EFFECTS OF PUROMYCIN ON THE STRUCTURE OF RAT INTESTINAL EPITHELIAL CELLS DURING FAT ABSORPTION

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ABSTRACT

This report provides information on the morphology of rat intestinal epithelial cells during fat absorption. In addition, the role of protein metabolism in this process has been evaluated by blocking its synthesis with puromycin and studying the fine structure of mucosal cells from rats at various times after fat intubation. The results indicate that SER-derived vesicles, containing fat droplets, migrate from the apical cytoplasm of the absorptive cell and fuse with saccules or vacuoles of the Golgi complex. Arguments are made that the Golgi complex is important in completing chylomicron formation and in providing appropriate enveloping membranes for the chylomicron. Such membranes may be necessary for Golgi vacuoles to fuse with the lateral cell membranes and release chylomicra. Puromycin treatment causes the absorptive cell to accumulate increased quantities of lipid that are devoid of membrane during fat absorption. In addition, puromycin-treated cells contain much less RER and Golgi membranes are strikingly decreased in number. In this paper we discuss the consequences of these abnormalities and suggest that continued protein synthesis by the RER is required in order to generate Golgi membranes. If such membranes are absent the cell's ability to discarge chylomicra is impaired and lipid accumulates.

INTRODUCTION

The process of fat absorption in the rat intestinal epithelial cell is well suited for microscopic analysis because droplets of triglyceride are visible in the light and electron microscope following appropriate staining. Thus, the pathway of lipid through organelles of the absorptive cell may be studied by identifying fat droplets at various time periods after administration of triglyceride (11, 24, 43, 57) or otherwise experimentally manipulating the animal during fat absorption (49, 51, 56, 59). From such morphological studies, much information has been obtained, and general hypotheses have been proposed for the movement of lipid from the apical cytoplasm to its discharge into the central lacteal of the villus (11, 24, 31, 43, 56, 57). In addition, a great deal of biochemical evidence is available on all aspects of the absorptive process, allowing morphologists to correlate their observations with this data so as to assemble a working hypothesis describing intestinal fat absorption (11).

Most workers agree that fat transport begins by passive diffusion of products of triglyceride breakdown in the gut lumen (8, 10, 21, 34, 35) into the apical cytoplasm of the absorptive cell (7, 11, 25, 26, 52, 56, 57). Within the cytoplasm, free fatty acids and 2-monoglycerides are reesterified into triglycerides by microsomal enzymes, and lipid is

complexed with protein, cholesterol, and phospholipid to form chylomicra (52). The role of the smooth endoplasmic reticulum in the resynthesis of triglyceride is recognized (11, 45, 52, 56, 57); however, the precise function of other cellular organelles in the formation of chylomicra is not clearly defined. For example, lipid droplets occur in vacuoles of the Golgi complex during fat absorption, suggesting a functional role for this organelle in fat transport, but the nature of this role is unclear.

The importance of protein synthesis by the intestinal epithelial cell during fat absorption has been emphasized (16, 51) even though the concentration of protein in chylomicra is relatively low (approximately 0.5%) (52). Apparently, the protein component is required for transport of fat particles (16, 51, 64), and its absence in patients with congenital β-lipoprotein deficiency causes malabsorption of lipid (16). Since protein synthesis is involved in many aspects of cellular activity, we considered it worthwhile to investigate further the effects of blocking protein synthesis at the ribosomal level with puromycin (15) and studying the effects of this antibiotic on the structure of intestinal epithelial cells during fat absorption. Thus, we have studied and compared the gross anatomy of the small intestine from fasted, fat-fed, and puromycin-treated rats fed fat. In addition, light and electron microscope studies of mucosal cells from 24-hr fasted rats, animals fed fat for 15, 30, and 60 min, and rats treated with puromycin and fed fat for similar periods of time have been completed.

In this paper, we describe changes in the organization of membranes of the smooth and rough endoplasmic reticulum of the intestinal epithelial cell during fat absorption. The structure of the Golgi complex in absorptive cells from fasted and fat-fed animals is contrasted, and a possible function for the Golgi complex proposed. This paper illustrates abnormal development of membranes of the endoplasmic reticulum and Golgi complex in absorptive cells from animals treated with puromycin. Based on these results, we argue that continual formation of membrane components by the rough endoplasmic reticulum is required for maintenance of the Golgi complex and smooth endoplasmic reticulum. Our results with puromycin treatment support the hypothesis developed by others (28, 51) that the primary defect in fat transport after treatment with this antibiotic is the inability of the intestinal absorptive cell to release triglyceride. We propose that puromycin treatment causes a deficiency of Golgi membranes which normally envelop the lipid. This defect blocks normal movement of lipid within the cell and is responsible for its inability to release chylomicra.

MATERIALS AND METHODS

Male rats (150–200 g each) obtained from Charles River Breeding Laboratories, Wilmington, Mass., were divided into three experimental groups: (*a*) eight fasted rats, (*b*) 27 fat-fed animals, and (*c*) 13 puromycin-treated rats fed fat. The fasted animals (group *a*) were deprived of food and maintained in cages which prohibited coprophagia for 24 hr before they were killed. Rats in group *b* were fasted for 24 hr, lightly anesthetized with ether, and fed 1.5 ml of Mazola corn oil (11, 43, 47, 51) by means of a thin polyethylene tube inserted via the esophagus into the stomach (43). Segments of upper jejunum were removed for study at intervals of 15, 30, and 60 min following feeding. Fasted animals in group *c* received 16 mg of puromycin (Nutritional Biochemicals Corporation, Cleveland, Ohio) dissolved in buffered salt solution (0.04 M phosphate buffer, pH 7.4 in 0.154 M NaCl) over a 10 hr period. Puromycin was injected (i.p.) hourly (2.5 mg/dose) for 4 hr followed by injection of 1 mg/dose each hour for 6 hr before fat intubation as described above, and intestinal segments were removed for study at intervals of 15, 30, and 60 min following feeding. Control saline-injected rats showed no morphological deviation from the pattern of fat absorption observed in uninjected rats.

The techniques for electron microscopy utilized in this study represent slight modifications of the procedures described by Cardell et al. (11); therefore, only a brief description is necessary. After the appropriate experimental procedure a small segment of the upper jejunum was exposed, removed rapidly, and placed in a drop of fixative. The specimen was cut into several pieces in such a manner as to produce small rectangular blocks of tissue with their long axes parallel to the longitudinal axis of the gut. Considerable care was exercised in the dissection method to insure proper longitudinal orientation of villi in the final embedding.

Three fixation procedures were employed: (*a*) cold 1% osmium tetroxide (at pH 7.3 in phosphate buffer) for 4 hr, (*b*) 3% glutaraldehyde (Fisher Biological Grade) buffered in 0.1 M cacodylate for 2 hr, or (*c*) glutaraldehyde in 0.1 M phosphate buffer at pH 7.3 for 2 hr (50). Following glutaraldehyde fixation, segments were rinsed with 10% phosphate-buffered sucrose and postfixed in phosphate-buffered osmium tetroxide for 2 additional hr. Dehydration was accomplished with a graded series of either

alcohol or acetone. Acetone dehydration permitted in-block staining with KMO_4, but alcohol dehydration yielded better lipid droplet preservation. Samples in 100% acetone were stained for 15 min in 0.5% KMO_4, rinsed with 0.04% (by volume) methyl acrylate and returned to 100% acetone (44).

With the use of a modification of Luft's technique (32), specimens were embedded in both Epon and a mixture of Araldite and Epon resins, and sectioned with a Porter-Blum MT-2 ultramicrotome equipped with a diamond knife. Thick sections (0.5–1.0 μ) prepared for light microscopy were stained with 1% toluidine blue in 1% sodium borate, while thin sections were doubly stained with 2% uranyl acetate (60) and lead citrate (48) and studied with a Philips EM 300 electron microscope. Additional light microscope studies were made on specimens fixed in acrolein (10%), embedded in glycomethacrylate, thin sectioned (0.5-1.0 μ), and stained with toluidine blue and acid fuchsin (1).

The ultrastructural aspects of this study are based on evidence obtained from 705 electron micrographs of intestinal absorptive cells from fasted and fat-fed rats, and 405 electron micrographs of cells from puromycin-treated animals fed fat. The micrographs represent selected images of absorptive cells predominantly from the upper one-third of the villus where fat absorption is considered most active (42).

OBSERVATIONS AND RESULTS

Our interpretations depend upon observations at three levels of organization: (*a*) gross morphology, (*b*) light microscopy, and (*c*) electron microscopy. It seems appropriate to present the results accordingly.

Gross Morphology

24 hr of fasting depletes the jejunum of its contents and causes the intestinal walls to appear collapsed. In the fasted rat the lymphatics and small blood vessels in the mesentery around the intestine are difficult to identify, but 60 min after corn oil administration they dilate and the lymphatics appear milky white. Fat feeding also causes the duodenum and upper jejunum to distend and to lose their collapsed appearance. The jejuna of the puromycin-treated rats fed fat expand similarly to untreated animals 60 min after corn oil intubation. However, the lymphatic vessels of the puromycin-treated animals are not dilated and do not appear white like those of untreated rats fed fat; in these respects they resemble the lymphatics of fasted animals.

Light Microscopy

FASTED RATS: In control animals, fasted 24 hr, the intestinal absorptive cells of the jejunal villus contain no conspicuous lipid (Fig. 1). However, at higher magnifications small inclusions resembling fat droplets are identified in the cytoplasm (Fig. 2). These inclusions are not localized to specific cellular regions and they appear in the intercellular spaces as well as in the lamina propria (Fig. 2). Attention is directed to the basophilic region of cytoplasm between the Golgi complex and terminal web area of the cell (Fig. 2).

FAT-FED RATS: 60 min after fat administration to fasted rats, lipid accumulates within the absorptive cells (Figs. 3, 4). Generally cells in the distal third of the villus contain more lipid than those proximally located (Fig. 3). Lipid occurs primarily in two regions of the cell: in the distal cytoplasm directly below the terminal web and in the Golgi complex (Fig. 4). It is significant to record that few lipid droplets are found in the basophilic region above the Golgi complex (Fig. 4). Fat droplets appear in the intracellular spaces between adjacent absorptive cells, illustrating lipid transport from these cells (Fig. 4).

PUROMYCIN-TREATED RATS FED FAT: 60 min after fat administration to fasted rats treated with puromycin, extensive amounts of lipid appear in the apical cytoplasm of the absorptive cells (Fig. 5). Indeed, fat droplets are so abundant in cells of the upper one-third of the villus (Figs. 5, 6) that many of the cellular organelles are obscured. Clearly, treatment with puromycin causes the absorptive cells to contain much more lipid than cells from untreated animals. We direct attention especially to the fact that lipid droplets are found throughout the apical cytoplasm including the basophilic region above the nucleus (Fig. 6). Furthermore, in the light microscope it is impossible to identify the Golgi apparatus in these lipid-laden cells (Fig. 6) because of the abundance of fat.

In addition, the central lacteal of the villus often appears more prominent after puromycin treatment than in untreated rats fed fat. It occupies a considerable portion of the lamina propria core and its lumen frequently exhibits small droplets of lipid interpreted as chylomicra (Fig. 5).

Electron Microscopy

FASTED RAT: Details of the ultrastructure of the intestinal absorptive cell may be found in numerous papers on this subject (*e.g.* 11, 24, 31, 43, 57); therefore, we restrict our comments here to those cellular components which are important for fat absorption and are modified by treatment with puromycin.

The apical cytoplasm displays a large quantity of rough endoplasmic reticulum (RER) whose cisternae appear in profile as paired lines studded with ribosomes (Fig. 7). Characteristically, the cisternae of the RER are directed parallel to the long axis of the cell and often similarly aligned filamentous mitochondria form a close association with the RER (Fig. 7). The location of RER coincides with the position of the basophilic region of cytoplasm identified earlier. On the other hand, smooth endoplasmic reticulum (SER) occurs in a relatively limited quantity in the distal cytoplasm directly beneath the terminal web (Fig. 7). It consists of a network of convoluted tubules which vary in both diameter (10–100 mμ) and direction. The SER often appears as isolateed segments; however, continuity of SER tubules with RER cisternae is frequently found (Fig. 7).

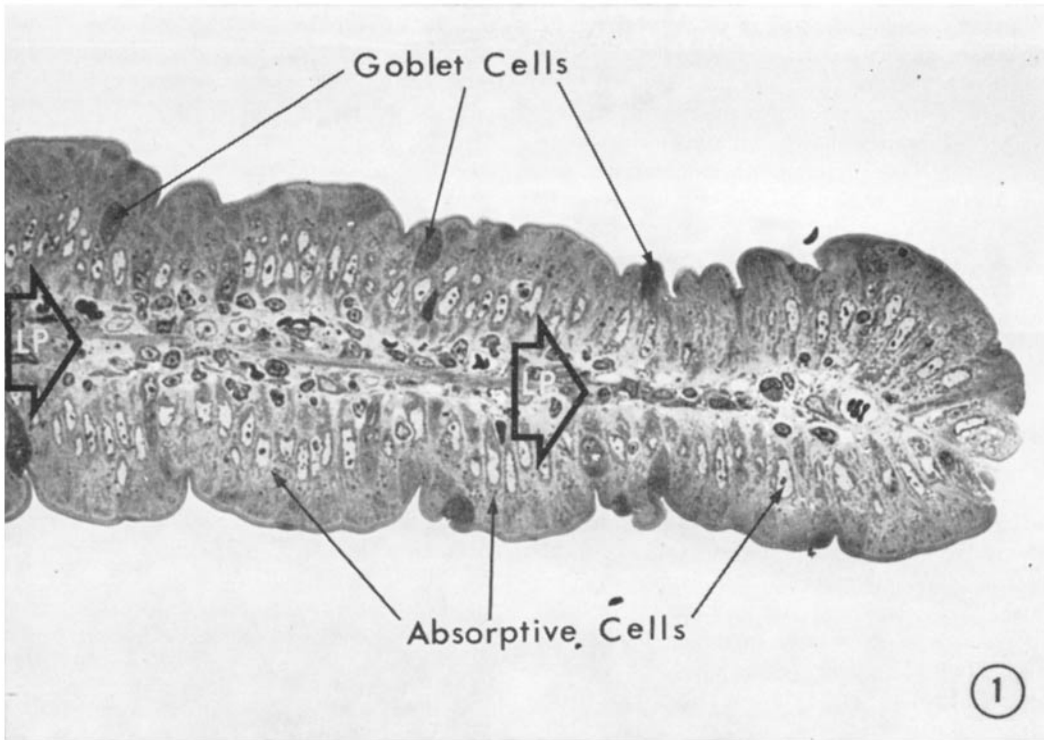
The Golgi complex resides in a supranuclear position and exhibits a characteristic structure of stacks of parallel flattened sacs (saccules), small vesicles, and large vacuoles which usually appear empty in the fasted rat (Fig. 9). Occasionally, however, the vacuoles may contain several droplets (500–1000 A in diameter) which resemble lipoprotein particles of the liver parenchymal cell (27). A maximum of six to eight parallel saccules per stack occur in the Golgi complex. The saccules are approximately 200–300 A apart and their lumina vary in width as described later, but most have a narrow cavity of 250 A (Fig. 9). Frequently, a single extremely narrow saccule is separated from the main stack by a distance of 600 A or more, and small vesicles are interposed between it and the other saccules. Large elongated vacuoles (approximately 0.3–0.5 μ in diameter) reside on one side of the saccules, while small spherical vesicles (600-1000 A in diameter) lie on the other (Fig. 9). Some of the vesicles are coated; others are not. In order to facilitate discussion of the Golgi complex, and to be consistent with the literature, the vesicular side of the Golgi complex is referred to as the immature or forming face, and the side containing vacuoles as the mature face (37). The saccules are about 2.5–3.0 μ in length; but the width of the saccule cavity varies depending on its position relative to the mature face (Fig. 9). Saccules close to the mature face display irregular widths along their lengths and prominent dilations occur at the ends of their cisternae. The saccule adjacent to the Golgi vacuoles exhibits the greatest dilation (Fig. 9). Those saccules nearer the vesicles are very narrow, and often their lumina are not distinct.

As noted above, the Golgi complex occupies a supranuclear position in the absorptive cell; in longitudinal sections, it appears in the central part of the cell just above the nucleus and in the lateral cytoplasm, near the plasma membrane. Usually, in the laterally located Golgi complex the maturing surface is located on the side facing the plasma membrane, and the forming face toward the central region of cytoplasm. Images of the Golgi complex in the central part of the cell, directly above the nucleus, show vacuoles on the cytoplasmic face (mature face) and vesicles (forming face) between the saccules and the nuclear envelope.

Cisternae of RER extend from the apical cytoplasm and associate with the forming face of the Golgi complex. These RER profiles are often directly adjacent to the outermost saccule and

FIGURE 1 Longitudinal section through an intestinal villus from a 24 hr fasted rat. The lamina propria (*LP*) and epithelium consisting of absorptive cells and dark-staining goblet cells are shown. In this study, our observations were restricted to cells of the upper one-third or distal portion of the villus where fat absorption is most active. Light micrograph. × 170.

FIGURE 2 Higher magnification of cells from the distal third of a villus from a fasted rat, showing striated border (*SB*), terminal web (*TW*), mitochondria (*M*), nucleus (*N*), and nucleolus (*Nu*) of the absorptive cell. Note dense, lipid-like inclusions (*LD*) in the apical cytoplasm (*AC*) of the absorptive cells and lamina propria (*LP*). Occasional lymphocytes (*LY*) penetrate the epithelium, and capillaries (*C*) lie in close proximity to the absorptive cells. Basement membrane (*BM*). Light micrograph. × 1700.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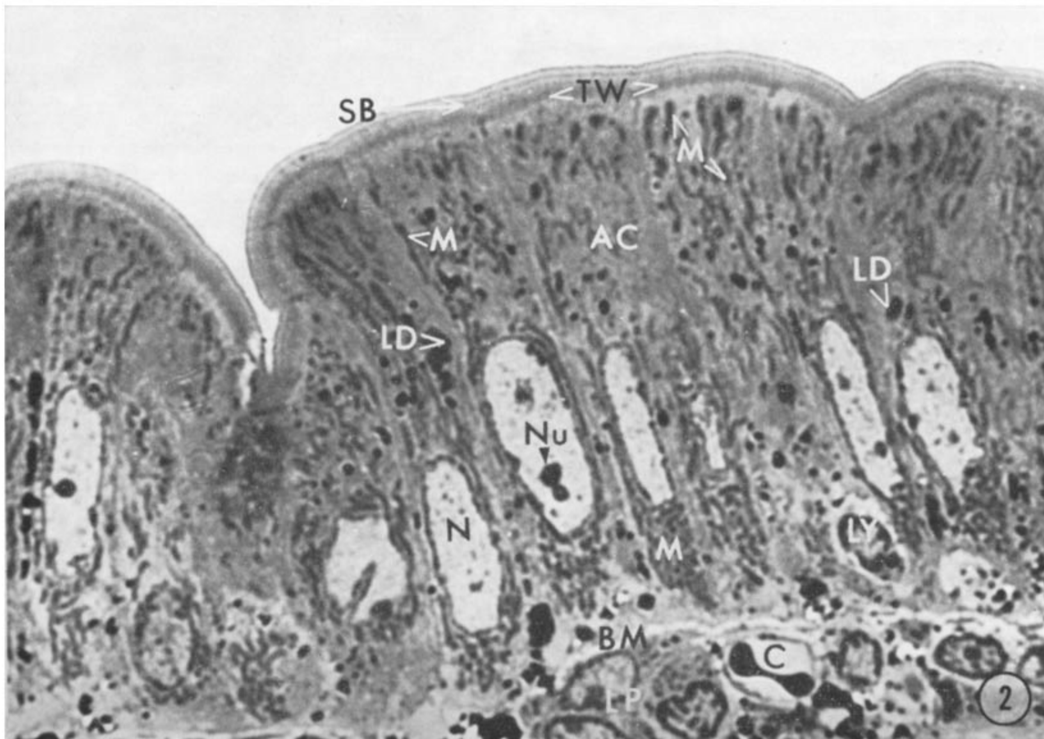
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frequently display a peculiar configuration with ribosomes absent on the membrane surface opposite the forming face of the Golgi complex (Fig. 9). Furthermore, in fortuitous sections the RER membranes show continuity with membranes that lack ribosomes on both surfaces and are in close apposition to Golgi saccules (Fig. 9). Such images are similar to those described by Claude (12) in liver cells and interpreted as important in providing membranes for the formation and maintenance of the Golgi complex. The RER not only shows the specializations described above, but often images are seen which suggest the formation of vesicles from the cisternae of RER. Such vesicles are similar to the ones found near and frequently fused with the saccules of the forming face of the Golgi complex (Fig. 9).

The lateral plasma membrane of the intestinal absorptive cell interdigitates with plasma membranes of adjoining cells, and desmosomes are found along the lateral membranes. Intercellular spaces are not conspicuous above the level of the nuclei (apical portions of the cells), but appear wider lateral to and below the nuclei. A basal lamina separates the absorptive cells from the underlying lamina propria.

FAT-FED RATS: 15 min after fat administration, small lipid droplets (approximately 100 mμ in diameter) appear in the tubules of the SER located in the apical cytoplasm of the intestinal absorptive cell (Fig. 8). Furthermore, the distal ends of these tubules frequently display bulbous expansions containing lipid droplets, 500-1000 A in diameter (Fig. 8). Occasional ribosomes are found attached to the SER membranes enclosing the fat droplets (Fig. 8). Lipid also occurs in the large vacuoles and adjacent saccules of the mature face of the Golgi complex (Fig. 10).

As the absorptive process continues (60 min after fat intubation), the lipid droplets appear larger (0.3–0.5 μ in diameter) in the SER. Often the droplets occur in bulbous expansions of the SER which are attached to the RER (Fig. 11). In other cases, there is no apparent attachment of the membrane-enclosed droplets to either tubules of SER or cisternae of RER. Such images are interpreted as free vesicles of SER or sections through attached bulbous expansions which do not include the zone of attachment within the plane of section. Lipid occupies the central region of the SER vesicle, and a lucent area is present between the lipid droplet and the enclosing membrane (Fig. 11).

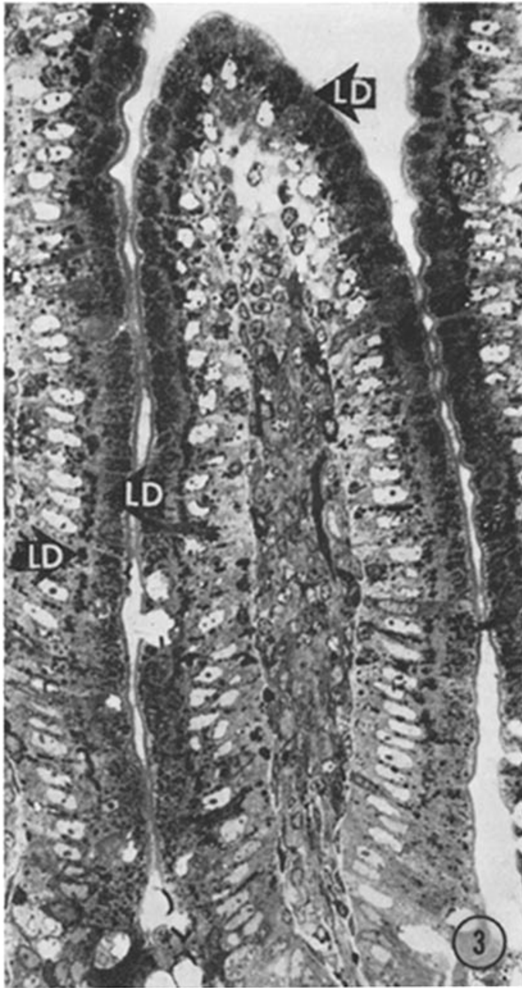
In addition to the droplets of fat in the SER, lipid occurs as free or "matrix" droplets (Fig. 11). These droplets are not enclosed by a membrane, and they may attain a diameter of 0.5 μ. The

FIGURE 3 Intestinal villus from fasted rat fed fat 1 hr before sacrifice. Lipid droplets (*LD*) are present in all cells along the length of the villus, but cells in the distal one-third of the villus show the greatest concentration of fat. Light micrograph. × 160.

FIGURE 4 Higher magnification of absorptive cells from the distal one-third of the villus of fasted rat fed fat 1 hr before sacrifice. Lipid droplets occur primarily in two areas: the apical cytoplasm (indicated at *a*) and the Golgi complex, *b*. Note absence of lipid droplets in the basophilic region between the Golgi apparatus and the apical cytoplasm (region *c*). Lipid droplets occur in the intercellular spaces between adjacent absorptive cells and presumably represent chylomicra (large arrow). Such extracellular lipid droplets are found only in the intercellular spaces at the level of the Golgi complex and toward the base of the cell. They appear also in the lamina propria and lacteals. Light micrograph. × 1400.

FIGURE 5 Intestinal villus from puromycin-treated rat, fasted for 24 hr, and fed fat 1 hr before sacrifice. Comparison of the absorptive cells of this villus with those from the untreated rat shown in Fig. 3 demonstrates that puromycin treatment causes the absorptive cells to contain far greater quantities of lipid (*LD*) than cells from normal animals. Note the dilated lacteal (*L*) in the lamina propria which contains particles interpreted as chylomicra. Light micrograph. × 160.

FIGURE 6 Higher magnification of absorptive cells from the distal one-third of a villus from a puromycin-treated, fasted rat fed fat 1 hr before sacrifice. This micrograph illustrates the abundance of lipid in these cells and shows that fat droplets (*LD*) are found throughout the apical cytoplasm from the nucleus to the terminal web. Thus, it is impossible to distinguish the Golgi complex and basophilic regions of the absorptive cells in puromycin-treated animals fed fat for 1 hr. This is in sharp contrast to the situation in absorptive cells from untreated rats fed fat a similar period of time (Fig. 4). Light micrograph. × 1300.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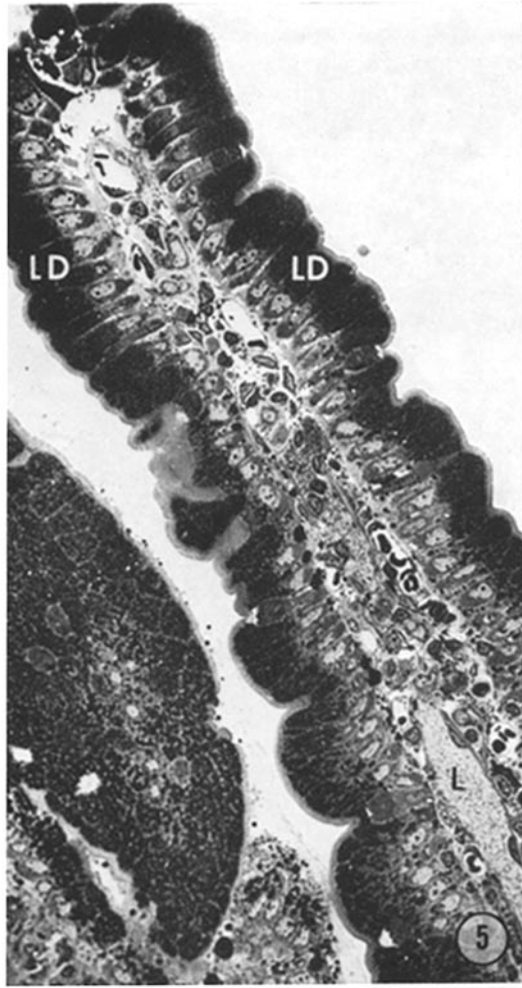
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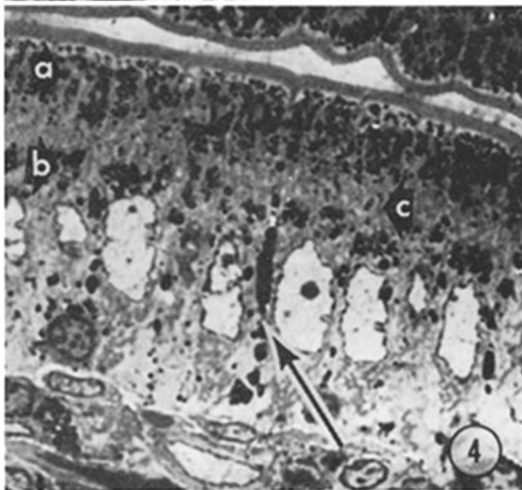
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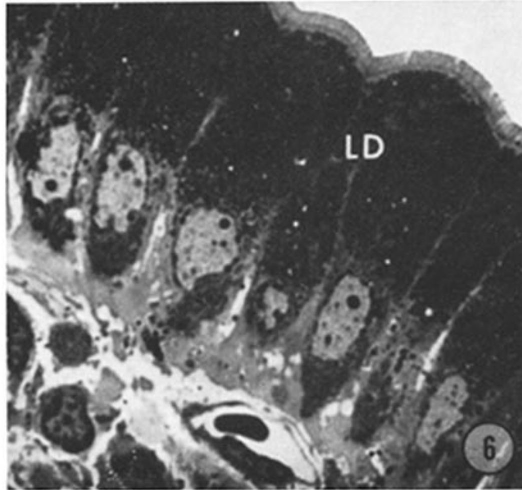
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matrix droplets are usually found in the apical cytoplasm; however, they are not nearly as abundant as the SER-bounded droplets (Figs. 11, 12).

As fat absorption proceeds there is an apparent increase in the amount of SER in intestinal cells from fat-fed rats when compared to fasted animals. The SER, consisting of tubules and vesicles containing lipid droplets, is located predominantly in the apical cytoplasm, and here it occupies almost all of the cytoplasm between the remaining RER and the terminal web (Fig. 12). It is obvious that after 60 min of exposure to lipid the intestinal absorptive cell contains much less RER and much more SER. The remaining cisternae of RER are located immediately above the Golgi complex and below the SER (Fig. 12).

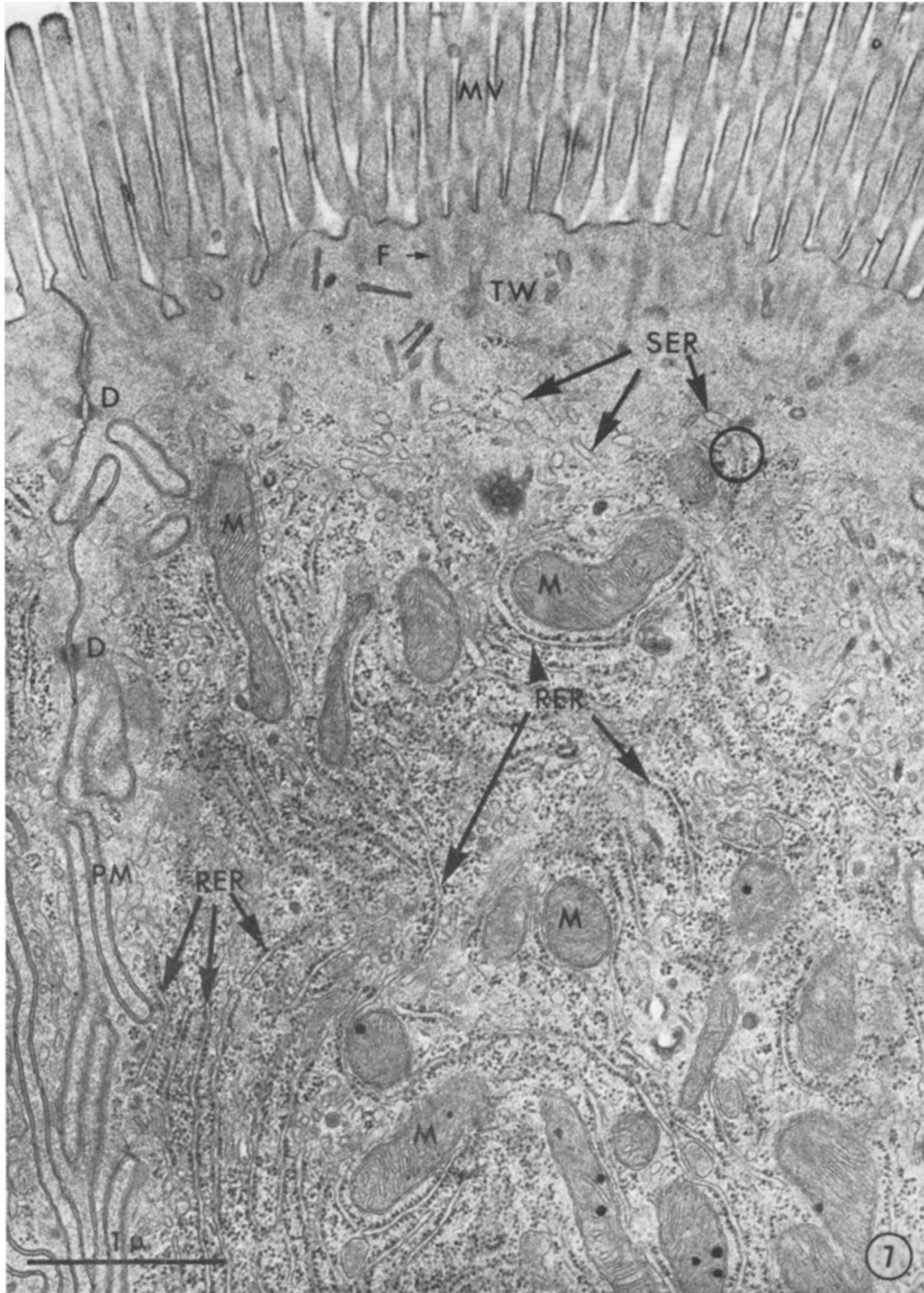
1 hr after fat administration, the Golgi complex contains many large lipid droplets (approximately 1–3 μ in diameter) and smaller droplets (Fig. 12). The smaller droplets resemble those in the SER whereas the larger ones probably result from fusion of several smaller droplets of lipid within the Golgi complex. Lipid droplets occur only in the large vacuoles and terminal expansions of saccules adjacent to the vacuoles; we have not seen droplets in the smaller vesicles or slender cisternae of the saccules located near the forming face of the Golgi complex. As fat absorption proceeds, the number of saccules per Golgi complex decreases to about three or four per stack (Fig. 13). Moreover, the length of the saccules decreases to approximately 1–1.5 μ. The diameters of the vacuoles, on the other hand, increase to approximately 2–3 μ (Fig. 12).

The intercellular space adjacent to the region of the Golgi complex and the area between it and the base of the absorptive cell contains lipid droplets approximately the same size as seen in the Golgi complex (Fig. 12). However, we have seen no lipid droplets in the intercellular space above the level of the Golgi complex. These droplets which lack enveloping membranes are found also in the lamina propria and lacteals after 1 hr of exposure to fat and are considered to represent chylomicra.

PUROMYCIN-TREATED RATS FED FAT. The apical cytoplasm of intestinal absorptive cells from puromycin-treated animals displays lipid droplets 15 min after fat administration (Fig. 14). These droplets are found predominantly in the SER and appear similar if not identical to those described in the apical cytoplasm of intestinal cells from untreated rats fed fat for a similar period of time. Likewise, lipid occurs in the vacuoles of the Golgi complex (Fig. 15). Usually two or three droplets are found per vacuole; however, some vacuoles contain a single large droplet (Fig. 15). Occasionally, a stack of saccules (consisting of three or four saccules) is found in the Golgi complex, but usually only isolated saccules are present (Fig. 15).

After 30 min of exposure to fat, intestinal absorptive cells show an increase in lipid within the SER (Figs. 16, 17). Moreover, the SER appears increased in quantity and often extends from the Golgi complex to the terminal web area of the cell. Conversely, the rough endoplasmic reticulum is greatly reduced in amount and is practically absent in some cells. Free ribosomes abound in the apical cytoplasm of the cell (Fig. 17). Matrix lipid droplets (maximum diameter 1.5 μ) are numerous in the apical cytoplasm intermingled with the SER (Fig. 16), but it should be noted that they also occur near the Golgi complex (Fig. 17). The Golgi complex is characterized by many vacuoles containing lipid droplets

FIGURE 7 Electron micrograph of the apical cytoplasm of an intestinal absorptive cell from a rat fasted 24 hr. The most distal cytoplasm or striated border of absorptive cells consists of numerous microvilli (*MV*) which, in turn, contain filaments (*F*) that extend into the cytoplasm and intermingle with similar filaments of the terminal web (*TW*). Beneath the terminal web, rough (*RER*) and smooth (*SER*) endoplasmic reticulum and mitochondria (*M*) are the predominant organelles of the apical cytoplasm. RER is abundant and consists of numerous cisternae extending from the distal margin of the Golgi complex almost to the terminal web region of the cell. SER is sparse and occupies a narrow region between the terminal web and most distal border of the RER. Occasionally the two ER forms display membrane and cisternal continuity (circle). The mitochondria are filamentous and oriented parallel to the long axis of the cell, often in close apposition to RER membranes. The plasma membranes (*PM*) of adjacent cells interdigitate and are reinforced along their length with desmosomes (*D*). × 29,000.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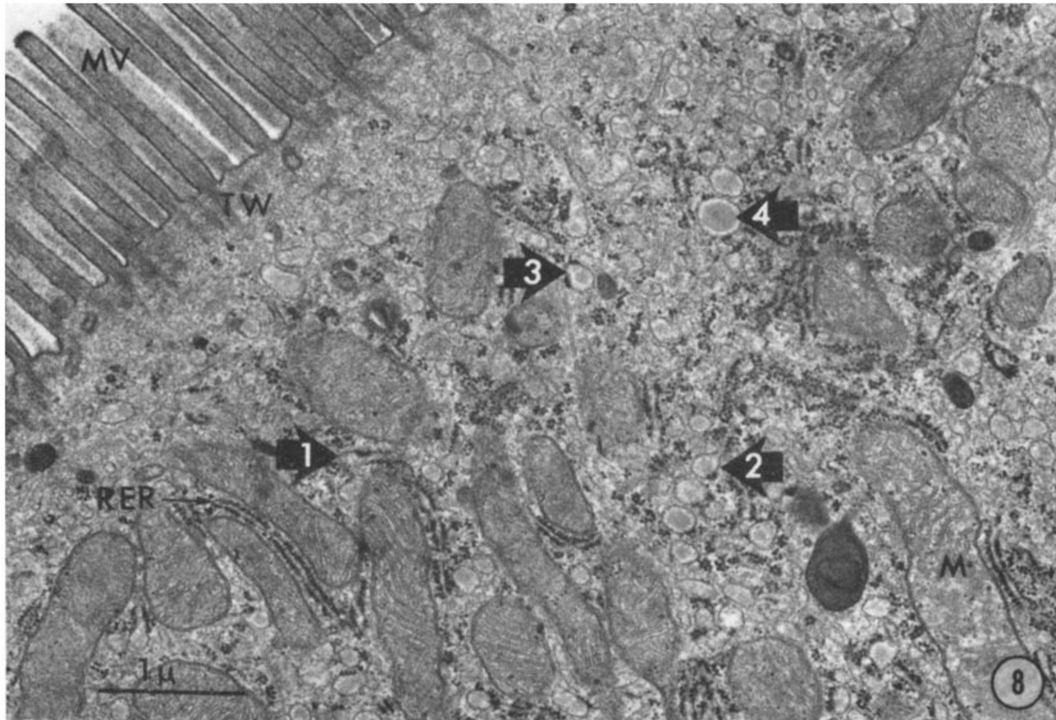


FIGURE 8 Apical cytoplasm of intestinal absorptive cell 15 min after corn oil intubation. This electron micrograph illustrates the pathway of triglyceride synthesis and movement within the apical cytoplasm of the absorptive cell. In response to the stimulus of fat absorption, the SER increases in amounts. Its membranes are often seen in continuity with membranes of the RER, suggesting that the RER transforms into SER (arrow *1*). Utilizing microsomal enzymes, triglycerides are resynthesized and appear as small lipid droplets in bulbous expansions of the SER (arrow *2*). The terminal expansions of SER detach and appear in the cytoplasm as isolated SER vesicles containing the newly formed fat droplets (arrow *3*). Often the SER vesicles show occasional ribosomes attached to their membranes. The fat droplets within the SER vesicles vary in size, reflecting increased triglyceride synthesis (arrow *4*). Microvilli, *MV*; terminal web, *TW*; mitochondria, *M*; rough endoplasmic reticulum, *RER*. × 22,500.

(Fig. 18). Occasionally, stacks containing a reduced number of saccules are found, but most often either they are absent or only isolated saccules are present (Figs. 18, 19). The width of the saccules is similar to that described in the untreated rat fed fat, but their length rarely exceeds 0.5 μ.

1 hr after fat administration, matrix lipid droplets are prominent in the apical cytoplasm of the absorptive cells (Fig. 19). These droplets are similar to matrix droplets described in untreated absorptive cells after fat administration and to the droplets identified in intestinal cells from puromycin-treated rats fed fat for 15-30 min (see above description). The matrix droplets are approximately 1.5-3.0 μ in diameter and not bound by an enveloping membrane; thus, they are easily distinguished from the smaller SER-bound lipid droplets (Fig. 19). Matrix droplets in absorptive cells from puromycin-treated rats exposed to fat for 1 hr differ from those in cells of either untreated rats fed fat for 1 hr or puromycin-treated animals exposed to fat for shorter periods of time in the following ways: (*a*) the matrix droplets are larger, (*b*) they are much more numerous per cell, and (*c*) they show a greater tendency to fuse with each other (Fig. 19).

In cells located near the distal third of the villus (Fig. 19), the Golgi complex is highly disorganized, and it is very difficult to identify characteristic components of this organelle. Rarely are any saccules seen and large vacuoles containing lipid

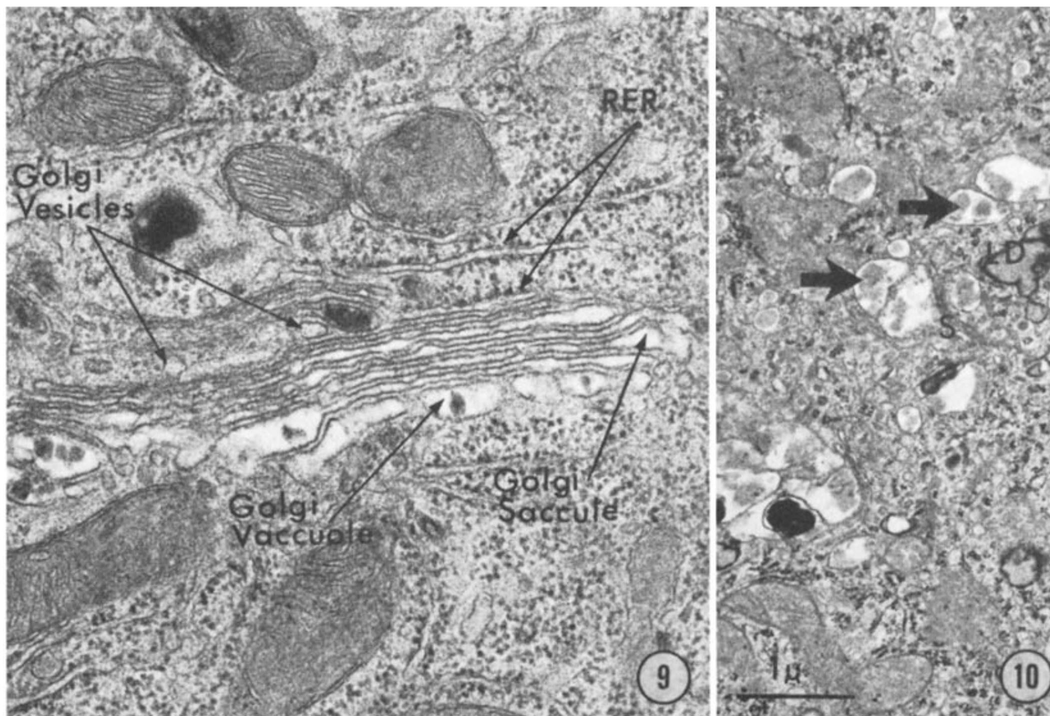


FIGURE 9 Electron micrograph of the Golgi apparatus in an intestinal absorptive cell from a 24 hr-fasted rat. The Golgi complex lies directly above the nucleus and consists of a membranous system of parallel flattened saccules, small vesicles, and large vacuoles. The saccules are arranged in lamellar stacks of six to eight cisternae, while the vesicles and vacuoles lie on opposite sides of each stack. The vesicular side of the Golgi complex is referred to as the forming face and often is closely associated with terminal ends of cisternae of the endoplasmic reticulum. The side of the Golgi complex showing vacuoles is considered the mature face and often is found opposite the lateral plasma membrane. Rough endoplasmic reticulum, *RER*. × 29,000.

FIGURE 10 Electron micrograph of the Golgi complex of an intestinal absorptive cell 15 min after corn oil intubation. Golgi vacuoles are dilated and contain small droplets of lipid within their lumina (arrows). Usually two or three discrete droplets occupy one vacuole, but frequently a single large droplet fills an entire vacuole (*LD*). Golgi saccules (*S*) are reduced in number compared to the fasted state, and no lipid droplets appear in their lumina. × 15,500.

occupy the region of the Golgi complex (Fig. 19). Cisternae of RER are absent and many free ribosomes appear in the cytoplasm (Fig. 19). It is apparent that exposure to lipid for 1 hr in puromycin-treated rats has caused a marked decrease in the membranes of the RER and Golgi complex of the intestinal absorptive cell. Vesicles of SER containing fat appear throughout the apical cytoplasm of the cell.

Chylomicra were identified in intercellular spaces, lamina propria, and lacteals of all puromycin-treated rats fed fat. The size of the chylomicra was similar to that of the lipid droplets in the Golgi vacuoles of absorptive cells proximally located along the villus. However, when Golgi vacuoles were greatly distended with lipid (as in cells distally located) the chylomicra were relatively smaller (Fig. 19).

DISCUSSION

Normal Fat Absorption

In 1856, Claude Bernard (5) observed that lymphatics distal to the pancreatic duct of the rabbit appeared cloudy following a meal rich in lipid. This discovery, following the work of Aselli

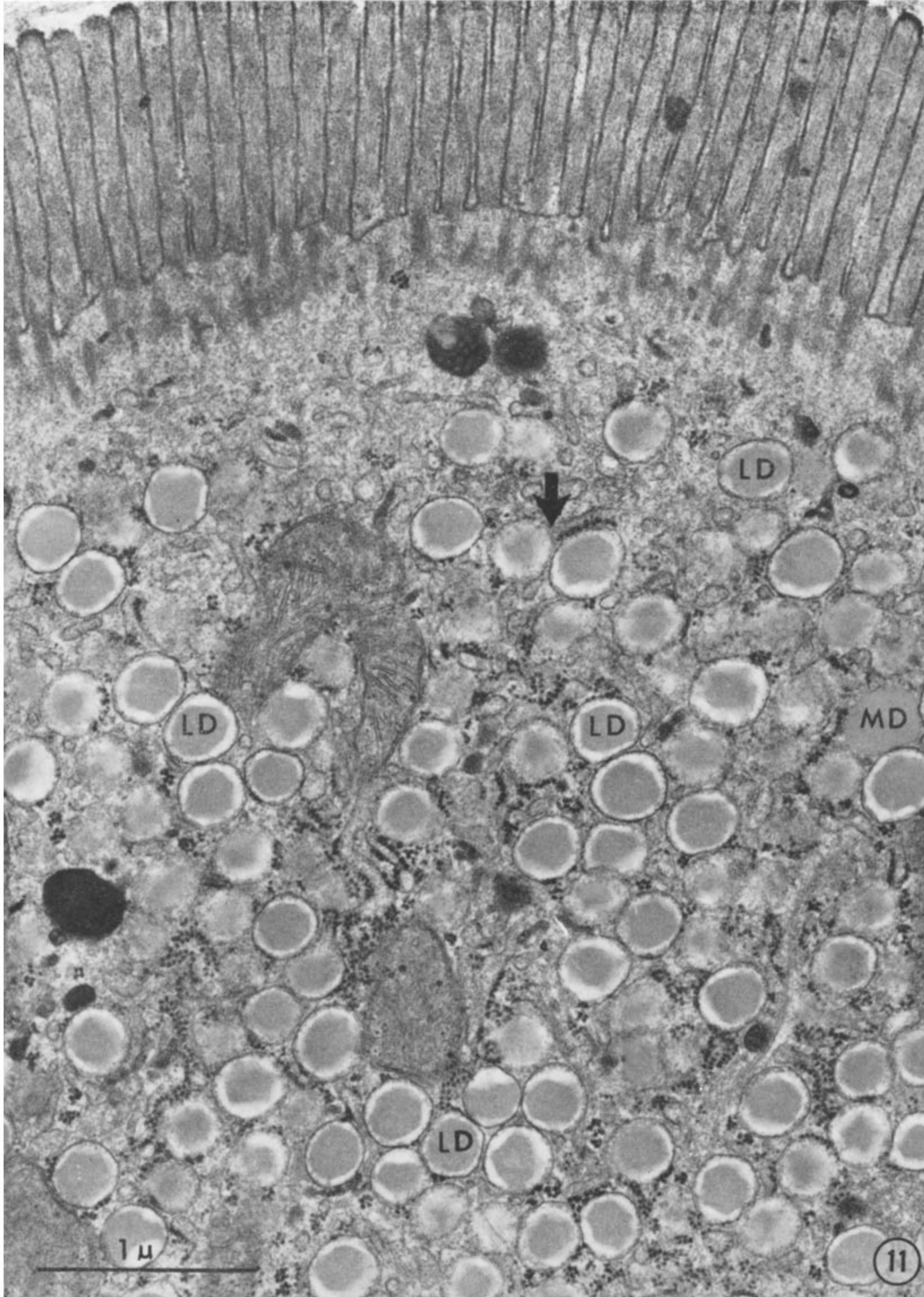


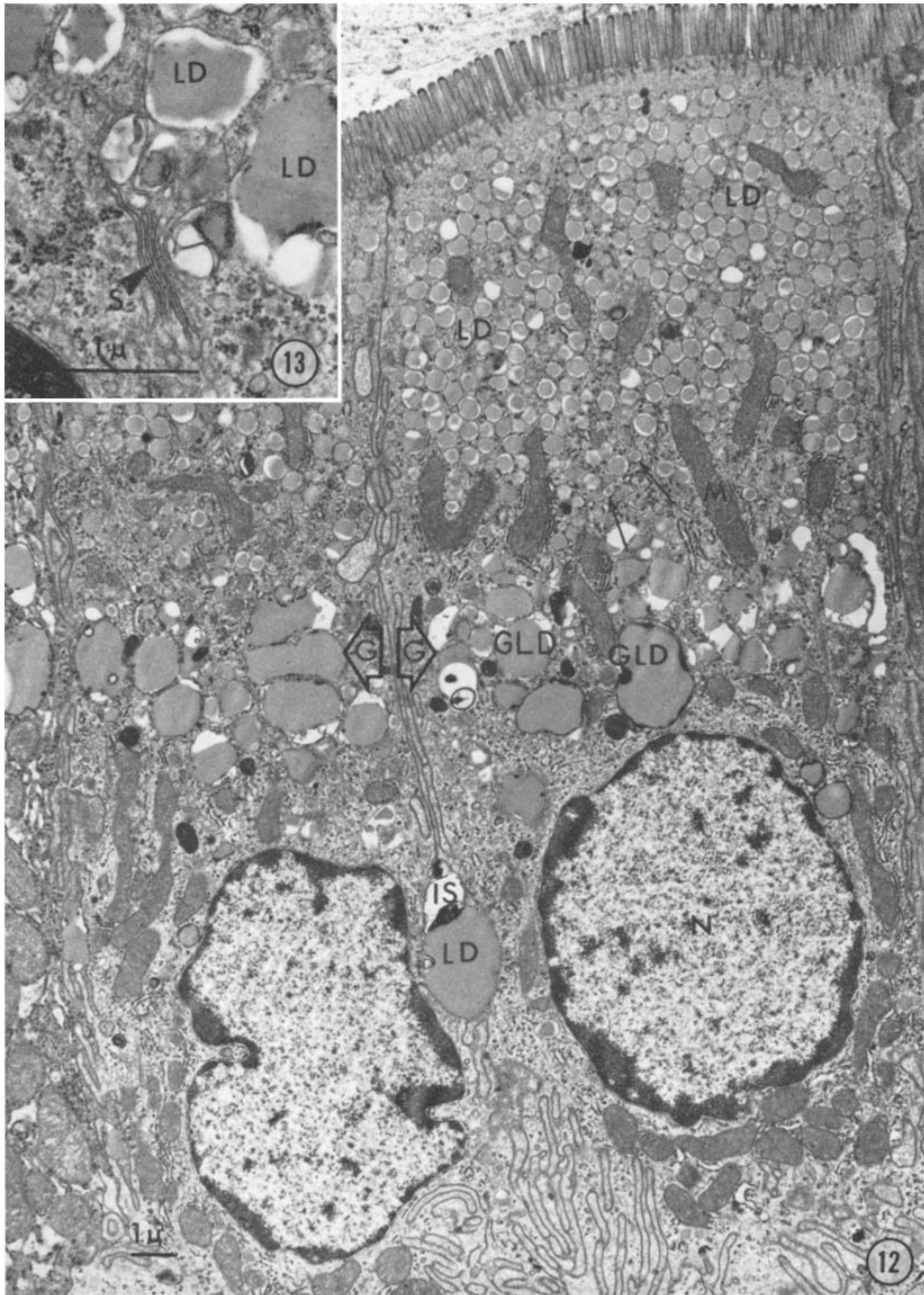
FIGURE 11 Electron micrograph of the apical cytoplasm of an absorptive cell 1 hr after corn oil intubation. As fat absorption proceeds, the SER increases in quantity and its contained fat droplets (*LD*) are larger than those seen after shorter exposure to lipid. Continuity between RER and bulbous expansions of SER containing lipid droplets is seen (arrow). The continuity of SER with RER and the presence of occasional ribosomes on membranes of the SER-derived vesicles containing fat suggest that the SER is derived from the RER. Most fat droplets in the apical cytoplasm are enclosed by a membrane (*LD*); however, other droplets lie free in the cytoplasmic matrix and are termed "matrix droplets" (*MD*). × 30,000.

in 1627 (2), implied that fat had passed from the lumen of the gut through the intestinal wall and into the lymph. Bernard's observation has been confirmed by many workers (6, 9, 54, 58) and is documented in this report on the gross morphology of the small intestine of the rat 60 min after corn oil administration. Thus, the lymphatic channels distend and appear milky white, indicating that fat transport is occurring from the gut lumen to the efferent lymph.

An important event in the appearance of exogenous lipid in the lacteals of the intestinal villus is its passage through the intestinal epithelial cell. Consequently, the mechanism of fat transport by this cell has been extensively studied and a number of excellent reviews on the subject have been published (25, 52, 55). The results of the above investigations have provided information on at least four major stages of intestinal fat absorption: (*a*) intraluminal digestion; (*b*) entry of fat into the mucosal cell; (*c*) synthesis of triglyceride within the mucosal cell; and (*d*) release of lipid from the intestinal cell. The third step of the process requires that triglycerides be resynthesized from free fatty acids and 2-monoglycerides in the cytoplasm of the cell. Enzymes important for the synthesis of triglycerides are located in the microsomal fraction of the absorptive cell (46, 49, 52, 53, 55), and since the smooth endoplasmic reticulum proliferates as fat is absorbed (11), it is generally accepted that this organelle is the site of synthesis of triglycerides in the absorptive cell. Indeed, it has been argued that enzymes for synthesis of triglyceride are located in the membranes of the smooth endoplasmic reticulum and, as triglyceride synthesis occurs, the product is sequestered within the tubules of the smooth endoplasmic reticulum (11, 55, 57). The triglyceride appears as electron-opaque droplets within the cavity of the smooth endoplasmic reticulum. Furthermore, the suggestion has been offered that vesicles containing triglyceride droplets pinch off from the tubules of smooth endoplasmic reticulum and transport fat within the cell (11, 31). Since fat accumulates in the Golgi complex during absorption, it is reasonable to believe that the fate of the SER vesicles is this organelle (24, 31, 62); however, it has been speculated that they may fuse directly with the plasma membrane of the intestinal epithelial cell (11, 43). Vacuoles from the Golgi complex migrate to the lateral cell boundaries and via reverse pinocytosis deliver the chylomicra to the intercellular space (31). Direct evidence for the latter event has not been obtained.

The observations on fat absorption in untreated rats reported in this paper in general confirm the events described above; however, some of our findings allow further interpretation of the cellular events important to this process. The decreased amount of rough endoplasmic reticulum and increased quantities of smooth endoplasmic reticulum per cell as fat absorption progresses suggest that the former is converted to the latter. It is important to note that the RER is not entirely depleted during fat absorption, an observation which implies that ribosomes on the rough endoplasmic reticulum are stimulated (by the events of fat absorption) to synthesize membrane proteins (both structural and enzymes) which are placed in membranes of this organelle in a manner similar to other cell types (3, 13, 14, 22, 23). Thus, the generation of RER membranes tends to replace those lost owing to the conversion to SER. We view the synthesis of membranes by the rough endoplasmic reticulum and their conversion to SER as independent but closely related events. In the fasted condition, the transformation to smooth endoplasmic reticulum is at a minimum; therefore, the cell contains much rough endoplasmic reticulum and little SER. The process of fat absorption by the intestinal cell causes an increase in the conversion of RER to SER. During this period, the RER decreases in amount because synthesis of new membranes is not rapid enough to replace their loss through conversion to SER.

As indicated above, two mechanisms for discharge of lipid from the absorptive cell have been proposed: (*a*) fusion of SER vesicles, containing fat, with plasma membranes; and (*b*) fusion of Golgi vacuoles, containing fat, with plasma membranes. We have obtained no unequivocal images of either Golgi vacuoles or SER vesicles attached to the plasma membrane suggesting discharge of chylomicra. However, we consistently find chylomicra in the intercellular space, approximately the same size as lipid droplets in the Golgi vacuoles. Moreover, we have observed no chylomicra in intercellular spaces above the level of the Golgi complex. Since the position of the SER and Golgi complex is relatively constant in this cell, it seems reasonable to expect that some fat droplets (chylomicra) would appear between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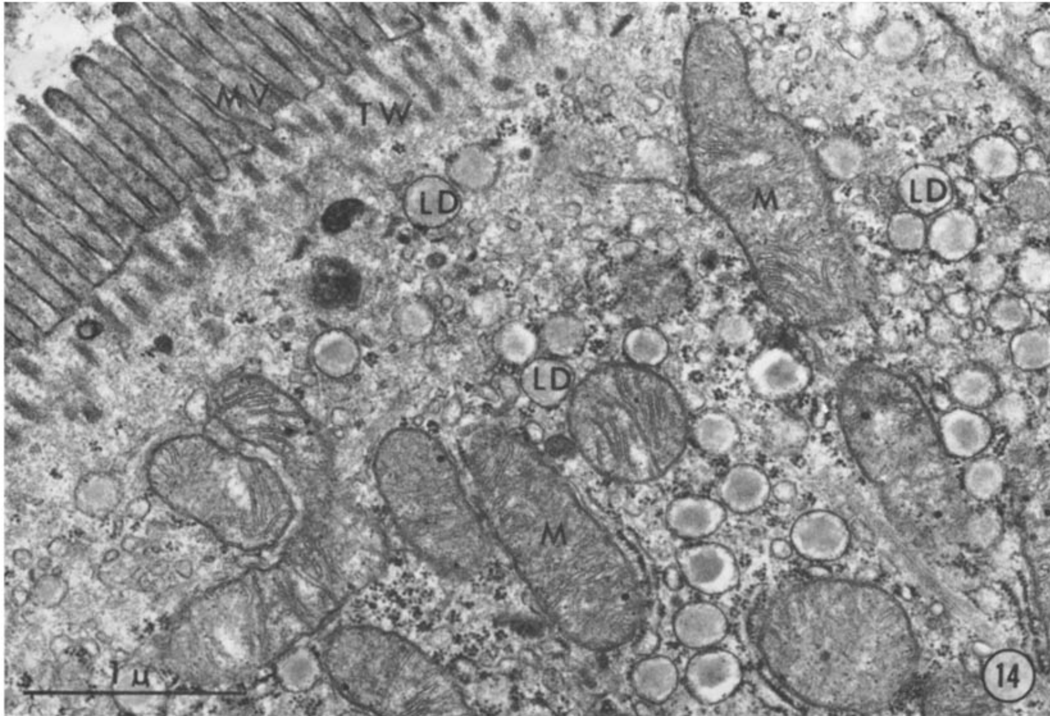


FIGURE 14 Electron micrograph of the apical cytoplasm of an intestinal absorptive cell from a puromycin-treated rat 15 min after corn oil intubation. The apical cytoplasm displays lipid droplets (*LD*) in a manner similar to untreated animals at this time period after feeding (compare with Fig. 8). It is important to note that the lipid droplets are enclosed in SER membranes, thus demonstrating that free fatty acids and 2-monoglycerides enter the absorptive cell and are synthesized into triglycerides in the puromycin-treated animal. Microvilli, *MV*; terminal web, *TW*; mitochondria, *M*. × 28,500.

cells at the level of the SER (apical cytoplasm), if the SER is capable of discharging fat directly to the intercellular spaces. Chylomicra were found in abundance in intercellular spaces at the level of the Golgi complex and toward the bases of the cells. These observations suggest that lipid must pass through the Golgi complex before discharge from the cell is possible.

FIGURE 12 Low-magnification electron micrograph of an intestinal absorptive cell 1 hr after corn oil intubation. The cell contains numerous lipid droplets (*LD*) within SER which occupies a considerable extent of the apical cytoplasm. The RER, on the other hand, is present in a rather narrow region between the Golgi complex (*G*) and the SER. Within this region of RER, smaller newly synthesized lipid droplets are observed (arrows). From the study of micrographs such as this, it is clear that as fat absorption progresses the SER develops at the expense of the RER. Lipid also is present in the Golgi complex (*G*), appearing in vacuoles as small droplets, similar in size to those in the SER, and larger single droplets (*GLD*). Note that the lipid droplet in the intercellular space (*IS*) is similar in size to those seen in the vacuoles of the Golgi complex. It is further important to point out that no lipid occurs in the intercellular spaces above the level of the Golgi complex. Mitochondria, *M*; nucleus, *N*. × 7000.

FIGURE 13 Electron micrograph of the Golgi apparatus of an intestinal absorptive cell 1 hr after fat administration. The Golgi saccules (*S*) are reduced in size and number with respect to those of the Golgi complex in cells from fasted rats, suggesting the conversion of saccules to vacuoles as fat absorption progresses. Golgi vacuoles are expanded with large lipid droplets (*LD*). × 26,000.

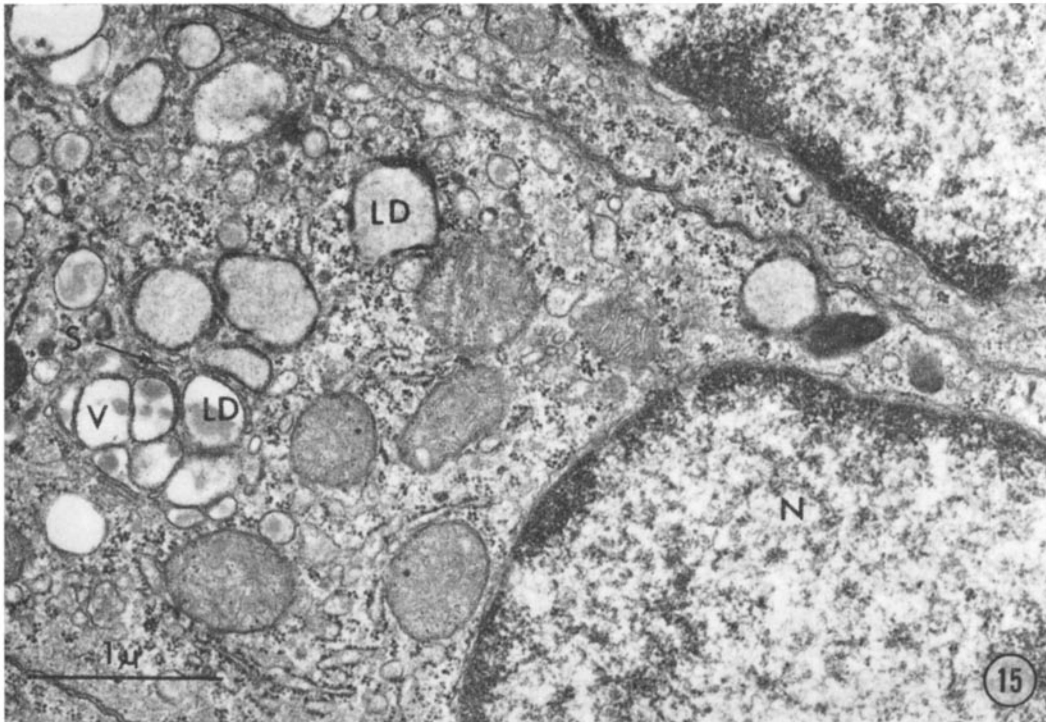


FIGURE 15 Electron micrograph of the Golgi complex in a cell from a puromycin-treated rat 15 min after fat administration. The Golgi vacuoles (*V*) are dilated and usually contain two or three lipid droplets per vacuole, but frequently a single large droplet (*LD*) fills an entire vacuole as in the untreated animals. Golgi saccules (*S*) are reduced in number with respect to those of untreated rats fed fat, and often only a single saccule is seen. Nucleus, *N*. × 25,000.

If the above conclusion is accepted, it immediately focuses attention on alterations of the Golgi complex during fat absorption. The organelle shows a distinct polarity in the intestinal cell with vacuoles located on one side and a variety of vesicles and tubules on the other. Six to eight narrow cisternae or saccules occur between these two components. Early in the process of fat absorption SER vesicles containing fat migrate from the apical cytoplasm and fuse with Golgi vacuoles, discharging into them their lipid content. This process continues as fat absorption proceeds up to 60 min. Concomitantly, the number of Golgi saccules decreases to about two or three per stack, apparently owing to their conversion to vacuoles and subsequent fusion of the vacuoles with the lateral cell membrane as chylomicra are discharged from the cell. This implies a constant loss of Golgi membranes as chylomicra are produced and released from the absorptive cell and suggests that maintenance of the Golgi complex requires replacement of its membranes during fat absorption. In other cell types, evidence suggests that ribosomes on the rough endoplasmic reticulum synthesize proteins required for the formation of new Golgi membranes (20, 61). These membranes are transferred to the Golgi complex via vesicles (22, 23, 29, 30, 63, 65) or tubules (12) from the rough endoplasmic reticulum (18). In the absorptive cell, it is suggested that the "forming face" of the Golgi complex is opposite the vacuoles (between the nucleus and saccules of the centrally located Golgi complex and near the central part of the cell in the laterally located Golgi complexes). Whether the membranes are transferred by vesicles or tubules from the rough endoplasmic reticulum is not clear, because we see both structures near the Golgi complex.

We consider it important to emphasize the observation that the SER-derived vesicles containing fat fuse only with the vacuoles or saccules

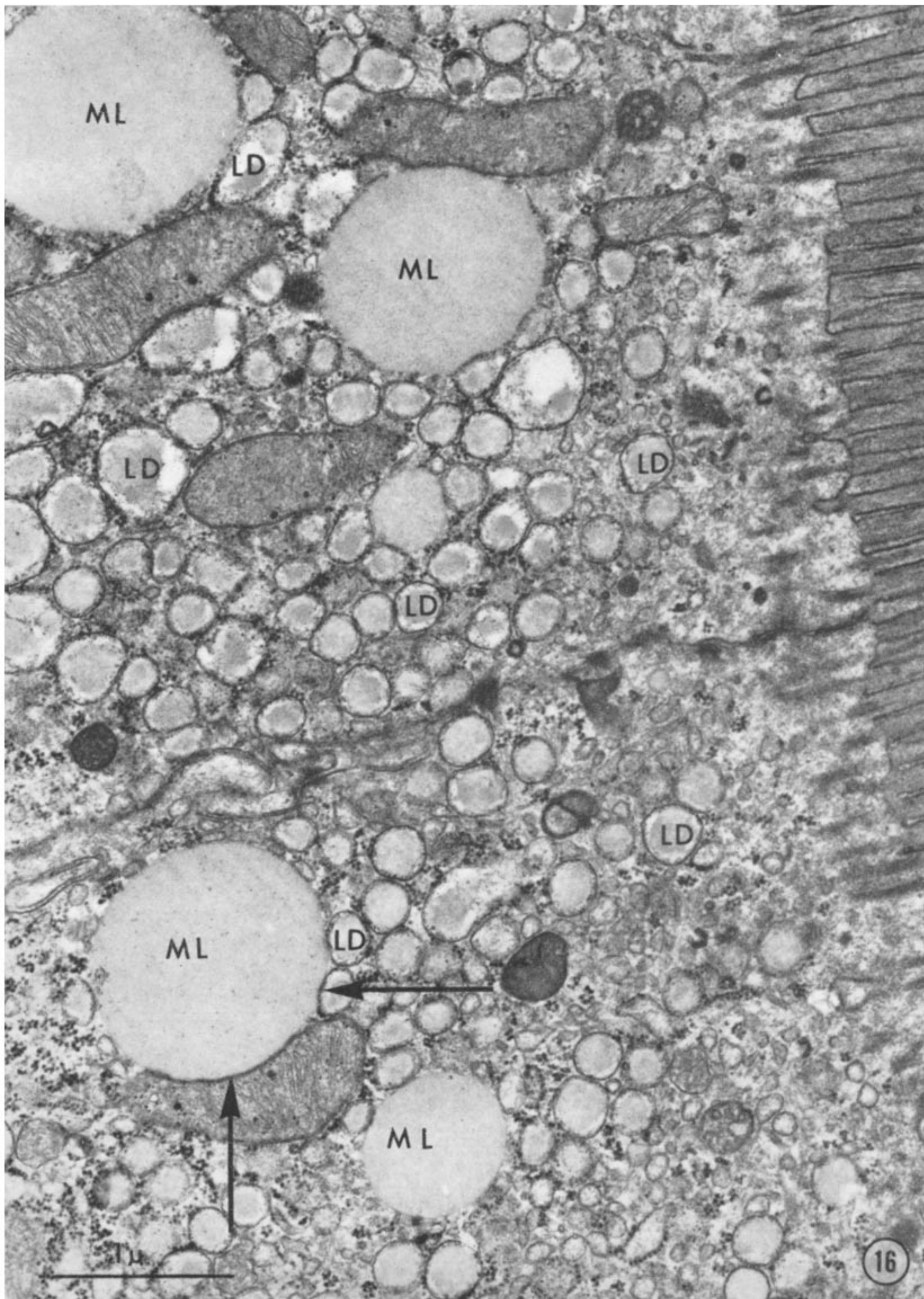


FIGURE 16 Electron micrograph of the apical cytoplasm of an intestinal absorptive cell from puromycin-treated rat 30 min after corn oil intubation. SER-bounded lipid droplets (*LD*) are numerous in the apical cytoplasm. The most prominent feature, however, is the presence of large unbounded lipid droplets (*ML*). These matrix droplets lack the characteristic smooth-surfaced membrane seen around the smaller droplets. The arrows indicate regions of apposition between the matrix lipid droplet and the membrane of an adjacent mitochondria and smaller membrane-bounded lipid droplet. × 27,500.

adjacent to vacuoles of the Golgi complex. No lipid droplets have been discovered in saccules located at the opposite pole or forming face of the Golgi complex. This observation implies that important maturation changes occur as the saccules move nearer the vacuoles. It has been recognized that changes occur in the staining characteristics (4, 17, 19, 37), enzyme content (19, 36, 41), dimensions of the saccules' membranes (20), and other properties of the saccules (4, 19, 36, 37), as they move toward the maturing face of the Golgi complex. It is not unreasonable to suggest that other properties (e.g. surface charge, protein content, carbohydrate components, etc.) of the membranes of the saccules may also change. One or all of these maturation changes may be required in order for membranes of the SER to fuse with membranes of the mature saccules or vacuoles of the Golgi complex. It could be further speculated that the maturation of the Golgi membranes is required in order for them to subsequently fuse with the lateral plasma membranes, thereby allowing discharge of chylomicra from the cell.

Thus, we envision the absorptive cell as possessing a variety of membranes each of which has the specific capability to fuse with certain other cellular membranes. SER membranes may fuse with mature Golgi membranes but not immature Golgi membranes or lateral plasma membranes. Mature Golgi membranes have the capacity to fuse with lateral cell membranes but not basal or apical plasma membranes. Therefore, the fate of fat droplets is determined by the specificity of their enveloping membranes (SER to Golgi to plasma membranes). It is apparent that this hypothesis is not restricted to the intestinal absorptive cell and its discharge of fat, but may apply as well to other types of cells which secrete a membrane-bounded product.

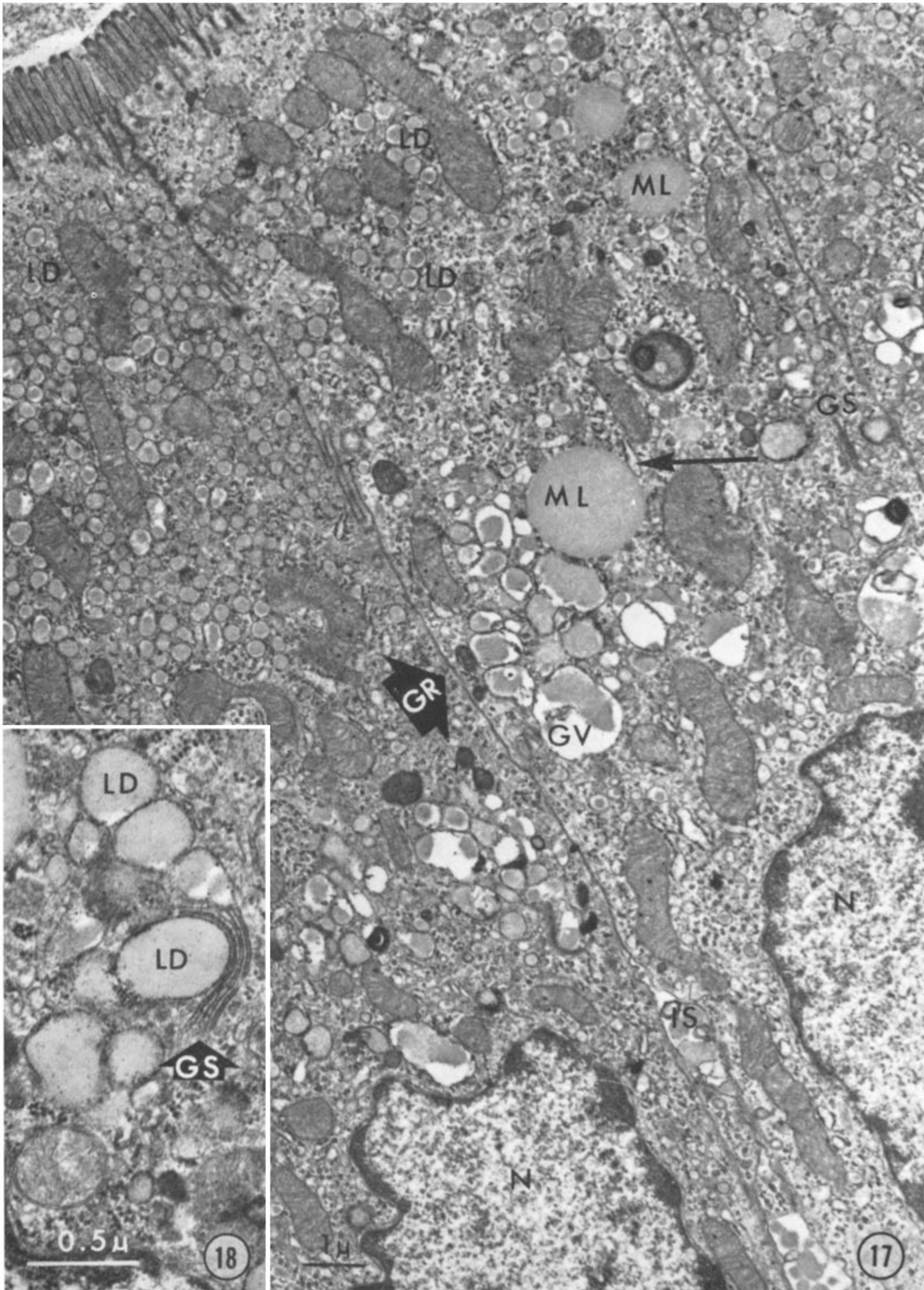
The Golgi apparatus may be implicated in fat absorption in yet another way. It has been recently shown that chylomicra contain a carbohydrate moiety (33) in addition to triglyceride, phospholipid, cholesterol, and protein (40, 52). Thus, the chylomicron is a complex glycolipoprotein as are other classes of serum lipoproteins (very low-density lipoproteins and high-density lipoproteins) (33). From studies already mentioned, it is reasonable to believe that triglyceride, cholesterol, and phospholipids are incorporated into the chylomicron in the SER. The protein component is probably synthesized in the RER and either complexed with the lipid in the SER (11, 52) or possibly transported to the Golgi complex and there associated with the forming chylomicron. Experiments of Neutra and Leblond (38, 39) implicate the Golgi apparatus as the site for complexing carbohydrate moieties to other substances. It is reasonable to suggest, therefore, that the carbohydrate component of the chylomicron is added in the Golgi complex, thus requiring passage of lipid through this organelle before export.

Effects of Puromycin on Fat Absorption

Our results and others (51) show clearly that puromycin treatment causes the absorptive cells to contain more and larger lipid droplets, especially 60 min after fat administration. Some of these droplets are bounded by membranes of the SER since occasional ribosomes are found on

FIGURE 17 Electron micrograph of the apical cytoplasm of absorptive cells from a puromycin-treated rat 30 min after corn oil intubation, illustrating the abundance of SER containing lipid droplets (*LD*) and the dramatic decrease in RER. The interpretation is offered that the stimulus of fat absorption causes the conversion of RER to SER, but in the puromycin-treated animals the cell is no longer able to synthesize membranes, and thus the RER is depleted as fat absorption progresses. Note the matrix lipid droplet (*ML*) near the Golgi vacuoles (*GV*); such droplets occur in the apical cytoplasm of the cell. The arrow again indicates an area of apposition between matrix lipid and cellular membrane. The intercellular space (*IS*) exhibits lipid droplets in the puromycin-treated animals. Golgi region, *GR*; Golgi saccules, *GS*; nucleus, *N*. × 32,000.

FIGURE 18 Golgi complex of an absorptive cell from a puromycin-treated rat 30 min after fat administration. Golgi vacuoles are dilated and contain large droplets of lipid. This image shows the maximum number of saccules (*GS*), three or four found in the Golgi complex of puromycin-treated rats fed fat for 30 min. Usually only one or no saccules are seen. Lipid droplet, *LD*. × 32,000.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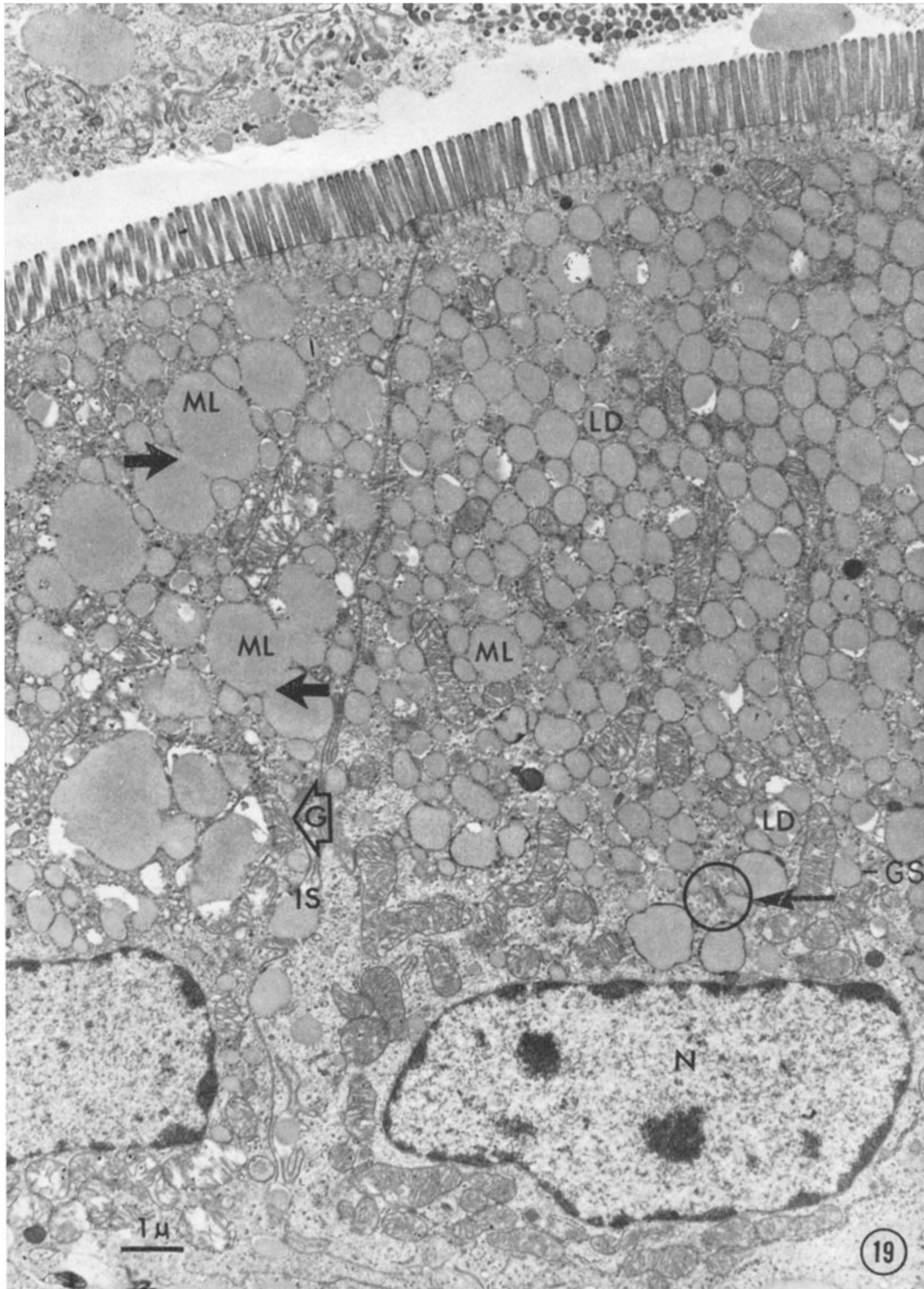
their surfaces; others show no ribosomes and, therefore, it is impossible to state whether they are bounded by SER or Golgi membranes. The droplets of fat enclosed by membranes are generally larger than those found in untreated animals. It is not clear whether these large membrane-bounded droplets result from fusion of smaller vesicles of SER containing fat, whether the SER vesicles enlarge owing to increased synthesis of triglyceride, or whether the large droplets originate from the Golgi complex.

It is also evident that puromycin treatment causes more matrix lipid droplets to appear in the absorptive cell. Their origin is obscure, but they are prominent in the apical cytoplasm, and we have obtained images which suggest fusion of both small matrix droplets and membrane-bounded lipid with large matrix droplets. We are unable, however, to determine if the matrix droplets result from fusion of SER-bounded lipid, Golgi membrane-bounded lipid, or arise from synthesis within the cytoplasmic matrix (57).

Since fat droplets occur in the absorptive cells from puromycin-treated rats, it is concluded that intraluminal digestion, fat entry into the cells, and lipid reesterification occur in the treated animals. However, the export of fat from the absorptive cell seems to be impaired by puromycin treatment, and this defect accounts for the accumulation of fat within the cells. Thus, Sabesin and Isselbacher (51) compared normal and puromycin-treated absorptive cells 4 and 6 hr following intubation with corn oil. They found that intestinal cells from untreated animals were depleted of lipid after 4 hr, whereas those from puromycin-treated animals contained large amounts of lipid. These workers concluded that puromycin blocked the synthesis of β-lipoproteins which are necessary for chylomicra transport (27, 51). Vodovar et al. (59) blocked protein synthesis with cycloheximide and observed lipid accumulations within the cytoplasm of the absorptive cells. Finally, Kayden and Medeck (28) cannulated the thoracic duct and measured the transport of long-chain fatty acids from the gut lumen to the lymph of control and puromycin-treated animals. They found that puromycin inhibited lipid transport into the lymph. However, Redgrave and Zilversmit (47) attribute decreased triglyceride absorption in puromycin-treated animals to an impairment of gastric emptying and intestinal motility. Our results do not support this hypothesis because if less lipid is presented to the absorptive cells during the early stages of absorption, it seems reasonable to expect less lipid to appear in the cytoplasm of the cell. Our data show, on the contrary, that the cells contain much more lipid than do untreated cells exposed to fat for a similar period of time.

Results from most studies in which protein synthesis is inhibited during fat absorption attribute the defect in fat transport to constituents of the chylomicron, particularly its protein component (51, 59). We should like to direct attention to yet another cellular function that is important for fat absorption and is altered by puromycin treatment, namely, the biosynthesis of cellular membranes. Our data clearly show that the amount of RER is strikingly decreased in cells treated with puromycin and exposed to fat for 1 hr when compared with untreated cells. This observation is illustrated in electron micrographs and in the light microscope by the decreased basophilia in the apical cytoplasm of the absorptive cells from puromycin-treated rats. It is suggested that the decrease of RER membranes is due to inability of the ribosomes to synthesize membrane components in the presence of puromycin. Thus, as the RER is converted to SER during fat ab-

FIGURE 19 Intestinal absorptive cells from a puromycin-treated rat 1 hr after corn oil intubation. Lipid has accumulated to a striking degree in the cytoplasm of these cells (see Fig. 12) and is present in both membrane-bounded (*LD*) and matrix form (*ML*). In addition, the size of matrix droplets is much greater than seen at earlier stages following fat administration. This may be explained by the coalescence of smaller droplets to form larger ones (arrows). The region of the Golgi complex (*G*) is often difficult to identify because Golgi vacuoles are greatly dilated with lipid, and Golgi saccules (*GS*) are rare. Both forms of ER are virtually nonexistent except for membrane surrounding lipid droplets and an infrequent profile of RER. The interpretation is offered that all of the RER has been converted to membranes of either the Golgi complex or the SER during fat absorption and cannot be regenerated owing to the inhibition of protein synthesis by puromycin. Some lipid is present in the intercellular space (*IS*). $\times$ 8000.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sorption, there is no replacement of the RER and its amount decreases.

It is reasonable to believe that eventually the effect of puromycin on membrane synthesis would impair fat absorption by blocking the generation of new smooth endoplasmic reticulum (and its contained enzymes). Puromycin treatment does not, however, affect the early stages of fat absorption, because droplets of triglyceride were found in tubules of SER after 15 min of exposure to fat in puromycin-treated animals. This observation suggests that the required enzymes for triglyceride synthesis were present in the membranes of the RER before treatment with puromycin and before the cell was stimulated to absorb fat. Thus the stimulus for the absorptive cell to synthesize triglycerides results in the conversion of RER to SER, and the enzymes function in the synthesis of triglycerides. Droplets of fat accumulate in the tubules and vesicles of SER.

In the early stages of fat absorption in puromycin-treated animals, the transport of SER vesicles containing fat to the Golgi complex and their fusion with this organelle appear similar to that described for untreated cells. After 1 hr of fat absorption in the puromycin-treated animal, the Golgi complex is abnormal. The stacks of saccules are disorganized and decreased in length and numbers; often only a single saccule is found. It is apparent that puromycin treatment and fat absorption for 1 hr have caused a marked decrease in the membranes of the Golgi complex. In addition, the Golgi vacuoles contain fat droplets considerably larger than those found in untreated rats.

A Working Hypothesis (Refer to Fig. 20)

In an attempt to explain the effects of puromycin on fat absorption we should like to propose the following working hypothesis. Two primary

FIGURE 20 Summary of the observations on intestinal absorptive cells after 24 hr fasting (Fig. 20 A); 15 min after fat administration (Fig. 20 B); 60 min after fat administration (Fig. 20 C); and 60 min after fat administration to puromycin-treated rats (Fig. 20 D). In the fasted condition (Fig. 20 A), the RER occupies a considerable portion of the apical cytoplasm, extending from the SER to the Golgi complex. The apical portions of the cisternae of RER are continuous with tubules of SER, while the opposite ends of RER near the Golgi complex show modified cisternae devoid of ribosomes on one surface and many forming vesicles. The vesicles and modified cisternae associate with the immature or forming face of the Golgi complex, suggesting that they represent vehicles for the transfer of membranes from the RER to the Golgi complex. Thus, membranes added to the forming face of the Golgi complex are incorporated into saccules which are progressively converted to Golgi vacuoles at the mature face. During fat absorption (Fig. 20 B and C), the SER increases in amount and contains lipid droplets within its lumen; the RER decreases in amount. We interpret these observations to mean that RER is converted to SER during fat absorption. The SER vesicles containing fat droplets migrate to the Golgi complex where they fuse with Golgi vacuoles or saccules adjacent to the vacuoles. This observation suggests that alterations occur in the membranes of the Golgi saccules as they move toward the mature face of the Golgi complex. These maturation changes apparently are required for the SER membranes to fuse with the Golgi membranes. Moreover, the alterations in the Golgi membranes may be important for the fusion of Golgi vacuoles with plasma membranes, thus allowing discharge of chylomicra (*Ch*) from the cell. A possible sequence of these events (*1–7*) is shown in Fig. 20 B. Three possibilities have been proposed for the origin of matrix lipid droplets (*ML*): (*a*) from fusion of SER vesicles containing fat, (*b*) from fusion of Golgi vacuoles containing fat, or (*c*) from synthesis via enzymes located in the cytoplasmic matrix (Fig. 20 C). Puromycin treatment causes the intestinal absorptive cell to accumulate lipid and to show a striking decrease in intracellular membranes. After 60 min of exposure to fat (Fig. 20 D), the RER is almost entirely absent and the Golgi complex shows only isolated saccules. It is argued that the cell becomes deficient in membranes because their replacement through synthesis is blocked by puromycin, whereas their utilization during fat absorption continues. Accumulation of lipid may be related also to a deficiency of Golgi membranes. As pointed out above, appropriate Golgi membranes are required for fusion of SER vesicles containing fat. If such membranes are lacking in the cell, the SER vesicles are unable to fuse with the Golgi complex and may remain in the cytoplasm or coalesce to form matrix droplets (Fig. 20 D). It is further possible that membranes of the Golgi vacuoles that form in the puromycin-treated animals are qualitatively different from those of untreated cells and are unable to fuse with the lateral plasma membranes; they either accumulate in the cell or fuse to form matrix droplets (Fig. 20 D).

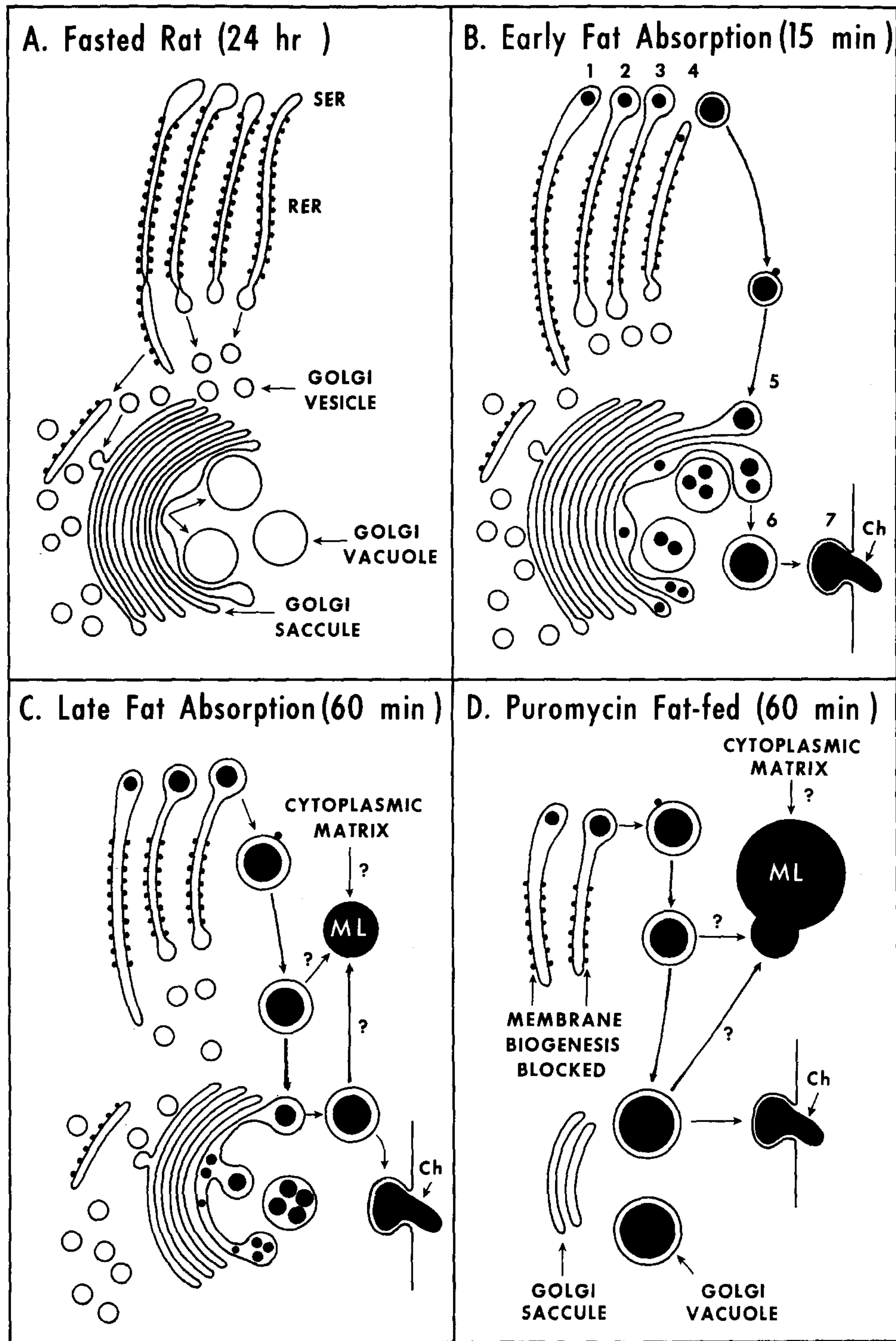

FIGURE 20

functions of the RER with regard to fat absorption are emphasized: (*a*) the generation of membranes containing enzymes required for triglyceride synthesis and (*b*) the synthesis of membranes required for the maintenance of the Golgi complex. It is our view that the RER synthesizes these components possibly in specialized regions of the RER (those destined for SER toward the apical pole of the cell and those destined for Golgi complex toward the nucleus). Therefore, in the fasting condition the cell contains an abundance of RER with the appropriate membrane components. This implies that membrane turnover is very low in the fasted condition. Fat absorption stimulates the intestinal cell to convert RER to SER, and the appropriate enzymes function in the synthesis of triglyceride. Fat accumulates in the SER, and vesicles derived from SER containing fat migrate to the Golgi complex where they fuse with vacuoles and saccules of this organelle. In turn, Golgi vacuoles containing fat migrate to the lateral cell membranes and release chylomicra. Thus, there is a conversion of RER to SER to Golgi membranes and finally fusion of Golgi membranes with plasma membranes. Replacement of Golgi membranes occurs by synthesis in the RER and transport via vesicles and tubules to the forming face of the Golgi complex. This implies a rapid turnover of membranes in the intestinal cell during fat absorption. The early phases of fat absorption apparently proceed normally in the presence of puromycin. We interpret this observation to mean that the absorptive cell utilizes the membrane components of the RER to form SER and to maintain the Golgi complex; however, as fat absorption proceeds and no new membrane synthesis occurs (blocked by puromycin), the membranes of the cell are depleted. It is suggested that the most important membranes for release of triglyceride from the cell are those of the Golgi complex. Thus, in the early stages of absorption when Golgi membranes are abundant, the puromycin-treated cell releases chylomicra similar to untreated cells. In the late stages (1 hr after fat administration) the Golgi membranes are decreased in amounts and the cell accumulates lipid. We argue that puromycin blocks the synthesis of membrane components in the RER that are destined for the Golgi complex; therefore, after utilization of the existing Golgi membranes the cell shows a deficiency of these membranes. Since there are few Golgi vacuoles or mature saccules with which the SER vesicles containing fat may fuse, they remain in the cell either as membrane-bounded fat droplets or matrix droplets.

The authors gratefully acknowledge the excellent technical assistance of Mrs. C. Y. Linsenmayer.

Fig. 20 was prepared by Mr. William E. Fairweather of the Division of Audio Visual Communications, University of Virginia.

This investigation was supported by United States Public Health Service Grant AM-11854.

Received for publication 17 May 1971, and in revised form 2 August 1971.

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