Regional specializations of the tegument of the metacercaria of *Timoniella imbutiforme*

H.E.M. El-Darsh and P.J. Whitfield*

Infection and Immunity Research Group, Division of Life Sciences, King's College London, Campden Hill Road, London, W8 7AH, UK

Abstract

The ultrastructure of the spinous body tegument of the metacercaria of *Timoniella imbutiforme* (Molin, 1859) has recently been described. Other regions of the metacercarial tegument, including those of the oral sucker, pharynx, and nephridiopore, demonstrate considerable specializations. The oral sucker tegument had an aspinous outer syncytial layer that possessed a pimpled apical surface as well as enclosing two types of secretory bodies. The pharyngeal tegument likewise lacked spines, but possessed only one type of secretory body, and a smooth but folded outer surface. The nephridiopore tegument, however, showed the greatest degree of specialization possessing a single type of secretory body specific only to this region of the tegument. Also associated with the syncytium here was a prominent long filamentous glycocalyx, and microtubules which were observed for the first time in this region of the tegument.

Introduction

The structure of the tegument of many trematode species is not regionally invariant, but is characterized by modifications linked with the functional requirements of the separate regions (Whitfield, 1979; Fried & Haseeb, 1991). Such modifications are frequently expressed as a change in the apical surface outline (Erasmus, 1972; McMichael-Phillips *et al.*, 1994), or the replacement of some, or all of the secretory body types normally associated with the body tegument, with distinct forms specific only to the region of tegument in which they are detected (Robinson & Halton, 1983; Mattison *et al.*, 1992). On rare occasions normal tegumental inclusions of the tegument, such as mitochondria, may disappear from specialized regions (Robinson & Halton, 1983), or organelles such as Golgi complexes (McMichael-Phillips *et al.*, 1994) or nuclei (Robinson & Halton, 1983), not normally associated with the outer syncytium, may be present.

Frequently altered regions of tegument include the sucker teguments (Bibby & Rees, 1971; Hockley, 1973), the oesophagus and pharynx (Dike, 1971; Bennett & Threadgold, 1973; Hockley, 1973; Dunn *et al.*, 1987; McMichael-Phillips *et al.*, 1994), and the uterine tegument (Hockley, 1973; Robinson & Halton, 1983). Few accounts are available on the excretory tegument, although there were several on the excretory bladder epithelium (Erasmus, 1967; Powell, 1977; Matthews & Matthews, 1993).

The tegument of the metacercaria of *Timoniella imbutiforme* was examined and found to possess regional specializations. The structure of spinous body tegument was described by El-Darsh & Whitfield (1999), and the remaining regional specializations encountered in this species are compared and considered further in the present study.

Materials and methods

Details of the collection and preparation of specimens for transmission electron microscopy examination are described in El-Darsh & Whitfield (1999).

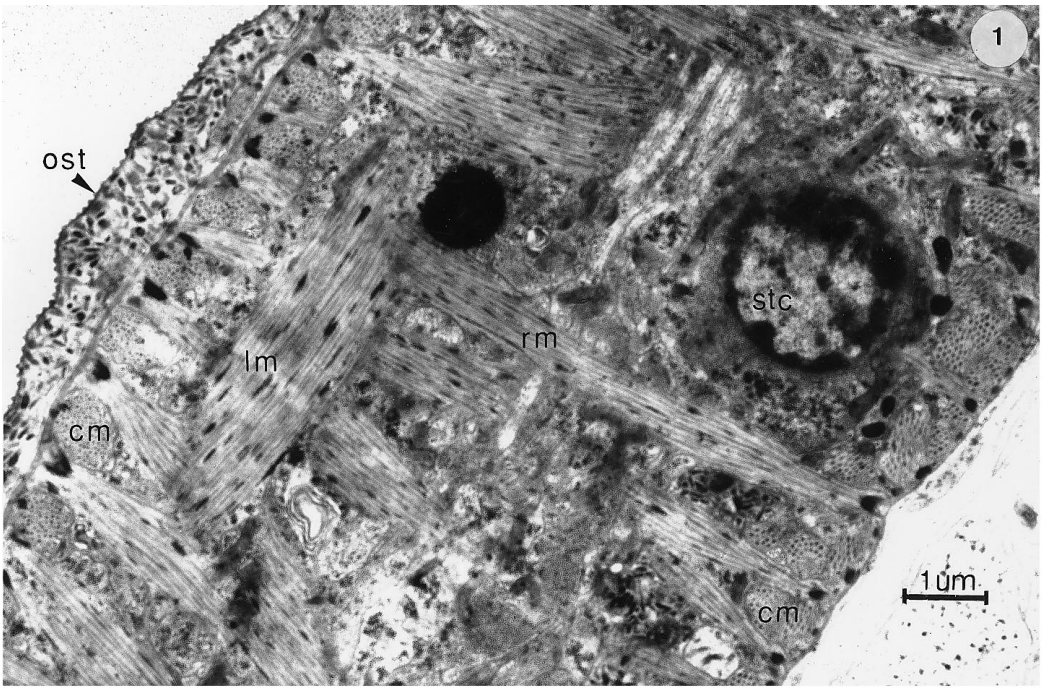
Results

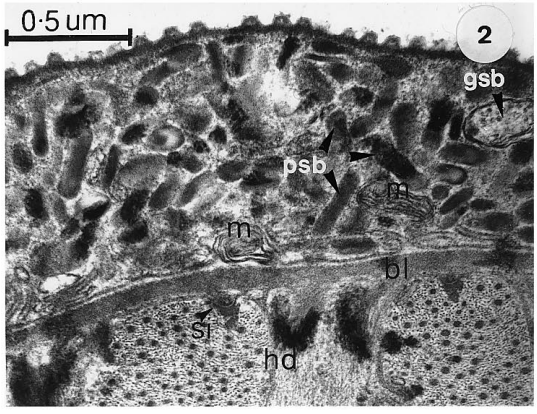
Oral sucker tegument

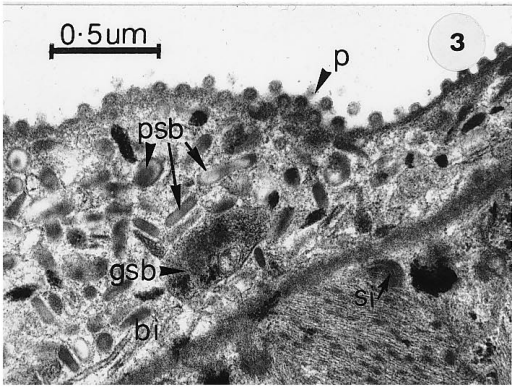
The oral sucker tegument conspicuously lacked spines (fig. 1). The outer surface was covered in small pimples similar in appearance and size (mean diameter 48 nm) to those of the aspinous areas of the body tegument. The

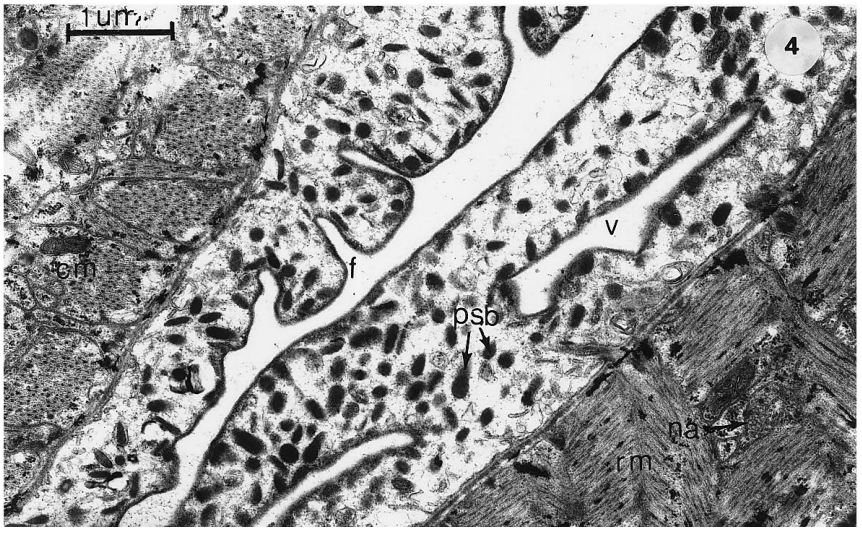
* Author for correspondence
Fax: 0171 333 4500
E-mail: phil.whitfield@kcl.ac.uk

https://doi.org/10.1017/S0022149X00700095 Published online by Cambridge University Press









https://doi.org/10.1017/S0022149X00700095 Published online by Cambridge University Press

mean overall thickness of this tegumental region was 0.66 μm, which was comparatively thinner than that of the spinous body tegument (mean thickness 1.3 μm). The basal membrane was frequently seen to invaginate into the tegumental matrix, producing finger-like processes (bi, fig. 3), lying parallel to the basal membrane and basal lamina. The matrix of the sucker tegument was less dense than that of the body tegument, and appeared granular (figs 2 and 3).

The basal lamina was discernibly thicker than that of the spinous body tegument, having a mean thickness of 74 nm. The interstitial layer associated with the basal lamina was absent from this site, and the basal lamina was directly attached to the subjacent sucker muscles by several large desmosomes, demarcated by areas of intense electron density (figs 1 and 2). The basal lamina was also attached to the basal membrane of the tegument by regularly spaced desmosomes, although these were considerably smaller.

The cytoplasmic inclusions of the sucker tegument included numerous secretory bodies, and a few mitochondria. The mitochondria occupied a basal position within the tegumental matrix (fig. 2), contrasting with the secretory bodies which were evenly distributed throughout the outer syncytium.

Two types of secretory bodies were encountered in the sucker tegument, a polymorphic electron dense secretory body (psb, figs 2 and 3), and a large oval shaped body with a distinctly granular matrix (gsb, figs 2 and 3). The polymorphic secretory bodies, as the name suggests, exhibited a wide variety of shapes ranging from elongate to oval and round. They were, nevertheless, the most abundant of the two types of bodies and smaller (elongate forms had a mean length of 0.22 μm, and a mean width of 0.06 μm) in relation to the granular form which ranged between 0.45 μm long, and 0.24 μm wide. The granular secretory bodies also exhibited marked differences in electron density, varying from electron lucent (fig. 2), to electron dense (fig. 3).

Although the outer tegumental syncytium of this region contained abundant numbers of secretory bodies, no disruptions of the basal membrane or basal lamina were detected to indicate the presence of cytoplasmic connectives from underlying subtegumentary cells. Subtegumentary cells were observed embedded in the muscle blocks of the suckers and not subjacent to them, as is characteristic of those associated with the body tegument. The cells appeared limited by the available space and could be seen elongating along the base of the suckers and frequently overlapped by radial muscles (fig. 1).

Pharyngeal tegument

The tegument of *T. imbutiforme* extends posteriad from the oral sucker to form the lining of the pharynx. At this site the tegument becomes thicker and more lobulated, and invaginations of the apical plasma membrane form deep pits which appear as large, elongated 'vacuoles' or spaces in certain sections (fig. 4). The tegument in this region was visibly thicker than the tegument of the suckers, ranging between 1.72 μm and 1.11 μm.

The outline of the apical membrane was smooth and no longer raised into pimples, although the surface area was increased by the formation of deep folds. The muscle layer subjacent to the pharyngeal syncytium was also as well developed, but not as thick as that of the oral sucker. Invaginations of the basal membrane were also detected in this region of the tegument, but the basal lamina was thinner (fig. 4) compared to that found associated with the oral sucker (fig. 2). The mean thickness of the lamina was 42 nm.

Mitochondria were few in the pharyngeal tegument, and the matrix of the syncytium was significantly less electron dense, and less granular than the previously described tegumental regions. Secretory bodies were loosely scattered throughout the pharyngeal tegument, and some were in direct contact with the apical membrane at the surface (fig. 4). The secretory bodies resembled the polymorphic secretory bodies of the sucker tegument with similar dimensions. The shapes ranged between round and oval, to pear shaped, and rod shaped. Most were electron dense with a homogenous matrix.

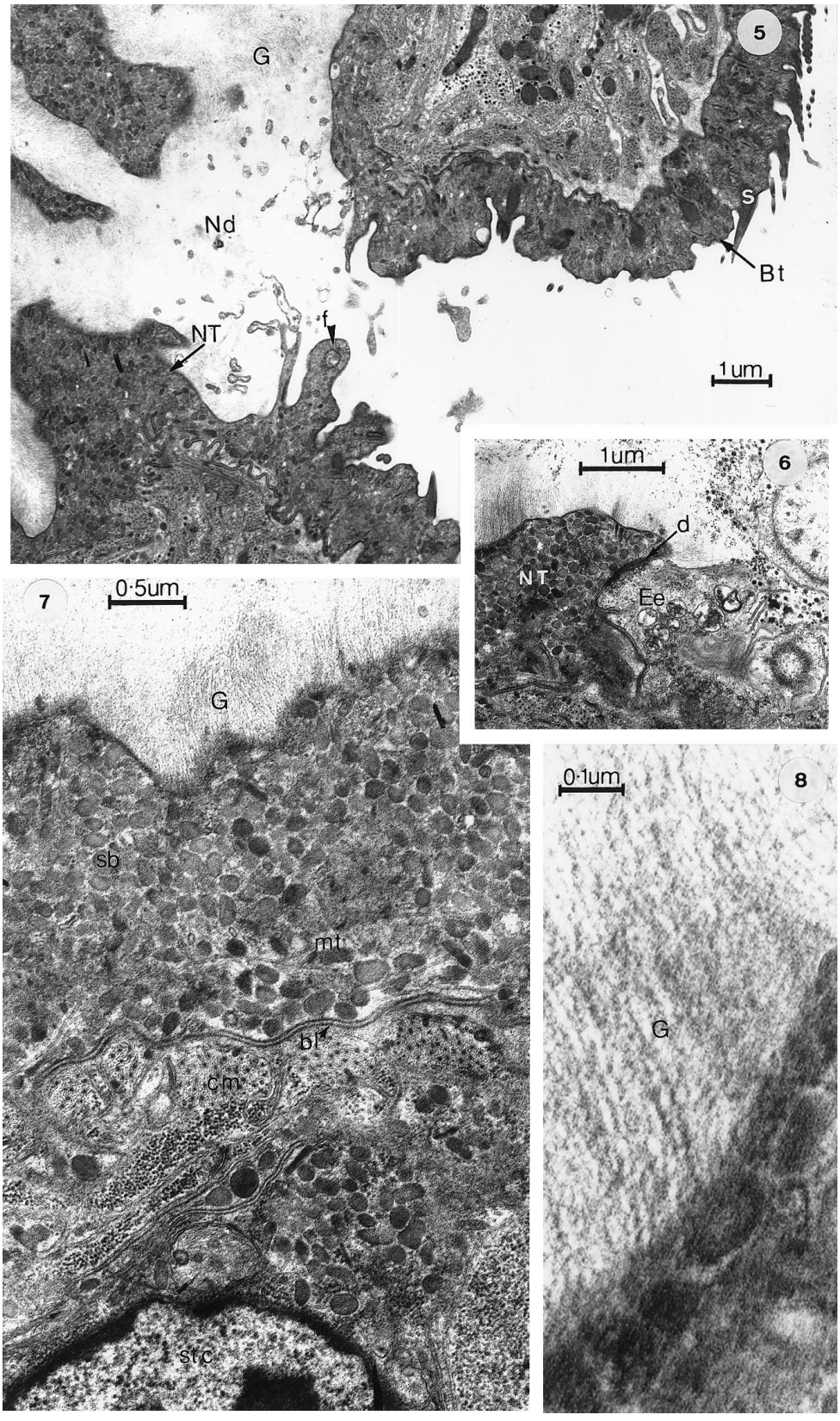
No disruptions of the basal membrane or lamina were detected in this area of the tegument and consequently no subtegumentary cell connectives were detected. The subtegumentary cells presumably associated with this region of the tegument were also not encountered in the micrographs.

The tegument of the nephridiopore duct

The most significantly modified area of tegument of *T. imbutiforme* was that associated with the nephridiopore and the nephridiopore duct which opened internally into the excretory bladder (lined by an epithelial layer) (see fig. 6). The tegument of the duct varied from that of the body tegument mainly by the absence of spines and the substitution of the latter's secretory bodies with an alternate type of secretory body specific to the duct region only (figs 5 and 7). The change in the secretory body content was also correlated with the presence of a filamentous glycocalyx, which appears to be emanating from individual secretory bodies in direct contact with the tegumental surface (fig. 8). The entrance of the nephridiopore duct was demarcated by the presence of guarding 'flaps' or attenuations of the tegumental syncytium of opposing sides, which left an aperture of only 1.4 μm wide between them (fig. 5). Larger extensions of the nephridiopore tegument also occurred within the lumen of the duct which resulted in an increment in the surface area, as well as a thickening of this region. The mean thickness of the tegument in the duct was 1.9 μm

Figs 1–4. Transmission electron micrograph of the metacercaria of *Timoniella imbutiforme*. 1. Tegument and subjacent muscle block of the oral sucker. 2. Tegumental syncytium of the oral sucker showing the secretory body inclusions. 3. Pimpled apical surface of the tegumental syncytium of the oral sucker. 4. Tegument and subjacent muscle block of the pharynx. Basal invaginations (bi), basal lamina (bl), circular muscles (cm), folds (f), granular secretory body (gsb), hemidesmosome (hd), longitudinal muscles (lm), mitochondria (m), neural axon (na), oral sucker tegument (ost), pimples (p), polymorphic secretory bodies (psb), radial muscles (rm), sarcolemmal indentation (si), subtegumentary cells (stc), vacuoles (v).

https://doi.org/10.1017/S0022149X00700095 Published online by Cambridge University Press



https://doi.org/10.1017/S0022149X00700095 Published online by Cambridge University Press

(range 1.5–2.2 μm) (fig. 7). This, however, becomes 2.4 μm when extensions are taken into account (range 1.5–3.6 μm). The nephridiopore tegument abutted the epithelial layer lining the excretory bladder, and was attached to it by a large desmosome at the apical part of the tegument (fig. 6).

The change from body tegument to nephridiopore tegument was clearly not an abrupt, but rather a gradual process (fig. 5). The spinous body tegument begins to lose its spines and most of its common secretory bodies at about 8 μm distance from the nephridiopore tegument. These are gradually replaced by the secretory bodies of the nephridiopore tegument. Below the syncytium, the basal lamina is also changing in thickness, reducing to 14 nm thick at the duct entrance, similar in size to the overlying basal membrane. Concurrently the interstitial layer is also diminishing, becoming almost completely lost beneath the guarding 'flaps' of the nephridiopore (fig. 5).

The only inclusions present in this region of the tegument were the secretory bodies, of which only one type was distinguished, occasional vacuoles, and microtubules near the base of the syncytium (fig. 7). The microtubules lay parallel or slightly oblique to the basal membrane. No mitochondria were observed in the tegumental syncytium.

The secretory bodies encountered in this region were oval in form (mean diameter 0.13 μm, range 0.09–0.23 μm) and of moderate electron density, although some did appear more or less dense. They were present in great numbers in the syncytium, and some were observed in direct contact with the surface membrane. Those found at the surface, appeared to disrupt the continuity of the apical membrane, allowing strands of what appeared to be a thick, electron-dense glycocalyx to emerge (fig. 8). The glycocalyx extended a mean distance of 0.88 μm into the lumen of the duct.

The lumen of the nephridiopore duct also contained large 'islands' of tegument, resulting from sections through the long, and apparently large tegumental extensions (fig. 5). The contents of these 'islands' were the same as those sections of tegument lining the duct. The glycocalyx arising from them, together with that from the tegumental lining, occupied most of the duct lumen (fig. 5).

The subtegumentary cells were directly subjacent to a single layer of circular muscle fibres arranged in round bundles beneath the basal lamina (fig. 7). The cells each contained a nucleus, free ribosomes, smooth endoplasmic reticulum, and secretory bodies similar to those found in the syncytium (fig. 7).

Discussion

Several areas of the body of the metacercaria of *T. imbutiforme* were covered with specialized regions of the tegument, which is not uncommon in digenean species (Fried & Haseeb, 1991). These regions were obviously all continuous with each other, as is invariably characteristic of the tegument of other digeneans so far described (Robinson & Halton, 1983; Dunn *et al.*, 1987).

The spinous body tegument covering the external face of the fluke was found to enclose up to four types of secretory bodies in various proportions, as well as mitochondria, vacuoles and spines. The presence of at least two discrete types of body spines indicates the further diversity of the body tegument (El-Darsh & Whitfield, 1999). The oral sucker tegument was characterized by the absence of spines, the presence of different types of secretory bodies, a pimpled outer surface, and the lack of an interstitial layer. In a manner similar to that in other juvenile digeneans (Dunn *et al.*, 1992a) the subtegumentary cells of this region were also present within the muscle blocks of the oral sucker, unlike the body tegument where the subtegumentary cells were found subjacent to the superficial muscles. The modifications of the pharyngeal tegument were characterized by the absence of pimples on the surface, an increase in the apical surface area brought about by multiple deep invaginations, and an obvious reduction in the number of secretory bodies (see also Bennett and Threadgold, 1973; Dunn *et al.*, 1987). The region that demonstrated the greatest degree of variation from the remaining tegumental areas was that lining the nephridiopore duct. The absence of any membranous barriers between the body tegument and the nephridiopore tegument showed that this system was truly a continuous syncytium, where a gradual reduction of inclusions of one region occurred as the features of the next appeared. The nephridiopore also contained very characteristic secretory bodies, not present in any other tegumental region, and possessed a very clearly defined, and unusually thick, filamentous glycocalyx. The presence of a large desmosome separating this tegument from the adjacent epithelial layer of the excretory bladder was evidence indicating the discontinuity between these two regions, but affirming the continuity of the tegument since no such structures were ever observed between the various tegumentary regions.

What is intriguing about the diversity of the specialized regions of the tegument is that they all occur in a single syncytium (Whitfield, 1979; Robinson & Halton, 1983; Dunn *et al.*, 1987). All the manifestations of each region, the surface alterations, the different secretory bodies, the various other inclusions such as the different spines, are all present in an outer cytoplasmic layer that is continuous over the whole body forming a single unbroken covering for the fluke. The cover also extends in *T. imbutiforme*, as far in as the pharynx and possibly further and, at the posterior of the fluke, lines the nephridiopore. Therefore there is no external region of the fluke that is exposed and not covered by this continuous tegumental layer. The question then arises as to what causes this diversity and differentiation in an obviously continuous cytoplasmic system?

Figs 5–8. Transmission electron micrographs of the tegument of *Timoniella imbutiforme*. 5. Transitional region between the body tegument and the nephridiopore tegument. 6. Junction between nephridiopore tegument and excretory bladder epithelium demarcated by a large desmosome. 7. Nephridiopore tegument. 8. Glycocalyx of the nephridiopore tegument. Basal lamina (bl), body tegument (Bt), circular muscles (cm), desmosome (d), excretory epithelium (Ee), 'flap' (f), glycocalyx (G), microtubules (mt), nephridiopore duct (Nd), nephridiopore tegument (NT), secretory body (sb), spine (S), subtegumentary cell (stc).

https://doi.org/10.1017/S0022149X00700095 Published online by Cambridge University Press

The functional role of each region is presumably correlated with the types of secretory bodies and other inclusions in the corresponding tegumental area (Fried & Haseeb, 1991). It is assumed here that the most probable regulators of the regional attributes of the tegument will be the nucleated components, the subtegumentary cells (Whitfield, 1979), since they are the source of the various inclusions found in the outer cytoplasmic layer. The simplest assumption is that each regionally differentiated zone of tegument demonstrates the different inclusions it does in the outer cytoplasmic layer because of particular and differentiated patterns of gene expression in the subtegumentary cells that connect with it. In the regions where the characteristics of one zone blend into those of another, the boundary zone is presumably affected by gene products from two groups of specialized subtegumentary cells.

These specialized types of subtegumentary cells were probably differentiated in the embryonic cercariae within the rediae, since it is at this stage that the cells of the various regions of the body undergo differentiation (Dunn *et al.*, 1992b). The cercarial (also metacercarial and adult) tegument is probably produced from a mass of ectodermal nuclei in which the differentiation patterns occur at this period of development. The eventual outcome is the various regional specializations of the tegument with their own specific sets of expressed genes, and characteristic synthetic products.

Acknowledgements

We would like to thank Mr Alan Maple for his technical help during the preparation of electron microscopical samples, Mr John Pacey and Ms Jane Storey for their invaluable assistance in the production of micrographs, Mr Reg Reed for assistance with fish sampling and the staff at Lots Road Power Station for making the fish sampling possible.

References

Bennett, C.E. & Threadgold, L.T. (1973) Electron microscope studies of *Fasciola hepatica*. XIII. Fine structure of newly excysted juvenile. *Experimental Parasitology* **34**, 85–99.

Bibby, M.C. & Rees, G. (1971) The ultrastructure of the epidermis and associated structures in the metacercaria, cercaria, and sporocysts of *Diplostomum phoxini* (Faust, 1918). *Zeitschrift für Parasitenkunde* **37**, 169–186.

Dike, S.C. (1967) Ultrastructure of the ceca of the digenetic trematodes *Gorgodera amplicava* and *Haematoloechus medioplexus*. *Journal of Parasitology* **53**, 1173–1185.

Dunn, T.S., Hanna, R.E.B. & Nizami, W.A. (1987) Ultrastructural and histochemical observations on the foregut and gut caeca of *Gigantocotyle explanatum*, *Gastrothylax crumenifer* and *Srivastavaia indica* (Trematoda: Paramphistomidae). *International Journal for Parasitology* **17**, 1141–1152.

Dunn, T.S., Dang, P.H., Mattison, G., Hanna, R.E.B. & Nizami, W.A. (1992a) Ultrastructural observations on the redial tegument of *Paramphistomum epiclitum* from the planorbid snail, *Indoplanorbis exustus*. *Journal of Helminthology* **66**, 167–176.

Dunn, T.S., Dang, P.H., Mattison, G., Hanna, R.E.B. & Nizami, W.A. (1992b) Embryological development of the cercarial tegument of *Paramphistomum epiclitum* in the planorbid snail, *Indoplanorbis exustus*. *Journal of Helminthology* **66**, 243–254.

El-Darsh, H.E.M. & Whitfield, P.J. (1999) Ultrastructure of the tegument of the metacercaria of *Timoniella imbutiforme*. *Journal of Helminthology* **74**, 57–66.

Erasmus, D.A. (1967) Ultrastructural observations on the reserve bladder system of *Cyathocotyle bushiensis* Khan, 1962 (Trematoda: Strigeoidea) with special reference to lipid excretion. *Journal of Parasitology* **53**, 525–536.

Erasmus, D.A. (1972) Metabolism and the host–parasite interface, pp. 214–261 in *The biology of trematodes*. Belfast, Edward Arnold.

Fried, B. & Haseeb, M.A. (1991) Platyhelminthes: Aspidogastrea, Monogenea, and Digenea. pp. 141–209 *in* Harrison, F.W. (*Ed.*) *Microscopic anatomy of invertebrates*. New York, Wiley-Liss, Inc.

Hockley, D.J. (1973) Ultrastructure of the tegument of *Schistosoma*. *Advances in Parasitology* **11**, 233–297.

Matthews, R.A. & Matthews, B.F. (1993) *Cryptocotyle lingua* in mullet, *Chelon labrosus*; significance of metacercarial excretory proteins in the stimulation of the immune response. *Journal of Helminthology* **67**, 1–9.

Mattison, R.G., Hanna, R.E.B. & Nizami, W.A. (1992) Ultrastructure and histochemistry of the digestive tract of juvenile *Paramphistomum epiclitum* (Paramphistomidae: Digenea) during migration in Indian ruminants. *International Journal for Parasitology* **22**, 1089–1101.

McMichael-Phillips, D.F., Lewis, J.W. & Thorndyke, M.C. (1994) Ultrastructure of the digestive system of adult *Sanguinicola inermis*. *Journal of Helminthology* **68**, 149–154.

Powell, E.C. (1977) Ultrastructural development of the excretory bladder in early metacercariae of *Ochetosoma aniarum* (Leidy, 1891). *Proceedings of the Helminthological Society of Washington* **44**, 136–140.

Robinson, R.D. & Halton, D.W. (1983) Functional morphology of the tegument of *Corrigia vitta* (Trematoda: Dicrocoeliidae). *Zeitschrift für Parasitenkunde* **69**, 319–333.

Whitfield, P.J. (1979) *The biology of parasitism: an introduction to the study of associating organisms*. London, Edward Arnold.

(Accepted 28 August 1999)