Intestinal zinc transfer by everted gut sacs from rats given diets containing different amounts and types of dietary fibre

BY C. J. SEAL AND J. C. MATHERS*

Department of Agricultural Biochemistry and Nutrition, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU

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Two experiments were carried out in which rats were offered diets containing different amounts and types of dietary fibre, i.e. commercial stock diet and three semi-purified diets containing no fibre, 200 g wheat bran or 200 g pectin/kg. Dietary inclusion of fibre, and especially pectin, stimulated large bowel fermentation, as indicated by caecal hypertrophy and reduced caecal pH. After 3 weeks, mucosal:serosal zinc transfer and Zn accumulation by tissue were measured using the everted-gut-sac technique. In Expt 2, incubations were carried out in the presence and absence of 0·25 mM-ouabain to assess the importance of transfer by Na^+,K^+-ATPase-dependent mechanisms, and some observations on glucose transport were also made. Ouabain reduced rates of transfer of both Zn and glucose and also tissue Zn accumulation. There were no significant differences in rates of Zn transfer by everted sacs from duodenal, ileal and colonic sites, but accumulation of Zn by tissue was a more important fate than transfer across the serosal surface, and accumulation by duodenal tissue was approximately twice as great as by other tissues. Mucosal:serosal transfer of glucose by ileal tissue was much more sensitive to ouabain than was Zn transfer. Previous diet appeared to alter the capacity of the intestinal tissue to transfer Zn, with the highest rates of transfer being by colonic tissue from pectin-fed rats.

Dietary fibre: Zinc: Glucose: Rat.

Zinc is transported across the enterocyte brush-border membrane from the intestinal lumen by a carrier-mediated, saturable process (Cousins, 1986; Solomons, 1986) which may be assisted by binding of the metal to a low-molecular-weight organic ligand such as citrate (Lonnerdal *et al.* 1980) or 2-picolinate (Evans & Johnson, 1981; Seal & Heaton, 1983, 1985). Zn absorption is under homeostatic regulation and is stimulated by dietary Zn depletion (Evans *et al.* 1973; Hoadley *et al.* 1987). The site(s) of Zn absorption within the gastrointestinal tract has not been established unequivocally. Whilst Underwood (1977) concluded that 'in rats, zinc is absorbed mainly from the duodenum, ileum and jejunum with very little being absorbed from the stomach and colon' and Davies (1980) found negligible absorption of Zn from the caecum and colon, others have reported that substantial Zn absorption may occur in the large intestine of rats (Meneely & Ghishan, 1982; Ghishan & Sobo, 1983; Wapnir *et al.* 1985), pigs (Partridge, 1978), sheep (Grace, 1975) and cattle (Bertoni *et al.* 1976). Within the small intestine, the relative importance of proximal and distal regions is also controversial.

Consumption of foods rich in dietary fibre (non-starch polysaccharides; NSP) and phytate has been associated with reduced availability of minerals including calcium (McCance & Widdowson, 1942*a*,*b*) and Zn (O'Dell & Savage, 1960; Davies & Nightingale, 1975; Davies & Reid, 1979) in some but not all studies (Fairweather-Tait & Wright, 1985). The mechanism of this reduced availability is believed to include (1) binding of minerals to NSP components (James *et al.* 1978) and (2) co-precipitation as Zn–Ca–phytate complexes (Oberleas *et al.* 1966) in which forms the minerals are unavailable for absorption. When

* For reprints.

Table 1. *Composition (g/kg) of semi-purified diets*

Diet...	No fibre	+Bran	+Pectin
Sucrose	660	498	478
Casein	200	162	182
Wheat bran	0	200	0
Pectin	0	0	200
Maize oil	80	80	80
Cod-liver oil	20	20	20
Salt mixture*	45·3	45·3	45·3
Vitamin mixture*	0·77	0·77	0·77

* For full details of salt and vitamin mixtures, see Seal & Heaton (1983).

digesta reach the large bowel they are subject to extensive attack by bacteria which ferment much of the NSP (Cummings, 1981) and hydrolyse phytate (Wise, 1983). There have been suggestions that compensatory absorption of minerals from the large bowel may then follow (Cummings *et al.* 1979).

The present study was designed to investigate possible effects of giving diets varying in content and type of NSP on the potential for absorption of Zn by different sites within the small and large intestine of rats. Some observations on glucose transport are also included.

A brief account of part of the present work has been published (Seal & Mathers, 1987).

MATERIALS AND METHODS

Expt 1

Animals and diets

Four diets containing different types and amounts of dietary fibre were used in the study. They included a commercial pelleted rat diet (stock; Oxoid 41B) and three isonitrogenous semi-purified diets based on sucrose and casein (Seal & Heaton, 1983). The semi-purified diets contained either no added fibre (no-fibre diet), 200 g wheat bran (+bran; Prewetts, Byfleet, Surrey)/kg or 200 g pectin (+pectin; 7·7% methoxy content; Sigma)/kg. The formulation of the diets is shown in Table 1. The complex carbohydrate contents (g/kg as used) of the stock diet and of the wheat bran used to prepare the +bran diet were respectively: starch 282 and 204, NSP 187 and 370, the monosaccharide components of the NSP being (mg/g): rhamnose 1·2 and 2·0, arabinose 13·2 and 82·1, xylose 65·1 and 147·3, mannose 0·1 and trace, galactose 3·7 and 3·9, glucose 99·6 and 125·9, uronic acids 4·0 and 8·8 respectively. The stock, no fibre, +bran and +pectin diets contained 41, 15, 30 and 17 μg Zn/g dry matter (DM) respectively.

Male Wistar albino rats weighing approximately 150 g were randomly allocated to diets and cages, and were housed in pairs (semi-purifed diets) or groups of three (stock diet only) with six rats per diet. The diets were provided *ad lib* for 3 weeks before the rats were used for gut sac experiments.

Gut sac incubations

Rats were killed by cervical dislocation at approximately 11.00 hours and sections of the intestine removed immediately and rinsed free of digesta with physiological saline (9 g NaCl/l). Segments of 30–40 mm length were cut from each region of the intestine. Duodenal segments were taken from the first 120 mm posterior to the common bile duct and ileal segments from the region immediately anterior to the ileo-caecal junction. Colonic

segments were taken from the region immediately posterior to its junction with the caecum. The caecum from each rat was also removed and weighed with its contents. Two segments from each region were placed in previously aerated Zn-free Tris-Krebs buffer, pH 7·3 at 37° (Seal & Heaton, 1983). A third segment was retained for Zn analysis. The two segments were everted and filled with Zn-free Tris–Krebs buffer and then incubated for 30 min in the same buffer containing 20 mg Zn as zinc sulphate/l. At the end of the incubation period the sacs were rinsed, blotted and the contents and tissue retained for Zn analysis.

Expt 2

Effect of ouabain

Six Wistar rats, initial weight 220 g, were randomly allocated to each of the four diets described previously, except for the group fed on the +bran diet (*n*4), and housed in pairs. Stock diet was fed *ad lib.*, and semi-purified diets were fed at 20 g/rat per d at approximately 16.00 hours daily for 22–28 d before measurements were made. Sections of gut were removed and everted gut sacs prepared as described previously. One gut sac from each region was incubated in Tris-Krebs buffer containing 20 mg Zn as $ZnSO_4$/l and 11·5 mM-glucose. The second sac was incubated in the same buffer containing 0·25 mM-ouabain. After incubation for 30 min, gut-sac contents and tissues were retained for Zn analysis. The caecum from each rat was removed, weighed with its contents and the pH of the contents measured directly with a pH meter fitted with a glass micro-electrode (Corning, Halstead, Essex).

Analytical methods

Intestinal sacs and unincubated tissue samples were oven-dried at 65° to constant weight. Dried sacs and diets were wet ashed for 16 h at 60° in a mixture of concentrated nitric, sulphuric and perchloric acids (3:3:1, by vol.). Zn was determined in sac contents and wet-ashed samples using a Pye Unicam SP9 atomic absorption spectrophotometer (Pye Unicam Ltd, Cambridge). Glucose in gut-sac contents was measured enzymically using a YSI 23A Glucose Analyser (Clandon Scientific Ltd, Aldershot, Hants). Complex carbohydrates in foods were determined and characterized by the methods of Englyst & Cummings (1984, 1985).

Statistical methods

Values were examined by analysis of variance in which diets were considered as main plots and their effects tested against the between-animals within-diets variance. Sites within the intestine were considered as a sub-plot and the effects of sites and diets × sites interaction were tested against the between-animals within-diets and sites variance (error 2); see Table 2. *A priori*-nominated orthogonal contrasts were used to distinguish between diets, between sites and between diets × sites interactions as indicated in Table 2. The contrast S *v.* (N + B + P) compared the value for the stock diet (S) with the mean for the three semi-purified diets (N, B and P) whilst N *v.* (B + P) compared the no-fibre diet (N) with the mean for diets containing added fibre (B and P). Similarly, the contrast (D + I) *v.* C compared the mean for the small intestinal sites (D and I) with that for the colon (C). Each of the diets × sites interactions examined, systematically, possible differences in effects of diets at the various intestinal sites. For example, (B *v.* P) × (D *v.* I) tested whether the difference between +bran and +pectin differed between the duodenum and ileum. For selected variables, additional *post hoc*-nominated between-diet comparisons, i.e. N *v.* B and N *v.* P, were also carried out. For Expt 2, values from incubations performed in the absence (−O)

Table 2. *Expts 1 and 2. Partition of degrees of freedom in analysis of variance of experimental values*

Source of variation	df
Between diets	3
S *v.* (N+B+P)	1
N *v.* (B+P)	1
B *v.* P	1
Between-animals within-diets (error 1)	19(16)*
Main plots total	22(19)*
Between sites	2
(D+I) *v.* C	1
D *v.* I	1
Diets × sites interaction	6
(S *v.* (N+B+P)) × ((D+I) *v.* C)	1
(N *v.* (B+P)) × ((D+I) *v.* C)	1
(B *v.* P) × ((D+I) *v.* C)	1
(S *v.* (N+B+P)) × (D *v.* I)	1
(N *v.* (B+P)) × (D *v.* I)	1
(B *v.* P) × (D *v.* I)	1
Error 2	38(32)*
Grand total	68(59)*

S, stock; N, no fibre; B, +bran; P, +pectin; D, duodenum; I, ileum; C, colon.
* Values for Expt 2.
For interpretation of contrasts, see p. 153.

and presence (+O) of ouabain and the difference between −O and +O values (O dep) were analysed separately. Contrasts significant at $P < 0.05$ or better are shown in tables and figures.

RESULTS

Animal health, growth and caecal characteristics

Apart from one rat on the +bran diet in Expt 1 which developed a lung tumour and was removed from the study, the rats remained healthy. In both experiments, rats given the semi-purified diet including pectin grew less well than the other rats (Table 3). Caecal weight (in absolute terms and relative to body-weight) was lowest for the no-fibre diet, increased only slightly with the inclusion of bran, was approximately twice as great for the stock-diet-fed rats and three to four times greater for the +pectin animals. Changes in caecal tissue weight with diet were similar to those for mass of the whole organ. Caecal pH was inversely related to caecal mass, with the hydrogen ion concentration ten times higher on the +pectin than on the no-fibre diet (Table 3).

Expt 1

In this initial experiment, rates of Zn transfer into everted gut sacs ranged from 6·4 to 16·8 μg/g tissue DM per 30 min (Table 4). When considered across all intestinal sites, rates of Zn transfer were significantly ($P < 0.01$) greater with pectin as the NSP source than with bran. Sacs from +pectin animals also transported Zn faster ($P < 0.05$) than those from no-fibre animals. There were no significant intestinal site effects, but there were significant ($P < 0.01$) sites × diets interactions due largely to the almost three times greater Zn transfer across colonic tissue with the +pectin diet than with the no-fibre diet (Table 4).

Table 3. *Growth rates, caecal weights, caecal tissue weights and caecal pH of rats given diets varying in type and content of dietary fibre*

(Mean values with pooled standard errors of means for each group of rats)

	Growth rate (g/d)		Caecal wt (g)		Caecal wt as % body-wt		Caecal tissue (g)	Caecal pH
Diet	Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2	Expt 2	Expt 2
Stock (S)	4·1	5·7	7·3	9·1	2·9	2·5	1·90	6·1
No-fibre (N)	5·6	4·9	3·7	3·8	1·4	1·1	0·99	7·0
+Bran (B)	5·6	4·5	4·4	5·1	1·6	1·5	1·4	6·2
+Pectin (P)	1·9	2·9	9·3	12·6	4·7	4·2	2·54	6·0
SE of mean (*n*)	0·38 (3)†	0·23 (3)†	0·49 (6)	0·36 (6)	0·21 (6)	0·08 (6)	0·168 (6)	0·19 (6)
Diet effects								
S *v.* (N+B+P)	NS	***	**	***	NS	NS	NS	NS
N *v.* (B+P)	**	**	***	***	***	***	***	**
B *v.* P	***	**	***	***	***	***	***	NS

NS, not significant.
** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.
† Based on weights of pairs of rats except stock diet, Expt 1 (three rats/cage).

Table 4. *Expt 1. Zinc transfer into everted gut sacs (μg Zn/g tissue dry matter per 30 min) from rats given* ad lib. *access to diets varying in content and type of dietary fibre**

(Each value is a mean from six animals except for +bran diet, where there were five animals)

Diet...	Stock (S)	No fibre (N)	+Bran (B)	+Pectin (P)
Duodenum (D)	7·6	8·3	9·0	8·8
Ileum (I)	8·6	10·5	6·9	10·2
Colon (C)	7·2	6·4	8·7	16·8

Mean squares were 35·30 and 13·58 for error terms 1 and 2 respectively (for details of analysis of variance, see Table 2).

Significant effects: (1) between diets: B *v.* P; $P < 0.05$; (2) sites × diets interaction: ((D+I) *v.* C) × (N *v.* (B+P)) $P < 0.001$, ((D+I) *v.* C) × (B *v.* P) $P < 0.01$.

* For details of diets, see p. 152 and Table 1.

Table 5. *Expt 2. Zinc transfer into everted gut sacs (μg Zn/g tissue dry matter per 30 min) from rats given diets varying in content and type of dietary fibre† and where incubations were performed in the absence (−O) and presence (+O) of ouabain*

(Each value is a mean for six animals except for +bran diet where there were four animals)

Ouabain treatment...	−O			+O			O dep		
Diet Intestinal site...	D	I	C	D	I	C	D	I	C
Stock (S)	6·6	9·0	12·4	5·2	8·2	9·2	1·4	0·8	3·2
No fibre (N)	7·4	8·3	7·6	4·8	7·8	3·1	2·6	0·5	4·5
+Bran (B)	9·1	11·6	12·6	5·8	10·4	11·1	3·5	1·3	1·6
+Pectin (P)	14·6	15·3	16·1	9·5	12·9	6·6	5·1	2·4	9·5
Mean squares									
Error 1		46·79			37·85			14·58	
Error 2		30·81			10·69			25·04	
Significant contrasts									
Diet effects		N *v.* (B+P)*			N *v.* (B+P)*			B *v.* P*	
Site effects					D *v.* I**			(D+I) *v.* C*	
Diet × site effects					(B *v.* P) × (D *v.* I)**				

O dep, Na^+,K^+-ATPase-dependent transfer calculated as difference between −O and +O values; D, duodenum; I, ileum; C, colon.

* $P < 0.05$, ** $P < 0.01$.

† For details of diets, see p. 152 and Table 1.

Expt 2

To ascertain the importance of energy-demanding Na^+, K^+-ATPase-dependent transfer by the intestinal everted sacs, incubations were carried out in the presence and absence of 0·25 mM-ouabain.

Zn

In the absence of ouabain, rates of Zn transfer by the everted gut sacs were similar to those found in Expt 1, ranging from 6·6 to 16·1 μg/g tissue DM per 30 min (Table 5). For all intestinal sites, rates were highest on the +pectin and lowest on the no-fibre diets with this difference significant at $P < 0.01$. When considered across all diets, mean rates of Zn

Table 6. *Expt 2. Zinc content of unincubated gut tissue (μg Zn/g tissue dry matter) from rats given diets varying in content and type of dietary fibre*

(Each value is a mean for six animals except for +bran diet where there were four animals)

Diet...	Stock (S)	No fibre (N)	+Bran (B)	+Pectin (P)
Duodenum (D)	123	96	93	106
Ileum (I)	208	212	149	195
Colon (C)	131	149	92	178

Mean squares were 1921 and 1283 for error terms 1 and 2 respectively (for details of analysis of variance, see Table 2).

Significant effects: (1) diet effects: B *v.* P, $P < 0.01$; (2) site effects: D *v.* I, $P < 0.001$.

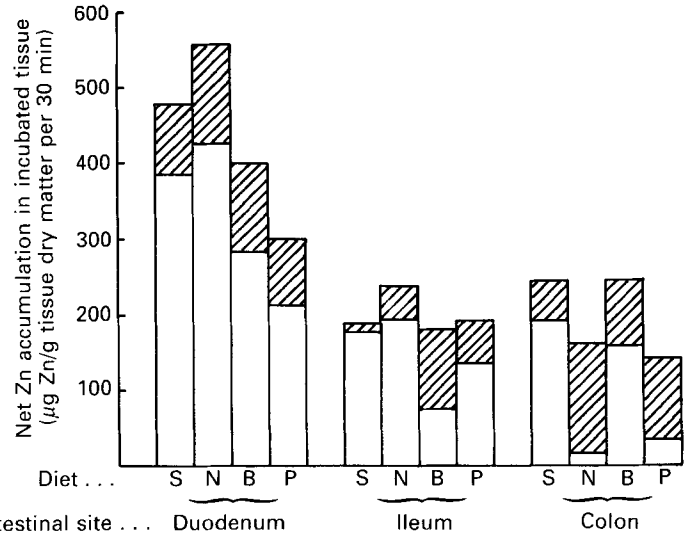

Fig. 1. The net accumulation after in vitro incubation of zinc in duodenal (D), ileal (I), and colonic (C) tissue from rats given diets varying in content and type of dietary fibre. Diets were stock (S), no fibre (N), +bran (B) and +pectin (P) (for details of diets, see p. 152 and Table 1). Incubations were performed in the presence (□; +O) and absence (□ + ▨) of 0·25 mM-ouabain with Na^+,K^+-ATPase-dependent accumulation (▨; O dep) calculated by difference. Each value is the mean for six animals except for +bran where there were four animals.

Statistical analysis (for details of analysis of variance, see Table 2)

Ouabain treatment...	−O	+O	O dep
Mean squares			
Error 1	23942	26297	10898
Error 2	23902	17650	13595
Significant contrasts			
Site effects	(D+I) *v.* C**	(D+I) *v.* C***	
	D *v.* I***	D *v.* I***	
Diet × site effects		(N *v.* (B+P)) × ((D+I) *v.* C)*	

* $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$.

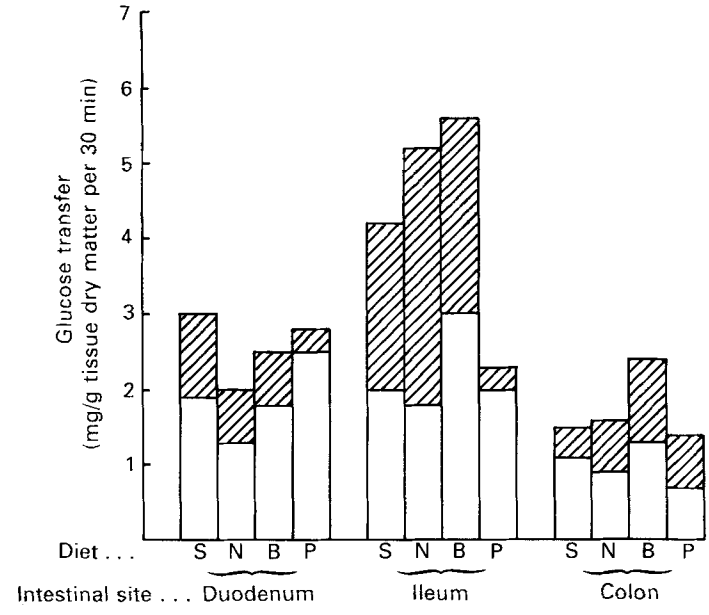

Fig. 2. Glucose transfer into everted gut sacs from rats given diets varying in content and type of dietary fibre. Sacs were prepared from duodenal (D), ileal (I) and colonic (C) tissue. Diets were stock (S), no fibre (N), +bran (B) and +pectin (P) (for details of diets, see p. 152 and Table 1). Incubations were performed in the presence (□; +O) and absence (□+▨) of 0·25 mM-ouabain with Na^+, K^+-ATPase dependent accumulation (▨; O dep) calculated by difference. Each value is the mean for six animals, except for +bran where there were four animals.

Statistical analysis (for details of analysis of variance, see Table 2)

Ouabain treatment...	−O	+O	O dep
Mean squares			
Error 1	17664	5387	14362
Error 2	23015	5123	19359
Significant contrasts			
Diet effects	B *v.* P*	N *v.* (B+P)*	B *v.* P*
Site effects	(D+I) *v.* C*** D *v.* I**	(D+I) *v.* C**	D *v.* I**
Diet × site effects	(B *v.* P) × (D *v.* I)*	(B *v.* P) × (D *v.* I)*	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

transfer were 9·4, 11·1 and 12·2 μg/g tissue DM per 30 min for duodenum, ileum and colon respectively. There were no significant site or diet × site effects.

When considered over all sites and diets, inclusion of ouabain in the incubation medium reduced rates of Zn transfer by 28%, but the reduction was greater for the duodenum and colon (33 and 38% respectively) than for the ileum (11%), indicating the reduced importance of Na^+, K^+-ATPase-dependent steps in Zn transfer by the terminal small intestine. In the presence of ouabain, Zn transfer was significantly ($P < 0.05$) higher by tissue from +pectin rats than from those on the no-fibre diet, and rates of transfer were greater ($P < 0.01$) by ileal than by duodenal tissue.

Rates of Na^+, K^+-ATPase-dependent (O dep) transfer of Zn were significantly ($P < 0.05$) higher for +pectin than for +bran or no-fibre rats and rates of transfer were greater for colonic than for duodenal and ileal tissue (Table 5) when considered across all diets.

The Zn content of unincubated tissue from +bran-fed rats was significantly lower ($P < 0.01$) than from those given the no-fibre or the +pectin diet, and was significantly ($P < 0.001$) lower for duodenal tissue than for ileal tissue (Table 6). In the absence of ouabain, net accumulation of Zn by intestinal tissue (calculated as the difference between Zn content of unincubated and incubated tissue) was unaffected by diet, but was significantly ($P < 0.001$) greater for duodenal than for ileal tissue, with accumulations by ileal and colonic tissue being similar (Fig. 1). Zn accumulation by Na^+, K^+-ATPase-independent routes (+O) showed a similar pattern and was significantly ($P < 0.001$) greater for duodenal than for ileal tissue (Fig. 1). There were no significant ($P > 0.05$) treatment effects on Zn accumulation by Na^+, K^+-ATPase-dependent (O dep) routes.

Glucose

Rates of glucose transfer by everted gut sacs were in the order ileum > duodenum > colon. Total glucose transfer and transfer by Na^+,K^+-ATPase-dependent routes were greater for tissue from rats given the bran-containing diet than that from those given the +pectin diet (Fig. 2). Over all sites and diets, inclusion of ouabain reduced the rate of glucose transfer by 41%, but the effect was greater for the distal intestine (ileum and colon 49 and 42% respectively) than for the duodenum (27%). When considered over all diets, Na^+,K^+-ATPase-dependent glucose transfer rate by ileal tissue was approximately three times greater than that by either duodenal or colonic tissue, despite a low rate for ileal sacs from +pectin rats (Fig. 2). This contrasts with the relative unimportance of Na^+,K^+-ATPase-dependent routes for Zn transfer by ileal tissue.

DISCUSSION

Following its development for measurement of sugar absorption (Wilson & Wiseman, 1954), the everted-gut-sac procedure has been validated and used extensively for studies of intestinal uptake of several minerals (Aldor & Moore, 1970; Arduser *et al.* 1985) including Zn (Pearson *et al.* 1966; Seal & Heaton, 1983). In the present study, rates of mucosal:serosal transfer of both glucose and Zn, and tissue accumulation of Zn were reduced by the inclusion of 0·25 mM-ouabain in the incubation medium, indicating the presence of active, energy-demanding, Na^+,K^+-ATPase-dependent transport mechanisms in the gut preparation used. In addition, these tissue preparations showed differential responses in mucosal:serosal transfer for the two nutrients, with 0·45 of glucose but only 0·10 of Zn transfer by ileal tissue being Na^+,K^+-ATPase-dependent.

As used in the present study, the everted-gut-sac procedure is useful for investigation of treatment effects on the potential capacity of intestinal mucosa to take up Zn and to retain it within the tissue or to transfer it across the serosa. Changes in accumulation or transfer associated with different diets are, therefore, due to changes in the disposition of the mucosa induced by diet rather than to lumen events, since the gut segments were washed free of digesta before eversion and incubation.

Tissue Zn concentrations

Concentrations of Zn in intestinal tissue were higher in the ileum than in the duodenum or colon (Table 6) in confirmation of earlier reports by Ghishan & Sobo (1983) and by Emes & Arthur (1975). Since mucosal metallothionein concentrations are higher in the duodenum than in the ileum (Flanagan *et al.* 1983), it seems likely that other Zn-binding proteins may be more important in ileal tissue.

Sites of Zn absorption

There were no significant differences in rates of Zn transfer by everted sacs from duodenal, ileal and colonic sites, but there was a tendency towards higher rates of transfer at more distal sites in both experiments. However, in agreement with the work of Kowarski *et al.* (1974), accumulation by intestinal tissue was a much more important fate for Zn removed from the incubation medium than was transfer across the serosal surface, and it should be noted that accumulation of Zn by sacs from the duodenum was approximately twice as great as that by tissue from either the ileum or colon. The form and location of Zn accumulated within gut tissue was not elucidated in the present study, but some may have been bound to metallothionein which occurs in higher concentrations in the duodenum than in the ileum (Flanagan *et al.* 1983) and to other Zn-binding proteins (Seal & Heaton, 1987). Such binding appears to be important in regulation of Zn absorption (Cousins, 1986; Solomons, 1986) and in Zn transport across the brush border (Seal & Heaton, 1987).

Higher rates of Zn transport or absorption by the colon or ileum, or both, than by the duodenum in rats have been reported by Kowarski *et al.* (1974), Emes & Arthur (1975), Antonson *et al.* (1979), Meneely & Ghishan (1982) and Wapnir *et al.* (1985), but this is in contrast to observations by Methfessel & Spencer (1973), Davies (1980) and Seal & Heaton (1983). Indeed, Davies (1980) reported that 60% of ^{65}Zn absorption occurred in the duodenum, with only negligible absorption from the caecum and colon. It is difficult to reconcile these disparate observations, but some of the differences may relate to the experimental technique, strain of rat or diet used. Our present observations, together with those demonstrating substantial absorption of Zn from the large bowel of other species (pigs: Partridge, 1978; sheep: Grace, 1975; cattle: Bertoni *et al.* 1976), suggest that this organ has the potential to absorb nutritionally important amounts of Zn, but that this potential may not always be expressed. For example, the relatively small proportion of ^{65}Zn absorbed from the colon after installation into the caecum of patients undergoing colonscopy for evident or suspected polypoid adenoma may have been due to the preparative treatment of the bowel preceding colonscopy or to the prevailing ailment (Sandstrom *et al.* 1986).

Effects of dietary fibre

Contrary to earlier beliefs that NSP provided inert 'bulk' in the diet, it is now clear that NSP are fermented in the large bowel, resulting in important changes in the anatomy, physiology and biochemistry of the gut (Cummings, 1981, 1984) and may influence the metabolic activities of the enteric flora (Wyatt *et al.* 1986; Cheng *et al.* 1987; Key & Mathers, 1987). The concentrations of NSP (g/kg diet) in the diets used in the present study were stock 187, no fibre 0, +bran 74 and +pectin 200. When compared with animals given the NSP-free no-fibre diet, there was substantial hypertrophy of the caecum and a reduction in pH with the +pectin, stock and, to a lesser extent, +bran diets, indicating that these diets promoted microbial fermentation in this organ (Table 3).

Changes in total caecal weight were accompanied by changes in caecal tissue weight. Wyatt *et al.* (1988) reported that the extent of hypertrophy of the rat caecum in response to dietary inclusion of NSP differed for different NSP sources. Whilst the reasons for these differences were unclear, Wyatt *et al.* (1988) observed a close quadratic relation between caecal contents and caecal tissue masses. Using the equation of Wyatt *et al.* (1988) and the caecal contents masses observed in the present study, the caecal tissue masses predicted for animals given the stock, no-fibre, +bran and +pectin diets were 1·8, 0·9, 1·0 and 2·4 g respectively, which are in fair agreement with our observed findings in Table 3. In the present study, higher dietary NSP concentrations were associated with greater caecal masses.

Very little is known about the effects of stimulation of large bowel fermentation on mineral absorption. In a feeding trial with healthy male students, Cummings *et al.* (1979) observed that pectin which binds Ca in vitro had no effect on Ca balance. Whilst pectin is not digested by mammalian enzymes, it is very readily fermented by the large-bowel flora (Goodlad & Mathers, 1988), and Cummings *et al.* (1979) speculated that Ca bound to pectin and carried to the large bowel may have been released therein and absorbed from this organ when this NSP source was fermented. The large bowel of both rats (Ammann *et al.* 1986) and man (Sandstrom *et al.* 1986) has the potential for Ca absorption, and rats adapted to a diet rich in fermentable NSP absorbed twice as much Ca from the caecum as did those given a fibre-free diet (Demigne & Remesey, 1985).

In the present study, the highest rates of Zn transfer were those by colonic tissue from +pectin rats (Tables 4 and 5), whilst tissue from animals given diets containing less-fermentable NSP (stock and +bran) resulted in slightly higher Zn transfer rates than the no-fibre diet in both experiments. Rats given the +pectin diet grew least quickly, and it might be anticipated that reduced growth would be accompanied by reduced rates of Zn transfer, but the opposite effect was observed in the present study. There is evidence that giving pectin (but not wheat bran) may produce ultrastructural modifications of colonic mucosa (Vahouny *et al.* 1981), but other fermentable NSP sources which also cause caecal enlargement did not alter gut morphology (Anderson *et al.* 1986). The functional significance of any morphological changes in mucosa with pectin feeding are unclear, but Shiau & Chang (1986) observed reduced apparent permeability of the intestine in pectin-fed rats which suggests that the increased potential for Zn absorption by colonic tissue observed in the present study is unlikely to be due to a general increase in 'leakiness' of the mucosa.

Inclusion of pectin in the diet of rats may increase the length of the small intestine (J. C. Mathers and H. J. Finlayson, unpublished results) and increase the weight of mucosa per unit length (Brown *et al.* 1979), with the latter effect a possible consequence of increased epithelial proliferation rate (Jacobs, 1983). Increased Zn transport rates observed in the present study might, therefore, be a result of greater mucosal mass per unit weight of intestine. Such measurements were not made in our study, but the findings of Brown *et al.* (1979) indicate little effect of dietary pectin in this regard, with mucosal wet weight as a proportion of total intestinal wet weight for rats given basal (no NSP) and pectin-containing diets being 0·46 and 0·53, 0·45 and 0·47 and 0·37 and 0·35 for upper jejunal, mid-jejunal and ileal tissue respectively. It, therefore, seems unlikely that by expressing our transfer values per unit total tissue DM (rather than mucosa weight), we have obscured possible dietary effects, but further studies of this area and especially of the colon are warranted.

In conclusion, the present study has demonstrated that the colon appears to have considerable capacity to absorb Zn by Na^+, K^+-ATPase-dependent mechanisms and that this capacity may be enhanced by consumption of pectin, a source of fermentation NSP.

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