Effects of Intravenous Infusions of Acetate, β -Hydroxybutyrate, Triglyceride and other Metabolites on the Composition of the Milk Fat and Blood in Cows

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1. The effects in the cow of intravenous infusions of sodium acetate, butyrate, propionate, β -hydroxybutyrate, malonate, citrate or succinate, of glucose or of an emulsion of cottonseed oil on the secretion of the component fatty acids of milk fat and on the composition of the blood plasma of the jugular vein have been studied. 2. Glucose and cottonseed oil were the only metabolites consistently to affect the yield of milk fat. Glucose decreased the yield of milk fat through a diminished secretion of the C_{18} fatty acids and in two out of three cows also of the steam-volatile fatty acids (C_4 – C_{10}). The cottonseed oil caused an increase in the yield of milk fat through an increased secretion of linoleic acid, the major component acid of the cottonseed oil. In three out of four cows, acetate caused an increase in the yield of milk fat through an increased secretion of mainly palmitic acid. 3. The effects of the infusions on milk-fat secretion are discussed in relation to existing knowledge on the origin of the fatty acids of milk fat.

The changes in the secretion of the component fatty acids of milk fat and in the composition of the blood plasma that result from the intraruminal infusion of volatile fatty acids into lactating cows (Storry & Rook, 1965*b*) suggest that milk-fat synthesis may be sensitive to the concentration of fatty acid precursors in the blood supplying the mammary gland. This possibility has now been investigated by measuring the effects of continuous intravenous infusions into lactating cows of acetate, β -hydroxybutyrate or triglyceride, or of a number of other metabolites that could conceivably affect fat synthesis, on the secretion of the component fatty acids of milk fat. The infusions were given to animals receiving diets with a normal ratio of hay to concentrates, and infusions of acetate, β -hydroxybutyrate and triglycerides were given also to animals receiving diets containing a low proportion of hay and a high proportion of concentrates rich in flaked maize. Diets low in hay and high in flaked maize cause a fall in the milk-fat content of about 50%, and in view of the decreased concentration in the blood of precursors of milk fat observed with these diets (Storry & Rook, 1965*a*) it was thought that animals receiving them might respond more markedly to the infusions.

Preliminary findings with the intravenous infu-

sion of triglycerides have already been published as a short communication (Storry & Rook, 1964).

EXPERIMENTAL

Animals and their management. Lactating Friesian cows were housed in metabolism stalls and were established on the appropriate diets before the start of an experiment. The normal diet consisted of 14–16 lb. of hay/day and a concentrate mixture balanced for milk production given at the rate of 3–4 lb./gallon of milk produced. The low-roughage diet consisted daily of 2 lb. of hay, 15 lb. of balanced concentrates and 15 lb. of flaked maize.

Experimental design. The materials were infused for periods of 2–8 days between initial and final control periods of 2–8 days. Full experimental details are given in Table 1. Effects of the infused materials on milk-fat secretion and on blood composition were assessed by comparing mean values for the period of infusion with mean values for the control periods, but for milk fat excluding the first 2 days of the final control period to avoid any carry-over effect from the infusion.

Intravenous infusions of materials. Except for the triglyceride, which was a proprietary emulsion of cottonseed oil (Infonutrol; Astra Hewlett Ltd., Watford, Herts.), the infused materials were of A.R. grade (British Drug Houses Ltd., Poole, Dorset) and were given as an iso-osmotic solution in water. Acids were infused in the form of their sodium salts. The solutions were held in a glass reservoir fitted with an electric heating element and, except for the Infonutrol emulsion, were sterilized *in situ* before infusion. The solutions were given as a continuous infusion through

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Table 1. *Details of the animals used, the diets offered, the amount and duration of infusions and the length of preliminary and final control periods*

Expt. no.	Cow	Diet	Infusion (g./day)	Length of infusion period (days)	Length of control periods (days)	
					Preliminary	Final
1	Bugle 6	Normal	Glucose (2000)	6	4	4
	Glee 11	Normal	Glucose (1000)	2	6	4
	Grouse	Normal	Glucose (1000)	4	4	2
	Sylph	Normal	Glucose (1500)	8	4	6
2	Bride 15	Low-roughage	β -Hydroxybutyrate (500)	4	8	2
	Glee 11	Normal	β -Hydroxybutyrate (500)	4	4	4
	Katie	Normal	β -Hydroxybutyrate (500)	4	4	2
	S3	Low-roughage	β -Hydroxybutyrate (500)	4	4	4
3	Glee 11	Normal	Acetate (500)	4	4	4
	Katie	Normal	Acetate (750)	4	4	4
	M98	Low-roughage	Acetate (700)	3	4	4
	S3	Low-roughage	Acetate (500)	5	2	6
4	Bride 20*	Low-roughage	Triglyceride (700)	3	5	6
	Glee 13	Normal	Triglyceride (1000)	2	4	8
	S39	Normal	Triglyceride (1000)	2	5	5
5	Brilliant	Normal	Succinate (700)	4	5	4
	Glee 6	Normal	Succinate (750)	4	4	2
	Katie	Normal	Succinate (500)	4	6	4
6	Brilliant	Normal	Malonate (500)	4	4	4
	Gallant	Normal	Malonate (500)	4	4	—
7	Gallant	Normal	Citrate (500)	4	4	6
8	Brilliant	Normal	Butyrate (320)	3	4	5
9	Bugle 6	Normal	Propionate (250)	4	4	4

* Refused a small amount of food, and slight fall in milk yield on the first day of infusion.

a polythene cannula into the jugular vein by means of a diaphragm-operated micro pump (type S with multiple capacity selector; The Distillers Co. Ltd., Epsom, Surrey).

Sampling of blood and milk. The yield of milk was recorded, and samples of milk were taken at each milking and composite samples representing 1-day or 2-day periods were prepared for milk-fat analysis. Samples of blood were taken, through an indwelling cannula inserted in the jugular vein opposite to that used for infusion, at 09.00, 12.00 and 15.00 hr. on certain days during the control and experimental periods.

Analyses of blood and milk. Blood plasma was prepared and analysed for steam-volatile fatty acids, glucose, ketone bodies and, with infusions of glucose and triglyceride, the major lipid fractions, as described by Storrey & Rook (1965b). The fatty acids of the triglycerides of milk, and of blood plasma in the experiments in which triglyceride was infused, were determined by chromatographic separation of the acids as their methyl esters (Storrey & Rook, 1965b). In the experiments where succinate, malonate, butyrate and propionate were infused, the milk-fat content only was measured.

RESULTS

Acetate (Tables 2, 3 and 8). Except for one cow, Glee 11, the infusion of sodium acetate increased

the concentration of total volatile fatty acids and of acetate in blood plasma, but the effect was not statistically significant ($P > 0.05$). No other changes in blood composition were observed, except that with cow Glee 11 there was a significant ($P < 0.001$) decrease in glucose concentration and with cow Katie a significant ($P < 0.05$) increase in β -hydroxybutyrate concentration.

Increases in the yield of milk fat were observed in both cows receiving the normal diet and in one of the cows receiving the low-roughage diet, and in the latter the increase was highly significant ($P < 0.001$). These increases were the result mainly of an increase in the yield of palmitic acid, but in one cow the yields of butyric acid, myristic acid, stearic acid and, to a smaller extent, oleic acid and linoleic acid also were increased. There was, however, no effect on the secretion of the C_6 – C_{12} acids.

β -Hydroxybutyrate (Tables 2, 3 and 7). Significant ($P < 0.05$) increases in the concentration of β -hydroxybutyrate in blood plasma were invariably observed during the infusion of sodium β -hydroxybutyrate. There was also a tendency for the concentrations of total volatile fatty acids and of

Table 2. *Effects in lactating cows of intravenous infusions of glucose, β -hydroxybutyrate, acetate and triglyceride on the concentrations of glucose and β -hydroxybutyrate in the plasma*

Experimental details are given in the text and in Table 1. Values are means $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, where P is the probability that the observed difference could have arisen by chance.

Infusion	Cow	Glucose (mg./100 ml.)		β -Hydroxybutyrate (mg./100 ml.)	
		Control	Experimental	Control	Experimental
Glucose	Glee 11	63 $\pm$ 1.8	75 $\pm$ 2.5***	7.9 $\pm$ 0.5	3.3 $\pm$ 0.2***
	Sylph	69 $\pm$ 1.1	74 $\pm$ 2.7	6.5 $\pm$ 0.2	5.3 $\pm$ 1.1
	Bugle 6	59 $\pm$ 2.4	79 $\pm$ 3.3***	9.6 $\pm$ 1.2	6.0 $\pm$ 0.3**
β -Hydroxybutyrate	Glee 11	66 $\pm$ 1.2	62 $\pm$ 1.6	7.9 $\pm$ 0.6	18 $\pm$ 1.1***
	Katie	73 $\pm$ 1.2	74 $\pm$ 1.9	9.2 $\pm$ 1.1	28 $\pm$ 2.6***
	S3	72 $\pm$ 3.8	80 $\pm$ 4.1	5.1 $\pm$ 0.8	19 $\pm$ 1.7***
	Bride 15	68 $\pm$ 3.2	65 $\pm$ 3.3	6.4 $\pm$ 1.4	13 $\pm$ 1.9*
Acetate	Glee 11	76 $\pm$ 1.7	67 $\pm$ 0.1***	9.4 $\pm$ 0.9	8.3 $\pm$ 0.6
	Katie	67 $\pm$ 1.8	66 $\pm$ 0.9	9.2 $\pm$ 1.1	14 $\pm$ 1.2*
	M98	81 $\pm$ 1.9	81 $\pm$ 1.5	8.3 $\pm$ 1.0	6.1 $\pm$ 0.9
	S3	72 $\pm$ 3.7	64 $\pm$ 2.1	6.4 $\pm$ 0.7	6.1 $\pm$ 1.1
Triglyceride	S39	71 $\pm$ 2.1	82 $\pm$ 2.7**	5.4 $\pm$ 0.4	8.7 $\pm$ 0.9
	Glee 13	59 $\pm$ 2.9	75 $\pm$ 5.0*	7.2 $\pm$ 0.9	11 $\pm$ 2.0

Table 3. *Effects in lactating cows of intravenous infusions of glucose, β -hydroxybutyrate, acetate and triglyceride on the concentrations of the total and individual steam-volatile fatty acids in the plasma*

Experimental details are given in the text and in Table 1. Distillates from determinations of total volatile fatty acids were combined on a period basis and used for the determination of individual acids. Values for total volatile fatty acids (T.V.F.A.) are means $\pm$ S.E.M. * $P < 0.05$, where P is the probability that the observed difference would have arisen by chance.

Infusion	Cow	T.V.F.A. (m-equiv./l.)		Acetate (m-equiv./l.)		Propionate (m-equiv./l.)		Butyrate (m-equiv./l.)	
		Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
Glucose	Glee 11	0.8 $\pm$ 0.11	0.5 $\pm$ 0.08	0.78	0.49	0.02	0.01	0.02	0.01
	Sylph	0.8 $\pm$ 0.05	0.7 $\pm$ 0.05	0.74	0.65	0.02	0.02	0.02	0.02
	Bugle 6	1.5 $\pm$ 0.19	1.0 $\pm$ 0.16*	1.45	1.08	0.03	0.03	0.03	0.02
β -Hydroxybutyrate	Glee 11	0.8 $\pm$ 0.10	0.9 $\pm$ 0.15	0.75	0.82	0.02	0.02	0.02	0.02
	Katie	1.2 $\pm$ 0.18	1.3 $\pm$ 0.26	1.17	1.21	0.03	0.04	0.03	0.03
	S3	0.7 $\pm$ 0.11	1.0 $\pm$ 0.12*						
	Bride 15	0.8 $\pm$ 0.15	0.9 $\pm$ 0.28	0.71	0.77	0.08	0.08	0.05	0.05
Acetate	Glee 11	1.2 $\pm$ 0.16	1.1 $\pm$ 0.26	1.37	1.05	0.03	0.04	0.06	0.03
	Katie	0.8 $\pm$ 0.12	1.4 $\pm$ 0.32	0.86	1.36	0.03	0.03	0.03	0.03
	M98	1.1 $\pm$ 0.16	1.6 $\pm$ 0.17	0.99	1.49	0.04	0.05	0.02	0.02
	S3	0.7 $\pm$ 0.10	0.9 $\pm$ 0.13	0.65	0.84	0.03	0.03	0.02	0.02
Triglyceride	S39	0.6 $\pm$ 0.06	0.7 $\pm$ 0.09	0.52	0.64	0.01	0.01	0.02	0.02
	Glee 13	0.8 $\pm$ 0.05	0.9 $\pm$ 0.15	0.75	0.83	0.02	0.02	0.03	0.04
	Bride 20	0.8 $\pm$ 0.07	0.7 $\pm$ 0.10	0.77	0.71	0.03	0.03	0.03	0.03

acetate to be increased, but the effect was significant ($P < 0.05$) only in one cow. No consistent effect on glucose concentration was observed.

A measurable change in fat yield was observed only with cow S3, which was receiving a low-roughage diet, and this effect was not statistically significant ($P > 0.05$): there was an increased secre-

tion of nearly all component acids but the effect was most marked with myristic acid, palmitic acid, stearic acid and oleic acid. For cow Glee 11 the effect of β -hydroxybutyrate on the yields of the milk fatty acids was not measured, but the daily yields of milk fat were 756 $\pm$ 34.7 and 727 $\pm$ 7.5 g. during the control and infusion periods respectively.

Table 4. *Expts. 1 and 4: Effects in lactating cows of intravenous infusions of glucose and cottonseed oil on the concentrations of the various lipid fractions in plasma*

Experimental details are given in the text and in Table 1. Values are means $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.01$, where P is the probability that the observed difference could have arisen by chance.

Infusion	Cow	Triglyceride (mg./100 ml.)		Cholesterol ester (mg./100 ml.)		Cholesterol (mg./100 ml.)		Phospholipid (mg./100 ml.)	
		Control	Experi- mental	Control	Experi- mental	Control	Experi- mental	Control	Experi- mental
Glucose	Glee 11	6.9 $\pm$ 1.5	4.2 $\pm$ 0.1	257 $\pm$ 7.1	225 $\pm$ 17.5	39 $\pm$ 0.7	37 $\pm$ 0.4*	178 $\pm$ 7.1	137 $\pm$ 3.7**
	Sylph	5.5 $\pm$ 0.6	4.5 $\pm$ 0.6	187 $\pm$ 4.4	163 $\pm$ 3.6**	30 $\pm$ 1.4	28 $\pm$ 2.1	91 $\pm$ 16.0	71 $\pm$ 11.0
	Bugle 6	6.3 $\pm$ 1.0	4.6 $\pm$ 1.4	154 $\pm$ 12.0	132 $\pm$ 4.3	22 $\pm$ 1.6	20 $\pm$ 1.4	96 $\pm$ 6.5	56 $\pm$ 0.4**
Triglyceride	Glee 13	6.8 $\pm$ 0.9	45 $\pm$ 32.0	231 $\pm$ 8.9	221 $\pm$ 2.3	37 $\pm$ 1.3	41 $\pm$ 6.4	158 $\pm$ 4.6	184 $\pm$ 33.4
	S39	10.1 $\pm$ 1.3	51.1	303 $\pm$ 3.2	314	46 $\pm$ 0.7	57	185 $\pm$ 7.7	242
	Bride 20	6.7 $\pm$ 1.1	10.9 $\pm$ 0.8*	158 $\pm$ 25.0	190 $\pm$ 2.3	28 $\pm$ 1.4	31 $\pm$ 0.0	113 $\pm$ 8.6	132 $\pm$ 0.0

Table 5. *Expt. 4: Effects in lactating cows of a cottonseed-oil infusion on the fatty acid composition of the plasma triglycerides*

Experimental details are given in the text and in Table 1.

Cow ...	Fatty acid composition (% by wt.)					
	Glee 13			S39		
	Pre-control	Experimental	Post-control	Pre-control	Experimental	Post-control
Fatty acid						
14:0	3.0	1.0	3.8	6.5	1.9	4.6
16:0	23.7	20.3	18.2	25.6	28.1	22.5
16:1	3.0	0.9	2.6	1.9	0.9	1.5
17:0	4.4	2.0	12.7	6.3	3.3	6.2
18:0	31.3	16.0	16.0	25.3	21.7	32.8
18:1	22.6	18.7	20.6	22.9	17.2	13.0
18:2)						
18:3)	12.0	41.0	26.2	11.5	26.8	19.1

Table 6. *Expt. 1: Effects in lactating cows of intravenous infusions of glucose on the yields of milk fat and of the major individual fatty acids of milk fat*

Experimental details are given in the text and Table 1.

Cow ...	Mean yield (g./day)					
	Sylph		Bugle		Grouse	
	Control	Experimental	Control	Experimental	Control	Experimental
Milk fat...	457 $\pm$ 63	440 $\pm$ 10	486 $\pm$ 13	447 $\pm$ 47	2490 $\pm$ 50	2140
Fatty acid						
4:0	11	11	16	12	77	68
6:0	9	9	10	9	58	52
8:0	5	6	6	6	33	31
10:0	9	12	9	11	39	48
12:0	12	13	12	16	43	48
14:0	40	48	39	35	173	179
16:0	107	130	139	154	535	475
18:0	34	24	29	17	246	198
18:1 <i>cis</i>	86	64	83	52	565	416
18:1 <i>trans</i>	14	10	14	10	77	65
18:2)						
18:3)	9	6	9	6	59	38

Table 7. *Expt. 2: Effect in lactating cows of intravenous infusions of β -hydroxybutyrate on the yields of milk fat and of the major individual fatty acids of milk fat*

Experimental details are given in the text and in Table 1.

Cow ...	Mean yield (g./day)					
	Katie		Bride 15		S3	
	Control 650 $\pm$ 12	Experimental 670 $\pm$ 40	Control 531 $\pm$ 42	Experimental 460 $\pm$ 19	Control 272 $\pm$ 17	Experimental 366 $\pm$ 29
Milk fat...						
Fatty acid						
4:0	24	19	10	5	5	6
6:0	18	15	7	5	4	4
8:0	11	11	5	3	3	3
10:0	16	17	9	6	6	8
12:0	17	20	8	5	7	10
14:0	64	66	32	21	25	38
16:0	161	177	70	47	55	84
18:0	44	48	12	8	15	19
18:1 <i>cis</i>	110	101	44	33	52	67
18:1 <i>trans</i>	16	16	7	5	11	11
18:2	14	14	18	10	13	14
18:3						

Table 8. *Expt. 3: Effects in lactating cows of intravenous infusions of acetate on the yields of milk fat and of the major individual fatty acids of milk fat*Experimental details are given in the text and in Table 1. *** $P < 0.001$, where P is the probability that the observed difference could have arisen by chance.

Cow ...	Mean yield (g./day)							
	Glee 11		Katie		M98		S3	
	Control 1446 $\pm$ 6.7	Infusion 1480 $\pm$ 0.0	Control 660 $\pm$ 27	Infusion 730 $\pm$ 10	Control 116 $\pm$ 8.2	Infusion 104 $\pm$ 4.8	Control 281 $\pm$ 14	Infusion 416 $\pm$ 16***
Milk fat...								
Fatty acid								
4:0	43	48	20	23	3	3	6	9
6:0	33	37	15	16	2	2	4	6
8:0	21	20	9	10	1	1	3	3
10:0	33	32	15	15	3	2	7	7
12:0	39	40	17	18	3	3	7	8
14:0	143	144	63	71	10	10	28	41
16:0	406	435	160	183	33	31	61	102
18:0	106	102	43	53	6	4	16	26
18:1 <i>cis</i>	235	205	106	115	25	18	55	83
18:1 <i>trans</i>	31	29	14	17	3	3	8	16
18:2	21	25	13	13	4	4	13	15
18:3								

Triglyceride (Tables 2, 3, 4, 5 and 9). The infusion of triglyceride caused a markedly increased concentration of plasma triglycerides, and the concentration of other lipid fractions also tended to be increased. The Infonutrol contained 1.2% (w/v) of soya-bean phosphatides as an emulsifying agent, which may have contributed to the increased concentration of plasma phospholipids. The make-up

of the fatty acids of the Infonutrol was as follows: myristic acid, 0.5%; palmitic acid, 16.0%; palmitoleic acid, 0.4%; stearic acid, 1.6%; oleic acid, 18.0%; linoleic acid plus linolenic acid (almost entirely linoleic acid), 63.5%. There was, consequently, a definite alteration in the make-up of the fatty acids of the plasma triglycerides. The proportion of linoleic acid plus linolenic acid was markedly

Table 9. *Expt 4: Effect in lactating cows of intravenous infusions of cottonseed oil on the yields of milk fat and of the major individual fatty acids of milk fat*

Experimental details are given in the text and in Table 1. *** $P < 0.001$, where P is the probability that the observed difference could have arisen by chance.

Cow ...	Mean yield (g./day)					
	Glee 13		S39		Bride 20	
	Control	Experimental	Control	Experimental	Control	Experimental
Milk fat...	760 ± 2.1	814 ± 31	586 ± 19	654 ± 42	396 ± 17	555 ± 15***
4:0	25	19	22	23	8	10
6:0	19	16	14	14	5	7
8:0	11	10	8	8	4	4
10:0	18	18	13	12	7	7
12:0	24	24	13	10	8	6
14:0	76	69	41	30	35	28
16:0	207	194	133	122	115	112
18:0	56	46	44	40	13	11
18:1 <i>cis</i>	114	101	122	95	74	70
18:1 <i>trans</i>	22	24	17	18	13	9
18:2	16	108	19	138	28	94
18:3						

Table 10. *Expts. 5-9: Effects in lactating cows of infusions of succinate, malonate, citrate, butyrate and propionate on the yield of milk fat*

Experimental details are given in the text and in Table 1. Values are means ± S.E.M. ** $P < 0.01$, where P is the probability that the observed difference could have arisen by chance.

Infusion	Cow	Yield of fat (g./day)	
		Control	Experimental
Succinate	Katie	641 ± 13	580 ± 12**
	Brilliant	827 ± 29	828 ± 56
	Glee 6	609 ± 10	603 ± 25
Malonate	Gallant	1400 ± 30	1415 ± 45
	Brilliant	712 ± 27	677 ± 14
Citrate	Gallant	1556 ± 20	1568 ± 4
Butyrate	Brilliant	755 ± 19	681 ± 32
Propionate	Bugle 6	410 ± 7	389 ± 15

increased, the proportions of myristic acid, palmitoleic acid and stearic acid were decreased and those of palmitic acid and oleic acid were little affected.

A significant ($P < 0.05$) increase in plasma glucose concentration, which may have resulted partly from the glucose (4%, w/v) present in the emulsion, and an increase in β -hydroxybutyrate also were observed.

The yield of milk fat was invariably increased, within the range 7-40%, and the effect was most marked with the cow receiving the low-roughage

diet. The increase was due specifically to an increase in the secretion of linoleic acid plus linolenic acid. There was a compensatory decrease in the secretion of particularly myristic acid, oleic acid and stearic acid; the effects on palmitic acid and lauric acid were less marked and no consistent effect on the short-chain (C_4 - C_{10}) acids was observed.

Glucose (Tables 2, 3, 4 and 6). The infusion of glucose invariably increased the concentration of glucose in blood plasma and decreased those of β -hydroxybutyrate and of acetate. In two out of the three cows studied the effects on glucose and β -hydroxybutyrate, and in one cow the effect on acetate, were significant ($P < 0.05$). There was also a decrease in the concentration of plasma triglycerides, in confirmation of the observations of McClymont & Vallance (1962), but in no instance was the effect statistically significant ($P > 0.05$). The concentrations of other major lipid fractions (free cholesterol, esterified cholesterol and phospholipid) also tended to decrease and in certain of the cows the changes were statistically significant ($P < 0.05$).

The infusion of glucose consistently caused a decrease (4-14%) in the yield of milk fat, but none of the falls were significant ($P < 0.05$). A definite pattern of change in the secretion of individual fatty acids was found in which the saturated and unsaturated C_{18} acids were invariably decreased, and in two of the cows a decrease in the yield of C_4 - C_8 acids also was observed.

Other metabolites (Table 10). The infusions of malonate, citrate, propionate, butyrate and, except

for one cow, succinate were without significant effect on the yield of milk fat.

DISCUSSION

For economic and technical reasons, the intravenous infusions were of comparatively short duration. The considerable variations that occur from day to day in the yield of milk fat, which are unavoidable with a normal milking technique, may therefore have obscured real variations in milk-fat secretion. Additionally, the need to give the organic acids in the form of their sodium salts restricted the quantities that could be infused because of the danger of imposing a sodium imbalance. Nevertheless, distinctive responses in milk-fat secretion were observed with certain of the metabolites and it seems probable that, of the materials investigated, only the concentrations in blood plasma of glucose, triglyceride and possibly acetate have an important effect on milk-fat secretion.

The intravenous infusion of a triglyceride emulsion in the present experiments was not accompanied by the clinical signs of distress reported elsewhere (Glascok, McWeeny & Smith, 1958; Leat & Gillman, 1964), but in one cow there was a temporary refusal of a small amount of food and a slight fall in milk yield. Tove & Mochrie (1963) infused cottonseed-oil emulsions intravenously into lactating cows without untoward clinical signs and they observed a massive increase in the concentration of linoleic acid in milk fat. Our results confirm that an increase in the linoleic acid of triglycerides in the plasma is accompanied by an increased secretion of the acid in milk fat. There is evidence (Barry, Bartley, Linzell & Robinson, 1963) that under normal physiological conditions the triglycerides of the chylomicra and β -lipoproteins of the plasma are selectively taken up by the mammary gland for milk-fat synthesis. It is not known whether under the conditions of the present experiment the uptake of triglycerides was confined to this accepted physiological route. Only a comparatively small proportion (5–20%) of the infused material was secreted in the milk fat and it is probable that much of the triglyceride was removed from the circulation by the reticuloendothelial system. Histological studies (Leat & Gillman, 1964) have shown that sheep and lambs eliminate some of the fat given as intravenous emulsions by phagocytosis in the liver, lung and spleen.

The diminished secretion of particularly myristic acid, stearic acid and oleic acid, and to a smaller extent of lauric acid and palmitic acid concomitantly with the increased secretion of linoleic acid probably arose from a decrease at high plasma concentrations in the completeness of mammary hydrolysis of

plasma triglycerides. The concentration of each of the acids as triglycerides in the plasma was in fact increased, but the effect was considerably more marked with linoleic acid than with other acids. The absence of a change in the secretion of C_4 – C_{10} acids and the slight change in the secretion of lauric acid and palmitic acid are consistent with other evidence (Barry, 1964; Rook & Storry, 1964) that these acids are largely synthesized within the udder.

The decrease in the secretion of milk fat observed during the infusion of glucose agrees with the findings of Vallance & McClymont (1959). That it was the result mainly of a decreased secretion of C_{18} acids and occurred in association with a decrease in the concentration of plasma triglycerides is consistent with their hypothesis (McClymont & Vallance, 1962) that glucose infusion exerts its effect on milk-fat synthesis by diminishing the uptake by the mammary gland of plasma triglycerides, since there is previous evidence (Barry, 1964; Rook & Storry, 1964) that it is mainly the C_{18} acids that are derived from the triglycerides. In addition, however, in two of the three cows there was a lowered secretion of C_4 – C_8 acids. This, it might be argued, was the result of the decrease in these two cows in the plasma concentration of β -hydroxybutyrate. Arteriovenous-difference studies (Shaw & Knodt, 1941; Barry *et al.* 1963; Hartmann & Lascelles, 1964) have shown that β -hydroxybutyrate is taken up by the mammary gland, and the incorporation of labelled β -hydroxybutyrate into mainly C_4 – C_{10} acids has been demonstrated in the isolated perfused cow's udder (Kumar, Lakshmanan & Shaw, 1959) and in tissue preparations of the goat mammary gland (Kumar, Singh & Keren-Paz, 1965). In the present series of experiments, however, there was no consistent response in milk-fat yield, or specifically in the secretion of C_4 – C_{10} acids, to infusions of β -hydroxybutyrate and a resulting increase in its concentration in plasma, either in cows receiving a normal diet or in those receiving a low-roughage diet and in which the concentration of β -hydroxybutyrate in the plasma had been depressed. A mixture of D- and L-forms was infused and the extent to which the concentration in the plasma of the naturally occurring D-form was increased is not known.

Responses in milk-fat secretion with the infusion of acetate were much less certain than with the infusion of triglyceride or glucose. Increases in fat yield were observed both in cows receiving normal diet and in cows receiving the low-roughage diet. This was the result mainly of an increased secretion of palmitic acid, but in cow S3, which received a low-roughage diet and showed the most marked response in fat yield, measurable increases in the yields of butyric acid, myristic acid, stearic acid

and oleic acid also were obtained. The acetic acid equivalent (220–330 g./day) of the infused acetate would, however, be small in relation to that absorbed from the gut (possibly about 3–4 kg./day). This was reflected in the comparatively small increase observed in the concentration of acetate in blood plasma. A more critical evaluation of the role of acetate is obviously required.

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