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ELECTRON MICROSCOPE OBSERVATIONS OF THE SPOROCYST OF POSTHARMOSTOMUM GALLINUM WITENBERG, 1923 (BRACHYLAIMIDAE)

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Abstract. The tegument of *Postharmostomum gallinum* sporocyst forms numerous microvilli and contains a large number of mitochondriae. There occur occasionally crystalline inclusions and in some parts also spherical, electron-dense, membrane-bounded bodies. In the parenchymal part of sporocyst wall, the subtegumental cells form processes on the side turned towards the body cavity of the sporocyst. These processes, in form of primitive epithelium, temporarily surround the developing cercariae. As soon as the cercariae start to form their own definitive tegument, the primitive epithelium degenerates. Cells with large lipid vacuoles and a high content of glycogen often occur in the parenchymal part. There are also unique cells with membranous or filamentous bodies and very rarely cells with geometrically arranged membranous structures known in the sporocysts of the genus *Leucochloridium*.

This paper is a continuation of the histochemical studies on larval stages of *P. gallinum* (Žďárská and Soboleva 1984b) and of electron microscopical studies on sporocysts of other species of the superfamily Brachylaimoidea (Žďárská et al. 1982, Žďárská and Soboleva 1982, 1984a). It is a part of complex studies on the adaptation to the existence under terrestrial conditions.

MATERIAL AND METHODS

Sporocysts of *P. gallinum* were obtained from spontaneously infected terrestrial snails *Ponsadenia semenovi* (Martens, 1864) and *Monachoides candacharica* Pfeiff, 1846 collected in the vicinity of Alma-Ata. The material was fixed with 3 % glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, at 4 °C for 2 h and postfixed with 1 % OsO_4 for 2 h. Then it was dehydrated through an alcohol series and embedded into Epon or Araldit through aceton. Ultrathin sections were cut by Reichert's OM-U2 ultramicrotome, contrasted with 20 % uranyl-acetate and Reynold's solution of lead acetate and examined in JEM 100 B electron microscope.

RESULTS

The body wall of *P. gallinum* branched sporocyst (Plate I, Figs. 1, 2, Plate III, Figs. 1, 2; Plate IV, Figs. 1, 2) is covered with a tegument projecting into short or long microvilli. The syncytial layer of tegument is connected through fine processes with subtegumental cells situated under lamina basalis. The tegument contains numerous mitochondria (Plate I, Figs. 1, 2; Plate IV, Figs. 1, 2; Plate VI, Fig. 1) with conspicuous cristae, minute vacuoles, and rough endoplasmic reticulum. Single spherical electron-dense bodies surrounded by a membrane (Plate I, Fig. 2) and crystalline inclusions of oval shape (Plate III, Fig. 1) with regularly or irregularly arranged crystalline lattice occasionally occur in some parts. The tegument is bordered with outer and basal plasmalemma. The basal plasmalemma closely adheres to lamina basalis. Feebly developed muscle fibres (Plate I, Fig. 1; Plate IV, Figs. 1, 2; Plate VI, Fig. 1) are situated under the lamina basalis. Under the thin layer of these fibres, there are the cells of sporocyst body wall, in which the subtegumental cells form processes towards the tegument, and at the opposite end processes forming the primitive epithelium (Plate V, Fig. 1) surrounding the developing embrya of cercariae. This primitive epithelium degenerates in older embrya and numerous vacuoles appear in it. At the time when the germ balls form the difinitive tegument, the primitive epithelium separates from their surface (Plate V, Figs. 1, 2). The subtegumental cells contain a large number of mitochondria, α- and β-glycogen particles and a large nucleus with electron-dense nucleolus. Conspicuous cells with large lipid vacuoles (Plate VI, Fig. 1; Plate IV, Figs. 1, 2) and a number of α- and β-glycogen particles are contained in the body wall of the sporocyst.

Another type are the cells localized on the inner side of sporocyst wall. Degenerative changes occur in them: there appear membranous (Plate III, Fig. 2; Plate IV, Fig. 1) and filamentous (Plate II, Fig. 2) bodies and lysosomes. The inner layer contains also single cells with geometrically arranged membranous structure (Plate II, Fig. 1) the membranes of which appear like undulate tubules.

DISCUSSION

The general tegument structure of *P. gallinum* branched mother sporocyst is identical with that of mother sporocyst of *Schistosoma mansoni* (Smith and Chernin 1974, Meuleman et al. 1978, Meuleman et al. 1980). There is a large number of microvilli on its surface and the subtegumental cells are situated under a feebly developed muscle layer. In some parts, the tegument of *P. gallinum* mother sporocyst contains spherical electron-dense bodies surrounded by a membrane. Meuleman et al. (1980) found them only in daughter sporocyst, but not in mother sporocyst of *S. mansoni*, and they regarded them as lysosomes.

The sporocyst of *P. gallinum* resembles in its ultrastructure also the sporocysts of the genus *Leucochloridium* (Žďárská et al. 1982, Žďárská and Soboleva 1984a), but it differs from them in poorly developed longitudinal and circular muscles and in the absence of pigments. Its tegument, like that in the genus *Leucochloridium* (Bakke 1982, Žďárská et al. 1982), contains crystalline inclusions, membranous bodies in parenchymal cells, and single, geometrically regularly arranged membranous structures, which are supposed by Bakke (1982) to perform a photoreceptive function. Like in the sporocysts of other members of the superfamily Brachylaimoidea, *Hasstilesia ovis*, *Leucochloridium paradoxum*, *L. perturbatum* (Žďárská et al. 1982, Žďárská and Soboleva 1982, 1984a), *L. holostomum* and *L. variae* (Bakke 1982), no typical sensory receptors could be demonstrated in the sporocyst of *P. gallinum*. They have not been reported either in mother sporocysts of the genus *Schistosoma*. Bakke (1982) assumes that the geometrically arranged membranous structures in *L. variae* sporocysts have a photoreceptive function. If they have the photoreceptive function in the genus *Leucochloridium*, in which the rhythmical contractions of sporocyst sacs are related with the light intensity, then there is no reason why they should be developed in the wall of sporocyst sacs of *P. gallinum*, which are completely localized in the hepatopancreas, never grow into snail tentacles and do not react to the light. Obviously these structures are typical of the superfamily Brachylaimoidea. We do not think that they perform a photoreceptive function, though their significance in *P. gallinum* cannot be explained for the time being.

The primitive epithelium surrounding the developing embrya of cercariae, which was demonstrated earlier in some members of the order Strigeata, was found also in the sporocysts of *P. gallinum*. Some authors consider this epithelium to be a part of cercaria body (Hockley 1972, 1973), others assume that it is a part of sporocyst body (James et al. 1966, James and Bowers 1967, Belton and Belton 1971, Meuleman and Holzman 1975, Meuleman et al. 1980). The formation of the definitive tegument is thus preceded by primitive epithelium representing a transitory envelope. Since only mother sporocysts develop in the superfamily Brachylaimoidea (daughter sporocysts are lacking), the primitive epithelium in *P. gallinum* may be considered to be identical with the primitive epithelium in mother sporocysts of *S. mansoni*, which surrounds the developing daughter sporocysts (Meuleman and Holzman 1975, Meuleman et al. 1980). The primitive eipithelium in *P. gallinum* is derived from tegumental cells of mother sporocyst, but in contrast to mother sporocyst of *S. mansoni*, it does not cover the embrya of daughter sporocysts, but those of cercariae. The primitive epithelium surrounds the cercarial embryo and performs a protective and nutritional function. When the definitive cercarial tegument starts to develop, the primitive epithelium degenerates (Bils and Martin 1966, Belton and Belton 1971, Matricon—Gondran 1971, Hockley 1972, Meuleman and Holzmann 1975), similarly as the primitive epithelium of *S. mansoni* daughter sporocysts (Meuleman et al. 1980). Like in the sporocysts and cercariae of *S. mansoni*, numerous vesicles and vacuoles appear in the primitive epithelium of *P. gallinum* and *Brachylaimus aequans* sporocysts (see Žďárská 1983 — Plate II, Fig. 2). Previously we assumed that an early definitive tegument was involved in *B. aequans* cercariae, but now it is evident that it is derived from sporocyst tegument and represents in both species a transitory envelope which is later replaced by the definitive tegument.

ЭЛЕКТРОННОМИКРОСКОПИЧЕСКОЕ ИЗУЧЕНИЕ СПОРОЦИСТ *POSTHARMOSTOMUM GALLINUM* WITENBERG, 1923 (BRACHYLAIMIDAE)

З. Ждярска и Т. Н. Соболева

Резюме. Тегумент спороцисты *Postharmostomum gallinum* образует многочисленные микроворсинки и содержит большое количество митохондрий. В нем встречаются отдельные кристаллоидные включения и в некоторых частях круглые, эллектронноплотные тельца, окруженные мембраной. В паренхимной части стенки спороцисты, на стороне, обращенной в полость тела спороцисты, субтегументальные клетки образуют отростки, временно окружающие, в форме примитивного эпителия, формирующиеся церкарии. Как только церкарии начнут образовать собственный дефинитивный тегумент, примитивный эпителий дегенерирует. В паренхимной части часто встречаются клетки с большими липидными вакуолями и большим количеством гликогена. Встречаются также отдельные клетки с мембранными и нитевидными тельцами и очень редко клетки с геометрически расположенным мембранным образованием, раньше описанным у спороцист рода *Leucochloridium*.

REFERENCES

BAKKE T. A., Histology and biology of the larval stages of *Leucochloridium* Carus, 1835 (Trematoda, Digenea) as revealed by light and electron microscopy. Fauna norv. Ser. A 3: 41—56, 1982.

BELTON J. C., BELTON C. M., Freez-etch and cytochemical studies of the integument of larval *Acanthatrium oregonense* (Trematoda). J. Parasitol. 57: 252—260, 1971.

BILS R. F., MARTIN W. E., Fine structure and development of the trematode integument. Trans. Amer. Micr. Soc. 85: 78—88, 1966.

HOCKLEY D. J., *Schistosoma mansoni*: the development of the cercarial tegument. Parasitology 64: 245—252, 1972.

—, Ultrastructure of the tegument of *Schistosoma*. In: B. Dawes (Ed.), Advances in parasitology, Acad. Press, London—New York 11: 233—305, 1973.

JAMES B. L., BOWERS E. A., Reproduction in the daughter sporocyst of *Cercaria bucephalopsis haimeana* (Lacaze-Duthiers, 1854) (Bucephalidae) and *Cercaria dichotoma* Lebour, 1911 (non Müller) (Gymnophallidae). Parasitology 57: 607—625, 1967.

—, —, RICHARDS J. G., The ultrastructure of the daughter sporocysts of *Cercaria bucephalopsis haimeana* Lacaze-Duthiers, 1854 (Digenea: Bucephalidae) from the edible cockle, *Cardium edule* L. Parasitology 56: 753—762, 1966.

MATRICON-GONDRAN M., Origine et différenciation du tégument d'un trématode digénetique: étude ultrastructurale chez *Cercaria pectinata* (larve de *Bacciger bacciger*, Fellodistomatidés). Z. Zellforsch. 120: 488—524, 1971.

MEULEMAN E. A., HOLZMANN P. J., The development of the primitive epithelium and true tegument in the cercaria of *Schistosoma mansoni*. Z. Parasitenkd. 45: 307—318, 1975.

—, —, PEET R. C., The development of daughter sporocysts inside the mother sporocysts of *Schistosoma mansoni* with special reference to the ultrastructure of the body wall. Z. Parasitenkd. 61: 201—212, 1980.

—, LYARUN D. M., KHAN M. A., HOLZMANN P. J., SMINIA T., Ultrastructural changes in the body wall of *Schistosoma mansoni* during the transformation of the miracidium into the mother sporocyst in the snail host *Biomphalaria pfeifferi*. Z. Parasitenkd. 56: 227—242, 1978.

SMITH J. H., CHERNIN E., Ultrastructure of young mother and daughter sporocysts of *Schistosoma mansoni*. J. Parasitol. 60: 85—89, 1974.

ŽĎÁRSKÁ Z., SOBOLEVA T. N., Ultrastructure of the tegument of *Hasstilesia ovis* larval stages. Folia parasit. (Praha) 29: 367—370, 1982.

—, —; Ultrastructure of the metacercaria and sporocyst sac of *Leucochloridium perturbatum* Pojmanska, 1969. Folia parasit. (Praha) 31: 133—139, 1984a.

—, —, Histochemistry of the cercaria and sporocyst of *Postharmostomum gallinum* (Brachylaimidae). Folia parasit. (Praha) 31: 333—338, 1984b.

—, —, OSIPOVSKAYA L. L., Ultrastructure of the tegument and associated structures of *Leucochloridium paradoxum* sporocyst and metacercaria. Folia parasit. (Praha) 29: 247—251, 1982.

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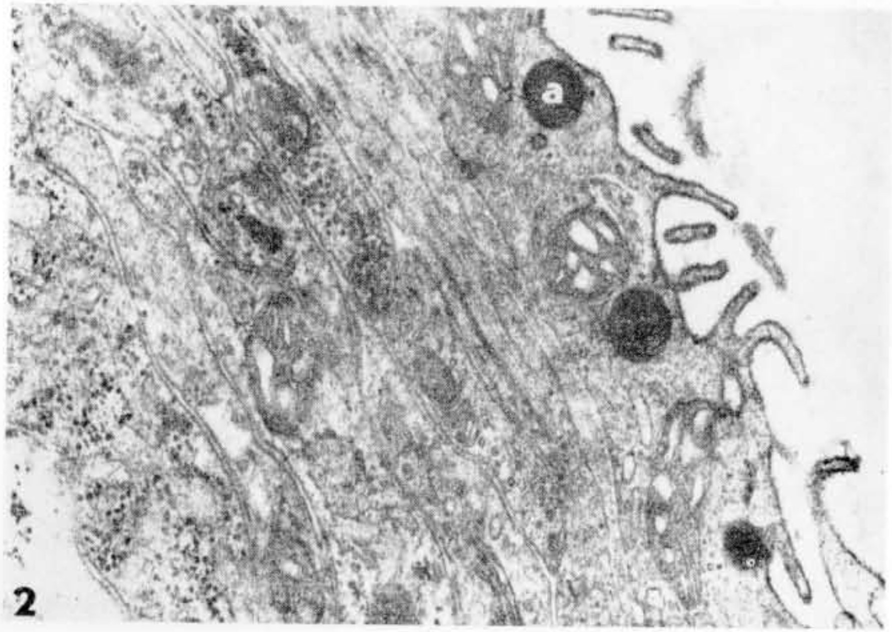
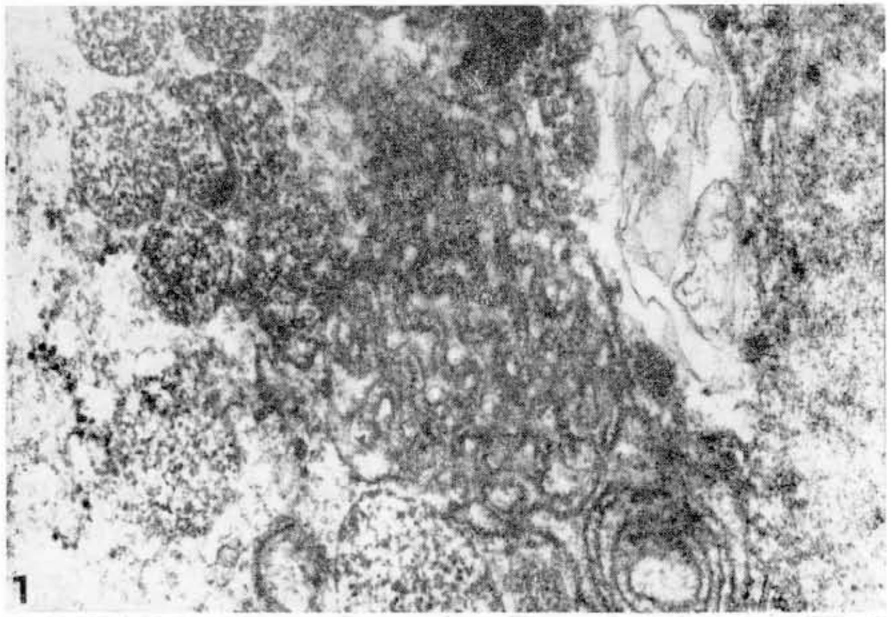


Fig. 1. Tegument of the sac wall of *P. gallinum* sporocyst forming numerous microvilli (at the top) and containing a large number of mitochondria. Basal plasmalemma of tegument adheres to thin lamina basalis (arrows). Single muscle fibres (a) occur under lamina basalis. G, Os, UAc, Pb (× 20,000). **Fig. 2.** Section through sac wall of *P. gallinum* sporocyst at the site where electron-dense spherical bodies (a) occur in the tegument (right). G, Os, UAc, Pb (× 45,000).



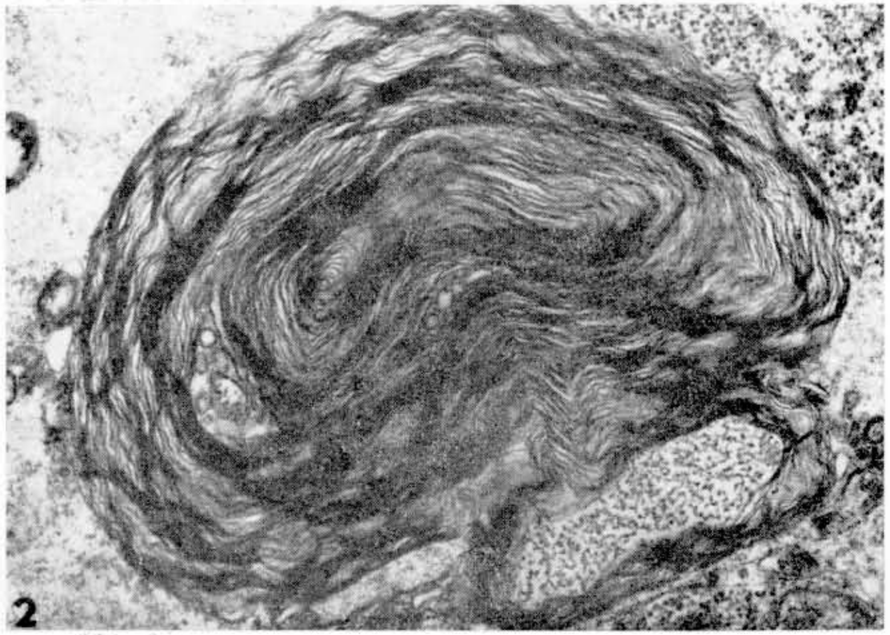


Fig. 1. Geometrically arranged membranous structure in limiting layer of the sac wall of *P. gallinum* sporocyst. G, Os, UAc, Pb (× 59,000). **Fig. 2.** Filamentous body in a cell of inner layer of the sac wall of *P. gallinum* sporocyst. G, Os, UAc, Pb (× 42,100).

Fig. 1. Crystalline inclusions in the tegument of *P. gallinum* sporocyst. G, Os, UAc, Pb (× 70,000). **Fig. 2.** Section through sac wall of *P. gallinum* sporocyst at the site of degenerating parenchymal cell containing large electron-dense bodies (a) and a membranous body (b). c — tegument, arrows — lamina basalis. G, Os, UAc, Pb (× 35,000).

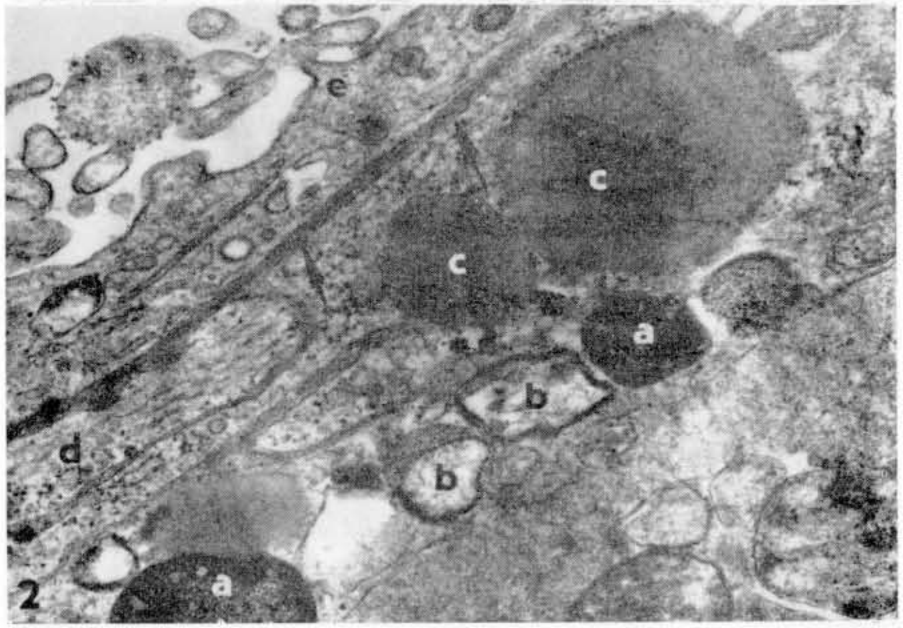
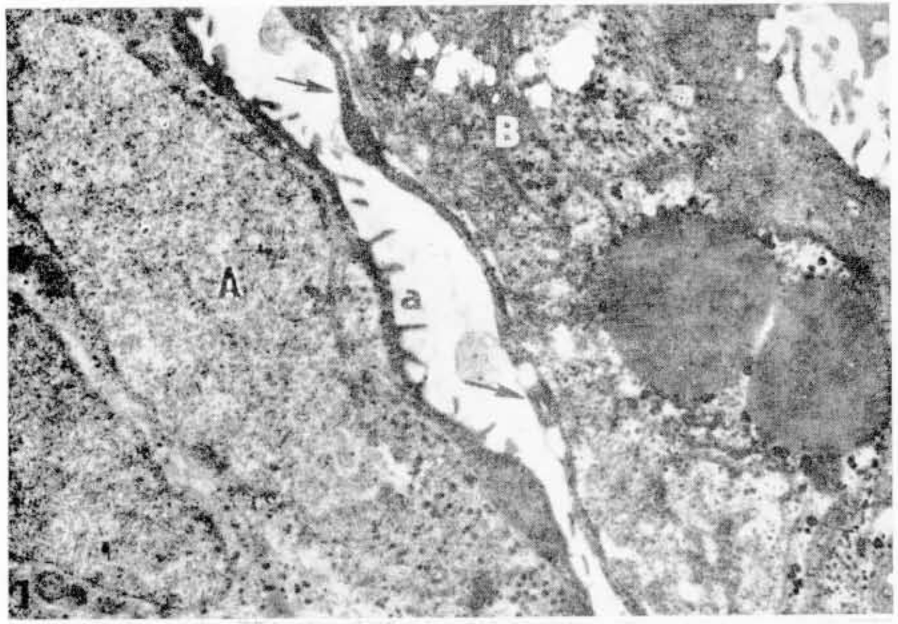


Fig. 1. Sac wall of *P. gallinum* sporocyst. a — microvilli, b — tegument, arrows — lamina basalis, c — circular muscle fibre, d — longitudinal muscle fibre, A — cell with high content of α- and β-particles of glycogen and a lipid vacuole (e). B — degenerating cell bordering with sporocyst cavity which contains irregular, electron-dense bodies (f) and a membranous body (g). G, Os, UAc, Pb (× 20,000). **Fig. 2.** Degenerating cell of sporocyst wall of *P. gallinum* situated immediately under lamina basalis (arrows) and containing irregular, electron-dense bodies (a), markedly bordered vacuoles (b) and lipid vacuoles (c). d — muscle fibre, e — tegument, G, Os, UAc, Pb (× 35,000).



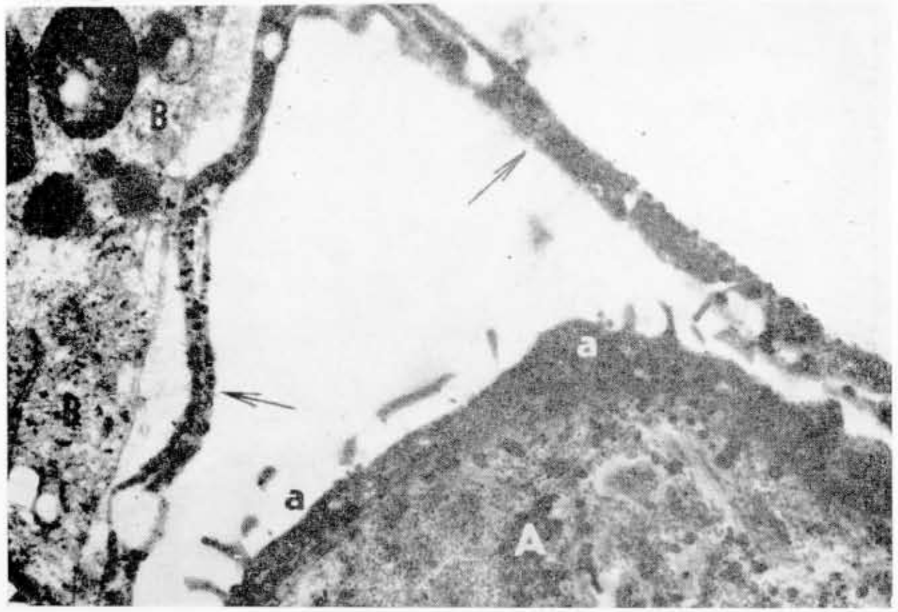


Fig. 1. Primitive epithelium (arrows) separated from the surface of germ ball (A) adheres to the sporocyst wall (B). Definitive tegument with microvilli (a) is forming on the surface of germ ball. G, Os, UAc, Pb (× 17,000). **Fig. 2.** Primitive epithelium (arrows) separated from the arising definitive tegument (a) of very young cercaria (A) of *P. gallinum*. B — limiting layer of sporocyst sac. G, Os, UAc, Pb (× 20,000).

Fig. 1. Parenchymal cell (A) of *P. gallinum* sporocyst sac filled with α- and β-particles of glycogen and lipid vacuoles (a) and parenchymal cell (B) containing a large number of α- and β-glycogen particles and mitochondria (b). c — tegument, arrows — lamina basalis, d — muscle fibres. G, Os, UAc, Pb (× 24,600).