

THE FINE STRUCTURE OF THE TRANSITIONAL EPITHELIUM OF RAT URETER

R. M. HICKS, Ph.D.

From the Bland-Sutton Institute of Pathology, The Middlesex Hospital Medical School, London, England

ABSTRACT

The fine structure of the transitional epithelium of rat ureter has been studied in thin sections with the electron microscope, including some stained cytochemically to show nucleoside triphosphatase activity. The epithelium is three to four cells deep with cuboidal or columnar basal cells, intermediate cells, and superficial squamous cells. The basal cells are attached by half desmosomes, or attachment plates, on their basal membranes to a basement membrane which separates the epithelium from the lamina propria. Fine extracellular fibres, *ca.* 100 Å in diameter, are to be found in the connective tissue layer immediately below the basement membrane of this epithelium. The plasma membranes of the basal and intermediate cells and the lateral and basal membranes of the squamous cells are deeply interdigitated, and nucleoside triphosphatase activity is associated with them. All the cells have a dense feltwork of tonofilaments which ramify throughout the cytoplasm. The existence of junctional complexes, comprising a zonula occludens, zonula adhaerens, and macula adhaerens or desmosome, between the lateral borders of the squamous cells is reported. It is suggested that this complex is the major obstacle to the free flow of water from the extracellular spaces into the hypertonic urine. The free luminal surface of the squamous cells and many cytoplasmic vesicles in these cells are bounded by an unusually thick plasma membrane. The three leaflets of this unit membrane are asymmetric, with the outer one about twice as thick as the innermost one. The vesicles and the plasma membrane maintain angular conformations which suggest the membrane to be unusually rigid. No nucleoside triphosphatase activity is associated with this membrane. Arguments are presented to support a suggestion that this thick plasma membrane is the morphological site of a passive permeability barrier to water flow across the cells, and that keratin may be included in the membrane structure. The possible origin of the thick plasma membrane in the Golgi complex is discussed. Bodies with heterogeneous contents, including characteristic hexagonally packed stacks of thick membranes, are described. It is suggested that these are "disposal units" for old or surplus thick membrane. A cell type is described, which forms only 0.1 to 0.5 per cent of the total cell population and contains bundles of tubular fibres or crystallites. Their origin and function are not known.

INTRODUCTION

The transitional epithelium lining the ureters and urinary bladder in the mammal is normally in contact with strongly hypertonic urine. This epithelium must, therefore, present an efficient per-

meability barrier to prevent a continual inflow of water and dilution of the urine from the intra- and extracellular fluids. No recent physiological studies of this permeability barrier in the rat ureters and

bladder have been published, and little evidence is available to show whether the barrier is active, as in the toad bladder (1-5), or a passive, mechanical structure.

The histology of the transitional epithelium lining the ureter has been described at the light microscope level (6-8), and it is apparently identical to that of the epithelium lining the bladder. This epithelium is three to four cells deep and is separated from the lamina propria by a thin basement membrane. The entire epithelium projects into the lumen of the ureter in a series of folds and ridges, so that the lumen presents a stellate appearance with long fissures projecting laterally between the epithelial folds.

The fine structure of mammalian bladder transitional epithelium has been documented (8-11), but it was considered that a further electron microscopic investigation of the ureteral epithelium, with special reference to a possible permeability barrier, would provide a useful morphological baseline for physiological studies. The ureters, rather than the bladder, were selected for study, to eliminate the variable factor of mechanical distension and collapse, and also to facilitate orientation of the tissue in the electron microscope.

EXPERIMENTAL

Animals

Adult male and female albino rats of the Wistar strain, weighing between 180 and 250 gm, were used. The animals were killed by dislocation of the neck, and the ureters immediately removed.

Fixation

The ureters were cross-sectioned under cold (0 to 4°C) 4 per cent osmium tetroxide buffered with phosphate (12) into approximately 0.5- to 1.0-mm lengths. Fixation was continued for 1 hour and the tissue was then dehydrated in ethanol and embedded in Epikote 812 (Shell Chemical Co. Ltd., London) essentially by the method of Luft (13).

Cytochemistry

For cytochemistry, ureters were fixed for 8 hours in cold (0 to 4°C) 3 per cent glutaraldehyde buffered to pH 7.4 with sodium cacodylate (14), then washed for 16 hours in 0.25 M sucrose buffered with 0.1 M sodium cacodylate to pH 7.4. The cross-sections were bisected longitudinally to give two hemicylindrical sections, thus exposing the epithelium lining the lumen. These pieces were incubated in Wachstein and

Miesel's (15) adenosine triphosphate-containing medium for 30 minutes, washed in 0.25 M sucrose, post-fixed in osmium tetroxide for 1 hour, then dehydrated and embedded as above. The incubation medium contained 125 mg per cent (w/v) adenosine triphosphate, 2 per cent (w/v) lead nitrate, and 0.1 M magnesium sulphate in 0.2 M Tris maleate buffer pH 7.2.

Light Microscopy

To show the general morphology of Epon-embedded tissue, 1- to 2- μ sections were cut with glass knives on a Porter-Blum microtome, mounted on glass slides, and stained for 2 to 5 minutes at 50°C with 1 per cent (w/v) aqueous toluidine blue.

Electron Microscopy

Sections showing silver-to-gold interference colours were cut with glass knives on a Porter-Blum microtome, mounted on copper electro-mesh grids, stained with uranyl acetate (16) in 50 per cent ethanol for 5 minutes followed by a lead salt (17) for 5 minutes, or with uranyl acetate alone for 30 minutes, and examined in a Siemens Elmiskop I.

OBSERVATIONS

The ureter in a 200-gm rat has an over-all diameter of approximately $\frac{1}{3}$ mm, or 350 μ , and a stellate-shaped lumen varying between 75 and 150 μ in diameter. The arrangement of the various strata in the ureter wall is shown in Fig. 1 and Fig. 2. The transitional epithelium lining the ureter is 3 to 4 cells deep and varies considerably in thickness, from 40 to 80 μ , between the base and peak of the ridges. At the base of the epithelium, and in very close contact with it, are blood capillaries running parallel to the ureter lumen. About 50 of these axial capillaries may be counted in a complete cross-section of the ureter, and they connect at intervals through radial channels with the larger circumferential vessels which lie between the lamina propria and the outer muscle coat. The axial capillaries adjacent to the transitional epithelium have unusually thick endothelial cells with plentiful cytoplasmic contents (Fig. 3). The lamina propria is composed mainly of collagen fibres and fibroblasts. This connective tissue layer supports the blood vessels, a few unmyelinated nerve fibres and, towards the periphery, longitudinal muscle cells. Peripheral to this there is a compact circular layer of smooth muscle, about 25 μ thick, and the whole is surrounded by an adventitial connective tissue sheath carrying other blood vessels and nerve fibres.

The epithelial cells can be conveniently classified

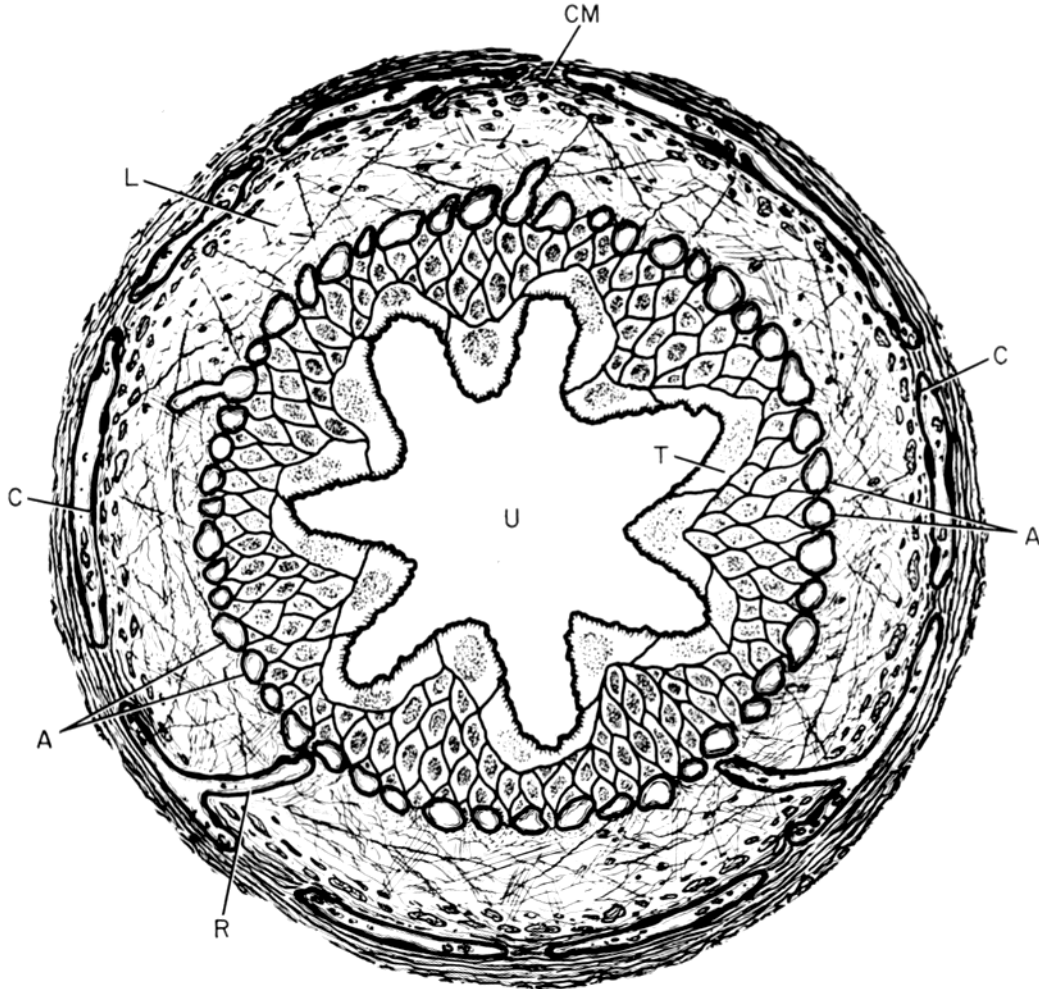


FIGURE 1 Representation of a cross-section through the rat ureter. $\times 420$. The stellate lumen (*U*) is surrounded by the ridged and fissured transitional epithelium (*T*) which is three to four cells deep. Immediately below this epithelium are axially arranged blood capillaries (*A*) which connect through occasional radial vessels (*R*) with large, more peripheral, circular blood vessels (*C*). Supporting the blood vessels and filling most of the area between them is the lamina propria (*L*), a connective tissue layer composed of collagen fibres, fibroblasts, and a few unmyelinated nerve fibres, and carrying, at its periphery, longitudinally arranged smooth muscle cells. Peripheral to this is a compact circular layer of smooth muscle (*CM*), and surrounding the whole ureter is an adventitial-connective tissue sheath which carries other blood vessels and nerve fibres. The adventitia is not illustrated in this diagram. Compare with Fig. 2. See also Maximow and Bloom (7).

as basal, intermediate, and squamous, with the squamous cells covering the entire free surface of the ureteric folds. With the electron microscope it can be seen that all the cells have deeply infolded and interdigitated lateral borders (Figs. 4 and 5) which are connected at intervals by desmosomes or maculae adhaerentes (Fig. 5). Lines of pinocytotic vesicles are frequently seen extending into

the cytoplasm from infoldings of one of the lateral plasma membranes (Fig. 4). Between the lateral membranes are many extracellular spaces which may vary considerably in size (Figs. 3 and 5). The extracellular spaces frequently contain an electron-scattering, finely granular material, (Figs. 3 and 5), and similar material is sometimes seen in the lumen of the ureter (Fig. 4). The extracellular

fluid between epithelial cells is separated from the interior of blood capillaries in the lamina propria by little more than the basement membranes of the ureter epithelium and capillary endothelium (Fig. 3).

The Basal Cell

The basal cells may be cuboidal or columnar, and are typical of many mammalian epithelial cells. Their cytoplasm is rich in tonofilaments and contains many free ribonucleoprotein particles, mitochondria, and small dense bodies, but little organised rough-surfaced endoplasmic reticulum (Fig. 3). They rest on a thin basement membrane and frequently have a deeply infolded base (Fig. 3). Disc-like attachment plates, resembling half desmosomes, attach the basal plasma membrane to the basement membrane at frequent intervals (Figs. 3 and 10). These half desmosomes on the epithelial cell membrane are associated with a

local condensation of cytoplasmic tonofilaments. They are matched by a localised thickening and increased density of the basement membrane at these points, and a condensation of small fibres in the underlying connective tissue layer (Figs. 3 and 10). These extracellular fibrils are approximately 100 A in diameter, and do not resemble mature collagen (Fig. 3).

The Intermediate Cell

The intermediate cells form a layer one or two cells deep between the basal and the squamous cells. Their cytoplasmic contents are comparable to those of the basal cells and include tonofilaments, mitochondria, free ribonucleoprotein particles, and a prominent Golgi region (Figs. 4 and 5). In addition, they may contain a variety of membrane-bounded vesicles and heterogeneous bodies seen more frequently in the squamous cells described below.

Abbreviations for Micrographs

<i>T</i> , transitional epithelium.	<i>er</i> , endoplasmic reticulum.
<i>B</i> , basal cell.	<i>es</i> , extracellular space.
<i>I</i> , intermediate cell.	<i>n</i> , nucleus.
<i>S</i> , squamous cell.	<i>m</i> , mitochondria.
<i>Z</i> , bundle cell.	<i>g</i> , Golgi complex.
<i>E</i> , capillary endothelial cell.	<i>l</i> , lysosomes.
<i>U</i> , lumen of ureter.	<i>p</i> , pinocytosis vesicles.
<i>J</i> , junctional complex.	<i>t</i> , tonofilaments.
<i>A</i> , axial blood capillary.	<i>r</i> , ribonucleoprotein particles.
<i>L</i> , lamina propria.	<i>f</i> , extracellular fibrils.
<i>C</i> , circular blood vessel.	<i>c</i> , collagen fibres.
<i>V</i> , adventitia.	<i>h</i> , heterogeneous bodies.
<i>cm</i> , cell membrane.	<i>b</i> , bundles of crystalline material.
<i>tm</i> , triple-layered unit membrane.	<i>o</i> , zonula occludens.
<i>bm</i> , basement membrane.	<i>a</i> , zonula adhaerens.
<i>dv</i> , dilated vesicles.	<i>d</i> , desmosome.
<i>fv</i> , fusiform vesicles.	<i>bp</i> , basal attachment plate.
<i>mv</i> , multivesicular body.	

All photographs, except Fig. 2 which is a light micrograph, are electron micrographs of rat ureter epithelial cells. All except Figs. 13 and 14 show tissues fixed in osmium tetroxide and embedded in Epon. The tissue shown in Figs. 13 and 14 was fixed in glutaraldehyde and stained to show adenosine triphosphatase activity before postfixing in osmium tetroxide and embedding in Epon. Figs. 7 and 18 illustrate parts of thin sections which have been stained for 30 minutes in alcoholic uranyl acetate. The tissue shown in Figs. 13 and 14 was stained for 30 minutes with a lead salt, and the tissues in the remaining figures with both uranyl acetate and a lead salt for 5 minutes each. The electron micrographs were taken with a Siemens Elmiskop I.

"Bundle Cells"

In the intermediate cell layer a few cells are found which contain aggregates, or bundles, of electron-scattering material (Figs. 6, 15 and 16); these cells comprise only 0.1 to 0.5 per cent of the epithelial cell population. Pending identification of their contents, they will be called here "bundle cells" for convenience. The material has the appearance of bundles of fine tubular crystallites, with a long axis up to 1μ in length and a short axis about 70 \AA across (Figs. 6, 15, and 16). In some cells the crystallite bundles are completely retained in membrane-bounded vacuoles while in others the membranes of the vacuoles are incomplete and fragmented (Figs. 6, 15, and 16). Alternatively, the bundles may lie free in the cytoplasm, although a relatively clear halo around them suggests that they were previously retained within a vacuole (Fig. 6). It is suggested that

these images show various stages in the development of the bundle cells. Other cytoplasmic organelles in these cells are sparse, but a few profiles of the endoplasmic reticulum and ribonucleoprotein particles are seen between the crystallite-containing vacuoles, and at all stages normal-looking mitochondria are present (Fig. 6). The plasma membrane of the bundle cell frequently appears to be incomplete (Figs. 3 and 6) so that the cell contents may be in direct contact with the fluid in the extracellular spaces. Such cells may be seen apparently discharging their contents into the extracellular spaces, and crystalline material is sometimes seen in the space between two cells (Fig. 3). Bundle cells have also been seen in the squamous cell layer, retained within the extracellular space formed by the lateral walls of the squamous cells and held back from the ureter lumen only by the terminal junctional complex between the squamous cells. In two

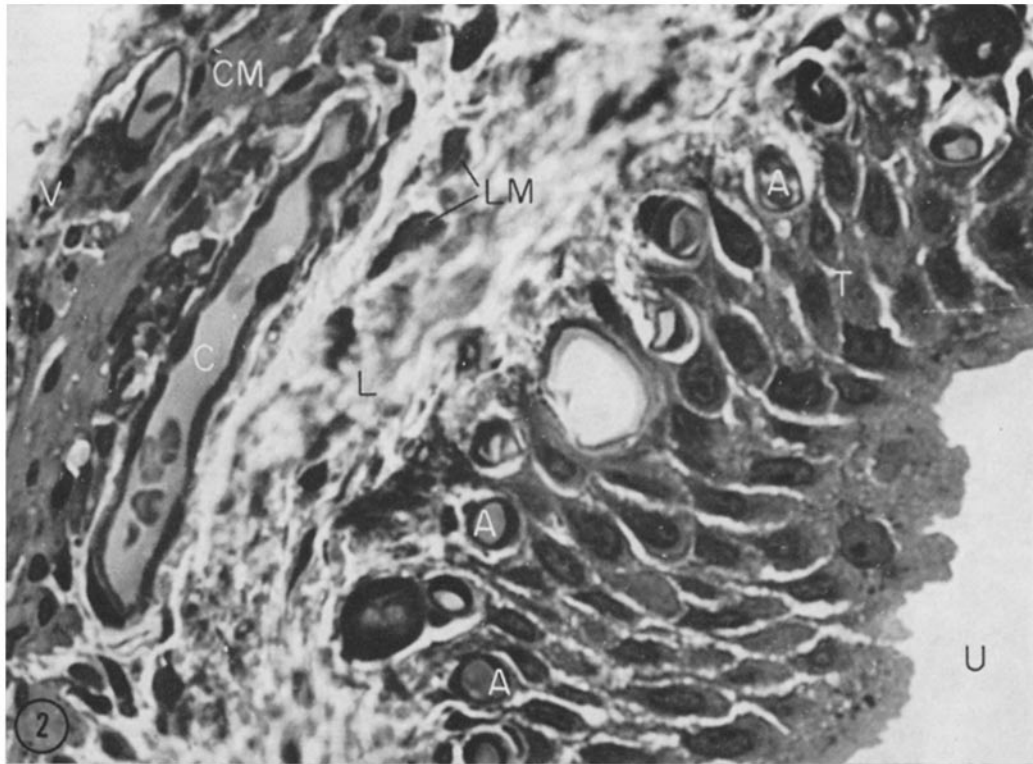


FIGURE 2 This photomicrograph is of a $1\text{-}\mu$ section of OsO_4 -fixed, Epon-embedded ureter. It shows the lumen of the ureter (*U*), transitional epithelium (*T*), axial capillaries (*A*), the lamina propria (*L*), longitudinal muscle cells (*LM*), a larger circular blood vessel (*C*), and the circular smooth muscle layer (*CM*). Most of the adventitia (*V*) has been stripped away. Compare with Fig. 1. $\times 1,050$.

instances, bundle cells have been seen projecting into the lumen of the ureter, lying loosely between squamous cells to which no desmosome attachments could be seen in the plane of section.

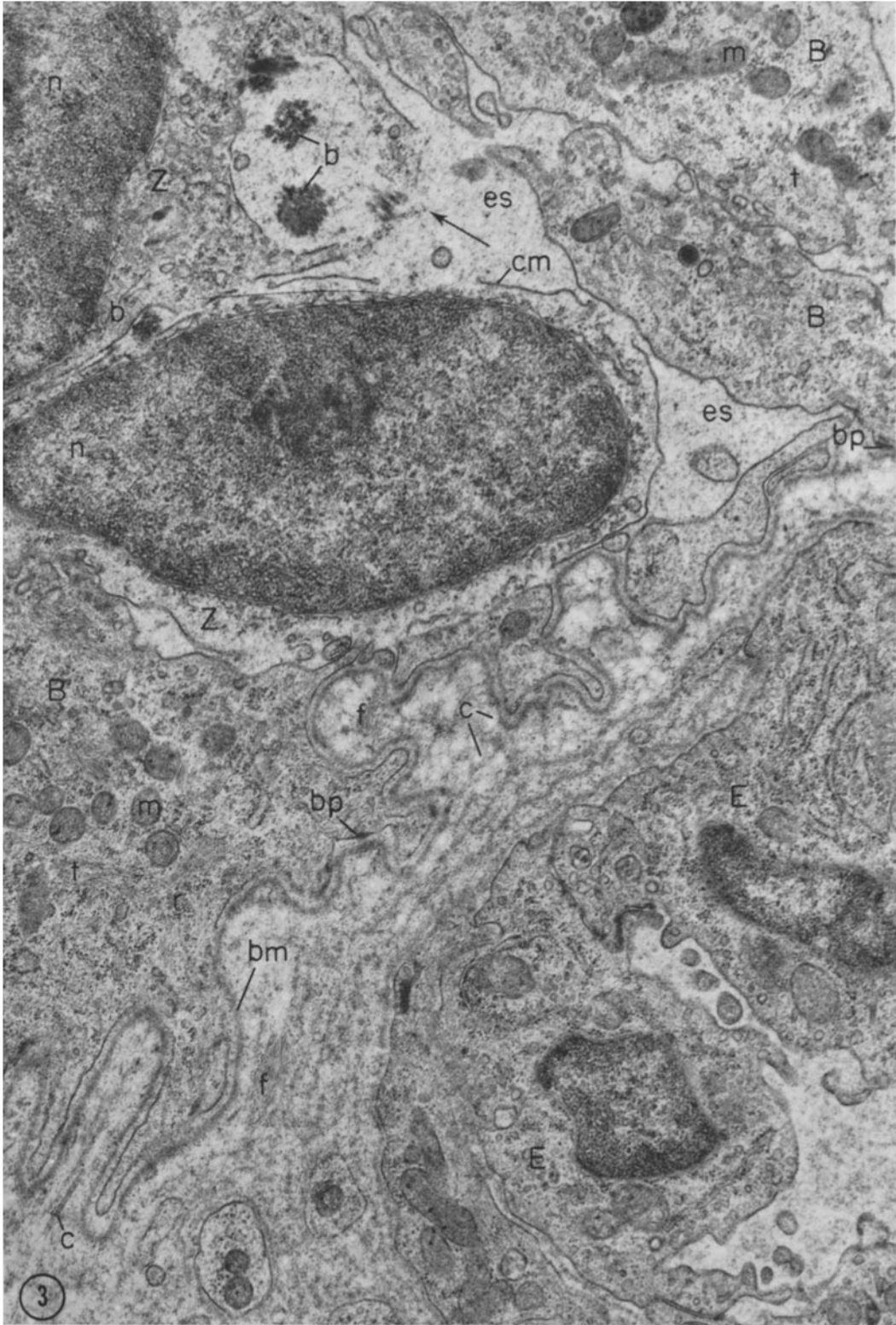
The Squamous Cell

The squamous cells are the most peripheral of the ureteric epithelial cells and cover the whole free surface of the irregularly ridged and fissured epithelium (Figs. 4, 5, and 7). The plasma membrane on the luminal free surface of these cells has an easily resolved, markedly trilaminar unit membrane (Fig. 7). A similar unit membrane is seen to limit the numerous angular cytoplasmic vesicles (Fig. 7), which may be either flattened and fusiform in shape, or dilated and roughly spherical (Figs. 4, 5, 7, and 11). The dilated vesicles do not have smooth contours, but retain an angular conformation and frequently contain smaller circular profiles (Figs. 4 and 11). They might, therefore, be classed as multivesicular bodies, although they differ, by having a thick boundary membrane, from multivesicular bodies of more usual appearance in the same cells (Figs. 4 and 11). A comparable angularity is seen at the free cell surface (Figs. 5 and 7) as if the thick membrane is unusually rigid. At higher magnifications, the two dense components of the unit membrane triple structure are seen to be of unequal width. The innermost leaflet of the membrane of the fusiform vesicles and the outer leaflet of the plasma membrane are thicker than the membrane leaflet adjacent to the cytoplasm (Figs. 8 and 9). The

asymmetry in the unit membrane is more readily seen after double staining with both uranyl and lead salts (Figs. 8 and 9) than it is after staining with uranyl acetate alone (Fig. 7). The fusiform vesicles are most numerous towards the luminal border of the squamous cells, but are seen throughout the cytoplasm right down to the base of the cell (Figs. 4 and 5). The distribution of these vesicles along the luminal edges of the cells is uneven, many being clustered together in some places (Fig. 7) while in other areas relatively few are to be seen (Fig. 4). These vesicles also occur, but in fewer numbers, in the cytoplasm of the intermediate cells (Fig. 4).

The unit membrane of the free cell surface is readily seen to be thicker than the convoluted lateral and basal borders of the squamous cells and the plasma membranes of the intermediate and basal cells. Cytochemical staining shows that the thick unit membrane, both at the cell surface and around the vesicles, has no adenosine triphosphatase activity (Fig. 13). However, the lateral and basal membranes of the squamous cells do dephosphorylate this substrate, as do the plasma membranes of the basal and intermediate cells (Figs. 13 and 14). No significant deposit of the reaction product, lead phosphate, has been observed in the area of the junctional complex, described below. A dense feltwork of fine tonofilaments, each about 60 Å in diameter, is present within the cytoplasm of the squamous cells. These filaments are primarily oriented parallel to the cell surface (Fig. 4) and are most prominent in

FIGURE 3 The field shows an area at the base of the ureter epithelium at a relatively low magnification. Parts of two bundle cells (*Z*) and basal cells (*B*) in the ureteric epithelium are separated from the lamina propria by a thin basement membrane (*bm*). Parts of the endothelial cells (*E*) of a blood capillary below the ureter epithelium can be seen. The basal cell cytoplasm contains bundles of tonofilaments (*t*), mitochondria (*m*), and free ribonucleoprotein particles (*r*). A few half desmosomes, or basal plates (*bp*), attach the plasma membrane of the basal cells to the underlying basement membrane. The connective tissue layer below the basement membrane of the ureteric epithelium contains small fibrils (*f*) and collagen fibres (*c*). The collagen fibres appear in negative contrast in cross-section, but the combined uranyl acetate and lead stain shows up their surface structure when they are sectioned longitudinally. The nuclei (*n*) of two bundle cells are shown at the top left of the field. Bundles of dense crystalline material (*b*) are present in the cytoplasm of the upper cell and in the extracellular space between two cells. The plasma membrane (*cm*) of the bundle cells is interrupted at several places. At one point (*arrow*) the dense crystalline material of the upper cell is in direct contact with a large extracellular space (*es*). $\times 25,000$.



the apical half of the cell. They make intimate contact with the thick membrane bounding many of the cytoplasmic vesicles, as can be seen when the face of the membrane is revealed by an oblique section (Fig. 11).

The cells are joined at the luminal end of their lateral borders by a characteristic tripartite junctional complex (Figs. 5 and 12), as has been described by Farquhar and Palade in numerous other epithelia (18, 19). A zonula occludens, zonula adhaerens, and macula adhaerens or desmosome can be identified where the plane of section is favourable (Fig. 12), and the tonofilaments are concentrated in this area. This complex maintains a tight junction between adjacent squamous epithelial cells even when the cells are separated along the entire remaining length of their lateral borders by a distended extracellular space (Fig. 5). The granular material contained in the extracellular space may then be separated from the lumen of the ureter by only a thin strip of cytoplasm and the junctional complex of two cells (Fig. 5).

In addition to vesicles and tonofilaments, the cytoplasm of the squamous cell has a very prominent Golgi complex (Figs. 4 and 5). Many smooth profiles are seen but little rough-surfaced endoplasmic reticulum. There are a few clumps of free ribonucleoprotein particles, a few small mitochondria, and a great variety of dense bodies and other cytoplasmic vesicles, some of which have irregular and angular profiles. In addition to vesicles with the characteristic appearance of multivesicular bodies and lysosomes, a type of body with heterogeneous contents (Figs. 17 to 19) is

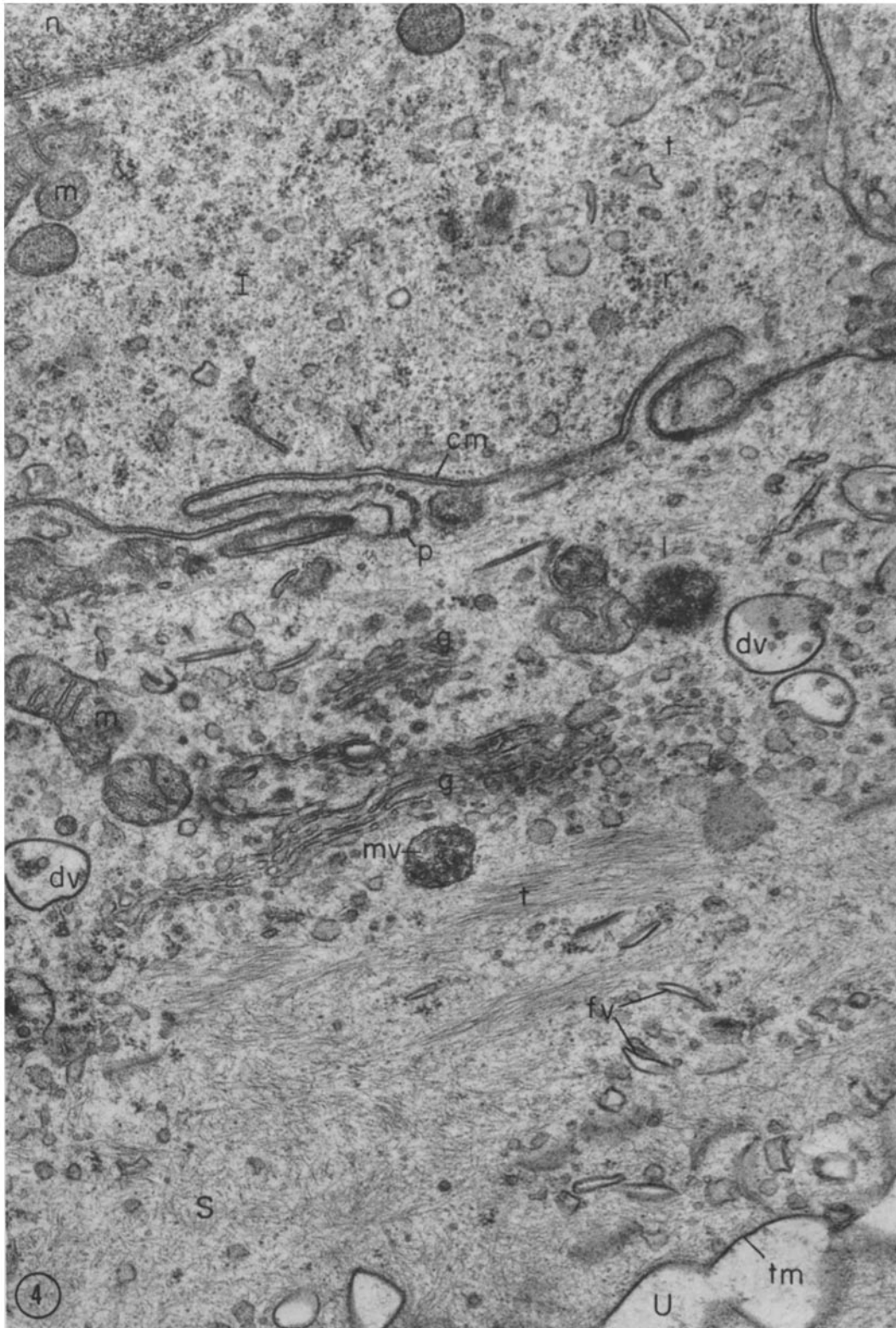
regularly seen grouped in clusters at the base of the squamous cells near the Golgi apparatus. These bodies are also seen, but less frequently, in the intermediate cells. Some of them are irregular in outline and contain fragments of thick unit membrane disposed in geometrical patterns within an otherwise homogeneous granular matrix (Fig. 17). In others, small, very dense areas are surrounded by hexagonally arranged light lines, believed to be the negative images of unit membranes (Figs. 17 and 18). Another form contains relatively "empty" vesicles randomly mixed with droplets of homogeneous lipid-like material (Fig. 19). Vestiges of thick unit membrane can be seen partially surrounding these droplets (Fig. 19). Intermediate stages between these three forms are regularly seen and all appear clustered together in the same region of the cell. They range in size from 0.5 to 5.0 μ in diameter and may be smooth or irregular in outline (Figs. 17 to 19).

DISCUSSION

This study was undertaken primarily to provide a morphological baseline for subsequent physiological investigations. However, the details of fine structure described here are in themselves indicative of function, and, therefore, call for comment.

The three- to four-cell-thick layer of transitional epithelium in the rat ureters and bladder is the only barrier between the isotonic extracellular fluids and the strongly hypertonic urine. Permeability barriers must, therefore, exist within the epithelium which prevent the flow of water from the subepithelial tissue both between and through the cells into the urine. A comparable epithelial

FIGURE 4 The field shows part of a squamous cell (*S*) bordering a cleft-like extension of the ureter lumen (*U*) which reaches deep into the epithelium. The lumen contains a little fine granular material. The upper third of this field is occupied by part of an intermediate cell (*I*). The plasma membranes (*cm*) separating the cells are deeply convoluted, and pinocytotic vesicles (*p*) extend from the plasma membrane into the squamous cell cytoplasm. An extensive Golgi complex (*g*), bundles of tonofilaments (*t*), mitochondria (*m*), and a lysosome (*l*) are present in the squamous cell cytoplasm. Cytoplasmic vesicles are present in the squamous cells, some of which (*fv*) are flattened or fusiform in shape while others are dilated (*dv*). A few fusiform vesicles may be seen in the intermediate cell and some of the dilated vesicles contain smaller circular profiles. All these vesicles are limited by a thick angular membrane, and the plasma membrane bordering the ureter lumen appears to be formed from a similar rigid, angular, thick membrane (*tm*). A few tonofilaments (*t*), many free ribonucleoprotein particles (*r*), a portion of the nucleus (*n*) and a few mitochondria (*m*) are shown in the intermediate cell. $\times 36,000$.



permeability barrier, but with reversed polarity, exists in the amphibian bladder in which large volumes of very dilute urine may be stored for long periods of time. The dynamics of water flow through the single-cell-thick epithelium of toad bladder have been extensively studied by Leaf and his coworkers (1-3), who have conclusively shown the permeability barrier to be under hormonal control and to be affected only by hormones applied to the serosal, and not to the mucosal, surface of the epithelium. Related electron microscopic studies of toad bladder by Choi (4) and Peachey and Rasmussen (5) have not revealed any obvious morphological counterpart of the physical permeability barrier, but the authors believe that the plasma membrane at the mucosal surface together with either its associated external filamentous coat or its underlying granular component is the most likely candidate.

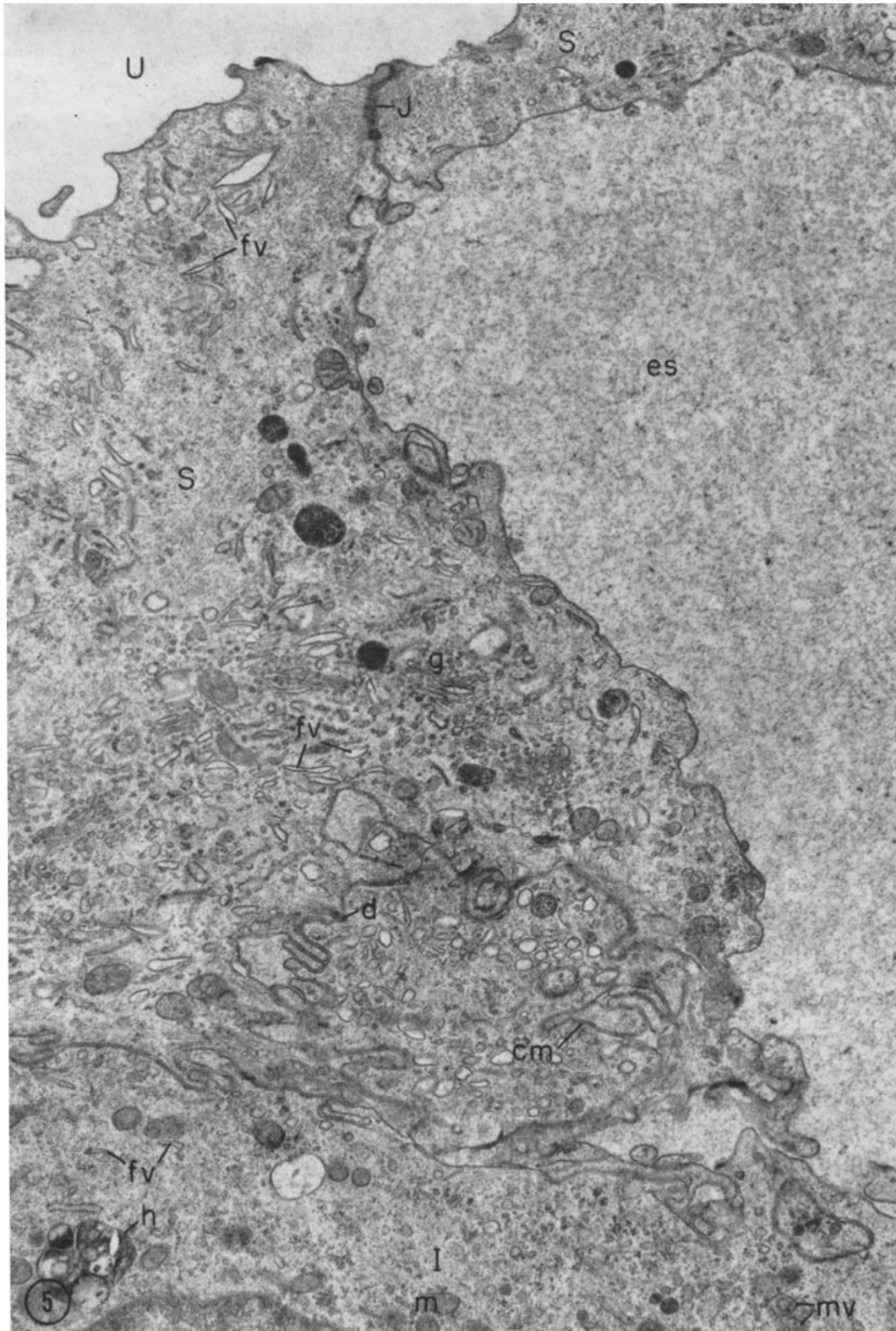
No comparable study of the dynamics of water flow through the transitional epithelium of mammalian urinary bladder or ureters has yet been made, although it is known that water and certain ions move only slowly from one side of the mammalian bladder to the other (21, 22). It is not known whether the permeability barrier is located at the luminal or basal surface of the epithelium. The junctional complex described here, between the lateral borders of the superficial squamous cells towards their luminal edge, is the probable barrier to water flow *between* cells. This complex, composed distally of a zonula occludens, then a zonula adhaerens, and basally of a macula adhaerens or desmosome, has not previously been reported for transitional epithelium although it has been described for many other epithelia including amphibian skin by Farquhar and Palade (18, 19). These authors note that the complex is found regularly in mucosal epithelia lining

cavitary organs. They show that it forms a barrier to the free passage of concentrated protein solutions from the lumina into the intercellular spaces, and quote evidence to suggest that the occluding zonules may also be impermeable to small molecules and possibly to water. The appearance of this complex in the rat ureter epithelium as illustrated here (Figs. 5 and 12) suggests that in this situation also it acts as a barrier but in the opposite direction, preventing the free passage of water from the isotonic fluid in the intercellular spaces into the hypertonic urine in the lumen.

The site of the intracellular barrier to water diffusion into the urine from cytoplasmic fluids still has to be considered. If it is accepted that the junctional complex is the primary obstacle to loss of extracellular water, then the morphological site of the intracellular barrier must lie at the same level or distal to this complex in the cell, or the barrier would be by-passed (see Fig. 20). The only structures to be found parallel with or distal to the junctional complex are first, bundles of tonofilaments, which are also found throughout the squamous, intermediate and basal cell cytoplasm, and second, the thick unit membrane which limits the vesicles and forms the plasma membrane lining the ureter. Leeson (8) proposed that the tonofilaments in rat bladder squamous cells might function as a permeability barrier, but he based this suggestion on the belief that the tonofilaments were confined to the "surface crust" of the cells, whereas they actually extend throughout the whole epithelium. The unusual thick unit membrane appears to be a structure more likely to provide a water permeability barrier.

The cytochemical experiments, using adenosine triphosphate as a marker substrate for nucleoside triphosphatase activity, show the thick surface membrane of the squamous cells to be in a different

FIGURE 5 A large extracellular space (*es*) between squamous (*S*) and intermediate (*I*) cells is separated from the ureter lumen (*U*) by a thin border of squamous cell cytoplasm and a junctional complex (*J*). The extracellular space contains finely dispersed, granular material. The plasma membranes (*cm*) between the cells are interdigitated, and a desmosome (*d*) can be seen connecting the membranes between a squamous and an intermediate cell. An extensive Golgi complex (*g*) of vesicles and cisternae is present at the base of one of the squamous cells, and fusiform vesicles (*fv*) can be seen throughout the cytoplasm of this cell. The intermediate cell at the bottom of the field contains a large dense body (*h*) with heterogeneous contents, mitochondria (*m*), a multivesicular body (*mv*), and a few fusiform vesicles (*fv*). $\times 18,000$.



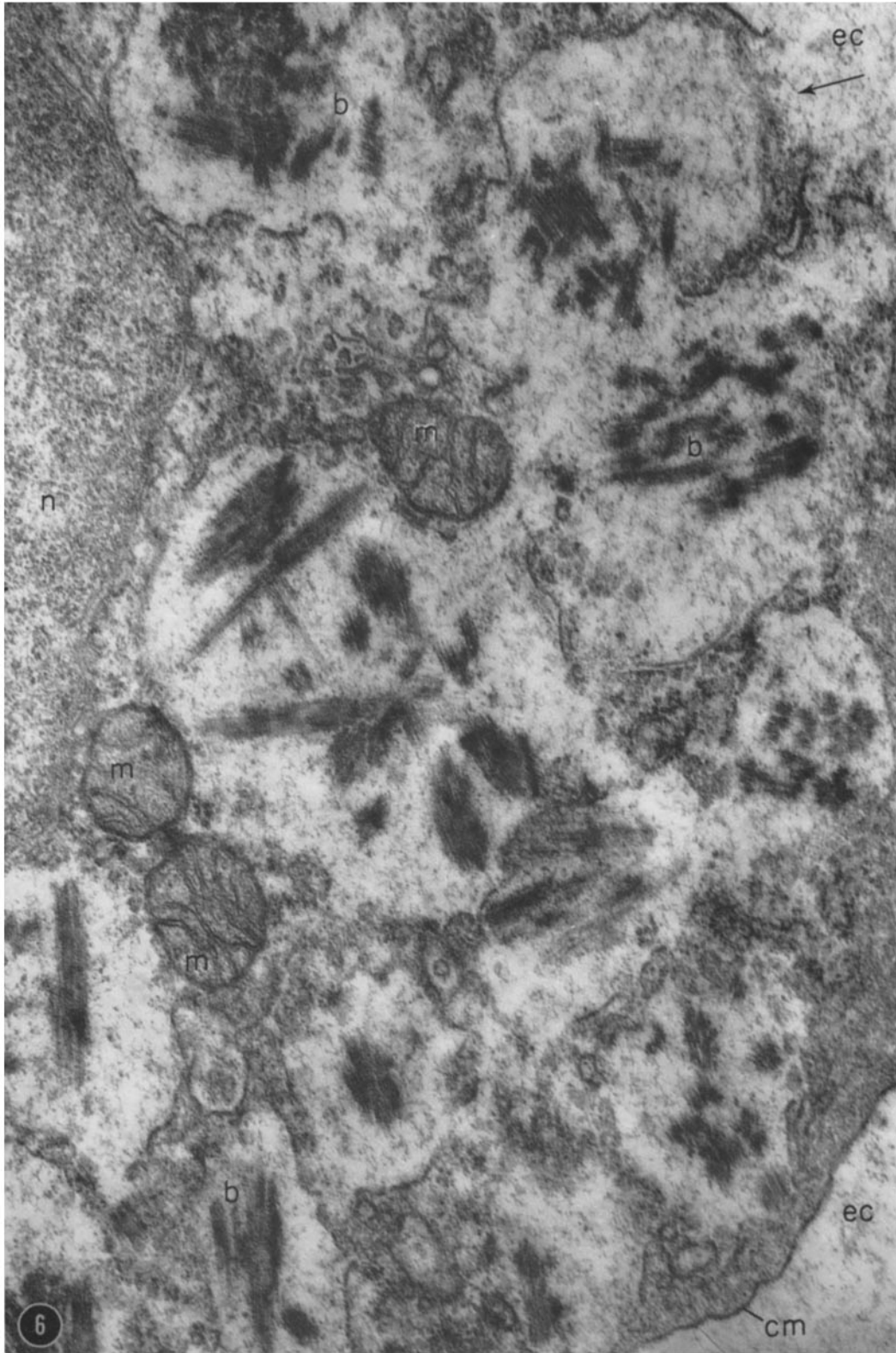
functional state from the lateral and basal plasma membranes of the same cells. The absence of this enzyme from the surface membrane suggests that there is no active transport of cations across it, but that, instead, it may be either freely permeable or impermeable to such small ions. From this it follows that, if this membrane is indeed the site of the intracellular permeability barrier, it is a *passive* rather than an active barrier. This is in marked contrast to the amphibian urinary bladder, in which the luminal surface membrane, thought to be the site of an *active* permeability barrier (1-5), has recently been shown to possess strong nucleoside triphosphatase activity (20). The association of nucleoside triphosphatase activity with the plasma membranes between the squamous epithelial cells and around the basal and intermediate cells in the rat transitional epithelium, is comparable to the enzyme location described by Farquhar and Palade for frog skin, in which it is believed to be indicative of active regulation of the cation balance between the extra- and intracellular fluid compartments (19).

There have been recent reports (18, 23) of triple-layered unit membranes in which the thickness of the three leaflets deviates from the original 20-, 35-, and 20-A dimensions first reported by Robertson (24) for nerve myelin. Moreover, asymmetric plasma membranes have been observed in glandular epithelium in which the outer leaflet of the unit membrane is finer and less dense than the inner one (18). In two instances, namely the luminal borders of colonic and gastric epithelia, it has been reported that the unit membrane shows asymmetry in the opposite direction, with the outer leaflet thicker than the inner one which is adjacent to the cytoplasm (18). The ureteric plasma membrane illustrated here (Figs. 8 and 9) is an extreme example of this last situation. The outer leaflet appears to be about twice the thickness of the inner one, but this difference is far more readily apparent after double staining of the

membrane with lead and uranyl salts than with uranyl salts alone. Very recently, Robertson (25) has attributed variations in the unit membrane pattern to probable differences in the molecular species making up the membrane, a necessary assumption if the different functions of different membranes are to be explained.

It is interesting to consider what molecular species might confer upon this membrane the property of a passive permeability barrier to water. The molecular species making up the plasma membrane in the ureteric squamous cell cannot, of course, be deduced from this direct morphological examination, but there is indirect evidence to suggest that keratin may be involved in its structure. The water-proofing properties of keratin have been known for a long time, and the importance of keratin in the evolutionary development of impermeable cuticles, necessary for water control in the colonisation of fresh water and dry land, has been discussed by Mercer (26). The permeability barrier of human epidermis, which prevents undue loss of water by evaporation, is dependent upon the integrity of keratin in the skin and can be destroyed and renewed by treatments removing and replacing keratin in the epidermal cells (27). One of the physical characteristics of keratinised structures is their mechanical rigidity, *e.g.* hair, nails etc., and the remarkable angular conformations of the ureteric cytoplasmic vesicles and free plasma membrane suggest that their thick membranes in the normal ureter are rigid and may, therefore, be constructed from keratin. Capurro and Angrist (28) observed, in normal untreated rat bladders, low but consistent levels of sulfhydryl and disulfide staining which was most prominent at the luminal edge of the epithelium, and they attributed this to the presence of keratin precursors in the epithelium. The cytoplasmic tonofilaments which are generally believed to be keratin (26, 29, 36) could well account for this diffuse staining, and keratin in the thick membrane

FIGURE 6 The field shows portion of a bundle cell, with the nucleus (*n*) at the upper left. The cytoplasm contains aggregates of dense crystalline material (*b*) retained within large vacuoles whose limiting membranes show a variable degree of fragmentation. Three mitochondria (*m*) are also included in this field. The cytoplasm is separated from the extracellular spaces (*ec*) by the cell membrane (*cm*) at the lower right of the field, but at the top right the cell membrane appears to be ruptured (*arrow*) and the cytoplasm is in direct contact with the fine granular material in the extracellular space. $\times 56,000$.



of the fusiform vesicles and free plasma membrane for the increased intensity of stain at the luminal margin of the squamous cells. Gross keratinisation of bladder epithelium occurs in various pathological and experimental conditions (30-33) and, if keratin is indeed a normal product of metabolism in transitional epithelium, the hyperkeratinisation associated with these pathological states indicates hyperactivity of an existing synthetic mechanism in response to stress.

The foregoing considerations lend support to the concept that the thick unit membrane in the normal ureter contains keratin as its protein moiety.

It has been suggested (8, 9) that the cytoplasmic vesicles have an excretory function and remove excess water from the epithelial cells into the urine. It is proposed here that there is normally a barrier to the outflow of water from the cells into the urine, and that water excretion is not, therefore, the primary function of the vesicles. It has also been suggested (10) that in the bladder the vesicles act as a readily available source of plasma membrane to allow a rapid increase in cell surface as the bladder dilates. In the ureters, which are not subject to the same degree of dilatation and collapse as the bladder, the vesicles may have some other additional function.

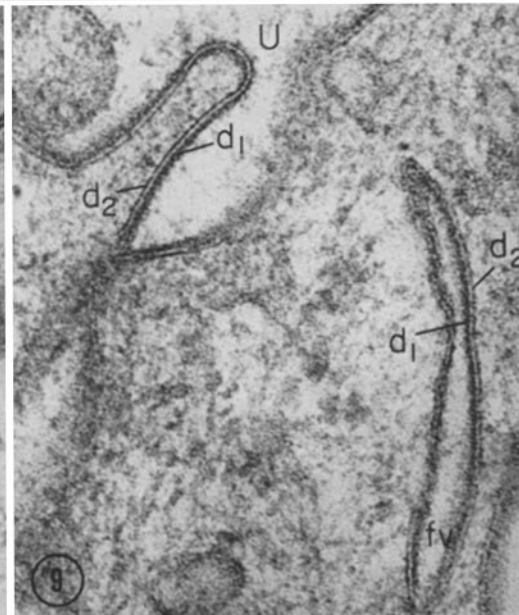
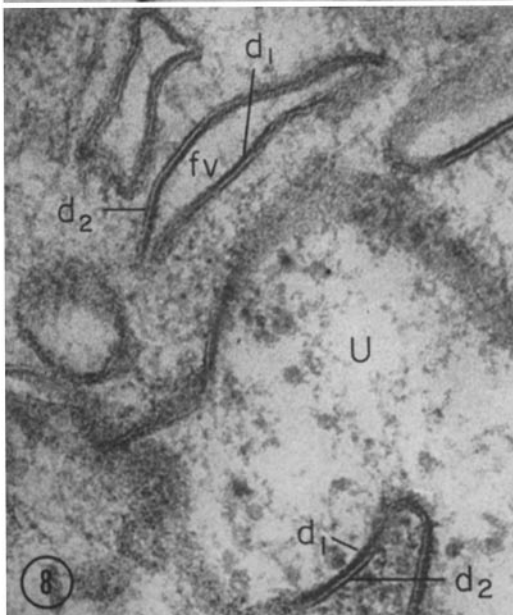
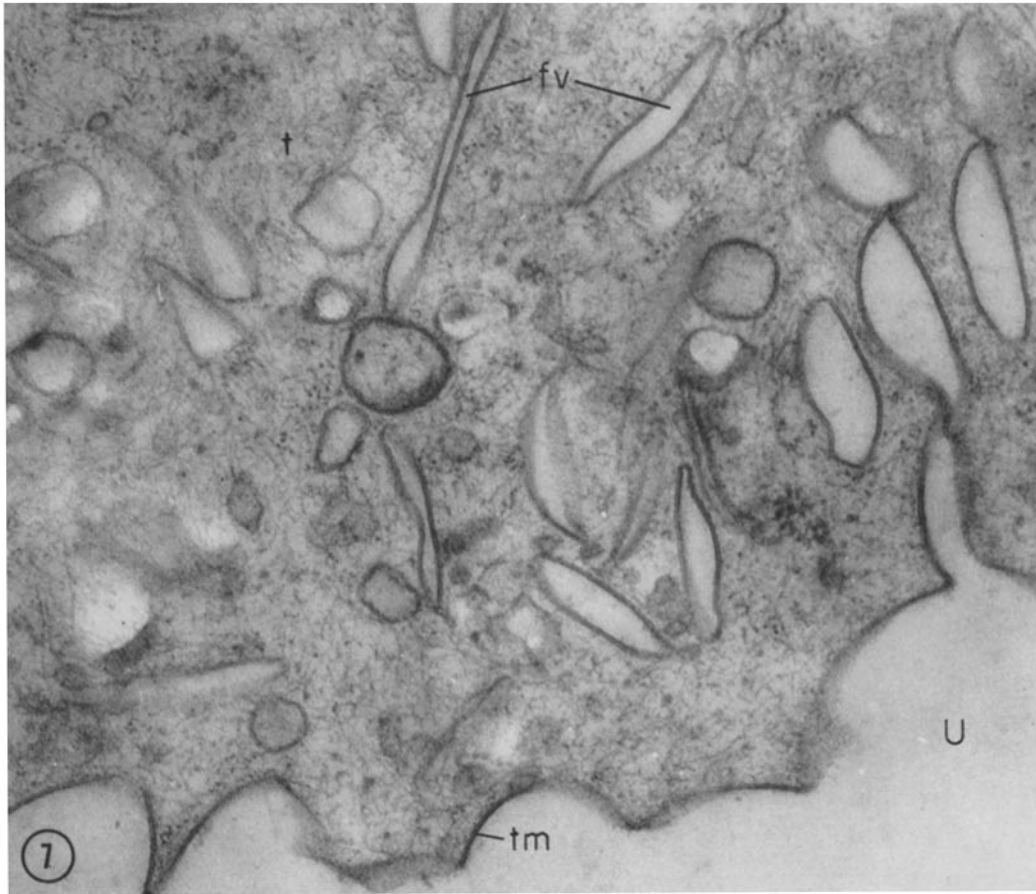
The similarity in appearance of the thick vesicular and plasma membranes suggests that they may have a common origin in the cell. Whaley and his coworkers have shown that vesicles produced from the Golgi complex in the epidermal cells of the maize root tip move through the cytoplasm to the cell surface in which their membranes are incorporated into the plasma membrane and their contents spread out to form part of the outer cell wall (34). In the ureter, the Golgi

complex is very prominent at the base of the squamous cells (Figs. 4 and 5), and in this region cisternae, small spherical vesicles, other vesicles with more angular profiles, and vesicles clearly limited by the thick asymmetric membrane described above, are in close juxtaposition. The asymmetric membrane which limits the fusiform vesicles and forms the free plasma membrane is always orientated with the thick leaflet on its luminal face and the thin leaflet adjacent to the cytoplasm (Figs. 8 and 9). This is consistent with a functional continuity of the vesicular and plasma membrane, in which vesicles could be formed from indentations of the free cell surface or could fuse with and become part of it (Fig. 7). It is also consistent with the probable synthesis of the plasma membrane at the base of the cells in the complex of vesicles associated with the Golgi region, in a way comparable to that described by Whaley for the plasma membrane of maize epidermal cells (34). The membranes of the Golgi cisternae and vesicles do not show the same marked asymmetry as do those of the fusiform vesicles and plasma membrane, but a progressive oxidation of keratin precursors, segregated within the Golgi vesicles, would lead to a deposit of mature keratin being formed on the inner walls as the vesicles move out towards the surface of the cell. A comparable morphological change is seen in the pancreatic acinar cell, in which the transition from Golgi vesicle to mature zymogen granule is accompanied by an increasing density and homogeneity of the vesicles' contents (35).

The nature and function of the large bodies with polymorphic contents is not known. The hexagonal images seen within them suggest at first sight the formation of regular crystals within their matrix (Fig. 18). A more likely explanation is that the

FIGURE 7 A portion of the apical cytoplasm of a squamous cell adjacent to the lumen of the ureter (*U*) is shown in this field. The cell is bounded by a plasma membrane in which the triple-layered unit structure can be clearly seen where the membrane is perpendicular to the plane of section (*tm*). The free surface of the cell is ridged and angular as if this membrane has some rigidity. Fusiform vesicles (*fv*) in the cytoplasm are also limited by a thick triple-layered membrane, and one at the right of the field is in close contact with the free cell membrane. The cytoplasm around the vesicles is filled with tonofilaments (*t*), most of which are cross-sectioned in this field. $\times 60,000$.

FIGURES 8 AND 9 These two figures show, at higher magnification, portions of the triple-layered membranes adjacent to the ureter lumen (*U*) and around fusiform vesicles (*fv*). The membranes are asymmetric and their dense leaflets (*d₁*) adjacent to the ureteric and vesicular lumina are thicker than the dense leaflets (*d₂*) adjacent to the cytoplasm. The ureter lumen contains a little electron-scattering material. $\times 125,000$.



images seen are of dense lipid material contained within geometrically arranged thick unit membranes which appear in negative contrast by comparison with the lipid. The hexagonal packing of the membranes is a regular feature of these bodies. The type of body shown in Fig. 19 in which the contents are less dense and the membranes less distinct is usually found in close association with the others and is thought to be derived from them. In the absence of organised rough-surfaced endoplasmic reticulum or any other sign of secretory activity in the squamous cells, it is unlikely that these polymorphic structures are secretion granules of any sort. It is possible that they are disposal units for surplus specialised unit membrane, or membrane which is due for removal due to normal wear and tear. The dense heterogeneous contents could then represent various stages in lipid production from breakdown of the membranes.

It is not possible to define the significance of the "bundle cells" (Fig. 6). They form a very small percentage of the total cell population in the epithelium, and the bundles appear to be composed of some crystalline material, possibly protein. The cells always contain normal-looking mitochondria and they do not have the usual appearance of a dying cell. The vacuole membranes surrounding the crystallites may be complete, fragmented, vestigial, or absent, and the cell plasma membrane is often interrupted at intervals. As a result, these cells are often seen with their cytoplasmic contents in direct contact with the extracellular spaces (Figs. 3 and 6), and they may well contribute to the dispersed, granular material frequently seen in the extracellular fluid (Figs. 3 and 5). However, it is possible that the regularly observed breaks in the plasma membrane around this cell type are a fixation artefact to which this

cell is selectively susceptible. In two instances, bundle cells have been seen projecting into the lumen of the ureter as if they were being expelled whole into the lumen. These cells also had incomplete plasma membranes and their cytoplasmic contents were exposed to the urine.

The dispersed granular material frequently seen in the lumen of the ureter in the rat may represent plasma protein not retained by the glomerular filter, for this species normally has proteinuria. Alternatively, it may be in part derived from extracellular material following local ruptures in the junctional complex between squamous cells.

The existence of basal attachment plates, or half desmosomes (Fig. 10), joining the basal cells to the basement membrane, is interesting. Basal attachment plates have been reported in epidermal epithelia (19, 36, 37) and epidermal derivatives such as sweat glands (38). The transitional epithelium of the ureters, or metanephric ducts, is mesodermal not ectodermal in origin, and these half desmosomes are not usually observed in epithelia of mesodermal origin.

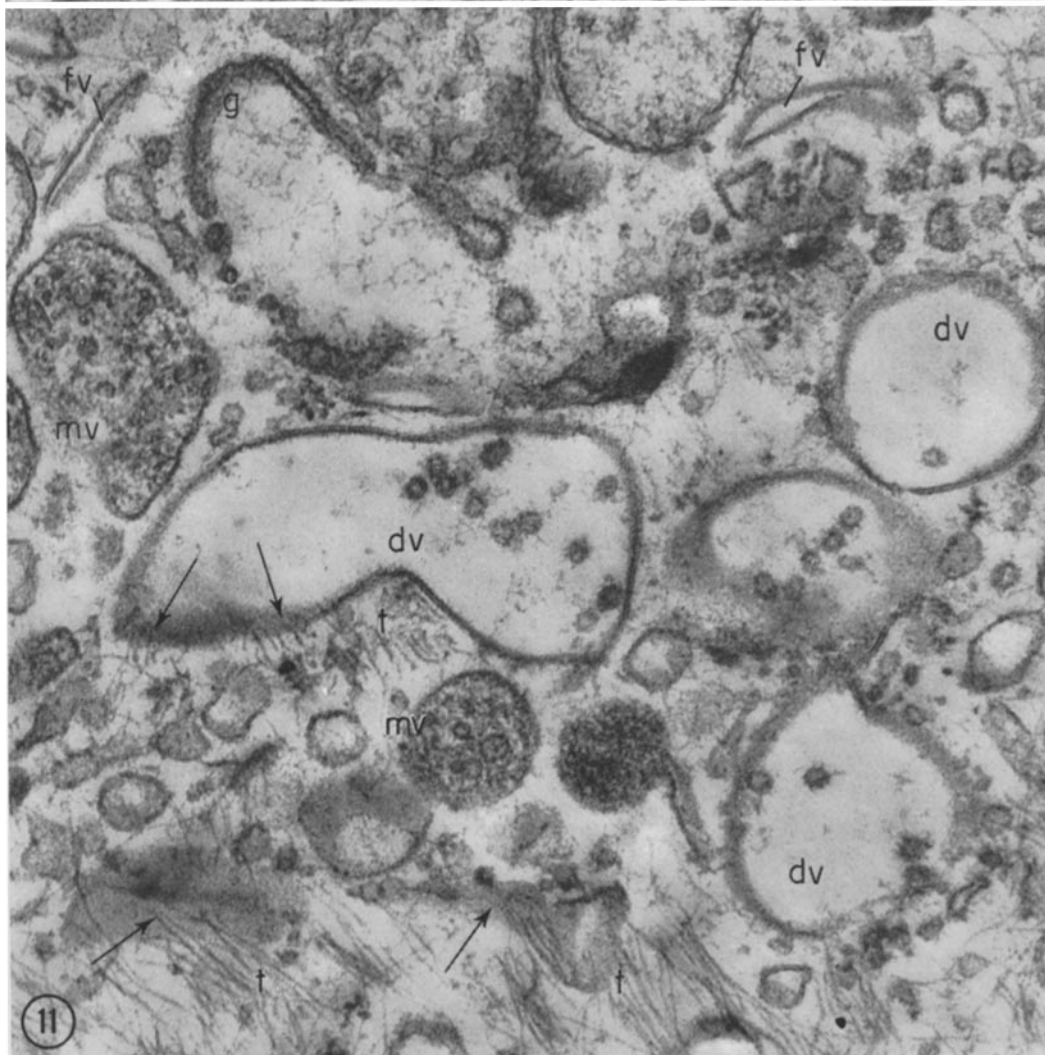
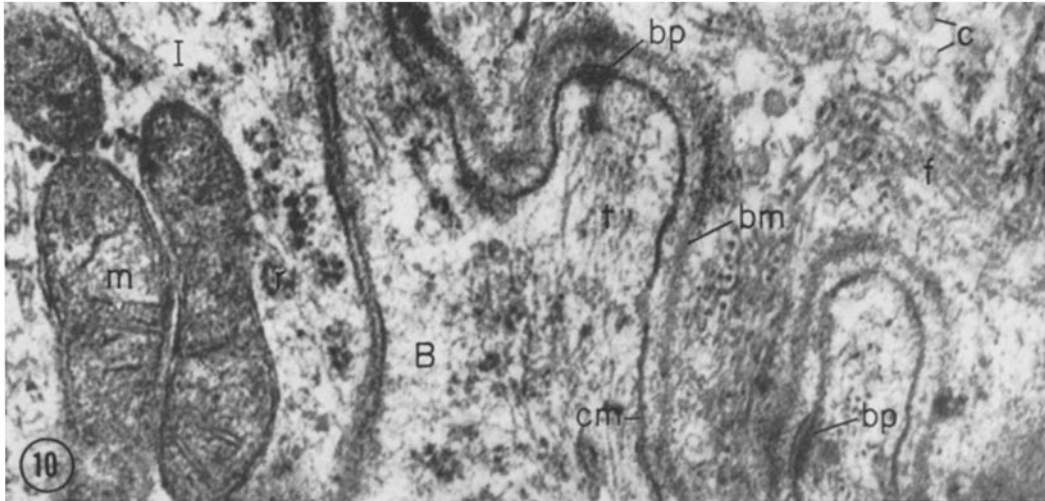
The nature of the small, 100-Å diameter, extracellular filaments seen below the basement membrane is not known, but their close proximity to the basement membrane of the ureteric epithelium and their absence from the deeper layers of the lamina propria suggest that they may play some part in anchoring the epithelium to the underlying connective tissue.

This investigation was supported by the British Empire Cancer Campaign. I wish to thank Dr. M. A. Epstein and Dr. S. J. Holt for their interest and encouragement, Mr. G. Ball for his technical assistance, and Mr. H. S. Drury for his help with the line drawings.

Received for publication, July 28, 1964.

FIGURE 10 Part of the folded cell membrane (*cm*) and underlying basement membrane (*bm*) of a basal cell (*B*) are shown. An adjacent intermediate cell (*I*) contains two mitochondria (*m*) and some free ribonucleoprotein particles (*r*). Two half desmosomes or basal attachment plates (*bp*) can be seen on the basal cell membrane, and the cytoplasm contains tonofilaments (*t*). Cross-sections of collagen fibres (*c*) and many small fibrils (*f*) are present in the subepithelial connective tissue. $\times 75,000$.

FIGURE 11 The field shows a small area of squamous cell cytoplasm which contains multivesicular bodies (*mv*), large dilated (*dv*), and fusiform (*fv*) vesicles. Tonofilaments (*t*) appear to connect with or overlap the obliquely sectioned thick membrane in some places (*arrows*). The large dilated vesicles (*dv*) contain other small, membrane-bounded vesicles; a Golgi cisterna (*g*) is present in this field. $\times 70,000$.



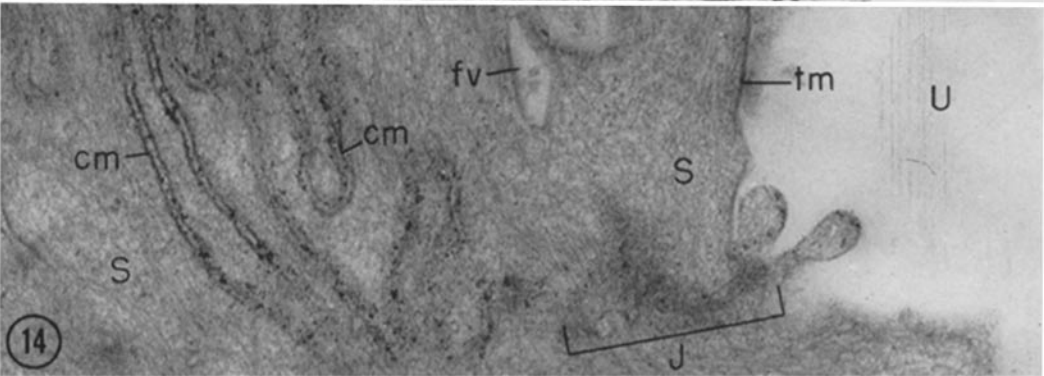
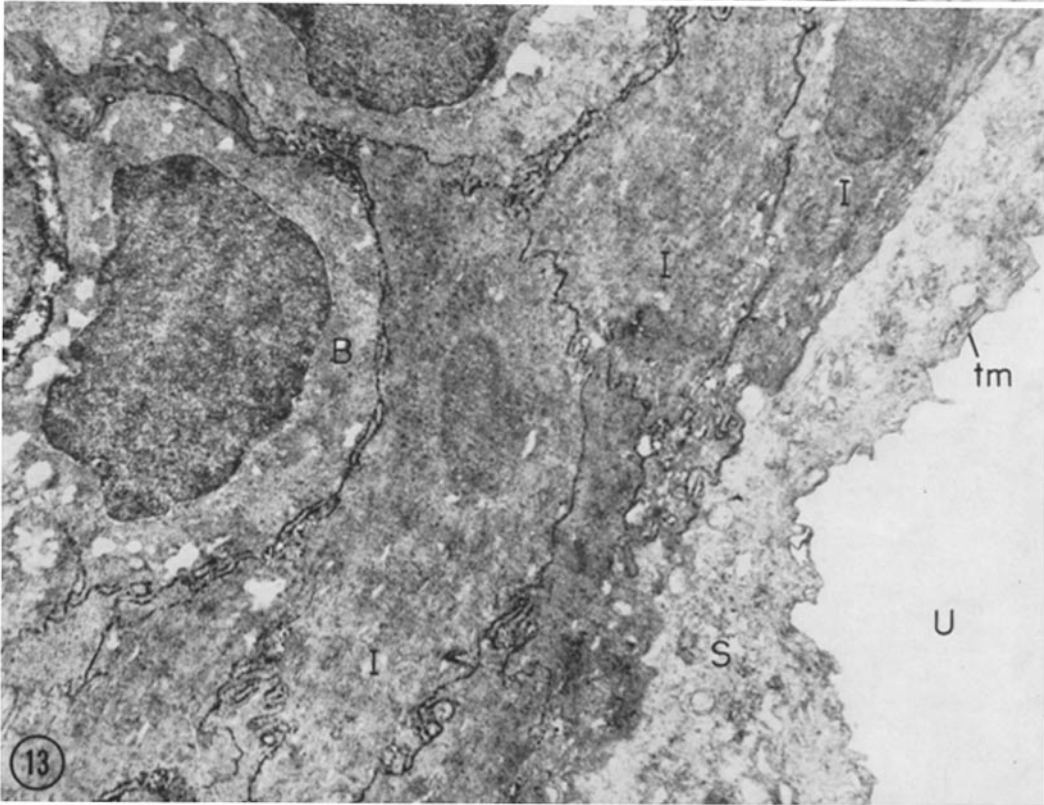
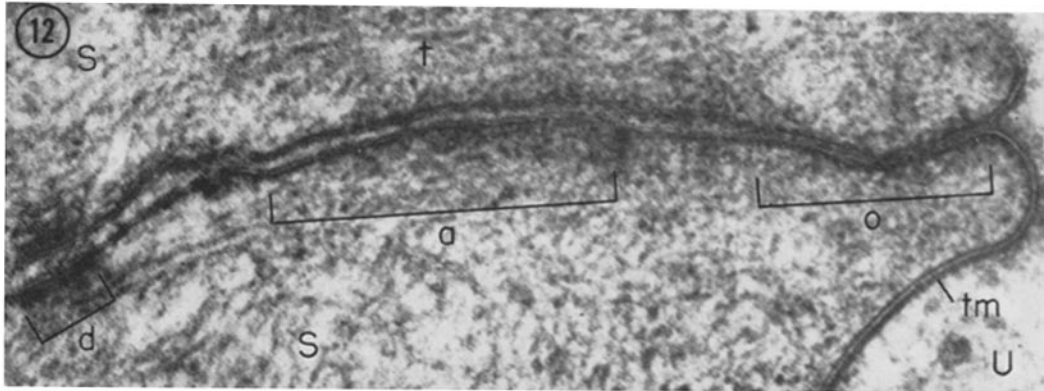
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FIGURE 12 The junctional complex formed by the lateral cell membranes of two squamous cells (*S*) bordering the ureter lumen (*U*) is illustrated here. The cell membranes are closely applied at their luminal ends to form a zonula occludens (*o*). Basal to that is a zonula adhaerens (*a*) and then a macula adhaerens or desmosome (*d*). The junctional complex is the same as that described in other epithelia by Farquhar and Palade (18). Parts of the triple-layered free cell membranes (*tm*) can be seen adjacent to the ureter lumen, and the squamous cell cytoplasm contains a thick feltwork of tonofilaments (*t*). $\times 130,000$.

FIGURE 13 The tissue illustrated in this figure was incubated in Wachstein and Meisel's adenosine triphosphate-containing medium (15) before postfixing in osmium tetroxide and embedding in Epon. Lead phosphate has been deposited between the opposing lateral and basal cell membranes of the intermediate (*I*) and basal (*B*) cells, as a result of enzymatic dephosphorylation of the substrate. The free plasma membrane (*tm*) of the squamous cell (*S*) adjacent to the lumen of the ureter (*U*) does not dephosphorylate adenosine triphosphate. Mitochondrial adenosine triphosphatase activity is not apparent in this tissue which was fixed in glutaraldehyde, but not frozen, before incubating in the cytochemical staining medium. $\times 10,000$.

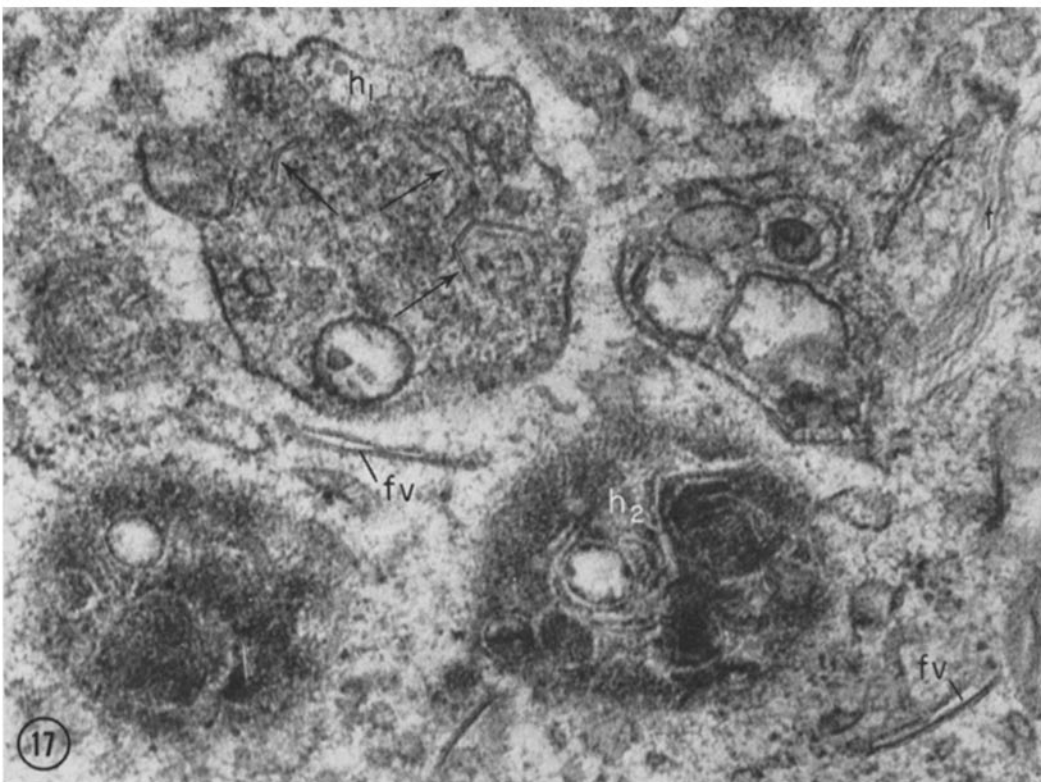
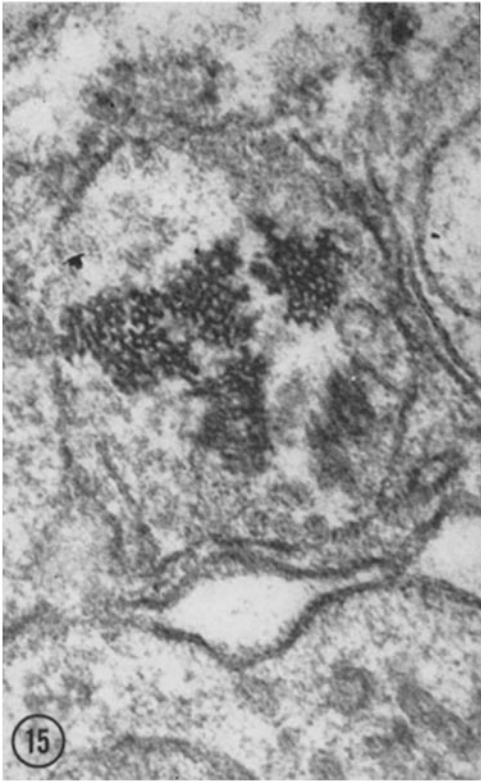
FIGURE 14 The tissue illustrated here was prepared in the same way as that shown in Fig. 12. Lead phosphate has been deposited between the opposing lateral cell membranes (*cm*) of two squamous cells (*S*), but not on the thick membrane around a fusiform vesicle (*fv*) or on the free cell surface (*tm*) adjacent to the lumen of the ureter (*U*). The area of the obliquely-sectioned junctional complex (*J*) has no significant deposit of lead phosphate associated with it. $\times 52,000$.



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FIGURES 15 and 16 These figures show two of the dense crystalline aggregates in a bundle cell at higher magnification. The bundle in Fig. 16 appears to have been sectioned in the plane of the long axis of the crystallites, while that in Fig. 15 shows a cross-section through the bundle. $\times 110,000$.

FIGURE 17 The field shows a small area of squamous cell cytoplasm containing four large bodies with polymorphic contents. At the top left of the field is a body (h_1) with an irregular profile, which contains short lengths of triple-layered membrane (arrows) in a finely granular matrix. Another body (h_2) contains small, very dense areas which are enclosed by hexagonally arranged light lines, believed to be the negative images of unit membranes. Bundles of tonofilaments (t) and fusiform vesicles (fv) are present between these bodies. $\times 65,000$.

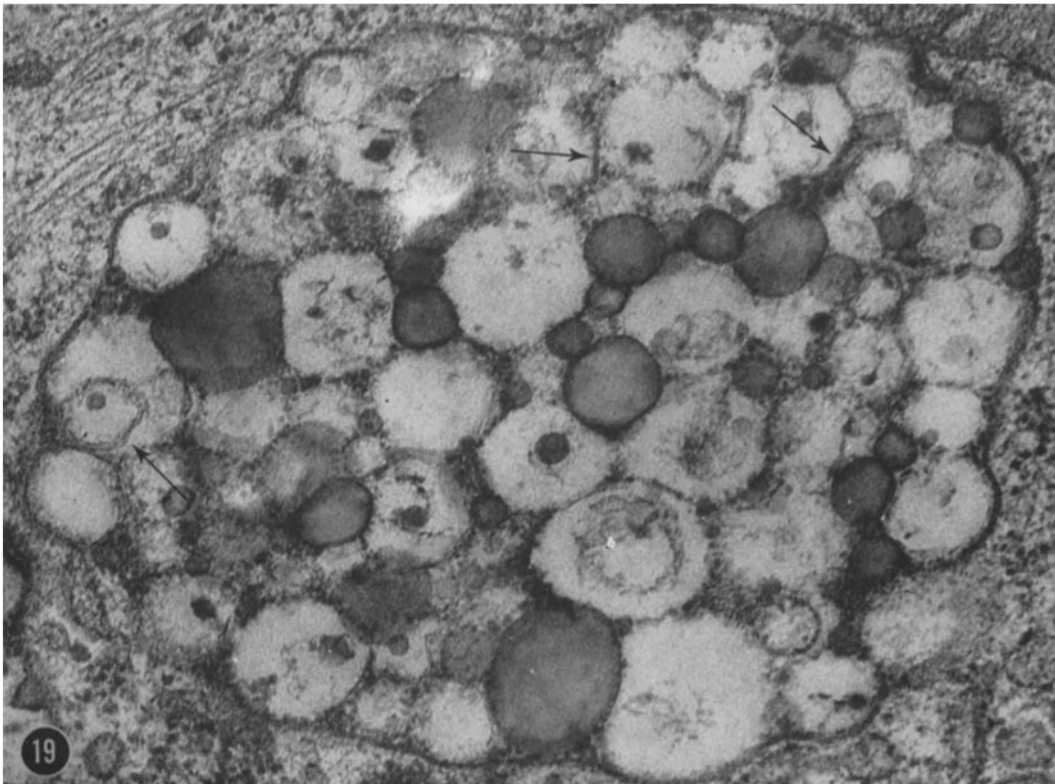
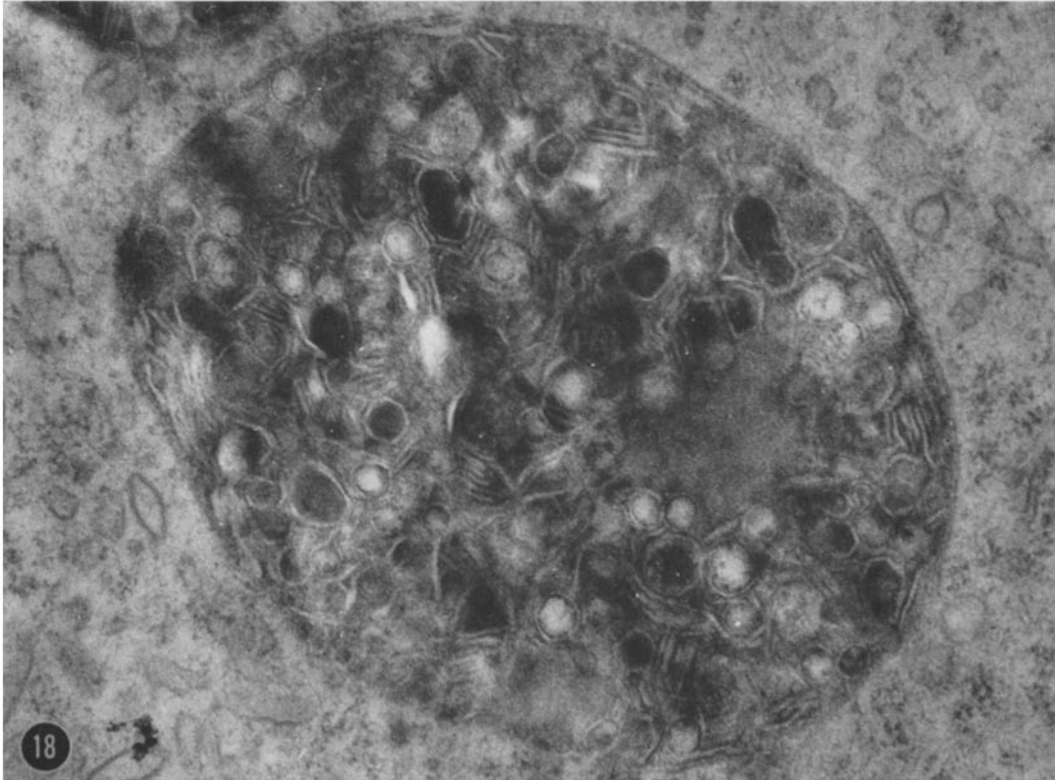


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FIGURE 18 A large dense body, about $2\ \mu$ in diameter, is shown in this figure. The light lines, thought to be negative images of unit membranes, are arranged in geometrical stacks sometimes forming hexagonal patterns around areas of variable density. $\times 60,000$.

FIGURE 19 Another large body, about $2\ \mu$ in diameter, is shown here. It contains relatively clear or empty vesicles randomly mixing with droplets having homogeneous lipid-like contents. In places fragments of triple-layered membrane (*arrows*) are present between the vesicles. $\times 75,000$.



LUMEN OF URETER

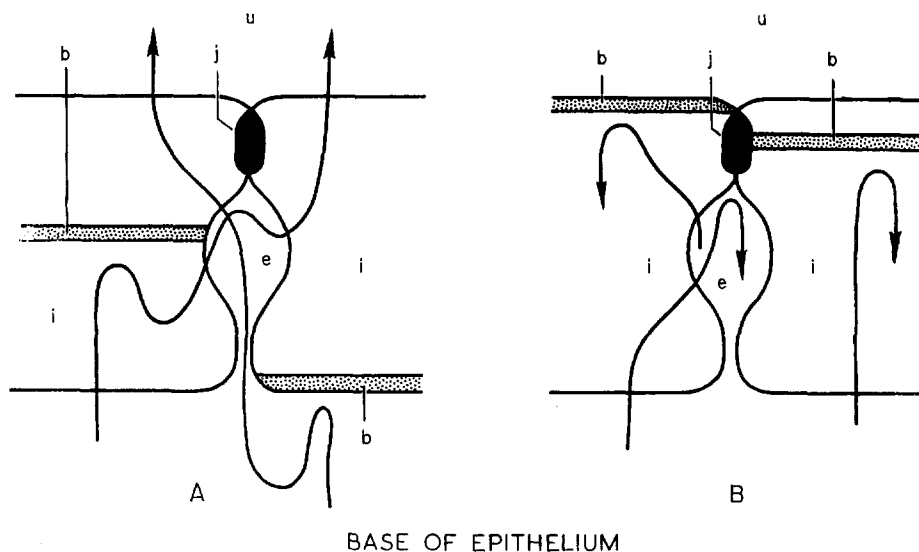


FIGURE 20 Schematic representation of a hypothetical permeability barrier located at different levels in the cell relative to the junctional complex.

A, Intracellular permeability barriers (*b*) are located basal to the junctional complex (*j*). Water from the intra- (*i*) and extracellular (*e*) fluids can bypass the barriers and be lost to the urine (*u*).

B, Intracellular permeability barriers (*b*) are located at the same level or distal to the junctional complex (*j*). There can be no loss of water across the barriers to the urine.