DYNAMICS OF ALBUMIN TRANSPORT THROUGH WALLS OF MESENTERIC VENULES IN RATS

K. T. Zaitsev

UDC 616.383-005-008.939.6-076-092.9

KEY WORDS: albumin; permeability; venules; mesentery

Microcirculation of proteins is a very important process in the system maintaining water balance. This process can be divided conventionally into several components, among which protein transport through the wall of blood vessels is possibly the most important, and that which determines the dynamics of all the rest.

Intravital observations [9] and the study of electron-dense traces [1] have shown that plasma proteins of albumin type are transported from blood into tissue mainly at the level of the venular section of the microcirculatory bed. This section comprises venules of various types: postcapillary, collecting, and muscular [2]. The fine structure of the wall of these vessels differs. With an increase in the diameter of the venules the thickness of their wall and the complexity of its organization have been shown to increase because of the appearance of cellular and fibrous components [10]. However, it is not clear how the exchange properties of the vessels change under these circumstances.

The aim of this investigation was to compare the transport characteristics of different mesenteric venules in relation to albumin.

EXPERIMENTAL METHOD

Experiments were carried out on 27 male noninbred albino rats weighing about 300 g. Under pentobarbital anesthesia (4 mg/100 g body weight) a catheter was introduced into the femoral vein of the animals and laparotomy performed. The mesentery of the small intestine in the region of the ileocecal angle was straightened out on a transparent plastic platform and irrigated with Ringer's solution containing 1% gelatin at 37°C and pH 7.4. Exposure of the mesentery under the microscope did not exceed 15-20 min. Albumin labeled with fluorescein isothiocyanate (FITC-albumin) was prepared from human serum albumin ("Reanal") and FITC of Soviet origin by the method in [5]. The conjugate was purified by gel-filtration on a Sephadex G-25 column and lyophilized. Before use the FITC-albumin was dissolved in sterile physiological saline up to 10% concentration. The tracer (25 μg/100 g body weight) was injected during microscopy through the catheter located in the animal's femoral vein.

Microscopy was carried out on a large "Leitz" intravital microscope, using an Hg-200 mercury lamp, a BG-12 fluorescence excitation filter and a K-530 emission filter. Photographic recording began immediately after injection of the tracer, with intervals of 10 sec between frames, and with constant exposure ("Orthomat" camera, RF-3 film). The films were developed under standard conditions.

The FITC-albumin concentration in the tissues was estimated by densitometry of the negatives with an IFO-451 microphotometer. The concentration of the tracer was assumed to be a linear function of the intensity of fluorescence of its solution [6]. On assessment of the level of fluorescence on the basis of the results of densitometry, a conventional scale was used: the "0" point was taken to be the optical density of unexposed regions of the negative, and "1" was taken to be the optical density in the lumen of large venules, in the juxtamural layer of plasma.

EXPERIMENTAL RESULTS

Between 5 and 10 sec after the beginning of injection of the tracer, its yellow-green fluorescence was observed in the mesenteric vascular bed. Against the general dark back-

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Laboratory of Electron Microscopy and the Microcirculation, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 11, pp. 522-526, November, 1989. Original article submitted August 26, 1988.

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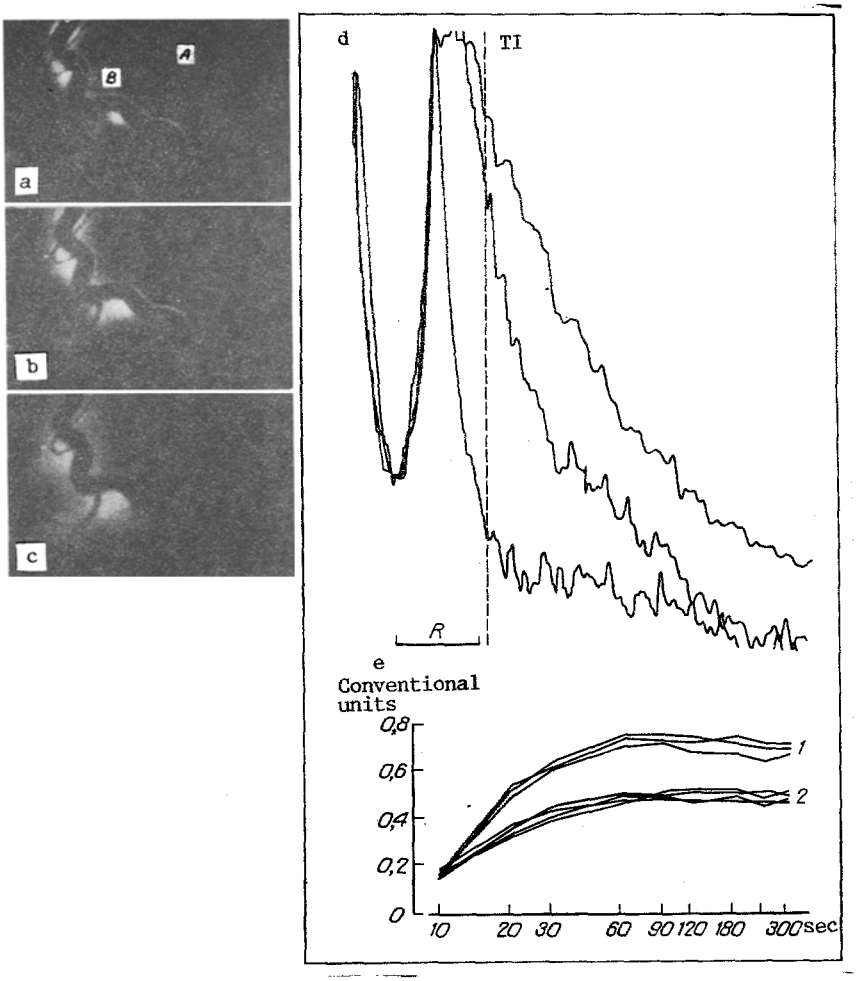

Fig. 1. Protein transport from blood into tissue through vessel wall. a-c) Successive phases of albumin accumulation in wall of venule, after 10, 30, and 60 sec; d) data of densitometer of corresponding negatives; e) curves reflecting accumulation of protein near walls of vessels about 60 (1) and 30 (2) μ in diameter.

ground, all components of the microcirculatory bed "appeared" in turn. Once it has filled the vascular bed, the labeled protein grandually began to escape through the vessel walls into the surrounding tissue. A fluorescent halo appeared on the outer surface of those vessels whose walls were permeable for albumin. With the passage of time the area of interstitial tissue occupied by escaping FITC-albumin increased (Fig. 1: a-c). Densitograms obtained by scanning along a line perpendicular to the axis of the vessel (Fig. 1d) enabled the increase in fluorescence near the wall of the venule and also at various distances from it to be estimate Comparison of curves plotted from photometric data and reflecting the dynamics of the tracer concentration near the walls of venules of different diameter reveals that these vessels differ in their transport properties. The rate of protein accumulation near the wall was determined by the angle of slope of the curve to the abscissa and was expressed as a value numerically equal to the tangent of that angle. This value was significantly greater in the larger venule (Fig. 1e). Scanning of the interstitial space along the axis of the vessel not far from its wall enabled the dynamics of the distribution of the tracer in the tissues for a short length of the venule to be determined. As Fig. 2 shows, different parts of the vessel differ in permeability. This heterogeneity was greatest in the initial period of escape of FITC-albumin. After a few minutes the differences in the levels of fluorescence largely disappeared and sites of greatest permeability of the vessel wall could no longer be precisely identified.

Visual analysis showed that 5-10 min after injection of the tracer its concentration and distribution in the tissues as a whole had stabilized. In this period it became particularly clear that the escaping protein was located mainly near the venules and also near

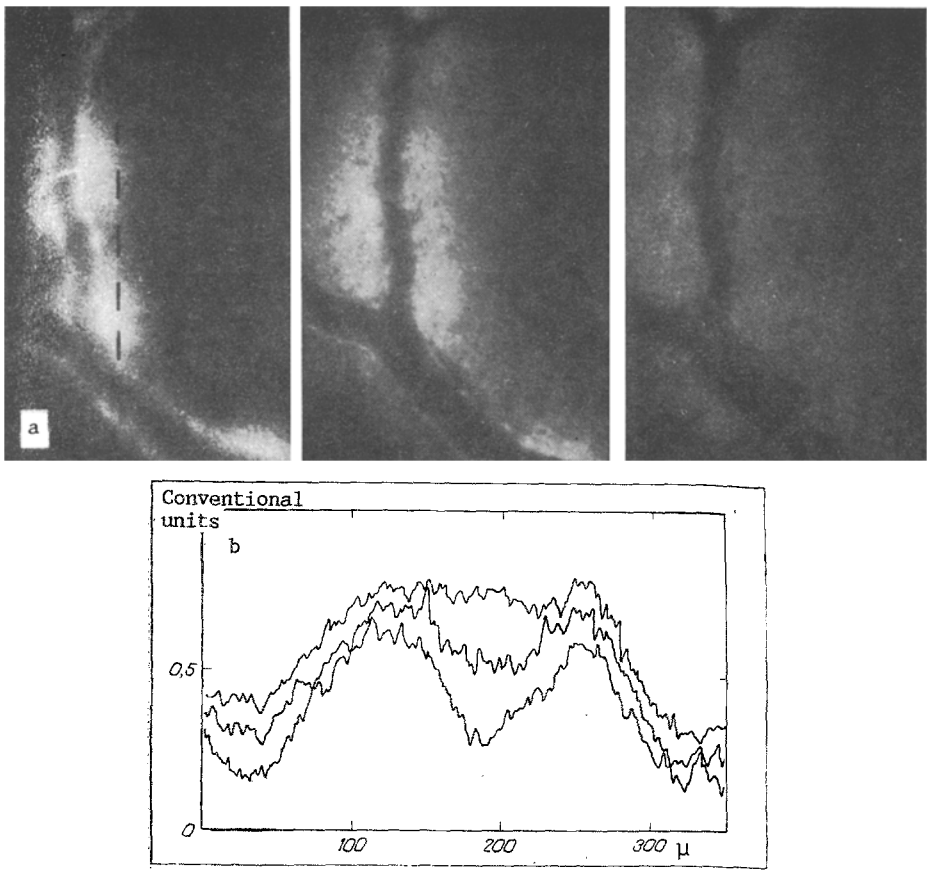

Fig. 2. Diffusion of albumin in interstitial connective tissue along vessel wall. a) Successive phases of protein transport after 1, 2, and 3 min; b) densitograms of distribution of protein along wall.

certain capillaries, as a rule near their venous part. Among venules whose walls were readily permeable for the tracer, most were of small and average diameter, mainly postcapillary venules. The study of the numbers of vessels of a particular diameter relative to the total population of venules showed that the proportion of venules present in the mesentery decreases with an increase in their diameter (Fig. 3a). When the number of vessels of a particular diameter, and in the region of which the tracer appeared, was compared with the frequency of vessels of that particular group in the rat mesentery in general, it was found that the proportion of "flowing" vessels was greater in the group of venules of comparatively large diameter (Fig. 3b). The highest protein concentration near the walls of the venules (a plateau on the curves in Fig. 1e) of different sizes increased in direct proportion to the increase in their diameter (Fig. 3c). To compare permeability per unit of surface area of the wall, the data of photometric analysis were normalized relative to the diameter of the vessels (Fig. 3d). Thus with an increase in diameter of the venules there was an absolutely significant tendency for the permeability of their wall for albumin to increase.

The curves showing changes in protein fluorescence near the vessel walls and at more distant points of the interstitial space are similar in shape, but differ in the steepness with which they flatten out on a plateau and in the level of maximal luminosity (Fig. 3e). If a line is drawn on the graphs parallel to the abscissa and connecting certain chosen values of fluorescence, it intersects the curves at points indicating the time when the chosen value of fluorescence is reached at a certain distance from the vessel wall. The graph plotted from these points reflects the dynamics of protein transport into the interstitial space (Fig. 3f).

By using the method of luminescence microscopic analysis [12], including injection of a fluorescent tracer into the blood stream and subsequent study of its concentration in the perivascular tissue, the investigation described above thus yielded data demonstrating the unequal transport properties of different vessels of venular type in the rat mesentery. The rate of entry of protein into the interstitial tissue and also its final concentration near the vessel wall depend on the diameter of the venule. The greater the diameter of the

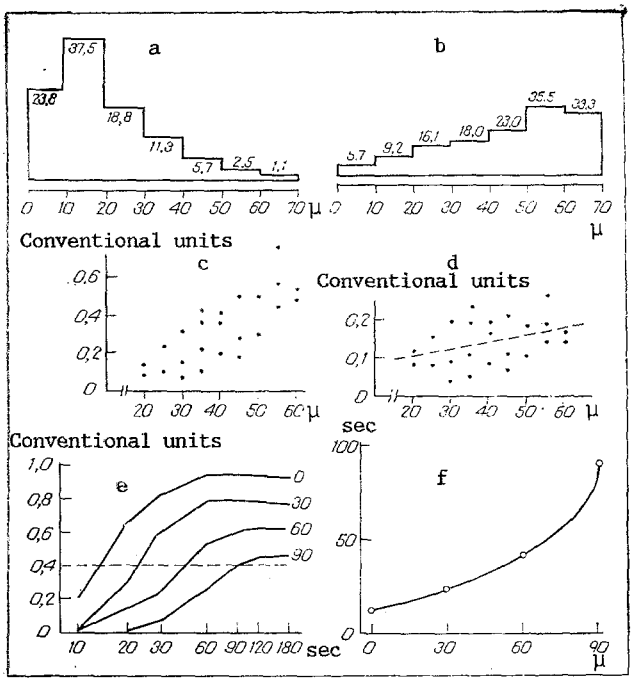

Fig. 3. Dependence of protein concentration near the wall of venules on diameter of vessels and dynamics of protein transport into interstitial tissue. a) Frequency of occurrence of vessels of different sizes, per cent; b) distribution of "flowing" venules depending on their diameter, per cent; c) protein concentration near walls of venules of different diameter; d) normalized values of protein concentration depending on diameter; e) curves reflecting accumulation of albumin at different distances from vessel wall; f) relationship between distance from vessel wall and time of protein accumulation.

vessel studied, the higher the value of these parameters. According to the results of analysis, the relationship observed was associated not only with an increase in area of the exchange surface of the vessel, but also with a regular increase in permeability of the walls of protein. This change of permeability can be interpreted as a manifestation of the phenomenon known as the "vascular permeability gradient" [7], in the comparatively narrower spectrum of microcirculatory vessels and, specifically, in venules between 20 and 60 μ in diameter. Since an important role in the regulation of protein transport through the walls of microvessels is played by endogenous mediators of vascular permeability [4], the increase in the transport powers of these vessels may be based on ultrastructural transformations such as an increase in the concentration of histamine receptors on the surface of the endothelium and "weakening" of interendothelial junctions [8, 11].

The subsequent fate of the albumin which enters the interstitial space of the mesentery through the walls of the venules depends not only on the transport properties of the vessel itself, but also on other factors. An important role in its transport begins to be played by the possible throughput of the interstitial matrix [3] and also of the mesothelial layers of the mesentery [6]. With an increase in the distance from the wall, the dynamics of protein transport thus reflect to an ever decreasing degree the true transport properties of the particular vessels.

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