Concurrent studies of the flow of digesta in the duodenum and of exocrine pancreatic secretion of calves.

7.* Influence of milk substitutes on abomasal lipolysis and biliary secretion

BY J. H. TERNOUTH†, S. Y. THOMPSON AND J. D. EDWARDS-WEBB

National Institute for Research in Dairying, Shinfield, Reading RG2 9AT

(*Received* 10 *July* 1979 – *Accepted* 26 *October* 1979)

1. The abomasal hydrolysis of lipids and the flow of endogenous (biliary) lipids was studied in two Friesian calves given four milk-substitute diets, by sampling the duodenal digesta. The diets were: reconstituted, mildly preheated, spray-dried skim-milk powder with (SKF) or without (SK) margarine fat or with 500 g/kg skim-milk powder in diet SKF replaced by soya-bean flour (ASKF) or fish-protein concentrate (BSKF) together with dried whey. The diets were given *ad lib.* twice daily from 13 to 37 d of age, each diet being given for six consecutive days. Collections of duodenal digesta from the re-entrant cannula situated caudal to the bile duct were made for 12 h after feeding the 6th and 12th meals for each diet. Samples from one collection only were subjected to detailed analysis of the lipid classes.

2. The inclusion of non-milk protein (ASKF and BSKF) not only increased the rate of passage of lipid through the abomasum but also the proportion of the lipid present as triglyceride particularly, in the first 2 h after feeding.

3. In a 12 h period, 2·3–6·3 g 'polar' lipids (mainly biliary phospholipids) were estimated to have been secreted. The rate of flow was high during the first hour after feeding and constant thereafter. The quantity of 'polar' lipid was not related to the type of milk fed or the duodenal flow of lipid.

4. When diet SK was fed, the small amounts of lipid present were extensively hydrolysed so that free fatty acids represented 700 g/kg lipid of dietary origin passing through the duodenum. When margarine fat was included in the diets (SKF, ASKF and BSKF), the free fatty acids represented only 210 g/kg lipid of dietary origin.

5. The quantities of lipid and nitrogen passing through the duodenum were poorly related to the quantities ingested at the beginning of the 12 h experimental period but were closely related to each other.

It is now well established that the rate at which protein and lipid pass out of the abomasum is influenced by the ability of the dietary proteins to clot in the abomasum (Ternouth *et al.* 1974; Ternouth *et al.* 1975). Casein forms a rubbery coagulum which entraps the fat globules so that the passage of casein and fat is delayed. Subsequently, progressive hydrolysis and liquefaction of the casein from the surface of the coagulum results in a relatively constant flow of protein and lipid through the pylorus. The use of heat-damaged milk proteins or the substitution of non-milk proteins alters normal clot formation and allows, at least initially, a more rapid abomasal outflow of protein and lipid.

To investigate the effect of these changes in lipid flow rate on the hydrolysis of dietary fat, three diets SKF, ASKF and BSKF, based on skim milk and margarine fat were fed to two Friesian calves. To vary the rate of abomasal outflow, part of the highly coagulable milk protein was replaced in two of the diets by either soya-bean (ASKF) or fish-meal protein (BSKF). A fourth diet, low in fat and based on skim milk only (SK) was used to study the effect of the level of dietary fat on the course of lipid digestion. The lipids present in digesta samples collected over a 12 h post-prandial period were analysed for triglyceride, diglyceride, monoglyceride, free fatty acids and polar lipids.

* Paper no. 6: *Br. J. Nutr.* (1978), 40, 553.

† Present address: Department of Animal Production, University of Queensland, St Lucia, Queensland 4067, Australia.

0007-1145/80/3371–2610 $01.00

In addition, information was obtained about the effects on bile secretion of the different diets and levels of dietary fat, by placing the re-entrant duodenal cannula 300 mm distal to the Sphincter of Oddi but before the pancreatic duct. Other aspects of the experiment have been reported elsewhere (Ternouth *et al.* 1975; Ternouth *et al.* 1976).

EXPERIMENTAL

Animals, diets and design

Two Friesian bull calves (A and B) were surgically prepared with duodenal re-entrant cannulas (triple technique) at 3 d of age as described by Ternouth & Buttle (1973). They were two of the four animals whose care and management were described by Ternouth *et al.* (1975). The detailed analyses reported here were completed on diet and digesta samples from one collection period for each diet. The ages of the calves at each collection period are given in Table 1. The diets with margarine fat (SKF, ASKF and BSKF) contained 210 g lipid/kg dry matter and the skim-milk diet (SK) contained 13 g lipid/kg dry matter. [^{3}H]lysine and polyethylene glycol were incorporated in the milks fed at the beginning of the collection period.

Analyses

The lipids were extracted from the diets and digesta samples by the technique of Folch *et al.* (1957) as described by Ternouth *et al.* (1975). The solvents were removed by heating on a steam bath under a stream of nitrogen. After weighing, the lipids were redissolved in a small quantity of chloroform–methanol (2:1, v/v) and stored at $-25°$.

For separating the lipid classes and estimating the fatty acids in each lipid class, the stored extracts were diluted with chloroform–methanol to give a concentration of 10 mg/ml. A 1 ml portion was used for thin-layer chromatography as described by Tuckley & Storry (1974). The fatty acids in each lipid class were estimated by gas–liquid chromatography (Edwards-Webb, 1975). For calibration, a standard solution of methyl esters of ten fatty acids ($C_{8:0}$–$C_{18:3}$) was made in proportions which simulated the composition of the margarine fat. Methyl heptadecanoate was added to the samples as an internal standard. A correction was made to each fatty acid for the small mechanical losses (approximately 10%) which occurred during the thin-layer chromatography stage. Replicate analyses showed that even for the small amounts of fatty acids present in the dietary 'polar' lipid fraction meaningful results were obtained, but the still smaller quantities of palmitoleic and linolenic acids that were observed in nearly all the lipid fractions have been excluded from the results.

The weights of chloroform–methanol extracts were corrected for the polyethylene glycol they contained, but in digesta other contaminants were evidently present, indicated by the green colour of the extracted lipids and by a greater proportion of 'polar' lipid than in the diet. For these reasons the chloroform–methanol extracts, after correction for the polyethylene glycol, are referred to as 'crude' lipid.

'Non-polar' lipid weights were calculated from the amounts of fatty acids found by thin-layer and gas–liquid chromatography by summing the calculated weights of triglyceride (TG), diglyceride (DG) and monoglyceride (MG) after allowing for the presence of the glycerol moiety, and the free acids (FFA) after correction for the loss of the short-chain acids at the evaporation stages.

The weight of fatty acids in the 'polar' lipids found on the 'thin-layer' plate was converted to the equivalent weight of phosphatidyl choline as this was considered to be the predominant polar lipid (Leat & Harrison, 1975). A 'total' lipid weight was obtained by

Table 1. *Intake and duodenal flow of lipid* (g/12 h) and recovery of nitrogen and [^{3}H]lysine by two calves when offered twice daily four reconstituted skim-milk powder diets; one without (SK) and three with added fat (SKF, ASKF, BSKF)†*

(In diet ASKF soya-bean protein, and in diet BSKF fish protein concentrate replaced 500 g/kg skim-milk powder in diet SKF)

			Intake				Duodenal flow				Recovery Duodenal flow: intake		
Diet	Calf	Age (d)	Crude	Polar	'Non-polar'	Total	Crude	Polar	'Non-polar'	Total	'Non-polar'	N	[^{3}H]-lysine
SK	A	22	14·48	0·10	8·93	9·03	19·13	3·56	3·75	7·31	0·42	0·66	0·36
	B	30	7·45	0·04	4·49	4·53	15·77	2·62	3·37	5·99	0·75	0·97	0·42
	Mean	26	11·20	0·07	6·71	6·78	18·00	3·09	3·63	6·72	0·59	0·82	0·39
SKF	A	31	93·11	0·09	78·65	78·76	81·06	6·37	52·45	58·82	0·67	0·79	0·46
	B	39	52·38	0·03	43·25	43·28	63·41	3·68	46·61	50·29	1·08	1·13	0·70
ASKF	A	19	91·77	0·22	79·74	79·96	100·76	6·28	77·22	83·50	0·97	0·93	0·89
	B	24	108·04	0·15	86·36	86·51	90·28	3·25	68·61	71·86	0·79	0·90	0·91
BSKF	A	37	52·52	0·03	42·26	42·29	68·19	4·25	47·67	51·92	1·13	1·37	0·56
	B	21	67·40	0·04	55·02	55·06	82·95	2·35	64·66	67·01	1·17	1·23	1·03
	Mean	28	77·54	0·09	64·21	64·31	81·10	4·36	59·54	63·90	0·93	1·05	0·76

* For details of lipid groups, see p. 142. † For details of diets, see p. 141.

summing the 'polar' and 'non-polar' weights. [^{3}H]lysine, polyethylene glycol and total N analyses have been described previously (Ternouth *et al.* 1975).

RESULTS

'Total' and 'non-polar' lipids

In the four diets, the quantity of 'total' lipid was consistently lower than the quantity of 'crude' lipid, the differences being 40·0 and 16·2% of the 'crude' lipid values for diet SK and the fat-containing diets (SKF, ASKF, BSKF) respectively (Table 1). In the duodenal digesta the differences between the 'crude' and 'total' lipids were greater than in the diets and indicate that approximately 3–8 g solvent-extractable material of endogenous origin appears in the duodenum in a 12 h experimental period. As described elsewhere (Ternouth *et al.* 1975), there is a lack of agreement between the intake and duodenal flow of either 'crude' or 'total' lipid.

There were only small quantities of 'polar' lipids in the diets although there was somewhat more in soya-bean diet (ASKF), but an additional 2·3–6·3 g 'polar' lipids of endogenous origin appeared in the duodenum in a 12 h period. The flow of 'polar' lipids was not related to the diet fed or to the flow of 'non-polar' lipid over the 12 h period, but was always higher for calf A than calf B. During the 12 h period the flow was high in the first hour after feeding (mean 360 mg/h) and constant thereafter (mean 250 mg/h).

There was a close relationship between the outflow:inflow value for 'non-polar' lipid (Y) and N (X). The regression was:

$$Y = 1{\cdot}026X - 0{\cdot}1511 \quad (\pm 0{\cdot}178) \quad (\text{SD } 0{\cdot}11, \text{ df } 6; P < 0{\cdot}01).$$

In the diets with added fat the pattern of duodenal flow of 'non-polar' lipid showed a high rate of outflow during the first hour after feeding, the highest occurring with diet ASKF and the lowest with diet SKF. This difference in flow rate of 'non-polar' lipid was subsequently maintained, and by 6 h, 0·5 of the weight of lipid ultimately appearing in 12 h had been collected with diets ASKF and BSKF but only 0·4 with diet SKF. These differences in flow rate were not associated with the size of the experimental meal or with calf age.

In diet SK the rate of flow of lipids was more constant throughout but increased somewhat in the 8–12 h period. These patterns of flow for palmitate, the typical long-chain fatty acid, are shown in Fig. 1(*c*). The patterns of lipid flow were not related to the flow of fluids (Ternouth, 1971).

Fatty acids in 'non-polar' and 'polar' lipids

For the 'non-polar' lipids, the fatty-acid composition of diet SK was similar to that found in butterfat with, in order of abundance, 16:0, 18:1, 18:0, 14:0 and 12:0 representing 898 g/kg fatty acids, whereas in the three diets containing margarine fat (SKF, ASKF, BSKF), the fatty acids 18:1, 16:0 and 12:0 were the most abundant and represented 732 g/kg fatty acids (Table 2). The fatty-acid composition of the duodenal digesta closely resembled that observed in the diet except that when diet SK was given there was less octanoic and decanoic acid.

The fatty-acid composition of the 'polar' lipids in the milk and digesta consisted almost entirely of four fatty acids: $C_{18:1}$, $C_{16:0}$, $C_{18:0}$ and $C_{18:2}$ (Table 3) in approximately this order of abundance. The fatty-acid composition of the gall bladder bile of two composite samples from six calves given other milk-substitute diets is also shown in Table 3 and indicates that considerable variability in bile fatty-acid composition does occur. The fatty-

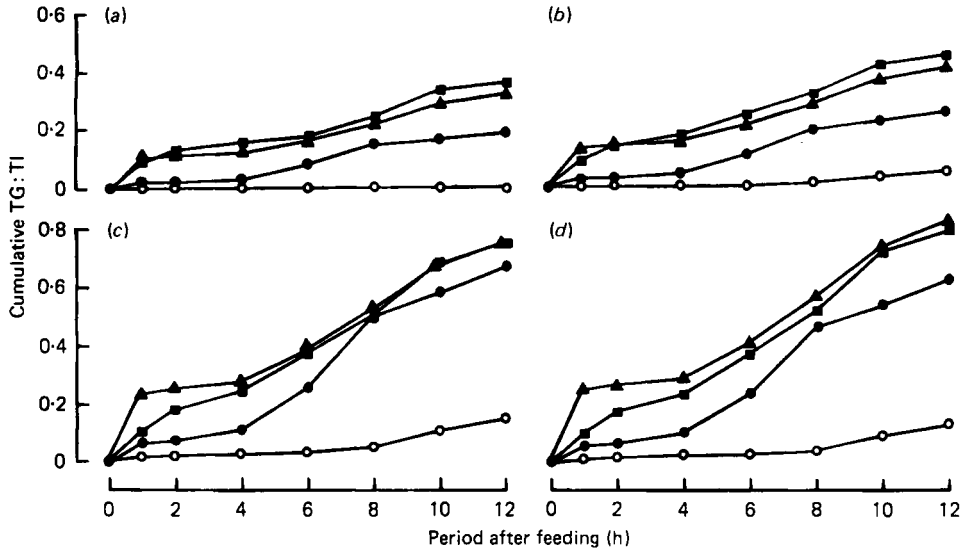

Fig. 1. Accumulating triglyceride (TG) as a proportion of the 'non-polar' lipid (Tl) collected in 12 h for (*a*) $C_{8:0}$, (*b*) $C_{12:0}$, (*c*) $C_{16:0}$, (*d*) $C_{18:1}$ fatty acids in the duodenal digesta of calves offered four diets. (○), Diet SK; (●), diet SKF, (■), diet ASKF, (▲), diet BSKF; for details of diets, see Table 1 of Ternouth *et al.* (1975).

Table 2. *Fatty-acid composition (g/kg total fatty acid) of 'non-polar' lipids of diets and duodenal digesta, for two calves offered twice daily four reconstituted skim-milk powder diets; one without (SK) and three with added fat (SKF, ASKF, BSKF)**

(In diet ASKF soya-bean protein and in diet BSKF fish-protein concentrate replaced 500 g/kg skim-milk powder in diet SKF)

Fatty acid...	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2
Diet								
SK								
Intake	22	53	112	134	285	149	218	27
Digestas	15	40	112	128	300	144	233	29
SKF, ASKF, BSKF								
Intake	23	22	166	76	198	61	368	86
Digestas	23	22	167	74	198	62	368	86

* For details of lipid groups, see p. 142.

acid composition of digesta 'polar' lipids approximated more closely to that of bile than that of diets.

Lipid classes

TG, DG, MG and FFA provided 936, 29, 1 and 34 g in diet SK and 959, 38, 1 and 3 g/kg 'non-polar' lipid in diets SKF, ASKF and BSKF. The corresponding values in the duodenal digesta were 115, 90, 94 and 701 g, and 662, 92, 38 and 207 g/kg 'non-polar' lipid respectively, showing that relatively more lipid was hydrolysed when the calves were given diet SK. The quantities of FFA and TG were inversely related as the concentrations of DG and MG together in any one sample never exceeded 250 g/kg 'non-polar' lipid for diet SK and 200 g/kg 'non-polar' lipid for diets SKF, ASKF and BSKF.

After feeding diet SK the digesta TG:'non-polar' lipid value remained low (< 100 g/kg) for the first 6 h after feeding and < 200 g/kg for the following 6 h. When margarine fat

Table 3. *Fatty acid composition (g/kg total fatty acid) of the major 'polar' lipids in diets and digesta of two calves offered four reconstituted skim-milk powder diets; one without (SK) and three with added fat (SKF, ASKF, BSKF)**

(In diet ASKF soya-bean protein and in diet BSKF fish-protein concentrate replaced 500 g/kg skim-milk powder in diet SKF)

Fatty acid...	16:0	18:0	18:1	18:2
Diet				
SK				
Intake	171	243	449	95
Digestas	237	189	413	150
SKF, ASKF & BSKF				
Intake	282	148	366	151
Digestas	280	126	308	259
Calf gall bladder bile†				
Sample A	250	172	199	320
Sample B	250	173	302	188

* For details of lipid groups, see p. 142.

† The two samples of gall bladder bile were each obtained from six milk-substitute-fed calves, approximately 10 weeks old at slaughter.

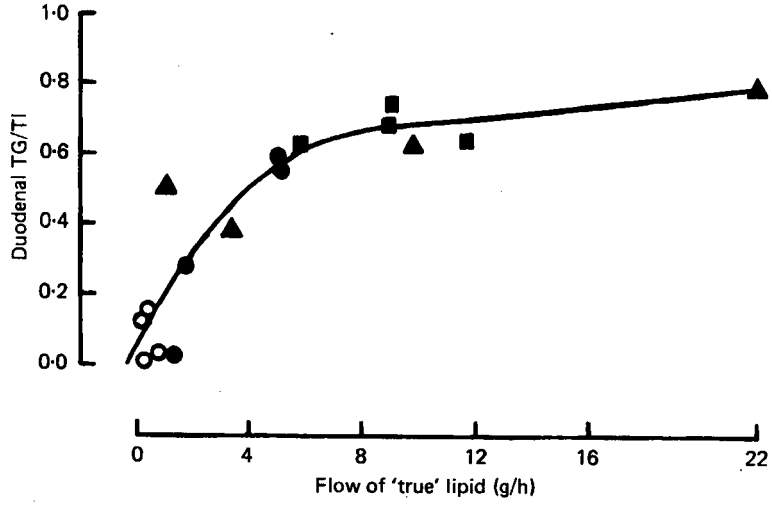

Fig. 2. Relationship during the first 2 h after feeding between duodenal digesta triglyceride:'non-polar' lipid (TG:Tl) and the flow of 'non-polar' lipid (g/h). The diets offered were, (○), diet SK; (●), diet SKF; (■), diet ASKF; (▲), diet BSKF; for details, see Table 1 of Ternouth *et al.* (1975).

was fed there was a higher TG:'non-polar' lipid value (500 g/kg) in the period 1–4 h after feeding, particularly when the diets contained non-milk proteins.

For all diets during the first 2 h after feeding there was a close relationship between the TG:'non-polar' lipid value and the 'non-polar' lipid flow rate (Fig. 2). Later in the post-prandial period the values were higher at comparable flow rates.

Fig. 1 shows for four fatty acids how the proportion of accumulating triglyceride to the 'non-polar' lipid collected in 12 h increases with time after feeding. As the fatty-acid carbon chain lengthens, the amount of fatty acid remaining as TG increases. It also shows that by

Table 4. *Linear regressions* (Y = a + bX) *relating the mean abomasal retention time* (X) *to the extent of hydrolysis of the margarine fat triglyceride* (Y) *for the individual fatty acids*

	Linear regression				In vitro half-life from Edwards-Webb & Thompson (1977)	
Fatty acid	*b*	SE	Residual standard deviation	Abomasal† triglyceride hydrolysis half-life (h)	Expt 1 (h)	Expt 3 (h)
8:0	2·90*	0·81	6·60	2·98	4·06	3·64
10:0	2·90*	0·82	6·70	4·76	4·03	3·57
12:0	2·60*	0·71	5·85	6·58	3·62	3·38
14:0	2·50*	0·76	6·26	10·87	8·33	7·57
16:0	1·03*	0·30	2·50	36·70	17·85	17·24
18:0	0·89*	0·23	1·87	37·53	31·25	26·31
18:1	0·68^{NS}	0·36	2·99	58·00	23·81	20·83
18:2	1·01^{NS}	0·49	4·00	40·80	19·23	14·71

NS, not significant.
* $P < 0·05$.
† Estimated from the regression equation when $Y = 0·5$.

the end of the 12 h collection period no $C_{8:0}$ had been collected as TG when diet SK was fed whilst more than 0·8 of the $C_{18:1}$ was still present as esterified TG when margarine fat was present in the diets. The inclusion in the diets of non-milk protein (ASKF and BSKF) increased the proportion of $C_{8:0}$ and $C_{12:0}$ appearing as triglyceride in the first 2 h after feeding.

The 12 h recoveries of [^{3}H]lysine ([^{3}H]lysine digesta/intake) (Table 1) were used to calculate the mean abomasal retention time (MART) for the 'non-polar' lipids assuming that the lipid would move with the casein fraction of the [^{3}H]lysine and that the outflow was completed in two 12 h post-prandial periods. As half the fat and radioactive material recovered in the 12 h period was collected in the first 6 h, the average retention time of the collected materials was 6 h. On the assumption that the lipid or radioactivity not collected in the first post-prandial period would be collected in the subsequent post-prandial period, then if X is the proportion of radioactivity collected in the first post-prandial period, MART is:

$$\text{MART} = 6 \times X + 18\,(1 - X),$$

when 6 and 18 are the mean recovery times for the lipids leaving the abomasum in the first and second 12 h post-prandial periods. This simplifies to:

$$\text{MART} = 18\,(1 - 0·67X).$$

Thus the MART represents an estimate of the period of time a typical long-chain fatty-acid group remained in the abomasum. When the retention times were compared with the extent of hydrolysis of the individual fatty acids from the margarine fat in the duodenal digesta, significant linear regressions were found for $C_{8:0}$–$C_{18:0}$ fatty acids (Table 4). Oleic and linoleic acids had non-significant regressions between abomasal TG half-life and retention time but $C_{16:0}$ and all the C_{18} acids had very long TG hydrolysis half-lives.

Since in digesta, assuming that all the lipid in the diet was initially present as TG, 'non-polar' lipid minus TG represents the TG hydrolysed and the latter value minus DG represents diglyceride hydrolysed, it is possible to calculate for each fatty acid the proportions of TG and DG hydrolysed, and the values obtained are shown in Table 5. In addition Table 5 gives the values for DG hydrolysed:TG hydrolysed, and these show that when

Table 5. *Proportion of triglyceride (TG) hydrolysed to diglyceride (DG) and DG hydrolysed to monoglyceride for individual fatty acids in the duodenal digesta of calves given skim milk (SK) and three milks containing margarine fat (SKF, ASKF and BSKF)**

	Fatty acids							
	C_8	C_{10}	C_{12}	C_{14}	C_{16}	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
Diet								
SK (*n* 2)								
TG hydrolysed†	1·00	0·98	0·95	0·90	0·85	0·85	0·87	0·91
DG hydrolysed‡	1·00	1·00	0·95	0·87	0·79	0·76	0·87	0·94
DG hydrolysis:TG hydrolysis	1·00	1·02	1·00	0·97	0·92	0·89	1·00	1·04
SKF, ASKF and BSKF (*n* 6)								
TG hydrolysed	0·71	0·66	0·63	0·53	0·27	0·32	0·21	0·22
DG hydrolysed	0·93	0·90	0·77	0·65	0·44	0·36	0·45	0·49
DG hydrolysis:TG hydrolysis	1·33	1·38	1·25	1·23	1·63	1·10	2·19	2·30

* For details of lipid groups, see p. 142.

† TG hydrolysed = (Tl–TG)/Tl, where Tl and TG are the molar quantities of non-polar and TG passing through the duodenum in a 12 h period.

‡ DG hydrolysed = (Tl–TG–DG)/(Tl–TG), where Tl, TG and DG are the molar quantities of non-polar, TG and DG passing through the duodenum in a 12 h period.

small amounts of fat are present (diet SK) similar proportions of DG and TG were hydrolysed, whereas when margarine fat was added to the diets (SKF, ASKF and BSKF) relatively more DG than TG was hydrolysed. This was especially marked for oleic and linoleic acids.

DISCUSSION

Recoveries in the duodenal digesta of 'non-polar' lipid and N varied between 0·42 and 1·17 and 0·66 and 1·37 respectively of the amounts ingested at the beginning of the 12 h experimental period. Comparisons have been made between the fatty-acid composition of diets and digesta; when the margarine fat was fed the proportions of the individual fatty acids in the digesta were the same as in the diets. However, when diet SK was fed, the proportions of C_8 and C_{10} in the digesta were less, and of $C_{16:0}$, $C_{18:1}$ and $C_{18:0}$ greater than in the diets. As the small quantities of 'non-polar' lipid in the digesta when diet SK was fed were mainly present as FFA, either the short-chain acids C_8 and C_{10} were being absorbed (Edwards-Webb & Thompson, 1978) or the longer-chain acids increased from endogenous sources.

Some of the differences between the 'crude' and 'total' lipids in the duodenal digesta were likely to be due to pigments, conjugated and unconjugated bile acids, cholesteryl esters and cholesterol, which are all extracted by chloroform–methanol (Lennox *et al.* 1968). Our extracts, because they were washed with saline (9 g sodium chloride/l), contained less conjugated bile acids than might have been expected from the value of 14:1 for conjugated bile acids:phospholipids found in the cranial duodenum of sheep by Lennox *et al.* (1968). Nevertheless the residual chloroform–methanol extractable material may explain the discrepancy between 'crude' and 'total' lipids in duodenal digesta.

The 'polar' lipids in the digesta were predominantly biliary phospholipids as judged by their fatty-acid composition relative to calf bile (Table 3), lamb and steer bile (Adams & Heath, 1963) and sheep bile (Lennox *et al.* 1968). The quantity of endogenous 'polar' lipids flowing through the duodenum (2·3–6·3 g/12 h) is comparable to the 10–15 g/d calculated for sheep (Adams & Heath, 1963). 'Polar' lipids (phospholipids) represented nearly half the total lipid flowing through the duodenum and hence available for absorption in the skim-milk-fed calf as suggested by Shannon & Lascelles (1969). The level of 'polar'

(biliary) lipid in the digesta was not influenced by the level of fat ingested. Calf A had not only more 'polar' lipids in the duodenal digesta but more sodium and chloride ions (mainly of abomasal origin). Calf A also secreted more pancreatic fluid, trypsin, chymotrypsin, protease, amylase and lipase than calf B (Ternouth *et al.* unpublished observations).

Our in vivo results agree with previous in vitro studies (Hamilton & Raven, 1973; Edwards-Webb & Thompson, 1977) that salivary lipase strongly prefers to hydrolyse the ester bond of the shorter-chain fatty acids. The estimate of the time taken to liberate half the shorter-chain ($< C_{16}$) fatty acids is less than the mean abomasal residence time for all the lipids of coagulating milk substitutes, whereas $C_{\geqslant 16}$ fatty acids have considerably longer hydrolysis half-lives. Hence for these longer-chain acids salivary lipase does not appear to be important at least when the levels of margarine fat in the diets are similar to those in this experiment (20 g/l). When skim milk (diet SK) was fed the small quantities of longer-chain fatty acids present were extensively liberated in the time the lipid remained in the abomasum due probably to the greater relative amount of lipase present.

The hydrolysis of the TG to MG is a two-step process, so that accumulation of DG would indicate that salivary lipase had a preference for TG over diglycerides. Both Richardson & Nelson (1967) and Edwards-Webb & Thompson (1977) have found that in vitro salivary lipase more readily hydrolyses TG than DG. Analysis of the abomasal content of calves (Siewert & Otterby, 1968; Edwards-Webb & Thompson, 1978) and gastric contents of rats and human infants (Olivecrona *et al.* 1973; Fredrickson & Hernell, 1977) shows relatively large concentrations of DG but little MG. Gooden (1973) found that there was little difference in the weights of FFA and DG released by either salivary or pancreatic lipase from washed milk globules, indicating considerable hydrolysis of the DG by both enzymes. The duodenal digesta samples in the present experiment contained considerably more FFA than DG with appreciable amounts of MG. Further, values for DG hydrolysis:MG hydrolysis greater than unity indicated that considerable DG hydrolysis had occurred. We have no explanation for these apparently conflicting results.

The extent of abomasal proteolysis of milk protein has been shown to be influenced by the period of time the proteins remain in the abomasum (Tagari & Roy, 1969; Ternouth *et al.* 1975, 1976; Johnson & Liebholz, 1976). In our experiments, failure of the diet to clot due to the inclusion of non-milk proteins results in the abomasal outflow of large amounts of lipid in the early post-prandial period, so that lipid outflow and the TG:'non-polar' lipid values were related. The peak flow of esterified fatty acids in the thoracic duct of young calves given fatty whey with little coagulable protein has been shown to occur 3 h earlier than when whole milk was given (Gooden *et al.* 1971). In addition, Gibney & Walker (1977) found for preruminant lambs that digestibilities of the fatty acids of coconut oil were lower (especially the longer-chain acids) when soya-bean-protein-based diets rather than milk-protein-based diets were fed. These digestibilities may be related to those for abomasal lipolysis, as Hamilton & Raven (1973) have concluded that hydrolysis by salivary lipase is of major importance in determining over-all digestibility. Over one-third of the fatty acids in our margarine fat had chain lengths shorter than C_{16}. For these shorter-chain fatty acids the differences in the quantity of lipids hydrolysed in the abomasum early in the post-prandial period were maintained throughout the experimental period, so that the difference in the ability of the salivary lipase to hydrolyse lipid is dependent upon the period of time the lipid remains in the abomasum. The longer-chain fatty acids are only poorly liberated within the MART. Thus the hydrolysis of a margarine fat by calves is aided by long MART values, high content of shorter-chain fatty acids and probably by the positional arrangement of the fatty acids on the glycerol molecule (Nelson *et al.* 1977).

REFERENCES

Adams, E. P. & Heath, T. J. (1963). *Biochim. biophys. Acta* **70**, 688.
Edwards-Webb, J. D. (1975). *J. Sci. Fd Agric.* **26**, 1943.
Edwards-Webb, J. D. & Thompson, S. Y. (1977). *Br. J. Nutr.* **37**, 431.
Edwards-Webb, J. D. & Thompson, S. Y. (1978). *Br. J. Nutr.* **40**, 125.
Folch, J., Lees, M. & Stanley, G. H. S. (1957). *J. biol. Chem.* **226**, 497.
Fredrickson, B. & Hernell, O. (1977). *Acta Paediat. scand.* **66**, 479.
Gibney, M. J. & Walker, D. M. (1977). *Aust. J. agric. Res.* **28**, 703.
Gooden, J. M. (1973). *Aust. J. biol. Sci.* **26**, 1189.
Gooden, J. M., Brandon, M. R., Hartmann, P. E. & Lascelles, A. K. (1971). *Aust. J. biol. Sci.* **24**, 1309.
Hamilton, R. K. & Raven, A. M. (1973). *J. Sci. F d Agric.* **24**, 257.
Johnson, R. J. & Leibholz, J. (1976). *Aust. J. agric. Res.* **27**, 903.
Leat, W. M. F. & Harrison, F. A. (1975). In *Digestion and Metabolism in the Ruminant*, p. 481 [I. W. McDonald and A. C. I. Warner, editors]. Armidale: University of New England Publishing Unit.
Lennox, A. M., Lough, A. K. & Garton, G. A. (1968). *Br. J. Nutr.* **22**, 237.
Nelson, J. H., Jensen, R. G. & Pitas, R. E. (1977). *J. Dairy Sci.* **60**, 327.
Olivecrona, T., Hernell, O., Egelrud, T., Billstrom, A. Helander, H., Samuelson, G. & Fredrickson, B. (1973). In *Dietary Lipids and Postnatal Development*, p. 77 [C. Galli, G. Jacini and A. Peccile, editors]. New York: Raven Press.
Richardson, G. H. & Nelson, J. H. (1967). *J. Dairy Sci.* **50**, 1061.
Shannon, A. D. & Lascelles, A. K. (1969). *Aust. J. biol. Sci.* **22**, 197.
Siewert, K. L. & Otterby, D. E. (1968). *J. Dairy Sci.* **51**, 1305.
Tagari, H. & Roy, J. H. B. (1969). *Br. J. Nutr.* **23**, 763.
Ternouth, J. H. (1971). Studies of the role of the abomasum and pancreas in digestion in the young calf. PhD Thesis, University of Reading.
Ternouth, J. H. & Buttle, H. L. (1973). *Br. J. Nutr.* **29**, 387.
Ternouth, J. H., Roy, J. H. B. & Shotton, S. M. (1976). *Br. J. Nutr.* **36**, 523.
Ternouth, J. H., Roy, J. H. B. & Siddons, R. C. (1974). *Br. J. Nutr.* **31**, 13.
Ternouth, J. H., Roy, J. H. B., Thompson, S. Y., Toothill, J., Gillies, C. M. & Edwards-Webb, J. D. (1975). *Br. J. Nutr.* **33**, 181.
Tuckley, B. & Storry, J. E. (1974). *Lipids* **9**, 493.

Printed in Great Britain