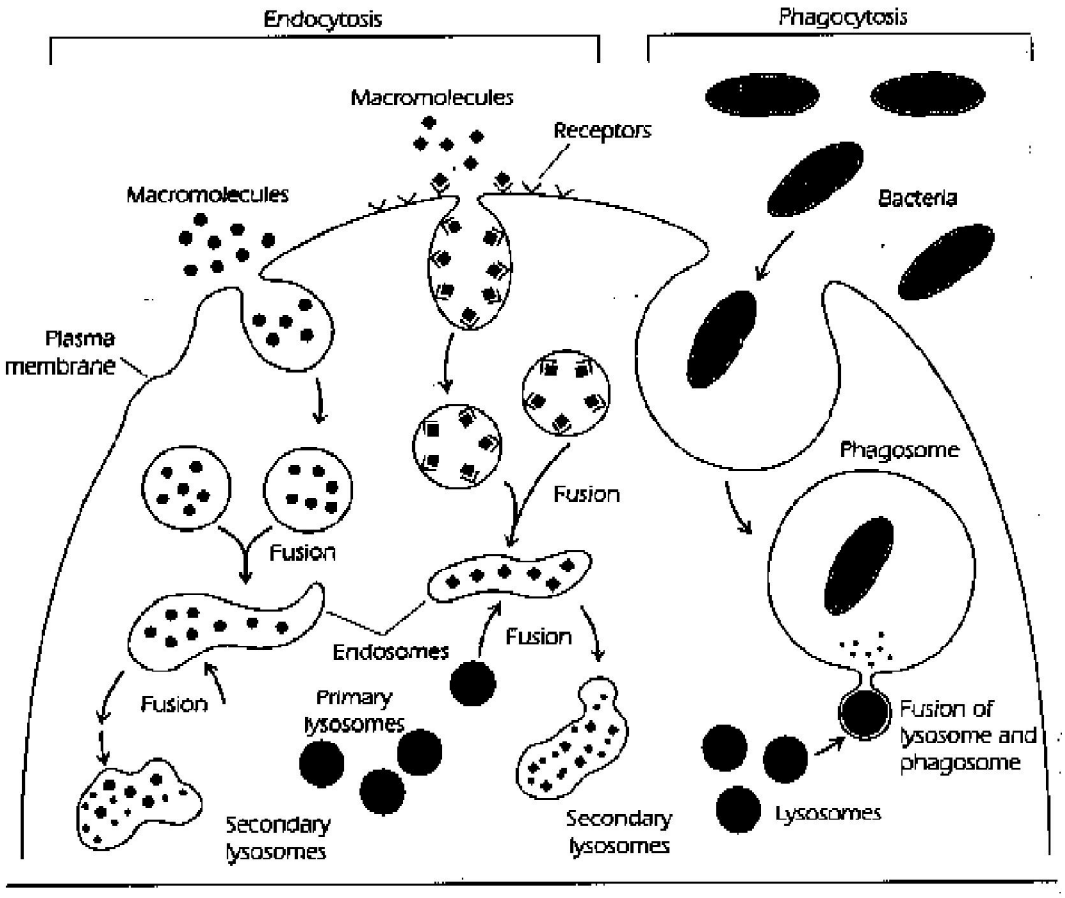


Fig. 4.2 **Endocytosis and phagocytosis by macrophages.**

(b) **Properdin:** It is a euglobulin present in normal serum. It causes lysis of Gram negative bacteria with the help of Mg^{++} and complement. It constitutes 0.02% of serum protein. It is not an antibody. Its level remains constant in new born and elderly individuals in both sexes. Its molecular weight is over one million.

(c) **Beta lysine:** It is relatively thermostable substance active against *Anthrax bacillus*. It is liberated from platelets during clotting.

(d) **Basic polypeptides:** They are bactericidal substances active at high pH (7 to 8). They act upon cell wall causing cell disintegration e.g. Lukens from leucocytes and plakin from platelets.

(e) **C-reactive proteins:** The sera of patient with pneumococcal and other diseases give a precipitate when mixed with somatic polysaccharide C of pneumococcus in presence of calcium ions. These nonspecific substances (C reactive protein) appear in blood of a person with tissue necrosis and inflammation. Characters of C reactive protein are:

1. Calcium is essential for the reaction.
2. Reactive substance β globulin is not detectable in normal serum. It is demonstrated in sera, pleural, and peritoneal and joint fluids of patient.

The demonstration of C reactive protein is useful in the diseases like rheumatic fever, and rheumatoid arthritis etc.

(f) **Bactericidin:** It is non specific serum factor active against *Neisseria*, *Streptococcal pyogenes* etc.

(g) **Complement:** It is thermolabile substance present in serum and tissue fluid. It enhances phagocytosis and kills most of Gram negative bacteria sensitized by specific antibodies.

(h) **Non specific hyaluronidase inhibitors:** In tissue damage non specific inhibitor hyaluronidase appears in blood. It is heat labile and requires magnesium ion for its activity.

Production of antibodies against antigens of microorganisms may induce resistance because they:

1. Neutralize toxin or cellular products.
2. Have direct bactericidal or lytic effect with complement.
3. Block the infective ability of microorganisms.
4. Agglutinate microorganisms thus subjecting them to phagocytosis.
5. Opsonize microorganisms.

4.2.14 LYMPHOCYTES – NATURAL IMMUNITY

Natural killer (NK) cells are large granular lymphocytes that appear to have nonspecific immune response.

NK cells are spontaneous in their presence in the body.

NK cells are cytotoxic for tumor cells and virally infected autologous cells.

NK cells release various cytokinins during their interaction with target cells (Fig.4-3).

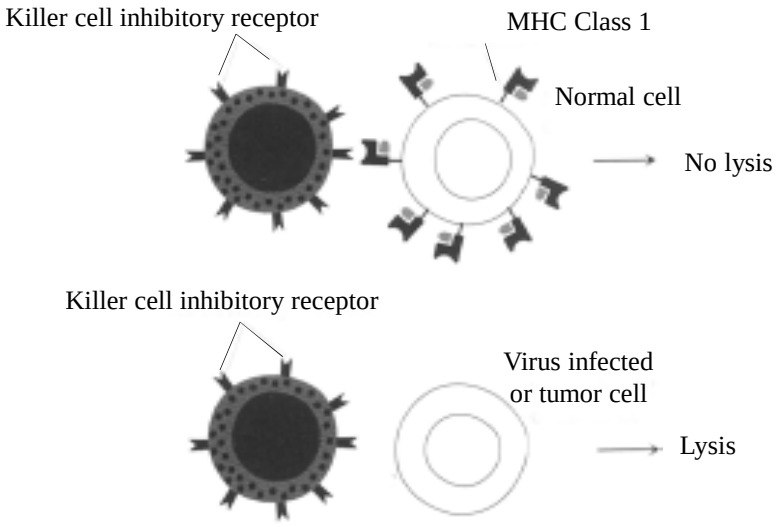


Fig. 4-3. Schematic representation of NK cell inhibitory receptors and killing.

4.2.15 SUMMARY

Resistance to infection is termed immunity.

Immunology is the science, which deals with the body's response to antigenic challenge.

Immunological mechanisms are involved in the protection of the body against infectious agents.

Immunity against infectious organisms is primarily divided into two types: Innate immunity and Acquired immunity.

Innate immunity is basic immunity that can be genetically transferred from one generation to other generation.

Natural immunity is the other name of innate immunity.

Natural immunity does not depend on previous contact with microorganisms.

Natural immunity is non specific immunity.

Innate immunity can be divided as under:

Species Immunity, Racial Immunity and Individual Immunity.

The appearance of same pattern of susceptibility to different infectious agents by individuals of same species is called species immunity.

The difference of physiological and biological mechanisms among the host species determines the multiplication capacity of the infectious organisms like poliomyelitis, measles, and leprosy in man.

When different races of a single species show differences in resistance mechanisms, it is termed as racial immunity. For example Negroes are resistant to yellow fever and malaria.

Homozygous twins show variation in the immunologic response to microbial infection; this is called as individual immunity.

4.2.16 KEY TERMINOLOGY

Antigen: An antigen is a substance which when introduced into the body induces the production of antibody (ies) and which react with antibody (ies)

Immune system: The system of the body, which governs all types of immune responses, is called the immune system.

Immune response: A specific immunological response in an individual brought about by antigens or immunogens.

4.2.17 SELF ASSESSMENT QUESTIONS

1. Explain about the non specific defense mechanisms.
2. Describe different types of immunity with suitable examples.
3. Write in detail about natural immunity.
4. Write short notes on:

a) Phagocytosis
b) Mechanical barriers
c) Role of chemical and biochemical inhibitors of immunity.

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UNIT – IV

4.3 ACQUIRED IMMUNITY

4.3.1 OBJECTIVES

The purpose of this lesson is to:
understand the nonspecific resistance mechanism to infection
know the mode of active and passive immunity
exemplify the genetic influences on acquired immunity
discuss the history of development of vaccine
list out vaccination agents

4.3.2 INTRODUCTION

The resistance showed by the host to the harmful effects of microbes and/or metazoan parasites is known as immunity.

Immunity is divided primarily into two types: Natural immunity and Acquired immunity.

Natural immunity is also called nonspecific immunity. It may be genetically passed from one generation to next generation. It does not depend upon the previous contact with any micro or macro organisms (pathogens).

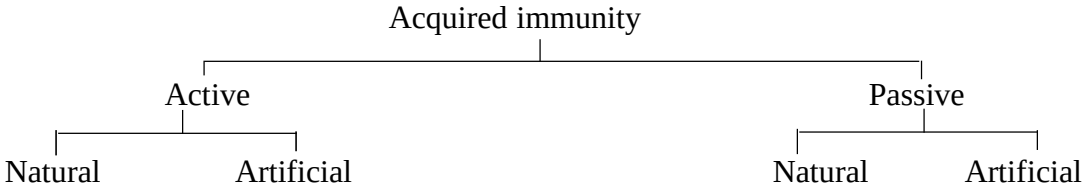
Acquired immunity is the immunity acquired during the lifetime of an individual due to the response of antigens or microbes or metazoan parasites. It is specific immunity. The hosts are able to develop the antibodies specific to the infection. Acquired immunity may differ from the natural immunity in the following aspects:

(1) It is not inherent in the body but is acquired during lifetime due to the exposure of antigens.

(2) It is specific for a single type of microorganism or metazoan pathogenic parasite.

4.3.3 DIFFERENT TYPES OF ACQUIRED IMMUNITY

Acquired immunity is divided into two types.



Acquired immunity may be:

(1) Active

(2) Passive

(1) Active immunity: It is the resistance developed due to antigenic stimulation. Active immunity may be attained as given below:

(A) Naturally acquired immunity: This is acquired by the host after one infection or recovery from disease or sub clinical infection after repeated exposure to small doses of the infectious organism.

(B) Artificially acquired immunity: It may be acquired artificially by inoculation of bacteria, viruses or their products. The mode of inoculation is as follows:

(a) Living organisms can be used after proper attenuation e.g., smallpox, BCG. Attenuation may be done as given below:

(i) Subjecting the organism to drying e.g. rabies virus vaccine.

(ii) Growing the organism at temperature higher than optimum e.g. Pasteur's

Anthrax vaccine is prepared by cultivating the organism at 42°C

(iii) By passing through animals of different species, e.g. variola virus through rabbit and calf.

(iv) By continued cultivation in the presence of antagonistic substance e.g. B.C.G Vaccine is prepared by prolonged cultivation of *Tubercle bacillus* (*Bacillus, Calmette* and *Guerin)* in medium containing bile.

(v) By making subcultures in artificial media e.g. *Streptococci*

(b) Organisms may be killed by heat or phenol without changing the antigenic structure of bacteria e.g. typhoid vaccine, cholera vaccine.

(c) Autovaccine

(d) Non specific protein therapy: Local cellular elements concerned with defensive mechanism respond to injection of non-specific protein substances like milk injection. They cause increased proliferation of cells resulting in the release of more antibodies.

(e) Toxoid: Bacterial inactivated toxin. The toxoid may be injected repeatedly in increasing doses e.g. diphtheria and tetanus toxoid.

(2) Passive immunity: Immunity is induced by immunization through prepared antibodies and body cells do not take any active part in the production of immunity. It may be obtained by the following ways:

(A) Natural passive immunity: Transmission of antibodies from the mother to fetus can occur through placenta. It may be by way of colostrum of mother and milk during first few months of life. Breast-fed infants resist establishment of enteroviruses in alimentary tract. These antibodies last for few weeks and protect infants from diphtheria, tetanus, measles, mumps, smallpox etc.

(B) Artificial passive immunity: Here immunization is passive and produced by injection of serum that has been obtained by active immunization. Antibodies remain protective for 10 days only. Serum of the following may be used:

(i) Antitoxic serum: It is produced by injection of toxoid into horse in increasing doses till the blood is rich in circulating antibodies. The animal is bled and serum is separated. This serum contains prepared antibodies. Examples of antitoxic sera are diphtheria, tetanus, etc.

(ii) Antibacterial serum: It is obtained by injection of bacteria into animals and serum is collected after the production of antibodies e.g. pneumococcal, meningococcal, anthrax, dysentery etc.

(iii) Convalescent serum: It is obtained from convalescent (persons who suffered from infection and come to normal life) patient. It is also called convalescent serum. Such serum is used in the treatment of measles, poliomyelitis, infective hepatitis etc.

Acquisition of passive and active immunity is as follows:

Type	Acquired through
Active immunity	Natural infective vaccines
	Attenuated organisms
	Inactivated organisms
	Purified microbial macromolecules
	Toxoids
Passive immunity	Natural maternal antibody
	Immunoglobulin
	Antitoxin

4.3.4 DIFFERENCES BETWEEN ACTIVE AND PASSIVE IMMUNITY

	Active immunity	Passive immunity
1	Host's immune system actively participates in the production of antibodies.	No participation by host's immune system. Host receives antibodies passively.
2	Induced by infection or by contacts with immunogen e.g. vaccines.	Conferred by the administration of ready-made antibodies.

3	Long lasting and effective protection.	Temporary and less effective protection.
4	Immunity effective after lag phase.	Immunity effective immediately.
5	Cells acquire immunological memory. Subsequent challenge is more effective.	No immunological memory retained by cells.
6	Negative phase may occur.	No negative phase.
7	Not applicable to immuno-deficient hosts.	Applicable to immuno-deficient hosts.
8	Used for prophylaxis to increase the resistance of body.	Used for treatment of acute infection.
9	Both cell mediated and humoral immunity are involved.	Exclusively humoral immunity is involved.
10	No inheritance of immunity.	May be acquired from mother.

4.3.5 GENETIC INFLUENCES

Natural and acquired immunity are under the influence of genetic components. Disease susceptibility is in some way, related to genes closely associated with the major histocompatibility complex particularly the HLA-D region located on chromosome 6 in humans. This region is suspected of carrying immune response genes. An example of correlation is association of HLA-B27 with ankylosing spondylitis and juvenile arthritis in humans. The basis for this association of specific genes with specific disease susceptibility is not clear. The possible implications are as given below:

(a) HLA antigens may serve as cell surface receptors for viruses or toxins.
(b) HLA antigens may be incorporated into viral coat protein.
(c) HLA antigens may be linked to immune response genes
(d) HLA antigens may cross-react with the antigens of bacteria, viruses or other agents to trigger autoimmune responses.

4.3.6 LOCAL IMMUNITY

When infections are localized or when they are combating with other infections at the primary entry of pathogens – the humoral immune response may be operative. The antibody titer may be increased but it is not high enough to prevent infection.

E.g. influenza immunization with killed vaccine elicits humoral response but can not prevent the entry of pathogen. Natural infection or live virus vaccine given intranasally may provide local immunity.

4.3.7 HERD IMMUNITY

The overall level of immunity in a community in the control of epidemic diseases is called herd immunity. When large proportion of individuals in a community are immune to certain epidem-

ics, it means the community is immune certain pathogens - the suitable level and role of herd immunity. When herd immunity is low, epidemics spread in the community.

4.3.8 HISTORICAL ASPECTS OF VACCINATION

It is interesting to know the history of development of vaccine. The history of development of immunization is as follows:

1721 – Variolation by Lady Montague

1793 – Smallpox immunization

1881 – Development of Pasteur's antirabic vaccine and also demonstration of efficacy on anthrax vaccine.

1891 – Development of diphtheria antitoxin

1896 – Vaccine against typhoid fever

1904 – Production of tetanus antitoxin

1920 – Diphtheria antitoxin floccules

1930 – Diphtheria toxoid

1951 – Yellow fever live vaccine

1955 – Polio killed vaccine (SALK)

1960 – Polio live vaccine (SABIN)

1970 – Live vaccine of Rubella and Mumps. Also measles vaccine (killed as well as live).

4.3.9 VACCINATION IN PRACTICE

The practice of vaccination or immunization began with the observation that individuals who recovered from certain diseases were often protected against the same illness. Edward Jenner (1798) first introduced the use of vaccination. Vaccine of cowpox was prepared to protect individuals against small pox. Later Louis Pasteur observed that attenuated rabies virus would protect individuals against rabies.

Several routes can administer agents used for immunization.

Injection is the most common method used for vaccination. Injections can be given intramuscularly or intradermally or subcutaneously.

Immunizing agents can be administered orally (through mouth). Savin polio vaccine, alive attenuated virus preparation is often given orally.

Agents of immunization can also be administered through intranasal route.

Common agents employed for passive immunization are as given below:

DISEASE	AGENT
Diphtheria	Horse antitoxin
Hepatitis A and B	Pooled human immune gamma globulin

Measles	Pooled human immune gamma globulin
Rabies	Pooled human immune gamma globulin
Snake bite	Horse antivenin
Tetanus	Pooled human immune gamma globulin or horse antitoxin

4.3.10 VACCINES

Live attenuated or dead organisms may be used for artificial active immunization. Killed vaccines produce relatively less immunity. The live vaccine contains major immunizing antigen and longer prolonged immunity as compared to killed vaccine. Live vaccine is quite cheap and may be given in small dose. The vaccines are classified as given below:

(A) Live attenuated vaccines: Attenuation of organisms is achieved by aging of culture, culture at high temperature, passage through another host species, drying (rabies) and selection of mutants (temperature sensitive). E.g. small pox, yellow fever, polio, BCG, plague, brucellosis, mumps, rubella and measles.

(B) Killed vaccines: heat, formalin, phenol, and alcohol and ultraviolet light kill Organisms. Killed vaccines may be preserved in phenol, alcohol or Merthiolate. e.g. whooping cough, TAB, cholera, polio (Salk), antirabic and measles.

(C) Toxoids: Toxoids are exotoxins. These are treated with formalin to destroy their toxicity but retaining their immunogenecity e.g. formal toxoid of diphtheria and tetanus.

4.3.10.A REPRESENTATIVE CURRENTLY USED BACTERIAL VACCINES

1. Diphtheria toxoid

   It is available as a fluid toxoid of *Corynebacterium diphtheria.* It is commonly combined with tetanus toxoid and pertussis vaccine as DPT vaccine.

2. Pertussis vaccine

   It is administered for whooping cough vaccination. It is heat-killed preparation of *Bordetella pertussis* organisms.

3. Tetanus toxoid

   It is available as fluid toxoid. It is a preparation of inactivated *Clostridium tetani.*

4. Bacille Calamette –Guerin (BCG) vaccine

   This is a vaccine used against tuberculosis. It is a live attenuated strain of *Mycobacterium bovis.*

5. Typhoid vaccine

   This vaccine is effective against typhoid fever. It is a suspension of heat or acetone killed *Salmonella typhi.*

6. *Haemophilus influenza*

   This vaccine is prepared from the polysaccharide capsule of *H. influenza* type b. This is used against bacterial meningitis.

4.3.10.B REPRESENTATIVE CURRENTLY USED VIRAL VACCINES

1. Rubella (German measles) vaccine
   This vaccine contains live attenuated virus grown in tissue culture (e.g. Rabbit kidney, duck embryo and human diploid cells). This is used to provide immunity against measles.

2. Measles and mumps vaccines

   These vaccines are live attenuated viruses grown in chick embryos. Available to provide immunity against measles and mumps.

3. Influenza virus vaccine

   This vaccine consists of whole virus or disrupted virus products grown in chick embryo. Formalin or UV light inactivates the viruses or virus products.

4. Polio myelitis vaccine

(a) Salk vaccine

   Also called inactivated polio vaccine (IPA). The virus is grown in tissue culture (e.g. monkey kidney) and inactivated by formalin or UV light.

   This form of the vaccine is given intramuscularly to provide immunity against paralytic or systemic disease. It cannot offer protection against poliovirus infection.

(b) Sabin vaccine

   It is a live attenuated oral vaccine (opv) with virus grown in tissue culture (e.g. monkey kidney or human diploid cells). This vaccine is able to confer both intestinal and humoral immunity. This vaccine provides more complete protection than Salk vaccine.

5. Rabies vaccine

   This is available in two forms

a) Virus grown in duck embryo. Virus is inactivated with –propolacetone for use as vaccine. Sometimes this vaccine may bring neurologic complications

b) Virus grown in nerve tissue (e.g. human lung fibroblasts). Virus is inactivated with tributyl phosphate. It is the preferred vaccine to protect against rabies.

6. Hepatitis B vaccine

   It is composed of inactivated, alum-adsorbed hepatitis B surface antigen (HB-Ag) particles. The antigen particles are purified from plasma of human carriers. HB.Ag (vaccine) produced from a genetically engineered strain of *Sacharomyces cerevisiae* is also used to protect against hepatitis B.

4.3.11 SIDE EFFECTS OF VACCINATION

Local sepsis, serum hepatitis, fever, soreness at injection site, arthralgia after rubella vaccine, convulsions in pertussis vaccine and allergic reaction may occur as side effects after vaccination.

Vaccination schedule that can be adopted is as follows:

0 to 1 month	BCG
3 months	DPT I, oral polio vaccine I
4 to 5 months	DPT II, oral polio vaccine II
5 to 6 months	DPT III, oral polio vaccine III
1 1/2 years	Booster DPT , oral polio vaccine IV
2 years	Typhoid vaccine
5 years	Tetanus toxoid
School entry	Typhoid vaccine
12 to 14 years	Tetanus toxoid
	Diphtheria toxoid
	Rubella vaccine

4.3.12 RECOMMENDED SCHEDULE TO ACTIVE IMMUNIZATION OF CHILDREN AND ADULTS

Age	**Vaccine**
1 month	Hepatitis B
2 months	Hepatitis B, Diphtheria and tetanus and pertussis (DTP) Haemophilus influenza b (Hib), inactivated polio virus (IPV), Rota virus (RV)
4 months	DTP, Hib, IPV, Rotavirus(RV)
6 months	Hepatitis B, DTP, Hib, IPV, RV
12 months	Hepatitis B, Hib, Oral polio virus vaccine (OPV), Measles, mumps, rubella (MMR)
15 months	Hepatitis B, Hib, OPV, MMR, Varicella vaccine for susceptible children
4 – 6 years	DTP, OPV, MMR
11 – 12 years	Hepatitis B, MMR, Varicella
25 – 64 years	Measles, rubella
> 65 years	Influenza, pneumococcal disease

4.3.13 SUMMARY

Acquired immunity is a specific immunologic response.

Specific immunity is acquired following response to a particular infectious agent.

The infectious agent may be administered deliberately (vaccination) or accidentally (infection).

Acquired immune response can be mounted by the induction of antibodies or by the production of sensitized lymphocytes specific for that microbe.

Natural or artificial processes may acquire acquired immunity.

When a person recovers from disease it is naturally acquired immunity

Vaccination (immunization) for the induction of immune response is artificially acquired immunity.

Recovery from disease is naturally acquired, active immunity.

Placental transfer of antibody is natural acquired, passive immunity.

Vaccination is artificially acquired, active immunity.

Administration of antitoxin is artificially acquired, passive immunity.

Specific immunologic responses are mediated by humoral and cell mediated immunity (CMI).

The humoral and CMI are interrelated and interdependent mechanisms.

Bursa confers humoral immunity (HI) or bone marrow derived (B) lymphocytes or B cells.

Cell mediated immunity (CMI) is conferred by thymus derived (T) lymphocytes or T cells.

In 1798, Edward Jenner introduced the use of vaccination with cowpox to protect individuals suffering due to smallpox.

Active or passive immunization can achieve immunity to infectious microorganisms.

In developing a vaccine, the immune system must be activated by infectious agents or their products.

Immunizing agents can be administered by injection or orally or intranasally.

The agents for inducing active immunity include the inoculation o microbes either in killed or in attenuated states; they do not cause disease but stimulate the production of antibody.

Active immunization can be induced by natural infection with a microorganism or by artificial administration of vaccine.

Three types of vaccines are currently used in humans: attenuated microorganisms, inactivated (killed) microorganisms or purified macromolecules.

Currently used Bacterial vaccines are Diphtheria toxoid, pertussis vaccine, Tetanus toxoid, Bacilli Calamette-Guerin (BCG) vaccine, Typhoid vaccine, Haemophilus influenza etc.

Currently used viral vaccines are Rubella vaccine, Measles and mumps vaccines, Influenza virus vaccine, poliomyelitis vaccine (Salk vaccine and Sabin vaccine), Rabies vaccine, Hepatitis B vaccine etc.

4.3.14 KEY TERMINOLOGY

Acquired immune response: The response of antigen-specific lymphocytes to antigens.

Bone marrow: The site of hematopoiesis. Within the bone marrow the stem cells give rise to the cellular elements of blood, including red blood cells, monocytes, polymorphonuclear leukocytes, platelets and lymphocytes.

Bronchus associated lymphoid tissue (BALT): Secondary lymphoid organs connected to the bronchial tree.

Burrs fabrics: It is an out pocketing of cloaca. Site of development of B cells in birds.

Cell mediated immunity (CMI): Immune reaction mediated by T cells. It is also called delayed hypersensitivity.

H-Z complex: The major histocompatability complex (MHC) situated on chromosome 17 of the mouse. It contains sub regions K, J, D and L.

Histocompatability: The ability of tissues to get along immune response; the ability to identify in all transplantation antigens. Those antigens are collectively referred to as histocompatible antigens.

Major histocompatability complex (MHC): A cluster of genes encoding polymorphic cell molecules. These are identified as MHC class I and MHC class II. These are involved in interactions with T cells. These molecules play an important role in transplantation injection.

Sensitization: Stimulation of immune response to antigen, prior to immunization.

Toxoid: A non-toxic derivative of a toxin used as an immunogen. This can be used for the induction of antibodies capable of cross-reacting with the toxin.

Vaccination: Any protective immunization against a pathogenic microbe or macrobe. The common reference is to immunization against smallpox with the less virulent cowpox virus.

Virus: An organism having a protein coat and DNA or RNA genome. It requires a host cell for replication.

4.3.15 Self Assessment Questions

1. What are the advantages of Sabin polio vaccine compared with the Salk vaccine?
2. What are the advantages of using vaccines?
3. Explain the acquired immunity.
4. List the vaccines which are currently used to protect against various diseases
5. Write short notes:
   a. BCG vaccine
   b. CMI
   c. Active immunization

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UNIT – IV

4.4 ANTIGENS – STRUCTURE AND MAJOR CLASS OF ANTIGENS

4.4.1 OBJECTIVES

The purpose of this lesson is to:
understand the structure of antigen
know the properties of immunogenecity
describe the antigen specificity.

4.4.2 INTRODUCTION

An antigen is a substance which when introduced into an animal/human body it will provoke the production of antibodies and/or reacts with antibodies in the body. Immunogens are substances, which can induce a detectable immune response when introduced into an individual. This property of immunogen is known as immunogenecity.

4.4.3 PROPERTIES OF ANTIGENS

An antigen must possess 2 properties:

1. Immunogenecity: It is the inherent ability of a substance (immunogen) to induce a specific immune response, resulting in the formation of antibodies or immune lymphocytes.
2. Specific reactivity or antigenicity: It is the property of a substance (antigen) to result specifically with the (preformed) antibodies or cells (sensitized) – immunological reactivity.
    a. This property of antigen is more important in biology.

b. One must remember that immunogenic substances are always antigenic, whereas antigens are not necessarily immunogenic.

4.4.4 HAPTANS

Haptans are partial antigens that are antigenic but not immunogenic (dinitrophenol and penicillin).

1. Haptans can not cause the production of immunolymphocytes or antibodies, but can react.
2. Haptans are usually smaller molecules, which are too small to be considered as immunogens. If they are coupled to a larger carrier molecule, however, they become immunogenic. Carrier molecules may be albumins, globulins or synthetic polypeptides.

4.4.5 ANTIGENIC DETERMINANTS OR EPITOPES

The smallest unit of antigenicity is known as antigenic determinant or epitope. These epitopes or determinant groups are present on the antigen or within the antigen. Antibodies react with these epitopes. These epitopes may be composed of sequential (Fig. 4- 4) or non-sequential amino acids (Fig.4 – 5).

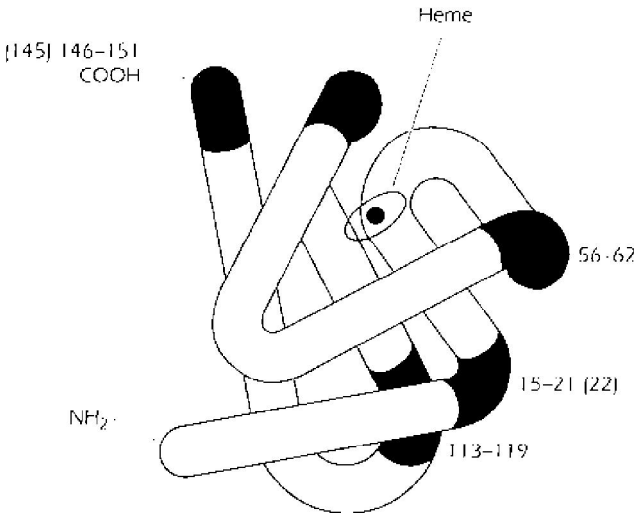

Fig. 4-4. An example of an antigen (sperm whale myoglobin) containing five sequential B-cell epitopes shown in black.

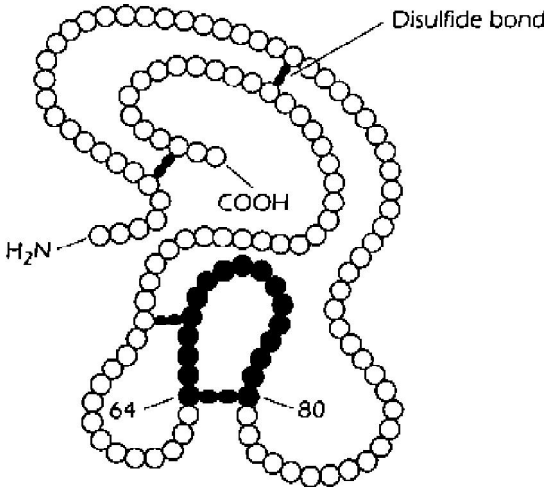

Fig. 4-5. Diagram of an antigen showing amino acid residues (circles), which form a nonsequential epitope "loop" shown in black resulting from a disulfide bond between residues 64 and 80.

1. Epitopes determine the specificity of the molecule and are somewhat induce an antibody response.
2. Haptans and epitopes are similar, but while a haptan is artificially added to a molecule. An epitope is an integral part of a native molecule. Both of them induce antibody formation.
3. Antigens are multivalent – that is they may have hundreds of epitopes, some specifying antibody 'A', others antibody 'B' and so on. The valence of the antigen will be equal to the total number of epitopes the antigen possesses.
4. Antigen molecules can be artificially manipulated by adding or taking away epitopes. When the epitope is changed, its antigenicity is altered.

a. Altering these epitopes produces new antigens. Conjugating haptans to the molecule can do this.

b. A classic example in human medicine is the allergic response of some persons to penicillin.

   A derivative of penicillin, penicilloic acid acting as haptan, combines with body protein and elicit an immune response that can be harmful, even life threatening.

5. The epitopes on an antigen may be linear or conformational.

4.4.6 PROPERTIES OF IMMUNOGENECITY

The degree of immunogenicity of a molecule is influenced by several factors. The relationship can be expressed algebraically by the following formula:

Immunogenicity = (Foreigness) (Chemical complexity) (molecular size)

4.4.6.A CHEMICAL NATURE OF IMMUNOGENS

Macromolecular proteins are mostly immunogenic. But polysaccharides, lipoproteins, lipopolysaccharides, synthetic polypeptides and other synthetic polymers are also immunogenic under suitable conditions. Few nucleic acids and lipids do not appear to be immunogenic.

4.4.6.B FOREIGNNESS

An antigen must be foreign (alien) to the host when it is administered. Basing on the nature and characters, antigens are divided into 6 types:

1. Autologous antigens are found within the same individual, i.e. it is not foreign. A skin graft from an individual's thigh to his own back is autograft.
2. Syngeneic antigens are found in individuals of an inbred strain (or between identical twins) who are, then, genetically identical. A graft between members of an inbred strain is a syngeneic graft or an isograft.
3. Allogeneic or homologous antigens are found between same species but between different individuals. For example, a kidney transplant from mother to daughter is called a homograft or allograft. Isoantigens (alloantigens) occur in certain members of the same species and not in others. This term is used to designate the and B blood group antigens.
4. Xenogenic or heterologous antigens are found across species boundaries. For e.g. a transplant of monkey kidneys to human is called a xenograft.

   Heterogenetic (heterophil) antigens are those which occur in different species. The spirochete that causes Syphilis has a heterogenetic antigen similar to a haptan called cardiolipin found in beef heart muscle.
5. Sequestered antigens: Antibodies are not ordinarily made to autologous brain or cornea protein because these substances do not come in contact with antibody producing cells since they can not reach the antibody forming tissues (i.e. they are sequestered (kept away or apart from other antigen)
6. Tissue specific antigens: Certain antigens are commonly present in certain organs and particularly or specially in certain other organs.

Thyroid has an organ specific antigen - thyroid antigen, thyroglobulin. Any thyroid from any species contains this unique antigen.

Another example is basic protein. It is present only in the brain (it does not exist in any other organ) tissue regardless of species.

4.4.6.C CHEMICAL COMPLEXITY

With the exception of pure lipids, most organic chemical groupings can be immunogens.

1. Majority of immunogens is proteins. Because they have the largest array of potential building blocks (amino acids),
    a) Bacterial and mammalian cells are also strong immunogens.
    b) Lipoproteins are special types of protein immunogens.
2. Polysaccharides: Most polysaccharides are haptans or incomplete immunogens because they do not possess sufficient chemical diversity.
3. Glycoproteins: the blood group antigens A and B and the Rh antigens best illustrate the immunogenecity of glycoproteins.
4. Polypeptide immunogens include hormones (insulin and growth harmones) and synthetic compounds such as polylysine. These are usually weakly immunogenic.
5. Nucleic acids are non-immunogenic. Nucleoproteins are stronger immunogens because the nucleic acid is coupled to protein.
6. Lipids (E.g. cardiolipin) are also non-immunogenic, although a few can function as haptans.

4.4.6.D MOLECULAR SIZE

Although there are exceptions, the large molecule acts as a better immunogen.

4.4.7 ACTIVE IMMUNOGENS

Active immunization is due to the production of protective antibodies against the virulent pathogens.

A. There is 4 types of vaccines:

1. Killed organisms (e.g. *Typhoid bacilli*)
2. Attenuated or altered live agents (e.g. Sabin polio virus)
3. Detoxified toxins (e.g. diphtheria and tetanus toxoid)
4. Artificially assembled microbial components (e.g. hepatitis B virus sub units)

B. Examples of active antigens:

1. All gram-negative, flagellated bacteria (e.g. *Salmonella typhi*) contain 2 types of antigens, to which the host organism makes different antibodies. The two antigens are:

a. The ‘H’ antigens, referring to the flagella

b. The ‘O’ antigens, referring to the body of the organism

2. Any bacterial cell is a good immunogen. Actually, hundreds of different antibodies are formed in response to the bacterial cell.
3. Diphtheria and tetanus toxins are also good immunogens. Protection from them can be obtained if antibodies to these toxins are produced – since the toxins cause the disease.

4.4.8 ANTIGEN SPECIFICITY

A. Haptans: Haptans can be side chains of benzene rings, or substituted benzene rings, that are attached to protein molecules, and, hence rendered immunogenic.

B. Capsular antigens: Pneumococcus, one of the major causes of local pneumonia consists of capsular antigens. There are above 80+ different immunologic types of *Pneumococcus*. An antibody of one type does not react with an antigen of another type. These capsular polysaccharides are structurally different which accounts for their antigenic differences.

C. Adjuvants: Adjuvants are substances that enhance the immunogenecity of molecules without altering their chemical composition. Non specific stimulation of the immune response can occur via adjuvants.

4.4.9 SUMMARY

Antigen is a substance which when administered into the body stimulates the production of an antibody or it reacts with antibody.

The property of the antigen is determined by a number of properties:

Foreignness, Size, Chemical nature, Antigen specificity, Tissue specificity etc.

Acquired immune responses arise as a result of exposure to antigens.

The compound that evokes the response is referred to either as "antigen" or as "immunogen".

An antigen is any agent capable of binding to lymphocytes and antibodies.

Immunogen is any agent capable of inducing, an immune response.

All immunogens are antigens.

But not all antigens are immunogens.

The smallest unit of antigen that is capable of binding with antibodies is called an antigenic determinant or epitope.

Epitopes are capable of reacting with immune components.

The immune response against antigens involves the production of antibodies or the generation of cells with specificity directed against most or all the epitopes.

Substances called haptans fail to induce immune response.

Haptans are not immunogenic unless they are conjugated to high molecular weight, physico-chemical complex carriers.

Al biochemical families of compounds – carbohydrates, lipids, proteins, and nucleic acids –as well as drugs, antibodies, food additives, cosmetics and small synthetic peptides induce immune response.

Polysaccharides are not always immunogenic.

Glycoproteins elicit an immune response.

An example of antigenecity of polysaccharides is the immune response associated with ABO blood groups.

Lipids are rarely immunogenic.

Nucleic acids are poor immunogens by themselves. But they become immunogenic when they are conjugated to protein carriers.

All proteins are immunogenic.
The most common immune responses are those to proteins.

4.4.10 KEY TERMINOLOGY

Allogenic: Genetically dissimilar within the same species.

Antibody: Serum protein formed in response to immunization.

Antigen: Any foreign substance that is specifically bound by antibody or lymphocytes

Antigen-binding site: The part of an immunoglobulim molecule or T-cell receptor that binds antigen specifically.

Antigenic determinant: A single antigenic site or epitope on a complex antigenic molecule or particle.

Antigen receptor: The specific antigen binding receptors on B or T lymphocytes.

Autograft: A tissue transplant from one area to another on a single individual.

Autologous: Derived from the same individual, self.

Determinant: Also termed epitope. Part of the antigen molecule that binds to an antibody-combining site or to a receptor on T cells.

Epitope: An alternative term for antigenic determinant.

4.4.11 SELF ASSESSMENT QUESTIONS

1. Describe the properties of antigens
2. Explain in detail the major classes of antigens.
3. Describe the details of requirements for immunogenecity
4. Write short notes on
   a. Epitope
   b. Haptan
   c. Immunogen

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Unit - V

5.1 ANTIBODIES –STRUCTURE AND PROPERTIES OF IMMUNOGLOBULIN IgG

5.1.1 OBJECTIVES

The purpose of this lesson is to:

Describe the structure of immunoglobulin G
Discuss the biological properties of IgG
Know the chemical properties of IgG
Exemplify the importance of IgG in human system

5.1.2 INTRODUCTION

Antibodies are globulin proteins. Antibodies are able to bind antigen. Tiselius (1939) first shown the association of antibody activity with the gamma globulin fraction of serum by hyperimmunizing rabbits with pneumococcal polysaccharides to produce high titers of circulating antibodies.

The glycoproteins present in the serum gamma globulins are called immunoglobulins (antibodies). Antibodies are produced by B-lymphocytes (B cells) or plasma cells in response to exposure to antigen.

5.1.3 STRUCTURE OF THE BASIC UNIT OF IMMUNOGLOBULIN

The basic structural unit of immunoglobulin (Ig) molecules consists of 4 polypeptide chains linked covalently by disulfide bonds (Fig. 5.1). The basic structural unit is termed as monomere. Polypeptide chains are composed of amino acids and the amino acids are arranged in a sequence. The sequence of amino acids identifies a given protein and distinguishes it from any other molecule. The four-chain structure is composed of two identical light (L) and two identical heavy (H) polypeptide chains.

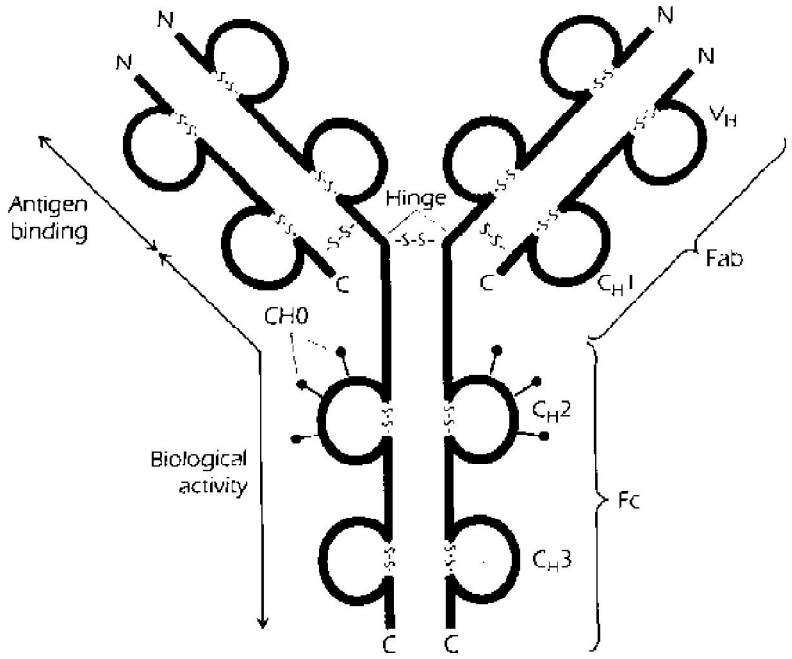

Fig. 5.1. Schematic representation of an immunoglobulin molecule showing immunoglobulin-fold domains formed by intrachain disulfide bonds.

5.1.3.A LIGHT CHAINS

Light chains have a molecular weight of approximately 23000.

Light chain is composed of 200 amino acids.

Light chains are common to all immunoglobulin classes.

Light chains are off two types – kappa(κ) or lambda (λ). These are different in their structure.

In humans the proportion of κ to λ chains in immunoglobulin molecule is about 2:1.

An immunoglobulin molecule may contain either identical κ or λchains but never both.

5.1.3.B HEAVY CHAINS

Heavy chains have a molecular weight approximately twice that of light chains (50000 – 75000) and twice the number of amino acids (about 400). There are five antigenitically distinct (isotypes) heavy chains are present: gamma (γ), alpha (α), mu (μ), detta (δ) and epsilon(e) chains. These are having different structures in the carboxy terminal portion of heavy chains.

In man, five major isotypes can be distinguished:

immunoglobulin G (IgG – contains γ chain), IgA (contains α chain), IgM (contains μ chain), IgD (contains δ chain), IgE (contains e chain).

Heavy chain classes are subdivided in subclasses of molecules.

5.1.3.C DISULFIDE BONDS

Immunoglobulins possess two types of bonds.

1. Interchain bonds:

   These bonds occur between heavy chains (H – H), heavy and light chains (H – L) and light chains (L – L).

   H – H bonds occur primarily in the hinge region.

   These bonds vary in number from 1 – 15.

   The bond variance depends on the class and subclass of the Ig molecule.

   These bonds also occur in the carboxyterminal portion of the heavy chain.

   H – L bonds occur in most Ig molecules. This bond is absent in IgA_2 molecule.

   Heavy and light chains are attached by one disulfide bond.

   L – L bonds occur only in pathological conditions.

2. Intrachain bonds:

   These bonds are stronger than interchain bonds.

   The number of these bonds varies depending on the type of chains.

   For eg. light chains have two, human γ, α, δ heavy chains have four and human μ and e chains heavy chains have five. Basing on the number of intrachain disulfide bonds, the Ig molecule is divided into a number of regions or domains.

5.1.4 IMMUNOGLOBULIN SEGMENTS

Each heavy and light chain consists of 2 segments: the variable (v) region and the constant (c) region.

1. The variable region

   It shows a wide variation in amino acid sequence in the amino or N terminal portion of the molecule.

2. The constant region

   It shows an unvarying number of amino acid sequences in the carboxy or C terminal portion of the molecule, except for minor inherited differences.

3. Hyper variable regions

   These are the areas of high variability in the variable region of heavy (V_H) and light (V_L) chains. These hyper variable regions are also called hot spots. Hyper variable regions are most intimately involved in formation of antigen binding site.

   There are at least three hypervariable regions in both V_H and V_L regions (hv1 – hv3)

5.1.5 DOMIANS

Each immunoglobulin chain consists of a series of globular homology regions or domians enclosed by disulfide bonds.

1. Each heavy chain possesses 4 or 5 domians – one in the variable region (V_H) and three or four in the constant region CH^1, CH^2, CH^3 and CH^4.

   The γ, α and δ chains have 4 domians (one variable and three constant)

   The μ and e chains have 5 domians (one variable and four constant)

2. Each light chain consists of two domians- one in the variable region (V_L) and one in the constant region (C_L)
3. Domians consist of about 110 amino acid residues

5.1.6 FRAGMENTS

All immunoglobulin molecules consist of a basic unit of four polypeptide chains, two identical H chains and two identical L chains, held together by a number of disulfide bonds.

Proteolytic enzymes can be used to degrade immunoglobulin molecules into 2 or 3 segments. The structure can be studied in detail with their fragments. The primary agents (enzymes) used are papain and pepsin.

When monomeric basic unit is treated with the enzyme papain, it splits into three fragments of approximately equal size at the hinge region – two Fab fragments (fragments-antigen binding) and one Fc fragment (fragment crystallizable)

Each Fab fragment contains an entire light chain and V_H and C_H1 domians of the heavy chain (or F_d fragment). These fragments can bind but can not precipitate the antigen. Therefore, these fragments are monovalent (possessing only one combining site each. Fd, the amino terminal half of the heavy chain – it binds with the Fab fragment) (Fig. 5-2).

One Fc fragment contains the carboxy terminal portion of the heavy chain. This portion of molecule contains carbohydrate. It is capable of binding compliment. It dictates whether a given immunoglobulin cross the placenta.

When monobasic unit of immunoglobulin is treated with the enzyme pepsin, it results in the digestion of most of the Fc fragment leaving one large fragment.

The $F(ab)_2$ fragment has two antigen-combining sites. It is bivalent. It has got the capacity to bind and precipitate the antigen.

5.1.7 HINGE REGION

The hinge region is the portion of the heavy chain between the C_H1 and C_H2 domians. The hinge region is considered as a separate domian. Because it is not homologous to any of the other known domians.

This region is highly susceptible to fragmentation when treated with enzymes:

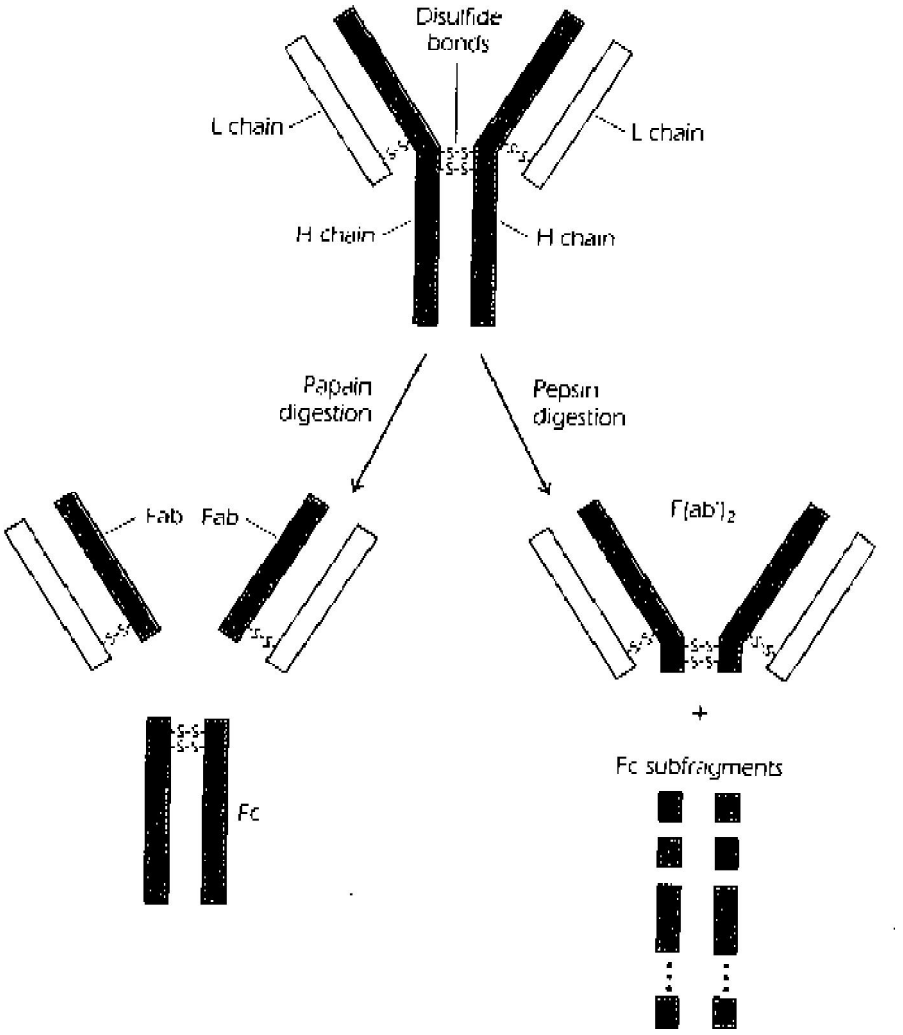

Fig. 5.2. Proteolytic digestion of immunoglobulin using papain and pepsin.

When treated with papain, the enzyme directly acts on the hinge region cleaving the molecule on one side of the disulfide bonds. When treated with pepsin, it acts on the opposite side of the disulfide bonds, degrading the molecule just below the hinge region.

5.1.8 S VALUE

The sedimentation coefficient or S value of protein molecule will be determined by via ultracentrifugation. The sedimentation coefficient is expressed in Swedberg units (S).

The S values of immunoglobulins range from 7S to 19S.

The S value depends on the size and density of the particle, as well as the density and viscosity of the medium.

5.1.9 ANTIBODY DIVERSITY

It has been reported that a man can produce upto 109 different antibodies. Immunologists have argued that whether man is born with the ability to make these antibodies or if during life the human body learns to make these proteins.

Isotypes: Immunoglobulinsa fall into 5 classes or isotypes based on certain structural differences. In man, five major isotypes can be distinguished: Immunoglobulin M (I_gM), I_gG, I_gA, I_gE and I_g D.

Allotypes: These are genetically inherited variations. Genetic variants of plasma proteins existing in different individuals within the same species that are inherited in a mendelian manner. Allotypes are found both in light and heavy chains and are localized in constant region.

Idiotypes: Genetic variants associated with the immunoglobulin antigen combining sites. Each idiotype is clonally derived i.e. one B lymphocyte alone expresses one unique idiotype.

5.1.10 CHARACTERISTICS OF IMMUNOGLOBULIN G

I_gG is the major immunoglobulin found in human and animal serum. It accounts approximately 80% of serum immunoglobulins.

5.1.11 STRUCTURE

IgG is a monomere consisting of identical pairs of heavy and light chains linked by disulfide bridges. There are four subclasses, which have been identified based on heavy chain differences. Heavy chains are Y_1, Y_2, Y_3 ,and Y4, which correspond to IgG_1, IgG_2, IgG_3 and IgG_4.

5.1.12 BIOLOGICAL PROPERTIES (TABLE 1)

Immunoglobulin G is the only class of antibody that clearly passes through placenta. The ability of IgG to cross the placenta provides a major line of defense against infection for the first weeks of a child's life. Normally, the human fetus begins to receive significant quantities of maternal IgG transplacentally at around 12 week's gestation. Small amounts of IgG can be found as early as 8 weeks.

IgG is the major antibody produced in secondary immune response.

IgG has the capacity to diffuse into the extravascular body spaces more readily than other immunoglobulins.

It plays an important role in phagocytosis.

The phagocytic cells (e.g. macrophages and neutrophils) have receptors for the Fc fragment of IgG, primarily IgG_1 and IgG_3.

5.1.13 CHEMICAL PROPERTIES

IgG has a molecular weight of 150000

It has an S value of 7 S

IgG molecules are capable of fixing compliment, except for Ig4

The compliment binding site is in the CH^2 domian.

Table 1: Structural Characteristics of immunoglobulin G

H chain	Y
H chain sub classes	Y_1, Y_2, Y_3, Y_4
Molecular weight	150,000
S value	7
Valence	2
J chain	-
Gm allotypes	+
Am allotypes	-
Km allotypes	+
L chain	r, λ

Abbreviations: H chains – heavy chain; S value –Sedimentation coefficient, Gm –genetic marker on the chain, Am –genetic marker on the α chain, Km –genetic marker on the χ chain, L chain –light chain

5.1.14 GENE ORGANIZATION

Immunoglobulin gene organization is studied in order to establish the role of immunoglobulin inheritance. Variability in the structure of immunoglobulin if the individual chains.

1. Separate diversity exists for each chain since they are coded for on separate chromosomes.

In humans they are:

Heavy chain chromosome 14
Light (λ) chain chromosome 22
Light (χ) chain chromosome 2

2. The heavy and light chains vary markedly in amino acid composition of their amino terminal portion (variable region).

   Analysis of immunoglobulin genes has revealed that the variable and constant regions are separately encoded and located on different fragments of DNA.

3. Light chain gene organization:

   Three genes code for each immunoglobulin light chain

   a) One gene controls the variable domian
   b) A second gene codes for a small peptide that occurs at the junction of the variable and constant regions.
   c) A third gene dictates the amino acid sequence of the constant region.

4. Heavy chain gene organization:

   Three genes code for heavy chain gene organization.

   Heavy chain genes are similar to that of light chain genes.

   Heavy chain gene organization is more complex. 3 segments of DNA join to generate a gene coding for the variable portion of the heavy chain: V_H, D_H, and JH. The additional germ line gene segment is designated the diversity (D_H) gene region.

The D_H segment accounts for the third hypervariable region of the heavy chain. The D segment in configuration with the variable and joining segments, is utilized to generate the enormous diversity of the heavy chain.

5.1.15 THEORIES OF ANTIBODY FORMATION

The ability of the immune system to recognize antigens depends on the antibodies generated by B cells and on the antigen receptors expressed by T cells. The ways by which B cells and T cells recognize the antigens are different, but both cell populations are capable of recognizing a wide range of antigens. This means that the immune system is capable of generating a great diversity of antibodies and T cell antigen receptors (TCRs).

Genetic mechanisms contribute to the generation of the total pool of antibody specificities of a given host.

1. Germ line theory: As a result of somatic recombination in the DNA and of RNA Splicing – more than 1500 varieties of light chains and more than 5000 varieties of heavy chains can be produced.

2. Somatic mutation theory: During lymphocyte differentiation – a small number of gene diversity by point mutations or by recombination events.

Present evidence suggests that both germ line genes and somatically mutated genes contribute to antibody diversity.

5.1.16 SUMMARY

Immunoglobulins of all classes have a fundamental four-chain structure.

Each chain consists of two identical Light (L) and two identical Heavy (H) chains.

Disulfide bonds link two heavy chains to each other.

Each light chain is linked to a heavy chain by disulfide bond.

Intrachain bonds occur within the individual chains.

These are stronger than interchain bonds.

Immunoglobulins are expressed in two forms: a membrane-bound antibody present on the surface of B cells and a secreted antibody produced by plasma cells.

Each heavy and light chain consists of 2 regions: the variable region (v) and the constant (c) region.

Each immunoglobulin chain consists of a series of domians encircled by disulfide bonds

The proteolytic enzymes used for degrading immunoglobulin molecules are papain and pepsin

Treatment of monomere with papain results into 3 fragments of approximately equal size at the hinge region- two Fab fragments and one Fc fragment

Fab fragments (Fragment antigen binding) are monovalent

Fc fragment (Fragment crystallizable) contains the corboxy terminal portion of the heavy chain

Treatment of monomere with pepsin results in digestion of most of the Fc fragments leaving one large Fab fragment (consisting of of two Fab fragments) joined by covalent bonds, termed $F(ab)_2$ fragments

The hinge region lies in the heavy chain between CH^1 and CH^2 domians

Hinge region is considered as a separate domian; it is not homologous to any of the other known domians

The sedimentation coefficient or S value is expressed in Svedberg units.

IgG is capable of carrying out numerous biological functions

Neutralization of toxins to activation of compliment and opsonization

IgG is only class of immunoglobulin that passes through the placenta and confers maternal immunity on the fetus

The half life of IgG (23 days) is the largest of all the immunoglobulin classes

IgG is the major immunoglobulin in normal human serum accounting for approximately 80%

IgG has a molecular weight of 150000

IgG has an S value of 7S

5.1.16 KEY TERMINOLOGY

Clone: Upon stimulation, the B cell undergoes successive mitosis and eventually produces a clone of identical cells that secrete the antibody. Moreover, antibody secretion may continue even if the antigen is no longer present. In this manner organisms produce antibodies only to the antigens to which they were previously exposed

Genome: The genetic complement of a cell or virus is refereed to as a genome, though in eukaryotes the term is commonly used to refer to one complete (haploid) set of chromosomes

Locus: Each gene occupies a well defined site or locus in its chromosome

5.1.17 SELF ASSESSMENT QUESTIONS

1. Describe the structure and properties of immunoglobulin G
2. Describe the chemical and biological properties of immunoglobulin G
3. Write short notes on:

a. Light chain

b. Heavy chain

c. Disulfide bonds

d. Antibody diversity

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Unit - V

5.2 THE ORGANS OF THE IMMUNE SYSTEM

5.2.1 OBJECTIVES

The purpose of the less on is to:
Understand the importance of the lymphoid system
Know the role of primary lymphoid organs
Know the role of secondary lymphoid organs
Exemplify the traffic of lymphocytes in the immune system

5.2.2 INTRODUCTION

The lymphoid organs and lymphocytes constitute the immune system of the human body. There are two facets of the immune response system – (1) Thymus dependent system (T-cell system) and (2) Thymus independent system (B-cell system)

The cells produced by the thymus dependent system are called T lymphocytes (T cells). The thymus dependent system is responsible for the expression of cellular immunity. For the development and maturation, the (T) lymphocytes require thymus. The lymphocytes, which undergo development and maturation under the influence of thymic hormones (e.g. Thymopoietin) or depended on thymus for development, are called T lymphocytes. If the thymus is removed in young experimental animals, the immune response (primarily cell-mediated immunity) is depressed.

The cells produced by a lymphoid organ in chickens that is known as the Bursa Fabricus are called B-lymphocytes. The B cell system (thymus dependent system) is responsible for antibody production. For the development and maturation, the B-lymphocytes require the presence of Bursa Fabricus (in birds) or Bursa equivalence tissue. In human beings the Bursa equivalent tissue is distributed

diffusely in the lymphoid tissue. (But there is argument about the location of this tissue). The cells, which arise through the pathway of Bursa Fabricus (in birds), or Bursa equivalent tissue (in humans) is referred to as thymus independent system or B cell system. The lymphocytes, which undergo maturation in Bursa Fabricus or its equivalent tissue (in humans), are called B-lymphocytes. The B-cell system is responsible for the production of circulating (humoral) antibodies.

In humans lymphoid tissue is found in association with gut (Gut Associated Lymphoid Tissue-GALT) and mucosa (Mucosa Associated Lymphoid Tissue- MALT). The GALT is perhaps the Payer's patches of the intestine, tonsils and appendix. The total liver or bone marrow may also be involved.

5.2.3 CHARACTERISTICS OF THE LYMPHOID SYSTEM

The lymphoid system involves lymphoid organs and lymphatic tissues. Lymphocytic cells originate as lymphocyte precursors in the lymphoid organs, Then, the lymphocyte precursors mature and differentiate and finally lodge in the lymphoid organs or move into circulatory system or lymphatic system of the body. (Fig. 5-3).

The functional development of the lymphocytic cells forms the basis to classify the lymphoid organs into these groups:

1. Lymphoid organs involved in the origin of precursor cells (or in the genesis of lymphocytes) e.g. yolk sac, liver, spleen or Bursa Fabricus (or its mammalian equivalent, the bone marrow) in an embryo or fetus.
2. Central or primary lymphoid organs e.g. thymus and the Bursa or Bursa equivalent tissue (bone marrow).
3. Peripheral or secondary lymphoid tissues (organs) e.g. Lymph nodes, spleen, GALT, MALT

5.2.4 LYMPHOID ORGANS INVOLVED IN THE IMMUNE SYSTEM

These organs contain a pool of undifferentiated stem cells. The stem cells migrate from these organs to the primary lymphoid organs for circulation. There is a constant renewal of dividing cell population in all the

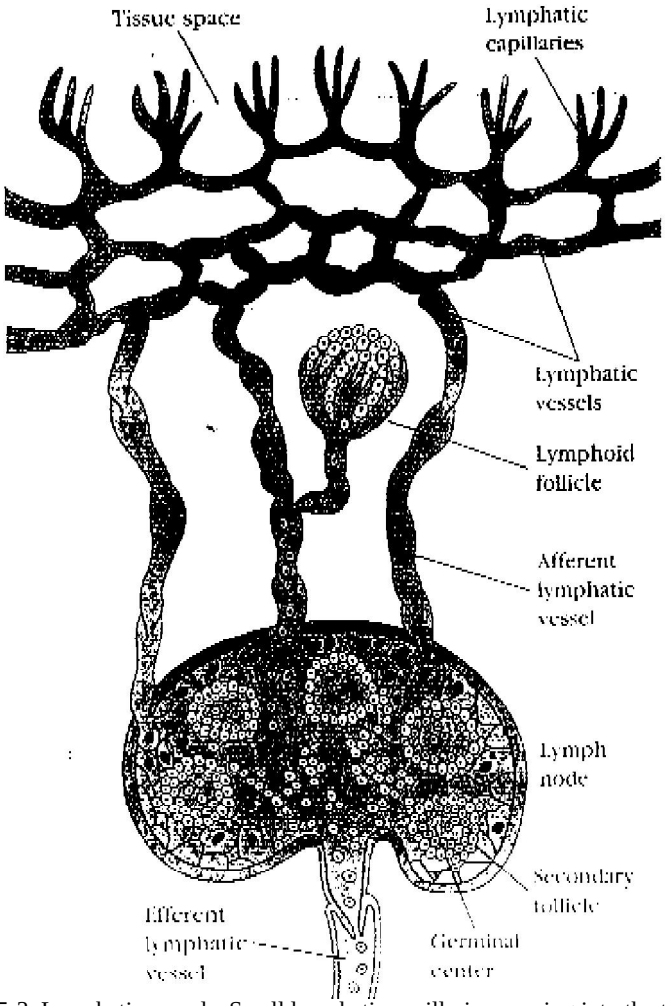

Fig. 5-3. Lymphatic vessels. Small lymphatic capillaries opening into the tissue spaces pick up interstitial tissue fluid and carry it into progressively larger lymphatic vessels, which carry the fluid, now called lymph into regional lymph nodes. As lymph leaves the nodes, it is carried through larger efferent lymphatic vessels, which eventually drain into the circulatory system at the thoracic duct or right lymph duct.

primary lymphoid organs. These lymphocytes are not immunologically competent cells. But the constant traffic of these cells is vital to the functional integrity of the protective immune system.

The yolk sac of the embryo contains blood islets. Stem cells originate from these islets, these include the stem cells of the hematopoietic tissue and lymphoid cells. These cells colonise in fetal liver and bone marrow. The cells, which migrate to the bone marrow, persist in bone marrow in later period.

Bone marrow remains as the chief source of stem cells for the entire life. Lymphopoiesis along with genesis of other hemopoietic cells occurs in the red bone marrow, which is found in most of the bones in fetal and early postnatal life. In adults, it is found in flat bones, in short bones and in ends of the long bones of vertebrates. Flat cells replace the remaining marrow; it is known as yellow marrow. When needed it can change to red marrow. Bone marrow is also an important peripheral lymphoid organ containing mature T and B cells.

The primary or central lymphoid organs are those in which the maturation of T and B-lymphocytes into antigen-recognizing lymphocytes occurs. Mature B and T lymphocytes migrate from the bone marrow and thymus respectively, through the blood stream to the peripheral lymphoid tissues, including the lymph nodes, spleen and gut-associated lymphoid tissues such as the tonsils (Fig. 5-4).

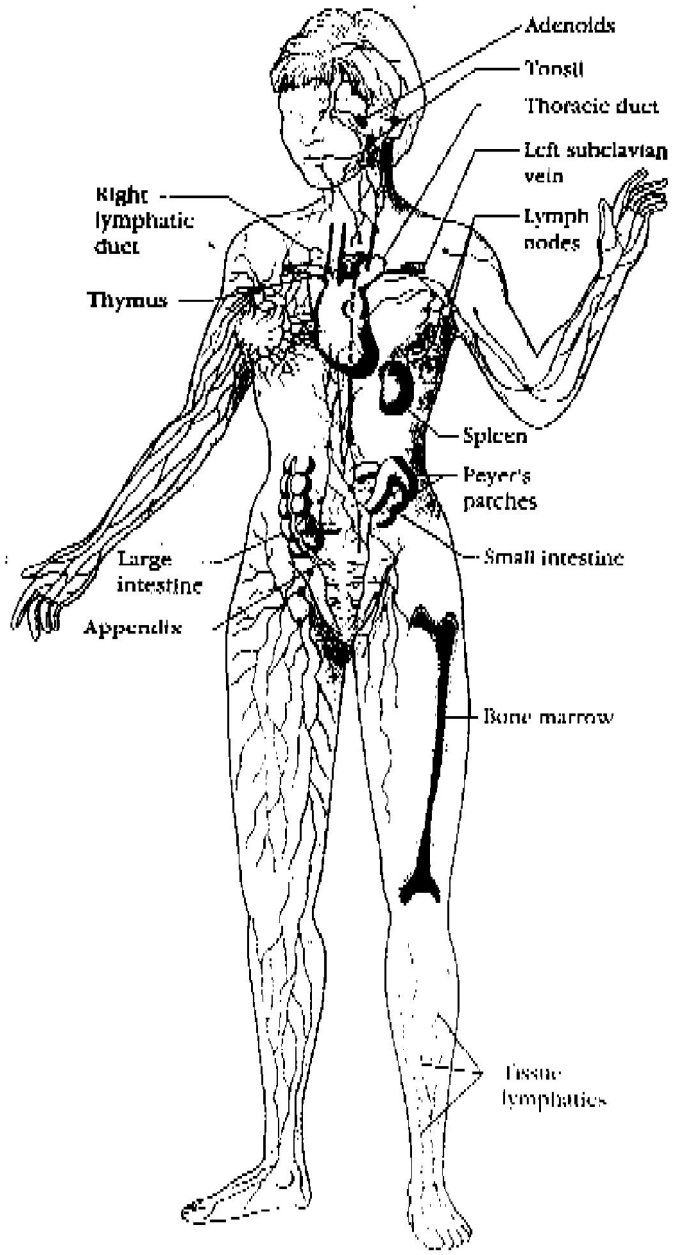

Fig. 5-3. The human lymphoid system. The primary organs (bone marrow and thymus) are shown in black; secondary organs and tissues are also shown in black. These structurally and functionally diverse lymphoid organs and tissues are interconnected by the blood vessels and lymphatic vessels through which lymphocytes circulate. Only one bone is shown, but all major bones contain marrow and thus are part of the lymphoid system. (Adapted from Lodish et al, 1995, Molecular Cell Biology, 3rd ed., Scienctific American Books.)

5.2.5 CENTRAL OR PRIMARY LYMPHOID ORGANS

These organs are concerned with immunological differentiation of lymphocytes. Stem cells from the bone marrow or embryonic tissues migrate and mature into lymphocytes in the central or primary lymphoid organs. After maturation /multiplication the small lymphocytes enter the circulation and colonize in peripheral lymphoid organs. These organs possess epithelial cells, the secretions of which may be responsible for this high rate of mitosis.

5.2.5.A THYMUS GLAND

The thymus gland is a flattened bilobed structure. Thymus is derived from the third and fourth pharyngeal pouches. During fetal development, it increases in size. It continues to grow until puberty. Thereafter, thymus undergoes atrophy with aging.

The thymus lies in the thorax, immediately below the sternum. The whole organ is encapsulated by connective tissue and is divided into lobules by connective tissue septa, which surround the lobules completely.

The thymus is a lymphoepithelial organ consisting of epithelial cells and lymphocytes. The epithelial cells are organized into cortical (outer) and medullary (central) areas that are infiltrated with lymphoid cells (thymocytes). Various sizes of lymphocytes (most of which are immature), and flattened macrophages are found in cortex. T lymphocytes mature in the cortex and migrate to the medulla. (Fig. 5-5). Here they undergo thymic selection – lymphocytes undergo development and mature as functional T cells. Through blood circulation they enter into secondary lymphoid organs.

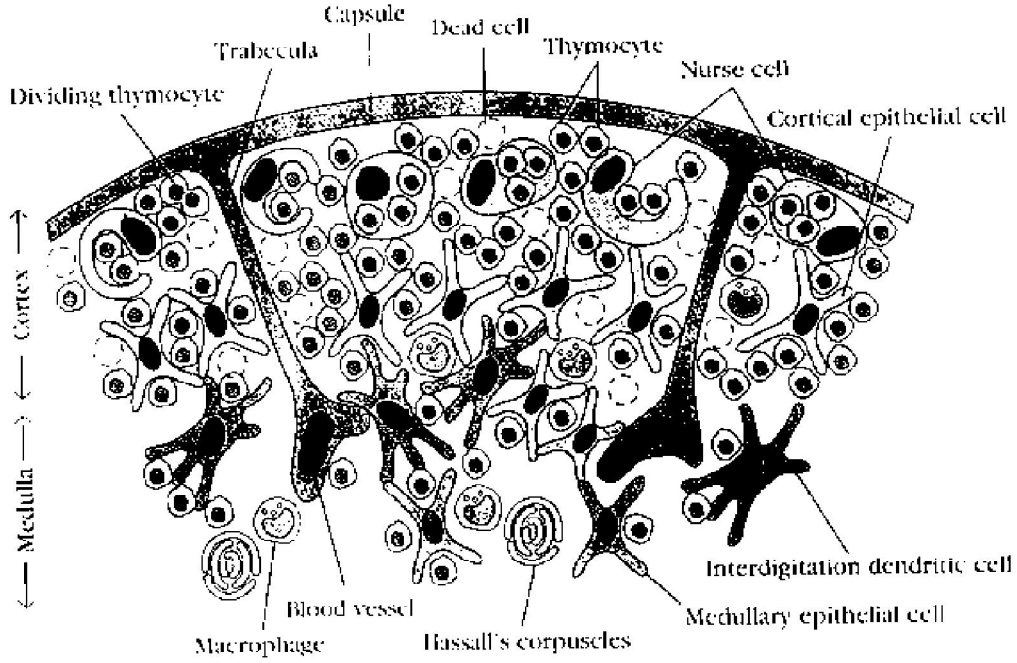

Fig. 5-5. Diagrammatic cross-section of a portion of the thymus, showing several lobules separated by connective tissue strands (trabeculae).

In the secondary lymphoid organs these cells respond to foreign antigens. Mature T lymphocytes in the medulla respond to foreign antigens in the same way they respond in the secondary lymphoid organs.

Removal of the thymus from an adult generally has little effect on the quality and quantity of lymphocytes. If there is no thymus, there may not be repopulation of new T lymphocytes into secondary organs

Only 5 – 10% maturing lymphocytes survive and eventually leave the thymus. The remaining 90 – 95% all thymocytes die in the thymus.

5.2.5.B BURSA FABRICUS

It is the primary lymphoid organ in birds. B cells undergo maturation in the Bursa Fabricus (in birds). Bursa Fabricus is situated near the cloaca. This organ consists of lymphoid centers that contain epithelial cells and lymphocytes. The lymphocytes in this organ are antibody producing B cells or B-lymphocytes.

5.2.5.C BONE MARROW

Bursa Fabricus is absent in mammals. In embryonic life, B cells differentiate from hematopoietic stem cells in the fetal liver in mammals. Bone marrow is considered as the primary lymphoid organ; with functions it is equivalent to the avian Bursa. B cells develop and mature in this lymphoid organ. The mature B cells migrate to the secondary lymphoid organs via blood. B-lymphocytes respond to foreign antigens staying in the secondary lymphoid organs.

5.2.6 SECONDARY LYMPHOID ORGANS

The major secondary lymphoid organs are the spleen and the lymph nodes. Tonsils, appendix, clusters of lymphocytes distributed in the lining of small intestine (Payer's patches), and aggregations of lymphocytes present throughout mucosal tissue are considered secondary lymphoid organs. Basing on their presence, the mucosal lymphoid organs have been given different names:

MALT (Mucosal Associated Lymphoid tissue) (Figure 5 - 6).

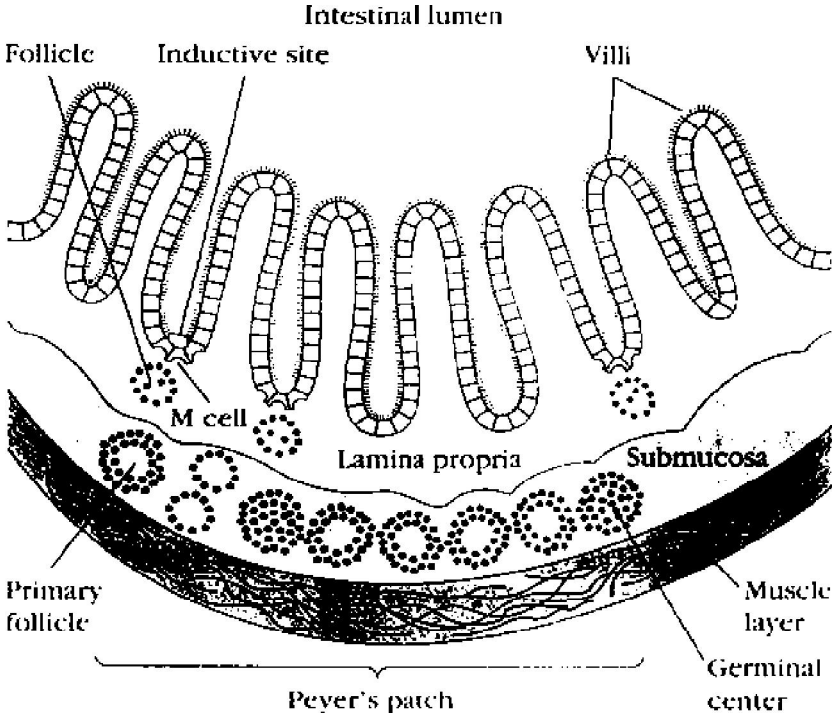

Fig. 5-6. Cross-sectional diagram of the mucous membrane lining the intestine showing a nodule of lymphoid follicles that constitutes a Peyer's patch in the submucosa. The intestinal lamina propria contains loose clusters of lymphoid cells and diffuse follicles.

GALT (Gut Associated Lymphoid tissue) and

BALT (Bronchus Associated Lymphoid tissue)

The secondary lymphoid organs (SLO) are also found in the genitourinary tracts, the conjunctiva and the salivary glands.

The SLO have two major functions: they are highly reactive to antigens and they are the main centers of antibody production and antigen specific T lymphocytes.

5.2.6.A THE SPLEEN

The spleen is the largest secondary lymphoid organ (Figure 5 - 7). It is the major organ in which antibodies are produced and from which they are released into the circulating blood. The spleen is composed of white pulp and red pulp. The white pulp is rich in lymphoid cells. The red pulp contains many sinuses, large quantities of erythrocytes and macrophages, some lymphocytes and a few other cells.

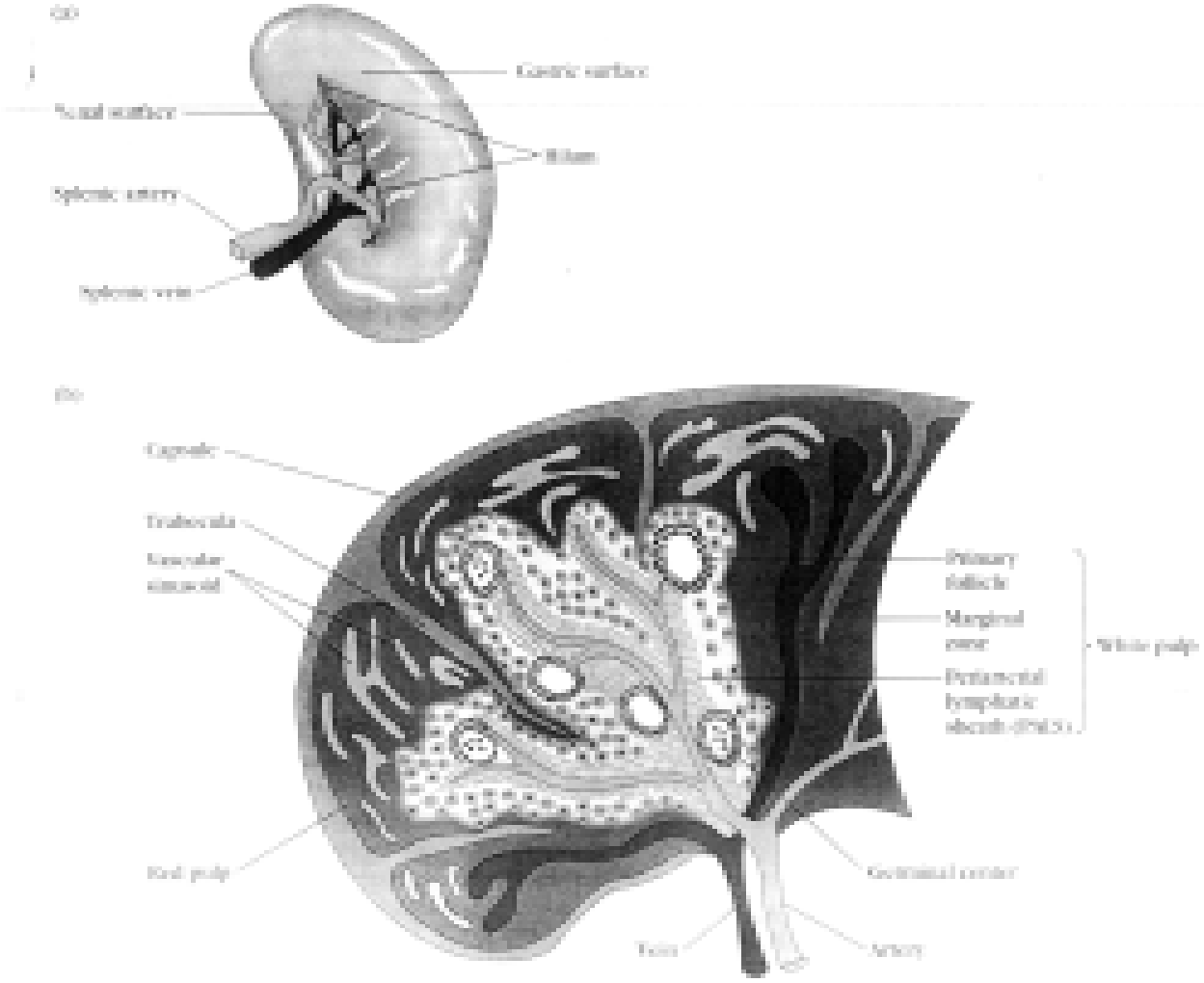

Fig. 5-7. Structure of the spleen. a) The spleen, which is about 5 inches long in adults, is the largest secondary lymphiod organ. It is specialized for trapping blood-borne antigens. b) Diagrammatic cross section of the spleen.

Small arterioles are richly surrounded by the areas of white pulp – the peripheral regions of these areas (white pulp) are rich in T cells and B cells. B-lymphocytes are present mainly in germinal centers. These centers contain large numbers of B cells and plasma cells (following antigenic stimulation). B cells and plasma cells synthesize and release antibodies. The spleen cells include: approximately 50% B-lymphocytes and 30 – 40% are T lymphocytes.

5.2.6.A LYMPH NODES

Lymph nodes are small ovoid structures (Figure 5 -8). These are normally less than 1 cm in diameter. Lymph nodes are present in various regions throughout the body.

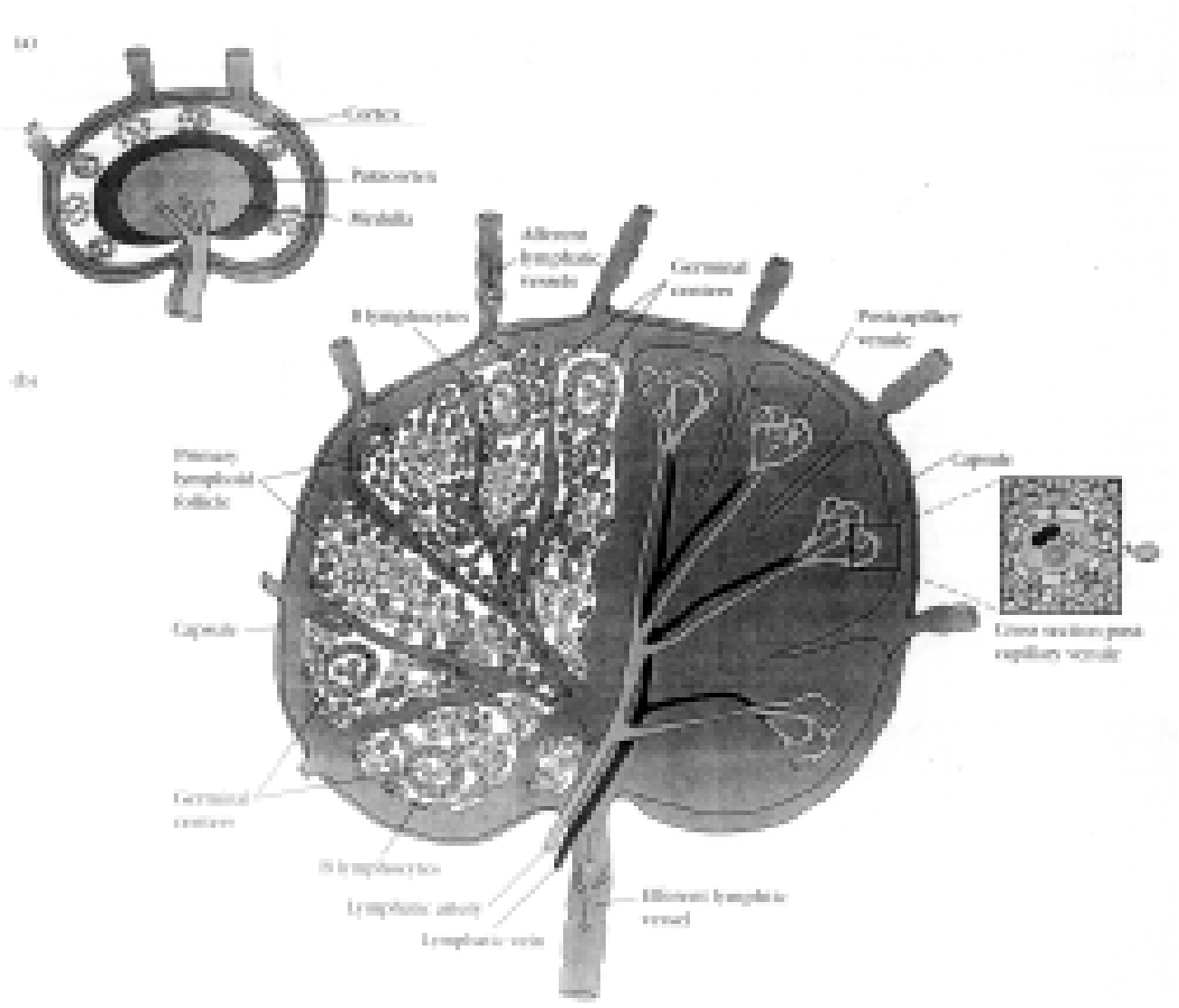

Fig. 5-8. Structure of a lymph node. (a) The three layers of a lymph node support distinct microenvironments. (b) The left side depicts the arrangement of reticulum and lymphocytes within the various regions of a lymph node. Macrophages and dendritic cells, which trap antigen, are present in the cortex and paracortex. The right side of (b) depicts the lymphatic artery and vein and the postcapillary venules.

A capsule of connective tissue surrounds the lymph node. The lymph node is divisible into an outer cortex and an inner medulla (with many sinuses). The cortical region contains many primary lymphoid follicles. Following antigenic stimulation, the primary lymphoid follicles enlarge to form secondary follicles. The secondary lymphoid follicles have central dense populations of lymphocytes (mostly B cells) which are undergoing mitotic division. Later, phagocytic cells appear in these centers.

Antigen-specific B cells, when stimulated by antigen, proliferate in these germinal centers. Also, they undergo a process known as affinity maturation to generate clones of cells.

The tissue, which separates the follicles from each other and also from the medulla, is known as the diffuse cortical tissue of the lymph nodes (or diffuse cortex). The diffuse cortex consists of packed small T lymphocytes. The deep cortical area (or paracortical region) contains T cells and dendritic cells.

The antigen-nonspecific B cells are pushed to the outside of germinal centers to form the mantle zone. Dendritic cells brought antigen and present antigen fragments to T cells in the mantle and paracortical areas.

The medullary region of the lymph node contains antibody-secreting plasma cells. These cells are migrated from the cortex via lymphatic vessels.

Usually antigen enters the lymph node through the afferent lymphatic vessels. Lymph nodes are the highly efficient centers for trapping antigen. The immune response is brought out by T cells, B cells and the interaction of T cells and B cells. Lymphocytes, antibodies and lymph leave the lymph node throughout the efferent lymphatic vessel. The lymphatic vessel is found just below the medullary region.

5.2.7 LYMPHOCYTE RECIRCULATION

The spleen functions like a lymph node. It contains both afferent and efferent blood vessels. Arterial blood lymphocytes enter the spleen through the hilum. Then they pass into the trabecular artery, which along its course become branched. The lymphocytes return to venous circulation by trabecular vein.

Lymph passes through efferent lymphatic vessels into the lymphatics; from the lymphatics the cells continue their recirculation through the body and back to the afferent vessels of the spleen.

Similarly, blood lymphocytes enter the lymph nodes through post capillary venules and leave the lymph node through efferent lymphatic vessels. These vessels converge in the thoracic duct. This duct opens into the vena cava. (this is the vessel that returns the blood to the heart). Thus, a continued recirculation of lymphocytes occurs in the body.

5.2.8 SUMMARY

The lymphoid organ and lymphocytes constitute the lymphoid system.

Based upon the functional development of lymphocytes, the lymphoid organs are classified into three groups:

1. Organs involved in the genesis of lymphocytes.
2. Primary or central lymphoid organs.
3. Secondary or peripheral lymphoid organs.

Yolk sac of embryo, fetal liver and bone marrow are the lymphoid organs involved in the genesis of lymphocytes.

The primary or central lymphoid organs are concerned in the maturation of T and B-lymphocytes.

There are two major lymphoid organs: the thymus and the avian Bursa Fabricus or Bone marrow (Bursa equivalent tissues) in mammals.

T cells develop in thymus.

B cells develop in Bursa fabricus or in bone marrow.

The secondary or peripheral lymphoid organs include lymph nodes, spleen, gut associated lymphoid tissue (GALT), MALT and BALT.

The thymus is a bilobed structure.

The thymus is a lymphoepithelial organ.

The thymus consists of epithelial cells organized into cortex (outer) and medulla (central); these areas are infiltrated with lymphoid cells (thymocyts)

Lymphocytes undergo maturation in thymus.

B and T lymphocytes develop in primary lymphoid organs.

B cells differentiate in the bone marrow. They leave the bone marrow as functional cells.

T cells differentiate partially in the bone marrow.

The partly differentiated cells mature in the thymus.

The mature T cells migrate to peripheral lymphoid organs.

Following antigenic stimulation mature B and T lymphocytes undergo differentiation and proliferation.

Differentiation and proliferation of T and B cells occur in secondary lymphoid organs.

B-lymphocytes synthesize and secrete antibodies.

T lymphocytes cannot synthesize and secrete antibodies.

B cells participate in antibody mediated immunity.

T cells participate in cell mediated immunity.

T cells help B cells to make antibodies by providing them cytokines needed for B cell activation.

Lymphocyte recirculation occurs continuously.

It occurs between the blood, lymph, lymphoid organs and tissues.

5.2.9 KEY TERMINOLOGY

B-lymphocytes: Cells, which are involved in the production of antibodies and are also the precursors of plasma cells.

Bone marrow: It is the Bursa-equivalent lymphoid tissue in human beings. It is a primary lymphoid organ which functions like Bursa fabricus in aves.

Bursa Fabricus: Lymphoepithelial tissue situated in the wall of the hindgut of chickens. It is a primary lymphoid organ in aves.

Cell mediated immunity (CMI): In this type of immune response, the primary event is the interaction of sensitized small lymphocytes with the antigen. It is a specific immune reaction.

Cytokines: Soluble growth and differentiation factors secreted by T cells. These are meant for B cell activation.

Immune system: The system of the body that is responsible for all types of immune responses is called the immune system.

Immunologically competent cells: The cells that are directly involved in immune response.

Lymphoid follicles: These are special aggregations of lymphocytes and reticular cells.

Red pulp: The spleen consists of red pulp and the red pulp consists of plasma cells. These are the sites of antibody production. White pulp (or islands of lymphatic tissue) is present in a sea of red pulp.

T lymphocytes: Cells involved in cell mediated immune response.

White pulp: The spleen consists of white pulp. It is having diffuse lymphoid tissue and lymphoid follicles. It contains T lymphocytes.

5.2.10 SELF ASSESSMENT QUESTIONS

1. Explain in detail about the importance of lymphoid system.
2. Describe the primary lymphoid organs and their role in the production of lymphocytes.
3. Enumerate the secondary lymphoid organs with suitable diagrams. Add a note about lymphocyte recirculation.
4. Write short notes on:
   a. Thymus
   b. Lymph nodes
   c. GALT
   d. T and B lymphocytes

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Unit - V

5.3 THE CELLS OF THE IMMUNE SYSTEM

5.3.1 OBJECTIVES

The purpose of the lesson is to:
understand the cells of the immune system
know about the origin and differentiation of cells

5.3.2 INTRODUCTION

Man lives in a hostile environment, literally immersed in a sea of pathogens ranging from viruses to worms; they try to invade and destroy tissues and cells. Yet most humans manage to survive or to expel them from their bodies. This *immunitis* (Latin, freedom from disease) is dependent on the existence of a complex and highly sophisticated defense system. Immunity is divided into 2 types as innate or natural and adaptive or acquired. The innate resistance system is viewed as the first line of defense. Its cellular components are ideally suited for this role because their actions do not depend on prior sensitisation and further more they do not possess the fine antigen specificity that is characteristic of the adaptive (or specific) immune responses. Complement is the major humoral component of innate immunity.

The principal cellular components of the adaptive immune responses are T and B lymphocytes. Each T and B lymphocyte possesses an antigen-specific receptor on its surface, by means of which they are genetically programmed to recognize only one antigenic determinant. Human body is not merely a bag of lymphocytes. When challenged with an antigen, say in the form of microbial infection, antigen-specific lymphocytes divide and expand to effectively handle the offending organism. Such clonal expansion of T and B lymphocytes is a complex multistep process involving both differentiation and cell division. This process may be completed within few days or sometimes weeks. Because of this requirement (cell division), specific immune responses are better suited as the second line of defense, which eventually becomes the major protective mechanism.

Although distinctions between innate and induced immunity and the cellular and humoral effector systems are helpful in resolving the complexity of the host defense systems, it is extremely important to remember that there are multiple interconnections and 'cross talk' among the various components. Complement, in turn plays an important role in modulating the function of neutrophils and macrophages. Macrophages are not only important effector cells in the expression of innate immunity, but also as antigen presenting cells, they play an important role in triggering specific immune responses mediated by T and B lymphocytes.

5.3.3 GENERAL FEATURES OF THE IMMUNE SYSTEM

The cells of the immune system are those of the lymphoid system. All lymphocytes are derived from the undifferentiated pool of stem cells. Precursor cells originate from the yolk sac, liver, spleen or bone marrow (or its avian equivalent Bursa of Fabricus) in an embryo or fetus. Stem cells from bone marrow or embryonic tissues migrate and mature into lymphocytes in the primary lymphoid organs (thymus and the bursa or bone marrow). The stem cells are not immuno competent cells. (Fig. 5-9). Upon maturation, the lymphocytes migrate to the secondary lymphoid tissue (i.e. the lymph

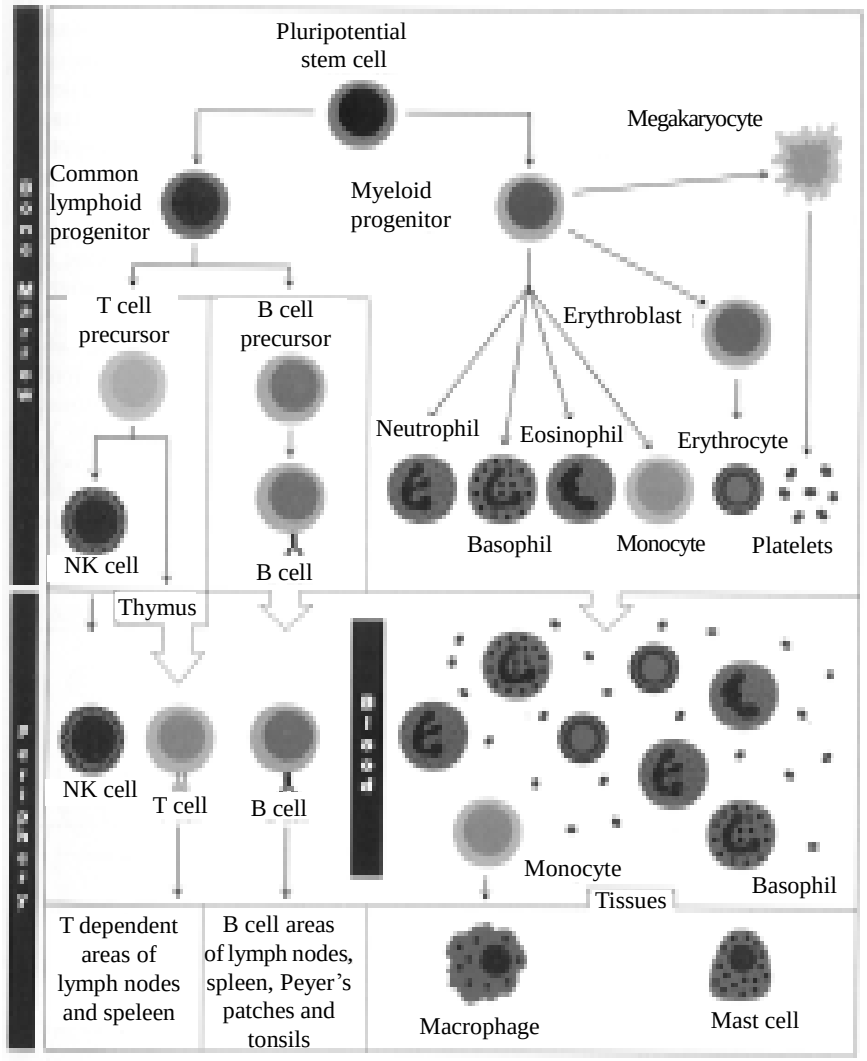

Fig. 5-9. The developmental pathway of various cell types from pluripotential bone marrow stem cells.

nodes, spleen and lymphoid follicles); they undergo further maturation in these organs either to produce antibodies or sensitized to produce lymphocytes.

Immunologically lymphocytes are of two types:

T lymphocytes: Which are involved in cell mediated immune response

B lymphocytes: Which are involved in the production of antibodies and are also the precursor of plasma cells.

The two types of lymphocytes are indistinguishable in peripheral blood smear and tissue sections on routine microscopic examination. However, electron microscopic examination reveals differences between T lymphocytes (relatively blind surface) and B lymphocytes (with surface projections).

Upon antigenic stimulation T lymphocytes are transformed to lymphoblasts. Antigenic stimulation of corresponding B lymphocytes leads to the transformation into plasma cells and finally produces large quantities of antibodies.

There are two facets of the immune response system – thymus dependent system and thymus independent system.

Thymus dependent system:

If the thymus of experimental animals is removed, the primary immune response is depressed. A thymus graft from another animal may restore immunocompetence and cell mediated immunity. The thymus cells (epithelial cells) produce soluble molecules; these cells are thymic harmones (thymopoietin). The cells produced/matured by the Thymus dependent system are called T lymphocytes.

Thymus independent system:

If the lymphoid organ in chicks (known as Bursa of Fabricus) is removed, the production of humoral (circulating) antibodies or humoral immunity is suppressed (but not cell mediated immunity). The cells produced by the B-cell system or thymus independent system are called B lymphocytes (B cells).

5.3.4 T LYMPHOCYTES

T lymphocytes arise from stem cells in the bone marrow, migrate to the thymus, undergo maturation into T cells and then leave the thymus. Mature T cells circulate in the blood and also enter into the peripheral lymphoid tissues, such as the paracortical areas of lymph nodes and periarteriolar sheaths of the spleen.

T cell functions are classified into 2 broad categories:

a) Cellular immune reactions:

Cytotoxic (killer) T cells react against virally infected cells and foreign histocompatability antigens. T cells play a vital role in cell mediated immunity or delayed hypersensitive reactions.

b) Regulatory functions:

T cells regulate the function of other T cells, B cells and haematopoietic stem cells, as well as

several non haematopoietic cells. This immuno-regulatory functions facilitate or supress the immune response. Primary T cells are found associated with CD 4 antigen and helper-T cells induce cells provide 'help' in the generation of cytotoxic T cells and antibody secreting B cells. These cell mediated systems function by the secretion of soluble factors, called lymphokines.

It is clear that T and B lymphocytes are genetically programmed to recognize specific antigens by means of antigen-specific cell-surface receptors.

5.3.5 B LYMPHOCYTES

B lymphocytes are found in the blood and lymphoid tissue, including bone marrow. They constitute 10 to 20% of the lymphocyte population in the human blood. B cells are found in lymph nodes and spleen. In lymph nodes they are present in the superficial cortex forming lymphoid follicles. In spleen, they are found in white pulp forming as lymphoid follicles, usually called as germinal centers. B cells express surface immunoglobulins.

5.3.6 MACROPHAGES

Macrophages are a part of the mononuclear phagocytic system. They process and present the antigen to immunocompetent T cells. Because T cells (unlike B cells) cannot be activated by soluble factors, including interleukin–I, which promotes the differentiation of both T and B lymphocytes.

Macrophages lyse tumor cells by secreting toxic metabolites and proteolytic enzymes and as such may play a role in immunosurveillance. Macrophages are important effector cells in delayed hypersensitivity reaction. (Refer Fig. 4-2).

5.3.7 NK CELLS

Natural killer cells are capable of lysing a variety of tumor cells, virus infected cells, and some normal cells, without previous sensitization. Man and a variety of animal species possess NK cells in the peripheral blood and lymphoid tissues. NK cells share some cell surface antigens with T cells and macrophages; but NK cells are distinct from mature T cells, B cells or macrophages. NK cells are larger than small lymphocytes and possess granular cytoplasm. Hence, they have been described as large granular lymphocytes. NK cells (Refer Fig. 4-3). lyse their target cells by means of 2 distinct mechanisms.

a. Antibody-dependent cellular toxicity (ADCC), utilizes NK cell surface Fc-receptors and antibodies directed against the target cell antigens.

b. Direct interaction between NK cells and their target cells and utilizes NK cell receptors that have not been fully characterized.

1. NK cells bear a small amount of the T-cell membrane marker, Thy – 1, but lack most of the other membrane characteristics of T cells; although they are weakly reactive with SKBC
2. NK cells have a membrane receptor for the Fc portion of the body but will kill targets in the absence of antibody

3. They are not restricted MHC complex (i.e. NK cells do not require matching of MHC molecules between effector and target cells).

4. NK cells release numerous cytokines during their interaction with the target cells, including alpha, and gamma interferons, interleukins 1 and 2, B cell growth factor and lymphotoxin.

5. The lymphokine activated killer (LAK) cell is another naturally occurring cytotoxic cell. It is a quiescent lymphocyte that is induced into an active cytotoxic state by the lymphokine interleukin-2.

5.3.8 DENDRITIC AND LANGERHAN'S CELLS

These cells possess dendritic cytoplasmic processes and large amounts of class II molecules (on their cell surface). Dendritic cells are found in lymphoid tissues, and some what similar cells are present within the epidermis – they are called as Langerhan's cells. They are extremely efficient in antigen presentation. They share with macrophages in antigen presenting capacity, but they are weak or not at all phagocytic.

5.3.9 HISTOCOMPATIBILITY ANTIGENS

Histocompatibility antigens are important in the regulation of immune response and in resistance or susceptibility to a growing list of diseases. The histocompatibility antigens and their corresponding genes are complex in structure and organization and are still incompletely understood.

When an individual receives an organ transplant obtained from a genetically dissimilar donor, the transplanted organ is rejected by immunologic mechanisms. During this process of rejection, the recipient's immune system recognizes the histocompatibility antigens displayed on the cell surfaces of the donor organ. Several genes code for histocompatibility antigens, but those that code for the most important transplantation antigens are clustered on a small segment of chromosome 6. This cluster of genes constitute the human major histocompatibility complex (MHC) and is also known as the HLA complex. It is equivalent to the murine H-2 complx.

The initials HLA stand for human leukocyte antigens. The HLA system is highly polymorphic – that is, there are several alternative forms (allels) of a gene at each locus. Based on their chemical structure, tissue distribution and function, the MHC gene products are classified into 3 categories:

1. Class I antigens: HLA-A, HLA-B, and HLA-C. These are present on virtually all nucleated cells and platelets.

2. Class II antigens: HLA-D. These antigens were initially defined by a phenomenon called the mixed lymphocyte reaction. This reaction occurs between lymphocytes of two individuals who have different HLA-D regions.

3. Class III proteins: These are the components of complement system.

5.3.10 RECIRCULATION OF LYMPHOCYTES:

Lymphocytes are not permanent residents of any one lymphoid tissue. They move throughout in all tissues of the body. As many as 80% of small lymphocytes are found in the blood of adult animals or humans. Small lymphocytes are long lived. Both small T and B lymphocytes recirculate. The pathway of recirculating lymphocytes takes them from the blood stream to the extravascular connective tissue spaces and peripheral blood via lymphatic vessels and back to the blood via the lymphatic system.

5.3.11 SUMMARY

The undifferentiated lymphoid stem cells are derived from fetal liver of bone marrow. They migrate to the thymus or Bursa of Fabricus (or bursa equivalent organ (bone marow) in mammals); they get differentiated into T lymphocytes or B lymphocytes respectively.

After maturation they migrate to blood or to secondary lymphoid organs.

The two types of lymphocytes can be distinguished by routine microscopic examination. However, they can be distinguished only by electronic microscopic examination.

Antigenic stimulation of T cells results in division and differentiation.

Antigenic stimulation of B cells leads to antibody production and transformation of B lymphocytes to plasma cells.

T lymphocytes are involved in cell mediated immune response.

B lymphocytes are involved in the production of antibodies and are also the precursor of plasma cells.

Macrophages are large mononuclear phagocytic cells. Some of these are fixed and found in liver, spleen, lymph nodes, bone marrow etc.

Some are moving-wandering histiocytes and monocytes.

Tissue macrophages have dendritic processes by which they have contact with lymphocytes in lymphoid tissues.

Macrophages do not take part directly in the immune response. They present the antigen to immunocompetent cells probably to T cells.

Macrophages produce interleukins (IL-1) when they present antigen to the appropriate T cell.

Interleukin has also pyrogenic effect.

About 5% of lymphocytes in the peripheral blood cannot be classified as T cells or B cells, they have been termed as “Null cells”.

Some of the Null cells are involved in a type of cytotoxic immune response and are known as killer cells.

These killer cells are called Natural Killer cells (NK cells), these are cytotoxic to target cells already coated with the antibody. Their role in mounting immune response has not been clearly identified.

5.3.12 KEY TERMINOLOGY

Cell mediated immunity: This is an immunogenic response. The primary event in this reaction is the interaction of sensitized small lymphocytes with the antigen. This is an immunologically specific reaction.

Immune system: the system of the body, which is responsible for all types of immune responses, is called the immune system.

Immunocytes: A mature immunologically competent cell is known as immunocyte.

5.3.13 SELF ASSESSMENT QUESTIONS

1. Describe the cells involved in the immune response.
2. Explain how various types of lymphocytes govern the immune system.
3. Write short notes on:

a. T lymphocytes
b. B lymphocytes
c. Phagocytes

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Unit - V

5.4 ANTIGEN –ANTIBODY INTERACTIONS, THE COOMBS TEST

5.4.1 OBJECTIVES

The purpose of this lesson is to:
describe the antigen-antibody interaction
discuss the utilization of in vitro reaction between antigen and serum antibodies.
understand the consequences of the interaction of antigen with antibodies
know the mechanism and use of the Coombs test.

5.4.2 INTRODUCTION

In vitro antigen-antibody reactions are called serological reactions. In the body these reactions form the basis of antibody mediated immunity in infectious diseases. In the lab, these are useful in diagnosis of various diseases and in the identification and quantitation of antigens and antibodies.

5.4.3 STAGES OF ANTIGEN-ANTIBODY INTERACTIONS

The reactions between antigens and antibodies occur in three stages. The primary, secondary and tertiary stages.

The primary stage is the initial interaction between the two, without any visible effects. This reaction is rapid, occurs even at low temperatures. In most cases, but not all, the primary stage is

followed by the secondary stage leading to demonstrable events such as precipitation, agglutination, lysis of cells, killing of live antigens, neutralisation of motile organisms and enhancement of phagocytosis. Some antigen-antibody reactions occurring *in vivo* initiate chain reactions that lead to neutralization or destruction of injurious antigens, or to tissue damage. These are tertiary reactions.

A. The titer or level of antibody in serum can be measured by using known antigens.

B. Various environmental factors affect the antigen-antibody reactions.

1. Physiologic pH and salt concentration promote optimal union. Forces of attraction tend to be weaker in acid (below pH 4.0) and alkaline (about pH 10.0) conditions.
2. Temperature also plays an important role. The higher the temperature (upto a maximum of 50 - 55°C) the more rapid the rate.

C. Different types of antibodies are responsible for different types of reactions. The physical state of the antigen is responsible, in general, for the identification of antigen-antibody reactions and the naming of antibodies.

1. Antibodies causing agglutination (aggregation) of cellular antigens are called agglutinins and the corresponding antigens agglutinogens.
2. Antibodies causing dissolution of cell membrane are called lysins.
3. Antibodies which form precipitates with soluble antigens – precipitins and the corresponding antigen precipitinogens.
4. Antibodies that neutralize toxins are called antitoxins.

A single antibody can cause precipitation, agglutination and other serological reactions. An antigen can stimulate the production of different classes of immunoglobulins. They differ in their reaction capacities as well as in other properties (Table 1)

Table-1: Comparative efficiency of immunoglobulin classes in different serological reactions

Reaction	IgG	IgM	IgA
Precipitation	Strong	Weak	Variable
Agglutination	Weak	Strong	Moderate
Complement fixation	Strong	Strong	Negative
Lysis	Weak	Strong	Negative

5.4.4 GENERAL CHARACTERS OF ANTIGEN –ANTIBODY REACTIONS

1. The reaction is highly specific
2. Entire molecules react and not fragments
3. There is no denaturation of antigen or antibody during the reaction.
4. The combination occurs at the surface. Therefore, it is the surface antigens that are immunologically relevant. Antibodies to the surface antigen are generally protective.

5. The combination is firm but reversible. The affinity and avidity of the reaction influence the firmness of the union. Affinity refers to the intensity of attraction between antigen and antibody molecules. Avidity is the strength of bond after the formation of antigen-antibody complexes.
6. Both antigens and antibodies participate in the formation of agglutinates or precipitates.
7. Antigens and antibodies can combine in varying proportions, unlike chemicals with fixed valencies. Both antigen and antibodies are multivalent. Antibodies are bivalent, though IgM molecules may have 5 or 10 combining sites. Antigens may have valencies upto hundreds.

5.4.5 PRECIPITATION REACTION

Precipitation occurs when the antigen is soluble instead of cellular when soluble antigen combines with its antibody (specific) in the presence of electrolyte (NaCl) at a suitable temperature and pH, the antigen-antibody complex forms an insoluble precipitate.

Precipitation can takes place either in liquid media or in gels such as agar, agarose or polyacrylamide. The amount of precipitate formed is greatly influenced by the relative proportions of antigens and antibodies. (Fig. 5-10).

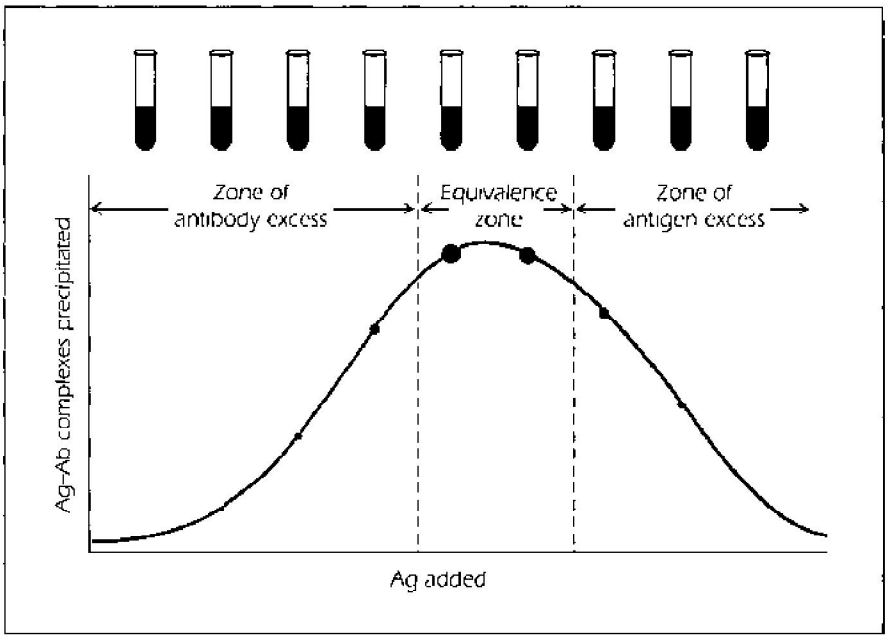

Fig. 5-10. A representation of the precipitin reaction.

If same amount of antiserum is kept in different tubes, when antigens are added in increasing quantities, precipitation will be found to occur most rapidly and abundantly in one of the middle tubes, in which the antigen and antibody are present in optimal or equivalent proportion.

In the preceding tubes, in which the antibody is in excess, and in later tubes in which the antigen is in excess, the precipitation will be weak or absent.

If the amounts of precipitate in different tubes are plotted on a graph, the resulting curve will have 3 phases: an ascending part (prozone or zone of antibody excess), a peak (zone of equivalence) and a descending part (post zone or zone of antigen excess). This is called Zone phenomenon.

Zoning occurs in agglutination and other serological reactions also. The prozone is of importance in clinical serology, as sometimes sera rich in antibody may give a false negative precipitation or agglutination result, unless several dilutions are tested.

5.4.5.A MECHANISM OF PRECIPITATION

Two sites on the antibody and multiple sites on the antigen result in antigen-antibody lattice formation that can build up into increasingly large complexes. This is possible only in the zone of equivalence. The lattice hypothesis holds good for agglutination also.

5.4.5.B APPLICATIONS OF PRECIPITATION REACTION

The precipitation test may be carried out either as a qualitative or quantitative test. It is very sensitive for detecting antigens and as little as 1μg of protein can be detected by using precipitation tests. This test can be used for the identification of blood and seminal strains and in testing for food adulterants. Precipitation is less sensitive for the detection of antibodies.

The following types of precipitation and flocculation tests are in common use:

5.4.5.C RING TEST

This is the simplest type of precipitation test, consisting of layering the antigen solution over a column of antiserum in a narrow tube. A precipitate forms at the junction of two liquids.

E.g.: C –reactive protein test,

Ascoli thermo-precipitation test and

The grouping of Streptococci by Lancefield technique.

5.4.6 AGGLUTINATION REACTION

Agglutination reaction serves to detect and quantitative agglutinins and identify cellular antigens. Agglutinins are antibodies that agglutinate cellular structures, including bacterial cells, white blood cells and red blood cells etc. When the cells interact with appropriate antibody, they clump together and eventually form masses that become large enough to be seen. When antibody agglutinates bacteria in the body, opsonization occurs.

1. Agglutination occurs because antibodies are bivalent (i.e. they have at least two combining sites).
2. Two sites on the antibody and multiple sites on the antigen result in antigen-antibody lattice formation that can build up into increasingly larger complexes. The aggregates may be seen in the test tube or under the microscope.
3. This reaction can be applied in the diagnosis of typhoid fever – the Widal test.

The term complement (C) refers to a system of factors occurring in normal serum, that are activated characteristically by antigen-antibody interaction and subsequently mediate a number of biologically significant consequences.

Complement is not a single substance, but a complex if nine different fraction called C1 to C9.

Thus C is made up of a total of 11 different proteins. Complement constitutes 10 –15% of human serum globulins.

5.4.7 LYSIS

In the presence of complement, an antigen-antibody reaction on a cell membrane may result in membrane damage leading to cell lysis, presumed to be due to the enzymatic activity of the activated complement. This phenomenon is probably of importance in the host's defense against microbial infections, cancer and so forth.

1. Hemolysis: Hemolysis in which the hemoglobin is released from the red blood cell, is a requisite phenomenon for the complement fixation test.
2. Bacteriolysis: Under certain condition cells of gram-negative bacteria also undergo immune lysis.
3. Cytolysis: Under appropriate conditions in the presence of specific antibody and complement, destruction of other cell types (e.g. tumor cells) occur.

Many immune assays are based on the *in vitro* reaction between antigen and serum antibodies. The interaction of antigen with antibodies may result in a variety of consequences, like precipitation (if the antigen is soluble), agglutination (if the antigen is particulate) and activation of complement. The consequences of antigen-antibody interaction listed above do not represent the primary interaction (between antibodies and a give epitome) but, rather, depend on secondary phenomena (between multivalent antigens and antibodies). Cross linking of various antigen molecules by antibody is required for precipitation, agglutination or complement activation and it is possible only if the antigen is multivalent and the antibody is divalent (either intact or F $(ab^1)_2$

Primary interactions between antibody and antigen

In the interaction between antibody and an epitope, no covalent bonds are involved. Consequently, the binding forces are relatively weak.

Secondary interactions between antibody and antigen

The reactions of antibody with a multivalent antigen that is particulate (i.e. an insoluble particle) results in the cross-linking of the various antigen particles by the antibodies. This cross-linking eventually results in the clumping or agglutination of the antigen particles by the antibodies.

5.4.8 THE COOMBS TEST

The Coombs test employs antibodies to immunoglobulins; hence it is also called the anti-immunoglobulin test. It is based on two important facts: (1) that immunoglobulins of one species (e.g. human) are immunogenic when injected into another species (e.g. rabbit) and lead to the production

of antibodies against the immunoglobulins, and (2) that many of the anti-immunoglobulins bind with antigenic determinants present on the Fe portion of the antibody, and leave the Fab portions force to react with antigens (In this process, rabbit anti-human immunoglobulins can be used).

Thus, for example, if human antibodies are mixed with erythrocytes, antibodies will be attached to their respective epitopes on erythrocytes (No agglutination occurs). To this mixture the addition of rabbit antibodies to human IgG will result in their binding with the Fe portion of the human antibodies bound to the erythrocytes by their Fab protion (Figure 5 –11). These rabbit antibodies bind with the human antibodies (that are bound to the erythrocytes).In binding with erythrocytes they cross-link

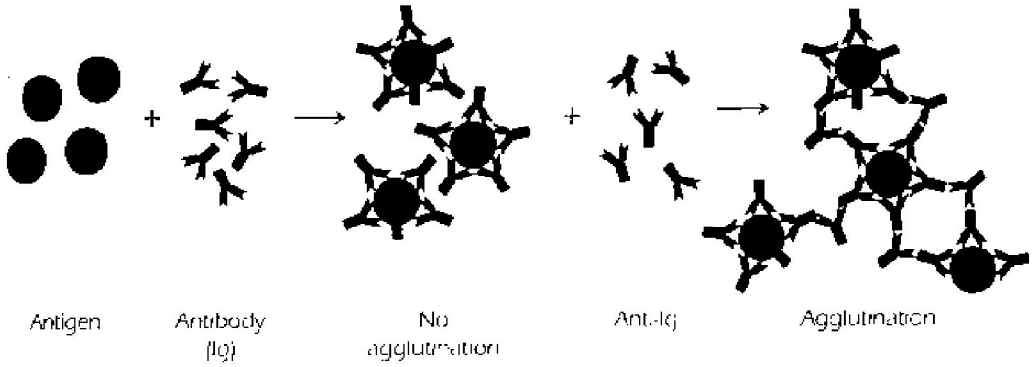

Fig. 5-11. A representation of the anti-immunoglobulin (Coombs) test.

between human IgG on relatively distant erythrocytes (erythrocytes become distant because of the separation caused by the Zeta potential). The binding between rabbit antibodies with human antibodies cause agglutination. The addition of anti-immunoglobulin brings about agglutination, even if there is high concentrations of antibodies. This agglutination is caused even if the antibodies directed against the antibodies present at sufficiently high concentrations to cause the prozone phenomenon.

5.4.8.A THE DIRECT COOMBS TEST:

There are two versions about the Coombs test: the direct Coombs test and the indirect Coombs test. Both tests differ in the mechanisms of the test but based on the same principle.

The principle of these tests: using heterologous anti-immunoglobulins to detect a reaction between immunoglobulins and antigen.

In the direct Coombs test, anti-immunoglobulins are added to the particles (e.g. red blood cells). These RBC are suspected of having antibodies bound to antigens on their surfaces.

For example, a new born baby suspected of having hemolytic disease can be tested by using direct Coombs test.

In the newborn hemolytic disease may be caused by maternal anti-Rh IgG antibodies when bound to baby's erythrocytes.

If the suspicion of having hemolytic disease in the newborn baby is correct, the direct Coombs test may give the following results:

the addition of anti-immunoglobulin to a suspension of the baby's erythrocytes would result in the binding of the anti-immunoglobulin to the maternal IgG on the surface of the erythrocytes and would cause agglutination.

5.4.8.B THE INDIRECT COOMBS TEST:

This test is useful to detect the presence of antibodies in the serum specific to antigens on the particle. The serum antibodies, when added to the particles, may fail to cause agglutination because of the zeta potential. The subsequent addition of anti Ig will cause agglutination.

Anti-Rh IgG antibodies in the blood of an Rh-negative woman can be detected by applying the indirect Coombs test.

5.4.9 SUMMARY

THE UTILIZATION OF THE IN VITRO REACTION BETWEEN ANTIGEN AND SERUM ANTIBODIES SERVES AS THE BASIS FOR MANY IMMUNE ASSAYS.

The interaction between antigen and antibodies in vitro is widely used for diagnosis.

In these clinical tests, antigen or antibodies can be detected and identified.

The interaction of antigen with antibodies may result in a variety of consequences including precipitation, agglutination and activation of complement.

Precipitation occurs if the antigen is soluble.

Agglutination occurs if the antigen is insoluble and is particulate.

The reaction of antibody with a multivalent antigen that is particulate (i.e. an insoluble particle) results in the cross-linking of the various antigen particles by the antibodies.

This cross-linking eventually results in the clumping or agglutination of the antigen particles by the antibodies.

This test consists, first, of the reaction of the woman's serum with Rh^+ erythrocytes, and then the addition of the anti-immunoglobulin reagents (as in the direct Coombs test). Thus, the direct Coombs test measures bound antibody while the indirect test measures serum antibodies.

Thus, the Coombs test is used for the detection, by the use of anti-immunoglobulin, of any Ig that is bound to antigen.

5.4.10 KEY TERMINOLOGY

Adhesion molecules: Mediate the binding of one cell to other cells. These molecules are important in the operation of the immune system.

Allergy: A term covering immune reactions to nonpathogenic antigens, which lead to inflammation and deleterious effects in the host.

Antibody: Serum protein formed in response to immunization.

Antiserum (plural: antisera): The fluid component of clothed blood from an immune individual. The clothed blood contains a heterogeneous collection of antibodies against the molecule used for immunization. Such antibodies bind the antigen used for immunization.

Complement: A series of serum proteins involved in the mediation of immune reactions. The complement cascade is triggered classically by the interaction of antibody with specific antigen.

$F(ab^1)_2$: A fragment of an antibody containing two antigen-binding sites; generated by cleavage of the antibody molecule with the enzyme pepsin, which acts at the hinge region C-terminally to the inter-heavy-chain disulfide bond.

Fab: Fragment of antibody containing one antigen-binding site; generated by cleavage of the antibody with the enzyme pepsin, which acts at the hinge region N-terminally to the inter-heavy-chain disulfide bond and generates two Fab fragments from one antibody molecule.

Plasma: Fluid component of unclothed blood.

Precipitin reaction: The mixing of soluble antigen and antibody against at different proportions that can result in the precipitation of insoluble antigen-antibody complexes.

Prozone: Agglutination may not occur at high concentrations of antibody. Even though it takes place at high dilutions of serum. The tubes with high concentration of serum, when agglutination does not occur, represent a prozone. In the prozone antibodies are present in excess.

Serum: Residual fluid derived from clothed blood; contains antibodies.

Titer: The highest dilution of serum that still causes agglutination, but beyond which no agglutination occurs is termed the titer.

5.4.11 SELF ASSESSMENT QUESTIONS

1. Explain the antigen and antibody interactions. Add a note on Coombs test.
2. Describe Coombs test.
3. Write short notes on:
   a. Precipitin reaction.
   b. Agglutination reaction.
   c. Direct Coombs test.
   d. Indirect Coombs test.

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