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# Parallelism Between Sulfur and Selenium Amino Acids in Protein Synthesis in the Skin of Zinc-Deficient Rats (37935)

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Similarity between the metabolism of methionine (Met) and selenomethionine (Se Met) has been demonstrated in two separate series of experiments. First, it was established that the conditions for active transport of Se Met across the intestine of the hamster were nearly identical with those for active transport of Met (1, 2). Since Se Met could inhibit the transport of Met and vice versa, it was suggested that Se Met and Met utilize the same transport system. Secondly, using a cell-free protein-synthesizing system, we have observed that selenomethionyl-tRNA participates in polypeptide synthesis to about the same extent as methionyl-tRNA and in other experiments there is competitive inhibition between Met and Se Met (3–5) in the loading of the amino acid onto tRNA. These results suggest that Se Met is incorporated into protein via the sulfur pathways. Since uptake of various amino acids, which include L-Met and L-Cyst, in the skin and hair of zinc-deficient rats (ZnD) was significantly lower than in zinc-supplemented (ZnS) controls (6–8), studies were made to investigate the parallelism between sulfur (Met and Cyst) which has been reported (7, 8) and selenium (Se Met and Se Cyst) amino acids in protein synthesis in the skin from Zn-D rats. Results clearly establish a parallelism between sulfur and selenium amino acids in protein synthesis in the skin of normal and ZnD rats.

*Experimental.* Male weanling rats of the Sprague–Dawley strain, 45–50 g, were housed individually in stainless-steel cages in a temperature-controlled room. One group was fed the basal diet composed of 65.9% sucrose, 15.0% dried egg albumin, 3.0% salt-free casein hydrolysate, 10.0% corn oil, 5.74% salt mixture,[1] and 0.29% vitamin supplement[2] (8). This diet assayed approximately 2 ppm zinc and is referred to as the "zinc-deficient" diet. The second group, used as controls, received the same zinc-deficient diet supplemented with 65 ppm zinc as zinc carbonate. In some experiments a third group, used as pair-fed controls, received the same diet as rats in the second group in a quantity equivalent to that which the first group (ZnD) consumed. Feed and deionized, distilled water were offered *ad lib.* in all experiments except to the pair-fed control rats.

In all experiments, after an overnight fast, each rat was weighed and injected intramuscularly (5 $\mu$Ci/100 g body weight) with the labeled compounds.[3] Radioactivity of $^{75}Se$ was measured in a Packard Model 3002 Tri-Carb Gamma Scintillation Spec-

[1] Furnished per 100 g diet (in g): $CaHPO_4$, 2.716; $CaCO_3$, 0.957; $Na_2HPO_4$, 0.670; NaCl, 0.383; KCl, 0.670; $MgSO_4$, 0.287; $FeC_6H_5O_7 \cdot 5H_2O$, 0.019; $MnSO_4$, 0.021; $KIO_3$, 0.001; and $CuSO_4 \cdot 5H_2O$, 0.001.

[2] Furnished per 100 g diet (in mg): thiamine–HCl, 0.24; riboflavin, 0.60; pyridoxine HCl, 0.30; Ca pantothenate, 2.00; niacin, 4.00; inositol, 10.0; biotin, 0.60; folic acid, 0.20; vitamin $B_{12}$, 0.002; choline chloride, 100.00; 2-methyl-1-1-4-napthoquinone, 1.00, and $\alpha$-tocopherol, 6.00; (in IU) vitamin A, 2500 and vitamin D, 300.

[3] L-$^{75}$Se-selenomethionine (SpA 1–10 mCi/mg) and L-$^{75}$Se-selenocystine (SpA 100–500 mCi/mmole) were obtained from Amersham/Searle Corp., Arlington Heights, IL.

trometer. The significance of the difference between the means of two groups of values was determined by the Student's $t$ test (9).

Immediately after decapitation of the rats, the liver, pancreas, muscle, kidney, and shaven skin were excised, weighed, and portions of each tissue were homogenized in a Potter–Elvehjem glass homogenizer (7). One milliliter homogenate was added to 2 ml ice-cold 10% trichloroacetic acid (TCA) and the TCA suspensions were kept at 4° overnight. Protein was prepared for measurement of radioactivity as follows: The TCA suspension was centrifuged and the sediment was washed thrice with 8 ml of 5% TCA containing 4.74 mg of unlabeled Se Met or Se Cyst. The residue was then dissolved in 3 ml of 88% formic acid and 0.6 ml of 30% hydrogen peroxide and the mixture allowed to stand 30 min at room temperature. To the formic acid-treated solution 5 ml of 10% TCA was added and the resulting precipitate was separated by centrifugation. To remove the residual TCA as well as some of the lipid, the precipitate was extracted with 5 ml of 95% ethanol saturated with sodium acetate. Further lipid extraction was completed with 5 ml of a 3:1 ethanol–ethyl ether mixture and 5 ml of anhydrous ethyl ether. The excess ether was evaporated by drying the precipitate in air at room temperature for 5–10 min. While the protein precipitate was still slightly wet, 4.0 ml of a 0.4 $N$ KOH solution was added, and the mixtures were incubated for 30 min at 40°C. An aliquot was used for the determinations of protein radioactivity and protein content.

Tissue protein concentrations were estimated by the method of Lowry *et al.* (10) with bovine serum albumin as standard. Results are expressed as cpm $^{75}$Se per mg protein. The concentrations of zinc were measured with an atomic absorption spectrophotometer. Selenium was determined by neutron activation analysis (11).

*Results and Discussion.* In 2 weeks feeding, the average weight gain was 20 ± 5 g for ZnD rats, 34 ± 4 g for ZnS pair-fed rats, and 65 ± 5 g for ZnS *ad lib.* rats. One-third of the ZnD rats showed such external symptoms as alopecia and scabby skin lesions (8). Table I presents the results of radioactive distribution after $^{75}$Se Met injection and Table II after $^{75}$Se Cyst injection. The amount of $^{75}$Se found in the skin of the ZnS pair-fed rats was 3.7× and 1.7× greater than that of the ZnD rats for $^{75}$Se Met and $^{75}$Se Cyst, respectively. The uptake of $^{75}$Se in the liver, kidney, muscle, and pancreas did not show any significant differences among the three groups. Less Met and Cyst were incorporated into the skin proteins of the ZnD rats than in the ZnS pair-fed rats. The uptake of Met and Cyst in the liver, kidney, muscle, and pancreas was not different in the ZnD and ZnS. Figure 1 shows the normalized results comparing the dis-

TABLE I. Twenty-four Hour Incorporation of L-Selenomethionine-$^{75}$Se into Tissue Proteins of the Rat.

| | Type of diet | Specific activity (cpm/mg protein) | | | | |
|---|---|---|---|---|---|---|
| | | Skin | Pancreas | Liver | Kidney | Muscle |
| 1 | Zn-supplemented (ad lib.) | 195 ± 52[a] | 696 ± 28 | 209 ± 10 | 593 ± 51 | 107 ± 21 |
| 2 | Zn-supplemented (pair-fed) | 103 ± 33 | 643 ± 36 | 232 ± 24 | 544 ± 36 | 94 ± 44 |
| 3 | Zn-deficient | 28 ± 14[b] | 622 ± 25[c] | 209 ± 26[c] | 590 ± 51[c] | 102 ± 77[c] |

[a] Mean ± standard error of the mean. Five to six rats in each group were on the experimental diet for 15 days.

[b] Difference between Zn-supplemented (ad lib. and pair-fed) and Zn-deficient rats is statistically significant ($P < 0.05$).

[c] Difference between Zn-supplemented and Zn-deficient rats was not statistically significant.

TABLE II. Twenty-four-Hour Incorporation of L-Selenocystine-$^{75}$Se into Tissue Proteins of the Rat.

| | Type of diet | Specific activity (cpm/mg protein) | | | |
|---|---|---|---|---|---|
| | | Skin | Pancreas | Liver | Kidney |
| 1 | Zn-supplemented (*ad lib.*) | 175 ± 20[a] | 186 ± 4 | 271 ± 8 | 810 ± 13 |
| 2 | Zn-supplemented (pair-fed) | 139 ± 23 | 263 ± 5 | 275 ± 2 | 1040 ± 15 |
| 3 | Zn-deficient | 81 ± 17[b] | 201 ± 20[c] | 266 ± 14[c] | 1173 ± 32[c] |

[a] Mean ± standard error of the mean. Five to six rats in each group were on the experimental diet for 15 days.

[b] Difference between Zn-supplemented (*ad lib.* and pair-fed) and Zn-deficient rats is statistically significant, ($P < 0.05$).

[c] Difference between Zn-supplemented and Zn-deficient rats was not statistically significant.

tribution of skin Met and Se Met and Cyst with Se Cyst in the ZnD and the ZnS rats. These results are essentially in agreement with previous findings (7, 8) which established that in the ZnD rat there is a significantly reduced incorporation of various amino acids in skin compared to ZnS controls.

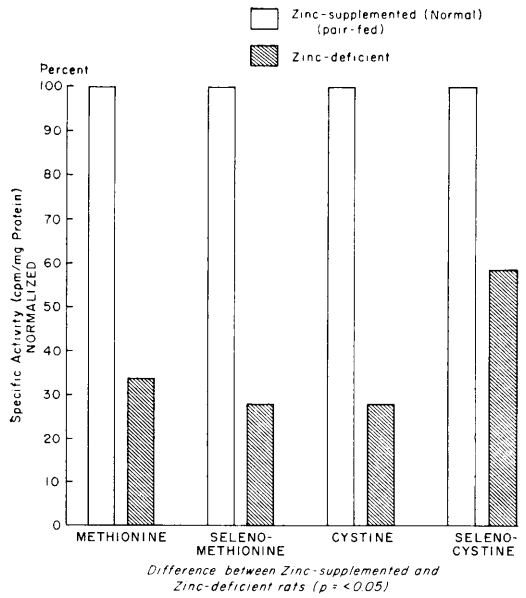


FIG. 1. Incorporation of methionine and selenomethionine, cystine, and selenocystine into skin proteins of zinc-deficient and normal rats. Values for L-Cyst and L-Met were obtained from the following: L-Cyst$^{35}$S J. Nutr. **101**, 445 (1971). L-Meth$^{35}$S J. Nutr. **102**, 1181 (1972).

As far as we have been able to ascertain, the only studies relating the metabolism of methionine and cystine to Zn deficiency are those carried out by one of us (J. Hsu (7, 8)). Further, this is the first time that the metabolism of Se Met and Se Cyst has been reported in relation to protein synthesis in the Zn-deficient rat. The similar metabolism between Se Met and Met as well as between Se Cyst and Cyst is worthy of note. The similarity of metabolism between Se Met and Met is not new as this phenomenon has been observed previously (1–4).

An explanation of the impairment of the normal pathway of skin protein synthesis in the ZnD rats may be found in the study of DNA synthesis in the skin (12). Suppression of DNA formation was clearly demonstrated in rats receiving a ZnD diet.

Studies (13) reported elsewhere have indicated that Zn is a component of DNA-dependent RNA polymerase, which is necessary for activity in *E. coli*, and thus in the Zn-deficient state protein synthesis should be affected by decreasing the amount of mRNA available for translation. It Zn is also necessary for activity of this enzyme in the mammalian system, this would be an explanation for decreased incorporation of amino acids into skin proteins in the Zn D rat.

It was of interest to determine the resid-

TABLE III. Selenium Content of Tissues from Zinc-deficient and Zinc-supplemented Rats.

| Type diet | $\mu$g Se[a] per g dry wt | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Skin | Skin and hair | Hair | Liver | Kidney | Muscle | Spleen | Heart |
| Zn-supplemented (*ad lib.*) | — | -- | — | 3.26 ± 0.18 | 4.69 ± 0.31 | 1.38 ± 0.53 | 2.24 ± 0.11 | 1.75 ± 0.24 |
| Zn-supplemented (pair-fed) | 0.60 ± 0.15 | 0.47 ± 0.04 | 0.81 ± 0.16 | 2.91 ± 0.23 | 4.62 ± 0.25 | 1.00 ±0.06 | 2.22 ± 0.22 | 1.65 ± 0.07 |
| Zn-deficient | 0.69 ± 0.13[b] | 0.53 ± 0.05[b] | 0.63 ± 0.27[b] | 3.11 ± 0.03[b] | 5.17 ± 0.21[b] | 0.98 ± 0.161[b] | 1.80 ± 0.16[b] | 1.95 ± 0.22[b] |

[a] Selenium determinations were made by Neutron activation analysis, courtesy A. Blotcky, VA Hospital, Omaha, NB.
[b] Differences between Zn-supplemented and Zn-deficient rats were not statistically significant. Mean ± standard error of the mean.

ual nonradioactive selenium and zinc in tissues of the ZnD and ZnS rats. The selenium content of skin, hair, liver, kidney, muscle, spleen, and heart and the zinc content of skin and hair in the ZnD rats was about the same as in the ZnS pair-fed rats (Tables III, IV). The tissue levels of Zn, Cu, and Mn in the Zn-deficient animal are not significantly different[5] from those in the normal animal (8).

Probably the most interesting information in these studies is the similarity between methionine and selenomethionine in protein synthesis. This has been demonstrated in earlier experiments (3, 5). Much attention has been directed toward the presence of selenium in protein components as a starting point for defining the biochemical role of this element. After the administration of selenium to various organisms, it appears in proteins, apparently having been metabolized via sulfur pathways to the selenoanalogs of Met and Cyst. We have recently examined the pathway of Se Met incorporation into protein using cell-free extracts from *E. coli* (3) and rat liver homogenates (5). Se Met is readily activated and attached to all species of methionine tRNA. The word "readily" is used since we found that the $K_m$ for selenomethionine was only 1.5 times greater than for methionine using a partially purified methionyl tRNA synthetase. To the best of our knowledge this represents the closest similarity of the $K_m$ of an amino acid analog to that of the natural amino acid in any such studies on aminoacyl-tRNA synthetases.

Similarity between Met and Se Met metabolism has been demonstrated in still another way. In recent studies it has been established that there is an active transport of L-$^{75}$Se Met across the intestine of the hamster (1, 2). DL-Se Met (10 m$M$) can reduce the rate of active transport of $^{14}$C DL-Met (1 m$M$), the corresponding sulfur analog, by 78%, suggesting that Se Met and Met utilize the same transport system.

[5] Possibly our analytical methods were not sensitive enough to detect the subtle differences in the Zn level that may exist in the tissues examined.

TABLE IV. Zinc Content of Skin and Hair of Zn-deficient and Zinc-Supplemented Rats.

| | μg Zn[a] per g dry wt | | | | | |
|---|---|---|---|---|---|---|
| Type diet | Skin | Molar ratio Se:Zn | Hair | Molar ratio Se:Zn | Skin and hair | Molar ratio Se:Zn |
| Zn-supplemented (pair-fed) | 30.0 ± 5.0 | 1:60 | 236.0 ± 18.0 | 1:350 | 65.0 ± 11.0 | 1:169 |
| Zn-deficient | 57 ± 12.0[b] | 1:99[b] | 237.0 ± 19.0[b] | 1:450[b] | 94.0 ± 15.0[b] | 1:214[b] |

[a] Zn determinations were made by atomic absorption, Perkin-Elmer Model 303, courtesy A. Blotcky, VA Hospital, Omaha, NB.

[b] Differences between Zn-supplemented and Zn-deficient rats were not statistically significant. Mean ± standard error of the mean.

The occurrence of the sulfur–selenium similarity and antagonism in relation to active transport was demonstrated.

This paper represents a third example of the definite similarity between the metabolism of sulfur and seleno amino acids. Results reported here definitely established a parallelism between Se Met and Met and Se Cyst and Cyst in protein synthesis in the skin of normal and ZnD rats.

*Summary.* Feeding animals a diet low in either zinc or selenium causes an abnormal condition of skin and hair. It has been established (7, 8) that the uptake of L-cystine-$^{35}$S and L-methionine-$^{35}$S in the skin protein of the ZnD rats was significantly lower than in the zinc-supplemented pair-fed controls (ZnS), i.e., 30 and 60%, respectively. Studies were made to investigate the parallelism between the sulfur (Met and Cyst) and selenium (Se Met and Se Cyst) amino acids in protein synthesis in the skin from ZnD rats. Results indicate that at 24 hr the specific activity (cpm/mg protein) present in the skin of the ZnD rats was 25 and 58% of that of the pair-fed controls, after injection of L-$^{75}$Se Met and L-$^{75}$Se Cyst, respectively. No significant differences were observed in the $^{75}$Se content of the liver, kidney and muscle between the two groups, which was also true with the sulfur amino acids. These results clearly establish a parallelism between sulfur and selenium amino acids in protein synthesis and strongly suggest that zinc plays a role in the metabolism of Se Met and Se Cyst in protein synthesis in the skin.

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