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THE ROLE OF EXTERNAL COMPRESSION AND MOVEMENT IN LYMPH PROPULSION IN THE SHEEP HIND LIMB

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SUMMARY

1. Pressure fluctuations and lymph flow were measured in metatarsal lymphatics in anaesthetized sheep.

2. Intermittent compression significantly increased lymph flow when this was applied over the hoof but did not increase flow significantly when applied over the metatarsal region.

3. In a second preparation a 15 cm length of metatarsal lymphatic was cannulated at both ends and measurements were made of the ability of the duct to pump saline from an inflow reservoir through an outflow at the same height.

4. In the absence of external forces fluid was propelled by the lymphatic's intrinsic contractions but when intermittent compression was applied over the metatarsal region flow increased almost fourfold.

5. When animals with the doubly cannulated duct were allowed to recover, the effect of normal limb movements on fluid propulsion was examined. Under these conditions flow only occurred in response to intrinsic lymphatic contractions and appeared to be unaffected by the animal moving round the cage.

6. These results suggest that the effects of external forces on lymph flow are more dependent on compression of tissues in the lymphatic drainage area than on compression of the main lymphatic ducts. External compression can increase fluid propulsion by these vessels but, since forces of adequate magnitude appear not to be encountered in normal hind-limb movements, lymph propulsion in this region must depend on intrinsic lymphatic pumping.

INTRODUCTION

The relationship between limb movement and lymph flow has been known at least since the latter part of the nineteenth century. Generish observed in 1871 that very little lymph flowed from resting limbs while Heidenhain (1981) found a threefold increase in thoracic duct flow in response to passive movement of the dog's hind limb. Since that time many measurements have been made confirming the role of muscular contraction or passive movement in promoting lymph flow (White, Field & Drinker, 1933; McCarrel, 1939; Morris, 1968). These authors considered that the role of

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movement was to enhance propulsion by a 'muscle pump' action similar to that found in veins. Early reviews argued that this was the essential motor of lymph transport (Drinker & Yoffey, 1941; Courtice & Simmonds, 1949). Other investigators, however, observed that lymphatics could propel lymph by their intrinsic contractions (Webb & Nicoll, 1944; Smith, 1949) and the view is gaining ground that this is the most important mechanism of lymph propulsion (Hall, Morris & Woolley, 1965; Campbell & Heath, 1973; Olszewski & Engeset, 1985). The observed increase in lymph flow with passive movement appears at first sight to be inconsistent with this view but the two could be reconciled if one assumes that the role of movement is to facilitate entry of fluid into the terminal lymphatics. There is indeed a concensus emerging that some movement is essential for lymph formation (Allen & Vogt, 1937; Guyton, Granger & Taylor, 1971; Reddy, Krouskop & Newell, 1975; Elhay & Caseley-Smith, 1976).

The purpose of the present study was to assess the relative importance of intrinsic and extrinsic forces in lymph propulsion in the sheep hind limb. First the effect of intermittent compression over the metatarsal lymph duct was compared with the same stimulus applied over its drainage area. Having demonstrated that the latter was much more effective, suggesting an effect on lymph formation, a preparation was used (similar to that described by McHale & Thornbury, 1986) in which inflow pressure to a segment of metatarsal duct was kept constant. It was then of interest to examine the effect of external compression and of normal hind-limb movements on fluid propulsion by this 'isolated' segment of lymphatic.

Preliminary accounts of part of this work have been communicated to the Physiological Society (McGeown, McHale & Thornbury, 1986*a*, *b*).

METHODS

Ewes weighing 45–55 kg were anaesthetized with intravenous pentobarbitone (20–30 mg/kg) and 1–2 % halothane in 0_2 and one of two surgical procedures was followed.

Intact lymphatic preparation

Evans Blue (1 % in buffered saline) was injected into the foot-pad to outline the lymphatic vessels in the metatarsal region. One such duct was cannulated, against the direction of flow, with a polythene tube (outer diameter, 0·8 mm; inner diameter, 0·4 mm) inserted about 2 cm distal to the hock joint. Lymph flow was measured (Fig. 1*A*) by allowing lymph to accumulate on the lever of a Statham UC3 tension transducer. Lymphatic contractile activity was estimated by measuring side-arm pressure with a Statham P23 transducer. Recordings were made with a Gould 2400 chart recorder.

'Isolated' preparation

A preparation similar to that described by McHale & Thornbury (1986) was also used. A second cannula was inserted, this time with the direction of flow, so that an approximately 15 cm length of lymphatic duct was cannulated at both ends (Fig. 1*B*). Side branches were tied off so the segment was isolated from the rest of the lymphatic system. The outflow tube was connected to the flow- and pressure-measuring devices described above while the inflow tube was connected to a saline reservoir. The reservoir and outflow were set at the same level (about 15 cm above the duct) so that there was no hydrostatic gradient favouring flow through the preparation.

External compression was achieved by placing a pneumatic cuff (10 cm wide) either over the metatarsal region midway between the hock and fetlock joints (to compress the duct) or over the hoof distal to the fetlock (to compress the soft tissue in the interdigital cleft between the keratinous toes and the area between that and the accessory digits which together include the main drainage area of the metatarsal duct). The cuff was inflated intermittently to a pressure of 160 mmHg for

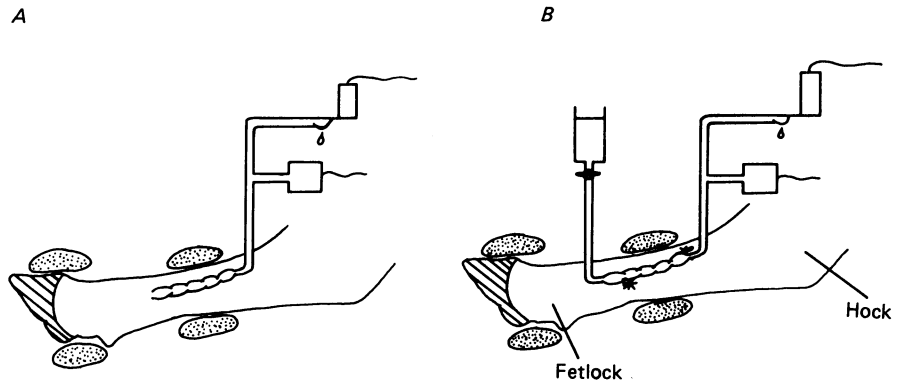

Fig. 1. Diagram of the experimental set-up for the 'intact' preparation (*A*) and for the 'isolated' preparation (*B*).

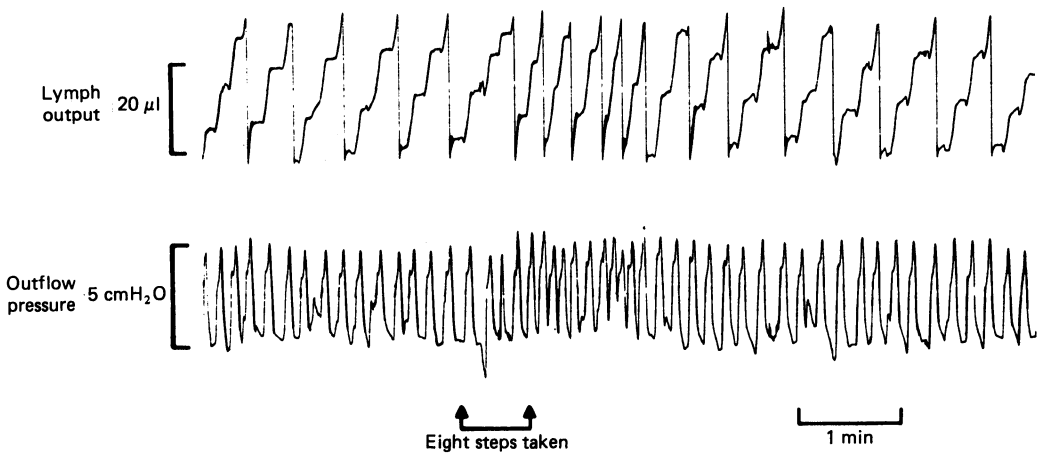

Fig. 2. Lymph flow in a conscious animal (single cannulation). The upper record is of lymph output while the lower is of lymphatic outflow pressure. When the sheep walked the length of the cage and back, flow almost doubled.

either 1 s on, 5 s off or 2 s on, 2 s off over a period of 5 min in each case. The cuff pressure was recorded using an air-filled transducer.

RESULTS

'Intact' lymphatic

Most of the experiments in this study were carried out under anaesthesia but some animals were allowed to recover in order to demonstrate the effects of normal hind-limb movements on lymph flow. Fig. 2 shows such an experiment. The lower record shows the regular phasic pressure changes resulting from spontaneous lymphatic contractions. Each expelled a quantity of fluid on to the transducer lever (seen as a step in the upper record). When enough had accumulated to form a drop this fell off and a new ramp began. Thus the slope of the ramps in the upper record was an index of flow. At the beginning of the period shown the sheep was standing still and lymph flow was steady at 60 µl/min. In the period between the two arrows

the animal walked the length of the cage and back taking a total of eight steps. This had the effect (after a short delay) of increasing the frequency of lymphatic contractions while flow went up to a peak of 120 μl/min 1·5 min after the beginning of movement and then gradually returned to the resting value.

The first part of this study was an attempt to examine the effect, in anaesthetized animals, of compression applied over the lymphatic's drainage area (cuff over hoof) or over the duct itself (cuff over the metatarsal region). Fig. 3 shows an experiment where a 1 s on, 5 s off compression cycle was applied over the hoof. The top record represents cuff pressure while the lower two records are of lymph output and pressure as in Fig. 2. It is interesting to note that there was no relationship between the frequency of cuff inflation (10 /min) and that of lymphatic contraction (4 /min). The resting flow of 12 μl/min increased to a maximum of 85 μl/min within 2 min of the

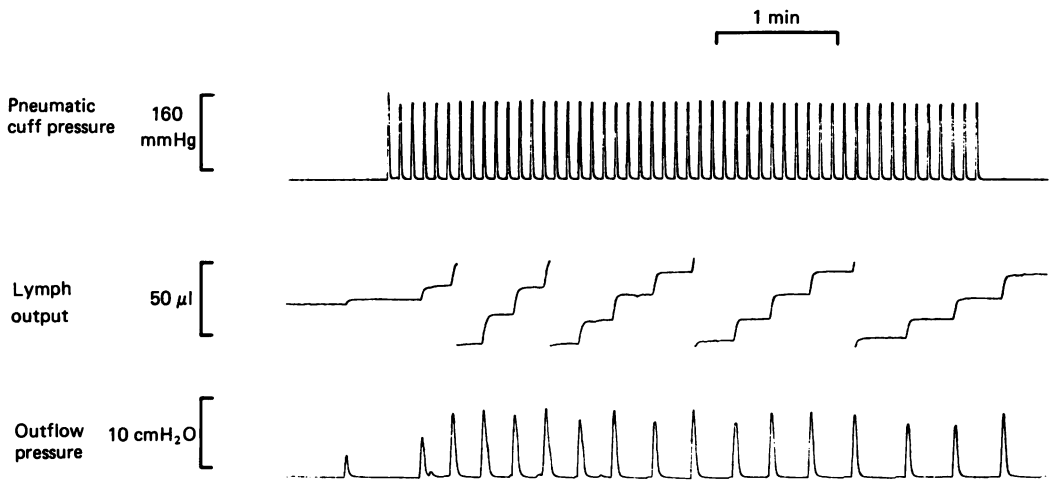

Fig. 3. The effect of intermittent compression applied over the hoof. The top record shows cuff pressure (1 s on, 5 s off cycle) while the lower two records are of lymph output and pressure (single cannulation).

start of external compression and then gradually declined during the rest of the 5 min period. In some experiments when intermittent compression was applied for 1 h flow continued to decline during the entire period.

Fig. 4 shows the effect of intermittent compression at 2 s on, 2 s off over the hoof (*A*) and the metatarsal region (*B*). Compression over the hoof increased flow from a resting value of 14 μl/min to a peak of 45 μl/min in the first 2·5 min of the compression period. This appeared to result from an increase in both frequency and force of intrinsic lymphatic contractions since there was no evidence of direct transmission of the external pressure to the lymphatic outflow. When compression was applied over the metatarsal region lymph flow increased from a control value of 12 μl/min to a peak of 20 μl/min. This time some evidence of direct conduction of the compression wave to the lymphatic was evident in the outflow pressure record.

Fig. 5 is a summary of seven experiments where intermittent compression at either 1 s on, 5 s off (upper panels) or 2 s on, 2 s off (lower panels) was applied either over

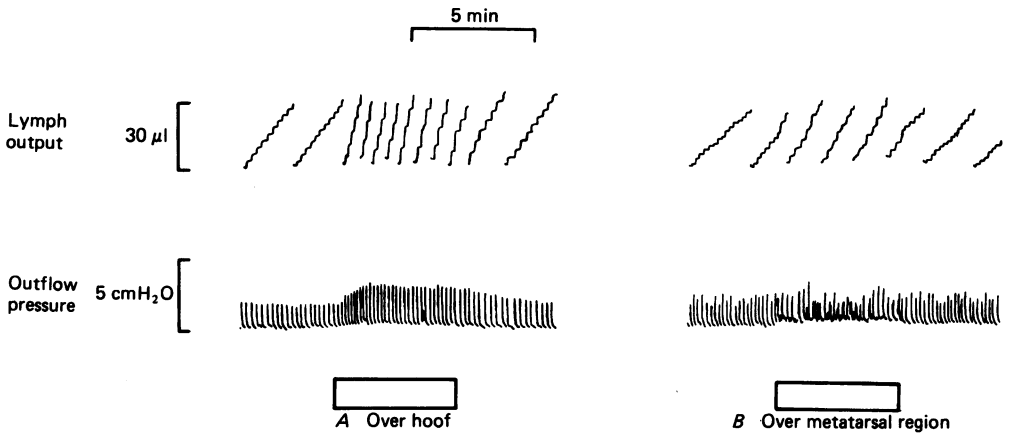

Fig. 4. Effect on lymphatic contractions and flow of intermittent compression at 2 s on, 2 s off (open bar) over the hoof (*A*) and the metatarsal region (*B*). In *A* there was no evidence of direct transmission of external pressure to the lymphatic (single cannulation).

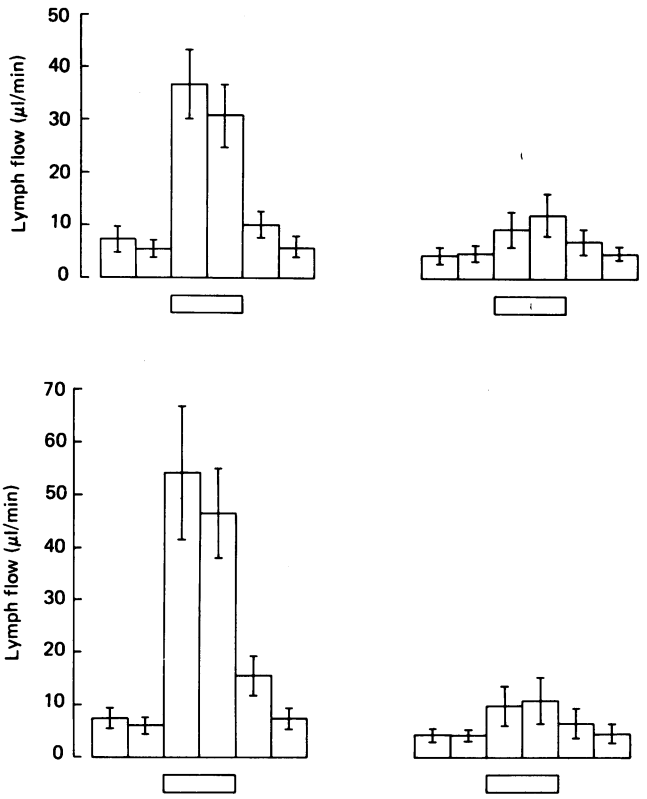

Fig. 5. Summary of seven experiments where intermittent compression at either 1 s on, 5 s off (upper panels) or 2 s on, 2 s off (lower panels) was applied either over the hoof (left-hand panels) or over the metatarsal region (right-hand panels). The six bars in each case represent the mean flow averaged over the two 2·5 min periods before, the two 2·5 min periods during and the two 2·5 min periods after intermittent compression. The vertical bars represent ± S.E. of mean (single cannulation).

the hoof (left-hand panels) or over the metatarsal region (right-hand panels). The six bars in each case represent the mean flow averaged over the two 2·5 min periods before, the two 2·5 min periods during and the two 2·5 min periods after intermittent compression. Compression over the hoof at 1 s on, 5 off increased flow from a control value of about 6 μl/min to a maximum of 36 μl/min in the first 2·5 min period. This declined to about 30 μl/min in the second 2·5 min period and declined to control level within 5 min after stimulation. The pattern was similar with the 2 s on, 2 s off protocol except that peak flow went up to over 50 μl/min in this case. Increases with both compression cycles were statistically significant (paired t test; $P < 0{\cdot}05$). When intermittent compression was applied over the metatarsal region increases in flow were much smaller and were not statistically significant.

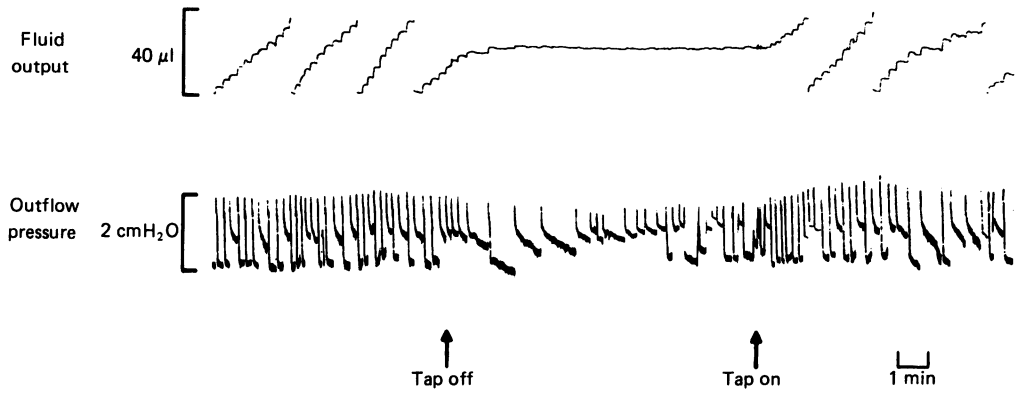

Fig. 6. Demonstration that the duct was isolated from the rest of the lymphatic system. Closure of the tap on the inflow reservoir stopped flow within a few minutes. When the tap was opened flow returned to approximately control level (doubly cannulated vessel).

The 'isolated' preparation

The above observations seem to demonstrate that intermittent compression of the large lymph ducts was fairly ineffective in promoting lymph flow. However, it could still be argued that there was little effect only because fluid supply to the ducts was limited. It was therefore of interest to re-examine the efficacy of this stimulus under conditions where filling of the duct was clearly not limited. To achieve this the second- or double-cannulation technique was used. The usefulness of this preparation is critically dependent on the absence of inflow other than from the reservoir. Confirmation that all input from side branches had been removed was demonstrated by closing the tap on the inflow reservoir. This resulted, in successful experiments such as that shown in Fig. 6, in cessation of flow within a few minutes. When the tap was turned on again flow returned to control values within 2 min. Spontaneous lymphatic contractions continued, at a lower frequency, during the period of tap closure but these did not produce flow, presumably because the lymphatic had too little fluid in its lumen.

Effect of intermittent compression in anaesthetized animals. Fig. 7 shows a record of an experiment where two 5 min periods of intermittent compression were applied over the metatarsal region. The 1 s on, 5 s off stimulus increased flow more than

fourfold while compression at 2 s on, 2 s off more than doubled it. In this preparation the effects of the external pressure pulses were clearly visible in the outflow pressure record and each one produced a clear step on the flow ramp. This is in contrast to the much smaller effect seen in metatarsal compression in the intact preparation (Fig. 4*B*) and was presumably due to the fact that filling of the vessel was not limited in this 'isolated' preparation. The pressure wave would appear to be conducted more readily along the lumen of a full than a partly empty vessel.

Intermittent compression was also applied over the hoof in these experiments, not with the expectation of seeing an increased flow, since all of the branches from this region should have been tied off, but to provide reassurance that even when flow from this region was increased there would be no increase in the 'isolated' segment. That no increase was observed is evident from the summary of five such experiments (upper panels, Fig. 8). The four bars in each case represent the mean flow averaged over two

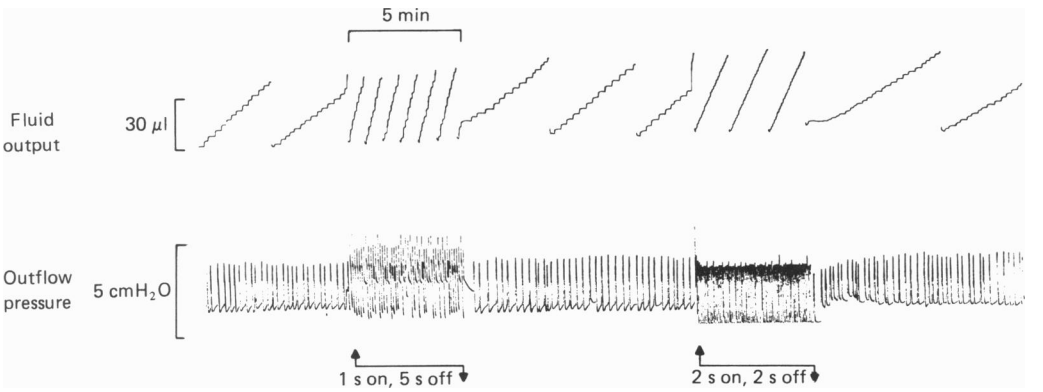

Fig. 7. The effect on flow in the 'isolated' duct of two 5 min periods of intermittent compression applied over the metatarsal region. In this preparation the effects of external pressure pulses were clearly visible in the outflow pressure record and each one produced a clear step on the flow ramp (doubly cannulated vessel).

2·5 min periods before and the two 2·5 min periods during intermittent compression at 2 s on, 2 s off (left-hand panels) and 1 s on, 5 s off (right-hand panels). The lower two panels represent a summary of five experiments such as that illustrated in Fig. 7. Flow increased from a mean control value of 16 µl/min to a peak of 56 µl/min during compression at 2 s on, 2 s off and to a peak of 66 µl/min at 1 s on, 5 s off.

Effect of normal limb movements on fluid propulsion through the 'isolated' duct in the conscious animal. Some sheep were allowed to recover from the anaesthetic. The following day, after checking that the preparation was still functioning (closing the inflow tap, etc.) the effect on fluid propulsion of walking was examined. Four animals were found to be satisfactory and an example of the type of record obtained is shown in Fig. 9. Each time the lymphatic vessel contracted a phasic increase in outflow pressure was evident. This occurred four times in the record shown and each time fluid accumulated on the transducer arm producing a step in the upper record. Between the two arrows the animal walked the length of the cage and back taking a total of eight steps. This produced the movement artifact seen in the outflow pressure record but resulted in no fluid being added to the transducer lever.

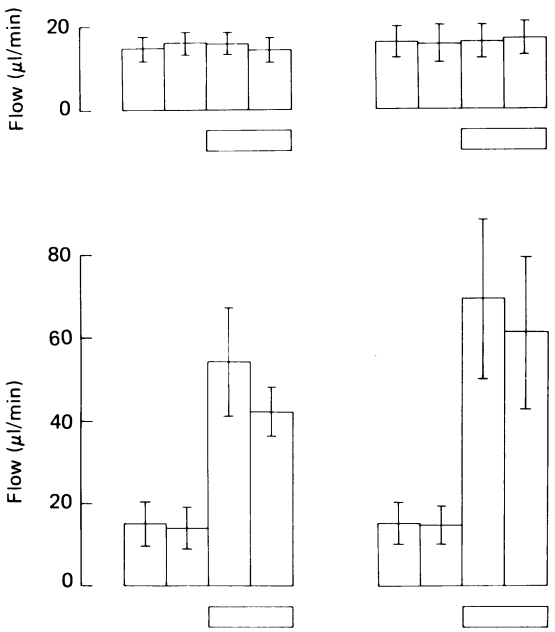

Fig. 8. Summary of five experiments where intermittent compression at either 2 s on, 2 s off (left-hand panels) or 1 s on, 5 s off (right-hand panels) was applied either over the hoof (upper panels) or over the metatarsal region (lower panels). The four bars in each case represent the mean flow averaged over two 2·5 min periods before and the two 2·5 min periods during compression. The fact that no increase was observed when compression was applied over the hoof provided confirmation that the duct was isolated from the rest of the lymphatic system.

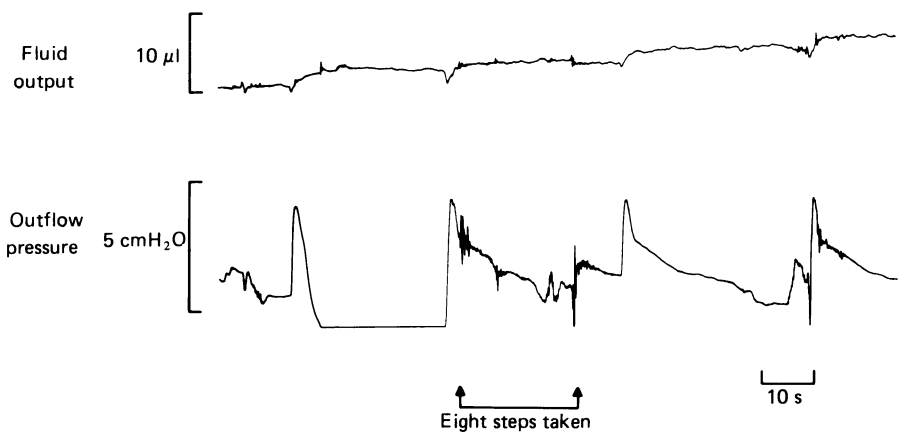

Fig. 9. The effect of walking on fluid propulsion by the 'isolated' duct. When the animal walked the length of the cage and back no fluid was added to the transducer lever (doubly cannulated vessel).

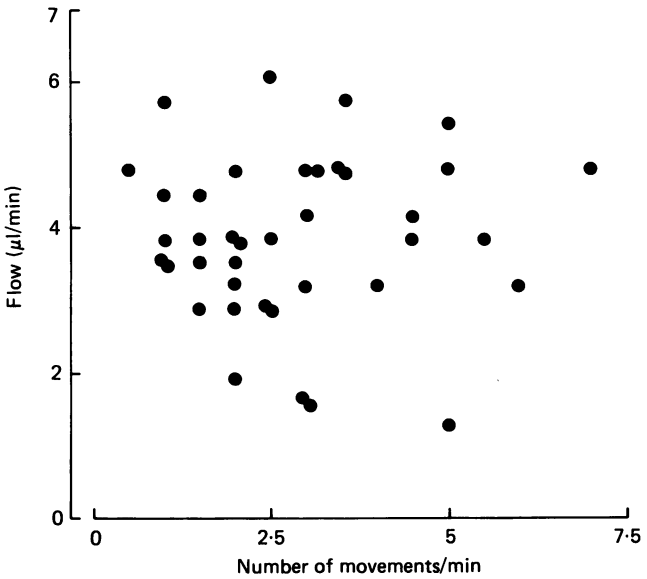

Fig. 10. Scatter diagram of the relationship between the number of times the sheep moved and fluid propulsion by doubly cannulated vessel. There was clearly no relationship between movement and flow and this is confirmed by the low correlation coefficient ($r = 0{\cdot}016$; not significant).

Experiments were analysed by dividing them into 2 min periods and noting the number of movements and the fluid output during each period. Fig. 10 shows the type of scatter diagram resulting from this analysis of one experiment. There is clearly no relationship between number of movements and flow and this is confirmed by the low correlation coefficient ($r = 0{\cdot}016$; not significant). The correlation coefficients for the other three experiments were $r = -0{\cdot}333$ ($P < 0{\cdot}05$), $r = -0{\cdot}14$ (not significant) and $r = -0{\cdot}58$ (not significant).

DISCUSSION

In 1941, Drinker & Yoffey wrote 'The lymphatic system in the mammal possesses no intrinsic mechanism for the movement of lymph. Accumulation of tissue fluid and formation of lymph depend on capillary permeability and capillary pressure. Movement of lymph depends upon forces outside the lymphatic system'. This conviction seems curious bearing in mind that Hewson (1774) and Florey (1927) had already observed that lymphatic ducts were rhythmically contractile. However, even today, the idea that a small pressure gradient from lymph capillary to central veins (*vis a tergo*) and the massaging effects of arterial pulsation and muscular contractions are primarily responsible for lymph propulsion is so intuitively appealing that it has been a difficult one (in the words of Staub & Albertine, 1985) 'to extirpate from the literature'. Although it is now widely accepted that lymphatics can propel lymph by their intrinsic contractions, no direct assessment of the relative importance of intrinsic and extrinsic mechanisms has been made. The reason for this is that, again to quote Staub & Albertine (1985), 'unless one has rigid control of the rate of

transvascular liquid filtration and delivery to initial lymphatics, there is no way to make any useful estimates of the effects of passive forces on lymph propulsion'. In the present study we have not controlled transvascular filtration but we have controlled fluid input to the segment of collecting duct under study. It has thus been possible to study the effects of external compressive forces and to compare these with the duct's intrinsic pumping activity. The results showed that intermittent compression was extremely effective in propelling fluid. This was particularly true at 1 s on, 5 s off (presumably due to the extra filling time) where flow increased to more than four times that produced by lymphatic contractions. There are, however, two reasons to believe that this extrinsic mechanism is not important under normal conditions. The first is that, while arterial pulsations were often apparent in the lymphatic outflow pressure record (these lymph ducts frequently lie alongside arteries and veins), these never had any effect on fluid output. Secondly, and more importantly, no correlation was found between fluid propulsion and normal walking movements in the conscious animals. It could be argued, of course, that the absence here of a 'muscle pump' action is not very surprising since there is very little muscle in this region. The problem remains, however, that lymph must be transported against gravity and in the absence of assistance from extrinsic forces it would appear that this is normally achieved by intrinsic lymphatic pumping.

Nevertheless, the fact remains that movement does increase lymph flow; however it appears increasingly likely that this is a result of increased transfer of interstitial fluid into lymphatic capillaries (lymph formation) rather than any effect on propulsion. The many observations of the effect of movement in promoting lymph flow can be explained in these terms although the authors often invoked extrinsic duct compression as the explanation. Thus Skalak, Schmid-Schonbein & Zweifach (1984) argued, on the strength of morphological evidence, that vasomotion increased lymph flow by intermittent compression of adjacent collecting ducts although there is no reason to exclude an effect on lymph formation. Similarly the observations by Calnan, Pflug, Reis & Taylor (1970) that flexion of the metetarsal–phalyngeal joint in the dog caused the same increase in lymph pressure as skin massage allow a similar interpretation. Parsons & McMaster (1938) noted that pulsatile perfusion of an isolated rabbit ear resulted in a much greater lymph flow than non-pulsatile perfusion. They attributed this to a combination of increased lymph formation and increased propulsion.

The observations in the present study that compression of the hoof fifteen times a minute was more effective than ten times a minute (in contrast to the result with the 'isolated' duct) might suggest that the effect was on lymph formation rather than on propulsion. This suggestion is, however, a very tentative one for it has not been possible, in this study or in those cited, to distinguish effects of movement on either capillary filtration or facilitation of entry of fluid into the terminal lymphatics from those on compression of the terminal network. A precise study of the role of movement in lymph formation will require a new technical approach.

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REFERENCES

ALLEN, L. & VOGT, E. (1937). A mechanism of lymphatic absorption from serous cavities. *American Journal of Physiology* **119**, 776–782.

CALNAN, J. S., PFLUG, J. J., REIS, N. D. & TAYLOR, L. M. (1970). Lymphatic pressures and the flow of lymph. *British Journal of Plastic Surgery* **23**, 305–317.

CAMPBELL, T. & HEATH, T. (1973). Intrinsic contractility of lymphatics in sheep and in dogs. *Quarterly Journal of Experimental Physiology* **58**, 207–217.

COURTICE, F. C. & SIMMONDS, W. J. (1949). Absorption of fluids from the pleural cavities of rabbits and cat. *Journal of Physiology* **109**, 117–130.

DRINKER, C. K. & YOFFEY, J. M. (1941). Lymph flow and lymph pressure. In *Lymphatics, Lymph and Lymphoid Tissue*, pp. 112–145. Cambridge; Harvard University Press.

ELHAY, S. & CASLEY-SMITH, J. R. (1976). Mathematical model of the initial lymphatics. *Microvascular Research* **12**, 121–140.

FLOREY, H. (1927). Observations on the contractility of lacteals. Part II. *Journal of Physiology* **63**, 1–18.

GENERISH, H. (1871). Die Autnahme der Lymphe durch die Sehen und Fascien der Skelettmuskeln. *Arbeiten aus der Physiologische Ansteiger Leipzig* **5**, 53.

GUYTON, A. C., GRANGER, H. J. & TAYLOR, A. E. (1971). Interstitial fluid pressure. *Physiological Reviews* **51**, 548–563.

HALL, J. G., MORRIS, B. & WOOLLEY, G. (1965). Intrinsic rhythmic propulsion of lymph in the unanaesthetized sheep. *Journal of Physiology* **180**, 336–349.

HEIDENHAIN, R. (1891). Versuche und Fragen zur Lehre von der Lymph Bildung. *Pflügers Archiv* **49**, 209–301.

HEWSON, W. (1774). A description of the lymphatic system. *Experimental Enquiries Part II*, p. 126. London.

MCCARREL, J. D. (1939). Cervical lymph pressure in the dog. *American Journal of Physiology* **127**, 154–160.

MCGEOWN, J. G., MCHALE, N. G. & THORNBURY, K. D. (1986*a*). Intermittent compression as a stimulus to lymph flow in the sheep hind limb. *Journal of Physiology* **380**, 35*P*.

MCGEOWN, J. G., MCHALE, N. G. & THORNBURY, K. D. (1986*b*). Assessment of the relative contribution of extrinsic and intrinsic pumping to fluid propulsion in sheep lymphatics. *Journal of Physiology* **380**, 33*P*.

MCHALE, N. G. & THORNBURY, K. D. (1986). A method for studying lymphatic pumping activity in conscious and anaesthetized sheep. *Journal of Physiology* **378**, 109–118.

MORRIS, B. (1968). Lymphatic contractility and its significance in lymph propulsion. In *Progress in Lymphology II*, ed. VIAMONTE, M., pp. 60–62. Stuttgart: Georg Thieme Verlag.

OLSZEWSKI, W. L. & ENGESET, A. (1985). Studies on the lymphatic circulation of humans. In *Experimental Biology of the Lymphatic Circulation*, ed. JOHNSTON, M. G., pp. 395–422. New York: Elsevier Science Publishers.

PARSONS, R. J. & MCMASTER, P. D. (1938). The effect of the pulse on the formation and flow of lymph. *Journal of Experimental Medicine* **68**, 353–376.

REDDY, N. P., KROUSKOP, T. A. & NEWELL, P. H. (1975). A note on the mechanism of lymph flow through the terminal lymphatics. *Microvascular Research* **10**, 214–216.

SKALAK, T. C., SCHMID-SCHONBEIN, G. W. & ZWEIFACH, B. W. (1984). New morphological evidence for a mechanism of lymph formation in skeletal muscle. *Microvascular Research* **28**, 95–112.

SMITH, R. O. (1949). Lymphatic contractility – a possible intrinsic mechanism of lymphatic vessels for the transport of lymph. *Journal of Experimental Medicine* **90**, 497–509.

STAUB, N. C. & ALBERTINE, K. H. (1985). Biology of lung lymphatics. In *Experimental Biology of the Lymphatic Circulation*, ed. JOHNSTON, M. G., pp. 305–326. New York: Elsevier Science Publishers.

WEBB, R. L. & NICOLL, P. A. (1944). Behaviour of lymphatic vessels in the living bat. *Anatomical Record* **88**, 351–367.

WHITE, J. C., FIELD, M. E. & DRINKER, C. K. (1933). On the protein content and normal flow of lymph from the foot of the dog. *American Journal of Physiology* **103**, 34–44.