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Ultrastructural and Cytochemical Investigations on the Renal Appendages and Their Concrements in Dibranchiate Cephalopods (Mollusca, Cephalopoda)*

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Summary. The renal appendages of the octopods *Octopus vulgaris* Lam., *Eledone moschata* Lam. and of the decapods of the order Sepioidea *Sepia officinalis* L., *Sepia elegans* d'Orb. and *Sepietta obscura* Naef have been investigated using different methods of light and electron microscopy and of cytochemistry. The cells of the excretory epithelium are polarised; in all species, they show a basal labyrinth with a thick positive PAS and alcian blue reaction. The cells contain numerous mitochondria, which show the crista type of internal structure, and in the basal as well as in the apical part there are lysosomal dense bodies with high phosphatase activity. The fine structure, the osmolality and the ionic composition of the blood, the urine and of sea water suggest osmotic filtration combined with active ion transport and apocrine excretory secretion and reabsorption. In *Sepia* only, crypts of the epithelium were found to contain spherical mixed crystals which contain Ca, K, Na, Mg, Cl, S, P according to the electron probe and cytochemical analysis; the matrix shows concentric layers, it gives a positive PAS and alcian blue reaction. The chemical composition, the genesis and the possible function of these crystals are discussed.

A. Introduction

Excretory secretion and ion transport in cephalopods are very important processes that maintain a rather constant internal medium as compared to conditions in other molluscs. It has been shown by different authors (Potts, 1965, 1967; Harrison and Martin, 1965; Schipp and Boletzky, 1974) that various organs take part in these regulatory transport processes in cephalopods; in addition to the renal appendages, these are the branchial hearts and their appendages, the "pancreatic" appendages (Boucaud-Camou, 1972), the coelomic epithelium, the ductus reno-pericardialis, the gills and probably also the midgut gland. The bulk of excretion, however, is no doubt carried out by the voluminous renal appendages (Potts and Todd, 1965; Harrison and Martin, 1965); rightly

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these are therefore considered as the actual renal organs of the cephalopods. The hitherto unknown ultrastructure of these organs is the main subject of the present study, in which structural and cytochemical findings are compared with the physiological data of the afore-mentioned authors.

B. Materials and Methods[1]

Animals. The following species have been investigated: *Octopus vulgaris* Lam., *Eledone moschata* Lam., *Eledone cirrosa* Lam., *Sepia officinalis* L., *Sepia elegans* d'Orb., *Sepietta obscura* Naef. These were represented by juvenile and adult animals. Of *Sepia officinalis*, newly hatched animals and young aged 3, 4–5, 12, 18–20, 35, 42–44 and 57 days, which had been reared in the laboratory, were studied. Juvenile *Sepia*, 42–44 days old, which had continually been underfed, were also used in this study (c.f. Boletzky, 1974).

Light Optical Methods. Material fixed with glutaraldehyde and formalin was either embedded in paraffin or frozen; sections were stained with Masson's trichrome after Goldner, with alcian blue, or they were treated for the PAS reaction. One micron sections were studied in the phase contrast microscope.

Electron Microscopical Methods. normal tissue was pre-fixed in 4% glutaraldehyde in a phosphate buffer; 1000 mOsm; pH 7.2; 2 hrs; 6–8° C; this was followed by a post-fixation in 1.5% OsO_4 in phosphate buffer, 1–2 hrs. The material was embedded in Araldit. Sections were cut on a LKB ultramicrotome; they were stained with uranyl acetate and lead citrate and viewed in a ZEISS EM 9A or a PHILIPPS EM 300.

For *scanning electron microscopy*, tissue fixed in glutaraldehyde was freeze dried, mounted on Al-blocks with silver paste, and finally coated with gold. The SEM investigations were performed on a Cambridge Stereoscan 4 microscope. For the element analysis of the urinary concrements of *Sepia officinalis*, an Ortec Multichannel Analyzer Model 6200 was used.

Cytochemical Studies on *Sepia officinalis*

a) Demonstration of Carboanhydrase (Hausson modific. of Yokota, 1969). Fixation: 4% glutaraldehyde in 0.2 M cacodylate buffer and 0.1 M sucrose; pH 7.2; 3 hrs. Rinsing: cacodylate buffer. Incubation of tissue pieces or freeze sections: 20 min at 20° C in a solution of *A*: 0.1 M $CoSO_4$ 1 ml + 0.5 M H_2SO_4 6 ml + $^1/_{15}$ M KH_2PO_4 10 ml + aqua dest. 17 ml, and, in the ratio of 1 to 1, *B:* $NaHCO_3$ 0.75 g in 40 ml aqua dest. Rinsing in buffer for 10 min. Post-treatment in 0.5% $Pb(NO_3)_2$ for 20 min; post-fixation in 1% OsO_4–0.1 M cacodylate buffer (pH 7.2) for 1 hr at 4° C. Controls: after first fixation tissue pieces in Diamox 10^{-4} M in 0.25 M sucrose for 20 min. Dehydration: acetone. Embedding: Araldit (Fluka).

b) Demonstration of Ca-ions (Carasso and Favard modific. of Komnick, 1969). Fixation: 5% glutaraldehyde—veronal acetate buffer; 870 mOsm (pH 7.1) with 0.0125 M ammonium acetate for 1 hr. Rinsing: 0.025 M ammonium oxalate—veronal acetate buffer for 2.5 hrs. Post-fixation: 1% OsO_4-veronal acetate buffer with 2.5×10^{-4} M ammonium oxalate for 1 hr. Rinsing: 0.025 M ammonium oxalate—veronal acetate buffer (a few minutes). Dehydration: acetone. Embedding: Araldit. Controls were fixed without oxalate.

[1] The technical assistance of B. Albat, A. Bleichner, M. Heller, B. Hindorf and U. Luft is gratefully acknowledged.

c) Demonstration of Ca-Salts (Carasso and Favard, 1966). Fixation: 2.5% glutaraldehyde in 1.7 M veronal acetate buffer (pH 7.2) for 45 min. Rinsing: aqua dest. with NaOH at pH 8 for 3 × 3 min. Incubation: 5% solution of lead acetate for 20 min. at 37° C. Rinsing: aqua deat. Post-fixation: 1% OsO_4 in veronal acetate buffer at pH 7.2 for 30 min. Controls: material treated with citrate buffer (pH 4.5) after first fixation.

d) Demonstration of Mucopolysaccharides by Alcian Blue (Tice and Barnett, 1962). Fixation: 4% glutaraldehyde in phosphate buffer (pH 7.2) for 2 hrs at 4° C. Rinsing: buffer for several hours at 4° C. Pre-incubation: 0.05 M acetate Cl buffer (pH 2.5) for 2 hrs; freeze sections. Incubation: 0.5 % alcian blue (pH 2.5) for 2 hrs. Rinsing: 0.05 M acetate HCl buffer (pH 5) for 30 min. Dehydration, embedding. No OsO_4 fixation.

C. Results

External Morphology and Development of the Renal Appendages. The renal appendages, or vein saccules, of dibranchiate cephalopods are clusters formed by the vena cava, or vena cephalica, which carries the blood from the head via the two posterior branches to the branchial hearts (Vigelius, 1880; Grobben, 1883). The appendages lie on the (physiological) ventral side, within the paired renal sacs. The material excreted by them is collected in these sacs and is then discharged through the paired renal openings into the mantle cavity, together with the material excreted by the renal appendages of the pericardial coelom (which communicates with the renal sac), *i.e.* the branchial hearts with their appendages ("pericardial gland"), and in the Sepioidea also the dorsal renal sac with the pancreatic appendages (Schipp and Boletzky, 1974) (Fig. 1).

At early developmental stages already, the close relationship between the coelom, the rudiment of the renal sacs and the circulatory system is apparent in all the cephalopod species so far studied (Naef, 1909, 1912; Boletzky, 1968; Meister, 1972; Fioroni and Meister, 1974). In *Octopus vulgaris*, the paired rudiment of the renal sacs appears at stage XII of Naef (1923) as part of the coelomic mesoderm (Marthy, 1968); it is in close contact with the vena cava branches which are formed as part of the "schizocoel" making up the circulatory system (Boletzky, 1968). During later embryonic development, the wall of the vena cava branches forms ramifications interdigitating with the wall of the renal sacs, and the latter differentiates as a layer of high, transport active cells; this is the renal epithelium (Figs. 1 and 2a) (Marthy, 1968). The rest of the wall of the renal sacs has a similar structure, but the cells are flattened.

Scanning Electron Microscopical Observations. The scanning electron micrographs show that the ramifications that can be made out macroscopically are composed of numerous lobuli surrounding the larger branches. The fungoid lobuli, which have different sizes, are made up

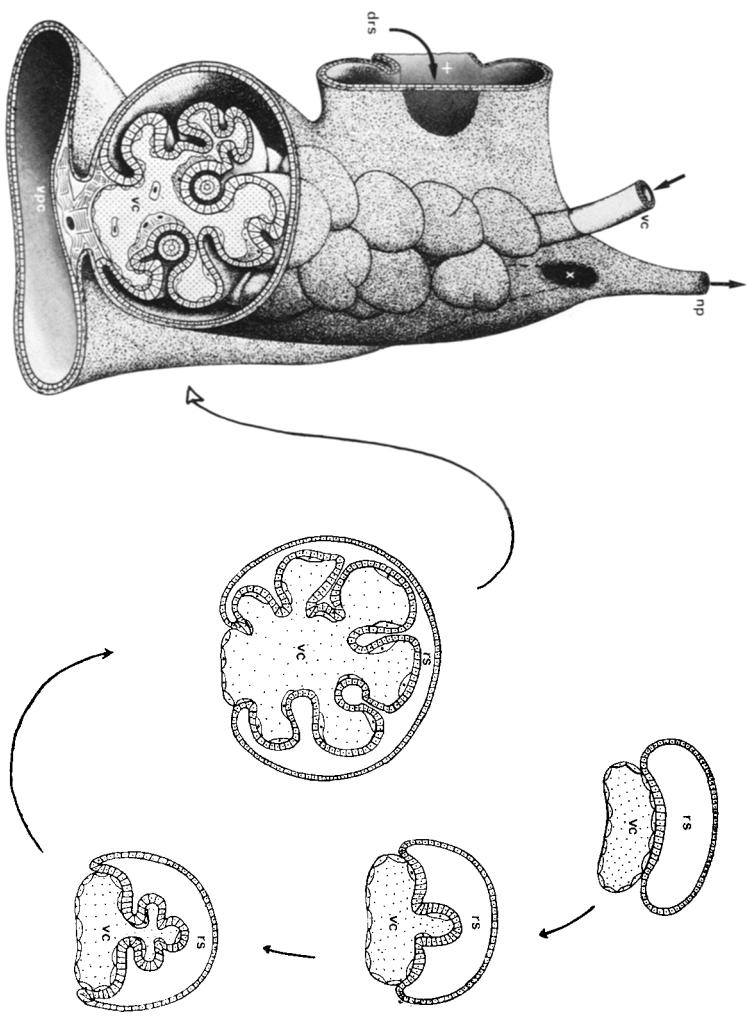

Fig. 1. Schematic diagram of the renal sac surrounding the renal appendages of the vena cava system *(vc)* in ventral view (left); in the right: schematic cross sections to the ontogenesis of the organ; opening (+) of the dorsal azygous renal sac *(drs)* into the ventral part; nephroporus *(np)*; vena cava *(vc)*; viscero-pericardial coelom *(vpc)* and its opening *(x)* into ventral renal sac *(rs)*

of a large number of small saccules of irregular sinuate shape (Fig. 3a and b). These are closely spaced, so that there are only very narrow interstices which deeply penetrate the organ. At high magnification (Fig. 3c), the surface of the saccules shows a lamellar sheathing which covers parts of the actual surface of the epithelium, i.e. of the apical region of the brush border.

Light Microscopical Findings. Observation in the phase contrast microscope reveals the high similarity in the structure of the renal epithelium in all the species investigated. The epithelial cells show a

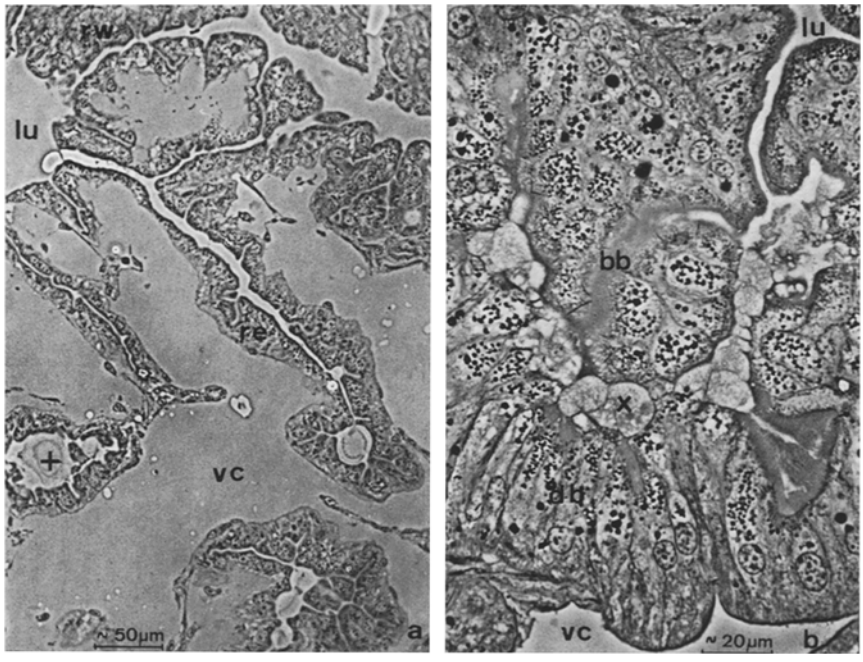

Fig. 2. (a) Part of a cross section of the renal appendages of *Sepia officinalis* at age of 42–44 days p.p. with interdigitations between the vena cava system *(vc)* and the renal epithelium *(re)* (in phase contrast); epithelial crypts with spherical bodies (+), wall of the renal sac *(rw)*. (b) Inner area of the renal appendages of *Eledone moschata* (adult) in phase contrast; intercellular dense bodies *(db)*; secretory protuberances *(x)* in brush border *(bb)*; lumen *(lu)*

polar structure, with a brush border at the side of the organ lumen and a continuous basal lamina next to the sinusoid and lacunar blood spaces (Fig. 2b). Both structures show a positive alcian blue and PAS reaction. In the apical region of the epithelial cells, an accumulation of dense bodies can already be seen in juvenile animals; they are particularly massive in adults (Fig. 2b). Moreover in all the species investigated, the apical region of the epithelium is caracterized by comparatively large protuberances of the plasma membrane which project into the lumen of the renal sac. These protuberances which show a basal constriction contain a fine granular material, occasionally also dense bodies mentioned above (Fig. 2b). They seem to disintegrate as soon as their contents are released into the lumen.

A peculiarity was observed in the renal appendages of the Sepioidea. In juvenile animals already, at an age of 18–20 days, the basal part of the narrow interstices mentioned above forms crypt-like enlargements

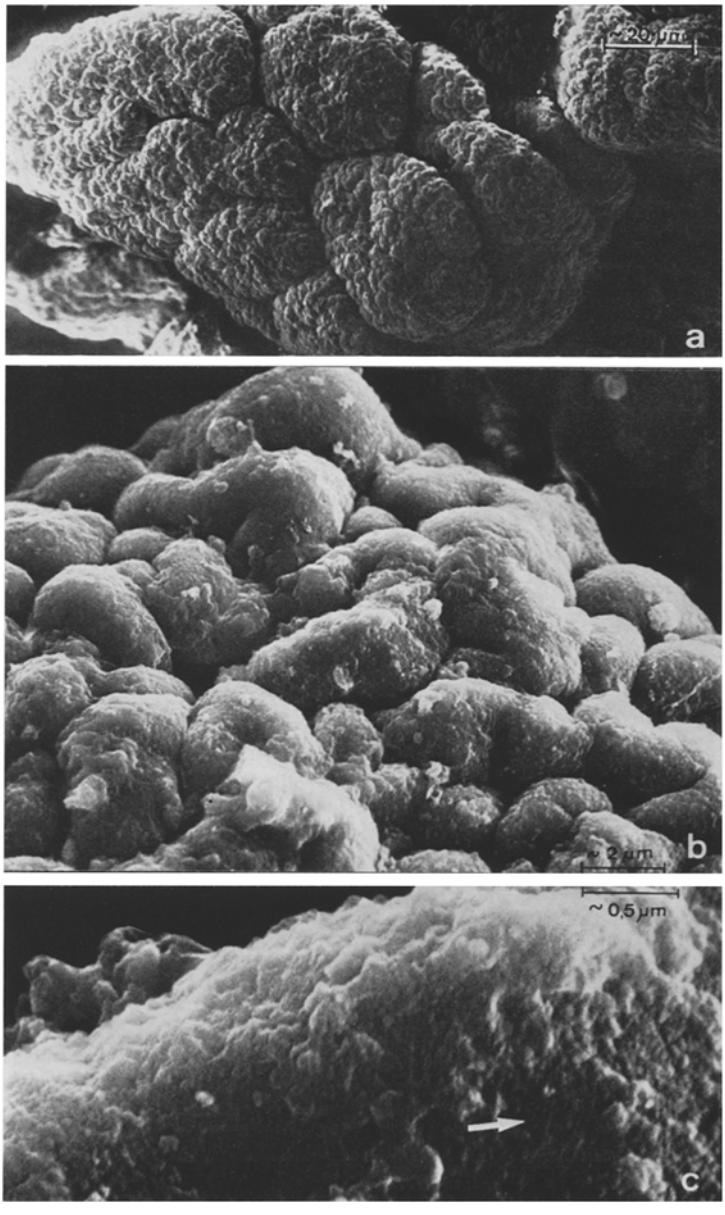

Fig. 3a—c. Scanning electron micrographs of the surface of renal appendages of *Sepia officinalis*; pattern of the surface of the brush border (arrow) partially covered by glycocalyx material

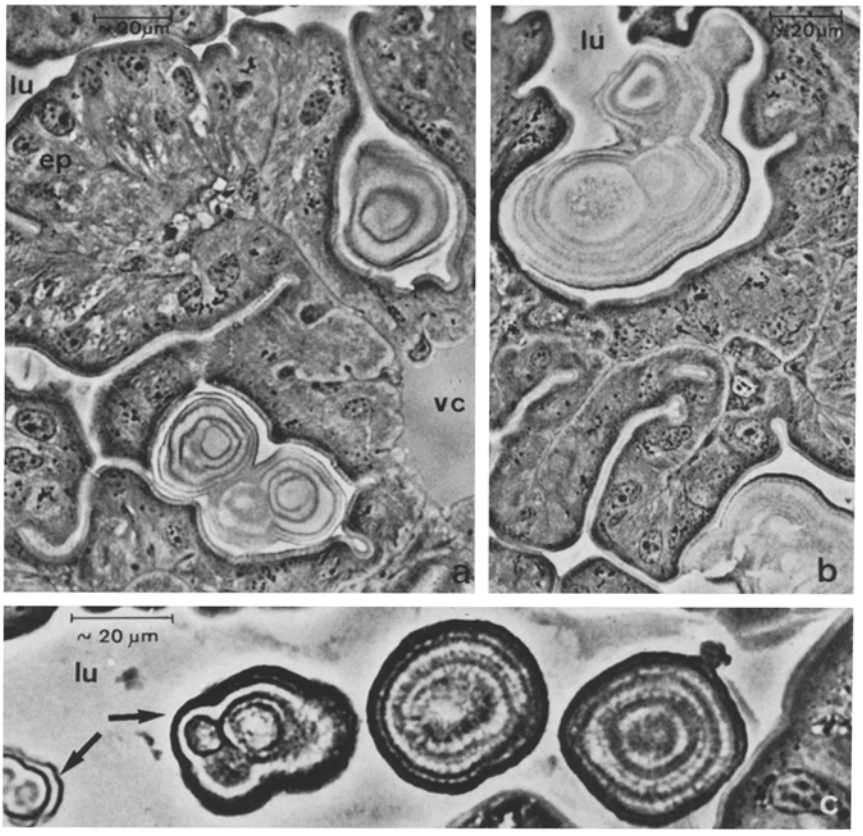

Fig. 4. (a) and (b) Phase contrast micrographs of spherical bodies with concentric layering in crypts of the epithelium *(ep)* of *Sepia officinalis*; some of the bodies in the peripheral renal sac lumen *(lu)* show solution tendency (c, arrows); vena cava system *(vc)*

which appear as circular structures on sections (Fig. 2a). These cavities contain spherical, stratified bodies, spherites (diameter 40–60 µm) which fill the space of the crypt entirely or nearly so (Fig. 4a-c). They are rarely seen in larger widenings of the middle and inner region of the renal appendages, still more rarely among the urinary crystals of the outer renal fluid (Fig. 5a und b). If they appear there, they generally show a tendency to disintegrate, so that often only fragments in form of distorted layers remain (Fig. 4c). The spherites frequently are duplicate or even multiplex (Fig. 4b); their colour generally is white, sometimes light orange. The concentrically stratified lamellae give positive PAS and alcianblue reaction, like the apical brush border.

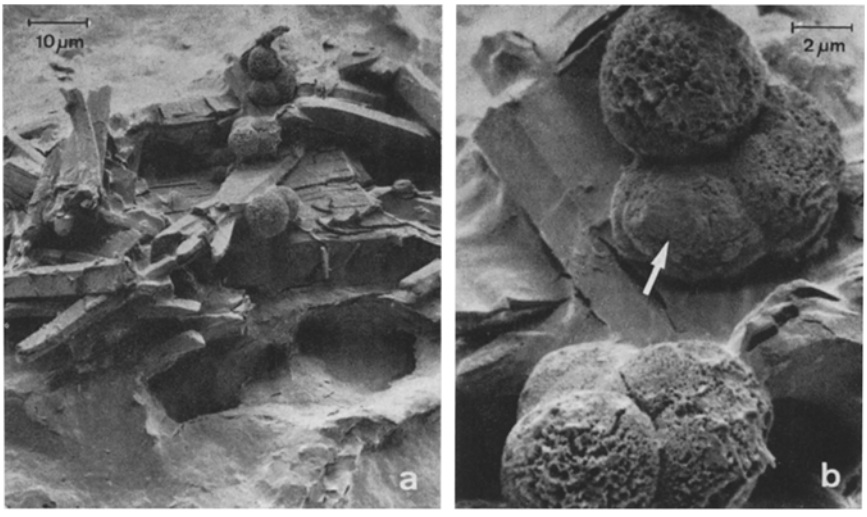

Fig. 5a and b. Scanning electron micrographs of the spherical renal bodies in contact with organic renal crystals, taken from the peripheral renal sac fluid of *Sepia officinalis;* the spongious surface—probably produced by solution processes in acid urine—is partly covered by smooth coats (arrow)

Cytological Findings. The electron microscopical investigations confirm the phase contrast microscopical findings on the structure of the renal epithelium which is basically identical in all the species studied. This epithelium proves to be a layer of cells with polar differentiation showing a basal labyrinth with a basal lamina and an apical border of long microvilli with a glycocalyx next to the lumen (Fig. 6a und b). The microvilli generally have a regular shape; there are also clavate forms, particularly in the inner part of the organ. All microvilli contain longitudinal filaments which are connected with the terminal web. The apical part of the microvilli shows a caplike thickening (Fig. 10b). Signs of intense micropinocytosis between the microvilli were not observed. Laterally the cells interdigitate with one another; towards the lumen,

Fig. 6a and b. Electron micrographs (sagittal sections) of peripheral lobuli of the renal appendages of an adult *Eledone moschata* (a) and *Sepia officinalis* at age of 44 days (b); note the numerous dense bodies (inset on the left) and mitochondria *(mi)* in the apical epithelium and the basal labyrinth; lumen of the renal sac *(lu)*, blood sinus *(bs)*, lamina basalis (arrows), microvilli with glycocalyx *(mv)*, lipid droplets *(ld)*, septate desmosome (inset on the right). × 40800 (left inset), × 46750 (right inset)

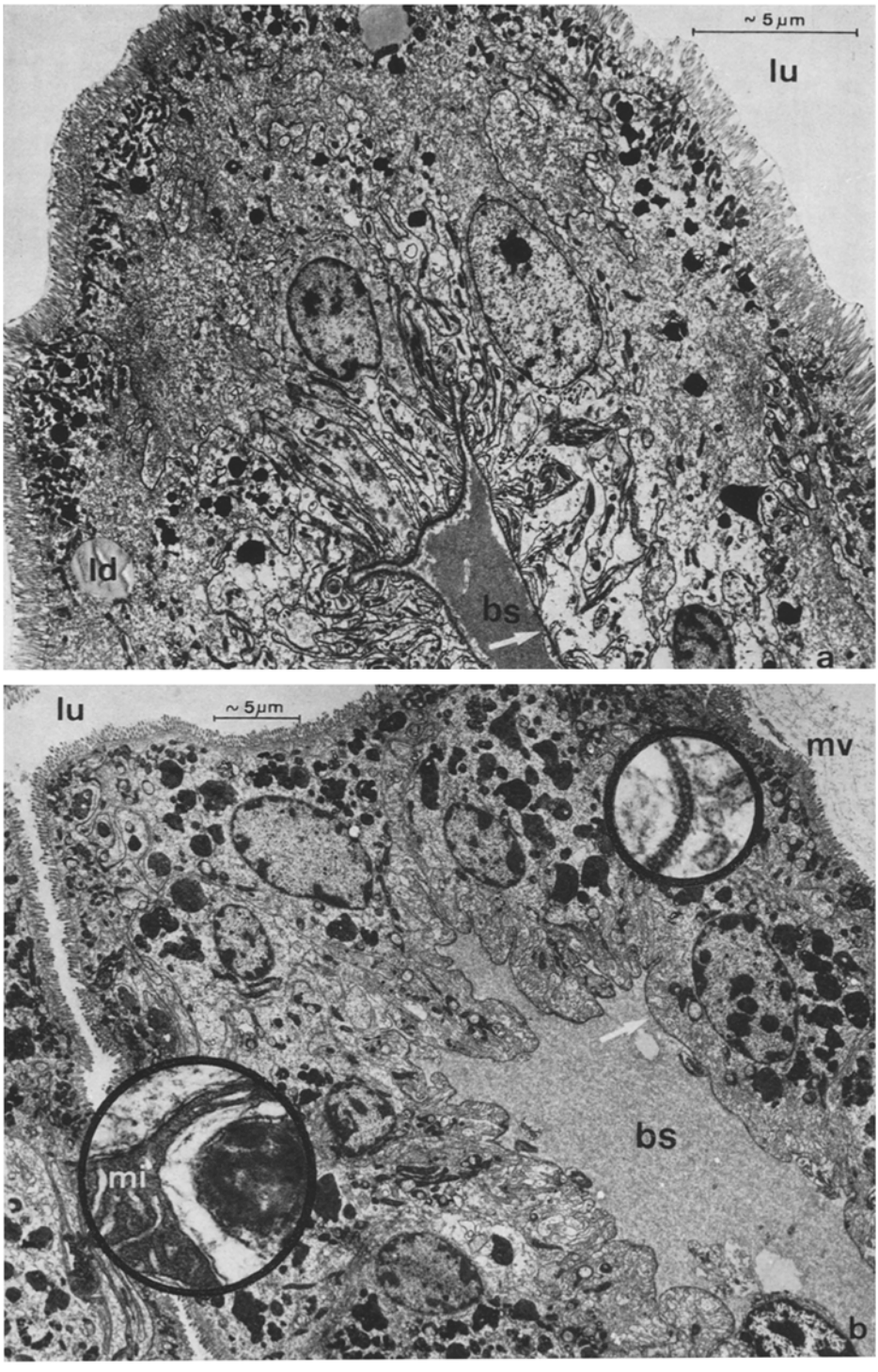

Fig. 6 a and b

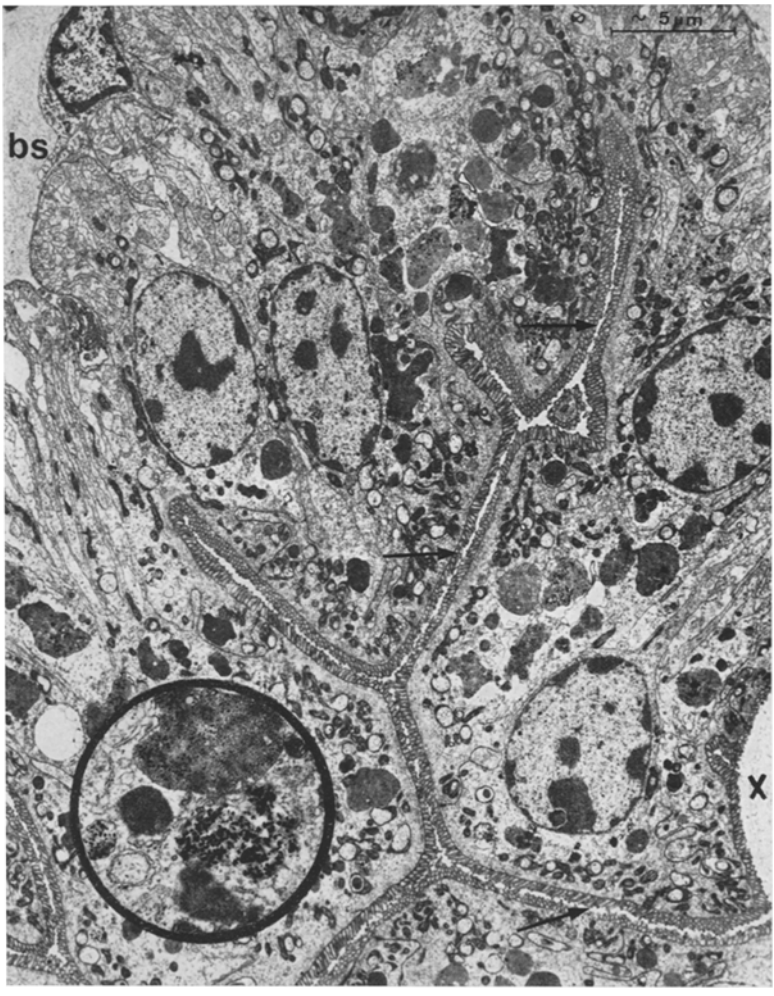

Fig. 7. Inner area of the renal appendages of a juvenile *Sepia officinalis;* a system of thin gaps (arrows) connects the crypts *(X)* with the surface of the organ; note the great number of mitochondria and dense bodies with a variable shape, structure and diameter (inset); blood sinus *(bs)*. × 9010 (inset)

there is regularly a rather wide area of septate desmosomes (Fig. 6b) which terminates in a zonula adhaerens.

On the side of the blood lacunae and sinuses, the basal lamina is accompagnied by endothelial cells containing filaments and by obliquely striated muscle cells. The latter serve the motion of the renal appendages, which can be observed *in vivo* and which is likely to support the transport

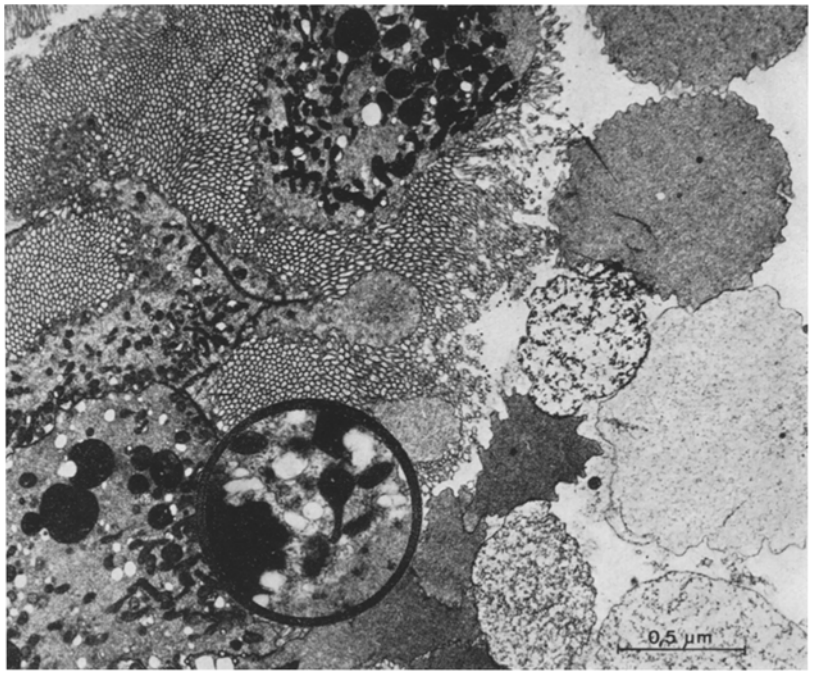

Fig. 8. Surface of the renal epithelium of an adult *Eledone moschata* with apokrine secreting mechanisms. × 9265 (inset)

of the blood and excreted material. The muscle cells are connected with nerve endings containing granula. It is likely that these are primarily monoaminergic fibers. They also occur sporadicly between the epithelial cells within the basal labyrinth. The assumption that the epithelium is composed of highly transport active cells is endorsed also by the observation that in the apical region as well as in the basal labyrinth there are large accumulations of mitochondria with an electron dense matrix; they lie close to the plasma membrane (Fig. 6b). The dense bodies of various sizes and shapes, which can already be seen in the light microscope, are formed in derivates of the rough endoplasmic reticulum; they are accumulated, often together with lipid droplets, in the area of the nucleus and in the apical region (Fig. 6a). They are enclosed in a membrane; their generally dense matrix appears mostly amorphous; sometimes lamellar stratification, granulation or presence of irregular inclusions can be seen (Fig. 7). In preparations stained with lead citrate, some of these dense bodies show particular affinity to lead salts (Fig. 7). In the apical region of the cells, the dense bodies often show a tendency

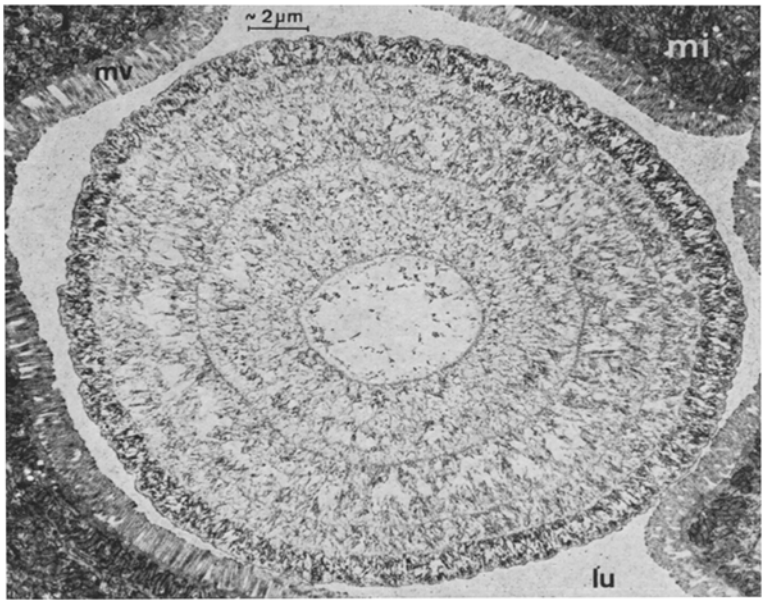

Fig. 9. Spherical body in a crypt of the renal appendages of *Sepia officinalis;* mitochondria *(mi)*, microvilli *(mv)* of the renal epithelium lumen *(lu)*

to disintegrate by forming concave grooves and vacuoles. This is particularly so in those which have penetrated into the protuberances observed in the inner part of the renal appendages. As mentioned above, these protuberances are considered to be a sign of apocrine secretion (Fig. 8).

In addition to the larger dense bodies, small electron dense particles (⌀ 600–700 Å) and myelin bodies are found, mainly within and beneath the terminal web (Fig. 10a). Particles of similar shape and size are sometimes found in the granular material excreted into the interstices and crypts of the organ lumen. This material is the product of the secretory processes mentioned above, as well as of the disintegration of cells; this cell moulting is regularly observed.

The spherical bodies enclosed in the crypts of the inner region of the renal appendages in Sepioids are generally composed of several (3–6) concentric layers showing low electron density. At higher magnification, a reticular and also micellar structure becomes apparent; there are no membranes, however (Fig. 9). Between the concentric lamellae, reticular structures as well as spicular crystalline elements are found (Fig. 10a); the latter show more or less distinct radial orientation. The contents of these crystalline elements seem largely to be dissolved by the fixation

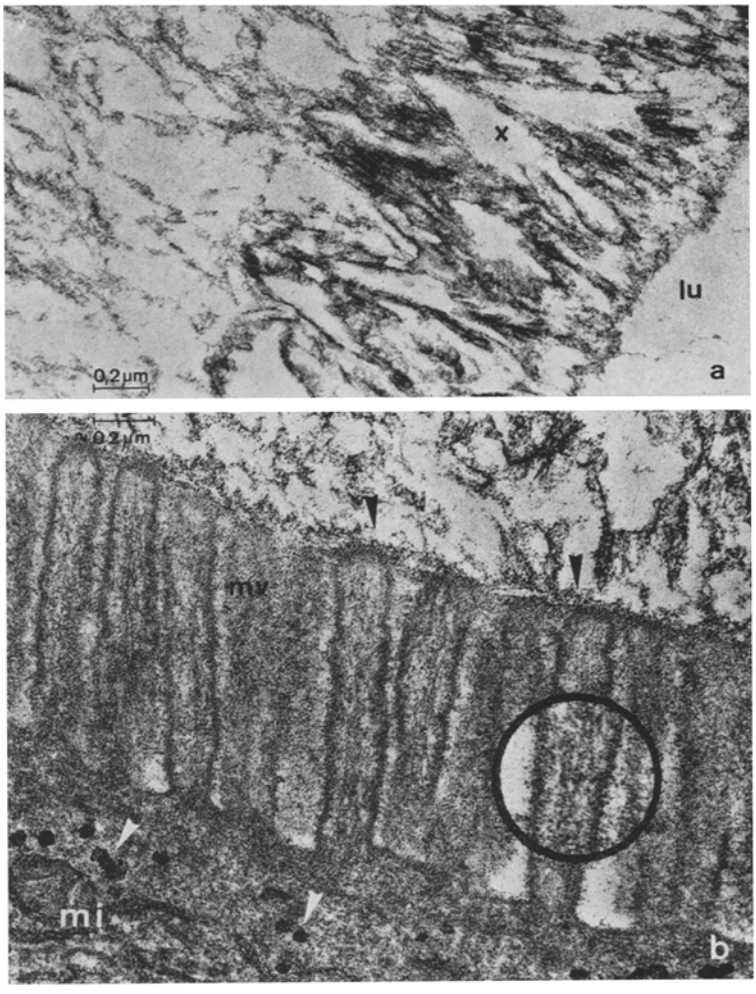

Fig. 10a and b. Part of the peripheral area of a spherical body (*Sepia officinalis*) with needle like crystalline elements in a radial orientation *(X)* within the organic matrix; the content substances seem to be solved partly (a); lumen *(lu)*; the area of contact between the microvilli *(mv)* and the surface of the body (black arrows) is the site of formation (b); note the small electron dense granula in the apical cytoplasm (white arrows); mitochondria *(mi)*

and dehydration of the tissue, so that the "spicules" are generally represented by their negative. The spherites are formed at the surface of the microvilli; the glycocalyx seems to separate from the plasma membrane and to be integrated in the layer being formed (Fig. 10b).

In accordance with the morphogenetic relationships presented before and with the light microscopical findings, the outer wall of the renal sac shows an ultrastructure fundamentally comparable with that of the renal epithelium of the saccules. The cells of the outer wall are therefore also transport active, but at a lower quantitative level as compared to the cells of the renal appendages (Fig. 11). In particular the number of mitochondria and dense bodies seems to be lower, and the basal labyrinth is less elaborate. In the outer wall, the pathway of extracellular transport is also longer because of the greater distance between the basis of the epithelium and the blood vessels which are comparatively sparse among the musculatur cells and the connective tissue.

Experimental and Cytochemical Findings. In partly starved young *Sepia officinalis,* a few days old, which had grown very little, rudiments of the spherites were found. These do not increase in size, however, and they may partially be dissolved. Thus in underfed animals aged 42–44 days, the concentric layers may still be recognized, the spaces between them are completely empty, however (Fig. 12a). The number of dense bodies within the epithelial cells is also markedly lower than in animals fed normally.

The cytochemical experiments supplement earlier and current histochemical studies (Schipp and Boletzky, 1974; Donaubauer, 1975, unpublished results); they are a contribution to the functional analysis of the spherites.

Carboanhydrase was demonstrated mainly in the microvilli; precipitation was particularly high in the apical region. The terminal part of the intercellular gap also generally shows a strong positive reaction, whereas precipitation within the cell was limited to the terminal web and was less strong than in the brush border (Fig. 12b and c).

Precipitation of Calcium by Oxalic Acid (Carasso *et al.*, 1969) gave a positive reaction at the surface of the microvilli, in the glycocalyx and in the spherites, mainly in the vicinity of their concentric layers (Fig. 13a and b). The precipitates (Ca-oxalate) often appear to be dissolved by fixation and dehydration. More stable reaction products were obtained by using the *lead salt method* (Carasso and Favard, 1966); this method allows the demonstration of Ca salts mainly. The reaction sites are found in the apical part of the brush border, the glycocalyx, in the layers of the spherites, in the apical part of the intercellular gap (Fig. 14a and b); they are very little pronounced within the cells (mitochondria, endoplasmic reticulum).

The results of the *alcian blue reaction* (Tice and Barnett, 1962) for demonstration of acid mucopolysaccharides on a cytological basis correspond to the histochemical findings obtained by the PAS and alcian blue reaction; the basal lamina, the glycocalyx and the peripheral

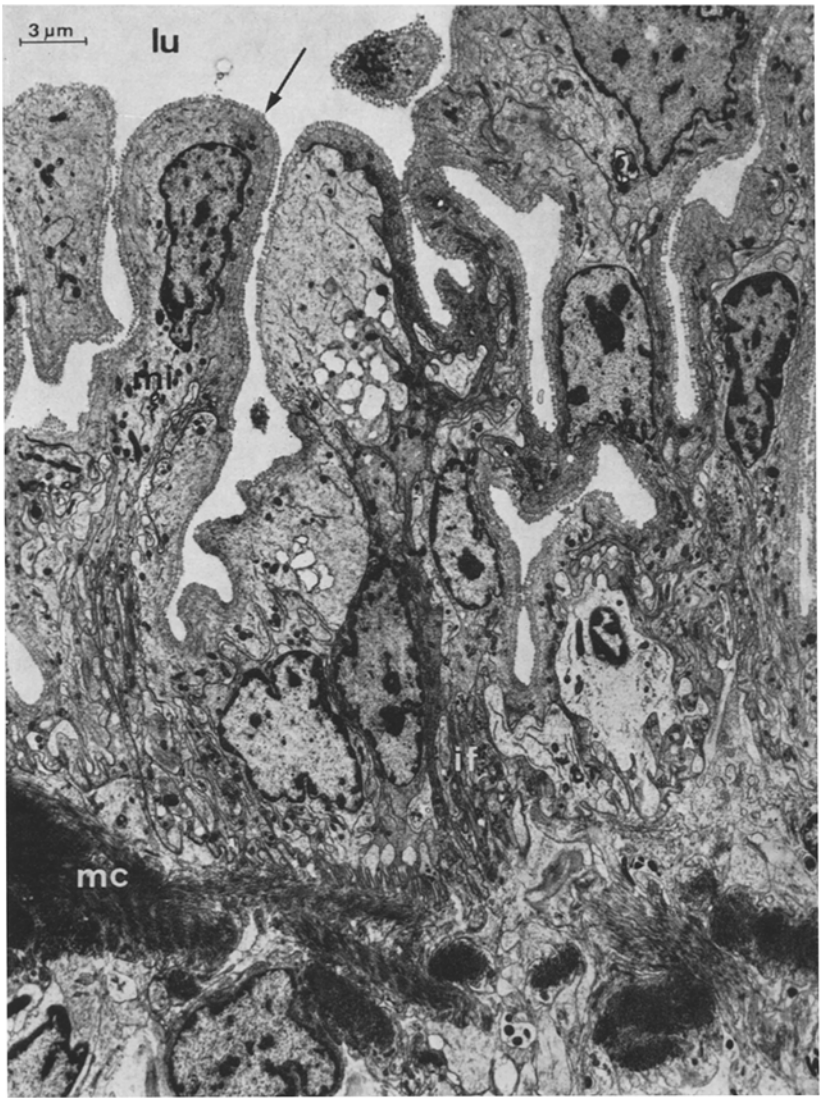

Fig. 11. Laterodorsal wall of the renal sac of a juvenile *Sepia officinalis*. Lumen of the renal sac *(lu)*; border of low microvilli (arrow), obliquely striated muscle cells *(mc)*, system of basal infoldings *(if)* with few mitochondria *(mi)* and electron dense granula

layers of the spherites show a precipitation of stain (Fig. 14c). A less pronounced reaction is sometimes observed in the peripheral part of the intercellular gap.

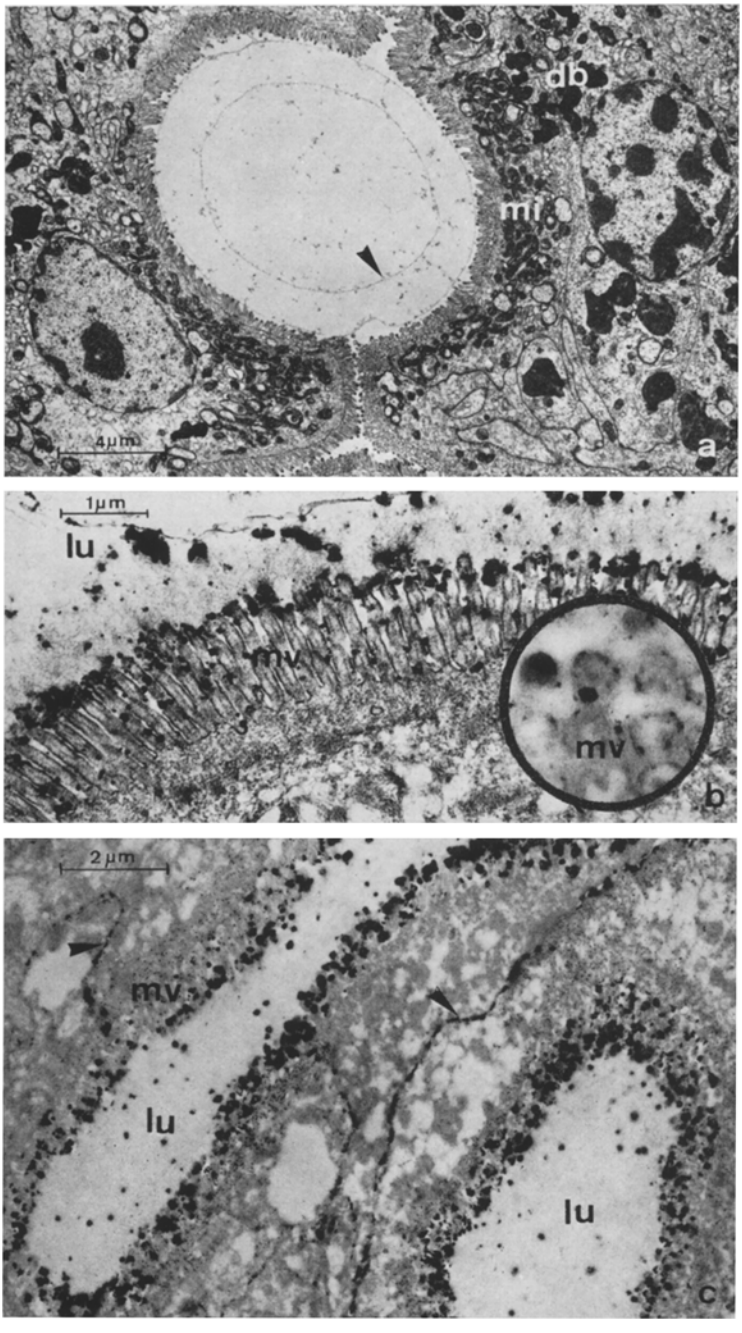

Fig. 12 a–c

Results of the Scanning Electron Probe Analysis. A qualitative X-ray microanalysis was made on spherites taken from the peripheral part of the renal sac. Fig. 15 shows the X-ray spectrum of an isolated spherite (*cf.* Fig. 5a and b); the most prominent peaks represent the elements K, Cl, S, Mg and Ca in the K α-line; the peaks of Na and Mg as well as K, Kβ and Ca Kα seem to overlap.

The other crystalline urinary sediments—colourless spicular crystals sometimes showing a druse shape (monocline crystal systems) and red cubical or amorphous crystals—were also analysed qualitatively. They seem to be composed of organic material (probably non-protein nitrogen compounds in the form of uric or hippuric acid, δ-lacton *e.g.*; *cf.* Delaunay, 1931; Emmanuel, 1957a; Potts, 1967) or of NaCl. An exact determination of the organic compounds was not possible with the method applied.

D. Discussion

The present findings on the histogenesis and ultrastructure of the renal appendages in dibranchiate cephalopods emphasize that in all the species considered an epithelium showing always the same type of structure is intercalated between the ramified blood space of the vena cava system and the lumen of the renal sac. As a continuous endothelial layer is lacking on the side of the blood spaces, the bulk of urine preparation is performed by this epithelium with its continuous basal lamina. The apical brush border, the basal labyrinth, the high content in mitochondria and lysosome-like dense bodies are the ultrastructural characteristics of a polarly differentiated layer of transport active cells very similar to those described in the renal organs of vertebrates and invertebrates. Despite the considerable uniformity in the ultrastructure of this folded epithelium, however, there are structural differences among different parts of the epithelium; they probably reflect functional differences.

The absence of podocyte-like cells, which are typical of the branchial heart and its appendage, both forming primary urine (Schipp *et al.*, 1969, 1971; Witmer and Martin, 1973), and also the structure of the blood vessels suggest that pressure filtration does not occur within the renal appendages. Despite the contractility of large parts of the blood vessels which may locally increase the blood pressure, the latter is likely to be

Fig. 12. (a) Inner region of renal appendages of an underfed and undersized *Sepia officinalis* at age of 42–44 days; in the extracellular crypt rests of the matrix of a spherical body (arrow), dense bodies *(db)*, mitochondria *(mi)* (b and c). Sections of the apical renal epithelium of a juvenile *Sepia officinalis* (c not contrasted) demonstrating the activity of carboanhydrase on the surface of the microvilli *(mv)*, and apical intercellular gap (arrows); lumen *(lu)*. $\times$ 31110 (inset)

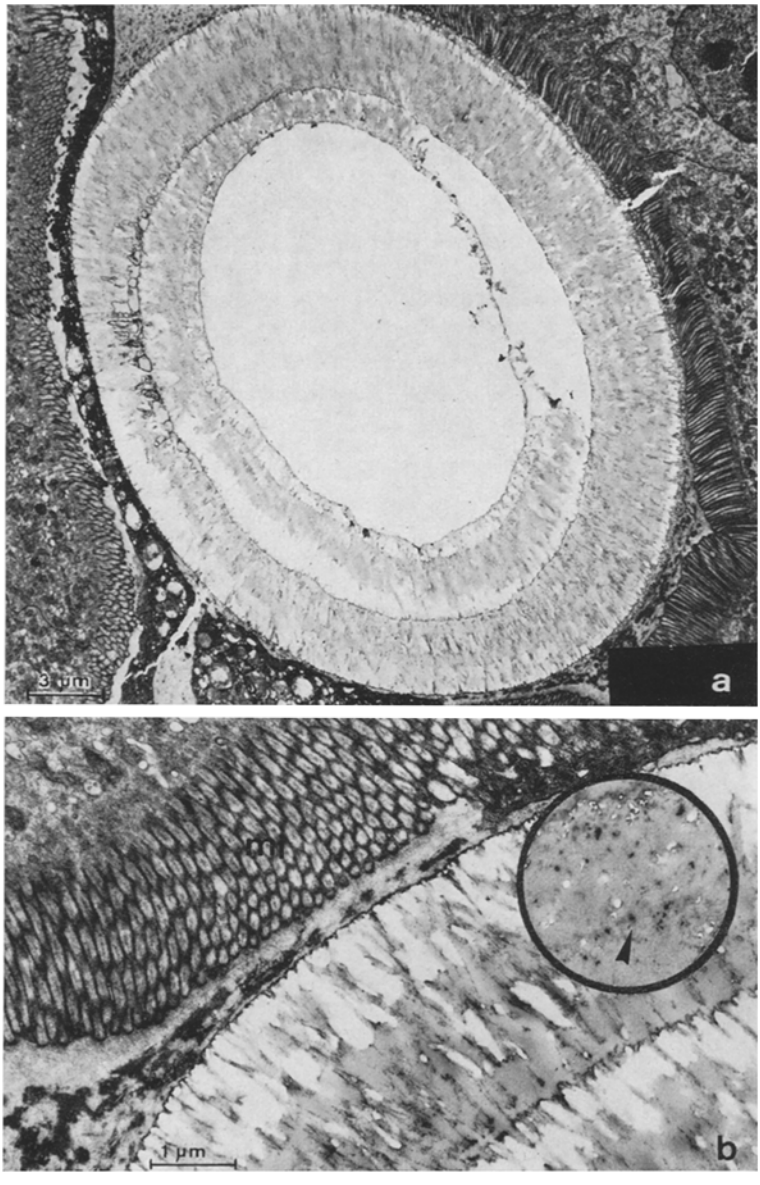

Fig. 13 a and b. Spherical body in an inner crypt of renal epithelium of *Sepia officinalis* (adult) after the precipitation of Ca with oxalic acid; the rests of the reaction products can be seen near the spherical layers and in the peripheric area of the bodies (arrow); microvilli *(mv)*. × 11050 (inset)

insufficient for this mode of filtration. The hydrostatic pressure of the blood would have to be higher than 4–5 cm H_2O, as measured colloid-osmotically in *Octopus dofleini* (Potts, 1967). Such values might be reached in some vessels lying close to the heart, as the Arteria renalis, for instance, with its renal appendages (Schipp and Boletzky, 1974), but they are not likely to be general.

A comparison of the ionic composition of the blood, the pericardial primary urine, the fluid of the renal sac, in *Octopus* and *Sepia*, and of sea water (Fig. 1) shows that these fluids contain the same ions, but that some of these show significant differences in their respective concentrations, e.g. H_3O^+, Ca^{++}, K^+, SO_4''. Accordingly the osmolality of the three components mentioned above is at the same level. Distinct differences are observed in *Sepia officinalis* (Schipp and Fischer, 1974, unpublished results), particularly between the nearly isotonic blood (933 ± 32.37 mOsm) and sea water (922.4 ± 15.3 mOsm), on the one hand, and the fluid of the pericardial (846.8 ± 19.5 mOsm) and the renal sac (805.2 ± 18.4 mOsm ventrally, 823.8 ± 22.7 dorsally), on the other hand. It seems likely therefore that, in addition to excreted material and water, particularly those ions which are represented by different concentrations in the blood plasma and in the fluid of the renal sac, are transported following, or against, the osmotic gradient blood $\leftrightarrow$ renal sac.

The present data on the ionic concentration in the blood and in the renal fluid, and the structure of the epithelium suggest that in large parts of the renal appendages osmotic filtration (Berridge and Oschman, 1972) combined with active ion transport takes place; this corresponds to the mechanism supposed in the Malpighi tubuli, which function under similar conditions and show a similar structure (Wessing, 1965; 1974; Eichelberg and Wessing, 1971), and in the aglomerular kidneys of fishes (Olsen and Ericsson, 1968; Ericsson and Olsen, 1970). A filter for this process would be the basal plasmalemm and the basal lamina which is impenetrable for hemocyanin; a transport path for ion shifting and ionic exchange diffusion (Berridge and Oschman, 1972) would be the basal infoldings of the epithelium with their numerous mitochondria, the microvilli and the lateral interdigitations which are also accompagnied by mitochondria. In the apical region of the cells, there may be cytopempsis and transport of material within the endoplasmic reticulum, both contributing to the transcellular transport.

The question of which ions are transported and exchanged against one another at the membrane surface cannot yet be answered definitively. In view of the positive carboanhydrase reaction and the distinct difference in pH between urine (pH 5) and blood (pH 7.3), it seems certain that H_3O^+ is exchanged against other cations (possibly Na^+, K^+). Furthermore, the main function of the renal appendages, i.e. elimination of nitrogenous

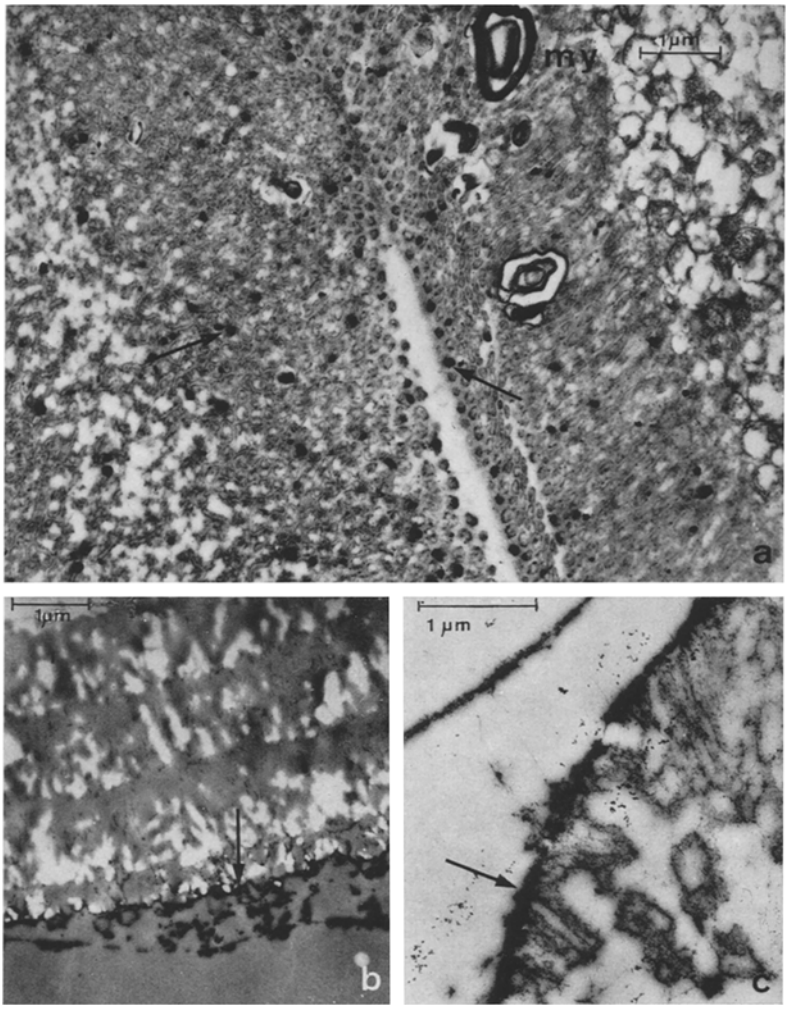

Fig. 14a—c. Brush border (a) resp. spherical body (b) in renal epithelium of *Sepia officinalis* (adult) after the precipitation of Ca with Pb-acetate; myelin body *(my)*. (c) Demonstration of acid mucopolysaccharides on the surface of a spherical body with alcianblue; the reaction products (arrows)

material, particularly of NH_3 and NH_4^+ (concentration in the urine of *Sepia officinalis* 990 mg/l; Schipp and Fischer, unpublished results) should be recalled. According to Potts (1956), this occurs by diffusion of NH_3 towards acid urine, where NH_4^+ is formed ($NH_3 + H^+ \rightleftharpoons NH_4^+$).

The experimental studies of Harrison and Martin (1965) show that in *Octopus dofleini* the vein appendages, like the appendages of the branchial hearts, are able to secrete phenolsulphonphtalein, p-aminohippuric acid and urea; intravasal injection of these is rapidly followed by an increase of their concentration in the renal sac over the concentration in the blood. Ultrastructural indications of secretory processes taking place in the renal appendages are mainly found in the apical protuberances of the epithelium, in the inner parts of the organs; these protuberances with their granular and vesicular contents seem to be tied off and to separate from the cell. We interpret these structures as the expression of an apocrine secretory mechanism and refer to corresponding findings on the branchial heart appendage of *Sepia officinalis* (Schipp *et al.*, 1971), the cephalopod gills (Schipp, 1970, unpublished results) and the epithelium of the proximal tubulus in elasmobranch nephrons (Bargmann and von Hehn, 1971). It cannot be excluded that ammonia is eliminated from the cells also by this mechanism, not only by diffusion (Potts, 1965). The intracellular passage of NH_3 is probably based on glutamin acting as a carrier, as in vertebrates. This assumption is endorsed by the fact that in *Octopus dofleini* the blood shows a high loss in glutamin when passing through the renal organs and that the renal epithelium has a high glutaminase activity (Potts, 1965). The high monoaminoxidase activity (Schipp and Boletzky, 1974) would also suggest this mode of transport. One may admit that it is also responsible for the emission into the renal sac of high molecular constituents such as hypoxanthin, tyramin, glycin-betain and of the possible NH_3 carriers guanin and δ-lacton, in *Octopus hongkongensis* (Emmanuel and Martin, 1956, 1957a, b).

The problem of the rôle played by the various dense bodies in the apical part of the cell is still open. It is conceivable that particularly the smaller bodies having a diameter of 600–800 Å take part in the transcellular transport. As to the larger "dense bodies", which may show lamellular stratification, it is likely that they are auto- as well as ambilysosomal elements that are responsible for the decomposition of cytoplasm in the apical protuberances and also in decaying cells discarded by the epithelium (Ericsson and Olsen, 1970). The high phosphatase activity in this cell region observed in *Octopus vulgaris* would also endorse this assumption (Schipp and Boletzky, 1974).

The ultrastructure of the epithelium suggests that reabsorption takes place in the peripheral interstices and in the superficial region of the organ. The very similar ammonia concentration in the urine and pericardial fluid observed in *Sepia officinalis* (Schipp and Fischer, 1974, unpublished results) indicates, however, that reabsorption is of minor importance for the concentration of NH_3 in the coelomic fluid (Harrison and Martin, 1965).

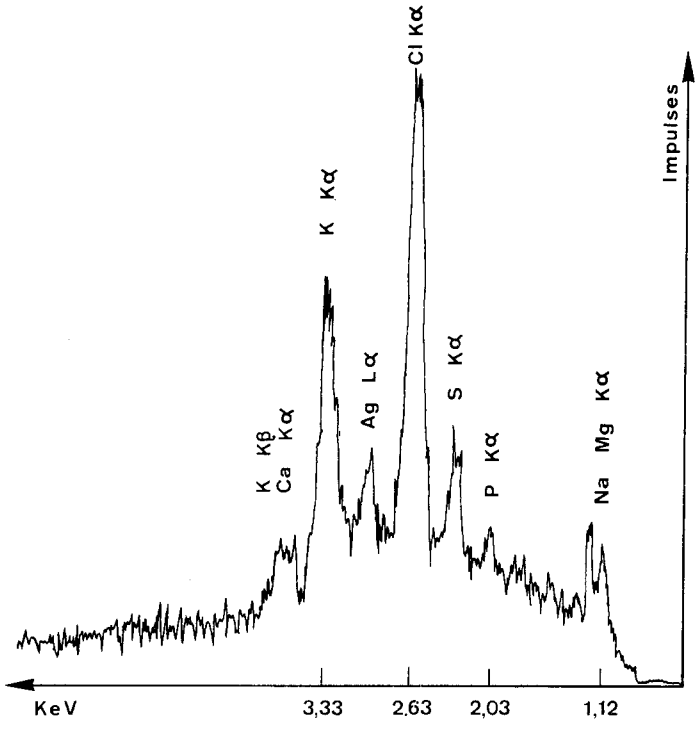

Fig. 15. Diagram of a qualitative x-ray microanalysis of a spherical body from the peripheral renal sac concrements of *Sepia officinalis* (adult)

In the following, we shall deal with the formation and conceivable functions of the spherites which have till now only been found in the crypt-like cavities of the renal appendages in *Sepia*. The concentric layers of these extracellular bodies give a positive PAS and alcian blue reaction, as do the glycocalyx and the brush border. We therefore suppose that these layers are composed mainly of acid mucopolysaccharides, like similar bodies described from the Malpighi tubuli (Wolburg *et al.*, 1973; Hevert *et al.*, 1974); these mucopolysaccharides would be secreted at regular time intervals by the microvilli. The cytochemical and micronalytical investigations have shown the presence of various cations, Ca^{++}, K^{+}, Na^{+}, Mg^{++}, in the mucopolysaccharide matrix where they are bound with corresponding negatively charged elements (*e.g.* hyaluronic acid). This may partly explain the low concentration of these ions in the urine fluid, as compared to the blood (Table 1; Robertson, 1953); but Ca might also be present in the form of carbonate (Roinel *et al.*, 1973), as suggested by the strongly positive Kossa reaction of the spherites (Donaubauer, 1974, unpublished results).

Table 1. Concentration of the major inorganic ions (mM/kg H_2O)

Ions	Fluids	*Sepia officinalis* Robertson (1953)	*Octopus dofleini* Potts and Todd (1965)	*Octopus hongkongensis* Emmanuel and Martin (1956)
Na	Sea water	492.0	410.0	409.3
	Blood plasm	460.0	402.0	—
	Pericardial fluid	—	405.0	—
	Urine	463.0	395.0	409.3
K	Sea water	10.5	9.2	9.2
	Blood plasm	23.8	11.2	—
	Pericardial fluid	—	9.2	—
	Urine	11.9	15.0	11.4
Ca	Sea water	10.8	9.0	9.6
	Blood plasm	10.8	8.9	—
	Pericardial fluid	—	8.4	—
	Urine	7.5	4.7	8.7
Cl	Sea water	574.8	479.0	486.2
	Blood plasm	588.6	484.0	—
	Pericardial fluid	—	483.0	—
	Urine	588.0	453.0	431.5
SO_4	Sea water	29.6	24.7	24.8
	Blood plasm	4.9	19.7	—
	Pericardial fluid	—	19.3	—
	Urine	10.5	36.3	56.6
NH_4	Urine	146.3	—	40.7–88.7
Mg	Sea water	56.0	—	47.6
	Blood plasm	56.9	—	—
	Urine	38.5	—	39.6

For the interpretation of the microanalytical findings on the Ca content of the spherites, it should be kept in mind, however, that bodies taken from the acid urine (pH 5) have been analysed, whereas the actual Ca content of the spherites *in situ*, according to the histochemical findings, is higher than indicated in the diagram (Fig. 15). The elements P and S, the presence of which has also been demonstrated, probably take part in the formation of the organic matrix, e.g. S in the form of chondroitin sulfate present in mucopolysaccharides. Cl might be bound by electro adsorption in this matrix, or—more likely—it forms NaCl by ional binding with Na.

We suppose that the ions mentioned above are reversibly bound by ional adsorption and salt formation (and therefore are osmotically ineffective) particularly in the spicular radial elements lying between the concentric mucopolysaccharide layers; as mentioned earlier on electron

micrographs, these elements are generally represented by their negatives because of the dissolution processes taking place during fixation and dehydration. By the contractions of the renal appendages, larger spherites may be expelled from the crypts, which probably are alcaline, to the surface of the organ and finally into the strongly acid urine, where they are partly dissolved. Possibly their elements there take part in the formation of the secondary crystals, or they may be dissolved in the secondary urine and thus be eliminated via the renal opening. We suppose that the spherites primarily serve a storage excretion, and that they are comparable to similar formations found in the mantle of bivalves (Istin and Girard, 1970; Roinel *et al.*, 1973), in the hepatopancreas of the blue crab (Becker *et al.*, 1974), in the excretory organs of crayfish (Riegel, 1970a, b) and *Drosophila* (Wolburg *et al.*, 1973; Hevert *et al.*, 1974). It is nevertheless likely that ions bound in the spherites may be remobilized and reabsorbed via the epithelium into the blood, if there is an encreased demand, *e.g.* of Ca^{++} for shell formation.

The hypothesis of a Ca storage is supported by the following points: 1. the topographical vicinage of Ca reserves and carboanhydrase, which has been demonstrated in the brush border and the spherites (Istin and Girard, 1970), 2. the early appearance of the spherites, a few days only after hatching of the animal, 3. the absence of Ca deposits in the spherites of animal reared under continuous malnutrition, 4. the exclusive presence of spherites in forms having a calcified shell (as far as is known to date).

It should finally be recalled that the inner lining of the renal sac shows an epithelial structure corresponding to that of the renal appendages, although there are quantitative differences (smaller number of mitochondria, absence of dense bodies, rather pour elaboration of the basal labyrinth, lack of direct contact with blood vessels). We therefore suppose that this epithelium may also take part in the preparation of urine, *e.g.* by reabsorbing H_2O and ions.

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