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The effect of intravenous infusions of sterculic acid on milk fat synthesis

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1. The effects of intraduodenal infusions of sterculic acid, a naturally occurring inhibitor of desaturase activity, on the yield and composition of milk were examined in a lactating goat.

2. Sterculic acid administration increased the percentage of stearic acid in milk fat, reflecting inhibition of mammary desaturase activity. Milk yield was not affected, but milk fat output decreased. Possible explanations are discussed.

3. No evidence was obtained for an alternative pathway of oleic acid synthesis from acetate.

In ruminants, short-chain fatty acids up to a chain length of C_{14} and part of the C_{16} in milk fat originate by *de novo* synthesis from acetate and β-hydroxybutyrate absorbed from circulating blood (see Linzell, 1968). The longer-chain fatty acids with 18 carbon atoms and part of the fatty acids with 16 carbon atoms in milk fat are derived direct from plasma lipids (Annison, Linzell, Fazakerley & Nichols, 1967; Bishop, Davies, Glascock & Welch, 1969). Substantial quantities of absorbed stearate are converted into oleate in the mammary tissue of lactating goats (Annison *et al.* 1967; Bickerstaffe & Annison, 1970) and cows (Lauryssens, Verbeke, Peeters, Garton, Lough & Duncan, 1960), by a desaturase enzyme located in the microsomal particles (Bickerstaffe & Annison, 1968, 1970). The desaturase enzyme in plant tissues (James, Harris & Bezard, 1968) and animal tissues (Raju & Reiser, 1967; Allen, Johnson, Fogerty, Pearson & Shenstone, 1967; Donaldson, 1967*a*) is inhibited by the cyclopropene fatty acid, sterculic acid. On the basis of experiments with adult rats in which the desaturase system was inhibited by sterculic acid, oleic acid has been proposed to be synthesized from acetate by β-γ-desaturation of synthesized lauric acid followed by elongation rather than desaturation of stearic acid (Raju & Reiser, 1969). According to some workers this pathway is inducible in chicks (Donaldson, 1967*b*). Doubts have been expressed regarding the existence of such a pathway (Pearson, Fogerty, Johnson & Shenstone, 1972; Coleman & Friedman, 1971).

In the present work we have examined the effect of sterculic acid on the desaturase enzyme system in mammary tissue and on milk fat synthesis by measuring arteriovenous differences of fatty acid concentration across the mammary gland and the fatty acids secreted in milk in a surgically prepared lactating goat. Milk yield was not affected but milk fat synthesis was reduced on infusing intravenously sterculic acid. Increased concentrations of stearic acid in milk fat reflected the inhibition of the desaturase enzyme by sterculic acid, but an alternate pathway to oleic acid, as assayed by the incorporation of [U-^{14}C]acetate into milk fatty acids, was not observed.

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EXPERIMENTAL

Animal. A lactating goat was prepared for mammary arteriovenous sampling and measurement of udder blood flow as described by Linzell (1960). The goat was given 1100–1200 g/d of a concentrate (kg/100 kg: maize 25, barley 22, decorticated cotton cake 15, malt culms 4, tallow 2, distillers' grain 10, meat meal 3, rice bran 3, milo 10 and molasses 5) in twenty-four hourly feeds, and 500 g hay/d at 09.30 and 16.00 hours in two feeds. The goat was housed in a metabolism crate and water was freely available.

Infusion of sterculic acid. Methyl sterculate was prepared from *Sterculia foetida* L. seeds by the procedure of Kircher (1964) and its purity established as 98% by thin-layer chromatography on silver nitrate impregnated silica gel followed by hydrogenation and identification of the products by gas–liquid chromatography (Johnson, Murray, Fogerty, Kennett, Pearson & Shenstone, 1967). A band at 1000–1010 cm^{-1} in the infrared spectrum of the methyl sterculate, as a liquid film, confirmed the purity and existence of the cyclopropene structure. Portions (500 mg) of the ester were saponified with a 10% excess of KOH in methanol at 50°; the methanol was removed and 100 ml 0·9% sterile saline was added and the volume made up to 200 ml with plasma previously obtained from the goat. The final solutions were continuously infused at 0·13 ml/min for up to 17 d into a catheterized jugular vein. Two experiments were carried out; in one the sterculate, after the purity had been checked by its infrared spectra, was infused for 2 d and in the other, for up to 17 d. Essentially the same results were obtained from both experiments.

Infusion of radioactive fatty acids. A mixture of [11, 12-^{3}H]stearic acid (100–200 μCi) and [U-^{14}C]acetate (175–380 μCi) was saponified as above and 80 ml of the plasma–saline sterculate solution were added. This radioactive infusion solution was infused at 0·13 ml/min into the catheterized jugular vein for 280–350 min, and during the last hour of infusion four pairs of arterial and mammary venous blood samples (30 ml each) were taken simultaneously at 15–20 min intervals as described by Annison *et al.* (1967). In some experiments [U-^{14}C]acetate only in plasma–saline was infused into the animal.

The animal was milked immediately before the infusion and at intervals of 1 h during the infusion, and for 4–6 h after the infusion, using oxytocin (200 m-units) injected intravenously to aid milk ejection. Mammary blood flow was estimated from the milk yield (see Linzell, 1971). Weight of the udder was determined by measurement of the udder volume by displacement of water (Linzell, 1966) and assuming that the specific gravity of mammary tissue is 1·035.

Chemical methods. Plasma and milk lipids were analysed and assayed for radioactivity as described by Annison *et al.* (1967). The level of blood acetate was determined as described by Freeman, Noakes & Annison (1970) and blood gases, β-hydroxybutyrate and udder weight as described by Annison, Linzell & West (1968).

Radioisotopes. [11,12-^{3}H]stearic acid (0·4 mCi/mg) was a generous gift from our colleagues at the Vlaardingen laboratory, Holland. [U-^{14}C]acetate (0·56 mCi/mg) was obtained from the Radiochemical Centre, Amersham, Bucks.

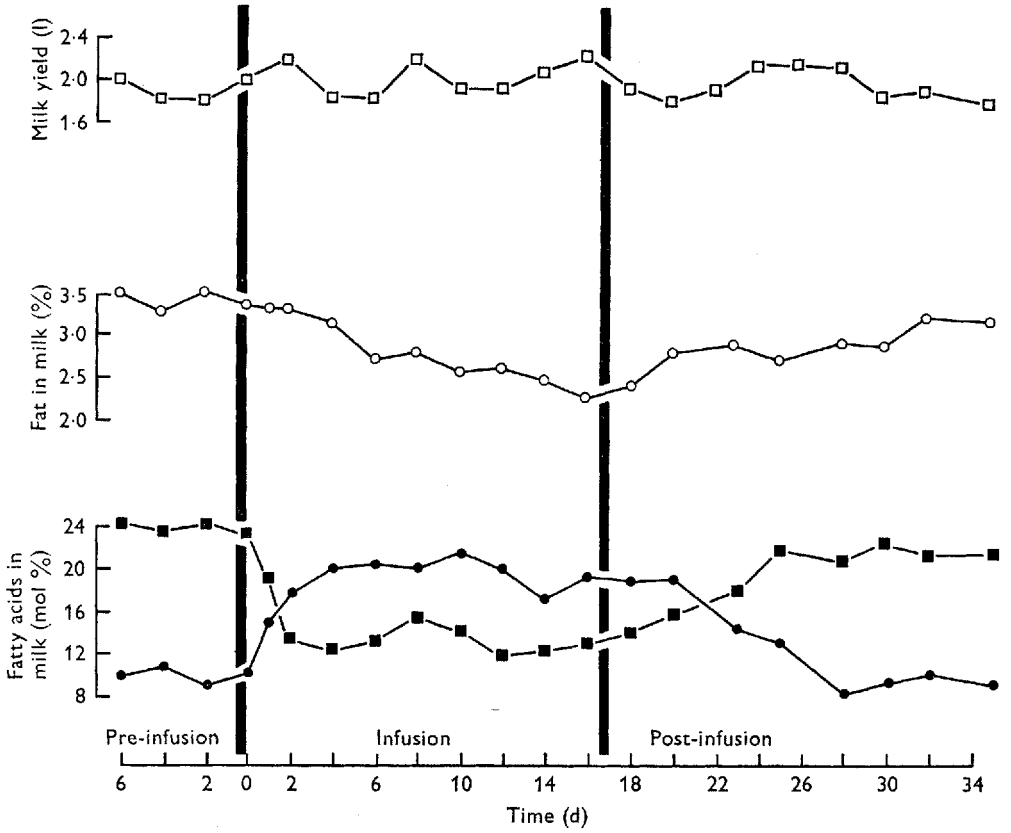

Fig. 1. Effect of an intravenous infusion of sterculic acid on milk yield (□) and fat content (○) and the proportion of octadecanoate (18:0) (●) and octadecenoate (18:1) (■) in milk fat in a goat.

RESULTS

The changes in milk yield, fat content of milk and fatty acid composition of milk fat at given time-intervals before and after intravenous infusions of sterculic acid into a lactating goat are shown in Fig. 1. The milk yield was largely unaffected by the infusion of sterculic acid; though the fat content of the milk was reduced during the infusion, it subsequently returned to pre-infusion values.

The mean daily intakes of dietary constituents, milk yield and composition of milk in each experimental period are given in Table 1. The infusion of sterculic acid had little effect on the intake of dietary constituents, milk yield, lactose or protein content of milk.

Measurement of the arterial levels and arteriovenous differences in β-hydroxybutyrate, glucose, acetate and lipids in each experimental period (Table 2) suggested that a reduction in the mammary uptake of triglyceride, acetate and β-hydroxybutyrate accompanied the infusion of sterculic acid, with a slow recovery in the post-infusion period. When a continuous intravenous infusion of [U-^{14}C]acetate was given in each experimental period the specific radioactivity of blood acetate and CO_2 reached constant values after 240 min. From these values the entry rate and oxidation of

Table 1. *Mean daily intake of dietary constituents, milk yields and composition of milk of a goat before, during and after sterculate infusion*

Dietary intake (g/d)	Before infusion	During infusion	After infusion
Concentrate	1080	1200	1200
Hay	500	500	500
Output of			
Milk (l/d)	1·71	1·96	1·97
Fat (g/d)	74·0	48·4	59·1
Lactose (g/d)	81·4	90·3	90·0
Protein (g/d)	49·4	57·0	58·1
Percentage in milk of			
Fat	4·33	2·47	3·00
Solids not fat	8·47	8·05	8·27
Lactose	4·76	4·61	4·57
Protein	2·89	2·91	2·95
Solids	12·80	10·52	11·27
Live weight (kg)	60·0	57·0	54·0

acetate in the whole animal and the amount of acetate oxidized in the mammary gland were calculated (Table 3). The infusion of sterculic acid had little effect on the absorption, utilization and oxidation of acetate.

The fatty acid compositions and concentrations of the free fatty acid and triglyceride fractions in arterial and mammary venous plasma, determined in each experimental period are shown in Table 4. Sterculic acid had little effect on the fatty acid composition of the plasma lipids. In contrast, there was a substantial increase in the proportion of stearic acid, accompanied by a reduction in the concentration of oleic acid in the milk fat lipids on infusing sterculic acid (Table 5) implying the inhibition of the desaturase enzyme system in mammary tissue. A comparison of the uptake of individual fatty acids from plasma triglycerides and their secretion into milk fat during each experimental period (Table 6) suggested that stearic acid was in fact converted into oleic acid and that palmitic acid was synthesized endogenously. The results also verify that the desaturation of stearic acid to oleic acid was inhibited by intravenous infusion of sterculic acid.

Determination of the specific activity of plasma free stearic acid after intravenous infusion of [11,12-^{3}H]stearic acid in the control and sterculic acid experiments (Table 7) showed that the entry rates of stearic acid were 18·1 and 14·8 mg/min respectively, which is within the range obtained by previous workers (Annison *et al.* 1967). In both experiments, the absence of a concentration difference (Table 4), but a fall in specific activity (Table 7) of free stearic acid across the mammary gland confirmed previous observations that there is substantial uptake of stearic acid by mammary tissue and that stearic acid is released from plasma triglycerides by capillary lipoprotein lipase. The presence of tritium-labelled oleic acid in the arterial and mammary venous plasma indicated that desaturation of intravenously infused stearic acid may occur in extra-mammary tissues.

Examination of the distribution of radioactivity in milk fat confirmed that stearic

Table 2. *Concentration of plasma phospholipids (PL), cholesterol esters (CE), free fatty acids (FFA), triglycerides (TG), blood glucose (Glu), acetate (Ac) and β-hydroxybutyrate (βOH) in arterial (Art) and mammary venous (MV) blood of a goat*

(Mammary blood flow, mammary gland uptake of blood constituents and the output of milk triglycerides are also shown before, during and after sterculate infusion. The results are the means of four observations)

Experimental period	Blood sample	Plasma lipids (mg/100 ml)				Blood constituents (mg/100 ml)			Blood flow (ml/min)	Mammary uptake (mg/min)				Milk TG output (mg/min)	
		PL	CE	FFA	TG	Glu	Ac	βOH		TG	Ac	Glu	βOH	Mean (4 days' pre-expt)	On day of expt
Before infusion	Art	NA	NA	3·3	12·4	NA	7·5	6·8	425	28·5	20·4	—	17·9	45·8	44·5
	MV	NA	NA	5·9	3·2	NA	2·7	2·6	—	—	—	—	—	—	—
During infusion	Art	18·1	19·0	3·5	9·3	54·4	6·4	4·7	430	20·8	17·2	60·2	12·0	31·2	32·0
	MV	19·1	21·1	5·6	3·0	40·4	2·4	1·9	—	—	—	—	—	—	—
After infusion	Art	20·1	21·2	3·9	9·1	54·4	6·0	4·9	470	23·4	17·4	81·8	12·7	38·8	37·0
	MV	20·1	21·7	7·1	3·1	37·0	2·3	2·2	—	—	—	—	—	—	—

NA, not analysed.

Table 3. *Substrate metabolism of [U-^{14}C]acetate by a lactating goat before, during and after sterculate infusion*

	Before infusion	During infusion	After infusion
Time acetate infused (min)	310	350	275
Infusion rate (μCi/min)	1·216	0·499	2·170
Week of lactation	8	13–15	17
Arterial concentration of substrate (mg/100 ml)	7·5	6·4	6·0
Specific activity of arterial acetate (μCi/g carbon)	11·258	4·085	18·973
Specific activity of arterial CO_2 (μCi/g carbon)	2·733	1·422	5·523
Entry rate of acetate into circulation mg/min	270	312	286
mg/min kg	4·5	5·5	5·3
Total CO_2 from substrate (%)	24·3	34·8	29·1
Udder weight (kg)	0·85	0·85	0·85
Mammary uptake of substrate (mg/min kg tissue)	24·0	20·2	20·5
Mammary CO_2 derived from substrate (%)	24·3	34·8	29·1
Substrate oxidized (%)	63·1	77·5	57·3
Mammary substrate uptake as % of total entry rate	7·6	5·5	6·1
Respiratory quotient	1·13	1·18	1·34

acid was actively incorporated into milk triglycerides and that stearic acid was desaturated to oleic acid. Determination of the specific radioactivities of stearic and oleic acids showed that 37% of the stearic acid was desaturated to oleic acid in the pre-infusion period, whereas in the sterculate infusion period only 24% was desaturated.

The distribution of radioactivity in the milk fatty acids after infusion of [^{14}C]acetate (Table 5) showed that acetate was incorporated into fatty acids up to chain length C16 but not into C18 fatty acids. The pattern of incorporation was not affected by infusion of sterculic acid.

DISCUSSION

In the present experiments desaturation of stearic acid in mammary tissue was inhibited by intravenous infusions of sterculic acid, leading to an increased proportion of stearic acid in milk fat. Milk fatty acids are normally 72% saturated but the infusion of sterculic acid increased the saturation to 84%. Sterculic acid had no significant effect on the fatty acid composition of the plasma free fatty acids or fatty acids of the plasma triglycerides. As the intravenously infused sterculic acid could pass through all organs, the level of desaturase activity in the liver and other tissues of the goat must be very low, with the exception of that of the mammary gland. Thus, as the oleic acid formed by desaturation of stearic acid was retained in the milk fat and not released into the circulation to any extent, and as the mammary gland is a major site for the desaturase enzyme, this enzyme probably controls the ratio of stearic acid to oleic acid in milk fat.

Table 4. *Relative proportions and amounts of the major components of the free fatty acids and triglycerides of arterial (ART) and mammary venous plasma (MV) before, during and after sterculate infusion*

	Before infusion				During infusion				After infusion			
	ART		MV		ART		MV		ART		MV	
	mol %	mg/100 ml	mol %	mg/100 ml	mol %	mg/100 ml	mol %	mg/100 ml	mol %	mg/100 ml	mol %	mg/100 ml
Free fatty acids												
16:0	26·9	0·8	26·7	1·5	25·1	0·8	28·9	1·5	23·2	0·8	27·4	1·8
18:0	40·6	1·2	41·0	2·4	41·6	1·5	42·6	2·4	37·0	1·5	38·8	2·9
18:1	20·8	0·6	20·9	1·2	19·2	0·6	15·4	0·9	19·0	0·8	16·1	1·2
Triglycerides												
16:0	37·4	3·4	35·7	1·0	35·8	3·0	34·4	0·9	35·3	3·0	33·3	1·0
18:0	39·1	5·0	37·0	1·1	39·0	4·0	34·2	1·1	38·2	3·6	29·0	0·9
18:1	14·7	2·6	17·7	0·7	15·3	1·5	18·9	0·6	15·7	1·5	19·2	0·6

Table 5. *Relative proportions and specific radioactivities (SRA) (μCi/g fatty acid) of fatty acids from milk fat of a goat obtained in each experimental period after infusion of [U-^{14}C]acetate*

	Before sterculate infusion		During sterculate infusion		After sterculate infusion	
Fatty acid	mol %	SRA	mol %	SRA	mol %	SRA
4:0	7·4	NA	9·2	NA	7·2	NA
6:0	3·9	NA	3·1	NA	3·4	NA
8:0	2·6	NA	1·8	NA	2·6	NA
10:0	7·1	2·24	5·4	1·84	8·0	6·70
12:0	2·1	1·83	2·7	2·21	3·1	9·02
14:0	8·3	1·23	10·4	1·94	10·8	7·14
15:0	0·7	NA	0·9	NA	0·8	NA
16:0	26·0	0·41	28·6	0·51	29·9	4·21
16:1	1·2	NA	NA	NA	1·2	NA
18:0	14·1	ND	21·6	ND	10·9	ND
18:1	24·4	ND	13·0	ND	19·7	ND
18:2	2·2	ND	3·4	ND	2·5	ND

NA, not analysed; ND, not detected.

Table 6. *Goat mammary gland uptake (from plasma triglyceride) and secretion (as milk triglyceride) of hexadecanoate* (16:0), *octadecanoate* (18:0) *and octadecenoate* (18:1) *before, during and after sterculate infusion*

	Uptake (mg/min)			Secretion (mg/min)			Production by gland (mg/min)	
Experimental period	16:0	18:0	18:1	16:0	18:0	18:1	16:0	18:1
Before infusion	7·4	12·1	5·9	13·0	8·4	14·4	5·6	8·5
During infusion	6·9	9·6	3·0	10·0	8·4	5·0	3·1	2·0
After infusion	7·8	10·5	3·5	13·5	5·5	9·8	5·7	6·3

Sterculic acid had no effect on the entry rate or oxidation of acetate, or on milk yield. There was a reduction in the synthesis of milk fat as a consequence of decreased secretion of both long- and short-chain fatty acids and was accompanied, to a certain degree, by a reduction in the uptake of acetate, β-hydroxybutyrate and triglyceride. There are at least two possible explanations for the reduction in triglyceride synthesis. Maximum activity of the enzyme synthesizing triglycerides could be dependent on the availability of unsaturated fatty acids which normally occupy the 2-position of the glycerol. An excess of saturated fatty acid, i.e. stearic acid, may inhibit the synthesis of triglycerides. Alternatively it is possible that sterculic acid itself could inhibit –SH enzymes, e.g. the glycerol-3-phosphate acyltransferase, responsible for the synthesis of milk fat.

The existence of an alternative pathway for the biosynthesis of oleic acid that does not involve desaturation of stearic acid has been postulated (Raju & Reiser, 1969), and it has also been suggested that this pathway is induced in chicks when the desaturase enzyme is inhibited by sterculic acid (Donaldson, 1967*b*). It is suggested that acetate

Table 7. *Specific radioactivities (μCi ^3H/g fatty acid) of the C18 fatty acids in the free fatty acids and triglycerides of arterial (ART) and mammary venous (MV) plasma and the triglycerides of milk fat of a goat in the final stages of perfusing [11,12-^3H]stearic acid*

	Before sterculate infusion		During sterculate infusion	
Infusion time (min)	310		350	
Rate of infusion (nCi/min)	631		266	
	ART	MV	ART	MV
Plasma free fatty acids				
18:0	34·8	12·2	18·2	6·5
18:1	0·3	0·3	0·2	0·2
Plasma triglycerides				
18:0	0·8	1·2	0·5	2·0
18:1	ND	ND	ND	ND
Milk triglycerides				
18:0	4·29		3·16	
18:1	1·60		0·75	

ND, not detected.

is incorporated into lauric acid, which is then desaturated at the β-γ position and chain-elongated to oleic acid. Mammary tissue of ruminants does not synthesize C18 fatty acids from acetate. The stearic and oleic acids are absorbed direct from the plasma and substantial amounts of oleate in milk fat are derived from the desaturation of stearic acid in mammary tissue. Therefore, as the goat does not normally possess the postulated alternative pathway, evidence for its induction should be obtainable if the desaturase system is inhibited by sterculic acid. Such evidence was not obtained; even after 17 d of continuous infusion of sterculic acid, acetate was not incorporated into oleic acid. Thus it is unlikely that the alternative pathway for the synthesis of oleic acid exists, at least in the goat mammary gland.

Unilever gave permission for A. R. Johnson to work as a visiting scientist.

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