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MORPHOLOGY OF THE CYSTICERCOID OF THE TAPEWORM CORONACANTHUS INTEGER (HAMANN, 1891) (HYMENOLEPIDIDAE)

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Abstract. Histological methods were first used for the studies on the morphology of cysticercoid of *Coronacanthus integer* recovered from body cavity of *Gammarus* (*Rivulogammarus*) *fossarum*. The cysticercoid is lens-shaped, evidently adapted to the host body which is flattened from sides. The species is characterized by the presence of 65—75 hooks measuring 12—15 μm in length. The outer part of the cyst tegument contains 2—18 μm long microvilli. Calcareous bodies were not found in the cyst wall, but only in the parenchyma of neck and scolex. Descriptions and measurements of individual layers of the cysticercoid are given.

The cysticercoid of *C. integer* was first described by Hamann (1891) under the name *Taenia integra*. Joyeux and Baer (1952), Spassky (1954), Prokopič and Groschaft (1961) and Prokopič et al. (1970) assigned this cysticercoid to the cestode species parasitic in *Neomys* in the Palaearctic subregion. Cysticercoids from *Gammarus* (*Rivulogammarus*) *pulex*, *G.* (*R.*) *fossarum* and *G.* (*R.*) *komareki* were described by Prokopič and Groschaft (1961) and Prokopič (1972). Adult specimens of *C. integer* were found by Prokopič (1972) in *Neomys fodiens*, *N. anomalus* and *Sorex araneus*. The present paper deals with the morphology of cysticercoid as revealed by histological methods. The results will serve for a comparative study of cysticercoids of cestodes belonging to the families Hymenolepididae Fuhrmann, 1907 and Diploposthidae Poche, 1926 developing in crustaceans. This group of cestodes includes specimens infecting economically important water fowl bred in large-scale breeding farms.

MATERIAL AND METHODS

The cysticercoids were recovered from the body cavity of *Gammarus* (*Rivulogammarus*) *fossarum* Koch, 1835 collected in the vicinity of Pyšely, Kamenný Újezd, Písek and Vimperk in the years 1979—1981. The cysticercoids were fixed in neutral formaldehyde (Pearse 1968) and 4% formaldehyde, embedded in paraffin and cut to 6 μm sections. The following histological methods were used for staining: Meyer's and Weigert's haematoxylin-eosin, van Gieson's method, Masson's and Goldner's trichrome, Mallory's PTAH method, aldehyde fuchsin, Gomori's impregnation method, PAS reaction and Kóssa's method for the detection of calcium (for the description of the methods see Pearse 1968).

RESULTS

The cysticercoid is lens-shaped and its cyst wall, cyst cavity and scolex are adapted to this shape. The parasite is evidently adapted to the host body which is also flattened from sides. The cysticercoid consists of a tailed cyst, the cavity of which contains the neck and scolex with invaginated rostellum. The scolex is usually surrounded by a neck which coalesces with the cyst at the site of invagination. In older cysticercoids, the neck is separated from the cyst wall so that it does not form a layer around the scolex, but it is contracted and coiled in the cyst cavity.

CYST

The cyst has a characteristic shape of a lens. In longitudinal section through the wider area (Plate I, Fig. 1), it is almost spherical (564—649 × 592—667 μm), in longitudinal section through the narrower area (Plate I, Fig. 2) it is elliptical (564—649 × 272—329 μm). The cyst wall in the wider portion measures 141—188 μm and gradually narrows to 23—19 μm. The cyst cavity measures 254—273 × 291 to 329 μm. The tail is 310 μm long and 108 μm wide. It is often torn off for the greater part (Plate I, Fig. 1). The outer limiting layer on the cyst surface is 1 μm thick, refractile, often damaged or absent. It stains only a faint pink colour by PAS reaction. The tegument measures 14.1—19 μm in the wider portion of cyst, gradually becoming thinner up to 9.4 μm, 4.7 μm and 2 μm in the narrow portion. The amorphous substance of tegument in the whole cyst is 1 μm high and structure-less in the light microscope. The microvilli on the tegument surface are finger-shaped and of various lengths. The longest ones (18 μm) are in the wider portion of cyst (Plate II, Fig. 1; Plate III, Fig. 2), and their length gradually decreases towards the narrow portion up to 1 μm (Plate I, Fig. 2). The microvilli stain pink by haematoxylin-eosin, yellow by van Gieson's method, red by Masson's and Goldner's trichrome, dark blue by aldehyde fuchsin and black by Gomori's method. The amorphous substance of tegument stains faint pink by haematoxylin-eosin and Masson's and Goldner's trichrome and deep pink by Gomori's method. The tail tegument is 1 μm high and stains similarly as the inner portion of cyst tegument. The basement layer measures 1 μm and stains faint grey only by Gomori's method.

The subtegument, like the tegument, is of different heights in different portions of the cyst. In the wider portion, it is 122—170 μm high and gradually lowers to 14.8—19.5 μm in the narrow portion (Plate I, Fig. 2). The outer part of subtegument consists of a circular and under it a longitudinal muscle and connective tissue layer. The connective tissue fibres (Plate III, Fig. 2) are 2—3 μm thick and stain pink by haematoxylin eosin, van Gieson's method and Mallory's PTAH method, blue by Masson's trichrome and green by Goldner's trichrome. Muscle fibres are arranged in parallel with connective tissue fibres and are 1—2 μm thick. They stain red by haematoxylin-eosin, dark red by Masson's and Goldner's trichrome, yellow by van Gieson's method, pink by PAS method and blue-grey by Gomori's method. The muscle and connective tissue fibres are covered with 1 μm thick fine connective tissue fibres staining brown-black by Gomori's method and red by PAS method. The connective tissue and to some extent also the muscle fibres in the outer part of subtegument are branched so that they, together with the fine argyrophilic fibres, form a dense network in the middle part of subtegument (Plate IV, Figs. 1, 2). Tegument-forming cells and numerous fibroblasts or their nuclei are situated there. The tegument-forming cells are pyriform and they are arranged in a row under the longitudinal layer of muscles and connective tissue (Plate II, Fig. 1). It was demonstrated in some sections that they are coalescing by their narrow anterior portion with the amorphous substance of tegument. These pyriform cells measure 18—27 × 12—18 μm, their nuclei 3—5 μm and nucleoli 2 μm. The plasma is strongly granulated, the nucleus is situated excentrically in the posterior part of cells. The pyriform cells stain like the tegument blue by Mallory's PTAH method. In older cysts, these cells are sac-shaped, measuring 20—24 × 14—18 μm, with a non-granulated plasma and pycnotic nuclei staining blue-black by haematoxylin-eosin and Gomori's method. Spherical to elliptical nuclei, measuring 3—4 μm and with a conspicuous nucleolus, were visible below the pyriform cells. The plasma situated around these nuclei was not distinctly demarcated. Numerous spindle-shaped nuclei of fibroblasts were found among the connective

tissue fibres. They measured 4—8 × 1—2 μm and contained a small nucleolus measuring 1 μm in diameter. The nucleus contained dispersed chromatin. In the inner portion of subtegument, connective tissue fibres and some muscle fibres are arranged longitudinally in a 4—9 μm thick layer. Calcareous bodies were not found in the cyst wall. The tail subtegument consists of a net of connective tissue and muscle fibres, the connective tissue fibres being more numerous. A plasmatic reticulum with nuclei is situated among the fibres. The pyriform cells and fibroblast nuclei occur only sporadically. The inner limiting layer of cyst was not demonstrated.

NECK

The neck forms another 10—20 μm thick layer freely adhering to the inner wall of cyst and coalesced with it only at the site of invagination. It surrounds the scolex in such a manner that the neck tegument is turned to the scolex tegument. This position is enabled by the cavity formed in the neck parenchyma at the phase of differentiated larva before invagination (Neradová-Valkounová 1971). Since both the scolex and neck are movable, the neck is gradually torn off the cyst wall (Plate II, Fig. 2; Plate I, Fig. 1) so that in older cysticercoids the neck is contracted and coiled in the posterior part of cyst cavity (Plate II, Fig. 1). The neck is usually up to 404 μm long, 38—141 μm wide and regularly tapers behind the scolex (Plate I, Fig. 1).

The outer limiting layer of the neck is 1 μm thick. The neck tegument immediately behind the scolex is 2 μm thick and may be differentiated into a 1 μm thick amorphous substance and 1 μm long microtriches. In the parts more distant from the scolex, the microtriches become shorter so that finally they are invisible in the light microscope and the tegument is only 1 μm high. The basement layer is also 1 μm thick. The circularly and longitudinally arranged muscle and connective tissue fibres (2—3 μm in diameter) form a 4—5 μm thick layer. The tegument, basement layer and muscle and connective tissue fibres of the subtegument stain in the same manner as in the cyst wall. The arrangement of neck parenchyma is the same as in adult cestodes and contains sparse calcareous bodies measuring 2—3 μm in diameter and staining brown-black by Kossa's method for the detection of calcium.

SCOLEX

The scolex is 112—141 μm long. In longitudinal section through its wider area it is 141—160 μm wide and in longitudinal section through the narrower area it is 103—132 μm wide. The suckers measure 28—38 μm in diameter. The rostellum is always invaginated, 65—85 μm long, 85—104 μm wide in longer axis and 47—65 μm wide in shorter axis. It bears 65—75 hooks measuring 12—15 μm in length (Plate III, Fig. 1).

The outer limiting layer of scolex is 1 μm high. The tegument consists of outer layer of microtriches (1—2 μm long) and inner layer of amorphous substance (1 μm thick). The microtriches on the rostellum and suckers are 2 μm long, in the distal portion of scolex they gradually become shorter, reaching only 1 μm. The basement layer is 1 μm thick. The layer of muscles and connective tissue of the subtegument is 3 μm thick and consists of outer circular and inner longitudinal fibres measuring 1—2 μm in diameter. The tegument-forming cells are sparse; they are sac-shaped and measure 8—10 μm. Their nuclei measure 3 μm and nucleoli 1 μm in diameter. Under the subtegument is the parenchyma which does not differ from that of adult cestodes. It contains calcareous bodies measuring 2—3 μm. All above-mentioned layers stain in the same manner as in the cyst wall.

DISCUSSION

The shape of cysticercoids of *C. integer* is very variable according to their age. After invagination of scolex and neck into the cyst cavity the cysticercoids are oval, the subtegument is almost evenly thick in all parts of the cyst and the maximum length of microvilli is 2 μm. During 5—20 days the middle portion of cyst subtegument grows in such a way that the cysticercoid becomes lens-shaped and the microvilli differentiate in their length (in our case from 2 to 18 μm).

Some of the cysticercoids developing in *Gammarus* possess typical, relatively long microvilli. They were already noted by Mrázek (1891) in the cysticercoid of *Triodontolepis hamanni* (Mrázek, 1891) who termed them "a dense garb of undulated cilia". In previously studied cysticercoids of *Rodentotaenia crassiscolex* (Linstow, 1890), *Hymenolepis erinacei* (Gmelin, 1790) and *Diplopylidium noelleri* (Skrjabin, 1924) (Valkounová and Prokopič 1978, 1980, Valkounová 1982a), the microvilli were not detected in the light microscope. Ubelaker et al. (1970) and Ubelaker (1980), who used an electron microscope, described microvilli in the outer part of cyst tegument of *Hymenolepis diminuta* (Rudolphi, 1819) and Baron (1971) in the cyst tegument of *Raillietina cesticillus* (Molin, 1858). In the larvae of cysticercoid type, the microvilli are typical of the cyst tegument and microtriches of the tegument of scolex and neck. Schramlová and Blažek (1981) in their studies on bladder tegument of *Cysticercus bovis* described microvilli at early stages of development, but the microtriches differentiated only at the time of scolex formation.

The subtegument of *C. integer* cyst did not contain any calcareous bodies, whereas in the previously studied cysticercoid of *R. crassiscolex*, *H. erinacei* and *D. noelleri* (Valkounová and Prokopič 1978, 1980, 1981, Valkounová 1982a,b), calcareous bodies were always demonstrated in the cyst subtegument. They were found also in cysticercoids developing in Copepoda and Ostracoda (Neradová-Valkounová 1971, Valkounová 1972a,b,c). Mrázek (1891) described calcareous bodies in the cyst of *Triodontolepis hamanni* and Prokopič (1972) in the cyst of *T. sumavensis* Prokopič, 1957 from *Gammarus*.

It is characteristic of older cysticercoids of *C. integer* that the neck is separated from the cyst wall at the site of invagination. Prokopič (1972) observed specimens which possessed an outline of segmentation on the separated neck, like strobilocercus of *Hydatigera taeniaeformis* (Batsch, 1786), which seems to be due to a longer stay of cysticercoids in the body cavity of host.

Note. In this paper we use the correct name *C. integer* instead of *C. integrus*.

МОРФОЛОГИЯ ЦИСТИЦЕРКОИДОВ ЦЕСТОДЫ *CORONACANTHUS INTEGER* (HAMANN, 1891) (HYMENOLEPIDIDAE)

Й. Валкоунова

Резюме. При помощи гистологических методов впервые изучена морфология цистицеркоидов цестоды *Coronacanthus integer*, выделенной из полости тела *Gammarus* (*Rivulogammarus*) *fossarum*. Цистицеркоиды имеют чечевицеобразную форму, по-видимому они приспособлены телу хозяина, которое также сплющено с обеих сторон. Для этого вида характерно присутствие 65—75 крючьев, длиной в 15 μм. Во внешней части тегумента цисты обнаружены микроворсинки длиной в 2—18 μм. Известковые тельца отсутствуют в стенке цисты, они встречаются только в паренхиме шейки и сколекса. Приведены описания и размеры отдельных слоев цистицеркоида.

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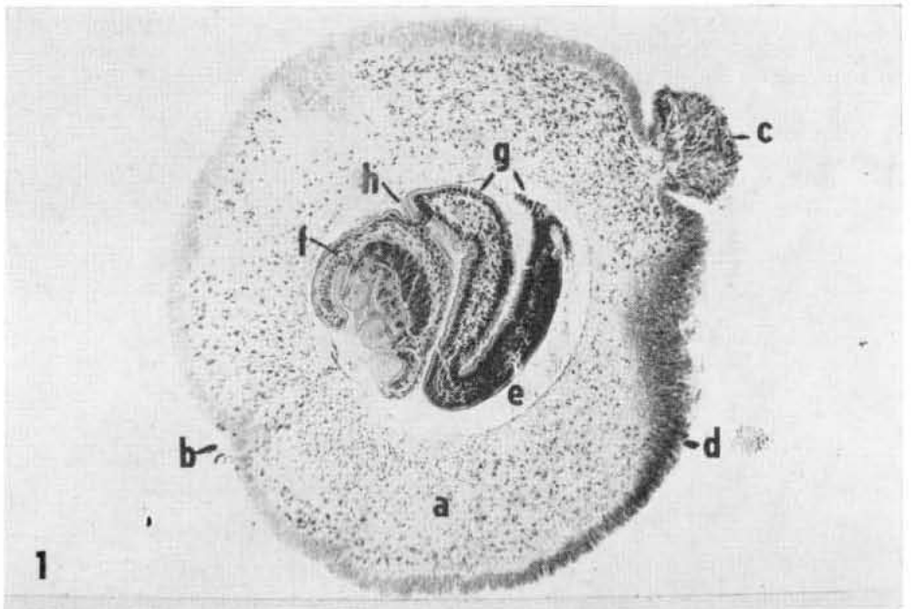


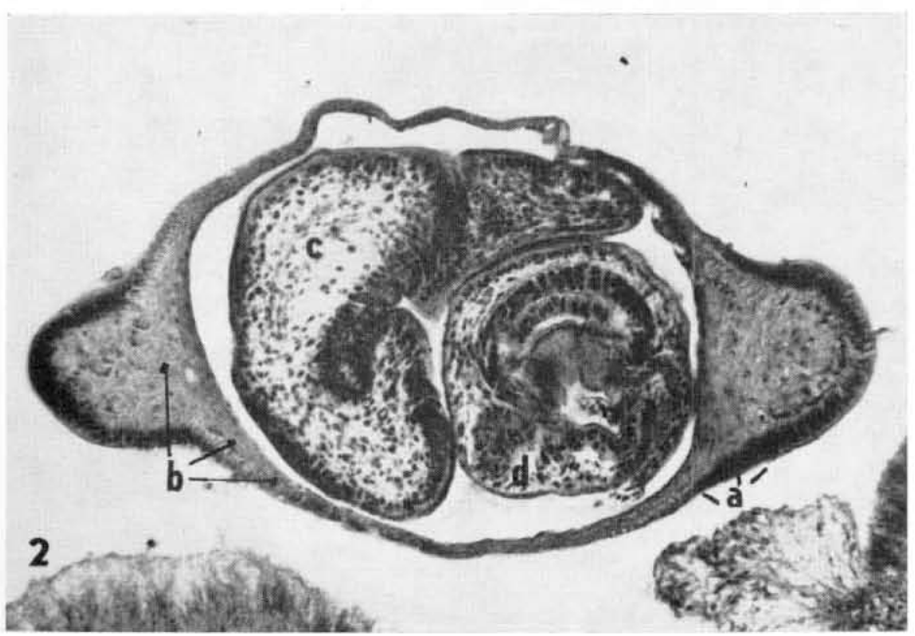
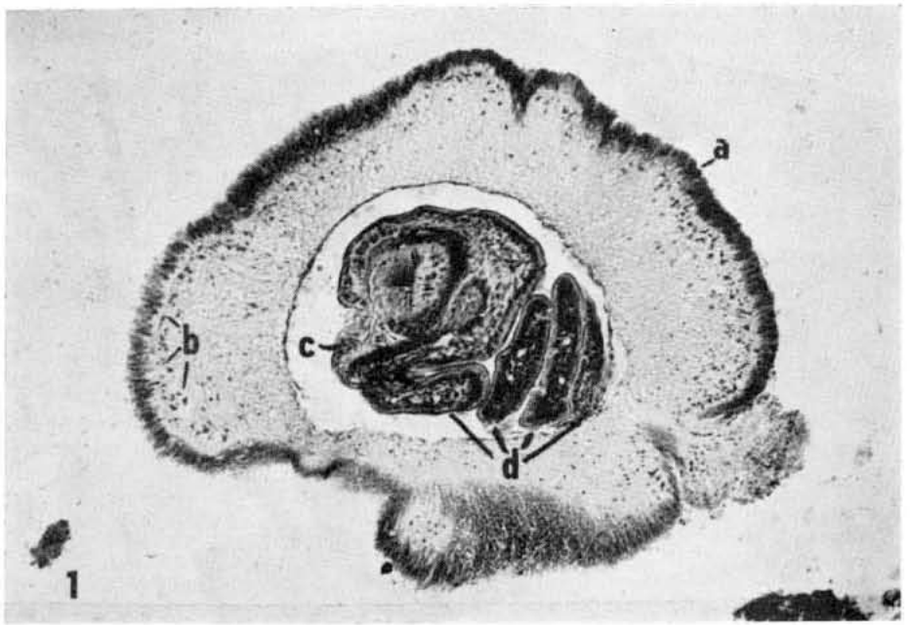


Fig. 1. Longitudinal section through wider area of cyst. Cyst (a), site of invagination (b), tail (c), microvilli (d), cyst cavity (e), scolex (f), neck separated from the cyst wall (g), neck narrowed behind the scolex (h). Haematoxylin-eosin (×130). **Fig. 2.** Longitudinal section through narrower area of cyst. Gradually shortening microvilli (a), cyst subtegument gradually becoming thinner (b), neck (c), scolex (d), Masson's trichrome (×180).



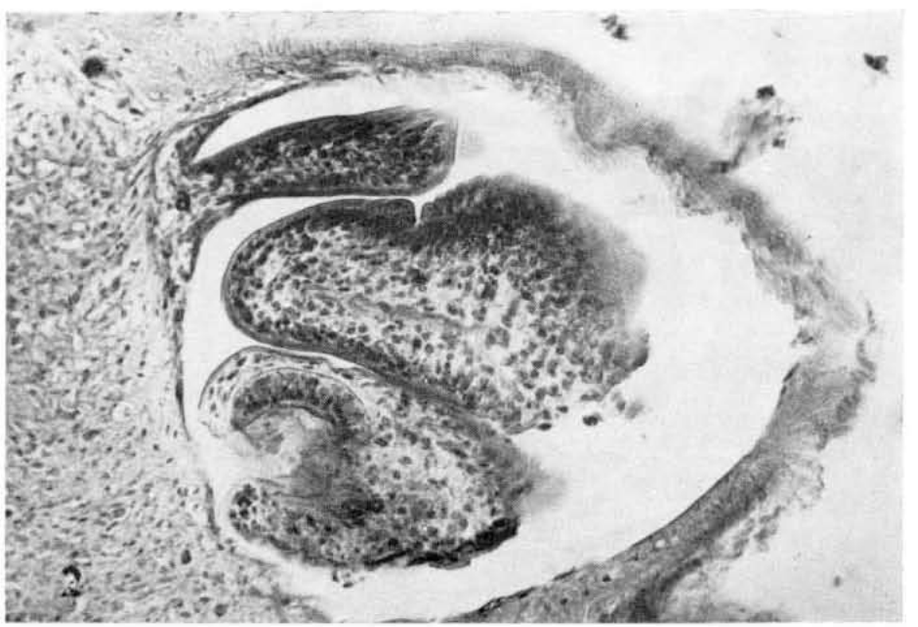


Fig. 1. Longitudinal section through wider area of cyst. Microvilli (a), pyriform cells of cyst subtegument (b), scolex (c), neck (d). Aldehyde fuchsin (×150). **Fig. 2.** Section through cyst at the site where the neck still adheres in a thin layer to its inner wall (a). Masson's trichrome (×280).

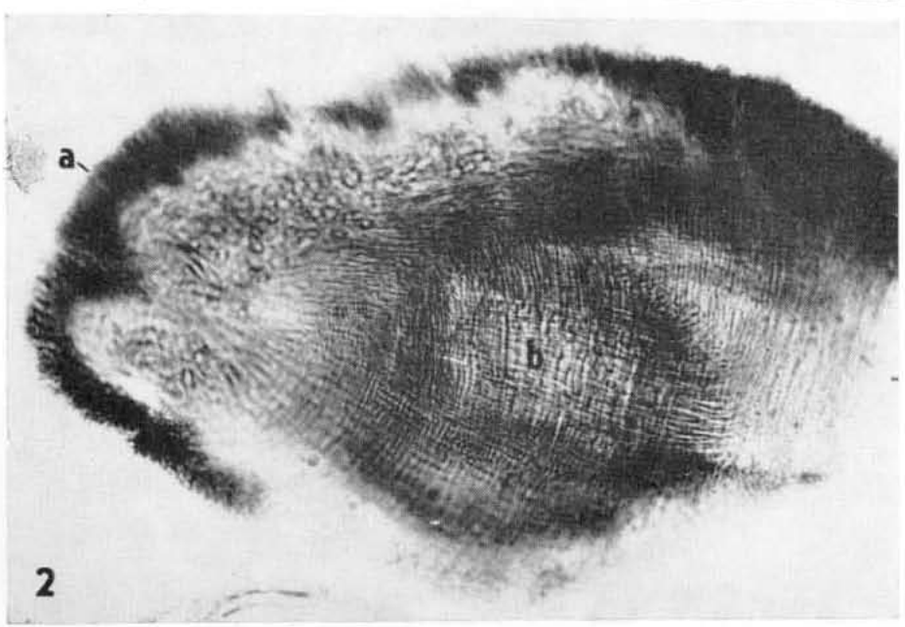
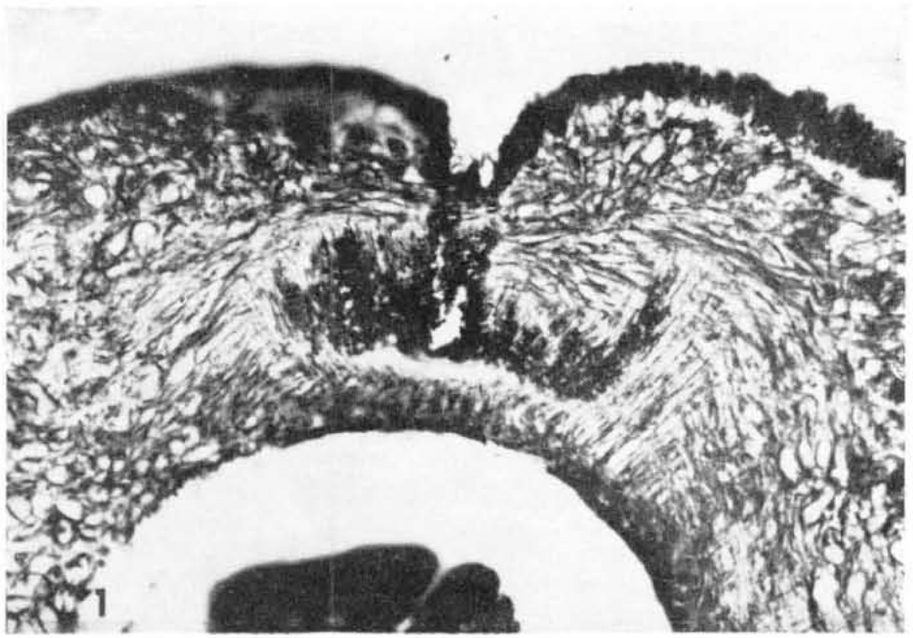


Fig. 1. Hook. Haematoxylin-eosin (×900). **Fig. 2.** Microvilli of cyst tegument (a), circular and longitudinal connective tissue fibres in outer part of subtegument (b). Masson's trichrome (×330).



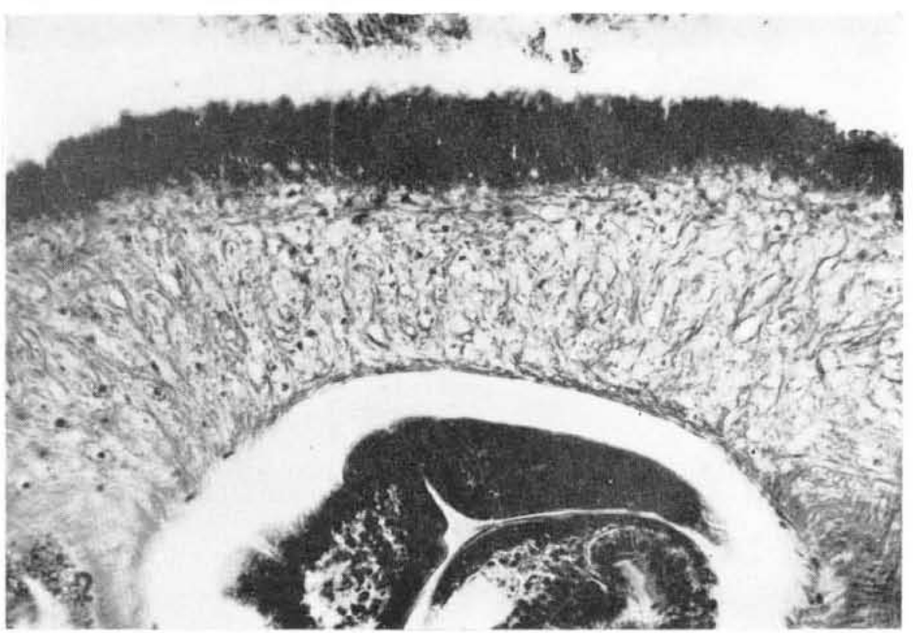


Fig. 1. Longitudinal section through cyst wall at the site of invagination. Fine connective tissue fibres of subtegument. Gomori (×350). **Fig. 2.** Connective tissue fibres of cyst subtegument. Mallory's PTAH method (×320).