J. Physiol. (1968), **197**, *pp.* 25–36
With 7 text-figures
Printed in Great Britain

RESPONSE OF LYMPHATICS OF CANINE HIND LIMB TO SYMPATHETIC NERVE STIMULATION

By N. L. BROWSE

From the Department of Surgery, St Thomas' Hospital, London, S.E. 1

(*Received* 20 *October* 1967)

SUMMARY

1. The changes in lymphatic pressure in a limb whose circulation was temporarily arrested with a pneumatic cuff have been studied.

2. Stimulation of the lumbar sympathetic chain caused an increase in lymphatic pressure. It has been shown that this is a primary not a secondary phenomenon, due to an active lymphomotor mechanism.

3. The increase of lymphatic tone is proportional to the rate of stimulation; peak values are reached between 5 and 9 impulses/sec.

INTRODUCTION

One of the first detailed descriptions of fine nerve endings in and around the walls of the large lymphatic vessels, particularly the thoracic duct, was by Quenu & Darier (1887). This study was stimulated by the experiments of Bert & Laffont (1882), who had, five years earlier, described a constriction of the lacteals following electrical stimulation of the mesenteric nerves, a dilatation after stimulating the splanchnic nerves and a dilatation of the cisterna chyli during stimulation of the caudal end of the divided vagus nerve. A little later Camus & Gley (1894) reported that stimulation of the distal end of the left splanchnic nerve caused dilatation of the cisterna chyli. Florey (1927) stated that the lacteals of the mesentery contracted when the splanchnic sympathetic nerves were stimulated and Acevedo (1943) described a constriction of the thoracic duct in response to stimulating the vagus nerve. Rusznyák, Földi, & Szabó (1950) reported spasm of the lymphatics of the dog's leg during electrical stimulation of the lumbar sympathetic chain. Most of these studies were based upon visual observations of the width of the vessels under study. However, visual observations take no account of alterations in calibre due to changes of pressure and flow so although the presence of nerve endings, smooth muscle fibres and apparent changes in diameter following nerve stimula-

tion constitute circumstantial evidence for postulating a neurogenic lymphomotor mechanism, they do not prove that it exists.

The simplest way to demonstrate an active change in the tension of the wall of a fluid conducting tube is to show a change in its resistance to flow. This technique is easily applied to the arteries and veins, but is extremely difficult to apply to the lymphatics since normal lymph volume-flow is very small and the changes of resistance minute. However, it is possible to detect a change of tone when flow is zero provided that the intraluminal volume does not change. In such conditions an increase in tone will produce an increase of intraluminal pressure. This fact has been used to study nerve mediated changes of venous tone (Browse, Lorenz & Shepherd, 1966; Browse, Donald & Shepherd, 1966) and it seemed worth while to attempt to apply it to the lymphatics. The required conditions, zero flow and constant volume, can be achieved either by isolating a short segment of a vessel, as in the 'isolated vein technique' (Burch & Murtadha, 1956) or by isolating a whole vascular bed as in the 'occluded limb technique' (Samueloff, Bevegard & Shepherd, 1966; Browse, Lorenz & Shepherd, 1966).

This study describes the responses of the lymphatics in the dog's hind limb to electrical stimulation of the sympathetic nerves whilst the circulation was temporarily arrested by a pneumatic cuff inflated to a supra-systolic pressure.

METHODS

The occluded limb technique. Large greyhounds (15–25 kg) were used because their hind legs, and hence their lymphatics, are large. They were anaesthetized with sodium pentobarbitone (Abbott Laboratories Ltd.), 20–25 mg/kg. I.V., intubated with a cuffed endotracheal tube, paralysed with gallamine triethiodide ('Flaxedil', May & Baker Ltd.), 3 mg/kg I.V., and ventilated with a Palmer Pump, 0–+12 cm H_2O. The abdomen was opened through a mid line incision and a bipolar platinum electrode placed on the undivided left sympathetic chain above the last lumbar ganglion.

The animal was then turned on to its right side and 0·4 ml. Patent Blue Violet (Guerbet Laboratories Ltd.) injected between the webs of the toes of the left hind limb. A short incision was made over the short saphenous vein on the outer aspect of the limb midway between knee and ankle to display the lymphatics on either side of the vein. The blue dye appeared in the lymphatics immediately the paw was massaged. A short length (0·5 cm) of the largest lymphatic was dissected and its central end cannulated, taking care not to damage the other lymphatics. The cannula was advanced 3–4 cm so that it was well up the lymphatic but not so far that it jammed into the popliteal lymph node. Part of the cannula (30 cm) was polythene (0·5 mm i.d., 1·0 mm. o.d.) swagged on to a 21-gauge needle, the last 10 cm was Teflon (0·6 mm o.d., 0·35 mm i.d.) inserted into and sealed to the polythene with epoxy resin. The central ends of a tributary of the saphenous vein and the posterior tibial artery were also cannulated with polythene cannulae (0·5 mm i.d.) just above the ankle. The venous and arterial cannula were filled with heparinized saline and connected to Statham transducers (23 De), calibrated with identical sensitivities to the same zero (tip of the cannula). The lymphatic cannula was similarly filled but a more sensitive transducer, Statham 23 BB, was used. All recordings were made on a Honeywell 1508 (Ultraviolet) Visicorder. In some experiments second cannulae were inserted into each set of vessels, i.e.

into another lymphatic, another tributary of the short saphenous vein and into another branch of the posterior tibial artery.

The patency of the pressure recording systems was checked by rapidly flushing the catheters with a high pressure. A square wave return to preflushing pressure indicated an acceptable pressure transmission. Another method of checking the adequacy of lymphatic pressure measurement was found when it was noticed that squeezing the paw when the cuff was deflated produced a marked rise in lymphatic pressure (Fig. 1).

A pneumatic cuff was placed around the thigh, as high up as possible, and covered with a Plaster of Paris bandage. It was then connected to a pressure reservoir so that it could be rapidly inflated (less than 0·5 sec) to a pressure of 300 mg/Hg when required.

The sympathetic chain was stimulated with a square wave stimulus of 15 V 0·5 msec duration, zero delay. The frequency of stimulation was varied between 0·5 and 15 impulses/sec (Medelec Stimulator, Model TS 2). Artifacts due to local spread of the stimulus to nearby motor nerves thus causing voluntary muscles in the leg to contract were abolished by the muscle relaxant.

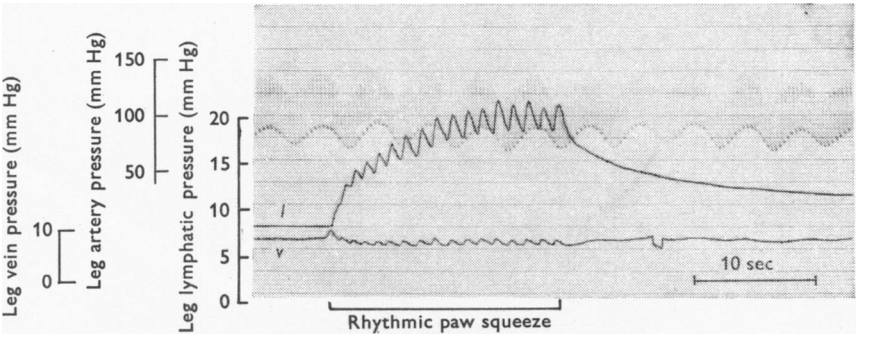

Fig. 1. The effect of rhythmic squeezing of the paw, cuff *not* inflated. Each rise in lymphatic pressure (*l*) corresponds to a squeeze. Venous pressure (*v*) rose with each squeeze but returned to the resting level afterwards. A response like this was interpreted as an indication of acceptable lymphatic pressure measurement.

The routine for each stimulation was as follows. All three catheters were flushed. The paw was then squeezed rapidly 4 or 5 times to cause a rise in lymphatic pressure coincident with each squeeze. If the responses to flushing and to squeezing the paw were adequate the cuff was inflated to 300 mg Hg. The lymphatic catheter was flushed again. After 1½–2 min the pressures within the leg stabilized and the sympathetic chain was stimulated. Although the venous and arterial responses reached their maximum within 30–50 sec, the lymphatic response was much slower, consequently the chain was always stimulated for 2 min. Before deflating the cuff the catheters were flushed again and after deflation the paw-squeeze test was repeated. A rest period of at least 10 min was given between stimulations.

The frequent checking of adequate pressure transmission from vessel to transducer was essential. Many experiments had to be discarded because the flush or paw-squeeze test was unacceptable; this happened most often when the lymphatic was small and had no tributaries.

Studies were made of the degree of damping caused by such fine catheters. It was found that the lymphatic catheter and Statham 23 BB gauge together prolonged a square wave change of 50 mg Hg to the extent that 95 % of the pressure change took 0·25 sec to record, a frequency response of 2 c/s. The frequency response of the larger arterial and venous catheters was greater than 50 c/s. Thus the lymphatic pressures shown in the figures are slightly damped but as the lymphatic response had a very low frequency the damping did not affect their accuracy.

RESULTS

Effect of inflating the cuff. After inflating the cuff the arterial pressure fell and the venous pressure rose, becoming stable after 1½–2 min. The lymphatic pressure rose by 1–2 mm Hg as the cuff expanded and then remained quite steady for as long as the cuff was inflated. If the lymphatic pressure had been altered by nerve stimulation (Fig. 2), paw squeezing or an intralymphatic injection (Fig. 5), it usually took 4 or 5 min to return to the control level. This was considerably longer than the time taken by the arterial and venous pressures to return to resting levels.

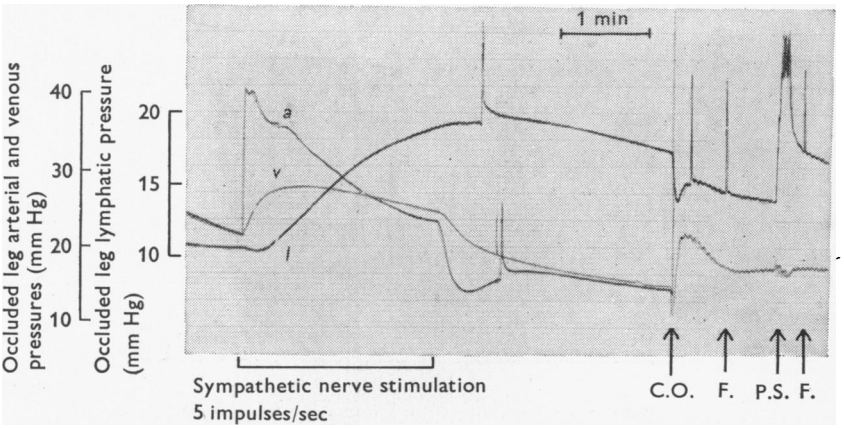

Fig. 2. Typical response of the vessels in the occluded hind limb to sympathetic nerve stimulation. Stimulation was commenced 1½ min. after inflating the cuff. The small pulsations are movement artifacts due to the positive pressure ventilation. The leg artery pressure rose off the top of the recording when the cuff was deflated. *a*, *v* and *l* = arterial, venous and lymphatic pressures. C.O. = cuff off; F = flush; P.S. = paw squeeze.

Response to sympathetic nerve stimulation. Figure 2 is typical of the responses of the vessels in the occluded hind limb to sympathetic nerve stimulation. The arterial pressure rose rapidly, almost a square wave, the venous pressure somewhat more slowly; these changes have been fully documented elsewhere (Browse, Lorenz & Shepherd, 1966). The lymphatic pressure increased slowly, the rise beginning after 5–10 sec and reaching its asymptote in 1½–2 min. The magnitude of the pressure changes in all three types of vessel was related to the rate of stimulation.

The increase of lymphatic pressure was maintained throughout the period of stimulation. The rise of pressure with rates of stimulation less than 0·5 impulses/sec. was too small to measure accurately. Although the maximum response varied from animal to animal (3–9 mm Hg) it was

generally achieved by rates of stimulation between 5 and 9 impulses/sec. Stimulation at higher rates did not increase the response.

The stimulus–response curves of nine successful experiments are to be seen in Fig. 3. The minimum number of stimuli in any experiment was four. The heavy line is the curve that best fits the arithmetical mean of

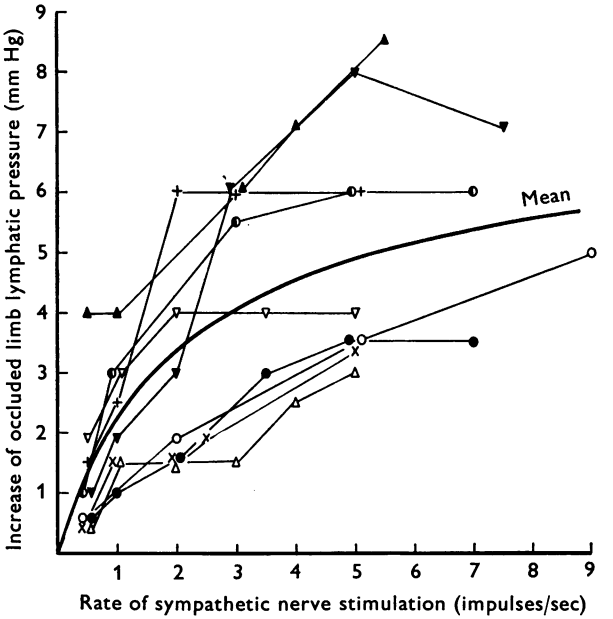

Fig. 3. The stimulus–response curves of nine experiments; minimum number of stimuli in any experiment was four. The heavy line is the best fit curve of the arithmetical means of the responses for each rate of stimulation, it is thus a crude mean stimulus response curve. Note that the curve reaches its asymptote between 7 and 9 impulses/sec.

the responses for each of the different rates of stimulation. It is thus a crude mean response curve. Further statistical evaluation of these results would be pointless.

The responses of all three types of vessel were abolished by dividing the sympathetic chain below the electrode (Fig. 4).

The intravenous administration of 4 mg atropine sulphate, a dose sufficient to abolish the vasodilator effect of sympathetic nerve stimulation, had no effect on the lymphatic response. The repeated injection of small doses (10 mg; maximum 100 mg) of Dibenyline (Smith, Kline & French) until the arterial and venous responses to stimulation were abolished also abolished the lymphatic response.

The effect on occluded limb lymphatic pressure of changing the arterial or venous pressures. The slow rise in lymphatic pressure during sympathetic

nerve stimulation, compared with the more rapid changes in arterial and venous pressure, suggested that the rise in lymphatic pressure might have been secondary to the rise in pressure in the other vessels. Therefore in an attempt to mimic the effect of sympathetic stimulation on occluded limb arterial and venous pressures, saline was injected into these vessels through additional catheters. Figure 5 shows the effect on lymphatic

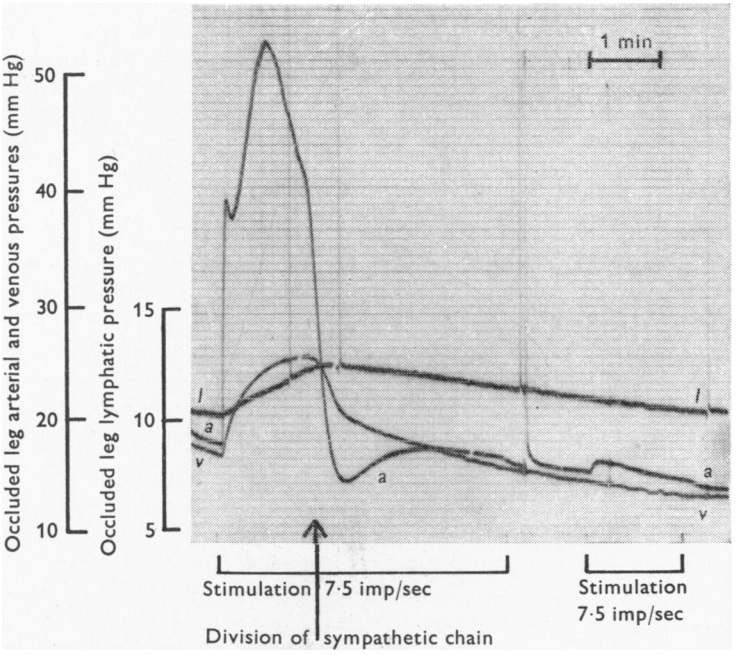

Fig. 4. The effect of dividing the sympathetic chain below the electrode during stimulation. The arterial, venous and lymphatic pressures all immediately begin to fall. The lymphatic shows the typical slow return to resting levels. A further stimulus has no effect on the lymphatic and venous pressures and only a minute effect on arterial pressure, the latter is probably due to spread of the stimulus to the chain below the transection via surrounding soft tissues.

pressure of 2·0 ml. saline being suddenly injected into the short saphenous vein of the occluded limb. There was a transient increase of venous and arterial pressure but no change of lymphatic pressure. A similar response was seen following an intra-arterial injection. However, both the injection of 2·0 ml. saline into the lymphatic and a very gentle paw squeeze produced a marked change of lymphatic pressure.

A sustained increase of venous or arterial pressure could only be produced by the continuous injection of saline from a pressure reservoir (Fig. 6), a procedure which added 20–30 ml. to the intravascular compartment of the limb. Such injections did cause a rise in lymphatic pressure

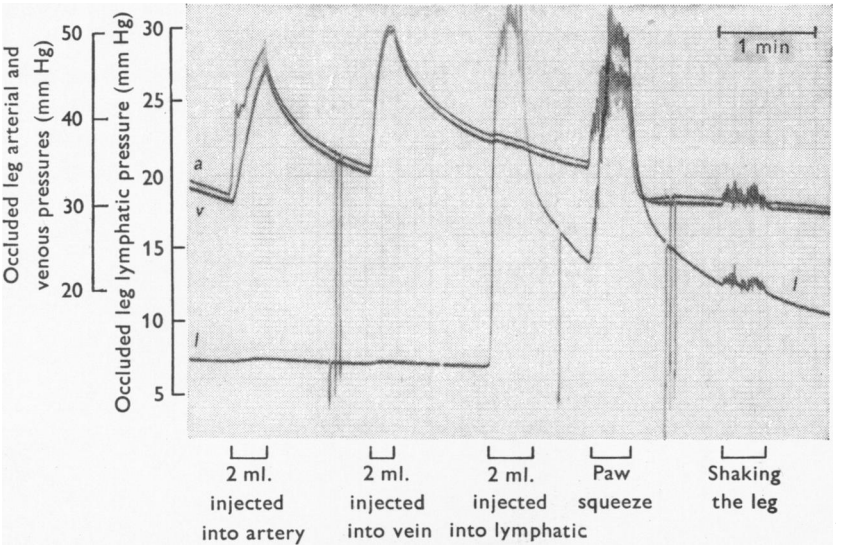

Fig. 5. The effect of injecting small volumes of saline into the vessels of the occluded hind limb, two catheters in each set of vessels. Note the absence of any change in lymphatic pressure during the injection of 2 ml. into the artery and vein and the large changes when fluid was injected into or squeezed into the lymphatics. Shaking the leg did not affect lymphatic pressure.

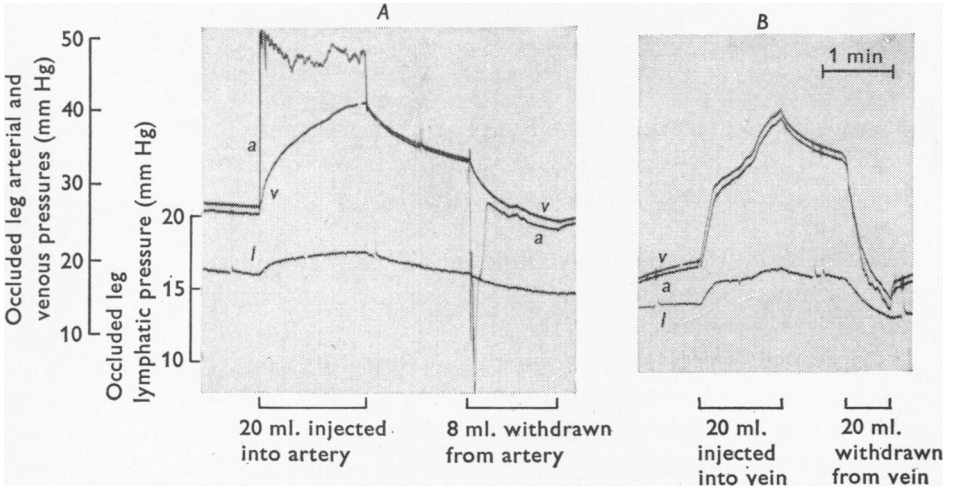

Fig. 6. *Left-hand panel* (*A*). The effect of injecting and withdrawing large volumes of saline into and from the artery of the occluded hind limb over 1–2 min to produce sustained changes of arterial pressure. The lymphatic changes are small and tend to follow the pattern of the changes of venous pressure. *Right-hand panel* (*B*). The effect of injecting and withdrawing large volumes of saline into and from the vein of the occluded hind limb over 1–2 min to produce sustained changes of venous pressure. Note the small increase of lymphatic pressure and the way in which the pattern of the lymphatic pressure change mimics that of the vein.

whether the injection was into the artery or the vein, but the increase of lymphatic pressure was much smaller than that seen in response to a rate of sympathetic nerve stimulation that caused an identical or smaller increase of arterial or venous pressure. For example, the increase of occluded limb lymphatic pressure during nerve stimulation in Fig. 2 is 9 mm Hg, the increases of arterial and venous pressure are 18 and 7 mm Hg respectively; in Fig. 6*A* the injection of 20 ml. into the artery raised its

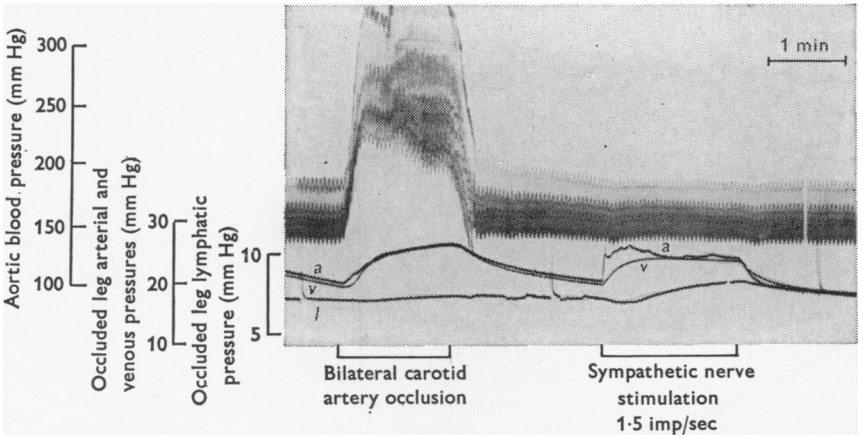

Fig. 7. The effect on the vessels of the occluded hind limb of bilateral carotid artery occlusion and sympathetic nerve stimulation. Similar changes of arterial and venous pressures have been achieved but the lymphatic response is much greater during nerve stimulation.

pressure by 25 mm Hg but the lymphatic pressure only rose by 2 mm Hg, and in Fig. 6*B* the injection of 20 ml. saline into the vein increased venous pressure by 20 mm Hg but the lymphatic pressure increase was only 3 mm Hg.

Not only did the injection of fluid into the artery and vein fail to reproduce lymphatic pressure changes of a similar magnitude to those seen during nerve stimulation but the shape of the response was quite different. The lymphatic pressure change during injection tended to follow the pattern of the venous pressure change (Fig. 6), whereas during sympathetic stimulation it had a shape and time course of its own (Fig. 2).

If the second arterial and venous catheters were disconnected there was a steady drip of blood from their open ends. Although the pressure within the artery and vein was not zero in these circumstances the size of the arterial and venous responses to stimulation was reduced yet the lymphatic response to stimulation was unchanged. Similarly, stimulation whilst the arterial or venous pressure was artificially raised also failed to prevent or alter the lymphatic response.

The effect on occluded limb lymphatic pressure of changing the arterial and venous pressures within the limb by carotid sinus hypotension. Figure 7 shows the responses of the vessels of the occluded hind limb to carotid sinus hypotension. Both vagus nerves had been divided in the neck and sinus hypotension achieved by bilateral carotid artery occlusion. Typical increases of arterial and venous pressure (6 mm Hg) are seen (Browse, Donald & Shepherd, 1966) but there is almost no change of lymphatic pressure. Subsequent stimulation of the sympathetic chain at a rate which produced an almost identical change in the arterial and venous pressure caused an increase of lymphatic pressure of 2·5 mm Hg.

DISCUSSION

The demonstration of nerve endings in and around the lymphatics makes it seem probable that these vessels have an autonomic motor innervation. The finding that an increase in lymphatic pressure during sympathetic nerve stimulation occurred in a limb whose circulation had been arrested by a suprasystolic occluding cuff supports this hypothesis, provided it can be shown that the intravascular volume below the cuff remained constant and that the increase of lymphatic pressure was a primary and not a secondary phenomenon.

It has been shown in both the dog (Browse, Lorenz & Shepherd, 1966; Browse, Shepherd & Donald, 1966) and man (Samueloff *et al.* 1966) that the venous volume remains constant below a suprasystolic occluding cuff. It is reasonable to assume that this is also true of lymphatic volume for these vessels do not communicate directly with either artery or vein. This assumption is supported by the finding that the lymphatic pressure of the occluded limb remained constant over periods of 10 min or more. Loss of lymph beneath the cuff would presumably have caused a gradual reduction of pressure and a gain of lymph an increase of pressure. In view of the previous studies concerning the veins and the stability of the lymphatic pressure after occlusion, no further studies were made of this aspect of the method and intralymphatic volume was assumed to remain constant during the control period. However, during sympathetic nerve stimulation conditions suddenly alter in the arteries, veins and other tissues below the cuff presenting three ways in which changes outside the lymphatics could change the pressure within them.

Firstly, an increase of skeletal muscle tone in the limb could squeeze the lymphatics and raise lymphatic pressure. This possibility has been carefully examined with respect to venous pressure in previous publications (Browse, Lorenz & Shepherd, 1966; Samueloff *et al.* 1966). No changes of skeletal muscle tone could be detected provided the animal was paralysed

with a neuromuscular blocking agent. Without a muscle relaxing drug changes of skeletal muscle tone do occur but they cause a totally different type of pressure change to that of nerve stimulation. Thus if the effect of the relaxant does wear off during the experiment any change of pressure due to changes of skeletal muscle tone is easily recognized.

Secondly, the lymphatics lie close to the veins and changes in the size and shape of the latter during stimulation might affect the lymphatics. Figure 5 shows however that the rapid injection of saline into the veins, with the object of suddenly distending them, did not alter lymphatic pressure. It is therefore unlikely that the changes seen in the lymphatics during nerve stimulation are secondary to alterations in the physical configuration of nearby veins.

Thirdly, the lymphatic pressure change could be due to movement of fluid (plasma) out of the capillaries into the tissue spaces and on into the lymphatics, or to direct movement of blood or plasma from the veins to the lymphatics if there were any large lympho-venous connexions. The experiments depicted in Fig. 6 suggest that movement of fluid is an unlikely cause for it was necessary to infuse a very large volume of saline, with a correspondingly large change of arterial or venous pressure, to produce a small change of lymphatic pressure.

No experiments were performed to detect the presence of lympho-venous connexions as many studies have been made of this problem, e.g. by Pentecost, Burn, Davies & Calnan (1966), all of which have failed to show any significant connexions in the normal limb of the dog. In a few experiments a radio-opaque material was injected, under fluoroscopic control, into the lymphatics after inflating the cuff but none was ever seen to enter the veins or leak out above the cuff.

The above experiments all suggest that the change in lymphatic pressure during nerve stimulation was a primary response of the lymphatics.

The carotid sinus hypotension experiment supports this for in Fig. 7 one can see identical increases of arterial and venous pressure in the occluded hind limb produced by different means but via the same route, viz. the sympathetic nerves. There was a lymphatic response to electrical stimulation but not to reflex stimulation. The change in lymphatic pressure following nerve stimulation must therefore have been a primary response of the lymphatics and not secondary to the changes in the arteries and veins.

The graded response of the lymphatics to the rate of sympathetic nerve stimulation shows that they behave in a similar way to the arteries and veins (Folkow, 1952; Mellander, 1960) although the maximum response was usually obtained with a slightly lower rate of stimulation. The time taken to achieve the peak of the response during any stimulation was

much greater for the lymphatics than for the arteries and veins. This is presumably a function of the amount of smooth muscle in the vessel wall and the richness of the nerve innervation.

The magnitude of the lymphatic response to sympathetic nerve stimulation should partly depend upon the initial length of the individual fibres in the vessel wall, i.e. upon the degree of filling. On the assumption that resting tone was constant it was assumed that an identical equilibration pressure indicated identical filling. The equilibration pressure could be controlled by varying the time at which the cuff was inflated after filling the lymphatics and raising their pressure with a paw squeeze. Thus the volume of the lymphatics before each stimulation was probably almost identical. Nevertheless, the necessity for such assumptions makes it unrealistic to draw any further conclusions from the results other than the crude mean stimulus–response curve.

The marked increase of lymphatic pressure during paw squeezing is of interest. It has been reported before (Drinker & Field, 1933) and is presumably due to the increased lymph flow caused by tissue compression and the high outflow resistance of the small lymphatic vessels, the lymph nodes and the pneumatic cuff. This feature of lymphodynamics is to be the subject of a future paper but it is worth noting that if lymphatic and tissue pressures are as high as 60 mm Hg during exercise, then little filtration of fluid from capillaries to tissue spaces can occur.

The abolition of the response to nerve stimulation by dividing the sympathetic chain below the site of stimulation and by the administration of an α-adrenergic blocking agent prove that the response was mediated through α-adrenergic sympathetic nerves.

These experiments do not shed any light on the existence of 'lymphatic tone' but the carotid sinus experiment (Fig. 7) suggests that the lymphatics are not much involved in peripheral vasomotor reflexes.

Whether the ability of the lymphatics to constrict in response to sympathetic stimulation has any functional importance to the lymph circulation is not known. There are many reports of 'spontaneous contractions' of lymphatics and it is possible that these are neurogenic contractions and play a role in lymph transport. It might also be envisaged that a change in the calibre of these vessels would alter their resistance to flow, a function which might be of value in controlling the absorption of chyle if the mesenteric lymphatics were similarly innervated.

I wish to thank Mrs A. Taylor for skilled technical assistance and the Endowment Fund of St Thomas's Hospital for financial support.

REFERENCES

ACEVEDO, D. (1943). Motor control of the thoracic duct. *Am. J. Physiol* **139**, 600–603.

BERT, P. & LAFFONT, X. Y. (1882). Influence du système nerveux sur les vaisseaux lymphatiques. *C. r. hebd. Séanc. Acad. Sci., Paris* **94**, 739.

BROWSE, N. L., LORENZ, R. R. & SHEPHERD, J. T. (1966). Response of capacity and resistance vessels of dog's limb to sympathetic nerve stimulation. *Am. J. Physiol.* **210**, 95–102.

BROWSE, N. L., DONALD, D. E. & SHEPHERD, J. T. (1966). Role of the veins in the carotid sinus reflex. *Am. J. Physiol.* **210**, 1424–1434.

BROWSE, N. L. SHEPHERD, J. T. & DONALD, D. E. (1966). Differences in the responses of veins and resistance vessels to the same stimulus. *Am. J. Physiol.* **211**, 1241.

BURCH, G. E. & MURTADHA, M. (1956). A study of venomotor tone in a short intact venous segment of the forearm. *Am. Heart J.* **51**, 807–828.

CAMUS L. & GLEY, E. (1894). Recherches experimentales sur les nerfs des vaisseaux lymphatiques. *Archs Physiol. norm. path.* **6**, 454–459.

DRINKER, C. K. & FIELD, M. E. (1933). *Lymphatics, Lymph and Tissue Fluid.* Baltimore: Williams and Wilkins.

FLOREY, H. W. (1927). Observations on the contractility of lacteals. *J. Physiol.* **63**, 1–18.

FOLKOW, B. (1952). Impulse frequency in sympathetic vasomotor fibres correlated to the release and elimination of the transmitter. *Acta physiol. scand.* **25**, 49–76.

MELLANDER, S. (1960). Comparative studies on the adrenergic neurohormonal control of resistance and capacitance blood vessels in the cat. *Acta physiol. scand.* **50**, suppl. 176, 1–86.

PENTECOST, B. L., BURN, J. I., DAVIES, A. J. & CALNAN, J. S. (1966). A quantitative study of lymphovenous communications in the dog. *Br. J. Surg.* **53**, 630–634.

QUENU, E. & DARIER, J. (1887). Note sur l'existence d'un plexus nerveux dans la paroi du canal thoracique du chien. *C. r. Séanc. Soc. Biol.* **39**, 529–530.

RUSZNYÁK, I., FÖLDI, M. & SZABÓ, Gy. (1950). Lymphangiospasm. *Acta med. scand.* **137**, 37.

SAMUELOFF, S. L., BEVEGARD, B. S. & SHEPHERD, J. T. (1966). Temporary arrest of the circulation to a limb for the study of venomotor reactions in man. *J. appl. Physiol.* **21**, 341–346.