

## 1. INTRODUCTION

### 1. Reproductive Organs of Pulmonate Gastropods

The pulmonates are in general simultaneous hermaphrodites. The reproductive system is complicated and its details constitute important taxonomic characters. They possess rather large and complex accessory sex organs. The ovotestis embeds in the midgut gland and consists of a variable number of follicles, related to the size of animal. From the ovotestis a hermaphroditic duct carries both the eggs and sperms to a point where the male and female systems separate. Along its course the hermaphroditic duct bears a number of small diverticula in which sperms are stored for which they are considered to be seminal vesicles. The hermaphroditic duct continues to a complicated area termed the carrefour into which opens a large albumen gland and small fertilization pouch. The sperm duct and the oviduct are difficult to ascertain and vary with species.

After separating from hermaphroditic duct at the carrefour, the sperm duct proceeds to male gonopore usually located behind the right tentacle. In its course to the tentacle the sperm duct is an open ciliated groove in Pythia and possibly in other Ellobiidae.

Along its course the sperm duct is provided with prostatic material, either in the form of a few to many diverticula or as a swollen region or as a large appended body. Beyond the prostatic region the sperm duct enters the proximal end of penial complex, which typically consists of the penis sheath or sac, the penis enclosed in the sheath, the prepuce, which continues the sheath and a long blind tubular appendage, the flagellum. The penis is simple, conical or cylindroid projection, armed at its tip with a stylet in some planorbidae. The penis is pierced centrally by sperm duct, which may open symmetrically at the penis tip or bend laterally to open at the side of tip.

The muscular portion of the sperm duct prior to the penis sheath is called epiphallus. It participates with the flagellum to form spermatophores. These are generally limited to terrestrial pulmonates. The oviduct separates from the hermaphroditic duct at the carrefour, where the attached albumen gland may be regarded as belonging to the female system. In many pulmonates the oviduct and the sperm duct after leaving the carrefour become closely applied to each other or even partially fused forming what is called by many authors as spermoviduct. Distally the two duct separate and pursue their courses to the penial complex and the vagina, respectively.

## 2. General Histology of Reproductive Organs :

Histological details of the reproductive system have been furnished by many authors but as they differ with species a general account must suffice. The hermaphroditic duct is lined with a 'cuboidal to high columnar ciliated epithelium, often glandular sometimes followed by muscle coat. In Lymnaea stagnalis (Holm, 1946) the hermaphroditic duct has on one side a ciliated groove lined with high epithelial cells. The fertilization pouch is lined with columnar ciliated cells or with columnar cells interspersed with small ciliated cells. The sperm duct is lined with low to high ciliated epithelium or with such cells interspersed with cuneiform ciliated cells. The epithelial cells may contain distally granules or globules of secretion. Outside the epithelium the duct is provided with a thick layer of circular muscle often followed by longitudinal fibers and connective tissue containing chondroid cells. The prostate is composed of epithelial cells packed with globules or with granules, acidophilic or basophilic and interspersed with cuneiform ciliated cells. The penial complex is characterised by the thick muscular walls, the lining epithelium is glandular, often with goblets. The penis is armed with thorns in Chilina, Hattonella and Ariophanta in which the inner surface of penis sheath is also thorny (Dasen, 1933).

### 3. Information of Male Accessory Sex Organs :

There are three important accessory sex organs i.e. prostate gland, dart gland and penial complex, attached to the male reproductive part of the hermaphrodite gastropod pulmonates. The following paragraphs give the information about these organs in detail.

#### Prostate Gland :

✓ The prostate gland of most stylommatophora is a thin, long, tubular gland connected to the sperm groove along much of the length of the spermoviduct. Most prostate tubules are similar in having two distinct types of cells. 1. Basal, triangular, secretory prostate gland cells and 2. Smaller, nonsecreting cells that intercalate between the gland cells and convey the secretory products with their cilia. Quattrini (1967), examined the ultrastructure of the prostate gland in Milax gagates, in the Soleoliferans, Vaginulus (Veronicella), Borellianus and in Laevicaulis alte and found them all to be very similar. Under the electron microscopic studies of prostate gland, Quattrini (1967) found two interesting observations in his work with Milax gagates, (1) the nuclei of the gland cells appear to have a great deal of DNA in many small granules, so that they may be polypoid; and (2) considering large amount of protein secretory material in the cytoplasm, there is very little RNA present, suggesting that the prostate may have a continuous, slow and nearly uniform activity.

Numerous other workers, using histological methods, have noted that the secretions are apocrine (Sirgel, 1973; Stears, 1974).

Prostate gland is generally assumed to provide fluid for autosperm during mating. The issue is complicated, however by the fact that the prostate glands seem to be active during the female phase of the reproductive process also (Lusis, 1961; Bayne, 1967; 1973). Ghose (1963), too found in Achatina fulica that the prostate secretes fluids during egg laying. In this same snail, Ramasubramaniam (1979) found some histochemical evidence indicating that both the shell membrane and egg shell receive prostatic secretions contrary to Els (1978). Thus, it is possible, although not yet proved, that the prostate may have more than one function : (1) it can help convey autosperm but not provide nourishment, because prostate secretions lack polysaccharides, which are provided by the flask cells instead; Els (1978); (2) it can help move allosperm after mating; (3) it can be involved in sperm capacitation in some way (for autosperm or allosperm?); or (4) it may take part in egg shell formation (Nanaware, 1974).

A supposed further complication is the observation that the prostate of Heteurethrans and Orthurethrans appears to be entirely different morphologically (Steenberg, 1925), and it is claimed that they may not be homologous with those of most of the Sigmurethrans nor with those of the Basommatophora, (Visser, 1977). In Succinea, the prostate consists of a mass of branching

tubules arranged radially, forming six channels before combining into the single duct leading into the vas-deferens. Other snails are also striking in their tendency to compact the prostate tissue into a single, spherical gland at the most posterior part of the reproductive ducts, near the carrefour in Pseudaneita gigantea and Ailya camerunensis (Van mol, 1978; Burton, 1980). Nevertheless in a comparison of veronicellid and stylommatophoran prostates, Quattrini (1967) showed that the cell types appear to be very similar and homologous despite differences in gross morphology. The prostate invariably empties into the vas defrens in all these cases, where the spermooviduct is completely separated. In many cases, ovoviviparity probably causes spermooviduct shortening, forcing the compaction of the prostate (Tompa, 1984). ♂

In the Soleoliferans, Veronicella and Laevicaulis, which always have a separate male duct with it's own distinct prostate gland. The prostate appears to be complete normal and active despite the fact that these species can reproduce indefinitely by self fertilization inside the hermaphroditic duct or even inside the gonad (Quattrini, 1967). In such cases, the prostate may still be need to activate, transport, or nourish a self fertilizing animal's own sperm. Nevertheless, it is interesting to note that although a prostate gland does occur in normal. Acanthinula lamellata, it is absent in Aphallics (Boycott, 1917). Similar observations have been made for some but by no means all aphallic or hemiphallic species (Boycolt, 1917; Watson, 1923b; 1934; Pilshry, 1939; 1948).

Pupillids such as *vertigo* are especially interesting because of their great variation in this respect.

A number of workers have found that in addition to the prostate gland, the posterior spermoviduct of many species contains another set of male gland, the flask glands. They are unicellular and discharge their products between the epithelial cells in groove. The histochemistry of two flask gland cell types has been described by Smith (1965) and Els (1978). Smith (1965) found that in Arion alter, flask glands (as well as prostate gland) secrete fluid into the sperm duct at copulation, enveloping and mixing with large sperm mass, which moves down the duct.

The flask-gland secretions appear to contain polysaccharides (Els, 1978), possibly providing an energy source for the sperm. Both flask-gland cell types and the prostate gland are found in snails that never form spermatophores, so that their secretions need not contribute to spermatophore formation.

### **Dart Gland :**

The existence of darts in certain snails of the family Helicidae has been known and documented for more than a hundred years (Ashford, 1883). Taylor (1894, 1914) divided stimulatory structures in land snails into two major groups : (1) the sarcobelum, a fleshy stimulator arising from the area of the penis, and (2) the gypsobelum, a sharp, hard, calcified or chitinous structure usually

arising from the female side but from the male side in zonitids, whose darts are thought to be not homologous with helicid darts of the reproductive system, used for piercing the mating partner's body. Taylor (1914) further subdivided the gypsobelum into 4 types on the basis of the presence or absence of blades on the shaft and on the basis of the number (two or four) and shape (simple or flanged) of the blades.

In the review of darts and dart biology by Tompa (1980), 10 families of land snails were found to form darts (Helicidae, Zonitidae, Philomycidae, Ariophantidae, Urocyclidae, Helicellidae, Helminthoglyptidae, Bradybaenidae, Parmarionidae, and Vitrinidae) and the major functions possibly associated with the dart apparatus were discussed. In the analysis of comparative dart sac morphology by Shileyko (1973), the group 'Helicodea' was discussed.

Most snails like Helix, lose the dart during mating, when it is extruded through the genital pore and pushed against (and into) the mating partner's body, usually on the right side of the foot, it then detaches. It is usually regenerated completely before the next primary mating session, this process requires 1 week (Tompa, 1982). Other snails, such as Philomycus, Ventridens, Parmarion and Helminthoglyptids, pierce the partner but then retract the dart back into the sac for later reuse (Tompa, 1980).

The method of dart formation was examined with X-ray radiography in Helix aspera by Tompa (1982). The long, mostly hollow



cone forms first, starting on the very first day of loss. This cone grows anteriorly from a fleshy tubercle inside the base of dart sac. The four blades are then elaborated and finally, the most posterior structure, the corona, is added. The whole process takes about 6-7 days, and it is the dart sac lumen probably determining the specific dart shape. Calcium for the heavily mineralized dart comes from sources outside the dart sac itself (Dillaman, 1981; Tompa, 1982). Dillaman (1981) has examined the histology of the dart sac epithellium in Helix aspera during dart formation.

Only the darts of Helix (Hunt, 1979) and Philomycus (Tompa, 1980), have been analysed - mineralogically; they are both made of aragonite, a form of  $\text{CaCO}_3$ , as is the body shell. If a calcified dart from the Helix is placed into acid, it dissolves, leaving behind an organic ghost. Hunt (1979) found that a protein occurs in the dart as external sheath and as a lining of the tubular core, preventing the dart from being easily demineralized. The matrix of the dart has a different protein constituent. Hunt (1979) gave the amino acid compositions of these two types of proteins. He noted, as did Taylor (1894, 1914) earlier, that the four simple blades may be not for cutting but for buttressing or supporting the dart, much as an H-section steel construction beam does, so that it is stronger in resisting breaking but lighter than if it were solid. This idea may find support in the observation that spiral dart (e.g., Spirotoxon) also occur. A completing hypothesis, however, can be made here, that the blades provide additional surface area for holding and

carrying mucous gland secretions on the dart meant to be introduced inside the recipient's body during mating (Tompa, 1984). The more elaborate the blades (e.g. Capaca nemoralis, with 4 blades, each flanged), the more glandular secretions it can hold.

Dart shape is rather constant within a given species. The dart morphology is classically used for distinguishing Cepaea nemoralis from C. hortensis. Hybrid between these two species have an intermediate dart morphology (Lang, 1908). Dart characters are sometimes used in taxonomic studies, as in the Trichotoxon group (Sensulato) of Africa, in which dart are elaborate and are partly calcified, partly chitinous. The subgenus Atrichotoxon lacks darts. Trichotoxon has 4-6 darts arranged by pairs. Polytoxon has 8 or more slender, needle like darts. Spirotoxon has single, spiral dart and Anisotoxon has 70 darts (Pilsbry, 1919; Van Goethem, 1977). The largest known darts occur in relatives of this group; Trichoioxon robustum sports 14 darts, each almost 3 cm long (Simroth, 1896; Verdcourt and Polhill, 1961).

Snails that possess a dart (e.g. Helix aspera) seem to have it throughout the entire year except for the first week after mating. Following it's loss, in Philomycus carolinianus whose dart is not lost at mating, the larger the animal, the larger its dart. The dart shows concentric lines, suggesting that it grows (Tompa, 1980). In Trichotoxon thickense, Verdcourt and Polhill (1961) found that small darts are present even in juvenile, sexually immature slugs;

whether they grow or are instead replaced by darts of ever increasing size is not clear.

Ashford (1883) listed British snails that have one dart sac with one dart, two dart sac with a dart in each sac or a pair of bilobed sacs with one dart in each larger sac. The outer two 'dart sacs' may yet turn out to be the glands of the two real sacs (at least in some cases).

Some Helicids appear to have undergone some degree of evolutionary dart degeneration. In Ochthephila turvicula, the dart sac has diminished in size to become a mere hemispherical evagination on the vaginal wall. In some species of Theba, degeneration is even more extensive, and finally, snails such as Monacha (ashfordia) granulata lack all these organs completely (Watson, 1923). Bornchen (1967) noted that there are few snails that lack a dart sac yet possess glands (e.g., Helicodonta and Carocollina) and also noted that even when there is a dart sac, occasionally it may lack a dart gland (e.g. Microcystis). However caution is indicated; in some better studied cases (e.g., Philomycus carolinianus). Although the dart sac appears to lack an attached gland. There is an 'atrial' gland nearby that could actually be the dart gland. It is also entirely conceivable that dart glands may be completely internal or not visible externally. In the vast majority of cases, the dart sac is clearly associated with a dart gland (a finger form gland). In those cases were the entire dart sac apparatus in

undergoing evolutionary degeneration, the sac and the associated gland certainly may disappear at somewhat different rates.

The glands usually open by ducts slightly posterior to the dart sac opening, but they may also open into the junction of the sac and the vagina, into the dart sac through a special mucous gland chamber e.g. Bradybaena (Eulota), or at various positions behind the sac attachment. In some species, two different types of glands appear to be associated with the dart apparatus e.g. Polymita (Moreno, 1950) and Cepolis and Micraionta spp. (Pilsbry, 1939; 1948). It is possible that one gland is responsible for dart formation (similar to glands in the dart sac wall of Helix) and that the other gland is analogous to Helix dart glands, which elaborate secretion for mating.

In only a few cases the dart is obviously a hypodermic apparatus, such that the gland products are unequivocally injected directly into the partner through the dart itself. These include Microparmarion malayanus, M. strubelli, Wiegnoannla beccani and some other species of the Parmarionidae; in these cases the dart itself is hollow throughout with a subterminal opening (Hoffman, 1940). Another such clearly hypodermic function is apparent in Semilimax (Vitrinopugio) of the family Vitrinidae. In the Asian Ariophanta lingula, Dasen (1933) noted that the so called amatorial organ also has a hollow stylet capable of injecting fluid during mating. In the soleoliferan Onchidium peronii, a hollow stylet

injects some material into the mating partner (Webb, et al., 1969). These latter two examples do not appear to be homologous with Helix darts.

Major studies of the histochemistry and ultrastructure of the dart glands include those by Nolte (1959), Hollande (1966), Bornchen (1967), Ovtracht (1967) and Nanaware and Varute (1973a). Filhol (1938) has made a comparative histological study of dart glands from several snails. In general, the gland cells are characterized by large amounts of rough endoplasmic reticulum and Golgi bodies. Bornchen (1967) found two types of secretory products; one is merocrine and one is apocrine. Ovtracht et al. (1969) have isolated and characterized the Golgi from these cells.

*other*

The exact origin and function of dart gland is still unclear. Simroth (1896) derived their evolutionary origin from the lip glands, also called as Simroth's glands. There have been many points of view. Von Jhering (1875) thought that the dart gland formed the dart. Lang (1900) and Filhol (1938) thought it formed part of the egg-shell. Moquin-Tandon (1855) thought it was for lubrication during copulation. Siebold (1848) thought it forms the spermatophore and Meisenheiner (1912) thought it facilitates dart expulsion. Dorello (1925) injected dart gland secretions experimentally into snails and found that this had two effects (1) it coagulated blood at the wound where the dart had pierced the skin and (2) it also showed a 'stimulatory effect' on the 'nervous' and

reproductive systems. Such work still needs confirmation and elaboration, although Bornchen (1967) generally supported these observations. Bornchen (1967) also noted that dart gland shows activity not only during copulation but also during egg laying at other specific times, so this gland may have more than one function. Filhol (1938) found that these glands are controlled by the ovotestis. Castration prevents their growth. Experiments and observations by Lind (1976) and Teppesen (1976) would indicate that dart-extirpated animals are not only able to copulate successfully but actually have a slightly higher rate of successful copulation than do control specimens, because the 'trauma' of being stabbed by the dart often causes potential mates to cease mating activity, and yet, Helix dart extrusion is accompanied by a globule of white mucus from the glands which definitely suggests a function. Tompa (1980) suggested that darts may not be involved with sperm transfer; dart receipt could be the stimulus for synchronizing or accelerating the maturation and release of new ova for a new clutch of egg or for more eggs than would otherwise result. In these snails, which can mate many times in a season, yet can store allosperm from a single encounter for several years e.g. Cepaea for 4 years (Duncan, 1975). The darts could function to increase the chances of the dart donor's sperm meeting more ova of the dart recipient at the right time and place for fertilizing eggs. This hypothesis would explain, on the one hand, why mating can continue without dart release and at the same time, could explain

the advantages of evolving and maintaining this system. Lind and Jeppesen (1976) did not investigate the timing and intensity of egg laying, nor did they look into changes in fecundity or sperm precedence or investigate possible egg laying stimulation after mating in dart extirpated compared to normal snails.

**Penis :**

Amongst the pulmonates, the stylommatophoran land snail have attended maximum complexity of structure of the reproductive apparatus and complicated procedures for the mating (Hyman, 1967), which was attracted the attention of many investigators, who have studied these organs from the morphological, anatomical, histological and ultrastructural point of view (Carabb, 1927; Dasen, 1933; Holm, 1946; Duncan, 1958; De Rezende and Lanzieri, 1964; Nordisieck, 1966; Luise, 1971). Some workers have also studied this reproductive organ from functional point of view (Thompson, 1961; Goddard, 1962), and during their investigations they have observed that the dart induces the penial activity at copulation and the whole penial complex ( *viz.*, dart, dart gland and penis with penis sheath) shows seasonal rhythmicity in it's activity (Goddard, 1962; Duncan, 1964; Berrie, 1966; Nanaware, 1974). They tested the rhythmic activity by employing various tissue extracts and chemicals on the isolated on a situ penial apparatus. The mechanism of spermatophore insertion and sperm exchange during the copulation of many species have been observed by several investigators,

(Lunzieri and de Rezende, 1965; Beeman, 1970). Beeman (1970), has studied exchange of sperm during copulation and storage of sperms autoradiographically.

The penis is highly muscular organ that is everted at copulation and is typically inserted into the genital atrium and vagina of the mate. The vas deferens enters it terminally or subterminally. Posterior to the penis, there may exist an epiphallus, a modification of the vas deferens or penis for spermatophore formation and even accessory flagella, and appendices or caeca can occur. The epiphallus may also have a calcareous gland or sac attached to it; all these structures serve to form parts of the spermatophore. The penis has undergone a tremendous amount of evolutionary modification and elaboration so that its morphology is one of the classic taxonomic characters to consider in identifying land snails. Unfortunately, the real biological significance of such structures as penial glands, plates (e.g. *Ventridens*), pilasters, hooks (eg., *striatura*), suckers (e.g., *Parmacella* and *Vitnopugio*), pads, calcareous spurs (e.g., *Phrixolestes*), etc., are not known, although they generally seem to have holdfast purpose. The "penis" is often actually a penial sheath, which may enclose a fleshy penial papilla, the verge. Unlike all the rest of the genital tract, which is invested by the visceral ganglion (Hochpochler, 1979) and this may originally have been a pedal nerve (Visser, 1981).



Those species that have penis, insert it simultaneously or sequentially into the partner's genital pore during mating; in some slugs like Limax maximus, however the penes entwine and exchange sperm at their tips without intromission completely outside of the body. Penes are often very long relative to total body length in the 5-8 inches long in slug Limax cinereoniger, the uncoiled penis in action may be more than 30 inches in length.

On the other hand, many species, especially some slug groups like Arionidae, normally lack penes altogether; mating in these forms may be accomplished by appressing the genital pores together directly or by using non homologous penis like structures (derived from other parts of the terminal genitalia) for sperm transfer. Apophallation, the apparent gnawing off of the entire penis as an aspect of mating, apparently is common in the giant slug Ariolimax (Mead, 1943). Individuals that have thus lost their penis cannot regenerate another, so that at best they remain obligate 'females'.

After normal use, the extruded penis is withdrawn back into the body by a specialized muscle, the penial retractor, whose morphology is very important in systematic considerations. This single, large, long muscle has also found considerable favour with physiologists for pharmacological experiments.

#### **4. Regulation of Functions of ASO :**

##### **Optic tentacles :**

According to Fretter and Graham (1962) the reproductive activities in the hermaphrodite pulmonates are controlled by two factors - one external or environmental factors and the second internal and genetic factors. The latter perhaps acts primarily by making the animal sensitive to changes in the former, although more recent work tends to make it much the more important factor. The important environmental factors include latitudes, temperature, humidity, photoperiod, food supply, etc. Newell (1965) has pointed out that the microflora and microfauna are important in their diet. These microorganisms might well be influenced by the variable periods of wetting and exposure by strong and weak tides and so influence the food supply of the snail and their reproductive condition.

*in* The control of the reproductive activities by hormones is even less well known <sup>in</sup> pulmonates. As Boer and Joosse (1975) state, there is 'to date no histological evidence for the production of hormones in the gonad of the pulmonates'. Yet it does seem possible that in some pulmonates the state of the reproductive tract is governed by gonadal hormones and that the gonad might itself be governed by hormones originating in or near the central nervous system (Golding, 1974; Boer and Joosse, 1975). Such organs include optic tentacles, cerebral and other ganglia, etc. The following is the brief

resume of the effects of such neurohormones, on the reproductive activities of pulmonate gastropods.

Pelluet and Lane (1961) were the first to suggest an endocrine function of the optic tentacles of stylommatophora. Since then, many controversial studies have been published in this field. However, the experiments of Watzek (1973, 1975, 1978, 1980) with Arion subfuscus clearly indicate that in this species and thus most probably also in other terrestrial pulmonates, the optic tentacles contain and release a masculinizing substance. §

Up till now, all efforts to identify the endocrine centers in the optic tentacles have failed. This is great disadvantage for the reproductive endocrinology of pulmonate gastropods, as little is known about production centers of androgenic substances in these hermaphrodite snails. This contrasts clearly to the detailed knowledge of the control centers of female reproductive activity. To stimulate further research in this area, a survey of the morphological studies is presented.

The results of the investigations of Tuzet *et al.* (1957), Sanchez and Bord (1958), Lane (1962, 1964), Smith (1966), Bierbauer and Torok (1968), Rogers (1969) and Bierbauer and Vigh-Teichmann (1970) suggest that in the optic tentacle tentacular ganglion is located close to the eye. This ganglion is connected with the procerebrum of cerebral ganglion, sending 6 nerve branches to the eye and the skin of the tip of the tentacle. Primarily

on the basis of their reactions with classic neurosecretory stains, 4 cell types, located outside the ganglion, were assumed to be hormone producing cells, the collar cells, two types of lateral cells and the gland cells of the dermatomuscular layer. Further histochemical and electron microscopic studies indicate that all these cell types are sub-epidermal gland cells (Lane, 1964; Rogers, 1969; Rohlich and Bierbauer, 1966). Of these, the collar cells have an intimate relation-ship with the tentacular ganglion, which is clear from their final cytoplasmic projections into the ganglion (Bierbauer and Torok, 1968, Lane, 1964). However, the collar cells have a process, which runs between the epidermal cells to the surface of the tentacle where external secretion occurs, and their secretion granules differ greatly from neurosecretory elementary granules (Rogers, 1969). In the future, attention should be focussed (as suggested by Boer and Joosse, 1975) on the tentacular ganglion, because surprisingly, no detailed studies have been devoted to this possibly neuroendocrine structure.

However, the neuroendocrine structure and function of the optic glands have been demonstrated and studied in detail in the cephalopod molluscs.

From the elegant experiments of Wells and Wells (1959, 1969), we know that in the coleoid cephalopods the optic glands control reproductive activity. These glands are located upon the optic tract, which connect the brain with the large optic lobes. The

glands are highly vascularized and are innervated by a nerve from the subpedunculate lobe of the supraoesophageal part of the brain. The activity of the glands is controlled by an inhibitory nerve supply; removing the subpedunculate lobe or cutting the nerves, which run to the glands, results in a rapid enlargement of the optic glands, which become bright orange. At the same time, sexual maturation starts (Wells and Wells, 1959). This can also be induced by implantation of optic glands in intact immature recipients (Wells and Wells, 1975) or in organ cultures of optic glands with gonadal material (Durchon and Richard, 1967). These experimental data prove that the optic glands and endocrine organs involved in the control of reproduction. The structure and innervation of the optic glands have been studied in detail by various authors (Bjorkman, 1963; Boycott and Young, 1956; Defretin and Richard, 1967; Wells and Wells, 1959). They contain only one type of granular cell, the stellate cell. In some species, these cells have long projections which run along the wall of blood capillaries but remain separated from the lumen by the pericytes of the capillaries (Froesch, 1979). The stellate cells contain large number of tubular mitochondria and free ribosomes. Defretin and Richard (1967) gave a clear description of the presence of typical secretion granules. Their origin from golgi zone and their change in quantity in relation to the activity of the gland in Sepia officinalis. On the other hand, Mangold and Froesch (1977) were unable to observe changes in activity of the cells in the optic glands of Octopus vulgaris and Eledone moschata at any

moment in their life cycles. They suggest that the presence of lipofuscin and haemocynin in the cells of the optic glands indicates that the stellate cells of O. vulgaris take up injected horse spleen ferritin. Froesch (1979) found that the stellate cells respond to a variety of foreign proteins by a mass production of particulated material. He suggested this to be the optic gland hormone. This hormone then would be steroid, as it is not stored in granules but produced in cells rich in tubular mitochondria. The hormone would be involved not only in the control of sexual maturation but also in defence mechanisms against foreign proteins.

In conclusion, it can be said that the endocrine nature of optic glands is undisputed, but that the opinions on the chemical structure of the hormone and its processing in the optic glands are still controversial. Moreover, the optic glands may also have nonendocrine functions.

There is clear evidence that the juvenile gonad of pulmonates contains only one type of gonadal stem cell (Hogg and Wijdenes, 1979). Both the sex cells and their supporting cells (sertolicells and follicle cells) originate from this stem cell. From organ culture experiments with juvenile gonads of H. aspera (Gomot, 1970; Guyard, 1971) and Arion subfuscus (Wattez, 1980), it seems that the female cells arise from by autodifferentiation. Differentiation of male cell is stimulated by factor from the optic tentacles as well as the cerebral ganglion of young animals. This factor suppresses

the development of female cells. Wattez (1980) has shown in vivo by tentacle (extirpation) and injection experiments, that in *A. subfuscus* the masculinizing factor is found only in optic tentacle of snails in the <sup>3</sup>male phase. This factor stimulates the spermatogonial mitosis. From organ culture experiments, it is clear that, this factor acts directly on sex cells. The cellular origin of the masculinizing factor is still unknown.

The investigation of Wattez (1980) has given the clearest answer to the question in the literature of the optic tentacles on reproduction in stylommatophora, which was raised by the tentacle (extirpation) experiments of Pelluet and Lane (1961), Pelluet (1964), Boer and Joose (1975) and Wattez (1980).

#### Cerebral ganglia :

Further research in this area has been greatly facilitated by the introduction of Limax maximus by Sokoiove and McCrone (1978) and McCrone and Sokolove (1979) as a model gastropod for studies concerning the start of puberty. These authors have shown that long day photoperiods (16L-8D) induce sexual maturation in Limax. In this process, the cerebral ganglia play a decisive role, because transplantation of these ganglia from long day donors to short day acceptors induces maturation in the latter animals. Maturation includes growth of the gonad which involves gametogenesis, and of the ASO. Most probably, a masculinized as well as an oocyte-stimulating factor is released by these cerebral

ganglia. The apparently irreversible activation of the cerebral ganglia will allow investigators to trace the cellular components involved.

Cerebral ganglia taken from specimens of H. aspera (Guyard, 1971) or A. subfuscus (Wattez, 1980) which are in the female phase of their reproductive activity, stimulate the growth of the oocytes in ovotestes kept in organ cultures. This growth may be mainly due to vitellogenesis. On the basis of extripation and implantation experiments, Geraerts and Joosse (1975) have shown that the DBH of L. staqualis stimulates vitellogenesis in this species. No changes of spermatogenesis were observed. Wijdenes and Runham (1976) demonstrated that DBH has a similar effect on oogenesis in Deroceras (Agriolimax) reticulatum in slug deprived of their DB, the maturation of the oocytes was retarded.

Effects of brain extracts on oocyte maturation in basommatophoran Helisoma duryi and H. tribolis have been demonstrated by Saleuddin et al. (1980). Stimulation was apparent from the appearance of endocytotic profiles on large oocytes and from the activation of oviposition within a few days. These effects were attributed to DBH, which was present in the ganglionic extract.

The above results clearly indicate that in all pulmonates DBH stimulates vitellogenesis in the oocytes, the effect of total cerebral ganglia extract being due to presence of the DB on these ganglionic extract.



In young specimens of L. stagnalis which are deprived of their LL, the maturation of the oocytes is delayed (Geraerts, 1976). This effect can be restored by LL implantation. In adult snails extirpation of the lobes results in a lower level of egg mass production. This effect is mostly probably exerted indirectly via the DB. In snails without LL the growth of the DB is delayed. Moreover, the synthesis and release of the Secretion product of the DB cells are reduced (Roubos et al., 1980). thus in juvenile snails the LL affect the male and female lines, both of which are accelerated and in the adults the ovipository activity, which is stimulated. Similar effects of the LL were observed in Bulinus truncatus (Geraerts and Mohamed, 1981).

As mentioned above, there is evidence that the Sertoli cells in the gonad of basommatophora produce steroids. These steroids would act only intragonadally. A possible function could be the synchronization of spermatogenesis of the male cells attached to them. A negative feedback effect from the gonad on DB activity could not be demonstrated in castration experiments in B. truncatus (Boer et al., 1976). Thus in Basommatophora, control of the DB occurs via neuroendocrine pathways, in which the LL might play an important role.

It is tempting to suggest that in basommatophora, where simultaneous hermaphroditism is preceded by a very short protandric period, the start of sexual activity is controlled by one

centre, the LL, which stimulates both sexes. On the other hand, in stylommatophora, where hermaphroditism is more successive (male cells reach maturity first, followed later by female cells), such as A. subfuscus (Wattez, 1980), D. reticulatum (Runham and Laryea, 1968), and H. aspera (Guyard 1971), the onset of reproductive activity is controlled by a factor which stimulates male and inhibits female line. The release of this factor decreases gradually after puberty. In all pulmonates female cell maturation is controlled by the exclusively female DBH.

### **Gonads :**

In basommatophora, the possible endocrine controlling effect of the gonad on the ASO has been studied nearly exclusively in planorbid species. In these animals, the gonad lies in the apical coils of the shell, posterior to the digestive gland. This makes castration a rather easy procedure, as contrasted to other groups, in which the lobes of the ovotestis are embedded between those of digestive gland, castration experiment in young but sexually mature snails were performed by Harry (1965) and Vianey-Liaud (1979) in Biomphalaria glabrata and by Brisson (1971), Boer et al. (1976), de Jong-Brink et al. (1979), and Geraerts and Mohamad (1981) in B. truncatus. Contrary to expectation, the castrated snails continued to produce egg masses, although at lower rate. Such egg masses consist of the secretion product of the albumin gland (perivitellin fluid), relatively normal egg membranes and an egg mass capsule

secreted by the oothecal gland, but of course no egg cells are present. The female ASO grow normally and are apparently able to synthesize their products (de Jong-Brink *et al.* 1979). In view of these results, it is highly improbable that the ASO of freshwater pulmonates are controlled by the gonad.

On the other hand, in stylommatophora, gonadal control of the ASO has been shown. Here again a particular group of slugs is exceptionally suitable for castration experiments as their gonad has a less intimate contact with the digestive gland. Castration experiments were performed on L. maximus (Abeloos, 1943; Laviolette, 1954), Arion spp. (Laviolette, 1954) and D. reticulatum (Runham *et al.*, 1973). The fate of juvenile ASO implanted into castrated snails was also studied. Runham *et al.* (1973) have showed that in castrated P. reticulatum implanted juvenile ASO did not develop, whereas in intact animals the male part of implants developed when the acceptors were in the male phase and the female part in acceptors which were in the female phase of the reproductive cycle. This points clearly to a sex-specific control by the gonad. For the male organs, this includes the penis (Wijdenes, 1981). The gonadal hormone of stylommatophorans may be of a steroid nature. Because Takeda (1979) and Gottfried and Dorfman (1970) could stimulate oocyte development and egg production in D. reticulatum and L. flavus by injections of steroids particularly estrogens respectively. However, the doses used (1 mg/g snail)

were very high. Thus in stylommatophora, a dual endocrine control of the ASO by the gonads seems well established.

However this is only part of the picture. Bailey (1923) obtained survival and growth of the prostate gland of D. reticulatum in an organ culture medium only if gonadal tissue, together with cerebral ganglia and optic tentacles, was present. In L. stagnalis the female ASO do not grow in juvenile animals deprived of the DB, whereas the male organs develop normally (Geraerts and Joosse, 1975). Juvenile tracts implanted in adult snails without DB show cellular differentiation and growth of male parts only. In intact snails the male and female structures of implants develop normally (Geraerts and Algera, 1976). Moreover, the synthesis of galactogen, an important component of the albumin gland secretion product, is very low in pond snails deprived of the DB (Veldhuijzen and Cuperus, 1976). Also in young specimens of the stylommatophoran slug D. reticulatum, cauterization of the DB results in retarded growth of the female ASO (Wijdenes and Runham, 1976).

Thus, there is convincing evidence that the DBH has a stimulating effect exclusively on the female ASO acting on cellular differentiation, growth and synthetic activity. This may true for all pulmonate snails.

An important question arising from the above discussion is whether the DBH action is exerted indirectly by stimulating the

release of a female gonadal hormone? For basommatophorans this seems improbable, as castration does not seriously affect egg mass production. The problem was solved in organ culture experiments. Goudsmit (1975, 1978) demonstrated that galactogen synthesis in albumin gland pieces of H. pomatia is stimulated by a factor of the cerebral ganglia. The origin of the factor is not yet clear. Absence of the effect in a calcium-free medium points to an active secretion by exocytosis. In L. stagnalis the cerebral ganglia also stimulate the polysaccharide synthesis in albumin glands kept in vitro. The effect could be attributed to the DBH and the caudodorsal cell hormone (CDCH). Albumin glands of young, sexually mature snails cultured with isolated DB or in the presence of DB homo-genate or of an extract of intercerebral commissures. All showed a significant rise in polysaccharide synthesis (Wijidenes et al., 1983). The DBH is probably specific at the genus level (Wijidenes et al., 1981).

Thus it suggested that in basommatophoran both DBH and CDCH act directly on the female ASO. In stylommatophora female ASO are controlled by gonadal hormone and by DBH. Most probably DBH acts via a gonadal hormone on the cellular differentiation and growth of the ASO, whereas it has a direct effect on the synthetic activity of these organs. There are no indications of a neuroendocrine control of the gonadal hormone secretion in stylommatophora. An endocrine organ analogous to the DB, for the control of male activity probably does not exist.

## 5. Statement of the Problem :

The survey of the literature on the reproduction in pulmonate gastropods, shows that the reproductive physiology in these animals is very complicated. The reproduction in these animals is influenced by three factors - (I) Environmental (2) Genetic and (3) Hormonal. Our knowledge of these three factors is very limited. There are some studies on the effects of hormones and neurohormones on the gonads. But very scanty information is available on the effects of the hormones on the accessory sex organs especially attached to the male part of the reproductive system. There are indications about the involvement of neurohormones elaborated in the optic tentacles and cerebral ganglia and the hormones elaborated in the gonads in the reproductive functions of male ASOs. Therefore, the present investigation was undertaken to confirm the effects of neurohormones elaborated by optic tentacles and cerebral ganglia and of hormones elaborated by ovotestis on the reproductive activities in the three important male accessory sex organs i.e. the prostate gland, the dart gland and the penis of a locally available slug, Semperula maculata.

The study is concerned with the histological, histochemical and biochemical alterations in these male ASOs in the presence and/or in the absence of neuro-hormones elaborated by optic tentacles and cerebral ganglia and in the presence/absence of hormones of ovotestis.

- 1) In the present investigation, it was decided to carry out the following experiments in the various groups of slugs after ablation of their optic tentacles and after injections of extracts of optic tentacles, cerebral ganglia and ovotestis to slug groups with and without optic tentacles in order to assess the effect of neurohormones on the male ASOs like the prostate gland, the dart gland and penial complex. Analysis of histological alterations in the prostate gland, the dart gland and penial complex after ablation of optic tentacles and after injections of the extract of optic tentacles, cerebral ganglia and ovotestis by employing important histological techniques.
- 2) Analysis of alterations in the polysaccharides in the prostate gland, dart gland and penial complex after ablation of optic tentacles and after injections of extracts of optic tentacles, cerebral ganglia and ovotestis by employing important histochemical techniques.
- 3) Analysis of alterations in the polysaccharides, proteins and cholesterol in the prostate gland, dart gland and penial complex after ablation of optic tentacles and after injections of extracts of optic tentacles, cerebral ganglia and ovotestis by employing biochemical techniques.

For the convenience of presentation and ease of understanding, the dissertation is divided into five chapters. The first chapter is on introduction, which gives the detailed information

on the reproductive organs of pulmonate gastropods, general histological structures of their reproductive organs, the information on male accessory sex organs, the regulation of functions of these ASOs and statement of the problem. The second chapter gives in detail the anatomy of the male accessory sex organs and the histological, histochemical and biochemical methods employed in the present study. Chapter third is the observations on the histological, histochemical and biochemical alterations in the male ASOs like the prostate and dart gland and penial complex. In the fourth chapter the observations have been discussed in the light of existing information and certain ideas have been arrived at and the conclusions are put forward. The chapter five gives the general summary and describes the importance of this study in the control of reproduction in these animals and conclusion. The last chapter is followed by the bibliography.