J. Membrane Biol. 71 – 89 (1978)

Morphological Aspects of Some Sodium Transporting Epithelia Suggesting a Transcellular Pathway Via Elements of Endoplasmic Reticulum

K. Møllgård and J. Rostgaard

Anatomy Department A & C, University of Copenhagen, Universitetsparken 1, 2100 Copenhagen Ø, Denmark

Received 30 November 1977

Summary. Electron microscopic studies of sodium transporting epithelia from frog skin, sheep choroid plexus, rabbit gallbladder and small intestine, and rat kidney revealed the presence of a complex intracellular system of tubulo-cisternal endoplasmic reticulum which appeared to connect apical (luminal) and baso-lateral cell surfaces. The system was present in the tight epithelium of frog skin but was most abundant in leaky epithelia with low transepithelial resistance and isotonic transport. The basic structural features of the system and its relationship with some associated components are described.

Our result, coupled with preliminary physiological studies, indicate that developmental and seasonal (hormone-induced) changes in the configuration of the tubulo-cisternal endoplasmic reticulum may be closely correlated with specific changes in epithelial permeability. The findings are discussed in the light of the hypothesis that epithelia possess two sodium transporting systems: One based on pump sites in the plasma membrane producing a hypertonic transportate and another located in the membranes of the tubulo-cisternal endoplasmic reticulum which, due to its extensive surface, would be well suited for producing an isotonic transportate.

Essential to the polarized function of most epithelia is the occurence of a characteristic intercellular junctional complex that serves to maintain a layer of cells as both a coherent sheet and a diffusion barrier (Farquhar & Palade, 1963). The junctional complex serves as a borderline between the apical and the baso-lateral cell membrane, a borderline between part of the cell membrane engaged in solute transport in opposite directions. The so-called tight junction or *zonula occludens* of this complex was originally assumed to act as the barrier to diffusion of substances through the intercellular space, but several recent morphological findings have established that the tightness of tight junctions is not always complete and that the permeability of the junctions varies considerably among different epithelia (*see*, e.g., Martinez-Palomo & Erlij, 1975).

0022-2631/78/S040-0071 $03.80

Epithelia can be classified according to their transepithelial electrical resistance in tight (high resistance) and leaky (low resistance) varieties (*see* reviews by Diamond, 1974; Ussing, Erlij & Lassen, 1974; Erlij, 1976). It is assumed that the major difference reflects the sealing capacity of the tight junctions; in other words, the so-called tight junctions are leaky in low resistance epithelia but really tight in high resistance epithelia. Solutes have therefore in principle two parallel routes available for transversing an epithelium, (i) the intercellular (paracellular) route via the tight junction and lateral intercellular space and (ii) the transcellular route via apical and basolateral cells membranes and intervening cytoplasm. The intercellular route has been the subject of several morphological studies during the last few years, whereas only few investigators have focused attention to a possible structural basis for transcellular transport which will be dealt with in the present study.

Recently it was reported that a short circuited frog skin (a tight epithelium) exposed to a hydrostatic pressure head of 25–50 cm H_2O on the inside developed morphological changes in the first living cell layer (Voûte, Møllgård & Ussing, 1975). In the light microscope, multiple "vesicles" were observed in the cytoplasm, and the number of "vesicles" increased in proportion to the rate of sodium transport. Electron microscopic studies showed the "vesicles" to be membrane bounded sacs ("scalloped sacs") intimately associated with the endoplasmic reticulum (ER) which was dilated when the the number of scalloped sacs was increased. As a working hypothesis it was suggested that the ER in the frog skin possesses an active transport mechanism which takes up sodium from the cytoplasm whenever the cell is overloaded with this ion.

The leaky epithelium of the choroid plexus from early sheep fetuses exhibits an intracellular system of tubules and cisternae of ER that appears to be involved in transcellular transport (Møllgård & Saunders, 1975), and specific changes during development in nonelectrolyte permeability across the fetal choroid plexus epithelium has been shown to be correlated with structural changes in the tubules and cisternae of the ER (Møllgård & Saunders, 1977). The morphological changes were identified by electron microscopy, using freeze fracture replicas, thin sections, and "thick" sections of material impregnated with heavy metals, according to the technique of Thiery & Rambourg (1976).

This report deals with morphological studies of intracellular systems of tubules and cisternae of endoplasmic reticulum and associated components in a variety of tight and leaky epithelia. The information has

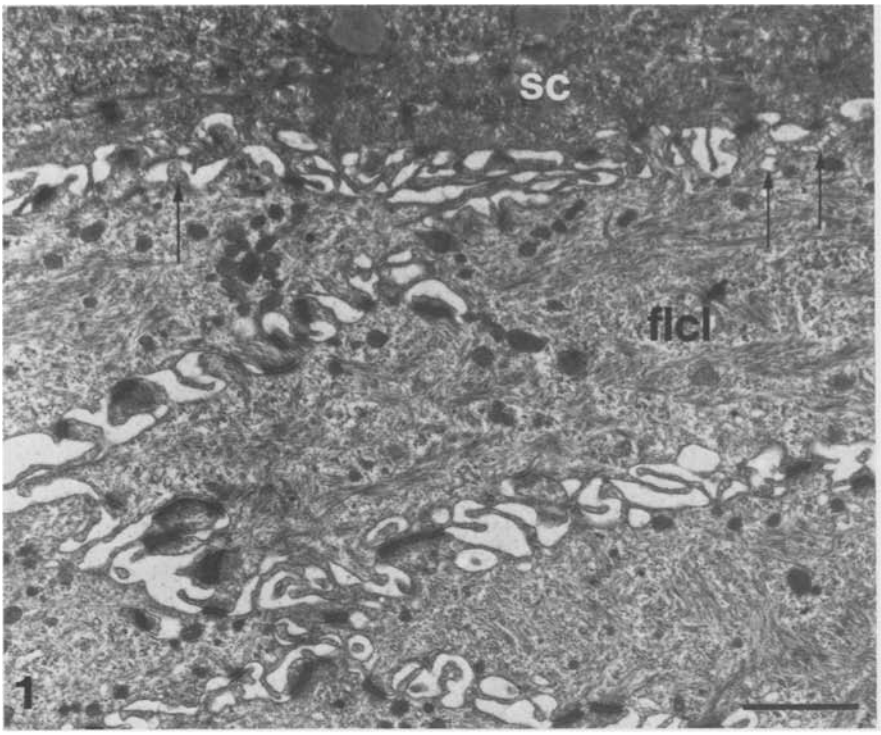
been obtained using freeze fracturing, thin-section electron microscopy and scanning electron microscopy. In addition, we used a metal impregnation technique, which was found especially valuable in revealing the distribution and continuity of intracellular tubules and cisternae. Our results show that a tubulo-cisternal endoplasmic reticulum (TER) is a characteristic feature of sodium transporting epithelial cells. The TER takes various forms and shows various degrees of development in the epithelia studied, being most highly developed in isotonically transporting epithelia. The TER may provide an additional and/or alternative structural basis for some of the different permeability properties of various leaky and tight epithelia, which cannot be fully accounted for by current models for transepithelial transport.

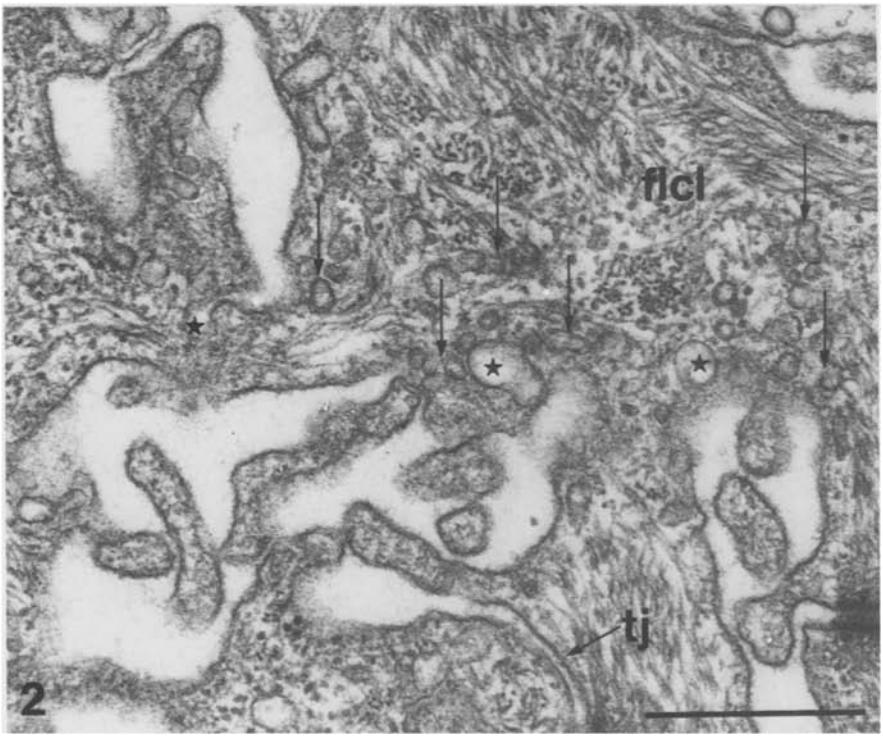
Materials and Methods

Frog skin (*Rana temporaria*), choroid plexus from late sheep fetuses (125 days gestation) and adult sheep, gallbladder and small intestine from rabbit, and kidney from rat were used in this study. The material was examined by scanning electron microscopy and by transmission electron microscopy of thin sections, of thick sections of metal impregnated specimens and of freeze-fracture replicas. After decapitation specimens were rapidly dissected out and immersed in a fixative containing 2.5% glutaraldehyde in 0.1 M cacodylate buffer or, in the case of choroid plexus, in a modified Karnovsky's fixative, as previously described (Møllgård & Saunders, 1975). For scanning microscopy the specimens were postfixed in 2% OsO_4 buffered in 0.1 M cacodylate buffer (pH 7.3) for 2 hr, rinsed in the same buffer, and dehydrated in acetone. The tissues were then critical-point dried in CO_2, mounted on specimen stubs, and coated with carbon and gold. Specimens were observed in a Jeol JSM-U3 scanning electron microscope using 15 kV. A detailed description of tissue processing for conventional electron microscopy and freeze fracturing has recently been given (Møllgård & Saunders, 1977). Following the glutaraldehyde fixation, specimens were impregnated with metals according to the technique of Thiery and Rambourg (1976) and embedded in Epon. Thin (600 Å) and thick (2,000–4,000 Å) sections were examined in a Philips EM 300 electron microscope operated at 60 or 100 kV and in a Hitachi HS8 electron microscope operated at 50 kV.

Results

The general morphology of the TER, which will be described first, very closely depicts the pattern of the system in epithelial cells of choroid plexus, gallbladder, and small intestine. The TER consists of an apical polygonal network of tortuous tubules oriented in parallel with the apical (luminal) cell membrane, in continuity with a complex system of flattened cisternae which is prevalent along the lateral (sometimes baso-lateral)


sc
flcl
1


flcl
tj
2

cell membrane. Occasionally, tubules are found in the baso-lateral part and cisternae in the apical part of the system. The TER is in close contact with both the apical and the baso-lateral cell membranes. Slender tortuous tubules or rows of vesicles pass from the apical TER-network towards the apical cell membrane, and a subsurface cisterna-like portion of the TER is closely associated with the basolateral cell membrane.

The rough ER is visualized by the metal impregnation technique to a varying degree but often it exhibits quite a faint reaction, whereas the Golgi complex shows the same reactivity as the TER. The intercellular space may in some instances be filled with reaction product, but this is found occasionally in all tissues and seems to depend on the geometry of the tissue block.

Frog skin: The structure of the frog skin is complicated by the presence of four regions and several cell types, but only the cells of the first living cell layer (flcl), which swell roughly in proportion to the amount of sodium transported, will be dealt with here. In conventional thin sections perpendicular to the epithelial surface (Fig. 1), it is difficult to visualize both apical surface invaginations and the TER which interweaves the apical cytoplasm. However, when a frog skin is cut parallel with the surface, the TER system and the apical cell membrane invaginations are easily identified (Fig. 2). Freeze fracture replicas also reveal some tortuous slender tubules arranged parallel with the apical cell membrane (Fig. 3), whereas the entire TER is difficult to visualize in thick sections of metal impregnated specimens (Fig. 3, insert). So far we have not been able to obtain selective impregnation of the system in frog skin epithelium. Thus the TER of frog skin epithelium shows some differences from the general pattern. It is less developed and exhibits a weak "stainability", but by appropriate methods it can be clearly demonstrated.

Sheep choroid plexus: Choroid plexus epithelial cells from metal impregnated specimens exhibit in thick sections (2,000 Å) a complex system

On all figures bars indicate 0.5 μm.

Figs. 1–3. Electron micrographs of the first living cell layer (*flcl*) of frog skin epithelium

Fig. 1. In a thin section cut perpendicular to the epithelial surface both surface invaginations and apical tubular elements of TER are inconspicuous, but can be identified (arrows). *Stratum corneum* (*sc*). 30,000 ×

Fig. 2. This micrograph demonstrates the apical surface invaginations (stars) and elements of the TER (arrows) in a thin section cut parallel with the epithelial surface through the uppermost part of cells of the first living cell layer (*flcl*). A tight junction (*tj*) characteristic of this part is indicated. 58,000 ×

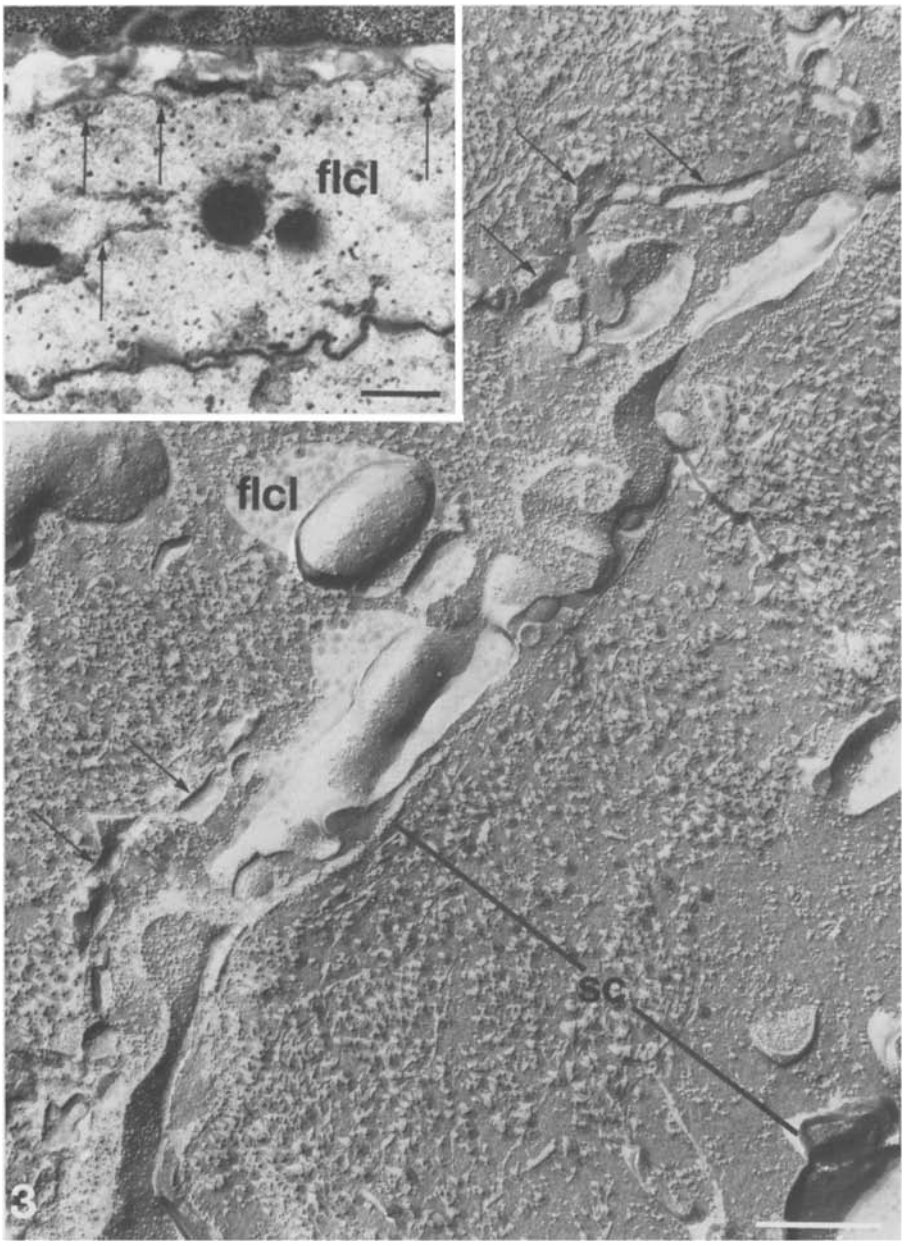
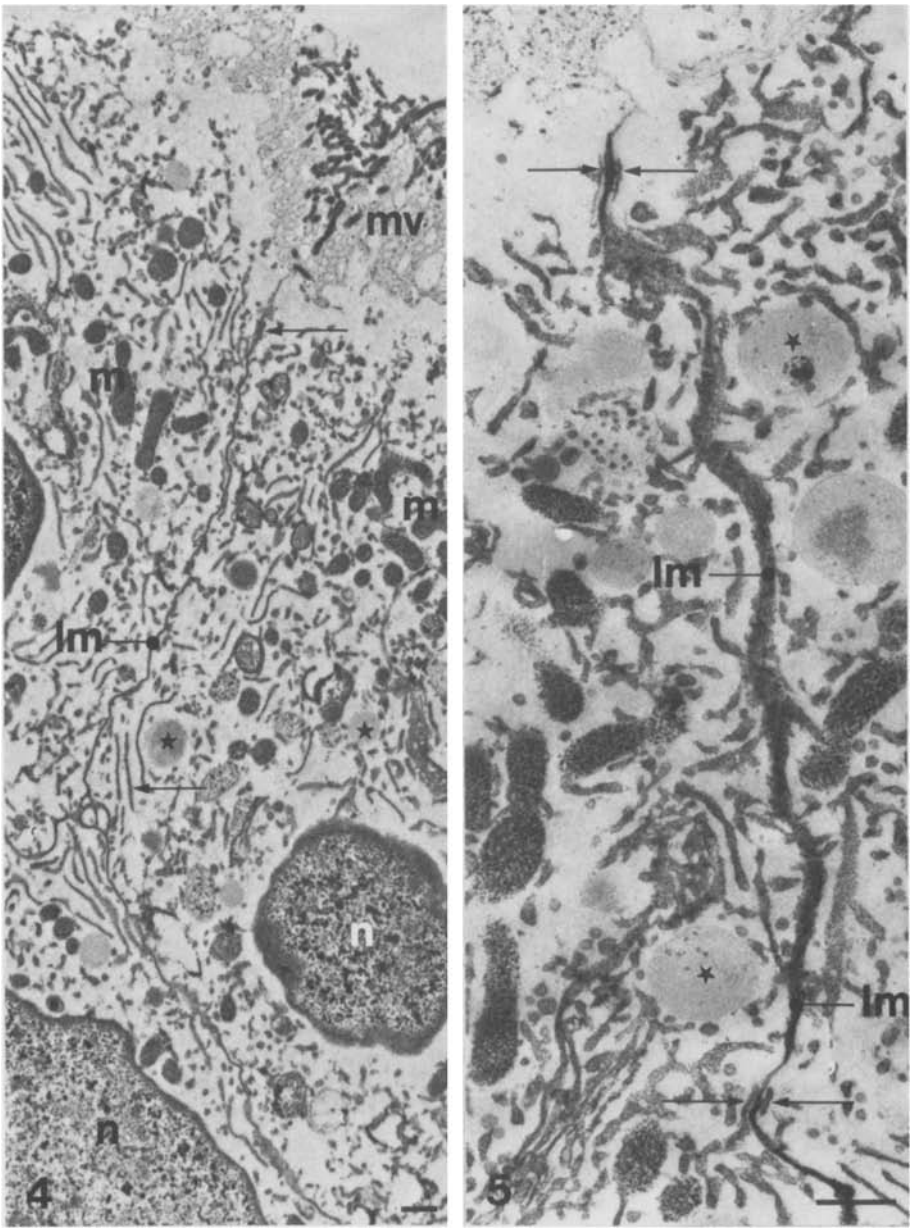


Fig. 3. Freeze fracture of frog skin epithelium. The lower right part of the picture shows a cross-fractured cell of the *stratum corneum* (*sc*); the free epithelial surface is seen in the lower right corner. The apical portion of a cell of the first living cell layer (*flcl*) exhibits cytoplasmic tortuous tubular elements of TER (arrows), mainly arranged parallel with the apical cell membrane. 34,000×. Insert shows the first living cell layer in a thick section of metal impregnated frog skin. *Stratum corneum* is seen in the top part of the picture. Few elements of faintly "stained" TER are marked with arrows. 20,000×



Figs. 4–7. Epithelium of sheep choroid plexus. Transmission electron microscopy of thick sections of tissue blocks metal impregnated with uranyl acetate, lead, and copper citrate

Fig. 4. The supranuclear portion of epithelial cells of the choroid plexus. The microvilli (*mv*), the opposing lateral cell membranes (*lm*), and the nuclei (*n*) are seen. Mitochondria (*m*) and TER (arrows) are intensely "stained", lysosomes (stars) are faintly "stained". Note the apparent lack of continuity between the various elements of the TER-system in this semithick section (1,500 Å). 9,500 ×

Fig. 5. Higher magnification of a similar area as shown in Fig. 4 demonstrates the intimate relation of the TER with the lateral cell membrane (*lm*), with mitochondria (*m*) and with lysosomes (stars). Some subsurface cisterna-like elements are indicated with arrows. Microvilli (*mv*). 21,000 ×

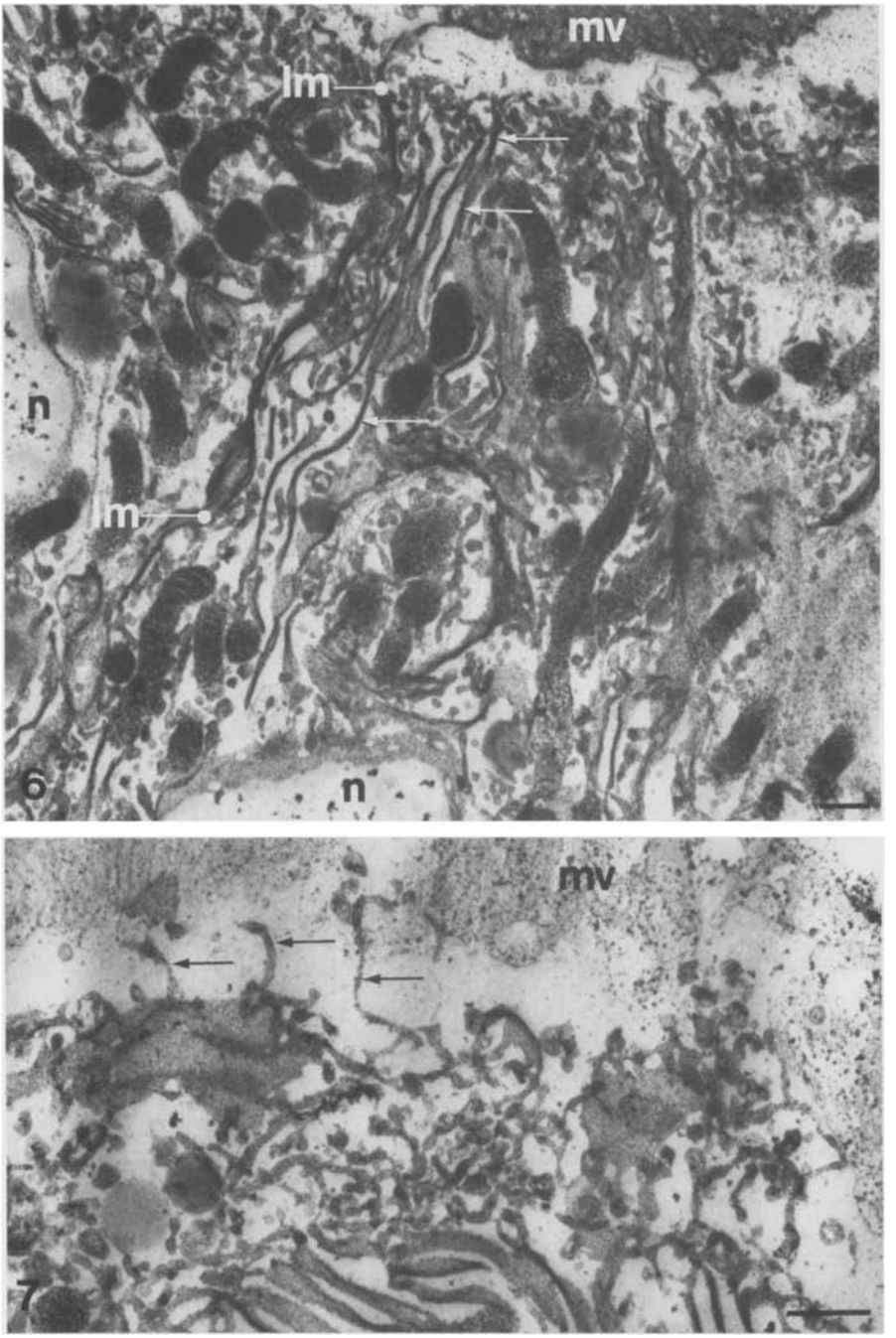


Fig. 6. This very thick section (4,000 Å) demonstrates the existence of continuity of apical and baso-lateral portions of the TER (arrows). The vast and complex TER is visualized. Nuclei (*n*), lateral (*lm*), and apical cell membrane with microvilli (*mv*) is shown. 13,500 ×

Fig. 7. Apical part of epithelial cell of choroid plexus demonstrating microvilli (*mv*) and the apical TER which exhibits tubular connections (arrows) with the apical cell surface. 21,000 ×

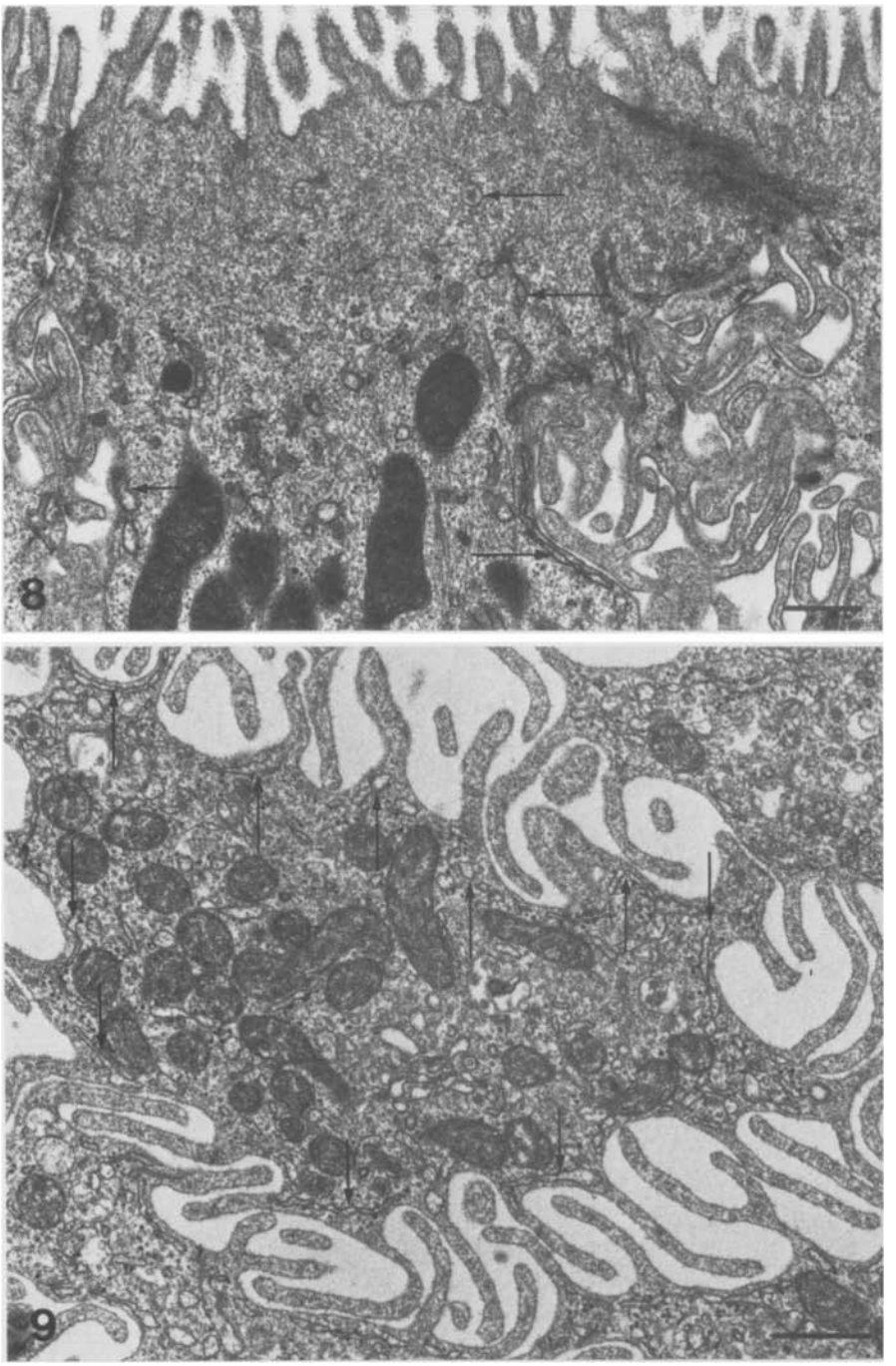


Fig. 8. Conventional electron micrograph of thin section of rabbit gallbladder epithelial cells. Subsurface cisterna-like elements and apical tubular elements are marked with arrows. 21,000 ×

Fig. 9. Cross section of the mitochondria-rich supranuclear zone of a gallbladder epithelial cell. Subsurface cisterna-like elements (arrows) are localized in or basal to the microplicae of the lateral cell surface. 26,500 ×

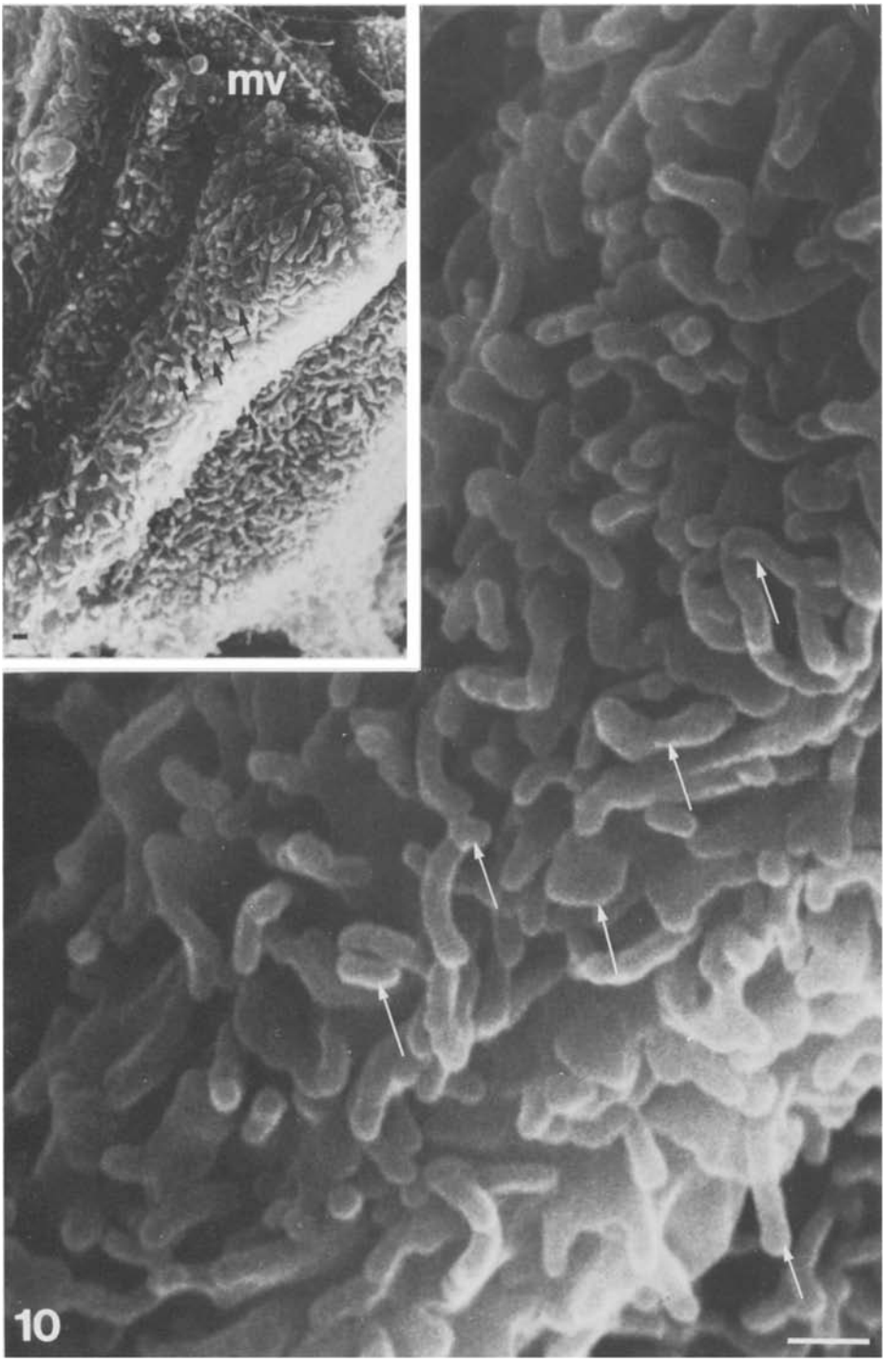


Fig. 10. Scanning electron micrograph of the lateral cell surface of a gallbladder epithelial cell demonstrating the many microplicae (arrows) which are a characteristic feature of the lateral cell surface. 23,000×. Insert demonstrates the microvilli (*mv*) of the apical and microplicae of the lateral cell surface (arrows). 3,800×

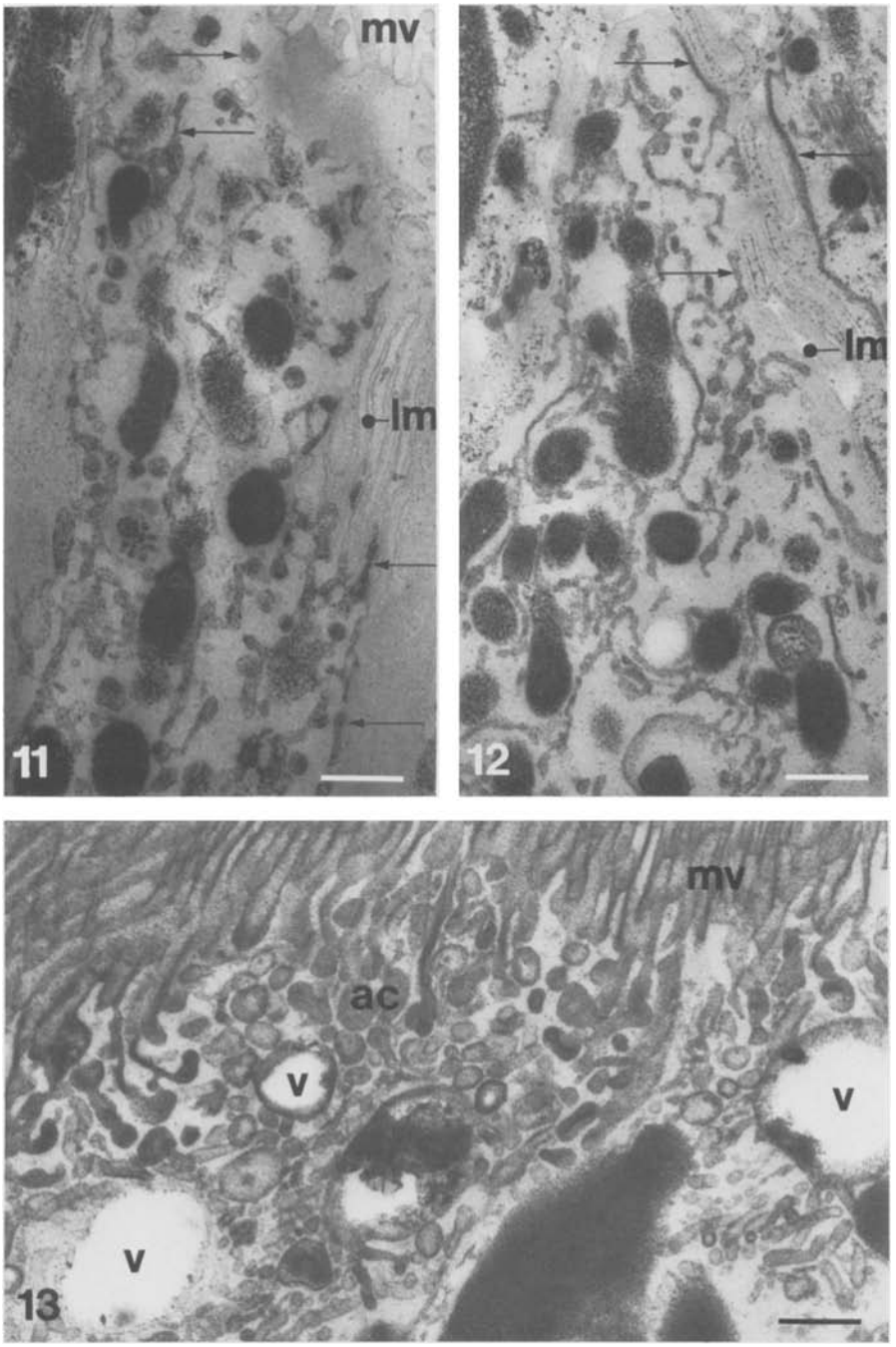
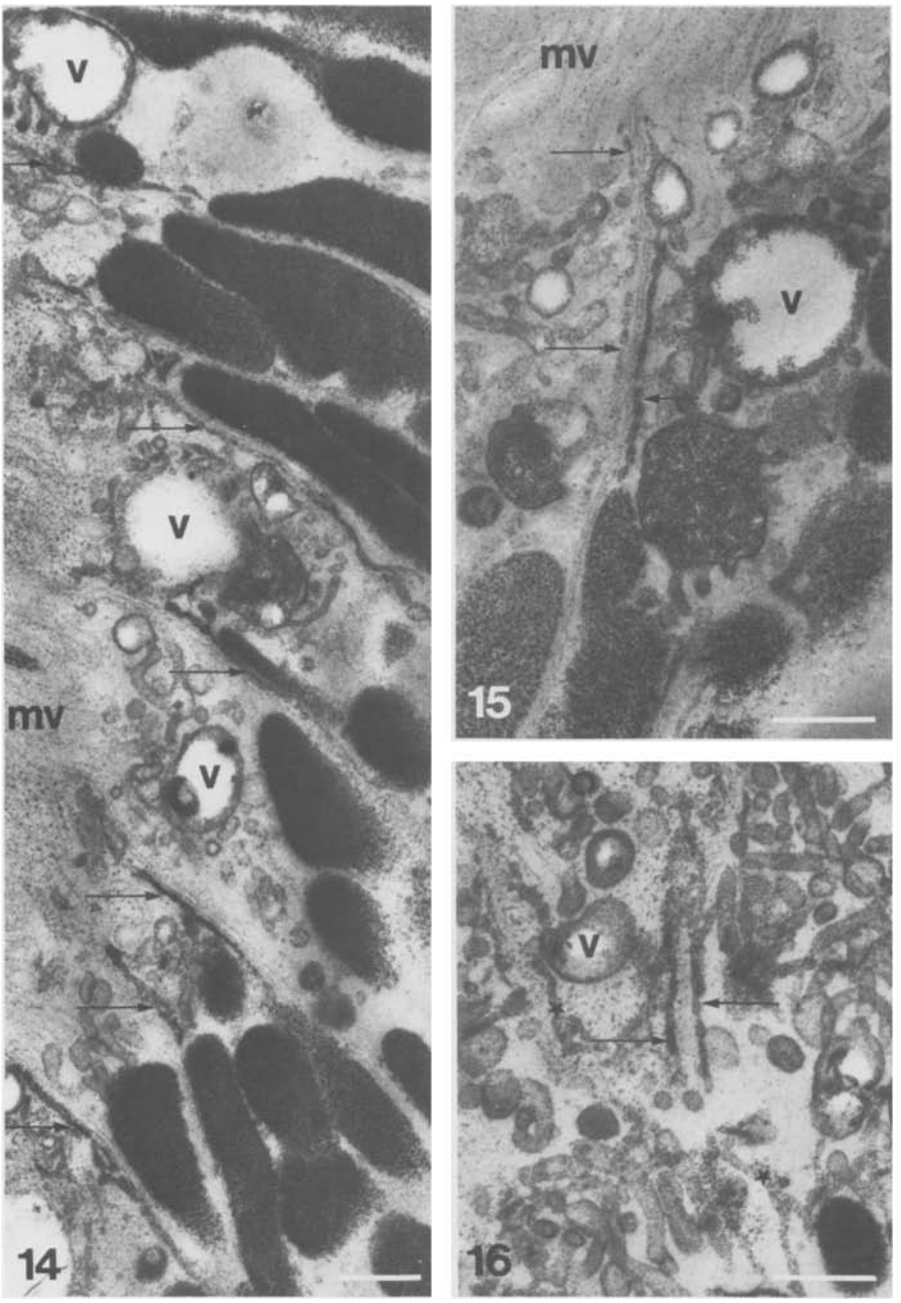


Fig. 11. Electron micrograph of thick section of metal impregnated gallbladder epithelial cells. Subsurface cisterna-like elements and tubular parts of TER are marked with arrows. Microvilli (*mv*), lateral cell membrane (*lm*). 22,000 ×

Fig. 12. Thick section of metal-impregnated intestinal epithelial cells. Subsurface cisterna-like elements and tubular parts of TER are marked with arrows. Lateral cell membrane (*lm*). 22,000 ×

Fig. 13. Electron micrograph of thick section of metal-impregnated proximal tubule epithelial cell showing the apical part containing a vast and complex system of apical canaliculi (*ac*) and associated vacuoles (*v*). Microvilli (*mv*). 22,000 ×



Figs. 14–16. Electron micrographs of metal-impregnated proximal tubule epithelial cells. Typical subsurface cisterna-like elements (arrows) follow the lateral cell membrane right to the junctional complex. Tubular TER intermingled with apical canaliculi, are marked with stars. Microvilli (*mv*), vacuoles (*v*). Fig. 14, 21,000×; Fig. 15, 27,500×; Fig. 16, 27,500×

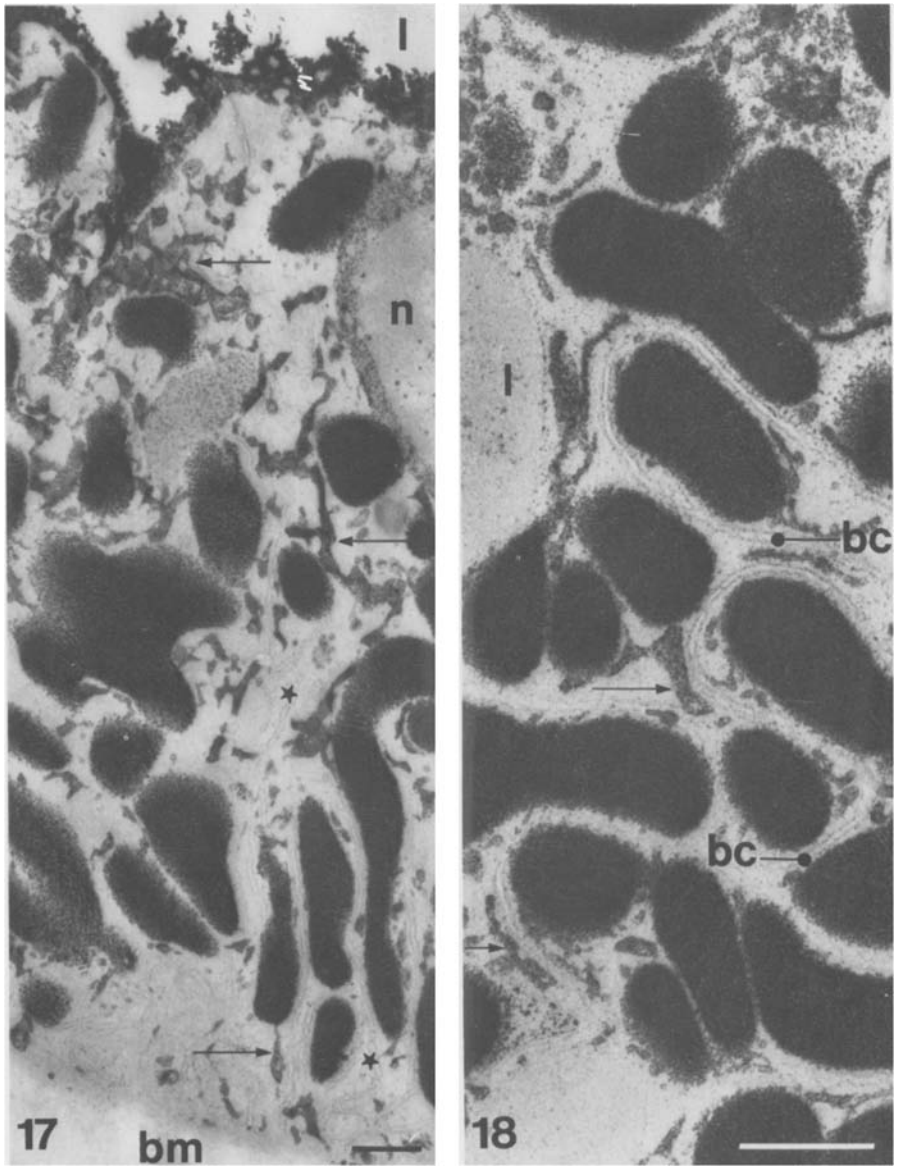


Fig. 17. Thick section of metal impregnated renal collecting duct. The TER is marked with arrows. Lumen (*l*), nucleus (*n*), basal infoldings (stars), basement membrane (*bm*). 18,000 ×

Fig. 18. Basal infoldings in distal tubule epithelial cell. Subsurface cisterna-like elements are marked with arrows. Basal cell membrane (*bc*), lysosome (*l*). 35,000 ×

of tubules and cisternae of endoplasmic reticulum (Figs. 4 & 5), and the use of very thick sections (up to 4,000 Å) demonstrates the continuity of the various parts of the tubulo-cisternal endoplasmic reticulum (Fig. 6). The organization of the TER in this epithelium closely fits the general description already given. Both mitochondria and lysosomes are often observed in close contact with elements of TER (Fig. 5). A distinct feature of the TER is the existence of slender tubules which extend from its most apical part towards the apical cell membrane (Fig. 7).

Rabbit gallbladder: Conventional electron microscopy of thin sections of gallbladder epithelial cells reveals a characteristic TER consisting of both apical tubular and subsurface cisterna-like elements (Fig. 8). Cross sections of the mitochondria-rich supranuclear zone exhibit numerous cisternae along a major part of the lateral cell membrane (Fig. 9). Some of these cisternae are seen to protrude into the base of the microplicae, and by serial sectioning it can be shown that practically all microplicae are underlined by a cisterna of the TER. The very complex organization of the lateral cell surface and the numerous microplicae are most clearly demonstrated by scanning electron microscopy (Fig. 10). Apart from this special relationship between TER and lateral cell surface, the system follows the general description already given (Fig. 11).

Rabbit small intestine: The organization of the TER in intestinal epithelial cells (Fig. 12) closely follows the general pattern and resembles that of gallbladder epithelial cells (*compare* Figs. 11 and 12).

Rat kidney: Thick sections of metal impregnated proximal tubule epithelial cells reveal the vast and complex system of apical canaliculi and associated vacuoles (Fig. 13). The characteristic TER is also present; it is intermingled with the apical canaliculi and vacuoles, and typical subsurface cisterna-like elements follow the lateral cell membrane right to the junctional complex (Figs. 14–16). Apart from the interrelationship with apical canaliculi and vacuoles the TER follows the general pattern.

Thick sections of metal impregnated collecting ducts (Fig. 17) and distal tubules (Fig. 18) also reveal a characteristic TER. The cell membrane of the basal infoldings is underlined by subsurface cisterna-like elements which often are squeezed in between mitochondria and cell membrane (Fig. 18). Special features of the TER in distal tubules and collecting ducts are currently under investigation.

Discussion

These studies indicate that an intracellular channel system of tubulo-cisternal endoplasmic reticulum (TER) exists in a wide variety of epithelia. Although the TER takes various forms, its basic pattern of construc-

tion is similar in different epithelia. The system seems to be most abundant in leaky epithelia with low transepithelial resistance and isotonic transport, but it is also identifiable in the tight epithelium of frog skin. These findings indicate that the TER may be a common feature of sodium transporting epithelial cells.

A continuous membrane system of smooth ER has been observed in nerve cells and implicated in fast axonal transport (Droz, Rambourg & Koenig, 1975), and a rather similar complex system of ER has very recently been described in several other cell types (spinal ganglion, spermatocytes, intestinal epithelial cells, Sertoli cells) by Thiery & Rambourg (1976). In epithelial cells from rat proximal tubules Ericsson (1964) has observed a "paramembranous tubular system" (PTS) situated along the lateral cell membranes. In some instances the system was in continuity with the apical canaliculi. The structural similarity of the PTS and the ER led Ericsson (1964) to suggest that the PTS might be involved in intracellular transport functions. These findings and the results presented in this report indicate that the tubulo-cisternal ER (TER) perhaps is a common feature of most cells.

Terminology: We have chosen the abbreviation TER which, in a broad sense, may stand for transport-functioning ER. In specialized epithelia involved in transepithelial transport mechanisms, the TER may define a system of transcellular (transport-functioning) ER. Until this is somewhat more substantiated we use the term TER as a designation for the tubulo-cisternal ER.

It is surprising that the TER has not been recognized as an anatomical entity in transporting epithelia before now, but examination of sections of increasing thickness from the same material (*see,* e.g. Figs. 4 and 6) clearly demonstrates why it is difficult, in the first place, to conceive the complicated structure of the entire system. Most of the numerous rounded profiles ("vesicles"), small tubules, and flattened cisternae as seen in a thin section actually correspond to a cross-sectioned complicated continuum of TER as seen to some extent in thick sections. However, it is also evident from these figures (4 and 6) that different planes of sectioning are necessary for the demonstration of the continuity of the entire TER. Various surface invaginations and intracellular membrane systems have been implicated in specific functions of the different epithelia (e.g., lipid absorption in small intestine, protein absorption in proximal tubules, HCl secretion in parietal cells), but the possible existence of a more or less complete transcellular channel system has not been adequately investigated.

In general, the TER consists of an apical polygonal network of tortuous tubules oriented in parallel with the apical (luminal) cell mem-

brane, in continuity with a complex system of flattened cisternae which is more prevalent along the baso-lateral cell membrane. Various parts of the system may in some instances be associated with the cell surface, but this important aspect requires further study (Møllgård, Rostgaard & Saunders, *in preparation*). However, both the apical tubules and the baso-lateral cisterna-like portions of the TER, with or without points of direct contact with the cell membrane, may have functional implications, since such structural elements may function as sites of entry into and exit from a transcellular TER and thus may be related to some of the permeability properties of an epithelium that cannot be accounted for by variation in tight junction permeability.

Close contact between TER and some other intracellular elements are also present. Abundant mitochondria (e.g., in the mitochondria-rich zone) are found in intimate contact with the TER, often virtually encaged in a polygonal tubular network. Also the mitochondria, responsible for basal striation in the kidney, are in close contact with basal extensions of the TER. Various vacuoles and lysosomes characteristically found in the supranuclear portion of the TER are also in close contact with elements of the polygonal tubular network. The relationship to both rough ER and Golgi complex should be mentioned. The role of the ER and Golgi complex in the synthesis, transport, and storage of proteins has been thoroughly studied (reviewed recently by Palade, 1975). Little is known about the nature of the flow through the ER, but it has been suggested that solute transport into the ER may bring about osmotic water flow into and through the cisternae, which could create a stream that carries protein toward the Golgi complex (*cf.* Oschman, Wall & Gupta, 1974). From the findings of the present study and observations in the literature, it is tempting to envisage the rough ER as a specialized part of the TER engaged in protein synthesis and not primarily involved in transport. The condensation process in the Golgi complex may well be accomplished by an ion transport coupled with water movements out of the system, so that the TER and Golgi complex would have opposite pumping directions.

Although the TER exhibits rather similar patterns of construction in the different epithelia, there are a few striking differences. The TER of the frog skin can be identified in thin section electron microscopy and in freeze fracture replicas, but the system is very difficult to impregnate. Long series of preliminay experiments with varying fixation procedures and incubation times and temperatures have not so far led to consistent results. The reason for this is not clear at present. In proximal

tubules the apical tubular portion of TER is replaced to a large extent by another channel system (the apical canaliculi and associated vacuoles). A TER is, however, clearly present also in the apical cytoplasm, where it is in close contact with both apical canaliculi and associated vacuoles. This modification of the intracellular transport system of proximal tubules can perhaps be related to the role of the proximal tubules in absorption of protein from the glomerular filtrate and in secretory functions.

By correlated biochemical and cytochemical methods, Straus (1975) investigated the reabsorption of protein (horseradish peroxidase) by proximal tubules of rat kidney. He reported that a decrease in protein uptake was correlated with a decrease in sodium and water uptake and discussed a possible direct relationship between sodium and protein transport. Based on his experiments and on a number of other published data (for references, *see* Straus, 1975), it was mentioned that cytoplasmic vesicles, in addition to their well-known function in protein transport, might also participate in fluid transport.

From a theoretical point of view, backwards fluid-transporting epithelia clearly differ from forwards systems (Diamond & Bossert, 1968). If the basic notion of TER as a transport system is correct, it is somewhat surprising to find the same polarity and the same general pattern of construction of TER from choroid plexus and gallbladder which exemplify a backwards and a forwards fluid-transport system, respectively. However, the transport direction in the system may well depend on localization (apical or baso-lateral) and/or orientation of the active solute transport mechanism. Our preliminary results indicate that the TER is more abundant in backwards systems than in forwards systems.

In an attempt to correlate the configuration of various TER elements with specific permeability properties of a given epithelium, two very recent studies appear to be relevant. In frog skin, Møllgård, Voûte & Ussing (*in preparation*) have shown a close correlation between sodium transport and apical surface invaginations and tubulo-cisternal ER; the higher the sodium transport, the more abundant the ER and the invaginations. In high resistance – low transporting winter skins the apical membrane of the first living cell layer is smooth and virtually no cytoplasmic tubules are found in the apical cytoplasm. In low resistance – highly transporting spring and summer skins the apical membrane exhibits abundant sinuous invaginations which extend into the apical cytoplasm and appear to contact the well-developed tubular ER. The degree of expansion of this system is strongly dependent on the rate of sodium transport as measured by the short-circuit current.

In the choroid plexus some characteristic differences in the configuration of the tubulo-cisternal ER at early and late stages of development have very recently been observed, and correlated with permeability differences at the same stages (Møllgård & Saunders, 1977). In the early, very leaky stage the apical polygonal network is situated close to the apical cell surface where points of contact are frequently seen, whereas the network usually is separated from the apical cell membrane by a distinct organelle-free zone in the late and less permeable stage of development. Relatively few slender tubules connect this network, and the apical cell membrane, and many lysosomes, are now in close contact with the TER. Previously, it has been shown that the configuration of the tight junctional network as revealed by freeze fracturing exhibits roughly the same junctional depth and strand number at early and late stages of development (Møllgård, Malinovska & Saunders, 1976). Taken together these studies suggest that a transcellular pathway via the tubulo-cisternal ER is also involved in transepithelial permeability in a leaky epithelium as the epithelium of the choroid plexus. The seasonal variations in frog skin and the developmental changes in choroid plexus indicate that alterations in the morphology of the tubulo-cisternal ER may be closely correlated with at least some of the hormone-induced changes in epithelial permeability.

The present results coupled with preliminary physiological studies (Møllgård, Voûte & Ussing, *in preparation*) and with findings described in the literature make us propose the following working hypothesis: Epithelia possess two sodium transporting system – one is based on pump sites on the cell surface (apical and/or baso-lateral cell membranes) and produces a hypertonic transportate; the other is located in the membranes of the TER which, due to its large surface, would be well suited for producing an isotonic transportate. In the frog skin where the transportate usually is strongly hypertonic the intracellular system may well be of minor quantitative importance, whereas in isotonically transporting epithelia, like proximal tubules, small intestine, gallbladder and choroid plexus, the TER might be of great importance.

We find that the concept of a transcellular pathway via the TER may make a contribution towards the solution of some of the many controversial and unknown features of transepithelial transport, but we realize that extensive morphological studies of each epithelium in various controlled functional states are crucially needed.

It is a pleasure to acknowledge our debt to professor H.H. Ussing for numerous stimulating discussions of this work. We thank Mrs. Yael Balslev and Miss Kirsten Sjöberg

for excellent technical assistance and Dr. Ebbe Eldrup for his help with freeze fracturing. Kjeld Stub and Vibeke Jørgensen gave expert assistance with the preparations of illustrations and Mrs. Kathe Juhler with the typing and retyping of the manuscript. Finally, we should like to thank Dr. Norman Saunders and his group at the University College London for providing the sheep material used.

References

Diamond, J.M. 1974. Tight and leaky junctions of epithelia. *Fed. Proc.* **33**:2220

Diamond, J.M., Bossert, W.H. 1968. Functional consequences of ultrastructural geometry in "backwards" fluid-transporting epithelia. *J. Cell Biol.* **37**:694

Droz, B., Rambourg, A., Koenig, H.L. 1975. The smooth endoplasmic reticulum: Structure and role in the renewal of axonal membrane and synaptic vesicles by fast axonal transport. *Brain Res.* **93**:1

Erlij, D. 1976. Solute transport across isolated epithelia. *Kidney Int.* **9**:76

Ericsson, J.L.E. 1964. Absorption and decomposition of homologous hemoglobin in renal proximal tubular cells. Acta Path. Microbiol. Scand. **168 (Suppl.)**:1

Farquhar, M.G., Palade, G.E. 1963. Junctional complexes in various epithelia. *J. Cell Biol.* **17**:375

Martinez-Palomo, A., Erlij, D. 1975. Structure of tight junctions in epithelia with different permeability. *Proc. Nat. Acad. Sci. USA* **72**:4487

Møllgård, K., Malinowska, D.H., Saunders, N.R. 1976. Lack of correlation between tight junction morphology and permeability properties in developing choroid plexus. *Nature (London)* **264**:293

Møllgård, K., Saunders, N.R. 1975. Complex tight junctions of epithelial and of endothelial cells in early foetal brain. *J. Neurocytol.* **4**:453

Møllgård, K., Saunders, N.R. 1977. A possible transepithelial pathway via endoplasmic reticulum in foetal sheep choroid plexus. *Proc. R. Soc. London* **199**:321

Oschman, J.L., Wall, B.J., Gupta, B.L. 1974. Cellular basis of water transport. *Symp. Soc. Exp. Biol.* **28**:305

Palade, G. 1975. Intracellular aspects of the process of protein synthesis. *Science* **189**:347

Straus, W. 1975. Altered reabsorption of protein by the renal cortex in rats treated with hypertonic saline or mannitol. *J. Histochem. Cytochem.* **23**:707

Thiery, G., Rambourg, A. 1976. A new staining technique for studying thick sections in the electron microscope. *J. Microscop. Biol. Cell.* **26**:103

Ussing, H.H., Erlij, D., Lassen, U. 1974. Transport pathway in biological membranes. *Annu. Rev. Phys.* **36**:17

Voûte, C.L., Møllgård, K., Ussing, H.H. 1975. Quantitative relationship between active sodium transport, expansion of endoplasmic reticulum and specialized vacuoles ("scalloped sacs") in the outermost living cell layer of the frog skin epithelium (Rana temporaria). *J. Membrane Biol.* **21**:273