# The Effect of Passive Motion on the Flow and Formation of Lymph

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*Summary*

The effect of different rates of passive movement on the flow and composition of paw lymph was studied in anesthetized dogs. Lymph flow was halved by decreasing the frequency of paw pumping from 100 cpm to 10 cpm. The lymph: plasma concentration ratio of total protein ($R_{TP}$) was not affected initially by the decrease in pumping rate. With continued pumping at 10 cpm, average lymph flow increased toward control (100 cpm) levels and $R_{TP}$ decreased. Resumption of pumping at 100 cpm, significantly increased lymph flow but did not change $R_{TP}$. Lymph flow then declined toward control flows as $R_{TP}$ rose toward the values observed at 100 cpm. Steady state lymph flows during initial 100 cpm pumping, during 10 cpm and during the resumption of 100 cpm pumping were not significantly different. When paw pumping was stopped, average lymph flow decreased quickly and remained at a very low value for at least 90 minutes. Resumption of paw pumping increased lymph flow above initial control values but did not affect $R_{TP}$.

We conclude that widely differing rates of passive paw movement give similar steady-state lymph flows from isolated paw lymphatics of anesthetized dogs. The interstitial space of the paw apparently acts as a volume buffer which stores lymph during transient periods of low lymph removal and empties during high rates of lymph removal.

There are many factors involved in the movement of lymphatic fluid centrally. Lower vertebrates possess lymph hearts through which by rhythmically contracting they are able to maintain a steady flow of lymph from lymph vessels and sacs to the blood stream. The "lymph hearts" have not been described in higher mammals. In man all lymphatics other than terminal capillary networks contain smooth muscle cells and nerves in their walls. Spontaneous lymphatic contractility has been demonstrated in anesthetized rats, mice and guinea pigs (1, 2); in pigs (3); in sheep and in man under pathologic conditions (4, 5). Muscular contraction (6, 7) is thought to contribute significantly to lymph propulsion. The passive compliance of a lymphatic is much larger than a vein or an artery (8, 9), implying greater sensitivity to small pressure changes; i.e. internal and external pressures could be influenced by surrounding tissues and thereby affect the flow of lymph. *McMaster* (6) demonstrated in man that heat, massage, and exercise increase the flow of lymph. Cyclic intrathoracic and intraperitoneal pressure changes also aid in propelling lymph centrally (3, 10). The pulsations of nearby blood vessels have been shown to have an augmentative role in the movement of lymph (6, 11). Gravity increases the formation of lymph while it initially decreases the returning flow (12).

This paper examines the effect of different rates of passive limb movement on the composition and flow of lymph from the paw lymphatics of anesthetized dogs.

## Methods

Mongrel dogs of either sex (weight range 10–20 kg) were anesthetized with sodium pentobarbital (30 mg/kg). Supplementary anesthesia and an infusion of mammalian ringer solution (0.42 ml/min) were administered through a cannula placed in an external jugular vein. The dogs were tracheotomized and had a cannula inserted into the common carotid artery for systemic blood pressure measurement. Each animal was placed on its sternum with the dorsal surfaces of both hind legs resting on a well padded metal rod. The rod was positioned to allow the dog's feet to pivot at the hock through an angle of approximately 60 degrees. Both paws were passively flexed by a system of nylon monofilament and pulleys which coupled a toenail of each paw to a cam driven by an electric motor. The flexion frequency of each paw could be controlled independently by varying the speed

of its motor. The flexion frequency varied ± 5 %. The total excursion of the paws did not vary significantly over the frequencies of passive movement which were used in this study (0–100 cycles per minute or cpm).

Prenodal lymphatics on either side of the lateral saphenous vein were exposed with a dermal flap and ligated just distal to the popliteal node. One lymphatic in each leg was cannulated with PE10 or PE50 siliconized tubing.

Lymph samples were collected into weighed vitals containing a dried heparin film. Lymph flow was estimated from the weight gain of the vial during 30 minute lymph collection periods, except during periods of no pumping when 60 minute collection periods were used. At the midpoint of each period, blood samples were drawn from the jugular cannula. The total protein concentration of plasma and lymph was estimated by the method of Lowry.

The right and left legs of each dog were usually subjected to different protocols, or to the same protocol displaced in time. In several instances, an experimental protocol was repeated several times in the same leg. Results from these repetitions were averaged to a single set of values before they were included in the final data.

*Protcol 1*

In four experiments, paw pumping begun at 100 cpm. After a minimum of 3 lymph collection periods, the pumping frequency was reduced to 10 cpm for at least 180 minutes. The paw pumping was then resumed at 100 cpm.

*Protocol 2*

After control lymph collection for a minimum of 3 collection periods (1 experiment at 100 cpm, 3 experiments at 10 cpm) passive paw movement was stopped completely. Paw pumping was resumed at the initial frequency at least three 30 minute collection periods later. These dogs were given systemic heparin to prevent lymph from clotting in the cannulas during period of low lymph flow. The hepari-

nization did not appreciably change the control flows or protein concentrations.

In the experiments following Protocol 1, the control flow (dotted line in Figure 1) and the control lymph, plasma protein concentration ratio ($R_{TP}$) was determined using a weighted average of pre and post experimental values. The actual flows and $R_{TP}$ were then compared to the control values using the paired -t test. Significant differences in data are marked by an asterisk. A similar comparison was made on data from protocol 2, however, pre-experimental steady state values were used as the control for this comparison.

*Results*

It was initially established that it takes 90 minutes from lymphatic vessel cannulation to achieve steady state lymph flows, it takes 60–90 minutes to achieve steady state flow after lowering pumping rate, and it takes 30–60 minutes (rarely longer) for the lymph flow to reach steady state levels upon returning to the control rates.

As shown in Fig. 1, lymph flow declined significantly when the paw pumping rate was decreased from 100 cpm to 10 cpm. The lymph plasma concentration ratio of total protein ($R_{TP}$) was unchanged initially. With continued pumping at 10 cpm, lymph flow rose toward the 100 cpm value and $R_{TP}$ fell significantly. An abrupt increase in paw pumping rate from 10 to 100 cpm increased lymph flow but did not change $R_{TP}$. Despite further pumping at 100 cpm, lymph flow then declined toward its previous level. The average $R_{TP}$ increased nonsignificantly (p .06) during this decline in flow. The flow "debt", the difference between control and actual flows, which occurred during 10 cpm pumping (approximated by the shaded area under the dotted line in Fig. 1) was less than the flow "payment", the difference between actual and control or steady state flows, (shaded area above the control flow line).

When paw pumping was stopped completely, as shown in Fig. 2, lymph flow declined to very low values (3 x $10^{-6}$ ml/sec) and did not increase for at least 90 minutes. Resumption

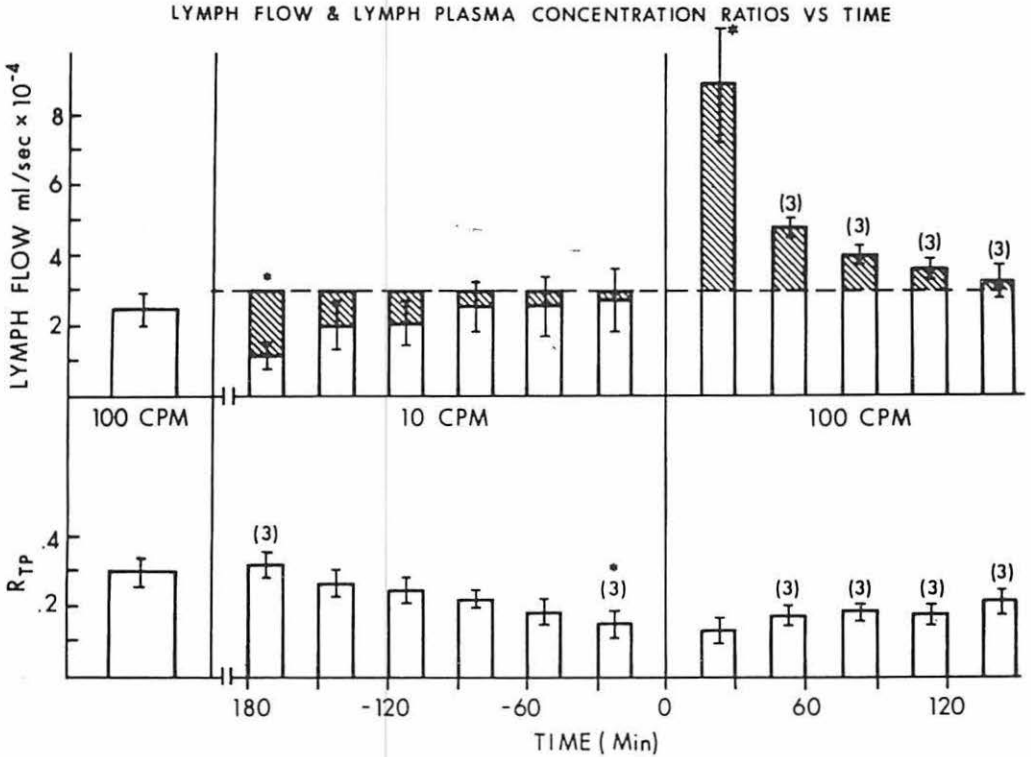LYMPH FLOW & LYMPH PLASMA CONCENTRATION RATIOS VS TIME



Fig. 1 Paw lymph flows (L) and lymph : plasma concentration ratios of total plasma proteins ($R_{TP}$) as functions of the frequency of passive paw pumping. The wide columns in the left panel represent the average of the last two 30 minute collection periods before the pumping rate was reduced from 100 cpm to 10 cpm. All data have been plotted from a common time (0 min.) of resumption of 100 cpm paw pumping. Asterisks above the bars denote data groups which were significantly different (paired -t P = .05). The dotted line at an L of 3 ml/sec X $10^{-4}$ marks the approximate steady state lymph flow determined using the weighted averages of pre and post experimental control flows. Shaded areas below and above this line mark the lymph flow "debt" and "repayment" which occurred when pumping was stopped and then resumed at its initial rate. The bars indicate plus or minus one standard deviation. Numbers in brackets above the columns indicate the number of experimental observations if different from the stated number of experiments.

of paw pumping at the previous rate significantly increased lymph flow above control levels for at least 90 minutes. In this case, approximately one-half of the lymph flow "debt" had been repaid within 90 minutes of the time that pumping had been resumed.

*Discussion*

Steady state lymph flow from the dog paw remains remarkably constant when the paw pumping rate is varied between 10–100 cpm. Although lymph flows were similar, pumping frequencies of 100 cpm were associated with

higher $R_{TP}$'s. Assuming an unchanged permeability surface area product and equal protein transport across the capillaries in each case, the higher lymph plasma protein concentrations observed during high rates of paw pumping must be due either to diminished lymph water or to mobilized extravascular protein. Conversely, the low pumping rates must increase the proportion of water in the lymph or permit sequestration of transported protein by extravascular stores. The precise mechanisms responsible for these changes in lymph composition are not known. Our results are quite different from those reported by
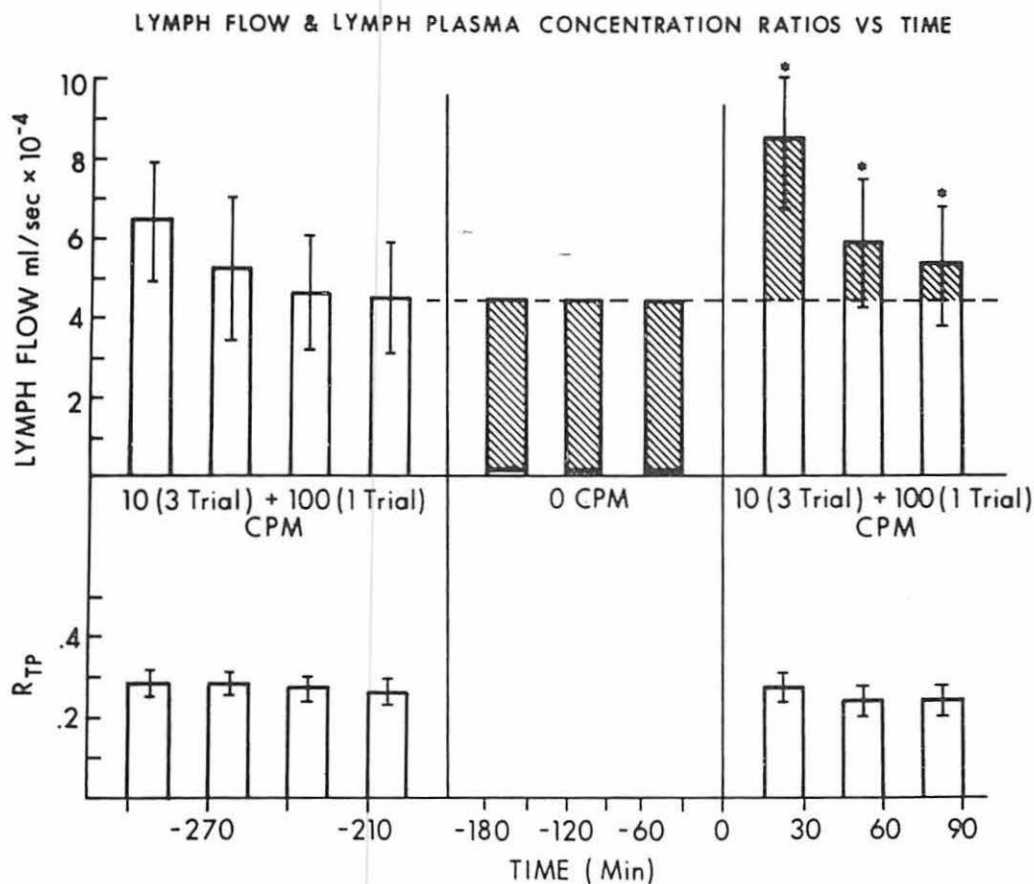
## LYMPH FLOW & LYMPH PLASMA CONCENTRATION RATIOS VS TIME



Fig. 2  Changes in lymph flow (L) and lymph : plasma concentration ratio of total plasma protein ($R_{TP}$) produced by total cessation of paw pumping. The control pumping rate (left panel) was 10 cpm in three experiments and 100 cpm in one experiment. Lymph flow after resumption of pumping (right panel) remained significantly above control flow (final bar in left panel) for at least 90 minutes. The dotted line marks the approximate steady state lymph flow. Shaded areas below and above this line mark the lymph flow "debt" and "repayment" which occurred when pumping was stopped and then resumed at its initial rate. Asterisks above the bars denote data groups which were significantly different (paired -t; P = .05). The bars indicate plus or minus one standard deviation. Control flows for these experiments are based on pre-experimental steady state lymph flows.

White et al. (13). In their experiments, leg lymph flow increased and lymph protein concentration fell or was unchanged when dogs walked or ran. Lymph flow practically stopped and its protein concentration increased if the dogs remained motionless. Our results show a similar decrease in lymph flow but no change in $R_{TP}$ when pumping was stopped and then started. However, our results also show a much greater post control lymph flow than White's data. These differences can be attributed to: 1) Different physiologic states. White's experiments were done using awake dogs initially anesthetized with ether followed by local anesthetic supplementation. Our experiments were done using Pentabarbital anesthesia with I.V. supplementation; and 2) Differences in sampling. Our experiment collected all the lymph from the paw region while White sampled a fraction of the draining lymph.

During periods of a zero pumping rate, low values of lymph flow were measured. These could have been due to spontaneous lymph flow or to superimposed respiratory movement which was occasionally observed to cause minimal movement of the leg.

We conclude that widely differing rates of passive paw movement give similar steady-state lymph flows from isolated paw lymphatics of anesthetized dogs. The interstitial space of the paw apparently acts as a volume buffer which stores lymph during transient periods of low lymph removal (flow "debt" incurred during periods of decreased passive movement) and empties the stored lymph during periods of high removal rate (flow "repayment").

## References

1 *Smith, R.O.:* Lymphatic Contractility. J. Ex. Med. 90 (1949) 497–509

2 *Godart, S.:* Studies of the physiology of lymphatic vessel by microcirculation methods. Lymphology 1, 3 (1968) 80–87

3 *Calnan, J.S., J.J. Pflug, N.D. Reis, L.M. Taylor:* Lymphatic pressures and the flow of lymph. Brit. J. Plastic Surg. 23 (1970) 305–317

4 *Kinmouth, J.B., G.W. Taylor:* Spontaneous rhythmic contractility in human lymphatics. J. Physiol. 133 (1956) 3

5 *Szegvari, M., A. Lakos, F. Szontagh, M. Földi:* The active function of the subcutaneous lymphatic vessels of the human lower extremity. Acta Med. Acad. Sci. Hung. 20 (1964) 209–213

6 *McMaster, P.D.:* Conditions in the skin influencing interstitial fluid movement, lymph formation and lymph flow. Ann. N.Y. Acad. Sci. 46, 8–10 (1946–47) 743–787

7 *Hyman, C.:* Physiologic functions of the lymphatic system. Cancer Chemotherapy Reports 52, 1 (1968) 25–30

8 *Nisimaru, Y.:* Lymphatics and lymph flow. Hiroshima J. Med. Sci. 17, 2 (1968) 53–76

9 *Nisimaru, Y.:* Structure and function of the lymph capillaries. Hiroshima J. Med. Sci. 17, 2 (1968) 77–91

10 *Irisawa, A., R.F. Rushmer:* Relationship between lymphatic and venous pressure in leg of a dog. Amer. J. Physiol. 196 (1959) 495–498

11 *Parsons, R.J., P.D. McMaster:* The effect of the pulse upon the formation and flow of lymph. J. Exp. Med. 68 (1938) 353–376

12 *Entrup, D.* et al.: Effect of posture on formation and evacuation of lymph. Am. J. Physiol. 210, 5 (1966) 943–949

13 *White, J.C., M.E. Field, C.K. Drinker:* On the protein content and normal flow of lymph from the foot of the dog. Amer. J. Physiol. 103 (1933) 34–44

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