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THE EFFECT OF THE CANINE POPLITEAL NODE ON THE COMPOSITION OF LYMPH

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SUMMARY

1. The composition of afferent lymph draining into the canine popliteal lymph node was compared with that of the efferent lymph leaving the node. Both the protein and cellular composition were studied.

2. In twenty-five greyhounds the protein concentration of efferent lymph was greater than that of afferent lymph collected from the same limb. Although the absolute level of protein varied greatly between dogs, in a particular animal there was a constant ratio between the protein content of afferent and efferent lymph. The concentration of protein in efferent lymph was approximately double that of afferent lymph.

3. Chromatographic analysis of lymph and the use of radio-iodinated canine albumin indicated that the reason for the increased level of protein in the efferent lymph is that the popliteal node concentrates the protein in afferent lymph.

4. Afferent lymph contained less than 3×10^3 cells/ml; efferent lymph contained between $0{\cdot}5 \times 10^6$ and $4{\cdot}3 \times 10^6$ cells/ml, 98 % of which were lymphocytes. In different dogs there was no correlation between efferent lymphocyte density and afferent or efferent protein concentration; however, when an afferent lymphatic was perfused with solutions of different protein concentration, the lymphocyte number in the efferent fluid became greater as protein concentration in the afferent perfusate was increased.

5. The concentrating effect of the node is discussed in terms of its significance to both fluid balance and immunological surveillance.

INTRODUCTION

The lymphatic system in higher vertebrates performs two distinct functions. The first is to provide a means for the return of plasma proteins from tissue interstitium to vascular compartment. This need arises from the unidirectional movement of proteins across the capillary endothelium. Tissues require perfusion with plasma proteins in order to supply nutritional elements as well as to remove waste products. Having crossed the capillary endothelium into the tissue interstitium, proteins above a certain size cannot re-enter nearby blood vessels. Fluid containing these proteins enters small collecting lymphatics which themselves drain into larger vessels. Lymph

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thus formed passes through a number of different lymph nodes before it reaches the thoracic duct (Yoffey & Courtice, 1970).

The second role of the lymphatic system is concerned with the immune system; it provides the anatomical structures which contain the cellular elements involved in this system and provides the necessary circulatory pathways for interaction between these cells and other tissues (Roitt, 1980).

There is a large population of resident lymphocytes within the lymph node; these lymphocytes move from the node into efferent lymph where they travel to other lymph nodes or to the blood via the thoracic duct. Similarly, lymphocytes leave the vascular compartment at the capillaries within the node to enter the lymphatic compartment (Gowans & Knight, 1964). These processes are normally balanced since lymph nodes do not change in size with time. Under normal circumstances there is only a low rate of division of lymphocytes within the node (Hall & Morris, 1965). However, when a tissue becomes infected an antigenic stimulus is delivered to the local node into which that tissue drains. There is a pattern of movement within the node, and the antigen, with the aid of T-helper or macrophage cells, can mitogenically stimulate specific clones of B-lymphocytes; these will then differentiate into plasma cells which are able to synthesize immunoglobulins that will recognize that antigen. Both immunoglobulins and some of the stimulated lymphocytes will then enter the efferent lymphatics and find their way into systemic circulation.

The two roles of the lymphatic system should not be considered in isolation, since it is likely that the system has evolved to be optimally suited for both functions. The following studies were carried out to compare the protein and cellular composition of afferent and efferent lymph derived from the hind leg of greyhound dogs; some of the results have been presented in a preliminary communication (Pflug, Knox & Hadjis, 1982).

METHODS

Cannulation of prenodal and post-nodal lymphatics

Greyhound dogs of either sex (approximate weight 30 kg) were maintained under Fluothane anaesthesia throughout the experiment. Fluothane (1 %) was combined with oxygen:nitrous oxide (3:4). Prenodal lymphatics were cannulated on the dorsum of the hind paw. A longitudinal incision 1 cm long was made above the dorsal communis digital vein III and this was extended by blunt dissection to the medial and lateral side. The prenodal lymphatic trunk can be identified on each side of the dorsal communis digital vein. The identification is made easier by the injection of 0·05 ml Patent Blue solution into the web space between the second and third digits. We have established that dye administered in this way does not alter the composition of afferent or efferent lymph. The lymphatic vessels were cannulated with lymphangiogram catheters (Macarthy's Surgical Ltd., London) or with polythene cannulae depending on the size of the individual vessel. All cannulae were first flushed with heparin solution. Afferent lymph was collected in LP3 tubes previously treated with heparin (1000 u. per tube). In most animals flow of lymph was spontaneous; in cases where flow was not spontaneous, mechanical flexion of the paw was effected.

Cannulation of the post-nodal lymphatic trunk was carried out on the medial aspect of the thigh, just above the knee. A 6 cm incision was made along a line from the knee joint, crossing the sartorius muscle at a sharp angle. The fascia covering the sartorius muscle was incised along the border of the proximal part of the sartorius muscle. By blunt dissection the proximal part of the sartorius muscle was retracted distally. The femoral artery became visible at the segment between the medial caudal femoral and the caudal femoral branches. After retraction of the artery, the femoral vein was exposed and on its proximal aspect the main trunk of the post-nodal lymphatic was identified. Polythene cannulae, pre-treated with heparin were used.

Blood samples were taken from the femoral and jugular veins and collected into lithium–heparin.

Measurement of total protein

Blood and lymph samples were centrifuged in the cold for 20 min at 2000 *g*. The supernatants were taken and total protein concentration determined by the methods of Lowry (Lowry, Rosebrough, Farr & Randall, 1951) and Maxwell (Maxwell, Hars, Bieber & Tolbert, 1978). The latter assay was carried out to ensure that lipoproteins did not interfere with the colorimetric method. In order to establish absolute values, standards of crystalline bovine serum albumin (Sigma type A7638) were used. For the determination of lymph/plasma ratios, a standard curve was contructed with different concentrations of plasma, and the lymph/plasma ratios for a given lymph sample determined by direct extrapolation.

Chromatography of lymph and plasma

High-performance gel filtration was carried out using an L.K.B. TSK-G 4000 SW column with a TSK-GSWP pre-column (L.K.B. Instruments Ltd., Selsdon, Surrey). Protein elution was measured by ultra-violet absorption at 206 nm with a Uvicord-S (L.K.B.). The eluant was phosphate-buffered saline (0·2 g KCl/l; 0·2 g KH_2PO_4/l; 8 g NaCl/l; 1·15 g Na_2HPO_4/l; 0·1 g $MgCl_2$/l; pH 7·4) containing 1 mM-sodium azide. The sample to be applied was brought to a total volume of 50 μl before application to the pre-column.

Preparation of canine albumin

2 ml aliquots of canine plasma were applied to a 95 × 2·7 cm column of Sephacryl 300 (Pharmacia, London). Protein was eluted with phosphate-buffered saline (PBS) and the fifth elution peak which contains albumin was concentrated back to starting volume using Amicon Centriflo membranes (CF25). The solutions were pooled four at a time and applied to a 23 × 2·3 cm column of Blue Sepharose CL-6B (Pharmacia) pre-equilibrated with PBS. After washing unbound protein through with 200 ml PBS, albumin was eluted with 1·5 M-KCl in PBS. Protein-containing fractions were pooled and exhaustively dialysed against PBS.

Iodination of canine albumin

Albumin was iodinated using the solid-phase method outlined by Fraker & Speck (1978). 100 μl albumin solution (1 mg/ml) was mixed with 1 mCi carrier-free $Na^{125}I$ (Radiochemical Centre, Amersham) in a tube pre-coated with iodogen. After 2–3 min the reaction mixture was applied directly to a 5 × 2 cm column of Sephadex G-25 (Pharmacia) in order to separate protein from free iodide. PBS was used as elution buffer. Gamma counting was carried out using an L.K.B. 1275 Minigamma.

Perfusion of the popliteal node via an afferent lymphatic

In order to perfuse the popliteal node through an afferent lymphatic, the prenodal lymphatics were first exposed as described in the cannulation section above. A pre-filled lymphangiogram catheter was inserted into the lymphatic in an anterior direction. All other afferent lymphatics were ligated. The perfusate was heparinized greyhound plasma diluted with Krebs–Heinseleit Ringer solution to give a final protein concentration of 10 g/l. Perfusion was effected with a Schuco constant infusion syringe pump, Model 'B' (Schuco Scientific Ltd., London, N12.). The perfusion rate was 10 μl/min, which we had previously established was the mean flow in single lymphatics.

Determination of cells in lymph

To measure cell density in lymph, samples were applied without dilution to a rhodium-plated improved Neubauer haemocytometer. To determine cell type, lymph samples were centrifuged at 1000 *g* for 15 min, resuspended in one drop of PBS and smeared on to microscope slides. After air-drying and then fixing with 95% methanol, cells were stained with May-Grunwald stain.

RESULTS

The anatomy of the popliteal node and its afferent and efferent lymphatic vessels have been described (Pflug & Calnan, 1969). The lymphatics draining the paw of the greyhound can be divided into a superficial and a deep system. Only the superficial system drains to the popliteal node. This contrasts with the lower limb of man where

vessels from both the superficial and deep system drain into the popliteal node (Pflug & Calnan, 1971).

Fig. 1 shows a schematic representation of the greyhound popliteal node and its afferent and efferent lymphatics. The diagram indicates the positions at which

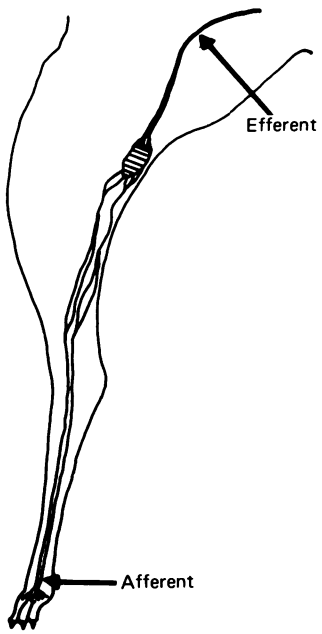

Fig. 1. Sites of lymphatic cannulation in the greyhound hind limb.

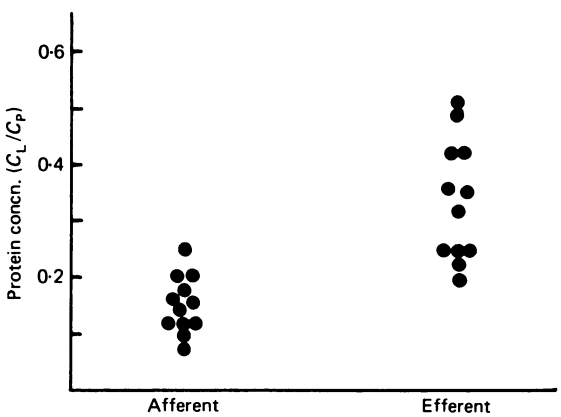

Fig. 2. Total protein concentration of afferent and efferent lymph. Each point represents the protein concentration of initial samples of lymph collected from a single afferent or efferent vessel. Only one lymphatic vessel was cannulated in each leg.

lymphatics were cannulated. These sites were chosen so that the dissection and cannulation could be achieved with the minimum of trauma to the lymph node. However, the lymph collected at the positions indicated did not differ in composition from lymph that was collected close (2 cm) to the node. This is true for both afferent and efferent lymph.

In an initial study, afferent lymph was collected from twelve greyhounds and compared to efferent lymph collected from twelve different greyhounds. Only one lymphatic was cannulated in each dog in order to minimize the effect of trauma on the composition of lymph. Fig. 2 shows the results of these studies expressed in terms of lymph/plasma ratios (C_L/C_P) for total protein concentration. There was large variation in the values obtained for afferent and efferent lymph but the mean value for efferent lymph was significantly higher than that for the afferent C_L/C_P.

Surface skin temperature of greyhounds was variable, and in order to investigate

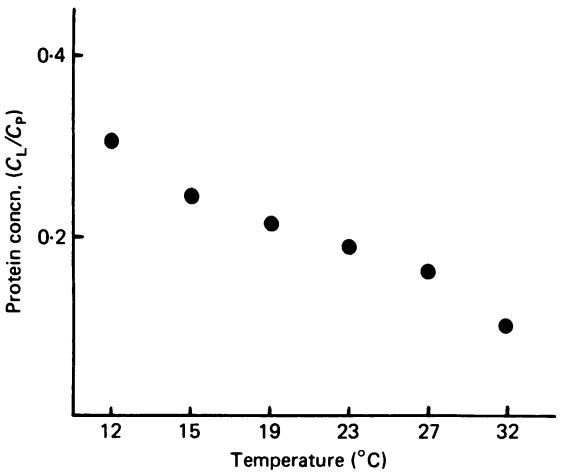

Fig. 3. The effect of surface skin temperature on the protein concentration of afferent lymph. Surface temperature of the paw was altered using water packs or a radiant lamp. The paw was kept at a given temperature for 45 min; the lymph collected during most of this time was discarded and a single sample was taken at the end of the 45 min period.

the possibility that this might affect lymph composition the skin temperature of the paw was artificially altered, using radiant lamps to raise the temperature and water packs to reduce it. Fig. 3 shows a typical result in a single dog. As skin temperature was elevated, the C_L/C_P ratio for total protein was reduced while flow increased; conversely, as skin temperature was lowered, C_L/C_P increased and flow decreased. The total protein entering the lymphatic system remained essentially unaltered.

In order to make a direct comparison, afferent and efferent samples were collected from the same leg. An inherent problem with this approach is that cannulation of an afferent lymphatic will reduce flow into the node. For this reason the first 0·15 ml of afferent and efferent lymph was always taken for cell and protein analysis. In fact, under these experimental conditions, C_L/C_P remained unchanged for several hours. Fig. 4 shows the relationship between afferent and efferent C_L/C_P for total protein in twenty-five greyhounds. The results are expressed as afferent C_L/C_P against efferent C_L/C_P for each dog. There was good correlation between the afferent and efferent lymph/plasma ratios for total protein, and for a given animal the total protein in efferent lymph was approximately double that in the afferent lymph.

There are at least three lymphatic trunks in the bundle around the digital vein, and in two experiments three vessels were cannulated in the same paw. There were significant differences in protein concentration in the lymph samples collected from

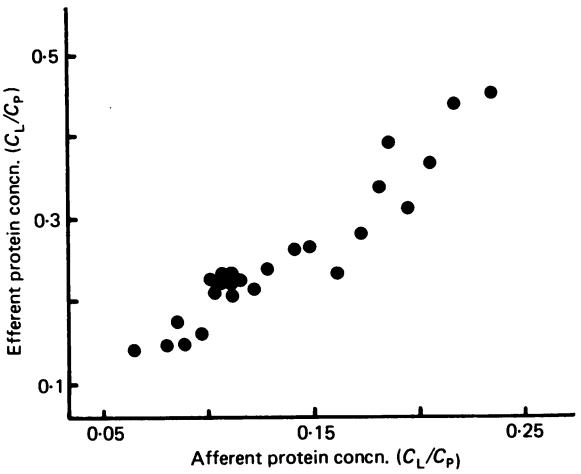

Fig. 4. Relationship between the protein concentration of afferent and efferent lymph. A single afferent and a single efferent lymphatic were cannulated in the same leg. Each point represents the values obtained from a single leg of different animals. The results are expressed as the afferent protein concentration against the efferent concentration for each animal.

TABLE 1. Cells in afferent and efferent lymph. The results are the values obtained from twelve afferent and twelve efferent lymph samples. The analyses were carried out on the first samples of lymph obtained following cannulation

	No. of cells/ml	
	Afferent	Efferent
Lowest value	0	$0{\cdot}5 \times 10^6$
Mean value	$0{\cdot}8 \times 10^2$	$2{\cdot}2 \times 10^6$
Highest value	$3{\cdot}0 \times 10^3$	$4{\cdot}3 \times 10^6$

the different vessels within one paw; however, the maximum variation between the highest and lowest values was 12%.

The cellular content of lymph was determined at the time of collection using a haemocytometer. At the completion of the experiment, cells were centrifuged, spread on to slides and stained. Table 1 shows the cellular content of afferent and efferent lymph. More than half of the afferent lymph samples contained no detectable cells when counted using a haemocytometer. In the samples that did contain cells the composition was variable, with lymphocytes, polymorphonuclear leucocytes and veiled cells present in varying proportions. In the case of efferent lymph, cell number varied between $0{\cdot}5 \times 10^6$ and $4{\cdot}3 \times 10^6$/ml (mean $2{\cdot}2 \times 10^6$/ml). However in all cases, > 98% were lymphocytes. In contrast to the results reported by others (Quin & Shannon, 1977) there was no correlation between the number of lymphocytes in efferent lymph and the concentration of total protein.

Protein composition of lymph

The protein composition of lymph was characterized using high-pressure gel filtration chromatography. As with any form of molecular sieve chromatography,

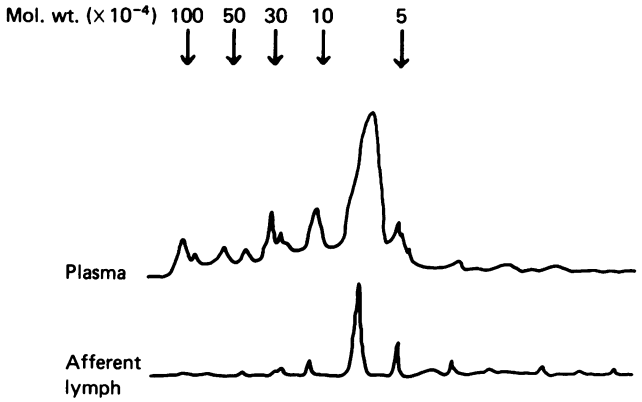

Fig. 5. Chromatographic elution profiles of plasma and afferent lymph 10 μl plasma or afferent lymph were brought to 50 μl with buffered saline, applied to a TSK-G 4000SW column and eluted with buffered saline. Protein concentration in the eluate was continuously monitored at 206 nm. The column was calibrated with α_2-macroglobulin, ferritin, catalase, IgG, albumin and cytochrome c.

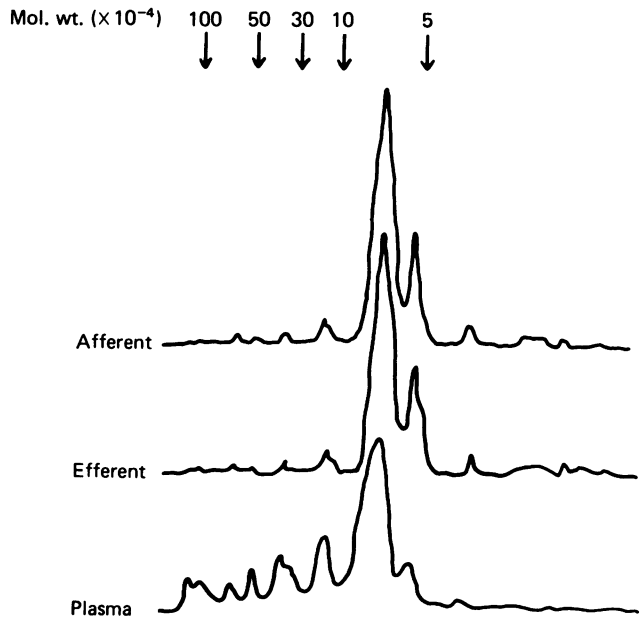

Fig. 6. Elution profiles of plasma, afferent lymph and efferent lymph. Samples of plasma, afferent lymph or efferent lymph were diluted with saline so that 50 μl contained 0·5 mg protein. 50 μl samples were then applied to a TSK-G 4000SW column and eluted with buffered saline. Protein concentration was continuously monitored at 206 nm.

proteins are separated on the basis of size. When prenodal lymph was compared to plasma, as expected the larger molecular weight proteins were present at relatively lower concentration. Fig. 5 shows the elution profiles of plasma and afferent lymph when the same volume of each fluid was applied to the column. In the set of elution profiles shown in Fig. 6, identical amounts of total protein were applied to the

column. When afferent lymph was compared to efferent lymph, elution profiles were not significantly different. This result demonstrates that, although absolute levels of protein were different, the ratios of concentrations of different proteins were the same in afferent and efferent lymph. To test the sensitivity of the technique, plasma was added to afferent lymph to bring it to the same total protein concentration as the efferent lymph collected from the same animal. The resulting solution, when analysed by high-pressure gel chromatography, was different from efferent lymph.

TABLE 2. The concentration of albumin in afferent and efferent lymph. The results show four experiments in which the total protein concentration of lymph samples were at the higher and lower end of the range encountered

	Albumin concentration (g/l)		
Experiment	Afferent	Efferent	Plasma
1	5	9	29
2	7	10	33
3	14	24	33
4	13	25	31

TABLE 3. Concentration of [^{125}I]albumin in afferent and efferent lymph after subcutaneous injection. After cannulation of one afferent and one efferent lymphatic, 30 μl of tracer [^{125}I]albumin in buffered saline was injected subcutaneously into the paw. Afferent and efferent lymph were collected at 5 min intervals

	Maximum [^{125}I]albumin (ct/min . ml $\times 10^{-4}$)		
Experiment	Afferent	Efferent	% increase
1	2·0	2·8	40
2	1·0	1·8	80
3	2·2	3·6	64
	Total protein (g/l)		
Experiment	Afferent	Efferent	% increase
1	12	22	83
2	14	25	79
3	14	24	71

It has been suggested that the increased levels of protein in efferent lymph (in comparison to afferent lymph) are due to the addition of plasma proteins within the lymph node. The results shown in Fig. 6 indicate that if protein is added in the node then there must be filtration within the node which is qualitatively similar to that which occurs in the capillaries of the paw. Table 2 gives the albumin concentrations measured in four experiments. These particular experiments were chosen since they represent the highest and lowest lymph/plasma ratios obtained in different experiments. The albumin concentrations found in the samples with the lower lymph/plasma ratios might be compatible with a mechanism in which plasma proteins are added to lymph within the node. In the case of the samples with higher ratios, the concentration in efferent lymph was almost as high as that in plasma.

Another explanation for the increased protein in efferent lymph is that the node

brings about a concentration of afferent lymph. In order to investigate this possibility two series of experiments were carried out using radiolabelled canine albumin. In the first, tracer levels of iodinated albumin were injected subcutaneously into the paw and afferent and efferent samples collected. Table 3 shows the results of four such experiments. Afferent and efferent lymph had a different time course for the appearance of isotope. Levels of isotope were highest in afferent lymph at 10 min and highest in efferent lymph at 20 min. For this reason results are expressed as the

TABLE 4. *In situ* perfusion of popliteal node with [^{125}I]albumin. A single afferent vessel was cannulated and continuously perfused with diluted canine plasma containing tracer [^{125}I]albumin; all other visible afferent lymphatics were tied-off. An efferent lymphatic was cannulated and efferent fluid collected at 5 min intervals

	[^{125}I]albumin (ct/min . ml × 10^{-4})		
Experiment	Perfusate	Efferent fluid	% increase
1	4·4	6·7	34
2	4·8	7·2	46
3	3·6	4·9	36
4	5·4	9·1	69
	Total protein (g/l)		
Experiment	Perfusate	Efferent fluid	% increase
1	14	22	57
2	14	20	43
3	14	20	43
4	14	24	71

maximum level of isotope, irrespective of time. In each case the maximum concentration of isotope in the efferent lymph was greater than that of afferent lymph. It is true that the apparent increased concentration of isotope did not correlate with the increased concentration of total protein. However, here again cannulation at the prenodal site will reduce the amount of lymph draining to the node. This problem can be avoided by perfusing the node through a single afferent lymphatic with all other visible lymphatics tied off. The lymphatic was perfused with diluted plasma containing tracer levels of radio-iodinated canine albumin. An efferent cannula was also implanted and the afferent fluid compared with the efferent. Table 4 shows that there was an increased concentration of radiolabelled albumin in the efferent fluid. The perfusate contains a constant level of radio-iodinated albumin and thus there are no problems associated with time course. The results in Table 4 are the steady-state values that were achieved.

An unexpected finding in the perfusion studies was that efferent lymphocyte concentration was influenced by the perfusate. Fig. 7 shows a typical result. As the protein concentration of the afferent perfusate increased the lymphocyte density in efferent fluid was increased. The effect is reversible in that the concentration of protein in the perfusate could be raised and lowered sequentially and there were corresponding changes in efferent lymphocyte density.

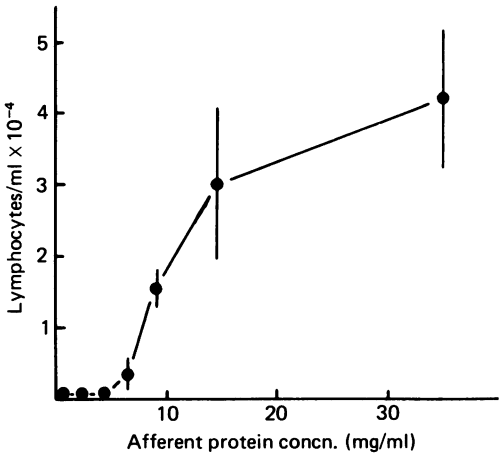

Fig. 7. The effect of afferent protein concentration on the number of lymphocytes in efferent lymph. The popliteal lymph node was perfused via an afferent lymphatic; all other visible lymphatic vessels were tied off. The protein concentration of the perfusate was altered using different concentrations of heparinized canine plasma diluted with bicarbonate-buffered saline. For each protein concentration, the node was perfused for 30 min, after which time four efferent samples were collected at 5 min intervals. The results are the mean ± 1 S.D. of the cell densities measured in the four samples.

DISCUSSION

There is considerable variation in the protein content of peripheral lymph obtained from different dogs even though inbred greyhounds of similar size were used throughout the study. One of the factors that seems to affect protein concentration is temperature. As skin temperature becomes raised then protein concentration becomes lower; conversely, as skin temperature is lowered protein concentration is elevated. There is a concomitant alteration in lymph flow. At higher temperatures flow is greater. These effects may be due to an alteration in blood perfusion. In a recent report, Renkin, Sloop & Joyner (1981) have also shown that at physiological temperatures the level of protein is reduced as temperature is raised; these authors also demonstrated that above physiological temperatures the relationship no longer holds, due to the breakdown of capillaries. Quin & Shannon (1977) have reported that in the sheep, post-popliteal lymph protein concentration is higher than the corresponding prenodal value. They postulate that this is due to the leakage of plasma proteins across the capillaries within the node. This conclusion is based on two observations. First, they describe a correlation between post-nodal protein levels and lymphocyte density; secondly, when all visible afferent lymphatics were ligated there was still efferent flow. Our observations in the greyhound were quite different. There was no correlation between lymphocyte number and protein concentration. Also it is impossible to ensure that all afferent lymphatics have been ligated; often a number of small afferent lymphatics join the node on its lateral aspect. Under physiological conditions the contribution of these vessels to total lymph output is low, but they continue to flow when the main trunk of afferent lymphatics is ligated.

While the results reported here do not rule out the possibility that some protein

from the vascular compartment enters the lymphatics within the node, it cannot be the most significant reason for the different protein concentrations of afferent and efferent lymph. Most of the results can only be explained in terms of a concentrating mechanism within the node.

Gel filtration shows that proteins are present at the same concentration ratios in afferent and efferent lymph. This could be explained in terms of the capillaries in the node filtering plasma proteins in a qualitatively similar fashion to those in the paw. However the absolute concentrations of all proteins would have to be significantly greater. A comparison of the albumin concentrations in lymph and plasma is indicative. Taking an example of an individual greyhound from Table 3, the concentrations of albumin in afferent lymph, efferent lymph and plasma were 13, 25 and 31 g/l respectively. Quin & Shannon (1977) suggest that in the sheep popliteal node the contribution of the nodal microcirculation to the efferent lymph is 30–50 %. Based on two observations, we found that when all afferent lymphatics in the paw were ligated then the popliteal efferent flow was reduced to less than 15 %. However, even if there were a contribution of 50 % from the node then the concentration of albumin in that contribution can be calculated as follows.

$$\text{Efferent concn.} = 0{\cdot}5 \times (\text{afferent concn.} + \text{nodal concn.}).$$

Therefore:

$$\text{nodal concn.} = (2 \times \text{efferent concn.}) - \text{afferent concn.}$$

By the use of the above values, the concentration of albumin in the nodal contribution would be 37 g/l; this is greater than the concentration of albumin in the plasma. If the contribution of the node is less, then the concentration of albumin would have to be even higher.

The results obtained by the use of iodinated albumin can only be explained in terms of a concentrating mechanism since the radiotracer is more concentrated after passage through the lymph node than before it. It is true that the degree of concentration of the tracer does not equal the degree of concentration of total protein. This is due in part to the contribution from the small lymphatics that enter the node on its lateral aspect. There are other inherent problems associated with the use of radio-iodinated proteins. Both the purification and the iodination of albumin cause changes in the nature of the protein; this is likely to give misleading results. Commercial preparations of albumin are isolated by a method that involves precipitation with organic solvents. This results in an albumin that is not in its native form. It is well established that denaturation of albumin leads to a rapid uptake by hepatic tissue (Benacerraf, Biozzi, Halpern, Stiffen & Mouton, 1975) and we have shown (S. Griffiths & P. Knox, unpublished results) that a number of non-hepatic cell types rapidly take up commercial preparations of albumin. For this reason an affinity binding method has been used to prepare albumin from canine plasma. This material is much closer to the native form and is less readily metabolized. The iodination procedure can also affect the subsequent response of cells and tissues to that protein. In a different system, iodinated commercial albumin does not behave like the native counterpart (Gamble, Hawkins, Penn & Spencer, 1982).

Lymph nodes have a high level of blood perfusion. The concentrating effect of the node presumably occurs as a result of the movement of water (and accompanying

salts) down an osmotic gradient from the lymphatic into the vascular compartment. The vessels where this process occurs must be relatively impermeable to protein since, in order to achieve the concentrating effect, only small molecular weight components must pass from lymph into the vascular compartment. This property of the lymphatic system has already been demonstrated by Mayerson, Patterson, McKee & Mayerson (1962); small tracer molecules diffused rapidly into the bloodstream while tracers with a molecular weight above 2,300 were mostly retained within the lymphatic system. The blood vessels within the node have an atypical endothelium. The post-capillary venules have a thick so-called 'high endothelium' (Smith & Henon, 1959; Wenk, Orlic, Reith & Rhodin, 1974) and the endothelial cells are known to synthesize unusual macromolecules (Andrews, Milsom & Ford, 1982). It is often suggested that the high endothelium is involved in the passage of lymphocytes from the blood into the lymph node (Gowans & Knight, 1964). At the ultrastructural level, lymphocytes have been shown to insinuate themselves between the endothelial cells (Schoefl, 1972; Anderson, Anderson & Wyllie, 1976). Although some electron microscopic data have been interpreted as showing that plasma components accompany the lymphocytes across the endothelium (Mikata & Niki, 1971) other workers have pointed out that the overlapping reticular cells underneath the endothelial cells act to prevent vascular loss (Anderson *et al.* 1976).

Andrews *et al.* (1982) have pointed out that other tissues in which there are also high rates of lymphocyte movement, i.e. spleen, do not have a high endothelium. It may therefore be that the high endothelium has a completely different function.

The density of lymphocytes in efferent lymph is variable; the range that we have encountered is $0{\cdot}5 \times 10^6$–$4{\cdot}3 \times 10^6$. One reason for the higher levels in some dogs may be the presence of subclinical levels of infection in tissues draining to the popliteal node. As outlined above, when the results from a number of dogs are compared, there is no correlation between lymphocyte number and protein concentration. However, for a given dog, when the popliteal node is artificially perfused, increased levels of protein in the perfusate lead to an increased number of lymphocytes in the fluid leaving the node.

It is important not to divorce the two functions of the lymphatic system, namely fluid balance and the immune surveillance mechanism. It is tempting to speculate on a role for the effect of afferent protein concentration on efferent lymphocyte number. During an infection there will be an increased level of protein in the afferent lymph due to the effects of local inflammatory agents on capillary function. The increased protein might lead to a non-specific mobilization of lymphocytes in the node and these would then find their way into systemic circulation. In addition, the concentrating mechanism would enable the level of antigens in afferent lymph to be doubled within the node. This would increase the rate of interaction between antigens and T-cells or macrophages.

During the course of these investigations we became aware that another group had also arrived at the conclusion that the popliteal node is able to effect a concentration of afferent lymph by the removal of low molecular weight components within the node. These workers perfused the node and measured the differences in afferent and efferent fluids in terms of flow and protein concentration; in addition they studied the effect of varying afferent flow and protein concentration. They found that the node is able to concentrate protein when the afferent fluid is within physiological

range; however, there is a limit above which no concentration is achieved. They also found that the level of concentration is dependent on afferent flow. A preliminary report of this work has appeared (Adair, Moffat & Guyton, 1981).

The afferent lymph that is formed when interstitial fluid enters collecting lymphatics passes through a number of nodes before it is returned to the vascular compartment at the thoracic duct (Yoffey & Courtice, 1970). One question that needs to be answered is whether there is any further concentration of lymph as it passes through subsequent nodes. Nevertheless, whatever the degree of concentration in subsequent nodes, it is clear that the measurement of flow at the thoracic duct will not accurately reflect the total amount of lymph that is formed in all tissues within its drainage area.

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