An electron microscopic study of endogenous very low density lipoprotein production in the intestine of rat and man

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ABSTRACT Previous studies have shown that in the absence of dietary lipid, intestinal lymph contains endogenous very low density lipoproteins (VLDL) which are identical to those in plasma in size, flotation rate, composition, and electrophoretic mobility. In order to document that these particles are produced in the mucosa of the small intestine itself, electron microscopic studies of rat and human intestinal mucosa were carried out.

Small intestinal absorptive cells from rats fasted and restrained for 48 hr were rich in osmiophilic particles of the size of VLDL (300–1000 A). These particles were present in the endoplasmic reticulum and Golgi apparatus, and in intercellular spaces and lacteals; they were most abundant in mucosa from mid-jejunum. Similar particles were seen in jejunal mucosal biopsy specimens obtained from normal human volunteers after a 40-hr fast. After 6 hr of bile diversion or cholestyramine administration to fasted rats, the VLDL-sized particles virtually disappeared from the mucosa, suggesting that they were produced in the mucosa itself and depended upon the absorption of endogenous intralumenal lipid.

These studies provide further evidence for the production of VLDL in absorptive cells of fasting rat and human intestine, and support the concept that the small intestine is a source of endogenous plasma VLDL.

SUPPLEMENTARY KEY WORDS bile diversion · bile phospholipids · cholestyramine · endoplasmic reticulum · Golgi apparatus · intestinal absorptive cell · jejunal biopsy · lacteals · osmiophilic particles

THE LIVER has long been recognized as a major source of very low density lipoproteins. However, the importance of the intestine in the production of nondietary lipid–protein complexes that fulfill the criteria for VLDL has been emphasized only recently (1–6). Previous ultrastructural studies from our laboratories (6) confirmed earlier observations of the close similarity between lymph and serum VLDL in the fasting rat (4, 5), and supported the concept that the triglyceride in lymph VLDL was derived from the intestinal lumen. Although other workers demonstrated that the intestine from fasted humans or animals contained lipid-rich particles the size of VLDL (7–12), their physiological significance was not established.

In order to provide direct evidence concerning the role of the intestinal absorptive cell in endogenous lipoprotein production, we have undertaken a detailed ultrastructural study of these cells during fasting and after bile diversion and cholestyramine administration in rats. In addition, we have examined the intestinal epithelial cells in human volunteers by means of peroral intestinal biopsy. Portions of this work have been reported in abstract form (13, 14).

MATERIALS AND METHODS

Animals and Experimental Procedure

Male albino Sprague-Dawley rats (Bioscience Animal Laboratories, Oakland, Calif.), 350–400 g in weight, were maintained prior to study on standard laboratory diet (FP Standard Rat and Mouse Food, Feedstuffs Processing Co., San Francisco, Calif.). The operative procedures included common bile duct and duodenal cannulation. A loop of the common bile duct cannula

Abbreviations: VLDL, very low density lipoproteins.

was exteriorized and the distal end was inserted into the duodenum at the time of surgery, permitting uninterrupted flow of bile until the start of the experiment. After surgery, all animals were placed in restraining cages and received 0.85% NaCl by continuous intraduodenal infusion until the conclusion of the experiments. In some experiments, rats received a continuous intraduodenal infusion of cholestyramine (Questran, Mead Johnson), 80 mg/hr, for the 6 hr prior to killing. The animals with reentrant bile fistulas had their bile cannulas severed 6 hr prior to killing. The animals were killed by cervical dislocation. Samples of small intestine were removed from areas located 15, 35, and 60 cm from the pylorus and were processed for electron microscopy.

Studies in Man

Four normal male volunteers, fasted 40 hr, had intestinal biopsies taken from the proximal jejunum immediately distal to the ligament of Treitz and in addition, in some cases, from the mid-jejunum. In none of the subjects was there a personal or family history or physical evidence of metabolic or lipoprotein disorder. Routine laboratory studies as well as liver function tests, plasma lipids, and lipoprotein electrophoretic patterns on agarose gel were normal. Biopsies were performed with a Quinton multipurpose hydraulic biopsy tube (15).

Electron Microscopic Procedure

Intestinal tissue samples were cut into approximately 10 small segments and fixed in either half-strength Karnovsky's glutaraldehyde–paraformaldehyde mixture (16) or phosphate-buffered 1% osmium tetroxide. The glutaraldehyde-fixed material was postfixed in a phosphate-buffered osmium tetroxide solution. In all cases the specimens were embedded in Epon 812 according to Luft (17), and thin sections stained with lead were examined by electron microscopy (RCA-EMU 3F or a Philips EM 300). At least 30 sections from each small tissue segment were examined. Absorptive cells along the entire length of the villus were studied, although in the bile diversion and cholestyramine groups most intestinal cell comparisons with the normal fasted animals were made near the villus tips.

RESULTS

Mammalian intestinal absorptive cells are tall, columnar cells having a microvillus-rich lumenal or apical surface and a flattened basal surface separated from the lamina propria by a basal lamina. The two organelles important to the present study, the endoplasmic reticulum and the Golgi complex, are well developed. The Golgi complex is supranuclear and consists of saccules, small vesicles, and large vacuoles. In the rat, as well as in man, the endoplasmic reticulum is abundant in the apical region of the cell between the Golgi apparatus and the terminal web. This membrane-limited system of minute intracellular canals consists of both ribosome-studded membranes (rough-surfaced endoplasmic reticulum) and smooth-surfaced membranes (smooth-surfaced endoplasmic reticulum). Continuity between the smooth and rough endoplasmic reticulum is common.

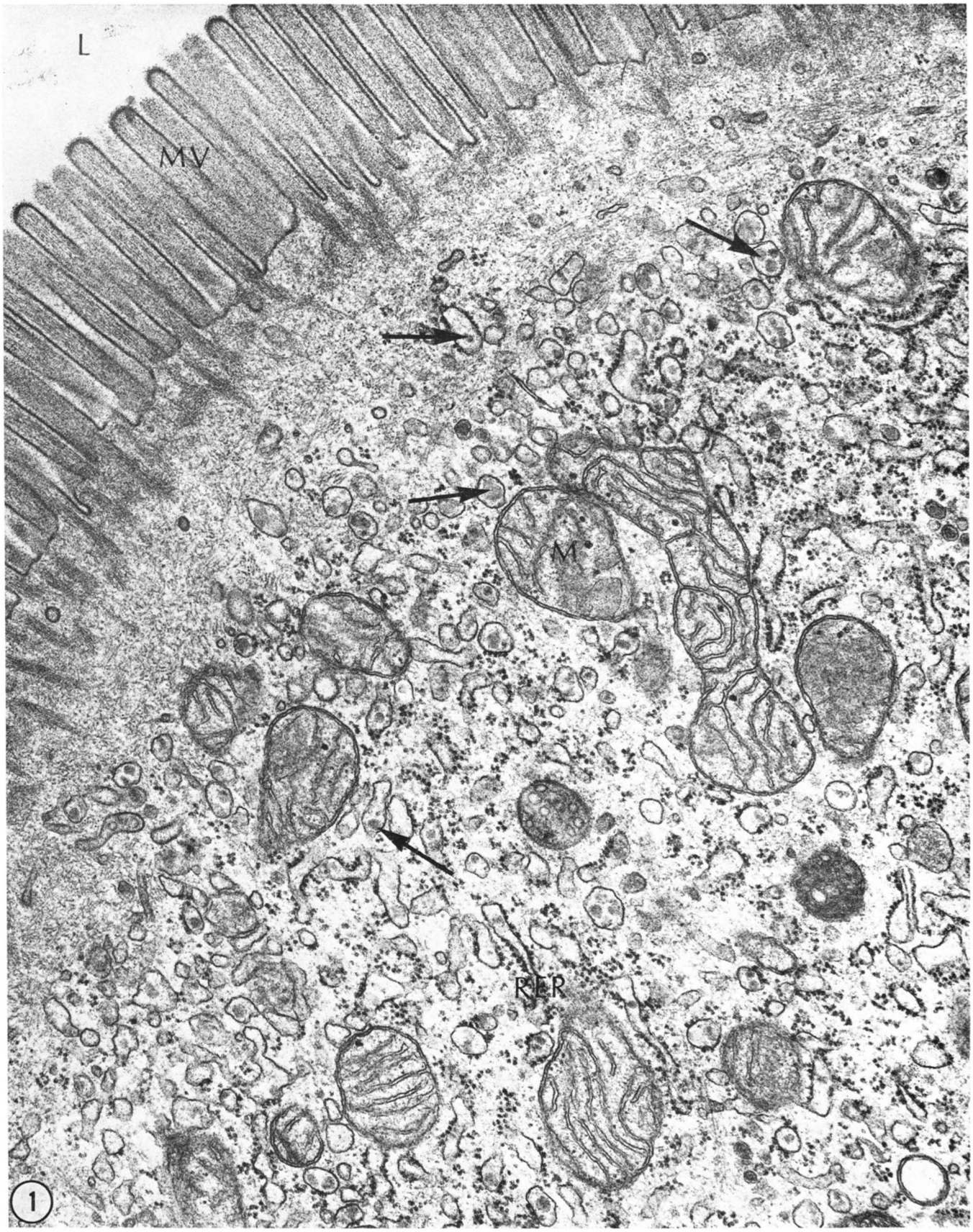
Ultrastructure of Absorptive Cells after Prolonged Fasting

Rat Intestine. After 48 hr of fasting, most absorptive cells throughout the small intestine contain lipid particles within the Golgi apparatus (Fig. 3), an observation originally made by Palay and Karlin (7). In addition, these VLDL-sized particles (300–800 A) are noted with surprising frequency within the cisternae of the endoplasmic reticulum (Figs. 1 and 2). Many of the membrane-bounded particles are found just beneath the fine filaments of the terminal web in the apical region of the epithelial cell. In contrast to the liver, in which similar VLDL particles are localized exclusively in the smooth endoplasmic reticulum or Golgi complex (18, 19), the particles in the intestine are often enclosed (alone or in small clusters) by membranes which contain both a smooth-surfaced and a rough-surfaced component (Figs. 1 and 2). Although a few invaginations or pits can be seen between the microvilli at the cell surface, there is no evidence that these structures contain lipid material. Large cytoplasmic lipid droplets which are often noted during dietary fat absorption (20–22) were not observed in these animals.

In addition to the particles located within the cells, there are many free, unbounded particles in the intercellular spaces between adjacent epithelial cells (Fig. 4). In general, both the intracellular and the extracellular particles are far more numerous in tissue from the mid-jejunum (35 cm distal to the pylorus) than in the other regions of the intestine examined.

Human Intestine. Absorptive cells in man were remarkably similar to those in the rat with the following exceptions: (*a*) the cells appeared more dense; (*b*) the mitochondria were smaller; and (*c*) the rough reticulum was present almost exclusively in the form of long, flattened cisternal profiles. Again, there was evidence of direct continuity between the rough- and smooth-surfaced reticulum.

Lipid particles of the size of VLDL, and even of the size of the smaller β-lipoproteins (<250 A), were seen frequently within the Golgi apparatus (Fig. 7) and also within the tubules of the smooth-surfaced endoplasmic reticulum (Figs. 6 and 7). These particles were abundant within the intercellular spaces between adjacent epithe-



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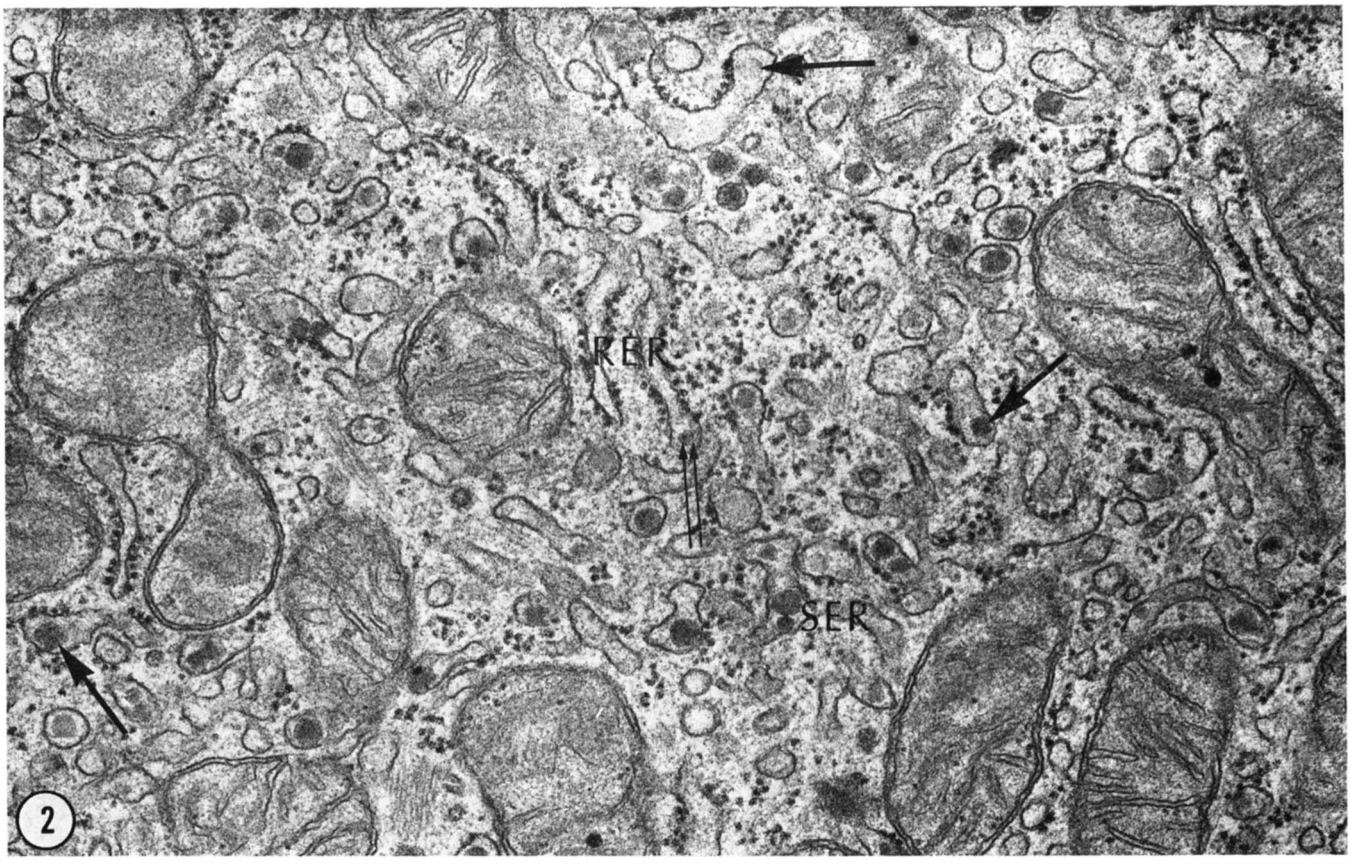


FIG. 2. Electron micrograph at higher magnification of an intestinal epithelial cell from the mid-jejunum of a fasted and restrained rat showing an area particularly rich in VLDL-sized particles. Here again, some of the particles are bounded by both rough and smooth membranes (single arrows). This may represent a transitional zone between the rough-surfaced endoplasmic reticulum (*RER*) and the smooth-surfaced reticulum (*SER*), since some particles are located clearly (double arrow) at the smooth-surfaced terminal end of the rough reticulum. Glutaraldehyde. × 48,000.

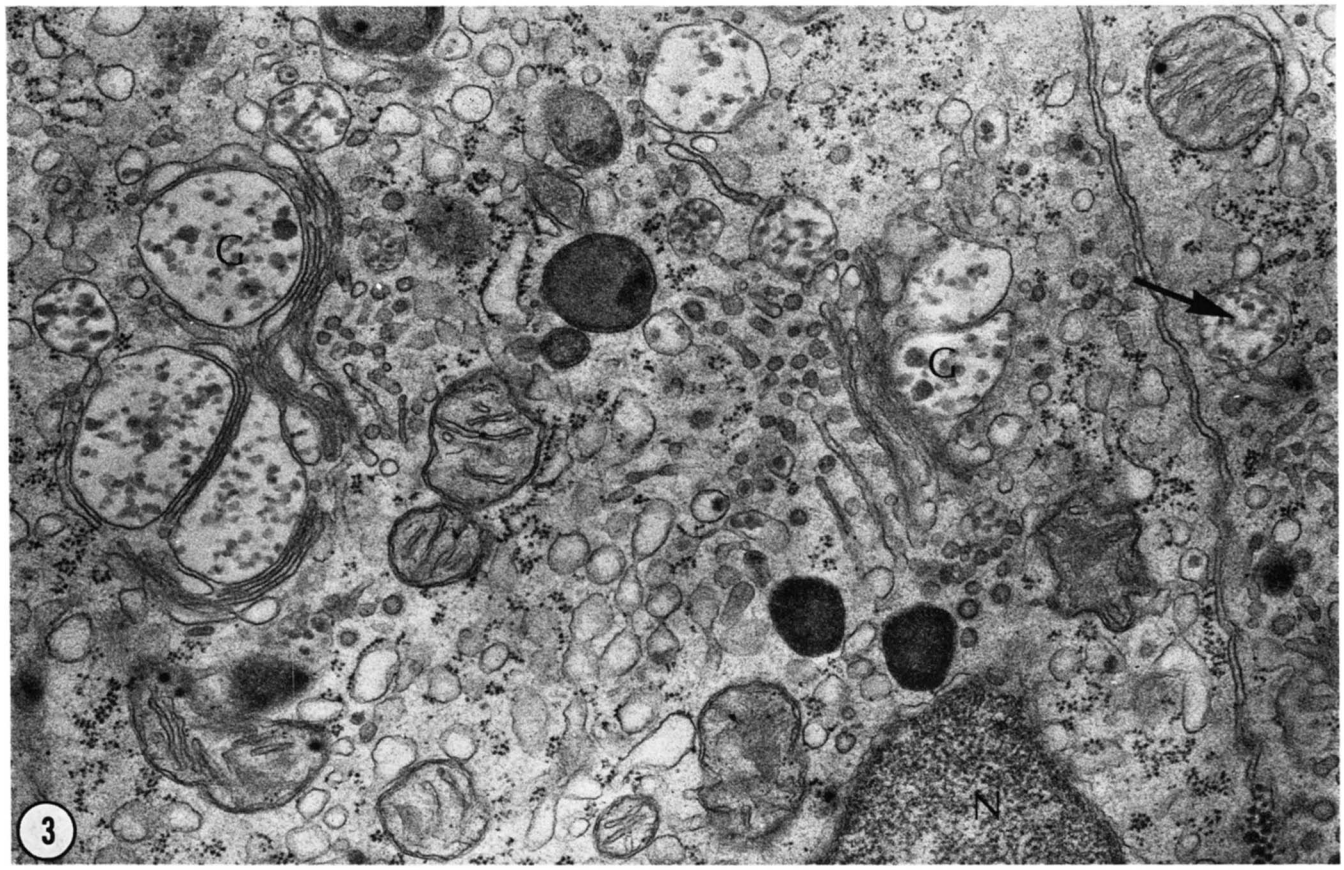
lial cells (Figs. 6 and 7) and within the lacteals. Moreover, electron microscopic examination of intestinal lymph obtained from three fasted human subjects during abdominal surgery revealed the presence of numerous lipoprotein particles in the size range of VLDL.[1] In contrast to the rat, the most abundant accumulations of lipoproteins in man were found in the proximal jejunal epithelium. In general, the lipoprotein particles in the human intestine were very difficult to stain with osmium tetroxide; therefore, they were not as electron opaque or conspicuous as those observed in the normal fasting rat. This may reflect differences in lipid composition between the two species.

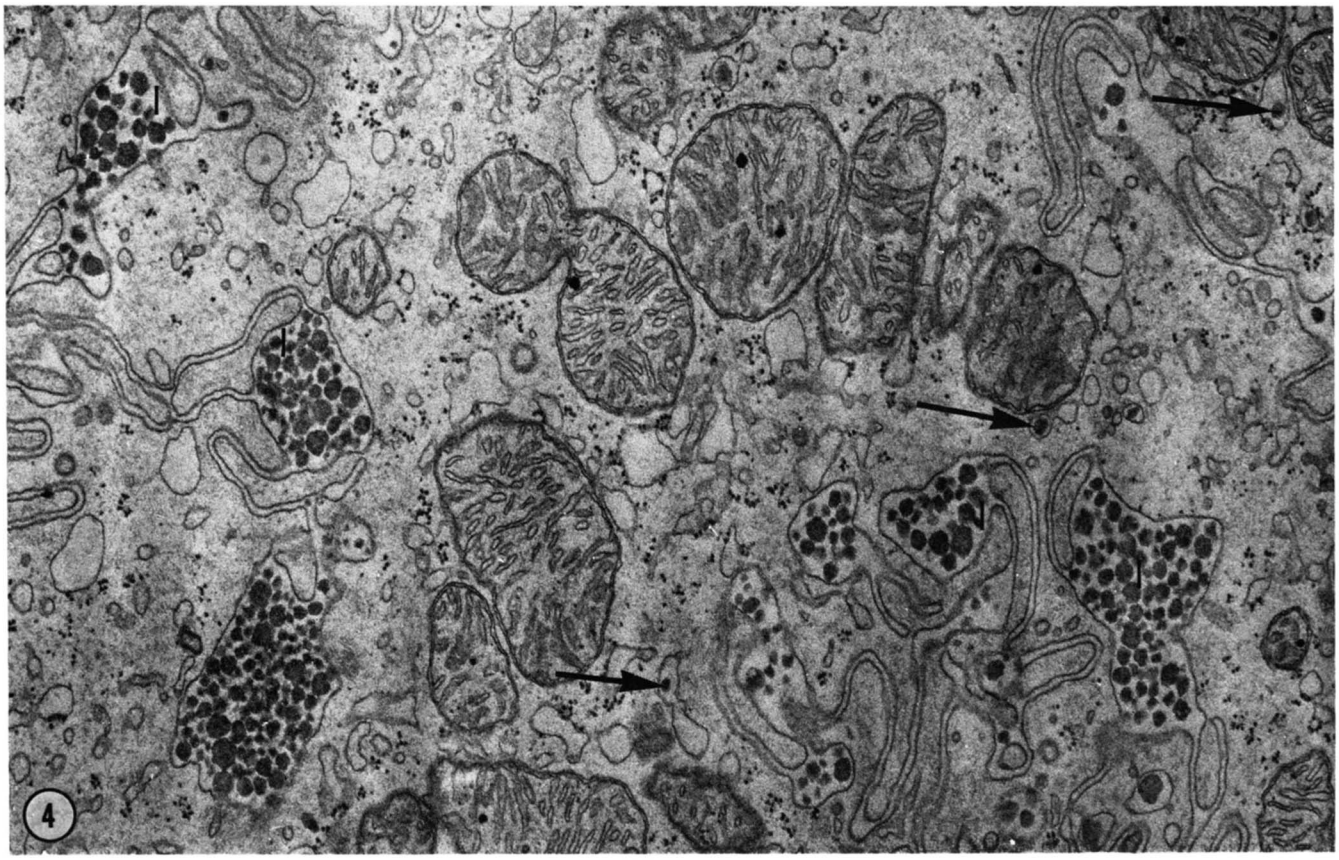
[1] Jones A. L., L. W. Way, and R. K. Ockner. Unpublished observations.

Bile Diversion and Cholestyramine Treatment

Because earlier studies had shown that bile diversion or cholestyramine administration rapidly led to the virtual disappearance of VLDL from intestinal lymph (5, 6), the effect of these manipulations on mucosal cell ultrastructure was examined. It was found that bile diversion and cholestyramine administration produced a picture which differed markedly from that seen in the fasting rats, as described above. At the end of 6 hr of bile diversion or cholestyramine infusion, there were essentially no lipoprotein particles in the examined segments of the rat intestine. This finding was most striking in specimens from the bile-diverted animals (Fig. 5), in which almost all of the Golgi material in every cell appeared dilated and free of particles. Similarly, the intercellular spaces, lamina propria, and lacteals were devoid of particles.

← FIG. 1. Electron micrograph of an apical portion of an absorptive cell from the mid-jejunum of a rat fasted and restrained for 48 hr. The intestinal lumen (*L*) and microvilli (*MV*) are observed. Note the striking number of 300–900-A electron-dense particles within cisternae of the endoplasmic reticulum (arrows). Some particles appear to be bounded entirely by smooth-surfaced endoplasmic reticulum, whereas others are bounded by both a ribosome-studded and a smooth membrane. The appearance of these lipid-rich particles in this location suggests that free fatty acid esterification occurs immediately within the endoplasmic reticulum at the lumenal surface of the cell. Numerous mitochondria are also observed (*M*). *RER*, rough-surfaced endoplasmic reticulum. Glutaraldehyde. × 38,500.





DISCUSSION

This study extends our previous observations of the fine structure of VLDL particles in intestinal lymph (6) and provides direct evidence that these particles are synthesized and released by intestinal epithelial cells during fasting in both rat and man. The sites of synthesis and intracellular transport of endogenous VLDL produced in the intestine are similar to those involved in the production of VLDL in the liver (18, 19, 23) and in the formation of chylomicrons during *exogenous* fat absorption (9, 10, 20–22). These two classes of intestinal lipoproteins (chylomicrons and VLDL) differ primarily in size, which, in all likelihood, is determined in large part by the lumenal fat load. With the increase in lipid absorption that follows a fat meal, more triglyceride is associated with the acceptor apoproteins, and the particles tend to become larger (> 800 A) than those produced when only endogenous lipid is available. This phenomenon occurs also in the perfused liver preparation as the amount of perfusate free fatty acid is increased (24). In the case of the intestine, however, lymph particle size is also influenced by the type of exogenous fatty acid entering the cell (6, 25).

Source of Lipid in Endogenous Intestinal VLDL

Since the work of Rony, Mortimer, and Ivy (26) almost four decades ago, which indicated that thoracic duct lymph in fasting dogs had an intestinal origin, questions have arisen as to whether the lipid precursor is derived from a lumenal or nonlumenal source. The fact that bile diversion (5, 6, 26–29) or intraduodenal cholestyramine administration (5, 6) markedly decreases the amount of intestinal lymph lipid and absorptive cell particle formation (present data) strongly supports the concept that the lipid precursor is lumenal. The phospholipid of the bile (secreted at a rate of about 5 mg/hr in the rat) probably is a major source of lumenal lipid, although other sources, including digestion of shed gastrointestinal epithelial cells, also contribute. In this regard it was noted[2] that when a constant intraduodenal infusion of bile salts was started immediately after bile diversion, intestinal lymph triglyceride fell to about 40% of basal levels. Therefore, it appears that a substantial proportion of lipid in intestinal VLDL comes from a source other than bile, most likely sloughed intestinal epithelial cells. The reabsorption of endogenous lumenal lipid is an important mechanism for conserving this nutritionally valuable material, which otherwise would be excreted.

Intracellular Lipoprotein Synthesis and Transport

After the fatty acids from hydrolyzed bile phospholipid or sloughed epithelial cells have entered the intestinal absorptive cell, presumably by a non-energy-dependent process (21, 22), they are thought to come into contact with the apical tubules and vesicles of the endoplasmic reticulum. Several lines of evidence indicate that this organelle plays a principal role in the reesterification of fatty acid and the subsequent development of the fatty core of the lipoprotein: (*a*) lipid particles in the apical region of the cell are found only within the cisternae of the endoplasmic reticulum; (*b*) fatty acid-esterifying enzymes, both in the liver and gut, are known to be localized in the microsomal fraction (30–33); and (*c*) Sjöstrand and Borgström (34) observed by cell fractionation after intraduodenal infusion of labeled fatty acid that the apical vesicles of the intestinal epithelial cells contained most of the absorbed lipid.

In contrast, the sequence of events following esterification and leading to the elaboration of the lipoprotein particle remains open to question. In the liver, the *rough endoplasmic reticulum* has been implicated in the production of the specific apolipoprotein of the VLDL (18, 19, 35) formed in response to free fatty acid uptake (24), whereas the *smooth reticulum* has been thought to play a principal role in the synthesis and addition of the lipid moiety (18, 19, 36). Similarly, in the intestinal absorptive cells of fasted animals, lipid particles also appear within the cisternae of the endoplasmic reticulum, although quite often they are observed within a portion of endoplasmic reticulum formed from both smooth- and rough-surfaced membranes. This association may allow the efficient synthesis of the lipid–protein complex.

Also open to question in the process of lipoprotein formation is the role of the Golgi complex. To consider the Golgi complex as a "packager" of lipoproteins for secretion (37) is tempting because it allows one to regard this secretory process as analogous to the transport of protein by pancreatic acinar cells (38, 39). In the liver, however, there is evidence suggesting that lipoproteins can be secreted without involving the Golgi apparatus

[2] Ockner, R. K., and A. L. Jones. Unpublished observations.

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FIG. 3. Golgi apparatus (*G*) of an intestinal absorptive cell from a rat fasted and restrained for 48 hr is shown to contain a number of VLDL-sized particles. Note the cluster of particles (arrow) in what appears to be a Golgi secretory vesicle near the cell margin. However, particles are also found in the endoplasmic reticulum near the cell margin (see Fig. 4). *N*, nucleus. Osmium tetroxide. × 25,000.

FIG. 4. Lipoprotein particles are located not only within the absorptive cells of fasted and restrained rats but are also observed in the intercellular spaces (*I*). In this electron micrograph an area is shown which is particularly rich in these extracellular particles. Note also that there are particles (at the arrows) within the endoplasmic reticulum and vesicles near the lateral surfaces of epithelial cells. Observations such as this lend strong support to the suggestion that not all lipoproteins need to pass through the Golgi apparatus prior to secretion. Osmium tetroxide. × 29,000.

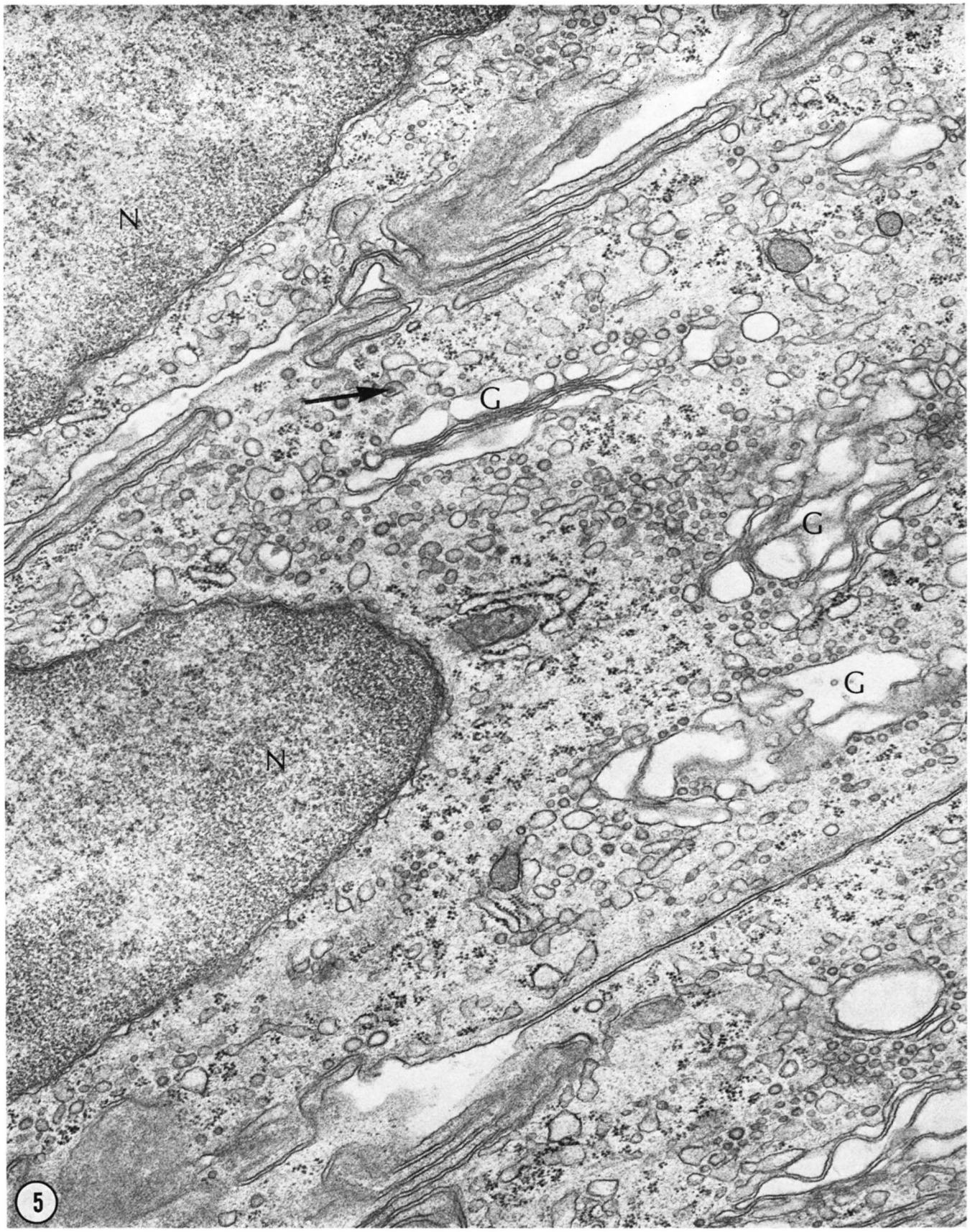
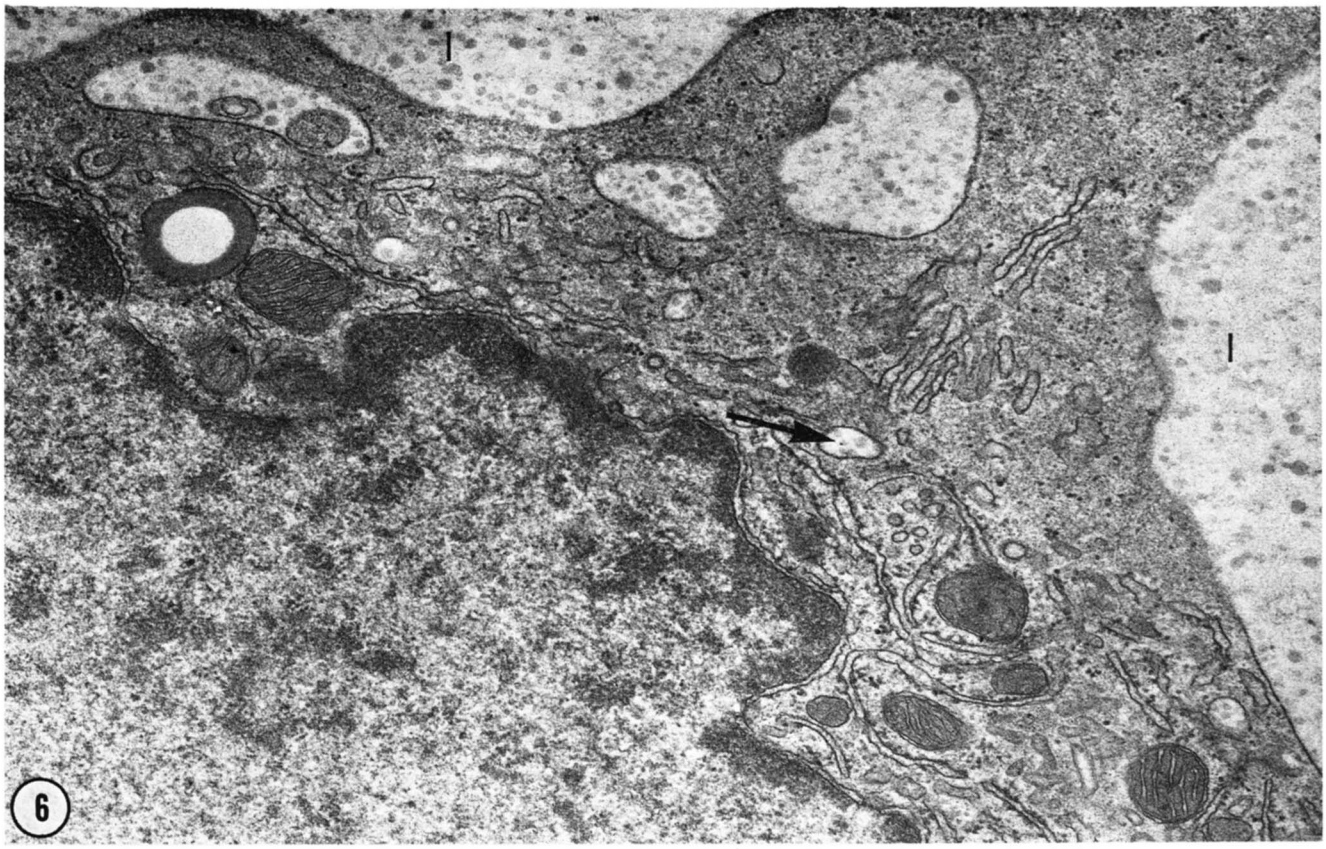


FIG. 5. Portions of three mid-jejunal epithelial cells from a 48-hr fasted and restrained rat after bile diversion for 6 hr. Note the virtual absence of lipoproteins within the Golgi apparatus (*G*) and endoplasmic reticulum. Only an occasional lipoprotein can be found in any of these cells (arrow). *N*, nucleus. Osmium tetroxide. × 31,000.



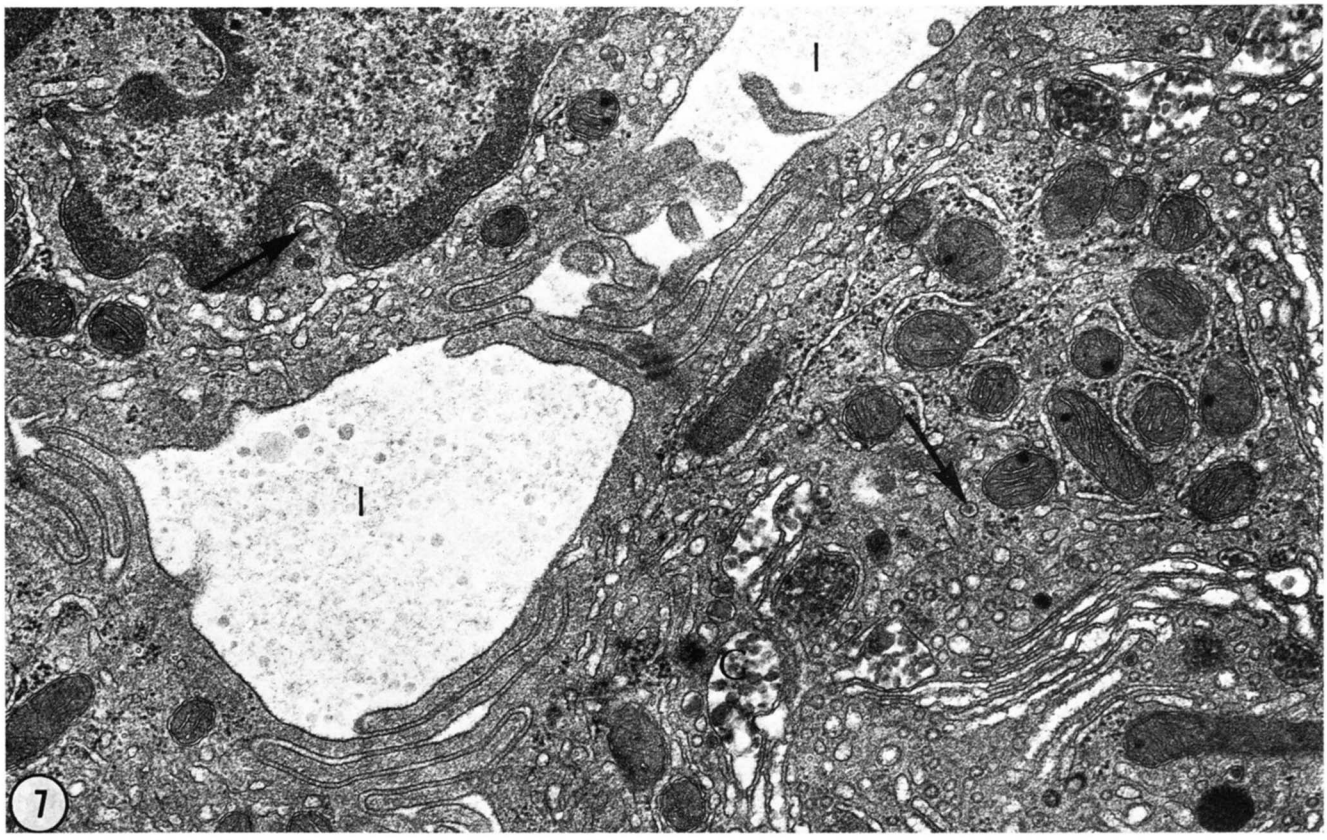


FIG. 6. Jejunal epithelial cell from human intestine after a 40-hr fast. Note the abundance of lipoprotein particles within the endoplasmic reticulum and intracellular vacuoles as well as those in the intercellular spaces (*I*). Glutaraldehyde. × 38,500.

FIG. 7. Epithelial cells from human intestine showing the large number of particles that can be observed normally in the Golgi apparatus (*G*) and endoplasmic reticulum (arrows) in human subjects fasted 40 hr. Glutaraldehyde. × 31,000.

(19). Nevertheless, as suggested previously (19), if the Golgi does have a specific function in lipoprotein metabolism, it may be related to the addition of a carbohydrate moiety to the lipoprotein peptides (40, 41).

Concluding Remarks

Although the details of the events leading to the formation and secretion of lipoproteins remain uncertain, the present experiments have provided further evidence in both rat and man that in the absence of dietary lipid the epithelial cell of the gut produces VLDL. The magnitude of this source relative to liver has not been established. However, recent studies (42, 43) have shown that, in rats, ethanol leads to enhanced production of *endogenous* intestinal VLDL and that this source may participate in the pathogenesis of ethanol-induced fatty liver and hyperlipidemia. The possibility that endogenous intestinal lipoproteins are important in other conditions associated with abnormalities of lipoprotein metabolism warrants further exploration.

The authors wish to acknowledge the helpful comments of Dr. Elinor S. Mills during the preparation of the manuscript, and the technical assistance of Mrs. Gabrielle Rouiller Neises, Mrs. Jan Yager, Miss Joan Hahn, and Mr. Jack Quan. The research was supported in part by grants AM-13328 and AM-05598 from the National Institutes of Health, and by the Veterans Administration Hospital, San Francisco, Calif. These studies were carried out in part in the General Clinical Research Center, University of California, San Francisco, with funds provided by the Division of Research Resources, RR-79, U.S. Public Health Service.

Manuscript received 7 January 1971; accepted 27 May 1971.

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