Odonatologica 13 (2): 249-258 *June 1, 1984*

METAMORPHOSIS OF THE MALPIGHIAN TUBULES OF *LIBELLULA QUADRIMACULATA* L.: STRUCTURE AND PHYSIOLOGY (ANISOPTERA: LIBELLULIDAE)

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Received April 28, 1983 / Accepted August 2, 1983

The ultrastructural organisation and physiology of the Malpighian tubules of larval and adult stages are described. The main structural difference between larval and adult tubules is the presence of elongate mitochondria inserted into the luminal microvilli of the adults. This correlates with an increase in the basal rate of fluid secretion by the adult tubules, which is some 3-4 times higher than in the larvae.

INTRODUCTION

The physiology of some insect excretory and osmoregulatory systems has been examined intensively (see, for example, reviews by WALL & OSCHMAN, 1975; MADDRELL, 1977), but these studies have been limited to a relatively small number of species. However, structural studies have extended the range somewhat, so that, even though the total number of species investigated still remains small, it is becoming clear that there are many interesting variations, (imposed by the physiological requirements of the insect), on the basic pattern. This basic pattern is one of secretion of a primary urine by the Malpighian tubules, and its subsequent modification during its passage through the gut. Variations include ultrastructural differences in the rectal pad cells, to enable the retention of either ions or water from the urine, depending on whether the insect is aquatic or terrestrial (reviewed by NICHOLLS, 1982); or physiological adaptations of the Malpighian tubules in blood-sucking insects to enable them to secrete urine rapidly after a massive influx of Na^+ -rich vertebrate plasma has grossly altered the balance of ions in the haemolymph (reviewed by MADDRELL, 1977). Amongst aquatic insects there are numerous variations in

the sites of uptake of ions from the external medium, to replace those lost by diffusion and in the urine (reviewed by KOMNICK, 1978).

The excretory system of the Odonata is of particular interest for a number of reasons. Firstly, the haemolymph of palaeopteran insects is known to be much richer in sodium and chloride than it is in other insect groups (SUTCLIFFE, 1963), and, in the Odonata at least, this difference is reflected in the mode of urine formation by the Malpighian tubules. In most insects fluid flow is linked to active potassium movements, whilst a few haematophagous species can utilise either potassium or sodium, depending on which ion predominates (reviewed by MADDRELL, 1977). However, the tubules of *Libellula quadrimaculata* are entirely dependent on sodium ions for urine secretion, and can produce urine at normal rates even in the total absence of potassium (NICHOLLS, 1982). Secondly, the rectum, which plays an important role in the modification of the primary urine in most insects, has become adapted for other purposes in the Anisoptera. The original rectal pad cells are now responsible for the uptake of ions from the external medium (KOMNICK, 1977, 1978) and the rectum also houses the gills (WICHARD & KOMNICK, 1974) and is used for propulsion. All of these functions require that the rectum is ventilated (e.g. HUGHES & MILL, 1966) and thus it can no longer play a role in the modification of the primary urine. This function has been transferred anteriorly to the pre-rectal ampulla and anterior hindgut (MOENS, 1980; NICHOLLS, 1982). Finally, since the larvae are aquatic and the adults terrestrial, this change in environment and in physiological requirements at metamorphosis must be reflected in changes in the excretory system. Metamorphosis of the gut has been described elsewhere (NICHOLLS, 1982), and the present work describes the ultrastructural and physiological changes that occur in the Malpighian tubules.

METHODS

Ultrastructure

Specimens of the last larval instar of *Libellula quadrimaculata* were collected from a small pond on the Mendip Hills, in Somerset, England. Some larvae were immediately dissected under saline and a number of Malpighian tubules removed. For light microscope observations these were fixed in Bouin's fixative for 12 hours, before dehydrating and embedding in paraffin wax (M.P. 54° C). Sections were cut at 10 μm and stained with Mallory's triple stain. For electron microscopy, tubules were fixed in 5% glutaraldehyde in 0.05M phosphate buffer at 20° C for one hour. A suitable osmotic pressure for the fixative was obtained by adding sucrose to a final concentration of 3% by weight. After washing in the buffer, the tissue was post-fixed in phosphate-buffered osmium tetroxide for two hours. The material was then given a pre-stain rinse in veronal acetate/HCl and stained *en bloc* for 12 hours in similarly buffered uranyl acetate (GIBBONS & GRIMSTONE, 1960). Ultrathin sections were cut on a Porter Blum ultramicrotome and these sections were subsequently stained with a 1% aqueous solution of lead citrate for 20 min. Observations were carried out on a Phillips 300 E.M.

Tubules were also removed from mature adults collected from the field and treated in a similar manner.

Physiology

The rate of secretion of the Malpighian tubules was measured for a number of different stages: last-larval instar, early and late-stage pharate adults, teneral adults and mature adults. Tubules were excised from the animals and placed one each to a 2 μl drop of haemolymph under liquid paraffin. This haemolymph had been removed previously from the experimental animal and allowed to stand under liquid paraffin for 20 min, so that the haemocytes could coagulate and settle out. The cut ends of the excised tubules were pulled free of the drops and allowed to adhere to small glass pegs situated about 1 mm from the drops.

Surface tension forces were sufficient to hold the tubules in this position for the duration of the experiment. After about 10 min the tubules recover from handling and begin to secrete fluid, which accumulates at the cut ends. Routinely, secretion was allowed to continue for 1 hour, after which time the drop of accumulated secretion was removed and allowed to float in the liquid paraffin. Under such conditions the drop can be assumed to be spherical and, therefore, measurement of the diameter enables the volume to be calculated as $\frac{4}{3}\ \pi\ r^3$. The length of the tubule within the haemolymph was measured and rates subsequently expressed as nl/mm tubule/hour, to offset variations in the lenght over which secretion was occurring. It has already been shown that secretion takes place along the whole lenght of the tubule (NICHOLLS, 1982).

RESULTS

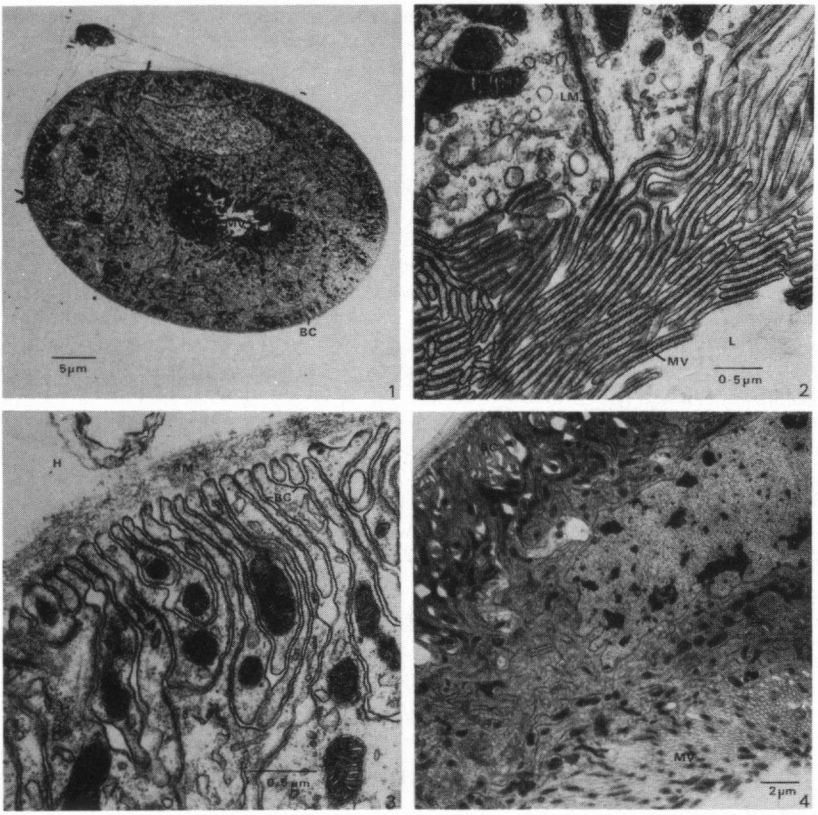
ULTRASTRUCTURE

The light microscope reveals that there is only one cell-type present, and that the tubule is similar along its entire length. The electron microscope confirms these observations.

The larval cells are 10-15 μm tall and surround a lumen 1.5-15 μm in diameter. At the luminal border, the plasma membrane forms a series of short microvilli (1--1.5 μm). These microvilli appear flattened and leaf-like in shape, and are only 20--25 nm across (Figs 1,2). They are stacked closely together, each being separated from its neighbour by a 10 nm gap. The microvilli contain no mitochondria nor any other organelles.

Just inside the microvillar border there is a 3-5 μm wide zone containing abundant mitochondria. Microtubules are also observed in this region.

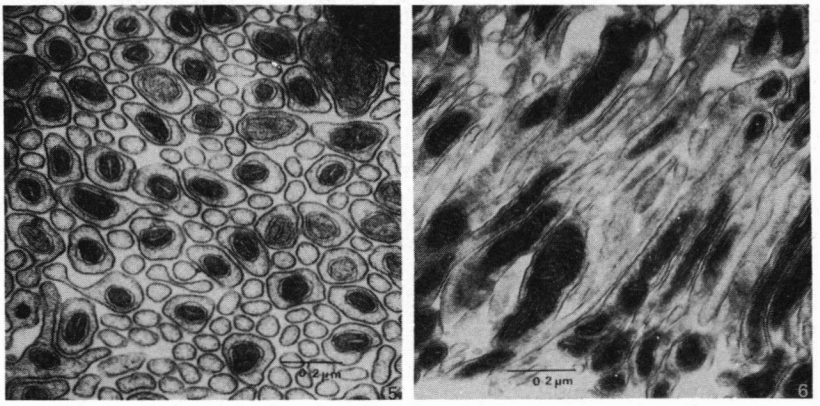
The lateral cell membranes are relatively straight and linked by septate junctions over a 5 μm section in the microvillar half of the cell. Profiles of autophagic vacuoles, multi-vesicular bodies and rough endoplasmic reticulum are also frequently observed in the central area of the cells. Adjoining cells send out numerous interdigitating processes on the side facing the haemolymph. These interdigitations form a series of basal channels, about 20 nm wide, which extend for about 0.5 μm (Figs 1,3). The cells rest on a 200 nm basement membrane, which consists of two distinct layers. The inner layer is of variable thickness and



Figs 1-4. *Libellula quadrimaculata:* (1) Transverse section through the distal portion of a larval tubule. At their basal borders, the cells interdigitate with their neighbours, forming a series of basal channels (BC). At their luminal borders, the cells possess closely packed microvilli (MV). There are two zones rich in mitochondria, one just inside the basal channels, and one just inside the microvillar border. — (2) Detail of luminal borders of larval tubule cells. (L: lumen; — LM: lateral cell membranes; — MV: microvillus). — (3) Detail of basal border of larval tubule cell. (BC: basal channels; — BM: basement membrane; — H: haemocoel). — (4) Transverse section through adult tubule. When compared to that of the larvae, the basal channels (BC) are more extensive, and the luminal microvilli (MV) now contain mitochondria. There are still two zones rich in mitochondria, just inside the basal channels and just inside the microvillar border.

consists of about 150 nm of largely amorphous material. The outer layer consists of a zone of fibrils. The tracheole which accompanies each tubule passes between these two layers, and is thus bound to the tubule by the outer fibrillar layer.

The adult tubules have the same overall dimensions as those of the larvae, but there are a number of important differences. The microvilli of the adult tubules are 4-7 μm long, compared with only 1-2 μm in the larva. Furthermore, the



Figs 5-6. *Libellula quadrimaculata*: (5) Transverse section through the microvilli of an adult tubule, showing contained mitochondria. — (6) Longitudinal section through the microvilli of an adult tubule, showing contained mitochondria.

microvilli of adult tubules contain elongate mitochondria within their length (Figs 4-6). This feature was never observed in the larvae. Adult microvilli are also wider than those of the larvae, i.e. 50-100 nm compared to 25 nm. This diameter increases to 150-200 nm over the portion of the microvilli into which the mitochondria are inserted.

The basal folds are 3-4 μm long, and in many sections appear to be dilated.

PHYSIOLOGY

Figure 7 illustrates the secretion rates of the Malpighian tubules of a number of different stages. The rate in the last-larval instar is slow (1.4 nl/nm of tubule/hour) and in the one early-stage pharate adult examined secretion had almost ceased. In this individual the tubules had lost their usual translucent appearance and had become milky and opaque. Subsequent to this the tubules resume their normal appearance, but the rate is much increased (some 3-4 times greater than that of the larvae). This increased rate is maintained throughout adult life.

DISCUSSION

The main function of the Malpighian tubules is to produce a primary urine, iso-osmotic (though not necessarily iso-ionic) with the haemolymph. This urine then passes into the gut where selective reabsorption of water or ions may occur (PHILLIPS, 1964) depending on the physiological needs of the animal. However, despite this similar basic role, the Malpighian tubules of different

species may differ considerably. Firstly, they may be differentiated into distinct regions. In *Rhodnius,* for example, there are two such regions which differ in the structure of the apical border of the cells (WIGGLESWORTH, 1931). Such structural differentiation is extreme in the case of *Ecdyonurus dispar,* where there are four very distinct regions (NICHOLLS, 1983a), and in the case of insects which have the distal ends of the tubules bound to the rectum as a cryptonephridial complex (e.g. IRVINE, 1969).

These different regions appear to have different physiological functions. In *Rhodnius* the upper region is concerned with the production of the primary urine, whilst the lower portion has been implicated in KCl reabsorption (reviewed by MADDRELL, 1977). Similarly, in cryptonephridial tubules, the distal portion is involved with producing a steep osmotic gradient down which water moves from the rectum, whilst more proximal portions are concerned with K^+ reabsorption and possibly Na^+ secretion (IRVINE, 1969).

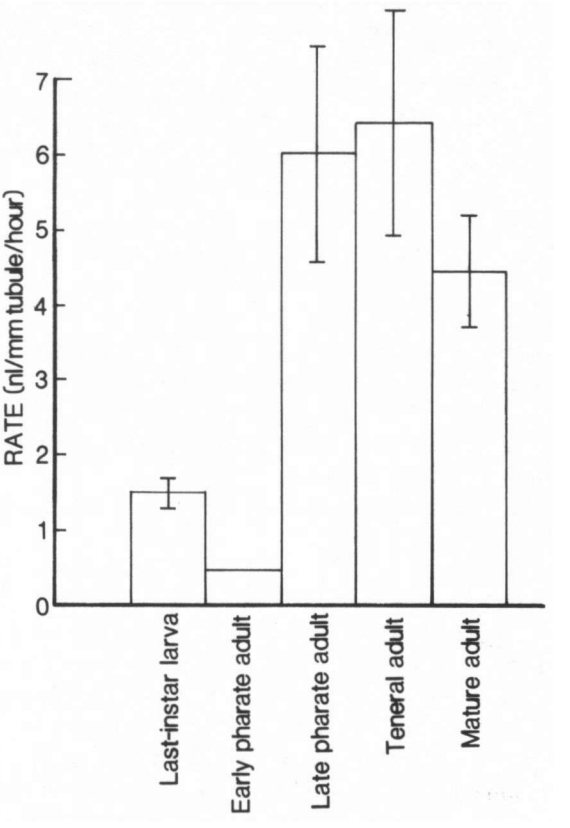


Fig. 7. Basal secretory rates of the Malpighian tubules of various developmental stages of *Libellula quadrimaculata.* For each developmental stage except the early pharate adult, four individuals were examined (6 tubules from each), and the bars represent 1.96 standard errors of the means. The rate for the early pharate adult stage represents one individual.

In tubules which are less regionally differentiated, the situation is often still complicated by the presence of a second cell-type distributed amongst the primary cells (e.g. TAYLOR, 1971; WALL et al., 1975). The function of this second cell type remains equivocal, although TAYLOR (1971) considers that it may be involved in ion reabsorption. Thus, in addition to their main function of producing a primary

urine, many tubules have become adapted in various ways to modify the urine before it reaches the gut.

In *L. quadrimaculata* no regional specialisation is obvious and only one cell--type is present. Furthermore, the tubules produce urine along their entire length. It is therefore tempting to think of these tubules as primitive. Such a view is given additional support by a study of the physiology of urine production by larval tubules (NICHOLLS, 1982). In all species fluid flow into the lumen is driven by active ion transport, but *L. quadrimaculata* is entirely dependent on sodium ions, whereas in other species potassium or both sodium and potassium ions are involved. Such a difference is probably a result of the primitive, Na^+-rich haemolymph of dragonflies (SUTCLIFFE, 1963; NICHOLLS, 1983b). Indeed, the physiological situation in larval *L. quadrimaculata* resembles that in the millipede, *Glomeris marginata,* which has a similar haemolymph composition (FARQUARSON, 1974).

The manner in which fluid secretion is coupled to this active ion pumping is by no means clear, and a number of models have been proposed. For example, it has been suggested that coupling could be via standing osmotic gradients, a model originally developed by DIAMOND (1962) and DIAMOND & BOSSERT (1967) to explain fluid movements across rabbit gall bladder. As applied to Malpighian tubules, ion transport across the microvillar border could increase the osmotic pressure in the narrow spaces between the microvilli, and the osmotic gradient thus formed could then serve to drive fluid movements (MADDRELL, 1977). Alternatively, coupling of solvent to solute movements could be via an electro-osmotic mechanism (HILL, 1977; MADDRELL, 1977). A third alternative is that ion pumping across the microvillar border serves to elevate the osmotic pressure in an unstirred compartment of the lumen which lies in contact with the lateral cell interspaces. Under such circumstances fluid coupling could occur through the lateral interspaces (NICHOLLS, 1983a).

The main point to emerge from all of these models is that ion pumps located on the microvilli are of prime importance in fluid secretion. In many ion-transporting tissues, mitochondria lie in close proximity to the membranes which bear the ion pumps (e.g. the basolateral membranes of the chloride cells in the dragonfly rectum (KOMNICK, 1978), and often form distinct junctions with the membrane (e.g. mitochondrial-scalariform junctions (NOIROT & NOIROT-TIMOTHEE, 1968). It is not surprising, therefore, to find that in adult *L. quadrimacuiata* tubules (and indeed in those of many other species) mitochondria are inserted into these microvilli. However, whilst all adult tubules examined possessed such inserted mitochondria, none of the larval ones did, and this difference was reflected in the secretory rates of the two groups of tubules. Such a difference in mitochondrial distribution could affect fluid secretion, via ion pumping, in a number of ways. Firstly, insertion of mitochondria into the microvilli places a source of ATP close to the ATP-ases of the ion pumps and, secondly, the

mitochondria may actually alter the ionic environment around the pumps (and hence the transmembrane potential) by active ion transport across their own membranes.

Short-term changes in rates of fluid secretion by Malpighian tubules are generally governed by diuretic hormones (reviewed by GEE, 1977) and, in one instance, stimulation by a diuretic hormone has been shown to affect mitochondrial distribution. In the blood-sucking bug, *Rhodnius*, the lower portions of the tubules are concerned with KCl reabsorption, and differ ultrastructurally from the upper, fluid-secreting portion in their lack of mitochondria within the microvilli. However, following release of the diuretic hormone, mitochondria are inserted into these microvilli, where they presumably serve to increase the reabsorption of valuable solutes (BRADLEY & SATIR, 1977). These observations again suggest that the distribution of the mitochondria in ion-transporting tissues may have important effects on the rate of transport.

A diuretic factor has been extracted from the thoracic ganglia of *L. quadrimaculata* (NICHOLLS, 1982), but it is unlikely that secretion of this factor in the adults is responsible for the ultrastructural and physiological differences described above, since all adults examined showed increased rates, and this rate continued even if the adult tubules were isolated in larval haemolymph in which larval tubules were secreting at their normal low rate. Rather, the difference is interpreted as a permanent increase in basal secretory rate in the adult stage, which is necessary because of the high level of adult activity, and consequent need to remove more excretory products. It is thus distinct from the short-term changes induced by diuretic hormones. The effects of the thoracic ganglia diuretic factor on the ultrastructure of the larval tubules has not been examined, but in this context it is interesting to note that in the larvae of another species of dragonfly, *Uropetala carovei*, the luminal microvilli have mitochondria inserted in them (GREEN, 1979).

The single case of an early-stage pharate adult with a very slow secretion rate is of interest. This is presumably a short-lived phase, during which reorganisation of the tubules is taking place. In two other insects in which metamorphosis of the tubules has been studied (*Calpodes* and *Tenebrio*) there is also a short period of tubule reorganisation which, in *Calpodes* at least, coincides with a temporary cessation of fluid production (BYERS, 1971a, 1971b; RYERSE, 1977a, 1978b).

ACKNOWLEDGEMENT

I would like to thank Dr L. STRONG for his useful comments on the manuscript.

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