THE FUNCTION OF THE GOLGI COMPLEX IN TRANSITIONAL EPITHELIUM

Synthesis of the Thick Cell Membrane

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ABSTRACT

The superficial squamous cells of rat transitional epithelium are limited, on their luminal face, by an asymmetrically thickened membrane. Patches of similar thick membrane are found in the walls of the Golgi cisternae and it is suggested that the Golgi system is the site of assembly of the thick plasma membrane. This implies membrane flow from the Golgi apparatus to the cell surface, and there is indirect evidence that the membrane is transported in the form of fusiform vacuoles, derived from the Golgi cisternae, which fuse with, and become part of, the free cell membrane. Uptake of injected Imferon shows that similar, large, thick-walled vacuoles may be formed by invagination of the free cell surface. Some of these vacuoles are subsequently transformed into multivesicular bodies and autophagic vacuoles. The formation of other large heterogeneous bodies is described, and some of these are shown to have acid phosphatase activity.

INTRODUCTION

The membrane which limits the luminal face of the superficial cells in transitional epithelium acts as a barrier to water diffusion (1, 2). This membrane is unusually thick, about 115 A across, and its unit structure is asymmetric, with a thinner dense leaflet adjacent to the cytoplasm and an outer dense leaflet on the luminal face of the cell (1, 2). Its barrier function appears to depend in part upon keratin (2, 3, 4) and it has been suggested that this thick keratinized membrane is synthesized in the Golgi complex at the base of the cells (1, 4).

In other cell types, morphological polarization of the Golgi apparatus has been observed. Secretory material in membrane-bounded vesicles appears to arise from the cisternae of the concave "maturing" face of the stack, while the outer convex surface of the stack is regarded as the "forming" face, where the new cisternal membranes and secretory materials are assembled (5–8). In these instances, morphological polarity is correlated with maturation of the cisternal contents.

In this paper, an investigation of the Golgi complex in rat transitional epithelial cells is reported, and morphological evidence is presented to support the suggestion that in this tissue the maturation process affects the membrane rather than the contents of the cisternae.

MATERIALS AND METHODS

ANIMALS: Adult male and female albino rats of the Wistar strain, weighing 180 to 250 g were killed by dislocation of the neck, and the ureters and bladder were immediately removed.

FIXATION: The ureters and bladder were

sectioned into small pieces under cold, 1% (w/v) osmium tetroxide, buffered with phosphate (9). Fixation was continued for 1 hr, and the tissue was then dehydrated in ethanol and embedded in Epikote 812 (Shell Chemical Co. Ltd., London) essentially by the method of Luft (10).

CYTOCHEMISTRY: For cytochemistry, tissues were fixed for 8 hr in cold 4% glutaraldehyde buffered to pH 7.4 with 1.0 M sodium cacodylate (11), then washed for 16 hr with 0.25 M sucrose also buffered with 1.0 M sodium cacodylate to pH 7.4. Sections 50 μ thick, cut with a freezing microtome, were incubated at 37° C for 20 min in Gomori's acid phosphatase staining medium, prepared as described by Gomori (12) but with the addition of 7.5% (w/v) sucrose. After staining, the tissue was washed in cold 0.05 M acetate buffer containing 7.5% (w/v) sucrose, then postfixed for 1 hr in 1% osmium tetroxide (9), dehydrated, and embedded as above.

INJECTION OF IMFERON: Imferon, an iron-dextran complex, was supplied as a solution containing 50 mg of iron per ml, by Benger Laboratories Ltd, Holmes Chapel, Cheshire. Rats were anesthetised and the left ureter and bladder exposed. The bladder, if not already collapsed, was emptied by gentle pressure. Approximately 0.25 ml of Imferon

Key to Symbols

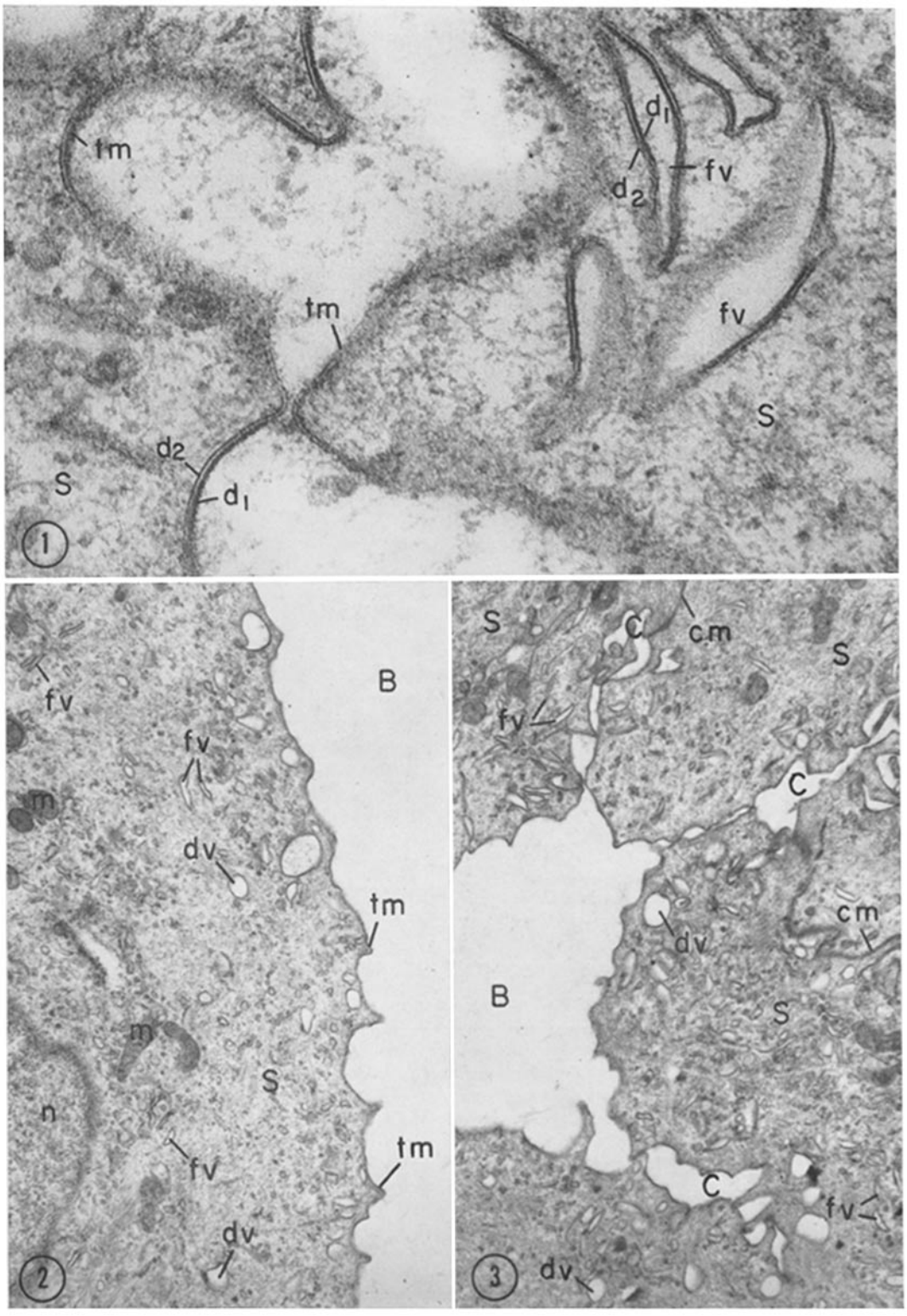
B, bladder lumen
U, ureter lumen
S, squamous epithelial cell
E, extracellular space
G, Golgi apparatus
F, proximal face of Golgi stack
M, distal face of Golgi stack
h, large heterogeneous bodies
n, nucleus
m, mitochondria
r, free ribosomes
t, tonofilaments
v, small cytoplasmic vesicles
cv, small coated vesicles
dv, dilated vacuoles
fv, fusiform vacuoles
cm, lateral or basal cell membrane
tm, thick membrane
rer, rough-surfaced endoplasmic reticulum

All photographs are electron micrographs of rat bladder or ureter epithelial cells. All except Figs. 8, 32, and 33 show tissues fixed in osmium tetroxide and embedded in Epon. Thin sections were contrast-stained with both uranyl acetate and a lead salt for 5 min each. The tissues shown in Figs. 8, 32, and 33 were fixed in glutaraldehyde before postfixing in osmium tetroxide and embedding in Epon. For Figs. 32 and 33 the tissue was stained between the two periods of fixation to show acid phosphatase activity. Sections of this material were not contrast-stained with lead or uranyl salts. The micrographs were taken with a Siemens Elmiskop 1.

FIGURE 1 The field shows the luminal edge of a squamous epithelial cell (*S*) of rat transitional epithelium. The structure of the 115 A thick unit membrane (*tm*), which limits he cell, may be seen where it is perpendicular to the plane of section. It is asymmetric and has a thick dense leaflet (d_1) on its luminal face and a thinner dense leaflet (d_2) on its cytoplasmic face. The large fusiform vacuoles (*fv*) in the cytoplasm are also bounded by asymmetrically thickened membrane, which has a thick luminal leaflet (d_1) and a thinner leaflet (d_2) on the cytoplasmic border. × 125,000

FIGURE 2 The field shows part of the luminal border of a squamous epithelial cell (*S*) in the dilated bladder, at low magnification. Although the cell is flattened and has a large, luminal surface area, the plasma membrane (*tm*) still retains some angular crests. Large, dilated (*dv*) and fusiform (*fv*) vacuoles are present in the cytoplasm. The nucleus (*n*) and a few mitochondria (*m*) can also be seen. × 12,000.

FIGURE 3 The luminal borders of three squamous cells (*S*) in the contracted bladder are shown here, at the same low magnification as Fig. 2. The lateral membranes (*cm*) separating the cells are indicated. These cells present a small surface area to the bladder lumen (*B*). The thick cell membrane is invaginated to form long channels (*C*) which extend from the bladder lumen (*B*) deep into the cytoplasm. Numerous fusiform (*fv*) and dilated (*dv*) vacuoles are present in the cytoplasm. × 12,000.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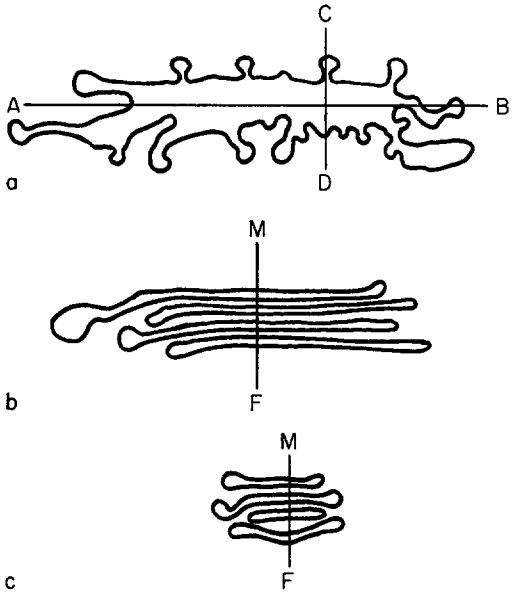


FIGURE 4 Diagrammatic line drawing of Golgi cisternae. Fig. 4 *a* shows a section in the plane of the cisterna. Fig. 4 *b* and *c* are cross-sections through stacks of cisternae cut in the directions *AB* and *CD* shown in Fig. 4 *a*. *F* marks the proximal face of the stack, and *M* the distal face.

was injected into the upper end of the ureter until the bladder was slightly distended. A clamp was placed over the lower end of the ureter before the needle was withdrawn, thus trapping some of the Imferon in the ureter. After 5 min, the ureter and bladder were injected with osmium tetroxide and removed for further fixation and preparation for electron microscopy.

ELECTRON MICROSCOPY: Sections showing silver-to-gold interference colors were cut with glass knives on a Porter-Blum microtome, mounted on copper electromesh grids, double contrast-stained (1) with uranyl acetate (13) and a lead salt (14), and examined in a Siemens Elmiskop 1. Sections of tissue which had been stained to show acid phosphatase activity were not contrast-stained with lead or uranyl salts.

OBSERVATIONS

The Plasma Membrane

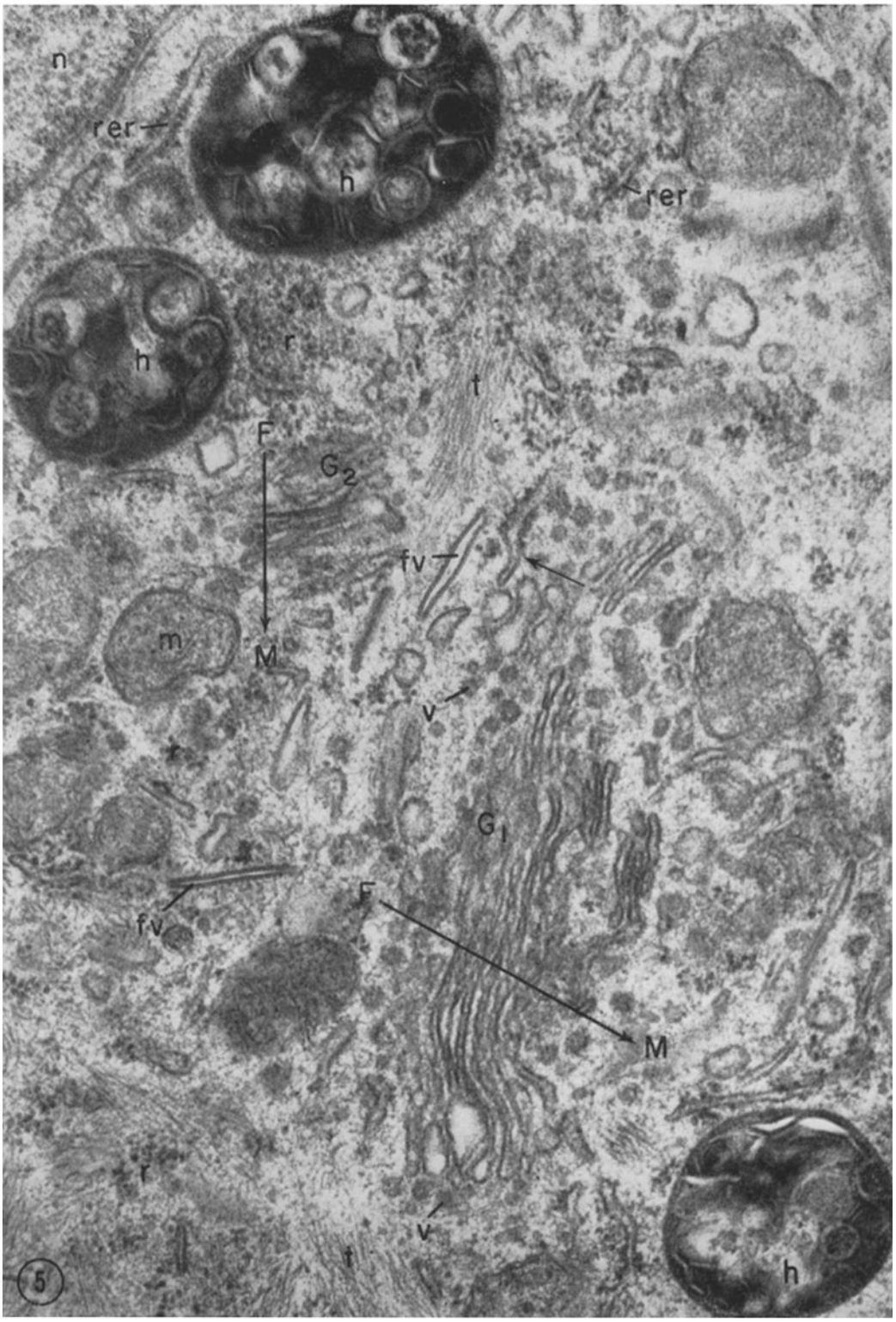
The squamous cell is limited on its luminal face by an asymmetrically thickened unit membrane measuring 115 A across (Fig. 1). The thick lamina of this unit membrane is on the luminal face, as has been reported previously (1, 2). In the dilated bladder, the squamous cell presents a large, relatively flat, luminal surface but the membrane, because of its rigidity (1), still maintains some angular ridges or crests on the face of the cell (Fig. 2). In the contracted bladder, the area of the luminal face of the squamous cell is greatly reduced, and the membrane infolds to form channels which extend deep into the cytoplasm (Fig. 3).

The Golgi Apparatus

As in other cell types (15) the Golgi complex in squamous epithelial cells consists of cisternae and associated small vesicles. In these cells, the small vesicles are 50 to 90 mμ in diameter. Some are actually attached to cisternae while others appear free, although they too may have connections beyond the plane of section (Fig. 6).

The Golgi apparatus occupies a juxtanuclear position near the base of the squamous epithelial cells. Each Golgi stack may be likened to a pile of flattened bags of irregular shape from which small evaginations, or blebs, form at the periphery (Fig. 4). In Fig. 5, two stacks of Golgi cisternae are cross-sectioned, one in the direction which

FIGURE 5 The field shows an area at the base of a squamous epithelial cell at a relatively low magnification. Part of the nucleus (*n*) is shown at the top left, and the free cell surface (not illustrated) lies diagonally opposite it, beyond the lower right corner of the field. Two stacks of Golgi cisternae are shown, one (G_1) cross-sectioned in the direction corresponding to *AB* in Fig. 4, and the other (G_2) in the direction *CD*. The arrows across the stacks mark the direction of maturation from the proximal face (*F*) to the distal face (*M*). Large, heterogeneous, dense bodies (*h*), fusiform vacuoles (*fv*), tonofilaments (*t*), mitochondria (*m*), and clumps of free ribosomes (*r*) are present in the cytoplasm. There are a number of short lengths of rough-surfaced endoplasmic reticulum (*rer*), and in one place (arrow) a rough-surfaced cisterna is continuous with a smooth cisterna. Around the stacks of Golgi cisternae are many small vesicles (*v*). × 50,000.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corresponds to *AB* in Fig. 4, and the other in the direction *CD*. In Fig. 6, two stacks of Golgi cisternae may be seen, one of which has been sectioned in the plane of the cisterna and shows an approximate plan view, while the other shows a cross-section in the direction *AB*. In plan view, the cisternae appear to be elongated rather than spherical.

The stacks are mostly orientated with their long axes (*AB* in Fig. 4) roughly parallel to the free cell surface, or, if the stack is cup-shaped, with the concave face towards it. For convenience, the stack may thus be said to have a proximal face (*F*) towards the foot or base of the cell and a distal face (*M*) towards the luminal surface. The orientation is marked in this way in Figures 4, 5, 7, 9, and 12.

The cisternal membranes are morphologically heterogeneous. In places they have a normal 75 A thick unit structure without any obvious specialization (Figs. 6 to 8). In other places they are modified and thickened so that the unit structure changes from 75 A to over 100 A thick (Figs. 7, 8, and 12). This thickening is multifocal both within the stack and in individual cisternae. Thus, more than one cisterna may be thickened (Figs. 9 and 12) and the thickening may not extend the full length or involve both faces of the same saccule (Figs. 7, 9, and 12). The thickening is more marked in those cisternae towards the distal face (*M*) of the stack than in those at the proximal face (*F*) (Figs. 9 and 12). At high magnification the thickening in some places appears to be asymmetric, so that one leaflet of the unit membrane structure is thicker than the other (Fig. 10, cf. d_1 and d_2). These thick membranes of the Golgi cisternae are thus comparable to the thick, asymmetric membranes of the fusiform vacuoles and free cell surface (Fig. 1), which have also been described elsewhere (1). The patches of thickened membrane are found most frequently in the main body of the cisternae, rather than in the peripheral blebs which are formed of normal membrane (Figs. 6, 7, 9, and 12). From some aspects, the thick-walled cisternae look remarkably like fusiform vacuoles and, indeed, can only be recognized as elements of the Golgi system by their arrangement in stacks and association with other small vesicles (Figs. 13 and 14).

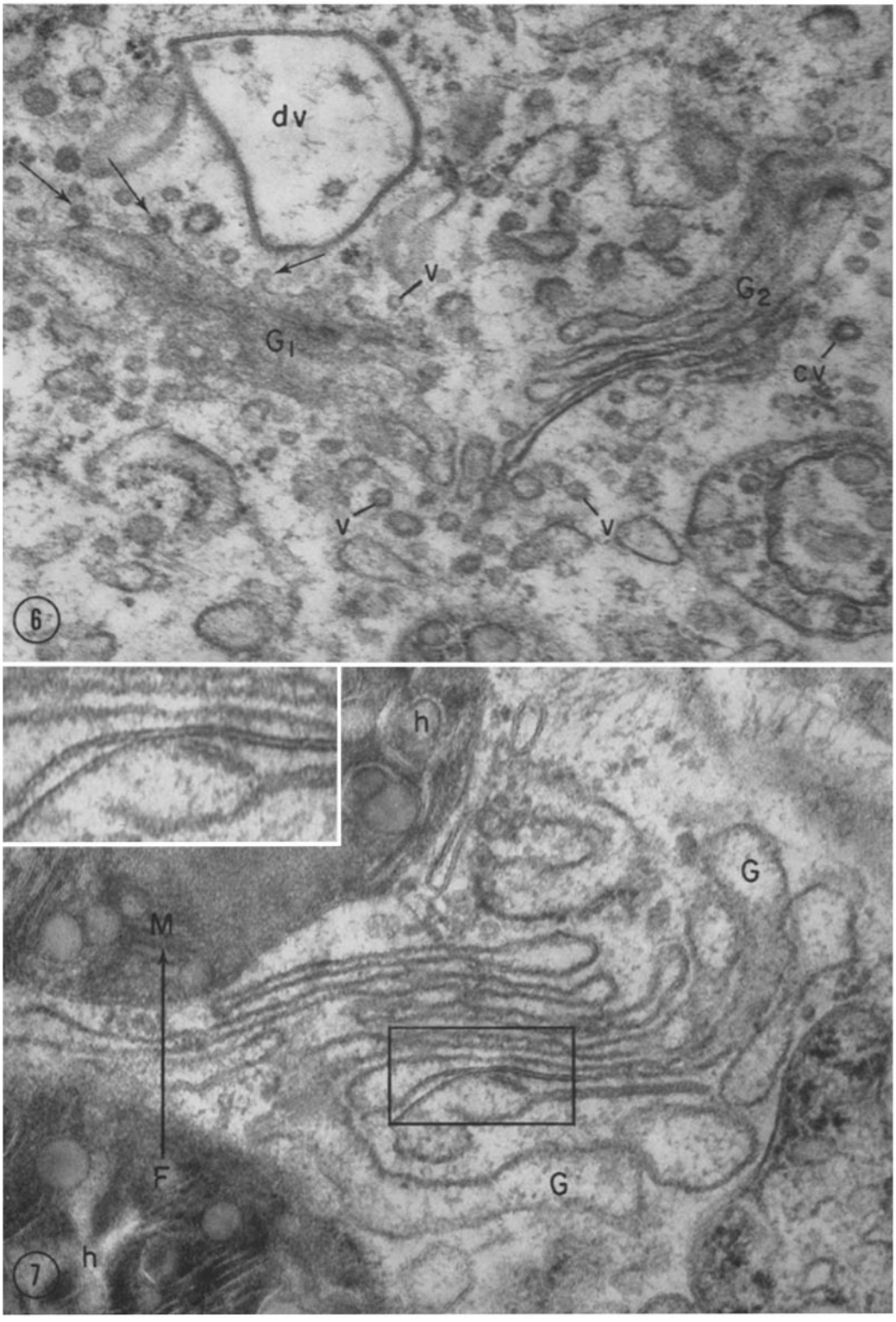
Thick-Walled Cytoplasmic Vacuoles

The squamous cell cytoplasm contains a variety of large, angular vacuoles, bounded by asymmetrically thickened unit membrane, measuring 115 A across. The structure of this membrane, which has a thick lamina on its luminal face, appears to be the same as that of the membrane which limits the luminal face of the squamous cell (Fig. 1). For convenience in description these thick-walled vacuoles may be subdivided into various groups, based on their morphological appearance.

FUSIFORM VACUOLES: These are characteristically flattened and empty (Figs. 1 to 3, 5, 15, 17, 22, 27, and 31) and have been described previously as being fusiform or discoidal (1, 16, 17). They occur throughout the squamous cell cytoplasm, between the Golgi complex at the base of the cell and the luminal border. Fusiform vacuoles are frequently observed with their limiting membranes continuous with the

FIGURE 6 The field shows a portion of the cytoplasm at the base of a superficial squamous cell. The section passes in the plane of a Golgi cisterna (G_1) at the left of the field, which thus presents an approximate plan view comparable to Fig. 4 *a*. The stack of cisternae (G_2) at the right of the field has been sectioned perpendicularly, in approximately the same direction as that illustrated in Fig. 4 *b*. Many small vesicles (*v*) lie around the Golgi stacks, and some of them are attached to a cisterna (arrows). To the right of the field is a coated vesicle (*cv*) which may be distinguished from the Golgi vesicles (*v*) by its distinct outer halo of fibrillar material. A large dilated vacuole (*dv*), containing a few small vesicles and sparse, fibrillar material, lies at the top of the field. × 70,000.

FIGURE 7 The stack of Golgi cisternae illustrated here lies at the base of the cell near two large heterogeneous bodies (*h*). The field shows part of the cytoplasm between the nucleus and the base of the squamous cell. The end portions of the cisternae (*G*) are dilated and contain a little fine granular material. The central portions lie parallel in the stack and are cross-sectioned here. Part of the membrane of the central portion of one cisterna is thicker than the rest (in box). The inset, top left, shows an enlargement of this area. The thick portion of the membrane has an easily resolved unit structure, with a wide, central light lamina, but the thickening is not asymmetric in this instance (cf. Fig. 10). Because of the position of the stack in the cell and the fact that only one of its cisternae is thickened, this is regarded as an immature Golgi complex. × 100,000; inset, × 170,000.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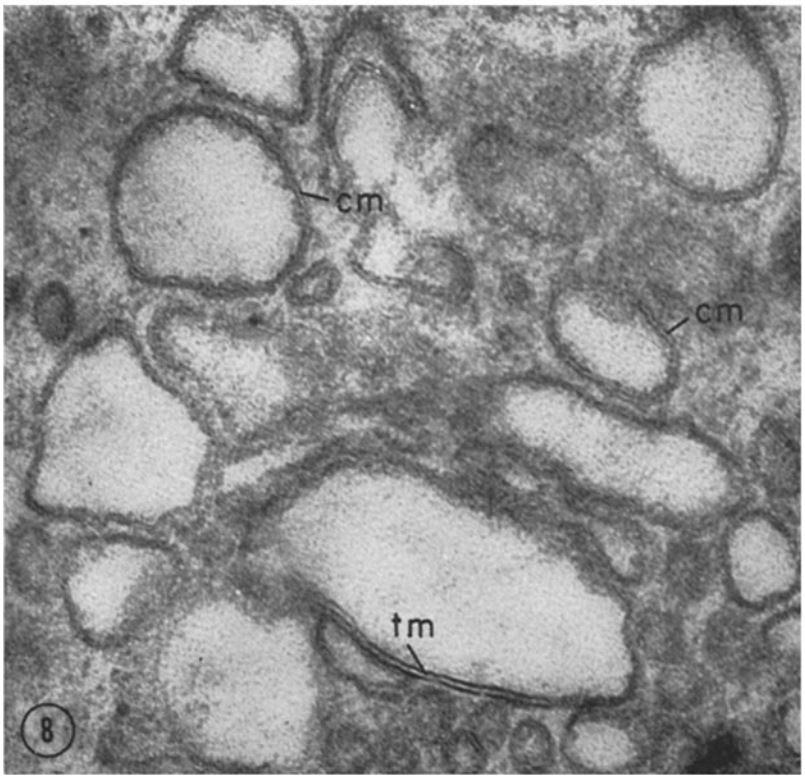
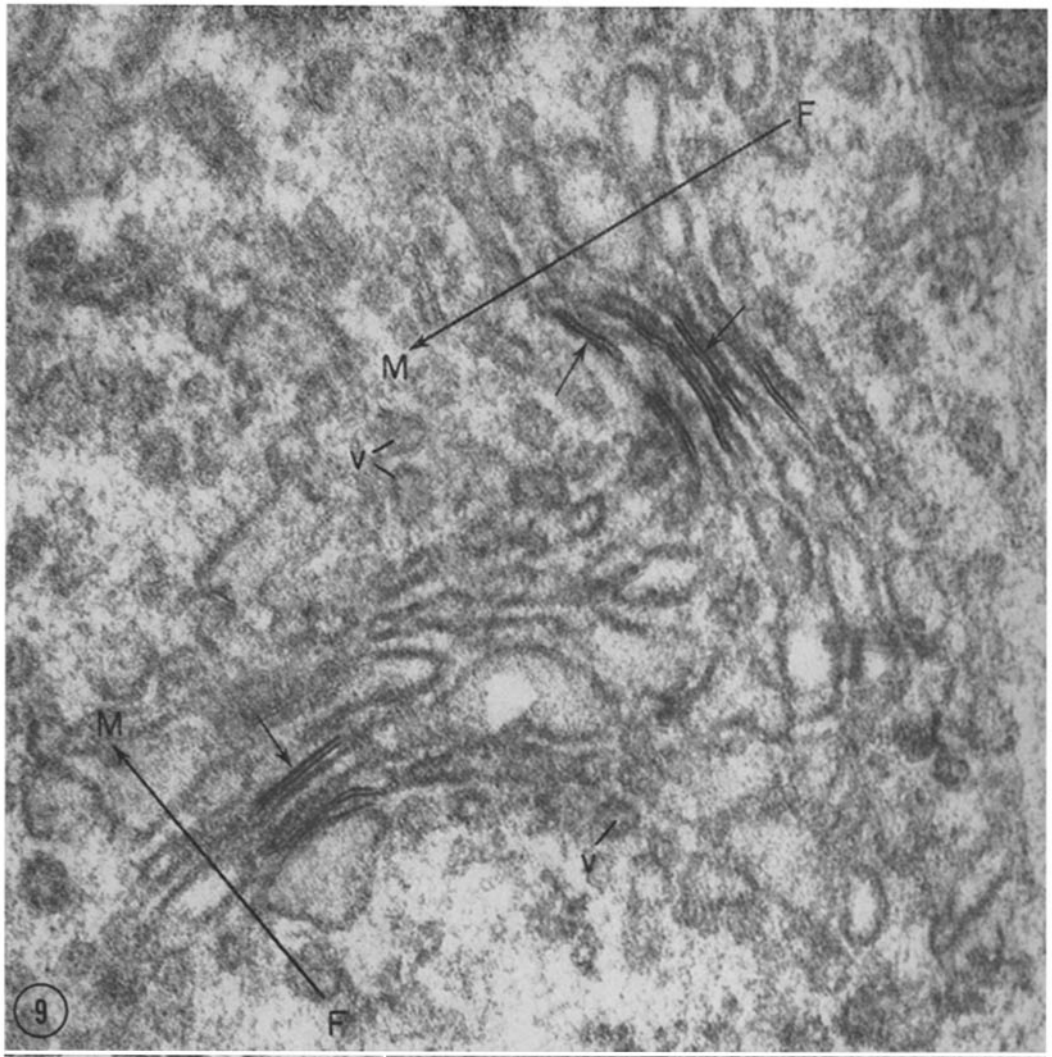


FIGURE 8 The field shows a cross-section through a stack of Golgi cisternae. The tissue was fixed in glutaraldehyde and, as a result, the cisternae appear more dilated than they do after osmium tetroxide fixation. The structure of the normal 75 A thick unit membrane (*cm*), which forms most of the cisternae, is resolved, and it can be seen that the wall of one saccule has a thickened patch (*tm*) where the cross-section of the membrane is about 100 A across. × 145,000.

FIGURE 9 The Golgi complex illustrated here is composed of two stacks of parallel cisternae and their associated vesicles (*v*). In both stacks, thickened membrane (short arrows) can be seen in the central portion of some of the cisternae. The direction of maturation of each stack from the proximal face (*F*) to the distal face (*M*) is marked by the long arrows. × 130,000.

FIGURE 10 A portion of Fig. 9 is shown here at higher magnification. The unit structure of the thickened cisternal membrane is now seen to be asymmetric, with one dense leaflet (d_1) of the trilaminate structure thicker than the other (d_2). × 240,000.

FIGURE 11 In this field, a fusiform vacuole (fv_1) is in contact with the luminal surface of a squamous cell. The lumen of this vacuole is open to the bladder lumen (*B*), and the thick cell membrane (*tm*) is continuous with the membrane of the vacuole. A second vacuole (fv_2) is close to the surface of the cell and may be in contact with it beyond the plane of section. × 65,000.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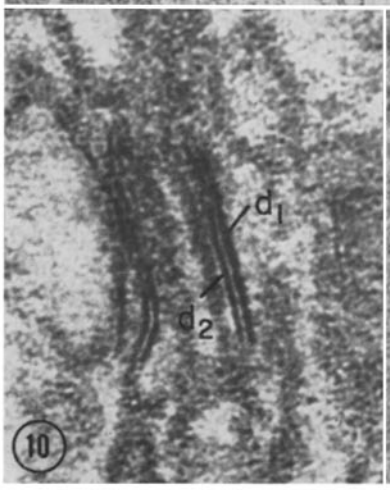
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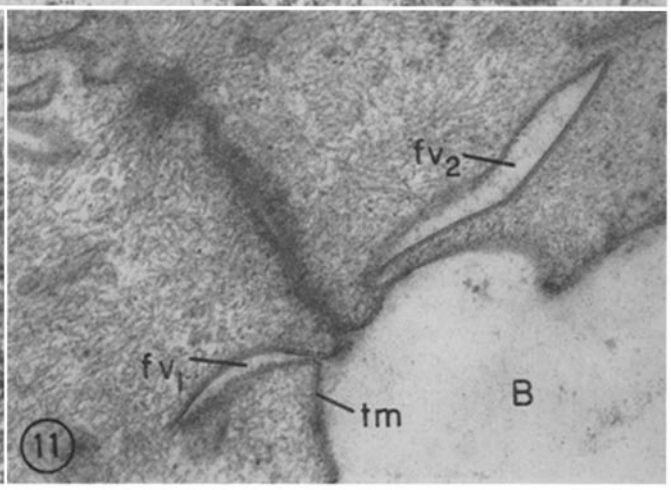
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membrane of the cell surface, and their lumen open to the bladder or ureter lumen through a narrow neck (Fig. 11). They are more numerous in the contracted (Fig. 3) than in the dilated (Fig. 2) bladder epithelium.

DILATED VACUOLES: These are large, irregularly shaped vacuoles which also have angular profiles, but which are larger than fusiform vacuoles (Figs. 2, 3, 6, 15 to 17 and 30). They are dilated and may either be empty or contain smaller, circular profiles of normal membrane, 60 mμ in diameter (Figs. 6, and 15 to 17). The small vesicles which they contain may arise as invaginations of a portion of the wall of the vacuole itself. Alternatively they may enter the vacuole from the cytoplasm; in some sections, small vesicles may be seen in the cytoplasm around (Fig. 15) or in contact with (Fig. 16) a dilated vacuole, while in other instances a small vesicle within the dilated vacuole is in contact with the vacuole wall (Fig. 17). Some dilated vacuoles contain, in addition to small vesicles, other cytoplasmic organelles such as fusiform vacuoles, endoplasmic reticulum, and short lengths of membrane (Figs. 18 and 19), and these dilated vacuoles could be designated autophagic, dilated, thick-walled vacuoles.

MULTIVESICULAR BODIES: Roughly spherical bodies are seen which contain small vesicles and an irregularly dense matrix (Fig. 20). Their limiting membrane, although thick, does not appear to be rigid, for the profile is spherical rather than angular (1). It is possible that these bodies arise from multivesicular dilated vacuoles, since intermediary morphological types may be found. In one such intermediary structure, the limiting membrane is angular but the internal vesicles are closely packed in a dense matrix (Fig. 21). Moreover, the limiting membrane, although observably thick, has lost its sharp definition by comparison with the thick asymmetric membrane of an adjacent fusiform vacuole (Fig. 20). The existence of such transitional forms between dilated vacuoles and multivesicular bodies suggests that no sharp distinction can be made between these structures.

Endoplasmic Reticulum

Short lengths of rough- and smooth-surfaced endoplasmic reticulum are present in the cytoplasm at the base of the squamous cells (Figs. 5, 15, 24, and 31). In places, the membrane of a rough-surfaced cisterna may be seen to be continuous with that of a smooth cisterna (Fig. 5). The rough-surfaced endoplasmic reticulum in this tissue is not arranged in stacks such as are seen in the protein-secreting cells of the liver or pancreas.

Coated Vesicles and Vacuoles

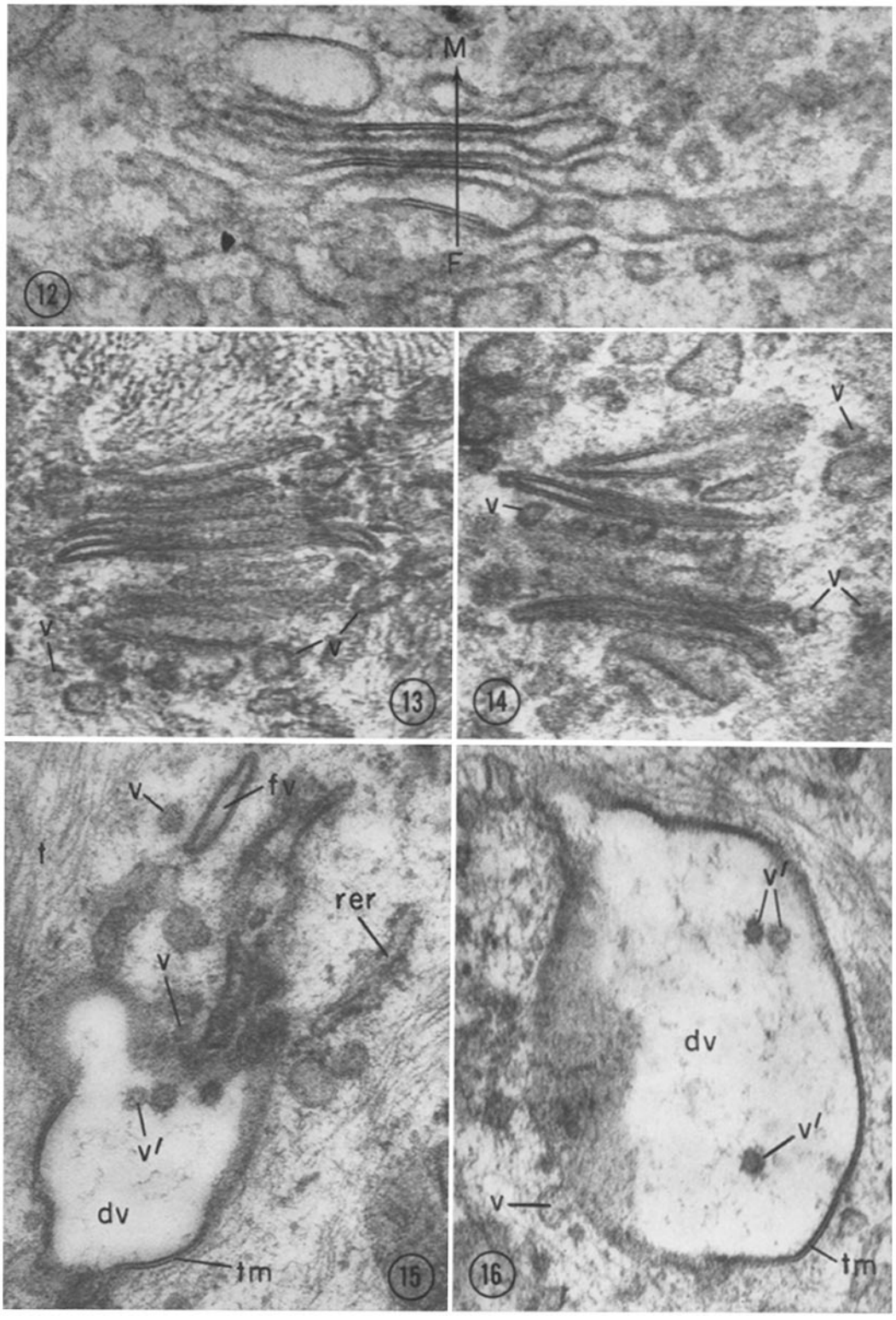
Irregular vacuoles are found in the squamous cells, of which the membrane is coated with a halo of fine fibrillar material (Fig. 22). They occasionally occur in groups, as illustrated, but are more frequently seen singly. Coated membrane also forms small cytoplasmic vesicles (Figs. 23 and 24), which may be observed throughout the cytoplasm (Figs. 6 and 29). Where these occur in the same cytoplasmic area as the Golgi complex, they may be distinguished from the normal small Golgi

FIGURE 12 A cross-sectioned stack of parallel Golgi cisternae is shown here. The cisternal membranes are thickened in a number of places, but the thickening does not extend the full length or involve both faces of every saccule. × 100,000.

FIGURES 13 and 14 Two fully matured stacks of Golgi cisternae are shown in these two figures. The parallel saccules now have the appearance of thick-walled, fusiform vacuoles and may only be recognized as Golgi cisternae by their arrangement in a stack and close association with small vesicles (*v*). The latter are presumed to have been derived from the terminal, unthickened portions of the same cisternae. × 100,000.

FIGURE 15 A large dilated vacuole (*dv*) with an angular profile and an asymmetrically thickened membrane (*tm*) is shown. In the cytoplasm nearby are small vesicles (*v*) which are similar in appearance to those (*v'*) within the dilated vacuole. Also in the field are a fusiform vacuole (*fv*), tonofilaments (*t*), and a short length of rough-surfaced endoplasmic reticulum (*rer*). × 80,000.

FIGURE 16 A large dilated vacuole (*dv*) containing three small vesicles (*v'*) is shown. Its thick, asymmetric membrane (*tm*) appears to be continuous with the normal membrane of a small cytoplasmic vesicle (*v*). At this point, the thick wall of the vacuole is sectioned tangentially and the unit structure cannot be seen. × 80,000.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vesicles by their coating (Fig. 6). Coated vesicles were not observed in contact with thick-walled Golgi cisternae. The same coated membrane is found as invaginations of the lateral and basal plasma membranes of these cells (Fig. 25).

Other Membrane-Bounded Polymorphic Structures

Membrane-bounded structures, characterized by their polymorphic shapes and dense granular contents, are regularly found in the squamous cell cytoplasm (Figs. 26 and 27). They are limited by membranes which are approximately 100 A thick but which do not appear to be either rigid or asymmetric, unlike the membranes of the thick-walled cytoplasmic vacuoles, Golgi cisternae, and free cell surface. These structures may be tubular, spherical, or bent into a variety of bizarre shapes (Figs. 26 and 27), and they may represent transitional stages in the formation of the large, dense, heterogeneous bodies illustrated in Fig. 5 and described in greater detail elsewhere (1). For example, in Fig. 27 a tubular form lies around a group of circular profiles, while in Fig. 28 an area of cytoplasm is completely enfolded by a dense saccule to form a heterogeneous body (h_1). The arrangement of membranes within another body (h_2) in the same field suggests its formation by a similar mechanism (Fig. 28). In this way a transitional series, based purely on morphological appearance, can be constructed from h_1 in Fig. 27 and h_1 and h_2 in Fig. 28, through h_1 and h_2 in Fig. 29, and h_2 and h_3 in Fig. 27, to the large heterogeneous bodies in Fig. 5.

Uptake of Imferon from the Bladder or Ureter Lumen

After injection of Imferon into the bladder or ureter, the material was observed inside both the fusiform and dilated thick-walled vacuoles even though the tissue had been fixed only 5 min after the Imferon injection (Fig. 30). Imferon-containing vacuoles were found throughout the cytoplasm, even near the base of the cells (Fig. 31).

Acid Phosphatase Activity in the Superficial Squamous Cells

After staining transitional epithelium by the Gomori technique (12) for acid phosphatase activity, the reaction product, lead phosphate, was observed in some but not all of the large, dense, heterogeneous bodies (Figs. 32 and 33). No re-

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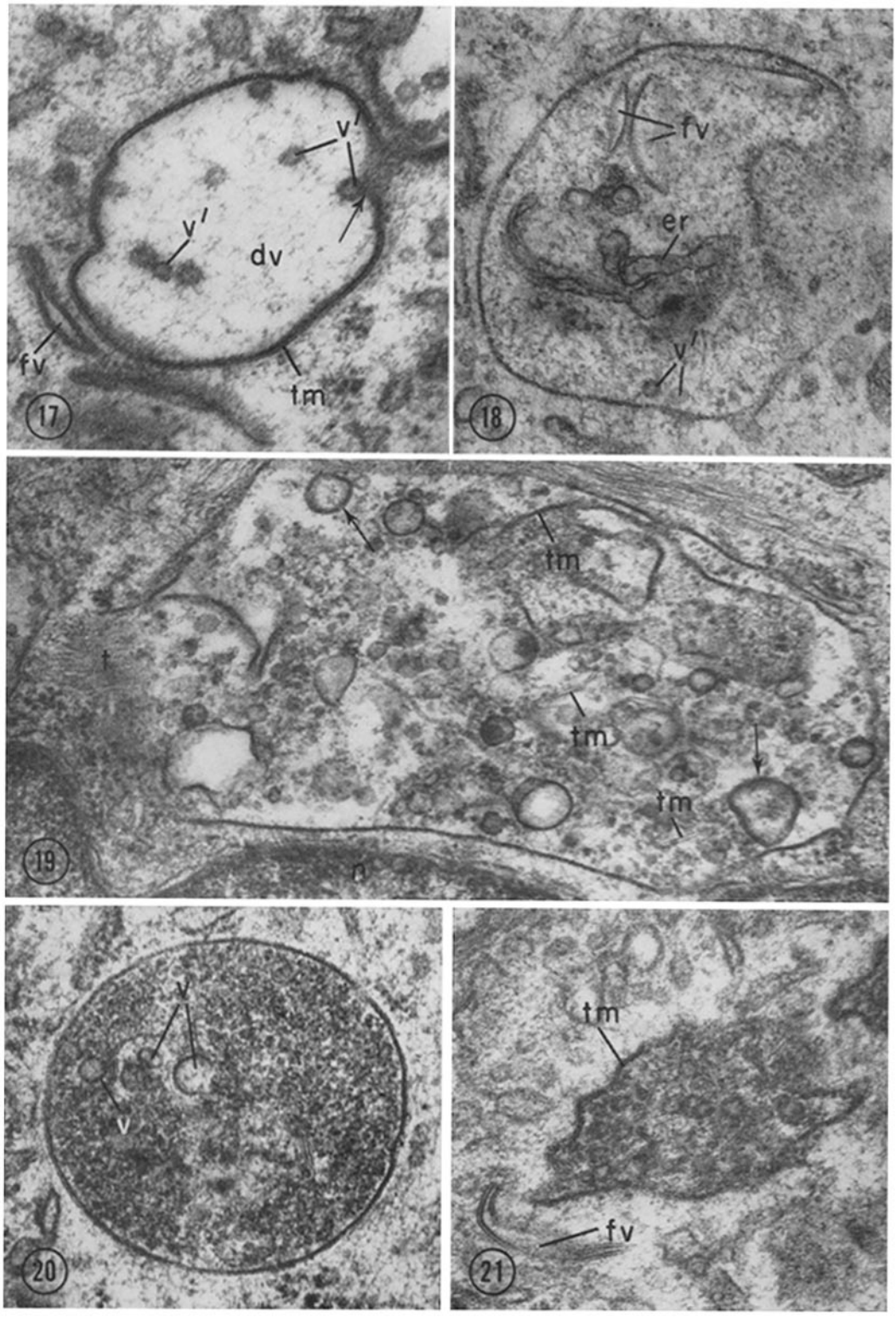
FIGURE 17 In this field, the membrane of one of the small vesicles (*v'*) within a large, angular, dilated vacuole (*dv*) appears to be in contact (arrow) with the thick membrane (*tm*) of the vacuole. A fusiform vacuole (*fv*) is also included in this field. × 80,000.

FIGURE 18 This figure shows, at a fairly low magnification, a thick-walled vacuole which contains small vesicles (*v'*), two fusiform vacuoles (*fv*), and what appear to be tubules of smooth-surfaced endoplasmic reticulum (*er*). × 50,000.

FIGURE 19 Close to the nucleus (*n*), a very large, thick-walled, dilated vacuole with an angular profile is shown. The vacuole contains many spherical profiles of different diameters, some of which (arrows) are composed of thick membrane. Short, straight lengths of thick membrane (*tm*) which appear attenuated and less dense than normal may also be seen. An irregular granular material lies between the membranous structures. × 55,000.

FIGURE 20 This figure shows a multivesicular body with a roughly spherical profile. It differs in this respect from the angular appearance of the thick-walled, dilated vacuoles illustrated in Figs. 15 to 17. It contains small vesicles (*v*) which are surrounded by a dense, granular matrix. × 70,000.

FIGURE 21 The structure illustrated here is thought to be at an intermediate stage of development between a dilated vacuole and a multivesicular body. Like the multivesicular body shown in Fig. 20, its matrix is dense and granular and it also contains small vesicles, but, like the dilated vacuoles in Figs. 15 to 17, it has an angular profile and its wall is composed of thick membrane (*tm*). However, this membrane is not so clearly asymmetric as that of the adjacent fusiform vacuole (*fv*). × 75,000.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action product was observed in the Golgi complex or other cytoplasmic organelles.

DISCUSSION

The concept of polarity of the Golgi complex, proposed for plant cells by Mollenhauer and Whaley (5), has been developed and extended to plasma cells and epithelial cells of Brunner's glands and epididymis (6) and to rat liver parenchymal cells (7, 8). In these cells, the Golgi apparatus is described as having an incoming or "forming" face at which new cisternal membranes and contents are assembled, and an outgoing or "mature" face from which vesicles which may contain secretory material are formed.

A different type of functional polarity is reported for the Golgi apparatus of polymorphonuclear leukocytes (18) in which two types of secretory granule are produced, one from each of the two faces of the Golgi complex. In this instance there can be no flow of material across the stack from a "forming" to a "maturing" face, but rather functional specialization of individual cisternae (18).

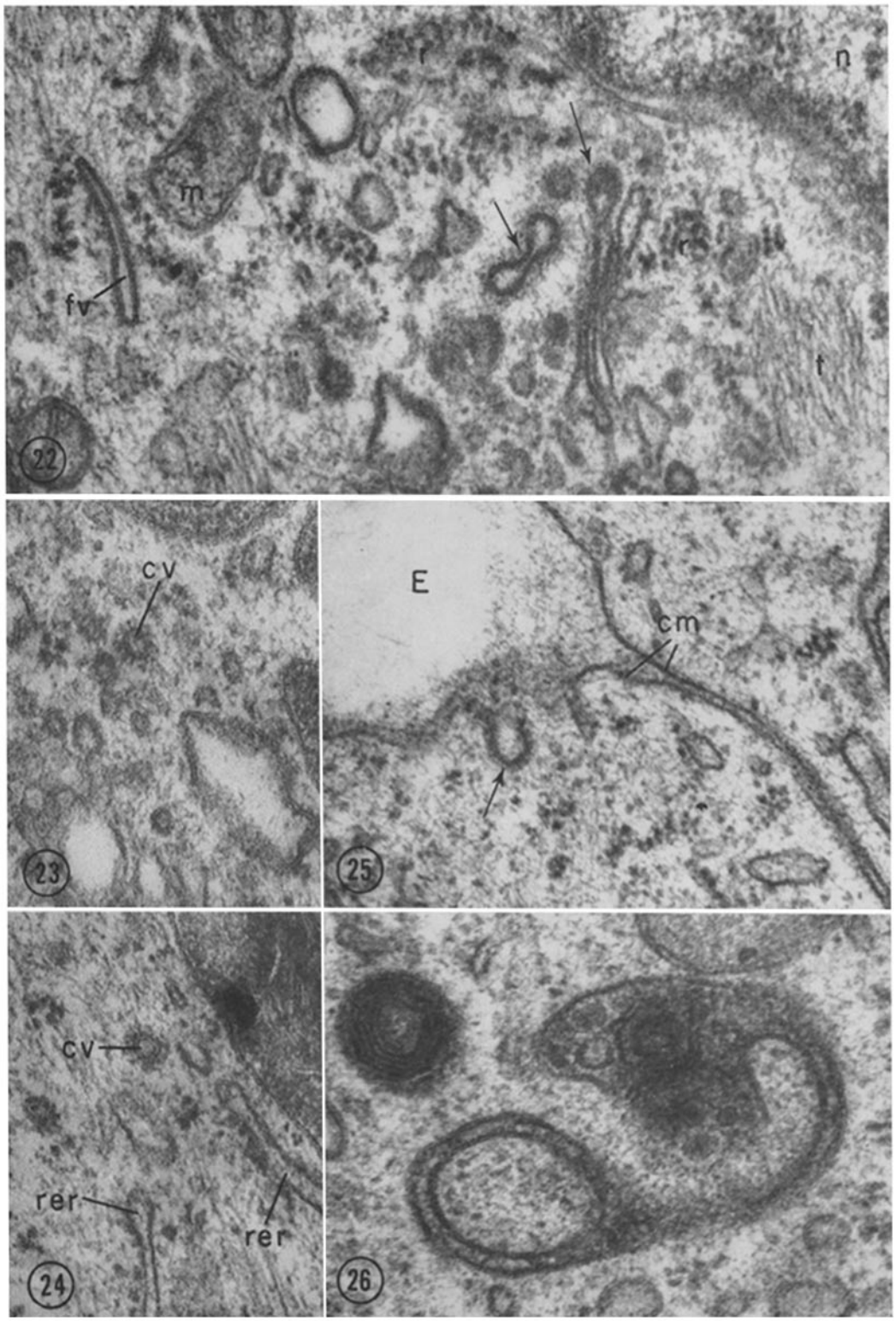
The Golgi apparatus of the superficial squamous cells in rat transitional epithelium exhibits yet another type of morphological and functional specialization. In these cells there is no biochemical or morphological evidence for a secretory function. It is possible, however, to detect a morphological change in the stacks of cisternae between the proximal and distal faces. The membranes of the Golgi cisternae undergo a multifocal transformation in which the unit structure changes from 75 A to about 115 A thick (Figs. 7 to 9, and 12). The maturation or thickening of the membrane appears more frequently in the cisternae near the distal face than in those near the proximal face of the stack. The patches of thickened cisternal membrane may sometimes be seen to have an asymmetric unit structure (Fig. 10), comparable to that of the membranes of the fusiform vacuoles and luminal cell surface (Fig. 1). In some stacks, the individual thickened cisternae exhibit a striking resemblance to thick-walled, fusiform cytoplasmic vacuoles (Figs. 13 and 14). It is concluded that free fusiform vacuoles are formed directly from the asymmetrically thickened body of each Golgi saccule, as small vesicles of normal membrane pinch off from the periphery. Fusiform vacuoles are also seen near the luminal border of the cell, and in places may be observed attached to the cell surface so that their membranes are continuous and the lumen of the vacuole connects with that of the bladder or ureter (Fig. 11). The thick lamina of the vesicle membrane is then continuous with the thick lamina on the luminal face of the cell membrane.

FIGURE 22 An area of the cytoplasm at the base of a superficial squamous cell, adjacent to the nucleus (*n*), is shown in this figure. Two vesicles or tubules, composed of membrane coated with a fine, fibrillar material (arrows) lie near the nucleus. Also present in this field are a fusiform vacuole (*fv*), tonofilaments (*t*), mitochondria (*m*), and clumps of free ribosomes (*r*). × 80,000.

FIGURES 23 and 24 A small, cytoplasmic vesicle (*cv*) is shown in each of these figures, in which the membrane is surrounded by a halo of fine, fibrillar material. In Fig. 23 this coating has the appearance of radially arranged spokes. Two short lengths of rough-surfaced endoplasmic reticulum (*rer*) are shown in Fig. 24. × 110,000.

FIGURE 25 Parts of two adjacent squamous epithelial cells are shown, with parallel lateral cell walls (*cm*). At the base of the cells is a large, extracellular space (*E*) which contains a little electron-opaque material. In one cell, the plasma membrane adjacent to the extracellular space has invaginated (arrow) and the invagination is coated, on the cytoplasmic side, with a fine fibrillar material like that of the coated vesicles in Figs. 23 and 24. × 100,000.

FIGURE 26 A strangely shaped, membrane-bounded structure with dense granular contents is shown. The limiting membrane is thick but not asymmetric, and the contents include small vesicles and other membrane complexes. A myelin figure lies at the top left of the field. × 100,000.


n
m
fv
t
22
cv
23
E
cm
25
cv
rer
rer
24
26

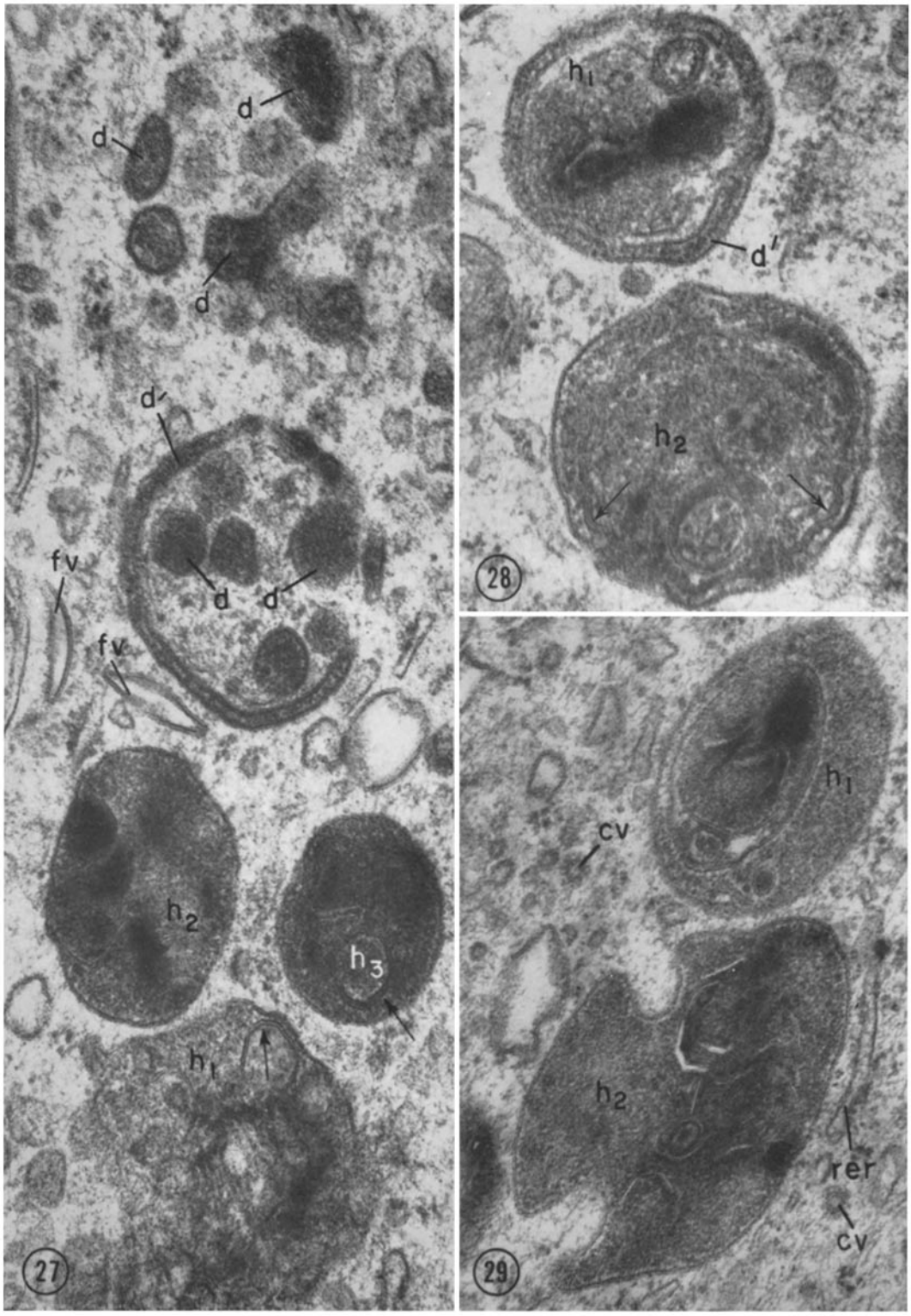
It has been suggested by Porter et al. (17) that the fusiform vacuoles are formed by an infolding of the cell surface. This is confirmed here by following the uptake of Imferon into thick-walled, cytoplasmic vacuoles. This infolding of the cell membrane is thought to occur during or very shortly after bladder contraction (17) and to function as a device for reducing the cell surface area. That this is a very rapid process is illustrated here (Figs. 30 and 31) by the finding of Imferon-containing vacuoles deep in the cytoplasm of cells fixed only 5 min after the Imferon injection. These Imferon-containing vacuoles may be the terminal portions of long channels, still in contact with the cell surface, which are formed by deep invaginations of the cell membrane. Such channels are found in the epithelial cells of the contracted (Fig. 3) but not of the dilated (Fig. 2) bladder. Alternatively, the Imferon may be in closed vacuoles formed from pinched-off lengths of the angular cell membrane. The persistence of such vacuoles, even though in reduced numbers, in the dilated bladder (Fig. 2) coupled with evidence from serial sections makes it clear that many of these vacuoles are completely isolated from the cell surface. If invagination of the cell membrane is the mechanism for decreasing the luminal surface area of the bladder, it is probable that, as the bladder gradually dilates, the reverse process occurs, and that the surface area is increased both by the unfolding of the long channels and by incorporation into the cell membrane of some of the thick-walled vacuoles. Since, however, the surface membrane of the bladder is subject to a certain amount of stress, both from the complex chemical environment of the urine and from the physiological dilatation and collapse of the organ, a mechanism must also exist in the cell for the regular replacement of old membrane by new. It is apparent that new membrane can be supplied to the cell surface by incorporating some of the thick-walled, fusiform vacuoles, newly formed from the Golgi cisternae in the manner described above. In this way, the Golgi complex can supply new cell membrane, and the assembly of this unique barrier membrane (2) can be regarded as the "export" function of the Golgi apparatus in these cells. It is perhaps relevant here that fusiform vesicles are just as numerous in the transitional epithelium of the ureter (1), which is not subject to the same degree of dilatation and collapse, as they are in the bladder. This implied flow of membrane through the cell from the Golgi system to the cell surface is in the direction opposite to that proposed by Daniels for membrane flow in amebae, where the Golgi cisternae are thought to be formed from invaginated cell membrane (19).

It is evident from the mode of uptake of Imferon by the superficial cells that the irregularly shaped, thick-walled cytoplasmic vacuoles are formed by pinched-off invaginations of the cell barrier membrane. This barrier membrane is rigid and angular (1) and the exact size and shape of the vacuoles formed from it will depend on the length of membrane involved (Fig. 30). Such vacuoles will at first contain only material derived from the ureter

FIGURE 27 The field shows part of the cytoplasm at the base of a superficial squamous cell. At the bottom of the field are three large heterogeneous bodies (h_1, h_2, and h_3) which contain lengths and complexes of membranes (arrows) within a very dense matrix. At the top and center of the field are two groups of round, oval, or elongated membrane-bounded structures (d) with dense granular contents. The lower group is partially encompassed by a similar crescent-shaped structure (d'). Also present in this field are two fusiform vacuoles (fv). × 80,000.

FIGURE 28 Two dense heterogeneous bodies are shown here. In the upper one (h_1) the dense central components appear to have been surrounded by another dense, membrane-bounded structure (d') which is comparable to the crescentic profile (d') in Fig. 27. In the lower body (h_2) the arrangement of membranes (arrows) within the dense matrix suggests that it was formed by a similar encircling process. × 80,000.

FIGURE 29 In this field there are two large, dense heterogeneous bodies (h_1 and h_2), two small coated vesicles (cv), and a short cisterna of rough-surfaced endoplasmic reticulum (rer). The large dense bodies contain membrane complexes similar to those within the dense structures illustrated in Figs. 26 to 28. × 80,000.


d
d
d
d
d'
fv
fv
d
d
h2
h3
h1
27
h1
d'
h2
28
h1
cv
h2
rer
cv
29

and bladder lumen, e.g. Fig. 30. The presence of small vesicles within them (Figs. 6, and 15 to 17) therefore suggests either subsequent invasion of thick-walled vacuoles by small vesicles from the cytoplasm, or invagination of small blebs from the vacuolar wall itself. It is not possible to tell, from the morphological evidence (Figs. 15 to 17) which interpretation is correct. The progressive condensation of small vesicles within the thick-walled vacuoles (Fig. 21) and subsequent loss of rigidity of the thick limiting membrane (Fig. 20) may represent early stages in the formation of digestive vacuoles. However, the evidence for this is equivocal. Attempts to follow the process, with acid phosphatase as a marker, were unsuccessful; either the method is too insensitive to reveal low states of enzyme activity, or this enzyme is not involved in this system. On the other hand, the finding of thick-walled vacuoles that may be termed autophagic vacuoles because they contain other cytoplasmic organelles as well as small vesicles (Figs. 18 and 19) may indicate that this is one way in which digestive vacuoles are formed. The attenuated appearance and reduced density of the short lengths of thick-walled membrane contained in one such vacuole (Fig. 19) may represent an early stage of breakdown of the phospholipoprotein complex.

An alternative mode of formation for autophagic vacuoles in liver cells has been described by Novikoff and Shin (7). A region of endoplasmic reticulum with increased electron opacity surrounds an island of cytoplasm; its outer surface remains as the delimiting membrane, and the permeability of its inner face alters to allow the release of enzymes from the cisterna into the vacuole which it has now formed (7). The morphological counterpart of this system is found in the transitional epithelial cells and is illustrated in Figs. 26 to 29. Bizarre, membrane-bounded structures with dense, granular contents apparently transform into large heterogeneous bodies which are comparable in appearance to residual bodies in other cell types. It was disappointing to find that this process also could not be clarified by employing acid phosphatase as a marker enzyme. Some, but not all, of the large dense bodies showed acid phosphatase activity (Figs. 32 and 33), but no activity was observed in any other organelle. However, it has been noted, in other cell types, that acid phosphatase is more frequently found in late residual bodies than in the Golgi complex or endoplasmic reticulum (20).

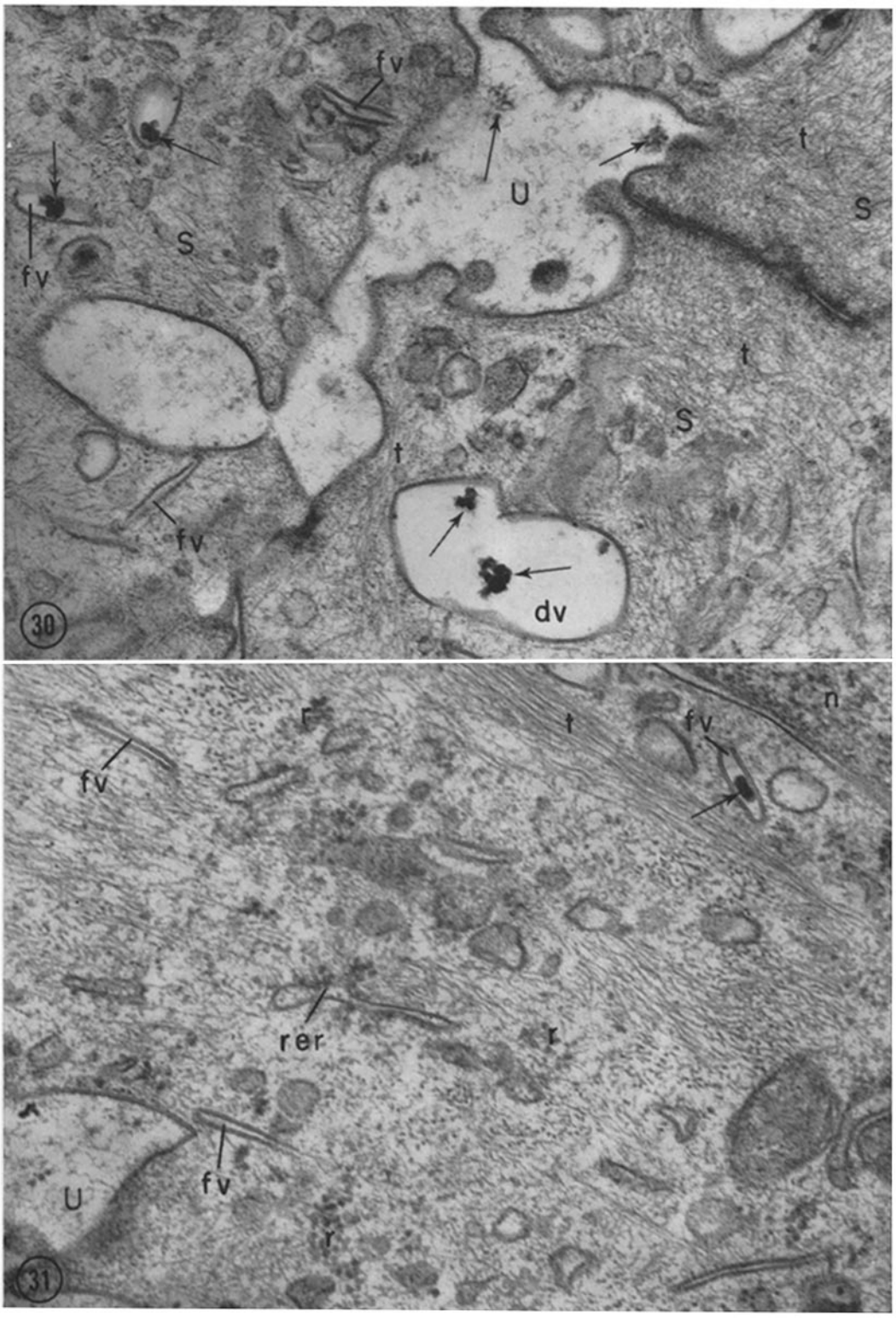
Large granules with acid phosphatase activity had previously been demonstrated by light microscopy in rat transitional epithelium (21), and it is now apparent that these granules are the large, dense, heterogeneous bodies illustrated here (Figs. 5, 27, and 29) and in a previous paper (1). Large granules with acid phosphatase activity have also been isolated in cell fractions from bovine and canine bladder epithelium (22).

Associated with these granules are large amounts of lipid, a yellow-brown pigment, and β-glucuronidase activity (22). These findings support a previous suggestion that the heterogeneous bodies are disposal units for surplus or worn-out thick membrane (1). Their polymorphic appearance would then correspond to different stages of membrane breakdown and the accumulation of lipid end products.

In rat transitional epithelium, the small Golgi vesicles around the cisternae do not appear to have specialized, coated membranes such as have been

FIGURE 30 The field shows parts of three superficial squamous cells (*S*) bordering the ureter lumen (*U*). A little, finely divided Imferon is present in the ureter (arrows), and larger aggregates of this substance are also shown (arrows) within large, thick-walled, cytoplasmic fusiform (*fv*) and dilated (*dv*) vacuoles. Many tonofilaments (*t*) and a number of empty fusiform vacuoles (*fv*) are also present in this field. × 50,000.

FIGURE 31 An area of squamous cell cytoplasm is illustrated between the ureter lumen (*U*) at the bottom left and the nucleus (*n*) at the top right of the field. A fusiform vacuole (*fv*) containing Imferon (arrow) is near to the nucleus, well away from the free cell surface. Also present in this field are tonofilaments (*t*), empty fusiform vacuoles (*fv*), a cisterna of rough-surfaced endoplasmic reticulum (*rer*), and clumps of free ribosomes (*r*). The tissue shown here, and in Fig. 30, was fixed 5 min after the Imferon was injected into the ureter lumen. × 60,000.


fv
U
t
S
fv
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rer
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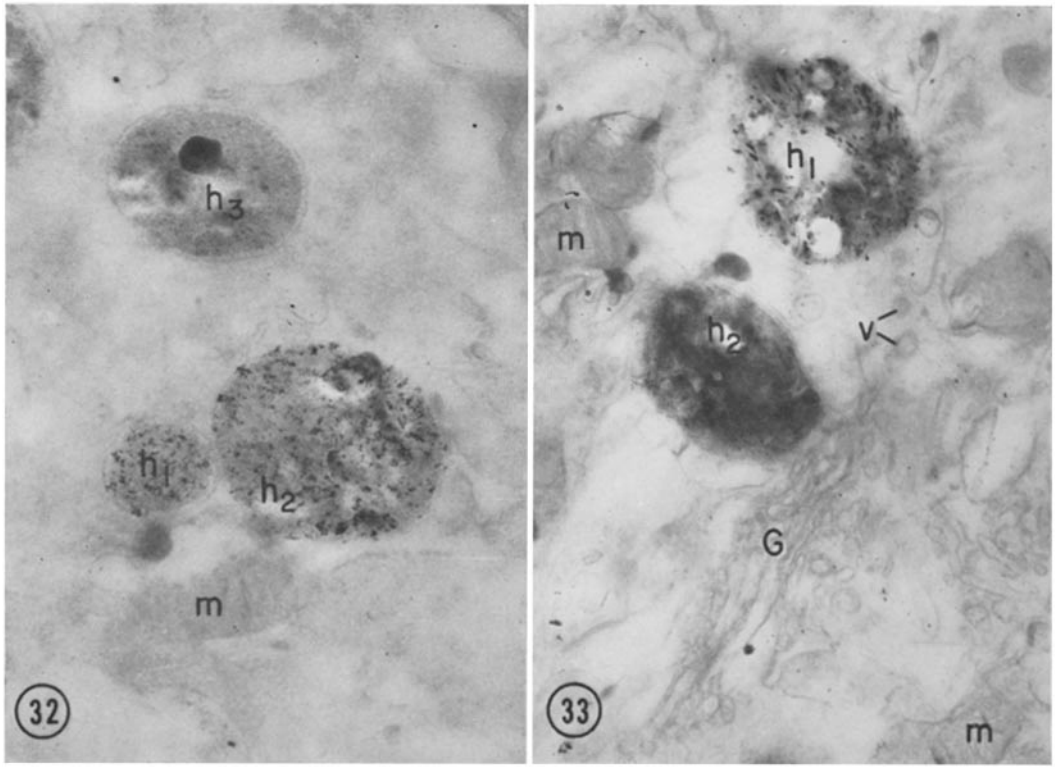
U
fv
r
31



FIGURES 32 and 33 The tissue illustrated here was stained to reveal acid phosphatase activity. The reaction product, lead phosphate, is present in two dense, heterogeneous bodies (h_1 and h_2) in Fig. 32, but not in a third (h_3). In Fig. 33 one dense body (h_1) shows enzyme activity but the other (h_2) does not. No reaction product is present in Golgi cisternae (G) or small vesicles (v).

This tissue was not contrast-stained with lead or uranyl salts. Such staining renders the dense heterogeneous bodies very electron-opaque (see Figs. 5 and 29), thus obscuring the lead phosphate where present. × 40,000.

observed in other cell types (8). A few coated vesicles may be seen in the Golgi zone (Fig. 6), but they are also to be found randomly distributed throughout the rest of the cytoplasm. It is probable that they originate as coated invaginations of the lateral and basal cell membranes (Fig. 25). It is known that coated invaginations of this type are the sites of protein uptake in the mosquito (23) and cockroach (24) oocyte, and by analogy it may be suggested that the coated vesicles in transitional epithelium bring new material into the cell from the extracellular environment.

In summary, thick asymmetric patches are found in the membranes of the Golgi cisternae in the superficial squamous cells of rat transitional epithelium. It is suggested here that this is the site of assembly of the thick barrier membrane which limits the luminal surface of the cells. Indirect evidence that fusiform vacuoles are formed from Golgi cisternae and are then incorporated into the cell membrane supports the postulate that membrane flow is in this direction, namely, from the Golgi complex to the cell surface. Large fusiform vacuoles, and others of more irregular shape, may also arise from pinched-off lengths of invaginated thick cell membrane. Some of these initially empty vacuoles are transformed into multivesicular bodies; small vesicles either invaginate from the vacuole wall or invade the vacuole from the cytoplasm. The demonstration of acid phosphatase activity in some of the large, heterogeneous bodies which are peculiar to this tissue supports an earlier suggestion (1) that these structures are responsible for the breakdown of old or damaged membrane.

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