Electron microscopic studies of the assembly, intracellular transport, and secretion of chylomicrons by rat intestine

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Abstract A detailed ultrastructural investigation of the assembly, intracellular transport, and secretion of chylomicrons by rat proximal jejunal intestinal cells was performed in rats fed corn oil. Following fat feeding the smooth endoplasmic reticulum of the absorptive cells becomes laden with triglyceride droplets which are transported through channels of the endoplasmic reticulum to the Golgi apparatus. The Golgi zones become extremely prominent due to the accumulation of osmiophilic droplets, similar in size and configuration to chylomicrons, within proliferated Golgi vesicles. Golgi-derived secretory vesicles, containing nascent chylomicrons, migrate towards the lateral cell membrane. The secretory vesicle membranes fuse with the lateral plasmalemma and nascent chylomicrons are then discharged into the intercellular spaces. Alterations of specific domains of the secretory vesicles were prominent, appearing as coated pits. Coated pits were apparent in the lateral plasmalemma in areas of active chylomicron exocytosis suggesting their derivation from secretory vesicle–membrane fusion. Chylomicrons, within the intercellular spaces, pass through the basement membrane that lines the basal surfaces of the epithelial cells, traverse the cellular elements of the lamina propria, and finally gain access to the lymphatics entering these channels through gaps between adjacent endothelial cells. These observations indicate that nascent chylomicrons accumulate within Golgi vesicles as a prerequisite to secretion and that secretion occurs by exocytosis resulting in the release of nascent chylomicrons from secretory vesicles.

Supplementary key words intestinal lipid absorption · endoplasmic reticulum · Golgi apparatus · exocytosis · lipoproteins · apoproteins · triglycerides · lymphatics

In recent years studies of intestinal lipid absorption have defined many of the biochemical and ultrastructural features of the uptake by the small intestinal absorptive cells of the lipolytic products of intraluminal fat digestion and the subsequent formation of chylomicrons (1). The experiments of Strauss (2) and Cardell, Badenhausen, and Porter (3) have settled the long controversy (4, 5) regarding the importance of pinocytosis in the uptake phase of fat absorption. Their work has provided unequivocal evidence that the major products of intraluminal lipolysis are absorbed into the epithelial cells by passive diffusion from bile salt micellar solutions rather than by pinocytosis. In other studies, it has been shown that the rough and smooth endoplasmic reticulum are the sites of chylomicron apoprotein biosynthesis and triglyceride esterification, respectively, and that nascent chylomicrons accumulate within the channels of the smooth-surfaced endoplasmic reticulum prior to secretion (1, 5, 6).

Despite this information there are still many morphological aspects of chylomicron assembly, intracellular transport, and secretion that have been described incompletely. Thus, it is not known whether or not the Golgi apparatus subserves an obligatory function in the final assembly and secretion of chylomicrons (3, 6). In earlier studies it was suggested that triglyceride droplets, within vesicles derived from the smooth endoplasmic reticulum (SER) are secreted directly without first being "processed" in the Golgi (1, 6). It seems likely that entry of nascent chylomicrons into the Golgi apparatus is essential if secretion is to occur since, after fat feeding, the Golgi zones become very prominent due to an accumulation of triglyceride droplets within proliferated Golgi vesicles (7–10). Evidence regarding the importance of the Golgi in chylomicron secretion is derived from studies of human betalipoprotein deficiency (11–13) and animal experiments with protein synthesis inhibitors (9, 14, 15), which indicate that B-apoprotein availability is essential for fat secretion. In these situations, ultrastructural studies (9, 12) reveal triglyceride droplets within the SER but entry of nascent chylomicrons into the Golgi apparatus is apparently prevented, resulting in a massive accumulation of tri-

Abbreviations: VLDL, very low density lipoproteins; SER, smooth endoplasmic reticulum; RER, rough endoplasmic reticulum.

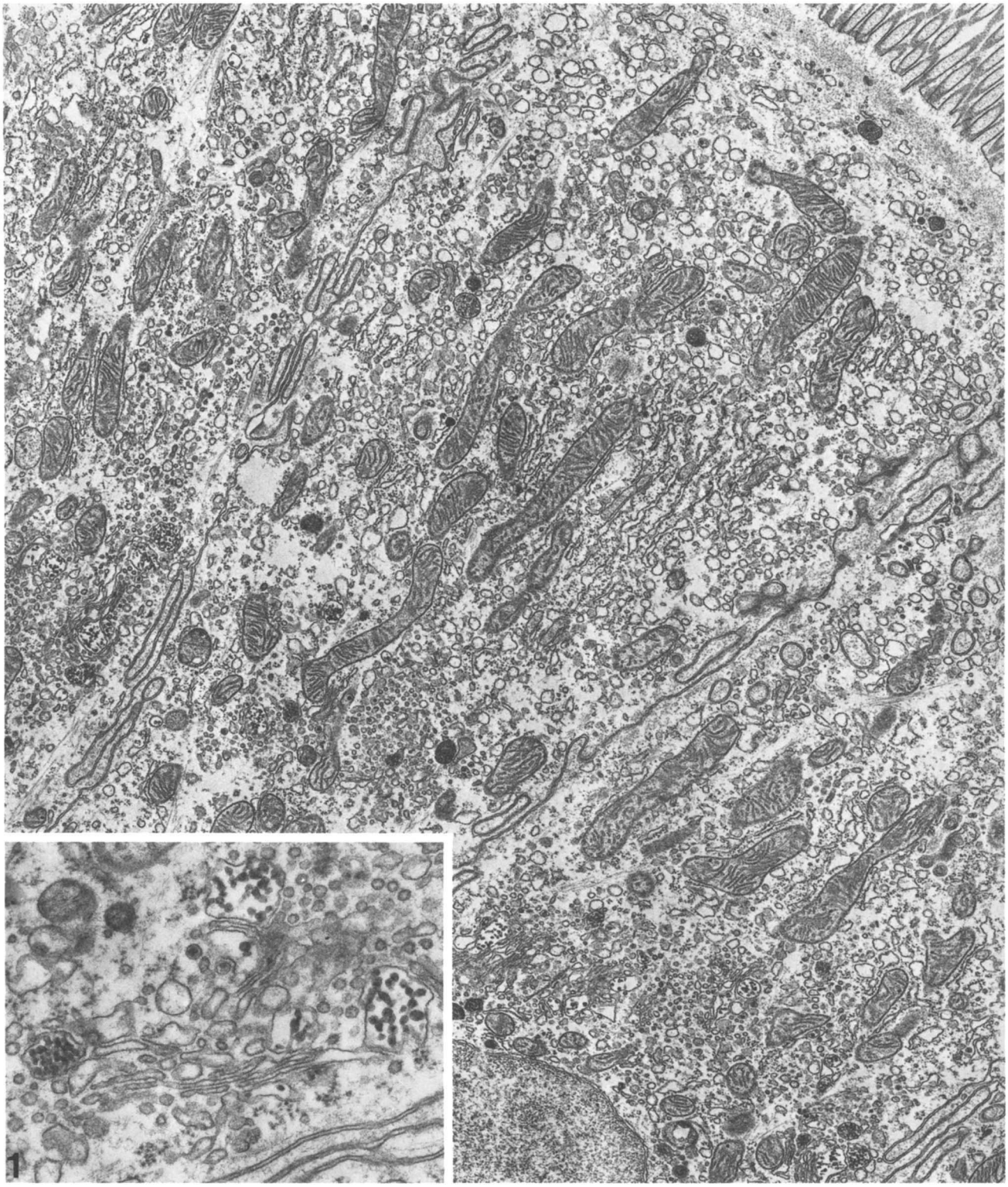

Fig. 1. Intestinal epithelial absorptive cells of a fasted rat illustrating the ultrastructural organization of nonabsorbing cells. Even in fasted animals the Golgi (insert) are filled with osmiophilic droplets reflecting endogenous VLDL synthesis. ×11,860. Insert ×19,100. The tissues in all of the figures were fixed for electron microscopy with osmium tetroxide.

glyceride-rich droplets within markedly distended channels of the SER.

The mechanism of chylomicron secretion from the intestinal absorptive cells into the intercellular spaces has eluded precise ultrastructural definition. The secretion of nascent chylomicrons is thought to involve exocytosis, or the fusion of lipid-laden secretory vesicles with the lateral plasmalemma, resulting in the release of chylomicrons into the intercellular spaces. Despite careful work by many investigators (1, 6) electron microscopic images consistent with this process have not been published; thus, the importance of exocytosis in chylomicron secretion has heretofore not been proven.

In the present study a detailed ultrastructural investigation of the normal process of fat absorption, by rat intestinal epithelial cells, was performed in an effort to more completely define the functional interrelationships between intestinal subcellular organelles in the assembly and intracellular transport of chylomicrons and to obtain additional information regarding the processes by which nascent chylomicrons are secreted into the intercellular spaces and transported into the lymphatics.

METHODS

Male Sprague-Dawley (Charles River stock) rats weighing 180–220 g were fed a standard laboratory diet prior to all experiments. Studies of intestinal fat absorption were performed by feeding 1.5 ml of corn oil to rats, fasted for 14 hr, by means of a thin polyethylene tube inserted into the stomach. Groups of animals were killed at intervals between 30 min and 4 hr, following corn oil feeding, and segments of upper jejunum were rapidly processed for electron microscopy. Jejunal tissues were fixed in 2% osmium tetroxide at pH 7.3 in 0.1 M phosphate buffer for 4 hr at room temperature. After fixation, the tissues were washed repeatedly in the 0.1 M phosphate buffer, dehydrated in a graded series of ethanol, and embedded in Maraglas. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Zeiss EM-10 electron microscope.

RESULTS

A detailed consideration of the ultrastructure of the rat jejunum in the fasting state is included in the review by Strauss (6) and in the study of fat absorption by Cardell et al. (3); however, a few specific points concerning normal morphology (16), important for appreciating the alterations that occur following fat feeding, are worthwhile considering. The intestinal villous epithelial cells are separated from each other by intricate interdigitations of adjacent lateral plasmalemma membranes, which also form the boundaries of the intercellular spaces between adjacent absorptive cells (**Fig. 1**). The intercellular spaces, inconspicuous in fasting animals, widen during fat absorption due to an accumulation of chylomicrons released from the absorbing epithelial cells. The Golgi complex, located in the supranuclear region, consists of flattened cisternae and vesicles that may contain VLDL particles, reflecting endogenous VLDL synthesis by the intestinal mucosa (Fig. 1). Elements of the smooth (SER) and rough (RER) endoplasmic reticulum are relatively inconspicuous in jejunal specimens obtained from fasting animals (Fig. 1).

A definite basement membrane, present beneath the basal poles of the epithelial cells and the intercellular spaces, forms a barrier between the epithelium and the lamina propria. The lamina propria contains a heterogeneous cellular population including mononuclear cells, lymphocytes, plasma cells, and eosinophils. Lymphatics and capillaries are readily identified adjacent to the cellular constituents of the lamina propria.

The following description of the alterations in intestinal ultrastructure following corn oil feeding represents the results of an examination of upper jejunal specimens since this area is quantitatively most important in fat absorption. Although the progress of fat absorption was systematically followed by examination of biopsies obtained between 30 min and 4 hr after feeding 1.5 ml of corn oil to fasted rats, it was soon evident that both early and later phases of fat absorption could be observed at almost all time intervals. Thus, in the earliest specimens, obtained 30 min after corn oil feeding, chylomicrons were observed within intercellular spaces and lymphatics. The following results and the accompanying electron micrographs are a description of the entire absorptive process regardless of the time after corn oil feeding; however, at 2 hr all of the features of uptake, intracellular chylomicron assembly, and secretion were readily seen. Therefore, the electron micrographs, chosen for illustration, were obtained 2 hr after corn oil administration.

Within 30 min of the oral administration of corn oil there is a remarkable alteration in the appearance of the intestinal epithelial cells. The apical portions of the absorptive cells become filled with osmiophilic droplets which are visualized within vesiculated elements of the SER. The absorbed lipid appears as single and multiple droplets bounded by smooth membranes, which represent cross-sectioned profiles

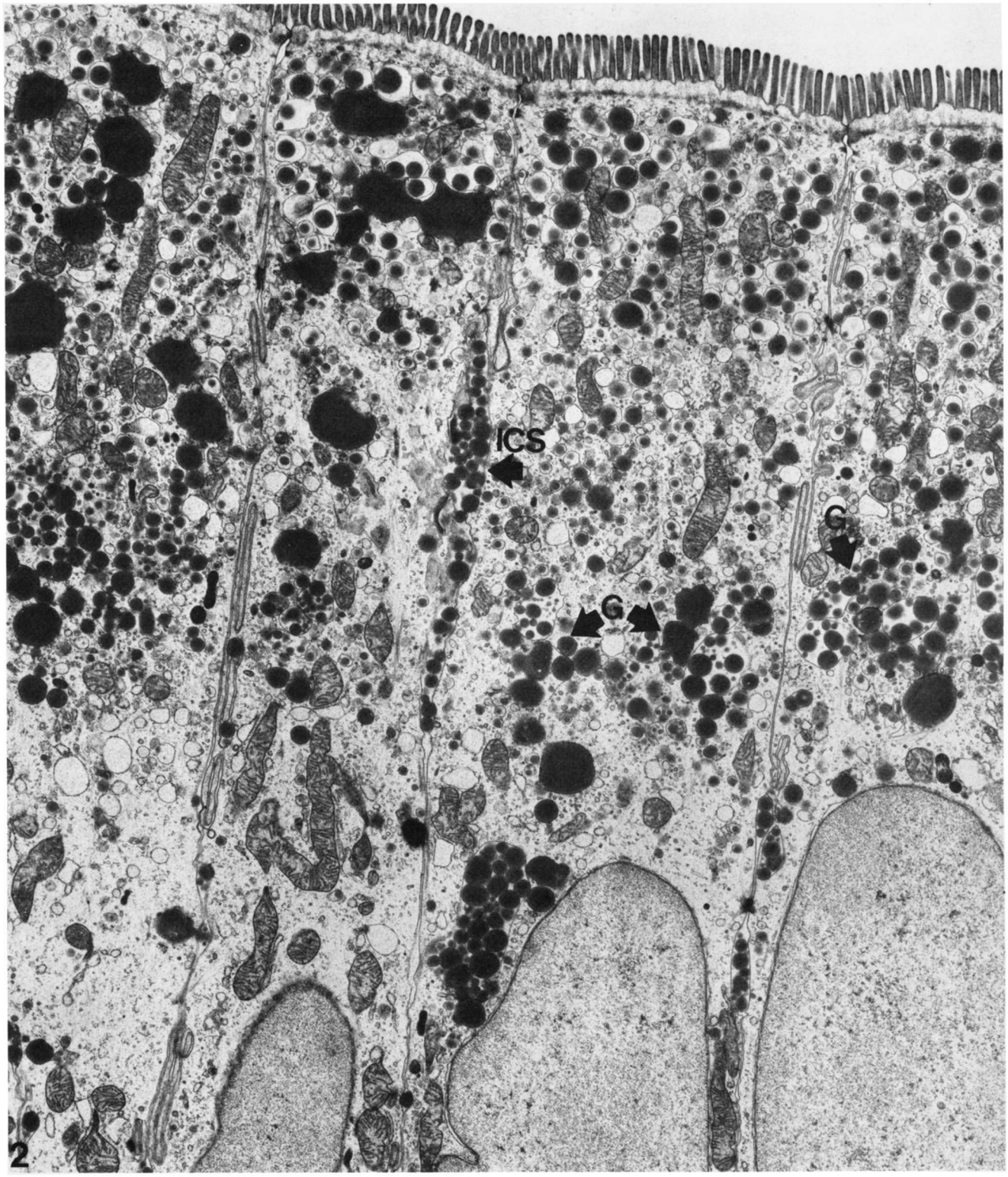

Fig. 2. In this electron micrograph taken of a specimen obtained 2 hr after corn oil feeding, the apical cytoplasm of the intestinal absorptive cells has become filled with osmiophilic droplets bounded by membranes of the smooth endoplasmic reticulum and the Golgi zones (G) are distended with nascent chylomicrons. The Golgi areas (G) contain osmiophilic droplets of chylomicron size and the intracellular spaces (ICS) are filled with lipid droplets of chylomicron size (1200–4800 Å) indicating that secretion has occurred. ×8,000. (This electron micrograph and all subsequent illustrations, except Fig. 17, are of specimens obtained 2 hr after corn oil feeding.)

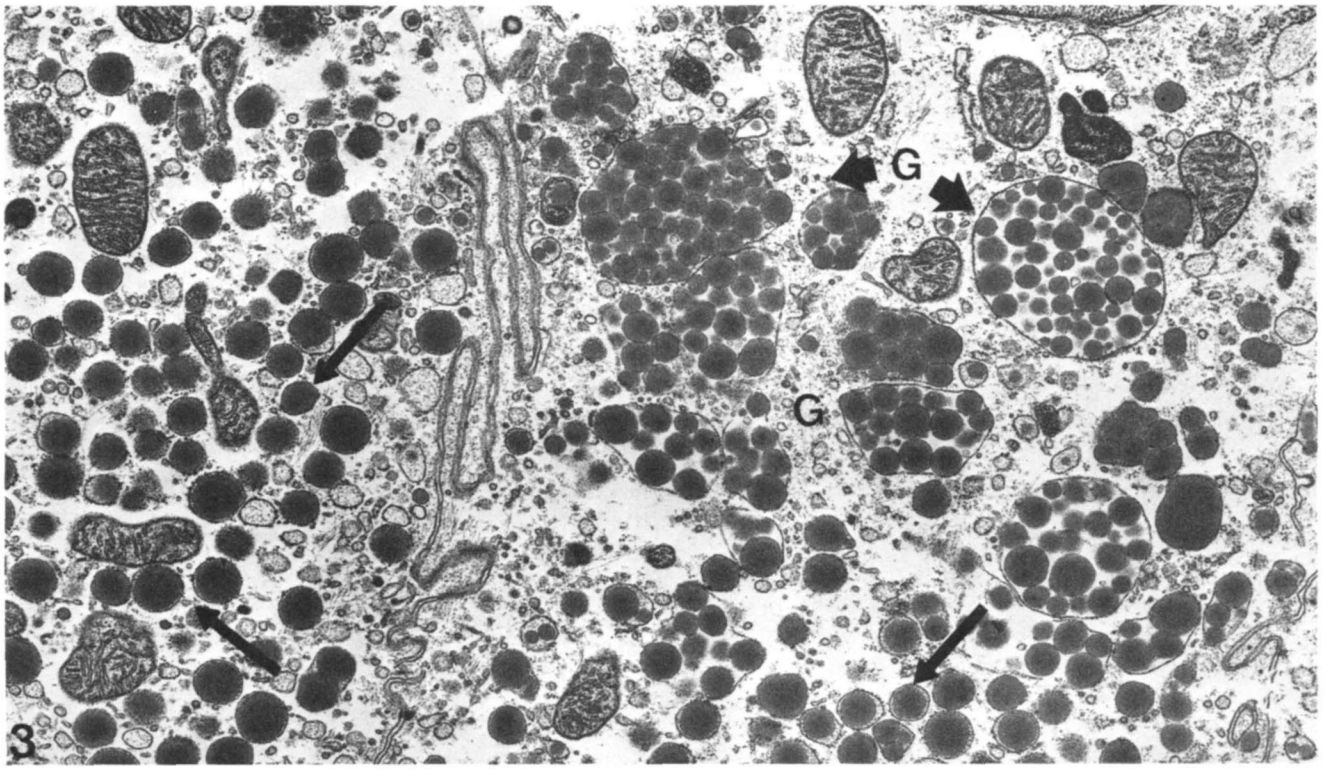

Fig. 3. This cell is filled with innumerable fat droplets located within vesiculated channels of smooth endoplasmic reticulum (long arrows). A Golgi zone (G) contains many vesicles filled with nascent chylomicrons measuring 600–3500 Å. ×13,377.

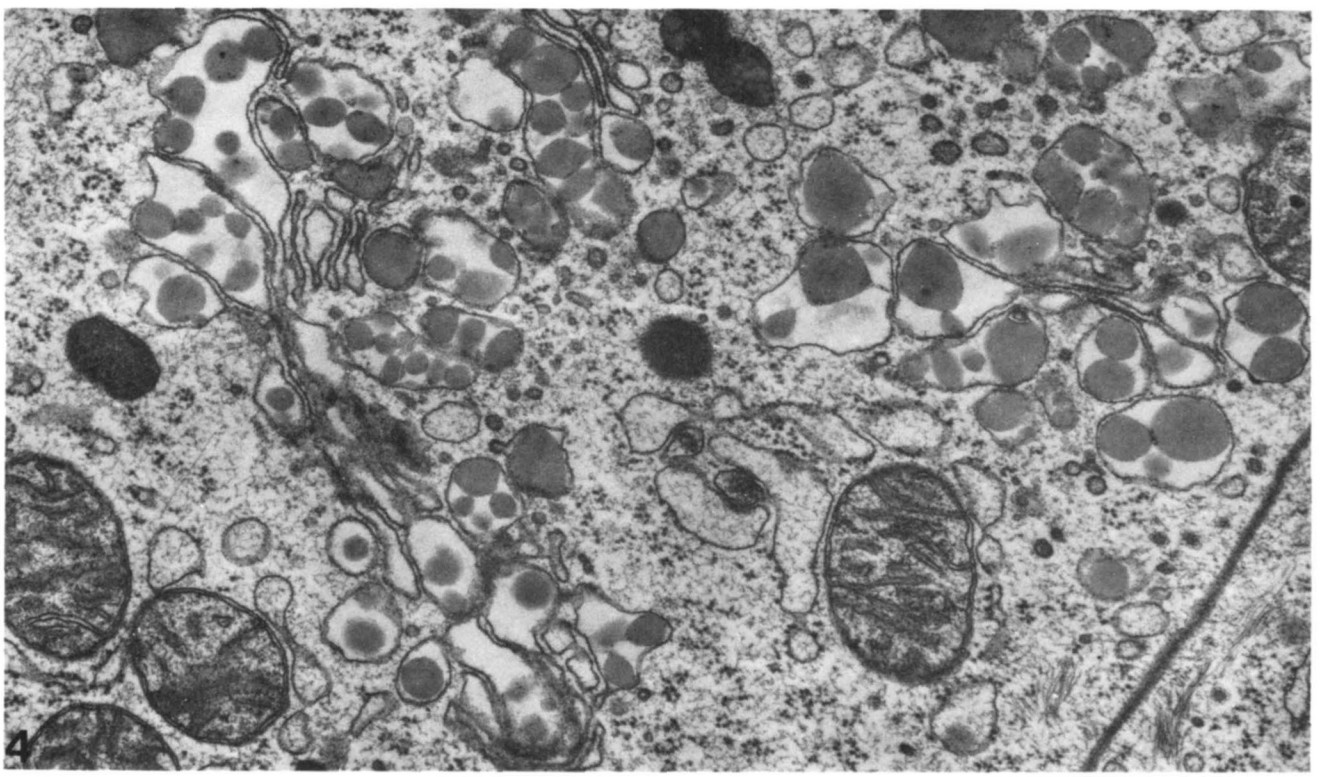

Fig. 4. The marked proliferation of the Golgi after corn oil feeding is evident in this electron micrograph. Golgi vesicles are filled with nascent chylomicrons. Note that multiple lipid droplets are present within Golgi vesicles. ×26,400.

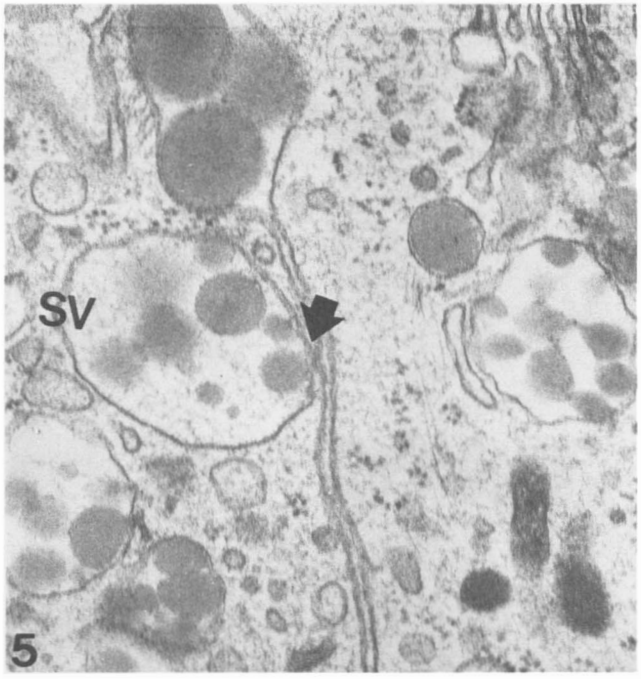

Fig. 5. A secretory vesicle (SV), containing nascent chylomicrons, has fused (arrow) with the lateral plasmalemma. ×44,160.

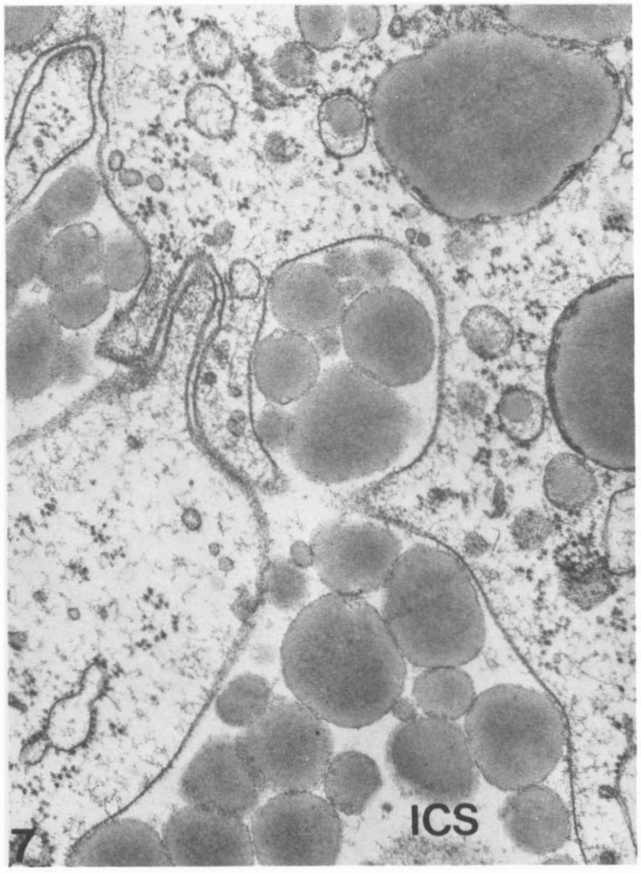

Fig. 7. The exocytosis of chylomicrons is evident in this electron micrograph. The nascent chylomicrons in the secretory vesicle are similar in size and morphology to the chylomicrons already present in the intercellular space (ICS). ×36,000.

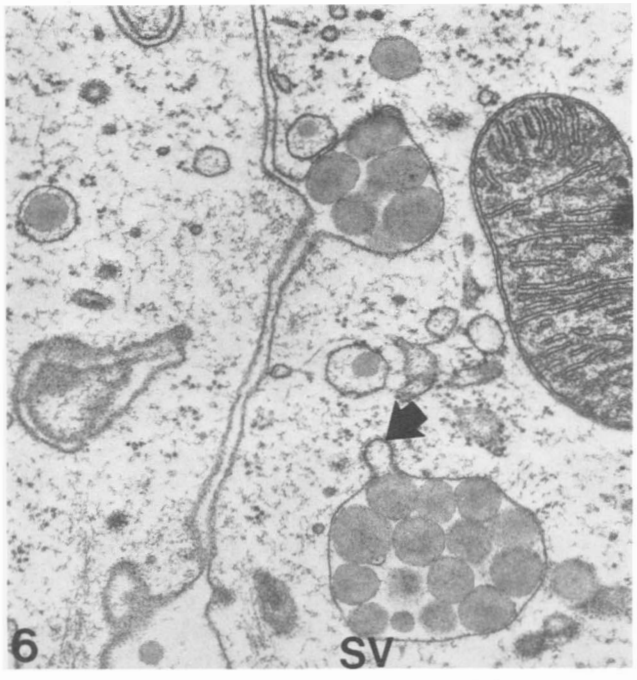

Fig. 6. This electron micrograph demonstrates the appearance of a secretory vesicle that has fused with the lateral plasmalemma. Note that the secretory vesicle contains several nascent chylomicrons that will be secreted together into the intercellular space. Another secretory vesicle (SV) containing a coated pit (arrow) is approaching the lateral plasmalemma. ×33,240.

of the dilated channels of the hyperplastic SER. In some samples the process of intestinal fat absorption proceeded extremely rapidly and, even at 30 min after feeding corn oil, chylomicrons were seen in the intercellular spaces, indicating that chylomicron formation and secretion had already occurred. **Fig. 2** illustrates the typical appearance of several intestinal absorptive cells 2 hr after feeding corn oil. Osmiophilic droplets within vesicles of the SER are abundant in the apical cytoplasm. In the supranuclear region nascent chylomicrons are seen within Golgi vesicles while secreted chylomicrons are evident in the intercellular spaces. At higher magnification it is obvious that the absorbed lipids have become sequestered within channels of the endoplasmic reticulum (**Fig. 3**). Although most of the lipid droplets are contained within the smooth surfaced endoplasmic reticulum, osmiophilic droplets were also present with the RER and transition zones between RER and SER were occasionally observed.

One of the most striking ultrastructural alterations in the absorbing cells is the proliferation of the elements of the Golgi apparatus. Although easily recognized in fasting epithelial cells, because of its characteristic morphology (Fig. 1) the Golgi assumes a striking prominence in cells that are absorbing fat (**Figs. 3, 4**). Golgi vesicles become distended with

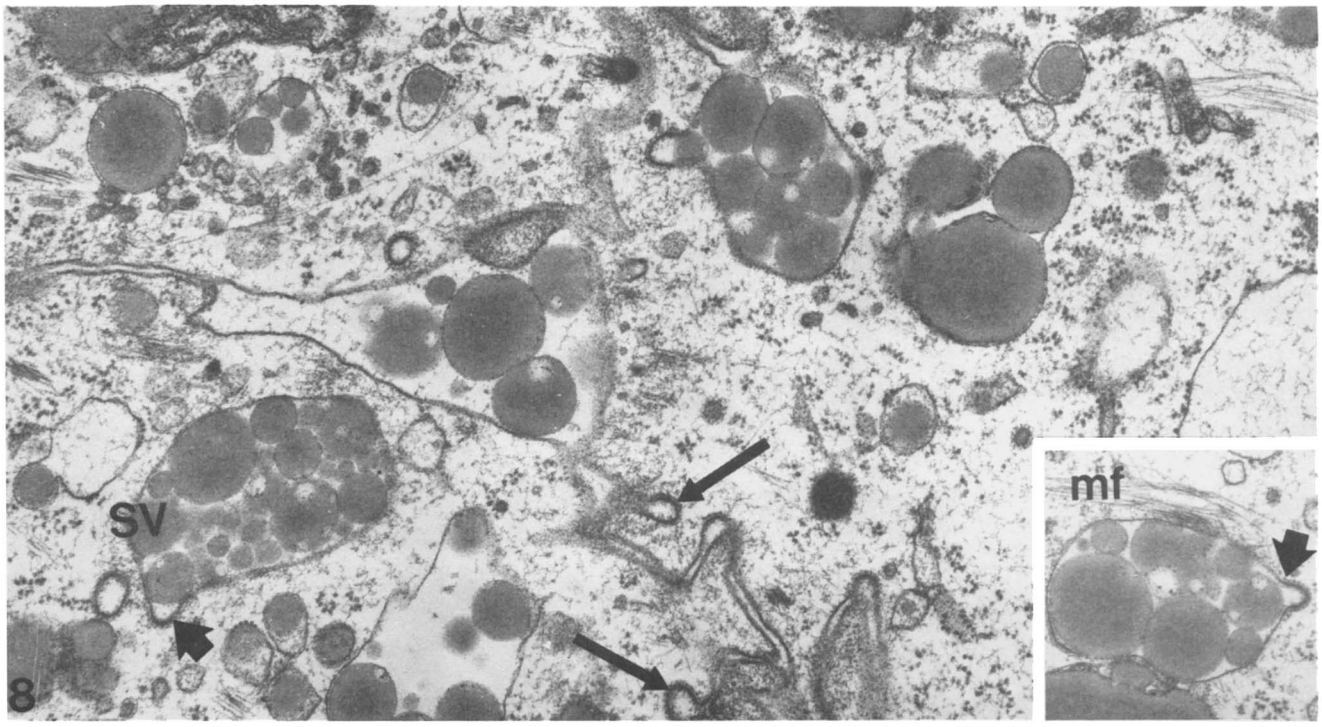

Fig. 8. This electron micrograph demonstrates the appearance of a coated area (short arrow) in the membrane of a secretory vesicle (SV). There is an impression that the coated areas of the secretory vesicle membrane are incorporated into the lateral cell membrane following vesicle–membrane fusion, since coated areas of morphology identical to those seen in the secretory vesicle are present in the lateral plasmalemma (long arrows). ×32,400. Insert—Microfilaments (mf) are abundant in close proximity to a secretory vesicle. Note the coated area (short arrow) on the secretory vesicle membrane. Insert ×27,450.

dense osmiophilic droplets which are the same size and configuration as the chylomicrons seen in the adjacent intercellular spaces. Because of the proliferation of Golgi elements and the accumulation of nascent chylomicrons within Golgi vesicles, the Golgi zones become very prominent, occupying a large portion of the supranuclear cytoplasm, in contrast to the usual observation in nonabsorbing cells in which a single inconspicuous Golgi complex is seen. The nascent chylomicrons engorge the vesicular elements of the Golgi complex but do not accumulate, to any great extent, in the flattened cisternae of the Golgi (Figs. 3, 4). The accumulation of nascent chylomicrons in Golgi vesicles results in the formation of secretory vesicles, which then migrate to the

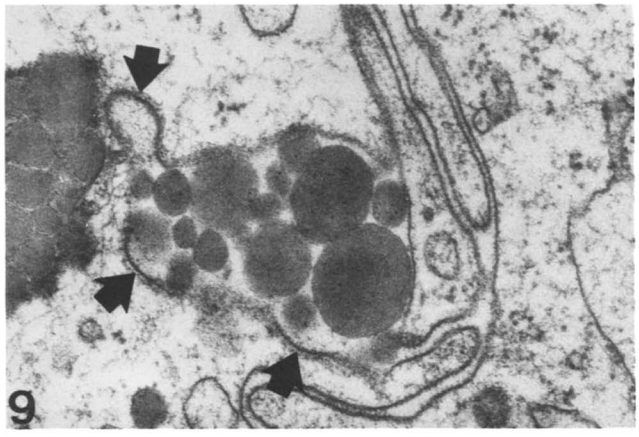

Fig. 9. A secretory vesicle containing nascent chylomicrons has fused with the lateral plasmalemma. Note the coated areas (arrows) in the vesicle membrane. ×43,320.

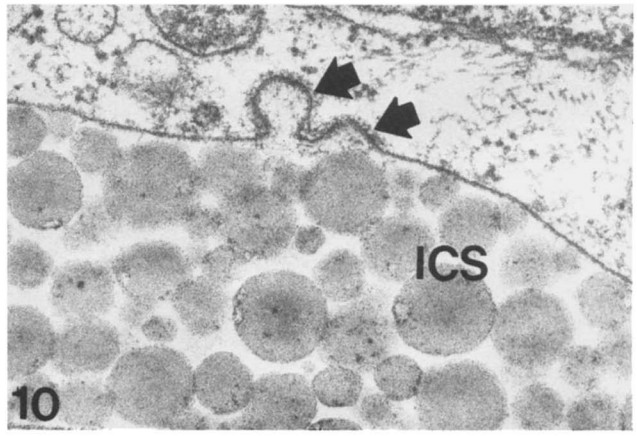

Fig. 10. In this electron micrograph the lateral cell membrane contains two discrete areas in which coated pits similar to those seen in the membrane of secretory vesicles are present (arrows). Chylomicrons are abundant in the intercellular space (ICS). ×54,000.

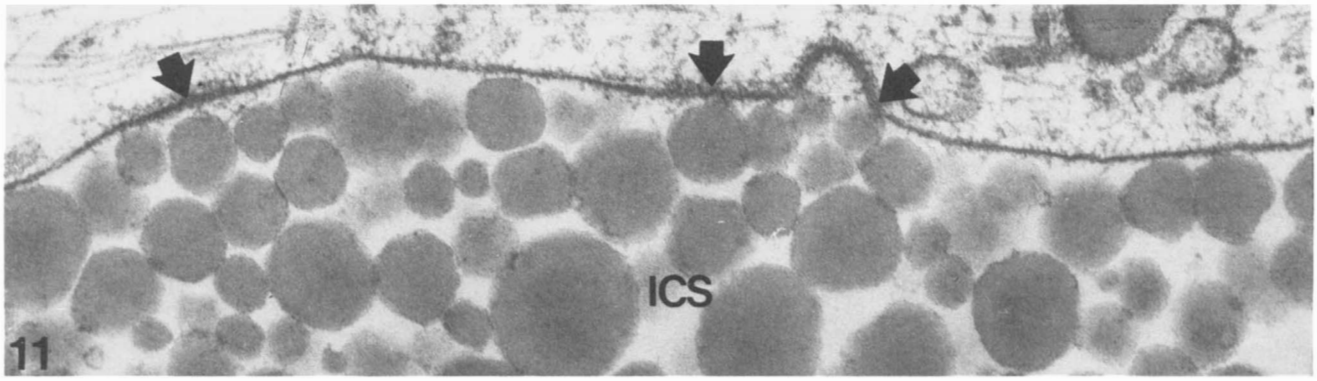

Fig. 11. A relatively lengthy portion of the lateral cell membrane contains coated areas (arrows). Note that the membrane appears thickened due to the incorporation of the rather amorphous material that imparts a coated or fuzzy appearance to the membrane. The intercellular space (ICS) is filled with chylomicrons. ×63,100.

region of the lateral plasmalemma where chylomicron secretion will occur.

Several important features of the process by which chylomicrons are secreted into the intercellular spaces are illustrated in **Figs. 5–7**. Although it has been assumed that secretory vesicles, containing nascent chylomicrons, fuse with the lateral plasmalemma and thus discharge their contents into the intercellular space, by a process termed exocytosis, electron microscopic images confirming this assumption have not been published (3, 6, 17). Progressive stages in chylomicron exocytosis are illustrated in Figs. 5–7. It is clearly shown in these electron micrographs that secretory vesicles containing nascent chylomi-

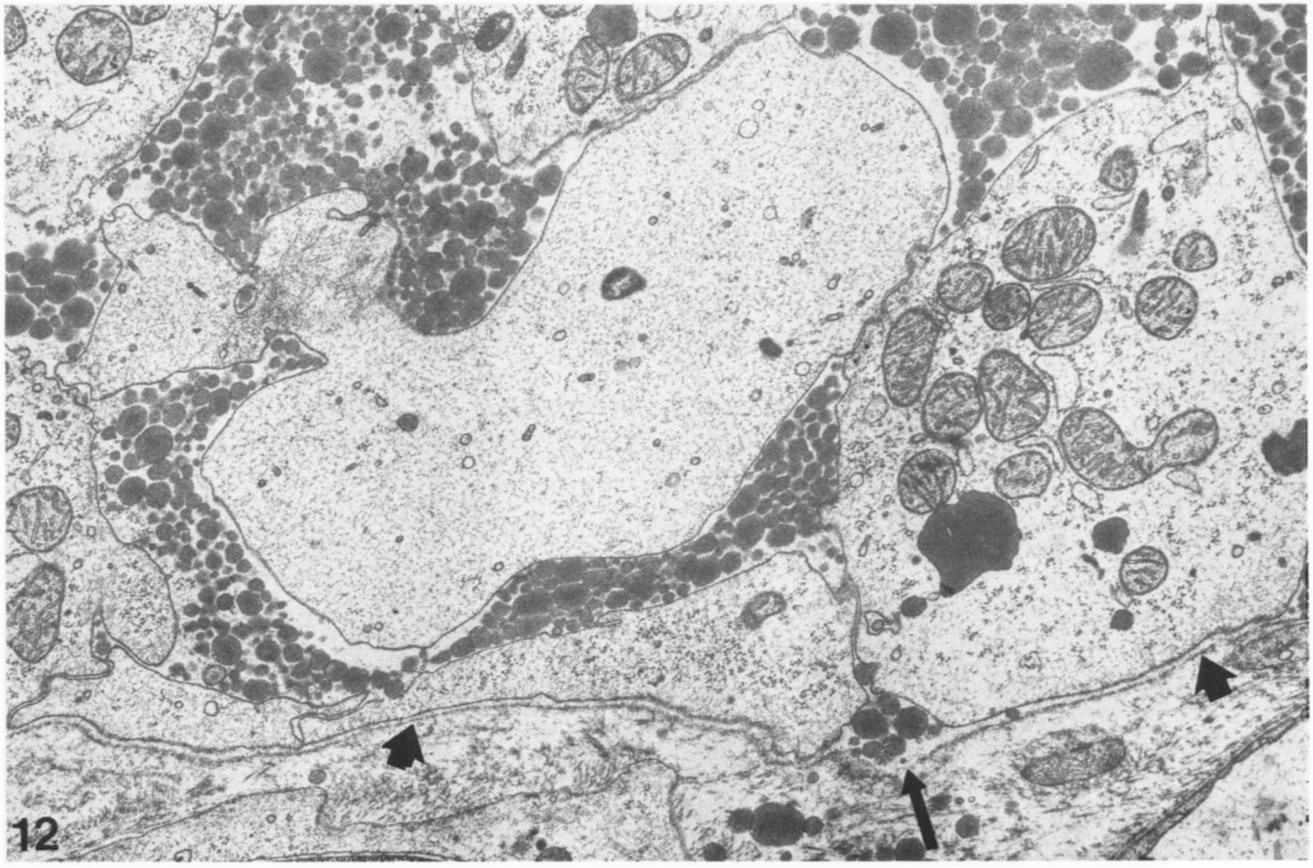

Fig. 12. Many chylomicrons are seen in the dilated intercellular spaces between adjacent epithelial absorptive cells. Note the basement membrane (short arrows) that underlies the basal portion of the epithelial cells. There is an apparent break (long arrow) in the basement membrane in an area where chylomicrons appear to be passing from the intercellular space into the lamina propria. ×54,000.

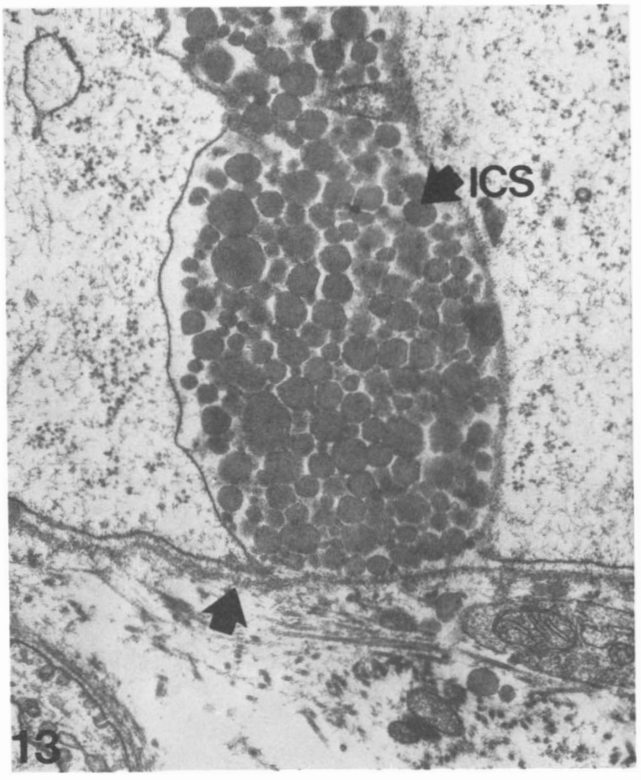

Fig. 13. The intercellular space (ICS) is distended due to an accumulation of chylomicrons. An intact basement membrane (arrow) forms a barrier separating the absorptive epithelial cells and the intercellular space from the lamina propria. ×27,550.

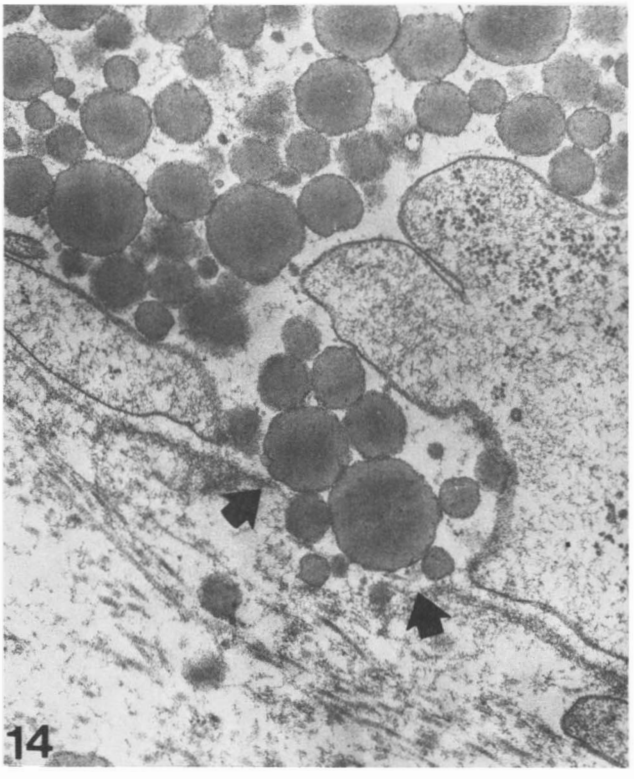

Fig. 14. Chylomicrons are entering the lamina propria through an apparent gap (arrows) in the basement membrane. ×30,000.

crons have migrated to the region of the intercellular space where first fusion of the secretory vesicle membrane with the lateral plasmalemma (Fig. 5) and then discharge of the vesicle contents into the intercellular space occur (Figs. 6, 7). The electron micrographs also demonstrate the striking morphological similarity between lipid droplets contained with the Golgi complex, the droplets within Goldi-derived secretory vesicles, and the chylomicron in the intercellular spaces.

Many secretory vesicles contained specialized alterations in discrete portions of the vesicle membrane (**Figs. 6, 8**). This localized membrane transformation appeared morphologically similar to the so-called coated pits or vesicles which have been observed in many secreting and absorbing cells (3, 9). Secretory vesicles containing coated areas were frequent in the region of the intercellular spaces (Figs. 6, 8) and similar areas were observed occasionally in vesicles that had fused with the lateral plasmalemma (**Fig. 9**). Similar coated areas were seen in the intercellular membranes, in areas in which chylomicron exocytosis had occurred (**Figs. 8, 10, 11**). These electron microscopic images suggest that the coated membrane portion of the secretory vesicle membrane is incorporated into the lateral plasmalemma during the process of vesicle–membrane fusion during exocytosis. In some cells the lateral cell membrane contained several coated areas (Fig. 10) and, in other cells, rather extensive portions of the membrane appeared altered by the accumulation of the coated material (Fig. 11). These membrane changes were confined invariably to areas of intense chylomicron secretion (Fig. 11). It is possible that the coated pits, in the lateral plasmalemma, could also reflect the absorption by endocytosis of other material from the intercellular spaces; however, the presence of coated areas on secretory vesicle membranes that have fused with the membranes lining the intercellular spaces suggests some role for these membrane alterations in chylomicron secretion.

Microfilaments were observed throughout the epithelial cell cytoplasm. Although, in some electron micrographs (Fig. 8), there appeared to be a specific localization of microfilaments in juxtaposition with secretory vesicles, their abundance made it difficult to determine if this represented a preferential localization that might be related to secretory vesicle movement.

Figs. 12–15 illustrate the process by which chylomicrons enter the lamina propria from the intercellular spaces. It would appear from these electron micrographs that the chylomicrons enter the lamina propria by passing through gaps in the base-

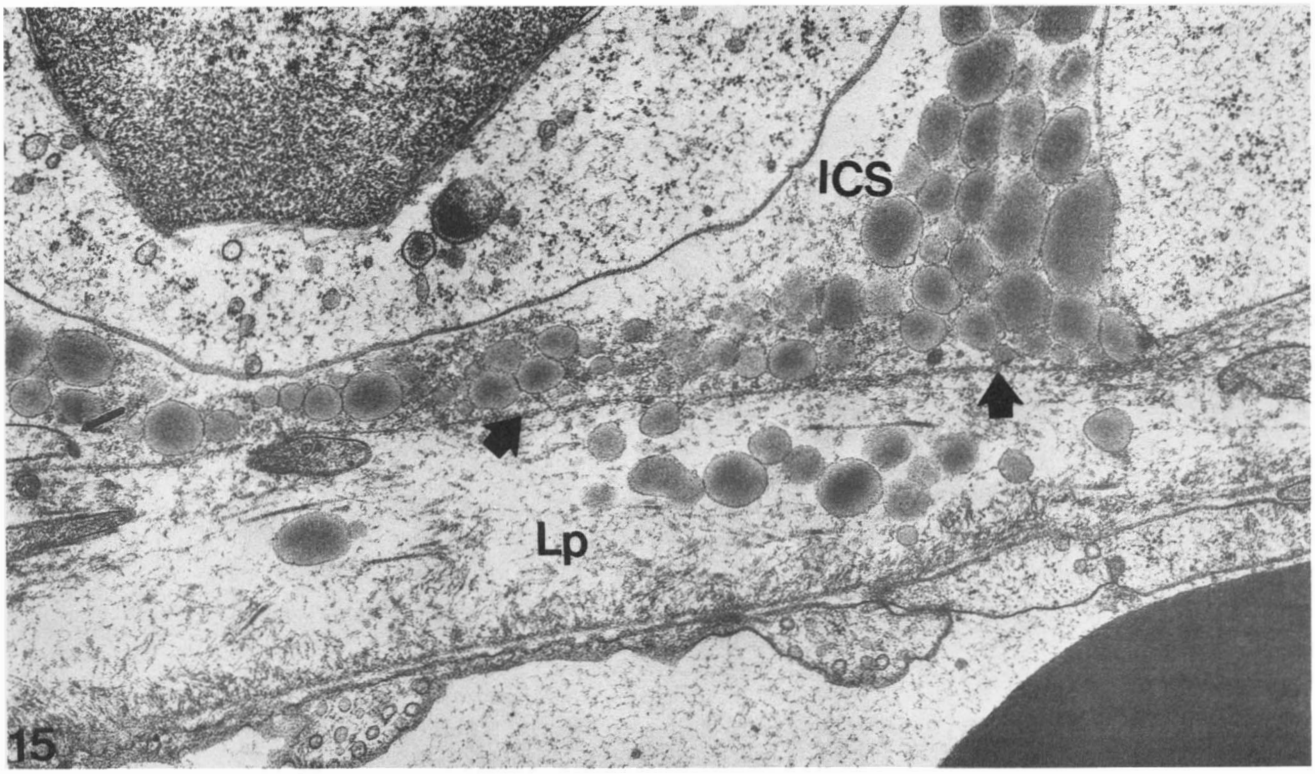

Fig. 15. Chylomicrons are present in the intercellular space (ICS) and also in the lamina propria (Lp). The basement membrane (arrows) appears intact even though chylomicrons have passed into the lamina propria (Lp). ×24,000.

ment membranes which, in nonabsorbing cells, form a continuous boundary along the basal portions of the absorptive cells and the intercellular spaces. Although occasional gaps in the basement membrane were seen (Figs. 12, 14), in many sections the basement membrane was intact despite the presence of chylomicrons in the lamina propria immediately beneath the basement membrane (Fig. 15). In actively absorbing cells the lamina propria became filled with innumerable chylomicrons (**Fig. 16**). The chylomicrons appeared to infiltrate between the dense cellular elements of the lamina propria and thus gradually move towards the lymphatics in the villous core.

In nonabsorbing cells the attenuated finger-like projections of the lymphatic endothelial cells overlap to form a continuous lining (**Fig. 17**). During active fat absorption gaps appear between the cytoplasmic extentions, thereby creating openings through which the chylomicrons pass into the lymphatic lumina (**Figs. 18, 19**).

DISCUSSION

The present ultrastructural investigation of intestinal lipid absorption has confirmed the studies of Strauss (2) and Cardell et al. (3), which demonstrated uptake of the intraluminal lipolytic products of fat digestion by passive diffusion from bile salt micellar solutions rather than by pinocytosis. Despite extensive examination of many proximal jejunal specimens, at several intervals after fat feeding, no increase was observed in the number of pinocytotic pits or vesicles.

The temporal aspects of the uptake of the lipolytic products of intraluminal fat digestion, the intracellular assembly and transport of chylomicrons, and their exocytosis and absorption cannot be followed in systematic fashion by examination of tissues in exact time sequence after fat feeding. In our experiments it was obvious that all phases of fat absorption could be observed within 30 min of fat feeding. Thus, at 30 min the absorptive cells were filled with smooth membrane-bounded osmiophilic droplets, Golgi vesicles were distended with droplets of chylomicron size, and the intercellular spaces were filled with secreted chylomicrons. At later time intervals (e.g., 4 hr) different phases of fat absorption were observed in adjacent villous epithelial cells. Some cells were actively engaged in fat uptake, visualized as a proliferation of smooth-surfaced vesicles containing osmiophilic droplets in the apical cytoplasm, Golgi vesicle engorgement with chylomicrons, and dilatation of the intercellular spaces with secreted particles. In other cells later phases of absorption

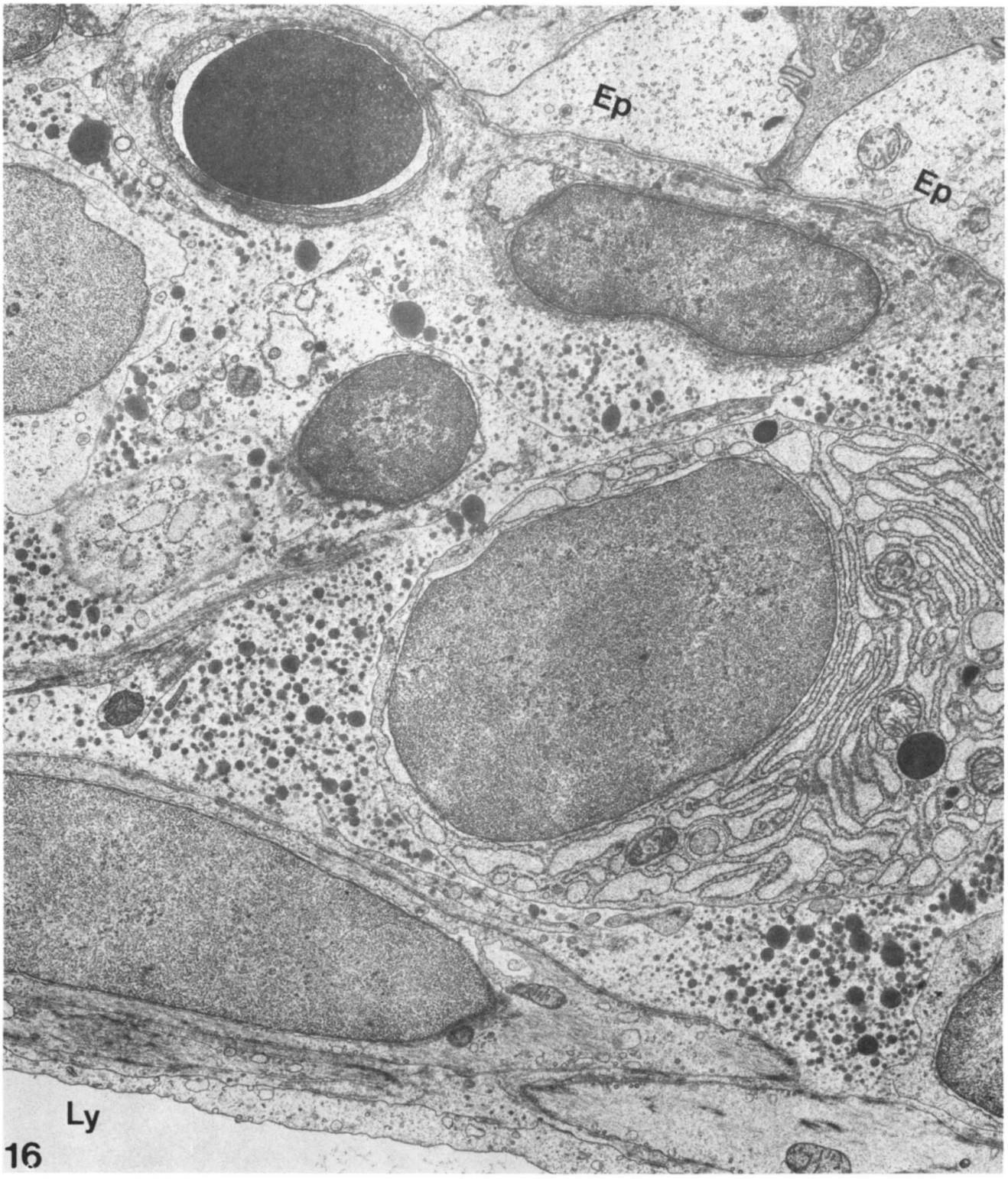

Fig. 16. The cellular components of the lamina propria are illustrated in this electron micrograph. The basal portions of the intestinal absorptive epithelial cells (Ep) are seen at the top of the picture and a lymphatic (Ly) at the bottom. Note the abundant osmiophilic chylomicrons that are infiltrating between the cells of the lamina propria. ×10,250.

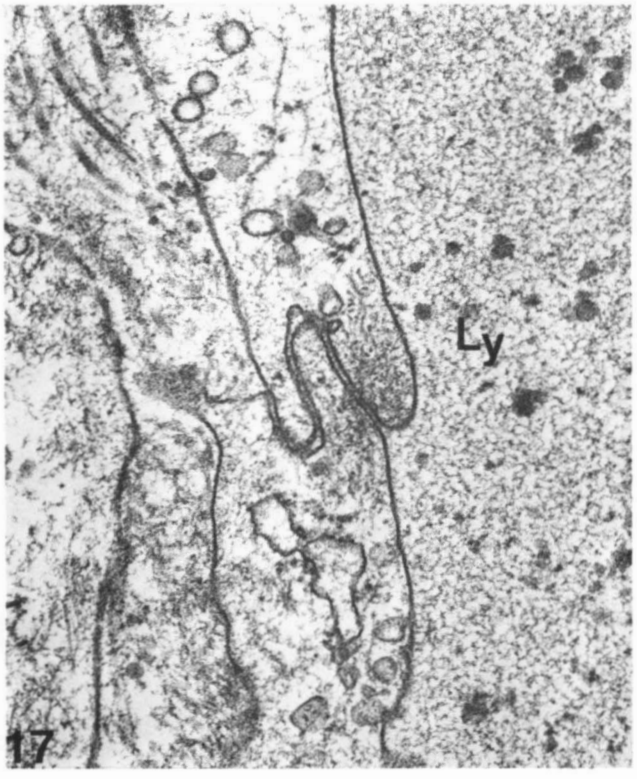

Fig. 17. Finger-like projections of adjacent endothelial cells overlap to form a continuous cellular lining of the lymphatics (Ly) in cells obtained from fasting animals. ×43,200.

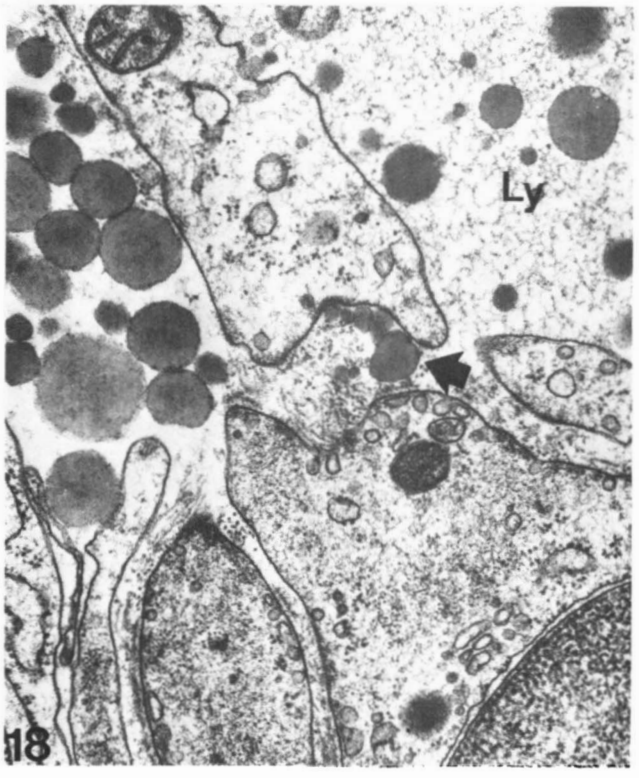

Fig. 18. Chylomicrons enter the lymphatic (Ly) by moving between gaps (arrow) that develop between the cytoplasmic extensions of the endothelial lining of the lymphatic (Ly). ×30,600.

were evident in that the apical cytoplasm was almost devoid of lipid and only the Golgi zones were filled with chylomicrons. Thus, there does not appear to be any obvious synchronous relationship between adjacent absorptive cells in regard to lipid uptake and chylomicron formation. Cells appear to function rather independently in this regard, although the usual picture, particularly at earlier times (30 min to 2 hr) after fat feeding, is of active uptake, chylomicron formation, and secretion in all epithelial cells that are exposed to the luminal contents.

The biochemical evidence regarding the 'activation' of absorbed fatty acids to form fatty acyl thiolesters, the synthesis of triglycerides, and the biochemical events involved in chylomicron biosynthesis have been extensively discussed (1, 5). The ultrastructural counterpart of this biotransformation involves the intimate relationship of absorbed fatty acids and monoglycerides to the substrates and enzymes required for triglyceride synthesis. This association is best provided by the smooth endoplasmic reticulum, which is known to contain the requisite enzymes for triglyceride synthesis as well as an appropriate surface area for diffusion of metabolites into channels that bear a functional continuity to the other subcellular organelles involved in chylomicron assembly and secretion.

After fat feeding, osmiophilic droplets in great quantity occupy the cisternae of the smooth endoplasmic reticulum. The cisternae are usually visualized as cross-sectioned tubules containing individual fat droplets. It is obvious from ultrastructural examination of many cell types that the endoplasmic reticulum represents a complex intracellular system of tubular structures, which channels biosynthetic products to other organelles while providing an environment that promotes biochemical transformations of the transported substances (18). This process is beautifully demonstrated in the case of fat absorption since progressive biochemical alterations occur in the absorbed lipids as they are transported within the endoplasmic reticulum, resulting in the synthesis and then secretion of chylomicrons. One can, therefore, envisage an orderly sequence of biochemical events culminating in the secretion of complete chylomicrons. Such an ordered sequence suggests that at least some of the chylomicron apoproteins are preformed by the rough endoplasmic reticulum in anticipation of triglyceride biosynthesis. In this regard Glickman, Khorana, and Kilgore (19), using

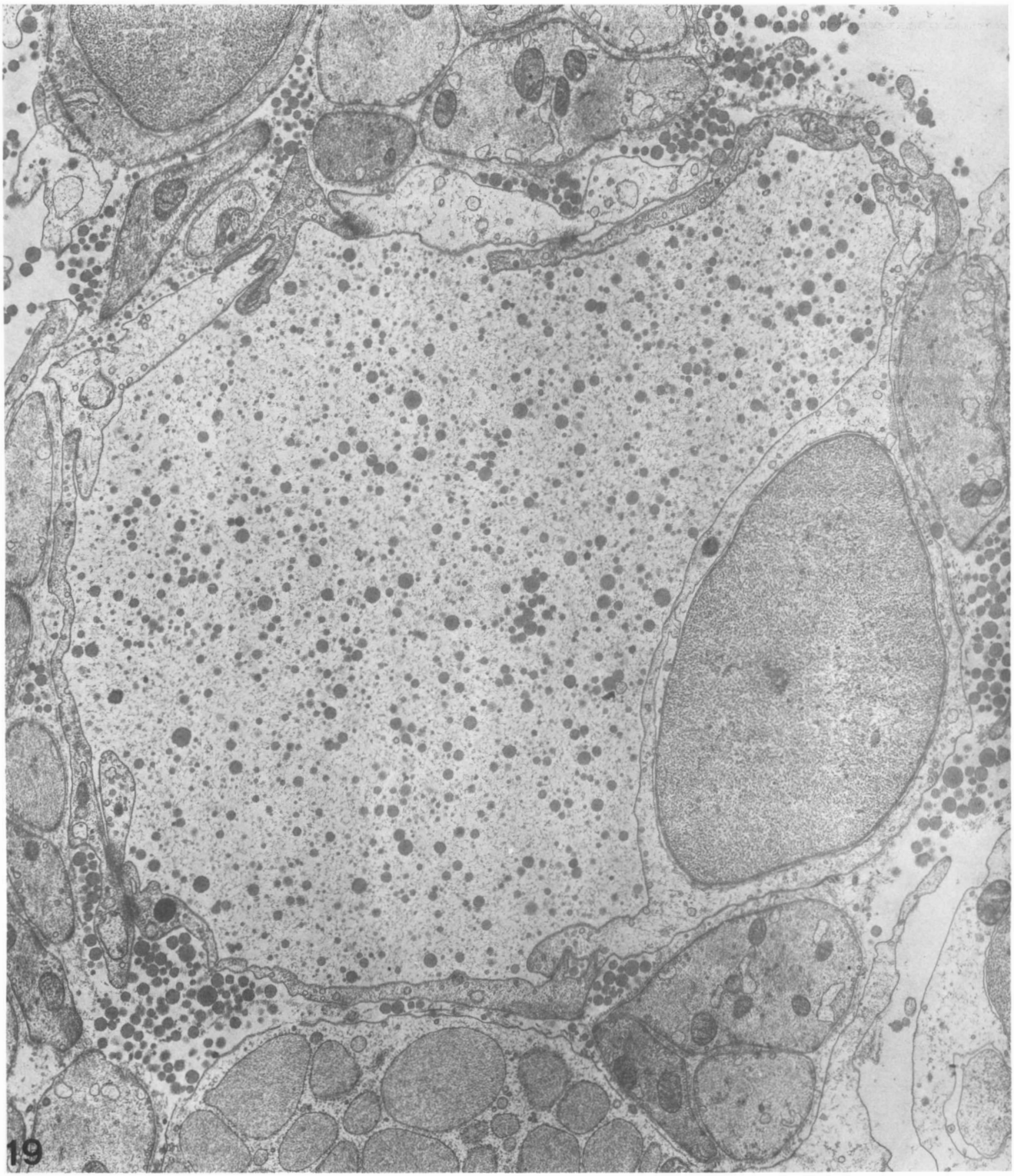

Fig. 19. Large numbers of chylomicrons are present within a lymphatic. Note the attenuated extensions of the endothelial cells that form the lymphatic lining. ×11,850.

immunofluorescent studies, have demonstrated a pool of apo-B and apo-A in small intestinal absorptive cells prior to fat feeding. Since the entrance of fatty acids into the cisternae of the smooth endoplasmic reticulum places them in the perfect environment for esterification and then for the addition of cholesterol and phospholipids, the addition of apoproteins, available initially as preformed moieties,

would result in the formation of an almost complete secretory product. The nascent chylomicrons are then transported within the channels of the endoplasmic reticulum to the Golgi apparatus in preparation for final assembly and secretion.

Since RER, SER, and the Golgi apparatus comprise an interconnected tubular system, a step-by-step assembly and transport of chylomicrons within the respective cisternal elements may occur. The electron microscopic images obtained in the present study are entirely consistent with this model. Although most lipid droplets are visualized within vesicles derived from the SER, it is evident that some are also within cisternae of the RER. The quantity of triglyceride droplets within these respective elements is not an important consideration since the SER is actually derived from the rough reticulum, thereby providing a continuous channel for intracellular transport (3, 9).

The observations presented here that indicate transport of nascent chylomicrons into the Golgi provide new interpretations concerning the importance of the Golgi apparatus in the final assembly and secretion of chylomicrons. It is evident from many investigations that the Golgi subserves an essential function in the final assembly of cellular secretory products (18). In the case of chylomicrons, assembly could involve the association of the lipid and protein moieties and/or the addition of sugar moieties. In addition to ultrastructural observations during normal chylomicron secretion, the importance of the Golgi apparatus in chylomicron assembly and secretion may be deduced from experimental evidence, in the rat, that the inhibition of intestinal protein synthesis by puromycin results in impaired intestinal lipid transport (14) and a defect in chylomicron entry into the Golgi complex (9). Furthermore, the importance of the Golgi in chylomicron processing can also be inferred from observations in the disease abetalipoproteinemia in which impaired chylomicron secretion occurs. In human abetalipoproteinemia (12) and in experimentally produced inhibition of chylomicron secretion (9), lipid droplets are retained within the SER and do not enter the Golgi complex, possibly because of defective Golgi membrane formation (9, 10).

Recent studies (20, 21) utilizing intestinal lipid perfusion have also suggested an important role for the Golgi complex in chylomicron assembly. Thus, differences in the rate of chylomicron secretion between proximal and distal rat intestine may be related to regional differences in Golgi membrane biosynthesis, vesicle formation, or glycosylation reactions resulting in impaired fat absorption from distal intestine under steady state conditions of lipid uptake (21).

The importance of the carbohydrate moiety in the secretion of chylomicrons by the intestinal epithelial cells has not been investigated extensively. It is known that the plasma lipoproteins are glycolipoproteins; however, the role of the glycosyl moieties in determining the structure, transport, or metabolism of lipoproteins has not been determined. Since it is known that the Golgi apparatus is the site of important terminal glycosylations, it is possible that secretion of lipoproteins by intestinal Golgi depends upon the addition of sugars. Thus it is apparent from studies of glycoprotein synthesis that the addition of certain sugar moieties to nascent glycoproteins is confined specifically to the Golgi apparatus (22). Indeed, it has been reported by Kessler (23) that apoprotein–lipid assembly occurs within the endoplasmic reticulum and that glucosamine is incorporated preferentially into chylomicrons by isolated Golgi fractions in vitro. Furthermore, using electron microscopic immunochemical techniques, Alexander, Hamilton, and Havel (24) have shown that the lipid and apoprotein moieties of hepatic VLDL are assembled together prior to the entry of nascent VLDL into the Golgi. The studies of Lo and Marsh (25) showed that, in the liver, 75% of the lipoproteins labeled with [^{14}C]glucosamine are recovered in the purified Golgi fraction. Using isolated cell fractions, they showed that [^{14}C]glucosamine is incorporated into lipoproteins and that microsomal fractions were also labeled with UDP-*N*-acetyl-[1-^{14}C]glucosamine; however, the specific activity of the sugar and the nucleotide precursor increased when purified Golgi fractions were used.

The present electron microscopic observations also relate to several additional points regarding Golgi function. Although it was recognized previously that lipid droplets appear within the Golgi after fat feeding (3, 7, 9, 26) it was considered likely that small triglyceride droplets coalesce into one large chylomicron which is then secreted (3). Our observations provide unequivocal evidence that multiple droplets, identical in size to chylomicrons, accumulate in Golgi vesicles prior to secretion. The occurrence of many chylomicrons within Golgi and secretory vesicles coincides with the presence of morphologically identical droplets in the intracellular spaces. These results provide additional evidence that the final processing of chylomicrons, prior to secretion, occurs within the Golgi complex.

Although a critical role of the Golgi apparatus in chylomicron assembly could relate to the addition of specific terminal sugars by glycosylating enzymes (25, 27, 28), the presence of many nascent chylomicrons with Golgi vesicles indicates that a bulk exocytosis of particles must occur if vesicle–mem-

brane fusion is the mechanism of chylomicron secretion. This concept varies somewhat from current thinking since it has been assumed that individual droplets are secreted and electron microscopic images of the actual phenomena of exocytosis have rarely been observed (2, 3, 9, 17). The present study provides considerable evidence regarding the importance of exocytosis in chylomicron secretion. Electron micrographs illustrated here (Figs. 5–7) clearly demonstrate fusion of secretory vesicles with the lateral cell plasmalemma and also illustrate the exocytosis of many chylomicrons from Golgi-derived vesicles into the intercellular spaces.

There are several additional aspects of chylomicron secretion that have been elaborated upon in the current investigation. It has been suggested that the secretory process is guided by the microtubular apparatus whose components may regulate and direct movement of secretory vesicles to the plasmalemma (29). Recent experiments with colchicine and other agents that interfere with the microtubular system have demonstrated inhibitory effects on VLDL and chylomicron release from the liver and intestine (30–33). These observations have important implications regarding the possibility that Golgi vesicle movement and vesicle–plasmalemma fusion are mediated by the presence of "recognition sites" located on specific areas of the plasmalemma (28). If this concept is valid, it is conceivable that microtubular–vesicle interactions could provide a mechanism by which vesicles are directed to specific recognition sites on cell membranes.

In previous ultrastructural studies of hepatic VLDL secretion (30, 31) and intestinal fat absorption (3), microtubules were identified infrequently and a specific localization of microtubules in relationship to secretory vesicles and areas of membrane fusion was not observed. The electron micrographs illustrated here demonstrate the occurrence of microfilaments in close proximity to secretory vesicles filled with nascent chylomicrons; however, microtubules were observed infrequently and then not in specific relationship to secretory vesicles. Additional research is obviously required to prove the functional importance of the intestinal microfilaments or microtubules in regard to chylomicron secretion.

It is intriguing to speculate that the coated pits and vesicles seen frequently in the membranes of secretory vesicles that contain chylomicrons and in lateral cell membranes, in areas of chylomicron exocytosis, may be concerned with modulating vesicle movement to specific sites on the plasmalemma thereby initiating exocytosis. Coated pits of similar ultrastructure have been seen in a variety of cells involved in active protein absorption where they presumably subserve an essential role in the absorptive process (34, 35). Although a specific function is yet to be determined for these structures, their frequent recognition in areas of active chylomicron secretion suggests a role relating to vesicle movement to specific membrane recognition sites where vesicle membrane fusion can then occur.

The appearance of chylomicrons in the intercellular spaces is followed by their passage into the lamina propria through gaps or discontinuities of the basement membrane (6, 17). Similar observations have been made in the present study (Figs. 12, 14), thus confirming that chylomicrons can enter the lamina propria by passing through discontinuities of the basement membrane. Nevertheless, the quantitative importance of basement membrane gap formation is difficult to determine since membrane breaks were observed infrequently and, in many electron micrographs, chylomicrons were evident on both sides of a basement membrane that appeared intact (Fig. 15).

In an excellent earlier study Casley-Smith (36) demonstrated chylomicron entry into lymphatics through gaps between the overlying processes of adjacent endothelial cells. Similar images were observed frequently in the present study and it is our impression that development of gaps between endothelial cells is the most likely explanation for the rapid flow of chylomicrons into the lymphatics.

This research was supported by NIH research grant number AM-17398, awarded by the NIAMDD PHS/DHEW, and by grants-in-aid from the American Heart Association, the Tennessee Heart Association, and the Veterans Administration.

Manuscript received 26 October 1976 and accepted 5 April 1977.

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