Increased Intrinsic Pumping of Intestinal Lymphatics Following Hemorrhage in Anesthetized Sheep


The return of fluid and protein to the bloodstream by the lymphatic circulation may play an important role in reconstituting intravascular volume following hemorrhage. In this study, we have defined the lymph-flow changes that occur in cannulated mesenteric lymphatics following a 25% blood loss in anesthetized sheep and investigated the effects of hemorrhage on the intrinsic contractile activity (lymph pumping) of these vessels in vivo using a new model system. The removal of 25% of the calculated blood volume resulted in increases in lymph flow over a 6-hour period, with peak changes to 3.5 times the prebleed levels. Systemic arterial pressures dropped to roughly 50% of control values immediately following the bleed and returned to control in 3 hours. To directly assess the effects of hemorrhage on lymphatic pumping, a segment of intestinal lymphatic was isolated from all lymph input and supplied with fluid from a reservoir. While there was no net pressure driving fluid through the duct, a transmural distending pressure was applied to the vessel, which stimulated resting lymphatic contractions and fluid pumping. A 25% blood loss resulted in increased activity of the lymph pump; up to 6 times more fluid was propelled through this "isolated" vessel in vivo than in similar preparations in sheep that were not bled (p<0.01). Measurements of fluid pulse pressures in this preparation indicated increased pumping frequency and/or force after hemorrhage compared with prebleed levels. We conclude that lymphatic contractile activity is stimulated after a blood loss independent of changes in lymph formation and speculate that this mechanism may play an important role in the reexpansion of the vascular space. (Circulation Research 1987;60:265-272)

The ability of an organism to attain hemodynamic stability following hemorrhage is imperative for survival. Acute compensatory mechanisms mediated by the sympathetic nervous system are well described and serve to maintain perfusion to vital organs. Restoration of intravascular volume and protein, while occurring over a more chronic period of time, is also important for survival. We believe that the lymphatic system plays an important role in this regard.

Lymph production determined primarily at the microcirculatory level is predicted by Starling's forces where a net positive filtration of fluid normally occurs from capillary to interstitium. The volume of lymph returned to the blood vascular system each day is approximately 50 to 100% of the plasma volume. This lymph contains greater than 100 g protein. Therefore, a large dynamic reservoir of circulating protein exists within the interstitial compartment and lymphatic vessels. Effective lymph transport of interstitial protein and fluid back to the blood stream may play an important role in reconstituting intravascular volume after a blood loss.

Reports in the literature of lymph-flow changes following hemorrhage have not yet provided a clear picture of this function. Methodological differences (severity of bleeding, animal species, etc.) make general conclusions difficult. While some studies have demonstrated increased lymph flow of varying degrees following blood loss, others have shown decreased flow rates. Often, an initial transient increase in flow was noted prior to a sustained fall in lymph output.

Complicating this issue is the fact that lymph-flow rates may be dependent not only on changes in lymph formation but also on the intrinsic pumping capabilities of the lymphatic vessels themselves (reviewed by Johnston). We have utilized a model that allows us to discriminate between these two physiological functions. Our results demonstrate that lymphatic contractile activity (lymph pumping) increases significantly after hemorrhage independent of any changes in lymph formation.

Materials and Methods

Cannulation of Intestinal Lymphatics for Lymph-Flow Measurements

Thirty-six sheep of either sex (25–35 kg) were used in these experiments. The animals were fasted for 48

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hours before surgery. Anesthesia was induced with intravenous administration of sodium pentobarbital (Somnotol, 20 mg/kg, MTC Pharmaceuticals, Hamilton, Ont., Canada) with additional smaller doses given as needed during surgery. All sheep were intubated (Portex, 6.5–7.5) and allowed to breathe unassisted. Through a transabdominal incision the main mesenteric efferent lymphatic was identified originating from the terminal lymph node lying within the ileocolic mesentery. Sterile Evan's blue dye (1% in phosphate-buffered saline, PBS) was injected into the terminal lymph node to clearly outline the lymphatic. A polyvinyl catheter (Microbore Tygon tubing; o.d. 0.070 in, i.d. 0.040 in, Cole Parmer Instrument Co., Chicago, Ill.) was inserted into the vessel against the direction of flow. This catheter was externalized, and the abdomen was closed securely using #1 Dexon (Davis and Geck, Cyanamid, Montreal, Quebec, Canada).

**Cannulation of Intestinal Lymphatics for Lymph Propulsion Measurements**

Reddy and Staub utilized an isolated segment of thoracic duct in the dog to study the physical and pharmacological properties of the intrinsic lymph pump. More recently, McHale and Thornbury have developed a mesenteric preparation in vivo that discriminates between changes in lymph flow due to alterations in lymph formation and changes in flow due to alterations in lymphatic contractile activity and lymph propulsion. A similar mesenteric preparation has been used in this study. Sheep were prepared and the main mesenteric efferent lymphatic identified as described earlier. Two polyvinyl catheters were inserted into the vessel: one in the direction of flow (close to the terminal node) and the second 10–15 cm downstream against the direction of flow. All lymphatic tributaries draining into the segment of lymphatic between the two catheters were ligated with silk ligatures (4–0, Davis and Geck). Surgical dissection was kept to a minimum in order to preserve local blood and nerve supply to the vessel. Both catheters were externalized and the abdomen closed securely. A reservoir of sterile lactated Ringer's solution was connected to the inflow catheter and provided the only source of fluid delivered to the segment (Figure 1). With no net driving pressure, i.e., with inflow (Pi) and outflow (Po) pressures equal, movement of fluid through the duct could only occur if the vessel contracted and pushed fluid in the direction allowed by the valves. However, by raising the height of both the reservoir and the outflow catheter relative to the lymph vessel (but keeping the height of the reservoir and outflow catheter equal to each other), the transmural distending pressure in the duct could be increased to the point where the vessel was "triggered" to contract and propel fluid.

To ensure complete isolation of the lymph vessel from extraneous lymph input, the reservoir stopcock was turned off, and if the flow rate did not return to 0, the preparation was not used. This procedure was repeated at the beginning and end of each experiment.

**Measurements of Flow Rates**

The outflow cannula was positioned so that lymph would flow onto the arm of an isometric tension transducer (Gould Statham, Schiller Park, Ill., Model UC-3), which was coupled to a separate channel on the Beckman dynograph recorder. As the drop of lymph formed on the transducer arm, an increase in tension was recorded. The slope of the tension line reflected lymph flow rate. As the drop fell off the arm, the transducer was automatically reset. Lymph flow was calculated using an on-line computer (Medac S100, CP/M) and expressed as microliters per minute.

**Measurements of Lymphatic Pressure**

Lymphatic pressures were monitored using procedures adapted from the literature. The outflow catheter was connected to a siliconized plexiglass T-tube and the side arm to a Beckman dynograph recorder (Schiller Park, Ill., Model R511-A). The remaining arm of the T-tube was connected to 10 cm of catheter of smaller diameter than the one in the lymphatic in order to provide a resistance so that pressures could be monitored through the other arm of the T-tube. The height of the outflow catheter was adjusted to be ap-

![Figure 1. Schematic representation of "double-catheter" preparation. Pi and Po represent inflow and outflow pressures, respectively. Height of outflow catheter was kept equal to that of fluid in reservoir to ensure that no hydrostatic gradient was applied, i.e., Pi = Po. Movement of fluid through duct can only occur via contractions of vessel with valves determining direction of flow.](http://circres.ahajournals.org/figure/1)
Experimental Protocol

Blood Pressure

Arterial blood pressure was monitored from the right carotid artery. The catheter was connected to a pressure transducer (Sanborne 267B) and preamplifier (Hewlett Packard 8805A) coupled to a strip chart recorder (Hewlett Packard 7706).

Lymph flow rates were continuously monitored for the first 75 minutes after the bleed and for the first 15 minutes of each hour thereafter. Similarly, blood pressures and pulse rates were taken every 15 minutes during the control period, for the first hour after the bleed, and at the beginning of each hour thereafter until the end of the study. In two of these experiments, the lymph flows were continuously monitored from intact intestinal lymphatics, lymphatic values were measured continuously for the first 75 minutes after the bleed and for the first 15 minutes of each hour thereafter. Similarly, blood pressures and pulse rates were taken every 15 minutes during the control period, for the first hour after the bleed, and at the beginning of each hour thereafter until the end of the study. In two of these experiments, the lymph flows were continuously monitored for the 6-hour period following the bleed. In the case of the "double-catheter" preparations, lymphatic parameters were monitored continuously for the 6 hours after hemorrhage, while blood pressures and pulse rates were taken every 15 minutes during the control period, immediately after completion of bleeding, and every 15 minutes thereafter until the end of the study.

Each animal was internally controlled (comparisons of flow changes with flows determined during control periods) and compared with external control animals (sheep that underwent catheter placement but were not bled).

Results

Effects of 25% Blood Loss on Intestinal Lymph Flow

Lymph-flow rates, arterial blood pressures, and heart rates were monitored in two groups of anesthetized sheep over a 7-hour period: a control group and a group in which 25% of blood volume was removed.

In 5 sheep, a rapid (10–15 minute) loss of 25% of the blood volume resulted in reductions in mean arterial blood pressure ranging from 100 mm Hg before bleed to 40–60 mm Hg immediately after the bleed was terminated. In most animals, the pressures had returned to prehemorrhage levels by the end of the third hour. Heart rates also demonstrated reduction in the range of 75% of control values but generally had returned to normal by the end of the first hour.

Lymph-flow rates from the mesenteric duct increased in each of the 5 sheep following hemorrhage (Figure 2). While the time course was different from animal to animal, the peak increases ranged from 200–700% of control levels. Figure 3 demonstrates the combined results of these experiments. The elevations in lymph-flow rates tended to be bimodal with peaks at 1 and 4 hours postbleed.

In comparison with these results, 3 animals that were treated similarly but not bled showed no significant deviation in any of the vascular or lymphatic measurements over a 7-hour monitoring period (data not shown).

Properties of Pumping Model

Measurements of lymph flow alone do not permit analysis of the role of lymphatic contractile activity (active lymph propulsion) in the dynamics of shock. For this reason, the lymphatic was completely isolated from all lymph input as described in "Materials and Methods." The segment was supplied with lactated Ringer's solution from a reservoir. With inflow and outflow pressures equal, fluid could move through the duct only after contractions of the isolated segment, with the valves controlling the direction of flow.

Before the "double-catheter" preparation could be used, a number of criteria had to be met. First, with no net driving pressure, the duct had to pump fluid. Second, the lymphatic segment had to be completely isolated from all lymph input. This was tested by shutting off the fluid from the reservoir. If the pumping of Ringer's lactate did not stop, it was assumed that one of the tributary vessels had been left untied. Generally, it took from 1–10 minutes for the flow through the duct to cease, probably reflecting the amount of fluid in the vessel at the time of reservoir closure. Third, the preparation had to remain patent for the duration of the

![Diagram showing changes in lymph flow rates and mean blood pressures](image-url)
Effects of 25% Blood Loss on Fluid Propulsion

Fluid pumping, arterial blood pressures, and heart rates were monitored in 2 groups of anesthetized sheep over a 7-hour period: a control group and group bled of 25% of blood volume. In 5 control animals, lymphatic pumping was fairly constant for the first hour and then usually dropped to approximately 60% of this level for the following 2 hours. In the remaining 4 hours, fluid pumping declined to a level approximately 40% of the first 1-hour period. In contrast, in each of the 7-hemorrhaged sheep, pumping increased above the prebleed levels. Pumped volumes ranged from 279-2,529 μl/hour in the prebleed periods and from 551-6,192 μl/hour in the sixth hour following hemorrhage. The time course of the increase was variable. In Figure 4, for example, fluid pumping increased almost immediately and was maintained at approximately twice the control levels for the full 6-hour period. In some animals, the pumping increased transiently in the first hour, then fell to control or lower levels over the next 2-3 hours, and finally increased again in the fifth and sixth hours. In 1 sheep, the maximum increase in pumping occurred at 3 hours postbleed. Figure 5 illustrates the results of the experiments with the “double-catheter” preparation, comparing pumped volumes relative to internal controls (i.e., prebleed activity) and relative to external controls (i.e., unbled animals). There was a pumping increased to approximately 15 cm H₂O and then declined (data not shown). All experiments were conducted at the submaximal-distending pressure of 10 cm H₂O. While it is evident that the values given for the transmural pressures are estimates, the only critically important factors allowing interpretation of the experimental results are 1) that the heights of the reservoir and outflow catheter be set equal to each other (no net driving pressure) and 2) that the transmural pressure be kept constant for the duration of the experiment.

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In most cases, arterial blood pressures showed a steady decline until death (data not illustrated).

**Lymphatic Pressure Changes Following Hemorrhage**

Lymphatic pressures monitored from the “double-catheter” preparation characteristically revealed rhythmic pressure fluctuations that could only be due to contractions of the lymphatic segments with attendant pulsatile movement of fluid to the flow transducer (Figure 7A). While it is theoretically possible that compression of the vessel during respiration of peristaltic movement of the intestines could result in fluid being expelled from the ‘duct, we could find no evidence that external compression forces contributed to fluid propulsion. Careful attention was paid to the relationship between respiration, lymphatic pressure, and fluid movement in this model, and it was clear that respiration had no effect on propulsion (respiratory rates were 30–60/min, whereas the frequency of pressure pulses ranged from 5–15/min). Superimposed on the rhythmic pulse pressures were slow changes in baseline pressures, which were most likely due to peristalsis of the intestinal tract. Neither respiratory movements nor the slow fluctuations of the baseline pressures were associated with movement of fluid to the transducer arm.

As can be observed in Figures 7A and B, the frequency and amplitude of the pulse pressures monitored from “double-catheter” preparations increased following hemorrhage, indicating enhanced intrinsic contractile activity. Similar increases in pulse pressure measurements were found in intact intestinal lymphatic vessels (Figures 7C and D).

**Effects of 50% Blood Loss on Lymph Flow and Fluid Propulsion**

The removal of 50% of blood volume resulted in variable changes in lymph-flow rates monitored from mesenteric vessels, but the dominant response was to decrease flow (Figure 6A). In 3 of the 5 animals there was an increase in lymph-flow rates immediately following the bleed, but in general, the flows declined to very low levels in the first 30 minutes. In one case, the animal died at the 30-minute period with flow rates still elevated.

The results with the pumping preparation illustrated a similar variability (Figure 6B). In 2 of the 4 sheep, fluid pumping increased initially, then fell to below control levels until death. In the remaining animals, pumping either remained elevated or, after a transient depression in pumping activity, increased to around control values immediately before death.

**FIGURE 5.** Effects of 25% hemorrhage on pumping activity. Each bar represents volume (μJ) pumped over 1-hour period expressed as a percentage of volume pumped during 1-hour control period (external control nonbled animals) or immediately preceding a 25% bleed (hemorrhaged group). Cross-hatched bars illustrate means ± SEM of volumes pumped over each 1-hour period from the 8 sheep in hemorrhaged group. Open bars represent means ± SEM of volumes pumped over each 1-hour period derived from the 5 sheep in nonbled group. Using an analysis of variance, there was a significant difference between the 2 groups (p<0.01). With an unpaired t test the hemorrhaged and external control values were different at 1, 4, 5 (p<0.025), and 6 hours (p<0.05). Bottom trace expresses ratio of volumes pumped over 1-hour periods between hemorrhaged/external control animals based on mean values shown above.

**FIGURE 6.** Effects of 50% hemorrhage on lymph-flow rates and lymphatic pumping. In each experiment, average flow rate over 10-minute intervals was determined for 1-hour control period. Following hemorrhage, lymph flow from intact intestinal vessels or volumes pumped from “double-catheter” preparation was calculated over 10-minute intervals and expressed as a percent of control rate (determined from the mean of 6 values). Upper graph (Panel A) illustrates results of intestinal lymph flows in 5 separate sheep bled 50% of their calculated blood volume. Lower graph (Panel B) denotes results of pumped volumes in 4 separate sheep bled to 50% of their calculated blood volumes.
Discussion

Despite the physiological significance of the lymphatic circulatory system, few investigators have considered a dynamic role for the lymphatic vessel in shock. This relative indifference may result from the popular notion that the lymphatic vessel is itself a passive conduit and therefore incapable of having any impact on flow rates. However, it is becoming increasingly evident that the intrinsic contractile properties of the lymphatics provide a major part of the propulsive force in resting states.\(^\text{131922-24}\) Unfortunately, the role of the lymph pump in pathophysiological conditions is less clear. Part of the problem is that it has been very difficult to assess the intrinsic lymph pump in vivo using existing models.

The "double-catheter" preparation used in this study, however, permits the analysis of the effects of hemorrhage on the pumping activity of a mesenteric lymphatic segment without the complication of variable lymph inputs. Following 25% blood loss, we were able to demonstrate increases in the flow of fluid through the "double-catheter" preparation, which, because of the nature of the model, could only be explained on the basis of increased activity of the lymph pump.

The mechanisms responsible for the increased lymphatic contractile activity have not yet been directly assessed; however, observations by us and others point to several possible regulatory factors. Inherent in lymph vessels is their ability to contract spontaneously depending on the degree of vessel wall distension (myogenic regulation).\(^\text{2425}\) In these experiments, however, the fluid load to the vessel in the form of Ringer's lactate from the reservoir (transmural distending pressure) remained constant. Therefore, increased pumping could not have been due to myogenic mechanisms.

Since surgical dissection during catheter placement was kept to a minimum in order to preserve local blood and nerve supply to the vessel, it is possible that autonomic discharge or humoral factors released into the bloodstream could modify pumping. Additionally, chemical substances produced in the tissues surrounding the duct may diffuse to the vessel and alter its contractile activity. Hemorrhagic shock is known to invoke a strong sympathetic response. Lymphatics possess noradrenergic innervation, and lymphatic motility can be markedly affected in vitro with catecholamines.\(^\text{202426-28}\) Many chemical mediators are known to be formed in hemorrhagic states, including products of arachidonic acid metabolism.\(^\text{29}\) It is interesting to note that several members of the prostaglandin and leukotriene family are among the most potent stimulators and inhibitors of lymphatic contractile activity in vitro, and their generation in the bloodstream or the local tissues may possibly alter the activity of the lymph pump.\(^\text{30-32}\)

Considering the large reservoir of fluid and protein in the interstitium and lymphatics, it seems likely that the lymphatic return of these components to a diminished vascular space is helpful in reestablishing vascular stability following a major blood loss. Pertinent to this is the observation that flows monitored from cannulated mesenteric vessels with lymph input intact also increased following 25% blood loss in the studies reported here. Indeed, Cope and Litwin\(^\text{13}\) noted a twofold to threefold increase in thoracic duct flow in the first hour after bleeding dogs of 30% of their total blood volume. More than 2 times the amount of protein removed in the bleed was returned to the circulation by the lymphatics over a 24-hour period. Much of this must have come from the bowel, since the thoracic duct lymph receives 70% of its volume and 60% of its
protein from the intestines.\textsuperscript{33} Therefore, it is tempting to speculate that stimulation of the lymph pump facilitates the return to the bloodstream of the contents of the lymphatic circulatory system and possibly the lymph-accessible fluid and protein in the interstitium as well. However, such a proposal introduces a number of difficulties that highlight our lack of understanding of several fundamental problems in modern lymphology.

For example, in the work reported here, 25\% blood loss resulted in increases in flow rates monitored from intact intestinal ducts despite the fact that mean arterial blood pressures were considerably depressed in the early stages of shock. Other investigators have observed diminished blood flow to the bowel under similar circumstances.\textsuperscript{34-36} Taken together, these observations suggest that lymph formation may be reduced under these conditions. If this is the case, where does the fluid come from? An increase in lymph flow might occur if the lymphatic vessel is capable of sucking fluid and protein from the interstitium, a view that has gained some support in recent years (reviewed in Adair and Guyton\textsuperscript{37}). Alternatively, the lymphatic may not act as a suction pump, and other forces may be responsible for driving or pulling the fluid into the lymphatic capillaries. Lymphatic pumping would be limited by the delivery of fluid from the tissues, which, in this case, would imply that sufficient fluid was available in the intestinal wall interstitium to maintain the increased flow rates. In addition, increases in lymph flows could result from local elevations in capillary pressures, perhaps as a consequence of venous constriction, or possibly might result from enhanced microvascular permeability.\textsuperscript{6,8,32,33} Whatever the mechanism, there is sufficient fluid to maintain elevated flow rates for at least the 6-hour monitoring period in the experiments reported here. Given the potential restrictions placed on the volume of fluid available to the vessels with lymph input compared to the unlimited supply of reservoir fluid available to the "isolated" preparations, it is not surprising that the time course of the changes in lymphatic pumping and lymph flows was not the same.

In animals bled to 50\% of their blood volumes, lymph flow from intact intestinal ducts did not increase but rather declined to well below control rates in most animals. Because of the rapid onset of cardiovascular failure and death in this series, it was difficult to assess the effects on pumping. Three of the 4 animals demonstrated a transient increase in activity, and then pumping declined. However, because of the short duration of the experiments (mortality was 100\%), it was impossible to determine the significance of these observations relative to the control animals. It is more than likely that with extremely severe bleeds all forms of compensatory mechanisms are overwhelmed, plunging the animal into intractable shock, and like all other organ systems, general lymphatic pump failure may ensue. Therefore, it is possible that the decline in flow was due to a suppression of the lymph pump, perhaps as a consequence of reduced blood flow to the vessel. In summary, the studies reported here have focused on the ability of lymphatic vessels to contract and propel fluid in vivo. We have demonstrated that lymphatic pumping in sheep increases following hemorrhage independent of changes in lymph formation. We speculate that stimulation of the intrinsic lymph pump plays an important role in reexpanding the vascular space.

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