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ARTERIAL PULSATION AND LYMPH FORMATION IN AN ISOLATED SHEEP HINDLIMB PREPARATION

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SUMMARY

1. Lymph flow was measured by cannulating metatarsal lymphatics in the sheep hindlimb. The region was perfused with warmed, heparinized, oxygenated blood via the tibio-cranial artery cannulated just distal to the hock. Constant and pulsatile perfusion pressures were compared. A cuff was inflated over the metatarsals to maintain venous pressure at 20 mmHg.

2. The lymph flow rates during constant perfusion at 80 mmHg were comparable to control flows in intact metatarsal preparations and oedema (monitored by measuring tissue volume) did not accumulate. Flow records were similar to those obtained from anaesthetized animals, lymph being expelled by regular lymphatic contractions.

3. Pulsatile perfusion using 120/60 mmHg (systolic cuff cycle 0·25 s inflation, 0·5 s deflation) did not increase the rate of lymph flow. Increasing the pulse pressure to 100 mmHg (150/50 mmHg) produced a 20% increase in flow but this was not statistically significant. The cuff deflation time was also increased (1 s inflation, 1 s deflation) using 120/60 mmHg but this had little effect.

4. It may be concluded that arterial pulsation is not important for lymph formation in this preparation.

INTRODUCTION

Lymph formation involves the transfer of fluid from the interstitium to the lumen of the small terminal lymphatics. Most of those who have recorded pressures at these sites, however, find that the average intralymphatic pressure is greater than the interstitial hydrostatic pressure (Clough & Smaje, 1978; Hogan, 1981). On the basis of this it has been proposed that some type of pumping must occur to overcome the hydrostatic barrier to lymph formation (Adair & Guyton, 1985). In previous studies we have shown that intermittent compression of the sheep's hoof increases lymph flow by stimulating lymph formation (McGeown, McHale & Thornbury, 1987*a*) and that this is the consequence of cyclical collapse and refilling of the lymphatics in the lymph-forming region (McGeown, McHale & Thornbury, 1988). It seems possible, therefore, that the arterial pulse might provide a physiological source of rhythmical external compression, thus promoting lymph formation. This would be in keeping with morphological findings in skeletal muscle showing that arteriolar dilatation is

correlated with lymphatic compression (Skalak, Schmid-Schönbein & Zweifach, 1984).

Parsons & McMaster (1938) reported work which supports the concept of a lymph-forming pump driven by vascular pulsation. They used the rate of spread of intralymphatic dye as an indirect estimate of lymph flow in the perfused rabbit's ear and their results suggested that lymph formation was greatly accelerated by arterial pulsation, with little or no lymph flow in its absence. The experiments described in this paper were designed to see if these findings could be confirmed and an isolated preparation was developed to this end from the distal sheep hindlimb. This allowed pulsatile and constant perfusion conditions to be alternated at will while the flow from a single lymphatic was recorded directly.

This work has previously been communicated in part to the Physiological Society (McGeown, McHale & Thornbury, 1987*c*).

METHODS

Ewes were anaesthetized with I.V. sodium pentobarbitone (20–30 mg kg^{-1}) and inhaled halothane (1–3 % in O_2). The metatarsal lymphatics were visualized by injecting Evans Blue (1 % in buffered saline) into the footpad and one vessel was cannulated about 8 cm distal to the hock and towards the hoof (Polythene cannula, outer diameter 0·8 mm, inner diameter 0·4 mm). The medial plantar artery (which anastamoses with the tibio-cranial artery) was exposed and ligated at the hock joint. 400–500 ml of blood were taken from the internal carotid artery into a heparinized saline bag (5 U heparin per millilitre of blood) which was used as the perfusion reservoir. The tibio-cranial artery was exposed 2 cm distal to the hock, tied and cannulated with a Vasculon 16G, I.V. cannula (Viggo Products) shortened to 6 cm overall length (Fig. 1). This had a measured resistance to blood flow of 0·4 mmHg ml^{-1} min. The cannulated limb was flushed with 10000 U of heparin in 5 ml of 0·9 % NaCl solution and was then attached to the blood reservoir. After 5 min perfusion with heparinized blood the inflow tube was detached for about 2 min to allow the preparation to be completely severed 5 cm above the hock joint. The animal was immediately killed using an overdose of I.V. sodium pentobarbitone and all further observations were made on the isolated hindlimb.

The arterial cannula was re-attached to the collapsible reservoir (Fig. 1). A thermocouple was placed in the blood flow at the arterial inflow and the temperature recorded using an electronic thermometer (Comark, Type 1621). This was held in the range 32–34 °C using a water-bath. Side-arm blood pressure was recorded proximal to the arterial cannulation point using a Statham P23 pressure transducer. This was calibrated in millimetres of mercury with the upper aspect of the hock joint as the zero reference level. A self-sealing side port at this point in the circuit allowed samples of 'arterial' blood to be taken 30 min after starting perfusion for blood gas analysis (Instrumentation Laboratory Systems, Model 1302). There was an acidosis in three out of five specimens (pH 7·28 ± 0·06; mean ± S.E.M.) due to CO_2 retention (P_{CO_2} 50 ± 8 mmHg). The P_{O_2} was greater than 100 mmHg in all five cases. In one experiment blood was sampled at the beginning and end of a 3 h period of perfusion. There was no change in plasma potassium concentration and the increase in free haemoglobin was equivalent to haemolysis of less than 0·5 % of the erythrocytes.

Blood from the cut veins was collected into a plastic funnel and returned to a Polythene beaker. This was stirred continuously using a magnetic stirrer and bubbled with O_2. Arterialized blood was siphoned from the base of the beaker and returned to the blood reservoir by means of a Watson–Marlow peristaltic pump (Type MHRE 200). Arterial pressure was controlled using pneumatic cuffs to pressurize the collapsible blood reservoir (Fig. 1). The outer cuff was inflated to provide constant perfusion pressure. Pulsatile pressure was generated by inflating the inner cuff intermittently, with the inflation and deflation times controlled by an electronic timer. Total blood flow was estimated using timed venous collections and this remained constant over several hours in satisfactory preparations. Venous pressure was not measured directly, but a venous cuff just

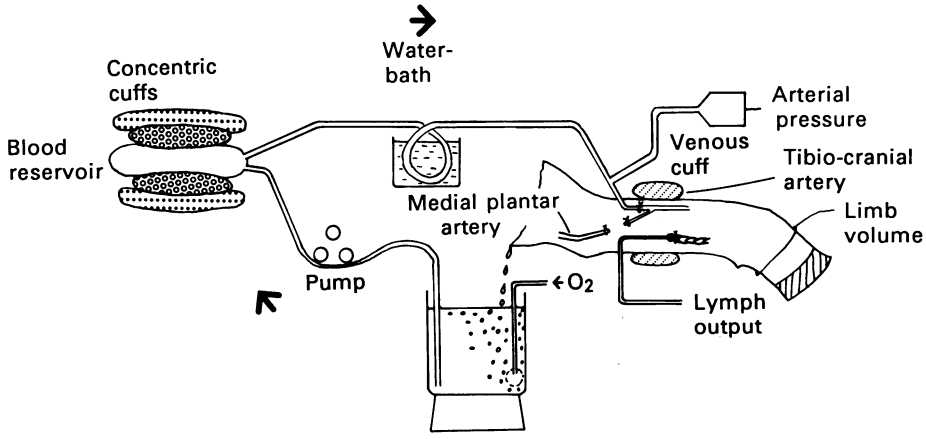

Fig. 1. Diagram of the isolated hindlimb preparation showing the perfusion circuit used.

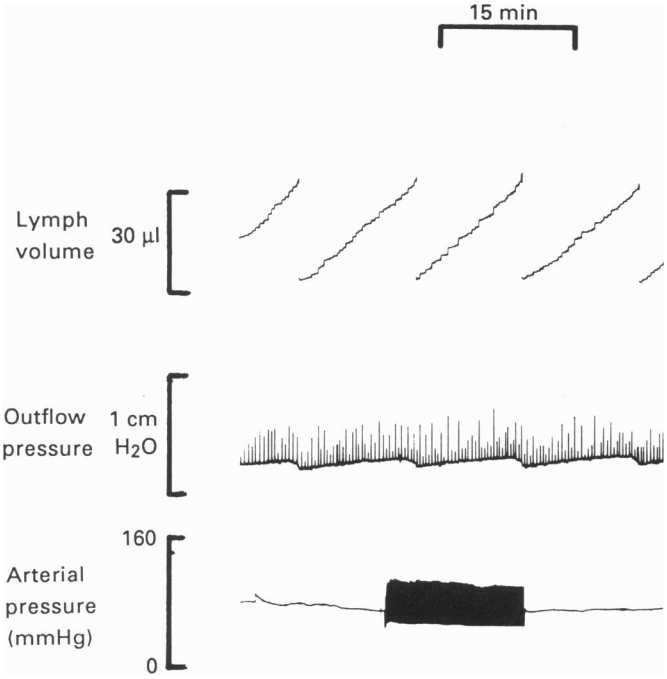

Fig. 2. Lymph flow (top trace) and output pressure (middle trace) during a 15 min period of pulsatile perfusion (120/60 mmHg, systolic cuff 0·25 s on, 0·5 s off) sandwiched between two 15 min periods at a constant perfusion pressure (80 mmHg; bottom trace).

distal to the hock was kept at 20 mmHg pressure throughout, close to the venous pressure in this region in the anaesthetized sheep (McGeown, McHale, Roddie & Thornbury, 1986).

Lymph flow was measured by allowing lymph to accumulate on the lever of a Statham UC3 tension transducer (McHale & Roddie, 1983). Side-arm pressure was also measured from the lymphatic outflow cannula using a Statham P23 transducer, providing a record of the lymphatic contractions (Fig. 2, middle trace). All records were made on a Gould 2400s chart recorder. Strain-gauge plethysmography (Medasonics, Vasculab SPG16) was used to monitor tissue volume distal

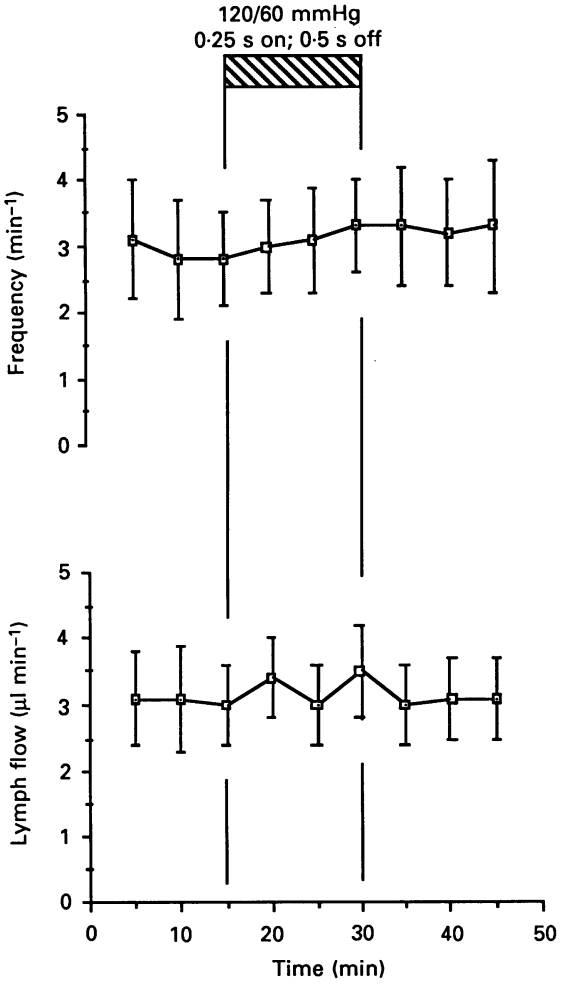

Fig. 3. Summary of lymph flow (below) and contraction frequency (above) for seven preparations exposed to constant arterial pressure (80 mmHg) for 15 min either side of the pulsatile perfusion (horizontal bar). Results have been averaged over 5 min blocks and means ± 1 S.E.M. are shown.

to the fetlock, the relevant area of lymph formation, and there was no indication of tissue fluid accumulation in any experiment even after 3–4 h of artificial perfusion.

Most of the protocols used compare lymph flow during pulsatile pressure with that during perfusion at the equivalent constant pressure. This was taken to be the time average mean pressure and produced very similar mean blood flow rates (96 ± 2% of that during pulsatile perfusion). Three pulsatile pressures were studied. A systolic pressure of 120 mmHg with a diastolic pressure of 60 mmHg was applied using an inner-cuff inflation time of 0·25 s followed by a 0·5 s deflation time (equivalent to 80 pulses min^{-1}). This was compared with a constant pressure of 80 mmHg and was chosen as close to physiological (McGeown, McHale & Thornbury, 1987*b*). A pulse pressure of 100 mmHg (150/50 mmHg; 0·25 s on, 0·5 s off) was also compared with 80 mmHg. Finally, 120/60 mmHg was used again but with a cuff cycle of 1 s on, 1 s off (30 pulses min^{-1}) and this was compared with a constantly applied 90 mmHg.

Lymph flow, contraction frequency and stroke volume (flow ÷ frequency) were averaged over 5 min periods. Summarized results represent the mean ± standard error of the mean (S.E.M.) for the

stated number of experiments, and the significance of apparent differences was tested with the paired Student's t test.

RESULTS

The first trace shown is typical of the records obtained in these experiments (Fig. 2). Lymph flow (top trace) was intermittent, a discrete volume of fluid being ejected

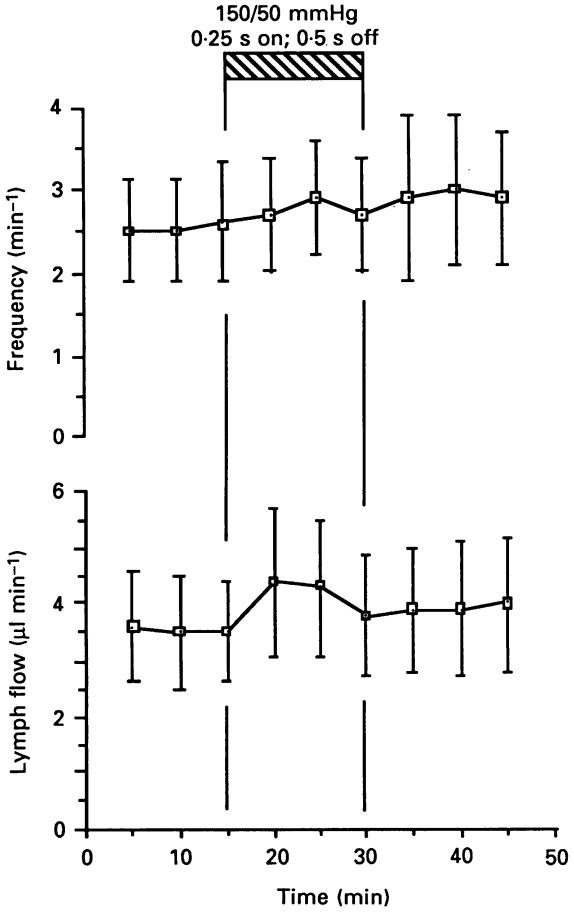

Fig. 4. Summary of 5 min averages for flow (below) and frequency in seven preparations exposed to a pulse pressure of 100 mmHg. A constant 80 mmHg was applied either side of the pulsatile perfusion.

by each of the lymphatic contractions which were recorded as regular peaks in outflow pressure (middle trace). When a drop of sufficient size formed on the tension transducer arm it fell off, returning the volume record to its basal level. This was associated with a decrease in the basal outflow pressure. These records are identical in form with those obtained from cannulated lymphatics in the intact sheep hindlimb (McGeown, McHale & Thornbury, 1987*a*, *b*). The current protocol extended over three consecutive 15 min periods. Initially the arterial pressure (bottom trace) was constant at 80 mmHg. Pulsatile perfusion was then introduced with a pulse pressure

of 60 mmHg and a frequency of 80 min^{-1} (120/60 mmHg, systolic cuff 0·25 s on, 0·5 s off) and finally the pressure reverted to 80 mmHg. Total blood flow was 55 ml min^{-1} throughout the 45 min. Lymph flow rose slightly from 3·2 to 3·8 μl min^{-1} at the start of pulsation, declined during it and fell from 3·4 to 2·6 μl min^{-1}, on returning to constant pressure. Contraction frequency also rose, but only from 2·6 to 2·8 min^{-1} on starting pulsation and fell from 3·2 to 3 min^{-1} on stopping it. Such small changes are

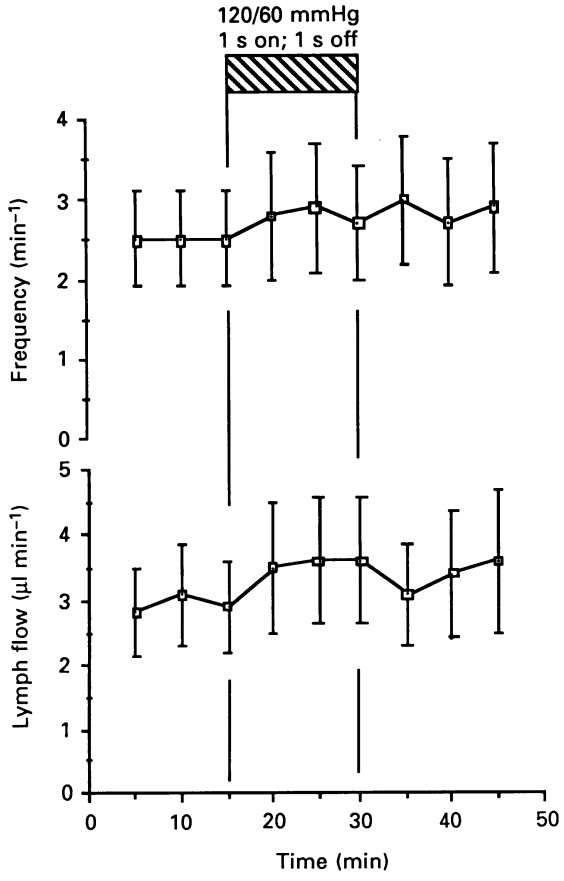

Fig. 5. Diagram summarizing flow and frequency in six preparations during two periods at a constant 90 mmHg bounding 15 min of pulsatile perfusion with prolonged systolic cuff inflation and deflation times.

unlikely to be of importance, as is confirmed by the summarized results showing flow (lower graph) and frequency (upper graph) for seven preparations under these conditions (Fig. 3). Both variables were averaged over 5 min time periods and remained constant regardless of the mode of perfusion. Mean total blood flow was 38 ± 9 ml min^{-1} and was also unaffected by pulsatile perfusion.

The possibility still existed that larger pulse pressures would produce an effect on lymph flow. To test this the experimental design just outlined was repeated, using the same inflation cycle for the inner cuff but with an arterial pressure of 150/50 mmHg. The mean results for seven such experiments (mean total blood flow

37 ± 12 ml min^{-1}) show a slight rise in flow rate at the start of pulsation (equivalent to 20% of control flow, Fig. 4, lower graph) but the difference from control is not statistically significant. Contraction frequency was unaffected (Fig. 4, upper graph).

Previous studies have shown that the effect of intermittent compression on lymph flow is dependent on the time available for lymphatic filling between compressions (McGeown *et al.* 1988). For this reason the initial pulse pressure (120/60 mmHg) was applied once more in six preparations, but using an inner-cuff cycle of 1 s on, 1 s off (blood flow 44 ± 11 ml min^{-1}). This produced no statistically significant changes in mean lymph flow or contraction frequency as compared to a constant pressure of 90 mmHg (the equivalent mean arterial pressure), even though the potential filling time for any lymphatics compressed by arterial pulsation was doubled (Fig. 5).

DISCUSSION

The first studies on arterial pulsation and lymph flow were carried out by Parsons & McMaster using the rabbit ear (1938). They found that intralymphatic dye always moved more quickly (over ten times more quickly) towards the base of the ear under pulsatile rather than non-pulsatile perfusion conditions. They also found that pulsatile perfusion led to more rapid diffusion of dye through the interstitium (McMaster & Parsons, 1938). They concluded that 'there was little or no formation of lymph in the non-edematous ears perfused at constant pressure' (Parson & McMaster, 1938). Studies during cardio-pulmonary bypass conflict with this inference, however, since a direct comparison of pulsatile and non-pulsatile bypass failed to demonstrate any differences in the thoracic duct flow rate of anaesthetized dogs (Anabtawi, Womack & Ellison, 1966). These findings would suggest that the overall rate of lymph flow is not greatly altered by non-pulsatile perfusion of the systemic circulation. This does not preclude a localized effect on lymph formation but it stands at variance with the suggestion that lymph propulsion through the thoracic duct is accelerated by vascular pulsation (Cressman & Blalock, 1939; Webb & Starzl, 1953). The latter conclusion was based on recordings of transmitted pulsation, however, in the absence of flow measurements.

Cardio-pulmonary bypass involves the entire systemic circulation and does not allow isolation of the mechanical effects of pulsation from possible central effects, such as reflex changes in vascular resistance (Giron, Birtwell, Soroff & Deterling, 1966). Perfusion of a single organ avoids some of these problems. With regard to peripheral lymph flow, Parsons & McMaster seem to be the only workers to have previously tackled the question directly. In their review of the lymphatic system, Yoffey & Courtice (1970) state that 'Whereas there seems no doubt that vascular pulsations cause some lymph movement, the effect is probably very small compared with that of other factors.' No evidence is quoted to substantiate this position, however. The present study would seem to provide such evidence, since arterial pulsation did not significantly accelerate lymph flow at physiological (Fig. 3) or increased pulse pressures (Fig. 4). Changing the pulse duration (and therefore frequency) did not alter the outcome (Fig. 5).

As with all isolated preparations, these results must be interpreted carefully.

Lymph flows were very similar to those measured under control conditions in intact limbs (MceGown *et al.* 1988). Vascular resistance sometimes changed suddenly in the first 30 min of perfusion and usually this was suggestive of an initial vasoconstriction, followed by vasodilatation. Thereafter, conditions generally remained stable for 2–3 h. Oedema, a common sign of deterioration in perfused systems (Bomzin & Naidu, 1985), did not develop in any of our experiments. In fact the limb volume tended to fall slowly in most cases. This also suggests that venous pressures were appropriate. In comparison Parsons and McMaster reported pitting oedema in over 30% of their preparations within 1 h, regardless of the type of perfusion used. This may have related to their perfusion procedure which included flushing the ear with 50 ml of Locke's solution over the initial 10–15 min of each experiment. Being free from colloid, this must have produced an imbalance in transcapillary filtration forces, favouring oedema (Michel, 1984). Such solutions also produce cell damage when used to perfuse living tissues (Belt, Smith & Whipple, 1920).

One experiment was carried out to test whether the effects of a crystalloid solution could explain the dramatic lymph flow response to arterial pulsation in the rabbit's ear. The hindlimb preparation was perfused in the normal way at 80 mmHg, but the blood was diluted with physiological saline solution (1 part saline to 2 parts blood). Tissue volume, which remained constant over a 15 min control period, began to rise steadily at a rate of 3% h^{-1} within 10 min of changing the perfusate. This was associated with an increase in lymph flow from a control rate of 4·5 to 9·8 $\mu l\ min^{-1}$ after 30 min. The diluted blood was then driven at a pulsatile pressure (120/60 mmHg, systolic cuff 0·25 s on, 0·5 s off). This produced a further increase in flow, but only to 11 $\mu l\ min^{-1}$, and flow continued to rise slowly on returning to constant pressure. This suggests that the different results cannot be explained on the basis of oedema alone.

Another worry in the present study was that the pulsatile pressure might not be transmitted to the most distal portions of the hindlimb. Tissue volume records were reassuring on this point since the strain gauge was sensitive enough to register rapid oscillations in output during pulsatile perfusion. These were similar to those recorded from the arterial pulse with an intact circulation in anaesthetized animals. Total blood flow was also high, as might be expected in a denervated system under acidotic conditions. There is therefore no reason to believe that the lymphatics did not experience genuinely pulsatile perfusion even though this had no effect on the lymphatic pressure record (Fig. 2). Previous studies have shown that pulsation due to intermittent compression applied directly over the region distal to the fetlock is completely damped out of the outflow pressure trace even though lymph flow is greatly increased (McGeown *et al.* 1987*a*). Calculations based on the resistance of the intra-arterial cannula and the measured blood flows suggest that the pressure at the cannula tip (5–6 cm proximal to the fetlock) would have been 90/50 mmHg with a side-arm pressure of 120/60 mmHg and 115/40 mmHg with the pressure set to 150/50 mmHg.

Further comparisons between the present study and those on the rabbit's ear must consider species and other technical differences. With regard to experimental technique, lymph flow has been measured directly in this study whereas the use of dyes can only record the maximum lymph velocity. Nevertheless, this should give a

qualitative measure of flow rate. It may well be, therefore, that the most significant differences lie in the tissues studied. The soft tissue distal to the fetlock in the sheep is likely to be more easily deformed than the less-compliant rabbit's ear, damping the pressure changes transmitted through the tissues from the artery. Lymph formation is assisted by the effects of movement in the hoof but it may be useful for the ear, which does not benefit from any such external massage, to experience a kind of 'internal compression'. Further studies, including the use of the current perfusion system with different tissues, would be necessary to give credence to such teleological speculation.

In conclusion, lymph flow in the isolated sheep hindlimb is not affected by arterial pulsation and continues at physiological rates for several hours during constant pressure perfusion. The mechanism for lymph formation is obviously not dependent on the pulse in this preparation. It may be that the terminal lymphatics pump fluid by spontaneous contraction as has been described in the bat's wing (Webb & Nicoll, 1944; Nicoll & Taylor, 1977; Nicoll & Hogan, 1978), although a role for vasomotion cannot be ruled out (Intaglietta & Gross, 1982; Skalak, Schmid-Schönbein & Zweifach, 1984).

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