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PERIPHERAL LYMPHATIC RESPONSES TO OUTFLOW PRESSURE IN ANAESTHETIZED SHEEP

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SUMMARY

1. Lymph flow and pressure fluctuations were measured by cannulating popliteal efferent and distal hind-limb afferent lymphatic vessels in anaesthetized sheep. The cannula outflow height was raised above the vessels to increase lymphatic outflow pressure.

2. Lymph flow decreased non-linearly as the outflow was raised. The rate of decrease increased with increasing outflow height.

3. Lymphatic contraction frequency rose and stroke volume fell with increasing outflow height.

4. The calculated power necessary to move lymph along the cannula initially increased with outflow height but it reached a peak and was reduced again by raising the outflow further. Calculated lymphatic stroke work followed a very similar pattern.

5. Lymph flow was maintained up to a greater outflow height in afferent than in efferent vessels. Curves relating frequency, power and stroke work to outflow height were shifted to the right in the afferent lymphatics.

6. These results are consistent with an intrinsic lymphatic pump which can be stimulated by increasing pressure. At high pressures, however, the pump fails.

INTRODUCTION

It is well known that the walls of lymphatic vessels contain smooth muscle which can produce organized contractions. This combines with the presence of frequent valves to produce a system capable of pumping fluid (Hall, Morris & Woolley, 1965; McHale & Roddie, 1976). One way of testing a pump's functional limitations is to increase the outflow pressure against which it is working and observe any changes in flow. Recent studies on lung lymphatics have suggested that lymph flow falls linearly with rising outflow pressure (Drake, Adcock, Scott & Gabel, 1982; Drake, Giesler, Laine, Gabel & Hansen, 1985), leading to a lymphatic model in which a constant driving pressure forces lymph through the lymphatic resistance. However, if a pump mechanism responsive to pressure changes exists this model cannot be used. Such a response could lead to a non-linear fall in lymph flow with outflow height (which largely dictates outflow pressure) as pump stimulation at lower pressures

progresses to pump failure at higher pressures with a more rapid decline in lymph flow. To see which model would best describe the behaviour of sheep hind-limb lymphatics, the response of lymph flow to increasing outflow pressure was studied in popliteal efferent lymphatic vessels and more peripheral afferent vessels in the metatarsal region.

A brief account of part of this work has been communicated to the Physiological Society (McGeown, McHale, Roddie & Thornbury, 1985).

METHODS

Sheep (mean mass 62 kg) were anaesthetized with either I.V. thiopentone (20–25 μg kg^{-1}) or pentobarbitone (20–30 mg kg^{-1}) followed by spontaneous inhalation of halothane (1–3 % in O_2). Lymphatic vessels were cannulated against the direction of flow with PVC tubing (Portex, internal diameter 0·5 mm, length 50 cm). This was made easier by injecting Evans Blue (1 % in buffered saline) into the drainage area of the relevant lymphatic to outline it. The cannula was connected to a Polythene outflow tube with a side branch. All tubing was pre-treated with TDMAC–heparin complex (2 %; Polysciences Inc.) and flushed clear with heparin sodium (5000 u ml^{-1}, Evans Medical) to prevent lymph clotting.

Lymph flow and pressure were measured as described by McHale & Roddie (1983) with the sheep in the supine position. Lymph was collected on a piece of filter paper attached to the side arm of a Statham UC3 transducer and the resultant tension record was calibrated for lymph flow in μl min^{-1}. Side-arm pressure from the outflow tube was recorded with a Statham P23 transducer and calibrated in cmH_2O. These pressure records were used to count contraction frequency. Frequency, flow and calculated stroke volumes were averaged over the last 4 min of each 5 min period. Recordings were made on a Gould 2400S recorder.

Two groups of lymphatic vessels were studied, popliteal efferents and more distal afferent lymphatic vessels. Efferent vessels were cannulated approximately 5 cm from the popliteal node and intervalvular distance was 5–10 mm. The afferents were cannulated over the metatarsal region of the hind-limb, about 10 cm proximal to the fetlock. At this site the valves were 3–6 mm apart. Control measurements were made with the outflow at the same level as the cannulated lymphatic (0 cm) and the outflow was then raised in fixed increments and measurements made for 5 min at each height. Experiments in which the same increases in height were maintained for longer periods of time indicated that equilibration was usually complete within 5 min. Outflow height was increased in 5 cm steps for the popliteal efferents and 10 cm steps for the hind-limb afferents.

The resistance of the cannula and outflow system used in these experiments was determined by attaching the inflow to a reservoir of artificial lymph (30 ml sheep plasma plus 20 ml N-saline) and measuring flow with the outlet at various heights below the reservoir. The flow varied linearly with perfusion pressure and the gradient of this relationship gave a total outflow resistance of 0·1 cm lymph μl^{-1} min.

The power necessary to move lymph at the observed rate was calculated at each outflow height. This was assumed to be equal to the pressure at the lymphatic cannulation point times the flow rate, i.e.

$$\text{power} = (Pdg)\,Q, \tag{1}$$

(power in 10^{-8} J min^{-1}; P = pressure in cm lymph; Q = lymph flow in μl min^{-1}; d = lymph density in kg l^{-1}; g = acceleration due to gravity in m s^{-2}).

The pressure factor was calculated as the sum of the pressure gradient along the cannula to the outflow due to resistance to flow and the hydrostatic gradient produced at each outflow height, i.e.

$$P = QR + h, \tag{2}$$

(R = outflow resistance in cm lymph μl^{-1} min; h = outflow height in cm).

Combining eqns. (1) and (2)

$$\text{power} = Qdg\,(QR + h). \tag{3}$$

The values used for the constants were, g = 9·8 m s^{-2}, d = 1·02 kg l^{-1} (Yoffey & Courtice, 1970) and R = 0·1 cm lymph μl^{-1} min (as determined experimentally). Power was converted to μW. Stroke work was calculated from the power output and the contraction frequency.

All results are expressed as the mean ± S.E. of mean unless otherwise stated.

RESULTS

The control (0 cm) records in Fig. 1 demonstrate well the intermittent flow seen in both types of lymphatic vessel studied. The volume of lymph expelled from the outflow increased in discrete steps and each step was associated with a peak in lymph pressure. This was interpreted as the consequence of a series of regular lymphatic contractions.

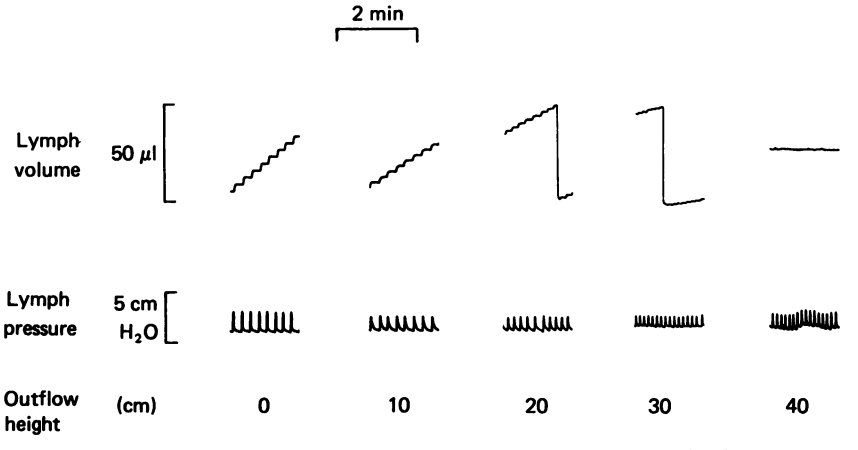

Fig. 1. Lymph flow (upper records) and side-arm pressure (lower records) for a popliteal efferent lymphatic vessel with the outflow at the indicated heights above the cannulation point.

Popliteal efferents

Samples of experimental record from an outflow-raising experiment for a popliteal efferent lymphatic vessel are shown in Fig. 1. The lower pressure record shows regular contractions at each height, associated with expulsion of lymph as recorded on the lymph record above it. Raising the outflow produced an increase in contraction frequency from 4·8 min^{-1} at lymphatic height 0 cm to 9·3 min^{-1} at a height 40 cm above this. Flow rate decreased in a non-linear manner with increased outflow height. At 0 cm flow was 19·2 μl min^{-1} and this had dropped by 14% at 10 cm. The same size of outflow height increment from 30 to 40 cm was associated with a flow reduction equivalent to 34% of the initial flow. Thus in this experiment, the initial increases in outflow height produced less of a decline in lymph flow than the final ones, whilst contraction frequency increased at heights above 10 cm. It can also be clearly seen from the upper record that the volume of lymph expelled with each contraction (the stroke volume) decreased as the outflow was elevated.

Not all lymphatic vessels responded identically and Fig. 2 summarizes the effects of raising the outflow on two efferent lymphatic vessels at either end of the range. Flow (bottom graph), frequency (middle graph) and stroke volume (top graph) are plotted against outflow height at 5 cm intervals. The results have been expressed as a percentage of the control values at 0 cm. In experiment no. 1 flow had only fallen by 16% at 30 cm. This was associated with a 4-fold increase in frequency and a reduction in stroke volume. However, despite a further small increase in frequency, flow fell rapidly to zero at 40 cm. The results for experiment no. 2, on the other hand,

show that even the initial increases in outflow height reduced flow, and this decline was slightly accelerated with further height increments. In this preparation there was little change in contraction frequency when the outflow was raised; the increase at 35 cm was less than 25%, while stroke volume decreased with outflow height in a manner similar to experiment no. 1.

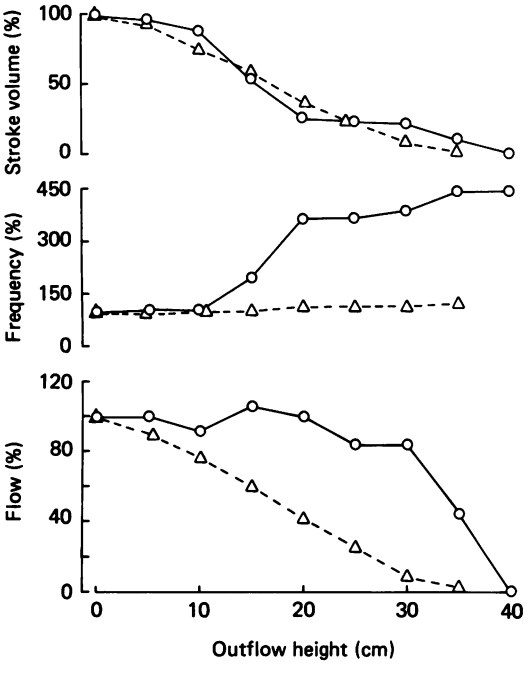

Fig. 2. The effect of outflow height on flow, frequency and stroke volume in two popliteal efferent lymphatic vessels in two experiments, no. 1 (○) and no. 2 (△). All three variables are expressed as a percentage of their control values as measured with the outflow at 0 cm.

Results for both efferent and afferent lymphatics are summarized as separate curves in Fig. 3. The combined results from experiments on ten different popliteal efferent lymphatic vessels have been plotted as the continuous curves which represent flow, frequency and stroke volume at each height. The control flow (outflow at 0 cm) was $20{\cdot}6 \pm 2{\cdot}6$ μl min^{-1}. This decreased non-linearly with increasing outflow height so that the gradient of the flow *vs.* height relationship becomes steeper at heights above 10 cm. The initial contraction frequency (0 cm) was $7{\cdot}3 \pm 0{\cdot}9$ min^{-1}. This was unchanged by raising the outflow through 5 cm and then rose rapidly with further steps up to 20 cm. Increasing the height beyond this produced a gradual rise in frequency to a maximum of $13{\cdot}9 \pm 2{\cdot}0$ min^{-1} at the highest outflow level of 40 cm. Mean stroke volume changes were similar to those described for individual lymphatic vessels (Fig. 2). Raising the outflow decreased the volume ejected by each contraction from the control value of $3{\cdot}2 \pm 0{\cdot}5$ μl. The rate of decrease was most rapid between 5 and 20 cm, thus corresponding with rapid frequency increases.

Hind-limb afferents

Experiments similar to those described above were carried out on six afferent lymphatic vessels cannulated over the metatarsal region of the hind limb. Fig. 3 shows the combined results for these preparations as dashed curves. Flow (bottom

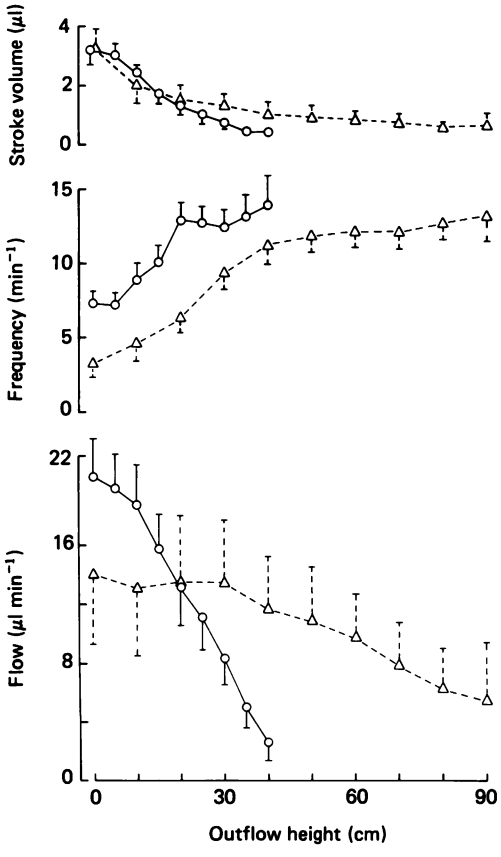

Fig. 3. Mean results comparing the effect of outflow height on flow, frequency and stroke volume in ten popliteal efferent lymphatic vessels (○) and six hind-limb afferent lymphatic vessels (△). Vertical bars represent 1 S.E. of the mean in each case.

graph), contraction frequency (middle graph) and stroke volume (top graph) have been plotted for outflow heights rising in 10 cm steps from the control level (0 cm) to 90 cm. Control flow was lower than in the popliteal efferents at $14{\cdot}0 \pm 4{\cdot}7$ μl min^{-1}, and flow was little affected by raising the outflow up to 30 cm but fell more rapidly with further height increments. The initial contraction frequency was $4{\cdot}6 \pm 1{\cdot}2$ min^{-1}, again below that in the efferents. This rose rapidly with height up to 40 cm and then more gradually to a maximum of $13{\cdot}1 \pm 1{\cdot}7$ min^{-1} at 90 cm in these experiments. Stroke volume fell with outflow height from a value of $3{\cdot}3 \pm 0{\cdot}6$ μl at 0 cm. Most of this reduction occurred between 0 and 40 cm, the range of outflow heights over which frequency changes rapidly.

Mechanical power output estimations

The power necessary to produce lymph flow at the observed mean rates was calculated at each outflow height for nine popliteal efferent lymphatic vessels using eqn. (3) as derived in the Methods. Under control conditions only $1{\cdot}1 \pm 0{\cdot}3$ nW was

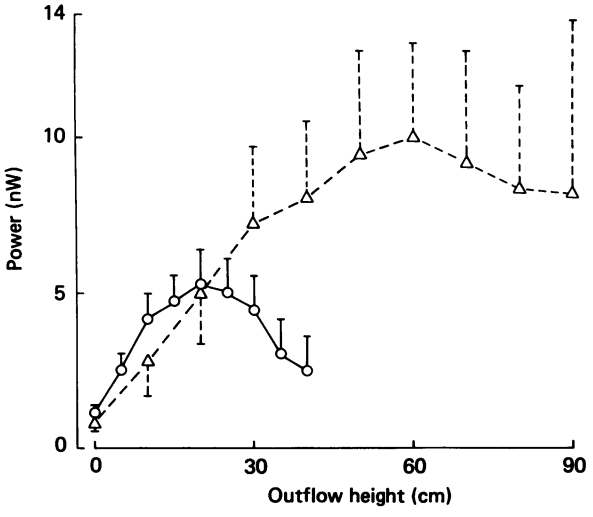

Fig. 4. Mean values showing the effect of outflow height on lymphatic power production in nine popliteal efferent lymphatic vessels (○) and six hind-limb afferent lymphatic vessels (△). Vertical bars represent 1 S.E. of the mean.

expended to overcome the resistance to flow of the outflow system. This rose rapidly with increasing outflow height to reach a peak of $5{\cdot}3 \pm 1{\cdot}1$ nW at 20 cm (Fig. 4, continuous curve). Raising the outflow further led to a reduction in power production.

Similar calculations were carried out for the six smaller, afferent lymphatic vessels. Power rose from $0{\cdot}9 \pm 0{\cdot}4$ nW at 0 cm to a peak of $10{\cdot}0 \pm 3{\cdot}2$ nW at 60 cm (Fig. 4, dashed curve). Further increases in height were associated with a tendency for power to drop.

Stroke work

The patterns of change in stroke work with outflow height were similar to those for power and are shown in Fig. 5. In the popliteal efferents (continuous curve) stroke work increased from 8 ± 2 nJ at 0 cm to a peak of 30 ± 8 nJ at 20 cm and then dropped off with further increases in height. In the case of the afferent lymphatic vessels (dashed curve) the initial stroke work was 14 ± 9 nJ rising to 51 ± 17 nJ at 60 cm before dropping off slightly as the outflow was raised further.

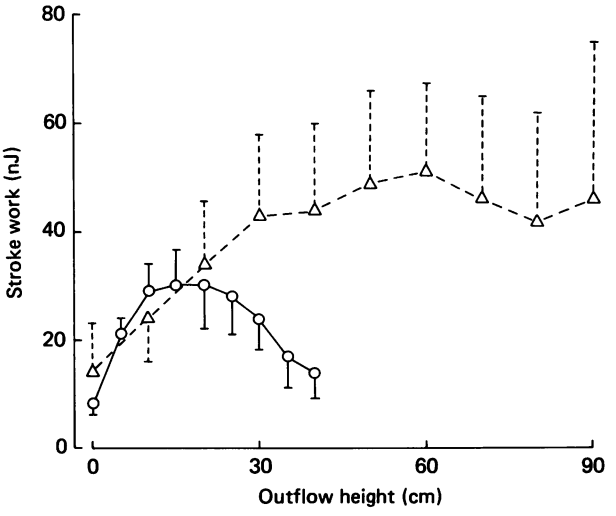

Fig. 5. The effect of outflow height on stroke work in nine popliteal efferent lymphatic vessels (○) and six hind-limb afferent lymphatic vessels (△). Results are plotted as the means with 1 S.E. denoted by the vertical bars.

DISCUSSION

The flow changes when the outflow was raised followed similar qualitative patterns in the efferent and afferent preparations. In both cases the response was non-linear, with a marked increase in gradient above a certain height (Fig. 3). This is consistent with an intrinsic pump whose activity is stimulated by raising the outflow pressure, thus tending to maintain lymph flow over the initial height-raising steps. At some point the lymphatic vessel's response to pressure might be expected to reach a maximum and further height increases would then cause more marked decreases in flow. This is in keeping with the response of isolated lymphatic segments to increased transmural pressure *in vitro* (McHale & Roddie, 1976).

Further evidence that increasing outflow pressure has an effect on the lymphatic vessel itself is seen in the dramatic changes in frequency of contraction, which rose to over eight times the control rate in one preparation. Hall, Morris & Woolley (1965) described similar increases in frequency when the outflow from a popliteal efferent lymphatic vessel was obstructed in a conscious sheep, while Hargens & Zweifach (1977) showed that occluding lymph vessels in rat and guinea-pig mesenteries again increased the rate of spontaneous contraction. These changes were associated with an increased pressure in the lymphatic vessel in each case. In our experiments frequency varied in a sigmoid fashion with height, rising rapidly over the lower outflow pressure range to reach a plateau value which was only slightly increased by raising the outflow further. Whether these responses are reflex or myogenic we cannot say from these studies. However, tetrodotoxin does not abolish the effect of transmural pressure on contraction frequency *in vitro* (Stewart, 1981), suggesting that reflex mechanisms are not vital.

A system involving a changing level of pumping activity with changing outflow pressures conflicts with the lymphatic model used by Drake *et al.* (1982) to analyse

their results from experiments on pulmonary lymphatic vessels in dogs. These workers found a linear decrease in lymph flow with outflow pressure, and this was interpreted in terms of a single driving pressure forcing lymph through a single lymphatic resistance. One assumption made was that changing the outflow pressure did not affect the total pumping activity of the system. Our experiments suggest that this assumption would not apply in peripheral lymphatic vessels at least, while others have shown that lung lymphatics can contract rhythmically with a frequency which increases when the outflow is occluded (Staub, 1974). Indeed, Drake *et al.* (1985) found evidence of lymphatic pumping in their sheep preparations but concluded that this was only significant at lymph flows approaching zero.

Although flow and frequency responses to increasing outflow pressure were qualitatively similar in the two types of lymphatic vessel in the present study, there were some interesting quantitative differences. The afferent vessels maintained their initial flows up to an outflow height of 30 cm, while efferent flow declined rapidly above 10 cm. This may relate to at least three factors. First, the popliteal lymph node provides a site where fluid may be exchanged between lymph and blood (Adair & Guyton, 1983) or shunted into other efferents. We have observed an increase in pre-femoral efferent flow from one control lymphatic vessel when another efferent from the same node had its outflow raised. In the case of the distal afferents, however, no increase in flow from a lymphatic vessel was observed when all the other afferents were blocked simultaneously; this suggested poor shunting through the tissues and few anastamoses between the afferent vessels.

Efferent and afferent lymphatic vessels also differ in their diameters. If pumping efficiency and frequency are influenced by the tension in the lymphatic wall they will also be affected by vessel diameter, in keeping with Laplace's Law. A given pressure will produce a lower resting tension in the wall of the smaller afferent lymphatics than in that of the efferent vessels. The frequency plateau was reached at an outflow height of 20 cm in the efferent vessels and at 40 cm in the afferents (Fig. 3). These two pressures would produce similar tensions in the walls of each type of vessel if the afferent vessels were half the diameter of the efferents, since tension is proportional to the distending pressure multiplied by the diameter. In these experiments the resting, external, afferent diameters were one-half to two-thirds of those for the efferents.

Both of these effects might help to explain why afferent vessels appeared to be able to maintain lymph flow against greater pressures than popliteal efferents. A third factor which may have contributed was the higher control flows in the efferent vessels. To maintain flow against an increased outflow pressure a pump must increase its work rate by an amount equal to the product of the flow rate and the rise in pressure. Lower initial flows mean that the work increase required of the afferent pump is smaller than that for the efferent with a given pressure increase. To explore this difference further, power (Fig. 4) and stroke work (Fig. 5) were calculated for the two types of vessel. Ideally, estimates of lymphatic power output should take account of the fluid pressure at the lymphatic inflow and the effective lymphatic resistance. In the absence of these measurements eqn. (3) has been applied over the range of outflow heights used. The calculations involve two obvious assumptions. First, the lymph is taken to gain all its energy from the lymphatic contractions. Our own records support

this since there was no flow between contractions (Fig. 1). This leads to the second assumption which is that calculations based on a continuous-flow model can be applied to a system producing the same mean flow discontinuously. Providing the outflow resistance does not vary with flow this seems a reasonable assumption, and in our experiments on cannula resistance flow varied linearly with pressure gradient over a wide range of flow rates. Unfortunately we have no way of knowing what length of a given lymphatic is affected by an increase in outflow pressure nor what proportion of the wall of any lymphatic vessel is made up of actively contracting muscle. These factors are likely to be important in dictating the maximum work limit for a lymphatic vessel.

The changes in power output with outflow height are consistent with a pump stimulated by an increasing pressure load (Fig. 4). Increases in power represent the product of both frequency (Fig. 3) and stroke work (Fig. 5) increases. Above a certain outflow height, however, stroke work drops off, leading to a decline in power production with a maintenance of high rates of contraction. This suggests that pressure can affect contraction frequency and strength separately, a point emphasized by the fact that in the afferents the frequency levels off above 40 cm while stroke work continues to rise up to 60 cm.

The differences between the stroke-work curves in the two types of vessel suggest that the lower initial flows alone do not account for the better lymph flow maintenance in the afferent vessels when the outflow is raised. The peak level of stroke work is reached at a higher pressure in these vessels. A model in which wall tension produces an increase in stroke work would again help to explain this difference, simply because of the difference in vessel diameter. However, the higher stroke-work peaks in afferents as compared to efferents suggest that either the bulk of smooth muscle involved in contraction is greater or that its mechanical efficiency is improved in these distal lymphatic vessels. No direct measurements of these variables are available.

An active lymphatic pump, capable of responding to a pressure load in such a way as to help maintain lymph flow, would clearly be of benefit should central venous pressure rise and might play a useful role during postural changes, especially in the limbs. The data discussed here are, we believe, in keeping with such a model of lymph propulsion.

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