The Functioning of Endothelial Fenestrae on the Arterial and Venous Limbs of Capillaries, as Indicated by the Differing Directions of Passage of Proteins

Fenestrated blood capillaries are found where there are likely to be accumulations of large molecules, and may permit their removal[1-8]. This may be very important when the lymphatics are few or absent, or functioning poorly[9-12].

Proteins slowly leave capillaries all along their lengths, via the vesicles, with the concentration gradient[1,2,11-14]; in some regions large molecules are also formed. They diffuse slowly and so are swept by the bulk-flow of fluid[15] which passes through the tissues from the arterial to the venous limbs of the capillaries in accordance with Starling's Hypothesis. Small molecules, but not large ones, can enter the venous limbs via the endothelial junctions[1-3,11-22]. Any inflow of fluid through the fenestrae on the venous limbs would carry large molecules in, too.

Simple gaps would destroy the osmotic pressure gradient and allow the escape of whole plasma e.g. when junctions were opened by injury[1,23]. Most fenestrae, however, have a diaphragm across them[1-7,11,24-26], particularly on the venous limbs[4,6,25-27]. It probably has a protein-polysaccharide nature[1,5,12] and, with the similar basement membranes, should be permeable to large molecules, but impede their passage according to their sizes, etc.[1,2,5,12,28]. (The differential permeabilities of fenestrae to peroxidase and ferritin[16] support this.) The diaphragms and basement membranes, therefore, should cause 'molecular-sieving'[15], making the passage of large molecules through them dependent on the bulk-flow of fluid. Hence large molecules would be carried into the venous limbs, but be prevented from diffusing outwards.

If so: a) Fenestrae must be permeable to large molecules. This was debated[1-3], but is now established for many regions[1-3,7,16,22]. (The early contradictory results are explained below.) b) The diaphragms must cause molecular-sieving. Theory and experiment support this[1,2,16]. c) Fenestrae must be mainly on the venous limbs. This has been shown for representatives of the tissues where they occur[4,6,25,27]. They are sometimes on the arterial limbs as well[4,24,25,28], allowing fluid carrying large molecules to leave the vessels. Such arterial fenestrae need not prevent the nett removal of large molecules from the tissues, provided there is a sufficient excess of venous ones with diaphragms[4].

These two functions of fenestrae have probably caused the dispute about their permeabilities[1-3]. Since tracers have always been injected into the blood[1-3,16,28], they have only been likely to pass through the arterial fenestrae and those venous ones without diaphragms. Both these are far less frequent than the venous ones which possess diaphragms. Hence different workers reported different findings, except for the renal glomerular fenestrae[1-3,22] which are all on arterial limbs. (Peroxidase[2,16] appears able to diffuse out of venous fenestrae – perhaps because of its small size and ease of detection.)

The possible inwards and outwards passage of ferritin through both limbs of capillaries have been studied in the jejunal villi, and in the renal glomerulae and medullae, of mice. (The methods for identifying the limbs of the jejunal capillaries, and other details are described elsewhere[4]; the glomerular capillaries are arterial, while the fenestrated ones in the rete of the medulla are venous[27].)

Cadmium-free ferritin was used at 5 g/100 ml (plus 0.1% Evan's blue) in Krebs-Ringer's solution. 0.2 ml were injected into the tail veins of ten 20 g mice, anaesthetized with Nembutal. Injections of 0.001 ml of the solutions were made, via 35 gauge needles, into the jejunal laminae propriae and renal medullae of 10 other mice. Blocks were taken after intervals of 1, 2, 4, 8 and 16 min. After glutaraldehyde fixation, they were trimmed so that only the edge of the injected region was examined, post-fixed in Osmium tetroxide, stained with Uranyl acetate and embedded in araldite.

There was the normal labelling of the endothelial vesicles[1-3,11-16], but fenestrae seemed quantitatively much more important[16]. When ferritin was in the blood, a few molecules could be seen in and just outside the arterial fenestrae at 2 min. These were more evident at 4 min (Figure 1), when vague concentration gradients could be seen outside individual fenestrae – as mentioned by CLEMENTI and PALADE[16]. Much more had passed through them at 8 and 16 min, but there was so much throughout the tissues that the gradients were obscured. While most arterial fenestrae showed this passage, few venous ones did – even at 8 min in the jejunum, or 16 min in the medullae. (After 16 min in the jejunum ferritin appeared outside the venous limbs – almost certainly some which had escaped from the arterial limbs and had been carried there by the flow of fluid.)

In the animals which had ferritin injected into the tissues, as time passed the amounts decreased near the

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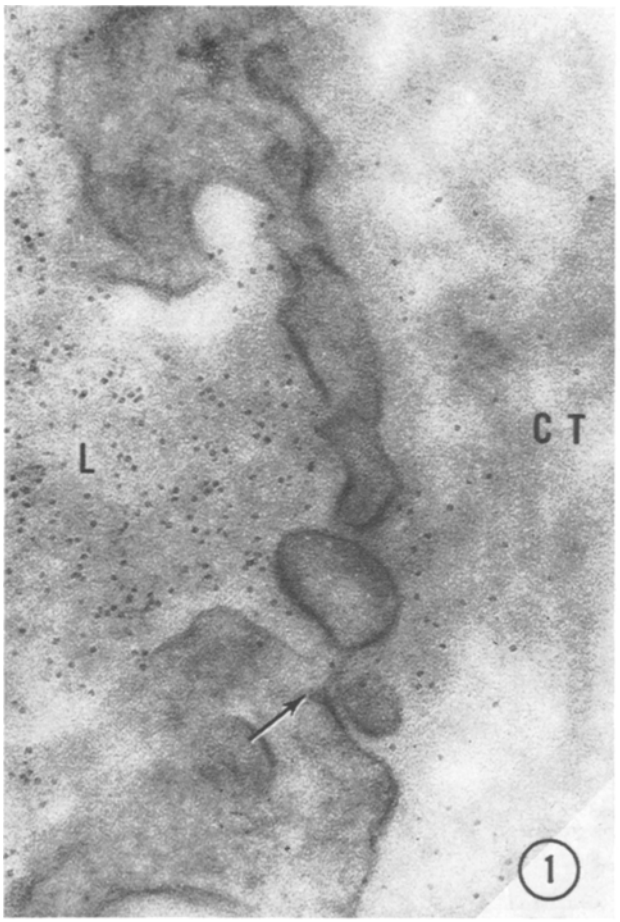

Fig. 1. Arterial limb of a capillary in the jejunum. Ferritin was injected into the blood 4 min previously. Molecules are passing from the lumen (L) to the connective tissue (CT). There are two fenestrae, one which appears to have no diaphragm, and one (arrow) where a diaphragm is visible. There is a concentration of ferritin in the connective tissue just outside the fenestrae. × 125,000.

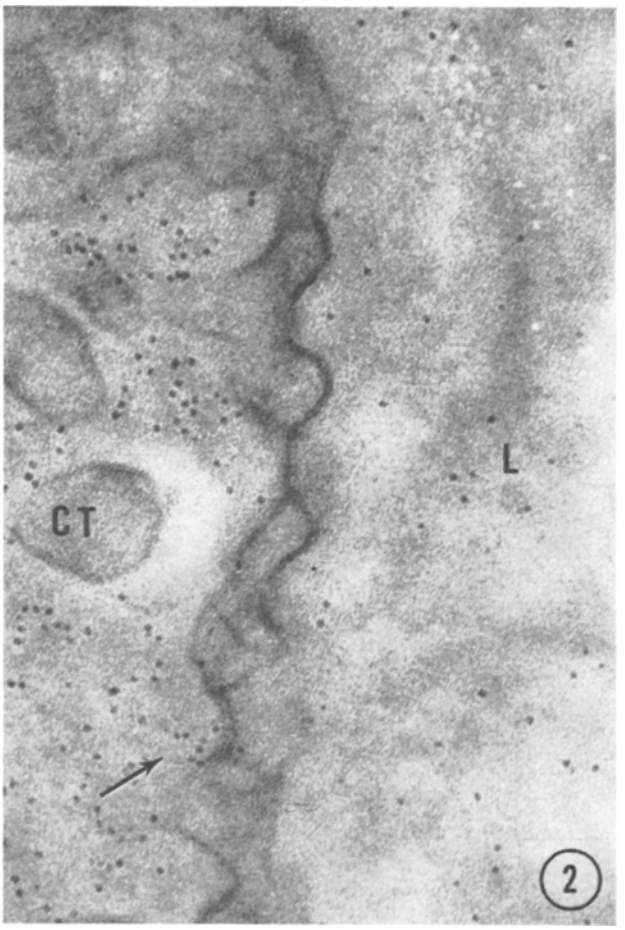

Fig. 2. Venous limb of a capillary in the medulla. Ferritin was injected into the connective tissue (CT) 8 min previously and is passing into the lumen (L). Some molecules may be seen in a fenestra, very close to its diaphragm (arrow). Another fenestra with a diaphragm is also visible. × 150,000.

jejunal arterial limbs, but increased at the venous limbs in both sites. Molecules could frequently be seen in the venous fenestrae (Figure 2), often appearing to be on or in the diaphragms.

All these results confirm that the ferritin passed out of the arterial fenestrae (and probably a few diaphragmless venous ones), was carried through the tissues by the bulk-flow of fluid, and passed inwards via the venous fenestrae with diaphragms. Measurements of capillary permeability relying on the amount of a substance appearing in the lymph[24,30], are therefore likely to be greatly in error if there are fenestrae present. This explains the inconsistencies[16] between such experiments and electron microscopical observations in fenestrated regions.

There are very few experiments showing the total amounts of material removed from the tissues by the blood system as against the lymphatic system. SZABÓ[31] has shown that in the renal cortex and medulla $>99\%$ of radio-albumen is removed by the blood, and $<1\%$ by the lymphatics. In subcutaneous tissues, where fenestrae are infrequent[1,26], the amounts are approximately equal[31]. Evidently the relative importance of the lymphatic system for protein removal varies greatly from tissue to tissue[32].

Résumé. Les molécules de ferritine s'échappent des capillaires sanguins par les fenestrae des rameaux artériels. Les grandes molécules pénètrent dans les capillaires veineux grâce à leurs fenestrae. On suggère le mode de fonctionnement de ces ouvertures. Leur diaphragme est très important pour enlever des tissus les grandes molécules et empêcher qu'elles ne s'échappent des capillaires veineux.

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