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Absorption, Excretion and Tissue Distribution of Natural Organic and Inorganic Zinc-65 in the Rat (38731)

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Events in recent years have established that the essential element, zinc, has considerable practical importance. Although there has been appreciable research on metabolism of zinc in the inorganic form, information on metabolism of zinc in the natural organic form of feeds is quite sparce. When fed with many plant proteins, zinc has a low availability to monogastric animals including rats, swine and poultry (1–3). This low availability which has been partially associated with phytate does not occur in ruminants (4). Forage (organic) ^{65}Zn and inorganic ^{65}Zn were equally well absorbed by calves; however, concentration in the most biologically active tissues was considerably higher in calves receiving the organic form (5). The objective of this study was to compare absorption, tissue distribution and excretion in the rat of ^{65}Zn grown into natural plant material (young corn and rye forage) with that from an inorganic form (as $^{65}ZnCl_2$).

Materials and Methods. In separate experiments, tracer amounts (about 1 g) of corn forage (experiment I) and rye forage (experiment II) containing ^{65}Zn were used for rat metabolism studies in comparison with $^{65}ZnCl_2$ mixed with comparable forage and and tissue paper (5). The ^{65}Zn containing forages were grown as previously described (5) with the corn harvested at 19–33 days after planting and the rye at 23 days. The inorganic forms were prepared by applying the ^{65}Zn solution to finely ground forage or tissue paper which was then dried and reground through a micromill.

The experimental ^{65}Zn-containing materials (about 1 g) were mixed with 9 g of the zinc-deficient purified diet to form the dosing material which was counted for ^{65}Zn[1] before being offered to each rat for 1 hr. Refusal ^{65}Zn also was measured.

In experiment I, 24 Carworth albino male rats initially weighing 155 g were housed individually in stainless steel cages, given redistilled water, and fed the zinc-deficient purified diet (5 ppm Zn) *ad libitum* for 7 days before dosing. This diet was the same as published previously (5) except that a B-complex vitamin premix was added. In experiment II, 18 Cherokee S-D albino male rats initially weighing 46 g were similarly fed and managed. After dosing, total feces were collected (for ^{65}Zn analysis) for 6 days (7 days for experiment II) at which time the rats were sacrificed and tissues taken for ^{65}Zn analyses.[1]

Results. Rats receiving ^{65}Zn grown into corn forage (organic form) excreted significantly ($P < 0.05$) less ^{65}Zn in feces than rats dosed with ^{65}Zn mixed with corn forage or tissue paper (inorganic forms) (Fig. 1A). The 6-day net absorption (retention) values (determined by difference from fecal excretion data) were 73.1, 65.2 and 64.1% of the dose (Fig. 1A). Similarly, the net ^{65}Zn absorption from that grown into rye forage averaged 13% higher than that mixed with rye forage and 20% higher than that mixed with tissue paper (Fig. 1B), the values being 78.4, 69.1 and 65.1% of the dose, respectively. In both experiments most of the treatment difference in fecal ^{65}Zn excretion occurred on day 1.

The average ^{65}Zn concentration in 12 tissues of rats dosed with ^{65}Zn grown into the corn forage was significantly higher ($P < 0.01$) than in those of rats dosed with ^{65}Zn mixed with corn forage or paper (Table I). Treatment differences were significant for 7 of the 12 tissues. Average ^{65}Zn in tissues of rats given ^{65}Zn mixed with forage was higher ($P < 0.01$) than in those given ^{65}Zn mixed with paper (Table I). The relative order of tissue ^{65}Zn concentrations was similar among the treatments with tibia having the

[1] The ^{65}Zn was determined with an automatic gamma ray test tube changer system with a 7.6 cm NaI (T1) well-type crystal. Model 709, manufactured by Baird Atomic, Inc., Cambridge, MA.

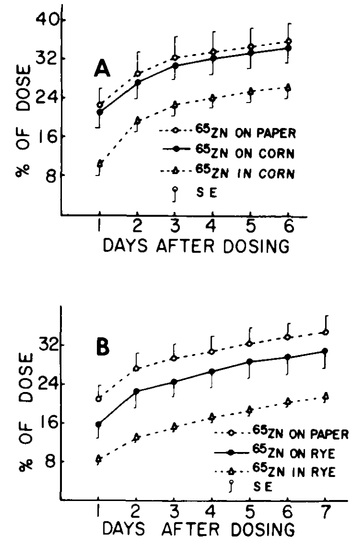

FIG. 1. Effect of chemical form of zinc on accumulated total fecal ^{65}Zn excretion following a single oral dose. A. Experiment I. ^{65}Zn grown into young corn plants (organic); and mixed with corn forage (inorganic) and tissue paper (inorganic). SE = standard error of mean (eight rats/treatment). B. Experiment II. ^{65}Zn grown into young rye plants (organic) and mixed with rye forage or tissue paper. SE with five, six and four rats per treatment, respectively.

highest level, followed by spleen, liver, lung, kidneys, small intestine, testes, heart, round muscle, skin, and blood.

Average ^{65}Zn in 12 tissues of rats dosed with ^{65}Zn grown into rye forage or mixed with rye forage was significantly higher ($P < 0.01$) than in those given ^{65}Zn mixed with paper (Table II). Every tissue from rats dosed with ^{65}Zn mixed with paper had lower ^{65}Zn than from the other two treatments (Table II). The relative order of tissue ^{65}Zn concentrations was similar to that in experiment I.

Discussion. In these studies, both the fecal excretion and tissue data indicate that ^{65}Zn grown into young corn and rye plants (organic form) was more readily absorbed and retained by rats than ^{65}Zn mixed with tissue paper. However, expecially the tissue data indicate that a part of this beneficial effect occurred when ^{65}Zn was mixed with the forage. Since only a tracer amount of forage (1 g) was used, the treatment effect must be ascribed to differences in the chemical combinations of the zinc and not to dietary effects. These forages were very young and highly digestible. Thus the same effect would not necessarily prevail with mature forages. Apparently zinc in young growing plants exists mostly as protein complexes having varying degrees of binding tenacity (6–8), with a small part reported occurring in cellulose and hemicellulose (8). Other research has shown that zinc may be absorbed as a zinc-ligand complex which may be further transported and metabolized as a unit (9). The zinc may be transferred to other (ligand) complexes during absorption, transport and metabolism (9). Whether the beneficial effects of the forage combination were due to the zinc per se or to a zinc ligand complex being absorbed in larger amounts is uncertain.

There appears to be an animal species difference in the metabolism of zinc in different chemical forms. In a previous experiment (5) in which a portion of this same radioactive corn forage was placed in the rumen of calves, there were no differences in ^{65}Zn absorption between calves dosed with ^{65}Zn grown into corn and ^{65}Zn mixed with paper; however, calves receiving the organic form had more ^{65}Zn in the most biologically active tissues.

In this and the previous study with calves (5), a zinc-deficient diet was used resulting in higher ^{65}Zn absorption and retention than would occur with a practical type diet (9). This approach makes "availability" of zinc the limiting factor rather than possibly being greatly reduced by homeostatic control factors (10). The threefold differences in ^{65}Zn tissue concentrations observed in experiment II over the ones in experiment I were related to the size of the rats involved.

Summary. The absorption and tissue distribution of organic and inorganic forms of ^{65}Zn were studied in the rat following single tracer oral doses. Rats dosed with ^{65}Zn grown into young corn and rye plants (organic form) absorbed and retained more ^{65}Zn in tissues than those given ^{65}Zn mixed with tissue paper (inorganic forms). A part of the beneficial effect was evident when ^{65}Zn was mixed with forage. The relatively high absorption values indicate that both forms were readily available, and thus in-

TABLE I. Effect of Chemical Form on ^{65}Zn Distribution in Tissues of Rats Fed a Zinc-Deficient Diet Six Days following a Single Oral Tracer Dose (Experiment I).

Tissues	Chemical form[a]		
	^{65}Zn grown into corn SE[b]	^{65}Zn mixed with corn SE[b]	^{65}Zn mixed with paper SE[b]
		— of dose/g fresh tissue	
Tibia	1.28[c] ± 0.09	1.05[d] ± 0.05	0.91[d] ± 0.07
Spleen	0.86[e] ± 0.05	0.69[f] ± 0.03	0.62[f] ± 0.06
Liver	0.82 ± 0.04	0.84 ± 0.06	0.71 ± 0.06
Lung	0.73[c] ± 0.05	0.63[cd] ± 0.04	0.57[d] ± 0.06
Kidney[h]	0.71[c] ± 0.04	0.66[cd] ± 0.03	0.57[d] ± 0.04
Sm. Int. 2[i]	0.56[e] ± 0.06	0.56[e] ± 0.05	0.45[f] ± 0.05
Sm. Int. 1[i]	0.54[e] ± 0.04	0.55[e] ± 0.03	0.47[f] ± 0.04
Testis[j]	0.54[c] ± 0.03	0.47[cd] ± 0.03	0.42[d] ± 0.03
Heart	0.52 ± 0.04	0.46 ± 0.02	0.43 ± 0.04
Muscle, round	0.27 ± 0.02	0.26 ± 0.01	0.22 ± 0.02
Skin	0.19 ± 0.02	0.17 ± 0.02	0.15 ± 0.008
Blood	0.16 ± 0.01	0.15 ± 0.02	0.13 ± 0.005
Av of above tissues	0.60[e]	0.54[f]	0.47[g]
SE[k]	± 0.033	± 0.027	± 0.026

[a] Chemical forms were: ^{65}Zn grown into young corn plants (organic); ^{65}Zn mixed with corn forage (inorganic); ^{65}Zn mixed with tissue paper (inorganic).

[b] Standard error of mean (8 rats/treatment).

[c, d, e, f, g] Treatment means on the same line having different superscript letters are significantly different: c, d = $P < 0.05$; e, f, g = $P < 0.01$.

[h] Both kidneys.

[i] Small intestine 1, first 7.5 cm from pylorus; small intestine 2, second 7.5 cm from pylorus.

[j] Both testes.

[k] Standard error of mean (96 observations/treatment).

TABLE II. Effect of Chemical Form on ^{65}Zn Distribution in Tissues of Rats Fed a Zinc-Deficient Diet Seven Days Following a Single Oral Tracer Dose (Experiment II).

Tissues	Chemical forms[a]		
	^{65}Zn grown into rye SE[b]	^{65}Zn mixed with rye SE[b]	^{65}Zn mixed with paper SE[b]
		% of dose/g fresh tissue	
Tibia	4.90[c] ± 0.86	4.68[c] ± 0.49	3.05[d] ± 0.55
Spleen	2.33 ± 0.19	2.39 ± 0.21	1.96 ± 0.23
Liver	2.06 ± 0.20	2.69 ± 0.69	1.66 ± 0.10
Lung	2.18[c] ± 0.20	2.03[c] ± 0.12	1.72[d] ± 0.15
Kidney[i]	2.02[c] ± 0.18	2.02[c] ± 0.13	1.55[d] ± 0.10
Sm. Int. 2[j]	1.77[e] ± 0.12	1.77[e] ± 0.13	1.25[f] ± 0.10
Sm. Int. 1[j]	1.74[c] ± 0.19	1.63[cd] ± 0.13	1.31[d] ± 0.09
Testis[k]	1.42 ± 0.22	1.33 ± 0.16	1.07 ± 0.15
Heart	1.76[ef] ± 0.20	1.72[e] ± 0.40	1.35[f] ± 0.10
Muscle, round	0.98 ± 0.13	1.07 ± 0.13	0.79 ± 0.13
Skin	0.59[ef] ± 0.10	0.69[e] ± 0.07	0.47[f] ± 0.05
Blood	0.40 ± 0.03	0.41 ± 0.04	0.33 ± 0.02
Av of above tissues	1.85[g]	1.87[g]	1.39[h]
SE[l]	± 0.16	± 0.14	± 0.11

[a] Chemical forms were: ^{65}Zn grown into young rye plants (organic); ^{65}Zn mixed with rye (inorganic); ^{65}Zn mixed with tissue paper (inorganic).

[b] Standard error of mean (see footnote, Fig. 1B).

[c, d, e, f, g, h] Treatment means on the same line having different superscript letters are significantly different: c, d = $P < 0.10$; e, f = $P < 0.05$; g, h = $P < 0.01$.

[i] Both kidneys.

[j] Small intestine 1, first 7.5 cm from pylorus; small intestine 2, second 7.5 cm from pylorus.

[k] Both testes.

[l] Standard error of mean (with 60, 72 and 48 observations per treatment, respectively.

corporating zinc into young forage protein is not of major practical importance.

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