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The Site of Zinc Absorption in the Rat Small Intestine (38481)

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(Introduced by S. W. French)

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The site of absorption of zinc in the small intestine of the rat has not been completely clarified. Using ligated intestinal segments *in vivo* (1) ^{65}Zn appeared to be absorbed most rapidly by the duodenum followed by the ileum and jejunum consecutively. These results were confirmed by measurement of the relative rates of absorption of ^{65}Zn by ligated rat small intestine (2). Investigation of zinc absorption by *in vitro* techniques produced conflicting results. Measurement of zinc uptake by isolated intestinal segments (3) showed that the ileum absorbed more zinc than the duodenum while the jejunum absorbed the least amount of zinc. A similar *in vitro* study (4) found that the duodenum was the least active site of zinc absorption and the ileum was most active. The effect of the dietary zinc status on the zinc content of the small intestine, and its possible value in determining the site of zinc absorption has not been extensively investigated. Rats fed a diet containing 58 μg/g of zinc had a relatively constant zinc content of 50 μg/g of tissue along the entire length of the small intestine (3). A lower zinc content in zinc-deficient rats when compared zinc-adequate controls was found in rat jejunum (5) and chick duodenum (6). More extensive studies of chick small intestine (7) showed that the duodenum and ileum responded rapidly to changes in the dietary zinc status but that the high duodenal content in zinc-adequate and zinc supplemented controls probably represented zinc in the process of excretion into the gut.

We have carried out two separate studies with different approaches to determine the site of zinc absorption and perhaps to explain some of the results which appear to be so much at variance. In the first we compared the distribution of zinc in the small intestine of zinc-deficient rats with those fed a diet adequate in zinc. In the second the absorption of ^{65}Zn by isolated intestinal segments was measured and expressed in terms of two different parameters; per unit weight and per unit length of intestine.

Material and Methods. The distribution of zinc in zinc-deficient and control rat small intestine. Male Wistar rats weighing between 45 and 65 g were divided into two groups of 15 and housed individually in stainless steel cages. Animals had free access to water and were fed *ad lib.* either a zinc-deficient diet containing 2.3 μg/g zinc or the same diet supplemented with 20 μg/g zinc as zinc chloride. Diets were prepared from a copper and zinc-deficient ration (General Biochemicals Inc., Chagrin Falls, OH) supplemented with 5 μg/g copper as $CuSO_4 \cdot 7H_2O$. After 6 wk the animals were sacrificed. Control animals weighed between 250 and 300 g; zinc-deficient animals between 75 and 125 g. Four animals in each group were examined postmortem. The small intestines of the remaining animals were removed, dissected free of adhering tissue, flushed with cold isotonic saline and divided into five segments of equal length. Each segment was weighed, wet ashed in a mixture of nitric and perchloric acids and the zinc content was determined by atomic absorption spectrometry.

The absorption of ^{65}Zn by segments of rat small intestine in vitro. Twelve male Wistar rats were fed *ad lib* a commercial ration containing 77 μg/g zinc. Animals weighing between 150 and 200 g were starved for 18 hr before use and killed with chloroform. The small intestine was removed, dissected free of adhering tissue, flushed with cold saline and everted. Each intestine was placed in a 50 ml Erlenmeyer flask with 25 ml of a solution containing 0.145 *M*-NaCl, 0.004 *M*-Tris, pH 7.4, 0.04 *M*-D-Mannose (4) and 0.1 μCi/ml $^{65}ZnCl_2$ (S.A. 1140 μCi/mg zinc) and incubated in a shaking water bath at 37° for 1 hr. The medium was gassed continuously with 95% oxygen, 5% carbon

dioxide. After incubation the intestines were removed, carefully blotted to remove excess fluid and cut into segments 2.5 cm long. Each segment was weighed and the ^{65}Zn uptake determined by counting in a gamma well detector. Absorption was expressed as the ^{65}Zn uptake (cpm) per g of fresh tissue and cpm per 2.5 cm segment. The uptake of ^{65}Zn was plotted against the position of the segment in the intestine and expressed as a percentage of the total length of the intestine.

Results and Discussion. Animals fed the zinc-deficient diet displayed lesions similar to those previously described (8). Growth was severely retarded (Fig. 1) and post-mortem examination showed extreme emaciation. Hyperkeratosis of the skin extended into the oesophagus, and keratinized cell debris was present in the lumen of the oesophagus and stomach. The entire wall of the small intestine was reduced in thickness and there was considerable degeneration of the pancreas. Microscopic examination showed degenerative changes of the mucosal epithelium and discontinuity of the brush border.

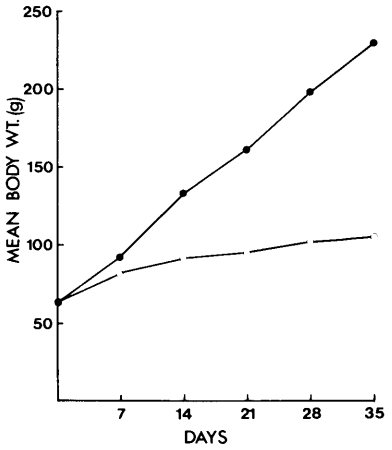

FIG. 1. The growth curves of rats fed a zinc-deficient diet (○) and the same diet supplemented with 20 μg/g zinc (●). Animals were fed *ad lib.* and had free access to water.

The distribution of zinc in zinc-deficient and control rat small intestine is presented in Table I. The control animals had the lowest zinc concentration in the second segment and the highest in the fifth segment, the distal ileum. The zinc-deficient rat small intestine exhibited a small but significant increase in zinc content from the second segment to distal ileum, although the concentration of zinc was reduced in each of the five segments examined. The control values differ from others reported (3) but since the zinc intake was lower they may reflect the reduced dietary zinc content. Chick small intestine (7) showed a rapid response to changes in the dietary zinc status suggesting that the difference between the zinc content of zinc-deficient, and control small intestine could represent zinc in the process of absorption. Change in the duodenal zinc content however, are complicated by the excretion of zinc into the intestine in this region. If the change in zinc content with dietary zinc status in the small intestine reflects the site of zinc absorption, our results suggest that the distal ileum is the most active region of zinc absorption and the jejunum is least active.

The *in vitro* accumulation of ^{65}Zn by segments of everted rat small intestine showed two distinctly different patterns of zinc

TABLE I. THE DISTRIBUTION OF ZINC IN THE SMALL INTESTINE OF RATS FED ZINC-DEFICIENT AND CONTROL DIETS.

Dietary treatment	Zinc content of intestinal segments (μg/g fresh tissue)				
	Proximal 1	2	3	4	Distal 5
Control	(5)[a] 27.0 ± 1.7[b]	(9) 19.8 ± 1.3	(9) 26.8 ± 1.3	(9) 29.7 ± 1.6	(9) 38.3 ± 1.4
Zinc-deficient	(5) 17.5 ± 0.4	(5) 17.3 ± 0.7	(5) 20.3 ± 0.6	(5) 21.3 ± 0.8	(5) 24.3 ± 0.6

[a] Number of animals.

[b] Mean ± standard error of the mean.

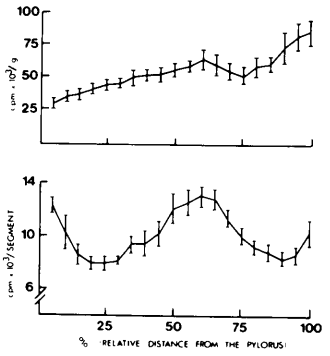

FIG. 2. The uptake of ^{65}Zn by rat in intestinal segments *in vitro* expressed per g fresh tissue and per 2.5 cm segment. The position of the segment is shown as a percentage of the total length of the small intestine. Each bar represents the mean and standard deviation of 12 segments.

uptake when compared per unit weight and per unit length of tissue (Fig. 2). On a weight basis, rat small intestine showed a steady increase in ^{65}Zn uptake from the duodenum to distal ileum. It is reasonable to assume that the absorption of ^{65}Zn is dependent, at least in part, on the area of the absorptive surface, and since the thickness of the wall of the small intestine and the structure of the mucosal epithelium varies along its length, the ^{65}Zn uptake per g of tissue may not reflect the true pattern of zinc absorption. Since it is difficult to precisely measure the mucosal surface area, results were expressed per unit length (2.5 cm segment) of intestine. A bimodal pattern of ^{65}Zn uptake was observed when presented per unit length of intestine but the range of values was narrower than when expressed per unit weight of tissue. Calcium uptake by chick small intestine (9) has been reported per unit weight and per unit area of intestine, but the difference between the patterns of absorption was not as great as found for zinc in the present study.

Neither result, however, may represent precisely the site of zinc absorption in the live animal. Competing ions (10, 11), pH (12), complex formation (13, 14) and other specific factors may restrict zinc absorption in particular intestinal locations *in vivo*. It is clear that the units by which absorption is expressed can dramatically alter the observed pattern of zinc uptake and may account for the discrepancies found by comparison of published *in vivo* and *in vitro* studies of zinc absorption.

Summary. Rats fed a zinc-adequate diet exhibited a nonuniform distribution of zinc in the small intestine. The zinc content of zinc-deficient rat small intestine was reduced, particularly in the duodenum and distal ileum. It is possible that the difference in the intestinal zinc content may reflect the rate of zinc absorption in the tissue. Uptake of ^{65}Zn by intestinal segments *in vitro* showed that the pattern of absorption obtained is dependent upon the parameters used for quantitation. This observation could account for the variation between the results obtained by different experimental methods of determining the site and rate of absorption of the nutrient.

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1. Van Campen, D. R., and Mitchell, E. A., J. Nutr. **86,** 120 (1965).
2. Methfessel, A. H., and Spencer, H., J. Appl. Physiol. **34,** 58 (1973).
3. Sahagian, B. M., Harding-Barlow, I., and Perry, H. M., J. Nutr. **90,** 259 (1966).
4. Pearson, W. N., Schwink, T., and Reich, M., *in* "Zinc Metabolism" (A. S. Prasad, ed.), ch. 14. Charles C Thomas, Springfield, IL. (1966).
5. Reingold, J. G., Kfoury, G. A., and Thomas, T. A., J. Nutr. **92,** 173 (1967).
6. Zeigler, T. R., Leach, R. M., Jr., Scott, M L., Huegin, F., McEvoy, R. K., and Strain, W. H., J. Nutr. **82,** 489 (1964).
7. Kasarskis, E. J., and Hoekstra, W. G., Proc. Soc. Exp. Biol. Med. **145,** 508 (1974).
8. Swenerton, H., and Hurley, L. S., J. Nutr. **95,** 8 (1968).
9. Bar, A., and Hurwitz, S., Poultry Sci. **98,** 1105 (1969).
10. Van Campen, D. R., J. Nutr. **97,** 104 (1969).
11. Starcher, B. C., J. Nutr. **97,** 321 (1969).
12. Emes, J. H., M. Sc. Thesis, Univ. Guelph (1970).
13. O'Dell, B. L., and Savage, J. E., Proc. Soc. Exp. Biol. Med. **103,** 304 (1960).
14. Becker, W. M., and Hoekstra, W. G., *in* "Intestinal Absorption of Metal Ions, Trace Elements and Radio nuclides" (S. C. Skoryna and D. Waldron-Edward, eds.) p. 229. Pergamon Press, Oxford (1971).

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