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Ultrastructure of the Reno-Pericardial System in the Pond Snail *Lymnaea stagnalis* (L.)

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Summary. The ultrastructure of the organs involved in urine production in the pond snail *Lymnaea stagnalis* is described.

The atrial wall, which has been assumed to act as an ultrafilter, shows little ultrastructural correspondence with other ultrafilters, such as the mammalian glomerulus. Thus, ultrafiltration probably can take place in systems lacking the typical podocytes. The atrium of the stylommatophore *Helix pomalia* appeared to differ only in quantitative aspects — it is thicker — from that of the basommatophores *L. stagnalis* and *Biomphalaria glabrata*.

The reno-pericardial duct consists of ciliated columnar cells, which contain considerable amounts of glycogen.

The cells of the kidney sac are characterized by the presence of large (5—20 μ) excretion granules, which are constricted off together with part of the cytoplasm. In degenerating nephrocytes great numbers of lipid granules, probably arising from mitochondria, were found. Deposits of glycogen are present in the nephrocytes as well as in the cells of the ureter, suggesting the kidney to be a glycogen storing organ. The presence of glycogen is accompanied by that of an elaborate agranular endoplasmic reticulum.

Although relative differences in the general ultrastructural pattern of the kidney sac and the ureter were found, some aspects of both epithelia—viz. the presence of numerous large mitochondria, a zone of microvilli at the free cell surface, and prominent infoldings of the basal and lateral cell membranes — suggest them to be involved in the reabsorption of solutes and in the transportation of ions and water.

Potts (1967) reviewed the literature on the structure and function of the excretory organs in the Mollusca. He clearly showed that during the past three decades considerable progress has been made in the understanding of the kidney function. Proportionally, only little information on the (ultra)structure is available. For the Gastropoda the only electron microscope investigation is that of Bouillon (1960) on the kidney of the snail *Helix pomatia*. Light microscope studies, on the other hand, have been carried out, especially on the Pulmonata. Of this group, the Stylommatophora have received more attention (e.g. Krahelska, 1910; Turchini, 1923; Wæchter, 1934) than the Basommatophora (e.g. Carriker and Bilstad, 1946). Extensive light microscope studies have been devoted to the stylommatophore *Helix pomatia* (Stiasny, 1903; Freitag, 1916).

In this laboratory van Aardt (1968) carried out a physiological investigation on excretion in the basommatophoran pond snail *Lymnaea stagnalis*. It was supposed that ultrastructural evidence might be of great value for the interpretation of the obtained physiological results. Therefore an electron microscope study of the reno-pericardial system of *Lymnaea stagnalis* was undertaken.

The kidney of *L. stagnalis* consists of a kidney sac and a primary ureter, opening into the lung cavity (Grobben, 1891); a secondary ureter, as found in Stylommatophora (Rolle, 1907), is absent.
In all gastropods the kidney sac is connected with the pericardial cavity by the ciliated reno-pericardial duct. The morphology of this duct in *L. stagnalis* has been described by Rolle (1907).

It now has generally been recognized that urine formation in gastropods is a process similar to that in vertebrates (cf. Potts, 1967). Thus, the kidney forms the final urine by reabsorption of water and solutes from, and by secreting waste products into an ultrafiltrate of the blood. In gastropods ultrafiltration has been established for the prosobranchs *Haliothis rufescens* (Harrison, 1962) and *Viviparus viviparus* (Little, 1965), for the stylommatophores *Archachatina ventricosa*, *Helix pomatia* (Vorwohl, 1961) and *Achatina fulica* (Martin *et al.*, 1965), and for the basommatophores *Lymnaea peregra* (Picken, 1937) and *L. stagnalis* (Van Aardt, 1968).

It is not quite clear where ultrafiltration takes place. In most cases the wall of the atrium probably acts as an ultrafilter. The pro-urine is then transported from the pericardial cavity to the kidney sac via the ciliated reno-pericardial duct. However, it has been assumed that in terrestrial forms (the Stylommatophora) ultrafiltration occurs somewhere in the kidney epithelium, the reno-pericardial duct being very narrow in these snails (Vorwohl, 1961; Martin *et al.*, 1965; Martin and Harrison, 1966; Potts, 1967).

Evidence for reabsorption of water and solutes (e.g. NaCl, glucose) from the pro-urine is available (Potts, 1967; Van Aardt, 1968). Furthermore, in all gastropods investigated — including *Lymnaea stagnalis* (Spitzer, 1937) — kidney cells appeared to excrete nitrogen containing waste products like uric acid, urates and purines.

Thus, very probably in *L. stagnalis* the atrium, the reno-pericardial duct, the kidney sac and the ureter are involved in urine production. These structures were ultrastructurally investigated.

**Materials and Methods**

For the investigation 50 specimens of *L. stagnalis* (shell size 15—35 mm), 5 specimens of *Biomphalaria glabrata* (shell size 13—15 mm), and 5 specimens of *Helix pomatia* (shell size 36—42 mm) were used. The basommatophores were taken from stock reared in the laboratory for many generations. The specimens of *H. pomatia* had been kept in the laboratory for several months before use.

For light microscopy the reno-pericardial system was fixed in Stieve or Zenker (Romeis, 1948), and embedded in paraffin wax (m. p. 58° C). Five μ thick sections were cut and stained with various methods. Furthermore, 2 μ thick sections of Epon embedded material (see below) were studied with a phase-contrast microscope.

For electron microscopy the reno-pericardial system was fixed in Stieve or Zenker (Romeis, 1948), and embedded in paraffin wax (m. p. 58° C). Five μ thick sections were cut and stained with various methods. Furthermore, 2 μ thick sections of Epon embedded material (see below) were studied with a phase-contrast microscope.

For electron microscopy the reno-pericardial system was, after decapitation of the snail, fixed *in situ* for 20—30 min in a Sörensens-buffered glutaraldehyde solution (pH: 7.3; osmolality: 240 mOsm). In a number of cases the pericard was opened prior to fixation in order to fix the atrium in an expanded state. After glutaraldehyde fixation the reno-pericardial system was dissected out, washed during 40—60 hrs in Sörensens's buffer (pH: 7.3) to which sucrose was added to attain an osmolality of 240 mOsm, and fixed in a 2% solution of OsO₄ (with adjusted pH and osmolality) for 2 hrs. The above treatments were carried out at 4° C. Then, at room temperature, the tissues were dehydrated and embedded in Epon-812 (Pease, 1964). Ultrathin sections were cut with glass knives on a Reichert ultramicrotome, picked up on copper grids, stained with Reynold's lead citrate, and studied with a Zeiss EM-9 electron microscope.
**Observations**

The kidney of *L. stagnalis* can, at the dorsal side of the animal, be seen through the shell as a bright yellow, semi-circular organ (Fig. 1). Frontal to its proximal end, i.e. at the left side of the snail, the heart is located. At the distal end, the transparent, folded ureter opens into the lung cavity near the pneumostome, on a muscular papilla. At the dorsal side the reno-pericardial complex is attached to the epidermis. Ventrally it protrudes into the lung cavity (Fig. 2). Thus, the roof of the lung lies partly in front of and partly behind the kidney.

![Fig. 1. Dorsal and ventral view of *L. stagnalis*, showing the position of the reno-pericardial system. At I, II and III cross sections were made, which are diagramatically represented in Fig. 2. a atrium; lc lung cavity; ks kidney sac; pn pneumostome; u ureter; ve ventricle; vp vena pulmonalis](image)

The blood vascular system of lung and kidney seems to be rather complicated. Its pattern is hard to establish from light microscope sections, especially because the venules do not possess a well differentiated, closed, endothelial lining (cf. Carriker and Bilstad, 1946). However, in general the vascular system would not seem to be very different from that in *Helix pomatia* (Freitag, 1916). Alongside the front of the kidney, the large vena pulmonalis runs to the atrium.

**The Atrium**

As mentioned in the introduction, there are strong indications that the atrial wall in the genus *Lymnaea* acts as an ultrafilter (Picken, 1937; van Aardt, 1968). The same may be expected to hold for other Basommatophora. In terrestrial snails the atrium probably plays no part in ultrafiltration (Martin and Harrison, 1966). For these reasons, not only the ultrastructure of the atrial wall of *L. stagnalis*, but also that of another fresh water snail (*Biomphalaria glabrata*), and that of a land form (*Helix pomatia*) was investigated.

1. *Lymnaea stagnalis*. The wall of the atrium is composed of two layers separated by a basal lamina (Fig. 3). The outer layer, the 0.5—3 μ thick epicard, consists of extremely flattened, partly overlapping cells, which contain elongated
nuclei. The usual cell organelles (mitochondria, Golgi-complex, endoplasmic reticulum) are very poorly developed. In adult snails (shell length 30—32 mm) the epicard has a fairly electron dense appearance when compared to that of young snails (shell length 16—18 mm). In both age groups, however, it obviously consists of inactive cells.

The epicard is only at certain points closely attached to the basal lamina. Its remaining basal surface area is separated from the lamina by spaces, in which, apposed to the basal lamina, irregular shaped, electron lucent cells can be observed (Fig. 3). They contain some granular endoplasmic reticulum; other cell organelles are scarce. Probably these cells serve to replace epicardial cells.

The inner layer of the atrial wall is composed of connective tissue elements and of muscle cells. Directly against the basal lamina collagen fibrils occur in an amorph matrix. Furthermore, fibroblasts and cells containing pigment granules (\( \varnothing 0.2—0.5 \mu \)) were observed. The connective tissue layer is covered by smooth
Fig. 3. Wall of the atrium of *L. stagnalis*. The pericardial cavity (*Pc*) is lined by extremely flattened epicardial cells (*ep*). Between the epicard and the basal lamina (*b*) extensions of electron lucent cells (*el*) can be observed. *L* lumen of the atrium; *cl* layer of connective tissue; *mf* muscle fibre

Fig. 4. Wall of the atrium of *Helix pomatia*. *b* basal lamina; *ep* epicardial cell; *f* fibroblast; *mc* muscle cell; *ne* nerve ending containing transmitter granules; *pc* pericardial cavity. × 8,700
muscle cells. They form a network of trabeculae, which traverse the atrium. Among the smooth muscle cells an occasional cross-striated muscle fibre is present. An endothelium, to separate the connective tissue and muscular elements from the blood, appeared to be absent. In atria fixed in a contracted state, the epicard as well as the connective tissue layer were strongly folded.

2. Biomphalaria glabrata. The structure of the atrium in this species shows only minor differences with that of L. stagnalis. Contrary to the situation in Lymnaea, the epicard is over its entire basal surface attached to the basal lamina. Furthermore, the epicardial cells, which are less electron dense than in Lymnaea, contain more and better developed cell organelles. The inner layer of the atrial wall is, in its general structure, not dissimilar to that of L. stagnalis.

3. Helix pomatia. Although composed of the same layers and elements as in the fresh water snails, the atrial wall of Helix has a different appearance, which is mainly caused by the greater thickness of the connective tissue layer. The epicardial cells, which contain mitochondria, granular endoplasmic reticulum and electron transparent vacuoles, are not as flattened as in the investigated basommatophores. They do not form a closed layer. Many basal cell projections connect them tightly to the connective tissue layer. Between the muscle cells of the atrial wall nerve endings laden with electron dense elementary granules were observed (Fig. 4). The granules probably contain neurotransmitter substance (cf. Baxter and Nisbet, 1963).

The Pericard

The pericardial wall, which is continuous with the epicard, is composed of a single layer of flattened cells (height 1—3 μ). The cells contain only few cell organelles. A basal lamina separates the epithelium from underlying connective tissue.

The Reno-Pericardial Duct

In unfixed, full-grown L. stagnalis the length of the reno-pericardial duct approximates 1.5 mm; its diameter varies from 250—500 μ. The duct is lined by a 15—20 μ high, ciliated, columnar epithelium. The cilia (length 60—100 μ) have a strong beat directed towards the kidney sac.

In fixed specimens the epithelium is folded due to contraction of muscle fibres, which form a circular layer around the duct. The epithelial cells stain positively with periodic-acid-Schiff (PAS) and with Best’s carmine, indicating the presence of glycogen.

Ultrastructurally the cilia have the normal 9 + 2 fibril structure (cf. Fawcett, 1961). Their basal bodies are anchored in the cytoplasm by a cross-striated rootlet (Fig. 5). The epithelial cells are interconnected by zonulae adherentes (cf. Farquhar and Palade, 1963), located at the apical parts of the lateral apposed cell surfaces. The nucleus, which possesses a large nucleolus, is situated in the cell centre. Other cell organelles are arranged alongside the cell membrane (Fig. 6). The endoplasmic reticulum is mainly of the agranular type; the mitochondria are elongated; the Golgi complexes consist of 4—8 sacculi surrounded by small Golgi vesicles.

A characteristic feature of the epithelium is the presence, in the central parts of the cells, of large amounts of glycogen, which could be recognized as
Fig. 5. Apices of two cells of the reno-pericardial duct. Cilia (ci) extend into the lumen (l) of the duct. Striated rootlets (r) anchor the basal bodies (bb) of the cilia; er smooth endoplasmic reticulum; lm lateral cell membrane; mi mitochondria. × 20,000

Fig. 6. Basal parts of two cells of the reno-pericardial duct. Large amounts of glycogen are present as α-rosettes (α) and β-particles (β). The mitochondria (mi) are arranged alongside the lateral (lm) and basal cell membrane. × 12,600
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α-rosettes (cf. Drochmans, 1962; Revel, 1964; Perry, 1967), 1,000—2,000 Å in diameter, composed of small (200 Å) β-particles.

The Kidney Sac

The kidney sac is — in particular at its dorsal side — strongly folded, forming lamellar and villilike invaginations into the lumen (Figs. 2, 7). A thin basal lamina separates the epithelium from a loose reticulum of connective tissue cells and fibres, present inside the villi and between the lamellae. The meshes of the reticulum are continuous with blood lacunae in the connective tissue surrounding the kidney.

The epithelium is composed of only one cell type, the columnar nephrocytes, excepting for small parts near the opening of the reno-pericardial duct. Here, at the bases of the folds, areas consisting of squamous epithelial cells, comparable in shape to those of the pericard, occur (Fig. 7).

Light microscope observations reveal the nuclei (5—7 μ) of the nephrocytes to be located in the cell centre. At the free cell surface a brush border is observed. The cell basis has a striated appearance. However, the most characteristic feature of a nephrocyte is the presence of a large apical vacuole, containing an excretion granule, measuring 5—20 μ in diameter (Fig. 7). Especially in cells at the top of a kidney fold, osmiophilic granules (1—2 μ) were found (Figs. 7, 10). Furthermore, in most cells, small (1—3 μ) non-excretory vacuoles occur. The nephrocytes stain positively with methods for indicating the presence of glycogen (PAS, Best’s carmine).

The brush border of the light microscope image is resolved into a great number of microvilli by the electron microscope (Fig. 8). At the top of these microvilli, antennulae microvillares were observed. Near the apices of contiguous cells, the apposed lateral cell surface membranes contribute to the formation of intercellular junctions (zonulæ adherentes). The basal cell membrane shows prominent infoldings. In the cytoplasm between these infoldings, elongated mitochondria, orientated with their long axis perpendicular to the cell basis, occur.

The endoplasmic reticulum is almost exclusively of the agranular type (Fig. 9). It is absent from the basal cell parts. In the remaining areas, in particular alongside the lateral cell surface and around mitochondria, it is observed as vesicles and branched tubules. Single ribosomes as well as polysomes occur. Golgi complexes consist of 4—7 sacculi surrounded by small Golgi vesicles. The mitochondria usually contain electron dense intramitochondrial particles (Fig. 11).

Just as in the reno-pericardial duct, considerable amounts of glycogen are present in the nephrocytes as α-rosettes and β-particles. The size of the rosettes is approximately 625 Å. Thus, they are smaller than those of the reno-pericardial duct.

The large apical vacuole is delimited from the cytoplasm by an unilaminar membrane. The excretion granules inside the vacuoles are composed of alternating electron transparent and electron dense layers, concentrically arranged around an amorphous core. In some cases two or three granules were present in one vacuole; moreover, granules possessing two or three cores were observed.
Fig. 7. Camera lucida drawing of part of the kidney sac of *L. stagnalis* near the opening of the reno-pericardial duct. At the top (*T*) of a kidney fold degenerating cells are seen; at the basis (*B*) squamous epithelial cells (*sq*) occur. *a* amoebocyte; *ex* excretion granule; *f* fibroblast; *L* cell of Leydig; *li* lipid granule

To release the granules into the urine, the vacuole together with part of the cytoplasm, in which cell organelles could be distinguished, is constricted off. This was not only established from many electron micrographs (Fig. 8), but also by investigating urine under the phase-contrast microscope.

Nephrocytes constricting off vacuoles were found all over the epithelium. Degenerating cells were only seen at the top of the kidney folds (Fig. 7). These observations suggest that a cell has the ability to produce an excretion granule more than once. Indications to assume that the release of granules from different cells is synchronized, were not obtained.

At the basis of the microvilli invaginations occur, suggesting endocytosis. In the zone underneath the microvilli vacuoles (0.2—1.5 µ) are present, which probably originate by fusion of endocytotic vesicles, since they possess a triple-layered membrane, comparable to the cell membrane. According to **De Duve** and **Wattiaux** (1966) intracellular digestion of material may occur after fusion of endocytotic vesicles with pre-lysosomes. Indications for the occurrence of this
process were obtained, in that lysosomes containing various material were observed (Figs. 10, 11).

Only in the basal area of a kidney fold sometimes mitoses were observed, indicating the place of cell differentiation. As signs for cell degeneration — at the top of the folds —, were regarded the presence of many lysosomes and osmiophilic granules, and the absence of well developed microvilli and an apical excretion vacuole. The number of osmiophilic granules per cell increases in cells nearer to the top of the kidney folds (Fig. 7). They show similarity with the "grains jaunes" described by Turchini (1923) and Bouillon (1960) in the nephrocytes of Helix pomatia. The latter author considered the granules to consist of oxidation products of phospholipids (lipofuscins). Ultrastructurally they appear to be composed of a ring of homogeneous, fairly electron dense material around an electron transparent core (Figs. 10, 12). However, granules lacking this core were also found. These may be regarded as the last stage in lipid granule formation (cf. Smith and Farquhar, 1966).

It seems not improbable that, in the nephrocytes of L. stagnalis, as in those of Helix pomatia (Turchini, 1923; Bouillon, 1960), part of the lipid droplets originates from degenerating mitochondria. First, the regular pattern of the mitochondrial cristae is lost; they are transformed into concentrically arranged membranes (Figs. 10, 11). Then, these membranes fuse to constitute an osmio-
philic layer around the electron transparent matrix, from which the intramitochondrial particles have disappeared (Fig. 12).

The Ureter

The most conspicuous feature characterizing the sharp transition of the kidney sac into the ureter is the absence of excretion granules from the ureter epithelium. As in the kidney sac, epithelial folds, mainly longitudinally orientated, occur in the ureter. Well defined areas of cell proliferation and cell degeneration could not be distinguished. The connective tissue capsule of the ureter, which is quite similar to that of the kidney sac, contains only a few smooth muscle cells, excepting for the ventral side, where a small (\( \varnothing 0.5 \text{ mm} \)) longitudinal muscle is present, which is continuous with the well developed circular muscle of the ureter papilla.

The ureter epithelium consists of 12—15 \( \mu \) high, glycogen containing (i.e. PAS- and Best’s carmine positive) columnar cells. At the ultrastructural level the cells differ in some respects from the cells of the kidney sac. Thus, the apical zone of microvilli is less well developed in the ureter (Fig. 13). On the other hand, the infoldings of the basal cell surface extend further into the cytoplasm — in some cases they even almost reach the apical cell membrane — whereas, in addition, infoldings of the lateral cell surface were observed. Just as in the reno-pericardial duct, the cells of the ureter are near the apices interconnected by zonulae adherentiae. The nuclei are situated in the centre of the cells. Numerous large mitochondria,
Figs. 10—12. Degenerating nephrocytes from the top of a kidney fold. The microvilli (mv) of the free cell surface have almost disappeared (Figs. 10, 11). In the cells lysosomes (ly) containing various (e.g. crystalline) materials can be observed (Figs. 10, 12). Obviously lipid droplets (li) originate from mitochondria (mi). In some mitochondria intramitochondrial particles (ip) can still be seen (Fig. 11), although the normal arrangement of the cristae has already been lost.

Fig. 11: $\times 15,500$; Fig. 12: $\times 14,000$; Fig. 13: $\times 22,000$
in particular located in the basal folds, are present (Fig. 13). The Golgi-complexes consist of 3—6 sacculi surrounded by small Golgi vesicles. Agranular endoplasmic reticulum occurs in the apical cell parts. Glycogen is present as α-rosettes (1,800 Å) and β-particles.

Contents of the Kidney Sac

In the lumen of the kidney sac of three 35 mm snails, out of a group of ten, very large concretes (6—10 mm) were observed, in addition to the usually occurring smaller ones (0.5—3 mm). These concretes probably consist of the same material as the excretion granules. Very likely the main constituent is uric acid, as Spitzer (1937) found this compound to be accumulated in the kidney lumen. Because excretion of large quantities of uric acid is considered as an adaptation to terristrial life, it has been supposed that the uricotelism of L. stagnalis indicates that the pond snail has descended from a land form (Needham, 1935). However, this explanation for the high production of uric acid by L. stagnalis seems still open for discussion (Potts, 1967).

In the kidney lumen furthermore cell debris, probably remnants of degenerated nephrocytes, occurs. Moreover, 18 hrs after injection of 50 μl of a 1% aqueous trypan-blue solution (Romeis, 1948), numerous dye-laden amoebocytes were found in the lumen of the kidney sac. These cells, which could be identified as last stages in the development of the amoebocyte as described by Müller (1956), were also
observed in between the nephrocytes. Thus, apparently whole cells pass through the kidney epithelium (cf. Trip, 1961; Martin and Harrison, 1966) to be excreted in the urine.

**Discussion**

Ultrastructurally the cells of the outer layer of the atrial wall in *L. stagnalis* appeared to consist of rather inactive cells. Rolle (1907) regarded this layer as homologous with the pericardial glands of prosobranchs described by Grøbben (1891). Although both authors recognized that the epithelia concerned do not have a gland-like appearance, they supposed — unacquainted with ultrafiltration mechanisms — that these "glands" contribute to urine production by active secretion. In Lamellibranchia, on the other hand, pericardial glands consisting of genuine glandular cells have been described (cf. Potts, 1967). Such structures were not found in the investigated pulmonate species.

Observations on the pericardial fluid of basommatophores (Picken, 1937; van Aardt, 1968) suggest the atrial wall to act as an ultrafilter. However, its ultrastructure in *L. stagnalis* and *Biomphalaria glabrata* shows little correspondence with that of known ultrafilters, such as the mammalian glomerulus (Müller, 1958; Menefee and Müller, 1967), or the coelomic sac of the crustacean *Cambarus affinis* (Kümmel, 1964). In the glomerulus the blood is separated from the ultrafiltrate by three layers, viz. a fenestrated endothelium, a basal lamina, and an epithelium consisting of podocytes, which are cells possessing numerous small feet apposed to the basal lamina. The typical podocytes were also found in the coelomic sac of *Cambarus*. In the atrial walls of the investigated pulmonates, on the other hand, neither endothelial cells nor podocytes occur.

Interpreting these morphological data, it is not quite clear whether or not the atrial wall has to be considered as an ultrafilter. If it is one — as the physiological evidence suggests — then obviously ultrafiltration can be brought about in systems which may differ ultrastructurally.

The main difference between the atrial wall of *Helix pomatia* and that of the investigated basommatophorans is quantitative in nature: in *Helix* it is much thicker. Thus, on a morphological basis, it might perhaps be supposed that in *Helix*, too, ultrafiltration can take place through the atrial wall, but at a much lower rate. This would be in accordance with the fact that urine production in *Helix* is rather low when compared to that in *L. stagnalis* (cf. van Aardt, 1968). However, it has been assumed that ultrafiltration in *Helix* occurs at places in the kidney sac (cf. Vorwohl, 1961; Martin and Harrison, 1966). If this were also true for *Lymnaea*, then perhaps the basal areas of the kidney folds near the opening of the reno-pericardial duct should be considered as such places, since here the epithelium consists of extremely flattened cells. It is difficult, however, to understand how the hydrostatic pressure in the lacunar venous system of the kidney could be adequate for ultrafiltration.

The direction of the ciliary beat in the reno-pericardial duct suggests that fluid is transported from the pericardial cavity to the kidney sac. Obviously the epithelium of the duct is furthermore involved in the storage of glycogen, deposits of which were also found in the kidney sac and the ureter. It seems reasonable to assume that this glycogen is synthesized of glucose reabsorbed from the ultra-
filtrate: uptake of glucose by the kidney has, among others, been reported for *Achatina fulica* (Martin et al., 1965). Moreover, very probably the differentiation of the apical cell surface into a zone of microvilli, in both the kidney sac and the ureter, may be regarded as the ultrastructural expression of the capability to reabsorb solutes, because in other epithelia, *e.g.* of the intestine and the proximal convoluted kidney tubule of vertebrates, the presence of microvilli has been related to absorption.

In all parts of the kidney the presence of glycogen is accompanied by that of an elaborate agranular endoplasmic reticulum, supporting the view that smooth endoplasmic reticulum is involved in glycogen metabolism (Threadgold, 1967). These ultrastructural data suggest that in *L. stagnalis* the kidney is a glycogen storing organ.

The cells of the kidney sac and the ureter of *L. stagnalis* have two ultrastructural aspects — *viz.* a) the presence of numerous large mitochondria and b) the strongly folded basal and lateral cell membrane — in common with ion- and water transporting epithelia, such as occur in the kidney of *Helix* (Bouillon, 1960), the proximal and distal tubules of the mammalian kidney (GoldfisHER et al., 1964), the gall bladder (Tormey and Diamond, 1967), the avian salt gland (Doyle, 1960: Komnick, 1963), and the Malpighian tubules of insects (Berridge, 1968). According to Diamond and Bossert (1967) these aspects — perhaps apical microvilli also play a role — may be considered as the structural basis for active ion transport, water following passively along an osmotic gradient, which is maintained in the long, narrow channels, formed by the infoldings of the basal and lateral cell membrane. Thus, the ultrastructure supports the view, based on physiological evidence (van Aardt, 1968), that solutes as well as water are reabsorbed by the kidney of *L. stagnalis*. However, relative differences in the ultrastructure of both kidney parts of *Lymnaea* — in the kidney sac the microvilli are more prominent, in the ureter the basal infoldings — suggest differences in reabsorption capacity and/or function. Perhaps the two parts may be compared to the proximal and distal convoluted tubules of the mammalian kidney, respectively (see also Potts, 1967).

The excretory function of the kidney sac finds its morphological expression in the presence and release of excretion granules. The granules are constricted off with part of the cytoplasm. Release of granules after rupture of the apical vacuole was not observed in the present material. Obviously, rupture of the vacuoles (Freitag, 1916) is artificial, and can be avoided by proper fixation and embedding.

References


Goldfischer, S., E. Essner, and A. R. Novikoff: The localisation of phosphatase activities at the level of ultrastructure. J. Histochem. Cytochem. 12, 72—95 (1964).


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