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EFFECTS OF VARYING PATTERNS OF EXTERNAL COMPRESSION ON LYMPH FLOW IN THE HINDLIMB OF THE ANAESTHETIZED SHEEP

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SUMMARY

1. Lymphatics draining the region distal to the fetlock were cannulated in anaesthetized sheep. A pneumatic cuff was used to intermittently compress the drainage area.

2. Intermittent inflation of the cuff accelerated lymph flow. This effect increased dramatically as the frequency of inflation was increased.

3. With a constant inflation time, increasing the time between inflations increased the additional volume of lymph expelled per external compression threefold over the range of 0·5–8 s. Longer intervals produced little further change.

4. There was no significant difference between the effects on lymph volume per compression of intermittent cuff inflations lasting from 1 to 18 s with a constant time between inflations.

5. A cuff pressure of 20 mmHg produced a significant rise in lymph flow. Increasing the pressure applied during cuff inflation increased the effect up to the maximum pressure used (320 mmHg).

6. These studies demonstrate that the promotion of lymph formation by tissue compression is related to the number of compressions applied and the period of time between them. Increasing the pressure exaggerates the effect but increasing the length of each compression does not. This suggests that compression empties the terminal lymphatics centripetally. These vessels refill after compression is released and in our preparation this is complete in about 8 s.

INTRODUCTION

In a previous paper (McGeown, McHale & Thornbury, 1987) the increases in lymph flow due to intermittent compression of the sheep hindlimb were described with relation to the effects of normal limb movement. One conclusion was that these treatments exert their main influence through increases in lymph formation rather than lymph propulsion. This has been further studied by observing the changes in lymph-promoting effect due to alterations in the size and time course of the pressure pulses applied over the lymph-forming region. The experimental outcome was analysed in terms of two different models of tissue compression, each of which might promote the entry of fluid into terminal lymphatics (as reviewed by Adair & Guyton, 1985). One proposition is that compressing the tissues raises the interstitial fluid

pressure and drives fluid directly into the terminal lymphatics. Alternatively, the lymphatics may be collapsed by compression, emptying their contents into the larger collecting ducts. Unidirectional flow would be ensured by the presence of endothelial flap valves in the terminal lymphatic walls (Allen, 1967; Leak & Burke, 1968) and by luminal valves in the larger vessels. With release of the external pressure the lymphatics should return to their resting volume, drawing fluid into themselves as they do so.

Whilst both of the mechanisms outlined above could account for a type of pumping at the terminal lymphatics, the time dependence of each would be very different. On the basis of the first model the amount of fluid moved into the lymphatics per unit time would depend only on the product of the applied pressure and the total compression time. If, however, external compression collapses the small lymphatics then the effect will tend to decrease if there is inadequate time for the lymphatics to refill between compressions. These predictions have been tested by varying the duration of cuff inflation and the time between inflations both synchronously (by changing the frequency of compression) and individually. Such a study is of physiological significance since lymph formation may normally occur against a pressure gradient due to a negative interstitial fluid pressure (Guyton, Granger & Taylor, 1971). There is evidence that this process may be assisted by tissue compression due to limb movement (McGeown *et al.* 1987), muscle contraction (Zweifach & Schmid-Schonbein, 1985) or vascular pulsation (Parsons & McMaster, 1938; Skalak, Schmid-Shonbein & Zweifach, 1984). The model of pumping which is suggested by the following experiments is likely to apply in these physiological forms of intermittent compression.

METHODS

Ewes were anaesthetized with intravenous pentobarbitone, 20–30 mg kg^{-1}, and 2–3% halothane in O_2. Metatarsal lymphatics were visualized by injecting 0·5 ml of Evans Blue into the hoof-pad and one was cannulated towards the hoof. The outflow from the cannula was placed 0–5 cm above the lymphatic and the lymph was collected on a piece of filter paper attached to the lever of a Statham UC3 tension transducer. The recorded weight of lymph was used as a measure of flow (McHale & Roddie, 1983). Outflow pressure was measured from a cannula side branch to a Statham P23 transducer. Intermittent external compression was applied through a pneumatic cuff, sited distal to the fetlock, over the region responsible for formation of more than 90% of the lymph flowing through the cannulated duct (J. G. McGeown, N. G. McHale & K. D. Thornbury, unpublished observation). Cuff pressure was measured with an air-filled transducer and all recordings were made on a Gould 2400S recorder.

Cuff inflation was controlled with an electronic timer. The effects on lymph flow of (a) compression frequency, (b) duration of cuff deflation between inflations, (c) duration of cuff inflation and (d) pressure applied during cuff inflation were investigated. In each case the time period containing intermittent compression lasted less than 2·5 min. Since flow returned to control levels 2·5 min after the end of intermittent compression in an earlier study (McGeown *et al.* 1987) it was assumed that all the flow changes due to intermittent compression would be apparent within 5 min of the start of cuff inflation. Flow for that period was therefore compared with flow in the preceding (control) 5 min in each case.

Summarized results have been expressed as the mean $\pm$ 1 standard error of the mean (S.E.M.) and the statistical significance of comparisons determined by using the paired Student's t test.

RESULTS

Compression frequency

The effect of compression frequency was studied by dividing a cumulative cuff inflation period of 64 s into varying numbers of individual compressions with a cuff inflation pressure of 160 mmHg. Cuff inflation and deflation times were equal in each case and the range of frequencies ran from one pulse (64 s on, 64 s off) to sixty-four pulses (1 s on, 1 s off) in a period of 128 s. Figure 1 shows a section from the

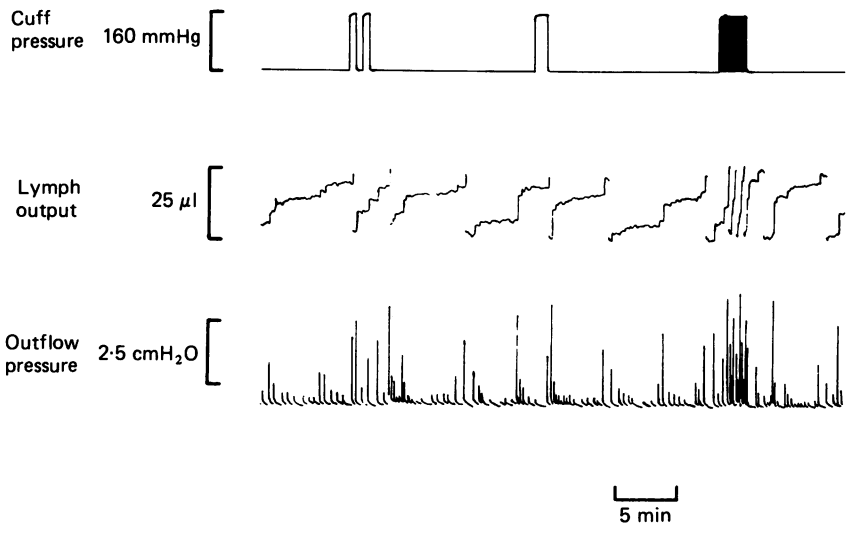

Fig. 1. Pneumatic cuff pressure (top trace), lymph flow (middle trace) and lymph pressure (bottom trace) are shown for a hindlimb afferent lymphatic. The cuff was placed distal to the fetlock and inflated to 160 mmHg. A total inflation time of 64 s was first applied as two pulses (32 s on, 32 s off), then one pulse (64 s on, 64 s off) and thirdly sixty-four pulses (1 s on, 1 s off).

record obtained in one such experiment. Lymph flow (middle trace) occurs as a series of steps, each associated with a peak in lymphatic pressure (bottom trace) due to lymphatic contraction. The first compression sequence of two pulses (32 s on, 32 s off; top trace) produced a clear increase in lymph flow, from 1·4 μl min^{-1} in the control 5 min to 7·0 μl min^{-1} in the 5 min containing the compressions. After a further 10 min a single pulse (64 s on, 64 s off) was applied. This had little, if any, effect on flow (3·4–3·7 μl min^{-1}). In marked contrast to this the third compression sequence (sixty-four pulses) produced a dramatic increase in flow from 4·8 to 20·9 μl min^{-1}. These differences occurred despite the fact that the same total cuff inflation time was used in each case.

Results from five experiments following the protocol outlined have been summarized in Fig. 2. Lymph flow is plotted against compression frequency (number of compressions per 128 s) for the seven inflation patterns used. The filled circles (continuous line) represent the mean flows for the 5 min containing compression and the open circles (dashed line) those in the 5 min preceding these periods, i.e. the control flows. Compression produced an increase in flow at all the frequencies studied

but the effect increased with increased frequency. The gradient of this frequency relationship, however, decreases with increasing frequency.

One explanation of the frequency effects described above could be that external compression over the region of lymph formation empties the terminal lymphatic vessels towards the collecting duct. For the small vessels concerned to contribute further to lymph formation they would have to refill during the period of cuff

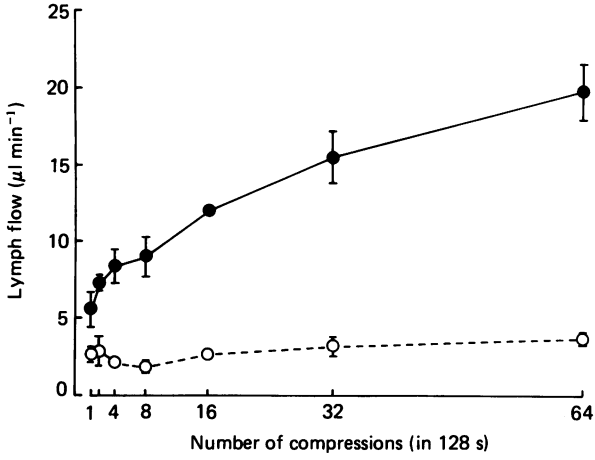

Fig. 2. The effect of compression frequency on lymph flow in five lymphatics. The mean flow for the 5 min containing the compressions (●) is plotted against the number of compressions applied. Controls (○) represent the mean flows for the 5 min preceding intermittent compression at each frequency. Vertical bars represent ±1 S.E.M.

deflation. According to this model the increase in lymph flow due to intermittent compression would depend on the time available between compressions for refilling of the terminal lymphatics as well as the number of compressions. This time decreases as frequency of compression increases. A series of experiments was carried out to study this by controlling the cuff deflation time independently so as to isolate its effects from those of the other changes inherent in the frequency study (duration of compression per pulse and total number of pulses).

Duration of cuff deflation

If intermittent compression is commenced with the cuff deflated there is a prolonged period of cuff deflation prior to the first inflation. To circumvent this problem the cuff was inflated to a constant pressure throughout the control periods (Fig. 3, top trace). At the start of the test period the cuff was deflated for a fixed number of seconds and then reinflated to the original pressure. In this way each inflation was preceded by the same deflation period. A low cuff pressure (40 mmHg) was chosen to minimize the effects of compression on blood flow during the control period since these might alter lymph flow (Pippard & Roddie, 1986). Each compression pulse was maintained for 8 s regardless of the deflation time. After the release of the fourth compression the cuff remained deflated for the rest of the 5 min test period. It was then reinflated to 40 mmHg and the procedure was repeated using a different deflation period. Lymph output and output pressure were recorded

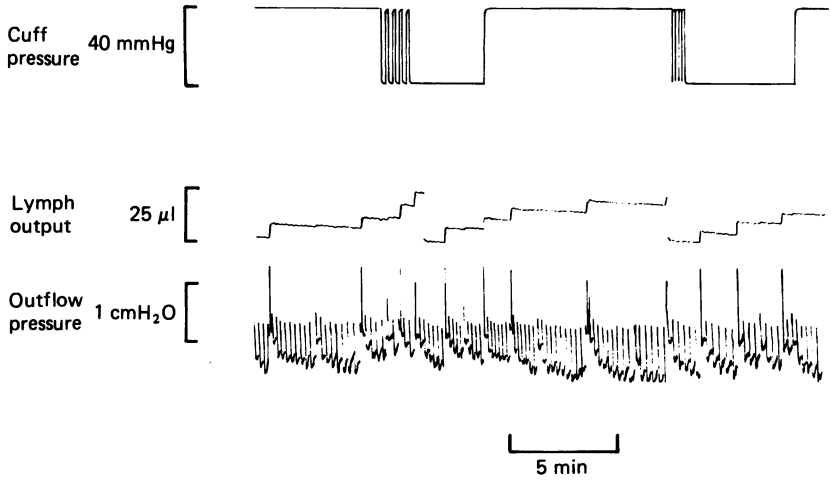

Fig. 3. The effect of four cuff inflations lasting 8 s each on lymph flow (middle trace) and lymph pressure (bottom trace). The deflation time between inflation was 12 s in the first period and 1 s in the second. The cuff was inflated to a constant 40 mmHg during the control periods preceding intermittent compression (top trace).

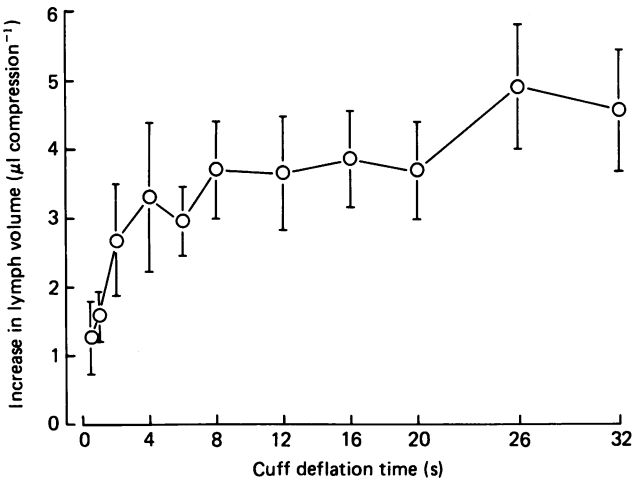

Fig. 4. The mean increase in lymph output due to each applied compression has been plotted against the deflation time between compressions for eight lymphatics. The cuff was inflated four times to 40 mmHg for 8 s in each case. Vertical bars represent ± 1 S.E.M.

throughout (Fig. 3, middle and lower traces, respectively). In the example shown, four compressions separated by 12 s (left-hand compressions) more than doubled lymph flow while four compressions of the same magnitude and duration had almost no effect when separated by only 1 s (right-hand compressions).

The protocol outlined above was applied to eight preparations using deflation times from 0·5 to 32 s. The results have been plotted in Fig. 4. The volume of lymph expelled during the 5 min control period was subtracted from that collected over the subsequent 5 min experimental period for each deflation time. This difference has been divided by 4 to give the increase in lymph output due to compression in

microlitres per compression and the mean value is plotted against the relevant deflation time. The increase in lymph flow on compression rises rapidly from $1{\cdot}3 \pm 0{\cdot}5$ μl per compression ($P < 0{\cdot}05$) with 0·5 s between inflations to $3{\cdot}9 \pm 0{\cdot}7$ μl per compression ($P < 0{\cdot}001$) at a deflation time of 8 s. The curve plateaus out with longer intervals so that lymph output was increased by $4{\cdot}5 \pm 0{\cdot}9$ μl per compression ($P < 0{\cdot}0001$) with a deflation time of 32 s. This was not significantly greater than the effect at 8 s despite the fourfold increase in deflation time. In a series of control experiments simply deflating the cuff from a steady 40 mmHg failed to significantly alter lymph flow in the absence of any further inflations.

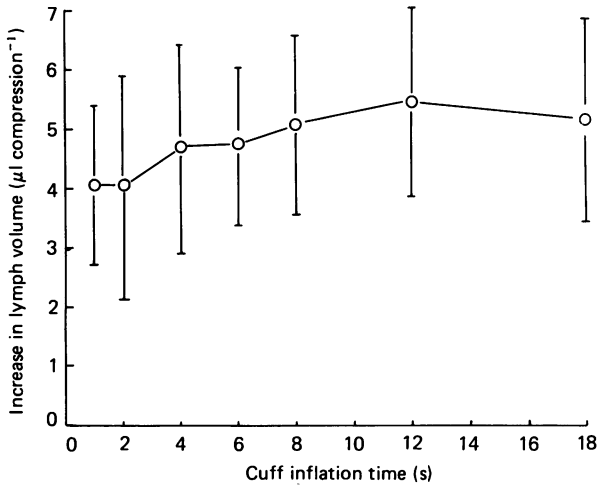

Fig. 5. Mean increases in lymph output due to each applied compression have been plotted against the duration of cuff inflation for five lymphatics. The cuff was inflated four times to 40 mmHg in each case and there was 8 s between inflations. Vertical bars represent ± 1 S.E.M.

Duration of cuff inflation

Figure 5 summarizes results from a series of five experiments in which the effect of varying the inflation time was studied using a protocol similar to that described for deflation time changes. Flow during 5 min containing four compressions to 40 mmHg was compared with flow in the previous 5 min, during which the cuff was held at that pressure. The cuff deflation time was held constant at 8 s and cuff inflation time varied from 1 to 18 s. There was a statistically significant ($P < 0{\cdot}05$) increase in flow due to compression for all pulse durations except 2 s. The results have been expressed as the increase in lymph output per applied compression and increasing the cuff inflation time had no effect on the resultant increase in flow with an increase of $4{\cdot}1 \pm 1{\cdot}3$ μl per compression at 1 s only rising to $5{\cdot}1 \pm 1{\cdot}7$ μl compression when each inflation lasted 18 s.

Cuff inflation pressure

In the final series of experiments in this study, the effects of varying the cuff inflation pressure were examined. The cuff was inflated eight times from zero

pressure and inflation and deflation times were both 8 s. The results from six animals have been summarized in Fig. 6 in which the mean flows for the 5 min containing intermittent compression and the control flows for the preceding 5 min are plotted against the applied pressures. Each of the pressures, except 40 mmHg, produced a statistically significant increase in flow ($P < 0{\cdot}05$). The size of this flow increase rose steadily with inflation pressure (from $+3{\cdot}7 \pm 1{\cdot}3$ μl min^{-1} at 20 mmHg to $+29{\cdot}9 \pm 9{\cdot}4$ μl min^{-1} at 320 mmHg).

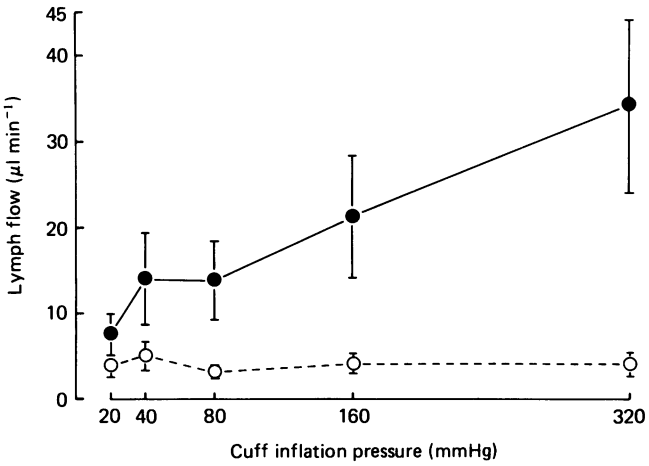

Fig. 6. Mean flow rates from five lymphatics for the control 5 min preceding (○) and test 5 min containing (●) intermittent compression have been plotted against the inflation pressure used. Eight compressions (8 s on, 8 s off) were applied at each pressure. Vertical bars represent ± 1 S.E.M.

DISCUSSION

Having established that tissue compression increases lymph flow largely due to its effects on lymph formation (McGeown *et al.* 1987) the more detailed experiments described in this paper were designed to throw light on the mechanisms involved. In particular, it was hoped that they would distinguish between two models which might account for the effects of compression. In one the applied pressure increases the pressure of the interstitial fluid favouring its movement down a hydrostatic gradient into the terminal lymphatics. This explanation has been put forward by several workers to account for the dependence of peritoneal fluid absorption by the diaphragmatic lymphatics on respiration (McCallum, 1903; Florey, 1927). A similar argument has been applied to explain filling of the subendocardial lymphatics (Patek, 1939) and the lymphatics of the intestinal villi (Drinker & Yoffey, 1941).

Another possibility is that compression acts to collapse the lymphatics, producing centripetal flow because of the valve action of the endothelial flaps in the walls of the terminal lymphatic (Leak & Burke, 1968; Leak, 1980). When the external pressure is released tissues and lymphatics passively recoil to their original dimensions. The volume of the terminal lymphatics can only be restored by the entry of fluid from the tissues since lymphatic valves prevent backflow from the ducts (Zweifach & Prather, 1975). Repeating compression after the lymphatics refill should further augment

lymph flow. Just such a mechanism for lymph formation has been proposed by some investigators who have studied diaphragmatic lymphatics (Allen & Vogt, 1937; Allen, 1956).

Each of these models of compression leads to different predictions of the time dependence of lymph flow responses to external compression. If there is an increase in tissue fluid pressure forcing lymph into the terminal lymphatics then the increase in flow should depend on the total time over which pressure is applied. This was clearly not the case in these experiments (Fig. 5). Furthermore, when the frequency of compression was altered (Fig. 2), the increase in flow depended on the number of compressions applied even though the total period over which the pressure was increased remained constant. This suggests that the effect of tissue compression on flow cannot be explained in terms of increased tissue pressure forcing fluid into lymphatics.

It is, however, much easier to reconcile the observed time dependence of compression-induced rises in lymph flow with the second model outlined above. If each pressure pulse rapidly empties the affected lymphatics then the greater the number of pulses the greater will be the change in flow. This will be limited by the volume of fluid contained by the lymphatics at the time of compression which will in turn depend on the time available for filling between compressions. This dependence was clearly demonstrated in experiments in which only the time between compressions varied (Fig. 5), with a fourfold increase in effect between the shortest and the longest deflation period used. The shape of this curve is also interesting since it plateaus out at an inflation interval of about 8 s, suggesting that filling of the lymphatics in the cuff region is complete in this time.

Increasing the applied pressure increased the effect on lymph flow (Fig. 6). This can be explained using either model of compression and lymph flow. Accepting that the time course of the responses described makes it hard to accept the 'pressure pushes fluid in' hypothesis, it seems likely that increasing pressure leads to compression of lymphatics in anatomical pressure shadow areas (e.g. between the keratinous toes or subjacent to the fetlock) which are protected from collapse at lower cuff pressures. On this basis increasing the pressure might effectively compress a greater number of lymphatics without altering the effect on each compressed vessel. It is interesting to note that pressures as low as 20 mmHg significantly increased lymph flow. This is within the range of pressure fluctuations which might be produced in the tissues by arterial pulsation, an observation of possible significance since vascular pulsation may provide a physiological source of intermittent compression. Indeed, Parsons & McMaster (1938) have shown that lymph flow is faster in the rabbit ear when it is perfused in a pulsatile rather than a non-pulsatile fashion.

These studies make it clear that tissue compression is a potent stimulus to lymph formation, the increases in flow probably being the consequence of lymphatic collapse with refilling during the periods in which pressure is released. They suggest that the lymphatics distal to the sheep's fetlock refill totally in about 8 s after release of compression. This information is likely to be physiologically relevant since lymphatics may be regularly compressed by weight-bearing movement (McGeown

et al. 1987), cardiac or skeletal muscle contractions (Zweifach & Schmid-Schonbein, 1985), arterial or arteriolar pulsation (Skalak *et al.* 1984) or microvascular vasomotion (Intaglietta & Gross, 1982).

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