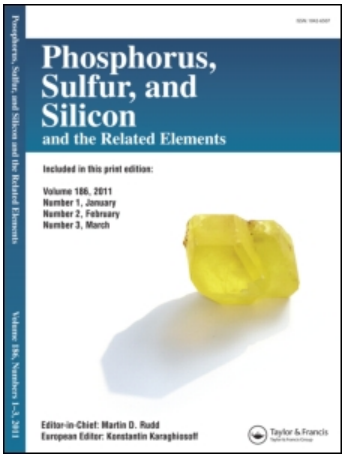

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J. L. Martin[ab]; J. A. Hurlbut[ab]
[a] Department of Biochemistry, Colorado State University, Fort Collins, Colorado, USA [b] Department of Chemistry, Metroipolitan State College, Denver, Colorado

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TISSUE SELENIUM LEVELS AND GROWTH RESPONSES OF MICE FED SELENOMETHIONINE, SE-METHYLSELENOCYSTEINE OR SODIUM SELENITE

by

J. L. Martin† and J. A. Hurlbutt†

Department of Biochemistry, Colorado State University, Fort Collins, Colorado 80523 USA

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ABSTRACT

Mice fed diets containing selenomethionine at a level of 20 ppm selenium and raised to 30 ppm selenium at 3 weeks on experiment showed (1) delayed response to selenium toxicity, (2) slow recovery from the toxicity after removal of selenium from the diet and (3) relatively high deposition and retention of tissue selenium. These data suggest that selenomethonine initially becomes incorporated in to the primary structure of proteins and as such is not particularly toxic. However, upon its slow removal from protein, selenomethionine becomes toxic by forming selenium IV compounds through a pathway similar to that followed by methionine.

Mice fed diets containing sodium selenite or Se-methylselenocysteine at the same level of selenium as the selenomethionine diet showed (1) immediate response to selenium toxicity (2) rapid recovery from the toxicity after removal of selenium from the diet and (3) relatively low deposition and relatively rapid depletion of tissue selenium. These data suggest that sodium selenite and Se-methylselenocysteine ultimately follow similar metabolic pathways and do not become part of the primary structure of proteins. A possible metabolic route for Se-methylselenocysteine is that it is oxidized to toxic selenium IV compounds through an oxidative pathway similar to that followed by S-methylcysteine.

Introduction

Most metabolic and toxicological studies on selenium have involved the use of inorganic selenium compounds such as sodium selenite (SS). Only a few studies relating to the relative toxicities of organic selenium compounds[1,2] and to tissue deposition of selenium[3] have been carried out using organic selenium compounds. This is unfortunate considering that undoubtedly it is organic selenium compounds which are most likely encountered by grazing livestock. Furthermore, clear distinctions should be made between the various organic selenium compounds likely to be encountered by livestock. All existing evidence suggests that the organic selenium compounds found in plants are various selenoamino acids. The selenoamino acids in crop plants are of the protein type; i.e., selenocystine and selenomethionine (SM)[4-6] and the selenoamino acids of seleniferous weeds are of the non-protein type; i.e., Se-methylselenocysteine (SMSC)[7] and selenocystathionine.[8] This difference in amino acid composition probably accounts for the striking differences between the toxic manifestations resulting from ingestion of selenized grain or from ingestion of seleniferous weeds. Blind staggers, characterized by poor coordination in affected animals, results when animals consume seleniferous weeds. Alkali disease results from the prolonged consumption of selenized grain.[9] The toxicological responses of animals fed inorganic selenium tend to resemble those of blind staggers and do not resemble the classic symptoms associated with alkali disease; i.e., deformed hoofs or loss of hair, etc. The unique symptoms of alkali disease may be attributed to the incorporation of the selenoamino acids in selenized grains into the primary structure of proteins, for it has been shown that selenomethionine, a protein amino acid, is readily incorporated into the primary structure of proteins[10-12]

Selenite, on the other hand, follows a different pathway of incorporation into proteins. Selenite is probably not converted into selenoamino acids to any great extent, if at all, in most animal systems[12,14] but

† *Present Addresses: Department of Chemistry, Metropolitan State College, Denver, Colorado 80204.*

finds its way into protein structure through the formation of selenotrisulfide bonds.[14–16]

Virtually no research has been carried out to determine the metabolic fate of Se-methylselenocysteine even though this compound is undoubtedly the causative agent of blind staggers. Does this selenoamino acid resemble metabolically the protein selenoamino acids found in selenized grain or does it more nearly resemble the metabolism of selenite?

Experimental Section

Mouse Care

Seventy weanling male albino mice were randomly separated into seven equal groups. One group was fed unsupplemented Purina mouse chow and served as the control group. Three groups were fed rations containing 10 ppm Se as either SS, SMSC* or SM†. Another three groups were fed rations containing 20 ppm Se, again as SS, SMSC or SM. After three weeks the selenium content in all toxic rations was increased by 10 ppm. From the end of 6 weeks to the termination of the study at 8 weeks, all mice received the control mouse chow ration. All mice comprising a single group were housed in a single cage. The temperature of the mouse room was thermostatically controlled at 68 degrees F. Weight gains were recorded after 4 days, one week and weekly thereafter throughout the 8 week period.

Tissue Analysis

At the end of the sixth week, three mice from each group fed the 30 ppm selenium diets and three from the control group were sacrificed. The selenium content of hair, liver, kidney and spleen was determined in each mouse by using the method of Cummins *et al.*[17] This procedure was repeated at the end of the seventh and eighth week on three mice from each of the selenium supplemented groups. Selenium content in the muscle was also determined at the end of the eighth week. However, when the tissue selenium content of mice fed a particular selenium toxic diet reached the levels observed in control animals, no further analyses were carried out on the remaining mice in that group.

Results

Two levels of selenium were employed so that a level of selenium could be established that would elicit maximum toxicity as measured by poor growth, yet allow for survival of most of the experimental animals. Growth patterns as presented in Figure I show very little initial shock to selenium toxicity in mice fed the 10 ppm Se ration regardless of the chemical nature of the selenium compound supplemented in the ration.

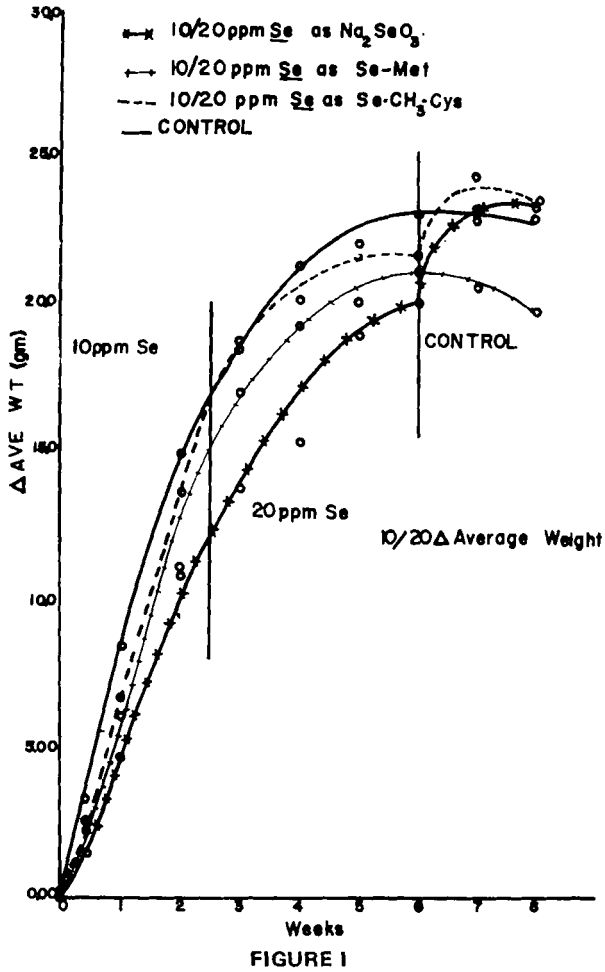

FIGURE I

Effect of various selenium compounds on the growth response of mice. The level of selenium supplemented to Purina mouse chow was 10 ppm for the first 3 weeks, then elevated to 20 ppm. At the end of 6 weeks all animals received control diets.

However, the growth patterns as presented in Figure II show that mice fed the 20 ppm selenium ration as either SS or SMSC experienced considerable initial shock to selenium toxicity whereas those mice fed a 20 ppm selenium ration as SM exhibited much less immediate response to the selenium. It is interesting to observe, however (Figure II), that by the end of the six-week period mice fed a diet containing SMSC, even when increased to a level of 30 ppm selenium, appeared to adjust to the SMSC so that by the end of 6 weeks these mice had gained as much weight as the control mice. On the other hand, mice fed diets supplemented with SM for 6 weeks showed a total weight gain comparable to that of mice fed SS supplemented diets even though initially they exhibited much less immediate response to selenium toxicity.

After SS and SMSC were removed from the diets, mice in these groups experienced a rapid gain in weight

* *Amend Drug and Chemical Co., Inc., New York, New York.*
† *Cyclo Chemical, Loss Angeles, Calinfornia.*

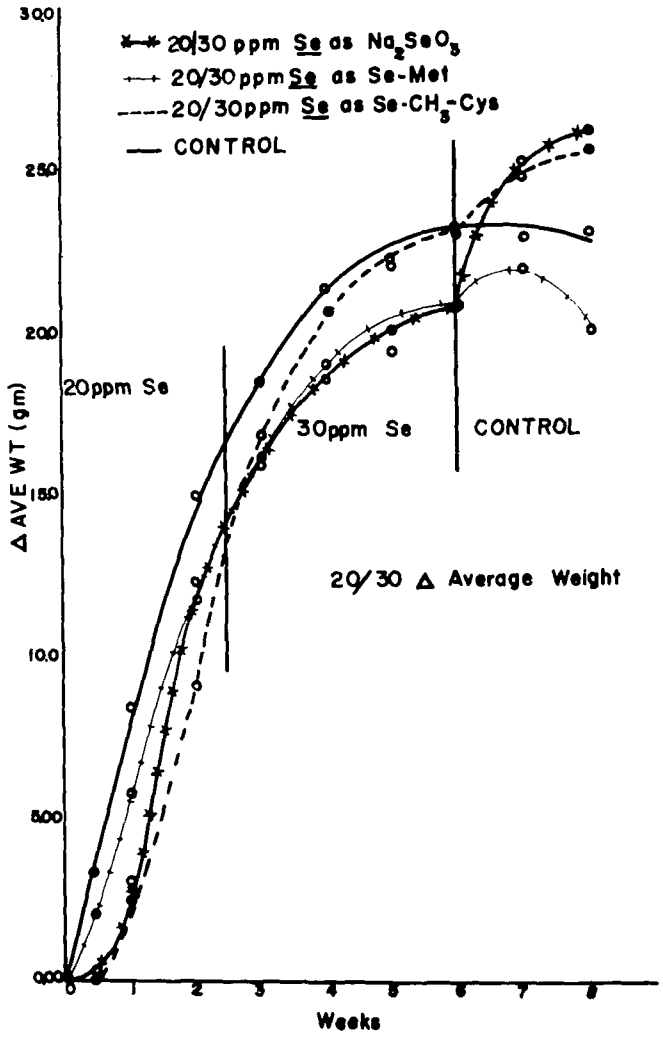

FIGURE II

Effect of various selenium compounds on the growth response of mice. The level of selenium supplemented to Purina mouse chow was 20 ppm for the first 3 weeks, then elevated to 30 ppm. At the end of 6 weeks all animals received control diets.

whereas the removal of SM from the diet did not elicit such an increased growth response (Figures I and II). The selenium content (Table I) in all tissues examined is much higher in mice fed SM than that found in mice fed diets containing equivalent amounts of selenium as either SS or SMSC. Except for hair, which is non-metabolizing tissue, the selenium content in the tissues of mice fed diets containing either SS or SMSC returned to control levels one week after removal of selenium from the diet. Conversely, except for the spleen, the selenium concentration in tissues of mice fed diets supplemented with SM remained significantly high 2 weeks after removal of selenium from the diet.

Discussion

The similarity between the rapid response to selenium toxicity in mice fed SS supplemented diets with that of mice fed SMSC supplemented diets (Figure II) suggests that these two compounds eventually follow similar metabolic routes. Did the toxicity result from SS being reduced to an SMSC-like compound or from SMSC being oxidized to an SS-like compound? Even though it is well established that SS is reductively methylated in animals to dimethyl selenide[18] and trimethylselenonium[19,20] such reductions occur as a means of detoxification of selenite. Both dimethylselenide and trimethylselenonium are relatively nontoxic[18,21] and, therefore, it would be expected that SMSC would also be relatively nontoxic *per se* because the chemical environment of selenium atoms in the molecules of these three compounds is somewhat similar in that all contain alkylated selenium in the −2 oxidation state. However, SMSC would become toxic after its metabolic oxidation to higher oxidation state selenium compounds. The question is, what might the oxidation product or products of SMSC be? We postulate that SMSC is catabolized in a manner similar to that of its sulfur analog, S-methylcysteine which is catabolized predominantly to sulfate[22] but cysteine is not an intermediate in this process. It has been shown that S-methylcysteine is metabolically cleaved on the amino acid side of sulfur atom rather than on the methyl side yielding methyl mercaptan[23] which is rapidly oxidized, presumably to sulfate[24] by as yet an unidentified pathway. A similar metabolic pathway of oxidation of SMSC would be expected except that selenite rather than selenate would be formed. A suggested metabolic pathway for the oxidation and subsequent detoxification of SMSC is given in Figure III. A portion of this scheme representing the pathway by which selenides are synthesized from selenite in rat-liver slices has recently been proposed by Ganther.[25]

This proposed metabolic pathway for SMSC would also explain the observation that mice fed SMSC supplemented diets adjust to selenium toxicity better than mice fed SS supplemented diets since fewer and less complicated steps are required for formation of excretable selenides from SMSC than from SS.

The toxicity resulting from the administration of SM is initially less pronounced. This observation suggests that SM is initially incorporated into the primary structure of proteins resulting in little immediate toxicity response. Eventually, however, an equilibrium is reached between pool SM and protein SM at which time any additional SM becomes toxic, presumably by being oxidized to toxic selenium IV compounds, such as selenocysteic acid and selenotaurine. We have

TABLE I

Tissue Deposition and Retention of Selenium (ppm)*

Tissue	Week	Control†	Na_2SeO_3	Se-methyl selenocysteine	Seleno-methionine
	6	1 ± 0.6	5 ± 1	6 ± 0.2	22 ± 4
Liver	7	–	2 ± 0.4	2 ± 0.5	6 ± 0.3
	8	–	1 ± 0.1	1 ± 0.3	4 ± 1
	6	1 ± 0.2	3 ± 0.8	4 ± 0.7	25 ± 4
Kidney	7	–	1 ± 0.8	2 ± 0.9	10 ± 0.4
	8	–	–	1 ± 0.2	4 ± 0.7
	6	1 ± 0.8	13 ± 2	14 ± 2	31 ± 1
Hair	7	–	11 ± 2	12 ± 2	31 ± 7
	8	–	9 ± 1	8 ± 1	23 ± 4
	6	2 ± 1	2 ± 2	3 ± 0.5	14 ± 2
Spleen	7	–	1 ± 1	2 ± 1	5 ± 2
	9	–	–	2 –	1 ± 1
	6				
Muscle	7				
	8	< 1	< 1	< 1	9 ± 1

* Average of 3 animals.

† Control-values for all tissues were determined for the sixth week period only, except muscle which was determined the eight week.

evidence from our laboratory that in mice and chicks SM is converted to these higher oxidation state selenium compounds.[26]

The observation that mice fed SS and SMSC supplemented diets show an immediate recovery from toxicity after removal of selenium from the diet demonstrates that selenium is present in the body of these animals in a chemical and biological state which is readily available for elimination. Conversely, mice on the SM supplemented diet show very little recovery after removal of selenium from the diet indicating that the selenium is in a form that is not promptly eliminated. Since SM becomes incorporated into the primary structure of proteins, obviously the selenium in this form would be slowly released for catabolism. On the other hand, selenium administered as either SS or SMSC probably becomes bound to proteins as –S–Se–S– bonds in which form the selenium is much more readily available for elimination. Furthermore, tissue data (Table I) show that there is much greater retention of selenium in the tissues of mice fed SM supplemented diets than in the tissues of mice fed diets containing equivalent quantities of selenium as SS or SMSC. These data again demonstrate that selenium as SM is present in the body in a chemical and biological state less readily available for elimination than that of either SS or SMSC. Previous investigations have shown that selenium administered as SS is rather quickly eliminated,[27] whereas selenium administered as SM is retained for much longer periods of time.[28] Such results would be expected if SM is incorporated into the primary structure of proteins, but selenium as SS does not become so incorporated either directly or indirectly. The pattern of tissue deposition and retention of selenium administered as SS is quite similar to that of SMSC but different from that of SM again suggesting that SS and SMSC eventually follow a similar metabolic route which is different from that of SM.

It thus appears that the two selenoamino acids most likely to be encountered by livestock follow different metabolic routes which possibly accounts for the difference between the toxicity symptoms of alkali disease with those of blind staggers. SM is first incorporated into the primary structure or protein and as such is not particularly toxic. To become toxic SM

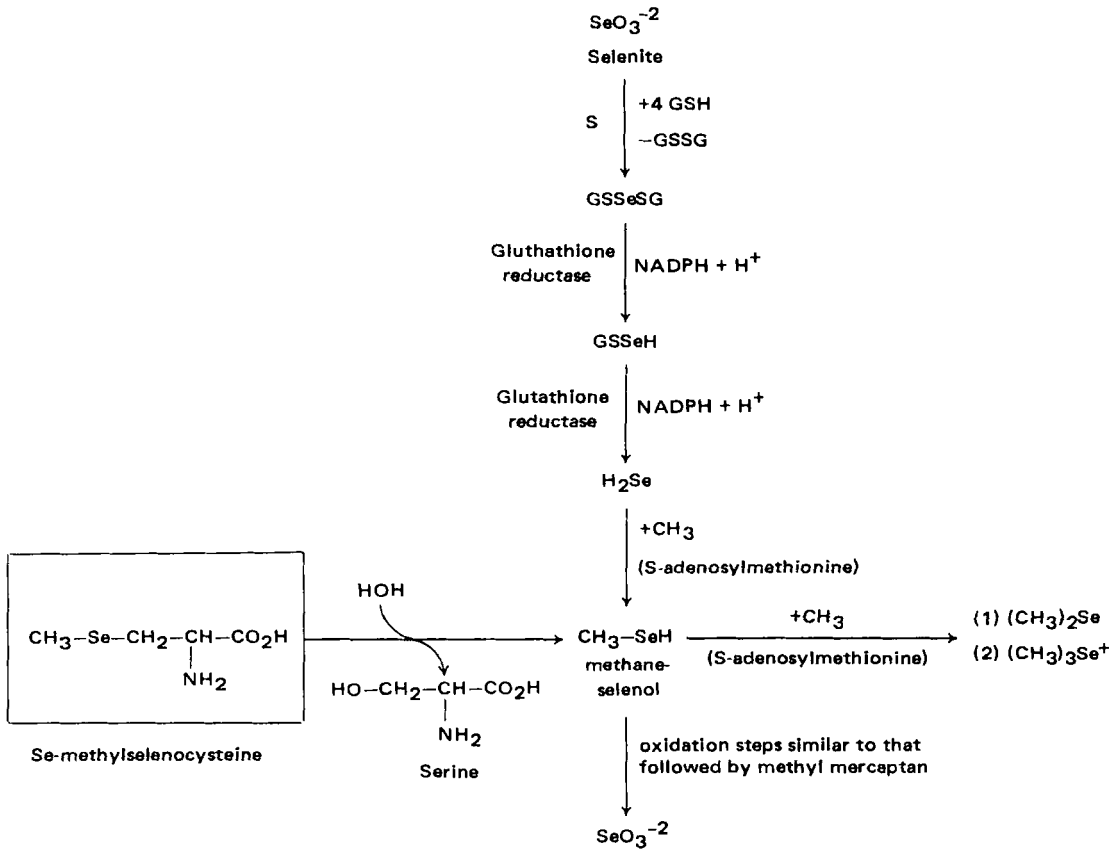

FIGURE III

Possible metabolic route of Se-methylselenocysteine

is slowly removed from proteins and then oxidized to higher oxidation state selenium compounds via a pathway similar to that of methionine. On the other hand, SMSC becomes toxic rather quickly by its rapid oxidation to higher oxidation state selenium compounds via a pathway similar to that of S-methylcysteine.

The results of this investigation extend our present knowledge of selenium metabolism in animals, and demonstrate that as new information on selenium metabolism becomes available, there is an ever increasing awareness of the great similarity between the biochemistry of sulfur and selenium.

References

1. A. L. Moxon, D. P. Dubois, and R. L. Potter, *J. Pharmacol. Exptl. Therap.*, 72, 184 (1941).
2. H. L. Klug, D. F. Peterson, and A. L. Moxon, *Proc. South Dakota Acad. Sci.*, 28, 117 (1949).
3. M. I. Smith, B. B. Westfall, and E. F. Stohlman, *U.S. Public Health Dept.*, 53, 1199 (1939).
4. K. W. Franke, *J. Nutr.*, 8, 609 (1934).
5. E. P. Painter and K. W. Franke, *Cereal Chem.*, 13, 172 (1936).
6. E. E. Olson, E. J. Novacek, E. I. Whitehead, and I. S. Palmer, *Phytochem.*, 9, 1181 (1970).
7. S. F. Trealease, A. A. DiSomma, and A. L. Jacobs, *Science*, 132, 3427 (1960).
8. M. J. Horn and D. B. Jones, *J. Biol. Chem.*, 139, 649 (1941).
9. I. Rosenfeld and O. A. Beath, "Selenium", Academic Press, New York and London, (1964) 146.
10. E. Hansson and M. Blau, *Biochem. Biophys. Res. Commun.*, 13, 71 (1963).
11. D. Lebez, A. Maretic, F. Gubensek, and J. Kristan, *Toxicon*, 5, 261 (1968).
12. A. Ochoa-Solano and C. Gitler, *J. Nutr.*, 94, 243 (1968).
13. L. M. Cummins and J. L. Martin, *Biochem.*, 6, 3162 (1967).
14. K. J. Jenkins, *Can. J. Biochem.*, 46, 1417 (1968).
15. H. E. Ganther, *Biochem.*, 7, 2898 (1968).
16. H. E. Ganther and C. Corcoran, *Biochem.*, 8, 2557 (1969).

17. L. M. Cummins, J. L. Martin, and D. D. Maag, *Anal. Chem.*, **37**, 430 (1965).
18. K. P. McConnell and O. W. Portman, *Proc. Soc. Exptl. Biol. Med.*, **79**, 230 (1952).
19. J. L. Byard, *Arch. Biochem. Biophys.*, **130**, 556 (1969).
20. I. S. Palmer, D. D. Fischer, A. W. Halverson, and O. W. Olson, *Biochem. Biophys. Acta*, **177**, 336 (1969).
21. B. D. Obermeyer, I. S. Palmer, O. E. Olson, and A. W. Halverson, *Toxicol. Appl. Pharmacol.*, **20**, 135 (1971).
22. V. DuVigneaud, H. S. Loring, and W. A. Craft, *J. Biol. Chem.*, **105**, 481 (1934).
23. F. Binkley, *J. Biol. Chem.*, **186**, 287 (1950).
24. E. S. Canellakis and H. Tarver, *Arch. Biochem. Biophys.*, **42**, 387 (1953).
25. H. E. Ganther, *Biochemistry*, **5**, 1089 (1966).
26. J. L. Martin and M. L. Gerlach, *Annals N.Y. Acad. Sci.*, **192**, 193 (1972).
27. C. Blincoe, *Nature*, **186**, 398 (1960).
28. K. A. Lathrop, *Strahlentheraphie Sonderb.*, **67**, 436 (1968).