

MORPHOLOGY OF THE LARVAE OF DIPLOPYLIDIUM NOELLERI (SKRJABIN, 1924)

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Abstract. The larva of the cestode *D. noelleri* lies in a capsule consisting of muscle connective tissue fibres. It is localized on the outer side of intestine and mesentery of snakes (*Tarbophis obtusus*). The capsule is filled with a gelatinous matter surrounding the larva. The larva measures 620—720 \times 300—480 μm , its scolex is not invaginated and well developed microtriches are present on its whole surface. The subtegument consists of outer circular layer and inner longitudinal layer of muscle and connective tissue fibres. Beneath the longitudinal layer are pyriform cells forming the tegument and joined to the amorphous substance of the tegument by their anterior narrowed parts. The rostellum is completely developed and bears 3—4 rows of 16—18 hooks each, i.e., 48—72 hooks altogether. Two anastomosing excretory canals run on both sides of body and open into a common opening in the middle of posterior end of body.

The present paper is another of a series of papers dealing with the development of larval stages of cestodes belonging to the families Hymenolepididae Fuhrmann, 1907, Dilepididae Fuhrmann, 1907 and Dipylidiidae (Mola, 1929) (Valkounová and Prokopič 1978, 1979, 1980, 1981). The material was collected by Academician B. Ryšavý during the parasitological expedition in Egypt organized by the Institute of Parasitology, Czechoslovak Academy of Sciences in 1971. I would like to thank him for providing me the larval cestodes for further examination. A report on the finding of larvae of three cestode species belonging to the genus *Diplopylidium* Beddard, 1913 and their descriptions based on whole mounts have already been published by Ryšavý (1973).

MATERIAL AND METHODS

The cestode larvae in capsules were isolated from the snakes *Tarbophis obtusus* caught in the environs of the village Abu Rawash near Giza pyramids. The capsules were localized on the outer side of intestine and on the mesentery. The material was fixed with Baker's neutral formaldehyde, embedded in paraffin, cut in 6 μm thick sections and stained by the following methods: Maeyr's, Böhmer's and Weigert's haematoxylin-eosin, van Gieson's method, Masson's and Goldner's trichrome, Mallory's PTAH method, impregnation method after Gomori, PAS reaction and Kossa's method for the detection of calcium. Detailed descriptions of the methods used were published by Pearse (1968).

RESULTS

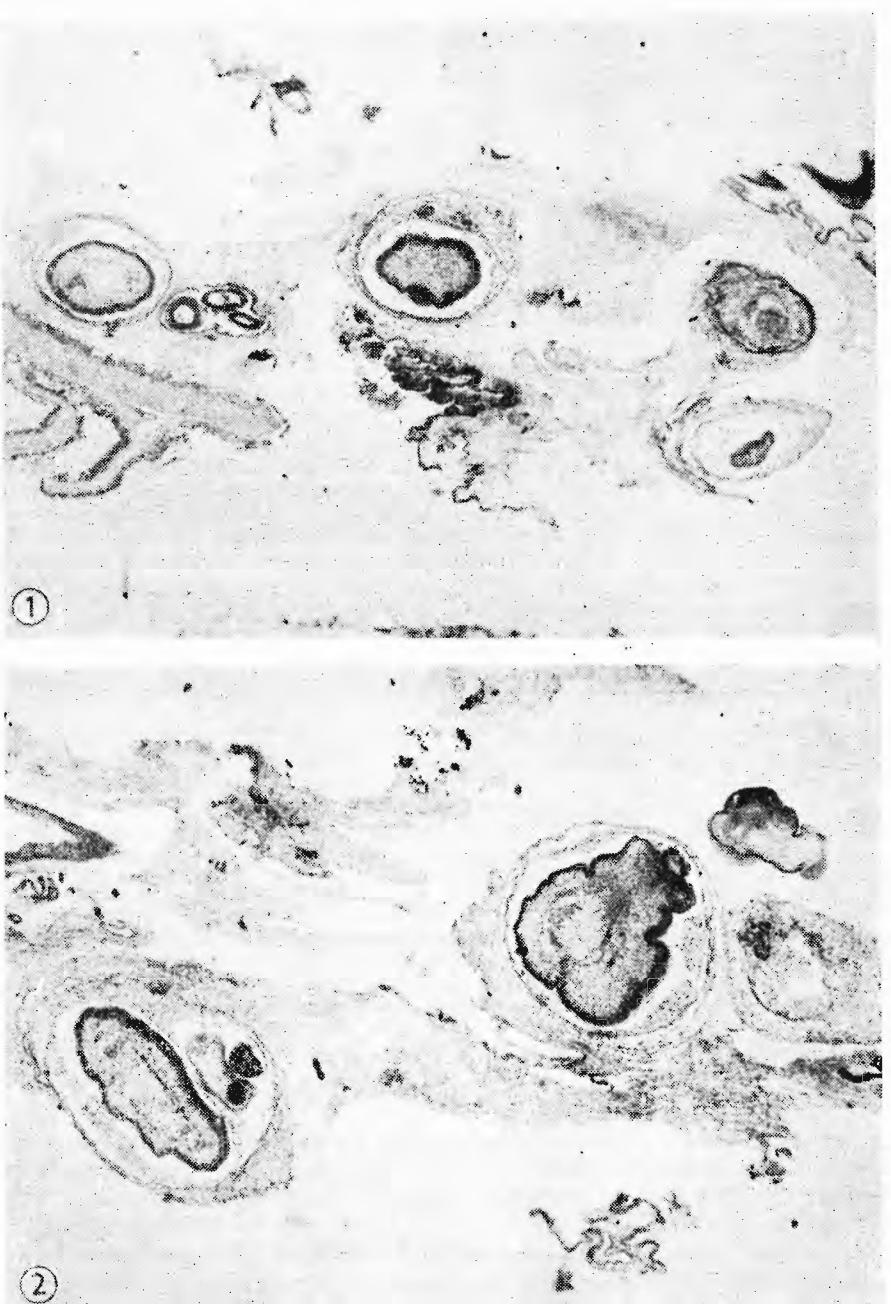
The larva of *D. noelleri* is situated inside a yellow-white sac consisting of muscle and connective tissue fibres. The sac is termed "capsule" and is considered to be a product of the host tissue (Figs. 1, 2). The cyst or its tail-shaped appendage known in cestodes of the family Hymenolepididae (Neradová-Valkounová 1971, Valkounová and Prokopič 1980, 1981) or tail-less cyst occurring in the family Dilepididae (Valkounová and Prokopič 1978, 1979) have not been found. The larva is not invaginated, though it possesses completely developed suckers, rostellum and hooks corresponding in their shape and size to the hooks of adult worms. The larva lies free in the capsule cavity and is not connected with it at any site. Not even a trace of forming cavity in which the scolex could invaginate is visible in the posterior part of larva (Plates I and II).

CAPSULE

The capsule 700—1,320×600—750 μm) is oval, pointed at both poles or egg-shaped (Plate I; Plate II, Fig. 2). The thickness of its wall is 90—250 μm on poles and 24—76 μm on sides. Histological examinations revealed three layers, outer, middle and inner one. The outer layer is 3.5—4.5 μm thick, consisting of outer circular and inner longitudinal layer of muscle and connective tissue fibres arranged regularly in parallel and upright. The circular connective tissue fibres measure 1—1.5 μm , the longitudinal connective tissue fibres 2—3 μm and the muscle fibres (both circular and longitudinal) 1—1.5 μm in thickness. The connective tissue fibres are stained rose with yellow tinge by haematoxylin-eosin, blue by Masson's trichrome, light red by Weigert's and van Gieson's methods, green by Goldner's trichrome and rose by Mallory's PTAH method. The Gomori's impregnation method allows to distinguish in the connective tissue fibres the amorphous substance which is stained deep rose, and fine fibrils situated in it and stained grey-black. The muscle fibres are stained rose with violet tinge by haematoxylin-eosin, red by Masson's trichrome, yellowish by van Gieson's method and light red by Goldner's trichrome. The connective tissue fibres contain numerous nuclei of fibroblasts measuring 5—8×1—1.5 μm and 7.5—11.5×2—2.5 μm , which are stained dark violet by haematoxylin-eosin, Masson's trichrome and van Gieson's method. The muscle fibres consist of muscle cells with light nuclei stained rose by haematoxylin-eosin, Masson's trichrome and van Gieson's method and measuring 2—3×1—1.5 μm . Both muscle and connective tissue fibres in this layer are surrounded by fine argyrophilic fibres stained violet by haematoxylin-eosin and Masson's trichrome, red by PAS and reducing silver on their surface in Gomori's method. The fibres in the outer layer branch and run towards both the host tissue (20—50 μm) and middle layer of the capsule.

The middle layer of the capsule is 24—220 μm thick (24—56 μm on sides and 97 to 220 μm at poles), being always thicker at one pole (e.g., 120 and 190 μm at poles and 24 μm on sides). If the capsule is ovoid, then one layer is thicker at the pointed pole. This layer contains irregularly distributed and 1—3 μm thick muscle and connective tissue fibres with numerous fine argyrophilic fibrils not only on the surface of muscle and connective tissue fibres, but also free-lying ones. Among the fibres are remnants of plasma without distinct cellular borders with spherical to oval nuclei measuring 4—5×5 μm and stained light blue by haematoxylin-eosin, Masson's trichrome and van Gieson's method and rose to red by Gomori's method. This layer contains another two types of nuclei: small and spherical, measuring 2.5—2.7 μm and stained dark blue to violet by haematoxylin-eosin, Masson's trichrome and van Gieson's method and red to red-violet by Gomori's method, and sparsely distributed nuclei of fibroblasts, measuring 11.5×2.5 μm and stained violet by haematoxylin-eosin, Masson's trichrome and van Gieson's method and red by Gomori's method. In longitudinal sections, this layer was found to contain spherical structure of various sizes (Plate III, Fig. 2) measuring 95—114 μm in diameter and bordered with connective tissue fibres with numerous nuclei of fibroblasts. Inside them are accumulated variously twisted muscle (prevailing) and connective tissue fibres and dark violet nuclei (stained by haematoxylin-eosin and Masson's trichrome) measuring 2—3 μm .

The inner layer of the capsule consists of longitudinal muscle and connective tissue fibres branching into the middle layer and numerous nuclei of fibroblasts. The circular layer of fibres was not detected. The inner limiting layer of the capsule not stained by common histological methods was observed in some sections.



Figs. 1—2. Localization of larvae in the host tissue. Fig. 1. Haematoxylin-eosin ($\times 30$). Fig. 2. Masson's trichrome ($\times 45$).

The gelatinous matter (Plate I, Fig. 1; Plate II, Fig. 2) fills the capsule cavity between its inner wall and larva surface. In the sections it is very damaged (shrinking of tissue during fixation) so that it does not form a continuous layer around the larva. However, remnants of this layer adhere to the surface of microtriches. The gelatinous matter is of cellular structure. The cells adhering to the inner layer of capsule are elongated, towards the surface of larva they are wider and of irregular shape. In the section, the cells are most often 4—5-sided, their walls are adjacent so that they conform in shape to one another. The plasma is granular, stained rose by haematoxylin-eosin, light blue by Masson's trichrome, faint rose by van Gieson's method and greyish by Gomori's method. The nuclei of these cells are spherical, measure $4 \times 4 \mu\text{m}$ and they are stained either compactly dark blue by haematoxylin-eosin and Masson's trichrome or they contain large nucleolus of dark blue colour occupying approximately $3/4$ of the nucleus. Some nuclei are larger, spherical ($5-6 \mu\text{m}$) or oval ($4 \times 6 \mu\text{m}$), stained light blue by haematoxylin-eosin and Masson's trichrome, with dispersed chromatin or with a nucleolus occupying about $1/10$ of the nucleus.

LARVA

The larva is mostly situated across the capsule, rarely longitudinally, and is usually coiled in form of a horse-shoe or contracted. However, scolex invagination into posterior part of larva has never been observed. The isolated larva measures $620-720 \mu\text{m}$ in length and $300-480 \mu\text{m}$ in width. The anterior portion of larva is occupied by a spherical scolex measuring $210-240 \mu\text{m}$. The suckers are also spherical to slightly oval and measure $72-86 \mu\text{m}$ in diameter. The rostellum ($70-92 \times 72-89 \mu\text{m}$) is conical and bears 3—4 rows of hooks consisting of 16—18 hooks each (total number of hooks 48—72). The rows of hooks (Plate IV) are arranged in form of a chessboard, i.e., the hooks of the 1st row are below those of the 3rd row and those of the 2nd row are below those of the 4th row. The hooks of the 1st row are the largest and measure 44 and $52 \mu\text{m}$, those of the 2nd row 34—42 μm , of the 3rd row 16—19 μm and of the 4th row (there are only spines and often they lack) 11.5—14 μm . The hooks were drawn in the paper by Ryšavý (1973). The larva is somewhat narrowed behind the scolex (Plate II, Fig. 1), measuring 144 to $228 \mu\text{m}$ in width. The posterior part is the widest, up to $480 \mu\text{m}$. The outer limiting layer is very damaged on the tegument surface and it was observed only in some places due to the active movement of the larva in the capsule. The tegument is 3—5 μm high. The microtriches are distributed on the whole surface of the scolex. They measure 3 μm , only in the region of rostellum their size decreases to 1—2 μm . In the posterior part of larva, the microtriches are longer, reaching up to 4 μm . They are stained rose to red by haematoxylin-eosin, red by Masson's trichrome, yellow and sometimes grey by van Gieson's method and rose-grey or blue-grey by Gomori's method. The amorphous substance of the tegument is 1 μm thick, only on the surface of rostellum where the hooks are formed it reaches 2 μm in thickness. It is stained deep rose by Gomori's method. The basement layer is 1 μm thick and is stained light blue only by Masson's trichrome.

The subtegument consists of the outer circular and inner longitudinal layer of connective tissue and muscle fibres, which are regularly arranged and 2—3 μm thick. The connective tissue fibres alternate with the muscle fibres and are surrounded by fine argyrophilic fibrils. Both the muscle and connective tissue fibres are approximately of the same thickness and the spaces between them are 1—4 μm according to the wider dilatation of fibres. The pyriform cells forming the tegument are situated by their

portion containing the nucleus beneath the muscle and connective tissue layer and by their stem-shaped necks they penetrate among the fibres of the subtegument and open into the amorphous substance of the tegument. They measure $4-8 \times 2-3 \mu\text{m}$ and contain a light nucleus measuring $2-2.5 \times 1.5 \mu\text{m}$. The connective tissue and muscle fibres are stained in the same way as those in the capsule. The parenchyma is divided into two portions. The outer one is densely filled with cells with spherical nuclei measuring 2—3 μm and rich in chromatin. The plasma of these cells contains eosinophilic granules. The nuclei are placed under the subtegument in two rows. They are particularly accumulated beneath the suckers and rostellum. Most probably these are the nuclei of embryonal cells from which the rostellum and suckers are formed and which are pressed during their growing to the middle portion of the scolex. There are numerous calcareous bodies among the parenchyma cells. They measure up to $20 \mu\text{m}$ and are stained brown-black by Kossa's method (Plate II, Fig. 2). The inner portion of the parenchyma is separated from the outer one by bundles of longitudinal muscle fibres among which are numerous argyrophilic fibres. In this part of larva are two longitudinal excretory canals, the lumen of which measures 2—4 μm (Plate II, Fig. 1; Plate III, Fig. 1). They run from the anterior part of scolex (beginning at the level of suckers) to its posterior part. There they unite in a common opening (lumen $7-8 \mu\text{m}$) situated in the middle of posterior end of larva. A network of fine canals (lumen about 1 μm) opens into the main excretory canals. The lumen of the final portion of the excretory system is formed by the walls of large cells ($11.5 \times 5 \mu\text{m}$) with indistinct nuclei and light plasma. These cells are surrounded by both fine argyrophilic fibrils and thicker connective tissue fibres. Outside this fibrous envelope are radially arranged cells which become larger in the direction from the excretory canal. They possess dark nuclei measuring 2—3.5 μm , with a conspicuous nucleolus of about 0.5 μm . The inner part of the parenchyma filling the middle of the larva consists of sparsely distributed cells connected with one another by plasmatic processes. Their nuclei measure also 2—3.5 μm , but they are lighter and their plasma contains numerous eosinophilic granules. The calcareous bodies are sparse in this part of larva.

DISCUSSION

During the studies of the morphology of *D. noelleri* larva we came across the problem the final solution of which will require histological studies of younger stages of larval development of this or a relative species. On the basis of the material available, i.e., morphologically already fully formed larva, it cannot be demonstrated whether the larva of this species is of a cysticercoid type, as it is reported in the literature. It is not evident from the data obtained whether the sac, in which the larva is situated, is a capsule (formed from the host organism) or a cyst, i.e., posterior part of body of developing larva of cysticercoid type, where a cavity is formed into which invaginates morphologically fully formed scolex in the final stage of larval development (Neradová-Valkounová 1971). Matevosyan (1963) published a survey of the larval development of this species after Joyeux (1923), Skryabin (1924), López-Neyra (1928) and Wittenberg (1932), who place the larva to the cysticercoid type on the basis of studies of whole mounts. The most detailed description of the development of larva was presented by Wittenberg (1932), but this author did not solve the above problem.

Joyeux (1923) and Skryabin (1924) described a spherical or oval cyst measuring about 1 mm and a free-swimming cysticercoid with invaginated scolex inside. López-Neyra (1928) and Wittenberg (1932) described a spherical cyst measuring up to 5 mm in diameter. It contained up to 13 cysticercoids not connected with the cyst wall but swimming freely in a transparent fluid. Each cysticercoid was oval, measured 0.5—1 mm

and possessed an invaginated scolex with fully developed hooks. The authors described also two anastomosing excretory canals beginning in the scolex region and uniting in a common opening in the posterior part of cysticercoid. This description corresponds almost entirely to our results except the fact that neither in our material nor in that studied by Ryšavý (1973) the sacs with more than one scolex were observed.

From the viewpoint of morphogenesis it is necessary to elucidate of what origin is the sac in the cavity of which lies the larva (according to the above-cited authors the larva was invaginated, consequently a cysticercoid was involved). The site of scolex invagination into the cyst cavity at one of the poles of the larva is typical and occurs also in *Hymenolepis erinacei* (Gmelin, 1789) (Valkounová and Prokopič 1980, 1981). At this site the scolex again evaginates after the digestion of the cysticercoid in the body of the definitive host (Valkounová 1979) or after a mechanical press on the cyst. The capsule is an envelope formed from the host tissue which mostly remains connected with the host tissue after damage. Inside the capsule swims the cysticercoid surrounded by a dense fluid, i.e., a cyst with invaginated scolex as in the member of the family Dilepididae, the species *Rodentotaenia crassiscolex* (Linstow, 1890) (Valkounová and Prokopič 1978, 1979).

This problem cannot be solved on the basis of the material available. The sac in which lies the larva is termed by us "capsule" and not "cyst" for the following reasons: 1) after damage its outer layer remains connected with the host tissue, 2) we have not found any site where scolex invagination could occur, 3) when the capsule is pressed, it breaks in the thinnest place, i.e., in the lateral part and not like the cyst, 4) no calcareous bodies commonly occurring in the cyst have been found in the capsule. The larva inside the capsule is not termed "cysticercoid", as there was no layer corresponding in its structure to the cyst wall as described by Voge (1960), Ubelaker et al. (1970), Baron (1971) or Valkounová and Prokopič (1978, 1980), and no scolex was invaginated in the posterior part of larva. If we admit that the scoleces may be again evaginated after the invagination, e.g., due to ageing of the larva, the structure of the posterior part of larva does not correspond to that of the cyst wall (Valkounová and Prokopič 1978, 1979, 1980, 1981). For example, the microtriches are on the whole surface of the larva and there is no cavity in its posterior part, but it is filled with the parenchyma. Also the excretory canals uniting in the posterior part of larva in a common opening are not typical of the cyst.

Since no younger stages of larvae were available for a comparison, it can be only supposed that only the outer layer of the capsule described by us might be the capsule and the middle and inner layer of the capsule might be the cyst which was changed, e.g., by a long stay in the host body. It is also possible that the cyst was not formed and the scolex was invaginated into the posterior part of larva containing no cavity, but only a thin parenchyma. This would explain the formation of the matter surrounding the larva inside the capsule, as it is common, e.g., in *Rodentotaenia crassiscolex*. There are no literary data demonstrating that the snakes are intermediate hosts and not, e.g., reservoir hosts of the cestodes. The papers dealing with the evolution of cestodes (e.g. Freeman 1973) should be referred to in this respect. This problem will be the subject of some of our further papers.

МОРФОЛОГИЯ ЛИЧИНОК *DIPLOPYLIDIUM NOELLERI* (SKRYABIN, 1924)

Й. Валкоунова

Резюме. Личинка цестоды *D. noelleri* помещена в капсуле, состоящей из фибрill мышц и соединительной ткани и локализированной на внешней стороне кишечника и ме зентерия пресмыкающихся вида *Tarbophis obtusus*. Полость капсулы выполняет студен образное

вещество, окружающее личинку. Размер личинки 620—720×300—480 мкм. У личинки нет инвагинированного сколекса и по всей её поверхности имеются хорошо развитые микротрихи. Субтегумент состоит из внешнего пиркулярного и внутреннего продольного слоев мышечных фибрill и фибрill соединительной ткани. Под продольным слоем расположены грушевидные клетки, образующие тегумент, которые своей передней, суженной частью соединяются с основным веществом тегумента. Хоботок вполне развит и несёт 3—4 ряда крючьев. В каждом ряду 16—18 крючьев, т. е. в общем 48—72 крючьев. На обеих сторонах тела тянутся два анастомозирующих экскреторных канала, входящие в совместный проток в центре заднего конца личинки.

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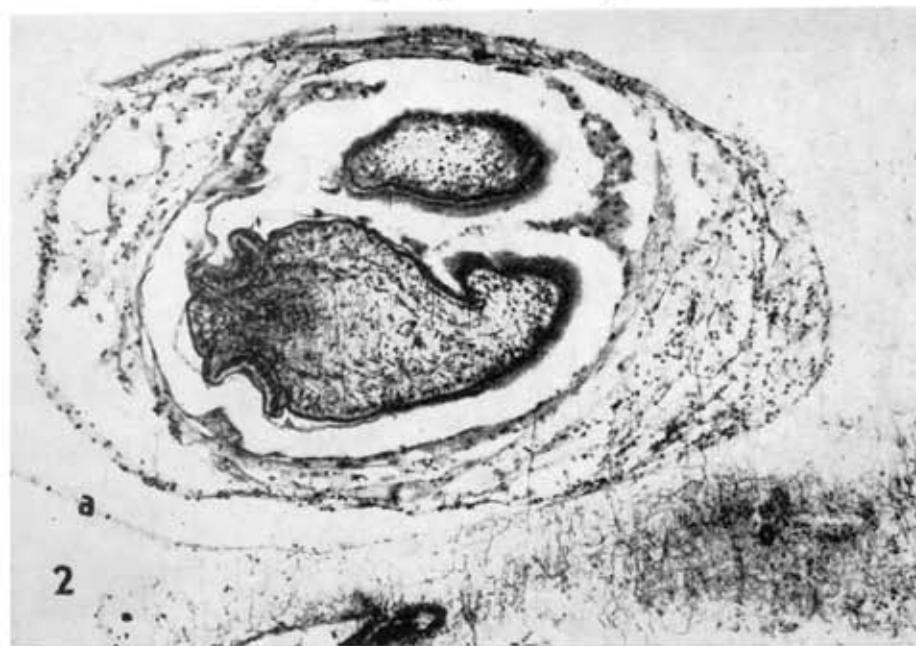
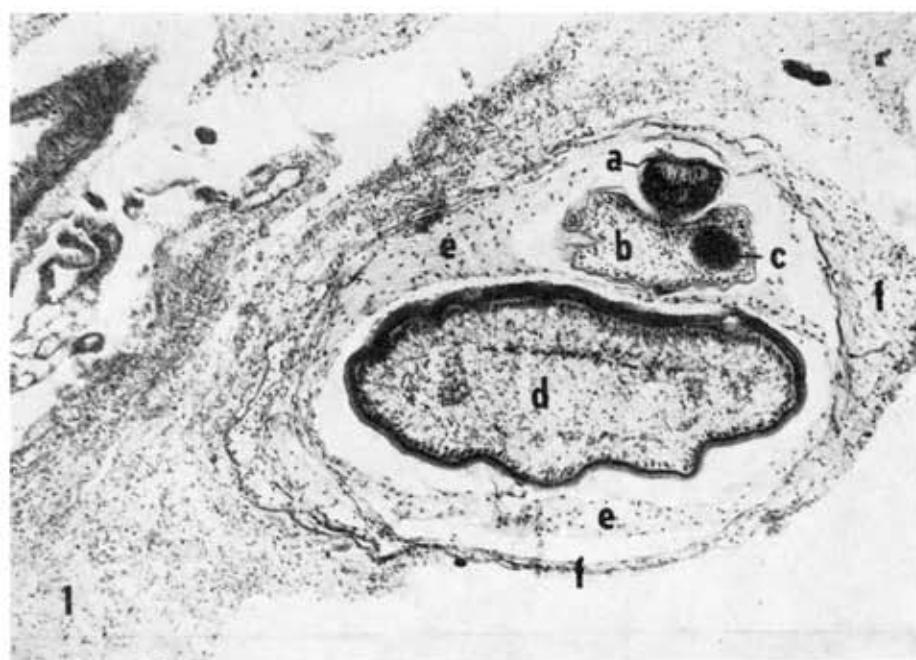


Fig. 1. Longitudinal section through the capsule. Larva with developed rostellum and hooks (a), scolex (b), and suckers (c); posterior part of larva (d), gellatinous matter (e), capsule (f). Masson's trichrome ($\times 130$). Fig. 2. Longitudinal section through the capsule with its separated upper layer (a) joined to the host tissue. Gomori ($\times 140$).

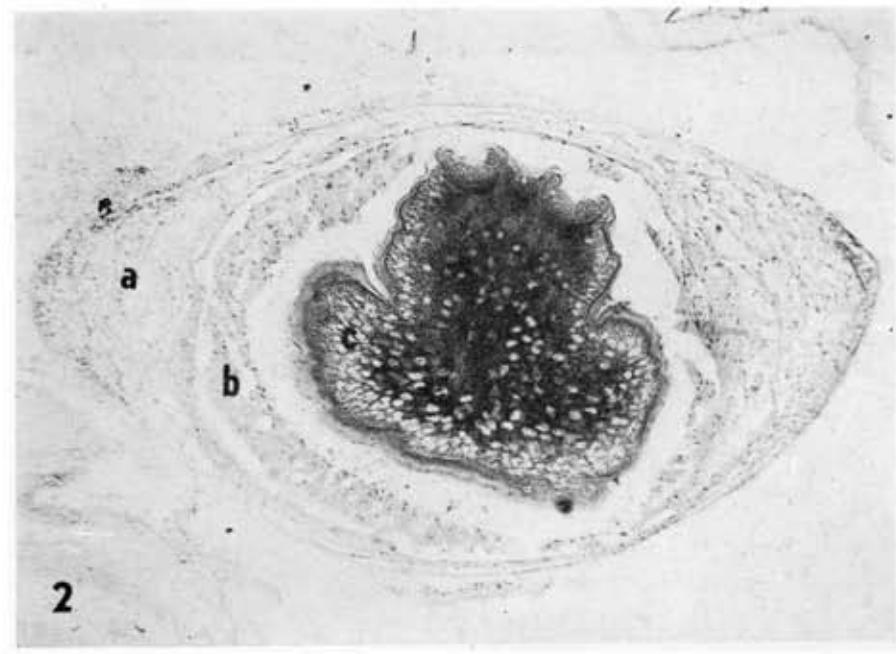
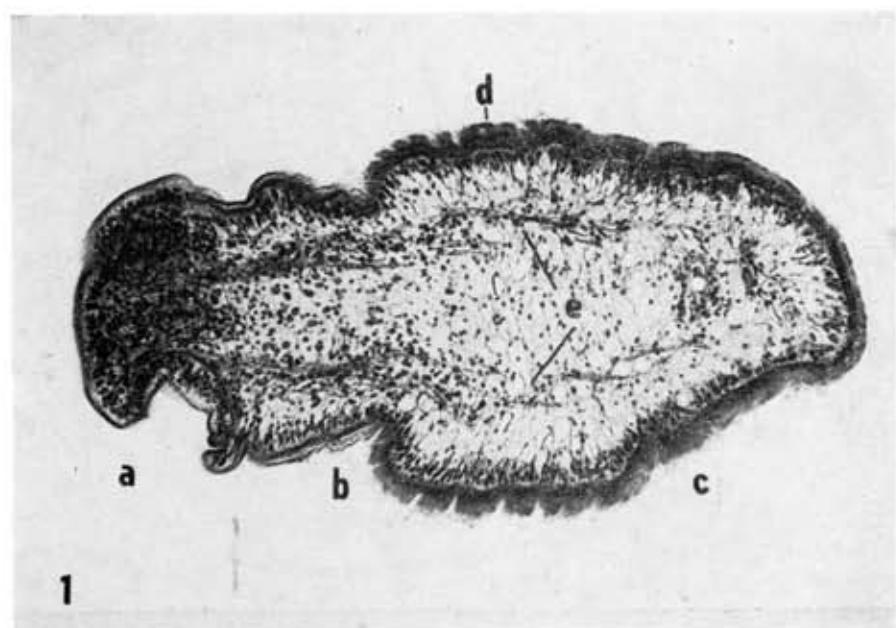


Fig. 1. Larva released from the capsule. Scolex (a), narrowed part (b), posterior part of larva (c), oblique section through the tegument with conspicuous microtriches (d), excretory canals (e). Masson's trichrome ($\times 170$). Fig. 2. Longitudinal section through the capsule (a), gellatinous matter (b) and larva (c) containing a large number of calcareous bodies. PAS ($\times 100$).

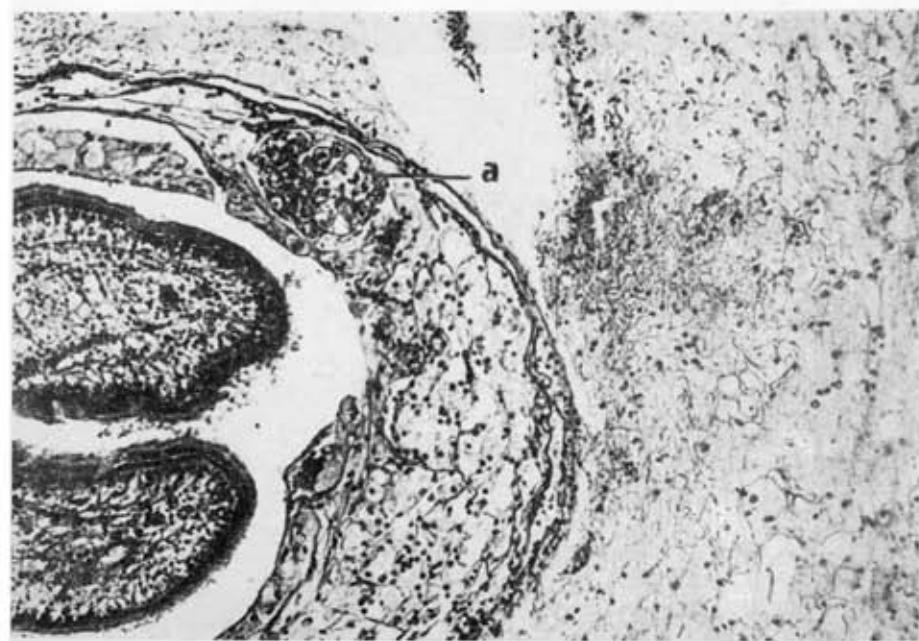
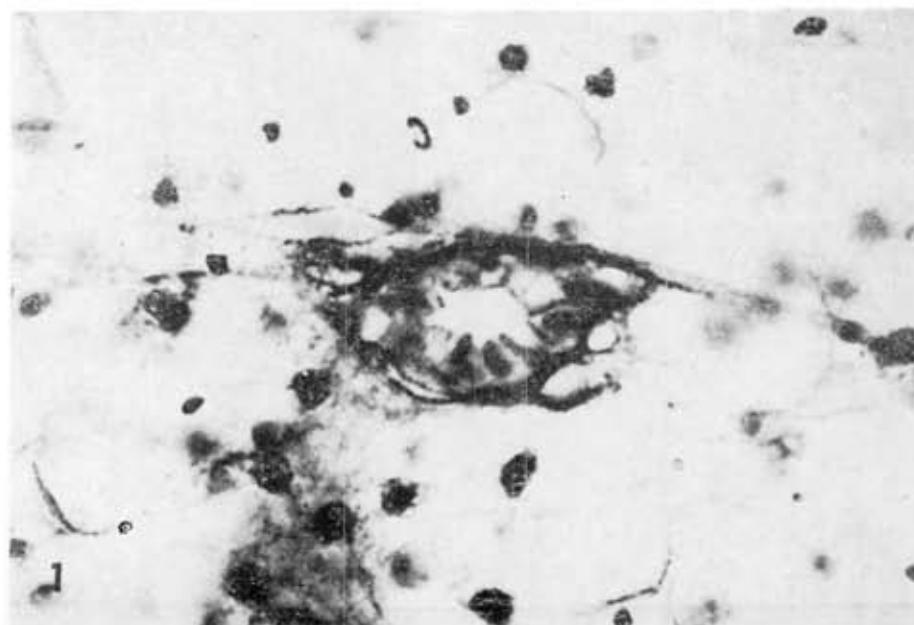


Fig. 1. Section through the excretory canal the lumen of which is bordered by cells with large nuclei rich in chromatin and with connective tissue fibres and numerous fine argyrophilic fibrils. Gomori ($\times 1300$). Fig. 2. Longitudinal section through the capsule with spherical structure (a). Masson's trichrome ($\times 75$).

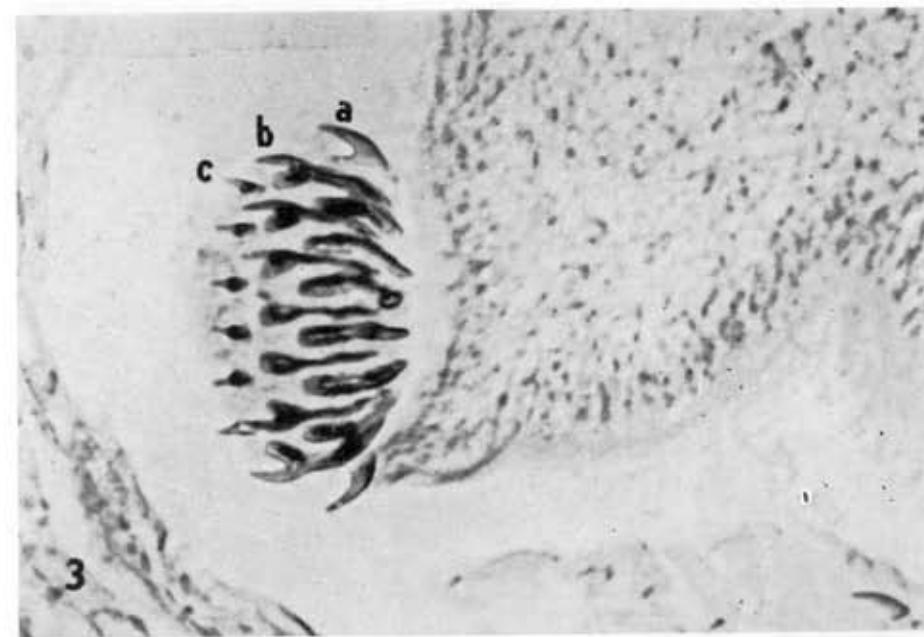
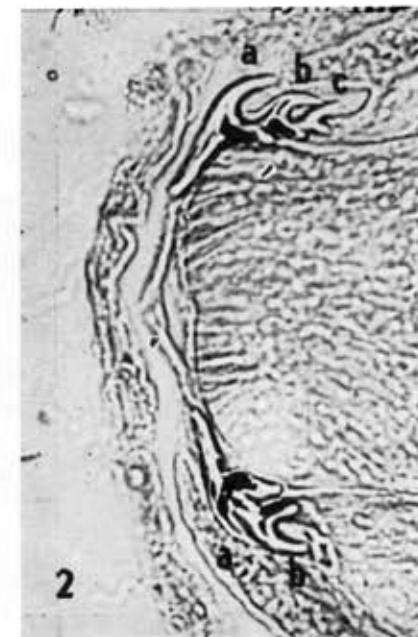


Fig. 1. Longitudinal section through the rostellum. Hook of 1st row (a), hook of 2nd row (b), hook of 3rd row (c). Gomori ($\times 430$). Fig. 2. Same as in Fig. 1. PAS ($\times 430$). Fig. 3. Tangential section through the rostellum showing hooks arranged in a chessboard form. Hooks of 1st row (a), hooks of 2nd row (b) and hooks of 3rd row (c). AB pH 2.6 ($\times 380$).