THE IMPORTANCE OF LIPOLYTIC ENZYMES IN MILK-FED AND RUMINATING CALVES

By J. M. GOODEN*

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Abstract

An attempt has been made to compare the activity and specificity of pregastric esterase and pancreatic lipase from calves 1–2 weeks of age in an *in vitro* system using washed milk-fat globules as substrate. In addition, the changes in activity of pancreatic lipase and pancreatic phospholipase have been investigated as milk-fed calves change from a monogastric to a ruminant type of digestion.

The results showed that pregastric esterase and pancreatic lipase have a similar specificity for long-chain fatty acids in milk triglyceride, and that on a unit volume basis the activity of pregastric esterase is approximately 10 times lower than that of pancreatic lipase. In the absence of pancreatic juice, pregastric esterase activity in the duodenum of milk-fed calves was negligible. The lipase activity per unit volume of pancreatic juice from 2-day-old calves was not significantly greater than that from 2-week-old calves. However, there was a substantial increase in flow in the latter calves, resulting in a threefold increase in the output of pancreatic lipase activity per kilogram body weight. In ruminant calves (6–7 months old) there was a further threefold increase in output of pancreatic lipase per kilogram body weight.

The conversion of lecithin to lysolecithin occurred much more rapidly in intestinal contents of ruminating calves compared with 1–2-week-old calves, although *in vitro* studies revealed that there was a rapid conversion using pancreatic juice and bile from both groups of calves at pH 7·5 and 6·2, but not at pH 5·2. The addition of large quantities of milk triglyceride to incubation mixtures containing pancreatic juice and bile from calves 1–2 weeks of age substantially inhibited the conversion of lecithin to lysolecithin. Inactivation of pancreatic lipase from ruminating and young calves resulted in a large reduction in the hydrolysis of biliary lecithin *in vitro*.

Evidence presented suggests that pancreatic lipase is the major enzyme involved in hydrolysis of lecithin and that conditions of low pH or the presence of triglyceride inhibit this reaction.

I. INTRODUCTION

It is well known that monoglyceride plays a major role in the digestion and absorption of lipid in monogastric animals, not only in micelle formation, but also as a precursor for the synthesis of chylomicron triglyceride in the intestinal mucosa (Hofmann and Borgström 1962; Mattson and Volpenhein 1964; Kayden *et al.* 1967). The young milk-fed calf is essentially monogastric with regard to its digestive physiology, but by 3–4 months of age under normal husbandry conditions the calf develops a digestive system which is essentially ruminant in character.

* Dairy Research Unit, University Farms, Camden, N.S.W. 2570; present address: Applied Biochemistry Division, D.S.I.R., Palmerston North, N.Z.

Recent studies from this laboratory have shown that pregastric esterase, which is secreted in large amounts in very young calves (Grosskopf 1965), and pancreatic lipase have an additive effect on the lipolysis of milk lipid in week-old calves (Gooden and Lascelles 1973). It was apparent from these studies that at this age pregastric esterase activity alone is sufficient to hydrolyse 65–70% of the lipid offered in a milk diet. The fatty acid composition of jejunal contents from calves deprived of pancreatic juice, compared with normal calves, suggested that the specificity of pregastric esterase was similar to that of pancreatic lipase. This is not in agreement with the results of Grosskopf (1965), who reported an absolute specificity of pregastric esterase for butyrate linkages, but is consistent with the suggestion that both enzymes exhibit an intermolecular specificity for glycerides containing short-chain fatty acids (Sampugna *et al.* 1967; Siewert and Otterby 1968; Pitas and Jensen 1970). One of the aims of the work described here was to compare activity and specificity of the two lipolytic enzymes in calves 1–2 weeks of age in an *in vitro* system where washed milk-fat globules were used as the substrate.

In the adult ruminant the majority of dietary lipid entering the small intestine is in the form of free fatty acid released within the rumen as a result of the hydrolytic activity of rumen microorganisms (Garton 1960), and any function that pancreatic juice may have in the further digestion of lipid has remained obscure. However, it was suggested recently that lysolecithin, formed from biliary lecithin in the presence of pancreatic phospholipase A, may replace the function of monoglyceride in micelle formation in the ruminant (Leat and Harrison 1967, 1969; Lennox *et al.* 1968). An additional aim, therefore, has been to follow the changes in activities of the enzymes pancreatic lipase (E.C. 3.1.1.3) and pancreatic phospholipase A (E.C. 3.1.1.4) as milk-fed calves change from a monogastric to a ruminant type of digestion.

II. Materials and Methods

(*a*) *Surgery*

Shunts between the pancreatic duct and duodenum were established in 28 calves of varying ages as described by Gooden and Lascelles (1973). Intestinal cannulae were also inserted in eight of these calves at varying distances distal to the pancreatic duct. The gall bladder in each of four additional 1–2-week-old calves was cannulated by inserting a polyvinyl tube (2·0 mm internal diam., 3·5 mm external diam., Dural Plastics Pty. Ltd., Dural) into the lower extremity of the organ. Calves were allowed 2–3 days to recover from surgery before pancreatic juice or bile was collected.

(*b*) *Collection of Pancreatic Juice and Bile*

Pancreatic juice was collected into cold glass containers by interrupting the shunt for a few minutes approximately 5 hr after feeding. Bile was collected into cold glass containers from calves 1–2 weeks old and from the gall bladders of slaughtered 7–12-month-old steers. Samples of pancreatic juice and bile were kept for periods of up to 30 min until used in incubation mixtures.

(*c*) *Collection of Saliva*

Saliva from calves 2–3 days and 1–2 weeks of age was collected under general anaesthesia (Fluothane, I.C.I. Ltd., Melbourne). The saliva was allowed to drain into a container packed in ice and used in incubation mixtures within 30 min of collection.

(*d*) *Collection of Intestinal Contents*

Samples of intestinal contents from the duodenum adjacent to the entrance of the pancreatic duct were collected in glass containers packed in ice, the pH measured, and the samples used in

incubation mixtures within 10 min of collection. Samples of contents (5 ml) taken from cannulae inserted in the intestine at various distances (0·3, 1·5, and 3·0 m) distal to the pancreatic duct were heated at 80°C for 10 min (to inactivate the lipases present) then stored at −15°C under nitrogen until analysed.

(e) Preparation of Incubation Mixtures

Washed milk-fat globules prepared by the method of Gooden *et al.* (1971) were suspended in isotonic saline and used as the substrate for measuring lipolytic activity in pancreatic juice and saliva. Preliminary experiments carried out to test the suitability of using this substrate revealed the following:

(1) Spontaneous release of free fatty acids (FFA) was significant with unwashed milk-fat globules compared with the very small release from washed globules. When corrections were made for spontaneous release of FFA, the differences between the lipolytic activities of saliva and pancreatic juice were similar with either washed or unwashed globules as substrate.

(2) The rate of lipolysis was not significantly greater in the presence of bile.

The amount of milk triglyceride in each incubation mixture was sufficiently high (approximately 40 mg) to ensure that the concentration of substrate was not limiting the rate of the enzyme reaction. Mixtures contained 0·2 ml of pancreatic juice or 1 ml of saliva, and 2 ml of milk-fat globules. The total volume was made up to 12 ml with phosphate buffer (0·1M, pH 6·2) and the release of FFA was monitored during the course of incubation. Clearly, the large volume of buffer used in incubation mixtures would overwhelm any possible differences in pH or ionic composition of saliva and pancreatic juice.

A comparison of lipolytic activity was made on contents collected from the duodenum adjacent to the entrance of the pancreatic duct in calves deprived of pancreatic juice for 12 hr and during normal pancreatic flow. The duodenal contents (1 ml) were incubated with 2 ml of milk-fat globules and 9 ml of phosphate buffer (0·2M, pH 6·2) and the release of FFA measured during the course of incubation.

Phospholipase activity at pH 5·2, 6·2, or 7·5 was determined by incubating 2 ml of pancreatic juice with an equal volume of bile and the mixture made up to 12 ml with 0·2M phosphate buffer (pH 6·2 or 7·5) or 0·2M citrate buffer (pH 5·2). The rate of conversion of lecithin to lysolecithin was used as an estimate of phospholipase activity.

All incubations were carried out in a shaking water-bath at 37°C and aliquots removed at various time intervals for analysis. The reaction in incubation mixtures containing washed milk-fat globules was stopped by quickly adding aliquots (1 ml) to Dole extraction mixture (Dole 1956). In cases where lipid classes were required for further analysis total incubation mixtures were added to cold chloroform–methanol. The reaction in incubation mixtures containing bile was stopped by adding aliquots (3 ml) to cold chloroform–methanol containing antioxidant (2,6-di-t-butyl-*p*-cresol, 0·08% w/v).

(f) Analytical Techniques

Free fatty acids were determined by the method of Dole (1956). Lipid extraction of intestinal contents and incubation mixtures containing bile or milk-fat globules were carried out using the method of Folch *et al.* (1957). Lipid classes from incubation mixtures containing milk-fat globules were separated by thin-layer chromatography carried out as described by Gooden and Lascelles (1973), and methyl esters prepared by sealed-tube methanolysis before analysis were separated by gas–liquid chromatography (Gooden and Lascelles 1971). The method of Allen (1940) was used to determine total concentrations of phospholipid in samples of intestinal contents and bile. The separation of lecithin and lysolecithin from the above samples was achieved using silica gel G (Merck) into which 5% aluminium ammonium sulphate (w/w) was incorporated. The solvent system was chloroform–methanol–water (60 : 30 : 5 v/v) and the phospholipid spots were made visible with iodine vapour. Standard lipids, which included lecithin and lysolecithin (Applied Sciences), were included on each plate. The spots containing lecithin and lysolecithin were scraped from the plates into glass tubes, 0·45 ml of perchloric acid (70% w/v) was added, and the mixture boiled until clear. After cooling, 4 ml of distilled water was added to each tube which was then shaken vigorously and centrifuged at 2500 rev/min for 10 min. The supernatant was removed and the concentration of inorganic phosphorus measured as described by Allen (1940). Similar quantities of silica gel from areas of the plate containing no phospholipids were taken through the same procedure and values subtracted from those obtained above. Recoveries of phospholipid were between 85 and 95%.

III. Results

(*a*) *Comparison of the Lipolytic Activities of Pancreatic Juice and Saliva from Calves 1–2 Weeks of Age*

The long-chain fatty acids released following incubation of pancreatic juice (0·2 ml) or saliva (1 ml) with washed milk-fat globules are presented in Figure 1. It is evident from the rates of release of fatty acid that pancreatic juice contained either a greater lipolytic activity per volume than saliva or alternatively that it had a greater specificity for long-chain fatty acid.

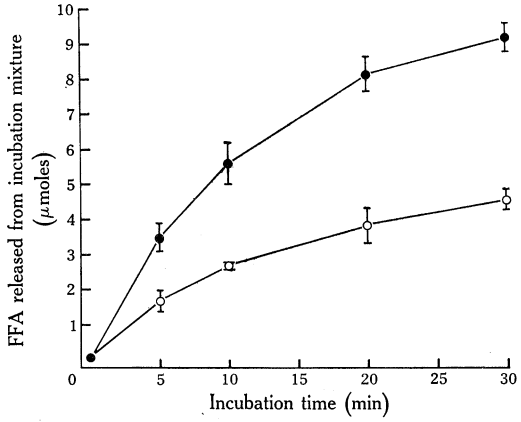

Fig. 1.—Long-chain fatty acid released following incubation of pancreatic juice (0·2 ml, ●) or saliva (1 ml, ○) from 1–2-week-old calves with washed milk-fat globules as substrate. Values plotted are means ± standard errors for three calves.

(*b*) *Fatty Acid Specificity of Pancreatic Lipase and Salivary Pregastric Esterase*

Either pancreatic juice (2 ml) or saliva (10 ml) from a 2-week-old calf was incubated for 30 min with washed milk-fat globules. The concentration and fatty acid composition of each lipid fraction obtained from the incubations are presented in Table 1. It is evident that there was a close similarity between the fatty acid composition of lipid fractions obtained from incubation mixtures containing pancreatic lipase or pregastric esterase, although there was a difference between the FFA fractions with respect to fatty acids 10:0, 12:0, and 18:1. A striking feature of the results in Table 1 was the low levels of fatty acids 4:0 and 6:0 in the residual triglyceride and diglyceride and the absence of detectable concentrations of these acids in monoglyceride fractions, and it was concluded that the majority of short-chain fatty acids were released during the course of incubation. It is important to stress that the absence of fatty acids <10:0 in the FFA fractions was due to the lipid extraction procedure which failed to extract these water-soluble acids. It was calculated that only 20–30% of the long-chain fatty acid (>10:0) was released during the course of incubation.

(*c*) *Lipase Activity in Duodenal Contents Incubated* in vitro

It has been shown that there is a rapid release of fatty acids when milk and saliva are mixed either *in vitro* (Ramsey and Young 1961) or in the abomasum of young calves (Otterby *et al.* 1964). It has been suggested that the activity of pregastric esterase in saliva may be considerable in the upper small intestine, where the pH is higher than in the abomasum (Siewert and Otterby 1971). In order to determine the

relative activities of pregastric esterase and pancreatic lipase in the upper small intestine the rate of release of FFA from samples of duodenal contents of milk-fed calves was

TABLE 1

FATTY ACID COMPOSITION OF LIPID CLASSES FROM *IN VITRO* INCUBATIONS OF MILK FAT WITH PANCREATIC JUICE OR SALIVA FROM A CALF 2 WEEKS OF AGE

Washed milk-fat globules were incubated for 30 min with 2 ml of pancreatic juice or for 60 min with 10 ml of saliva. Each value is the average of duplicate estimations expressed as percentage of total fatty acids. TG, triglyceride; DG, diglyceride; MG, monoglyceride. Values in parentheses are amounts (mg) of each lipid fraction in the incubation mixture

Fatty acid	Amount (as % of total fatty acids) in:					Fatty acid	Amount (as % of total fatty acids) in:				
	Milk TG	TG	FFA	DG	MG		Milk TG	TG	FFA	DG	MG
	Pancreatic juice						**Saliva**				
	(357·0)	(151·1)	(79·0)	(85·5)	(33·5)		(351·5)	(160·5)	(62·0)	(82·5)	(39·5)
4:0	3·1	0·8		0·5		4:0	3·0	0·6		0·4	
6:0	2·1	0·5		0·7		6:0	2·2	0·6		0·6	
8:0	1·4	0·8		0·6	0·8	8:0	1·6	0·7		0·5	0·3
10:0	3·6	1·4	1·5	1·1	1·4	10:0	3·6	1·5	5·1	0·7	0·5
12:0	3·8	2·5	3·2	3·0	2·9	12:0	4·0	3·0	7·3	1·9	0·8
14:0	11·3	9·1	9·0	13·4	13·2	14:0	10·3	8·9	10·9	6·8	6·9
14:1	1·0	1·1	1·3	2·1	1·4	14:1	1·2	2·1	3·2	1·7	0·8
15:0	1·3	1·4	1·4	2·2	1·7	15:0	1·2	2·5	2·0	2·0	2·2
16:0	27·0	29·6	33·0	35·4	42·0	16:0	27·2	26·1	32·4	38·2	42·0
16:1	3·1	2·5	3·2	2·2	2·2	16:1	3·5	2·6	3·2	2·6	3·2
18:0	14·0	13·9	13·9	12·2	10·1	18:0	14·1	15·5	9·1	14·9	13·8
18:1	25·7	32·8	31·3	23·4	21·8	18:1	25·9	31·4	22·9	26·3	27·7
18:2	1·6	2·1	1·5	1·6	1·4	18:2	1·4	2·6	2·2	2·4	1·4
18:3	1·0	1·5	0·7	1·6	1·1	18:3	0·8	1·9	1·7	1·3	0·4

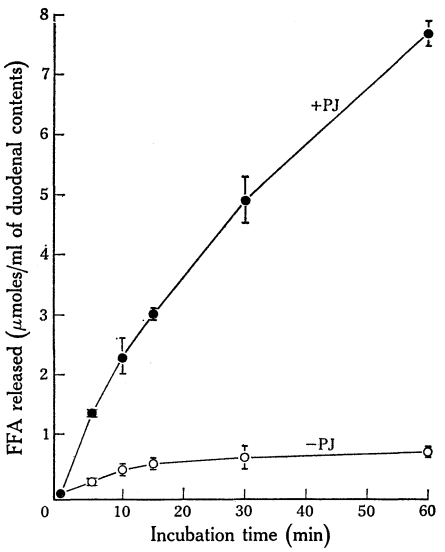

Fig. 2.—Long-chain fatty acid released from duodenal contents (1 ml) collected under +PJ or −PJ conditions (see text) from 1–2-week-old calves and incubated with washed milk-fat globules. Values plotted are means ± standard errors for three calves.

measured under conditions of normal pancreatic flow (+PJ) or when pancreatic juice was prevented from entering the duodenum for 12 hr (−PJ). The quantities of long-chain fatty acids released under these conditions are presented in Figure 2. It is

evident that pancreatic lipase is the major source of lipolytic activity in the upper small intestine and that the contribution of pregastric esterase is relatively small.

(*d*) *Changes in Output of Lipase Activity of Pancreatic Juice with Age*

The flow and lipase activity of pancreatic juice was measured in calves 2, 14, and 200 days of age. The lipase activities for the three age groups are illustrated in Figure 3. Although there is an apparent increase in activity with age, analysis of covariance of the results of log–log transformed data revealed that there was no significant difference for calves 2 and 14 days old.

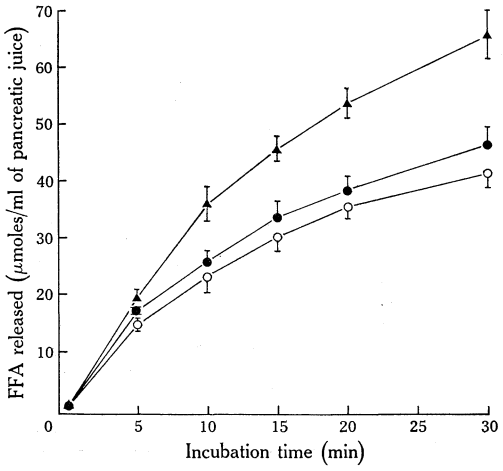

Fig. 3.—Long-chain fatty acid released from incubation mixtures containing pancreatic juice (0·2 ml) from calves 2 (○), 14 (●), or 200 (▲) days old. Washed milk-fat globules were used as substrate. Values plotted are means ± standard errors for three calves.

The means and standard errors for flow of pancreatic juice in calves 2, 14, and 200 days of age were 3·0±0·2, 11·9±0·9, and 91·3±6·1 ml/hr, which are equivalent to 0·1, 0·3, and 0·9 ml per kilogram body weight per hour for the respective age groups. It is clear therefore that output of pancreatic lipase activity per kilogram body weight increased substantially with age.

(*e*) *Changes in Pancreatic Phospholipase Activity with Age*

An attempt to estimate the activity of pancreatic phospholipase in calves 1–2 weeks and 5–7 months old was carried out by measuring the concentration of lecithin and lysolecithin in intestinal contents collected at various levels along the upper small intestine under +PJ and −PJ conditions. The mean concentration of total phospholipid and lecithin, and the lecithin : lysolecithin ratio of intestinal samples taken at positions 0·3, 1·5, and 3·0 m distal to the pancreatic duct are shown in Table 2. It is apparent that the concentration of total phospholipid in intestinal contents at 0·3 m from 1–2-week-old calves was approximately four times that of calves 5–7 months of age. The changes in ratios of lecithin : lysolecithin reflect the relative increase in concentration of lysolecithin with increasing distance along the intestinal tract under +PJ conditions for both groups of calves, the increase being more pronounced in older calves. In the younger calves the ratios were 10·2 : 1 and 6·2 : 1 at 0·3 and 3·0 m respectively, while for the older calves the corresponding ratios

were 6·5 : 1 and 1·7 : 1. It should be noted that in calves 1–2 weeks old under +PJ conditions the total phospholipid concentration increased by 14% at 1·5 m but decreased by 10% at 3·0 m relative to the value obtained at 0·3 m distal to the pancreatic duct. In calves 5–7 months of age there was a progressive decline in the concentration of total phospholipid with increasing distance along the intestinal tract. Values were 10% less at 1·5 m and 40% less at 3·0 m than the value obtained at 0·3 m.

TABLE 2

CONCENTRATION OF LECITHIN AND THE RATIO OF LECITHIN : LYSOLECITHIN IN INTESTINAL CONTENTS OF CALVES 1–2 WEEKS AND 5–7 MONTHS OLD

Calves 1–2 weeks old were fed 2 litres of milk twice daily and intestinal contents were sampled 4–5 hr after feeding. 5–7-month-old calves were fed lucerne chaff–crushed oats (3 : 1 w/w) *ad libitum*. +PJ, −PJ, in the presence or absence of pancreatic juice respectively (see text). Values are means ± standard errors, with total phospholipid and lecithin expressed as mg/100 ml of intestinal contents

Age of calves	1–2 weeks			5–7 months		
Distance (m) of cannula distal to pancreatic duct	0·3	1·5	3·0	0·3	1·5	3·0
No. of calves	4	4	4	2	2	2
+PJ conditions						
pH	4·9±0·3	5·6±0·2	6·3±0·3	3·8±0·4	5·4±0·4	6·9±0·2
Total phospholipid	154±2·9	175±2·2	138±2·6	42±2·0	38±4·1	25±3·3
Lecithin	122±1·7	155±2·1	112±2·3	26±1·5	22±2·9	12±1·7
Lecithin : lysolecithin	10·2±0·8	11·0±0·6	6·2±0·5	6·5±0·4	2·4±0·7	1·7±0·2
−PJ conditions						
pH	4·3±0·5	5·3±0·5	6·1±0·3	3·1±0·7	5·3±0·6	6·9±0·2
Total phospholipid	163±3·3	190±4·1	228±8·3	44±3·0	48±2·4	54±2·9
Lecithin	135±3·0	145±4·2	185±6·1	25±1·9	29±2·2	32±1·1
Lecithin : lysolecithin	8·4±0·9	9·7±1·1	10·4±1·5	8·3±0·5	4·8±0·5	3·6±0·4

Under −PJ conditions no conversion of lecithin to lysolecithin occurred in the section of intestinal tract between 0·3 and 3·0 m distal to the pancreatic duct in calves 1–2 weeks old. Although the ratio of lecithin : lysolecithin decreased approximately twofold with progression along the intestinal tract of calves 5–7 months of age, it can be seen that the values remained much higher than those observed under +PJ conditions (Table 2). In both groups of calves there was a progressive increase in the concentration of total phospholipid with increasing distance along the intestinal tract.

(*f*) In vitro *Studies on the Conversion of Lecithin to Lysolecithin from Calves 1–2 Weeks and 7–12 Months of Age*

In order to investigate the lipolysis of phospholipid under more controlled conditions, *in vitro* studies were carried out using pancreatic juice and bile taken from milk-fed (1–2-week-old) and ruminating (7–12-month-old) calves. The concentrations of lecithin and the ratios of lecithin : lysolecithin following incubation of equal volumes of pancreatic juice and bile at different pH levels (7·5, 6·2, or 5·2) are shown in Table 3. It is evident that at pH 7·5 and 6·2 considerable lipolysis of lecithin occurred during incubation of pancreatic juice and bile from milk-fed and ruminating calves. Recoveries of phosphorus in lecithin + lysolecithin after 60 min

incubation were between 95 and 102% of the values at zero time. These high values suggest that only one fatty acid from each lecithin molecule was hydrolysed during the incubation period, since the loss of two fatty acids would render the residue insoluble in the extraction procedure. The magnitude of the ratios of lecithin : lysolecithin indicate that hydrolysis was more rapid during the incubation of pancreatic juice and bile from calves 1–2 weeks old than from ruminating animals. At pH 5·2 there was little conversion of lecithin to lysolecithin for both groups of calves.

TABLE 3

EFFECT OF pH ON THE CONVERSION OF LECITHIN TO LYSOLECITHIN FOLLOWING INCUBATION OF PANCREATIC JUICE AND BILE FROM MILK-FED (1–2-WEEK-OLD) AND RUMINATING (7–12-MONTH-OLD) CALVES

Values are means ± standard errors for two calves

pH	7·5			6·2			5·2		
Incubation time (min)	0	30	60	0	30	60	0	30	60
Pancreatic juice and bile from milk-fed calves									
Lecithin (μg P/ml bile)	500	94	54	552	188	130	591	585	580
	±40	±29	±19	±32	±28	±12	±24	±31	±30
Lecithin : lysolecithin	8·6	0·2	0·1	8·1	0·4	0·3	7·9	7·8	7·3
	±0·3	±0·1	±0·1	±0·2	±0·1	±0·1	±0·5	±0·3	±0·2
Pancreatic juice and bile from ruminating calves									
Lecithin (μg P/ml bile)	584	442	326	568	454	344	580	575	558
	±20	±26	±32	±11	±15	±22	±18	±26	±12
Lecithin : lysolecithin	8·3	2·2	1·1	8·4	2·6	1·4	8·2	7·4	5·9
	±0·3	±0·2	±0·3	±0·4	±0·2	±0·2	±0·4	±0·5	±0·3

There would appear to be a discrepancy in the results presented in Tables 2 and 3. It is evident that the apparent conversion of lecithin to lysolecithin occurs at a relatively slow rate in intestinal contents collected under +PJ conditions from the small intestine of the young calf (Table 2), whereas there is a rapid and substantial conversion when pancreatic juice and bile are incubated *in vitro* (Table 3). It was considered that the presence of large quantities of triglyceride found in the upper small intestine of milk-fed calves may inhibit the conversion of lecithin to lysolecithin *in vivo*, and the results presented in the following section represent an attempt to investigate this situation.

(*g*) *Lipolysis of Biliary Lecithin in the Presence of Milk Triglyceride* in vitro

The concentration of lecithin and the ratio of lecithin : lysolecithin following the incubation of pancreatic juice and bile from calves 1–2 weeks of age in the presence of 0, 45, and 90 mg of milk triglyceride per millilitre of bile are presented in Table 4. There was a decline in the rate of conversion of lecithin to lysolecithin with increasing concentration of triglyceride. The concentrations of lysolecithin following 60 min incubation were 84, 31, and 21% of the concentration of total phospholipid at zero time for incubations containing 0, 45, and 90 mg of triglyceride per millilitre of bile respectively.

(h) Phospholipase Activity following Inactivation of Pancreatic Lipase

It has been shown that heating pancreatic juice at 70°C for 5 min inactivates lipase but not phospholipase A from rats (Boucrot and Clement 1971), man (Belleville and Clement 1968), pig (De Haas *et al.* 1968), and ox (Rimon and Shapiro 1959). Using this criterion it was of interest to determine whether phospholipase A in pancreatic juice from young and ruminating calves was responsible for part or all of the hydrolysis of biliary lecithin observed in Tables 3 and 4.

TABLE 4

LIPOLYSIS OF LECITHIN DURING INCUBATION OF PANCREATIC JUICE AND BILE FROM 1–2-WEEK-OLD CALVES IN THE PRESENCE OF MILK TRIGLYCERIDE

The pH of each incubation mixture was 7·5. Bile contained 16±1 mg of phospholipid/ml of bile. Phospholipid values are mean ± standard errors for two calves

Triglyceride added (mg/ml of bile)	0		45		90	
Incubation time (min)	0	60	0	60	0	60
Lecithin (μg P/ml bile)	564±37	95±6	543±52	418±42	562±43	498±26
Lecithin : lysolecithin	7·3±0·2	0·2±0·1	7·5±0·2	2·1±0·1	7·5±0·2	3·9±0·4

The concentrations of lecithin and the ratios of lecithin : lysolecithin in incubation mixtures containing equal volumes of heat-inactivated pancreatic juice and bile from calves 1–2 weeks and 7–12 months of age are presented in Table 5. The

TABLE 5

EFFECT OF INACTIVATING PANCREATIC LIPASE ON THE LIPOLYSIS OF LECITHIN FOLLOWING INCUBATION OF PANCREATIC JUICE AND BILE FROM 1–2-WEEK- AND 7–12-MONTH-OLD CALVES

Values are means ± standard errors for two calves

Age of calves	1–2 weeks		7–12 months	
Pancreatic juice	Heated*	Normal	Heated*	Normal
No incubation				
Lecithin (μg P/ml bile)	576±28	514±41	604±32	598±23
Lecithin : lysolecithin	9·6±0·3	8·6±0·2	9·4±0·2	8·8±0·1
30 min incubation				
Lecithin (μg P/ml bile)	570±37	88±14	600±50	448±26
Lecithin : lysolecithin	8·4±0·3	0·2±0·1	8·1±0·2	2·2±0·1
60 min incubation				
Lecithin (μg P/ml bile)	562±14	44±18	596±41	326±32
Lecithin : lysolecithin	6·4±0·2	0·1±0·1	6·3±0·2	1·1±0·1

* Pancreatic juice was heated at 70°C for 5 min to inactivate pancreatic lipase.

pancreatic lipase activity in heat-inactivated pancreatic juice was measured using washed milk-fat globules as the substrate and found to be only 5% of the activity of unheated pancreatic juice. The results in Table 5 demonstrate that in both groups of calves there is only a small conversion of lecithin to lysolecithin in the absence of lipase activity. Thus these results raise the question of whether pancreatic lipase itself is responsible for the conversion of biliary lecithin to lysolecithin in young and ruminating calves.

IV. Discussion

Although the results in Figure 1 illustrate that there is a greater lipolytic activity per unit volume of pancreatic juice than of saliva, it is evident that the fatty acid specificities of the salivary and pancreatic enzymes (pregastric esterase and pancreatic lipase respectively) are similar, at least for the long-chain fatty acids of milk triglyceride (Table 1). It is important to note that Gooden and Lascelles (1973) have shown recently that approximately 70% of dietary long-chain fatty acids were absorbed following the feeding of milk to young calves deprived of pancreatic juice, and that the efficiency of absorption appeared to be dependent largely upon the time of sojourn of lipid in the abomasum. Although the pH of the contents of the small intestine may be more favourable to the action of pregastric esterase than that found in the abomasum (Siewert and Otterby 1971), the virtual absence of lipolytic activity in intestinal contents of calves deprived of pancreatic juice (Fig. 2) suggests that most of the long-chain fatty acid must have been released prior to reaching the duodenum. Since pregastric esterase has been detected in duodenal contents of young calves (Otterby *et al.* 1964), it would appear that a factor present in intestinal contents inhibits the action of this enzyme. It is possible that the inhibitory factor is sodium taurocholate in bile (cf. Ramsey and Young 1961).

Although the lipase activity per unit volume of pancreatic juice was not significantly different in calves 2 and 14 days of age (Fig. 3), the increase in flow of pancreatic juice seen in the latter calves resulted in a threefold increase in the output of pancreatic lipase activity per kilogram body weight per hour. At 2 days of age, therefore, it is possible that the output of pancreatic lipase may not be adequate to hydrolyse sufficient milk triglyceride for efficient absorption of dietary lipid. In this connection it has been shown that pregastric esterase activity is high during the first few days of life (Grosskopf 1965), and that the feeding of colostrum results in a very firm casein curd in the abomasum, and as a consequence the entrapped fat remains in the abomasum for a long time (Gooden *et al.* 1971). These observations suggest that pregastric esterase plays a major role in lipid digestion in the abomasum of the newborn calf.

It is apparent from the results in Table 2 that virtually all the phospholipase activity in the small intestine of milk-fed calves is derived from pancreatic juice. However, in ruminating calves it would appear that conversion of lecithin to lysolecithin continues under −PJ conditions but at a much reduced rate compared with +PJ conditions. The source of lipolytic activity under −PJ conditions is not known.

In vitro studies indicated that considerable conversion of biliary lecithin to lysolecithin occurred using pancreatic juice from both pre-ruminant and ruminant calves (Table 3) when the pH of the incubation mixture was between 6·2 and 7·5, but at pH 5·2 conversion was severely depressed. The apparently small phospholipid conversion taking place in the first 3 m of small intestine of young milk-fed calves compared with ruminating animals may have been due to differences in pH conditions in these two groups of calves, although the actual pH values observed suggest that this was not an important factor. However, it is clear from Table 4 that the presence of large amounts of triglyceride in incubation mixtures containing pancreatic juice and bile from milk-fed calves severely inhibits the conversion of biliary lecithin to lysolecithin. It seems reasonable to suggest that a similar inhibition would occur *in vivo* since it has been estimated that the ratio of triglyceride : phospholipid in the upper

small intestine of milk-fed calves is approximately 4 : 1 at 4 hr after feeding (Gooden and Lascelles, unpublished data). In contrast, the intestinal contents of ruminating animals have been shown to contain predominantly FFA (Garton 1960; Lennox *et al.* 1968).

The fact that there was only a small conversion of lecithin to lysolecithin in the absence of lipase activity (Table 5) suggests that lipolysis of biliary lecithin by phospholipase A is negligible, although the possibility that the latter enzyme was in an inactive form, at least *in vitro* (Rimon and Shapiro 1959), cannot be discounted. It is reasonable to suggest that pancreatic lipase exhibits a greater substrate affinity for the 1,3-positions of milk triglyceride, which contains both long- and short-chain fatty acids (Pitas *et al.* 1967), than for the C1-ester linkages of biliary lecithin, which contains exclusively long-chain fatty acids (Adams and Heath 1963). This may be the explanation for the relatively slow rate of conversion of lecithin to lysolecithin in the milk-fed calf.

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VI. References

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