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The Proteins of the Thoracic-Duct and Intestinal-Duct Lymph of Sheep

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The composition of lymph has been reviewed by Yoffey & Courtice (1956). Early investigations were made on lymph of man and small animals, and samples were usually obtained under anaesthesia. More recently attention has been given to the lymph of ruminants, mainly in relation to the secretion of milk and to lipid absorption. Oxen, sheep and goats have been studied, with samples obtained *post mortem* or samples from continuously flowing or recirculating cannulae in conscious animals. The data available show that the protein concentration is lower in the lymph than in the plasma and varies with the region of the body from which it was obtained. The proteins present in lymph have been compared with those of plasma in small animals by Courtice & Morris (1955), in calves by Perlmann, Glenn & Kaufman (1943), in oxen by Heyndrickx (1959), in goats by

Linzell (1960) and in sheep by Lascelles & Morris (1961). The main electrophoretic components of plasma have been found in lymph and there is some evidence for discrimination against larger molecules passing into the lymph from a higher albumin:globulin ratio and the absence noted by Perlmann *et al.* (1943) of the β -anomaly on boundary electrophoresis of this fluid. However, Papp & Kisfaludy (1958), working with cats and dogs, reported that the concentration of γ -globulin could be higher in the lymph than in the corresponding sera.

As the data available on the proteins of sheep lymph are limited, the opportunity was taken to make an electrophoretic study of samples of thoracic-duct and intestinal-duct lymph from sheep cannulated in the lipid-absorption experiments of Feliński, Garton, Lough & Phillipson (1964).

Table 1. Protein concentration and related data on sheep lymph and blood serum

Details are given in the text.						
	Concn. of protein (g./100 ml.)	Conductivity (ohms ⁻¹ cm. ⁻¹ at 20°)	Flow rate* (ml./hr.)	Average protein flow (g./hr.)	Concn. of lipid* (mg./100 ml.)	Lipid:protein ratio (mg./g.)
Lymph						
(a) Thoracic duct	2.30	0.0127	30-50	0.9	37.5	16.3
(b) Intestinal duct (1)	3.81	0.0123	80-100	3.4	—	—
(2)	3.77	0.0120				
Serum	4.85	0.0123	—	—	14.6	3.01

* Data from Feliński *et al.* (1964): these refer to unclotted samples from the same animal.

METHODS

Samples were collected from a conscious animal about 24 hr. after the ducts were cannulated; a blood sample was obtained from the same animal for comparison. The thoracic-duct lymph samples did not contain the normal contribution of intestinal-duct lymph, since the intestinal duct was cannulated separately. All samples, obtained without anticoagulant, were allowed to clot and the clear sera from the lymph and blood were used for analysis. The intestinal lymph was freed from chylomicrons by ultra-centrifuging.

Protein concentration was estimated from measurements of refractive increment with a differential refractometer, based on the design of Cecil & Ogston (1951) by using the mercury yellow lines, and the average value for the refractive increment of serum albumin and globulin from the data of Adair & Robinson (1930): the instrument was calibrated with potassium chloride, by using the data of Brodsky & Filippowa (1936). Lymph and serum samples were diluted 1:5 (v/v) with 0.9% NaCl with which they were compared. This procedure assumes that lymph and sera have similar electrolyte compositions, an assumption supported by the data summarized by Yoffey & Courtice (1956), by the measurements of specific conductivity of the present samples and by the data of Heyndrickx (1959) on the refractive index and protein content of ox lymph and plasma. Solutions for electrophoresis were compared with the buffer against which they had been dialysed. Conductivity was measured at 3000 cycles/sec. at 20°.

Boundary electrophoresis was carried out in a Tiselius apparatus at +1° with sodium veronal buffer, pH 8.6 and *I* 0.1. Paper electrophoresis was conducted on horizontally suspended Whatman 3MM paper in sodium veronal buffer, pH 8.6 and *I* 0.05, for 16 hr. at 0.4 ma/cm. width of paper. Starch-gel electrophoresis was carried out by the method of Smithies (1955), but with the phosphate buffer system, pH 7.8, of Ashton (1957); polyacrylamide gel (7.5 g./100 ml.) was made as described by Cruft (1962) but with the above phosphate buffer.

The paper electrophoretograms were stained with Lissamine green [0.5 g./100 ml. of 5% (v/v) acetic acid in 25% (v/v) ethanol]; the starch and polyacrylamide gels were stained with Naphthalene Black 10B, and caeruloplasmin was detected in the gels by the oxidase stain with *p*-phenylenediamine as described by Uriel (1958).

The electrophoretic diagram was obtained by a semi-automatic transmission photometric scan of a polyacrylamide gel, in which the samples had been adjusted to about the same protein concentration (1 g./100 ml.) before being inserted in the gel, by using a red filter (Chance ORI).

Fig. 1. Paper electrophoretograms of (i) lymph proteins and (ii) serum proteins in veronal buffer, pH 8.6 and *I* 0.05: (a) serum; (b) thoracic-duct lymph; (c) and (d) intestinal-duct lymph. A, Albumin; α , β and γ , globulins; S, starting position. The arrow indicates the direction of migration, + and - the electrode disposition. The current was 0.4 ma/cm. of paper width for 16 hr.

RESULTS

The protein concentrations and the specific conductivities of the sera of lymph and blood, together with additional data from Feliński *et al.* (1964) on the flow of lymph and on the lipid contents of lymph, are given in Table 1. The protein concentration in the thoracic-duct lymph is less than 50% of that in blood serum, and the corresponding value for intestinal-duct lymph is about 75%.

The paper electrophoretograms of the lymph and serum proteins are shown in Fig. 1. The thoracic-duct lymph and intestinal-duct lymph contain the main electrophoretic components of the serum. The similarity of the patterns is shown by the electrophoresis of thoracic-duct lymph and

serum at the same protein concentration in polyacrylamide gel (Fig. 2). Some additional results of these electrophoreses are given in Table 2. The mobility of the fastest component of the thoracic-duct lymph is similar to that of the serum albumin and its relative proportion is nearly the same. The starch-gel electrophoresis showed in addition that thoracic-duct lymph and intestinal-duct lymph contained the same two transferrins as the serum but probably less of the slow α -globulin. The appropriately stained gels showed caeruloplasmin to be present in the α_2 -globulin region.

Fig. 2. Diagram of electrophoresis of (a) thoracic-duct lymph and (b) serum proteins in polyacrylamide gel in phosphate buffer, pH 7.8, from photometry of gel. A, Albumin; β and γ , globulins; S, starting position. The arrow indicates the direction of migration or of increasing light transmission. The current was 4 mA/cm. of gel width for 18 hr.; the gel thickness was 6 mm.

Table 2. *Electrophoretic examination of sheep lymph and blood serum*

Details are given in the text.

	$10^5 \times$ Mobility of albumin from boundary electrophoresis in veronal buffer, pH 8.6 and I 0.1, at 0° ($\text{cm.}^2 \text{sec.}^{-1} \text{V}^{-1}$)	Albumin as proportion of total protein from photometry of polyacrylamide gel (% w/w)
Lymph (thoracic duct)	-6.8	47
Serum	-6.5	43

DISCUSSION

The present results show that in the sheep as in other animals that have been studied the lymph proteins are closely related to those of the serum and have a lower concentration. The relatively higher protein concentration in the intestinal-duct lymph compared with that in thoracic-duct lymph has not been investigated further, but Table 1 shows that the calculated flow of protein is much greater for the intestinal-duct lymph than for the thoracic-duct lymph. The combined secretion yielded 103 g. of protein in 24 hr. This is in agreement with the estimate of Lascelles & Morris (1961) of 100 g. in 24 hr. and with the conclusion of Heath & Morris (1962) that the output of protein in the thoracic-duct lymph is largely due to the intestinal-duct lymph flowing into the thoracic duct.

The present results are inconclusive as to whether there is a selective transfer of proteins of lower molecular weight from serum to lymph. There is a deficiency of slow-moving α -globulins in the lymph indicated by starch-gel electrophoresis, but the proportion of albumin in the proteins of the two fluids is about the same. Further, as Feliński *et al.* (1964) observed that the composition of the thoracic-lymph lipid is reminiscent of high-density lipoprotein, the lipid:protein ratio in the two fluids has been calculated and included in Table 1 as a guide to the distribution of lipoprotein and is seen to be much greater in the thoracic-duct lymph than in the serum.

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