The Oxidation and Utilization of Palmitate, Stearate, Oleate and Acetate by the Mammary Gland of the Fed Goat in Relation to their Overall Metabolism, and the Role of Plasma Phospholipids and Neutral Lipids in Milk-Fat Synthesis

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1. Measurements were made of milk yield, mammary blood flow and arteriovenous differences of each plasma lipid fraction, and their specific radioactivities, during the infusion of [U-^{14}C]stearate, [U-^{14}C]oleate, [U-^{14}C]palmitate and [1-^{14}C]acetate into fed lactating goats. 2. Entry rates of fatty acids into the circulation were 4·2mg./min./kg. body wt. for acetate, and 0·18, 0·28 and 0·42mg./min./kg. for stearate, oleate and palmitate respectively. Acetate accounted for 23% of the total carbon dioxide produced by the whole animal, and contributed to the oxidative metabolism of the mammary gland to about the same extent. Corresponding values for each of the long-chain acids were less than 1%. 3. There were no significant arteriovenous differences of phospholipids, sterols or sterol esters, and their fatty acid composition showed no net changes during passage through the mammary gland. 4. There were large arteriovenous differences of plasma triglycerides, and their fatty acid composition showed marked changes across the gland. The proportions of palmitate and stearate fell, and that of oleate increased. 5. Arteriovenous differences of plasma free fatty acids (FFA) were small and variable, but a large fall in the specific radioactivity of each of the long-chain acids examined indicated substantial uptake of plasma FFA, accompanied by roughly equivalent FFA release from mammary tissue. The uptake of FFA was confirmed by the extensive transfer of radioactivity into milk. The FFA of milk were similar in composition and radioactivity to the milk triglyceride fatty acids, and quite unlike plasma FFA. 6. The formation of large amounts of oleic acid (18–21mg./min.) from stearic acid was demonstrated. 7. During the terminal stages of the [^{14}C]acetate infusion, milk triglyceride fatty acids of chain length C_4–C_{14} showed specific radioactivities that were 75–90% of that of blood acetate, and that of palmitate was roughly one-quarter of this value. Oleate and stearate were unlabelled. 8. The results confirmed that milk fatty acids of chain length C_4–C_{14} arise largely from blood acetate, and palmitate is derived partly from acetate and partly from plasma triglyceride, the latter fraction being almost the sole precursor of oleate and stearate.

The precursors of the fatty acids of ruminant milk fat are known to be largely acetate, which gives rise to acids of chain length C_4–C_{16}, and plasma triglycerides, which supply most of the C_{18} acids and a proportion of the other long-chain acids. Plasma FFA,* a lipid fraction of considerable metabolic significance (Steinberg, 1963; Annison, 1964), have attracted attention as possible precursors of milk fat, but recent studies have shown only small and variable arteriovenous differences of FFA across the lactating mammary glands of fed goats and cows (Barry, Bartley, Linzell & Robinson, 1963; Kronfeld, 1965; Hartmann & Lascelles, 1964). However, mammary blood flow is high during lactation so that small arteriovenous differences could represent substantial uptake of substrate. In the present studies the metabolism of FFA by the lactating mammary gland of the normal conscious fed goat has been examined in conjunction with measurements of FFA entry rate into the circulation in the whole animal. Isotope-dilution techniques were combined with measurements of arteriovenous differences and mammary blood flow (Annison & Linzell, 1964) to assess the uptake, oxidation and transfer into milk of plasma

* Abbreviation: FFA, free fatty acids.

FFA. Changes in the concentration, fatty acid composition and specific radioactivity of the major plasma lipids across the mammary gland, and the transfer of radioactivity into milk, were also followed. Similar measurements were made during the infusion of [^{14}C]acetate.

This work was carried out with trained lactating goats surgically prepared to allow the venous drainage from a single mammary gland to be sampled (Linzell, 1960*a*,*b*) for the assay of blood constituents, and for blood flow measurement. The use of fully trained undisturbed animals was essential to avoid the changes in FFA concentration that accompany even mild excitement. The plasma FFA concentrations observed in this work were uniformly low.

A preliminary report of this work was given to The Biochemical Society (Annison, Fazakerley, Linzell & Nicholls, 1965).

MATERIALS AND METHODS

Experimental animals. The surgical and infusion procedures were as described by Annison & Linzell (1964). The ^{14}C-labelled fatty acids were infused intravenously for 4–8 hr. at 2 μc/min. by an infusion arrangement that allowed the goats to move freely in their own pens during the experiment. Trained lactating goats were used that had been surgically prepared some years earlier to allow the collection of arterial and mammary venous blood, and the measurement of blood flow through one mammary gland, by exteriorizing one carotid artery, one main mammary vein and dividing blood vessels crossing between the two glands.

Catheters were inserted under local anaesthesia into one jugular vein and an exteriorized carotid artery on the morning of the experiment. Four pairs of simultaneous samples of arterial and mammary venous blood were taken at 15 min. intervals during the last hour of infusion. Mammary venous samples were taken from the vein loop with the precaution of manually clamping the other main vein to obtain a representative mammary venous sample (Linzell, 1960*a*,*b*, 1966). Since the two glands forming the udder were of equal size and giving an equal volume of milk, total uptake by the udder could be calculated. The animals were given hay *ad lib.*, and about 1 kg./day of a concentrate was fed in roughly two equal parts at the times of milking (09.30 and 16.00 hr.). The infusions were carried out on fed animals during the period 11.00–16.00 hr. They were milked hourly until 5 hr. after the end of the infusion, an intravenous injection of oxytocin (200 milliunits) being used to aid milk ejection.

In the experiments now reported labelled stearate was infused into goat Jill (body weight 63 kg.) when she was giving 2·92 l. of milk daily (59% of peak yield) of 4·4% fat, mean rate of blood flow through the udder 1140 ml./min., haematocrit 23%. Goat Balham was used for three experiments with labelled oleate, palmitate and acetate. She weighed 68·5–73 kg., the haematocrit was 23–27% and milk fat 4%. On the days of the experiments the corresponding milk yields (l./day) and rates of blood flow through the udder (ml./min.) were: 2·67, 950; 2·07, 790; 2·24, 790 (55–69% of peak yield).

^{14}C-labelled fatty acids. [U-^{14}C]Stearic acid, [U-^{14}C]oleic acid, [U-^{14}C]palmitic acid and sodium [1-^{14}C]acetate were obtained from The Radiochemical Centre, Amersham, Bucks. The long-chain acids were bound to goat albumin (prepared by the method of Kekwick & Mackay, 1954) by the procedure of Laurell (1957) and the resultant clear solutions diluted with 0·9% NaCl (without addition of carrier fatty acid) to adjust the solution to 2 μc/ml. Sodium [1-^{14}C]acetate was dissolved in 0·9% NaCl and carrier sodium acetate added to adjust the solution to 0·5 mg. and 2 μc/ml.

Extraction and fractionation of blood lipids. Blood samples (30 ml.) were immediately centrifuged (3000*g*) at 2° and the concentration of FFA in 2 ml. of the separated plasma was determined by the method of Dole (1956). Lipid was extracted from the remainder of the plasma by the method of Folch, Lees & Sloane-Stanley (1957). Plasma FFA was isolated from a portion of the isolated lipid by the procedure of McCarthy & Duthie (1962) and stored in solution in light petroleum (b.p. 40–60°) at −10° until examined by gas radiochromatography. The major part of the plasma lipid was separated into phospholipid and a neutral lipid fraction (containing triglycerides, free sterol and sterol esters) by silicic acid chromatography (Hirsch & Ahrens, 1958).

The component lipids of these major fractions were separated by thin-layer chromatography on silicic acid (silica gel G; E. Merck A.-G., Darmstadt, Germany). Phosphatidylcholine, phosphatidylethanolamine, sphingomyelin and lysolecithin were isolated from the phospholipid fraction by the solvent system chloroform–methanol–water (65:25:4, by vol.), and the neutral lipid fraction yielded triglyceride, free sterol and sterol esters when fractionated with the solvent system hexane–diethyl ether (17:3, v/v). Separated lipids were recovered from thin-layer plates by scraping off the silicic acid containing each fraction and eluting with chloroform–methanol (1:1, v/v) and then pure methanol. Yields of isolated lipids were determined by analysis with the following methods: phospholipid fraction (as total phosphorus after digestion; Allen, 1940); triglyceride (Carlson & Wadstrom, 1959); free and esterified sterol (with the modified Lieberman–Burchard reagent of Huang, Chen, Wefler & Raftery, 1961). Isolated lipid fractions were stored in chloroform–methanol (2:1, v/v) at −10° until assayed for radioactivity and fatty acid composition.

Extraction and fractionation of milk lipids. Extracted fat was subjected to column chromatography (Hirsch & Ahrens, 1958) to yield phospholipid and neutral lipids. The neutral lipid was separated into triglyceride and sterol (free plus esterified) fractions by thin-layer chromatography. Milk-fat FFA was isolated from a portion of the extracted lipid by the method of McCarthy & Duthie (1962).

Analysis of fatty acids. Methyl esters of plasma FFA were prepared by treatment with an excess of freshly prepared diazomethane in ether and examined by gas–liquid chromatography. The analytical data supplied by gas radiochromatography (see below) were supplemented by additional analyses with systems in which β-ionization detectors were used, but with column stationary phases and operating conditions similar to those used in the radiochromatographs. The fatty acid composition of lipid fractions was determined after preparation of the methyl esters of the component fatty acids by transesterification.

Lipids were refluxed with 5ml. of methanol–benzene–conc. H_2SO_4 (20:5:1, by vol.) for 120min. and the methyl esters extracted with light petroleum (b.p. 40–60°). Milk triglyceride was saponified with KOH, and the fatty acids were separated by partition chromatography on A.R. silicic acid (100 mesh; Mallinckrodt Chemical Works, New York, N.Y., U.S.A.) with methanol–water (10:1, v/v) as stationary phase. Fatty acids were eluted in two stages by using first *n*-hexane (100vol.) saturated with stationary phase (10vol.), followed by a mixture of *n*-hexane (50vol.) and toluene (50vol.) saturated with stationary phase. A mixture of higher fatty acids (including the C_{12} acid) emerged first, followed by well-separated fractions containing acids of chain length C_{10}, C_8, C_6 and C_4. Strict temperature control (25°) was necessary for good fatty acid separations. The solvent system allowed fractions collected from the column to be assayed for radioactivity by liquid scintillation after adding a suitable amount of phosphor in toluene. Quenching corrections were made by using internal standards. Alternate fractions were titrated with 0·01N-NaOH under CO_2-free conditions with 1ml. of Nile blue (0·001% in ethanol) as indicator. The remaining fractions were assayed for radioactivity. The higher acid fraction eluted from the column was methylated with diazomethane and examined by gas radiochromatography.

Assay of radioactivity. Lipids were dissolved in toluene containing 2,5-diphenyloxazole (4g./l.) and 1,4-bis-(5-phenyloxazol-2-yl)benzene (0·01g./l.) and assayed for ^{14}C content in a Tritomat liquid-scintillation counter (Isotope Developments Ltd., Beenham, Berks.). Corrections for quenching were made by adding known amounts of radioactivity (*n*-[^{14}C]hexadecane) as internal standards.

The ^{14}C-labelled long-chain fatty acids (chain length greater than C_{10}) were assayed for specific radioactivity by gas radiochromatography (James & Piper, 1963). Methyl esters of fatty acids were transferred to the gas radiochromatograph as described by Hitchcock & James (1964). The esters were separated on 130cm. straight or coiled columns (internal diam. 4mm.) containing 100–120-mesh Celite supporting 20% Apiezon L grease at 200° or 80–100-mesh Celite supporting 20% ethylene glycol adipate polymer at 170°.

The radiochromatographs used in these investigations were standardized daily by running methyl palmitate of known specific radioactivity.

Other methods of analysis. Concentrations of acetate, O_2 and CO_2 in blood, the specific radioactivities of CO_2 and acetate and the concentrations of total fat in milk were measured as described by Annison & Linzell (1964).

RESULTS

Mammary uptake of plasma triglyceride. As reported previously (Barry *et al.* 1963) there was a consistent and significant fall in the concentration of plasma triglyceride across the mammary gland. Uptakes of plasma triglycerides by the mammary gland were calculated from flow rates of plasma through the mammary gland and arteriovenous-difference data, and in the four experiments were equivalent to 63–82% of the output of milk triglycerides (Table 1). When [U-^{14}C]stearate was infused there was no detectable labelling of plasma triglyceride, but when [U-^{14}C]oleate and [U-^{14}C]-palmitate were infused appreciable amounts of these labelled acids were incorporated into plasma triglycerides (Table 2). In each pair of samples the oleic acid content of the venous plasma triglyceride increased and the stearic acid content decreased relative to arterial plasma triglyceride (Table 2). The plasma triglyceride-palmitate content fell during passage across the gland in two of the three experiments, but the specific radioactivity was largely unchanged, indicating that triglyceride arising in the gland, which would contain unlabelled palmitate synthesized in the gland, is not in equilibrium with plasma triglyceride. This conclusion is strongly supported by the absence of short-chain acids (which occur in milk triglycerides) in venous plasma triglyceride.

Mammary uptake of sterol and sterol esters. There was no significant uptake of sterol, free or esterified (Table 1). Slight labelling of the sterol ester fraction occurred during the infusion of [U-^{14}C]-palmitate, but the specific radioactivity of palmitate was similar in the arterial and mammary venous samples. The fatty acid composition of plasma sterol ester showed only slight changes during passage through the mammary gland (Table 3), but the possibility of fatty acid exchange in the gland cannot be ruled out. The sterol ester fraction, which accounts for the major portion of plasma lipid, was rich in linoleic acid, as observed by previous workers (Garton, 1963).

Mammary uptake of plasma phospholipid. In agreement with the observations of Blackwood (1934) no evidence was obtained for the uptake of significant amounts of phospholipid by the mammary glands (Table 1). Examination of individual phospholipid fractions (phospholipid choline, sphingomyelin, phosphatidylethanolamine and lysolecithin) revealed only small and variable arteriovenous differences. The extent of incorporation of infused labelled fatty acids into plasma phospholipids was too low for assay by gas radiochromatography. Fatty acid analyses were carried out on all isolated phospholipid fractions. No evidence was obtained of significant changes in the fatty acid content of any phospholipid fraction during passage through the mammary gland, but since the fractions were not labelled the possibility of fatty acid exchange cannot be excluded.

Uptake and oxidation of fatty acids by mammary tissue. (*a*) Long-chain acids. Although only small positive or negative arteriovenous differences in FFA concentrations were observed, in each instance the specific radioactivity of the labelled fatty acid declined markedly across the gland (Table 4). Oxidation accounted for only a small fraction of the radioactive fatty acid removed by the gland since the specific radioactivity of carbon dioxide

Table 1. *Mean values for the concentration of plasma lipids in four pairs of arterial and venous blood samples taken during* ^{14}C-*labelled fatty acid infusions*

The uptakes of triglyceride and acetate by the mammary gland are compared with the output of milk triglyceride.

Expt.	Sample	Sterol (mg./100ml.) Total	Sterol (mg./100ml.) Free	Phospholipid (mg. of P/100ml.)	FFA (m-equiv./l.)	Volatile fatty acid (m-equiv./l.)	Triglyceride (mg./100ml.)	Plasma flow (ml./min.)	Milk yield (l./day)	Mammary uptake: Triglycerides (mg./min.)	Mammary uptake: Acetate (mg./min.)	Output of triglycerides in milk (mg./min.)
[U-^{14}C]Stearate	Arterial	139	22	6·2	0·15	2·0	21·2	877	2·92	74	82	97
	Venous	123	28	5·6	0·14	0·7	12·7					
[U-^{14}C]Oleate	Arterial	172	34	9·4	0·18	1·4	27·1	693	2·67	63	48	81
	Venous	173	33	8·9	0·18	0·6	18·0					
[U-^{14}C]Palmitate	Arterial	121	43	7·5	0·24	1·2	30·8	608	2·07	63	18	76
	Venous	135	42	8·5	0·23	0·8	20·5					
[1-^{14}C]Acetate	Arterial	97	37	5·7	—	1·2	24·2	608	2·24	51	48	81
	Venous	104	38	5·7	—	0·3	15·8					

Table 2. *Composition and specific radioactivities of the fatty acids of arterial and mammary venous plasma triglycerides during the infusion of* [U-^{14}C]*palmitate*, [U-^{14}C]*stearate* and [U-^{14}C]*oleate*

Mean values for three pairs of samples taken during the last hour of infusion are shown. The shorthand nomenclature of Dole, James, Webb, Rizack & Sturman (1959) was used in this and subsequent Tables to indicate chain length, number of double bonds and branched-chain acids (br).

Substrate infused.....	[U-^{14}C]Palmitate				[U-^{14}C]Stearate				[U-^{14}C]Oleate			
	Arterial		Venous		Arterial		Venous		Arterial		Venous	
Fatty acid	(%)	Sp. act. (μc/g.)	(%)	Sp. act. (μc/g.)	(%)	Sp. act. (μc/g.)	(%)	Sp. act. (μc/g.)	(%)	Sp. act. (μc/g.)	(%)	Sp. act. (μc/g.)
10:0	0·4	—	0·6	—	0·2	—	0·4	—	2·7	—	4·1	—
12:0	1·4	—	1·6	—	0·3	—	0·7	—	2·6	—	3·9	—
12:br?	0·4	—	—	—	0·1	—	0·3	—	0·4	—	0·3	—
14:0	1·2	—	2·0	—	1·8	—	1·7	—	1·8	—	1·1	—
14:1	—	—	0·1	—	0·4	—	0·1	—	1·8	—	1·1	—
14:br?	0·7	—	0·1	—	1·4	—	0·2	—	0·7	—	0·7	—
15:0	0·6	—	0·1	—	1·5	—	0·5	—	0·4	—	0·3	—
16:0	27·9	14·1	21·1	12·7	23·4	—	14·8	—	21·7	—	21·3	—
16:1	3·4	—	5·1	—	5·2	—	3·6	—	6·6	—	5·0	—
16:br?	—	—	—	—	3·5	—	5·1	—	0·6	—	0·9	—
17:0	—	—	—	—	2·9	—	1·4	—	3·0	—	2·1	—
18:0	36·5	—	31·4	—	34·6	—	31·6	—	28·8	—	21·8	—
18:1	23·3	—	31·7	—	21·8	—	35·5	—	24·5	20·6	32·1	14·6
18:2	3·2	—	6·2	—	2·9	—	4·3	—	4·4	—	3·8	—

Table 3. *Fatty acid composition of the sterol esters of arterial and mammary venous plasma samples withdrawn simultaneously during the infusion of labelled long-chain fatty acids*

The results for a representative pair of samples from each experiment are shown.

Substrate infused.....	[U-^{14}C]Stearate		[U-^{14}C]Oleate		[U-^{14}C]Palmitate	
Fatty acid	Arterial (%)	Venous (%)	Arterial (%)	Venous (%)	Arterial (%)	Venous (%)
10:0	0·4	0·4	0·3	0·5	0·5	0·6
12:0	0·6	0·9	0·5	0·4	0·7	0·9
12:br?	2·0	4·3	—	—	—	—
14:0	0·7	0·9	0·5	0·9	0·4	0·4
14:1	0·6	0·9	0·4	0·5	0·3	0·2
14:br?	0·3	0·5	0·3	0·7	0·4	0·2
15:0	0·7	0·9	0·2	0·3	0·2	0·2
16:0	8·9	10·2	10·4	12·1	7·7	6·9
16:1	3·8	4·5	2·1	1·9	1·1	0·9
16:br?	0·1	0·1	0·1	—	0·6	0·6
17:0	2·2	3·3	0·7	0·5	0·4	0·4
18:0	2·4	4·2	3·2	4·3	5·5	2·6
18:1	28·1	26·6	27·9	30·0	24·3	24·9
18:2	48·0	41·0	50·4	46·9	54·6	56·9
18:3	1·2	1·3	3·0	1·0	3·3	4·3

in mammary venous blood was not significantly different from that in arterial blood in any of the experiments with long-chain fatty acids (Table 5).

Changes in the composition of plasma FFA across the mammary gland were evident when [^{14}C]stearic acid was infused, but only minor differences were observed in the experiments with labelled oleic acid and palmitic acid (Table 4).

The uptake of radioactivity by the mammary gland and the amount of radioactivity secreted in milk at the end of the period of the infusion are shown in Fig. 1. The ^{14}C-labelled milk fat of declining specific radioactivity continued to be secreted for some hours after the end of the ^{14}C-labelled fatty acid infusion (Fig. 2).

The extensive conversion of stearic acid into oleic acid in the mammary gland was clearly shown when the uptake and production of long-chain fatty acids by the mammary gland was calculated from the arteriovenous differences, and the rates of blood flow and milk production (Table 6). This was confirmed in the stearate experiment, where labelled oleic acid appeared both in the milk and in the mammary venous plasma FFA (Tables 4 and 9). Values for the production of milk C_{18} fatty acids were closely similar to the amounts taken up by the mammary gland, confirming that blood triglycerides are almost the sole precursors of the C_{18} acids in milk (Table 6).

(*b*) Acetate. Mean concentrations of acetate in arterial and mammary venous blood during the infusion of [1-^{14}C]acetate (Table 5) indicated an acetate uptake of 0·95m-mole (57mg. of free acid)/min. The contribution of acetate to carbon dioxide produced by the mammary gland was 28%, and 33% of the acetate taken up by the gland was directly oxidized. These values are very similar to those reported previously (Linzell, 1960*b*; Annison & Linzell, 1964).

Rates of entry and oxidation of fatty acids. (*a*) Long-chain acids. Total plasma FFA concentrations and FFA specific radioactivities were sufficiently constant during the terminal stages of the labelled fatty acid infusions to allow calculation of rate of entry of fatty acids into the plasma (Table 5). The low concentrations of plasma FFA reflected the use of fed undisturbed animals, where FFA mobilization from adipose tissue is minimal (Steinberg, 1963). The specific activity of arterial blood carbon dioxide increased only slowly during the final hour of infusion, and comparison of this specific activity with that of plasma fatty acid showed that the contribution of long-chain fatty acid to total oxidative metabolism was low (Table 5).

(*b*) Acetate. The mean concentration and specific radioactivity of acetate in arterial blood was 1·5m-moles/l. and 17·8μc/g. of C respectively. The acetate entry rate was 4·2mg./min./kg. body wt. and the contribution of acetate to carbon dioxide production in the whole animal was 23% (Table 5), both values being within the range reported by Annison & Linzell (1964).

Incorporation of plasma FFA into milk. When ^{14}C-labelled fatty acids were infused there was a lag period of about 2hr. before milk fat became

Table 4. *Fatty acid composition and specific radioactivities of plasma FFA in arterial and mammary venous blood obtained during* ^{14}C-*labelled fatty acid infusions*

Mean values for three pairs of samples taken during the last hour of infusion are shown.

Substrate infused.....	[U-^{14}C]Stearate				[U-^{14}C]Oleate				[U-^{14}C]Palmitate			
	Arterial		Venous		Arterial		Venous		Arterial		Venous	
Fatty acid	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)
10:0	—	—	—	—	0·5	—	0·2	—	0·1	—	0·2	—
12:0	0·9	—	0·3	—	1·6	—	0·7	—	0·2	—	0·1	—
12:br	0·2	—	0·2	—	—	—	0·5	—	1·8	—	0·9	—
14:0	2·5	—	2·9	—	3·4	—	2·0	—	2·2	—	2·0	—
14:1	0·4	—	—	—	0·3	—	1·0	—	0·3	—	0·3	—
14:br?	—	—	—	—	0·4	—	0·7	—	0·8	—	0·9	—
15:0	3·2	—	3·4	—	3·6	—	0·2	—	0·9	—	1·2	—
16:0	25·1	—	28·0	—	15·4	—	19·7	—	17·6	90	17·5	45
16:1	4·0	—	2·4	—	3·8	—	3·4	—	3·5	—	4·2	—
16:br?	—	—	—	—	1·8	—	1·4	—	1·7	—	1·5	—
17:0	1·0	—	1·0	—	2·3	—	0·7	—	0·7	—	0·5	—
18:0	33·7	252	40·4	84	35·2	—	37·6	—	31·4	—	30·1	—
18:1	25·7	4	19·5	49	25·6	166	27·3	100	36·1	—	37·3	—
18:2	3·0	—	3·1	—	2·9	—	7·5	—	2·7	—	3·3	—
18:3	—	—	—	—	4·4	—	—	—	—	—	—	—

Table 5. *Mean volumes for the concentrations and specific radioactivities of plasma fatty acids in four pairs of arterial and venous blood samples obtained during* ^{14}C-*labelled fatty acid infusions*

Entry rates of stearic acid, oleic acid, palmitic acid and acetic acid are shown with estimates of their contribution to CO_2 production in the whole animal.

Expt.	Sample	Plasma concn. of infused substrate (m-equiv./l.)	Sp. act. of infused substrate (μc/g. of C)	Sp. act. of blood CO_2 (μc/g. of C)	Entry rate (mg./min./kg.)	Contribution to total CO_2 (%)
[^{14}C]Stearate	Arterial	0·047	252	0·25	0·18	0·1
	Venous	0·060	84	0·24		
[^{14}C]Oleate	Arterial	0·051	166	0·40	0·28	0·2
	Venous	0·063	100	0·41		
[^{14}C]Palmitate	Arterial	0·042	90	0·58	0·42	0·6
	Venous	0·042	45	0·59		
[^{14}C]Acetate	Arterial	1·5	17·8	4·2	4·2	23
	Venous	0·3	14·9	4·3		

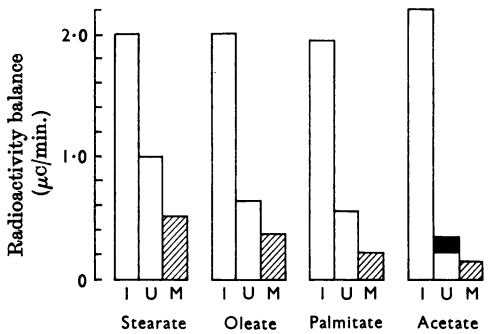

Fig. 1. Radioactivity balance (μc/min.) at end of infusion: I, rate of infusion into animal; U, uptake of whole udder; M, radioactivity in milk. The black bar in the acetate experiment represents the amount of acetate oxidized by the mammary glands.

appreciably radioactive (Fig. 2). In the experiment with long-chain acids maximum specific radioactivity occurred 1–2hr. after the end of the infusion (Fig. 2).

(*a*) Acetate. Sodium [1-^{14}C]acetate was infused at 2·2μc/min. for 450min. Maximum specific radioactivity of milk fat was observed after 7hr. although 210min. after the end of the infusion the milk fat showed 80% of this maximum value (Fig. 2). The mean specific radioactivity of acetate in arterial blood during the infusion was 17·8μc/g. of C. There was little change in fatty acid composition during the experiment, and the specific radioactivities of the even-numbered acids C_{12}–C_{16} reached maximum values after 7hr. (Table 7). Examination of the short-chain acids of the triglyceride isolated from this sample showed that the specific radioactivities of the even-numbered acids C_4–C_{10}, like those of the C_{12} and C_{14} acids, were 75–95% of that of circulating acetate, but that of palmitate was approx. 25% of this value (Table 7). These results confirm that acetate is the major precursor of the C_4–C_{14} acids, but that palmitate arises both from acetate (and ketone bodies) and by transfer from plasma triglyceride.

(*b*) Long-chain acids. The fatty acid composition of the milk showed only slight changes during the infusion, but the specific radioactivity of the fatty acid being studied increased steadily until 1–2hr. after the end of the infusion (Tables 8, 9 and 10).

The milk-fat sample of highest specific radioactivity obtained during each infusion was fractionated into triglyceride and free fatty acids and the component fatty acids were examined by gas radiochromatography (Tables 8, 9 and 10). The specific radioactivities of each fraction were of the same order in each experiment. The milk FFA fraction was similar in composition to that of the corresponding milk triglyceride, and obviously dissimilar in composition and specific radioactivity to plasma FFA.

DISCUSSION

The combination of arteriovenous-difference studies and isotope-dilution techniques allowed measurement of the rates of entry and oxidation of individual plasma free fatty acids in the whole animal, and in the mammary gland, since mammary blood flow was known. The low specific radioactivity of $^{14}CO_2$ in arterial and mammary venous blood relative to that of circulating ^{14}C-labelled fatty acid showed the limited extent of FFA oxidation in both the mammary gland and in the whole animal. Low overall oxidation of plasma FFA and low entry rates were consistent with the low plasma FFA concentration, which is characteristic of fed, unexcited animals (Steinberg, 1963).

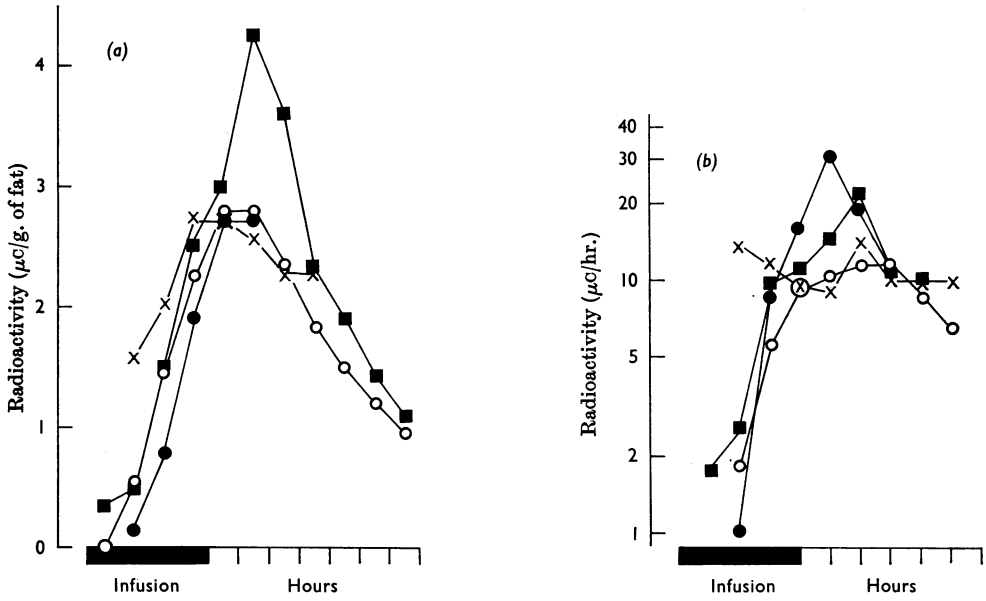

Fig. 2. Time-course of the appearance of radioactivity in milk fat, after the infusion of long-chain fatty acids for 4hr. and acetate for 7·5hr. The end of infusion is synchronized. ×, Acetate; ■, oleate; ●, stearate; ○, palmitate.

Table 6. *Mammary uptake (from plasma triglyceride) and secretion (as milk triglyceride) of stearate and oleate*

Substrate infused	Uptake across mammary gland (mg./min.)		Secreted in milk (mg./min.)		Net production of oleic acid (mg./min.)
Fatty acid.....	18:0	18:1	18:0	18:1	
[U-^{14}C]Stearate	24·5	4·2	9·1	21·4	21·4
[U-^{14}C]Oleate	23·6	2·6	8·2	16·3	17·4
[U-^{14}C]Palmitate	26·1	3·6	7·8	17·7	17·7

The small positive or negative arteriovenous differences of plasma FFA suggested the absence of significant FFA removal by mammary tissue, but the marked decline in the specific radioactivity of labelled FFA across the gland indicated substantial uptake of plasma FFA. This was confirmed by the considerable transfer of radioactivity into milk fatty acids, since the specific radioactivity of other possible precursors of milk fat was far too low to account for the radioactivity appearing in milk. The extent of the reduction of the specific radioactivity of ^{14}C-labelled FFA across the gland and the absence of net uptake indicated release of FFA from mammary tissue. The origin of FFA produced by mammary tissue is uncertain, but, if plasma triglycerides are hydrolysed before transfer across the surface membrane of capillary cells, liberated fatty acids would equilibrate with plasma FFA, and the fraction escaping absorption would appear in mammary venous plasma. It is known that mammary venous plasma in the goat contains more lipoprotein lipase than arterial plasma (Barry *et al.* 1963). The composition of mammary venous FFA resembled that of the fatty acids of mammary venous-plasma triglyceride (Tables 2 and 5), but was unlike that of mammary tissue, which, like milk fat, contains short-chain and medium-chain fatty acids. When [U-^{14}C]stearate was infused, labelled oleate appeared in mammary venous-plasma FFA (Table 5). This is difficult to explain since the dehydrogenation of stearate is almost certainly an intracellular process, and there was no evidence that fatty acids synthesized in the gland from acetate (chain length C_4–C_{16}) escaped from the mammary gland. A consistent increase in the oleic acid content of the mammary venous-

Table 7. *Composition and specific radioactivities of the fatty acids of milk fat secreted during and after the infusion of* [1-^{14}C]*acetate* (3·3μc/*min. for* 7·5*hr.*)

Only the long-chain acid fraction was examined in the total milk fat analyses. Sample numbers refer to hours after the start of infusion. Specific radioactivity of blood acetate, 17·8μc/g. of C.

	Total milk fat						Milk triglyceride	
	Sample 5		Sample 8		Sample 11		Sample 7	
Fatty acid	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)
4:0							1·3	13·3
6:0							2·8	13·8
8:0							8·3	15·3
10:0							12·9	13·9
12:0	5·1	10·3	4·5	14·0	4·3	6·5	3·6	16·8
12:1	0·6	—	—	—	0·5	—	0·4	—
12:br	0·6	—	—	—	—	—	—	—
14:0	12·5	9·1	11·8	11·3	13·4	4·9	10·2	12·6
14:1	1·0	—	0·5	—	0·7	—	0·4	—
15:0	—	—	—	—	—	—	—	—
16:0	29·2	3·1	30·1	4·0	32·1	1·7	24·5	4·3
16:1	1·0	—	1·0	—	1·0	—	0·9	—
17:0	—	—	—	—	—	—	—	—
18:0	10·0	—	12·2	—	12·3	—	9·8	—
18:1	30·7	—	29·1	—	28·5	—	23·3	—
18:2	2·7	—	2·2	—	1·5	—	1·5	—

Table 8. *Incorporation of radioactivity into the long-chain fatty acids of milk triglycerides and free fatty acids during the infusion of* [U-^{14}C]*palmitic acid* (1·95μc/*min. for* 4*hr.*)

Time after start of infusion (hr.).....	1		3	5				10	20	
	Triglyceride		Triglyceride	Triglyceride		Free fatty acid		Triglyceride	Triglyceride	
Fatty acid	(%)	Sp. act. (μc/g. of C)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)
10:0	1·4			6·3		—	—	—	4·8	—
12:0	3·9			5·2		5·1	—	—	5·2	—
14:0	12·4			14·1		9·2	—	—	11·7	—
14:br?	0·2			—		—	—	—	—	—
15:0	0·9			—		0·9	—	—	—	—
16:0	28·2	0·8	7·5	30·7	13·1	42·8	15·0	8·3	27·2	5·8
16:1	1·5			—		—	—	—	—	—
17:0	0·7			—		—	—	—	—	—
18:0	10·7			18·0		13·1	—	—	16·7	—
18:1	36·7			23·6		26·1	—	—	32·4	—
18:2	3·0			1·9		2·7	—	—	1·9	—

plasma triglyceride fraction suggested either the preferential removal of triglycerides not containing oleic acid, or the resynthesis and release from mammary tissue of triglycerides containing oleic acid arising from the dehydrogenation of stearic acid. In the present experiments the extent of incorporation of labelled stearic acid into plasma triglyceride was too low for changes in triglyceride specific radioactivity across the gland to be assessed, and further work with labelled triglycerides is necessary to resolve these problems.

Comparison of the rates of plasma triglyceride uptake (calculated from arteriovenous differences and mammary blood flow) with those of milk triglyceride production has clearly shown that the C_{18} acids of milk could be derived almost entirely

Table 9. *Incorporation of radioactivity into the long-chain fatty acids of milk triglycerides and free fatty acids during the infusion of* [U-^{14}C]*stearic acid* (2·02 μc/*min. for* 4 *hr.*)

Time after start of infusion (hr.).....	0		1·5		2·5		4		5		6			
	Triglyceride		Triglyceride		Triglyceride		Triglyceride		Triglyceride		Triglyceride		Free fatty acid	
Fatty acid	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)
10:0	5·3	—	—	—	—	—	3·5	—	—	—	6·3	—	5·9	—
12:0	6·6	—	—	—	—	—	7·0	—	—	—	7·2	—	6·7	—
14:0	15·6	—	—	—	—	—	15·6	—	—	—	16·4	—	9·6	
14:br?	2·2	—	—	—	—	—	3·4	—	—	—	2·5	—	0·8	—
15:0	1·1	—	—	—	—	—	1·8	—	—	—	—	—	—	—
16:0	31·0	—	—	—	—	—	34·1	—	—	—	37·3	—	35·8	
16:1	1·2	—	—	—	—	—	3·3	—	—	—	1·9	—	—	—
18:0	7·3	—	—	1·9	—	20·5	5·9	30·9	—	34·0	5·0	28·7	9·9	8·6
18:1	29·3	—	—	0·6	—	4·8	24·7	10·4	—	16·1	22·1	16·7	28·5	9·9
18:2	1·8	—	—	—	—	—	0·8	—	—	—	1·3	—	2·8	—

Table 10. *Incorporation of radioactivity into the long chain fatty acids of milk triglycerides and free fatty acids during the infusion of* [U-^{14}C]*oleic acid* (2·06 μc/*min. for* 4 *hr.*)

Time after start of infusion (hr.).....	1		2		3		4		5		6				8	
	Triglyceride		Triglyceride		Triglyceride		Triglyceride		Triglyceride		Triglyceride		Free fatty acid		Triglyceride	
Fatty acid	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)	(%)	Sp. act. (μc/g. of C)
10:0	7·2	—	3·4	—	4·7	—	7·6	—	7·6	—	5·0	—	2·3	—	9·6	—
12:0	4·9	—	3·8	—	4·9	—	5·8	—	4·4	—	5·7	—	4·8	—	5·6	—
14:0	12·3	—	12·7	—	13·2	—	13·7	—	13·8	—	13·8	—	9·8	—	13·2	—
14:br?	1·9	—	—	—	2·0	—	—	—	1·4	—	—	—	1·0	—	—	—
16:0	16·6	—	28·8	—	28·3	—	29·4	—	30·5	—	30·0	—	29·7	—	30·4	—
16:1	1·6	—	2·7	—	1·3	—	2·5	—	2·7	—	2·4	—	7·7	—	2·3	—
17:0	0·9	—	—	—	0·9	—	—	—	2·2	—	—	—	—	—	—	—
18:0	13·0	—	13·1	—	13·9	—	11·9	—	8·0	—	13·8	—	12·7	—	13·0	—
18:1	28·6	—	32·7	3·1	28·0	7·9	26·5	20·1	26·7	30·8	24·8	30·6	26·8	15·1	23·1	22·2
18:2	2·7	—	2·8	—	2·9	—	2·5	—	2·6	—	2·7	—	5·1	—	2·6	—

from plasma triglyceride (Table 5). The extensive conversion of stearic acid into oleic acid, first reported in udder fat of the perfused cow's udder by Lauryssens *et al.* (1960), was convincingly demonstrated *in vivo* (Table 5). Glascock & Reinius (1956) reported the occurrence of tritium-labelled oleic acid in the milk fat of cows given tritium-labelled stearic acid.

There was no detectable uptake of free or esterified cholesterol by the mammary gland, confirming the early observations of Lintzel (1934), but the amounts secreted in milk are so small that removal of an equivalent amount from arterial plasma would not be detectable. Patton & McCarthy (1963) demonstrated rapid uptake of palmitate and linoleate by the cholesterol ester fraction of milk when the acids were infused through the teat canal, suggesting that the cholesterol esters of milk originate in the gland. The present studies have not implicated the plasma cholesterol fraction in any aspect of milk lipid synthesis, but only experiments with isotopically labelled esters would provide unequivocal evidence on this point. Clarenburg & Chaikoff (1966) have estimated that in the rat 80% of milk cholesterol is synthesized in the mammary gland.

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