

Lymph Pools in the Basement, Sump Pumps in the Attic: The Anuran Dilemma for Lymph Movement

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ABSTRACT

Amphibians are a vertebrate group transitional between aquatic and terrestrial environments. Consequently, both increases and decreases in blood volume are a natural biological stress associated with aquatic and terrestrial environments. In comparison with other vertebrate classes, anuran amphibians have the most rapid compensation and greatest capacity to compensate for changes in blood volume and survive dehydration. Unlike in mammals, a Starling transcapillary uptake mechanism does not account for this fluid mobilization because lymph flow is a substantial and important additional factor. The role of the lymphatic system in flux of fluids back into the circulation varies interspecifically in anurans and is an order of magnitude greater in anurans than in mammals. Current models of lymph movement in anurans are centered on the role of lymph hearts, but we suggest that these models are untenable. We present a new hypothesis for lymph movement involving (1) pressure differences created by compartmentalization of the hind limb lymph spaces into sacs of serially graded compliance to move lymph horizontally and (2) both negative and positive pressure differences created by contraction of skeletal muscles to move lymph vertically. The primary function of some of these skeletal muscles may be solely for lymph movement, but

some may also be involved with other functions such as pulmonary ventilation.

Introduction

This article has an unusual title, but it reflects the difficulty that anurans face in maintaining lymphatic return and hence blood volume. We present the presumed transcapillary mechanism responsible for blood volume maintenance in mammals and point out the difficulties in its application to anuran amphibians and potentially other vertebrates. We then present preliminary data that support a model involving pressure differences created by compartmentalization of the lymph spaces in conjunction with the contraction of skeletal muscles.

Transcapillary Fluid Flux

The short-term transcapillary redistribution of blood volume in mammals has always been interpreted from the well-known Starling fluid flux equation, presented here for flux across a capillary:

$$\text{plasma flux} = F_C[(P_{\text{cap}} - P_{\text{ist}}) - \sigma(\pi_{\text{cap}} - \pi_{\text{ist}})],$$

where F_C is the filtration coefficient of the capillaries ($\text{mL min}^{-1} \text{kPa}^{-1}$), P_{cap} is the capillary hydrostatic pressure (kPa), P_{ist} is the interstitial hydrostatic pressure (kPa), σ is the capillary osmotic reflection coefficient for plasma proteins, π_{cap} is the colloid osmotic pressure of the plasma (kPa), and π_{ist} is the colloid osmotic concentration of the interstitial fluid (kPa).

Normally, the balance of hydrostatic forces favors efflux of fluid from the capillary, while the balance of colloid forces favors influx. On average, the hydrostatic forces dominate in the circulation, and there is a consequential overall efflux of fluid from the vasculature across the capillaries into the interstitium. The fluid filtered is known as lymph. If water is added to the plasma space, then P_{cap} will increase and π_{cap} will decrease; both lead to greater efflux of fluid and a return toward the original plasma volume. If water is lost from the plasma space, then P_{cap} will decrease and π_{cap} will increase; both changes promote increased absorption of fluid from the interstitium and a return toward the original plasma volume. Consequently, there is a mechanism inherent in this balance of forces that

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tends toward homeostatic redistribution of blood and interstitial volumes independent of a regulated negative feedback loop.

Because F_C and σ vary in different organs (see Taylor and Townsley 1987), the use of a capillary-focused equation to describe blood volume regulation would require an integration of both the blood flow and Starling variables for every organ in the body, an impractical approach. There is a relationship between the fluid filtered and both P_{cap} and P_{ist} that is determined by both the vascular compliance and the interstitial compliance of different organs (see Tanaka 1979). For instance, organs encased by inelastic connective tissue show large increases in P_{ist} in response to filtration (see Taylor and Townsley 1987). The increase in P_{ist} will immediately oppose further hydrostatic filtration. Consequently, the Starling fluid flux equation is an excellent vehicle for describing fluid fluxes in an individual capillary in a particular organ, but it has limited utility in interpreting redistribution of fluid at an organismal level.

A more useful model for understanding the short-term effects of volume loading and hemorrhage on blood volume in mammals is a derivation of the Starling fluid flux equation that emphasizes whole-body characteristics and incorporates the interaction of volume flux on pressures. This approach entails incorporating the compliances (capacitances) of the vascular space and interstitial space (intercellular and lymphatic spaces) as well as delineating a whole-body filtration coefficient. It essentially examines the kinetics of fluid flux using an electrical circuit analogy that is described by the following equation (Tanaka 1979):

$$\text{flux} = F_C(V_{\text{vas}}/C_{\text{vas}} - V_{\text{ist}}/C_{\text{ist}}),$$

where F_C is whole-body filtration coefficient, V_{vas} is the volume of fluid in the vasculature, C_{vas} is the compliance of the vasculature, V_{ist} is the volume of filtered fluid in the interstitium, and C_{ist} is the compliance of the interstitium. Note that with this approach, fluid shifts can be analyzed without knowing the

absolute capillary hydrostatic pressure, interstitial fluid pressure, or plasma or interstitial colloid osmotic pressure of the whole body, and flux is based on relative changes in pressure related to the compliances of the vasculature and interstitium. In this approach, the hydrostatic pressure and oncotic pressure terms of the Starling balance are embedded into net vascular pressure ($P_{\text{vas}} - \pi_{\text{vas}} = V_{\text{vas}}/C_{\text{vas}}$) and interstitial pressure ($P_{\text{ist}} - \pi_{\text{ist}} = V_{\text{ist}}/C_{\text{ist}}$).

At present, we can only compare two species of anurans with trout and a variety of mammals for these parameters (Table 1), all measured using the approach of Tanaka (1979). For fishes, it is debatable whether the plasma turnover reflects capillary filtration or mixing of primary and secondary circulatory fluids (Olson et al. 2003; Skov and Steffensen 2003). What seems clear from these few comparisons that are possible is that ectotherms have a greater F_C than do mammals, C_{ist} is very high in anurans, and C_{vas} is as variable between ectotherms as it is within mammals.

Models emphasizing a transcapillary mechanism of fluid flux appear to adequately explain blood volume maintenance in mammals largely because plasma has a slow turnover; hence, lymphatic flux is a small fraction of the total fluid flux and can be ignored. How useful a generalized mechanism this is for explaining blood volume regulation in other vertebrate classes is the thrust of this perspective.

The Anuran Problem

How fast is the net fluid exchange between the vasculature and interstitium in anurans? One approach to answering this is to measure the rate of turnover of labeled protein in anurans and compare it with that of other vertebrates (Fig. 1). Anuran plasma turnover values are 3%–5% of plasma volume min^{-1} (DeGrauw 1998) as compared with 0.8%–0.9% for fish (Nichols 1987) and 0.03%–0.1% for mammals (Bianchi et al. 1970; Aukland and Reed 1993).

Why should anuran plasma have such quick turnover? There are really three reasons for this higher turnover compared with

Table 1: Vascular and interstitial compliance and filtration coefficients in vertebrates

Species	Vascular Compliance (mL kg ⁻¹ kPa ⁻¹)	Filtration Coefficient (mL kg ⁻¹ kPa ⁻¹ min ⁻¹)	Interstitial Compliance (mL kg ⁻¹ kPa ⁻¹)
Trout	26 ^a	37 ^b	80 ^b
Bullfrog	28 ^c	25 ^d	2,000 ^e
Cane toad	47 ^c	25 ^d	2,000 ^e
Mammals	25–50 ^f	2–7 ^f	40 ^f

^a Zhang et al. 1995.

^b Olson et al. 2003.

^c T. M. Hoagland, personal communication.

^d Hancock et al. 2000.

^e Hillman et al. 2002.

^f Tanaka 1979; Brace and Gold 1984; Aukland and Reed 1993.

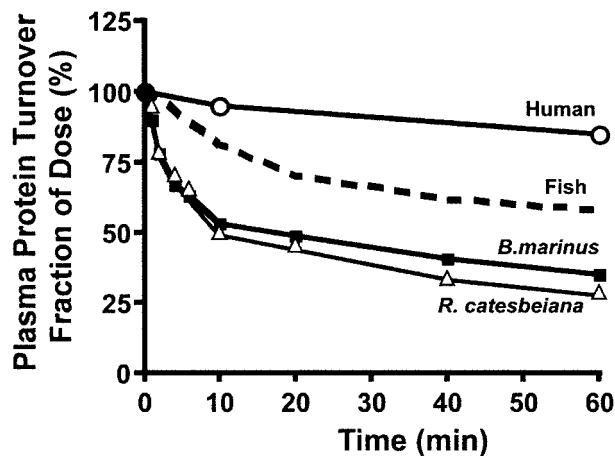


Figure 1. Plasma turnover in a variety of vertebrate species. The anuran data are taken from DeGrauw (1998), fish data are from Nichols (1987), and human data are from Bianchi et al. (1970).

fish and mammals: a high whole-body F_C , a leaky vasculature (to protein), and a very compliant interstitium. The whole-body F_C (Table 1) determined for amphibian vascular systems by two independent methods (gravimetrically from whole-body perfusion [Hancock et al. 2000] and short-term volume perturbation using the Tanaka [1979] approach [Hillman et al. 2002]) are about three- to sixfold higher than mammalian values (Tanaka 1979; Aukland and Reed 1993) but lower than those for trout (Olson et al. 2003). Couple this low-resistance pathway for fluid into an interstitial space that is greater than an order of magnitude more compliant (Hillman et al. 2002) than either the fish or mammalian interstitium (Tanaka 1979; Olson et al. 2003; see Table 1) with a low reflection coefficient, and then one has a physical system where little counterbalancing pressure to the capillary pressure will develop in the interstitium as a consequence of lymph formation. The fish data presented in Figure 1 present an interesting example of the interplay between these two variables. Trout have a greater F_C than do anurans (Olson et al. 2003), and that, without considering C_{ist} would predict a higher plasma turnover than in anurans, yet fish plasma turnover is lower. Trout have a much lower C_{ist} than do anurans (Olson et al. 2003), and this prevents fluid loss from a circulatory system because P_{ist} increases with any filtration, particularly with a higher F_C . The high C_{ist} of anurans would favor storage of extracellular fluid volume in the interstitium without increasing P_{ist} or P_{cap} , but it entails high filtrational losses and plasma turnover and requires a nontranscapillary mechanism for fluid return to the circulation. Anurans are unique relative to other vertebrate groups in having such a high C_{ist} . Storage of lymph may be another adaptation to handling the blood volume stress of dehydration as a consequence of high evaporative water loss in

a terrestrial environment, particularly in estivating species, but this has not been tested.

The addition to the whole-body interstitium of a volume equivalent to 4% of a toad's plasma volume (their minute turnover of plasma) will generate an increased interstitial pressure of only 1–2 Pa. Recognize that this pooling of lymph in the ventral-dependent reaches of the interstitium (or “basement”) is at a pressure about 200 Pa below the lymph hearts (or “sump pumps”). One has to look only at the anatomy of anurans to understand why their interstitial compliance is so much greater than that of either fish or mammals—anurans have large subcutaneous lymph sacs that underlie most of the skin (see Carter 1979). Anuran lymph sacs communicate with various numbers of paired anterior (beneath the scapula) and posterior (lateral to the urostyle) lymph hearts. The lymph hearts are situated on the dorsal aspect of the body; that is, they are “sump pumps in the attic.” They communicate via one-way valves to veins and functionally generate a pressure sufficient to move lymph back into the veins. There are excellent reviews of anuran lymphatic structure and function (e.g., Toews and Wentzell 1995), and further summary is unnecessary and beyond the scope of this perspective.

Estimates of fluid volume flux via the lymphatic system range from 0.7 to 1.6 mL kg⁻¹ min⁻¹ in control and posthemorrhage for *Bufo marinus* (Baustian 1988). Toews and colleagues subsequently reported lower fractional outputs from 0.25 to 0.64 mL kg⁻¹ min⁻¹ using cannulated lymph heart outputs (Jones et al. 1992), but more recent direct measurements of 1.7 mL kg⁻¹ min⁻¹ using implanted Doppler flow probes in *B. marinus* (Jones et al. 1997) confirm the estimates from Baustian's (1988) data. Malvin et al. (1995) report lymph heart output for cannulated hearts of 16 mL kg⁻¹ min⁻¹ for *Bufo woodhousei*, which appears inconsistent with all other data. Rapid values range from 0.7 to 0.9 mL kg⁻¹ min⁻¹ (Baldwin et al. 1993).

How important is the lymphatic pathway for the return of this lost plasma to the vascular volume relative to Starling's transcapillary balance? We can approach this question from three perspectives. (1) Do Starling forces describe known fluxes across the circulation? (2) What happens when the lymphatic pathway is blocked? (3) How do measured lymphatic fluxes compare with measured plasma fluxes from a budgetary perspective?

The transcapillary fluid balance explained by Starling forces does not adequately account for circulatory fluid flux in the following two examples. First, filtration rate in gravimetric preparations of anesthetized anurans have a much higher isogravimetric pressure than would be predicted from Starling variables (Hancock et al. 2000). Anurans can maintain blood volume over some degree of dehydration or hemorrhage by mobilizing lymph (Hillman et al. 1987; Hillman and Withers 1988). During the period of lymph recruitment to maintain blood volume during dehydration, the Starling forces actually favor filtration (Hillman et al. 1987), indicating that transcap-

illary uptake based on Starling forces cannot account for the fluid return into the vasculature that is occurring (via lymphatic return).

Second, blocking the lymphatic pathway by thermocautery of the lymph hearts leads to death within a couple of days as a consequence of a shift of fluid from the blood to the interstitial space (Zwemer and Foglia 1943). Middler et al. (1968) present conflicting data on the significance of the lymph hearts in mobilizing interstitial fluid following hemorrhage. More recently, Baustian (1988) has shown that anurans with no bladder reserves and cauterized lymph hearts are unable to replace fluids lost through hemorrhage. Clearly, lymph heart transport is critically important in maintaining blood volume. Consequently, anurans without functional lymph hearts are unable to maintain plasma volume at rest and when challenged hemorrhagically.

Third, a comparison of lymph flow to the flux of filtered plasma is a useful budgetary approach for establishing the significance of the lymphatic pathway for the return of plasma lost to the interstitium. We know from plasma turnover data in *B. marinus* that plasma is lost at $1.9 \text{ mL kg}^{-1} \text{ min}^{-1}$ (DeGrauw 1998). We also know that lymph flows are $1.7 \text{ mL kg}^{-1} \text{ min}^{-1}$ (Baustian 1988; Jones et al. 1997). Consequently, about 90% of filtered plasma appears to be returned via the lymphatic route. Nephrostomal (peritoneal funnel) flux in *B. marinus* is about $0.1 \text{ mL kg}^{-1} \text{ min}^{-1}$ (Morris 1981) and would account for the remaining flux of interstitial fluid back into the circulation. This budgetary analysis demonstrates that all of the known plasma loss can be accounted for by lymphatic and nephrostomal return pathways; hence, a near Starling transcapillary balance is not supported from a budgetary perspective. Because lymphatic return accounts for about 90% of the known return of interstitial fluid in anurans, understanding the control of lymphatic flux is crucial for understanding the control of blood volume. This is a conclusion also reached by Baldwin et al. (1993) and Malvin et al. (1995).

Lymph heart output can be rapidly modulated by both rate and stroke volume changes (see Jones et al. 1997). Lymph heart rate is modified by spinal nerve efferent activity (Priestley 1878; Okada 1956; Del Castillo and Sanchez 1961). There is a negative feedback control loop between arterial baroreceptors and lymphatic heart rate (Yamane 1990; Crossley and Hillman 1999). Natural variation in arterial pressure is inversely related to lymph heart rate, and this relation is lost following denervation of the baroreceptor nerves. Stimulation of the baroreceptor afferent nerves, which mimics an increase in arterial pressure, shuts off the lymph hearts without a change in blood pressure (Crossley and Hillman 1999). Little is known about the effect of physical factors, such as interstitial fluid pressure or venous pressure on lymphatic heart stroke volume, even though lymph stroke volume shows a wide range of variation (Jones et al. 1997). Clearly, lymph heart stroke volume (like cardiac heart stroke volume) is in part determined by lymph return; the

lymph heart cannot pump what it does not have. This limitation is apparent from the following three findings: (1) lymph heart stroke volume declines with dehydrational fluid loss despite no change in lymph heart systolic pressure (Jones et al. 1997); that is, there is a preload rather than an afterload problem; (2) postural shifts in anesthetized animals that favor pooling of lymph around the posterior hearts caused a dramatic rise in stroke volume (Jones et al. 1997); and (3) lymph heart output in general has been reported to correlate with the rate of lymph formation (Baldwin et al. 1993).

The question of what determines lymph flux then becomes, What determines lymph flow to the lymphatic hearts rather than, What controls the lymph hearts? The current hypothesis is "lymph is presumably redistributed within and passed through the lymph sacs by postural movements and by the continuous pumping of (and the probable slight negative pressure created by) the lymph hearts," and the "directional movement must be implemented by the modest aspiration of the lymph hearts" (Toews and Wentzell 1995, pp. 204–205). This hypothesis is predicated on a stretching of the elastic components of suspensory ligaments of the lymph hearts during systole and this stored energy being released during diastole to create a negative pressure within the lymph heart. This negative pressure presumably aspirates lymph to the heart. However, three lines of evidence contained in Toews's own experimental work contradict this physical mechanism for lymph suction as the primary mechanism for lymph return via the lymphatic sacs.

The first is that Toews and colleagues present little or no evidence for negative pressure inside lymph hearts in two articles that specifically report lymph heart pressures (Jones et al. 1992, 1997). The second is that the lymph hearts are located on the dorsal surface and would require a suction force to pull lymph up to them (depending on animal size; 100–200 Pa in the case of *B. marinus*), but this far exceeds the few small negative pressures reported. Third, they report increased lymph heart output with slight disturbances of animals but no change in lymph heart pressures (Jones et al. 1997). This is consistent with increased filling of the lymph hearts raising their output but is inconsistent with increased aspiration providing the mechanism for lymphatic return. Consequently, we feel that other forces are involved in moving lymph toward and filling the lymph hearts. Postural movements are alluded to by Toews and Wentzell (1995) as a potential pressure mechanism for lymph return, yet lymph is returned in quiescent animals, so physical activity per se is not a prerequisite for lymph flow.

From these considerations, it became clear to us that an understanding of lymph flux to the lymph hearts is probably more important than studying the lymph hearts in isolation because their output has to match input to fully understand how these amazingly high lymph fluxes are maintained in anurans and how they might vary interspecifically.

Preliminary Analyses

We present preliminary analyses of what is known and what we have discovered about the function of lymphatic flow to the posterior lymph hearts in *Bufo marinus*. The current level of understanding as expressed in the recent literature is summarized in Figure 2. Recognize that this pivots around the lymph hearts; lymph heart input is determined by the diastolic pressure creating a suction force for lymphatic return (Toews and Wentzell 1995), whereas lymph heart output is determined by lymph heart systolic pressure exceeding venous pressure (Jones et al. 1997) to return lymph to the circulation.

Anatomy

The anuran lymphatic system consists of interconnected subcutaneous sacs separated by connective tissue walls that have one-way valves. The valves appear to have muscular control and are not simple passive flaps (Jolly 1946). The general arrangement of the lymph sacs has been described by Ecker (1889), and interspecific variation in the arrangement of various lymph sacs has been described by Carter (1979). The only hypothesis presented to date for the significance of lymph sac compartmentalization is to alleviate difficulties of gravitational pooling that would be associated with postural changes for a single sac (Toews and Wentzell 1995). The driving force for

fluid movement between the sacs has never been investigated, although the lymph sacs are at a negative pressure relative to atmospheric pressure (Scholander et al. 1968; Hillman et al. 1987).

Consider, for example, the lymphatic compartments of the hind limb. The pattern of lymph flow from the distal reaches of the hind limb would follow a subcutaneous route of plantar (around the foot), to crural (around the calf), to femoral (around the thigh), to interfemoral (a ventral subcompartment of the femoral that covers the pelvic patch), to the pubic sac that connects to the posterior lymph heart (Fig. 3). When we examined the anatomy more carefully and tried to delineate lymph flow in the hind limb, we found that there is also an intermuscular pathway for lymph flow to the iliac sac in addition to the subcutaneous sac route. In fact, the predominant pathway for dye-labeled saline injected into the crural sac (the subcutaneous lymphatic sac of the calf) appears to be this intermuscular pathway of the thigh rather than the femoral lymph sac (the expected subcutaneous pathway on the basis of the current literature). This intermuscular pathway is connected to the subcutaneous sac system at the plantar sac and crural sac level but is independent of the femoral and interfemoral sacs. This intermuscular sac is probably the ilio-fibular sinus described by Gaup (1899), and although acknowledged in other early literature (Muller 1833; Priestley 1878; Jolly 1946), it has

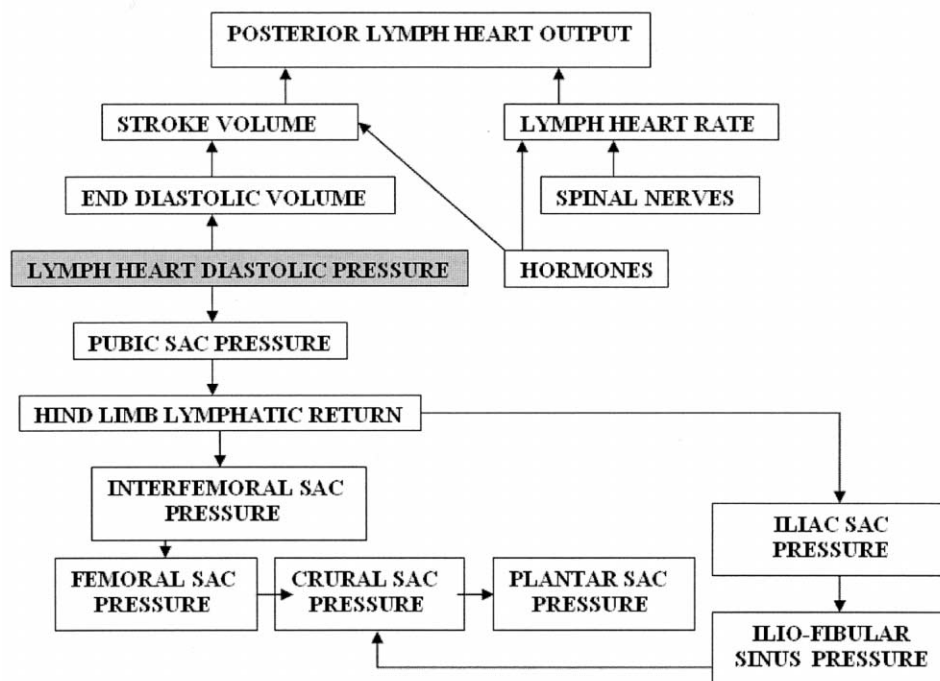


Figure 2. Current hypothesis for lymph movement (see Toews and Wentzell 1995; Jones et al. 1997). Arrows indicate the direction of influence, starting with lymph heart end-diastolic pressure (gray box). Recognize that both lymph heart output and input are dependent on end-diastolic pressure.

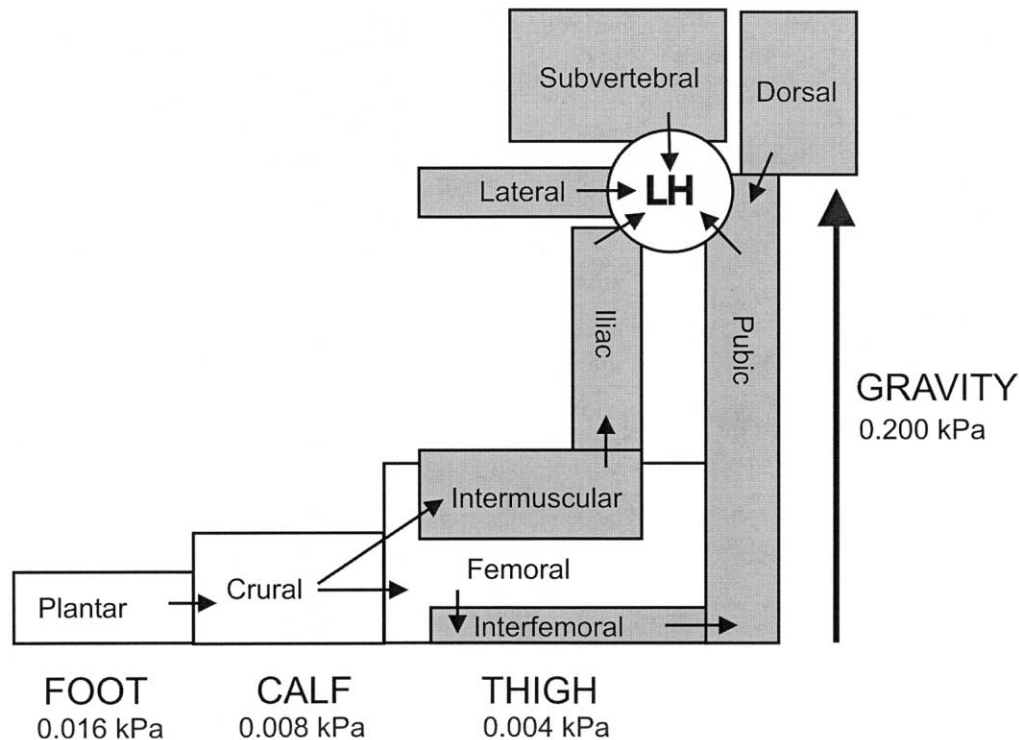


Figure 3. Schematic diagram of anatomical arrangement of hind limb lymph sacs for *Bufo marinus*. The relevant hydrostatic pressures are shown where possible.

neither been carefully described nor even been considered in more recent work.

Physiology

Two physiological characteristics determine the movement of lymph: the first is the compliance ($\Delta\text{volume}/\Delta\text{pressure}$) of the various sacs, and the second is capillary pressure. The addition of fluid by transcapillary filtration into lymph sacs increases the pressure, and the magnitude of the increase in pressure depends on the compliance of the lymph sacs. Partitioning the subcutaneous lymph space into discrete lymph sacs, and with the lymph sac compliance increasing in the direction of flow, is one potential mechanism for creating a pressure difference and driving lymph flow between sacs in series. The most distal sacs would have to be the least compliant. In such a system, lymphatic fluid influx to each sac would create a higher pressure in more distal sacs as a consequence of their lower compliance, and lymph would flow toward the lymphatic hearts from the distal reaches of the hind limb. Compliance is the product of lymph sac volume and distensibility of the lymph sac. Because each sac is surrounded by the highly collagenous dermis, which is not very distensible, the major variable in determining lymph sac compliance is probably the initial volume of the sacs. The extensive sinus structure of anuran lymphatic sacs would infer

a very compliant system as a consequence of both the obvious significant volume and relative ease with which the skin can separate from the underlying musculature. Carter (1979) has inferred from anatomical studies of frogs that large lymph sacs are correlated with an aquatic habit and that reduced lymph sacs are associated with a terrestriofossorial habitat. Because volume is a component of compliance, Carter's (1979) result suggests a greater compliance for lymph sacs of aquatic species compared with terrestriofossorial species; hence, a lower pressure is developed for the same lymphatic influx. The functional significance of this is not obvious and merits further study.

We have measured the compliance of lymph sacs for the hind limbs of *Bufo marinus* (Table 2). We calculated the pressure change associated with lymph flux in the ventral region of each subcutaneous sac leading to the posterior lymph heart from the hind limb by making the following assumptions: (1) the lymph sacs are arranged horizontally; (2) the total lymph flow pumped by each of the paired posterior lymph hearts originates from its ipsilateral hind limb, and the rate of lymph formation by filtration in the hind limb is equal to the lymph heart output; and (3) the rate of lymph formation in each segment of the limb is equal to the proportional segment mass relative to the total limb mass. We probably overestimate hind limb lymph flow using assumption (2) because there are potentially other

Table 2: Compliance (\pm SE, with sample size), lymph flow, and passive pressure change in hind limb lymph sacs for *Bufo marinus* ($n = 6$)

Lymph Sac	Compliance (mL kg ⁻¹ kPa ⁻¹)	Lymph Filtration (mL kg ⁻¹ min ⁻¹)	Filtered Pressure Change (kPa min ⁻¹)	Accumulated Lymph Flow (mL kg ⁻¹ min ⁻¹)	Lymph Sac Pressure Change (kPa min ⁻¹)
Plantar	4 \pm 1 ($n = 13$)	.112	.027	.112	.027
Crural	13 \pm 2 ($n = 11$)	.096	.008	.208	.017
Femoral ^a	76 \pm 12 ($n = 11$)	.19	.003	.398	.005

^a Note that there is an intermuscular lymphatic pathway in parallel with the femoral sac, and so not necessarily all of the crural lymphatic fluid passes through the femoral sac (and so the femoral pressure would be lower than calculated).

(lateral, ventral, dorsal, and subvertebral) lymph sac inputs to the lymph heart, and hence we probably overestimate the magnitude of hind limb lymph sac pressures, but this does not change our conclusions.

The pressure necessary to move the lymph to the dorsally located hearts is on the order of 0.1–0.2 kPa because the lymph hearts are 1–2 cm above the ventral surface of the animal. The magnitude of the pressure changes in hind limb lymph sacs by local plasma filtration is approximately 0.002–0.016 kPa min⁻¹ (Table 2). Even if all the plasma flow to the hind limbs was filtered into the femoral sacs, the actual pressure change would be only about 0.08 kPa; plasma flow to the hind limbs was estimated from the hind limbs representing 23% of body mass (S. S. Hillman, unpublished data) and a systemic blood flow of 50 mL kg⁻¹ min⁻¹ (Hedrick et al. 1999) distributed in proportion to a percentage of body mass. We conclude that the rate of formation of fluid in the hind limbs cannot possibly create a sufficient pressure for lymphatic return to the dorsally located lymph hearts.

Three important conclusions can be reached from these calculations of the pressure change created by lymph flux in each subcutaneous sac of the hind limb (Table 2). First, the compartmentalization of the sacs creates a sequential pressure head with the formation of lymph. Second, the pressure is higher in the more distal sacs, creating a series of pressure differences that would move lymph horizontally toward the lymph hearts. Finally, neither the pressure head generated by formation of lymph nor the reported diastolic pressures within lymph hearts are sufficient to move the lymph from ventral regions of the hind limbs (“basement”) to the dorsally located lymph heart (“attic”). We are left to consider another mechanism for vertical movement of lymph.

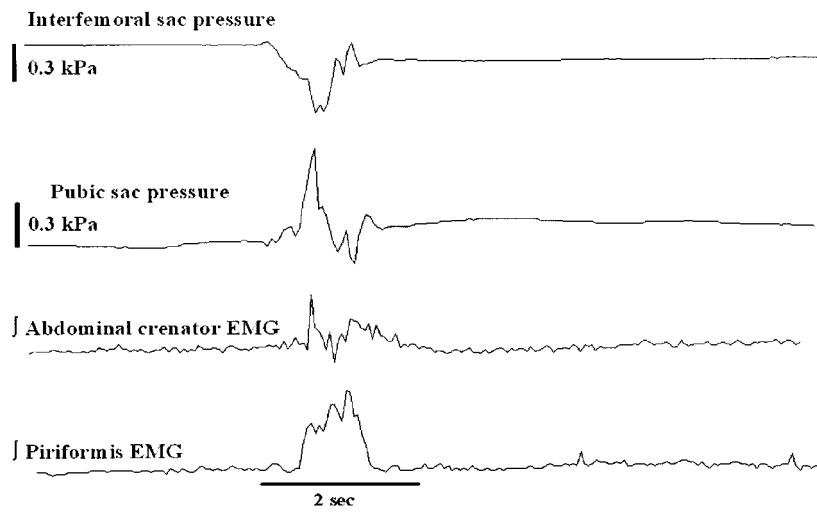
Lymph sac pressure could be varied actively by muscular contractions, independent of postural or activity changes that directly or indirectly alter lymph sac volume and pressure. For instance, Winokur and Hillyard (1992) have described muscles that originate on the skeletal system (gracilis minor and abdominal crenator) and insert on the dermis of the interfemoral and femoral sacs. Contraction of these muscles could change the pressure of the femoral and interfemoral sacs. The piriformis muscle, which is considered to be involved primarily in

locomotion (Emerson and De Jongh 1980), is in a position to influence pubic and iliac sac volume and, hence, lymphatic return to the lymph hearts. The piriformis muscle originates on the femur and inserts on the urostyle and surrounds part of the pubic sac, and presumably its contraction would depress the urostyle, thus decreasing volume and increasing pressure within the pubic sac. Because the subvertebral, ventral, lateral, and dorsal sacs surround the lungs, these sacs may be sensitive to variations in body volume that result from lung inflation and deflation. Similarly, the volume of the intermuscular sac could be varied by postural changes associated with the contraction of the thigh and calf musculature.

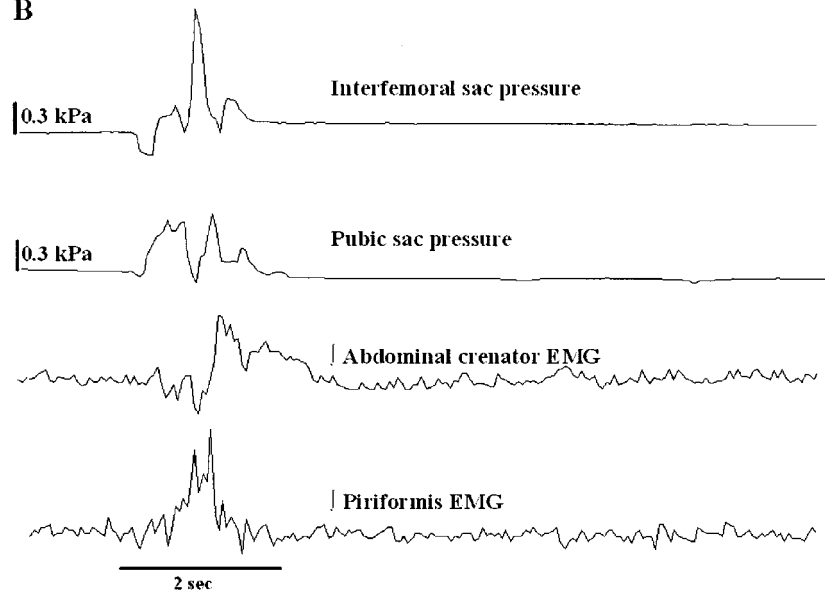
To test the hypothesis that skeletal muscles directly and indirectly affect subcutaneous sac pressures and thus mobilization of lymph, we have implanted EMG leads in a number of skeletal muscles while measuring pressure in lymph sacs of *B. marinus*. We examined whether these muscles were coordinated during activity in resting animals and whether the muscular activity is associated with pressure changes sufficient to move lymph from ventrally located lymph sacs (e.g., femoral sac and interfemoral sacs) up to the dorsally located posterior lymph heart. To test whether lung inflation/deflation cycles may be involved in changing subcutaneous lymph sac pressures, we have taken two approaches. First, we have measured changes in lymph sac pressures with changes in intrapulmonary pressures in anesthetized *B. marinus*. Second, we have also measured EMG activity of the abdominal oblique muscle simultaneously with lung pressures during graded hemorrhage in resting toads to determine whether hypotension results in ventilatory activity that could reduce lymph sac compliance, thus mobilizing lymph during acute hypovolemic stress.

There are several important conclusions from our preliminary results. First, there is considerable EMG activity in several skeletal muscles at rest; this muscular activity is independent of any apparent postural or activity changes in the animal. For example, EMG activities in the gracilis minor/abdominal crenator and piriformis muscles are clearly associated with pressure changes in the interfemoral sac and pubic sacs at rest (Fig. 4A, 4B). Piriformis activity is also associated with large negative pressures in the iliac sac (Fig. 4C). Second, the aspirational pressures generated by this muscular activity are about 200 Pa

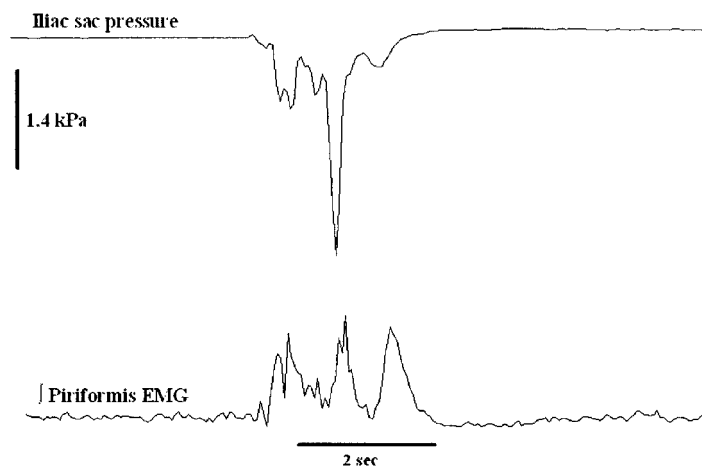
A



B



C



or lower, which are of sufficient magnitude to move lymph from the ventral surface (where the interfemoral sac is located) to the dorsally located pubic sac and lymph hearts. The iliac sac, a dorsally located sac that leads directly to posterior lymph hearts, has negative pressures that would allow suction of lymph from ventrally located sacs. Third, passive lung inflation in anesthetized toads increased pressure in surrounding lymph sacs, such as the lateral lymph sac (Fig. 5A). In awake animals, abdominal oblique EMG activity and lung ventilation cycles are substantially elevated by hypovolemic stress generated by hemorrhage of 5% of body mass (Fig. 5B). Furthermore, mean lung pressure approximately doubles (190 Pa at rest vs. 360 Pa with hemorrhage), and this pressure would be transmitted to lymph sacs surrounding the lungs (e.g., lateral sac; Fig. 5A), thus mobilizing lymph.

We present our working hypothesis for how the lymph moves to the posterior lymph hearts in *B. marinus* and the physiological variables determining the rate of lymph movement in Figure 6. We would anticipate that lymph would be moved to the (dorsally located) anterior lymph hearts in a similar fashion. Recognize the sharp contrast from the model summarized from Toews and Wentzell (1995) where the lymph heart itself determines both its output and input. In our model, skeletal muscle pumps, both aspirational and positive pressure, move lymph to the posterior lymph hearts. However, movement of lymph into the lymph heart from the dorsal regions of the lymph sacs would nevertheless be due to aspiration during diastole, as described by Toews and Wentzell (1995).

Our model involves a strong functional role of hind limb muscles and the urostyle/piriformis muscles modifying the pressure in the pubic sac. Interestingly, some muscles appear to function only to move lymph (e.g., abdominal crenator and gracilis minor), while others may have multiple functions (e.g., piriformis and respiratory muscles). The skeptic might argue that multiple function muscles are only incidentally and indirectly involved with lymph flow and that their control is independent of lymph flow. For example, it seems heretical to propose that lung ventilation is intimately associated with a lymphatic function in amphibians rather than the singular function of regulating blood P_{O_2} and P_{CO_2} . However, blood pH, P_{CO_2} , and P_{O_2} levels only partially account for ventilatory drive in amphibians (Wang et al. 1999a, 1999b), and ventilation is inhibited if the primary baroreceptor nerve is stimulated (Van Vliet and West 1986; Martinez and Hedrick 2000). This is an appropriate negative feedback control response if ventilation is an effector for the control of blood volume. Furthermore, the large increases in lung ventilation and lung pressure with hem-

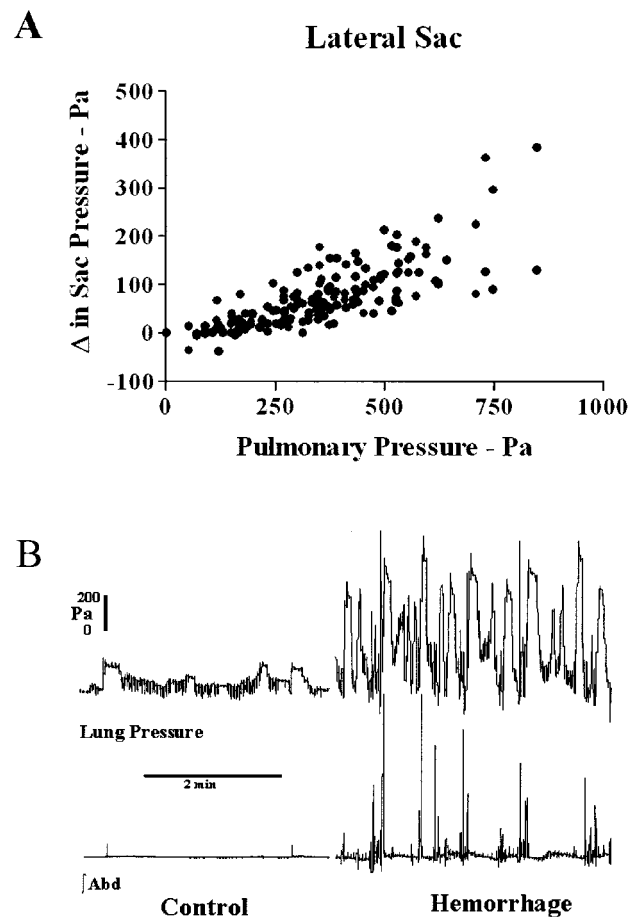


Figure 5. A, Lateral sac pressure as a function of pulmonary pressure in anesthetized *Bufo marinus*. B, Integrated abdominal oblique EMG and pulmonary pressure in control and hemorrhaged resting *B. marinus*.

orrhage in toads (Fig. 5B) are unlikely to be the result of changes in blood oxygen content because experimental anemia that reduces hematocrit to 50% of normal produces no change in ventilation in resting animals (Andersen et al. 2003). Our interpretation of these results is that increased lung ventilation cycles and lung pressure during severe hypotension reduce compliance of the lymph sacs surrounding the lungs, thus facilitating the movement of lymph from the sacs to anterior and posterior lymph hearts.

Figure 4. Lymph sac pressures and muscle activity in *Bufo marinus*. A, Interfemoral and pubic sac pressures (kPa) are out of phase with respect to each other but coincide with integrated abdominal crenator and piriformis EMGs. B, Same as in A, but note that pressures are largely in-phase and coincide with integrated muscle EMGs. C, Iliac sac pressure exhibits a large negative pressure (>2 kPa) with integrated piriformis EMG activity. Pressures are relative, not absolute.

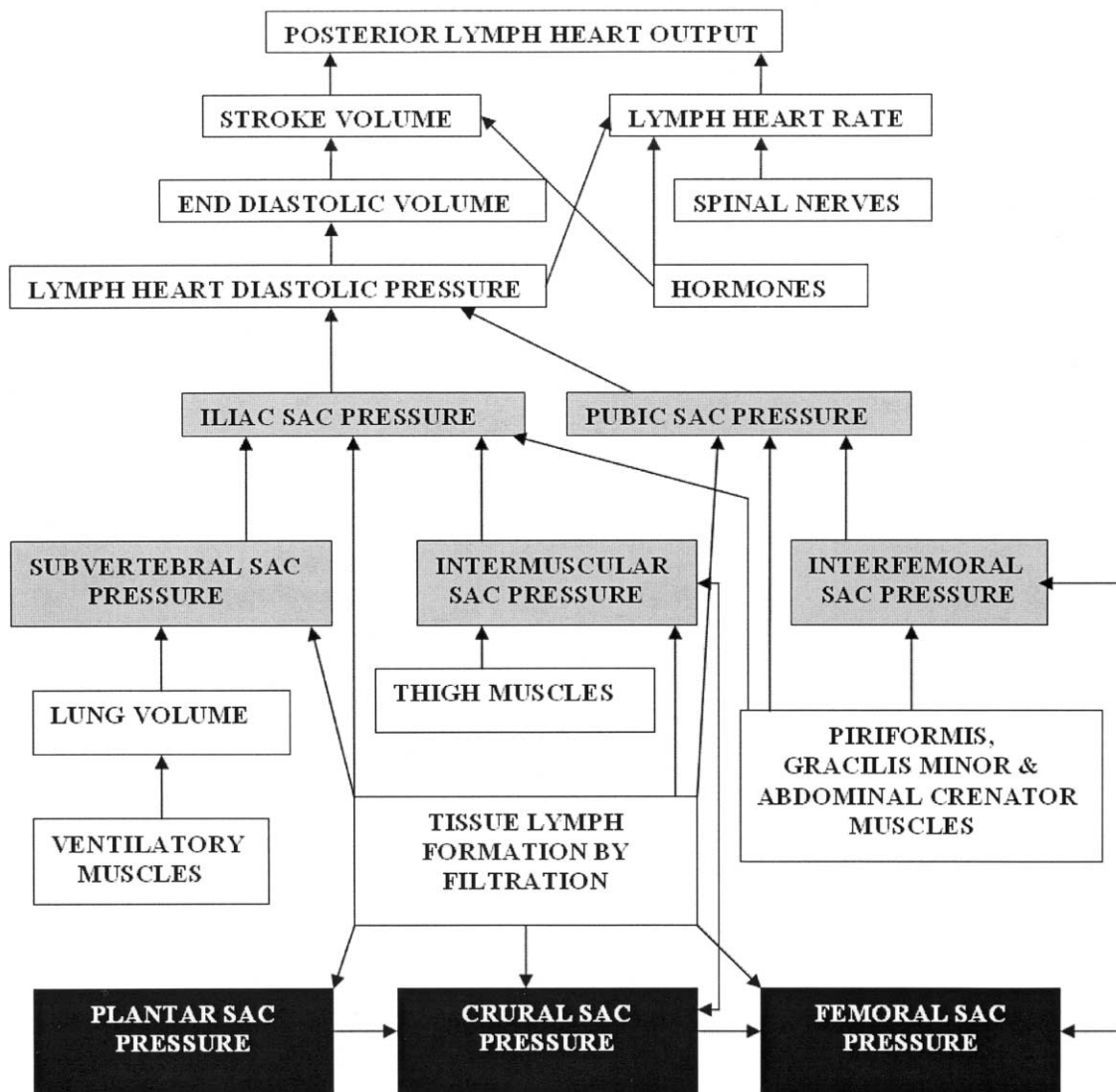


Figure 6. Proposed model of posterior lymph heart function for *Bufo marinus*. Shading indicates lymph sacs, dark boxes indicate filtration pressure forces for lymphatic return, and light gray boxes indicate direct muscular pressure forces.

Role of Lymphatic Return in Other Vertebrates

Our proposed mechanisms for lymphatic return in anuran amphibians are likely to be less applicable in the other amphibian orders, Urodela and Apoda. Both salamanders and caecilians have a pair of dorsal lymph hearts per body segment and lack subcutaneous lymph sacs (Kampmeier 1969). Their dermis is tightly connected by fascia to the underlying musculature; hence, they most likely have a low whole-body interstitial compliance. Consequently, even if their vasculature was as leaky as that of anurans, their lymph space pressures would more easily return fluid to the lymph hearts.

Do our observations on lymph return in anuran amphibians have applicability to other vertebrate classes? Probably not for

mammals because their blood volume maintenance appears to be primarily determined by transcapillary exchange, as predicted by Starling mechanisms. What about reptiles and birds? Reptiles, like amphibians, have a remarkable capacity to maintain blood volume when hemorrhagically stressed (Lillywhite and Smith 1981; Lillywhite 1985, 1993). Many of these articles invoke a transcapillary Starling balance for this regulation (Lillywhite and Pough 1983; Smits and Lillywhite 1985), although in none of these invocations is it ever critically tested by evaluating the actual Starling forces. There is also evidence for species differences in tail interstitial compliance in response to tilt-induced hypotension (Lillywhite 1993). The contraction of skeletal muscles to move lymph also seems to occur in reptiles.

Hemorrhaged snakes inflate their lungs and frequently show rhythmic contractions along their body (Lillywhite et al. 1983; Lillywhite 1985). These responses were interpreted as enhancing venous return to the heart and hence raising arterial pressure, although they would also presumably assist lymphatic return. Even in mammals, there is a reflexive increase in ventilation with hypotension or carotid occlusion (Von Euler and Liljestrand 1943; D'Silva et al. 1966), and ventilatory responses to chemoreception are augmented during hypotension (Heistad et al. 1975), although there appears to be little or no involvement of skeletal muscles to lymph flow in mammals (Roddie 1990; Drake et al. 1996).

Birds also have a remarkable capacity to maintain blood volume with hemorrhage and dehydration (Djojusugito et al. 1968; Kovach et al. 1969; Ploucha and Fink 1986; Carmi et al. 1993, 1994). They have high arterial pressures and presumably high capillary pressures coupled with low plasma colloid forces. Consequently, it is difficult to imagine that a Starling-based balance of forces accounts for their blood volume regulatory capabilities during dehydration and hemorrhage, although it is the mechanism invoked (Djojusugito et al. 1968; Carmi et al. 1994) with no consideration of lymphatic involvement.

Why has the significance of lymphatic pathways never been considered or even acknowledged for reptiles and birds? Are we as comparative physiologists so beholden to the mammalian paradigm that we ignore alternative hypotheses and in doing so fail to critically evaluate the mechanisms we invoke? We suggest that some important circulatory system variables have remained in the recesses of comparative cardiovascular biology: lymphatic flux, vascular compliance, interstitial compliance, and whole-body filtration coefficients. Their determination is central to our ability to understand comparative blood volume regulation and the evolution of the vertebrate cardiovascular system.

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