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FUNCTIONAL MORPHOLOGY OF THE HINDGUT-MALPIGHIAN TUBULE-COMPLEX IN *POLYXENUS LAGURUS* (DIPLOPODA; PENICILLATA)

by

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ABSTRACT

The malpighian tubules of *Polyxenus lagurus* differ greatly from those of other diplopods investigated so far. They are closely attached to the hindgut and are ensheathed with the latter by an envelope which consists of several flattened cells. Thus a complex is formed which resembles very much the cryptonephric system in insects.

According to their morphology and their position four different tubule segments can be distinguished. On the basis of ultrastructural findings the possible function of this hindgut-malpighian tubule-complex and the mechanism underlying this function, are discussed.

INTRODUCTION

There are only a few reports in literature indicating that water uptake and osmoregulation take place in the hindgut region of diplopods. Highly specialized formations, like rectal papillae — these are present in many insects — could neither be found by light microscopy (Krug, 1906; Effenberger, 1909; Wernitzsch, 1910; Randow, 1924; B. Seifert, 1932; Wegelin, 1958/1959; Nunez & Crawford, 1977) nor by electron microscopy (Schlüter, 1980). This suggests that the entire hindgut is a transport organ. In a physiological study Moffett (1975) demonstrated that in the desert millipede *Orthoporus ornatus* (Girard) ion transport is achieved by the rectal epithelium.

It is the aim of the present study to describe the ultrastructure of the malpighian tubules of *Polyxenus lagurus* (Linnaeus) and of their attachment to the hindgut. The observations indicate that the system is morphologically similar to the "cryptonephric" system of insects (e.g. Ramsay, 1964; Maddrell, 1971; Edney, 1977). The observations will be discussed in the context of a possible osmoregulatory function of the system.

MATERIALS AND METHODS

The investigation was carried out on specimens of *Polyxenus lagurus* (Linnaeus, 1758) collected in the vicinity of Giessen from the fissured bark of osier trees. Since merely the parthenogenetic race is common in this region, only females (mostly adults) were caught (the developing stages did not differ significantly from the adults).

The anaesthetized animals were freed of the trichobothria with a fine brush, submersed in fixative and cut into two pieces. They were fixed in cold 2.5% glutaraldehyde/phosphate buffer (pH 7.2-7.4) for 3 hours, washed with buffer (+ 5% sucrose) and postfixed in 2% OsO_4/phosphate buffer for another 2 hours. After dehydration in an acetone series the specimens were embedded in Araldite. Semithin sections were stained with toluidine blue, ultrathin sections with uranylacetate and lead citrate. The ultrathin sections were studied with a Zeiss EM 9 A electron microscope.

RESULTS

1. *Topography*

Fig. 1. shows the position and the course of the malpighian tubules; the reconstruction is based on evaluation of serial sections. The malpighian tubules originate on both sides in the transitional region of midgut and hindgut. Cranially to the tubule mouth the gut is covered by a thin cuticle indicating that not only the caudal region, but also this pylorus region is of ectodermal origin. In this place the efferent ducts of

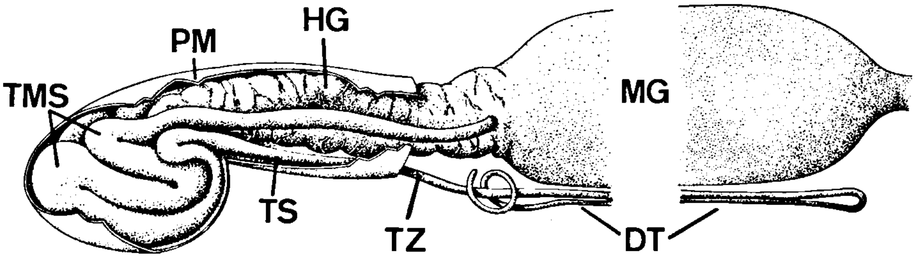

Fig. 1. Semischematic diagram of the gut and the malpighian tubules. Gut: MG, midgut; HG, hindgut. Malpighian tubules: DT, distal tubule segment; TZ, transition zone; TS, thin segment; TMS, thick meandering segment; PM, perinephric membrane.

exocrine glands release their product into the gut lumen. It is not intended to describe these glands within the scope of the present paper.

Starting from the pylorus the malpighian tubules run in caudal direction towards the anal region. Their outside diameter increases from about 5 µm up to 40 µm. In the anal sac region they meander, thus forming narrow loops which are closely attached to the hindgut. Together with the latter, the malpighian tubules form a complex which is separated from the haemocoel by a sheath of flattened cells: the so-called perinephric membrane. This complex is tightly closed caudally but opens at the level of the pylorus into the common haemocoel.

The thickened tubule segment eventually runs in a cranial direction while decreasing in diameter. At the level of the cardia (midgut-foregut transition zone) it is only 1/3 to 1/4 of the thickness as compared to the hindgut region. Up to the level of the pylorus both segments, the cranial and the caudal, remain in close contact with each other. After leaving the perinephric membrane the cranial segment runs along the gut towards the head, turns back like a hairpin, and runs downwards parallel up to the height of the pylorus, where it finally ends with some loops.

2. *Ultrastructure of the different malpighian tubule segments*

Examination of semithin serial sections shows that the distal malpighian tubules ranging from the blind endings up to the entrance into the perinephric compartment are of a rather uniform structure. This also applies to the ultrastructure. Within the perinephric compartment three further ultrastructurally different segments are discernible. In the following paragraphs a description of these four segments will be given.

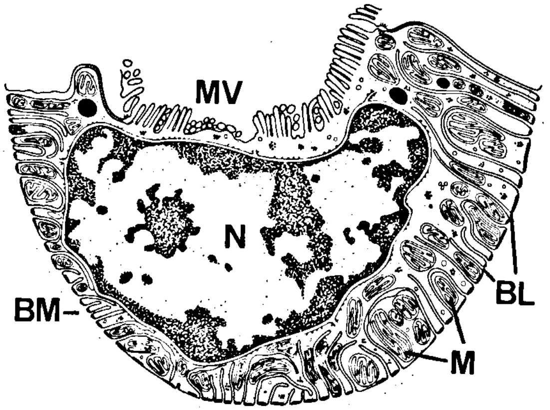

Fig. 2. Distal segment cell. BL, basal labyrinth; BM, basement membrane; M, mitochondria; N, nucleus; MV, microvilli.

a. The distal segment

This is the longest section of the malpighian tubule. In cross sections (fig. 2) it has a round to oval shape, its lumen is surrounded by 3-5 cells. The basement membrane surrounding the tubule is about three times as thick (270 nm) as that of all other parts of the tubule. The basal cell surface is at regular intervals deeply invaginated, thus forming a basal labyrinth. The

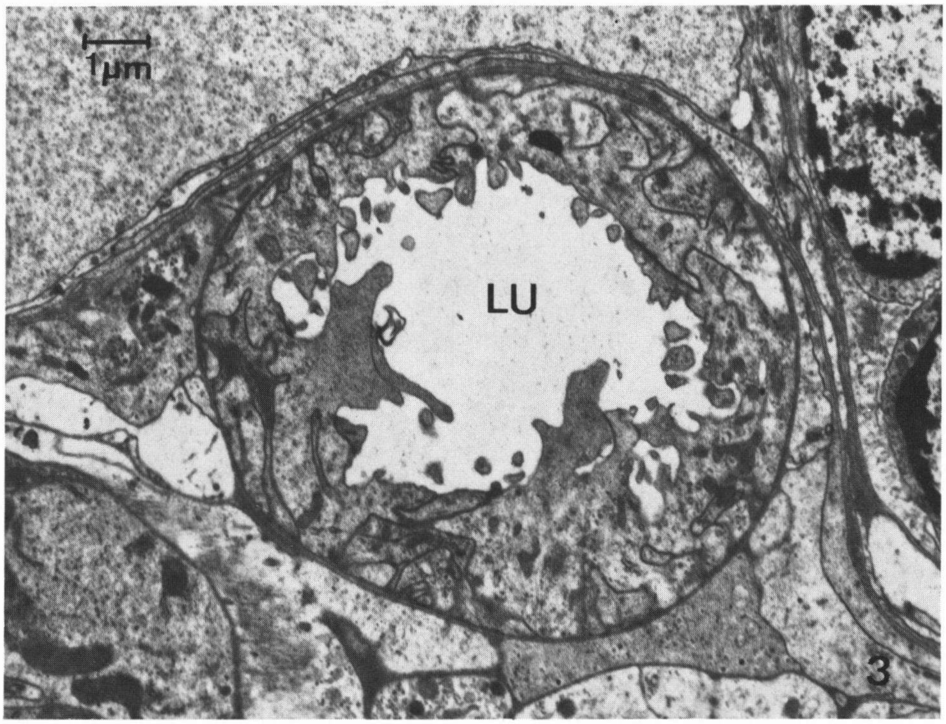

Fig. 3. Section through a transition-zone cell. Note the irregular luminal surface of the tubule cells. LU, lumen.

extracellular spaces of the labyrinth often reach 4/5 of the cell's height; thus they nearly reach the apex. The sites where the cells are attached to the basement membrane show hemidesmosome-like structures at the cytoplasmic side of the cell membrane. Between the infoldings of the basal labyrinth there are numerous voluminous and often elongated mitochondria associated with differing amounts of glycogen and lipid droplets. The lobed nuclei are mostly found in the apical cell region. They often show a compact nucleolus. Close to the nucleus there are a few Golgi bodies and some small cisternae of rough endoplasmic reticulum (rER). In the apical regions junctional complexes are always present between neighbouring cells. The apical cytoplasm shows many microtubules, which are not orientated in a particular direction. Microvillar groups vary in density and are therefore sometimes seen to be irregularly orientated in sections. The distal segment of the malpighian tubule thus represents the impression of a typical ions and water transporting epithelium.

b. Short transition zone

The cells are more flattened than those of the segment described above (fig. 3). The apices are marked by irregular protrusions of different extension. The cells are laterally connected by meander-like junctional complexes. A basal labyrinth is absent and the mitochondria are smaller in number and in size as compared with the distal section. Other organelles are less prominent as well, so that the cells seem to serve no special purpose other than forming the epithelium of a transporting duct.

c. Thin segment within the perinephric compartment

This segment is above all characterized by its extremely flattened cell regions (fig. 4). Within those places the 90 nm sized basement membrane is covered by a similarly sized

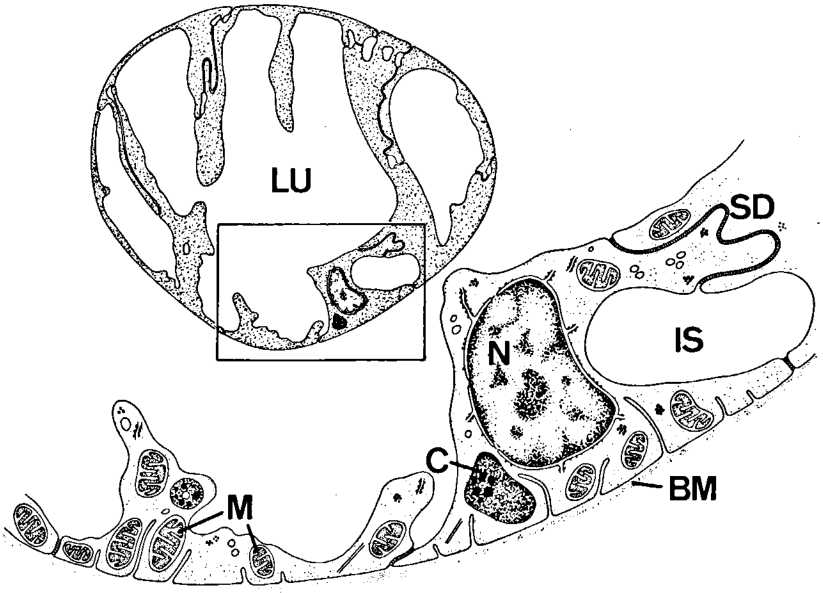

Fig. 4. Thin segment cell. BM, basement membrane; C, cytosome; IS, intercellular space; LU, lumen; M, mitochondria; N, nucleus; SD, septated desmosome.

cytoplasmic layer which is devoid of organelles. More voluminous cell regions elevate at irregular intervals, sometimes forming connections with protrusions of cells situated on the opposite side of the tubule, thus enclosing extracellular cavities which communicate with the tubule lumen. Junctional complexes are visible as well, but there are no microvillous projections at all. So far, organelles have only been observed within the voluminous regions. Some, but apparently not many, mitochondria occur, as do glycogen fields and lipid droplets. The nuclei, which are ovoid or spherical in shape, are occasionally marked by lobular protrusions. A prominent nucleolus is always present.

d. Thick meandering segment

When reaching the anal region the diameter of the malpighian tubules increases abruptly. The ultrastructure of this following segment differs significantly from the foregoing (figs. 5, 6). The increased thickness is not caused by a widening of the tubule lumen, but by a strong increase of the height of the cells. The underlying basement membrane is thinner (30-60 nm) than in all other regions. The basal cell membrane is extensively and deeply folded. The extracellular spaces are of a fairly constant wideness and extend up to the upper one-third of the cell. In the cytoplasm between the infoldings, elongated mitochondria with longitudinally orientated cristae are tightly packed together with lipid droplets. The mitochondria are closely attached to the plasmalemma, thus forming most effective membrane-mitochondria-complexes. Sporadically, glycogen fields are scattered between the lipid droplets. Tracheae and tracheoles are frequently present between the tubular loops in the vicinity of the cell bases. The nucleus is found within the upper parts of the tubule cells, while other organelles seem to be completely absent. It is rather interesting that a microvillous brush border is not present within this entire tubule segment.

e. Proximal (discharging) segment

The proximal segment is formed by ordinary tubule epithelial cells with occasional glandular cells. The latter will be described in a separate publication. This segment is rather short. In

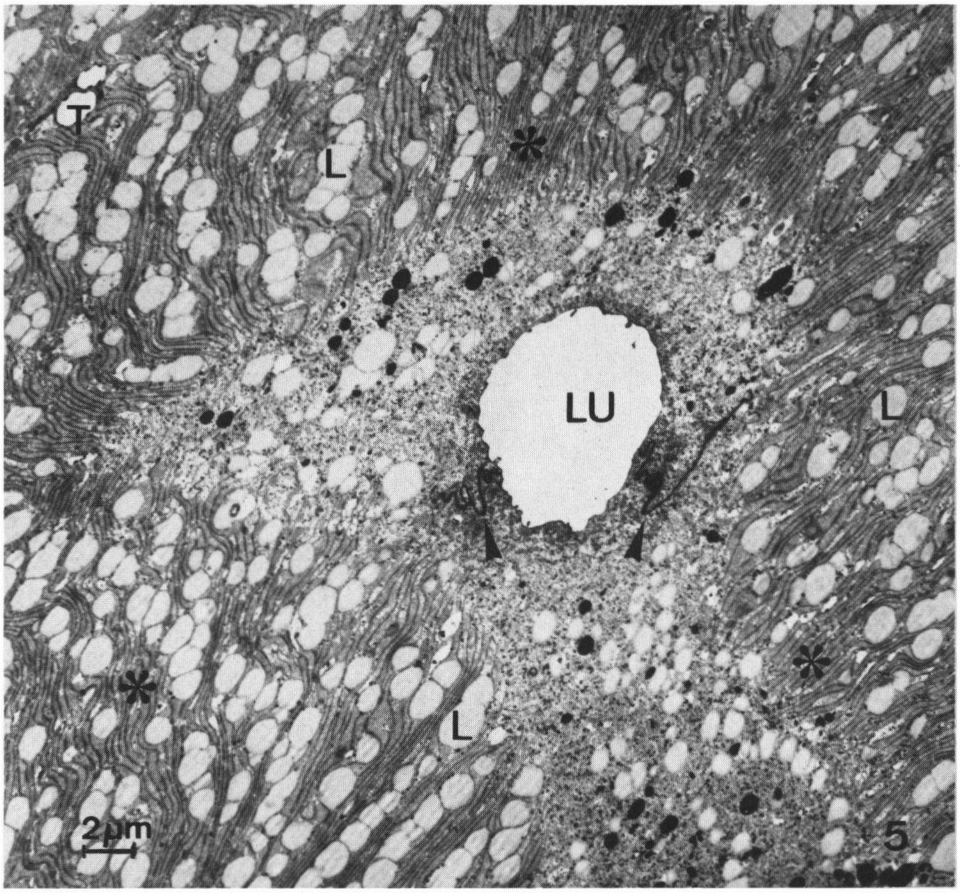

Fig. 5. Section through a thick tubule segment at the level of the anal sac. The basal region is occupied by membrane-mitochondria-complexes (*) and by lipid inclusions (L). LU, lumen; T, trachea.

contrast to the preceding segment the infoldings of the basal cell surface are less numerous and they do not extend as far. Additionally, the number of mitochondria is significantly lower and lipids are seldom observed, while an apical microvillous brush border is present. The tubule lumen is mostly observed as being filled with a foamy secretion, the origin and chemical nature of which was not studied.

3. *The perinephric membrane and the perinephric space*

The term "perinephric membrane" is taken from the more familiar formations in the cryptonephric organs of certain coleopteran and dipteran larvae. As has already been mentioned it comprises several layers of flattened cells (figs. 7, 8) which originate from the mesoblastema. There are two to five layers of cells which presumably form a consistent sheath. Interruptions like, for example, the "leptophragmata" known from the *Tenebrio molitor* cryptonephric complex (Ramsay, 1964) were not found. The flattened cells are almost devoid of organelles. Occasionally there are small mitochondria, and, even more seldom, short cisternae of rough endoplasmic reticulum. The basement membranes are extremely thin.

The perinephric membrane separates the cryptonephric complex (hindgut and proximal malpighian tubule segments) from the surrounding haemocoel and opens like a sack at the cranial end at the level of the pylorus. The

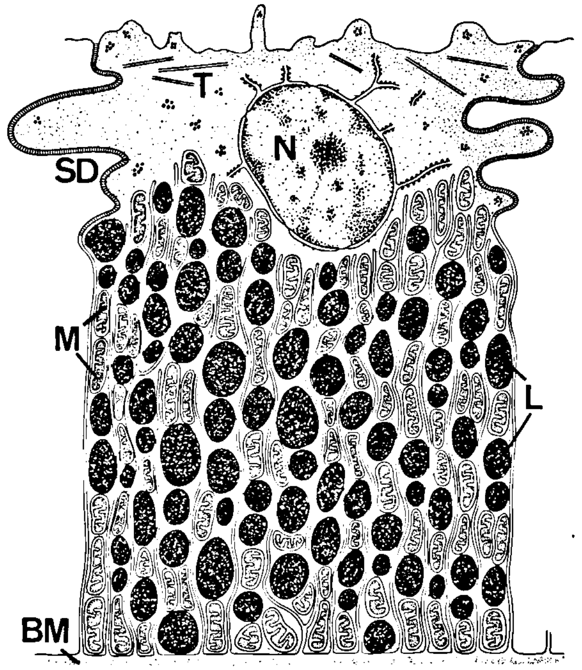

Fig. 6. Thick meandering segment cell. BM, basement membrane; L, lipid droplets; M, mitochondria; N, nucleus; SD, septated desmosomes; T, microtubules.

perinephric space is filled with a fine, homogenous stroma of moderate electron density (figs. 7, 8). In the vicinity of the opening of the perinephric space into the common haemocoel the substance appears to be less electron dense.

4. *The hindgut*

As far as the ultrastructure of the cells is concerned there are two different hindgut sections discernible. The anterior part, which is by far the longest, extends from the pylorus up to the beginning of the close association of the malpighian tubules. The cells of the anterior part are arranged in about 12 smaller pads and two bigger pads. They contain some but not very much mitochondria, lipid droplets and glycogen fields. The intercellular spaces as well as the basal infoldings are of constant diameter. The apical cell surface is irregular, a microvillous brush border is never seen. The overlying cuticle is homogeneous and has no special features (fig. 7). Closely attached to the basement membrane of these epithelial cells a circular layer of muscle cells can be observed. The cells have filament-free areas bordering the perinephric space. Two longitudinal muscle bundles are seen traversing each of the two bigger epithelial pads.

The cells of the posterior section, which may be called the anal sac (that is the chamber, where the faeces are formed and pressed), differ in two important points from those of the anterior region. Here, numerous basal infoldings form a labyrinth which extends almost up to the apex of the cells. The diameter of the infoldings is of differing width, thus forming numerous continuous lacunae. The cytoplasm is tightly packed with mitochondria and glycogen fields. The apex is formed by highly organized microvilli. The cells are joined by extended junctional complexes. The most interesting feature of the anal sac seems to be the cuticle overlying the epithelium. As in the anterior region and in the integument (G. Seifert, 1967) it consists of an epi- and a procuticle. The extraordinary thing is the presence of a proximal subcuticle which has about the same thickness as the procuticle. It is formed by a foamy flocculent material (fig. 7, inset).

DISCUSSION

The cryptonephric complex is not widely distributed among insects. It is mainly found in species that live in dry habitats (Maddrell, 1971; Edney, 1977). As far as lower tracheates are concerned, this report on *Polyxenus lagurus* is new. The description of the topography confirms Reinecke's (1910) observations, which showed the close association between the malpighian tubules and the hindgut. He also discerned the perinephric membrane, but did not attribute any function to it.

In contrast to the insects, where the cryptonephric complex comprises the rectum and the distal endings of the malpighian tubules, in *Polyxenus lagurus* the proximal tubule

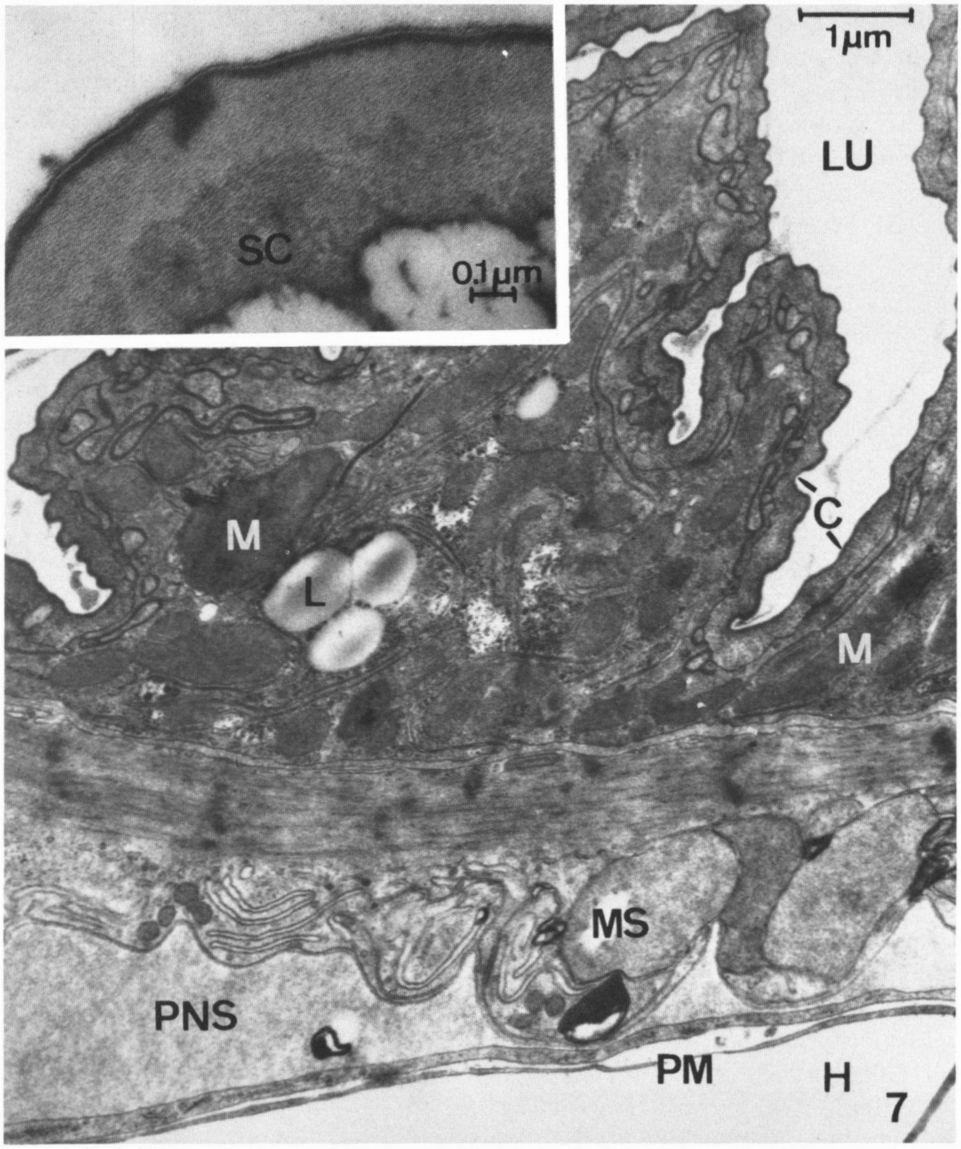

Fig. 7. Section through the anterior hindgut region. The epithelial cells rest on a layer of circular musculature (MS). Within the perinephric space (PNS), which is delimited to the haemocoel (H) by the perinephric membrane (PM), a flocculent substance is present. C, cuticle; L, lipid droplet; LU, lumen of the hindgut; M, mitochondria. Inset: Cuticle of the anal sac epithelium showing a distinct subcuticle (SC).

Fig. 8. The perinephric membrane (PM) is built up of four cells. LU, lumen of the thin segment; PNS, perinephric space.

segments form part of the complex. But this difference is considered to be without functional significance (see below).

The perinephric membrane of *Polyxenus lagurus* is a continuous layer. In contrast, this layer of the mealworm *Tenebrio molitor* Linnaeus shows openings, which are only covered by a basement membrane (Ramsay, 1964). These leptophragmata are supported by malpighian cells bearing the same name. The leptophragma cells are marked by a smooth basal cell surface without invaginations and are extremely flattened. It is supposed that K + -ions might be actively pumped from the haemocoel into the tubule lumen without being followed by water influx, thus producing an osmotic gradient which causes water-reabsorption from the hindgut lumen back into the haemolymph. Leptophragmata are found in several species (e.g. in the larva of the mycetophilid *Arachnocampa luminosa* (Skuse) (cf. Green, 1980). Whether in this case flattened cells are present at all in a particular region, is not known. In *Polyxenus lagurus* these are found within the entire thin-walled segment. During its course ions which certainly do not come from the haemocoel but from the perinephric compartment, could be transported into the tubule lumen to form a gradient increasing in proximal direction.

The thick rectally associated segment of the malpighian tubules can hardly be compared with a similar structure in literature because of its richness in lipids. Only Green (1980) found leptophragma cells as well as other cells containing a lot of lipids and a highly invaginated basal labyrinth in *Arachnocampa luminosa.* In this case, over a rather small area, nearly all necessary structures occur, which are, contrastively, in *Polyxenus lagurus* separated in different and long sections.

This tubule segment has, without any doubt, a high transport capacity while needing high energy supply. The ultrastructure supports the idea of a similar transport activity in the rectal cells. Especially in the caudal region, similarities with the proctodaeum of *Lepismodes inquilinus* Newman (cf. Noirot & Noirot-Timothée, 1971a) become apparent. The same is true of the typical rectal papillae of pterygote insects (e.g. Berridge & Gupta, 1967; Wessing & Eichelberg, 1973; Wall & Oschman, 1975; Eichelberg, 1976; Flower & Walker, 1979). The formation of a special subcuticle should be emphasized. A similar structure is often observed in organs involved in water- (and ion-) uptake (e.g. Noirot & Noirot-Timothée, 1971a, b; Eisenbeis, 1974; Noble-Nesbitt, 1976; Rosenberg & Seifert, 1977; Neuhaus et al., 1978; Rosenberg, 1982, 1983). Unfortunately, only little is known of its specific function or its chemical nature, but it should be engaged in water-binding. A similar function may be assumed for the stroma which fills the space between the hindgut, the malpighian tubules and the perinephric membrane. Ramsay (1964) found a similar substance in high concentrations and assumed it to be very much involved in water-uptake. Additionally, Green (1980) observed a ''sparse amorphous matrix'' within the perirectal space. However, she was not able to assert anything definite as does Ramsay (1964) of *Tenebrio* or as we do in the case of *Polyxenus lagurus.*

Based on the electron microscopical findings and on the comparison with the cryptonephric complexes of insects and the functional interpretation of the latter given by Ramsay (1964), the following hypothesis is submitted for discussion. These ideas are presented in the scheme in fig. 9.

1. Ions from the perinephric space enter the thin tubule segment via the extremely flattened cells. This causes within the lumen a concentration gradient in proximal direction. Water does not follow as it normally does according to the osmotic gradient, because it is bound by the stroma and because of the very small basal surface of the cells.
2. The highly prismatic cells of the thickened segment likewise pump ions with high energy turnover against a (proximad increasing) concentration gradient from the perirectal space into their lumen. Water in-

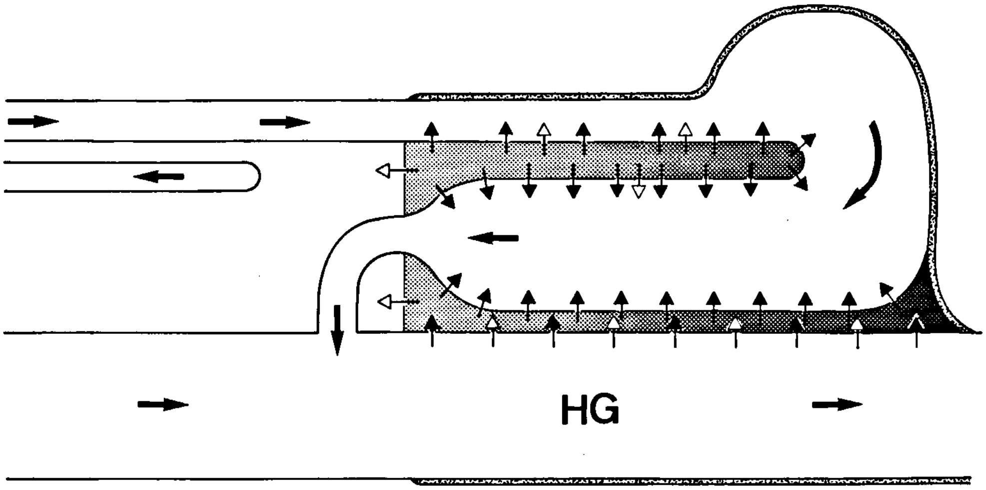

Fig. 9. Scheme of the assumed mechanism of ion- and water-recycling in the hindgut region of *Polyxenus lagurus*. The solid arrows indicate ion movements, the empty arrows indicate water movements. HG, hindgut.

flux is mainly inhibited by the water-retaining stroma.

3. The highly concentrated liquid in the proximal tubule lumen finally enters the pylorus. The caudally situated rectal cells repump ions back into the perinephric space. Resulting from this and especially from the high ionic concentration of the closely associated tubule loops, the rectal lumen is deprived of water. Possibly, specific structural details of the rectal intima or water-binding of the subcuticle and the stroma within the perinephric space play an important role.
4. When the stroma is saturated with water, water runs off into the common haemocoel according to the osmotic gradient. Within this transition zone the ionic concentrations should be considerably lower, possibly lower than in the adjacent haemocoel. For, on the one hand, both thin and thick tubule segments remove ions; on the other hand the pylorus epithelium is not particularly capable of pumping ions from the gut lumen into the perinephric space.

Thus it could be possible to recover water from the rectum. It seems likely that atmospheric water uptake by the anal sac underlies quite similar mechanisms. In fact, Eisenbeis & Wichard (1985:162) published some data showing that *Polyxenus lagurus* is able to sequestrate water from the atmosphere. This process may be accomplished by the highly specialized arrangement of the anal sac and the malpighian tubules.

With reference to the cryptonephric system, *Polyxenus lagurus* represents a species, which has — unique among diplopods so far — developed special adaptations for dry terrestrial habitats. Because of the similarity with corresponding organs in insects, the related connexions between Penicillata and insects are again indicated.

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