# THE TOXICITY OF SELENIUM ANALOGUES OF CYSTINE AND METHIONINE[1,2]

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The identification of selenium-cystine and selenium-methionine in seleniferous grain hydrolysates, as important selenium carriers, led to experiments involving the toxicity of each of these two selenium analogues of sulfur amino acids. The minimum fatal dosages and the effects on the liver succinoxidase system of these two compounds, using the rat as the experimental animal, are reported in this paper.

## Experimental

A dosage killing 75 per cent of the rats within 48 hours was considered to be the minimum fatal dose (MFD). For selenium-cystine, considerable data were available in the literature (1,2) and only enough animals were used to check the compound and the technique. It is clearly established that the MFD of selenium-cystine for the albino rat is 8.44 mg. (4.0 mg. of selenium) per kg. of the body weight when injected intraperitoneally. This is much more toxic than the majority of other known organic selenium compounds and is comparable to sodium selenite when administered by injection. It was further shown (2) that L-selenium-cystine was more toxic than the D- form when given orally and that arsenic gave full protection against the toxicity of the analogues just as it does against the toxicity of the selenium in seleniferous grains. From these results, it appears that selenium-cystine is one of the more important carriers, if not the most important, of selenium in seleniferous cereals.

Using 16 rats, weighing from 236 gm. to 400 gm., and of both sexes, the MFD of selenium-methionine was found

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to be 10.55 mg. (4.25 mg. of selenium) per kg. of body weight when injected intraperitoneally. This indicated that selenium-methionine is slightly less toxic than selenium-cystine. Each compound, when injected, showed the characteristic picture of selenium poisoning (3) except for the fact that the heart appeared to stop in systole rather than diastole as reported in many instances in the literature.

While the above work describes the over-all effects of these analogues on the animals, it gives no indication of the mode of action. In this laboratory, selenium as selenite, has been shown to react **in vitro** with sulfhydryl groups and as succinic dehydrogenase is considered to be a sulfhydryl enzyme, the effects of these selenium analogues on the succinoxidase system were investigated. Stotz and Hastings (4) had shown in 1937 that succinic dehydrogenase was the portion of the succinoxidase system inhibited by selenite.

The enzyme system as described by Schneider and Potter (5) was used with the delayed substrate addition technique. The results are shown in Table I.

TABLE I

The effects of Se-Cystine and Se-Methionine on Succinoxidase

| Compound | Inhibitor Concentration (final) | Inhibition per cent |
|---|---|---|
| Se-cystine | M/800,000 | 27.3 |
| | M/400,000 | 40.8 |
| | M/200,000 | 76.6 |
| | M/100,000 | 100.0 |
| L-Se-cystine | M/400,000 | 62.0 |
| | M/200,000 | 89.0 |
| D-Se-cystine | M/400,000 | 20.0 |
| | M/200,000 | 83.0 |
| L-cystine | M/33,000 | 12.0 |
| Se-methionine | M/5,000 | none |
| | M/1,000 | 17.0 |
| DL-Methionine | M/50 | none |

Enzyme system. A 5 per cent isotonic KCl homogenate of rat liver was the source of the enzyme. The sodium salts of all acidic compounds, adjusted to pH 7.4 were used. To each flask were added; water to make the final volume 3 ml.; 1 ml. of 0.1 M phosphate buffer at pH 7.4; 0.2 ml. of 2 × $10^{-4}$M cytochrome c; 0.3 ml. of inhibitor solution; 0.1 ml. of a solution containing 0.012 M $CaCl_2$ and0.012 M $AlCl_3$; 0.2 ml. of a 5 per liver homogenate and 0.3 ml. of 0.5 M sodium succinate was placed in the side arm. The center cup contained 0.2 ml. of 2 N NaOH. Temperature, 37° C. Gas phase— air. Substrate added at 20 minutes.



The selenium-cystine is shown to be very toxic to the system while selenium-methionine is to be noted for its lack of toxicity. As was shown in the feeding experiments, L-selenium-cystine was more toxic to the enzyme than the D- form. Much more concentrated solutions of cystine than of the selenium-cystine were required to give an inhibition while methionine at M/50 gave none at all. This emphasized the importance of the selenium as the causative agent yet the importance of the molecular structure was indicated in that the toxicities of the analogues were independent of the selenium contents. For example, the selenium in 0.3 ml. of M/20,000 selenium-cystine, to give a final molarity of $5\times10^{-6}$ in the flask and a 76% inhibition is 2.37 micrograms while 0.3 ml. of M/10,000 of selenium-methionine (to give a final molarity of $1\times10^{-5}$) gave no inhibition even though it contained the same amount of selenium.

Although this is only one sulfhydryl enzyme of the many involved in body functions, it indicates a point of attack especially if the animal receives the selenium as selenium-cystine. How the enzyme is inactivated by the selenium-cystine is not known but it may well be an oxidation to the SS form or even denaturation of the protein portion of the enzyme. Why selenium-methionine should lack the extreme toxicity of the cystine analogue for this enzyme is unknown. Both compounds should release selenium at the body pH but the selenium-methionine has the protective methyl group that may readily aid in the detoxifying process. Recent work with complexes formed from the reactions of selenous acid and methionine together with the reported ability of methionine to alleviate the effects of selenium poisoning in yeast (6), may furnish possible explanations.

**Summary**

The minimum fatal dose for rats of selenium-cystine is 4 mg. per kg. of body weight and for selenium-methionine is 4.25 mg. per kg., reported as selenium. Selenium-cystine has been found to be very toxic to the liver succinoxidase system while selenium-methionine is to be noted for its lack of toxicity. L-selenium-cystine is more toxic to the system than the D- form. L-cystine and DL-methionine produce only slight inhibitions of succinoxidase.

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