Fine Structure of the Gut Epithelium of *Schistosoma mansoni*

It has recently become apparent that the digenetic trematodes possess 2 surfaces capable of taking up nutrients, namely the gut and the tegument. Although tegument fine structure has been described for several species of trematodes[1,2], little is yet known about the ultrastructure of the gut epithelium.

In order to study the fine structure of the gut epithelium of *Schistosoma mansoni*, single worms and paired male and female worms were fixed in glutaraldehyde, osmicated, and embedded in Araldite or Maraglas according to standard methods. Tissue to be tested for acid phosphatase activity was incubated in a modified, freshly prepared GOMORI acid phosphatase medium for 40 min at 37 °C. Control material was incubated in a medium containing 0.01 *M* NaF. Thin sections were cut with glass knives, stained with uranyl acetate and lead citrate and examined on an A.E.I. EM6B electron microscope.

Figure 1 illustrates semi-diagrammatically the fine structure of generalized gut epithelium. No obvious differences in fine structure are detectable between the gut epithelia of male and female worms. In both the epithelium is syncytial and, so far as can be determined by the method used, without marked differences between anterior and posterior regions. The surface of the syncytium is thrown into periodic irregular projections (Figure 1) but does not possess regular large folds or villi. Numerous sheet-like lamellae extend from the surface and project a short distance into the lumen. These lamellae occasionally branch and may rejoin the gut surface to form loops. There is considerable variation in the thickness of the epithelium (compare Figures 2 and 3) and lamellae are longer and more numerous on the thicker projecting regions.

A finely filamentous coat can be seen external to the surface plasma membrane. Small surface depressions or caveolae, presumably indicative of micropinocytosis, can also be seen on the gut surface. The basal plasma membrane is separated from a layer of fibrous interstitial material by a basal lamina. The basal plasma membrane is thrown into numerous long invaginations which penetrate almost the entire depth of the epithelium (Figures 2 and 3). It is probable that these invaginations are slot-like and not tubular.

Contact between the gut epithelium and the parenchyma is provided by numerous junctional complexes

[1] L. T. THREADGOLD, Q. Jl microsc. Sci. *104*, 505 (1963).
[2] P. BURTON, J. Parasit. *52*, 926 (1966).

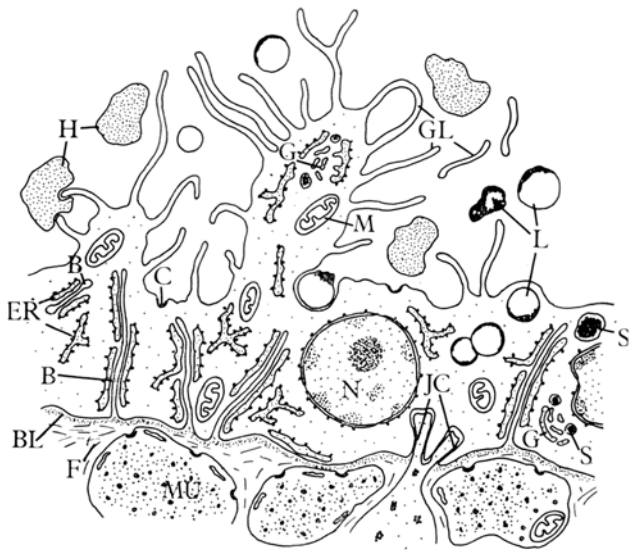

Fig. 1. Line drawing illustrating the fine structure of generalized *S. mansoni* gut epithelium. B, basal invagination; BL, basal lamina; C, micropinocytotic caveolus; ER, endoplasmic reticulum; F, fibrous layer; G, Golgi complex; GL, gut lamella; H, hemoglobin; JC, junctional complex; L, lipid-like droplet; M, mitochondrion; MU, muscle; N, nucleus; S, dense secretion body.

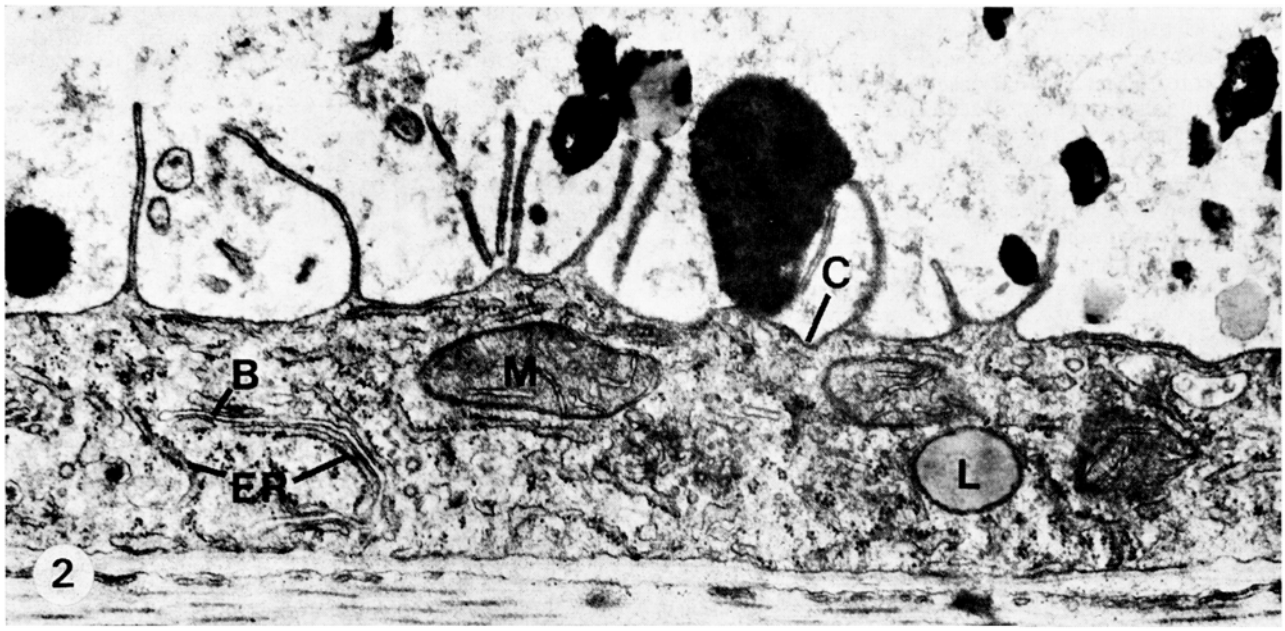

Fig. 2. Thin epithelium from mid-region of a female worm. Numerous dense configurations are visible in the lumen. × 25,000.

(see Figure 1). Parenchymal projections penetrate the fibrous layer and basal lamina and are coupled to the base of the gut epithelium by junctional complexes which are apparently identical to those described previously for *Fasciola hepatica*[3].

At its anterior end the gut is separated from the highly folded and much modified tegument which lines the oesophagus[4] by a septate desmosome. Red blood cells are apparently lysed immediately upon ingestion, but white blood cells in varying states of degeneration can be seen throughout the length of the oesophagus and in the anterior gut lumen. Since dark 'secretion bodies' can be seen in the oesophagus lining and since white blood cells in that region show signs of degeneration, it is probable that extracellular digestion begins there and continues in the gut.

Numerous mitochondria and an extensive endoplasmic reticulum are present in the gut epithelium. The basal invaginations are lined over much of their length by endoplasmic reticulum, and it is of interest to note that ribosomes are absent from the membrane most closely applied to the surface of the basal invagination (see Figures 1 and 2). The cisternae of the endoplasmic reticulum are often distended and contain an amorphous material of appreciable density. The presence of protein synthesis in the epithelium is indicated by the observation that electron-dense bodies enclosed by a unit membrane are found in association with scattered Golgi complexes (Figure 3).

Numerous lipid-like droplets can be observed both in the gut lumen and within the epithelial cytoplasm. While many of these droplets are intact, others are in various stages of breakdown (see Figure 5). Some of the droplets

[3] S. S. E. Gallagher and L. T. Threadgold, Parasitology, in press (1967).

[4] G. P. Morris and L. T. Threadgold, unpublished.

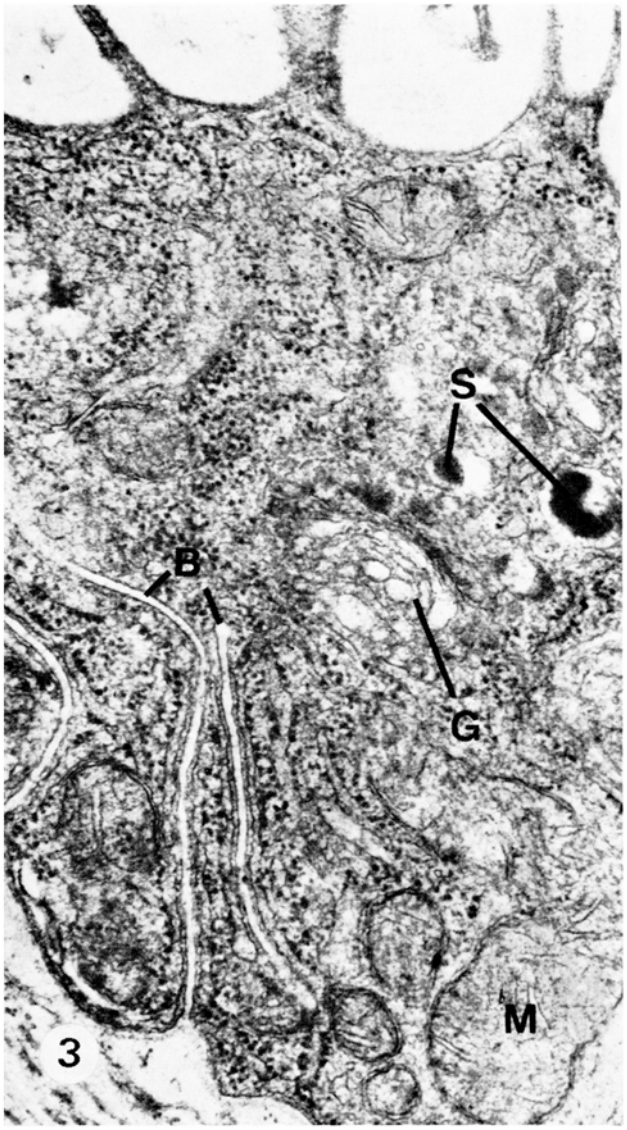

Fig. 3. Epithelium from anterior region of a male worm. × 40,000.

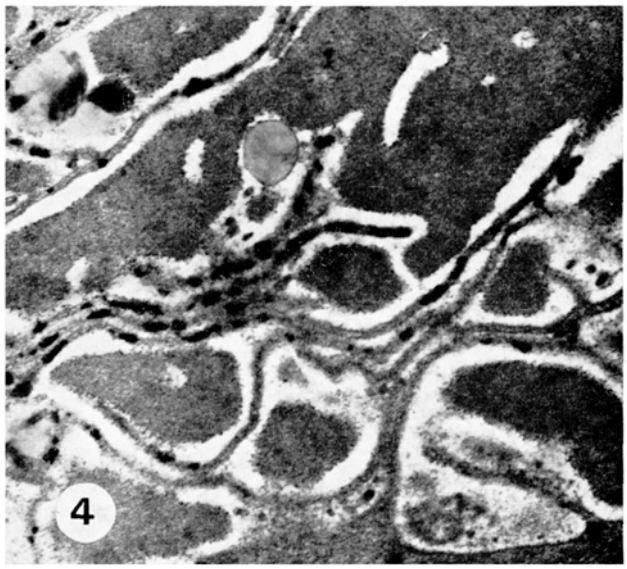

Fig. 4. Acid phosphatase activity in gut lamellae of a female worm. × 34,000.

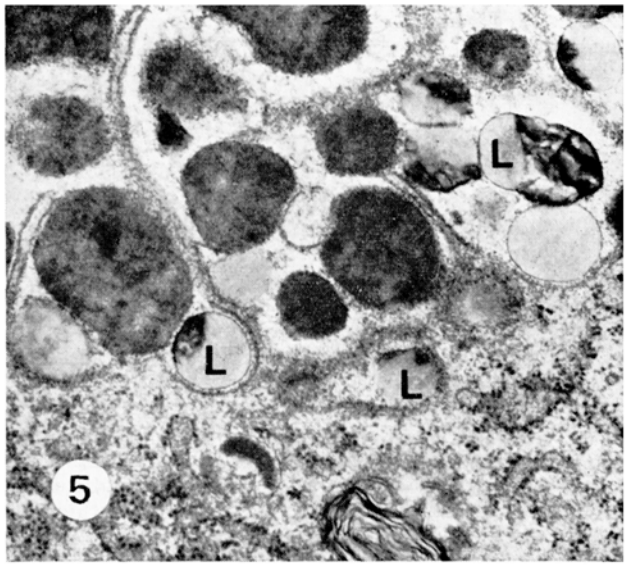

Fig. 5. Epithelial surface from posterior tip of a female worm showing lipid-like droplets in varying states of degeneration. × 32,000.

have a dense 'cap' around all or part of their surface. In others, this densification includes progressively greater quantities of the droplet matrix producing as a terminal configuration a dense wrinkled particle. The dense wrinkled particles are found only in the gut lumen, particularly in the posterior regions where they are often the predominant component of the gut contents. Furthermore, there is morphological evidence for uptake by the epithelium of entire droplets, but only of those showing little or no evidence of degradation. A droplet in the apparent initial stage of engulfment can be seen in Figure 5.

The dense wrinkled material is entirely removed when thin sections are incubated for $1^1/_2$ h in saturated picric acid in 95% alcohol. Incubation in alcohol alone has no effect. This solubility is characteristic of hematin which has been previously identified in the gut of *S. mansoni*[5]. It is postulated that the dense material is hematin and that the lipid-like material is some intermediate of hemoglobin degradation. Close examination of micrographs from the anterior gut lumen does, in fact, show an apparent merging of the hemoglobin matrix with that of the lipid-like material. It may be that 2 types of hemoglobin digestion exist – an extracellular type in which hematin is the end product and an intracellular type in which an intermediate is phagocytosed and broken down to simple end products. It may be speculated that extracellular digestive enzymes, perhaps produced by the oesophagus, initiate the digestion of hemoglobin. The digestive process may then be completed after uptake of the droplet by the epithelium.

Other than the apparent uptake of the lipid-like droplets and the existence of micropinocytotic caveolae there is no obvious sign of phagocytotic activity. The edges of the gut lamellae are often observed inserted into depressions in the matrix of the numerous pieces of hemoglobin found in the lumen. This would indicate that the gut lamellae may perform a digestive function as well as possibly serving to increase the area of the absorptive surface.

The gut lamellae react strongly when tested for the presence of acid phosphatase activity (Figure 4). The reaction is confined to the inner core of the lamellae and acid phosphatases may be localized on the inner portion of the plasma membrane. A similar condition also exists on the tegument surface of *S. mansoni* where acid phosphatase activity is restricted to the inner side of the surface plasma membrane[4,6].

Résumé. L'épithélium de l'intestin de *Schistosoma mansoni* est un syncyte de structure pareille dans les 2 sexes. Des lamelles hérissent la surface et de nombreuses invaginations basales en fentes rayent l'épithélium de la paroi basale plasmique. On constate l'existence d'une digestion extracellulaire, ainsi que l'évidence morphologique d'une utilisation des gouttes lipides entières et des micropinosyncytes. Des procès cytoplasmiques du parenchyme s'attachent par des complexions jonctionnelles («junctional complexes») à la base de l'épithélium de l'intestin et mettent en connection les 2 systèmes. La localisation de l'activité de la phosphatase acide dans les lamelles de la paroi intestinale est précisée.

G. P. MORRIS

Department of Zoology, Queen's University of Belfast (Northern Ireland), 4 October 1967.

[5] D. W. HALTON, Parasitology *57*, 639 (1967).

[6] Acknowledgment. This research was carried out while the author was in receipt of a Wellcome Trust Overseas Studentship.

Ultrastructure of the Pineal Gland after Hypophysectomy

Experimental investigations show that the anterior pituitary controls the structure and function of the thyroid gland, adrenal cortex and gonads; the part played by the anterior pituitary in the control of the parathyroid or insular pancreas is a matter of dispute and its control of the pineal gland is as yet unknown.

Although it has been demonstrated that the pineal is an endocrine gland secreting polypeptide hormones (LERNER, CASE and TAKAHASHY[1]; FARRELL and ISAAC[2]) very little is known about the factors controlling its secretory activity.

In a previous report (MILCU and LUPULESCU[3]) the presence of degenerative cellular and nuclear changes in the pineal gland of hypophysectomized rats observed by means of the light microscope were described.

The present paper deals with the ultrastructural changes of the pineal gland after hypophysectomy in rats.

Material and Methods. The experiments were carried out on adult male rats, which were divided in 2 groups. The first was formed of totally hypophysectomized adult male rats, weighing 260–280 g; the second one of control adult rats. The rats were sacrificed by ether anaesthesia at intervals of 7, 15, and 30 days after hypophysectomy and the pineal gland was removed. For study by the electron microscope, the pineal glands were collected immediately and fixed in PALADE's fixative[4], then postfixed in an aqueous uranyl-acetate solution, and embedded in Vestopal-W[5], or in Epon-812[6]. The ultrathin sections were cut in the LKB-ultrotome, and then stained with lead citrate[7] and examined under a JEM-7C or Hitachi-11-electron microscope.

Results. Electron microscope observations showed clustered, oval, parenchymal cells (pinealocytes) in the pineal gland of the control rats. The fine structure of the epiphyseal cells was variable in appearance. The nuclei were oval or round and in the cytoplasm, as in other endocrine glands, the endoplasmic reticulum was made up of numerous pleomorphic vesicles; within the cytoplasmic matrix the ribosomes were free or disposed in rosettes or clusters. A higher magnification revealed 2 distinct types

[1] A. LERNER, I. CASE and Y. TAKAHASHY, J. biol. Chem. *235*, 1992 (1960).

[2] G. FARRELL and W. MCISAAC, Archs. Biochem. Biophys. *94*, 543 (1961).

[3] ST. MILCU and A. LUPULESCU, Communs. Neurol. Endocr. *1*, 11 (1957).

[4] G. PALADE, J. exp. Med. *95*, 285 (1952).

[5] A. RYTER and E. KELLENBERGER, J. ultrastruct. Res. *2*, 200 (1958).

[6] J. LUFT, J. biophys. biochem. Cytol. *9*, 409 (1961).

[7] E. REYNOLDS, J. Cell Biol. *17*, 208 (1963).