RELATIVE IMPORTANCE OF PANCREATIC LIPASE AND PREGASTRIC ESTERASE ON LIPID ABSORPTION IN CALVES 1–2 WEEKS OF AGE

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Abstract

An attempt has been made to determine the relative importance of pancreatic lipase and pregastric esterase on lipid absorption in calves 1–2 weeks of age. This was carried out by measuring the quantity of esterified lipid in lymph and the products of lipid hydrolysis in jejunal contents of 10 calves fed either milk or fatty whey in the presence or absence of pancreatic juice.

The results showed that lipid was absorbed somewhat less efficiently during the feeding of fatty whey than milk and that a substantial reduction in lipid absorption was associated with the deprivation of pancreatic juice. It was also shown that malabsorption of lipid of calves deprived of pancreatic juice was almost entirely attributable to lipase deficiency rather than to a loss of buffering activity.

The markedly reduced efficiency of lipid absorption in the absence of pancreatic juice, especially during fatty whey feeding, was associated with a low free fatty acid : triglyceride ratio of jejunal contents. The results suggested that the lipolytic activity remaining in the gastrointestinal tract in the absence of pancreatic juice was almost entirely attributable to pregastric esterase and that activity of the latter was substantially diminished once the digesta entered the intestine.

I. INTRODUCTION

Although the role of pancreatic lipase in lipid digestion of monogastric animals is well known, its importance to the young milk-fed ruminant has not been conclusively established. Heath and Morris (1963) showed unequivocally that bile plays a major role in digestion and absorption of lipid in milk-fed lambs. However, their studies on the effect of deprivation of pancreatic juice on recovery of lipid in lymph were by no means conclusive.

It has been suggested that a salivary lipase (pregastric esterase), found only in young ruminants, serves as a supplement to pancreatic lipase during the first 2 months of life (Grosskopf 1965). Pregastric esterase has been shown to hydrolyse up to 20% of the ester linkages of milk triglyceride in the abomasum of young calves (Otterby *et al.* 1964), but its quantitative importance relative to pancreatic lipase has not been established. In the present studies an attempt has been made to determine the relative importance of these lipolytic systems to the young calf. This has been carried out by measuring the quantity of esterified lipid in lymph and the products of lipid hydrolysis in the jejunum of calves fed either milk or fatty whey in the presence of absence of pancreatic juice.

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II. Materials and Methods

(a) *Animals and Surgery*

Ten 1–2-day-old calves were used in the experiments. Anaesthesia was induced and maintained with a mixture of Halothane (Fluothane, I.C.I., Melbourne) and oxygen.

In six of the calves shunts were established between the thoracic duct and the left common jugular vein by way of the communicating branch of the cephalic vein, as described by Shannon and Lascelles (1967). After a recovery period of 2 days, these calves were subjected to a second operation in which a shunt between the pancreatic duct and the duodenum was made. Briefly, the pancreatic duct was located as it entered the duodenum near the caudal extremity of the pancreas and cannulated with polyvinylchloride tubing (1·5 mm internal and 2·7 mm external diameter) obtained from Dural Plastics Ltd., Dural, N.S.W. A similar tube (2·7 mm internal and 4·5 mm external diameter) was inserted into the lumen of the duodenum directly opposite the pancreatic duct and secured with purse-string sutures. The wound was closed with the two cannulae emerging from the centre of the incision. Each cannula was sutured to the skin in the form of a loop and the ends were joined together to allow pancreatic juice to flow into the duodenum. These calves were used to study the quantity of lipid absorbed into lymph during normal flow of pancreatic juice and during pancreatic deprivation (see Table 1).

TABLE 1

DETAILS OF EXPERIMENTAL SURGERY, EXPERIMENTAL PROCEDURES, AND FAT CONTENT OF THE DIETS FED TO THE CALVES

Surgery	No. of animals	Order of treatments*	Milk fat† (g/100 ml)
Lymphatic and pancreatic shunts [Section III(*a*)(i)]	2	Milk +PJ, milk −PJ, FW +PJ, FW −PJ	4·9±0·5
	2	FW +PJ, FW −PJ, milk +PJ, milk −PJ	4·0±0·1
Lymphatic and pancreatic shunts [Section III(*a*)(ii)]	1	FW +PJ, FW −PJ + inact PJ, FW −PJ	4·7
	1	FW −PJ, FW −PJ + inact PJ, FW +PJ	5·4
	2	Milk +PJ, milk −PJ, FW +PJ, FW −PJ	4·3±0·3
Pancreatic shunt and jejunal cannula [Section III(*b*)]	2	FW +PJ, FW −PJ, milk +PJ, milk −PJ	4·5±0·4

* +PJ, in the presence of pancreatic juice; −PJ, in the absence of pancreatic juice; FW, fatty whey; inact PJ, pancreatic juice heated at 70°C and infused into duodenum.

† Milk fat values are means ± standard errors.

The other four calves were fitted with a shunt between the pancreatic duct and duodenum as described above and an additional cannula in the upper jejunum approximately 1 m distal to the entrance of the pancreatic duct. This cannula was led out through the lower extremity of the incision and sutured to the skin. The end of the cannula was kept occluded except during collection of samples of jejunal contents. These calves were used for lipid analyses on duodenal contents during normal flow of pancreatic juice and during pancreatic deprivation (see Table 1).

Recovery from anaesthesia was rapid and the calves were able to stand within 2 hr of the operation. The calves were allowed several days to recover from the operation before the start of the experiment, by which time they were approximately 1 week old.

The collection and subsequent treatment of lymph samples were essentially the same as described by Shannon and Lascelles (1967). Jejunal samples were collected into glass tubes packed in ice. After the pH of each sample was measured, the sample was immediately heated at 70°C for 10 min, to prevent further lipolysis of lipid in it, then stored at −15°C until analysed.

(*b*) *Analytical Techniques*

Total esterified fatty acid (TEFA) in lymph was determined by the method of Stern and Shapiro (1953) and total fat content of each diet by the Babcock method (Davis and MacDonald 1953). Extractions of lipid in lymph and jejunal contents were carried out by the method of Folch *et al.* (1957) and individual lipid classes separated by thin-layer chromatography. Triglyceride, free fatty acid (FFA), and partial glycerides were separated by thin-layer chromatography as described by Hartmann and Lascelles (1965), and the partial glycerides obtained from this fractionation separated into monoglyceride and diglyceride by the solvent system described by Hawke and Robertson (1964). After fractionation each lipid band was recovered from the silica gel by scraping the appropriate band into a small glass column and eluting with solvent. Triglyceride and FFA were eluted with chloroform, and partial glycerides with chloroform–methanol (1 : 1 v/v). Gravimetric determinations were made on dried lipid fractions after correction for blank values (equal areas of silica gel treated in a similar manner). Triglyceride fractions were checked for purity by measurement of the esterified fatty acid present (Stern and Shapiro 1953) and FFA fractions were checked by the method of Dole (1956). In each case there was close agreement with the gravimetric determination. The fatty acid composition of lipid fractions obtained after thin-layer chromatography was determined using methyl esters (Gooden and Lascelles 1971) or butyl esters as described by Sampugna *et al.* (1966).

(*c*) *Preparation of Diets and Experimental Procedure*

Fatty whey was prepared from whole milk as described by Gooden *et al.* (1971). It was used in order to subject the lipolytic system to conditions of maximum load, as it is known that lipid fed in this form enters the small intestine rapidly (Gooden *et al.* 1971).

The efficiency of absorption of lipid in lymph was determined over a period of 12 hr during which lymph flow was measured hourly. All samples were kept at −15°C for subsequent analysis. Methods for estimating the quantities of long-chain fatty acid fed, and fatty acid absorbed into the lymph, have been described previously (Gooden *et al.* 1971).

Estimates of efficiency of lipid absorption were obtained for four calves starting at 1 week of age. Two calves were fed milk followed by fatty whey, and in the other two the order of feeding was reversed (see Table 1). Animals were fed a particular diet at the rate of 2 litres every 12 hr for a period of 2–3 days prior to estimation of efficiency of lipid absorption. The calves showed no obvious ill-effects to changes in dietary regime. The effect of pancreatic deprivation on lipid absorption during milk or fatty whey feeding was determined by comparing efficiencies of absorption when pancreatic juice was allowed to recirculate into the duodenum (+PJ) with values obtained when the pancreatic shunt was interrupted (−PJ) and pancreatic juice collected in a plastic container strapped to the side of the animal. Prior to the start of the −PJ absorption period the shunt was interrupted for a period of 12 hr.

In the two remaining calves fitted with a thoracic duct cannula, only fatty whey was fed and estimates of efficiency of lipid absorption were obtained under the following conditions: (1) +PJ; (2) −PJ, with inactivated pancreatic juice (heated rapidly to 70°C for 10 min) constantly infused into the duodenal cannula at 15 ml/hr using a peristaltic pump; (3) −PJ. In one calf treatments were applied in the above order whereas in the second calf the order was reversed (Table 1).

The four calves fitted with a pancreatic duct–duodenal shunt and a jejunal cannula were fed milk and fatty whey as described above for the four calves on which efficiency of lipid absorption was measured.

III. Results

(*a*) *Efficiency of Lipid Absorption*

The mean value for the output of esterified fatty acid in lymph 12 hr after feeding fatty whey was 0·29 g/hr for the four calves. At this time the animals were considered to be in a post-absorptive state and accordingly the above value was taken to represent the contribution of lipid from non-dietary sources. Thus in computation of the output of dietary lipid in lymph for both milk- and fatty whey-fed calves, 0·29 g was subtracted from hourly estimates of TEFA output in lymph.

(i) *Effect of Pancreatic Deprivation*

Estimates of the quantity of long-chain fatty acid fed in the diet, the output of dietary fatty acid in lymph, and the efficiency of absorption of lipid in four calves fed milk and fatty whey under the treatments +PJ and −PJ are presented in Table 2.

TABLE 2

EFFICIENCY OF LIPID ABSORPTION FOR CALVES FED MILK OR FATTY WHEY IN THE PRESENCE OR ABSENCE OF PANCREATIC JUICE

Values presented are means ± standard errors from results of four calves. Efficiency of lipid absorption was determined by expressing the estimated quantity of dietary fatty acid absorbed in lymph as a percentage of long-chain fatty acid fed (Gooden *et al.* 1971). +PJ, −PJ denotes presence or absence of pancreatic juice respectively

	Milk		Fatty whey	
	+PJ	−PJ	+PJ	−PJ
Long-chain fatty acid fed (g)	72·4±5·9	66·8±3·8	75·6±11·6	71·6±13·5
Dietary long-chain fatty acid absorbed (g)	69·9±6·3	46·0±2·1	60·3± 3·6	23·8± 3·3
Efficiency (%)	96·5±3·1	69·1±3·3	82·7± 6·5	37·5±10·5

It is evident from the results that lipid was absorbed less efficiently during the feeding of fatty whey than milk and that a substantial reduction in lipid absorption was associated with deprivation of pancreatic juice. The effect of the latter was particularly striking during the feeding of fatty whey. Analysis of variance of the results for the efficiencies of lipid absorption (Table 3) showed that the effect of deprivation of pancreatic juice was highly significant.

(ii) *Effect of Infusing Inactivated Pancreatic Juice*

In accordance with the results of Mylrea (1966), the pH of jejunal contents was extremely variable, but there was a suggestion that the pH was somewhat lower with the feeding of fatty whey in the absence of pancreatic juice than with the remaining diet–treatment combinations. The possibility was therefore considered that the very low efficiency of lipid absorption under these conditions (Table 2) may have been associated with the loss of the buffering effect of pancreatic juice rather than with the absence of pancreatic lipase itself. Accordingly, the effect of infusing inactivated pancreatic juice into the two additional calves fed fatty whey under −PJ conditions

was determined. The pancreatic juice was inactivated by heating at 70°C for 10 min and infused by way of the duodenal cannula at a rate (15 ml/hr) slightly higher than

TABLE 3

SUMMARY OF ANALYSIS OF VARIANCE FOR EFFICIENCY OF LIPID ABSORPTION IN CALVES FED MILK OR FATTY WHEY DIETS

"Treatments" under "Source of variation" refers to the efficiencies obtained in the presence or absence of pancreatic juice. The variance ratios for diets and treatments were computed using, as denominator, mean squares for calves × diets and calves × treatments respectively, while the residual variance was used for computation of the variance ratios of the remaining components

Source of variation	D.F.	Mean squares	Variance ratios
Diets	1	2061*	5·06
Treatments	1	5263***	82·23
Calves	3	170	
Diets × treatments	1	319*	5·70
Calves × diets	3	407*	7·27
Calves × treatments	3	64	
Residual	3	56	

* $P < 0{\cdot}10$. *** $P < 0{\cdot}005$.

the normal flow of pancreatic juice in calves of this age. It is evident from Table 4 that infusion of inactivated pancreatic juice in calves deprived of pancreatic juice did not substantially increase the efficiency of lipid absorption (see also Table 2).

TABLE 4

EFFECT ON EFFICIENCY OF LIPID ABSORPTION OF INFUSING INACTIVATED PANCREATIC JUICE INTO THE DUODENUM OF CALVES FED FATTY WHEY IN THE ABSENCE OF NORMAL PANCREATIC JUICE

Values are means ± standard errors from results of two calves. +PJ, in the presence of pancreatic juice; −PJ, in the absence of pancreatic juice; inact PJ, pancreatic juice heated at 70°C and infused into duodenum of calves in the absence of normal pancreatic juice

	Whey +PJ	Whey+ inact PJ	Whey −PJ
Long-chain fatty acid fed (g)	80·8±5·6	80·8±5·6	80·8±5·6
Dietary long-chain fatty acid absorbed (g)	69·3±6·1	31·7±2·5	24·5±5·5
Efficiency (%)	85·6±1·6	39·2±0·4	30·0±4·7

(*b*) *Lipid Composition of Jejunal Contents from Calves Fed Milk and Fatty Whey*

The proportions of triglyceride, FFA diglyceride, and monoglyceride (expressed as percentage of total lipid) for jejunal contents are shown in Table 5. Samples were taken 5 hr after feeding for the milk diet and 2 hr after feeding fatty whey, these times corresponding with the periods of maximum concentration and output of

lipid in lymph for the two diets (cf. Gooden *et al.* 1971). The results presented in Table 5 show that the proportion of lipolysis products (FFA, diglyceride, and monoglyceride) in jejunal contents was higher under treatment +PJ than −PJ, especially during the feeding of fatty whey. Conversely the proportion of triglyceride was higher during treatment −PJ than +PJ. It is interesting to note that the highest value for the FFA : triglyceride ratio was obtained during milk feeding under +PJ conditions and the lowest during the feeding of fatty whey under −PJ conditions (Table 5). This difference corresponds with the marked difference in efficiency of lipid absorption on these two diet–treatment combinations (Table 2).

TABLE 5

PROPORTION OF EACH LIPID FRACTION (EXPRESSED AS A PERCENTAGE OF LIPID IN ALL FRACTIONS) IN JEJUNAL CONTENTS OF FOUR CALVES FED MILK AND FATTY WHEY

Values presented are means ± standard errors. +PJ, in the presence of pancreatic juice; −PJ, in the absence of pancreatic juice

	Triglyceride	Free fatty acid	Diglyceride	Monoglyceride	Free fatty acid / Triglyceride
Milk					
+PJ	39·8±1·6	27·6±1·1	18·4±0·9	14·2±1·2	0·7
−PJ	53·0±1·7	18·8±0·7	15·4±0·7	12·8±0·5	0·4
Fatty whey					
+PJ	56·0±1·3	17·5±1·3	15·7±1·1	10·8±1·1	0·3
−PJ	77·4±0·7	7·7±1·6	9·0±0·5	5·9±0·7	0·1

(*c*) *Fatty Acid Composition in Jejunal Contents*

Fatty acids in the various lipid fractions (Table 5) from jejunal contents collected after the feeding of milk under +PJ and −PJ conditions were determined as described previously (Gooden and Lascelles 1971). The results of these analyses showed that the fatty acid composition of jejunal contents was essentially the same under these conditions (Table 6).

IV. DISCUSSION

The results obtained in the present experiments demonstrate conclusively that the efficiency of lipid absorption in calves 1–2 weeks old is substantially diminished in the absence of pancreatic juice (Table 2). Although attention has been drawn to the possible importance of the buffering activity of pancreatic juice in lipid absorption in rats (Masarei and Simmonds 1966), the results presented in Table 3 indicate that the malabsorption of lipid in calves deprived of pancreatic juice is almost entirely attributable to lipase deficiency.

Nevertheless it was evident that significant lipase activity remained in the gastrointestinal tract after diversion of pancreatic juice from the intestine. This was borne out by the finding that considerable quantities of lipid were absorbed in the absence of pancreatic juice, especially during milk feeding. Moreover, significant quantities of FFA, monoglyceride, and diglyceride were found in upper jejunal contents under these conditions.

The extremely low lipolytic activity in jejunal contents of calves deprived of pancreatic juice—determined by measuring FFA release from incubation mixtures of duodenal contents and washed milk fat globules (Gooden, unpublished data)—compared with the high activities under +PJ conditions, indicates that virtually all the pancreatic juice was being collected through the cannula. It also suggests that most of the lipolysis products present in the jejunal contents of calves deprived of pancreatic juice had been released before reaching the duodenum. Pregastric esterase activity may have been solely responsible for the lipolysis observed in these circumstances although the production of lipase from another source must also be considered. In this connection it has been reported that a lipase secreted by the gastric mucosa is present in the suckling rat (Helander and Olivecrona 1970), although in young ruminants a comparable lipase is apparently absent (Otterby *et al.* 1964).

TABLE 6

FATTY ACID COMPOSITION OF JEJUNAL CONTENTS FROM CALVES FED MILK IN PRESENCE AND ABSENCE OF PANCREATIC JUICE

Values presented are the mean percentages (of the total fatty acids) of two samples. Jejunal contents were collected 5 hr after feeding milk. TG, triglyceride; FFA, free fatty acid; DG, diglyceride; MG, monoglyceride; +PJ, in the presence of pancreatic juice; −PJ, in the absence of pancreatic juice

Fatty acid	Milk fat	+PJ				−PJ			
		TG	FFA	DG	MG	TG	FFA	DG	MG
4 : 0	3·2	1·0				1·9			
6 : 0	2·1	0·8		*		1·3		*	
8 : 0	1·0	0·5		0·6	*	0·6		0·7	0·6
10 : 0	2·5	1·0	0·6	0·5	0·4	1·1	0·5	0·7	0·7
12 : 0	3·0	1·4	1·3	0·7	0·8	1·2	1·2	1·2	0·8
14 : 0	10·6	6·9	8·6	5·0	9·4	7·0	7·1	6·1	8·2
14 : 1	2·0	1·6	1·9	1·7	1·5	1·9	1·8	1·5	1·2
15 : 0	0·5	1·6	2·0	2·0	2·2	2·0	1·9	1·8	1·8
16 : 0	27·4	24·9	25·2	28·2	31·1	25·0	26·8	29·9	35·5
16 : 1	3·1	3·4	4·2	4·8	4·5	4·1	4·5	3·8	3·6
18 : 0	13·3	18·1	15·1	20·0	20·4	15·8	16·6	20·1	16·3
18 : 1	28·3	35·7	37·6	34·6	27·1	34·7	37·4	32·1	29·3
18 : 2	2·0	1·7	2·5	1·2	1·8	2·3	1·6	1·7	1·4
18 : 3	1·0	1·4	1·0	0·9	1·0	1·3	0·7	0·8	0·9

* Trace only.

The low efficiency of lipid absorption (together with the markedly reduced FFA : triglyceride ratio of jejunal contents) under −PJ conditions during the feeding of fatty whey compared with milk (Table 2) may have been associated with the short sojourn of lipid in the abomasum (Gooden *et al.* 1971). It is possible that pregastric esterase activity is substantially diminished once the digesta enter the intestines. Indeed, it has been shown that sodium taurocholate inhibits the action of pregastric esterase *in vitro* (Ramsey and Young 1961) and, according to Peric-Golia and Socic (1968), taurine-conjugated bile acids predominate in both young and adult ruminants.

On the other hand the somewhat lower efficiency of absorption of lipid during the feeding of fatty whey compared with milk under +PJ conditions suggests that

there was insufficient pancreatic lipase activity to cope with the rapid influx of lipid entering the intestines. In support of this suggestion, the individual data for the four calves showed that the higher the fat content of the diet under +PJ conditions the lower was the efficiency of lipid absorption in lymph. In this connection it was reported earlier (Gooden *et al.* 1971) that the efficiency of lipid absorption was similar in newborn calves fed fatty whey or milk under +PJ conditions. However, it is perhaps significant that the fat content of the fatty whey diet used in the earlier study was 25% less than in those used here.

The finding that the fatty acid composition for each lipid fraction in jejunal contents collected under +PJ and −PJ conditions was not significantly different, suggests that if pregastric esterase were largely responsible for the lipolysis observed under −PJ conditions then its fatty acid specificity was similar to that for pancreatic lipase. It has been reported that pancreatic lipase displays an intermolecular rather than an intramolecular specificity (Sampugna *et al.* 1967). These workers showed that release of short- and long-chain fatty acids from triglycerides containing both types of fatty acids occurred at similar rates, and also that fatty acid release was more rapid than was observed from triglycerides containing exclusively long-chain fatty acids. The substantial proportion of long-chain fatty acid observed in upper jejunal contents, together with the relatively high efficiency of lipid absorption from milk-fed calves during −PJ conditions, indicate that pregastric esterase is capable of hydrolysing long-chain as well as short-chain fatty acids from milk triglyceride.

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