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FLOW AND PROTEIN CONTENT OF TESTICULAR LYMPH IN CONSCIOUS RAMS

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The highly convoluted arrangement of the spermatic artery has been described for a number of species (Harrison, 1949; Harrison & Weiner, 1949; Kirby, 1953). In the ram the degree of convolution is extreme and the close proximity of the spermatic veins to the artery in the region of the pampiniform plexus provides a mechanism for the rapid exchange of heat between arterial and venous blood. Waites & Moule (1961) have shown that changes in temperature at the surface of the scrotal skin of the ram are rapidly followed by changes in temperature of the substance of the testis. This occurs by way of the returning venous blood by countercurrent thermal transfer between artery and vein in the region of the pampiniform plexus. These authors have also drawn attention to the marked diminution of systolic pressure as the blood courses through the coiled portion of the spermatic artery (Waites & Moule, 1960).

These unusual features in vascular anatomy might be expected to influence the formation and composition of lymph in this organ. It was therefore decided to study the rate of flow and protein content of lymph and to determine how these may be influenced by heating or cooling the scrotum. A technique has recently been described by Lindner (1963) for the collection of testicular lymph over short periods of time from anaesthetized rams, but we have found no reference to its collection from conscious animals over prolonged periods.

METHODS

Animals. One Dorset Horn and three Merino rams of mature age were used in the experiments.

Surgical procedure. Anaesthesia was induced with pentobarbitone and maintained with cyclopropane and oxygen in closed circuit. The animals were placed on the operating table with the uppermost hind leg fixed in a position outwards and backwards, to allow good access to the region of the inguinal canal. An incision 7–9 cm long, centred over the external inguinal ring and following the course of the spermatic cord, was made in the skin. The

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incision was continued through the connective tissues until the tunica vaginalis and external inguinal ring were located. At this point the spermatic cord was maintained in a moderately stretched position by gently pulling the testis. In this way the pampiniform plexus was maintained in a position well distal to the point of cannulation. The external and internal reflexions of the tunica vaginalis were carefully incised for a distance of 2–3 cm immediately below the external inguinal ring. The lymphatic ducts (2–8 in number) were located along the external surface of the spermatic vein; these were dissected free and ligated. One or two of the largest ducts were cannulated with Transflex tubing (Minnesota Mining & Manufacturing Co., New York; internal diameter 1·2–1·5 mm). The cannulae were passed through a tunnel beneath the skin to emerge just in front of the coxal tuber. The lymph was collected in a plastic bottle, which was sutured to the skin of the flank. After the operation the rams were confined in small pens 6 ft. × 6 ft. and given a ration of crushed oats and lucerne hay. Jugular blood and testicular lymph samples were collected simultaneously and the cells were removed by centrifugation.

Albumin transfer studies. 25 μc of radioactive iodinated human serum albumin (^{131}I-HSA) was injected intravenously in 10 ml. of normal saline, and blood and lymph samples were collected at regular intervals after the injection. Radioactive assay was carried out on 0·2 ml. samples of plasma and lymph as described by Lascelles & Morris (1961*a*). Specific activities were expressed as counts/min/g of albumin. Confirmation that the ^{131}I-radioactivity was protein-bound was obtained in a preliminary experiment, in which treatment of plasma and lymph with an excess of anion-exchange resin (Deacidite FF (Cl^-)) had no effect on the radioactivity/ml. ($t = 0{\cdot}47$, d.f. = 19, $0{\cdot}6 < P < 0{\cdot}7$). The size of the interstitial fluid pool drained by the lymphatics of the testis was computed according to the method described by Lascelles & Morris (1961*a*).

Scrotal heating and cooling. The scrotum was heated by immersing the lower third to half in water at $40 \pm 0{\cdot}5°$ C for periods of up to 1½ hr. Similarly, the scrotum was cooled by immersing approximately one half in water at $5 \pm 1°$ C for periods of up to 1 hr 15 min. In one animal the scrotum was cooled by spraying ethyl chloride intermittently on a 5 × 5 cm area of the anterior scrotum of the cannulated side. During these experiments the animals were kept in elevated metabolism cages and were sufficiently accustomed to the experimental conditions to stand quietly, eating lucerne hay.

Histological methods. Sections for histological examination were taken from both the testis and epididymis of the treated and control sides of two rams at autopsy. These were stained and appraised as outlined by Scott, Wales, Wallace & White (1963).

Electrophoresis. The plasma and lymph proteins were separated into six fractions by electrophoresis on Whatman 3 MM paper strips in veronal buffer, $\mu = 0{\cdot}075$, pH = 8·6. The electrophoretic cell used was of the Durrum (1950) design and was operated at 5 mA constant current for 16 hr. The strips were stained with bromphenol blue as described by Block, Durrum & Zweig (1958) and the patterns evaluated from the tracings of a Spinco 'Analytrol' reflectance scanner.

Total protein and albumin. The method of Gornall, Bardawill & David (1949) was used.

RESULTS

Surgical preparation. Lymph was collected from one of the testes in three rams and from both testes of a fourth ram at different times. The rams recovered from the anaesthetic 1–2 hr after completion of the operation. The rate of lymph flow during the operation and for some time after was low, but 24–36 hr later much faster and constant rates of flow were observed. Twenty-four to 48 hr after the operation the animals were clinically normal and so remained during the entire period of lymph

collection. Semen collected during and for several months after the period of cannulation in three of the rams was normal with respect to volume, colour, density and motility of spermatozoa. There were no differences in the proportion of cells staining with supravital dyes when epididymal spermatozoa from the treated and control sides were compared by this technique. There were also no abnormalities evident in histological sections of testis and of epididymis taken from the experimental animals. Measurements of the tubule epithelial height, of the tubule diameter and extent of fibrous tissue in the epididymis, and inspection of the germinal epithelium and interstitial tissue of the testis gave no indication of any tissue damage arising from the cannulation. Palpation of the testis during the course of these experiments did not reveal any difference in size, as would be expected if impedance of lymphatic drainage had occurred.

TABLE 1. Means and standard errors of rates of flow (ml./hr) of lymph from the testis of rams

Ram 1, left testis,	7·25 ± 0·36 (6)
Ram 2, left testis,	9·2 ± 1·87 (5)
Ram 3, left testis,	11·2 ± 1·14 (5)
Ram 3, right testis,	10·8 ± 0·68 (7)
Ram 4, left testis,	10·5 ± 1·21 (5)

In three of the rams 1 mg of T-1824 (Evans Blue) in 0·5 ml. saline was injected intradermally or into the dartos of the scrotum of the cannulated side. Lymph collected during the ensuing hours and days showed no trace of blueing. On the other hand, a similar injection of dye into the substance of the testis during the course of an operation on one of the rams resulted in its appearance in the lymph in high concentrations within a few minutes. It was thus concluded that most, if not all, the lymph collected by way of the cannulated testicular lymph ducts was derived from the testis and probably the epididymis.

Lymph flow. The rates of flow of lymph which were computed from collections taken each day for a number of days in the various rams are given in Table 1. Flow rates recorded during the first 24–36 hr after the operation are not included in this table because it was considered that at this stage either lymph formation was depressed or there was some impedance to lymph flow because of clotting, which occurred frequently at this time. The data in Table 1 show how constant the flow rates were between rams. When the scrotum was handled an immediate increase in lymph flow resulted, presumably due to more rapid propulsion rather than greater formation of lymph. Momentary increases (six- to tenfold) in lymph flow were observed when the testes were drawn up abruptly by the cremaster muscles during ejaculation. This was true whether ejaculation was during normal service to a ewe (or artificial vagina) or was elicited by

electrical stimulation with the bipolar rectal electrode of Blackshaw (1954).

Exercise and lymph flow. After establishing resting flow rates the rams were exercised in a paddock for half an hour, during which period lymph samples were collected frequently. At the end of the exercise the rams, now tired and panting, were returned to their pens and further flow rates were taken. The flow responses observed in a typical experiment on one of the rams are shown in Fig. 1. It can be seen that while the flow increased markedly during the first 1–2 min of exercise it then progressively

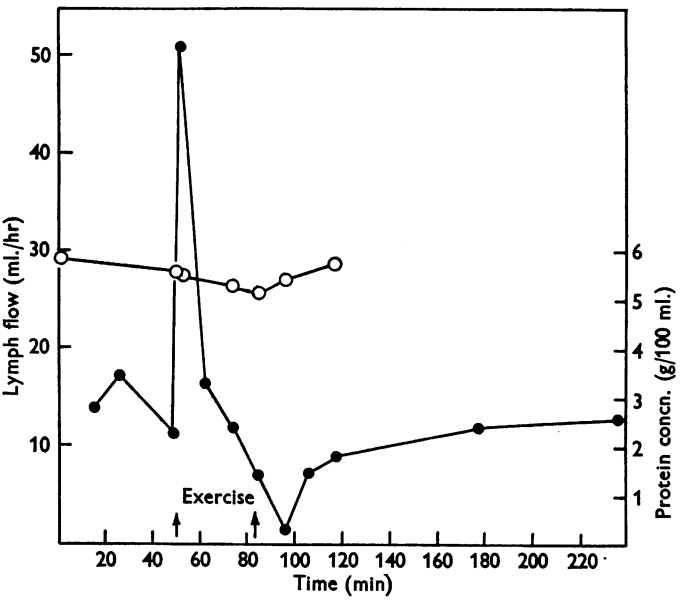

Fig. 1. The effects of exercise on the rate of flow and protein concentration of testicular lymph. ●——● Rate of lymph flow (ml./hr); ○——○ protein concentration (g/100 ml.)

decreased to very low levels. The protein content of the lymph did not alter significantly during the course of the experiment.

Scrotal heating and lymph flow. On heating the scrotum there was a marked increase in flow rate within 15–30 min. Maximum flow rates, 2–3 times the control level, were recorded 30–45 min after the application of heat, and were maintained at this level until heating was stopped (see Fig. 2). The flow slowly subsided to normal levels within 1–2 hr. Scrotal heating did not cause any significant change in protein content of the lymph during this period.

Effect of cooling the scrotum and lymph flow. There was a pronounced fall in the rate of lymph flow soon after cooling was started and minimum

rates of flow were recorded approximately 45 min later (see Fig. 2). Again there was no significant alteration in the protein content of the lymph collected during the experimental period.

Albumin transfer studies. Figure 3 shows the specific activity curves in the plasma and lymph following the intravenous injection of labelled

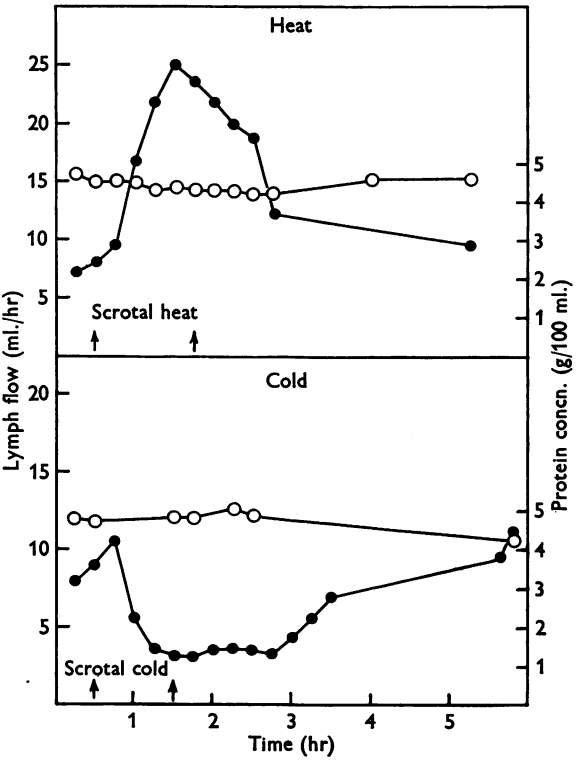

Fig. 2. The effect of scrotal heating (40° C) and scrotal cooling (5° C) on the flow and protein concentration of testicular lymph. ●——● Rate of lymph flow (ml./hr); ○——○ protein concentration (g/100 ml.)

albumin. The labelled albumin was transferred very rapidly from plasma to lymph and in the three rams studied the specific activity of the lymph equalled that of the plasma between 2 and 3 hr after the injection. The amount of newly filtered albumin and the volume of tissue fluid required to replace that in the interstitial pool at the beginning of the experiment was computed as described by Lascelles & Morris (1961*a*), and the values are presented in Table 2. The average testicular weight of the experimental rams was approximately 220 g and on this basis 4–7·5 % of the testis would be occupied by interstitial fluid.

Protein content. The results of the protein analyses of plasma and lymph

are set out in Table 3. The protein content of testicular lymph was high, varying between 59 and 72 % of plasma values in the different rams. The albumin:globulin ($A:G$) ratios of both plasma and lymph, determined by 'salting-out' the globulins, were confirmed by paper electrophoresis.

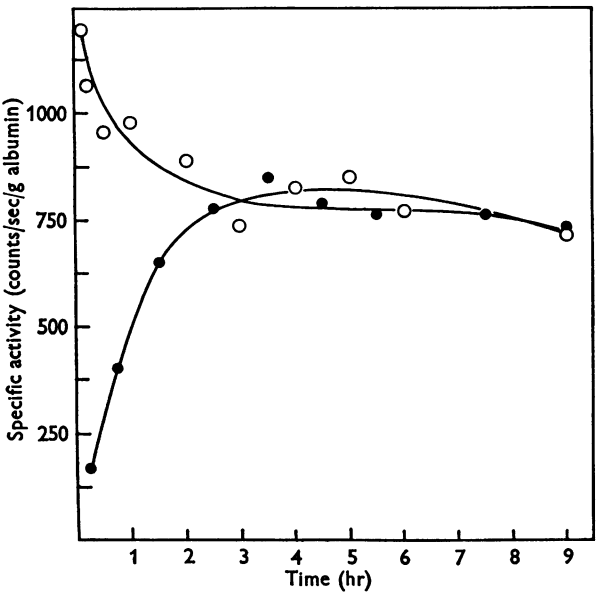

Fig. 3. The specific activities in plasma and testicular lymph of one of the rams following the intravenous injection of ^{131}I-HSA. ○——○ Specific activity of plasma; ●——● specific activity of lymph

TABLE 2. The estimates of the amount of filtrate albumin required to replace the albumin in the interstitial pool of the testis. The calculated volumes of the interstitial pool are also presented together with the mean albumin concentration of the lymph

Ram no.	1	2	3
Filtrate albumin required to replace that in interstitial fluid pool (g)	0·26	0·35	0·18
Mean concentration of albumin of lymph (g/100 ml.)	2·03	1·91	1·82
Size of interstitial pool (ml.)	10·9	18·3	10·14

Since this ratio was considerably higher in lymph than in plasma, there is clearly a greater capillary leakage of albumin than globulin. However, all the major plasma protein components were present in the lymph.

DISCUSSION

Technique. By means of the technique described it has been possible to collect lymph from the testis of the ram for as long as 20 days. The tendency for clotting in the cannula, particularly during the first day

TABLE 3. The concentrations of total proteins and albumin, together with *A*:*G* ratios in the blood plasma and testicular lymph of rams. Values presented are means ± S.E. with the number of samples shown in brackets. *P*, plasma value; *L*, lymph value

Constituent	Sample	Ram 1	Ram 2	Ram 3*a*	Ram 3*b*	Ram 4
Total proteins (g/100 ml.)	*P*	8·21 ± 0·01 (10)	9·10 ± 0·07 (9)	10·67 ± 0·16 (5)	9·87 ± 0·55 (3)	6·90 ± 0·39 (2)
	L	5·57 ± 0·02 (10)	5·59 ± 0·09 (7)	6·37 ± 0·13 (7)	5·82 ± 0·27 (3)	4·26 ± 0·07 (2)
Albumin (g/100 ml.)	*P*	2·74 ± 0·01 (10)	2·45 ± 0·08 (5)	2·43 ± 0·05 (5)	2·03 ± 0·06 (3)	3·27 ± 0·10 (2)
	L	2·40 ± 0·03 (10)	1·88 ± 0·07 (4)	1·80 ± 0·04 (7)	1·71 ± 0·14 (3)	2·58 ± 0·14 (2)
A:*G* ratio	*P*	0·48 ± 0·01 (10)	0·37 ± 0·01 (5)	0·30 ± 0·03 (5)	0·26 ± 0·02 (3)	0·90 ± 0·17 (2)
	L	0·76 ± 0·03 (10)	0·51 ± 0·03 (4)	0·40 ± 0·01 (7)	0·41 ± 0·02 (3)	1·54 ± 0·14 (2)

after the operation, is probably related to the high protein content of the lymph. In order to maintain the patency a careful inspection of the cannula was made twice daily and developing clots removed. Two days after the operation the rate of lymph flow had increased to a high steady rate and the cannula could be kept patent without difficulty. With the possible exception of one ram, it was considered for the following reasons that most if not all the lymph formed in the testis and epididymis drained through the one or two cannulae inserted into the largest ducts. First, no detectable oedema or loss of function of the testis, as evidenced by the volume and quality of semen, occurred although all visible lymphatics were ligated at operation. Secondly, the rate of lymph flow varied very little between rams. Thirdly, in one ram alternate blocking of one of the two cannulae led to an increase in flow in the other cannula and 3–4 hr later lymph flowed at rates similar to that which had been recorded when both cannulae were patent.

Lymph flow. Flow rates varied between 7 and 11 ml./hr for the conscious rams. The flow rate during anaesthesia was very much less than this. It is uncertain whether the low rates of flow observed during the operation were related to the effects of the anaesthetic or the low environmental temperatures which may have caused pronounced scrotal cooling. Our observations on flow rate during anaesthesia are therefore not entirely in agreement with those of Lindner (1963), who, using gentle suction, obtained average flow rates of 7·5 ml./hr in sheep during prolonged anaesthesia.

The transitory rise in lymph flow observed immediately after exercise commenced is clearly a reflexion of an increase in the rate of propulsion of lymph in the lymphatic vessels. The marked depression in lymph flow which followed the initial increase was sustained for a considerable time after exercise was stopped. It is probable that blood flow through the testis was depressed during this period, owing to the diversion of a considerable proportion of the blood volume to the muscles.

The rapid change in lymph flow following the application of heat and cold to the scrotal skin is of special interest in relation to what is known about the function of the scrotal skin in regulating the temperature of the blood flowing through the testis (Waites & Moule, 1961). It seems certain that these changes in lymph flow reflected changes in the actual formation of lymph rather than an alteration in its propulsion, because they were maintained over such long periods of time. In fact, during the periods of scrotal heating the volume of lymph collected was about equal to the volume of the testicular interstitial fluid pool.

Protein leakage from capillaries is a function of capillary filtration area, filtration pressure and capillary permeability (Yoffey & Courtice, 1956),

and since the protein content of the lymph during the period of the experiments was fairly constant (see Fig. 2) the alteration of lymph flow must have reflected to a large extent alterations in capillary filtration area or capillary blood flow. Similar conclusions were drawn by Cross & Silver (1962), who found that changes in oxygen tension in the testis and epididymis occurred following scrotal heating and cooling. Since thoracolumbar anaesthesia did not block these effects, Cross & Silver suggested that cutaneous thermal sense organs are involved in a type of axon reflex. In view of the very rapid changes in testicular oxygen tension following very brief periods of scrotal heating or cooling this explanation is feasible, but it may not be the complete explanation in our experiments, in which the heat or cold were applied over a much longer period of time. Under these conditions there was sufficient time for the temperature change at the skin surface to be reflected in the substance of the testes by way of the superficial testicular veins and the counter-current heat transfer via the incoming arterial blood in the region of the pampiniform plexus. Thus Waites & Moule (1961) found that a significant change in deep testicular temperature occurred 5–10 min after continuous heating or cooling of the scrotum. The altered temperature of the incoming arterial blood would be expected to cause a direct vasoconstrictor or vasodilator action on the small circulation of the testis. The much larger alterations in lymph flow, and presumably in capillary blood flow, following scrotal heating or cooling, observed in our experiments compared with the responses described by Cross & Silver (1962), may well be due to the direct effect of thermal stimuli on the small circulation of the testis in addition perhaps to the rapidly acting reflex mechanism.

The almost instantaneous responses following scrotal heating or cooling described by Cross & Silver (1962) were not observed in the experiments described in this paper. This is not surprising, however, because a change in lymph formation following changes in capillary blood flow would be 'buffered' by the testicular interstitial fluid pool, which has a volume approaching 20 ml.

In the ram in which cooling was achieved by spraying the scrotum with ethyl chloride, the chilled area subsequently became inflamed. This was associated with a sustained elevation in flow rate approximately 50% above basal level, and this persisted until the inflammation subsided. This effect may have been related directly to the increase in scrotal temperature as a consequence of the inflammation or to a temporary loss of function of the sweat glands of the affected area impairing the normal cooling mechanism.

The protein content of testicular lymph was found to be 59–72% of plasma values and is higher than that of lymph collected from other

regions of the body, with the exception of the liver. The $A:G$ ratio of testicular lymph was considerably higher than in plasma. It is surprising that the testicular lymph collected in anaesthetized rams by Lindner (1963) had a protein content, as assessed by visual examination of starch-gel electrophoretic patterns, about equal to that of plasma, except for the slow moving α_2 macroglobulin of Schultze (1958), which was present in plasma in higher concentrations.

Rate of protein transfer. There was a rapid rate of transfer of ^{131}I-HSA between plasma and testicular lymph; equilibration of the specific activity curves occurred within 3 hr after the injection. Similar rapid rates of transfer of labelled albumin have been observed only in the liver, the sinusoids of which are highly permeable to protein (Yoffey & Courtice, 1956).

Thus in the testis as in the liver there is a comparatively high rate of production of lymph with a high protein content and the rate of transfer of protein between plasma and lymph occurs very rapidly. It is therefore reasonable to propose that the permeability of testicular capillaries is higher than that of most other regions of the body. In view of the dual function of the testis in both the secretion of androgens and the production of vast numbers of spermatozoa (Ortavant, 1959) it seems likely that its demand for substrates from the blood would be high. However, the blood flow through the testis is quite low compared with that of many other organs. In order that an abundant supply of substrates should be available to the testicular cells it may be that a high degree of capillary permeability, together with a high filtration pressure, is necessary to ensure a rapid bulk transfer of fluids and solutes between the plasma and tissue-fluid compartments.

The volume and protein output of testicular lymph on a unit weight basis is approximately three times as high as that from the liver of the sheep (Lascelles & Morris, 1961*b*). In view of the comparatively low blood flow through the testis of the ram (8 ml./min/100 g testis) reported by G. M. H. Waites and B. P. Setchell (personal communication), the differences in testicular and hepatic lymph flow and protein output would be even greater if comparisons were made on the basis of unit volume of blood flow through these organs. The difference is probably related to the very low filtration pressures which are known to exist in the liver, compared with the rather high pressures in the testis of the ram (Waites & Moule, 1960). In this connexion it is significant that substantial increases in lymph flow and protein output in the liver lymph of the cat occur when the pressure in the hepatic portal system is elevated by partial occlusion of the hepatic veins (Morris, 1956).

SUMMARY

1. A technique is described for the collection of lymph from the testis and epididymis of the ram.

2. Lymph flowed continuously for periods of up to 20 days. The rate of lymph flow during anaesthesia was relatively low, but higher sustained levels, which varied between 7 and 11 ml./hr, were observed 24–36 hr after the operation.

3. There were pronounced increases and decreases in lymph flow following scrotal heating and cooling respectively. Since the protein concentration did not alter to any extent during the course of these experiments it was considered that lymph-flow responses largely reflected changes in capillary blood flow.

4. The protein content of testicular lymph was high, varying between 59 and 72% of plasma values for the different rams.

5. ^{131}I-labelled albumin was transferred very rapidly between plasma and lymph, and in the three rams studied the specific activity of the lymph equalled that of the plasma 2–3 hr after the injection of the isotope.

6. It was calculated that 4–7 % of the testis was occupied by interstitial fluid.

7. The dynamics of protein transfer between plasma and lymph in the testis are compared with those of the liver and other regions of the body of the sheep.

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