THE FINE STRUCTURE OF THE GALL BLADDER EPITHELIUM OF THE MOUSE*

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INTRODUCTION

Recent advances in cytology have brought to light a number of submicroscopic structural components which have hitherto been unrecognized or poorly characterized (Porter (36), Palade and Porter (24, 25), Palade (23), Palay and Palade (26), Dalton and Felix (7), Sjöstrand and Hanzon (44, 45)). The special manner in which such components are represented in simple columnar epithelium has not yet been described in detail. This paper presents such a description, based on the gall bladder epithelium of the mouse, using the electron microscope and thin sectioning techniques.

Our current concepts of the structure of this epithelium are based largely on light microscope studies. These stem from the classical description of Virchow (47), who described the appearance of the cells during the process of absorption. The primary function of the mucosa, as established by Rous and McMaster (40) in 1921, is the concentration of hepatic bile (from 8 to 10 times). This is accomplished by the transfer of water and inorganic ions to the rich vascular network which fills and underlies the rugae. It is doubtful whether there is any significant resorption of any other ingredients of the bile by this epithelium. The literature dealing with the cytology of the mucosa is reviewed up to the year 1932 by Pfuhl (28). Subsequent studies worthy of mention are those of Nagahiro (17) and of Ferner (11). Dalton *et al.* (7) have studied gall bladder epithelium with the electron microscope, but their description is very brief and incomplete, and the techniques available at the time of their work did not reveal as much structure as can be demonstrated by present procedures.

Materials and Methods

Adult mice were perfused through the heart with Palade's (19) buffered osmium tetroxide solution (pH 7.4) without preliminary flushing of the blood vessels with salt solution. The

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perfusion was carried out by means of a 10 ml. syringe connected by rubber tubing to a glass cannula. 5 to 10 ml. was injected manually as fast as possible. Well perfused tissue showed even blackening almost instantly, and such portions were used for study. After perfusion the tissues were excised, cut into small pieces less than 1 mm. on a side, and immersed in fixative for 4 hours. The pieces were then washed in Ringer's solution, dehydrated with ethanol, and embedded in a mixture of 90 per cent *n*-butyl methacrylate and 10 per cent methyl methacrylate. Polymerization was achieved at 45°C., using 1 per cent peroxide catalyst (Luperco CDB[1]). Sections were cut on an ultramicrotome built locally on a design modified from that of Porter and Blum (37). An RCA EMU-2C microscope with compensated objective and 35 micron aperture was used. Micrographs were taken at electronic magnifications of 2000 to 11,000, with subsequent photographic enlargement as desired.

Observations and Interpretation

General Features

Fig. 1 presents a general survey picture of the simple columnar epithelium of the mucous membrane of the gall bladder of the mouse. At the bottom of the picture a small area representing the loose connective tissue of the tunica propria can be distinguished. It can be seen that the general appearance of the cells corresponds to classical descriptions based on light microscope studies. The "striated border" described by Virchow (47), Shikinami (42), Jurisch (15, 16), Pfuhl (28), Nagahiro (17), Sommer (46), and Ferner (11), is seen to be composed of minute cellular processes similar to those termed "microvilli" by Borysko and Bang (4). This is in confirmation of Virchow (47), Nagahiro (17), and Ralph (38), who also recognized, on the basis of light microscope studies, that the striated border of the gall bladder epithelium consisted of numerous delicate processes. Dalton *et al.* (7) were the first to observe them with the electron microscope.

Ferner (11) on the basis of his light microscope studies, has described the gall bladder epithelial cells as displaying a series of zones or strata, disposed in planes parallel to the surface of the epithelium. He proposes to recognize a cuticular zone, which appears to include the striated border, the free cell membrane itself, and the immediate underlying cytoplasmic zone which appears in Figs. 1, 2, 4, 6–8 as a clear area about 0.4–0.5 μ thick, nearly devoid of granules or other structures. Examination of Figs. 1, 2, and 4, reveals that this cuticular zone of Ferner can conveniently be subdivided into two zones, which are designated the zone of microvilli and the clear zone. Beneath this superficial cuticular area, Ferner describes a subcuticular or dark zone, which is recognizable in the electron micrographs (Figs. 1, 2, 4) as a broad band containing many dense granules, mitochondria, and other bodies, occupying one-quarter to one-third of the height of the cell. Ferner then describes a supranuclear zone, likewise clearly displayed in the electron micrographs (Figs. 1, 5, 9–13). This zone lacks the dense granules characterizing the dark

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zone, but displays a fine granular component, mitochondria, endoplasmic, reticulum (Porter (35, 36), Palade and Porter (24, 25)) and smooth membranes corresponding to the Golgi membranes of Dalton and Felix (5, 6) and of Sjöstrand and Hanzon (45) and the "agranular reticulum" of Palay and Palade (26). Additional structures of an unrecognized nature are encountered in this zone. These appear in Figs. 5 and 9 as complete or incomplete ring-like structures 0.3 to 0.8 μ in diameter. They may correspond to the "ring figures" described in this zone by Ferner (11), but his description, based on the light microscope, is insufficiently detailed to permit a certain identification. Pending further work clarifying their nature, Ferner's term of ring figures is tentatively adopted to designate these structures. Ferner next describes a nuclear zone, also evident in Fig. 1. Between the nucleus and the base of the cell Ferner recognizes a basal zone, revealed in Figs. 1 and 3, and characterized by many mitochondria, lipoid bodies, endoplasmic reticulum, and nerve endings.

The various structural components of the cell will now be described in greater detail.

The Free Surface of the Cell

The surface of the cell presenting to the lumen is somewhat convex (Fig. 1), and is adorned with numerous minute, unbranched cytoplasmic processes or microvilli. A thin, delicate cell membrane, here resolved as a single structure about 80 A thick, can be seen overlying the intervillous cell surface, and extending without important modification over the surfaces of the microvilli themselves (Fig. 4). The microvilli vary in length and in profusion from area to area. Thus in Fig. 1, taken from a portion of the epithelium facing the main lumen of the gall bladder, the microvilli are short (0.3 to 0.6 μ) and spaced somewhat irregularly and sparsely (10 to 20/μ^2). In contrast, Figs. 2, 4, and 8 represent areas in recesses of the epithelium where a luxuriant display of closely spaced microvilli of greater length (0.5 to 0.7 μ) is evident. In such regions the numbers of microvilli per unit area are 80 to 100/μ^2. Except for length and for numbers per unit area, microvilli from all regions of the gall bladder are very similar. They range from 0.08 to 0.1 μ in diameter. In Fig. 8 it can be seen that individual microvilli vary in diameter to some extent from place to place along their length. They appear to be invested by a simple unmodified extension of the cell membrane which, when cut tangentially shows a very delicate granular and fibrillar texture (Fig. 4). This cell membrane investment seems to embrace a core of finely granular cytoplasmic material similar to that in the immediately adjacent cytoplasm just below the cell surface proper. Fig. 4 shows the base of several microvilli cut perpendicularly to the axis of the villi, and tangentially to an included portion of the cell membrane. The bases of the microvilli are

recognized as dense ring-like structures. However, this density does not appear to betoken a true increase in mass of unit area of the cell membrane at the base of the villi, but rather represents an appreciable thickness of membrane appearing as a cut edge. In Fig. 8 the tips or heads of the microvilli appear as blunt club-like endings with a density appreciably greater than that of the main shafts of the microvilli. Such an ending is here designated as the *capitulum microvilli.* Extending from the tips and from the distal portions of the shafts of the microvilli into the neighboring portions of the lumen are many very delicate lace-like filaments, less than 40 A in diameter. These are termed the *antennulae microvillares.* They show most clearly in Fig. 8, where they form a delicate frond-like tracery radiating irregularly from the cell membrane covering the surface of the microvilli. The longest antennulae extend out for distances of nearly 0.2 μ from the surface of the villus. They are most prominent and longest in the region of the tip, and microvilli cut transversely through the capitulum show a coronet of lace composed of these filaments (Fig. 4). The antennulae diminish in length and in number towards the base of the microvilli, but occasionally single ones may be seen here and at the base or even on the intervillous cell surface. In tangential slices the relatively dense cell membrane obscures structures lying within the microvilli (Fig. 8). However, the inner structure is revealed in instances where a longitudinal section passes through the central axial portion of a villus. Microvilli cut in this manner show the cell membrane only in cross-section, and display it as presenting irregularities in profile and in density, with occasional breaks in the profile leading into small recesses or caves. Occasionally thin-walled vesicle-like structures are seen in such areas. Some of these vesicles seem to communicate with the lumen through openings in the cell membrane covering the microvillus. These small cave-like indentations of the cell wall on the microvilli resemble similar structures described along the inner and outer cell membrane of capillary endothelial cells by Palade (22). Such recesses or vesicles opening through stomata or tunnels in the cell membrane are being encountered with increasing frequency in cytological studies made with the electron microscope, and a general term to designate them seems desirable. It is here proposed to speak of such a recess or pocket as a *caveola intracellularis*, or "intracellular cave," or "pit." This is defined as a small pocket, vesicle, cave, or recess communicating with the outside of a cell, and extending inward, indenting the cytoplasm and the cell membrane. Such caveolae would be lined with extensions of the cell membrane, but could be pinched off to form free vesicles within the cytoplasm, as postulated by Palade (22). The caveolae intracellulares of the microvilli are very delicate and minute, ranging from 150 to 400 A in diameter, extending 500 to 600 A into the substance of the villus, and presenting stomata or mouths from about 200 to 400 A in diameter.

Larger and coarser caveolae intracellulares of a very different nature are encountered in relation to the cell membrane lying between the bases of the microvilli (Figs. 4 and 8). These large caveolae are about 800 A in diameter and open to the lumen of the gall bladder through tunnel-like stomata about 200 to 800 A in diameter. The tunnel-like opening may be about 0.1 μ long, and may connect the lumen with a dilated space containing a dense material. The material within the dilated recesses of these caveolae does not show any well organized structure, but resembles closely in its irregular texture and density material seen in granules found in the dense stratum of the cell. The experimental work of Virchow (47), Policard (29, 30, 32, 33), and Aschoff (2) demonstrating an absorption function for the gall bladder, suggests that an attempt be made to explain these granules and caveolae in terms of this absorption function. Direct evidence is lacking, but the appearances in Figs. 4 and 8 suggest that the dense granules mentioned above may be derived from the material in the extended recesses of the caveolae by a process involving a pinching off and obliteration of the stomata of the caveola and a movement of the granule to the dense stratum after its isolation from the cell membrane. This interpretation assumes that these granules and caveolae have a role in one of the absorption functions of the epithelial cell, and leads to a tentative designation of these structures as large absorption granules and large absorption caveolae respectively. These granules are not associated with endoplasmic reticulum or Golgi membranes. Thus a secretory role cannot reasonably be assigned to them.

The Lateral Cell Membranes

Each epithelial cell is surrounded on all sides by a continuation of the cell membrane described in detail in the previous section. The delicate fibrillar texture mentioned in connection with the free surface cell membrane is also recognizable in favorable areas along the sides of the cell (Fig. 3). The lateral cell walls, which appear as straight lines under the light microscope, are resolved as displaying a more complicated configuration (Figs. 1, 3, 5, and 11). The configuration is most simple near the free surface of the cell, and becomes more and more tortuous and complicated as one follows the lateral cell border towards the base of the cell.

Immediately adjacent to the free surface, the lateral cell walls show special modifications which have long been known to light microscopists as "terminal bars." The electron micrographs resolve certain details of structure in this region which are not evident with the light microscope (Figs. 1, 2, 6–8). Each terminal bar appears to consist of portions of the cell membranes of two adjacent cells with associated intracellular dense material and some sort of a dense material lying in the narrow intercellular space between the two cell membranes. The membranes themselves in this area likewise display

a density greater than that which characterizes them elsewhere. In Fig. 7 a terminal bar is cut longitudinally. Near the center of this bar is a region in which the two cell membranes are cut nearly perpendicularly. Each membrane appears to be about 150 A thick, and to be separated from the other by an intercellular space about 100 A wide. This intercellular space appears more dense than does the corresponding space appearing in Figs. 5 and 11. In the cytoplasm immediately adjacent to the specialized dense cell membranes in the terminal bars is a cloud of dense granular or fibrillar material fading off into lighter areas of cytoplasm. The cloud of density associated with each of these cell membranes is about 0.1 μ thick, but has no sharp inner limit.

Beyond the specialized region of the terminal bar, the cell membrane appears unspecialized and runs a relatively straight course towards the base as far as the inner limit of the dense stratum of Ferner. The membrane here is about 80 A thick. The intercellular space is of low density and varies from 90 to 150 A in width. A light scattering of indefinite cytoplasmic densities accompanies the membranes. However, even in this area the cell membranes are not perfectly straight, but show profiles of small folds or protuberances (Figs. 5 and 6).

These irregularities become more pronounced in the portions of the cell membrane opposite the supranuclear stratum where the sections show the membrane as a line manifesting a sequence of recurrent double hairpin-like curves representing profiles of pleat-like folds. These folds limit shelf-like extensions of cytoplasm running in planes parallel to the side of the cell. The shelves of cytoplasm so enclosed range in thickness from 700 to 800 A, and in width from 0.2 to 0.3 μ. In Figs. 5 and 11 one can see isolated profiles of such folds cut so as to exclude from the section the connection between the enclosed cytoplasm and the main portion of the cell. The majority of these plications do not fit into corresponding recesses in the cells immediately adjacent, and hence to not have a role in locking cells together (Figs. 5 and 11). In the nuclear and basal zones of the cells these folds appear in increasing complexity (Figs. 1 and 3) but still do not interlock appreciably with irregularities of adjacent cells.

The Basal Cell Border

The basal cell border is shown in Figs. 1 and 3 as a fairly straight regular structure. In Fig. 3 a double layer is seen along the base of the epithelium, one representing a profile of the cell membrane itself, continuous with the lateral cell membrane described above. Outside the cell membrane is a second membrane, the limiting membrane, which does not follow the lateral contour of the cell, but is spread alike over the basal cell membrane and the narrow triangular grooves marking the limits of the lateral and basal cell membranes. This limiting membrane is about 80 A thick and is separated

from the basal cell membrane by a space about 120 A wide. Details of this region are not well resolved in the present micrographs, though irregular dense bodies can be seen associated with the membranes. Occasional peak-like protuberances are seen in which these two membranes pout together forming a little conical papilla near the center of the base of a cell. Such protuberances may be associated with some condensation of neighboring connective tissue elements (Fig. 1). The basement membrane of the light microscopist is thought to consist of this limiting membrane and adjacent connective tissue elements.

Associated Non-Epithelial Structure

In Fig. 3 one can distinguish a granular structure interposed between portions of three epithelial cells. This structure appears to be surrounded by a cell membrane, to contain a portion of a mitochondrion, and to be packed with numerous granules or vesicles about 400 A in diameter. This structure resembles the synaptic nerve terminals described by De Robertis and Bennett (8), and is thought to represent a functional terminal of an unmyelinated nerve in synaptic contact with the epithelial cells. The structures packed within it correspond to the synaptic vesicles of De Robertis and Bennett (8). In the enlarged insert one can see a caveola intracellularis extending into an adjacent epithelial cell in close relation to the synaptic ending. A prominent synaptic vesicle can be seen in the nerve terminal close to this caveola.

Large Absorption Granules

These granules (about 0.1 μ diameter) can be seen in Figs. 1, 2, 4–8. Few of them are found in the region of the cytoplasm immediately underlying the free cell surface. They are most concentrated in the outer portion of the dense zone of Ferner, diminishing gradually in abundance as one approaches the inner portions of this zone. They are not found in the supranuclear, nuclear, or basal zones. These granules lack a well defined membrane and appear to undergo a series of transitions involving a loss of density and size. The largest, most dense, and most discrete granules of this class are found in the outer region of the dense zone. As one progresses towards the base these granules are represented with less and less density and with the appearances of fragmentation (Figs. 2 and 4). If the assumption be correct that these represent granules of absorbed material, it would follow that after the granules become separated from the cell membrane by a pinching off of the tunnel openings of the caveolae, the granules move rapidly across the clear cytoplasmic zone between the dense zone and the free cell membrane. After taking positions in this outer portion of the dense zone, the granules would then seem to move more slowly towards the base of the cell, disinte-

grating as they move, surrendering or dissipating their substance to the surrounding cytoplasm.

Mitochondria

The fine structure of the mitochondria seen in the gall bladder epithelial cells corresponds to that which has been described for mitochondria in general by Palade (19–21). The mitochondria show the double membrane, cristae, matrix, and dense bodies, and the profiles encountered are consistent with the concept that most of the mitochondria are filamentous in nature (about 0.3 μ diameter), as described by Policard (31) and Nagahiro (17). The mitochondria are most abundant in the basal portion of the cell and in the inner portion of the dense zone. In the nuclear and supranuclear zone mitochondria are scanty and tend to occur only near the lateral cell walls. No morphological differences were detected in mitochondria located in different regions of the cell.

The "Golgi" or "Agranular" Membranes

Agranular membranes similar to those described by Dalton and Felix (5, 6), Sjöstrand and Hanzon (45), and by Howatson and Ham (14) as Golgi elements, and by Palay and Palade (26) as "agranular reticulum," are encountered in the present series of micrographs in regions of the gall bladder epithelial cells in which the "Golgi apparatus" has been described on the basis of classical light microscope technique (d'Agata (1) and Nagahiro (17)) (Figs. 1, 5, 9–12). In confirmation of these authors, Golgi elements are encountered in a cap-like region overlying the nucleus, occupying positions in the central portion of the supranuclear zone and the lateral margins of the nuclear zone. In this epithelium the Golgi structures are not associated with large clear vacuoles such as are pictured in close relation to Golgi membranes by Dalton and Felix (5, 6) and by Sjöstrand and Hanzon (45). The general relationships observed here are not significantly different from those described by Palay and Palade (26) and by Howatson and Ham (14), and by Nagahiro (17). The profiles of the Golgi membranes and their dimensions are consistent with the models of the agranular reticulum proposed by Palay and Palade (26). The Golgi membranes in the gall bladder epithelium are associated with numerous small vesicles about 400 A in diameter (Figs. 9–12), similar to those found in association with Golgi membranes of earthworm nerve cells by De Robertis and Bennett (9). In addition to the small Golgi vesicles, the micrographs reveal a larger species of vesicular structure, the large Golgi vesicles, shown in Figs. 5 and 11. These appear as somewhat irregularly oval profiles interpreted as representing a dense membrane surrounding a less dense somewhat granular material. They resemble in general density and contrast the ring figures described below, but differ from them

by virtue of smaller size, lack of an internal dense core and surrounding light zone, and close association with Golgi elements. The large Golgi vesicles range from 0.1 to 0.15 μ in diameter. The membrane appears to be about 80 A thick.

The Endoplasmic Reticulum

This newly recognized submicroscopic component of cells has been well characterized with respect to its appearance in the electron microscope by Porter (35, 36), Palade and Porter (24, 25), and Palade (23). Under the name "ergastoplasm" it has been fruitfully studied by Weiss (49) and by Bernhard *et al.* (3). This present study has nothing to add to our knowledge of the endoplasmic reticulum except to report its distribution and appearance in the epithelial cells of the gall bladder. It is found in the basal, nuclear, and supranuclear zones, but does not invade the dense zone appreciably, and is absent entirely from the cuticular zone. It is most evident in the supranuclear zone, between the Golgi cap and the lateral cell walls (Figs. 5 and 11). It is seen as single or multiple paired profiles with associated dense particles (Palade (23)). Occasional dilatations of the intermembranal space appear to be representations of the "cisternae" of Palade and Porter (25). They show a characteristic association with mitochondria (Fig. 5) recognized by Palade (23). Some of the endoplasmic reticulum membranes are also associated with the ring figures, to be described below.

The Ring Figures

In central portions of the supranuclear zone, covered by the Golgi cap, and amongst the Golgi membranes are conspicuous, complete or incomplete, ring-shaped oval profiles from 0.3 to 0.8 μ in diameter. These may correspond to the ring figures of Ferner (11), and are hence tentatively assigned this term. The ring figures are surrounded with a membrane about 80 A thick, on the inner surface of which are numerous fine granules about 200 A in diameter (Figs. 5, 9, and 10). In Fig. 5 two incomplete ring figures are seen, the membranes of which are double, and show characteristics of endoplasmic reticulum. Within each ring figure is a cloud of finely reticular material, most dense in the center, surrounded by a relatively clear zone just inside the membrane with its associated granules. These ring figures are sometimes associated with structures resembling endoplasmic reticulum. They are not found in the basal, nuclear, dense, or cuticular zones. The distribution and appearance of these bodies are in general confirmatory of Ferner (11), who noted the membrane and the central dense core, and reported that these two portions differed from each other in staining characteristics. Certain granular structures in the supranuclear zone which are strongly positive to the periodate-Schiff reaction (Fig. 1, insert) appear to correspond to the ring figures of Ferner described here.

Nucleus

Characteristic nuclear profiles encountered in these cells are seen in Fig. 1. An indentation of the nuclear membrane is seen presenting its convexity to the base of the cell. Within the recesses of this indentation some dense cytoplasmic material of an unknown nature is encountered. Several nucleolar bodies are seen in most nuclei. Often nucleolar densities are seen immediately adjacent to the nuclear membrane. The nuclear membrane is double, as described by Hartmann (13), and presents "pores" similar to those pictured by Watson (48) (Fig. 13).

Unidentified Cytoplasmic Structure

In the basal region of the cell are seen irregular dense bodies such as the one shown in Fig. 3. They are surrounded by an irregular cloud of particles and vesicles. It is thought that these large irregular dense bodies may be lipoid in nature.

DISCUSSION

Three characteristic features of the cell membrane of these epithelial cells deserve some comment. These are the plications or pleats of the lateral cell membranes, the thickening at the terminal bars, and the microvilli.

Concerning the folds or pleats of the lateral surface, it is noteworthy that this pleating is not characterized by extensive interdigitation. Since it is well known that leucocytes can migrate easily through the epithelium between the cells, the plications mentioned here would provide a means whereby a passage could be made for migrating cells by an unfolding of the pleats, without necessitating any great stretching of the molecular components of the cell membranes.

The classical concept of terminal bars based on light microscope studies holds that these structures are composed of a dense, intensely staining, intercellular cement substance which contributes to the mechanical attachment of one cell to its neighbor and which prevents the escape of the intercellular fluid (Schaffer (41)). The electron micrographs presented here, however, show that the terminal bar is better regarded as characterized by thickenings of the cell membrane itself, with an associated cloud of dense submembranal cytoplasmic material. It is possible that there is a small amount of fairly dense intercellular material besides (Figs. 6–8), but this does not contribute importantly to the over-all appearance of the "terminal bar." There seems to be no reason to question the concept that this structure does represent a region of specially strong mechanical adhesion between adjacent cells. The terminal bars thus resemble closely, and appear to be homologous in structure and function with the "desmosomes" of the "intercellular bridges" of stratified squamous epithelium, as described recently by Porter (34).

The microvilli seen on the gall bladder epithelial cells resemble similar structures encountered on the chorioallantoic membrane of the chick embryo by Borysko and Bang (4), on ciliated epithelial cells by Fawcett and Porter (10), on intestinal epithelium by Granger and Baker (12), and Palade (19), on mesothelial cells by Odor (18), in the brush border of kidney tubule cells by Pease and Baker (27), Sjöstrand and Rhodin (43), and Rhodin (39), and on various cells by Dalton *et al.* (7). Fawcett and Porter (10) have shown clearly how microvilli differ from cilia.

Microvilli appear to adorn free cell surfaces of epithelia active in absorption processes, or perhaps more generally, active in exchange between the two fluid phases separated by the epithelium. This paper presents some details of structure of microvilli which have not been described previously.

SUMMARY

Sections of mouse gall bladder epithelium fixed by perfusion with buffered osmium tetroxide have been studied in the electron microscope as an example of simple columnar epithelium.

The free surface presents many microvilli, each presenting a dense tip, the *capitulum*, and displaying a radiating corona of delicate filaments, the *antennulae microvillares*. Very small pit-like depressions, representing *caveolae intracellulares*, are encountered along the cell membrane of the microvilli.

The free cell surface between microvilli shows larger cave-like depressions, likewise representing caveolae intracellulares, containing a dense material.

The lateral cell borders are extensively folded into pleats, which do not interdigitate extensively with corresponding folds of the adjacent cell membrane.

The terminal bars are shown to consist of thickened densities of the cell membrane itself in the region of insertion of the lateral cell wall with the free cell surface. This thickening is associated with an accumulation of dense cytoplasmic material in the immediate vicinity. The terminal bar is thus largely a cytoplasmic and cell membrane structure, rather than being primarily intercellular in nature.

The basal cell membrane is relatively straight except for a conical eminence near the center of the cell, projecting slightly into the underlying tunica propria. The basal cell membrane itself is overlain by a delicate limiting membrane, which does not follow the lateral contours of the cell.

Unmyelinated intercellular nerve terminals with synaptic vesicles have been encountered between the lateral walls of epithelial cells.

A division of the gall bladder epithelial cell into five zones according to Ferner has been found to be convenient for this study.

The following cytoplasmic components have been noted, and their distribution and appearance described: dense absorption granules, mitochondria,

Golgi or agranular membranes, endoplasmic reticulum or ergastoplasm, ring figures, and irregular dense bodies, perhaps lipoid in nature. The nucleus of these cells is also described.

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EXPLANATION OF PLATES

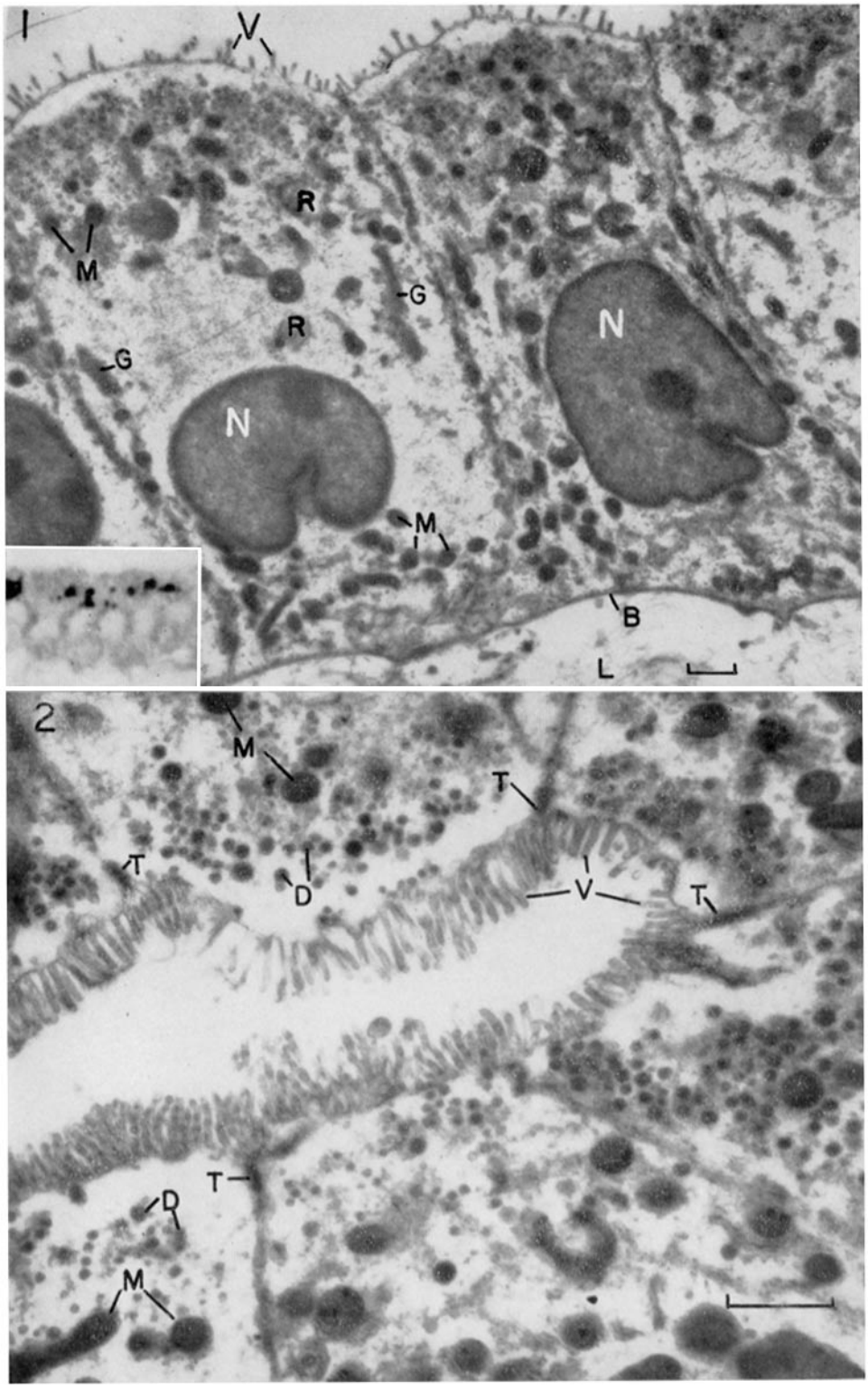
B, limiting membranes.
C, caveola intracellularis.
D, large absorption granules.
E, endoplasmic reticulum (or ergastoplasm).
F, antennula microvillaris.
G, Golgi bodies, or agranular reticulum.
Gm, Golgi membranes.
Gv, Golgi vesicles.
H, capitulum microvilli.
I, probable lipide body.
L, tunica propria mucosae.
M, mitochondria.
N, nucleus.
Ne, nerve terminal.
P, nuclear membrane pore.
R, ring figure.
S, synaptic vesicle.
T, terminal bar.
V, microvilli.
W, lateral cell wall.

All the figures except the insert in Fig. 1 represent prints of electron micrographs of sections of gall bladder epithelium of the mouse, fixed by perfusion with Palade's buffered osmium tetroxide solution.

PLATE 117

FIG. 1. General appearance of the simple columnar epithelium of the gall bladder. A series of zones parallel to the free surface of each cell can be distinguished somewhat as described by Ferner. These zones are the cuticular zone of Ferner which includes the zone of microvilli and the clear zone; the dark zone, supranuclear zone, nuclear zone, and basal zone, all as described by Ferner. Between the base of the epithelium and the tunica propria there can be recognized a delicate limiting membrane (*B*). The microvilli in this section vary considerably in length and arrangement. × 6600. Insert: Photomicrograph of section cut from the same block and stained with periodate-Schiff. The dark-staining granules appear to correspond to the ring figures (*R* in main figure) of Ferner. × 950.

FIG. 2. Higher power detail of superficial portions of gall bladder epithelial cells, showing zone of microvilli, clear zone, dark zone, and part of supranuclear zone. Microvilli in this region are more regular in length and in arrangement than in the area shown in Fig. 1. Numerous large absorption granules (*D*) are seen immediately under the clear zone, or even in the clear zone, close to the surface cell membrane. Some of these granules are seen to lie in cave-like indentations of the cell membrane, shown in greater detail at *C* in Figs. 4 and 8. × 15,000.

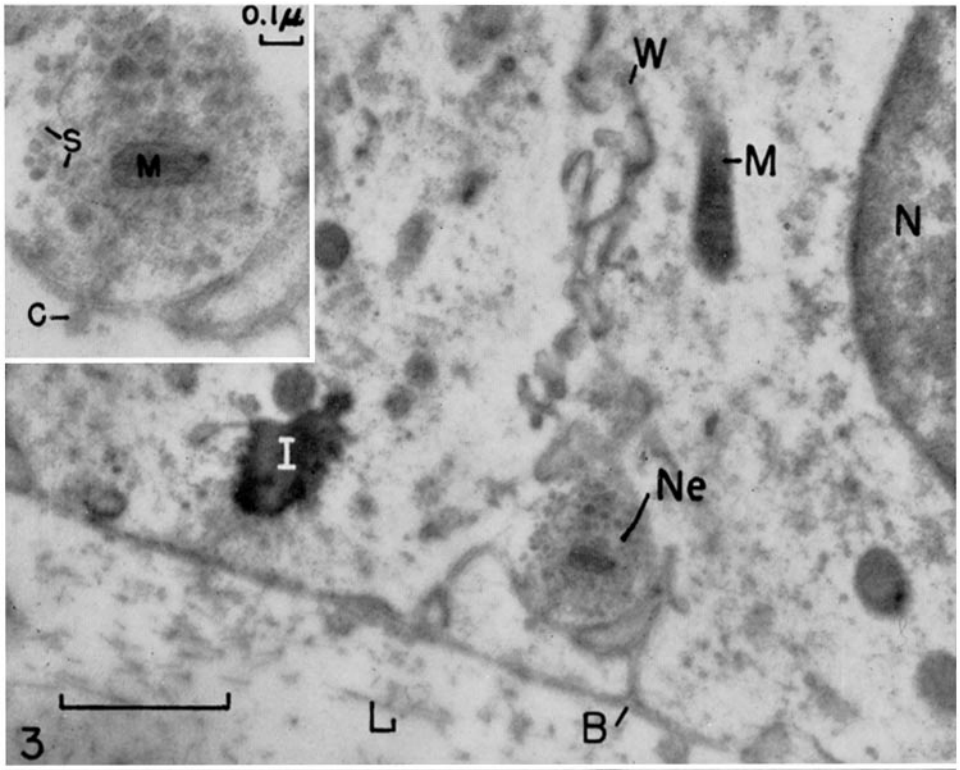


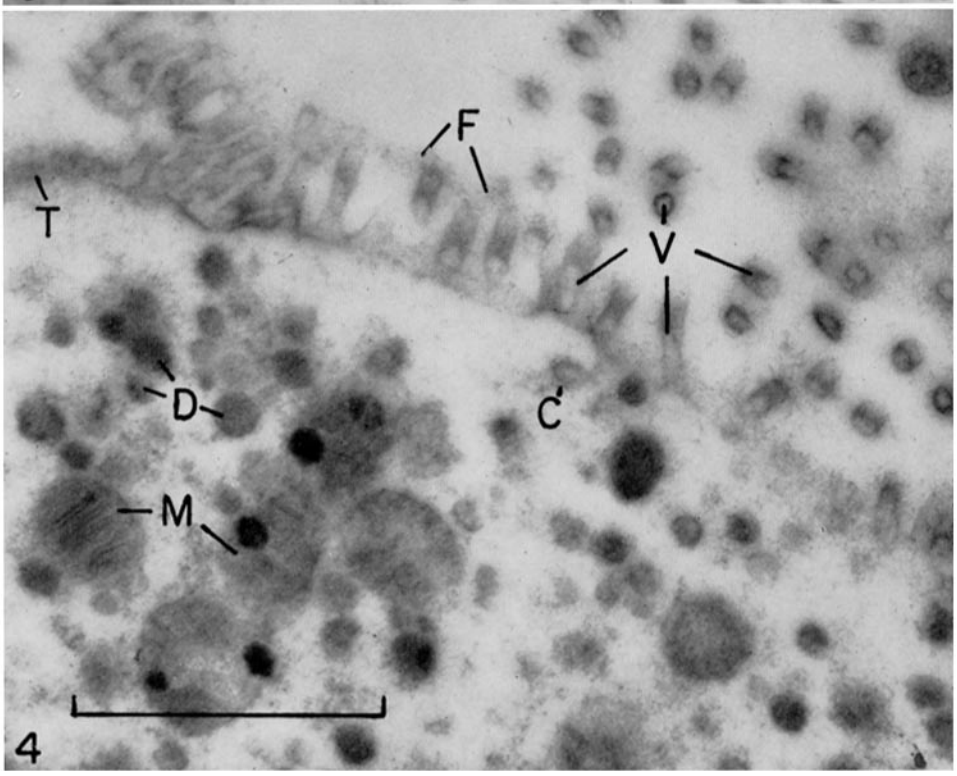
(Yamada: Fine structure of mouse gall bladder epithelium)

PLATE 118

FIG. 3. Detail of basal portion of gall bladder epithelium. The lateral cell wall (*W*) shows complicated plications, many of which do not appear to be in corresponding recesses in the adjacent cell. The limiting membrane (*B*), lying between the basal cell border and tunica propria (*L*), does not extend up between the cells. A structure thought to represent an interepithelial nerve terminal (*Ne*) is shown in the main figure and in the insert. This structure includes a mitochondrion (*M*) and numerous vesicles, thought to represent synaptic vesicles (*S*). In the insert a caveola intracellularis (*C*) in the basal region of an epithelial cell is seen in close relation to a synaptic vesicle. Main figure, × 22,000; insert, × 51,000.

FIG. 4. Detail of free surface of gall bladder epithelium, showing numerous microvilli (*V*) from which radiate the antennulae microvillares (*F*). A tangential section through the base of the microvilli, including a surface view of the cell membrane proper, appears along the upper right margin. × 41,000.

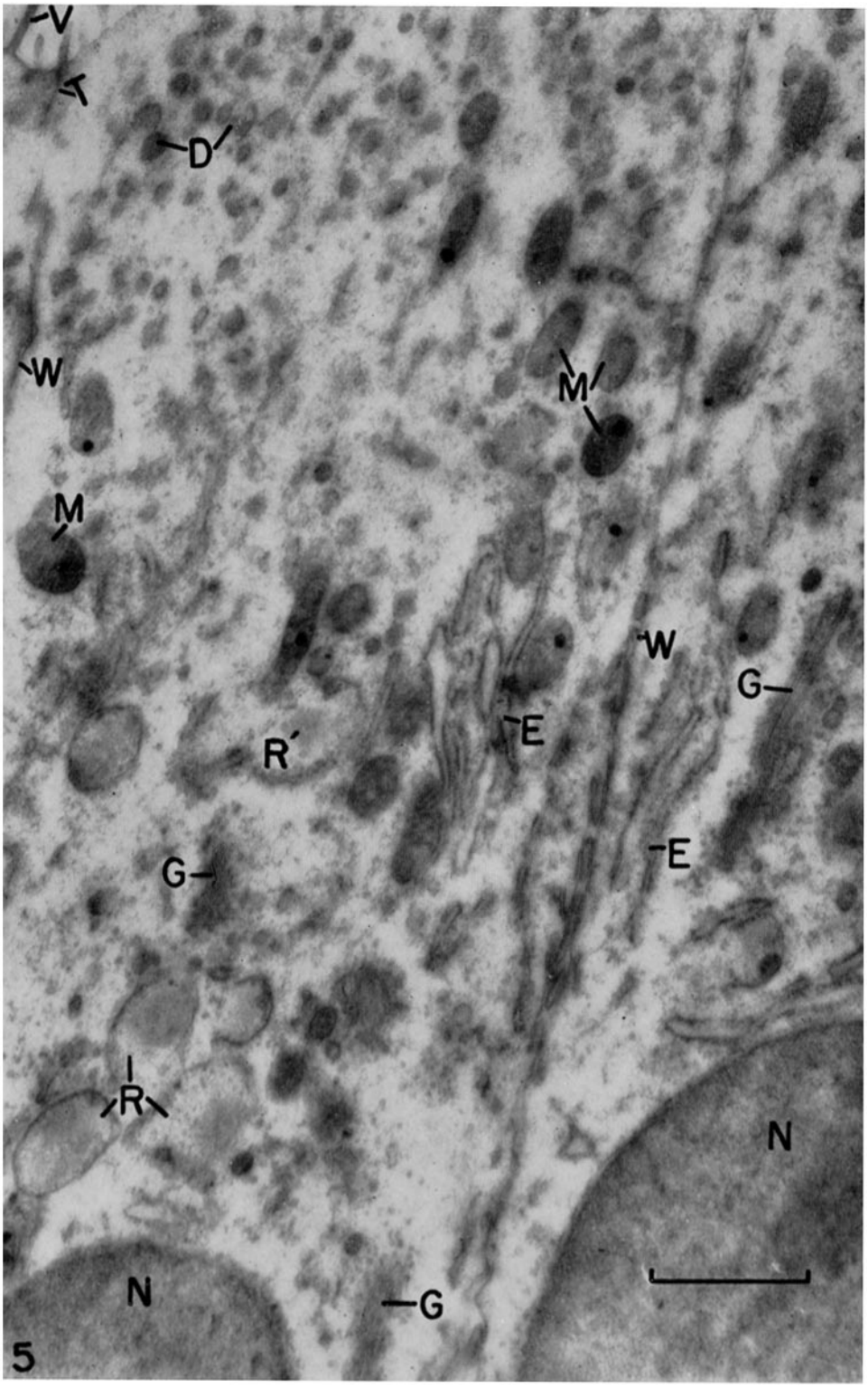




(Yamada: Fine structure of mouse gall bladder epithelium)

PLATE 119

FIG. 5. Detail of portions of nuclear, supranuclear, and dark zones, together with a small portion of the clear zone and zone of microvilli in the upper left corner. The supranuclear zone displays mitochondria (*M*), endoplasmic reticulum (*E*), Golgi bodies (*G*), and ring figures (*R*). *R'* shows structure resembling incomplete ring figure with associated granular membranes resembling endoplasmic reticulum. Characteristic pleat-like folds as seen in the lateral cell walls (*W*). × 23,000.



(Yamada: Fine structure of mouse gall bladder epithelium)

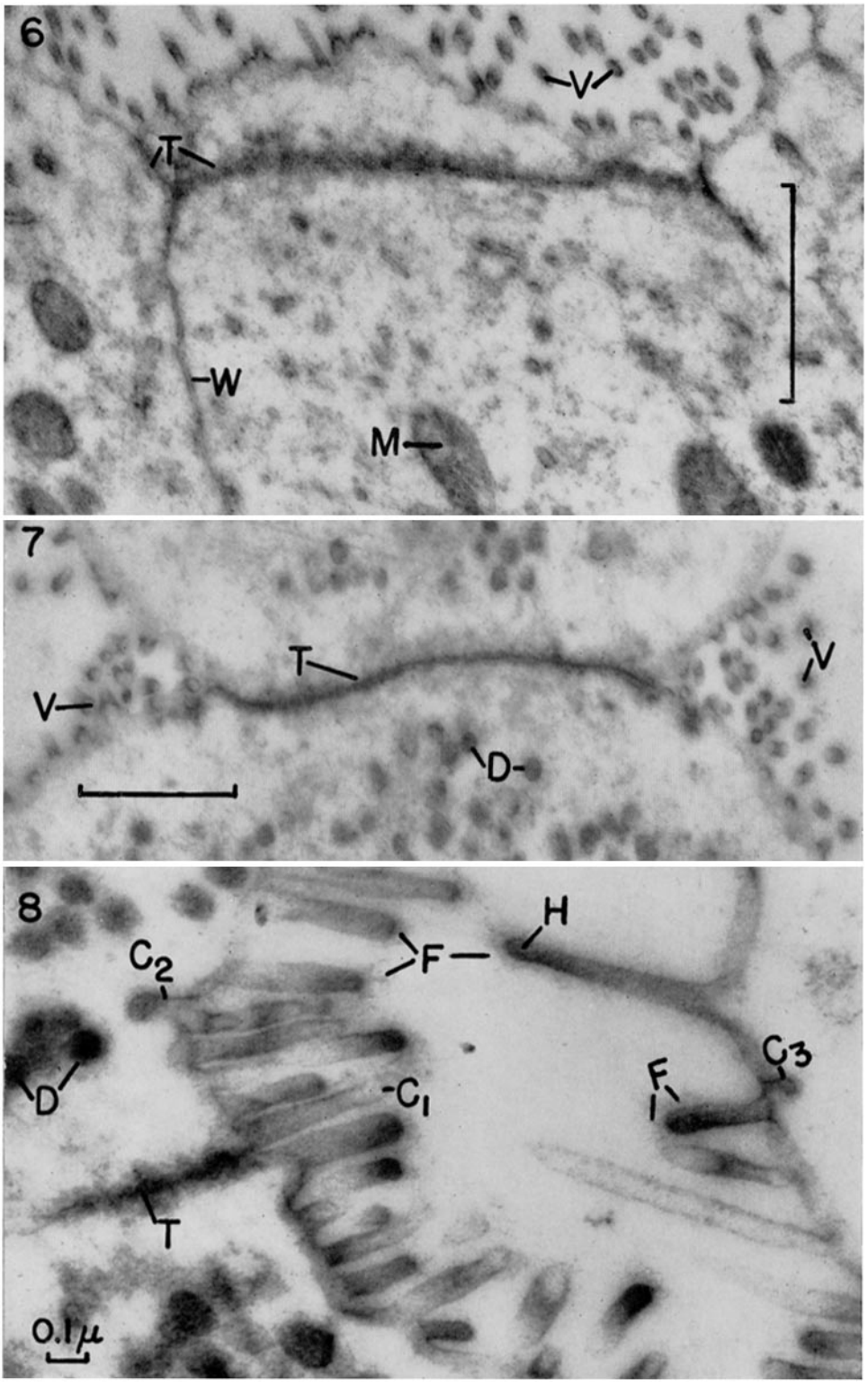
PLATE 120

All figures on this plate show details of portions of gallbladder epithelial cells adjacent to the free cell border. Note details of terminal bars (*T*) and microvilli (*V*).

FIG. 6. A section cut parallel to the terminal bar (*T*), but obliquely through the cell membrane. The lateral cell wall is almost straight in this region. The terminal bar shows dense cytoplasmic material arranged in a cloud close to the cell membrane. × 33,000.

FIG. 7. A similar section, perpendicular to the cell membrane in some areas. Here the two cell membranes are separately resolved, and are seen to be more dense than the cell membranes elsewhere. The cloud of associated cytoplasmic dense material is clearly shown. The intercellular space can be seen between the dense cell membranes in favorable places. × 23,000.

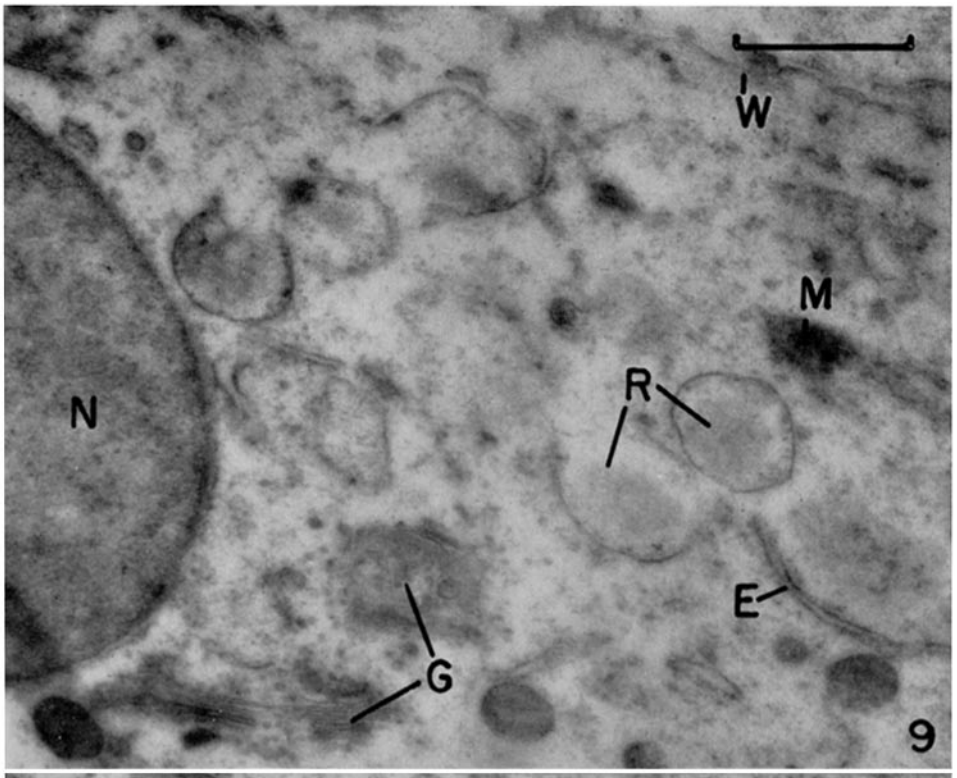
FIG. 8. Details of microvilli and of the free cell surface. The dense club-like tip of the microvilli, the capitulum (*H*), is clearly demonstrated. Radiating from the surface of the microvilli are the delicate antennulae microvillares (*F*). A cave-like depression or vesicle in the surface of a microvillus is shown at C_1. A more conspicuous depression or caveola intracellularis (C_2) containing a dense absorption granule is seen in the cell membrane between the bases of the microvilli. × 51,000.

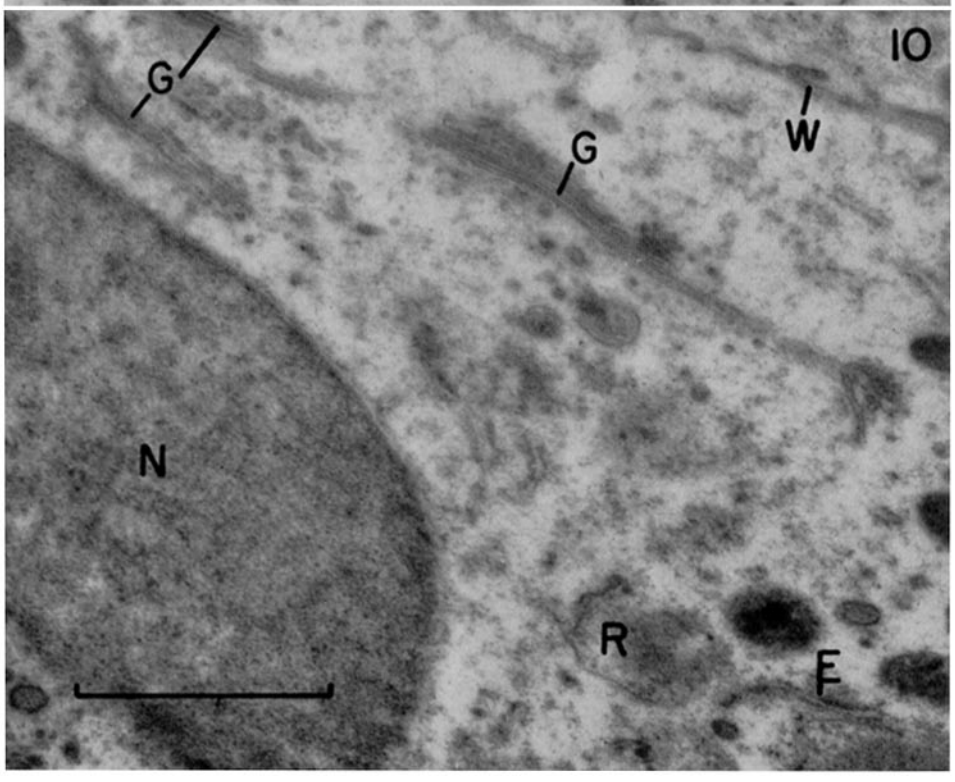


(Yamada: Fine structure of mouse gall bladder epithelium)

PLATE 121

FIGS. 9 and 10. Details of supranuclear zone, showing mitochondria (*M*), Golgi bodies or agranular reticulum (*G*), endoplasmic reticulum (*E*), and ring figures (*R*). In the upper right corner of Fig. 10 the pleated nature of the lateral cell wall is clearly demonstrated. Fig. 9, × 23,000; Fig. 10, × 33,000.





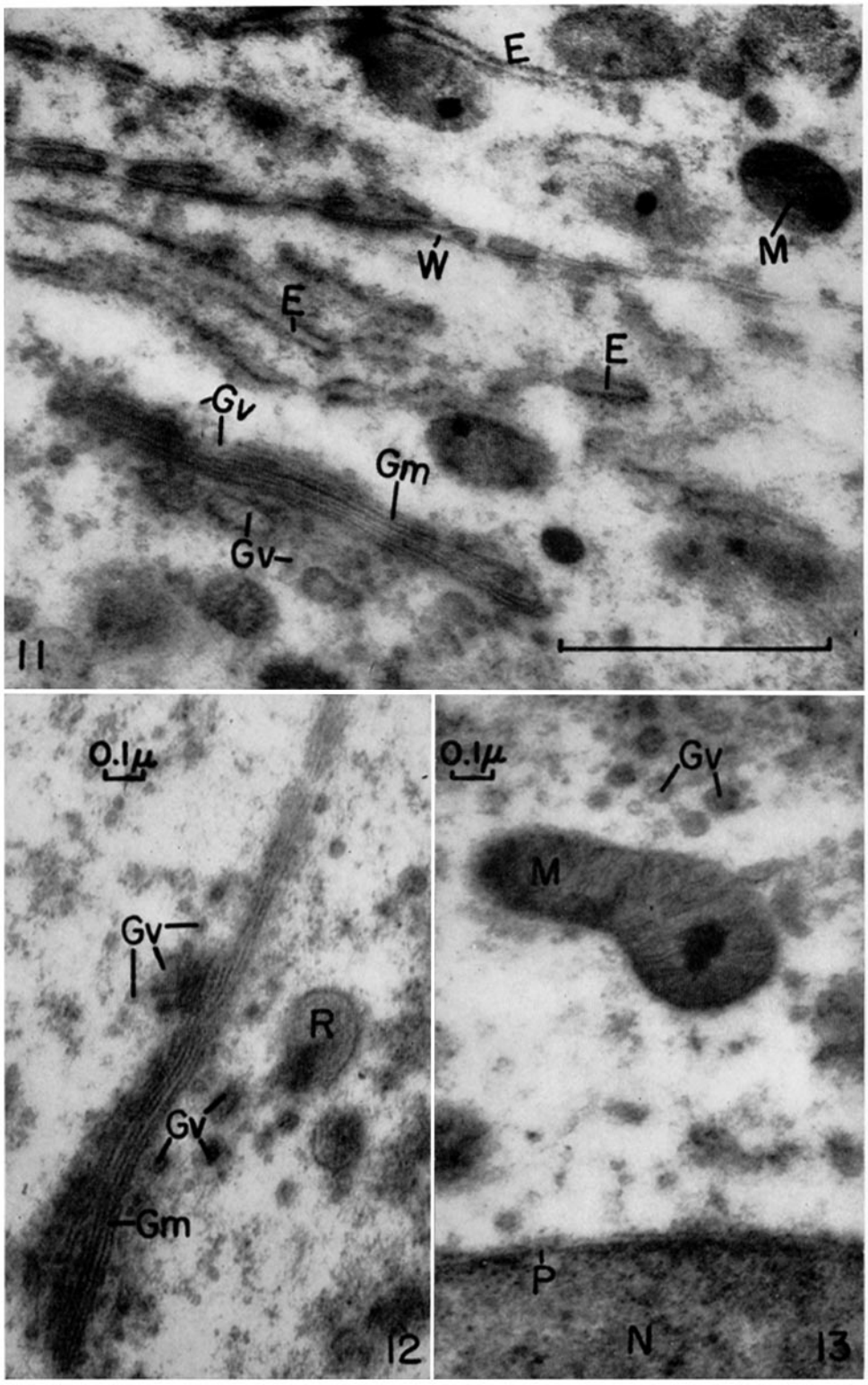
(Yamada: Fine structure of mouse gall bladder epithelium)

PLATE 122

FIG. 11. Detail of supranuclear zone, showing plicated lateral cell wall (*W*), endoplasmic reticulum (*E*), mitochondria (*M*), Golgi bodies with the agranular or smooth membranes (*Gm*), and large and small vesicles (*Gv*). × 41,000.

FIG. 12. Detail of Golgi bodies, showing an array of parallel double membranes (*Gm*) and vesicles of various sizes (*Gv*). Two structures taken to represent portions of ring figures are seen. × 59,000.

FIG. 13. Detail of portions of nucleus (*N*), mitochondria (*M*), and Golgi vesicles (*Gv*). The nuclear membrane shows a pore (*P*). × 59,000.



(Yamada: Fine structure of mouse gall bladder epithelium)