Further characterisation of the 'ileal brake' reflex in man – effect of ileal infusion of partial digests of fat, protein, and starch on jejunal motility and release of neurotensin, enteroglucagon, and peptide YY

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SUMMARY Previous studies have shown that ileal infusion of partially digested triglyceride inhibits jejunal motility. The partial digest used in those studies contained a mixture of glycerol, free fatty acid, mono-, di-, and triglycerides. In Part I of the present study we have separately infused emulsions containing either glycerol 3.1 g (n=6), oleic acid 9.6 g (n=6), triolein 10 g (n=12), or medium chain triglycerides 10 g (n=6) into the ileum and have recorded the effect this has on jejunal motility. Five further subjects received infusions of partial hydrolysates of corn starch 10 g and lactalbumin 7 g. Marked inhibition of jejunal pressure wave activity was seen after all three lipid infusions, per cent activity falling from a control of 37.7 (7.7) to 6.2 (2.1) and 22.4 (8.2)% 30 min after completing the oleic acid and triolein infusions respectively, and from a control value of 39.5 (4.1) to 17.7 (4.7) after MCTs (all $p < 0.05$). No significant fall occurred after infusion of glycerol, protein or carbohydrate. All three lipid infusions raised plasma concentrations of neurotensin, enteroglucagon and peptide YY equally effectively, although only the rise in peptide YY correlated significantly with the inhibition of jejunal pressure wave activity ($r = 0.80$, $n = 6$, $p < 0.05$). In Part II of this study six subjects received a 3 ml/min jejunal infusion of an isotonic carbohydrate saline solution followed after three hours by a similar infusion of a partial digest of lipid. During each infusion flow and transit time was measured by marker and dye dilution. Jejunal infusion of the carbohydrate-saline solution was associated with low jejunal flow, 4.7 (1.0) ml/min and a mean transit time through the 50 cm study segment of 36.5 (7.1) min. By contrast jejunal infusion of partially digested triglyceride was associated with a markedly increased flow, 9.0 (1.2) ml/min, a fall in mean transit time to 20.3 (2.6) min and significant rises in pancreaticobiliary secretions. Jejunal triglyceride also increased the incidence of prolonged high amplitude jejunal pressure waves in four of six subjects. These studies suggest that there are important differences in the jejunal response to ileal *versus* jejunal lipid. While long and median chain free fatty acids infused into the ileum exert an inhibitory effect on jejunal motility, when infused directly into the jejunum partially digested triglyceride accelerates transit, increases jejunal flow and subtly alters the pattern of jejunal contractions.

Patients with steatorrhea caused by mucosal disease have excessive quantities of partially digested fat

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passing through the distal ileum and colon. This is associated with abnormally high postprandial concentrations of a number of fat-sensitive peptides as well as a number of subjective features such as anorexia, bloating, and diarrhoea. Using an experi-

mental model of steatorrhoea in man we have previously shown that an ileal infusion of 10 g of partially digested triglyceride produces an inhibitory effect on jejunal motility and delays transit through the upper small intestine,¹ an effect which we have termed the 'ileal brake'. Other authors using very different methods have also reported inhibitory effects on gastric emptying and small bowel transit in man using an ileal infusion of undigested triglyceride² and similar effects have now been reported in animals.³ As our initial infusion was a mixture of undigested triglyceride as well as free fatty acid and substantial amounts of glycerol it was not possible to identify the chemical component responsible for the inhibition of jejunal motility. The aim of the present study was to characterise further the nature of the ileal stimulus initiating the 'ileal brake' by separately infusing the various components of the initial infusion. We also compared the effect of long chain triglycerides with medium chain triglycerides. These were of special interest, as they are much more rapidly hydrolysed and absorbed than long chain triglycerides,⁴ and would be expected to exert a more immediate effect if absorption is a necessary preliminary before fats can initiate the 'ileal brake' reflex. We also studied the effect of isotonic solutions of partial hydrolysates of corn starch and milk protein to determine the chemical specificity of this inhibitory reflex. Simultaneous measurements of the plasma concentrations of peptides known to be stored in the ileal mucosa and released by ingested lipid, i.e. neurotensin (NT), enteroglucagon (EG), and peptide YY (PYY) were performed with the aim of identifying a possible peptide mediator of the jejunal inhibition. Finally in separate studies in which partially digested triglyceride emulsion was infused into the jejunum we evaluated the differing jejunal response to ileal *versus* jejunal lipid.

Methods

SUBJECTS

After an overnight fast, 23 healthy volunteers, 13 men and 10 women, aged 20–39 years were intubated with one of two multilumen radioopaque polyvinylchloride tubes, which were passed through the pylorus under fluoroscopic control aided by a 10 g tungsten weight. Subsequent passage of the tubes through the small intestine was accelerated by inflating a small latex rubber balloon at their tip.

The study was divided into two parts. In Part I (17 subjects), the effects of various ileal infusions on jejunal motility were recorded, while in Part II (six subjects), the effect of jejunal infusion of fat or carbohydrate on jejunal flow and transit was studied.

All subjects gave their informed consent to take

part in these studies which had the approval of the Brent District Ethical Committee, 25 February 1981. Radiation exposure was from fluoroscopy (<60 sec), skin entrance dose <1 rad, testes/ovaries <0.1 rad, and from ¹⁴C-PEG, <10 mrad for the small and large intestine.

Part I

PROCEDURES

A 6-lumen combined manometry and perfusion tube was passed until the proximal infusion port P1 (Fig. 1) lay just beyond the duodenojejunal flexure, so that the distal infusion port P2 lay approximately 170 cm from the mouth, 70 cm from P1. Intraluminal pressures were recorded through three side opening jejunal ports J1, J2, and J3, sited 15, 25, and 35 cm beyond P1. Serial blood samples for peptide assay

Fig. 1 6-lumen combined infusion and manometry tube in situ. Jejunal infusion of fat-free nutrient solution at 2 ml/min through P1, ileal infusions of test infusates through P2 at 5 ml/min over 30 min. Numbers against each port indicates distance in centimetres from P1. The 6th lumen (not shown) provided an air line to the terminal balloon.

were collected through an indwelling intravenous cannula into prechilled heparinised tubes to which Aprotinin 200 ul (Trasylol, Bayer, W. Germany) had been added to inhibit proteolysis.

EXPERIMENTAL DESIGN

After basal blood samples had been collected, the jejunum was perfused through P1 at 2 ml/min with a fat free, isotonic nutrient solution providing 1 kcal/min. After a 30 minute equilibration period, pressures were recorded for an hour control period, after which one of six nutrient solutions (Table 1) was infused at 5 ml/min for 30 minutes through P2. During this period isotonic saline was simultaneously infused at 5 ml/min through J3 in order to minimise proximal reflux of ileal test solutions into the jejunum. All solutions were stirred continuously, maintained at 37°C, and were infused using a Watson Marlow constant rate infusion pump (Model HRE 2000, Watson-Marlow, Falmouth, Cornwall, UK). Pressure recordings were made for up to three hour periods after completion of the ileal infusion. The sequence of the infusions in experiments 1–6 were as shown in Figure 3, glycerol being infused at one hour, oleic acid two and a half hours, and triolein five hours after the start of the study. In experiments 7–12 subjects received infusions of triolein and medium chain triglycerides, the two infusions being separated by three hours, three subjects receiving triolein first and three medium chain triglycerides. In experiments 13–17 they received either a partial digest of milk protein (solution 5) or a partial digest of corn starch (solution 6) followed after one and a half hours by the other infusion using an alternating order of infusion.

PREPARATION OF SOLUTIONS

Unless otherwise specified, chemicals used were

Fig. 2 8-lumen tube used in Part II in situ. Jejunal infusion at 3 ml/min through port A and aspiration through port B. Slow marker infusion at 2 ml/min through port M 15 cm proximal to B. Jejunal pressure waves measured through manometry ports J1–3. Numbers again indicate distance from P1. The 7th lumen was an air lines (not shown) to the terminal balloon and the 8th lumen provided an air bleed for aspiration port B.

Table 1 Composition of ileal test solutions

Solution	
1	*Glycerol 225 mmol/l (3.1 g), NaCl 25 mmol/l
2	*Oleic acid 227 mmol/l (9.6 g), glycerol 203 mmol/l (2.8 g), NaCl 25 mmol/l
3	*Triolein 75 mmol/l (10 g), glycerol 203 mmol/l (3.1 g), NaCl 26 mmol/l
4	*Medium chain triglycerides: 98 mmol/l triolein and 19 mmol/l tridecanoin (10 g), glycerol 188 mmol/l (2.6 g), NaCl 29 mmol/l
5	Partial hydrolysate of lactalbumin 47 g/l (7 g)
6	Caloreen 67 g/l (10 g), NaCl 125 mmol/l

The total infused dose of the nutrient components is indicated in parentheses. All solutions were isotonic and were adjusted to pH 7.0 with 1 Mol/l NaOH before infusion at 5 ml/min for 30 minutes;

*10 mmol/l sodium taurocholate and 5.0 g/l egg lecithin added to aid emulsification; medium chain triglycerides (Liquigen, Scientific Hospital Supplies Ltd, Liverpool) have 81% by weight of fatty acid content as octanoic acid (C8) and 16% decanoic acid.

obtained from British Drug Houses, Poole, Dorset, UK and were of AnalaR grade. The fat free nutrient solution for jejunal infusion was prepared by dissolving 87 g of a partial hydrolysate of corn starch (mean chain length 6 glucose monomers; Caloreen, Rousell Laboratories, UK) and 40 g of a partial hydrolysate of milk protein (Casilan, Farley Health Products, UK) in 1 l water and adjusting the solution to isotonicity (290 mosmol/kg) by adding NaCl (final concentration 122 mmol/l).

The ileal test solutions 1–4 (Table 1) were prepared in emulsion form by vigorous agitation of the components, adjusting the pH to 7.0 using 1 mol/l NaCH. Before infusion the osmolality of all solutions was adjusted to 290 mosmol/kg as assessed by freezing point depression adding distilled water as required. Solution 5 was prepared from a partial hydrolysate of lactalbumin containing 50% by weight

Fig. 3 Jejunal % activity after ileal infusion of glycerol (solution 1), oleic acid (solution 2), and triolein (solution 3) (experiments 1–6). Hatched columns represent the periods of ileal infusion. Data are summarised by showing the mean (SE) to the right of the individual data points. * indicates significant difference from the control period ($p < 0.005$).

of peptides of molecular weight < 1000 , 47 g being dissolved in 1 l distilled water, and solution 6 was prepared by dissolving 10 g of Caloreen in 150 ml water and adding NaCl to a final concentration of 125 mmol/l.

Part II

PROCEDURES

Six subjects were intubated with an 8 lumen tube (Fig. 2), which was passed until the proximal infusion port A lay just beyond the duodenojejunal flexure with the distal aspiration port B 50 cm further distally. The jejunal infusion was given at 3 ml/min and jejunal transit was measured by infusing 50 mg non-absorbable dye, bromosulphthalein (BSP) through port A and measuring its appearance in intestinal fluid aspirated through port B. Flow past B was calculated from the dilution of ^{14}C -labelled polyethylene glyco (PEG, mol wt 4000, Amersham International, Amersham, Bucks, UK) which was infused as a 155 mmol/l NaCl solution containing $4 \mu\text{Ci/l}$ ^{14}C -PEG, at 2 ml/min through port M, sited 15 cm proximal to B. Rapid and frequent sampling through B was aided by an air bleed as described by Dillard *et al.*⁵ Intraluminal pressure was recorded through ports J1–J3 sited 15, 25, and 35 cm distal to A.

EXPERIMENTAL DESIGN

Once the tube was positioned, the jejunal infusion was started at 3 ml/min with an isotonic Caloreen-

saline solution (Caloreen 150 g/l, NaCl 82 mmol/l) which also contained 5 mmol/l sodium taurocholate. After an hour equilibration period BSP 50 mg was infused over 10 minutes as 60 ml solution containing 1.3 mmol/l BSP, lactulose 227 mmol/l, rendered isotonic by adding NaCl 33 mmol/l. The jejunal infusion thereafter reverted to 3 ml/min as before. Intestinal fluid aspirated through B was pooled in 5 minute aliquots until no further dye could be detected (usually two hours). The Caloreen-saline jejunal infusate was then replaced by a partial digest of soya bean triglyceride and after a further hour equilibration period the transit of BSP was again measured.

PREPARATION OF SOLUTIONS

The partial digest of soya bean triglyceride was prepared by incubating 132 ml lipofundin 20% (Braun, Melsungen, W Germany) with pancreatin 6 g (Grade IV, Sigma Chemical Co., Poole, Dorset, UK), sodium taurocholate 0.88 g, NaCl 2.24 g and distilled water 268 ml at 37°C for two hours. The final emulsion was diluted as necessary with water until the osmolality reached 290 mosmol/l. The final concentrations were: partially hydrolysed triglyceride 66 g/l, lecithin 5 g/l, glycerol 90 mmol/l, sodium taurocholate 5 mmol/l, NaCl 96 mmol/l, and pancreatin 15 g/l.

ANALYTICAL METHODS

Na^+ concentrations were measured by flame photometry using a multichannel Technicon SMA Plus autoanalyser (Technicon Instruments Inc, Tarrytown, New York). ^{14}C -PEG concentration was measured by liquid scintillation counting using a Beckman LS 7500 counter (Beckman Instruments Inc, High Wycombe, UK), counting in the gel phase. Quench correction was made using the H number method.⁶ Bromosulphthalein was measured by diluting 200 μl intestinal fluid with 4.8 ml 50 mmol/l NaOH containing 10 g/100 ml of the non-ionic detergent 'Brij 35', which effectively removed the non-specific absorbance of the opalescent fat emulsion. The resulting clear violet solutions were assayed for BSP using a Pye Unicam SP6-500 UV spectrophotometer (Pye Unicam, Cambridge, UK) with a 1 cm light path, measuring absorbance at 580 nm. Reproducibility was good, the coefficient of variation of repeated assays (CV) being 1.5% ($n=10$). Bilirubin was similarly assayed spectrophotometrically using the absorbance at 460 nm after clarifying the samples in 10 g/100 ml 'Brij 35', CV being 2% ($n=10$). Amylase was measured from the amount of dye liberated after incubation with a commercially available starch dye tablet (Phadebas Amylase Test, Pharmacia Diagnostics, Uppsala, Sweden), CV was

acceptable at 4% ($n=10$). Osmolality was assessed by freezing point depression, using an Advanced Osmometer Model 3W (Advanced Instruments Inc, Mass, USA), a method which is strictly only valid for dilute aqueous solutions; thus the values obtained must be regarded as approximations applying only to the aqueous phase of the emulsions. Plasma concentrations of neurotensin, enteroglucagon, and peptide YY were assayed using previously published radio-immunoassays.⁷⁻⁹

INTRALUMINAL PRESSURE RECORDINGS

Intraluminal pressures were measured through side opening ports which were constantly perfused with water at 0.1 ml/min using a pneumohydraulic capillary infusion system, Model DIP3 (Mui Scientific, Mississauga, Canada) and connected to external pressure transducers (Consolidated Electrodynamics, England), their output being recorded on a multichannel pen recorder (Grass Model D, Grass

Instrument Co, Mass, USA) at a paper speed of 1 cm/min, pen deflection being 1 mm/2.3 mmHg. The manometry tubes had an internal diameter of 1 mm and, at the infusion rate used, had a good dynamic response, end occlusion of the ports producing a brisk response of 16–45 mmHg/sec. *In vivo* this system recorded pressure events at 10 cycles/min without showing fusion or failure to return to baseline between pressure spikes. The pressure recordings were analysed manually in 30 minute epochs for the per centage of time that phasic pressure wave activity was present, excluding waves less than 10 mmHg. Records from J1, J2, and J3 were averaged to give an overall assessment of jejunal motor activity. Recordings during the infusions were not analysed as J3 was being used to infuse saline and hence during these periods the records were not comparable.

Records were independently analysed by two of the authors one of whom was unaware of the nature of the solutions being tested. These independent scores showed excellent correlation, $r=0.97$ ($n=84$ separate one hour records). The recordings from Part II were also analysed for the area under the curve yielding a motility index in mmHg.min.

Table 2 Plasma NT, EG, and PYY (pmol/l) after ileal infusions

Experiments 1–6						
	Control	Glycerol	Oleic acid		Triolein	
Time†	60	60	60	120	60	120 min
NT	24.0 (1.5)	26.7 (2.0)	49.7* (2.0)	39.3* (6.5)	31.5* (3.0)	35.8* (4.9)
EG	77 (6)	97 (11)	197* (21)	156* (15)	148* (8)	138* (4)
PYY	19.8 (4.1)	14.6 (1.8)	66.0* (10.5)	56.2* (7.8)	53.6* (8.5)	61.0* (12.4)
Experiments 7–12						
	Control	MCTs		Triolein		
Time	60	60	120	60	120 min	
NT	31.2 (3.9)	48.7* (10.7)	29.8 (7.0)	43.1* (3.6)	36.3 (1.7)	
EG	123 (14)	187* (18)	212* (46)	227* (23)	218* (19)	
PYY	9.9 (1.9)	36.7* (6.0)	41.0* (6.4)	64.3* (7.2)	48.0* (8.3)	
Experiments 13–17						
	Control	Protein	Caloreen			
Time	60	60	60 min			
NT	28.4 (2.6)	30.8 (4.3)	33.6 (3.5)			
EG	91 (8)	121 (24)	118 (18)			
PYY	16.8 (6.5)	18.2 (4.4)	16.6 (3.4)			

*Were significantly different from control values, $p<0.05$, unmarked values were not significantly different; †time after start of each infusion.

CALCULATIONS AND STATISTICS

Intestinal flow past port B was calculated from the dilution of ¹⁴C-PEG using the steady state equation¹⁰ applied to the 15cm mixing segment. Knowing flow and dye concentration it was possible to calculate the cumulative passage of dye past port B. The validity of these calculations was supported by the finding that on average 99.4 (6.2) % of the injected dose was accounted for during each transit measurement ($n=12$). Mean transit time through the segment AB was expressed, as the time for 50% of the dye bolus to pass port B.

Results in the text are expressed as mean $\pm$ standard error of mean (SE). The significance of differences were assessed using the non-parametric Mann Whitney U and Wilcoxon's matched pairs signed rank tests for unpaired and paired data respectively.

Results

PART I: EFFECT OF ILEAL INFUSIONS ON JEJUNAL MOTILITY

The 1 cal/min nutrient solution infused into the jejunum maintained a fed pattern of intestinal motility throughout and no migrating motor complexes characteristic of the fasting state were seen.

Ileal infusion of glycerol, bile salts and lecithin (solution 1) did not significantly alter jejunal motility, per centage activity being 26 (5.6) and 37.2 (7.2) % in the first and second 30 minute period after completing the ileal infusion compared with control

Fig. 4 Jejunal % activity after ileal infusion of medium chain triglycerides (MCTs) and triolein (experiments 7–12). The horizontal axes showing the time in hours from the completion of each infusion. Mean (SE) is again shown to the right of the data points. * indicates significant difference from the control period $p < 0.05$.

values of 33.0 (5.2) and 42.3 (10.2) (differences not significant). By contrast both oleic acid and triglyceride emulsions (solutions 2 and 3) significantly depressed per centage activity, which fell from a control value of 37.7 (7.7) % to 6.2 (2.1) and 22.4 (8.2) % respectively (both $p < 0.05$) in the 30 minutes after completion of the infusion (Fig. 3). At this time the inhibition seen after the oleic acid infusion was significantly greater than that seen after triolein ($p < 0.05$).

In experiments 7–12 medium chain triglyceride infusion produced a rapid inhibitory effect (per centage activity falling from the control value of 39.5 (4.1) to 17.7 (4.7) % at 30 min). This inhibition completely disappeared within one to two hours of completing the infusion (Fig. 4). The onset of the inhibitory effect after triolein was slower than after MCT infusion, maximum inhibition being seen in the second 30 minute period after completing the ileal infusion, per centage activity reaching a nadir of 15.9 (7.6). Recovery of activity was also slower, activity in the third 30 minute period after completing the ileal triolein infusion being 18.6 (7.7) compared with 46.8 (9.4) % during the corresponding period after MCT infusion ($p < 0.05$).

In contrast with the ileal lipid infusions, infusion of the partial hydrolysates of corn starch and lactalbumin did not inhibit jejunal motility (solutions 5 and 6, experiments 13–17). Percentage activity in

successive 30 minute periods being 39 (6.8) and 40.2 (5.1) after ileal protein and 18.2 (5.2) and 38.4 (6.2) after ileal starch, showing no significant difference from the control values of 39.0 (5.0) and 37.8 (8.7) ($n = 5$).

NEUROTENSIN, ENTEROGLUCAGON AND PEPTIDE YY CONCENTRATIONS AFTER ILEAL INFUSIONS (Table 2)

There was no significant change in plasma NT during the one hour control period but as expected, oleic acid infusion was followed by a substantial rise in plasma NT, peak concentrations of 49.7 (2.0) pmol/l being reached 30 min after completing the ileal infusion, these peaks being significantly greater than the basal preinfusion values of 24.0 (1.5) pmol/l ($p < 0.05$). Similar rises were seen after triolein and MCTs (Table 2). Ileal infusion of glycerol alone did not alter plasma NT nor did infusion of the carbohydrate or the protein hydrolysates. The release of EG and PYY by the other solutions tested closely paralleled the release of NT, as can be seen in Table 2.

CORRELATION BETWEEN PEPTIDE RELEASE AND INHIBITION OF MOTILITY

Considering the six ileal test solutions it was apparent that those which released the three peptides also inhibited motility, there being a weak but significant correlation between the mean fall in per centage activity in the first hour and mean rise in plasma peptide concentrations at one hour being 0.48, 0.50, and 0.80 for NT, EG, and PYY respectively ($n = 6$, only the correlation with PYY being significant $p < 0.05$).

PART II: EFFECT OF JEJUNAL INFUSIONS ON JEJUNAL FLOW AND MOTILITY

Jejunal infusion of the Caloreen-saline solution was associated with low flow rate which averaged only 2.9 (0.6) ml/min in the second half of the one hour equilibration period. Flow rose significantly as the dye and lactulose appeared averaging 5.8 (1.0) ml/min in the subsequent 30 min ($p < 0.05$), declining significantly in the following 30 min period to 4.8 (1.0) ml/min ($p < 0.05$). By contrast jejunal perfusion of partially digested lipid strikingly increased the flow rates, which reached 12.8 (4.0) ml/min in the second half of the one hour equilibration period, significantly greater ($p < 0.05$) than that observed during Caloreen infusion. Arrival of the dye was not associated with any noticeable further increase in flow, which averaged 9.4 (1.6) ml/min and 10.7 (1.4) ml/min in the two successive 30 minute intervals after the dye appeared in the aspirate.

Considering the 60 min period after injecting the

dye, average flow was significantly greater during jejunal lipid infusion than during the carbohydrate infusion ($9.0 (1.2) \nu 4.7 (1.0)$ ml/min, $p < 0.05$). Furthermore, over the same period, transit of the dye was markedly accelerated during lipid infusion, the time for 50% of the dye to pass port B being $20.3 (2.6)$ min compared with $36.5 (7.1)$ min during jejunal carbohydrate infusion ($p < 0.05$).

This marked increase in flow during jejunal lipid infusion was associated with significant increases in bilirubin and amylase output, which rose from $0.5 (0.3)$ to $7.8 (2.0)$ $\mu\text{mol/min}$ and from $40 (9)$ to $201 (49)$ IU/min respectively.

There was no obvious change in jejunal per centage activity which was $33.6 (7.1)$ and $32.7 (10.1)$ % during the Caloreen and lipid infusions respectively (difference not significant). Although the corresponding motility indices increased from $1.6 (0.3)$ to $3.7 (2.2)$ mmHg.min $\times 10^3$ these values were not significantly different owing to wide inter-individual variability ($p > 0.05$). In four of six subjects, however, lipid did appear to stimulate the

appearance of abnormal, prolonged (> 5 sec, mean $7.3 (0.9)$ sec), high amplitude ($49.5 (6.6)$ mmHg) contractions (Fig. 5). These contractions did not, however, appear to propagate to the other ports situated only 10 cm away. Similar contractions were observed during Caloreen infusion but were infrequent, only four being recorded in the 60 min after injection of the dye, whereas a total of 37 were recorded during the same period of lipid infusion. The two subjects who did not show any obvious response each had one of these contractions, one during the infusion of Caloreen and one during the infusion of lipid. These prolonged contractions were often followed by a period of quiescence so that when averaged out over time the appearance of such contractions altered neither the percentage activity nor the motility index.

Discussion

The first part of this study showed that ileal infusion of equicaloric amounts of a range of lipid emulsions

Fig. 5 Pressure recording during jejunal infusion of lipid. The three traces are recorded from ports J1, J2, and J3 each separated by 10 cm. The upper trace shows two abnormally prolonged high pressure peaks which were rarely seen during infusion of the fat free solution.

markedly but reversibly inhibited jejunal motor activity. This effect has previously been shown to be associated with a marked delay in jejunal transit.¹ These previous studies¹ had also shown that ileal infusion of isotonic saline produced no inhibition implying that the effect is not simply caused by intestinal distension but is a response to the chemical nature of the infusion. In our previous studies we used a partial digest of long chain triglyceride which contained large amounts of glycerol and undigested triglyceride together with free fatty acid. The present study indicates that the glycerol and bile salts in our original ileal infusions contributed little to the inhibitory effect. The initially smaller effect of triolein, which lasted longer than the effect of oleic acid and medium chain triglycerides, supports the idea that lipid must be digested and absorbed before it can exert its inhibitory effect. Further studies using nonhydrolysable ether analogues of triglyceride would be necessary to definitively prove this point.

The long chain oleic acid (C18) and the medium chain caproic acid (C8, the major component of the medium chain triglycerides) were equally inhibitory and in both cases the effect appeared to last less than two hours. This rapid reversal of inhibition may reflect rapid absorption of these lipids from the lumen. Recovery of activity after triolein infusion was slower, perhaps because our ileal infusions diluted luminal lipase and thus impaired hydrolysis and absorption which in these circumstances is slower for triolein than for medium chain triglycerides.⁴ As luminal levels of lipid were not measured, however, this must remain speculative.

Our use of a non-random order of perfusion in the first six experiments raises the possibility of a carry over effect. We choose this order as the most economical study design because in pilot studies it was apparent that while glycerol produced little or no effect and the effect of oleic acid was readily reversible, the effect of triolein was the most long lasting. Thus we believe that the sequence used minimised the chances of carry over effects. It seems unlikely that the slower recovery of motor activity after triolein in experiments 1–6 was caused by a carry over effect as a similar time course was noted in the three studies (experiments 7, 9, and 11) in which triolein was infused as the first infusate. We cannot exclude the possibility that some of the lipid infusion reached the colon but think that it is unlikely that substantial amounts did because our subjects did not experience diarrhoea or abdominal cramps as have been observed during colonic infusion of oleic acid.¹¹ Even if some did, it is unlikely that this would have altered small bowel motility because when 12 g of oleic acid was directly infused into the colon there was no change in ileal motility.¹¹

The ileal infusion of the protein hydrolysate did not affect jejunal percentage activity nor was there any significant inhibitory effect seen after ileal infusion of Caloreen (10 g). This lack of effect may, however, merely reflect an inadequate dose of carbohydrate, since when we subsequently infused a further three subjects with 15 g of Caloreen they all showed an obvious inhibition of motility when compared with the one hour control period, percentage activity being 23.9, 47.8, and 58 v 2.9, 21.9, and 31.7% respectively. Unfortunately as administration of this larger dose was associated with right iliac fossa discomfort and nausea, symptoms not seen with the other infusions, we did not think it reasonable to pursue this further. Just why 15 g of partially digested starch produced pain is uncertain, but these glucose oligomers are rapidly hydrolysed to glucose producing a markedly hypertonic solution whose osmotic effect may induce substantial water secretion and pain because of associated intestinal distention. In these same three studies we did note substantial rises in NT (90, 78, and 96 pmol/l), EG (540, 480, and 360 pmol/l), but not PYY (15, 18, and 11 pmol/l) at 1 h.

It is possible therefore that lipid is not the only nutrient capable of eliciting the 'ileal brake' but, using our method larger doses of carbohydrate could not be readily studied.

Some comment is necessary here on the fat concentration in our test solutions. The initial partial digest¹ was an attempt to approximate postprandial ileal contents in marked steatorrhoea and hence contained pancreatin, bile salts 5 mmol/l and partially digested fat (66 g/l). In the present studies the ileal infusate was diluted by endogenous secretions and by the 5 ml/min saline infusion *via* J3, giving an estimated ileal luminal concentration about half that infused – that is, 33 g/l. How closely this mimics the ileal contents in the various diseases characterised by steatorrhoea is uncertain, but it lies within the range (26.0 (6.5) mg/ml, mean (SE)) found postprandially in the ileum of patients with modest steatorrhoea due to vagotomy and gastroenterostomy.¹² Ileal fat concentrations have not been directly measured in other conditions but when stool output is substantial, faecal composition should tend to approximate to caecal contents and in these circumstances patients with pancreatic steatorrhoea have faecal fat concentrations of 100–240 g/l.¹³ This suggests that terminal ileal concentrations could well reach 33 g/l in severe steatorrhoea.

When comparing the different forms of lipid we choose to use equicaloric rather than equimolar doses as by analogy with the control of gastric emptying¹⁴ we anticipated that the calorie content of our emulsions would be the most important factor influencing their inhibitory effect. This was indeed

supported by our finding that 73 mmol/l of triolein exerted a similar degree of inhibition as 223 mmol/l oleic acid and approximately 117 mmol/l medium chain triglycerides. In testing the effect of our partial digests of starch and protein we used the most concentrated solutions which were tolerated without undue abdominal discomfort but it should be noted that these had a lower calorie content than the lipid emulsions so that no precise statement can be made about the relative effectiveness of these forms of nutrients without further dose response studies using lower calorie loads.

The lipid emulsions tested in the present study, like those used previously,¹ contained 66 g/l of lipid and 5 mmol/l Nataurocholate. Owing to the use of 20% Lipofundin in the present study rather than 10% Lipofundin used previously, however, there were differences in concentrations of lecithin (5 g/l instead of 10 g/l), glycerol (90 mmol/l instead of 181 mmol/l), NaCl (96 mmol/l instead of 37 mmol/l), and Pancreatin (15 g/l instead of 5 g/l). However, as solution I containing glycerol, pancreatin, and lecithin did not significantly alter motility we do not think that differences in these components invalidate our comparisons.

These studies support previous observations in showing the effectiveness of ileal lipid in releasing NT, EG, and PYY. They also show that MCTs infused directly into the ileum are as effective as long chain triglycerides in releasing these peptides. Although the potency of the infusates for releasing NT, and EG roughly paralleled their inhibitory effects on the jejunum correlations were poor in individual subjects and neither hormone appears to mediate the inhibition observed. Infusions of NT at doses known to produce blood concentrations similar to those seen after ileal lipid delay gastric emptying,¹⁵ but such doses merely abolish the fasting cycle¹⁶ of the small bowel and do not inhibit its motility in the way that lipid did. Furthermore in other studies ileal fat infusions have been shown to delay gastric emptying and small bowel transit without raising plasma NT or EG.² Enteroglucagon is not at present available for intravenous infusion but after ileal lipid infusion we found plasma EG concentrations remained raised for greater than two hours, while the jejunal inhibition after oleic acid and medium chain triglycerides infusion had completely disappeared by this time, a fact which again makes it unlikely that EG causes the jejunal inhibition observed.

PYY a more recently described peptide,¹⁷ which is found in ileal and colonic mucosal endocrine cells,¹⁰ is capable of inhibiting gastric emptying¹⁸ when infused into man at a dose (2 pmol/kg/min) which produces rises in plasma PYY (60 pmol/l) similar to those seen after our ileal lipid infusions. These plasma concen-

rations are similar to those seen postprandially in patients with steatorrhoea as a result of tropical sprue and chronic pancreatitis.¹⁹ More recently PYY infusions have been shown to delay both gastric emptying and mouth-caecum transit.²⁰

PYY has also been shown to act centrally to induce emesis in dogs²¹ which may be relevant to the anorexia and bloating often seen in patients with severe malabsorption caused by coeliac disease in whom we have recently noted markedly raised plasma PYY (up to 100 pmol/l) after a test meal.²² Others have suggested that PYY may be a mediator of the 'ileal brake'²³ and our data provide some support for this contention. Although normal meals are followed by only modest rises in PYY, the blood concentrations seen after gorging (4500 kcal meals) are similar to those seen after ileal infusion of fat⁹ implying that after large meals fat may reach the distal ileum in sufficient quantities to activate the 'ileal brake reflex'. This idea is supported by the work of Booth *et al* who showed that while little or no triglyceride entered the ileum after a small fat load, as the fat content of a meal rose so increasing amounts of fat entered the distal ileum.²⁴

Part II of our study shows that the inhibitory effect of an ileal infusion of partially digested fat is site specific as inhibition of upper small intestinal motility is not seen when the same concentration of fat is infused into the jejunum. Thus ileal lipid is likely to act locally rather than through the systemic effects of absorbed lipid. Increased jejunal flow during jejunal fat infusion is partly explained by increased biliary and pancreatic secretion and partly by jejunal secretion stimulated by luminal free fatty acids.²⁵ By contrast, the partially digested starch stimulated water absorption without any marked increase in pancreatic or biliary secretions, and was thus associated with much lower jejunal flow. This difference was made more obvious by using a protein free control infusate thus avoiding stimulation of pancreatico-biliary secretions.

Although percentage activity was not altered compared with the control period, there did appear to be a qualitative change in the pattern of jejunal contractions, lipid stimulating the appearance of prolonged contractions which others have also recently reported,²⁶ and which may have contributed to the faster dye transit we observed. Stimulation of propulsive activity has recently been reported in the ileum in response to boluses of short chain fatty acids²⁷ but as we did not measure ileal motility we cannot comment on the ileal response to long chain fatty acids nor whether such a response is a generalised one seen with all fatty acids.

Combining the results of the two parts of our study it would appear that in health after a mixed meal the

outpouring of pancreatic and biliary secretions produces a high flow in the jejunum. Flow will fall further down the small intestine as hydrolysis is followed by rapid absorption of nutrients, water, and electrolytes. Where mucosal disease prevents or inhibits absorption substantial amounts of unabsorbed nutrients will enter the distal ileum thereby activating the ileal brake effect and delaying jejunal transit allowing more time for absorption to take place.

The studies reported here have further characterised the nature of the stimulus activating the 'ileal brake'. They suggest that long and medium chain free fatty acids stimulate the ileum to exert an inhibitory effect on the motility of the proximal small intestine. This could be mediated *via* the activation of specific receptors or could be caused by the non-specific detergent effect of free fatty acids. Our studies do not permit differentiation between these possibilities which will probably require more systematic dose-response studies in an animal model. Circumstantial evidence supports the idea that peptide YY could be at least partly responsible for this effect but definite proof must await further studies.

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