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Fat Absorption by the Enterocytes of the Carp (*Cyprinus carpio* L.)*

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Summary. In the carp, the absorption of fat from the food occurs at the level of the enterocytes of the proximal region of the intestine. The absorbed fat gives rise to the presence of two forms of inclusions: lipid particles and lipid droplets. These two forms, whose precise significance is unknown, definitely play different roles in fat absorption. Only lipid particles are involved in direct transport of absorbed fatty acids into the blood circulation. Lipid droplets seem to be involved in the temporary storage of fatty acids.

Key words: Intestine (Teleost) — Enterocytes — Absorption — Lipid.

Introduction

In previous reports, we gave an account of the cytophysiology of two segments of the carp intestine (*Cyprinus carpio* L.). In its distal part, epithelial cells of the Middle Segment (Middle 2) possess the differentiations usually described in epithelial specialized for the absorption of macromolecules, and these cells absorb the alimentary proteins by pinocytosis (Gas and Noaillac-Depeyre, 1973; Noaillac-Depeyre and Gas, 1973). The Posterior Segment or Rectum, the enterocytes of which display an ultrastructure similar to that of the renal tubule, is involved in ion transport (Noaillac-Depeyre and Gas, 1973).

Here the authors present a study of the proximal part of the intestine, *i.e.* the Anterior and First Middle Part defined by Al Hussaini (1949a, b). These areas ensure absorption of fatty foods. Al Hussaini (1949b), Broussy and Serfaty (1958), Iwai (1969), Gauthier and Landis (1972) noted the presence of lipids in the enterocytes, but gave no information on absorption and transit of lipids through the intestine. Therefore, we emphasize the structural and ultrastructural characteristics of the mucosa, related to this physiological function.

Material and Methods

1. Animals Used

Common carps (*Cyprinus carpio* L.), average weight 250 g, living in fresh water ponds, received granules of a special carp food (JOF, Toulouse).

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2. Cytological Study

The intestines were fixed for light and electron microscopic investigations. The sections (5 μ thick) were stained with PAS-haematoxylin.

For electron microscopy intestinal fragments were fixed in 3 per cent glutaraldehyde in 0.1 M, pH 7.2 phosphate buffer, for 2 hours at 4°C. After rinsing for 18 hours in the same buffer solution to which 1.15 per cent sodium chloride was added, the specimens were fixed in a 1 per cent osmium tetroxide solution in 0.1 M, pH 7.2 phosphate buffer for 30 minutes, and then embedded in Epon 812.

The ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds, 1963), or by the OTO method (Seligman *et al.*, 1966).

3. Administration of Arachis Oil

5 ml of arachis oil containing saturated oil red 0 were delivered to the anterior part of the intestine. A rigid polyethylene tube attached to a syringe permits one to inject the solution beyond the pharyngeal sphincter. Twenty-four hours later, the animals were sacrificed and the intestine rapidly removed. For observation under the electron microscope, the specimens were fixed as above.

Frozen sections (8 μ thick) from the intestinal fragments fixed with glutaraldehyde, were then observed under the light microscope.

Results

1. Fine Structure of the Intestinal Mucosa

Particular attention was paid to the structural characteristics of the enterocytes and the subepithelial connective tissue.

a) The Enterocytes. The surface area of the apical pole of the enterocytes is considerably increased by the numerous microvilli (Fig. 1 A). These microvilli are regularly disposed as shown by sections parallel to the cell surface. We were thus able to assess their density in electron micrographs: there were about 70 per square micron at this level of the intestine. The microvilli have a cylindrical shape, a rectilinear course and a rounded tip. Their length is about 1.2 μ and their diameter 0,12 μ; they amplify the cell surface by a factor of about 32. Fine filaments reinforce the microvilli and pass into their apical cytoplasm. The membrane of the microvilli has a filamentous external covering especially at the tips. This "glycocalyx" is similar to that of mammals, as indicated by its PAS-positive reaction.

The apical cytoplasm does not contain organelles. Sometimes at this level tubules (Fig. 1 B) seem to link the bottom of the inter-microvillous space to a complex network of endoplasmic reticulum situated at the lower limit of this apical zone. This network appears to comprise smooth, irregular vesicles, variable in number (Fig. 1 A). Micropinocytotic invaginations of the plasmic membrane are rarely observed.

The subapical cytoplasm contains numerous mitochondria together with an extensive endoplasmic reticulum, which generally extends parallel to the main axis of the cell. Granular reticulum and agranular reticulum are frequently continuous with one another. Multivesicular bodies (Fig. 1 C) together with dense bodies (Fig. 2) are observed fairly constantly.

The Golgi apparatus, located in the supranuclear region, is formed of numerous saccules and vesicles. The nucleus, which has an elongated shape, occupies a basal

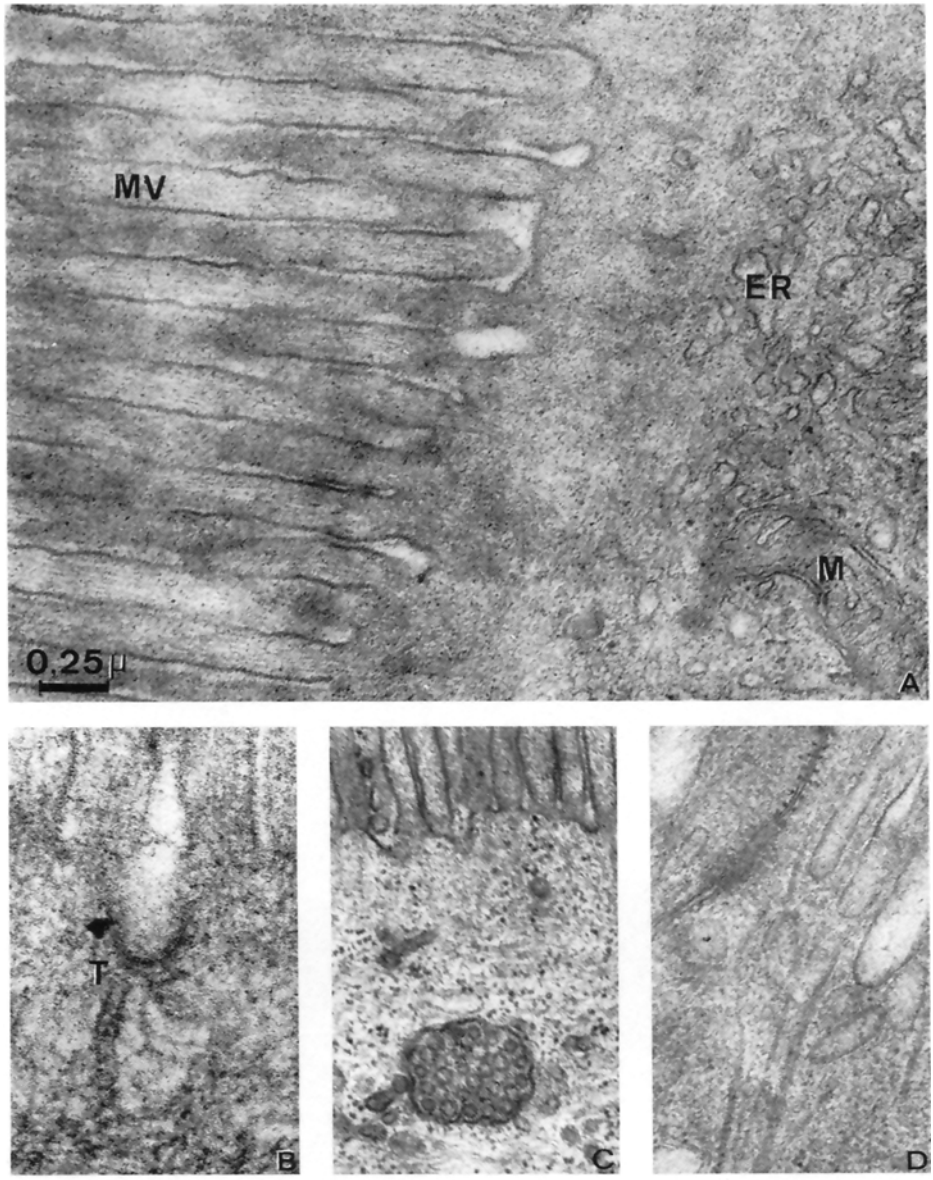

Fig. 1 A—D. Electron micrographs of the apical cytoplasm of an intestinal absorptive cell from a carp fed on granules of special carp food. The striated border consists of numerous microvilli (*MV*). Beneath the terminal web, rough and smooth endoplasmic reticulum (*ER*) and mitochondria (*M*) are the predominant organelles (Fig. A, ×44000). Sometimes tubules (*T*) seem to reach the bottom of the intermicrovillous space (Fig. B, ×95000). Multivesicular bodies are occasionally encountered (Fig. C, ×36000). At the level of the upper portion of the terminal web small desmosomes link adjacent cells (Fig. D, ×36000)

position. The cytoplasmic area situated below the nucleus, contains numerous mitochondria together with invaginations of the lateral and basal plasma membranes (Fig. 4 A).

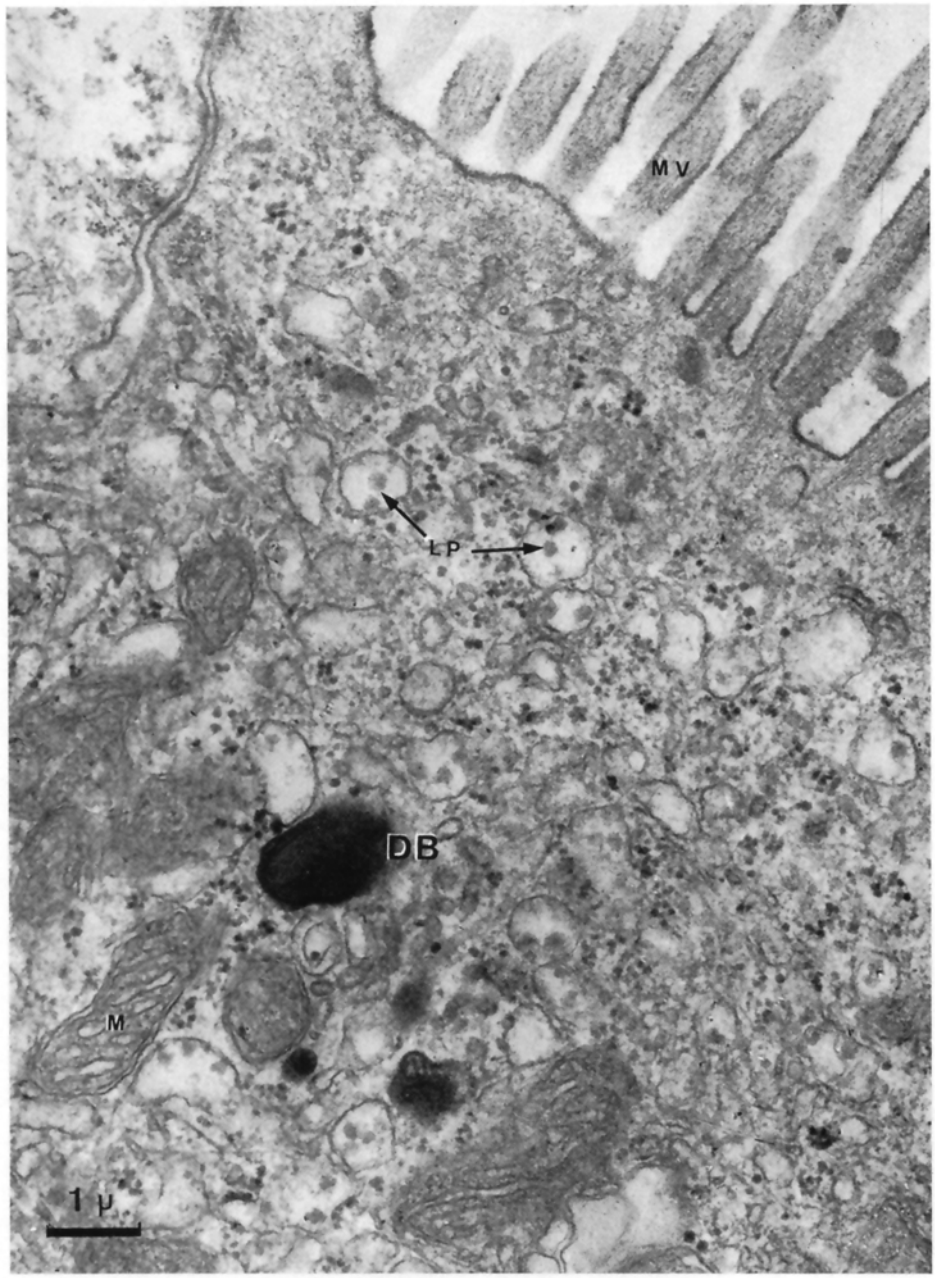

Fig. 2. Apical cytoplasm of enterocyte from carp fed on granules of special carp food. The newly formed lipid droplets (*LP*) appear in endoplasmic reticulum vesicles. *DB* dense bodies; *M* mitochondria; *MV* microvilli ×12000

The apical portions of adjacent epithelial cells are linked by five to seven closely spaced structures resembling small desmosomes (Fig. 1D). Below these,

other isolated and larger desmosome-like structures (generally three) are distributed along the lateral membranes of the enterocytes. There is no interdigitation between contiguous cells at this level.

Interposed between the epithelial cells and the lamina propria is a basal lamina (Fig. 3B), 0.03 to 0.07 μ thick. It is separated from the plasma membrane by a low contrast layer of fairly constant thickness.

b) The Lamina Propria. Beneath the basal lamina are fibroblasts, smooth muscle cells, nerve fibres and granule-containing cells. This area is supplied by fenestrated blood capillaries (pores approximately 100 nm in diameter) often in contact with the epithelial basal lamina. The endothelial cells bear small cytoplasmic processes projecting into the capillary lumen. The endothelial wall is partly related to pericytes. A loosely organized interrupted basal lamina surrounds the capillary (Fig. 4A).

2. Absorption of Alimentary Fat

a) Light Microscopy. Twenty-four hours after oil administration large fat droplets (up to several μ in diameter) are observed in the enterocytes. More diffuse red areas are also visible in the blood capillaries situated in the lamina propria. Fat particles are disseminated within the connective tissue but the experiment did not permit us to demonstrate lymphatic vessels.

b) Electron Microscopy. When animals are fed on granules of special carp food, the enterocytes contain lipid inclusions of two types: (1) particles of less than 100 nm diameter (*L.P.*); (2) droplets which may reach several microns in diameter (*L.D.*).

Localisation of Lipid Particles (*L.P.*). The lipid particles are present in the form of small homogeneous droplets between 30 and 100 nm in diameter. They are found: (1) within the tubules of endoplasmic reticulum at all levels of the enterocyte (Fig. 2); during fat absorption, the endoplasmic reticulum appears to play a very important role, occupying almost the entire supranuclear region of the cell; (2) within the vesicles of the Golgi apparatus which show an increase in number and size (Fig. 3A); (3) within certain macrophages and lymphocytes; (4) within the invaginations of the lateral and basal plasma membranes; (5) within the intercellular spaces of the epithelium where the lipid particles may be observed immediately below the band of apical desmosomes. Their number is greater closer to the base of the cells (Fig. 3). However, lipid particles do not generally accumulate at this level; finally, they are seen between the enterocytes and their basal lamina (Fig. 3B).

In the subepithelial connective tissue, lipid particles may be observed: (1) within the intercellular spaces where considerable accumulations sometimes occur (Fig. 4C); (2) between the basal lamina of the enterocytes and that of the capillaries (Fig. 3B; Fig. 4A); (3) against the endothelial cells, within their pinocytotic vesicles (Fig. 4B) and (4) within the lumen of the blood capillaries (Fig. 4A).

Localisation of the Lipid Droplets (L.D). The lipid droplets present in the form of large inclusions with a circular or oval shape. They may vary from 1 to 5 μ in diameter within the same cell (Fig. 5). These droplets are mainly found within the basal part of the enterocytes. They appear limited by a dense border but signs of fusion are sometimes observed. The droplets are frequently in close contact with

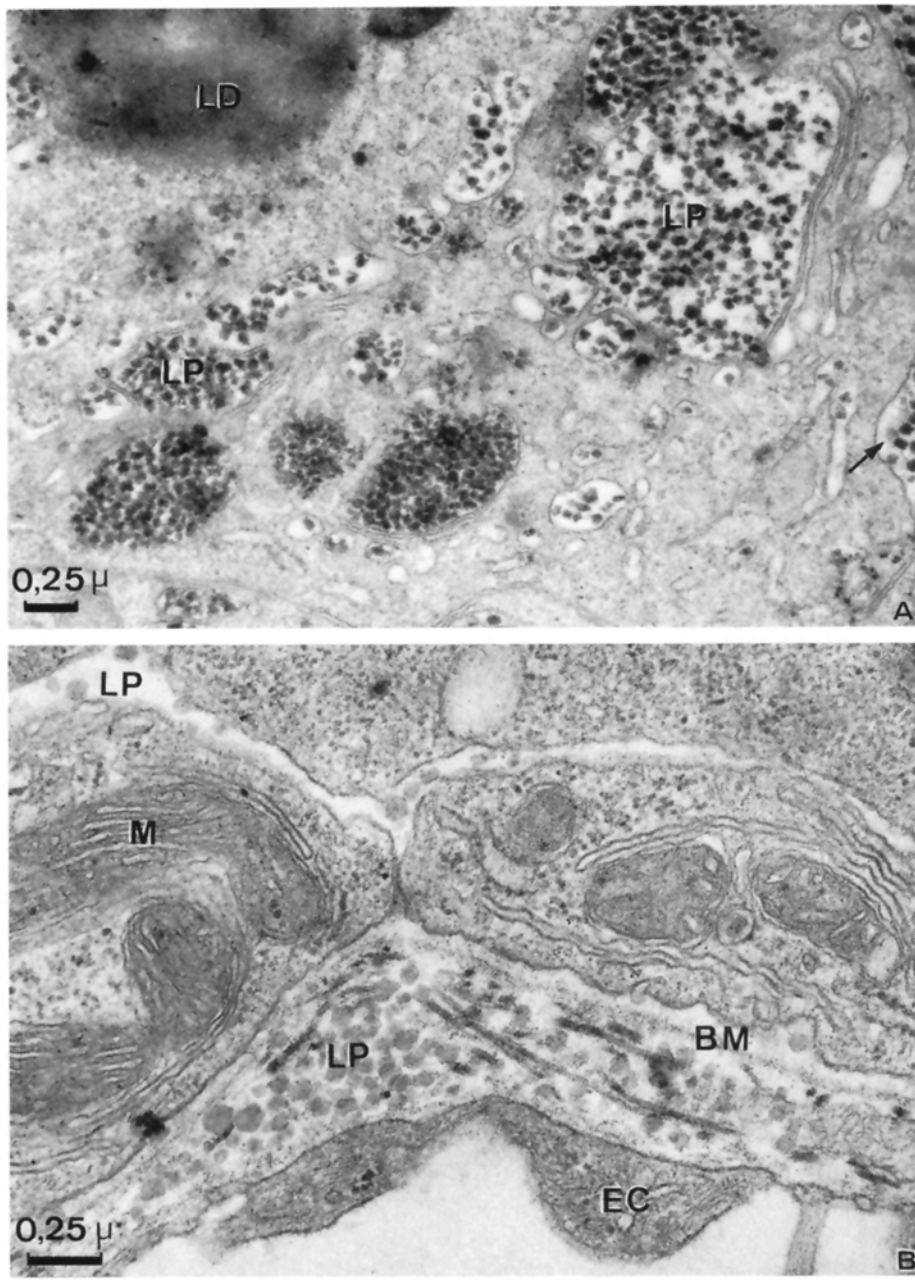

Fig. 3. (A) Electron micrograph of the Golgi apparatus of an enterocyte from carp fed on granules of special carp food. Golgi vacuoles are dilated and contain numerous lipid particles (*LP*) within their lumina. Note the presence of similar lipid droplets in the intercellular space (*arrow*). *LD* lipid droplets. (OTO method.) ×26000. (B) Basal portion of epithelium from carp fed on granules of special carp food. Lipid particles (*LP*) can be seen in the intercellular space and close to the endothelial cell (*EC*). Compare the epithelial and pericapillary basement lamina; the pericapillary basement lamina is less well developed. ×39000

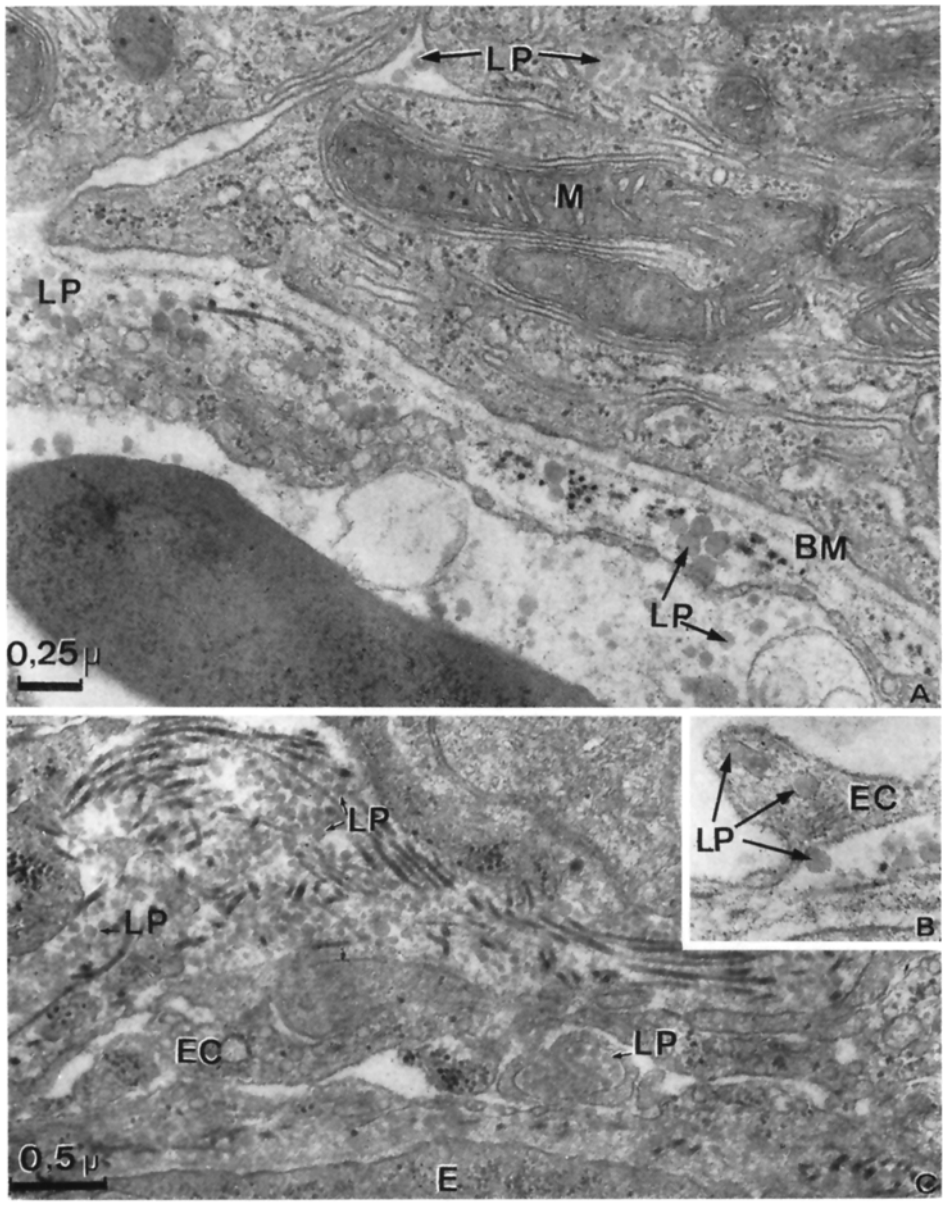

Fig. 4. (A) Blood capillary from a carp fed on granules of special carp food. In the lumen there are numerous lipid particles. (*LP*). ×32000. (B) Lipid particles in pinocytotic vesicles of an endothelial cell. ×46000. (C) Lipid particles in the subepithelial connective tissue. *BM* basement lamina; *E* enterocyte; *EC* endothelial cell; *M* mitochondria, ×24000

the smooth or granular reticulum, and certain appearances suggest that the lipid droplets may form within the lumen of the endoplasmic reticulum. In contrast with the lipid particles, lipid droplets are not observed within the intercellular spaces of the epithelium, or in the subepithelial connective tissue.

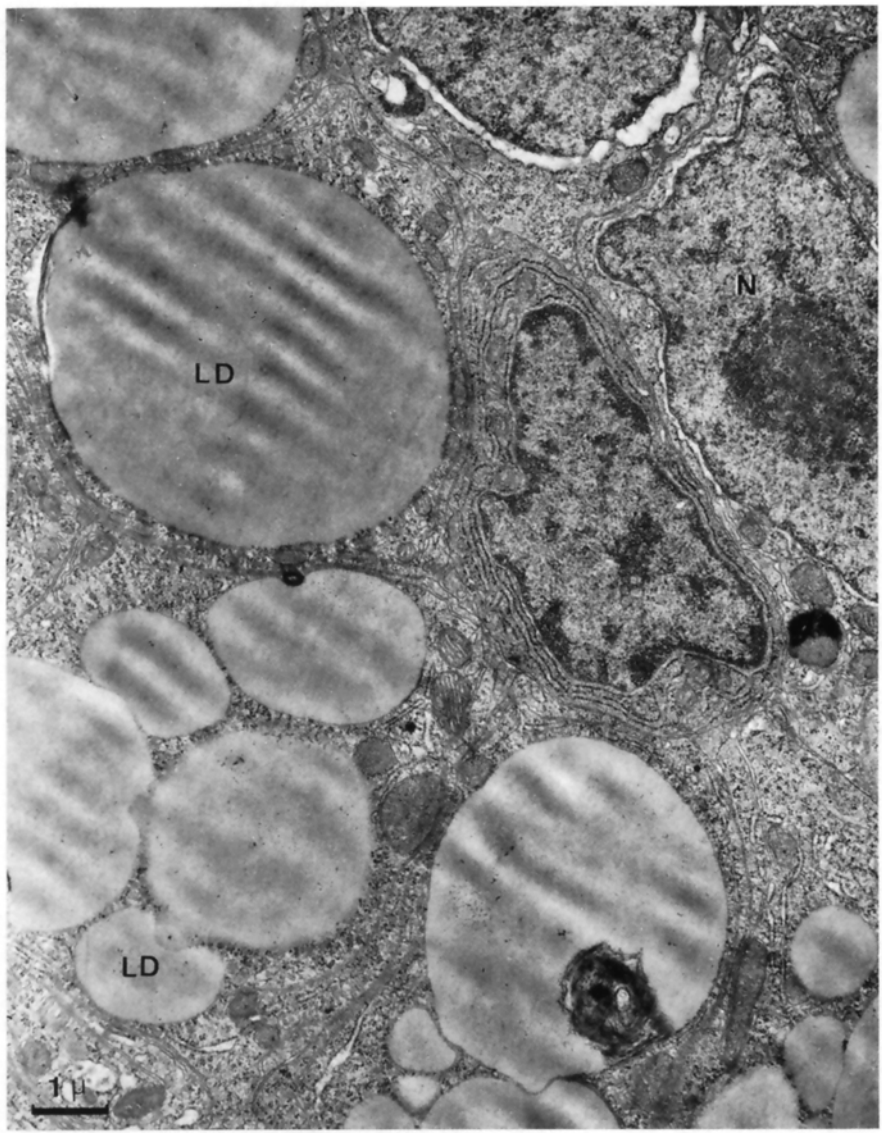

Fig. 5. Transverse section through the basal region of enterocytes from carp fed on granules of special carp food. Note the considerable variation in size of lipid droplets (*LD*). ×10000

Twenty-four hours after oil administration the enterocytes contain numerous lipid droplets (*LD*), the size and distribution of which correspond to those of the large drops observed under the light microscope. These lipid droplets are mainly located within the supranuclear area and their diameter increases close to the brush border. Sometimes at this level, the lipid droplets reach a very large size, pushing the cytoplasmic organelles towards the periphery of the cell. Within the intercellular spaces, a few lipid particles (*LP*) are also observed (Fig. 6).

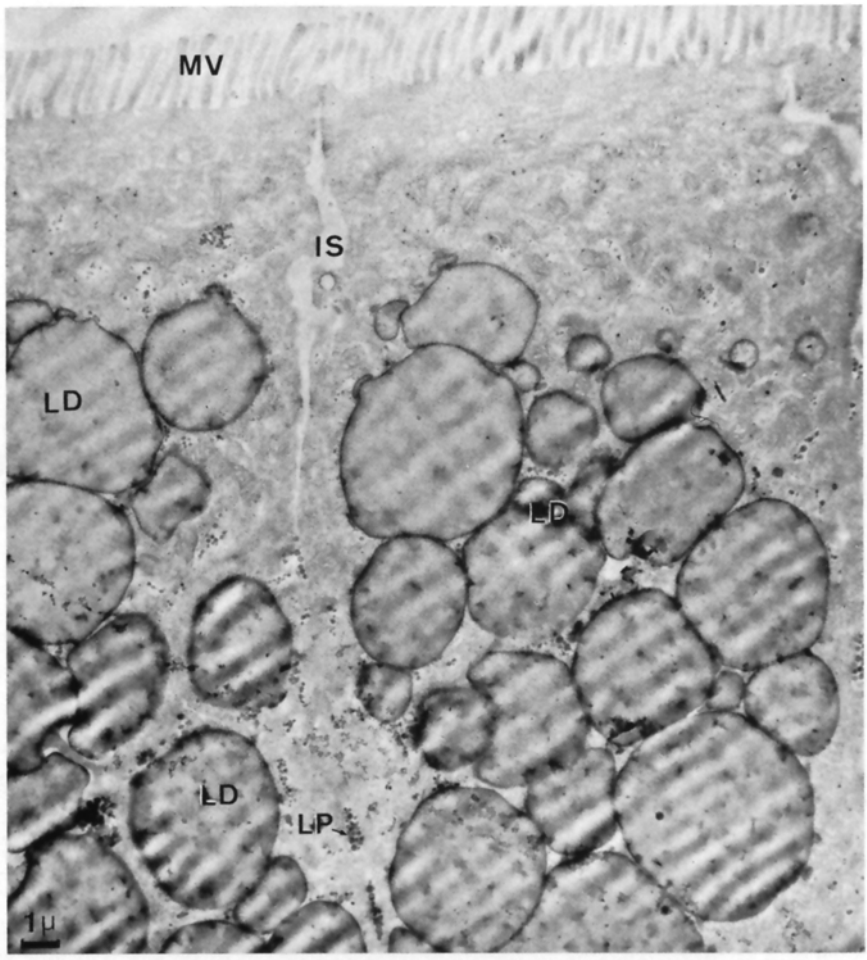

Fig. 6. Longitudinal section through enterocytes of anterior intestine 24 hours after ingestion of arachis oil. Numerous lipid droplets (*LD*) are closely packed in the apical cytoplasm. *IS* intercellular space; *LP* lipid particles; *MV* microvilli. (OTO method), ×4000

Discussion

The mucosa of the Anterior and Middle 1 Segments of the carp intestine appears different from that of mammals both in structure and for lipid absorption function.

1. Ultrastructural Characteristics of the Enterocytes of the Anterior and Middle 1 Segments

The enterocytes of the Anterior and Middle 1 Segments in many ways resemble those of mammals particularly with respect to the microvilli (Trier and Rubin, 1965; Vodovar and Flechon, 1966).

Similarly, the distribution of the organelles (mitochondria, Golgi apparatus, endoplasmic reticulum) and the position of the nucleus are similar.

We have, however, noted several special characteristics. In the first place, the sub-microvillous apical cytoplasm does not contain organelles and shows higher contrast than that of the subjacent cytoplasm. This terminal web differs in its ultrastructural appearance from that described in mammals as it does not contain filaments linking the roots of the microvillous processes and is not attached to zonulae adhaerentes.

Secondly the apical junctional complex of the carp is very different from that described in the intestinal epithelium of mammals in which a series of zonulae occludentes, zonulae adhaerentes and maculae adhaerentes occurs.

Finally, the lamellar structures deriving from the lateral and basal membranes of the enterocyte have not been described in mammals. They have previously been noted in other teleosts, such as the trout (Bergot and Flechon, 1970a) and the goldfish (Yamamoto, 1966).

The blood capillaries observed under the epithelium of the carp show endothelial pores, an interrupted basal lamina and an incomplete pericyte investment. They correspond to type B-2-alpha according to the classification of Bennet *et al.* (1959). They are thus different from those described in mammals by Papp *et al.* (1962) (interrupted basal lamina, very electron-opaque).

2. Absorption of Alimentary Fat by the Intestinal Mucosa of the Carp

In the enterocytes, fat absorbed from the intestinal lumen is found in two forms: in fat particles (*LP*) and in lipid droplets (*LD*). A comparable situation was noted in the trout by Bergot and Flechon (1970a, b). In mammalian enterocytes, large fat droplets have also been described in addition to chylomicrons (Taylor and Adamstone, 1965; Strauss, 1966; Vodovar and Flanzy, 1966; Cardell *et al.*, 1968; Friedman and Cardell, 1972a).

The latter may be differentiated from the fat particles by their diameter which is always greater (100 to 200 nm).

a) Localisation and Route of Transport of Fat Particles. The fat particles, present in the lumen of the endoplasmic reticulum and the vesicles of the Golgi apparatus, constitute a form of transport of alimentary fat. They are related by their size to low density lipoproteins of hepatic origin, described in mammals (Baglio and Farber, 1965; Hamilton *et al.*, 1967; Jones *et al.*, 1967; Trotter, 1967).

At the level of the enterocytes of the Anterior and Middle 1 Segments, the pinocytotic vesicles are rare and this suggests that fats do not enter the cells by micropinocytotic uptake. It thus seems possible that there is an intra-luminal hydrolysis of fat by pancreatic lipase. The absorption of the products of this hydrolysis may occur, as in mammals, in the molecular form from micelles (Hofman and Borgstrom, 1962).

In the carp, the endoplasmic reticulum is the organelle which undergoes the most apparent changes during absorption of lipid. One may observe marked development of the endoplasmic reticulum. In the apical part of the enterocytes, the fat particles are only visible within the lumen of the smooth endoplasmic reticulum, situated beneath the sub-microvillous zone. The resynthesis of triglycerides from absorbed fatty acids and monoglycerides, may thus occur, as in mammals, at the level of the endoplasmic reticulum (Senior, 1964; Cardell *et al.*, 1968; Porter, 1969; Carlier, 1971; Higgins and Barnett, 1971; Oledzka-Slotwinska,

and Desmet, 1971). The precise mechanism by which the fatty acids and monoglycerides after absorption reach the lumen of the reticulum, is not yet known. Ladman *et al.* (1963), and Vodovar and Flanzy (1966) have demonstrated an osmiophil substance within the smooth reticulum of the apical zone, close to the microvillous processes. The latter authors suggest the existence of a direct relationship between the bottom of the intermicrovillous spaces and this reticulum. However, according to Robins *et al.* (1959), the free fatty acids are partly re-esterified into triglycerides at the level of the microvillous membrane.

The precise role of the Golgi apparatus during the absorption of alimentary fat is still much debated. According to certain authors (Friedman and Cardell, 1972b; Oledzka-Slotwinska and Desmet, 1971), this cell organelle may be a compulsory intermediate between the cell and the intercellular spaces during trans-epithelial transport of fat. According to others, the endoplasmic reticulum which ensures the transport of fat particles may also channel them into the intercellular spaces (Strauss, 1966; Cardell *et al.*, 1968). The lamellar structures observed in the basal zone of the enterocytes seem to be simply an accessory route leading to the intercellular spaces. The considerable accumulation of lipid particles noted by Bergot and Flechon (1970a) close to the basal lamina, was not found in our material. The basal lamina of the carp intestinal epithelium does not appear to present an obstacle to the passage of fat particles.

The location of the fat particles within the lamina propria of the carp resembles that described by Bergot and Flechon (1970a) in the trout. On the other hand, it is very different from that observed in mammals, where chylomicrons are not present within the space between the capillary basal lamina and the endothelial cells, nor within their lumen. In the carp, the above-mentioned location may be explained by the thinness and irregularity of this membrane, whose structure resembles that of capillaries of new-born mammals (Suter and Majno, 1965). Now, it has been shown that in these animals, chylomicrons are present within the lumen of the blood vessels (Graney, 1967). After crossing the basal lamina the lipid particles seem to reach the capillary via the pinocytotic vesicles of the endothelial cells.

Although Bergot and Flechon (1970a) have described lymph vessels by electron microscopy within the lamina propria of the trout, we have not, so far, found such structures in the carp. The only route of evacuation of fat thus seems to be via the portal blood vessels.

The lamina propria of the carp seems to play a role in storage. We have often noted that there occurs a marked accumulation of fat particles within the intercellular spaces. This was also observed by Lovern and Morton (1939) in the halibut (*Pleuronectes hippoglossus*).

b) Nature and Role of Lipid Droplets. The lipid droplets observed were comparable to those described within the enterocytes of carp fry (Iwai, 1969) and of the trout (Bergot and Flechon, 1970b). The latter authors showed, furthermore, that the fatty acids were in the esterified form. Several theories have been formulated concerning the site of synthesis of the triglycerides contained within the lipid droplets. According to some, synthesis may take place outside the endoplasmic reticulum, using the enzyme systems present on the external surface of the reti-

culum or other hyaloplasmic enzymes distinct from those of the reticulum (Bergot and Flechon, 1970b). Other authors consider that the lipid droplets result from coalescence of lipid particles within the vesicles of the Golgi apparatus (Cardell *et al.*, 1968). Finally, it is possible that synthesis of these triglycerides may occur within the endoplasmic reticulum as shown in particular by Vodovar *et al.* (1969), who administered an inhibitor of protein synthesis. The absorption of fatty acids is then shown by the presence within the enterocytes of lipid structures similar to lipid droplets. Our observations seem to be in agreement with the interpretation given by the latter authors. In fact, we have sometimes noticed, around the lipid droplets, the presence of a membrane of endoplasmic origin.

Iwai and Tanaka (1968) have described the lipid droplets as being a form of transport of alimentary fat. During our observations, we have not encountered this type of lipid outside the absorbing cell. Thus, we believe in agreement with Bergot and Flechon (1970b), that the presence of these structures is linked to marked absorption of fatty acids by the enterocyte as is shown by their accumulation following exclusively fatty food administration. They thus represent a form of temporary storage of fatty acids. Slow passage of triglycerides towards the lamina propria, where protein synthesis could be insufficient for the formation of fat particles, may favour the formation of lipid droplets.

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