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Quantitative Aspects of Fatty Acid Biohydrogenation, Absorption and Transfer into Milk Fat in the Lactating Goat, with Special Reference to the *cis*- and *trans*-Isomers of Octadecenoate and Linoleate

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1. Surgically prepared lactating goats were used to obtain quantitative information on the biohydrogenation and absorption of dietary fat, and on the mammary uptake and transfer into milk fat of the complex mixture of *cis*- and *trans*-isomers of octadecenoate that arise during ruminal biohydrogenation. 2. About 90% of dietary linolenate, linoleate and oleate was hydrogenated in the rumen, and the availability to the animals of the essential fatty acid, linoleate, represented only 0.5–1.5% of the total dietary energy. 3. The intra-ruminal administration of ^{14}C-labelled linolenate and linoleate showed that these acids were not absorbed from the rumen, in agreement with previous work. 4. No selectivity was observed in the metabolism of the geometrical and positional isomers of octadecenoate: their rates of absorption from the small intestine, transfer into lymph, uptake by the mammary gland and appearance in milk fat were similar. 5. The desaturase activity of intestinal epithelium was demonstrated by the appearance in lymph of [1-^{14}C]oleate after the addition of [1-^{14}C]stearate to the small intestine.

In ruminants ingested food is subjected to microbial attack in the rumen. Complex lipids are rapidly hydrolysed, and the unsaturated fatty acids, which normally predominate in plant lipids, suffer extensive biohydrogenation and double-bond migration to yield a mixture of geometrical and positional isomers (see Dawson & Kemp, 1970). These fatty acids pass out of the rumen adsorbed on the solids of digesta, which consist largely of partly digested plant material (see Lough, 1970). Limited data are available on the flow of fatty acids into the duodenum of sheep (Scott *et al.*, 1969) and on the extent of biohydrogenation of unsaturated fatty acids (Ulyatt *et al.*, 1966). Lennox & Garton (1968) used sheep prepared with duodenal re-entrant cannulae in different parts of the small intestine to obtain quantitative information on lipid absorption, a topic that has been reviewed by Garton (1969).

In the present studies we have used lactating goats with duodenal cannulae to obtain quantitative information on the extent of ruminal biohydrogenation in lactating animals with particular reference to the availability of the essential fatty acid *cis*,*cis*-$\Delta^{9,12}$-linoleate in relation to its output in milk and the requirements of body tissues. Quantitative information on the positional and geometrical isomers of octadecenoate was also obtained. The absorption of specific fatty acid isomers from the small intestine was measured by collecting thoracic lymph, and their uptake by the mammary gland and incorporation into milk fat was examined by an arteriovenous-difference technique (Linzell, 1960). Results were also obtained on the rates of entry of [9,10-^{3}H]oleate and [1-^{14}C]-elaidate in the whole animal, and on their mammary uptake and rate of secretion in milk.

The possible absorption of linolenate and linoleate from the rumen, omasum and abomasum was studied in view of the observation by Moore *et al.* (1968, 1969) that intra-abomasal infusions of linoleate in sheep resulted in an increased concentration of the acid in plasma triglycerides within 2h. The quantitative significance of the desaturase activity of ruminant intestinal epithelium demonstrated *in vitro* (Bickerstaffe & Annison, 1968, 1969) was also examined.

Materials and Methods

Experimental animals

Five lactating goats were each prepared with a rumen fistula and a duodenal re-entrant fistula immediately caudal to the pyloric sphincter (Harrison & Hill, 1962), and three of the goats also had exteriorized carotid artery and mammary vein skin loops (Linzell, 1960). The animals were fed 'continuously' (at intervals of 1h) on a pelleted diet (dried grass, 50%; barley, 22%; soya-bean meal, 5%; extracted decorticated cottonseed meal, 5%; molasses, 5.5%; soya-bean oil, 5.5%; and a balanced mixture of minerals and vitamins) containing 7% total lipid

(see Tables 1 and 2). Feed intakes were adjusted according to milk yield (basal diet 500g/day plus 100g/250ml of milk). Water was supplied *ad libitum*. The goats were housed in crates, and measurements of milk output were carried out for 7 days before the collection of duodenal digesta and lymph.

Digesta flow into the duodenum was measured over 24h periods as described by Freeman *et al.* (1968). Portions of digesta were thoroughly mixed with a top-drive emulsifier (Silverson Machines) and a sample was retained for analysis before the remainder was returned to the small intestine through the caudal cannula. To minimize interference with the flow of digesta into the lower gut, digesta obtained 2–3 days earlier from the same animal were returned to the small intestine during the first collection period at the rate at which digesta were flowing out of the fistula. The initial replacement digesta were stored at −10°C, and heated to 37°C before use. Measured rates of flow were corrected for any variation in normal flow rates by the use of a chromic oxide marker (see Macrae & Armstrong, 1969) incorporated into the diet as a bread (1.2% of the diet) consisting of Cr_2O_3–wheat flour (3:17, w/w) that had been mixed and heated in the oven at 100°C overnight.

Lymph was collected from a catheter inserted into the thoracic lymph duct under local anaesthesia immediately before use. At 1h intervals a 20ml sample was retained for analysis and the remainder was infused into the jugular vein at about the rate of thoracic lymph production. The lymph catheter remained patent for periods of only 2–7 days, which limited the number of experiments that could be carried out on each animal.

Addition of radioactive fatty acids to rumen and duodenum

[1-^{14}C]Stearic acid (40μCi; 240μg), [(*n*)9,10-^{3}H]-oleic acid (440μCi; 170μg), [1-^{14}C]linoleic acid (219μCi; 65μg) and [1-^{14}C]linolenic acid (230μCi; 80μg) were saponified with KOH (50% excess) and made up to 100ml with water and strained rumen fluid (1:1, v/v) before introduction into the rumen.

[1-^{14}C]Stearic acid (32μCi; 190μg) and [(*n*)9,10-^{3}H]oleic acid (314μCi; 120μg) were dispersed in micellar form in a solution of sodium glycodeoxycholate (10mM) in Krebs–Ringer phosphate buffer (Freeman *et al.*, 1968) and injected into the small intestine through a duodenal fistula.

Intravenous infusion of radioactive fatty acids

A mixture of [9,10-^{3}H]oleic acid (0.3–1.2μCi; 125–500μg) and [1-^{14}C]elaidic acid (30–120μCi; 125–500μg) was bound to albumin, made up to 250ml and infused intravenously into lactating goats (1ml/min for 200min) as described by Annison *et al.* (1967). The uptake of fatty acids by the mammary gland was examined by the arteriovenous-difference technique, in which four pairs of blood samples (30ml each) were taken simultaneously at 15–20min intervals from the carotid artery and mammary vein (Annison *et al.*, 1967). The animal was milked immediately before the infusion and at 1h intervals during the infusion, and for 4–6h after the infusion with oxytocin (400 milliunits) being injected intravenously to aid milk ejection. Mammary blood flow was measured by the thermal-dilution technique (Linzell, 1960).

Extraction and assay of lipids

Abomasal digesta (5g), lymph (5ml), plasma (5ml) and milk (5ml) were extracted with chloroform–methanol (2:1, v/v) by the method of Folch *et al.* (1957). Feed samples (10g) were treated with 200ml of chloroform–methanol (2:1, v/v), and the whole mixture was evaporated to dryness, re-extracted with chloroform–methanol (2:1, v/v), filtered and weighed. The lipid extracts were methylated and the proportion of individual fatty acid methyl esters was determined by g.l.c. as described by Bickerstaffe & Annison (1970). The addition of appropriate concentrations of

Table 1. *Monoglyceride, diglyceride, triglyceride, phospholipid, free fatty acid and cholesteryl ester content of dietary lipid and of the lipid entering the duodenum of a lactating goat*

Experimental details are given in the text. The results are the means±S.E.M. of six analyses in two experiments with goat 1. (Mean lipid intake 86.7g; digesta lipid 78.8g/day.)

	Composition of lipid (% of total by wt.)					
	Monoglyceride	Diglyceride	Triglyceride	Phospholipid	Free fatty acid	Cholesteryl ester
Feed	3±0.1	7±0.2	51±1.6	15±0.7	22±0.6	2±0.1
Digesta	<1	<1	1±0.1	2±0.1	93±1.6	3±0.1

Table 2. *Content of the major fatty acids in the lipids of feed, digesta and lymph of a lactating goat*

Experimental details are given in the text. The results are the means ± S.E.M. of six analyses in two experiments with goat 1.

Fatty acid	Fatty acid composition (mol/100 mol)															
	Total lipid			Triglyceride			Free fatty acid			Phospholipid			Cholesteryl ester			
	Feed	Digesta	Lymph	Feed	Digesta	Lymph	Feed	Digesta	Lymph	Feed	Digesta	Lymph	Feed	Digesta	Lymph	
Hexadecanoate	15±0.5	16±0.8	22±0.5	15±0.4	28±2.6	28±0.6	20±0.6	16±0.5	15±0.5	21±0.5	26±0.7	20±0.5	32±0.9	20±0.9	13±0.8	
Hexadecenoate	<1	<1	2±0.1	1±0.1	2±0.1	2±0.1	<1	<1	4±0.1	2±0.1	4±0.1	1±0.1	<1	1±0.1	4±0.1	
Octadecanoate	5±0.1	65±1.5	38±1.4	5±0.1	15±0.8	47±0.6	5±0.1	74±0.6	20±0.6	2±0.1	18±1.0	24±0.7	8±0.2	51±1.4	21±1.4	
Octadecenoate	21±0.8	12±0.4	25±0.9	23±0.9	19±1.2	15±0.4	22±0.9	7±0.1	35±0.5	4±0.1	12±0.6	13±0.5	13±0.5	7±0.3	18±0.9	
Octadecadienoate	41±1.3	3±0.1	8±0.4	45±1.4	26±0.8	3±0.1	41±0.8	<1	13±0.4	21±0.7	26±0.8	28±1.0	35±1.2	10±0.6	29±1.7	
Octadecatrienoate	16±0.7	<1	<1	6±0.2	3±0.1	2±0.1	9±0.2	<1	3±0.1	45±1.4	4±0.1	3±1.0	5±0.2	<1	5±0.1	

internal marker, heptadecanoic acid, before extraction, was used to calculate the total fatty acid concentration of the lipid extracts (West & Rowbotham, 1967; Kuksis, 1966) after correction for the small amounts of heptadecanoate and phytanate normally present in the digesta. The methyl ester of phytanic acid had the same retention time as methyl heptadecanoate in the chromatographic system used and constituted 0.5% of the total long-chain acids. The total radioactivity in milk, plasma and lymph was determined by counting the radioactivity of 1ml samples in toluene–Triton X-100 (2:1, v/v) (15ml) containing 2,5-diphenyloxazole (0.4%). The specific radioactivities of the total lipid extracts were measured as described by Bickerstaffe & Annison (1970).

Separation of lipid classes

The extracted lipid was dissolved in a known volume of chloroform–methanol (2:1, v/v), and a suitable sample (20mg) was transferred to a glass plate coated with silica gel (0.4mm thick) and developed in diethyl ether–light petroleum (b.p. 40–60°C)–acetic acid (15:35:1, by vol.). The various lipid classes were eluted and analysed for fatty acids as described by Bickerstaffe & Annison (1970).

Separation of saturated and cis and trans unsaturated fatty acids

The saturated and *cis*- and *trans*-monoenoic acids and -dienoic acids were separated and analysed by t.l.c. on $AgNO_3$-impregnated silica gel as described by Bickerstaffe & Annison (1970). The amount of *cis,cis*-dienoic acid was determined by g.l.c. analysis of all the methyl ester fractions isolated from the $AgNO_3$-impregnated silica-gel plate.

Determination of double-bond positions in monoenoic acids. Monoenoic acid (0.5mg) was oxidized with $KMnO_4$ by the method of Davidoff & Korn (1963), modified to remove contaminants from the reaction products. Ether extraction of the mono- and di-carboxylic acids in the reaction mixture was followed by t.l.c. of the methyl esters (produced with diazomethane) on silica gel G in diethyl ether–light petroleum (b.p. 40–60°C) (3:7, v/v) before g.l.c. This purification step eliminated a number of materials that appeared as extraneous peaks during g.l.c.

Determination of double-bond positions in dienoic acids. The method involved random hydrogenation of the double bonds of dienoic acids (2mg) with hydrazine hydrate (50μl) in the presence of acetonitrile (400μl) and acetic acid (40μl). The resultant monoenoic acids (maximum yield 40%) were separated from saturated fatty acids and unchanged dienes by t.l.c. on $AgNO_3$-impregnated silica gel as described

above, and subjected to the standard 'double-bond' oxidation.

Radiochemicals

[1-^{14}C]Elaidic acid was prepared by isomerization of [1-^{14}C]oleic acid as described by Bickerstaffe & Annison (1970). [1-^{14}C]Oleic acid (57.2mCi/mmol), [(*n*)9,10-^{3}H]oleic acid (744mCi/mmol), [1-^{14}C]stearic acid (37.1mCi/mmol), [1-^{14}C]linoleic acid (52.9mCi/mmol) and [1-^{14}C]linolenic acid (40.8mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K., and purified by t.l.c. on $AgNO_3$-impregnated silica gel as described above.

Results

Flow of lipid into the duodenum

Comparison of the amount of lipid reaching the duodenum with that supplied in the diet (Table 3) showed that the extent of synthesis of lipid in the rumen was variable (0–20g/day). In agreement with previous work (see Garton, 1969), relatively little dietary triglyceride or partial glycerides reached the duodenum (Table 1). The predominant lipid fraction in digesta was free (unesterified) fatty acids, indicating that extensive hydrolysis of dietary lipids had occurred in the rumen. The rate of transfer of free fatty acids from the rumen into the duodenum was examined by adding a mixture of [1-^{14}C]stearic acid (40μCi) and [9,10-^{3}H]oleic acid (440μCi) to the rumen and assaying the digesta entering the duodenum for ^{14}C and ^{3}H radioactivity (Fig. 1). Since free fatty acids are adsorbed on solids in the rumen, the results may be equated with the rate of flow of solids from the rumen to the duodenum; about 40% of the radioactivity reached the duodenum within 24h.

Biohydrogenation of dietary fat

The extensive biohydrogenation of dietary fatty acids reported by other workers (see Dawson & Kemp, 1970) was confirmed by comparing the amounts of individual fatty acids in the feed and digesta (Table 4). Considerable loss (80–95%) of dietary linolenate and linoleate occurred in the process of rumen biohydrogenation, as indicated by the substantial increase of stearate and decreased concentrations of octadecadienoate and octadecatrienoate flowing into the duodenum. Analysis of the octadecadienoate fraction showed that 90–95% consisted of *cis,cis*-octadeca-9,12-dienoate (linoleate). The amounts of linoleate reaching the duodenum accounted for 0.5–1.5% of the total energy of the diet, and between 25 and 75% of the available linoleate was secreted in milk/day. Octadeca-11,15-dienoate, previously detected in cow rumen contents

Table 3. *Rates of flow and fatty acid content of digesta entering the duodenum, the volume and fatty acid content of thoracic lymph, and the volume and fatty acid output of milk in relation to the intake of dietary fatty acids in lactating goats*

Experimental details are given in the text. The results are given as the mean of two experiments (the ranges are in parentheses). N.A., Not analysed.

Goat no.	Body wt. (kg)	Dietary fatty acids (g/day)	Digesta flow into duodenum		Lymph flow		Milk yield	
			Volume (ml/day)	Fatty acids (g/day)	Volume (ml/day)	Fatty acids (g/day)	Volume (ml/day)	Fatty acids (g/day)
1	48	79 (75–83)	7500 (7200–7800)	77 (75–79)	6116 (5946–6286)	83 (80–86)	920 (910–930)	30 (29–31)
2	36	61 (59–63)	9240 (8910–9570)	74 (71–77)	5420 (5250–5590)	81 (79–83)	1297 (1250–1344)	44 (42–46)
3	46	71 (67–75)	10070 (9790–10350)	76 (73–79)	4290 (4050–4530)	110 (106–114)	1228 (1157–1299)	48 (46–50)
4	50	112 (107–115)	11040 (10765–11315)	136 (129–143)	N.A.	N.A.	1638 (1597–1679)	64 (61–67)
5	56	120 (114–126)	12840 (12527–13153)	141 (137–145)	N.A.	N.A.	1643 (1614–1672)	66 (63–69)

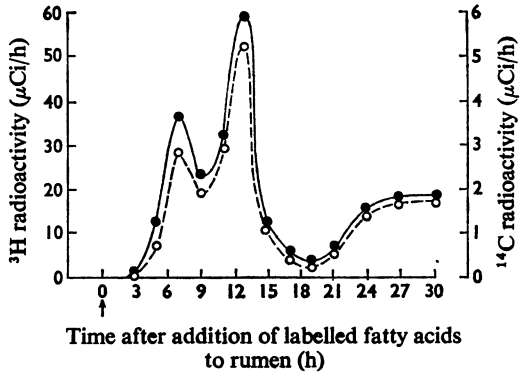

Fig. 1. *Amounts of ^{14}C radioactivity and ^3H radioactivity in digesta leaving the abomasum of a lactating goat after ruminal administration of [1-^{14}C]stearate (40μCi) and [9, 10-^3H]oleate (440μCi)*

Similar results were obtained with another lactating goat. Experimental details are given in the text. o, ^{14}C radioactivity; ●, ^{3}H radioactivity.

(Katz & Keeney, 1966), was present in the digesta octadecadienoate fraction in amounts less than 2%.

Analysis of the geometrical isomers of octadecenoate in the diet and digesta showed that most (80–85%) of the octadecenoate reaching the duodenum was of the *trans* configuration, in contrast with dietary octadecenoate, which was mainly (>90%) of the *cis* configuration. Determination of the double-bond position in the *cis*- and *trans*-octadecenoates (Table 5) indicated that, although double bonds occurred in every position between C-6 and C-16, in the *cis*- and *trans*-octadecenoates in digesta the major *cis*-isomer was octadeca-9-enoate (oleate) and the major *trans*-isomer was octadeca-11-enoate (vaccenate).

The extent of hydrogenation of individual fatty acids was also examined by adding labelled linolenate, linoleate or oleate to the rumen and examining the distribution of radioactivity in the fatty acids isolated from the digesta entering the duodenum 3h and 15h later (Table 6). The time of transfer of the fatty acids from the rumen to the duodenum was not necessarily an indication of residence time in the rumen, since passage through the omasum and abomasum must also be considered, but in any case the distributions of radioactivity in the 3h and 15h samples were not markedly different. The appearance in stearate of 76% of the radioactivity added to the rumen as [1-^{14}C]linolenate in the 3 h sample confirmed the rapidity of ruminal biohydrogenation reported previously (see Dawson & Kemp, 1970).

Table 4. *Quantitative interrelationships of the fatty acids in feed, digesta, thoracic lymph and milk in lactating goats fed at 1 h intervals*

Experimental details are given in the text. The results are given as the means of two experiments (the ranges are in parentheses). N.A., Not analysed.

		Individual fatty acids (g/24h)					
Goat no.	Sample	$C_{14:0}$–$C_{16:0}$ acids	$C_{18:0}$ acid	*cis*-$C_{18:1}$ acid	*trans*-$C_{18:1}$ acid	$C_{18:2}$ acid	$C_{18:3}$ acid
1	Feed	12.7	3.6	16.8	0.1	32.4	12.5
	Digesta	14.7 (14.3–15.1)	54.5 (53.1–55.9)	0.8 (0.77–0.83)	5.4 (5.2–5.6)	1.3 (1.2–1.4)	0.1 (0.96–1.04)
	Lymph	22.9 (22.1–23.7)	32.1 (30.9–33.3)	12.0 (11.6–12.4)	4.7 (4.9–5.1)	7.6 (7.3–7.9)	1.2 (1.17–1.23)
	Milk	7.7 (7.5–7.9)	5.0 (4.8–5.2)	9.5 (9.2–9.8)	1.2 (1.19–1.21)	1.0 (0.99–1.01)	0.2 (0.19–0.21)
2	Feed	18.2	1.6	12.4	0.1	25.8	2.1
	Digesta	20.2 (19.4–21.0)	36.1 (34.6–37.6)	3.4 (3.3–3.6)	6.1 (5.9–6.3)	5.1 (4.9–5.3)	0.1 (0.96–1.04)
	Lymph	26.5 (25.8–27.2)	23.4 (22.8–24.0)	15.0 (14.6–15.4)	4.6 (4.5–4.7)	8.3 (8.1–8.5)	0.7 (0.68–0.72)
	Milk	10.2 (9.7–10.7)	1.9 (1.8–2.0)	5.5 (5.3–5.7)	0.5 (0.48–0.52)	1.3 (1.2–1.4)	0.6 (0.58–0.62)
5	Feed	21.9	4.4	24.1	0.1	50.8	17.0
	Digesta	27.6 (26.8–28.4)	81.7 (79.2–84.2)	6.6 (6.4–6.8)	13.4 (13.0–13.8)	8.6 (8.3–8.9)	1.8 (1.7–1.9)
	Milk	35.7 (33.9–37.3)	8.7 (8.3–9.1)	13.4 (12.7–14.1)	2.5 (2.4–2.6)	2.6 (2.5–2.7)	0.5 (0.47–0.53)

Table 5. *Proportions of the positional isomers of cis- and trans-octadecenoate in the lipids of feed, digesta, lymph, milk and in the triglyceride and free fatty acid fractions of arterial plasma and mammary venous plasma*

Experimental details are given in the text. The results are the means ± S.E.M. of six analyses in two experiments with goat 1. N.D., Not detected.

		cis- or *trans*-Octadecenoate (% of total)							
					Arterial plasma		Mammary venous plasma		
	Position of double bond	Feed	Digesta	Lymph	Triglycerides	Free fatty acids	Triglycerides	Free fatty acids	Milk
cis-$C_{18:1}$ acid	6	N.D.	N.D.	1±0.1	1±0.1	1±0.1	1±0.2	2±0.2	1±0.2
	7	1±0.2	2±0.2	1±0.2	1±0.2	2±0.2	1±0.2	2±0.2	1±0.3
	8	1±0.2	3±0.3	1±0.2	3±0.2	5±0.4	3±0.4	6±0.5	2±0.2
	9	84±1.8	43±0.7	86±1.7	57±1.4	60±0.9	57±1.5	55±0.9	86±1.6
	10	1±0.2	3±0.2	1±0.1	2±0.3	3±0.2	2±0.2	3±0.3	1±0.2
	11	12±0.4	13±0.5	6±0.5	11±0.9	8±0.5	11±1.2	10±1.1	3±0.4
	12	1±0.1	17±0.6	4±0.3	12±1.1	9±0.6	12±1.4	9±0.8	2±0.2
	13	1±0.2	4±0.2	1±0.2	5±0.4	5±0.3	5±0.4	7±0.4	1±0.2
	14	N.D.	3±0.3	1±0.1	3±0.2	4±0.4	3±0.2	4±0.3	1±0.3
	15	N.D.	5±0.5	N.D.	4±0.3	4±0.3	5±0.5	2±0.2	2±0.4
	16	N.D.	6±0.4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
trans-$C_{18:1}$ acid	6	N.D.	N.D.	1±0.2	1±0.2	1±0.2	2±0.2	1±0.2	1±0.2
	7	N.D.	1±0.2	1±0.2	1±0.2	2±0.3	5±0.4	3±0.4	1±0.2
	8	N.D.	1±0.3	1±0.2	2±0.3	4±0.5	3±0.3	5±0.4	2±0.3
	9	N.D.	2±0.3	8±0.5	4±0.5	7±0.4	5±0.5	10±0.8	8±0.7
	10	N.D.	4±0.4	3±0.4	3±0.3	6±0.7	8±0.5	4±0.4	8±0.6
	11	N.D.	57±1.7	61±1.4	22±1.2	23±1.1	30±1.5	27±1.7	28±1.8
	12	N.D.	6±0.4	7±0.6	7±0.5	2±0.2	6±0.4	6±0.6	8±0.5
	13	N.D.	6±0.5	4±0.4	11±0.9	10±1.1	10±1.0	8±0.7	8±0.7
	14	N.D.	8±0.9	6±0.7	15±1.2	13±0.9	11±0.9	14±1.1	10±1.1
	15	N.D.	7±0.5	4±0.5	14±1.1	13±1.3	9±1.0	11±1.2	7±0.8
	16	N.D.	9±0.8	5±0.5	21±0.9	19±1.2	10±0.8	12±0.9	8±0.6

Table 6. *Distribution of radioactivity in fatty acids isolated from digesta entering the duodenum 3 h and 15 h after the addition of [1-^{14}C]linolenate (230 μCi; 80 μg), [1-^{14}C]linoleate (219 μCi; 65 μg) or [9,10-^{3}H]oleate (2.35 mCi, 920 μg) to the rumen of lactating goats*

Experimental details are given in the text. The results are the means ± S.E.M. of three experiments with three lactating goats. N.D., Not detected.

Fatty acid fraction	Distribution of radioactivity (%)					
	[1-^{14}C]Linolenate		[1-^{14}C]Linoleate		[9,10-^{3}H]Oleate	
Time (h) ...	3	15	3	15	3	15
Saturated	74 ± 3.5	76 ± 4.0	85 ± 2.0	85 ± 2.3	87 ± 1.7	88 ± 2.3
trans-Monoenoates	20 ± 2.3	18 ± 2.9	6 ± 0.6	6 ± 1.2	3 ± 0.4	3 ± 0.4
cis-Monoenoates	2 ± 0.6	2 ± 0.9	3 ± 0.6	2 ± 0.6	10 ± 1.5	9 ± 2.4
Dienoates	4 ± 0.4	4 ± 0.4	6 ± 1.5	6 ± 0.6	N.D.	N.D.
Trienoates	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Possible absorption of linolenate, linoleate and oleate from the rumen, omasum and abomasum

A mixture of [1-^{14}C]linolenate (230 μCi; 80 μg), [1-^{14}C]linoleate (219 μCi; 65 μg) and [9,10-^{3}H]oleate (2.35 mCi; 920 μg) was added to the rumen during the collection of digesta from the duodenal fistula, but the digesta were replaced by the addition of a similar volume of digesta collected from a donor goat fed on the same diet. This procedure ensured that no labelled digesta entered the duodenum but that normal gut function was maintained. Blood samples were withdrawn from the jugular vein at 30 min intervals, but examination of the plasma lipids showed that only 0.03% of the total dose of radioactivity appeared in blood plasma (assumed to be 7% of body weight). These results indicate that the fatty acids were not absorbed in significant amounts from the rumen, omasum and abomasum.

Absorption of fatty acids from the duodenum

The collection and analysis of thoracic lymph confirmed that about 80% of the *trans*-fatty acids entering the duodenum appeared in lymph (Table 4). The various positional isomers of *trans*-octadecenoate were present to the same extent in lymph and digesta, indicating that there was no selective absorption of any particular isomer (Table 5). In contrast, the amount of *cis*-octadeca-9-enoate in lymph was substantially higher than that in digesta, suggesting that octadeca-9-enoate may be synthesized in the intestinal epithelium by the desaturation of stearate.

The time-course of the uptake of stearate and oleate from the duodenum and their transfer into lymph and milk triglyceride was examined by adding to the duodenum a mixture of [1-^{14}C]stearate and [9,10-^{3}H]-oleate in micellar form. Both acids were rapidly absorbed, and the ^{14}C/^{3}H ratio remained unchanged

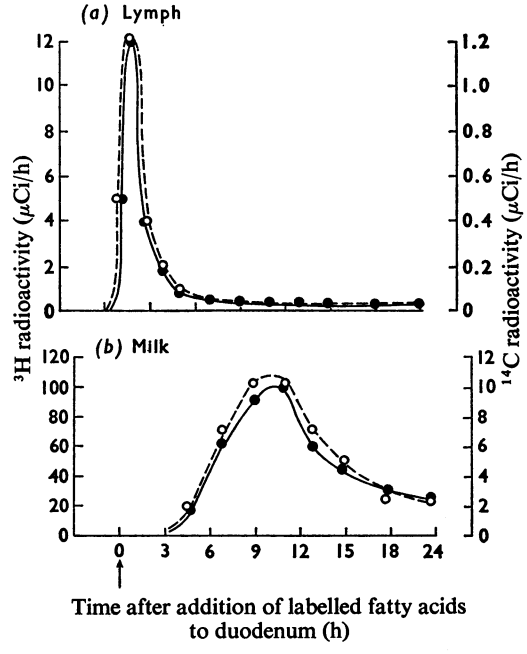

Fig. 2. *Amounts of ^{14}C radioactivity and ^{3}H radioactivity in lymph and milk of a lactating goat after duodenal administration of [1-^{14}C]stearate (33 μCi) and [9,10-^{3}H]oleate (33 μCi)*

Similar results were obtained with another lactating goat. Experimental details are given in the text. (*a*) Radioactivity in lymph; (*b*) radioactivity in milk. o, ^{14}C radioactivity; ●, ^{3}H radioactivity.

in lymph and milk. Maximum radioactivity was observed in lymph after 2.5 h and in milk after 6 h (Fig. 2). The percentages of the [1-^{14}C]stearate and

Table 7. *Extraction rate of individual fatty acids from plasma triglycerides*

Experimental details are given in the text. The extraction rate is defined as the arteriovenous differences as a percentage of the arterial concentration. The results are the means ±S.E.M. of three experiments with three goats.

Fatty acid	Arterial concn. (mg/100ml)	Extraction rate (%)
Hexadecanoate	3.15±0.14	59±2
Octadecanoate	3.30±0.42	71±5
cis-Octadecenoate	2.95±0.24	68±2
trans-Octadecenoate	0.45±0.11	69±2
Octadecadienoate	0.58±0.17	72±3
Concn. of plasma triglyceride (mg/100ml)	11.72±1.31	71±2
Milk yield (litres/day)	1.85±0.08	
Triglyceride output (mg/min)	42.2 ±2.7	
Triglyceride uptake (mg/min)	31.9 ±6.1	
Blood flow (ml/min)	523±77	

[9,10-^{3}H]oleate added to the duodenum that appeared in milk within 24h were 16.5% and 14.5% respectively. About 5% of the ^{14}C radioactivity in lymph was present as oleate, which confirmed previous results obtained *in vitro* showing that *cis*-octadecenoate is derived from the desaturation of stearate in ruminant intestinal epithelium (Bickerstaffe & Annison, 1968). This point was investigated further in experiments in which only [1-^{14}C]stearate was added to the duodenum in micellar form. In two experiments 7% and 9% of the added radioactivity appeared in lymph as oleate.

Mammary metabolism of fatty acids

The uptake of individual fatty acids from plasma lipids by the mammary gland and their incorporation into milk fat was examined in four goats by the arteriovenous-difference technique. Pairs of blood samples were withdrawn simultaneously from the carotid artery and mammary vein and assayed for plasma lipids. In agreement with previous work (Annison *et al.*, 1967) the only plasma lipid fraction to show a significant arteriovenous difference was triglyceride. The plasma triglycerides are largely hydrolysed during uptake, and the liberated fatty acids equilibrate with the plasma free fatty acid fraction during passage through the mammary gland (West *et al.*, 1972). Determination of the extraction rate of individual fatty acids from plasma triglycerides failed to demonstrate any significant difference in the selective uptake of any of the fatty acids, including the *cis*- and *trans*-isomers of octadecenoate, and linoleate (Table 7). Further, the proportions of the octadecenoate isomers in milk fat were closely similar to those in arterial and mammary venous blood (Table 5), except for the *cis*-Δ^9-isomer, which is produced in goat mammary tissue by the desaturation of stearate (Lauryssens *et al.*, 1960; Annison *et al.*, 1967).

The mammary uptake and incorporation into milk fat of *cis*- and *trans*-fatty acids was examined further by the intravenous infusion of a mixture of [9,10-^{3}H]-oleate and [1-^{14}C]elaidate. The fall in the specific radioactivity of [9,10-^{3}H]oleate and [1-^{14}C]elaidate across the gland (Table 8) was consistent with the intravascular hydrolysis of circulating triglycerides and equilibration of the released fatty acids with the plasma free fatty acids. The entry rates of the *cis*- and *trans*-octadecenoates into the plasma were calculated from the specific radioactivities of the arterial plasma free fatty acids and the infusion rate. The low entry rate of elaidate (3–14μg/min per kg body wt.) was similar to the amounts of this acid reaching the duodenum and confirmed the absence of significant synthesis of *trans*-fatty acids in mammalian tissues. Although the amounts of oleate in duodenal digesta were of the same order, the much higher entry rates of oleate (60–150μg/min per kg body wt.) reflected the extensive synthesis of this acid in tissues. Comparison of the specific radioactivity of arterial blood CO_2 with that of plasma free fatty acids showed that the contribution (0.05–0.10%) of elaidate to total oxidative metabolism was low.

The uptake of [9,10-^{3}H]oleate and [1-^{14}C]elaidate by the mammary gland and the amount secreted in milk are shown in Fig. 3. Both acids were taken up by the mammary tissue and incorporated into milk fat at closely similar rates.

Discussion

Surgically prepared lactating goats have been used in an integrated study of the quantitative aspects of biohydrogenation in relation to the production,

Table 8. *Composition and specific radioactivities of the fatty acids of arterial and mammary venous plasma triglycerides and free fatty acids during the intravenous infusion of* [9,10-^{3}*H*]*oleate* (1.142 μ*Ci/min*) *and* [1-^{14}*C*]*elaidate* (0.098 μ*Ci/min*) *into a lactating goat*

Experimental details are given in the text. The results are the means ± S.E.M. of three analyses. Specific radioactivity values in parentheses refer to a second experiment with another goat in which the rate of infusion of [9,10-^{3}H]oleate and [1-^{14}C]elaidate were 4.226 and 0.364 μCi/min respectively.

	Triglyceride				Free fatty acids			
	Arterial plasma		Mammary venous plasma		Arterial plasma		Mammary venous plasma	
Fatty acid	Composition (mol/100 mol)	Sp. radioactivity (μCi/g of acid)	Composition (mol/100 mol)	Sp. radioactivity (μCi/g of acid)	Composition (mol/100 mol)	Sp. radioactivity (μCi/g of acid)	Composition (mol/100 mol)	Sp. radioactivity (μCi/g of acid)
Tetradecanoate	2±0.2	0	2±0.2	0	2±0.2	0	3±0.2	0
Pentadecanoate	2±0.2	0	3±0.4	0	1±0.2	0	1±0.2	0
Hexadecanoate	22±1.8	0	22±2.4	0	17±1.2	0	20±1.4	0
Hexadecenoate	4±0.5	0	4±0.5	0	2±0.2	0	2±0.2	0
Octadecanoate	20±2.1	0	16±1.8	0	32±2.1	0	32±2.3	0
cis-Octadecenoate	21±1.6	52±3.4 (13±2.8)	20±1.7	38±1.9 (17±1.1)	19±1.8	326±27 (174±21)	20±1.9	198±22 (79±17)
trans-Octadecenoate	9±1.1	10±1.6 (8±0.9)	9±0.9	7±0.6 (14±0.9)	9±0.7	66±3.5 (104±14)	9±0.8	30±2.2 (24±2.0)
Octadecadienoate	17±1.3	0	20±1.7	0	13±1.4	0	10±1.0	0
Octadecatrienoate	3±0.3	0	1±0.2	0	4±0.3	0	2±0.2	0
Concn. (mg/100 ml)	13.5±1.2		10.3±0.8		4.1±0.3		3.8±0.4	

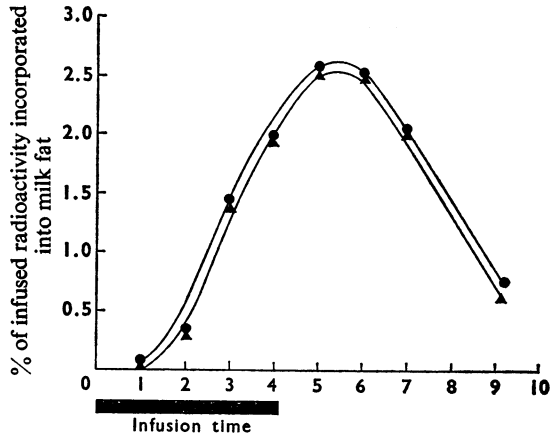

Fig. 3. *Time-course of the appearance of radioactivity in milk fat after the infusion of* [9,10-^{3}*H*]*oleate* (1.142 μ*Ci/min*) *and* [1-^{14}*C*]*elaidate* (0.098 μ*Ci/min*) *into a lactating goat*

Similar results were obtained with another lactating goat. Experimental details are given in the text. ▲, ^{14}C radioactivity; ●, ^{3}H radioactivity.

absorption and transfer into milk fat of *cis*- and *trans*-octadecenoate. In confirmation of previous work, unsaturated dietary fatty acids were extensively hydrogenated to give a complex mixture of *cis*- and *trans*-isomers (see Keeney, 1970). The predominant *trans*-isomer in all cases was *trans*-octadeca-11-enoate (vaccenate) and the major *cis*-isomer was *cis*-octadeca-9-enoate (oleate). No selectivity was observed in their absorption from the small intestine, transfer into lymph, mammary uptake or incorporation into milk fat.

Although substantial amounts of *trans*-octadecenoate and relatively low proportions of *cis*-octadecenoate were present in duodenal digesta, examination of the geometrical-isomer content of lymph showed that substantial changes had occurred in the relative proportions of the two isomers. The desaturase activity of ruminant epithelial tissue, previously demonstrated *in vitro* (Bickerstaffe & Annison, 1969), was confirmed by the appearance of ^{14}C-labelled oleate in lymph after the addition of [1-^{14}C]stearate to the duodenum. This pathway accounted in part for the increased proportion of *cis*-octadeca-9-enoate in lymph. Similarly the increase in the amount of *cis*-octadeca-9-enoate in milk fat relative to that in plasma is also due to the desaturation of stearate in mammary tissue (Lauryssens

et al., 1960; Annison *et al.*, 1967). Comparison of the positional and geometrical isomers of octadecenoate in the free fatty acid and triglyceride fraction of arterial and mammary venous blood and the rate of labelling of milk fat during the intravenous infusion of [9,10-^{3}H]oleate and [1-^{14}C]elaidate showed that the *cis*- and *trans*-fatty acids were absorbed and incorporated into milk fat to the same extent. The absence of discrimination against any particular isomer must reflect the equal susceptibility to lipoprotein lipase of triglycerides containing octadecenoates and the similar rate of uptake by mammary tissue of the products of hydrolysis. This finding is in line with work showing that *trans*-fatty acids are utilized by tissues at similar rates to the corresponding *cis*-isomers (see Bickerstaffe & Annison, 1970). Further, the hypothesis that *trans*-octadecenoates are involved in the aetiology of the low-milk-fat syndrome in dairy cows by adversely affecting either the mammary uptake of fat or milk-fat synthesis (Davis & Brown, 1970) is not supported by the present results.

Moore *et al.* (1968, 1969) showed that the intra-ruminal or intra-abomasal infusion of vegetable oils in sheep influenced the polyunsaturated fatty acid content of plasma lipids. In the latter procedure the rumen is by-passed to avoid biohydrogenation, but the results of the intra-ruminal infusions implied either that significant amounts of linoleate and linolenate escaped biohydrogenation, or that some absorption of these acids had occurred from the rumen. The possible absorption of radioactive linolenate, linoleate and oleate from the rumen, abomasum and omasum was checked in the present experiments by using a duodenal fistula to prevent the entry of radioactive gut contents into the small intestine. No evidence of the absorption of any of the fatty acids was attained, in agreement with previous work on the goat (McCarthy, 1962), sheep (Wood *et al.*, 1963) and calf (Cook *et al.*, 1969). Up to 95% of the dietary linolenate, linoleate and oleate were hydrogenated in the rumen to stearate. The stearate that reached the small intestine was readily absorbed, in contrast with the poor digestibility of stearate observed in non-ruminants (Carroll & Richards, 1958).

The extent of fatty acid synthesis in the rumen measured in five goats was in the range 0–24g/day (Table 3). Variable results were also reported by Ulyatt *et al.* (1966) and Scott *et al.* (1969). In three goats the rate of appearance of fatty acid in lymph exceeded that reaching the duodenum (Table 3), indicating that the secretion of biliary lipid (a major part of the enterohepatic circulation of fat) more than made up for the possible incomplete absorption of fatty acids flowing from the abomasum.

The amounts of linoleate flowing from the abomasum accounted for only 0.5–1.5% of the total dietary energy. In non-ruminants intakes of essential fatty acids of less than 1% of dietary energy may give rise to deficiency symptoms (Holman, 1968), but this condition has not been reported in adult ruminants. Considerably less linoleate is available to the tissue of the lactating ruminant, where 25–75% of the linoleate leaving the abomasum may be secreted in milk. There is no evidence that the supply of essential fatty acids is limiting for milk production or carcass growth in ruminants, but, since the availability is so low, ruminants have either a smaller need for essential fatty acids or an increased capacity to retain them for specific tissue functions other than oxidative metabolism. It has been shown that linoleate acid may be synthesized from *cis*-octadeca-12-enoate in the lactating goat (Gurr *et al.*, 1972) but the present data indicate that the amount of *cis*-octadeca-12-enoate in digesta flowing from the abomasum is too low to make a significant contribution to the supply of linoleate.

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