Urinary Excretion of Radiosulfur Following Taurine-^{35}S Injection in Zinc Deficient Rats (36033)

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The oxidation of sulfur in cystine to inorganic sulfate and taurine is well established (1). Previously (2), we reported that the excretion of total ^{35}S, inorganic sulfate-^{35}S, and taurine-^{35}S in urine of zinc-deficient rats was several times higher than control rats following injected cystine-^{35}S. These findings suggest that an increased catabolism of cystine exists in zinc-deficient rats. Since inorganic sulfate can be derived from taurine (3–6), it was of interest to determine whether the increased urinary excretion of inorganic sulfate-^{35}S was related to the increase of taurine degradation. To test this possibility, we studied the effect of dietary zinc deficiency on taurine-^{35}S metabolism in rats.

Experimental and Methods. Animals and diets. Commercially purchased male rats (40 to 50 g; 3 weeks old); of the Sprague-Dawley strain were divided into two groups. Group 1 was kept on a basal diet, a zinc-deficient diet as described previously (2). The zinc content of the diet was 2 mg of zinc/kg diet. Group 2, used as control rats, were pair-fed to the rats in Group 1 and were given the same basal diet supplemented with 80 mg of zinc in the form of zinc carbonate per kilogram of zinc-deficient diet. All rats were housed individually in stainless steel cages. Deionized water was offered *ad libitum* in all experiments.

Injection of ^{35}Sulfur Labeled Taurine.[2] Each rat was injected intramuscularly with 5 μCi/100 g of body weight after a fasting period of 16 hr and was transferred to individual stainless steel metabolism cages for fecal and urinary collection. A total of 24 rats (12 deficient and 12 zinc supplemented) were divided into two groups. Group A: 12 animals were sacrificed 24 hr after taurine-^{35}S injection; urine was collected and made up to volume. Group B: The remaining 12 rats were sacrificed 6 days following isotope injection; urine collection was made for each 24 hr period; fecal collections were pooled from days 1, 2, and 3, and again collections were pooled from days 4, 5, and 6; feces were dried over P_2O_5 until later analysis. During period of urine and fecal collection, the dietary regime remained the same.

Chemical analysis. Inorganic sulfate was assayed by precipitation with benzidine hydrochloride, to give benzidine sulfate, and determined colorimetrically, according to method of Kahn and Leiboff (7). Radioactive inorganic sulfate was determined by dissolving benzidine sulfate in 0.5 ml of Hyamine.[3]

Taurine was isolated and determined by the method of Pentz *et al.* (8). Taurine-^{35}S was identified by descending paper chromotography after being passed through Dowex 50, 8% cross-link, H^+ form ion exchange resin. Eighty percent aqueous phenol was used as moving solvent (9).

The dried feces were extracted with 1:1 methanol–chloroform in Soxhlet glass apparatus for 60 hr (10). Samples of lipid extract were counted for total ^{35}S.

Radiochemical measurements. All specimens were measured by dissolving aliquots and counting them in scintillation fluid (11). Corrections for quenching and decay were made.

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[2] Obtained from Amersham/Searle Corporation, Arlington Heights, IL. (16.1 mCi/mmole).

[3] Obtained from the Packard Instrument Company, Incorporated, Downers Grove, IL.

TABLE I. Twenty-Four Hour Urinary Excretion of ^{35}S, $^{35}SO_4^{2-}$, and Taurine-^{35}S Following Taurine-^{35}S Injection of Zinc-Deficient and Zinc-Supplemented Pair-Fed Rats.

Type of diet	No. of rats	% Injected radioactivity		
		^{35}S	$^{35}SO_4^{2-}$	Taurine-^{35}S
Zinc-deficient	6	20.6 ± 5.2[a]	2.50 ± 0.90	2.52 ± 0.80
Zinc-supplemented pair-fed rats	6	4.5 ± 1.7	1.28 ± 0.41	0.66 ± 0.37
Statistical comparison of values		$p < .005$	$p < .02$	$p < .005$

[a] Mean ± SD.

TABLE II. Twenty-Four Hour Urinary Excretion of Total ^{35}S For 6 Days, Following Taurine-^{35}S Injection in Zinc-Deficient and Zinc-Supplemented Pair-Fed Rats.

Days	% Injected radioactivity		Statistical comparison of values (p)
	ZnD[a]	ZnS[b]	
1	12.8 ± 4.8[c]	4.28 ± 2.56	< .005
2	3.91 ± 1.99	1.13 ± 0.85	< .01
3	4.97 ± 1.72	2.23 ± 1.04	< .05
4	1.85 ± 0.43	1.06 ± 0.40	< .01
5	2.09 ± 0.87	1.04 ± 1.00	ns
6	4.16 ± 0.97	1.91 ± 1.80	< .025

[a] ZnD = 6 zinc-deficient rats.
[b] ZnS = 6 zinc-supplemented rats.
[c] Mean ± SD.

Comparisons of differences in means were tested for significance by the t test (12).

Results. Urinary excretion of total ^{35}S, inorganic sulfate-^{35}S and taurine-^{35}S, 24 hr after injection of taurine-^{35}S are given in Table I. The total ^{35}S, total inorganic sulfate-^{35}S and taurine-^{35}S for the zinc-deficient rats were two times or more than the values obtained from control rats.

Using a new group of rats, Table II shows daily urinary excretion of total ^{35}S over a 6 day period. With the exception of day 5, the ^{35}S recovered from the zinc-deficient rats was significantly higher than controls.

Table III illustrates daily urinary excretion of inorganic sulfate and inorganic sulfate-^{35}S over the 6 day period. A significant increase in the concentration of inorganic sulfate was observed for days 1, 3, and 6. Significant increased inorganic sulfate-^{35}S were also noted during days 1 and 3. No significant differences in the specific activity expressed as percentage

TABLE III. Twenty-Four Hour Urinary Excretion For 6 Days of Total Inorganic Sulfate and Inorganic Sulfate-^{35}S After Taurine-^{35}S Injection in Zinc-Deficient and Zinc-Supplemented Pair-Red Rats.

Days	Total inorganic sulfate excreted (mg)		Total inorganic sulfate-^{35}S excreted (% IR[a])		Sp act (% IR/mg)	
	ZnD[b]	ZnS[c]	ZnD	ZnS	ZnD	ZnS
1	8.3 ± 2.2[d]	3.0 ± 0.6[e]	0.80 ± 0.49	0.21 ± 0.11[f]	0.104 ± 0.044	0.089 ± 0.026
2	7.9 ± 2.5	6.1 ± 2.0	0.82 ± 0.59	0.37 ± 0.25	0.080 ± 0.042	0.060 ± 0.027
3	11.2 ± 4.4	5.3 ± 2.5[f]	1.06 ± 0.56	0.28 ± 0.12[e]	0.099 ± 0.041	0.056 ± 0.026
4	9.6 ± 3.5	5.9 ± 3.2	0.29 ± 0.19	0.15 ± 0.10	0.023 ± 0.012	0.030 ± 0.007
5	12.9 ± 5.3	8.0 ± 3.1	0.32 ± 0.16	0.27 ± 0.18	0.025 ± 0.013	0.031 ± 0.012
6	18.1 ± 4.8	8.2 ± 3.3[e]	0.60 ± 0.24	0.36 ± 0.17	0.037 ± 0.014	0.044 ± 0.013

[a] IR = injected dose of radioactivity.
[b] ZnD = 6 zinc-deficient rats.
[c] ZnS = 6 zinc-supplemented pair-fed rats.
[d] Mean ± SD.
[e] Comparison between zinc-supplemented pair-fed rats and zinc-deficient rats is significant: $p < .01$;
[f] $p < .025$.

TABLE IV. Twenty-Four Hour Urinary Excretion For 5 Days of Total Taurine and Taurine-^{35}S After Taurine-^{35}C Injection in Zinc-Deficient and Zinc-Supplemented Pair-Fed Rats.

Days	Total taurine excreted (mg)		Total taurine-^{35}S excreted (% IR[a])		Sp act (% IR/mg)	
	ZnD	ZnS	ZnD	ZnS	ZnD	ZnS
1	6.19 ± 2.31[d]	3.14 ± 1.23[g]	3.87 ± 0.79	1.98 ± 0.28[e]	0.765 ± 0.298	0.643 ± 0.160
2	11.58 ± 1.24	3.72 ± 1.07[e]	1.20 ± 0.46	0.54 ± 0.34[g]	0.115 ± 0.040	0.135 ± 0.049
3	10.24 ± 2.74	5.73 ± 0.34[e]	1.39 ± 0.32	0.81 ± 0.08[f]	0.146 ± 0.019	0.127 ± 0.012
4	9.06 ± 1.76	4.77 ± 2.82[f]	0.48 ± 0.15	0.19 ± 0.04[f]	0.049 ± 0.010	0.039 ± 0.021
6	9.99 ± 2.08	3.96 ± 1.56[f]	0.59 ± 0.11	0.27 ± 0.14[f]	0.055 ± 0.008	0.062 ± 0.011

[a] IR = injected dose of radioactivity.
[b] ZnD = 6 zinc-deficient rats.
[c] ZnS = 6 zinc-supplemented pair-fed rats.
[d] Mean ± SD.
[e] Comparison between zinc-supplemented pair-fed rats and zinc-deficient rats is significant: $p < 0.01$; [f] $p < 0.025$; and [g] $p < 0.05$.

of injected radioactivity per milligram of inorganic sulfate were observed between 2 groups. This latter observation can be attributed to large standard deviations. In some instances, among the controls, no measurable trace of urinary inorganic sulfate-^{35}S could be found.

Table IV shows that the urinary taurine excretion and its ^{35}S form, but not specific activity, of zinc-deficient rats were significantly higher than those of the controls. The consistent high levels of urinary taurine resulting from zinc deficiency has been repeatedly observed in this laboratory. Another indication of an enhancement in taurine metabolism is the marked increase in the excretion of 24 hr urinary ^{14}C by zinc-deficient rats following injection of taurine-1,2-^{14}C (Table VI). Total ^{35}S recovered in the fecal lipid extract, predominantly bile acid conjugates is very low and shows no differences between 2 groups (Table V).

TABLE V. ^{35}S Excretion in Fecal Lipid Extract of Zinc-Deficient and Zinc-Supplemented Pair-Fed Rats.

Type of diet	% IR[a]	
	1st 72 hr	2nd 72 hr
ZnD[b]	0.300 ± 0.138[d]	0.086 ± 0.038
ZnS[c]	0.231 ± 0.127	0.032 ± 0.016

[a] IR = injected dose of radioactivity.
[b] ZnD = 6 zinc-deficient rats.
[c] ZnS = 6 zinc-supplemented pair-fed rats.
[d] Mean ± SD.

TABLE VI. Excretion of Urinary ^{14}C, 24 hr After Taurine-1,2-^{14}C Injection in Zinc-Deficient and Zinc-Supplemented Rats.

Type of diet	Total ^{14}C (% IR)[a]	Statistical comparison of values
ZnD[b]	24.43 ± 4.94[d]	$p < .05$
ZnS[c]	17.68 ± 2.47	

[a] IR = injected dose of radioactivity.
[b] ZnD = 6 zinc-deficient rats.
[c] ZnS = 6 zinc-supplemented rats.
[d] Mean ± SD.

Discussion. The apparent association between taurine and zinc deficiency was strongly supported in this study using ^{35}S-labeled taurine. Although urinary excretion of taurine is consistently increased in zinc-deficient rats, the knowledge concerning taurine content in various tissues is needed to determine the effect of zinc deficiency on taurine synthesis. Since cystine is the principal precurson of taurine, the excess taurine-^{35}S found in the urine of zinc-deficient rats after cystine-^{35}S injection suggests a zinc requirement in the regulation of cystine–taurine pathways.

Schram and Crokaert (5) and Boquet and Fromageot (6) showed that significant amounts of taurine-^{35}S by feeding were converted to urinary inorganic sulfate-^{35}S in rats. These authors concluded that conversion was accomplished in the gut by bacterial action.

The findings of increased urinary excretion of inorganic sulfate-^{35}S and ^{14}C by zinc-deficient rats after respective injections of labeled taurine suggest zinc deficiency induces a rapid catabolism of taurine and also indicate a possible conversion from taurine to inorganic sulfate without bacterial involvement. It is uncertain whether the increased inorganic sulfate in zinc-deficient rats after injection of various ^{35}S-labeled compounds (2) and taurine-^{35}S is linked to a common mechanism. Nevertheless, present evidence indicates a close metabolic relationship between zinc and sulfur metabolism.

Summary. The effect of zinc deficiency on taurine oxidation was studied by comparing the amounts of labeled-^{35}S found in urinary inorganic sulfate and taurine following injection of taurine-^{35}S into zinc-deficient and zinc-supplemented rats. The results show that rats receiving a diet low in zinc excreted significantly more ^{35}S, more $^{35}SO_4^{2-}$ and taurine-^{35}S than control animals. These findings suggest zinc deficiency enhanced the rate of taurine oxidation. This conclusion is supported by data showing a significant increase in excretion of 24 hr urinary ^{14}C by zinc-deficient rats over zinc-supplemented control rats following injection of taurine-1,2-^{14}C. The radioactivity in the fecal lipid extract was not affected by zinc deficiency, indicating that zinc-deficient rats were capable of forming taurine–bile acid conjugate.

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