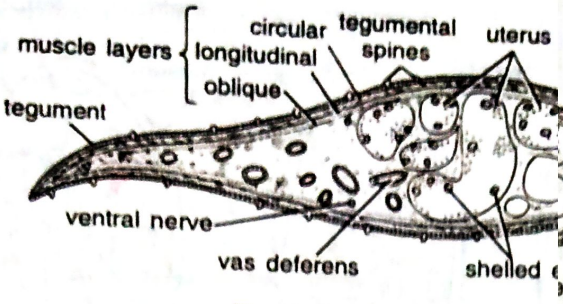

Fig. 13. *F. hepatica.* T.S. body th rough uterus.

secretion helps in lubricating uterus for smooth passage of eggs and in activating sperms. This secretion, perhaps, also causes release of shell globules from vitelline gland cells.

Life Cycle and Development

1. Digenetic life cycle. Life cycle of *F. hepatica* is complex and completed in two hosts. *Primary host,* in which the adult fluke lives, is sheep. While the *intermediate host,* in which numerous larval stages are passed, is a snail (*Lymnaea, Planorbis,* etc.). This type of life cycle, involving two different kinds of hosts, is termed *digenetic.*

2. Copulation. Self-fertilization is of rare occurrence in liver flukes though they are hermaphrodite. *Cross-fertilization* preceded by *copulation* is of normal occurrence. In *F. hepatica,* copulation takes place in bile ducts of the host.

Two flukes in copulation bring their genital pores in opposition. Cirrus of one fluke, everted through its gonopore, penetrates the Laurer's canal of the other through the latter's temporary opening, and injects spermatozoa. Secretion of prostate glands, and perhaps also of the Mehlis's glands, keep the sperm active for fertilization.

3. Fertilization. Fertilization is *internal.* In *cross-fertilization,* sperms received in Laurer's canal during copulation, enter the distal end of oviduct where fertilization occurs. During *self-fertilization,* sperms enter the uterus of same fluke through female genital aperture and pass down to fertilize the eggs.

4. Capsule formation. Each fertilized egg or zygote is surrounded by yolk cells, which provide yolk and shell material. Shell- globules of yolk cells contain proteins and a phenol. According to Stephenson (1947), phenol is oxidized to a quino ne in the proximal part of uterus. Quinone then t ans the protein, producing a hard, resistant and l eathery *sclerotin* like that of insects. This sclero tin forms the shell around fertilized eggs Above finding of Stephenson in liver flukes is perha ps true for all platyhelminths.

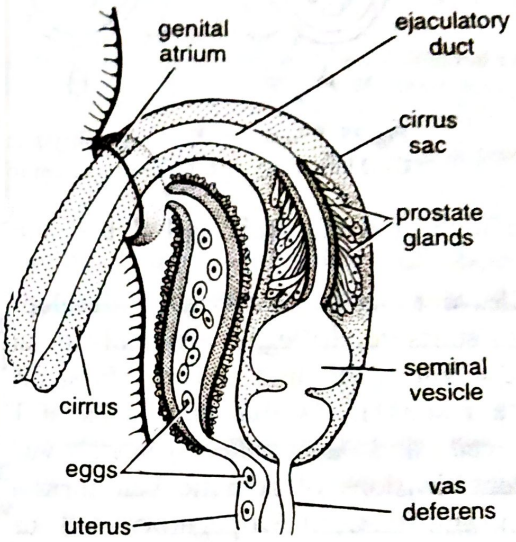

Fig. 14. *F. hepatica.* Cirrus protruding through gonopore.

5. Capsules. Shelled eggs are termed *capsules* or sir nply *eggs.* A shell or capsule is yellow or brow , in colour and oval in shape. It is about 130 t o 150μ long and 60 to 90μ wide. It is operc ulate, i.e., provided with a lid or *operculum.* Situat ed immediately beneath the operculum, at the te rminal end of egg is a viscous and granular cushi n. About 3,000 or more such capsules may occur at a time in the uterus of a single fluke. There may be as many as 200 flukes in the liver of or e sheep. If each fluke produces 500,000 eggs (in 10 years), a single infected sheep may dispe rse 100 million fertile eggs. This vast capac ity for egg production is necessary in view

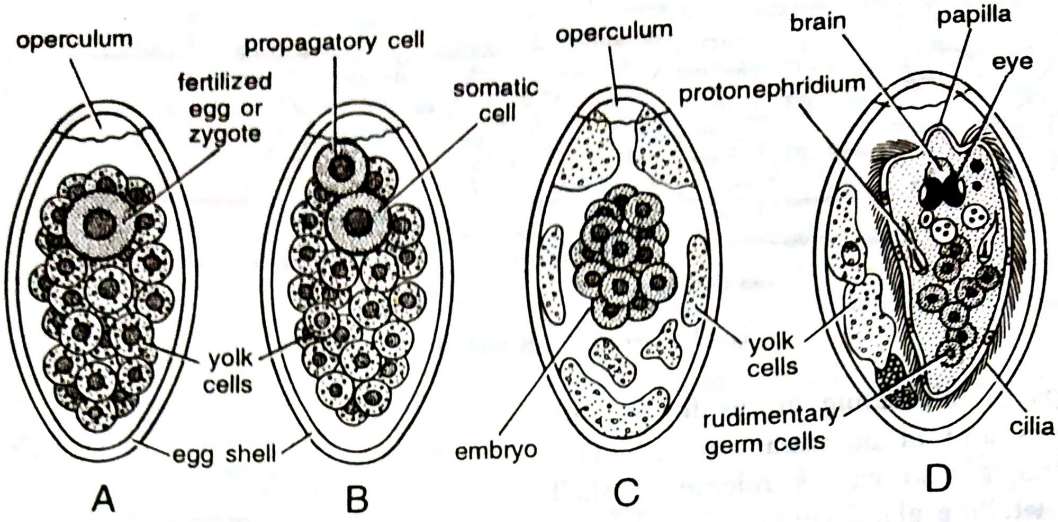

Fig. 15. *F. hepatica.* Early or embryonic development. A – Zygote in capsule. B – 2-cell stage. C – Many-cell stage. D – Miracidium in capsule.

of the complicated life cycle and slim chances of survival.

6. Cleavage and embryonic development. Cleavage starts while eggs are still in uterus. Cleavage is *holoblastic* and *unequal.* First division of zygote results in two *unequal* cells, a larger *somatic cell* and a smaller *propagatory cell.* Subsequent divisions of somatic cell form larval ectoderm and tissues. Propagatory cell divides further into two daughter cells. One daughter cell by its divisions finally produces the larval body. Other daughter cell divides several times to form a mass of smaller *germ cells* which cluster in the posterior part of larval body.

Encapsulated embryos or *capsules* or simply *eggs* do not develop further in fluke's uterus. A very large number of capsules leave fluke's body through its gonopore into host's intestine, and finally ejected out with its faeces. Further development takes place when capsules come in contact with water (or damp areas with at least 60% moisture content) which is slightly acidic (pH 6.5). Optimum temperature for development ranges from 22°C to 25°C.

7. Miracidium larva. It is the first larval stage involved in life cycle. When suitable conditions become available, the encapsulated embryo, in 4-15 days, differentiates into a *miracidium larva.* It hatches out and swims in water. Hatching is initiated by a proteolytic *hatching enzyme.* It dissolves the cementing material by which operculum is attached, thus releasing the operculum.

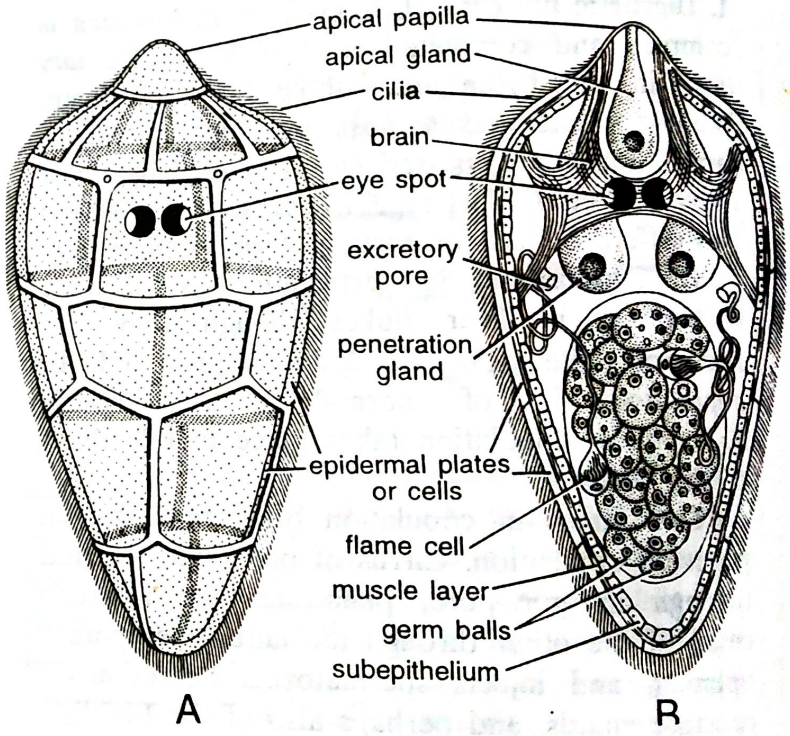

Fig. 16. *F. hepatica.* Miracidium larva. A – External structure. B – Internal structure.

(a) External structure. Miracidium is a minute, about 0.07 mm long, oval, elongate and richly ciliated active creature. Its broader anterior end is produced into a mobile and non-ciliated *apical papilla* or *terebratorium.* Miracidium is a multicellular organism. Its body is covered with flattened ciliated *epidermal plates* or cells, 21 in number and arranged in five rows or *tiers.*

Number and arrangement of cells in each tier is fixed. *First tier* (anteriormost) has 6 plates, two dorsal, two lateral and two ventral. *Second tier*

also has 6 plates, three dorsal and three ventral. *Third tier* has 3 plates one dorsal and two ventro-lateral. *Fourth tier* has 4 plates, two right and two left. *Fifth tier* (posteriormost) has 2 plates, one left and one right.

Beneath epidermal plates is a fine layer of *sub-epidermal musculature,* consisting of outer circular and inner longitudinal fibres. Below muscles is a layer of cells forming the *sub-epithelium.* Epidermal plates, sub-epidermal musculature and sub-epithelium together form the body wall of miracidium.

(b) ***Internal structure.*** Within the body of miracidium are present glands, nervous tissue, protonephridia and germ cells. A sac-like multinucleate mass of granular protoplasm is attached to the centre of apical papilla by a stalk. This structure, earlier thought to be rudimentary gut, is now regarded an *apical gland.* A pair of large, unicellular *cephalic* or *penetration glands* open by their narrow ends near the apical papilla. A large *brain* with several associated nerve fibres lies dorsally below epidermal cells of second tier. Situated above the brain is an "x" shaped larval *eye,* consisting of two crescentic pigmented cells or *eye spots,* with their concavities facing away from each other. The concavities contain a clear refractile material serving as lens. (It should be noted here that a photoreceptor of any sort is absent in adult due to its or the parasitic mode of life). A pair of long tubular *protonephridia* or *flame cells* open to the exterior through two *nephridiopores* or *excretory pores,* situated laterally in the posterior half of body. *Germ cells* lie in groups, called *germ balls,* in the rear part of body.

(c) ***Physiology.*** Miracidium does not feed. It swims about desperately as if *"seeking something with feverish haste"* (Barlow, 1925). Miracidia tries to penetrate any object or organism they may come across, but only those succeed that come in contact with a specific intermediate snail host (*Lymnaea, Succinea, Planorbis, Bulinus, Possaria, or Practicolella).* Those, which do not come across the suitable host, in about 24 hours after hatching, die invariably. For penetration, miracidium attaches its apical papilla and performs boring movements together with

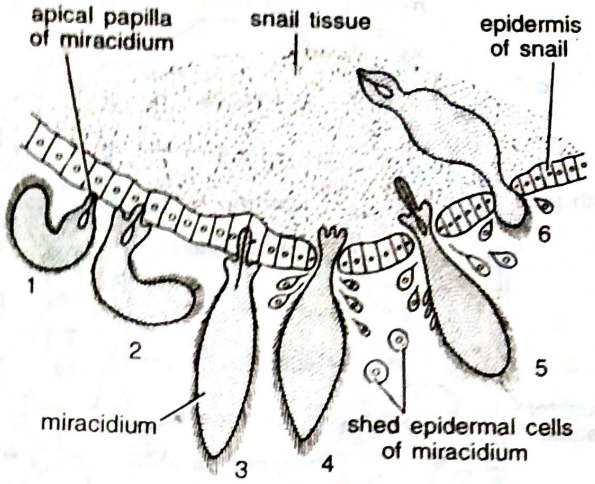

Fig. 17. Miracidia of *Fasciolopsis buski.* Stage of penetration through snail epidermis.

contractions and expansions of body. This process, aided by the action of flesh-dissolving larval secretions, results in a minute opening in the host's tissue. The larva then squeezes through this opening, casting off its ciliated epidermis while doing so. It soon makes its way into the digestive gland of snail, where it undergoes various changes and, in about 14 days, develops into the second larval stage, the *sporocyst larva.*

8. Sporocyst larva. It looks like an elongated sac, about 0.7 mm long. Its body wall retains all the layers of body wall of miracidium except the ciliated epidermis, which is lost in the process of

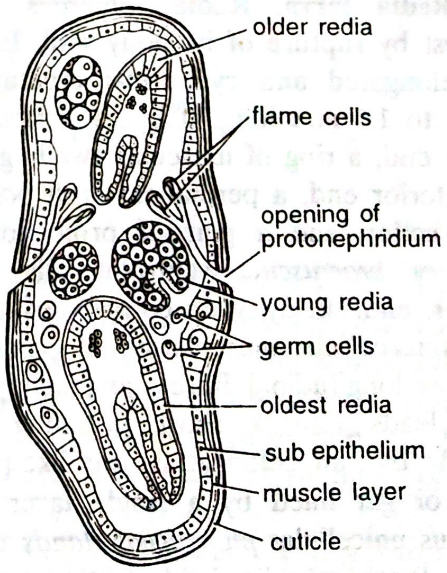

Fig. 18. *F. hepatica.* Sporocyst larva.

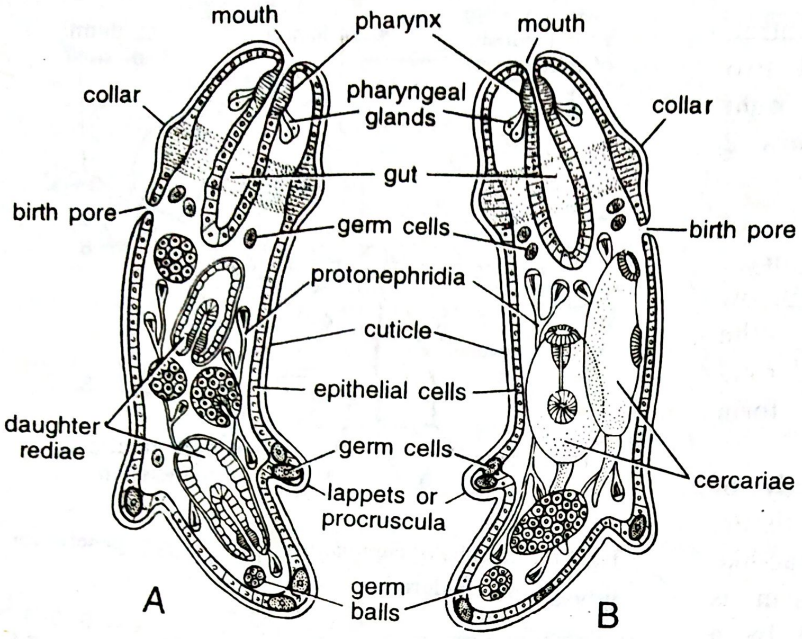

Fig. 19. *F. hepatica.* A – Redia with daughter rediae. B – Redia with cercariae.

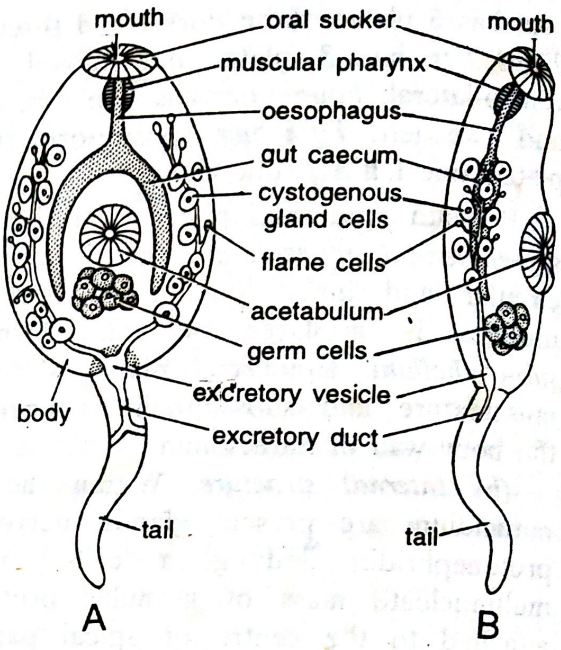

Fig. 20 *F. hepatica.* A – Cercaria in ventral view. B – Cercaria in lateral view.

penetration and soon replaced by a thin *cuticle.* Glands, brain, eye spots and apical papilla of miracidium degenerate and disappear in sporocyst. *Protonephridium* of each side divides into two *flame cells* which open outside by a common excretory pore. In addition, the sporocyst contains *germ balls.* Sporocyst moves about in the tissue of host, absorbing nutrition from it. Its germ balls develop into the next larval generation, the *rediae.* Each sporocyst produces 5 to 8 rediae.

9. Redia larva. Rediae emerges from the sporocyst by rupture of its body wall. Each redia is an elongated and cylindrical creature, about 1.3 mm to 1.6 mm long. It bears a *mouth* at the anterior end, a ring of muscular swelling or *collar* near anterior end, a permanent *birth pore* a little behind collar, and a pair of projections called *lappets* or *procruscula* ventrolaterally near the posterior end. Body wall consists of the usual layers, viz., cuticle, musculature of outer circular and inner longitudinal fibres, and subepithelium. Mouth leads into a short muscular *pharynx,* followed by an elongated sac-like *intestine, enteron* or *gut* lined by a single layer of cells. Numerous unicellular *pharyngeal glands* open into pharynx. *Protonephridia* divide further and form a much branched system. However, all the flame cells of each side open out through a common excretory duct. Body of larva is packed with *germ balls* and mesenchyme cells.

Redia moves through the host's tissues on which it also feeds. Movements are brought about by muscular contractions of body, aided by the collar and lappets. Moving rediae enter various organs of snail but prefer to migrate to its digestive gland. During summer months, when sufficient nourishment is available, the germ balls of rediae give rise to a *second generation of rediae,* morphologically identical to the parents. During winters, germ balls of rediae of second generation develop into larvae of the next stage, known as *cercaria larvae.*

10. Cercaria larva. Each redia produces 14 to 20 cercaria larvae. They leave the body of redia through its birth pore and enters the snail's digestive gland. Morphologically, cercaria bears a close resemblance with the adult fluke. It has an oval body, 0.25 mm to 0.35 mm long, with a long simple tail for swimming. Layers of its body wall are the same as of sporocyst and redia. Cuticle bears backwardly directed spines. Below body wall lie numerous *cystogenous gland cells,* which secrete cyst for the next larva (metacercaria).

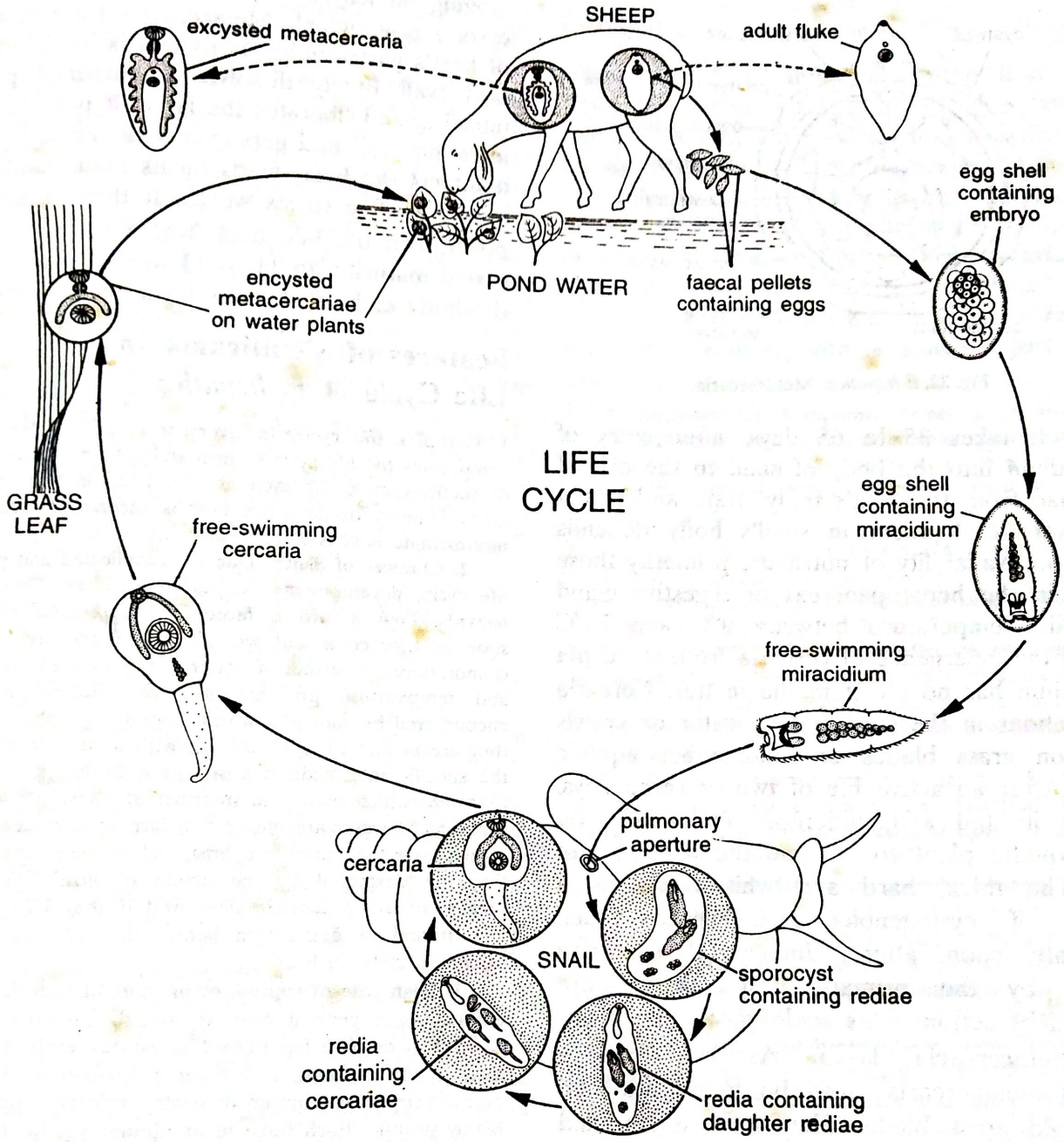

Fig. 21. *F. hepatica.* Life cycle.

Well formed *anterior (oral)* and *ventral suckers* like those of adult are present. Rudiments of adult's digestive, excretory and genital systems can be seen in cercaria. Mouth leads into a muscular *pharynx,* followed by oesophagus and intestine, the latter forking in front of the ventral sucker to form two tubular limbs. *Flame cells* occur in large numbers along the lateral zones, opening into a pair of *excretory tubules,* which unite in front of the tail to form an *excretory vesicle* or *bladder.* An *excretory duct* arises from bladder and extends into tail, where it bifurcates and opens out through a pair of *nephridiopores.* Lying in the body are groups of *germ cells,* which are direct descendants of propagatory cell of the capsule. These cells represent rudiment of adult's genital system.

Mature cercaria makes its way through the host's tissues, often migrates to its pulmonary sac, and from there escapes to the surrounding

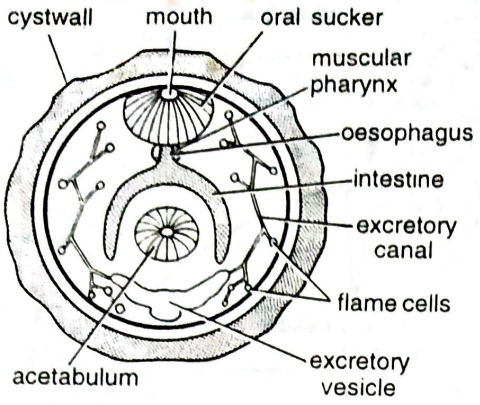

Fig. 22. *F. hepatica.* Metacercaria.

water. It takes 35 to 65 days, after entry of miracidium into the body of snail to the exit of cercariae from the host's body. Rate and extent of larval development in snail's body depends upon the availability of nutrients, primarily those stored in the hepatopancreas or digestive gland of snail. Temperature between 9°C and 26°C favours the emergence of cercaria from snail; pH of medium has no effect in the matter. Cercaria swims about in the surrounding water or crawls about on grass blades or some other aquatic plants. After an active life of two or three days, it loses its tail and undergoes encystment on some aquatic plant to become the *metacercaria larva.* The thick, hard and whitish cyst is a product of cystogenous gland cells which degenerate soon after. Unencysted carcariae ingested by the primary host (sheep) are destroyed by action of its acidic gastric juice.

11. Metacercaria larva. As many as a thousand metacercariae may be found attached to a single grass blade. They have a rounded form with a diameter of about 0.2 mm. Metacercaria is in fact the *juvenile fluke,* also called *marita.* It differs from cercaria in that it has a rounded form, a thick hard cyst and large number of flame cells. It lacks a tail and cystogenous gland cells and its excretory bladder opens out directly through a single pore. Germ cells or the genital rudiments are present as such. Cyst provides protection against short periods of desiccation.

12. Infection of primary host. Metacercaria develops into adult fluke only inside its definitive host or sheep. The latter gets infection by grazing on leaves and grass blades to which the cysts are attached. Metacercaria survives action of host's gastric juice as its cyst is insoluble in it. Cyst wall finally dissolves in proximal part of intestine and liberates the larva. It penetrates the intestinal wall and gets into coelomic cavity. Now it infects the liver, feeds on its tissue, and grows in size in five to six weeks. It then takes up its position in the bile duct, where it finally attains sexual maturity. In 11 to 13 weeks, after entering the body of host, it starts laying eggs (capsules.)

Features of Significance in Life Cycle of *F. hepatica*

1. Complex life cycle. Involvement of several larval stages complicates the life cycle of liver fluke. Further, liver fluke is digenetic, that is life cycle is completed in two alternating hosts. Primary or definitive host is sheep and secondary or intermediate host is a freshwater snail.

2. Chances of death. Due to complicated and prolonged life cycle, developmental stages have to encounter several hazards. First hazard is faced by *encapsulated embryos* as soon as they come out with faeces of primary host. They cannot develop further if water (or moisture), suitable pH and temperature are not available. Second harzard is encountered by *miracidia* which emerge from the capsules. If they are unable to make contact with a suitable soft part of the specific intermediate host within 24 hours, they perish. *Cercariae* which leave the intermediate host and swim into surrounding water are the next to face an uncertain future. If ingested by the vertebrate host, before they have encysted, they are destroyed by the action of host's gastric juice. Finally, *metacercariae* are destroyed if they have to face a long period of desiccation before they are ingested by a suitable vertebrate host.

3. High rate of reproduction. *Rate of reproduction* of an animal, as a general rule, is directly proportional to the *chances of death* it has to face at various levels. The ratio, in fact, is the deciding factor for perpetuation of a species. *Fasciola hepatica,* exposed to several threats to its survival, is highly prolific. Each fluke in its lifetime produces more than 200,000 eggs. Further, each sporocyst produces 5-8 rediae, each of which in turn produces 8-12 rediae in the second generation, each of which further produces 14 to 20 cercariae. Thus, each egg is *capable* of producing 1000 to 2000 cercariae but the actual number produced is far less due to high mortality. Out of those produced, a very small number successfully encysts and infects the final host.

4. Heterogamy. Grobben (1882) and some others believed that germ cells in the sporocysts and rediae were eggs which developed *parthenogenetically* into subsequent larval forms. This kind of asexual parthenogenetic reproduction by larval forms is known as *heterogamy.* Reproduction in immature or larval stages is called *paedogenesis* (Gr., *pais,* child; *genesis,* origin). This view, however, is now considered erroneous and has been given up in favour of the *polyembryony,* concept is described further.

5. **Polyembryony.** Germ cells in sporocysts and rediae are direct descendants of *propagatory cells* produced by first division of zygotes. These germ cells multiply mitotically and produce subsequent larval stages within sporocysts and rediae. In doing so, they (germ cells or propagatory cells) behave as several embryos. Thus, this process of reproduction in sporocysts and rediae has been looked upon as *polyembryony* by Ishii (1934), Chen (1937), Rees (1940) and Cart (1944).

6. **Metagenesis.** Life cycle of *Fasciola* involves a period of asexual reproduction during immature stages (sporocysts and rediae) followed by a period of sexual reproduction in the adult stage. Steenstrup (1942) and some others interpret this as an alternation of asexual and sexual generations in the life cycle. But Hyman is of the view that it is a continuous ontogeny (life history) involving asexual multiplication in larval stages.

7. **Advanced larval stages.** Miracidium and cercaria, being free-living larvae, exhibit more advanced features than the adult which has undergone degeneration in many respects to suit its parasitic mode of life. Body cavity, locomotory organs, sense organs, and a cellular epidermis are lacking in adult but are present in larvae.

Parasitic Adaptations of *Fasciola*

Liver fluke has undergone great modifications, morphological as well as physiological, to suit its existence as an endoparasite in the bile ducts of sheep.

(1) Outer *tegument* is thick, permeable to water, but enzyme-resistant, so that parasite is not digested by digestive juices of the host.

(2) Locomotory organs are absent as not required by adult. However, free-swimming larvae, such as miracidium has cilia and cercaria has a locomotory tail.

(3) Oral sucker, acetabulum and spines of body wall of adult worm serve as organs of attachment in the host's body.

(4) Alimentary canal is without anus as there is no undigested food for egestion. Suctorial pharynx helps in sucking bile etc. and much-branched intestine serves to distribute digested food to all parts of the body.

(5) Adult lacks circulatory, respiratory and sensory organs as they are not needed. Nervous system is poorly developed for the same reason. However, free-swimming miracidia has sensory eye spots.

(6) Respiration is *anaerobic* as free O2 is not available.

(7) Reproductive system is highly developed. Number of eggs produced is enormous (about 200,000 eggs per fluke). This is necessary to offset several hazards resulting in great mortality.

(8) Resistant egg shells around zygotes provide further safety from unfavourable environmental conditions.

(9) Hermaphroditism ensures self-fertilization even in the absence of another companion for copulation. It is necessary for survival of the species.

Liver Rot

When sheep are infected by the liver fluke *Fasciola hepatica,* the liver of sheep is seriously affected in structure and function. This disease is known as "Liver rot", or "Fascioliasis".

Infection. The vertebrate host (sheep, goat, etc.) gets the infection by grazing on grass, leaves and other vegetation to which metacercarial cysts are attached. The invertebrate host (snail) acquires infection when a miracidium, at random, establishes contact with a suitable part of its body.

Pathogenesis or Symptoms. Infection of invertebrate host (snail) results in a partial or complete destruction of the affected site, which is preferably the digestive gland (liver) or gonad. In case of heavy infections, snail considerably increases in size.

Of significant economic importance is the effect of *F. hepatica* on its vertebrate host, whose bile ducts as well as liver may be damaged. In bile ducts, it causes *inflammation* and *hepatitis,* resulting in loss of its epithelium and thickening of wall, followed by calcification and formation of *gall stones.* Heavy infections upset the normal metabolism of liver. This is due to *haemorrhage* caused and irritation inflicted by cuticular spines. The disease thus caused is called *liver-rot* or *fascioliasis.*

Symptoms of *liver-rot,* are more acute in lambs than in sheep, appear about a month after infection. Frequently, death may soon result due to *cerebral apoplexy.* However, if the host survives few weeks of infection, it falls a victim to acute *anaemia* and falls even at mild contact. Its appetite declines, rumination (chewing the cud) becomes irregular and at times there is fever and increase in respiratory activity. Conjunctiva becomes whitish-yellow, and dry and brittle wool falls off. After three months of infection comes the fatal period. Large *oedemas* or swellings ("watery poke") appear on jaws. Lactation and breeding are greatly reduced. Rarely does the host survive this period. In case it does, the fluke may migrate to the duodenum and finally escape to the outside world with faeces. When this happens, or when fluke somehow dies in *situ,* the host recovers considerably.

Infection by *F. hepatica* takes a huge toll of sheep annually. In England it caused the death of about one and a half million sheep in 1830 and about double in number in 1879-80. Ireland lost 60 per cent of its flocks in 1882.

Therapy or treatment. Treatment of infection is not easy because it is difficult to introduce drugs in bile passages of infected sheep. Anti-helminth drugs such as *hexachloroethane,*

carbon tetrachloride, filcin, emetine hydrochloride, phenothiazine and *tetrachloroethane* are being employed for treating cases of liver-rot. These drugs are fairly effective in killing stages of the parasite in liver.

Prophylaxis or Prevention. It is better to prevent infection by control of the vector or intermediate host. Preventive measures include : (i) killing heavily infected sheep, (ii) destroying eggs and manure of infected sheep, (iii) feeding infected sheep with salt and little dry food, and (iv) killing or checking snail population. Snails are killed by adding copper sulphate solution in ponds and ditches or by draining their pastures as they are unable to survive long dry periods. Ducks feed on snails and can be usually employed in removing their population. Breeding of snails can be checked by removing vegetation from ponds and streams they inhabit. Man can avoid infection by consuming thoroughly washed and adequately cooked vegetables.

IMPORTANT QUESTIONS

» Long Answer Type Questions

1. What is a digenetic life cycle ? Explain it with reference to the life history of *Fasciola hepatica.*
2. Give an account of the reproductive organs of *Fasciola hepatica.*
3. Give a detailed account of the parasitic adaptation in *Fasciola.*
4. Give a detailed account of the life cycle of *Fasciola hepatica* and discuss its economic importance.
5. Describe the anatomy of *Fasciola hepatica* with special reference to its parasitic mode of life.
6. Draw neat and fully labelled diagrams of the following : (i) T.S. of *Fasciola* passing through the ovary. (ii) V.S. of Body wall of *Fasciola.* (iii) Full page, well-labelled diagrams of life history of *Fasciola.* (iv) Reproductive system of *Fasciola hepatica.*
7. Write an essay on the economic importance of *Fasciola hepatica.*
8. Write notes on : (i) Cercaria, (ii) Control measures against *Fasciola* infection, (iii) Distinction between digenetic and monogenetic parasites, (iv) Effects of infection of *Fasciola* on primary host, (v) Flame cell, (vi) Metacercaria, (vii) Miracidium, (viii) Ootype, (ix) Polyembryony, (x) Redia, (xi) Sporocyst.

» Short Answer Type Questions

1. What is miracidium ?
2. What are flame cells ?
3. Where can you locate flame cells ?
4. In *Fasciola* write the name of the canal that leads from the junction of the oviduct and combined vitelline duct and open to the exterior on the mid-dorsal surface.
5. Explain the structure of the body wall in *Fasciola.* Draw and mention three adaptive features.
6. Define polyembryony and mention its example.
7. Define parthenogenesis. Explain it with reference to development in *Fasciola.*
8. Draw a labelled sketch of Miracidium of *Fasciola hepatica.*
9. Draw a neat labelled diagram T.S. of liver fluke through middle region.
10. In *Fasciola,* draw neatly stages from Redia to Metacercaria and label all the parts.
11. Draw a sketch showing the reproductive system of *Fasciola hepatica* and label it fully.
12. Give an account of the reproductive system of *Fasciola.*
13. Give an account of the life history of the common liver fluke.
14. Give a detailed account of the reproductive organs of *Fasciola.* How is it adapted to the parasitic mode of life ?

» Multiple Choice Questions

1. In *Fasciola* miracidium develops into the next stage inside :
   (a) *Bulimes* (b) *Limnea trunculata*
   (c) *Pila globosa* (d) *Planorbis*
2. *Planorbis* and *Lymnaea* are the intermediate host of :
   (a) *Fasciola* (b) *Schistosoma*
   (c) *Trichinella* (d) *Echinococcus*
3. Which of the infective stage to primary host of *Fasciola* ?
   (a) Miracidium (b) Sporocyst
   (c) Metacercaria
   (d) Cercaria
4. One of the following larval stage of *Fasciola* does not produce several larvae ?
   (a) sporocyst (b) secondary Redia
   (c) primary Redia (d) Cercaria
5. Which layer of *Dugesia* contains rhabdites :
   (a) epidermis (b) circular muscle
   (c) parenchyma (d) cuticle
6. Miracidium is one of the stages in the development of :
   (a) *Fasciola hepatica* (b) *Taenia solium*
   (c) *Ascaris lumbricoides* (d) *Planaria*