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MICROSCOPIC AND ULTRASTRUCTURAL STUDY OF DIFFERENT EPITHELIAL ELEMENTS IN THE ILEUM OF LARVAE OF ANISOPTERAN DRAGONFLIES

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Microscopic and ultrastructural studies of the ileum of anisopteran larvae reveal the existence of thick and thin epithelia. The thick epithelium apparently is organised for ion transport. The cells are covered with a multilayered cuticula. The apical cell membrane is much folded. Many mitochondria occur beneath these foldings. The basal cell membrane is also folded and closely associated with mitochondria. The lateral cell membranes are simularly folded. Septate desmosome structures are present. Transporting epithelia in the anterior part of the hindgut may play an important role in the osmoregulation of the haemolymph of the larvae by reabsorbing ions from the urine, produced by the Malpighian tubules.

INTRODUCTION

As all fresh water animals, dragonfly larvae are confronted with the problem of osmoregulation. Although their haemolymph is hypertonic to the external medium they maintain this osmotic pressure and keep constant the concentration of the major ions in the haemolymph (MOENS 1975a, 1975b; SCHMITZ & KOMNICK, 1976; KOMNICK, 1977). The influx of water, caused by osmotic phenomena is compensated for by urine production by the Malpighian tubules. However, active absorption of ions from the urine and from the external medium in the gill chamber is necessary to balance the loss of ions caused by diffusion and by the initial production of urine.

Special ion transporting epithelia in the basal pads of the rectal gills of anisopteran larvae have been investigated by GREVEN & RUDOLPH (1973), WICHARD & KOMNICK (1974) and SCHMITZ & KOMNICK

(1976). Similar epithelia are found in the anterior part of the hindgut, the ileum, and are described below.

MATERIAL AND METHODS

Larvae of *Libellula depressa* L., *Aeshna cyanea* (Müll.) and *Anax imperator* Leach were taken form different ponds in the N.E. part of Belgium (Limburg) and kept in aquaria with pond water. The animals were anaesthesised in water with a high carbondioxide content (soda water) for at least 7 min and dissected under a low power (8-40X) stereoscopic microscope.

For light microscopic observations, the whole ileum was fixed in Bouin. The paraffine sections were stained with trichrome (Masson).

For the ultrastructural study small pieces of the epithelia were removed and fixed either in 2% osmiumtetroxide in 0.1 M cacodylate buffer (pH 7.2) or in 3% glutaraldehyde in 0.1 M Veronal buffer (pH 7.2) and post-fixed in 2% osmiumtetroxyde in 0.1 M cacodylate buffer (pH 7.2). They were embedded in epon after dehydration in alcohols and propylene-oxide, sectioned on a Reichert UM 3 ultramicrotome, stained with uranylacetate and leadnitrate, and viewed with a Philips EM 400 electron microscope.

RESULTS

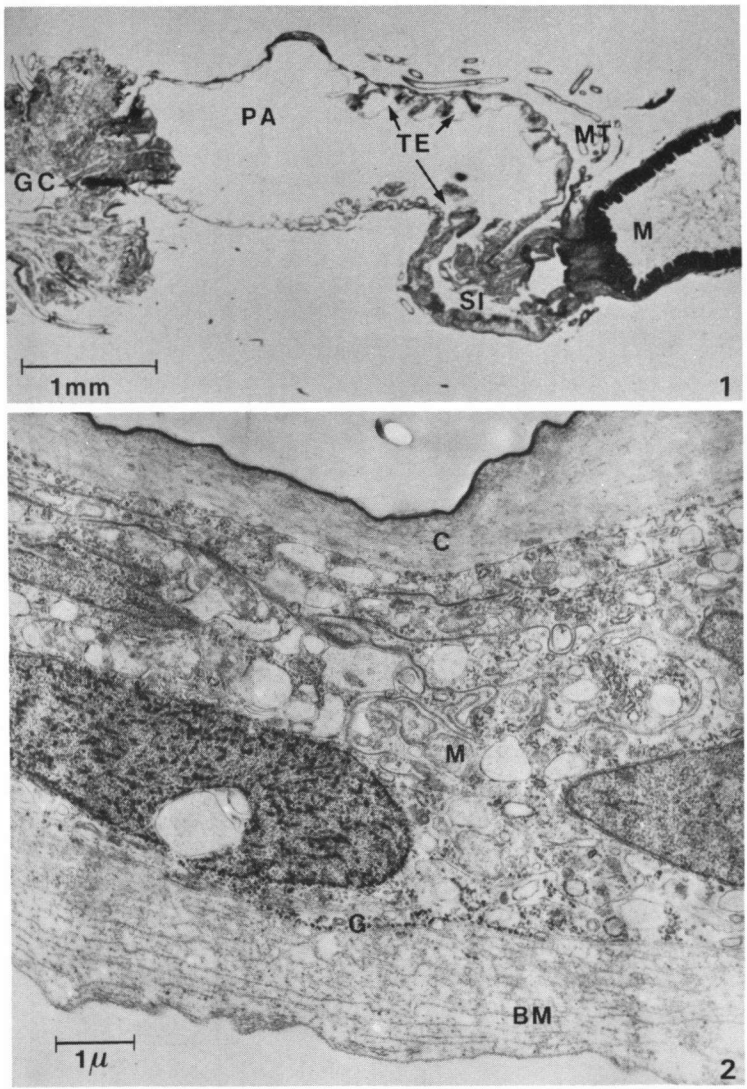
MACROSCOPIC AND LIGHT-MICROSCOPIC OBSERVATIONS

The ileum is S-shaped and guarded by a sphincter on either end. The Malpighian tubules discharge in the narrow anterior part. The posterior half is enlarged into a kind of ampulla or ileal sac (SNODGRASS, 1954). These parts have been named, respectively, "portion grêle" and "ampoule prérectale" by SADONES (1896) and "short intestine" and "prerectal ampulla" by TILLYARD (1917) and STRAUB (1943).

Viewed through the light microscope, the epithelium lining the lumen of the ileum is composed of a thin and highly folded layer, and a thicker layer containing enlarged cells (Fig. 1). Both epithelia are coated with a thin cuticula. The cells in the thin epithelium are nearly cuboidal, approximately 12 μm high, with a clear homogenous cytoplasm. Flattened nuclei which contain mosaiclike dispersed chromatine occur in the basal parts of these cells. Most nuclei have two nucleoli.

The thicker epithelium is composed of cylindrical cells which are spheric and spotted and occur more apically in the cell. An intensively stained zone is present just beneath the cuticula and in the basal part of these cells.

The arrangement of the thickened epithelium differs in the three species studied. The arrangement was visible after dissection and was confirmed in serial sections. In *Libellula depressa* only the ventral half of the short intestine is lined with the thicker epithelium. Two pads of thick epithelium project latero-ventrally into the prerectal ampulla. In *Aeshna cyanea* the ventral and lateral sides of the short intestine are coated with the thick epithelium. The



Figs. 1-2. *Anax imperator* Leach, larval ileum: (1) longitudinal section (cuticle of the transporting epithelium locally detached during preparation): GC, gill chamber; — M, midgut; — MT, Malpighian tubules; — PA, prerectal ampulla; — SI, short intestine; — TE, transporting epithelium; — (2) cross section of the thin epithelium: BM, basal membrane; — C, cuticle; — G, glycogen grana; — M, mitochondrium.

prerectal ampulla bears three pads of thick epithelium (one dorsal and two lateral). In *Anax imperator*, the short intestine is coated by the thick epithelium except for a small dorsal slit. The prerectal ampulla is coated mainly with the thicker epithelium, but the thin epithelium also covers a small part of the ventral and dorsal sides.

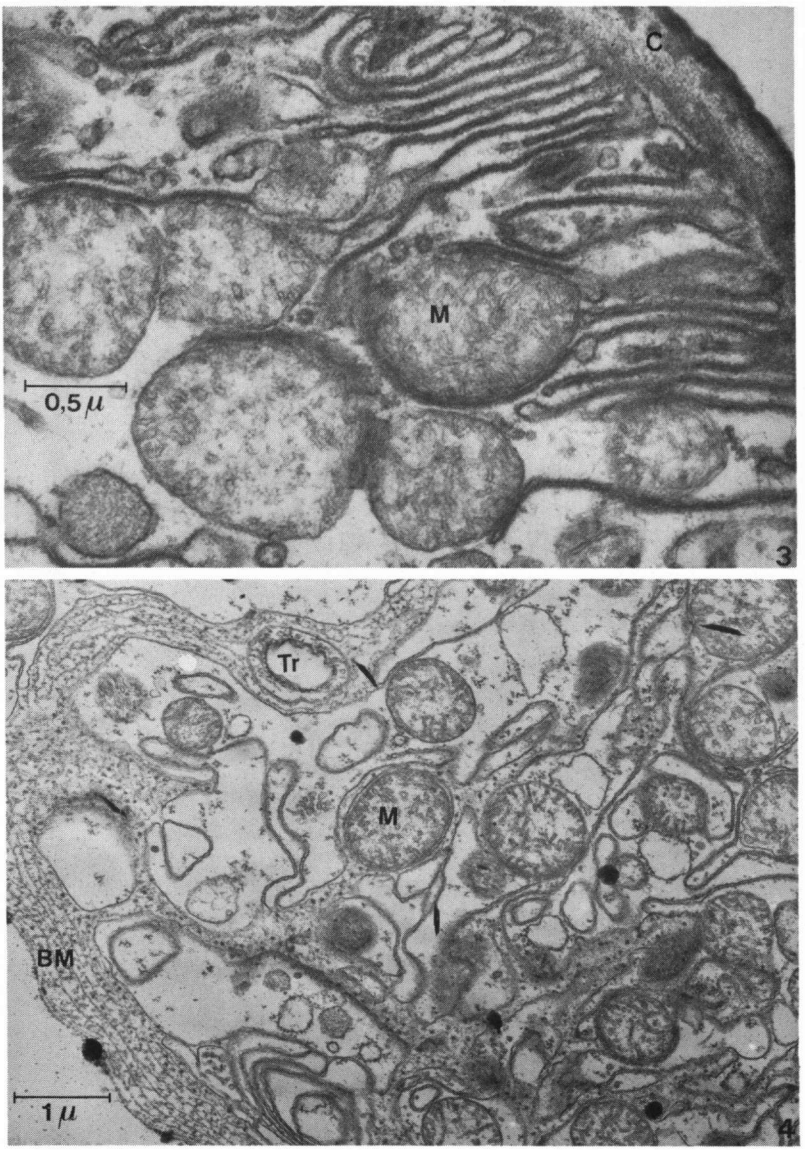
ULTRASTRUCTURAL STUDY

The apical side of the cells in the thin epithelium is coated with a relatively thick cuticula (0.6 μm). The cells have a homogenous distribution of mitochondria (Fig. 2). The oval nuclei are situated near the base of the cells and show a regular reticular chromatine. The well developed basal membrane is 1-2μm thick and contains fibers which are orientated in different directions. Glycogen grana occur in the basal side of the cells. The lateral cell membranes are highly folded.

The cells in the thicker epithelium differ from the above description. The apical side of these cells is coated by a multilayered thin cuticula (0.2μm) (Fig. 3). The apical cell membrane is much folded to form numerous microvilli--shaped structures, which are directed to the basal side of the cells. The intermembrane space is frequently enlarged towards the folded side. The extracellular space is filled by the subcuticula. Many mitochondria occur beneath the apical foldings. These foldings together with the mitochondria may represent the intensively stained apical zone observed by light microscopy. In the middle region of the cells, the density of mitochondria is lower. In this part of the cells glycogen grana occur (Fig. 4). On the basal side the cells are lined by a multilayered basal membrane ($\pm$ 0.5 μm) from which branched foldings penetrate almost to the center of the cell. (Fig. 4). However, these have no contact with the foldings of the apical membrane. The foldings of the basal membrane in which tracheoles occur are closely associated with numerous mitochondria, thereby forming a basal labyrinth. This basal labyrinth probably corresponds to the intensively stained basal zone observed by light microscopy. The lateral membranes are simularly highly folded and associated with mitochondria (Fig. 5). The intercellular space is sometimes enlarged. Septate desmosome structures are present.

DISCUSSION

The ultrastructure of the thick epithelium, which occurs in the short intestine and the prerectal ampulla of the gut of anisopteran larvae, suggest a role in ion transport. In the review paper by BERRIDGE & OSCHMAN (1972) ion transporting epithelia are described in different animals and they exhibit the following common features:



Figs. 3-4. *Anax imperator* Leach, ion transporting cells of the larval ileum: (3) apical region, showing the foldings of the apical cell membrane; — (4) basal region. — BM, basal membrane; — C, cuticle; — M, mitochondrium; — Tr, tracheole.

— villi-shaped foldings of the apical cell membrane and the occurrence of mitochondria just beneath it;
— branched foldings of the basal membrane closely associated with numerous mitochondria;
— extensively folded lateral membrane also closely associated with mitochondria.

These characteristic features were also found in the ion transporting epithelia of the basal pads of the rectal gills (GREVEN & RUDOLPH, 1973; WICHARD & KOMNICK, 1974a) and in the rectal chloride epithelia of damselfly larvae (WICHARD & KOMNICK, 1974b). The epithelia actively absorb ions from the external medium to compensate for ion loss due to excretion and diffusion (KOMNICK, 1977a, 1977b).

Fig. 5. *Anax imperator* Leach, apical region of the ion transporting cells of the larval ileum, showing the elaborate foldings of the lateral cell membrane with enlarged intercellular spaces (arrow): C, cuticle; — M, mitochondrium; — SD, septate desmosome.

Investigations of different insects and their larvae have led to the conclusion that the primary secretion, produced by the Malpighian tubules and discharged into the gut, is isosmotic to the haemolymph and therefore does not influence the osmotic pressure of the haemolymph (STOBBART & SHAW, 1974). However, production of hypotonic urine, by reabsorbing ions from the primary secretion of the Malpighian tubules aids the maintenance of hyperosmotic regulation. In most insects, reabsorption of ions from the urine takes place in the rectum by the rectal pads.

The special anatomical features of the hindgut of anisopteran larvae, necessitated by the respiratory function of the rectum, suggest that reabsorption of ions from the urine must take place elsewhere as the rectum is continuously ventilated. Our observations, which reveal the presence of ion transporting epithelia in the two parts of the ileum (the short intestine and the

prerectal ampulla), led us to the hypothesis that reabsorption of ions from the urine takes place in this part of the gut. An analogous mechanism probably exists in damselfly larvae because special transporting epithelia occur in the prerectal ampulla. Apparently, the ion transporting epithelia in the ileum (anterior to the hindgut) produce hypotonic urine which reduces the loss of ions by excretion and aids the maintenance of hypertonic regulation.

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