

An Electron Microscopic Study of the Intestinal Villus

II. The Pathway of Fat Absorption*

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ABSTRACT

The intestinal pathway for absorbed fat was traced in thin sections of intestinal villi from rats fed corn oil by stomach tube after a fast of 24 to 40 hours. For electron microscopy the tissues were fixed in chilled buffered osmium tetroxide and embedded in methacrylate. For light microscopy, other specimens from the same animals were fixed in formal-calcium, mordanted in $K_2Cr_2O_7$, and embedded in gelatin. Frozen sections were stained with Sudan black B or Sudan IV.

About 20 minutes after feeding, small fat droplets (65 $m\mu$ maximal diameter) appear in the striated border between microvilli. At the same time fat particles are seen within pinocytotic vesicles in the immediately subjacent terminal web. In later specimens the fat droplets are generally larger (50 to 240 $m\mu$) and lie deeper in the apical cytoplasm. All intracellular fat droplets are loosely enveloped in a thin membrane, the outer surface of which is sometimes studded with the fine particulate component of the cytoplasm. This envelope, apparently derived from the cell surface by pinocytosis, has at this stage evidently become a part of the endoplasmic reticulum. Just above the nucleus numerous fat droplets lie clustered within the dilated cisternae of the Golgi complex. As absorption progresses fat droplets appear in the intercellular spaces of the epithelium, in the interstitial connective tissue spaces of the lamina propria, and in the lumen of the lacteals. All of these extracellular fat droplets are devoid of a membranous envelope.

The picture of fat absorption as reconstructed from these studies involves a stream of fat droplets filtering through the striated border, entering the epithelial cell by pinocytosis at the bases of the intermicrovillous spaces, and coursing through the endoplasmic reticulum to be discharged at the sides of the epithelial cell into extracellular spaces. From the epithelial spaces, the droplets move into the lamina propria and thence into the lymph. If the lumen of the endoplasmic reticulum is considered as continuous with the extracellular phase, then the entire pathway of fat absorption may be regarded as extracellular. However, it is impossible to evaluate from the electron microscopic evidence thus far available the quantitative importance of particulate fat absorption by the mechanism described.

INTRODUCTION

In 1842 Gruby and Delafond (25, 26) deposited with the Academy of Sciences in Paris a paper reporting

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A preliminary report of this investigation was pre-

their observations on the intestinal villi and on the absorption of ingested fat from the intestinal lumen. They described the intestinal epithelial cells of fat-fed animals as crammed with small particles and globules of fat. According to their conception, the coarsely

sented before the American Association of Anatomists in Milwaukee, Wisconsin, April 1956 (47).

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emulsified fat in the intestinal lumen passed directly into the open epithelial cells, which converted it into a homogeneous and smooth emulsion of small particles and then transferred it to the central lacteal. Although this view was taken up by Brücke (12), it was soon convincingly disputed by Funke (24) and Kölliker (34), who recognized the integrity of the striated border of the intestinal epithelial cell, and who suggested that fat passes through the pores or channels which they thought were represented by the striations in the free border of the cells. This interpretation was strengthened by several reports that fat droplets can be seen passing through the striated border (6, 17, 18, 34, 56). However, biochemical studies of fat absorption toward the end of the nineteenth century brought forward evidence that ingested fat is actually not absorbed in this way (50). The biochemical evidence was so apparently conclusive that by 1916 Bloor (10) was able to write as follows in the introduction to his paper on fat assimilation:

“The way in which the food fat (or at least the greater part of it) gets from the intestine into the blood has been quite satisfactorily determined. It is saponified in the intestine, absorbed in water-soluble form as soaps and glycerol, resynthesized by the intestinal cells, and passed into the chyle and thence to the blood as neutral fat (glycerides suspended in the plasma in a very finely divided condition).”

This satisfying theory was modified when it was realized that the formation of soaps in the upper intestine must be seriously limited by the acid reaction of the intestinal contents (54, 57). Verzár and coworkers (57) emphasized the role of bile acids in forming water-soluble and diffusible complexes with the insoluble higher fatty acids released during hydrolysis of ingested triglyceride by pancreatic lipase in the intestinal lumen. Once taken into the epithelial cells, the bile acid-fatty acid complex was supposed to split apart and the fatty acids were recombined with glycerol via an intermediate phosphorylation, while the bile acids were returned to the cell surface where they entered into new complexes with more fatty acids. This notion of a minor circulation of the bile acids was intended to account for the disproportion between the small concentration of bile acids and the large concentration of fatty acids during absorption of a fatty meal.

The lipolytic theory of Pflüger and Verzár was challenged by Frazer and his colleagues (19-23) on the grounds that glycerides are only partially hydrolyzed in the intestinal lumen and that emulsification is an essential step in the absorption of lipides. Under the conditions prevailing in the intestinal lumen, a stable emulsion of triglycerides, with a particle size less than 0.5μ , is formed only with the triple combination of fatty acid, bile salt, and monoglyceride. They suggested that the particles of fat pass into the epithelial cells through the canals described by Baker in the striated border (3, 5).

Recent studies with isotopically labelled triglycerides (see review in reference 8) have shown that complete lipolysis of the long-chain glycerides is indeed limited, in the sense that only 30 to 50 per cent of available glycerol is set free. Practically all of the labelled fatty acids found in the lymph are in the form of triglycerides (a finding which confirms a frequently repeated observation since the days of Munk, 41). The liberated glycerol itself is rapidly absorbed via the portal blood and is not directly utilized to any large extent for resynthesis of triglycerides in the intestine. However, there is an extensive redistribution of fatty acids in the triglycerides of the lymph as compared to those ingested, *i.e.*, the ester bonds in positions 1 and 3 of glycerol are rapidly equilibrated with the free fatty acids made available either in the food ingested or by digestion. This means that considerable hydrolysis of triglycerides does occur in the intestine and is accompanied by resynthesis of triglycerides from mono- and diglycerides and free fatty acids, both in the intestinal lumen and in the epithelium. In general, it is now agreed that emulsification is necessary for fat absorption and that hydrolysis of triglycerides does occur in the lumen, at least to some extent (16). But these biochemical studies, comparing the composition of the dietary mixture administered or the luminal contents on the one hand with the composition of the lipides recovered from the intestinal lymph on the other, do not really attack the problem of absorption into the intestinal epithelial cells. Instead, they describe the net result that appears when fat passes from the intestinal lumen to the intestinal lymphatics. The composition of lipides in the derived lymph does not necessarily reflect the detailed events occurring at the surface of the intestinal epithelial cell.

Morphological studies of fat absorption have been equally as contradictory as the biochemical ones, and consequently morphological evidence has been adduced in support of each successive theory as it has appeared (see references 5, 18, 27, 49 for reviews). Even during the nineteenth century, when direct absorption of emulsified fat was the most highly favored theory, the entrance of fat into the intestinal epithelial cells was admittedly rarely seen (17, 34, 36, 56). The most persistent observation was of fat granules appearing within the deeper portions of the cytoplasm, enlarging to droplets, and eventually filling the entire cell (18, 24, 28, 34, 36). Kölliker (33, p. 97) stated:

“These observations demonstrate that fatty matters are absorbed as such and are not saponified; on the other hand, it cannot at present be certainly stated how it is possible that they penetrate the membrane of the epithelial cells, the parenchyma of the villi, and the walls of the lacteals. I should be most inclined to compare the whole process to the imbibition of an emulsion fluid, such as milk, by a porous body; and I believe that the fatty molecules of the chyme are absorbed simply

in consequence of their being carried along with its fluid part."¹

However, the prestige of Kölliker was not sufficient to establish this line of reasoning. In 1890 Krehl (36) was insisting on the interpretation that fat is absorbed in a soluble form and reconstituted by the cytoplasmic bioblasts or granules (1). In his extensive series of experiments he never saw fat droplets within or just beneath the striate border. As both Teichmann (56) and M. Heidenhain (27) pointed out, this position logically required that fat be split and resynthesized three times during absorption, once on its entrance into the cell, again on its entrance into the lamina propria, and lastly on its entrance into the lymph. For in each of these sites everyone agreed that fat was found in the form of droplets of triglyceride. However, Kölliker (34), Donders (17), Eimer (18), von Basch (6), and Teichmann (56) all reported that they had seen fat droplets, admittedly rarely, in the striated border or just beneath it in the apical cytoplasm. With the ascendance of Pflüger's lipolytic theory these occasional observations were ignored and emphasis was placed upon the first appearance of absorbed fat in the depths of the cell, near the nucleus or in the Golgi apparatus (2, 9, 15, 31, 39, 49, 51, 53, 57-59, 62).

In the present century, although several observers have reported seeing fat particles in the striated border during absorption (2, 32, 59, 64), the most convincing observations are those of Baker (5). He described elliptical particles of sudanophilic material within the striated border of the intestines of mice killed 1½ to 2¼ hours after a fatty meal. Baker's observations can be readily repeated if his procedure is carefully followed. They have been confirmed in the rat by Hewitt (29, 30), and in both rat and mouse by the present study. These results clearly indicate that fat can be absorbed in particulate form by the intestinal epithelium. Nevertheless, they do not indicate how the fat actually enters the cells, and one is driven again to think in terms of Kölliker's imbibition of a century ago.

In the only electron microscopic study of this problem previously published, Weiss (61) was unable to find any signs of particulate fat absorption in the striated border and concluded that fat is absorbed in soluble form and concentrated in the Golgi apparatus, where it appears in droplets. In

¹ In the 1867 edition of his textbook (p. 415), Kölliker (35) reaffirmed his conviction "dass das Fett nur in der Form unmessbar feiner Moleküle aufgesaugt wird, und kann . . . freilich in seltenen Fällen, solche Moleküle auch in der porösen dicken Basalmembran der Epithelcylinder beobachtet worden sind, kaum bezweifelt werden, dass diese Poren die Wege abgeben, auf denen die Fettmoleküle in die Zellen dringen."

the present investigation we found that fat droplets 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 membrane by pinocytosis (37).

Materials and Methods

After a 24- to 40-hour fast, 21 young adult Sprague-Dawley rats were lightly anesthetized with ether and then given a dose of 1.5 ml. of corn oil (mazola) by means of a thin polyethylene tube inserted through the oropharynx into the stomach. In a series of preliminary trials rats were given various fat-containing foods to eat, for example, ordinary food pellets (Purina lab chow), heavy cream, butter, or cacao butter. All of these materials were eventually discarded either because of their low fat content or because they interfered with good fixation of the intestinal mucosa. Corn oil was particularly well suited for tracing fat absorption with osmic acid fixation because of its high content of long-chain unsaturated fatty acids (38).

Following the administration of the corn oil, the rats were allowed to remain awake for various periods from 20 minutes to 3.5 hours. At the end of this interval they were anesthetized with an intraperitoneal injection of sodium pentobarbital (3.5 mg. per 100 gm. of body weight) and blocks of tissue were taken from their jejunums according to the procedures outlined in the first paper of this series (48).

Along with the tissue for electron microscopy, a nearby piece of intestine that had not been reached by the osmium tetroxide was removed from each animal, fixed in formal-calcium and mordanted in potassium dichromate according to Baker's method for fat stains (4), and embedded in gelatin. Frozen sections (5 μ thick) of these blocks were stained with Sudan black B or Sudan IV (13), mounted in gum arabic-sucrose medium, and examined in the light microscope.

In a few animals the procedure for obtaining tissue was modified in order to increase the chances of striking an area of the intestine that was undergoing the earliest stages of fat absorption. About 20 to 30 minutes after administration of the corn oil, the entire jejunum was fixed by filling the intestine with buffered cold osmium tetroxide from an injection into the duodenum. Simultaneously, the fixative was applied to the serosal surface. The entire strip of jejunum was then rapidly cut out in one piece and placed on a wax plate where it was covered with fresh osmium tetroxide. Five successive portions were cut from it at 1 to 2 cm. intervals. Each piece was then trimmed into fragments 1 mm. square and prepared for electron microscopy in the usual way (48).

In order to repeat Baker's observations (5) on fat absorption, five mice were fasted for 24 hours. Two

mice served as fasted controls and three mice were then allowed to eat as much butter as they desired. One hour and 45 minutes after their meal they were anesthetized with ether and sodium pentobarbital. Pieces of upper and middle jejunum were removed and fixed according to Baker's method (4). The tissues were embedded in gelatin and frozen sections were prepared and stained as described above.

OBSERVATIONS

Light Microscopy.—The intestinal epithelium of fasted animals contains little or no Sudan-positive material (Fig. 1). Occasional fat droplets occur in the supranuclear portions of the epithelial cells and in the lamina propria, and some of the larger lymphatics of the submucosa are filled with sudanophilic material. In animals that are just beginning to absorb fat, the epithelial cells at the tips of the villi contain more fat than do those on the sides. The fat is almost always restricted to the supranuclear parts of the cells. In this early stage the striated border contains ellipsoidal accumulations of fat oriented with their long axes normal to the free surface of the cells (Fig. 2). In sections passing parallel to the surface of the villi, the fat appears in thin streaks that form a reticular pattern over the surface. This finding indicates that the ellipsoidal bodies are profiles of thin streams of fat lying within the striated border. In the rat these ellipsoids are more common in cells that are just beginning to absorb fat than in those which have been exposed to fat for several hours. In those animals that were given fat 3 hours before fixation the supranuclear cytoplasm of the epithelial cells is loaded with fat droplets, but the striated border is clear (Fig. 3). At this stage fat droplets also accumulate between the cells at the level of the nuclei and below, and in the lamina propria.

The repetition of Baker's experiment (5) yielded results identical with his. Streaks of Sudan-positive material run at frequent intervals through the striated border of the intestines of mice fed butter about 2 hours before fixation, and the apical cytoplasm of the epithelial cells is filled with fat. Except for the more frequent occurrence of sudanophilic material in the striated border of cells that also have large quantities of it in their cytoplasm, the appearance of the intestinal epithelium in the mouse is similar to that in the rat.

Electron Microscopy.—In sections of intestine from rats that have been fed corn oil 1 to 3 hours before fixation, droplets of fat occur in all parts of

the mucosa, except in the striated border and in the terminal web just beneath it. The fat particles appear as small, round, adielectronic bodies, 50 to 100 $m\mu$ in diameter, scattered within and between the cells, both in the epithelium and in the lamina propria. Fat droplets sometimes adhere to the tips of the microvilli (Fig. 3) but usually all of the fat within the intestinal lumen has been washed away during the processes of fixation and dehydration.

During the stages of fat absorption observed in this study, the microvilli exhibit no discernible alteration in their dimensions or structure. Recently Sjöstrand and Zetterqvist (55) have reported that the plasmalemma covering the microvilli changes from a double-contoured structure into a single adielectronic layer, the width of which depends upon the type of food being absorbed. In the present study, although this aspect of absorption was not investigated in detail, the double-contoured structure of the microvillous surface was seen in fat-fed animals as well as in fasted animals. Earlier claims that the striations of the border disappear (11) and that the striated border becomes markedly reduced in height (11, 18) during absorption of fat are demonstrably incorrect.

Fig. 5 shows a section through the apical cytoplasm and striated border of an intestinal epithelial cell in a rat 70 minutes after administration of 1.5 ml. of corn oil. Because the section lies in a plane oblique to the surface of the villus, the microvilli appear in circular profile and the terminal web appears broader than in normal sections. The junction of the microvilli with the main body of the cell produces a scalloped appearance and some of the intermicrovillous spaces are continued into the cell as elongated profiles ending in rounded tips. Some of these profiles represent narrow furrows in the cell surface, as can be seen in Fig. 4 of the previous paper (48), where the section passes transversely across the terminal web. Small vesicles, about 60 $m\mu$ in diameter, are attached to some of these furrows or lie deeper in the apical cytoplasm. It is noteworthy that these vesicles are the only organelles occurring in this zone of the cytoplasm, which corresponds to the light streak under the striated border seen in light micrographs shown in Figs. 1 to 3.

Sometimes these vesicles carry within them a small spherule of fat (Fig. 4), but usually they are empty. These vesicles constitute evidence of pinocytosis by the intermicrovillous plasma membrane.

The pinocytosis appears to be a spontaneous process not requiring the presence of particulate material as a stimulus, for it is found in the intestinal epithelial cells of fasting as well as fed animals (see Fig. 5 in the previous paper, 48). In the fasting animals the vesicles are always empty. Although they also occur in animals that have been given fat as long as 3 hours before fixation, they are most numerous in intestinal cells that are just beginning to absorb fat. When a strip of jejunum is removed from an animal that has been given corn oil about 20 minutes previously, and pieces are removed from the strip at intervals of 1 to 2 centimeters, it is possible to find sections from three consecutive pieces of which the most distal is totally devoid of fat particles, the middle is just beginning to absorb, and the most proximal has large quantities of fat in the apical cytoplasm. In such material the greatest pinocytotic activity is found in the middle piece where active absorption is occurring. Furthermore, in such an area of active absorption it is common to find adjacent cells one of which contains moderate amounts of fat and the other of which is completely devoid of it. In this case the former usually displays more pinocytosis than does the latter.

Deeper in the cytoplasm are numerous single fat droplets, mitochondria, and elements of the granular endoplasmic reticulum. The fat droplets, 110 to 240 $m\mu$ in diameter, are round and of nearly uniform density. Each is enclosed within a thin envelope similar in thickness and density to the plasmalemma (Figs. 5 to 7). The droplet appears to be loosely enclosed, with a narrow space between it and the membrane. Careful inspection of Fig. 5 reveals that the envelope does not always conform in shape to the droplet within it. Although the droplets are nearly all circular in outline the vesicles enclosing some of them are elliptical, calyciform, or even tubular. This is especially noticeable in the more apical range of droplets (Fig. 5, arrows). A few of the vesicles have tubular extensions above and below or to one side. Occasional vesicles appear joined together by a narrow tubular membrane. Such appearances suggest that the fat droplets lie in a labyrinthine membranous system consisting of small vesicles intercommunicating by means of slender tubular bridges, in other words, the endoplasmic reticulum. This suggestion is further borne out by the observation that in some cells many of the fat droplets are enclosed in membranes that are studded with fine granules

apparently identical with those found in ergastoplasm (Fig. 7).

The earliest observed stage in the absorption of fat can be seen in Fig. 4, which represents a section of the striated border and apical cytoplasm of an intestinal epithelial cell from a rat that had been given corn oil 22 minutes before fixation. Small droplets of fat with a maximal diameter of 65 $m\mu$ are lodged in the intermicrovillous spaces at various depths in the striated border. Accumulations of droplets in this stage presumably correspond to the ellipsoids and streaks of fat seen by light microscopy (Fig. 2, and reference 5). In the terminal web more droplets can be seen, each enclosed within a membranous envelope. Apparently the droplets have entered the cell by means of pinocytosis. Critical intermediate stages in this process have not yet been observed; further work will be necessary before a definitive picture of the entrance of particulate fat into the cell can be constructed (see Discussion).

If a cell has been exposed to fat for about 3 hours, the apical cytoplasm is practically filled with fat droplets. Compared with those in cells that have been exposed to fat for shorter periods, the vesicles are usually larger and more numerous and are filled with large and small droplets. Presumably the larger droplets have formed by coalescence of small ones. In addition, large irregular masses of fat are sometimes found lying apparently free in the cytoplasmic matrix and not enclosed in a membranous envelope (Fig. 15). These masses resemble lipid material in other cell types and represent either absorbed fat resynthesized *in situ* or fat that has escaped from the membrane-limited channels.

Fat droplets are nearly always found in the Golgi complex, in amounts directly proportional to the amounts found in the apical cytoplasm. The quantity is striking in animals that were fed 3 hours before fixation (Fig. 8). The fat accumulates as discrete droplets of various sizes (40 to 150 $m\mu$) within the dilated cisternae. Except for its increased content of fat, the Golgi complex undergoes no obvious change in its pattern during fat absorption. In contrast, the ergastoplasm usually disappears. This change does not necessarily reflect a reduction in the amount of granular reticulum, for this is still abundant as single cisternae, tubules, or vesicles dispersed throughout the apical cytoplasm and often containing fat droplets (Figs. 7 and 15). But ordered arrays like those found in the

epithelial cells of fasting animals are not encountered after feeding of fat.

As shown in Figs. 9 and 10, almost all of the intraepithelial fat droplets at and below the level of the nuclei lie *between* the cells rather than within them. Streaming of fat through the interspaces between epithelial cells has been noticed in numerous light microscopic studies (6, 18, 28, 29, 32, 39, 53, 60), and the electron micrograph in Fig. 9 corresponds almost exactly to the stained preparation pictured in Fig. 78 (p. 109) of Patzelt's comprehensive review (49).

Aside from their extracellular position, the most noteworthy features of these droplets are their discreteness and their lack of an enclosing membrane. In the process of transfer from the intracellular to the extracellular position all of the fat droplets have been divested of their membranous envelope. How this maneuver is accomplished was not directly observed in the present study. That the tubules and cisternae of the endoplasmic reticulum may approach very close to the lateral surface membranes of the epithelial cells is demonstrated in Fig. 15, where three fat-containing profiles of the granular reticulum in neighboring epithelial cells are disposed near the interdigitating plasma membranes. Although direct continuity of the reticulum and plasma membrane has not been seen in the present study, this figure suggests that it may occur in these cells as in macrophages (44), and that the fat droplets enclosed in the lumen of the reticulum are in this way discharged into the intercellular spaces (see also Fig. 9 in the previous paper, reference 48).

The extracellular fat droplets collect in small clusters or in large heaps in the interstices between cells, at the tips of interdigitated flutings of adjacent cell surfaces, in places where the apposed surfaces diverge (Figs. 9 and 15; also Fig. 9 in previous paper, reference 48). At the base of the epithelium, above the basement membrane, the droplets tend to pile up, and the cells appear farther apart than they do in the fasted animal (Fig. 10). Fat droplets have not been seen in the basement membrane itself. In recently fed animals extracellular fat droplets appear scattered throughout the lamina propria, between neighboring cells of the connective tissue, among collagen fibers, and in the invaginated folds of macrophages (Figs. 11-14, 16). Rarely fat droplets are found in the crevices between the imbricated edges of endothelial cells of capillaries and even within capillaries (Figs. 13, 14). But in the central lacteals fat

droplets are numerous and streams of them can be seen between the endothelial cells (Fig. 16). Even in fasted animals droplets of fat still remain in the connective tissue of the lamina propria, and in electron micrographs of the macrophages they appear in rows and small clusters, entrapped among the folds of the cell surface and in pinocytotic vesicles lying just beneath it (Fig. 15 in previous paper, 48).

DISCUSSION

The Pathway of Fat Absorption.—The observations recorded in this paper delineate the pathway followed by fat droplets in their passage between the lumen of the intestine and the lymph. From the intermicrovillous spaces of the epithelial striated border, the droplets are received into vesicles produced by the pinocytotic activity of the apical cell surface. These vesicles join or empty into the endoplasmic reticulum, in the lumen of which the droplets pass toward the lateral surfaces of the epithelial cells. Here they are released, presumably by reversal of pinocytosis, into the intercellular spaces of the epithelium. The droplets then traverse the basement membrane of the epithelium into the interstitial spaces of the lamina propria, penetrate the basement membrane of the central lacteal, and finally slip between the overlapping endothelial cells to enter the lymph. The most noteworthy characteristic of this pathway is that so much of it is extracellular. Indeed, if it is permissible to think of the lumen of the endoplasmic reticulum as continuous with the extracellular phase (44, 45), even the epithelial portion of the pathway may be considered as extracellular.

The mechanism of the transport of absorbed fat droplets through these spaces is obscure. It may be assumed that gross movement through the connective tissue and into the lymphatic is a result of the mechanical pumping action of the villi.² But in this case, the *direction* of droplets moving passively across basement membranes, through areolar connective tissue, and between endothelial cells must reflect the architecture of these structures. For example, in the preceding paper (48), apparently open communications between the lymphatic lumen and the interstitial connective tissue spaces

² According to Verzář and McDougall (57), the leaf-like villi of the rat's intestine (unlike the finger-like villi of the dog, cat, and other animals) do not possess the property of contractility. This observation seems incongruous with the presence of abundant smooth muscle strands in the villi.

were occasionally noted in the lacteals of fasting animals. Thus, the endothelial cells of the lacteal seem to be less tightly held together than are those of the blood capillaries. The existence of stomata in the walls of lymphatic capillaries has been debated for many years since the early descriptions of von Recklinghausen (52), and the consensus now is that they are artifacts of silver preparations (40). However, the pathway taken by fat droplets, as shown in this study and by Weiss (61), reopens the question.

The apparent selectivity of the pathway for fat absorption is also a mystery. Triglycerides and long-chain fatty acids pass into the lymphatics, whereas short-chain fatty acids flow into the blood capillaries (8, 16, 20, 22). This discrimination may be related to several architectural characteristics of the villus. The relative positions of the capillaries and lacteal may have something to do with it. At the end of each contraction the passive distention of the villus by blood may result in differential pumping of particulate material into the lacteal. Another possibility is that the physicochemical properties of the basement membranes and the interstitial ground substance may control the diffusion of water-soluble materials through them and into the appropriate vessel. Finally, the highly attenuated and fenestrated endothelium of the blood capillaries, as opposed to the thicker wall of the lacteals (48), would favor diffusion of water-soluble substances into the blood. It should be remembered that a relatively constant concentration gradient is maintained between the stroma of the villus and the blood in its capillaries by means of the blood flow, whereas the lacteal is essentially a cul-de-sac and has only tidal flow.

Entrance of Fat Droplets into the Intestinal Epithelial Cell.—In the absorption of fat droplets from the intestinal lumen, the striated border of the epithelial cells appears to serve as a fine filter, the pores of which are the intermicrovillous spaces. In the present study droplets were found only in these spaces and never in the substance of the microvilli. Thus, it seems that the elaborate structure of the striated border, which increases the apical or free surface area of the cell some 24 times, has just the opposite significance in particulate fat absorption: it decreases the effective area available for absorption. This statement should not be construed as suggesting that the intestinal microvilli play no role in the absorption of soluble substances such as water, salts, monosaccharides, amino acids, and short-chain fatty acids. If, however, they do par-

ticipate in the absorption of such small molecules, they do not display signs of this activity in the fashion of microvilli on other epithelia. For example, during absorption, the microvilli of certain epithelia exhibit considerable individual variation in their dimensions and angle of inclination to the free surface of the cell, and vacuoles and vesicles appear both in the substance of the microvilli and opening upon their surface. This aspect of the problem is the object of a separate study.

It may be assumed that the fat droplets are pressed into or entrapped in the striated border as a result of peristaltic contractions of the intestine. Possibly they adhere to the plasmalemma of the microvilli and are carried into the intermicrovillous spaces by membrane flow as suggested by Bennett (7).

In any case, when the droplets reach the bases of the intermicrovillous spaces they are apparently engulfed by pinocytotic vesicles and enter the cytoplasm. A similar process has been described by Clark (14) for the absorption of protein from the intestines of young rats. Palade (42) and Wissig (63) have presented electron micrographic evidence for an important role of pinocytosis in the transport of substances across capillaries. Wissig (63) has shown that during the absorption of ferritin across capillaries in heart muscle, the particles of ferritin apparently stick to the surface of the endothelial cell, are engulfed within pinocytotic vesicles, and finally come to lie both within intracellular vesicles and free in the cytoplasm. In contrast to this observation on capillaries, the particles of fat followed through the intestinal epithelial cell in the present study are always enclosed within membrane-bound structures. The importance of this feature of fat absorption for general theories of the endoplasmic reticulum will be discussed below.

It would be gratifying to be able to state that the demonstration of fat absorption by pinocytosis at the base of the striated border resolves the conflicting opinions reviewed at the beginning of this paper, in agreement with the theory of particulate absorption (19, 22, 23). Unfortunately, the nature of the electron microscopic data does not permit this conclusion. In the first place, it can be argued that although pinocytosis was observed and does unquestionably occur, the frequency of the observation seems insufficient to account for all the fat that ultimately appears within the cell. Pinocytosis of fat droplets was seen only during the early stages of absorption, even though the continued accumu-

lation of fat within the cell indicates continuing absorption over some time. As was previously described, it is possible to find in a 4-centimeter strip of jejunum successive areas of epithelial cells that are completely devoid of fat, are just beginning to take in fat, and are filled with fat particles. Because such a transition occurs within a 4-centimeter strip of jejunum in an animal that had been given corn oil only 20 minutes before fixation, it is evident that fat must cross the striated border in large quantities very rapidly. If it crosses principally in a particulate form, by pinocytosis of droplets, such activity should be obvious in those areas where the cells are just beginning to take in fat. However, although pinocytosis is greatest at this stage, it never seems extensive enough to account for all the fat that has already been absorbed and will be absorbed. Even in areas of greatest activity, the majority of cells shows no sign of pinocytosis and only rarely does more than one vesicle appear to be forming in any one section of a single cell. The paucity of vesicles cannot be explained as due to the extreme thinness of the section, for even in the essentially two-dimensional section, the area of fat in pinocytosis seems totally inadequate to account for the area of fat within the apical cytoplasm. In order for pinocytosis to be important in fat absorption, it must be assumed, *first*, that it occurs so rapidly and is of such short duration that even if hundreds of thousands of vesicles form during the active stage, only a few can be captured by our current methods and, *second*, that once a cell has accepted a certain load of fat droplets the process must cease.

It is possible, however, that the line of argument pursued in the previous paragraph may be specious, because it does not take into account the dynamic properties of the cell. A few calculations show that the assumptions just mentioned are indeed plausible. A typical epithelial cell with a height of 20 μ and a cross-sectional area of 15 μ^2 has a volume of 300 μ^3 . A typical pinocytotic vesicle, 60 $m\mu$ across, has a volume of 10^{-4} μ^3 . In order to fill one-third of the volume of the cell (see Fig. 3) with fat at the end of 1 hour, one million such vesicles containing fat droplets must form at the surface and enter the cytoplasm during that hour. If the rate of pinocytosis is assumed to be uniform, this means that 277 new vesicles will be required each second. If, in addition, the vesicles are uniformly dispersed over the free surface of the cell (exclusive of the microvilli) and 40 sections, each 100 $m\mu$ thick are obtained from each cell, only 7 vesicles

need appear in each section. As a matter of fact pinocytosis is not uniformly dispersed over the surface of a cell. Lewis (37) observed that small vesicles form and enter in clusters of as many as 10 at a time and that they move very rapidly from the surface to the center of the cell. In one macrophage that he watched in tissue culture, he estimated that the volume of fluid taken in by pinocytosis during 1 hour amounted to one-third of the total volume of the cell. He suspected that cells in their normal sites are even more active, and he suggested that absorption of digestive products by the intestinal epithelium might occur by way of this mechanism.

The second important argument against particulate absorption is that fat particles are infrequently seen in the terminal web, which directly underlies the striated border. Krehl (36) especially stressed this point. Baker (5) states that he occasionally found fat particles in this zone of the cell. In the previous electron microscopic study of this problem, Weiss (61) saw no fat in the terminal web. This fact suggested to him that the absorbed fat traverses the striated border and apical surface of the cell in a dispersed, invisible form. It is not difficult to take such negative evidence as favorable to the lipolytic theory. If, however, the absorption of fat does proceed according to this theory, then there should be electron microscopic evidence of large quantities of soluble forms of fat in the apical cytoplasm of the cell. Since the osmiophilia of the administered fat is due to the unsaturated bonds of the constituent fatty acids and since this function is unaffected by the chemical reactions involved in solubilizing the fat, there should be a discernible increase in the diffuse density in the apical cytoplasm of cells absorbing fat.³ Furthermore, as the fat is converted into visible form, small particles of various sizes should appear in the apical cytoplasm. Actually neither diffuse density nor multiple particle sizes are seen in the electron micrographs. The smallest particle is about 50 $m\mu$ in diameter, and this is the size that fits into the small pinocytotic vesicles.

If we return to the calculations concerning a typical absorbing cell, the infrequent observation of fat droplets in the terminal web can be related to the time required for a vesicle to cross it. In our example, if we assume only one vesicle per section

³ In considering the same problem, Baker (5) observed no diffuse sudanophilia in the apical cytoplasm after feeding mice a meal of fatty acids.

at any one instant, or 40 over the whole free surface of the cell, the vesicles could persist in this zone only 0.14 second ($40 \div 277 = 0.14$). Since the web is of the order of 0.2μ in width, the vesicles would have to move at the rate of only 1.5μ per second. In view of these considerations it need not be surprising that so few fat droplets are captured in the terminal web in random sections.

On the basis of the actual observations, however, it is impossible to evaluate the relative importance of particulate and soluble absorption of fat. The evidence presented demonstrates that particulate absorption does occur by means of pinocytosis, but the electron microscope cannot now give decisive evidence concerning its importance. It is still possible that both methods proceed concurrently.

The Role of the Endoplasmic Reticulum.—Once having been taken into the epithelial cell, the fat droplets remain enclosed by a thin membrane that is frequently studded with fine granules. This membranous capsule is a part of the endoplasmic reticulum, which in the form of tubules, cisternae, and vesicles permeates the supranuclear region of the cell. Although the fat droplets are thus isolated within their capsules from the matrix, or the continuous phase, of the cytoplasm, they may still be subject to chemical alteration by enzymes situated in the membranes or in the circumambient substance in the lumen of the endoplasmic reticulum. In particular, they may receive a coating of phospholipide and protein while in this stage of absorption (22). The fact that the droplets in the supranuclear zone are several times larger than those encountered in the pinocytotic vesicles indicates that some coalescence occurs after their entrance into the cell. But these secondarily larger droplets remain discrete and independent even when several of them are close together within the same envelope. This observation suggests that the secondary emulsion discharged from the epithelium is already stabilized while inside the cells.

At the lateral surfaces of the epithelial cell the membranes of the endoplasmic reticulum apparently join with the surface membrane. Although this junction was never actually observed in the present study, it is reasonable to infer it because the enclosed droplets approach close to the surface and because the extracellular fat droplets are devoid of an envelope. We interpret the electron micrographic appearance as resulting from discharge of the fat droplets into the intercellular space by coalescence of the membrane of the endoplasmic reticulum with the surface membrane

of the cell. If this hypothesis is correct, the process of discharge, like pinocytosis, must be initiated and completed very quickly.

The over-all picture that emerges from these observations and considerations is that of a stream of fat droplets entering through the striated border by pinocytosis between the microvilli at the apical surface of the epithelial cell, and coursing through the endoplasmic reticulum to be discharged at the sides of the cell into extracellular spaces. This pathway illustrates one of the important functions of the endoplasmic reticulum in the general economy of the cell. It apparently serves as a transportation system by means of which substances either produced or delivered by the cell are directed to the appropriate external surface. A similar function is envisaged for the endoplasmic reticulum in gland cells (44–46). It should be noted that segregation within the lumen of the endoplasmic reticulum does not preclude interaction between the enclosed substance and the cytoplasm.

The pathway of fat absorption through the intestinal epithelium also illustrates a second point of general interest. The history of the capsules enclosing the fat droplets—their origin from the apical cell surface, their fusion with the endoplasmic reticulum, and disappearance at the lateral cell surface—betokens a circulation of membranous substance between the cell surface and the interior of the cell, as suggested by Palade (44). The observations reported here provide the first evidence, although incomplete, supporting this hypothesis.

The Role of the Golgi Complex.—In his electron microscopic study of fat absorption, Weiss (61) attributed an important role to the Golgi complex, asserting that the smooth surfaced vacuoles dispersed throughout the apical cytoplasm and enclosing fat droplets were a part of this organelle. This conclusion agrees with some earlier light microscopic interpretations (9, 15), but not with others (59). However, the fact that fat droplets also appear within granule-studded membranes, which are usually considered part of the ergastoplasm, indicates that the important organelle to be considered here is not the Golgi complex specifically, but, rather, the endoplasmic reticulum, of which it is a localized differentiation (43, 46). Since fat droplets occur in the cisternae of the Golgi complex of nearly every intestinal epithelial cell, even when the animal has been fasted for over 24 hours, it appears that this organelle is less active in fat absorption than the rest of the endoplasmic reticulum and is the last to yield its load of fat.

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EXPLANATION OF PLATES

PLATE 160

FIGS. 1 to 3. Photomicrographs of $5\ \mu$ frozen sections of intestinal villi, fixed in formal-calcium, mordanted in potassium dichromate, embedded in gelatin (4), and stained with Sudan black B in propylene glycol (13).

FIG. 1. Intestinal epithelium from a rat fasted 40 hours. Small, slightly sudanophilic particles (probably mitochondria) are distributed throughout the cytoplasm of the cells. The dense, black, round and elliptical particles are probably extracellular fat droplets. The striated border contains no fat, and the nuclei do not stain. The thin non-staining zone beneath the striated border is the site of the terminal web. $\times 2700$.

FIG. 2. Intestinal epithelium from a rat that had been given 1.5 ml. corn oil 19 minutes before fixation. The striated border now contains numerous slender masses of sudanophilic material, and a few droplets are visible in the apical cytoplasm of nearly every epithelial cell. The terminal web remains clear of fat. Compare with Fig. 4. $\times 2700$.

FIG. 3. Intestinal epithelium from a rat that had been given 1.5 ml. of corn oil 75 minutes before fixation. Although numerous fat droplets adhere to the free surface of the epithelium, the striated border is now clear of particulate fat. Each cell is packed with sudanophilic masses of fat. Compare with Fig. 5. $\times 2700$.

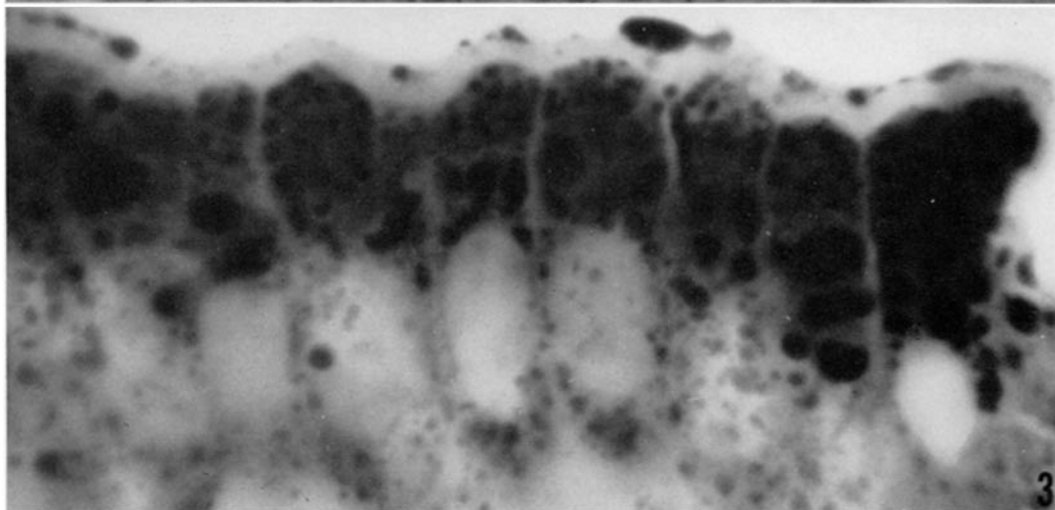
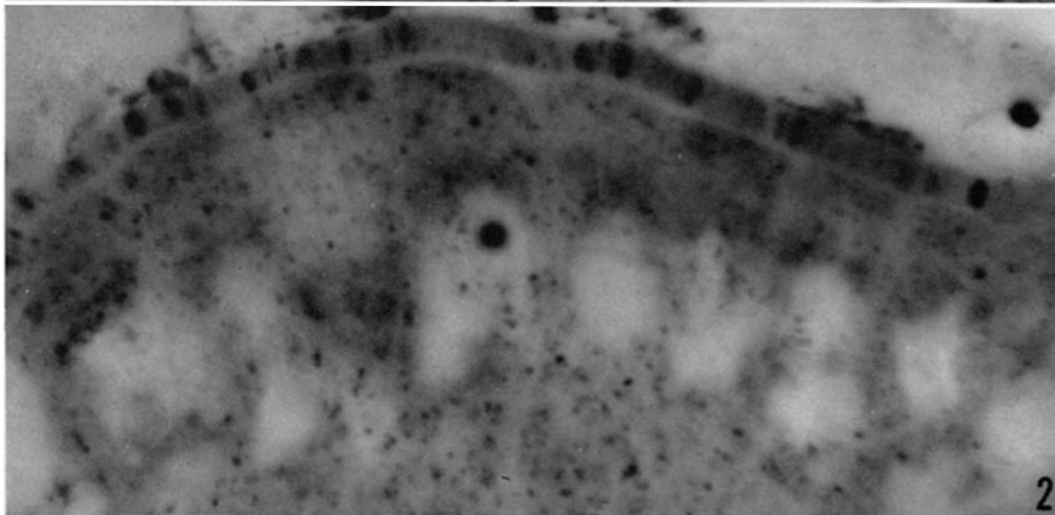
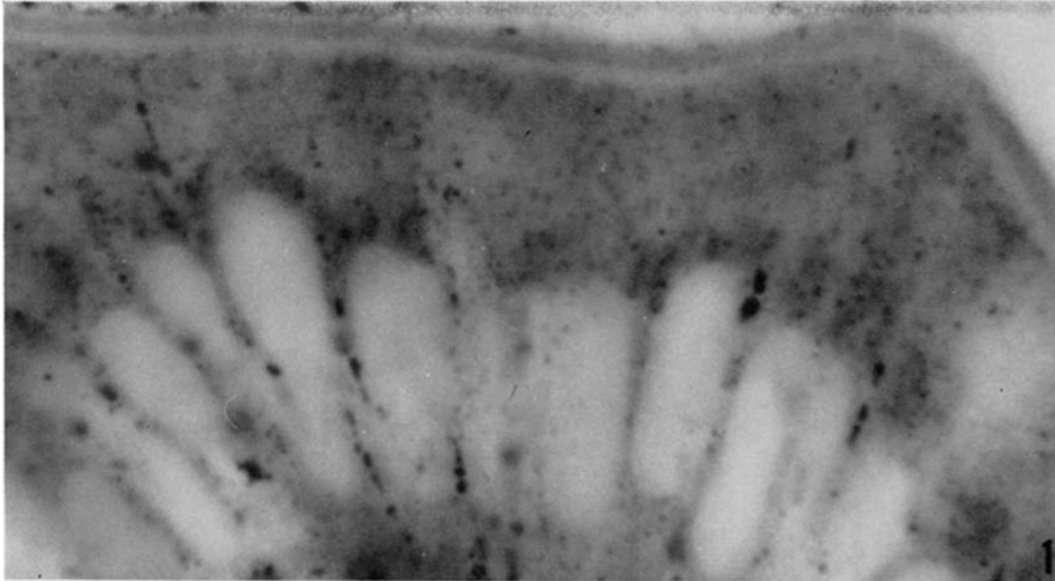
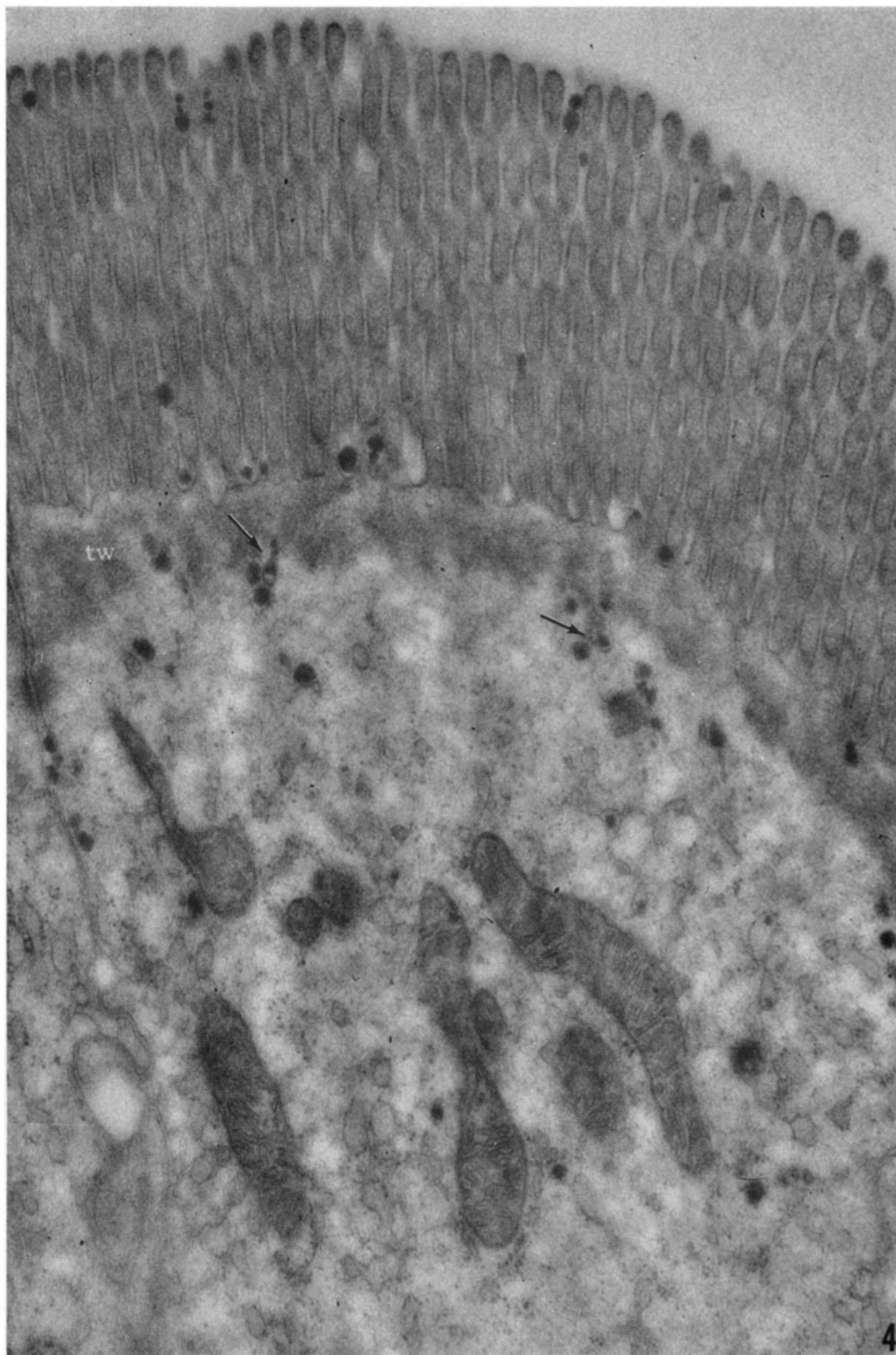


PLATE 161

FIG. 4. Striated border and apical cytoplasm of an intestinal epithelial cell from a rat that had been given 1.5 ml. corn oil 22 minutes before fixation.

Numerous small fat droplets in the intermicrovillous spaces of the striated border and a few droplets within vesicles (arrows) in the terminal web (*tw*) and just beneath it indicate that this cell is in the early stages of absorbing fat by pinocytosis. $\times 49,000$.

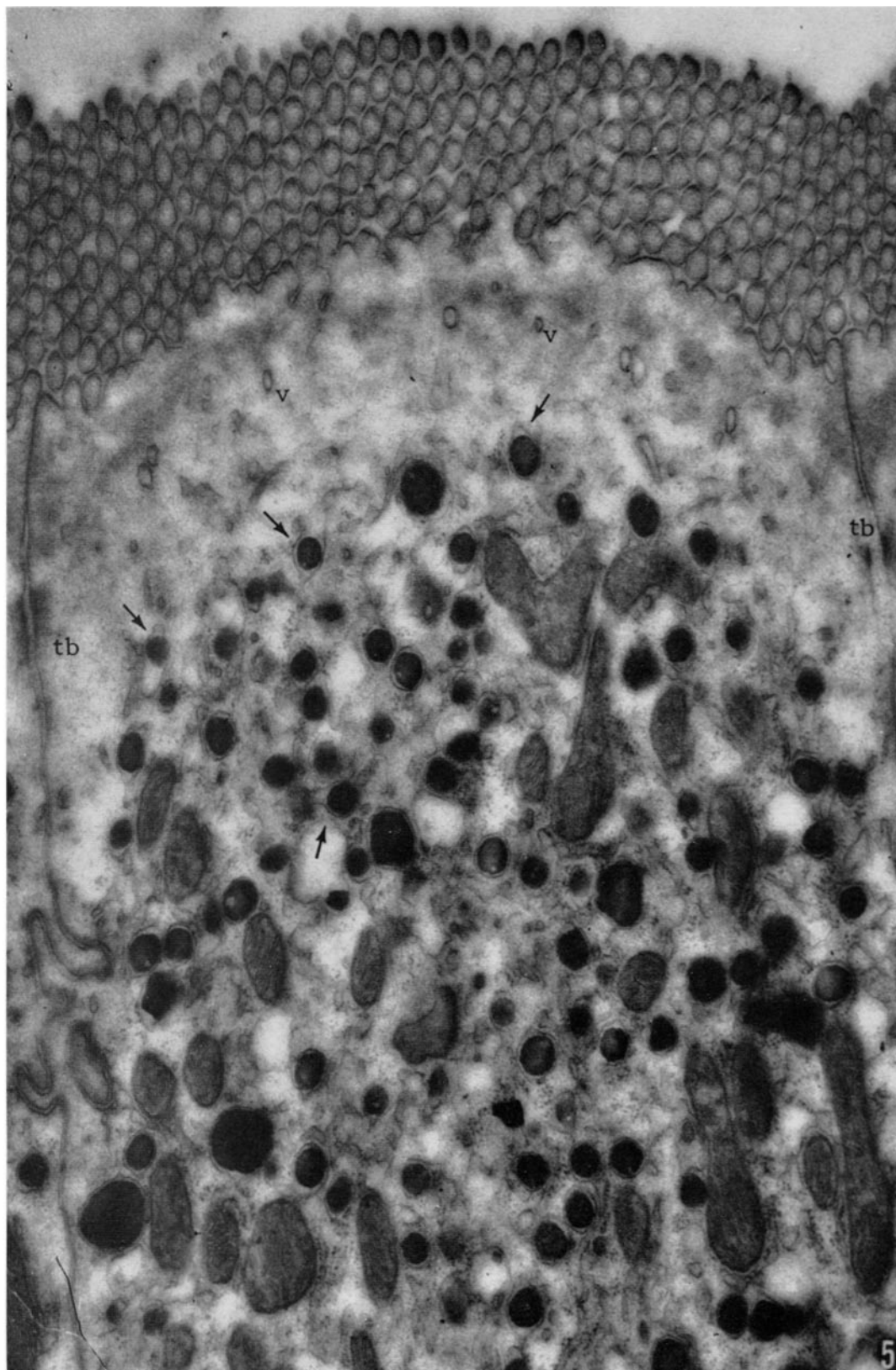


(Palay and Karlin: Pathway of fat absorption)

PLATE 162

FIG. 5. Striated border and apical cytoplasm of an intestinal epithelial cell from a rat that had been given 1.5 ml. corn oil 75 minutes before fixation.

The excessive breadth of the terminal web is a consequence of the oblique plane of the section with respect to the long axis of the cell. Note the terminal bars (*tb*) on each side. In the terminal web and the subjacent cytoplasm are small membrane-limited structures which are interpreted as pinocytotic vesicles (*v*) in the process of traversing the apical ectoplasm to join the endoplasmic reticulum. Deeper in the cytoplasm and filling most of the picture are individual droplets of fat. Each droplet is enclosed within a membranous envelope. Some of the profiles of these envelopes (arrows) indicate clearly that they are tubules probably joined together to form a continuous membrane-limited labyrinth in the cytoplasm, *i.e.* they are elements of the endoplasmic reticulum. Profiles of mitochondria are also present. Note the paucity and disorderly disposition of the granular endoplasmic reticulum compared with the appearance in the epithelial cells of the fasted animal (Figs. 1 and 2 in reference 48). $\times 39,000$.



(Palay and Karlin: Pathway of fat absorption)

PLATE 163

FIG. 6. Fat droplets in the apical cytoplasm of an intestinal epithelial cell from a rat that had been given 1.5 ml. corn oil 25 minutes before fixation. The droplets are of nearly uniform size and each is enclosed in an agranular membranous capsule. Many membrane-bound profiles are present which do not contain fat droplets. $\times 57,000$.

FIG. 7. Fat droplets in the apical cytoplasm of an intestinal epithelial cell from a rat that had been given 1.5 ml. corn oil 25 minutes before fixation.

These droplets are also of nearly uniform size although some appear crenated. Each droplet lies within a membranous envelope, and attached to the outer surfaces of some of these are small granules (arrows). These profiles demonstrate that the fat droplets are within elements of the granular endoplasmic reticulum. $\times 57,000$.

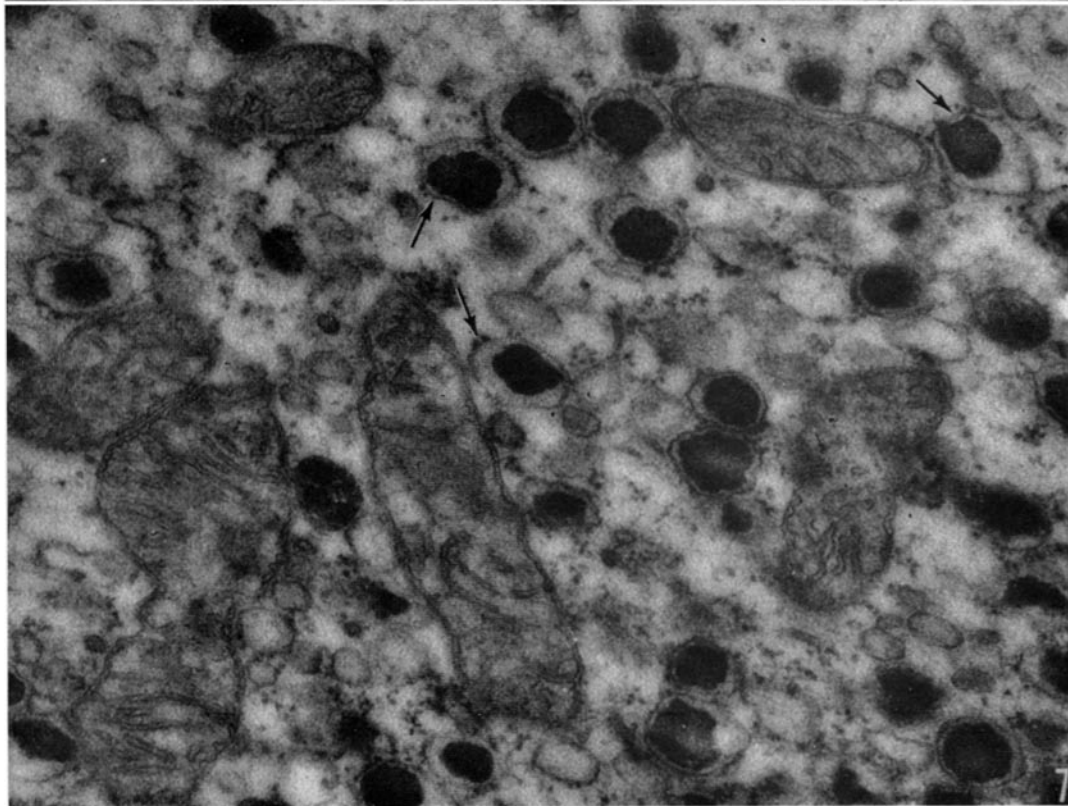
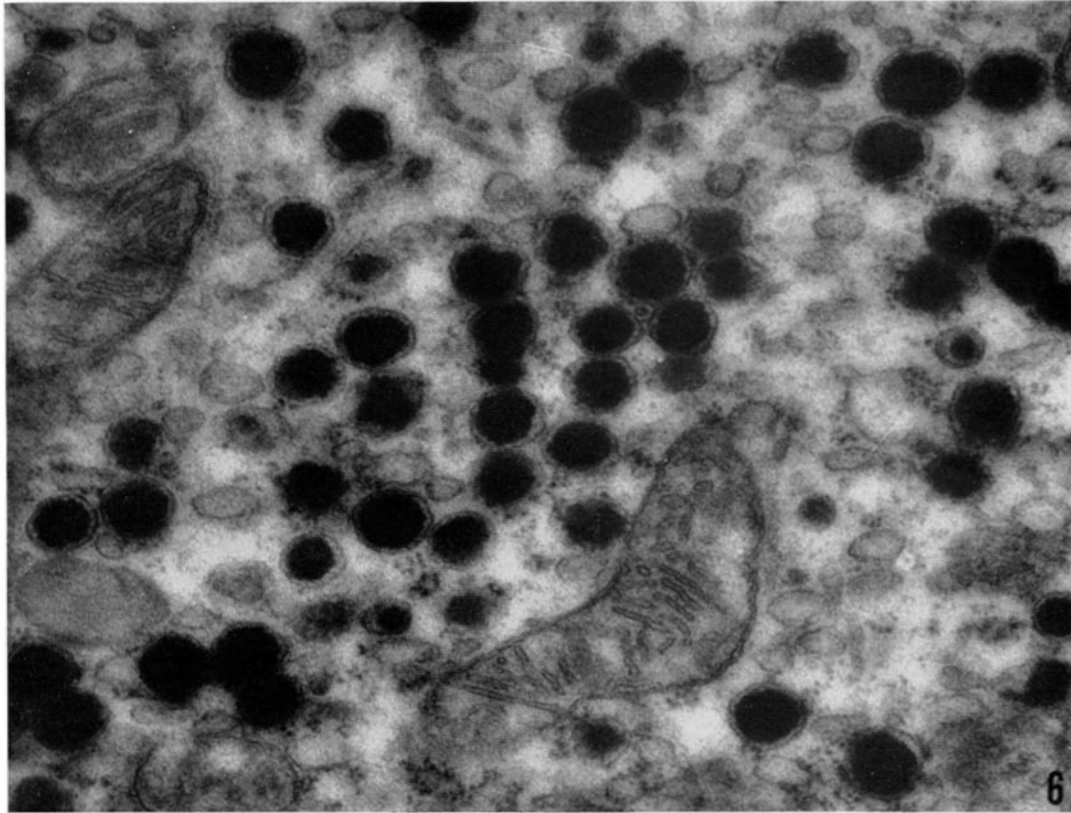
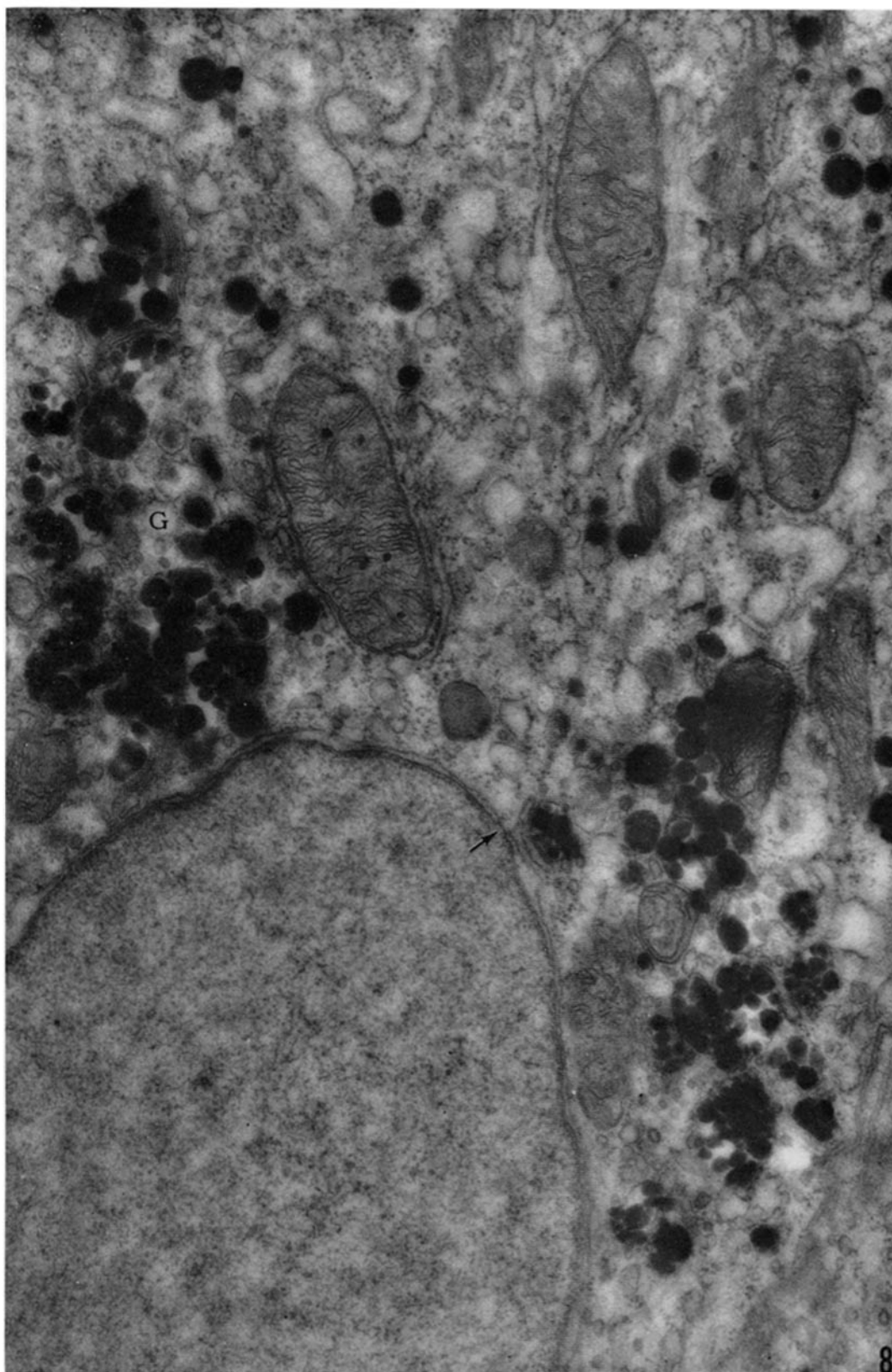


PLATE 164

FIG. 8. Nucleus and supranuclear cytoplasm of an intestinal epithelial cell from a rat that had been given 1.5 ml. corn oil 210 minutes before fixation.

The nucleus, at the lower left, consists of a nearly homogeneous mass of fine granules, some of which appear to be arranged in rows and threads, particularly near the center. The nucleus is bounded by a so called "double membrane" enclosing the perinuclear cisterna. A nuclear pore is indicated by the arrow. A row of granules is aligned immediately within the nuclear envelope.

Three of the mitochondria contain small adielectronic particles. A close relation between the elements of the granular endoplasmic reticulum and the mitochondria is illustrated particularly by the two mitochondria near the middle of the figure. The fat droplets in this cell are nearly all in the cisternae of the Golgi complex (*G*). The more apical cytoplasm was nearly free of fat. $\times 49,000$.



(Palay and Karlin: Pathway of fat absorption)

PLATE 165

FIG. 9. Intestinal epithelium from a rat that had been given 1.5 ml. corn oil 70 minutes before fixation. The section passes transversely across the cells at the nuclear level in a plane parallel with the free surface of the epithelium. The nucleus in the center of the cell pictured has the characteristics described in Fig. 8. The cytoplasm appears somewhat pale, probably because of the exceptionally long period of immersion in the fixative in this instance (3.5 hours). Intracellular fat droplets are rare at this level of the cell. Numerous fat droplets occupy the intercellular spaces of the epithelium. This micrograph confirms the interpretation of stained preparations studied in the light microscope (49). Note that the droplets are devoid of their membranous envelope. $\times 13,000$.

FIG. 10. Intestinal epithelium from a rat that had been given 1.5 ml. corn oil 60 minutes before fixation. The section lies in a plane perpendicular to the free surface and parallel with the long axes of the epithelial cells. The micrograph shows the infranuclear portions of the cells applied directly upon a delicate basement membrane. The nucleus and cytoplasm of a fibroblast lie just beneath (*fb*). Clusters of fat droplets have collected in the intercellular spaces of the epithelium above the basement membrane. $\times 22,000$.

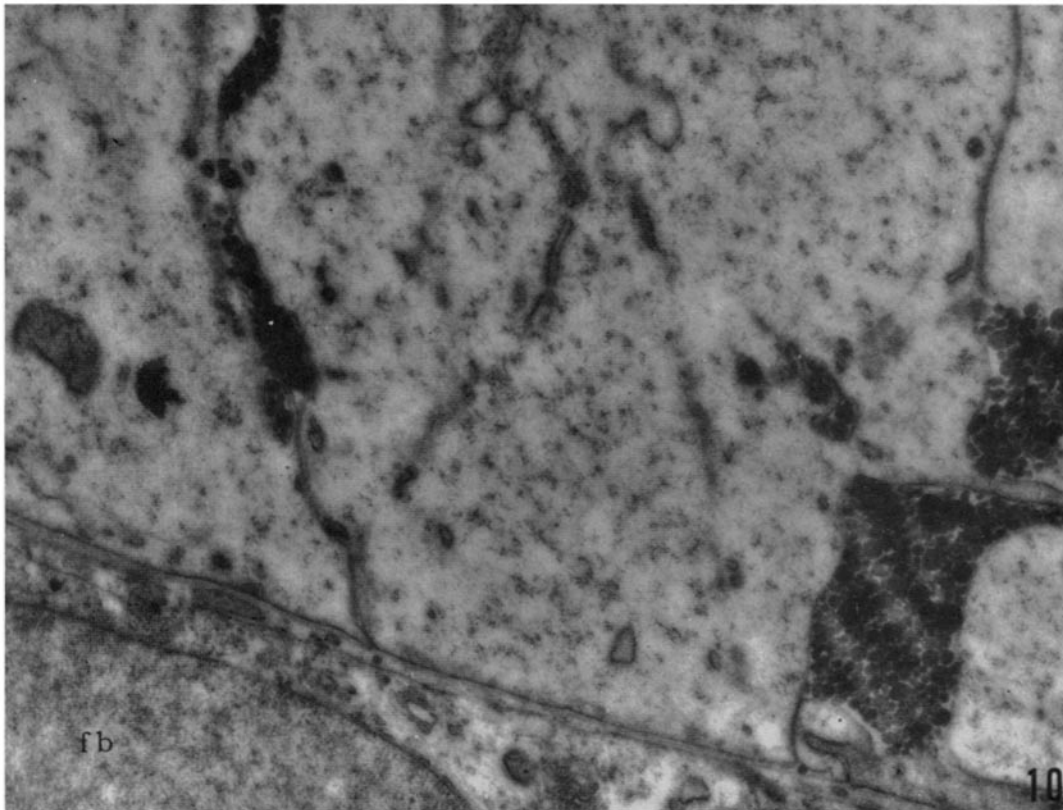
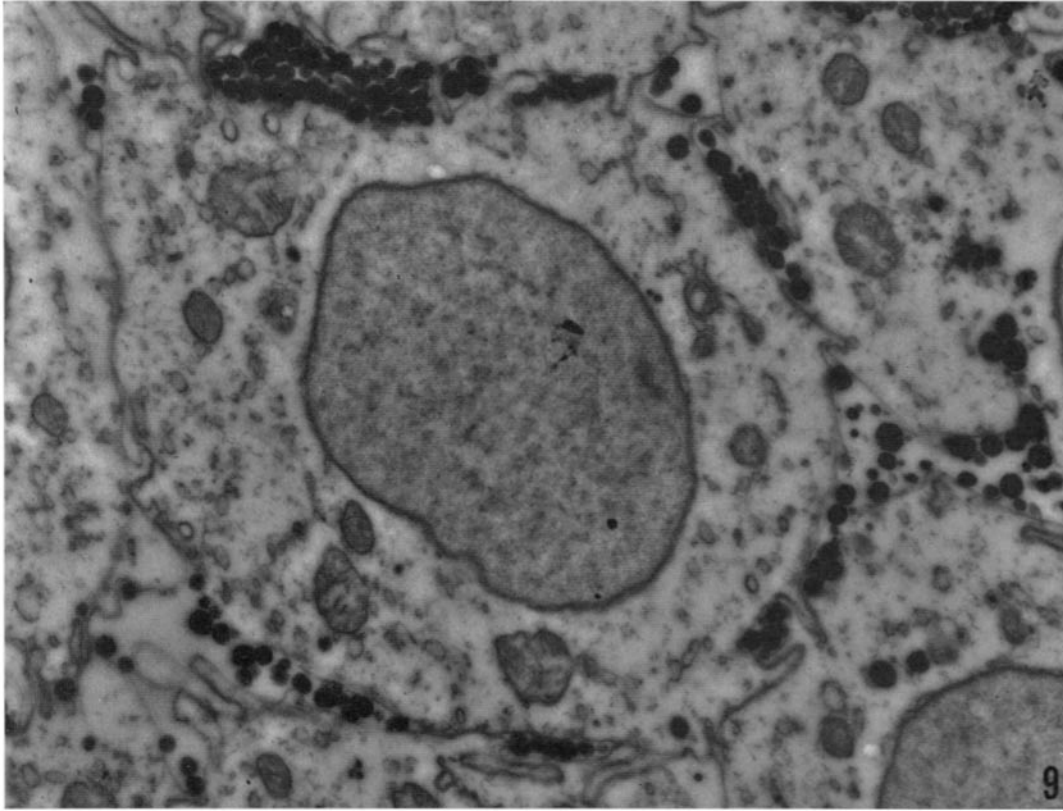


PLATE 166

FIG. 11. Junction between the lamina propria and intestinal epithelium from the jejunum of a rat that had been given 1.5 ml. corn oil 37 minutes before fixation. The bases of the epithelial cells occupy the right side of the figure. A thin basement membrane (bm_1) delimits the epithelium from the lamina propria. A blood capillary containing two erythrocytes courses from top to bottom of the figure. The endothelium of this vessel displays large numbers of pinocytotic vesicles and minute fenestrations. A second basement membrane (bm_2) completely surrounds it. Fat droplets are shown between the epithelial cells (f_1), within vesicles of the capillary endothelium (f_2), and among the collagen fibrils of the intervening connective tissue (f_3). It is exceedingly rare to find fat droplets either in the lumen of blood capillaries or in their endothelial cells (f_2). $\times 22,000$.

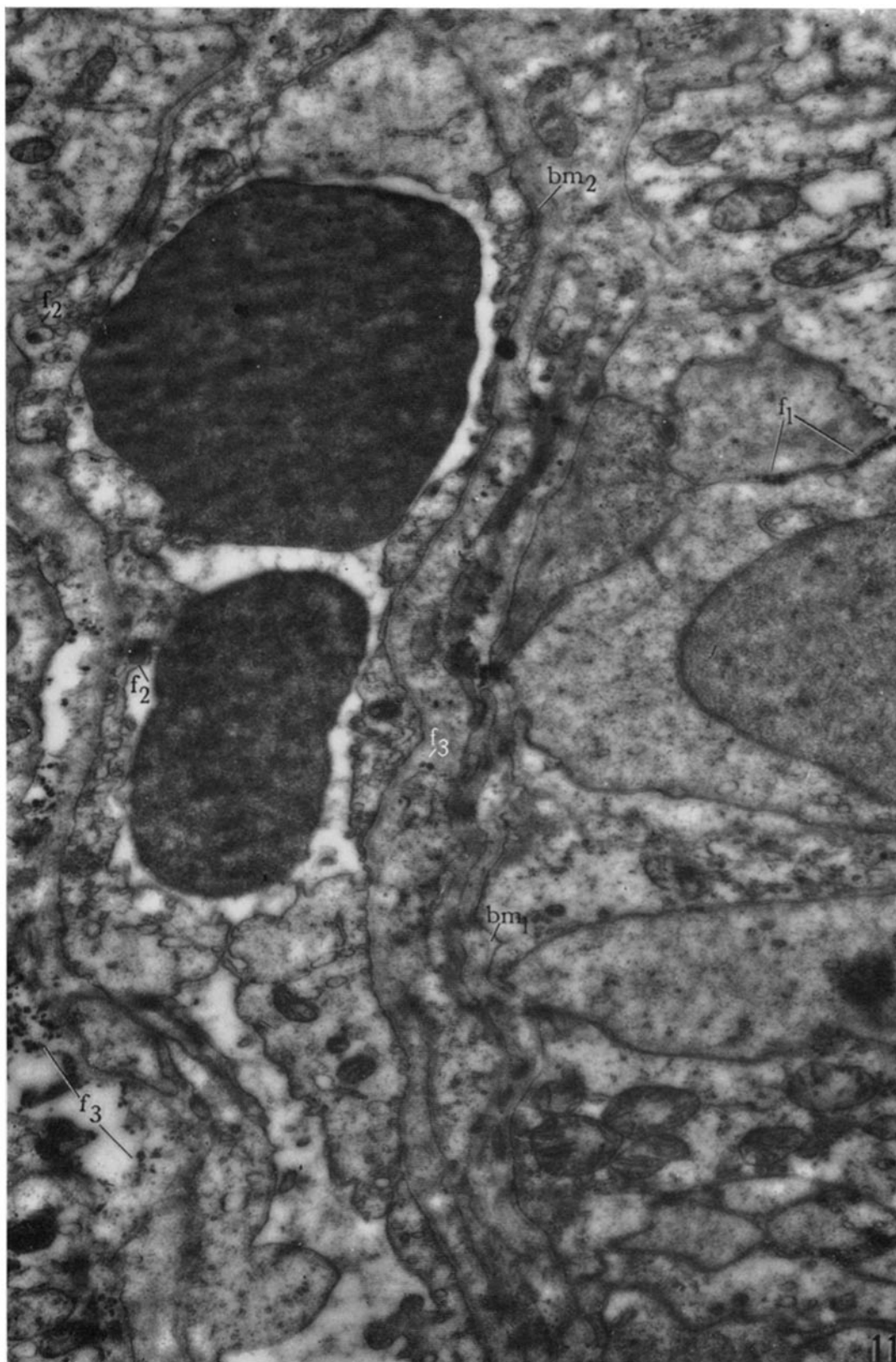


PLATE 167

FIGS. 12 to 14. Portions of capillary walls in intestinal villi from a rat that had been given 1.5 ml. corn oil 3.5 hours before fixation.

The figures illustrate the characteristic morphological features of capillaries in the lamina propria of the villi: extremely attenuated endothelial lining, the cells of which overlap at their edges (as at *ic* in Fig. 12), are frequently perforated, and display numerous pinocytotic vesicles (*pv* in Fig. 13). In the micrographs the thin basement membrane (*bm*) underlying the endothelium cannot always be distinguished from collagen fibrils lying alongside. In each of the pictures extracellular fat droplets are visible in the interstitial connective tissue.

The figures also show a rare observation: in Fig. 13 a single fat droplet is seen within the basement membrane and between two overlapping endothelial cells (arrow).

In Fig. 14, three fat droplets are seen within the lumen of the capillary, between an erythrocyte and the endothelium. Note in Fig. 14 that the fat droplets have an elliptical outline and that the erythrocyte is reciprocally indented. Perforations or fenestrations (*fe*) in the endothelial lining of the capillary are well shown in this figure.

In addition, the figures reveal several incidental observations in the erythrocytes. Each is bounded by a thin surface membrane which appears as an adielectronic line. The profile of a mitochondrion (arrow) is visible within one of the erythrocytes in Fig. 12. $\times 38,000$.

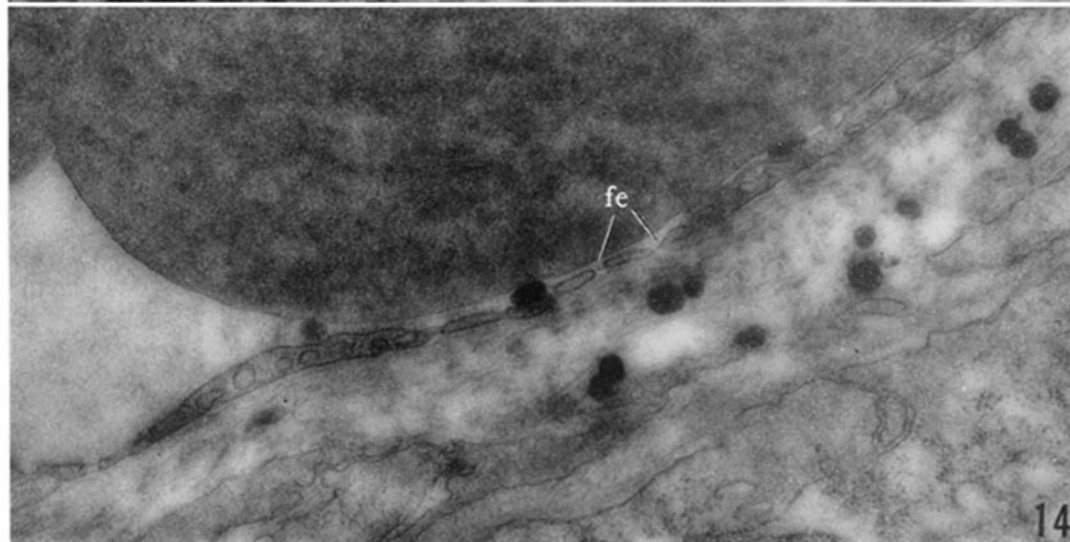
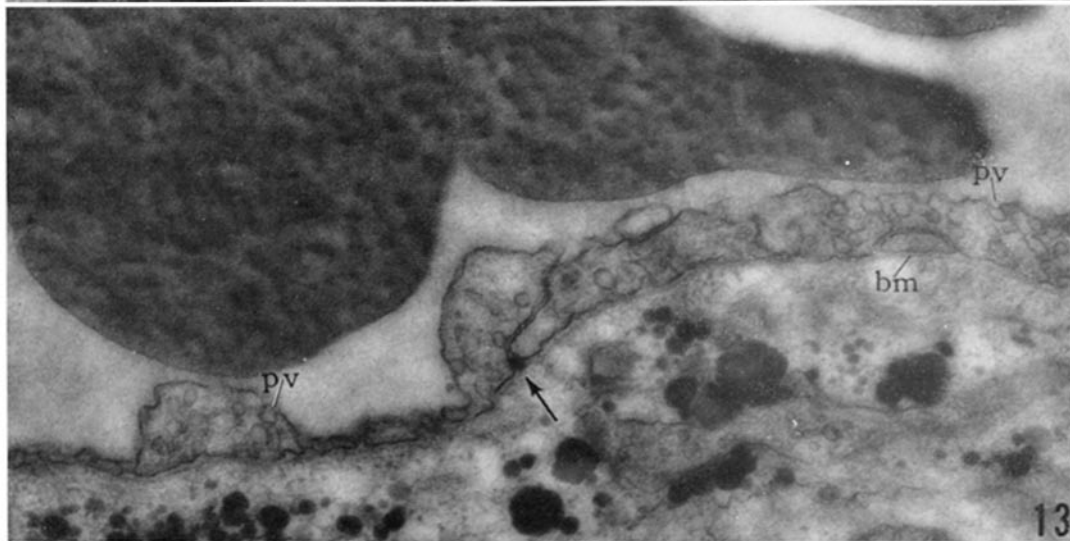


PLATE 168

FIG. 15. Portions of epithelial cells covering an intestinal villus from a rat that had been given 1.5 ml. corn oil 3.5 hours before fixation.

The field includes transverse sections of three epithelial cells at their interdigitating margins. Fat droplets lie in the intercellular spaces. Irregular profiles of the granular endoplasmic reticulum (*er*) lie close to the fluted plasmalemma of each cell. One of these profiles (*er*₁) is only about 20 m μ from the surface of its cell. This proximity suggests that the fat droplets contained in the lumen of the endoplasmic reticulum may be discharged into the intercellular spaces by coalescence of the plasmalemma with the membrane of the endoplasmic reticulum. An irregularly shaped lipide mass (*l*) in the cytoplasm of the cell at the left is representative of lipide material which is not enclosed in a membranous envelope and which appears in epithelial cells that are loaded with absorbed fat. *n*, nucleus of epithelial cell. $\times 46,000$.

FIG. 16. Lacteal in the center of a villus from a rat that had been given 1.5 ml. corn oil 75 minutes before fixation. The lumen of this vessel contains many fat particles of assorted sizes. A group of fat droplets lies in the interspace between overlapping endothelial cells (arrow), apparently entering the lumen. The interstitial connective tissue surrounding the vessel is packed with fat droplets. Note that all of these extracellular droplets lack membranous capsules. $\times 14,000$.

