in the same proportion. A possible explanation for the preferential utilization of the added palmitate is diminution of the rate of release of free fatty acids from endogenous triglycerides and phospholipids.

The metabolism of ^{14}C -labelled fatty acid incorporated into the liver lipids 20–30 min. before the cell suspensions were prepared is virtually the same as that of added $[\text{G-}^{14}\text{C}]$ palmitate (Table 7). The stoichiometric relationships between the ^{14}C -labelled products of oxidation are similar; but the specific activity of the $^{14}\text{CO}_2$ is much lower than that of the ketone bodies, showing that about half the carbon dioxide is produced from endogenous substrates other than fatty acids. The source of this carbon is not clear.

The use of $[\text{G-}^{14}\text{C}]$ palmitate has confirmed the conclusion (Exton, 1964) that addition of pyruvate or a citric acid-cycle substrate does not promote the oxidation of endogenous fatty acid to any significant degree. With all the substrates examined the decrease in acetoacetate was found to be almost entirely due to its reduction to β -hydroxybutyrate.

The low rate of oxidation of fatty acid to carbon dioxide indicates that the turnover rate of the citric acid cycle is low in isolated liver cells. It has been suggested (Exton, 1964) that this might be due to a low concentration of oxaloacetate. However, if this is the case it is surprising that malate and succinate exert such little effect on the oxidation of palmitate in the citric acid cycle. Since citric acid-cycle intermediates increase the NADH/NAD^+ ratio of the cells (Exton, 1964) and cause inversion of the acetoacetate/ β -hydroxybutyrate ratio, it is probable that they also increase the malate/oxaloacetate ratio. This would partly explain why malate, succinate and other intermediates produce only a slight decrease in ketone-body production by the cells.

SUMMARY

1. Palmitate at concentrations below 2 mM increased the oxygen uptake and carbon dioxide production of cells isolated from rat liver.

2. $[\text{G-}^{14}\text{C}]$ Palmitate added *in vitro* was utilized in preference to endogenous substrates. It was oxidized largely to ketone bodies and was not converted into other fatty acids or esterified to a significant extent.

3. Succinate, malate and pyruvate promoted the reduction of acetoacetate formed from $[\text{G-}^{14}\text{C}]$ -palmitate and endogenous substrates. The total ketone-body production was little altered by these substrates.

4. About 16 % of the ^{14}C injected in the form of albumin- $[\text{G-}^{14}\text{C}]$ palmitate complex was recovered in the fatty acids of the liver cells isolated 20–30 min. later. During incubation of the cells the isotope content of the phospholipids and triglycerides fell while that of the free fatty acids rose. The ^{14}C which disappeared from the lipids was equal to that which appeared in the carbon dioxide and ketone bodies.

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Blood Lipids

5. THE LIPIDS OF SHEEP PLASMA*

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Until fairly recently there was a dearth of information on the nature and fatty acid composition of the lipids in the plasma of ruminant animals. With respect to the ox, however, work in this Laboratory

has established the overall pattern of lipids in the plasma of cows during pregnancy and lactation (Lough & Garton, 1957; Garton, Duncan & Lough, 1961; Duncan & Garton, 1963) and in the plasma of non-lactating non-pregnant heifers (Duncan & Garton, 1962). As far as we know, no detailed study

* Part 4: Lough (1964).

has been reported on the lipids of sheep plasma though, incidental to other studies, Dole, James, Webb, Rizack & Sturman (1959) determined the fatty acid composition of the neutral lipids, phospholipids and free fatty acids obtained from the plasma of a single sheep. Our investigations of the digestion and assimilation of lipids in the sheep have included the effects of rumen micro-organisms on ingested lipids (Garton, Lough & Vioque, 1961) and the composition of the lipids present in lymph draining the sheep's intestine (Feliński, Garton, Lough & Phillipson, 1964); these investigations have now been extended to the lipids of sheep plasma.

EXPERIMENTAL

Animals and diet. Twelve Cheviot wethers, each aged about 6 months, were used. They had grazed pasture since weaning and, for a month before the blood samples were taken, each animal received a daily supplement of 1.5 lb. of a mixture of bruised oats and flaked maize.

Blood samples and preparation of lipids for analysis. Blood samples (about 150 ml.) obtained by jugular venepuncture were taken into heparin-treated receivers, pooled and centrifuged to yield the plasma.

The plasma (836 ml.) was poured slowly, with stirring, into 1.7 l. of absolute ethanol and the precipitated protein (containing most of the lipid) was removed by centrifuging. The supernatant aqueous ethanol was reduced to about 150 ml. by vacuum distillation at 40° and shaken in a separating funnel with 450 ml. of chloroform-methanol (2:1, v/v). The chloroform layer was removed and the extraction repeated with a second portion (450 ml.) of chloroform-methanol; the two chloroform layers were combined and washed with water. Lipids were extracted from the protein precipitate by adding 2.4 l. of chloroform-methanol (2:1, v/v). The mixture was brought to the boil, cooled and filtered; after two further such extractions the pooled filtrates, together with the chloroform extracts of the

supernatant aqueous ethanol, were distilled to dryness under reduced pressure in an atmosphere of nitrogen to give 1.91 g. of crude lipid.

Analysis of lipids. The crude lipid was separated into its component classes (cholesterol esters, triglycerides, free cholesterol, free fatty acids and phospholipids) as described by Garton *et al.* (1961); glycerol was determined in the triglyceride fraction according to Lambert & Neish (1950). Fatty acid methyl esters prepared from appropriate fractions (Duncan & Garton, 1962) were analysed by gas-liquid chromatography in an Argon Chromatograph (W. G. Pye and Co. Ltd., Cambridge) under conditions described in detail previously (Duncan & Garton, 1963). Thin-layer chromatography was used to check the homogeneity of the isolated lipids and of the fatty acid methyl esters derived therefrom; the adsorbent used was Kieselgel G (Merck A.-G., Darmstadt, W. Germany) and the appropriate solvents were those recommended by Malins & Mangold (1960).

The *trans*-unsaturated fatty acid content of methyl ester samples was determined by measuring the absorption at 962 cm.⁻¹ (in carbon disulphide) with an infrared spectrophotometer (Unicam model SP. 200); methyl elaidate was used as reference standard (cf. Jart, 1960). The results were calculated as elaidic acid; though mono-unsaturated C₁₈ acid predominated in the samples examined it is recognized that acids with more than one *trans* bond may also have been present.

Conjugated unsaturation was determined by measuring the degree of maximum absorption between 231–234 mμ (conjugated di-unsaturated acid) and 268–271 mμ (conjugated tri-unsaturated acid) of solutions in spectroscopically pure ethanol with a Hilger Uvispek photoelectric spectrophotometer, model H. 700 (cf. Pitt & Morton, 1957).

RESULTS AND DISCUSSION

Table 1 shows the results of the chromatographic separation of the plasma total lipids, together with analytical data for the different fractions. The

Table 1. *Separation and analysis of sheep plasma lipids*

For experimental details see text and Garton *et al.* (1961).

Chromatography on silicic acid of crude lipid (1.91 g.)

Eluting solvent	Wt. of material eluted (mg.)	Nature of fraction	Analysis of fraction
Light petroleum, b.p. 40–60°	26	? Hydrocarbons	Not examined
Light petroleum containing 0.5% (v/v) of ether	636	Cholesterol esters	Alkaline hydrolysis gave 386 mg. of cholesterol and 263 mg. of fatty acids
Light petroleum containing 3.0% (v/v) of ether, followed by light petroleum containing 10% (v/v) of ether	310	Triglycerides, free cholesterol, free fatty acids and a trace of monoglycerides	Alkaline extraction gave 55 mg. of free fatty acids; after alkaline hydrolysis of the remaining lipid 136 mg. of glyceride-bound fatty acids, 15.8 mg. of glycerol and 108 mg. of free cholesterol were recovered
Ether	14	Phospholipids plus non-lipid material	Acid methanolysis yielded 266 mg. of fatty acid methyl esters, equivalent to 253 mg. of fatty acids
Chloroform-methanol (1:1, v/v)	871		

results of these analyses, expressed in terms of the plasma content of each lipid component, are shown in Table 2, which for comparison gives corresponding values obtained in earlier work on ox plasma lipids. In this Table the values for phospholipids were calculated as lecithin since it is by far the most abundant phospholipid in sheep plasma, though sphingomyelin is also present together with very small amounts of other phosphorus-containing lipids including phosphatidylethanolamine and choline plasmalogen (Dawson, Hemington & Lindsay, 1960).

As Table 2 shows, the lipid content of sheep plasma was only just over half that in ox plasma and it seems likely that this observation applies generally since, in other (unpublished) studies, we have found values of a similar order to those relating to the respective pooled plasma samples in a number of individual sheep and oxen of different ages and fed on different rations. The difference in plasma lipid values between these two species of ruminants is largely a reflexion of the much higher content of cholesterol esters in ox plasma, though phospholipids and free cholesterol also contribute.

Table 2. *Component lipids of sheep and ox plasma*

Lipid fraction	Sheep plasma (present work) (mg./100 ml.)	Ox plasma (Duncan & Garton, 1962) (mg./100 ml.)
? Hydrocarbons	3	1
Cholesterol esters	76	166
Free cholesterol	13	28
Triglycerides	18	14
Free fatty acids	7	7
Phospholipids (as lecithin)	46	75
Total	163	291

Triglycerides and free fatty acids are of a similar order in the plasma of the sheep and the ox, as also is the ratio of free cholesterol to total cholesterol, the values being 0.22 (sheep) and 0.23 (oxen). Of the total fatty acids in the sheep plasma (85 mg./100 ml.), 37.3 % was esterified to cholesterol, 19.2 % was present in glycerides, 35.6 % was in phospholipids and 7.9 % was in the free state.

The fatty acid compositions of the appropriate lipid fractions of the sheep plasma are given in Table 3; if plasmalogens were present in the phospholipids they would yield dimethylacetals under the conditions used to prepare fatty acid methyl esters and so would be included in the components listed as unidentified. The overall pattern of fatty acid distribution in the triglycerides, free fatty acids and phospholipids resembles that in the corresponding fractions from ox plasma (Duncan & Garton, 1962), though the proportions of palmitic acid are somewhat lower and those of C_{18} mono-unsaturated acid are higher. However, the relative proportions of the fatty acids esterified to cholesterol show considerable differences between the sheep and the ox as is indicated by the values recorded in Table 4 for palmitic acid and C_{18} fatty acids. The differences in respect of the latter may be associated with differences in the nature or capacity of the rumen micro-organisms of the two species to hydrogenate C_{18} unsaturated acids of the feed lipids, of which acids the lipids of pasture and 'concentrates' of vegetable origin are rich sources (see Garton, 1961).

In addition to giving rise to stearic acid, microbial hydrogenation in the rumen of C_{18} unsaturated acids produces geometrical and positional isomers of oleic acid, linoleic acid and linolenic acid (Shorland, Weenink, Johns & McDonald, 1957) and these isomeric forms are found in the tissue lipids and milk

Table 3. *Component fatty acids of sheep plasma lipids*

Fatty acid*	Cholesterol esters	Triglycerides (%, w/w, of total recovered)	Free fatty acids	Phospholipids
< 16:	2.8	2.1	1.4	1.0
16:0	9.3	14.2	9.8	13.7
16:1	3.4	1.6	3.3	0.7
17:0	2.3	0.9	1.6	1.0
18:0	6.7	23.5	17.1	15.8
18:1	37.9	43.2	50.1	34.9
18:2	26.3	5.8	6.0	12.2
18:3	5.7	1.9	3.9	2.9
20:†	3.2	1.7	2.3	5.2
22:†	0.6	0.9	2.1	6.1
23:†	—	—	0.9	1.4
24:†	—	—	—	3.0
Unidentified‡	1.8	4.2	1.5	2.1

* Number of carbon atoms and number of double bonds (Dole *et al.* 1959).

† Saturated and unsaturated components of each chain length.

‡ Including at least three (saturated) components, probably having branched chains.

Table 4. *Palmitic acid and C₁₈ fatty acid content of the fatty acids esterified to cholesterol in sheep and ox plasma*

Fatty acid*	Sheep plasma	Ox plasma
	(present work)	(Duncan & Garton, 1962)
	(%, w/w, of total esterified fatty acids)	
16:0	9.3	5.5
18:0	6.7	1.5
18:1	37.9	5.6
18:2	26.3	52.4
18:3	5.7	22.9

* See footnote to Table 3.

Table 5. *trans- and conjugated unsaturated fatty acid content of sheep plasma lipids*

Lipid fraction	<i>trans</i> -acids	Conjugated acids
	(as elaidic acid)	(as C ₁₈ di-unsaturated acid)
	(%, w/w)	(%, w/w)
Cholesterol esters	5.7	1.7
Triglycerides	20.0	2.0
Free fatty acids	—*	0.9
Phospholipids	5.6	1.6

* Not determined owing to experimental loss.

lipids of ruminants (see Hartman, Shorland & McDonald, 1955; review by Garton, 1963). It was thus of interest to determine, in the lipids of sheep plasma, the proportions of unsaturated fatty acids which possessed *trans* double bonds and conjugated double bonds; the results of these determinations are given in Table 5. The highest content of *trans*-unsaturated fatty acid was present in the triglycerides and this presumably reflects the contribution to this plasma lipid fraction of chylomicron triglycerides resulting from the intestinal absorption of *trans*-acids produced in the rumen. Conjugated unsaturated acids appear as a small proportion of the total acids of each class of plasma lipid, though again the highest content is in the triglycerides; no conjugated tri-unsaturated acid was detected in any of the lipids and this is probably related to the observation that linolenic acid is more effectively hydrogenated in sheep-rumen contents than is linoleic acid (Shorland *et al.* 1957; Garton *et al.* 1961).

SUMMARY

1. Sheep plasma lipids were fractionated and the fatty acid composition of the fractions determined by gas-liquid chromatography; *trans*- and conjugated double bonds were also determined.
2. Sheep plasma contained only about half the total amount of lipid in ox plasma, due largely to a lower content of cholesterol esters in the former. These cholesterol esters contained more C₁₈ mono-unsaturated acid and less C₁₈ di- and tri-unsaturated acids than did the corresponding fraction of ox plasma lipids.
3. The nature of the unsaturated fatty acids in the plasma lipids is briefly discussed in relation to the microbial modification of feed lipids in the rumen.

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