FATTY ACID COMPONENTS OF OVINE TISSUE LIPIDS, AND THE RESPONSE TO PROLONGED PROTEIN DEPLETION

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Summary

The lipids of ovine liver and plasma have been separated by silicic acid chromatography into cholesterol ester, triglyceride, free fatty acid, and phospholipid fractions. Fatty acid constituents of these fractions have been determined by gas chromatography. The lipid content of these tissues was also determined for animals subjected to a prolonged protein depletion.

Several significant differences were caused by the experimental diet, with the liver showing more alterations than plasma. The unsaturated C_{18} fatty acids were affected to the greatest degree.

Implications of these results have been discussed in relation to comparative biochemistry and intermediary lipid metabolism.

I. Introduction

Numerous workers have reported results of fatty acid analyses for tissues of monogastric experimental animals (James *et al.* 1957; Dole *et al.* 1959; Lawrie *et al.* 1961; Okey *et al.* 1961; Swell *et al.* 1961*a*; Getz *et al.* 1962; Grande 1962) but, by comparison, the literature relating to ruminants is very small. Most of the results available for ovine tissues are from the period before gas chromatography analytical techniques were introduced, although Hartman and Shorland (1961) have published values obtained by this technique for the subcutaneous fats of ox and sheep, and Annison (1954) used this method in a study of the volatile fatty acids present in sheep blood.

In view of this paucity of information on the fatty acid constituents of ovine lipids, the present study was undertaken. The lipids of ovine plasma and liver have been separated by silicic acid chromatography into cholesterol ester, triglyceride, free fatty acid, and phospholipid fractions, and the fatty acid constituents determined by gas chromatography.

In addition, the specific fatty acid response to prolonged protein depletion has been studied. This was of interest (1) as an extension of previous research into the biochemical consequences of drought feeding (Masters and Horgan 1962*a*, 1962*b*; Masters 1963*a*, 1963*b*); (2) in view of the importance of fatty acid metabolism to energy production in the ruminant (Annison and Lewis 1959); and (3) in the light of the intimate connection between fat metabolism, rumen reactions, and dietary deficiency in the sheep. Liver and plasma were chosen as the tissues most likely to reflect changes in lipid metabolism under the experimental conditions.

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II. Methods

(a) *Experimental Animals*

The animals subjected to dietary restriction were well-matched adult Merino ewes, 3–4 years of age. After feeding for 3 weeks on lucerne chaff and water *ad libitum*, three of the animals (controls) were slaughtered. The diet of the other animals was changed to a ration of 3 kg per week of chaffed wheat straw (digestible crude protein 0·5%) and water *ad libitum* for a period of 8 weeks. This experimental diet was chosen to approximate that under drought conditions and, though representing an average *ad libitum* intake of feed under these conditions, it is a low protein, low energy ration. The duration was shown by preliminary experiments to approach closely the maximum consistent with survival.

Liver tissues required for the analysis of lipid fractions were obtained by excision from freshly slaughtered animals and stored in a closed vessel at −10°C in the dark until required. Blood samples were collected into tubes containing heparin. Plasma was separated as rapidly as possible and stored in the same manner as the liver samples.

(b) *Extraction and Fractionation of Lipids*

Lipids were extracted from the test materials with boiling ethanol–ether (3 : 1 v/v) as described by Creasy, Hamkin, and Handschumacher (1961). These extracts were evaporated at reduced pressure, taken up in petroleum ether, and separated into four fractions (cholesterol esters, triglycerides, free fatty acids, and phospholipids) by silicic acid chromatography (Lis, Tinoco, and Okey 1961). For ease in estimation, columns were run in duplicate—fractions collected from one column were methylated for separation by gas chromatography while those from the second column were assayed for lipid content by gravimetric means.

(c) *Preparation of Methyl Esters*

Methylation of the lipid fractions was attained by the inter-esterification and micro-sublimation method of Stoffel, Chu, and Ahrens (1959) with one slight modification, in that sulphuric acid was used as the catalyst instead of hydrochloric acid. The super-dry methanol required was prepared by fractionation as described by Vogel (1957).

The methyl esters were stored in excess petroleum ether (b.p. 40–60°C) at 4°C until analysed by gas chromatography.

(d) *Gas Chromatography*

Analyses of the methyl esters were carried out on a Pye argon gas chromatograph. The methyl esters were dissolved in a small volume of petroleum ether and added directly to the column by means of a micropipette. Gas flow was 40 ml/min, and two stationary phases were used in the analyses. Polyethyleneglycol adipate on 100-mesh "Celite" (10% w/w) at 175°C was used for routine separations. Additional analyses were carried out on some samples using "Apiezon L" on a 100-mesh "Celite" (10% w/w) column at 200°C to assist in identification. Calculation of the proportions

in each mixture was made directly from the relative peak area on the chromatograms, determined by the "triangulation" procedure (James 1960), after confirmation of the detector linearity.

TABLE 1

FATTY ACID COMPOSITION OF LIVER LIPIDS IN NORMAL SHEEP

Values for each component refer to the mean (± standard error of the mean) of percentage weight of the total fatty acid methyl esters in each fraction. Trace means that the amount of fatty acid present is less than 0·5%. No. of observations given in parenthesis. br., branched chain

Chain Length	No. of Double Bonds	Cholesterol Esters (3)	Triglycerides (3)	Free Fatty Acids (3)	Phospholipids (3)
12	0	Trace	Trace	Trace	Trace
14 (br.)	0	Trace	Trace	Trace	,,
14	0	0·7±0·2	1·8±0·5	0·9±0·3	,,
14	1	Trace	0·6±0·1	Trace	,,
15 (br.)	0	Trace	0·7±0·2	Trace	,,
15	0	0·7±0·2	0·7±0·1	0·9±0·2	,,
15	1	Trace	Trace	0·8±0·1	,,
16 (br.)	0	0·7±0·1	0·5±0·2	0·6±0·1	,,
16	0	16·5±2·5	26·3±2·3	19·6±2·6	16·2±2·9
16	1	3·8±0·1	3·5±0·2	3·7±1·2	1·4±0·1
17 (br.)	0	1·4±0·1	1·3±0·1	1·6±0·4	0·8±0·1
17	0	1·6±0·3	1·6±0·1	2·0±0·3	1·2±0·1
16	2	1·8±0·3	1·1±0·2	1·4±0·4	0·5±0·1
18 (br.)	0	Trace	0·6±0·1	0·9±0·3	0·6±0·1
18	0	9·0±0·5	10·3±2·6	9·6±3·2	32·5±3·3
18	1	28·5±0·5	38·3±4·8	32·4±1·9	21·5±1·2
18	2	7·0±0·6	4·4±0·6	7·3±2·2	7·8±1·0
18	2?	2·8±1·8	1·0±0·3	1·0±0·3	Trace
18	3	3·9±0·3	1·8±0·2	4·3±0·4	1·7±0·1
20	0	3·7±0·1	1·3±0·4	1·0±0·2	0·6±0·2
20	1	Trace	1·0±0·3	1·1±0·2	0·8±0·1
20	2	Trace	Trace	0·6±0·1	0·6±0·4
21	0	1·0±0·5	Trace	0·5±0·3	1·0±0·3
20	4	2·6±0·7	0·8±0·3	2·4±0·8	7·5±0·7
22	0	5·0±0·6	Trace	1·6±0·1	1·9±0·3

(e) *Identification*

The peaks obtained were identified in several ways:

(1) By comparing the retention volumes of the unknown fatty acid methyl esters with those of known acids run under similar chromatographic conditions.

(2) Relative retention volumes were calculated and compared with the values given by Farquar *et al.* (1959) (relative to stearic acid) and by James (1960) (relative to palmitic acid).

(3) The "carbon number" method of Woodford and van Gent (1960) also proved to be of value.

(4) By comparing the behaviour of the unsaturated acids on the polar polyethyleneglycol adipate and nonpolar "Apiezon L" columns.

(5) Microbromination as described by James (1960) was used to separate saturated and unsaturated acids.

TABLE 2

FATTY ACID COMPOSITION OF LIVER LIPIDS IN DEPLETED SHEEP

Values for each component refer to the mean (± standard error of the mean) of percentage weight of the total fatty acid methyl esters in each fraction. Trace means that the amount of fatty acid present is less than 0·5%. No. of observations given in parenthesis. br., branched chain

Chain Length	No. of Double Bonds	Cholesterol Esters (3)	Triglycerides (3)	Free Fatty Acids (3)	Phospholipids (3)
12	0	Trace	0·8±0·2	0·5±0·1	Trace
14 (br.)	0	Trace	Trace	Trace	,,
14	0	0·6±0·1	2·1±0·2	1·8±0·7	,,
14	1	Trace	0·7±0·2	Trace	,,
15 (br.)	0	Trace	1·2±0·1	0·8±0·2	,,
15	0	0·7±0·1	1·1±0·1	0·6±0·2	,,
15	1	0·6±0·2	Trace	Trace	,,
16 (br.)	0	1·0±0·4	0·7±0·1	Trace	0·6±0·1
16	0	21·5±1·3	17·7±0·6*	14·9±1·7	12·0±1·1
16	1	3·4±1·4	4·2±0·8	4·3±0·6	1·4±0·2
17 (br.)	0	1·9±1·0	1·3±0·3	1·0±0·4	Trace
17	0	2·3±0·5	1·2±0·2	0·9±0·3	1·0±0·3
16	2	0·7±0·3	1·1±0·1	0·6±0·1	Trace
18 (br.)	0	Trace	Trace	Trace	Trace
18	0	20·4±3·5*	12·2±1·7	11·1±0·9	28·2±0·7
18	1	33·0±4·0	45·7±0·6	42·9±1·6*	34·7±0·6*
18	2	7·7±1·9	4·5±0·3	10·6±2·6	11·5±1·8
18	2?	1·2±0·1	Trace	0·6±0·2	Trace
18	3	1·2±0·3*	0·6±0·1*	1·7±0·4*	1·0±0·1*
20	0	Trace*	0·9±0·2	0·9±0·2	0·8±0·3
20	1	0·5±0·2	Trace	0·7±0·1	Trace
20	2	Trace	Trace	Trace	Trace
21	0	1·0±0·2	0·5±0·1	Trace	Trace
20	4	1·2±0·1*	1·0±0·2	2·0±0·5	5·6±0·4
22	0	Trace*	Trace	0·8±0·2	0·5*

* Significantly different ($P < 0·05$) from the normal state.

III. RESULTS

The fatty acids from C_{12} to C_{22} of the four major lipid fractions have been estimated and values for liver and serum in the normal and depleted states are shown

in Tables 1, 2, 4, and 5. Table 3 shows comparisons of the percentage composition of the four major lipid fractions in liver and serum lipids.

In addition to the acids shown in the tables, further components were recognizable as trace quantities. The relative retention data and saturation of five of these methyl esters are consistent with a tentative identification of chain lengths as 13 (sat.), 15 (unsat.), 19 (sat.), 20 (branched), and 22 (branched). The identification of the acids C_{20} (1 double bond) and C_{20} (2 double bonds) must also be considered tentative, in view of the lack of suitable reference standards. The C_{18} (1 double bond) fraction besides containing oleic acid could contain other acids including the *trans* type, since the retention volumes of monoenoic acids on polyethyleneglycol adipate columns are not affected by the position of the double bond or by steric configuration (James 1959). From relative retention volume data and the behaviour following bromination the acid designated C_{18} (? 2 double bonds) is thought to be of the *trans* variety, also.

TABLE 3

RESULTS OF FRACTIONATION OF LIVER AND SERUM LIPIDS IN NORMAL AND DEPLETED SHEEP BY SILICIC ACID CHROMATOGRAPHY

Values for each fraction are mean percentages by weight (± standard error of the mean) of total liver or serum lipids. No. of observations made given in parenthesis

Fraction	Liver		Serum	
	Normal Sheep (3)	Depleted Sheep (3)	Normal Sheep (4)	Depleted Sheep (2)
Cholesterol esters	7±1	4±1	33±5	32±1
Triglycerides	49±2	25±2	8±2	7±2
Free fatty acids	10±2	13±2	15±2	16±2
Phospholipids	34±2	58±3	44±4	44±4

Comparison of Tables 1 and 2 show that the experimental diet produced a number of changes in the fatty acid patterns of the liver lipids. There is significant lowering of the percentage content of the C_{18} acid with 3 double bonds in all fractions of the depleted tissues and a general increase in the C_{18} (1 double bond) content. In addition, the cholesterol ester fraction of the depleted sheep show significant decreases in arachidic (C_{20}, sat.), arachidonic (C_{20}, 4 double bonds), and behenic (C_{22}, sat.) acids, with a marked increase of stearic (C_{18}, sat.) acid. There is also a significant decrease of the palmitic acid content of the triglyceride fraction. Whereas Table 1 shows that it is only in the phospholipid fraction of normal livers that the oleic acid content is less than the stearic, Table 2 shows that starvation has had the effect of reversing this ratio. This has been achieved mainly by an increase in the C_{18} (1 double bond) acid with a slight lowering of the C_{18} (sat.) content.

Table 3 shows that the percentage contribution of the cholesterol esters and triglycerides to the liver lipids has been almost halved in the depleted animals. The free fatty acid fraction is almost unchanged, while the phospholipid fraction shows a

large increase. Table 3 also shows that the percentage composition of the main lipid fractions of the serum do not demonstrate significant differences in response to the experimental treatment.

TABLE 4

FATTY ACID COMPOSITION OF SERUM LIPIDS IN NORMAL SHEEP

Values for each component refer to the mean (± standard error of the mean) of percentage weight of the total fatty acid methyl esters in each fraction. Trace means that the amount of fatty acid present is less than 0·5%. No. of observations given in parenthesis. br., branched chain

Chain Length	No. of Double Bonds	Cholesterol Esters (3)	Triglycerides (3)	Free Fatty Acids (4)	Phospholipids (4)
12	0	Trace	Trace	Trace	Trace
14 (br.)	0	Trace	Trace	Trace	,,
14	0	0·8±0·2	1·6±0·5	1·5±0·2	,,
14	1	Trace	Trace	0·6±0·1	,,
15 (br.)	0	Trace	1·0±0·4	1·0±0·2	,,
15	0	0·8±0·1	2·3±0·7	1·3±0·3	0·5±0·1
15	1	Trace	Trace	Trace	Trace
16 (br.)	0	0·7±0·2	0·9±0·3	0·5±0·4	0·6±0·2
16	0	13·5±0·7	21·8±2·4	14·4±1·7	18·9±1·5
16	1	5·0±0·7	6·0±0·8	5·5±1·3	2·1±0·2
17 (br.)	0	1·1±0·5	2·3±0·5	1·4±0·4	1·2±0·2
17	0	1·4±0·2	3·5±0·3	2·0±0·5	2·0±0·1
16	2	1·1±0·5	1·2±0·3	1·3±0·5	Trace
18 (br.)	0	Trace	Trace	Trace	Trace
18	0	3·0±0·6	16·2±0·9	10·6±2·0	22·7±3·5
18	1	33·2±6·1	27·4±4·3	41·1±5·9	28·2±3·3
18	2	26·3±4·5	6·6±0·6	7·0±1·2	11·4±0·7
18	2?	1·9±0·4	0·6±0·2	1·3±0·3	0·5±0·2
18	3	5·6±2·6	2·0±1·4	2·7±0·7	2·8±0·8
20	0	1·6±0·5	Trace	1·1±0·4	0·5±0·2
20	1	0·5±0·1	1·2±0·2	1·2±0·1	0·6±0·1
20	2	Trace	Trace	1·1±0·6	Trace
21	0	0·5±0·1	1·0±0·3	1·3±0·3	0·6±0·2
20	4	1·9±0·5	1·8±0·4	2·1±0·3	3·5±0·6
22	0	1·3±0·2	1·0±0·2	1·0±0·1	2·5±0·6

Comparison of Tables 4 and 5 show that changes in the serum fatty acids are not nearly as numerous or significant as those in the liver. The palmitic acid of the triglyceride fraction exhibits a significant decrease, there is a general decrease in the percentage of C_{18} (3 double bonds) in all fractions, and the arachidonic acid content of the cholesterol ester fraction shows a significant increase. An apparent increase of oleic acid occurs in plasma triglycerides.

In order to clarify the relevant part of Section IV, the percentages of the major C_{16} and C_{18} acids occurring in bovine ovine and human plasma have been compared in Table 6. With the C_{18} acids, there are marked differences in distribution between

TABLE 5

FATTY ACID COMPOSITION OF SERUM LIPIDS IN DEPLETED SHEEP

Values for each component refer to the mean (± standard error of the mean) of percentage weight of the total fatty acid methyl esters in each fraction. Trace means that the amount of fatty acid present is less than 0·5%. No. of observations given in parenthesis. br., branched chain

Chain Length	No. of Double Bonds	Cholesterol Esters (2)	Triglycerides (2)	Free Fatty Acids (2)	Phospholipids (2)
12	0	Trace	Trace	Trace	Trace
14 (br.)	0	Trace	Trace	Trace	,,
14	0	0·6±0·1	1·0±0·2	1·1±0·1	,,
14	1	Trace	Trace	Trace	,,
15 (br.)	0	Trace	Trace	0·6±0·1	,,
15	0	0·8±0·1	1·2±0·3	0·8±0·1	,,
15	1	Trace	0·9±0·2	Trace	,,
16 (br.)	0	0·8±0·2	Trace	0·5±0·1	0·7±0·3
16	0	12·0±0·5	16·9±0·9*	19·0±4·0	14·3±1·4
16	1	3·0±0·3	4·0±0·8	4·5±0·2	2·2±0·5
17 (br.)	0	1·9±0·8	1·0±0·2	1·5±0·1	0·5±0·1
17	0	1·3±0·7	3·3±0·5	1·6±0·1	0·9±0·1
16	2	1·9±1·2	Trace	1·6±0·1	0·5±0·2
18 (br.)	0	Trace	Trace	Trace	Trace
18	0	4·5±0·9	24·8±1·4*	10·0±0·7	28·3±1·4
18	1	37·3±3·1	40·2±5·0	41·7±3·5	29·1±0·3
18	2	26·5±0·4	6·2±0·8	10·5±0·9	11·0±0·8
18	2?	1·9±0·8	0·6±0·2	1·2±0·4	0·6±0·3
18	3	2·5±0·2	0·9±0·2	1·6±0·1	1·1±0·2*
20	0	0·8±0·1	Trace	Trace	0·6±0·3
20	1	0·6±0·2	1·2±0·2	0·6±0·1	0·7±0·1
20	2	0·6±0·3	Trace	0·5±0·1	Trace
21	0	1·0±0·5	1·2±0·4	1·3±0·5	0·8±0·2
20	4	4·4±0·2*	1·2±0·3	2·1±0·6	5·2±0·2
22	0				1·6±0·5

* Significantly different ($P < 0·05$) from normal state.

the saturated and unsaturated forms in the three species. The cholesterol ester fraction of sheep plasma is only one-half as unsaturated as this fraction in bovine plasma, and, in particular, contains considerably less tri- and di-unsaturated acid, with the monoenoic form predominant. Whereas the ratio of C_{18} unsaturated to C_{18} saturated acids in the sheep is similar to that in humans, the diethenoid to monoethenoid ratio is less than for human cholesterol esters. Comparison of the C_{18} acids

of the triglyceride, free fatty acid, and phospholipid fractions in these three species is characterized by a gradation from human $\gg$ ovine $>$ bovine in the ratio of unsaturated to saturated acids. This is particularly obvious in the triglyceride fraction where the comparative ratios are of the order 12 : 2 : 1. Differences between cattle and sheep are largely concentrated in the stearic acid percentages (triglycerides and free fatty acids) and monoethenoid percentages (phospholipids).

IV. Discussion

The fatty acid components of ovine plasma and liver lipids display features which are characteristic of ruminant metabolism, i.e. the presence of geometric and positional isomers, and the occurrence of odd-numbered carbon chains in quantities greater than are usual in monogastric animals (Shorland 1962). In addition, the proportional distribution of the various C_{16} and C_{18} acids is dissimilar to that in other experimental animals (Table 6).

Table 6

COMPARISON OF FATTY ACID COMPOSITION OF BOVINE, OVINE, AND HUMAN PLASMA LIPIDS

These results have been derived from Table 4, and from the following references: Chlouverakis and Harris (1960); Lawrie *et al.* (1961); Duncan and Garton (1962). Percentages by weight of total acids are given. B, bovine; O, ovine; H, human

Chain Length*	Cholesterol Esters			Triglycerides			Free Fatty Acids			Phospholipids		
	B	O	H	B	O	H	B	O	H	B	O	H
16(0)	6	13	14	24	23	21	18	14	25	16	19	27
18(0)	2	3	3	30	16	4	24	11	9	27	23	10
18(1)	6	33	22	24	28	26	39	41	45	16	28	16
18(2)	52	26	35	5	7	12	5	7	7	14	11	17
18(3)	23	6	—	2	2	—	3	3	—	2	3	—

* No. of double bonds given in parenthesis.

The overall pattern of greater saturation of C_{18} acids in ruminant triglycerides, free fatty acids, and phospholipids is to be expected in view of the microbial hydrogenation occurring in ruminants (Garton 1961); but it appears anomalous that the fatty acid moiety of bovine cholesterol esters should have a considerably higher unsaturated content than is the case in sheep and humans.

The presence of C_{18} tri- and di-unsaturated acids as significant components of bovine plasma cholesterol esters has been ascribed to the high content of linolenic acid in feed lipids, partial hydrogenation of this acid in the rumen, and subsequent preferential esterification to cholesterol (Duncan and Garton 1962). This proposal would explain the differences between the fatty acids of bovine cholesterol esters and those of other monogastric species (Swell *et al.* 1960), but leaves the bovine–ovine situation somewhat clouded. Since the feed lipids are comparable in these two instances and since the other lipid fractions demonstrate a more complete hydrogenation of bovine fatty acids than ovine, an explanation of the highly unsaturated nature

of the bovine cholesterol esters would seem to require either a marked species difference of selectivity in the esterification of cholesterol, or a consideration of further factors, such as the interrelationships of plasma lipids with lymph (Swell, Field, and Treadwell 1960).

In the case of the fatty acid moieties of liver lipids, comparative information is meagre. Hilditch (1956) and Futter and Shorland (1957) have compared the hepatic fatty acid composition in several animals by means of a fractionation into "glycerides" and phospholipids. Considering that this "glyceride" fraction contained both triglycerides and cholesterol esters, there is a reasonable agreement with the values for ovine liver obtained by silicic acid fractionation and gas chromatography, and the broad interspecies comparisons drawn by Garton (1960) remain unchanged.

A more detailed basis for comparison has been provided by Getz *et al.* (1962) in their results for rat liver lipids. The cholesterol esters and triglycerides of the rat contain higher percentages of palmitic acid than ovine liver, and a greater degree (5–10 times) of unsaturated C_{18} acids. Whereas the predominant unsaturated C_{18} acid in ovine liver lipids is monoenoic, rat lipids display a much higher C_{18} (2 double bonds) : C_{18} (1 double bond) ratio throughout. Also, ovine liver triglycerides and phospholipids do not possess the high content of C_{20}–C_{22} polyunsaturated fatty acids that rat liver exhibits.

It is apparent, then, that the constituent fatty acids of both liver and plasma reflect the hydrogenation occurring in the alimentary tract of ruminants. The difference between these two tissues in the sheep presumably indicate selective retention and utilization of certain components by the liver, in relationship to metabolic requirements, and the maintenance of physical characteristics (Okey *et al.* 1961). It is noteworthy that both the liver and plasma fractions contain a high percentage of the C_{18} monoene. Especially relevant may be the high content in both hepatic and plasma free fatty acids, as this may indicate preferential mobilization and extraction of the C_{18} (1 double bond) acid by sheep tissues (Jeanrenaud 1961).

The experimental diet of low protein content was chosen to resemble that prevalent under drought conditions. Consequently the results reported in the experimental sheep probably bear a close relationship to the changes occurring under natural grazing conditions, characteristic of large areas of Australia, and accompanying the transition from green pasture to a low quality, dry grass diet.

Two of the main responses to the experimental diet were the lowering of the tri-unsaturated C_{18} acid content, and the increase in the monoenoic C_{18} fatty acids of ovine hepatic lipids. The first of these changes may be a reflection of the reduced intake on the experimental diet, since linolenic acid comprises approximately 60% of the total fatty acids of pasture lipids (Shorland, Weenink, and Johns 1955).

A probable contributing factor to the increase in the liver C_{18} (1 double bond) acid, is a lessened ruminal hydrogenation under the experimental conditions. Other workers have found a significant decrease in rumen microorganisms when sheep were fed on a low quality diet (Moir 1951), and diminished microbial reduction of linolenic acid might be expected to cause the intermediate monoethenoid acid to become more predominant at the expense of stearic acid (Shorland *et al.* 1959). This proposal

would also explain the inversion of the C_{18} (sat.) to C_{18} (1 double bond) ratio occurring in hepatic phospholipid during the experimental treatment.

Hilditch and Pedelty (1941) reported a preferential mobilization of palmitic glycerides with sheep in a low plane of nutrition, and the liver and plasma levels in this investigation support their finding. Preferential mobilization may also be a relevant factor with the C_{18} (sat.) and C_{18} (1 double bond) acids, but the experimental data do not provide a clear indication in this matter.

Another interesting response to the experimental treatment was the lowering of the arachidonic acid content of the liver cholesterol ester fraction, along with an increase of this acid in the plasma cholesterol esters. Arachidonic acid is of entirely endogenous origin (Mead, Steinberg, and Howton 1953) and is formed from linoleic acid by a process thought to involve pyridoxal phosphate, and taking place mainly in the liver (Steinberg *et al.* 1956; Swell *et al.* 1961*b*; Wakil 1961; Witten and Holman 1952). It has previously been shown that this particular experimental diet caused a deficiency of pyridoxal phosphate in sheep tissues (Masters and Horgan 1962*a*). Consequently, the observed changes in the hepatic and plasma cholesterol ester levels of arachidonic acid probably reflect both a reduced synthesis of this acid and an increased mobilization.

The marked reduction in the percentage of triglycerides in the liver lipids is consistent with the severe state of depletion of the animals (Shorland 1962); and the rise in the phospholipid fraction could be accounted for either by increased hepatic synthesis, or by transport from other organs and tissues. In this connection, Gillman, Gilbert, and Savage (1962) found that a kwashiorkor infant baboon synthesizes significantly greater amounts of phospholipid fatty acids than does a normal infant.

Differences between the normal and depleted status of serum lipids are not, in general, as marked as those shown by the liver. Okey *et al.* (1961) reported that in their experiments with rats, the fatty acid components of the liver lipids showed a greater capacity for variation with diet than those of the plasma.

V. Acknowledgments

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