Hexose transport and mucosal morphology in the small intestine of the zinc-deficient rat

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(*Received 19 December 1983 – Accepted 16 April 1984*)

1. Immature male Wistar rats were given a zinc-depleted semi-synthetic diet (2 mg Zn/kg diet) for 4 weeks. Control groups received a similar diet containing 60 mg Zn/kg diet, either *ad lib.* or in amounts matched to the consumption of the Zn-deficient group. Rates of growth and food conversion efficiency were markedly lower in the Zn-deficient group compared to controls.

2. The phloridzin-sensitive uptake of galactose and 3-*O*-methyl glucose by everted jejunal rings from each group was measured over a range of concentrations. Kinetic analysis revealed that the maximum transport rate (V_{max}) for the uptake of both sugars was significantly higher in the Zn-deficient group than in the control groups.

3. The villi, microdissected from samples of proximal jejunum, were significantly shorter and narrower across the base in the Zn-deficient group than those of the control groups, but were present in greater numbers per unit serosal area.

4. The present study shows that Zn deficiency leads to significant morphological changes in rat small intestine, which are accompanied by an increase in the capacity of the tissue to take up sugars across the mucosal membrane.

Zinc deficiency is associated with a wide variety of physiological and biochemical defects, marked depression of appetite, and severely retarded growth and development (Mills *et al.* 1969). Previous studies have established a role for Zn in nucleic acid metabolism (Sandstead & Rinaldi, 1969; Prasad & Oberleas, 1974) and it has been suggested that inhibition of DNA synthesis in rapidly-dividing tissues accounts for the poor growth observed in animals given a low-Zn diet.

Another possibility is that the intestine, which is a site of continuous renewal of absorptive cells and enzymes, may be particularly sensitive to Zn deficiency. Growth failure might therefore be a secondary consequence of a reduced capacity for nutrient absorption. Although there are several previous reports of minor ultrastructural abnormalities in the intestinal mucosa of Zn-deficient rats, and evidence of impaired triglyceride absorption (Koo & Turk, 1977*a*, *b*), there has been no thorough investigation of small intestinal structure and function. In the present study the intestinal uptake of active-transported hexose sugars and the growth and morphology of the small intestinal mucosa were investigated in groups of Zn-deficient, feed-restricted and *ad lib.*-fed rats.

MATERIALS AND METHODS

Animals

Fifty-four immature, male Wistar rats, weighing 95–100 g, were randomly divided into three groups of eighteen and housed in pairs in polypropylene cages with stainless steel gridded bottoms and tops. The Zn-deficient group (ZD) received a semi-synthetic diet containing 2 mg Zn/kg diet *ad lib.* The second and third groups were controls receiving a similar diet containing 60 mg Zn/kg. The first of these control groups received the amount of food consumed on the previous day by a matched pair of Zn-deficient rats. This group was designated feed-restricted (FR) rather than pair-fed, because they tended to consume their

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Table 1. *Composition of semi-synthetic diet (g/kg)*

Casein*	168
Starch	326
Sucrose	326
Maize oil	80
Solka floc	40
Minerals†	40
Vitamin mix‡	20

* To remove zinc, casein was washed with EDTA solution (10 g/l), rinsed thoroughly with distilled water and freeze dried before inclusion in the diet.

† Minerals (g/kg diet); $CaHPO_4$ 13·00, $CaCO_3$ 8·20, KCl 7·03, Na_2HPO_4 7·40, $MgSO_4 . H_2O$ 4·00, $MnSO_4 . H_2O$ 0·18, $ZnCO_3$ 0·10 (excluded from the low-Zn diet), $FeSO_4 . 7H_2O$ 0·144, $CuSO_4$ 0·015, KIO_3 0·001.

‡ Vitamin mix (mg/kg diet); nicotinic acid 60, cyanocobalamin in mannitol 50, calcium D-pantothenate 40, thiamin hydrochloride 10, riboflavin 10, pteroylmonoglutamic acid 5, D-biotin 1, menadione 1, Rovimix E-25 (Roche) 300, Rovimix A-500 (Roche) 25, Rovimix A.500/D3 (Roche) 15, choline bitartrate 1800.

ration much more quickly than the Zn-deficient animals. The third group (AL) was fed *ad lib*. The composition of the semi-synthetic diet is shown in Table 1. All three groups received distilled deionized water *ad lib*. Food intakes were measured daily and body-weights recorded at twice weekly intervals. On days 28, 29 and 30 of the study, ten rats from the Zn-deficient, feed-restricted and *ad lib*.-fed groups respectively were killed and the small intestines removed for studies of hexose uptake and morphological examination. The remaining rats in each group were killed and bone samples were taken for the assessment of Zn status.

Transport study

Animals were killed by a blow to the head followed by cervical dislocation. The entire small intestine was removed, flushed with Krebs–bicarbonate buffered saline (9 g sodium chloride/l), everted and extended on the bench with minimal stretching. The extended intestine was measured and the first twelfth (duodenum) was discarded. A small sample (5–10 mm) was removed and placed in fixative, and the following six-twelfths of the total length was cut into rings (2–3 mm) and placed in saline. Three further 50 mm lengths were removed for measurements of mucosal weight, DNA and protein content.

The rings from each animal were distributed randomly amongst sixteen conical flasks (25 ml) containing 10 ml Krebs–bicarbonate buffered saline. The incubation flasks were divided into two sets of eight, containing either galactose or 3-*O*-methyl glucose at concentrations of 2, 5, 10, 20, 40 and 80 mmol/l. The remaining two flasks in each set contained the appropriate sugar at a concentration of 20 or 80 mmol/l, together with phloridzin (phloretin-2-β-D-glucoside; Sigma, Poole, Dorset) at a concentration of 0·1 mmol/l. The flasks also contained either [^{3}H]galactose or 3-*O*-[^{3}H]methyl glucose (Amersham International, Amersham, Bucks) at activities of 1000 or 2500 μCi/l. The incubation period was 4 min at 37°; flasks were shaken at 120 cycles/min and gassed continuously with oxygen/carbon dioxide (95:5 v/v). After incubation the rings were collected on a Buchner filter, rinsed with ice-cold saline, placed in glass vials and dried to constant weight overnight at 85°. The vials were securely capped and the dried residues were dissolved in concentrated nitric acid (0·4 ml) and mixed with 3·6 ml of 0·75 M-Trizma base (Sigma), portions (0·5 ml) were diluted to 1 ml with distilled water and added to 9 ml scintillation fluid (Cocktail T, Scintran; BDH, Poole, Dorset). The ^{3}H activities of the samples were determined by counting on a Philips PW4700 liquid-scintillation spectrometer.

Mucosal weight. The first of the 50 mm samples taken from mid-intestine was slit open lengthwise and laid on a glass plate, mucosa uppermost. The mucosa was stripped from the underlying muscle layers by scraping with a microscope slide, the separated tissue samples were placed in pre-weighed glass vials and reweighed before and after drying.

Mucosal DNA. This was determined in fresh mucosa from the second 50 mm sample by a fluorometric technique (Fiszer-Szafarz *et al.* 1981). DNA content per unit dry weight of mucosa was calculated using the wet weight: dry weight value obtained from the first 50 mm sample.

Mucosal protein. The analysis was carried out on homogenates of fresh mucosa from the third 50 mm sample, by a modification of the Lowry method (Lowry *et al.* 1951), and the protein content per unit dry weight calculated as above.

Zn status. This was assessed by measurement of femur Zn concentration. The femur was removed, freed of skeletal muscle, dried and ground to a powder. Samples were dry-ashed at 480° in a muffle furnace and the ash dissolved in 6 M-hydrochloric acid (Analar). Zn was measured by atomic absorption spectroscopy.

Morphological study

Samples of whole intestine obtained from animals in the transport study were fixed by immersion in a mixture of absolute ethanol–glacial acetic acid (75:25, v/v) for 24 h, followed by storage in ethanol–water (75:25, v/v). Subsequently, each such sample was slit open and measured under a dissecting microscope fitted with a graduated eyepiece to provide estimates of width across the serosal surface and total thickness. Samples were then divided approximately equally and a total of ten individual villi were removed from one half by microdissection with sharpened needles. Estimates of the maximum height and basal width of each villus were made using the graduated eyepiece. The remaining half of each sample was stained in bulk by the Feulgen reaction (Clarke, 1970). Stained samples were examined, serosal surface uppermost, under the dissecting microscope. Five separate estimates of the numbers of villi and crypts per mm^2 serosal surface were made and averaged to give a single estimate for each animal.

Statistical analysis of results

The concentration-dependence of phloridzin-sensitive sugar uptake was analysed by fitting the Michaelis–Menten equation to the experimental values. The indices for the curve of best fit were estimated by means of the nonlinear regression program of Duggleby (1981). The values were weighted assuming that the standard deviation of y (sugar uptake) was approximately proportional to y, and by means of 'bisquare weighing' (Mosteller & Tukey, 1977) which is an option available in the program. The significances of differences between means were assessed by Student's unpaired t test.

RESULTS

General effects of Zn deprivation

A detectable reduction in food consumption had occurred in the animals fed on a low-Zn diet by day 2 of the study and this was followed by a decline in the growth rate as judged by body-weight. The growth rate of the feed-restricted group of animals was significantly greater than that of the Zn-deficient group from day 5 of the experiment onwards. The growth curves for all three groups of rats over the first 25 d of the study are illustrated in Fig. 1. The total weight gain, food consumption and overall food conversion ratio for the three groups over this 25-d-period are shown in Table 2. The Zn-deficient group exhibited a progressive onset of other symptoms during this period, including noticeable lethargy, loss of hair, and skin lesions.

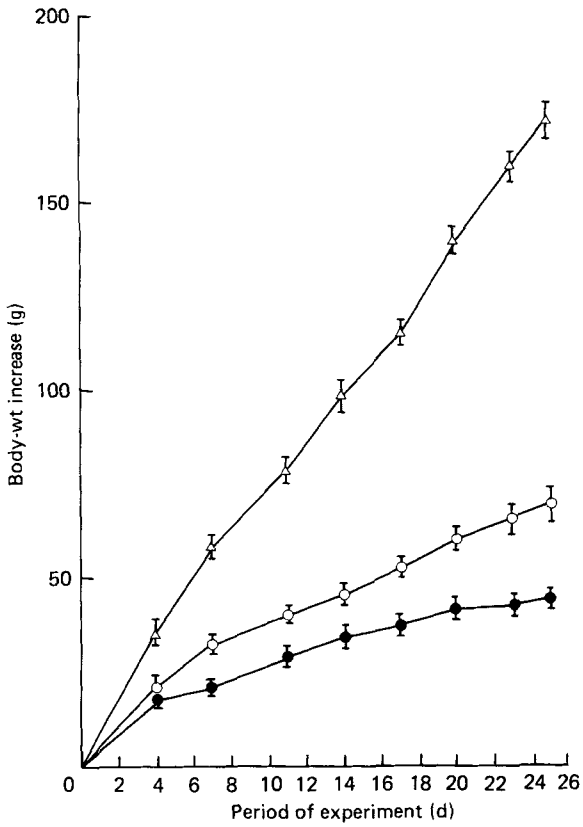

Fig. 1. Growth curves of zinc-deficient (●), feed-restricted (○) and *ad lib.*-fed (△) rats. Points are mean values with their standard errors represented by vertical bars for eighteen rats.

Table 2. *Body-weight, food consumption, food conversion ratios (body-weight gain × 100/food intake) and femur zinc concentration in Zn-deficient (ZD), feed-restricted (FR) and* ad lib.*-fed (AL) rats*

(Mean values with their standard errors: no. of animals in parentheses)

	Wt gain over 25 d (g) (10)		Food consumed (g/rat per 25 d) (10)		Food conversion ratio (10)		Femur Zn (μg/g dry wt) (8)	
Dietary group	Mean	SE	Mean	SE	Mean	SE	Mean	SE
AL	171·3	4·7	456	7·3	37·6	0·7	228·1	9·4
FR	69·5	2·2	234	3·1	29·7	0·8	237·7	6·8
ZD	43·8	2·9	228	5·6	19·2	1·3	75·7	2·9
	Statistical significance of differences: $P <$							
ZD *v.* FR	0·001		NS		0·001		0·001	
ZD *v.* AL	0·001		0·001		0·001		0·001	
FR *v.* AL	0·001		0·001		0·001		NS	

NS, not significant.

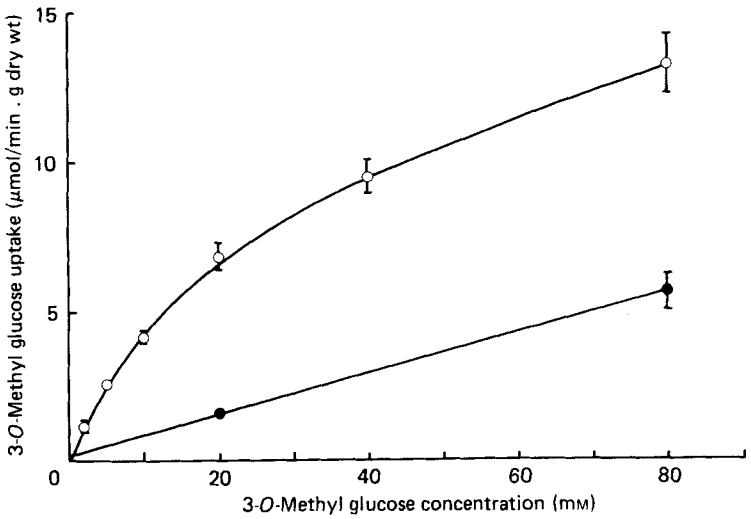

Fig. 2. Uptake of galactose *v.* concentration for zinc-deficient rats, in the presence (●) or absence (○) or phloridzin. Points are mean values with their standard errors represented by vertical bars for ten rats. The curve is fitted by eye.

Femur Zn concentration, measured as an index of Zn status, was reduced from approximately 230 μg/g dry weight in the control groups to 76 μg/g dry weight in the Zn-deficient animals (Table 2).

Transport study

A curvilinear relationship was observed between rate of uptake and concentration for both galactose and 3-*O*-methyl glucose in the phloridzin-free incubations. In the presence of phloridzin, which is a specific and highly effective inhibitor of carrier-mediated sugar transport at the intestinal brush-border membrane (Newey *et al.* 1959), the uptake of both sugars was substantially reduced. Typical results are given in Fig. 2, which illustrates the uptake of galactose by tissue from Zn-deficient rats. The small intercept on the *y* axis, obtained by extrapolating the phloridzin-insensitive uptake to zero concentration, was regarded as indicating a negligible deviation from linear kinetics; therefore, by subtracting the slope of this linear component from the raw uptake values, an estimate of phloridzin-sensitive, carrier-mediated uptake was obtained. The pooled results for each sugar in the three groups of animals are shown in Fig. 3. It can be seen that the phloridzin-sensitive components follow saturable kinetics resembling the Michaelis–Menten model. For analysis, this equation was fitted to the corrected values for each individual animal, and the means of the estimated indices for the curves of best fit were compared between groups (Table 3). For both sugars there was a significant increase in maximum transport rate (V_{max}) in the Zn-deficient animals compared to both control groups, and in the feed-restricted animals compared to the *ad lib.*-fed group. There was no indication, however, of any alteration in carrier affinity (K_m). For all groups the V_{max} for galactose uptake tended to be higher than for 3-*O*-methyl glucose, whilst the K_m was significantly lower.

Since the values for sugar uptake are expressed in terms of dry weight of whole intestine, they cannot be interpreted fully without reference to the probable variation in structure and composition of the gut between the experimental groups. Samples from the mid-intestine were analysed to provide an indication of the proportional contribution of mucosal cells

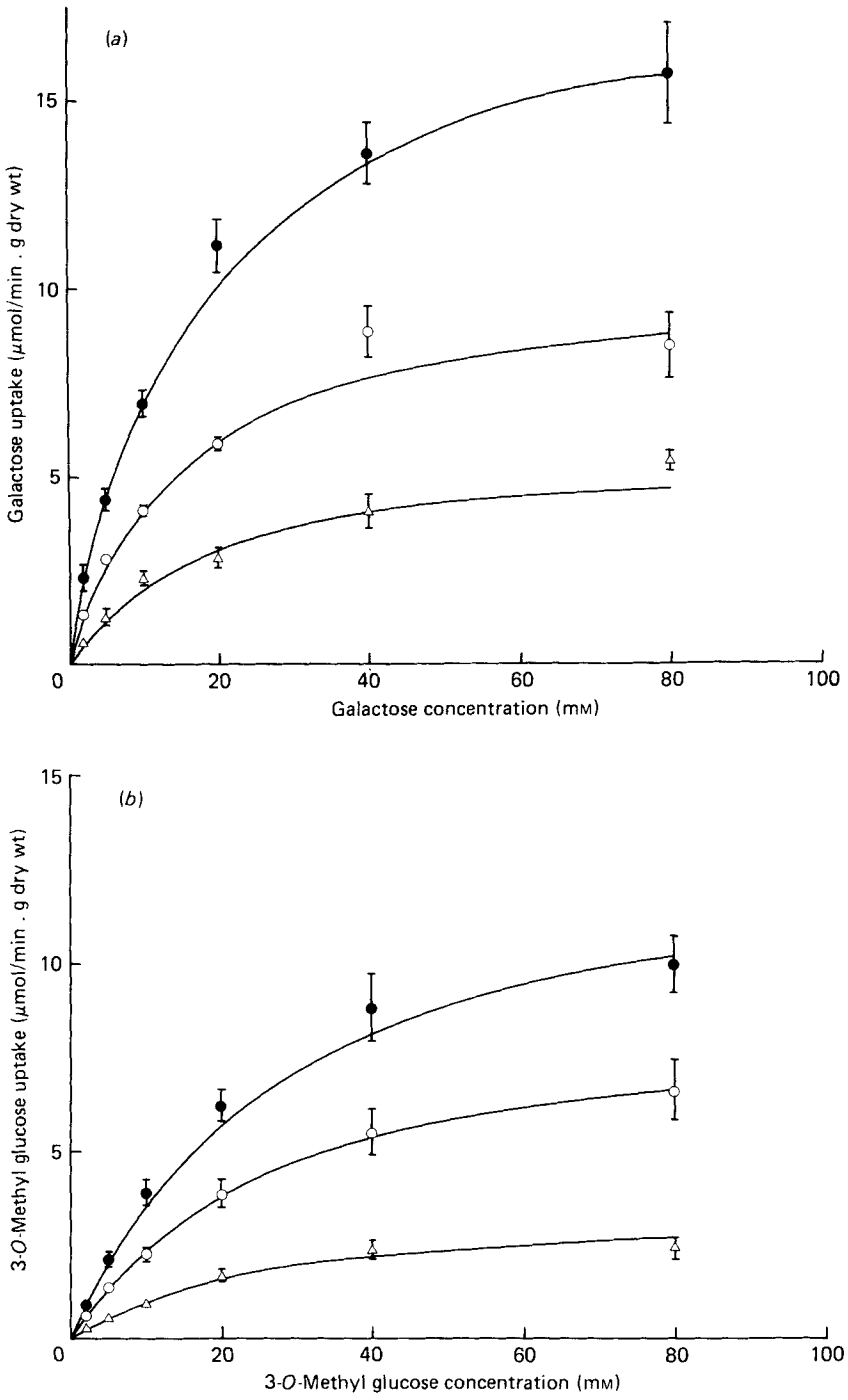

Fig. 3. Uptake of (*a*) galactose and (*b*) 3-*O*-methyl glucose *v.* sugar concentration for zinc-deficient (●), feed-restricted (○) and *ad lib.*-fed (△) rats. Points are mean values with their standard errors represented by vertical bars for ten animals in each group. Curves are derived from the Michaelis–Menten kinetic equation, using the indices shown in Table 3.

Table 3. *Kinetic indices for phloridzin-sensitive galactose and 3-O-methyl glucose uptake by everted jejunal rings from zinc-deficient (ZD), feed-restricted (FR) and* ad lib.*-fed (AL) rats*

(Mean values with their standard errors for ten estimations)

	Galactose				3-*O*-methyl glucose			
	V_{max} (μmol/min per g)		K_m (mM)		V_{max} (μmol/min per g)		K_m (mM)	
Dietary group	Mean	SE	Mean	SE	Mean	SE	Mean	SE
AL	5·7	0·6	17·3	2·0	3·6	0·6	27·4	5·4
FR	10·4	0·9	14·7	1·7	9·1	1·7	28·5	5·1
ZD	19·1	1·6	17·5	2·3	13·9	1·1	28·5	2·5
	Statistical significance of differences: $P <$							
ZD *v.* FR	0·001		NS		0·005		NS	
ZD *v.* AL	0·001		NS		0·001		NS	
FR *v.* AL	0·001		NS		0·001		NS	

NS, not significant; V_{max}, maximum transport rate; K_m, carrier affinity.

Table 4. *Weight of intestine and relative contributions of mucosa and its constituents in zinc-deficient (ZD), feed-restricted (FR) and* ad lib.*-fed (AL) rats*

(Mean values with their standard errors; no. of animals in parentheses. All values are for dry weight)

	Wt (mg/50 mm)		Mucosal wt (mg/g whole gut)		DNA (mg mucosal DNA/g whole gut)		Protein (mg mucosal protein/g whole gut)	
Dietary group	Mean	SE	Mean	SE	Mean	SE	Mean	SE
AL	66·9	5·2 (10)	719	26 (10)	17·9	1·0 (9)	364	17 (10)
FR	38·7	1·8 (10)	711	21 (10)	22·7	1·2 (10)	433	32 (10)
ZD	43·6	2·1 (10)	736	14 (10)	18·5	1·6 (9)	407	21 (10)
	Statistical significance of differences: $P <$							
ZD *v.* FR	NS		NS		NS		NS	
ZD *v.* AL	0·001		NS		NS		NS	
FR *v.* AL	0·001		NS		0·01		NS	

NS, not significant.

to the dry weight of rings of whole gut (Table 4). The intestines of the *ad lib.*-fed animals were significantly heavier per unit length than those of the other groups, and there was a tendency for the intestine from feed-restricted animals to contain a higher proportion of mucosal DNA and protein.

Morphological studies

There were significant differences in the total lengths of the small intestine from each of the groups, the Zn-deficient animals having the shortest and the *ad lib.*-fed control group the longest. Measurements of small intestinal length are somewhat arbitrary, but examination of the tissue under the dissecting microscope revealed a similar order of differences in all

Table 5. *Morphology of small intestine and microdissected villi from zinc-deficient (ZD), feed-restricted (FR) and* ad lib.*-fed (AL) rats*

(Mean values with their standard errors for ten animals in each group)

	Intestine						Villi					
	Total length (mm)		Width (mm)		Thickness (mm)		Height (μm)		Width (μm)		Density (no./mm^2)	
Dietary group	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
AL	968	19	8·9	0·3	1·00	0·03	538	10	648	22	8·4	0·2
FR	911	12	8·2	0·3	0·96	0·03	525	19	590	21	9·8	0·6
ZD	846	25	7·3	0·2	0·92	0·02	468	9	446	12	12·9	0·9
	Statistical significance of differences: $P <$											
ZD *v.* FR	0·02		0·05		NS		0·02		0·001		0·01	
ZD *v.* AL	0·001		0·002		0·005		0·01		0·001		0·001	
FR *v.* AL	0·01		NS		NS		NS		NS		NS	

NS, not significant.

of the dimensions measured, although they were not always significant (Table 5). Villi from all the groups were of the leaflike form described by other workers (Clarke, 1970), but those from the Zn-deficient animals were shorter and smaller in basal width than those from the control groups. Though smaller, the Zn-deficient villi were present in larger numbers per unit area of serosal surface.

DISCUSSION

In view of the fact that growth failure and diarrhoea are characteristic symptoms of Zn deficiency in man and experimental animals, it is perhaps surprising that relatively little attention has previously been paid to the influence of reduced dietary Zn on gastrointestinal function. There have been occasional reports of minor histological changes and possible enzyme deficiencies in acrodermatitis enteropathica but no clear picture has emerged (Moynahan *et al.* 1962; James *et al.* 1969; Rayhanzadeh & Dantzig, 1974; Baudon *et al.* 1978). One previous study in rats has demonstrated evidence of impaired triglyceride transport, associated with ultrastructural abnormalities of the enterocytes (Koo & Turk, 1977*a*, *b*) and a more recent report by Elmes & Gwyn-Jones (1980) also described relatively minor ultrastructural changes, but found no abnormalities during routine light microscopy. The present study reveals a distinct shortening and narrowing of the jejunal villi which appears to be associated with Zn deficiency in the growing rat, rather than with restricted nutrient intake. This subtle difference has not previously been reported, probably because only villous height is easily measurable in conventional histology using sectioned material, and the relatively slight shortening we have observed would not be obvious without a systematic survey.

The transport studies show a marked enhancement of carrier-mediated hexose uptake in the jejunal mucosa of our Zn-deficient rats, and the kinetic analysis demonstrates that this is due to an increase in maximum transport rate rather than in carrier affinity. The implication is that larger number of carrier sites are present per unit dry weight of intestine. Comparisons of intestinal functions between groups of animals differing greatly in body-weight and condition are notoriously difficult because of the necessity to express

results in terms of some physical index which may vary between the groups (Levin, 1967). In the present study we have expressed our uptake results in terms of dry weight of whole gut wall which includes both mucosal cells and non-absorptive elements such as muscle cells and fat. In theory an apparent enhancement of V_{max} might stem from a reduction in the proportion of these non-transporting tissue components. From Table 5 it can be seen that the weight per unit length of mid-intestine is indeed reduced in the feed-restricted and Zn-deficient groups, and there is an increase in mucosal DNA and protein content which probably reflects the presence of a higher proportion of mucosal cells in the tissue. However, although this difference may account for some of the increase in V_{max} in the feed-restricted group compared to the *ad lib.*-fed group, it clearly contributes nothing to the increased uptake in the Zn-deficient group compared to the feed-restricted controls. It seems justifiable to conclude, therefore, that our results indicate a real increase in the capacity of the mucosal cells to take up substrate.

Hexose transport is only one aspect of enterocyte function, but clearly the present results offer no support to our initial view that some of the symptoms of Zn-deficiency might be secondary consequences of intestinal malabsorption. Indeed, we are faced with the problem of accounting for an enhancement of cellular function as an apparent consequence of Zn deficiency. One possibility is that increased rates of small intestinal transport occur as an adaptive response to malnutrition, and that this is somehow intensified by the stresses of Zn deficiency. Several previous authors have claimed that restricted food intake gives rise to increased intestinal absorption of sugars in the rat (Levinson & Englert, 1972; Wilson & Schedl, 1979), but this has been disputed (Debnam & Levin, 1973). It is interesting to note the enhancement of uptake rate in our feed-restricted control animals compared to the *ad lib.*-fed group. Although Debnam & Levin (1973) report a reduction in V_{max} following partial starvation in rats, their animals were restricted for only 9 d compared to 28 d in the present study and the experiments are therefore not strictly comparable.

A second possibility is that the changes we have observed are a result of altered mucosal cell proliferation. There is firm evidence that the absorptive capacities of mammalian enterocytes develop progressively as they mature and ascend the villi, so that there is a gradient of absorptive function with the most active cells confined to the villous tips (Kinter & Wilson, 1965). A slowing of cell migration might allow more time for the development and expression of carrier capacity, with a consequent increase in carrier sites per unit weight of tissue. Since Zn metalloenzymes are known to be involved in cell division, some modification of cell proliferation might be expected to occur in Zn deficiency. The findings of our morphological study support this view. It should be noted too that our observations are unusual in that other workers studying factors which modify small intestinal function have usually reported that a reduction in villous height is associated with a smaller V_{max} (Roche *et al.* 1970). Clearly, further work will be necessary to determine the distribution of transporting cells in the mucosa, and the effects of Zn nutriture on mucosal proliferation.

Apart from the problem of understanding the role of Zn in cell and tissue physiology, the results of the present study may have wider implications. The purpose of these experiments was to study the uptake of sugars by the jejunal mucosa, via the phloridzin-sensitive pathway located in the brush-border membrane. Further studies are necessary to determine whether an enhancement of this process necessarily implies that transfer of glucose into the blood is accelerated in vivo. This is a matter of some importance as several reports have described reduced glucose tolerance in Zn-deficient rats given glucose by intraperitoneal injection, but not when the dose was given orally (Quarterman *et al.* 1966; Hendricks & Mahoney, 1972). Another aspect of abnormal glucose metabolism in Zn-deficient rats has been described recently by Reeves & O'Dell (1983) who have shown a reduced incorporation of glucose from an oral dose into fatty acids, but increased

incorporation into liver glycogen. These authors also claimed that glucose absorption rates were slightly lower in their Zn-deficient rats, but this conclusion was based only on measurements of residual glucose in the small intestine 90 min after an intragastric dose. Since the regulation of pancreatic insulin release is under the partial control of gastrointestinal hormones (Walsh, 1981), an understanding of these changes in glucose metabolism cannot be achieved without a fuller picture of gastrointestinal function in the Zn-deficient rat.

The authors thank Mr M. Gee for atomic absorption analyses, Mrs J. Cooke and Mr P. Muddel for skilled animal care, Miss J. Brown and Mrs Z. Piper for technical assistance.

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Printed in Great Britain