THE ABSORPTION OF FAT BY INTESTINE OF GOLDEN HAMSTER *IN VITRO*

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ABSTRACT

Everted sacs of intestine from golden hamsters were incubated at 37°C for at least 1 hour *in vitro* with emulsified lipid after removal of both pancreatic lipase and bile salts. The fine structure of intestinal epithelium is well preserved under these conditions. Absorption of fat by the intestinal mucosa *in vitro* closely resembles lipid absorption *in vivo*, as observed by both light and electron microscopy. The physiological significance of these observations is discussed. Tubular elements of the agranular endoplasmic reticulum are often strikingly abundant in the apical cytoplasm of intestinal absorptive cells. These have a role in the intracellular transport of fat since they frequently contain droplets of lipid derived from the incubation medium. The rate of fat accumulation in the epithelium appears to be proportional to the concentration in the medium.

During digestion, triglycerides of long-chain fatty acids are physically and chemically modified in the intestinal lumen by pancreatic lipase and bile salts. The actions of these agents result in a complex mixture of glycerides and fatty acids in a finely divided state. There has long been considerable uncertainty as to the nature of the lipids that are finally absorbed from this mixture, and as to the mechanism by which these compounds traverse the epithelium to gain access to the lacteals of the lamina propria.

Interest in the pathway traversed by lipid in its passage through the intestinal epithelium has been greatly stimulated by electron microscopic studies of Palay and Karlin (1) and Palay (2) on the intestinal mucosa of rats fed corn oil. Their observations strongly indicated that minute droplets of fat were taken in at the free surface of the cell by pinocytosis and transported through membrane-limited canalicular elements of the endoplasmic reticulum to the Golgi region and from thence to the lateral surfaces of the cells. There, the lipid appeared to be discharged into the space between adjacent cells and to be extracellular for the remainder of its path through the epithelium. These observations have been confirmed (3) and extended to include man (4). The participation of pinocytosis in lipid absorption, although still not conclusively demonstrated, has gained widespread acceptance. In the *in vivo* studies reported by others, the presence of pancreatic lipase makes it difficult to exclude the possibility that lipolysis may have taken place before absorption.

MATERIALS AND METHODS

These experiments employed the method of Wilson and Wiseman (6) in which small sacs of everted intestine are incubated in various media while being agitated on a shaker. The eversion of the gut appears to permit adequate oxygenation of the intestinal mucosa in the absence of a circulation.

Male hamsters weighing 100 gm were fasted overnight, killed by a blow on the head, and the abdominal cavity was opened. The contents of the small intestine were washed out with 50 ml of 0.9 per cent NaCl and the small bowel except the duodenum was stripped from the mesentery and placed in a Petri dish containing 0.9 per cent NaCl. The intestine was everted with a stainless steel rod and

washed for 1 minute in each of three beakers containing 100 ml of 0.9 per cent NaCl. A segment of the everted intestine (usually about 4 cm in length) was tied at one end and a blunt needle attached to a tuberculin syringe introduced into the open end. The intestine was tied loosely to the needle, 0.5 to 1.0 ml of Krebs-Henseleit bicarbonate-saline (7) introduced, the needle withdrawn, and the thread tightened. The sac of everted intestine was then placed in a 50 ml Erlenmeyer flask with the appropriate medium and gassed with 5 per cent CO_2 and 95 per cent O_2. Incubation was carried out for 1 hour in a Dubnoff shaking incubator at 37°C. Control sacs were incubated in 5 ml of Krebs-Henseleit bicarbonate-saline containing 8 mg/ml glucose but no lipid. The mucosa of experimental sacs was exposed to 5 ml of medium consisting of bicarbonate-saline with 30 mg/ml cottonseed oil, 2 mg/ml lecithin, 8 mg/ml glucose, and 0.6 mg/ml of an ethylene oxide polypropylene glycol polymer as a co-emulsifier[1]. Lipid was present as an emulsion with droplets having an average diameter of less than 0.5μ. The addition of this mixture to the medium gave it a milky appearance which persisted throughout incubation.

Tissues were fixed for electron microscopy with 1.3 per cent osmium tetroxide in *s*-collidine buffer (8) with sucrose (9) at pH 7.6 in the cold for 6 hours, dehydrated rapidly with ethyl alcohol, and infiltrated with methacrylate which was polymerized with ultraviolet light using 2 per cent Luperco CDB as catalyst. Sections were cut with a Porter-Blum microtome and stained by method A of Karnovsky (10). Electron microscopy was performed with RCA model EMU-3E and 3F. Tissues were fixed for light microscopy with 10 per cent cold phosphate-buffered formalin, embedded in gelatin, sectioned with a freezing microtome, and stained with Sudan Black B for 7 minutes.

[1] These components, aside from the bicarbonate-saline, may be obtained from the Upjohn Company, Kalamazoo, Michigan, as "Lipomul," an emulsion used for intravenous feeding of patients.

LIGHT MICROSCOPE OBSERVATIONS

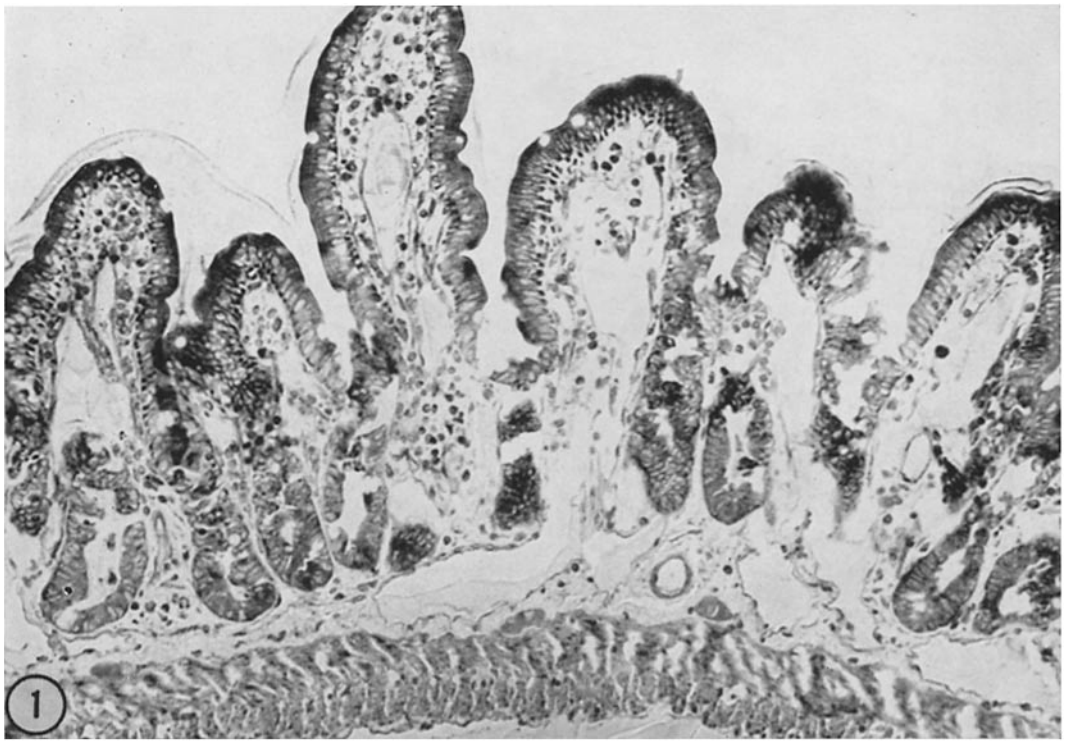
The present investigation (5) was undertaken to determine whether the isolated intestinal epithelium could absorb emulsified fat *in vitro* and to what extent the cytological events would parallel those already recorded by others for absorption of lipid *in vivo*. Doubt is frequently expressed as to the validity of conclusions drawn from *in vitro* studies on the supposition that one is dealing with cells deprived of their blood supply and undergoing rapid cytological deterioration. Therefore, a further objective of the present study was to assess with the electron microscope the degree of persistence of normal fine structure in intestinal epithelium maintained for a few hours under conditions of agitation in oxygenated physiological salt solution. The results provide the basis for new confidence in the validity of *in vitro* studies by showing that near normal cell structure is preserved throughout rather long incubation periods. It is also demonstrated that fat absorption takes place after removal of bile salts and pancreatic lipase and that it follows much the same pathway that it appears to follow during absorption *in vivo*. Frozen sections of control sacs after incubation in the lipid-free medium showed normal villous structure (Fig. 1). The epithelium stained gray owing to the sudanophilia of mitochondria and other membranous components of the cells but there was relatively little difference in depth of staining from the tips to the bases of the villi. Black staining droplets of lipid in the cells were uncommon. Similar preparations of sacs, in-

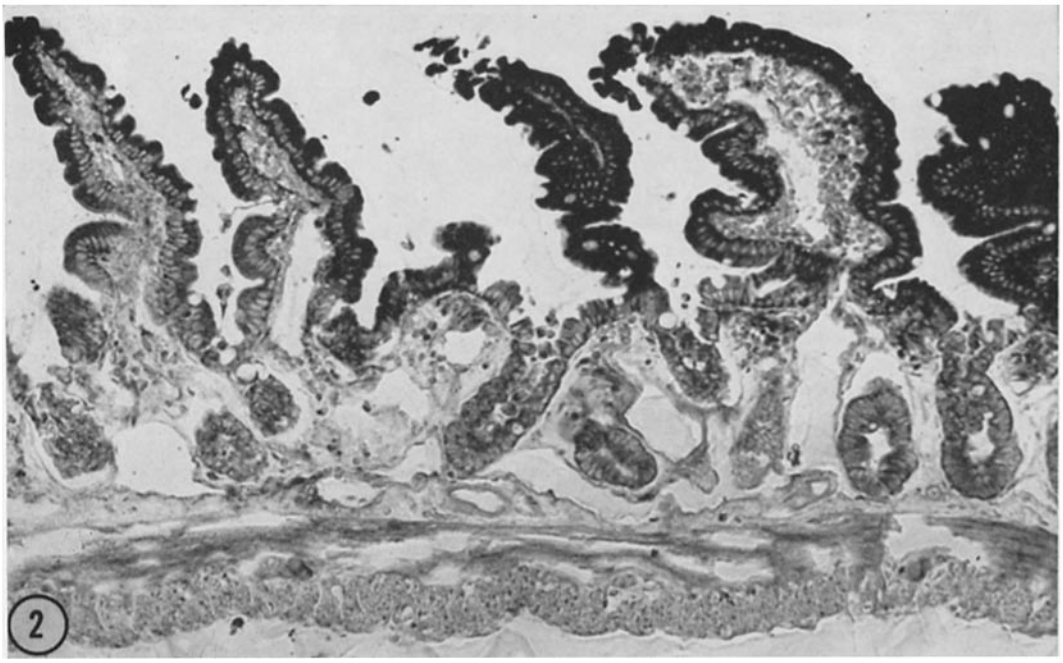
FIGURE 1

Photomicrograph of frozen section from control intestinal sac after incubation for 1 hour at 37°C in bicarbonate-saline *in vitro*. The slight sudanophilia of various types of cells is not extractable by acetone and is attributed to the phospholipid in membranous organelles. × 220.

FIGURE 2

Photomicrograph of frozen section from intestinal sac after incubation in bicarbonate-saline plus emulsified lipid for 1 hour at 37°C *in vitro*. There is an intense staining of epithelial cells on the distal parts of villi by Sudan Black B, due to the presence of absorbed fat. However, there is little absorbed lipid in epithelium on the bases of villi and none is present in the crypts of Lieberkühn. At higher magnifications of the light microscope absorbed lipid may be seen as sudanophilic droplets which are almost completely extractable by acetone. × 220.





cubated in medium containing the fat emulsion, showed a marked blackening of the epithelium of the villi but no appreciable change in that of the crypts of Lieberkühn (Fig. 2). The tips of the villi were very black and the intensity of staining diminished from the tips toward the bases of the villi. At higher magnification the Sudan staining could be seen to reside in numerous droplets of varying size in the cytoplasm of the epithelial cells. Droplets of lipid were also seen in the connective tissue of the lamina propria and in the lumen of lymphatics. Both intracellular and extracellular droplets were almost completely extracted before staining by exposure of the sections to 100 per cent acetone for 30 minutes. Similar treatment of sections of the control sacs incubated in lipid-free medium resulted in no significant change in their sudanophilia. Thus it is clear at the light microscope level that everted sacs of intestine can absorb emulsified lipid *in vitro*. The distribution of the absorbed fat as well as its rate of accumulation by the epithelium are influenced by the concentration of lipid in the medium. When sacs were incubated in medium containing one-tenth the usual concentration of emulsified fat, only the cells on the distal ends of the villi contained lipid droplets and then only in small numbers.

ELECTRON MICROSCOPE OBSERVATIONS

Electron micrographs of hamster intestinal mucosa fixed immediately after death (Fig. 3) closely resembled normal intestine of rat described by other authors (11). The striated border consists of closely packed microvilli in orderly array. The cytoplasm immediately subjacent to these is free of organelles but rich in fine filaments which have a preferred orientation parallel to the luminal surface and are closely compacted, constituting the so called terminal web. In the apical cytoplasm beneath this differentiated ectoplasmic layer is an accumulation of elongated mitochondria and a well developed plexiform system of anastomosing tubules having an amorphous content of appreciable density and limiting membranes that are free of associated ribosomal particles. This agranular reticulum appears to communicate with granular elements and also with smooth surfaced membranes of the Golgi complex in the supranuclear region. Occasional tubular invaginations of the plasmalemma between microvilli penetrate the region of the terminal web but, although these may communicate with the underlying agranular reticulum, the existence of continuity has not been demonstrated.

On their lateral surfaces the columnar epithelial cells are in close apposition and may deeply interdigitate in their upper portions but toward the base they frequently diverge leaving sizeable intercellular spaces. These usually appear empty but may occasionally be occupied by lymphocytes migrating through the epithelium. The basal surface is smooth contoured and rests upon a thin basement membrane which is continuous across the gaps between the bases of adjacent epithelial cells.

The foregoing description would apply equally well to the epithelium of sacs of intestine incubated *in vitro* for at least 1 hour. The changes in the striated border and the cytoplasmic organelles are very slight in the 1st hour or two *in vitro* if care is taken in fixation, dehydration, and embedding. There is some indication that the sensitivity of the tissue to induced artifacts of specimen preparation increases with the passage of time after death, and some of the distortions observed in electron micrographs may give a misleading picture of the state of the tissue at the time of fixation.

Electron micrographs of the mucosa from control sacs, incubated in bicarbonate-saline alone, fail to disclose the presence of lipid droplets within the endoplasmic reticulum and in the spaces between lateral plasma membranes of absorptive cells (Fig. 4), common sites for accumulation of

FIGURE 3

Electron micrograph of absorptive cells on a villus from unincubated, promptly fixed intestine of golden hamster. This animal was not fasted and a few small dense droplets, presumably of lipid, are present within the abundant profiles of the endoplasmic reticulum. Note how the apical plasma membrane is arrayed in evenly spaced microvilli and also invaginates (*Inv*) for short distances into the terminal web. The lateral plasma membranes of the apical parts of these cells are closely apposed throughout their elaborate interdigitations. × 20,000.


Inv
Reticulum
3

lipid during fat absorption. The scarcity of absorbed lipid at these places or elsewhere in the tissue indicates that there was only minimal transport of fat by the control sacs.

Electron micrographs of epithelium from sacs incubated in lipid-enriched medium occasionally reveal minute droplets in small numbers between microvilli of the striated border and within the invaginations of the plasma membrane in the terminal web. Droplets of somewhat larger size are present in considerable numbers in the apical cytoplasm (Fig. 5). These are almost invariably enclosed by a membrane and in some planes of section it is clear that they reside within vesicular expansions of the tubular elements of the endoplasmic reticulum (Fig. 6). This reticulum is well developed in the hamster intestinal epithelium and is of the agranular variety. Although ribosomes are abundant in the cytoplasmic matrix they are only infrequently associated with membranes. The reticulum has a close-meshed organization and a moderately dense content. Its tubular elements characteristically show abrupt changes of caliber and angular profiles that are rarely seen in typical granular endoplasmic reticulum.

In addition to the small droplets located within the agranular reticulum, accumulations of larger droplets are frequently found within vacuoles of the Golgi complex (Fig. 5) and are less commonly found in the basal cytoplasm as well. Numerous droplets of lipid are also crowded into the extracellular spaces between the lateral surfaces of adjacent cells at the base of the epithelium.

DISCUSSION

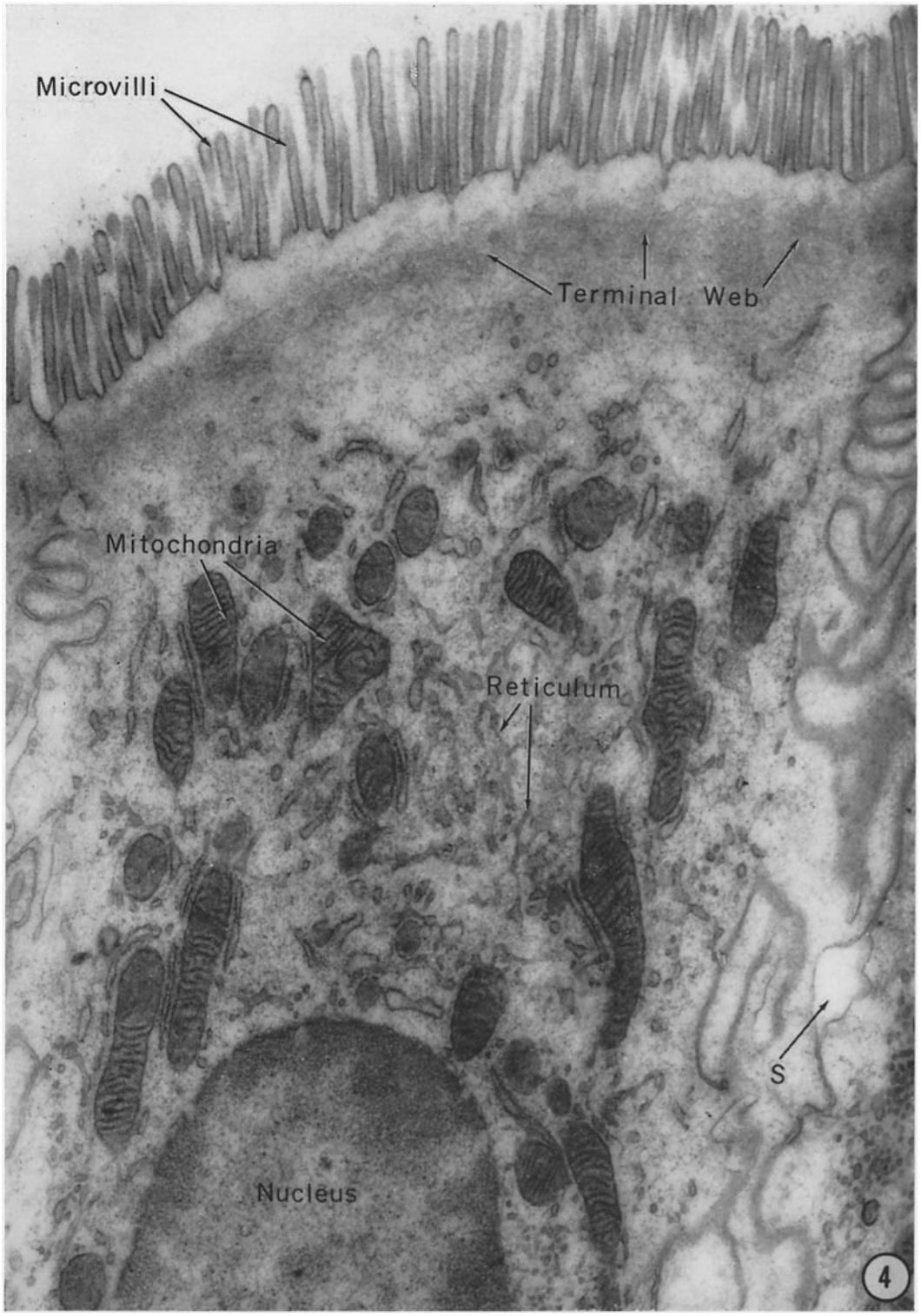
It has been demonstrated that isolated intestinal epithelium can absorb an artificially emulsified fat *in vitro* and that distribution of lipid in the villus, as seen with the light microscope, and its localization within the cells, as viewed with the electron microscope, are strikingly similar to that described for lipid absorption in the intact animal. The metabolic integrity of a similar preparation of intestine has been previously demonstrated by means of chemical techniques (24) showing transport of fatty acid from a palmitic acid-albumin solution.

There have been two conflicting views concerning the mechanism of fat absorption by the intestine, one holding that hydrolyzed fat is taken up in particles of molecular dimensions or micelles formed by the actions of pancreatic lipase and bile salts (12, 13), the other contending that it is incorporated largely as triglyceride in the form of small droplets (14). The results reported here tend to support the latter view in that they indicate that the pathway for lipid absorption is the same *in vitro*, after removal of both pancreatic lipase and bile salts, as it is *in vivo*, in their presence. It is tempting to assume, as others have (1, 3), that during fat absorption lipid droplets penetrate between the microvilli of the striated border, are then taken into the cell by pinocytosis, and are transported across the terminal web in vesicles or tubules that become continuous with the endoplasmic reticulum. On the other hand, the small number of lipid droplets present within the striated border, and the fact that they are infrequently found in invaginations of the plasma membrane or in membrane-limited canalicular structures traversing the terminal web, make it difficult to believe that such a mechanism could account for the substantial amounts of lipid absorbed without revealing itself more clearly in electron micrographs in the form of droplets in passage through the absorbing surface.

Studies employing isotopic tracers indicate that both free fatty acids and glycerides are absorbed after feeding triglyceride (15). Perhaps it is erroneous to assume that the mechanism of fat absorption involves *either* the uptake of the extremely small particles of solubilized lipid *or* incorporation in bulk. It may well be that both occur. In primitive Metazoa in which extracellular digestion is not highly developed, the evidence of uptake of material, including lipid by pinocytosis, is compelling (16). In mammals the evidence is equivocal. One may speculate that pinocytosis is a primitive mechanism which persists to some ex-

FIGURE 4

Apical part of an absorptive cell from a control sac, incubated for 1 hour at 37°C in bicarbonate-saline. Droplets of absorbed lipid are notably absent from cytoplasmic components such as the endoplasmic reticulum or in the intercellular spaces (*S*) between lateral plasma membranes. × 27,000.


Microvilli
Terminal Web
Mitochondria
Reticulum
S
Nucleus
4

tent in higher forms, but that concurrently with the evolution of special accessory digestive glands secreting lipases and other enzymes into the lumen of the gastrointestinal tract it may have come to play a subsidiary role. While both mechanisms may coexist, absorption of products of hydrolysis of fat may well be quantitatively more important in the intact animal than uptake of fat in bulk. Whatever the mechanism of entry of fat into the cell, this study has confirmed the observation of Palay and Karlin that the agranular endoplasmic reticulum plays an important role in the intracellular transport of lipid. There are indications, too, that this form of the reticulum may be involved in the handling of lipid in other ways than merely as a pathway directing its movement to the Golgi region and to the lateral surface of the cell. Agranular reticulum has been found to be well developed in a variety of other cell types active in biosynthesis of steroids (17–19) and in other types of lipid metabolism (20, 21). Moreover biochemical studies have demonstrated, in the microsome fraction of intestinal mucosa, a thiokinase for activation of long-chain fatty acids (22).

Numerous problems relating to intestinal absorption of fat remain to be solved. The results reported here lend confidence in the value and validity of *in vitro* methods for pursuing these problems by showing that cells remain essentially normal in appearance at the ultrastructural level throughout periods of incubation in excess of 1 hour. These findings are consistent with the results of other recent studies which have shown that the mitochondria and other membranous structures of the cytoplasm persist for longer periods after removal from the animal than is generally supposed (23).

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FIGURE 5

Apical and supranuclear portions of several absorptive cells from an experimental sac incubated in bicarbonate-saline plus emulsified fat for 1 hour at 37°C *in vitro*. Numerous interconnected tubular forms belonging to the endoplasmic reticulum are present. There are many electron-opaque droplets of fat within tubular (*T*) and vesicular (V) profiles of the organelle, but there is little evidence of absorbed lipid in the terminal web. Vacuoles (*G*) in the supranuclear Golgi region contain large droplets of fat. × 22,000.

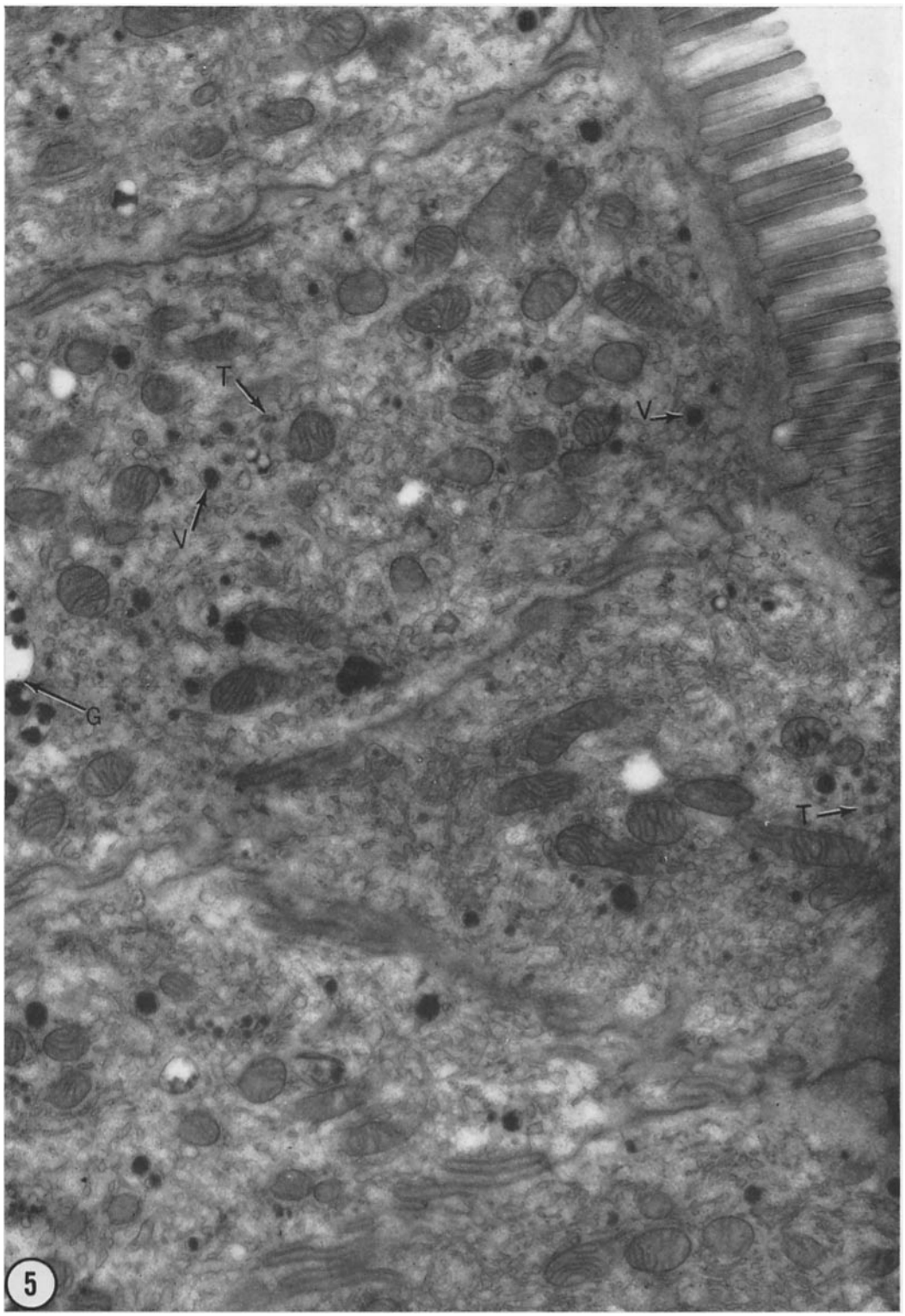
T
V
V
G
T
5

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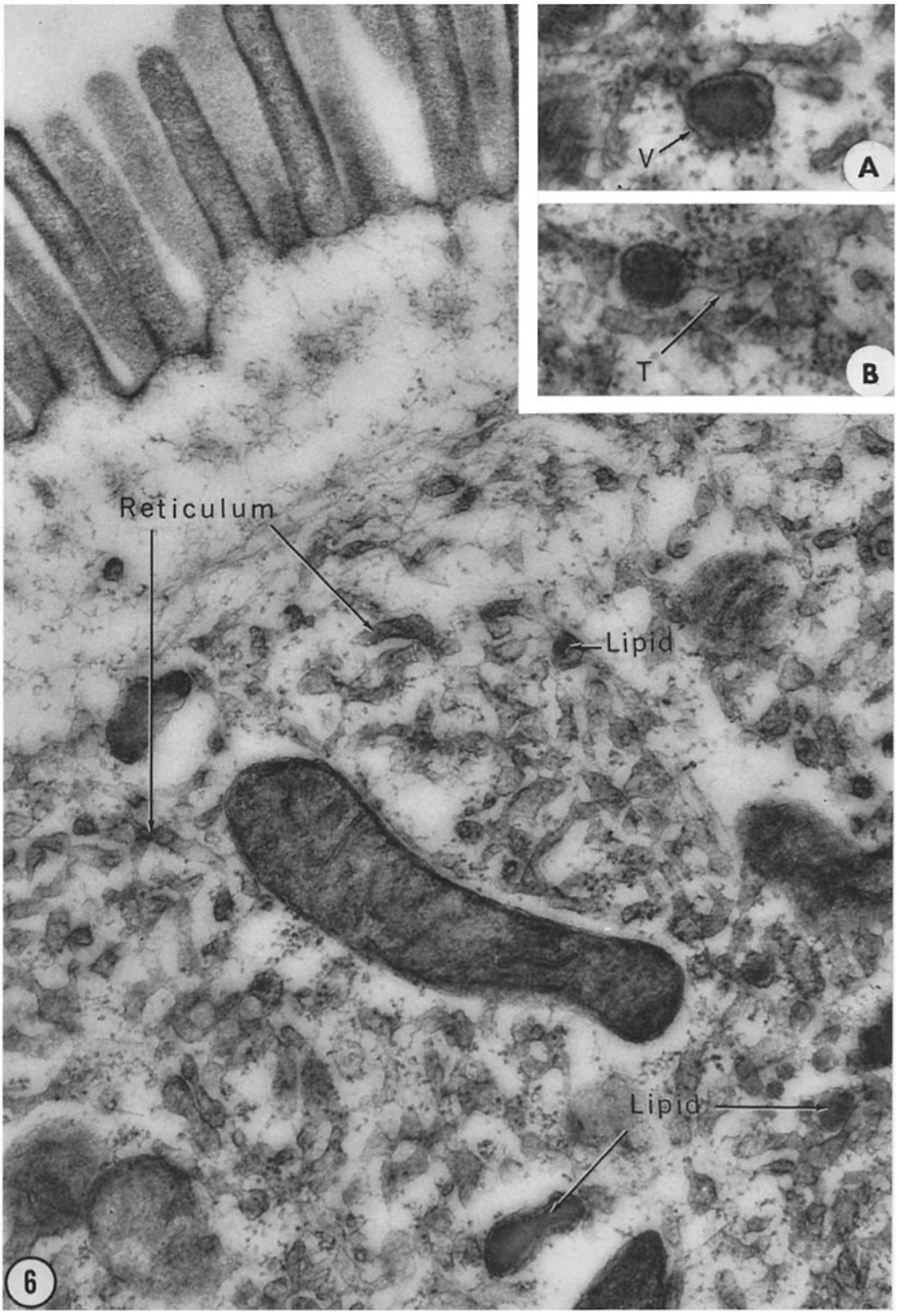
FIGURE 6

Apical portion of absorptive cell from experimental sac incubated with emulsified fat. In many regions like this the endoplasmic reticulum consists of a fine meshwork of tubular elements which have localized segments of abruptly increased calibre. Some of the dilated regions contain lipid droplets. Larger droplets are within vesicular profiles which do not have tubular connections in the plane of this section. This reticulum has a content of moderate density and its membranes are largely agranular. Numerous ribosomal granules seem to be free in the cytoplasm. × 45,000.

The inset contains two other regions from the apical cytoplasm of the same cell. In *A* there is a vesicular profile (*V*), and in *B* a tubular form (*T*) with vesicular expansions at its ends. A droplet of lipid within each profile is separated from the limiting membrane by material of moderate density. The close resemblance between the upper and lower lipid-containing profiles suggests that these belong to similar structures but that the tubules attached to the vesicular form (*V*) were outside of the plane of this section. × 63,500.


V
A
T
B
Reticulum
Lipid
Lipid
6