The physiology of lymph production and propulsion

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The principal roles of the lymphatic system are: (1) the prevention of oedema, achieved by returning capillary filtrate and plasma proteins to the circulation; (2) immunosurveillance and the circulation of lymphocytes; and (3) the transport of particulate matter such as chylomicra. This chapter focuses chiefly on the first of these functions and emphasizes recent advances in understanding the lymphatic pump and the formation of interstitial fluid, the precursor of lymph. For reviews on interstitial fluid formation, the reader is referred to Aukland and Reed (1993),¹ Michel (1997)² and Levick (2003);³ and for reviews of lymphatic function to Yoffey and Courtice (1970),⁴ Nicoll and Taylor (1977),⁵ Olszewski (1985),⁶ Schmid-Schönbein (1990)⁷ and McHale (1995).⁸

THE TURNOVER OF EXTRACELLULAR FLUID

Water, small solutes and small amounts of plasma protein pass continuously from the microcirculation into the interstitial spaces to form interstitial fluid. At the same time, interstitial fluid is removed by a network of initial lymphatic vessels, thereby preventing the accumulation of fluid. The ionic and protein composition of prenodal lymph indicates that it is essentially interstitial fluid that has drained into the initial lymphatic network.⁹⁻¹¹ The initial lymphatic plexus drains via 'collectors' into afferent lymph trunks, which possess smooth muscle and actively pump the lymph to the regional lymph nodes. Here some fluid is absorbed, antigens are processed and lymphocytes are added. The modified, efferent lymph is actively pumped back into the circulation, chiefly via the thoracic duct, which drains into the subclavian vein in the neck. Smaller cervical trunks return lymph from the head to the neck veins. There is also evidence for lymphatic-venous communications at more peripheral locations,¹² but the amount of fluid returned by these pathways is probably small (less than 2 per cent) under normal conditions. The brain and eye lack a lymphatic system but have other specialized fluid drainage systems.

Magnitude of net lymph flow and concentration of lymph in lymph nodes

Fistulae of the human thoracic duct pour out 1–3 L lymph per day. From this it has been estimated that an adult produces up to approximately 4 L efferent lymph per day, inclusive of cervical lymph.¹ This flow of efferent lymph was traditionally taken as a measure of the rate of formation of afferent lymph and hence the net microvascular filtration rate. It now appears, however, that afferent lymph flow may be up to double the efferent lymph flow,
i.e. up to 8L per day (Fig. 3.1). This upward revision arose from the discovery that as much as half of the water in lymph can be absorbed directly into the bloodstream as the lymph passes through the regional lymph nodes. The lymph proteins are not absorbed at the same time so the protein concentration in effenter lymph is about twice that in afferent lymph. The proteins are chiefly plasma proteins that have leaked from the microcirculation into the interstitial compartment.

**Turnover times**

The human circulation contains approximately 3 L plasma. Since afferent lymph production is of the order of 4–8 L per day, the entire plasma water mass must circulate through the interstitial compartment (in the region of 12 L) and lymphatic system in 9–18 hours. In other words, the extravascular circulation of fluid is relatively rapid. Because of this, the lymphatic system has a major impact on interstitial and plasma volumes. This is demonstrated clinically by the rapid formation of lymphoedema following lymphatic obstruction. Conversely, pharmacological arrest of the dorsal lymph ‘hearts’ in amphibians causes a rapid fall in plasma volume.

**Regional differences in lymph flow and composition**

Roughly 30–50 per cent of human thoracic duct lymph is derived from the liver. Hepatic lymph contributes disproportionately to the plasma protein content of thoracic duct lymph because hepatic capillaries are discontinuous (sinusoidal) and therefore unusually permeable to protein. Table 3.1 summarises the lymph flow and composition from various tissues. The marked regional differences in lymph protein content arise from differences in local capillary permeability and filtration rate.

As well as plasma-derived solutes such as electrolytes, urea and glucose, lymph contains a low concentration of hyaluronan that has leached out of the interstitial matrix. The hyaluronan is avidly taken up and cleared in lymph nodes by highly specific receptors, probably the recently discovered receptor LYVE-1.

<table>
<thead>
<tr>
<th>Region</th>
<th>Flow [% of total thoracic duct flow]</th>
<th>L/P ratio (concentration of plasma protein in lymph relative to plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoracic duct</td>
<td>1–3 litres per day</td>
<td>0.66–0.69</td>
</tr>
<tr>
<td>Liver</td>
<td>30–49%</td>
<td>0.66–0.89</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>~37%</td>
<td>0.50–0.62</td>
</tr>
<tr>
<td>Kidneys</td>
<td>6–11%</td>
<td>0.47</td>
</tr>
<tr>
<td>Lungs</td>
<td>3–15%</td>
<td>0.66–0.69</td>
</tr>
<tr>
<td>Limbs and cervical trunks</td>
<td>&lt;10%</td>
<td>0.23–0.58</td>
</tr>
</tbody>
</table>

Adapted from reference 4.

**FORMATION OF MICROVASCULAR FILTRATE: LYMPHATIC ‘LOAD’**

In the steady state, i.e. when a tissue is neither swelling nor shrinking, the volume of fluid drained by the afferent lymphatic system must equal that produced by microvascular filtration. The filtration rate is thus the ultimate determinant of lymph flow in the steady state. Advances in understanding capillary filtration have so far passed largely unreported in standard textbooks, and are reviewed below.

**Starling principle of capillary filtration**

In his seminal paper of 1896, Starling pointed out that microvascular endothelium forms a semipermeable membrane, albeit a slightly leaky one, between plasma and interstitial fluid. Consequently, the rate and direction of
fluid exchange in capillaries and venules depends in principle on four pressures. Filtration is driven by capillary blood pressure, $P_c$, minus the opposing pressure of interstitial fluid, $P_f$. Absorption of fluid, when it occurs (see below), is driven by the colloid osmotic pressure of plasma ($\pi_p$), minus $\pi_f$. $\pi_c$ is defined here as the colloid osmotic pressure of fluid at the abluminal surface of the semipermeable pores. $\pi_c$ has traditionally been assumed to be the same as interstitial fluid colloid osmotic pressure, which is quite large as a consequence of the leaked plasma proteins. Recent evidence of pore exit microgradients has raised doubts about this assumption, as discussed below.

The crucially important Lands–Starling equation tells us that the microvascular filtration rate $J_e$ and hence lymph flow in the steady state, is determined by the algebraic sum of the four pressures multiplied by the hydraulic conductance of the wall (hydraulic permeability, $L_p$) and the wall area ($A$). Thus:

$$J_e = L_p A (|P_c - P_f| - \sigma (\pi_p - \pi_f))$$

The sum of all the $L_p A$ values in 100 g tissue is called the tissue's capillary filtration capacity (CFC). The term $\sigma$ is the reflection coefficient, which is a measure of the osmotic 'effectiveness' of the membrane. For a perfect semipermeable membrane, the reflection coefficient is 1 (100 per cent reflection of the plasma proteins), whereas for a totally leaky membrane it is zero. Most estimates of $\sigma$ for healthy capillaries lie in the range 0.80–0.95.

**Effects of microvascular pressure and inflammation**

Figure 3.2 shows how filtration into the interstitial compartment depends on microvascular pressure and plasma colloid osmotic pressure, and how the relationship is altered during acute inflammation. The control results in the lower panel show that filtration rate increases linearly with microvessel blood pressure above a certain value but is zero at a certain, relatively high pressure. The positive pressure intercept shows that there must be a pressure opposing filtration, namely the plasma colloid osmotic pressure.

The slope of the relation represents the endothelial hydraulic permeability $L_p$. The hydraulic permeability is increased many fold by inflammatory agonists such as serotonin, histamine and bradykinin because of the formation of $\mu$m-wide gaps through and between the endothelial cells. In addition, the pressure intercept shifts to the left during inflammation, indicating that the effective osmotic pressure across the wall is reduced. This is caused by a fall in the reflection coefficient $\sigma$ as gaps form in the endothelial layer. As a consequence of these changes, fluid with a high protein content pours rapidly into inflamed tissue, causing local oedema and increased lymph flow. The exudate and lymph are particularly enriched in the larger plasma proteins such as fibrinogen, which can lead to adhesions in certain clinical situations.

**Pathways across microvascular endothelium**

Recent experimental and theoretical work indicates that the microstructure of the transendothelial pathway is likely to be an important factor slowing the formation of interstitial fluid and lymph (see below). In the continuous capillaries of the skin, muscle, connective tissue and lung, the water and small hydrophilic solutes (glucose, salts, etc.) pass through a long, narrow paracellular pathway consisting of clefts approximately $20\,\mu$m wide between adjacent endothelial cells – the intercellular junctions.
In the fenestrated capillaries of the synovium, intestinal mucosa, kidney and all endocrine and exocrine glands, water and small solutes also pass through circular, ultra-thin windows in the cell body called fenestrae. The entrances to the intercellular clefts and fenestrae are covered by the glycocalyx, a layer of fibrous molecules. There is evidence that the glycocalyx is so dense (effective pore radius in the region of 4 nm) that it reflects the plasma proteins. The glycocalyx has therefore been proposed as the anatomical site of the 'small-pore system' that confers the property of semipermeability.24

A separate pathway is responsible for the slow transfer of plasma proteins across the endothelium into the interstitial fluid and lymph; this is called the 'large-pore system.'24,25 The physical identity of the 'large pores' is controversial because pores as such are rarely seen. There is considerable evidence for the transcytosis of proteins by endothelial vesicles approximately 60 nm in diameter, but other structures, such as rare transendothelial pores, may also contribute.24,25

HYPOTHESES OF LYMPH FORMATION, OLD AND NEW

We have adopted above the simple view that capillary filtration generates the fluid that enters the lymphatic system, but there is a tenaciously held, albeit poorly substantiated, alternative hypothesis of lymph formation. This is the traditional view that most of the filtrate produced by the arterial capillaries, where pressure is high, is continuously reabsorbed by the downstream, venous microvessels, where pressure is low, leaving behind a small, concentrated fraction of the original filtrate to drain away as lymph.

There is now considerable evidence against the concept of continuous downstream reabsorption in most tissues, as described below.

Adding up the four Starling pressures in venous microvessels

The first issue is whether the four traditional Starling forces actually add up to a net absorptive force in venous microvessels. Figure 3.3 shows on the left the conventional schema for lymph formation in human skin at heart level, based on micropuncture measurements of human skin capillary pressure.24 Blood pressure falls along the capillary because of the resistance to flow. By the time the blood reaches the venous capillaries, its pressure is less than the plasma colloid osmotic pressure so a sustained downstream reabsorption has been assumed. This would only be true, however, if the interstitial hydraulic and colloid osmotic pressure were negligibly small or cancelled each other out, and modern measurements of the interstitial forces show that neither is the case.24,28

The right side of Fig. 3.3 incorporates interstitial forces measured directly in the human arm.26 Interstitial pressure measured by a subcutaneous wick-in-needle is negative relative to atmospheric pressure (−2 mmHg), in keeping with the seminal studies on dogs by Guyton et al.29 Interstitial colloid osmotic pressure measured on samples obtained by a soaked subcutaneous wick is far from negligible, namely 15.7 mmHg. The combined interstitial term \( \pi_i - \sigma_i \) tips the conventional sum of the Starling pressures in the venous skin capillary towards a slight net filtration pressure (Fig. 3.3, rightside).

The example in Fig. 3.3 is just one of many data sets obtained from a variety of tissues and species in which

Figure 3.3 Traditional (left) and recent (right) hypotheses of interstitial fluid formation in the human arm at heart level. See text for symbols. (Left) Capillary blood pressures measured by micropuncture of nailfold capillaries. The two interstitial Starling pressures were thought to be roughly equal and opposite, and thus had little overall effect on exchange. Lymph formation is viewed as being a slight imbalance between upstream filtration and downstream reabsorption. Based on reference 24. (Right) Net filtration force along the microvascular axis when measurements of subcutaneous interstitial pressure (−2.1 mmHg, wick-in-needle) and interstitial colloid osmotic pressure (15.7 mmHg, soaked wick method) are taken into account. Same capillary pressure and plasma colloid osmotic pressure as on left. A, arterial end of capillary; V, venous end of capillary. Data from reference 26.
summarization of the conventional four Starling pressures reveals no net absorptive force in venous capillaries and venules. Figure 3.4 compares the blood pressure in the venules with the sum of the other three conventional Starling pressures for 13 sets of data. Without exception, the data refute the traditional hypothesis of lymph formation through upstream filtration and sustained downstream reabsorption. Instead, the balances indicate that, in most tissues, lymph is produced by a net filtration force that dwindles along the microvascular axis (Fig. 3.3, right side). The magnitude of the net filtration force is, however, probably less than the values calculated above using conventional Starling pressures because of the microgradients at the small pore exits (see below).

**Direct observations of the direction of fluid exchange at low capillary pressures**

An important test of the filtration–reabsorption hypothesis was conducted by Michel and Phillips (Fig. 3.5). These authors measured fluid exchange at controlled pressures in individual mesenteric capillaries by observing the motion of red cells after stopping the blood flow. At pressures typical of arterial capillaries, filtration was observed, as expected. When the pressure was reduced to venous levels well below the plasma colloid osmotic pressure, the direction of fluid exchange altered with time.

**Figure 3.5 Effect of reducing pressure to venular levels upon fluid exchange across single capillaries in the saline-superfused frog mesentery. Absorption occurred transiently at capillary pressures below the plasma colloid osmotic pressure (approximately 30 cmH₂O) (open circles). When the pressure was held at venular levels for several minutes to establish a steady state, absorption no longer occurred (filled circles). This was attributed chiefly to a rise in pericapillary $\pi$, in response to the change in transendothelial flow (inset sketches: thin arrows, water flow; thick arrow and dots, protein). Data from reference 30.**
Immediately after the pressure reduction, a transient absorption of interstitial fluid was observed, in accordance with the Starling principle. Transient absorption is medically important during hypotensive episodes such as haemorrhagic shock because it contributes to an 'internal fluid transfusion' that boosts the depleted plasma volume. When the capillary was perfused at the same, low pressure for several minutes, however, the process of absorption dwindled and ceased, even though fluid was freely available for absorption, since the mesentery was superfused with saline. Sustained absorption could not be produced no matter how much the microvascular pressure was reduced.

**Abluminal colloid osmotic pressure: why downstream absorption is not sustained**

The reason that downstream absorption cannot in general be sustained is that the colloid osmotic pressure on the abluminal side of the capillary pores is not a fixed quantity but a variable whose magnitude depends on the capillary filtration rate (Fig. 3.6). This is well established on the macroscopic scale; that is to say, bulk interstitial protein concentration varies inversely with microvascular filtration rate, \(^{13,27}\) which is in accordance with the predictions of the molecular sieving theory. \(^{30}\) The greater the filtration rate, the greater the dilution of the interstitial proteins (and \(\pi_i\)), as explained in the upper panel of Fig. 3.6. Conversely, the lower the filtration rate, the higher the interstitial protein concentration and \(\pi_i\). (see insets to lower panel, Fig. 3.6). At zero filtration rate, the plasma and interstitial concentrations will eventually equilibrate if the capillary permeability to protein is finite (Fig. 3.6, lower panel). If fluid is reabsorbed, \(\pi_i\) will rise even more quickly because reflected interstitial protein begins to accumulate around the capillary (see insets to Fig. 3.5) and also inside the paracellular pathway (see below). Thus, the absorption term \(\pi_p - \pi_i\) falls when filtration is reduced or reversed, until a net absorption force no longer exists. Therefore, unless the tissue has certain structural specializations (see the following section), fluid absorption is a self-cancelling process, contrary to the filtration-reabsorption hypothesis of lymph formation.\(^{2,3,31}\)

This new perspective on fluid exchange, namely a state of dwindling filtration along the microvascular axis, reinforces the importance of lymphatic function because lymphatic drainage emerges as the only effective means of removing capillary filtrate from most tissues in the steady state.

**Exceptions to the rule: microvessels that can sustain fluid absorption**

In contrast to the microcirculations in Figs 3.3–3.5, the fenestrated renal peritubular capillaries and gut mucosa capillaries (after water ingestion) absorb fluid for long periods, this being an inherent part of the normal function of these tissues. Sustained absorption is made possible by breaking the coupling between the interstitial colloid osmotic pressure and the capillary filtration rate so that the relation in Fig. 3.6 no longer applies. The coupling is broken by a second source of water input into the interstitial compartment. Intestinal colloid osmotic pressure is prevented from rising during fluid absorption because the interstitial space is continuously flushed by an independent stream of fluid. The independent stream comprises water pumped through the interstitial compartment by epithelium in the kidney and mucosa, and the afferent lymph stream pumped through lymph nodes.

A second factor that helps to sustain the absorptive process in the above tissues is the presence of fenestrations. Interstitial plasma proteins that are reflected during the reverse ultrafiltration (fluid absorption) across the fenestrations can readily diffuse away from the unconfined fenestrations into the interstitium. This is very different from the situation deep inside the long,
narrow intercellular clefts of non-fenestrated capillaries, as described in the next section.

**Paradox of low lymph flow and insights into pore exit microgradients**

As noted earlier, the production of afferent lymph may be as much as double the earlier estimates (see Fig. 3.1), a finding that is in keeping with the absence of sustained, downstream reabsorption in most tissues. The rate of afferent lymph production is nevertheless so small that its explanation presents a serious challenge. Overnight lymph production by the human foot, for example, is only 0.22 mL/100 g per hour. Calculations of the expected microvascular filtration rate, based on the relation \( J_e = CFC \times (\text{sum of Starling pressures})\), give values that are an order of magnitude bigger than the observed lymph flows. This can be called the 'low lymph flow paradox.' Several factors may contribute to this paradox.\(^{31}\)

**Arteriovenous gradient of permeability**

The magnitude of CFC is usually determined by a venous congestion experiment and may be unduly weighted by venular permeability. The latter is up to seven times greater than capillary permeability. In arterial capillaries, where the filtration pressure is highest, the wall permeability is low. Thus, filtration calculations using a global CFC and averaged Starling pressures may overestimate the net filtration rate.

**Arteriolar vasomotion**

Vasomotion may influence fluid balance in certain tissues. Intermittent contraction of the arterioles at 2–3 cycles per minute repeatedly stops the blood flow through the capillaries in some muscle. During this cessation of flow, the capillary pressure will inevitably fall towards venular levels. This could cause short but repeated periods of transient fluid absorption.\(^{32}\) If this idea proves correct, the traditional view of spatial (axial) filtration–reabsorption near-balance would be replaced by one of temporal (intermittent) filtration–reabsorption near-balance. The intermittency of capillary flow is, however, much less marked in most tissues than it is in skeletal muscle. In the skin of supine adults, for example, blood flow in individual capillaries stops for only 4 per cent of the time, rising to 35 per cent in the skin of the foot during orthostasis.\(^{34}\)

**Pore exit microgradients**

A step towards resolving the low lymph flow paradox came with advances in understanding the protein concentration gradients at fenestral exits\(^{35}\) and within the paracellular clefts of continuous capillaries.\(^{2}\) Colloid osmotic pressure is generated across small pores of radius approximately 4 nm (see above). Since the pore exit is probably the underside of the glyocalyx, which overlies both the long, deep intercellular cleft and the very shallow fenestration, the relevant liquid osmotically is not the bulk interstitial fluid per se but the fluid at the pore exit, which, in the case of continuous capillaries, means the fluid deep inside the 750 nm-long intercellular cleft (Fig. 3.7). To reach this point, interstitial protein has to diffuse up the cleft against a continuous stream of filtrate. Because of the continuous washout of proteins, the protein concentration and osmotic pressure within the cleft at the pore exit, during filtration across vessels of high hydraulic permeability, is up to an order of magnitude smaller than in the interstitial fluid.\(^{36}\) The effective osmotic pressure across the pore is then closer to plasma colloid osmotic pressure, i.e. \( \pi_p - 0 \), than to the conventional Starling term \( \pi_p - \pi_i \) bulk interstitial colloid osmotic pressure. The true filtration rate is consequently lower than that predicted by the Landis-Starling equation, and lymph production is much reduced. Analogous reasoning also applies, albeit to a less extreme degree, to the exit

![Figure 3.7 Sketch of the endothelial cell junction (left on the left). The small-pore exit on the underside of the glyocalyx 'sees' intracleft fluid of lower protein concentration (C<sub>pore exit</sub>) and osmotic pressure (\( \pi_i^p \)) than bulk interstitial fluid (C<sub>interstitial</sub> = \( \pi_i \)). See text for details. Plasma proteins reach the interstitial fluid chiefly via the 'large-pore' system (probably vesicles). The true force opposing filtration, \( \pi_p - \pi_i^p \), is larger than \( \pi_p - \pi_i \) bulk. This may contribute to the low rate of lymph formation rate. Adapted from references 2 and 36.](image-url)
region around the fenestrations and to capillaries of lower hydraulic permeability such as muscle capillaries.

If the above reasoning is correct, it follows that bulk interstitial colloid osmotic pressure should have less effect on filtration rate than does $\pi_c$. This inference is supported by experimental evidence. Changes in bulk interstitial colloid osmotic pressure have less effect on fluid exchange than do changes in $\pi_c$, in synovial joints, where the capillaries are fenestrated. In an elegant and dramatic test of the glycoalyx/junctional strand model, Hu et al. showed that raising the bulk interstitial colloid osmotic pressure caused almost no change in filtration rate across continuous mesenteric capillaries, in agreement with the pore exit microgradient model.

If the above ‘pore-in-cleft’ model is correct, the traditional filtration–reabsorption hypothesis becomes increasingly untenable for such capillaries. Interstitial plasma protein will be washed into the cleft during the early, transient stage of fluid absorption. Reflection at the interface between pore and cleft raises the local protein concentration and osmotic pressure in the confined space. This gradually reduces the osmotic pressure difference that is driving the absorption process. Whether an equally extreme process would occur in continuous capillaries of lower hydraulic permeability, such as those in skeletal muscle, awaits investigation.

### PHYSIOLOGICAL AND PATHOLOGICAL FACTORS AFFECTING LYMPHATIC LOAD

Tissues with a high capillary density (large endothelial area) and high hydraulic permeability (fenestrated and discontinuous capillaries), such as liver, gut and kidneys, generate more lymph than those with a low density of continuous capillaries, such as skin, fat and connective tissue.

### Inflammation

Inflammation greatly increases local microvascular filtration (see Fig. 3.2). As a result, the local lymph flow is increased. In the case of snake or spider envenomation, the increase in lymph flow caused by the bite is highly dangerous. Lymph flow from the bitten limb can be greatly reduced by proximal compression of the lymphatic trunks using a cuff or bandage, although compression pressure of 40–70 mmHg is required to overcome the contractile power of the lymphatics.

### Hypoproteinaemia

Hypoproteinaemia raises lymph flow because plasma colloid osmotic pressure is the only force that acts to retain water within the plasma compartment. Experimental reductions in plasma protein concentration cause marked increases in lymph formation. The severe hypoproteinaemias associated with the nephrotic syndrome, hepatic failure and malnutrition cause the capillary filtration rate to exceed the maximum lymphatic drainage rate, leading to clinical oedema.

### Increased capillary pressure

Raised capillary pressure is a common cause of increased microvascular filtration and thus lymph flow. Capillary pressure is increased by exercise, gravity (posture) and many pathological conditions, as follows.

- **Exercise** causes arteriolar dilatation, which raises the local capillary pressure. This leads to marked increases in lymph flow from exercising human legs and a 3–6-fold increase in the clearance of labelled interstitial colloid.

- **Pathological conditions** that raise microvascular pressure chronically and lead to oedema include cardiac failure, deep venous thrombosis, portal hypertension and overt transfusion. The enhancing effect of venous congestion on lymph flow has been demonstrated many times since the seminal work of Heidenhain in 1891 for example by Olszewski et al., Aarli et al. and Renkin et al.

Leg lymph flow alters during orthostasis. Gravity raises the filtration pressure in the microvessels below heart level, which form the majority of vessels in man. The weight of the vertical column of blood raises the local arterial and venous pressures in proportion to the vertical distance. Micropuncture studies on human toe capillaries show that capillary pressure increases with distance below heart level but does not rise by as much as the arterial and venous pressures. This is because an active, precapillary vasoconstriction (postural vasoconstriction) helps to protect the capillaries from the increased arterial pressure. Postural vasoconstriction shifts the capillary pressure towards its lowest limit, which is the current venous pressure. Even so, $P_c$ reaches around 120 cmH$_2$O (95 mmHg) in the human foot during standing. This increases the lymph formation rate and raises the initial lymphatic pressure 2.5-fold.

The lymph protein concentration falls because of the ultrafiltration mechanism illustrated in Fig. 3.6.

The dermal lymphatic plexus in the human leg appears to be more highly developed than in the forearm, which implies that the lymphatic system has adapted anatomically to cope with a high lymph load in the human leg. If the lymphatic system fails to remove the interstitial fluid as fast as it forms, oedema of the leg develops. This increases further the local interstitial fluid pressure and raises the initial lymphatic pressure in the leg.

### Safety factors that limit lymphatic load

Several factors help to protect tissues against excessive capillary filtration and oedema. Postural cutaneous vasoconstriction has been mentioned above. The resulting sluggish blood flow allows a substantial local haemoconcentration to develop during plasma transit through the capillary. This raises the colloid osmotic
pressure of the plasma entering the distal microcirculation, reducing the venular filtration rate.

Another important protective mechanism in the legs is the calf muscle pump, in which movement lowers the venous pressure (and with it capillary pressure) to around 30 mmHg during walking. The pump fails if the venous valves become incompetent, resulting in chronic ambulatory venous hypertension and oedema. Reduction of the interstitial colloid osmotic pressure by dilution has to date been seen as an important factor opposing filtration (see Fig. 3.6). Whereas this view may still be true for some tissues, the issue may require re-evaluation for other tissues in view of the dramatic findings of Hu et al., described earlier. Finally, a rise in interstitial fluid pressure by 1 mmHg or so opposes filtration. Despite the above protective mechanisms, however, some increase in local filtration is inevitable in response to venous congestion, orthostasis, etc.

We next consider how the microvascular filtrate crosses the interstitial compartment to reach and enter the lymphatic system.

INTERSTITIAL COMPARTMENT: PRESSURE, VOLUME, FLOW AND EXCLUSION

Interstitial compliance curve (pressure–volume relation)

Interstitial fluid pressure, $P_f$, affects both capillary filtration and drainage into the lymph vessels. Interstitial fluid pressure is a function of interstitial fluid volume. A plot of pressure $P_f$ as a function of volume is highly non-linear in a loose connective tissue such as the subcutis. The relation is steep at normal tissue hydration, small changes in fluid volume causing marked changes in the subatmospheric $P_f$. This ‘buffers’ the fluid exchange across the microcirculation. At the increased volumes typical of overhydration and oedema, however, the value of $P_f$ is just above atmospheric pressure and the compliance curve is very flat, so that large volumes of fluid can accumulate with little increase in $P_f$ to oppose filtration. Interstitial pressure is typically around $-2$ mmHg in normally hydrated human subcutis (a common site for oedema) and between $+1$ and $+2$ mmHg in oedematous subcutis.

Interstitial resistance to flow, effective ‘pore’ size and pitting

Although water accounts for two thirds or more of the interstitium, it cannot normally be easily displaced because the interstitial matrix has a low hydraulic conductivity. This arises from the hydraulic drag exerted by long chains of glycosaminoglycans and other interstitial biopolymers. The polymer chains subdivide the $\mu$m-wide spaces between the cells into submicroscopic, interconnected voids. The average size of these irregular spaces can be characterized by the ratio of the fractional water content (void volume) to the surface area of the polymer chains. This ratio is called the ‘mean hydraulic radius’ of the matrix and ranges from 3 nm in articular cartilage to approximately 300 nm in vitreous humor. Because the mean hydraulic radius is so small in most tissues, the interstitial hydraulic conductivity is likewise small, namely $10^{-10}$ to $10^{-12}$ cm/s per dyn. This is nevertheless sufficient to allow the capillary filtrate to percolate slowly through the matrix to the lymphatic plexus under a small pressure gradient, which can be as little as 0.004 cmH$_2$O/$\mu$m. The low interstitial conductivity, however, prevents the rapid displacement of the fluid when an external force is applied. Consequently, normal tissue does not ‘pit’ in response to brief (1 minute) applications of external pressure.

Protein transport and exclusion in the interstitium

Escaped plasma proteins and water are transported through the interstitial matrix towards the draining lymphatic system by convective transport or ‘wash-along’. The characteristic transport distance is determined by the width of a unit ring in the ‘chicken wire’ network of initial lymphatic capillaries, namely in the region of 500–1000 $\mu$m in human skin. Small solutes such as fluorescein (379 Da) diffuse quickly across this space, passing from the plasma to the initial lymphatic in 2–3 minutes in rat mesentery. Macromolecules take much longer; labelled intravascular albumin, for example, takes 2–3 days to reach a steady-state concentration in the interstitium and lymph of the rabbit leg. Such macromolecules can experience considerable restriction to movement through the interstitium because of the narrowness of the voids between the matrix polymer chains.

The narrowness of the interchain voids not only restricts the diffusional velocity of macromolecules, but also excludes macromolecules from some of the interstitial water space. Albumin, for example, is excluded from 20–50 per cent of the interstitial water space in subcutis and muscle. As a result, the effective interstitial protein concentration, and with it the interstitial colloid osmotic pressure, is greater than the apparent concentration calculated as protein mass/total water volume. The greater the matrix glycosaminoglycan concentration, the smaller the average void size and the greater the degree of macromolecular exclusion. Conversely, when glycosaminoglycan concentration falls as a consequence of oedema formation, the exclusion effect is reduced. An increased fraction of the water space is then available to the proteins so that the fall in effective protein concentration is ‘amplified’, i.e. the dilution is greater than expected from the increase in tissue water seen. This causes an amplified fall in interstitial colloid osmotic pressure, which may help to attenuate the filtration rate in some tissues.

It has been suggested that, rather than fluid percolating diffusely through the interstitial matrix, flow might be carried preferentially by definable prelymphatic,
trans-interstitial pathways. The structural evidence for such pathways has, however, been challenged. In addition, the magnitude of interstitial conductivity provides no grounds for postulating such a pathway. The observation that large proteins pass across the interstitial compartment into the lymphatic system in a shorter time than small proteins is sometimes cited as indirect evidence of preferential flow channels, but the difference in transit time may simply be related to the greater distribution volume that is available to the smaller solute. In support of this, solute transit time is found to be size dependent even in a homogeneous glycosaminoglycan solution in vitro.

FILLING OF LYMPHATIC CAPILLARIES

Having followed the capillary ultrafiltrate through the interstitial compartment, we consider next how it enters the lymphatic system. It needs to be stated plainly at the outset that, although plausible inferences can be drawn concerning how lymphatics fill, the supporting evidence is often sketchy.

Openings in the vessel wall

The initial lymphatic network is usually a blind-ending system of anastomosing lymphatic capillaries approximately 50–200 μm in diameter (e.g. skin), or sometimes a system of closed end-bulbs (e.g. bat wing). A notable exception is provided by the open-ended lymphatic stomata of parietal pleura, especially over the diaphragm. These drain away the pleural fluid. The wall of a lymphatic capillary consists of a thin layer of endothelium and an incomplete basal lamina, with no smooth muscle investment in most tissues. Crucially, some of the endothelial intercellular junctions are 14 nm or more wide. This renders the wall highly permeable to water, interstitial plasma proteins and even fine particulate matter. Human skin microlymphatic vessels are freely permeable to small solutes and 40 kDa dextran, but less so to dextrans of 150 kDa or more.

Endothelial flap valves and tethering filaments

The endothelial cell junctions run very obliquely in lymphatic capillaries, as a result of which the margins of adjacent cells overlap. It is thought that this enables the junctions to act as flap valves. When the interstitial pressure exceeds the intraluminal pressure, the flap on the luminal side is pushed open, and interstitial fluid flows into the lymphatic lumen. Conversely, when the lymph pressure rises above the interstitial fluid pressure, the luminal flap is pressed onto the abluminal flap, preventing a reflux of lymph into the tissue.

The outer surface of the lymphatic capillary is tethered to the surrounding tissue by radiating fibrils called anchoring filaments. These probably act to prevent lymphatic collapse when the interstitial pressure is high, as in oedema.

Fluid velocity in lymph vessels

Fluid velocity in lymphatic capillaries is 5–500 μm/s in human skin and mouse tail. The initial network drains into collecting vessels, where semilunar valves first appear, directing the flow centrally. The larger vessels acquire a contractile coat of smooth muscle and have fluid velocities of around 33–50 μm/s (2–3 cm/min) in human and dog limbs.

What force fills the lymphatic capillary?

Initial lymphatic pressures

The answer to the above question is poorly resolved in most tissues. The interstitial fluid is probably driven through the lymphatic intercellular gaps by a very small, intermittent, hydraulic pressure gradient (1 cmH₂O or less), such as that illustrated in Fig. 3.8. Supporting evidence has been found in bat wing and rabbit pleura. The lymphatic end-bulbs in the bat wing are, however, actively contractile, unlike most lymphatic capillaries. In the rabbit pleural space, the normal liquid pressure is around −3.9 mmHg, and the diaphragmatic lymphatics can suck in fluid down to −(8–9) mmHg. Their capacity to pump fluid out of the pleural cavity may be powered by the contraction of the diaphragm.

In contrast, in the exposed rat mesentry, which has neither contractile initial lymphatics nor contiguous
skeletal muscle, the pressure gradient does not appear to favour filling; the measured lymphatic pressures of 0–4 mmHg are slightly higher than the interstitial fluid pressure. Occasional, transient dips of mesenteric initial lymphatic pressure to −1 cmH$_2$O have been noted, which might induce intermittent filling. In human skin, the interstitial fluid pressure is between −2 mmHg (chest and arm) and +1 mmHg (foot). Although the mean pressure in fluorescein-filled dermal lymphatic capillaries is higher than this (2.6 ± 2.8 mmHg), pressure oscillations with transient dips as low as −7 mmHg have been observed. Some of the difficulty experienced in demonstrating a gradient in favour of fluid uptake may arise from the high conductance of the initial lymphatic wall; because of the high wall conductance, the required pressure difference may be as little as 0.12 cmH$_2$O. The mechanisms proposed for the intermittent generation of a favourable pressure gradient across the walls of non-contraction lymphatic capillaries are as follows:

1. The vessel may fill intermittently by a recoil process akin to the filling of a rubber teat. An external squeeze resulting from tissue movement first empties the lymphatic capillary proximally. Then elastic recoil, aided by the special tethering filaments, creates a transient, low luminal pressure that establishes a filling gradient.

2. An intermittent, subatmospheric pressure might be created in contractile, proximal vessels as they relax and recoil. Flow into the relaxing segment from the periphery might then reduce the pressure in the feeding lymphatic plexus. Set against this, however, is the observation that pulsating pressures in the contractile lymphatics of exposed mesentery are suprather than subatmospheric, and increase by approximately 2 cmH$_2$O (1.5 mmHg) as lymph progresses centrally from one intersegment to the next.

3. Many small lymphatic vessels, including initial lymphatics in muscle, lie alongside pulsating arterial vessels. Transmitted pulsations may help to pump fluid along non-contraction lymph vessels.

4. A complex uptake mechanism based on translymphatic osmosis was proposed by Casley-Smith, but this currently lacks experimental support.

**COUPLING BETWEEN LYMPH FLOW AND CAPILLARY FILTRATION RATE**

A fixed rate of lymph drainage cannot guarantee tissue volume homeostasis. To achieve this, the lymph flow has to be coupled (linked) to the net microvascular filtration rate. In other words, the lymphatic system needs, somehow, to be responsive to the microvascular filtration rate. Coupling is an important property because it enables a change in filtration rate to be matched in a relatively short time by a change in lymphatic drainage rate. Coupling has been demonstrated in studies of thoracic duct lymph flow, canine hindlimb, lung, diaphragm, bat wing, mesentery and rat tail. In all cases, an increase in fluid load on the interstitium, caused by increased filtration, is followed by an increase in lymph flow from the tissue.

What mechanism produces this coupling? Interstitial fluid pressure is one possible coupling factor because it is affected by microvascular filtration rate and is itself the 'uphill' end of the pressure gradient into the lymphatic capillary. The increase in local lymph flow in response to increased fluid pressure in the pleural cavity is shown in the upper panel of Fig. 3.9. The lower panel of Fig. 3.9

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**Figure 3.9** (a) Effect of subatmospheric fluid pressures on lymph drainage from the pleural and peritoneal cavities in rabbits. Based on reference 72. (b) Femoral lymph flow during progressive experimental oedema of the tissue around a rabbit knee. Fluid was driven from the joint cavity into the peri-articular tissue at increasing intra-articular pressure over several hours. Unpublished data, S. Saboratnam and J.R. Levick.
shows that, when flow into an interstitial compartment is increased to the point of oedema, the lymphatic drainage rate responds non-linearly and tails off at high fluid loads. This presumably underlies oedema formation since oedema develops only when the lymphatic drainage rate is slower than the input of fluid to the interstitial compartment.

Several groups have argued that interstitial volume rather than pressure is the coupling factor, at least in oedematous tissue.11,64,80 Interstitial fluid volume and pressure are themselves interlinked through the compliance curve described earlier. As a result of the non-linearity of the compliance curve, there is little change in pressure in response to large increases in volume in an oedematous tissue, so pressure would be a poor coupling mechanism in oedema. Increased interstitial fluid volume may pull open the intercellular junctions by increasing the tension in the anchoring filaments,89 thus facilitating lymphatic filling.

Because the lymphatic system comprises differing elements plumbed in series (non-contractile initial lymphatics and contractile trunk segments), it is vital that some form of coupling should also exist between the initial lymphaticplexus and the contractile draining vessels, as well as between one segment of contractile vessels and the next. This aspect of coupling involves an active 'distension-pumping' relation, and is described next.

**ROLES OF PASSIVE AND ACTIVE TRANSPORT IN INITIAL LYMPHATIC PLEXUS VERSUS CONTRACTILE LYMPH TRUNKS**

Three mechanisms have been proposed to explain lymph propulsion in mammals: (1) 'vis a tergo' (force from behind), i.e. lymph might flow from a region of higher pressure in the periphery to one of lower pressure in the central veins, (2) extrinsic pumping and (3) intrinsic pumping.

**Vis a tergo**

This mechanism seems unlikely for at least two reasons. First, even at the relatively low flow encountered in the lymphatic system, a pressure gradient as high as 30 mmHg between lymphatic capillaries and central veins could be required to overcome the resistance to flow. This is unrealistic under normal conditions. Second, this mechanism implies that lymph pressure should drop as one moves from the lymphatic capillaries to the thoracic duct. Where direct measurements have been made, such as those of Zweifach and Prather,85 the opposite has been found to be true. These authors found that pressure rose from 0.6 mmHg in ducts near the terminals to more than 40 mmHg in the larger collecting ducts. Such measurements are consistent with a pump rather than a vis a tergo. The next question is therefore whether the pump is an extrinsic or an intrinsic one.

**Extrinsic pumping**

The idea that the massaging effects of muscular contractions and arterial pulsation are primarily responsible for lymph propulsion is so intuitively appealing that it has been widely, if uncritically, accepted as the main mechanism of lymph flow. Thus, Generisch observed in 187192 that very little lymph flowed from resting limbs, whereas Heidenhain found, in 1891,93 a threefold increase in thoracic duct flow in response to passive movement of a dog's hind limb. Many other studies have confirmed the role of muscular contraction or passive limb movement in promoting lymph flow.93-95 These results are usually cited as evidence that the role of movement is to enhance propulsion by a 'muscle pump' action similar to that found in veins, and it was on this basis that early reviews, such as those of Drinker and Yolfe96 and Courtois and Simmonds,97 argued that lymph transport was essentially passive. An alternative interpretation of the above evidence is, however, that passive movement enhances the entry of interstitial fluid into the lymphatic terminals, lymph propulsion thereafter depending on the intrinsic pumping of the larger lymph ducts.

**Intrinsic pumping**

Many observations have been made of spontaneous contractility in lymphatic vessels.98-100 Smith101 argued convincingly, on the strength of experiments conducted on mice, rats and guinea-pigs, that spontaneous contractions are an important means by which lymph is propelled. He suggested that even if lymph were propelled from the extremities by passive movement, it would remain pooled in the proximal, freely distensible lymph vessels of the leg and thigh in the absence of an intrinsic propulsive mechanism.

Studies with anaesthetized and conscious sheep strongly support this argument.102 Intermittent compression of the hoof region significantly increased lymph flow from a cannulated metatarsal vessel, but when the intermittent compression was applied over the lymphatic itself, there was no significant increase in flow. This was taken to mean that the lymph duct's intrinsic contractions were keeping it fairly empty so that there was little fluid upon which the external compressive forces could act. When the metatarsal duct was cannulated at both ends and the inflow connected to a constant pressure reservoir of saline, intermittent compression over the (now filled) lymphatic vessel was very effective in promoting flow. These results indicate that external compression is likely to be more effective when the intrinsic pump fails than when it is functioning normally. It is interesting to note, however, that when these animals were allowed to recover, there was no correlation between walking movements and fluid propulsion. This indicates that normal walking
movements in sheep do not assist pumping in the metatarsal lymphatics, even when these are filled with fluid.

**PUMP ACTIVITY OF LYMPHATIC SEGMENTS**

**The pump cycle**

It is now generally agreed that, in normal healthy tissue, passive movements are important only in the initial or pre-contraction lymphatic vessels. Once the fluid has entered the muscular collecting ducts, it is propelled mainly by the intrinsic contractions of the smooth muscle in their walls. The pumping cycle has interesting similarities to that of the heart (Fig. 3.10). A given segment first enters a diastolic filling phase during which the peripheral inflow valves are open and the proximal outflow valves are closed. As contraction begins, a small rise in pressure closes the peripheral inflow valves and isovolumetric contraction quickly raises the pressure until this forces open the proximal outflow valves. This is followed by the ejection phase, which delivers a ‘stroke volume’ into the upstream segment. As relaxation supervenes, closure of the outflow valve marks a brief isovolumetric relaxation phase, during which the pressure quickly falls. The inflow valves then open, and diastolic filling recommences.

How are the force (stroke volume) and frequency (rate) of intrinsic pumping controlled? How is the rate of fluid delivery from the initial vessels (which may be to a large extent determined by random extrinsic movements) coupled to the pumping rate of the contractile vessels? The main mechanism appears to be an autoregulatory one, although this can be modulated by extrinsic regulatory factors such as autonomic nerve impulses and circulating vasoactive substances.

**Autoregulation**

Lymphatic vessels respond to increased distension by increasing the force and frequency of their spontaneous contractions. The elevation in transmural pressure can be caused by either an increased preload (an increase in the rate of lymph production) or an increased afterload (an increase in the upstream resistance to flow). There is an optimum value for distending pressure beyond which flow decreases as a result of a drop in stroke volume (Fig. 3.10), which is reminiscent of the decompensation phase of the cardiac Starling curve. Flow falls even though frequency continues to increase.

Figure 3.11 shows the effect of changing the transmural pressure from 0 to 21 cmH\(_2\)O in an isolated, doubly cannulated bovine mesenteric lymphatic. Flow increased with increasing transmural pressure up to a maximum at 8 cmH\(_2\)O as a result of an increase in both frequency of contraction and stroke volume. A further increase in transmural pressure above 10 cmH\(_2\)O caused flow to decline because the stroke volume decreased, despite a continued increase in frequency of contraction. These results have been confirmed in the conscious sheep, except that considerably higher pressures could be tolerated before the pump began to fail. Human leg lymph vessels can pump to at least 40–50 mmHg, and an external pressure of 40–70 mmHg is required to prevent the transmission of snake venom up the human limb lymphatic system.

![Figure 3.10](image-url)  
**Figure 3.10** Pressure-volume cycles in sheep mesenteric lymph vessel at increasing diastolic distensions. TP, transmural pressure. The dashed loop shows increased contractility and ejection fraction after a haemorrhage. Adapted from reference 103.

![Figure 3.11](image-url)  
**Figure 3.11** Flow, stroke volume and frequency of contraction in a spontaneously pumping isolated bovine mesenteric lymphatic during an experiment in which transmural pressure was increased from 0 to 21 cmH\(_2\)O. Data of N. McHale.
Nerves and circulating vasoactive substances

It has been known, at least from the eighteenth century, that lymph vessels are innervated, but the significance of this is not yet fully understood. Lymphatic trunk vessels are known to have noradrenergic, purinergic, cholinergic and peptidergic nerves. The first two of these are known to be excitatory, whereas the role of the last two is unclear.

Stimulation of the sympathetic nerves, both in isolated lymphatic vessels and in the intact animal, increases the frequency of spontaneous contractions and hence lymph flow. This occurs even when blood flow is depressed, indicating that sympathetic stimulation can enhance pumping even when input to the initial lymphaticplexus is reduced. A direct effect of nerve stimulation on lymphatic pumping can be confirmed by using a preparation of the sheep main mesenteric lymphatic in situ, in which fluid input pressure is held constant. Under these conditions, stimulation of the splanchnic nerve causes a significant increase in frequency and fluid propulsion, confirming a direct neural effect on lymphatic smooth muscle.

Similar results were obtained using isolated bovine vessels, in which field stimulation shifted the pressure–flow curve to the left. Figure 3.12 shows the effect of pressure on flow under control conditions and during field stimulation at 4 Hz. Flow was significantly increased at each distending pressure by the positive chronotropic effect of sympathetic stimulation. In contrast to the heart, however, there is no positive inotropic effect of sympathetic nerves on the lymph pump. It therefore seems that stimulation of the excitatory nerves renders the autoregulatory mechanism more sensitive to stretch.

These results lead to the proposal that the lymph pump is important in the control of interstitial volume. In normal circumstances, the interstitial fluid volume is held constant by a balance between capillary filtration and the rate of filling of the initial lymphatic plexus. This in turn determines the degree of distension, the rate and force of contraction of the collecting vessels and thus the lymph flow. If the lymph pump could be made more sensitive to distension, this would result in the same lymph flow being achieved at a lower filling pressure; thus, the interstitial fluid volume could be set to a lower value. In other words, the tissues would be maintained in a relatively dehydrated condition. This would be consistent with the body adjusting its set point for interstitial fluid volume to a lower value during a “fight or flight” emergency in order to increase the available circulating fluid volume. The same mechanism might help to explain the increased lymph flow observed in response to haemorrhage. Recently, an increase in lymphatic ejection fraction has also been reported after haemorrhage (see Fig. 3.10 above).

Conversely, the lymph pump can be made less sensitive to distension, a condition that might be called lymphatic pump failure. Elias and Johnston showed that the effect of distension on flow in a doubly cannulated sheep mesenteric lymphatic vessel was shifted to the right when the vessels were perfused with lymph taken from a sheep that had been treated with endotoxin. Figure 3.13 shows the dependence of flow on transmural pressure in a doubly cannulated ‘isolated’ intestinal lymphatic of a recipient sheep that was pumping lymph taken from a donor sheep. The open circles on the left form the pressure flow curve before the donor sheep was treated with endotoxin, the closed squares on the right indicating the corresponding curve after endotoxin treatment.

LYMPHATIC ELECTROPHYSIOLOGY: IONIC CURRENTS AND PACEMAKERS

Origin of lymphatic rhythmicity

The above account of the lymph pump suggests that it is a relatively simple system that adjusts its output in response