Studies on lipid digestion in the preruminant calf

3*. The action of salivary lipase on milk fat in the abomasum

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1. The lipolysis of cow's milk fat by salivary lipase (*EC* 3.1.1.3) in the preruminant calf has been studied in vitro by a simulated abomasal digestion, and also in vivo by examining the abomasal effluent collected over 12 h after giving whole milk to a calf.

2. In the in vitro experiment the liquid drained from the clot contained a higher proportion of short-chain fatty acids than the abomasal effluent in the in vivo experiment. This was considered to indicate the absorption of short-chain free fatty acids from within the abomasum.

3. Preferential release of short-chain fatty acids both in vitro and in vivo was observed.

4. The outflow of butyric acid from the abomasum of the calf was initially rapid, but had levelled off at approximately 6 h, whereas the outflow of a typical long-chain fatty acid (palmitic) was fairly constant over the 12 h.

Butyric acid predominated in the free fatty acids of abomasal effluent 0·5 h after feeding (668 mmol/mol total free fatty acids) but had become a minor component by 12 h (15 mmol/mol total free fatty acids).

5. The mean amounts of free and esterified fatty acids (mmol/mol fatty acid ingested) present in the abomasal effluent from the 12 h collection period were: triglyceride 465, diglyceride 215, monoglyceride 68, free fatty acid 252. These values showed that only one-third of esterified fatty acids ingested are lipolysed to absorbable products by salivary lipase.

It is now well established that calf salivary lipase (*EC* 3.1.1.3) has little ability to liberate long-chain fatty acids from milk fat (Grosskopf, 1965) and that its action differs in this respect from that of calf pancreatic lipase (Harper, 1957; Ramsey & Young, 1961; Edwards-Webb & Thompson, 1977). We have shown that the extent of lipolysis in vitro of long-chain ester bonds by salivary lipase was low at several different enzyme concentrations, and periods of incubation up to 2 h, yet Gooden & Lascelles (1973) reported that the milk-fed calf was able to absorb quite large amounts of long-chain fatty acids from milk fat in the absence of pancreatic lipase action; the greater part of the ingested milk fat must therefore have been lipolysed to free fatty acids and monoglyceride, which are known to be the forms in which fats are absorbed in the non-ruminant or preruminant animal (Mattson & Volpenhein, 1964; Harrison & Leat, 1975). We have shown (Toothill, Thompson & Edwards-Webb, 1976) that only salivary lipase is responsible for lipolysis in the abomasum. It is possible that in vivo the pattern of lipolysis might differ from that previously observed in vitro and lead to the production of relatively more long-chain free fatty acids and monoglycerides. For instance, in the abomasum the presence of proteolytic enzymes alters the physical state of the substrate and as the milk clots the fat droplets are trapped in the curd and emptying into the duodenum is delayed. Thus the lipid is not only exposed for longer to the action of salivary lipase but is at a more favourable pH for the action of the enzyme (Hill, Noakes & Lowe, 1970).

We have therefore carried out a further in vitro experiment under conditions simulating those in the abomasum. We have also studied the contribution of salivary lipase to fat digestion in the calf by determining the pattern of lipolysis of milk fat in the abomasum by following the outflow of fatty acids into the duodenum over a 12 h period and the

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distribution in abomasal effluent of free and esterified (triglyceride, diglyceride and monoglyceride) fatty acids.

EXPERIMENTAL

Expt 1. *Simulated abomasal digestion in vitro*

For this procedure the following materials were used: salivary lipase reconstituted by redissolving (1:10, v/v) the product obtained by freeze-drying the effluent collected from an oesophageal fistula while a calf was drinking water.

Abomasal fluid was obtained via an abomasal fistula from a 127-d-old milk-fed calf 8 h after giving a feed of separated milk. This served as a source of proteolytic enzymes and of acid.

To 250 ml whole milk at 39° was added 12·5 ml reconstituted calf saliva, followed by 50 ml abomasal fluid. A clot formed immediately and the mixture (pH 5·9) was poured into a glass tube measuring approximately 270 × 48 mm, lined with a Terylene-net bag which allowed liquid but not clot particles greater than approximately 0·5 mm to pass through. The lower end of the tube was constricted and fitted with a short length of PVC tubing and a screw-clip. The upper end was closed with a rubber bung which, because it was fitted inside the net bag, held the latter against the walls of the glass tube.

The mixture was incubated at 39°, with occasional shaking, for 8 h and at intervals material was withdrawn via the lower outlet, and 0·1 M-hydrochloric acid was added through a closable hole in the bung. The experimental procedure was as follows.

Period of incubation (h)	0·1 M-HCl added (ml)	pH of mixture	Volume of liquid removed (ml)
0	—	5·9	—
1	14	5·0	60
2	14	4·0	60
4	14	3·0	60
8	14	—	60

After 8 h the mixture remaining in the Terylene-net bag was removed and blended. All samples were treated with methanol in the same way as the calf digesta samples (see below).

Expt 2. *Study of abomasal effluent in vivo*

Surgery and collection of digesta. Digesta samples were taken from a male Friesian calf at 101, 108 and 115 d of age via a re-entrant duodenal cannula. This had been fitted immediately posterior to the pyloric sphincter when the calf was 4 d old by the method of Brown, Armstrong & MacRae (1968). The calf was given four feeds of separated milk in the 2 d before the experimental meal to ensure a low level of fat in the abomasum at the beginning of each collection period. The experimental meal consisting of 5 l whole milk was given at 09.00 hours, and the volume of digesta flowing from the proximal cannula was measured over the next 12 h. Digesta was returned through the distal end of the cannula during the course of collection at approximately the same rate as it was collected, but 'spot' samples of approximately 50 ml were taken at 0·5, 1, 2, 4, 6, 8, 10 and 12 h after feeding. To compensate for the digesta removed, an equal volume of a solution consisting of sodium chloride (80·6 g) glucose (50·0 g) and 11 M-HCl (1·75 ml) made up to 1 l in distilled water, was added to the digesta returned via the distal cannula. The samples of digesta were made up to twice their volume with methanol to prevent further enzyme action before storage at 2° until required for analysis.

Table 1. *Expts* 1 *and* 2.* *Proportions (mmol/mol) (A) of total fatty acids originally present, recovered during a simulated abomasal digestion of cow's milk with salivary lipase (EC* 3.1.1.3*) and abomasal fluid for* 8 *h (Expt* 1*) and in abomasal effluent collected during* 12 *h after giving cow's milk to a preruminant calf (Expt* 2*) and (B) of fatty acids found as free fatty acids in liquid removed (Expt* 1*), and in abomasal effluent (Expt* 2*)*

(Values obtained in Expt 2 are the means for three feeding experiments)

Fatty acid ...	Expt no.	4:0	6:0	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3†	10:0–18:3	4:0–18:3
						(A)									
Recovered in liquid removed:															
+Clot	1	1007	858	868	895	912	880	872	992	902	784	864	670	—	880
Alone	1	624	412	354	360	395	345	347	324	353	317	333	270	—	379
Recovered in abomasal effluent	2	277	340	465	519	526	527	516	450	534	488	513	404	—	475
SE		26	42	64	73	80	79	72	43	85	51	45	65	—	61
						(B)									
Proportion as free fatty acid:															
In liquid removed	1	966	702	408	381	423	241	114	336	62	92	137	290	168	360
In abomasal effluent	2	897	505	359	401	433	275	138	276	85	111	166	429	188	252
SE		22	21	21	12	12	13	9	48	14	8	11	36	11	12

* For experimental details, see p. 126.

† Relative retention time identical with that of authentic linolenic acid, but may contain 18:2 isomers.

Analytical methods

Lipid and fatty acids analyses were carried out as described by Edwards-Webb & Thompson (1977). Fatty acids were identified by comparing relative retention times with those of authentic standards. Such tests do not necessarily differentiate between linolenic acid ($C_{18:3}$) and some $C_{18:2}$ isomers.

Statistical analysis

For each set of results in Expt 2 (e.g. values for $C_{6:0}$ (Fig. 2 and Table 2), the triglyceride values (Fig. 3) analyses of variance were performed and standard errors calculated from the 'period after feeding × experiment' interaction mean square with 6 df (Fig. 2 and Table 2) or 14 df (Fig. 3).

RESULTS

Expt 1

When the results for the liquid removed at the different intervals and those for the clot remaining in the incubation tube were considered together (Table 1 (A)) it was found that the total recovery of the fatty acids was 880 mmol/mol originally present. Losses appeared to be unrelated to chain length. Values for butyric acid, caproic acid and caprylic acid were 1007, 858 and 868 mmol/mol respectively. However, when only the liquid removed was considered the recovery of short-chain acids appearing in the 8 h period were (mmol/mol fatty acid originally present): butyric 624, caproic 412, whereas for all acids the corresponding value was only 379.

The proportions of fatty acids found as free fatty acid in the liquid removed are given in Table 1(B). The values for butyric and caproic acids were 966 and 702 mmol free fatty acid/mol total fatty acid respectively. However, when all fatty acids were considered the proportion as the free fatty acid was only 360 mmol/mol total fatty acids, and for $C_{10:0}-C_{18:3}$ acids it was lower still at 168 mmol/mol total fatty acids.

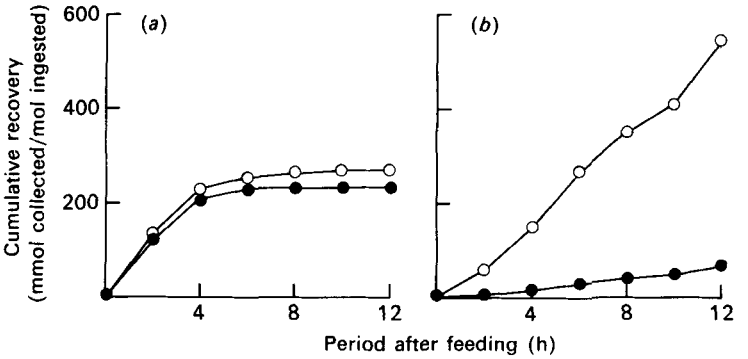

Fig. 1. Expt 2. Cumulative recovery (mmol collected/mol ingested) of butyrate (*a*) and palmitate (*b*) in abomasal effluent, from a calf in 12 h after giving a single feed of cow's milk. ○, Total fatty acid; ●, free fatty acid. For experimental details, see p. 126.

Table 2. *Expt 2.* Molar proportions (mmol/mol) of the free fatty acid present in abomasal effluent at 1, 4, 8 and 12 h after feeding cow's milk to a preruminant calf*

(Values are means of three feeding experiments)

	Period after feeding (h)				
Fatty acid	1	4	8	12	SEM (6 df)
4:0	668±30†	411±41†	56±22†	15±3†	
6:0	127	126	45	22	5·0
8:0	17	33	43	35	2·3
10:0	29	62	122	117	5·9
12:0	28	64	119	121	4·9
14:0	38	97	194	213	7·9
16:0	52	116	234	267	8·9
16:1	nd	12	30	32	2·9‡
18:0	14	19	28	35	2·8
18:1	20	45	100	112	5·2
18:2	6	11	22	24	1·2
18:3‖	nd	4	7	7	1·2‡

nd, Not detected.

* For experimental details, see p. 126.

† Standard errors of the individual means are given since there was a suggestion of variance heterogeneity.

‡ 4 df.

‖ Relative retention time identical with that of authentic linolenic acid, but may contain 18:2 isomers.

Expt 2

Proportion of fatty acid intake recovered in 12 h. Table 1 (A) gives the proportions of intake of fatty acids that were recovered in abomasal effluent in 12 h. The total recovery of fatty acids was 475 mmol/mol ingested, and for long-chain acids, except palmitoleic and 'linolenic', the value was approximately 520 mmol/mol. However, for butyric and caproic acids the values were considerably lower: 277 and 340 mmol/mol ingested respectively.

Proportions of fatty acids collected as free fatty acids in 12 h. The proportions of fatty acids that were found unesterified during the entire 12 h collection are shown in Table 1 (B). Although most of the butyrate and approximately half the caproate entering the duodenum were present as the free fatty acids, the proportion for all fatty acids was only 252 mmol/mol total fatty acids, and for $C_{10:0}-C_{18:3}$ acids the proportion was 188 mmol/mol total fatty acids.

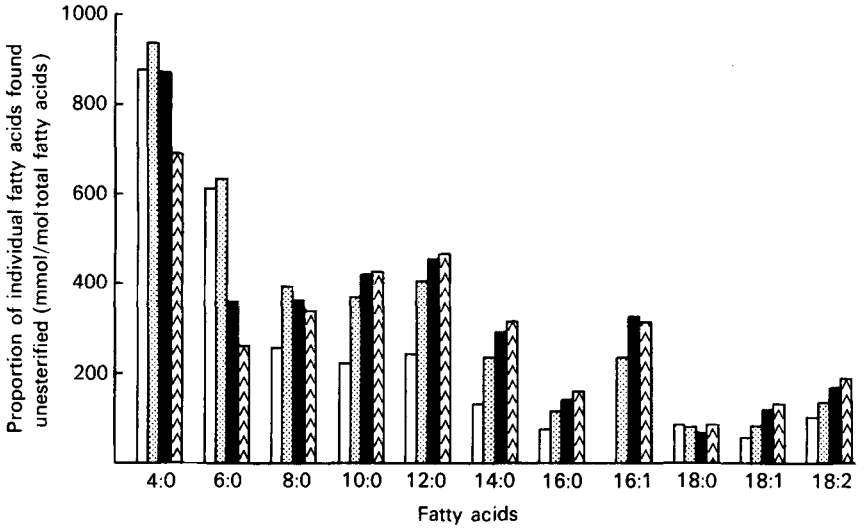

Fig. 2. Expt 2. Effect of period after feeding (h) on the molar proportions (mmol/mol total fatty acid) of individual fatty acids found unesterified in abomasal effluent taken 1, 4, 8 and 12 h after giving cow's milk to a preruminant calf. □, 1 h; ▨, 4 h; ■, 8 h; ▨, 12 h. Standard errors were 41 (4:0), 44 (6:0), 33 (8:0), 22 (10:0), 22 (12:0), 14 (14:0), 7 (16:0), 61 (16:1) (based on 3 df), 13 (18:0), 6 (18:1) and 14 (18:2). Differences between values for different periods after feeding were significant ($P < 0.05$) for all fatty acids except 16:1 and 18:0. 16:1 had a missing value at 4 h. For experimental details, see p. 126.

Patterns of outflow of butyrate and palmitate. In order to show the difference in outflow patterns between butyric acid and a typical long-chain fatty acid the cumulative recoveries of butyrate and palmitate, both the total and as free fatty acid for a single feeding experiment are shown in Fig. 1. The outflow of butyrate was rapid during the first 4 h of the collection, and then levelled off, the acid occurring mainly in the free form. Palmitate by contrast appeared at a fairly constant rate, with only a small proportion as the free fatty acid.

The effect of period after feeding on the relative proportions of free fatty acids. The relative proportions of the free fatty acids at 1, 4, 8 and 12 h are shown in Table 2. Initially butyric acid (668 mmol/mol total free acids) was the predominant fatty acid, but gradually it contributed a smaller proportion, until at 12 h it was only a minor constituent (15 mmol/mol total fatty acids). The concentration of longer-chain acids gradually increased during the 12 h, particularly capric acid and lauric acid which increased approximately fourfold, and myristic, palmitic and oleic acids which increased approximately fivefold. Stearic acid although doubling in concentration remained low throughout.

The effect of period after feeding on the proportion of individual fatty acids found as free fatty acids. For individual fatty acids the amount of free fatty acid expressed as a proportion of the total amount of that fatty acid (free and esterified) is given in Fig. 2. At all sampling intervals butyrate was mainly present as the free fatty acid. Initially the proportion (mmol free fatty acid/mol total fatty acid) was 900, but by 12 h this value had decreased to 700. The proportion of free caproic acid (mmol free fatty acid/mol total fatty acid) also decreased, from approximately 600 at 1 and 4 h to 350 at 8 h, and 250 at 12 h. All other fatty acids, except stearic acid, showed increases in the proportion present as free fatty acid passing from the abomasum, although none approached the proportion found for butyric acid. For stearic acid the proportion remained fairly constant at less than 100 mmol free fatty acid/mol total fatty acid throughout the 12 h period.

Distribution of fatty acids in lipid classes. The change in molar distribution of fatty acids

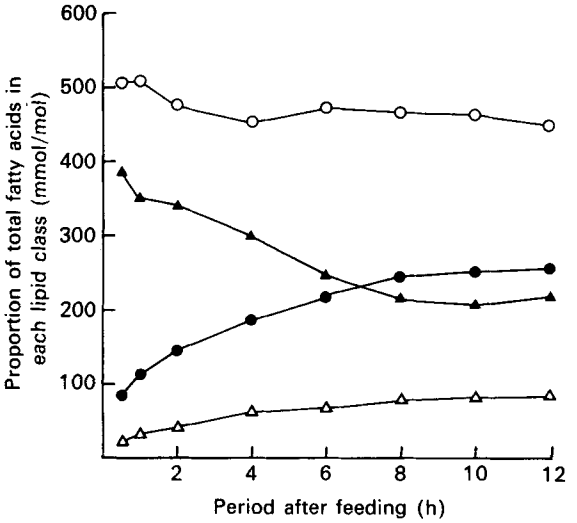

Fig. 3. Expt 2. Effect of period after feeding (h) on the molar proportions (mmol/mol total fatty acids) of fatty acids present in the lipid classes of abomasal effluent of a calf in 12 h after feeding cow's milk. ○, Triglyceride; ●, diglyceride; △, monoglyceride; ▲, free fatty acid. Standard errors were 18·3 (triglyceride), 7·6 (diglyceride), 2·2 (monoglyceride) and 25·2 (free fatty acid). Except for triglycerides the proportions of total fatty acids in each class changed significantly with the period after feeding ($P < 0.001$). For experimental details, see p. 126.

present as triglyceride, diglyceride, monoglyceride and free fatty acid in abomasal effluent with the period after feeding is shown in Fig. 3. The triglycerides contained about half the total fatty acids at all sampling intervals. The free fatty acids initially accounted for almost 400 mmol/mol total fatty acids, but this proportion gradually decreased to slightly more than 200 mmol/mol total fatty acids at 8 h and remained at this level up to 12 h. By contrast the proportion of diglyceride-fatty acid (mmol/mol total fatty acid) increased from less than 100 to approximately 250 mmol/mol total fatty acids, and monoglyceride showed a small increase from 20 to 80 mmol/mol total fatty acids.

DISCUSSION

The results of the in vitro experiment confirmed the previous finding of a preferential release of the short-chain fatty acids by salivary lipase (Edwards-Webb & Thompson, 1977), and the high over-all recoveries of the individual fatty acids established the validity of the analytical technique.

For the in vivo experiment, to ensure as far as possible that lipid material collected in the abomasal effluent was derived mainly from the experimental meal, the calf was maintained on skim milk for the previous 2 d. In this way the lipid content of prefeeding digesta was reduced from a normal value of approximately 40 to 2·6 g/l. The results obtained using this technique showed that approximately half the total fatty acids in the meal fed appeared in the abomasal effluent in the subsequent 12 h, a finding that was in good agreement with that reported by Ternouth, Roy, Thompson, Toothill, Gillies & Edwards-Webb (1975). However, recoveries differed for individual fatty acids and although approximately half the individual longer-chain fatty acids were recovered, for butyric and caproic acids the proportions recovered were only 0·28 and 0·34 respectively. The concentrations of these fatty acids in digesta were initially high, but decreased to low values in the last 4 h of the collection period, suggesting that little was left in the abomasum at 12 h. This was confirmed (Edwards-

Webb & Thompson, unpublished observations) by analysis of the abomasal contents of a preruminant calf, slaughtered 12 h after a feed of cow's milk. The butyric acid in the fat in the stomach clot of this calf was present as the free fatty acid and its concentration was only 12 mmol/mol total fatty acids, compared with 110 mmol/mol total fatty acids in the milk fat given.

The marked differences between the recoveries of the short-chain fatty acids in the in vitro and in vivo experiments were unexpected, and suggest that absorption of these fatty acids occurs in the abomasum. Indeed the rapidity and near completeness of lipolysis of butyrate may be facilitated, not only by early outflow, but by rapid removal by absorption. It is noteworthy that evidence for absorption of short-chain fatty acids from the abomasum of the young steer has recently been reported (Edrise, Smith & Buttle, 1977).

The major proportions of the long-chain fatty acids remained esterified in abomasal effluent, although there was some increase with the period after sampling in the proportions of fatty acids present unesterified. It is clear that approximately half the dietary fatty acid entered the duodenum as triglyceride, approximately one-quarter as free fatty acid, rather less than one-quarter as diglyceride, and only a relatively small proportion as monoglyceride. Somewhat similar patterns of products of lipolysis due to salivary lipase, with approximately equal amounts of diglyceride and free fatty acid, and rather lower amounts of monoglyceride, were observed by Siewert & Otterby (1968) in calves, Olivecrona, Billström & Helander (1973) in rats, and Hamosh, Klaeveman, Wolf & Scow (1975) in humans.

Summation of the amounts of fatty acids found unesterified and as monoglyceride shows that less than one-third of the long-chain fatty acids in the meal entered the duodenum in an absorbable form (cf. Mattson & Volpenhein, 1964; Harrison & Leat, 1975). It therefore seems likely that in the normal calf salivary lipase makes only a relatively small direct contribution to the release of the long-chain fatty acids. These findings are difficult to reconcile with those of Gooden & Lascelles (1973) who showed that 69 % of the long-chain fatty acids from a milk feed were absorbed by a calf which had an exteriorized pancreatic duct and therefore relied on salivary lipase to break down triglycerides to absorbable products.

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