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THE EFFECT OF INTRAVENOUS ADRENALINE AND NORADRENALINE INFUSION ON PERIPHERAL LYMPH FLOW IN THE SHEEP

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SUMMARY

1. Pressure fluctuations and lymph flow were measured in popliteal, prefemoral and mesenteric efferent lymphatic vessels in conscious sheep.

2. Intravenous adrenaline infusion increased frequency of lymphatic contraction and lymph flow in all three vessels. In the case of the prefemoral vessels flow remained high after the infusion had stopped.

3. Intravenous noradrenaline infusion also increased frequency of contraction and lymph flow in all three vessels but prefemoral flow was depressed after the infusion had stopped.

4. Lymphatic frequency of contraction and lymph flow increased when the animals were startled.

5. Anaesthetizing the animals with pentobarbitone did not abolish lymphatic contractions, nor did it prevent the response to adrenaline and noradrenaline infusions.

6. The most obvious interpretation of these results is that adrenaline and noradrenaline act by increasing frequency and force of pumping of lymphatic vessels.

INTRODUCTION

Intravenous infusions of adrenaline and noradrenaline are known to increase thoracic duct lymph flow in many animals (Doemling & Steggerda, 1962, Fujii & Wernze, 1966, Schad, Folwaczny, Brechtelsbauer, Birkenfeld & Kramer, 1977). The mechanism of this increase is not clear since thoracic duct flow is the resultant output of many different lymphatic vessels, some of which could be increasing their flow rates while others were decreasing, the net result still being an increase. Nor is it clear whether these drugs are acting to increase lymph production or to increase lymph propulsion. Adrenaline for example is known to increase muscle blood flow and this may well be accompanied by an increase in lymph production and thus flow.

In this study we have looked again at the effects of noradrenaline and adrenaline on peripheral lymph flow in sheep, measuring that from popliteal, prefemoral and mesenteric efferent vessels. The first drains mainly skin, the second muscle and skin, while the last drains mainly the intestine and mesentery. Thus if adrenaline and

noradrenaline were changing lymph flow by an effect on blood flow, one might expect the lymph output from these three vessels to be affected differently.

A brief account of part of this work has been communicated to the Physiological Society (McHale & Roddie, 1983*a*).

METHODS

Sheep of either sex weighing 45–55 kg were anaesthetized by inducing with pentobarbitone (20–30 mg/kg) and maintained with 1–2% halothane. Sterile Evans Blue (1% in buffered saline) was injected into the drainage area of the lymphatic in question to outline the vessels clearly. The efferent vessels were cannulated against the direction of lymph flow with polythene tubing (Portex P.P. 50, internal diameter 0·58 mm for the popliteal and prefemoral vessels and P.P. 90, internal diameter 0·86 mm for the mesenteric vessels). Animals were housed in metabolism cages and had unlimited access to food and water. They were allowed to recover for 24 hr before experiments were done.

Measurements were made of lymphatic pressures as described by Hall, Morris & Woolley (1965). The polythene tube from the sheep was connected to a T-piece the side arm of which was connected to a Statham P23 pressure transducer. The remaining limb of the T-piece was connected to a 30 cm length of outflow tubing the tip of which was set approximately at the level of the head of the humerus in the standing sheep. This tube served the dual purpose of providing a small aribitrary resistance to enable pressure measurements to be made at the side arm and of carrying fluid to the flow measuring device described by Johnston, McHale & Gordon (1983). This consisted simply of a Statham UC3 transducer with a small piece of blotting paper attached to the tip of its lever. A fibre teased from this allowed a fluid bridge to form between the tip of the polythene tube and the lever. Lymph could thus accumulate on the transducer arm with a minimum of surface tension artifact so that the tension measured was an accurate reflection of the volume of lymph leaving the cannula. When enough had accumulated to form a drop this fell off and the voltage output reset to a new level. Lymph density was measured and the output calibrated in μl. For the purpose of summarizing experiments, flow was expressed as ml. hr^{-1} and represented the mean flow over the period specified. The frequency of contraction was also expressed as the average for the specified period. Because of the complex wave forms of some of the pressure recordings, frequency was measured independently by the two authors and the results averaged. The two estimates, however, rarely differed by more than 10%.

Blood pressure was recorded by cannulating the femoral artery and connecting the cannula to a Statham transducer (P23), the output of which was both directly recorded and fed via a Devices Ratemeter to give heart rate. Recordings were made on a Devices M4 recorder.

Saline was infused continuously via a cannula in the saphenous vein during the course of the experiment at a rate of 1 ml. min^{-1} except during the period of drug administration when the inflow cannula was moved to an identical infusion pump delivering, at the same rate, either noradrenaline acid tartrate (Levophed Winthop Laboratories) or adrenaline tartrate (Evans Medical) of appropriate concentration.

RESULTS

The pattern of outflow pressure fluctuations was variable but in all cases clearly pulsatile. Pressures recorded from popliteal and prefemoral vessels often showed single discrete pulses as though representing activity in a single lymphatic vessel, presumably the length of duct between the node and the cannula. This would imply that the pulses generated by the afferent vessels were effectively damped in their passage through the node. The most complex wave forms were those of the mesentric lymphatics, due likely to the summed activity of the lymphatic cannulated and that of its main branches. The resting frequency of contraction in popliteal vessels ranged between 2·7 and 8·5 contractions per minute, mean 5·9, S.D. 1·5, $n = 11$ giving a mean resting flow of 4·7 ml. hr^{-1}, S.D. 2·8, $n = 11$.

Popliteal vessels

The record shown in Fig. 1*A* is reasonably typical of the activity of popliteal efferent vessels. The vessel contracted in a fairly but not absolutely regular fashion at a frequency of about 8 min^{-1}. Contractions are represented by the pressure increases of the lower record. Each contraction resulted in a volume of lymph being added to the isometric transducer lever resulting in the stepwise increase in tension shown in the upper record. The upper record then remained flat until the next

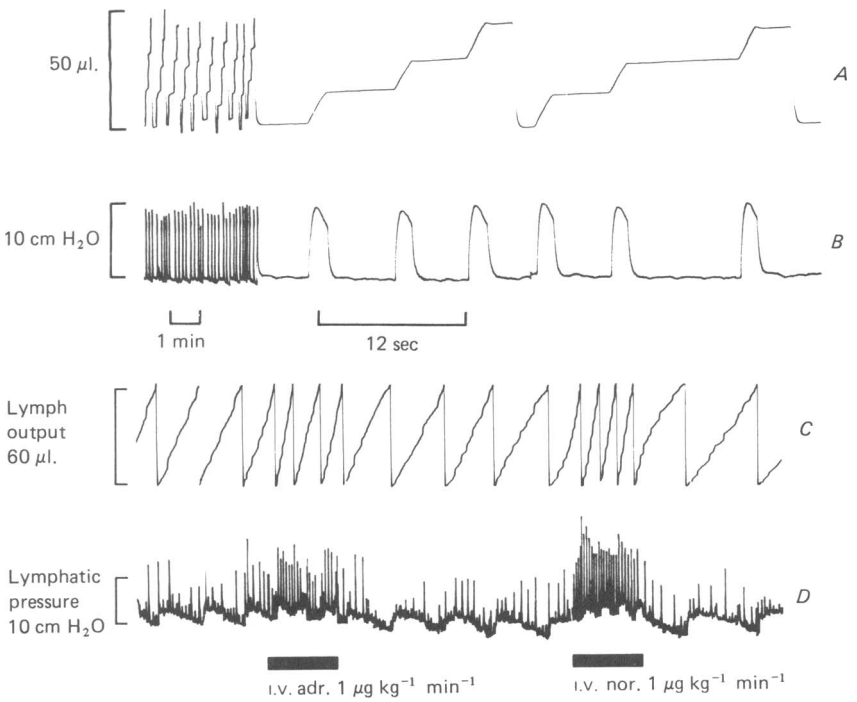

Fig. 1. Traces *A* and *B* show simultaneous recordings of lymph flow and pressure respectively, obtained from a popliteal efferent vessel. When the lymphatic contracted, the outflow pressure increased and this led to a tension increment in the flow record due to lymph accumulation on the transducer lever. Traces *C* and *D* show simultaneous recordings of lymph flow and pressure respectively from a popliteal efferent lymphatic during intravenous infusions of adrenaline (adr.) and noradrenaline (nor.). The bars indicate the 5 min periods of drug infusion.

contraction expelled a further quantity of lymph and so on. The volume of lymph expelled during each contraction although fairly constant in this record varied between 10 and 15 µl.

Fig. 1*B* shows the effect of 5 min consecutive infusions of adrenaline and noradrenaline in a dose of 1 µg kg^{-1} min^{-1} on lymph flow from a popliteal efferent vessel. Prior to adrenaline infusion flow was 1·4 ml. hr^{-1} at a frequency of 1·6 contractions per minute. During adrenaline infusion, flow increased to 2·0 ml. hr^{-1} while frequency of contraction increased to 4 min^{-1}. Flow and frequency then returned to pre-drug level. During infusion of noradrenaline frequency increased to

5 min^{-1} while flow increased to 2·8 ml. hr^{-1}. Following noradrenaline infusion, flow was depressed for a period of about 15 min. The greater increase in flow produced by noradrenaline was due to a greater increase in both frequency of contraction and in the amount expelled per contraction.

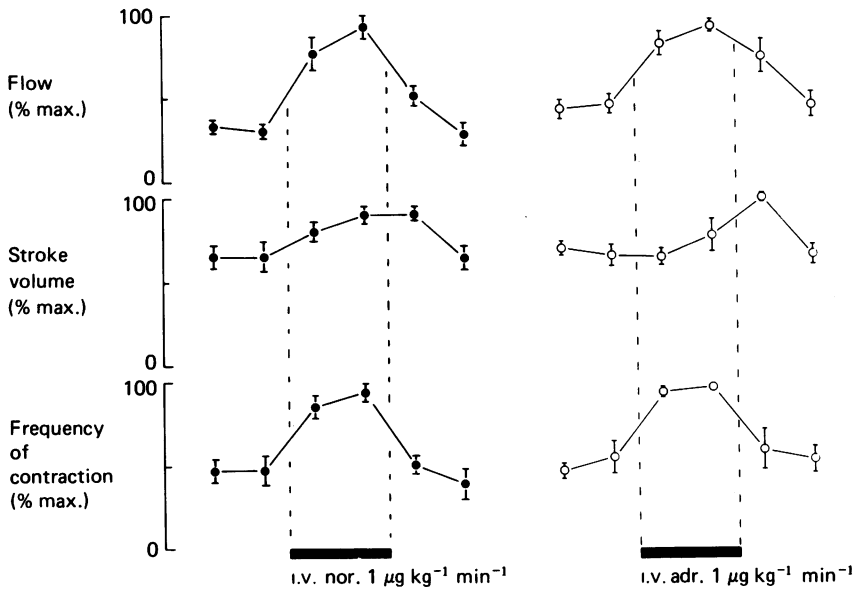

Fig. 2. Summary of the effects of noradrenaline (left hand panel) and adrenaline infusions (right hand panel) on popliteal lymph flow, stroke volume and contraction frequency in two different sets of five sheep. Frequency and flow were averaged over six 2·5 min periods, two before, two during and two after drug infusion. Stroke volume was obtained by dividing flow by frequency. Results were expressed as percentage of the maximum for any given sheep. Each point represents the mean ± S.E. of mean for the five values.

A summary of the results of noradrenaline infusions in five sheep is shown in the left-hand panel of Fig. 2. Frequency and flow were averaged over 2·5 min periods, two before, two during and two after noradrenaline infusion. The results were then expressed as a percentage of the maximum for any given sheep and the means calculated. The vertical lines represent plus or minus one standard error of the mean in each case. Noradrenaline almost doubled the frequency of contraction and this was accompanied by an almost three-fold increase in flow. The original values of stroke volume for the second and fourth points were compared by a paired t test and the difference was found to be significant ($P < 0{\cdot}01$). The second panel of Fig. 2 shows the results of adrenaline infusion in five different sheep. The method of presentation is the same as for noradrenaline. Frequency of contraction approximately doubled as before but stroke volume was not significantly increased with the result that flow was not increased as much as with noradrenaline.

Prefemoral lymphatics

The effect of intravenous infusion of 1 μg kg^{-1} min^{-1} noradrenaline on prefemoral vessels is shown in Fig. 3*A*. In addition to lymphatic pressure and lymph output

(middle two records) arterial pressure (top) and heart rate (bottom record) are shown. Prior to drug infusion the frequency of contraction averaged 3 min^{-1} and this increased during the latter part of drug infusion to a maximum of 7 min^{-1}. Lymph flow increased from a pre-drug level of 2 ml. hr^{-1} to a maximum of 9 ml. hr^{-1} in the latter part of the drug infusion period. Mean arterial pressure increased from about 100 to 140 mmHg, while heart rate decreased from 110 to 80 beats min^{-1}. After drug infusion lymph flow was often depressed for 10–15 min. Fig. 3*B* shows a similar experiment in which adrenaline was infused for 5 min. The main differences were that

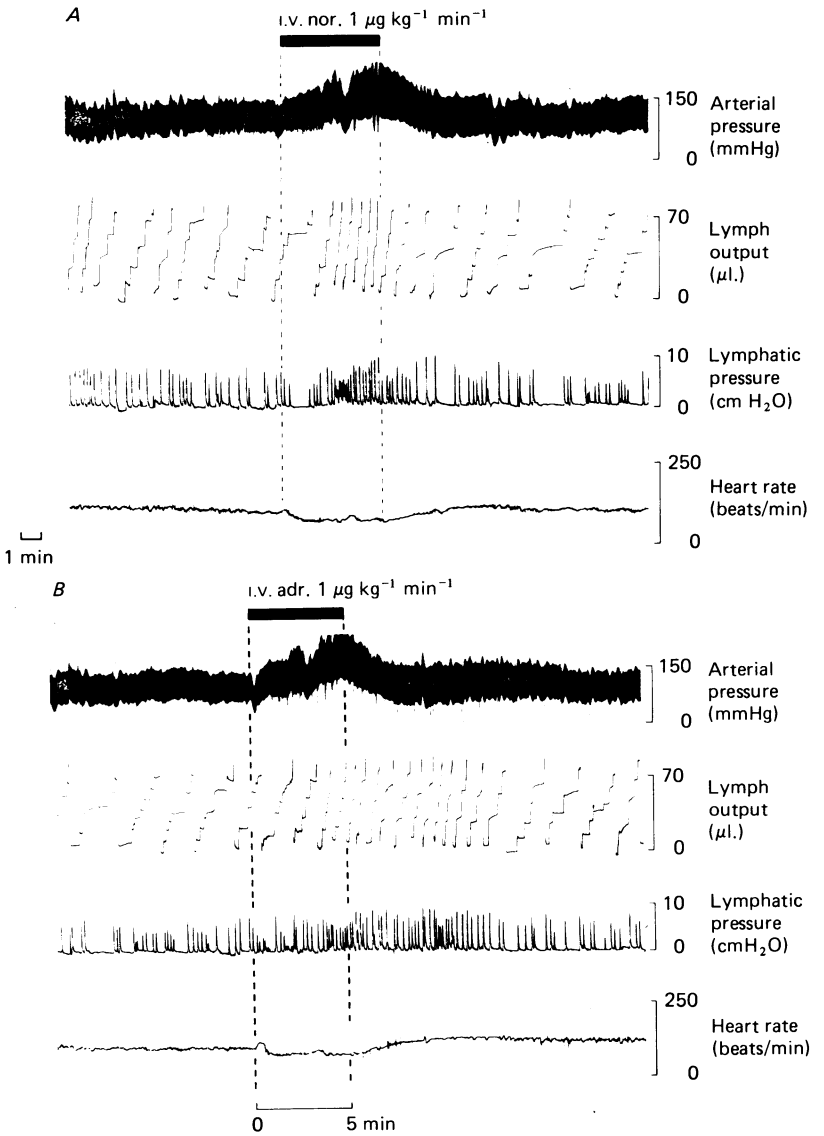

Fig. 3. A comparison of the effect of infusion of noradrenaline (*A*) and adrenaline (*B*) (1 μg kg^{-1} min^{-1}) on prefemoral lymph flow and pressure, arterial pressure and heart rate in a conscious sheep.

there was a transient increase in heart rate of about 10 beats min^{-1} followed by a decrease of about 20 beats min^{-1} below resting level. Blood pressure showed a transient fall followed by a rise to 150 mmHg mean pressure. Lymph flow did not increase much during the first 3 min of the adrenaline infusion but remained high for more than five minutes after infusion, in contrast to the response to noradrenaline when flow was depressed after the infusion.

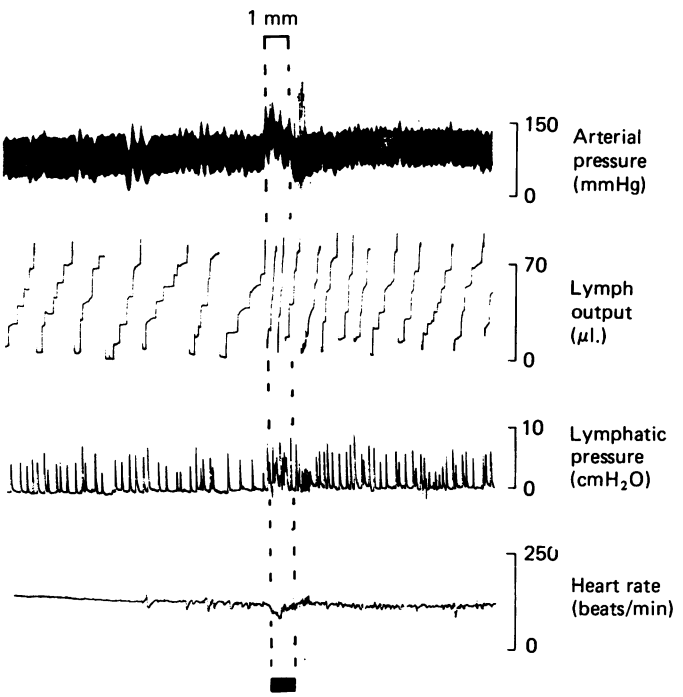

Fig. 4. The effect on prefemoral contractions and flow of restraining the sheep's head for the 1 min period between the dotted lines on prefemoral lymph flow and pressure, arterial pressure and heart rate in the conscious sheep.

Response to emotional stimuli

In early experiments it was observed that lymph flow appeared to increase when the animal was startled, so an attempt was made to standardize this stimulus. This was done by restraining the animal's head manually for a period of 1 min. During this time lymph flow increased dramatically (Fig. 4) from a resting value of 2·5 ml. min^{-1} to 9·6 ml. min^{-1} during the period of restraint. Flow also remained high for several minutes after the animal was released. This response seemed almost like a mixture of the noradrenaline and adrenaline effects. Arterial pressure was also transiently increased while heart rate fell but these effects were very short lived.

In experiments on five sheep, flow was averaged over the 5 min period prior to restraining the animal, the 1 min period of restraint and the 5 min period after restraint. Flow increased from a mean value of 2·7 ml. $hr^{-1} \pm 0·7$ (S.E. of mean) to $7·3 \pm 1·2$ ml. hr^{-1} during restraint and then fell to $4·7 \pm 0·7$ after the fright stimulus.

The effect of adrenaline and noradrenaline infusions in anaesthetized animals

In the light of the previous experiments it could be argued that adrenaline and noradrenaline were having their effect by increasing anxiety in the conscious animals, perhaps resulting in increased movement. For this reason the infusion experiments were repeated on anaesthetized animals. Fig. 5 shows the effect of 0·5 μg kg^{-1} min^{-1} noradrenaline on an animal anaesthetized with pentobarbitone (the lower noradrenaline dose was used because the pressor effect of noradrenaline appeared to be magnified in the anaesthetized animals). Flow was again increased during the period of drug infusion and depressed afterwards. When adrenaline was infused the flow increased more during the infusion period but returned to a level after infusion that was still greater than before.

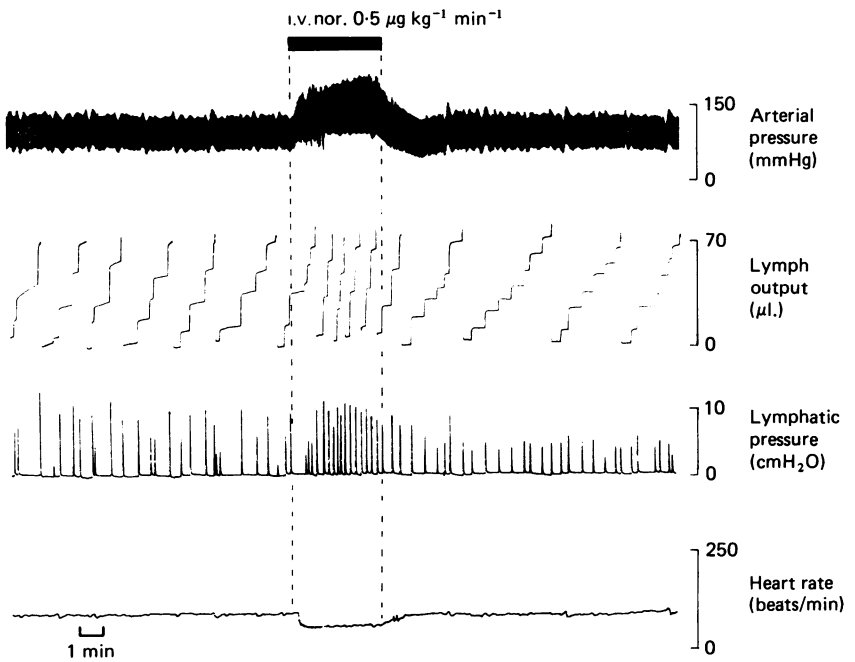

Fig. 5. The effect of a 5 min infusion of noradrenaline 0·5 μg. kg^{-1} min^{-1} on prefemoral lymph flow and pressure, arterial pressure and heart rate in an anaesthetized sheep.

Mesenteric vessels

Flow in mesenteric vessels was less regular than in popliteal and prefemoral vessels. The pattern fluctuated between periods of quiescence and rapid flow. Pressure changes consisted either of discrete pulses or sustained elevations, the latter coinciding with the periods of rapid flow. When adrenaline 1 μl. kg^{-1} min^{-1} was infused (Fig. 6 bottom panel) the resting frequency of contraction increased from 10 to 16 min^{-1} while flow increased from 7·5 to 39 ml. hr^{-1}. After the infusion had stopped, flow remained high for several minutes but then declined to a level lower than that seen before the drug was administered. Noradrenaline infusion (Fig. 6, bottom panel) also increased frequency and flow but the effect was less dramatic than

with adrenaline. Frequency of contraction increased from 9 min^{-1} before infusion to 12 min^{-1} during, while flow increased from 10·9 ml. hr^{-1} before to 14 ml. hr during infusion.

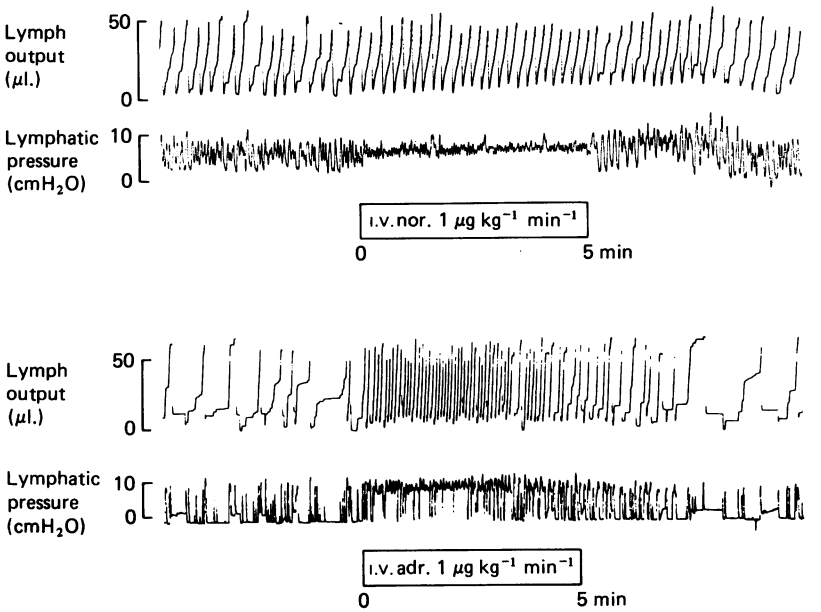

Fig. 6. The effect of a 5 min infusion of noradrenaline (upper panel) and adrenaline (lower panel) on mesenteric lymphatic contractions and lymph flow in two conscious sheep.

DISCUSSION

There is little concensus on the effect of catecholamines on lymph flow. Hall, Morris & Woolley (1965), for example, found that a 15 min infusion of adrenaline (50 μg min^{-1}, a dose similar to that used in the present study) had no effect on thoracic duct pressure or lymph flow in sheep. Foldi & Zoltan (1966) found that noradrenaline depressed thoracic duct flow in the dog while adrenaline enhanced it. In contrast, DeMicheli & Glasser (1975) found that both adrenaline and noradrenaline increased canine thoracic duct flow, the latter being more potent. This view was shared by Schad *et al.* (1977). These contradictions are less surprising than at first sight they might appear when the potential complexity of the response is considered. There may be at least three sites of action of these drugs; they may affect blood flow to the region drained and thus lymph production, they may act directly on the lymphatic vessels themselves or they may reflexly activate the autonomic nervous system which could in turn act by either or both of the above means.

The results of this study are difficult to reconcile with an action solely on blood flow although this might represent one component of the response. Adrenaline is well known to have the capacity to increase skeletal muscle blood flow (Allen, Barcroft & Edholm, 1946) so this could account at least in part for the increase in lymph flow from the prefemoral vessel. In this context it is interesting to note the difference in time course between the prefemoral response to adrenaline infusion and that of the

popliteal. In the latter case lymph flow increased almost immediately at the beginning of the infusion period and similarly decreased at the end and this was followed by a period when flow was depressed. In contrast prefemoral flow reached its peak more slowly and remained high for some time after the infusion had stopped. It would be more difficult to argue that adrenaline increased popliteal and mesenteric lymph flow by dilating arterioles since adrenaline's constrictor effect on the skin and intestinal vasculature is also well known (Green & Kepchar, 1959), Nor are the effects of noradrenaline readily explicable in terms of blood flow changes since it is known to decrease blood flow to all the regions drained by the lymphatics under investigation. (Barcroft, Gaskell, Shepherd & Whelan, 1954, Green, Deal, Bardhanabaedya & Denison, 1955, Green & Kepchar, 1959). The possibility cannot be ruled out, however, that increased lymph flow was a passive consequence of increased arterial pressure. There is evidence that intravenous noradrenaline infusions can produce a transient increase in blood flow which can be prevented by inserting a pressure compensator, indicating that it results from the increased arterial pressure (Cobbold & Vass, 1953). An increased blood flow of this type does not readily explain the lymph flow changes in the present study since their onset was too rapid. Lewis & Winsey (1970), for example, found that blood flow increased within seconds when acetylcholine was infused in the cat hind limb while lymph flow reached its peak more than 10 min later.

All of these results, on the other hand, can readily be explained in terms of a direct action on the lymphatic vessels. Lymphatic smooth muscle is known to increase its frequency and force of contraction in response to exogenous noradrenaline and this can increase fluid propulsion in isolated vessels (McHale & Roddie, 1983*b*). Increased frequency and force of pumping could account for the increased lymph flow in the face of decreased blood flow. This would be limited, of course, to the expulsion of lymph already in the lymphatics and may indeed explain the depression in lymph flow after noradrenaline infusion.

The involvement of the autonomic nervous system in lymph flow changes is potentially complex. Lymphatic vessels are known to be innervated (Florey, 1927, Browse, 1968, McHale, Roddie & Thornbury, 1980). Bert & Laffont (1882) described a dilatation of the cisterna chyli during stimulation of the caudal end of the vagus and Acevedo (1943) found that the thoracic duct was constricted when the vagus was stimulated. However the bulk of the evidence points to a noradrenergic motor innervation. Browse (1968), for example, found an increase in lymphatic tone in anaesthetized dogs proportional to frequency of sympathetic stimulation and this could not be explained by any indirect mechanism. Similarly field stimulation of isolated bovine mesenteric lymphatics leads to an increase in frequency and force of contractions with an accompanying increase in flow (McHale *et al.* 1980) and a similar response may well occur in the living sheep. This could account for the increase in flow seen when the animal was frightened and for its rapid onset. However the precise way in which the response is mediated is probably complex. Adrenaline and noradrenaline infusions may also exert their effects reflexly through the autonomic nervous system.

All the lymph collected in the course of these experiments had to pass through at least one lymph node. In its passage through the node, lymph can be profoundly modified (Adair, Moffatt, Paulsen & Guyton, 1982) due to exposure to the very rich

nodal vasculature. Very little is known about the effects of catecholamines and sympathetic nerves on these blood vessels but it is possible that lymph flow could be greatly altered by such effects. If this were the case yet another mechanism could be proposed to explain the results of this study.

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