Functional Fine Structure

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In recent years the electron microscope, and tracer techniques associated with it and interpreted through information gained from it, have added immeasurably to our understanding of the functioning of the whole blood vascular-tissue-lymphatic system. Perhaps the most important finding which has emerged is that what used to be thought of as three separate systems are now becoming increasingly revealed as merely three subsystems of a single system: the state of any one part can affect all the others. The blood vascular system[1–9], the interstitial tissue[3,4,10–12] and the lymphatic system[2,3,13,14] have all been recently reviewed. While there are still considerable differences of opinion and many investigations waiting to be performed, there are substantial areas of agreement. What follows attempts to present some of these differences, but is of necessity brief and somewhat didactic; more details are presented in the reviews.

Blood vessels

While a few workers[6] consider that both the small pores of PAPPENHEIMER[15] and the large pores are represented by continuous chains of vesicles crossing the endothelium, most now consider that, in continuous capillaries, the small pores are the 'close' junctions, where there is a gap of ~6 nm between the adjacent endothelial cells. Recent quantitative work[5] has shown that these occupy ~7% of the intercellular junctions and that, using the normal laws of physics, the dimensions of these slits can be used in Poiseuille's and Fick's equations to yield results for vascular permeabilities very close indeed to those obtained by physiologists working with whole regions. It is usually held[2,3,6] that macromolecules are transported across the endothelium via numerous small vesicles (~50 nm diameter), which are moved randomly by Brownian motion: this is diffusion in quanta. There is considerable evidence for this, both from electron microscopical tracer experiments and from physiological work with whole legs (see reviews and[16–18]). It appears, however, that the smaller macromolecules sometimes pass through the junctions and sometimes via the vesicles[18]. The vesicular system is quantitatively quite unimportant for the crystalloids, but accounts for the slow leakage of proteins from continuous capillaries.

Not all blood capillaries are continuous: many, especially in the viscera possess holes or 'fenestrae' (~50–100 nm diameter), which are predominantly on their venous limbs. There is increasing evidence[2–4] that these permit a considerably greater turn-over of both small and macro-molecules through the tissues, and also permit most macromolecules which originate in such a tissue to pass directly to the blood rather than indirectly via the lymphatics. The interrelationships of these two systems will be discussed later. While the fenestrae and the basement membrane seem to act as the large pore system, it is very probable that they are far too permeable to control the passage of small molecules through the wall of fenestrated capillaries. This is likely to be a function of the channels through the interstitial tissue[4]. A hypothetical analysis by INTAGLIETTA and DE PLOMB[12] showed the difference between 'tube-capillaries' where the pores in the wall controlled small molecular passage, and 'tunnel-capillaries' where the wall was so permeable that this role passed to the surrounding tissues. Evidence has since been presented indicating that fenestrated capillaries are tunnel-capillaries and that in the tissues which possess many of them it is the permeability of the tissues which is being measured[4]; quantitative morphology plus this analysis yields the same values for permeability as are obtained by physiologists[4]. Injured blood vessels are a special case where vessels which are normally tube-capillaries (which term includes the post-capillary venules) are temporally made

[1] M. J. KARNOVSKY, in *Capillary Permeability* (Eds. C. CRONE and N. A. LASSEN; Academic Press, New York and London 1970), p. 341.
[2] J. R. CASLEY-SMITH, in *The Inflammatory Process* (Ed. B. W. ZWEIFACH; Academic Press, New York and London 1973), vol. 2, p. 161.
[3] J. R. CASLEY-SMITH, in *The Microcirculation* (Eds. G. KALEY and B. M. ALTURA; University Park Press, Baltimore, USA 1976), in press; and Experientia *32*, 1 (1976).
[4] J. R. CASLEY-SMITH, P. J. O'DONOGHUE and K. W. J. CROCKER, Microvasc. Res. *9*, 78 (1975).
[5] J. R. CASLEY-SMITH, H. S. GREEN, J. L. HARRIS and P. F. WADEY, Microvasc. Res. *10*, 43 (1975).
[6] N. SIMIONESCU, M. SIMIONESCU and G. E. PALADE, J. cell. Biol. *57*, 424 (1973).
[7] N. SIMIONESCU, M. SIMIONESCU and G. E. PALADE, J. cell. Biol. *64*, 586 (1975).
[8] B. W. ZWEIFACH, in *The Inflammatory Process* (Ed. B. W. ZWEIFACH; Academic Press, New York and London 1973), vol. 2, p. 3.
[9] W. J. CLIFF, *Biological Structure and Function of Blood Vessels* (Cambridge University Press 1976), in press.
[10] T. C. LAURENT, in *Capillary Permeability* (Ed. C. CRONE and N. A. LASSEN; Academic Press, New York and London 1970), p. 341.
[11] T. C. LAURENT, Pflügers Arch. Suppl. *336*, 21 (1972).
[12] M. INTAGLIETTA and E. P. DE PLOMB, Microvasc. Res. *6*, 153 (1973).
[13] J. RUSZNYAK, M. FÖLDI and G. SZABO, *Lymphologie, Physiologie und Pathologie der Lymphgefässe und des Lymphkreislaufes* (Gustav Fischer Verlag, Stuttgart 1969).
[14] J. M. YOFFEY and F. C. COURTICE, *Lymphatics, Lymph and the Lymphomyeloid-Complex* (Academic Press, London and New York 1970).
[15] J. R. PAPPENHEIMER, Physiol. Rev. *33*, 387 (1953).
[16] M. PERRY and D. GARLICK, Microvasc. Res. *9*, 119 (1975).
[17] R. D. CARTER, W. L. JOYNER and E. M. RENKIN, Microvasc. Res. *7*, 31 (1974).
[18] E. M. RENKIN, R. D. CARTER and W. L. JOYNER, Microvasc. Res. *7*, 49 (1974).

into tunnel-capillares by the opening of many of their intercellular junctions[19]. When these close again the tube-capillary state is resumed once more. However, even then there is still an increased permeability to macromolecules[17,18], which is probably due to the presence of many large vacuoles (~200 nm diameter) in the endothelium, rather than to any alteration in the small vesicles[19].

Interstitial tissues

Relatively little is known about this from a structural viewpoint, but it appears that this usually consists of a relatively course mesh of collagen fibres, together with a finer system of fibrils (perhaps protocollagen) all embedded in a gel-sol mixture of mucopolysacharide ground substance. It is now often considered that there are actual channels through the ground substance. Measurements and calculations from physiological data indicate that their mean diameters are 60–120 nm in muscle and the intestine[4]. It was also suggested that these channels were preferentially oriented in relation to the vessels, but much more work needs to be done in these areas, especially in relation to variation with the site, and with physiological and pathological conditions.

Prelymphatics

One frequently sees channels of protein, or other marker, ending at a lymphatic endothelial junction, and it would seem that these are part of the interstitial system of channels, all over the body. There are some specialized tissues where there is no tissue movement to cause normal lymphatics to function and, thus, where they do not exist: the brain, retina and bone marrow. In these regions the tissue channels are still vital for drainage[20] and become a tributory system of continuous spaces, and potential spcases, reaching from the depths of the tissue to outside the boney case. They occur in the basement membrane region of the smaller vessles and the adventitia of the larger ones, eventually draining into the true lymphatics which occur in the vascular adventitia outside the skull. Thus instead of being only some 10–100 μm long, these systems of channels are up to 20 cm. So important are these non-endothelialized 'vessels' in the drainage of the brain etc. that, while they are similar to and continuous with the other interstitial channels in the body, they are often called 'prelymphatics'.

Lymphatics

A small proportion of the intercellular junctions of the initial (terminal, capillary) lymphatics can open widely. This is the way that almost all material enters these vessels. There are many connective tissue fibrils attached to the endothelium so that during oedema, when the tissues are stretched, the initial lymphatics are held open, allowing the raised tissue hydrostatic pressure to force fluid etc. into them. How they fill in most tissues[2,3] under normal condition is, however, much more difficult to understand.

The hydrostatic pressure in the initial lymphatics is almost certainly atmospheric or slightly above; the tissue hydrostatic pressure is now often held to be negative in many tissues. A possible explanation for lymphatic filling under these conditions, for which there is increasing evidence[2,3,21,22], is that it occurs due to raised colloidal osmotic pressure of the proteins in the initial lymphatics. It has been shown that the slowly diffusing macromolecules can exert considerable pressures even in the presence of very large pores, and there is theoretical and experimental evidence that the fluid which flows in because of this will cause more proteins to enter with it thus replacing those which are forced into the collecting lymphatics during tissue compression. It is suggested that fluid is forced out of the junctions, which act as flapvalves and are sealed to macromolecules, during tissue compression. (This is possible because it is the raised total-tissue-pressure which is transmitted to the lymph via the endothelium, and this is much greater than the tissue hydrostatic pressure.) Thus the remaining lymph becomes concentrated and more fluid, and protein, flows in via the newly opened junctions as soon as the compression is relaxed. This hypothesis has been attacked[23] because it was held that the fluid forced out would merely dilute the proteins in the adjacent tissues and so the inflowing fluid would be more dilute than normal. This suggestion overlooked the facts that there are many other junctions (away from the openable ones) through which fluid could also leave, and the presence of the protein in tissue channels. The walls of these are relatively impermeable to protein, but readily so to water. Hence the proteins in the channels adjacent to the initial lymphatics should retain their normal concentrations inspite of the outrush of small molecules.

As the lymph passes along the collecting lymphatics, the macromolecules are retained by the endothelial junctions becoming all closed, although the small molecules can probably exchange quite readily[13,14]. This would cause the concentrated lymph to rapidly be diluted again, but the extent of this would no doubt depend on the hydrostatic pressure exerted by the contracting collecting lymphatic walls, and the relative macromolecular concentrations outside the vessels. It is likely that a considerable exchange of both pro-

[19] J. R. Casley-Smith and J. Window, Microvasc. Res., submitted for publication (1976).
[20] J. R. Casley-Smith, E. Földi-Börcsök and M. Földi, Br. J. exp. Path., in press (1976).
[21] J. R. Casley-Smith, Microvasc. Res., in press (1976).
[22] W. Perl, Microvasc. Res. *10*, 83 (1975).
[23] A. E. Taylor, W. M. Gibson, H. J. Granger and A. C. Guyton, Lymphology *6*, 192 (1973).

tein and fluid also occurs in the lymph nodes, but some controversy exists about the net direction of both of these.

Blood-lymph interrelationships

In regions with only continuous capillaries, apart from protein which is normally lysed in the tissues and which may be up to about a third of that which leaves the blood vessels (vide infra), the remaining protein which leaves them is returned via the lymphatic system. If this does not function properly considerable oedema results. A relatively small amount of fluid is also returned, but this amount is still very important in avoiding tissue oedema[2,3,23].

In regions where there are many fenestrated capillaries, it is becoming increasingly probable that there is a considerable circulation of plasma proteins, through the tissues, which re-enter the blood via the fenestrae on the venous side of the circulation[2,3,4,12]. It appears, however, that the lymphatics in this situation still are vital for the avoidance of oedema[24]. The amount of protein they remove may only amount to about 10–30%, but because this is in a concentrated form the mean concentration in the tissues is considerably reduced, and with it the colloidal osmotic pressures and tissue hydrostatic pressures. It appears that this reduction is essential for the avoidance of a mean positive tissue pressure and oedema.

Evolution

Primitive chordates have continuously lined great vessels, but the endothelial cells gradually become further and further apart in the smaller vessels, until even the basement membranes of the vessels may partly disappear and the tissue spaces are directly continuous with the blood vessels[2,3]. The higher animals have more continuous endothelial vascular linings, until in the elasmobranch the venous intercellular junctions in the body wall can still be opened by muscular movements, but in the viscera fenestrated capillaries appear, allowing macromolecular uptake there. In the teleosts the increased blood hydrostatic pressure no longer allows any blood vascular intercellular junctions to be openable, but also necessitates a raised plasma protein concentration. A separate lymphatic system now develops, which is filled by variations in the solid tissue pressure, but which propels the lymph and discharges it into the blood by means of the contractions of muscle in its walls. Thus, aside from part of the raised protein circulation through the tissues in fenestrated regions, protein is now returned to the blood via a separate system of pumps – the initial lymphatics, the segments in the collecting lymphatics and the lymph hearts, when these exist.

[24] J. R. CASLEY-SMITH, Microvasc. Res., submitted for publication (1976).

Active Contractility of the Lymphangion and Coordination of Lymphangion Chains

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A 'lymph drainage capable of compensation' (FÖLDI[1]) is always based on the functional interplay between differentiated lymph drainage mechanisms, and specially effective importance is shown to be attached to the vasomotoric lymph drainage[2]. The initial lymphatics do not show any active contractions because of lack of muscle in their walls. The lack of that autorhythm of course does not mean the lacking of contractility. First information about open junctions in the lymphatic capillaries is given by CASLEY-SMITH and FLOREY[3]. My collaborator SCHIPP[4] observed in the peripheric lymph vessels intraendothelial filaments, which could be interpreted in the sense of a contractility of the endothelium. SCHIPP and SCHÄFER[5] found no open junctions inspite of experimental lymphostasis. LEAK[6] postulated in his studies on the permeability of lymphatic capillaries, that these small vessels are able to open and close the interendothelial cell joints by active contraction an relaxation of the endothelium. The larger musculous lymphatic vessels have a pronounced autorhythm. From the morphological point of view, the lymphatic vessel is characterized by its segmentation; it consists of valve segments with central muscle collars and directed multiple innervation of the smooth muscles. Physiologically the valve segment, we called it lymphangion[7], is an autochthonous efficiency element with its typical action potential[8]. The in vivo experiments of SMITH[9] and our own in vitro experiments demonstrated that the single

[1] M. FÖLDI, in *Handbuch der Allgemeinen Pathologie* (Springer Verlag, Berlin, Heidelberg, New York 1971), vol. 3/6, p. 329.
[2] H. MISLIN, in *Handbuch der Allgemeinen Pathologie* (Springer Verlag, Berlin, Heidelberg, New York 1971), vol. 3/6, p. 219.
[3] J. R. CASLEY-SMITH and H. W. FLOREY, Q. Jl. exp. Physiol. *46*, 101 (1961).
[4] R. SCHIPP, Acta anat. *71*, 341 (1968).
[5] R. SCHIPP and A. SCHÄFER, Zool. Anz. Suppl. *33*, 407 (1969).
[6] L. V. LEAK, J. Cell Biol. *50*, 300 (1971).
[7] H. MISLIN, Experientia *17*, 19 (1961).
[8] H. MISLIN, *Lymphographie und Pharmakolymphographie* (Gustav Fischer Verlag, Stuttgart 1975), p. 10.
[9] R. O. SMITH, J. exp. Med. *90*, 498 (1949).