

Ultrastructure of the primitive epithelium of *Echinostoma revolutum* (Digenea: Echinostomatidae) cercaria

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Abstract. Ultrastructure of the primitive epithelium of *Echinostoma revolutum* (Digenea: Echinostomatidae) cercaria was studied. The germinal balls and developing cercariae are covered with the primitive epithelium in daughter rediae. When the definitive tegument of the cercaria is differentiated, the primitive epithelium degenerates. The last remnants of the primitive epithelium in a cercaria can be detected at the stage when the lateral gland cells have released their secretion into the definitive tegument.

In several cercaria species the formation of the definitive tegument has been found to be preceded by a transitory epithelium variously named by different authors (supporting cells, embryonic membrane, germinal cyst, syncytial supporting layer, primitive epithelium, investing syncytium), for details see Meuleman and Holzmänn (1975) and Al-Salman and James (1988). The definitive cercarial tegument is formed from the peripherally located somatic cells developing beneath the original primitive epithelium which eventually degenerates.

This paper describes for the first time in an echinostomatid cercaria the development and degeneration of the primitive epithelium and the first developmental phases of the definitive tegument. Later phases of the development of the definitive tegument of the cercariae of *Echinostoma revolutum*, characterized by the deposition of three types of secretory granules, have been described earlier (Žďárská 1989).

MATERIALS AND METHODS

Rediae with developing cercariae of *Echinostoma revolutum* (Froelich, 1802) obtained from the naturally infected snails *Planorbis corneus* at České Vrbné, Czech Republic, were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) at 4°C for 2 h and postfixed in 1% OsO₄ in 0.1 M cacodylate buffer. They were dehydrated through an alcohol series and embedded through acetone into Durcupan. Series of ultrathin sections were cut with a Reichert OM-U2 ultramicrotome, contrasted with 20% uranyl acetate and Reynold's lead citrate solution, and examined in a Philips 420 electron microscope.

RESULTS

The germinal balls of *Echinostoma revolutum* cercaria are surrounded by a primitive epithelium (syncytium) at a very early stage in development (Fig. 1). The outer plasma membrane is smooth and the cytoplasm contains numerous vesicles, vacuoles, residual bodies, mitochondria and α -glycogen particles. The development of the definitive tegument (Fig. 2) beneath the primitive epithelium occurs before any other differentiation in the germinal ball cells. The nuclei of the flat superficial cells are surrounded by a very fine cytoplasmic layer. The cytoplasmic extensions of these cells beneath the primitive epithelium join later to form a very narrow cytoplasmic layer. At this stage no traces of muscle development are evident (see Fig. 2). The primitive epithelium persists for a long time in the form of a very fine cytoplasmic layer with large vacuoles, the nuclei were not present. In cercariae with developed circular and longitudinal muscles below the definitive tegument, the primitive epithelium is well preserved (Fig. 3). The last traces of the primitive epithelium in the form of disrupted cytoplasmic strips are recognizable in cercariae at the stage with developed spines, and secretion of the lateral gland cells released into the definitive tegument (Fig. 4).

DISCUSSION

The formation of the primitive epithelium in the cercarial embryo has been described in species of the following 8 families – Schistosomatidae (Rifkin 1970,

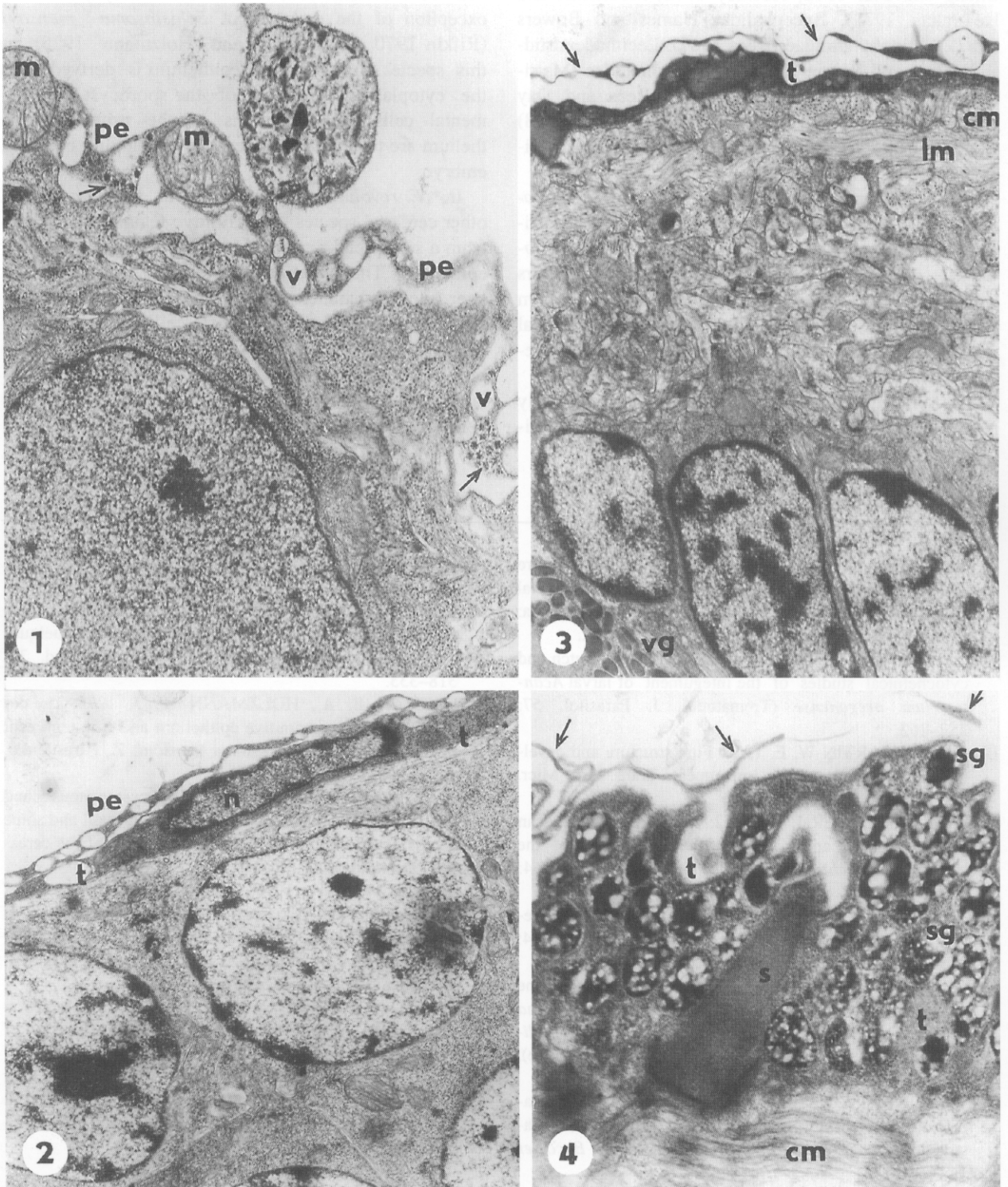


Fig. 1. Detail of the primitive epithelium (pe) of a germinal ball without any trace of the definitive tegument; m – mitochondria, v – vacuoles, arrows – α -glycogen particles ($\times 21,000$). **Fig. 2.** The definitive tegument (t) tightly connected to the underlying tissue develops beneath the primitive epithelium (pe); n – nucleus of the definitive tegument ($\times 10,200$). **Fig. 3.** Attenuated primitive epithelium (arrows) above the definitive tegument (t) in an older cercaria with developed ventral gland cells (vg); in comparison with Fig. 2 the circular (cm) and longitudinal (lm) muscle layers under the definitive tegument are well developed ($\times 13,800$). **Fig. 4.** Remnants of the primitive epithelium (arrows) on the surface of the definitive tegument (t) of a cercaria with developed spines (s) and discharged secretion of the lateral gland cells (sg); cm – circular muscles ($\times 23,000$).

Cheng and Bier 1972, Hockley 1972, Meuleman and Holzmann 1975), Bucephalidae (James and Bowers 1967), Philophthalmidae (Rees 1967), Lecithodendriidae (Belton and Belton 1971), Fellodistomatidae (Matricon-Gondran 1971a), Heterophyidae (Rees and Day 1976), Gymnophallidae (Al-Salman and James 1988) and Echinostomatidae (Bils and Martin 1966, Matricon-Gondran 1971b).

Bils and Martin (1966) in the cercaria of *Acanthoparyphium spinulosum* Johnston, 1917, and Matricon-Gondran (1971b) in the cercaria of *Paryphostomum segregatum* Dietz, 1909, are the only authors who described the presence of the primitive epithelium in echinostomes. However, these authors did not deal with the development of this structure, which is first described in the present paper.

The primitive epithelium, which has been variously named (see Meuleman and Holzmann 1975), is derived

from somatic cells within the embryo, with the exception of the embryos of *Schistosoma mansoni* (Rifkin 1970, Meuleman and Holzmann 1975). In this species the primitive epithelium is derived from the cytoplasmic extensions of the sporocyst subtegumental cells. The functions of the primitive epithelium are to feed and protect the developing cercarial embryo.

In *E. revolutum* cercaria as in the majority of other cercarial species the definitive tegument develops from a superficial nucleated layer beneath the primitive epithelium. The nuclei and surrounding cytoplasm sink later into the parenchyma resulting in an outer syncytium connected to subtegumental cells by cytoplasmic bridges. In *E. revolutum* cercaria the remnants of the primitive epithelium disappear at the stage when the lateral gland cells release their secretion into the definitive tegument.

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