INTRINSIC PUMP-CONDUIT BEHAVIOR OF LYMPHANGIONS

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ABSTRACT

Lymphangions, segments of lymphatic vessels bounded by valves, have characteristics of both ventricles and arteries. They can act primarily like pumps when actively transporting lymph against a pressure gradient. They can also act as conduit vessels when passively transporting lymph down a pressure gradient. This duality has implications for clinical treatment of several types of edema, since the strategy to optimize lymph flow may depend on whether it is most beneficial for lymphangions to act as pumps or conduits. To address this duality, we employed a simple computational model of a contracting lymphangion and predicted the flows at both positive and negative axial pressure gradients, and validated the results with in vitro experiments on bovine mesenteric vessels. This model illustrates that contraction increases flow for normal axial pressure gradients. With edema, limb elevation, or external compression, however, the pressure gradient might reverse, and lymph may flow passively down a pressure gradient. In such cases, the valves may be forced open during the entire contraction cycle. The vessel thus acts as a conduit, and contraction has the effect of increasing resistance to passive flow, thus inhibiting flow rather than promoting it. This analysis may explain a possible physiologic benefit of the observed flow-mediated inhibition of the lymphatic pump at high flow rates.

Keywords: lymphangion, time-varying elastance, shear stress induced dilation
INTRODUCTION

The relatively simple structure of collecting lymphatic vessels yields complex behavior. Like veins, lymphatic vessels are remarkably thin-walled and contain unidirectional valves. Whereas the initial lymphatics are typically passive structures, the conducting lymphatics contain smooth muscle that cyclically contracts at a rate of 1-15 cycles per minute (2, 13, 27, 42, 53). The section of vessel between valves forms a functional unit, called a lymphangion (32). Lymphangions are arranged to form a converging structure that returns interstitial fluid, called lymph once it enters the lymphatic system, to the general circulation through the great veins of the neck. Cyclical contractions of lymphangions usually actively pump lymph against a local pressure gradient. The concept that lymph is actively transported by lymphatic vessels took years to be accepted (17), and has become the focus of investigators interested in the role of lymphatic function in the genesis and resolution of edema (2, 4, 6, 25, 27, 42, 53).

This pumping behavior has lead investigators to describe lymphangion function in terms typically applied to ventricular function. For instance, lymphangions have discernable systolic and diastolic periods, and ejection fraction, stroke volume and stroke work have been used to describe active lymphangion output per cycle (3, 25). Borrowing the terms describing ventricular function is not unwarranted. Transmural pressure-volume loops, for instance, reveal behavior similar to that of ventricles, including an identifiable end-systolic pressure-volume relationship (25). Furthermore, lymphangion output increases with preload like the Frank-Starling effect (28). Lymphangion and lymphatic vessel output also is sensitive to afterload (6, 23). Characterization of lymphatic function using ventricular analogies is complicated, in part, because the afterload of one lymphangion forms the preload of the next one or more (16), yielding complex and variable interactions among lymphangions within a lymphatic vessel (53).
The use of ventricular analogies to describe lymphangions, however, is insufficient because lymphangions exhibit some behaviors best described by analogy to muscular arteries. Although lymphangions cyclically contract, they can exhibit a basal tone (26) that is sensitive to vasoactive substances such as NO donors (43), prostaglandins and thromboxane (22). Furthermore, high-flow conditions cause lymphatic vessels to relax (15), much like the shear stress-induced vasodilation found in muscular arteries. Wall tension-length relationships are similar to that of muscular arteries, with an optimal radius that yields an optimal developed tension (10, 42). Interest in this constellation of artery-like behaviors has not been reconciled with description of lymphangions as pumps.

Interpreting lymphatic vessels only as a series of pumps becomes problematic when they are exposed to conditions common to arteries. Both high transmural pressures, leading to pump failure (4, 25, 28, 36), and the high flow rates, leading to shear stress-induced relaxation (15), can inhibit active lymphatic pumping. Since these behaviors occur with edema, they may be perceived as maladaptive. However, if the inlet pressure of a lymphatic vessel rises significantly higher than its outlet pressure, a fundamentally new situation arises. In this case, the lymphatic inlet pressure is high enough that lymphangions are no longer pumping against a local pressure gradient. Lymphatic function, assessed by lymph flow, has not been sufficiently investigated for the special case in which inlet pressure is greater than outlet pressure. Since lymph flow depends on preload, afterload, contractility, temperature, time delay between lymphangion contractions, tissue pressure, and lymph viscosity, it is understandable that many investigators have restricted themselves to studying flow through lymphatic vessels with a zero axial (end-to-end) pressure gradient (8, 19, 28, 29).
The complexity arising from the interaction of lymphangions is contrasted with the comparative simplicity of lymphatic vessel structure and fluid flow. The mechanics of fluid flow through vessels is fairly well understood, and computational models characterizing this flow have been well established (35, 52). Furthermore, ventricular contraction also has been well characterized, and computational models have been used successfully to describe their function in a variety of loading conditions (45, 49, 50). Computational modeling, combining models of arteries and ventricles, provides the means to characterize lymphatic function without neglecting many dynamic complexities, while focusing in particular on the response to changes in axial pressure gradients.

The purpose of this study was to determine the effect of reversing the axial pressure gradient on lymph flow using a computational model of a single lymphangion, and to verify predicted results with focused in vitro experiments. We propose that under normal conditions, lymphangions act primarily as active pumps, but with elevated inlet pressure they can begin to act as passive conduits.

**THEORY**

*Characterizing lymphangion contraction.* The need to characterize the inherent contractility of a lymphatic vessel independent of its loading conditions is similar to the historical need to characterize ventricular contractility independent of both preload and afterload. Suga et al., in a series of seminal papers (47-49), developed a relatively simple description of the heart that related ventricular chamber pressure, \( P_t \), to chamber volume, \( V \). This relationship, named the time-varying elastance, \( E(t) \), is a function of time, and can be calculated from measured \( P_t \) and \( V \),
\[ E(t) = \frac{P(t)}{V(t) - V_o} \]  

(1)

where \( V_o \) is the dead volume (the theoretical volume at zero pressure). The maximum value, \( E_{max} \), is the slope of the end-systolic pressure-volume relationship, and the minimum value, \( E_{min} \), is the slope of the end-diastolic pressure-volume relationship. The value of \( V_o \) can be calculated from the intercept of the end-systolic pressure-volume relationship. \( P_t \) is a transmural pressure, the difference in luminal and external pressures, and thus can be modified by external compression. Although it has been subject to numerous criticisms and improvements since its inception (37, 44), this simple description formed the basis for successful models of heart-arterial system interaction (3, 11), and will be used in the present work to describe lymphangion contraction.

**Characterizing lymph dynamics.** The need to characterize pulsatile lymph flow as a function of pressure is similar to the historical need to characterize the pulsatile pressure-flow relationship in arteries. Reddy et al. recognized the need for a fundamental basis from which to predict lymph flow and derived equations from simplifications of the Navier-Stokes equation (39, 40). The results are equivalent to equations described by Noordergraaf (35) for blood flow in arteries. In essence, the resulting relationship depends on tube radius, \( r \), length, \( L \), fluid density, \( \rho \), and viscosity, \( \eta \). Assuming a cylindrical shape, the pressure drop across a segment of vessel, \( \Delta P \), depends on a resistive term and a term related to fluid inertia.

\[
\Delta P = \frac{8\eta L}{\pi^4} Q + \frac{\rho L}{\pi^2} \frac{dQ}{dt}
\]  

(2)

Unlike Eq. 1, the pressure in this case is a difference in upstream and downstream *luminal pressures*. We add the inertial term to the model to ensure that the model accounts for fluid acceleration during ejection, even though it is expected to have a smaller effect than the resistive
term. Continuity is assured if the rate at which volume is stored is equal to the difference of the flow into the vessel, $Q_{in}$, and the flow out of the segment, $Q_{out}$.

$$\frac{dV}{dt} = Q_{in} - Q_{out}$$  (3)

In addition, a small resistance is added before and after the inlet and outlet of the vessel, in order to simulate the resistance of the tubing used for in vitro experiments, and is analogous to upstream and downstream resistances in vivo. These equations have been successfully used as a basis to describe the interaction of multiple vessels in a vascular network (21, 51), and will be used in the present work to describe lymph flow. The fundamental difference, however, is that radius in Eq. 2 is considered a variable, rather than a constant.

**Description of valves.** The valves in lymphangions are very thin, and prevent, or at least minimize, retrograde flow (12). In concert with computational descriptions of ventricular valves, the lymphatic valves are assumed to open when the pressure immediately proximal to the valve is greater than the pressure immediately distal to the valve. The valve is assumed to close when the pressure immediately distal to the valve is greater than the pressure immediately proximal to the valve. Because there is a rigid annulus surrounding the valve structure, it is explicitly assumed to depend only on the axial pressure gradient, and not transmural pressure.

**External compression.** Notably, the axial pressure gradient in Eq. 2 is expressed in terms of luminal pressures only, but the pressure in Eq. 1 is expressed in terms of transmural pressure (luminal pressure minus external pressure). Characterizing lymphangion contraction in terms of transmural pressure allows this system of equations to predict the effects of both intrinsic and extrinsic propulsion of lymph. The underlying assumption, however, is that external compression is uniform. This may be a questionable assumption for compression by skeletal
muscle \textit{in vivo}, but is a fair approximation for compression due to submersion of a lymphangion in a vessel bath \textit{in vitro}.

\textit{Boundary conditions and solution technique.} With lymphangions described by Eqs. 1-3, the flow resulting from a pressure gradient can be predicted. To simplify, the inlet and outlet pressures were set to mimic those \textit{in vitro}. All inlet and outlet pressures were maintained below 8 mmHg to ensure that transmural pressure remains below a level that would be expected to induce pump failure (13). All equations were solved using a multistep solver (implementing Gear’s Method) in MatLab, which is a particularly stable solver for these nonlinear equations (Eqs. 1-3). Flow was solved as a function of time, and the solution was obtained after the system achieved steady-state. To estimate the sensitivity of the solution to the particular parameters, the ranges of parameters necessary to cause a 10% change in predicted mean flow were calculated.

\begin{flushleft}
\textbf{EXPERIMENTAL METHODS}
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Post-nodal mesenteric lymphatic vessels were isolated from euthanized cattle and placed in a tubular organ bath (Harvard Apparatus, Holliston, MA). Vessel segments containing no valves (allowing measurement of transmural pressure) were used for experiments to establish elastance parameters. Vessel segments containing two valves were used for experiments to demonstrate the effect of axial pressure gradient on flow. The vessels were perfused and bathed with a balanced buffered polyionic solution with 1% albumin, gassed with room air and set to 37°C and pH of 7.4 as shown in Fig. 1. Because the vessel was submerged, there was an external pressure of 1 mmHg acting on the vessel. Thus all inlet and outlet pressures ($P_{in}$ and $P_{out}$) are expressed in terms of transmural pressures (i.e., inlet and outlet pressures – 1 mmHg).
In a spontaneously contracting vessel segment without valves, transmural pressures were raised from 0 to 6 mmHg and the resulting radii were recorded. Pressure-volume loops were recorded, and an end-systolic pressure-volume relationship was determined. Data from this one vessel segment provided an illustrative slope and intercept used for determining a time-varying elastance curve for the mathematical model according to Suga et al. (49).

In a spontaneously contracting vessel segment with valves, outlet pressure was set to 4 mmHg, and inlet pressure was set incrementally between 2.5 and 5.5 mmHg to set the axial pressure gradient ($P_{in} - P_{out}$) between -1.5 and 1.5 mmHg. Instantaneous vessel diameter was measured using a custom video dimension analyzer (IMAQ, National Instruments Corp., Austin, TX). Lymphangion stroke volume was calculated from the volume (estimated from length $\cdot \pi r^2$) of the lymphangion at end-diastole minus the volume at end-systole. Instantaneous flow was measured using a calibrated low-Reynolds Number, pressure-gradient flowmeter at the outlet end. Instantaneous flow at each setting was recorded for one minute and averaged. After setting the axial pressure gradient to a new level, the preparation was allowed to equilibrate for three minutes prior to the onset of recording. Data for all measured parameters were digitally collected and recorded from the vessel under baseline conditions. This protocol was repeated after making the vessel passive with calcium-free luminal and bath solutions.

RESULTS

Model construction: time-varying elastance calculated from in vitro data. Transmural pressure and volume in a segment of lymphatic vessel without valves is plotted in Figs. 2A and B as a function of time. As volume at end-diastole increased, maximum developed pressure increased. Equation 1 was applied to calculate time-varying elastance from the pressure and
volume curve for a representative cycle. \( V_o \) was calculated from linear regression from the end-
systolic pressure-volume relationships of the pressure-volume curves. The resulting elastance
curve is illustrated in Fig. 2C, and forms the basis for the lymphangion model.

*Instantaneous flow for different pressure gradients.* Figure 3 reports the flow predicted
from the computational model (Eqs. 1-3) for two illustrative different pressure gradients. In the
case that \( P_{in} < P_{out} \), there was no flow in diastole (Fig. 3A). Flow increased when diameter
decreased. In the case that \( P_{in} > P_{out} \), there was flow during diastole (Fig. 3B). Flow first
increased in early systole, and then decreased with further decreases in diameter. Predictions can
be compared to experimental data recorded in a lymphangion for the same boundary conditions
(Figs. 4A and B).

*Average flow for different axial pressure gradients.* To generalize the specific results
illustrated in Figs. 3 and 4 for a full range of pressure gradients, Fig. 5A illustrates the average
flow predicted by the computational model for outlet pressure = 4 mmHg and varied axial
pressure gradient. When inlet pressure was less than outlet pressure, flow was relatively small.
When downstream pressure was less than upstream pressure, mean flow increased markedly.
Fig. 5B illustrates the measured mean flow from a representative bovine mesenteric vessel.
Compared to the computational model, the absolute values of measured flow are different, yet
the same fundamental behavior is illustrated.

*Lymphatic pump inhibition.* To simulate the effect of pump inhibition, also illustrated in
Fig. 5A, the flow is predicted when \( E(t) \) is set to a constant value equal to \( E_{min} \). Inhibition of
pumping yields zero flow when the axial pressure gradient is less than 0 mmHg, but actually
increases flows above control values when the pressure gradient is larger than 0.2 mmHg. Figure
5B illustrates the measured mean flow from a representative bovine mesenteric vessel with and
without calcium-free perfusate. In the calcium-free case, spontaneous contraction ceased. Inhibition of pumping yielded zero flow when the pressure gradient is less than 0 mmHg, but increased above control flows when the pressure gradient increased sufficiently. Compared to the computational model, the same fundamental behavior was observed.

*Calculated versus measured flow.* To illustrate how a pressure gradient can influence the accuracy of a calculated flow (stroke volume x contraction frequency), flow was calculated from the model (Fig. 6A) and from the data from a representative lymphangion (Fig. 6B). For both the computational model and the *in vitro* data, when outlet pressure was higher than inlet pressure, there was no flow in diastole, and the calculated flow corresponded to the total flow. When inlet pressure was greater than outlet pressure, however, flow occurred during diastole, and the calculated flow greatly underestimated total flow.

*Valve behavior at different pressure gradients.* The amount of time valves are open during a contraction cycle cannot easily be determined from the *in vitro* analysis of the bovine mesenteric collecting lymphatic vessels, but can be investigated by use of the computational model. Figure 7 illustrates the percentage of the contraction cycle that the valves are in the open position as a function of pressure gradient. When outlet pressure is higher than inlet pressure, the valves are closed during most of the contraction cycle. As inlet pressure approaches outlet pressure, the valves remain open a larger portion of the cycle. When inlet pressure rises above outlet pressure, there is some flow in diastole, and the percent of time the valves are open increases markedly. At a critical pressure gradient, the valves remain open for the entire contraction cycle, indicating that the lymphangion is acting as a conduit. This prediction is difficult to confirm experimentally in our study given the difficulty viewing valves through the lymphangion wall in the *in vitro* preparation.
Parameter sensitivity. To determine the sensitivity of the model to assumed model parameters, the range of parameters resulting in a 10% change in mean flow were calculated for the case of a pressure gradient of 1.5 mmHg. Experimental values for elastance used for the model include both $E_{\text{max}}$ and $E_{\text{min}}$. $E_{\text{min}}$ can be varied up to 5% before altering calculated flow by 10%, and $E_{\text{max}}$ can be varied up to 11% before altering calculated flow by 10%. The experimental error in $E_{\text{max}}$ and $E_{\text{min}}$ measurement are estimated to be less than 5%, given that pressure measurement error is less than 2% and radius measurement error is likewise less than 2%. Because input and output resistances were small, changes in their resistances had negligible effects on flow.

DISCUSSION

This work utilized multiple approaches to illustrate that lymphangions can behave as either pumps or conduits. Under conditions in which inlet pressure is less than outlet pressure, flow through a particular lymphangion only occurs when vessels actively pump. Under conditions in which inlet pressure exceeds outlet pressure, however, there can be flow during diastole, and contraction may only partially augment flow. When the inlet pressure is high enough for the valves to be open the entire contraction cycle, lymphangions become conduits. When vessel segments are thus acting like conduits, cyclical contraction is unnecessary to propel lymph and, in fact, cyclical contraction can impede flow. Because lymphangions decrease pumping and decrease baseline tone with flow down a pressure gradient (14, 15), it is likely that lymphangions act as self-regulating units that become better conduits under high flow conditions. Whereas early reports described lymphatic vessels as conduits, and newer functional
studies describe them as pumps, the present work attempts to reconcile these two descriptions and delineate the conditions in which one description is better than the other.

**Conditions that may lead to conduit behavior in vivo.** The present work used experimental and theoretical approaches to predict behavior of lymphangions when the normal axial pressure gradient reverses. Although the scope of the present work did not include *in vivo* studies, four cases likely result in an altered lymphatic axial pressure gradient: interstitial edema, limb elevation, extrinsic compression, and contraction of upstream lymphangions.

1. **Interstitial edema.** Many forms of interstitial edema will not result in a reversal in the lymphatic axial pressure gradient because interstitial fluid pressure may increase very little (1), especially with inflammatory edema (34, 38). However, several notable cases have suggested that interstitial pressure can rise significantly: (A) Mortillaro and Taylor (33) showed that intestinal interstitial fluid pressure increased from -0.56 ± 0.193 to 7.3 ± 0.63 mmHg as a result of venous hypertension, (B) Laine et al. (24) showed that hepatic interstitial fluid pressure increased from 5.8 ± 0.87 to over 20 mmHg in response to inferior vena caval hypertension, (C) Stewart and Laine (46) showed that cysterma chili pressure increased from 1.0 ± 0.2 to 6.0 ± 1.0 mmHg following partial occlusion of the inferior vena cava and (D) Granger et al. (18) estimated that intestinal interstitial fluid pressure increased from 0.82 ± 0.72 to 4.4 ± 0.76 mmHg during intestinal lipid absorption. In these cases, it may be possible for average lymphangion inlet pressures to be higher than average outlet pressures, favoring the conduit behavior described in the present work.

2. **Limb elevation.** Even if edema does not act to reverse the normal pressure gradient in lymphatic vessels, a common treatment may. Limb elevation has long been recognized as an effective treatment of local edema (31). In this case, the inlet lymphangion pressure can increase
an amount equal to the pressure head generated from elevation. When a limb is elevated above the great veins of the neck, the axial pressure gradient could favor the passive flow of lymph (Fig. 7) and thus yield conduit behavior.

3. *External compression.* Cyclical lymphangion compression resulting from locomotion, intestinal peristalsis, or respiration (20, 41) can augment or even obviate the need for intrinsic lymphatic pumping. External compression, however, may also alter the inlet lymphangion pressure. McGeown et al. illustrated that lymphatic outflow can increase fourfold with intermittent compression over metatarsal regions of sheep (30).

4. *Contraction of upstream lymphangions.* Lymphangions can generate pressures at least 20 mmHg, a value much higher than normal central venous pressure. Although this may appear to preclude the possibility for the conduit behavior explored in the present work, it is the local axial pressure gradient that determines whether a particular lymphangion acts as a conduit. If, for instance, an upstream lymphangion cycles between 3-15 mmHg and a downstream lymphangion cycles in phase from 2-14 mmHg, Fig. 7 suggests this axial gradient would be enough (i.e., 1 mmHg) to ensure passive lymph flow. Valves separating intermediate lymphangions may never close, and cyclical contraction of intermediate lymphangions may inhibit this passive flow. Similarly, if contractility of an upstream lymphangion is stimulated, or contractility of a downstream lymphangion is inhibited, lymphangions could generate a local pressure gradient that favors conduit behavior. Since the pressure gradient between lymphangions is typically small (7), and regional variations in contractility can be quite large (14), there may be numerous opportunities for a 1 mmHg pressure gradient to arise, favoring local conduit behavior.
Transition from a pump to a conduit. Most lymphatic studies in vitro or in situ are performed with the axial pressure gradient set to zero. In our validation experiment with a bovine vessel, the flow at an axial pressure gradient of zero was 0.4 ml/min. This value corresponds to reported flows of 0.27-1.3 ml/min (29), 0.2-1.0 ml/min (8) and 0.2-1.0 ml/min (19). Also, McHale and Roddie (28) reported the flows from one vessel ranging from 0.38-0.8 ml/min. The high sensitivity of lymphangions to axial pressure gradients, especially near a zero pressure gradient, allows an abrupt transition from a low-flow pump to a high-flow conduit, and may explain in part the high degree of variability of lymphatic vessel behavior. Not only does the transition from pump to conduit result in very large changes in flow, there is a range of low pressure gradients where the behavior is not completely that of a pump or a conduit. Under these conditions, flow occurs during diastole, yet vessel contraction augments total flow. Many investigators have characterized lymphatic vessel function in vitro or in situ with a zero axial pressure gradient. Because the pressure gradient across each lymphangion is temporally variable due to the cyclic contraction of upstream and downstream lymphangions, this type of experiment would ensure that some lymphangions would be acting like pumps, some like conduits, and some would be in transition from one to the other.

New direction for treatment of edema. This work suggests a new direction in clinical research focusing on treatment of some kinds of edema, especially those caused by increased microvascular pressures or permeability. Typically, lymphatic vessels are viewed only as pumps, and thus only pump failure is understood to lead to edema (4, 5). It follows that an increase in the cyclical contraction of lymphangions will increase lymph flow and thus is necessary to resolve edema. This could be true for edema resulting from increased downstream resistance or pressure for given lymphatic bed (e.g., for post-inflammatory partial lymphatic
obstruction). However, if interstitial edema were to cause inlet pressure in a given lymphatic bed to rise above outlet pressure, vessels may become conduits, thus vessel radius determines the resistance to passive flow. In fact, from the computational model, validated by *in vitro* experimentation, flow can increase when cyclical contraction is inhibited. If the clinical goal in edema treatment for such cases is to increase drainage of fluid from the interstitium, then promoting vessel relaxation, not lymphatic pumping stimulation, may best achieve this goal.

*From apparent paradox to new anti-edema mechanism: reinterpreting pump failure.*

Gashev et al. reported that small, transport vessels relaxed when presented with progressively increasing axial pressure gradients and, presumably, high endothelial shear stresses (15). This behavior is consistent with shear stress-induced dilation found in arteries. This pump inhibition could be viewed as a maladaptive response to edema. This work, however, provides evidence that pump inhibition is instead “a reasonable physiological mechanism” (15) to save energy by reducing contractions and, thus, reducing resistance to lymph flow. Similarly, the observation that stroke volume of lymphatic vessels diminishes when transmural pressure rises above a critical value (28) may be described as “pump failure.” Since lymphatic transmural pressure increases in edema, this behavior has also been viewed as a maladaptive response to edema formation. Of course, the underlying assumption has been that pumping is always necessary to resolve edema. The present work provides evidence that “pump failure” may be a beneficial adaptation that decreases resistance to passive lymph flow—a manifestation of a previously unappreciated anti-edema mechanism.

*Increasing pumping verses pump inhibition in response to edema.* The question of whether “pump failure” is detrimental or beneficial for resolving edema may not be answerable using *in vitro* experiments where blood-borne or lymph-borne humoral factors, innervation, and
tethering of the vessel to the surrounding extracellular matrix are removed, and every effort is made to keep inlet and outlet pressures constant. In addition, because the lymphangion interacts with its environment, the critical pressures causing a transition from pump to conduit cannot be determined from *in vitro* experiments—lymphangions can alter their own inlet and outlet pressures. Furthermore, the transition point from pump to conduit is not immutable, since it depends on a lymphangion’s ability to contract. For instance, endotoxin can inhibit lymphatic pumping at a particular transmural pressure (9), suggesting that the response to a significant edemagenic challenge is to become a conduit. However, endotoxin causes lymphangions to be better pumps at higher pressures (9), possibly optimizing pumping for the higher interstitial pressures that result from endotoxin. Pump-conduit duality of lymphangions complicates the interpretation of both *in vivo* and *in vitro* data, and puts into bold relief the need to take into consideration the complex interaction of lymphatic vessels and their environment.

*Apparent versus true lymph flow.* Because flow is difficult to measure *in vivo*, it is possible that the effect of pump inhibition in edemagenic states may have been misinterpreted. Lymph flow is difficult to measure directly with intravital microscopy in the microlymphatics because lymph for the most part is transparent. To deal with this limitation, an apparent flow has been calculated from stroke volume multiplied by contraction frequency (2). From this method, for instance, Benoit et al. calculated that in response to volume-loading, the calculated flow from small collecting lymphatics in the mesentery of a rat increased by 3-fold. The underlying assumption of this calculation is that there is no passive flow in diastole, and that all flow out of a lymphangion is equal to the volume change of the lymphangion. However, the current study presents clear evidence that it is possible for flow through a lymphangion to occur in diastole when inlet pressure is higher than outlet pressure. In fact, flow-induced pump inhibition can
cause the calculated lymph flow to actually decrease in edema. Failure to describe lymphatic vessels as conduits in edema can yield misleading results.

Broadening the scope. A pragmatic approach informed the scope of the current work. The model and the experiment were designed to be complementary, so that the transition from pump to conduit behavior could be studied in the most elemental functional unit of the lymphatic system—the lymphangion. To interpret experimental results fully and place the model on a firm basis, the mathematical model was designed to mimic the in vitro system rather than a portion of the lymphatic vasculature in vivo. This model also focused on intrinsic lymphatic pumping due to smooth muscle activity, rather than the important effect of extrinsic pumping arising from cyclical external compression (20, 30). Equation 1, however, was formulated in terms of transmural rather than luminal pressure, and could be used to further study the effects of cyclical compression. Nonetheless, the fundamental behavior of interest—the transition from a pump to a conduit—can be expected in portions of a lymphatic vessel segment, a network of lymphatic vessels, and an entire lymphatic network. Each of these three hierarchical levels has been modeled by Drake et al. (5), Stewart et al. (46), and Reddy et al. (40), respectively. Only Drake et al. (5) tangentially described this transition. Potentially, pump-conduit behavior of lymphangions could be complex, at each hierarchical level. For instance, at the level of a single vessel, it is possible that an upstream lymphangion forces flow through a downstream lymphangion, causing it to transition from a pump to a conduit. Similarly, with two convergent lymphatic vessels, the pumping in one vessel could raise the outlet pressure of the other vessel, causing it to transition from a conduit to a pump. An entire lymphatic system could therefore have multiple sections that are acting like both pumps and conduits. By limiting the scope of the present work to intrinsic contraction of lymphangions, vessels, networks and the entire system
can now be modeled with more confidence, and the physiological and clinical implications of pump-conduit behavior can be broadened.

**Computational model caveats.** This work predicted the function of lymphangions based upon known properties of lymphangions and the principles governing fluid motion. Although this computational model is based on fundamental principles, several simplifications were made. First, the characterization of lymphangions with the time-varying elastance concept was convenient, and provided a simple representation of the contraction of the lymphangion wall (54). The use of experimentally derived time-varying elastance parameters $E_{\text{max}}$ and $V_o$ is not particularly sensitive to measurement error. Rather, time-varying elastance, used extensively to model the function of ventricles, has been criticized for its simplicity. This approximation to describe lymphangions in particular neglects the high degree of temporal variability in the contractility and contraction frequency found in intact lymphatic vessels (25). This simple description, furthermore, does not fully account for “pump failure” at higher pressures, as described by McHale and Roddie (28) or the flow-induced dilation described by Gashev et al. (15). Both behaviors are important aspects that effect lymphangion contractility, but do not determine whether contraction itself increases or decreases total lymph flow. In fact, this simplicity helps illustrate the effects of axial pressure gradient without the confounding effects of these complications. Second, the computational model used simplified equations to describe the fluid motion. Following the derivation of Noordergraaf et al. (35), these simplifications notably neglect second-order terms and fluid momentum in the radial direction. These descriptions were derived by Reddy et al. from a simplification of the Navier-Stokes equations, which is justified for vessels with low Reynolds Numbers (39). The results are consistent with the *in vitro* measurements which were performed to validate the results. Figure 7 presents information from
the computational model that is particular difficult to obtain experimentally. We maintain that when computational models based on fundamental principles result in the same basic behavior observed in actual physiologic systems, the interpretation of the data is more secure.
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FIGURE LEGENDS

Figure 1: Schematic illustration of organ perfusion bath with lymphangion. Diameter recorded by video dimension analyzer (VDA), and flow recorded by flowmeter (grey box) on the outlet of the lymphangion. Height of fluid reservoirs set to favor passive flow, and inlet pressure, Pin, and outlet pressure, Pout, recorded. Unidirectional valves ensured flow was from left to right.

Figure 2:  A. Example of pressure recording in a segment of bovine mesenteric lymphatic vessel in vitro.  B. Volume calculated from simultaneously recorded radius. Vessel segment lacked valves, making it possible to measure luminal pressure.  C. Time-varying elastance calculated with Eq. 1 applied to A and B. Like ventricles, pressure increases even as volume decreases. To ensure stability in the simulation, two additional points were added so that beginning and ending elastance values matched.

Figure 3:  A. Flow and vessel diameter predicted by the computational model for an axial pressure gradient of 1.5 mmHg (pumping against a pressure gradient).  B. Values predicted by the model for an axial pressure gradient of 1.5 mmHg (pumping down a pressure gradient).

Figure 4:  A. Example of observed flow and vessel diameter for a bovine lymphatic vessel in vitro for an axial pressure gradient pumping against a pressure gradient of 1.5 mmHg.  B. Values measured for an axial pressure gradient pumping down a pressure gradient of 1.5 mmHg. Compare to Fig. 4.

Figure 5: The effect of active contraction. Mean flow out of a lymphangion when outlet pressure is set to 4 mmHg and variable axial pressure gradient.  A. Computational model results with cyclical contraction (●) and with cyclical contraction eliminated
by setting $E_{max}=E_{min}$ ($\circ$). 

B. Mean flow measured from a representative bovine lymphatic vessel with (●) and without (○) contraction (i.e., contraction eliminated with calcium-free perfusate). Mean flow at a zero axial pressure gradient was 0.4 ml/min, consistent with other reports (8, 19, 28, 29).

Figure 6: Flow as a function of axial pressure gradient. True flow was measured by flowmeter (●) and apparent flow was calculated from stroke volume x contraction frequency (○) in computational model (A) and representative lymphangion studied in vitro (B).

Figure 7: Percent of contraction cycle that the valves are open. Predicted from computational model under conditions similar to Fig. 6. Valves open completely during the entire cycle when inlet pressure is significantly higher than outlet pressure.
Figure 1: Schematic illustration of organ perfusion bath with lymphangion. Diameter recorded by video dimension analyzer (VDA), and flow recorded by flowmeter (grey box) on the outlet of the lymphangion. Height of fluid reservoirs set to favor passive flow, and inlet pressure, $P_{in}$, and outlet pressure, $P_{out}$, recorded. Unidirectional valves ensured flow was from left to right.
Figure 2:  
A. Example of pressure recording in a segment of bovine mesenteric lymphatic vessel *in vitro*. 
B. Volume calculated from simultaneously recorded radius. Vessel segment lacked valves, making it possible to measure luminal pressure.  
C. Time-varying elastance calculated with Eq. 1 applied to A and B. Like ventricles, pressure increases even as volume decreases. To ensure stability in the simulation, two additional points were added so that beginning and ending elastance values matched.
Figure 3:  
A. Flow and vessel diameter predicted by the computational model for an axial pressure gradient of 1.5 mmHg (pumping against a pressure gradient).  
B. Values predicted by the model for an axial pressure gradient of 1.5 mmHg (pumping down a pressure gradient).
Figure 4:  
A. Example of observed flow and vessel diameter for a bovine lymphatic vessel *in vitro* for an axial pressure gradient pumping against a pressure gradient of 1.5 mmHg.  
B. Values measured for an axial pressure gradient pumping down a pressure gradient of 1.5 mmHg. Compare to Fig. 4.
Figure 5: The effect of active contraction. Mean flow out of a lymphangion when outlet pressure is set to 4 mmHg and variable axial pressure gradient. A. Computational model results with cyclical contraction (●) and with cyclical contraction eliminated by setting $E_{\text{max}} = E_{\text{min}}$ (○). B. Mean flow measured from a representative bovine lymphatic vessel with (●) and without (○) contraction (i.e., contraction eliminated with calcium-free perfusate). Mean flow at a zero axial pressure gradient was 0.4 ml/min, consistent with other reports (8, 19, 28, 29).
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