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The Functioning and Interrelationships of Blood Capillaries and Lymphatics*

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Summary. The structure and function of blood capillaries, as related to permeability, depends on tight, close and (in injured vessels) open junctional regions, small vesicles, vacuoles (in injured vessels) and fenestrae. The basement membrane presents a hindrance to the larger macromolecules, at high flow rates, but not to small molecules. The connective tissue channels are probably the paths by which macromolecules, and most of the small ones, pass from the arterial-limbs to the venous ones, and to the lymphatics. In some regions these channels are grouped in special systems: the prelymphatics. The initial lymphatics take up material via open junctions, which close during tissue-compression. The collecting lymphatics retain the lymph because they do not have open junctions.

In the close junctional regions the motive force for water flow is the result of Starling's forces; diffusion is very important for other small molecules. The small vesicles transport macromolecules slowly by Brownian motion, as may the vacuoles, but possibly these latter are moved actively.

There is much evidence that colloids can develop high effective osmotic pressures even across pores much larger than their molecules, and that proteins can be dragged up a concentration gradient by the resultant fluid flow. On the basis of this, hypotheses have been developed about the functioning of venous-limb fenestrae and the initial lymphatics, for which there is much theoretical, in vitro, and in vivo evidence. Thus, in fenestrated regions there is held to be a large local circulation through the tissues, of which a quantitatively small, but qualitatively vital, part goes to the lymphatics. Material is considered usually to enter these latter because of the relative concentration of the lymph.

It is becoming increasingly evident that in the study of the microvasculature, as with other systems, there is much to be gained by quantifying fine structural observations and by combining and contrasting this data, via physical laws, with that obtained by other methods where the characteristics of whole organs and regions are studied. Thus one can obtain interrelated information, which is not possible by either method alone, and which gives us a vital, comprehensive, perspective of the ways in which whole systems function, and how different systems interact. In this paper I shall show how this approach has yielded much that is new about the functioning of different kinds of blood capillaries, of the tissue channels, of the whole lymphatic system, and of the ways they affect each other.

The structure and functioning of the blood capillaries

These vessels have been reviewed recently[1–5]. The permeabilities of the following structures are important: endothelial intercellular junctions, small vesicles, vacuoles and, in some regions, fenestrae.

The junctions occur between adjacent endothelial cells (Figure 1). The gap is normally $< \sim 1$ nm (in 'tight junctions'), which is usually considered to be impermeable to all molecules. However (except in a few regions, e.g. the brain), for about 5% of their lengths the junctions are 'close' with a gap of $\sim$6 nm

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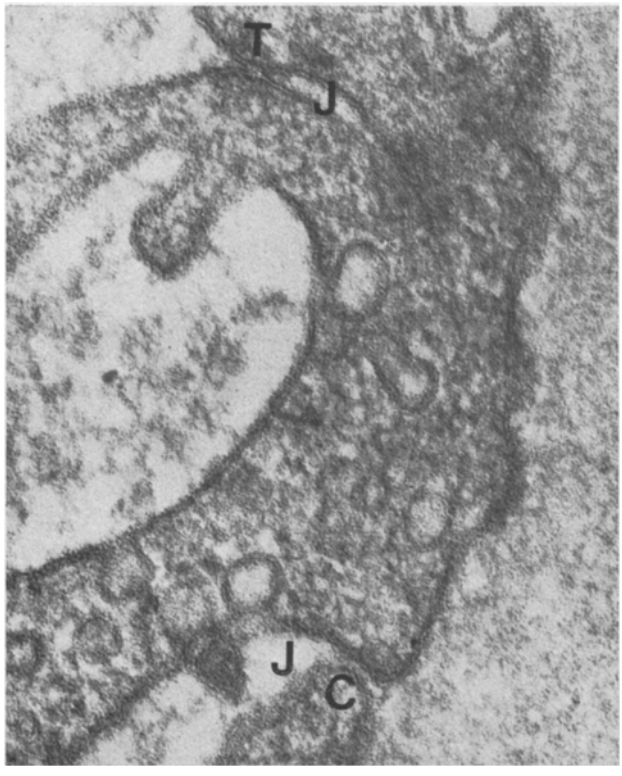

Fig. 1. Two junctions (J) are shown in the wall of a blood capillary in dog gastrocnemius[6]. The upper one shows a tight region (T) where the two outer laminae of the plasma membranes fuse; the lower one is close (C) with a ~4 nm gap between them. ×120,000.

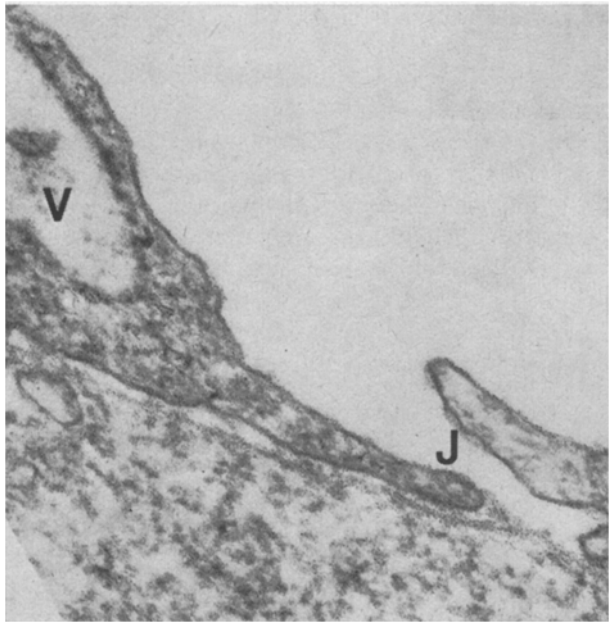

Fig. 2. An open junction (J) and part of a vacuole (V) are visible in the wall of a capillary from a mouse diaphragm[14], 10 min after it was treated with histamine. ×120,000.

for ~30 nm of their depths (from the lumen to the exterior); the remaining ~500 nm of the depth is ~20 nm wide all along the junction[2,6]. It is now almost certain that the junctional lengths where this 6 nm gap occurs (the 'close junctions') correspond to the small pores of PAPPENHEIMER[7]. The gap is now considered to be ~6 nm, not ~4 nm as is actually measured, because of statistical considerations when measuring from electron micrographs[8]. Quantitative data, combined with normal physical laws, give very good estimations of capillary filtration coefficients (CFC) and diffusion coefficients (CDC), when compared with those actually obtained[6]. Thus continuous capillaries are 'tube-capillaries', in the sense of INTAGLIETTA and DE PLOMB[9], with the endothelium controlling their permeabilities to small molecules.

If capillaries are injured[1,10–14] much of the junctional lengths become temporarily open with gaps (Figure 2) of ~50 nm for up to ~30% of their lengths[14], probably because of endothelial contraction[15] plus junctional injury. The presence of these gaps corresponds to a temporary increase in the CFC and CDC, but calculations show that actually they should be 50–100 times greater than is observed: thus it was concluded that here the permeabilities of the vessels are determined not by the endothelium, but by the connective tissue[14]. Thus these 'tube-capillaries' temporarily become 'tunnel-capillaries'[9].

The small vesicles (Figure 1) have internal diameters of ~60 nm and have frequently been shown to transport material across cells, including blood and lymphatic endothelium[4,5,16–20]. Experiments[18–21] show that vesicular transport is quite slow (~5 vesicles discharge/μm^2/sec), and can readily account for the slow leak of proteins out of continuous capillaries, but not for the much more rapid passage of smaller molecules. It has been suggested, however, that inter-

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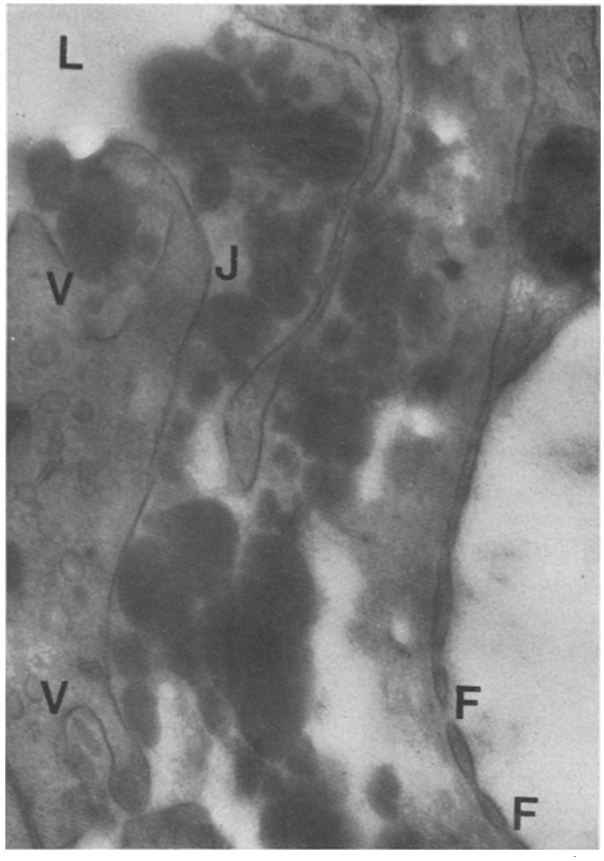

Fig. 3. Chylomicra, produced by feeding maize (corn) oil[26], are entering a lacteal (L) in a rat, via an open junction (J) and vacuoles (V). They are obviously too large to enter the fenestrae (F) in a nearby blood capillary, although smaller macromolecules do. × 40,000.

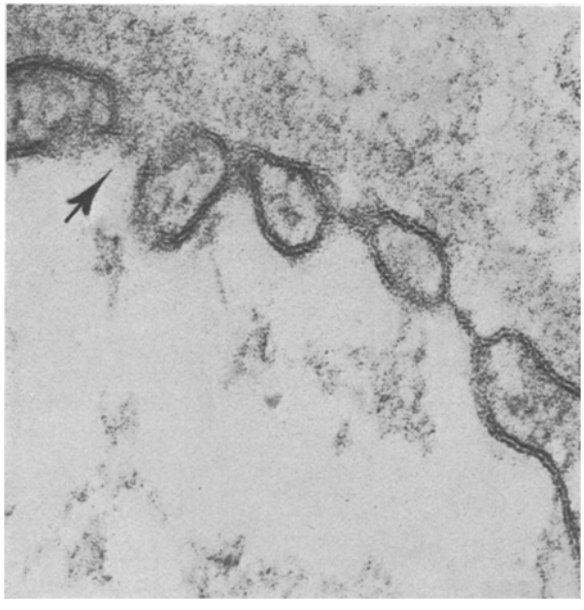

Fig. 4. Fenestrae in a capillary in the villus of a mouse[34]; one (arrow) has almost no diaphragm visible. × 100,000.

connected systems of vesicles may form channels right across the cells[22]. This appears somewhat unlikely since such channels would not produce the observed molecular sieving of variously-sized small molecules (as is found with the 'close' junctional regions[6]), and there is an error in the calculation of the necessary tilting which has to be done to distinguish between interconnected and simply overlapping vesicles[23]. However some single vesicular channels are also shown where this does not apply[22].

The vacuoles, ~200nm in internal diameter, are occasionally seen in normal vessels[24,25], where it has been considered that they might transport material across the endothelium. This has never been established here, although it has for chylomicra crossing lymphatic endothelium[26–28]. Undoubtedly much confusion has been caused by sections of simple indentations, by phagocytosis, and by dilated endoplasmic reticulum. On the other hand in injured capillaries, (Figures 2 and 10), such vacuoles are very frequently seen[1,10,14]. They remain for much longer than the temporarily opened junctions, and could well explain[14] the deductions from physiological experiments[29–31] that protein transport under these conditions occurs in 'vesicles' some twice the size of the normal small vesicles; these latter actually remain their normal sizes and are, if anything, reduced in number[14,32]. There is some direct evidence that such transport does indeed occur via vacuoles[10], but this has at present certain technical deficiencies[14].

Fenestrae (Figures 3 and 4) are holes (~50 nm) through thin portions of the endothelium of capillaries in certain regions; these are usually visceral – where proteins are produced, or concentrated in the tissues by the excretion of water[4,5]. It has been suggested[5,33] that they are devices for allowing much more fluid and proteins to reach the tissues than is possible from continuous capillaries, and for once more removing these and cellular products from the tissues. Fenestrae are very much more frequent on the 'venous-limbs' (used in a statistical sense) of capillaries, than on the

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'arterial-limbs'[4,5,34]. While many are probably sealed[35] by a diaphragm (Figure 4), variable proportions (according to site) do not possess one[1,4,5,33,34]. They are very permeable to proteins[1,2,4,5,36–39], perhaps even if there is a diaphragm[38], but this may possibly be an appearance caused by the short lives of fenestrae. In fact, nothing is known of the permanency of fenestrae. Possibly they form from vesicles[40]; even if they do not, it appears that their diaphragms are analogous to those briefly sealing vesicles when they unite with a plasma membrane[41]. If so, since the median duration of attachment of a vesicle is ~6 sec[6,19] and the diaphragms disappear from many of these[41], it would seem that the fenestrae and their diaphragms are likely to be equally short-lived.

Quantitative data, combined with physical laws, have shown that discontinuous, fenestrated capillaries should have CFC's $\sim 5 \times 10^4$ and CDC's ~200 times greater than are actually observed[33]. This shows that such capillaries are permanently tunnel-capillaries[9], with their permeabilities to small molecules controlled by the connective tissue.

It has been shown[36] that large proteins mostly pass out of the fenestrae on the arterial-limbs and in at those on the venous-limbs. This directional passage was responsible for early reports that the fenestrae were largely impermeable[1], since the tracers were usually injected into the blood and the most frequent fenestrae were observed, i.e. those on the venous-limbs, except in the glomerulae. Tracers introduced into the tissues[36,39], or smaller blood-borne ones with substantial back-diffusion against fluid flow[2,4,5,37,38] have since revealed fenestrae to be permeable in many regions.

The venous-limb fenestrae probably remove many macromolecules (as well as fluid) from the tissues, in fact, many more than pass out via the lymphatics[4,5,35,39,42–44]. There is a great difference between fenestrated and non-fenestrated regions (where relatively more of the material enters the lymphatics[42,43]). While some of these experiments used artificially introduced tracers, others used naturally occuring molecules – thus avoiding the possibility of vascular damage. (Similarly, fenestrated regions have been shown to be much more permeable to macromolecular tracers injected into the blood[42,45], presumably via the arterial-limb fenestrae.) There is, however, a difficulty: while evidence of high blood/lymph uptake ratios shows what happens to proteins appearing de novo in the tissues, they only trace protein movements in one direction; they do not show that there is a net uptake of normal plasma proteins from the tissues at the venous-fenestrae. It can be shown, however, that much more albumin leaves the blood in fenestrated regions than returns via the lymph[33,45,46]; proteolysis in the tissues, while probably occurring[47,61], is unlikely to be able to account for more than a fraction of this, so the rest must represent venous-limb uptake, as was strongly suggested by the other experiments. Calculations[35] indicate that this occurs and is much greater, quantitatively, than lymphatic removal.

The basement membranes

The structure of these has been reviewed[1,3,48,49] and for our purposes we can take it as a fibrilar matrix with pores of ~5 nm[37,48,49] although it has been shown that there is an exclusion of molecules of this size in vivo in the glomerulus[50], probably due to layering effects. Calculations show that the membrane has a negligible effect on the passage of small molecules[6]. For molecules the size of albumin it is likely to cause some resistance, depending on the rate of fluid flow[35]; e.g. its effect is negligible in continuous capillaries[51], but appreciable in fenestrated ones, although more at the arterial-limbs[35,51] (Figure 16). Its effect becomes more and more important as molecular sizes increase – as has been seen by tracer studies[48].

Connective tissue channels

More and more the intercellular matrix is being viewed, not as a homogenous, formless mixture of mucopolysaccharide, but as being separated into distinct regions[52–55]. Vital microscopy[55], electron

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microscopy[18,38,56–58] and calculations[33,35] indicate that there is a network of channels (~100 nm) through it. The calculations were able to be performed using the observed CFC and CDC data, once it had been shown that fenestrated capillaries are tunnel-capillaries so that these data were seen really to relate to the channels in the tissue[33]. No doubt the numbers and dimensions of these channels vary with the tissue and the conditions. Presumably they form an essentially random network; this assumption allows calculations which fit the experimental data better than two other alternatives[35]. It has been shown, however, that there is probably a preferential orientation, since ~60 times more channels (than would be expected randomly) can be calculated to end at fenestrae[35]: this is perhaps to be expected, since flow through the tissues should cause it, just as draining surface water produces runnels in a field.

These channels obviously offer easy routes for proteins, as has been observed[55,59], but they must also offer the easiest, although not the only, routes for the filtration, and diffusion, of small molecules. This is because of the r^4 term in Poiseulle's Law, and because of the effects of varying dimensions of the intestices, blind pockets, etc. on diffusion.

Prelymphatics

Everywhere in the body there are tissue channels, some of which lead to the initial lymphatics – often to their open junctions[60]. Such channels are normally only ~0.1 mm long; however, in certain regions (e.g. the brain, the retina and bone marrow) they are some *tens* of centimetres[58]. They form a system of non-endothelialized spaces, and potential spaces, lying adjacent to the basement membranes of capillaries and in the adventitia of the larger vessels. They pass from the depths of the cerebral cortex to discharge their contents into the cervical lymphatics near the great vessels in the neck[58]. One wonders if this system has developed because of the lack of varying total-tissue pressures in such enclosed cavities. Ligation of the cervical lymphatics causes lymph stasis and lymphoedema in the regions drained by the prelymphatics[58]. Although such channels occur throughout the body, it would appear that such a distinct and important system deserves a specific title: the prelymphatic system.

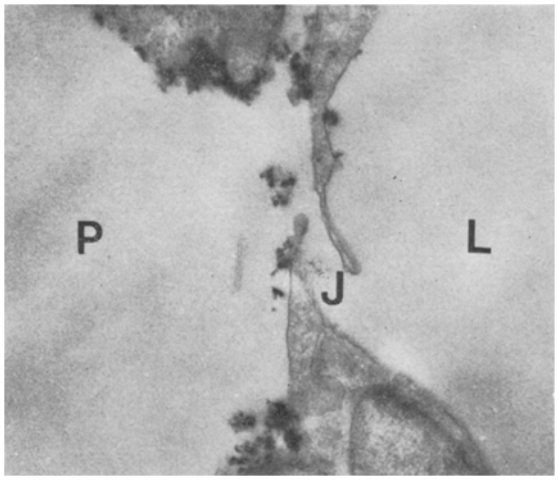

Fig. 5. In the diaphragm, here of a mouse[27], the peritoneal cavity (P) and the lacunes (L) are in continuity via the open lymphatic endothelial junctions (J). The overlapping shows how the endothelial flaps can function as inlet valves. ×20,000.

The structure and functioning of the lymphatic system

The initial lymphatics (or terminal, peripheral, capillary lymphatics) have been reviewed[4,5,61–65]. The passage of macromolecules into them is facilitated by their tenuous basement membranes. Although there is vesicular transport[4,5,26,27,32], there is much evidence that the only important passage is via open (~0.1–1.0 μm) endothelial junctions[4,5,26,27,32,65] (Figures 3 and 5). These constitute ~2% of the junctional length; another ~10% consists of 'close' regions; the remainder are tight[28,60]. The amount of open junction is considerably higher in active regions, or if there is tissue injury, especially if there is oedema[4,5,32,65]. The junctions tend to open, because[4,5,65]: they are poorly supported by adhesion devices and basement membrane; inflowing fluid pushes the inner flap of cell aside; filaments (which are under tension in oedema) are mainly attached to the outer cell – pulling it out, and also elongating the vessel; and the cells contract if injured[66]. The open regions are only open during tissue relaxation (Figure 5) – the filling-phase; during tissue compression – the emptying-phase – they are closed (Figure 6), and are permeable only to small molecules[4,5,65].

With the intra-lymphatic valves, the initial lymphatics thus are able to act as millions of tiny force pumps – albeit rather leaky ones (vide infra).

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The collecting lymphatics (Figures 13 and 15), as they pass centrally, have fewer and fewer open junctions, until there are none[4,5,65,67]. In addition, the thickness of the basement membrane and of the whole wall increases. Hence these vessels come to have only a slight permeability to macromolecules[4,5,62–65,67–70], which slowly traverse the endothelium via vesicles. On the other hand, their permeability to small molecules is still quite high[4,5,63,68–72]. The intermittent contractions of the muscle in their walls, and varying external pressures, can raise the intra-lymphatic pressures to high levels[64,73]; with the aid of the intra-lymphatic valves, this propels the lymph towards the blood, with each segment ('lymphangion'[74]) contracting according to its load – similar to Starling's Law of the Heart.

The forces and mechanisms involved in vascular functioning

In the *close junctional regions*, where the gap is ~6 nm, the net force for water flow is the result of internal, and tissue, hydrostatic pressures (HP) and colloidal osmotic pressures (COP), as has frequently been established using single capillaries, and in other ways[3,63,64,75]. Thus Starling's Hypothesis has been established, although it is now well-known that the arterial-limbs and venous-limbs are only statistical concepts, since the capillaries frequently form a network, and since at times whole regions seem to be given over to filtration or absorption[3,9]. Diffusion is the important force for small molecules other than water, but convection gains in importance as their size increases[7,75]. Molecular sieving[7,49,75,76] is also increasingly important with the larger molecular diameters.

The *small vesicular* transport of material is not affected by cold or poisons[4,5,17,77,78], and it appears that it is a random process powered by Brownian motion[79,80]. Two theoretical models for this have been made[81, cf. 80, 82], which differ in the probability they give for a vesicle fusing with a plasma membrane if it approaches it; experiments[6,19] favour that with a low probability (~0.004), as might be deduced from the fact that the exterior of the vesicles and the interior of the membrane have similar charges, which are therefore mutually repelling, and quite large. Even the union and detachment of vesicles from the plasma membranes (and each other) can be explained by random thermodynamic activity[19]. The effect is the same as diffusion, except being in quanta[18].

The vacuoles may also be moved by this mechanism[6]. While the coated vesiclels (rhopheosomes) have been shown to be directed in cells[82,83] and therefore, presumably, are moved by cellular activity, as are the large phagocytic vesicles[78], the vacuoles have not been investigated so far. Certainly they resemble phagocytic vacuoles (i.e. the 'pinocytotic vesicles' of LEWIS[84]) and may be actively formed and moved, but experiments to differentiate between this and Brownian motion have not yet been performed.

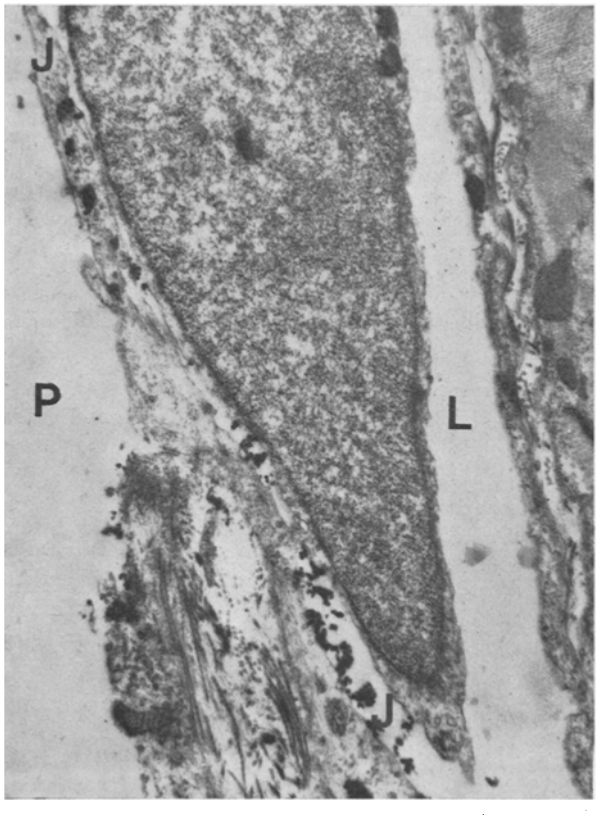

Fig. 6. As for Figure 5, but here the diaphragm was fixed during contraction. The junction (J) is effectively closed to macromolecules, although it would still be permeable to small ones. × 15,000.

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[84] W. H. LEWIS, Bull. John Hopkins Hosp. *49*, 17 (1931).

Effective colloidal osmotic pressures (ECOP) are developed (Figure 7) across pores considerably larger than the molecules, when these latter are proteins[85]. It appears that the inflowing fluid pushes back the slowly, outwardly-diffusing, macromolecules so that a 'virtual membrane' (Figure 8) is formed at the inner end of the pore[86]. This sharp concentration gradient gives rise to this pressure – which may even be measured across a piece of filter paper! This effect must be clearly differentiated from that of smaller molecules whose rapid diffusion causes very much lower ECOP's, related to their reflection coefficients[49,73,74]. Apart from the in vitro experiments[85], it is easy to calculate[5] that if this effect did not occur we would lose ~3 l of fluid each hour into our intestines alone, via the fenestrated capillaries. Also, the movements of proteins – caused by fluid flow – towards venous-limb fenestrae, show that fluid is taken-up there[36,39].

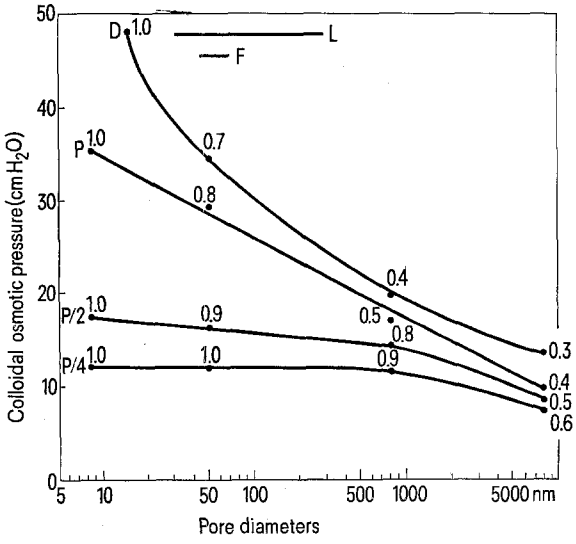

Fig. 7. The effective colloidal osmotic pressures are recorded (D) for 6% dextran (M.W. 110,000) and (P, P/2, P/4) for human plasma, whole, and diluted 1:1 and 1:3[85]. Various pore diameters are shown, and the approximate dimensions of fenestrae (F) and lymphatic open junctions (L) are indicated. It can be seen that very high ECOP's are retained even with quite large pores – many times greater than the molecular diameters.

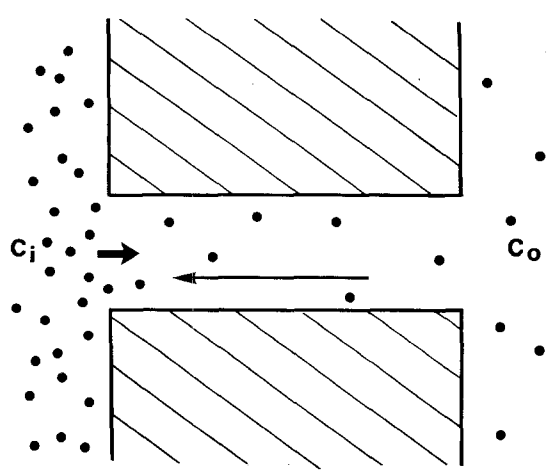

Fig. 8. To illustrate the formation of a virtual membrane[86] at the inner end of a large pore between two solutions of protein ($C_i > C_o$). Because of the difference in ECOP, the water flows in (thin arrow) and drives back the protein, in spite of its tendency to diffuse out (broad arrow).

The uptake of proteins against a concentration gradient, because of the solvent-drag of the inflowing fluid, has been suggested as one of the consequences of this ECOP[4,5,86]. There are theoretical[86,87] reasons (and limits) for this, which overcome previous objections[88]: the net inflow will always be less than the protein concentration outside the vessel. There is in vitro evidence[89,90] for this mechanism. In vivo evidence is harder to obtain, but protein movements confirm it[36,39] and it is implied by all the evidence presented, and to be presented, relating to the hypotheses about the functioning of venous-limb fenestrae and initial lymphatics. This concept is fundamental to both hypotheses.

The arterial-limb fenestrae allow a great out-flow of fluid and proteins into the tissues[35], as they do in the glomerulae. The forces are similar to those operating across close junctional regions, except that we must use the ECOP, taking into account the size of the pore[85], and remembering that the basement membrane can cause a considerable banking-up, thus probably increasing the concentration of larger molecules in the peri-vascular space to higher than that in plasma[35,51]. Similar forces, of course, will apply across the basement membrane, except that the differing sizes of the pores will cause a different ECOP across these pores – for the same concentration – as compared with across the fenestrae. Protein is largely moved by fluid flow, but diffusion may also be important if the flow is slow enough[35].

The venous-limb fenestrae take up much fluid and protein from the tissues (vide supra). The calculations[35] indicate that here also the resultant force is composed of the tissue and blood HP's and their ECOP's, again taking account of the fenestral diameters. As mentioned earlier, it is considered that the flow of fluid thus caused will usually drag proteins in with it, although at very low flows, diffusion would be important if the concentrations were different; this does not appear to be the case[35,51].

[85] J. R. Casley-Smith and T. Bolton, Microvasc. Res. *5*, 213 (1973).
[86] J. R. Casley-Smith, Microvasc. Res. *9*, 43 (1975).
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[89] J. R. Casley-Smith, in *Proc. IV Int. Congr. Lymphology, Tuscon* (Eds. M. and C. Witte; Univ. Arizona Press, Tuscon press 1975), in press.
[90] J. R. Casley-Smith, in preparation (1975).

The basement membranes and tissue channels may have similar forces affecting flow through them as affect flow through the fenestrae. Here it is likely that there are great concentration differences[35,51], but the velocities of flow usually appear to be large enough to prevent diffusion being of much importance, although it cannot be neglected for the membranes.

The initial lymphatics certainly remove fluid and macromolecules from the tissues; why these enter the vessels has been a problem. It used to be thought that there was a slight HP gradient into them[64,91]; although this was often questioned[62,63]. It is fairly certain that the HP inside the vessels is slightly positive[4,5,63,64,91,92], so that the concept of a normal negative tissue HP[93,94], means that this gradient cannot exist (except in oedema) and indeed would normally be directed outwards. It has been mentioned that the vesicles are not active, so they cannot pump fluid in, as has sometimes been suggested[28]. Again, suction by the collecting lymphatics has been proposed[94a], but these do not have many attached filaments to cause suction from tissue recoil after contraction[4,5,67]. This also would not dispose of the facts that measurements show the initial lymphatic HP's to be greater than those in the tissues, and that lymph needs varying total-tissue pressure to be formed, even if the collecting vessels are still actively contracting. By exclusion, the only possibility is the ECOP's of the lymph[95]. Even if the concept of negative tissue HP were found not to be correct[91,96], although it probably usually holds true[93,94], this mechanism could still contribute substantially. Calculations[97] indicate that only when the tissue HP's are quite high – as in oedema and in a few organs – is this ECOP force negligible; at lower HP's it is very significant; at negative HP's it is the only one. The hypothetical initial lymphatic cycle has been described elsewhere[4,5,51,65] and is shown in Figure 9.

In brief, during the filling-phase, fluid and protein enter because of the high ECOP of the concentrated lymph, which becomes diluted. When the lymph is so dilute that the net pressure across the open junctions becomes just less than O, the junctions will be closed to protein by the start of fluid outflow. Thus the protein of the lymph, and hence the fluid, is kept indefi-

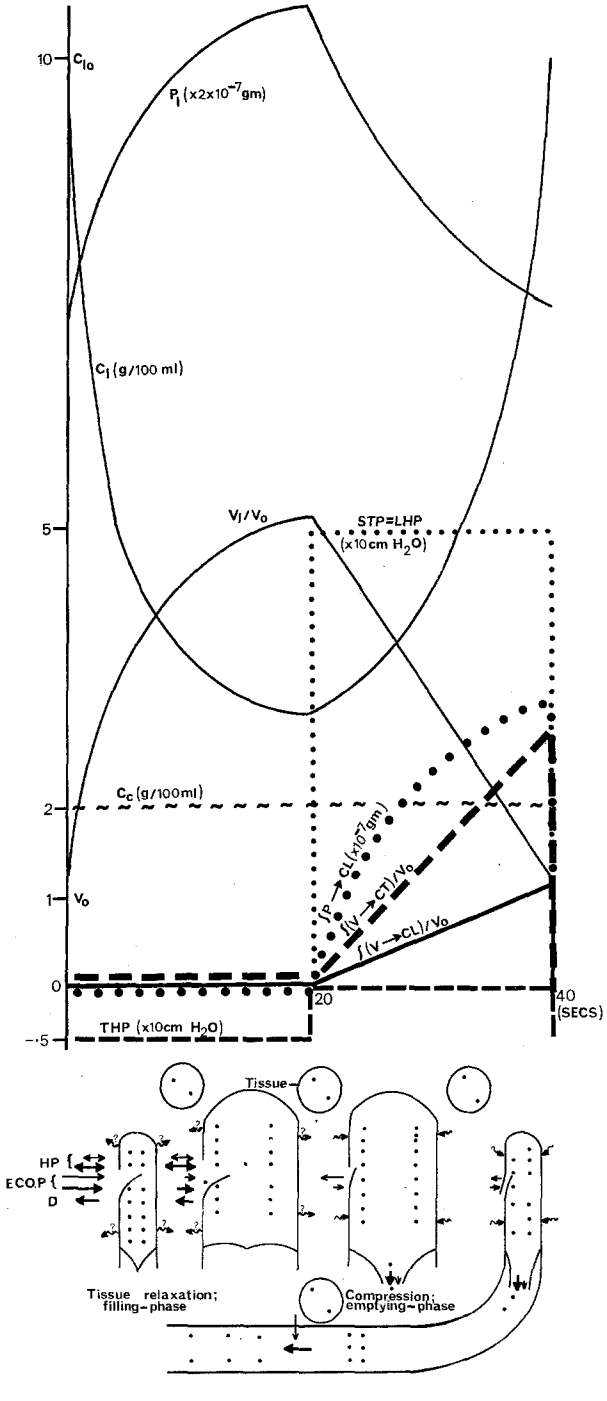

Fig. 9. At the bottom of the illustration is a representation of the initial lymphatic cycle[4,5,65]. On the left is the filling-phase, during tissue relaxation; on the right is the emptying-phase, during tissue compression. The intermediate-phase (not shown) occurs between these two, with V_l/V_o, C_l and P_l maintaining their values from the end of the filling-phase to the start of the emptying-phase. The proteins are shown as dots, in the tissues and in the initial lymphatics – at their varying concentrations. They are shown being diluted during filling, reconcentrated during emptying, some ejected into the collecting lymphatic at about a mean concentration, and finally rediluted in the remote collecting lymphatic. The forces acting on the initial lymphatic are shown as wavy arrows, possibly outwards during filling (if there is oedema), and inwards as the total-tissue-pressure during compression. The forces acting on the water are shown as thin arrows, and on the proteins as thick arrows. They are: HP, hydrostatic pressure; ECOP, effective colloidal osmotic pressure; D, diffusion. The lengths of the arrows indicate the net amounts of these at varying times during the cycle.

At the top of the illustration are shown various pressures, flows, concentrations, etc., derived from the calculated model[97]. P_l is the amount of protein in the initial lymphatic, C_l its concentration, with C_{lo} that at 0 sec (the start of the fillingphase), C_c is the concentration in the tissue channels. THP is the tissue hydrostatic pressure, STP is the total-tissue-pressure. V_l/V_o is the ratio of the volume of the initial lymphatic to that at time 0. $\int P \rightarrow CL$ is the total protein transferred to the collecting lymphatics by any time; $\int (V \rightarrow CT)/V_o$ is the total volume ultrafiltered into the tissues; $\int (V \rightarrow CL)/V_o$ is that passed to the collecting lymphatics.

These varying pressures explain the functioning of the initial lymphatic cycle, which is powered by the STP, accumulating energy as concentrated protein in the lymph. The essential functioning of the cycle is indicated by the transfers of protein and fluid.

nitely in the vessels, until tissue compression occurs. This is the intermediate-phase[97]. During tissue compression, the total-tissue-pressure rises to much greater than tissue HP, and is transmitted to the lymph[97,97a]. This closes the open junctions to protein, but ultrafilters the lymph – thus concentrating it again – and also forces some into the collecting vessels. The regurgitated water temporarily dilutes the proteins in the adjacent tissue channels, but rapidly passes through their walls so that the fluid which will next enter the lymphatics is no longer diluted. Also, about half this water actually passes through close junction regions which will not open in the next phase[97]. These two factors dispose of one argument against the hypothesis[98]: that the escaping fluid would only dilute the adjacent protein. Calculations (Figure 9) indicate that such a cycle is feasible, according to physical laws and the concept of the uptake of protein caused by an ECOP[97]. They also indicate that only a small part of the lymph is propelled into the collecting lymphatics; most fluid is returned to the tissues. This has been confirmed by observations which indicate that the

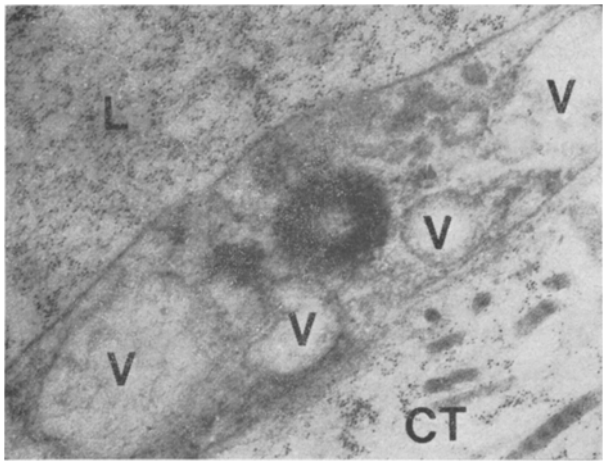

Fig. 10. There are some vacuoles (V) in the endothelium of a lymphatic in a mouse ear, injured by heat, and into whose connective tissue ferritin was injected $2^1/_2$ h before[57]. There is a much higher concentration of the macromolecules in the lumen (L) than in the connective tissue (CT). × 50,000.

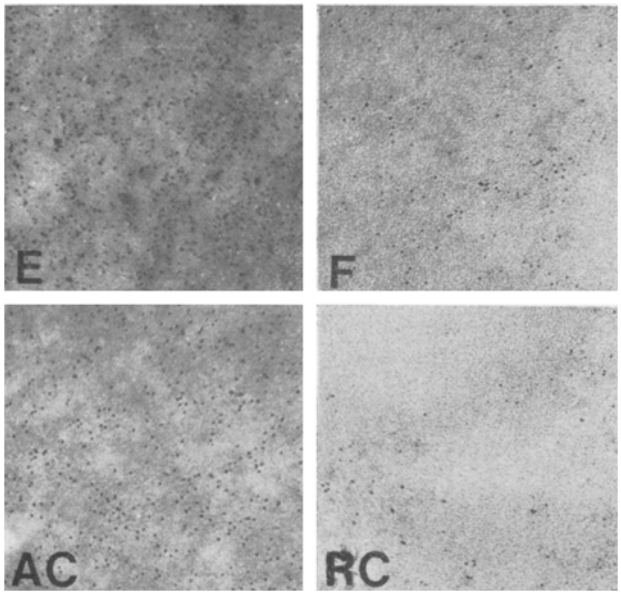

Fig. 11. Ferritin and 4% albumin were injected into the peritoneal cavity of mice[57]. E shows the concentration of ferritin (and of protein) in the lumen of a lacune fixed during the emptying-phase; F that in one fixed during the filling-phase; A.C. that in an adjacent collector; RC that in a remote collecting lymphatic. It will be seen that the concentration in E is much greater than in F, in AC is about the mean of these, and in RC is much more dilute than in the others. This indicates that there is concentration of the lymph during emptying and dilution during filling, and that the adjacent collectors receive approximately the mean of the concentration of the initial lymphatics, while this is greatly diluted in the remote collectors. ×65,000.

[91] C. A. Wiederhielm, J. gen. Physiol. *52*, 29s (1968), and in *The Pulmonary Circulation and Interstitial Space* (Eds. A. P. Fishman and H. H. Hedt, University of Chicago Press 1969), p. 59.
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[94] A. C. Guyton, H. J. Granger and A. E. Taylor, Physiol. Rev. *51*, 527 (1971).
[94a] N. P. Reddy, T. A. Krouskop and P. H. Newell, Microvasc. Res. *10*, 214 (1975).
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[97] S. Elhay and J. R. Casley-Smith, Microvasc. Res. submitted for publication (1976).
[97a] A. C. Guyton, personal communication (1975).
[98] A. E. Taylor, W. M. Gibson, H. J. Granger and A. C. Guyton, Lymphology *6*, 192 (1973) and *8*, 43 (1975).

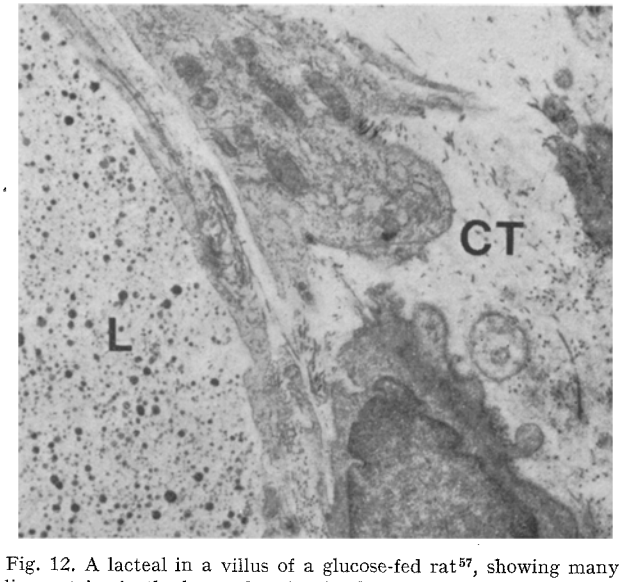

Fig. 12. A lacteal in a villus of a glucose-fed rat[57], showing many lipoproteins in the lumen but few in the connective tissue, i.e. the lymph is more concentrated than the tissue fluid. × 5,000.

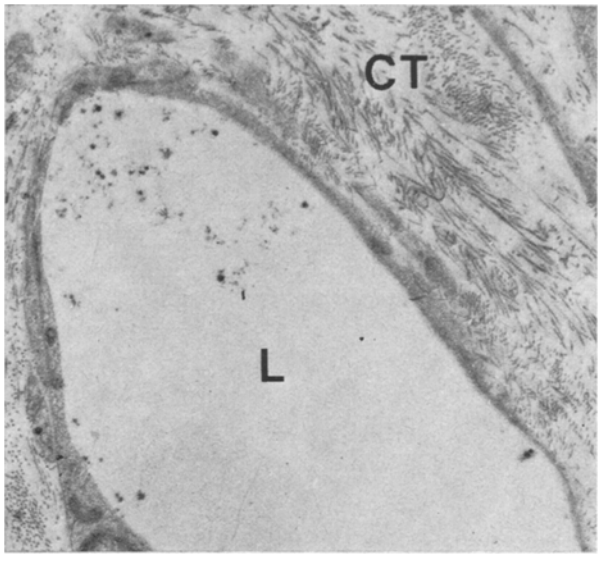

Fig. 13. A remote collecting lymphatic, as for Figure 12, showing that the lymph is much diluted compared with the initial lymphatics. ×4,000.

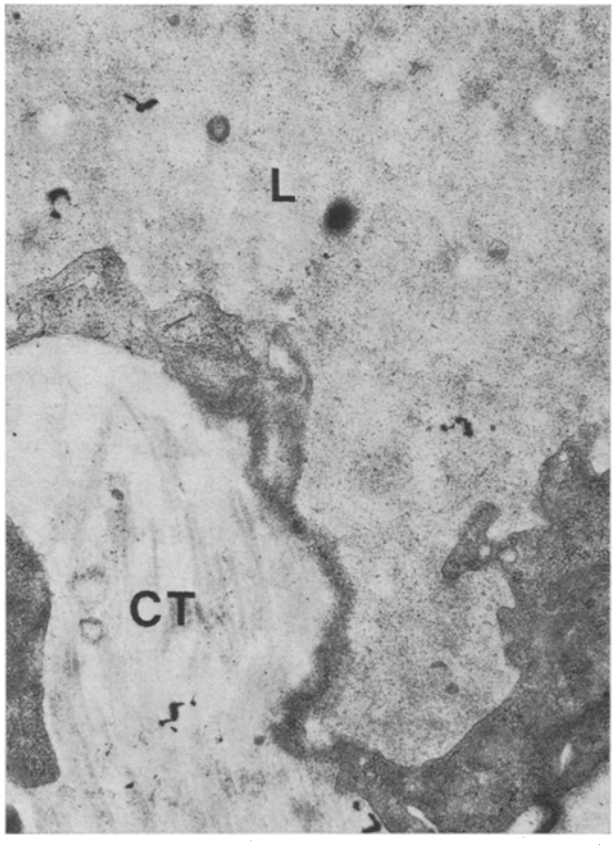

Fig. 14. A lacteal in a villus of a mouse, which had been given RISA 24 h before[51]. There is a greater specific activity in the initial lymphatic than in the connective tissue, and the mass-densities of protein is also greater, although many more micrographs are necessary to establish the former, and measurement on the plate to establish the latter. ×16,000.

efficiency of the initial lymphatics is[99] only ~1.0–10%, and also disposes of a second argument against this hypothesis[98], where much higher efficiencies were assumed.

Other evidence in favour of the hypothesis is that in a number of regions and species, and using 5 quite different techniques, it has been shown (Figures 10, 12 and 14) that the mean concentration of protein in the lymph is ~3 times that in the adjacent tissues[57,59,63,89,95,100], after allowing for any exclusion by the mucopolysaccharides. Not only this, but it has been shown (Figure 11) that the concentration during the compression-phase is much higher than during the filling-phase[57]. There is also some indirect evidence in its favour[101].

The collecting lymphatics will, according to the hypothesis, receive concentrated lymph[4,5,65] (Figure 9). Because their walls are quite permeable to small molecules though not to large ones (vide supra) and because the pressures in them will be similar to those in the adjacent tissues (except during contractions of their walls), the ECOP of the lymph will cause fluid to enter and redilute it. This will not occur in the 'adjacent collectors' (Figure 11) which are in the same region as the initial lymphatics[51,57] and are similarly compressed during the compression-phase, but in the 'remote collectors' (Figures 11, 13 and 15) which are outside of this region[51,57]. Both of these deductions have been shown to occur by 3 different methods[51,57]. This distinction between adjacent and remote collectors is an important one, and counteracts another argument raised against the hypothesis[102,103], when no concentration difference was found along adjacent collectors. The fact that the adjacent collectors in the jejunum are quite short, so that their impedance to lymph outflow is low, also accounts for the relatively higher efficiencies of the lymphatic system there[99].

The interrelationships of capillaries, channels and lymphatics

In continuous capillaries there appears little doubt that there is continual leakage of proteins from the capillaries. These traverse the channels suffering some molecular sieving[35,49,75,76] and, except for local proteolysis[47], are removed by the lymphatics (Figure

[99] J. R. CASLEY-SMITH, Microvasc. Res., submitted for publication (1975).

[100] J. JONSSON, K.-E. ARFORS and H. C. HINT, in *VI Europ. Congr. Microcirculation, Aalborg* (Karger, Basel 1971), p. 214.

[101] P. C. JOHNSON and D. R. RICHARDSON, Microvasc. Res. *7*, 296 (1974).

[102] N. C. STAUB, G. NICOLAYSEN and A. NICOLAYSEN, in *Proc. V Int. Congr. Lymphology, Buenos Aires and Rio de Janeiro* (Ed. C. A. GRANDVAL; Int. Soc. Lymphology 1975), p. 49, and Microvasc. Res. *10*, 138 (1975).

[103] C. E. VREIM, R. H. DEMLING and N. C. STAUB, in *Proc. V Int. Congr. Lymphology, Buenos Aires and Rio de Janeiro* (Ed. C. A. GRANDVAL; Int. Soc. Lymphology 1975), p. 47.

19). Since they traverse the capillary endothelium via vesicles, and since this is a diffusion process (although in quanta) they must follow the concentration gradient, so that they have to be eventually removed by the lymphatics, together with some fluid. If these do not work, lymphoedema results.

When the filtration from the capillaries increases (e.g. in plasmaphaeresis), the lymphatics certainly do not remove all the fluid. They do, however, to a large extent[104] prevent oedema by removing some of it. Here they are quantitatively unimportant (in that much more fluid still passes to the venous-limbs), but qualitatively vital[4,5,98,104]. The alteration in Starling forces tends to move the zero-flow position too far towards the arterial-limb; the increased activity of the lymphatics restores the balance.

In *fenestrated capillaries* the situation is similar, but more complex. Here there is probably a quantitatively very large local circulation of proteins (and fluid) from the arterial-limb fenestrae to those on the venous-limbs[4,5,35,51], but calculations (Figures 16–18) show that there is also some qualitatively vital removal by the lymphatics[35]. Since the venous-limb fenestrae can only remove proteins in a solution less concentrated than in the adjacent fluid, the absence of lymphatics can be shown to be likely to cause such elevations of the mean protein concentrations in the tissue that the tissue HP will become positive, with resulting oedema[35]. Even the difference between the initial lymphatics concentrating the lymph, or not, will result in the model in a 50% drop in the lymphatic safety factor against oedema[98]. Thus we can see that, in fenestrated regions also, the lymphatics are vital to prevent oedema, although here they only remove a small part of the protein traversing the tissues.

The concentration profiles of protein calculated for the model[35] have been largely confirmed by experiments[51]; they show (Figure 16) that the concentrations in the arterial-limb peri-capillary space are greater than in the blood, fall abruptly across the arterial-limb basement membrane, rise slowly along the tissue channels, have a variable slight rise or fall (according to conditions) at the venous-limb membrane, and in

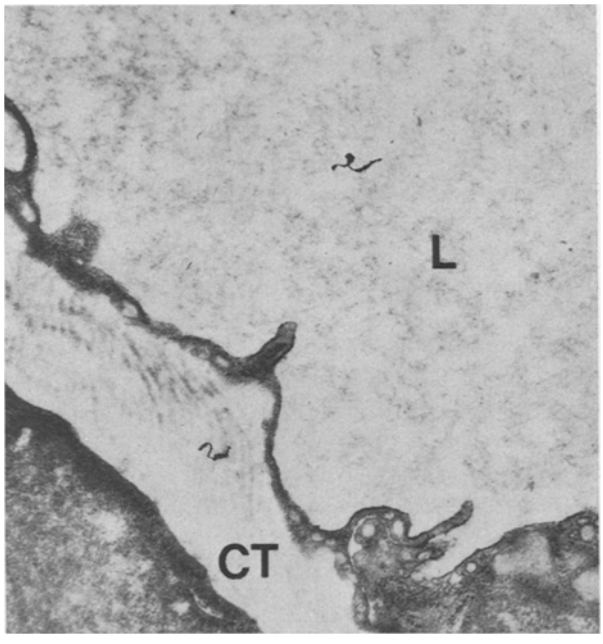

Fig. 15. As for Figure 14, but a remote collector – where the specific activity of the lymph is considerably reduced, as is the mass-density of the protein in it. × 16,000.

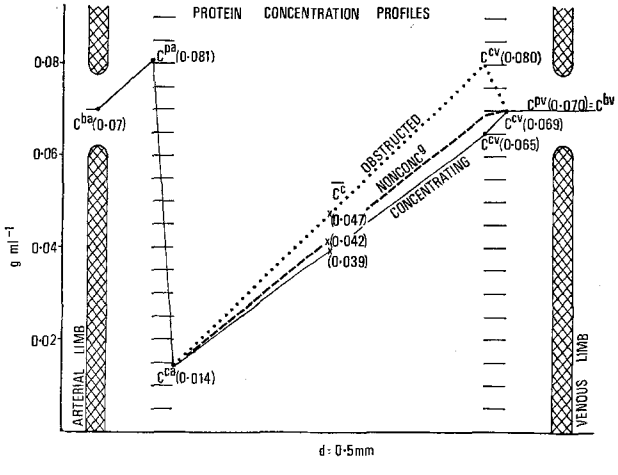

Fig. 16. The results of calculations[35] on the protein concentrations (mg ml^{-1}) at various sites in the cat jejunum. The arterial-limb basement membrane causes a banking-up (C^{pa}), and a sharp fall through it (C^{ca}). There is a gradual rise along the tissue channel to just outside the venous-limb basement membrane (C^{cv}), while concentrations are equal in the venous peri-capillary space (C^{pv}) and the blood (C^{bv}). If the lymphatic system is obstructed, or even just nonconcentrating there is a rise in the mean concentrations of protein in the tissue channels ($\overline{C^c}$). Relatively small as these are, it will be seen, Figures 17 and 18, that they are very important.

[104] M. Földi, *Diseases of the Lymphatics and Lymph Circulation* (Thomas, Springfield, USA 1969).

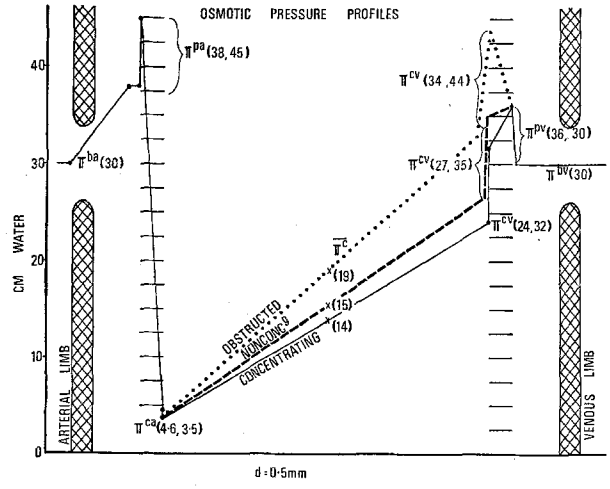

Fig. 17. Here the ECOP's corresponding to the protein concentrations are plotted. 2 pressures are shown in a number of sites, referring to the differently sized pores on their two sides[85].

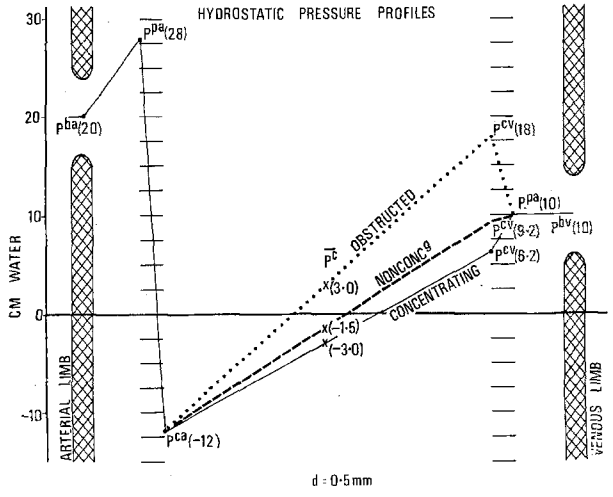

Fig. 18. Hydrostatic pressure profiles are shown, produced by homeostatic mechanisms, from the ECOP's given in the previous figure. It can be seen that if the lymphatics are obstructed the mean HP in the tissue channels ($\overline{Pc}$), which is normally negative, becomes positive – which would imply oedema[93, 94]; even if the initial lymphatics just did not concentrate the lymph, 50% of the lymphatic-safety factor[94] is lost. This shows that although the lymphatics only remove a small amount of the protein (20% in this model), this amount is vital to prevent oedema.

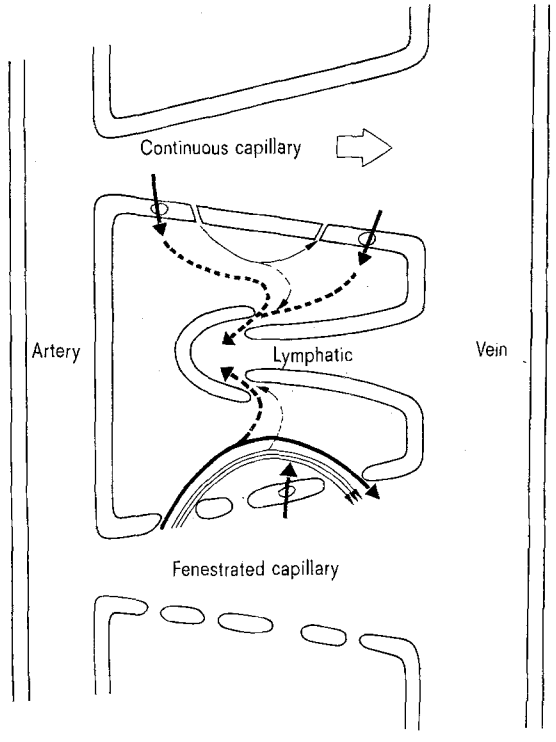

Fig. 19. This diagram illustrates the roles of the lymphatic system[4, 5] in regions with continuous (top) and fenestrated (bottom) capillaries. In the first all the protein (apart from that lysed in the tissue[47]) is removed by the lymphatics after it has crossed the capillary wall in vesicles and traversed the tissue channels; most of the water, having passed out of arterial-limb close junctions, returns via venous-limb ones. In the fenestrated region there is a very considerable local circulation of water and protein, via the fenestrae, from the arterial limbs to the venous limbs. Some of this, however, is still removed via the lymphatics. This is quantitatively small, but qualitatively vital.

the venous-limb peri-capillary space are equal to that in the blood. These varying concentrations give rise to varying ECOP's (Figure 17) which, in turn, probably cause varying HP's (Figure 18) by local homeostasis.

The evolution of the fenestrae and the lymphatics

In the previous sections we have seen the different, although similar roles, played by the fenestrae and the lymphatics. It is interesting to trace their evolution. In primitive vertebrates and invertebrates the junctions of the blood vessels are largely open[105]. With increasing development they became more and more closed, centripetally. In the invertebrates the junctions which control permeability are outside the basement membranes; in the vertebrates they are inside. The increasing size of the animals and their increasing activity necessitated higher blood pressures, which needed higher plasma protein levels to stop excessive fluid outflow. These in turn demanded more closed junctions, at first centrally where the HP's are high, then peripherally. Because of vesicular protein leakage, some return mechanism was necessary. At first this was open, or openable, vascular junctions (as in the elasmobranch[106]). When these were no longer enough, a separate lymph system evolved, with lymph hearts or – later and better – lymphangions to propel the lymph and cause it to re-enter the blood. However the need for high local fluid and protein flows also developed (or remained) in the viscera. Fenestrae seem not to have evolved in the hagfish[107] but are extensive in the elasmobranches which also retain openable venous junctions in their body walls[106]; while the true lymphatic system is present in the teleosts[63, 108].

Conclusion

In both continuous and fenestrated regions the lymphatic system removes the excess proteins (Figure 19), thus avoiding their stagnation, and oedema. In the fenestrated regions there is probably also a large local circulation from the arterial-limb fenestrae to those on the venous-limbs, where they are taken up by a similar mechanism to that operating in the initial lymphatics, except that the continuous flow of blood obviates the need for periodic reconcentration of the plasma as occurs with the lymph in the lymphatics. It is uncertain whether the fenestrae developed because of the need for extra protein passage to, and removal from, the tissues, or because of the need for extra fluid flow.

[105] J. R. CASLEY-SMITH, Lymphology *4*, 49 (1971).
[106] J. R. CASLEY-SMITH and P. E. MART, Experientia *26*, 508 (1970).
[107] J. R. CASLEY-SMITH and JUDITH R. CASLEY-SMITH, Revue Suisse Zool., *82*, 35 (1975).
[108] O. F. KAMPMEIER, *Evolution and Comparative Morphology of the Lymphatic System* (Thomas, Springfield, USA 1969).