J. Physiol. (1976), **261**, *pp.* 255–269
With 10 *text-figures*
Printed in Great Britain

THE EFFECT OF TRANSMURAL PRESSURE ON PUMPING ACTIVITY IN ISOLATED BOVINE LYMPHATIC VESSELS

BY N. G. MCHALE* AND I. C. RODDIE
From the Department of Physiology, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL

(*Received 2 November 1975*)

SUMMARY

1. Isolated preparations of bovine mesenteric lymphatics containing about seven valved segments were cannulated and set up in a perfusion system so that, when the preparation was not contracting, the inflow and outflow pressures were exactly equal and there was no flow through the preparation.

2. Transmural pressure was varied by raising or lowering the inflow and outflow pressures simultaneously by the same amount.

3. The isolated vessels showed rhythmic spontaneous activity; it consisted of quick contractions which spread rapidly over the entire preparation, each followed by a rapid relaxation and a diastolic pause.

4. With each contraction, the preparation decreased in both length and diameter and generated an outflow pressure which pumped fluid in the direction determined by the orientation of the values.

5. Raising the transmural pressure in the preparation increased the output of the preparation; this was achieved by an increase in both the frequency and force of the individual contractions.

6. It was concluded that bovine lymphatics could propel fluid by their intrinsic activity at a rate which was related to the degree of distension of their walls.

INTRODUCTION

Work on isolated mesenteric lymphatic vessels from the cow has shown that they are capable of regular spontaneous contractions (Mawhinney & Roddie, 1973). Contractions occurred regularly and briskly and each contraction was followed by a diastolic pause. The form of the contractions and the presence of valves suggested that the vessels should be able to propel lymph by their intrinisic activity in the manner of a number of

* M.R.C. Student.

primitive hearts in series. The present experiments were designed to see if this were the case. A short account of this work has already been published (McHale & Roddie, 1975*a*).

METHODS

Lymphatic vessels can be seen as small opalescent tubes running on the surface of bovine mesentery immediately under the peritoneum. They usually travel separately from the mesenteric veins and arteries. Fresh mesentery was obtained as soon as possible (usually 30–40 min) after the cattle had been slaughtered. A 20 × 5 cm segment which included a suitable lymphatic was excised and immersed in Krebs solution at 37° C and the subsequent dissection was also carried out under Krebs solution at this temperature; this was done to prevent the fat from solidifying and making gentle dissection impossible.

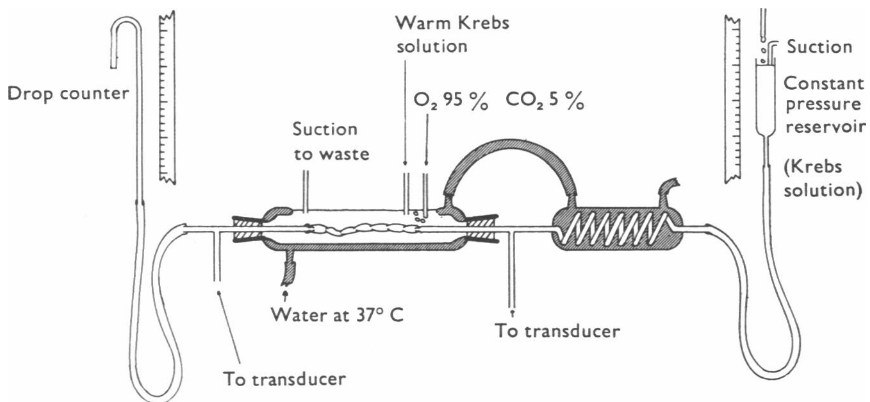

Fig. 1. The apparatus used for perfusing isolated lymphatic vessels. Transmural pressure was varied by raising or lowering both the constant pressure reservoir and the drop counter outflow.

A length of lymph duct, about 8 cm in length and about 1–3 mm in diameter, was dissected free and cannulated at both ends with glass cannulae. The vessel, which contained about seven valued segments, was then arranged in a horizontal open-topped organ bath (Fig. 1) containing a modified Krebs solution (mM): NaCl, 120; $NaHCO_3$, 15·0; KCl, 5·9; NaH_2PO_4, 1·2; $CaCl_2$, 2·5; $MgCl_2$, 1·2; glucose, 11·0;) gassed with a 95 % O_2, 5 % CO_2 mixture and maintained at a constant temperature in the range 30–37° C. The inflow cannula was connected via a heat exchanger to a pressure reservoir whose height could be varied. The outflow cannula was connected to a drop counter whose height could also be varied. Pressure was measured at both proximal and distal ends of the preparation using Statham transducers (model P23H) and recorded on a Devices recorder (model M4). The surface of the liquid in the bath was taken as the zero reference level since the lymphatic segment lay less than 0·5 cm below the surface. Flow was measured by means of a drop counter and recorded simultaneously. Flow was intermittent, each contraction resulting in the expulsion of a number of drops. The output of drops was integrated by the drop counter and the counter was reset at regular time intervals. The heights of the inflow reservoir and the outflow drop counter outflow were adjusted so that, when the vessel was not

contracting, the inflow and outflow pressures were exactly equal and there was no flow through the vessel. When the vessel was first placed in the organ bath the transmural pressure was set at 4 cm H_2O and the preparation was allowed to equilibrate for half an hour; spontaneous activity had usually developed within this time.

A Shackmann Mk. 3 Auto Camera was mounted above the organ bath to take serial photographs of the active lymphatic at rates up to 4 frames per second on Ilford FP_4 35 mm film. A marker on the Devices M4 recorder was activated when the camera was switched on, and again when it was switched off which permitted the film frames to be correlated with the recordings of pressure and flow. The resulting photographs were used to analyse pumping activity in two ways. Firstly, it was possible to follow the movement of fluid through the vessel and make a detailed visual inspection of the pumping action when Evans Blue dye was injected into the inflow tube as a marker substance. Secondly, the negatives were projected and the area of the lymphatic outline was measured using a planimeter. This together with the length (measured using a piece of thread) was used to make a rough estimate of vessel volume at any given time in the contraction cycle. For this it was assumed that the vessel was cylindrical.

In some experiments, a second inflow pressure reservoir was arranged which could be raised and lowered rhythmically by a piston attached to the shaft of a modified Watson Marlow flow inducer (model M.H.R.E. 7). The amplitude of the pressure variations was kept at 2·5 cm H_2O while the frequency was varied (using the speed control of the pump) from 1·8 to 7·2 cycles min^{-1}. The outflows from both the steady and the oscillating pressure reservoirs were connected to a two-way tap system to permit a rapid change from a steady to an oscillating input pressure.

In some experiments segments of lymphatic 2–3 mm in diameter and 2–3 cm in length were mounted in a water-jacketed organ bath and attached to a modified Statham mechanotransducer (model UC3) which detected changes in longitudinal tension. The output from the transducer was amplified and recorded on a Devices M4 pen recorder. The organ bath was perfused as before with a modified Krebs solution and maintained at a temperature of 34° C. After the lymphatic was set up, the resting tension was adjusted to 100 mg and the preparation was allowed to equilibrate for an hour. If at the end of this time regular spontaneous activity had developed the tension was reduced to zero and then increased by steps of 50–200 mg allowing the tension to remain at each level for 4 min.

RESULTS

(a) *Spontaneous activity*

Though most vessels set up in the horizontal organ bath showed spontaneous activity, not all showed the regular well co-ordinated contractions necessary for efficient pumping; some preparations began by contracting regularly, but changed to irregular multipeaked contractions which decreased in amplitude within 5 or 10 min. With satisfactory preparations, the amplitude and frequency of the contraction were reasonably constant and the rate of flow through the preparation remained fairly steady under the equilibration conditions. It is likely that the irregularity of the contractions seen in some preparations was due to damage inflicted upon them during dissection. In the first few months of the experiments about 90 % of

the preparations were unsatisfactory in that their contractions were irregular and short lived. After 3 years of practice in setting up lymphatics, more than 90 % of preparations were satisfactory in that they contracted regularly and for periods of a few hours. Fig. 2 is part of a record obtained from such a preparation. When the vessel became active at the equilibration transmural pressure of 4 cm H_2O, contraction of the lymphatic resulted in a small increase in inflow pressure, probably as a result of back pressure on the first valve. The outflow presure showed a rapid rise followed by a more gradual fall which was interrupted in the middle by a notch. Flow was intermittent; when the rise in outflow pressure overcame the resistance in the outflow tube a series of six drops was expelled. This was followed by a

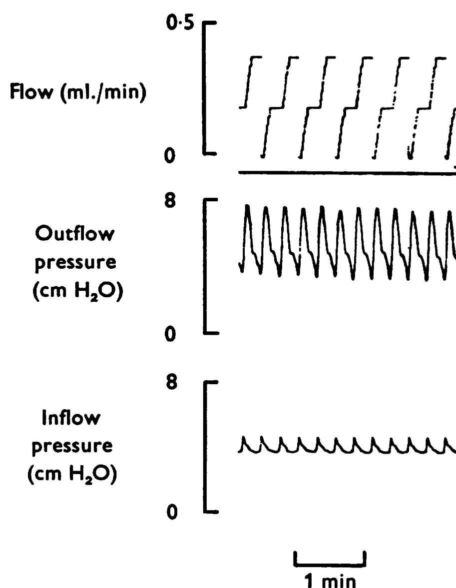

Fig. 2. The changes in inflow pressure, outflow pressure and flow rate during spontaneous activity in an isolated lymphatic segment at 30° C with a resting transmural pressure of 4 cm H_2O. Flow was intermittent, a series of drops being expelled with each contraction. The flow record shows the cumulative drop count integrated over 30 sec periods.

pause until the next contraction produced a further six drops. With each contraction, all the segments in the preparation contracted near simultaneously and the lymphatic decreased in both diameter and length with a writhing movement. Visual inspection did not suggest that a peristalic wave passed along the lymphatic or that each valved segment contracted in turn to expel its contents into the adjacent distal segment.

Fig. 3 shows a record made using a similar preparation at a faster

recording speed. The lymphatic cycle had many features in common with the 'cardiac cycle'. The cycle could be divided into a systole lasting about 8 sec and a diastole lasting about 24 sec. 'Systole' commenced with an *isometric contraction phase* in which the outflow pressure rose rapidly but the volume of the segment remained nearly constant. The inflow pressure

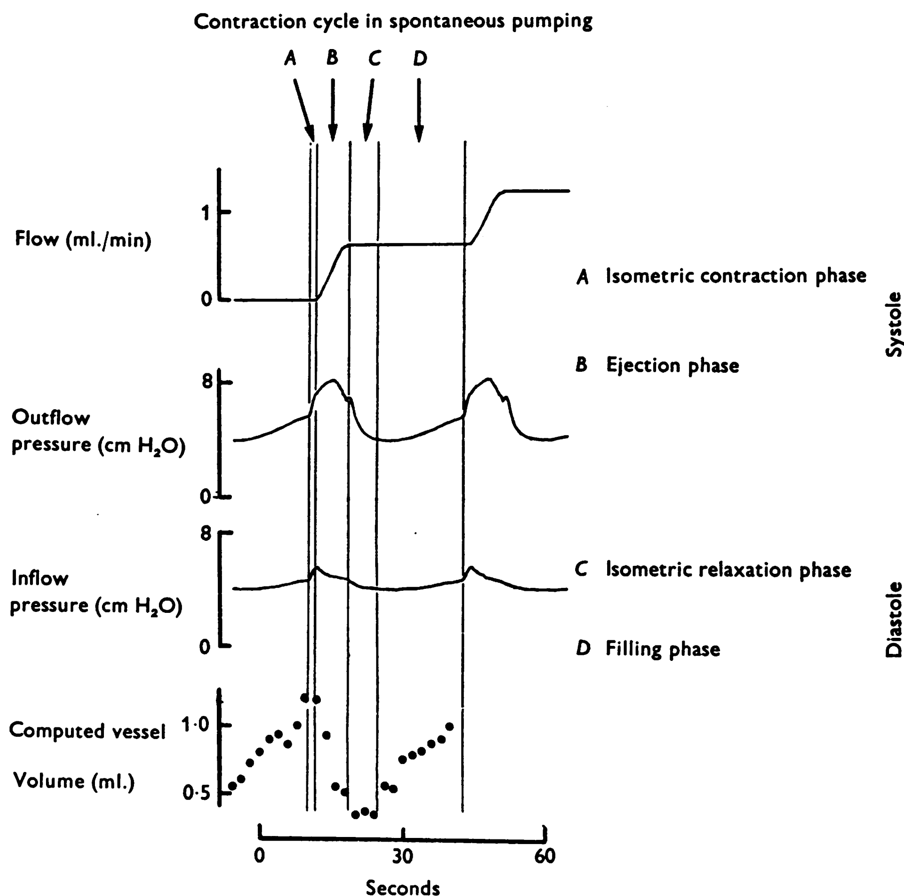

Fig. 3. Changes in flow rate, outflow pressure, inflow pressure and computed lymphatic volume during cycles of spontaneous lymphatic activity. *A*, Isometric contraction phase; *B*, Ejection phase; *C*, Isometric relaxation phase; *D*, Filling phase. The bath temperature was 30° C.

also rose sharply during this phase, presumably because the rise in intra-lymphatic pressure caused the valves at the proximal end of the segment to close and press back against the inflow. During the second phase of systole, the *ejection phase*, the outflow pressure reached a level where it

overcame the resistance of the outflow tube and fluid was pumped out as drops, rapidly at first and then more slowly. The volume of the segment as computed from serial photographs fell rapidly during this phase.

Diastole began when the outflow pressure fell below that needed to overcome the outflow resistance. At that time there was a pressure fluctuation reminiscent of the 'dicrotic notch' in arterial pressure records.

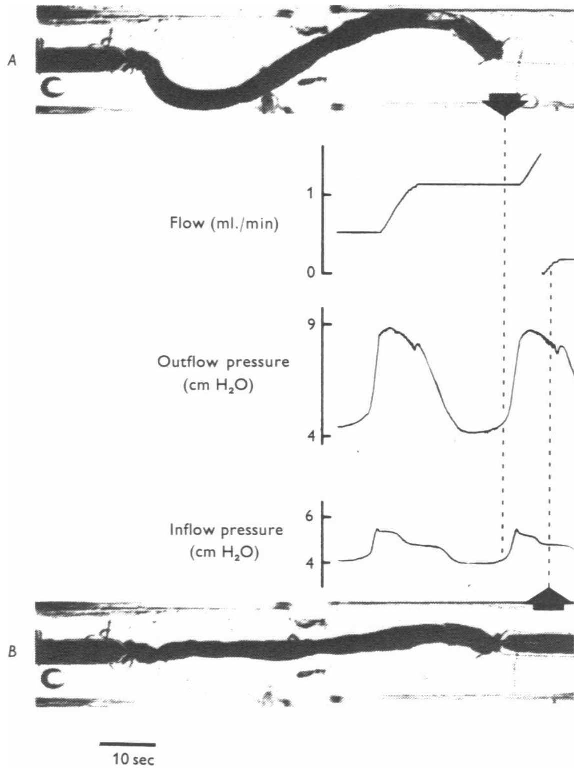

Fig. 4. Changes in the dimensions of a lymphatic vessel during a cycle of contractile activity. Photograph (*A*) was taken at the end of the filling phase (first arrow) and photograph (*B*) was taken near the end of the ejection phase (second arrow). Contraction resulted in a decrease in both the length 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.

This fluctuation was presumably due to vibrations set up by the closure of the valves at the distal end of the segment when the intra-lymphatic pressure fell below the pressure in the outflow tube. There then followed an *isometric relaxation phase* when the outflow pressure fell rapidly but the volume of the lymphatic segment did not change appreciably. The main

part of diastole was taken up with a *filling phase* as the walls of the lymphatic gradually relaxed to accommodate more fluid. During this time the inflow and outflow pressures rose slowly and gradually until the commencement of the next systole.

Fig. 4 shows a similar record together with two photographs of the lymphatic taken at different stages in the cycle. Photograph *A* was taken at the time indicated by the first arrow at the end of the filling phase. The fluid perfusing the preparation from left to right contained Evans blue dye which at this stage had just reached the outflow cannula. At the end of the filling phase the vessel was relaxed and, with its increased length, took a tortuous course between the inflow and outflow cannulae. Photograph *B* was taken at the time indicated by the second arrow, near the end of the ejection phase. The contraction of the vessel drove dye-stained fluid into the outflow cannula. During contraction the length of the preparation decreased by about 13 %; the vessel took up an almost straight course between the inflow and outflow cannulae. The mean diameter also decreased by about 20 %. Vessel volume estimated from area and length measurements was 0·93 cm^3 at the end of the filling phase *A* and 0·48 cm^3 at the end of the ejection phase. The computed volume which was ejected was thus 0·45 cm^3 which correlated quite well with the ejected volume measured by the drop counter (0·51 ml.). A similar correlation was obtained when the exercise was repeated for two other contractions. This suggested that the assumptions made in estimating lymphatic volume from area and length measurements were reasonably valid.

Fig. 5 is a sequence of line drawings made from photographs taken at different stages in the contraction cycle. The first frame, taken in the latter part of the filling phase, shows the dye front in the inflow cannula about to enter the proximal end of the lymphatic. In frame 2 taken seven seconds later, dye had moved about 1·5 cm into the vessel. This marked the end of the filling phase since between frames 2 and 3 there was a rapid increase in both inflow and outflow pressure. Three seconds later (frame 4) the vessel began to shorten its length and narrow its diameter. This contraction expelled fluid through the drop counter and also pushed the dye front in the proximal valued segment back into the inflow cannula (frame 5). This fluid proximal to the first valve was forced back into the inflow cannula while fluid distal to the first valve was forced towards and into the outflow tube. Between frames 6 and 7 there was a fairly rapid fall in outflow pressure with little increase in volume of the vessel. In frames 8 and 9 the dye front advanced rapidly into the relaxing vessel which increased both in length and diameter. The dye front was subsequently ejected through the outflow cannula (frame 10) by the following contraction.

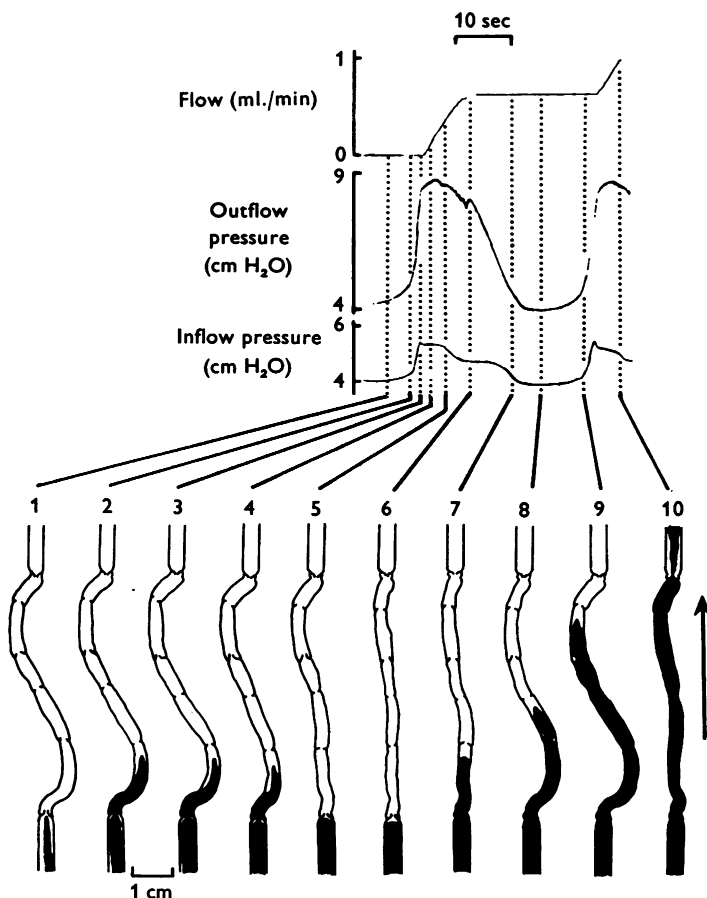

Fig. 5. Sequence of line diagrams made from photographs which were taken at different stages of the lymphatic contraction cycle. Direction of flow is indicated by the arrow. The bath temperature was 30° C.

(*b*) *Changing transmural pressure*

Fig. 6 shows the result of an experiment in which the transmural pressure of the lymphatic vessel was raised from 1 to 2 cm H_2O in 0·5 cm steps. Flow was 0·38 ml./min at a frequency of 8 beats/min at 1 cm H_2O. When the transmural pressure was increased to 1·5 cm H_2O, flow increased to 0·6 ml./min while frequency rose to 9 beats/min. Further increase of transmural pressure to 2 cm H_2O resulted in a flow of 0·8 ml./min at a frequency of the increase in flow; the concurrent increase in stroke volume (i.e. the number of drops expelled per contraction) was partly responsible. Stoke volume rose from three drops per contraction at 1 cm H_2O to about six drops per contraction at 2 cm H_2O.

Fig. 7 shows the mean result of four experiments at 37° C where transmural pressure was increased from 0 to 6 cm H_2O. Flow increased gradually with increasing transmural pressure reaching a maximum between 4 and 5 cm H_2O. This was due to an increase in both stroke volume and frequency. At 6 cm H_2O, however, stroke volume declined and resulted in a fall in output despite the continued increase in frequency of contraction.

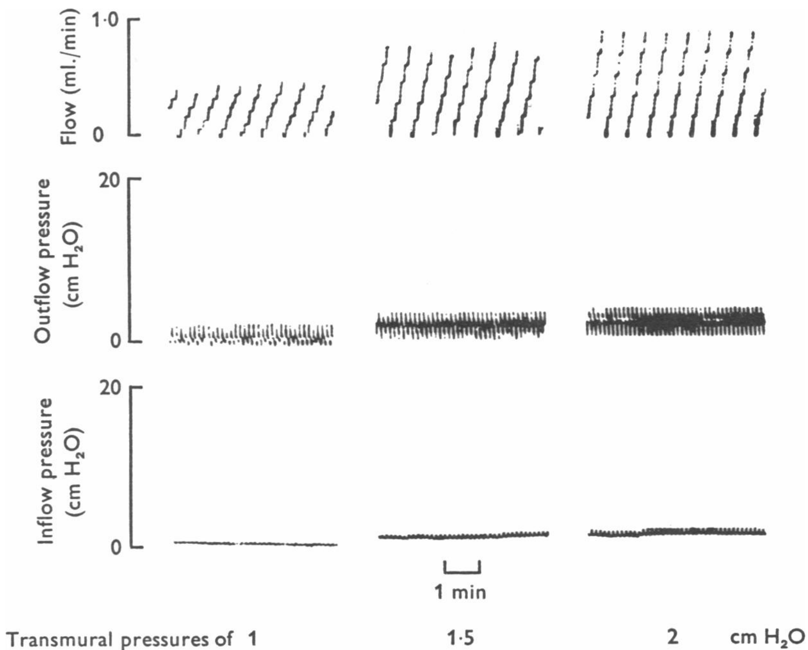

Fig. 6. The effect of raising transmural pressure from 1 to 2 cm H_2O in 0·5 cm H_2O steps on flow rate outflow pressure and inflow pressure in an isolated lymphatic vessel. The bath temperature was 30° C.

In some experiments it was found that the frequency of contraction increased spontaneouly with time even though there was no change in the transmural pressure. The experiment shown in Fig. 8 shows that the increase in frequency seen with increasing transmural pressure could not be explained by this type of occurrence. In this experiment the transmural pressure was raised and lowered in a random fashion from time to time. Raising transmural pressure always increased frequency and vice versa.

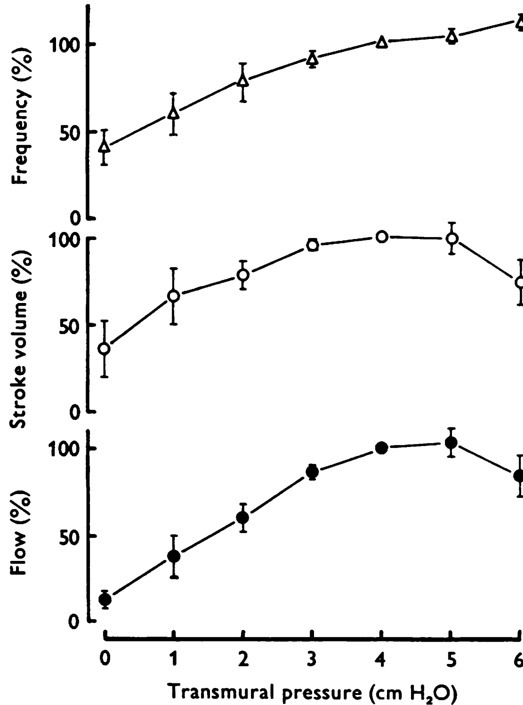

Fig. 7. Mean results of four experiments where transmural pressure was increased from 0 to 6 cm H_2O in 1 cm steps. Flow (●), stroke volume (○) and frequency (△) are plotted as percentages of their values at a transmural pressure of 4 cm H_2O. Vertical lines represent plus or minus 1 S.E. of the means. The bath temperature was 37° C.

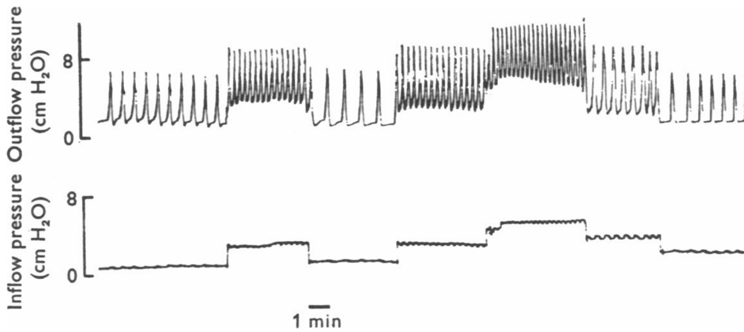

Fig. 8. The effect of random changes in transmural pressure on the frequency of contraction in an isolated lymphatic vessel. The bath temperature was 30° C.

(c) *Pulsatile transmural pressure*

Fig. 9 shows the result of an experiment where the preparation was exposed to a pulsatile as opposed to a steady increase in transmural pressure. The sinusoidal pulsation increased the frequency of contractions but the increases seemed to depend on the fact that the transmural pressure was raised, since the increases in the frequency of contraction of contractions were not related to the frequency of the applied pulsation. When the lymphatic was pulsed with sinusoidal waves which did not alter the mean resting transmural pressure, the pulsations had little effect on the frequency of contractions.

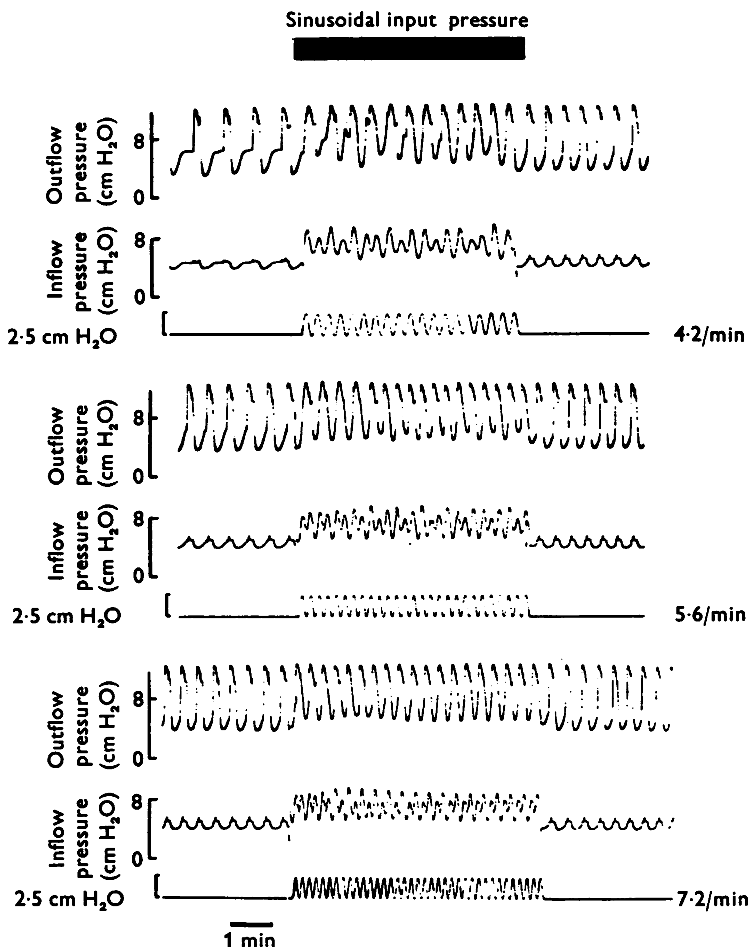

Fig. 9. The effect of pulsatile increases in transmural pressure on contractions in an isolated lymphatic vessel. The bath temperature was 30° C.

(*d*) *Longitudinal stretch*

Whereas increasing transmural pressure increased both the force and frequency of lymphatic contractions, longitudinal stretching of the vessel caused an increase in force only. Fig. 10 shows the mean results of three such experiments in which the preparation was stretched so that the resting longitudinal tension increased from 0 to 200 mg in 50 mg steps. Frequency and force were plotted as a percentage of the maximum response for each lymphatic. There was a linear increase in the amplitude of the spontaneous contractions with increasing longitudinal tension but no significant change in their frequency.

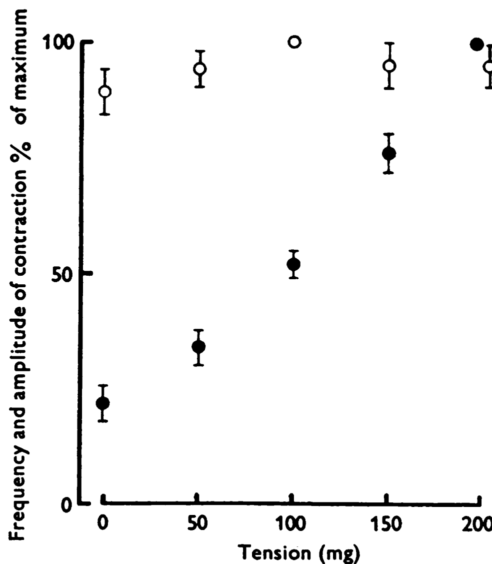

Fig. 10. Mean results of three experiments where resting longitudinal tension was increased from 0 to 200 mg in 50 mg steps. Frequency (○) and amplitude (●) of contraction are plotted as a % maximum value of each. Vertical lines represent plus or minus 1 S.E. of the means. The bath temperature was 34° C.

(*e*) *Effect of tetrodotoxin*

To investigate the possible role of nerves in coordinating the contractile activity of lymphatics, the effect of tetrodotoxin was studied on the pumping preparation. The preparation was exposed to gradually increasing doses of tetrodotoxin over a 30 min period. In these concentrations, tetrodotoxin did not affect either the inflow and outflow pressures or the

flow. Frequency increased slightly from 4 to 5 beats/min over the period. However, this was balanced by a slight decrease in the stroke output so that flow remained fairly constant at about 1·2 ml./min.

DISCUSSION

Rhythmic contractions have been observed in many varieties of lymph vessels *in vivo* (Hewson, 1774; Florey, 1927; Smith, 1949; Kinmonth & Taylor, 1956). Hall, Morris & Wooley (1965) established lymphatic fistulae in conscious sheep and recorded pulsatile pressures ranging from 1 to 25 mmHg at frequencies from 1 to 30/min. Campbell & Heath (1973), using a similar technique, found a linear relationship between contractility and flow rate when they infused Ringer–Locke solution intravenously or saline directly into the lymphatics.

The activity of the smooth muscle in bovine mesenteric lymphatics differs considerably from that seen in bovine mesenteric veins and arteries (Roddie & Scott, 1969; Mawhinney & Roddie, 1973). Isolated mesenteric veins at 37° C show spontaneous contractions but these are irregular in force and frequency and normally pauses are not seen between contractions. Strips of mesenteric arteries in the organ bath at 37° C do not normally show spontaneous activity but can be excited to contract by electrical or pharmacological stimuli. The contractions of bovine mesenteric lymphatic muscle are much brisker than those seen in the corresponding arteries and veins. Relaxation of lymphatic muscle also tends to be brisker and is usually followed by a 'diastolic' pause before the next spontaneous contraction begins. This form of activity, a brisk contraction followed by a longer diastolic pause, together with the organization of the vessel into valved segments, makes the lymphatic well suited physiologically for propelling its own contents in the manner of a number of small hearts in series.

Under the conditions of the present experiments it was found that the segments of lymphatic could propel their own contents in the absence of a resting perfusion pressure. The pressures generated with each beat were not great, several centimetres of water, but they propelled fluid at rates of about 1 ml./min. All the valve segments in the preparation contracted nearly simultaneously, reducing the diameter and length of the preparation so that its contents were expelled as dictated by the orientation of the valves.

Rapid conduction of excitation along the preparation is necessary to explain the near simultaneous contraction of all the valve segments. Since tetrodotoxin in doses of up to 7μg/ml. did not affect the contractile behaviour of the vessels, it would seem that nerves are not involved in

coordinating the contractions of the individual cells. It is more likely that excitation spreads directly from cell to cell in a fashion analogous to that seen in cardiac muscle.

The rate of contraction was presumably determined by the dominant pace-maker and, in healthy preparations, the entire vessel segment followed its rhythm.

Presumably the spontaneous changes in rate were due to changes of activity in the pace-maker focus or to the onset of activity in other foci. Agents such as increases in local temperature (McHale & Roddie, 1975*b*) and noradrenaline (Mawhinney & Roddie, 1973) can increase the rate of pace-maker activity in bovine lymphatic muscle.

It is well known that stretch causes depolarization and increased excitability in many types of smooth muscle (Bozler, 1938; Bülbring, 1955; Maschima & Yoshida 1965). The findings in the present experiments are in keeping with this. Increasing the transmural pressure in a lymphatic segment increased both the rate and force of contraction so that the amount of fluid propelled forward from the segment arose. Rather curiously when the segment was simply stretched in a longitudinal plane, only the force of contraction was increased; the rate was little affected. The difference may lie in the fact that an increase in intraluminal pressure would subject the muscle fibres with a more circular orientation to more stretch than those fibres lying in a longitudinal plane. The reverse would be true when the vessel was subjected to longitudinal stretch. If the dominant pace-makers lay in the more circularly oriented cells, distension would be a more potent chronotropic stimulus than longitudinal stretch. However, this possible explanation is entirely hypothetical.

Pumping of fluid by isolated lymphatics was most effective when the basal tone in the lymphatic muscle was low and there were relatively long diastolic pauses between beats. This permitted a relatively greater degree of diastolic filling to occur. Agents which increased the basal tone in the vessels or caused a marked increase in the beat frequency tended to reduce the effectiveness of the pumping by reducing the amount of lymphatic filling during diastole.

In conclusion, the behaviour of lymph vessels *in vitro* and the increase in their ability to pump 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 the rate and force of lymphatic contraction and hence the transport of lymph back to the blood.

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