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THE REPRODUCTIVE SYSTEM OF **OPISTHOGONIMUS PHILODRYADUM** (WEST, 1896) (DIGENEA)

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RESUMO - O sistema reprodutor do adulto de **Opisthogonimus philodryadum**(West, 1896) foi estudado anatomica e histologicamente ao microscópio. Esta análise mostra que o canal de Laurer, metratermo, cirro e duto ejaculatório possuem um epitélio aprofundado. O metratermo e a franja citoplasmática do cirro ou tectum tem uma camada esclerótica diferenciada, semelhante em aspecto e cor às dos espinhos do tegumento do animal.

ABSTRACT - The reproductive system of adult **Opisthogonimus philodryadum** (West, 1896) has been anatomically and histologically investigated with light microscopy. This analysis shows that Laurer's canal, metraterm, cirrus and ejaculatory duct prossess a sunken epithelium. The metraterm and cirrus cytoplasmatic fringe or tectum has a sclerotical differentiated layer, similar in aspect and colour to the ones belonging to the spines of the animal's tegument.

INTRODUCTION

Among the Digenea of Argentina, **Opisthogonimus philodryadum** (West, 1896) (syn. **O. lecithonotus** (Lühe, 1900), is the most common ophidian parasite (Caubisens-Poumareau, 1968) Since the first description of the adult of **O. philodriadum** (West, 1896) its reproductive system has not been fully studied. Limitative techniques (Caubisens-Poumareau, 1968) or microanatomic sketches (Cordero & Vogelsang, 1928) have been published. Some work undertaken with restricted taxonomic point of view, does nevertheless not allow anatomical and histological comparisons (Pereira, 1929); Artigas **et al.** 1943 ;

Mañé-Garzon & Gortari, 1965; Travassos **et al.** 1969; Gomes 1979), which in other digeneans has proved important for taxonomical judgements (Bakke, 1976, 1977, 1978)

The genitalia play an important role in the classification of digenetic Trematoda (Bakke, 1976, 1977, 1978). It is not strange to find that the scant knowledge of the histological structure of the reproductive system in these animals leads to a misinterpretation of the physiology of reproduction (Bhatnager & Gupta, 1981) Interesting studies of the reproductive system in other digeneans at the photonic level have been published (Ebrahimizadeh, 1966; Reinhardt, 1969).

This study gives anatomical and histological information at the photonic level concerning the reproductive system of **Opisthogonimus philodryadum** (West, 1896) from Argentina.

MATERIALS AND METHODS

The specimens of **Opisthogonimus philodryadum** were collected from the mouths of "yarará" snakes, **Bothrops alternatus** (Viperidae) sent from El Cimarrón (Entre Ríos) to the snake collection of the Instituto Nacional de Microbiología "Carlos Malbrán". The Digenea where picked out alive and washed in NaCl physiological solution; 3% neutral formaldehyde, Bouin Hollande and Gendre solutions were used as fixing agents. Part of the material (10 specimens) was stained with Grenacher carmine and mounted "in toto". The rest (40 specimens) was embedded in paraffin wx 56-58°C and serially processed to 5-7 µm thick sections. The longitudinal (parasagittal and frontal) and transverse sections were stained with (1) Hematoxyline-eosine; (2) Groat-picro-indigo-carmine; (3) Masson's trichromic; (4) Mallory; (5) Dahlgren; (6) PAS and (7) Tri-PAS.

The length of the examined animals oscillated from 3 to 5 mm and their width form 0.9 to 1.5 mm.

RESULTS

The transverse plane that passes the centre of the acetabule divides the body of the animal into two regions; the preacetabular where no genital organs are found and the postacetabular which contains the whole reproductive system. This is of the hermaphrodite type with separate male and female independent genital organs.

The male system drains ventrally through its gonopore which is located 1/4 the lenght of the animal from the acetabular centre to the posterior end and on the parasagittal plane 50 µm to the left of the sagittal one.

The female system is connected with the outside by means of two pores: the dorsal pore of Laurer's canal and the ventral femenine gonopore. As the masculine gonopore, female

opens on the same transverse plane, 100 µm to the left of the sagittal plane.

The pore of Laurer's canal is located on the right parasagittal plane, 100 µm from the sagittal plane and 1/5 the length of the animal from the acetabular centre to the posterior end.

The vitellaria lies dorsal along the medial part of the animal's body from the ovarian region (ootype) up to a short distance behind the testis.

FEMALE SYSTEM

The ovary and its related annexed part lies right, dorsal, in the anterior part of the postacetabular region. These annexed parts are: the oocapt, the oviduct, the ootype with Mehlis' glands, the receptaculum seminalis, Laurer's canal, the right vitelline acini and the common vitelline duct.

The female genital pore as well as the left portion of the vitellaria and nearly all the uterus with the metraterm ocuppy other areas of the postacetabular region.

The ovary is an ovoid organ, about 220 x 165 µm diameter. Its wall consists of a layer of dense connective tissue having 6 x 3 µm diam. nuclei, with a few, irregularly displayed, thick chromatine granules. Few, thin muscular fibres are part of the wall. This is thicker in the oocapt region and thinner at the opposite site. The germinative epithelium is separated from the connective capsule by a basal membrane.

The ovary exhibits all the different stages of oogenesis. Mature and inactive oocytes are spherical cells of 7 µm diam. Their nuclei are 5 µm diam. They contain medium sized chromatine granules and non-central nucleolus 1.5 µm diam. Their cytoplasm is highly acidophilic. These are observed near the oocapt. An ameboid snaking aspect shows their exit from the ovary.

The capsular connective of the ovary is prolongued into the region of the sexual ducts up to the beginning of the uterus. All sexual ducts are surrounded by this connective. The oviduct, starting in the oocapt, meets at about 65 µm with the opening of the receptaculum seminalis and adjacent to it with the internal mouth of Laurer's canal. From this point, the oviduct bends downwards and forwards. Then turns to the right and upwards. The oviduct finally goes backwards to join the common vitelline duct, where it transforms itself into the ootype. This extends 55 µm before continuing into the uterus. (Figure 1) Ellipsoidal nuclei 4 x 5 µm diam. with thick chromatine granules and a 1 µm diam. nucleolus are observed in the connective of the oocapt and oviduct zones. These kind of nuclei are abundant. Other types are observed in the connective of the ootype zone and at he beginning of the uterus. The first are round, 5 µm diam., clear, with scanty chromatine in medium size granules. The second are small, pycnotic nuclei clustered.

The oocapt length is ca. 20 µm. Its epithelium contains two nuclei of 6 x 3 µm diam. with thick, irregularly dis -

played chromatine granules and a 1 µm diam. nucleolus. Spermatozoa are observed in its lumen close to the ovocytes leaving the ovary.

The oviduct, between the oocapt and the opening of the receptaculum seminalis, has in its connective wall an helicoidal muscle fibre. The oviduct epithelium is ciliated with 7 x 3 µm diam. nuclei and non-central nucleolus 1.2 µm diam. Chromatine is irregularly distributed in a few thick and thin granules.

The receptaculum seminalis is a short-necked sack limited by a thin connective wall with muscular fibres. Its epithelium has a few ellipsoidal nuclei 13 x 6 µm diam. Chromatine is scarce and located in thin peripherically distributed granules. The nucleolus is 3 x 2 µm diameter.

The external pore of Laurer's canal lies in a narrow, 50 µm deep depression of the tegument. Laurer's canal ends next to the opening of the receptaculum seminalis. It possesses a sunken epithelium with no apparent cell boundaries (Welsch & Storch, 1976), where pericaryons can be pointed out. These are 6 x 3 µm diam. with 4 x 2 µm diam. nuclei. They contain a 1 µm diam. nucleolus and thick chromatine granules. The muscle layers of the wall consist of a few fibres which have the same orientation as those from the body tegument. The external surface of the sunken epithelium is covered with a thin denticled cuticle. Spermatozoa can be seen in the canal's lumen. There are some peripherical spindle-shaped cells 12 x 4 µm diam. their pycnotic nuclei being 4 x 2 µm diam. Their cytoplasms contain erytrophilic granules and seen to pour through the sunken epithelium into the canal's lumen by a thin duct (Figure 3)

The vitellaria are formed by bundles of a few sack-shaped cylindrical glands, which drain into the two main vitelline ducts. These join into a common, relatively short duct that opens into the oviduct before the ootype. The vitelline cells found in the common vitelline duct are spheroidal, about 6 µm diam. The central pycnotic nucleus, 3 µm diam., is surrounded by a clear cytoplasmatic zone. The portion against the cell membrane is occupied with a layer of granules, ca. 1 µm diam., which stains yellow amber with Tri-PAS (globules of shell material) (Figure 3).

The ootype is 50 µm long and its epithelium shows 4 µm nuclei, with the chromatine packed in 3 to 5 thick granules. The Mehlis' glands open inside the cavity of the ootype. They are pyriform and have a collar that is longer the farther the ootype be from the cellular body. The collar length ranges from 6 µm to 40 µm according to where the cellular body is situated. The glands together with their collars may reach 60 µm diameter, considering the ootype as the centre. The cellular bodies closer to the ootype are small, 6 µm diameter, with 4 x 5 µm diam. nuclei and 1 µm diam. nucleolus and chromatine in medium sized granules uniformly distributed. Among the farthest ones, some stand out because of their 20 µm lenght and 10 µm width. The central nucleus has a 5 x 6 µm diameter and a 1.5 µm diam. nucleolus.

There is a type of intermediate sized cellular body -16 x 7 µm diam. with 5 µm diam. nucleus and 1.5 µm diam. nucleolus-closer and more abundant. The nuclei of the three morphological types of Mehlis' glands are slightly stained.

As it arrives at the ootype, the fertilized oocyte is surrounded by five vitelline cells which come out from the common vitelloduct one by one. Most of them are placed behind the oocyte. The peripherical granules are ejected from the vitelline cells and they are joined together in a mass of the same colour as the prospective egg shell. This mass encircles the oocyte and the vitelline cells forming the egg shell (Figure 4)

The uterus, following the ootype, presents two regions : a proximal one that lacks parietal muscles, and a distal one with an external layer of longitudinal muscle underlying the uterine epithelium. Beyond this, another layer of internal circular muscles is found. The distal region of the uterus can expand its walls considerably. The epithelium of the uterus is thin, with a few nuclei 8 x 5 µm diam., non-central nucleolus 0.5 µm diam. and scarce, dense chromatine granules.

The metraterm is a muscular duct which runs ventral-dorsally and forwards up to the transverse plane that bisects the acetabule. At this point, is becomes the continuation of the uterus. The transition from one into the other is abrupt (Figure 5) The wall of the metraterm undergoes a fundamental structural change. It exhibites a sunken epithelium with no apparent cell boundaries, which in relation to the basal membrane, presents two parts: an external cytoplasmatic region or tectum and an internal or deep portion. The latter consists of nuclei with their peripherical cytoplasms (pericaryons) and of their prolongations that go through the basal membrane (columnae) Two cellular types may be distinguished: 1) pyriform pericaryons with scarce cytoplasm and pycnotic nuclei 4 x 2 µm diam. and 2) oval pericaryons of 12 x 11 µm diam. without or with few (6-7) secretion granules, their nuclei being 4 x 5 µm diam. and their nucleoli 1 µm diam. The distal tectal portion is made up to villi or lamellae. The density of pericaryons in the metraterm is higher than that in the body tegument. A differentiated layer of sclerotical cytoplasm can be seen in the proximal tectal portion above the basal membrane. (Figures 6 and 7) This region stains bright yellow with Dalgren and orange-red with trichromics. A PAS positive substance secreted by the tectum can be found in the cavity of the organ and also pouring out of the gonopore together with the eggs. In some specimens, masses of foreign spermatozoa can be observed in the metraterm. (Figures 5 and 6) The metraterm has an external wide muscular layer ca. 8 µm in thickness with circular orientation and an internal one consisting of thick, isolated fibres, separated about 8 µm from one another (Figures 5, 6 and 7)

The embryonated eggs found in the metraterm are ellipsoidal. Their operculum is clearly distinguished and it presents its suture line in the first fifth of the total lenght of the eggs. The suture is stressed by a thickering of the shell.

The shell appears yellow amber in non-stained animals fixed in neutral formaldehyde. The eggs have the larger diameter between 21-26 µm and the smaller one between 11-16 µm (n = 100)

MALE SYSTEM

It consists of two testes with their deferent ducts draining at the same point in the proximal end of the cirrus pouch. This includes the vesicula seminalis, the pars prostatica, the ejaculatory duct and the cirrus. The distal end of the pouch builds up a proof of a small cavity formed by the invagination of the wall of the animal's body. The hollow or atrium, that communicates with the outside by means of the male gonopore, allows the evertion of the cirrus.

The right and left testes are ovoid bodies of 450 x 350 µm diameter situated behing the ovarian region. Each organ is limited by a dense connective capsule. An ellipsoidal nucleus (11 x 8 µm diam.) can be seen in this capsule, in the origin zone of the vas deferens. There are thick granules of scarce chromatine and a 1 µm nucleolus within the nucleus. That zone is the thickest portion of the capsule which gradually dimi - nishes its thickness towards the opposite region. Thin muscular fibres can be noticed following the organ's curvature.The germinative epithelium is settled over a basal membrane. The all different stages of spermatogenesis can be observed within the organ.

Though the deferent ducts are of small calibre, they can expand by expelling numerous spermatozoa. Their walls are provided with very thin, loosely helicoidal muscles fibres The epithelium has a few nuclei of 8 x 5 µm diam. and the non -central nucleolus is 2 µm diam. Due to the position of the testes which originates it, the left duct is longer than the right one. Both of them open together in the apex of the cirrus pouch forming a valvelike structure that enters the vesicula seminalis.

The cirrus pouch is placed neighbouring the bisectal plane of the acetabule. When it is observed "in toto", the cirrus pouch is visually proyected on horizontal optical planes and it seems to be U-shaped.

The cirrus pouch is anatomically a tube of convex ends of 900-1000 µm long and a diametre of 100-110 µm. It bends on different planes long its way, as shown on Figure 2. The proximal part, situated towards the left of the ovary, on the right side of the animal, is dorsal.

It is directed from the back forwards. One first loop turn the cirrus pouch to the left side, locating it perpendicularly to the sagital plane. A second concave loop turns it to the venter and to the left side of the animal. A third turn bends the pouch downwards and to the right, and after a fourth bend, it ends in the small atrium that communicates with the outside through the gonopore.

The cirrus pouch is separated from the corporal parenchyma by two muscular layers that define it as a discrete entity. The atrium is an invagination of the animal's body wall with its corresponding sunken epithelium and muscle system (Beklemishev, 1969). The muscular layers -keeping the original disposition of their fibres- are divided in two groups when they reach the roof of the atrium: one that limits the cirrus pouch and another associated with the sunken epithelium of the atrium's roof. The boundaries of the pouch are formed by an external and internal layer of muscular fibres, the former consisting of thick longitudinal fibres (in touch with the corporal parenchyma) and the latter of a single row of separated thin fibres, embedded in a ca. 1 µm thick connective sheet.

The connective tissue of the pouch is similar to the one in the corporal parenchyma of the animal, with two nuclei types. One is clear and large (7 x 5 µm diam.) with a 2 µm diam. nucleolus and a few small chromatine granules. Another measuring 4 µm diam. with a 1 µm diam. nucleolus and with medium sized chromatine granules. The vesicula seminalis, the prostate glands, the ejaculatory duct and the cirrus are located among this connective.

The internal vesicula seminalis lies from the apex of the pouch to the end of the first loop. It is a folded sack, covered with apparently helicoidal muscular fibres. Its epithelium is thin, containing scanty, round and flattened nuclei (6 µm diam. and 2 µm thick) that project into the vesicula lumen. Scarce chromatine is packed in medium size and irregularly distributed granules, the 1 µm nucleolus is non-central.

The vesicula seminalis is joined to the pars prostatica by a short 30 µm long duct, with thin helicoidal fibres in its wall Nearly a dozen epithelial nuclei are gathered in a wide portion at the entrance of this duct. The nuclei are 5 x 4 µm diam. with a non-central nucleolus 1 µm diam. The epithelium also projects into the prostate lumen, where two nuclei, similar to the ones already descripted, form a small valve.

The pars prostatica extends along 140 µm from the first loop. It contains a relatively big cavity surrounded by the prostate glands. (Figures 8 and 9)

The lumen's wall consists of an epithelium and the underlying muscles (the external are circular and the internal longitudinal) The nuclei of the epithelium are 4 x 3 µm diam. with a 1 µm diam. nucleolus and a clear cytoplasm.

The prostate glands are ellipsoidal, balloon-shaped cells with a long collar The perinuclear cytoplasm may be found far away from the prostate cavity. some of them have been found half way down the region of the second loop. Some of them lie among the folds of the vesicula seminalis. The nucleus of the glandular cell is central, round 5 µm diam., clear, with chromatine in a few thick irregularly distributed granules and the 1 µm diam. nucleolus is non-central. The cytoplasms contain granules. Their collars run through the wall of the prostate cavity and remain free in the lumen. (Figures

8 and 9) Phycnotic nuclei of 6 x 3 µm diam. are observed among the collars. Their nucleoli are non-central and 1 µm diam. and there are many medium sized chromatine granules (Figure 8) Longitudinal muscular fibres may be noticed among the cytoplasm of the prostate glands.

The ejaculatory duct starts in the second loop and stretches up to the third one. The cirrus begins in the latter and ends in the pore on the roof of the atrium. The walls of both ducts suffer deep structural changes. The epithelia are of the sunken type with no apparent cell boundaries, as the ones belonging to Laurer's canal and the metraterm.

The sunken epithelium of the ejaculatory duct has relatively few pericaryons with 3 x 2 µm diam. pycnotic nuclei. There are two thick muscular layers separated by a connective sheet: one internal, made up of longitudinal, separated fibres and the other external, of circular fibres.

The retracted cirrus presents a folded tract.(Figure 10). Its sunken epithelium has relatively abundant pericaryons of 10 x 12 µm diam.Secretion granules can be detected in some of their cytoplasms, which are poured into the lumen of the organ through the upper tectum. The nuclei are 5 µm diam.,the nucleolus is 1 µm diam. and the chromatine is found in thick granules. The tectum of the cirrus is formed by three layers: the external with a striated border (microvilli), a middle sclerotical sheet and a basal one with grooves. The sclerotical layer is thick and clear, staining bright yellow with Dahlgren and orange with trichromics. (Figure 11).

Under the cirrus basal membrane there is a single external layer of discrete circular muscle fibres, beyond which there is an internal layer of lax longitudinal muscle fibres. Another longitudinal muscular fibres run along the lacunar connective surrounding the sunken epithelium. Columns of three or five cells can be found against the sheath of the cirrus pouch. They are 10 x 4 µm diam. and contain 3 x 2 µm diam. nuclei with a few thick chromatine granules.

The wall of the atrium's roof continues into the cirrus pouch, becoming the cirrus wall with its characteristic differentiations.

During the erection, the cirrus projects through the expanded gonopore. The everted "piston-like" cirrus can be observed in specimens fixed during copulation. In this state, the ejaculatory duct reaches a diametre of 26 µm and the golbet-shaped cirrus of 63 µm.

DISCUSSION

The reproductive system of **Opisthogonimus philodryadum** (West, 1896) has a complex structure. The "in toto" mountings do not help completely to describe the structure of its genitalia. The reconstruction based on serial histological sections allows to accurately know the whole system.

The structures of the ejaculatory duct and of the cirrus differ from that of the pars prostatica because they have a

sunken epithelium, the latter is different in both structures; having the cirrus' sunken epithelium a sclerotical layer

The sclerotical layer of the sunken epithelium of the metraterm and cirrus reveals -with the stainings performed - a similar aspect and colour to the ones belonging to the spines of the animal's tegument.

By means of electron microscopy, Threadgold (1975) points out that the cirrus of **Fasciola hepatica** "is merely an extension of the general body surface and is covered by a modified tegument" In the specimens of **O. philodryadum,** all organs acting as entrance to the animal's genitalia (Laurer's canal and metraterm; the cirrus and the ejaculatory duct)have walls structured at he animal's tegument, i.e., a sunken epithelium with muscular layers. These organs may be regarded as modified invaginations of the tegument. It would be interesting to perform an electronic microscopical analysis with this species of Digenea in order to obtain in corroboration at this level.

The presence of spermatozoa in the oocapt together with mature oocytes coming out from the ovary would indicate that fertilization occurs in that region.

The development of the egg observed in the ootype indica te that the peripherical granules of the vitelline cells originate the egg shell.

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ABBREVIATIONS IN THE FIGURES

a = atrium
c = cirrus
cm = circular muscle layer
dd = deferent duct
e = egg
ed = ejaculatory duct
l = Laurer's canal
lm = longitudinal muscle layer
M = metraterm
m = muscles
mg = Mehlis' gland
o = ovary
ot = ootype
ov = oviduct
p = pericaryon of the sunken epithelium
pg = prostate gland
pl = lumen of the pars prostatica
pp = pars prostatica
rs = receptaculum seminalis
s = spermatozoa
se = sunken epithelium
t = tectum
U = uterus
vd = vitelline duct
vs = vesicula seminalis

Fig. 1 - Sketch of the female system.
Fig. 2 - Sketch of the cirrus pouch.

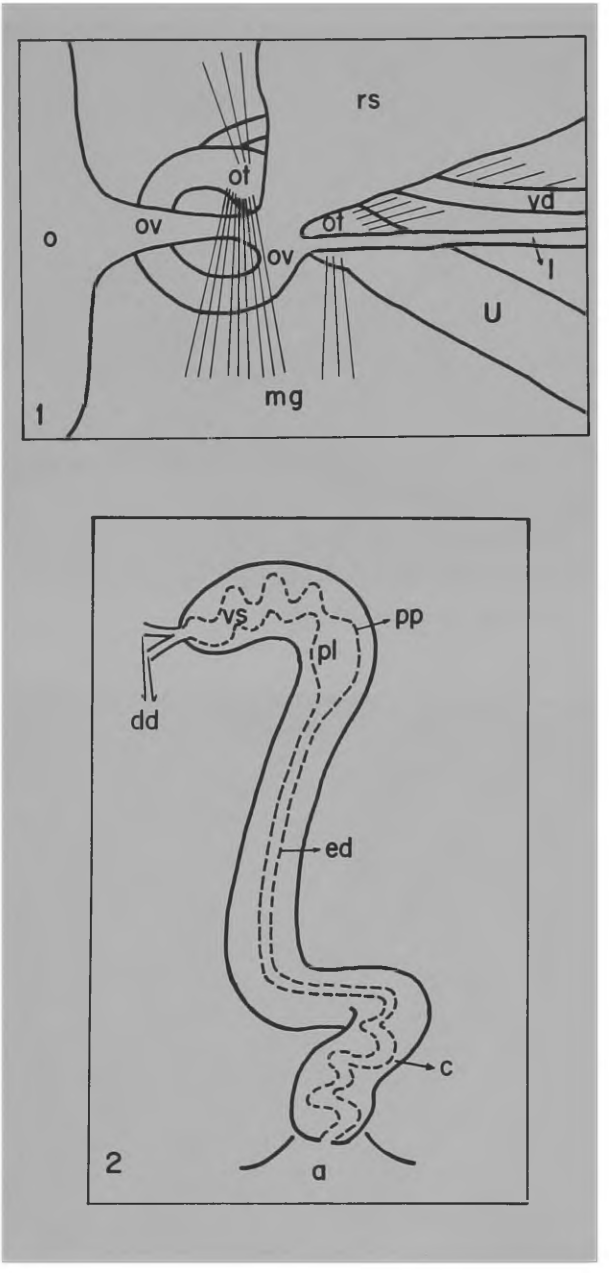
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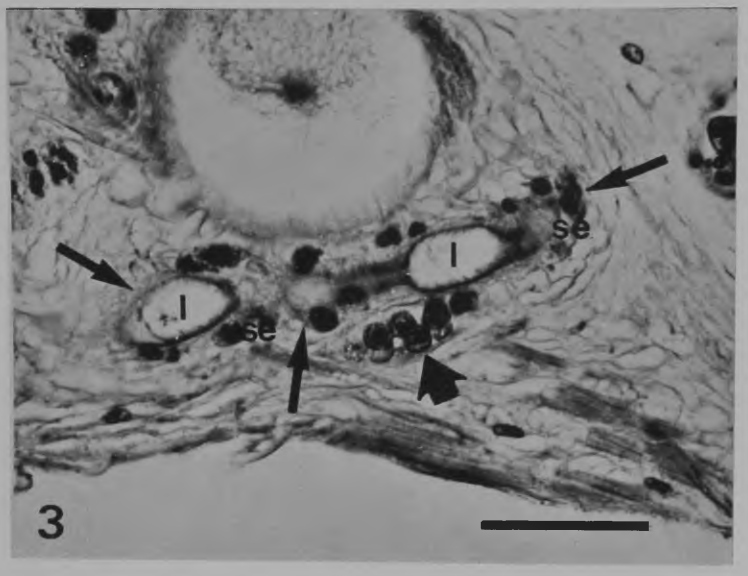
Fig. 3 - Oblique section of Laurer's canal (➡) and vitelline duct. Note the vitelline cells (➡) Tri-PAS. Scale: 38 µm.

Fig. 4 - Ootype with early stage of egg formation (➡). Tri-PAS. Scale: 25 µm.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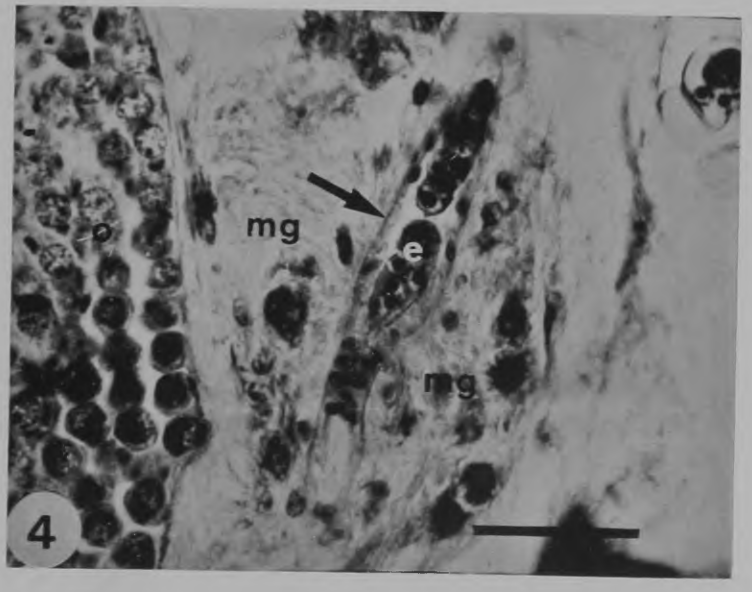
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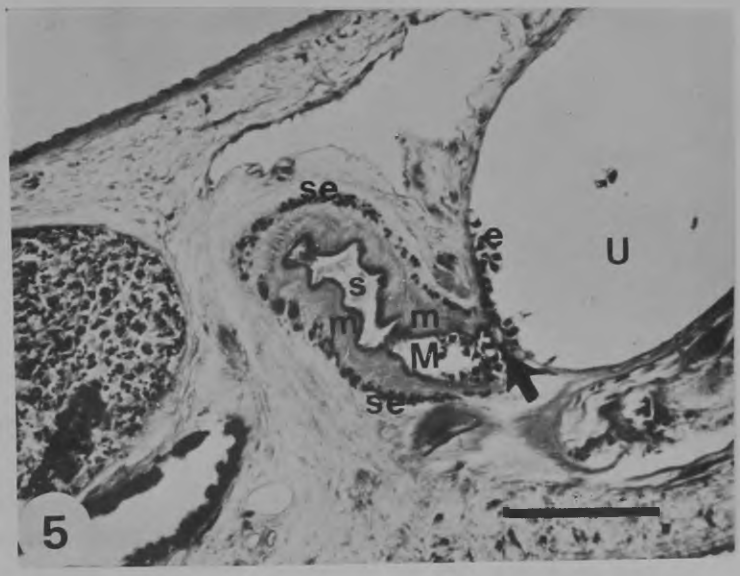
Fig. 5 - Passage from uterus to metraterm (➡). Tri-PAS. Scale: 70 µm.

Fig. 6 - Detail of metraterm. The sclerotic layer of the tectum (➡) and spermatozoa in its lumen can be seen. Tri-PAS. Scale: 6 µm.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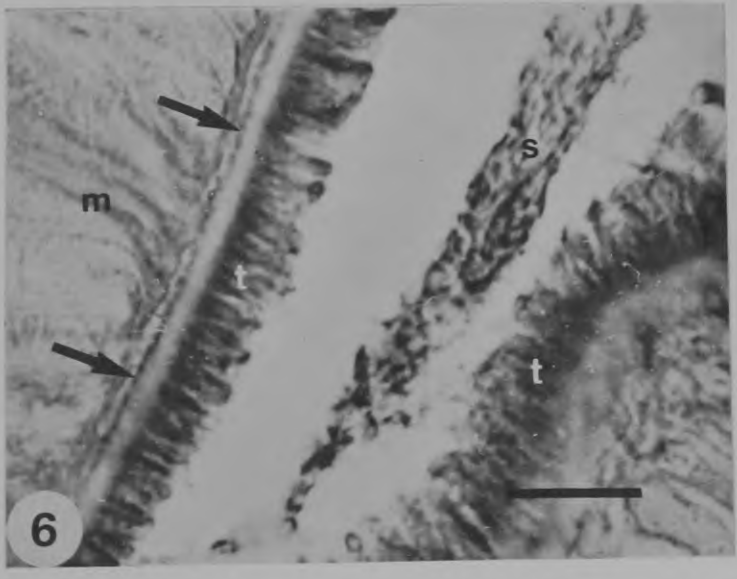
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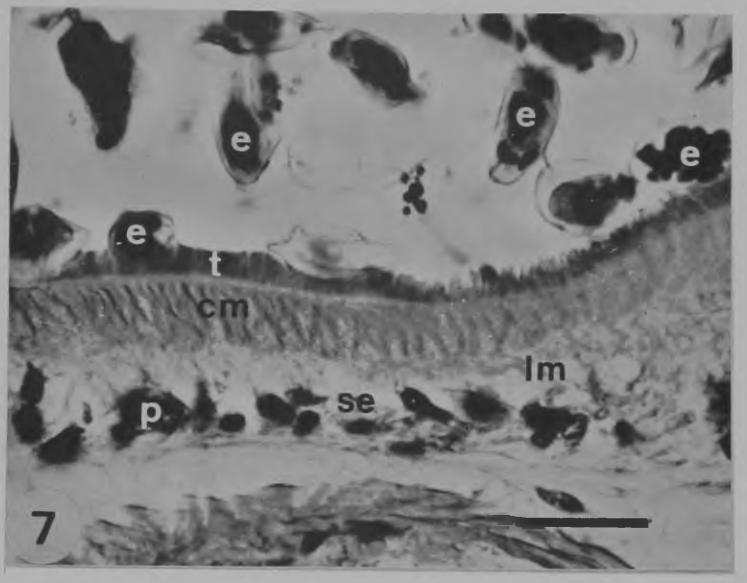
Fig. 7 - Detail of metraterm. Eggs in its lumen can be seen. Tri-PAS. Scale: 20 µm.

Fig. 8 - Oblique section of the cirrus pouch in the pars prostatica. The pycnotic nuclei between the collars of the prostata can be observed. Tri-PAS. Scale : 52 µm.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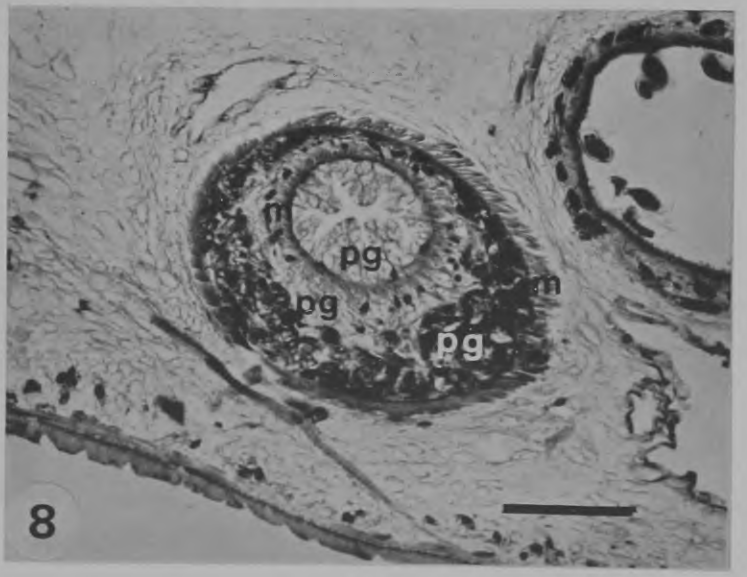
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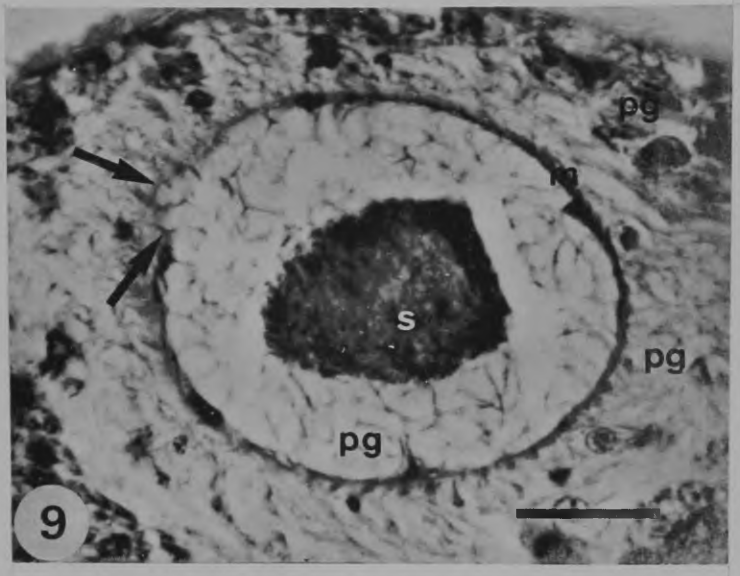
Fig. 9 - Details of the pars prostatica. The pasagge of the prostate glands collars through the wall of the pars prostatica may ben seen (➡) Tri-PAS. Scale: 15 µm.

Fig. 10 - Distal region of the cirrus pouch (➡) The cirrus may be seen folded at rest. Animal tegument (➡) Tri-PAS. Scale: 120 µm.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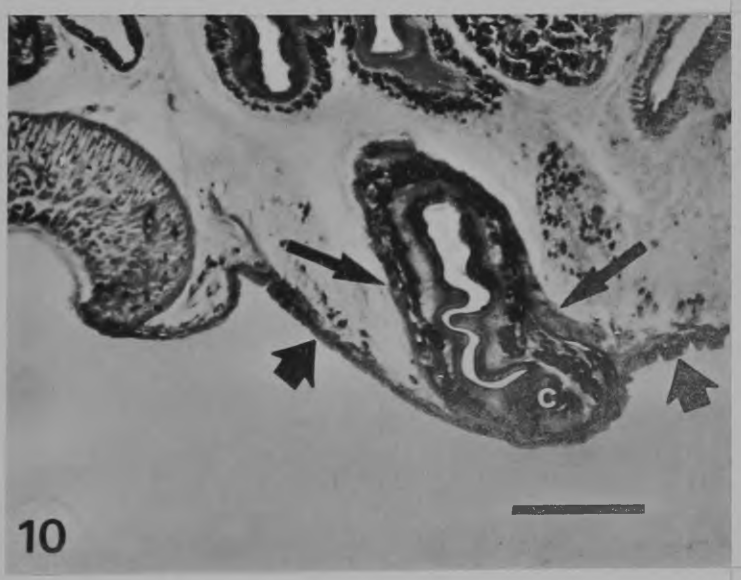
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Fig. 11 - Detail of the cirrus. The three layers of the tectum can be observed. Basal membrane (➡). In its lumen a PAS positive secretion is observed Tri-PAS. Scale: 20 µm.


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