

QUANTITATIVE APPROACHES TO THE STUDY OF LYMPHATIC CONTRACTILE ACTIVITY IN VITRO AND IN VIVO: POTENTIAL ROLE OF THIS DYNAMIC 'LYMPH PUMP' IN THE RE-EXPANSION OF THE VASCULAR SPACE FOLLOWING HEMORRHAGE

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ABSTRACT

Few investigators have considered a dynamic role for the lymphatic vessel in regulating the movement of fluid and protein from the interstitium back to the bloodstream. This view is based on the assumption that lymphatics are passive conduits and that hydrostatic pressure gradients and external compression forces acting on the vessels are primarily responsible for the movement of lymph. However, it is becoming increasingly evident that the intrinsic contractile capabilities of lymphatic vessels provide a major part of the propulsive force. Lymphatics have noradrenergic innervation and respond to a variety of humoral factors and inflammatory mediators suggesting that the pumping activity is centrally regulated and in addition, may respond to local factors. In this article, we will discuss what is known of the regulation of this 'lymph pump'. Techniques that permit analysis of contractile activity and fluid pumping in vitro and in vivo will be reviewed. Of particular interest is a sheep model that allows the quantitation of pumping activity in vivo without the complication of variable lymph inputs. While there is little information available at this time concern-

ing the potential role of the 'lymph pump' in pathophysiological states, some preliminary experiments from our own laboratory suggest that an independently regulated 'lymph pump' may play an important role in hemorrhagic shock.

The concept of a 'lymph pump' has developed from considerations of the mechanisms by which fluid and protein enter the terminal lymphatic capillaries from the interstitium and the factors responsible for generating the force required to propel these components back to the bloodstream (1). While the classic view holds that fluid is moved from blood to interstitium and into lymphatics by virtue of hydrostatic pressure gradients, the finding of negative pressures in the free tissue fluid in the interstitium of many tissue compartments has made this hypothesis unattractive since it is difficult to imagine how fluid can move against a pressure gradient. It has been postulated that tissue fluid may be sucked into the lymphatics by forces created by the lymphatic (either through contraction-relaxation of the vessel or compression-decompression created by in-

termittent tissue motion). Alternatively, the fluid may be drawn into the lymph capillaries by osmotic gradients between lymph and interstitium (2). Whatever the mechanism, the above proposals taken together have been loosely termed the 'lymph pump'.

Those who consider that the lymphatics are passive conduits assume that external compression forces such as muscular exercise, respiratory movements, peristalsis of the gastrointestinal tract, blood pressure pulses, etc. are responsible for lymph transport. It is likely that these forces play a role in some circumstances. However, if lymph formation and flow were regulated entirely by this random process, little provision would exist for regulating flow under conditions of physiological and pathophysiological stress. On the other hand, numerous investigators have observed lymphatics contracting *in vivo* in many species including man (reviewed in 3). In some animals (bats for example), all lymphatics from capillaries to the collecting ducts demonstrate contractile activity (4). The concept of an active 'lymph pump' allows for flexibility in that the vessels have the ability to adapt their propulsive activity in response to varying fluid loads.

Therefore our concept of the lymphatic vessel has evolved from a series of passive conduits to vessels capable of actively participating in lymph formation and propulsion. While the notion of a dynamic 'lymph pump' is an attractive one, few experimental studies have directly assessed the relative importance of lymphatic contractions and tissue compression forces. In addition, little is known of the lymph pump itself. How is it regulated? Is it an important factor in interpreting interstitial/vascular dynamics in disease? In the context of this brief review we will concentrate on lymphatic contractile activity since this would seem to offer the most interesting possibilities in terms of the regulation of fluid dynamics and pharmacologic manipulation in clinical medicine. We will discuss what is known of the regulation of lymphatic contractions and review techniques appropriate to the study of contractile activity *in vivo* and *in*

vitro. In particular we will develop the theme of a 'lymph pump' regulated independently from microvascular events and its possible significance in the pathogenesis of hemorrhagic shock.

Regulation of lymphatic contractile activity

The general consensus of opinion is that the control of contractions resides within the properties of the smooth muscle cells with distension of the vessel wall triggering changes in activity (i.e. control is myogenic, 5-7). Both cyclo-oxygenase and lipoxygenase inhibitors completely suppress the spontaneous contractions of isolated lymphatics (8-10) suggesting that the metabolism of arachidonate within the vessel (perhaps following a stretch stimulus) is involved in the modulation of the stimuluscontraction cycle. In addition, arachidonic acid has a number of pharmacological effects that are consistent with its conversion to various metabolites in the vessel wall (both stimulatory and inhibitory, 11), a fact that has been confirmed in recent studies (12,13).

This method of regulation implies that lymphatic pumping will be directly proportional to the amount of lymph formed, i.e. as lymph production increases or decreases, contractions of the lymphatic vessels will increase or decrease accordingly. However, in addition, lymphatic vessels respond to nervous stimuli and to a variety of humoral factors including many of the classical mediators of inflammation. This raises the possibility that other forms of regulation may override myogenic control.

Bovine lymphatics have noradrenergic innervation and contain alpha and beta receptors (14-16). Noradrenaline has a marked effect on the contractions of lymphatic vessels *in vitro* and this effect can be blocked with the appropriate receptor antagonists. In isolated preparations, noradrenaline has been found to alter lymphatic pumping (17). However, neurogenic mechanisms do not seem to support spontaneous activity since phasic activity in isolated preparations cannot be suppressed with alpha receptor antagonists, nor with

tetrodotoxin, a nerve blocker (6,15,18). Indeed, even *in vivo*, contractions persist after death (7,19,20). Nonetheless, it seems likely that neurogenic mechanisms have the potential to modulate contractile activity in some situations (reviewed in 21).

Similarly, blood-borne factors or mediators produced in the local tissues may alter lymphatic pumping. Lymphatic vessels, like their blood vascular counterparts, respond to a wide variety of pharmacological agents (reviewed in 3). These include the products of arachidonic acid metabolism. Not only do cyclo-oxygenase and lipoxygenase inhibitors abolish the contractile activity of sheep and bovine lymphatics *in vitro*, but several of the arachidonate metabolites including endoperoxide, a stable thromboxane analogue (compound U46619), and the leukotrienes B₄, C₄ and D₄ are among the most potent agonists when tested on these isolated preparations (8,9,11).

Techniques for studying lymphatic contractile activity *in vitro* and *in vivo*

Since the lymphatic vessel is essentially

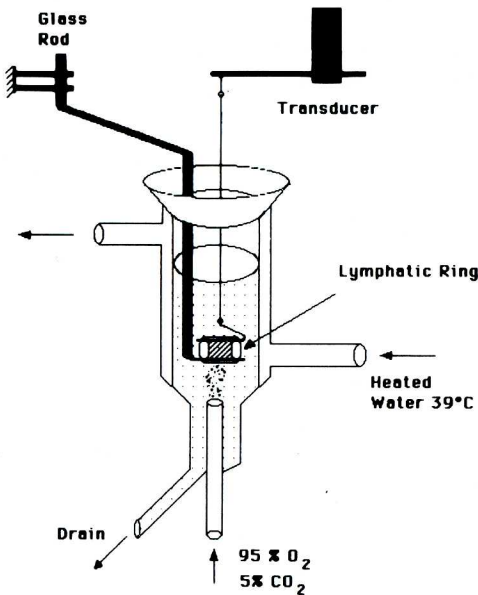


Fig. 1: Schematic representation of the preparation of monitoring lymphatic contractions *in vitro*. Contraction force is measured through an isometric transducer.

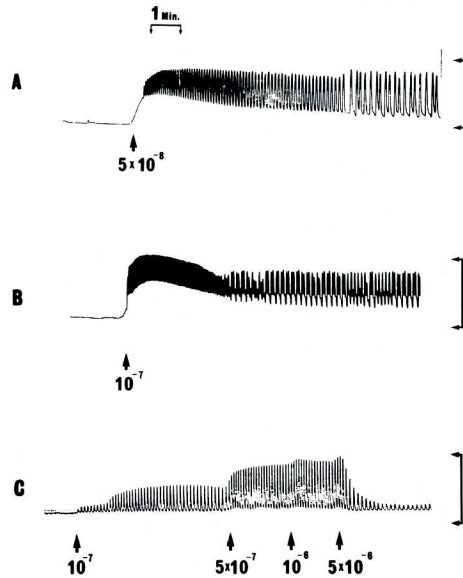


Fig. 2: Response of isolated bovine mesenteric lymphatic segments to the addition of pharmacologic agents. In each of the examples illustrated in this figure, the vessels were not spontaneously active. The following agents were added to the tissue bath in order to elicit phasic and tonic activity (concentrations are molar): A) Compound U46619 (thromboxane-endoperoxide analogue); B) Leukotriene C₄; C) Noradrenaline. The scales to the right of the traces represent 1.0 gram force.

a group of pumps (termed lymphangions) arranged in series, efforts have been directed at ways to quantitate either the force produced by the contractions or quantitate the flow of fluid through isolated ducts *in vitro*. In this latter case bovine mesenteric segments have been cannulated at both ends such that inflow and outflow pressures and fluid flow through the duct could be measured (6,17). Alternatively, one can monitor the contraction force generated by a vessel arranged longitudinally in a tissue bath (15) or produced by the circular contractions of a lymphatic ring as illustrated in Fig. 1 (11). Many of these isolated preparations are spontaneously active, i.e. they contract phasically without the addition of pharmacologic agents. On the other hand, phasic and tonic activity can be induced in quiescent preparations with the addition of several agonists in-

cluding noradrenaline, compound U46619 (thromboxane-endoperoxide analogue) and Leukotriene C4. Fig. 2 illustrates some examples of recordings obtained from various bovine lymphatic rings suspended in Krebs solution.

If lymphatic contractions generate a major part of the force required to move lymph one might predict that lymph flows and lymphatic pressures *in vivo* would be pulsatile. Hall and his colleagues (22) developed a method to monitor lymphatic pressures from indwelling catheters and they (and subsequently others-20,23,24) have found this to be the case. In this system lymphatic pressures are determined by fluid moving against the constant resistance provided by the outflow catheter distal to the T-piece (Fig. 3). Fig. 4 illustrates examples of lymph pressure-flow recordings taken from various popliteal lymphatic preparations in experiments from our laboratory. These examples demonstrated the pulsatile nature of lymph flow monitored from vessels 1) efferent and afferent to the lymph node, 2) from the efferent duct in nodectomized preparations, and 3) from efferent lymphatics in sheep that had been sacrificed prior to the time at which the recording was obtained. Obviously, in this latter case, lymph pumping persisted in the absence of external compression forces, neurogenic factors and cardiovascular activity. In our experience lymph flows and pulse pressures can be monitored for up to 30 minutes after death in this model.

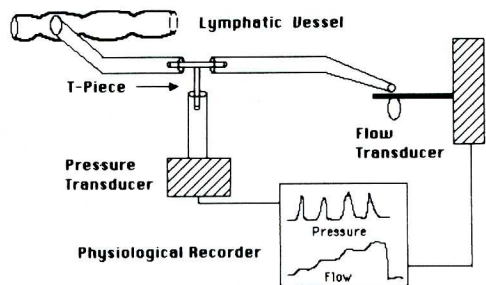


Fig. 3: Schematic representation of the system used to measure lymphatic pressure and flow rates in the sheep.

Considerable end-lymphatic pressures can be obtained from cannulated vessels. Olszewski and Engeset noted end-pressures up to 100mmHg in human lymphatics (23). In Fig. 5 pressures have been measured from a sheep popliteal vessel. At A, the

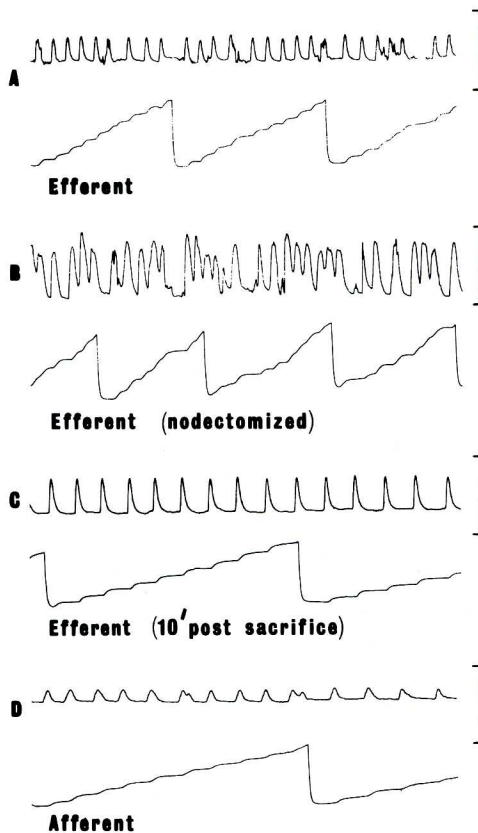


Fig. 4: Examples of pressure-flow relationships monitored from popliteal lymphatic preparations. In each case the pressure record is on the top and flow rate recording on the bottom. The scale to the right of the pressure trace represents 10mmHg. A) Pressure-flow recording obtained from a lymphatic efferent to the popliteal node; B) Pressure-flow recording obtained from an efferent (now afferent vessel) in a nodectomized preparation. In this example the lymph node was surgically excised six weeks prior to the time the record was taken; C) Pressure-flow recording obtained from a vessel efferent to the popliteal node in an animal that was sacrificed with an overdose of pentobarbital. The record was taken ten minutes after the heart had stopped beating. D) Pressure-flow recording obtained from a vessel afferent to the lymph node.

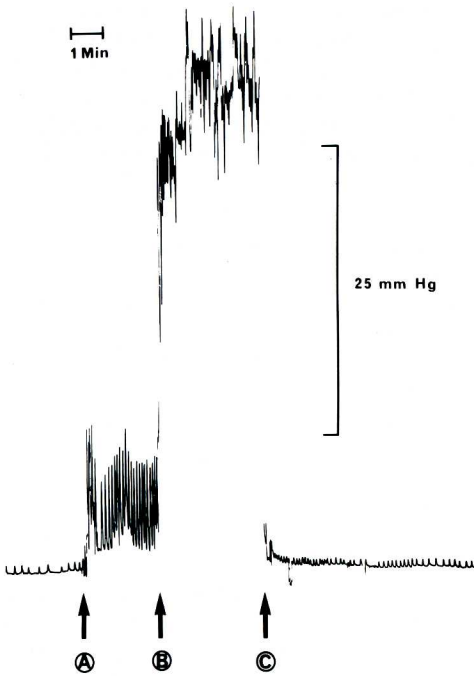


Fig. 5: Example of end-lymphatic pressure monitored from an efferent popliteal vessel in a conscious sheep. The outflow catheter was partially occluded at A and completely occluded at B. At C, the occlusion was removed.

outflow catheter was partially occluded and at B, totally occluded. This resulted in increases in pressures approaching 50mmHg as well as increases in the frequency and amplitude of the pressure pulses. It is interesting to note that the frequency of pulsations remained elevated above the pre-occlusion level even after the occlusion was removed, probably indicating enhanced contractile activity in an attempt to propel the accumulated fluid. This supports the contention that filling of the vessel triggers an increase in lymphatic contractile activity and lymph pumping.

While the pulsatile nature of the pressure recordings has been attributed to the contractile properties of the lymphatics (20,22-24), this approach is open to the criticism that lymph flow is dependent ultimately on lymph formation and variable lymph inputs may obscure lymph pumping caused by the contractions of the vessels

themselves. To get around this problem another model system has been developed which permits analysis of lymph pumping directly without the complication of changes in lymph formation. One of these monitors flow through a segment of thoracic duct (25) while the other utilizes a portion of the sheep mesenteric vessel (26,27).

In the latter case, a segment of the mesenteric lymphatic is isolated *in vivo* from lymph input by placing a catheter in the direction of flow close to the point where it emerges from the terminal mesenteric node and inserting a second catheter downstream from the node (10 to 15 cm) against the direction of flow (Fig. 6). All of the tributary vessels are tied off so that there is no lymph flow through the duct. A fluid reservoir containing saline or lymph provides the only input to the vessel. By raising the height of both the reservoir and the outflow catheter, the transmural distending pressure of the duct can be increased to the point where the vessel is triggered to contract and propel fluid. Fluid can move through the duct only if the lymphatic vessel contracts and propels its contents in

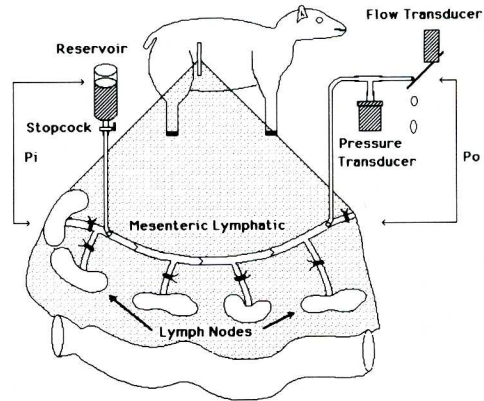


Fig. 6: Schematic representation of the preparation for monitoring the consequences of lymphatic contractile activity *in vivo*. The height of the reservoir (P_i) is set equal to that of the outflow catheter (P_o) to ensure that no hydrostatic gradient is applied to the system. Flow can occur only if the vessel contracts and propels fluid in the direction allowed by the valves.

the direction allowed by the valves provided that the heights of the reservoir and outflow catheters are set equal to each other (i.e. no net driving pressure).

This *in vivo* model allows the study of the effects of systemic perturbations (physiological, pharmacological, pathological) on lymph pumping. The only input to this 'isolated' duct would be nerves and blood supply (vasa vasorum/lymphorum). In this regard it is interesting to note that McHale and Thornbury (27) have demonstrated increased pumping from this model following a fright stimulus and have noted that intravenously administered noradrenaline has a direct effect on the contractions of the duct. These results are an elegant demonstration of the fact that systemic factors have the capability of directly controlling lymphatic contractile activity *in vivo*.

In light of the fact that the concept of a 'dynamic' lymph pump is still not firmly established, the potential contribution of lymphatic contractile activity to lymph propulsion in disease states is understandably obscure. With the application of new techniques such as the ability to grow lymphatic endothelial and smooth muscle cells in tissue culture (28) and the capability to assess the 'lymph pump' *in vivo* (26,27) it is hoped that some of these issues will be addressed. In particular, the *in vivo* pumping preparation described above would appear to be an extremely useful tool in addressing some fundamental questions regarding the role of the lymphatic vessel in pathophysiological conditions. To illustrate this point, we would like to relate some of our recent experience with the *in vivo* pumping model in hemorrhage experiments in sheep which suggest that lymphatic contractile activity is independently regulated and may contribute significantly to the reconstitution of the vascular volume following a blood loss.

Potential role of the dynamic 'lymph pump' in reconstituting plasma volume following hemorrhage

Following hemorrhage, re-expansion of the vascular space with fluid and protein is

of paramount importance. A potential endogenous source of these constituents is known to exist within the interstitium. The magnitude of interstitial fluid is considerable, accounting for 15% of total body mass or roughly three times plasma volume (29,30). Similarly, the mass of interstitial protein is large; variable estimates range between one and three times the amount found within the vascular pool (30,31). Lymph is derived from interstitial fluid and protein and under normal circumstances over a 24-hour period is returned to the circulation in volumes comparable in size to the intravascular pool (32). Given that the return of interstitial or extravasated protein is the major function of the lymphatic circulatory system, it is tempting to speculate that the dynamic 'lymph pump' may play a vital role in the re-expansion of the vascular space following a significant blood loss. However, little information is available at the present time to support such a proposal. One could argue that decreased tissue perfusion and subsequent reductions in lymph formation in many organ systems would simply reduce the fluid load to the lymphatics which would adjust their pumping accordingly.

Changes in lymph flow rates in hemorrhage have been reported but these are extremely variable and these variations may be due in part, to the methodologic differences in these studies. Flows have been noted to increase (33-37) or decrease (38-42). Occasionally, an initial transient increase in flow was noted prior to a sustained fall in output (38,39,42-44). Nonetheless, estimates of the volume of lymph returned to the vascular space suggest that lymphatic return is an important component. For example, Cope and Litwin (43) monitored dogs for 24 hours following a sublethal hemorrhage (30% of blood volume) and found that the amount of protein returned from thoracic duct lymph was twice the amount lost from the hemorrhage.

In order to determine whether a blood loss is accompanied by changes in the activity of the dynamic 'lymph pump' independent of alterations in lymph formation, we have begun to test the effects of a

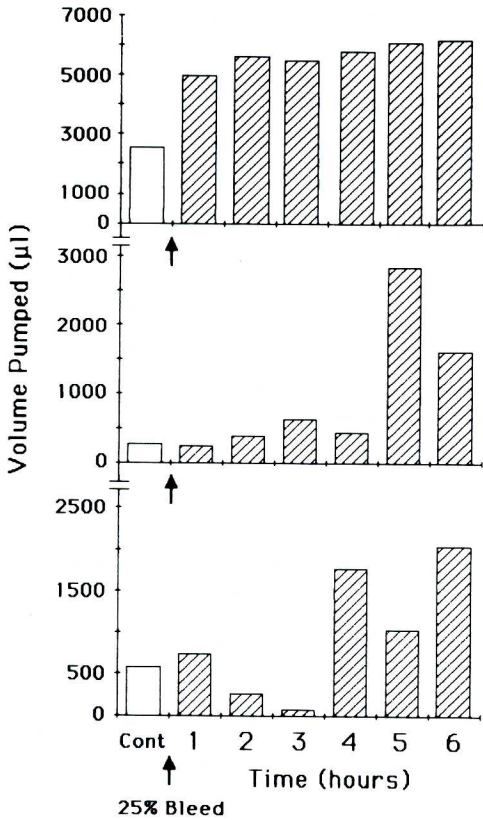


Fig. 7: Effects of a 25% hemorrhage on the pumping activity of mesenteric lymphatics in three representative experiments using the *in vivo* pumping model described in the text. In each case, the animals were anesthetized with sodium pentobarbital for the duration of the experiment. Each bar represents the volume of fluid (μl Ringers Lactate taken from the reservoir) pumped over a one hour period. The open bars illustrate the fluid pumped during the pre-hemorrhage control hour. The cross-hatched bars depict the fluid pumped for each hour after the bleed (indicated by the arrow).

25% blood volume bleed on the pumping activity of the isolated preparation described earlier. We found that a 25% bleed resulted in significant increases in the pumping of fluid by the isolated duct (examples from three experiments are illustrated in Fig. 7). Arterial pressures declined to approximately 50% of control levels but had usually returned to normal by 2-3 hours post bleed. Comparisons of fluid pumping between animals that were bled and control

animals that underwent catheter placement but had no blood withdrawn, revealed that up to six times more fluid was pumped through the duct in the hemorrhaged group. Measurements of lymphatic pressures from the outflow catheter of the *in vivo* pumping model revealed increases in the frequency and/or amplitude of pulses indicating enhanced contractile activity of the duct (example illustrated in Fig. 8). The mechanisms responsible for stimulating lymph propulsion are not clear at present. The only input to the vessel was its blood and nerve supply so one or both of these factors appear to be important.

It is interesting to note that the increasing pumping of the 'isolated' duct was accompanied by significant increases in lymph flow measured from intact cannulated mesenteric lymphatics. It could be argued that these changes were due to elevations in lymph formation. Microcirculatory changes resulting in an increase in transcapillary flux following hemorrhage may arise from an increase in permeability (34,45,46) or an increase in capillary hydrostatic pressures secondary to postcapillary venospasm (36,47,48). However, blood flow to the bowel has been

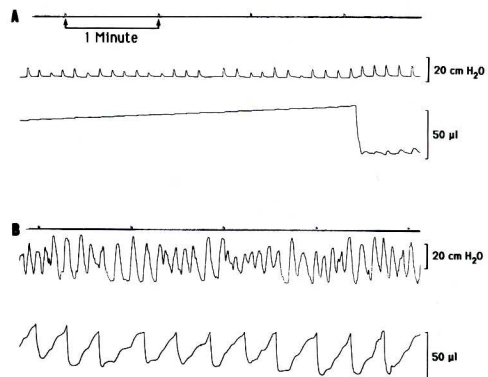


Fig. 8: Examples of pressure-flow relationships recorded from the *in vivo* pumping preparation in an anesthetized sheep before and after a 25% hemorrhage. In each case, the pressure trace is on the top and flow rate recording on the bottom. A) Recording taken during the pre-hemorrhage control period. B) Recording taken two hours and 30 minutes after the blood was removed.

found to decrease in hemorrhage (49-51) suggesting the possibility that lymph formation may also be reduced.

Future Directions

It is, of course, premature to hypothesize that the lymphatics may actively mobilize interstitial protein and fluid during hemorrhagic shock under conditions that may not favor increased lymph formation. Much more work will have to be performed to confirm and extend these observations and fully assess their implications in shock. However, given 1) the magnitude of the volume of fluid and the mass of protein that is returned to the bloodstream by lymphatic vessels each day, 2) the possibility that these vessels actively participate in the movement of lymph, and 3) the potential regulation of this dynamic 'lymph pump' by nervous and humoral factors, it is possible that contractions of the lymphatic vessels play an extremely important role in the movement of fluid and protein from one compartment to the next.

This may be true in hemorrhagic shock which has been used as an example in this review, but many other clinical problems may take on a new light when viewed from a lymphatic perspective. With the development of *in vivo* and *in vitro* techniques that permit analysis of these problems including the role of lymphatic contractile activity in fluid propulsion, the mechanics of contractions, and potential regulatory mechanisms, it is hoped that many of the important issues related to lymphatic function can be resolved. One potentially important problem relates to the question of drug effects. Do the various pharmacologic agents used in the clinics directly affect the 'lymph pump'? Is it possible to manipulate lymphatic pumping activity and fluid dynamics with compounds that stimulate or inhibit the contractile activity of the lymph vessels *in vivo*?

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