قســـــــــم : علم الحيوان ـ كلية العلوم ـ جامعة أسيوط.
رئيس القســـــم : أ.د / محمد خليل النفـــار.

دراسات مورفولوجية وهستولوجية على أعضاء التكاثر للقوقع بيللاميا يونيكلر

(طائفة : بطنقد ميات ، فصيلة فيفيبارد ى)

عبد الونيس بد ينى ، منى ابراهيم حماد ى

تم فى هـذا البحث د راسة التركيب البين والد قيق لأعضاء التكاثر الناضجة فى كل من ذكـــــر وأنثى قوقع " بيللاميا يونيكلر" المنتشرة فى المياه العذبة المصرية . وقد أشير فى هـذه الد راســـة الى الملائمة الوظيفية لكل عضو من هـذه الأعضاء .

وقد د لت هـذه الد راسة على صغر حجم الذكر البالغ بالنسبة للانثى فى هـذا النوع مـــــن القوقع ، كما أن اللامسة اليمنى فى الذكر مقوسة وتقوم بعمل عضو التلقيح . تتكون أعضاء التكاثـــر فى الذكر من خصية كبيرة نسبيا واقعة فى التجويف البرنسى ووعاء ناقل رفيع وغد ة البروســـــتاتا ووعاء ناقل طرفى ممتد داخل اللامسة اليمنى للذكر ليفتح عند قمتها الحرة .

تتكون أعضاء التكاثر فى الأنثى من مبيض صغير نسبيا وقناة مبيض د قيقة وأخرى سميكة غد يـــة ومن قابلة منوية غد ية وجيب توالد ومهبل . ومما هو جد ير بالذكر أن جيب التوالد معد لاحتضـان البيض حتى يفقس وتخرج القواقع الصغيرة من الفتحة التناسلية المؤ نثة فراد ى .

وقد لوحظت القناة التناسلية التامورية الد قيقة فى الأنثى فقط. وقد نوقشت نتائج هـــــذا البحث مع مثيلاتها فى قواقع أخرى منتمية لنفس الفصيلة والرتبة والطويئفة .

Dept. of Zoology,
Faculty of Science, Assiut University,
Head of Dept. Prof. Dr. M.K. EL-Naffar.

MORPHOLOGICAL AND HISTOLOGICAL STUDIES ON THE REPRODUCTIVE SYSTEM OF THE VIVPARID SNAIL BELLAMYA UNICOLOR (OLIVIER, 1804) (GASTROPODA : VIVIPARIDAE)

By

E.A.M. BEDDINY and M.I. HAMADA

(Received at 20/6/1982)

SUMMARY

The reproductive system of the mature male and female viviparid snail, Bellamya unicolor (Olivier, 1804) was studied by gross dissection and histological examination and a reference to the function of each part was made.

External differences between male and female were found in the relative size and the form of the right tentacle. The male genital organs consist of the well developed testis lying in the mantle cavity, characteristic of genus Bellamya, the thin proximal vas deferens, the prostate gland and the distal vas deferens extending within the right tentacle and opening at its tip. The female genital organs are composed of a remarkably small ovary, a thin oviduct, a U- shaped glandular and thick oviduct, an albumen gland, a glandular inverted U- shaped receptaculum seminis, a brood pouch and a vagina. The brood pouch is adapted for the incubation of the eggs till after hatching. The young snails emerge from the female genital opening singly. A gonopericardial duct was observed only in the female. The results of this study were discussed in comparison with those of other mesogastropods especially those belonging to the family Viviparidae.

INTRODUCTION

The present work deals with the macro- and microanatomy of the reproductive system of the viviparid snail Bellamya unicolor (Olivier, 1804). A review of earlier work on the reproductive system of viviparid snails has shown that it is brief and incomplete (SPEYER, 1855, BAUDELOT, 1863, ANNANDALE and SEWELL, 1921, ANKEL, 1921, ANNANDALE and RAO, 1925, NEUMANN, 1928 and ROHRBACH, 1937). It is but in the paper of the last mentioned author that the characters of the genital organs of B.unicolor are mentioned and this only briefly. Certain aspects of reproduction in viviparids have been investigated by FRETTER and GRAHAM (1962) in their study of British prosobranchs, including certain species of Viviparus which is a near relative to the genus Bellamya. Recent work in this field includes that of BARBATO (1971) on the seasonal variations of Viviparus ater, BOTTKE (1972 & 1973) on the light and electron microscopical structure of the ovary of Viviparus contectus and BERRY (1974) on the effect of certain physical factors on the reproductive activity of two Malayan viviparid snails of the genera Siamopaludina and Filopaludina, with brief reference to the macroanatomy of the reproductive system. More recently, VAIL (1977) has published a comparative study on the reproductive anatomy of three species of North American viviparids belonging to the genera Campeloma, Lioplax and Viviparus. He has compared the results of his study to those of other workers in this field to clarify the existing knowledge of viviparid reproductive anatomies and its role in the differentiation between the examined viviparid genera and in their classification into subfamilies.

Thus, the present work is a trial to elucidate the characters of the reproductive system of B.unicolor in order to contribute to the existing knowledge about the relationships between the different viviparid genera and species in this regard. Also, it may help in clarifying the evolution of the genus Bellamya and the family Viviparidae.

MATERIAL and METHODS

The specimens of B.unicolor (Olivier, 1804) used in the present investigation were collected form a region at the point of connection between the River Nile and the Ibrahimia canal at Assiut City in Egypt. This area is characterised by the presence of sand, soil and small rocks and by the absence of water plants. The living snails of B.unicolor are usually found crawling on the bottom or embedded either partially or completely in the substratum of the canal.

For collecting the snails, the normal scoop was used for scraping the bottom of the canal. The snails were kept alive in large plastic containers provided with dechlorinated tap water and were fed on boiled or fresh leaves of lettuce or greater plantain. The reproductive system of more than one hundred mature specimens of each sex was dissected under a binocular dissecting microscope. Dissected specimens were either fresh or preserved after relaxation with menthol in absolute alcohol (HUBENDICK, 1954). The average shell height of the dissected male and female specimens was 18.0 (± 0.21) and 26.6 mm (± 3.24) respectively and all measurements given in the following description are those normal to these sizes. Drawings of the reproductive system were done with the help of the camera lucida. The terms proximal and distal are in relation to the gonad.

For histological studies, mature specimens of both sexes were fixed in Bouin's fluid and paraffin sections, 5-6 um thick were stained with Harris' haematoxylin-eosin combination or Mallory's triple stain. For the differentiation of mucus secretory tissues, VASSOR and CULLING's (1959) method was adopted, using toluidine blue-veronal acetate solution.

KEY TO LETTERING OF FIGURES

alb. g.= albumen gland, alb. g. d. = albumen gland duct, b.m. = buccal mass, b. po. = brood pouch, ci. co. ep.= ciliated columnar epithelium,c.t.=connective tissue,ct.=ctenidium,cu. ce.=cuboidal cell,di. g.=digestive gland, epi.= epitaenia, ep. l. t.= epithelial layer of tentacle, e.s.= exhalent siphon, f. g.= food groove, ge. op.= genital opening, gm. ep.= germinal epithelium, gl. ce.= glandular cell, go. ce.= globulated cell, gop.= gonopericardial duct, gr.ce.= granulated cell, h.= head, ha.= heart, in. ep.= inner epithelium, int.= intestine, l.f.= left fold, l.t.= left tentacle, l.m. f.= longitudinal muscle fibres, lu.= lumen, m. ce.= mucous cell, m. c. t.= muscular connective tissue, oc.= oocyte, ou. ep.= outer epithelium, ov.= ovary, ovd. 1 = thin oviduct, ovd. 2= thick oviduct, pa.= papilla, pc.=pericardial cavity, pro. g.=prostate gland, py. ce.=pyramidal cell, rec. se. 1= first or proximal limb of receptaculum seminis, rec. se. 2= second or distal limb of receptaculum seminis, rec.= rectum, r.f.= right fold, r.t.= right tentacle, sec. ce.= secretory cell, sem. g.= seminal groove, sem. tu.= seminiferous tubule, spd.= spermatid, spc.= spermatocyte, spg.= spermatogonium, spz.= spermatozoa, ts.= testis, tr.= trabeculae, vc. ce.= vacuolated cell, va.= vagina, v. d.= vas deferens, vs. ef.= vasa efferentia, we. ce.= wedge cell.

RESULTS

The male and female B.unicolor specimens can hardly be differentiated externally by the difference in size. In well extended snails, the male may be differentiated by the tightly curved right tentacle which is longer than the left one. The female, on the other hand, has two slender, straight equal tentacles. After the removal of the shell, however, the mature female snail can be distinguished by the swollen brood pouch with the eggs and hatching juveniles within it appearing as irregularities on its external surface.

A- THE MALE REPRODUCTIVE SYSTEM

The male reproductive system in B.unicolor (Fig. 1) consists of the testis, the proximal vas deferens, the terminal part of the vas deferens and the prostate gland.

1- The testis:

It is a deep yellow, large, elongated and slightly curved structure with its greatest part lying on the right side of the mantle cavity, parallel to the rectum (Fig. 1). It has a small part embedded in the posterior limit of the mantle cavity, while its anterior tip ends at a point lying posterior to the mantle collar and the head region. In the examined specimens, it has a somewhat uniform width of about 2.4 mm except at its both narrow ends and its long axis is about 9.5 mm. The testis is a compact mass, formed of a large number of seminiferous tubules. They are connected together by thin strands of connective tissue, which together with scattered circular muscle fibres form the thin sheath of each tubule (Figs. 2 & 4). Just inside this sheath, there is the germinal epithelium which is a thin layer of flattened cells containing spindle shaped unclei (Fig. 2).

Within the lumen of the seminiferous tubule, primary and secondary spermatocytes, spermatids and spermatozoa can be easily differentiated. The primary spermatocytes have spherical nuclei showing the chromosomal activity of meiosis. They are smaller in size than the secondary spermatocytes. The spermatids are quite distinct due to their considerably small size and spherical dark appearance. The spermatozoa produced by the metamorphosis of some spermatids can be seen scattered in the lumen with their heads towards the centre (Fig. 2). The spermatozoa can be distinguished into a relatively long thread like type corresponding to eupyrenic spermatozoa of MEVES (1903), and a short type, corresponding to the oligopyrene designated by SIEBOLD (1836). In B. unicolor, the first type has a corkscrew shaped head with central nucleus and a long flagellum. The second type has a relatively thick oval head with elongated nucleus and a short thin flagellum (Fig. 3).

2- The Vas deferens:

The vas deferens can be differentiated into a proximal tubular distinct part (the proximal vas deferens) which extends from the testis to the prostate gland and a distal part (the terminal vas deferens) which emerges from the prostate gland and extends within the right tentacle (Fig. 1).

The proximal vas deferens is formed within the testis by the union of a number of minute ductules, i.e. the vasa efferentia carrying the sperms from the seminiferous tubules (Fig. 4). It emerges from the dextrolateral concave surface of the testis, at a point near the end of its proximal third, and extends ventrally and inwards within the mantle cavity to connect the proximal curved tapering end of the prostate gland. Along its course, the proximal vas deferens shows a distinct curvature, takes the form of a transluscent tube about 1.9 mm in length and increases gradually in width, being about 0.3 mm in diameter near the testis and about 0.6 mm close to the prostate gland.

Each vas efferens has a relatively wide lumen and a thin wall consisting of an epithelial lining, followed externally by muscular connective tissue (Fig. 5). The epithelial lining is formed of a single layer of unciliated columnar cells about 25 um in height, with granulated cytoplasm and large ovoid nuclei. The muscular connective tissue sheath is about 10 um in thickness and consists of compact connective tissue fibres and cells intermingled with circular muscle fibres. The latter layer is surrounded by the spongy network connective tissue of the testis.

In cross sections (Fig. 6), the proximal vas deferens has an oval outline, a relatively narrow lumen, being about 165 and 30 um in its longest and shortest axes respectively, and a thick wall, about 115 um in thickness. Its lining consists of an inner simple highly folded epithelial layer of ciliated columnar cells varying in height from 25 to 55 um. Each cell has a granulated cytplasm and an elliptical laterally located median nucleus. The epithelial lining is followed externally by a sheath of circular muscle fibres, about 70 um in thickness, which is covered by the meshwork of connective tissue of the lining of the mantle cavity.

The terminal vas deferens passes from the distal end of the prostate gland within the right tentacle and ends at its tip (Fig. 1). Like this, the right tentacle of the male acts as a penis, being tightly curved and long (more than double the length of the left tentacle).

Transverse sections of the right tentacle of the male, in a relaxed state, show that the terminal vas deferens has an almost oval, narrow lumen about 100 um and 35 um in the longest and shortest axes respectively (Fig. 7). It is lined with a simple epithelial layer of ciliated columnar cells and club shaped mucus secreting cells scattered among the former. The two types of cells are about 40 um high. The columnar cell is distinguished by long cilia, basal homogeneous cytoplasm, oval large nucleus with dense chromatin granules and apical granulated acidophilic cytoplasm. The epithelial lining is surrounded externally by a circular muscle layer about 20 um thick and an outer connective tissue layer about 250 um thick. The latter is richly supplied with isolated strands of circular, radial and obique muscle fibres and with black pigments dispersed between the connective tissue fibres. It has a more or less lattice appearance with the meshes occupied by numerous blood spaces, giving the tentacle a spongy erectile nature. Outside this layer, there is a thin circular muscle layer, about 12 um in thickness, covered externally by the simple ciliated columnar epithelial layer of the tentacle.

3- The Prostate gland:

The prostate gland occurs as a thick compact, elongated, pale-brown structure. It extends transversely on

the floor of the mantle cavity,from the distal end of the proximal vas deferens, for a short distance and bends forwards, passing embedded in the tissues below the epitaenia and the ridge bearing the food groove, till the base of the right tentacle. It has a nearly uniform width of about 1.3 mm along its whole length, which is about 10.3 mm, except at its two narrow ends (Fig. 1).

In cross sections, the prostate gland has a somewhat oval outline (Fig. 8a). It has a central seminal lumen connected with that of a large number of radially arranged tubules. The tubules are separated from each other by thin layers of connective tissue containing smooth muscle fibres. The whole tubules are surrounded externally by a sheath of connective tissue, richly supplied with circular muscle fibres and about 25 um in thickness.

Each tubule is lined with a simple layer of large secretory cells about 20 um in height and wedge-shaped ciliated interstitial cells (Fig. 8 b). The secretory cell is large, barrel shaped and heavily charged with large acidophilic spherules. Its nucleus is oval and basal with distinct nucleoli. Some of these cells appear opened, discharging their contents into the lumen of the tubule. The wedge-shaped interstitial cell is more or less pyramidal in shape with its broad ciliated free end directed towards the lumen of the tubule. It has a large cone-shaped nucleus with two refractive nucleoli. The secretion of the large cells flows into the central seminal lumen of the prostate gland by the action of the cilia, and passes, together with the sperms, by the action of the muscular sheath of the gland,to the central canal of the terminal vas deferens, during copulation.

B- THE FEMALE REPRODUCTIVE SYSTEM

The female reproductive system of B.unicolor (Fig. 9 a) consists of six different regions. These are ovary, oviduct. albumen gland, receptaculum seminis, brood pouch and vagina.

1- The Ovary:

The ovary can hardly be distinguished in macroanatomy of the mature female as few reddish spots close to the most anterior follicles of the digestive gland and connected to the very thin oviduct (Figs. 9 a&b). Examination of the gross anatomy and sections of the female shows that the ovarian follicles lie within the connective tissue of the mantle wall lining the floor of the mantle cavity. It is present between the oesophagus and the receptaculum seminis.

The ovary is covered by a simple epithelium of cuboidal cells containing large nuclei and black pigment granules (Fig. 10). Dispersed between these cells there are some mucus secreting cells. Interior to this epithelium there is a thin layer of circular muscle fibres,followed by a thick layer of connective tissue. The epithelial covering is continuous with that surrounding the digestive gland as well as the rest of the body.

In B.unicolor, each ovarian follicle appears oval or rounded in cross section and its diameter varies according to the state of oogenesis and the number of mature ova within it. In a follicle containing four well developed ova, the largest diameter is about 110 um. It has a wall composed of an outer layer of connective tissue surrounding a germinal epithelium. The latter consists of flattened or cuboidal cells with spindle shaped or oval nuclei, lying about the middle of the cells.

2- The Oviduct:

The oviduct arises from the ovary, extends anteriorly, and forms a loop before it opens into the receptaculum seminis (Fig. 9 b). In macroanatomy, it can be easily differentiated into two distinct parts. The proximal one, arising from the ovary, is a very thin pale yellow tube (the thin part of the oviduct) measuring about 600 um in length and 15 um in diameter. The second or distal part is a glandular U-shaped structure lying close to the distal end of the receptaculum seminis and can be designated as the thick part of the oviduct. It is obvious that the proximal limb of the latter, with respect to the ovary, is thinner than the distal one, the first being about 0.3 mm wide x 2.0 mm long and the second about 0.4 mm wide x 2.6 mm long. The two branches are close and parallel to each other. Sections show that the distal end of the thin part of the oviduct opens into the proximal limb of the thick part of the oviduct near its free end. Also, sections reveal that the free end of the same limb receives the albumen duct, while the distal limb of the thick part of the oviduct is connected with the receptaculum seminis by a small papilla, about 0.3 mm in length (Fig. 9 b). Careful examination of

serial sections through the region of the ovary, oviduct and heart has shown that the thin part of the oviduct is connected to the pericardium by a fine gonopericardial duct (Fig. 11).

The thin part of the oviduct has a very narrow irregular lumen, lined with thin columnar epithelial cells varying greatly in height, which ranges from about 5 to 11 um (Fig. 12). Each cell has an acidophilic cytoplasm and nearly central elongated nucleus. The epithelium is surrounded externally by a thin layer of circular muscle fibres, about 2 um in thickness and followed by a relatively thick layer of muscular connective tissue which is about 31 um in thickness.

The proximal limb of the thick part of the oviduct appears oval in cross section (Fig. 13). Its epithelial lining is formed of ciliated columnar cells which vary in height from about 23 to 32 um, giving the inner border of the wall a wavy appearance.The columnar epithelium can be differentiated into vacuolated and globulated cells which almost alternate. The first type has a wide central transparent vacuole with peripheral thin cytoplasm and slender elongated eccenteric nucleus. The second type is distinguished by the presence of large globules occupying most of the central part of the cell and acquiring blue colour with Mallory's triple stain. This colour indicates that they may be of a mucoid nature. The nucleus is oval in shape and basal in position. The epithelium is covered externally by a connective tissue layer, about 22 um in thickness and richly supplied with circular, longitudinal and oblique muscle fibres. This layer is continuous with the connective tissue of the mantle wall.

The wall of the distal limb of the thick part of the oviduct is composed of two layers, an outer thick sheath of muscle fibres and an inner layer of ciliated epithelium (Fig. 14). The outer sheath consists of an internal layer of longitudinal muscle fibres and an external one of circular muscle fibres. The thick muscular sheath (about 30 um thick) seems to act as a sphincter which may serve to hold the eggs. Therefore,this part of the oviduct may act as a place for fertilization. The epithelial lining of this limb shows many projections into the lumen which, therefore, appears to be branched. This is due to the great difference in the heights of several groups of epithelial cells (ranging from 20 to 75 um). The epithelium is composed of two types of cells, the first is of the ciliated columnar type which has vacuolated and acidophilic cytoplasm, the second of the glandular columnar type which almost appears as an inverted flask when the apical part is filled with large globules. The nucleus of each of the two types is slender,fusiform and embedded in the lateral thin cytoplasm. The papilla of this limb (Figs. 9 b & 15) which connects it with the receptaculum seminis, is a narrow tube that has the lining epithelium thrown into folds and includes columnar and cuboidal cells, with spherical basal nuclei. This lining is covered by a thick layer of circular muscle fibres, about 100 um thick, followed by the mantle wall.

3- The Albumen gland:

The albumen gland is an elongated curved, nearly cylindrical structure which is deep yellow in colour (Figs. 9 a & b). Its dorsal convex surface is bordered by the rectum, while its ventral surface adheres to the receptaculum seminis. It lies within the connective tissue of the mantle wall of the right side of the penultimate whorl. Thus, it is covered dorsally by the outer darkly pigmented epithelium of the mantle. In the sexually mature female,the gland has a nearly uniform thickness of about 0.6 mm and a length of about 9.7 mm. The albumen gland passes along the receptaculum seminis from its proximal extremity till its distal one.At this latter point, the albumen gland crosses transversely the receptaculum seminis to reach the tip of the proximal limb of the thick oviduct. If the distal end of the albumen gland is carefully raised to expose its ventral surface, a thin whitish duct can be seen extending from the gland to the tip of the proximal limb of the thick oviduct. This albumen gland duct appears emerging from the gland nearly at the beginning of its distal third. It measures about 2 mm in length and 60 um in diameter.

Sections show that the albumen gland is composed of numerous, closely packed follicles which are circular or oval in outline (Figs. 16 & 17). Each follicle has a central lumen which varies in diameter according to the amount of its secretory contents and the state of activity of the epithelial lining. Each follicle has an outer thin layer of circular muscle fibres, about 2.5 um thick. This layer is followed internally by a simple epithelial lining, formed of two types of cells. The first type is in the form of large glandular cells which vary in shape according to their state of activity. In the active phase, the cells mostly take a barrel shape with large central vacuoles or with clear rounded or ovoid acidophilous secretory granules. Their nuclei are frequently

basal in position and rounded or oval in shape. Some of these cells can be seen discharging their secretion into the central lumen. The unchanged cytoplasm of each cell seems scarce and lines its wall or fills up the narrow interstices between the secretory granules. Each cell measures abou 15 um in height and 12 um in width. Between the apices of these cells, small pyramidal interstitial cells, forming the second type of cells, are found (Fig. 17). Each interstitial cell has its broad edge towards the lumen of the follicle and a relatively large central triangular nucleus conforming to the space available.

Examination of serial sections of the albumen gland shows that the central canals of the follicles discharge through lateral ducts into a common albumen duct (Fig.16). The wall of the latter is formed of an epithelial lining of ciliated glandular columnar cells about 15 um in height, followed externally by a thin sheath of circular muscle fibres about 6 um in thickness. Most of the epithelial cells are granulated and there are few vacuolated cells scattered between them. The granules of few cells acquire a pale blue colour with Mallory's triple stain, or a purple colour with toluidine blue indicating their mucoid nature.

4- The Receptaculum Seminis:

The receptaculum seminis of B.unicolor is a large yellow glandular inverted U-shaped structure. In macroanatomy, it appears in the form of two parallel limbs, the first, or proximal limb, passes posteriorly from the oviduct till it reaches the beginning of the penultumate whorl and then bends sharply, anteriorly upon itself, forming the second or distal limb, which passes into the brood pouch (Fig. 9 a). The two limbs are elongated, smooth, cylindrical and closely connected structures, about 7.4 mm in length. The proximal limb is relatively thin, being about 0.5 mm thick, whereas the distal one is about 1.9 mm in thickness. They lie within the connective tissue of the dorsal mantle wall of the right side of the penultimate whorl of the visceral hump. They are close to the concave ventral surface of the albumen gland. Examination of serial transverse sections of the two limbs of the receptaculum seminis shows that the distal end of the second limb has a ventral groove which is continuous with the groove of the brood pouch.

Histologically, the wall of the two limbs of the receptaculum seminis is composed of three layers, which are an outer connective tissue layer, a middle muscular layer and an inner simple epithelial lining (Fig. 19). The outer connective tissue layer is continuous with that of the mantle wall. The muscular layer is composed of circular smooth muscle fibres. This layer is thicker in the proximal limb than in the distal one, being about 45 and 30 um in thickness respectively (Fig. 18). The epithelial and muscular layers are thrown into flods. These folds are more numerous and longer in the distal than in the proximal limb. The epithelium is formed of two types of cells: large columnar glandular cells, and wedge-shaped ciliated interstitial ones (Fig. 19). The columnar cells have basal, oval and relatively small nuclei. The shape and nature of each columnar cell varies according to its state of activity. Most of the glandular cells are narrow and granulated,about 40 um in height, and dispersed between them are the goblet cells with their secretion oozing out through apical pores into the lumen. The secretion in some goblet cells takes the form of large oval globules and gives positive reaction with toluidine blue, indicating its mucoid nature. Other scattered goblet cells have large central oval vacuoles. The secretory granules of most of the glandular cells acquire a pale blue colour with Mallory's triple stain and red colour with eosin,showing acidophilic nature. The wedge-shaped ciliated interstitial cells have oval or flattened central nuclei and the apical part of their cytoplasm is more acidophilic than the basal part. It is obvious, however, that the cilia of the epithelial lining of the proximal limb are longer than those of the distal limb.

Sections of the female reproductive system of ten sexually mature specimens, fixed at the four seasons of the year, show the presence of the eupyrene and oligopyrene sperms in the lumen of the two limbs of the receptaculum seminis.

5- The Brood Pouch:

The receptaculum seminis is connected distally with an elongated swollen cylindrical whitish part of the female tract. It forms the brood pouch and incubates the eggs till after hatching. The brood pouch lies on the right side of the pallial cavity, closely attached to the right mantle wall and bordered by the rectum on the left side.The dimensions of the brood pouch vary to a considerable extent according to the reproductive activity and the number of eggs incubated. However, in the average sized female of 26.6 mm shell height,it is about 15 mm long and 4 mm broad. Distally, it is continuous with the vagina (Fig. 9 a).

In gross anatomy, the eggs and newly hatched young snails, existing within the brood pouch, give its external surface an irregular appearance, indicating the flexibility of its wall. The egg is pear-shaped with a short stalk at the pointed end. Its wall is delicate and ruptures under any pressure (Figs. 9 c & d).

When an incision is made in the wall of the brood pouch of a gravid female, the eggs appear lodging within chambers formed by inner folds of the wall. In cross sections, this part of the female tract appears oval or circular in outline, with its lumen divided by trabeculae into chambers occupied by sections of the eggs or the embryos (Figs. 20 & 21).

Histologically, the wall of the brood pouch consists of an outer thin epithelial layer of flattened and cuboidal cells ranging from 3 to 5 um in thickness and an inner lining of almost the same structure with scattered glandular goblet cells. Between the two layers, there is a relatively thick layer of muscular vascular connective tissue about 30 um in thickness, with wide vacuoles (Fig. 22). The muscle fibres take mostly a circular orientation, but there are a few scattered oblique fibres. The circular muscle fibres are more concentrated close to the inner epithelial lining than to the outer epithelium, especially at the inner wall of the pouch, free from the mantle wall.

There is a longitudinal groove extending along the ventral wall of the brood pouch (Fig. 20). This groove is lined by ciliated columnar glandular epithelium about 40 um high. Such cells can be differentiated into granulated narrow cells and much swollen vacuolated barrel- shaped ones. The latter contain large oval or spherical globules and discharge the secretion into the lumen by the rupture of their free tips (Fig. 23). The two types of cells acquire a deep violet colour with toluidine blue, showing the mucus nature of their secretion. This groove is continued on one side along the distal limb of the receptaculum seminis, and on the other side along the floor of the vagina till the female genital opening.

6- The Vagina:

The vagina is a whitish or creamy coloured cylindrical terminal part of the female tract, connected to the distal end of the brood pouch. It is clearly marked off from the latter by its relatively thick muscular, shiny wall and its narrow diameter (Fig. 9 a). It extends anteriorly, closely attached to the ventral wall of the rectum, and curving towards the dorsal mantle wall. It measures about 2.1 mm in length and 0.5 mm in its widest diameter at the proximal end. It tapers gradually to terminate by a small thin tubular papilla about 1.7 mm long and 0.3 mm in diameter. The later opens by the female genital opening in the mantle cavity, just behind the mantle edge, a little behind the anus.

In transverse sections, the vagina has a circular outline, a thick wall about 35 um in thickness and an irregular narrow lumen. The vagina is lined with a folded epithelium made of ciliated columnar cells about 25 um in height. Some of these cells become centrally vacuolated, barrel-shaped and glandular, with lateral oval nuclei (Fig. 24). The vacuoles probably contain a mucus secretion because it gives positive reaction with toluidine blue. The apical part of these cells is filled with dense acidophilic cytoplasm. The epithelial lining is followed externally by a thick layer of vacuolated muscular connective tissue about 10 um in thickness and an outermost thin epithelial layer of flattened or cuboidal cells. The muscle fibres are mainly circular in position and the muscular layer increases gradually in thickness towards the female aperture, where it forms a sort of a sphincter to control the emergence of young snails.

DISCUSSION

Terminology:

The main parts of the reproductive system of B.unicolor show certain grades of similarity with those of other viviparid snails previously described. Yet, there are certain variations in the anatomical names applied to the viviparid reproductive organs. In the male reproductive system, the prostate gland has been misidentified as "reservoir seminal" by BAUDELOT (1863) in Paludina vivipara, as "seminal vesicle" by ERLANGER (1891) in the same species, and as "vesicula seminalis" by ANNANDALE and SEWELL (1921) in Viviparus bengalensis. Among females, the most common name used for the pallial part of the female tract, designated the brood pouch in the present study, is the "uterus". This latter name has been used by many authors such as ANNANDALE and SEWELL

(1921) and ROHRBACH (1937). Some recent authors such as BERRY (1974) and VAIL (1977) have referred to the corresponding part in other viviparid females as "the pallial oviduct". Since the viviparid snails are ovoviviparous and this part of the female tract is used for the incubation of the developing eggs till after hatching, the term "brood pouch" clarifies this function. In Campeloma ponderosum coarctatum, Van der Schalie (1965) has used the names "seminal receptacle" and "bursa copulatrix" to refer to the parts corresponding to the thick oviduct and the receptaculum seminis in B.unicolor respectively.

Taxonomic Characters:

In the present snail, the location of the bean shaped testis outside the spire and the inverted long U-shaped receptaculum seminis are in close agreement with the characters of the subfamily Bellamyinae mentioned by ROHRBACH (1937). Although several authors have studied the reproductive organs of certain vivparid snails, no work has been published concerning species of the African and Asiatic genus Bellamya except that of ROHRBACH (1937). He established the subfamily Bellamyinae and distinguished between it and the subfamily Viviparinae by the characters fo the testis, the receptaculum seminis and the embryonic shell. Also, MANDAHL- BARTH (1954) mentioned that the best character distinguishing the two genera Viviparus and Bellamya is the position of the "testicle", and that there are distinct differences between the females of the two genera, especially the shape of the receptaculum seminis. The two viviparid subfamilies Lioplacinae and Campelominae were not mentioned by ROHRBACH (1937). On comparing the reproductive system of B.unicolor and other bellamyine genera with that of other viviparid species belonging to each of the genera Lioplax and Campeloma (BAKER, 1928 and VAIL, 1977), it becomes clear that the first group is also unique in the position of the testis and absence of the distinct vesicula seminalis in the male and the shape and size of the receptaculum seminis in the female. Consequently, a distinct vesicula seminalis is lacking in the studied bellamyine genera, while it is present in the previously examined viviparine and lioplacine genera. As a result of this new taxonomic status of some viviparid snails, it can be concluded that the previous definition of the viviparid genera on the basis of the shell form, opercular features and radular characteristics is not sufficient for the differentiation between these genera.

On comparing the male and female reproductive systems of B.unicolor with those of B.capillatus (ROHRBACH, 1937), one can now differentiate between the two species by the difference in the shape of the testis and the penial tentacle as well as by the length of the thin oviduct. ROHRBACH (1937) did not record the dimensions of the different parts of the reproductive system, but the figures and description show that B.capillatus is characterized by the cone shaped testis, the cylindrical penial tentacle with uniform thickness, and the relatively short thin oviduct, designated by him the ovary.

Anatomy:

The male and female reproductive systems of B.unicolor are generally similar in gross anatomy to that of B. capillatus (1937) and to those of Filopaludina sumatrensis and Siamopaludina martensi (BERRY, 1974). The spermatozoa of B.unicolor as those of all Monotocardia in general and Viviparus viviparus in particular, (MEVES, 1900, 1903 and HANSON et al. 1952) are of two types designated eupyrene and oligopyrene. According to the previously mentioned authors and to PRASHAD (1925), the first type is capable of fertilizing ova, while the second one is inactive and probably has some secretory function.

The terminal vas deferens of B.unicolor passes through the modified right tentacle to open at its smooth tip. The histological structure of this tentacle, fixed during relaxation, shows that it is adapted for considerable increase in size due to the flow of considerable amount of blood within the spaces of the connective tissue at erection. VAIL (1977) has stated that the prostate gland tapers and extends within the right tentacle of the male in Campeloma geniculum and Lioplax pilsbry.

The present study shows that the oviduct can be differentiated into a thin duct emerging from the ovary and opening into the proximal limb of the U-shaped thick glandular oviduct. It is noticeable that ROHRBACH (1937) did not refer to the thin oviduct in the same species and in B.capillatus. He considered that the ovary is a fine duct with lateral and terminal branches. Similar results have been recorded by VAIL (1977) in Campeloma geniculum, Lioplax pilsbry and Viviparus georgianus. But BAKER (1928) has referred to the thin oviduct in Campeloma integrum, Lioplax subcarinata and Viviparus subpurpurus. Also, BERRY (1974) has described this duct in the bellamyine species Filopaludina sumatrensis and Siamopaludina martensi.

In B.unicolor, the connection of the thin oviduct with the pericardial cavity by a fine duct is in close agreement with that reported by BERRY (1974) in the two viviparid species. But VAIL (1977) has stated that the gonopericardial duct connects the ovary with the pericardial cavity in Campeloma geniculum and Lioplax pilsbry, while it is lacking in Viviparus georgianus. A similar duct occurs in other mesogastropods (FRETTER, 1946) and in some neogastropods (HOUSTON, 1976).

The thick U-shaped glandular oviduct of B.unicolor is nearly similar in shape to the corresponding duct of other viviparid species examined by ANNANDALE and SEWELL (1921), ANKEL (1925), NEUMANN (1928), ROHRBACH (1937), BERRY (1974) and VAIL (1977). This finding leads to the assumption that the thick U-shaped oviduct is a common structure among viviparid snails. But further studies on the reproductive system of other viviparid species are necessary. ANNANDALE and SEWELL (1921) have considered that this duct in Viviparus viviparus and V. bengalensis is responsible for the formation of the egg shell and thus they have named it the egg shell gland. The histological structure of the thick oviduct of B.unicolor does not show any evidence of this function. No sperms were noticed in the lumen of thick oviduct of the present species, but the presence of sperms in the corresponding part of the female tract of the viviparid Campeloma ponderosum coarctatum, named the seminal receptacle (VAN DER SCHALIE, 1965), shows that after copulation, the sperms can pass within the female tract till they reach the thick oviduct, where fertilization can occur. Further support of this assumption is the muscular sheath of the distal limb of the thick oviduct of B.unicolor helping control the passage of the eggs to the brood pouch via the receptaculum seminis.

In B.unicolor, there is a follicular albumen gland opening into the free edge of the proximal limb of the thick oviduct by an albumen gland duct. Such connection between the albumen gland duct and the thick oviduct is in agreement with that found by VAIL (1977) in the three North American viviparid species. However, according to BERRY (1974), the albumen gland duct opens into the thin oviduct in the two bellamyine species Filopaludina sumatrensis and Siamopaludina martensi.

The inverted U-shaped glandular receptaculum seminis of B.unicolor, with the ventral groove along its distal limb is used as a pathway for the sperms to the thick oviduct. The finding of the two types of sperms in the lumen of its two limbs indicates that it may also be used for storage of excess sperms and then absorption of the unwanted ones. Sperms were also found in the corresponding part of the female tract of other viviparids by VAN DER SCHALIE (1965), BERRY (1974) and VAIL (1977) in the previously mentioned species. Similar findings in other mesogastropods have been mentioned by PRASHAD (1925), ABOUL-ELA and BEDDINY (1969) and in Stenoglossa by FRETTER (1946). Thus, the absorption of the unwanted sperms carried out by this organ is common in prosobranchs.

In B.unicolor, the pallial part of the female tract, identified as the brood pouch has a glandular, muscular and vascular wall, with numerous trabeculae dividing its lumen into chambers. Similar results have been reported by ALYAKRINSKAYA (1969) showing the adaptations to viviparity in Viviparus viviparus. He also added that this part of the female tract, designated by him the uterus, performs wavy movements to facilitate the process of emergence of the juvenile snails. Furthermore, the location of the groove in the brood pouch and vagina of B.unicolor, and the mucous secretion of its glandular epithelial lining show that this groove is used as a pathway for the sperms, during and after copulation, till they reach the receptaculum seminis. Thus, the term "seminal groove" is appropriate.

Finally, it can be concluded that this morphological and histological study of the reproductive system of B.unicolor is a contribution to the previous studies on viviparid species in this field. Furthermore, on the basis of findings from this study and other similar studies, it appears that the basic organization of the reproductive system is uniform in all the examined bellamyine genera (Bellamya, Filopaludina, Siamopaludina and Idiopoma). In addition, the characters of the reproductive system of bellamyine genera reveal that this group has followed a special line of evolution with regard to the position of the testis, the absence of distinct seminal vesicle, and the relatively large size and shape of the receptaculum seminis. This result is in agreement with the consideration of PRASHAD (1928) that the family Viviparidae is of polyphyletic origin. Yet, further studies to reveal the evolutionary history of the different viviparid genera and subfamilies are needed.

REFERENCES

Aboul-Ela, I.A. and Beddiny, E.A.M. (1969): Morphological and histological studies on the genitalia of Lanistes bolteni Chemnitz, 1786. Ain Shams Sci., Bull., Cairo No. 13: 145-176.

Alyakrinskaya, I.O. (1969): Morphophysiological adaptations to viviparity in Viviparus viviparus (Gastropoda, Prosobranchia). Zool Zh. 48: 1608-1613.

Ankel, W.E. (1925): Zur Befruchtungsfrage bei Viviparus viviparus L. Nebst Bemerkungen über die erste Reifungsteilung des Eies. Senckenbergiana, 7:37-54, pl. 1-2.

Annandale, N. and Sewell, R.B.S. (1921): The banded pond- snail of India (Vivipara bengalensis). Records of the Indian Museum, 22: 215-292, pl. 1-3.

Annandale, N. & Rao, H.S. (1925): Further observations on the aquatic gastropods of the Inlé Watershed. Records of the Indian Museum, 27: 101-126.

Baker, F.C. (1928): The fresh water Mollusca of Wisconsin. Part 1. Gastropoda. Bulletin of the Wisconsin Geological and Natural History Survey, 70: xx+ 507, pl. 1-38.

Barbato, G. (1971): Studio sur comportamente stugionale di Viviparus ater (Crist. & Jan) dei laghi d'Iseo e di Gard a (Gastropoda, Viviparidae) Natura, Milano, 62: 65-74.

Baudelot, M. (1863): L'appareil generateur des mollusques gastéropodes. Ann. Sci. Nat., Ser. IV, 19: 135-222.

Berry, A.J. (1974): Reproductive condition in two Malayan freshwater viviparid gastropods. J. Zool., Lond. 174: 357-367.

Bottke, W. (1972): Zur Morphologie des Ovars von Viviparus contectus (Millet, 1813) (Gastropoda: Prosobranchia): I. Die Follikelzellen. Zeitschrift für Zellforschung und mikroskpische Anatomie, 133: 103-118.

Bottke, W. (1973): Zur Ultrastruktur des Ovars von Viviparus contectus (Millet,1813) (Gastropoda, Prosobranchia) II. Die Oocyten. Z. Zelforsch, 138: 239-259.

Erlanger, R.von (1891): Zur Entwicklung von Paludina vivipara. Morphologische Jahrbuch (Zeitschrift für Anatomie und Entwicklungsgeschichte), 17: 337-376 & pl. 20-23 (Teil I), 636-680 & pl. 32-33 (Teil II).

Fretter, V. (1946): The genital ducts of Theodoxus, Lamellaria and Trivia and a discussion on their evolution in the prosobranchs. Journ. Mar. Biol. Assoc. U. K. 26: 312-351.

Fretter, V. & Graham, A. (1962): British prosobranch molluscs. Roy. Soc. Lond., 755 pp.

Hanson, J., Randall, J.T. and Bayley, S.T. (1952): The microstructure of the spermatozoa of the snail Viviparus. Expl. Cell Res. 3: 65-78.

Houston, R.S. (1976): The structure and function of Neogastropod reproductive systems, with special reference to Columbella fuscata Sowerby, 1832. The veliger, 19, No. 1.

Hubendick,B. (1954): Viewpoints on species discrimination, with special reference to medically important snails. Proc. Malac. Soc. Lond., 31 (1): 6-11.

Mandahl-Barth, G. (1954): The freshwater mollusks of Uganda and adjacent territories. Sciences Zoologiques, 32: 23-37.

Meves, Fr. (1900): Uber den von V. la Valette St. George entdeckten Nebenkern (Mitochondrienkörper) der Samenzellen. Arch. f. mikr. Anat., 56: 553.

Meves, Fr.(1903): Uber oligopyrene und apyrene Spermien und über ihre Entstehung,nach Beobacht ungen an Paludina und Pygaera. Ibid., 61: 12.

Neumann, G. (1928): Zur Kenntnis des Baues der Mantelorgane und des Mantelrandes von Paludina vivipara Lamarck und Paludina ceylonica Dohrn,.Jenaische Zeitschrift für Naturwissenschaft, 63: 181-236, pl. 3-5.

Prashad, B. (1925): Anatomy of the common Indian apple snail Pila globosa. Mem. Ind. Mus. VIII, 3: 91-152, Pls. XVI- XVIII.

Prashad, B. (1928): Recent and fossil Viviparidae. A study in distribution, evolution and palaeogeography. Ibid. VIII, 3: 153-251, pl. 19.

Rao, H.S. (1925): On the comparative anatomy of Oriental Viviparidae. Rec. Ind. Mus. 27: 129-135.

Rohrbach, F. (1937): Oekologische Und morphologische Unter suchungen an Viviparus (Bellamya) capillatus Frauenfeld und Viviparus (Bellamya) unicolor Olivier, unter Berücksichtigung anderer tropischer Formen und

im Hinblick auf phyletische Beziehungen. Arch. f. Molluskenkunde, 69: 177-218.

Siebold, C. Von. (1836): Fernere Beobachtungen über die spermatozoen der wirbellosen Tiere. 2 Die spermatozoen der Paludina vivipara. Müller's Arch. Anat. Phys. pp. 240-255, pl. 2-10.

Speyer, O.W.C. (1855): Zootomie der Paludina vivipara. Inaugural-Dissertation, Marburg, Fischer, Cassel, 46 pp., 2 pls.

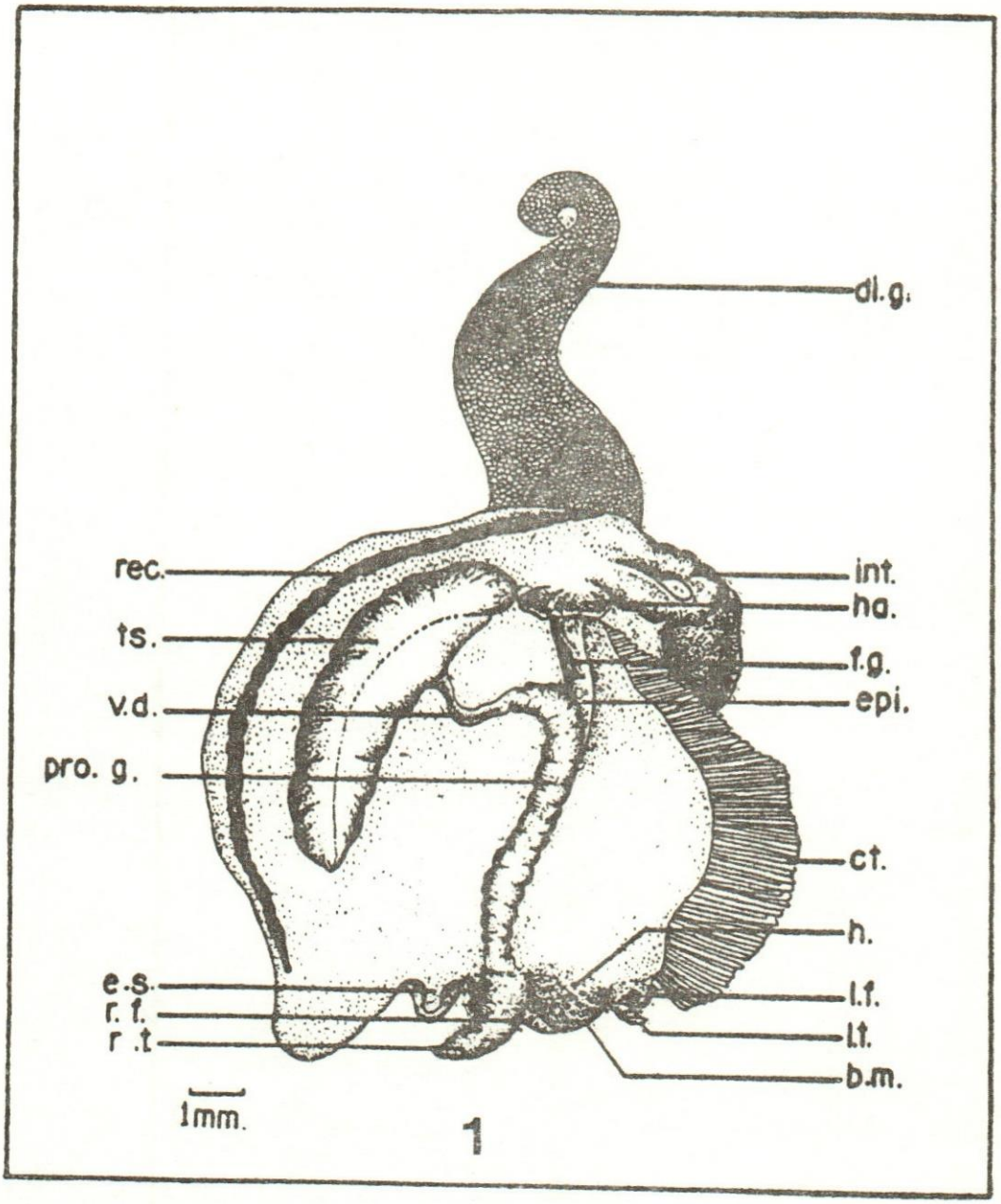
Vail, V.A. (1977): Comparative reproductive anatomy of 3 viviparid gastropods. Malacologia, 16 (2): 519-540.

Van der Schalie, H. (1965): Observations on the sex of Campeloma (Gastropoda, Viviparidae). Occasional Papers of the Museum of Zoology, University of Michigan, No. 611: 1-15.

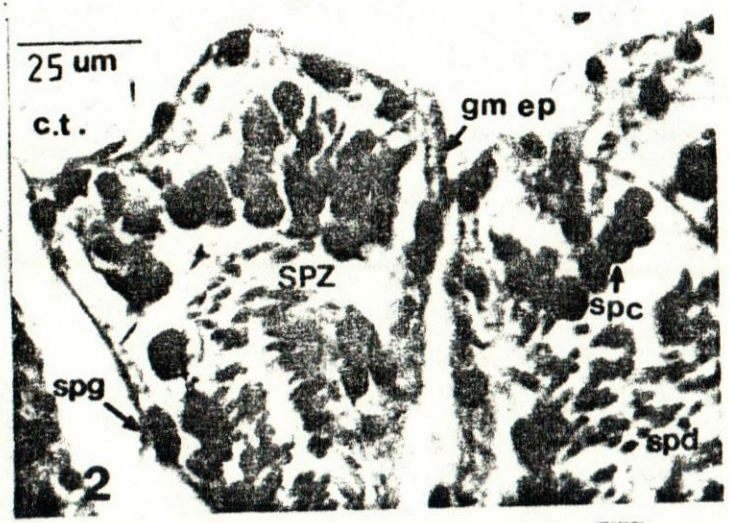
Vassor, P.S. & Culling, C.F.A. (1959): Carleton's histological technique, 4th ed. pp. 214-215, Oxford University Press, New York.

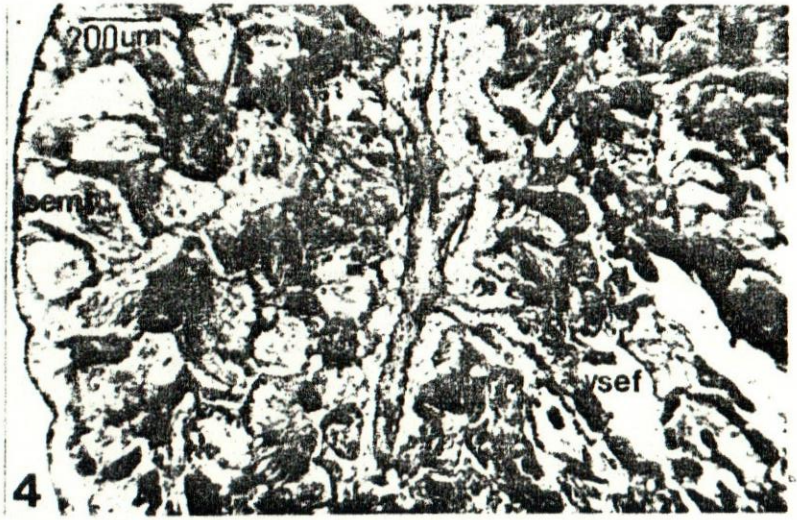
EXPLANATION OF FIGURES

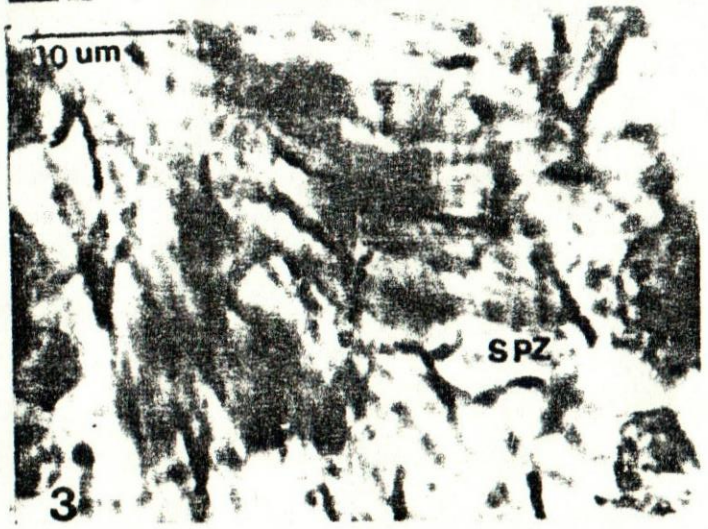
Fig. 1 : The roof of the pallial cavity of a male specimen cut open to expose the male reproductive system in situ.
Fig. 2 : Transverse section of the testis.
Fig. 3 : A magnified part of a section of the testis showing the spermatozoa.
Fig. 4 : Transverse section of the testis showing the vasa efferentia which lead to the vas deferens.
Fig. 5 : Transverse section of the vas efferens.
Fig. 6 : Transverse section of the proximal vas deferens.
Fig. 7 : Transverse section of the distal part of the right tentacle of a male, with the terminal vas defereas at its centre.
Fig. 8 a: Transverse section of the prostate gland.
Fig. 8 b: A magnified part of a transverse section of the prostate gland.
Fig. 9 a: The roof of the pallial cavity of a female out open to expose the female reproductive system in situ.
Fig. 9 b: The oviduct, albumen gland and its duct.
Fig. 9 c: Egg case.
Fig. 9 d: Shell of a young snail, just after emergence from the genital opening.
Fig. 10 : Transverse section of the ovary.
Fig. 11 : Transverse section of the visceral mass showing the gonopericardial duct.
Fig. 12 : Transverse section of the thin part of the oviduct.
Fig. 13 : Transverse section through the proximal limb of the thick part of the oviduct.
Fig. 14 : Transverse section through the distal limb of the thick part of the oviduct.
Fig. 15 : Transverse section of the papilla of the distal limb of the thick part of the oviduct.
Fig. 16 : Transverse section of the albumen gland and its duct.
Fig. 17 : Transverse section through few follicles of the albumen gland.
Fig. 18 : Transverse section of the two limbs of the receptaculum seminis.
Fig. 19 : A magnified part of a transverse section of the second or distal limb of the receptaculum seminis showing the structure of a fold.
Fig. 20 : Transverse section of the brood pouch with the seminal groove.
Fig. 21 : A magnified part of a transverse section of the brood pouch showing the trabeculae dividing its lumen.
Fig. 22 : A magnified part of a transverse section of the brood pouch showing the structure of its wall.
Fig. 23 : A magnified part of a transverse section of the brood pouch showing the structure of the epithelial lining of the seminal groove.
Fig. 24 : A magnified part of a transverse section of the vagina.

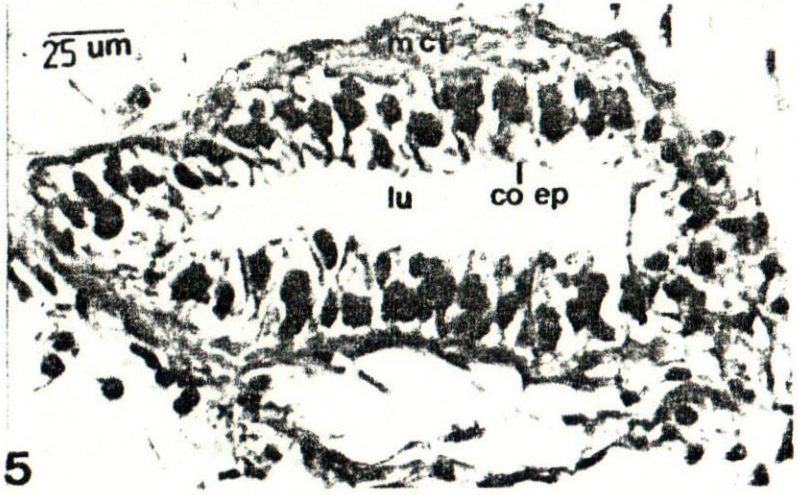


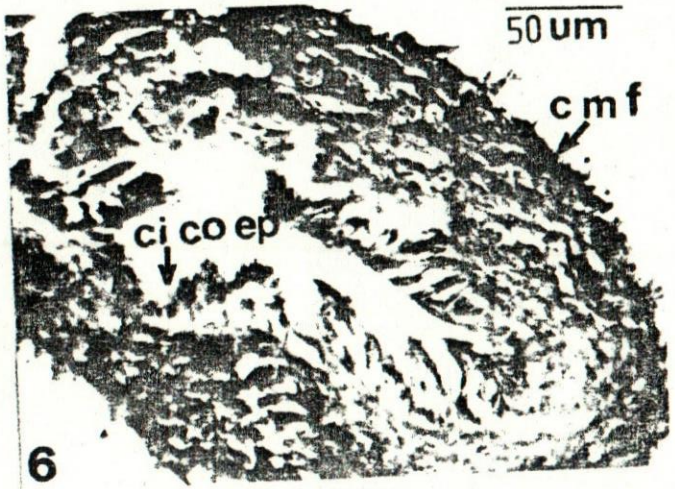
1


25 um
c.t.
gm ep
SPZ
spc
spg
spd
2

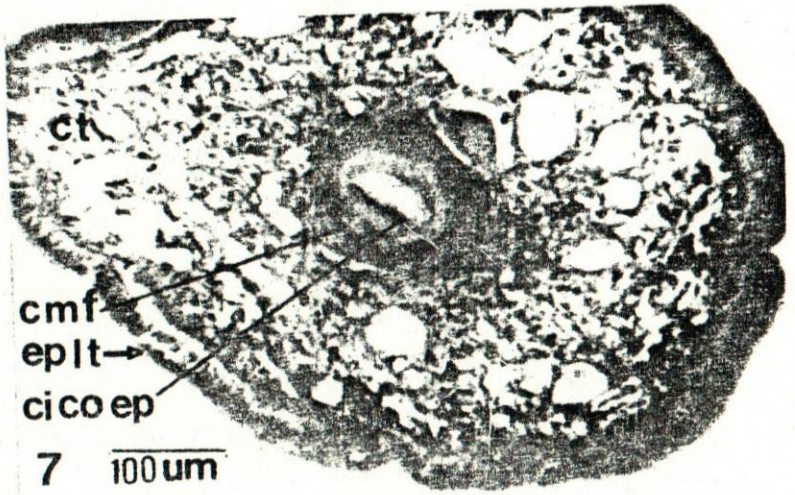

200um
vsef
4


10 um
SPZ
3

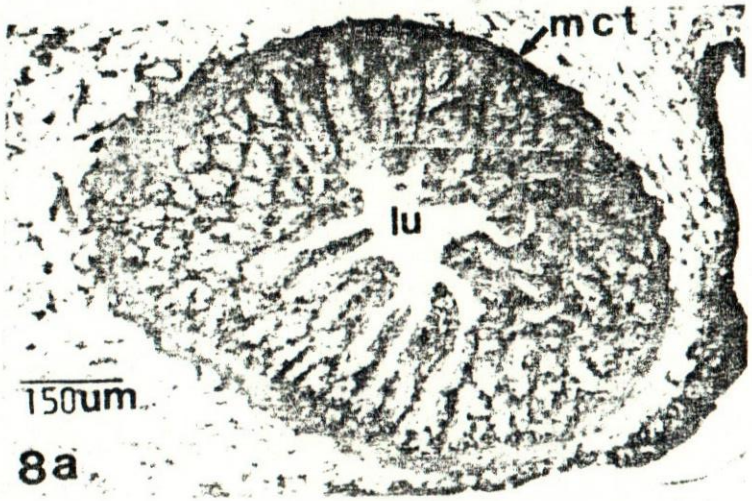

25 um
mcl
lu
co ep
5



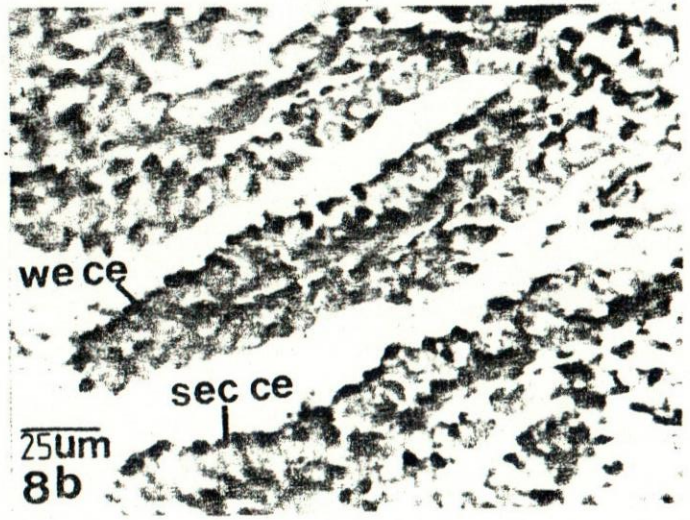
6



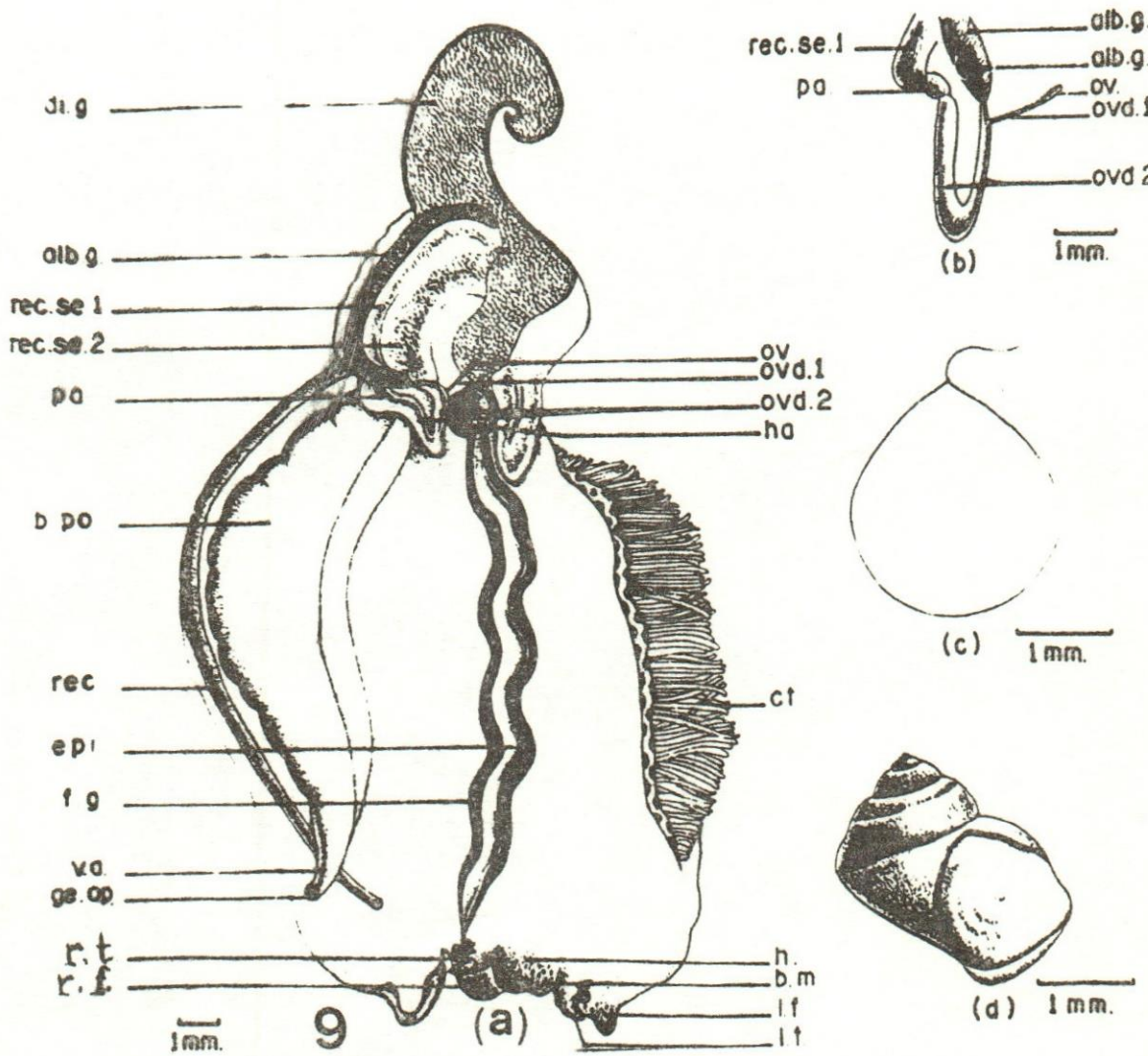
7 100 um

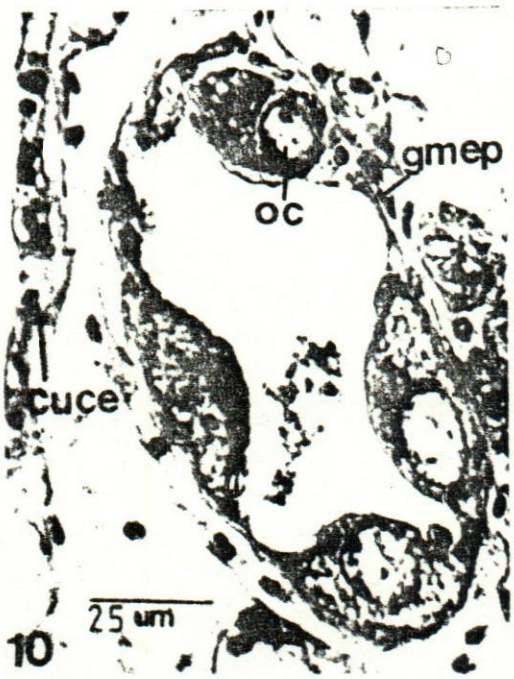


8a

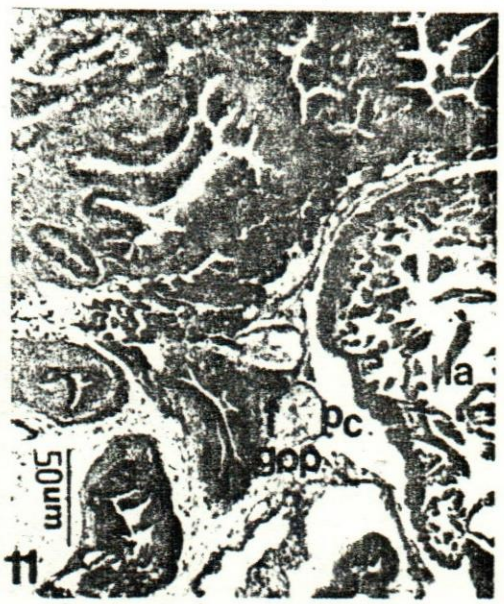


8b

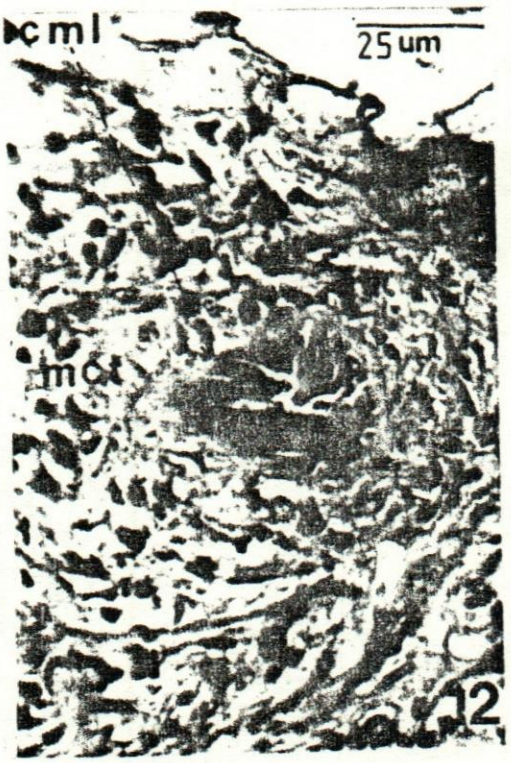

ut.g
alb.g
rec.se.1
rec.se.2
pa
b.po
rec
epi
f.g
va
ge.op
r.t
r.f
ov
ovd.1
ovd.2
ha
ct
h.
b.m
l.f
l.t
1mm.
9
(a)
rec.se.1
pa
alb.g.
alb.g.d
ov.
ovd.1
ovd 2
(b)
1mm.
(c)
1mm.
(d)
1mm.


gmep
oc
cuce
25 um

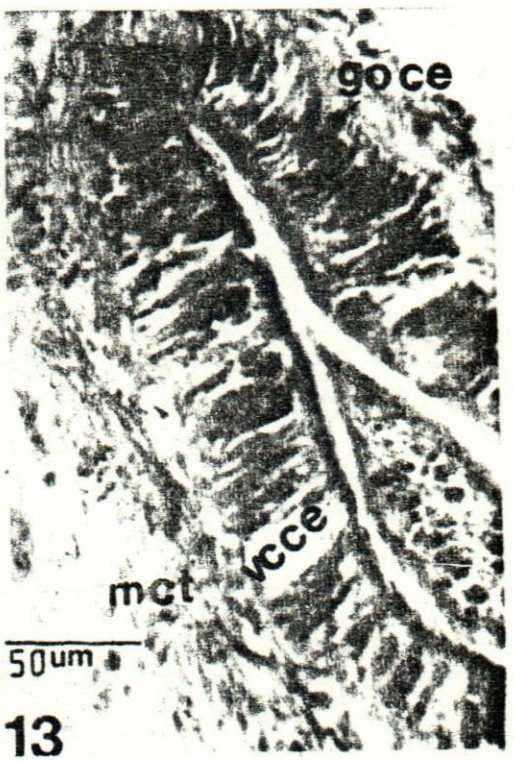
10


la
pc
gop
50um

11

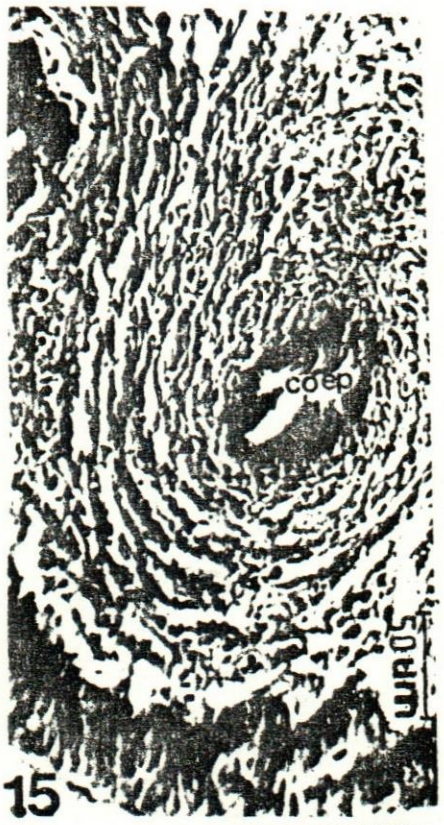

mcml
25 um
mct

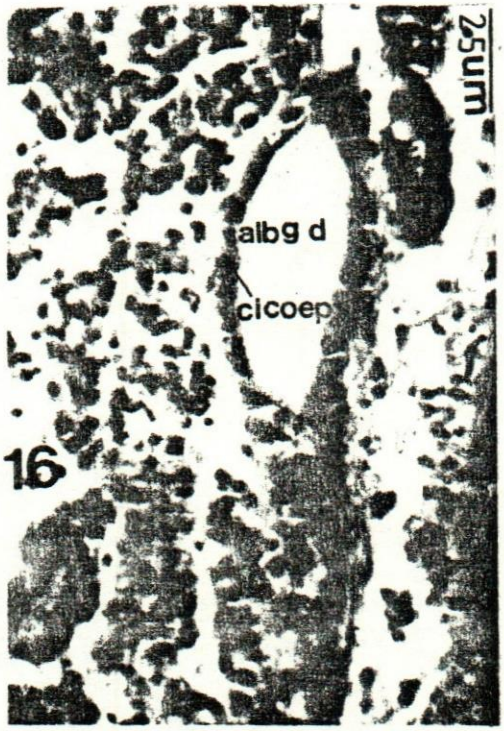
12

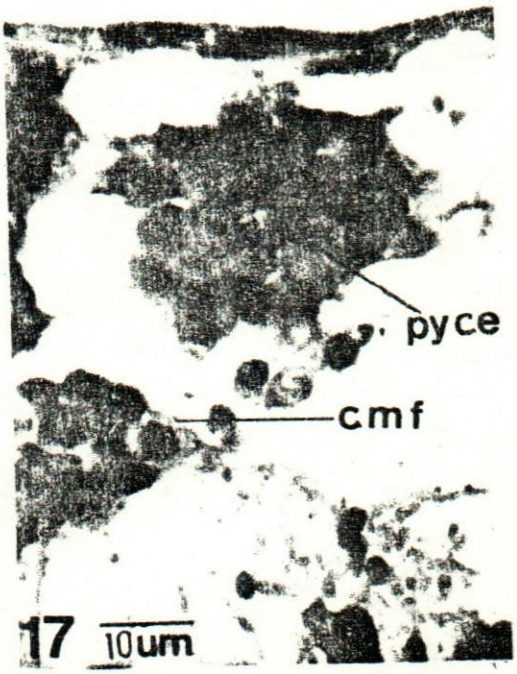

goce
vcce
mct
50um

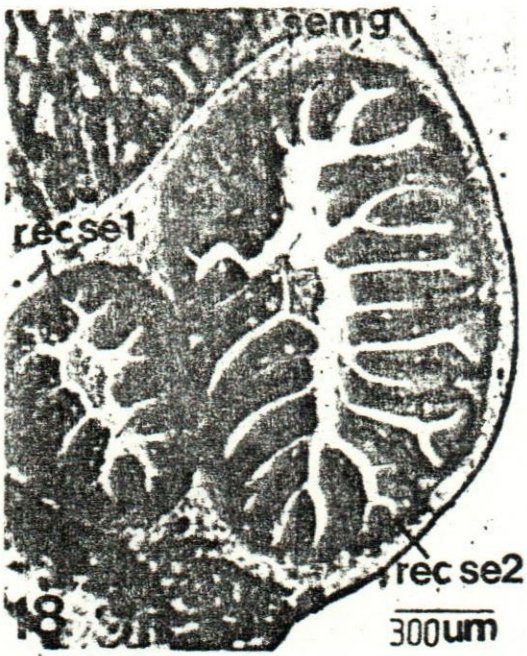
13


cmf
lmf
vcce
glce
25um
14

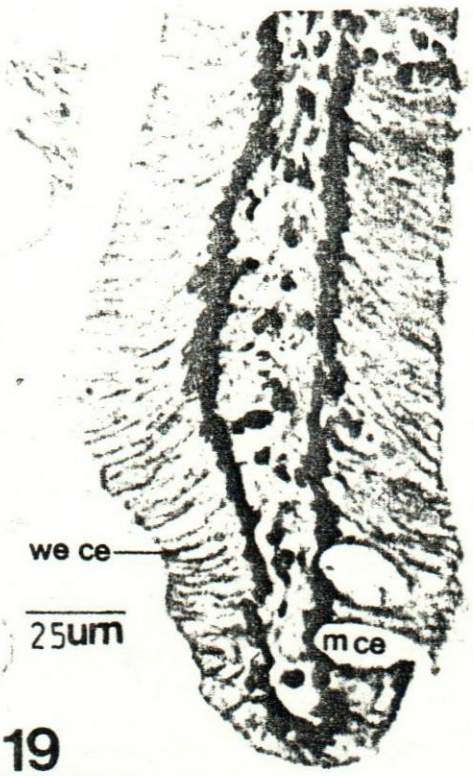

coep
25um
15


25um
albg d
cicoep
16

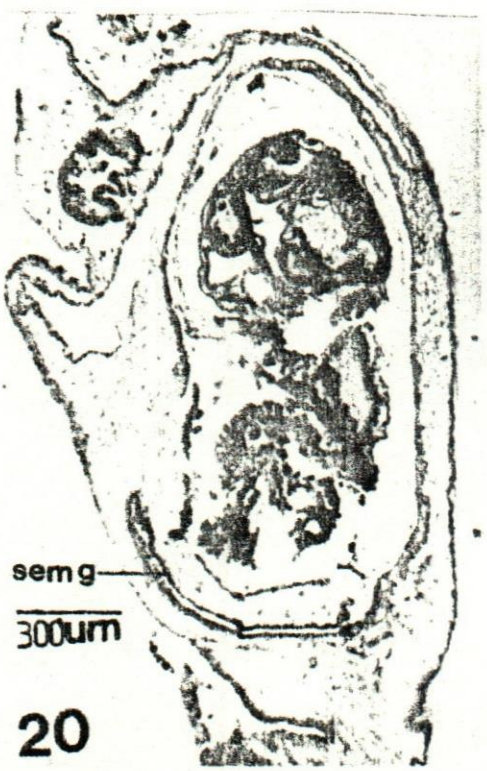

pyce
cmf
17
10um



18

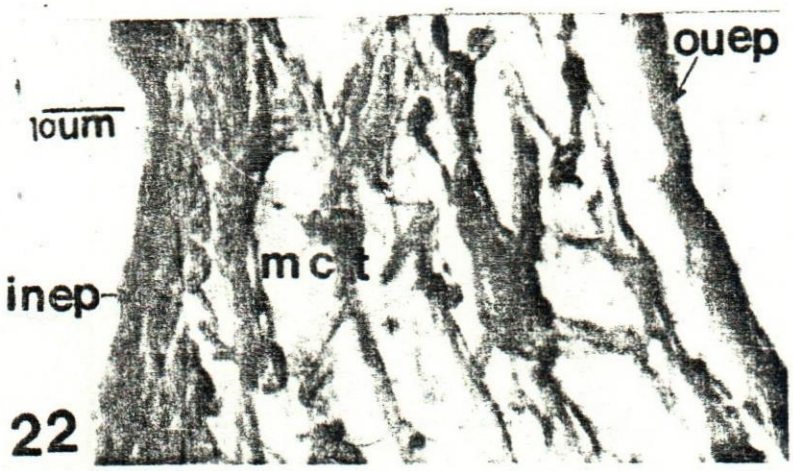


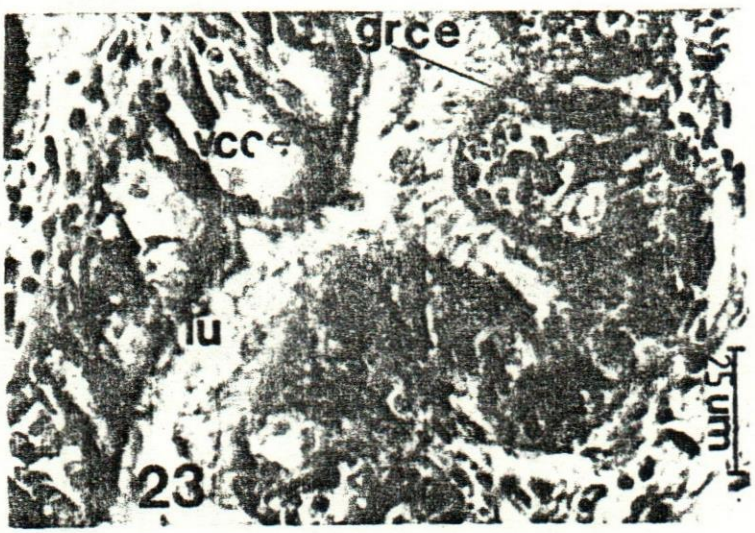
19

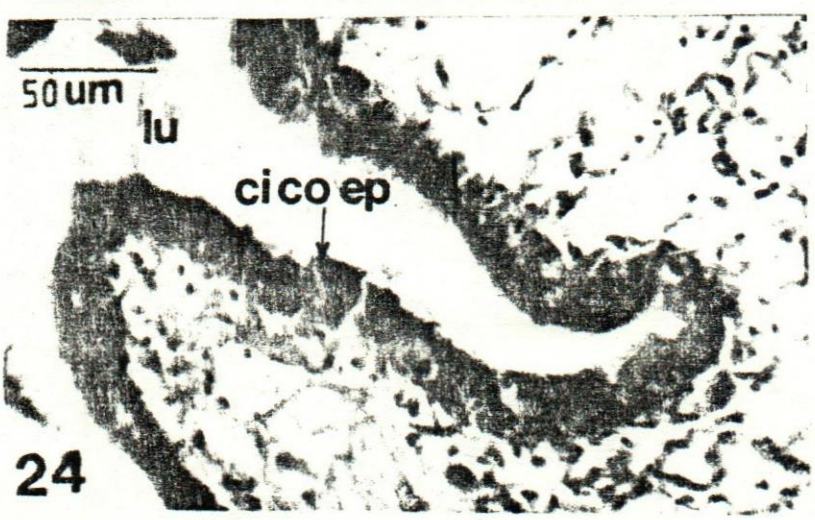


20

21


ouep
10um
inep
mcat
22


grce
vcc
lu
23


50um
lu
cicoep
24