PEROXIDASE TRANSPORT THROUGH ENDOTHELIOCYTES OF BLOOD MICROVESSELS IN RAT JEJUNAL VILLI

V. V. Kamyshova

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Compared with other organs, exchange between the intravascular and extravascular albumin pool takes place more intensively and rapidly in the small intestine [13] and this suggests that the endothelium of the exchange microvessels is highly permeable for protein. Recently, however, as a result of physiological investigations, doubts have been expressed on the higher permeability of fenestrated capillaries of the mucosa of the small intestine for plasma proteins compared with capillaries of somatic type [6]. Attempts to identify systems of "small and large pores" in the capillary wall have led some workers to conclude that intercellular junctions between capillary endotheliocytes in villi of the intestine are virtually impermeable for molecules over 2 nm in diameter [3, 7, 11]. The capillary wall in this organ is evidently a selective barrier, which can select and pass macromolecules in accordance with their size and electric charge [12]. An important place in transcapillary protein exchange is occupied by serum albumin, which is the principle fraction of interstitial protein. Among electron-microscopic markers, the molecular characteristics of the native form of horseradish peroxidase (mol. wt. about 40,000; effective radius a_e about 2.9 nm) are closest to those of albumin (mol. wt. about 69,000; a_e about 3.5 nm), although by contrast with albumin, peroxidase has a neutral or slightly positive charge (pH 7.4). It may be expected that its use as macromolecular tracer will enable the permeability and limiting size of the pathways of transendothelial exchange to be determined more accurately.

EXPERIMENTAL METHOD

Experiments were carried out on six albino rats. Under pentobarbital anesthesia horseradish peroxidase (from Reanal, Hungary) was injected into the femoral vein in a dose of 50 mg of enzyme/100 g body weight, in 0.5 ml of physiological saline. After 40 min fragments of the proximal part of the jejunum were fixed *in situ* in 2.5% glutaraldehyde solution in 0.1 M phospate buffer (pH 7.4). Thin strips of the organ, consisting of one row of villi, were washed in 0.1 M phosphate buffer. The reaction product was detected by the technique in [5], slightly modified [1]. After preincubation of the material in medium not containing hydrogen peroxide for 30 min at room temperature the samples were incubated for 2 h in changing portions of this medium with the addition of 1% H_2O_2 solution at 37°C. Subsequent processing of the material was carried out by the usual electron-microscopic method, and semithin sections were cut. Ultrathin sections were stained with uranyl acetate and lead citrate, then examined in the Hitachi 12A electron microscope.

EXPERIMENTAL RESULTS

After injection of peroxidase the contributions of paracellular and transendothelial protein transfer were found to be unequal, depending on the segmental level of the microvessels.

Interendothelial junctions in the precapillary arteriole of the jejunal villus had specialized connecting complexes in the form of local pentalaminar structures (maculae occludens) or not very extensive hepatalaminar membrane formations, with a gap 2-4 nm wide between the cells (gap junction, Fig. 1a). The peroxidase reaction product filled the intercellular space as far as the specialized complex. The interstitial portion of the intercellular space either contained no reaction products whatever, or there was much less of it than in the lumen. The distal portion of the intercellular junction was marked when plasmalemmal vesicles, filled

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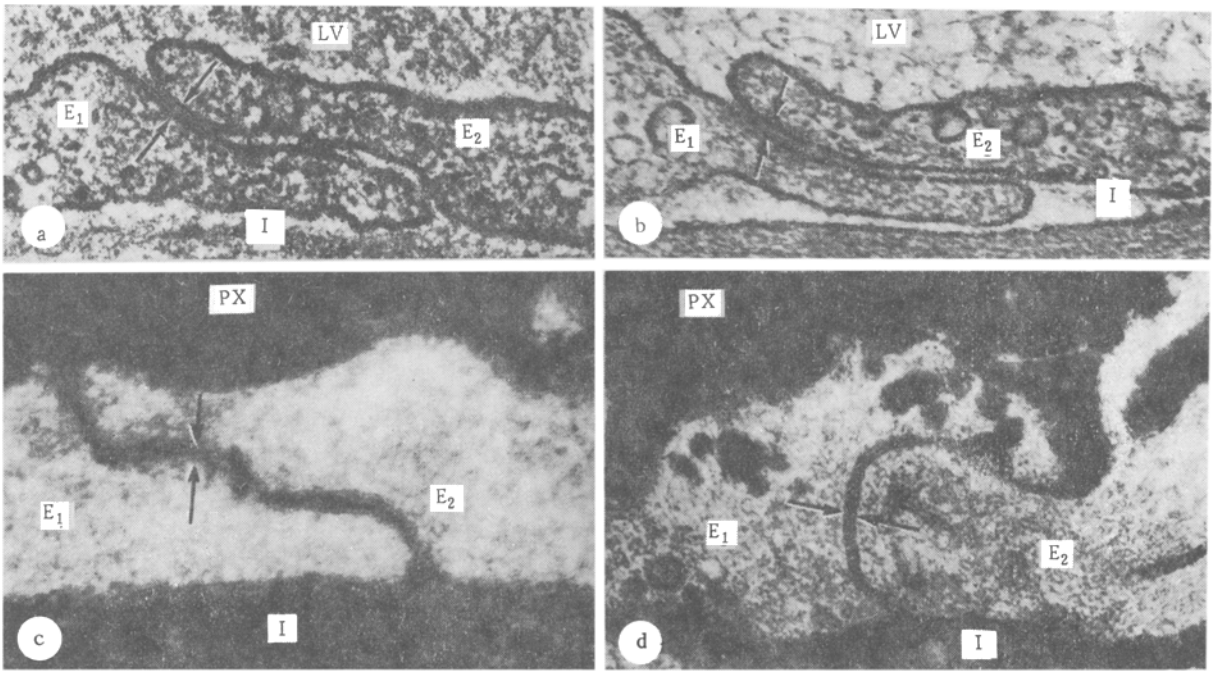

Fig. 1. Paracellular transport pathways in endotheliocytes of microvessels of albino rat jejunal villus. a) Gap junction of precapillary arteriole; b) junction in endothelium of blood capillary of zone of fusion type (control); c) permeability of capillary junction for horseradish peroxidase; d) peroxidase transport through open endothelial junction of postcapillary venule. Here and in Figs. 2 and 3: E_1, E_2) endotheliocytes; LV) lumen of vessel; PX) product of peroxidase reaction in lumen of vessel; I) interstitial tissue. Arrows indicate junction. Magnification: a) 42,000, b, c) 30,000, d) 18,000 ×.

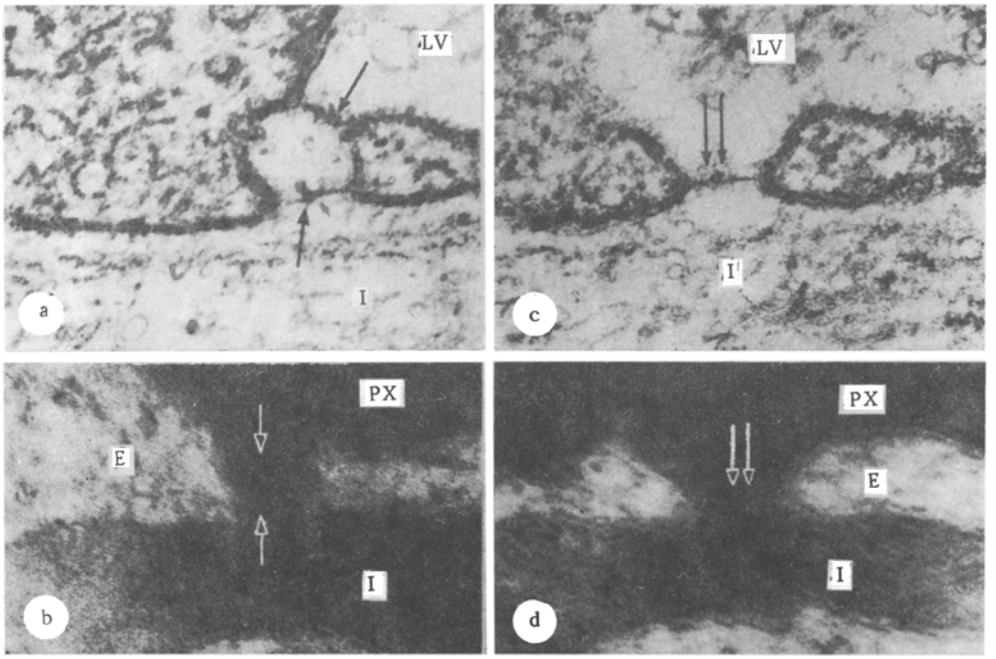

Fig. 2. Transcellular transport pathways in endotheliocytes of of blood capillaries of albino rat jejunal villus. a) Transendothelial canal (control); b) marking of transendothelial canal with peroxidase reaction product; c) fenestra (control); d) transport of horseradish peroxidase through fenestra. ↑) Transendothelial canal; ↑↑) fenestra. 30,000 ×.

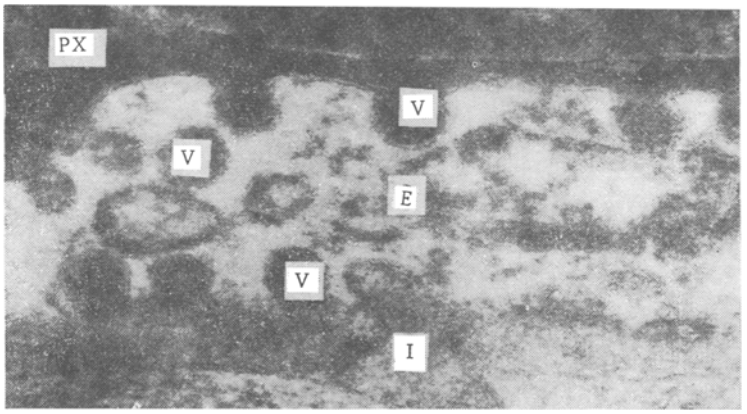

Fig. 3. Transcellular vesicular transport of horseradish peroxidase. V) Vesicles labeled with protein tracer. 18,000 ×.

with the tracer, opened into it. Interendothelial junctions of the subepithelial and marginal blood capillaries, which had more simply organized connecting complexes than the precapillary arteriole, frequently without fusion, often were marked with reaction product more or less uniformly over their whole extent (Fig. 1b, c). In the endotheliocytes of the postcapillary venule, among the intercellular junctions punctate pentalaminar structures (maculae occludens) were observed together with junctions not containing specialized occluding complexes. Just as in the blood capillaries, the junction gaps in this case were often filled with reaction product, differing little in density from that in the lumen of the microvessel (Fig. 1d).

Vesicles, transendothelial canals, and fenestrae are usually regarded as possible candidates for transcellular transport through endotheliocytes. Peroxidase reaction product was found in a few plasmalemmal vesicles in endotheliocytes of a precapillary arteriole. The endothelium of the jejunal villus contained transendothelial canals (Fig. 2a) and numerous fenestrae (Fig. 2c) in the subepithelial and marginal capillaries. Canals were formed by fusion, as a rule, of one vesicle with the plasmalemmas on both sides of the cell. Vesicles in the blood capillaries were observed in thickened zones of endotheliocytes, but were virtually absent in the fenestrated zones. A characteristic feature of the venular endothelium was the presence of a much greater number of single vesicles and vesicular clusters of the "rosette" type and of chains, than in the blood capillaries. Single fenestrae and transendothelial canals, formed as a rule by fusion of several vesicles, a fact connected with the considerable thickness of the cell, were observed in endotheliocytes of the postcapillary venule. Canals in the endothelium of the exchange microvessels were often marked with reaction product. Local accumulation of the tracer also was frequently found in the interstitial tissues in the zone of transendothelial canals and of several fenestrae (Fig. 2b, d). Most vesicles in endotheliocytes of blood capillaries and of the postcapillary venule contained reaction product (Fig. 3).

The opinion is held that horseradish peroxidase stimulates histamine secretion by mast cells in rats, and this facilitates "flow" of the endothelium [4]. We did not observe any difference in the ultrastructure of the mast cells after injection of peroxidase compared with the control.

The results of the present investigation demonstrated the absence of paracellular "leaks" in the endothelium of the precapillary arteriole. The most probable pathway for transendothelial transport in the precapillary arteriole is the very low level of vesicular transport.

The high permeability of endotheliocytes of the subepithelial and marginal capillaries, and also of the postcapillary venule of the jejunal villus for horseradish peroxidase can be attributed, evidently, to vesicular transport and to open junctions. The throughput of these junctions in endotheliocytes of blood capillaries of various organs has been described extremely contradictorily [8, 10, 11, 14]. Wissing [15], who in a more recent publication accepted the permeability of intercellular junctions in the epithelium of blood capillaries of the mouse diaphragm, explained his original negative result by the low dose of peroxidase. The role of junctions as "small pore" systems is denied by workers who have studied the per-

meability of interendothelial junctions of blood capillaries of the jejunal villus [3, 7, 11]. According to our own observations, the marker often filled the intercellular spaces in the endothelium of the blood capillaries and postcapillary venule, which are exchange microvessels, over their whole extent; no appreciable difference could be observed from the density in the lumen of the vessel. This may be evidence of the absence of occluding systems in the intercellular space, or of their permeability for protein. The problem of transport through transendothelial canals and fenestrae is difficult to decide unequivocally, for their diaphragms are masked by the reaction product. It was recently shown that structures connected with transmural protein exchange are evidently cationic components of the capillary wall in the intestine [9]. According to Simionescu et al. [12], the cell surface of the endothelium of the small intestinal mucosa has a differentiated chemical organization and a heterogeneous distribution of negatively charged loci. The latter are present on the luminal plasmalemma, with a particularly high concentration on the diaphragms of the fenestrae. The negative charge possibly limits transport of most plasma polyanions. Anionic sites are rarely observed or are absent altogether in vesicles, transendothelial canals, and also their diaphragms. On the basis of our own data and of the principles of distribution of the electric charge on the luminal surface of the endotheliocytes which have been reported, we regard vesicles and transendothelial canals as the more universal pathways of transport. Meanwhile fenestrae, which have a considerable negative charge, may provide a selective pathway for transendothelial protein transport; evidence that this is so is given by its accumulation near the interstitial surface of several fenestrae. On account of the size of the open intercellular junctions (about 6 nm), they can be classed in the system of "small pores." The patency of the intercellular junctions, tranendothelial canals, and single fenestrae, revealed by the present investigation, is evidence of the high permeability of the endotheliocytes of exchange microvessels of the intestinal villus, and it also suggests that the dimensions of the transport communications in their endotheliocytes may be sufficient to allow the passage of serum albumin.

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