Z. Zellforsch. 146, 525—541 (1973)

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

Ultrastructural and Cytochemical Study

J. Noaillac-Depeyre and N. Gas

Laboratoire d'Ecophysiologie Animale (Professor A. Serfaty)
Université Paul Sabatier, Toulouse, Cedex, France

Received September 13, 1973

Summary. In the adult carp, the ultrastructure of the enterocytes of the distal segment of the medium intestine is similar to that of the intestinal cells of certain mammals during the neonatal period. Frequent aspects of pinocytosis are visible at the base of a plateau of already developed microvillosities. The apical cytoplasm shows the presence of a dense tubulo-vesicular network. The vacuoles which separate off run together to form a voluminous supra-nuclear body. Their P.A.S. positive and orthochromatic contents are rich in alkaline phosphatases. The permeability of this epithelium to macromolecules is demonstrated by the administration of "Horseradish Peroxidase" (HRP). This protein penetrates by pinocytosis into the apical tubulo-vesicular system and reaches the blood circulation via the extra-cellular spaces. The presence of certain structures involved in protein transport following a period of experimental fasting of six months reflects their independence with regard to exogenous supplies.

Key words: Intestine (Teleost) — Enterocytes — Absorption of proteins.

In the cyprinidae, which are fishes lacking a stomach, the digestive system is, from the morphological point of view, one of the least differentiated among the vertebrates. In spite of this, four different regions that differ in their physiological functions may be distinguished in the intestine of the carp. (Al Hussaini, a and b). Above the opening of the bile duct, a dilated portion forms the anterior intestine. A long thin segment or small intestine extends from it into two different portions: a proximal portion (Medium 1) and the other distal portion (Medium 2). A short posterior intestine, or rectum, ends at the anus; an original aspect of its functions, independently of the strictly digestive mechanisms involved, has recently been described (Noaillac-Depeyre and Gas, 1973).

Yamamoto (1966), Gauthier and Landis (1972) in the golden Cyprin, as well as Iwai (1969) in the fry of the carp, underline the ultra-structural differences observable between the enterocytes of the anterior and medium 1 segments, and those of the medium 2 segment. They also mention the existence of a proximo-distal physiological gradient: the absorption of lipids occurring essentially in the first segment (Anterior and Medium 1) that of the proteins in the distal portion (Medium 2) by the mechanism of pinocytosis.

* This study has been carried out thanks to a grant by the C.N.R.S. (R.C.P. No 250), and with the electron microscope of the department of plant Biology (Professor Leredde) kindly placed at our disposal by Mr. B. Lugardon. We are also grateful to Mrs A. Rup for her technical assistance.

The purpose of our study is to establish the ultrastructures involved in the absorption of the macromolecules and to describe the means of their transport. In order to do so we have traced the intracellular development of an enzymatic protein "Horseradish Peroxidase" which is easily identifiable by cytochemical methods.

Elsewhere, within the wider context of a cytophysiological study on denutrition, this work led us to analyse the influence of six months fasting on the cell sites linked with protein absorption.

Materials and Techniques

1. Animals Used

Common Carps (*Cyprinus carpio* L.), of an average weight of 250 gm, living in tanks in running water, are subdivided into two batches:

a) The "control" animals are fed on granulated whole food for Carps provided by the Suppliers JOF, Toulouse;

b) other animals are submitted to an experimental fasting period, of six months (from November to May).

2. Cytological Study of the Intestinal Epithelium

The animals were decapitated, their intestine rapidly removed and fixed in Bouin Duboscq fluid mixture or in 10 per cent neutral formalin, for study under the light microscope. The histochemical tests such as P.A.S., amylase digestion followed by P.A.S., and Toluidine blue stain are performed on sections of 4 microns thickness.

For study under the electron microscope, the samples are treated: either by a 3 per cent glutaraldehyde solution (Suchardt Laboratory) in 0.1 M phosphate buffer (Sörensen) at pH 7.2, during two hours at 4° C then post fixed for thirty minutes in 1 per cent osmium tetroxide in the same buffer; or by osmium tetroxide alone during one hour.

The ultra-thin sections are stained with uranyl acetate and lead citrate (Reynolds, 1963).

3. Identification of Acid and Alkaline Phosphatase Activities

The samples are fixed in 3 per cent glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 during two hours, then rinsed for one night in cacodylate buffer to which 7.5 per cent saccharose is added (at 4° C). The aldehyde fixation enables the cytochemically detectable enzymatic activities to be preserved (Sabatini *et al.*, 1963). 50 μ thick sections are then cut and incubated either: *in order to detect the acid phosphatase activity*, in Gomori's medium, supplemented with 0.05 M phenylalanine, specific inhibitor of alkaline phosphatase activity (Hugon and Borgers, 1968), or in Hugon and Borgers' medium (1968) for the *identification of alkaline phosphatase activity*.

The sections used as controls are incubated in a similar medium but without substrate. All of the samples are then post fixed with osmium tetroxide and treated in the same way as those destined for the ultrastructural study. The ultra-fine sections are either observed directly or stained with uranyl acetate, and lead citrate.

4. Absorption of Peroxidase by the Enterocytes

Control Carps receive approximately 2 ml of a solution containing 10 mg/ml of "Horseradish peroxidase" (HRP: Sigma Type II) in the anterior region of the intestine. A syringe the end of which is attached to a rigid polyethylene tube enables the administration in the region just above the pharyngeal sphincter. An hour or two following ingestion, the animals are killed and portions of the intestine are fixed in the glutaraldehyde and cut frozen. The identification test is performed according to Graham and Karnovsky's technique (1966). The endogenous peroxidase activity is detected by the same technique in portions of intestine from animals that had not ingested HRP.

Results

1. Cytological Study of the Enterocytes in the Fed Animals

a) Light Microscopy: The epithelium of the second segment of the middle intestine shows considerable vacuolization in the region of the lateral walls of the folds. These vacuoles occupy the upper region of the cells, thrusting the nucleus in the neighbourhood of the basal membrane (Fig. 1a). Their contents are P.A.S. positive and the digestion by amylase does not modify this tinctorial affinity. Toluidine blue confers an orthochromatic colour to these formations whereas it produces a metachromatic colour with the secretion product of the goblet mucous cells. These histochemical tests thus reflect the presence of a glucide fraction associated with the vacuolar material in the form of neutral or slightly anionic glycoprotein complexes.

b) Electron Microscopy. As in the case of most absorbant surfaces (eg. intestine, kidney), the plasmic membrane possesses a fibrous covering ("cell coat") notably on the external surface of the microvillosities. This differentiation of the surface is again visible in the intermicrovillositary space, in the numerous invaginations of the membrane as well as in the vacuoles of pinocytosis from which they are derived.

Some filaments form the framework of the microvillosities (Fig. 3a) but may also be observed in the cell axis throughout the supranuclear area. It is believed that together with the microtubules it forms the cytoskeleton of this region, which is furthermore fairly rich in vacuoles.

A Complex Tubulo-vesicular Network in fact occupies the apical zone of the enterocyte (Fig. 1c), some of these structures contain a finely granular material. Connections between this network and the vesicles formed by pinocytosis may sometimes be observed (Fig. 3c). The size of these vesicles increases in the neighbourhood of the nucleus, probably by the running together of the primary vacuoles. They finally give rise to a voluminous supranuclear body (Fig. 2).

The *Golgi apparatus*, always highly developed, is situated next to the supranuclear body (Fig. 3d), and the numerous small vesicles that separate off are to be found throughout the enterocyte. Others probably of the same origin but larger in size (Fig. 3a, e) with a thicker membrane and covered, with short filaments externally, are comparable to the "coated vesicles". These connect up with the plasmic membrane in various places particularly in the region of the intestinal lumen (Fig. 3a). The mitochondria, the granular reticulum and numerous free ribosomes are situated principally in the infranuclear region where several invaginations of the plasmic membrane are also visible.

2. Absorption of a Heterologous Protein, Horseradish Peroxidase (HRP: Mol.Wt. 40000) by the Fed Animals

a) Light Microscopy. An endogenous peroxidase activity is only detectable in the erythrocytes and the leucocytes of the lamina propria. (Fig. 4a). On the other hand, the subjects that had ingested some HRP showed a positive reaction in the neighbourhood of the vacuoles of the supranuclear area. The comparison of the different regions of the folds revealed that absorption, which was reduced at the tip and the at the base was quite considerable in the region of the lateral walls (Fig. 4b).

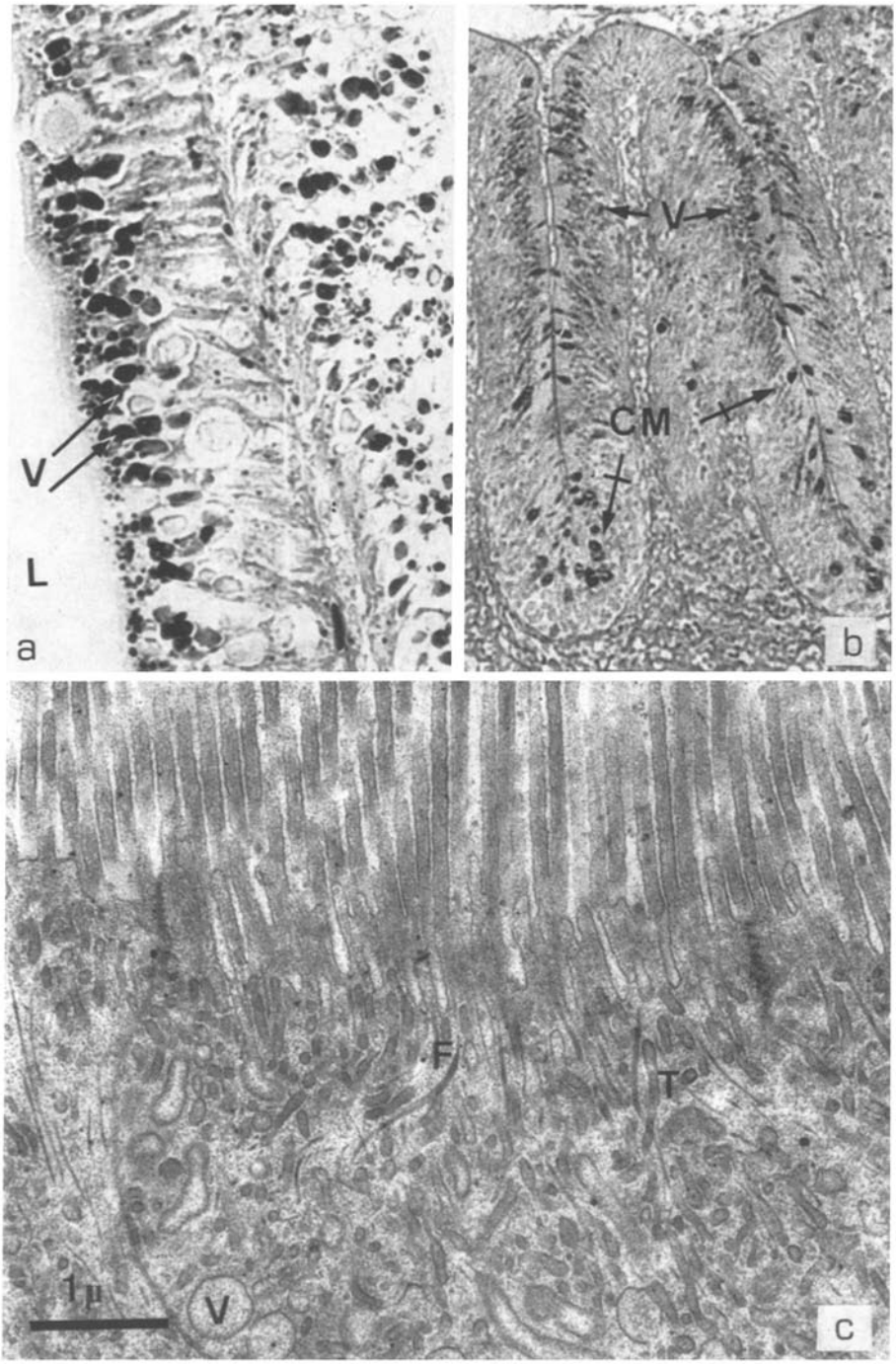

Fig. 1. a. Medium 2 segment of "control" animal. Apical vacuoles and supranuclear vacuoles (*V*) are observed in the cytoplasm of the absorptive epithelial cells. *L* lumen (×2000 — PAS — Hematoxylin). b. Medium 2 segment of carp fasted for six months prior to sacrifice. Apical vacuoles are still observed in the cytoplasm of the lining cells (*V*). *CM* mucus goblet cells (×1375 — PAS). c. Apical surface of epithelial cell from medium 2 segment. The microvillosi-

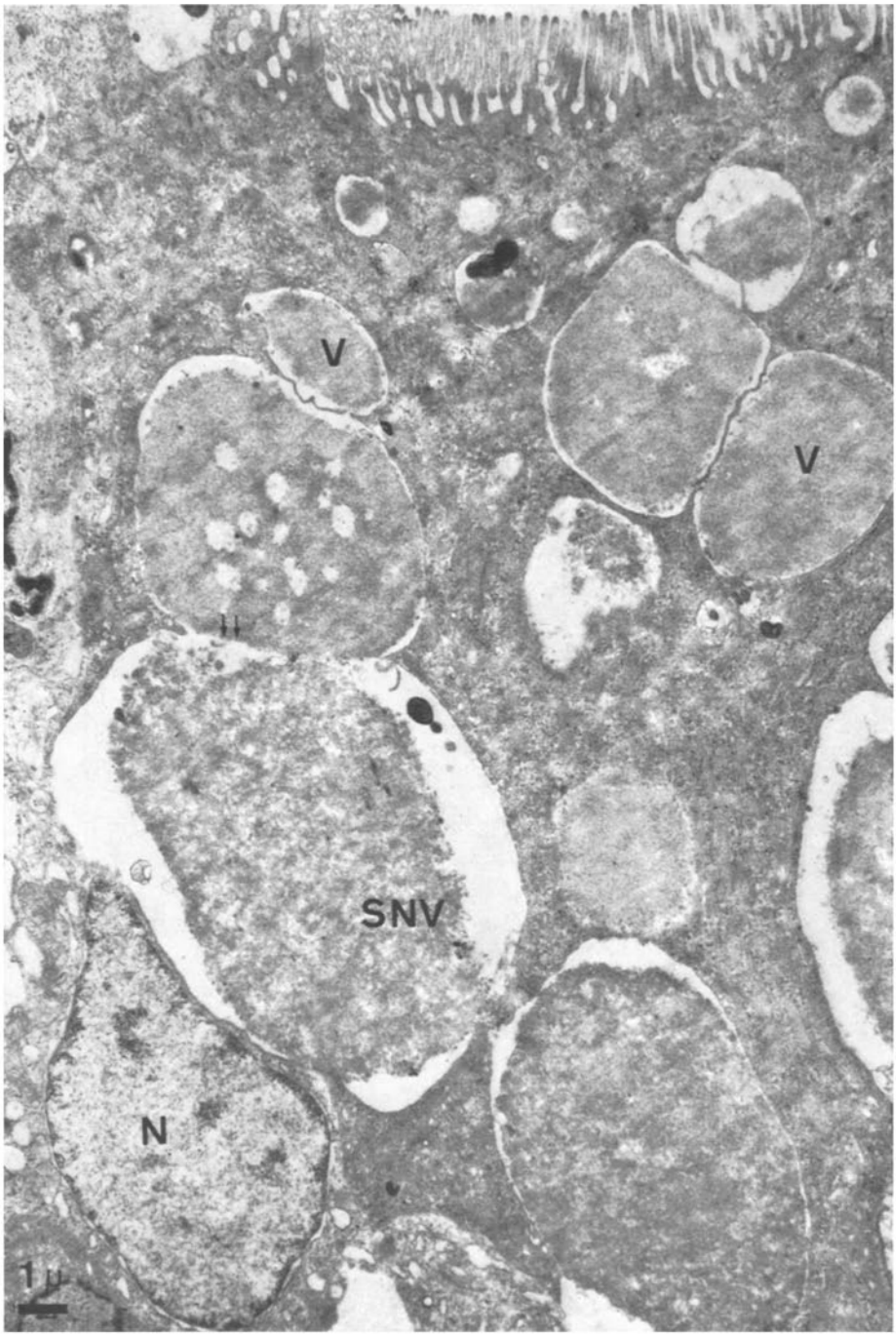

Fig. 2. Absorptive cells from medium 2 segment. The apical cytoplasm contains vacuoles (V) of various sizes. They appear to be coalescing in the supranuclear region (SNV ↓↓) ($\times 5950$)

ties, apical vacuoles (V) and tubules (T) are well developed. Rootlets (F) extend from the microvillosities into the apical cytoplasm. ($\times 20000$)

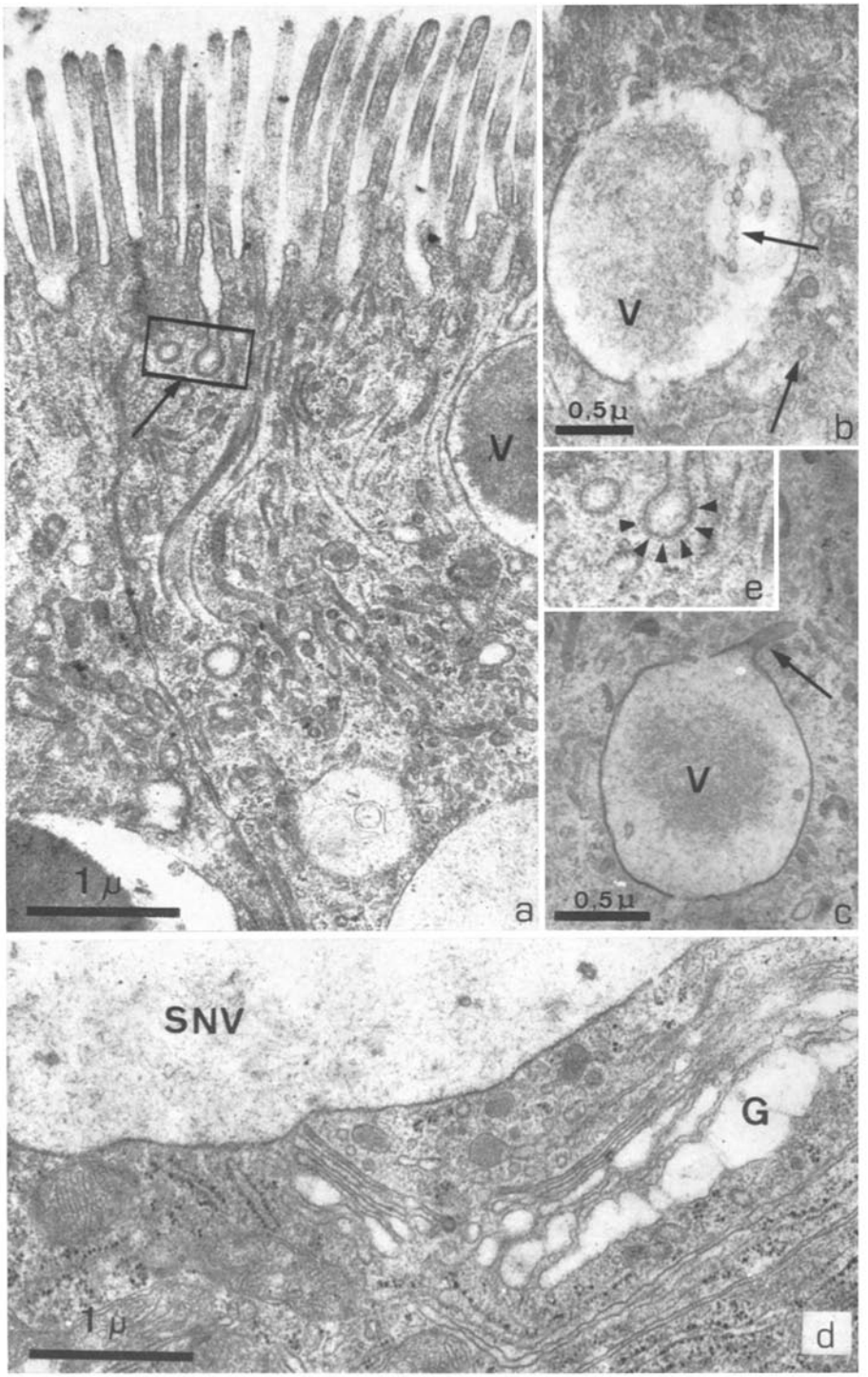

Fig. 3a—e

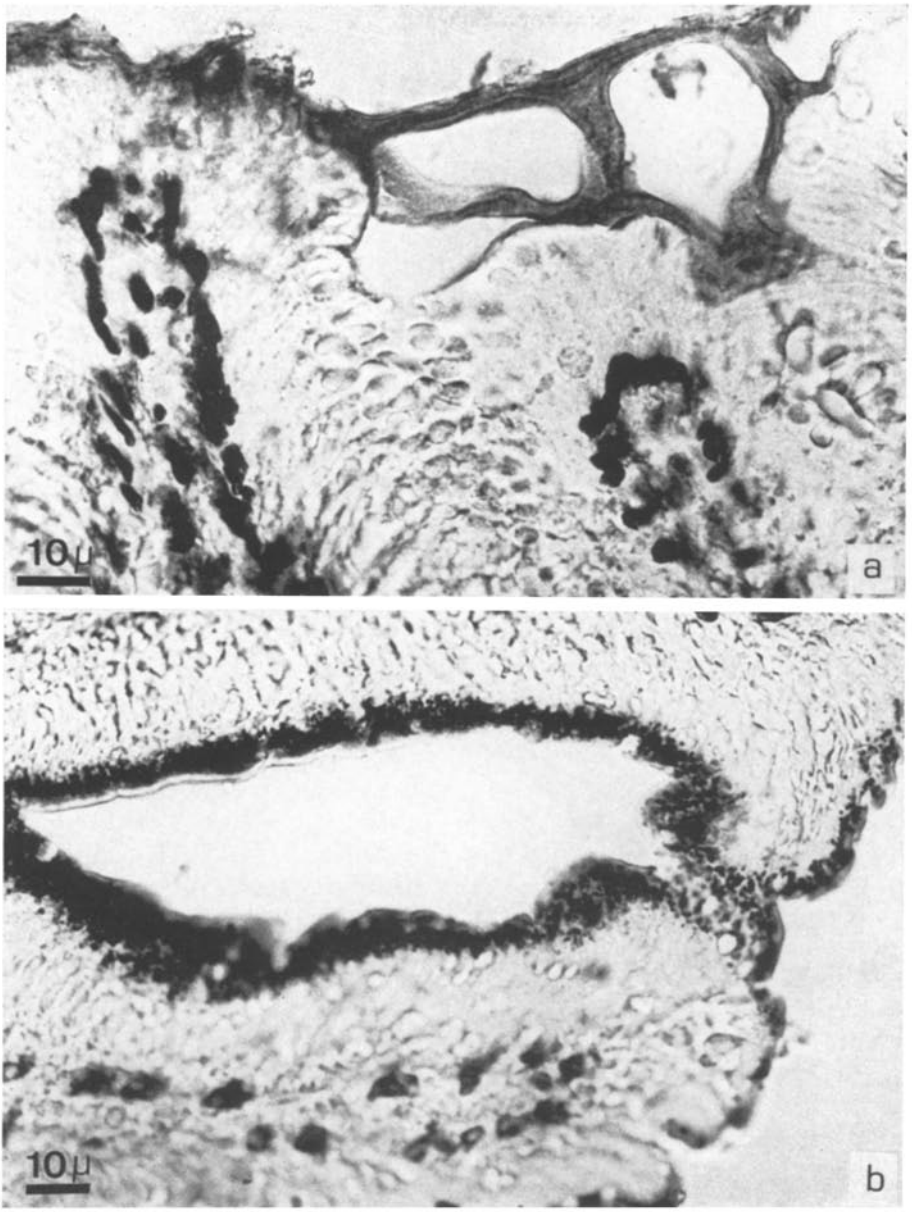

Fig. 4. Localization of the peroxidase activity. a. Anterior intestine 120 minutes following administration of HRP. Note the presence of exogenous protein in abundance in the lumen. Activity in the connective tissue reflects the presence of hemoglobin (×1150). b. Medium 2 intestine 120 minutes following administration of HRP. Exogenous peroxidase activity is evident in the apical cytoplasm of the epithelium. (×1000)

Fig. 3. a. Apical zone of enterocyte contains numerous absorptive vacuoles (*V*), and coated vesicles (arrow) (×24000). b. Numerous small vesicles are to be found throughout the enterocyte and in the absorptive vacuole (*V*) (arrow) (×25000). c. This micrograph shows vacuole with which tubule appears to be fusing (arrow) (×30000). d. The Golgi apparatus, highly developed is located next to the supranuclear body (*SNV*) (×25000). e. Insert from box in figure a: higher magnification

b) Electron Microscopy. Examination of fragments obtained from control animals made it possible to detect an endogenous peroxidase activity only in the small sized vacuoles of the zone just below the microvillosities and which was not visible under ordinary light microscopy.

In the animals killed one or two hours following the ingestion of HRP, this activity is localized in other cellular organelles. Present at the surface of the microvillosities, the protein penetrates at their base into the vesicles produced by pinocytosis (Fig. 5a). The sites of peroxidation in the region of the apical tubulo-vesicular network, the absorption vacuoles (Fig. 5b) and the supranuclear body (Fig. 5c) enable one to ascertain its outward transit. Since the axis of the microvillosities never contains any free HRP, the peroxidase thus penetrates into the epithelium exclusively by pinocytosis. Throughout its intracellular course, it remains included in the structures surrounded by a membrane. In the zone lying between the nucleus and the lower membrane of the enterocytes, no peroxidase activity could be demonstrated. However, the presence of a byproduct in the extracellular spaces reflects exit of the tracer. This certainly occurs through the intermediary of the apical tubules which can be seen to connect with the lateral membranes of the cells (Fig. 5a). The HRP reaches the basal layer of epithelium through the extracellular spaces, passes through it and reaches the lumen of the capillaries (Fig. 6a). Certain protein molecules are then phagocytosed by the circulating macrophages (Fig. 6b).

3. Localization of Acid and Alkaline Phosphatases Activities

These cytoenzymological research studies were undertaken in order to locate the alkaline phosphatases whose presence is generally related to mechanisms of absorption, and the acid phosphatases liable to intervene in the intracellular decomposition of the absorbed molecules. In the latter cases a positive reaction is detectable only in the region of the small non characteristic lysosomes of the cell type under study.

On the other hand, an intense alkaline phosphatase activity is observed in the apical tubulo-vesicular network (Fig. 7c), the absorption vacuoles, as well as in the saccules and Golgi vesicles (Figs. 7a, b, d). A weaker activity is detectable in the region of the granular endoplasmic reticulum. Finally, this enzyme is absent from the membrane of the microvillosities, whereas it is observable in this region in the anterior and medium 1 segments.

4. Morphology of the Enterocytes in the Animals Left Fasting

At the apical pole of the enterocytes, the microvillosities are considerably reduced in height (controls: 1.2 μ; fasting: 0.5–0.6 μ) but their distribution remains regular. The aspects of pinocytosis, as well as the absorption vacuoles disappear

Fig. 5a—c. Micrograph showing the localization of peroxidase reaction product 120 minutes after ingestion. Reaction product is present in the extracellular coat of cell membrane invaginations, in the small tubules (Fig. a), vesicles of the apical cytoplasm (Fig. b), and in large supranuclear vacuole (Fig. c). HRP is also present in the extracellular space; tubules can be seen to connect with the lateral membrane of the cell (arrows) (Fig. a). (Fig. a and c ×12500); Fig. b ×25000)

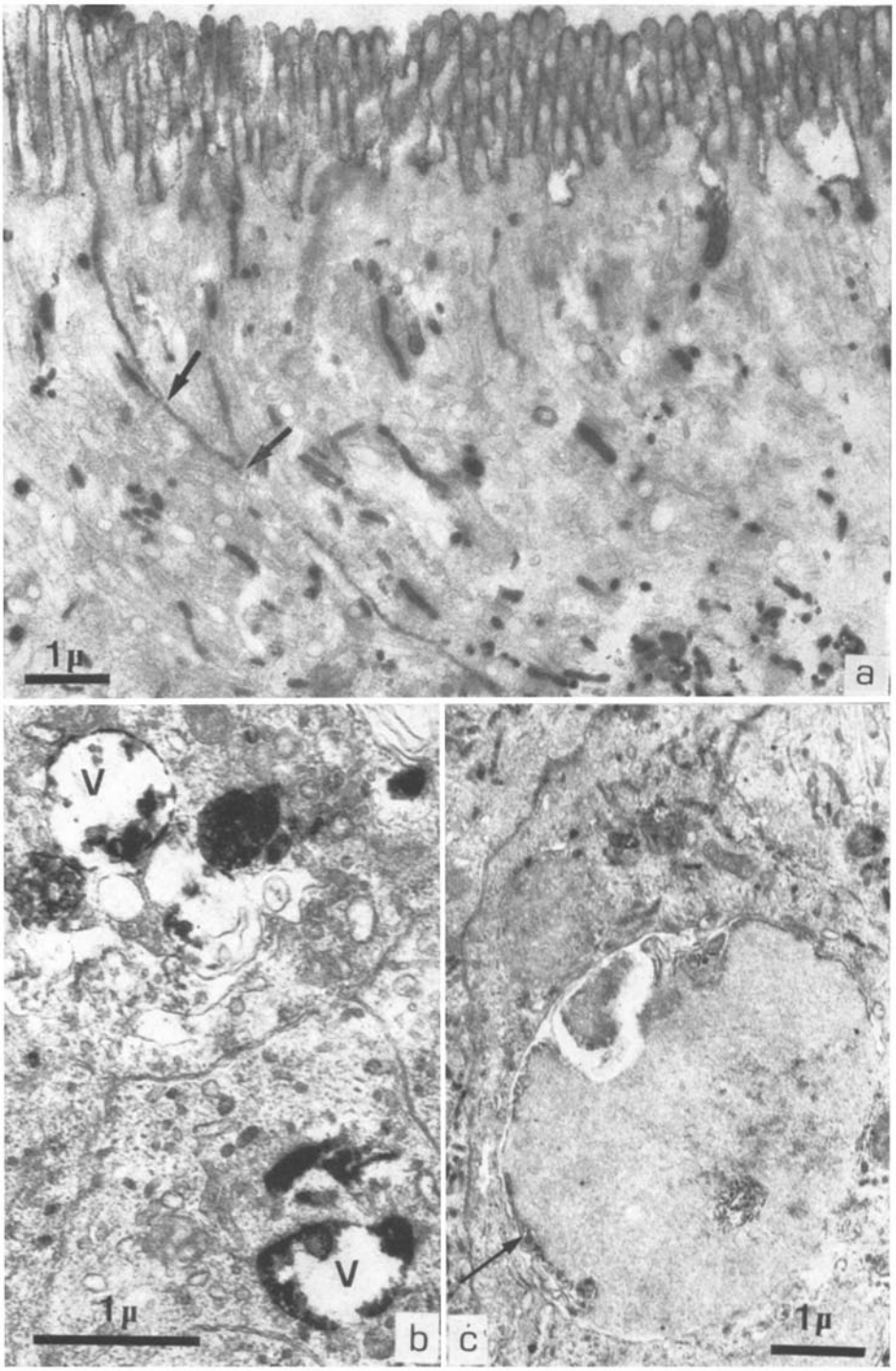

Fig. 5a—c

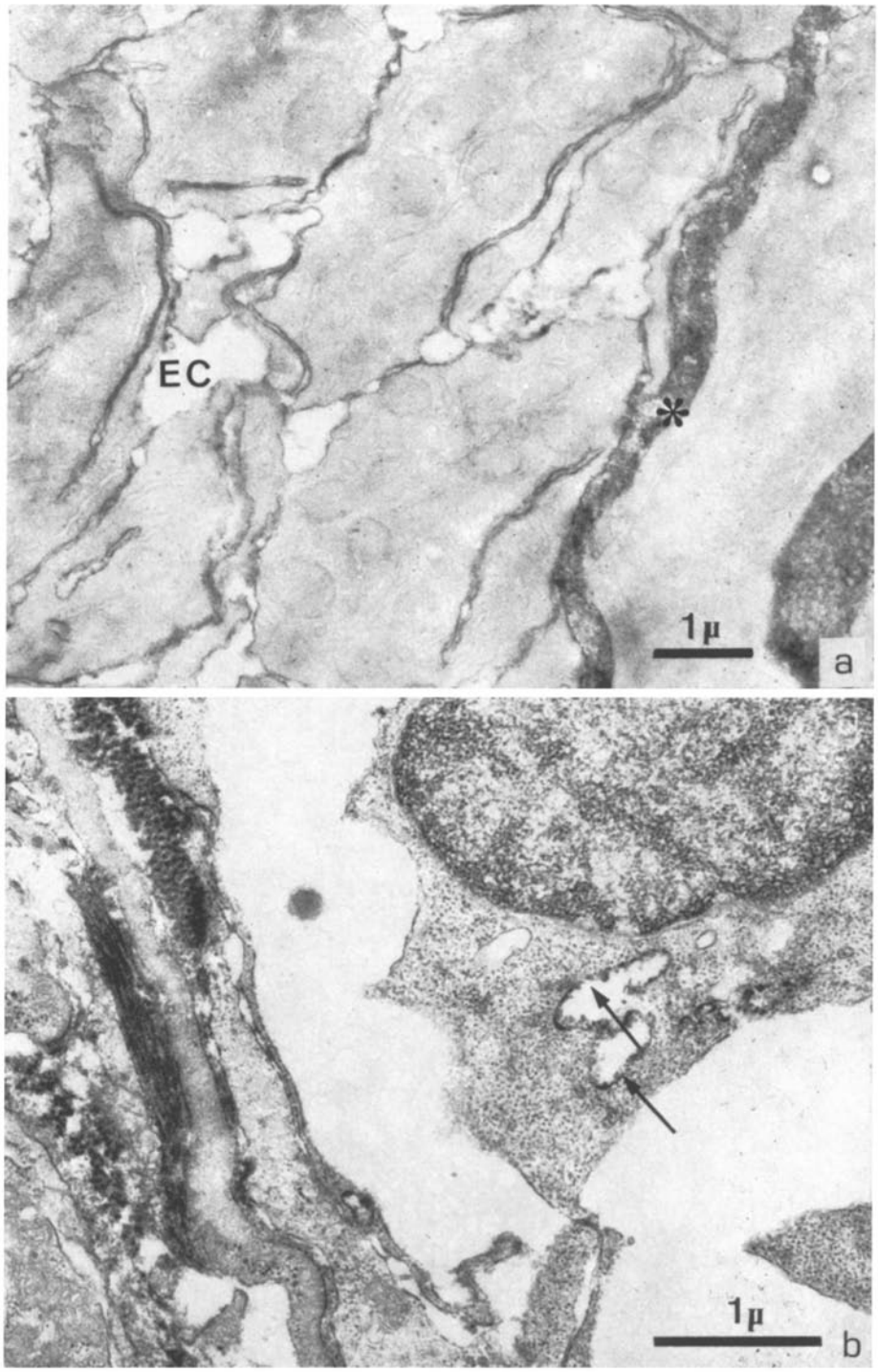

Fig. 6. a. Basal portion of epithelial cells. Peroxidase reaction product is observed in the extracellular spaces (*EC*) and in the basement membrane (*). (×15000). b. Certain protein macromolecules are phagocytosed by the circulating macrophages (arrows) (×25000)

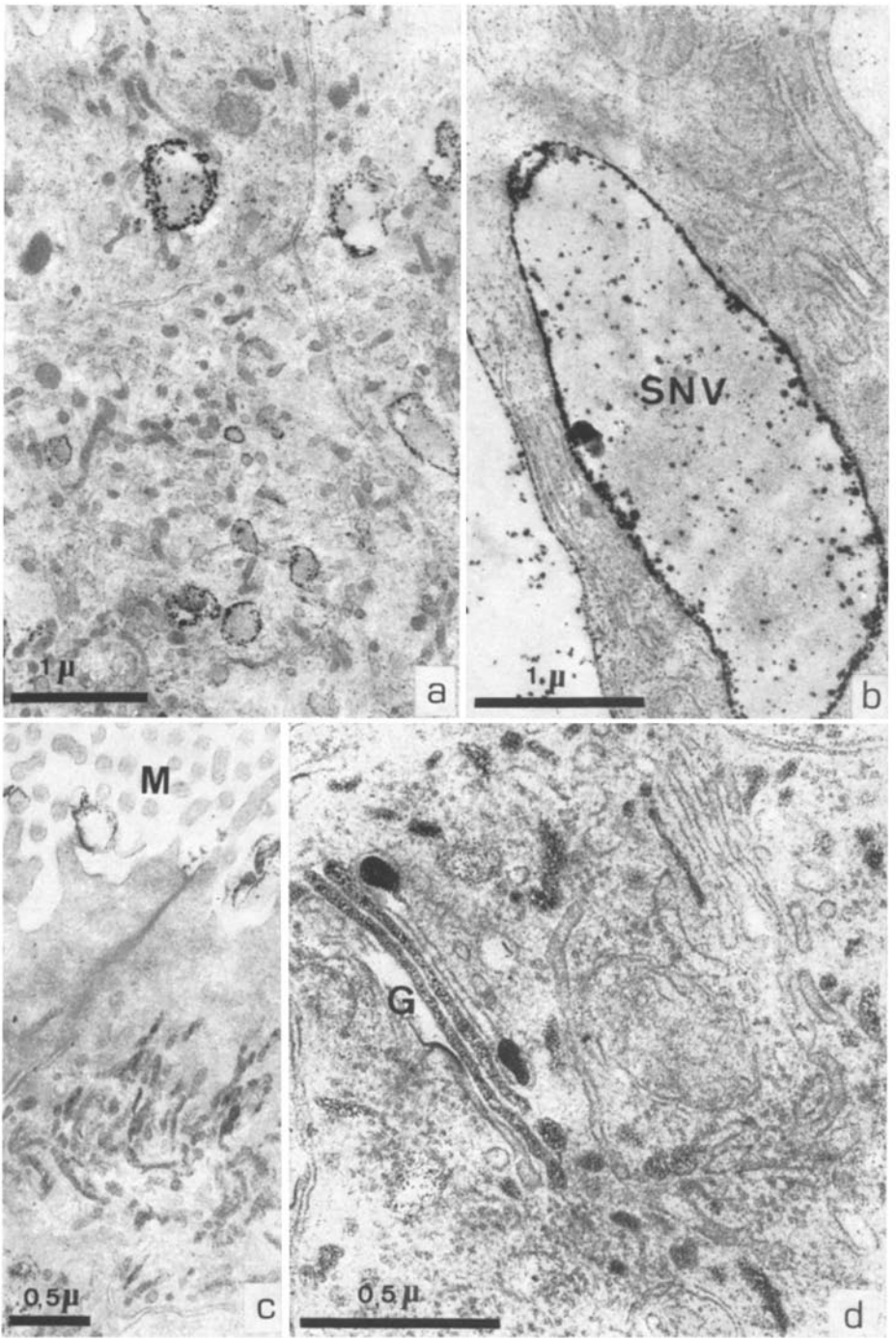

Fig. 7a—d. Alkaline phosphatase in the medium 2 intestinal segment. The precipitate of lead phosphate is prominent in apical small vacuoles (Fig. a, ×20000) and in the supranuclear body (*SNV*, Fig. b, ×25000). Numerous profiles of apical tubules show the reaction product (Fig. c, ×25000). The Golgi cisternae are also strongly loaded (Fig. d, ×60000)

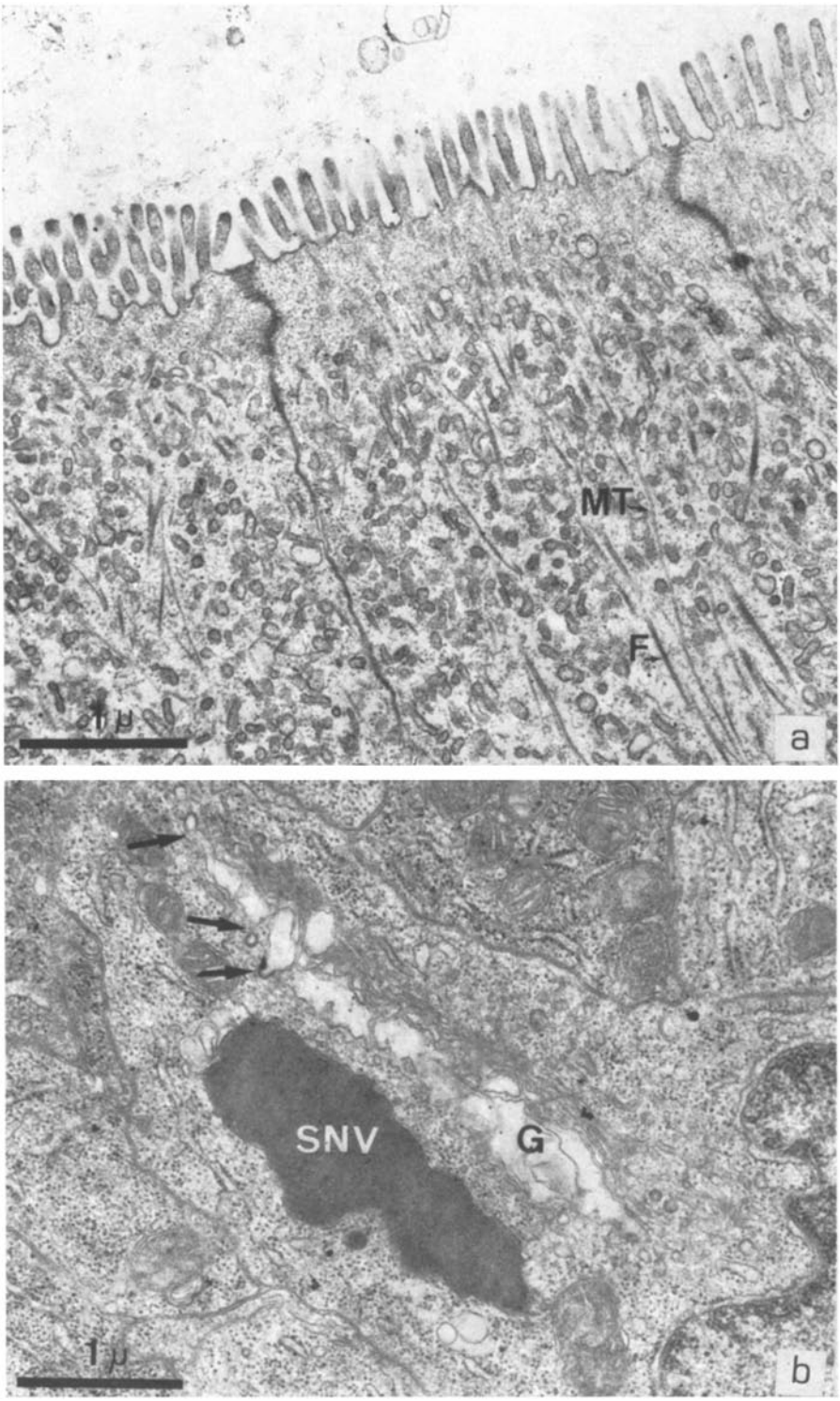

Fig. 8a and b

from the apical cytoplasm (Fig. 8). Whereas, the extensive network of tubules persists below the microvillosities. The cytoplasmic web thins out and the cytoskeleton consisting of filaments and microtubules becomes quite apparent. The supranuclear body is more reduced in size but its contents show a greater opacity to electrons. It nevertheless retains the same staining affinity (P.A.S.+ (Fig. 1b), basophilic reaction). The Golgi apparatus situated in the neighbourhood of the supranuclear vacuole shows signs of an intense metabolic activity.

Discussion

1. Structural Adaptations of the Enterocytes in Relation to Protein Absorption

The different aspects described above (i.e. microvillosities, pinocytosis, a tubular network and numerous vacuoles) are observed in many different types of epithelia but which are all adapted for protein transport: renal tubules (Graham and Karnovsky, 1966), vitelline sac (Padykula *et al.*, 1966) and intestine. They have been widely studied in this respect in various newly born Mammals such as the rat (Cornell and Padykula, 1969; Dunn, 1967; Shervey, 1966; Graney, 1968; Kraehenbuhl, 1967) the rabbit (Kraehenbuhl *et al.*, 1967) the pig (Moon *et al.*, 1973; Sibalin and Bjorkman, 1966; Staley *et al.*, 1972; Vodovar and Flechon, 1966) and the mouse (Hugon, 1971). These differentiations are already present in the foetus of the rat "in utero" (Orlic and Lev, 1973) where they allow for the absorption of immunizing proteins (γ globulins in particular, Mol. Wt. 160000). Among the Mammals, they disappear between the 15th and 21st day of postnatal development when peptic secretion is developing (Mosinger *et al.*, 1959). This type of absorption of macromolecules thus seems to be related to the lack of a complete extracellular digestion. Moreover, in Fish without stomachs, peptic secretion does not exist (Sarbahi, 1951) and the pancreatic trypsin present in the intestinal lumen (Vonk, 1937) does not produce complete hydrolysis of the protein material.

Various interpretations have been suggested concerning the part played by the different structures common to these different cell types. The fibrous covering observed in the region of the invaginations of the plasmic membrane represent a point of attachement for the protein material (Lambson, 1966). Furthermore, according to Cornell and Padykula (1969), the apical tubular system would consist of a differentiation of the plasmic membrane between the microvillosities, related to the mechanism of pinocytosis. Our observations do in fact show the persistence of a network of tubules in the animals allowed to fast, whereas there is no obvious pinocytosis, and the absence of alkaline phosphatases in the region

Fig. 8. Enterocytes of carp fasted for six months prior to sacrifice. a. At the apical pole of the enterocyte, microvillosities are considerably reduced in height. The absorption vacuoles disappear but the extensive network of tubules persists. The cytoskeleton filaments (*F*) and microtubules (*MT*) become quite apparent. b. Supranuclear body is reduced in size (its contents show a greater opacity to electrons). The Golgi apparatus appears well developped (*G*) and very active (arrows) (×25000)

of the microvillosities whereas they are present in the region of the tubules. Hence, we are of the opinion that there is a morphological and functional heterogeneity of the apical tubules as already suggested by Graham and Karnovsky (1966).

The supranuclear body is also of complex origin. Since it persists throughout fasting in spite of the absence of an exogenous supply, it does not seem to result solely from the running together of the food vacuoles. According to Cornell and Padykula (1969), it arises from the fusion of the Golgi vesicles. This structures would thus have a dual origin, endogenous through the hydrolytic enzymes which it contains, and exogenous through the proteins supplied by the food which are partially digested in this site.

The vesicles with an external coat, visible in all the regions of the enterocytes have been described among the cells of various other tissues: intestine (Sibalin and Bjorkman, 1966) deferent canal (Friend and Farquhar, 1967), macrophages (Lagunoff and Curran, 1972), and oocytes from mosquitoes (Roth and Porter, 1964). According to the latter authors, vesicles of this type are specialized for the transport of proteins. In fact, in the intestine of the carp, they are only occasionally in contact with the apical membrane. Hence, it seems unlikely that they have an important function in the transit of food proteins. They are probably more likely to take part in the transfer of enzymes as has been previously suggested by Friend and Farquhar, 1967). Furthermore, the different aspects of fusion of these structures with the apical cell membrane (Fig. 3a) would suggest that they might intervene in the renewal of the surface membrane lost through the mechanism of pinocytosis during protein absorption.

2. Means of Absorption and Transport of Peroxidase

The previous accounts of the intracellular localization of the sites of peroxidation enable one to suggest the following means of absorption: from the vesicles of pinocytosis, the protein passes into the tubular network, the vacuoles and the supranuclear body. Nevertheless, a fraction of the HRP ingested reaches the extracellular spaces without penetrating into the large size absorption vacuoles and reaches the sub-epithelial basal layers and the vascular system. Since the HRP is never observed in the subnuclear region, this region of the cell does not seem to participate in protein transit.

During the neonatal period in the rabbit, mouse and rat, the absorption of peroxidase and even ferritin (a protein of higher molecular weight: 800000) takes place in a comparable manner (Hugon, 1971; Kraehenbulh *et al.*, 1967; Graney, 1968). Other substances such as colloidal gold and latex (Sanders and Ashworth, 1961) are probably also absorbed by identical processes.

This ability to transfer exogenous proteins from the intestinal lumen towards the blood is thought to persist in the adult (Cornell *et al.*, 1971; Warshaw *et al.*, 1971). In the carp, we are of the opinion that most of the ingested macromolecules are concentrated in the supranuclear body where they dissociate, the non assimilable fraction remaining stocked in this structure.

The affinity of the vacuolar material for PAS described in the enterocytes of the distal portion of the medium intestine has already been mentioned by numerous research workers (Krause, 1972; Shervey, 1966; Cornell and Padykuka, 1969; Staley *et al.*, 1972). The latter author is of the opinion that the glucid fraction (PAS+) penetrates at the same time as a protein portion and would be even necessary for its absorption. According to Cornell and Padykula (1969), the basophilic reaction of the vacuoles and their affinity for PAS reflect their lysosomal nature. In the fasting Carp, the presence of a basophil supranuclear body and which is highly stained by PAS would be in favour of this hypothesis.

3. Acid and Alkaline Phosphatases Activities

Our studies have shown the presence of an alkaline phosphatase activity in the region of the apical tubules, the absorption vacuoles and of the Golgi complex. On the other hand, we were unable to reveal the presence of acid phosphatases in these structures.

The alkaline phosphatases have already been detected in the apical tubular network of the renal cells (Molbert *et al.*, 1960). These enzymes have also been described in the structures related to protein absorption in the newborn rat (Hugon, 1970; Cornell and Padykula, 1969; Shervey, 1966). According to these same authors, acid phosphatases may also be observed; however, the presence of these lytic proteins is not always demonstrable (Miller and Palade, 1964; Hayward, 1967); possibly their bond with the substrate or their presence in reduced amounts account for the fact that they are non detectable by the cytochemical method used.

Conclusion

From our study it seems clear that part of the intestinal epithelium (2nd medium segment) of the adult carp is specialized in the absorption of protein macromolecules thanks to a particular mechanism which we have described. The differentations (tubulo-vesicular system, supranuclear body) of the enterocytes of this region are moreover independent of the presence of food material in the intestinal lumen since they are also present in fasting animals.

The use of "Horseradish-Peroxidase" furthermore enabled us to show that this heterologous protein passes through the epithelium apparently without modification and may either enter directly into the blood circulation, or accumulate in the supranuclear vacuole where it becomes decomposed by the lytic enzymes identified in this region. The use of this cytochemical method thus enabled interesting observations to be made with regard to the structure-function relationships.

Finally, it is remarkable to find the presence of structural arrangements in the *adult Carp* that have been principally described in newborn Mammals. Among the latter, this structural arrangement disappears on round about the 21st day of post-natal development. It may represent the vestige of a mode of absorption existing in the adult state among some of the lower vertebrates, a hypothesis that is particularly interesting from the point of view of comparative physiology and phylogeny.

References

Al Hussaini, A. H.: On the functional morphology of the alimentary tract of some fish in relation to differences in their feeding habits: I. Anatomy and histology. Quart. J. micr. Sci. **90**, 109–139 (1949). II. Cytology and physiology. Quart. J. micr. Sci. **90**, 323–354 (1949)

Cornell, R., Padykula, H. A.: A cytological study of intestinal absorption in the suckling rat. Amer. J. Anat. **125**, 291–316 (1969)

Cornell, R., Walker, W. A., Isselbacher, K. J.: Small intestinal absorption of horseradish peroxidase : a cytochemical study. Lab. Invest. **25**, 42 (1971)

Dunn, J. S.: The fine structure of the absorptive epithelial cells of the developing small intestine of the rat. J. Anat. (Lond.) **101**, 57–68 (1967)

Friend, M., Farquhar, M. G.: Functions of coated vesicles during protein absorption in the rat vas deferens. J. Cell Biol. **35**, 357–376 (1967)

Gauthier, G. F., Landis, S. C.: The relationship of ultrastructural and cytochemical features to absorptive activity in the goldfish intestine. Anat. Rec. **172**, 4, 675–701 (1972)

Graham, R. C., Karnovsky, M. J.: The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney ultrastructural cytochemistry by a new technique. J. Histochem. Cytochem. **14**, 291–302 (1966)

Graney, D. O.: The uptake of ferritin by ileal absorptive cells in suckling rats. An electron microscopic study. Amer. J. Anat. **123**, 227–254 (1968)

Hayward, A. F.: Changes in fine structure of developing intestinal epithelium associated with pinocytosis. J. Anat. (Lond.) **102**, 57–70 (1967)

Hugon, J. S.: Ultrastructural differentiation and enzymatic localization of phosphatases in the developing duodenal epithelium of the mouse. II. The newborn mouse. Histochemie **22**, 109–124 (1970)

Hugon, J. S.: Absorption of horseradish peroxidase by the mucosal cells of the duodenum of mouse. II. The newborn mouse. Histochemie **26**, 19–27 (1971)

Hugon, J., Borgers, M.: Fine structural localization of acid and alkaline phosphatase activities in the absorbing cells of the duodenum of rodents. Histochemie **12**, 42–66 (1968)

Iwai, I.: Fine structure of gut epithelial cells of larval and juvenile carp during absorption of fat and protein. Arch. histol. jap. **30**, 183–199 (1969)

Kraehenbuhl, J. P., Gloor, E., Blanc. B.: Resorption intestinale de la ferritine chez deux espèces animales aux possibilités d'absorption protéique néo-natales différentes. Z. Zellforsch. **76**, 170–186 (1967)

Krause, W. J.: Light and electron microscopic studies on the gastrointestinal tract of the suckling Echidna (*Tachyglossus aculeatus*). Anat. Rec. **172**, 603–621 (1972)

Lagunoff, D., Curran, D. E.: Role of bristle-coated membrane in the uptake of ferritin by rat macrophages. Exp. Cell Res. **75**, 2, 337–347 (1972)

Lambson, R. O.: An electron microscopic analization of transport across rat visceral yolk sac. Amer. J. Anat. **118**, 21–51 (1966)

Miller, F., Palade, G. E.: Lytic activities in renal protein absorption droplets. J. Cell Biol. **23**, 519–552 (1964)

Mölbert, E., Duspiva, F., von Deimling, O. H.: Die histochemische Lokalisation der Phosphatase in der Tubusepithelzelle der Mäuseniere im elektronenmikroskopischen Bild. Histochemie **2**, 5 (1960)

Moon, H. W., Kohler, E. M., Whipp, S. E.: Vacuolation: a function of cell age in porcine ileal absorptive cells. Lab. Invest. **28**, 1, 23–29 (1973)

Mosinger, V., Placer, Z., Koldovsky, O.: Passage of insulin through the wall of the gastrointestinal tract of the infant rat. Nature (Lond.) **184**, 1245–1246 (1959)

Noaillac-Depeyre, J., Gas, N.: Mise en évidence d'une zone adaptée au transport des ions dans l'intestin de Carpe commune (*Cyprinus carpio* L.). C. R. Acad. Sci. (Paris) **276**, 773–776 (1973)

Orlic, D., Lev, R.: Fetal rat intestinal absorption of horseradish peroxidase from swallowed ammiotic fluid. J. Cell Biol. **56**, 1, 106–119 (1973)

Padykula, H. A., Deren, J. J., Wilson, T. H.: Development of structure and function in the mammalian yolk sac. I. Development morphology and vitamin B_{12} uptake in the rat yolk sac. Develop. Biol. **13**, 311–348 (1966)

Roth, T. F., Porter, K. R.: Yolk protein uptake in the oocyte of the mosquito *Aedes aegypti* L. J. Cell Biol. **20**, 313–332 (1964)

Sabatini, D. D., Bensch, K., Barrnett, R. J.: Cytochemistry and electron microscopy: the preservation of cellular structure and enzymatic activity by aldehyde fixation. J. Cell Biol. **17**, 19–58 (1963)

Sanders, E., Ashworth, C. T.: A study of particulate intestinal absorption and hepatocellular uptake. Use of polystyrene latex particles. Exp. Cell Res. **22**, 137–145 (1961)

Sarbahi, D. S.: Studies of the digestive tracts and the digestive enzymes the goldfish, *Carassius auratus* (Linnaeus) and the large mouth black bass, *Micropterus salmoïdes* (Lacepede). Biol. Bull. **100**, 244–270 (1951)

Shervey, P.: Observations on the development and histochemistry of the intestinal inclusion bodies of the suckling rat. Anat. Rec. **154**, 422 (1966)

Sibalin, M., Björkman, N.: On the fine structure and absorptive function of the porcine jejunal villi during the early suckling period. Exp. Cell Res. **44**, 165–174 (1966)

Staley, E., Corley, L. D., Bush, L. J., Wynn Jones, E.: The ultrastructure of neonatal calf intestine and absorption of heterologous proteins. Anat. Rec. **172**, 559–580 (1972)

Vodovar, N., Flechon, J. E.: La cellule épithéliale absorbante de l'intestin grêle du porc. Ultrastructure. Ann. Biol. anim. **6**, 13–32 (1966)

Vonk, H. J.: The specificity and collaboration of digestive enzyme in Metazoa. Biol. Rev. **12**, 245–284 (1937)

Warshaw, A., Walker, W. A., Cornell, R., Isselbacher, K. J.: Small intestine permeability to macromolecules. Transmission of horseradish peroxidase into mesenteric lymph and portal blood. Lab. Invest. **25**, 6, 675–684 (1971)

Yamamoto, T.: An electron microscope study of the columnar epithelial cell in the intestine of fresh water Teleosts. Goldfish and rainbow trout. Z. Zellforsch. **72**, 1, 66–87 (1966)

N. Gas
Université Paul Sabatier
38, Rue des 36 Ponts
F-31087 Toulouse, Cedex
France