Mechanisms Causing Initial Lymphatics to Expand and Compress to Promote Lymph Flow*

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Summary. Microlymphatics can be devided into two segments, initial lymphatics which are made up of irregular tissue crevices lined by a continuous attenuated endothelium, and collecting lymphatics with a smooth muscle media and the ability for spontaneous contractility. Virtually the entire array of mammalian organs with lymphatic drainage have initial lymphatics which are drained by collecting lymphatics, but in organs like skeletal muscle and intestine almost all lymphatics are of the initial type, and the muscular collecting lymphatics arise only outside the organs per se. How can interstitial fluid find its way into the sparsely positioned initial lymphatics? Initial lymphatics exhibit no detectable contractile activity. Their endothelium shows incomplete attachment between neighbouring cells, providing a mechanism to open and close lymphatic endothelial microvalves along the walls of the initial lymphatics. Current evidence suggests that lymph fluid formation in the initial lymphatics requires periodic expansion and compression of the initial lymphatics. Expansion of the initial lymphatics causes filling by percolation of interstitial fluid across the open endothelial microvalves. Compression causes closure of the endothelial microvalves and outflow along the lumen of the microlymphatics with eventual transport into collecting lymphatics, towards the nodes and into the thoracic ducts. This report is dedicated to Professor M. KOTANI. I am deeply honored to participate in the celebration of this imminent scientist and highly admired teacher. Professor KOTANI has covered in his long scientific record an amazing range of topics. Outstanding and numerous are his contributions to lymphocyte immunology, endocrinology, histochemistry, ultrastructure and biomagnetism. Fortunately he has also expressed strong interest over many years in lymphology and has made many pioneering contributions (see for example the following publications: KOTANI ET AL., 1968, 1977, 1979, 1980, 1982; EKINO, MATSUNO AND KOTANI, 1979).

Professor KOTANI has been especially interested in lymph transport, cell migration and permeability. Lymphatic transport depends on continuous collection of interstitial fluid and fluid movement along its delicate network of endothelialized channels, past the nodes, and via the lymphatic ducts back into the circulation. The process is life sustaining and the identification of its transport mechanisms is a basic problem in lymphology. It will be the focus of this discussion.

The vascular-tissue exchange process starts with filtration across the vascular barrier, passage along an interstitial space with preferential pathways but without guidance along cellular pathways, and eventual entry into a set of sparsely dispersed initial lymphatics. From here on the fluid passage is restricted to lymphatic vessels, it follows through contractile lymphangions towards the lymph nodes and via the central lymphatics ducts back into the vasculature. A large volume of fluid passes through the lymphatics every day, but in a way that depends on physiological activity of the individual organs. During rest low levels of lymph flow exist which can be enhanced by orders of magnitude during daily activity and exercise. What are the mechanisms that control this lymph activity? How is fluid collected into the initial

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lymphatics?

The fluid transport across the vascular wall into the interstitium can be attributed to Starling pressures. This has been subject of numerous investigations. The transport along the contractile segment of the lymphatics is achieved by the coordinated sequence of lymphatic smooth muscle contraction to propel fluid and by closure of valves to prevent reflow. This lymph transport mechanism derives its energy from muscle contraction. But as we will see in the following discussion, the majority of mammalian organs do not possess a contractile initial lymphatic system. That observation raises a more focused question: How does interstitial fluid find its way into the initial lymphatics? Several specific proposals have been advanced, in form of an osmotic pump (Casley-Smith, 1977), a hydrostatic retrograde pump (Reddy, Kronskop and Newell, 1975), pulse pressure (Parsons and McMaster, 1938) or vasomotion (Intaglia et al., 1982; Skalak, Schmid-Schönbein and Zwifach, 1984), but none can enjoy today general acceptance. Most investigators acknowledge that gentle tissue massage, walking or muscle contraction, respiration or intestinal motility leads to lymph formation. But no integrated view of lymphatic dynamics has been generated from these observations.

The starting point for dealing with lymphatic transport is the microanatomy, in as much as a clear picture of the parenchymal arrangement around the lymphatics will by itself support or negate a proposed mechanism. In the following we will present a summary of the architecture of microlymphatics in different organs and then discuss a mechanism of lymph formation in skeletal muscle.

**LYMPHATIC MICROANATOMY**

In order to discuss the lymphatic microanatomy it is convenient to divide the lymphatics into two large groups: the initial lymphatics and the collecting lymphatics. Initial lymphatics have a single endothelial lining but have no smooth muscle in their wall. Initial lymphatics may or may not have valves. In contrast, collecting lymphatics have a smooth muscle media and they generally have valves. Collecting lymphatics are frequently arranged in form of a chain of lymphangions, each lymphangion consisting of a contractile compartment and valve. During a recent

![Fig. 1. Initial lymphatic in the rabbit omentum with a single attenuated endothelium. The initial lymphatics have larger lateral dimension than the adjacent vasculature but are thin and easily collapsible. Infusion of albumin conjugated with Evans blue (right side) leads to diffusion across the lymphatic endothelial barrier into the interstitium. From Zweifach and Schmid-Schönbein (1985).](image)
review of the microanatomy of lymphatics in different organs (SCHMID-SCHÖNBEIN, 1989) it became apparent that the majority of organs have a noncontractile initial lymphatic system (Fig. 1). In fact, in most organs, like the heart, skeletal muscle, intestine, appendix, skin, lung, kidney, and others, the initial lymphatic system extends over all or major parts of the organ. In these organs we have currently no anatomical or functional evidence that the initial lymphatics are contractile via a mechanism that is intrinsic to the lymphatic endothelium and comparable to smooth muscle contraction in arterioles or elsewhere. Yet lymph fluid that is formed in the initial lymphatics is carried into collecting lymphatics and toward the nodes and central lymphatics by peristaltic contractions of smooth muscle in the collecting lymphatics. It is interesting to note that according to our current assessment, the bat wing is the only tissue with blind lymphatic endings that are contractile and endowed with a smooth muscle media (WEBB and NICOLL, 1944; NICOLL and TAYLOR, 1974). The bat wing stands in contrast to other organs in this important microlymphatic aspect. Furthermore the wing of the bat stands in contrast to the lymphatics in other organs of the same creature which consist of noncontractile initial lymphatics (AZZALI et al., 1988).

Many ultrastructural features of initial lymphatics in different organs and different mammals closely resemble each other. The lymphatic endothelium is highly attenuated, it rests on connective tissue, and has only a discontinuous basement membrane with large openings. The endothelial cytoplasm contains numerous vesicles, Weibel-Palade bodies, endoplasmic reticulum, centrioles, some microtubules and other cytoplasmic organelles. In contrast to vascular endothelium with a continuous tight junction region between cells, lymphatic endothelium has a discontinuous ring of adhesion complexes around individual cells and exhibits flap like cell junctions. These junctions can open during stretch of the lymphatics and during influx of interstitial fluid into the lumen (Fig. 2) (CASTENHOLZ, 1984), while anchoring filaments keep the endothelial cells tightly attached to the adjacent collagen network (LEAK and BURKE, 1966). The lymphatic endothelial flaps can be opened to dimensions of several micrometer and therefore exhibit little if any molecular selectivity. Edema in the initial lymphatics serves to open the endothelial microvalves (CASLEY-SMITH, 1977). Reflux of lymphatic fluid into the interstitium may be prevented by closure of the endothelial flaps. Initial lymphatics in the tissue parenchyma generally have rather irregular channel geometries to the point of partial or complete lumen closure. There is no evidence, however, that the initial lymphatics are contractile. The lack of lymphatic smooth muscle in these lymphatics poses an important question: What mechanism causes fluid filling of initial lymphatics?

INITIAL LYMPHATIC EXPANSION AND COMPRESSION

In the past major emphasis has been placed on pressure considerations to answer this question. Several attempts have been made to document a hydrostatic pressure drop from the interstitium into the initial lymphatics (CLOUGH and SMAJE, 1978; LEE, 1986; SKALAK et al., 1984). But no clearcut conclusion could be drawn that a positive pressure drop exists from the interstitium into the initial lymphatics. Interstitial pressures and pressures in the initial lymphatics are similar and in several studies the average lymphatic pressure was found to be a few cmH₂O higher (CLOUGH and SMAJE, 1978; LEE, 1986). Two issues need to be considered in regards to such experiments. First, even an optimistic order of magnitude estimate of the lymphatic aspiration pressure (with a single lymphatic surrounded by a large permeable tissue cylinder) indicates that only about 0.1 cmH₂O below the interstitial pressure is necessary to cause fluid percolation into the initial lymphatics (SCHMID-SCHÖNBEIN, 1990). In many situations even smaller aspiration pressures are sufficient for lymph filling. Second, whole organ experiments suggest that periodic pressures may lead to lymph formation. Micropressure measurements may not have been designed to document dynamic pressure variations in initial
lymphatics, (a) because the tissues have been immobilized to allow faithful pressure readings, which in turn depresses most periodic lymph pump mechanisms, and (b) the frequency response of many micro-pressure recorders may only marginally be sufficient to record dynamic pressure histories.

Instead of pursuing the question of mechanism exclusively by a pressure consideration, we like to pursue here another approach of investigation. What tissue deformation could lead to periodic expansion and compression of the initial lymphatic chalells? The idea is as follows: expansion of an initial channel causes filling from the interstitium since reflux within lymphatic channels is prevented by valves, whereas compression causes outflow of lymph fluid centrally towards the lymph nodes. Since the arrangement of parenchymal tissue surrounding the initial lymphatics is organ dependent, the answer to the mechanism of periodic lymphatic expansion and compression must be organ dependent.

**SKELETAL MUSCLE**

In rat skeletal muscle all lymphatics are of the noncontractile initial type throughout the perimysial spaces. The lymphatic channels closely follow the course of the arcade arterioles (Fig. 3), often accompanied by a nerve, mast cells and multiple collagen bundles. The entire vessel arrangement is deeply embedded in multiple muscle layers (Fig. 4). In the region of the rat spinotrapezius muscle the initial lymphatics continue further outside the organ along the feed arteries (Fig. 5). They remain positioned in the adventitia, they have no intrinsic smooth muscle and exhibit no spontaneous contractility. Occasionally larger veins may be accompanied by a lymphatic. Furthermore no lymphatic channels can be detected in the narrow capillary space of skeletal muscle.

The specialized arrangement of lymphatico-arteriolar pairs suggests that at least two mechanisms of lymphatic compression and expansion may operate. One is due to deformation of the arteriole, the other one is due to the skeletal muscle fibers deformation during contraction. If the arteriole contracts during vasomotion or is subject to elastic recoil from a transmural pressure reduction (like during transition from systole to diastole) the adjacent lymphatic channel is expanded and filled with fluid from the adjacent interstitium. Vice versa, if the arteriole expands the adjacent lymphatic is compressed and fluid is propelled outwards into the collecting lymphatics. In addition, if the surrounding muscle fibers contract or are subject to passive stretch, the lymphatics are subject to a volume compression.

We have tested these two possibilities in a series of experiments. In the first experiment the resting spinotrapezius muscle of the rat was suffused with norepinephrine to constrict the arterioles, then fixed and the lymphatic volume was estimated from cross-sectional area measurements of thin sections. The experiment was repeated in a similar fashion after dilation of the arterioles with papaverine. This experiment showed that during contraction of the arterioles...
ioles the lymphatics are expanded, and they are compressed after dilation of their paired arterioles (SKALAK, SCHMID-SCHÖNBEIN and ZWEIFACH, 1984). In the second experiment the arterioles were unstimulated and dilated, but instead the spinotrapezius muscle fibers were passively stretched over their resting length by an external tensile stress, or actively shortened by muscle stimulation. Under these conditions the lymphatic crossectional area measurements also indicate an initial lymphatic volume change. During stretch of the muscle the majority of its lymphatics are subject to volume expansion, and during active muscle shortening the lymphatics are compressed. For example, a 20% stretch of the muscle starting from its in-vivo resting length causes an expansion of the lymphatic lumen cross section by about 40%, contraction of the muscle fibers by 20% leads on average to about 30% compression (MAZZONI, SKALAK and SCHMID-SCHÖNBEIN, 1990). These values are smaller than the average volume expansion during arteriolar contraction, which is more than 100% of the lymphatic reference volume during arteriolar dilation (SKALAK, SCHMID-SCHÖNBEIN and ZWEIFACH, 1984).

The suggestion from these microscopic observations is that there are at least three different mechanisms to induce periodic expansion and compression of the initial lymphatics in skeletal muscle: arteriolar pressure pulsations, vasomotion, and skeletal muscle contraction during exercise. The three lymphatic compression mechanisms are additive, so that the lymph flow can be adjusted according to the physiological activity of skeletal muscle. Arteriolar pressure pulsations have a small amplitude but are driven at the heart rate, vasomotion is slower (about 1/10 to 1/100 of the heart rate) but has larger amplitude, and lymph compression due to muscle contractions depend of course on the nature of the exercise. Resting skeletal muscle has a relative low lymph flow rates which during anesthesia and suppression of arteriolar vasomotion and the pulse pressure reduces even to lower values. Exercising muscle in turn, which is subjected either to active or passive length change, produces also an increase in lymph flow rate, an observation reported repeatedly in the literature (JACOBSSON and KJELLMER, 1964; GARLICK and RENKIN, 1970; BACH and LEWIS, 1973).

LYMPH FORMATION IN OTHER ORGANS

How could the situation look like in other organs? The microanatomy and the mechanical properties

Fig. 4. Crossection of a lymphatic (LYM) in rat skeletal muscle. The lymphatic is positioned in the adventitia of an arteriole (ART) and exhibits an irregular crossection with partially collapsed lumen. The arterio-lymphatic pair is embedded in multiple layers of skeletal muscle (SKM) fibers (A). The lymphatic wall consists of only a thin endothelial lining (arrows, B) without smooth muscle media. At this level of the microcirculation the lymphatic vessel is considerably larger than the adjacent capillaries (CAP) or venules (VEN).
vary from tissue to tissue, so we cannot extrapolate but need to investigate each organ separately. For example, work of Ohtani (1987) and Unthank and Bohlen (1988) have provided a major clarification of the microlymphatics in the intestine. In this organ three layers of lymphatics can be distinguished, in the villi, the submucosa, and a separate lymph meshwork in the smooth muscle surrounding the mucosa. Each villus has a lymphatic ending, denoted as lacteal. Depending on the species there are on average one to five such lacteals per villus and they are not paired with any vascular structure. The lacteals are fused at the villus base to form a submucosal network. This network has dense interconnections and few, if any, valves are present. Microinjected tracers are free to flow to all parts of this network and into adjacent villi, suggesting that the lymphatics in this region permit unrestricted fluid flow within the network. Neither the lacteals nor the submucosal microlymphatics exhibit spontaneous contractions; they do not contain smooth muscle and in cross-section can be seen to have quite irregular shapes (Fig. 6). The lymphatics of the muscle layer form another densely interconnected network. The muscle layer lymphatics also have no functional valves and flow directions in the lymphatics are unrestricted, as seen clearly during microinjections (Unthank and Bohlen, 1988). The mucosal-submucosal and the muscle layer lymphatic networks have no connections but instead merge via large collecting lymphatics near the mesenteric border. This site represents the locus where the first spontaneous lymphatic contractions can be observed, independent of the intestinal smooth muscle motility. It can be considered the beginning of the collecting lymphatics in the intestine. The first lymphatic valves are found in the proximity of the intestine exit regions. More distally the lymph is drained via the so-called lymphatic conduits through the mesentery. Ohtani (1987) also reports that in the rat the few collecting lymphatics in the muscularis have their own smooth muscle and valves, but within a short distance drain into the mesentery lymph duct. The lymph ducts in the mesentery are contractile, they have valves that prevent reflow and they are

Fig. 5. Cross-section of the feeder arteries (ART) to the rat spinotrapezius muscle. The vessel is positioned outside the muscle fibers (SKM) in an endomysial space. It is paired with a venule (VEN), several nerves (N), and a large lymphatic (LYM). The lymphatics has a single endothelial lining without smooth muscle media, and almost completely embraces the arteriole.
frequently paired with the mesenteric sector artery and vein. In summary, almost the entire lymphatic network in this organ consists of initial lymphatics that depend on deformation of the tissue surrounding the lymphatics in order to be compressed or expanded. Contractile collecting lymphatics arise only at the point where the lymphatic channels leave the organ and enter into the mesenteric lymph ducts. At those sites no tissue support is available, but a chain of lymphangions provide the transport mechanism.

It is interesting to note, that when one fixes (for example with glutaraldehyde) a rat intestine in the relaxed state, the lacteals, submucosal or muscle layer lymphatics are found in an open state, rather than being collapsed (Fig. 6). This observation suggests that these lymphatics are supported by the surrounding tissue and require compression during emptying, but can be filled during expansion provided by compressive stresses in the tissue (i.e. passive recoil). Thus we can see that lymph flow in this organ is dependent on intestinal smooth muscle contraction associated with intestinal motility. Each contraction of the mucosal smooth muscle leads to compression of the enclosed tissue, including the villi, the submucosal lymphatics, etc.. Tissue compression causes lymph compression and fluid outflow towards the collecting lymphatic ducts in the mesentery. Following smooth muscle relaxation, the intestinal lymphatics expand and are filled by fluid aspiration from the intesrstitium and possibly across the brush border. Refill is possible as long as interstitial fluid is free to permeate across the initial lymphatic endothelium. Retrograde flow of fluid from the mesentery ducts is prevented by valves.

**CONCLUSION**

The current microscopic observations of lymph flow need to be supplemented in the future by systematic microscopic observations.
whole organ experiments. In such studies the pressure pulse, vasomotion need to be controlled independently of each other while vascular fluid filtration, i.e. the Starling pressures, are preserved. Separately the effects of muscle contraction need to be investigated in a systematic fashion with direct flow rate measurements. Thus we need to establish whether the mechanisms proposed by the microvascular studies are actually sufficient to explain normal lymph flow. Such an approach will provide a clear understanding of lymph transport.

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REFERENCES


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