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AN ELECTRON MICROSCOPE STUDY OF THE INTESTINAL CAPILLARIES IN LOACH *MISGURNUS FOSSILIS* (L.)

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En étudiant la structure électrono-microscopique des vaisseaux capillaires de la muqueuse intestinale du *Misgurnus fossilis* durant l'inanition, les auteurs ont constaté certaines particularités structurales: un endothélium capillaire avec une structure similaire aux mammifères, la présence des jonctions entre les cellules endothéliales (desmosomes) et de nombreux vésicules de pinocytose. On a suggéré que les vésicules de pinocytose constituent un mécanisme de transport actif chez les poissons, très répandu dans le monde animal.

The study of blood capillaries from different tissues made the object of many investigations carried out particularly in mammals. In all cases, in their organization, three different layers were described: endothelium, basement membrane and adventitia (external layer of connective tissue), the structure of which shows some particularities related to the species, the tissue nature and the physiological conditions (Palade, 1953; Farquhar, Vernier and Good, 1957; Bloom, et al., 1959; Donahue and Pappas, 1961; Bennett et al., 1959; Farquhar, 1961; Fawcett, 1962; Stehbens and Silver, 1965; Bruns and Palade, 1968). Moreover, it has been pointed out that basement membranes are produced by the endothelial cells with which they are in contact (Farquhar, 1978). It may be stated that the basement membrane represents a cell skeleton playing a particular role, characterized by a high "flexibility and adaptability" during morphogenesis (Hay, 1978).

Since the data concerning the ultrastructure of capillaries in the intestinal mucosa of lower vertebrates are scarce (Bennett et al., op. cit., Fawcett, op. cit.), in the present paper we refer to the ultrastructure of intestinal capillaries in *Misgurnus fossilis*. We have chosen the loach as study material taking into account the fact that in this species the intestinal mucosa plays an important role both in digestion and in breathing exchanges (Călugăreanu, 1907; Lupu, 1911; Bușniță, 1925; Meșter, 1973; Meșter and Babeș, 1977).

MATERIAL AND METHOD

Small fragments of posterior intestine of loaches kept under inanition conditions (10 days) were removed, fixed for one hour in glutaraldehyde 2,5% and then postfixed in 1% OsO_4 solution prepared in Tris-HCl buffer pH 7,4,

at cold. The pieces were washed with the same buffer solution, dehydrated in acetone and embedded in Epon. Ultrathin sections were prepared with an LKB ultratome and grids were stained with uranyl acetate and lead citrate. Our observations and photographs were made by means of an electron microscope Philips M 201.

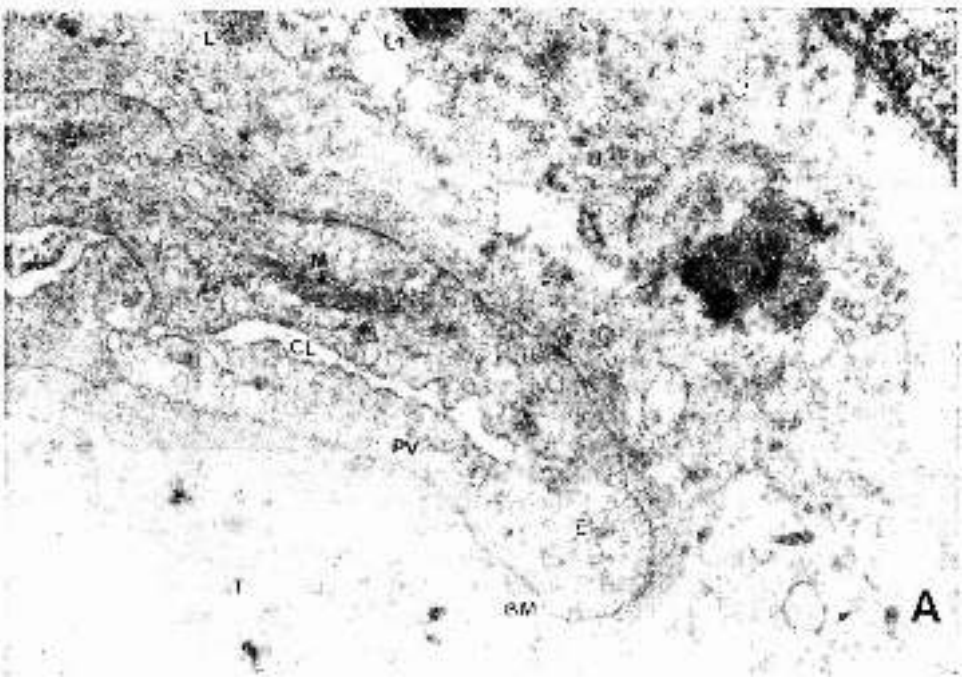
RESULTS AND DISCUSSION

The capillaries of the intestinal mucous membrane in loach show a heterogeneous structure. The electron microscopical observations identified, just as in the case of intestinal capillaries in mammals, the presence of three distinct layers: capillary endothelium, basement membrane and pericapillary connective tissue space.

Capillary endothelium comprises a layer of flattened cells with elongated nuclei. In the cytoplasm of these cells, the elements of rough endoplasmic reticulum, of smooth endoplasmic reticulum and relatively large and elongate mitochondria with tubular cristae can be seen. Also multivesicular bodies and dense granules with polymorphic content and sometimes lamellated structures are found (Fig. 1, A, B; Fig. 2, A). The rough endoplasmic reticulum consists of a low number of cisternae, covered with ribosomes (Fig. 2, A), particularly visible in the close vicinity of the nucleus, but also dispersed in the cytoplasm (Fig. 1). In agreement with Palade's morphological observations on endothelial cells of capillaries of muscular and intestinal tissues in mammals, the endothelial cells in fish intestine show free ribosomes dispersed in the cytoplasm (Fig. 1, A; Fig. 2, A, B). Mitochondria are scarce, large and ovoid in shape, with tubular cristae (Fig. 1, A; Fig. 2, B). On some images, the presence of some Golgi vesicles can be seen, without forming an obvious dictiosomal system. In the cytoplasm of endothelial cells numerous fibrillar elements with an irregular arrangement are found (Fig. 1, A, B). Their presence was reported by Bruns and Palade (op. cit.) in the endothelial cells of muscular capillaries, as well as in the vascular endothelium of amphibians (Hama, 1961).

A general characteristic of the endothelial cells of all capillaries studied is the presence of a high number of pinocytotic vesicles. These vesicles were reported for the first time by Palade (op. cit.) and afterwards by other authors, in the majority of studied capillaries (Farquhar, 1961; Fawcett, op. cit; Clementi and Palade, 1969). Fawcett (op. cit.) cited the presence of some pinocytotic vesicles in the capillary endothelium of fish, giving a single electrono-microscopical image representing a fragment of a capillary wall in toadfish.

Fig. 1 A — The ultrastructure of an intestinal capillary of loach. E — capillary endothelium; N — nucleus of the epithelial cell; CL — lumen of capillary; BM — basement membrane; M — mitochondria; PV — pinocytotic vesicles; T — tonofibrils; L — lysosomes. Magnification 40.000 x. Fig. 1 B — The fine structure of the intestinal capillary at the junctions'limit: CO — connective tissue fibres; D — desmosome; MB — multivesicular bodies; PA — pinocytotic vesicles; PCTS — pericapillary connective tissue space. Magnification 40.000 x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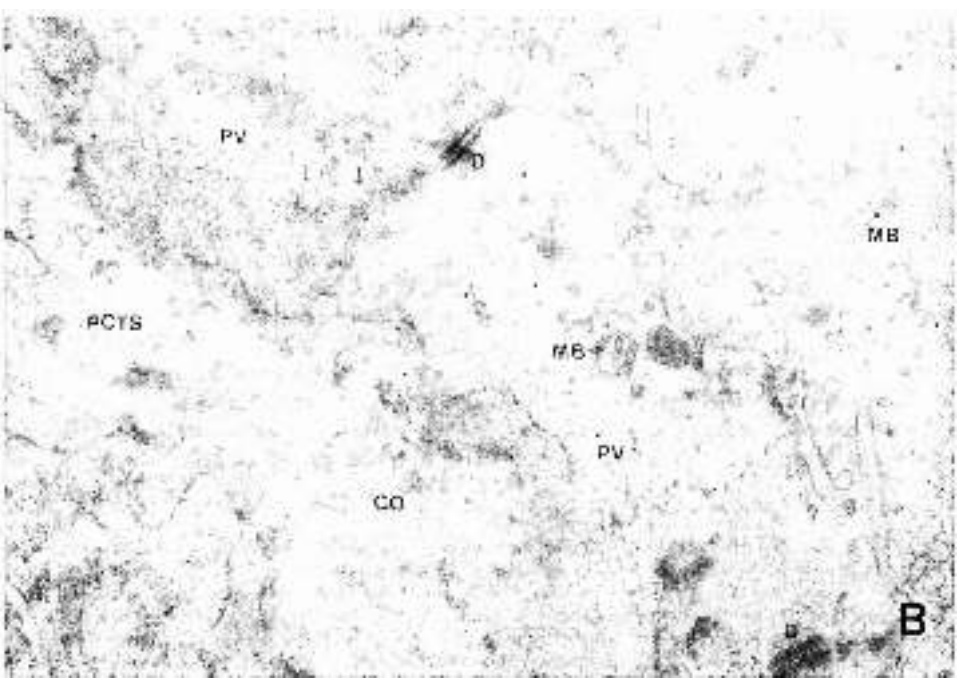
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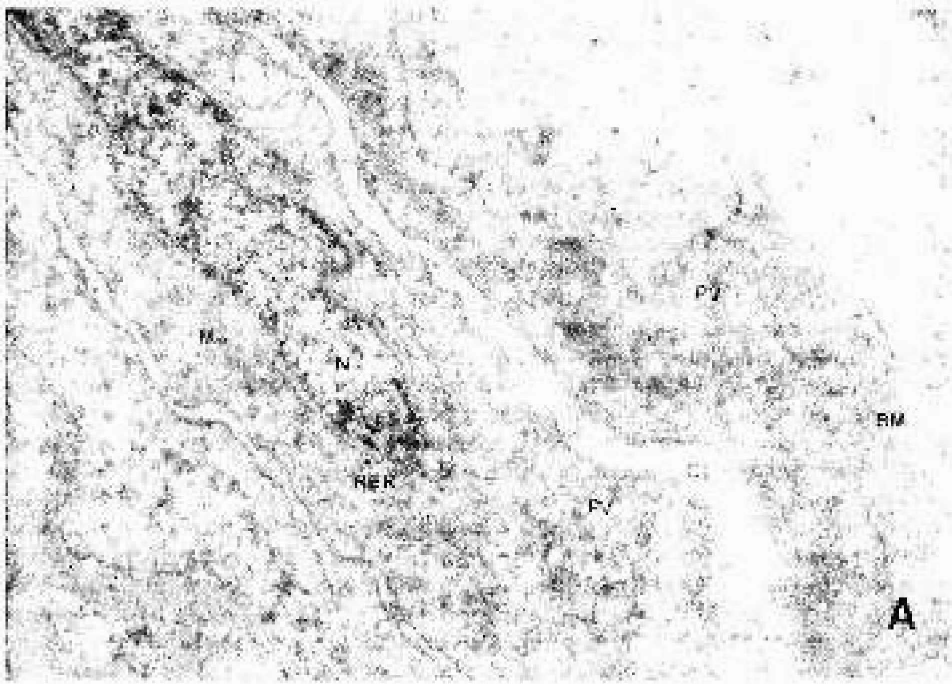
Our observations concur with those of the above authors, according to the electrono-microscopical images obtained in loach where the presence of pinocytotic vesicles can be found. As shown in figs 1 and 2, the endothelial cells of loach intestinal capillaries contain many vesicles, which, just like in mammals, are mainly concentrated in the vicinity of the cells' plasmatic membranes. Some vesicles open into the lumen of the capillary, some others are directed towards the basement membrane. In the case of loach capillary endothelium, the number of vesicles attached by a sort of neck to the plasmalema directed towards the basement membrane is 2—5 times as high as those directed towards the capillary lumen (Figs 1 and 2). The diameter of these vesicles ranges between 60—100 A.

Some images show two, three or more vesicles merged, leading to the formation of some larger intracellular structures (Fig. 2, B arrow). A similar observation was made by Fawcett (op. cit) in toadfish. In the endothelial cells of mammalian capillaries (Bruns and Palade, op. cit., Clementi and Palade op. cit.) no continuity of vesicles with structural elements of reticulum, Golgi complex or other intracellular components could be observed. On some ultrastructural images we obtained, some particularities appeared. Thus, some vesicles are found fused with multivesicular bodies (Fig. 2 B), or with cisternal elements characteristic of the smooth endoplasmic reticulum (Fig. 1 B). These particularities suggest supplemental functional mechanisms of the vesicles in the capillary endothelial cells, aside from those previously described for mammalian capillaries (Fawcett, 1961; Clementi and Palade, op. cit). A low number of vesicles was identified also in the area of intercellular junctions of endothelial cells (Fig. 1 B), which was not found in the endothelial cells of mammals (Bruns and Palade, op. cit).

Intercellular junctions. The endothelial cells of blood capillaries touch one another by the intermediary of different distinct structural formations. At the level of blood capillaries in Mammals, several junctional systems were described (Bennett et al., op. cit; Fawcett, 1961; Bruns and Palade op. cit). Our studies revealed the presence of two types of junctions between the endothelial cells of intestinal capillaries in loach. Thus, there are areas where the membranes of neighbouring cells are in close contact, without any fusion of membranes (Figs 1 B and 2 B). Around these occlusion areas, a dense fibrillar material is observed. In contrast with the mammalian capillaries, the endothelial cells of capillaries in fish show typical desmosomes (Fig. 1 B), which seem to represent a particularity of the endothelial cells of capillaries in low vertebrates (Fawcett, 1961, 1962).

Basement membrane. The basement membrane consists of a fibrilar structure bounding the basal face of endothelial cells. In the capillaries of the

Fig. 2 A — Blood capillary in the intestinal mucosa of loach. Enlarged endothelium full of pinocytotic vesicles and hypertrophic basement membrane exhibiting on its inner face pinocytotic vesicles, are noticed: N — nucleus of endothelial cell; BM — basement membrane; CL — capillary lumen; M — mitochondria; PV — pinocytotic vesicles; RER — rough endoplasmic reticulum. Magnification 40.000 x. Fig. 2 B — Intestinal capillary of loach. The same explanation as in Fig. 2 A. Arrow indicates fused pinocytotic vesicles. Magnification 40 000 x.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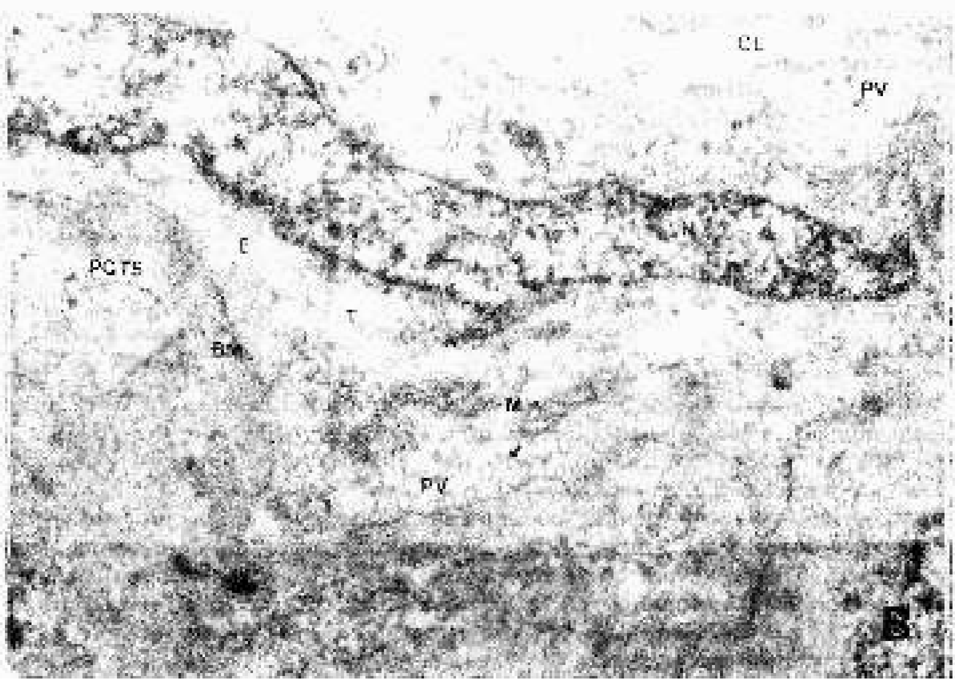
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intestinal mucosa in fish, the basement membrane is obvious, forming a quite thick compact structure (Figs 1 A, B and 2 A). Just like in the case of mammalian capillaries, its inner margin is separated from the endothelium by a subendothelial space. It consists of several layers with fibrilar structure. The basement membrane plays an important role in the permeability of capillaries. It consists of tropocollagen fibres and glycoproteins, forming a porous and amorphous matrix of the capillary endothelium.

Pericapillary connective tissue space. On the outside, the blood capillaries show the third characteristic structural element, the pericapillary connective tissue space. This latter consists of a net of fibrillary elements, enclosing macrophages and plasmocytes (Figs 1 A, 2 B). Also at this level, particular ultrastructural aspects are found. Thus, the cells neighbouring the capillaries show a micropinocytosis process in the vicinity of the basement membrane (Figs 1 B, 2 A). At the level of these cells the formation of some large vacuolar structures probably resulting from the fusion of vesicles, as well as the presence of some lysosomal structures are found (Fig. 1 B).

The transport systems of different substances through the capillaries represent a quite controversial issue. The electrono-microscopical data obtained by the analysis of capillaries under different experimental conditions led to the suggestion of several transport systems through the endothelial cells of capillaries: a) through micropinocytotic vesicles (Palade, 1953; Fawcett, 1962; Marchesi and Barrnett, 1963); b) by cellular junctions (Karnovsky, 1967); c) by vesicles forming canals in the endothelial cells of capillaries (Luft, 1965; Wissig and Williams, 1978). It is indisputable that the functionability of these ways may show different degrees at the level of blood capillaries in the different tissue types, since the local control factors of selective permeability have also an influence.

Our ultrastructural study on the blood capillaries of loach intestinal mucosa reveal the importance of the transport system through micropinocytotic vesicles. Even though they do not represent the single transport process through the endothelial membranes, our morphological data suggest, however, that this mechanism is active in fish, particularly in certain adverse conditions. The cooperation of other transport systems through the endothelial cells of fish intestinal capillaries cannot be excluded.

CONCLUSIONS

On the basis of electrono-microscopical studies on the capillaries of intestinal mucosa in loach, some ultrastructural particularities were described.

1. Capillary endothelium, consisting of a layer of flattened cells with elongated nuclei. These cells present lysosomes, large mitochondria with tubular cristae, multivesicular bodies and a scarce endoplasmic reticulum. Fibrillary formations similar with microtubules were distinguished. These structural elements seem to be similar to those of other endothelial cells, described for intestinal capillaries in mammals. By contrast, we found the presence of some junctions between the endothelial cells, represented by well-distinct desmosomes.

2. The basement membrane of the endothelial cells consists of a fibrillar material, much more developed as compared with the one described for the intestinal capillaries in mammals. The pericapillary connective tissue space consisting of a net of fibrillary elements was described.

3. The active micropinocytotic process found at the level of endothelial cells of fish shows the importance of this transport system in the transepithelial transport.

STUDIUL ELECTRONO-MICROSCOPIC AL CAPILARELOR INTESTINALE LA ȚIPAR, *MISGURNUS FOSSILIS* (L.).

REZUMAT

În alcătuirea capilarelor intestinale de la țipar s-au identificat trei straturi. similare cu cele descrise la mamifere: endoteliul capilar, membrana bazală și un țesut conectiv pericapilar. Celulele endoteliale se caracterizează prin prezența unor mitocondrii alungite cu creste tubulare, lizozomi, corpi multiveziculați și un reticul endoplasmic puțin dezvoltat. Celulele endoteliale prezintă un număr mare de vezicule de pinocitoză, ce sugerează existența unui mecanism transcelular foarte activ în capilarele intestinale ale peștilor. Spre deosebire de capilarele de la mamifere, celulele endoteliale ale capilarelor de țipar prezintă desmozomi tipici. Observațiile ultrastructurale au evidențiat de asemenea, prezența unei membrane bazale bine individualizate la nivelul capilarelor intestinale ale peștilor ținuți în inaniție.

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