# INTESTINAL TRIGLYCERIDE ABSORPTION IN THE RAT

## An Electron Microscopical Study

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ABSTRACT

This report provides information on the morphology of fat absorption in rat intestinal epithelial cells. Three types of experiments were performed: (*a*) intubation of corn oil into fasted rats, (*b*) injection of physiological fatty-chyme prepared from fat-fed donor rats into ligated segments of jejunum of fasted animals, and (*c*) administration of electron-opaque particles in corn oil and markers given concurrently with the fat. These results support the hypothesis that fat is absorbed by selective diffusion of monoglycerides and fatty acids from micelles rather than by pinocytosis of unhydrolized triglycerides. Evidence is presented that the pits between the microvilli, previously believed to function in the transport of fat, are not involved in this process. Instead they appear to contribute their contents to lysosomes in the apical cytoplasm. Arguments are offered that the monoglycerides and fatty acids diffuse from the micelle while the latter is associated with the microvillous membrane of the absorptive cell. These micellar components penetrate the plasma membrane and diffuse into the cytoplasmic matrix where they encounter the SER. Triglyceride synthesis occurs in the SER and results in the deposition of fat droplets within its lumina. The synthesis of triglycerides and their sequestration into the SER establishes an inward diffusion gradient of monoglycerides and fatty acids.

## INTRODUCTION

The mechanism of intestinal fat absorption, although extensively studied by several approaches, remains controversial. It is known that about 95–100% of ingested triglycerides are absorbed and appear as triglycerides or fatty acids in the circulation (118), but how these cross the barrier represented by the absorptive cell of the intestine, and more specifically, the plasma membrane of these cells, is in doubt. In what follows we report yet another study of these phenomena, a study which attempts to determine the relative importance of uptake by small droplets or particles of fat as opposed to uptake via micelles of much smaller dimensions.

Obviously, it is of prime importance to understand the characteristics of lipid dispersion in the gut lumen in order to explain adequately fat absorption by the mucosal cell. Indeed, the proponents of various theories accounting for lipid uptake have had as one of their basic differences the interpretation of the state of lipid in the gut lumen (4, 5, 9, 10–13, 15, 30, 32, 38–46, 52, 62, 67–69, 73, 82, 96, 97, 114). There is agreement that fat enters the duodenum from the stomach as

unhydrolyzed triglycerides in the form of small droplets which mix with two important secretions: the conjugated bile salts and pancreatic lipase. The bile salts stabilize the fat emulsion, and lipase hydrolyzes the triglyceride. But the extent of intraluminal triglyceride hydrolysis has remained unresolved and has been the subject of debate over a number of years (9, 10, 40, 44, 52, 73, 82, 97, 114, 117, 118).

One group of investigators has maintained that hydrolysis is completed in the intestinal lumen and that the resulting fatty acids and glycerol diffuse into the mucosal cell where they are resynthesized to triglycerides (10, 73, 82, 114). This is the basis for the "lipolytic theory of fat absorption". Frazer (40), on the other hand, has contended that hydrolysis is only partial, and the the combination of bile salts–fatty acids–monoglycerides is important in maintaining an emulsion of unhydrolyzed triglyceride in the gut lumen. According to him, this emulsion simply filters through the "pores" described, e.g. by Baker (4), in the striated border of the mucosal cell. This is referred to as the "particulate" theory of lipid absorption.

The idea of particulate uptake lost favor when it was demonstrated by electron microscopy that the striated border does not consist of pores, but rather of many small microvilli (47) which are enclosed by a continuous membrane. Nonetheless, the theory of absorption of unhydrolyzed triglyceride again gained popularity when small pits containing fat droplets were discovered between the microvilli (77, 78). The images depicting these small pits or invaginations were interpreted to mean that particulate fat droplets are engulfed by a process of pinocytosis and transported into the epithelial cell. Palay and Karlin (77, 78) provided important information on this process and other aspects of the fine structure of the intestinal mucosal cell under conditions of fasting and after fat-feeding. Particularly significant was their discovery that the fat droplets appeared within the cisternae of the endoplasmic reticulum and that these droplets moved through this system of the epithelial cell. As is now well known, the suggestion was offered that fat is engulfed in droplet form by pinocytosis and that the pinocytotic vesicles fuse with the endoplasmic reticulum and thereby transfer the fat to this system of the cell. It was their further suggestion that the fat is released from the cell to the intercellular spaces by a process of reverse pinocytosis. Accordingly, the fat does not traverse a cell membrane at any time or become exposed directly to the cytoplasmic matrix. Indeed, its route through the cell could be considered "extracellular" (78).

Images, which have been interpreted as further evidence for this type of pinocytotic uptake, have subsequently been described by several investigators (1–3, 49, 50, 61, 70, 74, 79, 95, 105). However, other observers have challenged this interpretation primarily because of the paucity of pinocytotic vesicles found during fat absorption (7, 60, 76, 83, 84, 87–89, 103, 104, 108). Further, it has been argued as highly improbable that fat, along with other crude materials in the gut lumen, would be introduced into the cisternae of the ER where sequestration of metabolites and products of cell synthesis normally occur (85). It may be reasoned as also unlikely that a cell with an apical specialization of microvilli, which increases its surface some 14–40 times (19, 47, 113, 119), would utilize only the relatively small membrane area between the microvilli for fat absorption.

But an even more compelling reason for rejection of the pinocytotic mechanism is the fact that much of the biochemical data (53–55, 58, 65, 97, 117) seems incompatible with pinocytotic uptake. Notable are the experiments which show fat uptake by the intestinal mucosal cells under such conditions as 0°C, after heat inactivation, and in the presence of metabolic inhibitors (58). Obviously, since pinocytosis is an active, energy-requiring process (93), it would not operate under these conditions. Finally, it has been shown that the enzymes necessary for triglyceride synthesis are present in the intestinal epithelial cell and more specifically in the microsomal fraction of this cell (54, 55, 97–101). These data argue strongly for the interpretation that the triglycerides of the intestinal epithelial cell are resynthesized, at the smooth ER, from free fatty acids and monoglycerides rather than taken up as unhydrolyzed droplets.

Recent studies on the physiochemical nature of lipids in the gut lumen also indicate that absorption is accomplished via diffusion of monoglycerides and fatty acids (11, 12, 15, 34, 51, 52, 97). Hofmann and Borgström (52), for example, analyzed the gut contents during fat absorption, and after centrifugation they found two distinct phases: (*a*) an oily phase consisting primarily of di- and triglycerides, and (*b*) a micellar phase made up of free fatty acids, monoglycerides, and conjugated

bile salts. These workers suggest that it is the micellar phase of the gut contents which comes into contact with the membrane limiting the microvilli and from which the fatty acids and monoglycerides diffuse through the membrane. The conjugated bile salts remain in the gut lumen and are available for reutilization in the formation of micelles. After diffusing into the mucosal cell, the fatty acids and monoglycerides are reassembled into triglycerides, a process catalyzed by enzymes found in the endoplasmic reticulum.

These conflicting views notwithstanding, the electron microscopical evidence that lipid droplets do occur within the pinocytotic pits seems quite convincing. Recently, Palay and Revel (79) have repeated the original experiments on fat absorption, and they report that more pinocytotic vesicles are present during fat absorption than were previously recognized. These authors feel that the new observations make the pinocytotic mechanism more compatible with the biochemical data; they also point out that biochemical changes could occur in the triglycerides while they are within the ER, and thus account for the known alterations in the ingested triglyceride during absorption (81).

Senior (97), in an attempt to reconcile these various observations, has suggested that perhaps both pinocytosis and micellar absorption are operative, but that the micellar form is the major and more important process, while pinocytosis is less involved and accounts for only a minor amount of total lipid uptake. He has reasoned that a droplet of unhydrolyzed triglyceride would be attacked by pancreatic lipase and thus would release monoglycerides and fatty acids. These form micelles which are absorbed by diffusion. As intraluminal hydrolysis continues the residual triglyceride droplets become smaller and, when they reach a size suitable for pinocytosis, they are engulfed by the pinocytotic pits between the microvilli. It should be recognized that even here hydrolysis could continue.

It is obvious, therefore, that the role of pinocytosis in fat absorption is unsettled, and certainly the quantitative contribution of pinocytosis to this process is undetermined. Since the mode of fat entry into the mucosal cell is important to an understanding of the total process of fat absorption, synthesis, and transport, and because an understanding of these events might further elucidate the functioning of the endoplasmic reticulum in this and other cells, the investigation of this problem, with the primary objective of providing information on the quantitative importance of pinocytosis to fat absorption, seemed warranted.

Two basic approaches were utilized in this study. First, we reasoned that, if pinocytosis contributed significantly to this process, then there should be an increase in the number of pinocytotic pits and vesicles during fat absorption. Accordingly, a study of rat intestinal epithelial cells from fasted and fat-fed rats was made with special attention to the pinocytotic pits and terminal web area of the cell. Incidental to this study, a number of observations were made on the fine structure of the rat intestinal cell during fat absorption and, because these seemed particularly pertinent to an understanding of the over-all process, they also are reported.

The second approach to the problem was to place electron-opaque markers in the ingested fat, and thus to allow a distinction to be made between fat absorbed by diffusion and fat absorbed by particulate uptake or pinocytosis. It is obvious that if any quantity of fat is taken up in particulate form an inert marker included with the fat should accumulate in the cells.

The results of this study do not support the hypothesis of particulate fat uptake; they suggest instead that fatty acids and monoglycerides diffusing into the intestinal epithelial cell represent the major, if not the sole, mechanism of fat absorption. No changes were detected in the microvilli or in the terminal web area which could be interpreted as evidence for intact fat micelles within the cytoplasm of this cell. It is evident that the synthesis of triglycerides occurs at the membranes of the smooth endoplasmic reticulum and it appears that chylomicra formation is completed within the cisternae of this subcellular system. After formation, the chylomicra are transported directly to the intercellular spaces or to the Golgi complex.

The results of this study were obtained from three separate groups of experiments and it seems preferable to consider the observations and results of each section separately. Thus we shall present the observations on the intestinal epithelial cell from fasted rats as Part I, intestinal epithelial cell from fat-fed animals as Part II, and the experiments utilizing electron-opaque markers as Part III. Some of this work has been reported previously in abstract form (21, 22).

# Part I. Intestinal Epithelial Cell: Fasted Rat

Despite the voluminous literature on the fine structure of the intestinal epithelial cell, there remain a number of details which are not clearly resolved. Since an understanding of the structure of the cell is basic to an understanding of how it functions, we feel it important to describe our observations in some detail. Some regions of the cell which do not seem to be involved in fat absorption directly are omitted from consideration, as are others on which we are able to offer no new information or interpretation. Thus our attention has been focused primarily on the apical cytoplasm of the mucosal cell. We, therefore, have nothing new to report on the lamina propria and can only refer the reader to recent papers on this important component of the villus (29, 113).

## MATERIALS AND METHODS

Sprague-Dawley (Harvard stock) rats were fasted for 48 hr, and anesthetized with ether. The upper jejunum was exposed. A small segment of the intestine was removed rapidly, placed in a drop of fixative, and immediately cut into several pieces of a size to favor good fixation. Care was taken to trim the specimen in such a way as to facilitate orientation during embedding.

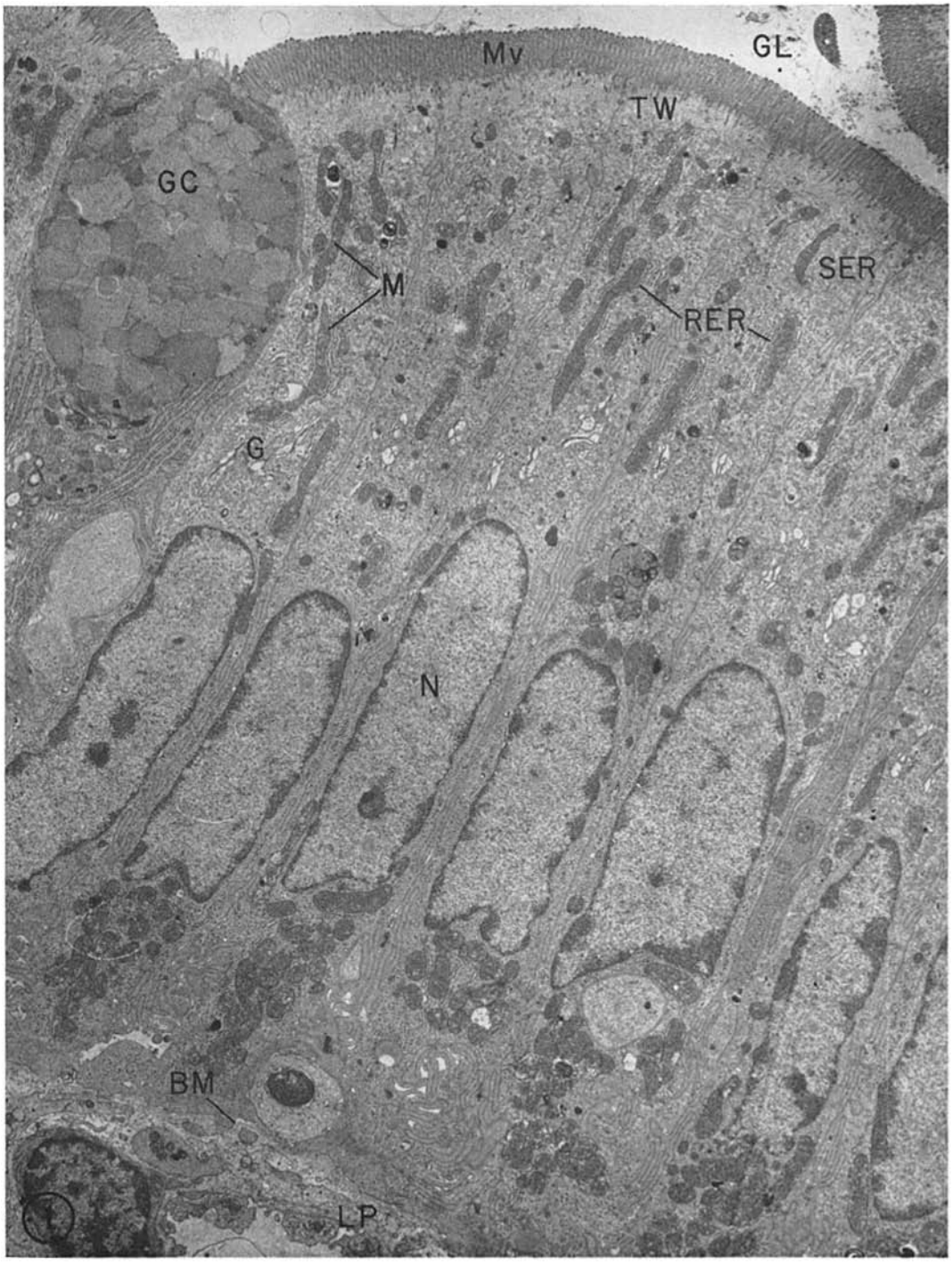
Two fixation procedures were employed: (*a*) 3% glutaraldehyde (Biological Grade, Fisher Scientific) (94) in either 0.1 M cacodylate or 0.1 M phosphate buffer at pH 7.3 for 1–4 hr at room temperature; (*b*) 1% osmium tetroxide (at pH 7.3 in phosphate buffer) for 1–2 hr (71, 72) at room temperature. Calcium chloride (2–4 mM) was added to all glutaraldehyde fixing fluids. After glutaraldehyde-fixation the

*Abbreviations*

*AL*, apical lysosome
*AP*, apical pit
*AV*, apical vesicle
*BM*, basement membrane
*CP*, coated pit
*Cm*, chylomicron
*D*, desmosome
*F*, filaments
*Fe*, fenestrations of RER
*FLD*, free lipid droplet
*G*, Golgi complex
*GC*, goblet cell
*GL*, gut lumen
*I*, interdigitations
*IS*, intercellular space
*LD*, lipid droplet
*LP*, lamina propria
*M*, mitochondrion
*Mv*, microvilli
*Mt*, microtubules
*N*, nucleus
*RER*, rough endoplasmic reticulum
*SER*, smooth endoplasmic reticulum
*TJ*, tight junction
*TW*, terminal web

FIGURES 1–14 are electron micrographs of rat intestinal mucosal cells fixed for 2 hr at room temperature in 3% glutaraldehyde, postfixed in $OsO_4$, dehydrated in ethanol, and embedded in Epon 812. The sections were cut with a diamond knife on a Sorvall MT1 and stained on the grids with uranyl acetate and lead citrate.

FIGURE 1. Micrograph showing, at low magnification, several absorptive cells and a part of a goblet cell (*GC*) from a fasted rat. The lumen (*GL*) of the intestine is at the top and a small area of the lamina propria (*LP*) is included at the lower left. A thin basement membrane (*BM*) which follows the contours of the basal surfaces of the epithelial cells, separates these cells from the lamina propria. A complex of interdigitations characterizes the lateral surfaces of these cells. Such infoldings are prominent near the basal poles of the cells and also along the upper half. An elongated nucleus (*N*) is situated mostly within the basal half of each cell. Basal to this is a dense cluster of mitochondria and a cytoplasm showing free ribosomes and a few profiles of RER. The apical half of the cells which contains the bulk of the cytoplasm, shows long slender mitochondria mostly with long axes parallel to the long axis of the cell. The rough endoplasmic reticulum (*RER*) is prominent in the form of long slender profiles closely associated with the mitochondria. Numerous smaller profiles of the smooth endoplasmic reticulum (*SER*) are evident throughout this region of the cell, but are concentrated mostly just under the terminal web (*TW*). A prominent Golgi component sits just apical to each nucleus. The free surface is covered by microvilli (*Mv*) making up the striated border. 48 hr fasted rat. × 5,200.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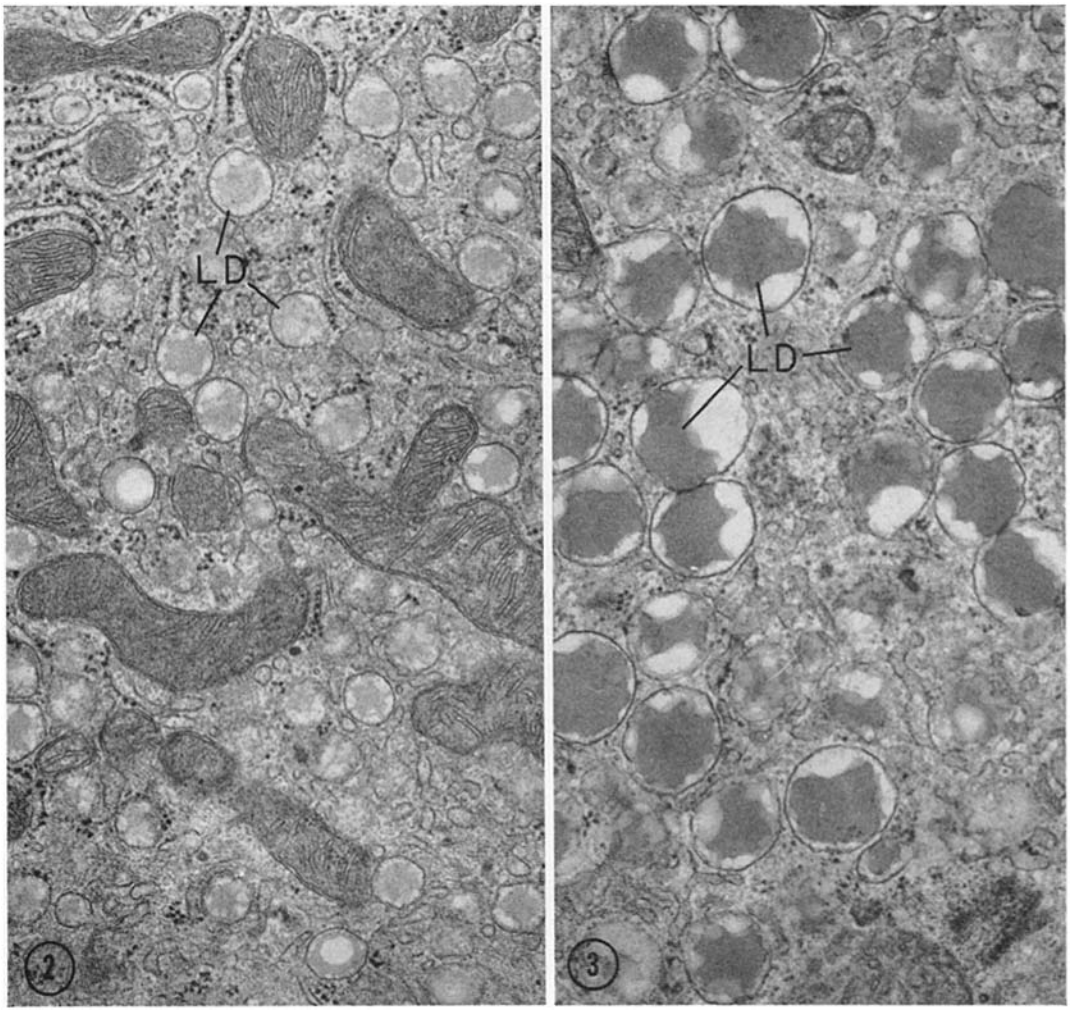



FIGURES 2 and 3 A comparison of the effects of varying the buffer used in the glutaraldehyde fixative. The lipid droplets (*LD*) are denser after phosphate-buffered glutaraldehyde (Fig. 3) than they are after cacodylate-buffered glutaraldehyde (Fig. 2) fixation. In other respects the quality of the fixation achieved with these two mixtures is very nearly identical. Fig. 2, fixation 1 hr after corn oil intubation. × 40,000. Fig. 3, fixation 45 min after corn oil intubation. × 33,000.

specimen was rinsed in multiple changes of the same buffer used in fixation with the addition of 10% sucrose and postfixed in 1% osmium tetroxide (pH 7.3 in phosphate buffer). The glutaraldehyde-osmium tetroxide resulted in recognizably superior fixation and was generally relied upon in these studies.

The tissues were dehydrated in a graded series of ethanol and embedded in Epon (66). Ultrathin sections were cut on a Porter-Blum I or II ultramicrotome, double stained with uranyl acetate (115) and lead citrate (86), and studied in a Philips EM-200 electron microscope.

## OBSERVATIONS AND RESULTS

### *General Morphology of the Rat Intestinal Epithelial Cell*

The intestinal mucosal cell of a *fasted* animal is columnar and is modified on its luminal surface to form a striated border consisting of many small microvilli (Figs. 1, 4). The basal surface of the cell rests on a thin basement membrane which separates the epithelium from the lamina propria. The lateral margins of the cell are characterized by

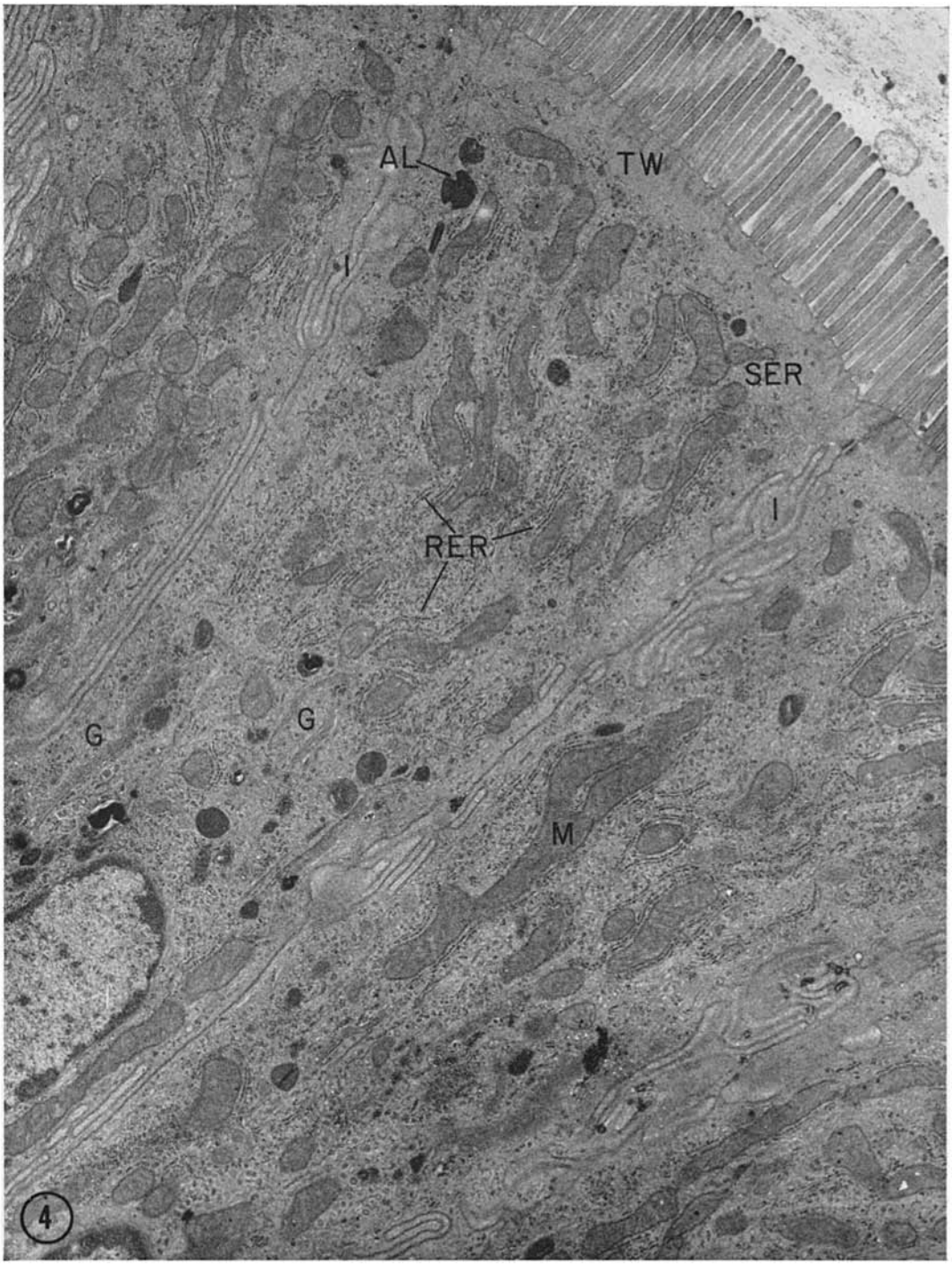



FIGURE 4 Apical cytoplasmic regions of three intestinal epithelial cells from a fasted rat. The rough endoplasmic reticulum is abundantly represented and appears in profile as slender cisternal units studded with ribosomes. The RER extends throughout the greater portion of the cytoplasm between the Golgi complex (*G*) and the terminal web (*TW*). The SER is present to a shallow depth below the terminal web area but it is not prominent in the epithelial cell from a fasted rat. Apical lysosomes (*AL*) and mitochondria (*M*) are shown. Apposing surfaces of adjacent cells show complex interdigitations (*I*). 48 hr fasted rat. × 12,000.

many deep foldings and interdigitations and by specialized regions of attachments with the adjacent cell, i.e. desmosomes, terminal bars, and tight junctions (Figs. 1, 4, 8).

The nucleus is located in the basal half of the cell, where the cytoplasm consists primarily of free ribosomes and mitochondria (Fig. 1). The Golgi complex occupies a position just apical to the nucleus, and the endoplasmic reticulum, consisting of both the smooth and rough, but predominantly rough, varieties extends from the Golgi zone to the region just below the terminal web area (Figs. 1, 4). Large mitochondria, usually aligned with their long axes parallel to the length of the cell, are interspersed with the cisternae of the ER (Figs. 1, 4).

The terminal web area consists of fine filaments oriented in a position predominantly parallel to the luminal surface of the cell. Some of these filaments insert on the terminal bars and thus form a continous web across the apical pole of the cell (Figs. 4, 8).

### *Striated Border and Terminal Web Area*

The striated border, as mentioned above, is composed of microvilli. Each microvillus is approximately 0.1–0.15 $\mu$ in diameter and 1.0–1.5 $\mu$ in length and is limited by an unusually thick (110 A) plasma membrane (Figs. 6 and 6 *a*). A coating of material, considered to be acid mucopolysaccharide (56), covers the plasma membrane on the outer or luminal surface. This "apical fuzz" is particularly prominent on the tips of the microvilli (Fig. 6).

The microvillous membrane, as it extends between the bases of the microvilli, frequently dips or invaginates into the cortical cytoplasm and thus forms pits (Figs. 9, 14). These are the structures believed to function in pinocytosis of fat, and they have been termed "pinocytotic pits" (77, 78). We prefer the name "apical pits" because this term does not assign specific functional properties where none are known. The apical pits are approximately 50–100 m$\mu$ deep and are bounded by a membrane identical with that which limits the microvilli. We shall return to this point later in this report.

The central core or shaft of each microvillus includes a bundle of approximately 25–30 fine parallel filaments (Figs. 6, 6 *a*, 7, 7 *a*). These filaments are about 60 A in diameter and extend from a region close to the tip of the microvillus into the zone of the terminal web filaments (Figs. 7 and 8). An individual filament is observed to be long and straight when it can be followed for a considerable distance in one section. The microvillous and terminal web filaments intermingle and are essentially identical in appearance (Fig. 8 and others), except that the terminal web filaments are not so straight as those of the microvilli; thus in a section many short segments of them are seen (Fig. 7). The length of an individual terminal web filament is unknown.

The junctional complexes between intestinal epithelial cells, i.e. the three types shown in Fig. 9, have been treated in detail by Farquhar and Palade (36). Thus, located just below the luminal surface of the cells there is the tight junction, zonula occludens, characterized by the fusion of the outer dense lines of the opposing unit membranes. Subjacent to this there is the intermediate junction, zonula adhaerens. Usually below the zonula adhaerens is a desmosome or macula adhaerens which is different from the two other junctional specializations in that it is a discontinuous structure and occurs as a "buttonlike" attachment only at certain points (35).

There are also within the terminal web zone of the intestinal epithelial cell (Fig. 8) a number of electron-opaque bodies showing a variety of shapes and sizes. These we shall refer to as apical vesicles. The majority are oblong or spherical and frequently appear to possess a lumen (Figs. 8, 8 *a*, 9). The unit membrane surrounding these bodies has the same thickness (110 A) as that limiting the microvilli and the apical pits (Figs. 8 *a*, 14); this fact among others suggests that these bodies are derived from the apical pits. (See evidence presented also in Part III reporting experiments with electron-opaque markers.) Apparently the apical pits pinch off to form these apical vesicles. A dense content is characteristic of these structures.

Two other types of bodies are seen frequently in the terminal web area of the cell: one is about 0.5 $\mu$ in diameter and contains within it many smaller vesicles; the other is similar except that it has fewer enclosed vesicles and the internal structure is much more dense (Figs. 4, 8, 8 *a*). These are the same bodies that have been shown in other studies of intestinal epithelial cells to possess acid phosphatase activity and are described as lysosomes (6, 8, 75, 102, 112). Because these bodies occur most frequently in the apical pole of the cell we refer to them as "apical lysosomes." The thickness of the membrane (110 A) limiting these lysosomes

and their enclosed vesicles is identical with that of the apical pits and apical vesicles (Fig. 8 *a*), a fact which can be interpreted to mean that the apical vesicles fuse to form the apical lysosome.

### *Endoplasmic Reticulum*

The smooth endoplasmic reticulum (SER) in these absorptive cells is concentrated immediately beneath the terminal web area and in fact seems to invade this zone of the mucosal cell (Fig. 8). It has the form of a loosely woven lattice of convoluted tubules which vary widely (10–100 mμ) in diameter and are limited by a membrane about 60 A thick. In the fasted animal the SER of the mucosal cell is not particularly abundant and usually appears in thin sections as isolated segments (Fig. 8). These do not intermingle with the large mitochrondria which, in these cells, show a preferred association with the cisternae of the rough ER (Figs. 1, 4, 8). For the most part the tubules and vesicles of the SER appear empty, but occasionally even in a 48 hr fasted rat, they contain dense particles resembling lipid.

Evidence of continuity between the SER and the rough endoplasmic reticulum (RER) is readily found (Fig. 8). In a favorably oriented section the RER cisternae can be traced for a considerable distance and their transition into SER tubules is encountered at their margins. In cells of the fasted animal the RER is prominent in the supranuclear cytoplasm between the Golgi zone and the terminal web zone. The profiles of these cisternae appear as fairly long and uninterrupted, paired lines studded with ribosomes and oriented parallel to the long axis of the cell. Apparently the large lamellar cisternae represented by these profiles is the preferred form of the RER in the intestinal cell during states of fasting. Mitochondria in large numbers intermingle with the RER cisternae.

### *Golgi Complex*

The Golgi complex in a 48 hr fasted rat is located between the nucleus and the RER zone of the intestinal absorptive cell (Figs. 1, 4). This complex consists of the flattened sacs, small vesicles, and larger vacuoles characteristic of this system in other cells (27) and as described previously in the intestinal mucosal cell (77, 112, 113, 116, 119). There are usually many smaller, coated vesicles apparently derived from coated pits of the Golgi saccules. In some cells, after 48 hr fasting, the Golgi vacuoles are dilated and contain electron-opaque droplets resembling lipid.

### *Microtubules*

Microtubules are present throughout the supranuclear cytoplasm and are generally aligned parallel with the long axis of the cell (Figs. 8, 10, 13). They have also been seen in the terminal web area where they assume an orientation more or less parallel to the terminal web filaments. The major morphological features of the microtubules are similar to those of tubules described in a variety of plants and animal cells (20, 63, 64, 109–111) and need not be commented on here. They show no consistent variation during fat absorption that we have been able to detect.

### *Lateral Cell Membranes and Intercellular Spaces*

The intercellular spaces are not prominent between intestinal epithelial cells when fat absorption is not in progress; however, when present, they usually occur at about the level of the nuclei. Interdigitations of adjacent cells are common especially in the apical region of the cell (Fig. 4). Where not involved in interdigitations the cell membranes of the same regions occasionally show coated pits (Figs. 12, 12 *a*). These pits are about 50 mμ in diameter and taper to a small neck at the junction with the lateral cell membrane. The membrane of the pit is rendered distinctive by its roughened appearance on both sides as is characteristic of these structures in other types of cells (16–18, 48, 90–92).

## Part II. Intestinal Epithelial Cell: Fat-Fed Rat

The upper jejunum is the area of the small intestine most active in fat absorption (14, 28, 57, 117), and so we have restricted our observations to this region. It is generally agreed also, based on re-

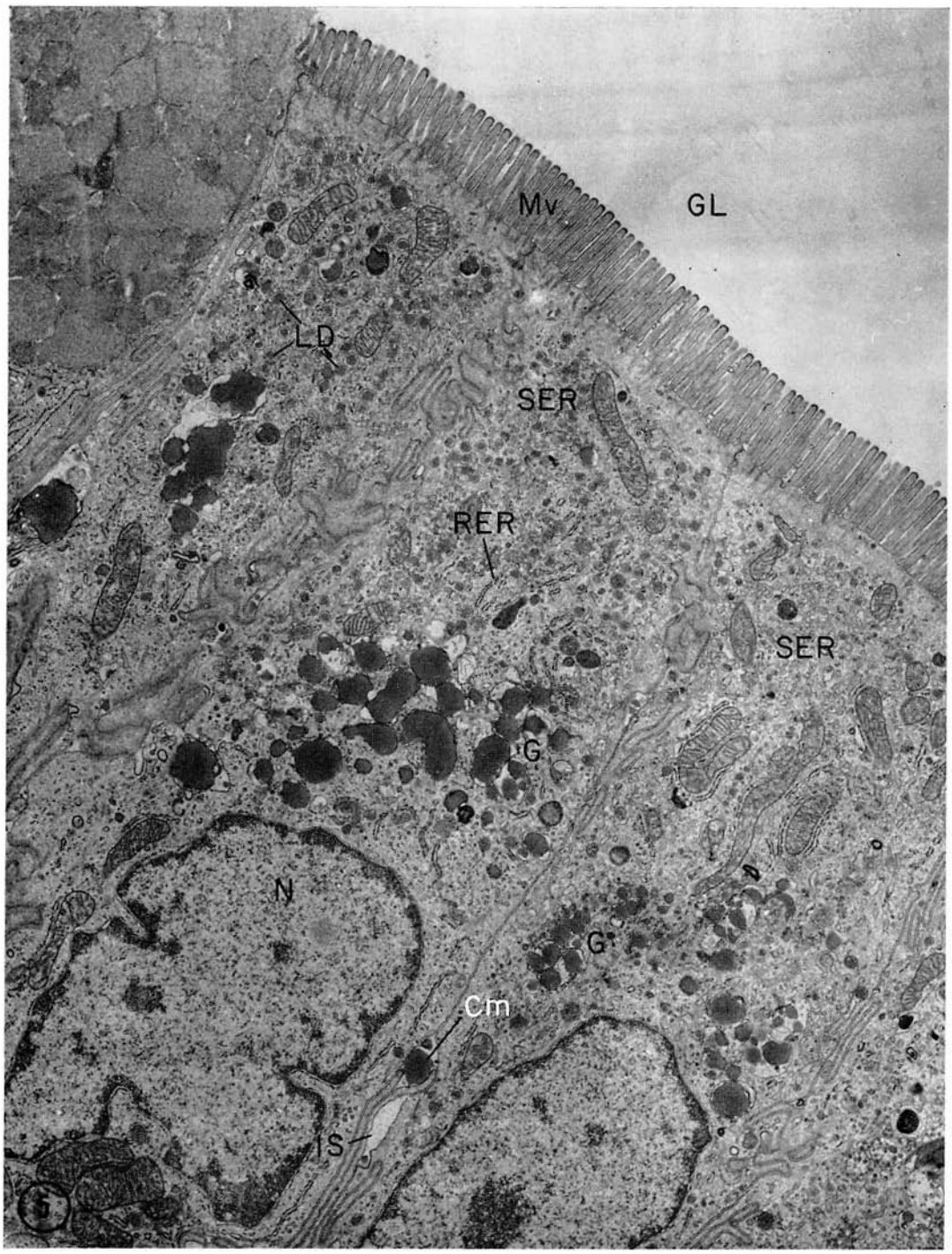



FIGURE 5 Intestinal epithelial cells located near the tip of a villus from a fat-fed animal showing accumulations of lipid (*LD*) as electron-opaque bodies within the apical cytoplasm. The RER is far less prominent than in Fig. 4 and appears as short cisternal profiles during phases of fat absorption. The SER and Golgi complex on the other hand are far more prominent than in the fasted state and contain numerous droplets of fat. The SER-derived vesicles containing fat droplets are distributed throughout the apical cytoplasm from the terminal web area to the Golgi zone of this cell and even beyond. The lipid droplets within the SER vesicles are uniform in size and smaller than those of the Golgi vacuoles. Chylomicra (*Cm*) appear in the intercellular spaces (*IS*) but very few such droplets are found in the cytoplasm lateral to the nucleus or in the basal cytoplasm. Fixation 45 min after corn oil intubation. × 9,000.

peated observations (117, 118), that the upper one-third of the villus is most active in fat absorption. Actually there is considerable variation in cell structure along the villus from the tip to the base, related presumably to the progressive maturation of cells along this axis. Again to avoid long and, for present purposes, unimportant descriptions of diversity we have chosen to limit this report to cells in the upper one-third of the villus facing the intestinal lumen.

## MATERIALS AND METHODS

In the experiments to be reported, fasted, lightly anesthetized rats were fed corn oil by means of a thin polyethylene tube (inside diameter 0.85 mm; outside diameter 1.50 mm) inserted via the esophagus into the stomach (79). Each animal was given 1.5 ml of corn oil, and thereafter segments of upper jejunum were removed for electron microscopy at intervals from 10 min to 1 hr. Procedures for electron microscopy were as described in Part I of this paper. One point about the fixation of lipid droplets merits special comment, however. The lipid droplets are rendered more electron-opaque after fixation with glutaraldehyde in phosphate buffer followed by osmium tetroxide, than they are after fixation in glutaraldehyde in cacodylate buffer followed by osmium tetroxide (compare Figs. 2, 3). The lipid droplets are, however, very easy to identify in the mucosal cell after either fixation procedure.

## OBSERVATIONS AND RESULTS

### *General Morphology of the Rat Intestinal Epithelial Cell*

The most conspicuous change in the morphology of the intestinal epithelial cell after fat administration is the appearance of dense spherical bodies throughout the apical cytoplasm of the cell (Fig. 5). These are properly interpreted as lipid droplets derived from fat digestion and uptake. The number and size of the fat droplets in the absorptive cell vary according to the position of the cell on the villus and the length of time the cell is exposed to the lipid. In cells located at the tip of the villus, the lipid droplets are larger than in those located laterally and somewhat removed from the tip. We have noted as well that the intercellular spaces between the tip cells are much larger after fat feeding, and that frequently the base of the cell is separated from the basement membrane. It is probable that some part of these latter features reflect the process of cell aging and extrusion occurring in this apical zone of the villus. This variation in morphological response according to the position of the cell on the villus is consistent in all villi which are absorbing fat; however, the cytological variation of absorptive cells, associated with time after fat administration, varies from villus to villus, and indeed there is variation within a single villus. Generally, the cells contain smaller lipid droplets early after fat administration, e.g., at the end of 10 min; and much larger and more numerous droplets later, e.g., at the end of 1 hr.

### *Striated Border and Terminal Web Area*

The microvilli of the striated border during fat absorption appear similar in fine structure to those on the mucosal cell during fasting. The unit membrane, the core of filaments, and the density of the microvillus are similar to, if not identical with, those described for the fasted rat. At the resolutions employed here we have noted no really striking differences between microvilli from fasted and fat-fed rats (compare Figs. 6 and 7).

*There is, moreover, no apparent increase in the number of apical pits or change in their structure except for extremely rare occasions when a droplet of fat is present in the cavity of the pit.* This observation is based on a study of 280 electron micrographs taken at random of the apical regions of the intestinal absorptive cells from fat-fed and fasted rats. It is also significant to record that out of 750 electron micrographs obtained in this study only two images of fat droplets in apical pits were recorded.

We have also observed no change in the number or structure of the apical vesicles (derived from the pits) or of the apical lysosomes. And the filaments of the terminal web area and the junctional complexes seem identical in controls and experimentals.

### *Endoplasmic Reticulum*

The above similarities do not extend, however, to all structures. For example, the smooth endoplasmic reticulum (SER) shows striking alterations during fat absorption. This system, which in the cells of the fasted animal is relatively insignificant, occupies a far greater portion of the cytoplasm and is more abundant per unit volume of cytoplasm in the absorptive cells of the fat-fed animal. Lipid droplets are frequently present within the tubules of the SER (Figs. 5, 9, 10), and very frequently they occur in bulbous expansions of this system (Figs, 10, 10 *a*). Usually a single lipid droplet is

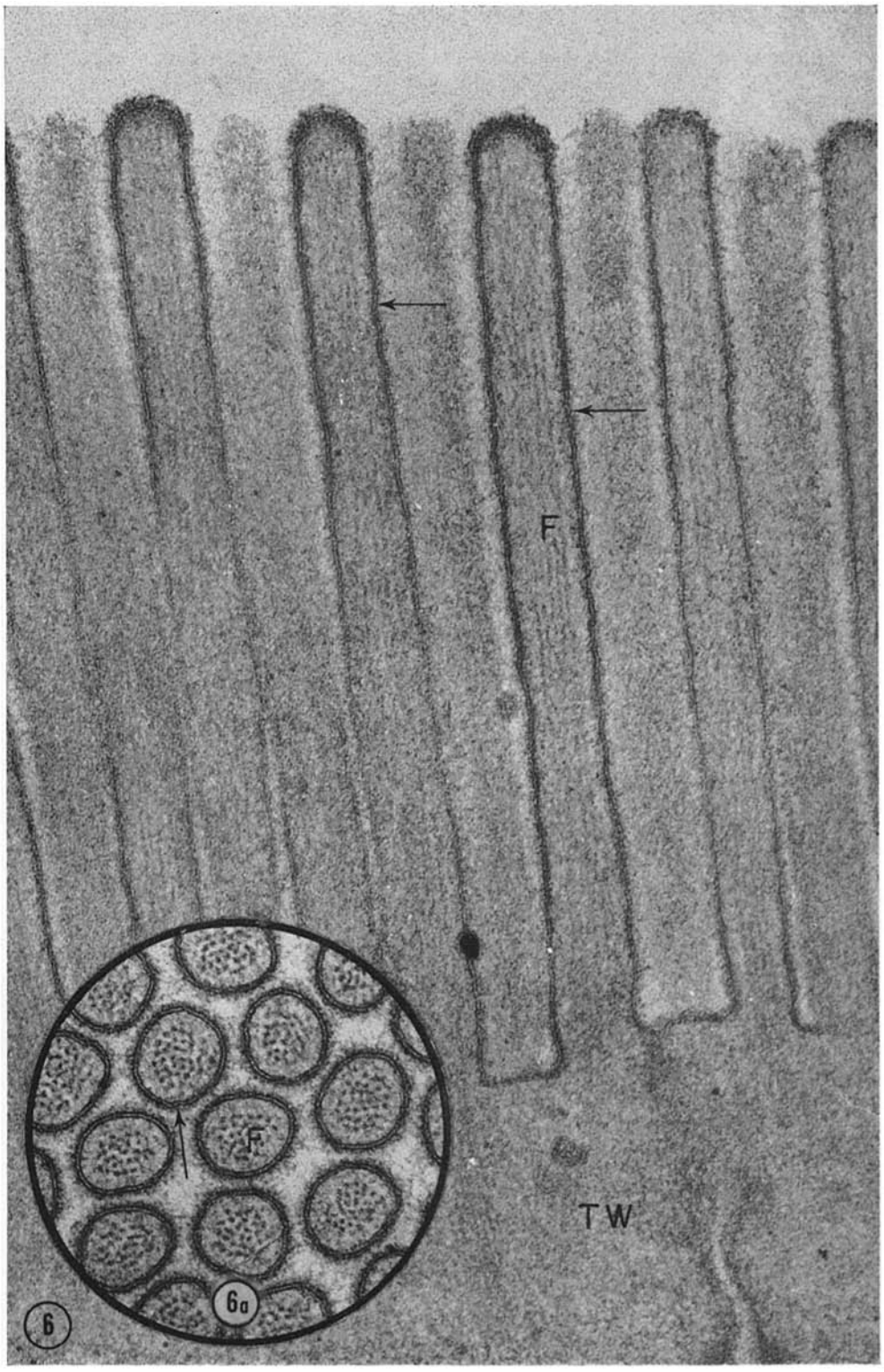
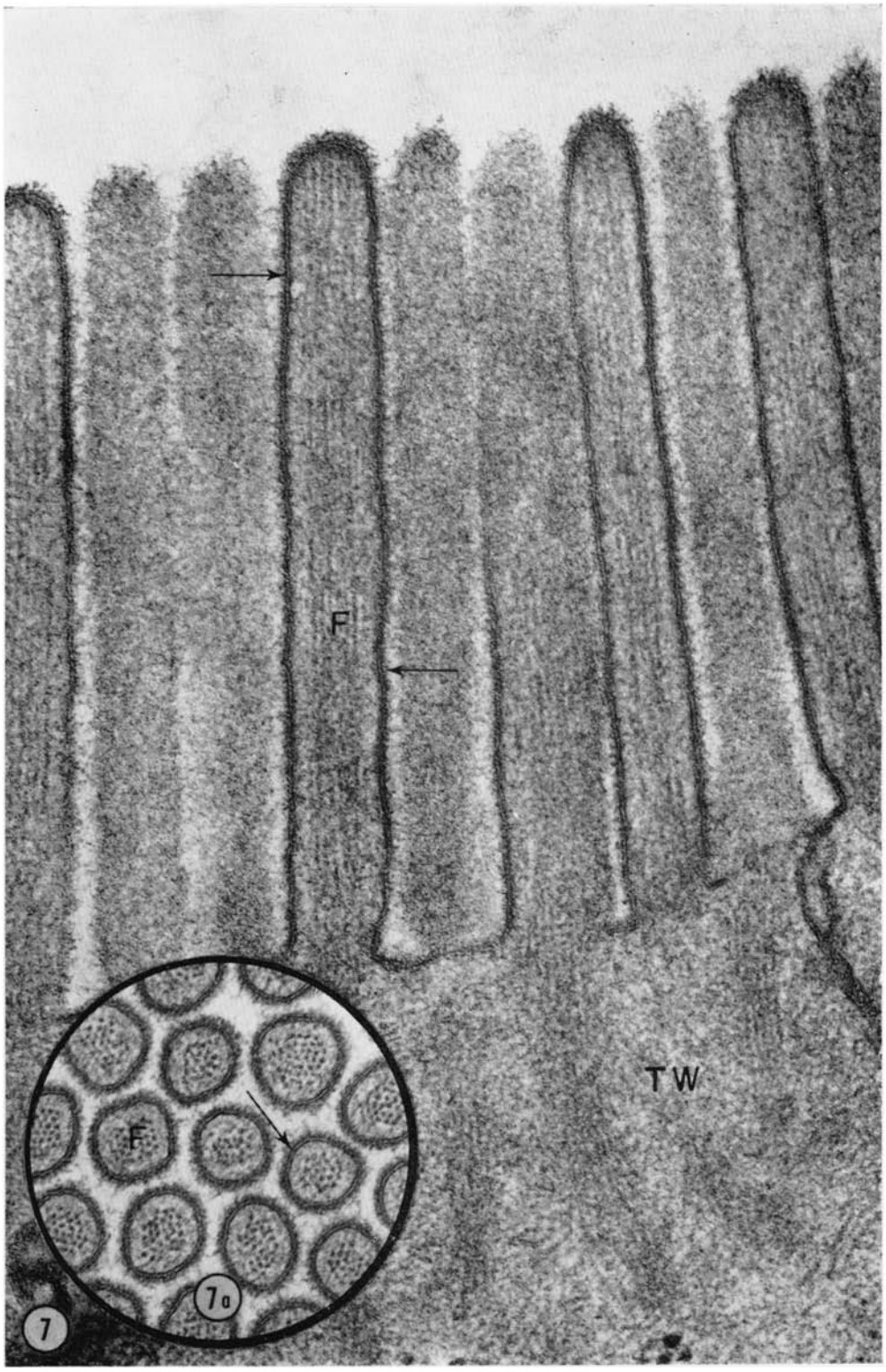



FIGURE 6 Microvilli from an intestinal epithelial cell of a fasted rat. The microvilli are limited by a unit membrane (arrows) which on its luminal surface is coated with a fine fuzzy material. The central region of the microvillus contains filaments (*F*) which extend from near the tips of the microvilli into the terminal web area (*TW*) where they mingle with the terminal web filaments. A cross-section of the microvilli (inset, Fig. 6 a) shows the disposition of the filaments within a microvillus. 48 hr fasted rat. × 104,000.




FIGURES 7 and 7 *a* Microvilli from an intestinal epithelial cell of a fat-fed rat. The microvilli with their limiting unit membrane (arrows) do not appear to differ in any significant respect from those of the fasted rat. This comparison is difficult, especially where it involves dimensions, because the microvillous structure and size vary greatly in different epithelial cells depending somewhat on their position on the villus. Fig. 7, 1 hr after corn oil intubation. × 104,000. Fig. 7 *a*, 20 min after corn oil intubation. ×104,000.

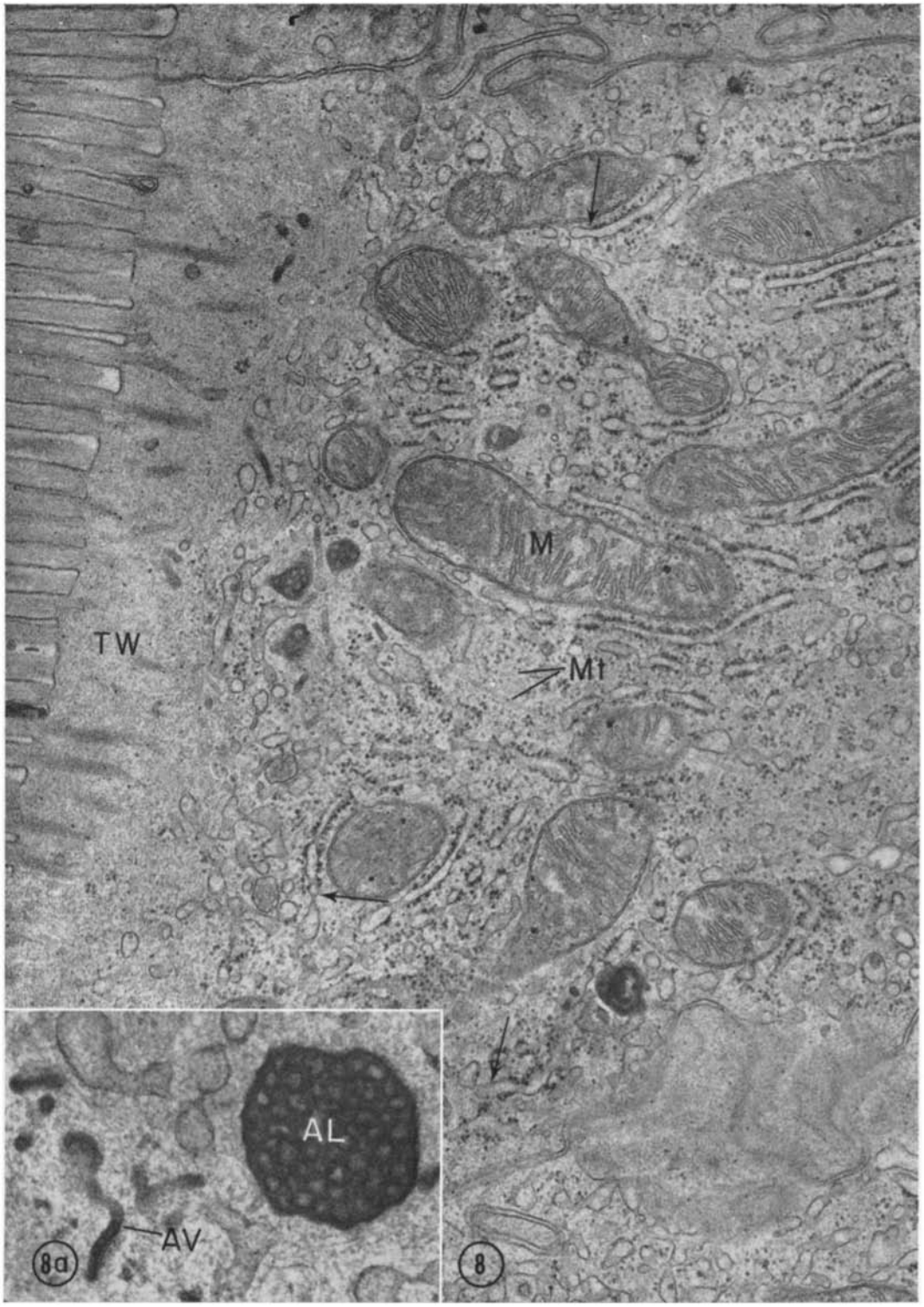



FIGURE 8 Apical cytoplasm of intestinal epithelial cell from a fasted rat. The tubular, reticulate form of the SER is shown and the localization of this system below the terminal web (*TW*) is clearly apparent. This is the region of the cell where continuities between the long cisternal units of the RER and the tubular SER can be identified (arrows). The amount of RER in this micrograph is strikingly greater than that present during fat absorption, as pictured in Fig. 10. Conversely the SER is far less evident in the fasted cell. Microtubules (*Mt*) are usually oriented parallel to the long axis of the cell. 48 hr fasted rat. × 31,500.

FIGURE 8 *a* Enlargement of the apical lysosome (*AL*) and apical vesicles (*AV*). Note the similarity of the unit membranes surrounding the vesicles and the lysosome. The vesicles enclosed within the apical lysosome also possess the 110 A thick unit membrane. × 51,000.

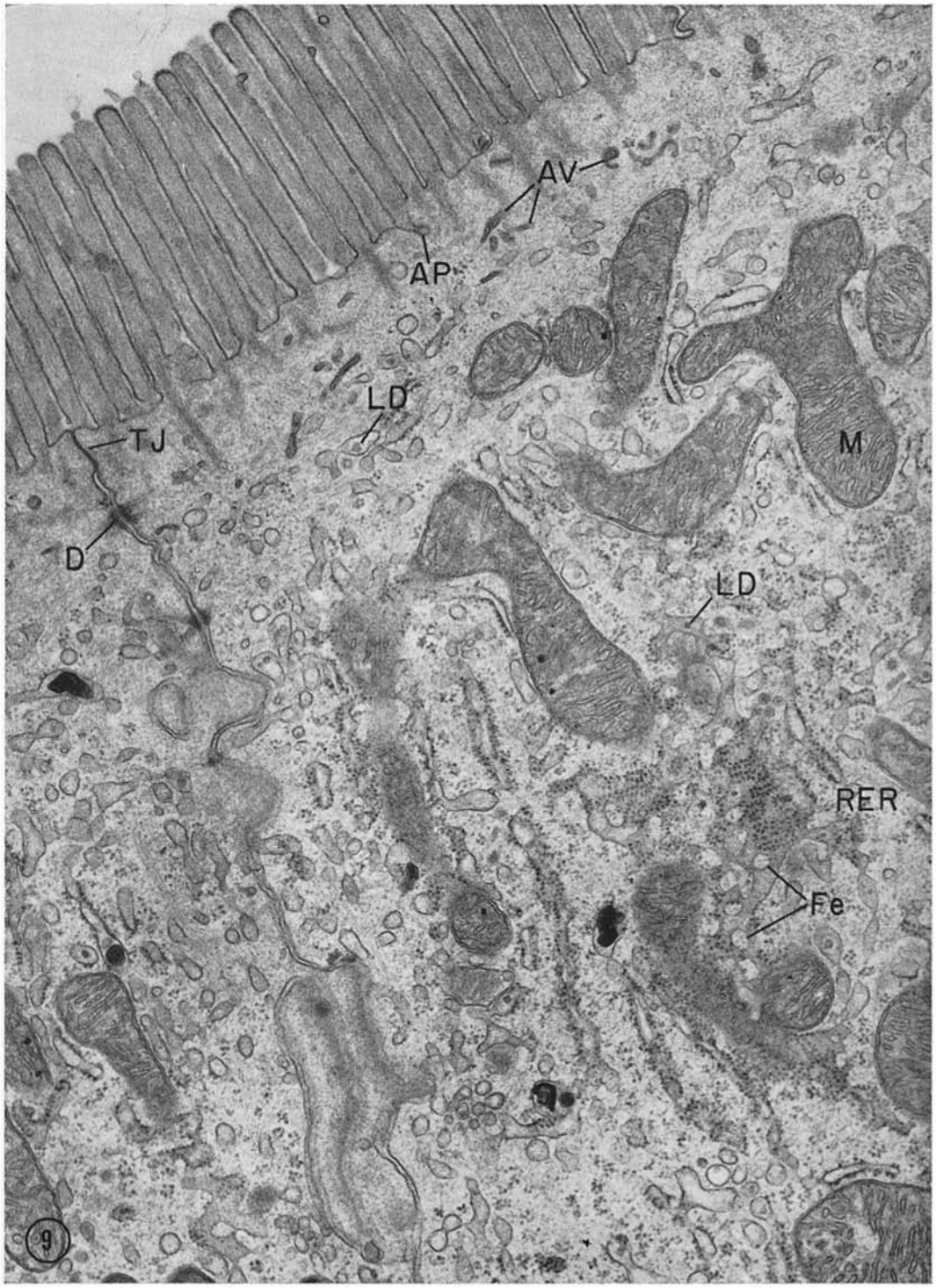



FIGURE 9 Apical portion of an intestinal epithelial cell located in the deeper region of a villus which is in the early phase of fat absorption. Very small lipid droplets occur in the tubular SER (*LD*) and there is little evidence of SER vesiculation (no fat droplets contained in SER-derived vesicles). Another feature particularly prominent in these cells is the fenestration (*Fe*) of the RER along the margins of the cisternae. Evidence of this is found in grazing sections as in this micrograph where many fenestrae are present. It is probable that the tubular SER is formed from the cisternal RER by the latter's undergoing extensive fenestration concomitant with the loss of ribosomes. The close relationship of the SER and RER to each other is thus explained. Fixation 45 min after corn oil intubation. × 26,500.

present in the profile of one bulbous expansion, but images are encountered when two and even three droplets occupy one vesicle. This association of lipid with the SER is evident at all levels in the apical cytoplasm of the cell.

As mentioned above, elements of the SER containing lipid are frequently seen to be continuous with the RER and this association is a very constant one. This continuity is especially apparent where a planar distribution of rough and smooth elements is caught in one section (Fig. 9).

The lipid droplets vary in size within the SER in such a way as to suggest that their size is related to the absorptive state of the cell. During the early phase of absorption and resynthesis, i.e. within 15 min after feeding, they are small and usually occur within the tubules of the SER. As absorption continues the droplets become larger and frequently occupy bulbous expansions of the SER as noted just above. Isolated vesicles containing lipid droplets, and apparently derived from the SER, are also present in the mucosal cell. They are located not only in the SER zone of the cell but throughout the apical cytoplasm (Fig. 10) and especially along the lateral cell membranes (Fig. 11). Dramatic differences in the sizes of lipid droplets within the SER exist between the absorptive cells of a given villus. The apical cells have large droplets whereas the less apically located units show a progressive decrease in size of the SER-contained fat droplets toward the base of the villus.

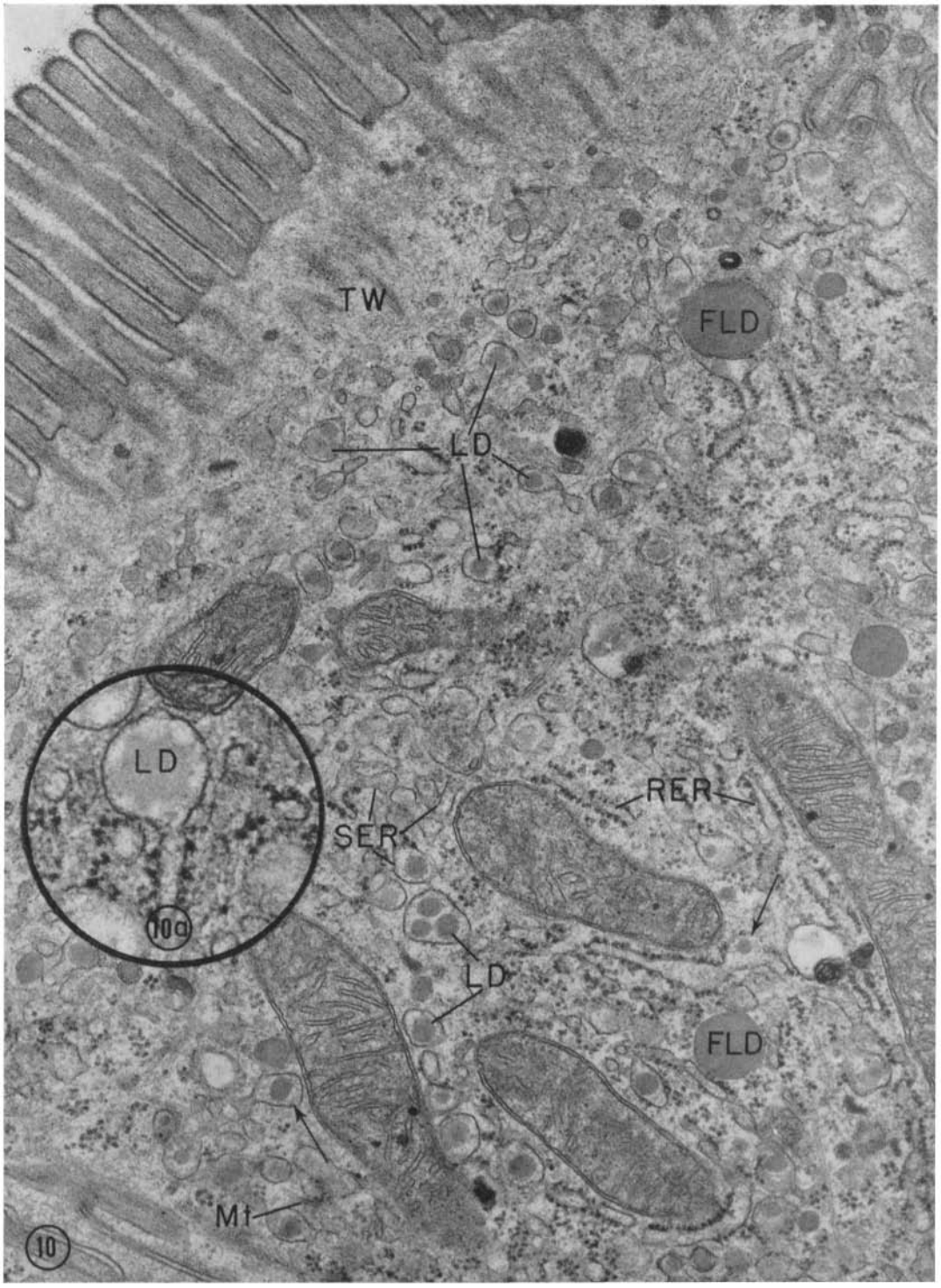
The rough endoplasmic reticulum, as noted earlier, is continuous with the smooth and the occasional lipid droplet within its cisternae serves to emphasize this fact. The presence of lipid within the RER is not common enough, however, to suggest that it is a major pathway for the transport of lipid from the SER. It is more probable in explanation of their presence in the RER that a few small droplets of fat actively form in the peripheral reaches of the RER cisternae adjacent to the point of continuity with the smooth.

We regard it important to record that during fat absorption the long profiles of RER evident in cells of the fasted animal greatly diminish in number and seem to persist only in close association with mitochondria and to be markedly fenestrated even here. Thus as the SER increases the RER decreases. This inverse behavior plus the obvious continuity of the two indicates that through fenestration and loss of ribosomes, the rough transforms nto the smooth during the early and subsequent phases of fat absorption.

### *Golgi Complex*

The Golgi complex responds to fat absorption by showing enlarged vacuoles containing fat droplets (Figs. 11, 11 *a*). In cells believed to be in the early phases of fat absorption, i.e. when only small lipid droplets are in SER, the Golgi vacuoles enclose several droplets about the size of the SER lipid droplets (500 A). However, as the cell accumulates larger droplets of lipid in the SER (later stage of uptake), the Golgi vacuoles show accumulations of much larger lipid droplets and usually only one per vacuole (Fig. 5). These are

FIGURES 10 and 10 *a* This micrograph illustrates the hypertrophy of the SER during fat absorption (compare with Fig. 8). The system is extensively developed beneath the terminal web area (*TW*) and extends throughout the apical cytoplasm. The lipid droplets (*LD*) occupy the lumina of the SER and very frequently are present in bulbous expansions of this system (arrows and inset, Fig. 10 *a*). The droplets are surprisingly uniform in size within the same cell, especially when one considers the factor of variation introduced by sectioning. Instances of SER continuity with the RER are observed frequently. While the SER-derived vesicles usually contain only one lipid droplet, some instances are evident where two or three are present within one vesicle. The lipid droplets within the SER are interpreted as resynthesized triglycerides from absorbed monoglycerides and free fatty acids. As the lipid accumulates within the SER, the cisternae dilate forming bulbous expansions containing lipid droplets (inset, Fig. 10 *a*). It is thought that these enlarged terminal ends of the SER tubules pinch off to form SER-derived vesicles which are important in intracellular transport of the fat. Free lipid droplets (*FLD*) occur throughout the apical cytoplasm and are easily distinguished by their larger diameters and absence of enveloping membranes. Fig. 10, fixation 40 min after corn oil intubation; Fig. 10 *a*, fixation 1 hr after corn oil intubation. Fig. 10, × 34,000; Fig. 10 *a*, × 65,000.


TW
FLD
LD
LD
SER
RER
LD
FLD
Mt
10a
10

on the average larger than the SER lipid droplets of the same cell and apparently are formed by fusion of the smaller droplets. The position of the Golgi complex is similar to that described in the fasted state; this brings it into close association with lipid-containing vesicles of the SER. But whether the latter are built into the developing Golgi apparatus is hard to decide (Figs. 11, 11 *a*). The larger cisternae of the Golgi complex usually contain one or two lipid droplets. Coated pits and vesicles are present on and around these Golgi saccules during fat absorption, and seem not to be more numerous in the fed than in the fasted animal.

### *Lateral Cell Membranes and Intercellular Spaces*

Vesicles containing single lipid droplets and apparently derived from the SER (or Golgi complex) are found abundantly along the entire lateral margins of the intestinal epithelial cell. Golgi vacuoles, on the other hand, containing several lipid droplets are not commonly present along the entire length of the lateral cell membranes, but occur near the cell membrane mostly in the supranuclear zone of the cell (Fig. 11).

The fat droplets varying in size from 50 to 500 m$\mu$ are present in the enlarged intercellular spaces, and are here devoid of their enveloping membranes (Fig. 12). How they reach this location is unknown but it has been suggested by Palay and Karlin (78) that the fat-containing vesicles fuse with the lateral cell membranes, and thereby place the lipid droplet in the intercellular space. This interpretation seems reasonable to us, but we have not seen unequivocal images of intermediate stages in this discharge process. Possibly the event is of such short duration that it is seldom if ever caught by the usual method of fixation. Coated pits, indistinguishable from those described on the lateral cell membranes of the fasted animal and on the Golgi saccules, occasionally contain a droplet of lipid (Fig. 12 *a*), but this is doubtless more a manifestation of coincidence than an expression of function.

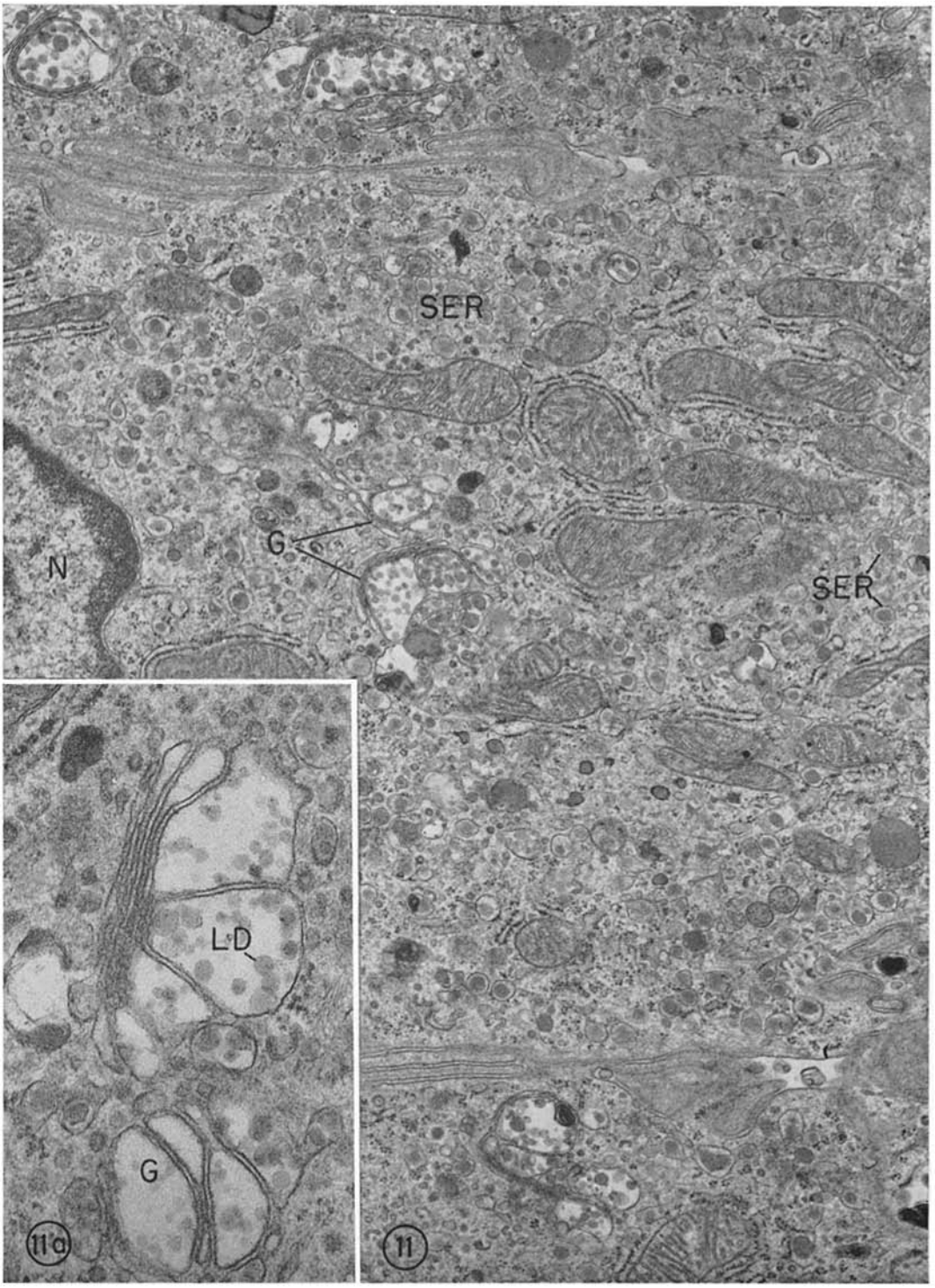
## Part III. Absorption of Lipid: Use of Electron-Opaque Markers

It was reasoned that if a colloidal suspension of a material opaque to electrons could be prepared in corn oil and presented to the absorptive cell it would be possible to evaluate better the quantitative importance of pinocytosis in fat uptake. The marker, if present in the administered fat, should remain in the smaller, incompletely hydrolyzed, lipid droplets destined for pinocytosis and would therefore be available for identification of the lipid taken in by pinocytosis as opposed to that absorbed by micellar diffusion.

### MATERIALS AND METHODS

A suitable suspension of a dense marker in corn oil should possess at least two properties: first, the particles should be less than 300 A and preferably

FIGURES 11 and 11 *a* Supranuclear region of an intestinal epithelial cell located deep in the villus during fat absorption, showing the Golgi complex (*G*). In micrographs of thin sections this system appears to consist of several stacks of flattened cisternae, of which some dilate during fat absorption and form fat containing vacuoles. The fat droplets which appear here during the early stages of fat uptake are approximately the same size as those within the SER, suggesting of course that the SER-derived vesicles containing fat droplets fuse with the Golgi cisternae thereby transferring their lipid to this system. It is of interest to compare the Golgi complex here with that in Fig. 5 where the cell is in a later absorptive phase and the Golgi vacuoles contain one large fat droplet per vacuole. Apparently the smaller lipid droplets coalesce into one large droplet while within the Golgi vesicles, whereas in the SER cisternae they remain small and separate. Fig. 11 *a* shows a Golgi complex at higher magnification. Figs. 11 and 11 *a*, fixation 40 min after corn oil intubation. Fig. 11, × 20,000; Fig. 11 *a* × 45,000.


SER
G
N
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LD
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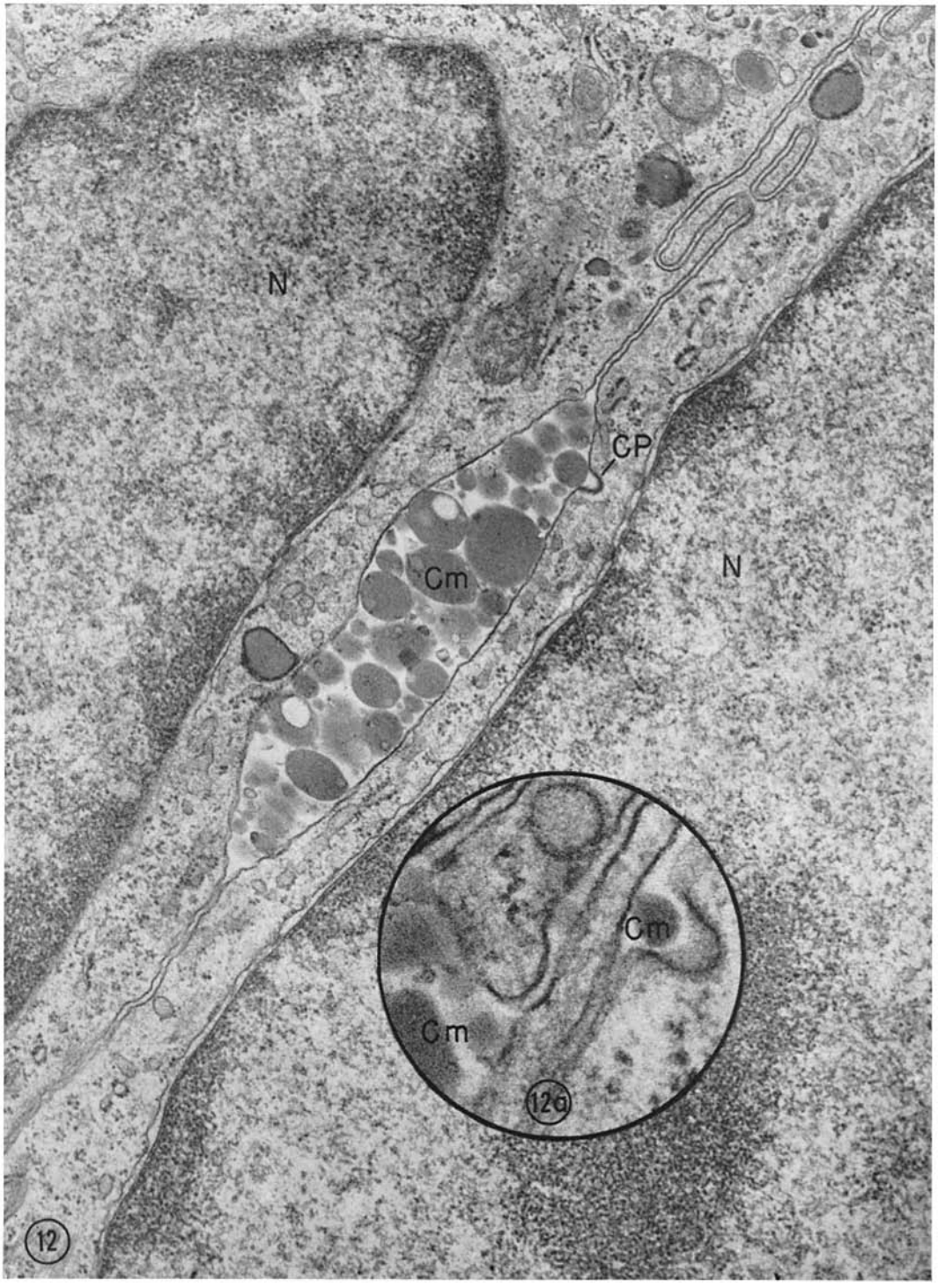
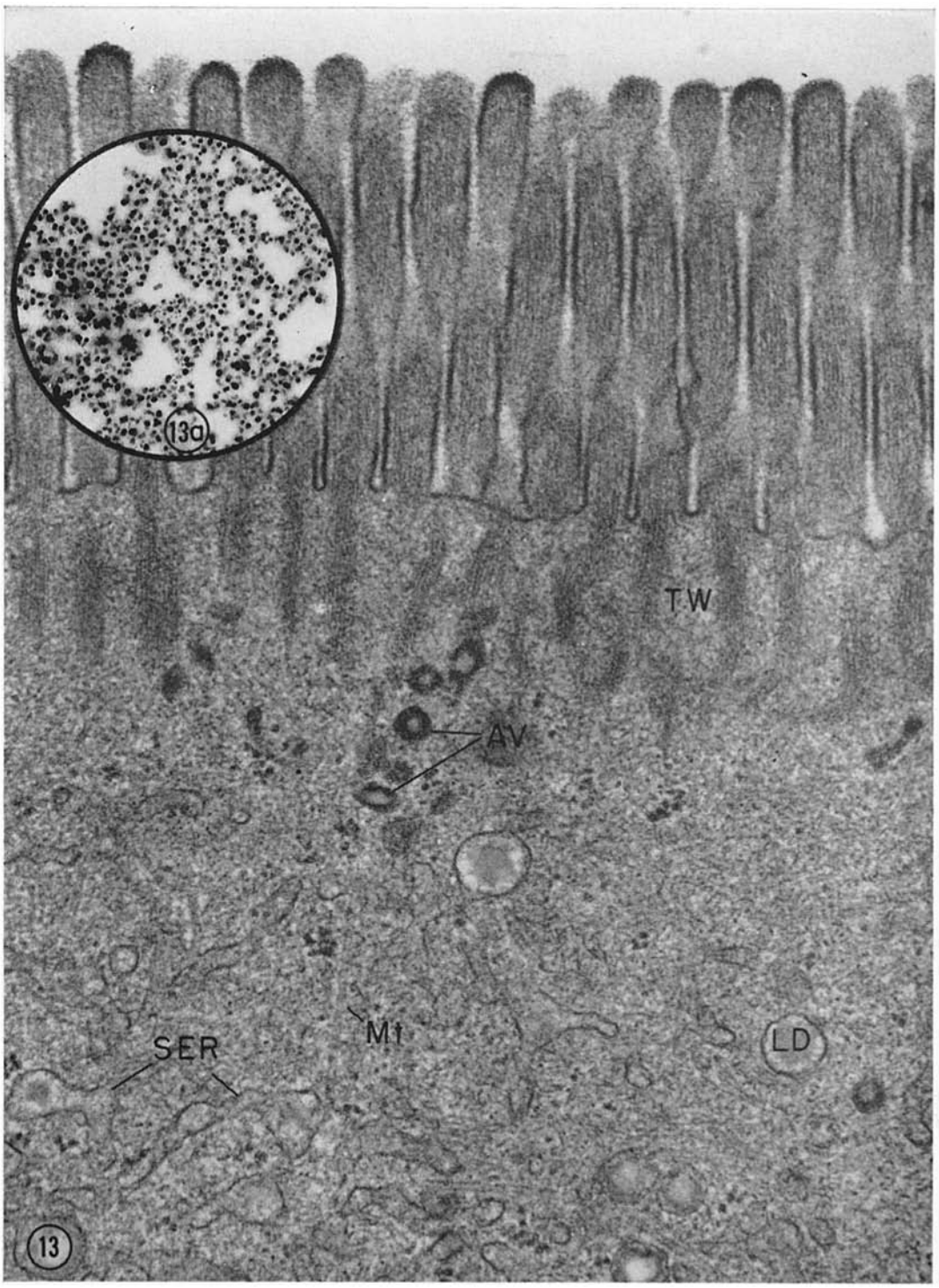
11a
11




FIGURES 12 and 12 *a* Intercellular spaces are frequently enlarged and prominent between intestinal epithelial cells during fat absorption. The enlarged spaces, especially common opposite the nuclei, contain several chylomicra (*Cm*) approximately the same size as the lipid droplets contained within the adjacent absorptive cells. Here between the cells the droplets are devoid of their enveloping membranes. The implication is that the intracellular SER vesicles containing lipid discharge their contents into the intercellular space. It is common to find coated pits (*CP*) bordering on these intercellular spaces. Figs. 12 and 12 *a*, fixation 40 min after corn oil intubation. Fig. 12, × 26,500; Fig. 12 *a*, × 72,000.




FIGURES 13 and 13 *a* Apical portion of an intestinal epithelial cell which was exposed to corn oil containing a suspension of silver particles (see insert). Fat absorption has occurred as evidenced by lipid droplets (*LD*) within the *SER* but no silver particles are present within the apical vesicles (*AV*) or the *SER*. Fig. 13 *a* is an electron micrograph showing the silver particles within the oily phase of the intestinal contents during fat absorption. This micrograph was obtained by removing the intestinal contents of a rat intubated with the corn oil–silver mixture and spreading the oily phase on the surface of water. The resulting film was picked up on a coated grid and examined in the electron microscope. Fig. 13, fixation 1 hr after intubation of metallic silver suspended in corn oil. × 61,000.

less than 100 A in diameter; and second, these particles should be completely dispersed in the corn oil. A number of markers[1] were examined, but the one best suited for our experiments was a colloidal suspension of silver in corn oil. This was prepared by mixing equal volumes of liquid organic silver[2] with corn oil and heating to 100°C. The metallic silver suspension that resulted has an excellent dispersion in corn oil and the particle size ranged from 70–100 A (although there were some 200–250 A particles in the suspension) as judged from thin films of the lipid examined in the electron microscope. These thin lipid films were prepared by placing a drop of the corn oil suspension on a water surface and allowing it to spread into a thin film. This film was picked up on a Formvar-coated grid and examined in the electron microscope. *The contents of the rat intestine after feeding by intubation were similarly examined for the presence of marker in the oil phase (Fig. 13 a).*

Another series of experiments was performed in which a 2 cm segment of the upper jejunum of a fasted rat was tied off, care being taken that the blood supply to the segment remained intact. Intestinal contents removed from a rat previously intubated with a colloidal silver suspension in corn oil were injected into the ligated segment of the fasted rat. The incision in the body wall was sutured and the animal was allowed a recovery period of 1 hr. The jejunum tissue was then removed and processed for electron microscopy.

A final series of experiments was performed in which colloidal ferritin was injected into a ligated intestinal segment, and colloidal ferritin in combination with intestinal contents from a rat previously intubated with corn oil was injected in a similar manner. After 1 hr the jejunal tissue was processed for electron microscopy.

[1] Titanium (Titanium white oil color, Winsor & Newton Ltd., England); Iron oxide (light red oil color, F. Weber Co., Philadelphia, Pa.); Iron cyanide (Prussian blue, Talens Son, Inc., Union, N.J.); Carbon (Ivory black oil color, Winsor & Newton, LTD, England). All prepared by mixing with corn oil.

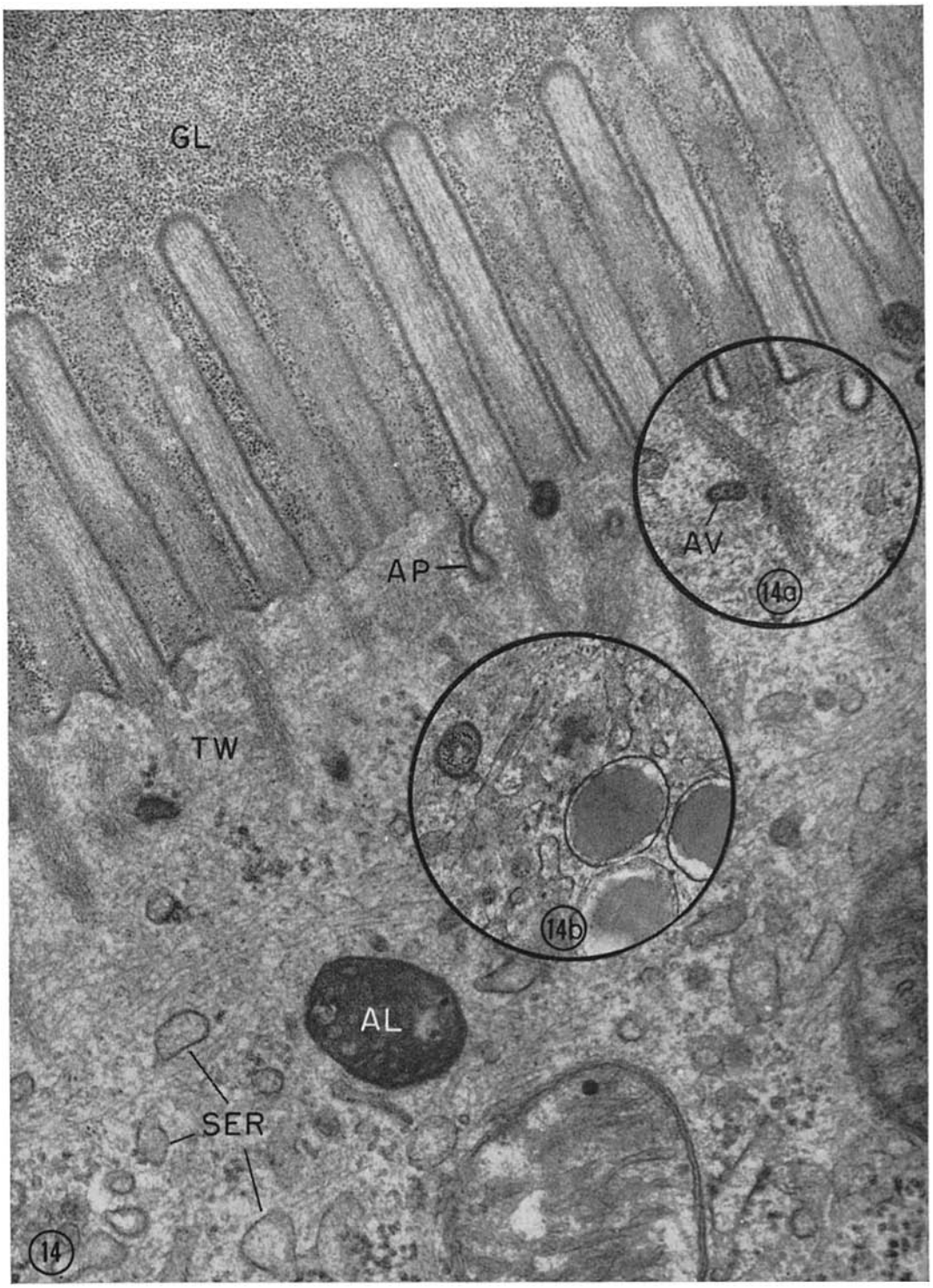
[2] Silver alkyl carboxylate dissolved in naphtha, Engelhard Industries, Inc., Hanovia Liquid Gold Division, East Newark, N.J.

## OBSERVATIONS AND RESULTS

The gut contents from animals presented with colloidal silver either by intubation or injection showed the presence of silver particles in the oil phase of the intestinal contents (Fig. 13 *a*). This procedure is necessary in order to establish that the dispersion of metallic silver in the fat was maintained during the hydrolysis of the triglyceride. We can only assume that any droplets of unhydrolyzed triglyceride of a dimension suitable for pinocytosis retained the metallic silver. If this assumption is valid, the conclusion that lipid of a dimension suitable for pinocytosis containing a colloidal suspension of silver was available to the mucosal cells for absorption reasonably follows. It is also known that fat absorption occurred, as evidenced by the presence of fat droplets within the epithelial cell (Fig. 13). However, *no silver particles were found in either the apical pits, apical vesicles, apical lysosomes, SER, or Golgi complex.*

The experiments with colloidal ferritin and an equal volume of corn oil, from a previously intubated rat, show that the ferritin, a marker *not dispersed in the fat* but present in the gut lumen while fat absorption was in progress, was present in the intermicrovillar spaces, apical pits, and apical vesicles (Figs. 14, 14 *a*). It is further significant

FIGURES 14, 14 *a*, and 14 *b* Apical portions of intestinal epithelial cells exposed to ferritin during fasting and fat absorption. Ferritin is abundant in the gut lumen (*GL*) and between the microvilli but is not evident at all in the SER. The ferritin is, however, found within the apical vesicles (*AV*) which are believed to be derived from the apical pits and further to represent formative stages of apical lysosomes (*AL*). Although not pictured here, ferritin has been observed in apical pits following similar experimental conditions. Obviously the significant fact here is that ferritin as a marker distributed in the gut contents of fat-fed and fasted animals is not taken up very rapidly in either situation and does not appear with the fat in vesicles of the SER in the fat-fed animal. Fig. 14 and 14 *a*, ferritin injected into a tied-off jejunal segment from a 48 hr fasted rat. The cells were exposed to the ferritin for 30 min. Fig. 14 *b*, ferritin injected simultaneously with physiologically fatty-chyme into a tied-off jejunal segment from a 48 hr fasted rat. The cells were exposed to the ferritin and the fatty chyme for 45 min. Fig. 14, × 61,000; Fig. 14 *a*, × 64,000; Fig. 14 *b*, × 51,000.


GL
AV
AP
14a
TW
14b
AL
SER
14

that the ferritin was present in apical vesicles that were much too large to be derived from a single apical pit (Fig. 14 *b*); such enlarged vesicles probably represent stages in the fusion of apical vesicles to form apical lysosomes. It is evident, at very least, that any particulate matter taken up by apical pits, including occasional fat droplets, finds its way then into lysosomes. *In no instance was ferritin found in the vesicles and tubules of the SER containing droplets of fat.*

## DISCUSSION

The experiments described in this paper were designed primarily to evaluate the role of pinocytosis in fat absorption. However, the observations reported are relevant to the several steps in the absorption of fat by the intestinal mucosal cell and should, therefore, be discussed in relation to those steps. Since an understanding of fat absorption relies upon an understanding of the state of lipid in the gut lumen, we have chosen to review the physiochemical evidence on the intraluminal digestion of fat in some detail. This is followed by sections on the entry of fat into the mucosal cell, synthesis of triglyceride within the mucosal cell, release of lipid from the intestinal cell, and finally an eclectic theory of intestinal triglyceride absorption.

### *Intraluminal Digestion of Fat*

Centrifugation of intestinal contents during fat digestion results in two distinct layers: a lower, clear phase and an upper, opaque phase (11, 34, 52) Analysis of these two layers by silicic acid chromatography reveals that the lower, clear layer consists primarily of 2-monoglycerides, conjugated bile salts, free fatty acids, and very little triglyceride. This layer is termed the "micellar phase." The upper, opaque layer consists primarily of triglycerides, most of the diglyceride, some free fatty acids, and very little monoglyceride. This layer is called the "oily phase." The volume of the micellar phase is 10–50 times that of the oil phase during fat digestion in the intestinal lumen (52).

Borgström and his colleagues (11–15, 51, 52, 58) have analyzed this system of intraluminal digestion of lipids and have concluded from extensive evidence that it proceeds in the following manner. The triglyceride is stabilized as a fine emulsion by the conjugated bile salts. Pancreatic lipase hydrolyzes the 1- and 3-ester linkages of the triglyceride molecule and this releases free fatty acids and 2-monoglycerides. The monoglyceride is resistant to lipase; therefore, the predominant products of the hydrolysis are free fatty acids and 2-monoglycerides. As triglyceride hydrolysis proceeds the gut contents consists of triglyceride, diglyceride, monoglyceride, free fatty acids, conjugated bile salts, and pancreatic lipase.

Hofmann and Borgström (52) have discussed the conditions present in the intestinal lumen during fat digestion, and they consider these conditions ideal for the formation of a micellar solution. Each micelle consists of monoglycerides, fatty acids, and conjugated bile salts and appears to be about 60–100 A in diameter (15). The suggestion is offered that micellar absorption of lipid occurs by the micelles' or their components' simply diffusing into the intestinal epithelial cell at the surface represented by the microvilli. It seems unlikely to us that intact micelles enter the absorptive cell, since the conjugated bile salts do not cross the plasma membrane (59). Rather, as the micelles encounter and very likely become associated with the microvillous membrane, the monoglycerides and fatty acids pass selectively through the plasma membrane.

### *Entry of Fat into the Mucosal Cell*

Currently then, two mechanisms of fat entry into the epithelial cell are recognized. One of these relies upon the diffusion of fatty acids and monoglycerides across a plasma membrane and into the mucosal cell where they are reassembled into triglycerides (15, 52). The other mechanism, mentioned previously, is the one of bulk engulfment of fat by a process of pinocytosis (1–3, 49, 50, 61, 70, 74, 77–79, 95, 105, 108). This mechanism relies upon the emulsification of triglycerides into droplets 50 mμ in diameter which filter between the microvilli and into the pinocytotic pits. According to this hypothesis the pits, with their engulfed lipid, pinch off and form vesicles which now enclose a fat droplet. These vesicles fuse with the endoplasmic reticulum thereby placing the fat droplet within the cisternae of the ER.

Senior (97) has pointed out that these two mechanisms are not necessarily mutually exclusive, and he suggests that perhaps both function. Thus he proposes that as intraluminal hydrolysis of large triglyceride droplets proceeds, many smaller, unhydrolyzed triglyceride droplets are formed and, as these droplets reach a size appropriate for pinocytosis, they are incorporated into the pinocytotic

pits. Accordingly, both processes may operate simultaneously during fat absorption, although it is thought that the diffusion mechanism is the more important.

The evidence obtained in our experiments does not support pinocytosis as an important process in fat absorption if, indeed, it functions at all. First, there is no increase in the frequency of pinocytotic pits during fat absorption, and the instances in which lipid droplets occur within a pit are too rare to make us believe that pits represent a major route for the entry of fat into the cell. The terminal web area in our micrographs does not contain any vesicles with enclosed fat droplets that could be considered derived from the apical pits; it should be easy to recognize such derived vesicles because the unit membranes surrounding them are much thicker than those limiting other vesicles in the cells. These facts are true even during the most rapid phase of fat absorption and resynthesis when the intestinal epithelial cells literally fill up with fat by 30 min after a feeding.

Second, the experiment with an electron-opaque marker (metallic silver) suspended in the administered lipid[3] gave the cell ample opportunity to accumulate marker if pinocytosis of lipid were an active process. Obviously the marker should accumulate in either the apical vesicles, lysosomes, SER, or Golgi vacuoles. However, no marker was found within any of these cellular systems or organelles. We demonstrated directly that the marker was present in the gut contents and further that the epithelial cells were absorbing fat. Since there is no reason to believe that the metallic silver particles would be completely segregated from the lipid and thus excluded from pinocytotic uptake as lipase hydrolyzes the large lipid droplets to much smaller ones, we can only conclude that pinocytosis of unhydrolyzed triglyceride does not occur.

Finally, although there is no unequivocal evidence on the point, it appears that the apical pits pinch off and fuse to form larger vesicles which eventually become lysosomes. The evidence available on this point is the similarity of the membranes of the apical pits, apical vesicles, and apical lysosomes. It should be emphasized that these are the only structures in the apical cytoplasm which show the 110 A thick unit membrane. The experiments reported here in which ferritin was identified in apical pits and also within apical vesicles further suggests a relationship of the pits to the apical lysosomes. Even though none was demonstrated in the lysosomes, ferritin was present in vesicles larger than could be drived from a single apical pit. Perhaps a longer exposure to the marker is necessary for them to accumulate in the lysosomes. A point of greater significance is that the ferritin did not accumulate in the SER with the fat droplets as would be expected if the apical pits and vesicles discharge their contents into this subcellular system.

Further experiments are necessary to clarify the lysosome-apical vesicle relationship. The evidence suggests, however, that the apical pits and derived apical vesicles are not related to fat absorption and either enlarge or fuse with other vesicles to form lysosomes. The specific function of the pits is obscure, but we believe that the fat droplets, which have been occasionally seen within the pits, are simply fortuitously entrapped, just as was the ferritin, and that they do not contribute to fat absorption in any significant way. It is possible that the apical pits are the adult equivalent of the structures employed in the suckling rat for the selective uptake of specific proteins (25, 26, 80). They might also be small sinks for the removal of particulates trapped in the small spaces between the basal ends of the microvilli. If, as seems reasonable, hydrolysis continues in the apical pits, they may represent merely small extensions of the intestinal lumen from which absorption is finally completed.

Regardless of what their true function may be, the apical pits and vesicles seem not to transport and discharge their contents into the vesicles of the SER where fat droplets appear because ferritin, although present in the apical pits, etc., was never found in these ER elements.

The biochemical evidence that simple diffusion accounts for the entrance of the great bulk of fat constituents is overwhelming. Johnston and Borgström (58), using intestinal slices, have shown that micellar solutions of radioactive fatty acids and monoglycerides are absorbed and resynthesized into triglycerides at 37°C. If the intestinal slices are heat inactivated or incubated at 0°C there is little effect on the absorption of fatty acids and monoglycerides, but the synthesis of triglycerides is blocked. Metabolic inhibitors impair triglyceride biosynthesis but have little effect on absorption

[3] It should be mentioned that the silver alkyl carboxylate is dissolved in naphtha; therefore other components are present in addition to the corn oil.

(58). More recently Strauss and Ito (107) in a classical study have reported similar results employing intestinal segments incubated in a micellar solution of 0°C. 50% of the fatty acids are absorbed and appear as fatty acids in the tissue. When, however, the period at 0°C is followed by postincubation at 37°C, the absorbed fatty acids appear in the tissue as triglycerides. Since pinocytosis requires metabolic energy (93), this mechanism could not account for lipid uptake under the conditions of their experiments. We interpret these various results and the observations reported in this paper to mean that pinocytosis is not quantitatively important in fat absorption and that absorption from micelles is probably the sole mechanism of lipid uptake.

It is reasonable to search for morphological alterations accompanying the micellar type of absorption, and some workers (7, 60, 87-89) claim that they have visualized micelles or aggregates of lipid within the microvilli. However, it must be stated that the relationship of the electron microscopical images to the in vivo state of the micelles is difficult to evaluate. We have seen no morphological changes in the microvilli, microvillous membrane, apical pits or vesicles, or lysosomes which could be correlated with micellar fat absorption. We envision the fatty acids and monoglycerides as evenly distributed throughout the microvilli and terminal web area rather than as discrete packets or micelles within the cytoplasmic ground substance.

### *Synthesis of Triglyceride within the Mucosal Cell*

The endoplasmic reticulum is the component of these cells which shows greatest alterations during fat absorption. The SER accumulates lipid droplets at a rapid rate, and the size of the droplets varies according to the absorptive state of the cell. Thus in a cell which is in the early phase of absorbing fat, the lipid droplets are small and usually occupy a position within the tubules making up the three-dimensional lattice which is the SER of the apical pole. This evidence is interpreted to mean that the fatty acids and monoglycerides, which have diffused through the microvillous membrane and into the terminal web area, are picked up at the SER membranes and synthesized into triglycerides. Triglycerides are segregated in the SER lumina and are visible in light and electron microscopical preparations as membrane-limited fat droplets.

A considerable amount of biochemical information is available on this synthesis as it occurs in the intestinal mucosal cell (97). One very important step is the "activation of free fatty acids" which converts fatty acids to fatty acyl thiolesters. The thiolesters are much more reactive than are the fatty acids, and they are more water-soluble (97). The enzyme necessary for this transformation has been identified and named "fatty acid:Co. A ligase," and many of its properties are known (97). In a study of mucosal cell fractions the activity of the enzyme was greatest in the microsomal fraction (98); therefore its subcellular location is interpreted to be the endoplasmic reticulum. Biochemical evidence is not available to determine whether this enzyme is located in the smooth or rough endoplasmic reticulum or whether it is associated with the membranes or the contents of the ER. It appears that the enzyme is finally present in the SER, and that it is probably associated with membranes of this system. The other enzymes necessary to complete triglyceride synthesis are also found in the microsomal fraction (100, 101) and likewise could occupy positions in either the membranes or the lumina of the SER. In either location these enzymes could effect the formation of di- and triglycerides.

Obviously, if a diffusion mechanism of absorption is to function, it is necessary to remove the fatty acids and monoglycerides from the cytoplasm, thus to establish and maintain an inward gradient of concentration from the gut lumen into the epithelial cell. As a device for picking up the fatty acids, like a chelator, and removing them, the SER seems well designed. It is present close to the apical absorbing surface, i.e. within short diffusion distances of that surface. Its surface area probably equals or exceeds that of the microvilli and thus allows ample surface for absorption of these substrates into the ER. This large surface also provides an adequate area for location of the enzymes necessary for triglyceride synthesis.

It follows, therefore, that the main functions of the SER in these cells are to provide a site and surface for the enzymes operative in fat synthesis, a large surface area for the absorption and diffusion of metabolites, and an intracellular compartment for sequestration of triglycerides. The synthesis and sequestration of triglycerides main-

tains the diffusion gradient and allows for continued absorption.

The RER is continuous with the SER in such a way that the membranes and cavities of the one are coextensions of the other. This relationship, evident in the cells of the fasted animal, is much more apparent in the cells of the fat-fed animal where the RER fenestrates and seems to decrease in prominence. The most obvious interpretation is that the rough becomes smooth by a change in form and a loss of ribosomes. It may be said that certain important ends are thus quite simply achieved. The change to the reticulate form of the SER and the associated increase in surface brings the system into more intimate contact with the ground substance of the apical cytoplasm. Enzymes and sites for triglyceride synthesis may be built into the membranes while ribosomes were still attached. And proteins for coating the chylomicra could have been stored in the ER cisternae in anticipation of the transformation and subsequent sequestration of triglycerides.

The endoplasmic reticulum which certainly provides a subcellular system for directed or contained diffusion, or even active transport in some cells (85), has been interpreted as a mechanism for the movement of fat droplets through the intestinal epithelial cell (78, 97). A careful search for images of fat droplets within the RER does indeed reveal some, but their frequency is low and does not suggest to us that the RER is a main channel for the transport of fat. It appears rather that the tubular form of the SER, and more especially the derived vesicles are the major elements involved in the intracellular transport of lipid.

The implication from the above interpretations is that chylomicra are completed while within the SER. The known constituents of this final product are triglyceride (85-90%), phospho-lipids (6-9%), cholesterol (3%), and protein (0.5-2.0%) (65). The site of triglyceride synthesis has been discussed; phospholipids are probably also synthesized in the SER since the necessary enzymes are present in microsomal fractions (97); and cholesterol biosynthesis occurs in the SER in a variety of other cell types (23, 24, 35, 37) and may also take place here in the intestinal cell. Protein, the only other component of the chylomicron, is added last and some investigators have assumed that the lipid droplets pick this up as they move through the RER (97). The alternative suggestion may be offered that proteins synthesized in the RER are probably present in the SER even before the fat droplets form. If this assumption is correct, then the triglyceride, as synthesized, would condense in a protein solution within the SER and thus would allow ample opportunity for the protein coating to be applied.

Fat droplets occur in the Golgi vacuoles during fat absorption and their presence here merits comment. During the early phases of fat absorption the droplets are about the same size as those in the SER; this might be interpreted to mean that they are transferred from the SER to the Golgi apparatus or that they form within the Golgi complex independently. This latter possibility seems unlikely since lipid does not accumulate in the Golgi complex of intestinal epithelial cells from patients with congenital $\beta$-lipoprotein deficiency, despite the fact that synthesis of triglyceride occurs (33). As the cell continues to absorb glycerides, the droplets in the Golgi vacuoles become greatly enlarged and their number per vacuole diminishes to one. This is interpreted to mean that the discrete droplets have fused into one large fat droplet and that the internal enviroment in these vesicles may differ from that in the SER, perhaps in the absence of the chylomicron protein.

The function of the Golgi apparatus in the intestinal epithelial cell during fat absorption is unclear, and the results described in this report provide no new information on this subcellular system. It is possible that the Golgi complex functions not only in fat transport, but also as a storage depot for fats important to the metabolism of the absorptive cell itself and they are released for the general metabolism of the animal after periods of extended fasting.

### *Release of Lipid from the Intestinal Cell*

Intercellular spaces are present between the mucosal cells at about the level of the nuclei. These in the fat-fed animals contain fat droplets which are generally about the same size as those within the adjacent absorptive cells. The fat droplets are devoid of the enveloping membrane which surrounds them during their passage through the epithelial cell. The implication from this is that the SER-derived vesicles open to the extracellular spaces through the plasma membrane, and thereby discharge the lipid to the intercellular space as already pointed out (78).

The presence of "coated pits" on the lateral

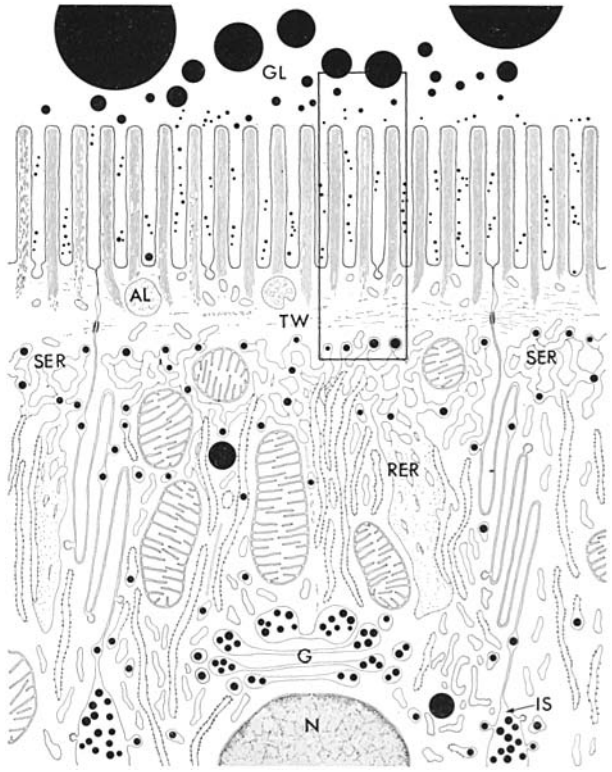



FIGURE 15 A diagrammatical summary of the apical portion of an intestinal epithelial cell during fat absorption. This drawing is based on the interpretations of the electron micrographs obtained in this study and the reader should refer to these figures and the text for details of this cell. The lower portion of the enclosed area shows several stages in formation of a SER-derived vesicle containing a fat droplet. This area is enlarged in Fig. 16 and the significant biochemical events in the initial phases of fat absorption shown.

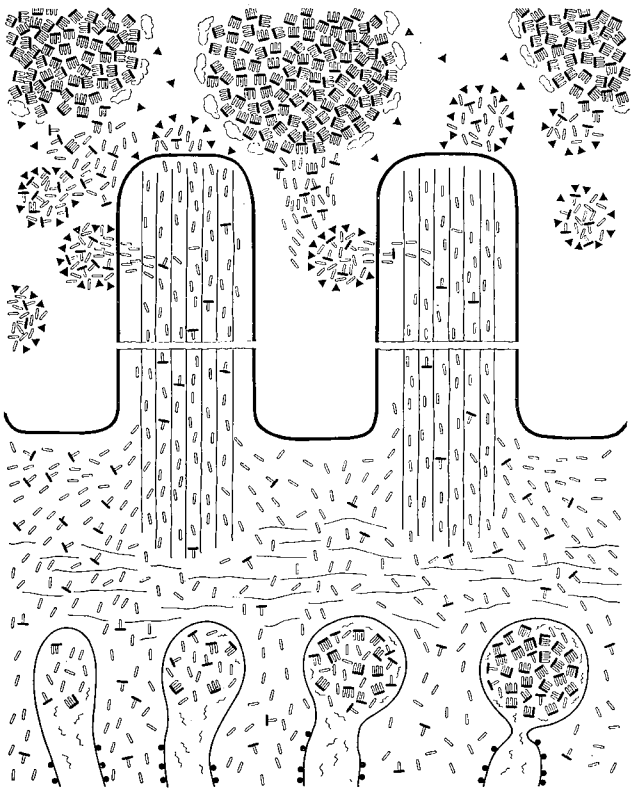



FIGURE 16. This drawing presents in a diagrammatical form the biochemical events in (*a*) the intraluminal digestion of triglycerides, (*b*) the selective absorption by the intestinal epithelium of monoglycerides and free fatty acids from micelles, and (*c*) the synthesis and segregation of triglycerides into the cisternae of the smooth endoplasmic reticulum. Refer to Fig. 15 for the morphological counterparts of this information and to the text for a discussion of these biochemical events. This information is based, to a large extent, on the excellent review of the biochemistry of fat absorption by J. R. Senior (97).

cell membranes in areas of intercellular lipid accumulation is interesting since this type of pit in other cells has been shown to be involved in the selective absorption of proteins (16, 90-92). This invites the speculation that they are performing a similar role for the intestinal cell and conserving for this cell a protein which might otherwise be lost to the circulation.

### *In summary: An Eclectic Theory of Intestinal Triglyceride Absorption* (Refer to Figs. 15 and 16)

The contents of the intestinal lumen, a short time after the ingestion of a meal rich in fat, consists of two phases: (*a*) an emulsion of di- and triglycerides stabilized by conjugated bile salts and (*b*) a micellar solution of free fatty acids, monoglycerides, and conjugated bile salts. The The former phase is the primary site of hydrolysis by pancreatic lipase, and the latter phase, because of its solubility properties, is important in penetrating the mucosal cell. This is accomplished by diffusion of monoglycerides and fatty acids from the micelle into and through the microvillous membrane. The diffusion thereby places them in the mucosal cell cytoplasm; the conjugated bile salts remain in the gut lumen. The monoglycerides and fatty acids now diffuse into the apical region of the cell, where they encounter an extensive development of the SER which incorporates them into the interior phase of this network, again probably by a physical process. Fatty acid:Co. A ligase present in the SER membranes preferentially catalyzes the reaction of fatty acids possessing more than fourteen carbon atoms with Co. A and ATP to produce highly reactive thiolesters. These react with surrounding monoglycerides, and with the aid of enzymes present in the SER membranes or vesicles, di- and triglycerides are produced. The unactivated fatty acids are free to diffuse out of the cell at the lateral and basal margins.

The synthesis and sequestration of triglycerides is important for establishing and maintaining an inward diffusion gradient of fatty acids and monoglycerides. Almost as soon as soluble substrates enter the cell, and become incorporated into insoluble triglycerides within the SER, they are pulled out of solution thus creating a "sink." This happens at all levels of the cell where SER exists. We envision the triglycerides, phospholipids, and cholesterol as forming droplets within a protein solution inside the SER and the completed chylomicron as ready for export to the extracellular environment. Toward this end the SER-derived vesicles containing lipid droplets (chylomicra) move to the lateral cell surfaces and discharge their contents probably by a process of reverse pinocytosis. Thus the completed chylomicron is delivered to the intercellular space from which point it moves into the lacteal.

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