Micromanipulation of pressure in terminal lymphatics in the mesentery

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ZWEIFACH, B. W., AND JOHN W. PRATHER. Micromanipulation of pressure in terminal lymphatics in the mesentery. Am. J. Physiol. 228(5): 1326-1335. 1975.—The terminal lymphatics are a network of highly pervious vessels that are distributed in a loose association with the blood capillary bed, in particular along with the collecting venules. The basilar pressure in these lymphatics is close to atmospheric, but after they converge to form valved collecting channels P\textsubscript{l} is increased with a pressure differential of 1-2 cmH\textsubscript{2}O built up across each valve. This increase in lymphatic pressure is clearly related to the presence of one-way valves, the contractile activity of the collecting channels, and the comparative impermeability of these channels. The pressure differential required to draw fluid from the interstitium into the lymphatics would appear to reside in the vasomotor activity of the collecting channels, although the data do not rule out changes in P\textsubscript{l} coincident with net capillary filtration or absorption.

lymphatic micropressures; lymphatic permeability; lymphatic vasomotion; tissue pressure; fluid exchange

UNLIKE THE BLOOD CAPILLARY NETWORK, whose structural features have been well characterized, the terminal ramifications of the lymphatic system have only been incompletely described. There has been a tendency to use the same nomenclature for the terminal lymphatics as for the microscopic blood vessels, despite substantial differences in their size and arrangement. The smallest lymphatics are thin-walled endothelial channels, but they are 5-8 times as wide as the blood capillaries. The terminal lymphatic network does not have a regular pattern of distribution and its full extent is difficult to recognize even in thin tissues such as the mesentery. In most instances, portions of the network of lymphatic vessels can be brought into view by injecting into the tissue a dye that is quickly taken up by the contiguous microscopic lymph channels (19). The vessels are wider than even the largest collecting venules, so that the term “capillary,” meaning hairlike, would be a misnomer here. Casley-Smith (2) has suggested the term “initial” lymphatics for the flattened, irregular network of channels that make up the beginnings of this system. We have adopted the term “terminal lymphatic” to cover all of the different lymphatic vessels believed to be involved in uptake of materials from the interstitium and the term “collecting lymphatic channels” where the vessels become confluent and begin to show the presence of valves.

In recent years, the work of Hauck (12) on the mesentery of the rabbit has emphasized the extraordinary richness of the lymphatic network and has described some of the details of their disposition and properties through the use of a modified dark-field procedure and the injection of fluorescent-tagged substances. Our own experience has shown that the complete network of lymphatic vessels at the microscopic level could best be determined by using a micropipette to inject a dye-colored protein solution directly into the network and taking serial photographs of its rapid spread (28). The present study deals with the structural and functional attributes of the terminal lymphatic system in the mesentery and omentum as established by micromanipulative procedures. Special emphasis was placed on the distribution of pressures in the lymphatic tributaries, in the hope that such measurements would shed some light on the question of tissue pressure.

METHODS

The observations reported in the main represent the study by intravital microscopy of the lymphatic origins in the mesentery of the cat and in the omentum of the rabbit. In addition, a small number of experiments were carried out on the mesentery of the rat because of the highly contractile nature of the collecting lymphatic channels in this tissue (1). Unless otherwise indicated, the microscopic observations were made after the animals were anesthetized with pentobarbital (30-35 mg/kg body wt). We have found that the anesthetic agent Inactin, a thiobarbiturate, is the agent of choice in the rat and rabbit (35-45 mg/kg) because it does not suppress respiration even at levels of deep surgical anesthesia, as may occur with sodium pentobarbital in these two species. The mesentery or omentum was exteriorized by well-established procedures that have been described in detail (29). The surface of the tissue was suffused with a balanced Ringer solution containing 1% gelatin at a temperature of 36-37°C. A magnification of 200-300 X was achieved with long working distance objectives 20 X, 32 X, and 40 X. Micropressures were recorded with sharpened micropipettes containing 1 M saline with the electrical servo-null procedure developed by Wiederhielm et al. (25) and modified by Intaglietta and Tompkins (15). Vessel dimensions were taken from Polaroid prints or from video tape recordings.

In view of the need to rule out untoward effects of technical factors such as the anesthetic agent or the nature of...
The suffusing solution, a number of studies were done with chloralose (25%) or chloralose-urethan mixtures (30 mg/kg each) in the rabbit and rat and with halothane or chloralose anesthesia in the cat. The pressure values recorded in the terminal lymphatics were essentially the same with all these anesthetic procedures. The mesentery and omentum were irrigated with several colloid-free, balanced electrolyte solutions, including Krebs-Henseleit or Tyrode solution, again without any effect on the lymphatic pressures that were recorded.

In most experiments, at least one micropressure was recorded in one of the terminal blood vessels, usually in the immediate postcapillaries. A second needle was placed in a terminal lymphatic. Systemic blood pressure was recorded in all the animals by a catheter in the interior portion of the aorta. A second catheter in the vena cava close to the heart on the right side was used to record central venous pressure. Where indicated, colloid osmotic pressures were determined on blood samples by a membrane osmometer method (21) developed in this laboratory.

**RESULTS**

**Structural features.** Ultrastructural studies in different tissues (18) have shown that the lymphatic terminal vessels have flattened, elliptical contours. These terminal portions are so thin in the mesentery that one could not always be certain (in many instances) whether a pipette tip was actually in the lumen or had pierced the wall of the lymphatic. Even gentle mechanical pressure tended to collapse the vessels. The position of the pipette therefore was routinely tested by use of the servo pump of the micropressure system to inject a small volume of saline. When the microprobe was in the interstitium, great resistance to injection was encountered; however, when the pipette tip was free in the lumen, the injected bolus flowed smoothly into the vessel and brought into view lymphatic channels in almost every area of the mesentery, much of which has been described.

With such a procedure and use of a dye-colored solution (0.1 M Evans blue), the majority of terminal lymphatics in the mesentery were found to be distributed as an interconnecting network. As seen in Fig. 1, the network of thin-walled lymphatics tends to follow the arcuate pattern of the small blood vessels. These flattened vessels have an uneven contour, varying in diameter from 25 µm to wider bulgings of 40-50 µm. The vessels in such networks usually are associated with the paired arterioles and venules and only infrequently follow the course of the capillaries proper.

Under higher magnification, the walls of the terminal lymphatics are seen to be extraordinarily thin and appear to consist only of a delicate endothelial membrane. With the light microscope no obvious discontinuities are seen, such as those described in electron-micrograph sections (18); as is described in a subsequent section, however, when dye-labeled protein is injected it leaks out at discrete points. The endothelial lining of these terminal lymph channels is strikingly demonstrated in vivo when dye-albumin solution is introduced into the vessel lumen with a micropipette.

In addition to the network pattern, a small number of blind lymphatic endings are seen, again with no distinctive pattern (Fig. 2, A and B). The number of such blind lymphatic terminals is comparatively small in comparison to the many interconnecting vessels. The distribution of the terminal lymphatics, as shown in Fig. 2A, is taken from the mesentery of an animal exposed to whole-body X-irradiation, where slight mechanical tension or stretching during preparation for in vivo microscopy leads to a rapid filling of the terminal lymphatics with red blood cells. These bulbous structures can be seen in the interstitium proper of the mesentery either away from the blood vessels or with the rounded portion abutting against a small venule. The blind endings are clearly demarcated by a thin endothelial wall (Fig. 2, B and C). We were unable to demonstrate instances where clearly identified lymphatics terminate as spaces or crevices in the ground substance without an endothelial barrier at the interphase (14).

The pattern and appearance of the terminal lymphatics are not the same in different tissues. For example in the omentum of the rabbit (Fig. 3) terminal lymphatics under low magnification appear as saccular structures that are much larger than the related network of blood vessels (Fig. 3A). When examined under higher magnification these saccular endings show a well-defined endothelial lining after being injected with an Evans blue-albumin solution (Fig. 3B). These, in turn, converge to form thin-walled collecting channels (30–40 µm wide) with prominent valves.

Many of the lymphatic terminations are essentially collapsed and do not show up against the translucent ground substance of the mesentery. They become quite prominent, however, under certain circumstances, such as an increase in blood volume produced by an infusion of 5% serum albumin. Histological cross sections of the mesentery fixed
in situ show the terminal lymphatics to be flattened tubes, with an average width of 25-40 \(\mu m\) and a depth of only 5-6 \(\mu m\).

An interesting structural configuration that has been described in different preparations (4), but has not been taken into account in a functional context, is the close contiguity of many of the terminal lymphatic vessels with the postcapillaries and venules. These configurations can be readily seen under intravital conditions (Fig. 4A), but the full extent of the wall-to-wall fusion was brought out in tissues prepared for electron microscopy (Fig. 4B). In particular, when sections are made tangential to the long axis of the microvessels, the contiguous lymphatic and vascular endothelial layers can be seen to consist of a thin barrier only 2-3 \(\mu m\) thick. The possible implications of this alignment are discussed in another section of this paper.

The networks of terminal lymphatics converge to form collecting channels. These begin as 40- to 50-\(\mu m\) vessels and in the mesentery may become as wide as 200 \(\mu m\). The collecting channels have a much more prominent wall (several microns thick) and are distinguished by the presence of valve leaflets (Fig. 5). These valve leaflets are almost transparent and are spaced at intervals of 400-1500 \(\mu m\), again with no discernible consistency, except that they are uniformly present where two such channels become confluent. Frequently in the region of the valve attachment to the vessel wall, the vessel appears to have a pinched appearance, not unlike a hooplike constriction. The leaflets are thin and appear to have a funnel-like shape. Despite their fragile appearance, the valves were found to withstand retrograde pressures up to 20 mmHg imposed by microinjection. As indicated later in this paper, these larger, lymphatic channels are muscular and show both spontaneous activity and localized responses to microneedle stimulation.

**FIG. 2.** Blind lymphatic endings in mesentery filled with red blood cells in rat exposed to whole-body X irradiation are shown in A (X 55). Under higher magnification (X 170) a typical blind ending or terminal lymphatic in mesentery is seen before injection of dye (B) and after microinjection of dye (C). Note sharply defined endothelial lining of such lymphatics. A = arteriole, V = venule.

**FIG. 3.** Saccular endings of terminal lymphatic in omentum of rabbit. A: under low power (X 65) the large size of such bulbous structures relative to the capillary network is illustrated. B: under higher magnification (X 200) the endothelial boundaries stand out sharply when lymphatic is filled with dye.
Lymphatic pressures. Micropressures could be recorded in any of the vessels described above. In Fig. 6 are shown representative tracings of pressures recorded simultaneously in the terminal lymphatic (P_L) and in an adjacent terminal arteriole and venule (P_v). The P_L tracings are flat, with none of the pulsatile characteristics corresponding to cardiac activity in the pressure tracings of blood capillaries and venules. The pressures in 53 initial or terminal lymphatics ranged between 0 and 3.5 cmH_2O and averaged 1.6 ± 2 cmH_2O. The absolute pressures were not the same in apparently similar lymphatic terminals in a given preparation, but 90% of the values were in the above range. Negative pressures of −0.5 to −1.0 cmH_2O were registered on occasion. There was no discernable relationship under steady-state conditions between the absolute levels of pressures in the adjacent precapillaries, postcapillaries, and collecting venules (exchange-type vessels between 8 and 25 μm wide) and terminal lymphatic pressures. In contrast, in experiments involving acute perturbations in blood pressures, P_L was affected in a predictable way.

In view of the high permeability of the mesothelial membrane covering the sheet of mesentery, the possibility was entertained that the nature of the solution suffusing the surface of the preparation might affect lymphatic vessel pressures. Various balanced electrolyte mixtures, including Ringer, Tyrode, and Krebs-Henseleit solutions, were used and found to have no effect on the range of P_L recorded. In the majority of our studies, the suffusing fluid contained sufficient colloid in the electrolyte solution to reproduce ascitic fluid. Additional experiments were carried out in which the colloid concentration was varied from 0.25% to 2% by adding either serum albumin or gelatin to the suffusing solution, again without any demonstrable effect on the pressures recorded in the terminal lymphatics.

As has been mentioned, a statistical sampling of steady-state pressures in terminal lymphatics (53 cats) showed P_L values to range from 0 to approximately 4.0 cmH_2O, with 68% of the pressures falling between 0.5 and 1.5 cmH_2O. When the pressure in a particular lymphatic was observed for 15–30 min, P_L tended to rise and fall; e.g., pressure would rise slowly from 0.5 to +3 cmH_2O over a period of 3–4 min and then would return more abruptly to its low point in 5–10 s.

In the rat mesentery, where the larger collecting lymph channels showed considerable spontaneous activity, the pressure fluctuations in the terminal lymphatics for the most part were related to the vasomotion of the contractile lymphatic channels. For example, pressure (P_L) fell to 0 or even −1 cmH_2O during the relaxation phase of such vasomotor excursions in the terminal lymphatics.

In the omentum, where the terminal lymphatics formed an extensive network, the pressure in these vessels remained reasonably constant. This may possibly be related to the fact that the total volume of these saccular vessels was calculated to be much larger (5–10 times larger) than the volume of the intervalve segment of the collecting lymphatic channel into which they drained.

Pressure in collecting channels. As the terminal lymphatics converged to form collecting channels, P_L rose progressively in each intervalve segment until in the largest channels, at the apex of the mesenteric sector, pressures of 12–18 cmH_2O were recorded. Not only were the absolute levels of P_L higher but the pressure tracings began to show a clear-cut pulsatile form. The accompanying composite (Fig. 7) shows the pressure distribution in the different-sized lymphatic vessels in the rat mesentery and characteristic pressure tracings for each segment.

It should be noted that the amplitude of pressure excursions increased as the lymphatic system was followed cen-
trally. These pressure excursions closely reflect the spontaneous contractile activity of these collecting channels, as well as mechanical movements of the tissue (respiratory movements, peristalsis of the small intestine).

By inserting one micropipette above the entrance to a valve leaflet and a second micropipette below the valve, it was possible to measure the pressure required to open or to shut these valves. This was accomplished in several ways. Recordings were made during the spontaneous opening and closing of the valve. Also, it was possible to use one pipette as the sensor and the second to deliver a pulse of fluid. In the latter experiments it was found that an increment in pressure of as little as 1.0–1.5 cmH$_2$O was sufficient to open a closed valve. In general, the pressure differential across successive valves was of this magnitude (Table 1).

Despite their delicate appearance, the valve leaflets were able to withstand retrograde pressures of up to 20 mmHg without becoming incompetent. When pressure in the collecting lymphatic channels was increased by the local injection of a minute bolus of fluid, the pressure rose sharply and the proximal valve closed briskly. In turn the distal valve opened and $P_l$ fell off smoothly.

Within the mesentery proper, the collecting lymph channels contained on the average four to five valves before they reached the apex or root of the mesenteric sector, where pressures as high as 12–18 cmH$_2$O were recorded in the most distal segment. Pressures were found to fluctuate more in valve-containing segments than in terminal lymphatic vessels, presumably due to active vasomotor excursions and to passive compression of these channels.

**Spontaneous vasomotion.** As in the case of the small blood vessels, a cyclic pattern of vasomotor activity was seen in the collecting lymphatics and ranged from partial narrowing of the vessel to complete obliteration of the vessel lumen. Spontaneous activity was best studied in the mesentery of the rat and guinea pig. There were considerable differences in both the rate and intensity of such periodic activity under different conditions (Table 2). In contrast, lymphatics in the mesentery of the cat and rabbit or in the omentum of the rabbit showed no spontaneous contractile activity. It was not possible to induce such activity by direct mechanical or chemical stimulation with musculotropic agents. This was in contrast to the lymphatics in the mesentery of the rat and guinea pig, where 80–85% of the preparations showed spontaneous contractions. In those preparations where the lymphatic channels appeared quiescent, activity could be induced by mechanical stretching of the lymphatics with a microneedle. It is our impression that such contractile behavior occurs only in those channels that have been shown to be endowed with smooth muscle. The vessels referred to here as terminal lymphatics have never been observed to undergo active contraction, nor have these endothelial structures responded to the neurogenic (bleeding), chemical (central hypoxia), or mechanical stimuli that have produced an active response in blood vessels with demonstrated smooth muscle cells. The changes in pressure observed in these terminal lymphatic vessels therefore must be attributed to other factors.

Two intrinsic factors could be identified on the basis of pressure measurements. The first was related to the periodic contraction and dilation of large vessels. A comparison of the time course of the pressure tracings to that of the contraction-relaxation activity could be made by superimposing the pressure recording on the video tape recording of the contractile activity. The close interdependence of the two phenomena strongly suggested that the spontaneous activity represented a form of myogenic activity. In any particular vessel, it was possible to predict precisely the onset of contraction by the level to which the $P_l$ rose. In other words, there was a threshold pressure above which there was an immediate contraction that raised the pressure by an additional 2–4 cmH$_2$O; the pressure then fell as the fluid was forced through the downstream valve into the next segment.

A second type of pressure fluctuation was seen in the intestinal mesentery, but over a narrower range of from 5 to 6 cmH$_2$O. It could best be distinguished in lymphatic vessels, where spontaneous activity was dampened, or during periods where the spontaneous contractions ceased.
Fig. 6. Representative tracings of pressure recordings in mesentery of cat. Arteriolar pressure (Pₐ), postcapillary pressure (Pₗ), and terminal lymphatic pressure (Pₗ). Note absence of pulsations in Pₗ tracing.

secondary fluctuations appeared to be related to the peristaltic movement of the intestinal wall and were quite irregular.

Closer analysis of a representative pressure tracing in a contractile lymphatic (Fig. 8) shows that Pₗ rose rapidly to a maximal level and then fell as the downstream valve was opened. As Pₗ begins to fall, the vessel relaxes; as a consequence, Pₗ may fall to as much as 3 cm H₂O below its original level. Contraction-relaxation cycles occur at between 10 and 18/min. An average cycle occupies about 3–4 s. The contractile event is short (0.8–1.0 s), whereas the relaxation phase develops more gradually. The spontaneous activity in these collecting lymphatics usually begins in the smallest tributaries and spreads centrally in a peristaltic wave that requires some 4–5 s to move from the smallest to the largest lymphatics in these preparations.

If one charts the percent change in vessel diameter for a given vessel segment (Fig. 9), it can be seen that the magnitude of the contractions becomes progressively less, until after 2–4 min spontaneous movement may cease completely. Then, after remaining quiescent for about 40–90 s, the contraction-relaxation activity is started up again. In some preparations after continuous spontaneous activity for 20–30 min, lymphatic vasomotion will stop completely. It is then possible to reactivate this behavior either by gently stretching the vessel lengthwise with a microneedle or by injecting a bolus of physiologic saline into the vessel several times.

Contraction-relaxation cycles are most rapid in the larger collecting channels (150–250 μm), with a periodicity of 15–18/min. In the smallest contractile channels (50–100 μm), the periodicity is somewhat slower, 6–10/min, and the amplitude of the contractions is more shallow. Thus, in the first and second interveal segments, the pulsatile changes in pressure during spontaneous activity are only 2–4 cm H₂O over the minimal pressure levels; however, in the larger downstream segments, the excursions are 10–20 cm H₂O above base-line levels.

Effect of systemic perturbations on Pₗ. It is well established that lymph flow from particular tissues varies considerably with perturbations in blood flow and may even cease completely (6). It therefore, was of interest to document the changes in pressure in the terminal lymphatics under such experimental conditions. In one set of experiments, the effects of acute blood loss and replacement were followed in the cat. A representative protocol shown in Fig. 10 includes measurements of plasma colloid osmotic pressure, which is an important feature determining the rate and direction of transcapillary fluid movement. When almost 45% of the calculated blood volume was removed over a period of about
TABLE 1. Pressure across valves in collecting lymphatics of rat mesentery

<table>
<thead>
<tr>
<th>Location</th>
<th>Proximal</th>
<th></th>
<th>Distal</th>
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<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Terminal lymphatic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st valve*</td>
<td>1-4</td>
<td>2.3</td>
<td>3-5</td>
<td>3.6</td>
</tr>
<tr>
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<td>3.0-7.0</td>
<td>3.6</td>
<td>4-8</td>
<td>5.5</td>
</tr>
<tr>
<td>3rd valve</td>
<td>4-8</td>
<td>4.8</td>
<td>5-9</td>
<td>6.8</td>
</tr>
<tr>
<td>4th valve</td>
<td>6-9</td>
<td>6.0</td>
<td>7-9</td>
<td>8.5</td>
</tr>
<tr>
<td>5th valve</td>
<td>5-10</td>
<td>8.1</td>
<td>8-11</td>
<td>10.5</td>
</tr>
<tr>
<td>6th valve</td>
<td>8-16</td>
<td>10.3</td>
<td>9-18</td>
<td>12.7</td>
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</table>

* Beyond terminal lymphatic.

TABLE 2. Lymphatic pressures during spontaneous contractions in lymphatic vessels of rat mesentery

<table>
<thead>
<tr>
<th>Diameter, μm</th>
<th>Relaxed, cmH₂O</th>
<th>Contracted, cmH₂O</th>
<th>Contraction Frequency, no./min</th>
<th>Δ Pressure, %</th>
<th>Δ Diameter, %</th>
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<tr>
<td>55</td>
<td>3.0</td>
<td>35</td>
<td>5.5</td>
<td>8</td>
<td>83</td>
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<tr>
<td>55</td>
<td>6.0</td>
<td>27</td>
<td>9.0</td>
<td>9</td>
<td>50</td>
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<td>20</td>
<td>7.0</td>
<td>6</td>
<td>180</td>
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<tr>
<td>90</td>
<td>6.3</td>
<td>60</td>
<td>14.3</td>
<td>15</td>
<td>123</td>
</tr>
<tr>
<td>90</td>
<td>8.0</td>
<td>50</td>
<td>16.0</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
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<td>9.5</td>
<td>69</td>
<td>11.5</td>
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<td>21</td>
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<tr>
<td>143</td>
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<td>7.0</td>
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<td>190</td>
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<tr>
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<td>200</td>
<td>7.0</td>
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</table>

8 min, the systemic pressure was brought down to 60 65 cmH₂O (or 45 mmHg) where it remained for almost 60 min. The Pₜ fell slowly but continuously from 1.3 cmH₂O down to 1.0 cmH₂O. Coincident with this fall in Pₜ, plasma colloid osmotic pressure dropped continuously, reflecting a net uptake of fluid from the interstitium equivalent to almost 20% of the original plasma volume. Within 2-3 min after blood replacement, Pₜ levels returned to normal, as did the microvascular variables that were measured.

An interesting counterpart of this situation (Fig. 11) was created by the intravenous injection of concentrated serum albumin sufficient to raise plasma colloid osmotic pressure by almost 15%. The colloid osmotic pressure remained elevated for over 60 min. It can be seen that Pₜ fell from a steady state of 3.0 cmH₂O to 0.5 cmH₂O over a period of 6-7 min but then gradually returned to control levels over a period of 30 min, well before plasma colloid osmotic pressure had returned to normal.

Permeability of terminal lymphatics. Two other features were
LYMPHATIC MICROPRESSURES

FIG. 11. Effect of transient elevation of plasma colloid osmotic pressure induced by intravenous injection of 30% albumin solution (cat mesentery). Plasma colloid osmotic pressure gradually falls as fluid is absorbed from tissue compartment. Capillary absorption is accompanied by a fall in P_L, which then returns to control levels before plasma colloid osmotic pressure is normalized.

Fig. 12. Permeability of terminal lymphatics (cat mesentery). A: diffuse outward movement of dye (0.1 M Evans blue in saline) across entire surface of lymphatic wall (2 s). B: when dye complexed to albumin was injected, spotty leaks were seen along vessel (2 s). C: dense diffuse coloration of tissue (8 s). × 150.

DISCUSSION

In addition to morphological considerations, the current studies were directed at several questions: 1) the distribution of pressures in the terminal lymphatics, 2) the relationship of these pressures under steady-state conditions and after acute perturbations to microvascular pressures, and 3) the implications of these findings to the Starling constitutive equation for blood-tissue fluid exchange.

Morphology. Except for the bat wing (5, 24, 25), there is no detailed information concerning either the distribution or functional characteristics of the smallest lymphatics at the level of their origin in the tissues. The present intravital studies were made on thin transparent mesenteric tissues and demonstrate the difficulty of recognizing and tracing the complete distribution of these delicate structures even under especially favorable circumstances. Histological studies of the initial or terminal lymphatics are based on abnormal conditions where the vessels become prominent or where the vessels are made visible by the local injection of dye-colored materials (17, 18). On the basis of the local uptake of dyes, the bizarre patterns and shapes of the lymphatic vessels have suggested a more or less random distribution (6). Our own studies, as well as those of Nicoll (19) in the bat wing, have shown that the terminal lymphatics are much more extensive than demonstrated by the dye-uptake data.

In both the mesentery and omentum, the terminal lym-
Red blood cells are seen in the terminal lymphatics (29). The nature and extent of such exchange need to be explored. The mechanism for fluid exchange between blood and lymph. The lymphatics are frequently in direct contact with the collecting venules of the microvascular system (4). We were able to corroborate this feature in the mesentery with intravital microscopy, as well as in specially osmium-fixed tissue prepared for electron microscopy. There are, however, many terminal lymphatics that course independently of the small blood vessels.

In a previous paper (15), it was pointed out that the Starling hypothesis is predicated on the exchange of fluid between two fluid compartments separated by a semipermeable barrier and that these conditions are not met as in the blood and interstitial per se are concerned. The close juxtaposition of some terminal lymphatics and the collecting venules seen in vivo has been corroborated by histological evidence. The structural arrangement whereby blood and lymph are separated by a thin permeable barrier on the venous side is highly suggestive of a secondary mechanism for fluid exchange between blood and lymph. The nature and extent of such exchange need to be explored.

The direct transfer of blood into the terminal lymphatics has been reported by several investigators (23), most strikingly in the rabbit ear chamber (4). A similar phenomenon is seen in the mesentery when the tissue is handled excessively and in the mesentery of rats exposed to whole-body X-irradiation at a time when they show a marked bleeding tendency and coagulation defect. It is at this stage that the venules show a predilection to form petechiae and numerous red blood cells are seen in the terminal lymphatics (29).

The observations on the larger vessels add little to the finer morphology of the collecting lymphatics, other than to support the observations of others (1) that these vessels are contractile. The vessel wall appears distinctly thicker than that of the terminal lymphatics, a fact confirmed by numerous ultrastructural studies that indicate the presence of smooth muscle (6). The presence of smooth muscle is suggested by the fact that the collecting channels can be made to contract by mechanical stimulation or stretching with microneedles, in contrast to the terminal lymphatics, which are unaffected by such manipulation.

**Pressure distribution.** Our measurements of terminal lymphatic pressures consistently show under steady-state conditions a small positive pressure, findings in accord with those reported by Cliff and Nicoll (5) and Wiederhelm and Weston (25) in the rat wing.

Under steady-state conditions, there is good reason to believe that the hydrostatic pressure in the terminal lymphatics (P_L) reflects the hydrostatic pressure in the surrounding tissue. Inasmuch as there is no agreement as to the applicability of measurements of tissue pressure made by direct methods (perforated capsule (9), cotton wick (16), micropipette (25)), there is no acceptable frame of reference that could be used to substantiate our contention that terminal lymphatic P_L in the mesentery should closely approximate tissue pressure.

Measurements of P_L in exposed organs, such as the mesentery or omentum, have been criticized since intravital microscopy involves exposure of these structures to the atmosphere and as a consequence tissue pressure may fall to atmospheric levels. However, it should be noted that identical pressures have been recorded in terminal lymphatics of the bat wing (21) and ear of the mouse (A. Hargens, personal communication) in their natural environment.

A further criticism stems from the need to bathe the exposed mesentery with an artificial electrolyte mixture. Presumably, small molecules diffuse freely across the mesothelial surface, but the extent to which proteins in the suffusing fluid can penetrate into the interstitium is essentially unknown. Our own data with protein concentrations ranging from 0.25 to 2.0% show no effect on terminal lymphatic P_L values. There remains the possibility nonetheless that in situ (intra-abdominal) interstitial and lymphatic pressures may be different from those observed in the intravital mesentery preparation. It has been proposed (10) that the osmotic and hydraulic pressures in the interstitium may be the decisive factors determining the exchange of fluid between the interstitium and the lymphatics. The development of a hydrostatic pressure in the tissue proper (P_t) would depend on the compliance of the interstitial gel. Conflicting evidence has been presented concerning the compliance of interstitial tissues in muscle (7) and in skin (8). For the most part, P_t has been considered to be small and to remain relatively unchanged, a concept essentially in line with the P_L measurements cited for the mesentery. There is a good possibility that P_t in other tissues, as well as P_L, may be quite different from that in the mesentery.

The pumping action of the larger lymphatics represents an obvious mechanism for drawing fluid into the terminal lymphatics as the contracted vessels relax. Our evidence suggests that the stimulus responsible for the spontaneous contractility of the collecting lymph channels may be myogenic in nature—e.g., the simultaneous pressure tracings that show a threshold level coincident with the onset of contraction. A striking increase in spontaneous activity occurs during hemorrhage in the rat mesentery and has been reported by others as well (1, 11). The hemorrhage experiments suggest that either a neurogenic or chemical mediator is involved, since the pressures in the lymphatics fall with the onset of hypotension so that the pressure threshold that is normally coincident with the onset of lymphatic vessel contraction is reduced in a given experiment to almost half.

A pressure gradient in the collecting lymphatics was found not only in the mesenteries of species where these channels display spontaneous contractile behavior, but also in the cat, where no spontaneous activity was seen. In the latter,
terminal lymphatic pressure values remained fairly constant. During periods where peristalsis developed in the intestine proper, lymph fluid movement and pressure fluctuations became striking. Under such conditions three to four valves in succession would be opened with each forward surge of lymphatic vessel fluid coincident with an increase in pressure of +4 to +6 cmH₂O.

In other tissues such as skeletal muscle, it has been shown that lymph flow is greatly increased by contraction of the muscle, providing a passive type of milking action (11). A somewhat similar situation is encountered in the intestinal wall, where peristaltic movements assist in driving lymphatic vessel fluid centrally. In the mesentery of the cat and in the omentum of the several species that were studied, however, there was no evidence of contractile activity in any of the microscopic lymphatic vessels. No substantive explanation for lymph flow has been demonstrated. Casley-Smith (3) favors an osmotic mechanism that would come into play as the vessel fills with fluid and the overlap between endothelial cells is closed. The premise is advanced that this phenomenon, together with vesicular transport of protein, sets up a concentration gradient that would move fluid into the lymphatics by osmosis.

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