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## The Effect of Anaesthesia and Surgery on Lymph Flow, Protein and Leucocyte Concentration in Lymph of the Sheep

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### Summary

Anaesthesia and the trauma of surgery, associated with the cannulation of lymphatic ducts in various regions of the body of the sheep, had a profound effect on lymph flow, protein concentration and leucocyte concentration of lymph. In general lymph flow was depressed and the protein concentration elevated in lymph collected at the time of cannulation, or within the first 24 hours of recovery from surgery. The changes in protein concentration in lymph draining the peripheral regions of the body appeared to be due to surgical interference in the region of drainage. The greatest changes in lymph flow were observed in lymph draining peripheral regions (skin, tendon, muscular areas) while lymph draining soft tissues in central regions (kidney, liver) was less affected by the anaesthesia and surgical stress.

A neutrophilia was observed in venous blood collected under anaesthesia while the overall numbers of lymphocytes in three sources of efferent lymph were depressed. It is suggested that corticosteroid hormones may play a role in the changes in leucocyte migration observed during anaesthesia and surgical stress. Changes observed in the cellular content of afferent lymph appeared to be due to a low grade inflammation associated with surgical interference in the region of lymphatic drainage.

### Introduction

Anaesthesia interrupts physiological functions and may result in biochemical and pathological changes in blood and tissues. The nature and duration of these changes are dependent upon the type of anaesthetic agent employed, together with the length and depth of anaesthesia.

The effect of anaesthesia on flow and composition of lymph is of some physiological importance since these parameters reflect the circulation of fluids and proteins between the vascular space and the interstitial space. The effect of anaesthesia on lymph flow in animals has been the subject of a number of studies (see 1, 2). In general, it has been found that anaesthesia depresses lymph flow with the exception of ether anaesthesia which results in an elevated lymph flow.

The circulation of plasma proteins between the vascular and interstitial pools is of physiological importance in view of the role plasma proteins play in blood clotting, wound healing, maintenance of fluid balance, humoral immunity and the transport of various molecules. To date, much of the knowledge on the lymphatic transport of proteins has been based on studies in the anaesthetised animal, or in animals only recently recovered from anaesthesia. In these experiments it has been assumed that anaesthesia and the trauma associated with surgery have had a minimal effect on protein transport into lymph. However several reports suggest that this assumption may not be valid. The protein concentration in lymph collected from both the intestinal and hepatic lymphatic ducts in anaesthetised calves was higher than that observed when the animals had fully recovered from the effects of anaesthesia (3). In addition, activity and movement have been shown to significantly alter the protein concentration in lymph from the lower limbs of dogs (4).

The effects of anaesthesia and surgery may also account for the discrepancies in protein content of afferent and efferent popliteal lymph reported in different species. Thus *Osogoe and Courtice* (5), *Roberts and Courtice* (6) and *Bach and Lewis* (7) observed no difference in the protein content of afferent and efferent popliteal lymph in the anaesthetised rabbit in direct contrast to the relatively large difference in these two sources of lymph reported for the conscious sheep (8, 9). The present study has been undertaken to ascertain the effects of anaesthesia and the recovery from its effects on protein transport in lymph of the sheep.

### *Materials and Methods*

#### *Animals*

Non-pregnant merino and merino cross-bred ewes aged between 2 and 4 years were employed in the experiments. The animals were housed in-doors in metabolism cages and fed a mixture of lucerne and oats. Water was provided *ad libitum*. Food and water were withheld for a period of 24 hours prior to surgery to prevent regurgitation of ruminal contents under anaesthesia.

#### *Anaesthesia*

Anaesthesia was induced with thiopentone sodium (Intraval sodium, May and Baker, Australia) and the animals intubated with a McGill cuffed endotracheal tube. Anaesthesia was maintained with a halothane (Somnothane, Hoechst Australia Ltd., Australia) -oxygen mixture in a closed circuit system.

#### *Surgical Procedures*

Various lymphatic ducts in 15 sheep were cannulated as follows: An efferent lymphatic duct of the popliteal node in each of 8 sheep was cannulated by the method of *Hall and Morris* (10). In each animal either one or two afferent lymphatic ducts of the popliteal node were cannulated in the opposite leg enabling simultaneous collections of efferent and afferent lymph. Following the surgical procedure described by *McIntosh and Morris* (11), 2 afferent and 2 efferent lymphatic ducts of the renal node were cannulated in 3 sheep. Both ducts were cannulated in one of the sheep, an afferent duct to the node on the left side and an efferent duct from the node on the right side. Afferent hepatic lymph was collected from 1 sheep according

to the procedure described by *Lascelles and Morris* (12). In the remaining 3 sheep, 3 afferent lymphatic ducts and 1 efferent lymphatic duct of the prescapular node were cannulated as described by *Pedersen and Morris* (13).

An indwelling jugular cannula was inserted in each sheep at the time of the lymphatic cannulations to facilitate blood collection without disturbance to the animal.

### *Protein Determinations*

Total protein concentrations in blood plasma and lymph samples were determined by the *Biuret* reaction (14). Pooled plasma from 6 sheep, standardised by the micro-kjeldahl digestion method (15), was used to draw up a standard curve. A sample of this standard sheep plasma was included in each series of determinations.

The single radial immunodiffusion technique (16) was used to determine the concentration of albumin and each of the immunoglobulin classes in lymph and blood plasma samples. Mono-specific antisera for each of the immunoglobulin classes were prepared as described by *Watson, Brandon and Lascelles* (17) and for albumin as described previously (18).

### *Total cell counts and differential counts*

Total white blood cells (WBC) in blood and lymph were counted using a model ZBI Coulter counter (Coulter Electronics, England). Smears were prepared, stained with Giemsa stain (G.T. Gurr, England) and differential cell counts carried out on the stained films.

### *Experimental Procedure*

Lymph samples were collected into graduated measuring cylinders containing 0.01 ml of a 1000 i.u./ml heparin solution (Pularin, Evan's Medical Australia Ltd., Brononia, Victoria). Lymph was collected in this manner at intervals for periods of 15 minutes to 1 hour and lymph flow estimated from the volume collected. Blood samples were taken in the middle of each period of lymph collection.

In each sheep lymph was collected immediately after the lymphatic duct had been cannulated, while the animal was still anaesthetised. Following recovery from anaesthesia, lymph was collected during the period 6-12 hours after the initial collection; subsequent collections were made at 1, 2, 3 and up to 20 days after anaesthesia. In 5 of the sheep with cannulated afferent and efferent lymphatic ducts of the popliteal node, collection of lymph was also made when the animals had been reanaesthetised 5 days after the initial period of anaesthesia.

## *Results*

### *Hind Leg Lymph*

(a) *Protein.* The total protein concentrations in lymph collected from the efferent and afferent lymphatic ducts of the popliteal node during anaesthesia and at various times after cannulation are shown in Fig. 1 (a). The protein concentrations of lymph from both ducts were considerably higher under anaesthesia than those observed 3 days later in the conscious animal. Indeed, the lymph:plasma concentration ratio (Cl:Cp ratio) for afferent lymph under anaesthesia was  $0.57 \pm 0.06$  whereas 3 days later the ratio was  $0.24 \pm 0.01$ , a decrease of 57%. The Cl:Cp ratio in efferent lymph collected under anaesthesia was  $0.64 \pm 0.06$  compared to  $0.42 \pm 0.04$  observed 3 days later, a decrease of 36%. A significant finding was the observation that the protein concentrations of efferent and afferent lymph of the popliteal node of the sheep were not statistically different when the lymph from both ducts were collected under anaesthesia. This would be in agreement with the observations of *Osogoe and Courtice* (5) and *Bach and Lewis* (7) in the anaesthetised rabbit. When the sheep had fully recovered from the effects of the ope-

ration, however, a 40% difference in protein concentration was observed in the lymph from these 2 ducts as has been reported previously (8, 9).

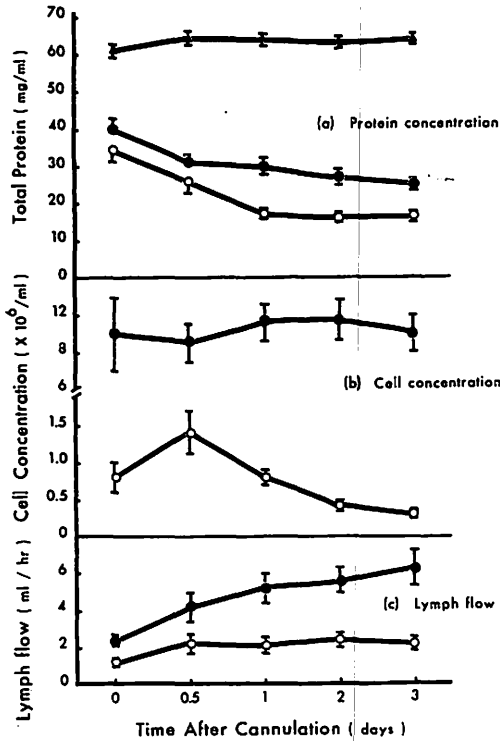


Fig. 1. (a) The total protein concentration observed in afferent (o—o) and efferent (●—●) lymph of the popliteal node, together with the concentration in blood plasma (▲—▲), collected under anaesthesia and at various times following cannulation of the lymphatic ducts.

(b) The leucocyte concentration observed in afferent (o—o) and efferent (●—●) lymph of the popliteal node collected under anaesthesia and at various times following cannulation of the lymphatic ducts.

(c) The lymph flow observed in afferent (o—o) and efferent (●—●) lymph of the popliteal node collected under anaesthesia and at various times after cannulation of the lymphatic ducts.

Values shown in the above 3 graphs are means of 8 sheep  $\pm$  the standard errors.

Table 1. The concentrations of albumin and immunoglobulins in efferent and afferent popliteal lymph and in blood plasma. Samples were collected under anaesthesia, immediately after cannulation, and again 3 days later. Values shown are the means of 5 sheep  $\pm$  standard errors.

Protein	Sample	Anaesthetised (immediately after cannulation)	Conscious (3 days after cannulation)	Percentage Change
Albumin (mg/ml)	Plasma	26.53 $\pm$ 1.54	27.65 $\pm$ 1.90	+ 4.2
	Afferent	12.68 $\pm$ 1.53	6.65 $\pm$ 0.61	- 47.6
	Efferent	17.37 $\pm$ 1.36	12.92 $\pm$ 1.17	- 25.6
IgG <sub>1</sub> (mg/ml)	Plasma	20.52 $\pm$ 2.93	20.68 $\pm$ 2.84	+ 0.8
	Afferent	7.61 $\pm$ 0.75	2.73 $\pm$ 0.47	- 64.1
	Efferent	10.88 $\pm$ 2.64	7.93 $\pm$ 1.81	- 37.2
IgG <sub>2</sub> (mg/ml)	Plasma	7.24 $\pm$ 1.44	7.88 $\pm$ 1.75	+ 8.8
	Afferent	2.51 $\pm$ 0.61	1.27 $\pm$ 0.24	- 49.4
	Efferent	4.19 $\pm$ 0.59	2.91 $\pm$ 0.54	- 30.6
IgM (mg/ml)	Plasma	2.29 $\pm$ 0.24	2.15 $\pm$ 0.16	- 6.1
	Afferent	0.74 $\pm$ 0.08	0.42 $\pm$ 0.03	- 43.2
	Efferent	1.19 $\pm$ 0.13	0.81 $\pm$ 0.08	- 31.9

Similar changes to those observed for total protein concentration were also observed in each of the individual proteins measured in Table 1, a difference of 43-64% in the afferent concen-

tration and 26-37% difference in the efferent lymph concentrations between samples collected under anaesthesia and samples collected 3 days later.

It is noteworthy that no significant change was observed in either the concentration of total protein or the concentration of the individual proteins in blood plasma between anaesthesia and the conscious state.

The results in Table 2 suggest that the changes in protein concentration in efferent and afferent lymph of the popliteal node, reported above, were not due to the effect of anaesthesia alone. Anaesthesia, in the absence of surgery, had no significant effect on the protein concentration of either blood plasma afferent or efferent lymph.

#### (b) Lymph Flow and Cell Concentration

Anesthesia induced with barbiturate and maintained with halothane resulted in a considerable reduction in lymph flow from the leg (see Fig. 1c and Table 2). Afferent lymph flow under anaesthesia was only 25% of the flow observed in the conscious animal, while efferent lymph under anaesthesia was 33-50% of normal values.

Changes in the concentration of leucocytes in efferent and afferent popliteal lymph between the time of cannulation and 3 days later are shown in Fig. 1 (b). Immediately after cannulation, the number and type of leucocytes in afferent lymph were similar to that observed 3 days later. However, soon after cannulation large numbers of neutrophilic granulocytes appeared in the afferent lymph and their numbers remained elevated for a period of 2-3 days. The total number of leucocytes in efferent lymph collected under anaesthesia was variable when compared with values observed 3 days later. In 3 sheep the number of leucocytes was depressed; in another 2 sheep the cell concentration was elevated and in the remaining sheep no change was observed. Of the leucocytes in efferent lymph, 98% were lymphocytes and anaesthesia and surgery had no significant effect on the differential count in efferent lymph.

During anaesthesia alone, the number of leucocytes in efferent popliteal lymph was depressed while there was no change in the number of leucocytes in afferent popliteal lymph (Table 2).

Table 2. The effect of anaesthesia (in the absence of surgery) on the total protein concentration, leucocyte concentration and lymph flow in efferent and afferent popliteal lymph. Values shown are means  $\pm$  standard errors with the number of sheep involved in each value shown in brackets.

		Conscious (5 days after cannulation)	Reanaesthetised	
			1 hour	2 hours
Protein Concentration (mg/ml)	Blood plasma (5)	62.80 $\pm$ 0.79	63.61 $\pm$ 1.04	62.89 $\pm$ 0.89
	Efferent lymph (4)	27.82 $\pm$ 1.81	26.06 $\pm$ 2.99	34.14 (1)
	Afferent lymph (4)	14.67 $\pm$ 1.24	14.50 $\pm$ 1.35	14.02 $\pm$ 1.18 (3)
Cell Concentration ( $\times 10^6$ /ml)	Efferent lymph (3)	12.84 $\pm$ 2.91	8.35 $\pm$ 2.15	15.94 (1)
	Afferent lymph (4)	0.61 $\pm$ 0.06	0.63 $\pm$ 0.12	0.50 $\pm$ 0.10 (3)
Lymph Flow (ml/hr)	Efferent lymph (3)	4.77 $\pm$ 1.97	2.73 $\pm$ 1.13	1.00 (1)
	Afferent lymph (4)	1.73 $\pm$ 0.24	0.40 $\pm$ 0.05	0.42 $\pm$ 0.07 (3)

#### Renal Lymph

##### (a) Protein and Cell Concentration

The results in Fig. 2(a) show that anaesthesia and recovery from surgery had little or no effect on the total protein concentration of efferent and afferent lymph of the renal node. In common with the 8 sheep in which popliteal lymph was collected, no change was observed in total protein concentration in blood plasma.

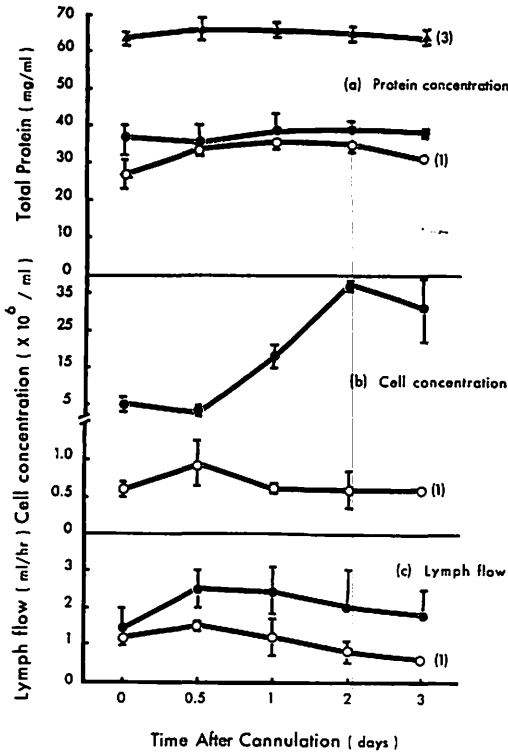


Fig. 2. (a) The total protein concentration observed in afferent (o-o) and efferent (●-●) lymph of the renal node, together with the concentration observed in blood plasma (▲-▲).

(b) The leucocyte concentration observed in afferent (o-o) and efferent (●-●) lymph of the renal node.

(c) The lymph flow observed in afferent (o-o) and efferent (●-●) lymph of the renal node.

Samples in each case were collected under anaesthesia and at several times after cannulation of the lymphatic ducts.

Values shown in the above 3 graphs are the means  $\pm$  standard errors (for 2 sheep). The total protein concentration in blood plasma is the mean of 3 sheep and where smaller numbers of animals were sampled at any one point the number is shown in brackets next to the value.

Anaesthesia and the trauma of surgery depressed the number of leucocytes in renal efferent lymph but had very little effect on afferent lymph (see Fig. 2b). The concentration of leucocytes in efferent lymph reached a stable level 3 days after surgery and remained stable for as long as the lymph flowed (up to 3 weeks). The leucocytes in both types of lymph were predominantly lymphocytes with some macrophages appearing in the afferent renal lymph (cf. 11).

The flow of efferent lymph from the renal node was depressed by 30% under anaesthesia compared to normal flow, however little effect was observed on the afferent lymph flow.

#### Prescapular Lymph

The prescapular node receives afferent lymph from the superficial and deep tissues of the neck in the sheep (13). The protein concentration, lymph flow and leucocyte concentration in the lymph flowing to and from this node at the time of cannulation and 3 days later are shown in Table 3. Both afferent and efferent prescapular lymph had higher protein concentrations, lower cell concentrations and lower flow rates at the time of cannulation when compared to the values observed 3 days later.

The changes in leucocyte concentration observed in afferent prescapular lymph in the first 3 days were similar to those reported for afferent lymph to the popliteal node. Large numbers of neutrophilic granulocytes were present in the lymph during the first 2 days after cannulation.

#### Hepatic Afferent Lymph

The protein concentration of afferent lymph from the liver was higher in the anaesthetised animal at the time of cannulation when compared to the concentration in the conscious animal 3 days later (see Table 3).

Table 3. Total protein concentrations, leucocyte concentrations and lymph flow in afferent and efferent lymph of the prescapular node and afferent lymph from the liver observed at the time of cannulation and 3 days later. The values shown are means  $\pm$  standard errors. The number of sheep involved in each value is shown by the number in brackets.

Sample		Anaesthetised (immediately after cannulation)	Conscious (3 days after cannulation)
Protein Concentration (mg/ml)	Blood plasma (3)	67.61 $\pm$ 1.74	67.71 $\pm$ 1.37
	Efferent prescapular (1)	47.84	44.03
	Afferent prescapular (3)	49.81 $\pm$ 8.05	41.53 $\pm$ 0.76
	Afferent hepatic (1)	65.56	55.45
Cell Concentration ( $\times 10^6$ /ml)	Efferent prescapular (1)	15.03	21.28
	Afferent prescapular (3)	0.67 $\pm$ 0.03	1.07 $\pm$ 0.22
	Afferent hepatic (1)	1.80	1.70
Lymph Flow (ml/hr)	Efferent prescapular (1)	7.50	9.70
	Afferent prescapular (3)	0.43 $\pm$ 0.07	0.60 $\pm$ 0.15
	Afferent hepatic (1)	4.00	1.90

#### *Effect of Anaesthesia on the WBC Counts*

The total number and type of WBC observed in venous blood collected under anaesthesia and in the conscious sheep is shown in Table 4. It can be seen that anaesthesia combined with surgery resulted in a marked neutrophilia. The neutrophilia was short lived and by 24 hrs after the cannulation the differential counts had returned to normal in most sheep. A similar result has been observed during nembutal anaesthesia and surgery associated with the cannulation of the thoracic lymphatic duct in the dog (19).

Table 4. The total number of white blood cells, neutrophilic granulocytes, lymphocytes and other cell types observed in venous blood samples during anaesthesia and surgery of 1 to 3 hr duration, and in the conscious animal. The percentage of the total WBC for each cell type is also shown. Values shown are means  $\pm$  standard errors with the number of sheep involved in each value in brackets.

		Conscious*		Anaesthetised	
Total white blood cells (8) ( $\times 10^6$ /ml)		7.44 $\pm$ 0.52		9.03 $\pm$ 0.90	
<u>Differential Count (6)</u>	Count ( $\times 10^6$ /ml)	Percentage	Count ( $\times 10^6$ /ml)	Percentage	
Neutrophilic granulocytes	2.20 $\pm$ 0.26	31.03 $\pm$ 2.42	4.07 $\pm$ 0.46	48.67 $\pm$ 3.56	
Lymphocytes	4.34 $\pm$ 0.51	60.82 $\pm$ 2.98	4.02 $\pm$ 0.42	48.67 $\pm$ 3.32	
Other cell types	0.53 $\pm$ 0.11	8.05 $\pm$ 2.12	0.22 $\pm$ 0.04	2.75 $\pm$ 0.47	

\*In the case of conscious animals, samples were obtained either prior to anaesthesia or 3-5 days after anaesthesia when the animals had fully recovered.

#### *Discussion*

The flow and protein concentration of lymph depends upon the balance between the tissue and capillary hydrostatic and colloidal osmotic pressures. These parameters in turn are inevitably influenced by changes in cardiac output, total peripheral resistance, and plasma and interstitial fluid volumes. Barbiturates result in an increase in plasma volume and a decrease in interstitial fluid volume (20, 21). These changes are possibly due to a net inflow of interstitial fluid into the vascular system (22). The changes in fluid distribution are undoubtedly influenced by the decrease in blood pressure which is known to occur during both barbiturate and halothane anaesthesia (23). In view of these changes, the observed decreases in lymph flow in the sheep

under barbiturate and halothane anaesthesia were not unexpected. However lymph flow is also influenced by blood flow and biological activity in the region of drainage; thus the absence of movement in muscular regions and the 24 hour starvation could in part account for the changes in lymph flow observed in the hind leg, hepatic and intestinal lymph reported in this paper and by others (4, 3).

Despite the changes in plasma volume, no changes in blood plasma protein concentrations have been observed with anaesthetic agents other than chloroform (22). In accord with this observation, there were no changes in the total or individual plasma protein concentrations in the sheep under the barbiturate and halothane anaesthesia employed in the present study. On the other hand, considerable changes were observed in the protein concentrations of lymph following surgical interference and recovery from anaesthesia. These changes may be due to changes in part in capillary permeability induced in the drainage area by surgical damage. This would be true for the popliteal and prescapular afferent ducts. Alternatively, or additionally, the changes could be due to changes in fluid migration between the vascular and interstitial pools, or hormonal influences association with the stress of surgery.

It is generally accepted that the concentrations of proteins and other large molecules in lymph, relative to plasma, are inversely related to lymph flow, provided capillary permeability remains constant (24). This relationship has been shown to hold for lymph collected from muscular areas (reviewed in 25) and the intestine (3). *White et al.* (4) suggested that this relationship is due to absorption of fluid from the interstitial space during periods of little activity. However, since flow was observed to decrease in efferent and afferent lymph from several regions without an increase in the protein concentration of this lymph it is unlikely that this relationship holds true in the anaesthetised animal. In general, the greatest changes in lymph flow were observed in lymph draining regions of low priority for survival, such as tissues associated with movement and digestion. On the other hand, lymph flow from regions of a higher priority for survival, such as the kidney and liver, was much less affected by anaesthesia.

The changes in protein concentration of the lymph appeared to be due to surgical interference in the region of drainage since anaesthesia alone failed to show any effect. The increases in the protein concentration in lymph from the afferent lymphatic ducts was greater than that observed in lymph from the efferent ducts since there was generally a greater degree of surgical interference to the region of lymph drainage during the cannulation of the afferent ducts.

The effect of anaesthesia and surgery on the number of leucocytes in lymph varied with the lymphatic duct cannulated. The overall results for efferent lymph suggested that anaesthesia and surgery tend to depress the number of lymphocytes in the lymph. The factors causing this change in lymphocyte migration could be due to either hormonal changes associated with the stress of anaesthesia and surgery or to inflammatory changes associated with the after effects of surgery. It is known that an increase in the blood levels of active adrenal cortical hormones occurs during surgery (27, 28) and it has been reported that corticosteroids influence lymphocyte migration patterns in the sheep (29) and in laboratory animals (30, 31). Indeed, corticosteroid hormones have been shown to produce a transient lymphocytopenia and neutrophilia in the blood of cows (32) and a significant lymphocytopenia in the lymph of sheep (30). These observations would tend to implicate the action of corticosteroids in producing the neutrophilia in blood and lymphocytopenia in lymph present in the sheep at the time of anaesthesia and surgery.

The changes in afferent lymph varied with the region cannulated. Renal and hepatic lymph showed little change in the WBC concentrations or differential counts either under anaesthesia or during the recovery period. Afferent lymph to the popliteal and prescapular nodes showed little change immediately after cannulation but the appearance of neutrophilic granulocytes in



the lymph shortly after surgery possibly points to an inflammatory state as described by *Morris* (26). This inflammation was undoubtedly associated with the surgical interference in the region of drainage.

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## Studies on the Immunochemical Composition of Human Thoracic Duct Lymph of Patients with Rheumatoid Arthritis and Polymyositis

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### Summary

Analysis of immunoglobulins, autoantibodies and third component of complement was carried out on the thoracic duct lymph of 6 patients with rheumatoid arthritis and 2 patients with polymyositis. It was found that the distribution of these immunoproteins between lymph and serum reflected the distribution of total protein and did not appear to be dependent on molecular size. There was no significant difference in the distribution of the macromolecule IgM as compared to the other immunoglobulins. IgD was an exception with an unanticipated and unexplained lower concentration in lymph and higher serum to lymph ratio when compared to the other immunoglobulins.

### Introduction

Lymphocytes are thought to play an important role in the pathogenesis of autoimmune disease. We have studied the effects of lymphocyte depletion by thoracic duct drainage (TDD) in patients with rheumatoid arthritis and have found that such patients undergo significant clinical improvement during prolonged continuous removal of thoracic duct lymphocytes through a surgical fistula (1). Immunoproteins such as immunoglobulins, autoantibodies and complement are also thought to play a role in the pathogenesis of autoimmune diseases.

Some studies of immunoprotein levels in the central lymph of humans have been previously published although nearly all in patients with non-rheumatic disease. *Dumont* and co-workers (2) showed that initial gamma globulin levels in lymph were about one half that in serum and declined from five to ten fold in 10 patients who underwent lymph depletion by TDD for up to 8 days with no reinfusion of lymph. Total protein and gamma globulin levels declined in both serum and lymph in these patients, with serum gamma globulin levels falling to less than one

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