

Intestinal absorption of medium chain fatty acids: *in vivo* studies in pigs devoid of exocrine pancreatic secretion

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(Received 15 March 1993 – Revised 4 January 1994 – Accepted 15 February 1994)

In order to study the influence of pancreatic enzyme secretion on the intestinal absorption of medium-chain fatty acids (MCFA), three growing pigs (mean body-weight 61 kg) with ligated and severed pancreatic ducts were fitted with a permanent fistula in the duodenum and with two catheters in the portal vein and carotid artery respectively. An electromagnetic flow probe was also set up around the portal vein. A mixture of octanoic and decanoic acids, esterified as medium-chain triacylglycerols, together with maltose dextrine and a nitrogenous fraction was continuously infused for 1 h into the duodenum. Samples of blood were withdrawn from the two vessels at regular intervals of time for 8 h and further analysed for their non-esterified octanoic and decanoic acid contents. The concentrations of non-esterified octanoic and decanoic acid in the portal blood increased slowly after the beginning of each infusion, reaching about 10 times higher values than the basal level. Only 26% of octanoic acid infused in the duodenum and 27% of decanoic acid were recovered in the portal flow throughout each experiment. The possible mechanisms underlying the appearance of MCFA in the portal blood in the absence of pancreatic enzyme secretions and the importance of duodenal absorption of MCT in such physiological conditions have been discussed.

Medium-chain triacylglycerols: Pancreatic enzyme deficiency: Pig

Medium-chain triacylglycerols (MCT) are known to be well absorbed in the small intestine, leading to a rapid appearance of their hydrolytic products (i.e. medium-chain fatty acids (MCFA) from eight to ten carbon atoms) in the portal blood (Hashim *et al.* 1964; Playoust & Isselbacher, 1964; Greenberger *et al.* 1966). In a previous experiment (Guillot *et al.* 1993) we studied the rate of appearance and the recovery of non-esterified octanoic and decanoic acids in the portal blood after their direct infusion into the duodenum as MCT. We found that the portal concentration of both MCFA in the non-esterified form showed a biphasic time-course. An early peak (15 min after the beginning of the infusion) might mainly result, according to previous studies (Playoust & Isselbacher, 1964; Greenberger *et al.* 1966), from the intraluminal hydrolysis of MCT by pancreatic lipase (EC 3.1.1.3). A later peak (between 90 and 120 min) led us to discuss a possible alternative absorption mechanism including direct MCT absorption and subsequent hydrolysis inside the intestinal epithelial cells thus bypassing the pancreatic lipase hydrolysis.

It has been found elsewhere that MCFA, when given orally as MCT to children suffering from pancreatic deficiency, seem to disappear completely from the digestive tract (Galabert

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et al. 1975). However, this latter experiment, using stool analysis, did not indicate (a) in which part of the digestive tract the MCT had been absorbed, (b) if they had been previously hydrolysed before absorption and (c) what amounts of non-esterified MCFA had truly reached the portal flow. Thus the influence of pancreatic enzyme secretions on the potency of intestinal absorption of MCT is questioned, and may be answered by a suppression of these secretions. Furthermore, since it has been shown (Rérat *et al.* 1992) that the interaction between absorption of amino acids and that of carbohydrates differs according to the physico-chemical nature of nitrogenous components of the diet (free amino acids or small peptides), this type of interaction between absorption of MCT and absorption of amino acids was also interesting to study.

In this present experiment we used conscious pigs in which the pancreatic duct had been severed and which were fitted with a permanent fistula in the duodenum enabling the infusion of a complex mixture containing both a MCT-rich lipid fraction, a carbohydrate and a nitrogenous component. Appearance of the non-esterified MCFA in blood from the portal vein and the carotid artery was recorded for 8 h after the beginning of the perfusion. This preparation allowed us to determine the time-course and efficiency of the MCFA intestinal absorption. The influence of the type of nitrogenous component (free amino acids or peptides) was also investigated.

MATERIALS AND METHODS

Animals

Three castrated Large White pigs (61 (SE 1) kg initial body weight) were used. They had been fed on a standard growing diet (UFAC, Limours, France). Under halothane anaesthesia, each animal was fitted with two catheters, one in the portal vein and one in the carotid artery, and with one electromagnetic flow probe around the portal vein. A permanent fistulation was also set up in the duodenum. Exocrine pancreatic deficiency was obtained by tying and cutting the pancreatic duct. The pancreatic tissues were carefully dissected around the pancreatic duct to isolate a 10 mm section. Two silk loops were placed at the end of the isolated part of the duct and tied. The pancreatic duct was cut between the two ligatures. This procedure results in a complete suppression of pancreatic exocrine sections, i.e. enzymes and salts, with a decrease in apparent digestibility of N (Corring & Bourdon, 1976). The modifications of digestibility were due to lack of enzymes but not of salts, since digestibility was the same when these were present or absent (Corring & Bourdon, 1976). All these surgical techniques have already been described (Rérat *et al.* 1980).

Infusion mixtures

Two infusion mixtures differing only in their nitrogenous fraction were used, i.e. either a peptide mixture (PEP) or a free amino acid mixture of the same composition as PEP; these mixtures have been described elsewhere (Rérat *et al.* 1988, 1992).

Each infusion mixture contained 110 g nitrogenous fraction, 440 g maltose dextrine and 110 g of a MCT-rich lipid fraction described in Table 1 in a 2 l total volume. Each mixture was supplemented with vitamins and trace elements. All these compounds were supplied by Sopharga (Puteaux, France).

Experiment design and infusion technique

After a 1 week recovery period from surgery, each animal was subjected to two successive 8 h experiments at 8 d intervals. During the interval, pigs were fed orally on the standard growing diet. The first infusion was always composed of PEP and the second of free amino

Table 1. *Composition and fatty acid pattern of the lipid fraction in the infusion mixture*

	g/100 g total lipid
Composition of the lipid fraction	
Medium-chain triacylglycerols*	37.33
Evening primrose (<i>Oenothera biennis</i> L.) oil	24.20
Soya-bean oil	16.20
Glycerol monostearate	9.00
Ascorbyl palmitate	0.07
Soya-bean lecithin	13.20
Fatty acid composition of the lipid fraction	
Octanoic acid	28.8
Decanoic acid	11.7
Myristic acid	0.6
Palmitic acid	5.7
Stearic acid	4.3
Oleic acid	6.2
Linoleic acid	24.5
Linolenic acid	1.6

* Octanoate and decanoate, obtained from copra oil, were randomly esterified on the glycerol moiety.

acids. Each experiment was preceded by an 18 h fast. At the beginning of each experiment the infusion mixture was prepared immediately before infusion as previously described (Guillot *et al.* 1993). The warm (30°) stirred mixture (2 litres) was then continuously infused into the duodenum for 1 h through the permanent fistula. Blood samples (4 ml) were withdrawn from each vessel at 15 min intervals during the first 2 h after the beginning of the infusion, every 30 min during the 3rd hour, and every hour until the 8th hour and poured into heparinized tubes. Blood plasma samples were prepared by centrifugation and stored at -80° until assay. Packed cell volume was also measured every 2 h and the portal flow rate was recorded continuously.

Analytical methods

The non-esterified octanoic and decanoic acid contents of each plasma sample were determined by gas chromatography of the corresponding butylesters. Lipid extraction from plasma, non-esterified fatty acid isolation and analysis have already been described (Guillot *et al.* 1993). The plasma MCFA concentrations were corrected using the packed cell volume coefficient and the results expressed in µg/ml blood, assuming negligible amounts of MCFA were carried in erythrocytes.

Calculations

The net appearance of MCFA (octanoic and decanoic acids) in the portal circulation was calculated, after concentration correction by packed cell volume coefficient, using the formulas of Rérat *et al.* (1980):

$$q_i = (C_{p_i} - C_{a_i}) D_i t, \quad Q_n = \sum_{i=0}^{i=n} q_i,$$

where q_i is the amount of MCFA absorbed within a time unit t of 5 min during which all concentrations and flows are considered as constant; Q_n is the total amount of MCFA

absorbed during n time units; C_{p_i} , C_{a_i} , are the portal and arterial blood concentrations respectively of MCFA; D_i , is the portal flow.

Differences between variables upon time (for example MCFA concentration in the portal blood compared with that in the arterial blood) were studied on a within-animal basis by fitting a linear regression (Kendal & Stuart, 1967) over selected time-periods, i.e. 0–2, 2–5 and 5–8 h. The significance of a difference was then tested by means of an analysis of covariance (Kendal & Stuart, 1968). The effect of a given factor of variation (i.e. the kind of blood, portal or arterial, upon MCFA concentration in the above example) and the cross effect of time \times factor were individually tested. Significance was decided when either the factor or time \times factor effect was significant at $P < 0.05$ or $P < 0.01$ over the time-period considered. This procedure, though not optimal, is far more powerful than crude comparisons of means at each time. The numerical results were obtained through the linear model specialized module of S (Chambers & Hastie, 1992).

RESULTS

Blood flow in the portal vein

The portal blood flow showed relatively constant values before and during the experiments and it was not modified by the infusion; the mean value was 2419 (SE 104) ml/min.

Blood concentrations of octanoic and decanoic acids

The blood concentrations of non-esterified octanoic and decanoic acids in the portal vein and the carotid artery *v.* time have been plotted in Fig. 1 (octanoic acid) and Fig. 2 (decanoic acid). For each of the three animals used (which will be called A, B and C), two separate graphs have been presented in each figure, corresponding to the first (PEP mixture) and second (free amino acids mixture, 8 d later) infusions respectively.

Before each infusion all basal levels of MCFA were very low, both in portal and arterial blood. Their concentration increased after the beginning of each infusion, slowly in the arterial blood and more rapidly in the portal blood, which generally showed a concentration peak about 2 h later, with the exception of portal blood from pig B in the experiment using the PEP mixture: the concentration peaks of the two MCFA were delayed to 4 (octanoic acid) and 6 (decanoic acid) h in this experiment, as seen in Figs 1(b) and 2(b).

As shown in Figs. 1 and 2, differences between the portal and arterial blood concentrations of MCFA were significant at $P < 0.05$ or $P < 0.01$ over the 0–5 h time-period in pigs A and C whatever the N source used and in pig B when given the free amino acid mixture, over the 2–5 h time-period in pig B given the PEP mixture and over the 5–8 h time-period in pig C given the PEP mixture. These significant differences between MCFA concentrations in the two vessels over given periods mean that significant amounts of MCFA were being absorbed from the intestine during these periods in our animals devoid of exocrine pancreatic secretion.

Amounts of MCFA absorbed

The calculated amounts of non-esterified MCFA that were absorbed per hour via the portal vein have been reported in Fig. 3, expressed as percentage of administered dose for each of the three animals and each of the infusion mixtures. Most of the total amount of MCFA absorbed over each experiment generally appeared within 5 h, with the exception of pig B which showed a more delayed MCFA absorption, i.e. in the interval between 2 and 8 h in the experiment using the PEP mixture. Pig C also showed substantial MCFA absorption in the interval between 5 and 8 h following infusion of the PEP mixture.

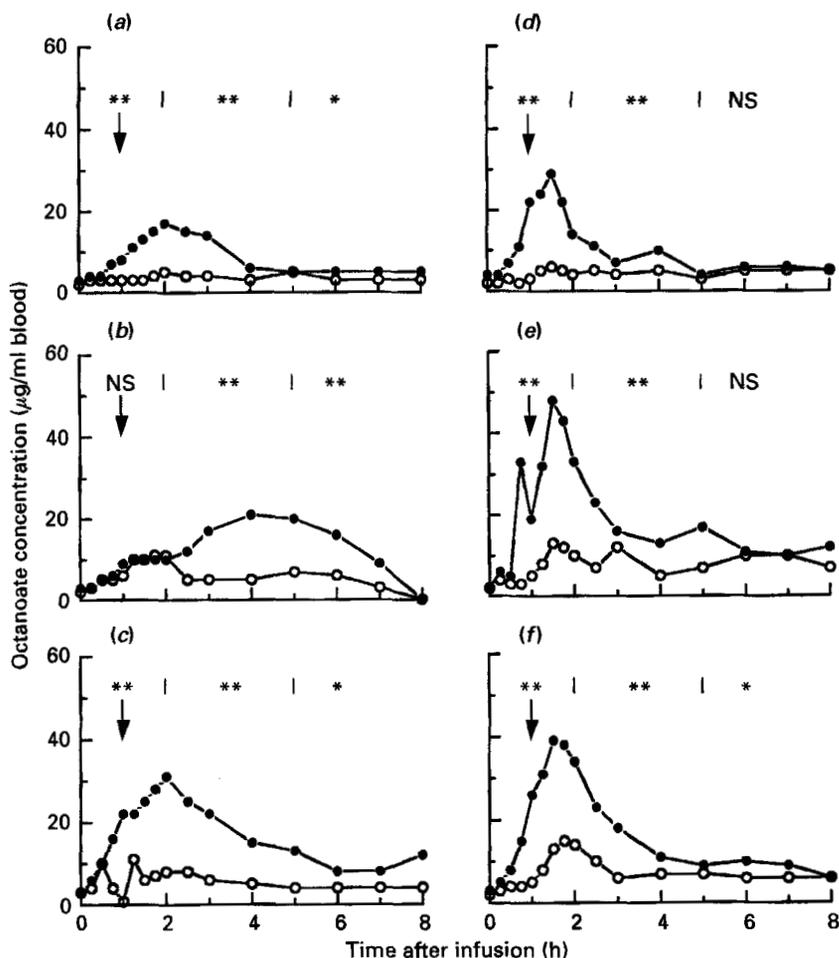


Fig. 1. Variations in octanoate concentrations in the portal blood (●) and arterial blood (○) of three pancreatic-deficient pigs v. time after infusion. Concentrations were determined after a duodenal infusion (for 1 h) of octanoic and decanoic acids esterified as medium-chain triacylglycerols, together with maltose dextrine and a nitrogenous fraction, i.e. either peptides or free amino acids. Arrows indicate the end of each infusion. (a), (b) and (c): individual results from pigs denoted A, B and C respectively, after the first infusion (peptide mixture as nitrogenous source). (d), (e) and (f): individual results from pigs A, B and C respectively, after the later infusion (free amino acids as nitrogenous source). The statistical significance of the within-animal differences between portal and arterial blood concentrations was studied by fitting a linear regression over selected time-periods (0–2, 2–5 and 5–8 h). * $P < 0.05$; ** $P < 0.01$; NS, not significant. For details of procedure, see pp. 546–548.

If we now compare the time-courses of MCFA hourly absorption within each animal as function of the N source used, significant differences may be shown. Amounts of MCFA absorbed over the time-period 1–2 h were generally higher following infusion of the free amino acid mixture than following that of the PEP mixture. The reverse was true over the time-period 2–8 h, at least in pig B and, to a lesser extent, in pig C. However, when calculating the total amount of MCFA absorbed over each whole course, this amount was practically the same whatever the N source used: mean values of 26% and 27% of the total quantities of octanoic and decanoic acids respectively infused as MCT were recovered in the portal blood, as shown in Fig. 4.

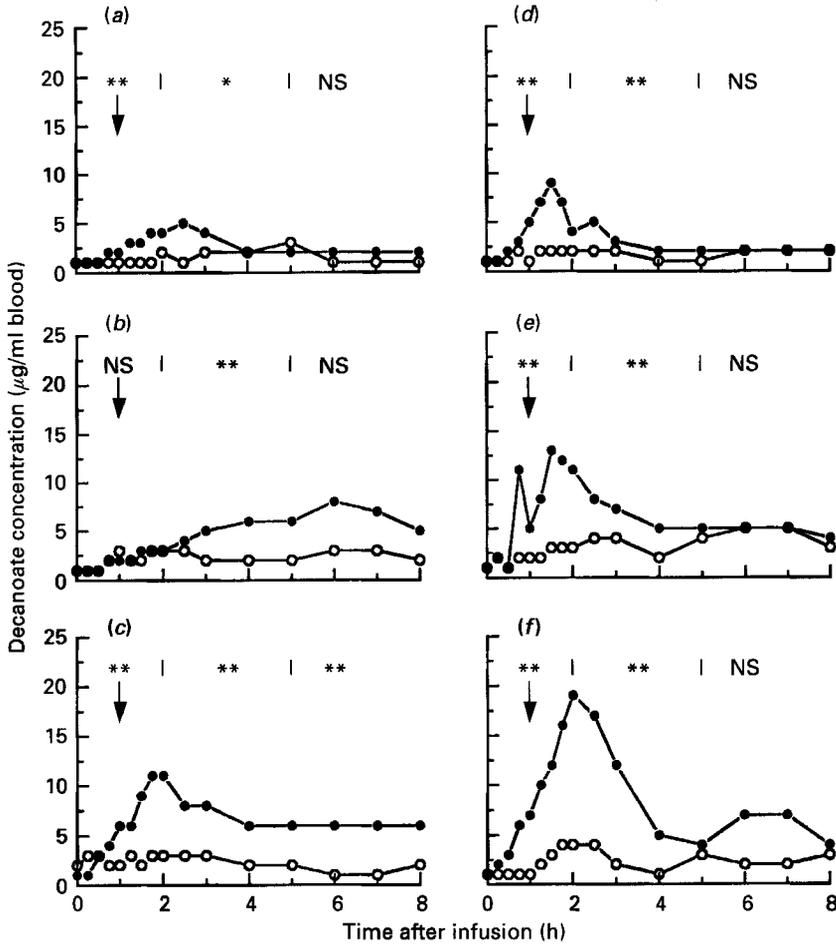


Fig. 2. Variations in decanoate concentrations in the portal blood (●) and arterial blood (○) of three pancreatic-deficient pigs *v.* time after infusion. Concentrations were determined after a duodenal infusion (for 1 h) of octanoic and decanoic acids esterified as medium-chain triacylglycerols, together with maltose dextrine and a nitrogenous fraction, i.e. either peptides or free amino acids. Arrows indicate the end of each infusion. (a), (b) and (c): individual results from pigs A, B and C respectively, after the first infusion (peptide mixture as nitrogenous source). (d) (e) and (f): individual results from pigs A, B and C respectively, after the later infusion (free amino acids as nitrogenous source). The statistical significance of the within-animal differences between portal and arterial blood concentrations was studied by fitting a linear regression over selected time-periods (0–2, 2–5 and 5–8 h). * $P < 0.05$; ** $P < 0.01$; NS, not significant. For details of procedure, see pp. 546–548.

DISCUSSION

This study of MCFA absorption, carried out in pigs devoid of any pancreatic enzyme secretion (that will be called 'pancreatic-deficient pigs') shows that the infusion of an octanoic- and decanoic acid-rich triacylglycerol emulsion into the duodenum led to the appearance of non-esterified octanoic and decanoic acids in the portal blood.

In the present study the concentrations of non-esterified octanoic and decanoic acids in the portal blood increased progressively after the beginning of the infusion, reaching maximum values generally between 90 and 120 min but, exceptionally, later on in pig B given the peptide mixture as N source. In healthy pigs under the same conditions of infusion, MCFA concentrations in the portal blood rose sharply, reaching maximum

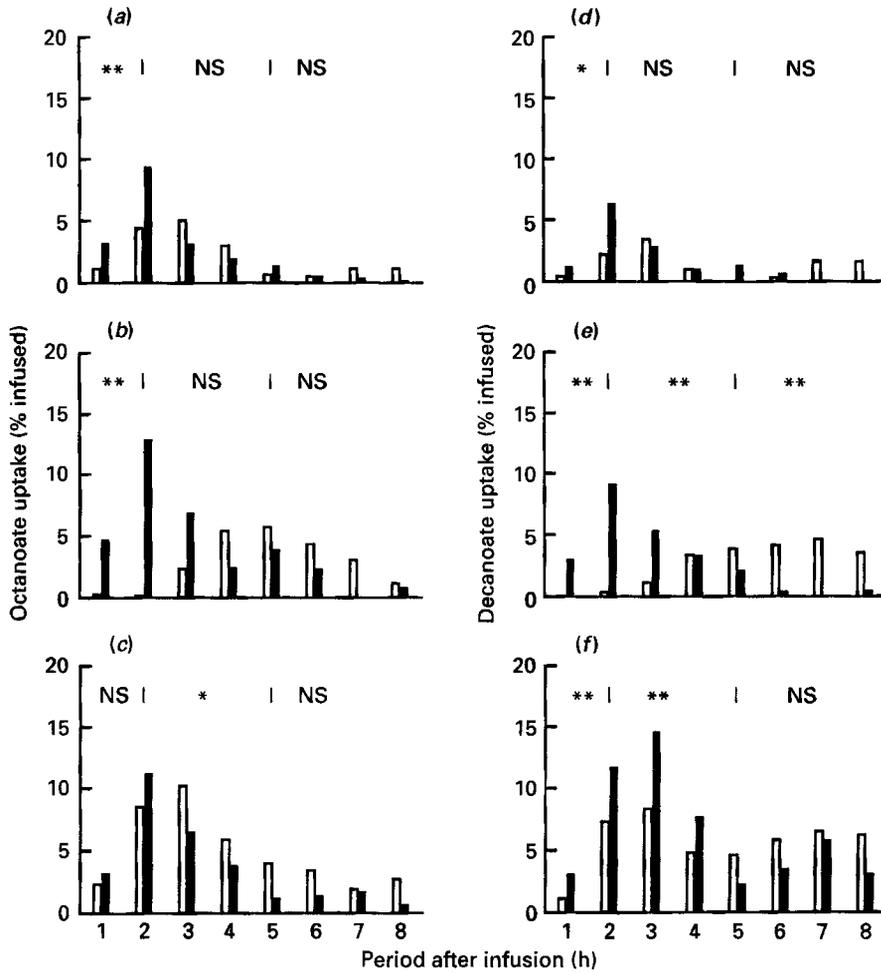


Fig. 3. Hourly intestinal absorption (as percentage of infused quantity) of octanoate ((a), (b) and (c), corresponding respectively to pigs A, B and C and decanoate ((d), (e) and (f) corresponding respectively to pigs A, B and C, after the duodenal infusion of octanoic and decanoic acids esterified as medium-chain triacylglycerol together with maltose dextrine and a nitrogenous fraction, i.e. either peptides (□) or free amino acids (■). The statistical significance of the within-animal differences between hourly absorbed quantities v. the N source used was studied by fitting a linear regression over selected time periods (0-2, 2-5 and 5-8 h). * $P < 0.05$; ** $P < 0.01$; NS, not significant. For details of procedure, see pp. 546-548.

values within the first 15 min short period after the beginning of the infusion (Guillot *et al.* 1993).

Obviously, this early concentration peak was missing in pancreatic-deficient pigs. Moreover, the upper concentrations reported in this study were about 2 to 3 times lower than those previously obtained in healthy pigs, whatever the infusion mixture used (peptides or free amino acids). As a consequence, the total octanoic and decanoic acid amounts absorbed during these 8 h experiments (about 26% of infused octanoic acid and 27% of decanoic acid) were lower than those measured using healthy pigs (61% and 47% respectively).

In contrast to healthy pigs in the same conditions of infusion (Guillot *et al.* 1993), the kind of N source used seemed to influence the time-course of MCFA absorption in

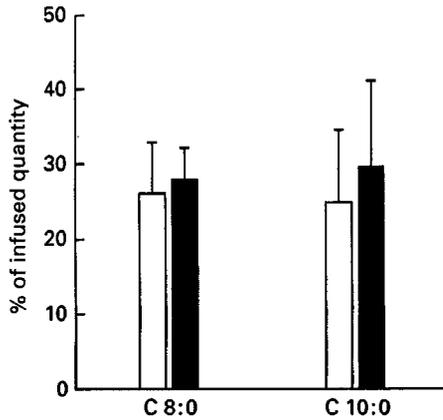


Fig. 4. Cumulated amounts of octanoate (C 8:0) and decanoate (C 10:0) absorbed by pigs after the duodenal infusion of octanoic acid and decanoic acid esterified as medium-chain triacylglycerols together with maltose dextrine and a nitrogenous fraction, i.e. either peptides (□) or free amino acids (■). Values are means with their standard errors represented by vertical bars for three animals. For details of procedure, see pp. 546–548.

pancreatic-deficient pigs, particularly in pig B and, to a lesser extent in pig C, which showed delayed MCFA absorption after infusion with the peptide mixture. However, owing to the low number of animals used in the present study, the peptide mixture was always given in the first infusion, and the free amino acid mixture in the second one, in order to compare all three animals under the same experimental conditions. It may therefore be asked whether the differences between the first and the second infusion were due to the kind of N source, or to a possible, unknown adaptative mechanism of MCFA absorption in these pancreatic-deficient pigs during the 8 d interval between them.

Since MCT in physiological conditions are rapidly hydrolysed by pancreatic lipase from pancreatic juice, leading to the subsequent liberation of non-esterified MCFA in the intestinal contents (Greenberger *et al.* 1966), and since MCFA introduced in the intestinal lumen of rats seem to appear at a maximum concentration in the portal blood within a short period (5 min; Bernard & Carlier, 1981, 1984), the absence of pancreatic lipase would explain the lower absorption rates observed in pancreatic-deficient pigs compared with those seen in healthy pigs.

However, the present study demonstrated that MCFA portal appearance under these pancreatic lipase-deficient conditions is not completely suppressed, which could indicate that alternative pathways, distinct from that following hydrolytic activity of the pancreatic lipase, may occur. A microsomal lipase has already been reported in the enterocyte (Serrero *et al.* 1975). In pancreatic-deficient rats (Playoust & Isselbacher, 1964; Greenberger *et al.* 1966; Clark & Holt, 1968) MCT are transported into the enterocyte, where intracellular hydrolysis occurs. This may explain the portal appearance of MCFA in our pancreatic-deficient pigs. A residual activity of gastric lipases may also be implicated. Clark *et al.* (1969) in rats and Cohen *et al.* (1971) in humans described considerable MCT hydrolysis by the gastric juice. Gastric lipase coming from the stomach to the duodenum, and for which maximum activity has been measured between pH 4 and pH 7 (Cohen *et al.* 1971, for review see Hamosh, 1979), might exhibit residual activity towards MCT in the intestine, particularly in pancreatic-deficient pigs since Abello *et al.* (1987) have demonstrated that the absence of pancreatic secretion in pigs fasted for 16 h induced a duodenal pH decrease (below 6).

In conclusion, the present study provides quantitative information on the absorption of

octanoic and decanoic acids when they are introduced as MCT into the duodenum, thus bypassing the stomach, in pancreatic-deficient pigs. Studies from Galabert *et al.* (1975) have shown that 3- to 5-year-old children suffering from cystic fibrosis of the pancreas seemed to absorb readily an orally administered MCT emulsion. Further studies would be necessary to determine the relative importance of preduodenal and intestinal MCT hydrolysis in cases of pancreatic exocrine deficiency.

The authors are grateful to P. Vaugelade, C. Désert and P. Lefébure for their technical assistance, and to G. Brachet for animal care. This work was supported by a grant from Spharga Laboratory.

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Printed in Great Britain