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Lipids of Sheep Lymph

TRANSPORT FROM THE INTESTINE

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The depot fats of ruminants are characterized by their relatively high content of saturated fatty acids (in particular of stearic acid) compared with the amounts present in the depots of other herbivorous animals such as the horse and the rabbit (for detailed information see Hilditch, 1956; Garton, 1960*a*). To account for the presence of these 'stearic acid-rich' triglycerides Hilditch & Sleight-holme (1931) and Banks & Hilditch (1932) suggested that preformed oleo-glycerides underwent hydrogenation in the tissues of the ruminant animal. Though this possible mechanism cannot be excluded it is rather to events in the rumen that attention has been directed. Studies such as those of Reiser (1951), Shorland, Weenink, Johns & McDonald (1957) and Garton, Lough & Vioque (1961) have shown that unsaturated fatty acids present in the lipids of the feed can be hydrogenated under the influence of the micro-organisms (mainly bacteria) that inhabit the rumen. Stearic acid is the final product of hydrogenation of the C_{18} unsaturated fatty acids (oleic acid, linoleic acid and linolenic acid) of which the lipids of pasture grasses and other herbage are rich sources (Weenink, 1959, 1961; Garton, 1960*b*). It has also been demonstrated that the feeding to oxen and goats of oils having a high content of C_{18} unsaturated fatty

acids (linseed oil, cottonseed oil) leads to the deposition of enhanced amounts of stearic acid in the depot fats (Willey, Riggs, Colby, Butler & Reiser, 1952; Reiser & Reddy, 1956). Further, when linseed oil was given to a sheep via a duodenal cannula, thereby by-passing the rumen, Ogilvie, McClymont & Shorland (1961) found that the perinephric fat contained much more linoleic acid and linolenic acid than are normally found in sheep depot fat, thus supporting the indirect evidence that the high stearic acid content of ruminant tissue lipids results from the intestinal absorption and subsequent assimilation of stearic acid produced in the rumen by microbial hydrogenation of unsaturated C_{18} fatty acids.

To obtain further information on this topic it was decided to examine the lipids present in the feed, the alimentary tract and, in particular, the lymph draining the sheep's intestine, with special reference to stearic acid. An account of these experiments was communicated to the International Conference on Biochemistry of Lipids, Birmingham, August 1962 (see Lough, Feliński & Garton, 1963).

EXPERIMENTAL

Animals and diet

The sheep used were four young adult animals, weighing 70–100 lb.; two (sheep 1 and 2) were castrated Scottish Blackface males, sheep 3 was a castrated Shetland male and sheep 4 was a Shetland ewe. Sheep 1 and 2 had previously been fitted with rumen fistulae and for several

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weeks before operation all were stall-fed, twice daily, on a ration that provided each animal daily with 600 g. of chopped hay, 60 g. of linseed cake meal and 30 g. of crushed oats; the same ration was given post-operatively. At all times the sheep had access to drinking water and a mineral 'lick'.

*Establishment of lymph-duct cannulae
and collection of lymph*

The animals were anaesthetized by the intravenous injection of Nembutal (sodium pentobarbital). The area at the base of the neck on the left side was shaved and a longitudinal incision, following the course of the left jugular vein, was made to the point of the shoulder. The jugular vein was cleaned of surrounding tissue and traced to its junction with the anterior vena cava. The thoracic duct was found to join with an ampulla which also received major lymphatic vessels from the head and neck. The ampulla discharged into the left jugular vein either at its junction with the anterior vena cava or slightly anteriorly to this junction; the position varied somewhat from sheep to sheep. A slight reflux of blood into the ampulla could be seen with each heart beat. The thoracic duct of each sheep was cannulated before its junction with the ampulla as indicated in Fig. 1, which shows (for sheep 3) the position of the cannula from which cervical-duct lymph was collected.

After sheep 3 was killed the left side of the thorax was opened by removing the last few ribs; injection of indian ink into the posterior part of the thoracic duct so exposed showed that there were no other anastomoses between the thoracic duct and the ampulla or between the thoracic duct and the cervical lymphatics, so that there was no possibility that the cervical-duct lymph was contaminated with thoracic-duct lymph.

To expose the cisterna chyli and the intestinal lymphatic duct which joins it a longitudinal incision was made in the left lumbar region of sheep 4, starting immediately behind

the last rib. The position of the intestinal duct was exactly as described by Lascelles & Morris (1961) and it was cannulated as it emerged from beneath the pancreas; during this operation the hepatic lymphatic duct was ligated. It was thus possible to measure the contribution which the intestinal-duct lymph would normally make to that in the thoracic duct.

All cannulations of lymphatic ducts are made difficult by the presence of valves. The polythene tubing used (0.5 mm. internal diam.) was inserted into the duct as far as possible and ligatures were tied though it was generally necessary to withdraw the tubing very slightly from its original position to free the end from valves in order to obtain a free flow of lymph. Thoracic-duct lymph and cervical-duct lymph were collected into polythene bottles suspended from the animals' necks with stout string; with a collection bottle in this position lymph could be collected whether a sheep was standing or lying down. To collect intestinal-duct lymph a collection bottle was attached to an abdominal 'harness' of surgical bandage.

To prevent the lymph clotting sodium citrate (3 ml. of a saturated solution/100 ml. of lymph) was added to the collecting bottles. To facilitate collection of lymph and measurement of its flow rate the animals were kept in wooden 'metabolism' crates for up to 3 days post-operatively; they were then killed by captive-bolt stunning and immediate exsanguination.

To maintain plasma electrolyte balance as nearly normal as possible the thoracic-duct lymph collected, less that required for analysis, was put into the rumen of the fistulated animals (sheep 1 and 2), together with a volume of 0.9% NaCl equal to that of the lymph retained for analysis; sheep 3 and 4 were given a corresponding amount of NaCl mixed with the ration.

Extraction and analysis of lymph lipids

Thoracic-duct lymph (about 200 ml.) obtained during the day after operation from sheep 1 and 2 was centrifuged at 1400g in the M.S.E. Major Centrifuge for 10 min. to remove lymphocytes and the few red cells which were present. Lipids were extracted under reflux, with a mixture of chloroform and methanol, from the lymph as it was collected or after it had been freeze-dried and stored at -1° . These extractions led to the formation of fatty acid methyl esters as artifacts (Lough, Feliński & Garton, 1962) which precluded a detailed study of the individual classes of lipid present in the lymph. The artifact-contaminated total lipid was saponified under reflux with excess of ethanolic 0.5N-KOH and the total fatty acids were recovered.

To avoid the formation of methyl esters during subsequent extractions, the lymph (100–200 ml.) collected from sheep 3 and 4 during the day after operation from each of the two cannulae was centrifuged as before to remove cells and then freeze-dried. The dried material was extracted in the cold with a two-phase mixture of chloroform, methanol and water (22:11:8, by vol.), similar to that used by Rhodes & Lea (1961) for the extraction of lipids from sheep liver. The extraction was made in a top-drive homogenizer (Measuring and Scientific Equipment Ltd., London) with 41 ml. of the solvent mixture/g. of freeze-dried lymph. The suspension thus obtained was centrifuged at 1400g in the M.S.E. Major Centrifuge for 10 min., after which the aqueous methanol was decanted and the chloroform layer

Fig. 1. Diagram showing positions of cannulae in sheep 3. Broken lines indicate ligatures. For clarity the lymph vessels and ampulla are shown about 3–4 times their true size in relation to that of the blood vessels. A, Cannulae; B, thoracic duct; C, cervical ducts; D, ampulla; E, internal jugular vein; F, left jugular vein; G, vein from left shoulder; H, carotid artery.

removed from beneath the protein 'plug'. A further such extraction of the protein 'plug' served to remove all the lipid from the freeze-dried lymph. The combined chloroform extracts were taken carefully to dryness in a stream of N_2 on a warm-water bath. Examination by thin-layer chromatography of the lymph total lipid so obtained showed no fatty acid methyl esters to be present (Lough *et al.* 1962).

The lipid was then applied, in solution in light petroleum (b.p. 40–60°), to a column of silicic acid (30 mg. of lipid/g. of silicic acid) (Garton & Duncan, 1957). Lipids were then eluted (Duncan & Garton, 1962) to yield sterol (cholesterol) esters, glycerides plus free sterol (cholesterol) and free fatty acids, and, finally, phospholipids. Only traces of mono- or di-glycerides were found by thin-layer chromatography of the glyceride fractions by the method of Privett, Blank & Lundberg (1961). The glyceride-containing fraction was then dissolved in ether and the free fatty acids were removed by washing with aq. 0.5% KOH, and the neutral lipid was saponified under reflux with excess of ethanolic 0.5N-KOH, followed by recovery of the fatty acids and of the unsaponifiable matter. Cholesterol was determined by the method of Schoenheimer & Sperry (1934) on this unsaponifiable matter and on that obtained after saponification of the sterol ester fraction. Fatty acid methyl esters were prepared as described by Duncan & Garton (1962) from appropriate lipid fractions.

Extraction and analysis of feed lipids and alimentary-tract contents

Feed. A sample of the feed was ground in a laboratory mill and a portion (2 g.) was extracted with chloroform-methanol-water mixture as described above for freeze-dried lymph. The total lipid thus obtained (75.5 mg.) was subjected to methanolysis by refluxing it with excess of methanolic 6N-HCl for 2 hr. followed by recovery of the fatty acid methyl esters produced (54.0 mg.).

Alimentary-tract contents. These were obtained from sheep 4 immediately after it was slaughtered; the rumen and upper half of the small intestine were ligated with string, taken out of the carcass and their contents removed. Samples (100 g.) of the contents of the rumen and small intestine were taken; each had a pH about 5.5, which was brought to 4.0 by the dropwise addition of dilute H_2SO_4 after the addition of 50 ml. of water to the semi-solid rumen contents. The material was then mixed with 200 ml. of methanol and filtered at the pump. The solid residue was twice extracted for 10 min. under reflux with 100 ml. of a chloroform-methanol mixture (2:1, v/v); the pooled extracts were concentrated *in vacuo* at 50° to about 30 ml. The aqueous methanol from the initial filtration was similarly concentrated. The two concentrated extracts were combined in a separating funnel to which 50 ml. of chloroform was added and the mixture was shaken. The chloroform layer was removed, washed with water and evaporated to dryness to yield the crude lipid. This was dissolved in chloroform and applied to a silicic acid (10 g.) column prepared as described by Garton & Duncan (1957), except that chloroform was used to prepare the slurry. Neutral lipids plus free fatty acids were eluted with chloroform and the retained phospholipids were subsequently eluted with chloroform-methanol (1:1, v/v). The chloroform eluate was evaporated to dryness and dissolved in 100 ml. of ether, and the free fatty acids were removed by washing with aq. 0.5% KOH. The acids were recovered and

converted into methyl esters by refluxing with excess of methanol containing 1% (w/v) of H_2SO_4 . The residual neutral lipids were combined with the phospholipids and the mixture was saponified with ethanolic 0.5N-KOH to yield the fatty acids which were converted into methyl esters. These esters and those of the free fatty acids were separately dissolved in 0.5% (v/v) ether in light petroleum and purified by passage of the solutions through columns of silicic acid to remove coloured impurities and methyl esters of bile acids, small amounts of which accompanied the free fatty acids extracted from the intestinal contents.

Analysis of fatty acid methyl esters. Fatty acid methyl esters were prepared and analysed by gas-liquid chromatography as described by Duncan & Garton (1962).

RESULTS

Appearance and flow-rate of lymph. As it issued from a cannula the thoracic-duct lymph was a very pale straw colour and opalescent and, in the absence of an anticoagulant, it clotted readily. The opalescence of the citrate-treated lymph increased on standing and cooling to give a milky pale-pink opaque fluid. The total cell count (almost entirely lymphocytes) was in the range 10 000–13 000/mm.³ and these cells sedimented completely when the lymph was centrifuged at 1400g in the M.S.E. Major Centrifuge for 10 min. The milky supernatant remained apparently unchanged after centrifuging for 15 min. at 7000g in the Micro High-Speed Centrifuge, model 54/002/01; microscopic examination showed it to contain very small, highly-refractile spherical particles (chylomicrons). The intestinal lymph was opalescent as it drained from the animal, but subsequently became milky and opaque; it contained no red cells but its lymphocyte count was double that of the thoracic-duct lymph, i.e. about 24 000/mm.³ The cervical-duct lymph and the thoracic-duct lymph that did not include the contribution of intestinal-duct lymph were clear and pale-amber coloured, and did not develop opalescence on standing and cooling; the cell content of both samples was about 7000/mm.³

Lymph flow from the thoracic-duct cannulae during the day after operation was in the range 50–175 ml./hr., though for periods of many hours a rate of 90–120 ml./hr. was observed in all the animals. From the cervical duct 30–50 ml./hr. was obtained and from the animal with cannulae in its intestinal duct and its thoracic duct the flow rates were 80–100 and 30–50 ml./hr. respectively. The above rates of lymph flow from the thoracic and intestinal ducts are similar to those observed by Lascelles & Morris (1961).

Composition of lymph lipids. The amounts and relative proportions of the lipids present in the lymph obtained from sheep 3 and 4 are shown in Table 1 and the fatty acid composition of the corresponding lipid fractions is given in Table 2.

Table 3 shows the stearic acid content of the thoracic-duct lymph of sheep 1, 2 and 3 and the intestinal-duct lymph of sheep 4, together with an estimate of the amount of stearic acid in daily trans-
port from the intestine of each animal. This estimate was calculated on the basis of a lymph flow rate taken as 100 ml./hr. in the thoracic duct of sheep 1, 2 and 3 and in the intestinal duct of sheep 4.

Table 1. Amounts and relative proportions of lipids in sheep lymph

Details are given in the text.				
Percentage (by wt.) of total lipid				
Nature of lipid	Sheep 3		Sheep 4	
	Thoracic-duct lymph	Cervical-duct lymph	Thoracic-duct lymph less intestinal-duct lymph	Intestinal-duct lymph
Total lipid (mg./100 ml.) ...	892	27	38	1029
Triglycerides	79.1	1.0	18.4	74.0
Phospholipids	15.9	51.3	31.6	17.5
Sterol (cholesterol) esters	2.3	25.7	31.6	5.7
Free sterol (cholesterol)	0.9	14.7	5.2	1.7
Free fatty acids	0.9	7.3	13.2	0.7
?Hydrocarbons	0.9	—	—	0.4

Table 2. Fatty acid composition of lymph lipids from sheep 3 and 4

Details are given in the text.				
Fatty acid	Source of fatty acids			
	Triglycerides	Phospholipids	Sterol (cholesterol) esters	Free fatty acids
	Percentage (by wt.) of total fatty acids in each lipid class			
Thoracic-duct lymph (sheep 3)				
Palmitic acid	14.2	18.2	8.4	18.7
Stearic acid	45.1	23.8	32.5	33.6
C ₁₈ mono-unsaturated acids	21.9	16.2	30.8	28.1
C ₁₈ di-unsaturated acids	4.8	20.2	13.3	3.9
C ₁₈ tri-unsaturated acids	4.5	8.4	4.8	5.8
Other acids*	9.5	13.2	10.2	9.9
Cervical-duct lymph (sheep 3)				
Palmitic acid	21.5	38.9	12.8	14.5
Stearic acid	32.0	21.6	7.8	20.5
C ₁₈ mono-unsaturated acids	25.5	18.6	35.8	48.1
C ₁₈ di-unsaturated acids	3.7	4.4	24.5	4.1
C ₁₈ tri-unsaturated acids	2.0	1.6	7.5	3.0
Other acids*	15.3	14.9	11.6	9.8
Intestinal-duct lymph (sheep 4)				
Palmitic acid	23.4	15.7	15.5	23.8
Stearic acid	29.0	25.1	15.4	28.9
C ₁₈ mono-unsaturated acids	25.5	21.3	32.2	27.9
C ₁₈ di-unsaturated acids	4.2	19.0	15.2	4.9
C ₁₈ tri-unsaturated acids	2.0	5.2	4.5	3.7
Other acids*	15.9	13.7	17.2	10.8
Thoracic-duct lymph from which the normal contribution of intestinal-duct lymph was excluded (sheep 4)				
Palmitic acid	22.8	19.4	16.0	19.4
Stearic acid	31.2	24.3	3.7	29.3
C ₁₈ mono-unsaturated acids	22.9	27.4	40.4	37.2
C ₁₈ di-unsaturated acids	3.5	8.6	23.0	3.7
C ₁₈ tri-unsaturated acids	2.8	2.2	4.1	1.2
Other acids*	16.8	18.1	12.8	9.2

* Includes small amounts of lauric acid, myristic acid, fatty acids with an odd number of C atoms/molecule, and components of uncertain structure, some probably having branched chains.

Feed lipids and alimentary-tract lipids. The fatty acid composition of the lipids of the feed consumed by the sheep and of the free and esterified fatty acids present, at slaughter, in the rumen and proximal half of the small intestine of sheep 4 are given in Table 4; the total fatty acid content of the digesta obtained from the rumen and small intestine was 229 mg./100 g. and 592 mg./100 g. respectively.

DISCUSSION

The proportion of each lipid class in the lymph of the thoracic duct and intestinal duct of the sheep (Table 1) is similar to that found in the corresponding chyle lipids of non-ruminant animals, e.g. cat (Bergström, Borgström, Carlsten & Rottenberg, 1950), rat (Borgström, 1951, 1952; Laurell, 1954) and man (Blomstrand & Ahrens, 1958; Blomstrand & Dahlbäck, 1960). The relative proportions of the lipids are characteristic of chylomicrons which consist of a central droplet composed essentially of triglyceride with a

'covering' of phospholipids, cholesterol and free fatty acids together with loosely adsorbed protein (see Dole & Hamlin, 1962). On the other hand, the lipids present in the lymph arising from parts of the body other than the intestine are derived from lipoproteins (cf. Yoffey & Courtice, 1956) and are characterized by their relatively high content of phospholipids and cholesterol esters and their low content of triglycerides.

Table 1 shows that almost all the lipid in the lymph of the thoracic duct originates from the intestinal duct. The lipid content of the thoracic-duct lymph from which the contribution of intestinal-duct lymph was excluded is of a similar low order to that found in the cervical-duct lymph, indicating that lymph entering the thoracic duct from other parts of the animal's body does not contribute significantly to the lipid content of thoracic-duct lymph. The intestinal duct may possibly contain lymph originating from parts of the alimentary tract other than the intestine, e.g. from the rumen and the abomasum, though it is doubtful if lymph draining these organs adds appreciably to the lipid content of intestinal-duct lymph. The fatty acid composition of the lipid fractions in the thoracic-duct lymph and cervical-duct lymph (Table 2) show that the triglycerides of each contained stearic acid as the principal component acid; that present in the glycerides of the cervical-duct lymph is of a similar order (about 30 % of the total fatty acids) to that found in sheep depot fat (Hilditch & Pedelty, 1941), whereas that in the glycerides of the thoracic-duct lymph was present to the remarkably high extent of 45.1 % of the total fatty acids. Stearic acid also represented a higher proportion of the total fatty acids of the phospholipids, the sterol esters and the free fatty

Table 3. *Stearic acid content of sheep lymph and amount in daily transport from the intestine*

Details are given in the text.

Sheep no.	Source of lymph	Stearic acid	
		(mg./100 ml. of lymph)	Estimated amount being transported/24 hr. (g.)*
1	Thoracic duct	250	6.0
2	Thoracic duct	247	5.9
3	Thoracic duct	312	7.4
4	Intestinal duct	232	5.6

* Flow-rate taken as 100 ml./hr. (see the text).

Table 4. *Fatty acid composition of feed lipids and of alimentary-tract contents of sheep 4*

Details are given in the text.

Fatty acid	Source of fatty acids				
	Total lipid of feed	Rumen contents		Upper half of small intestine	
		Free fatty acids*	Esterified fatty acids	Free fatty acids†	Esterified fatty acids
	Percentage (by wt.) of total fatty acids in each lipid class				
Palmitic acid	16.6	9.8	25.9	14.0	12.5
Stearic acid	2.6	73.1	14.9	50.6	22.0
C ₁₈ mono-unsaturated acids	25.7	11.1	18.9	15.6	27.2
C ₁₈ di-unsaturated acids	21.8	0.7	7.7	5.1	16.6
C ₁₈ tri-unsaturated acids	20.3	Trace	3.8	2.8	8.8
Other acids	13.0‡	5.3‡	28.8§	11.9§	12.9§

* 81% of total fatty acids of rumen contents.

† 51% of total fatty acids of small-intestinal contents.

‡ Mostly lauric acid, myristic acid and palmitoleic acid.

§ In addition to small amounts of lauric acid and myristic acid and fatty acids with an odd number of C atoms/molecule, branched-chain acids were apparently present.

acids of the lipids of the thoracic-duct lymph than it did of the total acids of the corresponding lipid fractions of cervical-duct lymph. Apart from palmitic acid, the remaining constituent fatty acids of the lipids of the lymph from both ducts largely comprised unsaturated C_{18} fatty acids. These are designated in the Tables as C_{18} mono-, di- and tri-unsaturated acids since they probably contained positional and geometrical (*trans*) isomers of oleic acid, linoleic acid and linolenic acid formed (together with stearic acid) in the rumen during the bacterial hydrogenation of these unsaturated acids of the feed lipids (Shorland *et al.* 1957; see also review by Garton, 1961).

Similar considerations relate to the fatty acid composition of the lipids of the intestinal-duct lymph and thoracic-duct lymph collected from sheep 4 (Table 2). The glycerides of the intestinal-duct lymph of this animal had a somewhat lower content of stearic acid than was observed in those of the thoracic-duct lymph of sheep 3, though it was nevertheless still of a similar high order compared with that present in sheep depot fat.

The amount of stearic acid in daily transport from the intestine was 5.6–7.4 g. (Table 3), and it seems likely that this was derived from the intestinal absorption of stearic acid produced, as Table 4 indicates, in the rumen by the microbial hydrolytic release and hydrogenation of C_{18} unsaturated fatty acids of which the feed lipids provided about 12 g. per sheep daily.

It appears that stearic acid is more readily assimilated by the sheep than by the non-ruminant animal. In the rat, for example, Carroll & Richards (1958) observed digestibility coefficients of 14% and 24% for tristearin and stearic acid. The apparently enhanced digestibility of stearic acid in the sheep may well be related to the nature of its digestive process which, unlike that in simple-stomached animals, is more or less continuous. This is associated with the 'hold up' of digesta in the rumen and the comminuted material passing from this organ to the lower gut contains a relatively small proportion of lipid (mainly free fatty acids) in a finely dispersed state which possibly facilitates its subsequent intestinal absorption.

The esterified fatty acids of rumen contents (Table 4) represent those of feed lipids that had not been released by bacterial lipolytic activity together with those of the structural lipids of the rumen micro-organisms from which the fatty acids with an odd number of carbon atoms/molecule and the branched-chain fatty acids present in this fraction are derived (Keeney, Katz & Allison, 1962). The free fatty acids isolated from the lipids present, at slaughter, in the upper half of the small intestine of sheep 4 contained similar component acids, indicating that microbial structural lipids

had undergone hydrolysis in the small intestine. A further feature of the lipids of the intestinal lumen (in addition to their expected high proportion of free stearic acid) is the enhanced proportions of unsaturated C_{18} acids in both the free- and esterified-fatty acid fractions compared with the proportions present in the rumen lipids. This evidently cannot be explained solely by the preferential absorption of other fatty acids from the intestinal lumen and it thus appears that the lipids of the intestinal digesta had been 'diluted' with less-saturated lipids of endogenous origin. Burr, McPherson & Tidwell (1960) observed the secretion of lipids into the intestinal lumen of the rat and McCarthy (1962) obtained evidence that indicated that unsaturated C_{18} fatty acids were secreted into the lumen of the goat's small intestine. Such lipids could be contained in a true secretion or, perhaps more likely, in shed epithelial cells.

SUMMARY

1. The thoracic duct of each of four sheep was cannulated, enabling lymph to be collected; in addition, lymph was also obtained from the intestinal duct of one of these animals and from the cervical ducts of another.

2. Thoracic-duct lymph contained very much more lipid than did cervical-duct lymph, and it was shown that this lipid originated in lymph draining into the thoracic duct via the intestinal lymph duct. Triglycerides constituted the major lipid component of thoracic-duct lymph and intestinal-duct lymph, and these were characterized by the high proportion of stearic acid that they contained; the amount of stearic acid in daily transport from the gut of four sheep was in the range 5.6–7.4 g.

3. The stearic acid was apparently largely derived from the intestinal absorption of that produced in the rumen by microbial hydrogenation of unsaturated C_{18} fatty acids of the feed lipids. Analysis of the contents of the upper small intestine of one sheep indicated that, in addition to lipid reaching this part of the alimentary tract in the digesta, lipid was also being contributed to the lumen contents from some endogenous source.

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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).