New views on intestinal absorption of lipids in teleostean fishes: an ultrastructural and biochemical study in the rainbow trout

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Abstract Lipid absorption in rainbow trout was studied after gastric administration of [1-^{14}C]linoleic and [1-^{14}C]-palmitic acids. The intestinal epithelial cells were isolated at various times of absorption and the major lipid classes were isolated. Radioactivity was found primarily in the triglycerides. Blood radioactivity was measured at different times after administration of the labeled acids. It was very low until after 6 hr. After 4 hr when it was detectable, it was located essentially in the triglyceride fraction. At various times after feeding (a meal with 60% of unsaturated long chain fatty acids) the absorptive epithelium of the anterior intestine and pyloric caeca were examined by electron microscopy. Surprisingly, the esterification of fatty acids corresponded to the formation of VLDL-like particles, seen in SER, RER, Golgi apparatus, lamellar structures, intercellular space, interstitial space of lamina propria and lumen of lymphatic vessels. The respective roles of endoplasmic reticulum and Golgi apparatus in the lipoprotein synthesis are discussed. The particles are delivered in the intercellular spaces by way of the lamellar structures, whose role until now was unknown. Though the absorption of dietary triglycerides is much slower than in mammals, the mechanism does not differ fundamentally. The long chain fatty acids are esterified by the intestinal cells and transferred as VLDL-like particles to lymph.—**Sire, M-F., C. Lutton, and J-M. Vernier.** New views on intestinal absorption of lipids in teleostean fishes: an ultrastructural and biochemical study in the rainbow trout. *J. Lipid Res.* **22:** 81–94.

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It is generally thought that no chylomicrons are found in the lymph or the blood of teleostean fishes. The question may then be asked: how does absorption of long chain fatty acids take place after intraluminal hydrolysis of alimentary triglycerides? Robinson and Mead (1) have studied the time course of absorption in rainbow trout fed [1-^{14}C]palmitic acid. They concluded that fish are incapable of delivering triglyceride to its circulation in the usual way (as chylomicrons), and they receive most of the absorbed lipid as free fatty acids, probably via the portal system. In 1976, Kayama and Iijima (2) confirmed this in studies with carp. They found that fatty acids enter the blood associated with albumin-like-proteins and are directly transported to the tissues, and that although chylomicrons are absent, the epithelial cells of intestine could form triglycerides which might be transported by plasma lipoproteins. Cowey and Sargent (3) stated that "the balance of evidence then is that fat assimilation and translocation in fish differ from mechanisms present in mammals but further data are desirable to identify the course of events in fish".

Endogenous lipid particles whose size approaches that of VLDL (4) have been shown in the epithelial cells of intestine from trout embryo or alevin. In an immunoelectrophoretic study of plasma lipoproteins in normally fed trout, we showed two precipitation lines due to VLDL (5), one of which corresponded to hepatic VLDL (6). By electron microscopy, VLDL-like particles have been seen in the intracellular membranes of the epithelial cells of intestine in the trout alevin 3 days after the first meal (7), in the young trout (8) and in the adult trout (9). In view of these data, we have reexamined the problem of lipid absorption in this species. The question arises as to whether VLDL might not play a part similar to that of chylomicrons in mammals. We have also undertaken an ultrastructural study of the trout intestinal cell during lipid absorption and have examined whether or not long chain fatty acids are reesterified intracellularly, by the use of [1-^{14}C]palmitic and [1-^{14}C]linoleic acids.

Contrary to the conclusions of Cowey and Sargent (3), we feel, at present, that the mechanisms involved

Abbreviations: VLDL, very low density lipoprotein; SER, smooth endoplasmic reticulum; RER, rough endoplasmic reticulum; RE, endoplasmic reticulum; MZ, mitochondrial zone.

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in the lipid absorption in fish are very similar to those previously described in mammals.

MATERIAL AND METHODS

Isotopes

[1-^{14}C]Palmitic acid (53mCi/mmol) was purchased from C.E.A. (France) and [1-^{14}C]linoleic acid (51mCi/mmol) was obtained from Amersham (England). Purity was checked by thin-layer chromatography on silica gel G plates developed in hexane–ether–acetic acid 50:50:2 (v/v/v) and was found to be ⩾98%.

Animals and diets

After fertilization, the animals were kept in the laboratory in constantly running water at 12 ± 1°C under optimal conditions of oxygenation. Every morning they received, ad libitum, one meal of commercial diet containing 8% lipid (fish oil, 6%, containing unsaturated fatty acids C16 to C22; and vegetable oil, 2%, containing primarily linoleic and linolenic acids). In this ration at least 60% of the fatty acids are unsaturated fatty acids, and the data of Castell et al. (10), Watanabe et al. (11), and Takeuchi and Watanabe (12) were taken into account.

Experimental procedures

On the day of the experiment, instead of the usual meal, the trout (400–500g) were slightly anaesthetized with ethylcarbamate and received 200 μl of an extract from trout adipose tissue containing 20 μCi of [1-^{14}C]-palmitic acid or 25–50 μCi of [1-^{14}C]linoleic acid. The lipid extract was introduced into the stomach at the pyloric flexure with the help of a micropipettor. The trout were then immediately replaced in their aquarium where they recovered and swam freely within a few minutes. Animals were killed at various times after lipid administration according to the type of experiment (epithelial cell analysis or plasma analysis).

Epithelial cell analysis. Trout were killed 4, 6, 12, and 18 hr after intubation of the labeled fatty acid. Stomach, caeca, and intestine were removed, and their contents were collected in saline solution; the walls were washed and weighed. The epithelial cells of intestine were obtained with citrate buffer (NaCl, 96 mmol; KH_2PO_4, 8 mmol; Na_2HPO_4, 14 mmol; KCl, 15 mmol; and sodium citrate 2 H_2O, 20 mmol, pH 7.4), according to the method of Towler, Pugh Humphreys, and Porteous (13).

Tissue lipids were immediately extracted overnight with 20 volumes of chloroform-methanol 2:1 (v/v) according to the method of Folch, Lees, and Sloane Stanley (14). After washing, the chloroform extract was evaporated to dryness under nitrogen and the residual lipids were redissolved in chloroform. Aliquots of the chloroform extract and of authentic lipid standards were spotted on thin-layer silicic acid plates (0.5 mm, silica gel G, Carl Schleicher and Schüll) and the major neutral lipids were separated using a solvent system of hexane–diethyl ether–acetic acid 50:50:2 (v/v/v). After visualization of the standards, the spots were scraped directly into scintillation vials. Radioactivity was measured in a scintillation mixture containing Cab-o-sil or in a conventional toluene solution with a liquid scintillation spectrometer (Intertechnique, Plaisir, France).

Plasma analysis. Blood was obtained by cardiac puncture at 30 min, 1, 2, 3, 4, 6, and 18 hr after administration of the labeled fatty acid in trout fasted for 3 days. After addition of 25 μl EDTA (4%)/MIA (monoiodo-acetamide, 3.7%) per ml of sample and centrifugation, the plasma was obtained and its radioactivity was measured either by direct counting of 20 μl samples in a scintillation vial or after lipid extraction of samples (1–2 ml) as described above.

Ultrastructural study

Normally fed trout (250–400 g) were killed at various times after the meal (4, 12, 18, 24, and 28 hr) and the segments of proximal intestine were removed just below the last pyloric caecum. The tissues were fixed at 4°C for 15 min in 4% paraformaldehyde buffered by sodium cacodylate (0.15 M, pH 7.3), rapidly washed in the same buffer, post-fixed for 1 hr in osmium tetroxide buffered by sodium cacodylate (0.15 M, pH 7.3), and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate.

The lipids were preferentially contrasted by the method proposed by Seligman, Wasserkrug, and Hanker (15). Thin sections of tissues fixed by osmium tetroxide (final concentration 2%) were collected on gold grids and successively placed on 1% aqueous solution of thiocarbohydrazide (1 hr at 50°C) and on 1% aqueous solution of osmium tetroxide (1 hr at 50°C). A similar study was performed on pyloric caeca.

RESULTS

Tracer studies

Four hours after the labeled palmitic acid was introduced by stomach tube, lipid radioactivity was 4% of the administered radioactivity in the wall of caeca and less than 1% in the intestine. It increased abruptly

TABLE 1. Percentage radioactivity in the lipids isolated from intestinal cell epithelium of trout[a] 6, 12, and 18 hr after intubation of [1-^{14}C]palmitic acid or 14 hr after administration of [1-^{14}C]linoleic acid

TLC Area	[1-^{14}C]Palmitic Acid			[1-^{14}C]Linoleic Acid
	6 hr	12 hr	18 hr	14 hr
1—Front and cholesteryl esters	0.5, 1.0	5.5, 6.2	2.9, 6.0	trace,trace
2—Triglycerides	62.5,66.2	70.0,77.1	68.1,76.6	77.6,92.6
3—Free fatty acids	9.4,22.6	20.2,15.1	22.5, 7.1	8.8, 3.2
4—Mono- and diglycerides	27.5,11.1	4.3, 1.4	6.5,10.3	13.6, 4.1

[a] Two trout per time period.

at 6 hr (at least 10 times more radioactivity was recovered at this time, for example 44 and 56% in the caeca) and, then, decreased slightly. Five and 18% of the administered dose was still found in caeca after 18 hr. The caeca appear to be the major site of lipid absorption, while the proximal intestine is a minor site in respect to their relative areas. Nevertheless, as the ultrastructure of the epithelial cells in the intestine is the same as that of the caeca, we performed the following study on intestinal cells which are easier to isolate.

Sixty to 77% of lipid radioactivity isolated from the intestinal epithelial cells was in triglycerides 6, 12, or 18 hr after the trout were fed the labeled palmitate (**Table 1**). Similar results were obtained after linoleic acid feeding. We have checked that lipid radioactivity from the caeca and intestine contents was entirely present in the free fatty acids.

During the first 4 hr after administration of [1-^{14}C]-palmitic acid or [1-^{14}C]linoleic acid, the radioactivity in the plasma was extremely low. It was detectable only after 4 hr (less than 0.04% of administered dose, **Table 2**). The lipid radioactivity in plasma was then almost entirely in the triglyceride fraction (**Table 3**).

TABLE 2. Appearance of radioactivity[a] (as a percent of administered dose) in plasma lipids of five trout receiving [1-^{14}C]palmitic or [1-^{14}C]linoleic acids

Time of Killing	Fatty Acid Administered				
	[1-^{14}C]Palmitic Acid		[1-^{14}C]Linoleic Acid		
	1	2	3	4	5
30 Min	n.d.[b]	n.d.	n.d.	n.d.	
1 Hr	n.d.	n.d.	n.d.	n.d.	0.001
2 Hr	0.003	n.d.	0.006	0.011	0.005
3 Hr		n.d.	0.009	0.020	
4 Hr		0.006			0.033
6 Hr					0.104
18 Hr		>1			

[a] Trout plasma volume was assumed to be 20 ml.
[b] n.d., not detected.

Ultrastructural study

In the trout fed a normal diet, the ultrastructure of intestinal epithelial cells exhibits few changes whatever the time of killing after the meal (**Fig. 1**).

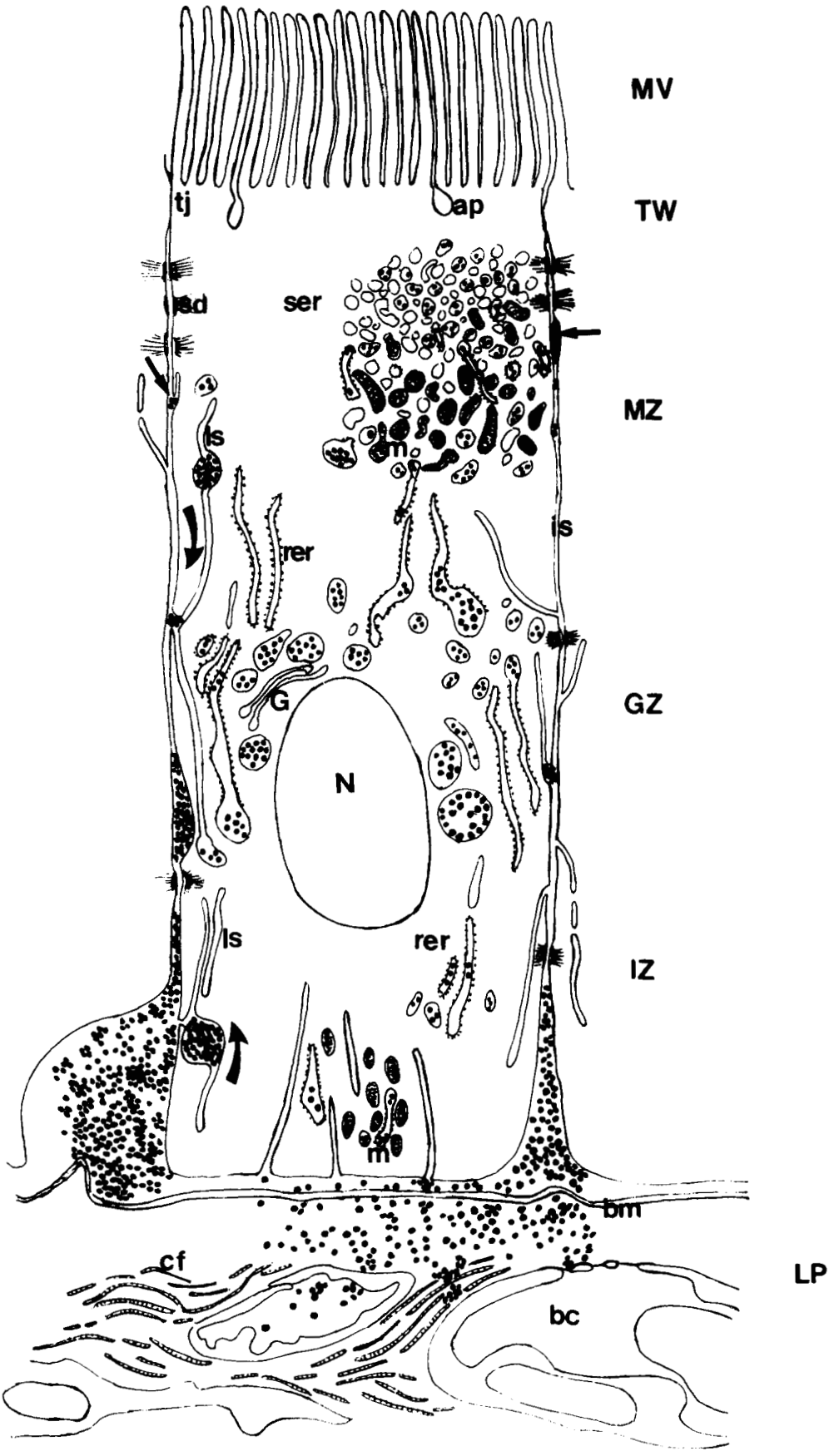
The mucosal component lines the intestine throughout with a simple columnar epithelium consisting of typical columnar epithelial cells with sparsely intermingled goblet cells. The columnar epithelial cell is very tall, 70 μm, narrow, 7–10 μm, and its luminal surface forms a striated border consisting of high microvilli, 2.5–3 μm. The basal surface of the cell rests on a thin basement membrane which separates the epithelium from the lamina propria. Contrary to mammals, the lateral margins of the cells are not characterized by foldings and interdigitations. The terminal bar between adjacent cells occurs at the terminal web level and consists of a typical tight junction and below it a belt desmosome. Immediately beneath the terminal web level, three or more rows of spot desmosomes completely encircle the cell.

The most striking feature of the proximal intestine cells is the presence of numerous flattened sacs derived from the lateral and basal membranes which have been designated "lamellar structures" by Yamamoto (9) and Iwai (7). These ribbon-like sheet structures, bound by two very regular parallel membranes about 350 Å apart, are generally oriented parallel to the longitudinal axis of the cell and are never associated with ribosomes. These lamellae, which were not encountered in mammalian intestine, are well developed in the whole cell.

In the basal portion, these lamellae are very nu-

TABLE 3. Percentage radioactivity in lipids isolated from plasma samples of a trout (No. 5, see Table 2) 4 and 6 hr after administration of [1-^{14}C]linoleic acid

TLC Area	4 Hr	6 Hr
1—Front and cholesteryl esters	trace	trace
2—Triglycerides	94.1	95.7
3—Free fatty acids	5.9	4.3
4—Mono- and diglycerides	trace	trace



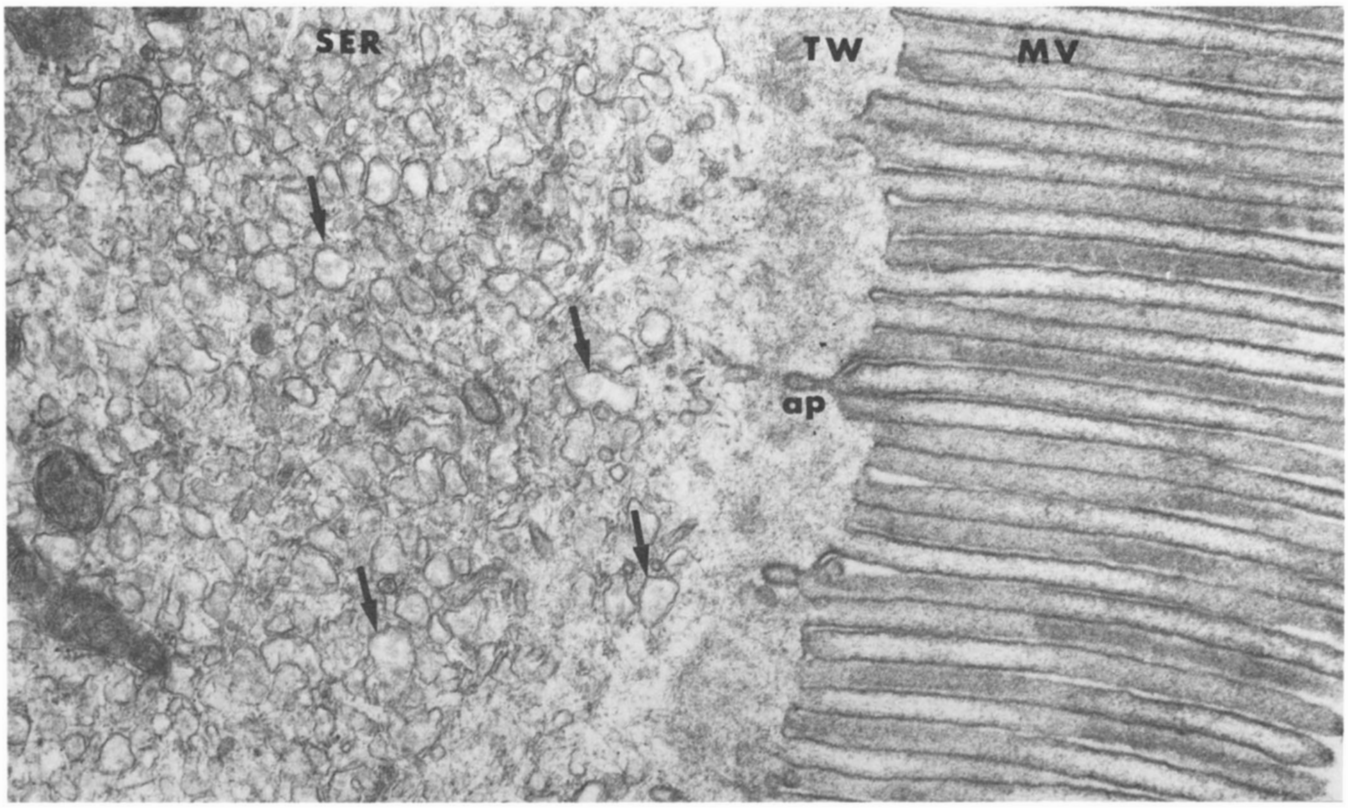


Fig. 2. Apical cytoplasmic region of an intestinal epithelial cell from a fed trout showing the hypertrophy of the SER during fat absorption. Beneath the high microvilli (MV) and terminal web (TW) area lipid granules occupy the lumen of SER (arrow). Note the apical pits (ap) whose role is unknown, and the abundance of microfilaments. ×23.000.

merous and separated by groups of mitochondria, like the infoldings of the plasma membrane in the basal cytoplasm of the proximal kidney tubule cell.

Lying beneath the microvilli, the terminal web area (TW) (**Figs. 2 and 3**), free of cell organelles, consists of fine filaments, some of them inserted on the terminal bar and on apical spot desmosomes.

Below the terminal web, the repartition of the organelles corresponds to a strict cell polarity. The SER is concentrated in the nearest underlying zone of apical cytoplasm (Figs. 2 and 3). It forms a network of tubules and vesicles which penetrate into the zone of mitochondria (MZ) (**Figs. 4 and 5**) and where it intermingles with cisternae of rough endoplasmic reticulum. The Golgi complex (**Fig. 6**) is located above and beside the nucleus, often separated from the MZ by some dense bodies (Fig. 5). Numerous reticular elements surround it (Fig. 6), spread under the nucleus, and invade the infranuclear cytoplasm up to the mitochondria-rich basal area.

The connective cells of the sub-epithelial lamina propria (fibroblasts, smooth muscle cells, etc.) (**Figs. 7, 8, and 9**) bathe in the interstitial fluid among very numerous collagen fibers.

Whatever the observation time after the single morning meal, the whole reticular and Golgi structures contain granules whose lipid nature is indicated by reaction with OsO_4. Up to 18 hr after the meal, their diameter ranges from 600 to 1000 Å. Between 18 hr and 24 hr, when the absorption peak seems to occur, the mean size increases and some granules can attain a diameter of 1500 Å. Beyond 24 hr, the size of the granules again ranges from 600 to 1000 Å and the emptying of the implicated structures begins and is completed after 3 days of fasting. No lipid particles can then be detected in the epithelial cells.

Fig. 1. A diagrammatic summary of the events in an intestinal epithelial cell during fat absorption. A thin basement membrane (bm), which follows the contours of the basal surfaces of the epithelial cells, separates these cells from the lamina propria (LP). This drawing is based on the electron micrographs obtained in this study. The reader should refer to these figures and the text for details. MV, microvilli; TW, "terminal web"; MZ, zone of mitochondria; GZ, Golgian zone; IZ, infranuclear zone; LP, lamina propria; G, Golgi; N, nucleus; ap, apical vesicle; tj, "tight junction"; sd, desmosome; ser, smooth endoplasmic reticulum; rer, rough endoplasmic reticulum; is, intercellular space; ls, lamellar structure; m, mitochondria; bm, basal membrane; cf, collagen fiber; bc, blood capillary.

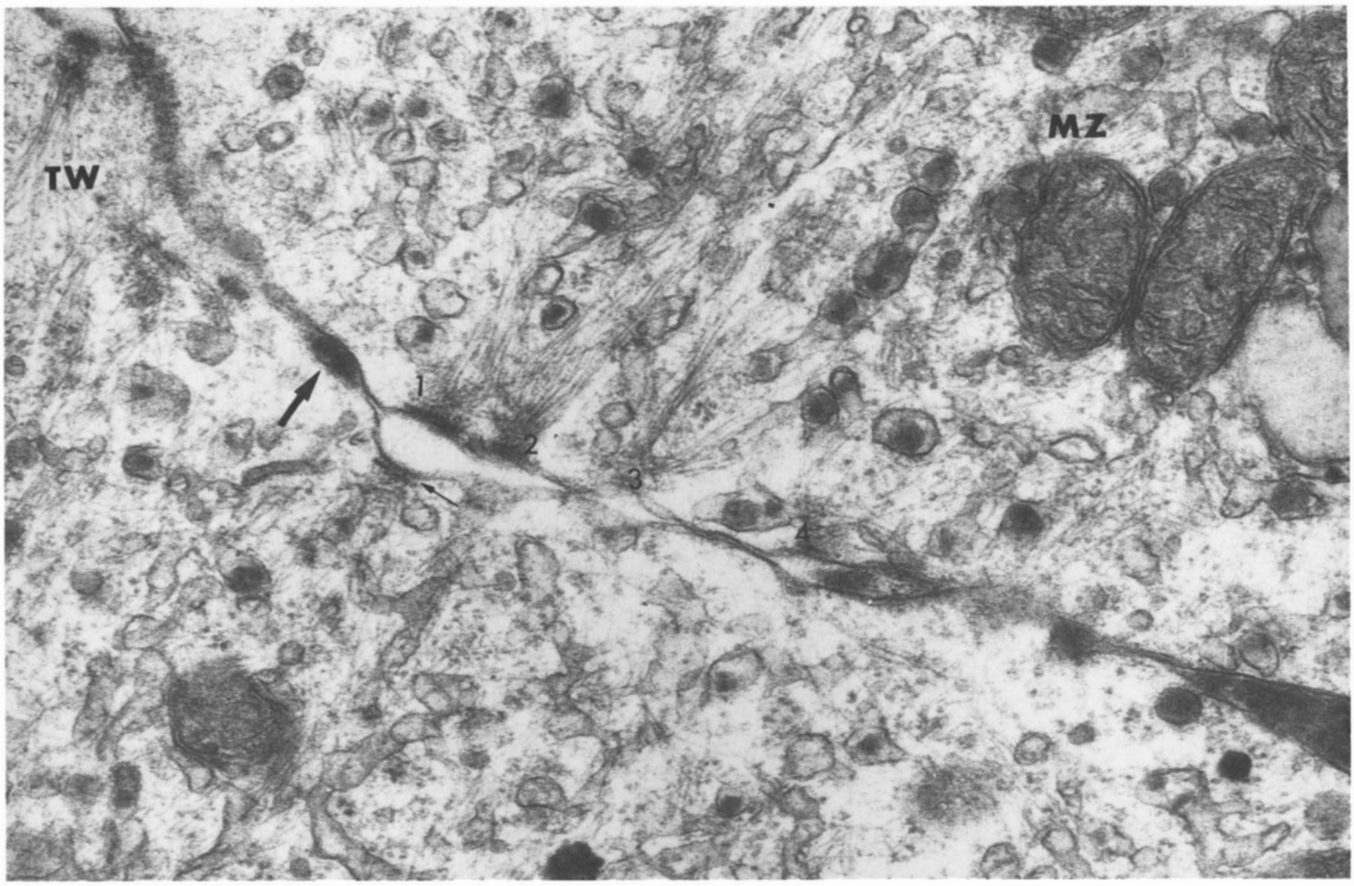


Fig. 3. Apical SER-zone between the terminal web (TW) and mitochondrial zone (MZ). The SER with lipid droplets is extensively developed. Lipid granules can already be seen in the intercellular space and particularly here just beneath the terminal web (thick arrow). The four spot desmosomes (1 to 4, in the center of the micrograph) belong to the rows of spot desmosomes which at this level completely encircle the cell. Very numerous cytoplasmic microfilaments form a network which intermingles with the tonofilaments attached to the cytoplasmic plaques (thin arrow) of desmosomes. ×42.000.

The granules can be observed in the SER tubules immediately beneath the terminal web (Figs. 2 and 3). At the MZ level they are encountered in the SER and also in the RER cisternae (Fig. 4). The cisternae often form vesicles (Fig. 5) and then lose their ribosomes, and some cisternae and vesicles inosculate with the lamellar structures (**Figs.** 4 and **10a,b**). The whole Golgi elements contain lipid granules (Fig. 6) and the "secretory granules" of Golgi origin are often indistinguishable from the SER vesicles. The reticulum of the infranuclear cytoplasm also contains granules (**Fig. 11**) and these latter can be seen everywhere in the intercellular space and especially just below the terminal web (Fig. 3), i.e., more than 15 μm above the Golgi apparatus. Above the basal membrane, the enlarged intercellular space exhibits a considerable accumulation of these granules (Figs. 7 and 11) and they are innumerable in the interstitial space of the lamina propria (Figs. 7, 8 and 9) and in the lumen of lymphatic capillaries. On the contrary, they are almost absent in the lumen of blood capillaries (Fig. 9). How do granules enter into the intercellular space? We never observed membrane fusion between a vesicle containing granules and the lateral plasma membrane. The passage occurs by means of the lamellar structures (**Fig. 12**).

All these observations are valid for the cells of intestinal caeca (**Fig. 13**). After the first meal of the alevin (stage 37 of Vernier's Table (16)), the intestinal cell ultrastructure is overturned. The juxtaluminal reticulum so far almost undiscernable then extends and forms lipid granules which are also found in the Golgi apparatus, the intercellular space, and the lamina propria. There is then concomitancy between the formation of lipid granules in the intestinal cells and the beginning of intestinal absorption.

The events in an intestinal epithelial cell during triglyceride absorption are summarized in Fig. 1. This

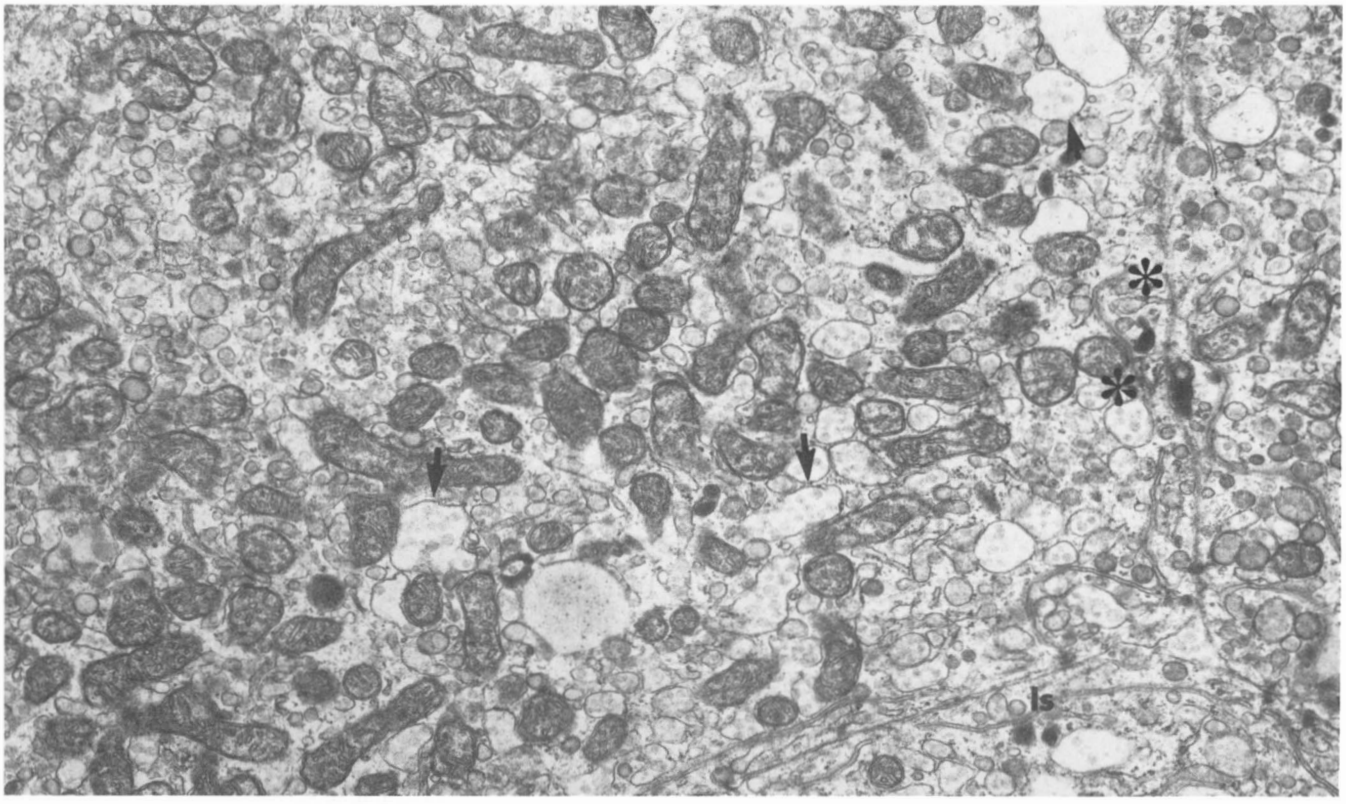


Fig. 4. Through the mitochondrial zone, an extensively developed reticulum (smooth and rough) containing very numerous lipid granules often presents bulbous expansions (arrows). Two reticular elements open into lamellar structures which are already very numerous at this level (ls). ×15.000.

drawing is based on the electronmicrographs obtained in this study.

DISCUSSION

Intracellular fatty acid esterification

Present biochemical study shows that the major part of labeled fatty acids (palmitic or linoleic) entering the trout intestinal cell is esterified (Table 1).

In fishes, during a natural meal, the intraluminal hydrolysis of the dietary triglycerides is complete. Indeed, either pancreatic lipase is not specific (17) or the activity of a β-monoglyceride lipase completes its activity (18). According to the composition of the meal used here, 60% of the liberated fatty acids would be unsaturated. Their intracellular esterification leads to the formation of VLDL-like particles. Several authors (19–22) have shown that in mammals, after intraduodenal infusion of mixed micelles, the nature of the lipoprotein particles which are secreted into the intestinal lymph depends on whether the micellar fatty acids are saturated or unsaturated. After feeding saturated fatty acids, the particle size is that of VLDL while it attains that of the chylomicrons after ingestion of unsaturated fatty acids. If these observations are extended to trout, chylomicron-like particles and not VLDL might be formed under our dietary conditions.

In mammals, triacylglycerol biosynthesis occurs almost exclusively in the endoplasmic reticulum (23, 24). In a variety of species it has been shown that, because triglycerides are the major source of dietary fat, the monoacylglycerol pathway is the major one for triacylglycerol synthesis in the intestinal mucosa while the glycerol-3-phosphate pathway is less important. In addition, monoacylglycerol may serve as an inhibitor for the acylation of glycerol-3-phosphate (25). These two pathways operate quite independently in the mucosal cell (26). Moreover, as a result of biochemical and morphological studies (27, 28) it was suggested that the monoacylglycerol pathway is associated with the SER while the glycerol-3-phosphate acylation takes place in the RER where glycerophospholipids might be synthesized as well as cholesteryl esters and proteins.

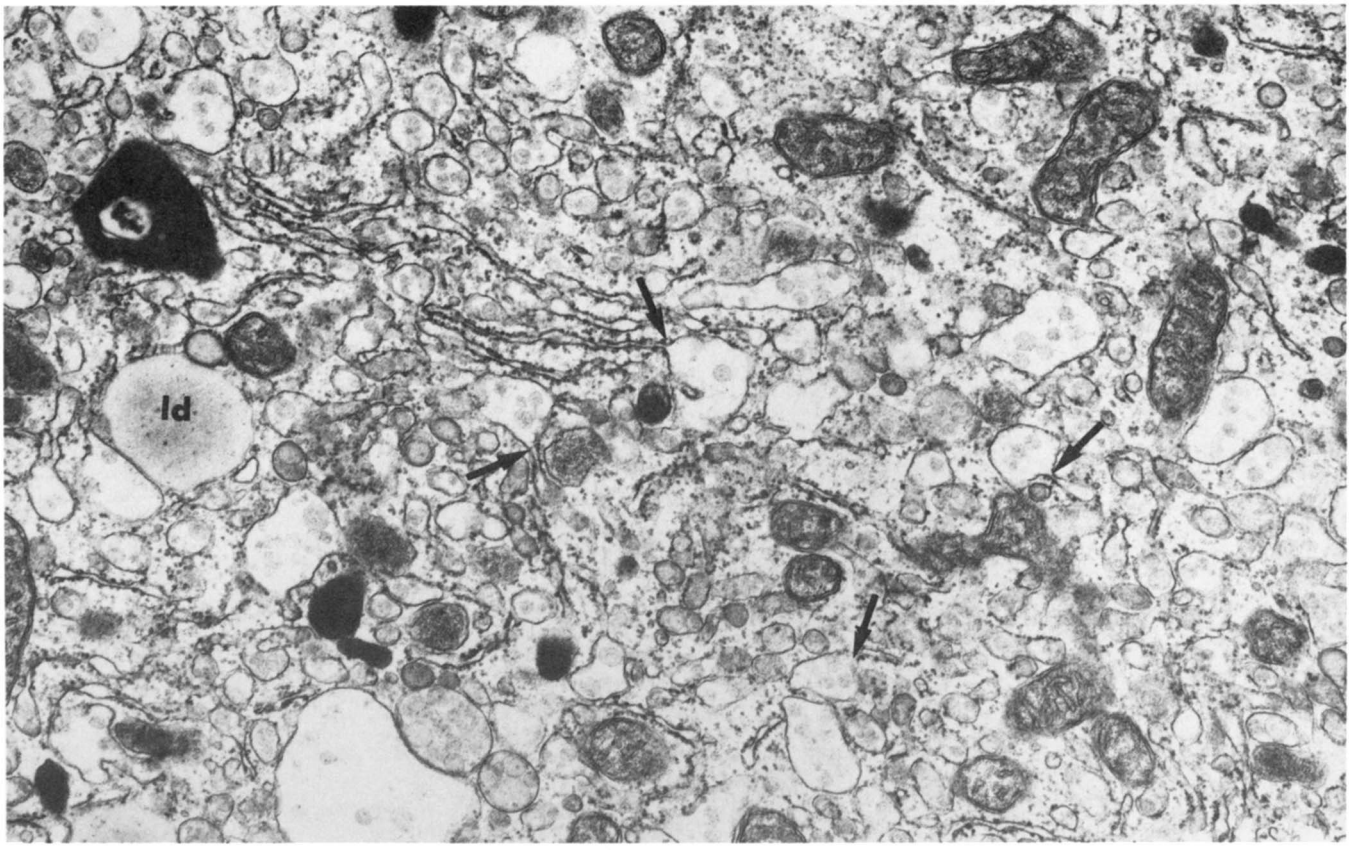


Fig. 5. Beneath the mitochondrial zone the ER is extensively developed and instances of SER continuity with the RER are frequently observed. The RER cisternae often dilates (arrows). Note a lipid droplet (ld) without surrounding membrane. ×22.000.

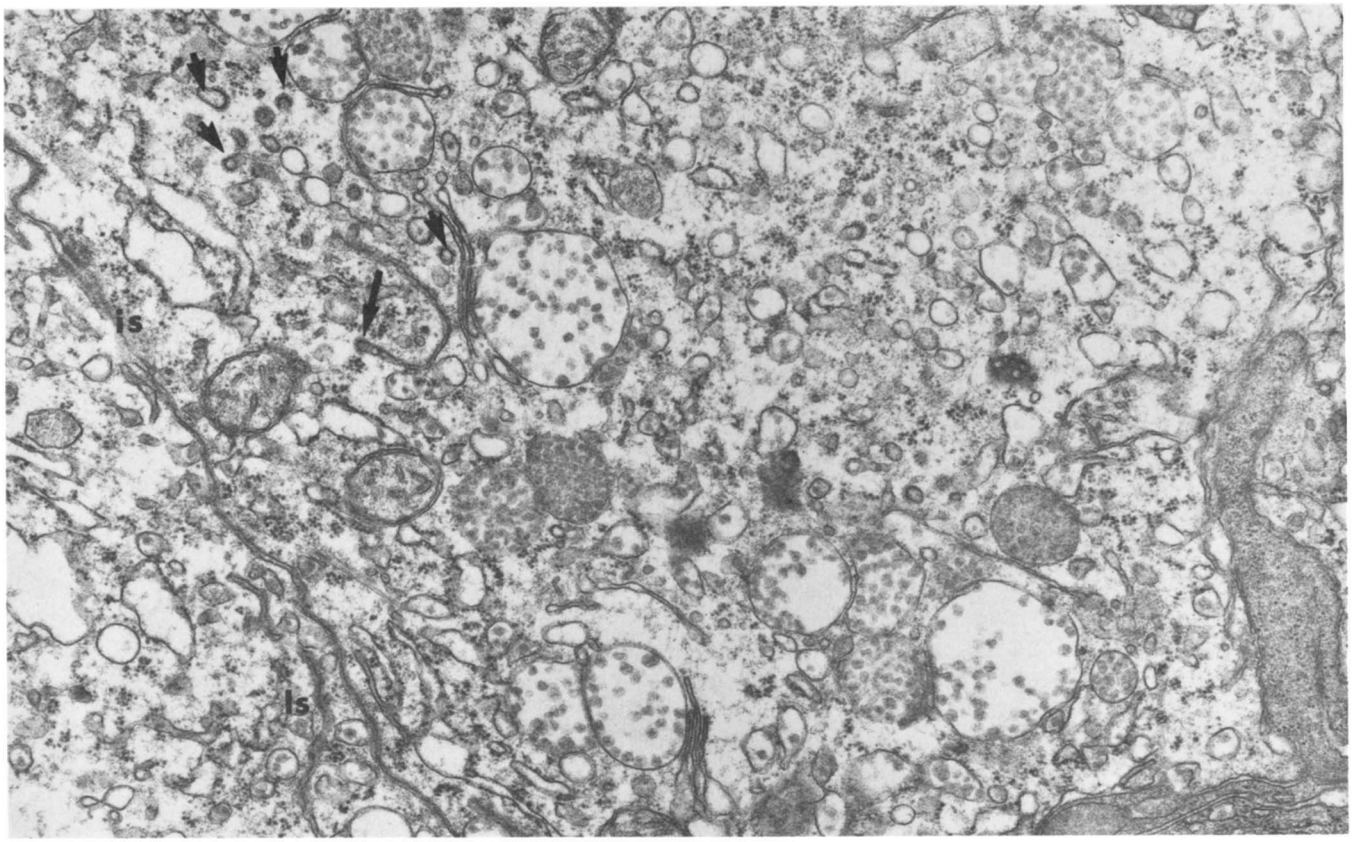


Fig. 6. Supranuclear region probably showing the Golgi complex. This view is favorable because usually no flattened stacks are visible and the supranuclear great lipoprotein-filled vesicles are considered as belonging to Golgi. Note very numerous "coated-vesicles" (short arrows) on the trans face of dictyosome and one of them on the end of the rigid lamellae (long arrow). ×20.000.

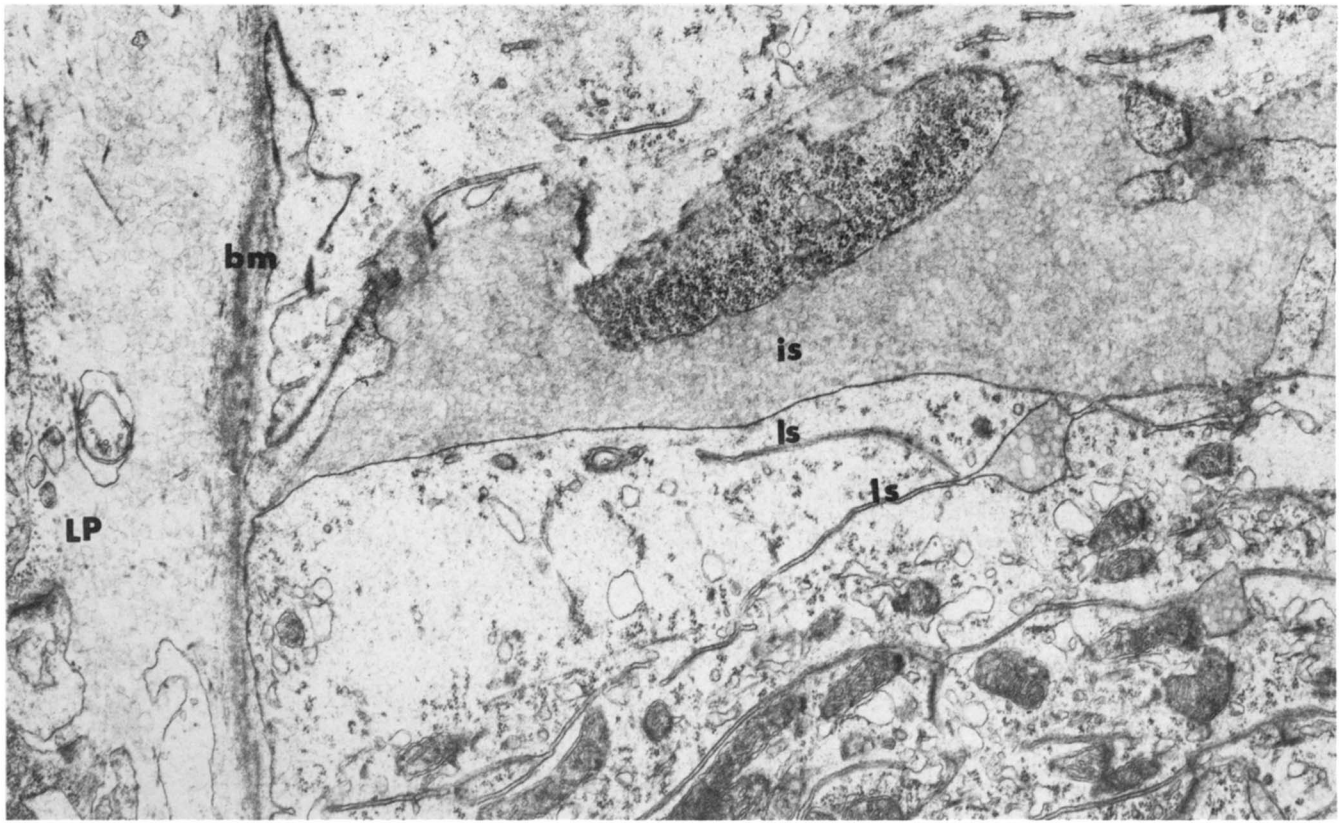


Fig. 7. In the basal zone of the cells, VLDL-like particles reach the intercellular space (is) also by way of the lamellar structures (ls) and subsequently attain the interstitial space of the lamina propria (LP). ×20.000.

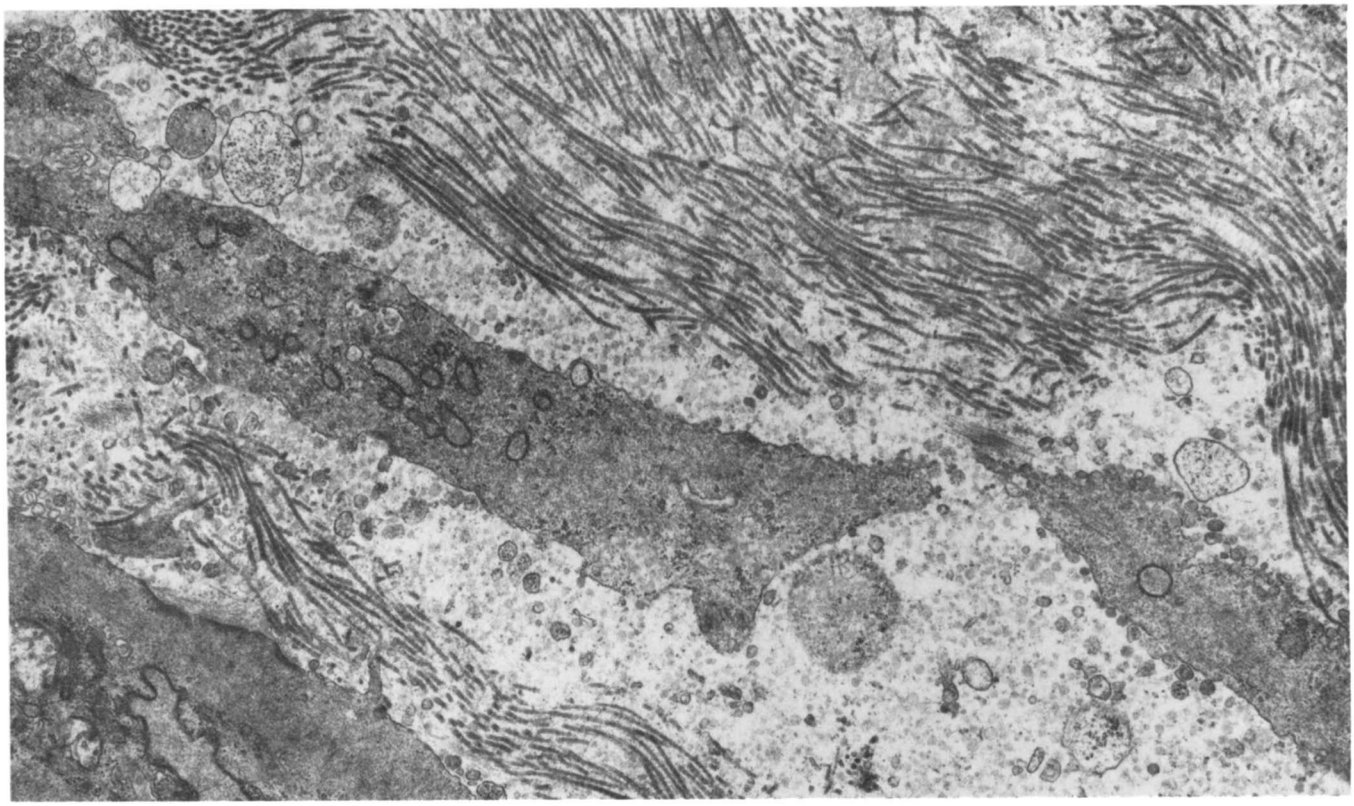


Fig. 8. The interstitial space of the lamina propria shows innumerable VLDL-like particles between the elements of the connective tissue and the collagen fibres. ×16.000.

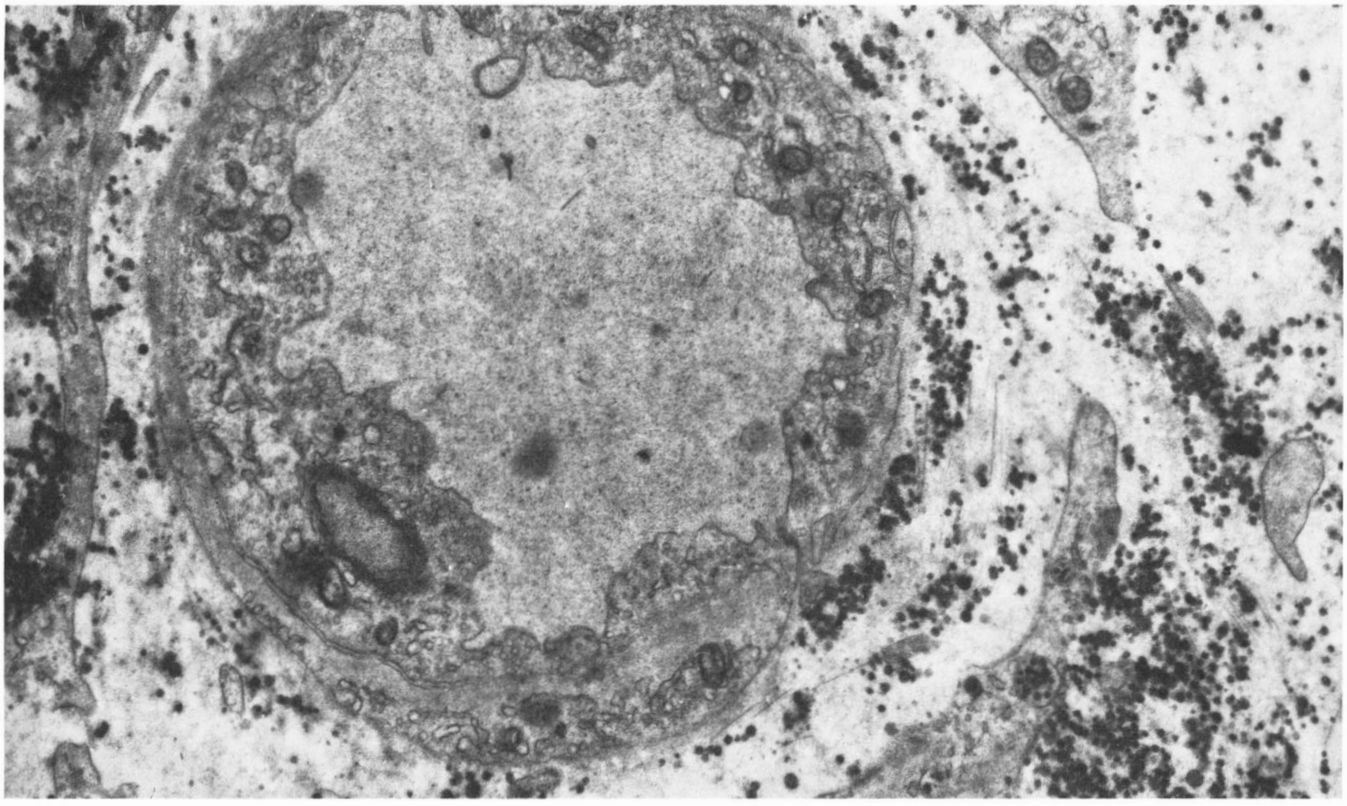


Fig. 9. Deep in the lamina propria, a cross section of a blood capillary with a thick wall shows no VLDL-like particles in the cytoplasm of the endothelial cells and none in the lumen. The lipid particles of the connective tissue are contrasted by the OsO_4 method. ×10.000.

In teleostean fishes, the glycerol-3-phosphate pathway appears to be the major one, indeed the only one, because of the total or the almost total hydrolysis of dietary triglyceride in the lumen. If one acknowledges, in agreement with Johnston et al. (26, 29), that the glycerol-3-phosphate pathway is utilized for both glycerophospholipid and triacylglycerol biosynthesis, our morphological studies show that this pathway does not occur exclusively in the RER. In particular, the glycerophosphate acyltransferase activity might be very important in the SER which intensively forms lipid particles just beneath the terminal web. As this pathway is prominent, glycerophospholipid synthesis is proportionally higher in fish than in mammals for a similar uptake of fatty acids. Moreover as our fish diet contained a high concentration of protein (49%) and the anterior trout intestine absorbs amino acids extensively (30), we are able to provide a likely explanation as to why the trout intestine synthesizes VLDL-like particles. As compared with mammals, the rate of formation of surface material is high and the resulting lipoproteins are small. This probably applies to all teleostean fishes. Indeed, for all species studied (sea or river fishes, including reputed omnivorous or herbivorous grass-eating species), there is at least 35% protein in the diet, pancreatic lipase is not specific (17), and the glycerol-3-phosphate pathway is, thus, prominent. The ultrastructure of epithelial cells reveals particles similar in size to VLDL in the RE and the Golgi apparatus from tench (31), carp (32), and grasscarp (33).

Endoplasmic reticulum and Golgi apparatus in VLDL-like particle formation

According to Ockner and Isselbacher (20) "it is not entirely clear that passage through the Golgi apparatus is an obligatory step in the secretion of lipoproteins". In their interesting morphological study, Sabesin and Frase (34) assert that "nascent chylomicrons accumulate within Golgi vesicles as a prerequisite to secretion". However they do not provide new data in support of their statement but extrapolate to the intestine the biochemical results concerning VLDL synthesis in the liver cell where glycosylation reactions are induced by the Golgi apparatus. Although in the trout we have observed that the Golgi apparatus is an obligatory step for synthesis of all hepatic VLDL, we feel that this is not the case for the synthesis of all

intestinal lipoproteins after dietary fat absorption. Cisternae of endoplasmic reticulum have been observed in continuity with lamellar structures. RER swells, the ribosomes then disappear, and the vesicles thus formed become indiscernible from Golgi vesicles if present. Generally, during absorption, it is difficult to differentiate the Golgi elements from the reticular ones with which a continuity is possible, contrary to the liver where differentiation is easy. Lipid granules can be seen in the intercellular space, just above the MZ, therefore very far from the Golgi zone. The endoplasmic reticulum of intranuclear cytoplasm contains numerous lipid granules up to the mitochondria-rich basal zone.

These morphological data can be envisaged together with some recent biochemical results obtained in mammals. The apoprotein content of both newly formed VLDL and chylomicrons changes dramatically as these lipoproteins enter the blood stream and interact with other circulating lipoproteins (35, 36). They appear to pick up apoproteins C and E which are synthesized in the liver. The apoproteins A and B, major constituents of chylomicrons, are synthesized in the intestine and the apoprotein B appears to be essential to the transport of triglycerides out of the intestine. It seems to contain a notable proportion (5%) of glycosyl moieties (galactose, mannose, glucosamine, and sialic acid) whose role has not been determined. For numerous cell types it has now been demonstrated that carbohydrate fragments can be bound to the proteins, either at the ribosomal level or at the RE level. Thus, for example, Inoue and Kurosumi (37) remark that "in gonadotrophs of rat anterior pituitary, in the normal and castrated conditions, glycoproteins were undoubtedly synthesized in rough endoplasmic reticulum". In consideration of present knowledge on lipid absorption, we feel it is wise to return to Cardell's statement (38): "After formation the chylomicra are transported directly to the intercellular spaces *or* to the Golgi complex". This allows a complete interpretation of our morphological observations.

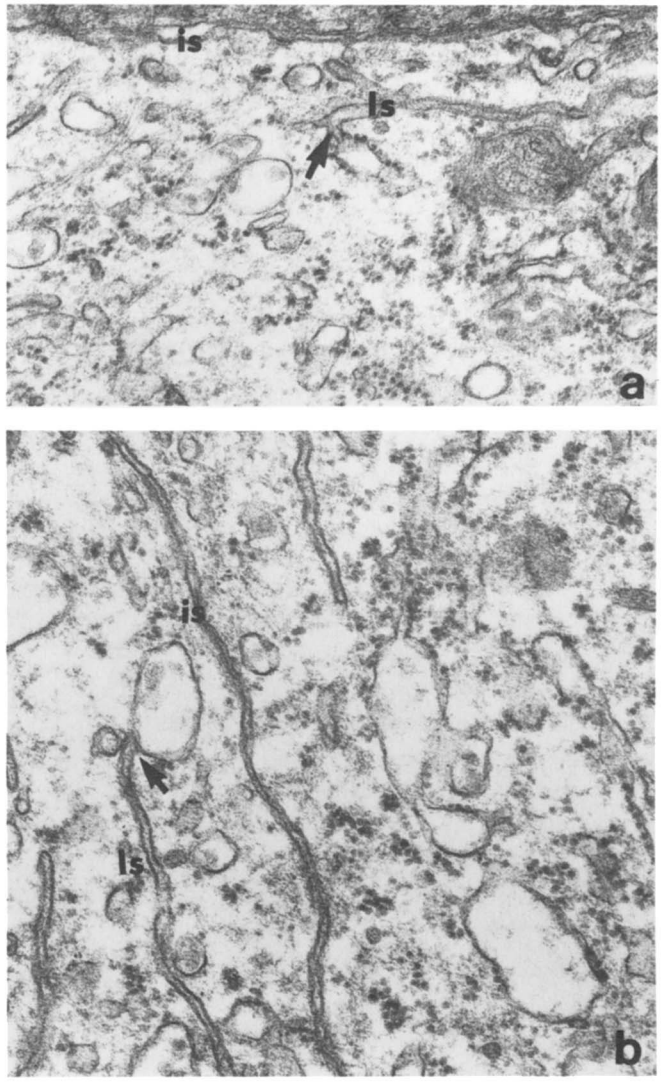


Fig. 10. Here, elements of RER (a) and SER (b) open to lamellar structures (ls), the most striking feature of intestinal cells of teleostean fishes. 10a: ×33.000; 10b: ×42.000.

Role of lamellar structures

Although the lamellar structures have been described as striking features of the intestinal epithelial cells in teleostean fishes, their role is still unknown. By analogy with the infoldings of the basal membrane observed in the cells of proximal kidney tubules, several authors have suggested that these structures are involved in osmoregulation. Only Yamamoto (9) notes, but without experimental demonstration, that "they are presumably the structure involved in transport of water or nutrients". We possess several dozen electron micrographs showing that these structures interfere with a transport of lipid granules up to the intercellular space. Although we never did observe a fusion between a reticular or Golgian element containing granules and the laterobasal plasma membrane, we cannot assert that transfer by lamellar structures is the only type. Although the role of lamellar structures in lipid transport is thus the first one established, it is probably not the only one.

Relative importance of the VLDL secretion in lipid absorption

We have demonstrated in the trout epithelial cells that long chain fatty acids are esterified in triglyc-

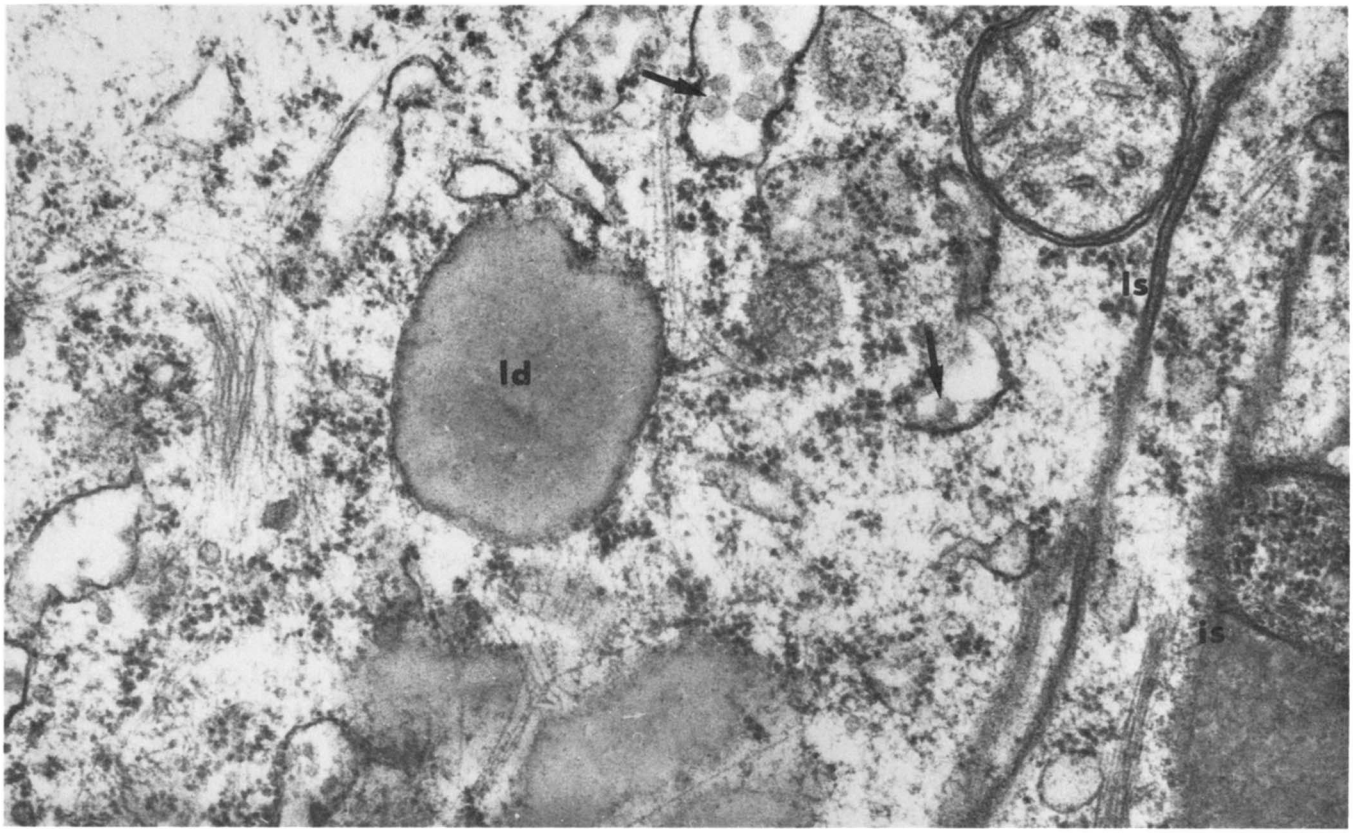


Fig. 11. Infranuclear cytoplasm of the epithelial cell shows extensively developed RER filled with VLDL-like particles (arrows). Note the fine network of microfilaments. ×42.000.

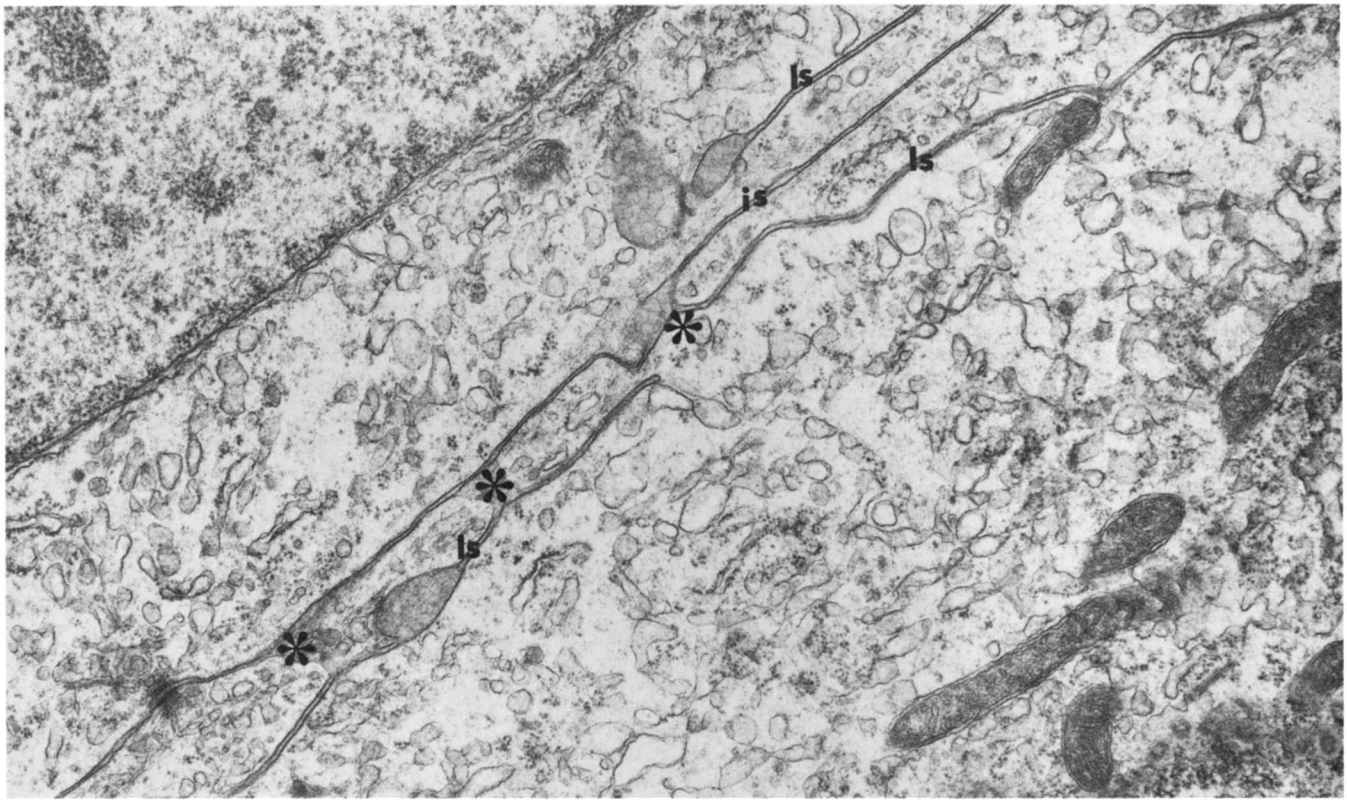


Fig. 12. The VLDL-like particles are transferred to intercellular space (is) by way of the lamellar structures (ls) which communicate with the lateral plasma membrane (*). ×20.000.

erides and that esterification leads to a VLDL-like particle formation (similar to production of chylomicrons in mammals). The relative contribution of this pathway remains to be determined. For Robinson and Mead (1), "the fish receives most of its absorbed lipid as free fatty acids, probably via the portal system". Our study shows that only traces of radioactivity appear in the trout plasma during the first 6 hr following intragastric administration of [1-^{14}C]palmitic or [1-^{14}C]linoleic acid. As soon as the radioactivities in plasma lipids could be determined accurately (after 4 hr in our experiments), we observed that the labeling was essentially in triglycerides (Table 3). In order to check whether the liver does not take up very rapidly labeled plasma fatty acids, we measured the liver radioactivity 2 hr after [1-^{14}C]palmitic acid administration. Again, the radioactivity found was extremely small (0.004% of the administered dose).

All these morphological and biochemical data invalidate the previous scheme proposed by Robinson and Mead (1) to explain the mechanism of absorption of dietary triglyceride in the fish. In fact, the absorption mechanisms of alimentary triglycerides are very similar in the trout and in mammals, although absorption may have been slower in the fish. The long chain fatty acids are esterified in the intestinal epithelial cells and transferred to the lymph within VLDL.

Moreover in fish these mechanisms seem to be extended to the absorption of hydrolyzed wax esters from zooplankton. Bauermeister and Sargent (39) and Bauermeister, Pirie, and Sargent (40) have indeed demonstrated that fatty alcohols and fatty acids which result from hydrolysis are transformed into triglycerides by the caecal epithelial cells in the trout. This ingestion of marine zooplankton is accompanied by lipoprotein synthesis in the form of granules which can attain 4000 Å in diameter.

In our present study we have not disproved the possibility that under appropriate conditions chylomicrons might be formed in trout. The results of Bauermeister, Pirie, and Sargent (40) and the fact that Skinner and Rogie (41) note in the trout plasma "a few large particles of diameter 3000–8000 Å which displayed sharp surface features characteristic of chylomicrons" led us to demonstrate chylomicron synthesis by modifying the load and the chemical composition of the lipid (triglyceride) fraction of the diet. The data show that chylomicrons can be obtained.

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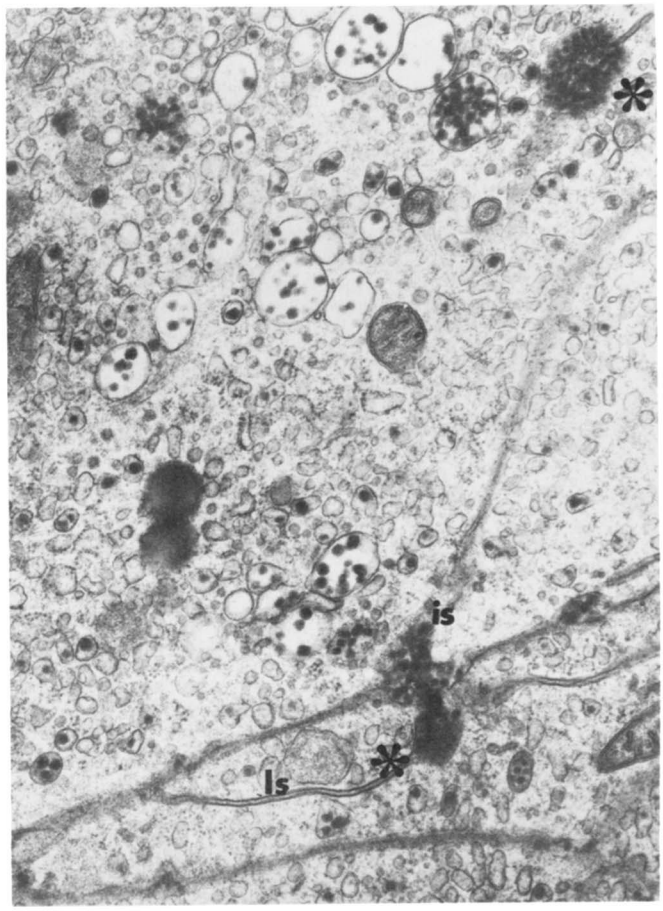

Fig. 13. Caecal epithelial cells. As in intestinal cells VLDL-like particles are seen in RE, secretory vesicles and intercellular space (is). Here, two secretory vesicles open (*) in lamellar structures (ls). ×18.000.

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