Intestinal Maturation: *in Vivo* Zinc Transport

FAYEZ K. GHISHAN¹⁽²⁰⁾ AND GWENN SOBO

Department of Pediatrics, Division of Gastroenterology-Nutrition, Vanderbilt Medical School,
Nashville, Tennessee, USA

Summary

We investigated with an *in vivo* single pass perfusion technique net absorption and the lumen to mucosa flux of zinc in the proximal and distal small intestine and in the colon segments of suckling (2-wk-old), weanling (3-wk-old) and adolescent (6-wk-old) rats. The concentration of zinc in the isotonic sodium chloride solution perfused through the intestinal segments was either below (10 μ M) or above (25, 50, and 100 μ M) serum zinc level. In suckling rats, the relationship between luminal zinc concentration and net absorption and lumen to mucosa flux was curvilinear in the proximal segment only; however, the relationship was linear in the distal small intestine and colon. In weanling rats, the relationship between luminal zinc concentration and net absorption and lumen to mucosa flux was curvilinear in the proximal and distal small intestinal segments and linear in the colon segments. In the adolescent rats, the relationship between luminal zinc concentration, net absorption and lumen to mucosa flux was curvilinear in all intestinal segments studied. The curvilinear relationships noted fits a Michaelis-Menten kinetics. Analysis of the kinetic data indicate a progressive rise in mean K_m values with age in the proximal segments of all age groups. Similarly, there was a rise in mean K_m values in the distal segments of the weanling and adolescent rats. These findings suggest that intestinal zinc transport is characterized by a maturational pattern which evolves with age.

Zinc is an essential trace element required for optimal growth and development (8). Studies on intestinal transport of zinc have yielding conflicting results (12, 15, 17). *In vivo* studies using ligated loops have shown the duodenum to be the site of maximal absorption (2–4). Whereas an *in vivo* recirculation study showed the ileum to be the maximum site of zinc absorption (1). *In vitro* studies using tissue bath preparations showed that the ileum absorbs zinc most rapidly (5, 13). A direct dosing technique showed no regional differences (7); furthermore, the role of the colon in zinc transport has been given little attention.

In adult rats the process of zinc transport was shown to be an active process requiring energy (10). Moreover, both mucosa to lumen and lumen to mucosa fluxes were shown to be active processes (10).

Studies on zinc transport during maturation are quite limited. Duncan and Hurley (4) demonstrated that a low molecular zinc binding ligand was present in human and rat milk which enhanced [⁶⁵Zn] absorption in preweaning rats (4).

The characteristics and the kinetics of intestinal zinc transport in the rat during maturation remains to be defined. Using *in vivo* single pass perfusion technique we studied net absorption and lumen to mucosa flux of zinc from segments of small and large intestine of suckling, weanling, and adolescent rats. Transport of zinc was measured *in vivo* from luminal solutions containing zinc at concentrations between (10–100 μ M).

MATERIALS AND METHODS

After birth, rat pups were distributed among mothers to maintain litter size at seven to nine pups until the time of study.

Animals were raised under usual laboratory conditions. Intestinal absorption was determined in suckling (14–15-day-old), weanling (21–22-day-old) and adolescent (42–45 day-old) rats. Content of zinc in the breast milk ingested by the suckling and weaning rats was approximately 9 ± 0.5 mg/liter (9). Content of zinc in the regular rat chow was 35 mg/kg. At the time of study, unfasted rats were anesthetized with intraperitoneal injection of an ethylurea phenobarbital mixture (6:1) (K and K Laboratories, Plainview, NY) at a dose of 0.2 ml/100 g body weight. After opening the abdominal cavity, 10–15 segments of proximal, distal small intestine, and the entire colon excluding the cecum were isolated, cannulated, flushed with 20 ml of warm normal saline and then with 20–30 ml of air. The proximal segment extended from the ligament of Treitz to 10–15 cm aborad. The distal segment extended from the ileocecal valve to 10–15 cm orad. The cannulated segments were then returned to the abdominal cavity. Body temperature of the rats was maintained at 36–37°C at all times during the perfusion. The cannulated segments were perfused *in situ* at a rate of 0.4 ml/min. The perfusion solution contained per liter: 154 mmole sodium chloride, 30 mg phenol red (as a nonabsorbable marker for volume changes) and zinc chloride in concentration either below (10 μ M) or above (25, 50, and 100 μ M) serum zinc level. After the start of the perfusion, a 30-min period was allowed for attainment of steady-state conditions. Perfusates were then collected during the next three consecutive 20-min periods. At the end of the perfusion periods, wet weight of the segments was determined after expressing the contents gently with forefingers. The segments were then dried in a vacuum oven at 95°C for 24 h and the dry weight was determined. Phenol red in the perfusion solutions and perfusates was determined by a spectrophotometer with a previously described method (14). Previous recovery studies of phenol red during perfusion in all segments at three age groups showed ~97–98% recovery of total phenol red perfused (19). Zinc concentration in the solutions and tissues was determined by an atomic absorption spectrometer. [⁶⁵Zn] radioactivity was determined by a gamma counter (Packard 3001 Model).

Disappearance of zinc from the lumen was taken to indicate net absorption. Disappearances of [⁶⁵Zn] from the lumen was taken to indicate the lumen to mucosa flux. The calculations are shown below:

$$\text{Net Zn absorption: } \mu\text{moles/20 min} = V_i [Zn_i - Zn_f PR_i/PR_f]$$

$$\text{Lumen to mucosa flux} = \frac{V_i}{\text{Sp. Act.}} [[^{65}\text{Zn}]_i - [^{65}\text{Zn}]_f (PR_i/PR_f)]$$

Where V is volume perfused in each segment in ml/20 min; [Zn] is total concentration of Zn as μ mole/ml; PR is phenol red concentration in μ g/mliter. [⁶⁵Zn] is radioactivity of [⁶⁵Zn] in dpm/ml; Sp. Act. is the arithmetic mean of specific activity of initial and final [⁶⁵Zn]/ μ mole of zinc. Subscript i and f refers to initial values (i) in the perfusion fluid before perfusion and in the perfusate collected (f), respectively. Absorption rates at 20-min periods did not vary more than 15% within each rat, indicating a steady state. They were added to obtain amount absorbed per h. In order to compare transport rates of zinc in segments from rats of different age groups, the quantities transported were normalized for the marked difference in the weights of the perfused segments.

Transport rates were expressed per unit dry weight to indicate "absorptive specific activity" of segment.

Statistical analysis. The unpaired student's *t* test was used for statistical comparison of differences between mean values obtained for the rats at the different ages. A *P* value (2) of less than 0.05 was considered to indicate a statistically significant difference between two mean values. For Figure 1, the straight lines were analyzed using the method of least square curves fit using the formula, $[Y = A + (Bx)]$, to determine the best linear function. For the curvilinear lines a lineweaver double reciprocal plot of the Michaelis-Menten kinetics was used to calculate K_m , J_{max} and correlation coefficient values.

RESULTS

Mean body weight and measurements of the perfused proximal, distal small intestine and colon in suckling (14–15-day-old), weanling (21–22-day-old), and adolescent rats (42–45-day-old) are shown in Table 1. At each age period, six to eight rats had all segments perfused with a solution containing tracer [^{65}Zn] and ZnCl_2 in concentrations of 10, 25, 50, and 100 μM .

Figure 1 depicts the rates of transport of zinc and its lumen to mucosa flux in the rats studied at the three age periods. Rates of transport and lumen to mucosa fluxes ($\mu\text{mole/h}$) are expressed on the basis of dry weight of the segments.

Findings in the proximal small intestine segments. The relationship between net transport rate, lumen to mucosa flux and luminal zinc concentration was curvilinear in the three age periods studied. A lineweaver double reciprocal plot of the transport data indicate that the curves at all age periods fits a Michaelis-Menten kinetics with K_m values of $(35 \pm 5 \mu\text{M})$ in the suckling rats which was significantly less than mean values in the weanling ($62 \pm 7 \mu\text{M}$) and adolescent rats ($99 \pm 7 \mu\text{M}$), respectively. J_{max} values were not significantly different between all age groups.

Findings in the distal small intestine segments. The relationship between net transport rate, lumen to mucosa flux, and luminal zinc concentration was linear in the suckling rats. The data fits a straight line when analyzed using the formula, $[Y = A + (Bx)]$, of the least square fit. The relationship between net transport rate, lumen to mucosa flux and luminal zinc concentrations was curvilinear in the weanling and adolescent rats. A lineweaver double reciprocal plot of the transport data indicate that the curves fit a

Fig. 1. The relation of rate of absorption of zinc and the initial concentration of zinc in the solution perfusing segments of the proximal and distal small intestine and the colon. Net absorption represents the rate of disappearance of zinc from the lumen during perfusion. The lumen to mucosa flux was determined from the rate of disappearance of tracer [^{65}Zn]. The symbols depict mean values and the bars the S.E. In some cases the S.E. was small and could not be shown on the graph. The data for the proximal segment in each age period fits a Michaelis-Menten kinetics. K_m values (μM) were 35 ± 5 , 62 ± 7 and 99 ± 7 , for suckling, weanling, and adolescent rats, respectively. Mean K_m values for suckling rats is significantly different from corresponding K_m values in weanling and adolescent rats ($P < 0.01$). The data for the distal segments in the suckling rats fits a straight line when analyzed using the formula ($Y = A + Bx$) of the least square fit, but, in the distal segment of the weanling and adolescent rats the relationship between net absorption, LM flux, and luminal zinc concentration showed a Michaelis-Menten kinetics with mean K_m values (μM) were 29 ± 4 and 45 ± 5 for weanling and adolescent rats, respectively. The data for the colon segments of the suckling and weanling rats showed a linear relationship using the formula ($Y = A + Bx$).

Michaelis-Menten kinetics with K_m values of $29 \pm 4 \mu\text{M}$ and $45 \pm 5 \mu\text{M}$ for the weanling and adolescent rats, respectively. The J_{max} values ($\mu\text{mole/h/g}$ dry weight) were (1.9 ± 0.1) and (2.7 ± 0.2) for weanling and adolescent rats respectively.

Findings in the colonic segments. The relationship between net transport rate, lumen to mucosa flux and luminal zinc concentration was linear in the suckling and weanling periods; however, a change to a saturable process was noted in the adolescent rats. A lineweaver double reciprocal plot of the transport data indicate that the curve fits a Michaelis-Menten kinetics with K_m of $(50 \pm 6 \mu\text{M})$ and J_{max} of $(1.2 \pm 0.1 \mu\text{mole/h/g}$ dry weight).

Table 2 depicts tissue concentration of zinc in the intestinal and liver tissues of the rats studied. Zinc concentration in the distal segment of the small bowel was similar at all age periods. But mean values in the distal segments were significantly greater than

corresponding mean values in proximal and colon segments at all age periods ($P < 0.01$). Zinc concentration in the liver of suckling rats was significantly greater than corresponding mean values in weanling and adolescent age periods ($P < 0.01$).

Table 3 depicts radioactive counts per min of [^{65}Zn] in the livers of the rats studied after 90 min of perfusion. The results are expressed as cpm per g of wet weight of tissue. These results confirm the transfer of [^{65}Zn] from the intestine to the body and provide evidence that the disappearance of zinc from perfusate does represent absorption. In suckling rats there were significantly greater counts of [^{65}Zn] per g of tissue during perfusion at all luminal zinc concentrations, compared to corresponding values in adolescent rats ($P < 0.001$).

DISCUSSION

The present study demonstrates that the proximal and distal small intestine and the colon segments of rats at all age periods studied transported zinc out of the lumen. Before this study, the process of zinc transport during maturation had not been defined. In the proximal segment of suckling rats, both net absorption and lumen to mucosa flux of zinc showed a saturable process, whereas the relationship between zinc transport and lumen concentration in the distal small intestinal segment and in the colon was linear, indicating a passive process. At the time of weaning, zinc transport in both the proximal and distal small intestinal segments showed a saturable process, whereas transport in the colon segments was by a passive process. In the adolescent rats zinc transport was by a saturable process in the small and large intestinal segments; moreover, there was progressive rise in mean apparent K_m values with age. A transport system with low K_m in the suckling period indicated a higher affinity to absorb zinc during periods of active growth. The change in K_m was evident in proximal intestine at all age groups as well as in the distal intestine of weanling and adolescent rats. The finding of a change in K_m values with age agrees with previously published data for glucose transport in the rat and rabbit.

K_m values for glucose transport in the small intestine of suckling rats was 7.2 mM compared to K_m of 16.7 mM in the postweanling rat (18). Increasing K_m was also noted by Thomson (16) for intestinal glucose transport in rabbits. He found apparent K_m of 0.5 mM at 1 wk of age and 4.0 mM in the adult. Two-week-old suckling and adolescent rabbits had k_m values which were intermediate between values of the 1-wk-old and the adult rabbits; thus, maturation in both rats and rabbits may be associated with a rise in K_m . The mechanism of the changing K_m for transport is not clear; it may be related to increased affinity for the permeant or to a lesser thickness of the unstirred water layer adjacent to the intestinal villi in the young animal as compared to the adult. The finding of a saturable transport process in all segments of the adolescent rats indicate a maturational phenomena. Duncan and Hurley (4) have reported that a zinc binding ligand in the intestinal mucosa was responsible for zinc transport. The binding ligand was absent in the first wk of life in the rat and appeared around the end of the second wk of life. It is possible that active zinc transport follows the appearance of the ligand, which also exhibits maturational characteristics along the intestinal tract. Our finding of a saturable zinc transport process in the adolescent rats agrees

Table 1. Age, body weight and measurements of the intestinal segments in the groups of rats studied¹

	Age (days)		
	14-15	21-22	42-45
Rats			
Number studied	32	30	24
Body wt (g)	29 ± 1	45 ± 1	178 ± 3
Measurement of segments			
Proximal			
Length (cm)	12 ± 0.5	10 ± 0.3	10 ± 0.4
Wet wt (g)	0.19 ± 0.01	0.34 ± 0.02	0.75 ± 0.04
Dry wt (mg)	44 ± 2	66 ± 3	150 ± 7
Distal			
Length (cm)	11 ± 0.5	12 ± 0.5	11 ± 0.5
Wet wt (g)	0.19 ± 0.01	0.3 ± 0.01	0.7 ± 0.02
Dry wt (mg)	39 ± 3	57 ± 4	136 ± 5
Colon			
Length (cm)	3 ± 0.1	6 ± 0.2	8 ± 0.2
Wet wt (g)	0.05 ± 0.002	0.26 ± 0.01	0.9 ± 0.03
Dry wt (mg)	10 ± 0.4	47 ± 2	164 ± 7

¹ Values are mean $\pm$ S.E.

Table 2. Native zinc concentration in the intestinal and liver tissues of the rats studied¹

	Age (days)		
	14-15	21-22	42-45
Intestinal tissue ²			
Proximal	0.47 ± 0.03^4	0.4 ± 0.009	0.35 ± 0.01
Distal	0.62 ± 0.01^3	0.58 ± 0.008^3	0.6 ± 0.02^3
Colon	0.47 ± 0.03^4	0.39 ± 0.009	0.33 ± 0.005
Liver tissue ²	1.2 ± 0.1^4	0.65 ± 0.05	0.6 ± 0.001

¹ Values are mean $\pm$ S.E.

² Tissue concentration of zinc is expressed as $\mu\text{mole/g}$ wet weight.

³ Mean values for zinc concentration in distal segments at all age groups are significantly different from corresponding mean values in proximal and colon segments ($P < 0.01$).

⁴ Mean values for zinc concentration in suckling rats are significantly different from corresponding mean values in adolescent rats ($P < 0.05-0.01$).

Table 3. Liver counts cpm/g wet weight at the end of perfusion period with different luminal concentration of zinc in the rats studied¹

Luminal Zn concentration	Liver counts cpm/g wet weight			
	10 μM	25 μM	50 μM	100 μM
Suckling rats	$9 \times 10^5 \pm 12 \times 10^3$	$15 \times 10^5 \pm 7.5 \times 10^2$	$28 \times 10^5 \pm 33 \times 10^3$	$34 \times 10^5 \pm 29 \times 10^3$
Weanling rats	$5 \times 10^5 \pm 4 \times 10^3$	$5 \times 10^5 \pm 3 \times 10^3$	$19 \times 10^5 \pm 9 \times 10^3$	$16 \times 10^5 \pm 9 \times 10^3$
Adolescent rats	$4 \times 10^5 \pm 3 \times 10^3^*$	$4 \times 10^5 \pm 3 \times 10^3^*$	$7 \times 10^5 \pm 6 \times 10^3^*$	$5.5 \times 10^5 \pm 5 \times 10^3^*$

¹ Values are mean $\pm$ S.E. Six (6) rats were used in each study.

* Mean values in adolescent rats are significantly different from corresponding values in suckling rats ($P < 0.01$).

with published *in vitro* studies which demonstrate an active process for zinc transport in the rat small intestine (10). Kowarski *et al.* (10) have demonstrated with everted gut sacs technique that zinc transport from the mucosa to the serosa was an active process. This active process was dependent on sodium, oxygen and a metabolizable hexose. Moreover, metabolic inhibitors as 2-4 dinitrophenol and iodoacetate markedly increased both mucosa to serosa and serosa to mucosa fluxes. More recently Davies (3) using an *in vivo* model in adult rats has shown that Zn transport was by a saturable process indicating a "carrier" mediated process.

The findings of zinc transport in the colon segment at all age groups indicates a role for the colon in zinc transport. These findings are the first to demonstrate transport of zinc from the colon in animals. The overall contribution of the colon in zinc homeostasis is not known. Because the luminal content of zinc in the normal colon may be minimal, it is reasonable to believe that the role of the colon in overall zinc homeostasis is minimal; however, under certain circumstances such as short bowel syndrome, the colon may contribute to zinc homeostasis.

To further document that disappearance of [^{65}Zn] from the lumen indicates absorption, we measured [^{65}Zn] radioactivity in the livers of the rats studied at the end of the perfusion period. The results indicate that indeed [^{65}Zn] was transferred to the liver (Table 3). In the suckling rats [^{65}Zn] counts per min per g of liver tissue was significantly greater, at all luminal concentrations of zinc, compared to [^{65}Zn] counts in adolescent rats reflecting the increased rate of [^{65}Zn] transfer in the younger rats. These findings are consistent with our previous studies on calcium (6) and magnesium (11) transport in the rat during maturation.

REFERENCES AND NOTES

1. Antonson, D. L., Barak, A. J., and Vanderhoff, J. A.: Determination of the site of zinc absorption in rat small intestine. *J. Nutr.*, **109**: 142 (1979).
2. Brown, W. B. and Hollander, M.: Statistics: a biomedical introduction. p. 90 (John Wiley & Sons, New York, 1977).
3. Davies, N. T.: Studies on the absorption of zinc by rat intestine. *Br. J. Nutr.*, **43**: 189 (1980).
4. Duncan, J. R. and Hurley, L. S.: Intestinal absorption of zinc: A role for zinc-binding ligand in milk. *Am. J. Physiol.*, **235**: 556 (1978).
5. Emes, J. H. and Arthur, D.: The site of zinc absorption in the rat small intestine. *Proc. Soc. Exp. Biol. Med.*, **148**: 86 (1975).
6. Ghishan, F. K., Jenkins, J., and Younoszai, M. K.: Maturation of calcium transport in the rat small and large intestine. *J. Nutr.*, **110**: 1622 (1980).
7. Hampton, D. L., Miller, W. J., Neatherly, M. W., Kincard, R. L., and Gentry, R. P.: Intestinal sites of zinc absorption as determined by direct ^{65}Zn dosing of intact rats. *Nutr. Rep. Int.*, **14**: 691 (1976).
8. Hurley, L. S.: The role of zinc in prenatal and neonatal development in trace elements. In: *Health and Disease*, N. Kharasch (ed). p. 167 (Raven Press, New York, 1979).
9. Keen, C. L., Lonnerdal, B., Clegg, M., and Hurley, L. S.: Developmental changes in composition of rat milk: Trace elements, minerals, protein, carbohydrate and fat. *J. Nutr.*, **111**: 226 (1981).
10. Kowarski, S., Blair-Stanek, C. S., and Schachter, D.: Active transport of zinc and identification of zinc binding protein in rat jejunal mucosa. *Am. J. Physiol.*, **226**: 401 (1974).
11. Meneely, R. L., Leeper, L., and Ghishan, F. K.: Intestinal maturation: *In vivo* magnesium transport. *Pediatr. Res.*, **16**: 295 (1982).
12. Methfessel, A. H. and Spencer, H.: Zinc metabolism in the rat: Intestinal absorption of zinc. *J. Appl. Physiol.*, **34**: 58 (1973).
13. Sahagian, B. M., Harding-Barlow, I., and Perry, H. M.: Uptakes of zinc, manganese, calcium, and mercury by intact strips of rat intestine. *J. Nutr.*, **90**: 259 (1968).
14. Schedl, H. P. and Clifton, J. A.: Small intestinal absorption of steroids. *Gastroenterology*, **41**: 491 (1961).
15. Schwarz, F. J. and Kirchgessner, M.: Experimental studies on the absorption of zinc from different parts of small intestine. *Nutr. Metab.*, **18**: 157 (1975).
16. Thomson, A. B.: Unstirred water layer and age dependent changes in rabbit jejunal d-glucose transport. *Am. J. Physiol.*, **223**: 685 (1979).
17. Van Capen, D. R. and Mitchell, E. A.: Absorption of ^{65}Cu , ^{65}Zn , ^{99}Mo and ^{59}Fe from ligated segments of the rat gastrointestinal tract. *J. Nutr.*, **86**: 120 (1965).
18. Younoszai, M. K.: Jejunal absorption of dextrose in infants and adults. *J. Pediatr.*, **85**: 446 (1974).
19. Younoszai, M. K., Saporio, R. S., and Laughlin, M.: Maturation of jejunum and ileum in rats: Water and electrolyte transport during *in vivo* perfusion of hypertonic solutions. *J. Clin. Invest.*, **62**: 271 (1978).
20. Requests for reprints should be addressed to: Dr. Fayed K. Ghishan, M.D., Department of Pediatrics, Vanderbilt Medical Center, Nashville, TN 37232.
21. This study was supported by Diabetes Training Grant #AM 20593-03.
22. Received for publication March 9, 1982.
23. Accepted for publication June 11, 1982.