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DIRECT MEASUREMENT OF FLOW FROM THE POSTERIOR LYMPH HEARTS OF HYDRATED AND DEHYDRATED TOADS (*BUFO MARINUS*)

J. M. JONES[1,*], A. K. GAMPERL[2,†], A. P. FARRELL[2] AND D. P. TOEWS[1]

[1]*Department of Biology, Acadia University, Wolfville, Nova Scotia, Canada B0P 1P0* and [2]*Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6*

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Summary

Flow from the posterior lymph hearts of *Bufo marinus* was measured using Doppler flow probes. These probes were placed on the posterior vertebral vein and recorded flow as lymph was ejected from the heart. In resting, hydrated toads, mean lymph flow from one of the paired posterior lymph hearts was 25.9±4.9 ml kg^{-1} h^{-1}, stroke volume was 8.9±1.4 μl kg^{-1} and lymph heart rate was 47.5±3.7 beats min^{-1}. We estimate that, together, the paired posterior lymph hearts are capable of generating flows that are approximately one-sixtieth of the resting cardiac output. Mean peak systolic pressure developed by the posterior lymph hearts was 1.62±0.08 kPa. Simultaneous measurements of lymph heart pressure development and flow revealed that the outflow pore of the heart opened at a pressure of 0.71±0.04 kPa, approximately 113±5 ms into systole.

When toads were moderately disturbed, stroke volume increased by as much as fourfold with little change in lymph heart rate (<5 beats min^{-1}). When toads were dehydrated, lymph flow decreased by 70 % at 12 h and by 80 % at 24 h. Since there was only a modest non-significant decrease in lymph heart rate (30 %), this reduction in flow was attributed to decreases in stroke volume (approximately 80 %). Lymph heart flow and stroke volume returned to control values 30 min after adding water back into the experimental chamber. Stroke volume was clearly more important in regulating lymph flow than lymph heart rate under these conditions in *Bufo marinus*.

Key words: Amphibia, toad, lymph hearts, lymph flow, dehydration, *Bufo marinus*.

Introduction

The lymphatic system is an important contributor to water balance in amphibians and has been shown to mediate fluid and solute return to the circulatory system during osmotic stress (Baustian, 1988; Hillman *et al.* 1987; Jones *et al.* 1992). Comprised of capacious, interconnecting lymph spaces found just beneath the skin and around the internal organs, and pairs of contractile lymph hearts, the lymphatic system can circulate large amounts of fluid apparently at rapid rates (Conklin, 1930; Müller, 1833). During diastole, the posterior lymph hearts, located on either side of the urostyle, receive lymph from the surrounding spaces through numerous afferent pores. With each beat, these hearts eject lymph through a single efferent pore into the posterior vertebral vein (Radwanska, 1906, cited in Kampmeier, 1969).

The importance of the anuran lymphatic system has been demonstrated numerous times by the inevitable death of animals 2–4 days after the destruction of their lymph hearts. These animals die from irreversible loss of plasma into the tissues because there is no appreciable return of lymph to the circulatory system except *via* functional lymph hearts (Baldwin *et al.* 1990; Baustian, 1988). In addition, if in a hydrating environment, these animals show a marked increase in weight and oedema as incoming ambient water contributes to the total amount of fluid pooled in the subcutaneous lymph sacs (Foglia, 1941).

The rapid onset of changes in blood composition following immobilization of the lymph hearts implies that lymph formation may be a rapid process. There is little information, however, on the actual rates of lymph flow. Earlier measurements of flow rates tended to be indirect and may be inaccurate. Using a visual estimation of stroke volume and by counting lymph heart rates, von Brücke in Winterstein's *Handbuch* (cited in Conklin, 1930) determined that, in an unnamed species of frog, 100 times the blood volume of 3 ml was produced each day, a volume of 340 ml kg^{-1} h^{-1}. Isayama (1924), using haematocrit and blood counts before and after destroying the lymph hearts, estimated that 50 times the total amount of plasma is passed from the blood each day; a total output of 154 ml kg^{-1} h^{-1}. Baldwin *et al.* (1993) estimated lymph flow using stroke volume and heart rate in resting *Rana*

*e-mail: dan.toews@acadiau.ca.
†Present address: Anesthesiology (129), VA Medical Center, 4150 Clement Street, San Francisco, CA 94121, USA.

pipiens and found total flow from the four lymph hearts to be 258 ml kg^{-1} h^{-1}. Only one direct measurement of lymph flow has been made. By chronically cannulating the posterior lymph hearts of *Bufo marinus* and measuring the drainage at negative pressure, Jones *et al.* (1992) determined that lymph flow from these paired hearts was 11 ml kg^{-1} h^{-1}. This value is considerably lower than that predicted from the earlier studies.

In the present study, flow from the posterior lymph hearts was directly measured by placing Doppler flow probes on the posterior vertebral vein in freely moving *Bufo marinus* under both hydrated and dehydrated conditions. In addition, by simultaneously monitoring lymph heart pressure, we investigated the relationship between lymph heart stroke volume, pressure and rate. In doing so, we hoped to establish an understanding of the mechanisms by which lymph heart output is regulated.

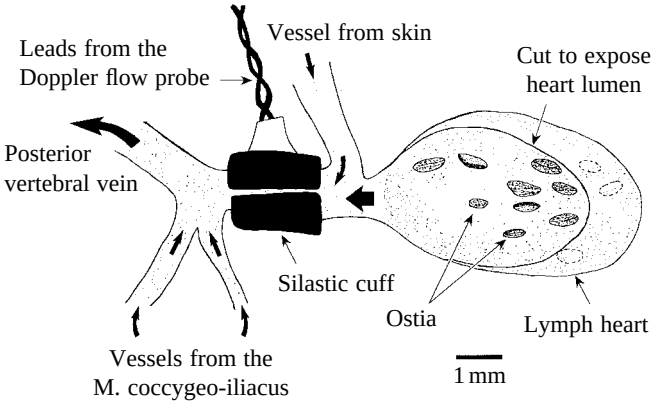

Fig. 1. Dorsal view of one posterior lymph heart and associated blood vessels showing placement of the Doppler flow probe.

Materials and methods

Cane toads (*Bufo marinus* L.; 325–780 g hydrated mass) from Charles D Sullivan Co. (Nashville, TN, USA) were maintained in large polypropylene containers (69 cm×123 cm×65 cm) filled with sand to a depth of 10 cm. The toads had free access to water and were force-fed beef liver (sprinkled with Biotin Stress Pak, Solvay Animal Health, Inc.) weekly. Temperature was maintained at 21 °C and photoperiod was 12 h:12 h light:dark. Toads were kept for at least 6 weeks prior to experimentation. Measurements of normal flows were conducted during February and March 1995; flows during dehydration/rehydration were measured between August 1995 and March 1996.

Surgical procedures

Toads were anaesthetized in dechlorinated tap water containing 2 g l^{-1} Tricaine methane (MS-222, Sigma Chemical Co.) and 2 g l^{-1} $NaHCO_3$. When the corneal reflex was no longer present, the animals were removed from the anaesthetic and placed on a surgical table.

One of the posterior lymph hearts (usually the right) was located by observing the pulses beneath the skin (approximately 0.5 cm lateral to the urostyle). A 2–3 cm incision was made over this location, and the skin and fascia were separated from the underlying tissue. The posterior vertebral vein, extending from the anterior region of the lymph heart, was dissected free and fitted with a Doppler flow probe (silastic cuff, i.d. 0.8 mm; Iowa Doppler Products, Iowa City, IA, USA). Although flow probes were positioned as close to the lymph heart as possible (Fig. 1), placement varied between individuals. Anastomosing veins from the skin or adjacent muscles often precluded probe placement directly adjacent to the lymph heart outflow pore (Fig. 1). Direct observations, however, confirmed that lymph expelled from the heart did not flow in a retrograde direction into these anastomosing veins. The flow probe was sutured securely to the adjacent musculature with 4-0 gauge silk. The leads (80 cm in length) from the flow probe were tied to the skin on the back of the animal at several locations, and the incision was closed with 3-0 gauge silk. During surgery, the subcutaneous space surrounding the lymph heart was periodically flushed with heparinized (100 i.u. ml^{-1}) MacKenzie's saline (in g l^{-1}: NaCl 6.72, $NaHCO_3$ 1.68, KCl 0.24, NaH_2PO_4 0.433, $MgSO_4$ 0.174; de la Lande *et al.* 1962) to prevent dehydration of the exposed lymphatic tissues and clotting of the lymph. Following surgery, which lasted between 1.5 and 2.5 h, the toads were placed under running water to recover and then transferred to covered plastic boxes (3.5 cm×20 cm×11 cm) containing 2–3 cm of dechlorinated tap water.

After the initial measurements of resting lymph flow had been completed (N=5; see below), the toads were re-anaesthetized and the lymph hearts were cannulated with flared PE 50 tubing (80 cm length) filled with heparinized saline as described by Jones *et al.* (1992). This permitted successful simultaneous measurement of intra-lymph-heart pressure and lymph heart flow in four of these individuals. For the dehydration experiments, lymph heart cannulation (with an 80 cm length of flared PE 60 tubing filled with heparinized saline) and flow probe placement were performed during the same surgical procedure.

Toads were allowed to recover from surgical procedures for at least 18 h, and a further 6 h if the probe placement needed readjustment.

Experimental protocols

Routine lymph heart flow and pressure development

Measurements of resting lymph heart flow and recordings of simultaneous flow and pressure development were obtained over 1 h periods. In addition, lymph heart flow was recorded for 10 min preceding, and 20 min following, a modest disturbance (removing the cover and shaking the experimental chamber) in several toads.

Lymph heart flow was recorded by connecting a pulsed Doppler flowmeter (model 545c-4; Bioengineering, University of Iowa) to a four-channel Gould amplifier/recorder (model RS 3400). Following experiments, each Doppler flow probe was calibrated while still in place around the outflow vessel. The lymph heart and vessel were perfused with dilute blood, *via*

the lymph heart cannula, using a peristaltic pump (HaakeBuchler Instruments, Saddle Brooke, NJ, USA). In this way, absolute flow rates (in ml h^{-1}) were determined for each probe and animal. During calibration, the animals were heavily anaesthetized to prevent contraction of the lymph hearts. MacKenzie's saline containing a small amount of blood (haematocrit 2–5 %) was used for perfusion to simulate the lymph/blood mixture normally pumped through the Doppler flow probe.

Lymph heart rate was determined by counting the number of pulses on the flow record during a 1 min period. Lymph heart stroke volume was determined by measuring the area of the flow recording contained within individual lymph heart beats and comparing this value with areas corresponding to known flows. Intra-lymph-heart pressure was measured by connecting the lymph heart cannulae to a Narco pressure transducer (model 320-1000c) and a Gould amplifier/recorder. Pressure was calibrated regularly against a static water column. The zero pressure level was initially set for each quiescent animal at the level of the lymph heart. Generally, the diastolic pressure of the lymph heart was close to the zero level as determined from the water column. As the animal changed position, however, this value sometimes changed slightly. Because of the difficulty in establishing a zero position, we measured total pressure change, reported as peak systolic pressure.

Recordings were usually collected at a chart speed of 25 or 50 mm min^{-1}. However, recordings used for the measurement of stroke volume and for the determination of the phasic relationship between lymph flow and lymph heart pressure were collected at 25 or 50 mm s^{-1}.

For each animal, mean values for lymph heart rate and peak systolic pressure development were calculated from three 1 min recordings chosen randomly from the 1 h of recording. Mean stroke volume was calculated from two randomly chosen 30 s records. Mean lymph heart flow was calculated from the product of mean heart rate and stroke volume. The mean pressure at which the lymph heart outflow pore opened and the delay between the onset of lymph heart pressure development and lymph ejection were measured for six pairs of pressure/flow traces per individual. The individual means were combined to calculate group means and standard errors.

Lymph heart flow and pressure development during dehydration and rehydration

Five toads were used for measurements of lymph heart flow and pressures during dehydration and rehydration. After a control period of at least 30 min, during which time the experimental chambers were supplied with a continuous flow of air (660 ml min^{-1}), water was removed from the chambers and the air flow routed through an 8 cm diameter glass cylinder filled with Drierite to remove moisture. After 24 h, which represents a modest level of dehydration (Jones *et al.* 1992), 2–3 cm of dechlorinated tap water was returned to the chambers.

The recording instruments and their calibrations were identical to those described above with the following exceptions: a Statham 23Db pressure transducer, a two-channel Gould amplifier/recorder (model 8188.2202) and a Sage multi-rate infusion pump (model M361) using whole blood for probe calibrations. To determine whether whole blood affected the flow signal, approximately 1 ml of blood was slowly injected into the space surrounding the lymph heart of an anaesthesized animal with functioning lymph hearts. As expected, there was no change in the flow signal when the lymph heart pumped blood as opposed to lymph.

For these experiments, recordings were collected at a chart speed of 60 mm min^{-1}. Mean values for lymph heart rate, pressure, flow and stroke volume in each animal were calculated as described above using three randomly selected 30 s recordings. These recordings were chosen from 15 min sections at 0, 1, 4, 8, 12, 16, 20 and 24 h after the onset of dehydration. Similar measurements were made at 1 and 2 h following rehydration. The opening pressures for the outflow pore were measured in the manner described above at 0 h and after 20 h of dehydration.

Statistical analyses

Statistical comparisons of the data were made using one-way repeated-measures analysis of variance (one-way RM ANOVA). Bonferroni's multiple-comparison method was used to identify the values significantly different from the control mean ($P<0.05$). A Student's *t*-test was used to compare the opening pressures for the outflow pore before and after dehydration. Statistical analyses were performed using SigmaStat (Jandel Scientific, Corte Madera, CA, USA). Values in the figures and throughout the text are mean ± S.E.M.

Results

Routine lymph heart flow and pressure

Stroke volume from one of the paired posterior lymph hearts, measured before the insertion of a lymph heart cannula, was 8.9±1.4 µl kg^{-1} (N=5) and lymph flow was 25.9±4.9 ml kg^{-1} h^{-1} (N=5). The presence of a cannula in the lymph hearts did not appear to affect the output volume since, following cannulation, stroke volume was 8.7±1.4 µl kg^{-1} (N=4) and lymph flow was 24±8 7.1 ml kg^{-1} h^{-1} (N=4). Lymph heart rate before heart cannulation was 47.5±3.7 beats min^{-1} and after lymph heart cannulation it was 44.5±6.6 beats min^{-1}. The mean peak systolic pressure generated by the lymph hearts was 1.62±0.08 kPa.

Simultaneous recordings of lymph heart pressure and stroke volume (e.g. Fig. 2) revealed that a substantial beat-to-beat variation in both parameters was exhibited by all animals. For example, stroke volume varied from 2 to 20 µl kg^{-1}, with most of the values falling between 6 and 12 µl kg^{-1} (Fig. 3). Although there was a strong positive correlation between stroke volume and peak systolic pressure (r^2=0.51 for all animals, $0.76 \leqslant r^2 \leqslant 0.97$ for individuals; Fig. 4A), there was no correlation between stroke volume and diastolic filling time (r^2=0.09; Fig. 4B).

Using expanded tracings and measuring the lymph heart

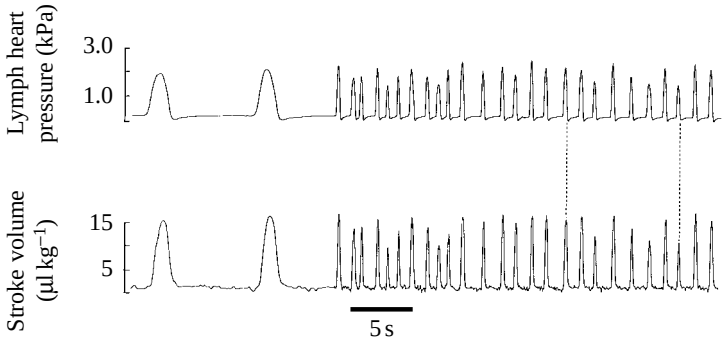

Fig. 2. Typical simultaneous recording of posterior lymph heart pressure (A) and stroke volume (B) in a hydrated *Bufo marinus*. A distinct beat-to-beat variation can be observed, with a direct relationship between pressure generation and stroke volume. This relationship is clearly illustrated in the section of recording between the vertical dotted lines.

pressure at the point where flow was just initiated (Fig. 5), we found that a lymph heart pressure of 0.71±0.04 kPa (N=4) was needed to open the outflow pore. This value is consistent with measurements of the minimum peak intra-lymph-heart pressure required to produce flow into the posterior vertebral vein. These latter measurements were possible because pressure development by the lymph heart was periodically insufficient to produce flow. The occurrence of such ineffectual contractions varied among animals, from less than 1 beat min^{-1} in some to 5–10 beats min^{-1} in others. With sufficient pressure development, lymph flow into the posterior vertebral vein occurred 113±5 ms after the onset of lymph heart contraction.

A dramatic, but short-lived (2–5 min), increase in lymph flow was observed following slight physical disturbances to the animals. For example, the stroke volume in one animal increased fourfold (from 5 to 20 µl kg^{-1}) (Fig. 6A), producing an increase in lymph flow from 8.5 to 51.0 ml kg^{-1} h^{-1}. In another animal, after an initial stoppage in lymph heart contraction lasting approximately 1 min, stroke volume doubled (from 7.5 to 18 µl kg^{-1}; Fig. 6B). This resulted in lymph flow increasing from 18.0 to 43.0 ml kg^{-1} h^{-1}. Since there was no appreciable change in the rate of lymph heart contraction (<5 beats min^{-1}), the increased lymph flow was largely attributable to the increase in stroke volume.

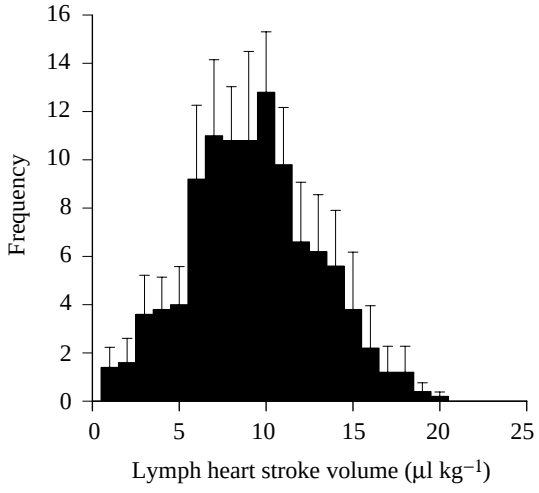

Fig. 3. Histogram showing frequencies of posterior lymph heart stroke volume for five hydrated toads. Stroke volumes were measured from a randomly selected 30 s section of recording for each toad. Values are means + S.E.M.

Lymph heart flow and pressure development during dehydration

Dehydration resulted in a progressive reduction of stroke volume and lymph flow (Fig. 7A,B). For example, 12 h after the onset of dehydration, lymph flow had decreased

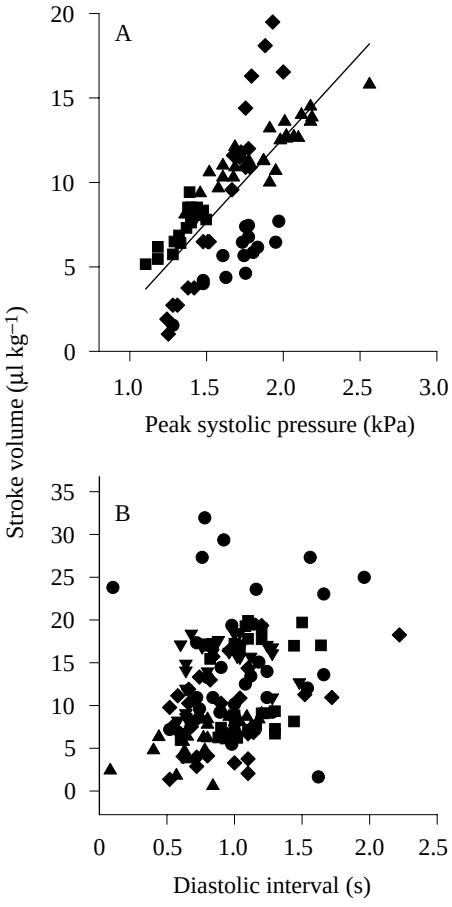

Fig. 4. Relationships between stroke volume (Vs) and peak systolic pressure (P) (A) (r^2=0.51), and between stroke volume and diastolic filling time (B) (r^2=0.089) for five toads. The regression equation for the former is as follows: Vs=−7.30+9.95P. Different symbols represent the individual toads ($0.76 \leqslant r^2 \leqslant 0.97$).

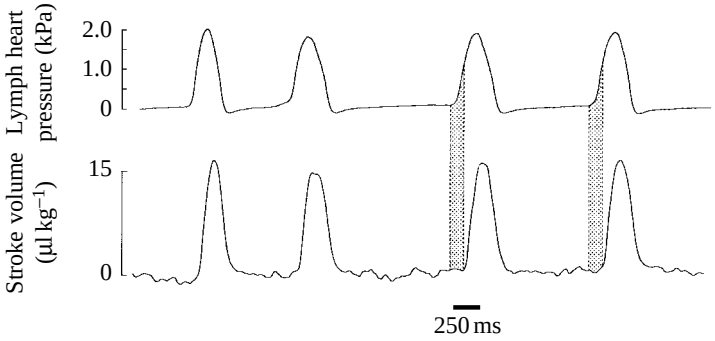

Fig. 5. Simultaneous recordings of posterior lymph heart pressure and stroke volume in a hydrated *Bufo marinus*. The pressure required to produce flow during each beat is assumed to be the pressure that just opens the lymph heart outflow pore, thus allowing lymph to enter the posterior vertebral vein (approximately 0.9 kPa). The delay in the opening of the outflow pore following the onset of pressure development is illustrated by the hatched sections.

significantly (by 70 %) from a control value of 18.48±3.87 ml kg^{-1} h^{-1} to 5.67±3.28 ml kg^{-1} h^{-1}. By 24 h, at the end of the dehydration period, it had decreased further to 3.52±1.42 ml kg^{-1} h^{-1} (*N*=5) (Fig. 7B). Again, changes in stroke volume were the major contributor to these alterations in lymph flow. Stroke volume decreased from the control value of 7.4±1.8 μl kg^{-1} to 2.1±1.0 μl kg^{-1} by 12 h, and then remained at this low level for the duration of the dehydration experiment (*N*=5) (Fig. 7A). Heart rate did not decrease significantly during this period (Fig. 7C).

The opening pressure of the outflow pore also changed during dehydration. It decreased significantly from a control value of 0.87±0.11 kPa to 0.40±0.06 kPa after 20 h of dehydration (*N*=5; *P*=0.025 Student's *t*-test). Lymph heart pressure development, however, did not change significantly over the dehydration period (Fig. 7D).

Lymph flow and stroke volumes returned to control values 30 min after adding water back into the experimental chamber (Fig. 7A,B).

Discussion

This is the first study to measure lymph flow directly as it is ejected from the lymph hearts in confined but unrestrained amphibians. Our measurements show that, when the toad *Bufo marinus* is in a hydrating environment, flow produced by a single posterior lymph heart is 26 ml kg^{-1} h^{-1}. If we assume

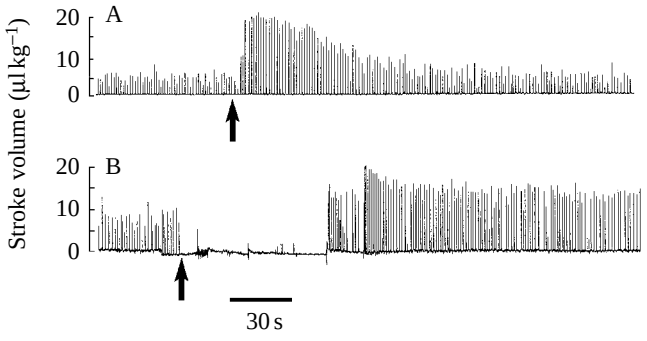

Fig. 6. Posterior lymph heart stroke volume in two toads following moderate disturbances. Arrows indicate the point at which each toad was disturbed. In one animal (A), stroke volume increased immediately and lymph heart flow increased from 8.5 to 51 ml kg^{-1} h^{-1}. After a stoppage in lymph heart function lasting approximately 1 min, stroke volume in the other animal (B) increased and lymph flow increased from 18 to 43 ml kg^{-1} h^{-1}.

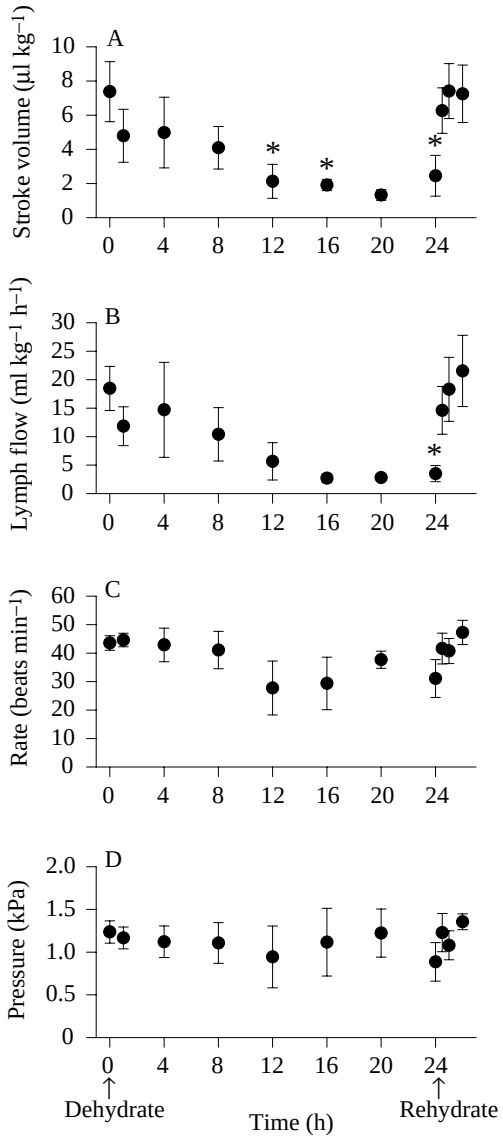

Fig. 7. Effects of dehydration and rehydration on posterior lymph heart function in *Bufo marinus*. (A) Stroke volume, (B) lymph heart flow, (C) lymph heart rate and (D) lymph heart pressure. An asterisk indicates a significant difference from the mean value at 0 h (P<0.05) (N=5). Values are means ± S.E.M.

that the two posterior lymph hearts have similar outputs, then the combined lymph flow (52 ml kg^{-1} h^{-1}) is estimated to be approximately one-sixtieth of that of the systemic heart (57 ml kg^{-1} min^{-1}; West and Smits, 1994). This value is five times that determined by draining lymph heart cannulae at negative pressures (Jones *et al.* 1992), but approximately half the indirect estimates presented by Conklin (1930) and Baldwin *et al.* (1993), if we assume that the posterior paired lymph hearts have a similar output to that of the anterior paired lymph hearts. Our results support the premise that the amount of lymph production in anurans is high and that the lymph hearts are important in returning this lymph to the blood.

Baldwin *et al.* (1993) suggested that lymph heart function in anurans is regulated according to the rate of lymph formation. In her recent study with *Rana pipiens*, she demonstrated that after moderate exercise there was an increase in blood pressure, resulting in an increase in lymph formation. She also reported an increase in lymph heart rate and concluded that this was to accommodate the extra volume of lymph to be returned to the circulation. In contrast, our results for *Bufo marinus* clearly show that lymph flow is regulated to a greater extent by changes in stroke volume; alterations in lymph heart rate play only a minor role. For example, in mildly agitated toads, stroke volume increased by as much as fourfold. In addition, after 12 h of dehydration, stroke volume declined almost fourfold. This 16-fold range for stroke volume in *Bufo marinus* contrasts sharply with the minor changes in heart rate. An exception to this generalization was the sometimes abrupt cessation of lymph heart contractions.

To facilitate changes in stroke volume of this magnitude, the structure of the lymph hearts must be able to accommodate large adjustments in end-diastolic volume and/or end-systolic volume. Since the lymph heart is not constrained by a pericardium, an increased filling pressure during diastole could lead to considerable distention and a concomitant increase in end-diastolic volume. Furthermore, the internal volume of lymph hearts in anaesthetized *Bufo marinus* (43±0.01 µl kg^{-1}, N=6; J. M. Jones, unpublished observations) is considerably larger than the mean stroke volume measured in this study (8 µl kg^{-1}). This is an end-systolic reserve of approximately 80 % of its volume. Observations of the opaque lymph hearts in *Rana catesbeiana* support this in that when contraction occurs there is only a small change in the circumference of the internal wall. In fact, a stroke volume of 5 µl kg^{-1} (as in Fig. 6A) results from a change in the diameter of the lymph heart lumen of only 5 % (assuming that lymph heart shape is approximated by an ellipsoid). A further 15 % decrease in lymph heart diameter would quadruple the output, producing a stroke volume of 20 µl. These small dimensional changes with each heart beat may partially explain why earlier indirect estimates of lymph output were higher than our direct measurements. The inability of all lymph heart contractions to develop sufficient pressure to open the outflow pore is surely another contributor to the earlier overestimates of lymph flow.

The significant correlation between lymph heart pressure and stroke volume and the lack of correlation between diastolic interval and stroke volume indicate that systolic pressure is more important than filling time in determining stroke volume in hydrated animals. The beat-to-beat variability in force development and therefore stroke volume is probably due to the recruitment of a different number of muscle fibres that comprise the tunica media of the lymph heart (Kampmeier, 1969). The mechanism(s) involved in fibre recruitment with each beat is not clearly understood. del Castillo and Sanchez (1961), however, reported a varying number (5–10) of impulse volleys, and action potentials per volley, in the motor neurones supplying the lymph heart with each lymph heart contraction. They proposed that the resulting mechanical activity of the lymph heart was the result of the summation of a number of acetylcholine-elicited postsynaptic potentials, thereby resembling the electrical activity of skeletal muscle. Our measurements, which show extensive beat-to-beat variation in contraction strength and stroke volume, support their findings as well as those of histological studies that show that the tunica media of amphibian lymph hearts is largely made up of modified skeletal muscle (Kawaguti, 1967; Satoh and Nitatori, 1980).

Pressure measurements were not taken during the time when the animals were disturbed. During dehydration, however, we observed only minor decreases in lymph heart pressure which coincided with a considerable drop in stroke volume. This suggests that, in addition to muscle recruitment, adjustments in stroke volume probably reflect lymph availability (lymph sac pressure), of which we know little.

Since the lymph heart is a pump, pressure differences created by each contraction force lymph from a low-pressure area (the sacs) to a higher-pressure area (the veins). Lymph flow occurs when the pressure in the lymph heart exceeds that in the outflow vessel, and the outflow pore opens. Filling occurs when the lymph heart recoils after contraction and the intra-lymph-heart pressure drops slightly below (by approximately 0.025–0.1 kPa) that in the surrounding lymph spaces. It is likely, therefore, that both ‘inflow’ pressure (pressure within the lymph sacs) and ‘outflow’ pressure (pressure within the posterior vertebral vein) influence lymph heart end-diastolic and end-systolic volumes.

Interstitial pressures of approximately −0.25 kPa have been documented in hydrated *Bufo marinus* (Scholander *et al.* 1968). In addition, Carter (1979) found that it was not possible to aspirate lymph from the subcutaneous space of hydrated frogs, suggesting a negative fluid pressure in the lymph sacs. With an increase in interstitial fluid pressure (e.g. following a rise in blood pressure and capillary filtration), a greater pressure differential would be created between the lymph space and the lymph heart at the beginning of diastole. Increased filling pressure and, consequently, greater end-diastolic volume and stroke volume would be the result. We frequently observed this effect during the surgical procedure when the anaesthetized animal was raised from a prone to an upright position. Lifting the animal caused the lymph in the sacs to pool quickly in the posterior lymph heart area, causing an immediate and dramatic rise in stroke volume that was probably due to an increase in lymph sac pressure.

Similarly, a decrease in interstitial fluid pressure would lead to lower lymph heart filling pressures and, therefore, a reduction in end-diastolic and stroke volumes. Scholander *et al.* (1968) demonstrated that, during dehydration, interstitial fluid pressure declines to a lethal value of approximately –1.0 kPa. These lower filling pressures provide a mechanistic explanation for the reduction in stroke volume observed during dehydration. The lymph heart muscle fibres were stimulated to generate pressures similar to those in hydrated animals, but filling of the lymph heart was inadequate. Obviously, the relationship between stroke volume and systolic pressure shown in Fig. 4A for hydrated animals should not be expected to hold for dehydrated individuals. A lower slope would be expected. In fact, different states of hydration may explain some the variation between the animals shown in Fig. 4A.

Hillman *et al.* (1987) found that during dehydration there was no decline in venous pressures in *Bufo marinus*. We observed a decrease, however, as indicated by the 50 % lower opening pressure of the lymph heart outflow pore in the posterior vertebral vein. While the lower venous pressure may have enabled an increased emptying of the lymph heart, an effect which would have countered the decreased inflow during dehydration, the steady decrease in stroke volume during dehydration indicates that lymph sac pressure has a greater effect on stroke volume than venous outflow pressure.

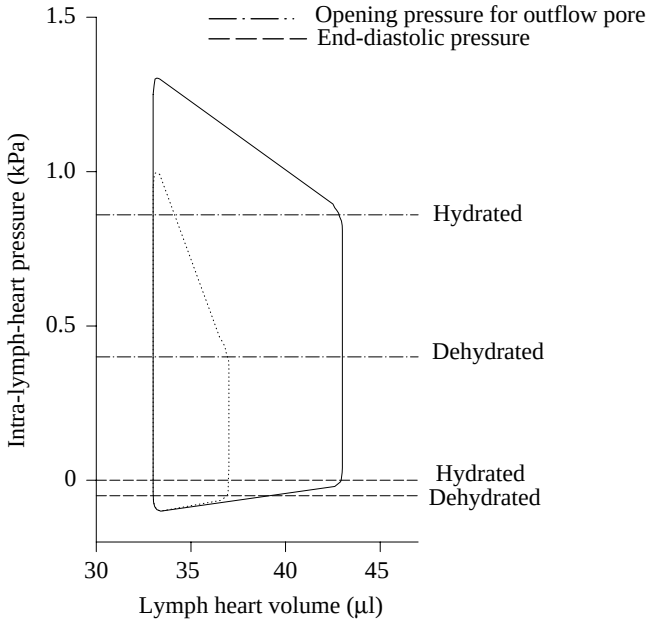

Fig. 8. Pressure–volume loops for the posterior lymph heart based on mean pressures and stroke volumes in *Bufo marinus*. The solid loop represents the pressure–volume loop for hydrated animals. The dotted loop shows the pressure–volume relationship in dehydrated individuals. Each loop represents one lymph heart contraction. Lymph flow from the posterior lymph heart occurs during contraction, when the lymph heart pressure exceeds that in the outflow vessel. Lymph heart filling occurs during diastole, when the pressure in the lymph heart becomes lower than that in the lymph sacs. Dehydration results in a decrease in stroke volume, a decrease in the pressure required to open the outflow pore (i.e. a decrease in venous pressure), a decrease in end-diastolic volume due to a decrease in lymph sac pressures, and a small non-significant change in peak systolic pressure. The negative pressures represented during diastole are estimates, and the point where normal intra-lymph heart and lymph sac pressures are equal is given as 0 kPa. We have also assumed that end-systolic volume is the same under both hydrated and dehydrated conditions. During dehydration, however, it may be lower, leading to a shift to the left in the loop.

To help illustrate the effects of changes in input and output pressures on stroke volume, we have derived a simple pressure–volume loop (Fig. 8) for the lymph heart based on the previously described observations. It should be noted, however, that many different shapes of the loop are possible given the considerable beat-to-beat variability in pressure and stroke volume. To show how a difference in end-diastolic volume and outflow pressure can alter the shape of the curve, a change in the pressure–volume loop during dehydration is also provided. If, at the beginning of diastole, the lymph sac pressure is more negative than the pressure in the lymph heart, the lymph hearts will not fill. This, in fact, occurred with two animals in the later stages of dehydration, when the lymph hearts were contracting with no corresponding flow.

Although we have only discussed the effect of venous pressure and the rate of lymph formation on lymph heart function, it should be noted that lymph hearts are innervated by sympathetic as well as motor nerves (Priestley, 1878), which also play a role in controlling lymph heart function (Day *et al.* 1963).

In summary, since various hydration states and blood pressure changes alter the amount of lymph formation, it follows *a priori* that the regulation of lymph heart function plays a vital role in the fluid homeostasis of the animal. We have shown that stroke volume is related to pressure generation by, and lymph availability to, the lymph heart. We have also shown that alterations in stroke volume are considerably more important than changes in lymph heart rate in regulating lymph flow in *Bufo marinus* under hydrated, dehydrated and slightly agitated conditions.

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