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Nitrogen losses from the human small bowel: obligatory losses and the effect of physical form of food

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SUMMARY **The amount and form of nitrogen lost from the human small intestine and the dietary factors which influence it have been studied in six ileostomists. Over a six day period the subjects were fed a series of diets including low nitrogen (LND) 0·17 g N/day, LND+soya beans (5·87 g N/day) and a high fibre diet (HFD) (10·6 g N/day). The soya beans were fed either whole or pureed to test the effect of physical form of food. Total N, protein, amino acids, urea, and ammonia were measured in ileostomy effluent which was collected throughout the study. Total N excretion was LND 0·91 (0·04) (SE) g/day; LND+whole soya beans (WSB) 2·26 (0·15) g/day; LND+pureed soya beans (PSB) 1·42 (0·12) g/day (WSB *v* PSB, p<0·001); and HFD 2·17 (0·11) g/day (HFD *v* PSB, p<0·001, HFD *v* WSB, NS). N losses as urea, ammonia, and free amino acids were less than 10–15% of total N, the remainder being protein (48–51%) and (by difference) peptides (20–30%). Eighty to 85% of effluent N was in the insoluble (pellet) fraction except on the low N diet where it was 66%. The physical form of food clearly influenced N digestibility in the soya beans whilst changes in dietary fibre seem not to have a significant effect.**

The human large intestine plays a significant role in the digestion of dietary carbohydrates especially starch and non-starch polysaccharides (dietary fibre).[1] It has only recently been appreciated that significant protein breakdown may also occur in the colon.[2] Total nitrogen reaching the human large bowel from the terminal ileum is in the range 0·5–4·0 g/day.[3–6] It is assumed that this is a mixture of endogenous nitrogen and dietary residues. The relative contributions from the two sources, and the form in which they enter the large bowel are not known. There are also few data available on the influence of dietary factors on small bowel nitrogen losses. Gibson *et al*[6] have shown that increasing dietary protein from 40–100 g/day increased mean daily nitrogen losses in ileostomy effluent from 1·8–2·6 g/day. In another study with ileostomists Sandberg *et al*[7] showed that supplementing a low fibre diet with 16 g wheat bran does not change nitrogen loss.

Nitrogen entering the large intestine may exert a significant effect on colonic function particularly production of ammonia, phenolic compounds and amines.[8,9] Because the factors controlling nitrogen loss from the small intestine are largely unknown we have, using ileostomy subjects as a model, attempted to (1) measure endogenous nitrogen losses, (2) determine the forms of nitrogen loss and the effect of diet on the different fractions, (3) test the effect of change in physical form of food, and (4) assess the role of dietary fibre.

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Methods

PATIENTS

Six healthy ileostomists aged 38–66 years (mean 53 years) were studied. All had total proctocolectomy with terminal ileostomy carried out for ulcerative colitis at least two years before the study. All subjects had less than 5 cm terminal ileum resected. They had normal dentition and were otherwise healthy with normally functioning ileostomies.

PROTOCOL

The study lasted six days during which time the subjects lived in a metabolic suite at the Dunn Clinical Nutrition Centre. On the day preceding the start of the study all subjects ate a low nitrogen diet

from 5 pm onwards. On days 1 and 2, the low nitrogen diet (LND) was continued, after which three further diets were given in random order. These diets were LND+whole soya beans (WSB) one day, LND+pureed soya beans (PSB) one day, and high fibre diet (HFD) two days. Throughout the study ileostomy output was collected every two hours from 9 am to 9 pm. The 12 hour overnight collection was pooled as a single sample. Samples of ileostomy discharge were collected directly from the stoma from all subjects for urea and ammonia estimation and microbial counts. Immediately after collection the sample was weighed and homogenised in a stomacher (Lab Blender 400, Seward Laboratory, London, UK) for four minutes. Aliquots were taken and stored at −20°C. A weighed aliquot was centrifuged at 3000 *g* for 30 min. The supernatant (soluble fraction) and pellet (insoluble fraction) were separated and stored at −20°C. The bacteria in the sample for urea and ammonia estimation were inactivated with absolute ethanol[10] (three parts fluid to one part ethanol) and stored at −20°C. The samples for microbial counts were collected in sterile containers and serially diluted in half strength nutrient broth. Aliquots (0·1 ml) were then plated on either Wilkins-Chalgren anaerobe agar (OXOID – Code CM 619) or nutrient agar (OXOID – Code CM 3/4) plates. The Wilkins-Chalgren plates had been preincubated for 48 h in an anaerobic cabinet (80% N_2, 10% CO_2, 10% H_2 atmosphere) at 37°C for up to five days. The nutrient agar plates were incubated anaerobically at 37°C for 48 h. Breath hydrogen was measured daily in all subjects using an electrochemical detector (Exhaled hydrogen monitor, GMI, Scotland) in the fasting state and two to three hours postprandially.

DIETS

All diets were prepared in the metabolic kitchen. The subjects did not eat any food other than that given from the kitchen. Any food left was weighed and recorded. Duplicates of each meal were homogenised and pooled aliquots frozen and stored at −20°C for nitrogen estimation. Details of the diet are given in Tables 1 and 2. Diet I was LND which provided 0·17 g N/day. It was used to measure endogenous nitrogen losses. Diet II had 100 g whole soya beans boiled at 100°C for one hour and given in the form of a salad in addition to LND. The subjects ate part of the soya beans with breakfast and the rest with lunch. Diet III was similar to Diet II except that the soya beans were ground in an electric blender before cooking (pureed soya beans). Diet IV was designed to contain about twice the average national fibre intake for the UK. The fibre was contributed by wholemeal bread, breakfast cereals, fruits and vegetables.

Table 1 *Diets*

	Low nitrogen diet (Diet I)		*High fibre diet (Diet IV)*	
9 am	Low protein bread (toasted)	40 g	Weetabix	40 g
			Wholemeal bread	50 g
	Margarine	10 g	Butter/Flora	50 g
	Marmalade	20 g	Milk	200 g
			Marmalade	15 g
11 am	Hycal ice cream	50 g	Flapjack	60 g
1 pm	Low protein bread	40 g	Wholemeal bread	100 g
	Tomato	30 g	Chicken	40 g
	Margarine	10 g	Tomato	50 g
	Hycal ice cream	50 g	Butter/Flora	20 g
			Pear	100 g
3 pm	Pear	50 g	Banana	100 g
	Hycal ice cream	50 g	Wholemeal bread	25 g
			Butter/Flora	5 g
5 pm	Low protein bread	40 g	Beef	50 g
	Margarine	10 g	Carrots	80 g
	Jam	20 g	Potatoes	150 g
	Hycal ice cream	50 g	Gravy	60 g
			Apricot crumble	110 g
7 pm	Hycal ice cream	50 g	Wholemeal bread	25 g
			Butter/Flora	5 g
	Sweets (daily)	43 g		
	Lemon juice (daily)	5 g		
	Black tea and coffee as wished		Black tea and coffee as wished	

On Diet II patients ate soya bean salad in addition to Low Nitrogen Diet. Ingredients of soya bean salad were as follows: Whole soya beans 100 g, tomato 50 g, French dressing 20 ml. Part of the salad was eaten at 9 am and the rest at 1 pm. Diet III was similar to Diet II except that the soya beans were pureed.

CHEMICAL METHODS

Nitrogen was measured in all samples of effluent homogenate and in soluble and insoluble fractions by the Dumas method[11] (Carlo Erba, Milan ANA 1500 nitrogen analyser). To estimate protein, the sample was first precipitated with 10% (w/v) Trichloroacetic acid (TCA) and centrifuged at 15000 *g* for 10 min. The pellets were solubilised and resuspended in 0·1 M NaOH. Proteins were then determined by the Lowry method.[12] To determine free amino acids samples from three subjects were precipitated with 4% sulphosalicylic acid and centrifuged at 3000 *g* for

Table 2 *Composition of diets**

		Low N diet (Diet I)	*Low N diet+soya beans (Diets II/III)*	*High fibre diet (Diet IV)*
Energy (Kcals)		1618	2157	2042
MJ		6·8	9·02	8·6
Nitrogen	g	0·17	5·87	10·6
Protein	g	1·06	35·6	65·2
Fat	g	68·8	101·1	81·3
Carbohydrate	g	258·1	278·1	281·7
Fibre	g	1·94	15·69	37·4

*Calculated from McCance and Widdowson, *The composition of foods*, 1978.

10 min. Free amino acids in the supernatant were estimated using an LKB 4151 Alpha plus amino acid analyser (LKB Instruments Ltd, South Croydon, UK). Urea and ammonia were measured using Sigma diagnostic kit no 640 (Sigma Chemical Co Ltd, Dorset, England). Total proteolytic activity was assayed by the method of Brock *et al*[13] using 3% azocasein (w/v) in phosphate buffer (pH 7·0) as substrate and incubation period for one hour. Amylase activity was assayed by the method of Dahlqvist.[14]

All subjects gave informed, written consent to the protocol approved by the Ethical Committee of the Dunn Nutrition Unit.

Statistical analysis was by analysis of variance using the SPP package for microcomputers, designed by Patrick Royston, London School of Hygiene and Tropical Medicine.

Results

The wet and dry weights of ileostomy effluent on the various diets are shown in Table 3, and all are within the range expected in healthy ileostomists.[15] Total effluent excretion on LND was 329 (range 226–481) g/day, and excretion of solids 26·0 (range 21·4–32·1) g/day. Total excretion was significantly greater on high fibre diet (LND *v* HFD t=8·6, p<0·001) but not on soya bean diets; effluent dry weight was greater on both soya bean diets (LND *v* WSB t=7·2, p<0·001; LND *v* PSB t=7·8, p<0·001) and high fibre diet (LND *v* HFD t=18·8, p<0·001). There was no difference in total effluent excretion or dry weights between the two soya bean diets, but the high fibre diet was greater than both. (Total effluent: WSB *v* HFD t=4·0, p<0·025; PSB *v* HFD t=5·4, p<0·005. Dry weight: WSB *v* HFD t=3·4, p<0·025; PSB *v* HFD t=5·8, p<0·005). The wet and dry weights of effluent were both significantly related to the amount of dietary fibre intake (total effluent r=0·88, p<0·001; solids r=0·85, p<0·001).

Table 3 *Ileostomy outputs*

Ileostomy output (g/24 h)		*Diet I*	*Diet II*	*Diet III*	*Diet IV*
Wet weight	Mean (SE)	329[a]* (45)	490[a] (44)	465[a] (34)	702[b] (29)
	Range	226–481	337–671	345–586	574–777
Dry weight	Mean (SE)	26·0[a] (1·6)	56·5[b] (5·9)	49·1[b] (3·2)	71·3[c] (2·1)
	Range	21·4–32·1	31·3–70·7	39·4–60·3	66·4–80·3

*Means in any row with different superscript letters are statistically significantly different.

Total nitrogen excretion (Table 4) in ileostomy effluent on the low N diet was 0·91 (0·04) g/day (mean (SE)), range 0·82–1·09 g/day and this has been taken as arising entirely from endogenous nitrogen secreted into the gut, since the small amount of dietary nitrogen (0·17 g) would probably have been absorbed. Despite the low protein intake, exocrine pancreatic function was normal[14] on the low N diet; effluent amylase 223 (48) units/ml; total proteolytic activity $4{\cdot}6\times10^4$ (0·7) μg azocasein hydrolysed/h/ml. When whole soya beans were added to low N diet, nitrogen losses increased to 2·26 (0·15) g/day (range 1·66–2·69 g/day), but were significantly lower when the same amount of beans was well ground and fed (pureed beans) N=1·42 (0·12) g/day; range 1·03–1·83 g/day (t=8·0, p<0·001). Nitrogen digestibility (%) (Table 4) on the pureed bean diet (90·1 (1·3)) was significantly higher than with whole beans (73·0 (3·3)). Nitrogen loss on the high fibre diet was 2·17 (0·11) g/day, range 1·80–2·35 g/day and digestibility was similar to pureed bean diet (88·5 (0·7)).

Eighty to 85% of effluent N was in the insoluble (pellet) fraction except on the low N diet where it was 66% (Table 4).

COMPOSITION OF ILEOSTOMY NITROGEN LOSS

Table 5 shows the composition of ileostomy effluent N on the various diets. The protein content of the homogenate mirrored total N loss and ranged from 2·69 g/day on low N diet to 7·45 g/day on whole soya bean diet. Protein loss on whole soya beans was significantly higher than pureed beans (t=5·5,

Table 4 *Effect of diet on ileostomy nitrogen losses (g/day), mean (SE)*

	Low nitrogen diet (LND)	*LND + whole soya beans (LND + WSB)*	*LND + pureed soya beans (LND + PSB)*	*High fibre diet (HFD)*
Total N	0·91[a] (0·04)	2·26[b] (0·15)	1·42[c] (0·12)	2·17[b] (0·11)
% N absorbed†		73·0[a] (3·3)	90·1[b] (1·3)	88·5[b] (0·7)
Insoluble N	0·60[a] (0·04)	1·92[b] (0·12)	1·13[c] (0·13)	1·74[b] (0·10)
Soluble N	0·31[a] (0·04)	0·34[a] (0·08)	0·30[a] (0·03)	0·43[a] (0·04)

*Means in any row with different superscript letters are statistically significantly different.

†$\frac{\text{(ileostomy N on diet} - \text{ileostomy N on low N diet)}}{\text{dietary N} - \text{dietary N on low N diet}} \times 100$

Table 5 *Composition of small bowel nitrogen losses, mean (SE)*

	LND	*LND+WSB*	*LND+PSB*	*HFD*
Protein (g/day)	2·69[a]* (0·11)	7·45[b] (0·48)	4·19[c] (0·21)	6·15[b] (0·29)
Free amino acid† conc (μmol/ml)	6·90[a] (0·65)	20·48[b] (2·42)	14·50[ab] (1·68)	12·17[a] (2·37)
Free amino acid† output (μmol)	2561[a] (201)	11410[b] (2813)	7177[b] (2813)	8364[b] (1833)
Urea (mmol/l)	3·01[a] (0·17)	3·41[a] (0·94)	3·19[a] (1·87)	4·07[a] (1·22)
Ammonia (mmol/l)	1·89[a] (0·31)	1·41[a] (0·60)	2·56[a] (0·92)	2·65[a] (0·84)

*Means in any row with different superscript letters are statistically significantly different. †n=3.

$p<0·005$). Urea losses were low and ranged from 3·01–4·07 mmol/l, whilst ammonia losses were similarly low at 1·41–2·65 mmol/l.

Total free amino acid concentrations and outputs on the different diets for three subjects are shown in Table 5 and Table 6 shows the concentrations and outputs of the individual amino acids. On LND, the amino acids with the highest concentrations are glycine, proline, alanine, valine and glutamic acid; and those with lowest concentration are D-L-allohydroxylysine, citrulline and methionine. Bearing in mind the statistical limitations of data for only three patients, analysis of variance for free amino acid concentrations between soya bean diets and high fibre diets showed significant differences in the following: arginine, beta-aminoisobutyric acid ($p<0·001$); leucine, phenylalanine, methionine, histidine ($p<0·005$); alpha-aminobutyric acid, gamma-aminobutyric acid ($p<0·01$), glutamine, proline, alanine, valine, and isoleucine ($p<0·05$).

In Table 7 the contribution of the various fractions towards small bowel N loss is shown. Proteins account for 45–51%, amino acids for 6–11% and urea and ammonia for approximately 5% of ileal N losses on the various diets.

Each subject provided a sample directly from the stoma for bacterial counts. Total aerobes were $5·5\times10^{7}$ (1·1), range $4·8\times10^{5}$–$8·2\times10^{8}$ and total

Table 6 *Free amino acid concentrations (μmol/ml) and outputs (μmols) in ileal effluent, mean (SE)*

	LND (n=6)		*LND+WSB (n=3)*		*LND+PSB (n=3)*		*HFD (n=6)*	
	Conc	*Output*	*Conc*	*Output*	*Conc*	*Output*	*Conc*	*Output*
Aspartic acid	0·24 (0·5)	103·2 (27·2)	0·55 (0·07)	307·9 (77·3)	0·43 (0·25)	230·7 (148·2)	0·66 (0·23)	456·6 (169·4)
Threonine	0·18 (0·03)	72·3 (15·8)	0·70 (0·15)	397·4 (130·9)	0·48 (0·11)	243·6 (82·9)	0·32 (0·10)	228·0 (73·6)
Serine	0·14 (0·03)	58·1 (17·2)	0·57 (0·17)	330·5 (138·7)	0·35 (0·14)	185·0 (89·8)	0·19 (0·09)	133·1 (66·6)
Asparagine	0·11 (0·05)	53·5 (26·5)	0·45 (0·32)	288·1 (219·2)	0·27 (0·27)	157·3 (157·3)	0·25 (0·16)	181·2 (114·7)
Glutamic acid	0·37 (0·05)	134·0 (10·3)	1·45 (0·15)	767·2 (44·0)	1·55 (0·25)	748·8 (119·9)	1·38 (0·26)	936·8 (192·2)
Glutamine	0·33 (0·08)	142·2 (39·7)	2·42 (0·46)	1372 (435·5)	0·81 (0·41)	424·7 (232·4)	0·70 (0·39)	509·1 (281·0)
Proline	0·62 (0·15)	227·4 (38·4)	1·36 (0·03)	734 (85·1)	1·11 (0·21)	537·2 (103·7)	0·62 (0·17)	438·2 (125·2)
Glycine	1·36 (0·31)	459·6 (57·9)	1·75 (0·31)	921·9 (117·6)	1·88 (0·33)	916·5 (174·7)	1·44 (0·20)	970·7 (137·8)
Alanine	0·48 (0·08)	170·5 (9·3)	1·43 (0·13)	783·7 (156·1)	1·11 (0·16)	532·1 (68·0)	0·79 (0·12)	538·2 (94·2)
Valine	0·38 (0·06)	136·2 (14·0)	1·02 (0·18)	577·1 (170·2)	0·80 (0·06)	389·5 (52·6)	0·47 (0·09)	326·0 (71·3)
Cystine	0·08 (0·02)	28·6 (6·9)	0·15 (0·01)	82·8 (12·7)	0·23 (0·07)	110·6 (27·5)	0·12 (0·02)	84·4 (18·3)
Methionine	0·06 (0·01)	24·1 (4·4)	0·20 (0·03)	107·0 (17·7)	0·16 (0·02)	79·8 (16·0)	0·09 (0·01)	59·8 (7·9)
Isoleucine	0·24 (0·02)	93·7 (12·6)	0·83 (0·17)	473·3 (153·3)	0·57 (0·05)	283·6 (56·6)	0·39 (0·08)	271·8 (59·8)
Leucine	0·34 (0·03)	133·0 (19·4)	1·40 (0·28)	797·1 (256·5)	0·89 (0·07)	440·9 (81·9)	0·50 (0·08)	347·4 (68·9)
Tyrosine	0·21 (0·03)	80·5 (16·0)	0·63 (0·12)	355·4 (111·5)	0·47 (0·06)	235·9 (56·7)	0·33 (0·07)	229·9 (53·6)
Phenylalanine	0·21 (0·02)	80·2 (11·5)	0·75 (0·09)	416·7 (103·4)	0·57 (0·06)	283·2 (70·0)	0·24 (0·05)	166·7 (41·0)
D.L. Allo-hydroxylysine	0·02 (0·01)	6·5 (2·9)	0·01 (0·01)	5·3 (3·6)	0·04 (0·02)	17·6 (9·1)	0·02 (0·01)	15·3 (5·5)
Lysine	0·15 (0·04)	55·1 (16·2)	0·61 (0·61)	408·3 (408·4)	0·37 (0·29)	209·6 (172·5)	0·48 (0·24)	337·3 (172·9)
Arginine	0·28 (0·04)	108·0 (17·7)	1·81 (0·30)	1019·0 (296·5)	0·71 (0·08)	354·0 (79·8)	0·45 (0·12)	316·1 (90·5)
Histidine	0·21 (0·03)	75·3 (9·6)	0·64 (0·03)	347·2 (56·9)	0·44 (0·01)	210·9 (16·2)	0·33 (0·05)	222·8 (36·6)
1-Methyl-histidine	0·36 (0·13)	128·2 (52·6)	0·89 (0·46)	426·3 (227·3)	0·55 (0·28)	238·2 (121·2)	0·42 (0·16)	282·6 (110·6)
3-Methyl-histidine	0·19 (0·02)	75·0 (11·0)	0·47 (0·13)	270·2 (106·4)	0·21 (0·12)	109·5 (68·4)	0·13 (0·08)	90·5 (57·3)
Citrulline	0·01 (0·01)	3·2 (2·3)	0·05 (0·03)	21·4 (21·4)	0·10 (0·04)	41·6 (25·1)	0·03 (0·02)	18·3 (10·6)
Ornithine	0·08 (0·02)	24·8 (4·5)	0·10 (0·05)	61·1 (38·4)	0·08 (0·03)	38·3 (9·1)	0·06 (0·01)	40·0 (5·5)
α-Amino butyric acid	0·08 (0·03)	24·2 (5·5)	0·23 (0·02)	123·8 (12·3)	0·30 (0·08)	142·0 (28·6)	0·11 (0·01)	70·7 (7·9)
β-Amino isobutyric acid	0·11 (0·05)	45·9 (23·0)	0·00	0·00	0·00	0·00	1·52 (0·25)	999·0 (143·2)
γ-Amino butyric acid	0·07 (0·04)	17·8 (9·2)	0·02 (0·02)	12·4 (12·4)	0·03 (0·03)	16·2 (16·2)	0·14 (0·02)	93·3 (8·8)

Table 7 *Composition of N losses on each diet (g/day), mean (SE)*

	LND	*LND+WSB*	*LND+PSB*	*HFD*
Total N	0·91[a]* (0·04)	2·26[b] (0·15)	1·42[c] (0·12)	2·17[b] (0·17)
Protein N	0·43[a] (0·03) (47%)	1·16[b] (0·13) (51%)	0·68[c] (0·06) (48%)	0·99[b] (0·08) (45%)
Free amino acid N	0·05[a] (0·001)	0·26[b] (0·002)	0·16[b] (0·001)	0·20[b] (0·001)
Urea N	0·04[a] (0·01)	0·06[a] (0·02)	0·05[a] (0·03)	0·08[a] (0·05)
Ammonia N	0·01[a] (0·001)	0·01[a] (0·005)	0·02[a] (0·004)	0·03[a] (0·004)

*Means in any row with different letters are statistically significantly different.

anaerobes $4{\cdot}2\times10^6$ (0·72), range $8{\cdot}0\times10^5$–$2{\cdot}6\times10^7$ which were within the range seen in other ileostomists.[16,17] Average breath hydrogen was 4 ppm and in no subject rose above 7 ppm.

Discussion

Small intestinal nutrient absorption in man has been studied by a variety of techniques including intestinal perfusion and using patients with terminal ileostomies. Intestinal intubation delays gastric emptying and shortens small intestinal transit time,[18] which in turn influences nutrient absorption.[19] So we have used healthy ileostomists to measure endogenous nitrogen losses and evaluate the effect of dietary factors on nitrogen losses from the small intestine. Healthy ileostomists have a mouth to caecum transit time identical to normal subjects.[18] Though microbial counts are higher in ileostomy effluent as compared with ileal fluid from normal subjects, there is little bacterial fermentation as breath hydrogen is always low, does not rise after meals, and short chain fatty acid concentrations are also very low.[20,21] In other respects, the digestive function of ileostomists resembles that of the normal gut in that ileal effluent contains substantial amounts of pancreatic enzymes[22,23] and ileal excretion of fat, protein and carbohydrate from mixed diets is no greater than faecal excretion in normal healthy subjects.[19,24]

Although there are numerous reports of total nitrogen losses from patients with a terminal ileostomy,[3–5] values range from 0·6–2·4 g/day, little is known of the relative contributions from diet and endogenous sources. We measured endogenous nitrogen losses from distal small bowel in healthy ileostomists by feeding them an essentially nitrogen free diet (0·17 g N/day). Total nitrogen loss in the ileal effluent was 0·91 g/day. Most of the endogenous nitrogen is contributed by secretions, mucus, and shed epithelial cells. As dietary proteins are known to stimulate pancreatic exocrine secretion[25] the low nitrogen diet may not have adequately stimulated the pancreas. Hence, there would be an underestimation of endogenous nitrogen loss measured when the patients ate such a diet. So, total proteolytic activity (TPA) and amylase activity in the ileal effluent were measured. Total proteolytic activity ($4{\cdot}64\times10^4$ μg azocasein hydrolysed/h/ml) and amylase activity (222·7 U/ml) on the low nitrogen diet were within the range seen in healthy ileostomists[24] indicating that there was adequate stimulation of pancreatic exocrine activity.

The importance of physical form of food on rate of digestion of carbohydrates in cereals and legumes has been shown.[26,27] We evaluated the effect of physical form (whole and pureed) of soya beans on nitrogen losses. Nitrogen losses with whole beans (2·3 g/day) is significantly higher than pureed beans (1·4 g/day) and percentage absorption of nitrogen from whole beans (73%) is significantly lower than pureed beans (90%). The probable reason for this difference in digestibility is the greatly increased surface area when the beans are pureed. This provides easy access to digestive enzymes and hence more rapid and complete digestion and absorption. The effect of physical form of food in determining nitrogen losses from the distal small intestine may be important because beans, which form a significant part of dietary protein in developing countries, are usually eaten whole.

Previous studies show that increase in intake of non-starch polysaccharides (NSP) is associated with increase in faecal nitrogen losses.[28,29] The increase in faecal nitrogen is mainly bacterial[30] and may be related to increased bacterial growth on high NSP (dietary fibre) diets. Do small intestinal events contribute to increased nitrogen losses? It has been shown by *in vitro* studies[31] that NSP interferes with pancreatic enzyme activity. Non-starch polysaccharides also increases the volume and weight of intestinal contents, may act as a physical barrier impairing digestion and absorption and cause morphological changes in the structure of the small bowel.[32,33] To gauge the effect of NSP on ileal nitrogen losses, we fed the patients a high NSP diet and compared the ileal nitrogen losses with those seen with the soya bean diets, containing only half the NSP, and with a larger group of free living ileo-

stomists consuming standard UK diets containing about 15 g NSP/day whom we had previously studied.[15] Ileal nitrogen losses in the free living ileostomists (2·1 g/day) is similar to the patients fed high NSP diet (2·2 g/day) and percentage absorption of nitrogen on high NSP diet (88·5%) is similar to pureed bean diet (90·1%). Similarly N losses on the HFD are no greater than with whole soya beans, although NSP intakes are more than double. These results suggest that dietary fibre as consumed in a normal high fibre mixed diet, plays a small role, if any in influencing small bowel nitrogen losses.

The exact forms of nitrogen entering the large bowel are largely unknown. Macfarlane *et al*[9] have shown that the solubility of proteins is an important factor determining the rate of their fermentation. By low speed centrifugation we separated ileostomy effluent into soluble and insoluble fractions. Insoluble fraction accounts for 66% of endogenous N losses and 80–85% of nitrogen lost on other diets. Protein accounted for the major portion of effluent nitrogen on all diets and ranged from 45% of total N on high fibre diet to 51% on the whole soya bean diet. Only small amounts of urea and ammonia were found, accounting for less than 5% of total N. Urea[34,35] and creatinine[36] concentrations in distal ileal fluid are usually similar to blood levels. Very little (about 100 mg) uric acid is reported to enter the large bowel each day[37] and only small amounts of amino acids are found in ileal fluids.[36,38] In the present study amino acids contribute a small fraction to ileal nitrogen loss ranging from 6·0% on low nitrogen diet to 11% on the pureed bean diet. High concentrations of beta-amino isobutyric acid are seen on high fibre diet (1·5 μmol/ml). Beta-amino isobutyrate is a degradation product of thymine[39] so the increased levels may be due either to increased small intestinal cell turnover, known to occur with high fibre diets, or be derived from plant cell nucleic acids. By difference, most of the unaccounted for nitrogen must be in the form of peptides (30–40%). This suggests that proteins and peptides are the major forms of nitrogen lost from the small bowel (80–85%).

In conclusion, this study suggests that obligatory N losses from the small intestine are about 1·0 g/day, and nitrogen loss is mainly in the form of proteins and peptides. Physical form of food influences N loss but not dietary fibre content.

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