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Effect of Alterations of Zinc Status on the Zinc Content of the Gastrointestinal Tract of Chicks[1] (37841)

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Although zinc deficiency has been produced in several species of experimental animal, the effect of dietary zinc status on the zinc content of the gastrointestinal tissues has received only limited attention. No significant effect of zinc deficiency on tissue zinc content was reported for chicken "small intestine" (1), chicken proventriculus (2, 3), squirrel monkey duodenum or stomach (4), pig duodenum (5) or jejunum (6, 7) and calf duodenum, jejunum (8), rumen mucosa, or stomach fundus (9). However, a higher zinc content in zinc-adequate controls when compared to zinc-deficient animals was observed for rat jejunum (but not ileum) (10) and for chick duodenum (3) (no analysis for statistical significance). In addition, significantly higher zinc contents of calf duodenum, jejunum (8), and pig jejunum (7) were observed in animals fed zinc in greater than adequate amounts when compared to their zinc-adequate controls.

Our preliminary experiments with chicks showed nearly a twofold difference in intestinal zinc content between zinc-deficient and zinc-adequate chicks. Since this observation was at variance with the majority of published studies, experiments were conducted to ascertain whether experimental variables such as fasting, acute changes in dietary zinc load, and anatomical site within the intestine could explain these discrepancies.

Materials and Methods. Two experiments were conducted with day-old White Leghorn cockerels randomly assigned to treatment groups and maintained at 37° in a stainless steel battery. Feed and distilled water were provided *ad lib.*, except as noted, from aluminum troughs. The basal diet was identical to the arginine-adequate casein diet described previously (11) except that the casein was extracted with EDTA (12) in order to lower the zinc content of the basal diet and that the supplemental iron was increased to meet the recently revised NRC requirements (13). The basal diet contained by analysis 0.65 ppm zinc on an air-dry basis.

Experiment 1 was designed to test the effect of fasting on the zinc content of plasma, duodenum, and ileum. Groups of chicks were fed *ad lib.* either the basal diet or the basal diet supplemented with 80 ppm zinc until 12 days of age. They were then fasted for 55 hr and subsequently refed *ad lib.* for another 18 hr. Distilled water was supplied *ad lib.* throughout the experimental period. Representative chicks were killed by exsanguination at the following experimental stages: the original *ad lib.*-fed state, after 24 or 55 hr fasting, and 1.5 or 18 hr after refeeding *ad lib.* Plasma was obtained from heparinized blood taken at the time of killing; duodenum and the ileum distal to the yolk stalk remnant were removed immediately, stripped of mesentery, and rinsed with deionized water. Duodenal, ileal, and plasma zinc contents were determined by atomic absorption spectrometry according to standard procedures.

In Expt 2, five dietary schedules were followed to observe the effect of acute

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changes in dietary zinc *per se* on zinc content of tibia, plasma, duodenum, and ileum in *ad lib.*-fed chicks. Three groups of chicks were maintained *ad lib.* for the entire experiment on either basal, basal plus 100 ppm zinc, or basal plus 800 ppm zinc diets. Two groups were fed the basal zinc-deficient diet *ad lib.* 12 days and then switched to either the basal plus 100 ppm or the basal plus 800 ppm zinc diet. Chicks were weighed and killed by exsanguination daily between 12 and 16 days of age in an *ad lib.*-fed state. Plasma, duodenum, ileum, and tibia were obtained and analyzed for their zinc content. Statistical analysis was by Duncan's multiple range test (14).

Results and Discussion. In Expt 1 (Table I), chicks fed basal diet were severely zinc deficient as indicated by significant ($P < 0.05$) growth retardation and depressed plasma zinc concentration. Fasting resulted in a significant ($P < 0.05$) loss in body weight over 55 hr, followed upon refeeding by a prompt recovery of body weight to the prior nonfasted level. Fasting for 24 or 55 hr resulted in a significant ($P < 0.05$) increase in plasma zinc in both deficient and zinc-supplemented controls. Plasma zinc did not further increase with additional fasting beyond 24 hr. Refeeding fasted chicks resulted in a rapid and significant ($P < 0.05$) decrease in plasma zinc to the *ad lib.* level in the zinc-supplemented controls and a somewhat more protracted decline in the zinc-deficient group.

There was no significant ($P > 0.05$) difference in the zinc content of duodenum between deficient and zinc-supplemented groups when the chicks were fed *ad lib.* In zinc-supplemented chicks, the zinc content of duodenum increased slightly but not significantly ($P > 0.05$) during the first 24 hr of fasting; fasting beyond 24 hr resulted in a further and significant ($P < 0.05$) increase in duodenal zinc content. In these chicks, the zinc content of duodenum decreased significantly ($P < 0.05$) toward nonfasted levels over 18 hr of refeeding. Similar trends of duodenal zinc content to fasting and subsequent refeeding were observed in the zinc-deficient chicks, but these changes were not significant ($P > 0.05$). Zinc content of duodena from zinc-deficient chicks were significantly ($P < 0.05$) lower than those from control chicks only after fasting for more than 24 hr. The zinc content of ileum was not affected by either dietary treatment or fasting.

In Expt 2 (Fig. 1), the zinc-deficient chicks had essentially ceased growing by 12 days of age (Fig. 1A). Feeding zinc-deficient chicks diets containing either 100 or 800 ppm supplemental zinc reestablished growth after a 1-day lag at the same rate as chicks maintained on these diets from the outset of the experiment. Supplying zinc in amounts

TABLE I. Effect of Fasting and Refeeding on Body Weight and Zinc Content of Plasma, Duodenum, and Ileum of Chicks Fed Casein-based Diets (Expt 1).

Dietary regimen[a]	Hours on dietary regimen	Dietary Treatment	Body weight (g)	Zinc concentration		
				Plasma (μg/100 ml)	Duodenum (ppm[b])	Ileum (ppm[b])
Fasting	0	Basal	55[c]D[d]	6[c]F[d]	103[c]D[d]	108[c]A[d]
	24	Basal	52 D	51 D	112 CD	106 A
	55	Basal	44 E	66 D	136 CD	118 A
Refed	1.5	Basal	52 D	50 D	121 CD	120 A
	18	Basal	56 D	27 E	116 CD	129 A
Fasting	0	Basal + 80 ppm Zn	101 A	101 BC	118 CD	128 A
	24	Basal + 80 ppm Zn	89 B	133 A	142 CD	103 A
	55	Basal + 80 ppm Zn	79 C	130 A	220 A	138 A
Refed	1.5	Basal + 80 ppm Zn	83 BC	100 C	201 AB	132 A
	18	Basal + 80 ppm Zn	103 A	117 AB	160 BC	123 A

[a] Day-old chicks were fed their respective diets for 12 days *ad lib.* prior to initiation of stated dietary regimen. During periods of fasting or *ad lib.* refeeding, chicks had free access to distilled water.

[b] Dry basis.

[c] Means of 7 to 8 animals per group.

[d] Means within a column not followed by the same letter are significantly different ($P < 0.05$).

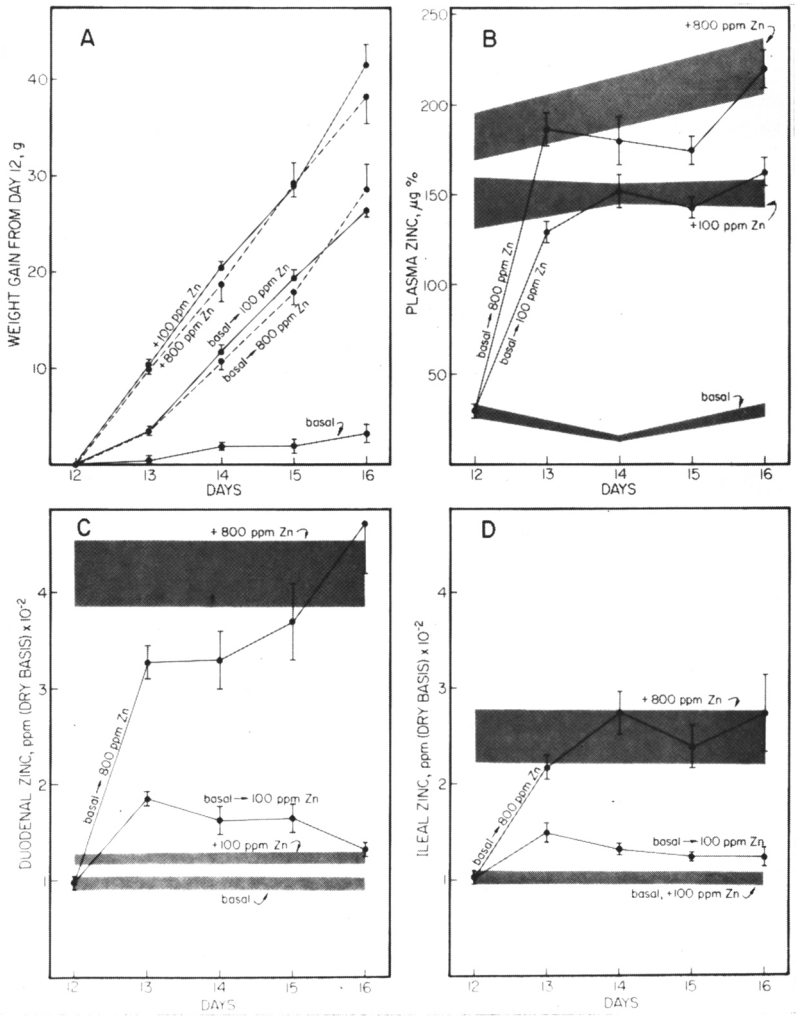

FIG. 1. Results of Expt 2. Effect of refeeding zinc-deficient chicks a diet containing either 100 or 800 ppm supplemental zinc. (A) Weight gain from Day 12. (B) Plasma zinc content. (C) Duodenal zinc content. (D) Ileal zinc content. Means are from 6–10 chicks per group. Bars represent the mean ± SEM; stippled areas are means ± SEM from chicks maintained on the specified diets ("basal", "+100 ppm Zn", or "+800 ppm Zn") from the start of the experiment. (→) indicates a change in diet on Day 12 of the experiment.

greater than 100 ppm did not further increase growth.

The resupplementation of either 100 or 800 ppm zinc to deficient chicks resulted in a prompt restoration of their low plasma zinc levels to those seen in chicks fed the respective diets from the start of the experiment (Fig. 1B). The zinc contents of duodenum (Fig. 1C) and ileum (Fig. 1D) were significantly ($P < 0.05$) higher in chicks fed 800 ppm supplemental zinc from the beginning of the experiment compared to these same tissues from zinc-deficient animals. The zinc content of duodenum was 1.7 times that of ileum in the groups supplemented with 800 ppm zinc. Feeding 800 ppm zinc to the zinc-deficient chicks resulted in a marked increase in duodenal and ileal zinc content over the subsequent 3 days (Fig. 1C, D). By comparison, 100 ppm supplemental zinc increased ($P < 0.05$) the zinc content of duodenum and ileum of zinc-

deficient chicks, but zinc concentration declined substantially over the next 4 days as growth was restored. Resupplementing zinc-deficient chicks with either 100 or 800 ppm zinc for 4 days resulted in an increase in tibial zinc content (data not shown) from 60 to 114 ppm and 198 ppm, respectively, whereas the zinc content of tibia was 172 and 373 ppm, respectively, in chicks maintained on these diets from the outset. This contrasts markedly with the rapid response to resupplementation observed for intestinal zinc content.

Our studies demonstate that the duodenum, and to a lesser extent the ileum, of the chick respond rapidly to acute increases in zinc load supplied either exogenously in the diet or endogenously during fasting. Bone is generally regarded as a good indicator of zinc status (1), but it responded slowly to changes in zinc load. In previous studies of the effect of zinc deficiency or excess on tissue zinc levels, the length of fasting has generally been disregarded and, in fact, was explicitly stated in only one of the studies cited (3). The data of the present experiments explain why zinc content of various portions of the intestine are sometimes correlated with dietary zinc intake but at other times not related. The large increase in plasma zinc that occurs with fasting may be most dramatic in experiments with rapidly growing animals in which fat stores are minimal and muscle catabolism is required for energy. The anatomical site of the intestine from which tissue is sampled is likewise important, since our experiments indicate that duodenum responds to a greater extent than ileum, especially when the zinc load is moderate as in the fasted animal.

The significance of the rise of intestinal zinc with high zinc load can only be speculated upon at this point. The zinc content of intestinal mucosa has been suggested to serve in part as a regulator of zinc homeostasis (18). Since the present studies showed no significant difference in duodenal zinc content in chicks fed *ad lib.* and those fasted 24 hr, it seems doubtful that the high zinc content is indicative of zinc in the process of absorption. In addition, large alterations in intestinal zinc content in the *ad lib.*-fed chicks occurred only when the dietary zinc was offered in amounts greatly in excess of the requirement. Severe zinc deficiency was not accompanied by a significant depression of intestinal zinc content in *ad lib.*-fed chicks compared to chicks fed diets containing zinc in amounts modestly above the requirement. Although zinc deficiency may lower the zinc content in some small compartment critical to the regulation of zinc absorption, it is unlikely that the difference in intestinal zinc content in our experiments is a reflection of zinc in this compartment. In view of the significant portion of zinc which is excreted in the duodenal secretions (15, 16), presumably by the Paneth cells (17), it is more likely that the high zinc content of duodenum during conditions of mandatory zinc excretion does in fact represent zinc in the process of excretion.

Summary. The zinc content of intestinal tissue of growing chicks depends upon the anatomical site of sampling, the dietary intake of zinc, and the extent of fasting. The zinc content of duodenum, but not ileum, increased after 24 hr fasting in both zinc-deficient and zinc-adequate chicks and rapidly decreased to prefasting levels after refeeding. Feeding diets either adequate or greater than adequate in zinc to zinc-deficient chicks resulted in a rapid increase in plasma, duodenal, and ileal zinc contents to levels found in animals maintained continuously on these diets. The zinc content of tibia rose more slowly over this same time period. Thus, duodenal zinc content accurately reflects acute increases in "available" body zinc and most likely represents zinc being excreted endogenously from the body tissues.

1. Savage, J. E., Yohe, J. M., Pickett, E. E., and O'Dell, B. L., Poult. Sci. 43, 420 (1964).

2. Kienholz, E. W., Sunde, M. L., and Hoekstra, W. G., Poult. Sci. 43, 667 (1964).

3. Zeigler, T. R., Leach, R. M., Jr., Scott, M. L., Huegin, F., McEvoy, R. K., and Strain, W. H., J. Nutr. 82, 489 (1964).

4. Macapinlac, M. P., Barney, G. H., Pearson, W. N., and Darby, W. J., J. Nutr. 93, 499 (1967).

5. Lin, C. W., Ph.D. Thesis, Univ. Wisconsin (1969).

6. Hoekstra, W. G., Lewis, P. K., Jr., Phillips,

P. H., and Grummer, R. H., J. Anim. Sci. **15**, 752 (1956).

7. Lewis, P. K., Jr., Hoekstra, W. G., and Grummer, R. H., J. Anim. Sci. **16**, 578 (1957).

8. Miller, W. J., Wells, E. S., Gentry, R. P., and Neathery, M. W., J. Nutr. **101**, 1673 (1971).

9. Miller, J. K., and Miller, W. J., J. Nutr. **76**, 467 (1962).

10. Reingold, J. G., Kfoury, G. A., and Thomas, T. A., J. Nutr. **92**, 173 (1967).

11. Coleman, B. W., Reimann, E. M., Grummer, R. H., Sunde, M. L., and Hoekstra, W. G., J. Nutr. **101**, 1695 (1971).

12. Davis, P. N., Norris, F. H., and Kratzer, F. H., J. Nutr. **78**, 445 (1962).

13. "Nutrient Requirements of Poultry, Sixth Revised Edition," National Academy of Sciences, Washington, D. C. (1971).

14. Steele, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics," p. 114, McGraw-Hill Book Co., New York (1960).

15. Montgomery, M. L., Sheline, G. E., and Chaidoff, I. L., J. Exp. Med. **78**, 151 (1943).

16. Pekas, J. C., Amer. J. Physiol. **211**, 407 (1966).

17. Millar, M. J., Vincent, N. R., and Mawson, C. A., J. Histochem. Cytochem. **9**, 111 (1961).

18. Evans, G. W., Grace, C. I., and Hahn, C., Proc. Soc. Exp. Biol. Med. **143**, 723 (1973).

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