Early Tissue ^{65}Zn Distribution After Duodenal Dosing in Calves Fed Zinc-Deficient and Control Diets[1,2] (35114)

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Tissue distribution of ^{65}Zn from 2 to 70 days after a single oral dose has been studied in ruminants fed normal and zinc-deficient diets (1–6). However, little is known concerning the effects of a zinc-deficient diet on the early accumulation and distribution of zinc in body tissues following absorption. In ruminants, orally administered ^{65}Zn is dispersed in ruminal contents with a variable time required for passage to the small intestine, the primary site of zinc absorption (7–9). Thus, oral dosing obscures accurate definition of early ^{65}Zn accumulation by ruminant body tissues. Intravenously injected ^{65}Zn has been employed for studying early distribution in rats (10, 11), but injected zinc (6, 12) is metabolized differently from that absorbed from the gastrointestinal tract.

The objective of this study was to determine the effect of a zinc deficient diet on the early accumulation of ^{65}Zn in various body tissues of calves following absorption of a duodenally administered ^{65}Zn dose.

Materials and Methods. Sixteen Holstein bull calves, averaging 118 kg and 162 days of age, were fed either a zinc-deficient (5 ppm zinc) or a control diet which contained 32 ppm of supplemental zinc as ZnO. The diets were fed *ad libitum* for 1 week and then at 3% of body weight for 1 week prior to dosing with 518.0 to 695.5 μCi of ^{65}Zn via a duodenal catheter. The rations and dosing procedure have been previously described (13).

Heparinized blood samples were taken at 0.5 and 1 hr after dosing in all calves and at 4, 8, and 24 hr from calves which had not been sacrificed. At 1, 4, 8, and 24 hr following ^{65}Zn dosing, 2 calves from each diet were injected with sodium pentabarbital and killed by cannulation of the carotid artery. Tissues were immediately removed and frozen for analyses. Tissues, sampled as previously described (2), included; liver, kidney, heart, lung, spleen, muscle (semitendinosus or round), pancreas, rumen, abomasum, small intestine, cecum, and colon. The small intestine (SI) was divided into 7 segments: SI 1, first 1.8 m; SI 2, second 1.8 m; SI 7, last 1.8 m; and SI 3, 4, 5, and 6, anterior to posterior end, respectively, divided into equal length segments of approximately 2.4 m each. After removal of digesta, each intestinal section was washed out twice with 0.9% saline.

The ^{65}Zn activity was determined with an automatic gamma test tube changer with a NaI (TI) well crystal. Tissue ^{65}Zn data were calculated as a percentage of administered dose/kg of fresh tissue. These values were adjusted to the mean body size for all the animals which has the effect of calculating the data to a uniform dosing level per kg of body weight (1).

Results. The gastrointestinal sections of deficient-fed calves accumulated ^{65}Zn more rapidly than controls (Table I). The first few small intestinal sections contained a high concentration of ^{65}Zn 1 hr after dosing, with far larger amounts in calves fed the deficient diet. At 4 hr, ^{65}Zn content in these tissue sections had decreased considerably and the decline continued through the 24-hr period. With both dietary treatments the ^{65}Zn concentrations of the lower small intestine, cecum, and large intestine peaked 4 or 8 hr

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TABLE I. Effect of Dietary Zinc Level on ^{65}Zn in Calf Gastrointestinal Sections with Time After Duodenal Dosing.[a]

	% of ^{65}Zn dose/kg of fresh tissue							
	Control diet (37 ppm Zn)				Zinc-deficient diet (5 ppm Zn)			
Tissue (hr):	1	4	8	24	1	4	8	24
Rumen	0.11	0.20	0.51	0.57	0.08	0.55	0.67	1.20
Abomasum (fundic)	0.24	0.32	1.07	0.53	0.49	1.57	1.26	1.33
(pyloric)	0.15	0.23	0.42	0.43	0.23	0.57	0.59	1.11
Small intestine 1	5.87	2.63	3.57	1.36	37.09	11.25	14.17	3.81
2	8.10	1.49	2.63	1.02	24.49	8.06	8.02	3.78
3	3.22	3.36	2.45	0.93	16.53	5.05	3.50	2.55
4	2.12	2.57	1.98	1.09	5.65	2.91	3.27	2.19
5	2.88	1.99	1.79	1.01	2.64	3.27	3.06	2.18
6	0.85	2.33	1.64	0.90	0.37	3.01	4.39	2.35
7	0.26	1.55	1.69	0.94	0.32	4.08	2.70	1.98
Cecum	0.14	0.98	3.18	0.55	0.13	0.83	2.94	1.44
Colon	0.17	2.14	1.18	0.53	0.15	1.09	0.99	1.21

[a] Each value represents an average of 2 animals.

after dosing. Rumen and abomasum tissue had peak ^{65}Zn levels at 8 hr after dosing in control calves, but continued to accumulate ^{65}Zn through 24 hr in deficient-fed calves. ^{65}Zn concentrations in these tissues were considerably higher in deficient-fed calves than in controls.

In control animals, ^{65}Zn accumulated in liver very rapidly after dosing, with concentrations in pancreas, kidney, spleen, and lung being progressively lower (Table II). ^{65}Zn levels peaked in these tissues of control calves 8 hr after dosing and were noticeably reduced at 24 hr. Tissues of deficient-fed calves accumulated more ^{65}Zn than controls. The relative ^{65}Zn concentrations in liver, kid-

TABLE II. Effect of Dietary Zinc Level on ^{65}Zn in Calf Tissues with Time After Duodenal Dosing.[a]

	Control diet (37 ppm Zn)					Zinc-deficient diet (5 ppm Zn)					
Tissue (hr):	0.5	1	4	8	24	0.5	1	4	8	24	SE[b]
Absorbed ^{65}Zn (%)[c]		30.1	58.0	66.9	65.9		50.0	73.4	79.6	86.9	6.01
	% of ^{65}Zn dose/kg of fresh tissue										
Liver		3.38	3.36	5.71	2.59		4.07	6.66	6.17	6.99	1.08
Pancreas		1.51	2.10	4.90	1.97		1.09	3.57	4.25	3.69	1.17
Kidney		1.13	1.37	2.65	1.53		1.61	5.02	4.46	4.99	0.69
Spleen		0.73	1.23	2.04	1.50		1.36	3.75	3.80	4.65	0.56
Lung		0.32	0.47	0.96	0.72		0.67	1.28	1.54	1.78	0.22
Heart		0.18	0.28	0.67	0.77		0.15	0.66	0.70	1.35	0.13
Muscle		0.02	0.04	0.10	0.15		0.01	0.07	0.06	0.16	0.02
Blood	0.16	0.19	0.16	0.13	0.09	0.23	0.17	0.16	0.12	0.13	0.06

[a] Each value represents an average of 2 animals, except blood which was 8, 8, 6, 4, and 2 calves for 0.5, 1, 4, 8, and 24 hr, respectively.

[b] Standard error; $n = 2$.

[c] Absorbed ^{65}Zn is defined as that which was not recovered in the intestinal contents and feces.

TABLE III. Effect of Dietary Zinc Level on the Stable Zinc Concentration of Various Body Tissues of Calves.[a,b]

Tissue	Zinc in fresh tissue (ppm)		
	Control diet (37 ppm Zn)	Deficient diet (5 ppm Zn)	SE[c]
Liver	31.8	26.2[d]	1.6
Kidney	16.6	16.4	0.6
Lung	17.5	17.8	0.7
Heart	16.8	17.0	0.4
Blood	2.6	2.2[d]	0.1
Sm. intestine 1[e]	16.4	15.8	0.6
7[e]	17.3	15.4[d]	0.5

[a] Each value represents an average of 8 animals, with the exception of small intestine 1 and 7 which were 5 and 7 calves for control and deficient treatments, respectively.

[b] Calves were fed the respective diets for 2 weeks prior to tissue collection.

[c] Standard error; $n = 8$, with the exception of small intestine 1 and 7 where $n = 6$.

[d] Significantly different from control, $p < 0.05$.

[e] Average dry matter contents of small intestine 1 and 7 were 16.7 and 15.6 for control; 17.1 and 15.3 for deficient treatment.

ney, spleen, and lung of deficient-fed calves followed the same order as in controls. However, in deficient-fed animals, relative to the other tissues, the pancreas accumulated less ^{65}Zn. In contrast to control animals, ^{65}Zn levels in liver, kidney, spleen, and lung of deficient-fed calves were either as high or higher at 24 hr than at earlier times. With both dietary treatments heart and muscle had very low ^{65}Zn levels at 1 hr but continued to accumulate the isotope through 24 hr.

Blood ^{65}Zn, peaked sharply at 0.5 or 1 hr after duodenal dosing (Table II). There was little difference in blood ^{65}Zn values between control and deficient-fed calves.

The stable zinc concentrations of several tissues are presented in Table III. After 2 weeks on diets, the liver, whole blood, and small intestine 7 of deficient-fed calves contained significantly less ($p < 0.05$) zinc than those from controls. Small intestine 1 had a lower, but nonsignificant, stable zinc concentration in calves fed the deficient diet, while kidney, lung, and heart were unaffected by dietary zinc level.

Discussion. Feeding a zinc-deficient diet to calves for 2 weeks had a pronounced effect on the early tissue accumulation of ^{65}Zn absorbed from the intestinal tract. The early peak ^{65}Zn concentration, as a percentage of the administered dose, in liver, pancreas, kidney, spleen, and lung of control animals suggest that with adequate dietary zinc, these softer tissues contain some labile zinc. A portion of this labile zinc subsequently is deposited in other tissues such as muscle. The continued ^{65}Zn retention by the softer tissues indicate that this labile zinc moiety was at least partially depleted in calves after 2 weeks on a zinc-deficient diet. In contrast to the softer tissues, heart and muscle have a slower and more consistent zinc turnover rate, which is not greatly affected by the zinc status of the body.

The 18% reduction in the stable zinc concentration of liver after 2 weeks on a zinc-deficient diet shows that the labile fraction was substantial. At least 4 different zinc pools have been identified in Japanese quail liver by a dialysis procedure with two of them greatly reduced by a low-zinc ration (14). In this study, failure of the zinc-deficient diet to reduce the kidney and lung zinc content indicates that the labile zinc fraction in these tissues is quite limited (Table III).

The results show that following absorption, ^{65}Zn accumulates rapidly in several body tissues but subsequently decreases. In contrast, following intramuscular injections in chicks ^{65}Zn continued to increase in all tissues through 32 hr (15). This continued ^{65}Zn accumulation may have been due to the slow mobilization of the injected ^{65}Zn from muscular tissue. While some differences exist, in general the order in which the different body tissues accumulate ^{65}Zn in both the chick and rat studies (10, 11, 15) are in agreement with those reported herein.

The early sharp peak and subsequent decline of blood ^{65}Zn reflect the rapid rate of zinc removal by other tissues from the blood following absorption. After intravenous injections, ^{65}Zn was rapidly removed from the blood of rabbits (16) and calves (6).

The low ^{65}Zn content of the lower small

intestine, cecum, and colon at 1 hr, relative to the upper small intestine, can be explained by most of the ^{65}Zn dose not having moved into the lumen of these segments. However, ^{65}Zn was never concentrated in these lower intestinal sections perhaps partially due to much of the dose being absorbed in the upper small intestine.

Presumably, the large quantities of ^{65}Zn contained in the first 4 sections of the small intestine 1 hr after duodenal dosing were concentrated in the mucosa. The rapid build-up and subsequent moderate removal rate of ^{65}Zn from these sections confirms the proposal that zinc transfer from the intestinal mucosa to the blood, rather than uptake from the digesta, is the rate-limiting step in zinc absorption (17). The early ^{65}Zn accumulation by blood and tissues following duodenal dosing indicates that considerable transmural passage also relatively rapidly occurs. Therefore, the early slow movement of ^{65}Zn from the intestinal lumen to the bathing fluid observed in *in vitro* perfusion studies (17), does not hold in the live animal. Even after 2 weeks on a zinc-deficient diet, transfer of ^{65}Zn to body tissues of calves was only moderately enhanced compared to a very large increase in ^{65}Zn uptake by intestinal tissue.

Summary. Following duodenal dosing, ^{65}Zn was rapidly accumulated by several calf tissues, with a considerable amount in the liver and other tissues within 1 hr. With adequate dietary zinc, liver contained a substantial quantity of labile zinc which was greatly depleted in calves fed a zinc-deficient diet for 2 weeks. Other soft tissues had a very limited amount of labile zinc. Zinc turnover rate was much slower in muscle than in the softer tissues, and was not greatly affected by dietary zinc level. Transfer of zinc from the intestinal mucosa to the blood appeared to be the rate-limiting step in zinc absorption. This step was accelerated by feeding a zinc-deficient diet for 2 weeks. However, ^{65}Zn uptake by the mucosa from the intestinal contents was vastly increased by the zinc-deficient diet.

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