Biochem. J. (1967) **104**, 34

The Incorporation of Acetate, Stearate and D(−)-β-Hydroxybutyrate into Milk Fat by the Isolated Perfused Mammary Gland of the Goat

By J. L. LINZELL, E. F. ANNISON, S. FAZAKERLEY and R. A. LENG
Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge, Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford, and School of Rural Science, University of New England, Armidale, New South Wales, Australia

(*Received* 26 *September* 1966)

1. Mammary glands of lactating goats were perfused for 12·5–15hr. with heparinized whole blood and infused with a substrate mixture of glucose, acetate and amino acids (and sometimes chylomicra) containing either [1-^{14}C]acetate, D(−)-β-hydroxy[1-^{14}C]butyrate or [U-^{14}C]stearate. 2. There was a substantial net uptake of acetate by the glands and transfer of radioactivity into milk fat. Acetate was extensively utilized for the synthesis of milk fatty acids of chain length up to C_{14} and to a smaller extent for the synthesis of palmitate. 3. There was a small and variable net uptake of stearate and β-hydroxybutyrate and negligible oxidation of these substrates. However, tissue uptake was demonstrated by a substantial fall in specific radioactivity across the glands and an extensive transfer of radioactivity into milk fatty acids. 4. With β-hydroxybutyrate the labelling of milk fat was very similar to that with acetate, but the distribution of radioactivity suggested a cleavage into C_2 fragments of about 40%. 5. Labelled stearate gave rise to highly labelled stearate and oleate in the milk fat. Small amounts of radioactivity were detected in stearate of plasma triglycerides and oleate of plasma free fatty acids. 6. In experiments where there was a decline in milk-fat secretion late in perfusion, the milk fatty acids showed a marked decline in the proportion of stearate and oleate and a rise in the proportion of myristate and palmitate. This did not occur in experiments where milk-fat secretion was maintained at a higher level. 7. The present results confirm that there is a large pool of long-chain fatty acids in mammary tissue that can act as an endogenous source of these substrates.

Recently quantitative data on the role of glucose and fatty acids in mammary-gland metabolism in the undisturbed conscious goat have been obtained by combining arteriovenous difference studies with isotope dilution (Annison & Linzell, 1964; Annison, Linzell, Fazakerley & Nichols, 1967). These procedures allow mammary-gland metabolism to be correlated with overall metabolism, but the infusion of radioactive substrates into intact animals inevitably involves considerable dilution with body substrate pools, which may obscure precursor–product relationships in the gland. There are large net arteriovenous differences across the udder for acetate (Linzell, 1960; Annison & Linzell, 1964) and triglycerides (Barry, Bartley, Linzell & Robinson, 1963), and when these substrates are labelled and injected into lactating goats a large amount of the radioactivity is recovered in the milk fatty acids (Popják, French & Folley, 1951; Lascelles, Hardwick, Linzell & Mepham, 1964). By contrast there is a negligible arteriovenous difference of FFA* in fed animals (Barry *et al.* 1963; Annison *et al.* 1967), and yet when labelled stearate, oleate or palmitate are infused a substantial amount of radioactivity is incorporated into the milk triglyceride fatty acids (Annison *et al.* 1967). Further analysis of this situation may be simplified by the use of the isolated perfused gland, where only one organ is involved and where the incorporation into milk of substrates of relatively high specific radioactivity can be studied for as long as 12–15hr. We have used this system to study the incorporation into milk fatty acids of [U-^{14}C]stearate, [1-^{14}C]-acetate and D(−)-β-hydroxy[1-^{14}C]butyrate.

There is also a mammary arteriovenous difference of β-hydroxybutyrate in cows (Shaw & Knodt,

*Abbreviations: FFA, free fatty acids; R.Q., respiratory quotient.

1941) and goats (Barry *et al.* 1963). The ^{14}C-labelled compound is incorporated into milk casein and fatty acids (Kumar, Lakshmanan & Shaw, 1959; Palmquist, Davis & Brown, 1966), but some doubt exists as to whether or not the mechanism of incorporation into fatty acids is similar to that of butyrate, i.e. as two C_2 units (Lauryssens, Verbeke, Peeters & Reinards, 1960). Studies with DL-β-hydroxy[3-^{14}C]butyrate in the lactating cow (Luick & Kameoka, 1966) and in cell-free systems of mammary tissue from lactating goats and rabbits (Kumar, Singh & Keren-Paz, 1965) suggested that the conversion of β-hydroxybutyrate into butyrate occurs largely without cleavage of the carbon chain. In the present work we have studied the incorporation of D(−)-β-hydroxy[1-^{14}C]butyrate into milk fatty acids because this is the naturally occurring isomer taken up by the goat's udder (Barry *et al.* 1963).

The amount of fat secreted by isolated perfused goat mammary glands is more variable than that of lactose and protein (Hardwick & Linzell, 1960), and less responsive to the availability of the precursors (acetate, triglycerides and β-hydroxybutyrate) that are taken from the plasma *in vivo* (Lascelles *et al.* 1964). Triglycerides and β-hydroxybutyrate are not usually necessary in the substrate mixture infused into isolated glands to maintain milk secretion, probably because the large pool of tissue triglycerides can serve as an endogenous supply of lipid substrate (Hardwick, Linzell & Mepham, 1963*a*). In some experiments after 8hr. of perfusion the secretion of milk fat falls, possibly owing to the exhaustion of endogenous substrates, but unfortunately this cannot be determined beforehand and may explain why the addition of triglycerides to the substrate mixture had little effect on the amount of fat secretion in two experiments performed by Lascelles *et al.* (1964). Another interest of the present experiments was therefore the assessment of the value of the isolated perfused lactating gland in studies of milk-fat secretion and synthesis, by comparing the uptake and incorporation of ^{14}C-labelled precursors into milk fat with that occurring *in vivo*.

MATERIALS AND METHODS

Experimental procedure. Isolated glands of lactating goats were perfused with heparinized whole blood for 12·5–15hr. according to the technique of Hardwick & Linzell (1960) and Hardwick, Linzell & Price (1961). A substrate solution of glucose, acetate and amino acids was infused continuously into the perfusate at a rate appropriate to the amount of milk being secreted. Milk was removed hourly, aided by an intra-arterial injection of oxytocin (40milliunits). Simultaneous arterial and venous blood samples were taken after 3–4hr. of perfusion and at hourly intervals thereafter. Blood flow was recorded every 10min.

In the experiment with acetate, both glands of one animal were perfused separately. Each gland received substrates containing [1-^{14}C]acetate in similar amounts but one gland received in addition goat chylomicra as a source of triglyceride (49ml., 3·1g. of fat infused in 9hr.). The chyle was collected 2 days before the experiment from a young goat with a cannula inserted in the main intestinal lymph duct by the method of Lascelles & Morris (1961).

The infusion of radioactive materials was started 2hr. after the start of the perfusion, and continued for the entire experiment with acetate, but only for 7 and 4·5hr. with stearate and β-hydroxybutyrate respectively. Stearate was bound to goat albumin as described by Annison *et al.* (1967).

Chemical methods. Analyses of glucose and acetate in the blood and artificial kidney fluid and of fat and lactose in the milk were carried out as reported by Hardwick & Linzell (1960). Analyses of plasma and milk fatty acids and of the concentration and specific radioactivity of blood CO_2 were carried out as described by Annison *et al.* (1967), except for the analyses on milk fats obtained during the [^{14}C]stearate and D(−)-β-hydroxy[^{14}C]butyrate infusions. Complete fatty acid analyses on these samples were made by temperature-programmed gas–liquid chromatography by using the method of DeMan (1964).

Isolation and degradation of short-chain acids. Isolated milk fat was saponified with 5N-KOH, and the short-chain fatty acid fraction was separated by steam-distillation. Butyric acid was isolated by column chromatography on silica gel (Moyle, Baldwin & Scarisbrick, 1948) and degraded stepwise by the method of Phares (1951), modified as reported by Leng & Annison (1963). Isolated $BaCO_3$ was assayed for specific radioactivity by suspending known weights of finely ground material in toluene containing low-density silica (38g./l.) and 2,5-diphenyloxazole (4g./l.) and the radioactivity measured in a liquid-scintillation counter (Packard Instruments Ltd. 4000 Series Tri-Carb).

Radioactive materials. [U-^{14}C]Stearate and [1-^{14}C]acetate were obtained from The Radiochemical Centre, Amersham, Bucks. D(−)-β-Hydroxy[1-^{14}C]butyrate was prepared by incubating [1-^{14}C]butyrate with sheep-liver slices, as described by Leng & Annison (1964): 82% of the radioactivity was in C-1 and 18% in C-3.

Calculations. (1) The hourly yield of fat secreted during perfusion is expressed as a percentage of the mean hourly yield before the experiment, determined by fat analysis of the total yield of milk for 3 days (hourly yield=daily yield/24).

(2) The R.Q. was calculated from the arteriovenous differences of O_2 and CO_2 in samples of arterial and venous blood taken simultaneously.

RESULTS

[1-^{14}C]*Acetate perfusion.* Two glands from one goat were perfused in separate systems for 15hr. Both glands received similar amounts of glucose, amino acids and acetate from the end of the second hour, together with [1-^{14}C]acetate at 0·6μc/min.; the left gland received in addition chylomicron lipid (3·1g. in 9hr.).

The quantities of milk fat obtained each hour, expressed as a percentage of the mean hourly yield for 3 days before the experiment, are shown in Fig. 1,

together with the time-course of the passage of radioactivity into the milk fat.

As in two previous perfusions (Lascelles *et al.* 1964) the differences in the amounts of milk fat secreted by the two glands cannot be confidently ascribed to the presence or absence of chylomicron lipid in the substrate mixture because such differences can occur in two glands of one animal treated identically (Hardwick & Linzell, 1960; Hardwick *et al.* 1961; J. L. Linzell, unpublished work). It is well known that after normal milking a small amount of milk, very rich in fat, is left in the udder. In perfusion experiments attempts are made to remove this residual milk fat by thorough milking with the aid of oxytocin at the start of perfusion, but it may be several hours before all this preformed milk fat is entirely cleared and variability in its removal makes it difficult to assess the true rate of fat secretion early in perfusion (e.g. see Fig. 1).

Other results were very similar for the two glands. The concentration of total acetate in the perfusates of both glands fluctuated over the range 0·7–1·4 m-moles/l. and there was a substantial uptake of acetate by both glands (7g. in 15hr., 1·6mg./100g. of tissue/min.), which was 70% of the mean value recorded by Linzell (1960) in conscious lactating goats. Throughout both experiments the R.Q. remained above 1 (mean ± S.E.M. 1·81 ± 0·37), which is characteristic of mammary glands actively synthesizing fatty acids (Folley, 1949). At the end of the perfusions there was still a substantial amount of total lipid in both glands, 3·1g./100g. in the gland that received chyle and 2·8g./100g. in the gland that did not, which supports the suggestion of Hardwick *et al.* (1963*a*) that mammary tissue has a large pool of fatty acids. The fatty acid composition and specific radioactivity of the tissue lipid were very similar to the milk fat, so that the tissue pool could have acted as an endogenous supply of substrate.

As in two similar perfusions reported by Hardwick, Linzell & Mepham (1963*b*), the magnitude of transfer of radioactivity from acetate to milk fat was considerable. The specific radioactivity of the milk fat produced by both glands rose steadily to a maximum after about 12hr. of perfusion, a value that was 64% of that of the acetate in the substrates, and between the tenth and eleventh hours of perfusion the total radioactivity recovered in the milk fat was 54% of the radioactivity being infused each hour (Fig. 1). The labelling of individual higher fatty acids (Table 1) was in agreement with the results obtained in the whole animal (Popják, French, Hunter & Martin, 1951; Annison *et al.* 1967). In spite of the high radioactivity of the infused acetate, there was negligible incorporation of radioactivity into fatty acids of chain length C_{17} and higher. In the final stages of the infusion the

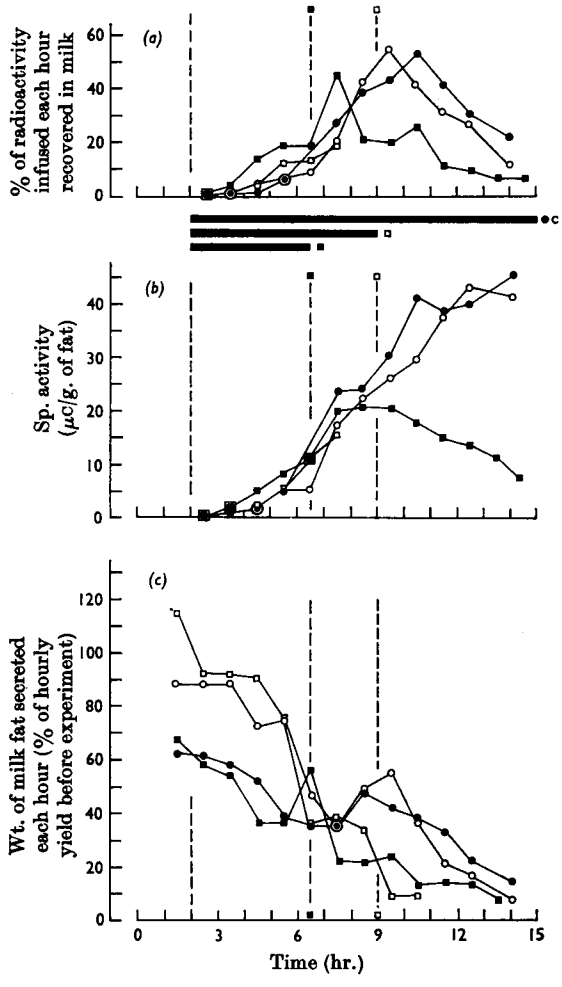

Fig. 1. Time-course of milk-fat secretion and incorporation of radioactivity into milk fat during perfusion of four goat mammary glands. A substrate mixture of glucose, acetate and amino acids was infused continuously. After 2hr. the infusion of labelled substrate was started. One gland received D(−)-β-hydroxy[1-^{14}C]butyrate (■) for 4·5hr., another [U-^{14}C]stearate (□) for 7hr. and the two glands of a third goat [1-^{14}C]acetate for 13hr. with (●) or without (○) chylomicra in the substrate mixture.

specific radioactivities of laurate, myristate and palmitate were roughly similar (Table 1).

The changes in fatty acid composition and changes in the quantities of the major components secreted in the milk each hour are shown for the gland receiving chyle in Table 1 and Fig. 2. The fatty acid composition of the milk of the gland not receiving chyle was very similar. The secretion of stearate was not as well maintained as that of the other major fatty acids, and at the end of perfusion

Table 1. *Composition and specific radioactivities of the higher fatty acids of milk secreted during the perfusion of an isolated mammary gland with* [1-^{14}C]*acetate*

The substrate mixture contained [1-^{14}C]acetate (67μc/g.) infused at about 0·5g./hr. and chylomicron lipid at 0·5g./hr. falling to 0·25g./hr. The fatty acid nomenclature in this and subsequent Tables is that of Farkquhar, Insull, Rosen, Stoffel & Ahrens (1959).

	Sample (time of perfusion)									
	3hr.		6hr.		8hr.		12hr.		Tissue lipid	
Fatty acid	Composition (%)	Sp. activity (μc/g.)	Composition (%)	Sp. activity (μc/g.)	Composition (%)	Sp. activity (μc/g.)	Composition (%)	Sp. activity (μc/g.)	Composition (%)	Sp. activity (μc/g.)
12:0	3·7	0·7	3·5	53·0	5·2	27·0	8·2	35·2	2·9	33·2
12:br	—	—	—	—	1·9	—	4·0	—	0·6	—
14:1	—	—	—	—	0·2	—	0·4	—	0·2	—
14:0	10·2	0·3	11·0	24·8	12·7	30·4	8·7	36·9	9·5	39·2
14:br	0·7	—	0·6	—	1·2	—	0·8	—	—	—
15:0	0·8	—	0·7	—	0·5	—	0·8	—	0·4	—
16:0	28·8	0·1	28·8	30·2	29·7	29·4	34·6	34·1	33·5	28·8
16:1	1·9	—	1·9	5·4	1·3	5·4	2·5	—	1·6	—
16:br	0·7	—	1·4	—	3·5	—	0·4	—	1·0	—
17:0	0·5	—	0·4	—	0·5	—	—	—	0·5	—
18:0	16·4	—	12·4	—	10·4	0·2	10·2	—	12·1	1·0
18:1	34·1	—	36·7	—	27·3	0·7	29·4	—	35·5	0·1
18:2	2·3	—	2·7	—	1·2	—	—	—	1·7	—

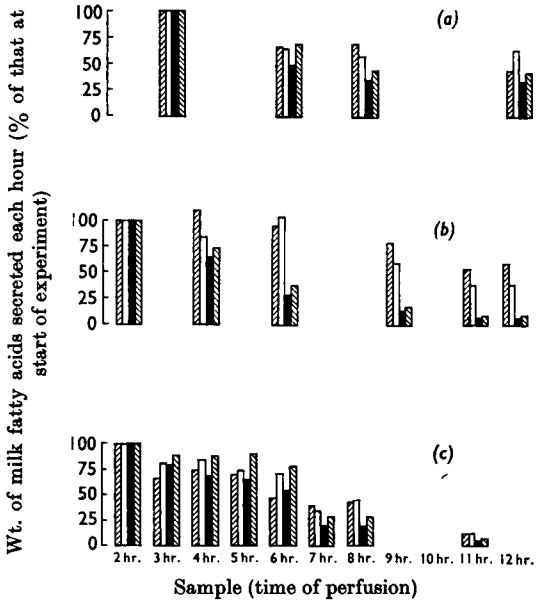

Fig. 2. Quantities of milk fatty acids secreted each hour in three perfusions, expressed as percentages of the quantities formed at the start of perfusion, i.e. after residual fat was eliminated. (*a*) Perfusion with [1-^{14}C]acetate, with the gland being given chylomicron lipid. (*b*) and (*c*), Perfusion of two glands given acetate but no source of long-chain fatty acids: (*b*) with carrier-free D(−)-β-hydroxy[1-^{14}C]butyrate; (*c*) with [U-^{14}C]stearate. ■, Myristate; □, palmitate; ■, stearate; ■, oleate.

Table 2. *Fatty acid composition and specific radioactivities of plasma free fatty acids in perfusate arriving at* (A) *and leaving* (V) *the perfused mammary gland after 6·5hr. of* [*U*-^{14}C]*stearate infusion*

	Composition (%)		Sp. activity (μc/g.)	
Fatty acid	A	V	A	V
12:0	2·2	6·1	—	—
14:0	5·7	7·0	—	—
15:0	1·7	1·0	—	—
16:0	31·6	34·7	—	—
16:1	10·4	7·4	—	—
17:0	3·8	2·9	—	—
18:0	17·1	14·0	2795	1145
18:1	25·1	24·9	263	209
18:2	2·8	1·7	—	—

the secretion of palmitate had fallen much less than that of myristate, stearate and oleate.

[*U*-^{14}C]*Stearate perfusion.* The labelled fatty acid was bound to goat albumin as described by Annison *et al.* (1967) and infused without added carrier into the perfusate at 0·5μc/min. for 7hr. from the second hour after the start of the perfusion, in an experiment lasting 13hr. Chylomicron fat was not included in the substrates. Plasma FFA concentrations remained low, but constant (0·15–0·16m-mole/l.), throughout the perfusion. There was a very small arteriovenous difference of FFA

Table 3. *Fatty acid composition and specific radioactivities of milk fat secreted by an isolated perfused goat mammary gland during the infusion of [U-^{14}C]stearate (32 μc/hr.) for 7 hr. from the start of the second hour*

	Sample (time of perfusion)												
	2 hr.	3 hr.	4 hr.		5 hr.		6 hr.		7 hr.		8 hr.		11 hr.
Fatty acid	Composition (%)	Composition (%)	Composition (%)	Sp. activity (μc/g.)	Composition (%)	Sp. activity (μc/g.)	Composition (%)	Sp. activity (μc/g.)	Composition (%)	Sp. activity (μc/g.)	Composition (%)	Sp. activity (μc/g.)	Composition (%)
4:0	1·8	1·6	1·6	—	1·7	—	1·5	—	1·4	—	0·9	—	0·9
6:0	2·1	2·2	1·9	—	1·9	—	2·0	—	2·2	—	2·0	—	1·8
8:0	2·5	2·5	2·5	—	2·3	—	2·3	—	2·5	—	2·5	—	2·7
10:0	8·6	7·1	7·5	—	8·0	—	8·0	—	9·8	—	8·7	—	9·6
?	0·2	0·2	0·2	—	0·3	—	0·3	—	—	—	—	—	—
12:0	3·7	3·5	3·6	—	3·8	—	4·3	—	5·2	—	5·0	—	5·6
14:0	9·0	7·5	8·4	—	8·1	—	6·5	—	11·5	—	12·5	—	14·2
14:br	0·5	0·5	0·4	—	0·4	—	0·5	—	0·5	—	—	—	—
15:0	0·6	0·5	0·5	—	0·5	—	0·6	—	0·5	—	—	—	—
15:br/1	0·5	0·8	0·7	—	0·8	—	0·7	—	0·7	—	0·7	—	0·8
15:br/2	0·3	0·5	0·4	—	0·2	—	0·3	—	0·3	—	—	—	0·3
16:0	22·1	22·7	23·5	—	21·3	—	24·1	—	24·6	—	30·9	—	33·6
16:1	1·8	1·8	1·9	—	2·1	—	1·8	—	1·9	—	2·1	—	2·0
16:br	0·9	0·8	0·8	—	0·8	—	0·9	—	0·8	—	0·9	—	0·8
17:0	0·8	0·7	0·7	—	0·7	—	0·6	—	0·6	—	0·5	—	0·5
?	0·5	0·4	0·5	—	0·4	—	0·4	—	0·4	—	0·4	—	0·4
18:0	13·0	12·8	11·2	3·8	10·9	6·4	10·9	17·6	8·5	105	7·5	126	4·9
18:1	26·3	29·4	29·2	1·3	30·9	4·3	31·6	14·6	25·4	41·5	24·2	67	20·3
18:2	2·7	2·6	2·7	—	3·0	—	3·0	—	2·6	—	3·0	—	1·6
>18:0	2·1	1·9	1·8	—	1·9	—	1·5	—	0·6	—	—	—	—

Table 4. *Fatty acid composition and specific radioactivities of milk fat secreted by the isolated perfused goat mammary gland during the infusion of* D(−)-*β-hydroxy*[1-^{14}C]*butyrate* (20 μc/*hr.*) *for* 4·5 *hr. from the start of the second hour*

	Sample (time of perfusion)										
	2 hr.		4 hr.		6 hr.		9 hr.		11 hr.	12 hr.	
Fatty acid	Composition (%)	Sp. activity (μc/g.)	Composition (%)	Sp. activity (μc/g.)	Composition (%)	Sp. activity (μc/g.)	Composition (%)	Sp. activity (μc/g.)	Composition (%)	Composition (%)	Sp. activity (μc/g.)
4:0	1·8	—	1·9	—	1·7	61·6	1·4	—	1·3	1·2	—
6:0	1·9	—	2·1	—	2·2	51·7	1·8	—	1·9	1·5	—
8:0	2·3	—	2·5	—	2·7	65·6	2·3	—	2·1	1·9	—
10:0	5·2	13·6	7·3	34·7	9·6	55·8	9·8	42·1	9·7	9·7	31·8
?	0·1	—	0·1	—	0·1	—	0·1	—	0·1	0·2	—
12:0	2·2	9·9	3·1	40·9	4·3	64·8	5·8	51·4	5·9	6·4	13·6
14:0	4·3	11·3	5·6	33·5	7·7	51·4	10·6	32·7	12·0	12·5	15·6
14:br	0·2	—	0·3	—	0·2	—	0·1	—	0·1	0·4	—
15:0	0·4	—	0·4	—	0·3	—	0·2	—	0·2	0·4	—
15:br/1	0·6	—	0·5	—	0·4	—	0·3	—	0·2	0·5	—
15:br/2	0·3	—	0·2	—	0·2	—	0·2	—	0·2	0·2	—
16:0	18·1	2·6	19·4	10·0	28·3	35·2	33·9	27·0	36·1	37·5	16·4
16:1	2·7	—	2·1	—	1·8	—	2·1	—	1·9	1·5	—
16:br	1·0	—	0·9	—	0·6	—	0·6	—	0·7	0·7	—
17:0	0·9	—	0·7	—	0·5	—	0·5	—	0·4	0·4	—
?	0·7	—	0·6	—	0·4	—	0·4	—	0·3	0·3	—
18:0	15·0	—	12·0	—	7·9	—	6·5	—	5·5	4·8	—
18:1	37·2	—	34·8	—	26·6	—	20·1	—	17·8	17·4	—
18:2	3·9	—	4·0	—	3·0	—	2·9	—	3·1	1·6	—
>18:0	1·2	—	1·5	—	1·5	—	0·4	—	0·5	0·9	—

across the mammary gland, and the composition of the FFA fraction showed only slight changes (Table 2). As in the whole animal, however, there was a marked decline in the specific radioactivity of stearate across the gland, indicating substantial uptake of FFA accompanied by FFA production by the gland. The uptake of FFA was confirmed by the extensive transfer of radioactivity into milk fat (Fig. 1), radioactivity being found in C_{18} fatty acids only.

Comparison of the specific radioactivities of arterial and venous blood carbon dioxide showed that the carbon dioxide produced by the gland (0·47 μc/g. of C) was very low relative to that of circulating [U-^{14}C]stearate (Table 2), indicating that this acid made only a small contribution to oxidative metabolism in the isolated perfused mammary gland. The R.Q. was 1·20 ± 0·18.

Labelled oleate appeared both in plasma FFA and in milk fat (Tables 2 and 3). Some radioactivity appeared in arterial and venous triglycerides, but the concentrations of this fraction were variable, and in some cases venous concentrations were higher than arterial, suggesting triglyceride production by the gland. There was negligible labelling of phospholipids, but the sterol ester fraction showed slight radioactivity (3 μc/g.).

The amount of milk fat obtained each hour was high in the first 6hr. of the perfusion, but then declined to about 50% of the pre-perfusion level during the last 3hr. Milk fatty acid composition showed marked changes (Table 3 and Fig. 2). There was a steady fall in the secretion of stearate and a fall in oleate after 6hr., so that after the seventh hour the proportions of myristate and palmitate in the total milk fatty acids had appreciably increased. At the end of perfusion the tissue contained 1·73g. of lipid/100g. The milk fat of highest specific radioactivity was fractionated to isolate the small amount of FFA. On analysis this was shown to be similar in composition and radioactivity to the fatty acids of the triglyceride fraction.

D(−)-*β-Hydroxy*[1-^{14}C]*butyrate perfusion.* The labelled material, essentially carrier-free, was infused into the perfusate at 0·36 μc/min. for 4·5hr. of a perfusion lasting 12·5hr. Blood concentrations remained at 0·6–0·8mg./100ml. throughout the perfusion, and arteriovenous differences were small and variable. Substantial uptake of β-hydroxybutyrate was shown by the fall in specific radioactivity across the mammary gland (660 to 544 μc/g. of C), and the extensive transfer of radioactivity into milk (Fig. 1). A comparison of the specific radioactivity of the carbon dioxide produced by the gland (from arterial and venous values) showed that only 1·4% of the carbon dioxide originated from the circulating β-hydroxybutyrate. The R.Q. was 2·67 ± 0·57.

During the perfusion the hourly milk yield was 50–60% of the mean hourly yield on the goat 3 days before the experiment, but the fat content fell sharply (Fig. 1). This was accompanied by a steady fall in the proportions of stearate and oleate, and compensatory increases in the proportions of palmitate, and acids of chain length C_{14}, C_{12} and C_{10}, but not of chain length C_8, C_6 and C_4 (Table 4 and Fig. 2). At the end of perfusion the tissue contained only 0·45g. of lipid/100g. The specific radioactivity of milk fat reached a maximum after 6–7hr. (Fig. 1), and the specific radioactivities of the short-chain and long-chain fatty acids of this sample were determined. Roughly similar values were obtained for each acid in the series butyrate to myristate, with palmitate about 30% lower. The acids of carbon chain length C_4, C_8 and C_{12} were somewhat higher in specific radioactivity than the C_6, C_{10} and C_{14} acids, which might suggest that β-hydroxybutyrate was incorporated into milk fatty acids to some extent as a C_4 unit. However, the distribution of radioactivity in the carbon chain of milk butyrate was 69% in C-1 and 31% in C-3, which must be compared with that of the labelled precursor, D(−)-β-hydroxybutyrate (82% in C-1 and 18% in C-3). These results are consistent with 41% cleavage of β-hydroxybutyrate to the C_2 units before conversion into butyrate.

DISCUSSION

The present results show that the isolated perfused mammary gland can give valuable data on milk-fat synthesis, in spite of the fact that it takes several milkings to remove completely preformed milk fat and it is not as immediately responsive to variations in the availability of lipid precursors as it is to precursors of lactose and protein.

The pattern of incorporation of labelled acetate and stearate into milk fat by the isolated perfused mammary gland was closely similar to that occurring *in vivo*, but the specific radioactivity of these materials in the perfusate was roughly tenfold greater than that achieved in the whole animal (Annison *et al.* 1967). This gave rise to highly labelled milk fat, and examination of the constituent fatty acids has confirmed conclusions reached in earlier work on the role of acetate and stearate in milk-fat synthesis. Acetate was extensively used for the synthesis of fatty acids of chain length C_4 to C_{14}, and contributed to a smaller extent to palmitate production. Negligible synthesis of acids of greater chain length was observed from acetate. Stearate was readily taken up by the mammary gland, transferred to milk fatty acids and gave rise to large amounts of oleate, some of which appeared in the milk fatty acids and in the blood leaving the gland in the plasma FFA.

The isotopic data support the hypothesis that, in ruminants, milk short-chain fatty acids are derived from plasma acetate and β-hydroxybutyrate and the long-chain ones from plasma long-chain fatty acids. The present experiments add more weight by showing that these processes are to some extent independent. In two of the four experiments the quantity of milk fat secreted each hour was well maintained for 12hr. and in these the milk fatty acid composition showed little change. The other two glands were secreting less than half the quantity of milk fat late in perfusion, and in these experiments, in which lipid was not included in the substrate mixture, there was a steady decline to very low rates in the hourly secretion of stearate and oleate in milk fat, whereas the secretion of myristate and palmitate (largely formed from acetate, which was supplied continuously) was well maintained for 6hr. and then declined more slowly than that of stearate and oleate (Fig. 2). In these experiments less fat was present in the tissue at the end of perfusion, and this confirms that lactating mammary glands have a large pool of long-chain fatty acids (Hardwick *et al.* 1963*a*), which can act as an endogenous source for the synthesis of milk fat, in those experiments where lipid is not included in the substrate. It is noteworthy that the fatty acid composition of mammary-tissue lipid is remarkably similar to that of milk fat (Table 1).

The magnitude of the mammary-gland fatty acid pool is probably responsible for the slow transit of radioactivity into milk fat, as it is *in vivo* (Hardwick *et al.* 1963*a*). Evidence for this is that the residual tissue lipid is low in those experiments where there is a marked fall in milk-fat secretion late in perfusion, suggesting that the tissue fatty acid pool was being used as a source of endogenous substrates. It is significant that, in such cases, the proportion of stearate and oleate in the milk fatty acids falls markedly, because these are the milk fatty acids that are believed to be incorporated from plasma long-chain precursors and the isotopic evidence is clear that mammary tissue cannot form these fatty acids from acetate.

The metabolism of β-hydroxybutyrate has not been previously studied with isotopes in the goat and in only one other study has the naturally occurring isomer been used in a lactating animal (Palmquist *et al.* 1966). In our experiment negligible amounts of the D(−)-β-hydroxy[1-^{14}C]butyrate were oxidized, but in spite of low circulating plasma concentrations, which were only 10–20% of that in the normal animal (Barry *et al.* 1963), a large proportion of the radioactivity infused was transferred to milk fat. The pattern of labelling in the milk fatty acids was very similar to that with acetate, but suggested that incorporation proceeds by at least two routes. The distribution of radioactivity in butyrate isolated from milk fat indicated about 40% cleavage to C_2 units, in contrast with the findings of Kumar *et al.* (1965) that conversion of β-hydroxybutyrate into butyrate proceeds without cleavage. The specific radioactivities of the lower fatty acids also suggested that some cleavage of β-hydroxybutyrate must have occurred in the perfused gland (Table 4), but the slightly higher specific radioactivity of C_4, C_8 and C_{12} fatty acids than of C_6, C_{10} and C_{14} fatty acids might suggest some incorporation of C_4 units. The lactating mammary gland is not usually regarded as a site of ketone-body production, since there is normally a positive arteriovenous difference of β-hydroxybutyrate and none of acetoacetate (Barry *et al.* 1963), but the relatively unchanged concentration of β-hydroxybutyrate in the perfusate indicated continuous production in our experiment. Kronfeld & Kleiber (1959) have suggested and Kronfeld, Raggi & Ramberg (1966) demonstrated that acetoacetate is produced by the udder of the ketotic cow *in vivo*.

The present experiments clearly demonstrate the extensive ability of the goat mammary gland to form oleate from stearate. At maximum the specific radioactivity of the milk oleate was above half that of the milk stearate and in all experiments the fall in oleate secretion was less than the fall in stearate secretion.

We are grateful to Mr I. R. Fleet, Mr R. D. Burton and Miss A. Boyd for skilful technical assistance.

REFERENCES

Annison, E. F. & Linzell, J. L. (1964). *J. Physiol.* **175**, 372.
Annison, E. F., Linzell, J. L., Fazakerley, S. & Nichols, B. W. (1967). *Biochem. J.* **102**, 637.
Barry, J. M., Bartley, W., Linzell, J. L. & Robinson, D. S. (1963). *Biochem. J.* **89**, 6.
DeMan, J. M. (1964). *J. Dairy Sci.* **47**, 546.
Farkquhar, J. W., Insull, W., Rosen, P., Stoffel, W. & Ahrens, E. H. (1959). *Nutr. Rev.* **17** (Suppl.), 18.
Folley, S. J. (1949). *Biol. Rev.* **24**, 316.
Hardwick, D. C. & Linzell, J. L. (1960). *J. Physiol.* **154**, 547.
Hardwick, D. C., Linzell, J. L. & Mepham, T. B. (1963*a*). *Biochem. J.* **87**, 4P.
Hardwick, D. C., Linzell, J. L. & Mepham, T. B. (1963*b*). *Biochem. J.* **88**, 213.
Hardwick, D. C., Linzell, J. L. & Price, S. M. (1961). *Biochem. J.* **80**, 37.
Kronfeld, D. S. & Kleiber, M. (1959). *J. appl. Physiol.* **14**, 1033.
Kronfeld, D. S., Raggi, F. & Ramberg, C. F. (1966). *J. Dairy Sci.* **49**, 733.
Kumar, S., Lakshmanan, S. & Shaw, J. C. (1959). *J. biol. Chem.* **234**, 754.
Kumar, S., Singh, V. N. & Keren-Paz, R. (1965). *Biochim. biophys. Acta*, **98**, 221.
Lascelles, A. K., Hardwick, D. C., Linzell, J. L. & Mepham, T. B. (1964). *Biochem. J.* **92**, 36.
Lascelles, A. K. & Morris, B. (1961). *Quart. J. exp. Physiol.* **46**, 199.

Lauryssens, M., Verbeke, R., Peeters, G. & Reinards, M. T. (1960). *J. Dairy Res.* **27**, 151.
Leng, R. A. & Annison, E. F. (1963). *Biochem. J.* **86**, 319.
Leng, R. A. & Annison, E. F. (1964). *Biochem. J.* **90**, 464.
Linzell, J. L. (1960). *J. Physiol.* **153**, 492.
Luick, J. R. & Kameoka, K. K. (1966). *J. Dairy Sci.* **49**, 98.
Moyle, V., Baldwin, E. & Scarisbrick, R. (1948). *Biochem. J.* **43**, 308.
Palmquist, D. L., Davis, C. L. & Brown, R. E. (1966). *J. Dairy Sci.* **49**, 738.
Phares, E. F. (1951). *Arch. Biochem. Biophys.* **33**, 173.
Popják, G., French, T. H. & Folley, S. J. (1951). *Biochem. J.* **48**, 411.
Popják, G., French, T. H., Hunter, G. D. & Martin, A. J. P. (1951). *Biochem. J.* **48**, 612.
Shaw, J. C. & Knodt, C. B. (1941). *J. biol. Chem.* **138**, 287.