The Composition of the Liver Lipids of Sheep and the Effect of Early Sporidesmin Poisoning

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Facial eczema, a disease of sheep and, to a lesser extent, of cattle, has long been known in the North Island, New Zealand. The disease is contracted by grazing in pastures infected with the fungus *Pithomyces chartarum* (Commonwealth Mycological Institute Herbarium no. 74473). Synge & White (1959) isolated from cultures of this fungus a metabolic product which they named sporidesmin. Crystalline sporidesmin was prepared (Done, Mortimer, Taylor & Russell, 1961; Ronaldson, Taylor, White & Abraham, 1963) and experimental poisoning of normal sheep was found to be similar to field cases of facial eczema in sheep (Done, Mortimer & Taylor, 1960). Publications on the experimental sporidesmin poisoning of sheep have dealt with clinical and post-mortem observations (Mortimer & Taylor, 1962), with biochemical changes in some serum constituents (Done, Mortimer & Taylor, 1962), with haematological and liver-function disturbances (Mortimer, 1962), and with histological and histochemical observations (Mortimer, 1963).

The pathological and biochemical changes that occur as the disease progresses are complex, but the earliest recorded symptom (2 days after dosing) is a fatty infiltration of the liver. Preliminary investigations (Mortimer, Taylor & Shorland, 1962) of these liver lipids indicated that the increases were confined to the neutral-lipid fraction.

The present paper describes a more detailed investigation than hitherto reported of various lipid methods, and their application in the quantitative isolation and identification of the individual liver lipids of normal sheep and of sheep dosed with sporidesmin. In addition, the mechanism of the observed changes is considered in the light of the available information. A preliminary note on this investigation has been published (Peters, 1963).

MATERIALS AND METHODS

Materials

Sporidesmin. This was prepared as described by Ronaldson *et al.* (1963), and administered as its benzene solvate. The doses were calculated on the basis of a non-solvated molecular weight of 475.5.

Animals. The sheep used in the present investigation and the method of dosing and slaughter were similar to those

described by Mortimer & Taylor (1962). Four out of eight animals were given an oral dose of sporidesmin (1 mg./kg. body wt.), the remaining four being used as controls. After 2 days the entire group was slaughtered and the livers were excised within 10 min. of death.

Preservation of livers. Each liver was cut into cubes which were submerged in liquid nitrogen for 5 min. The frozen cubes were collected in a polythene bag, placed in a Dewar flask packed with solid CO₂ and transported to the laboratory where they were stored at -20°, until required for analyses.

Reagents. All reagents and solvents were British Drug Houses AnalaR or Merck 'pro analysi' quality. Chloroform, containing 1% (v/v) of ethanol as preservative, was passed through a column of alumina (Brockmann grade I) immediately before use. Chloroform and methanol were deoxygenated under reduced pressure, which was then released with nitrogen.

Chemical analyses

Suitable samples of column fractions were evaporated under a stream of nitrogen. Lipid phosphorus was determined by the method described by Brown (1954). Total esterified fatty acids were determined by the hydroxamic acid method described by Morgan & Kingsbury (1959). Total cholesterol was determined by the method of Zak, Luz & Fisher (1957).

Extraction of liver lipids

The method of Bligh & Dyer (1959) was modified to provide for a more complete lipid extraction of tissues containing more than 1% of lipid. Dry-weight determinations of three sheep livers from a local abattoir gave an average moisture content of 71.6%. A knowledge of the tissue moisture content is required to obtain optimum conditions for the diphasic solvent system on which the Bligh & Dyer (1959) procedure is based.

Three extractions were made as follows. In the first extraction 100 g. of frozen liver cubes was homogenized for 3 min. in a Waring Blendor with a mixture of 100 ml. of chloroform, 200 ml. of methanol and 8.4 ml. of distilled water. To the homogenate was then added 100 ml. of chloroform, the mixture blended for 30 sec. and 100 ml. of distilled water added. The homogenate was filtered on a Buchner funnel through a Whatman no. 1 filter paper. The filtrate was transferred to a separating funnel. In the second extraction the residue was homogenized again as in the first extraction, except that 80 ml. of distilled water was used in the first stage and 50 ml. of distilled water in the second stage. The filtrate was transferred to a second separating funnel. In the third extraction the residue was homogenized with 150 ml. of chloroform, and the

filtrate was transferred to a third separating funnel. The chloroform phases were combined and evaporated in a rotary evaporator. The concentrated extract was redissolved in chloroform and filtered through a sintered-glass filter (porosity 3). The filtrate was evaporated and the yellow viscous lipid material dried to constant weight *in vacuo* in a desiccator previously flushed with nitrogen. Throughout this entire investigation all manipulations were performed, as far as practicable, under nitrogen.

Column chromatography

General considerations. The chromatographic fractionation of the total liver lipids in a three-column system was derived from investigations on brain lipids, described by Rouser, Bauman, Kritchevsky, Heller & O'Brien (1961*b*). The three-column system consisted of a DEAE-cellulose column, a silicic acid-silicate-water column and a silicic acid column. During preliminary investigations, to determine the reproducibility of the column-chromatographic procedures and the identity of the eluted lipids, duplicate columns were run under identical conditions. In addition, 20 ml. fractions were collected throughout the entire elution procedures, except with the silicic acid column, when 10 ml. fractions were collected. The fractions were then analysed for total esterified fatty acid content. Once the completeness of elution of each lipid or group of lipids had been established, the collection of individual fractions was discontinued and each eluate was collected in bulk. The eluates were filtered through a sintered-glass filter (porosity 3) and evaporated in a rotary evaporator. The lipid material was dried to constant weight *in vacuo* in a desiccator previously flushed with nitrogen. Progress during the elution was monitored by a solids test (Rouser *et al.* 1961*b*) on each fraction. During the bulk collection, solids tests were done only towards the end of the elution of each solvent. Similarly, the elution from the column of ninhydrin-positive material was monitored with a rapid ninhydrin test (Rouser, O'Brien & Heller, 1961*a*).

The columns (2.5 cm. internal diam. $\times$ 40 cm. long) for the DEAE-cellulose-column and the silicic acid-silicate-water-column chromatography were as described by Rouser *et al.* (1961*b*). A 500 ml. glass-stoppered reservoir was sealed on to the column. For the silicic acid-column chromatography a glass tube (1.0 cm. internal diam. $\times$ 40 cm. long) was fitted with a Teflon tap and a glass-wool plug. A 100 ml. glass-stoppered reservoir was sealed on to the column.

Diethylaminoethylcellulose-column chromatography. Whatman DE 50 DEAE-cellulose powder (16 g.) was placed on a sintered-glass filter and washed successively with 1 l. of *N*-HCl, 2 l. of distilled water and 1 l. of *N*-NaOH. The DEAE-cellulose was then suspended for 3 hr. in 800 ml. of acetic acid, after which it was filtered and washed with 2 l. of methanol. The methanol slurry was poured into the column and the adsorbent weighed down with a filter-paper disk and several glass beads. The column was then washed with 200 ml. of chloroform-methanol (9:1, v/v). Approx. 350 mg. of the total lipid mixture was dissolved in 10 ml. of chloroform-methanol (9:1, v/v) and applied to the column. The lipids were then eluted successively by the eluting solvents as described in Fig. 1. After solvent *E*, and preceding solvent *F*, the column was eluted with 300 ml. of methanol, until the effluent was free of acetic acid. This fraction contained no lipid material and was discarded. The

various eluates were evaporated, and the lipids were dried to constant weight, as described above. In addition to lipid material, eluates *D*, *E* and *F* also contained inorganic salts, eluted from the column. The concentrated lipid material in these eluates was therefore redissolved in 5 ml. of chloroform-methanol (2:1, v/v) and shaken with an equal volume of distilled water. After centrifugation, the chloroform phases were evaporated, and the lipids dried to constant weight.

Silicic acid-silicate-water-column chromatography. Silicic acid (325 mesh; BioRad Laboratories, Richmond, Calif., U.S.A.) (30 g.) was dried overnight at 120° and cooled *in vacuo* in a desiccator previously flushed with nitrogen. A suspension was prepared with 200 ml. of chloroform-methanol (1:1, v/v) containing 6 ml. of aq. ammonia (sp.gr. 0.88). The column was prepared as described above and washed with 300 ml. of chloroform. The lipids (245–280 mg.) eluted with solvent *A* from the DEAE-cellulose column were dissolved in 10 ml. of chloroform and applied to the column. The lipids were then eluted successively by the eluting solvents as described in Fig. 2. The eluates were evaporated, and the lipids dried to constant weight, as described above.

Silicic acid-column chromatography. Silicic acid (325 mesh; BioRad Laboratories) (6 g.) was dried overnight at 120° and cooled *in vacuo* in a desiccator previously flushed with nitrogen. A suspension was made with 50 ml. of diethyl ether, and the column prepared as described above. It was then washed with 50 ml. of hexane-benzene (17:3, v/v) followed by 50 ml. of hexane. The lipids (69–104 mg.) eluted with solvent *G* from the silicic acid-silicate-water column were dissolved in 5 ml. of hexane and applied to the column. The lipids were then eluted successively according to the procedure of Barron & Hanahan (1958), as described in Fig. 3. The eluates were evaporated, and the lipids dried to constant weight, as described above.

Infrared spectrometry

During preliminary investigations, the lipids of the combined fractions of each eluate were examined as solutions in carbon disulphide, chloroform or bromoform, or as films on potassium bromide disks, with a Perkin-Elmer model 21 infrared spectrometer. Subsequent routine identifications were made from films on potassium bromide disks with a Perkin-Elmer model 137 Infracord spectrometer.

Paper chromatography

The methods for the paper chromatography of lipids were derived from techniques described by Rouser, Bauman, Nicolaides & Heller (1961*c*).

Chromatography tanks. The internal dimensions were 13 cm. $\times$ 20 cm. $\times$ 26 cm. deep. The tanks were covered with a glass plate and sealed on the outside with silicone grease. Provision was made in each tank for two papers to be suspended from a glass-rod cradle so that each paper dipped 1.5 cm. into the solvent.

Papers. Whatman 3MM 'for chromatography' papers (23 cm. long $\times$ 18 cm. wide) were impregnated and prepared as three types: 'phosphate' paper (Rouser *et al.* 1961*a*), 'acid' silicic acid paper and 'neutral' silicic acid paper. Each paper accommodated six spots, placed 5 cm. from the bottom. Immediately before spotting, the neutral papers were dried for 10 min. at 100° and the acid papers

for 5 min.; the phosphate papers were not dried. All papers were placed between glass plates during the spotting procedure.

Spotting of chromatograms. The lipids were dissolved in chloroform-methanol (2:1, v/v) in concentrations of 5 or 10 mg./ml. The loads applied to the paper, though usually 50 µg./spot, were frequently varied from 25 to 200 µg./spot.

Solvents. The following solvents were used as a routine, and under the conditions described by Rouser *et al.* (1961b):

1, chloroform-methanol (7:1, v/v); 2, chloroform-methanol (4:1, v/v), containing 8 ml. of aq. ammonia (sp.gr. 0.88)/l.; 3, chloroform-acetone (4:1, v/v) (19 vol.) + acetic acid-water (9:1, v/v) (1 vol.); 4, chloroform-acetone (4:1, v/v) (17 vol.) + acetic acid-water (9:1, v/v) (3 vol.); 5, benzene; 6, hexane-diethyl ether (19:1, v/v); 7, hexane-diethyl ether (3:1, v/v).

Staining of chromatograms. The developed chromatograms were air-dried for 5 min. and stained in a 0.001 % solution of Rhodamine 6G in 0.25 M-dipotassium hydrogen phosphate (Rouser *et al.* 1961a). The stained chromatograms were examined, in both wet and dry condition, under ultraviolet light.

Standard reference compounds. Synthetic L- α -dimyristoyllecithin (LaMotte Chemical Products Co., Chestertown, Ind., U.S.A.), and synthetic L- α -dipalmitoylcephalin (Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A.) were hydrolysed to their lyso derivatives by the enzymic procedure described by Rouser *et al.* (1961a). The source of phospholipase A was venom of black snake (*Pseudechis porphyriacus*), obtained from the Commonwealth Serum Laboratories, Melbourne, Australia. Phosphatidylinositol, ceramide and sphingomyelin were prepared as described respectively by Hawthorne (1960), Tropp & Wiedersheim (1933) and Carter, Haines, Ledyard & Morris (1947). For the phosphatidylinositol, however, sheep liver was used in place of ox liver, and the sphingomyelin was further purified as outlined by Schwarz (1960). Cholesterol and cholesterol stearate were obtained from the Nutritional Biochemical Corp. A variety of pure, saturated and unsaturated triglycerides, diglycerides and monoglycerides were prepared as described respectively by Wheeler, Riemenschneider & Sando (1940), Hartman (1957) and Hartman (1960).

RESULTS

Extraction of lipids. The modified Bligh & Dyer (1959) procedure extracted the lipids almost completely, as Soxhlet re-extraction of the tissue residue with light petroleum (b.p. 60–80°)-methanol (9:1, v/v) yielded not more than 1.25 % of the total extractable lipids. When examined by infrared spectrometry and paper chromatography these Soxhlet-extracted lipids appeared to be similar in composition to the original lipid mixture. As, however, there was considerable streaking on the chromatograms, indicating deterioration of the Soxhlet-extracted lipids, no further attempts were made to recover these lipids from the tissue. The lipid extract, obtained from the modified Bligh & Dyer (1959) procedure, contained 0.5 %, or less, of

non-lipid material. Water-soluble lipids (gangliosides), if present in sheep liver, may have been removed with the aqueous methanol phases during extraction. These lipids, and also cerebroside, were not detected during this investigation.

Diethylaminoethylcellulose-column chromatography. The lipids from this and subsequent columns were identified by the procedures described above. Fig. 1 shows the elution pattern of the total lipids.

Solvent A eluted hydrocarbons, cholesterol, cholesterol esters, glycerides, ceramide, choline-containing phospholipids and some phosphatidylethanolamine. It was found that phosphatidylethanolamine appeared before the elution of the choline-containing phospholipids was completed. Thus the tail end of the elution curve consisted of fractions containing a mixture of decreasing amounts of lysophosphatidylcholine and increasing amounts of phosphatidylethanolamine. However, complete elution of phosphatidylethanolamine by this solvent was extremely slow. The change to solvent B was therefore made when no further lysophosphatidylcholine was eluted, as evidenced by the disappearance of the infrared covalent phosphate (P–O–C) band at 970 cm.⁻¹ (10.3 µ). Solvent B eluted the remaining phosphatidylethanolamine. Non-lipid material was eluted with solvent C. Solvent D eluted an unidentified lipid, together with a trace of free fatty acids. The lipid eluted with solvent E was identified as phosphatidylserine (Rouser *et al.* 1961b). Its infrared-

Fig. 1. Chromatography on DEAE-cellulose column of total lipids (from a normal sheep). Esterified fatty acid (hydroxamic acid) values are recorded from 5 ml. samples of 20 ml. fractions. Eluting solvents are: A, chloroform-methanol (9:1, v/v); B, chloroform-methanol (7:3, v/v); C, methanol; D, chloroform-acetic acid (3:1, v/v), containing sodium acetate (1 mM); E, acetic acid; F, chloroform-methanol (4:1, v/v), containing 10 ml. of aq. ammonia (sp.gr. 0.88)/l. Further details are given in the text.

absorption spectrum was identical with that reported by Kimura & Nagai (1960) for 'original' phosphatidylserine. From the investigations of Rouser *et al.* (1961b) it was expected that cardiolipin, cerebroside sulphate and phosphatidylinositol, if present, would be eluted by solvent *F*. Silicic acid-column chromatography was used to investigate further the lipids eluted with solvent *F* by employing chloroform-methanol (9:1, v/v) for cardiolipin (Gray & Macfarlane, 1958), chloroform-methanol (4:1, v/v) for cerebroside sulphate (Long & Staples, 1961) and chloroform-methanol (3:2, v/v) for phosphatidylinositol (Hawthorne, 1960). Each of these solvents eluted a lipid, but of these only phosphatidylinositol was firmly identified by comparison with authentic phosphatidylinositol by using infrared spectrometry and paper chromatography. For this reason the lipids eluted by solvent *F* were not further fractionated.

On the basis of gravimetric determinations, the recovery of the DEAE-cellulose-column load was 100–102%.

Silicic acid-silicate-water-column chromatography.

Fig. 2 shows the elution pattern of the lipids eluted by solvent *A* from the DEAE-cellulose column. Solvent *G* eluted cholesterol, cholesterol esters and glycerides. Solvent *H* eluted a lipid that behaved on paper chromatography like *N*-lignoceryl-sphingosine, but differed from it in its infrared-

Fig. 2. Chromatography on silicic acid-silicate-water column of lipids eluted with solvent *A* from the DEAE-cellulose column (Fig. 1). Esterified fatty acid (hydroxamic acid) values are recorded from 5 ml. samples of 20 ml. fractions. Eluting solvents are: *G*, chloroform; *H*, chloroform-methanol (19:1, v/v); *I*, chloroform-methanol (4:1, v/v); *J*, chloroform-methanol (4:1, v/v), containing 0.5% of water; *K*, chloroform-methanol (4:1, v/v), containing 1.5% of water; *L*, chloroform-methanol (4:1, v/v), containing 2.5% of water; *M*, methanol, containing 2% of water. Further details are given in the text.

absorption spectrum. A relatively weak ester carbonyl ($C=O$) band occurred at 1740 cm^{-1} (5.74μ), whereas the *trans* configuration band at 970 cm^{-1} (10.3μ) was not present. It has been provisionally identified as ceramide. Solvent *I* eluted phosphatidylethanolamine. Phosphatidylcholine made an abrupt appearance once the elution of phosphatidylethanolamine was completed. The change to solvent *J* was therefore made when the ninhydrin test on the effluent became negative. Most of the phosphatidylcholine was readily eluted with this solvent, but there still remained a small amount of this lipid which was only slowly eluted. The change to solvent *K* was therefore made before the phosphatidylcholine was completely eluted. The infrared-absorption spectrum of the phosphatidylcholine exhibited, at 1650 cm^{-1} (6.06μ), a distinct band which has been attributed to the $C=C$ stretching vibration of the plasmalogen phosphatidylcholine (Gottfried & Rapport, 1962). The paper-chromatographic procedures did not resolve these two types of phosphatidylcholine. Solvent *K* eluted a mixture of phosphatidylcholine and sphingomyelin. Solvent *L* eluted sphingomyelin. Although a negative solids test indicated that the elution of sphingomyelin with this solvent appeared complete, a trace of this lipid was nevertheless found with the lysophosphatidylcholine, eluted with solvent *M*. Quantitative paper chromatography was employed as the most convenient means of calculating the amounts of the individual lipids eluted with solvents *K* and *M*. A series of known amounts of the lipid mixtures were developed on paper chromatograms. The spots were cut out, the lipids were eluted in chloroform-methanol (2:1, v/v), and phosphorus was determined on the dried extracts. The amount of each individual lipid was then calculated as a percentage of the total lipid mixture. Thus the lipids eluted by solvents *K* and *M* respectively contained 30% and 50% of sphingomyelin.

On the basis of gravimetric determinations, the recovery of the column load was 100–103%.

Silicic acid-column chromatography. Fig. 3 shows the elution pattern of the lipids eluted by solvent *G* from the silicic acid-silicate-water column. Solvent *N* was judged, on the basis of the Barron & Hanahan (1958) procedure, to elute hydrocarbons, which were not further identified. Solvent *O* eluted cholesterol esters. Solvent *P* eluted triglycerides; no free fatty acids were detected in this fraction. This was taken as evidence that no hydrolysis of the neutral lipids had occurred during their passage through the three chromatography columns, the free fatty acids in the original total lipids having been eluted on the DEAE-cellulose column. Solvents *Q* and *R* respectively eluted, without overlapping, cholesterol and diglycerides. Solvent

S eluted an unidentified neutral lipid. Mono-glycerides were eluted by solvent *T*.

On the basis of gravimetric determinations, the recovery of the column load was 100–103%.

Evaluation of analyses. Table 1 summarizes the composition of the total liver lipids from the control sheep and the dosed sheep. The results are expressed as the mean percentage composition of the

Fig. 3. Chromatography on silicic acid column of lipids (from a dosed sheep) eluted with solvent *G* from the silicic acid-silicate-water column (Fig. 2). Esterified fatty acid (hydroxamic acid) values are recorded from 5 ml. samples of 10 ml. fractions (O). Cholesterol values are recorded from 1 ml. samples of 10 ml. fractions (●). Eluting solvents are: *N*, hexane; *O*, hexane-benzene (17:3, v/v); *P*, hexane-diethyl ether (19:1, v/v); *Q*, hexane-diethyl ether (17:3, v/v); *R*, hexane-diethyl ether (7:3, v/v); *S*, hexane-diethyl ether (1:1, v/v); *T*, diethyl ether. Further details are given in the text.

Table 1. Composition of liver lipids from normal sheep and from sheep dosed with sporidesmin

Experimental details are given in the text. The results are expressed as the mean values ($\pm$ S.D.) from four animals in each group. The *P* values (Student's *t* test) represent the levels of the statistical significance of the differences between each group as calculated from the mg./100 g. wet wt. of tissue. Levels of *P* < 0.05 are considered significant.

	Composition				Significance
	(% of total lipids)		(mg./100 g. wet wt. of tissue)		
	Control	Dosed	Control	Dosed	
Phosphatidylethanolamine	21.76 ± 2.74	18.92 ± 1.98	966.7 ± 106.5	1020.4 ± 120.1	0.5 < P < 1
Unidentified lipid + free fatty acids (trace)	4.46 ± 1.18	4.71 ± 1.08	198.0 ± 49.6	254.6 ± 61.3	0.1 < P < 0.25
Phosphatidylserine	2.60 ± 0.86	2.14 ± 0.58	115.8 ± 35.6	115.6 ± 31.7	0.5 < P < 1
Cardiolipin + cerebroside sulphate + phosphatidylinositol	5.13 ± 3.72	4.21 ± 1.67	226.0 ± 161.4	226.5 ± 89.1	0.5 < P < 1
Ceramide	2.35 ± 0.86	2.55 ± 1.80	105.0 ± 40.3	136.0 ± 94.6	0.5 < P < 1
Phosphatidylcholine	35.63 ± 4.35	34.89 ± 2.55	1587.7 ± 218.1	1879.6 ± 145.9	0.05 < P < 0.1
Sphingomyelin	6.08 ± 0.92	5.83 ± 0.86	270.3 ± 40.5	313.7 ± 45.5	0.1 < P < 0.25
Lysophosphatidylcholine	1.44 ± 0.55	2.62 ± 2.17	64.2 ± 24.6	139.4 ± 113.1	0.1 < P < 0.25
Hydrocarbons	2.01 ± 2.15	1.93 ± 1.00	90.1 ± 102.7	103.6 ± 53.7	0.5 < P < 1
Cholesterol esters	1.97 ± 0.95	2.01 ± 0.98	88.1 ± 44.3	108.8 ± 54.1	0.5 < P < 1
Triglycerides	7.02 ± 0.59	11.21 ± 1.90	311.9 ± 21.3	605.3 ± 112.3	P < 0.001
Cholesterol	7.15 ± 0.81	5.94 ± 0.84	317.7 ± 32.5	320.2 ± 46.3	0.5 < P < 1
Diglycerides	0.98 ± 0.49	1.30 ± 0.46	43.6 ± 22.3	69.8 ± 23.4	0.1 < P < 0.25
Unidentified neutral lipid	0.84 ± 0.45	1.15 ± 0.11	37.6 ± 20.4	61.8 ± 5.8	0.05 < P < 0.1
Monoglycerides	0.61 ± 0.43	0.59 ± 0.11	27.4 ± 19.6	31.9 ± 5.2	0.5 < P < 1
Total lipids	—	—	4449.3 ± 90.2	5387.0 ± 126.5	P < 0.001

total lipids, and also as the mean weight (in mg.)/100 g. wet wt. of tissue. Thus the elevated total lipid concentrations of the dosed group are shown to be due to a highly significant increase in the triglycerides, whereas no significant changes occur in the concentrations of other lipids.

DISCUSSION

One of the aims of the present investigation was to develop procedures by which the individual lipids from a total lipid extract could be quantitatively isolated and identified in their natural state. With lipids from a variety of animal tissues, it has been our experience (J. A. Peters, unpublished work) that separation of individual neutral lipids on a silicic acid column, according to the procedure of Barron & Hanahan (1958), was complete only when the phospholipids had been removed from the total lipid extract, before its application to the column. With regard to the phospholipids, silicic acid-column chromatography (e.g. Hanahan, Dittmer & Warashina, 1957) fails to separate phosphatidylserine from phosphatidylethanolamine. In addition, an overlapping of some lipids on elution from the column is difficult to avoid. In the three-column-chromatography procedure described, the above disadvantages are eliminated, except for a slight overlap of sphingomyelin with lysophosphatidylcholine. Thus quantitative isolation of lipids by consecutive column-chromatography procedures appears to be superior to isolation procedures involving only a single chromatography column.

To our knowledge, the composition of the total liver lipids from sheep has not been reported since an early contribution by Hilditch & Shorland (1937). Dawson (1960) determined the composition of the liver phospholipids from one sheep by chemical-hydrolysis procedures. Allowing for individual variations of the lipids in each liver, the present results are in agreement with those reported by Dawson (1960).

Because so few animals were involved in the present investigation the results must be interpreted with caution. It is clear, however, that the action of sporidesmin on the sheep liver results in a significant increase of triglycerides, without obvious changes in the other lipids. Similar fatty infiltrations of the liver have been induced in non-ruminant animals by a variety of hepatotoxic substances (Schotz & Recknagel, 1960; Harris & Robinson, 1961; Snyder, Cress & Kyker, 1960; Di Luzio, 1958; Henderson, 1963). Also, there is adequate evidence that the liver is the main site of synthesis for the circulating triglycerides, derived from endogenous sources (Stein & Shapiro, 1959; Byers & Friedman, 1960; Kay & Entenman, 1961;

Borgström & Olivecrona, 1961; Havel & Goldfien, 1961). Thus the increase in liver triglyceride concentrations, as the result of the action of hepatotoxins, has been interpreted as a malfunction of a hepatic triglyceride-secreting mechanism (Recknagel, Lombardi & Schotz, 1960; Heimberg, Weinstein, Dishmon & Dunkerley, 1962). In general, then, the early pathological changes induced by the action of hepatotoxins are reflected in a rapid increase of liver triglycerides, with a concomitant hypotriglyceridaemia (Harris & Robinson, 1961; Di Luzio, 1958; Olivecrona, 1962).

To our knowledge, the effects of hepatotoxins in lipid metabolism and transport in ruminants have not been investigated. Nevertheless, it appears that, on the basis of the present investigation and of previous reports (Mortimer *et al.* 1962; Done *et al.* 1962), the effect of early sporidesmin poisoning (i.e. 2 days after dosing) on the sheep liver resembles the effect of other hepatotoxins on non-ruminant animals. In this respect a further factor should be considered which takes into account the rise of liver triglyceride concentrations over a period. Harris & Robinson (1961) and Olivecrona (1962) have shown that, in ethionine-treated rats, the liver triglyceride concentrations are maximal at about 24 hr. after dosing, and then decline rapidly. Thus the liver triglyceride concentrations in sheep 48 hr. after dosing, as observed in the present investigation, may not be indicative of the maximum concentrations possible.

SUMMARY

1. The liver lipids of normal sheep and of sheep dosed with sporidesmin were isolated quantitatively by a three-column-chromatography procedure involving a DEAE-cellulose column, a silicic acid-silicate-water column and a silicic acid column.
2. The isolated lipids were identified by infrared spectrometry and paper chromatography, supplemented by chemical analyses.
3. The liver triglyceride concentrations rose very significantly ($P < 0.001$) in the sporidesmin-poisoned sheep. No changes were observed in the concentrations of other liver lipids.
4. The early effect of the heptatoxin-like action of sporidesmin on sheep was interpreted as a malfunction of a hepatic triglyceride-secreting mechanism.

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Biochem. J. (1964), **92**, 385

The Effect of β -Sitosterol on the Metabolism of Cholesterol and Lipids in Rats on a Diet Low in Fat

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Earlier studies by Peterson (1951) have shown that serum cholesterol of chicks, when elevated by dietary cholesterol, may be lowered by feeding with β -sitosterol and other plant sterols. This action of plant sterols on serum cholesterol in our opinion has

not been satisfactorily explained. At first Peterson, Schneour, Peek & Gaffey (1953) considered that β -sitosterol inhibited the formation of cholesterol esters in the intestinal mucosa, but the esterification of cholesterol was regarded by Glover & Green