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REGULATION OF WATER BALANCE BETWEEN BLOOD AND LYMPH IN THE FROG, *RANA PIPIENS*

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ABSTRACT

To determine the relative importance of the lymphatic system and transcapillary reabsorption in maintaining water distribution between blood and lymph in Rana pipiens, *the hematocrit (HCT) was measured (12 frogs) when each frog was: 1) under tricaine methanesulfonate (MS222) anesthesia, which inactivates the lymph hearts, 2) conscious at rest (control), and 3) conscious after activity. Arterial pressure (AP), lymph-heart rate (LHR), and plasma and lymph protein concentrations and colloid osmotic pressures (COP) were also measured. The average HCT during control state was 26.0±5.2% (SD), AP was 21.9±5.2 mmHg, and LHR was 86±13 bpm. MS222 increased HCT (42.6±7.9%), and did not affect AP. Experiments, in which plasma volumes were determined by intra-arterial injection of Evans blue-labeled albumin (EB-BSA) (16 frogs), showed that the increased HCT reflected a reduced plasma volume. Activity did not affect HCT, but increased AP (33.1±6.4 mmHg, $p<0.01$), and LHR (115±26 bpm, $p<0.01$). Thus lymph hearts are largely responsible for maintaining blood volume, and changes in transcapillary filtration rate are rapidly mirrored by changes in lymph heart function. Plasma and lymph COP values after MS222 or activity were not significantly different from control. This observation is consistent with the protein and fluid fluxes resulting from Starling forces if an average reflection coefficient to protein, σ, of 0.5 is assumed, which is consistent with our EB-BSA data.*

Control of water distribution between blood and lymph is vital for normal physiological function. If more fluid leaves the blood than is returned, the erythrocytes become densely packed within the capillaries causing stasis, and the excess fluid leaving the blood accumulates in the tissue, causing edema. In healthy subjects, fluid is returned to the blood system by means of the lymphatic system and osmotic reabsorption. Reabsorption depends on the relative hydrostatic pressures in the exchange vessels and in the tissue, and on the relative colloid osmotic pressures, and hence the protein concentration, of blood and lymph. In organs such as the kidneys or the intestinal mucosa, which are supplied by capillaries that are relatively leaky to proteins, the colloid osmotic pressure difference between blood and lymph will be low, and so reabsorption may not play a dominant role in fluid return to the circulation. There have been few studies on the relative importance of lymphatic function and reabsorption on fluid return within such systems. To gain some insight into this question we designed experiments to determine the effects of impaired lymphatic function and of changes in blood pressure on fluid distribution between blood and lymph.

For these experiments we used the frog, *Rana pipiens*. We have shown in previous experiments that the frog is a good model for a system with a high rate of protein turnover between blood and lymph (1). In addition, the

frog has two other advantages for our purposes. Firstly, the water turnover, at 9-10 ml/hr (2-5), is about three times the normal frog blood volume so any changes in the water balance will be quickly apparent. Secondly, the lymphatic system is driven by four lymph hearts which can be stopped within a few minutes by application of the anesthetic MS222 (6).

In a previous study, Hillman et al. (7) used two amphibians, *Rana catesbeiana* and *Bufo marinus*, to determine transcapillary forces during dehydration and found that the latter, more dehydration resistant species, mobilized more fluid to stabilize hematocrit over a wide range of water loss. Capillary exchange forces were not consistent with reabsorption, and so it was concluded that the fluid was provided by lymphatic return.

In another study, Middler et al. (8) found that acute blood loss in the toad resulted in a drop in hematocrit, coincident with a dilution of tagged plasma protein, indicating that peripheral lymph was the source of the mobilized fluid. Although lymph heart rate increased after blood loss, hemodilution still occurred when the lymph hearts were destroyed. Thus no definite conclusions could be drawn regarding the mechanism of fluid mobilization.

In older studies, Isayama (9), Ito (10) and Zwemer and Foglia (11) cauterized the lymph hearts of frogs and observed a rapid increase in hematocrit, indicating that capillary reabsorption does not maintain blood volume when the lymph hearts are destroyed. Consistent with these studies, Baustian (6) found that toads (*Bufo marinus*) that had had their lymph hearts destroyed by electrocautery did not recover their blood volume after hemorrhage, whereas the intact animals did recover blood volume.

None of the studies described above answer the question whether transcapillary reabsorption plays a significant role in maintaining plasma volume under physiological conditions (i.e. under normal conditions of hydration, and when the lymph hearts are working). The present study addresses this question, using frogs in the resting and active states. In our experiments we subjected each frog to temporary lymph heart stoppage, and after recovery, to increased blood pressure induced by activity. In both cases we monitored changes in fluid distribution by measuring blood HCT's. We measured plasma and lymph COP's in order to estimate the effective driving pressures for transcapillary reabsorption/filtration under the different conditions. Initially, to establish that changes in HCT induced by MS222 truly reflected changes in plasma volume, rather than changes in the number of circulating erythrocytes, we performed a study in which a known quantity of EB-BSA was injected intra-arterially into 10 MS222-treated and 6 control frogs, and plasma EB-BSA concentration was measured as a function of time to determine mean plasma volume in each case.

MATERIALS AND METHODS

Animals

The experiments were performed on 33 frogs (*Rana pipiens*), (male 2.5-3.0 in) supplied by J.M. Hazen, VT. All frogs were within the weight range 30g-40g. Sixteen frogs were used to determine plasma volumes by intravascular injection with Evans blue labelled albumin, either with lymph hearts functioning (6 frogs), or after immobilizing lymph hearts with MS222 (10 frogs). Seventeen frogs were used to determine intercompartmental fluid and protein distribution under different conditions.

Surgical Preparation

Each frog was immersed in 0.5 g% MS222 (Sigma, St. Louis, MO) for 8 minutes, rinsed in tap water, and removed for surgery. During surgery and throughout the experiment the frog was covered with a moist gauze pad to prevent water loss by evaporation. The left sciatic artery was exposed and cannulated

against the direction of flow. The cannula was fabricated from polyethylene tubing (PE50) which had been pulled in a flame to the appropriate diameter. The cannula was connected via a stopcock to a pressure transducer (Statham, Oxnard, CA) to monitor mean arterial blood pressure, pulse pressure and heart-rate. This operation was complete 15 minutes after removing the frog from MS222. In the 17 frogs used for measurement of fluid and protein transport, the right crural lymph sac was cannulated. Loose skin, just above the ankle, was pinched with forceps to prevent bleeding during incision and a 6 inch length of PE50 tubing was inserted through a small incision and slid up the leg under the skin until it rested between the peroneus and gastrocnemius muscles, and tied with a purse-string suture. The other frogs, used for measurement of plasma volumes, had both lateral lymph sacs cannulated. Loose skin along the side of the abdomen was pinched with forceps, and a 3 inch length of PE50 tubing was inserted through a small incision, slid under the skin towards the armpit, and tied with a purse-string suture. This procedure was repeated on the other side of the abdomen. In all frogs bladder reserves were drained by cloacal cannulation and suprapubic pressure.

Preparation of Evans Blue-labeled Albumin

Bovine serum albumin (BSA) obtained commercially (Sigma, St. Louis, MO) was purified by dialysis and then lyophilized. Eight grams of purified BSA and 0.96g of Evans blue dye were dissolved in 100 ml of frog Ringers solution at room temperature and the pH was adjusted to 7.4. Any free, unbound Evans blue in the solution was removed by ultrafiltration (Amicon) and the solution was concentrated down to 10 ml. This volume was then run through a fractionation column (10x220 mm) filled with G25 Sephadex (Pharmacia) to ensure that all the free Evans blue was removed from the solution. The pure EB-BSA fraction was then concentrated down to 10 ml and then brought back to 100 ml by addition of frog Ringers solution.

Experimental Procedure

The frogs were left until the 2 posterior lymph hearts were no longer beating. This condition, which was ascertained by examination through a stereo-microscope (Olympus, TOKYO), was reached about 40 minutes after start of MS222 immersion. (In preliminary experiments, after immersing the frog in MS222 for 8 minutes, we exposed the anterior pair of lymph hearts, which lie on the dorsal surface of the third vertebra, by making incisions along the medial margin of each suprascapula. These experiments demonstrated that the anterior lymph hearts, as well as the posterior pair, stopped beating after MS222 anesthesia.)

Plasma Volumes

In the 10 frogs to be used for plasma volume determination without functioning lymph hearts, a blood sample (70 µl) was taken from the sciatic artery and a lymph sample (70 µl) from the lateral lymph sacs, 20 minutes after lymph heart stoppage. These samples would later serve as blanks against which plasma and lymph EB-BSA concentrations could be measured. Next, 0.5 ml of 0.05 g/ml EB-BSA, prepared as described below, in frog Ringers solution, pH 7.4, was injected into the sciatic artery. Samples (70 µl) of blood and lymph were taken at approximately 5, 10, 20 and 30 min. after injection. Timing could not be exact because blood and lymph could not be drawn simultaneously. The plasma was separated by centrifugation at 2000g for 10 min. Each frog was then sacrificed with an intravascular injection of sodium pentobarbital (100 mg/ml) (Harvey Laboratories, Philadelphia, PA). EB-BSA concentrations of plasma and lymph were measured by spectrophotometry (Beckman) and plots of plasma and lymph EB-BSA concentrations versus time were

constructed for each animal. Each set of data was fitted with a polynomial function obtained from least squares analysis. The plasma EB-BSA concentration immediately after injection, C_0, was determined for each frog by interpolating the plasma plot. Since the exact mass of EB-BSA injected was known, the plasma volume could be calculated.

The 6 frogs to be used for plasma volume determination with functioning lymph hearts, were left until they became fully conscious (about 2 hours after MS222 immersion). These frogs were then injected intravascularly with 0.5 ml of 0.05 g/ml EB-BSA. Samples (70 µl) of blood and lymph were taken at 5, 10, 20 and 30 min. after injection. The samples were used as described previously and the frogs were sacrificed.

Cardiovascular Measurements under Three Conditions

In the set of 17 frogs to be used for measurement of fluid and protein distribution, the mean arterial blood pressure was noted and blood and lymph samples were taken using 70 µl heparinized tubes (Clay Adams, Parsippany, NJ); 3 for blood and 2 for lymph. These procedures were performed 20 minutes after lymph heart stoppage. Lymph was collected by raising the frog's upper body and gently massaging the leg. The blood sample was spun in a centrifuge (Clay Adams, Parsippany, NJ), the hematocrit read, and the plasma removed for measurements of colloid osmotic pressure in a membrane osmometer (Instrumentation for Physiology and

TABLE 1
Frog Plasma Volumes

Frog	Initial Protein Concentration (g%)	Plasma Volume (ml)
	Lymph Hearts Functional	
11	1.44	1.3
18	0.93	2.3
19	0.52	4.5
20	0.76	2.9
42	1.08	1.9
54	0.90	2.4
		2.6±1.0 (SD)
	Lymph Hearts Immobile	
23	1.13	1.8
24	1.86	0.9
27	1.37	1.4
28	1.86	0.9
34	1.18	1.7
36	0.84	2.6
37	1.12	1.7
51	1.13	1.8
52	0.96	2.2
53	1.53	1.2
		1.6±0.5 (SD)

Medicine, San Diego, CA). Protein concentrations were measured using the Lowry technique (12). The lymph sample was saved for similar measurements.

Twelve of the frogs were left until they became conscious and their lymph hearts could be seen under the skin beating strongly and regularly (the control condition); this usually occurred about one hour after taking the first samples. At this point the mean arterial pressure and rates of both lymph hearts were noted and blood and lymph samples were taken. One hour later each frog was restrained in the hand, causing it to kick and croak. This procedure caused an immediate increase in blood pressure which lasted as long as the period of restraint which was 10 minutes in these experiments. Mean arterial pressure and lymph heart rates were noted and blood and lymph samples taken. Finally each frog was sacrificed with an intravascular injection of sodium pentobarbital.

Hematocrit versus Time

The remaining 5 frogs were each injected with 0.1 ml curare (1.5 mg/ml) between the posterior pair of lymph hearts and 0.05 ml into each anterior lymph heart, 20 min. after lymph heart stoppage in order to keep the lymph hearts inactivated. In each case a blood sample was taken for hematocrit measurement every 10 min. for the next hour. These measurements were made to determine whether the hematocrit had reached its maximum value 20 minutes after lymph heart stoppage.

STATISTICS

The 2 mean plasma volumes, in frogs with lymph hearts functional and immobile, respectively, were compared using a Student t-test. For the set of frogs in which blood pressures, lymph heart rates, hematocrits and plasma and lymph colloid osmotic pressures and protein concentrations were measured, the mean values of these parameters were calculated for each of the three conditions (control, MS222, active). To determine whether the different experimental procedures significantly affected the above values we performed an ANOVA test for each parameter. For tests which were positive, we then compared the means using the Students t-test. If the Students t-test gave a $p<0.01$ for 2 sets of data, we considered the difference to be significant.

RESULTS

Plasma Volumes

The plasma volume results (*Table 1*) demonstrate that the mean plasma volume of frogs with inactivated lymph hearts was probably significantly smaller than that of control animals ($0.01<p<0.025$, Students t-test). Thus stoppage of the lymph hearts appears to result in a fluid shift from blood to lymph. *Fig. 1* shows the mean time course of plasma and lymph EB-BSA concentrations, normalized with respect to C_0, in frogs with normal lymph hearts. This figure was constructed by using the best fit polynomial plots obtained from each experiment, and averaging the normalized tracer concentrations obtained at 5, 10, 20 and 30 minutes. These data were used to test a theoretical model for transvascular protein transport as described in the Discussion.

Cardiovascular Measurements under Three Conditions

Arterial pressures and heart rates measured 15 minutes after removal of the frog from MS222, when the lymph hearts were still beating, were not significantly different from the values obtained 20 minutes after lymph heart stoppage.

Changes in arterial pressure, lymph heart rate and hematocrit, under the 3 conditions: impaired lymphatic function, conscious control, and conscious with increased arterial pressure, are shown in *Table 2*. The ANOVA test gave F-values of 13.27 and 22.92 for the

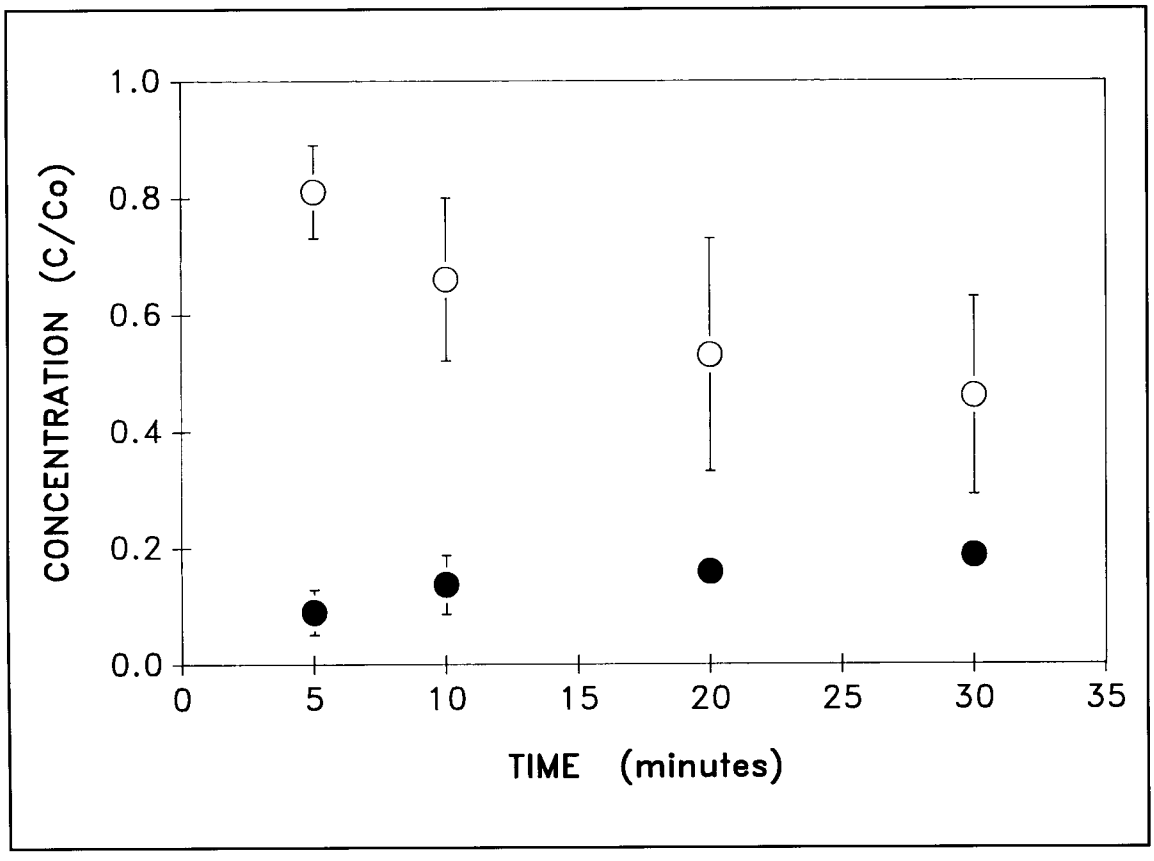

Fig. 1. Mean time course of concentration of Evans blue labeled bovine serum albumin in plasma (open circles) and lymph (filled circles). Concentrations are normalized with respect to concentration at zero time (C_0). Error bars represent standard deviations. To construct this plot, plots from 6 individual animals were used, and the concentrations at 5, 10, 20 and 30 minutes were obtained from the best-fit curves. These values were then averaged.

TABLE 2
Water Distribution between Blood and Lymph

Condition of Frog	Arterial Pressure (mmHg)	Lymph Heart Rate (bpm)	Hematocrit %
MS222	24.7±4.3	0	42.6±7.9
Conscious	21.9±5.2	86±13	26.0±5.2
Active	33.1±6.4	115±26	28.6±5.4

All values are expressed as Mean±SD where n=12

TABLE 3
Water Distribution between Blood and Lymph

Condition of Frog	Protein Concentration (g%)		Colloid Osmotic Pressure (mmHg)	
	Plasma	Lymph	Plasma	Lymph
MS222	2.4±1.0	1.4±0.6	6.3±4.7	3.3±1.9
Conscious	2.4±0.8	1.4±0.5	6.2±3.4	3.5±1.7
Active	2.3±0.8	1.5±0.6	5.8±3.2	3.3±1.7

All values are expressed as Mean±SD where n=11

TABLE 4
Hematocrit versus Time*

Time	Frog 1	Frog 2	Frog 3	Frog 4	Frog 5
0	1.000	1.000	1.000	1.000	1.000
10	0.994	0.974	0.973	0.955	1.025
20	1.012	0.959	0.947	0.961	0.994
30	0.901	0.936	0.950	0.905	0.919
40	0.901	0.879	0.941	0.855	0.919
50	NA	0.812	0.882	0.838	0.913
60	NA	0.797	0.876	0.810	0.931

*Expressed as a fraction of hematocrit measured 60 minutes after start of MS222 immersion

arterial pressure and hematocrit data, respectively, and the critical value for $p<0.01$ was 5.39. Thus, for these data, the variance among the groups was significantly greater than the variance within the groups. When the lymph hearts were stopped with MS222, the mean arterial pressure did not differ significantly from the control value, but there was a significant increase in hematocrit from 26.0±5.2% to 42.6±7.9% after only 40 minutes. A similar response has been observed after lymph heart cauterization (6,13,14).

Activity significantly increased arterial pressure from 21.9±5.2 mmHg to 33.1±6.4 mmHg. Mean lymph heart rate was significantly increased from 86±13 bpm to 115±26 bpm but occasionally a slight irregularity of the lymph heart beat, which lasted a few seconds, was observed in some animals during activity. From these values of

lymph heart rate, we calculated average lymph flow rates to be 0.43 ml/min/100g at rest, and 0.58 ml/min/100g after activity in 40g frogs (assuming the 4 lymph hearts each have a stroke volume of 0.0005 ml (2)). Hematocrit, measured ten minutes after the start of exercise, was not significantly altered by activity. This result demonstrates that changes in transcapillary filtration rate are rapidly mirrored by changes in lymph heart function in order to maintain a constant fluid distribution between blood and lymph under various conditions.

Mean colloid osmotic pressures and protein concentrations, for plasma and lymph, are shown in *Table 3*. Plasma and lymph colloid osmotic pressures (COP) were not significantly different between the 3 conditions for each frog. Protein concentrations showed a similar response.

Hematocrit versus Time

Values of hematocrit, expressed as a fraction of the hematocrit measured 60 min. after start of MS222 immersion (which was usually 20 min. after lymph heart stoppage) are shown in *Table 4*. Data from all 5 frogs showed that the hematocrit did not rise above the value measured 60 min. after start of MS222 immersion during the next 60 min. A gradual drop in hematocrit with time was observed in all cases. Hematocrits taken 40 min. after start time were significantly lower than those measured 10 min. after start time ($p<0.01$). This reduction in hematocrit must have resulted from transcapillary reabsorption of tissue fluid because the lymph hearts were inactive. Reabsorption would occur if the Starling forces were appropriate, i.e. if removal of blood samples had decreased mean transcapillary hydrostatic pressure to less than the transcapillary colloid osmotic pressure.

DISCUSSION

This study has demonstrated, in the frog, that the lymph hearts play a major role in maintaining vascular water content, both when the animal is at rest, and during exercise. The small role that reabsorption normally plays in returning fluid to the circulation is not surprising if we consider the relative size of the osmotic and hydrostatic components of the transcapillary pressure gradient. Our study showed that the mean difference in colloid osmotic pressure between plasma and lymph is 2.7 mmHg. If we assume that the mean reflection coefficient to plasma proteins, σ, is less than unity the effective osmotic pressure difference will be even lower. Although our lymph samples came from the leg, the measured colloid osmotic pressures are probably also typical of lymph from other parts of the body because the lymph spaces in the frog communicate with each other (4,13,15-17) and with the peritoneal cavity (4,13). To confirm this point we measured the colloid osmotic pressures of samples of peritoneal fluid from 6 frogs and found them similar to those of leg lymph [3.7±0.4 (SD)].

With regard to hydrostatic pressures Landis (14) showed that the mean hydrostatic pressures within arteriolar and venular capillaries of the frog mesentery are 10.6 mmHg and 7.4 mmHg respectively. These values both greatly exceed our value for the colloid osmotic pressure difference between blood and lymph. Thus if the hydrostatic pressures measured in the mesentery are typical of other frog capillary beds, we conclude that there is unlikely to be much reabsorption of fluid into the circulation. Landis (18) observed some reabsorption in his preparation of the frog mesentery but his suffusate was protein-free and so a higher transvascular colloid osmotic pressure was exerted.

However, if the lymph hearts are not functioning, and if the Starling forces are appropriate, then water can be returned to the circulation by capillary reabsorption, as suggested by our observation of a fall in hematocrit with time starting 20 min. after lymph heart stoppage. Middler et al. (8) also found that acute blood loss in toads with non-

functional lymph hearts resulted in a drop in hematocrit, as mentioned in the Introduction.

Our finding that lymphatic function is the primary mechanism for return of fluid to the circulation is contrary to results from the cat muscle (19). In these experiments lymph flow rates ranged between 0.013 ml/min/100g muscle (at rest) and 0.02 ml/min/100g muscle (during exercise), which when compared to transcapillary filtration rates in working muscle of 0.2 to 0.4 ml/min/100g, demonstrate that the lymphatics play a minor role in returning lymph to the circulation. One reason for this difference could be that in mammalian skeletal muscle the colloid osmotic pressure difference between plasma and lymph is about 20 mmHg (20) rather than 2 or 3 mmHg, so there is a much larger driving force for reabsorption. It is possible that in mammals the lymphatic system may play a greater role in returning lymph to the circulation in organs associated with a low colloid osmotic pressure difference between plasma and lymph, and hence in those organs invested with more permeable blood capillaries.

The fact that plasma colloid osmotic pressure was apparently unaffected by stoppage of the lymph hearts was surprising, since the accompanying large increase in hematocrit would tend to concentrate the plasma proteins if the exchange vessels are as impermeable to proteins as single vessel measurements suggest (21-25). Other authors (11) reported a similar phenomenon in the toad and they presumed that whole plasma was traversing the capillary walls.

An alternative explanation for the protein concentration data obtained from this study arises from consideration of the changes in pressure distribution and transvascular fluid flux which occur when the lymph hearts are immobilized. We know that inactivation of the lymph hearts results in a net fluid shift from the blood into the interstitium and lymph sacs which is completed when the blood reaches a hematocrit of about 40%. At this point, since there is no net convective fluid flux from blood to tissue, we assume that throughout the whole microvasculature, the hydrostatic transmural pressure balances the colloid osmotic transmural pressure. However, fluid will still be exchanged between the blood and the lymph because the effective transcapillary pressure difference (hydrostatic minus colloid osmotic) will be slightly positive in the arteriolar regions of the microcirculation and slightly negative in the venular regions. Since the protein concentration of the plasma exceeds that of the lymph, and hence the interstitium (26), the bidirectional fluid exchange will result in a net flux of protein from blood to tissue. The similarity between our values for plasma protein concentrations with and without functional lymph hearts could be explained by an initial increase in plasma protein concentration when the lymph hearts are first inactivated and plasma volume is reduced, followed by a decrease in concentration as protein is transported into the interstitium.

To determine the conditions under which the processes described above would result in the plasma protein concentration reaching its control value 20 to 30 minutes after lymph heart stoppage, as observed, we developed a simple, compartmental, theoretical model, based on the Patlak equation for molecular transport (27).

TRANSPORT MODEL

The following assumptions are made:

1. Two compartments, blood and lymph, separated by a combined barrier of capillary and lymphatic endothelium and interstitial matrix.

2. Rapid mixing between compartments.

3. When lymph hearts are immobilized, there is a net fluid loss from blood to lymph until the difference in hydrostatic pressure across the barrier equals the difference in colloid osmotic pressure across the barrier.

Protein transport from blood to lymph is described by the Patlak equation:

$$J_p = J_v(1-\sigma)(C_p-C_1e^{-x})/(1-e^{-x}) \quad (1)$$

where J_p is rate of protein transport, J_v is the net rate of water transport across microvascular walls, σ is reflection coefficient to plasma proteins, C_p and C_1 are protein concentrations in plasma and lymph, respectively, and x is the Peclet number:

$$x = (1-\sigma)\, L_p\Delta P/P \quad (2)$$

where L_p is microvascular hydraulic conductance, P is the mean permeability coefficient to plasma proteins, and ΔP is the integral mean total pressure difference, (hydrostatic-colloid osmotic), across microvascular walls. Protein transport from lymph to blood is equal to the product, $J_v.C_1$, assuming that lymph flow rate is equal to the net transvascular flow rate.

At steady state, (i.e. when protein transport from blood to lymph equals that from lymph to blood):

$$J_p/J_v = C_1 \quad (3)$$

Substituting (3) into (1) and rearranging gives:

$$C_1/C_p = (1-\sigma)/(-\sigma e^{-x}) \quad (4)$$

Equation (4) is derived by equating the protein concentration of lymph to that of the ultrafiltrate. It has been shown in the frog that this is a fair assumption (26). From equations (2) and (4) it is evident that in order to produce the observed physiological value for C_1/C_p, under steady state conditions, for a given P/L_p, and for a given effective transcapillary pressure, (ΔP), σ has a predetermined value. The calculated variation of σ with pressure for a given value of P/L_p is illustrated in *Fig. 2*. The curves were plotted using eqn. (4) for $C_1/C_p = 0.6$.

When lymph hearts are immobilized, water and proteins travel from blood to lymph until hydrostatic and osmotic pressures balance. At this point, there is no **net** water flow from blood to lymph, but there is still some water exchange, the direction of which is governed by local differences between hydrostatic and osmotic pressures. If C_p is greater than C_1, a net protein flux will ensue:

$$J_p = J_v(1-\sigma)(C_p-C_1)(1+e^{-x})(1-e^{-x}) \quad (5)$$

In the above equation, J_v represents the mean water flux from blood to lymph (or from lymph to blood). At this point, J_v is much smaller than the net water flow from blood to lymph before the lymph hearts are immobilized.

The above model was used to determine the values of σ and P/L_p necessary in order for the plasma and lymph protein concentrations to reach their control values 30 min. after lymph heart stoppage, as observed. The following initial, control values for the various parameters were used: capillary hydrostatic pressure: 10 mmHg [Landis (5), for frog mesentery], our measured values for plasma and lymph colloid osmotic pressures and protein concentrations. Three steps were involved:

1. Simulation of the fluid and protein transport between plasma and lymph in the conscious frog, and use of eqn. (4) to predict σ-P/L_p pairs resulting in the observed steady state C_1/C_p value (see *Fig. 2*).

2. Elimination of fluid return from the lymphatic system to the circulation until net filtration eventually stopped. The relationship between the mean transcompartmental hydrostatic pressure difference and net fluid loss from the circulation was defined so that the mean hydrostatic pressure difference was equal to the colloid osmotic pressure difference when the hematocrit reached its observed maximum value. We assumed a linear relationship between the fall in hydrostatic pressure difference and the net volume of fluid leaving the circulation.

Transcompartmental hydrostatic pressures and plasma and lymph protein concentrations (hence colloid osmotic pressures) were calculated at every second between stoppage of the lymph hearts and attainment of hydraulic equilibrium.

3. Continuation of protein transport under conditions of hydraulic equilibrium. [Eqn. (5)].

Of the possible σ-P/L_p pairs determined in step (1), only one pair produces the correct plasma and lymph protein concentrations when used in steps (2) and (3), as observed 30 minutes after lymph heart cessation. The only values of σ and P/L_p which fulfill the necessary condition are 0.5 and 4 mmHg, respectively. If a higher pressure (20 mmHg) is assumed, the required values of σ and P/L_p are 0.4 and 4 mmHg, respectively. For a σ of 0.8 [as is measured in frog mesenteric capillaries (22)], and accompanying P/L_p of 9 mmHg, the model predicts that 30 min. after lymph heart cessation, C_p and C_l will be 1.8 g% and 1.6 g%, respectively, rather than the observed values of 2.4 g% and 1.5 g%.

It is possible to re-express equation (1) (transport of protein from blood to tissue) in terms of a diffusive (first term) and a convective (second term) component:

$$J_p=J_v(1-\sigma)(C_p-C_l)/(e^x-1)+J_v(1-\sigma)C_p \qquad (6)$$

Use of values for σ and P/L_p of 0.5 and 4 mmHg, respectively, in the above equation, predicts that 83% of transcapillary protein transport is convective at a transmural hydrostatic pressure of 10 mmHg, and 96% is convective at 20 mmHg.

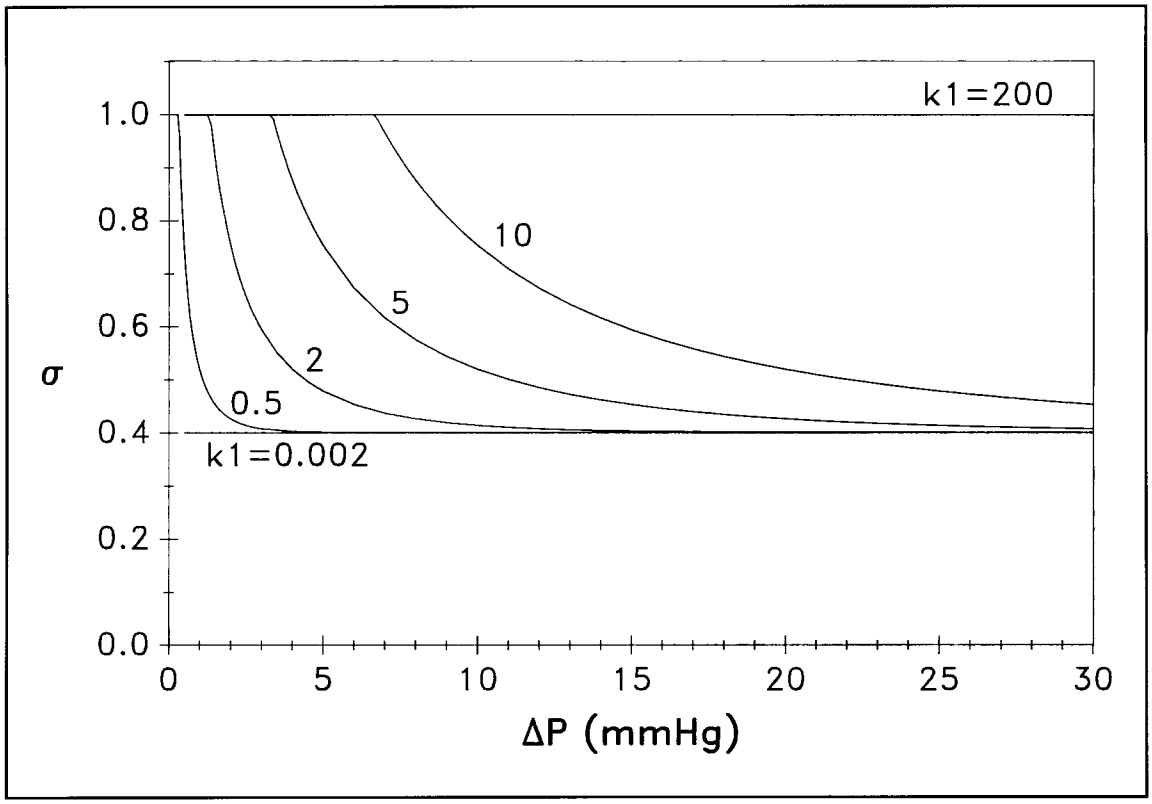

Fig. 2. Calculated variation of plasma protein reflection coefficient (consistent with observed value of C_l/C_p) with chosen effective transcapillary pressure for given values of K1 (where K1=P/L_p). Plots were constructed using eqn. (4).

To test the above transport model experimentally, we used the EB-BSA data obtained during this study as input values (initial EB-BSA concentrations in plasma), and obtained theoretical predictions of the variation in plasma and lymph EB-BSA concentrations with time *(Fig. 3)*. A best fit to the data (shown by solid lines) is obtained from the model if a reflection coefficient to plasma proteins, σ, of 0.5 is assumed. Thus our EB-BSA concentration data do not support the supposition expressed by Zwemer and Foglia (11) that whole plasma traverses the capillary walls. Leakage of whole plasma across the vascular walls would be consistent with a reflection coefficient to plasma proteins of zero. The best fit value of σ (0.5) is consistent with that required to approximate the time course of changes in protein concentrations when the lymph hearts are immobilized. A value of σ of 0.8 (as measured in frog mesenteric capillaries), and accompanying P/L_p of 9 mmHg, do not give a good fit to the data (broken lines).

No direct measurements of whole body vascular permeability to albumin have been made in the frog previously. The fact that our time course data for plasma EB-BSA concentrations after intravascular injection of EB-BSA could only be explained by assuming a reflection coefficient to albumin of 0.5, indicates that the microvessels most responsible for albumin exchange are much more permeable than capillaries in the frog

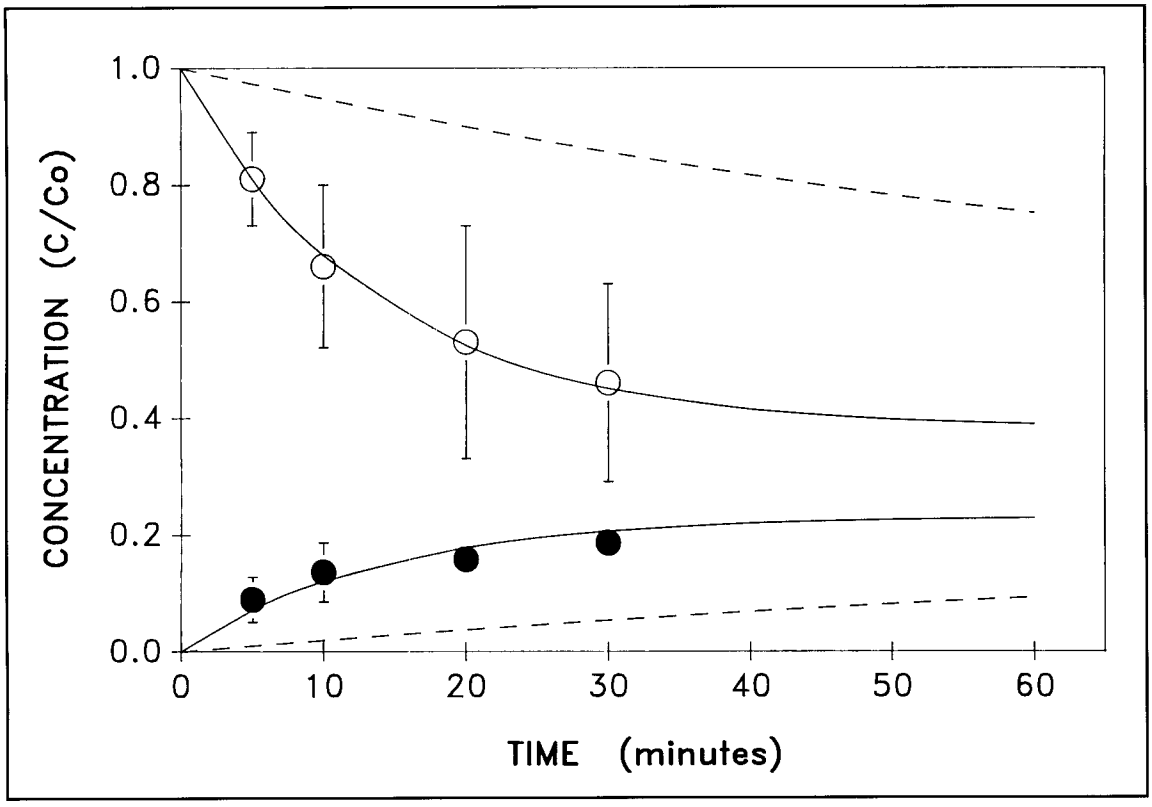

Fig. 3. Mean time course of concentration of Evans blue labeled bovine serum albumin in plasma (open circles) and lymph (filled circles). Concentrations are normalized with respect to concentration at zero time (C_O). Error bars represent standard deviations. The smooth curves are calculated from the theoretical model using σ=0.5, P/L_p=4 mmHg (solid lines), and σ=0.8, P/L_p=12 mmHg (broken lines).

mesentery, in which the measured value of σ to albumin was 0.8 (22). However, even in frog mesenteric capillaries permeability measurements suggest that medium sized molecules (T1824) are transported primarily (63%) by convection (28). This is consistent with our conclusion that for an "average" exchange vessel (which is more leaky to proteins than is the average mesenteric capillary), about 90% of transvascular albumin transport in the whole frog vasculature is convective.

Convective transport of albumin also seems to be important in mammals, as demonstrated by measurements on human muscle and subcutaneous fat (29) and by measurements on perfused rat hindquarters (30). However, Renkin et al. (31), working on the perfused dog paw, concluded that only 30% of albumin flux was convective unless the venous pressure was elevated. Two other studies on the dog paw (32,33) indicated that albumin transport was predominately diffusive, but the lymph flow rates employed were much lower than those seen in the frog. From the limited data available, it appears that capillary beds which are relatively permeable to protein tend to utilize convection as a means of transport, whereas those which are less permeable rely more on diffusion.

Our finding that in *Rana pipiens* hematocrit does not change after ten minutes of enforced activity has not been reported before. In fact, authors of two previous studies using the toad, *Bufo marinus* (34,35) reported that hematocrit increased from 23% to 33% after 30 minutes of exercise. However, Tufts and co-workers did not think that this change in hematocrit reflected changes in water distribution, but that it was probably due to an increase in the number of circulatory erythrocytes. Our results from *Rana pipiens* indicate that either the water balance is not disturbed by 10 minutes of activity, or that any initial perturbations are quickly corrected by changes in lymphatic function. Our finding that lymph heart rate increased after activity supports the latter hypothesis, i.e. that lymph hearts and lymphatic vessels are capable of adjusting their activity according to the rate of transcapillary fluid filtration. In another study, Jones et al (36) also observed an increase in lymph heart rate of toads, following slight movement of the animal, and concluded that the lymph heart rate increased in response to the greater fluid volume within the lymph hearts. Muscular exertion, per se, has previously been reported to cause increases in lymph flow in mammals (18,37-40). However, in these previous experiments blood pressure was not measured and so there was no experimental evidence of increased transcapillary filtration. In addition no attempts were made to simultaneously monitor fluid distribution between blood and lymph.

More convincing evidence that lymphatic function is regulated according to rate of lymph formation, at least in mammals, comes from studies on lymph vessels *in vivo* (41-43) and *in vitro* (44,45) in which lymphatic pumping activity was found to increase with increasing transmural pressure across initial lymphatics and collecting ducts. In addition, Jones et al. (36) demonstrated in toads that during dehydration when blood pressure, and hence capillary filtration, are reduced, lymph heart rate is decreased. These findings suggest that lymph hearts, and some lymphatic vessels, are capable of adapting their activity to changes in interstitial fluid volume. At present we are not aware of any mechanism which could account for such regulation. Ohhashi et al. (46) have suggested that in mammals the lymphatic contraction rate is regulated by a pacemaker site in the lymph vessel wall, but so far such a site has not been located.

Although frog lymph hearts are capable of beating when completely removed from the body, it has long been known that the lymph hearts are innervated (47), and are therefore likely to respond to a wide variety of humoral factors. McHale and Thornbury (43) have demonstrated that a fright stimulus to sheep can alter pumping in this preparation, and that the administration of intravenous noradrenalin stimulates pumping, while isoprenaline depresses flow. It is possible that

during our experiments on frogs such a stimulus was operating so as to increase lymph heart rate.

In summary, we have demonstrated the importance of the lymphatic system versus transcapillary reabsorption in maintaining fluid balance in the frog, *Rana pipiens*, under normal conditions of hydration, and have provided an explanation for this phenomenon in terms of the intercompartmental pressure distributions and fluid fluxes within the system. Most striking is the speed with which fluid balance is restored following a change in capillary filtration rate, demonstrating a precise regulation of lymph heart contractile activity. Similar mechanisms may operate in other organs/animals which support high fluid turnover and which show a low colloid osmotic pressure difference between plasma and lymph. In addition we have demonstrated that in the frog, transcapillary albumin exchange averaged over the entire microcirculation is driven predominantly by convection rather than by diffusion. This finding is consistent with the high rates of fluid turnover between blood and lymph which exist in the frog.

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