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Lymphatic System Flows

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Abstract

The supply of oxygen and nutrients to tissues is performed by the blood system, and involves a net leakage of fluid outward at the capillary level. One of the principal functions of the lymphatic system is to gather this fluid and return it to the blood system to maintain overall fluid balance. Fluid in the interstitial spaces is often at subatmospheric pressure, and the return points into the venous system are at pressures of approximately 20 cmH_2O. This adverse pressure difference is overcome by the active pumping of collecting lymphatic vessels, which feature closely spaced one-way valves and contractile muscle cells in their walls. Passive vessel squeezing causes further pumping. The dynamics of lymphatic pumping have been investigated experimentally and mathematically, revealing complex behaviours indicating that the system performance is robust against minor perturbations in pressure and flow. More serious disruptions can lead to incurable swelling of tissues called lymphœdema.

Keywords

Physiology; Oedema; Cancer; Immunology; Lymph

1 Anatomy and physiology

1.1 Basic introduction

The lymphatic system as a functional whole includes several organs whose association as a system is not readily apparent. Lymphoid organs include the spleen, thymus and tonsils; in addition, a vital component is the bone marrow where white cells are manufactured; see Figure 1. This review will concentrate on the lymphatic vascular system, which comprises a network of vessels extending to every part of the body except the brain and spinal cord. An alternative clearance system has been hypothesised for these tissues, since lymphatic vessels have been found only in the dura mater.

Functionally, the lymphatic vascular system runs in parallel to the blood venous system, in that both return fluids centrally (see Figure 2). Lymphatic vessels carry lymph, which is

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largely water gathered from interstitial tissue spaces. Fluid appears in the interstitial spaces because blood capillary walls are somewhat leaky, admitting part of the aqueous component of blood, along with some proteins. The leak passages are glycocalyx-covered intercellular clefts, acting overall as a semi-permeable membrane. As such, the volume filtered per unit time, J_V, is described by the Starling equation for fluid filtration (Levick 2010)

$$J_V = L_p S[(p_c - p_i) - \sigma(\pi_c - \pi_i)]$$

where p is hydraulic pressure, π is osmotic pressure, subscripts c and i denote capillary and interstitium, L_p is the hydraulic conductance of the wall, S is the capillary surface area, and σ is the osmotic reflection coefficient for membrane leakiness to solute. The principle here is that the hydraulic pressure difference between capillary blood and interstitial fluid drives plasma-derived fluid out, but is opposed by the oncotic pressure difference resulting from the greater concentration of protein in plasma. Capillary blood pressure is necessarily elevated [some 30 mmHg in skin at heart level (Levick 2010)] because it is responsible for returning blood to the heart via the veins. Fluid leakage out of capillaries has a physiological function, being responsible for tissue hydration and nutrition. In total, several litres per day of water seep into the interstitium.

The lymphatic vascular system scavenges this water and protein, ultimately returning it to the venous circulation via junctions with the subclavian veins at shoulder level (Figure 2). The maintenance of the interstitial milieu is one of its vital functions; if fluid is not returned to the blood system at the same rate as it leaves, the painful and debilitating condition of œdema can develop. Also scavenged are particles, viruses and bacteria. All lymph passes through at least one lymph node, where this potentially harmful foreign matter is mechanically sieved and neutralized by dendritic cells, macrophages and the T and B cells of the body's immune system. There are some 500−600 lymph nodes in the human body. There are also specialized blood vessels inside nodes, across which fluid, proteins, and cells may translate in either direction. Under normal conditions, some of the incoming (afferent) lymph fluid is taken up into blood, but very concentrated afferent lymph can also be diluted (Adair & Guyton 1983, Adair & Guyton 1985). Absorption of (mainly) water in this way results in post-nodal lymph usually having higher protein concentration (Hansen et al 2015, Knox & Pflug 1983). It is estimated (Renkin 1986) that capillaries lose to the interstitium some 8 L/day of fluid which becomes afferent lymph; after reabsorption at nodes (usually several nodes along a typical lymphatic vessel pathway), the total post-nodal (efferent) flow-rate is about 4 L/day.

While there are wide variations in lymphatic vascular anatomy between organs (Moriondo et al 2010, Schmid-Schönbein 1990a), two principal vessel types are distinguished. Initial lymphatics are the vessels which receive interstitial fluid; starting from blind stumps of some 50 μm in diameter (Schmid-Schönbein 1990a, Zweifach & Prather 1975), they form a converging manifold conveying lymph to the collecting lymphatics. Initial lymphatics have occasional internal one-way valves (Kampmeier 1928, Murfee et al 2007) and thin non-muscular walls. They lead to the collecting lymphatics, which are subdivided into short

segments called lymphangions by regularly spaced one-way valves, and have muscular walls capable of mounting more or less periodic contractions. The contraction of lymphangions, combined with the valve action, transports lymph by a mechanism (intrinsic pumping) analogous to that occurring in the chambers of the heart. A second transport mechanism (extrinsic pumping) results from intermittent squeezing by the relative motion of or pressure change in surrounding tissues, through skeletal muscle use, breathing (Moriondo et al 2005), cardiac-induced blood vessel pulsation (Causey et al 2012, Negrini et al 2004), intestinal peristalsis, external body compression, etc.

1.2 Interstitial fluid take-up – primary valves

The primary valves of the lymphatic vascular system are located at the entrance to initial lymphatics, the walls of which consist of a monolayer of endothelial cells and lack a continuous basement membrane (Bazigou & Makinen 2013, Bazigou et al 2014, Pflicke & Sixt 2009); see Figure 3. Elsewhere, i.e. in collecting lymphatics and adjacent blood vessels, the endothelial cells form a tighter but still permeable barrier by forming continuous zipper-like tight junctions. At the tips of initial lymphatics, the cells have a characteristic oak-leaf shape and form overlapping flaps (Baluk et al 2007, Leak 1971) with discontinuous button-like junctions. Furthermore, they are anchored to the surrounding extra-cellular tissue matrix by filaments consisting mainly of the protein fibrillin (Leak & Burke 1968). By pulling outward, these filaments prevent collapse of the initial lymphatic in the presence of elevated tissue pressure (Reddy 1986). In combination, these features (lack of basement membrane, discontinuous junctions, anchoring filaments) allow the initial lymphatic endothelial cells to act as flap valves, allowing interstitial fluid to enter but largely preventing the intra-lymphatic fluid from escaping back to the tissue. The gaps between cells can reach several μm (Baluk et al 2007, Trzewik et al 2001), allowing free ingress of protein, water, debris and cells (Ikomi et al 1996). Consequently, there is neither significant osmotic difference between interstitial fluid and this initial lymph, nor a significant steady pressure difference (Aukland & Reed 1993). However, net fluid ingress occurs because of the intermittent compression (when the flap valves close) and subsequent re-expansion (when they open) of initial lymphatics (Guyton et al 1971a, Schmid-Schönbein 2003, Trzewik et al 2001), taking advantage of the same processes involved in extrinsic pumping by collecting lymphatics. In skeletal muscle undergoing passive stretch then active contraction, it has been demonstrated that the volume change of blood vessels, by both their own active contraction and their passive stretch, also contributes to the filling and emptying of initial lymphatics (Causey et al 2012). The specialised endothelial cells that allow this fluid ingress are not confined to the extreme tips of initial lymphatics, but occur, probably to a diminishing extent, along their length (Baluk et al 2007). Models of primary valves have been proposed (Galie & Spilker 2009, Heppell et al 2013, Heppell et al 2015, Mendoza & Schmid-Schönbein 2003).

1.3 Intrinsic pumping

Utilizing a muscle type that is intermediate between vascular smooth muscle and myocardium (von der Weid & Zawieja 2004), collecting lymphatics can mount variably regular cardiac-like contractions at a repetition rate of some 10 /min (Zawieja et al 1993). In rat mesenteric lymphatics of some 100 μm in diameter, contraction propagates along the wall at a rate of 4−8 mm/s. A given contraction may take 1 s to reduce diameter maximally,

and of the same order to decay. Since the lymphangions in these vessels are typically of the order of ten or fewer diameters in length, the above figures mean that contraction is essentially simultaneous all along the length of a given lymphangion. Thus the contraction has the character of an almost completely synchronous reduction of lymphangion diameter everywhere; the lymphangion behaves as a short contractile chamber which reduces its volume, relying on closure of the inlet valve to cause forward flow rather than passage of a peristaltic wave. Further emphasizing that the mechanism of propulsion does not rely on peristalsis, contraction activation propagates along collecting vessels in the opposite direction to flow almost as often as in the same direction (Zawieja et al 1993).

Because the contractions thus embody the same mechanism of pumping as the cardiac chambers, terminology from cardiac physiology is used to describe the performance. Ejection fraction, which describes how much of the initial volume is ejected during a contraction, can reach an impressive 80% (Scallan et al 2012) in isolated segments of rat mesenteric collecting lymphatic vessel.

Adjacent lymphangions of isolated vessels usually constrict within 0 to 0.5 s of each other, with the most common interval lying between 0 and 0.25 s (Crowe et al 1997). Short intervals and retrograde propagation both suggest that an activation signal is propagated electrically within the lymphatic wall. The initial trigger for a lymphangion to contract may be filling; collecting lymphatics exhibit a myogenic response, with contraction being initiated when tension (rather than stretch) surpasses a threshold (Davis et al 2009a). This contraction may then be propagated along the chain of lymphangions in either direction, even across Y-junctions between vessels (Zawieja et al 1993). It is thus possible to reverse the direction of propagation of a contraction wave in an isolated two-lymphangion segment by manipulation of the pressures applied to the ends (unpublished data of M.J. Davis, analysed by CDB). However the electrically excitable cells in the muscle layer of the vessel wall also exhibit spontaneous pacemaker activity (von der Weid et al 2001). An alternative, purely mechanical mechanism of contraction propagation has long been proposed, whereby ejection from one lymphangion into the next would initiate a contraction there by distension, but this hypothesis does not explain retrograde propagation. The intramural electrical conduction is likely to be via gap functions between muscle cells formed of connexin proteins (Scallan et al 2016) A recent computer model (Baish et al 2016, Kunert et al 2015) which included representations of calcium-ion release (initiating contraction) and re-uptake, along with equations describing the evolution from LECs of the short-lived contraction inhibitor nitric oxide (NO), predicted that contraction waves would propagate forward when calcium dynamics dominated, and backward when NO dominated. However, direct evidence for this prediction is lacking so far.

The myogenic properties lead to contraction frequency being a very sensitive function of both distending pressure and its rate of change (Davis et al 2009b, Scallan et al 2012), ultimately reaching a plateau above 20 /min at high pressures. Again analogously to heart performance, collecting lymphatics also exhibit both a Starling response, with the extent of contraction increasing with the degree of lymphangion filling (although the degree of muscle shortening declines at transmural pressures above 3 cmH_2O) (Gashev et al 2004, Scallan et al 2012), and an Anrep effect, i.e. an increase in contraction strength with afterload (defined

as aortic pressure for the left ventricle of the heart, and as outlet pressure for a perfused lymphatic segment) (Davis et al 2012). In experiments on perfused single-lymphangion segments of rat mesenteric vessel involving gradually raising the outlet pressure, (Davis et al 2012) found that the pump failed to open the outlet valve when outlet pressure exceeded inlet pressure by an average of 11 cmH_2O (range 2−18 cmH_2O). The reader is encouraged to view the movies of lymphatic vessel pumping experiments contained in the supplemental material for that publication. For human leg subcutaneous lymphatics *in situ*, contractions generate pressures of 61 ± 26 cmH_2O (range 27−109 cmH_2O) in the upright position when outflow is prevented (Olszewski & Engeset 1980); this of course involves the summated efforts of multiple lymphangions in series.

Mention of the upright position demands brief examination of hydrostatic considerations. Ignoring all other factors, we would expect the pressure in blood vessels in the feet to be some 120 cmH_2O higher than in those at heart level. However, blood capillary pressure increases somewhat less than that because lower-limb arterioles constrict, in a regulatory response that limits the rise by increasing precapillary resistance to blood flow. Among other factors, this prevents interstitial fluid pressure being considerably greater in the feet than in the lung, say; Noddeland et al. measured ankle subcutaneous interstitial pressure in healthy volunteers as averaging just 0.13 cmH_2O (Noddeland et al 1984). This prevents tissue oedema in the feet, but means that there is little upstream pressure to aid lymphatic return. Fortunately, the downstream pressure faced by collecting lymphatics in the feet is also not the simple high hydrostatic-column value. The pressure opposing lymph flow from the foot does not alter greatly between recumbency and standing (Olszewski & Engeset 1980), because the fluid column above is interrupted by many competent valves.

Contrary to the effect of distending pressure, both the frequency and amplitude of lymphatic contractions are reduced by antegrade lymph flow, as was demonstrated in experiments involving equal and opposite changes in inlet and outlet pressure for perfused segments, thus altering the flow-driving pressure gradient while leaving the transmural pressure unchanged (Gashev et al 2004, Gashev et al 2002). Contractions also lower the resting tone, thereby increasing the diastolic diameter; both these effects are believed to benefit the total flow-rate by enlarging the conduit and reducing hydraulic resistance. For blood vessels it is well established that endothelial cells respond to raised levels of fluid shear at the glycocalyx by activating endothelial nitric oxide synthase (eNOS), causing release of nitric oxide (NO) which acts on vascular smooth muscle cells to depress tonic constriction. A similar eNOS mechanism exists in lymphatic endothelial cells (LECs). However, as might be expected, given that lymphatics mount both tone and short-lived contractions, the effect of NO release is more complex. The contraction-induced inhibition of tone is caused by NO release (Gasheva et al 2006), whereas, at least in isolated segments, the shear-dependent inhibition of contractions is apparently independent (Kornuta et al 2015) of both NO and histamine, another known lymphatic relaxing factor for which LECs have receptors (Kurtz et al 2014, Nizamutdinova et al 2014). Evidence also suggests that NO may increase lymphatic contraction strength by lowering contraction frequency at low concentration, and only depress contraction strength at higher concentrations (Scallan & Davis 2013). Overall, the mechanotransduction of fluid shear by LECs, and the wall-tension-derived triggering of contractions, emphasize that lymphatic vessels actively monitor their immediate fluid-

mechanical environment in terms of both pressure and flow, and interact with it through their locally organized contractile and tonic responses.

1.4 Extrinsic pumping

Mesenteric lymphatics are often the target of investigations of intrinsic pumping not only for their experimental convenience but also because they are among the most active vessels for muscular contraction (Gashev et al 2004). But lymph transport still occurs in vessels which do not contract, thanks to the extrinsic mechanism. The relative importance of extrinsic pumping is not known with any accuracy, and almost certainly varies widely both across the organs of the body and with the nature of physiological activity. It has been known at least since the 1930s that lymph flow can be greatly augmented by passive limb motion (Haynes 1932). In humans, (Engeset et al 1977) found that fast walking increases ankle lymph flow 15× over values measured in recumbent sleep (however the same group later concluded that leg subcutaneous lymph flow was principally the result of intrinsic contractions (Olszewski & Engeset 1980), whether calf muscles were active or not). The older literature documenting the existence of extrinsic pumping through respiration, intestinal peristalsis, passive and active limb movements, external compression and massage, and blood vessel pulsation and vasomotion has been comprehensively reviewed (Aukland & Reed 1993). Schmid-Schönbein (Schmid-Schönbein 1990a) makes the important point that extrinsic pumping can potentially generate sufficiently large intralymphatic pressures to propel fluid effectively regardless of nearby tissue pressure. It may therefore be the only means of generating contractions in the presence of elevated downstream pressures that would limit intrinsic pumping. McGeown et al. (McGeown et al 1987) obtained evidence that intermittent compression applied to initial lymphatics in the sheep hind limb is more effective than that applied to collectors.

1.5 Heterogeneity of lymphatic network topology

The architecture and topology of lymphatic vessel networks is heterogeneous, exhibiting departures from a purely converging vessel network structure shown in Figure 3. This suggests that, unlike blood vessel networks, minimisation of mechanical energy loss in fluid transport is not a dominant ‘design’ criterion. This topic has yet to be systematically explored, but one can readily identify many intriguing examples of lymphatic network structures where other, as yet unidentified, guiding principles are seemingly in control.

A potent example is the drawn reconstruction of subcutaneous lymph capillaries (initial lymphatics) and deeper-lying collecting lymphatics in the fœtal leg forming Figure 4. The initial lymphatics prominently form re-entrant loops at both the smallest scale (a single vessel splitting and rejoining) and on larger scales (circuits which include junctions with neighbouring vessels). In only a few of these latter circuits is flow direction obviously enforced by one-way valves; the illustration includes arrows indicating what must be the vessel flow direction in some of these cases. More recent work confirms the prevalence of such loops in subcutaneous initial lymphatics (Schmid-Schönbein 1990a, Soto-Miranda et al 2013). A high prevalence of initial lymphatic vascular loops is seen also in the submucosa of the small intestine (Unthank & Bohlen 1988) and the peritoneum of the liver (data of A.V. Borisov). In the diaphragm, which is also characterised by atypical ultra-large initial

lymphatics (Moriondo et al 2010), loops occur in collecting vessels (Moriondo et al 2013, Negrini & Del Fabbro 1999). A particularly highly organized, regular hexagonal lattice of initial lymphatics occurs in the skin of the mouse tail. These vessels eventually drain into a pair of collecting lymphatics that run the length of the tail. Roose & Swartz have shown that the hexagonal arrangement provides more efficient clearance of fluid for a given network density than square or parallel networks (Roose & Swartz 2012).

2 Clinical importance

2.1 Lymphœdema

One consequence of inadequate lymph transport is lymphœdema, in which protein-rich interstitial fluid accumulates in the tissues, leading to distension, inflammation, fatty tissue proliferation and fibrosis (ISL 2013). Lymphœdema usually involves swelling of a limb, but other areas, including the head, neck, breast or genitalia, may also be involved (Tiwari et al 2013). Generally considered an incurable condition, lymphœdema gravely affects quality of life. Because of the important role the lymphatic system plays in immune function, patients are at high risk of soft-tissue infections (Rockson 2013).

Primary lymphœdema arises from congenital disorders, with the most common forms being Milroy disease and distichiasis (which also causes aberrant eye-lashes). Both commonly affect the lower limbs, but Milroy is usually symmetrical and present at birth or soon after, whereas distichiasis lymphœdema appears in late childhood and may be asymmetrical. For people under the age of 20, the prevalence of primary lymphœdema is estimated at 1.15 in 100,000. Distichiasis results from pathogenic variants of the FOXC2 gene (Mansour et al 2012); other gene mutations causing lymphœdema are reviewed by Mortimer and Rockson (Mortimer & Rockson 2014).

Secondary lymphœdema often results from damage to or removal of lymph nodes, as a result of surgery, radiation, trauma or infection. The most common manifestation in developed countries is breast-cancer-related lymphœdema (BCRL) of the upper limb. Its extent correlates with how many axillary lymph nodes have been removed, and how much the axilla has been irradiated. Lower-limb secondary lymphœdema may result from treatment of melanoma, or of prostate or gynæcological cancers. Incidence rates of post-surgical lymphœdema are difficult to assess due to confounding factors such as deficiencies in patient follow-up and reliable, quantitative diagnostic tools. However, attempts at quantifying prevalence generally indicate a substantial problem with incidence rates of up to 77% reported in 2001 [65]. Adaptations in surgical practice to minimise node resection have reduced the risk of lymphœdema. In 2010, it was reported that 16% of patients undergoing a combination of lymph node resection and radiation therapy showed measurable signs of lymphœdema (Bar Ad et al 2010). Because surgical procedures that endanger the lymphatic system are so common, lymphœdema remains a substantial problem, with nearly three million sufferers in the USA alone (Padera et al 2016). Chronic lymphœdema can also result from such conditions as congestive heart failure and end-stage renal disease (Tiwari et al 2013). However in tropical regions of the world, the most common cause, affecting over 120 million people, is a parasitic roundworm which is spread by mosquito bite; the resulting filariasis can result in large-scale tissue swelling (‘elephantiasis’).

There is no cure for lymphœdema. Successful management, in the sense of limiting progression, can be achieved with early diagnosis. The current standard of care is Decongestive Lymphatic Therapy, which includes fastidious skin care, the almost constant wearing of compression garments, exercise, and manual lymphatic drainage. The latter consists of careful massaging by specifically trained therapists to encourage subcutaneous lymph flow. Challenges to lymphœdema management include the fact that many cancer surgeons fail to warn their patients about the risk of it developing, general ignorance of the lymphatic system in the medical community, and the psychological impact of the deformities in patients who have already suffered through cancer diagnosis and treatment.

2.2 Metastases

All of the deadliest forms of cancer spread because metastatic cells separate from the primary tumour and are easily taken into the lymphatic system. The immune system is in fact capable of eliminating these rogue cells, and even in healthy people serves to eliminate occasional genetic mutations. Unfortunately, this defence system can be overwhelmed or subverted, resulting in the establishment of secondary tumours in other parts of the body. It is these secondary tumours that are actually responsible for approximately 90% of cancer deaths.

Like most other immune functions, the important actions of identifying and eliminating metastatic cells occur in lymph nodes. The nodes in the axilla (arm pit) are responsible for monitoring the lymph coming from the breast, and so cancer surgeons remove them for histological examination to determine the likelihood of metastatic spread. There are some 30–40 nodes in the axilla, some of which drain the arm, which as mentioned above is at high risk for œdema development. It would therefore be preferable to remove only one ‘sentinel’ node for the tumour tissue, and indeed many surgeons are now mindful of the need to limit node removal. Unfortunately, the complex network organisation of the lymphatic system both reduces confidence in the identification of a sentinel node, and compels the removal of several nodes to satisfy the primary consideration of stopping metastatic spread. The risk of subsequent œdema increases with the number of nodes removed (Warren et al 2014).

2.3 Immune system dysfunction

Without the lymph-flow mediated transport of immune cells and antigens, there could be no adaptive immunity in large animals. Immune cells that have detected antigens find their way efficiently to the nearest lymphatic vessel by following concentration gradients of specific chemokines secreted by lymphatic endothelial cells. These gradients are established and maintained by a combination of diffusion, advection, binding to extracellular matrix, and multimodal scavenging by multiple cell types. Transport along the lymphatic flow stream delivers crucial information to lymph nodes within tens of minutes; much sooner than could be accomplished with active cell movement alone. Inside the node, chemokine gradients are again important in directing cognizant immune cells to distribute the information required to fight the infection (Ulvmar et al 2014).

3 Fluid-dynamic characterization

3.1 Lymph properties

Like interstitial fluid, the quantitative composition of lymph varies in different tissues. Pre-nodal lymph is interstitial fluid that has entered initial lymphatics, and so has the same composition of water, salts, plasma proteins (albumins, globulins and fibrinogen) and white blood cells; the plasma-protein concentration is typically < 0.01 gm/ml. The salt ions in aqueous solution include sodium, chloride, calcium, magnesium and bicarbonate; other constituents include sugars, fatty acids, amino acids, signalling molecules and cellular waste products. Water is usually absorbed from lymph into the blood supply of lymph nodes, so that post-nodal lymph has of the order of twice the plasma-protein concentration; post-nodal lymph also has a higher concentration of lymphocytes. Lymph from the intestine and liver is called chyle; it has between 0.02 and 0.06 gm/ml of protein and a sufficient concentration (> 0.01 gm/ml) (Allanson 2005) of triglyceride fat globules (chylomicrons) to give it a milky white colour.

In rat mesenteric lymphatics, (Dixon et al 2006) found a lymphocyte concentration of between 326 and 35,500 cells/μL, with an average of 12,000 ± 5,200 cells/μL. If these be assumed to be spherical leukocytes with a diameter of 6 μm, the average cell volume concentration is then some 0.0014. Thanks to this extremely low concentration, the cells do not significantly affect the bulk properties of the lymph, which to a good approximation is a Newtonian fluid which can be characterized by a single value of viscosity. However, like the plasma from which it is derived, lymph can clot (Burton-Opitz & Nemser 1917) through conversion of fibrinogen to fibrin strands, something which has to be considered in experimental design. In 10 dogs fed 4–6 hours previously, the viscosity of thoracic duct lymph (thus including a chyle component) at 37 °C was measured at 1.23 mPa s (Burton-Opitz & Nemser 1917), with a range of 1.08 to 1.36 mPa s. The density ranged from 1.005 to 1.016 gm/mL, averaging 1.0097 gm/mL.

3.2 Reynolds number

Initial lymphatics have a diameter of some 50 μm and individually tiny flow-rate, such that they definitely operate in the fully viscous flow regime. The greatest concentration of both experimental and modelling effort has been expended on rat mesenteric collecting lymphatic vessels. In such vessels, (Dixon et al 2006) found an average diameter of 91 μm and average volume flow-rate of 13.95 μL/h; with the same viscosity and density these figures translate to a Reynolds number of 0.045, i.e. viscous flow again. The largest lymphatic vessel in the human body, the thoracic duct, averages 2.2 mm inside diameter (Telinius et al 2010). This vessel is generally regarded as draining three-quarters of the human body's post-nodal lymph flow of 4 L/day. With the above figures for average viscosity and density, this mean flow implies a Reynolds number of 16. These figures may be greatly exceeded in circumstances of temporarily raised lymphatic flow-rate, as for instance when staving off interstitial œdema (Rahbar et al 2014).

Given that lymphatic flow is pulsatile rather than steady, the peak Reynolds number will be higher. (Dixon et al 2006) tabulated the maximum velocity they recorded in each of 7 rats;

taking these values as representing the centre-line of a parabolic profile, and using their values of average diameter in each animal, the peak Reynolds number reached 0.4, i.e. a factor of 10 higher than the time-mean value. A similar factor probably applies in the thoracic duct. On this basis, one can conclude with confidence that flow is always viscous in lymphatic vessels of diameter 100 μm or less, and always laminar in the largest vessel, the thoracic duct.

3.3 Womersley number

The contraction of a rat mesenteric lymphangion typically lasts some 2s from inception to end-decay (Dixon et al 2006, Zawieja et al 1993), but the period of relaxation between contractions is extremely variable, being controlled by regulatory factors (section 1.3). A 0.5 Hz sinusoid represents the 2s contraction reasonably well; along with the above diameters (Dixon et al 2006) and lymph properties (Burton-Opitz & Nemser 1917), this leads to values of Womersley number up to 0.1, for which quasi-steady flow is a good approximation.

(Telinius et al 2010) recorded complex contractions from ring segments of human thoracic duct, consisting of phasic contractions lasting some 60s or more, during which the tension oscillated at higher frequency. They reported spontaneous contractions at 1.39 ± 0.35 /min, increasing to 6.78 ± 1.56 /min under pharmacological activation. From traces they presented, the tension oscillated at 18 /min. If one assumes that such tension oscillations would influence the flow *in vivo*, this frequency can also be considered as indicating a Womersley number of 1.4.

At the level of rat mesenteric collecting vessels, the above values of Reynolds and Womersley number encourage the modelling assumption of Poiseuille flow. (Rahbar & Moore 2011) tested this assumption in an axisymmetric computational fluid-dynamic model of a 100 μm tube with a 450 μm-long segment that varied its diameter in the range 80 to 240 μm according to prescribed time-waveforms. Steady and time-varying aqueous flows were imposed, with some including a backflow component. Flow velocities were matched to measured values (Dixon et al 2006). Although the ratio of radial to axial velocity reached values as high as 1.3, fluid shear stress at the wall always remained within 4% of the Poiseuille-profile value. The largest deviations corresponded with peak radial velocities.

There may be larger deviations from the Poiseuille-flow predicted wall shear stress for larger Reynolds numbers such as those found in the human thoracic duct. Although Wo = 1.4 indicates profiles that do not differ greatly from the quasi-steady parabolic form, Re = 16 (steady) and perhaps 160 (pulsatile) indicates important inertial effects. Particularly in the context of valve opening and closure transients, events which have not been considered quantitatively in forming values of Wo, it is likely that flow would depart substantially from quasi-steady.

3.4 Diameter change

As a dimensionless number, it is conventional in lymphatic research to express diameter change as ejection fraction EF, a concept taken from cardiac mechanics. EF = $(V_{max} - V_{min})/V_{max}$, where V = volume. In the context of a lymphatic vessel, where diameter is measured and a lymphangion is assumed to remain cylindrical and of unchanging length

during contraction, EF = $1 - (d_{min}/d_{max})^2$, where d is inside diameter. It should be noted that this number is a measure of contraction amplitude and does not take into account backflow through lymphatic valves before closure, nor the possibility of flow due to a favourable pressure gradient; thus it cannot be directly related to lymph flow-rate. Impressive values of EF have been recorded for rat mesenteric collectors; in isolated segments (Davis et al 2012) measured values ranging up to 0.86 at low outlet pressure. Active contraction therefore seems capable of reducing the vessel diameter below the value that the relaxed vessel would have at zero transmural pressure. If the vessel were obeying the fully relaxed pressure/diameter relation, it would take on a non-circular cross-section in this circumstance, whereas in fact it appears to remain circular (Davis et al 2012).

3.5 Secondary lymphatic valves

Whereas inertial effects in the form of persistent vortices have been shown to play important roles in the operation and behaviour of cardiac valves, secondary (i.e. intravascular) lymphatic valves almost all inhabit a purely viscous flow regime (a characteristic they share with the vast majority of venous valves). Almost all are bileaflet, but tricuspid and monocuspid valves have also been found. The valve (see Fig. 5) has an approximately fusiform sinus, and the leaflets form a tubular structure which tapers to decreasing lumen cross-section going downstream, with the trailing edge of the leaflets providing a flattened orifice which extends across the whole width of the sinus, at close to its widest point (see Fig. 6). The whole valve thus has a circumferential orientation, and successive valves often differ in this orientation by 90° (Gashev 2008). The valve proper thus occupies the upstream half of the sinus, while the downstream half is unobstructed.

(Mazzoni et al 1987) have proposed a simple model for the operation of such a valve, based on the observation that only the pressure gradient and viscous terms of the Navier-Stokes equations are non-zero in this Stokes flow. They postulate that axial viscous pressure drop along the valve leaflets ensures that pressure p_1 upstream is greater than p_2 downstream when the valve is open and there is forward flow. Because there is little or no flow in the sacs behind the leaflets, the pressure there is also p_2, and the positive pressure difference $p_1 - p_2$ acts to keep the leaflets in the open position. If downstream pressure rises to the point where $p_2 > p_1$, a positive pressure difference $p_2 - p_1$ then exists to push the valve leaflets inwards, shutting the valve. The model suggests that the essential elements of the ‘design’ are the viscous pressure drop along the elongated tubular space between the leaflets, and sufficient flexibility of the leaflets to respond to the pressure differences thus set up across their thickness.

(Davis et al 2011) investigated the specific properties of valves in isolated segments of rat mesenteric collecting vessel. They found that opening and closure both occurred when there was an adverse trans-valvular pressure drop Δp_V, i.e. the valve has a bias to remain open. This bias increased with increasing positive transmural pressure Δp_{tm}, i.e. increasing vessel distension, such that at high Δp_{tm}, opening occurred while there was still 0.2 cmH_2O of adverse Δp_V. The measurements indicated substantial hysteresis, in that the threshold Δp_V for closure was more negative than that for opening, but this finding remains uncertain since the closing data did not account for pressure drop in the cannulating micropipettes. These

observations suggest that substantial regurgitant flow back through still-open lymphatic valves when downstream lymphangion contraction starts, as observed by (Dixon et al 2006), is inevitable. The apparent inefficiency is perhaps offset by the advantage of having valves open in advance, minimizing resistance to flow, when the overall flow-driving pressure gradient is in process of changing from negative to positive. Bias also allows adjacent, synchronously contracting lymphangions to operate as one pumping chamber, with minimal internal hindrance to flow (Bertram et al 2016a). The origin of the bias lies in the configuration of the valve, which in its relaxed state is already partially open as shown in Fig. 6. If the design is indeed optimal, and not simply an inevitable result of the shape and structure needed for operation in viscous flow, certainly the optimum is quite different from that exhibited by heart valves, the design of which allows them to close with little or no regurgitation.

Lymphatic vessels contain large numbers of secondary valves; for instance the mesentery of the mouse has up to 800 (Sabine et al 2015). The process of valvogenesis, whereby it is determined in the embryo exactly where valves will be located, involves the expression by lymphatic endothelial cells of molecular regulators (transcription factors) such as FOXC2 and CX37. Although the embryonic vessels in which these processes are observed biologically have not been subjected to detailed flow investigation, a widespread belief has arisen that these processes are triggered and localised by characteristics of the flow field. In particular, based partly on comparisons with flow fields that have been investigated in detail in blood vessels, the molecular processes leading to valve formation are believed to co-localise with sites of disturbed flow (Kazenwadel et al 2015, Sabine & Petrova 2014), oscillatory wall shear stress (Bazigou & Makinen 2013) or complex flow patterns (Sabine & Petrova 2014).

Direct evidence for this is lacking. The circumstantial evidence consists mainly of the old observation (Kampmeier 1928) that lymphatic valves often develop at vessel junctions (true also for venous valves (Franklin 1927)), and these are asserted to be sites of disturbed flow by analogy with blood vessel branchings such as the carotid bifurcation (Hahn & Schwartz 2009). It will be obvious to a fluid dynamicist that this argument ignores the problem that fluid flow does not scale to microscopic vessels without change. Nor is it evident that Stokes flow through a microscopic junction is necessarily any more disturbed than that in the vessels on either side, since the convective effects that might promote instability are lacking. However, as long as ‘disturbed’ is interpreted as no more than periodic flow reversal, the association may have merit, although it has yet to be properly established either experimentally or numerically.

Once established, lymphatic valves are themselves instigators of disturbed flow. Details of the flow through such valves are now emerging through numerical modelling (Wilson et al 2015, Wilson et al 2013). Of particular interest is the almost stagnant flow in the blind sac behind each leaflet. Because of the three-dimensional geometry, flow does not fully recirculate with closed streamlines, but residence times in some sac locations can be prolonged. As in veins (Bovill & van der Vliet 2011), this introduces some risk of clotting, but perhaps more importantly greatly affects the distribution of lymphatic endothelial cell-

derived nitric oxide, a short-lived but potent depressor and thus regulator of lymphatic pumping (Bohlen et al 2011).

In this context it is worth noting that recirculation does not depend on inertial effects; it survives in blind cavities under viscous flow conditions. An example was provided in a recent numerical model of a blind-ended blood capillary sprout, a stage in the formation of new capillary blood vessels (Stapor et al 2011). With an impermeable wall, flow within the sprout was shown to consist of three counter-rotating zones of recirculation, each of course being weaker than the more proximal one. Noting that recirculation zones do not imply closed streamlines in 3D, we see that even in this extreme cavity geometry, and with zero wall permeability, the residence time at the end is finite.

3.6 Mass transport of vasoactive substances

NO is present in concentrations of 200−400 nM in collecting lymphatic vessels, with the highest concentrations seen at the sinus region surrounding valves. This occurs because of the relatively stagnant flow behind the valve leaflets (Wilson et al 2013). It is not known if there are particular benefits to these higher concentrations, but at least two potential benefits have been hypothesised. The first is that valve function would be impeded by strong contraction of LMCs in the vessel surrounding the valve. The second is that that dendritic cells and other immune cells might be chemotactically drawn into collecting lymphatics in the valve sinus region, where there are fewer LMCs lining the wall, leaving gaps for transmigration.

4 Lumped-parameter modelling

Computational modelling of the lymphatic vascular system is confronted by a number of challenges. One is obviously the range of scales of the overall system; it is impractical to think of modelling the whole system except via a number of approaches, each addressing a different scale. A second severe difficulty is the paucity of experimental data. The lymphatic system has been little studied relative to the blood circulatory system, and data on quite basic aspects of vessel behaviour are only now becoming available. Lymphatics are small (the most-studied ones are microscopic) with thin transparent walls; they convey a transparent fluid at low velocity and flow-rate. In consequence there are severe measurement difficulties, particularly for non-invasive measurement in humans.

At the level of individual lymphatic vessels, one is confronted by the division into individually actively pumping elements (lymphangions), bounded by valves. The valves (see section 3.5) comprise strong nonlinearities which, owing to their hysteresis, bring the further complication of numerical solution discontinuities in time. Another source of strong nonlinearity is the elastic properties of the lymphangion wall, which exhibits much sharper and more dramatic change between its stiffnesses at low and at high distension than comparable arterioles and venules; see Figure 7.

Both the lack of data to support detailed distributed models and the abundance of strong nonlinearities mean that lumped-parameter modelling finds a useful role, and by number of publications dominates lymphatic vascular modelling thus far. The simplest models

amounted to little more than the fitting of experimental observations of mean pressure and flow-rate to a Thévenin equivalent circuit (Drake et al 1986), a concept from electrical engineering which specifies a two-terminal ‘black box’ by (in the hydraulic analogy) its source pressure and source resistance to flow. This idea was taken up and further developed in the first generation of true lumped models (Quick et al 2008, Venugopal et al 2007), which relate specified time-variables by ordinary differential equations and thereby encompass temporal events. Quick and co-workers also introduced the idea of modelling lymphatic contraction as a time-varying elastance, a concept adopted from cardiac mechanics (Sagawa et al 1988). An elastance is equivalent in electrical analogy to a capacitance; varying it, e.g. by a motor, requires external energy, which in the cardiac case (Elzinga & Westerhof 1980) is supplied by the oxidation of fats and carbohydrates in muscle. The resulting lymphangion model was incorporated into a network model which predicted a structure that would optimize lymph flow. This model predicted lymphangion lengths increasing in the streamwise direction, at a rate consistent with measurements of postnodal bovine mesenteric lymphatics (Venugopal et al 2009), and an optimal number of vessel confluences.

Further lumped-parameter modelling has been closely tied to biological experimental findings, mostly on rat mesenteric lymphatics. Whereas early lymphatic vascular models simulated valve function simply by disallowing reverse flow, the concept of valve resistance varying hysteretically (different opening and closing characteristics) with both the transvalvular and the transmural pressure (Davis et al 2011) is now encompassed (Bertram et al 2014a, Bertram et al 2014b), and both valve leakage at physiological pressure differences, and prolapse at pathological pressure differences, can be simulated. The highly nonlinear passive pressure-diameter relationship is incorporated, and the influence of wall muscle contraction (Zhang et al 2007) is included via an explicit time-varying active circumferential tension produced through the interaction of an imposed activation waveform with the constraint on tension of the instantaneous muscle length (Bertram et al 2014a, Bertram et al 2016a, Bertram et al 2016b). Such models have for instance recently been used (Bertram et al 2016a) to investigate how contraction timing variables (refractory period, inter-lymphangion delay) interact with the complex properties of lymphatic valves (*vide infra*) to determine what configurations of a multi-lymphangion segment occur, and which produce the greatest pumped flow. These are experiments that it would be impossible to conduct biologically, where the relative timing of contractions cannot be controlled, and the properties of adjacent valves may differ. A recent further extension attempts to incorporate generalized vascular regulatory properties (assumed similar to blood vessels) to simulate active local control of transmural-pressure dependent contraction rate, shear-dependent standing tone and stress-induced structural remodelling (Caulk et al 2016), in work linked to experiments on the mechanical behaviour of the rat thoracic duct (Caulk et al 2015).

Lumped-parameter models are in principle well suited to the modelling of extended chains of lymphangions (Bertram et al 2016a, Jamalian et al 2013) and networks of lymphatic vessels. A very early model (Reddy et al 1977) attempted simulation of the whole-body lymphatic network with one computational cell per lymphangion, but it was later concluded through a replication of the methods used (Macdonald et al 2008) that the results were probably afflicted with numerical artefact. A recent lymphatic vascular network model was

more modest, sticking to a three-generation convergence of four inlets to one outlet, with four lymphangions per vessel (Jamalian et al 2016). It was found that for favourable pressure differences, pumping was more efficient with fewer lymphangions. Further progress demands a good model of how lymphangion contractions are partially coordinated over extended networks, largely though local mechanisms (Baish et al 2016).

5. Applications of fluid-dynamic analysis to physiology and pathology

In the 1960s, the famed physiologist Arthur Guyton published measurements demonstrating that the pressures in some interstitial tissue beds are subatmospheric (Guyton & Coleman 1968, Guyton et al 1971b). While there was considerable resistance and debate on this issue, others eventually confirmed his findings with different measurement techniques. A particularly striking example is the pleural space, where subatmospheric pressures are necessary to inflate the lungs. Maintaining a favourable pressure gradient into the lymphatic vessels of the diaphragm results in pressures of −40 cmH_2O in initial lymphatics (Moriondo et al 2010, Moriondo et al 2005). Progression of lymph to the subclavian veins means overcoming an adverse pressure gradient of around 60 cmH_2O. An interesting physiological question naturally arises about how collecting lymphatics, which can only contract, perform their pumping function in the presence of upstream and internal subatmospheric pressures. Guyton was never able to resolve this question, but proposed that “lymphatic suction” could be responsible.

Recently, (Jamalian et al 2015) used a combination of isolated vessel measurements and lumped parameter modelling to explain the mechanisms behind draining these tissue beds. There is indeed a suction mode involved, in which the intralymphatic pressure transiently drops below the inlet pressure (Figure 8). Importantly, this facilitates the re-filling of the collecting lymphatic following contraction, which provides the opportunity for the next contraction to generate stroke volume. The key to the generation of suction is the existence of either a positive transmural pressure or tethering to external tissues to pull the vessel back open after contraction. This bears a loose similarity to the *vis a fronte* mechanism that is thought to play a role in filling the right atrium from the low-pressure vena cava.

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Terms and Definitions

Breast Cancer Associated Lymphœdema:
Pathologic swelling of the arm as an eventual result of lymph node removal from the axillary region as part of mastectomy procedures. Lymph nodes are removed as part of the surgery to determine and/or minimise the probability that the cancer has spread to other tissues, this being an important prognostic indicator.

Chemokine

Cytokines (cell signalling proteins) that are specific instigators to immune cell migration in peripheral tissues as well as lymph nodes. There are some 45 chemokines and 20 chemokine receptors identified so far.

Collecting Lymphatic
A medium- to large-sized lymphatic vessel having an inner lining of lymphatic endothelial cells, an outer lining of several layers of lymphatic muscle cells, and interspersed elastin (inner layers) and collagen (outer layers). Collecting lymphatic vessels also have bi-leaflet one-way valves at regular, short intervals. These vessels exhibit a highly nonlinear pressure-diameter behaviour (Figure 7).

Decongestive Lymphatic Therapy
A combination of medical procedures intended to minimise the further progression of Breast Cancer Associated Lymphœdema. Includes skin care, compression bandaging, exercise and specialised massage (manual lymphatic drainage).

Extrinsic Pumping
Results from lymphatic vessel compression due to movement of adjacent tissues.

Immune System
A major bodily function spanning several organs and systems that defends against disease. Key cell types are dendritic cells, macrophages, B cells and T cells.

Initial Lymphatic
The smallest of the lymphatic vessels, they take in interstitial fluid. Consist of a layer of lymphatic endothelial cells that are tethered to surrounding tissues (Figure 3). Sometimes called “lymphatic capillaries” or “terminal lymphatics.”

Interstitium
The spaces between cells and tissue-specific structures such as barrier membranes. Contains fluids, proteins, etc. that are taken up into initial lymphatics.

Intrinsic Pumping
Results from active contraction of lymphatic muscle cells in the walls of collecting lymphatic vessels.

Lumped-Parameter Modelling
Use of 0D models of fluid flow variables in the equations of motion and constitutive relations. When a lumped-parameter model consists of many segments, it is not essentially different from a finite-difference solution of 1D equations.

Lymph
The fluid that flows through lymphatic vessels. Contains mainly water, with suspended proteins and immune cells.

Lymph Node
Small (1−2 cm or less), kidney-shaped organs central to immune system function. Lymph from peripheral tissues is pumped into lymph nodes by afferent collecting lymphatic vessels.

Pathogens are then filtered and screened by immune cells within the node. Fluid and immune cells move across the walls of specialised blood vessels, which include high endothelial venules. There are some 500−600 lymph nodes in the human body.

Nitric Oxide

A powerful vasodilator substance first identified in blood vessels. Also secreted by lymphatic endothelial cells as a trigger for lymphatic muscle cells to dampen contraction.

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Summary Points

- The lymphatic system returns interstitial fluid to veins to maintain fluid balance.
- Lymphatic vessels feature primary valves at the smallest level (initial lymphatics) and bi-leaflet secondary valves in larger collecting vessels that actively pump.
- Pumping can occur through extrinsic means (adjacent tissue movement) or intrinsic contractility of specialised muscle cells in lymphatic vessel walls.
- Lymphatic flow is highly viscous, featuring maximum Reynolds numbers based on average flow of about 20.
- Initial lymphatics are highly porous, and take up noxious foreign materials that are filtered and neutralised by immune cells in lymph nodes.
- All of the deadliest forms of cancer spread by sending metastatic cells into the lymphatic system.
- Disruptions to lymphatic pumping can result in a presently incurable form of tissue swelling called lymphœdema.

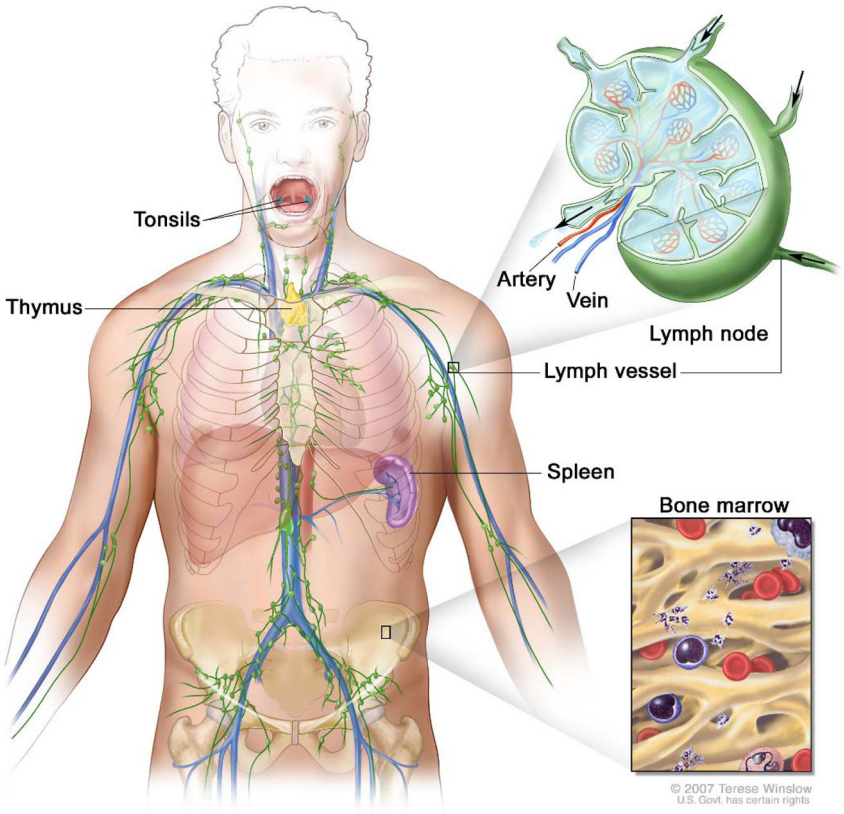

Figure 1.
The organs of the lymphatic system. Major lymph vessels in the trunk and upper limbs are shown in green (Institute).

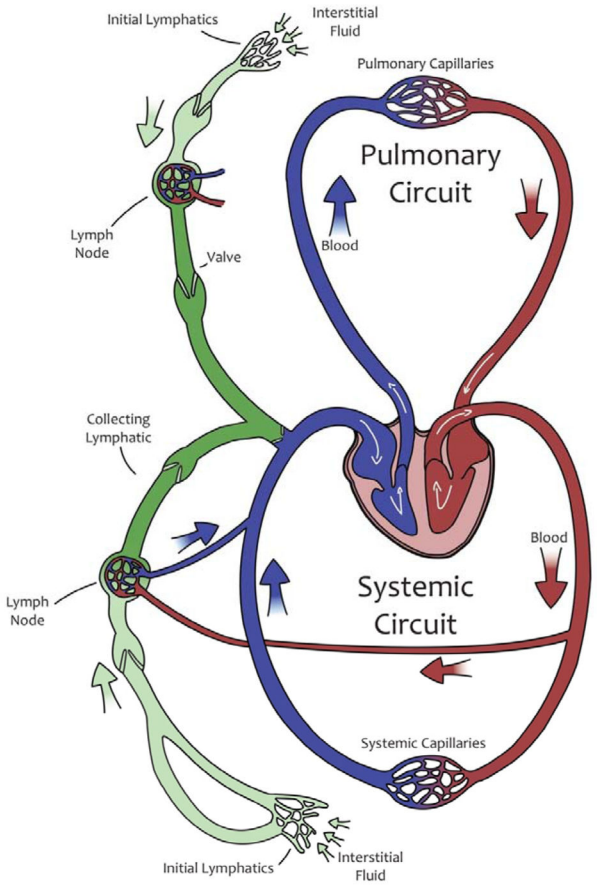

Figure 2.

Schematic of the blood circulation and lymphatic vascular system. Some lymph fluid is reabsorbed via the nodal blood circulation under normal conditions, resulting in post-nodal lymph having a higher protein concentration. Based on (Lubopitko).

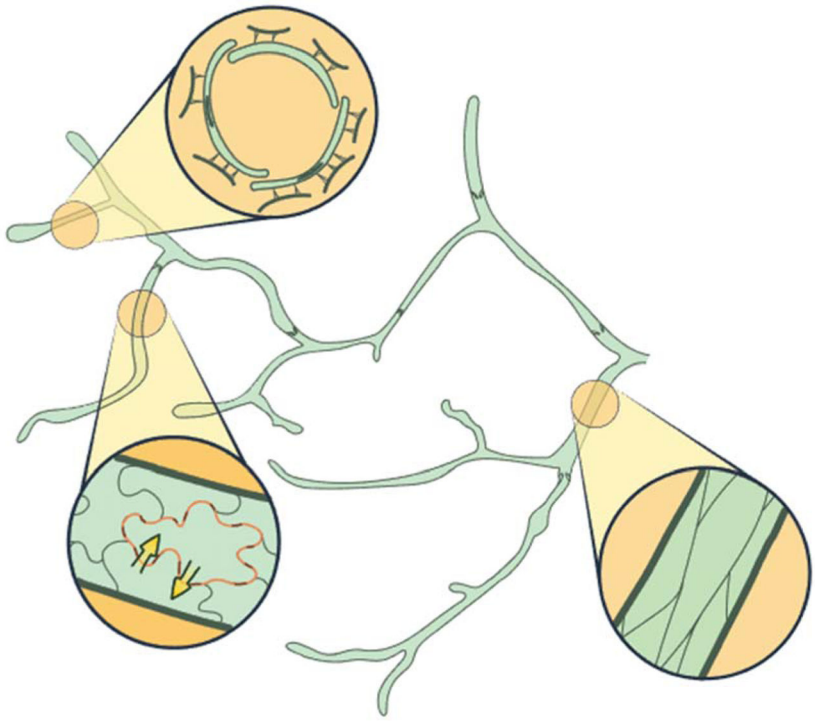

Figure 3.

A small network of initial lymphatics. Top inset shows endothelial-cell primary valves, consisting of unbonded overlaps between ECs, and anchoring filaments to surrounding fibrous tissue. Lower left inset shows characteristic oak-leaf EC configuration, with discontinuous button junctions. Little is known about where, i.e. how far along the network, such cells give way to ECs with continuous zipper junctions (inset at bottom right). Although not divided into lymphangions, initial lymphatics can also have sparse secondary (intravascular) valves. Based on published material (Baluk et al 2007, Galie & Spilker 2009, Murfee et al 2007, Schmid-Schönbein 1990a).

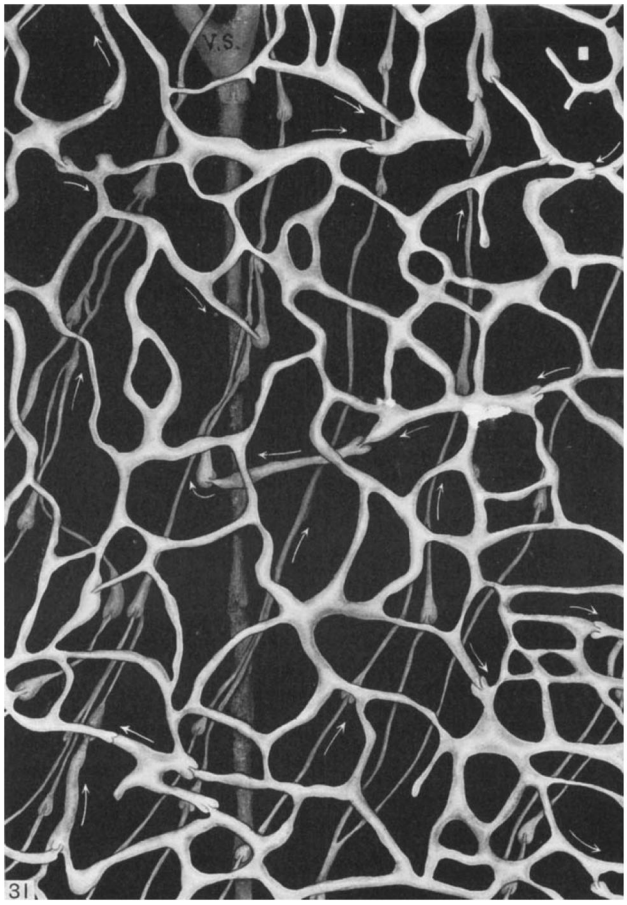

Figure 4.
Subcutaneous lymph capillaries and deeper-lying collecting lymphatics in the leg of a 130mm human fœtus. From (Kampmeier 1928). Scale: long edge = 4 mm approx.

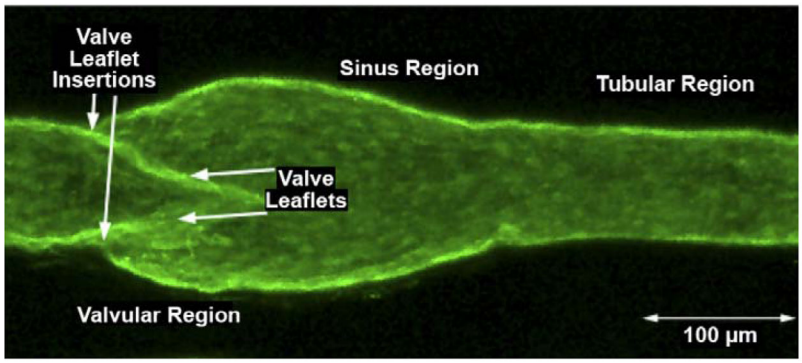

Figure 5.

A transverse section of a lymphatic valve from rat mesentery, showing the two leaflets, the sinus, and the lymphatic vessel continuing at each end. Visualisation by fluorescently tagged nitric oxide synthase expressed by the lymphatic endothelial cells. From (Bohlen et al 2009).

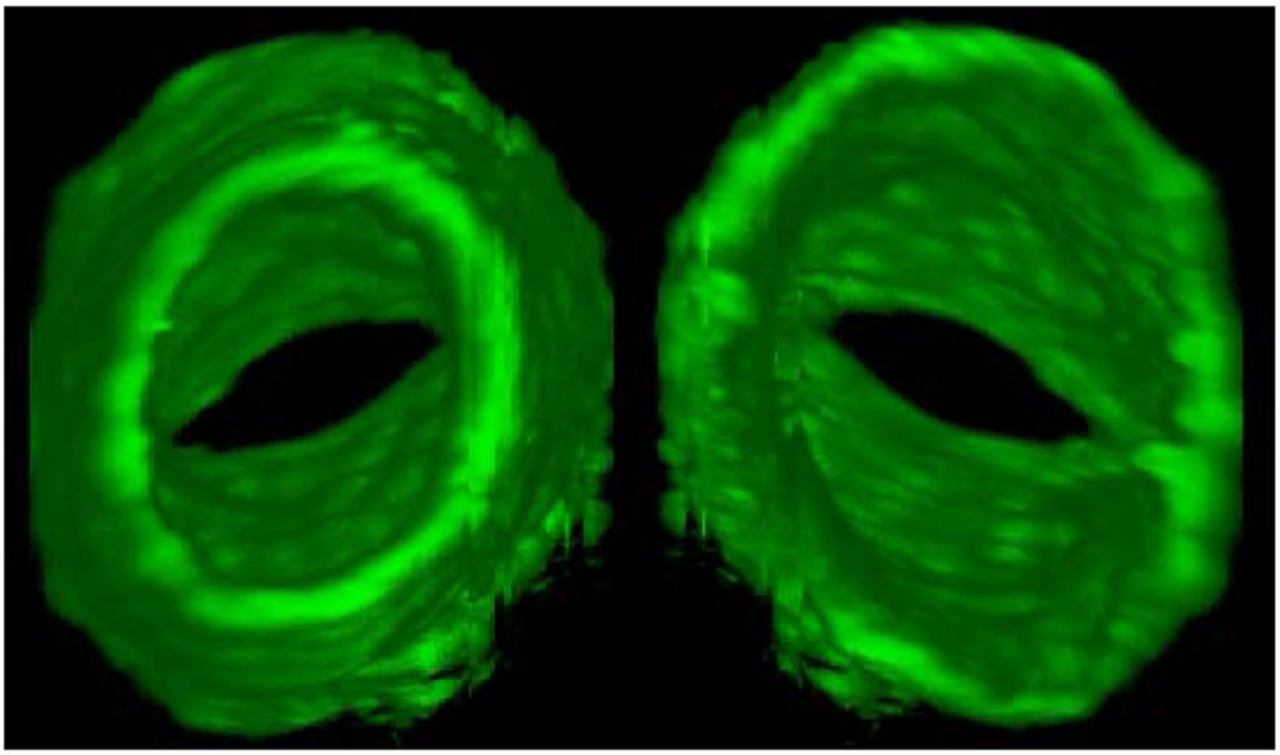

Figure 6.
Two orientations of a 3D reconstruction of a lymphatic valve from a stack of confocal images. Left, looking downstream, into the narrowing bore of the valve between the leaflets, and at the outside of the vessel wall as it tapers outwards to the middle of the sinus. Right: looking upstream, directly at the free trailing edge of the leaflets and the twin blind fluid sacs formed between the inside wall of the sinus and the outer surface of the leaflets. From (Zawieja 2009).

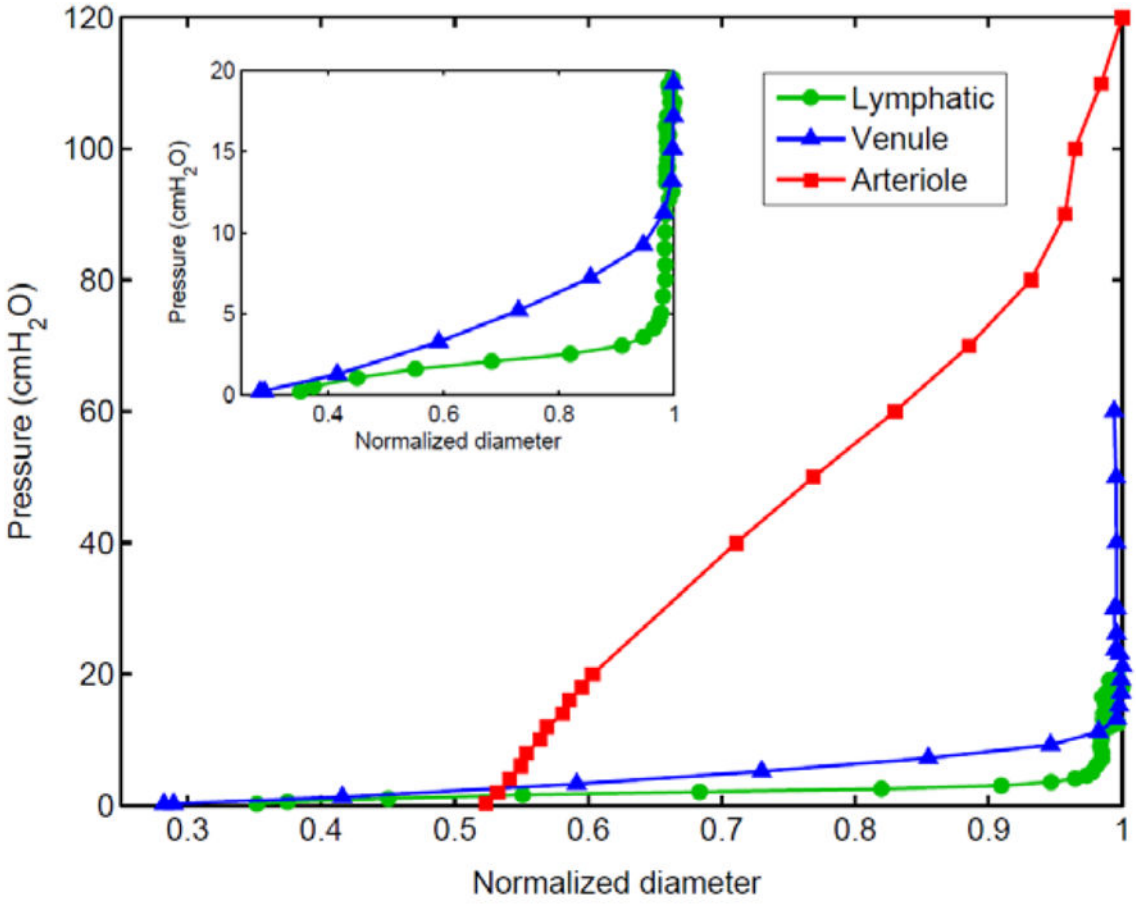

Figure 7.

Comparison of the passive pressure/diameter relation of a lymphatic, a venule and an arteriole, all from the mesentery of the same rat. Diameter is normalised to the maximum value in each case: lymphatic 267 μm, venule 278 μm, and arteriole 135 μm. The non-linearity of elastic stiffness is related to the increase in local slope between (say) $D_{norm} = 0.8$ and $D_{norm} = 1$. The increase at high distending pressure is much more for the venule and lymphatic than the arteriole, and (as shown in the inset) more for the lymphatic than the venule. Redrawn from (Rahbar et al 2012).

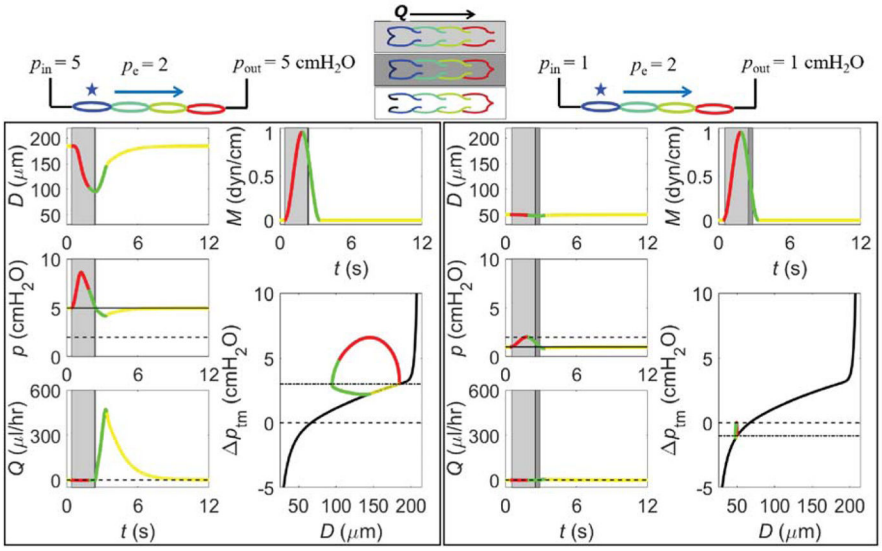

Figure 8.

Lumped-parameter modelling simulations of pumping by a chain of lymphangions contracting synchronously in the presence of positive (left) and negative (right) transmural pressure. D = diameter of the first lymphangion (indicated by star, at top), p = intra-lymphangion pressure, Q = flow-rate through the first valve in the chain, M = contraction activation, Δp_{tm} = transmural pressure, t = time. Pumping is initiated as shown in the curve of $M(t)$ by lymphatic muscle activation (red), followed by relaxation (green) and inactivation (yellow). These colours are then used in the other panels to indicate timing. Suction occurs just after 170 seconds, when the pressure inside the lymphangion dips below the inlet pressure p_a, after which the flow-rate through the first valve peaks. The loops of Δp_{tm} vs. D illustrate the time-course alongside the passive behaviour of the lymphangion (black), with the area of the loop defining the output work, and change in diameter indicating the flow-rate generated. In the presence of a negative transmural pressure (right), the Δp_{tm}-D loop is so small as to be barely visible, indicating pumping failure. Animations available in the on-line version.