MEMBRANE MODIFICATIONS IN THE APICAL ENDOCYTIC COMPLEX OF ILEAL EPITHELIAL CELLS

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ABSTRACT

Ileal lining cells of the suckling rat possess an "apical endocytic complex" capable of sequestering intact protein from the intestinal lumen. The complex consists of a network of invaginations of the apical plasma membrane, a number of subjacent small vesicles, and a giant supranuclear vacuole. The first two components initially incorporate material from the intestinal lumen and then transfer it to the giant vacuole where it is stored. Their limiting membrane displays striking structural modifications when viewed in various planes of section. Its lumenal dense leaflet appears discontinuous and consists of an ordered array of minute discrete plaques. A dense particle approximately 70 A in diameter is centered over each plaque. The particles are arranged in a two-dimensional square lattice with center-to-center spacing of approximately 120 A.

INTRODUCTION

From the standpoint of function, the terminal portion of the small intestine of the suckling rat differs from that of the adult in that it can take up protein from the intestinal lumen and transfer the protein, presumably via its lymphatic drainage, to the general circulation. This ability is an essential component of the over-all process by which antibodies from maternal milk are transferred into the circulation of the newborn (see review by Brambell, reference 1). The ultrastructure of differentiated lining cells in the ileum of the suckling rat differs markedly from that of the adult as a reflection of this difference in functional properties (2–5). The apex of the cell is permeated by numerous deep invaginations of the apical plasma membrane which extend into the underlying cytoplasm where they anastomose to form networks and, in a lower region, open into vesicles. A giant vacuole lies below the vesicles and occupies the entire length and breadth of the midportion of the cell, compressing the nucleus towards the base. The giant vacuole contains flocculent material of two types. One is finely fibrillar in texture and is dispersed throughout the vacuolar lumen; the other is more compact and dense and is usually aggregated into one or several large masses. The function of the apical complex consisting of invaginations, vesicles, and giant vacuole has been investigated in tracer studies employing fluorescence and electron microscopy. These studies have demonstrated that various colloids and proteins enter the vesicles from the network of invaginations of the apical plasma membrane and are transferred to the giant supranuclear vacuole which serves as a repository for ingested tracer (2–5). The complex appears, therefore, to be specifically adapted for endocytic function.

A detailed study of the fine structure of limiting

membranes of the apical endocytic complex in ileal lining cells of the suckling rat is reported in this paper. We have placed particular emphasis on a description of ordered substructure of the membranes of two components of the apical endocytic complex, the network of apical invaginations and the apical vesicles. A brief description of this substructure was reported earlier by Graney (6). This substructure has also been described by Porter et al. (7), but that group of investigators has interpreted their morphologic observations somewhat differently (see below).

MATERIALS AND METHODS

On the morning of the experiment 13–14-day-old suckling rats of the Long-Evans strain were separated from their mothers. Each suckling rat was anesthetized with a Nembutal solution prepared according to the method of Pilgrim and De Ome (8), and its abdomen was opened by a midventral incision. A short segment of ileum 2–3 cm proximal to the ileocecal junction was excised, placed on a wax plate, and immersed in fixative. The segment was transected into rings with a razor blade. After the rings had been stored in fixative for several hours, they were diced into small cubes approximately 1 mm on a side.

The following fixative solutions were used, either chilled or at room temperature, to preserve the specimens: (*a*) 2% osmium tetroxide, buffered at pH 7.5 with acetate-Veronal or *s*-collidine, with 1% sucrose added (9–11); (*b*) 3.125% redistilled glutaraldehyde, buffered at pH 7.5 with 0.1 M sodium cacodylate or 0.025 M Sörensen's ($Na\text{-}Na_2$) buffer (12), with 1% sucrose added; and (*c*) the glutaraldehyde-formaldehyde mixture of Karnovsky (13) at approximately one-half or one-quarter strength and buffered at pH 7.5 with 0.1 M sodium cacodylate, with 1% sucrose added.

The osmium tetroxide-fixed tissue remained in fixative for 1–1½ hr. The aldehyde-fixed tissue remained in fixative overnight and was then postfixed in osmium tetroxide for 1 hr. Prior to dehydration in acetone, the fixed tissue was stained en bloc with uranyl acetate by the method of Kellenberger et al. (14) modified according to Farquhar and Palade (15). The specimens were embedded in Araldite or Epon according to the method of Luft (16). Sections 0.5 μ thick were cut with a Servall Porter-Blum MT-2 microtome equipped with glass knives and were mounted on glass slides and stained with toluidine blue-borax (17) for examination and photomicrography with a Zeiss photomicroscope. Thin sections 400–800 A in thickness were cut with the microtome equipped with a diamond knife and were mounted on copper grids coated with a parlodion film stabilized with carbon. The thin sections were stained sequentially in 5% uranyl acetate at 37° or 60°C for 30–45 min and in lead citrate (18) for 15 min. The latter step was carried out in a humid oxygen atmosphere. The thin sections were examined in a Siemens electron microscope operated at an accelerating voltage of 80 kv. The microscope was equipped with a platinum condenser aperture 200 μ in diameter, a thin silver objective aperture 50 μ in diameter, and a decontamination device cooled with liquid nitrogen.

RESULTS

Light Microscopy

The topography and degree of development of the apical endocytic complex in individual ileal lining cells varies according to the cell's location on the villus. The most highly developed form of the complex is seen in cells located at the crests of villi. Its most striking feature is the giant supranuclear vacuole (Figs. 1 and 2). In three dimensions, its shape is that of an ellipsoid with its major axis coincident with that of the lining cell. The lining cell is approximately 45 μ in height, and almost its entire midportion is filled by the vacuole which may reach 30 μ in length. By virtue of its central position and great volume, the vacuole divides the rest of the cell into two aggregates displaced to the extreme apical and basal poles. The nucleus together with a cluster of mitochondria can be recognized at the basal pole. The lower surface of the vacuole and the nucleus are often in close contact, and, apparently as a result of the greater internal turgor of the vacuole, the upper surface of the nucleus is indented to form a shallow, cup-shaped depression.

The peripheral surface of the giant vacuole is smooth. In the anterior of the vacuole, individual dense masses dispersed amid a pale matrix material are spherical or discoidal in shape and are of varied diameter, a few being quite large (Fig. 2). Some of the masses adhere to the limiting membrane of the vacuole; their surface in the zone of contact conforms to the surface configuration of the membrane and is flattened (Fig. 1).

With regard to the other two components of the apical endocytic complex (the network of apical invaginations and the vesicles into which they empty), the apical invaginations are apparently too small in size to be clearly resolved by light microscopy. Along the apex of the lining cells, a layer of cytoplasm 1–2 μ in width separates the

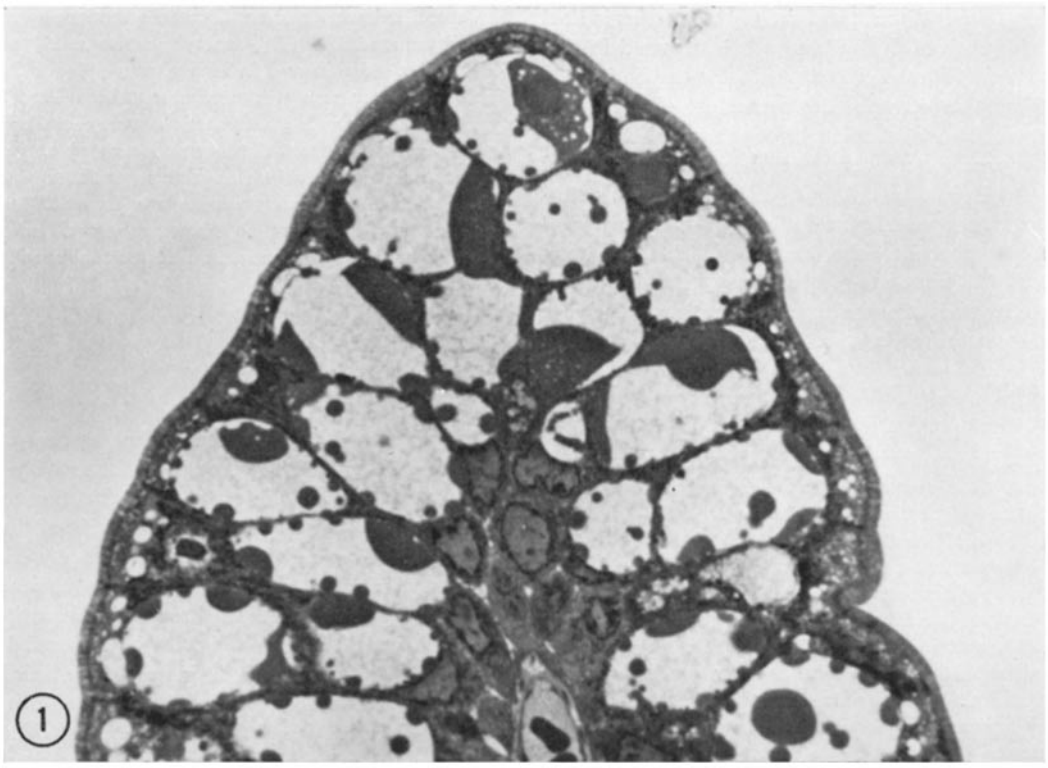


FIGURE 1 Tip of an ileal villus of a 14 day old suckling rat. The plane of section is oblique to the long axes of the epithelial cells. In the middle third of each cell lies a single giant vacuole filling the entire breadth of the cell. Its content consists of a pale matrix material of slightly mottled texture in which are dispersed homogeneous dense granules of spheroidal or discoidal shape. The discoidal granules are generally large and are flattened against the wall of the vacuole. Small spherical vesicles which appear empty are seen in the narrow zone of cytoplasm between the brush border and the giant vacuole. The nuclei of the epithelial cells are clustered below the giant vacuoles near the basement membrane. × 1,090.

brush border from the underlying zone containing the vesicles. The only indication of the presence of the network of invaginations permeating this layer is its barely discernible foamy texture (Figs. 1–3). The apical vesicles are clustered along the upper border of the giant vacuole. They are relatively few in number and are approximately 1–4 μ in diameter. The layer of cytoplasm separating the vesicles from the giant vacuole frequently is extremely attenuated and often appears vanishingly thin, which suggests that some of the vesicles are about to fuse with the giant vacuole (Fig. 2). The content of the vesicles resembles in density the matrix material in the giant vacuole, and the vesicles do not seem to contain material with a density approximating that of the large dense masses in the vacuole. Presumably these masses form by condensation of material after its entry into the vacuole. An unknown fraction of the vacuole's content presumably is maternal antibody which will be cleared from the vacuolar lumen and then pass into the general circulation. The means by which antibody is extracted from the lumen of the vacuole and transferred across the base of the lining cell is unknown, as is the ultimate fate of the remainder of the vacuole's content. The reader should consult reference 4 for a more extensive discussion of this subject.

Electron Microscopy

At relatively low magnification in the electron microscope, the layer of cytoplasm underlying the brush border can readily be seen to be honeycombed with many membrane-limited tubules and small vesicles of a wide variety of shapes and sizes

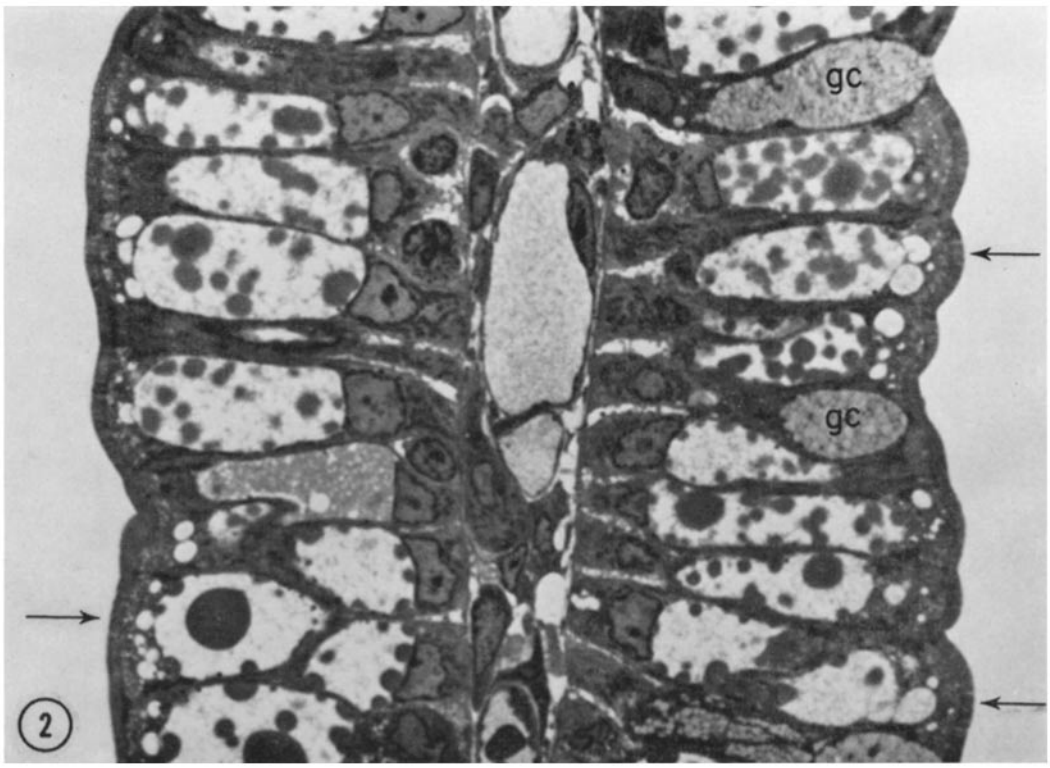


FIGURE 2 A region near the apex of an ileal villus of a 14 day old suckling rat. The plane of section is parallel to the long axes of most of the epithelial cells in the field. A few goblet cells (*gc*) can be recognized among the absorptive cells. A giant vacuole occupies the middle third of each absorptive cell and frequently flattens the apical pole of the nucleus underlying it. Some of the apical vesicles near the giant vacuole appear to be about to fuse with it (arrows). The cytoplasm immediately subjacent to the brush border is foamy in texture. Mitochondria are clustered in the basal cytoplasm below the nucleus. × 1,090.

(Figs. 4 and 5). The largest of these vesicles are usually round and lie close to the giant vacuole. These are the vesicles that can be discerned with the light microscope. In favorable sections (Fig. 5), it is possible to recognize that the tubules form a highly branched, randomly oriented network and that, at scattered points which are actually the introits to the network, the apical plasma membrane covering the microvilli is continuous with the limiting membrane of the tubules. The interior of the network appears, therefore, to be in open communication with the intermicrovillous space at the base of the striated border.

At high magnification, the plasma membrane covering the microvilli of the brush border has the structure of a conventional unit membrane (Fig. 6). The outer and inner dense leaflets of the membrane are of equal density, and the lumenal surface of the outer leaflet is coated with a layer of moderately dense amorphous material. This material is probably a rudimentary and poorly developed counterpart of the filamentous, carbohydrate-rich material that forms a prominent coating on the brush border of intestinal lining cells of cat, bat, and man (19). In normally sectioned membranes, the amorphous coating material infrequently appears aggregated into small clumps more or less regularly spaced along the membrane's surface, which suggests that there might be some orderly pattern in its distribution. However, corroborating evidence suggestive of order in the surface cannot be detected when the membrane is seen in fullface view in tangential sections.

It is of some interest to point out that a finely dispersed, particulate material of moderate electron opacity is occasionally observed in the inter-

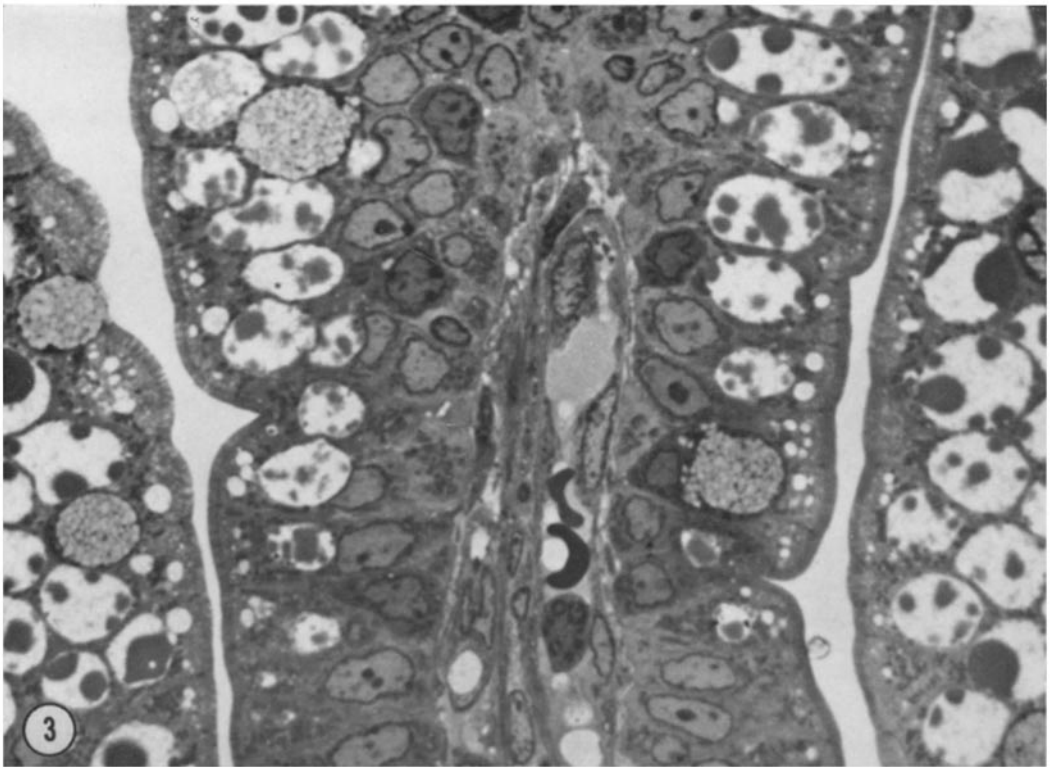


FIGURE 3 The basal portion of an ileal villus of a 14 day old suckling rat appears in the center of the field. Two components of the apical endocytic complex, specifically the apical vesicles and giant vacuole, can be observed in the cells near the upper margin of the figure. These components gradually become smaller and are no longer visible in the cells towards the base of the villus at the lower margin of the figure. × 1,090.

microvillous spaces of the brush border and also in the lumens of the apical invaginations (Fig. 6). The particles appear as tiny rods or spheres, all having approximately the same density. Their greatest dimension does not exceed 75 A. They are of the same order of size as that reported for micelles formed as an end product of the digestion of dietary lipid, but it seems unlikely, for several reasons, that they represent lipid in the process of being absorbed. In the first place, according to physiologic and morphologic data lipid absorption takes place under normal conditions in the first part of the small intestine, i.e. in the duodenum and initial segment of the jejunem. Consequently, this material should be extracted from chyme before it reaches the terminal portion of the ileum. In the second place, intestinal lining cells convert absorbed lipid into chylomicra which are released into the lamina propria and are taken up by lacteals. Chylomicra can be readily recognized by light and electron microscopy in all three of these locations. Since in this study chylomicra have not been observed anywhere in the terminal ileum, it seems reasonable to conclude that this region of the small intestine of the suckling rat, like that of the adult, does not play a significant role in the digestion and absorption of triglyceride. Alternatively, the size, shape, and density of the particles, as well as their localization among microvilli and in apical invaginations, are consistent with the possibility that the particles are protein molecules in the process of being absorbed.

In contrast to the conventional appearance in ultrathin sections of the unit-membrane structure of the apical plasma membrane covering the microvilli, the limiting membrane of the apical invaginations and vesicles displays a unique ultrastructural differentiation. This differentiation is

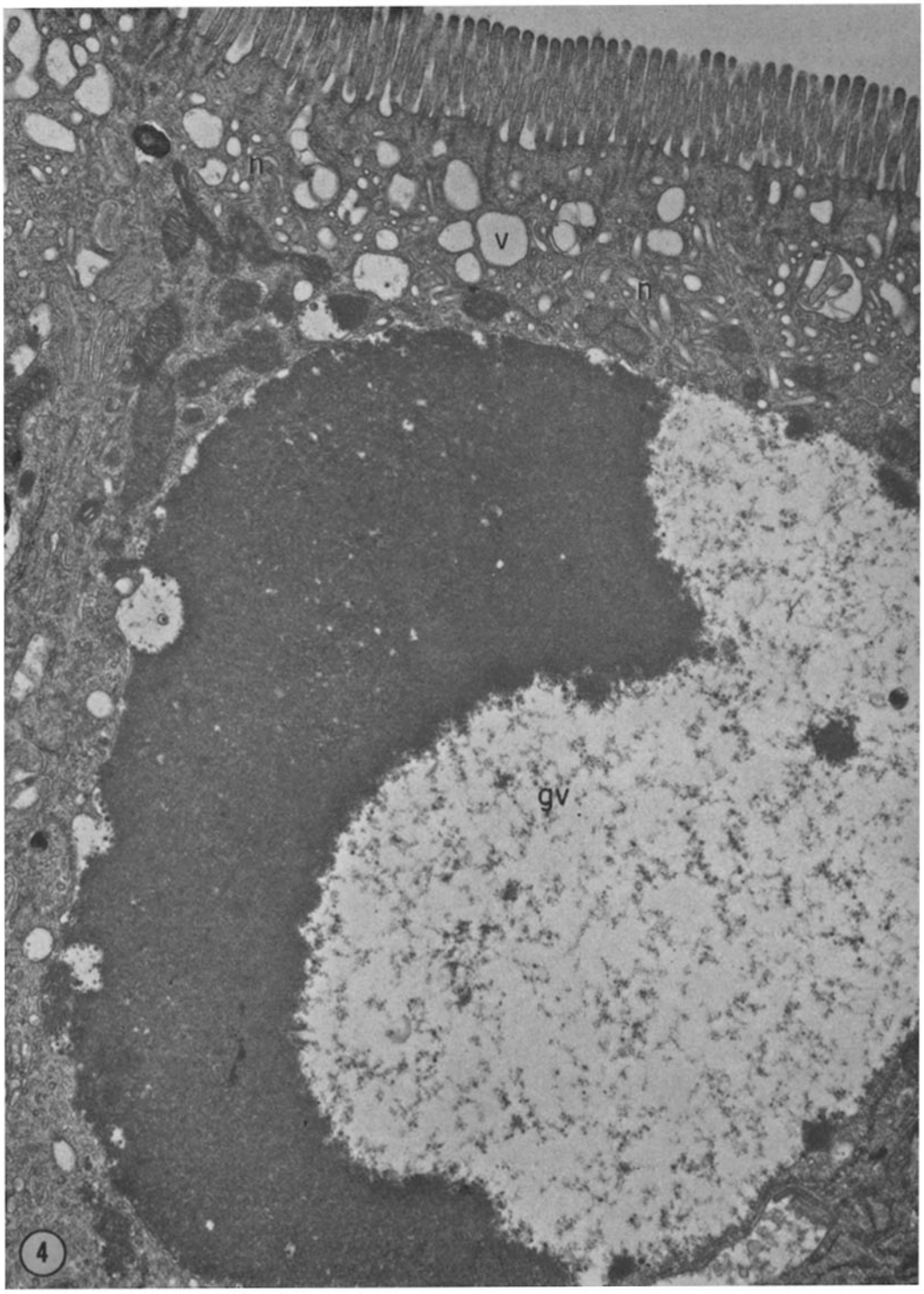


FIGURE 4 Electron micrograph (at low magnification) of the apex of an ileal absorptive cell of a 14 day old suckling rat. In the field appear the three components of the apical endocytic complex: the network of tubular and vesicular invaginations of the apical plasma membrane (*n*), apical vesicles (*v*), and the giant supranuclear vacuole (*gv*). The apical network is seen as many small circular or elongated membrane profiles which permeate the cytoplasm around the apical vesicles. A large dense granule is flattened against the wall of the giant vacuole, and the rest of the vacuolar lumen is filled with tiny clumps of finely fibrillar material. The vacuole's limiting membrane is not clearly visualized at this magnification. × 13,000.

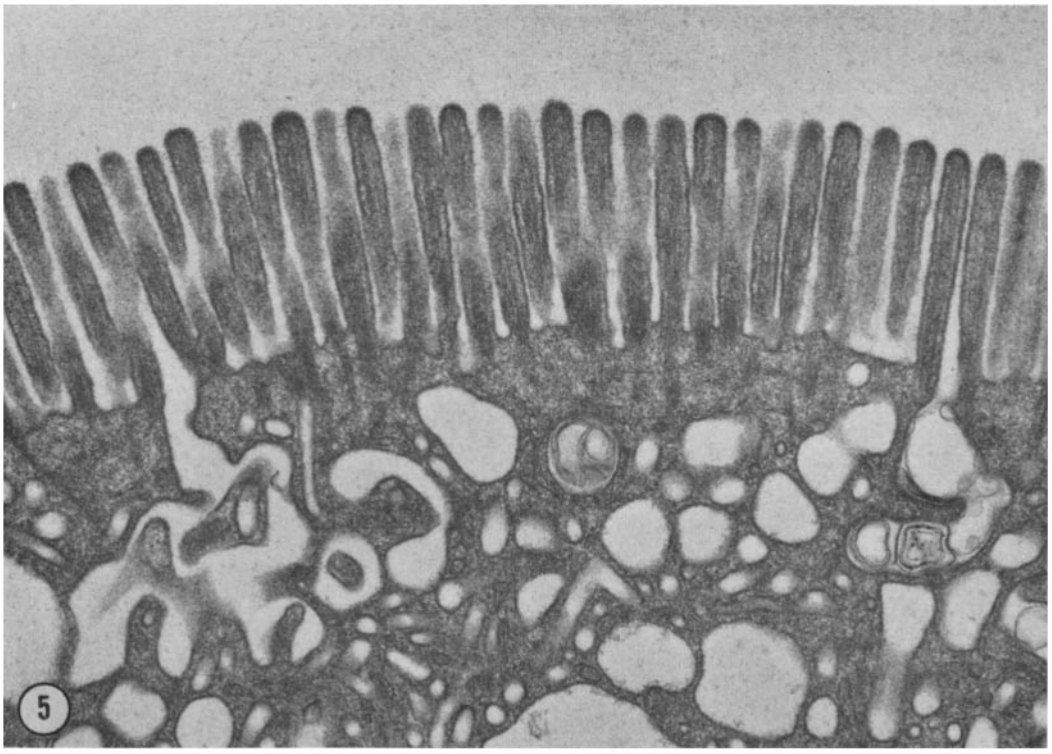


FIGURE 5 A portion of the apical surface of an ileal lining cell from a 14 day old suckling rat. The plane of section is perpendicular to the surface of the cell. The cytoplasm underlying the brush border is honeycombed with many membrane-limited clear spaces of pleomorphic outline representing the sectioned appearance of a network consisting of tubular and vesicular elements. Near the left and right margins of the figure the network opens at the surface of the cell between the bases of microvilli, and at these points the apical plasma membrane is continuous with the limiting membrane of the network. The extensive branching of the apical invagination near the left margin illustrates the degree of interconnection among elements of the network. Interconnections are not so apparent elsewhere in the field. The apical invagination near the right margin is filled with a myelin figure, an artifact which occurs in aldehyde-fixed but not in OsO_4-fixed specimens. × 27,000.

seen in all specimens examined, irrespective of whether the specimen was fixed initially with aldehyde or osmium tetroxide, and is therefore assumed not to be an artifact arising from special conditions of tissue preservation. It is most readily discerned in the membrane of the invaginations and the small vesicles close to the apical surface of the cell, and it is less apparent in the membrane of the larger vesicles situated deeper in the cytoplasm near the giant vacuole. The membrane of the giant vacuole itself has a conventional, unmodified unit-membrane structure.

The appearance of the differentiation depends upon the orientation of the membrane with regard to the plane of section. In profile view, the membrane may have one of three appearances. In most locales it resembles the limiting membrane of the microvilli and consists of four layers (*1* in Fig. 8). The inner three layers constitute a conventional unit membrane approximately 75 A thick, whereas the outermost layer is a relatively homogeneous one approximately 70 A thick which adheres closely to the outer dense leaflet of the unit membrane. This outermost layer is somewhat less electron opaque than the outer dense leaflet of the unit membrane. Although the unit-membrane component of the limiting membrane still appears continuous and uninterrupted in sections thinner than 800 A, the outermost layer commonly does not. Instead, it sometimes appears as a single row of

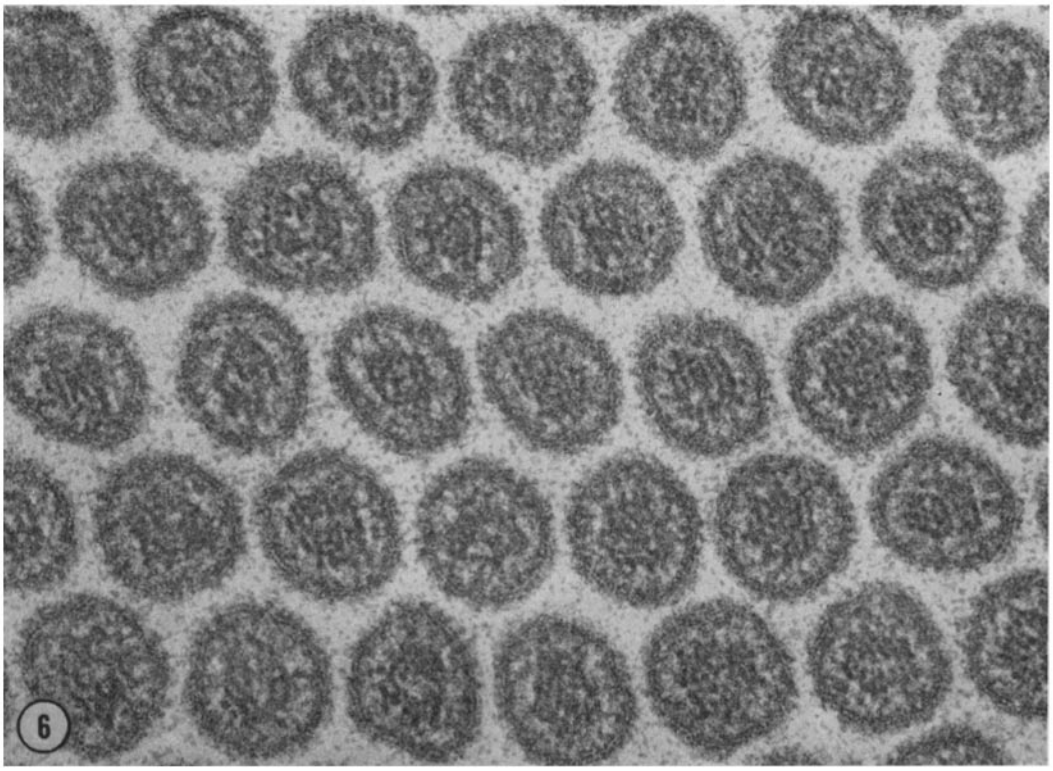


FIGURE 6 Microvilli on the apical surface of an ileal absorptive cell. The plane of section is normal to the long axes of the microvilli. The latter are arranged in precise hexagonal array. Each microvillus is limited by a typical unit membrane which is obscured owing to the density of the lumenal coating material and the cytoplasmic matrix apposed, respectively, to its outer and inner surface. The lumenal coating material seems to be irregularly globular in texture. The axial bundle of parallel fine filaments in the core of each microvillus is cut in cross-section and has a dense punctate appearance. The intermicrovillus space contains many small particles 75 A or less in diameter. × 108,000.

small particles 60–80 A in diameter affixed at regular intervals to the outer dense leaflet of the unit membrane (*2* in Fig. 8). The center-to-center spacing between adjacent particles in regions where they can be clearly visualized is approximately 120 A. Another feature of the limiting membrane makes its appearance in thin sections but with considerably less frequency. In this case, the particulate nature of the outermost layer is still apparent, and the appearance of the outer dense leaflet is altered; it appears interrupted or "hyphenated" at regular intervals along its length, and one particle is attached to each "hyphen" (*2* in Fig. 7; *3* in Fig. 8; arrows in Fig. 9). Particles never overlie interruptions of the outer dense leaflet.

Where the plane of the limiting membrane is oriented somewhat obliquely to that of the section, i.e. at an angle of approximately 45°, the limiting membrane usually has one of two appearances. On the one hand, it may appear as a homogeneous broad band of moderate electron opacity with no indication of periodic substructure within it. This is the appearance that one would expect of a conventional unit membrane observed under these conditions. On the other hand, it may appear as a broad band crossed by regularly spaced dense striations alternating with striations of fainter density (*4* in Fig. 8). The width of the dense striations closely approximates the diameter of the particles observed in profile view of the membrane, and, similarly, the center-to-center spacing of the dense striations corresponds to the center-to-center spacing of the particles. The aspect of the membrane in the latter orientation could create the impression

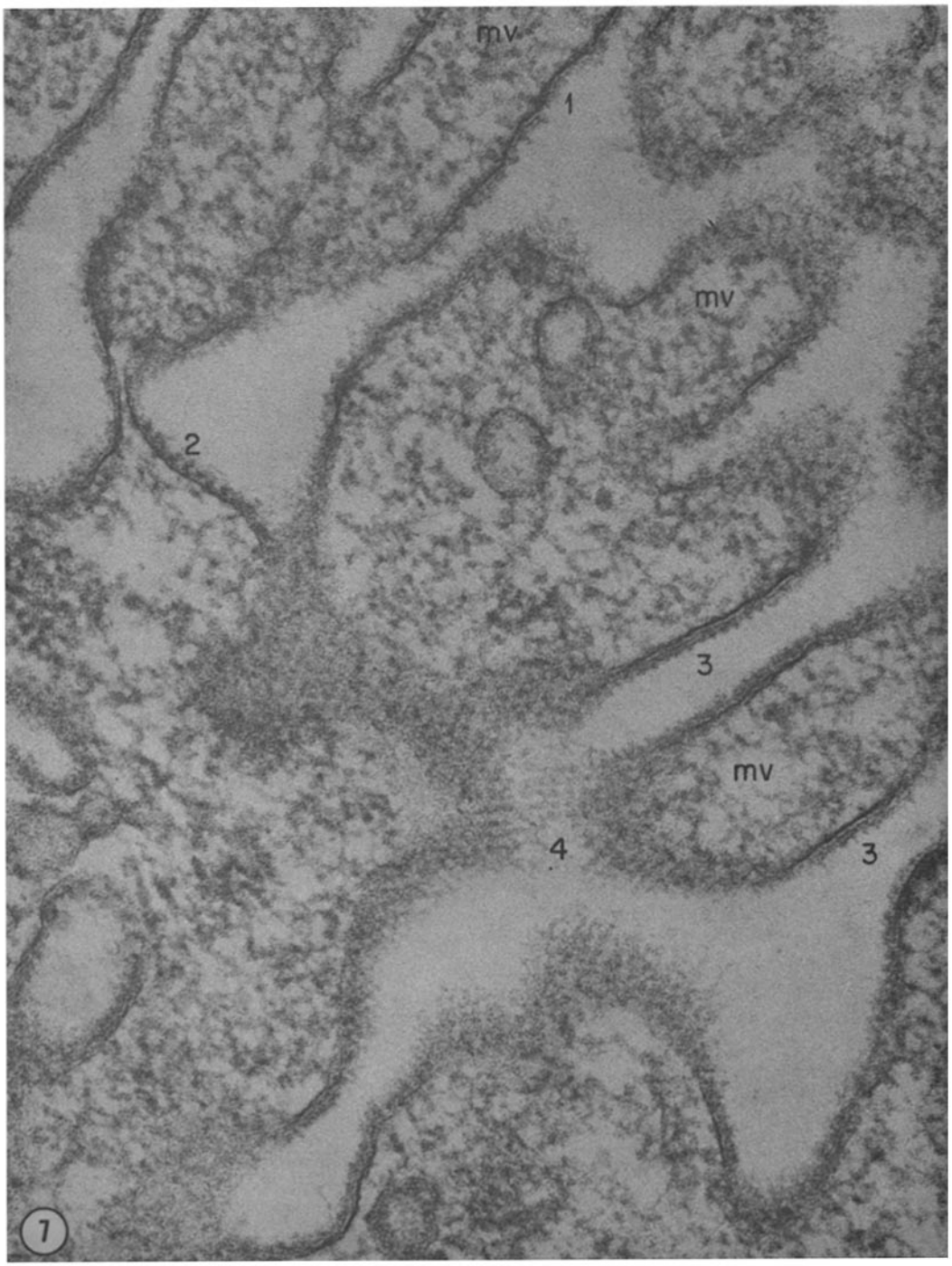


FIGURE 7 Introits into the network of tubules and vesicles formed by invaginations of the apical plasma membrane of an ileal absorptive cell. The bases of several microvilli (*mv*) appear in the upper right portion of the field. Their limiting membrane is coated with a coarse-textured material which, when the membrane is cut normally (*1*), shows no indication of substructure. At the introits near the base of the microvilli, the appearance of the plasma membrane and its external coat changes. At one point (*2*), only its cytoplasmic dense leaflet is continuous, and its lumenal face is lined with a row of regularly spaced particles of uniform size. In other regions the array of particles on the outer face is less clearly visualized (*3*), and the membrane appears to possess an additional or fourth layer external to the three layers comprising its unit membrane. Where the membrane is sectioned tangentially, particles are seen arranged in linear rows or in a two-dimensional square lattice (*4*). × 148,000.

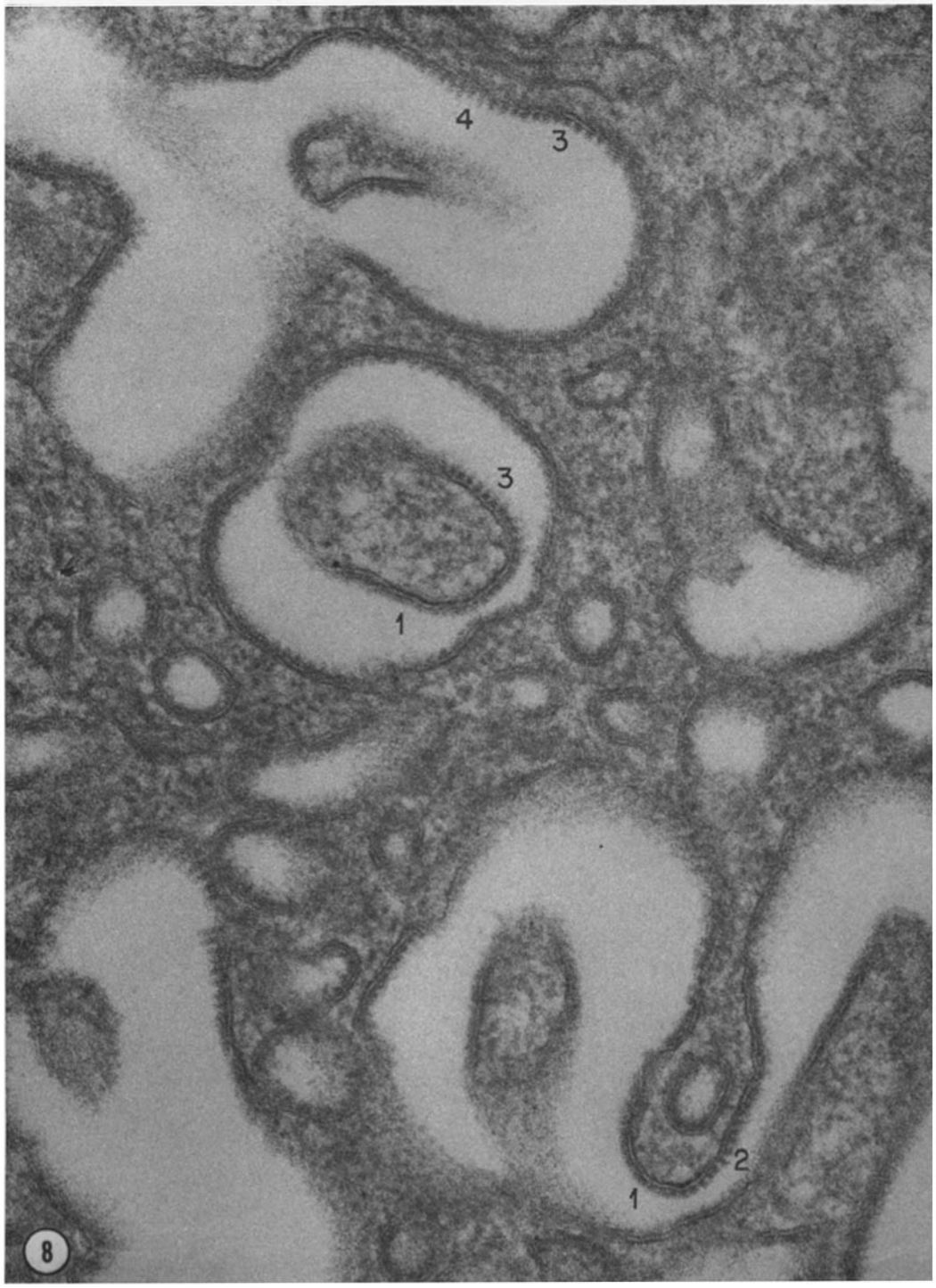


FIGURE 8 Part of a network formed by invaginations of the apical plasma membrane of an ileal absorptive cell. At certain points, the limiting membrane of the network is cut in normal section (*1*) and appears like a conventional unit membrane with the addition of a homogeneous dense layer applied to its lumenal face. Elsewhere the outermost layer assumes the form of a row of small particles affixed at regular intervals to the lumenal surface of the unit membrane (*2*). In a similar plane of section, only the cytoplasmic dense leaflet is hyphenated, and a small particle is attached to the lumenal face of each hyphen (*3*). Where the membrane is sectioned more obliquely (*4*), linear striae appear running at right angles to the profile of the membrane, indicating that the particles are aligned in rows. × 140,000.

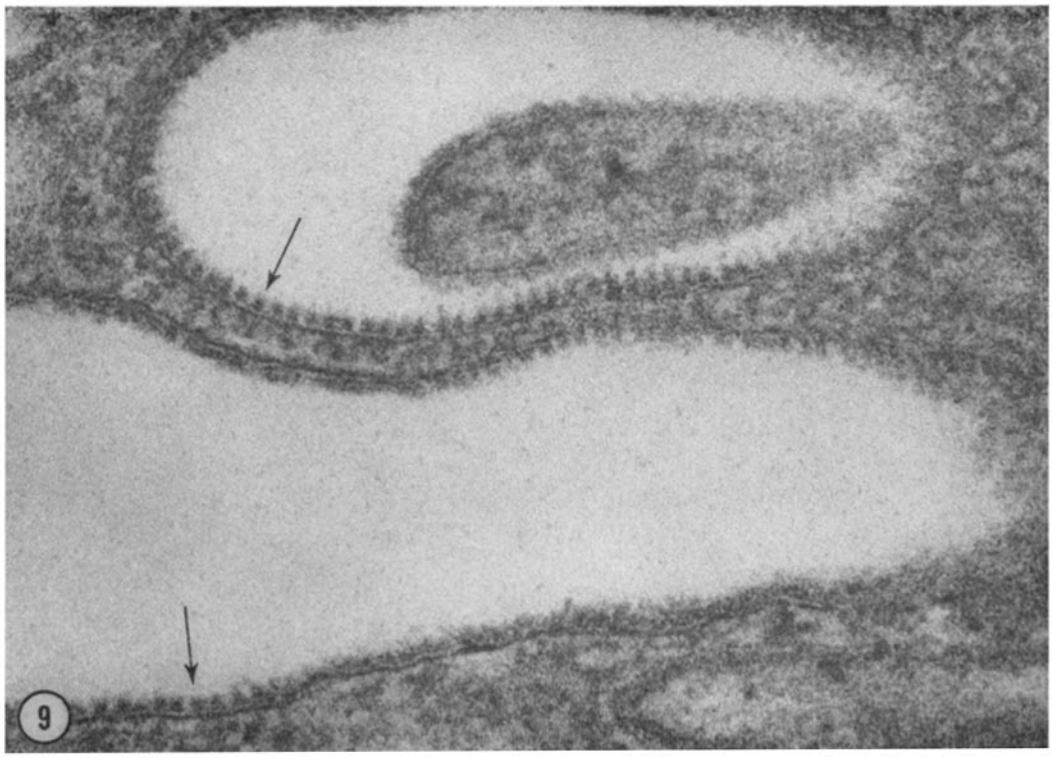


FIGURE 9 A segment of the apical network of tubules and vesicles in an ileal absorptive cell of a 14 day old suckling rat. At the points indicated by arrows, the plane is normal to the membrane and to the rows of particles on its lumenal face. At these points, the discrete nature of the particles, the hyphenation of the lumenal dense leaflet to which they are attached, and the continuity of the cytoplasmic dense leaflet can be visualized. × 190,000.

that it is covered by a regular array of parallel ridges (7). However, this interpretation is inconsistent with the appearance of the membrane when its surface is visualized in fullface view as described below.

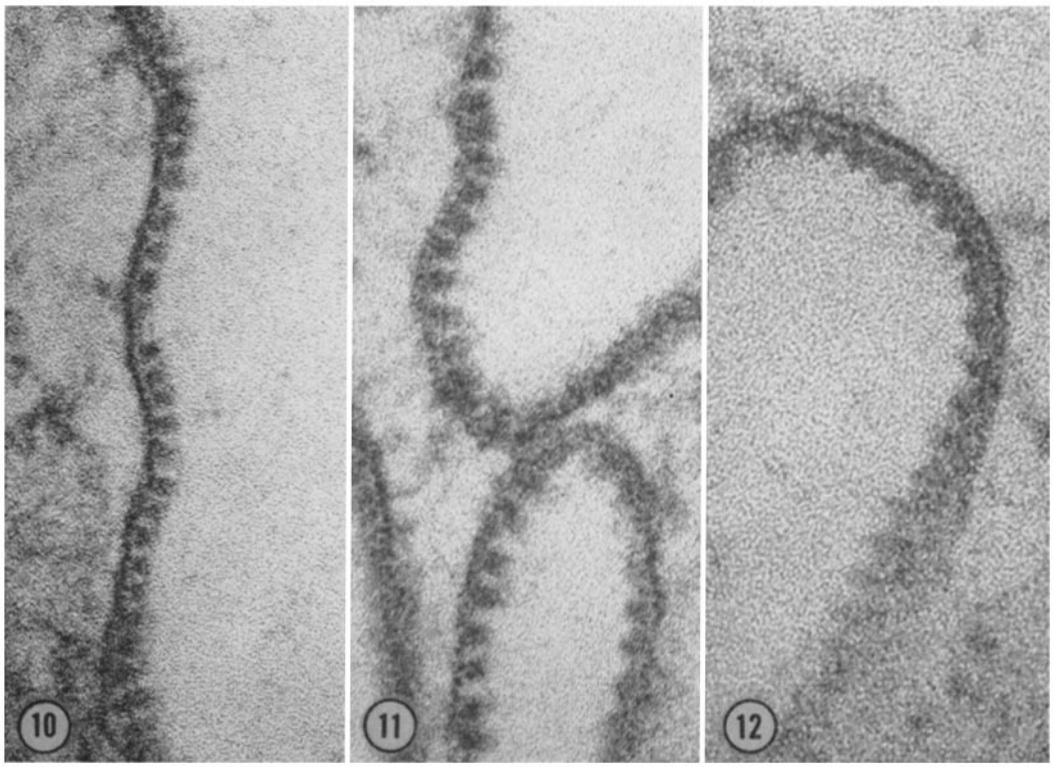
In regions where the orientation of the limiting membrane closely parallels the plane of section, the membrane has the appearance of a layer of particles of uniform size arranged in a regular repeating pattern (*4* in Fig. 7). The pattern appears to have the form of a square lattice in which the particles closest to any given particle are four in number and are equally spaced. This type of lattice would be formed by particles arranged in two sets of equally spaced and mutually perpendicular rows in which the particles of neighboring rows are in register. The distance between the rows of the lattice observed in sections corresponds roughly to the distance between particles observed in profile view of the membrane. Each particle, however, appears only as a poorly visualized node in the lattice, and one can neither visualize its limits clearly, nor, therefore, measure its diameter accurately.

In retrospect, the various appearances of the limiting membrane of the invaginations and vesicles in profile view can also be considered consistent with the interpretation that its lumenal face is coated with particles of uniform size arranged in two mutually perpendicular sets of rows. Where the three leaflets of the unit membrane are clearly visualized, one can assume that the plane of the membrane is oriented precisely at right angles to the plane of section. Taking into consideration the size of the particles, the space between them, and the thickness of the section, one can also assume that, at any given point, the depth of the section contains at least three or four particles arranged, with varied orientations, one behind the other. In those instances in which the particles

appear individually and clearly separated from one another, we conclude that one set of rows is oriented precisely perpendicular to and the other precisely parallel with the plane of section. The image of a single particle, then, actually represents three or four particles aligned one directly behind the other. When the orientation of the plane of the particles is perpendicular to the plane of section but the axes of the rows of particles are oblique to it, the particles no longer are situated one directly behind the other. In this case the layer of particles appears as a continuous stratum, and its thickness corresponds to the diameter of the particles.

The location of the periodic interruptions in the outer dense leaflet of the underlying unit membrane is related to the arrangement of particles on the surface of the membrane, and the width of each interruption in relation to the thickness of the section is minute. It is clear that the interruptions will be visualized only when they are arranged squarely one behind the other, i.e. when the particles attached to the membrane appear clearly individual owing to the fact that their rows are oriented precisely perpendicular to the plane of section.

We stated previously that the image of the membrane seen where the plane of the membrane parallels the plane of section constitutes the main evidence in support of the assumption that the structures attached to the membrane are particles arranged in a two-dimensional square lattice rather than a set of parallel electron-opaque rods.



FIGURES 10–12 Profiles of the limiting membrane of apical tubules and vesicles at higher magnification. In Figs. 10 and 11, individual particles and their associated underlying hyphen of the lumenal dense leaflet appear fused together to form a single composite structure with a diameter of 60–80 A. The center-to-center spacing of the particles is approximately 120 A. The cytoplasmic dense leaflet is continuous in both figures. In Fig. 12, the individuality of each particle is clearly recognizable, and both the lumenal and cytoplasmic dense leaflets appear continuous. The hyphenation of the former is probably masked by a slight tilt of the membrane away from a plane normal to the plane of section. Fig. 10, × 300,000; Figs. 11 and 12, × 350,000.

We also pointed out that the individual particles were indistinctly visualized under these geometric conditions. There are probably several reasons for their lack of clarity. First, each particle is being viewed individually and is embedded in a matrix of embedding medium that is generally 600–800 A thick. Second, the imaging electrons may also be passing through the three leaflets of the unit membrane and possibly through a layer of cytoplasmic matrix adjacent to the membrane. The presence of these additional components in the section contributes to the background density and undoubtedly obscures the particles by reducing their relative contrast. Another possible explanation for the obscure image of the particles is that the outer dense leaflet of the membrane appears hyphenated in profile view, and one particle is attached to each hyphen. This appearance may indicate that, in the plane of the membrane, the outer leaflet is not a continuous layer but is actually subdivided into a mosaic of plaques of uniform size, each plaque underlying one particle. If this should prove to be true, then the densities at the nodes of the square lattice seen in fullface view of the membrane are a summation of the density of a single particle and the segment of the outer dense leaflet to which it is attached. Although the superimposition has the beneficial effect of accentuating the density of nodes in the lattice, it also has the disadvantage of obscuring the outlines of the two components at each node.

Little can be said about the fine structure of each individual particle of the lattice (Figs. 10–12). "Single" particles are most distinctly outlined in profile views of the membrane when the rows of particles are oriented perpendicular to the plane of section. The image of each single particle under these conditions in reality represents a row of three or four particles positioned one directly behind the other. Its outline is usually round, and it is attached directly to the outer dense leaflet of the membrane; there is no indication of a stalklike structure linking the two. In most instances, the particle appears solid. On rare occasions, however, a pale center appears in the particle, and the outline of the particle resembles a square.

DISCUSSION

The limiting membrane of certain components in the apical endocytic complex of ileal lining cells is structurally modified in two respects. Its external dense leaflet is discontinuous and subdivided into an ordered planar array of minute discrete plaques of uniform size, and a small particle is attached to the outer surface of each plaque. The appearance of segmentation of the external dense leaflet can be interpreted in a number of ways. The major constituents of the external dense leaflet, presumably proteins, may actually be distributed as a mosaic in vivo, and the appearance in thin sections is, therefore, an accurate representation of their distribution in the living cell. Or, the leaflet could exist as a continuous stratum in vivo but consist of two classes of protein; one underlying the particles is insolubilized by fixation and becomes electron opaque following exposure to the heavy metal ions in the fixative and staining solutions, whereas the other underlying the space between particles may be either extracted during specimen preparation or, although still present, may not couple with sufficient heavy metal ions to become electron opaque and visible in the electron microscope. In the latter case, the appearance of segmentation would be artifactual. It is also conceivable that projections of the lipid constituents of the membrane, which lack electron opacity and are ordinarily visualized as a negative image in thin sections, extend from the middle leaflet of the membrane through the external dense leaflet as regularly arranged septa subdividing the dense leaflet. Other interpretations are also possible, but we need not dwell on this point since it is not possible at the present time to decide which alternative is the correct one.

Structural differentiation of the external dense leaflet, whether real or artifactual, has been observed in other cells. In disc-shaped vesicles of superficial epithelial cells lining the urinary bladder, parallel ridgelike thickenings spaced 130 A apart appear in the leaflet (7). The external leaflet of the axolemma in paranodal regions of nodes of Ranvier is subdivided into circular bands which form hoops or helices girdling the axon at right angles to its long axis (20, 21). Where the extracellular or lumenal faces of two apposed membranes come into intimate contact, ordered substructure either in the form of particles or septa attached to the membranes or in the form of an ordered pattern of linear thickenings of the conjoined external dense leaflets may appear in the line of fusion. Examples of the former are observed in septate desmosomes examined under high resolution (22–25) and in granules of the epidermal Langerhans cell (26–28). Examples of

the latter are observed in the synaptic discs of club endings on the giant Mauthner neuron (29). Descriptions of particles associated with the surface of an individual membrane have also been published. The most extensively studied of these particles are those associated with the internal membrane of mitochondria (30). These particles have recently been isolated in relatively pure form by Racker and Horstman (31) who report that the particles contain an ATPase activity essential for coupling phosphorylation with oxidation. Slautterback (32) recently described a highly ordered array of "pegs" and globules occurring on the lumenal face of the limiting membrane of apical invaginations and vesicles in absorptive cells of *Hydra*; these structures may also be considered components of an apical endocytic complex since they sequester tracer material introduced into the gastrovascular cavity.[1]

From this brief and incomplete review of the pertinent literature, it seems clear that structural differentiation of cell membranes may take a variety of forms. Constituents of the unit membrane itself may be altered, or an array of appendages may be attached to its surface. In rarer instances, as in the case of the ileal lining cell, a combination of both types of modifications may occur. From their variety we may conclude that the modifications do not conform to an underlying structural pattern. This suggests that the nature of the modification is related in some way to the particular functional properties of individual membranes.

[1] Slautterback refers to the invaginations and vesicles as "coated," on the basis of the presence of the particulate arrays on the lumenal face of their limiting membrane, though he recognizes that the cytoplasmic face of the membrane is smooth or "uncoated." His classification of the vesicles may be questioned since, in most articles published thus far, the chief criterion for applying the term coated to an invagination or vesicle has been the presence of a fibrillar or alveolate coating material along its *cytoplasmic* face. We can report, as an incidental observation from our study of the ileal lining cell, that the type of particulate substructure we describe in this paper also occurs in membranes of apical invaginations and vesicles that are coated in the conventional sense. The latter were seen only infrequently, and their morphologic and functionl relationship to the preponderant uncoated components is still not clear. We know from studies of other cells (38–40), for example, that the sequestering of tracer protein is not limited to coated vesicles but can also be carried out by ordinary pinocytic vesicles.

The functional role of the particles attached to membranes of the apical network and vesicles is currently a matter of speculation. Enzymes involved in the hydrolysis of small carbohydrate molecules and peptides have been detected in fractions of brush borders obtained from intestinal lining cells (33, 34). Morphologic study by negative staining of intestinal brush borders has shown that small particles 60 A in diameter are attached to the lumenal surface of the limiting membrane of their microvilli (35). These particles have been separated from the membrane by fractionation procedures and have been found to contain the aforementioned digestive enzymes (36, 37). Although the structure of the brush border of intestinal lining cells has been extensively studied in sectioned specimens, particles of this size have not been visualized on the lumenal surface of its limiting membrane. A sparse enteric coating (19) covers the microvilli in the brush border of the suckling rat's ileum, but does not extend into the underlying network of apical invaginations and vesicles despite the fact that the limiting membranes of these structures are continuous. At the point where the feltwork terminates at the base of each microvillus, particles which are similar in size to those observed on negatively-stained brush borders make their appearance and coat the lumenal surface of the underlying membranous network. The particles observed in this study may also coat the membranes of the microvilli, but their presence at the latter surface might be obscured by the enteric coat. If this proved to be the case, there would be reason to suspect that the particles associated with components of the apical endocytic complex contain digestive enzymes similar to those detected on the membrane of the brush border.

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