

Modulation of fluid absorption and the secretory response of rat jejunum to cholera toxin by dietary fat

F A Sagher, J A Dodge, R Moore, C McMaster, G McCaughey

Abstract

To study the effects of dietary fat on jejunal water and ion absorption and on cholera toxin-induced secretion, 3 week old Sprague Dawley rats were fed isocaloric diets. Forty per cent of the total calories were given as fat, as butter (high saturated fat), olive oil (high mono-unsaturated fat), or corn oil (high poly-unsaturated fat), with one group on low fat (10% of calories) standard laboratory diet as controls. During in vivo jejunal perfusion studies we found that (i) a polyunsaturated fat (corn oil) supplemented diet improves jejunal absorption of water and electrolytes and these changes are independent of the observed concentrations of luminal prostaglandins; (ii) high dietary fat appreciably reduced the secretory response to cholera toxin, probably without fundamentally changing the mechanism by which cholera toxin induces secretion. We conclude that dietary fat composition altered the permeability and transport characteristics of the small intestine. This observation might have relevance to some human diarrhoeal disorders.

Interest in dietary modification of intestinal ion transport and eicosanoid synthesis has arisen both as an alternative approach to studying their physiological importance and for possible therapeutic value. Evidence is gradually accumulating for a protective effect of dietary fat in diarrhoeal disorders. Epidemiological studies suggest a possible relation between low milk fat intake in children and an increased incidence of gastrointestinal illnesses, especially diarrhoea. Careful analysis failed to explain the association and it was concluded that there may be a non-immunoglobulin factor in milk fat which prevents diarrhoeal illness.¹

In a clinical study children with chronic non-specific diarrhoea who had no evidence of mal-absorption were found to be taking low fat diets, and they responded well to polyunsaturated fat supplementation.^{2,3} In the laboratory fractionation of human milk by ammonium sulphate precipitation and column chromatography yields a non-immunoglobulin fraction which has been shown to protect rabbits against enterotoxin-induced intestinal fluid secretion in vitro. Preliminary experiments showed that the inhibitory activity was extracted by chloroform and methanol, which suggests the involvement of lipid.⁴ A glycoprotein or a glycolipid compound in the non-immunoglobulin fraction of human milk has recently been shown to inhibit the adherence of certain enterotoxigenic *Escherichia coli* strains to guinea pig intestinal tract.⁵

The present study was designed to investigate

further the relations between diet and function and to explore the possible protective value of different types of dietary fat in a rat model of secretory diarrhoea.

Methods

PREPARATION OF ANIMALS AND DIETS

Three week old male Sprague Dawley rats were fed one of three synthetic diets. The diets contained 20% fat, which provided 40% of the total calories, as butter, olive oil, or corn oil. The diets were isocaloric and nutritionally adequate, providing all known essential requirements (Special Diet Service, Essex, England). A control group was maintained on a standard low fat (10% of calories) diet (Beta-Laboratory Diets - Witham) (Table I). The fatty acid composition of the diets is shown in Table II. To reduce coprophagy animals were housed individually with alternate 12 hours of light and dark, in cages with a mesh wire bottom, and their intake was calculated daily.

The diet high in saturated fatty acids (butter) provided 24.6% of the total fatty acids and 9.8% of the total calories (% wt/wt) as oleic acid, and approximately 2.5% of the total fatty acids and 0.95% of the total calories as linoleic acid, whereas only approximately 0.6% of the total fatty acids and 0.2% of the total calories were provided as α linolenic acid, with a w6/w3 ratio of 4.75.

The diet high in polyunsaturated fatty acids (corn oil) provided 29.78% of the total fatty acids and 11.9% of the total calories as oleic acid, approximately 58% of the total fatty acids and 23.2% of the total calories as linoleic acid, and only approximately 0.2% of the total fatty acids and 0.8% of the total calories were provided as α linolenic acid, with a w6/w3 ratio of 29.

The diet high in monounsaturated fatty acids (olive oil) provided 79.4% of the total fatty acids and 31.7% of the total calories as oleic acid, approximately 7% of the total fatty acids and 2.8% of the total calories as linoleic acid, but only approximately 1% of the total fatty acids and

TABLE I The basic diet per 1 kg of complete diet

	Synthetic (g)	Control
Fat*	200	35
Protein (casein) vitamin free	200	173
Carbohydrate:		
Corn starch	250	549
Sucrose	250	
Vitamin mix	20	11.3
Salt mix	40	43.2
Fibre (non-nutritive)	40	31

*Dietary fat as high monounsaturated (olive oil), or high saturated (butter), or high polyunsaturated (corn oil).

Department of Child Health, The Queen's University of Belfast, Belfast BT12 6BJ
F A Sagher
J A Dodge
R Moore
C McMaster
G McCaughey

Correspondence to: Professor J A Dodge.

Accepted for publication 15 January 1990

TABLE II Fatty acid profile of diets*

20% fat				
Fatty acid	Olive oil (HMU) (% wt)	Butter (HS) (% wt)	Corn oil (HPU) (% wt)	Standard diet: 3.5% fat (% wt)
6:0	—	0.28	—	—
8:0	—	0.86	—	—
10:0	—	2.82	—	—
12:0	—	2.89	—	—
14:0	0.12	17.02	0.26	0.57
14:1	—	1.32	—	—
15:0	—	1.21	—	—
16:0	9.22	31.25	8.46	13.87
16:1	—	1.72	—	—
17:0	—	0.86	—	—
18:0	2.94	10.38	1.76	2.15
18:1	79.36	24.58	29.78	22.51
18:2 w6	6.99	2.39	57.78	54.13
18:3 w3	0.89	0.58	1.97	6.28
20:1	—	—	—	—
Others	—	1.84	—	—
Saturated	12.28	67.57	10.48	16.59
Monounsaturated	80.16	27.62	29.78	23.01
Polyunsaturated	7.88	2.97	59.75	60.41

*Diets analysed by Department of Food Biochemistry, Faculty of Agriculture, Queen's University of Belfast.

HMU=high monounsaturated; HS=high saturated; HPU=high polyunsaturated.

0.4% of the total calories were provided as α linolenic acid, with a w6/w3 ratio of 7.

STEADY STATE JEJUNAL PERFUSION STUDIES

The non-stimulated perfusions were performed on adult male Sprague Dawley rats after an overnight fast (18–20 hours) during which water only was allowed ad libitum. Before laparotomy, anaesthesia was induced with intraperitoneal sodium pentobarbitone (60 mg/kg) and maintained when necessary with intramuscular doses of 0.3 mg.⁶ Rectal temperature was maintained throughout the experiment at 37(1)°C (mean (SEM)) by an electrically heated pad. The perfusion studies were essentially as described by Sladen and Harries⁷ with some modification as follows.

The abdomen was opened with a midline incision and about 20 cm length of jejunum from the duodenojejunal junction was prepared. The inlet catheter (polyvinyl tubing bore 0.63 mm, diameter 1.4 mm) and the outlet catheter (polyvinyl tubing bore 2 mm, diameter 3 mm) were inserted into the gut and tied firmly in position with silk ligature. Faecal material, if present, was removed from the loop by washing with the perfusate at 37°C, then the loop was returned to the abdominal cavity.

The final perfusate contained (mmol/l): Na⁺ 155, K⁺ 4, Cl⁻ 130, total CO₂ 21, glucose 1.6, polyethylene glycol (PEG 4000) 3 g/l as non-absorbable marker with 5 μ Ci/l of ¹⁴C PEG. pH was adjusted to 7.0 and the osmolality of the solution adjusted to (mean (SEM)) 299 (1.5) mOsmol/kg.

TABLE III The characteristics of animal growth

Diet	Weight at age 20 days (g)	Weight at end of experiment (g)	Duration of feeding (weeks)
Control (n=14)	42.1 (2.6)	277.2 (21.8)	8.4 (0.5)
Butter (n=14)	40.5 (0.8) NS	270 (17.9) NS	8.2 (0.5) NS
Olive oil (n=19)	38.3 (1.3) NS	289.3 (18.7) NS	8.4 (0.5) NS
Corn oil (n=12)	40.4 (1.7) NS	309 (21.2) NS	8.6 (1.08) NS

NS=not significant. Values are expressed as mean (SEM). Animals were fed from the age of 20 days with one of the synthetic diets (Table I).

The solution was perfused in a single pass perfusion at a rate of 0.45 ml/min using a Harvard infusion pump. The solution was warmed and kept warm through coils of polyvinyl tubing placed into a water bath and the water bath temperature adjusted so that the temperature of the perfusate was 35 (1)°C as it entered the intestine. After 30 minutes, which was sufficient to reach an equilibrium state in preliminary experiments using recovery of ¹⁴C PEG as an index, three consecutive 10 minute collections of effluent were obtained. Each collection was analysed for net absorption and net secretion of water and solutes using the standard formula.⁸

The results were expressed in μ l or μ mol/min per g wet weight. Negative results indicate net secretion. Positive results indicate net absorption.

In the cholera toxin experiment perfusions were performed on adult female Sprague Dawley rats. These were sibs of the male rats used in the steady state studies. Each group of experiments was performed on single sex animals to eliminate possible sex related variations in absorption, and in preliminary studies females had been shown to tolerate prolonged anaesthesia better than males. A blind loop was initially constructed from the jejunum as described by Harries and Sladen.⁷ Faecal material, if present, was removed from the loop by washing with the perfusate at 37°C. 75 μ g of cholera toxin dissolved in 0.75 ml of water was instilled into the loop, which was returned to the abdomen for two hours to allow adequate time for binding of the toxin to the mucosa. The loop was then rinsed with perfusate and prepared for perfusion as previously described.

Cholera toxin was obtained from Sigma Chemical Company (No C3012).

A very low (1.6 mmol/l) glucose concentration was used as an additional marker, because luminal disappearance will reflect active transport or mucosal metabolism, or both, and thus viability of mucosa.⁶ It was assumed that any effect of prolonged anaesthesia on the absorption would be similar to all four groups.

LUMINAL PROSTAGLANDIN ASSAY

One ml of each collection of good recovery from non-stimulated jejunal perfusions was reserved at -20°C after centrifugation at 2500 rpm for 10 minutes, extracted as described by Powell,⁸ and assayed for prostaglandin E₂ and F_{2 α} using prostaglandin radioimmunoassay kit (Seragen Inc, Boston) within three weeks. For each prostaglandin a standard curve of percentage binding v pg prostaglandin was plotted using several standards. The amount of PGE₂ and PGF_{2 α} contained in each sample was then calculated using the appropriate curve and corrected for 100% recovery. Each assay included non-specific binding standards – that is, no added antibody – and controls. The results were corrected for the flow rate and the wet weight of the perfused segment and expressed in pg/ml per min per g wet weight.

The cross reactivity of PGE₂ with PGF_{2 α} is only 0.3% and PGF_{2 α} with PGE₂ 1.3%. The

TABLE IV Dietary fat and ion transport

	Water ($\mu\text{l/g per min}$)	Na ($\mu\text{mol/g per min}$)	Cl ($\mu\text{mol/g per min}$)	K ($\mu\text{mol/g per min}$)	Glucose ($\mu\text{mol/g per min}$)
Control (n=8)	+7.67 (1.4)	+1.93 (0.245)	+0.452 (0.173)	-0.0456 (0.006)	+0.179 (0.011)
Olive oil (HMU) (n=8)	+6.99 (2.7) NS	+1.7 (0.34) NS	+0.32 (0.49) NS	-0.11 (0.036) NS	+0.184 (0.02) NS
Butter (HS) (n=6)	-4.78 (5.0)**	-0.163 (0.86)**	-0.818 (0.56)**	-0.213 (0.102) NS	+0.147 (0.03) NS
Corn oil (HPU) (n=6)	+12.9 (2.0)*	+2.57 (0.285) NS	+0.75 (0.35) NS	-0.067 (0.016) NS	+0.156 (0.014) NS

Values are expressed as mean (SEM). Values *v* control: * $p < 0.05$; ** $p < 0.025$. NS=not significant.

recovery of total counts is better than 95%. The coefficient of variation of PGE₂ is 8.5% (intra-assay) and 5.0% (interassay) and for PGF_{2 α} 8.1% (intra-assay) and 5.0% (interassay).

Rats were sacrificed at the end of the experiment by cardiac puncture and the perfused segment removed and weighed after stripping fluid from the lumen. In all the experiments the concentration of glucose in the effluent was checked by a specific glucose oxidase method (Beckman glucose analyser), Na⁺ and K⁺ by ion selective electrode (Beckman ASTRA), and Cl⁻ by the colorimetric titration technique (Corning 920). ¹⁴C-polyethylene glycol activity in 100 μl aliquots (in duplicate) were counted in Scinttran-fluoran HV (BDH) in a Beckman scintillation counter. Only 100 (5)% of ¹⁴C PEG levels were accepted as good recovery.

STATISTICAL ANALYSIS OF THE RESULTS

One way analysis of variance (Anova) and unpaired Student's *t* test were used to analyse the statistical significance of the differences between the means of water and electrolyte absorption. The values obtained were reported as mean (SEM).

The non-parametric Kruskal-Wallis test of variance and Mann-Whitney U test were used to analyse the statistical significance of the differences of prostaglandin concentration between the groups. The values obtained were reported as mean (SEM) and range.

Results

CHARACTERISTICS OF ANIMALS

The weight of animals at the beginning and at the end of the experiments and the duration of feeding did not significantly differ between the four groups (Table III). No change in the frequency or in the consistency of stool was observed during the feeding period.

EFFECTS ON JEJUNAL ION TRANSPORT

Feeding rats corn oil enhanced the absorption of water ($p < 0.05$ *v* control; $p < 0.005$ *v* olive oil and

butter), Na⁺ ($p < 0.05$ *v* olive oil; $p < 0.005$ *v* butter), and Cl⁻ ($p < 0.025$ *v* butter only), but did not significantly change the absorption of K⁺ or glucose.

Feeding butter fat reduced the absorption of water, Na⁺, and Cl⁻ ($p < 0.025$ *v* control) without significantly changing the absorption of K⁺ and glucose (Table IV). Olive oil did not significantly alter the transport of water or electrolyte during perfusion studies.

EFFECTS ON LUMINAL EICOSANOIDS

Feeding rats either butter or corn oil did not significantly change the luminal PGE₂ and PGF_{2 α} during in vivo steady state perfusion studies as compared to low fat chow diet.

Feeding rats olive oil increased the production of luminal PGE₂ ($p < 0.005$ *v* control; $p < 0.025$ *v* butter and corn oil) and also increased the production of luminal PGF_{2 α} ($p < 0.025$ *v* control and butter; $p < 0.005$ *v* corn oil). Comparing the effects of dietary fat on the mean PGE₂/PGF_{2 α} ratio, it seems that feeding rats olive oil increased both prostaglandins, particularly PGF_{2 α} , whereas feeding corn oil appreciably decreased PGE₂ compared to the low fat diet (Table V).

EFFECTS ON CHOLERA TOXIN-INDUCED SECRETION

Feeding rats high dietary fat did not significantly alter the absorption of glucose at the low concentration used. High monounsaturated and high polyunsaturated dietary fat, compared with low fat intakes, reduced the effects of cholera toxin on secretion of water and electrolytes (Figs 1 and 2).

Taking into consideration the baseline absorption of unstimulated jejunum, it seems that all three high dietary fat regimens reduced the net secretory response to cholera toxin by about 50%.

Discussion

The absorption values of these perfusion studies were lower than those previously reported.^{7,9,10} The reason behind these variations may be related to the variation in the Na⁺ content of the perfusate, the relatively higher osmolality, lower glucose, lower total CO₂, and lower flow rate in this study, the variation in the ages of the animals, and the units used to express the data. Since our aim was to compare the effects of different dietary fat on ion transport under the same conditions, however, the comparisons are valid.¹¹

Prostaglandin synthesis in the intestine occurs predominantly in the subepithelial cells, whereas the capacity to degrade prostaglandins lies mainly in the epithelial cells.¹² Prostaglandins are not stored in tissue but are continually formed and

TABLE V Effects of dietary fat on luminal prostaglandins (PG)

Diet (no of perfusions)	pg/ml per min per g wet weight of perfused segment	
	PGE ₂	PGF _{2α}
Control (n=8)	24.1 (3.6) (5.9-33.4)	2.9 (0.9) (0.5-5.4)
Butter (HS) (n=6)	23.7 (3.0) (11.8-33.2)	2.9 (1.3) (0.2-11.03)
Olive oil (HMU) (n=8)	37.04 (4.3) (21.6-52.1)*	7.4 (1.7) (1.9-14.7)**
Corn oil (HPU) (n=6)	17.9 (2.4) (9.1-26.2)	2.37 (0.4) (0.3-4.03)

Values are expressed as mean (SEM) and range.

* $p < 0.005$ *v* control, and $p < 0.025$ *v* butter and corn oil.

** $p < 0.025$ *v* control and butter, and $p < 0.005$ *v* corn oil.

All unstimulated perfusion studies were performed on male Sprague Dawley rats after feeding one of the synthetic diets (Table I).

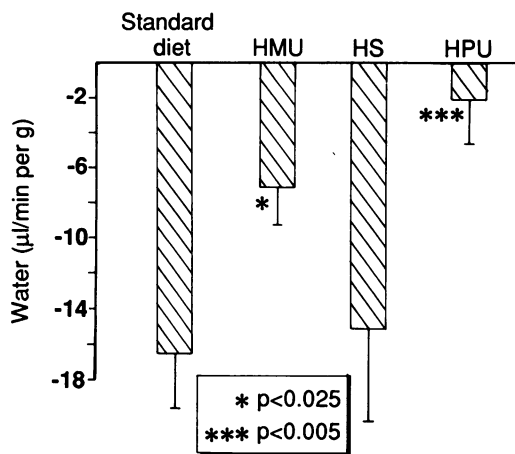


Figure 1: Secretory (water) response of jejunum to cholera toxin in rats fed diets with differing fatty acid contents. HMU = high monounsaturated (olive oil); HS = high saturated (butter); HPU = high polyunsaturated (corn oil). Values are shown as mean (SEM). The number of perfusions were 10 each for the control and corn oil groups and eight each for the olive oil and butter groups. *p* values are between control (standard diet) and other groups.

released. Determination of luminal PGE₂ concentrations provides a simple and reproducible index of intestinal prostaglandin production which may be used for studies of pathophysiological mechanisms.¹³ The use of a valid marker perfusion technique may provide a reliable index of local prostaglandin synthesis after exclusion of the luminal fluids from the stomach, pancreas, and bile.

The sequence of biochemical events that occurs between ingestion of dietary fat and tissue prostaglandin production is complex, with several steps of autoregulation, and the effects of dietary manipulation on prostaglandin synthesis in various body organs are difficult to predict.

Permeability properties of the lipid bilayer are greatly dependent on the nature and chain length of the fatty acid constituents of lipid molecules.¹⁴ High dietary polyunsaturated fat is known to increase the fluidity of the enterocyte plasma membrane,¹⁵ which in turn produces a more permeable membrane due to reduced attractive forces between adjacent lipid molecules.^{16,17} The observations that high polyunsaturated fat appreciably increased the activity of Na⁺/K⁺ ATPase in the basolateral membranes,¹⁵ significantly increased tissue concentration of somato-

statin in the proximal small intestine (submitted data),¹⁸ and appreciably increased villous heights in the jejunum (submitted data)¹⁸ might lead to an expectation of enhanced absorption.¹⁹ The alterations in prostaglandins produced by diet may be relevant, since luminal PGE₂ in rats fed corn oil was reduced to lower levels than in the control groups²⁰ (Table V). Polyunsaturated fat may therefore inhibit a prostaglandin mediated step in intestinal secretion beyond the generation of cAMP.²¹

In contrast, rats on high monounsaturated dietary fat showed no significant alteration in ion transport compared with low fat control groups. Their luminal PGE₂ and PGF_{2α} were significantly increased during perfusion (Table V), however, and the fact that an increase in luminal PGE₂ has been shown to increase the inhibition of Na⁺/K⁺ ATPase and stimulate adenylyl cyclase²¹ may explain the relative reduction in the absorption of water and electrolyte compared with the corn oil group.

High saturated fat reduces the permeability of the enterocyte plasma membrane,^{16,17} greatly reduces Na⁺/K⁺ATPase activities,¹⁵ and also reduces the jejunal villus height.¹⁸ It also increases total phospholipids in intestinal microvillus membranes and in particular membrane bound phosphatidylinositol,¹⁵ the hydrolysis of which is accompanied by more gating of Ca²⁺ across the basolateral membrane. This calcium-gating process increases ionised Ca²⁺ levels in the cell and this is thought to stimulate secretion through Ca²⁺ dependent protein kinases. This is under investigation in our laboratory. These factors in combination may explain the appreciable reduction in water and electrolyte transport that we observed in the high saturated fat group (Table IV).

We used a single, very low glucose concentration as a marker to show the viability of the mucosa. Further studies using different concentrations of glucose are required to elucidate the possible effects of fat on glucose absorption. Previous *in vitro* studies showed that the rate of jejunal uptake of D-glucose varied from 0.5–20 mmol and was higher in animals fed high polyunsaturated fat.²²

Cholera toxin-induced stimulation serves as a useful model of secretory diarrhoea in experimental studies. Cholera toxin is a protein molecule, which consists of a B subunit containing five non-covalently linked molecules and an A subunit containing two peptides, A₁ and A₂, linked by a disulphide bond.²³ In a comprehensive review of the pathogenesis of cholera, Hendrix²⁴ concluded that there is no evidence that cholera toxin increases epithelial permeability or osmotic or hydrostatic gradients, and the increased intestinal fluid secretion stimulated by cholera toxin arises from increased filtration through normal calibre 'channels.' The gross effect of cholera toxin is the production of water and electrolyte secretion by a complex biochemical mechanism, not yet well understood. It involves a rise in cAMP, which causes active secretion of Cl⁻ from the crypts and inhibits active electrogenic Na⁺-coupled glucose absorption.²⁵ It is not definitely known how the binding of the B subunit to a specific membrane glyco-

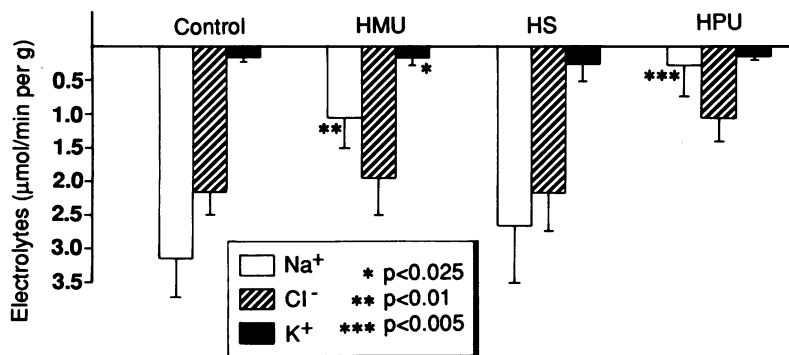


Figure 2: Secretory (electrolyte) response of jejunum to cholera toxin in rats fed diets with differing fatty acid contents. HMU = high monounsaturated (olive oil); HS = high saturated (butter); HPU = high polyunsaturated (corn oil). Values are shown as mean (SEM). The number of perfusions were 10 each for the control and corn oil groups and eight each for the olive oil and butter groups. *p* values are between control (standard diet) and other groups.

lipid mediates the entry of the A subunit or whether this is the only mechanism by which cholera toxin induces a secretory state.

In the present studies, if we can extrapolate from baseline studies in male rats to the cholera toxin-induced studies in female animals, it seems that the pre-existing absorptive/secretory state of the mucosa has a pronounced effect on the secretory response to toxin. Thus, bearing in mind that a butter diet produced a small net secretory state when unstimulated whereas corn oil apparently enhanced water and electrolyte absorption, all three fat supplements reduced the net secretory response by about half. This suggests that the fundamental mechanism by which cholera toxin activates secretion is unchanged, although a number of other theories may explain variations in both the unstimulated absorption and the toxin response.

The changes in membrane composition induced by dietary fat may determine the binding of bacteria or toxin to the intestinal cells. Studies on animals have shown that more cholera toxin binds to the microvillus membrane of the newborn rabbit compared with adults²⁶ and these effects may be related to the reduced ratio of protein/phospholipids commonly found in the immature membrane of the newborn.^{27, 28}

Although high polyunsaturated dietary fat reduces the total phospholipids in microvilli,²⁹ which may in turn reduce the binding sites available for cholera toxin, this is unlikely to be the only explanation for our observations. Polyunsaturated fat, however, can increase activity of Na⁺/K⁺ ATPase in the basolateral membrane,^{15, 30} and this could counteract the secretory effect of adenylyl cyclase induced by cholera toxin.

The Na⁺-coupled glucose absorption route is unlikely to be involved in the protective mechanism of unsaturated dietary fat because cholera toxin does not interfere with Na⁺-coupled glucose transport²⁵ and dietary fat did not appreciably alter the absorption of glucose at the low concentration used in this study.

Polyunsaturated fat significantly increased the tissue concentration of somatostatin in the proximal segment of rat small intestine compared to the low fat group (submitted data).¹⁸ Somatostatin inhibits *in vivo* jejunal secretion induced by PGE₁ and theophylline in rats,³¹ improves diarrhoea in human carcinoid syndrome³² and in severe refractory secretory diarrhoea,³³ and inhibits adrenergic and cholinergic neurotransmission in smooth muscle.³⁴ High tissue concentrations of somatostatin may therefore contribute to the protective effects of corn oil in secretory diarrhoea.

The protective mechanism of olive oil may involve different mediators. Rats maintained on high monounsaturated dietary fat (olive oil) had high levels of luminal PGE₂ and PGF_{2α} during unstimulated perfusion studies. This might be expected to alter the interdigestive motility pattern of the small intestine³⁵ and contribute to sodium and chloride secretion.^{36, 37}

The possibility exists that stimulation of adenylyl cyclase by prostaglandins³⁶ and by cholera toxin²⁵ is through different and antagonistic or competitive pathways.

The applicability of these studies to diarrhoeal disorders in humans is not known, but preliminary clinical observations suggest that the fatty acid composition of cell membranes may be related to the varying severity of childhood diarrhoea in different populations.³⁸

We thank Mr T McFarlane, Royal Belfast Hospital for Sick Children, and Mr T Oliver, Department of Food Biochemistry, Faculty of Agriculture, Queen's University of Belfast, for their technical help.

- Koopman JS, Turkisk VJ, Monto AS, Thompson FE, Isaacson RE. Milk fat and gastrointestinal illness. *Am J Public Health* 1984; 74: 1371-3.
- Cohen SA, Kirsty M, Hendricks KM, Eastham EJ, Mathis RK, Walker WA. Chronic non-specific diarrhea: a complication of dietary fat restriction. *Am J Dis Child* 1979; 133: 490-2.
- Cohen SA, Hendricks KM, Mathis RK, Laramie S, Walker WA. Chronic non-specific diarrhea - dietary relationships. *Pediatr Res* 1979; 64: 402-7.
- Otnaess AB, Svennerholm AM. Non-immunoglobulin fraction of human milk protects rabbits against enterotoxin-induced intestinal fluid secretion. *Infect Immun* 1982; 35: 738-40.
- Ashkenazi S, Mirelman D. Non-immunoglobulin fraction of human milk inhibits the adherence of certain enterotoxigenic *Escherichia coli* strains to guinea pig intestinal tract. *Pediatr Res* 1987; 22: 130-4.
- Sandhu BK, Tripp JH, Candy DCA, Harries JT. Loperamide studies on its mechanism of action. *Gut* 1981; 22: 658-62.
- Sladen GE, Harries JT. Studies on the effects of unconjugated dihydroxy bile salts on rat small intestinal function *in vivo*. *Biochim Biophys Acta* 1972; 288: 443-56.
- Powell WS. Rapid extraction of oxygenated metabolites of arachidonic acid from biological samples using octadecylsilyl silica. *Prostaglandins* 1980; 20: 947-57.
- Harries JT, Sladen GE. The effect of different bile salts on the absorption of fluid, electrolytes and mono-saccharides in the small intestine of the rat *in vivo*. *Gut* 1972; 13: 596-603.
- Powell DW, Malawer SJ. Relationship between water and solute transport from iso-osmotic solutions by rat intestine *in vivo*. *J Physiol* 1986; 215: 49-55.
- Ewe K, Wanitschke R, Staritz M. Intestinal permeability studies in humans. In: Csaky TZ, ed. *Handbook of experimental pharmacology. Vol 70. Intestinal permeation part II*. Berlin: Springer Verlag, 1984: 535-71.
- Smith SG, Worhurst G, Turnberg LA. Synthesis and degradation of PGE₂ in the epithelial and subepithelial layers of the rat intestine. *Biochim Biophys Acta* 1982; 713: 684-7.
- Bukhave K, Rask-Madsen J. Prostaglandin E₂ in jejunal fluids and its potential diagnostic value for selecting patients with indomethacin-sensitive diarrhoea. *Eur J Clin Invest* 1981; 11: 191-7.
- Jain MK, Wagner RC. *Introduction to biological membranes*. New York: John Wiley, 1980: 117-42.
- Brasitus TA, Davidson NO, Schachter D. Variation in dietary triglycerol saturation alters the lipid composition and fluidity of rat intestinal plasma membranes. *Biochim Biophys Acta* 1985; 812: 460-72.
- Boggs JM. Intermolecular hydrogen bonding between lipids: influence on organization and function of lipids in membranes. *Can J Biochem* 1980; 58: 755-70.
- Chapman D. Phase transitions and fluidity characteristics of lipids and cell membranes. *Q Rev Biophys* 1975; 8: 185-235.
- Sagher FA. The effect of variation in dietary fat in early life, with particular reference to the small intestine. PhD Thesis: 1988. The Queen's University of Belfast.
- Riecken EO, Lorenz-Meyer M, Sahfeld M, Bloch R. Relationship between structure and function in the human intestine. In: Robinson JW, ed. *Intestinal ion transport*. London: MTP Press, 1976: 371-5.
- Sagher FA, Dodge JA, Moore R. Modification of rat jejunal ion transport and luminal eicosanoids by dietary fat [Abstract]. *Gut* 1988; 29: A1439.
- Rachmilewitz D, Karmeli F, Okon E. Effects of bisacodyl on cAMP and prostaglandin E₂ content, Na⁺-K⁺ ATPase, adenylyl cyclase and phosphodiesterase activities of rat intestine. *Dig Dis Sci* 1980; 25: 602-8.
- Thomson ABR, Keelan M, Clandinin MT, Walker K. Dietary fat selectively alters transport properties of rat jejunum. *J Clin Invest* 1986; 77: 279-88.
- Kurosky A, Markel DE, Touchstone B, Peterson W. Chemical characterization of the structure of cholera toxin and its natural toxoid. *J Infect Dis* 1975; 133: 514-22.
- Hendrix TR. Effect of cholera enterotoxin on intestinal permeability. In: Csaky TZ, ed. *Handbook of experimental pharmacology. Vol 70. Intestinal permeation part II*. Berlin: Springer Verlag, 1984: 391-9.
- Serebro HA, Bayless TM, Hendrix TR, Iber FL, McGonagle T. Absorption of D glucose by rabbit jejunum during cholera induced diarrhoea. *Nature* 1968; 217: 1272-3.
- Bresson JL, Pang K, Udall J, Fritze L, Walker WA. Evidence for increased enterotoxin binding to newborn microvillous membranes: a possible explanation for enhanced toxicogenic diarrhoea in infancy [Abstract]. *Gastroenterology* 1980; 78: A1145.
- Chu S W, Walker WA. Development of the gastrointestinal mucosal barrier: changes in phospholipid head groups and

- fatty acid composition of intestinal microvillous membrane from newborn and adult rats. *Pediatr Res* 1988; **23**: 439-42.
- 28 Bresson JL, Pang K, Walker WA. Microvillous membrane differentiation: quantitative difference in cholera toxin binding to the intestinal surface of newborn and adult rabbit. *Pediatr Res* 1984; **18**: 984-7.
- 29 Brasitus TA, Davidson NO, Schachter D. Variations in dietary triglycerol saturation alter the lipid composition and fluidity of rat intestinal plasma membranes. *Biochim Biophys Acta* 1985; **812**: 460-72.
- 30 Charney AN, Gots RE, Giannella RA. (Na⁺-K⁺)-stimulated adenosinetriphosphatase in isolated intestinal villous tip and crypt cells. *Biochim Biophys Acta* 1974; **367**: 265-70.
- 31 Dharmasathaphorn K, Sherwin RS, Dobbis JW. Somatostatin inhibits fluid secretion in rat jejunum. *Gastroenterology* 1980; **78**: 1554-8.
- 32 Davis GR, Camp RC, Kreis GJ. Effect of somatostatin infusion on jejunal water and electrolyte transport in a patient with carcinoid syndrome and in healthy subjects [Abstract]. *Gastroenterology* 1979; **76**: A117.
- 33 Jaros W, Biller J, Greer S, O'Dovisio T, Grand R. Successful treatment of idiopathic secretory diarrhea of infancy with the somatostatin analogue SMS 201-995. *Gastroenterology* 1988; **94**: 189-93.
- 34 Cohen JL, Rosing E, Wiley KS, Slater IH. Somatostatin inhibits adrenergic and cholinergic neurotransmissions in smooth muscle. *Life Sci* 1978; **23**: 1659-64.
- 35 Tollstrom T, Hellstrom PM, Johansson C, Pernow B. Effects of prostaglandins E₂ and F_{2α} on motility of small intestine in man. *Dig Dis Sci* 1988; **33**: 552-7.
- 36 Rachmilewitz D, Karmeli F, Okon E. Effects of bisacodyl on cAMP and prostaglandin E₂ content, Na⁺-K⁺ ATPase, adenylyl cyclase and phosphodiesterase activities of rat intestine. *Dig Dis Sci* 1980; **25**: 602-8.
- 37 Mozsik G, Kutas J, Nagy L, Nemeth G. Inhibition of M¹-Na⁺-K dependent ATPase systems from human gastric mucosa by prostaglandins E₁ and E₂. *Eur J Pharmacol* 1974; **29**: 133-7.
- 38 McClean P, Sagher FA, Akhtar T, Dodge JA. Does dietary fat affect the severity of childhood diarrhea? [Abstract]. *Pediatr Res* 1989; **26**: 268.