Synthesis and Transport of Lipoprotein Particles by Intestinal Absorptive Cells in Man

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ABSTRACT The site of synthesis and some new details of lipoprotein particle transport have been demonstrated within the jejunal mucosa of man. In normal fasting volunteers, lipoprotein particles (88%, 150–650 A diameter) were visualized within the smooth endoplasmic reticulum and Golgi cisternae of absorptive cells covering the tips of jejunal villi. Electron microscopic observations suggested that these particles exited through the sides and bases of absorptive cells by reverse pinocytosis and then passed through the extracellular matrix of the lamina propria to enter lacteal lumina.

When these lipid particles were isolated from fasting intestinal biopsies by preparative ultracentrifugation, their size distribution was similar to that of very low density (S_f 20–400) lipoprotein (VLDL) particles in plasma.

After a fatty meal, jejunal absorptive cells and extracts of their homogenates contained lipid particles of VLDL-size as well as chylomicrons of various sizes. The percentage of triglyceride in isolated intestinal lipid particles increased during fat absorption. Our interpretation of these data is that chylomicrons are probably derived from intestinal lipoprotein particles by addition of triglyceride.

INTRODUCTION

The liver and the intestine are the only organs known to be capable of lipoprotein particle synthesis. Physical, biochemical, and morphological studies of animal intestinal mucosa and lymph have shown the small intestine to be a source of these small ($< 0.1\ \mu$), very low density ($d < 1.006$) lipoprotein particles (VLDL)[1] (1–7). Furthermore these VLDL particles play a major role in the transport of lipids in intestinal lymph of fasting rats (6).

In a previous report from this laboratory, numerous small particles of less than 0.1 μ diameter were described in various areas of the fasting jejunal mucosa in man: in the intercellular spaces between the absorptive cells, in the extracellular spaces of the lamina propria and within the lumina of lacteals (8). These particles were presumed to be lipoprotein because they were identical in appearance to those which Casley-Smith (9) found in the vicinity of rat intestinal lacteals and which he showed to be morphologically similar to those isolated from rat chyle by ultracentrifugation.

This study was designed to determine where the human intestine synthesizes lipoprotein particles and how these particles gain access to the circulation. Our approach was both morphological and biochemical. Fasting human jejunal biopsies were studied by electron microscopy after special staining to accentuate lipoprotein particles. In parallel studies lipoprotein particles were isolated from biopsy homogenates by preparative ultracentrifugation. Particle sizes were then measured after shadow casting and particle content analyzed to determine lipid composition.

METHODS

Subjects. 28 normal volunteers, aged 21–30 yr, were studied. Unless stated otherwise, volunteers were maintained for variable periods before biopsy either on ad lib. diets or on standardized isocaloric diets containing 40% of calories as fat, 45% as carbohydrates, and 15% as protein. All biopsies were taken in the fasting state after a 12–14 hr overnight fast.

Biopsy processing for electron microscopy. 180 intestinal biopsies were taken from the 28 normal volunteers either with a multipurpose (10) or a hydraulic biopsy tube (11);

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[1] *Abbreviations used in this paper:* VLDL, very low density lipoprotein; SER, smooth endoplasmic reticulum.

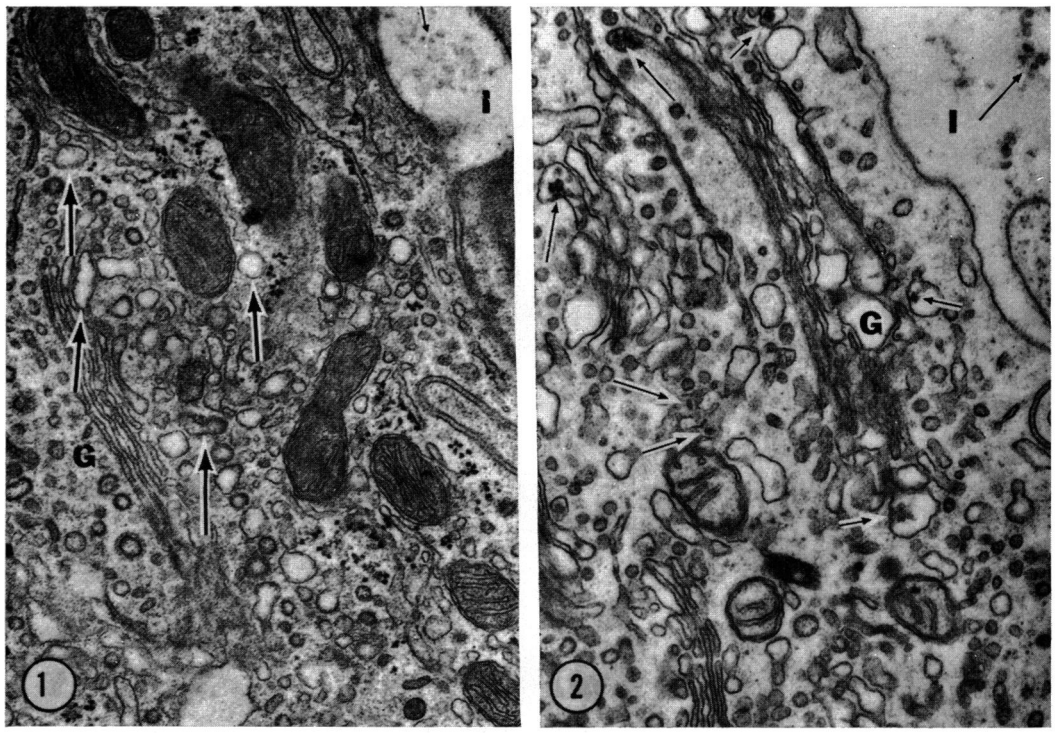


FIGURE 1 Golgi zone (G) and adjacent intercellular space (I) in an absorptive cell near the tip of a jejunal villus after "double" staining. Lipoprotein particles (small arrow, upper right) were barely discernible within the intercellular space (I). They could not be seen within Golgi membranes (G) or within membranes of the SER (larger arrows, pointing upward). Magnification, × 27,500.

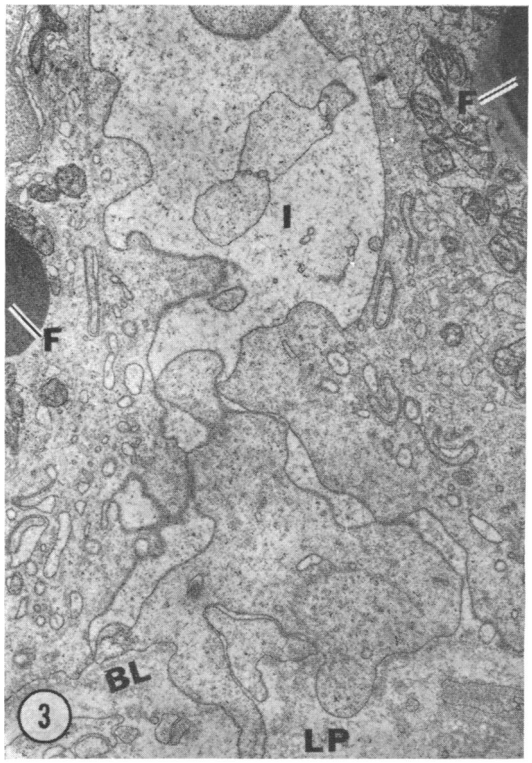
FIGURE 2 Golgi zone (G) and adjacent intercellular space (I) from the same location in a jejunal biopsy, at the same time and from the same patient as the one illustrated in Fig. 1. The only difference was the staining, which was Kushida-Fujita tissue block stain followed by Reynold's lead–citrate section stain. Note the lipoprotein particles (small arrows) which were more clearly evident within the intercellular spaces (I), and were also visible within the Golgi profiles (G) and the SER. Magnification, × 27,500.

all were processed for, and studied by, electron microscopy. The biopsies were mounted cut side down on monofilament plastic mesh and fixed in chilled bicarbonate-buffered (12) 3.3% osmium tetroxide containing 0.9 mM calcium ion. Fixation was started within an average of 30, to at the most, 60 sec after excision, and lasted 60–75 min. The biopsies were dehydrated in graded strengths of ethanol and before embedding were usually stained for 2 hr in uranyl nitrate and lead acetate by the method of Kushida and Fujita (13). The plastic mesh was then removed and the specimen carefully oriented and embedded in Luft's Epon (14). The biopsies were cut in half with a jewelers saw and mounted on two separate chucks for 1 μ "thick" sectioning with a glass knife. The sections were stained with Richardson's azure II-methylene blue (15) so that well-oriented tips of villi could be selected for trimming and thin sectioning with a Reichert ultramicrotome using diamond knives. Most thin sections were lightly stained with Reynold's lead citrate (16). Only 10 of the 180 biopsies were not stained according to Kushida and Fujita; these sections were "double" stained with a 3% aqueous solution of uranyl acetate (17) followed by Reynold's lead citrate (16). Almost all of the biopsies in this study were examined with an AEI 6B electron microscope (AEI Scientific Apparatus, Division of Picker Corp.), although, early in our study, a few biopsies were examined with a modified RCA 2C electron microscope.

After comparison of various fixative mixtures employing combinations of osmium with chromate, glutaraldehyde, formalin, and acrolein, we found buffered 3.3% osmium tetroxide alone to be most satisfactory because it produced the fewest artifacts and provided the best structural preservation. After comparison with other buffers, bicarbonate was chosen because it gave the most informative pictures, i.e. the contrast was adequate and visually little material appeared to have been leached out of the tissue. The addition of calcium ion decreased the amount of membrane breakage.

The Kushida-Fujita tissue block stain (13) was tried in the hope that contrast would be increased and stain contamination decreased. General tissue contrast was not particularly altered but stain contamination was decreased. Surprisingly, the density of lipid was considerably enhanced, especially that of the lipoprotein particles.

In fasting jejunal biopsies taken from patients with aβ-lipoproteinemia, no lipoprotein particles whatsoever are visible by electron microscopy after the usual "double" staining of thin sections (18). A fasting jejunal biopsy from



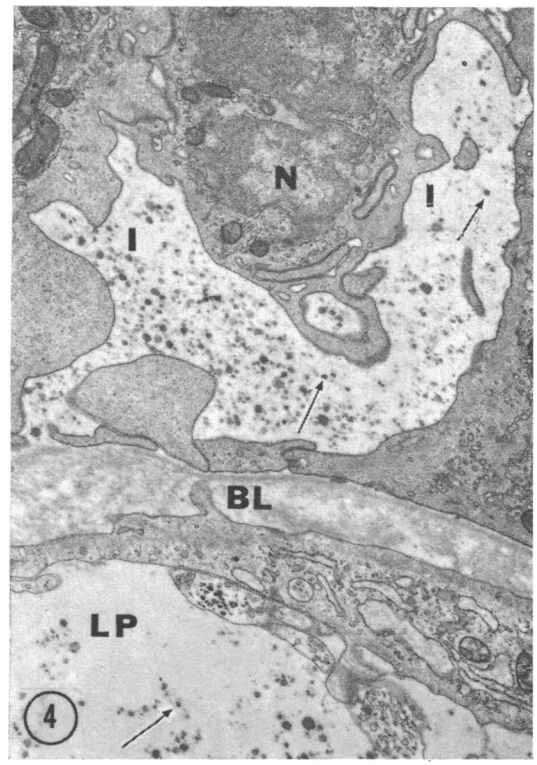


FIGURE 3 Intercellular space (I) and portions of bases of two adjacent absorptive cells in a jejunal biopsy from a fasting patient with aβ-lipoproteinemia. Despite staining of the tissue block according to Kushida and Fujita and staining of the section with Reynold's lead citrate, no lipoprotein particles were visualized in the absorptive cells, in the intercellular space (I) or beneath the basal lamina (BL) within the lamina propria (LP). The abnormal fat globules (F) nevertheless stained darkly. Note staining of the fine precipitate within the intercellular space and the lamina propria. Magnification, × 10,800.

FIGURE 4 Intercellular space (I) and portions of bases of two adjacent absorptive cells in a normal fasting patient, stained and processed identically to Fig. 3. Numerous lipoprotein particles (arrows) were stained darkly and were evident within the intercellular space (I) and in the lamina propria (LP). Nucleus (N), basal lamina (BL). Magnification, × 10,800.

such a patient was therefore used as a control to test the specificity of the Kushida-Fujita tissue block stain, i.e., to determine whether this stain produced artifacts, morphologically indistinguishable from lipoprotein particles.

Ultracentrifugation techniques. Lipoprotein particles were isolated from homogenates of proximal jejunal biopsies from normal fasting volunteers on six different occasions. Two of these volunteers were examined once and two were examined twice. All volunteers had no evidence of liver disease or malabsorption by history and physical examination. All volunteers had normal plasma triglycerides, cholesterol, and lipoprotein electrophoresis. 15–25 fasting biopsies (total wet weight 100–200 mg) were taken during each of the six examinations. They were ground in 2.0 ml of mannitol-EDTA-phosphate buffer (19) at 4°C in a motor-driven (400 rpm) all-glass homogenizer, using six up-and-down strokes. The homogenate was spun in an 873 rotor[2] at 3000 rpm for 10 min at 4°C. The sediment was resuspended in 1.5 ml of buffer (19) homogenized with three up-and-down strokes, and then centrifuged. The combined supernates were spun at 4°C in an 873 rotor at 15,000 rpm for 15 min (345,000 *g*-min); the sedimented mitochondria (20) were resuspended in buffer and were centrifuged at 345,000 *g*-min. 4 ml of the combined supernates, which had been raised to a density of 1.052 g/ml with NaCl, were overlayered with a NaCl density gradient (21) and were spun 2×10^8 *g*-min in an SW 41 Ti rotor[3] which concentrates $S_f > 20$ plasma lipoproteins in the upper 0.5 ml of the tube (21). After centrifugation the upper 0.5 ml was removed with the aid of a tube-slicer and portions were taken for electron microscopy.

Shadow casting procedure. Samples of VLDL and/or chylomicron particles, isolated from jejunal mucosal homogenates, were fixed in 10 volumes of 1% osmium tetroxide in 0.05 M Sorensen phosphate buffer (pH 7.4) for 30 min at room temperature. A small drop of this suspension was placed on a parlodion-coated grid and allowed to stand for 1–2 min. The grid was then gently washed with several drops of Millipore-filtered distilled water and air-dried. Grids were shadowed at an angle of approximately 15 de-

[2] International Equipment Co, Needham Heights, Mass.

[3] Beckman Instruments, Inc, Palo Alto, Calif.

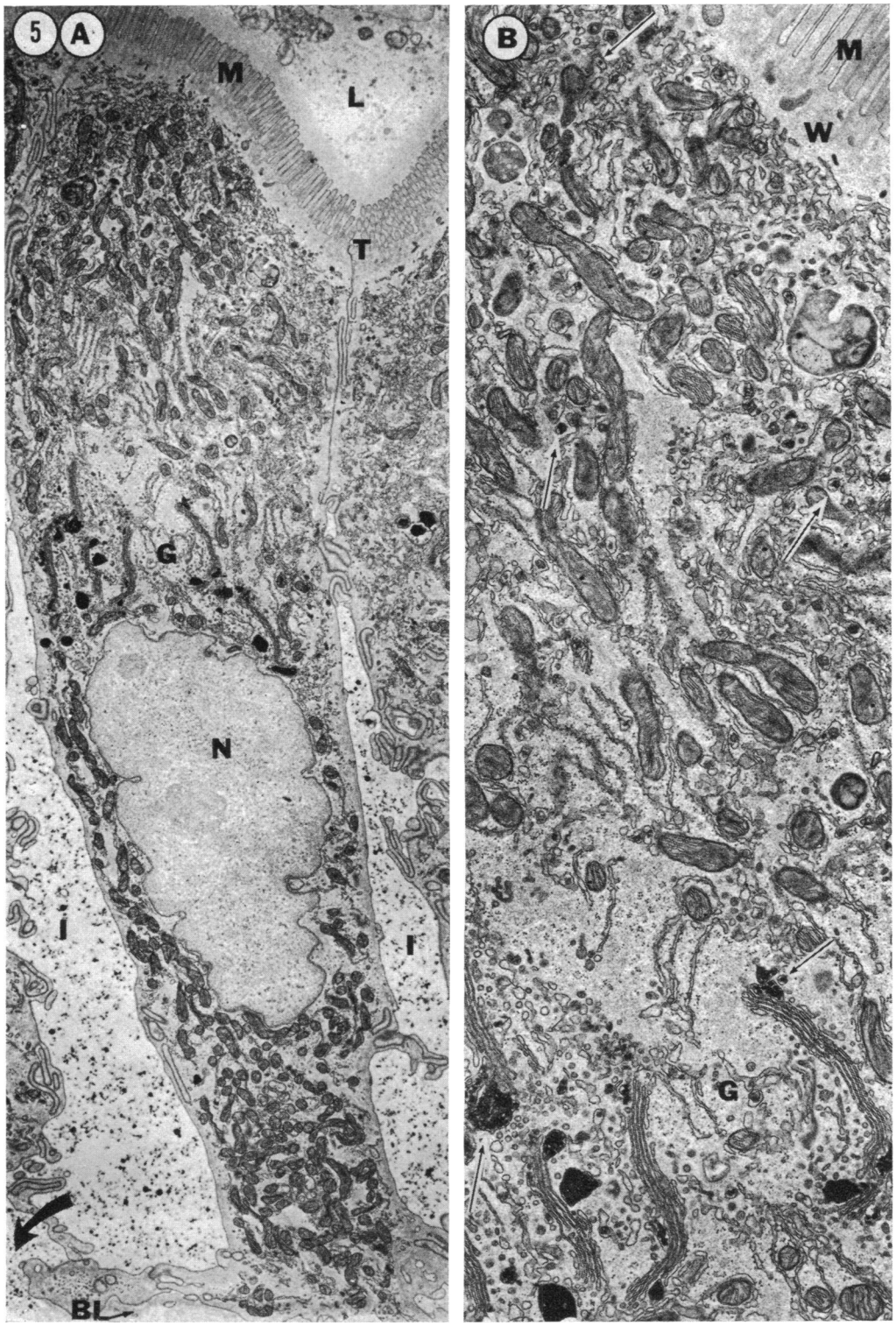


FIGURE 5 Absorptive cell at the extreme tip of a jejunal villus. A, The triangular intercellular spaces (I) on each side of the absorptive cell were filled with lipoprotein particles, some of which extended through a gap (black curved arrow) in the basal lamina (BL) into the lamina propria. The Golgi zone (G) above the nucleus (N) contained several black

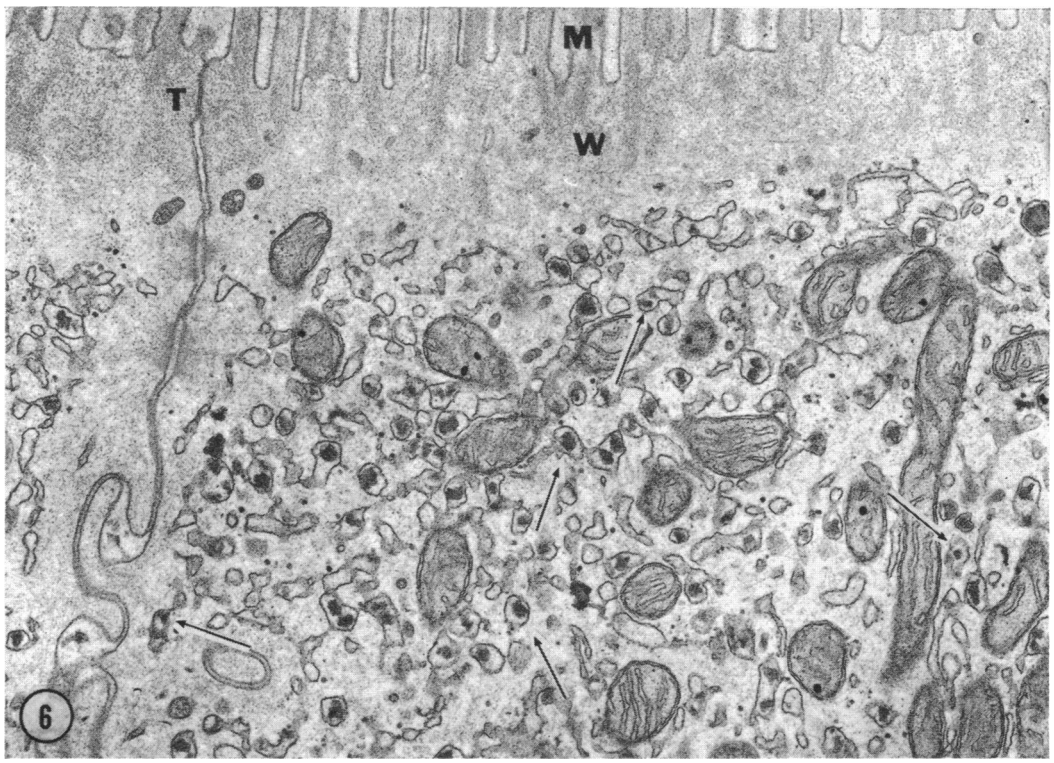


FIGURE 6 Apical cytoplasm subjacent to the terminal web (W) in a fasting absorptive cell near the tip of the villus. Note the absence of lipoprotein particles from the terminal web area (W). An unusually large number of lipoprotein particles (arrows) were clearly visible within profiles of the SER. Magnification, × 27,500.

grees by evaporation of chromium or carbon-platinum pellets[4] as described by Jones and Price (22). Particle sizes were measured on photographic negatives (× 40,500) or on corresponding prints (total magnification × 110,000) with an optical microcomparitor.[5] The maximum diameter of each particle was measured in the axis at right angles to its shadow (23). The diameters of 600 randomly chosen particles were measured after shadow casting of particles separated from pooled biopsy homogenates taken from six different fasting volunteers. Measurements were confined to individual particles with well-defined edges, nondeformed contours, and crisp shadows. Occasional excessively flattened particles were easily excluded because their shadows were greatly shortened. We also excluded small structures buried in the metal-shadowed background which might have been completely flattened particles. Clumps of particles were also excluded.

There were at least two sources of error which increased measured particle size: some flattening of the particles was always present after drying because particle height calculated from shadow length was less than the measured diameter rather than identical to it. If the particle had remained perfectly spherical, calculated height should have equaled measured diameter. No attempts were made to calculate the true particle diameters from the measured diameters because this would have involved unjustified assumptions regarding the constancy of three-dimensional geometry after flattening. Some minor size enlargement also resulted from the addition of osmium molecules during fixation (±5% size increase) (24).

Changes in intestinal lipoprotein particles after a fatty meal. The effect of a fatty meal on the size and on the composition of intestinal lipoproteins was defined in an experiment with a normal volunteer. Intestinal lipoprotein particles were isolated in an identical manner both in the fasting and in the postprandial state. To accentuate differences between fasting and postprandial intestinal lipoprotein particles, we sought to minimize large (> 1000 A) particles in fasting biopsies by feeding our subject a low (9 g) fat diet for 2 days before the overnight fast. After 47 fasting biopsies (wet weight, 379 mg) were taken from the subject's proximal jejunum, a liquid meal of 100 ml of water, two whole raw eggs, and 30 ml of linseed oil[6] was

[4] Ladd Research Industries, Inc, Burlington, Vt.

[5] Bausch and Lomb.

[6] Hain Pure Food Co, Los Angeles, Calif.

particles, the details of which can be studied at higher magnification in 5B. Lumen (L), microvilli (M), tight junction (T). Magnification, × 5670. B, Enlargement of supranuclear portion of the absorptive cell illustrated in Fig. 5A. Note lipoprotein particles (arrows) within SER, i.e. beneath the terminal web (W), further down within the apical cytoplasm and accumulated within Golgi profiles (G). Magnification, × 14,850.

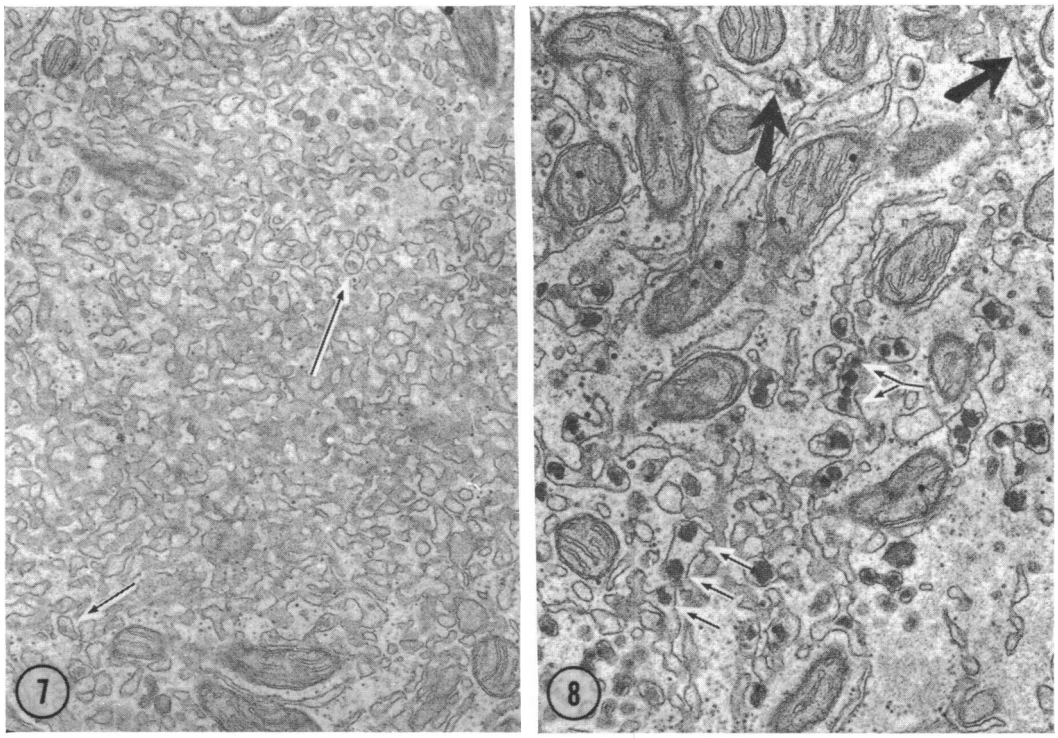


FIGURE 7 Fortuitous section revealing the interconnecting nature of the SER tubules within the apical cytoplasm of a jejunal absorptive cell. Occasional lipoprotein particles (arrows) were seen within SER profiles. Magnification, × 27,500.

FIGURE 8 Lipoprotein particles in a row (small arrows) within SER of apical cytoplasm of a jejunal absorptive cell. Some particles were also evident within profiles made up of both smooth and rough ER (two larger dark arrows, upper third of illustration). Magnification, × 27,500.

then infused intraduodenally over a 20 min period. The fatty acid composition (moles/100 moles) of this meal was: palmitic-16, stearic-6, oleic-33, linoleic-17, and linolenic-26. An additional 23 mucosal biopsies (wet weight, 184 mg) were then taken from the subject's proximal jejunum over the next 20 min.

Lipid particles were isolated in an identical manner from homogenates of both fasting and postprandial jejunal biopsies. The 345,000 *g*-min supernate from each set of biopsies was made up to 5 ml with buffer (19) of density 1.003, and was spun at 23°C for 3.08×10^6 *g*-min in a Beckman SW 65 Ti rotor. Portions of the upper 0.5 ml containing the isolated intestinal lipid particles were taken to measure their size by shadow casting, and their lipid composition by chemical analysis (25). 4 ml of each infranate were then spun under conditions designed to isolate $S_f > 20$ plasma lipoprotein particles (21).

Illustrations. All illustrations are of portions of cells located near or at the tips of jejunal villi in biopsies taken from fasting normal volunteers, with the exception of Fig. 3 which was taken from a fasting patient with aβ-lipoproteinemia, and Figs. 17A–C which were taken from a volunteer after a fatty meal. All biopsies were fixed in bicarbonate-buffered osmium tetroxide, stained with the Kushida-Fujita tissue block stain and then stained with Reynold's lead–citrate section stain, with the exception of Fig. 1 which was only "double"-stained with uranyl acetate and Reynold's lead citrate after sectioning. Figs. 15A–C, 16C, and 17C were shadow-casted particles extracted from jejunal biopsies.

90% of intestinal lipoprotein particles fall into a range of sizes whose upper and lower limits differ by a factor of five; thus there is a considerable variation in the sizes of lipoprotein particles in the following illustrations.

RESULTS

Electron microscopic technique for visualizing lipoprotein particles. In fasting intestinal biopsies an attempt was made to increase the electron density of lipoprotein particles by increasing their osmiophilia with a diet of unsaturated fat. There was no obvious improvement in electron microscopic visualization of fasting lipoprotein particles even though a 2 wk linseed oil diet (80 g linseed oil/day as sole fat source) had increased the unsaturation of total lipids extracted from fasting jejunal biopsies (the content of linolenic acid increased from 0 to 4.0 moles/100 moles fatty acids in the extracted lipids). Very low fat diets (4 g/day for 2 and 4 wk, two volunteers) or pure carbohydrate diets (36 hr, two volunteers) also had no consistent effect

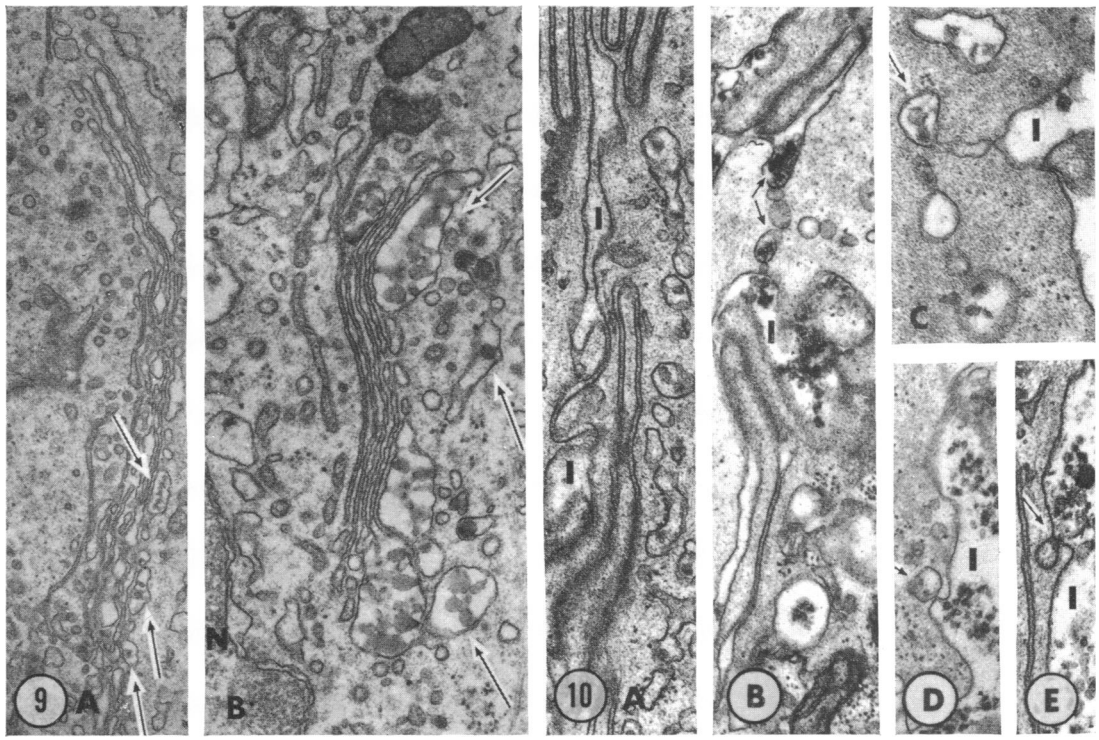


FIGURE 9 Lipoprotein particles (arrows) were seen within Golgi profiles of jejunal absorptive cells. The whole spectrum of size and density of lipoprotein particles was illustrated in Figs. 9A and B; A, small particles singly or in rows within flattened sacs; B, particles of varying sizes and densities both singly and in clusters within Golgi vacuoles. Magnification, × 27,500.

FIGURE 10 Examples of structural configurations near the lateral cell membrane, suggesting exit of lipoprotein particles (arrows) by reverse micropinocytosis into the intercellular space (I); A, row of vesicles and tubules extending along lateral cell membrane, often containing lipoprotein particles; B, row of vesicles containing lipoprotein particles (arrows) connecting above and below with lateral cell membrane and appearing to empty into intercellular space (I); C–E, vesicles suggesting various phases of exit (arrows) of lipoprotein particles into the intercellular spaces (I) by reverse micropinocytosis. Magnification, × 27,500.

on the number or electron density of visible lipoprotein particles in fasting biopsies, although residual chylomicrons were practically eliminated by this maneuver.

With the usual "double"-stained sections, lipoprotein particles could be visualized only with difficulty in extracellular areas of normal-fasting intestinal biopsies. Unquestionable visualization of lipoprotein particles within intestinal absorptive cells was almost impossible because the particles were so poorly stained (Fig. 1). After Kushida-Fujita staining of tissue blocks followed by Reynold's lead staining of sections, visualization of lipoprotein particles improved remarkably (Fig. 2) although the electron density of these particles still varied (Figs. 4, 9, 11, 12). Adequate studies of the morphologic details of lipoprotein particle synthesis and transport have only been possible since this staining method was adopted.

Using the Kushida-Fujita plus Reynold's stains, no lipoprotein particles were visualized in a fasting biopsy from a patient with aβ-lipoproteinemia. However, the large abnormal fat globules within the fasting absorptive cells of this patient were clearly stained (Fig. 3). On the other hand, lipoprotein particles were regularly found in identically-stained fasting jejunal biopsies from normal-fasting volunteers (Fig. 4).

Pathway of lipoprotein particles from absorptive cell to lacteal. Lipoprotein particles were clearly and consistently visualized by electron microscopy within fasting intestinal-absorptive cells and in the intercellular spaces between the cells covering the tips of proximal jejunal villi of volunteers (Fig. 5). Occasional lipoprotein particles were evident within profiles of the smooth endoplasmic reticulum (SER) of the apical halves of the absorptive cells (Figs. 5, 6, 8). More numerous collections of particles were often visualized within dilated portions of the Golgi cisternae (Figs. 5, 9B).

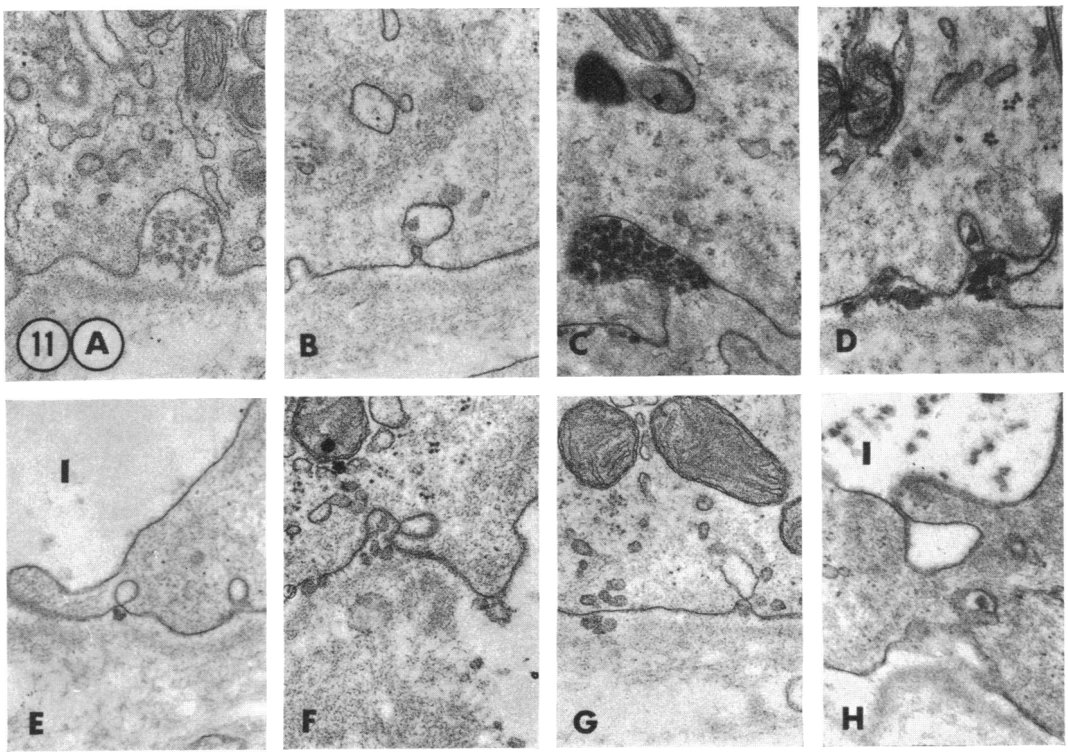


FIGURE 11 Examples of structural configurations near the basal cell membrane suggesting exit of lipoprotein particles by reverse micropinocytosis. In all illustrations the cytoplasm of the absorptive cell is above and the lamina propria below; separating the two is the basal lamina. A and C, numerous lipoprotein particles tightly packed within a vesicle whose opening faces the basal lamina; C, D, F, lipoprotein particles between the basal cell membrane and the basal lamina; A–H, configurations suggesting various phases of exit of lipoprotein particles from the cell. Magnification, × 27,500.

Large numbers of lipoprotein particles were also evident within some of the intercellular spaces between the bases of absorptive cells (Figs. 4, 5, 12) and within the lamina propria of the villus tip (Figs. 4, 12, 13A). A few lipoprotein particles were regularly seen within the lumina of lacteals of the villus trips (Fig. 13A).

Lipoprotein particles were never seen within the interior of microvilli; nor were they seen with certainty within vesicles of the terminal web area. Some lipoprotein particles were seen within profiles of the SER subjacent to the terminal web (Figs. 5, 6). The interconnecting nature of the SER network of tubules in absorptive cells was occasionally revealed in fortuitous sections (Fig. 7). The SER was especially abundant in the supranuclear half of the absorptive cell and lipoprotein particles were scattered within it (Figs. 5–8). At times several lipoprotein particles in a row were evident within tubular profiles of the SER (Fig. 8, small arrows). Continuity between the smooth and rough endoplasmic reticulum was readily demonstrated, and occasionally lipoprotein particles were located within such combined profiles (Fig. 8, large arrows, upper third of the illustration).

The largest intracellular collections of lipoprotein particles were always located within the Golgi cisternae (Figs. 5, 9). If the lipoprotein particles could be found in no other intracellular location, some were detected within profiles of the Golgi complex. Lipoprotein particles of varying density and size were seen singly within small Golgi vesicles, in clusters within larger vacuoles, or in rows within one of the flattened sacs (Fig. 9A, B).

Lipoprotein particles were seen less frequently below the nucleus. Large lipid aggregates in the basal portions of some of the fasting absorptive cells were easily distinguishable from the lipoprotein particles because of their large size (1–3 μ) and lack of a membranous envelope.

Roughly triangular intercellular spaces were located between the lower portions of adjacent absorptive cells and were characteristically seen at the extreme tips of the villi of fasting patients; they served as a landmark

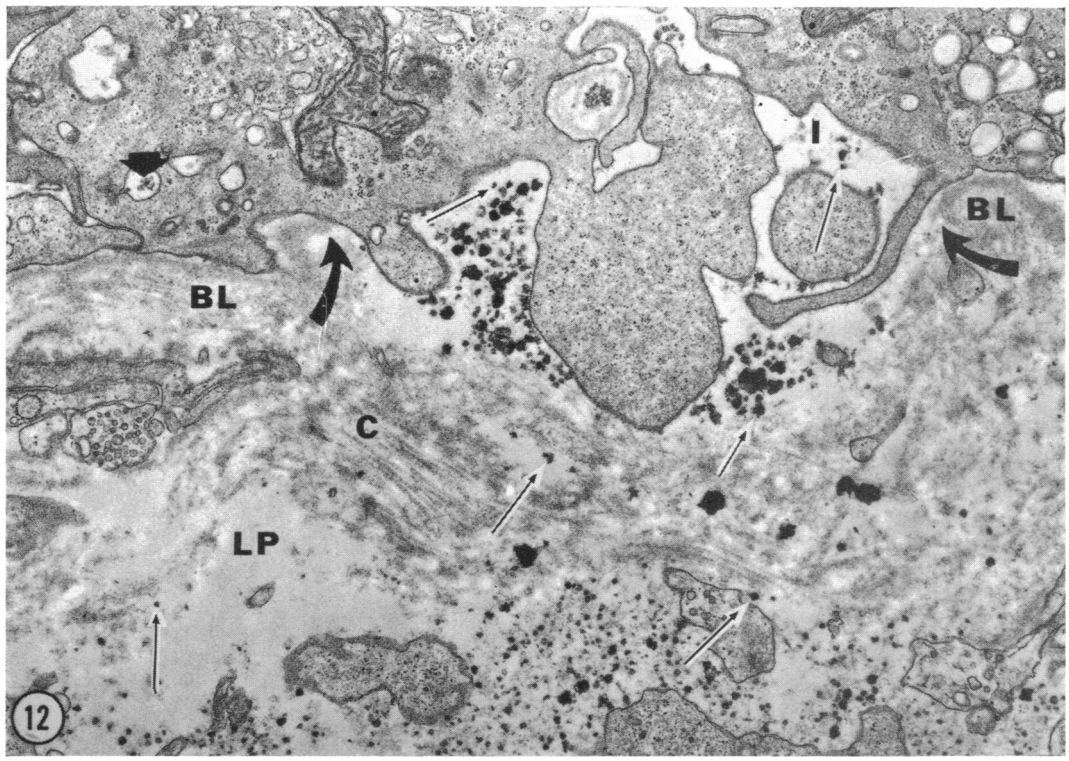


FIGURE 12 Intercellular space (I) and basal portions of two adjacent absorptive cells. Lipoprotein particles (small arrows) were seen within the intercellular space (I), within a gap in the basal lamina (BL-between large curved arrows) and in the lamina propria (LP). A cell process also extended from the absorptive cell on the left through the gap in the basal lamina (BL). Intracellular lipoprotein particles (short large arrow) were less dense than extracellular lipoprotein particles (small arrows). Collagen fibers (C) were seen in the lamina propria (LP). Magnification, × 10,800.

which assured the observer that the plane of section passed through the tip of the villus (Fig. 5A). It is within these spaces that the lipoprotein particles were most obvious (Figs. 4, 5A, 10B, D, E, 12). It was here that they were first discovered before the advent of adequate staining procedures (8). With the Kushida-Fujita tissue block stain it became possible to accumulate some evidence regarding the possible movement of lipoprotein particles between the absorptive cells and the intercellular spaces, and beyond them into the lamina propria.

Lipoprotein particles were observed within vesicles and vacuoles near the lateral and basal borders of the absorptive cells. Connections between these vacuoles and the SER tubules were occasionally seen. Furthermore, it was not uncommon to see a row of vesicles extending towards the lateral cell membrane (Fig. 10A, B). Less commonly the last of this row of vesicles connected with the lateral cell membrane and appeared to open into the intercellular space (Fig. 10B). Lipoprotein particles were seen anywhere within the intracellular vesicles and occasionally within a vesicle which appeared to connect with the intercellular space (Fig. 10B–E).

The basal border of the absorptive cell where it abutted on the basal lamina was an easier place to observe particles within vesicles opening out of the cell (Fig. 11A–H). Rows of lipoprotein particles often were seen between the basal plasma membrane and the basal lamina (Fig. 11C, D, F); furthermore, large numbers of particles packed within a single vesicle, whose opening faced the basal lamina, were only seen in this location (Fig. 11A, C).

The basal lamina hugged the undulations of the basal portions of the absorptive cells throughout most of the villus. Gaps in the basal lamina did occur and were often occupied by cell processes or wandering cells such as lymphocytes, which extended from the lamina propria into the intercellular space. Groups of lipoprotein particles were also seen extending from the intercellular spaces through gaps in the basal lamina into the extracellular spaces of the lamina propria (Figs. 12, 5A).

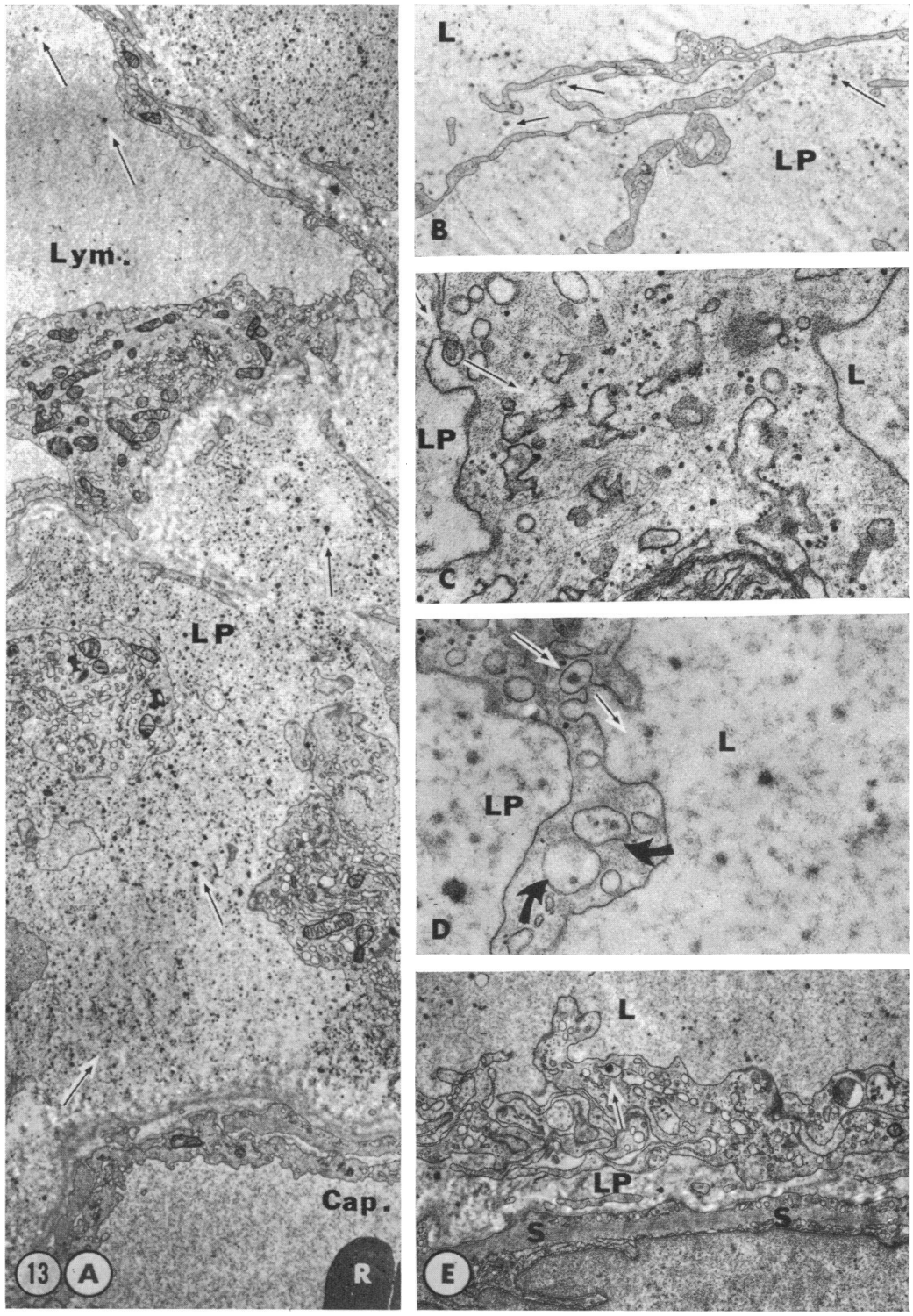


FIGURE 13 Structural configurations suggesting entry of lipoprotein particles into lacteals; A, survey picture of lamina propria (LP) showed numerous lipoprotein particles (arrows) in the extracellular spaces of the lamina propria, occasional particles in the lymphatic lumen (Lym.) and none within the capillary lumen (Cap.) which contained red cells (R), mag-

The lipoprotein particles which were seen throughout the lamina propria of the villus tip were mostly extracellular in location but some were evident within the cytoplasm of macrophages. Lipoprotein particles were not seen within the lumina of villus capillaries (Fig. 13A). Lipoprotein particles were evident within the lumina of lacteals but they were usually less numerous than those in the surrounding lamina propria (Fig. 13A).

The walls of the lacteals were generally thinner than those of the villus capillaries. Unlike the capillary walls, they lacked fenestra and had only rudimentary wisps of basal lamina. Closed, overlapping endothelial processes maintained the separation between the lacteal lumen and the extracellular space of the lamina propria. At times these endothelial processes were separated and a row of lipoprotein particles could be seen extending from the lamina propria into the lacteal lumen (Fig. 13B). The interdigitations between adjacent lacteal endothelial processes were often so complex (Fig. 13E) that it was hard to tell whether lipoprotein particles were actually within the endothelial cytoplasm or merely within an extracellular indentation whose plane of section was such that it appeared to be intracellular (Fig. 13D, large arrows). After extensive search, we were convinced that some lipoprotein particles were located within intracellular endothelial vesicles which opened either to the lamina propria (Fig. 13C) or to the lacteal lumen (Fig. 13D). Furthermore, lipoprotein particles were occasionally observed within a row of vesicles within the lacteal endothelium (Fig. 13E).

Shadow casting of lipoprotein particles. Lipid particles of density < 1.006 were isolated from six jejunal mucosal homogenates taken from four fasting normal volunteers. The structure of the particles obtained from these homogenates was investigated by electron microscopy using chromium or carbon platinum shadow casting. These were found to be spherical structures ranging from 180 to 1700 A in diameter. The great majority of these particles were in the size range between 150 and 650 A (Figs. 14, 15) which is similar to the size range of plasma lipoprotein particles (VLDL) (Fig. 15B) in man (21, 22, 26–31). Very few of the intestinal lipoprotein particles had diameters greater than 750 A. Most of the shadow-casted particles were distributed singly (Figs. 15A, 16C) but rare clumps of very small particles of uniform diameter were seen (250–350 A) (Fig. 15C).

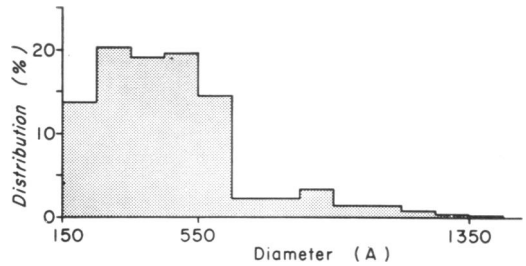


FIGURE 14 Size distribution of lipoprotein particles extracted from fasting human jejunal mucosal homogenates by preparative ultracentrifugation ($S_f > 20$) and measured after shadow casting. Distribution divided into 100 A increments.

After VLDL particles from human plasma were purified and characterized extensively,[7] they were shadow casted and found to be morphologically indistinguishable (Fig. 15B) from those which we had isolated from human intestinal biopsies.

Changes in intestinal lipoprotein particles after a fatty meal. After a low fat intake for 48 hr before intestinal intubation, lipid particles > 1000 A almost disappeared from fasting intestinal biopsies (Fig. 16A, B).

The 3.08×10^6 *g*-min top layer from the homogenate of fasting jejunal biopsies contained particles, 67% of whose diameters were in the 150–650 A range, and 89% were < 1000 A (Fig. 16C). The top layer of the 3.08×10^6 spin contained 134 nmoles of lipid, of which 73.2% was triglyceride, 4.5% was free cholesterol, and 22.5% was phospholipid. The 3.08×10^6 *g*-min infranate was subjected to further centrifugation under conditions designed to concentrate $S_f > 20$ plasma lipoproteins

[7] Human plasma VLDL were generously provided by Dr. William Hazzard from a fasting subject with mild endogenous (type IV) hyperlipoproteinemia (32): after removing $S_f > 400$ particles (7.95×10^6 *g*-min in a Beckman SW 41 Ti rotor), the infranatant material was spun for 18 hr at 45,000 rpm in a SW 50 rotor; the resulting sample of S_f 20–400 lipoproteins was then subjected to preparative starch block electrophoresis (33), and the purified VLDL was eluted from the α_2-lipoprotein zone. These VLDL were of normal triglyceride-rich composition (TG/cholesterol 4.2) (34).

nification, × 5250; B, lipoprotein particles (arrows) extended from lamina propria (LP) through open overlapping endothelial processes into the lacteal lumen (L), magnification, × 5250; C, thick portion of lacteal endothelial wall showed configuration suggesting entry (arrows) of lipoprotein particles from lamina propria (LP) into endothelial cytoplasm by micropinocytosis, magnification, × 27,500; D, thinner portion of lacteal endothelial wall showed configuration suggesting exit (arrows) of lipoprotein particle from endothelial cytoplasm into lacteal lumen (L) by reverse micropinocytosis; note other vacuoles with lipoprotein particles which might, or might not have been intracellular (heavy arrows), magnification, × 27,500; E, complex interdigitating lacteal wall containing row of cytoplasmic vesicles, the last of which contained a lipoprotein particle (arrow), suggesting possible particle movement through the cell cytoplasm. Smooth muscle (S). Magnification, × 13,500.

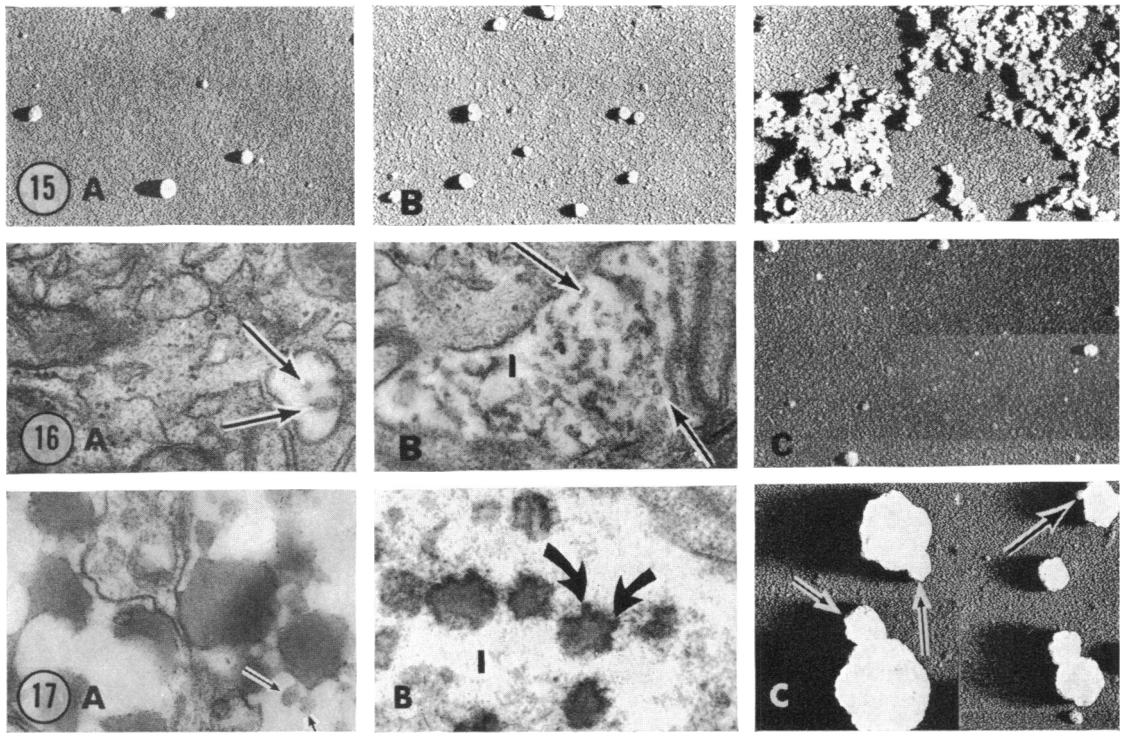


FIGURE 15 Shadow-casted lipoprotein particles like those measured in Fig. 14; A, shows representative single shadow-casted particles whose sizes were measured; B, shows human plasma VLDL (S_f 20–400) which were of the same size distribution as intestinal lipoprotein particles; and C, shows clumps of shadow casted particles of approximately 250–350 A diameter which could not be accurately measured. Magnification, × 40,500.

FIGURE 16 Intestinal lipoprotein particles in the fasting state (A–C). A, Example of lipoprotein particles (arrows) which were visualized within intracellular vesicles of this patient's fasting biopsies; B, accumulation of lipoprotein particles (arrows) within the intercellular space (I) of a fasting biopsy; C, lipoprotein particles extracted from fasting biopsies taken at the same time and in the same patient as biopsies A and B. Magnification, × 40,500.

FIGURE 17 Chylomicron plus lipoprotein particles after a fatty meal (A–C). A, chylomicrons and lipoprotein particles within the Golgi zone after the same patient as in Fig. 16 was given a fatty meal. Note satellite lipoprotein particles (arrows) around a chylomicron; B, chylomicrons within the intercellular space (I), note lipoprotein particles (arrows) apparently adherent to a chylomicron; C, chylomicrons and lipoprotein particles extracted from biopsy taken from the same patient at the same time as those illustrated in A and B, note small chylomicrons (small arrows) and lipoprotein particle (large arrow) adhering to the periphery of larger chylomicrons. Magnification, × 40,500.

into a top layer (21). This top layer contained particles, 91% of whose diameters were in the 150–650 A range; their lipid composition was not completely characterized because only 20 nmoles of lipid were present in this top layer (triglyceride–13 nmoles, phospholipid–7 nmoles, free cholesterol–undetectable).

Biopsies taken after the administration of intraduodenal fat contained chylomicrons as well as smaller particles in the VLDL range (Fig. 17A, B). The 3.08 × 10^6 *g*-min top layer from the homogenate of postprandial biopsies also contained chylomicrons of different sizes (48% > 1000 A) as well as particles in the 150–650 A VLDL range (38%) (Fig. 17C). The percentage composition of this chylomicron-rich top layer was (moles/100 moles): triglyceride-97.0, free cholesterol-0.6 and phospholipid-2.4. The 3.08 × 10^6 *g*-min infranate was subjected to further centrifugation under conditions designed to concentrate $S_f > 20$ plasma lipoproteins into a top layer (21) which was again found to contain particles predominantly in the 150–650 A range.

An interesting configuration was seen both in tissue sections (Fig. 17A, B, arrows) and shadow-casted particles (Fig. 17C, arrows). Lipoprotein particles and small chylomicrons could be seen adherent to the periphery of some of the chylomicrons in satellite clusters.

DISCUSSION

This study provides evidence regarding the site of lipoprotein particle synthesis within the human intestinal absorptive cell. It also provides new details regarding the transport of lipoprotein particles from the absorptive cells through the lamina propria into the lacteals. When these particles were separated from jejunal biopsies by preparative ultracentrifugation, they were proven to be very similar to plasma VLDL in size distribution.

In fasting patients, lipoprotein particles were seen within the smooth endoplasmic reticulum of the absorptive cell, but not within the lumen, microvilli, or terminal web area. Presumably lipoprotein particles were synthesized within fasting absorptive cells from absorbed fatty acids derived from hydrolysis of biliary lecithin (35, 36). The evidence which supports this belief is the almost complete disappearance of lipoprotein particles from fasting absorptive cells of rats (7) and men after biliary exclusion[8] and their absence from human jejunum after bile has been completely excluded by a short circuit operation for obesity.[9]

The morphological evidence suggests that lipoprotein particles follow the same pathway in the fasting state as do chylomicrons and lipoprotein particles after a fatty meal. Lipoprotein particles were evident within endoplasmic reticulum, some of which connected with Golgi cisternae. The Golgi membranes in turn connected with rows of vesicles of the endoplasmic reticulum; these vesicles extended towards, and opened through, the lateral and basal cell membranes. One cannot, however, rule out the possibility that these rows of vesicles were artifacts secondary to osmium fixation (37). Lipoprotein particles were seen at many points within these rows of vesicles; exit from the cell by reverse micropinocytosis was suggested by the occasional particle which seemed to be caught within a vesicle connecting with the extracellular space. Multiple particles within a single vesicle opening towards the basal lamina were occasionally seen, suggesting that these particles were leaving, rather than entering, the cell; i.e. random brownian movement should have favored particle movement from the more crowded vesicle into the less crowded lamina propria.

The number of lipoprotein particles was greatest in the intercellular spaces between the basal halves of the absorptive cells at the tip of the villus. Except for occasional gaps, the basal lamina seemed to serve as a temporary barrier between these intercellular spaces and the extracellular matrix of the lamina propria. The morphological appearance suggested that lipoprotein particles pass through such gaps from the intercellular spaces to the lamina propria. Numerous particles were also seen scattered throughout the lamina propria but none was evident within the capillary lumina. Particles within lacteal lumina were less numerous; lymph flow may have removed many of these particles shortly after they entered lacteals.

During absorption of a fatty meal, the size distribution of lipid particles extracted from jejunal biopsies changed from that of the smaller lipoprotein particles towards that of the larger chylomicrons. Nevertheless, small particles of lipoprotein size were still mixed in with the larger chylomicrons extracted from postprandial biopsies. These observations suggest that lipoprotein particles are synthesized continuously by human absorptive cells, both in the fasting state and after eating.

The main chemical difference which we could detect between lipid particles extracted from fasting jejunal biopsies and those extracted from postprandial biopsies was the higher percentage of triglyceride in the latter. Electron microscopy of postprandial jejunal biopsies, and of lipid particles extracted from them, often revealed satellite clusters of lipoprotein particles adhering to the periphery of larger chylomicrons. The morphological and chemical evidence is consistent with the hypothesis that lipoprotein particles expand and coalesce to form chylomicrons as triglyceride synthesis increases during fat absorption. In our view, there may be no essential difference between chylomicrons of various sizes and the smaller lipoprotein particles–chylomicrons may be lipoprotein particles whose triglyceride compartment has expanded.

[8] Porter, H. P., D. R. Saunders, G. Tytgat, O. Brunser, and C. E. Rubin. 1971. Fat absorption in bile fistula man—morphological and biochemical study. *Gastroenterology.* **60:** 1008.

[9] Personal communication, Stanley Shimoda.

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