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POSTERIOR LYMPH HEART PRESSURE AND RATE AND LYMPH FLOW IN THE TOAD *BUFO MARINUS* IN RESPONSE TO HYDRATED AND DEHYDRATED CONDITIONS

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Summary

Posterior lymph heart pressure, rate and flow were measured in chronically cannulated *Bufo marinus* during normal hydrated and dehydrated conditions. A new surgical technique was developed which allowed direct and constant measurement of the functioning of the posterior lymph hearts with minimal disruption to normal lymph drainage. The mean intra-lymph-heart systolic pressure was 2.29±0.12 kPa for hydrated animals at rest, decreasing to 1.01±0.10 kPa after 24 h of dehydration. Similarly, lymph heart rate, which was 48.2±1.7 beats min^{-1} under hydrated conditions, decreased to 31.8±4.6 beats min^{-1} after 18 h of dehydration. Lymph flow decreased almost to zero during dehydration from a hydrated rate of 1.11±0.04 ml h^{-1} 100 g^{-1}. This is the first study to measure directly and to correlate these variables in an amphibian and to show specifically that pressure, rate and lymph flow are significantly reduced during periods of dehydration.

Introduction

The amphibian lymphatic system is unique among the vertebrates, primarily because of its high rate of lymph production and circulation (Conklin, 1930*a*). The main function of this system appears to be the drainage of fluid from the tissues and its return to the circulatory system. The source of this fluid may be both net capillary filtration and cutaneous uptake. To accommodate this rapid exchange of fluid between the circulatory and lymphatic systems, amphibians have two distinct features: large, interconnecting lymph spaces into which lymph vessels drain; and two or more pairs of lymph hearts which propel lymph from the spaces into the circulatory system (Müller, 1833; Ecker, 1889; Conklin, 1930*a*; Kampmeier, 1969).

The lymph hearts are small, pulsatile organs usually located on the dorsal side of the animal's body. In most anurans, the anterior pair lie, one on each side, on the anterior surface of the transverse process of the third vertebra, beneath the serratus medius muscle and the scapula (Müller, 1833; Ecker, 1889; Kampmeier, 1969). The posterior lymph hearts, numbering from one pair in the common toad to two or more in certain frogs (Kampmeier, 1969) are found, one of each pair on

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each side, lying lateral to the end of the urostyle (Müller, 1833; Ecker, 1889; Kampmeier, 1969). Because of their accessibility, the posterior lymph hearts have been studied more frequently.

Lymph, formed by plasma filtration and cutaneous water uptake, collects in the large subcutaneous sacs and in sinuses among the viscera, from where it enters the lymph hearts through many afferent pores (Conklin, 1930*a*; Kampmeier, 1969). This is accomplished by the pumping action of the lymph hearts and the movements of the animal's legs (Müller, 1833; Conklin, 1930*a*; Carter, 1979). The anterior lymph hearts pump lymph drained from the viscera and axillary lymph sacs *via* the subscapular lymph sinus into the anterior vertebral vein, a branch of the interior jugular vein (Müller, 1833; Kampmeier, 1969). The posterior lymph heart collects lymph from the subcutaneous lymph spaces and pumps it into the posterior vertebral vein and, ultimately, into the renal portal vein (Müller, 1833; Kampmeier, 1969; Carter, 1979). The posterior lymph heart is also thought to drain water that enters through the skin when the animal is in water. Because this lymph ends up in the renal portal system, the kidney provides a means for the excretion of excess water (Carter, 1979).

Unlike the blood heart, the lymph heart has an irregular rhythm (Müller, 1833; Priestly, 1878; Conklin, 1930*a*; Kampmeier, 1969), with the right and left of each pair neither beating synchronously with each other nor beating continuously (Priestly, 1878). However, the anterior and posterior hearts of a pair on the same side have been shown, through external monitoring at least, to beat simultaneously (Pratt and Reid, 1939). The pattern and frequency of the lymph heart contractions may be influenced by a variety of factors, such as body position, fright, activity and temperature (Kampmeier, 1969). These variables are also known to affect the rhythm of the blood heart, but often not in the same manner or to the same degree. The lymph hearts may also, for no apparent reason, stop beating altogether for a short time (Priestly, 1878).

The resting lymph heart rate in the frog is 50–70 beats min^{-1} (Priestly, 1878) with a stroke volume estimated at 0.5–1.0 ml $beat^{-1}$ (Winterstein, 1925 in Conklin, 1930; Radwanska, 1906 in Kampmeier, 1969). Using these figures, lymph flow for a hydrated animal can be calculated to be between 1.5 and 4.2 ml h^{-1} $heart^{-1}$. Isayama (1924*a*,*b*) determined the rate of lymph production and estimated that fifty times the volume of the total blood plasma passes out of the blood and into the tissues in a 24 h period. The importance of the lymph hearts in the return of this fluid back into circulation was demonstrated by Foglia (1941). He showed that destruction of the lymph hearts resulted in the rapid onset of oedema, with a 60 % increase in body weight, eventually leading to death within 4 days for *Bufo arenarum* and *Leptodactyllus*. Additionally, Baustian (1988) showed that recovery from acute blood loss in *Bufo marinus* was rapid in animals with intact lymph hearts. When these organs were destroyed, however, death occurred within 1–3 days as a result of progressive haemoconcentration.

The above estimates of lymph production and flow assume that the stroke volume of the lymph heart is always within certain limits. Kampmeier (1969),

however, states that a lymph heart containing a minimal amount of lymph beats as often as one filled normally. Using an estimate of stroke volume and rate, therefore, may not give an accurate assessment of lymph flow through the lymph hearts. This study was undertaken to measure lymph flow directly using chronic cannulations of the lymph hearts in freely moving *Bufo marinus*. The relationships between lymph heart pressure and rate and lymph flow during hydration and dehydration were also investigated.

Materials and methods

Surgical procedure

Adult *Bufo marinus* of either sex (mass 191–572 g) were obtained from a commercial supplier (Charles D. Sullivan Co. Inc., Nashville, TN, USA). The animals were maintained in fibreglass aquaria with free access to water and were force fed raw liver once each week.

For each experiment, the animals were randomly chosen and maintained in 2–5 cm of water for 1 day prior to surgery. They were anaesthetized by partial submersion in a $2\,g\,l^{-1}$ solution of tricaine methane sulphonate (MS-222) buffered to pH 7 with $NaHCO_3$. Animals were removed when the corneal reflex was no longer present.

The posterior lymph hearts were located by observing pulses beneath the skin approximately 0.5 cm on either side of the urostyle. A 1 cm incision was made over this location, and the skin and underlying fascia were separated to allow access to the heart. The lightly pigmented connective tissue covering the lymph hearts was then located and it was into this that a very small incision (3 mm) was made, providing a portal to the interior of the lymph heart. A purse-string suture (CE-4 19 mm needle, 3.0 silk) was made around the opening and the flared end of a 0.8 m length of polyethylene (PE60) tubing, filled with heparanized saline, was inserted. The suture was secured around the cannula and the incision was closed, with the sutures arranged so that the cannula protruded vertically.

All toads were also occlusively cannulated in the isciatic artery, for the purpose of heparinized saline injection, using 0.8 m of polyethylene (PE60) tubing (Boutilier *et al.* 1979). In six of the animals used in the pressure experiments, the femoral vein in the opposite leg was also occlusively cannulated in the direction of the body using a 0.8 m length of PE90 tubing. In all cannulations the skin incisions were closed with ligatures and sealed with cyanoacrylate glue. The entire operation was completed in 1–2 h.

For an 18–24 h recovery period, and for the duration of the experiments, the animals were placed in darkened 2.5 l acrylic cylinder-shaped chambers and supplied with a continuous flow of air ($660\,ml\,min^{-1}$). During recovery and throughout the hydration experiments, 300–400 ml of tap water was maintained in the chambers. Conklin (1930*b*) has reported that the clotting protein fibrinogen has been identified in lymph, so a 0.5 ml injection of heparinized saline ($125\,i.u.\,ml^{-1}$) was administered through the arterial cannula at the beginning of

each experiment to prevent coagulation of the lymph within the lymph cannula. All experiments were conducted at room temperature (23°C). Dehydration periods were of sufficient length to subject the animals to moderate stress (Tufts and Toews, 1986).

Lymph heart pressure and rate measurements

Normal

Nineteen animals were used for intra-lymph-heart pressure measurements. In six of these animals, venous pressure was also recorded at the onset of the experiment. Cannulae were connected to two Statham 23 Db pressure transducers, which were attached to a Gould two-channel recorder, enabling the simultaneous recording of the left and right lymph heart pressures.

At the beginning of each experiment, the transducers were calibrated and zeroed using pressures obtained from static water columns. The zero level was set individually for each animal at the level of the lymph hearts, which was equal to the surrounding water level when the animal was being hydrated. For venous pressure recordings, the zero level for the posterior lymph hearts was used.

Normal pressures were recorded continuously for 1–2 h prior to dehydration. Lymph heart pressures were determined from a representative part of the recording, where the pulse peaks were well defined. At times, an animal would move and twist the cannulae around its body, causing the normally sharp peaks to flatten out. This movement probably changed the position of the cannula inside the lymph heart, resulting in a partial blockage of the lumen.

Only the systolic pressures were used in subsequent analyses. Generally, the diastolic pressure of the lymph heart corresponded to the zero level as determined by the water column; however, as the animal changed position throughout the experiment, this value sometimes drifted slightly to a different resting level. It was not feasible to adjust the water column constantly to the new position, so the value of diastole on the recording was always taken to be 0 kPa. The systolic pressure varied with every beat; therefore, the average pressure over a period of 20 s was used as the representative value.

Lymph heart rates were determined by counting the number of pulses to the nearest full beat in exactly 1 min, from the same section of the chart recording used in the pressure measurements.

Dehydration

To dehydrate the animal, the chamber was emptied of water and wiped dry. The air flow was routed through an 0.08 m diameter cylinder filled with a commercial drying medium (Drierite). The dehydration period lasted for 24 h, during which time lymph heart pressures were recorded continuously. The animal was then rehydrated by returning 300–400 ml tap water to the chamber, and water-saturated air was infused. Continuous readings were then taken for at least 2 h.

Data are shown as means±S.E. of the mean number of animals given in the

figure legends. Statistical comparisons of the data obtained during dehydration and rehydration were made using analysis of variance (ANOVA). Results were considered significant for $P<0.05$.

Lymph flow measurements

Normal

After the recovery period, during which time the lymph cannulae remained occluded, the water in the chambers was replaced. The lymph cannulae were then connected to a fraction collector (LKB 2070 Ultrorac II), either with both cannulae filling one set of tubes or with a separate set of tubes for each cannula.

Because of the difficulty encountered in attempting to identify and surgically obstruct the outflow pore of the lymph heart, the outflow ends of the cannulae were placed below the level of the lymph hearts of the animal, with the assumption that, because of gravitational pull, the lymph would be preferentially shunted down the tubing instead of through the heart outflow valve. The cannulae of individual animals were adjusted slightly up or down within a 5–7 cm range so as to provide maximal flow, as suggested by Adair *et al.* (1983), who recommended a distance of 5–10 cm to maintain the lymphatic hydrostatic pressure at a constant level. If the end of the cannula is placed too high, flow is diminished by pressure build-up within the tubing; if the tip is placed too low, the increased pressure may cause the interior of the heart to be sucked into the opening of the cannula, decreasing flow. When the cannula is adjusted for maximal flow, any loss of lymph through the outflow pore would necessarily be minimal.

It is also believed that, because the end of the lymph cannula was more widely flared in this set of experiments, the outflow pore might be partially or totally obstructed. This assumption appears to have been valid as at least six of the animals used in flow determinations were noticeably, although not excessively, oedematous after the recovery period. Because of this perceived accumulation of excess lymph in the body tissues, the first 1–2 h of collection data was discarded because it was believed that the elevated flow observed reflected the animal's attempt to rid itself of the extraneous fluid. Flow volumes during this drainage period were at first very large, but they decreased rapidly to a constant level, reflecting the resumption of normal lymph heart function. Animals observed at this point were no longer outwardly oedematous. Lymph flow continued at a relatively constant rate during the 24 h hydrated collection period, indicating that flow was not being exaggerated by excess body water.

Fractions were collected into tared test tubes over 15 or 30 min intervals for a period of 24 h.

Dehydration

The experiments on dehydrated animals were conducted as before, except that, prior to the connection of the lymph cannulae to the fraction collector, the chamber water was emptied. The air supply was channelled through a commercial

drying medium to prevent the introduction of moisture through the air vapour. Fractions were again collected over intervals of 15 or 30 min, for a total period of 30–50 h. Because it was observed that the cannulae were more prone to clogging when the lymph flow was diminished (as in dehydration), an additional 0.5 ml injection of heparinized saline was administered halfway through the experimental period *via* the arterial cannula. This injection did not affect the flow in any way.

Plasma proteins, specifically fibrinogen, albumin and globulin (Conklin, 1930*b*), are known to be a substantial component of lymph, so there was concern regarding the effect of protein depletion on the normal physiological functioning of the animal. Therefore, the animals were used for only one experiment and then killed.

After the experiments, the tubes were weighed and the net lymph volumes were determined. A paired *t*-test was performed on the dehydration data, with values identified as significant if $P<0.05$. Owing to the large amount of data, only the values recorded every 30 min were used in the statistical analyses. All values are expressed as means±S.E. of the mean number of animals given in the figure legends, except where noted.

Results

Lymph heart pressure and rate

Normal

Normal recordings of posterior lymph heart rate and pressure were taken when the animal was at rest following the recovery period. Because the animals for this part of the study did not become oedematous following surgery, the outflow pore was thought to be functional and, therefore, the recorded pressures were considered to be characteristic of normal lymph heart functioning.

The contraction of the lymph heart produced a wave form similar to that produced during the contraction of lymphatic smooth muscle in sheep, as observed by Thornbury *et al.* (1989). A sharply peaked systole was followed by a longer diastole, predominantly seen as a flat line on the tracing (Fig. 1). In most of the toads, the lymph heart produced single contractions at regularly spaced intervals. In others, however, a regular pattern was at times established with a brief pause every two or three beats. The pressures generated by the lymph heart exhibited a characteristic fluctuation in amplitude from one beat to the next, sometimes from a very weak deflection, to a maximum of 4.1 kPa.

The average lymph heart rate in 19 hydrated *Bufo marinus* was found to be 48.21±1.68 beats min^{-1}, with a range of 36 to 62 beats min^{-1}. For each animal, there was little variation in the overall rate throughout the hydration period. Occasionally, however, the regular rhythm was interrupted, for no apparent reason, by pauses lasting up to 30s. Additionally, there were other cessations ranging from a few seconds to a few minutes in response to the animal being touched or following room noises.

The average systolic pressure generated by the lymph hearts of 19 *Bufo marinus* under hydrated conditions was 2.29±0.12 kPa, with a range of 1.48–3.63 kPa. The

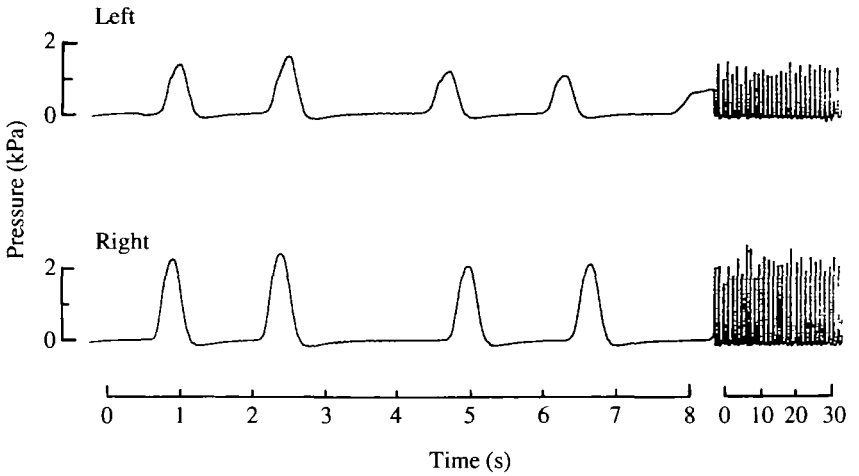

Fig. 1. Intra-lymph-heart pressure of the left and right posterior lymph hearts in a normal hydrated *Bufo marinus*.

highest systolic pressure recorded was 4.1 kPa; the lowest were pulses of less than 0.1 kPa.

There was no correlation between the mass of the animals and the systolic pressures or rates (r^2 values of 0.0004 and 0.0134, respectively). For this reason, values were not standardized to mass. Venous pressure in the femoral vein ranged from 0.73 to 2.75 kPa (29.2–106.6 % of lymph heart systolic pressure).

Dehydration

During dehydration, the average lymph heart rate decreased significantly (paired *t*-test $P<0.05$) from 47.5±3.1 beats min^{-1} at 0 h to 31.8±4.6 beats min^{-1} at 18 h. Within minutes of returning water to the chamber, the rates returned to pre-dehydration values (Fig. 2).

The same pattern can be seen in Fig. 3, which shows the effect of dehydration on intra-lymph-heart systolic pressure. The average pressure declined significantly ($P<0.05$) from 1.94±0.20 kPa at 0 h to 1.01±0.10 kPa at 24 h. Immediately upon rehydration, the pressure returned to values not significantly different from normal ($P<0.05$).

In most animals, the intermittent cessations of rhythm increased in duration and frequency during dehydration. There were also periods when the pulses became sporadic and weak (Fig. 4). Most often the pauses or 'slowdowns' occurred simultaneously in the right and left hearts. They usually lasted 3–10 min and could occur 2–3 times an hour for several hours. Pauses of up to 30 min were sometimes observed, interspersed with isolated weak contractions. The lymph heart, however, could at any time commence beating and continue for hours without another stoppage. The pauses in lymph heart contraction could not usually be attributed to

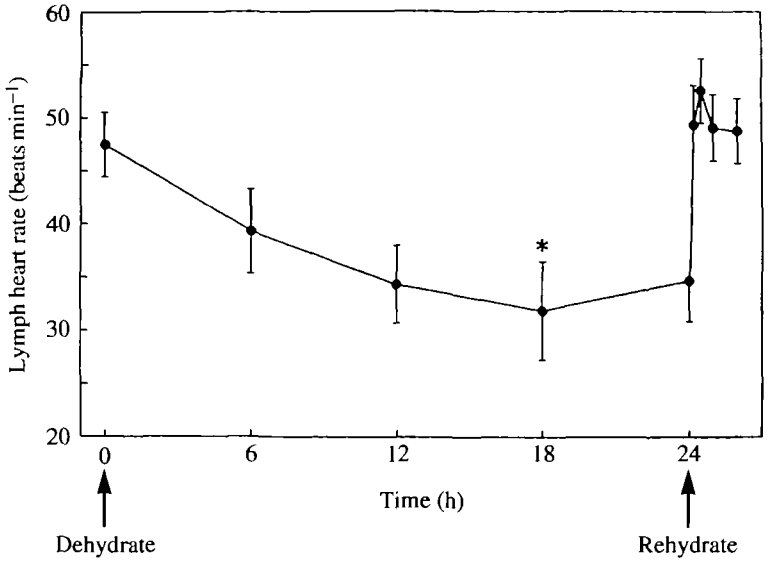

Fig. 2. Posterior lymph heart rate in *Bufo marinus* during dehydration and rehydration (N=8). Values are presented as mean±S.E.M. The asterisk indicates a significant (P<0.05) difference from the value of 0 h (start of dehydration).

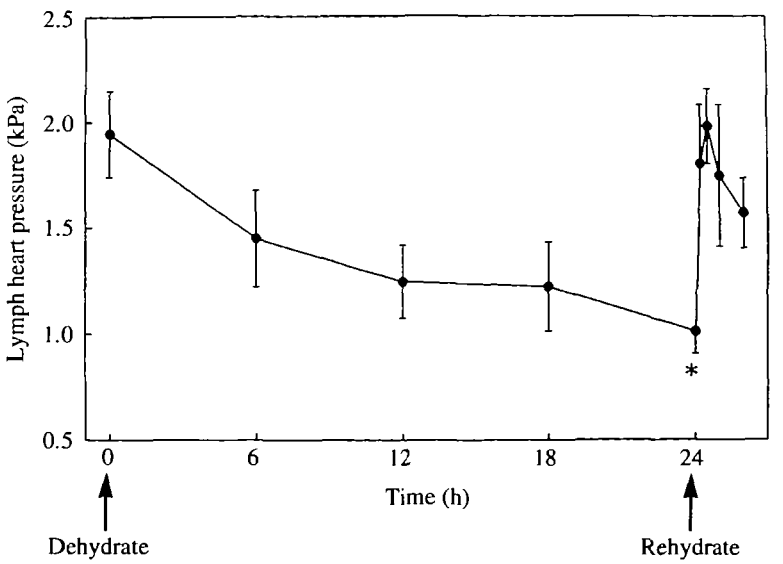

Fig. 3. Posterior lymph heart pressure in *Bufo marinus* during dehydration and rehydration (N=8). Values are presented as mean±S.E.M. The asterisk indicates a significant (P<0.05) difference from the value at 0 h (start of dehydration).

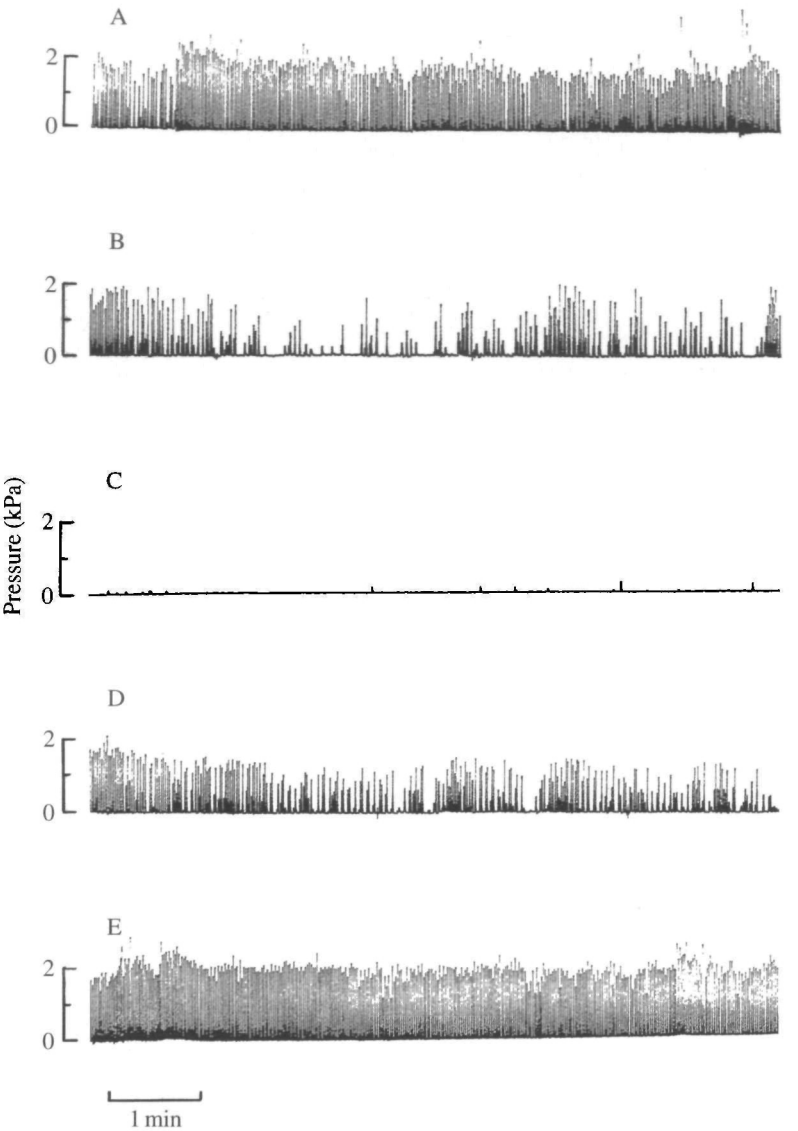

Fig. 4. Effect of dehydration on lymph heart pressure and rate in a representative *Bufo marinus*. (A) Normal hydrated animal. (B) A decrease in lymph heart function (after 3 h of dehydration). (C) Almost complete cessation of lymph heart function. This particular stoppage occurred at 7.5 h of dehydration and lasted 29.4 min. (D) Lymph heart function after 24 h of dehydration, illustrating a decrease in both pressure and rate when compared to the control (A). (E) Increase in pressure and rate after 30 min of rehydration.

a change in the animal's position since the animals were usually inactive during dehydration.

In most cases, immediately following a slowdown or cessation, there was a temporary increase in both the systolic pressure and rate compared to the average value for that time. The same response was observed when the animal breathed or changed its position, as long as the cannula did not become twisted. These effects were observed during hydration as well, but were more pronounced during dehydration.

Lymph flow

Normal

Initially, the lymph flow of fully hydrated *Bufo marinus* was determined from only one cannula for each animal over a short period of time. This value was roughly equivalent to that previously calculated (1.5–4.2 $ml\,h^{-1}\,heart^{-1}$). However, when lymph measurements were taken separately from both the left and the right cannulae, it was evident that the assumption of equal flow from each side was not always valid (Fig. 5). When one lymph heart is processing a large amount of lymph, the other is often much less active. Therefore, the mean lymph flow cannot be accurately determined using measurements from only one heart over a short period.

The cause of this flow 'mirror image' is not known; however, the physical position of the animal during lymph collection is a potential source of the discrepancy. The possibility that it is a surgical artefact also cannot be ignored. By

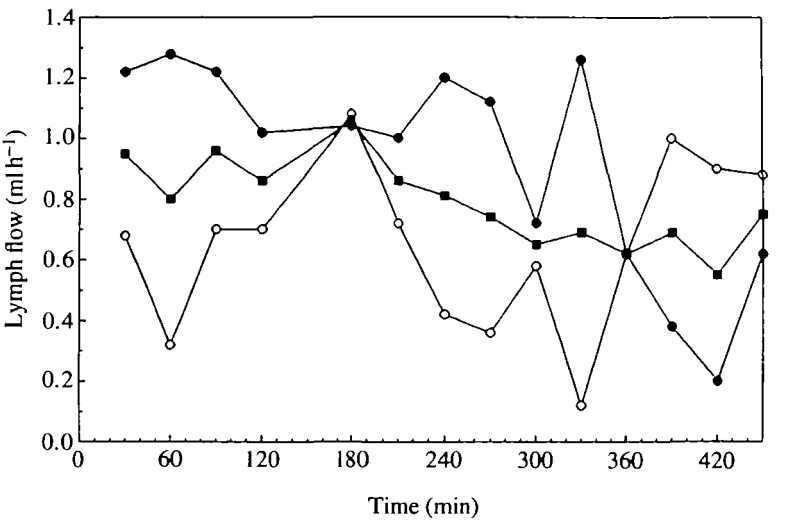

Fig. 5. Lymph flow from the posterior lymph hearts of a single *Bufo marinus* under normal hydrated conditions. Flows from the left heart (filled circles), the right heart (open circles) and the average of the two hearts (filled squares) are shown.

combining the flow from both cannulae, however, it is believed that an accurate assessment of lymph flow has been made.

Because it was observed that the size of the experimental animals (191–572 g) had a direct bearing on the rate of lymph production, the data for each animal were standardized to $ml\,h^{-1}\,100\,g^{-1}$ body mass. A regression analysis of flow *versus* body mass produced an r^2 value of 0.74, supporting the conversion. The mean combined lymph flow for both posterior hearts of ten hydrated *Bufo marinus* was thus determined to be $1.11\pm0.04\,ml\,h^{-1}\,100\,g^{-1}$, with a range of 0.74 ± 0.17 to $1.93\pm0.06\,ml\,h^{-1}\,100\,g^{-1}$.

Regular collection of lymph by this method will necessarily result in the depletion of proteins and ions from the interstitial spaces, and there was some concern regarding the effect of this drainage on the normal functioning of the toad lymphatic system. However, the consistency of the flow over the 24 h experimental period indicated that little effect was present. Protein recruitment from other stores is possible as a source for replenishment.

Dehydration

Although there were distinct variations in the lymph flow from animal to animal during moderate dehydration, the overall flow pattern was quite characteristic. Typically, after an initial above-normal efflux of lymph, the lymph flow began to decrease within 2–5 h of the start of dehydration, occasionally stopping altogether (Fig. 6). However, even after 48 h of dehydration, the lymph hearts continued intermittently to produce small quantities of lymph. Using the value at 1 h as a

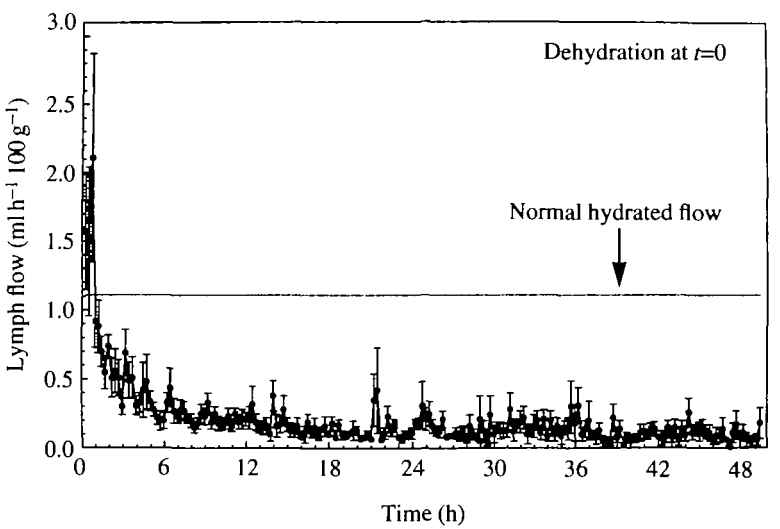

Fig. 6. Lymph flow of *Bufo marinus* during dehydration, standardized to the individual mass of the animal (N=8). Normal hydrated lymph flow is also shown (dashed line).

control, the decrease in rate was found to be significant at 2, 3, 4, 5, 5.5 and 6 h and at almost all times from 7 h after the start of dehydration on ($P < 0.05$). Because of fluctuations in the flows in the individual animals, however, a small number of non-significant values were found after this time (12.5, 14, 21.5, 29, 36, 37, 48.5 and 49.5 h).

Urine flow is known virtually to cease within 8–10 h of the onset of dehydration (Tufts and Toews, 1986). Because the lymph hearts pump fluid through the kidneys, presumably for removal, a reduction or cessation of activity in the lymphatic system, as was observed during these experiments, would account for a large portion of the decrease in urinary production. The effect of the loss of proteins and ions has previously been determined as minimal in the hydration experiments. Therefore, the reduction in lymph flow observed is presumed to be a real response to the moderate dehydrational stress, and not an effect of protein, ion or extracellular fluid depletion.

Discussion

The systolic pressure generated by the posterior lymph hearts (2.29±0.12 kPa) was much greater than was originally expected. In order to open the lymphaticovenous valve guarding the efferent pore of the lymph heart, the lymph pressure would have to be at least slightly greater than the venous pressure. The mean femoral pressure obtained in this study was 1.43±0.42 kPa. It is reasonable to assume that the pressure in the posterior vertebral vein, which drains lymph from the posterior lymph hearts into the renal portal system, is similar to this value, given its close proximity to the femoral vein. If so, the average lymph heart pressure is approximately 50 % greater than the minimum amount that would be required to open the valve.

If the pressure produced by the lymph heart was less than the pressure in the posterior vertebral vein, the lymphaticovenous valve would probably not open. Such ineffectual contractions were observed as small pulses on the pressure tracings, with more being observed during dehydration than when the animal was fully hydrated. Presumably, insufficient filling of the lymph heart has an effect on pulse pressure.

The average lymph heart rate of 48.21±1.68 beats min^{-1} obtained in this study was comparable to the value of 50–70 beats min^{-1} previously given for other amphibians (Müller, 1833; Conklin, 1930*a*). The observed cessation of contractions in response to fright or physical disturbance was also consistent with the findings of others (Priestly, 1878). The increase in rate following slight movement of the animal, however, contradicts the findings of Kampmeier (1969), who stated that an almost empty lymph heart beats as often as a filled one. Carter (1979) demonstrated that active movement causes lymph to flow towards the lymph heart. It would appear from our results that the lymph heart rate increased in response to a greater filling pressure and, therefore, a greater volume inside the lymph heart.

A lymph flow of 1.5–4.2 ml h^{-1} $heart^{-1}$ was previously calculated for the frog from the findings of Radwanska (1906, in Kampmeier, 1969) and Winterstein (1925, in Conklin, 1930). Assuming that the animals in their studies weighed 40 g, the values of 1.5–4.2 ml h^{-1} $heart^{-1}$ (3.0–8.4 ml h^{-1} for the pair of posterior hearts) can be converted to 7.5–21.0 ml h^{-1} 100 g^{-1} for both hearts. Because *Bufo marinus* is primarily a terrestrial species, the lymph flow might be expected to be lower than that of the chiefly aquatic frog because of the relatively slower cutaneous uptake of environmental water. The value of 1.11 ± 0.04 ml h^{-1} 100 g^{-1} determined in this study, however, is disproportionately lower, considering that the animals were fully hydrated. It must be emphasized that the previous findings were simply estimates of lymph production based upon indirect observation, and not direct measurements as have been made in this study. Additionally, many of the previous experiments were carried out on animals which had been anaesthetized with urethane, which is known to increase lymph heart rate greatly (Conklin, 1930*a*).

Kampmeier (1969) states that there is little difference in size between the posterior and anterior lymph hearts in toads. If it is assumed that the output from the anterior hearts is equal to that of the posterior hearts, the lymph output is equal to about 1/200 of the cardiac output over a 24 h period for a completely hydrated animal. This compares with the figure of 1/3000 in humans (Eckert and Randall, 1983), emphasizing the enormous contribution of the lymphatic pathway to fluid homeostasis in amphibians.

The results obtained during dehydration indicate a reduction in lymph volume in the extracellular space. Several factors are probably involved. It is well known that evaporative water loss from the blood and interstitial spaces through the skin occurs during dehydration (Shoemaker and Nagy, 1977). Removing the ambient water would eliminate a significant source of water uptake into the lymph sacs (Carter, 1979). Additionally, the mean arterial pressure decreases (Hillman *et al.* 1987), reducing the hydrostatic component of lymph production by capillary filtration.

During dehydration, lymph heart contraction, and thus lymph flow, rarely stopped altogether for any length of time, presumably because there was still a degree of capillary filtration by the tissues, even during bouts of prolonged dehydration. This fluid exchange results from hydrostatic and colloid osmotic pressure differences between the capillaries and the interstitial spaces. Hillman *et al.* (1987) found that the return of fluid and, concomitantly, protein to the blood *via* the lymphatic pathway was a necessary factor in dehydration tolerance in *Bufo marinus*.

The complexity of the relationship of the amphibian lymphatic system with the circulatory system, the kidney and the external environment necessitates its consideration when examining osmoregulatory processes. The implementation of the surgical techniques developed for this study would be invaluable for further assessment of the important role the lymph hearts play in maintaining internal fluid homeostasis. In particular, determination of the relative contributions of the

anterior and posterior lymph hearts to fluid return would be useful in providing estimates of total lymph space and lymph production.

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