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Electron microscopic study of the jejunum and lamina propria in fasting and fat fed white mice

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AN ELECTRON MICROSCOPIC STUDY OF THE JEJUNUM AND LAMINA PROPRIA IN FASTING AND FAT FED WHITE MICE

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INTRODUCTION

The introduction of the electron microscope into biological research heralded the onset of intensive investigation of the ultrastructure of the cell. Much of this investigational activity has been concerned with the morphology of the components of the gastrointestinal tract, especially the structure of the very specialized intestinal epithelial cell and cells within the lamina propria.

Renewed interest has developed in determining the mechanism involved in absorption of fats, carbohydrates and proteins. The mechanism involved in transfer of lipids from the intestinal lumen, through intestinal epithelial cells and thence into the general circulation has been the subject of great controversy over the last century. Essentially three schools of thought have developed: those who feel that absorption of fats is by biochemical breakdown and molecular transfer with later reconstitution; those who support the theory of particulate absorption of lipids from the intestinal lumen by a process similar to pinocytosis with little biochemical alteration of lipids other than emulsification; and finally, that group which believes that both biochemical alteration and mechanical absorption

can and does occur.

It is the purpose of this investigation to first review and present previous electron microscopic studies of the normal mouse jejunal epithelium and the underlying lamina propria; second, to present new electron microscopic studies of lipid absorption in the mouse jejunum with the goal of correlating these observations with other similar studies conducted with human volunteers and labroatory animals; and finally, to attempt to correlate and interrelate these findings with currently accepted biochemical concepts of lipid metabolism and absorption.

HISTORICAL REVIEW OF STUDIES ON LIPID ABSORPTION

In 1842, Goodsir published a paper in the Edinburgh New Philosophy Journal associating the presence of fat droplets in the intestinal epithelium with the concept of fat absorption (Wotton, '63). In the same year Gruby and Delafond presented a paper to the Academy of Sciences in Paris reporting their observations on the intestinal villi and on the absorption of ingested fat from the intestinal lumen (Palay and Karlin, '59$_b$). They described the intestinal epithelial cells of fat fed animals as packed with small particles and globules of fat. Their conception was that the coarsely emulsified fat in the intestinal lumen passed directly into the open epithelial cells where they were converted into a homogeneous and smooth emulsion of particles which were then transferred to the central lacteals.

Brücke in 1854 expressed the view that these cells possessed apertures on their surfaces which allowed passage of fat into the cell. This concept was disputed by Kölliker (1854) who maintained the integrity of the striated border of the intestinal epithelial cell. He suggested that the fat passes through small pores or channels which he thought were represented by

the striated border of the epithelial cells.

The conception of the absorption of fat in the emulsified form was disputed by Pflüger ('01) and Bloor ('16) in biochemical studies on fat absorption. They maintained that lipids were saponified and absorbed as water-soluble soaps and glycerol, resynthesized in the intestinal cells and then passed by way of the lymphatic vessels to the blood in the form of neutral fat chylomicrons. This theory was challenged by Verzár and McDougall ('36) who believed that bile acids were functional in producing water-soluble complexes with the insoluble fatty acids which were released by action of pancreatic lipase on the ingested triglycerides. They felt that saponification would be greatly limited by the presence of acid in the upper gastrointestinal tract. After entering the cell, the bile acid-fatty acid complex was thought to be broken with reconstitution of glycerol and fatty acids into neutral fats and with return of the bile salts to the intestinal lumen where they again formed complexes with more fatty acids.

Frazer ('54, '55 and '58) established that triglycerides are only partially hydrolyzed in the intestinal lumen, and that emulsification is an essential step in the absorption of lipids. He

suggested that emulsification and micelle formation in the intestinal lumen by action of bile salts may be the starting point of a particulate system which can be directly absorbed.

Others agree with the concept of micelle formation. As shown by Hofmann and Borgström ('62), monoglycerides and fatty acids liberated through pancreatic lypolysis have been shown to form mixed micelles in vitro similar to the "micellar phase" observed in normal human intestinal contents. Frazer ('54, '55 and '58) suggested that micelles may pass into epithelial cells by way of canals as described by Baker ('49 and '51).

Renewed interest in the theory of particulate absorption of emulsified lipids by epithelial cells again developed with the application of the electron microscope to cytological study. This theory was generally accepted in the nineteenth century, several investigators reporting the observation of fat droplets just beneath or within the striated border of epithelial cells (Köllicker, 1855; Donders, 1857). Krohl (1890) insisted however that fat must be absorbed in a soluble form. He based his statement on the fact that he never observed lipid droplets

within or beneath the striated border.

With the development of Pflüger's theory, emphasis was placed on biochemical modification and molecular absorption of fat (Bloor, '16 and Verzár and McDougall, '36). Although lipid droplets were occasionally observed in the striate border in light microscope studies (Wotton and Zwemer, '39' Baker, '51; Hewitt, '54 and '56; Wotton and Hairstone, '59 and Wotton, '63), most credence was given to the biochemical concept of triglyceride degradation, molecular diffusion into cells with reconstitution of di- and triglycerides within the cell.

The first electron microscopic study of fat absorption in the intestinal epithelium was conducted by Weiss ('55). He was unable to find any signs of particulate lipid absorption in the striated border or apical cell regions, and therefore concluded that lipids are absorbed in soluble form with reconstitution and concentration in the Golgi complex. Palay and Karlin ($59_{a,b}$) conducted a classical study in which they described the normal morphological appearance of the intestinal epithelial cell. A second study demonstrated the appearance of small lipid droplets in the striated border between microvilli and fat particles in what appear to be vesicles in the terminal

web of the cell. They also observed large granular and agranular membrances enclosing fat droplets lying in the cytoplasm. They concluded that fat droplets may enter the cell by passing through the intermicrovillous spaces of the striated border, in the depths of which they become enclosed within vacuoles apparently derived from the plasma membrance by pinocytosis.

Since 1959 numerous papers have been published on the subject of fat absorption. These will be considered when the results of this study are discussed.

MATERIALS AND METHODS

Much of the research on the ultrastructure of the intestinal epithelial cell and studies of fat absorption have employed either the mouse or the rat as an experimental animal. Because of the availability of mice, the ease of maintenance and low cost of upkeep, these animals were selected as the experimental animal.

A series of 30 adult white mice of both sexes weighing between 20 and 25 grams was used. The animals were fasted for 24 hours prior to feeding but were allowed free access to water. Five of these mice were selected at random to serve as controls. The remaining 25 mice were fed a test meal of Mazola oil (a polyunsaturated corn oil) stained previously with Sudan III acting as a tracer to enable gross observations for the presence of fat in the intestine after sacrifice. This solution was prepared by adding an excess amount of dye in powdered form to the oil, then filtering out all the dye remaining in suspension by passing the stained oil through filter paper.

The animals were anesthesized by placing them in a jar containing cotton saturated with diethyl ether. After they were unconscious, they were fed 1 to 2 ml. of stained corn oil administered with a glass pipette

gently introduced into the esophagus of the animal. The animals were then placed in a cage and allowed to recover from the anesthetic.

All animals were sacrificed after being fed a test meal at periods ranging from 20 minutes to 4 hours after feeding. They were rapidly reanesthesized using diethyl ether, then an H-shaped incision was made through the ventral abdominal wall. Flaps were developed exposing the abdominal contents. The stomach was isolated and the small intestine traced for 5 to 6 inches. Distal to this point 4 inches of bowel was removed and 5 to 6 specimens of bowel were taken at 3 cm. intervals and immediately immersed in a watch glass containing warm to room temperature 1% osmium tetroxide dissolved in phosphate buffer solution at a pH of 7.2 prepared according to the method of Millonig ('61). These specimens were immediately cut into 1 mm. pieces while still immersed using a new razor blade cleaned of its protective oil coating. The pieces of tissue were then immediately transferred to containers of 1% osmium tetroxide in buffered phosphate and allowed to fix for one hour with one change.

The fixed tissues were then washed for 2 minutes in buffered phosphate at a pH of 7.2 and transferred for dehydration to graded concentrations of ethyl

alcohol. Changes were made at 15 minute intervals until absolute alcohol was reached. After dehydrating for three one-half hour intervals in absolute alcohol, the tissues were prepared for embedding in methacrylate or Epon 812.

Preparation for metacrylate embedding consisted of the following procedures. Butyl and methyl methacrylate with inhibitor removed was mixed 15 parts butyl to 85 parts methyl with 2 gm. of LuperCo CDB added per 100 ml. of solution to act as a catalyst. The tissue was infiltrated with 2 changes of 50% absolute alcohol and 50% mixed methacrylate for one-half hour each. The tissue was then embedded in pure mixed methacrylate in 00 gelatin capsules and placed in a drying oven for 24 hours at 45°C. After this period the capsule was transferred to a 60°C. oven for 48 to 72 hours until hard.

Epon 812 was prepared according to the procedure outlined by Luft ('61). Solution A consisting of 62 ml. of Epon 812 mixed with 100 ml. of DDSA was combined with solution B consisting of 100 ml. Epon 812 mixed with 84 ml. NMA in a ratio of 7 parts solution A to 6 parts solution B. For each 10 ml. of the resultant mixture 0.15 ml. of DMP-30 was added to act as a

catalyst. The final solution was then mechanically mixed for at least one hour to assure complete blending of all constituents. In a departure from the procedure as outlined by Luft ('61), absolute acetone was substituted for propylene oxide because of the fat leaching properties of the latter chemical compound.

The tissue after removal from absolute alcohol was passed through three one-half hour changes of absolute acetone. The tissue was then immersed in a 50% absolute acetone-50% prepared Epon 812 mixture and allowed to infiltrate for a period of one hour. The tissue was then transferred to a second 50% absolute acetone-50% prepared Epon mixture and was allowed to stand uncapped for 15 to 18 hours allowing the acetone to evaporate, the Epon mixture slowly replacing the acetone within the tissue. The tissue was then transferred to 00 gelatin capsules and embedded in pure prepared Epon mixture. Capsules were placed in a 36°C. oven for 24 hours, a 45°C. oven for 24 hours and then to a 60°C. oven to remain until hard.

All capsules were trimmed and blocked and sections were cut in the silver to light gold range, approximately 60 to 90 mμ on a Sorvall Porter-Blum microtome with glass knives (Latta and Hartman, '50). Methacrylate sections were mounted on 200 mesh RCA copper

or nickel grids with a supporting membrane of formvar. These grids were examined unstained on the Phillips EM 100-B electron microscope.

Tissue embedded in Epon 812 was also sectioned at 60 to 90 mμ and were mounted on RCA copper or nickel 200 mesh grids. They were post-stained for one hour in a saturated solution of uranyl acetate dissolved in 95% ethyl alcohol, washed, dried and viewed on the Phillips EM 100-B electron microscope.

OBSERVATIONS

Normal Jejunal Epithelium

The columnar epithelial cells which comprise the lining of the intestinal lumen have flat bases applied to a thin homogeneous basement membrane (Figures 10, 18 and 19). The lateral surface of the epithelial cells exhibit multiple infoldings and invaginations which apparently interlock the cells together. These may be parallel to the long axis of the cells or perpendicular to the axis (Figures 4, 6, 7 and 9). The most intricate infoldings are noted at about the level of the nucleus (Figures 6, 7 and 9) and one may occasionally note pseudopodia-like projections interlocking and invaginating the cells (Figure 9).

The plasma membranes of adjacent cells are noted to run parallel to each other at a distance of about 95 to 120 Å (Palay and Karlin, '59$_a$), but at junctions between cells one may note widening of the intercellular space to a much wider and variable distance (Figure 8).

The terminal bar is a characteristic formation found normally just beneath the apex of adjacent cells (Figures 2 and 5). At this point the plasma membranes are more widely separated and appear thicker and denser.

Tonofibrils, numerous tiny filaments, may be noted curving away from the terminal bar into the cytoplasmic matrix. On rare occasion, terminal bars (or desmosomes) may be also noted at the base of epithelial cells (Figure 10). The terminal bar has been described as an intercellular bridge and as a formation probably encircling the cell (Palay and Karlin, '59$_a$), but this has not been definitely established. It is likely, however, that they, in combination with plasma membrane interdigitations and invaginations play some part in positioning and stabilization of adjacent cells. Sjöstrand ('63) has classified the terminal bar as a Type II attachment zone.

Above the terminal bar just below the microvilli at the cellular apex one may find an area of increased plasma membrane density associated with increased density of the terminal web substance (Figure 2). This structure is characteristically similar to the Type I attachment zone described by Sjöstrand ('63). In this high resolution electron microscope study, Sjöstrand described this structure as being composed of five layers of fused plasma membrane from both cells.

The apical surface of the intestinal epithelial cell is provided with an extensive elaboration of the surface membrane known as microvilli. They are smooth

surfaced, regularly spaced cylindrical projections of the apical surface of the cell with rounded terminations (Figures 1, 2, 11 and 12). Those at the tip of a villus are longer and more densely packed together, whereas as one proceeds down a villus into the crypts, the microvilli become shorter and more loosely arranged (Figure 12). The microvilli are arranged in a hexagonal manner, each unit equidistant from six other microvilli surrounding it. This may be demonstrated by obtaining sections through the microvilli in a plane perpendicular to their long axis (Figure 3). It becomes very obvious that the free surface area of the epithelial cell is greatly increased. This has been calculated by Palay and Karlin ('59_a) as an increase in surface by a factor of 14 over a smooth surfaced epithelial cell.

The plasma membrane encompassing the microvilli appears as a pair of thin lines separated by a finer light line (Figure 2). The plasma membrane has been described as being composed of three layers by Sjöstrand ('63). He describes the plasma membrane as a triple layered asymmetric structure all around the epithelial cell, thicker in the brush border region than at other parts of the cell. He states the plasma membrane is morphologically and structurally different in the brush

border region than elsewhere on the cell surface.

Occasionally one may note that the plasma membrane dips somewhat deeper into the underlying cytoplasm at bases of some intermicrovillous spaces, forming short tubules (Figures 1, 2 and 12). One may also see small vesicles at the tips of these short tubules which may be evidence of pinocytosis (Palay and Karlin, '59$_a$, $_b$; Oberti, '61).

The terminal web region is seen just below the microvilli as a fibrillar area of cytoplasm (Figures 1, 2, 11 and 12). It is characteristically devoid of the organelles and other structures typically found in other areas of cellular cytoplasm. A dense fibrillar meshwork is found in the core of the microvilli. It is usually oriented parallel to the long axis of the microvilli, but also may be found oriented in other planes as well. This fibrillar meshwork passes into the apical cytoplasm of the cell where it merges with the dense fibrillar meshwork of the terminal web proper just below the microvilli (Figures 1 and 2). On occasion, the fibrillar network in the microvillous core may appear to be separate and distinct from that of the terminal web (Figure 12). The reason for this variation is not clearly understood. Puchtler and Leblond ('58) have shown by histochemical means that

the terminal web is comprised primarily of protein with little or no lipids or carbohydrates present.

Just beneath the terminal web area in the apical cytoplasm is an area of many cytoplasmic organelles (Figures 1, 11 and 12). Mitochondria are most numerous in this area but may also be found in the perinuclear and basal part of the cell in lesser concentrations. The majority of mitochondria are filamentous, many branching or rod-like, but many may be spherical in shape. They tend to be longitudinally oriented to the axis of the cell, especially in the apical regions (Figures 1, 11 and 12), but may be more loosely arranged in the basal portion of the epithelial cell. Within mitochondria, dense small osmophilic bodies may be seen. Little is known of their origin or possible function. They have no apparent relationship with absorptive function of the cell.

Occasional other osmophilic bodies, probably secretory granules may be seen in the cytoplasm (Figure 1). Their origin, function and composition is unknown.

The endoplasmic reticulum is a system of intracytoplasmic tubular membranes. It is comprised of two distinct types: the agranular endoplasmic reticulum composed of a smooth tubular membrane system, and the

granular reticulum, a tubular membrane system encrusted with ribonucleoprotein particles (Figure 8). The granular reticulum is apparently most concentrated in the apical, peripheral and perinuclear regions. Numerous vesicles are also noted, some with and some without any encrusting layer of ribosomes. These may be due to a cross-sectional view of the endoplasmic reticulum in many cases.

Numerous ribosomes are noted throughout the cytoplasm unassociated with any membrane system. These tend to be concentrated in the apical and perinuclear regions, arranged in groups or in close association with mitochondria and the granular endoplasmic reticulum.

The Golgi complex is located in the immediate supranuclear region. In the normal fasting animal, the Golgi complex is composed of one or more groups of four to six agranular cisternae arranged in concentric layers. Many vesicles or dilated cisternae are usually present and may be empty or occasionally filled with a lightly osmophilic homogeneous substance. More rarely, a dense, moderately dark osmophilic homogeneous substance may be present. Contrary to studies by Palay and Karlin, ('59$_a$, $_b$) who reported the dilated cisternae usually contain lipid droplets even in fasted

animals, many micrographs were obtained in this study demonstrating what are apparently empty dilated cisternae (Figure 5). In fat fed animals, the dilated cisternae were very numerous and usually contained a moderately dense substance closely resembling lipids (Figures 11 and 13).

The nucleus is located near the base of the cell (Figures 11 and 12). It is round or ovoid. The nuclear membrane is composed of two osmophilic layers between which is a lighter layer (Figure 7). Frequent nuclear pores may be noted interrupting the membrane (Figures 6, 7 and 13). Mitochondria may be seen in close association with the necleus on many occasions (Figures 7, 9 and 15). On occasion the nucleolus is also visualized (Figure 7). Chromatin material appears as light and dark granular areas.

The lamina propria is separated from the epithelium by a thin basement membrane which appears as a thin, continuous homogeneous, moderately electron opaque line (Figure 10) which appears on close examination to be composed of a condensation of very fine fibrils. A space between the epithelial cells and the basement membrane may be noted. This space appears to be composed of a lighter homogeneous ground substance.

Scattered between cells comprising the lamina

propria are numerous strands of collagen fibers recognized by their periodicity. Other areas are occupied by a relatively structureless ground substance (Figures 10, 17, 18, 19 and 20). The lamina propria is chiefly occupied by an assemblage of various cells. They are fibrocytes, plasma cells, eosinophils, macrophages, occasional lymphocytes and smooth muscle cells. Blood and lymphatic vessels as well as unmyelinated nerve fibers are also noted in great numbers in this region.

Cells comprising the lamina propria may be crowded together in what may be called an epithelioid arrangement (Deane, '64). The majority of the cells lie in close association with each other, often with membranes in close approximation without ground substance or fibers in between them. Occasional cell bridges between similar cells may be seen (Figure 18).

One of the most common cells is the fibrocyte. They appear as extremely attenuated structures generally in fairly close apposition to the epithelial basement membrane (Figures 10 and 18). In Figure 10 they are noted interposed between the basement membranes of the epithelium and that of a lacteal lying in the lamina propria along with many collagen fibers. They will be found most often in close association

with collagen fiber bundles. Their nuclei are very rarely seen. When observed it will appear to be flattened. On occasion, small rod-shaped mitochondria may be seen. Vesicles may often be observed in these cells, especially during passage of foreign material such as ingested fat through the lamina propria (Figure 18).

The most common round cell is the differentiated plasmocyte (Bessis, '61; Deane, '64). The nucleus is ovoid in appearance. They typically have an abundunce of endoplasmic reticulum, the cisternae of which are often distended with a medium density secretory substance (dePetris, '63; Deane, '64). A plasmocyte containing a dense Russel body may be seen in Figures 16 and 17. Deane ('64) describes plasmocytes as occuring in close approximation to each other forming rows or partial sleeves along lymphatic vessels. Their functional relationship to the lymphatics is unknown.

Eosinophils are also present in moderate numbers (Figure 16). They may be recognized by the presence of their characteristic granules in the cytoplasm and their irregular nucleus (Bessis and Thiery, '62). The cytoplasm contains a simple endoplasmic reticulum and a few vesicles.

The other common cell of the reticuloendothelial

system is the macrophage (Figures 16 and 17). These cells tend to be rounded but their outline appears irregular and somewhat ruffled (Deane, '64). The nucleus is irregular in shape, the chromatin appearing slightly less dense than that of the plasma cell previously described. In fat fed animals these cells are filled with ingested lipid droplets. The cells may be identified with certainty only when filled with ingested material. Those without ingested material have been classified by Deane ('64) as undifferentiated stem cells. In Figure 18 two apparently undifferentiated stem cells containing no ingested material or vacuoles are noted exhibiting an intracellular bridge.

The lymphatic vessels have been described by Palay and Karlin (59_a) as being lined by a flattened endothelium which appears more substantial and less compactly joined than those of blood vessels. The luminal surface of the endothelium is deeply fluted and is five to six times thicker than that of the blood capillary endothelium. Many vesicles were noted within the cytoplasm. A basement membrane was not always discernable and no fenestrations were noted in their study of the lacteal endothelium.

In this study, lacteals appeared to have

an irregular collapsible lumen (Figure 10). As described by Palay and Karlin ('59_a), the endothelium is thick, quite fluted in appearance and the endothelial cell cytoplasm contains numerous vesicles. In Figure 10 a basement membrane may be seen surrounding the lacteal. In contrast to Palay and Karlin's study, fenestrations through the endothelial lining may be noted. The presence of fenestrations or spaces between endothelial cells may leave a passage of varying dimensions through which interstitial fluid, lymph or lipids may pass. Palay and Karlin ('59_a) also reported occasional strands of smooth muscle which may be found at varying distances from the lacteal.

Capillaries may most easily be identified by the presence of erythrocytes within the lumen (Figure 20). The endothelial lining appears more attenuated and the surface is much less fluted in appearance than that of the lacteal (Figures 19 and 20). Multiple fenestrations in the endothelial lining may be noted (Figure 20). The capillary tends to retain a much rounder appearance and a basement membrane surrounding the capillary is almost always noted (Figures 19 and 20). The endothelial cytoplasm usually contains fewer vesicles than that of the lacteal endothelium.

Fat Absorption

In segments of small intestine taken from mice sacrificed from 20 minutes to 4 hours after being fed fat meals, the majority of lipid within epithelial cells was found to be in the segments removed from the jejunal region Only a few droplets were noted in tissue from duodenum or ileum, these primarily being found in transitional areas. It is assumed therefore, that the greatest lipid absorption occurs in the jejunum as determined by grossly quantitative methods.

Jejunal tissue removed from mice 20 to 40 minutes after feeding a fat meal revealed a few small droplets of lipid in the apices of epithelial cells. The greatest number of lipid droplets were found in jejunal tissue removed one to two hours after feeding, Numerous small and medium sized droplets were noted in the apices and middle regions of epithelial cells (Figure 12). In the lamina propria, many small lipid droplets were also noted within macrophages and in the intercellular spaces between the cells (Figures 16, 17, 18, 19 and 20).

Jejunal tissue removed three to four hours after feeding of the lipid meal showed a great decrease in the number of lipid droplets in the cell apices, but many large irregular lipid masses were noted deeper in the cell cytoplasm, especially in the supranuclear regions

(Figure 11). Observations of the lamina propria at three to four hours were little different than those obtained at one to two hours after sacrifice and fixation. Many small lipid droplets were noted scattered throughout the lamina propria, some within engorged macrophages, some in the intercellular spaces between cells, and many concentrated around lymphatic and capillary vessels.

Several reports have revealed large amounts of lipid uptake by cells in periods of 10 to 20 minutes after feeding (Thomas and O'Neal, '60; Parmentier, '62; Ashworth and Johnston, '63), but in these reports feeding was accomplished by means of transgastric intubation near the biopsy site. This procedure eliminates the emptying time required by the stomach as well as the time required for the fat meal to traverse the duodenum. Gastric emptying time may vary greatly, consequently when feeding through an esophageal tube of pipette, best results are obtained at one to two hours after feeding.

Fat droplets are occasionally observed in the intestinal lumen (Figure 12) but usually all the fat in the lumen has been washed away during the process of fixation and dehydration. Although reported by several investigators (Palay and Karlin, '59$_b$; Ashworth and

Johnston, '63; Thomas and O'Neal, '60; Jones et. al., '62), no evidence of particulate fat in between microvilli or at the bases of intermicrovillous spaces was noted in this study.

First good evidence of intracellular lipid is found in the apical cytoplasm just below the terminal web area. No lipid droplets are noted above or in the area of the terminal web. Figure 12 demonstrates what appears to be a lipid droplet just below the terminal web area. This droplet appears to have the same consistency as that of larger lipid droplets deeper in the apical cytoplasm. Close examination will demonstrate the presence of a very thin membrane surrounding fat droplets. Although not measured, this membrane system appears to be thinner than that of the plasmalemma or microvillous border. At least two canaliculi are visible in Figure 12, penetrating the region above and coming in contact with the terminal web. These canaliculi are composed of a limiting membrane which appears thinner than the limiting membrane of the microvilli even though they appear as a continuous structure. This has also been observed by Sjöstrand ('63).

Larger fat droplets are observed quite readily in the apical cytoplasm below the terminal web

(Figure 12). A closely adherent very thin membrane may be seen enclosing many of the droplets. Several mitochondria are observed in close proximity to the lipids (Figure 13) and an increase in granular reticulum and ribosomes is also noted.

Figure 12 demonstrates the apparent coalesence of a larger and a smaller intracellular fat droplet. A membrane system still appears to separate these lipid masses which are so closely adherent. Figure 11 demonstrates a jejunal epithelial cell in a mouse three hours after administration of 2 ml. of corn oil. The apical region of the cell appears clearer of fat. Deeper in the subapical and supranuclear regions of the epithelial cells, large irregularly shaped lipid masses are found, apparently without a surrounding membrane. Clusters of small droplets are observed in the region of the Golgi complex, many enclosed within cytoplasmic vacuoles.

Several membrane enclosed fat droplets are seen in close association with the nuclear membrane, one of which is in direct contact with the nuclear membrane (Figure 13). The Golgi complex is filled with droplets of a uniform density appearing somewhat lighter than that of the large lipid masses. In several cases two or more droplets are noted within one dilated cisterna.

Other droplets are contained within a system of membranes which are covered with granular particles of ribonucleoprotein. Figure 14 demonstrates other groups of fat droplets, some in packets scattered throughout the cytoplasm. It becomes obvious that membranes surrounding the smaller droplets are being resorbed or altered in some unknown fashion enabling coalesence of the smaller droplets with formation of larger irregular masses.

At a later stage, clusters of fat droplets divested of their enclosing membranes may be observed in the intercellular space between the plasma membranes of adjacent cells (Figure 15). They are always observed at or below the level of the nucleus (Figures 11 and 15). They are never observed in the intercellular space in the cellular apical regions, the Type I and II cell attachment zones as described by Sjöstrand ('63) apparently blocking passage of fat droplets from the intestinal lumen. Numerous intercellular spaces can be observed between cells in fasting animals which may provide a ready pathway for the passage of fat droplets to the epithelial basement membrane (Figure 8).

The mechanism by which fat droplets are discharged and divested of their surrounding membranes is not known

at present, but it has been postulated that they pass by way of the endoplasmic reticulum which may be in direct contiguity with the plasma membrane (Palade, '56) or by a process of extrusion with loss of the containing membrane as described by Bennet ('56).

It must be emphasized that fat droplets have not been seen in the process of passing through the plasma membrane in this or any other study to date and the mechanism is still theoretical. The droplets pass down between cells and traverse the basement membrane probably by a process of diffusion, as many droplets appearing above the basement membrane are identical in density and size to those found free in the lamina propria.

Once past the basement membrane, lipid droplets may be found free within the interstitial space of the lamina propria, and also intracellularly within macrophages and fibrocytes comprising part of the cellular components of the lamina propria (Figures 16, 17, 18, 19 and 20).

Those lipid droplets lying free in the interstitial space are free of membranes, their spherical shape apparently derived from the high surface tension characteristic of lipids. Many are noted in close approximation to each other, or in the process of

coalesence into larger spherical droplets with greater mass and less surface area (Figures 18, 19 and 20).

Figures 16, 17 and 18 demonstrate fat within cells comprising the lamina propria. The macrophage apparently has the greatest affinity for ingestion of the lipid droplets. Many droplets may be observed enclosed singly or in groups in membranes within the macrophage (Figure 17). Still other droplets appear to be without membranes and lie free in the cytoplasm. Many appear to be in a process of coalesence within the macrophage cytoplasm.

In Figure 18, fat droplets may be noted within vesicles of a fibrocyte. In two instances small droplets appear to be entering a fibrocyte by a process of pinocytosis. The role of fibrocytes in phagocytosis thus appears still to be determined.

Many fat droplets are seen clustered around capillaries (Figures 19 and 20). Although primary transportation of lipids is by way of lymphatic vessels, it appears feasible that a moderate amount of lipids may enter capillaries in particulate form by way of fenestrations present in the endothelial lining. The absence of lipid droplets within the capillary and lacteal vessels is probably due to washing away of lipids during fixation and dehydration. In Figures 19

and 20 a few lipid droplets may be seen within the capillary lumen. The presence of fenestrations within lacteal and endothelial cells suggests that lipids may enter the lacteal lumen by a similar mechanism.

DISCUSSION AND CONCLUSIONS

The first electron microscopic study of fat absorption in the intestinal tract of mice was published by Weiss ('55). In 1959, Palay and Karlin presented their classical paper on the pathway of fat absorption in the intestine of the rat. Since this time numerous papers have been published on fat absorption using mice, rats and human volunteers. In this discussion, it is possible to consider only a few of the many papers which have already been published on this subject. Therefore, we have attempted to select papers which represent the most generally accepted biochemical and particulate ingestion concepts of intestinal fat absorption.

The physico-chemical state of lipids during digestion and absorption has been the source of much controversy over the years. Much of the debate has concerned itself with the degree to which dietary triglycerides must be emulsified or hydrolyzed for adequate absorption. Recent biochemical studies indicate that extensive but not complete cleavage of fatty acids from triglycerides occurs in the intestinal lumen, the result of enzymatic action of pancreatic lipase resulting in production of free fatty acids, beta monoglycerides and some free glycerol (Mattson, et. al., '52).

Some coarse emulsification of fats occurs as the result of gastric motility and mixture with other diet constituents, but the action of bile and pancreatic lipase on this emulsion results in the production of very small fat particles known as micelles. The micelles are composed of a large amount of free fatty acids, monoglycerides and conjugated bile salts. They contain only small amounts of tri- or diglyceride or cholesterol. They are in the order of 40 to 200 Å. in diameter, much smaller than that of the coarse emulsion released from the stomach (Hoffman and Borgström, '62; Hoffman and Borgström, '63).

Conjugated bile salts are not apparently absorbed with the fatty acids and monoglycerides in the distal duodenum or jejunum, but are absorbed in the ileum and returned to the liver by way of the mesenteric blood. They are then resecreted into the duodenum in combination with the bile (Lack and Weiner, '61).

Although micelles, as well as emulsified di- and triglycerides may be absorbed by a process of pinocytosis in part, Isselbacker and Senior ('64) feel molecular uptake of fatty acids and the lower glycerides may be the main mechanism responsible for intake of the hundreds of grams of fat daily absorbed. They emphasize the production of fatty acyl Coenzyme A

which may be directly reformed into diglycerides or indirectly reformed into diglycerides by intermediate conversion into phosphatidic acid with subsequent resynthesis of diglycerides.

A biochemical and electron microscopic study of intestinal fatty acid absorption using C^{14} labeled oleic acid conducted by Ashworth and Johnston ('63) demonstrated the presence of fatty acid droplets the same size as the micelle between microvilli of intestinal epithelial cells. Radiochemical findings revealed significant transfer of labeled C^{14} from fatty acid to triglyceride at some stage during absorption. They established that some of the lipid droplets within epithelial cells were triglycerides, and that as fatty acids are taken into the cell, they are converted to di- and triglycerides presumably by the phosphatidic acid pathway previously discussed.

Ashworth and Johnston ('63) emphasized the role of pinocytosis in uptake of fatty acids and suggested that this process could contribute significantly and quantitatively to cellular ingestion of lipids. They estimated the number of pinocytotic vesicles necessary for absorption of large amounts of lipids, but questioned whether a sufficient quantity of vesicular membranes could be formed to account for the large

amounts of lipids which must be absorbed daily.

Oberti ('61) in a study on young suckling rats observed a great increase in the number of pinocytotic vesicles formed in the post-prandial period 20 minutes after feeding. He noted that all vesicles appeared to start from the intermicrovillous membrane. Baker ('42); Hewitt ('54) and Palay and Karlin ('59$_b$) have all stressed the uptake of lipid droplets by pinocytosis, and all have noted a great amount of pinocytotic activity, but Palay and Karlin ('59$_b$) were able to demonstrate only an occasional droplet within the terminal web area, not a sufficient number to account for the great amount of fat absorbed per day.

Phelps, et. al. ('64) states that the rarity of particles of fat within the brush border region could be due to cessation of pinocytosis at the moment of cell death because the brush border of the actively absorbing cell is the first area to contact the fixative. Another explanation is, the process of pinocytosis is so rapid and sections are so thin that only a few vesicles may be noted at any one time and therefore pinocytosis as a mechanism may still be quantitatively important.

Clark ('59) has demonstrated absorption of protein by a process which strongly suggests

pinocytosis of protein and colloidal material by intestinal absorptive cells in suckling rats and mice. The lack of absorption of tiny plastic spherules of 0.01 to 2 ,u demonstrated by Juhlin ('59) has been cited as evidence against absorption by pinocytosis or phagocytosis, but such a conclusion may not be justified, as plastics are very different in deformability from lipid droplets. In contrast, Sanders and Ashworth ('61) were able to demonstrate the presence of what appears to be intracellular latex particles by electron microscopy after administering the inert particles intraluminally.

In a similar study, this time involving the cells comprising the proximal convoluted tubules of the mouse kidney, Miller ('60) was able to demonstrate the passage of particulate hemoglobin in between the microvilli comprising the apex of the tubular cell, passage through tubular invaginations at the base of intermicrovillous spaces and transportation in bulk into vesicles in the intermediate cell zone. This study demonstrates that particulate absorption may occur in cells similar in function to those of the intestinal epithelium.

The function of the microvilli appear to be of great significance in increasing absorptive area of

the luminal portion of the epithelial cell. Palay and Karlin ('59$_b$) compared the microvilli to a filter, the intermicrovillous spaces functioning as a pore system. They reported seeing lipid droplets only in the intermicrovillous spaces. Weiss ('55) states he has observed lipid droplets within microvilli and Wotton and Hairstone ('59) consider microvilli in the cat functional in absorption of fat. Wotton ('63) suggests that the microvilli are not rigid structures but may expand and contract, shortening being described as arising from contraction of protein fibers comprising the core of the microvilli. He suggests that lengthening of the microvilli may be possibly due to action of isolecethin on the microvillous plasma membrane, or to increase in intracellular pressure distending the microvilli. In a personal communication, Wotton ('64) postulated that tiny pores in microvilli, too small to be observed by present electron microscope resolution may allow entrance of fat, and that expansion and contraction of microvilli may act as a "pumping mechanism" forcing lipids into the cell.

In this study, no lipid droplets were noted within intermicrovillous spaces or within microvilli, although the former has been reported on many occasions by

others. In addition, no evidence of lengthening and shortening of microvilli was noted. Intracellular fat droplets were first noted enclosed in membranous structures shortly below the terminal web area. Other electron micrographs demonstrate the development of tubular extensions of intermicrovillous spaces into the terminal web area and occasional vesicle formation.

In summary, there appears to be substantial evidence that pinocytosis occurs, and it is one of at least two functional mechanisms active in ingestion of lipids by epithelial cells. There is still no indication of the degree of importance pinocytosis plays in the absorption of lipids under normal conditions.

There is overwhelming evidence that emulsification and encymatic hydrolysis of fats into mono- and diglycerides and glycerol occurs within the intestinal lumen (Mattson, et. al., '62; Isselbacher and Senior, '64; Hoffman and Borgström, '62). It seems quite probable that molecular transfer does occur across the cell membrane. A study by Barka ('63) has demonstrated that after ingestion of corn oil by mice, acid phosphotase activity diminishes in cytoplasmic corpuscular granules situated beneath the microvilli and the granular pattern is altered. He

also noted that enzyme activity occasionally surrounded lipid droplets in the cytoplasm. These processes cannot be observed by ordinary electron microscopic studies, therefore, the importance of these possible mechanisms of transfer remain to be established.

When an animal is fed a large fat meal, a very abnormal and unnatural situation is established. It may be that under normal physiologic circumstances with only a moderate amount of the diet comprised of fat, absorption is primarily on the basis of intraluminal hydrolysis followed by molecular transfer across the epithelial cell membrane. However, when a massive fat meal is fed or when a large amount of lipid is introduced at a biopsy site, as has been the case in all previously conducted studies, a secondary mechanism of transfer such as pinocytosis is activated to handle the heavy concentration of lipids in the intestinal lumen. Thus fat may be transferred from an area of high concentration to an area of lower concentration within the cell.

Phelps, et. al. ('64) established the feeding of Ahrens test meal consisting of a mixture of corn oil, skimmed milk, dextrose and water near the biopsy site produced confusing results. They found it difficult

to distinguish structural changes associated with absorption of fat from those attributable to protein and carbohydrate and they could not readily identify fat. However, when straight corn oil or corn oil emulsified in water was administered, evidence of fat absorption was consistent. Other investigators have also been required to use large amounts of fat in a similar manner to demonstrate fat absorption in electron microscopic studies. Therefore, the mechanisms involved in normal fat absorption still remain to be demonstrated.

Weiss ('55) concluded that vacuoles within the apical cytoplasm are morphologically similar to the large supranuclear vacuoles (or dilated cisternae) of the main Golgi complex. He suggested that these similar smaller vacuoles located in the apical cytoplasm should also be considered Golgi vacuoles. It was his opinion that the Golgi complex may be the site of resynthesis and elaboration of complex lipids, after traversing the striated border in a dispersed, invisible form.

Palay and Karlin ('59_b) argued however that if fat is absorbed as described by Weiss, there should be electron microscopic evidence of large quantities

of soluble forms of fat in the apical cytoplasm of the cell with an increase in density of the apical region. Furthermore, as fat is reconverted into its previous form, particles of various sizes should occur. They reported seeing particles no smaller than 50 μ.

Coalesence of lipid droplets has been reported by many investigators (Palay and Karlin, '59$_b$; Phelps, et. al., '64; Thomas and O'Neal, '60). Phelps, et. al. ('64) were able to demonstrate a closely applied smooth membrane around the larger, more irregular lipid masses. In this study lipid droplets were noted in the apical region, the majority encased in agranular membranes. Some were contained in vesicles with a granular membrane, probably part of the granular endoplasmic reticulum. Coalesence of smaller into larger droplets was observed, however, the presence of membranes around the large irregular lipid masses, formed as a result of coalesence, was not demonstrated.

Lipid droplets were noted within the Golgi complex, but they appeared to be less dense than those droplets contained in reticular membranes in the same area. A light microscope study on human volunteers by Parmentier ('62) using an improved carbowax technique to preserve lipids, demonstrated high concentration of lipids in the Golgi complex 15 minutes after instilla-

tion of corn oil in the area of the biopsy. At this time it is as yet impossible to determine the nature of lipids present within the Golgi complex or within the cytoplasm, but it is hoped that advances in lipid chemistry will be extended to histochemisty so that accurate identification of lipids within cells may be accomplished.

At a later stage, lipid droplets are observed in the intercellular space at or below the nucleus. These droplets are found in the intercellular space divested of their surrounding membranes and may be scattered singly or in groups from the level of the nucleus to the basement membrane. The process by which these membranes are lost is at present still an open question.

Bennet ('56) suggests that liquids or solid particles may be extruded from a cell by a reversal of pinocytosis. The enclosing membrane surrounding a vacuolated particle within the cytoplasm may come in contact with, and subsequently become part of the plasma membrane with extrusion of the particle into the extracellular space. He considers all cell membranes to be in a dynamic state of constant flow.

Palade ('56) suggested that the endoplasmic reticulum is a continuation of the extracellular space and the membranes forming the endoplasmic reticulum

are functionally a plasma membrane. If this is considered carefully, one must assume that liquids or solids contained within the endoplasmic reticulum are actually within an extracellular phase although microscopically they appear intracellular. If this is true, then the transfer of lipids may be extracellular in its entirety.

Lipid droplets are never found above the terminal bar area, ruling out the possibility of passage from the lumen between the plasma membranes of adjacent cells. Almost all appear in the intracellular space at the level of the nucleus. The droplets migrate toward the cell base and accumulate at the epithelial cell basement membrane (Parmentier, '62) and apparently diffuse through the basement membrane into the lamina propria.

Within the lamina propria they may be noted as free droplets lying in between cells, within and close to collagen bundles and in close proximity to the basement membranes of lacteals and capillaries.

Other droplets will be found in the process of, or already ingested by macrophages and fibrocytes. Macrophages may be filled with numerous lipid droplets. Some are enclosed in membranous structures and others lie free without surrounding membranes within the

cytoplasm. Their affinity for lipid droplets may be explained on the basis of their affinity for foreign material introduced into the body.

Contrary to Palay and Karlin's study ('59$_b$) numerous fenestrations were found in peripheral lacteals of fasting mice. They are usually located close to the epithelial cell basement membrane. In contrast, capillaries are generally located deeper in the villus and rarely are found closely associated with the base of epithelial cells.

It is difficult to explain the apparent selectivity of triglycerides and long chain fatty acids for the lymphatic system and the affinity for capillaries shown by short chain fatty acids (Frazer, '56; Frazer '58). One possible explanation is the more rapid diffusion of short chain, low molecular weight fatty acids in comparison to that of the larger triglyceride and fatty acids. The low molecular weight fatty acids would exhibit greater plasticity and a lower surface tension, allowing them to penetrate deeper into the lamina propria than droplets composed of the heavier fatty acids and triglycerides. This would result in a concentration gradient of the shorter chain fatty acid droplets deep within the lamina propria in regions where capillaries are more

numerous. The heavier, slower moving droplets would be taken up by the more superficial lacteals. A second explanation is, that the basement membrane of the epithelial cells and interstitial matrix act as a differential filter restricting or enhancing the passage of lipid droplets of different composition through its substance.

Much remains to be investigated in delineating the pathways of fat absorption. New techniques must be developed in the fields of biochemical assay and histochemistry before we can adequately investigate lipid absorption and metabolism. With application of these new techniques to electron microscopy great strides toward a good understanding of fat absorption may be obtained.

SUMMARY

The structure of the specialized absorptive cell of the intestinal epithelium of the jejunum and the cells comprising the lamina propria of mice were studied in the fasting state and after the ingestion of corn oil introduced by an esophageal pipette. The animals were sacrificed at periods from 20 minutes to 4 hours after administration of the test meal.

For electron microscopy, tissues were fixed in phosphate buffered osmium tetroxide at room temperature and embedded in methacrylate or Epon 812. Sections from methacrylate were viewed unstained and sections from Epon embedded tissues were stained with uranyl acetate.

Close observations of the striated border of jejunal epithelial cells were made. Canaliculi and associated vesicles were noted to extend from the intermicrovillous spaces into the terminal web. The membranes comprising these structures appeared thinner than those membranes investing the microvilli. The Golgi complex was noted to consist of layers of granular membranes with large numbers of dilated cisternae, many of which appeared empty.

The lamina propria consists of numerous fibrocytes,

macrophages, eosinophils, plasmocytes and many indifferentiated cells. Many collagen fibers and areas of amorphous ground substance were noted. The lacteals were observed to generally have an endothelial basement membrane. The endothelial lining was found to exhibit many fenestrations and are similar to capillaries in this characteristic. The lumen is generally irregular in shape.

Lipid absorption in intestinal epithelial cells is found to be most active one to two hours after introduction of the fat into the esophagus and absorption appears to be greatest in the jejunum. No fat droplets were noted in the intermicrovillous spaces or in the terminal web. Fat was first noted in the region just below the terminal web, the droplets contained within membranous structures. Many small droplets enclosed in membranes were noted in the apex of the cell at an early stage of absorption. Later, large irregular lipid masses, apparently without membranes were observed deeper in the cellular cytoplasm and in the supranuclear regions.

After emulsification and partial hydrolysis with micelle production brought about by the action of pancreatic lipase and bile salts, lipids may enter the cell either by a process similar to pinocytosis

or by molecular diffusion. It is hard to evaluate the importance of pinocytosis as an absorptive mechanism as it is observed only on occasion, and does not by itself appear to be an adequate means of absorbing the large amounts of fat ingested every day.

The Golgi complex was found to be filled with numerous lipid droplets lying in dilated cisternae. The Golgi complex apparently plays some role in the physico-chemical state of intracellular lipids, but its exact function is at this time unclear.

At a later stage the lipid droplets appear in the intercellular spaces divested of their membranes. They are apparently extruded from the epithelial cells by a process of reverse pinocytosis in which the membranes are resorbed or become part of the plasma membrane.

These droplets progress to the base of epithelial cells where they traverse the basement membrane and enter the lamina propria. Many may be seen lying free in the interstital spaces where many are ingested by fibrocytes or macrophages. They may be seen clustered around lacteals and capillaries and occasionally a few droplets may be seen within a lacteal or capillary lumen, probably entering through fenestrations in the endothelial lining. The selec-

tivity of short chain fatty acids for capillaries may possibly be explained on the basis of greater plasticity and more rapid diffusion to the region deep in the lamina propria where capillaries are most numerous. The slower moving, less plastic triglyceride and long chain fatty acid droplets then enter the more superficial lacteals. The epithelial basement membrane and interstitial matrix may also function as a differential filter to movement of lipid droplets.

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APPENDIX

FIGURE I

Photomicrograph of apex of intestinal epithelial cell. Microvilli (mv) form the luminal surface of the cell. An invagination of the intermicrovillous space (a) may be observed passing into the terminal web (tw). Many mitochondria (m) can be seen concentrated in the apical region of the cell. Three secretory granules (sg) are present. Epon embedded.

Approximate Magnification 18,800X.

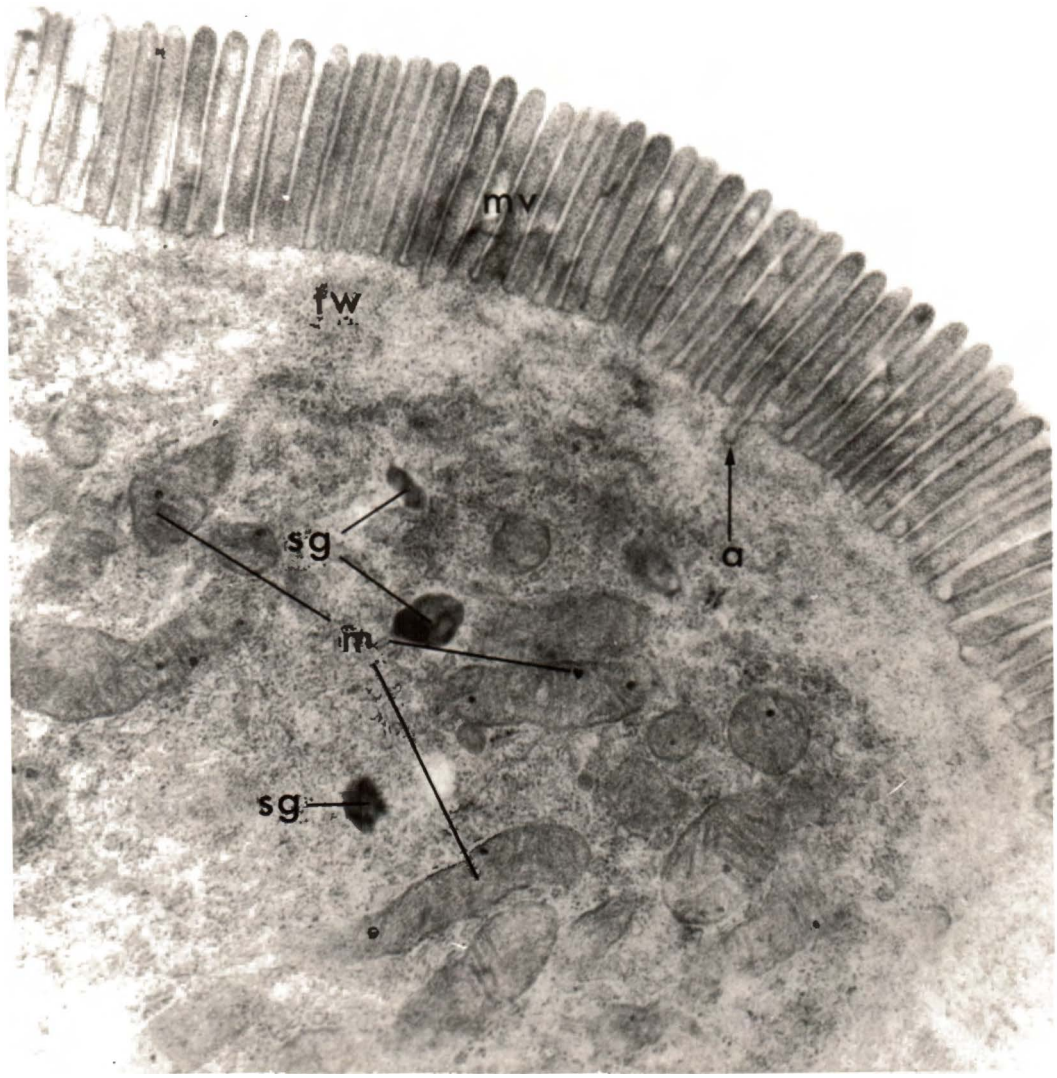

mv
fw
sg
a
m
sg

FIGURE 2

Photomicrograph of microvilli projecting into the intestinal lumen (L). The microvilli (mv) are bounded by a triple layered plasma membrane, two opaque layers with a lighter layer in between (arrows). An invagination (a) from the base of an intermicrovillous can be seen passing into the terminal web. A Type I attachment may be seen above the terminal bar (tb) and just below a microvillus. It appears to consist of fibrils associated with the terminal web. Epon embedded.

Approximate Magnification 33,100X.

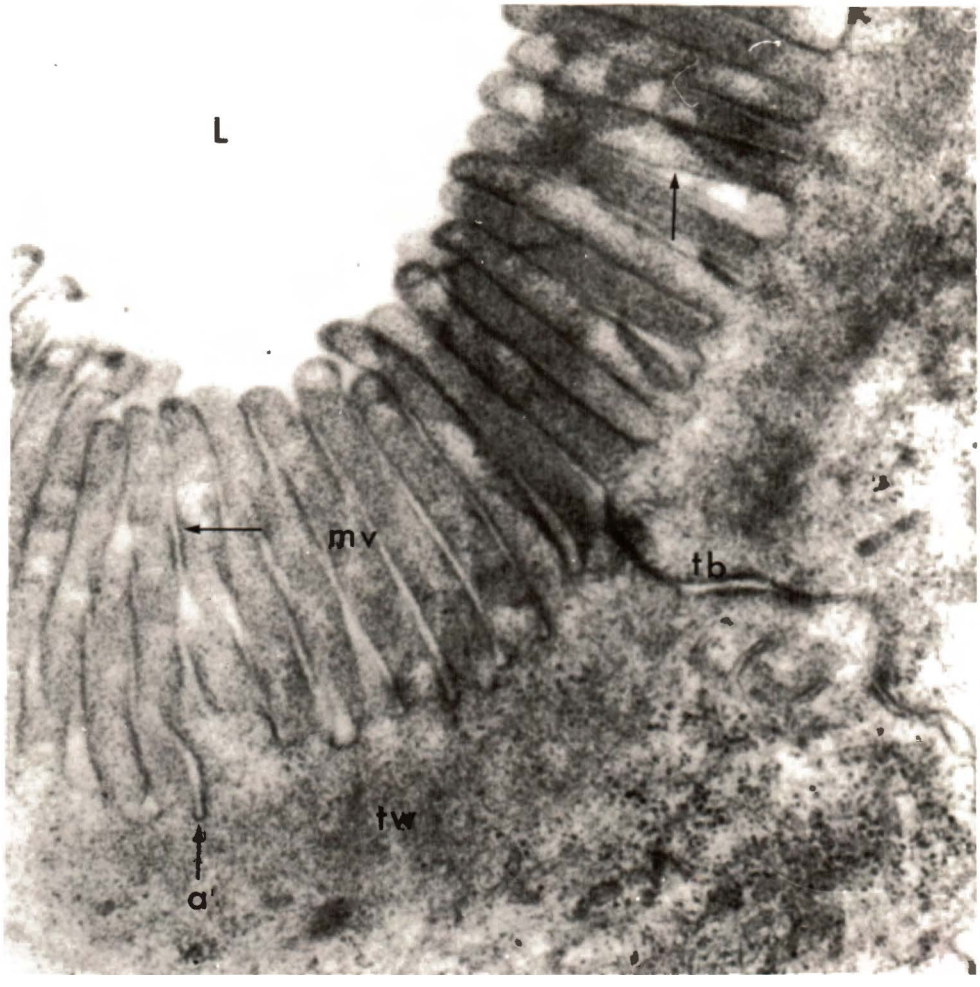

L
mv
tb
tw
a'

FIGURE 3

Photomicrograph of a cross section through microvilli. The hexagonal arrangement of the microville is quite apparent. Methacrylate embedded.

Approximate Magnification 62,000X.

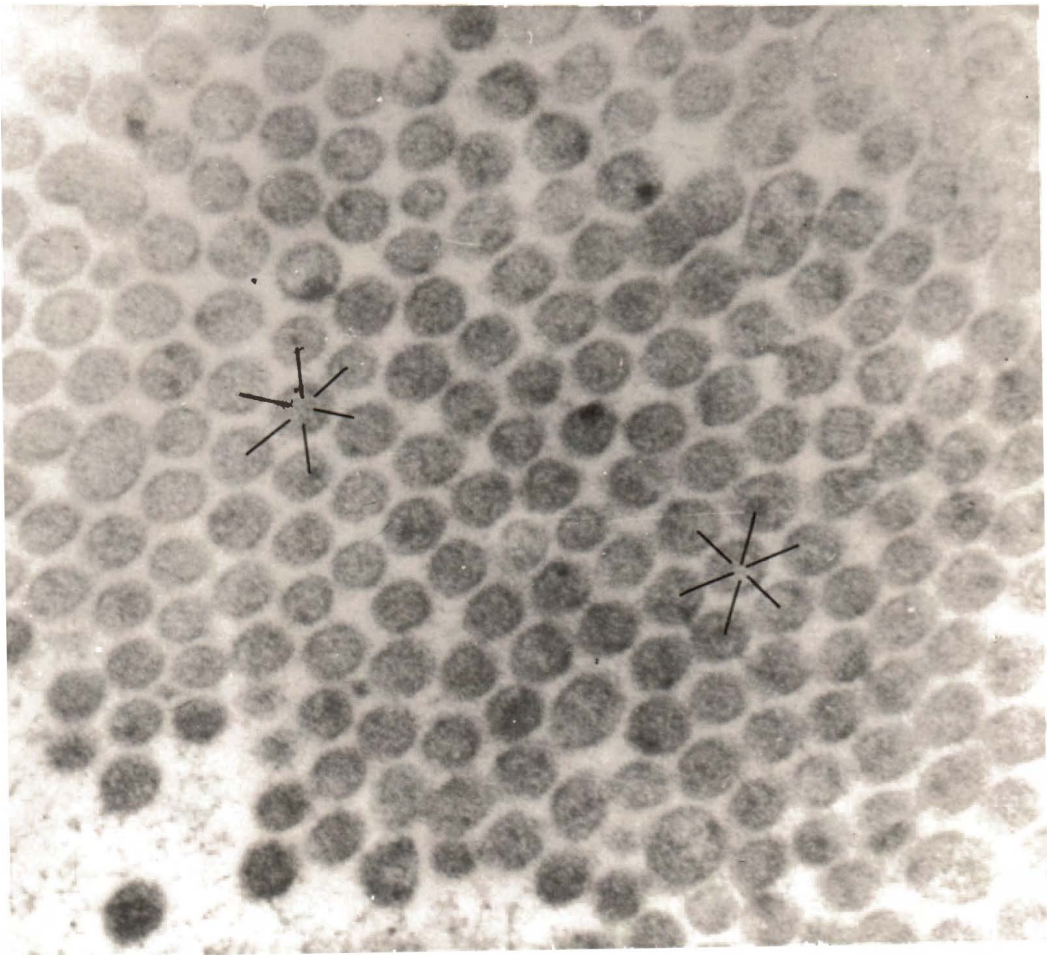


FIGURE 4

Photomicrograph demonstrating the infoldings of the plasma membranes (pm) of two adjacent epithelial cells. A nucleus (N) of one of the cells may be observed in the lower left corner. Many mitochondria (m) are found in the supranuclear region of the cell. Methacrylate embedded.

Approximate Magnification 18,400X.

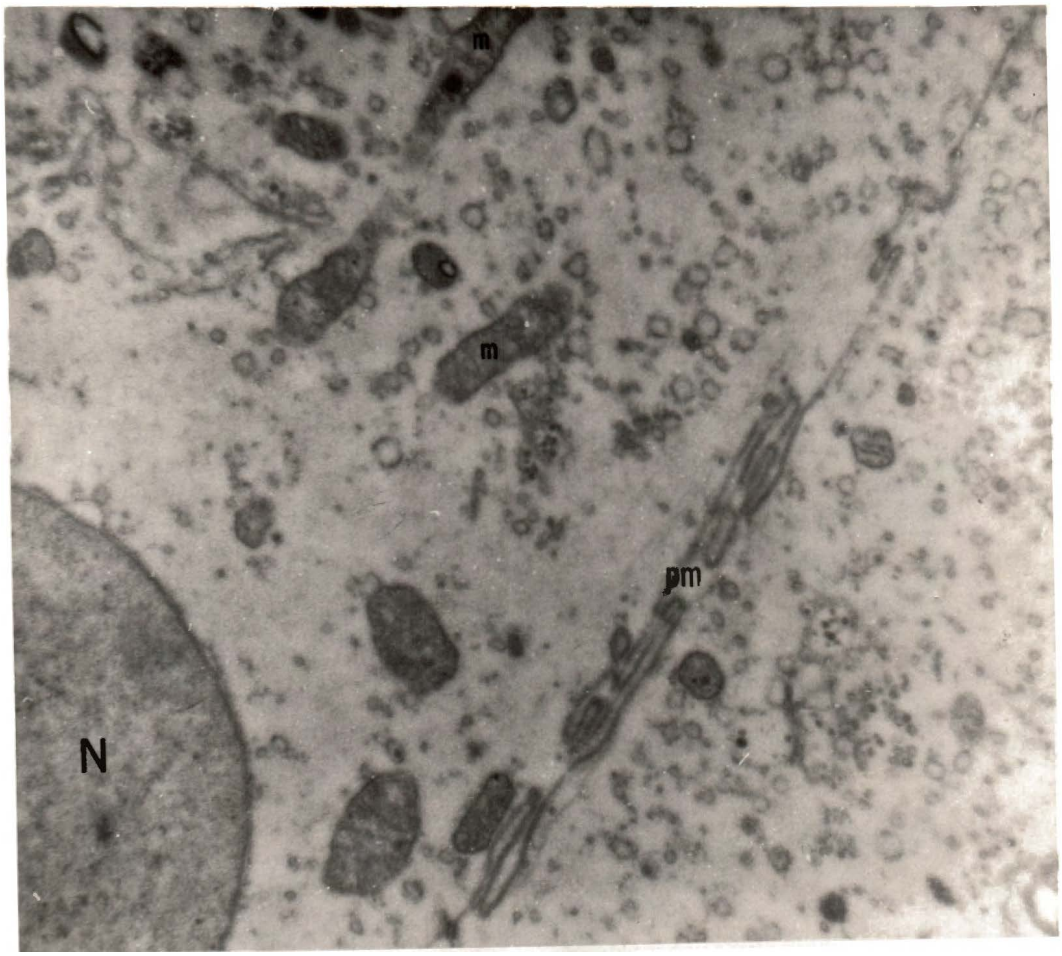

m
m
pm
N

FIGURE 5

Photomicrograph of two terminal bars (tb). Tonofibrils (tf) are seen extending into the cytoplasm. Three epithelial cells (EC) are observed in close apposition. Epon embedded.

Approximate Magnification 65,700X.

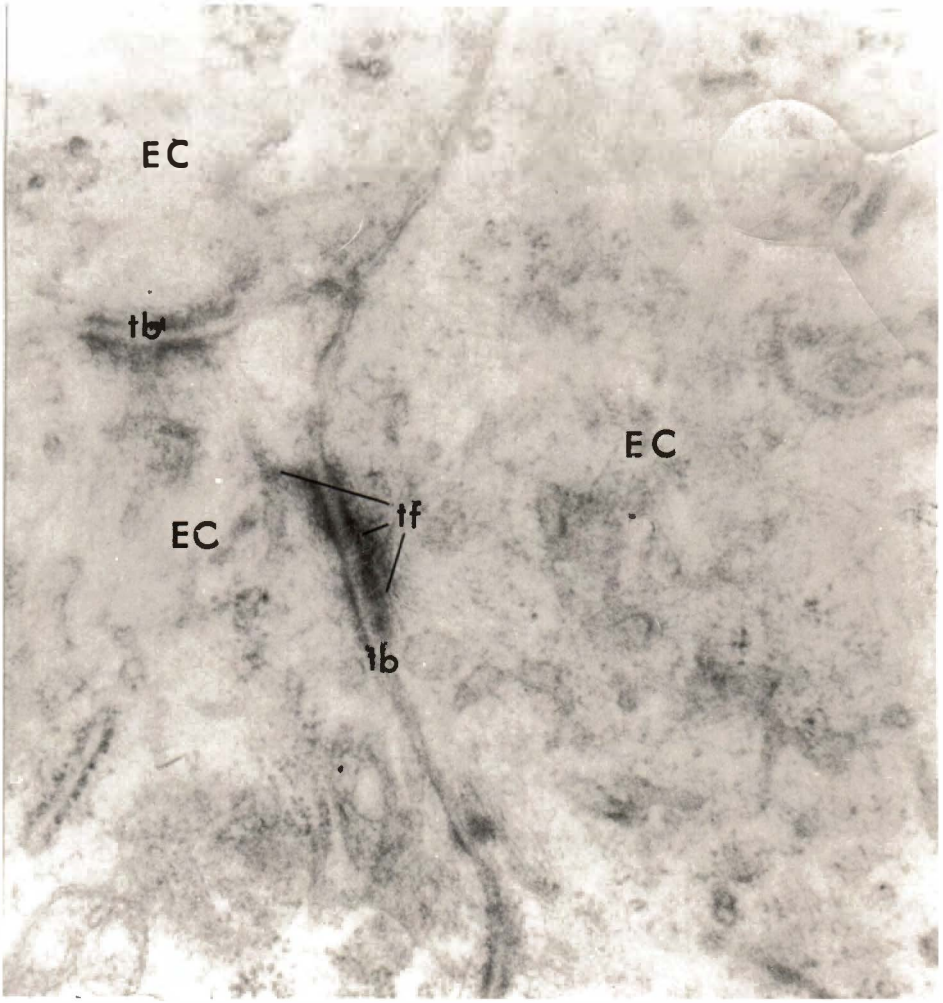

EC
tb
EC
EC
tf
tb

FIGURE 6

Photomicrograph of the supranuclear zone of an intestinal epithelial cell. Several interdigitating plasma membranes (pm) are shown. Nuclear pores (n) form openings in the bounding membranes of the nucleus (N). The Golgi complex consists of agranular membranes (c) arranged in layers with several dilated cisternae (v) evident. Epon embedded.

Approximate Magnification 20,500X.

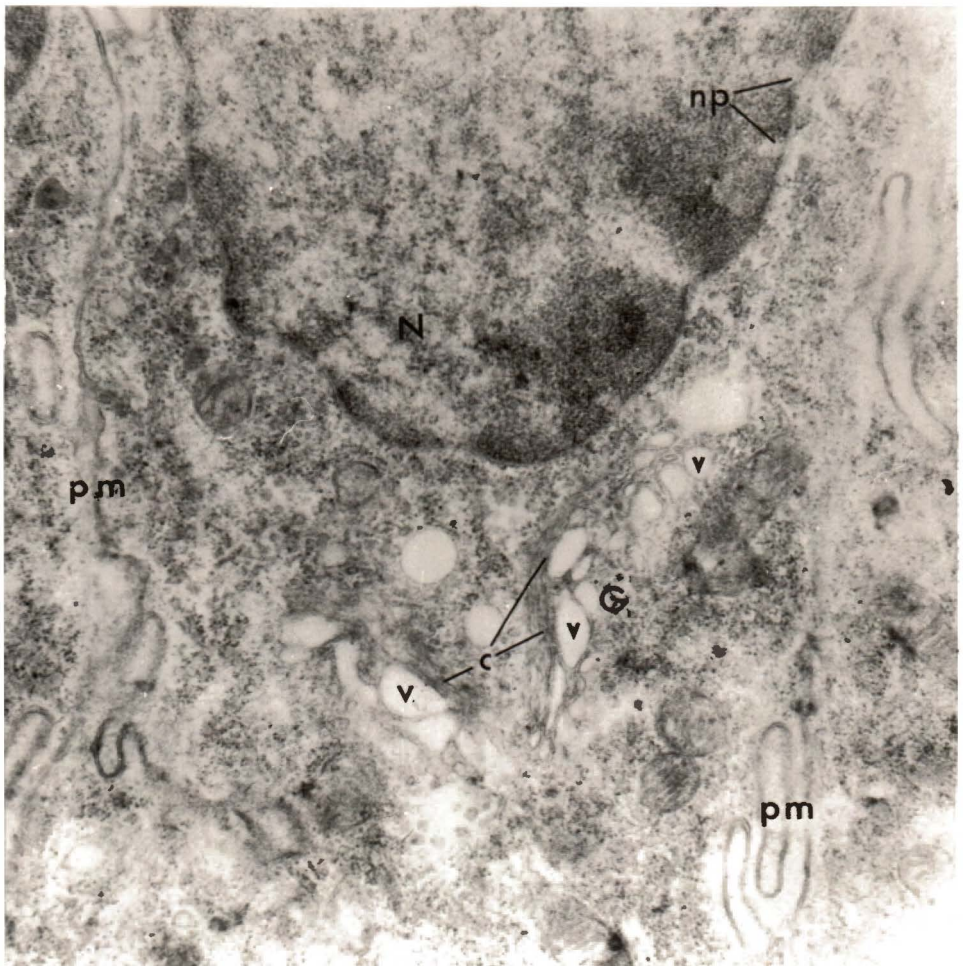

np
N
pm
v
G
v
c
v
pm

FIGURE 7

Photomicrograph of several intestinal epithelial cells at the level of the nucleus Many mitochondria are found in this area The extreme interdigitations of the plasma membranes of adjacent cells may be ob served Several nuclear pores (np) are evident A nucleolus (ne) and diffuse chromatin material may be seen in the nucleus (N) Epon embedded.

Approximate Magnification 11 500X

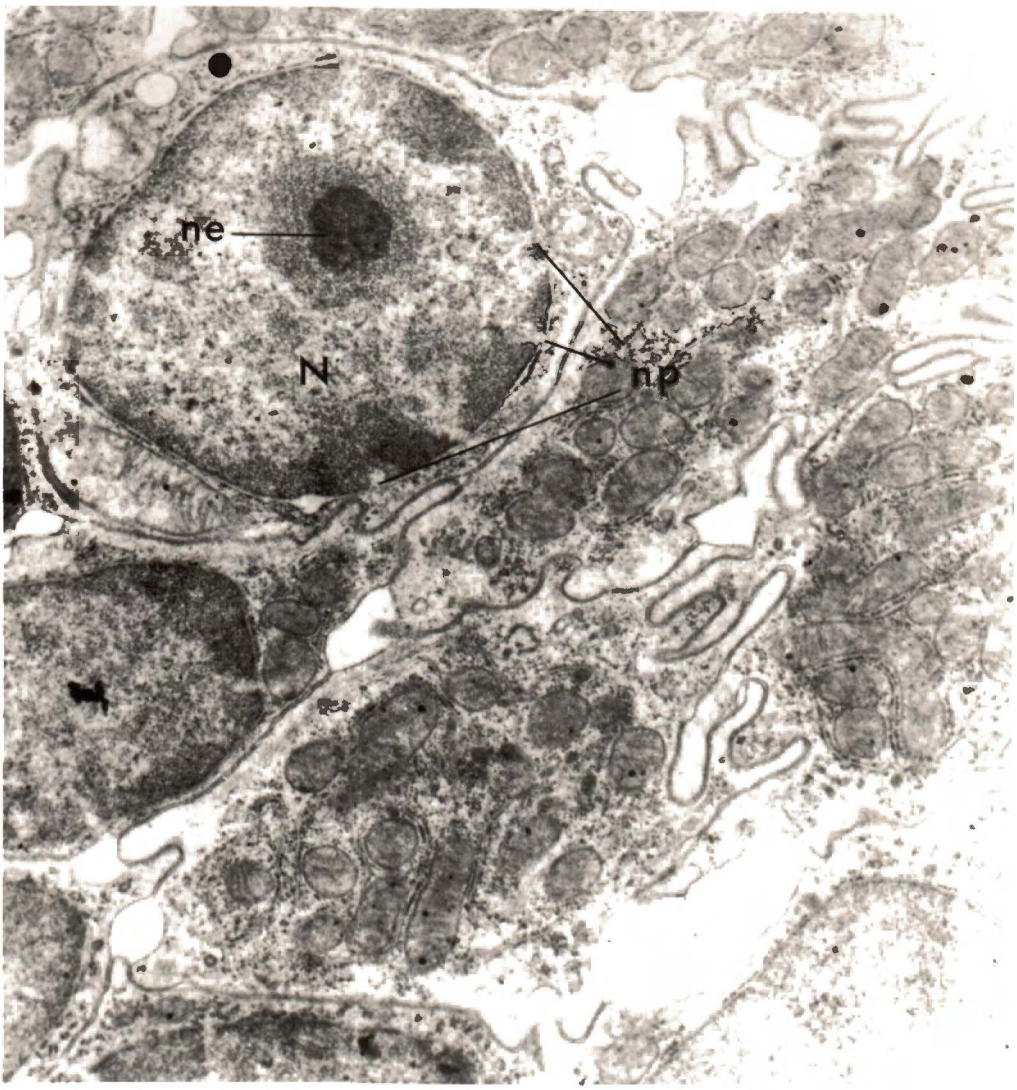

ne
N
np

FIGURE 8

Photomicrograph of three epithelial cells. At their junctions two intercellular spaces (ics) are formed. Granular endoplasmic reticulum (ger) with many ribosomes adherent to the membrane may be observed in one cell. Several mitochondria (m) are located along the periphery of two cells while in the third cell, the nucleus (N) is very evident. Epon embedded.

Approximate Magnification 22,000X.

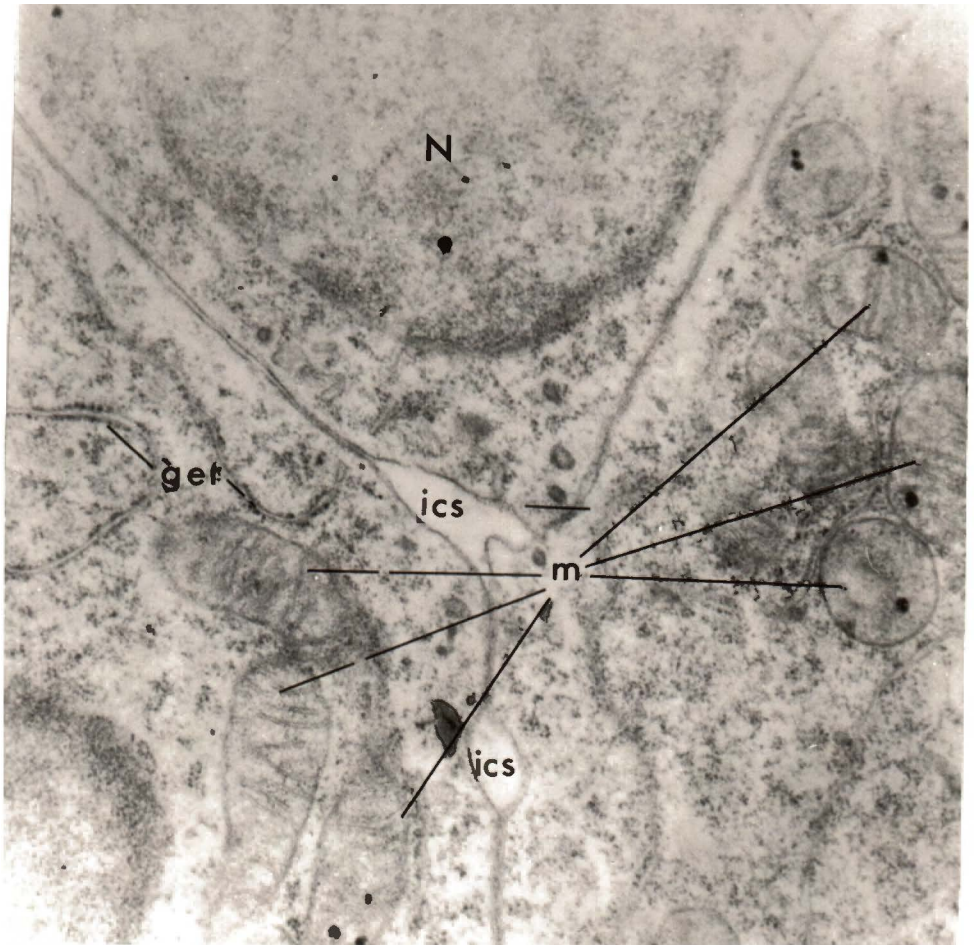

N
ger
ics
m
ics

FIGURE 9

Photomicrograph of two epithelial cells (EC) bordering a goblet cell (GO) filled with mucous. Plasma membranes (pm) exhibit the characteristic parallel configuration. A Golgi complex (G) with cisternae and a few vesicles may be seen in one epithelial cell. Fingerlike invaginations of cells (iv) are seen in cross section. Note the circular configuration and parallel plasma membranes. Epon embedded.

Approximate Magnification 15,500X.

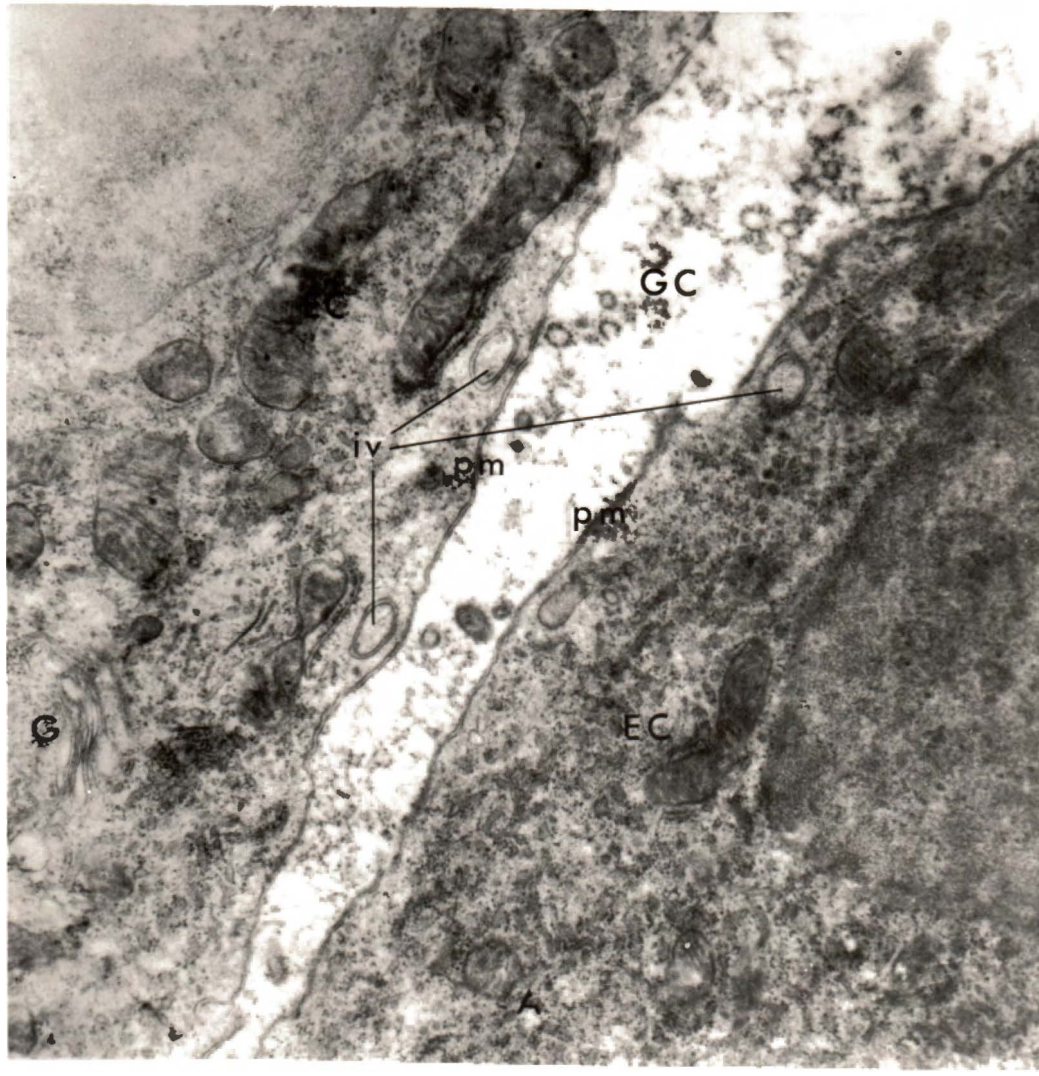

GC
iv
pm
pm
EC

FIGURE 10

Photomicrograph of a lymphatic vessel lying in the apex of a villus between two epithelial cells (EC). It lies in the lamina propria (LP) in close apposition to two epithelial basement membranes (bm_e). Two fibrocytes (F) with collagen fibers (c) nearby are also found in the lamina propria The lymphatic vessel is surrounded by a basement membrane (bm_l) and several fenestrations (fe) are noted through the lining endothelium. The nucleus (N) of the endothelial cell encroaches upon the tortuous and partly collapsed lumen. A terminal bar (tb) or desmosome is noted connecting two epithelial cells. Epon embedded.

Approximate Magnification 14,400X.

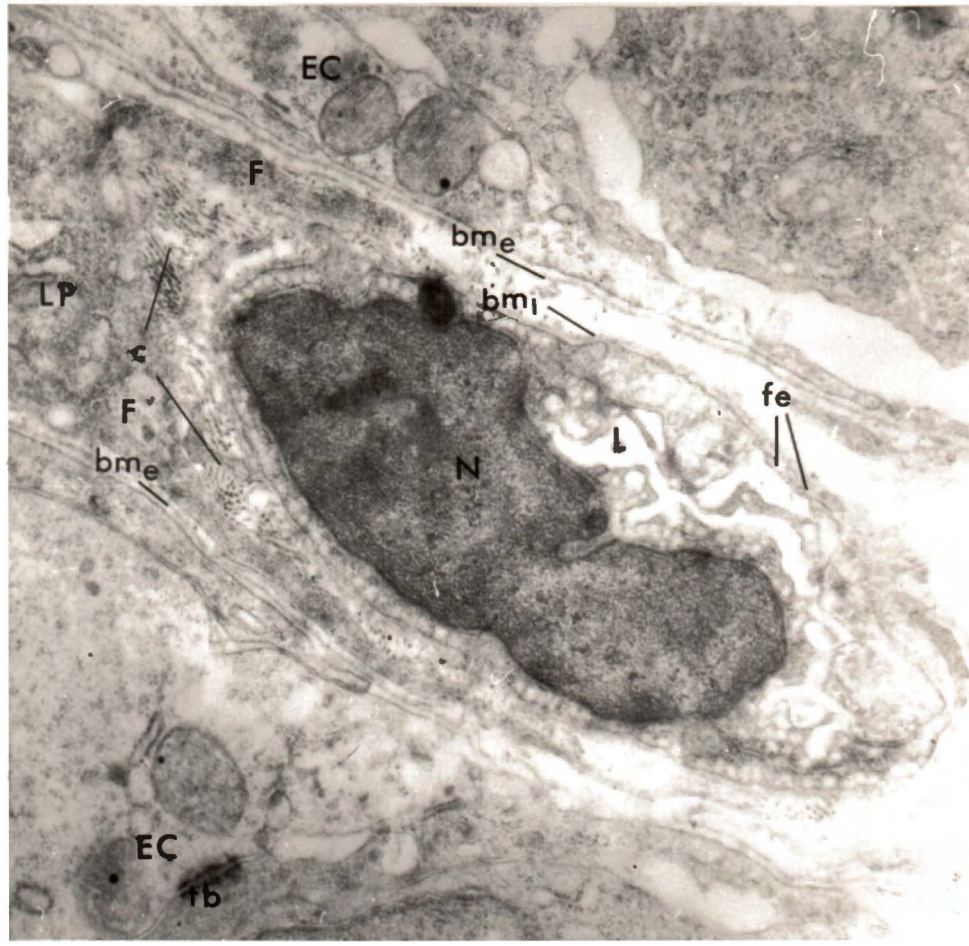

EC
F
bm$_e$
bm$_i$
LP
c
F
bm$_e$
N
L
fe
EC
fb

FIGURE 11

Photomicrograph of several epithelial cells of the mouse jejunum three hours after feeding a fat meal. Microvilli (mv) are quite evident lining the luminal border. Many mitochondria (m) may be seen most oriented perpendicular to the longitudinal axis of the cells. Many large irregular lipid masses (L) apparently without membranes are scattered throughout the cell. Many small lipid droplets (l) are also observed, many apparently in a membranous structure. A few lipid droplets may be observed between plasma membranes (pm) at the position of and below the nucleus (N). Methacrylate embedded.

Approximate Magnification 6,400X.


mv
mv
ptm
m
l
l
N
le

FIGURE 12

Photomicrograph of the apex of an epithelial cell one hour after a fat meal. Several lipid (l) droplets may be observed in the lumen (L) closely adherent to microvilli (mv). Still other lipid droplets (l) are observed within membranes in the apical region of the cell. A few small fat droplets (b) may be observed just below the terminal web (tw). Invaginations of the intermicrovillous spaces (a) are seen entering the terminal web (tw). A large and small lipid droplet are in the process of coalesence (c) although still separated by surrounding membranes. Epon embedded.

Approximate Magnification 12,800X.

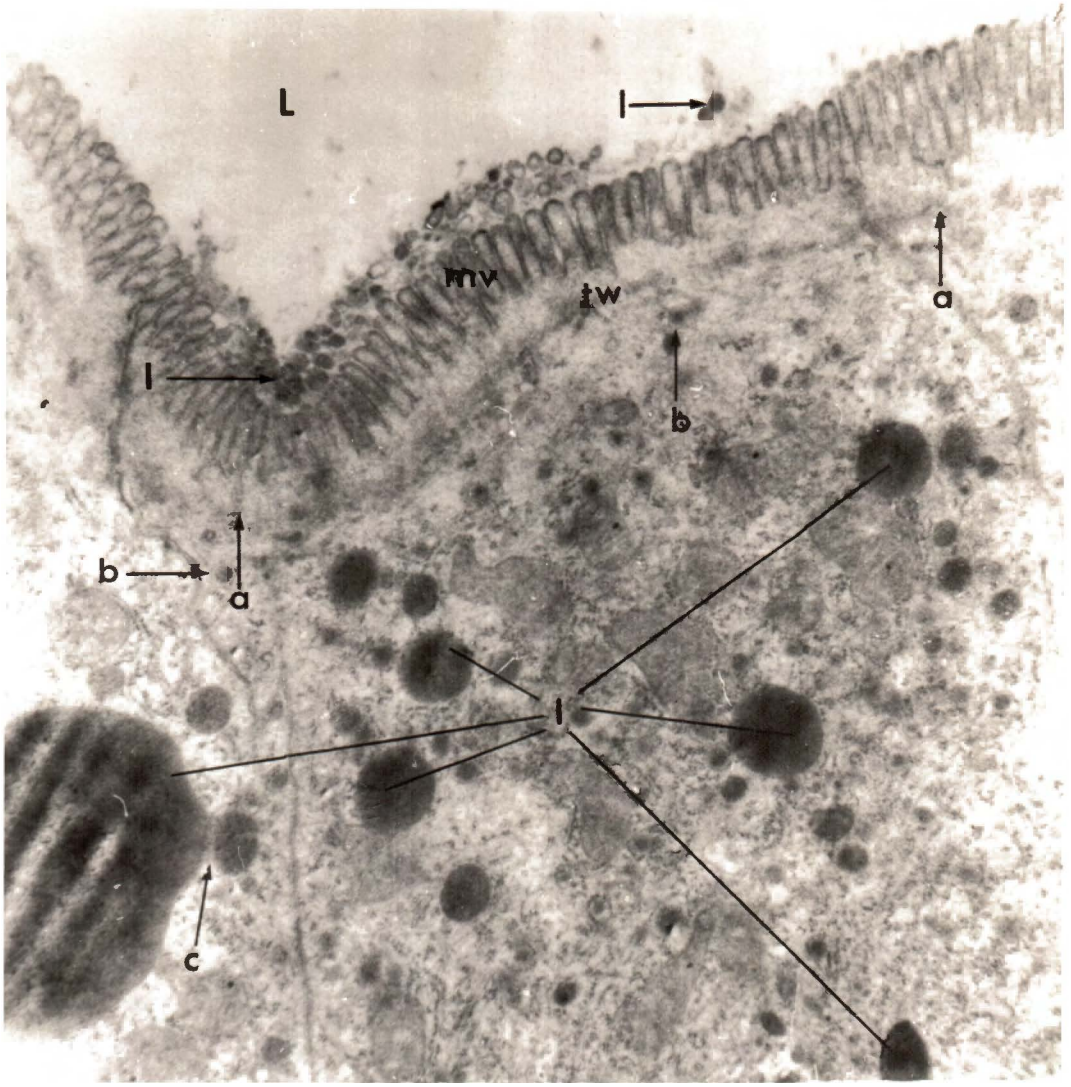

L
l
mv
tw
a
l
b
b
a
l
c

FIGURE 13

Photomicrograph of the supranuclear region of an intestinal epithelial cell. Numerous fat filled vesicles may be observed in the Golgi complex (G). Larger lipid droplets (l), one in close apposition to the nuclear membrane are also found in this area. Two large nuclear pores (np) interrupt the limiting membrane of the nucleus (N). Epon embedded.

Approximate Magnification 15,500X.

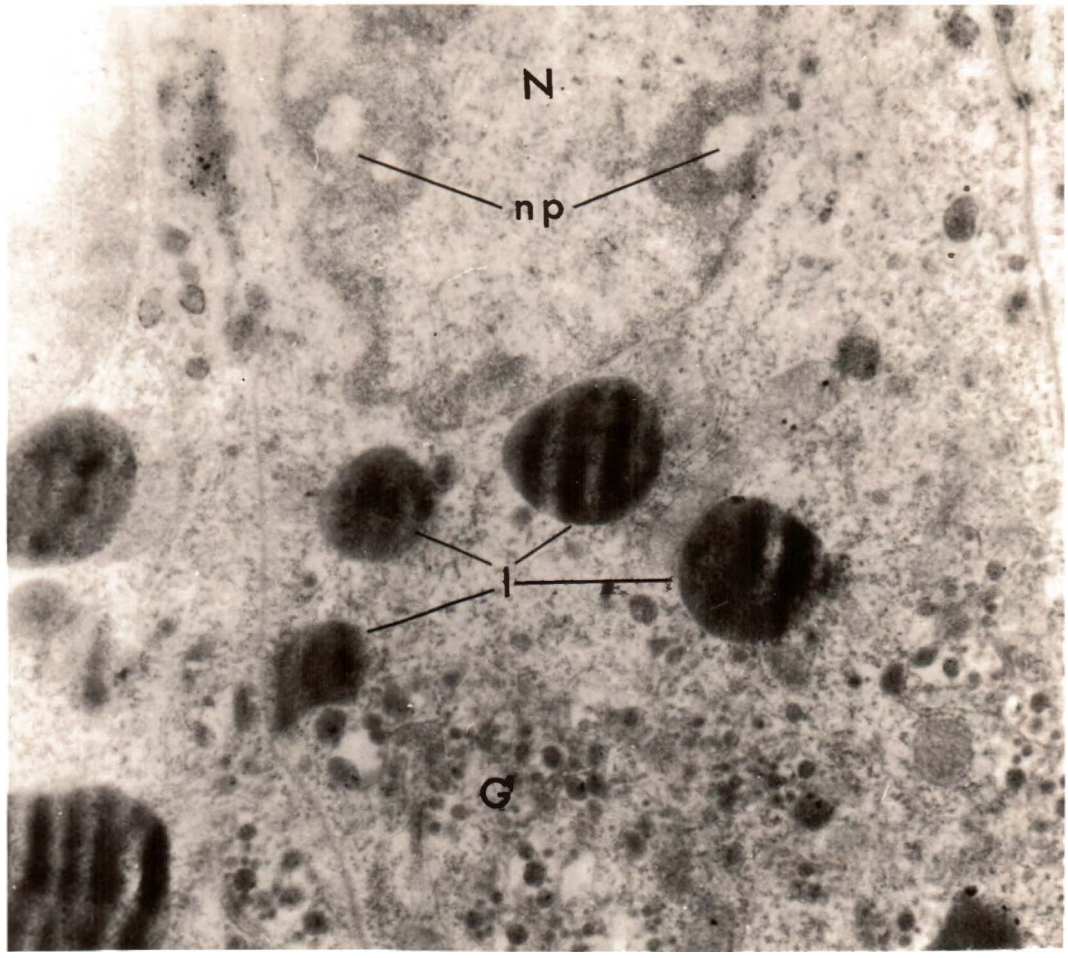

N
np
I
G

FIGURE 14

Photomicrograph of the cytoplasm of two epithelial cells. Large lipid masses (L) are scattered throughout the cell. No evidence of containing membranes can be noted around them. Many smaller lipid droplets (l) are scattered throughout the cytoplasm, several enclosed within a single membrane. Many of the smaller droplets are in a process of coalesence with other smaller droplets or larger lipid masses. Methacrylate embedded.

Approximate Magnification 26,300X.

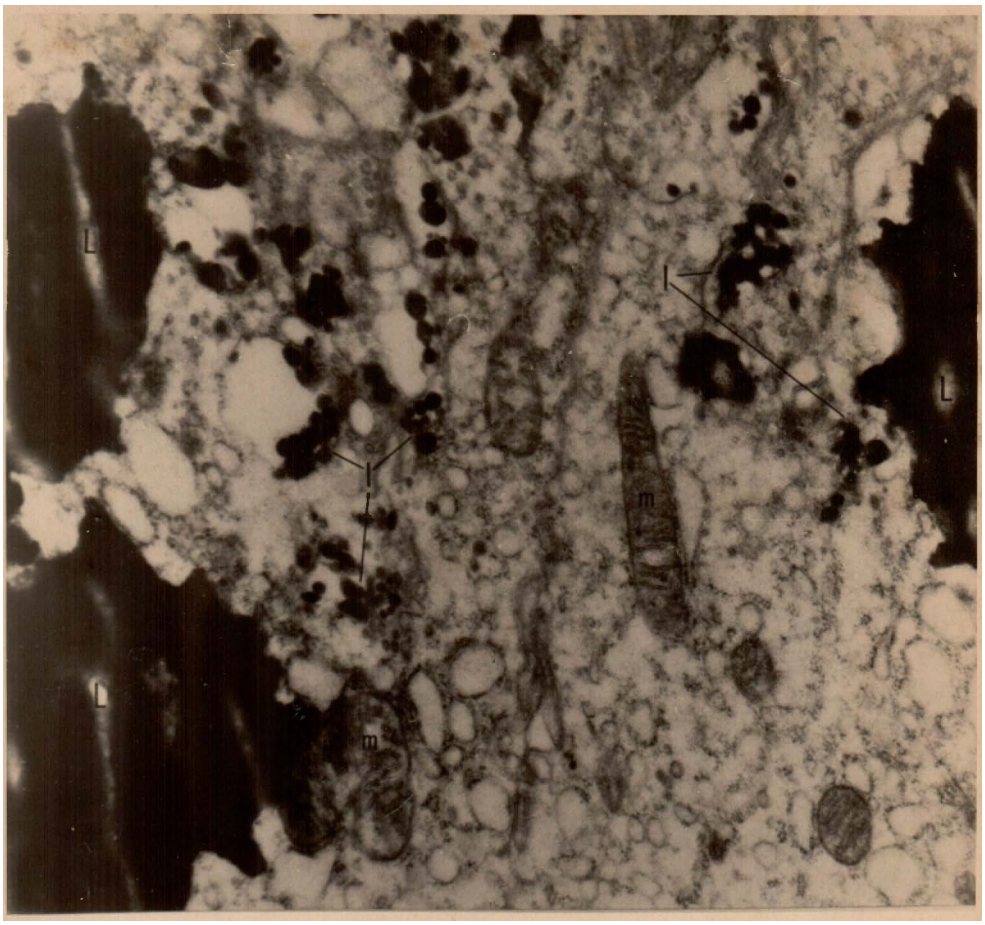

L
L
L
L
l
l
m
m

FIGURE 15

Photomicrograph of amembranous lipid droplets (l) in the intercellular space in between plasma membranes (pm). Many mitochondria are closely associated with the plasma membrane. On one side a large nucleus (N) may be seen. Methacrylate embedded.

Approximate Magnification 34,400X.

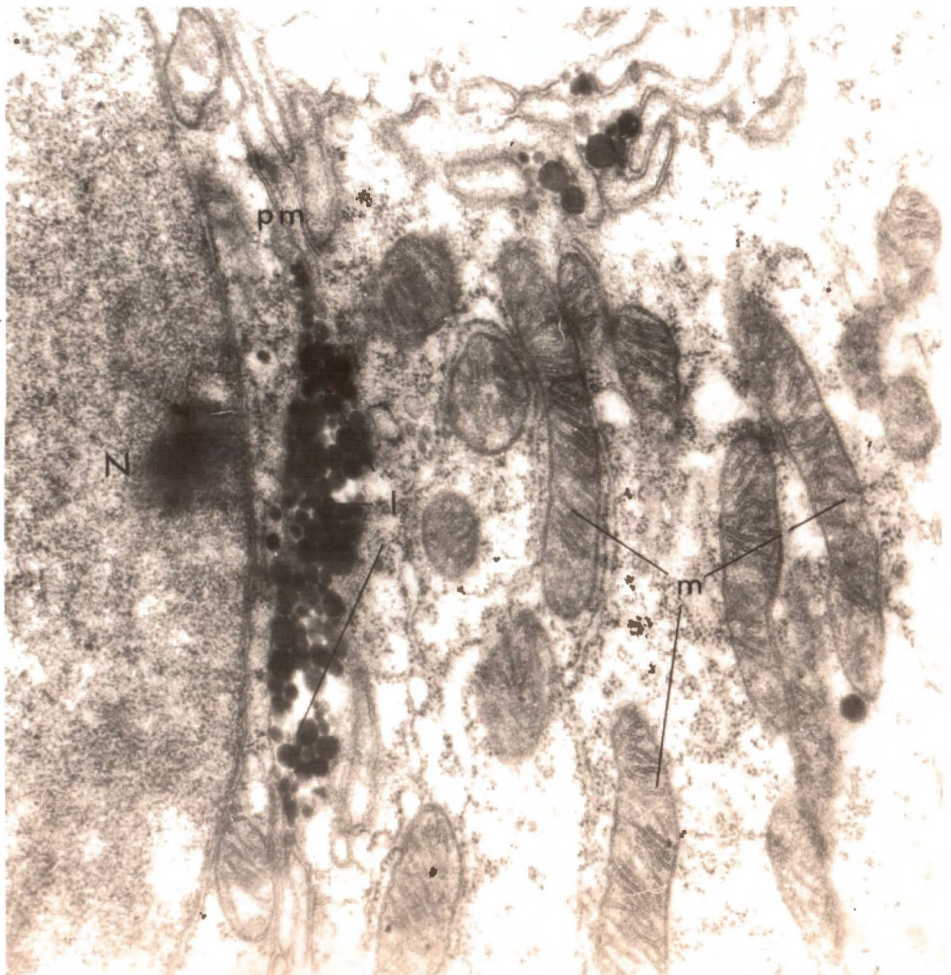

pm
N
l
m

FIGURE 16

Photomicrograph of the lamina propria. Several macrophages (M) may be seen. A plasmocyte (P) may be observed, its endoplasmic reticulum containing an amorphous substance. A Russel body is present in its cytoplasm. An eosinophil (Eo) may be observed in the lower right corner. Many lipid droplets are present in the interstitial space and in a macrophage in the upper portion of the photomicrograph. Epon embedded.

Approximate Magnification 4,000X.

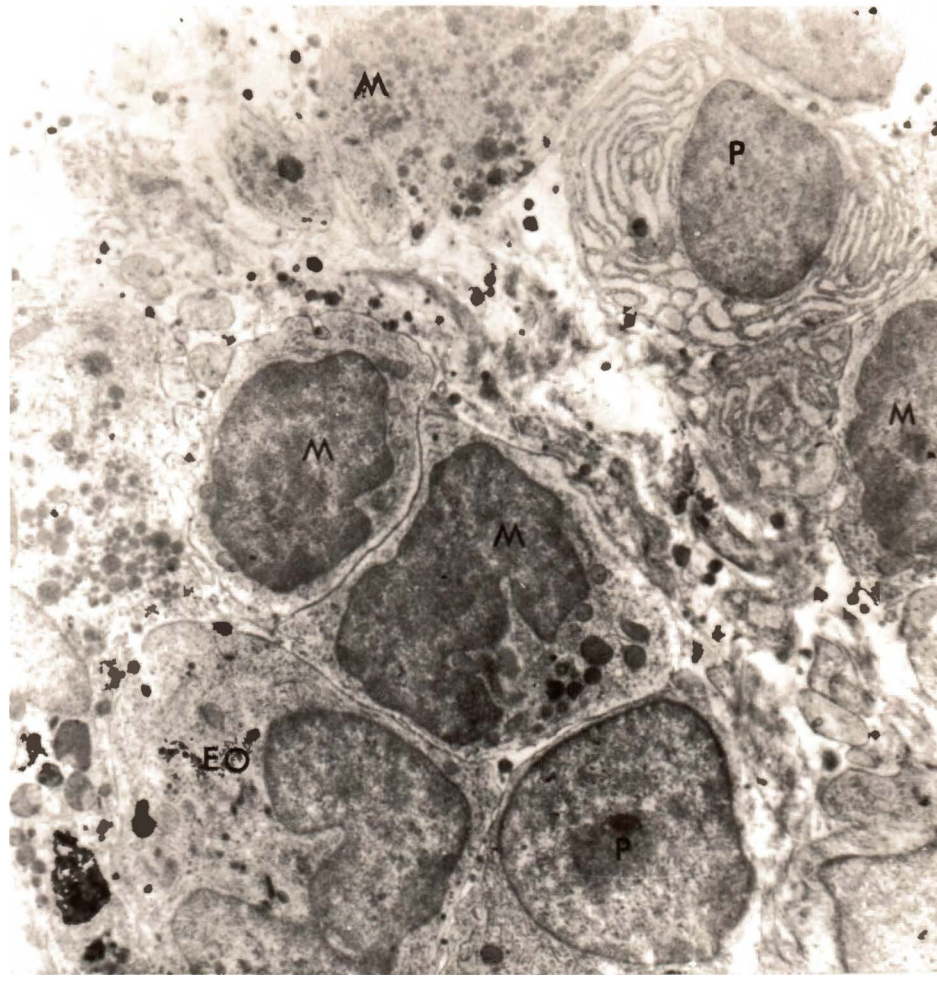

M
P
M
M
M
EO
P

FIGURE 17

Higher magnification of an area of Figure 16. Many lipid droplets may be seen in the interstitial space along with collagen fibers (c). Lipid droplets are found in great numbers in one of three macrophages (M), some contained in membranes (arrow) and many apparently lying free in the cytoplasm. Many are in a process of coalesence within the cell. An unidentified cell (U) may be seen containing a lipid droplet. Epon embedded.

Approximate Magnification 6,100X.

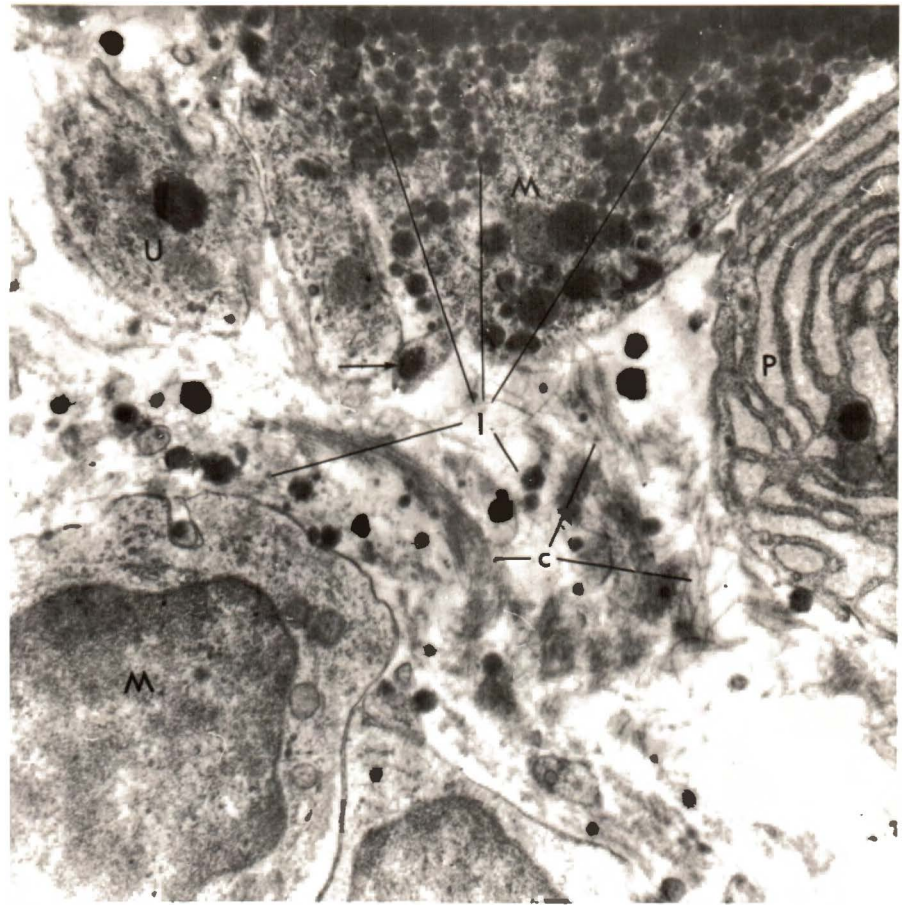

M
U
P
I
C
M

FIGURE 18

Photomicrograph of lamina propria. Many lipid droplets (l) some in a state of coalesence may be observed in the interstitial space in close association to collagen fibers (c). An undifferentiated cell (U) exhibits an intercellular bridge. A fibrocyte may be observed in cross-section (Fx). Two other fibrocytes (F) may be seen in the process of ingesting lipid droplets (arrows). Several vacuoles (v) are present in the fibrocytes. One vacuole (v^1) contains three lipid droplets. Epon embedded.

Approximate Magnification 6,100X.

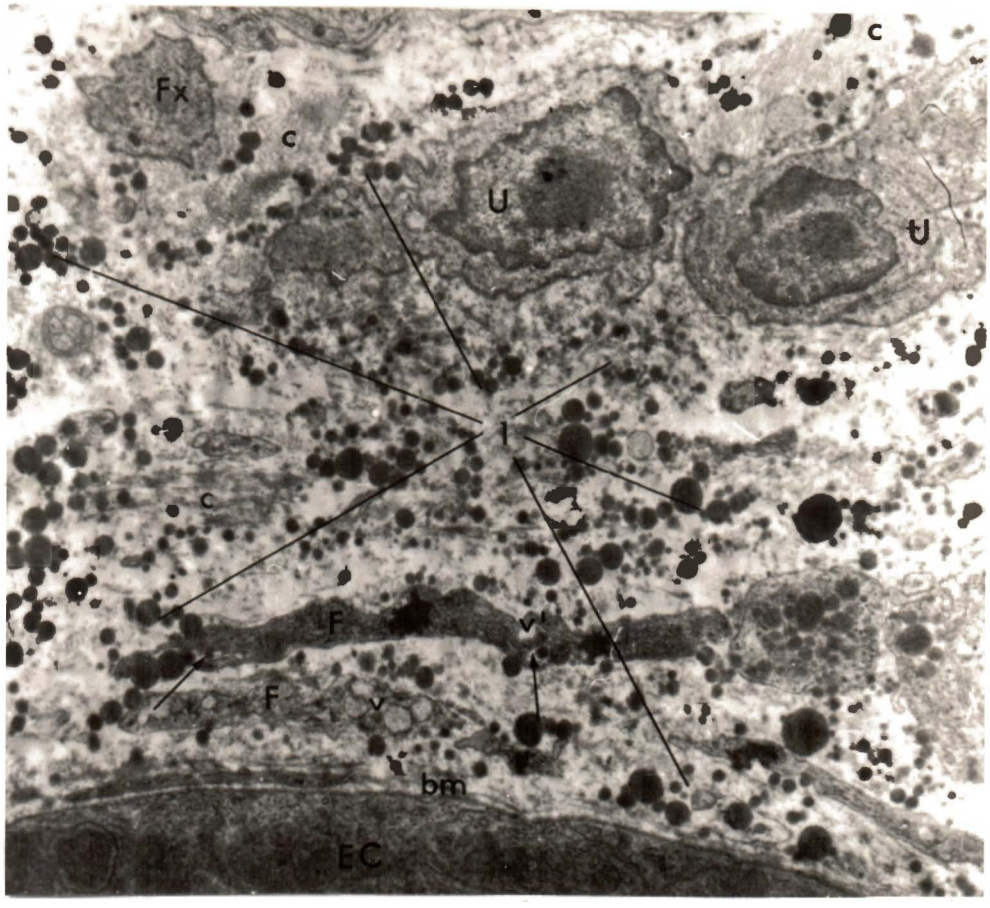

Fx
c
U
c
U
l
c
F
vl
F
v
bm
EC

FIGURE 19

Photomicrograph of the basal portion of three epithelial cells. Beneath the cell lies a capillary (C). The endothelium (end) and basement membrane of the capillary (bm_c) are depicted. The basement membrane of the epithelial cells (bm_e) is evident. Lipid droplets without membranes are grouped in the vicinity of the capillary, some droplets apparently coalescing. Methacrylate embedded.

Approximate Magnification 18,800X.

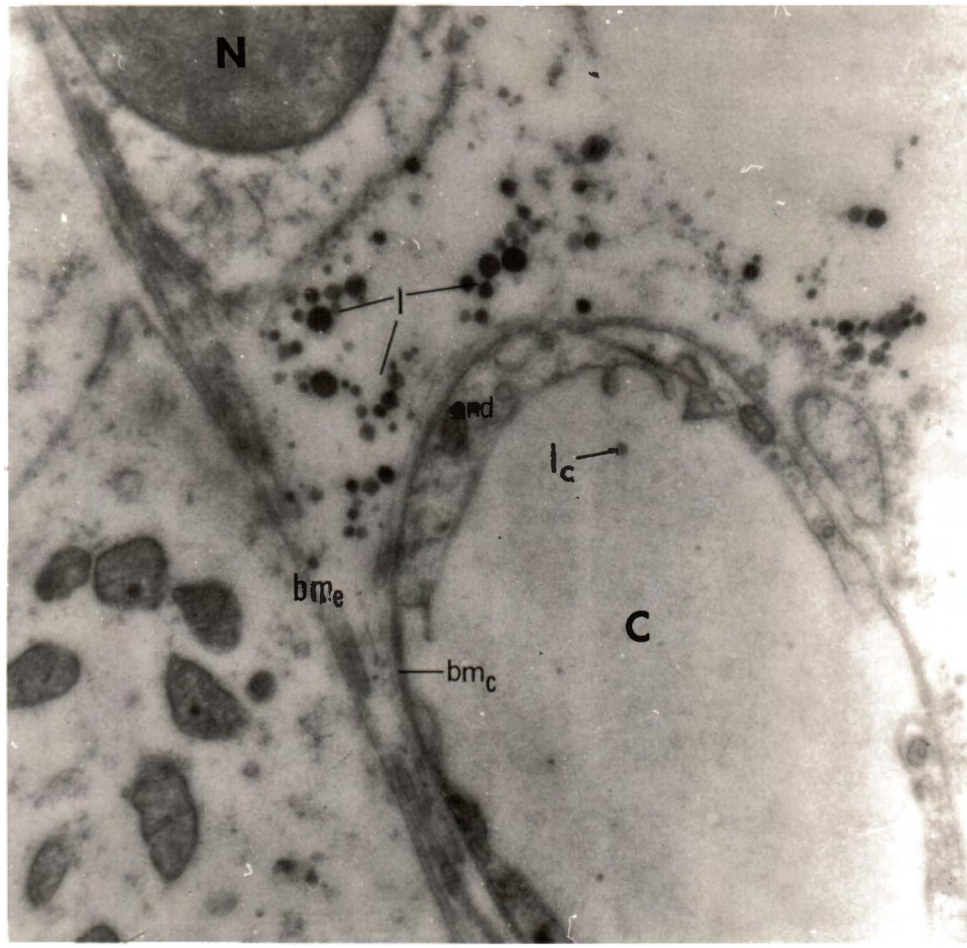
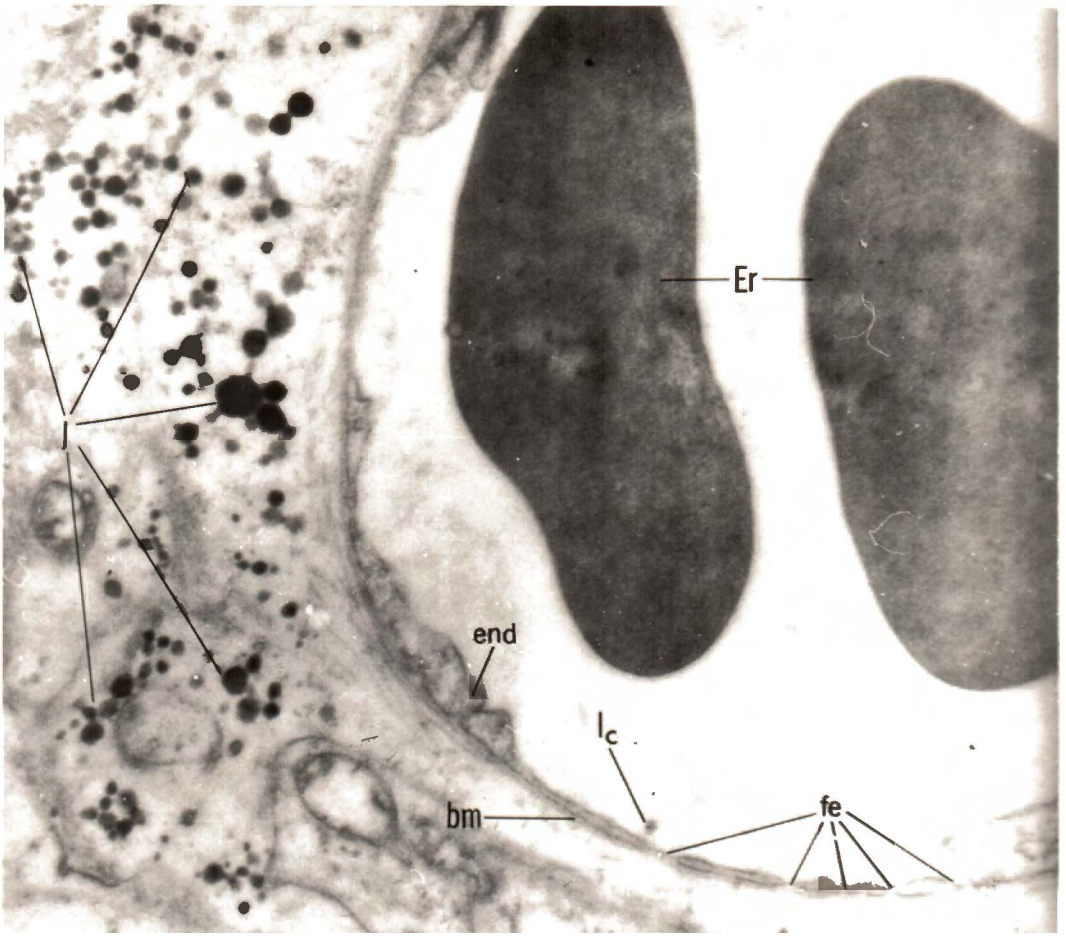

N
l
end
l_c
bm_e
C
bm_c

FIGURE 20

Photomicrograph of a capillary in the lamina propria. The endothelial lining (end) and basement membrane (bm) complete with fenestrations (fe) can be observed. Two erythrocytes (Er) lie within the capillary lumen. Around the capillary lie many lipid droplets, many of which are in a stage of coalesence. A lipid droplet (l_c) lies within the capillary lumen. Methacrylate embedded.

Approximate Magnification 20,000X.


Er
end
l_c
bm
fe