The connective tissue space in view of the lymphology

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1. *The Ludwig-Starling theory*

For its examination of the origin of the lymph fluid, modern lymph research is characterized by having enormously promoted the knowledge about the blood microcirculation: it did less, however, for the comprehension of the lymphogenesis. Methodological difficulties are obvious in the handling, even today, of this still somewhat mysterious system.

Carl Ludwig (1861) with the intent to elucidate lymphogenesis, was the first to recognize the hydrostatic capillary pressure as an essential force for the outward movement of blood fluid. This concept finally led to the fundamental formulation of the pressure components responsible for the effectltration pressure after Starling (1896), who with ingenious cognition, had perceived the second important force in the colloid-osmotic pressure:

$$P_{eff} = P_c - P_t - II_{pl} + II_t$$

(P = hydrostatic pressure, II = colloid osmotic pressure, c = capillary, pl = plasma, T = tissue)

2. *The protein permeability*

This relation or interaction of pressure forces guarantees the fluid balance in the extravascular space under physiological conditions, but only if no fluid drainage by the lymphatic system occurs, as was indeed recognized by Starling. However, this has been misunderstood or wrongly interpreted by numerous investigators. They concluded – as can be still read today in many publications – that the fluid drainage by the lymph system can be neglected quantitatively. This is an inadmissible generalization of a special situation which is found or is assumed to exist only in certain body regions where there happens to be hardly any protein permeation through the microvessel wall. Here, any protein leakage must diminish the colloid osmotic pressure of the plasma and therefore the removal of fluid. The consequence would be the edema formation. Under physiological conditions, therefore, an impermeability for proteins is postulated based on the semipermeable properties of the capillary wall. This concept has found also experimental confirmation, especially by the studies performed by the Pappenheimer group using the isogravimetric method in isolated organs of mammalians. This method, however, was not sensitive enough to detect the protein permeation and, furthermore, the lymph drainage was considerably impaired in isolated organs.

This was the starting point for our own investigations keeping in mind the importance of the lymph drainage for the maintenance of the fluid equilibrium in the tissue spaces. By means of fluorescent dye-marked proteins, we were able to show that a protein permeation of the microvessels exists and therefore the semipermeable nature of the capillary wall on principle is contradicted experimentally; body regional differences of the permeation of macromolecules across the microvessel wall remain to be considered. These findings agree with results obtained by Mayerson et al. (1960) who calculated the lymph-plasma quotient for albumins. In contrast to this indirect method, the vitalmicroscopical technique we used permits additional localization of the protein passage, whereby maximum permeation was found along the venous part of the microvasculature in the venous branch of the capillaries, the venules and in small collecting veins. These results demonstrate also that the true capillaries represent only a small part of the microvessels characterized by substances and fluid exchange, and furthermore that a uniformity of the wall properties does not exist. The highest wall permeability seems to exist in the venous microvasculature and, consequently, this region should be very important for the lymphogenesis. The body regional differences of the permeability for macromolecules again indicate a similar difference concerning the importance of the lymphdrainage for the maintenance of the fluid balance.

In these investigations also a study of the transinterstitial movement of the marked fluid and its appearance time inside the lymphatics and larger lymph vessels was possible. Approximately 15 min after the i.v. injection of the fluorescent dye the larger lymphvessels were visible. This time was considerably shorter after increase of the permeability by histamin, serotonin or bradykinin and after venous congestion. The time was prolonged with diminished wall permeation observed in older animals and also after thrombin infusion with subsequent defibrination of the blood (Hauck and Schröer, 1965).

3. *The elastic fibers and the 'low resistance pathway'*

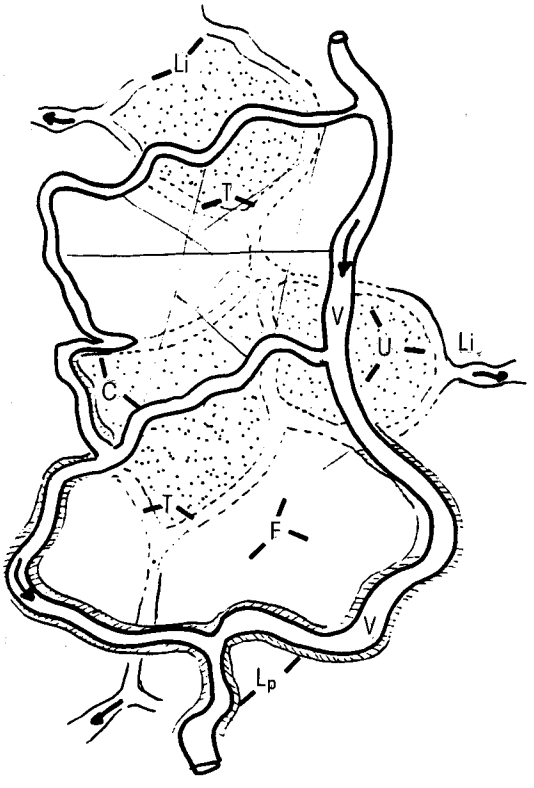
After the passage of fluorescence dye through the microvessel wall in the mesentery of the rabbit and cat, a typical fluorescent pattern of linear structures in the extravascular space was visible. Confirmed by studies using the incomplete dark field transillumination, the network pattern is formed mainly by the elastic fibers of the interstitial connective tissue. This also could be verified by means of the cathodoluminescence technique in the scanning electronmicroscopy (Hauck et al., 1978). The studies suggest the existence of a preferred fluid pathway along the surface of the elastic fibers – marked by a fluorescence halo along the fibers; this is also confirmed by the catholuminescence studies.

The collagen fibers do not exhibit these properties. Model experiments performed on gel with embedded fibrous material confirmed a preferred fluid movement along the artificial fibers. The nature of the mechanism is still not clear, but the transport occurs much faster than is possible by diffusion. The halo phenomenon along the single fibers (or bundle of fibers) can be interpreted as diffusion event from the fibers into the surroundings. So the elastic fibers, besides fulfilling their mechanical function, also have a passive transport or 'guide rail' function for fluid and solved molecules between microbloodvessels and drainage system (fig.) marking the 'low resistance pathway'. This has already been described by Kihara (1956) as an extravascular fluid pathway. Diminution of the elastic fibers as one finds in athropic changes of the skin leads to considerable slowing of the spreading of fluorescent dye in the tissue (Jäger et al., 1979).

4. *The prelymphatic tissue channels*

To the preformed system favoring the fluid drainage also belongs a network of prelymphatic tissue channels demonstrated in our own investigations using the incomplete dark field transilluminations in the mesentery of the rabbit and cat. Tissue channels were described recently also by Casley-Smith (1976) by means of electronmicroscopy. The diameter of these structures ranges between 0.1 and 10 µm and they do not show a microscopically recognizable wall structure. The beginning of a wall formation can be well marked microscopically by the dark field technique. The prelymphatic and initial lymphatic systems are continuous-

ly connected, forming a common converging drainage system to the larger lymph transport vessels. The results suggest the interpretation that the most peripheral part of the lymphvessel system is a completely open system of tissue channels. The traditional concept of the blind end - or beginning - of the lymphatics seems to be mainly the consequence of the retrograde filling methods used by the morphologists. The most peripheral parts are not reached in this way. Actually, the lymphatics diameters do not seem to be much bigger than those of blood capillaries, but they are essentially more extensible.



Semi-schematical diagram of the organization of the extravascular space in view of the tissue supply and drainage.

C, Capillaries; V, venules and small collecting veins; L_p, paravascular lymphatics or tissue channels; L_1, initial lymphatics; T, tissue channels; F, elastic fibers; U, tissue unit; scale = 10 µm.

The connection between tissue channels and lymphatics was mainly found in the paravascular region of the venous microvasculature, characterized by their high permeability for proteins (fig.). Here, the macromolecules can be taken up over a short distance directly and quickly into the lymph fluid coming from the capillary area. In the mesenteric area we could not observe any removal of proteins into the venous blood stream from the extravascular space. We assume, therefore, that the return of proteins into the blood in such regions occurs by the lymph - but not generally via ductus thoracicus. The close neighborhood of the venous microvasculature and tissue channels or initial lymphatics lets us take into account also direct connections between both systems. Corresponding observations could be made after artificial haemorrhages into the mesenteric tissue.

5. *The tissue unit*

As has already been mentioned, our studies suggest that the most peripheral part of the drainage system, forming a network pattern and converging to the initial lymphatics, represents the primary or elementary drainage system. This seems to be the beginning of a canalization system for fluid drainage whereby the tissue is divided by the tissue channels in elementary three-dimensional tissue units (fig.). The pattern, found by application of the non-invasive dark field transillumination, agrees with the findings obtained by intermesothelial injection of India ink suspension or fluorescein-isothiocyanate. The extension of the single units seems to be dependent on the local metabolism activity of the tissue. Measurements were performed by means of automatic video analysis with the Texture-Analysis System by Leitz. A diameter of about 50 µm was found near the intestinal wall. More peripherally, the diameter increases. The fields represent a unit drainage by the surrounding tissue channels.

So far our concept of the tissue organization for supply and drainage of this is essentially marked by the following preformed elements: microvessels, elastic fibers, tissue units, tissue channels, initial lymphatics (fig.). Our results could represent an experimentally confirmed part of the tissue unit, already defined by Letterer (1953) and nominated as histion.

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