I. Experimental problems

TRANSENDOTHELIAL VESICULAR MACROMOLECULE TRÀNSPORT IN LARGE BLOOD VESSELS

S. Nikolov, V. Vankov

Key-words: blood vessels — endothelial cells — macromolecular tracers — electron microscopy

Endothelium of large blood vessels is a barrier for macromolecular diffusion. That is why vesicular transport is of primary importance for their transcytosis. The study of the basic regularities of this process and of its structural manifestations creates a possibility for clarifying the pathways and speed of passage of various substances (nutritive, regulatory, pathogenic) with a macromolecular character from the blood flow into the vascular walls. As structural bases of vesicular transport are intensively investigated in capillary endothelium (2, 5, 6, 12, 15) similar studies of endothelium of blood vessels are scanty and concern aorta only (4, 8, 14).

The purpose of the present work is to study some aspects of this problem with the help of macromolecular tracers with different molecular weight, chemical composition and molecule size on material from aorta and pulmonary trunc of rat. They are a part of a all-out programme for studying endothelial permeability.

Material and methods

Our study covers 23 rats of Wistar breed which are i. v. injected under inhalation narcosis as follows: ferritin — 10 per cent solution (10 rats); dextran — 6 per cent solution (10 rats), and peroxidase — 10 per cent solution (3 rats). Circulation time — up to 30 min. After that the animals are killed. Fixation: glutaraldehyde 3 per cent (120 min) and osmium tetraoxide 1 per cent (90—120 min) in 0,1 mol phosphate buffer with pH 7,4. Dehydratation in alcohol and aceton. Embedding in Durcupan. Double contrasting with uranyl acetate and lead citrate. Electron microscope JEM 7A is used in our study.

Results and discussion

Iron-containing protein ferritin belongs to the most often used tracers. It has molecular weight of 500 000 and diameter about 10 nm. Our investigations demonstrate that ferritin molecules fallen into circulation group rapidly together and bind to the glycocallix of luminal cytolemma. From there they are taken by forming micropinocytotic vesicles (fig. 1) thus remaining bound to their inner envelope or located freely in their cavity. Ferritin particles pass through extranuclear parts of aortic endothelial cells (EC) for 30—60 sec. and settle down in subendothelium. In certain cases with both vessels ferritin molecules initially accumulate in large invaginations of luminal cytolemma (grooves) where they remain and are then taken by vesicles (fig. 2). While in aorta ferritin transpor-

ting vesicles bind only exceptionally to each other in pulmonary trunc EC they show very often different forms of close interrelations (1). In some cases there are vesicular chains forming transcellular channels but in other ones they are depositing vacuoles which hold the tracer for a certain time and delay its enter into the subendothelium.

An other macromolecular tracer is glucose polymer dextran (molecular weight 60 000—90 000, diameter about 12 nm). It is notable that a considerable quanti-

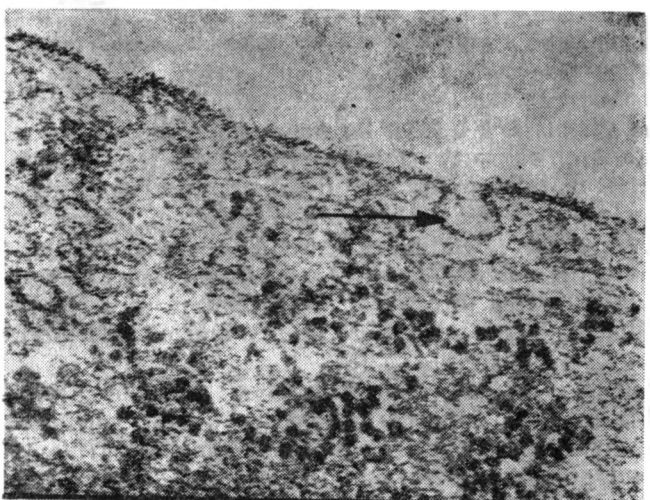

Fig. 1. EC luminal surface from the pulmonary trunc of rat. Ferritin uptake by a forming micropinicytotic vesicle (arrow). Circulation time 5 min. Magn. × 4000

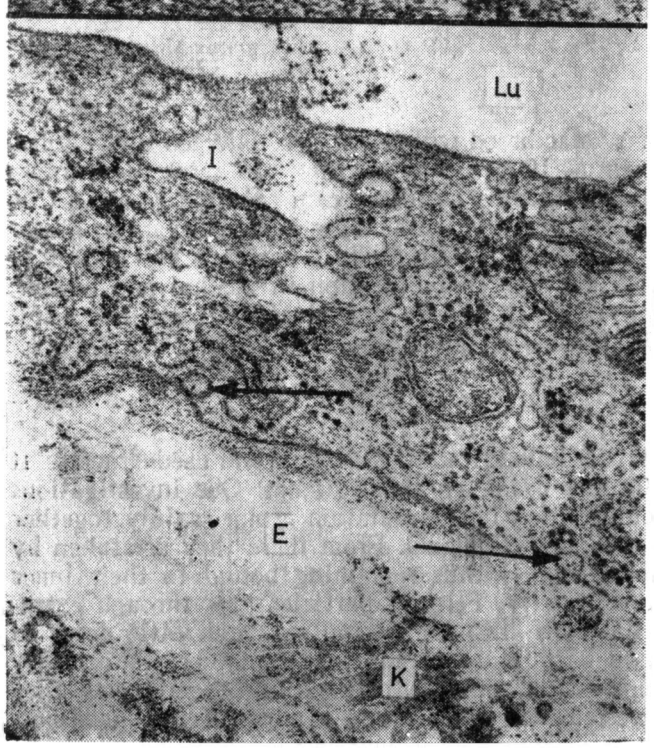

Fig. 2. Aortic EC. Invagination of the luminal cytolemma where ferritin-containing plasma (I) is restored. Lu — lumen. Basal ferritin exocytosis (arrows). Subendothelial elastic fibres (E) and collagen fibrilles (K). Circulation time 60 sec. Magn. × 30000

ty of dextran molecules aggregated or complexicated with plasma proteins remain a long time in vascular lumen in the form of finely granulated mass. There are no close interrelations between dextran particles and luminal cytolemma (fig. 3) as described with ferritin. They fall in groups or singly into forming ve-

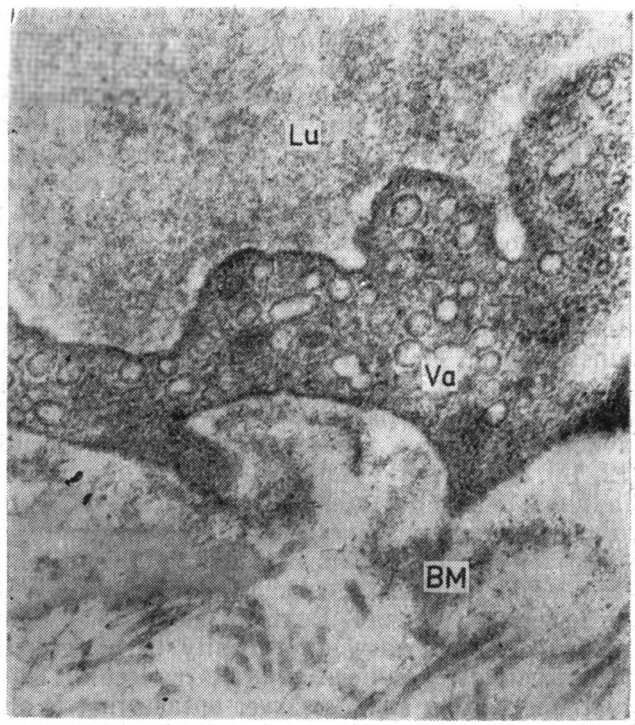

Fig. 3. EC from the pulmonary trunc of rat — extranuclear strongly vesiculated part. Va — vacuole in contact with micropinocytotic vesicles. In the lumen (Lu) — dextran substance with plasma; only single molecules can be established in the micropinocytotic vesicles as well as within the basal membrane (BM). Circulation time 15 min. Magn. × 30 000

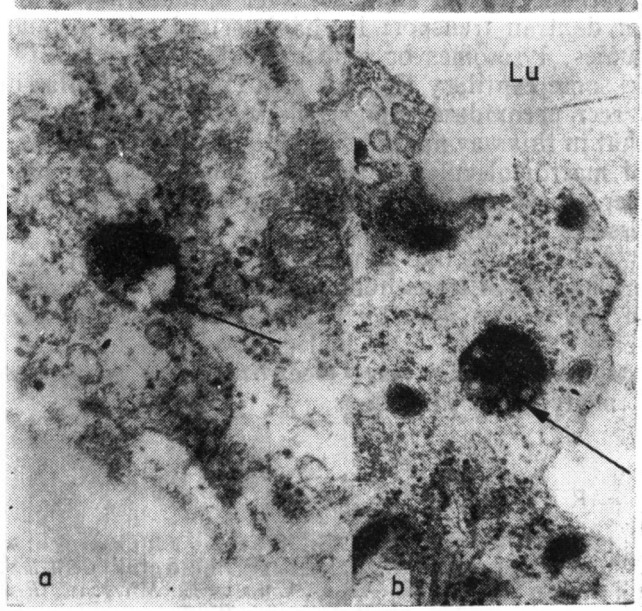

Fig. 4. Peroxidase-positive material (arrows) accumulated in a vacuole (Fig. 4-a) and in a multivesicular body (fig. 4-b). Aortic EC. Lu — lumen. Circulation time 7 min 30 sec. Magn. × 40 000 — fig. 4-a Magn. × 30 000 — fig. 4-b

sicles which internalize them. Most of them reach subendothelium after 5—10 min circulation. In EC of both vessels one can ctten establish vesicles which fuse to vacuoles or other ones which open into multivesicular bodies or lysosomes where dextran not only remains but also undergoes hydrolysis. Transcellular channels are formed rather seldomly.

Third we report some results of our investigation with peroxidase as tracer (molecular weight 42 000, diameter about 5 nm). With this ultracytochemical reaction peroxidase localization is visualized by means of reaction product and thus marks the pathway of the tracer. It is established that peroxidase falls into EC micropinocytotic vesicles of both vessels after 5 min circulation. Up to the 10th min a considerable part of the tracer accumulates in vacuoles and multivesicular bodies (fig. 4) without reaching subendothelium.

Macromolecular tracers ferritin, dextran and peroxidase pass through aortic and pulmonary trunc EC on vesicular way. Without any discussion of the theoretical fundamentals of this mechanism (16) we will pay attention to certain quantitative and qualitative peculiarities of the transport of the three molecule kinds. Heavy ferritin particles (almost 3 times heavier than high density lipoproteins or serum globulins) remain on luminal cytolemma prior to their taking by the vesicles (10). However, when fallen in them they rapidly get free from this so-called "endocapillary layer" when capillaries are concerned (9). It is probably favoured by some specific electrochemical peculiarities of luminal cytolemma (12, 13), too. Ferritin molecules cross EC by means of vesicles and reach subendothelium. Transcellular channels for accelerated passage are practically observed only in pulmonary trunc EC, and, exceptionally, in aortic EC where cytopempsis is rapider due to specific hemodynamic conditions (7). Ferritin accumulation in vacuoles in pulmonary trunc EC prolongs obviously its passage and depends on the low blood pressure. Dextran (molecular weight is near to that of plasma albumins) does not show any affinity to EC glycocallix, probably because of the electrochemical peculiarities of its molecule. This can be a reason for the long tracer circulation and its slower uptake that is already reported (11). Transcellular dextran transport is characterized with a tendency to accumulation into vacuoles, lysosomes or multivesicular bodies of its molecules. This is established in some capillary EC, too (3). There are similar data concerning mediomolecular tracer peroxidase comparable with those from the literature (14). It is probably that in this way not only a holding but also a destruction, respectively, inactivation of macromolecules is achieved which thus do not overcome the endothelial barrier at all. No tracer used, including peroxidase, can pass through intercellular fissures in the vessels studied.

We would like to point out in conclusion that vesicular transport is the only mechanism of transendothelial permeation of macromolecules with size and weight of the tracers used by us. Its ultrastructural peculiarities are determined by a series of factors, e. g. molecular weight and size of transported molecules, their chemical, respectively, electrochemical properties, circulation time and local hemodynamic conditions.

REFERENCES

1. Николов, С., В. Ванков. *Експерим. мед. и морфол.*, 1980, № 3, 131—135. — 2. Bruns, R. R., G. E. Palade. *J. Cell. Biol.*, 37, 1958, 277—299. — 3. Casley-Smith, J. R., D. B. Carter. *Microvasc. Res.*, 18, 1979, 319—324. — 4. Flo,

rey, H. W. *Q. J. Experim. Med.*, 49, 1964, 117—128. — 5. Florey, L., B. L. Sheppard. *Proc. Royal Soc. B*, 174, 1970, 435—443. — 6. Granger, D. N., A. E. Taylor. *Amer. J. Physiol.*, 238, 1980, 457—464. — 7. Holle, G., J. Massmann, H. Weidenbach. *Pathol. Europ.*, 9, 1974, 125—132. — 8. Hüttner, J., M. Boutet, R. H. More. *Labor. Invest.*, 28, 1973, 678—685. — 9. Luft, I. H. *Fed. Proc.*, 25, 1966, 1773—1781. — 10. Shirahama, T., A. S. Cohen. *J. Cell. Biol.*, 52, 1972, 198—206. — 11. Simionescu, N., M. Simionescu, G. E. Palade. *J. Cell. Biol.*, 53, 1972, 365—392. — 12. Simionescu, N., M. Simionescu, G. E. Palade. *J. Cell Biol.*, 90, 1981, 605—613. — 13. Skutelsky, E., D. Danon. *J. Cell Biol.*, 71, 1976, 232—241. — 14. Stein, O., Y. Stein. *Z. Zellforsch.*, 133, 1972, 211—222. — 15. Themann, H., F. Keuker, U. Westphal. *Cytobiologie*, 3, 1981, 13—24. — 16. Wagner, C. W., J. R. Casley-Smith. *Microvasc. Res.*, 21, 1981, 267—298.

ТРАНСЭНДОТЕЛИАЛЬНЫЙ ВЕЗИКУЛЯРНЫЙ ТРАНСПОРТ МАКРОМОЛЕКУЛ В КРУПНЫХ КРОВЕНОСНЫХ СОСУДАХ

С. Николов, В. Ванков

РЕЗЮМЕ

С помощью электронной микроскопии исследованы пути трансцеллюлярного транспорта макромолекулярных трассеров — феритина (мол. вес 50 000), декстрана (мол. вес 60 000—90 000) и пероксидазы (мол. вес 42 000) в эндотелиальных клетках аорты и легочного ствола взрослых крыс. Трассерные молекулы проходят через барьер эндотелия единственно везикулярным путем с помощью микропиноцитозных везикул. Ни одна из них не проходит через межклеточные щели. Переход феритина обоих сосудов быстрее по сравнению с переходом остальных двух трассеров. Последние аккумулируются в вакуолях, мультивезикулярных телах и даже в лизозомах. Различия в трансэндотелиальном везикулярном транспорте определяются природой трассеров при одинаковости остальных условий.