Parasitol Res (1996) 82:312–318

ORIGINAL PAPER

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Ultrastructure of the gut caecal epithelium of *Pricea multae* (Monogenea: Polyopisthocotylea)

Received: 20 June 1995 / Accepted: 3 November 1995

Abstract Ultrastructural observations of the gut caecal epithelium of *Pricea multae* revealed the presence of pigmented and non-pigmented digestive cells. The pigmented digestive cells were separated by a connecting syncytium and appeared elongated,with numerous vesicles appearing toward the apical cell surface. They were characterised by granular inclusions in the form of electron-dense pigments, which were eliminated by exocytosis. These cells and their lamellar connecting syncytium were observed projecting into the gut lumen. Transverse sections of the gut epithelium revealed intact portions of digestive cells lying in the lumen. Endocytosis at the apical surface of the pigmented digestive cells gave rise to the formation of granular inclusions, which appeared as electron-dense pigments confined to lysosomal vesicles within the digestive system. Electron X-ray microanalysis indicated these granules were primarily composed of iron, demonstrating that *P. multae* is a blood-feeder. The connecting syncytium, while probably involved in a structural, supportive role, may additionally function in the absorption of micromolecular nutrients from the host blood meal.

Introduction

The ultrastructure of the gut caeca of a number of monogeneans has previously been described (Smyth and Halton 1983). Monopisthocotyleans are known to feed on host epidermal cells and mucus, which is digested by single, columnar shaped digestive cells present in the gut caeca (Kearn 1963; Halton and Jennings 1965; Halton and Stranock 1976a, b), whereas the gastrodermis of polyopisthocotyleans, which feed principally on the host blood, consists of pigmented digestive cells known as haematin cells interconnected by a non-pigmented syncytium (Llewellyn 1954; Halton et al. 1968; Rohde 1975; Tinsley 1973; Halton 1975, 1976). Exceptions to these generalisations have also been observed, for example, *Euzetrema*, a monopisthocotylean, is a known blood-feeder (Fournier 1978), whereas some of the polyopisthocotyleans, such as *Polystomoides*, *Polystomoidella* and *Neopolystoma*, feed on host epithelial cells and mucus (Tinsley 1974; Allen and Tinsley 1989). The digestive cells, which are known to pinocytose the components of the host blood, are characterised by granular inclusions in the form of an electron-dense pigment, which is believed to be the result of intracellular digestion of the host haemoglobin (Halton 1974b, 1975, 1982). The indigestible residues, which are composed mostly of haematin, are eliminated by exocytosis and subsequently excreted via the mouth.

Another mechanism that has been proposed for elimination of these by-products is the sloughing off from the gut caecum of the intact digestive cells, which eventually disintegrate in the gut lumen, a process that involves cyclic loss and replacement of the digestive cells (Llewellyn 1954; Rohde 1975; Halton 1976). The latter hypothesis is based on the apparent discontinuous organisation of the gastrodermal cells and the infrequent occurrence of free, undamaged digestive cells in the lumen of the gut of several monogeneans (Rohde 1975; Halton 1976; Fournier 1978; Allen and Tinsley 1989). Autoradiographic pulse-chase experiments in *Diclidophora merlangi* revealed that if sloughing off and renewal of digestive cells did occur, then it did so only rarely or at an undetectably low rate (Halton 1976). Hence, no clear explanation to account for the numerous observations of intact haematin cells in the gut lumen has been suggested.

The present study describes the ultrastructural organisation of the gut caecum of *Pricea multae*. The light microscopic morphology of *P. multae* was described initially by Chauhan (1945) and later by Ramalingam (1952)

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and Rohde (1976), whereas the surface topography and ultrastructure of the tegument and haptor have been described by Ramasamy et al. (1986).

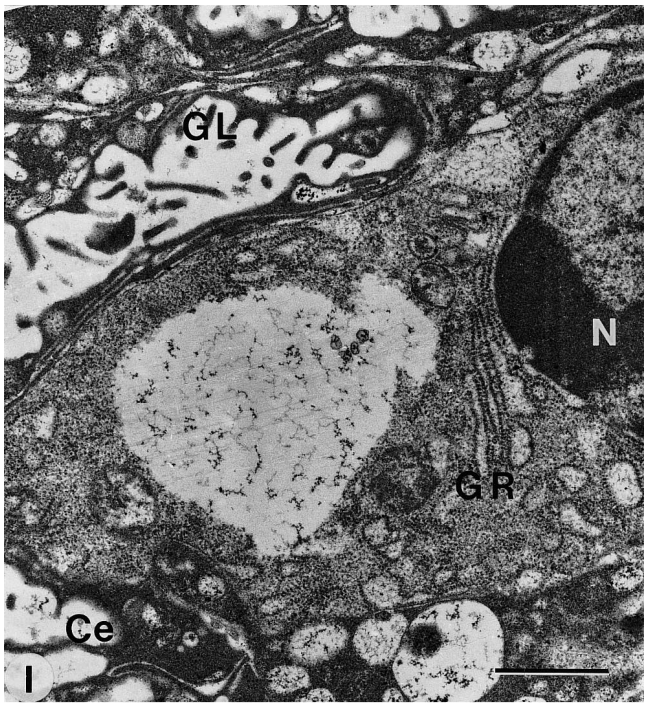
Materials and methods

Live adult specimens of *Pricea multae* were collected from the seer fish, *Scomberomorus commerson*, caught in the Bay of Bengal and landed at Marina Beach, Madras, India. Specimens were washed in seawater, fixed for 4 h at 4°C in 4% glutaraldehyde or glutaraldehyde-paraformaldehyde buffered to pH 7.2 with 0.1 *M* sodium cacodylate buffer containing 1% sucrose and 3% NaCl. The specimens were washed several times in sodium cacodylate buffer, post-fixed for 1 h in 1% OsO_4, dehydrated through alcohol to propylene oxide and infiltrated and embedded in Epon 812 resin for 48 h at 60°C. Thin sections (60–70 nm in thickness) were cut using a Reichert Ultracut E ultramicrotome with a diamond knife, collected on bare 200-mesh copper grids, double-stained with uranyl acetate (5 min) and lead citrate (8 min) and examined in a JEOL 100CX transmission electron microscope operating at 100 kV. For X-ray microanalysis the specimens were fixed for 2 h at 4°C in 2% double-distilled glutaraldehyde buffered with 0.1 *M* sodium cacodylate containing 1% sucrose and 3% NaCl at pH 7.2. The specimens were buffer-washed overnight, dehydrated in ethanol and embedded in Epon 812 resin. Semi-thin sections (100–150 nm in thickness) were cut on the Reichert Ultracut E ultramicrotome with a diamond knife, mounted on bare 200-mesh aluminium grids, coated with carbon and viewed unstained using a JEOL 100CX TEM SCAN transmission electron microscope fitted with a LINK energy-dispersive X-ray spectrometer system (EDS).

Results

Electron microscopy

The gut caecum of *Pricea multae* is extensive, with branches ramifying amongst the tissues of vitellaria, the testes and the protonephridial capillaries. Ultrastructural examination of the gut caecal epithelium of *P. multae* revealed that the gastrodermis consists of two types of cells, namely, the pigmented digestive (haematin) cells and the non-pigmented connecting syncytial cells (Figs. 1–4). The pigmented digestive cells occur singly or in groups of varying size, shape and number (Figs. 1–5). Between the pockets of digestive cells, a connecting syncytial epithelium is present throughout the gut caecum. This syncytium alone covers extensive areas of the caecum, even though digestive cells may be absent. The cytoplasmic surface of some of the pigmented digestive cells and connecting syncytium are visible projecting deep into the lumen of the gut. The lateral surfaces of each pigmented digestive cell are supported by a non-pigmented, lamellar connecting syncytium and, thus, the pinocytotic and/or exocytotic surfaces of the digestive cells are limited by a microvillus luminal surface (Figs. 3, 11). Each digestive cell contains a basally positioned nucleus, large mitochondria, a Golgi complex and abundant granular endoplasmic epithelium (GER) and is characterised by granular inclusions, in the form of an electron-dense pigment, which vary in size and number.



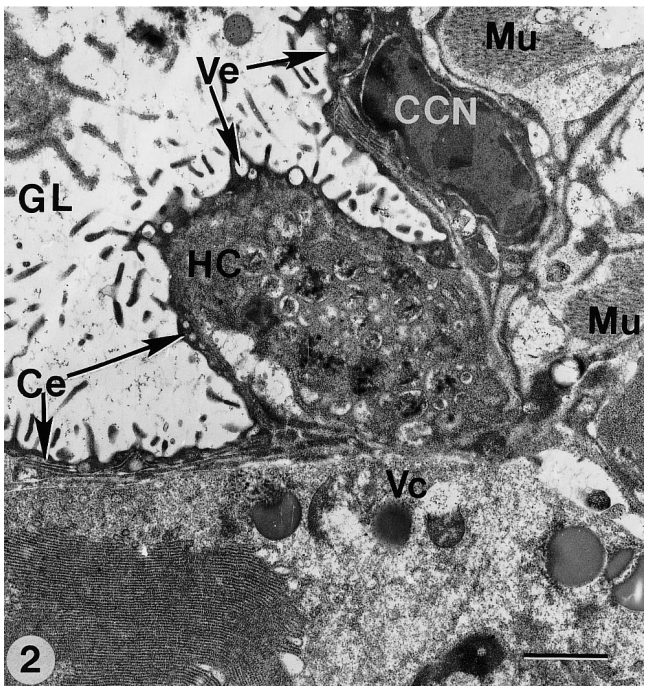


Fig. 1 *Pricea multae*: section through a digestive cell, showing a nucleus (*N*), granular endoplasmic reticulum (*GR*), connecting syncytium (*Ce*) and gut lumen (*GL*). *Bar*=1 μm

Fig. 2 *P. multae*: section through a portion of the gut caecum, showing the pigmented digestive cell (*HC*), a connecting syncytial cell (*CCN*) and the connecting syncytium (*Ce*) covering the surface of the digestive cell and lining the gut lumen (*GL* Gut lumen, *Vc* vitelline cell, *Mu* muscle, *Ve* connecting synctium vesicles). *Bar*=1 μm

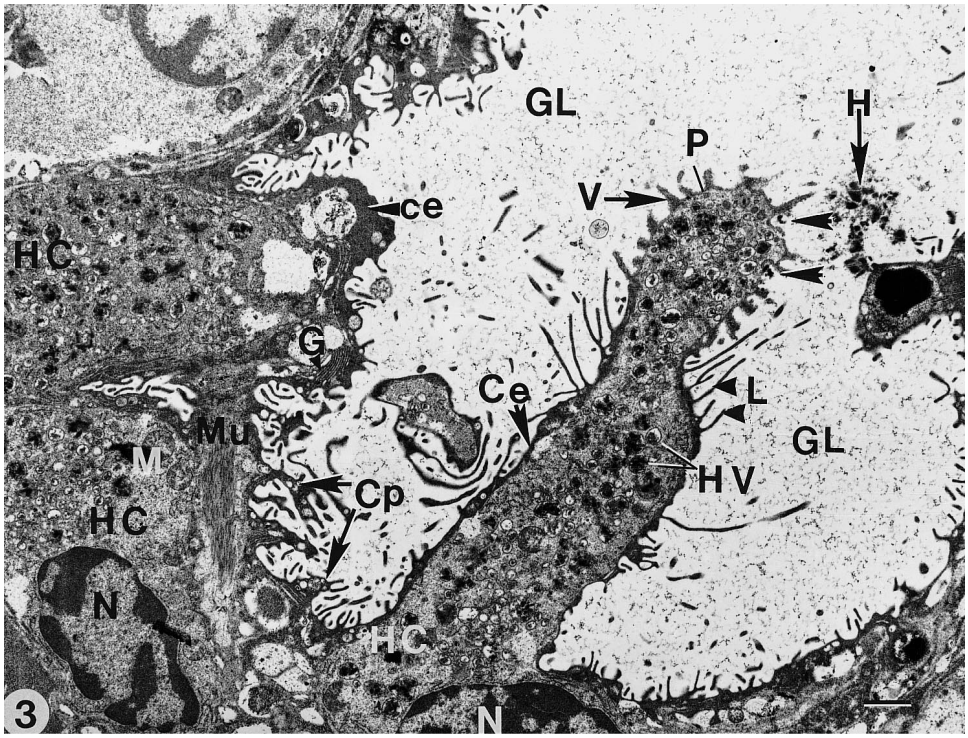


Fig. 3 *P. multae*: section through the gut caecum, showing an elongated digestive cell (*HC*) projecting its cytoplasm a considerable distance into the lumen of the gut (*GL*). Pinosomes (*P*) and presumed exocytotic vesicles are confined to the microvillous apical surface (*V*) of the digestive cell. Note the occurrence of adjacent digestive cells and their cytoplasmic inclusions. The connecting syncytium (*Ce*) is lamellar (*L*) and branched (*Cp*), projecting into the lumen of the gut and connecting the digestive cells on their lateral surfaces (*G* Golgi complex of the connecting syncytium, *H* free haematin, *M* mitochondrion, *N* nucleus, *P* pinosome, *HV* digestive vesicles, *V* microvilli, *Mu* muscles, *unlabeled arrowheads* exocytosis, *GL* gut lumen). *Bar*=1 μm

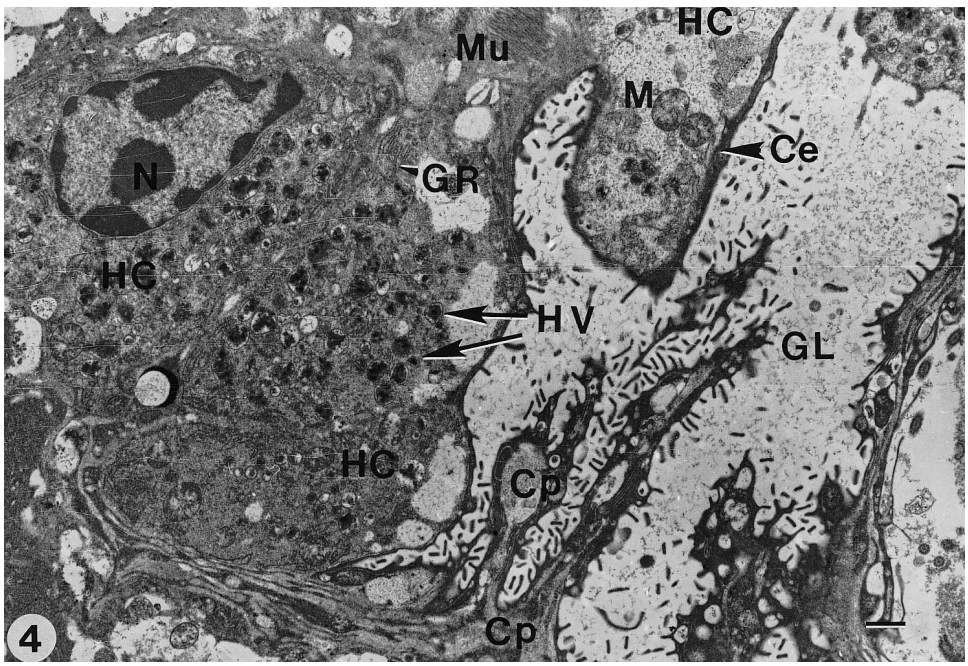


Fig. 4 *P. multae*: section through the gut caecum. Note the lamellar projections of portions of the connecting syncytium (*Cp*) into the lumen of the gut (*GL* Gut lumen, *HC* digestive cell, *HV* digestive vesicles, *GR* granular endoplasmic reticulum, *N* nucleus, *M* mitochondrion, *Mu* muscle, *Ce* connecting syncytium). *Bar*=1 μm

This pigment is confined to lysosomal vesicles within the digestive cell. Numerous vesicles are present towards the apical surface of the digestive cells, possibly as a result of pinocytosis and/or exocytosis. The connecting syncytium consists of occasional nucleated regions and of electron-dense cytoplasm possessing GER, a Golgi complex, mitochondria and many lucent or moderately electron-lucent vesicles. Pinocytotic and exocytotic vesicles are distinctly absent at the lamellar surface of the connecting syncytium.

Intact portions of pigmented digestive cells and cells containing electron-dense secretory granules resembling vitelline cells and exocytosed material occur freely in the lumen of the gut caecum (Figs. 6–9). Smooth muscles occur at the baso-lateral region of the digestive cells or beneath the connecting syncytium (Figs. 2–4). Nerve fibres and axons containing presumed neurosecretory vesicles, neurotubules and mitochondria are visible running up to the baso-lateral region of the digestive cells and beneath the connecting syncytium (Figs. 10, 11).

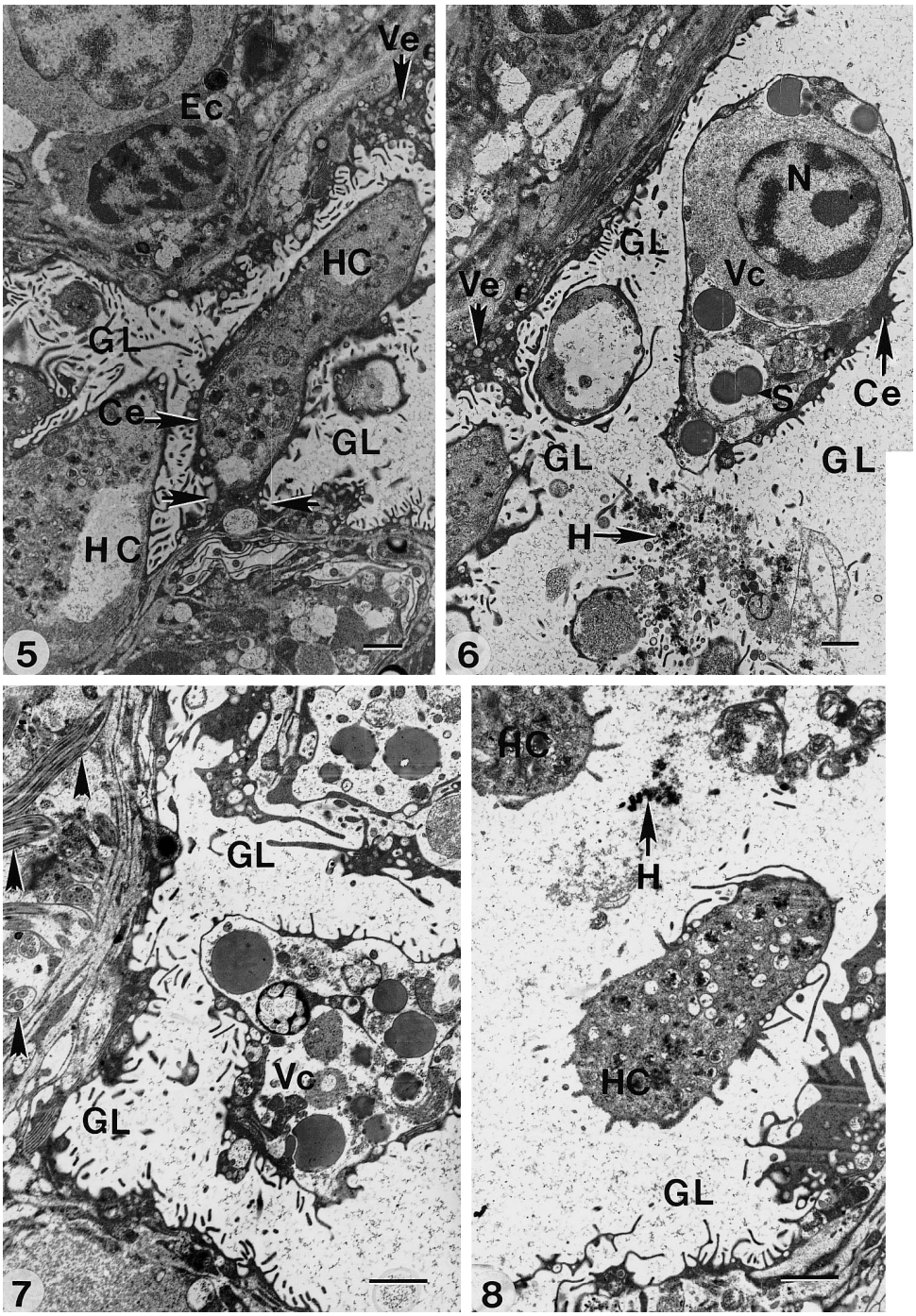


Fig. 5 *P. multae*: section through the gut caecum, showing a portion of digestive cell (*HC*) projecting into the gut lumen (*GL*) and covered by connecting syncytium (*Ce*). Note the nucleated cells, which may possibly represent connecting cells or embryonic haematin cells (*Ec*). (*Ve* connecting syncytium vesicles, *unlabeled arrowheads* point of attachment of the digestive cell) *Bar*=1 μm

Fig. 6 *P. multae*: section through the gut caecum, showing a possible nucleated vitelline cell (*Vc*) containing electron-dense secretory granules (*S*) lying in the gut lumen (*GL*). The cell is covered by a connecting syncytium (*Ce*). Note the free haematin (*H*) and other cellular debris present in the gut lumen, probably deriving from exocytosis of secretory granules, and the electron-lucent vesicles (*Ve*) of the connecting syncytium. *Bar*=1 μm

Fig. 7 *P. multae*: section through the gut caecum, possibly showing a portion of a vitelline cell (*Vc*) containing secretory materials. Note the lamellar wall of the gut caecum and spermatozoa (*unlabeled arrowheads*) beneath the connecting syncytium (*GL* Gut lumen). *Bar*=1 μm

Fig. 8 *P. multae*: section through the gut caecum, showing a cross-sectional view of a digestive cell (*HC*) in the lumen of the gut (*GL* Gut lumen, *H* Free haematin). *Bar*=1 μm

Electron X-ray microanalysis

X-ray microanalysis was carried out on semi-thin sections (150 nm) of *P. multae* to determine the nature of the observed electron-dense inclusions in the pigmented cells. Spectral analysis identified K_a and K_b X-ray emissions for the element iron (Fe; Fig. 12). The X-ray peaks for silicon (Si) and copper (Cu) are derived from the embedding resin and the specimen holder, respectively. The extraneous peak for aluminium (Al) is derived from the support grid.

Discussion

The current study on the gut caecal epithelium of *Pricea multae* clearly demonstrates the occurrence of single digestive cells and groups of digestive cells that are separated and supported on their lateral surfaces by a connecting syncytium as in common with many other polyopisthocotylean monogeneans. However, in the monogenean polyopisthocotylean *Diclidophora merlangi* the digestive cells were found to occur only singly and never in groups. In *P. multae*, ultrastructural examination of the

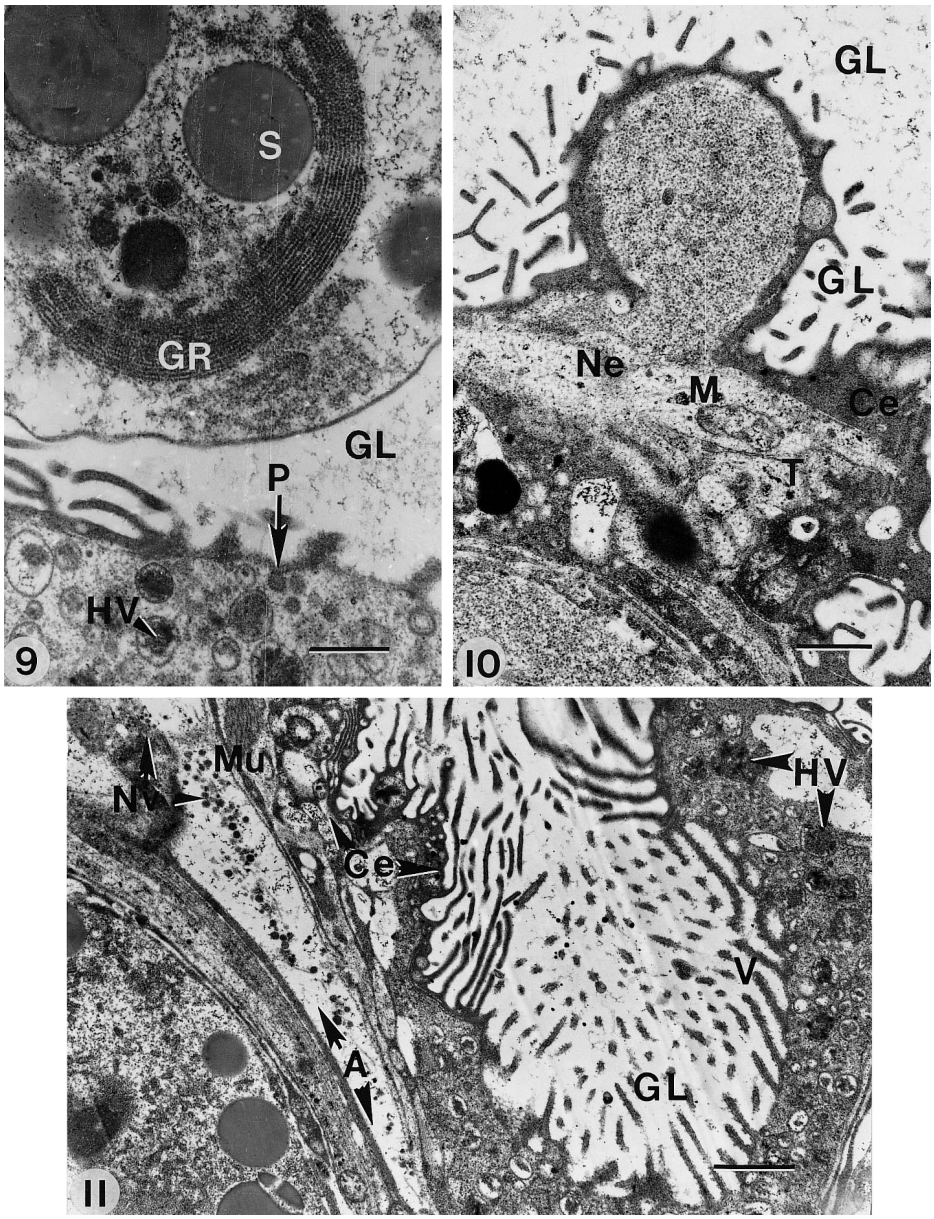


Fig. 9 *P. multae*: note the intact granular endoplasmic reticulum (*GR*) and secretory vesicles (*S*) of a cell in the lumen of the gut (*GL* Gut lumen, *P* pinosome, *HV* digestive vesicle). *Bar*=0.5 μm

Fig. 10 *P. multae*: neuronal process (*Ne*) at the base of a globular structure in the gut that runs up to the connecting syncytium (*Ce*). Note the mitochondria (*M*) and neurotubules (*T*). *Bar*=0.5 μm

Fig. 11 *P. multae*: a portion of the gut caecum, showing an axon (*A*) with presumed neurosecretory vesicles (*Nv*) running to the baso-lateral surface of the digestive cell. Note the gut lumen (*GL*), microvilli (*V*), muscles (*Mu*), digestive vesicles (*HV*) and connecting syncytium (*Ce*). *Bar*=1 μm

gut caecal epithelium revealed that digestive cells appear elongated and compartmentalizsed into an apical cytoplasmic region with coated pinocytotic and/or exocytotic vesicles, responsible for the transport of materials for intracellular digestion and, eventually, the removal of waste materials from the cell, respectively. A basal nucleated region consists of GER, a Golgi complex and mitochondria. Transverse sections of the gut caecum revealed portions of digestive cells lying in the gut lumen, suggesting they may possibly represent the cross section of the elongated digestive cells rather than cells that may have been sloughed off from the gastrodermis to lie free in the lumen. These observations in *P. multae* suggest that shedding and renewal of the digestive cells does not occur.

Shedding and disintegration of the digestive cells has been reported in *Polystomoides* sp. infecting frogs (Rohde 1975) but is absent in polystomatid monogeneans infecting chelomins (Allen and Tinsley 1989). Cellular disintegration and shedding has also been reported during the synchronous digestive cycle in the blood-feeding monopisthocotylean *Euzetrema knoepffleri* (Fournier 1978). In *D. merlangi*, sloughing off and renewal of digestive cells occurs only rarely and evidence of such an event is restricted to the occasional finding of a free and relatively undamaged cell in the lumen of the main caecum (Halton 1974a, 1975, 1976). According to Halton (1976), incidental sloughing off and replacement of digestive cells could occur in monogeneans in response to conditions of stress imposed by body movements and feeding or senescence, and to validate this point, stereological details of the so-called free digestive cells in the gut lumen would be necessary to clarify whether they are indeed free or attached to the gastroder-

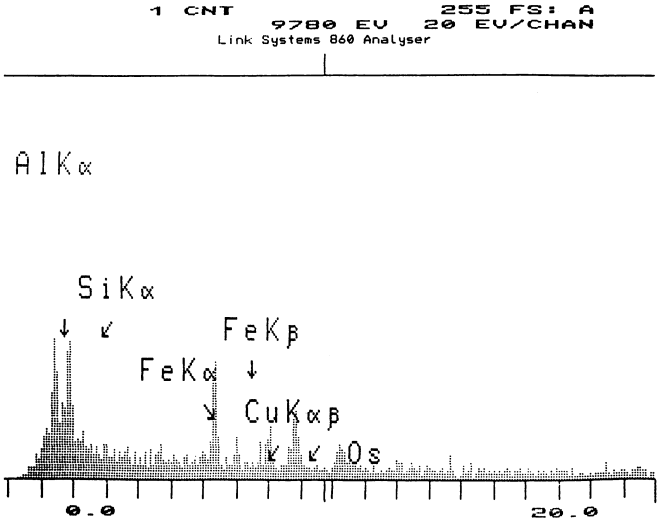


Fig. 12 *P. multae*: an X-ray energy spectrum from the analysis of an electron-dense granule in a pigmented cell. The K_a and K_b peaks for iron (*Fe*) represent the principal constituent element. The copper (*Cu*) and silicon (*Si*) peaks represent extraneous elements derived from the microscope column and the embedding medium, respectively

mis. Allen and Tinsley (1989) observed apparently free digestive cells in the lumen of the gut of *Polystomoides* sp.; however, serial sectioning revealed that the majority of these cells were attached to the gastrodermal syncytium.

The present study also reveals the occurrence of cells resembling vitelline cells in the lumen of the gut caecum of *P. multae*. These presumed vitelline cells may possibly have been released into the gut via the genito-intestinal canal, which is generally considered an outlet for excess shell material that is to be degraded and recycled (Smyth and Halton 1983). However, the presence of a lamellar epithelium limiting the presumed vitelline cell suggests that it may be the connecting syncytium of the gut caecum that projects into the lumen of the gut caecum, in which case these cells appear only in cross section.

The apical surface of the digestive cells in *P. multae* is elevated into microvilli, which undoubtedly increases the absorptive surface area, as in other platyhelminths (Smyth and Halton 1983). The presence of presumed coated vesicles and pits in the apical surface of the digestive cells suggests that endocytosis of host macromolecules occurs. In *D. merlangi*, haemoglobin and other host blood proteins are sequestered into the digestive cells by endocytosis (Halton et al. 1968; Halton 1974b, 1975, 1976). Similar observations for intracellular digestion, as described in *D. merlangi*, were also noted in *P. multae*. The digestive vesicles are membrane-bound and vary in size and electron density, suggesting the occurrence of different stages of intracellular digestion. Intracellular digestion is a primitive feature of monogeneans as compared with the highly specialized extracellular digestion of other evolutionary advanced flukes, worms and invertebrates. X-ray microanalysis of the electron-dense digestive cell pigments in the gut caecum of *P. multae* indicated they are primarily composed of iron, most probably derived from the digestion of host haemoglobin, similar to that identified in *D. merlangi* (Halton 1982). However, X-ray microanalysis of the digestive cell pigments in *Polystomoides* sp. failed to reveal any demonstrable iron-containing inclusions (Allen and Tinsley 1989). The present study clearly demonstrates that *P. multae* is a blood-feeder and that the end product of digestion is most likely the ferriporphyrin haematin, which probably occurs in the majority of polyopisthocotyleans, although the presence of other iron compounds such as haemosiderin should not be excluded (Smyth and Halton 1983). Additionally, monogeneans are capable of absorbing micromolecular nutrients transtegumentally (Halton 1978).

In *P. multae* the elimination of indigestible residues occurs principally by exocytosis. In *D. merlangi*, exocytosis of indigestible haematin is a continuous process, whereas in starved worms, total depletion of digestive cell pigments has been observed (Halton 1975). In *E. knoepffleri*, cellular disintegration, shedding and the apocrine secretions of the digestive cells are associated with the synchronous digestive cycle and elimination of indigestible waste products (Fournier 1978). Similarly, the involvement of apocrine- and holocrine-like processes has been suggested to be associated with the elimination of the indigestible pigments in *Polystomoides* sp. (Rohde 1975).

In *P. multae* the connecting syncytium covers extensive areas of the caecum, although pigmented digestive cells may be absent. It also ramifies amongst the vitelline cells, the protonephridial capillaries and adjacent to the testes. However, the syncytial epithelium did not extend to cover the apical region of the pigmented digestive cells, which is elevated into microvilli, as observed in other polyopisthocotyleans (Smyth and Halton 1983). This would suggest that the principal function of the connecting syncytium is involvement in a mechanical, structural or supportive role. The presence of lamellae on the luminal surface of the connecting syncytium, which projects into the gut caecum, together with the presence of vesicles, mitochondria, GER, and Golgi complexes, suggests an additional active role in the absorption of micromolecular nutrients from the host blood meal and the distribution of extracellularly digested products, an action that is probably regulated to some degree by the neurosecretory vesicle-containing nerve axons lying beneath the luminal surface. The cytoplasm of the connecting syncytium of *Polystomoides* sp. has been shown to contain numerous secretory vesicles and cell organelles and, hence, an active role in digestive physiology has been proposed (Allen and Tinsley 1989). Earlier ultrastructural observations on a number of monogeneans have shown an absence of secretory vesicles with only occasional GER and Golgi complexes in the connective syncytium, suggesting a less active role in absorption and digestion (Halton et al. 1968; Tinsley 1973).

Acknowledgements The authors are grateful for grants from the British Council, U.K., and UGC, New Delhi. Thanks are due Mr. George McCartney for photographic assistance and Mrs K. Moore for secretarial services.

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