AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 24, NUMBER 3, SEPTEMBER 1995

EDITOR: B.O. ONADEKO
ASSISTANT EDITORS:
B.O. OSOTIMEHIN and A.O. UWAIFO

SPECTRUM BOOKS LIMITED
Ibadan • Owerri • Kaduna • Lagos

ISSN 1116—4077

Afr. J. Med. med. Sci. (1995) 24, 219-225

Human bilharzial ureters: I. Fine structure of eggs deposited in the submucosa and muscularis

S.M. EL-SHOURA,[1] N.F. RAMADAN,[2] O. HASSOUNA,[3] H.I. MAHMOUD,[4] S. SHETTY[5] and A. WESTMUCKETT[6]

[1,6]*Department of Pathology,* [5]*Department of Urology, College of Medicine, King Saud University, Abha P.O. Box 641, Saudi Arabia;* [2]*Faculty of Science, Ain-Shams University and* [3]*College of Medicine, and* [4]*Faculty of Science, Al-Azhar University, Cairo, Egypt.*

Summary

The internal fine structure of the partially calcified *Schistosoma haematobium* eggs in the lower ureteral segments of Saudi patients with chronic urinary schistosomiasis is described. The egg shell is penetrated by cribriform pores and consists of the three previously described layers: outer microspinous, middle intermediately dense, and inner dense layers. The space between the egg shell and the developing embryo is partitioned by three layers. An outer acellular Reynolds' layer of unknown origin and function consists of a fribrillar material mixed with a finely granular matrix extending to the egg-shell pores via racemose channels. The middle von Lichtenberg's envelope consists of a single layer of flattened epithelial cells containing several mitochondria as in other previously described *Schistosoma* eggs suggesting an active, and perhaps selective, transport in or out of the egg shell. The inner fluid filled cavity, or Lehman's lacuna, between the von Lichtenberg's envelope and the embryo contains numerous lipoid bodies suggesting a relation to vitelline cells. Four systems (out of eight previously described for the free, mature *Schistosoma* miracidium) have been recognized for the first time in the developing miracidium within *S. haematobium* eggs and include: (1) ciliated epidermal plates representing the epithelial system, (2) an outer circular and an inner longitudinal muscle layer forming the musculatures, (3) lateral penetration glands, and (4) ciliated flame cells representing the excretory system.

Résumé

La structure fine interne des oeufs *schistosoma haematobium* partiellement calcifiés se trouvant dans les segments inférieurs urétéraux des patients Saoudites souffrant de schistosomiase urinaire chronique est décrite.

Le coquillage est pénétré de pores cribriformes et comprend trois couches déjà décrites: la couche microspineuse extérieure, la couche moyenne un peu dense et une couche intérieure plus dense.

L'espace entre le coquillage et l'embryon qui se développe est cloissonée par trois couches.

Une couche de Reynold accélulaire d'origine et de fonction inconnues se trouve à l'extérieur. Elle comprend un matériel fibrillaire mélangé avec un matrix de granules fines et s'étendant jusqu'aux pores du coquillage via les voies racimoses.

Au milieu se trouve l'enveloppe von Lichtenberg composée d'une couche unique de cellules épithéliales plates qui comprennent plusieurs mithochondria dans des oeufs de *Schistosoma* décrits au préalable; ce qui suggère un transport actif et probablement sélectif dans ou hors du coquillage. La cavité liquide intérieure ou la lacune de Lehman entre l'enveloppe Lichtenberg et l'embryon contient plusieurs corps lipoides, évidence d'un rapport avec les cellules vitellines.

Quatre systèmes (parmi les huit déjà décrits pour le Schistosoma miracidium maturé, libre) sont notés pour la première fois dans le miracidium développant à l'intérieur des oeufs de *S. haematobium* et ceci inclut: (1) des plants ciliés épidermaux représentant le système épithéliale, (2) des couches de muscles circulaires à l'extérieur et longitudinales à l'intérieur formant les musculatures, (3) des glandes de pénétration latérales, et (4) des cellules de flamme cilliées représentant le système excrétoire.

Introduction

The fine structure of the internal morphology of *Schistosoma* eggs has been described in previous studies but were limited to the egg shell proper[1-3]. Recently, the ultrastructure of the developing perimiracidial envelope and formation of the miracidial epidermal plates and ridges were studied

in *S. mansoni* eggs collected from mice intestine[4]. To our knowledge, nothing has been published on the fine structure of the internal morphology of *S. haematobium* eggs in general and on those deposited in the lower segments of human ureters in particular.

Eggs of *S. haematobium* deposited at the lower end of the human ureters and the urinary bladder release enzymes and antigens to facilitate their migration out of the host body. These parasite products sensitize the host lymphocytes which migrate to areas of egg deposition and recruit other cells including monocytes, macrophages, multinucleated giant cells, lymphocytes, plasma cells, neutrophils, mast cells, and blood platelets (El-Shoura, submitted to Annales de Parasitologie) through the secretion of lymphokines. As a result, a compact cellular infiltrate, or granuloma, is formed to destroy the parasite eggs[5]. These impede urine flow and cause hydroureter, bladder lesions and hydronephrosis. In severe infections, thick "sandy" patches coalesced to form a "calcified tissue" as a result of subepithelial deposits of calcified eggs. This study describes the ultrastructure of *S. haematobium* eggs deposits in the submucosa and muscularis of the lower segments of the ureters of Saudi patients with chronic urinary schistosomiasis to contribute to the understanding of the *in vivo* ultrastructural interactions between the parasite egg and host tissues. Results obtained herein are also compared with those previously described for other human *Schistosoma* eggs obtained from experimental hosts.

Materials and methods

Seven biopsies were taken from the lower end of the ureter, about 5 cm above the bladder junction, of Saudi patients aged 26-63 (mean 38.5) with chronic urinary schistosomiasis. All patients were from Asir Province, in the South-West of the Kingdom of Saudi Arabia. Each biopsy was cut into 2-3 mm cubes, and fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.2 at 4°C, for three hours. Specimens were decalcified in 1% EDTA in sodium cacodylate buffer for five days at room temperature. After washing thoroughly in the buffer, specimens were post-fixed in 1% osmium tetroxide in the sodium cacodylate buffer, for one hour, dehydrated in an ascending series of ethyl alcohol and embedded in Spurr's resin. Thick sections, 0.5 µm, were stained with toluidine blue. Thin sections were stained with uranyl acetate and lead citrate and examined in a Jeol 1200 EX transmission electron microscope (TEM) at 80 kV.

Results

Thick sections (Figs. 1 a, b) showed many eggs scattered in the submucosa with a few of them trapped in muscle layers and surrounded by cells forming granulomas. The infiltrate extended from the base of the epithelium downward where a richly vascularized granulation tissue was diffusely infiltrated by a massive number of these cells.

Fig. 1: Light micrographs of thick sections showing a) — *S. haematobium* eggs (empty arrow) scattered in the ureter submucosa (S) and muscle layers (M) and surrounded by the granuloma cell (solid arrow). b) — Higher magnification of the arrowed egg seen in Fig. 1a. X80, Fig 1b. X 800.

Fig. 2: Electron micrographs of a thin section of the egg seen in Fig. 1b showing the relative thickness of the egg shell at the tip of the spine pole, compared with rest of the egg shell. X6000.

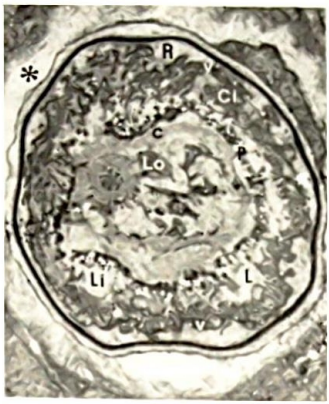

Fig. 3: Cross section through an egg showing satisfactorily preserved internal structure. Note the three layers forming the egg shell, Reynolds' layer (R), von Lichtenberg envelope (V) and Lehman's lacuna (L). These three layers fill the space between the embryo surface which is represented by epidermal plates (P), and the egg shell. Beneath the epidermal plates (P) are the circular (C) and longitudinal (Lo) muscle layers. Note also the continuous space between the outer granuloma cell and the egg shell. Cilia (Ci) arise from the plates. Li, lipoid droplets in Lehman's Lacuna. Asterisk represents a calcified area. X 8000.

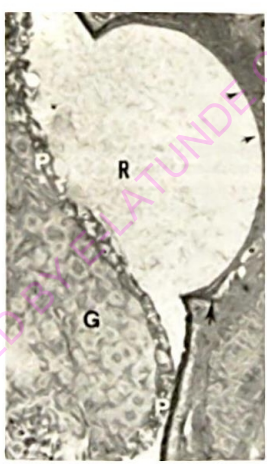

Fig. 4: Tangential section of an egg showing a lateral penetration gland packed with secretory granules (G) containing electron-dense cores. Note the close attachment of the outer granuloma cell to the egg surface where both the shell microspines and the separating space have disappeared (two small arrows). P, epidermal plates without cilia; R, Reynolds' layer. Single arrow points to the peeled part of the shell. X 25,000.

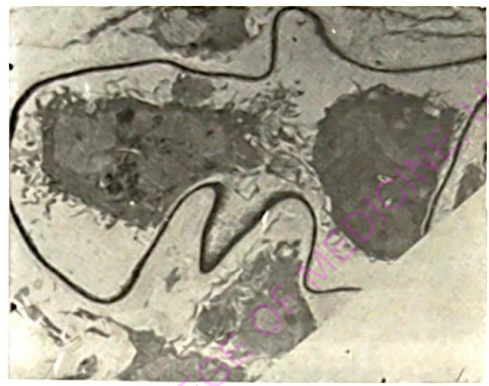

Fig. 5: An egg shell containing two macrophages. X 4000.

Thin sections revealed partially calcified eggs with variable degrees of preservation of both the egg shell and developing miracidium (Figs. 2-4). Several eggs were completely encircled by the granuloma cells or their long cytoplasmic extensions (Fig. 3). Eggs containing macrophages (Fig. 5), or cell debris (Fig. 6) were also seen. The egg shell (Fig. 7) consisted of an outer microspinous (250-350 nm thick), a middle intermediately dense (200-350 nm thick) and inner dense (60-70 nm thick) layers. In longitudinal sections (Fig. 7), the microspines were conical, up to 350 nm in length, with a base measuring 50-55 nm in diameter. These three layers were greatly thickened at the spinous pole (Fig. 2), apparently to provide firm penetration through the host tissues. Each microspine had a core with a basal diameter of 15-18 nm. The core was continuous with the middle intermediately dense layer. In cross sections, each core was surrounded by approximately 14 ridges running from base to apex of the cone (Figs. 8, 9).

The egg shell was transversed obliquely by minute cribriform pores with serpiginous branching and 30-35 nm diameter anastomosing channels. The channels were extended from the underlying Reynolds' layer. There was always a space filled with an intermediate dense material separating the egg shell from the granuloma cell membrane (Figs. 3, 10, 11); this space was almost defined by the spine length. Plasma membranes of the cells forming granuloma were in direct contact with the egg surface where the egg shell seemed to be peeled off externally (Fig. 4).

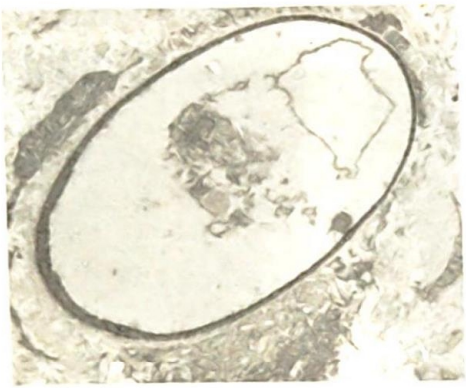

Fig. 6: An egg empty but for a few debris. X 3000.

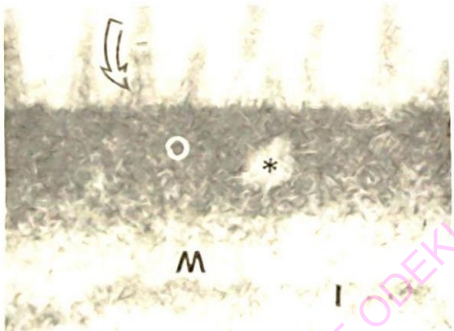

Fig. 7: Longitudinal section through the egg shell showing the outer (O), middle (M) and inner (I) layers. Arrow points to a microspine containing core. Asterisk represents a cribriform pore. X 90,000.

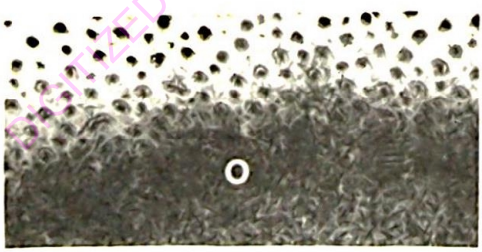

Fig. 8: Cross section through the microspinous outer layer (Q) of the egg shell. X 50,000.

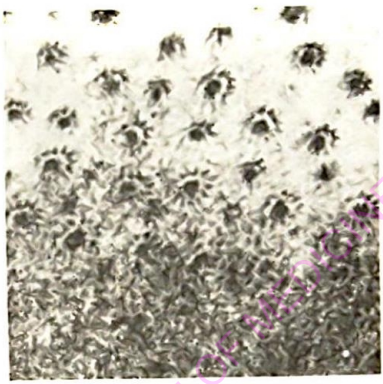

Fig. 9: Higher magnification of Fig. 7 showing that each microspine is surrounded by ridges. X 250,000.

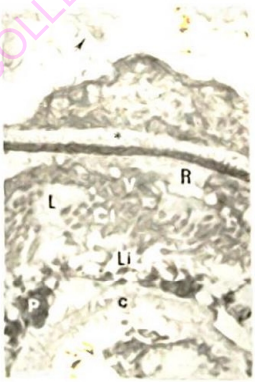

Fig. 10: Higher magnification of the top right zone in Fig. 2 showing the ultrastructural details of its tissue. Note the space (*) containing moderately dense material and separating the granuloma cell from the egg shell. This space is defined by the microspine's length. Letterings as in Fig. 2. The arrow indicates a basal lamina beneath the cell forming the granuloma and separating them from the "halo" calcified zone. X 12,000.

The space between the inner, electron-dense layer of the egg shell and the developing miracidium was morphologically partitioned into three layers (Figs. 3, 4, 10, 11): Reynolds' layer, von Lichtenberg's envelope, and Lehman's lacuna. The outer acellular Reynolds' layer, lying immediately beneath the egg shell, consisted of a fribrillar material mixed with a finely granular matrix (Figs. 3, 4, 10, 11). The intermediate single layer of flattened epithelial cells forming von Lichtenberg's envelope surrounded the inner contents of the egg. In early stages of

miracidia, the nuclei of the von Lichtenberg's cells were usually opposite to the spine pole, but deteriorated in the mature miracidia[5]. The inner fluid-filled cavity, or Lehman's lacuna, surrounding the developing miracidium, contained several dense bodies of variable sizes (Figs. 3, 10, 11).

In some specimens, the degree of preservation allowed the recognition of certain miracidial internal structures. These included typical motile cilia arising from electron-dense epidermal plates and were protruding into the lacuna (Figs. 3, 10, 11). Scattered among the ciliary rootlets were numerous electron-dense, membrane-bound, round to oval bodies measuring up to 350 nm in diameter (Figs. 10, 11). Outer circular and inner longitudinal muscle layers forming the musculature of the developing miracidium, were also visible (Figs. 3, 10, 11). In cross sections (Fig. 12), circular fibers appeared to encircle the entire developing miracidium. The circular myofibrils were attached to the base of the epidermal plates. One apical and two lateral large glandular cells, representing the presumptive penetration glands, were found to occupy much of the anterior area of the growing miracidia. Each gland was packed with round secretory granules of various sizes and containing electron-dense cores (Fig. 4).

The flame cells previously described in the free, mature miracidium and representing its excretory system[6] consisted of a cyton, a hollow cylinder or barrel, and numerous cilia within the cylinder. In the present study, cross sectioned cilia were found enclosed within the electron-dense cylinder wall (Fig. 13).

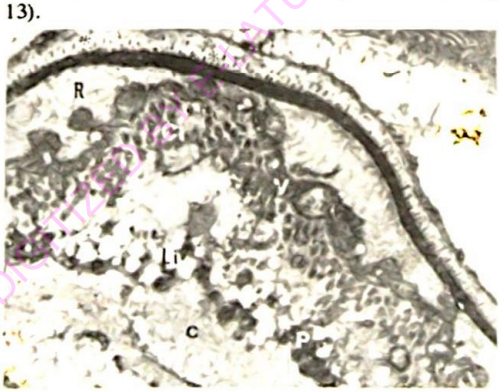

Fig. 11: Higher magnification of the bottom zone in Fig. 2 showing the ultrastructural details of its internal tissue. Lettering as in Figs. 2 and 9. X 12,000.

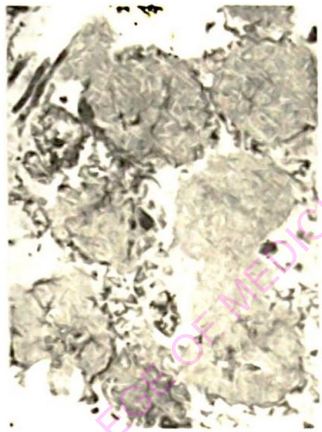

Fig. 12: Cross section of the egg longitudinal muscle fibers running beneath the circular muscle layer. X 16,000.

Fig. 13: Cross section through the cylinder wall (W) of a flame cell enclosing cilia. X 48,000.

Discussion

The egg shell proper of *S. haematobium* is structurally identical to that previously described for *S. mansoni* [3,4,7], but differs from *S. japonicum* [8] in which microspines are finer and more densely distributed over the surface. In addition, the central dense cores seen in the microspines of *S. haematobium*, and *S. mansoni* [4] are lacking in the individual spines of *S. japonicum* [8]. These differences do not clarify the function of the microspines. Hockly[9] has suggested three possible

correlates: a mechanical or abrasive action, a mode of increasing the shell surface area, or serving as conduits to localize egg or worm secretion on the shell surface. Accordingly, the relatively larger size of *S. haematobium* spines would suggest that here a mechanical or abrasive function is more likely than for the *S. japonicum*. In addition, microspines of the *S. haematobium* eggs observed by Schnitzer *et al.* [8] were bent beneath the surrounding granuloma cell membranes indicating less firm spines. However, the spine length seems to define the space separating the egg shell surface from the granuloma cell membranes. This space is usually filled with a material that could be derived from either the egg or the granuloma cells or both, and was suggested to be the functional interface between the host and parasite and responsible for the egg lesion. The cribriform pores and their serpiginous branching and anastomosing channels contiguous with the underlying Reynolds' layer are similar to those described for the egg shell of *S. mansoni* [4]. Existence of large perforations in *Schistosoma* eggs[1-3] makes it more difficult to explain the resistance of normal eggs to be penetrated by fixatives, clearing, and embedding agents. This has impeded progress in defining egg internal structure and development of miracidium for over 20 years.

The acellular Reynolds' layer, consisting of a fibrillar material mixed with finely granular matrix, permeates the pores in the egg shell proper. This layer is similar to "peri-vitelline material" described in *Fasciola hepatica* eggs[10], and corresponds to the inner layer of *Schistosoma* egg shells studied previously[11,12]. The origin of this layer is not known, and several possibilities have been suggested[4]. The most convincing explanation is that the antigen-antibody complexes formed when antibodies leak through the pores and combine with egg antigen produce a reverse Hoeppli phenomenon. Inside the egg shell[13], such a phenomenon has been seen microscopically in human and experimental *S. japonicum* infection. It has also been suggested that impregnation of Reynolds' layer by antigen-antibody complexes and their reaction with the fixatives and other media may play a role in the blocking of the egg pores against normal processing agents. However, the immunogenicity, tissue toxicity or function of the material comprising Reynolds' layer is not yet known.

The fine structure of von Lichtenberg's envelope is identical to that described in *S. mansoni* eggs[4], and corresponds to both the "outer envelope" of Swiderski[14-17], and the "vitelline membrane" described earlier[18,19]. Scanning electron microscope observations of newly hatched miracidia showed that the envelope remains within the shell upon hatching[20], while the earlier light microscope studies indicated that the envelope occasionally emerges surrounding the miracidium. Neill *et al.* [4] were able to detect a large number of mitochondria and several nuclei suggesting an indication of a high metabolic activity. This was consistent with active transport and subtentacular functions including a role in providing essential materials for growth and differentiation of the miracidium. Elimination of toxic products and maintaining chemical milieu occurred in Lehman's lacuna. The extracted lipoid bodies seen distributed throughout the Lehman's lacuna were also observed in *S. mansoni* eggs[4] and were suggested to be related to the vitelline glands.

The acellular organization of the free, mature miracidium of *S. mansoni* has been divided into eight major categories or systems[6]: terebratorium, nervous system, interstitial cells, germinal cells, epithelial system, musculature, penetration glands, and excretory system. The last four systems, represented respectively by ciliated epidermal plates, outer circular and inner longitudinal muscle layers, lateral penetration glands packed with secretory granules, and flame cells with cilia enclosed in a cylinder wall have been distinguished, for the first time at the TEM level, in the developing miracidium within *S. haematobium* eggs. Their fine structure is also identical to those described for the free, mature miracidium of *S. mansoni*. The present TEM observations have elucidated several ultrastructural similarities concerning the egg shell proper and certain organs or systems in the developing miracidium of *S. haematobium* eggs trapped in human ureters to eggs of other *Schistosoma* spp. This knowledge will assist biochemical studies to understand the geometric constraints in egg functions, but open up still, more questions than were initially posed for answer.

References

1. Race GJ, Michaels RM, Martin HH, Larsh JE, Matthews JL. *Schistosoma mansoni* eggs: an electron microscopic study of shell pores and microbarbs. Proc. Soc. Exp. Biol. Med. 1969; 130: 990-992.

2. Race GL, Martin JH, Larsh JE, Michaels RM. Scanning and transmission electron microscopy (EM) of *Schistosoma mansoni* ova, structure and topography of the shell pores (abst.) Proc. 2nd Int. Congr. Parasitol. 1970: 276.
3. Race GL, Martin JH, Moore DV, Larsh JE. Scanning and transmission electron microscopy of *Schistosoma mansoni* eggs, cercariae, and adults. Am. J. Trop. Med. Hyg. 1971; 20: 914-924.
4. Neil PJG, Smith JH, Doughtry BL, Kemp M. The ultrastructure of the *Schistosoma mansoni* egg. Am. J. Trop. Med. Hyg. 1988; 39: 52-65.
5. Beaver PC, Jung RC, Cupp EW. Clinical pathology. 9th edition. Philadelphia: Lea Febiger. 1984: pp 781.
6. Pan SC. The fine structure of the miracidium of *Schistosoma mansoni*. J. Invert. Pathol. 1989; 36: 307-372.
7. Eklu-Natey DT, Swiderski Z, Moczon T, Striebel HP, Huggel H. Ultrastructure and histochemistry of egg shell formation in *Schistosoma haematobium* (Abst.). Proc. 5 Int. Congr. Parasitol. Mol. Biochem. Parasitol. 1982; (Suppl.): 703.
8. Schnitzer B, Sodeman T, Sodeman W, Durkee T. Microspines on *Schistosoma japonicum* and *S. haematobium* egg shells. Parasitol. 1971; 62: 385-387.
9. Hockley DJ. Scanning electron microscopy of *Schistosoma mansoni* cercariae. J. Parasitol. 1968; 54: 1241-1243.
10. Wilson RA. The structure and permeability of the shell and vitelline membrane and the egg of *Fasciola hepatica*. Parasitol. 1967; 57: 47-58.
11. Von Lichtenberg F, Lindenberg W. An alcohol-acid-fast substance in eggs of *Schistosoma mansoni*. Am. J. Trop. Med. Hyg. 1954; 3: 1066-1076.
12. Von Lichtenberg F. The bilharzial pseudotubercle: a model of immunopathology of granuloma formation. In: Immunologic aspects of parasitic infections. PAHO Orig. Sci. 1967; 150: 107- 127.
13. Smith JH, Von Lichtenberg F. The Hoeppli phenomenon in schistosomiasis. II. Histochemistry. Am. J. Pathol. 1967; 50: 993-1007.
14. Swiderski Z, Eklu-Natey DT, Moczon T, Skubillia L, Huggel H. Embryonic development of *Schistosoma mansoni* and *S. haematobium* (abst.). Proc. 5 Int. Congr. Parasitol. Mol. Biochem. Parasitol. 1982; (Suppl.): 708-709.
15. Swiderski Z. Embryonic development of *Schistosoma mansoni* (abst.). S. Afr. J. Sci. 1984; 80: 434.
16. Swiderski Z. Blastomere differentiation during development of *Schistosoma mansoni* (abst.). Proc. 11 Int. Congr. Electron Microscopy, Kyoto 1986a: 3321-3322.
17. Swiderski Z. *Schistosoma matthei* egg-shell degradation in the liver of *Praomys (Mastomys) natalensis* during chronic infection (abst.). Proc. 11. Int. Congr. Electron Microscopy, Kyoto 1986b: 3567-3568.
18. Loss A. Beobachtungen uber die eier und embryonen del Bilharzia. Parasiten des Menschen 1893; 2: Abst: 521-528.
19. Cort WW. Notes on the eggs and miracidia of human schistosomes. Univ. Calif. Publ. Zool. 1919; 18: 509-519.
20. Higgins-Opitz SB, Evers P. Observations by scanning electron microscopy of miracidial hatching from *Schistosoma mansoni* ova. J. Parasitol. 1983; 69: 432-43.