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Lymphatic Motility

I.C. Roddie, H.J.D. Mawhinney, N.G. McHale, C.T. Kirkpatrick and K. Thornbury

Department of Physiology, The Queen's University of Belfast

The lymphatic system poses two main physiological questions. First of all, how is lymph formed? We have heard from Dr. *Guyton* that though there are many theories, the exact mechanism of lymph production is not well understood. The second main question is how lymph, once formed, is transported back to the circulation? Though this is a relatively more easy question, it has given rise to much controversy.

Transport Mechanisms

There are three main theories put forward to explain the transport of lymph back to the circulation. Their common denominator is that they generate a sufficient pressure head within the lymphatic system to overcome the resistance offered by the lymphatic vessels.

The first mechanism may be described as the *tissue pump*. The idea here is that the force which drives lymph into the lymphatic capillaries builds up a sufficient vis a tergo in the peripheral lymphatic bed to return the lymph to the blood. As mentioned earlier, the mechanism by which lymph is driven into the lymphatic capillaries is still a matter of controversy.

The second mechanism proposed is that *extrinsic forces* are responsible and that the lymphatis behave as passive tubes. When compressed by adjacent muscles or peristaltic movements of smooth muscle, the pressure of lymph within the lymphatic vessels rises to provide the pressure head to drive lymph back to the blood according to the orientation of the valves. The negative intrathoracic pressure which occurs with each inspiration would also act as an extrinsic force driving lymph centripetally. This general mechanism of lymph propulsion has been strongly supported by *Mayerson* (1963).

A third mechanism porposed is that lymph is driven centripetally by *intrinsic pumping*, i.e., by active contractions of elements within the lymphatic wall. In primitive animals, lymph hearts exist which pump lymph back into the circulation and if these are damaged, the animal dies from hypovolaemic circulatory failure. Active contractions have been observed in many varieties of lymph vessel in many species for many years. They have been well described by *Florey* in 1927, *Smith* in 1949 and at this Congress, Dr. *Baez* has shown us very impressive cinephotographs of lymphatic contraction in guinea-pig mesentery. A recent review of the subject has been made by *Nichol & Taylor* (1977).

Study of Intrinsic Transport

To study *intrinsic pumping* in lymph vessels there are many advantages in using isolated preparations since the results are not complicated by the effect of the tissue pumping and of extrinsic pumping. The difficulty is that lymphatics are very fragile and their life in vitro tends to be short and downhill. However, great credit must be given to *Mislin* (1971) who was first able to study the contractile behaviour of an isolated guinea-pig mesenteric lymphatic in vitro. He showed that single valve segments were capable of pumping lymph and produced evidence that the action of the smooth muscle was integrated by local nerve nets.

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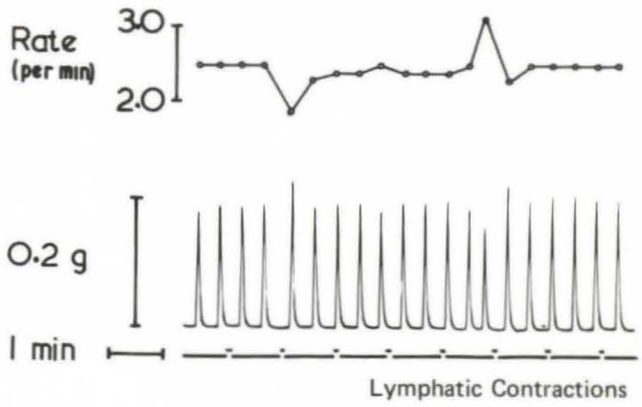

Fig. 1 The effect of spontaneous changes in rate on the strenght of contraction of a bovine lymphatic. A decrease in the rate of contraction was associated with an increase in amplitude and vice versa. (From *Mawhinney and Roddie*, 1973, J. Physiol. 229, 359–348)

Working on the principle that bigger preparations would be easier to keep in good condition than small preparations, we in Belfast worked on bovine mesenteric lymphatic (*Mawhinney and Roddie*, 1973, *McHale and Roddie*, 1976; *McHale, Kirkpatrick and Roddie*, 1979; *McHale, Roddie and Thornbury*, 1979). Pieces of bovine mesentery can be obtained within 20–30 minutes after cattle have been slaughtered at the abattoir and lymphatic vessels can be seen running within the mesentery towards the mesenteric lymph glands. These vessels which run separately from the veins and arteries can measure up to 5 mm in diameter. On histological examination it can be seen that they have a well defined coat of smooth muscle which is arranged in a somewhat haphazard fashion and there are no clear cut divisions into bands of longitudinal and circular muscle. At about 1 cm lengths, prominent valves occur whose competence is such that they prevent retrograde flow of fluid when fluid is injected into the distal end of the lymphatic. When stained with the Falck technique spots of fluorescence can be seen throughout the muscle suggesting that the muscle has a scanty adrenergic nerve supply.

Some Responses of Longitudinal Strips of Lymphatic Vessels

When a 4–5 cm strip is set up in an organ bath to measure longitudinal tension, the strip beats regularly and spontaneously at about 2–3 beats per minute (Fig. 1). There is a sharp contraction followed by a brisk relaxation followed in turn by a relatively long diastolic pause. Increasing the rate of beating tends to reduce the strength of subsequent contractions and vice versa. Epinephrine increases the rate of contraction in a dose dependent fashion but reduces the strength of the individual contractions (Fig. 2). With high doses of epinephrine, e.g., 25. ng/ml^{-1}, the rate can rise to about 10 beats per minute but the individual contractions are so small that they appear as ripples on the baseline. It is as if the lymphatic muscle was fibrillating. Isoproterenol has the opposite effect to epinephrine, i.e., it reduces the rate of contractions but causes some increase in the strength of the individual contractions. In large doses, i.e., 5 ng/ml, it causes complete suppression of spontaneous activity.

Acetylcholine, even when given in high doses, i.e., 100 ng/ml^{-1}, has practically no effect on spontaneous lymphatic contractions. On the other hand, lymphatic muscle is very sensitive to strech. With increasing stretch the lymphatic contracts much more vigorously (Fig. 3) and this response would have important implications for the transport of lymph by intrinsic activity.

The preparation can also be stimulated by field stimulation. When the preparation is stimulated with 300 μsec pulses at 35 v for 1 minute at different frequencies the rate of contraction is increased (Fig. 4). This effect is abolished by tetrodotoxin indicating that it is mediated through nerves. The following results suggest that the nerves involved were adrenergic.

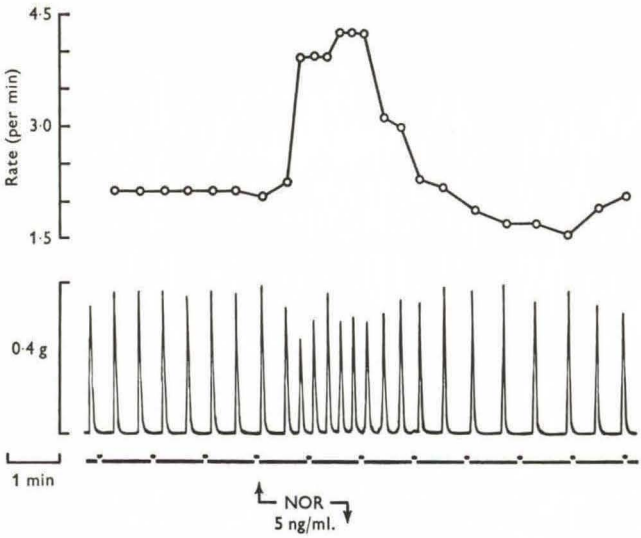

Fig. 2 The effect of noradrenaline (5 ng/ml) on the rate and strength of contraction of a bovine lymphatic. When norepinephrine (NOR) was added to the bath there was an increase in rate associated with a slight decrease in the amplitude of the contractions. (From *Mawhinney and Roddie*, J. Physiol. 229 (1973) 339–348

Phenoxybenzamine, which blocks adrenergic α-receptors, converts the excitatory effect of field stimulation into an inhibitory one (Fig. 4). Cocaine, which blocks the uptake of epinephrine into nerve terminals, potentiates the increase in contraction rate with field stimulation. Atropine has little effect on the response to field stimulation suggesting that cholinergic nerves do not play an important role in the regulation of mesenteric lymphatics.

Electrical Correlates of Lymphatic Contractions

When segments of lymphatics are set up in a sucrose gap apparatus so that membrane potential can be estimated and the tension of spontaneous contractions measured, the following results are obtained (Fig. 5). Each contraction is immediately preceded by a spike action potential.

The action potential, which has a duration of about 1 sec, is preceded by a pre-potential and is followed by a period of hyperpolarization. When epinephrine, 100 ng/ml^{-1}, is added to the preparation, the slope of the pre-potential increases so that the rate of contraction increases. There is a slight depolarization and an increase in the duration of the action potentials to about 2–3 sec. The strength of the individual contractions decreases. When the norepinephrine is washed away, the records return to their control values.

Propulsion of Fluid by Lymphatics

To see if lymphatic segments can actually propel fluid, a length of lymphatic containing about 6 valved segments was set up as indicated in Fig. 6. The proximal end of the lymphatic was connected to a pressure reservoir via a heat exchanger and the distal end was connected to a drop counter. The heights of the inflow

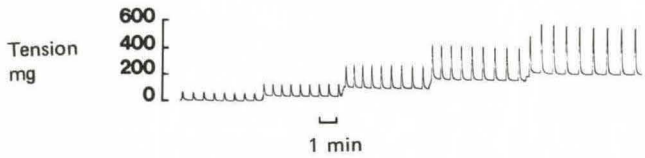

Fig. 3 The effect of increasing longitudinal tension on the strength of spontaneous contractions in a bovine mesenteric lymphatic

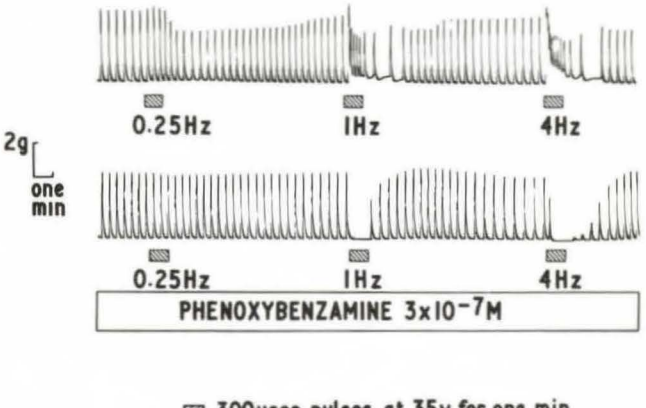

Fig. 4 Effect of electrical field stimulation on a spontaneously active lymphatic before (upper trace) and after phenoxybenzamine (lower trace). (From *McHale, Roddie and Thornbury*, J. Physiol., 1979, in press)

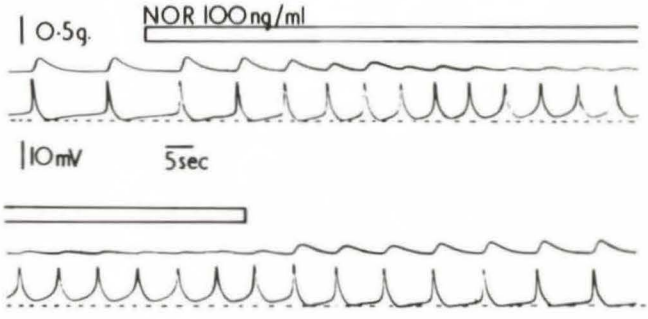

Fig. 5 The effect of noradrenaline on electrical and mechanical activity of a lymphatic segment. (From *McHale, Kirkpatrick and Roddie*, 1979, (Proc. IIIrd Symposium on Vascular Neuroeffector Mechanisms – in press))

reservoir and the outflow drop counter were adjusted so that, when the vessel was not contracting, the inflow and outflow pressures were equal and there was no flow through the vessel since there was no perfusion pressure. However, the levels of the drop counter and pressure reservoir could be adjusted together so that the transmural pressure in the lymphatic could be varied. Measurements were made of inflow and outflow pressures using transducers and of flow through the lymphatic using the drop counter. Cine-pictures were taken of the lymphatic at different stages of the contraction cycle.

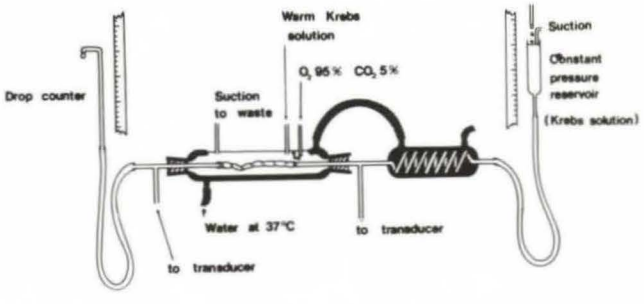

Fig. 6 The apparatus used for perfusing isolated lymphatic vessels. Transmural pressure was varied by raising or lowering both the constant pressure reservoir and the drop counter outflow. (From *McHale and Roddie*, J. Physiol. 261 (1976) 255–269)

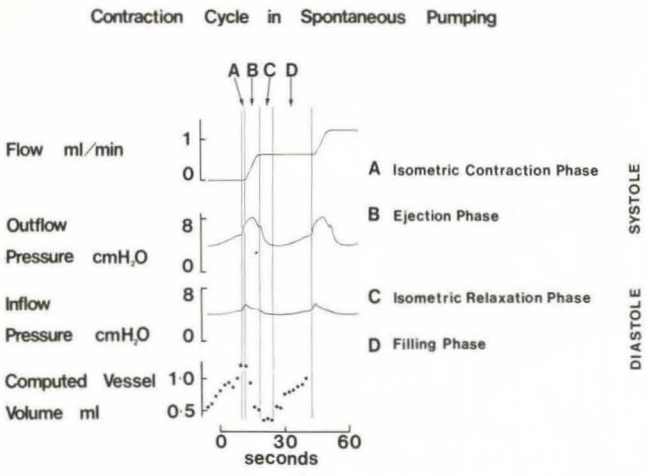

Fig. 7 Changes in flow rate, outflow pressure, inflow pressure and computed lymphatic volume during cycles of spontaneous lymphatic activity. A, Isometric contraction phase; B, Ejection phase; C, Isometric relaxation phase; D, Filling phase. The bath temperature was 30 °C. (From *McHale and Roddie,* J. Physiol. 261 (1977) 255–259)

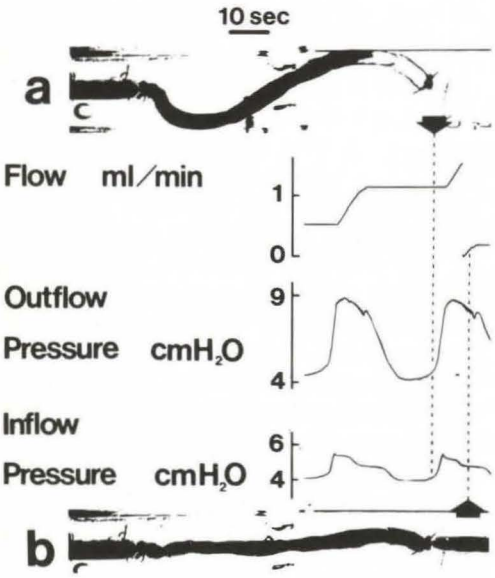

Fig. 8 Changes in the dimensions of a lymphatic vessel during a cycle of contractile activity. Photograph (A) was taken at the end of the filling phase (first arrow) and photograph (B) was taken near the end of the ejection phase (second arrow). Contraction resulted in a decrease in both the length and diameter of the vessel so that the dye-stained perfusion fluid was driven to the right into the outflow cannula. The bath temperature was 30 °C. (From *McHale and Roddie,* J. Physiol. 261 (1976) 255–269)

The stages of contraction cycle are shown in Fig. 7. During diastole, the computed volume contained in the vessel increased gradually, and the inflow and outflow pressures rose gently. At the beginning of systole there was a small increase in inflow pressure probably due to back pressure on the proximal valve. The outflow pressure rose sharply and when it reached a pressure sufficient to overcome the resistance of the outflow tubing, a series of drops were extruded from the drop counter, rapidly at first and then more slowly. During this phase the computed volume of the vessel fell rapidly. When the outflow pressure fell to a value where it was no longer able to overcome the outflow resistance, flow stopped and the record showed a pressure fluctuation reminiscent of the 'dicrotic notch' seen in arterial pressure records. Fig. 8 shows photographs of the segment taken at the end of diastole (A) and towards the end of systole (B). When the lymphatic contracted it shortened in both length and diameter. Inspection of the contraction indicated that it did not consist of one valve segment contracting, expelling its contents into the next which would then contract in turn. Nor was it due to a slow wave of peristalsis driving a bolus of lymph along the vessel. Rather it consisted of a near-synchronous contraction of all the valve segments in the preparation so that the

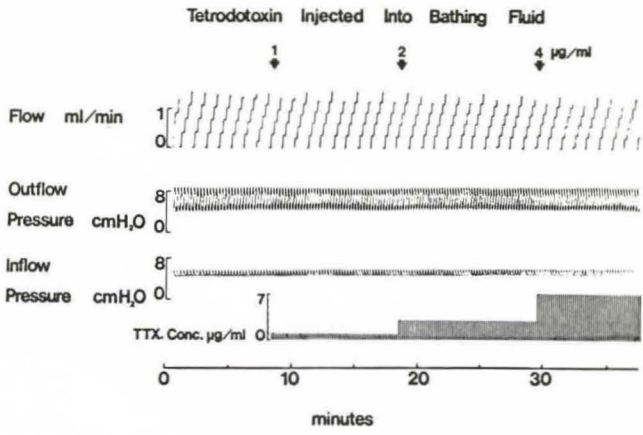

Fig. 9 The effect of increasing doses of tetrodotoxin on the pumping ability of a length of bovine mesenteric lymphatic

total volume of the preparation was decreased. For this to occur the lymphatic muscle must contain a good conducting system so that excitation can be spread rapidly to all the smooth muscle cells in the preparation. The spread is probably myogenic since the contraction of the vessel was not affected by tetrodotoxin (Fig. 9). In electron micrographs, tight junctions have been seen between adjacent lymphatic muscle. cells.

When the lymphatic was exposed to increasing transmural pressure (Fig. 10), the preparation increased its pumping output up to a certain value after which it began to fall off again. The increased ability to propel fluid was due to both an increase in the strength and rate of the contractions. The fall-off in output at high transmural pressure was due to a decrease in the strength of the individual contractions since the frequency of contractions continued to rise.

The increase in the ability of lymphatics to propel fluid when their transmural pressure is raised could explain, at least in part, lymph transport in vivo. An increase in lymph production in vivo would provide the adequate stimulus, i.e., a rise in transmural pressure, to increase both the rate and force of lymphatic contraction and hence the transport of lymph back to the blood. The transport function of the lymphatics could be modified by the effect of adrenergic nerves which exist within their walls.

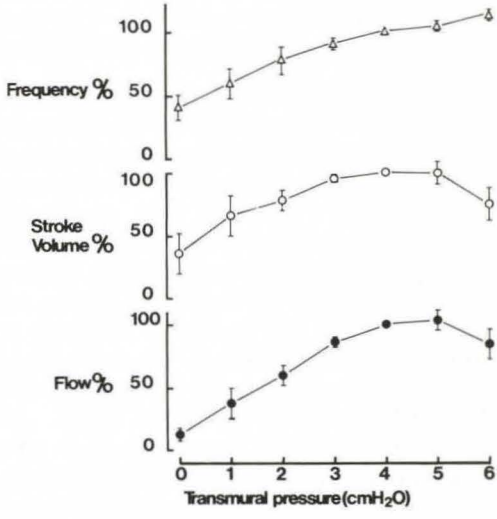

Fig. 10 Mean results of six experiments where transmural pressure was increased from 0 to 6 cm H_2O in 1 cm steps. Flow (●), stroke volume (○) and frequency (△) are plotted as percentages of their values at a transmural pressure of 4 cm H_2O. Vertical lines represent plus or minus 1 S.E. of the means. The bath temperature was 37 °C. (From *McHale and Roddie*, J. Physiol. 271 (1977) 255–269)

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Dr. I.C. Roddie, Department of Physiology, The Queen's University, Belfast