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MAMMARY AND WHOLE ANIMAL METABOLISM OF GLUCOSE AND FATTY ACIDS IN FASTING LACTATING GOATS

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SUMMARY

1. Measurements were made of milk yield, mammary blood flow and mammary arteriovenous differences during the measurement of substrate entry rate by the isotope dilution method using [U-^{14}C]glucose, acetate, palmitate, stearate or oleate in conscious lactating goats after 24 hr starvation.

2. As previously reported, in fasting, milk yield fell to 40 ± 3·4 (S.E.) %, lactose secretion to 31 ± 3·4 %, milk fat secretion to 81 ± 6·7 % and mammary blood flow fell to 53 ± 7·5 % of the values before fasting. Mammary O_2 uptake was only 45 ± 5 % of the mean value in fed animals and there were marked falls in the uptakes of glucose, acetate and triglycerides, a smaller fall in β-hydroxybutyrate uptake, and a large increase in free fatty acid uptake.

3. Glucose was found to enter the circulation of the fasting animal at 1–1·6 mg/min/kg body wt. (entry rate) and it gave rise to 3–5 % of the total CO_2. The udder took up 10·7–16·1 mg/min/kg of tissue and 8–10 % of mammary CO_2 was derived from glucose, although only 5–10 % was oxidized. Mammary uptake accounted for 35–43 % of the total glucose entering the circulation.

4. In the whole animal acetate entry rate was 1–1·4 mg/min/kg and 9–10 % of total CO_2 was derived from it. The udder used 0·8–2·4 mg/min/kg of tissue and 9–13 % of mammary CO_2 was derived from acetate, 46–79 % of that taken up being oxidized. Mammary uptake accounted for only 2–6 % of the total acetate entry rate. Negligible quantities of isotope were found in milk fatty acids and there was a fall in the proportion of milk fatty acids of chain length up to C_{14} which in fed animals are synthesized from acetate and β-hydroxybutyrate.

5. Palmitate, stearate and oleate entered the circulation as free fatty

acids at 0·94–6·8 mg/min/kg and 6–9 % of total CO_2 was derived from each. The udder took up 3·0–5·7 mg/min/kg of tissue and 4–8 % of mammary CO_2 was derived from each acid. In the udder 8 and 5·5 % of stearate and oleate were oxidized and 25 % of palmitate. Mammary uptake of stearate was 31·5 % of the total entry rate, palmitate 1 %, and oleate 7·5 %. Only long chain milk fatty acids were labelled.

6. During fasting the mammary R.Q. was $0{\cdot}85 \pm 0{\cdot}045$ compared with a value in fed animals of $1{\cdot}24 \pm 0{\cdot}02$, when the udder is synthesizing fatty acids from acetate. The total mammary uptake of lipid precursors was only 74 % of the rate of milk fat secretion and there was an 18 % shrinkage in empty udder volume, suggesting the use of endogenous mammary tissue substrates.

INTRODUCTION

In recent years the measurement of the rate of entry of substrates into the circulation by the isotope dilution technique has added considerably to our knowledge of the metabolism of conscious undisturbed animals. Ruminants have been widely studied in this way and, in the lactating goat, more information has been obtained by the simultaneous measurement of the rate of milk secretion, of mammary blood flow and of arteriovenous differences of plasma glucose and fatty acids (Annison & Linzell, 1964; Annison, Linzell, Fazakerley & Nichols, 1967). These studies have provided quantitative data on uptake, release and oxidation of these substrates by the udder in relation to their over-all utilization and oxidation in the whole animal and stress the magnitude of the mammary demand for substrates during lactation.

The same techniques have now been applied to lactating goats starved for 24 hr because marked changes in milk secretion and mammary substrate uptake occur at this time (Linzell, 1967*a*) suggesting that, although milk secretion continues at a reduced rate, mammary and general metabolism must be considerably altered.

METHODS

Experimental animals. The surgical and infusion procedures and the measurement of mammary blood flow were as described earlier (Linzell, 1966*a*; Annison, Linzell, Fazakerley & Nichols, 1967) using similar surgically prepared and trained lactating goats. The goats (aged 3–5 yr) were in their 3rd–5th lactations and experiments were carried out in the 8th–36th week of lactation. The animals were given hay *ad libitum*, and about 1 kg/day of concentrate given in two roughly equal parts at the times of milking (09.30 and 16.30 hr). On the day before an experiment food was removed at 09.30 hr, and the infusion started at 10.00–11.00 hr on the following morning. In some experiments the goats were milked hourly until 5 hr after the end of the infusion, an intravenous injection of oxytocin (200 m-u.) being used before each milking to aid milk ejection. The milk yields during the experiment are shown in Table 1.

Blood flow measurements were confined to one gland, and the total flow was calculated from the milk production of each gland assuming proportionality between milk secretion and blood flow (Linzell, 1960).

Radioactive materials [U-^{14}C]stearic, oleic, palmitic and acetic acids, and [U-^{14}C]glucose were obtained from The Radiochemical Centre, Amersham. The long-chain acids were bound to albumin and infused as described earlier (Annison, Linzell, Fazakerley & Nichols 1967), and the infusions of labelled glucose and acetate were carried out as described by Annison & Linzell (1964).

Chemical methods

Blood acetate specific radioactivity. Acetate was isolated from blood by steam distillation as described earlier (Annison & Linzell, 1964) and after titration the dried samples were assayed for radioactivity as described below.

Plasma glucose specific radioactivity. Glucose was isolated from plasma (1–5 ml.) as the penta-acetate (Jones, 1965) after the addition of carrier glucose (100 mg). Plasma glucose concentrations were measured with glucose oxidase (Huggett & Nixon, 1957).

Blood CO_2 specific radioactivity. Blood CO_2 was isolated as $BaCO_3$ (Annison & White, 1961) and assayed for radioactivity as described below.

Extraction and analysis of blood lipids. Blood samples (10 ml.) were immediately cooled in ice and centrifuged (3000 *g*) at 2° C. Lipids were extracted from plasma (5 ml.) by the method of Folch, Lees & Sloane Stanley (1957) using 0·1 M phosphate buffer, pH 6·0, instead of water. Internal standards (heptadecanoic acid and glyceryl tri-heptadecanoate) were added to the chloroform–methanol extract in the proportions of 60 and 20 μg/ml. plasma respectively. The lipids were separated on thin layers of silica gel G (West & Rowbotham, 1967). Portions (one fifth) of the separated triglyceride and free fatty acids (FFA) were transferred to scintillation counter vials and, after the removal of solvent, counted as described below. Methyl esters were prepared from the remainder of this lipid material by transmethylation as described by West & Rowbotham (1967). The proportion of the individual long chain fatty acid methyl esters were determined by gas chromatography as described below.

Extraction and analysis of milk lipids. Milk (3 ml.) was extracted with 60 ml. chloroform-methanol (2:1 v/v) by the method of Folch *et al.* (1957) and dried with anhydrous sodium sulphate. The milk triglyceride was purified by thin layer chromatography (West & Rowbotham, 1967). A portion of the triglyceride was transferred to a scintillation counting vial and after the removal of solvent weighed and assayed for radioactivity as described below. Methyl esters were prepared by transmethylation using a procedure devised to eliminate losses of the short-chain esters. Milk lipid was transferred to thick-walled glass vials, 2 ml. of methanol–benzene-conc. sulphuric acid (20:10:1 v/v/v) added, the vial sealed and then heated at 80° C for 90 min. After cooling to room temperature, all subsequent operations were carried out with all glassware and reagents in an ice-bath. The contents of the vial were transferred to a glass stoppered test-tube using 5 ml. of isopentane (A.R. grade). Saturated sodium carbonate (3 ml.) was added, the tube shaken for 1 min and the lower phase removed by aspiration. The isopentane phase was extracted with a further 5 ml. of saturated sodium carbonate and transferred to a fresh tube.

The analysis of fatty acids. The proportion of individual fatty acid methyl esters was determined using a gas chromatograph, fitted with a flame-ionization detector (Series 104, Model 14, Pye Ltd., Cambridge, U.K.) and a coiled glass column (120 cm long, 4 mm i. d.) packed with Celite (Chromasorb W, 60–80 mesh) coated with a polar stationary phase 10% polyethylene glycol adipate. The plasma lipid fatty acid methyl esters were separated at 167° C and those from milk lipids using temperature programming. (The temperature was kept at 70° C until methyl octanoate emerged (about 10 min) and then raised by 3–4° C/min to 180° C.) The areas under the peaks were calculated by triangulation and those corresponding to acids up to C_8 corrected for the detector response which had been determined using standard mixtures. The values, thus determined, proportional to the weight of

methyl esters, were used to calculate the proportion of fatty acids and the concentration of those from plasma lipids. This operation was carried out by computer as described by West & Rowbotham (1967).

Methyl esters of ^{14}C-labelled long chain fatty acids (chain length greater than C_{10}) were separated by thin layer argentation chromatography (Morris, 1964) into three groups (saturated esters, esters with one double bond, and poly-unsaturated esters) before the determination of the specific radioactivity of the individual fatty acids. The methyl esters were applied to thin layers (400 μ) of silica gel G impregnated with silver nitrate (5 g/100 g) and developed twice with diethyl ether-petroleum ether, b.p. 40–60° C (5:95 v/v). The spots were located by spraying with a solution of 2′,7′-dichlorofluorescein in ethanol (0·2 g/100 ml.) and viewing under U.V. light.

The labelled long chain fatty acids of milk fat were assayed for specific radioactivity by gas radiochromatography (James & Piper, 1963). Methyl esters of fatty acids which had been separated into classes on the basis of their degree of unsaturation were transferred to the gas chromatograph as described by Hitchcock & James (1964). The esters were separated in a 120 cm straight column (internal diameter 4 mm) containing 100–120 mesh Celite (Anakrom) supporting 15% polyethylene glycol adipate at 170° C. The radiochromatograph was standardized daily by running methyl palmitate of known specific radioactivity.

Methyl esters of labelled plasma FFA, after separation according to their degree of unsaturation, were isolated by preparative gas liquid chromatography. The column was packed with Celite (Chromasorb W, 60–80 mesh) coated with a polar stationary phase (15% polyethylene glycol adipate). The esters emerging from the column were trapped in a glass U tube packed with Celite (Chromasorb W, 60–80 mesh) and cooled with solid CO_2-isopropanol. The methyl esters were eluted from the column with 10 ml. of petroleum ether (b.p. 40–60° C). An aliquot of this material was transferred to a scintillation counter vial. The mass of methyl ester was determined spectrophotometrically on a further aliquot of the material using the method of Morgan & Kingsbury (1959).

Assay of D(−)-*β-hydroxybutyrate and acetoacetate.* The method of Williamson, Mellanby & Krebs (1962) was used.

Assay of radioactivity. All substrates were assayed by liquid scintillation counting using a Packard 4000 Series Spectrometer. Correction for quenching was determined using an external standard of ^{226}Ra. Lipids and glucose penta-acetate were assayed in solution in toluene containing 2,4-diphenyl oxazole (PPO) (0·4 g/100 ml.). Water-soluble materials (i.e. acetate) were dissolved in 1 ml. of water and mixed with 15 ml. of a mixture (2:1 v/v) of toluene containing PPO (0·4 g/100 ml.) and Triton X-100 (Lennig Chemical Co., Jarrow-on-Tyne), which gave a one phase system suitable for counting. Finely divided $BaCO_3$ (10–50 mg) was assayed in suspension in 15 ml. of a gel of low density silicic acid (Cab-O-Sil, Packard Co.) 3·4 g/100 ml. in toluene containing PPO (0·4 g/100 ml.).

Blood gases. Blood O_2 and CO_2 were measured on the same blood sample (1 ml.) by the procedure of Peters & Van Slyke (1932).

Measurement of udder weight. Udder volume was measured by displacement of water as described by Linzell (1966*b*), and converted to weight using a mean sp. gr. of udders of 1·035.

RESULTS

Mammary metabolism and secretion

As reported by Linzell (1967*a*) milk yield fell after 24 hr of fasting to $40 \pm 3·4$ (S.E.) % of the yield immediately before fasting and was maintained at this level for a further 12 hr. The rate of lactose secretion fell to $31 \pm 3·4$ %, fat secretion to $81 \pm 6·7$ % of previous and mammary blood

flow to $53 \pm 7{\cdot}5$ %. Mammary oxygen uptake was $10{\cdot}5 \pm 1{\cdot}24$ (S.E.) ml./min/kg tissue, which is significantly lower than the value for fed goats in full lactation of $23{\cdot}4 \pm 1{\cdot}0$ (Linzell, 1960). The R.Q. was $0{\cdot}85 \pm 0{\cdot}045$ compared with $1{\cdot}24 \pm 0{\cdot}02$ in fed goats of this herd (Linzell, 1960).

In all experiments the blood concentrations of the substrates remained constant and the isotope (virtually carrier free) was infused I.V. for 4 hr. In the 4th hr of the infusion the specific radioactivities of the substrate and the CO_2 were also constant and during this time 3–4 pairs of arterial and mammary venous blood samples were taken and mammary blood flow measured.

Metabolism of acetate. Labelled acetate was infused for 4 hr into two starved (24 hr) goats, and mammary blood flow was measured and simultaneous samples of arterial and venous blood were taken during the final hour of the infusion when blood acetate and CO_2 had attained constant specific radioactivities. As previously reported (Linzell, 1967*a*) arterial acetate concentration had fallen to 17 and 28 % of the mean value in fed goats and the mammary arteriovenous difference to 8 and 13 % respectively. As with fed goats (Annison & Linzell, 1964) the specific radioactivity of acetate was considerably lower in mammary venous blood (30 and 36 % respectively of the arterial blood values) indicating acetate release by mammary tissue. The rate of entry of acetate into the general circulation was only 21 %, the mammary acetate uptake only 6 % and the proportion of CO_2 derived from acetate was 33 % (whole animal) and 42 % (udder) of the corresponding figures for fed animals. The proportion of acetate oxidized by the mammary glands was similar to that in fed animals (Table 1). The uptake of acetate by the udder was only 2 and 6 % of the total entry rate (14–41 % in fed animals).

The incorporation of acetate into milk fat was low, as expected from the low acetate uptakes. As in fed animals examination of the milk fat showed the absence of radioactivity in fatty acids of chain length greater than C_{16} (palmitic acid). The maximum specific radioactivities of the milk fatty acids of chain-length C_4–C_{14}, which in the fed animal are extensively labelled from blood acetate, were only about 1 % of that of blood acetate.

There was no measurable transfer of radioactivity from blood acetate to milk lactose or milk triglyceride glycerol, or to plasma free fatty acids (FFA).

Metabolism of glucose. In two experiments infusions of labelled glucose were carried out on fasting goats, but in only one animal were the arterial glucose concentration and mammary arteriovenous difference less than in fed animals. However, owing to the fall in mammary blood flow, the uptakes of glucose by the udder were 17 and 25 % of the values in fed animals (Annison & Linzell, 1964). Moreover compared with corresponding

values in fed animals in fasting the entry rate of endogenous glucose into the circulation was reduced to 36 %, the amount of CO_2 derived from glucose to 45 % (whole animal) and 23 % (udder) and the amount of glucose oxidized by the udder to 29 % (Table 1). Glucose uptake by the udder accounted for 35 and 43 % of the total glucose entering the circulation (60–85 % in fed animals). Lactose output was 78 and 68 % of the glucose uptake but the maximum specific radioactivity of the lactose was only 30 % of that of the plasma glucose. This may be a minimum figure as milk samples after the end of infusion were not examined. Milk fatty acids were not labelled.

Metabolism of plasma fatty acids. [^{14}C]Palmitic, oleic and stearic acids were infused into three starved (24 hr) goats in separate experiments. In contrast to fed animals (Annison, Linzell, Fazakerley & Nichols 1967) the arterial FFA levels were raised, and there were large mammary arteriovenous differences (Tables 1, 2). The specific radioactivity of each fatty acid infused fell across the mammary gland to 74–78 % of the arterial values indicating release of endogenous FFA, but this fall was less than in fed animals. There was a large transfer of radioactivity into the corresponding milk fatty acids, confirming the uptake of FFA revealed by the positive arteriovenous differences. When [^{14}C]stearic acid was infused, 54 % of the radioactivity transferred into milk was present as oleic acid, reflecting the known ability of the goat mammary gland to desaturate stearic acid (Annison, Linzell, Fazakerley & Nichols 1967). Short chain milk fatty acids (C_4–C_{14}) were not labelled. The rates of entry of plasma palmitate, oleate and stearate into the circulation were 6, 19 and 9 times those in fed animals and both the udder and the whole animal oxidized far more of these substrates than the fed animal (Table 1).

Mammary uptake of triglycerides and ketones. The arterial concentrations and mammary arteriovenous differences of plasma triglycerides (Table 2) were both 30 % of the values reported earlier for fed goats (Annison, Linzell, Fazakerley & Nichols 1967). The specific radioactivity of the plasma triglyceride rose steadily during each labelled fatty acid infusion, the triglyceride fatty acid reaching a value about 2 % of that of the corresponding FFA infused. In all experiments the specific radioactivity of the triglycerides in mammary venous plasma was significantly higher than in the arterial plasma.

In four experiments the mean concentration of D (−)-β-hydroxybutyrate in arterial and mammary venous blood was 4·6 (range 3·6–6·6) and 1·6 (range 1·0–2·6) mg/100 ml. respectively. The mean net arteriovenous difference (3·0 mg/100 ml.) was greater than that of acetate, but the possible production of β-hydroxybutyrate by the mammary gland must be borne in mind. These figures are both 80 % of the values in fed animals

(Barry, Bartley, Linzell & Robinson, 1963). In three of the four experiments, levels of acetoacetate in arterial and mammary venous blood were less than 0·2 mg/100 ml., but in one experiment there was some production of acetoacetate by the udder, the arterial and mammary venous blood containing 0·26 and 0·44 mg/100 ml. respectively.

TABLE 1. Substrate metabolism by the whole animal and by the udder in lactating goats starved 24 hr

Experiment number	4	11	5	10	12	1	13
Radioactive substrate infused (carrier free)	Glucose	Glucose	Acetate	Acetate	Palmitate	Stearate	Oleate
Week of lactation	8	28	12	33	33	29	36
Whole animal							
Body weight (kg)	58·5	67·0	70·0	80·0	57·0	64·0	64·0
Arterial concentration of substrate (mg carbon/100 ml. of blood)	17·6	14·9	0·89	0·53	2·16	4·46	4·77
Entry rate of unlabelled substrate into circulation							
(mg/min/kg)	1·59	1·01	1·37	1·0	6·8	0·94	3·2
(mg/min/$kg^{0.75}$)	4·4	2·9	4·0	3·7	18·7	2·7	9·1
Total CO_2 derived from substrate (%)	3·0	5·0	10·0	9·0	8·0	6·0	9·0
Udder							
Milk fat secretion							
(mg/min)	84·5	38·1	69·5	34·6	47·4	31·8	48·4
(% fed value)	89·0	63·0	110·0	65·0	96·0	68·0	75·0
Milk lactose secretion							
(mg/min)	27·5	20·8	45·1	17·9	22·5	13·7	26·3
(% fed value)	25·0	26·0	44·0	32·0	—	29·0	—
Blood flow							
(ml./min/kg tissue)	13·4	19·8	13·7	8·8	18·6	18·4	23·4
(% fed value)	37·0	62·0	48·0	36·0	90·0	57·0	53·0
Arteriovenous difference of substrate (mg carbon/100 ml. of blood)	4·5	2·2	0·38	0·17	0·86	1·52	2·41
Uptake of substrate (mg/min/kg tissue)	16·1	10·7	2·4	0·81	3·0	5·7	4·3
Substrate oxidized (%)	5·0	10·0	46·0	79·0	25·0	5·5	8·0
O_2 uptake, (ml./min/kg tissue)	10·6	15·6	7·3	5·7	11·1	12·6	11·2
R.Q.	0·72	0·88	0·82	1·07	0·72	0·82	0·92
CO_2 derived from substrate (%)	10·0	8·0	13·0	9·0	8·0	4·0	5·5
Mammary substrate uptake as a % of total entry rate	43·0	35·0	6·0	2·0	1·0	31·5	7·5

Quantitative relationships in mammary gland fat metabolism. The contribution of plasma FFA, plasma triglyceride, blood acetate and blood β-hydroxybutyrate to milk fat production was calculated from the arteriovenous difference, change in specific radioactivity across the glands, substrate oxidation and mammary blood flow data. Since plasma triglyceride is known to be the main source of the FFA released by the gland (West, Annison & Linzell, 1967), the net uptakes of triglycerides and FFA were used. The assumption was made that the fatty acids of the triglycerides taken up by the gland were oxidized to the same extent as plasma FFA

(25 %). In the absence of data on the possible production of β-hydroxybutyrate net uptakes were used with corrections for oxidation which assumed that the extent of oxidation of this substrate was the same as for acetate. The carbon balance sheet (Table 3) indicates that only about 74 % of milk fat production can be accounted for by the uptake of blood FFA, triglyceride, acetate and β-hydroxybutyrate. Linzell (1967*a*) has observed that mammary gland uptake of plasma amino-acids in starved

TABLE 2. Concentration and molar proportions of the fatty acids of the free fatty acid (FFA) and triglyceride (TG) fractions of arterial (A) and mammary venous (MV) plasma in fasting lactating goats.

	Molar proportions (%)											
Expt.	12				13				1			
	FFA		TG		FFA		TG		FFA		TG	
Acid (*a*)	A	MV	A	MV	A	MV	A	MV	A	MV	A	MV
12:0	0·2	0·9	—	—	0·2	0·2	0·3	0·6	1·2	1·8	1·3	0·6
14:0	1·7	2·0	0·9	0·9	1·5	1·8	2·4	2·8	4·5	5·5	3·4	2·5
14:1	0·8	0·7	0·3	0·5	0·5	0·4	0·6	0·5	0·8	0·7	2·6	2·2
15:0	1·7	2·1	0·4	1·4	0·8	1·0	1·9	1·9	1·1	1·9	2·0	1·8
15:1	0·2	0·1	—	0·9	0·2	0·2	0·1	0·1	0·2	0·6	1·4	0·5
16:0	23·4	23·9	28·7	22·9	20·0	21·1	31·9	32·8	22·4	22·4	28·2	30·0
16:1	3·3	4·0	4·7	3·8	2·0	2·4	5·6	5·6	2·4	3·2	5·9	4·7
16:br	1·5	1·3	1·9	1·3	1·2	1·0	1·1	1·1	1·4	1·0	0·8	1·7
17 (*b*)	0·5	0·5	0·5	1·5	0·4	0·7	0·1	0·3	2·8	4·0	2·8	1·3
18:0	34·8	34·3	30·0	41·6	33·1	36·3	19·5	20·8	28·0	27·5	22·6	23·5
18:1	28·8	26·8	28·9	20·3	36·2	30·9	30·7	28·1	28·2	27·3	21·4	25·0
18:2	1·6	1·8	3·0	2·4	2·8	2·9	5·3	4·9	3·6	3·7	3·6	4·2
18:3	0·9	0·5	0·5	0·6	0·8	0·7	0·4	0·4	0·9	0·3	0·3	0·5
18:br	0·2	0·1	0·2	1·9	0·3	0·4	0·1	0·1	0·2	0·1	1·3	1·1
20 (*c*)	—	—	—	—	—	—	—	—	—	—	1·2	0·4
Other	0·4	1·0	—	—	—	—	—	—	2·3	—	0·2	—
Concn. (m-equiv/l.)	0·715	0·430	0·246	0·123	0·845	0·492	0·213	0·140	0·970	0·650	0·288	0·172

(*a*) Notation after Farquhar, Insul, Rosen, Stoffel and Ahrens (1959), denoting the number of C atoms and double bonds in the molecule. br = branched chains.

(*b*) Includes isomers of C_{17} and C_{18} other than 17:0.

(*c*) Includes saturated, unsaturated and branched chain isomers of C_{20}.

(24 hr) goats falls to 15–20 % of the normal values, suggesting that the contribution of ketogenic amino acids to milk fat synthesis is unlikely to be significant. The failure to account for more than 74 % of milk fatty acid carbon suggests that milk fat precursors stored in the secretory cells may contribute to milk fat production in early starvation. An observed decrease in the volume of the empty udder during starvation of 17 and 19 % in two goats (Table 4) lends support to this hypothesis.

There are two alternative methods of relating the uptake of fat precursors to milk fat production. Since stearic and oleic acids were not synthesized from shorter chain fatty acids in the mammary gland (see also Annison, Linzell, Fazakerley & Nichols, 1967) the direct comparison

of their uptake (corrected for oxidation in the gland) from blood with output in milk is valid. In three experiments, 76, 73 and 75% of the milk stearic and oleic acids were accounted for by mammary gland uptake of triglyceride and FFA from blood, in good agreement with the carbon balance data (Table 2). Furthermore, during the infusion of [U-^{14}C]stearic

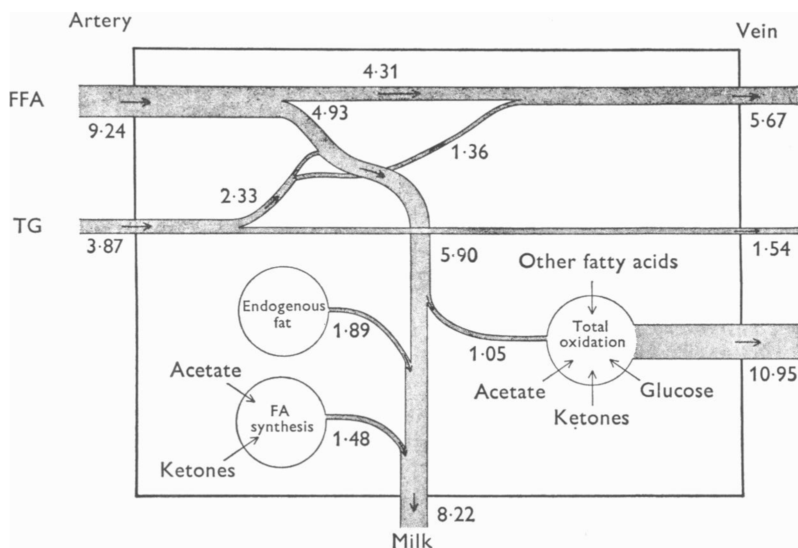

Fig. 1. Flow diagram of the quantitative interrelations of palmitate metabolism by the mammary gland of the starved (24 hr) goat. The oxidation of palmitate is shown in relation to over-all substrate oxidation by the gland. All values are expressed as mg carbon/min.

acid the specific radioactivity of the 'stearic + oleic' acid in milk fat was 66% of the maximum specific radioactivities of this fraction in plasma triglyceride and FFA. It is necessary to consider these acids as a single entity in view of the extensive conversion of stearic acid to oleic acid in the gland.

The complex interrelationships of palmitate uptake, release, oxidation, synthesis and transfer to milk fat in the mammary gland are illustrated in Fig. 1. The assumptions made in preparing this flow diagram were those made for the calculation of carbon balance data in Table 3.

Composition of milk fat. There were significant changes in the fatty acid composition of milk fat during starvation. The content of acids of chain-length C_4–C_{14} which are synthesized *de novo* in the gland in fed animals (Popjak, French, Hunter & Martin, 1951; Annison, Linzell, Fazakerley & Nichols, 1967) fell steadily over the whole period (Fig. 2). In contrast, the proportion of acids of chain-length C_{18}, which are transferred into milk fat

from blood, almost doubled. Palmitic acid, which is both transferred from blood, and synthesized in the gland, occupied an intermediate position (Fig. 2).

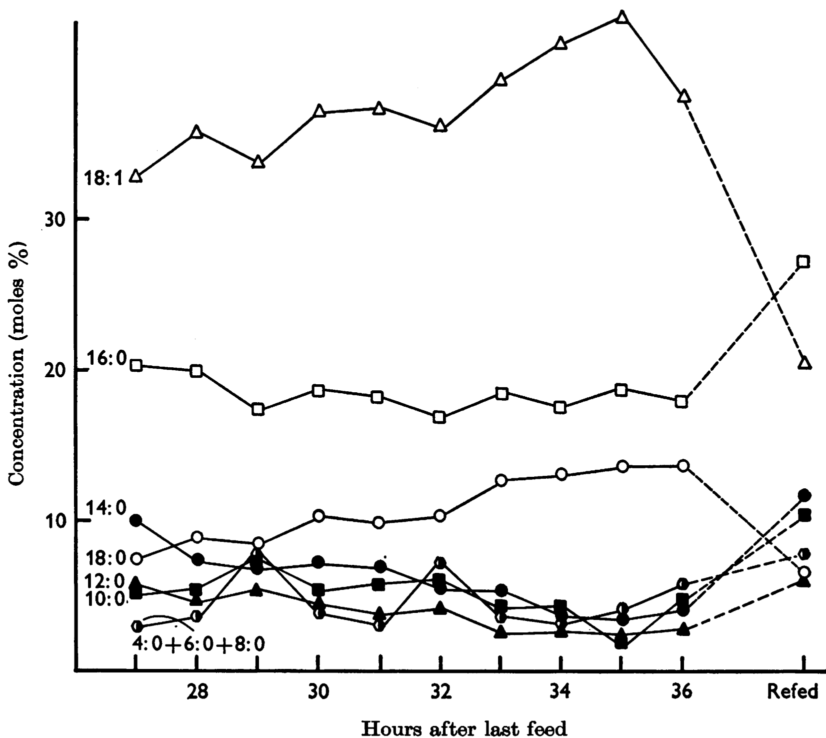

Fig. 2. Changes in milk fatty acid composition on fasting in a goat (Expt. 1). △ oleic (18:1), □ palmitic (16:0), ○ stearic (18:0), ● myristic (14:0), ▲ lauric (12:0), ■ capric (10:0), ◑ butyric (4:0) + caproic (6:0) + caprylic (8:0).

TABLE 3. Balance data relating milk fatty acid production to the mammary uptake of precursors (expressed as mg carbon/min)

	Experiment		
	1	12	13
	Mammary uptake		
FFA	15·2	13·6	23·7
Triglyceride	5·5	6·0	5·0
Acetate	2·4	1·0	2·1
β-Hydroxybutyrate	(3·4*)	4·5	2·4
Total	26·5	25·1	33·2
	Milk triglyceride fatty acid output		
	34·8	31·3	47·4
$\frac{\text{Uptake}}{\text{Output}} \times 100$	76·0	77·0	70·0

* Not measured-mean of values for Expts. 12 and 13.

TABLE 4. The effect of starving and re-feeding on empty udder volume and milk production. Each goat had one gland auto-transplanted to the neck where volume measurement is easier than *in situ*. All glands were thoroughly emptied of milk using oxytocin to aid milk ejection. Mean ± S.E. of mean of 3–5 measurements

	Mammary gland volume (ml.)				Mammary gland milk production (ml./hr)			
	Hazel		Rachel		Hazel		Rachel	
Interval since last fed (hr)	Control	Transplant	Control	Transplant	Control	Transplant	Control	Transplant
1	1180 ± 22	1066 ± 10·5	900 ± 15	655 ± 8·5	87	84	70	55
29	857 ± 20	940 ± 22	650 ± 14	636 ± 13	56	56	44	20
Refed, 26·5 hr	1058 ± 28	1125 ± 14	850 ± 17	690 ± 15	72	68	72	40

DISCUSSION

In ruminants the digestion of the large volume of food residues continues for some days of fasting. Nevertheless, Annison, Brown, Leng, Lindsay & West (1967) found that in sheep there is a marked fall, as early as 24 hr, in the utilization of those nutrients that are derived directly (acetate) and indirectly (glucose) from the gastro-intestinal tract and an increased utilization of fatty acids, mobilized from adipose tissue. The present results are therefore mainly of interest when compared with previous similar studies in fed lactating goats (Fig. 3).

The most striking effect of fasting the lactating goat is the rapid fall in milk yield to about half between 16 and 24 hr, accompanied by a fall in the mammary uptake of acetate, glucose and amino-acids and a rise in the uptake of FFA (Linzell, 1967*a*). The infusion of these substrates into fasted goats (Linzell, 1967*b*), suggests that the fall in the mammary uptake of glucose is the main reason for the fall in milk secretion in the early stages of fasting because only glucose infusions produce a rise in milk yield (Linzell, 1967*b*). The present results also show that the mammary glands and the whole animal obtain less energy from the oxidation of glucose and that the mammary glands are less of a drain on the glucose resources, using about 40 % of the total glucose entering the circulation, compared with 60–85 % in the fed animal (Annison & Linzell, 1964). In fed goats the arteriovenous differences of glucose and acetate are linearly related to the arterial concentration (Linzell, 1960). Since blood glucose does not fall as much as acetate in fasting, further work is necessary to discover the mechanism of the fall in mammary glucose uptake.

The present work has extended our knowledge of the changes in mammary fat metabolism in fasting by showing that major changes occur in the relative uptakes of the precursors of milk fat in early starvation. The uptake of triglycerides falls markedly, the uptake of acetate almost ceases, but there is a large rise in the uptake and oxidation of FFA. This complete

reversal of the situation characteristic of the fed animal is illustrated in Fig. 3.

The rate of utilization and oxidation of acetate by the starved (24 hr) lactating goat were about 20 % and 30 % respectively of the values re-

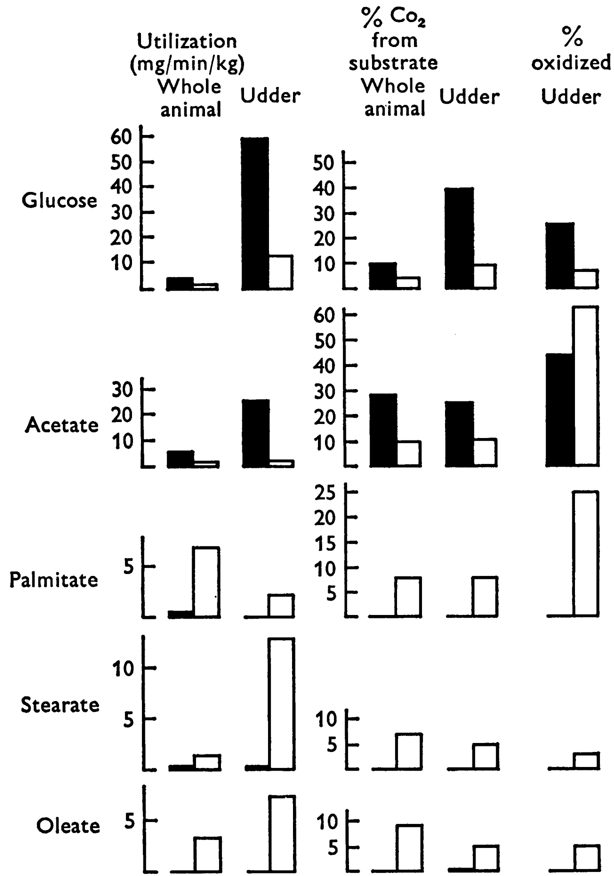

Fig. 3. A comparison of general and mammary metabolism of fed ■ and starved (24 hr) □ lactating goats. The data for the fed animals is taken from previous studies of the same animals (Annison & Linzell, 1964, Annison, Linzell, Fazakerley & Nichols, 1967).

ported earlier for fed lactating goats (Annison & Linzell, 1964), whereas the rates of entry and oxidation of long chain free fatty acids were markedly raised and more extensively used for milk fat synthesis.

The low specific radioactivities of the milk triglyceride fatty acids relative to that of blood acetate (< 1 %) were in marked contrast to the situation in fed goats (80 % for C_4–C_{14} acids, Annison, Linzell, Fazakerley & Nichols, 1967) and confirmed the minor role of acetate in milk fat synthesis

in the starved goat shown by the carbon balance data (Table 3). The lowered availability of acetate for milk fat synthesis was reflected in the progressive decline in the proportions of fatty acids of chain-length C_4–C_{14} in milk fat (Fig. 2), which, as previous work has shown, are synthesized from acetate and β-hydroxybutyrate.

In the fasted goat the fall in the secretion of milk fat is significantly less than the fall in lactose secretion (Linzell, 1967*a*). The present work confirms that this is largely due to the marked rise in mammary FFA uptake compensating for the fall in acetate and triglyceride uptake, whereas there appears to be no substitute for glucose, which the glands need to synthesize lactose, the main osmotic constituent of milk. Nevertheless in the present experiments, the milk fat output was more than the plasma lipid uptake and it is suggested that the udder shrinkage occurring in fasting means that it is utilizing its own reserves of lipid at this time. Hardwick, Linzell & Mepham (1963) calculated that goat mammary tissue contained enough lipid to provide all the precursors for milk fat synthesis for up to 15 hr and Linzell, Annison, Fazakerley & Leng (1967) showed that isolated perfused udders can indeed use their pools of long chain fatty acids, which are very similar in composition to milk fatty acids, as an endogenous source of milk lipid precursors. Furthermore, in the present work Fig. 2 shows that even after 26 hr of fasting milk fat still contained 21 % by weight of fatty acids C_4–C_{14} inclusive, and that there was a progressive fall to 8·4 % by 35 hr, by which time presumably the tissue was depleted of these fatty acids normally synthesized mainly from acetate.

The slight increase in the specific radioactivity of plasma triglycerides across the mammary gland during infusion of labelled FFA could have resulted from the release of triglyceride synthesized from labelled FFA, but more likely reflected the non-uniform uptake of the heterogeneous complex comprising plasma triglyceride. The preferential uptake of chylomicra and low-density lipoproteins by goat mammary glands has been demonstrated in other studies (Barry *et al.* 1963).

The flow diagram summarizing the metabolism of palmitate by the mammary gland (Fig. 3) demonstrates the utility of combining isotope dilution and arteriovenous difference techniques with measurements of blood flow, since these quantitative data on the uptake, synthesis, oxidation and release of palmitate could not have been obtained in any other way. Similar diagrams could be constructed for the mammary metabolism of stearic and oleic acids.

A feature of over-all and mammary metabolism in the starved lactating goat is that only about 50 % of the total carbon dioxide output can be accounted for in terms of the oxidation of acetate, plasma FFA and glucose (Table 1). The accuracy of estimating the contribution of several substrates

to total CO_2 production by the comparison of the specific radioactivities of the substrate and blood CO_2 has been investigated in fed and fasted sheep by Annison, Brown, Leng, Lindsay & West (1967). The method is only approximate, because of partial exchange of blood CO_2 with CO_2 produced by fermentation in the rumen, and because of the overlap of metabolic pathways, which, however, is slight for glucose and fatty acids in ruminants. The results in fasting sheep suggest that only about 10 % of the remaining 50 % of CO_2 could come from the oxidation of β-hydroxybutyrate. Therefore it seems likely that in the early stages of fasting a substantial part of the tissue is mobilized and oxidized without equilibration with the blood pools of acetate, glucose and FFA.

The present experiments demonstrate that, in the early stages of fasting in lactating goats, when the rate of milk secretion has fallen to about half, major changes in the metabolism of the animal and its udder have also taken place. The main changes for the udder are a fall in metabolic rate and over-all substrate uptake, the almost complete cessation of acetate metabolism, a marked rise in long-chain fatty acid metabolism and the probable utilization of endogenous substrates for milk secretion. The results are also consistent with the view that the availability of glucose is rate limiting for milk secretion.

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