PREPARATION OF SELENIUM DERIVATIVES OF SULFHYDRYL COMPOUNDS[1] [2]

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During the course of investigation of the mechanism of selenium poisoning, it was found that acute toxicity produced by single subcutaneous injections of Na_2SeO_3 into rats lowered the reduced gluthathione (GSH) content in blood, liver, and kidney. This suggested that GSH was being bound by injected selenium and hence could not be measured as reduced GSH. Therefore an attempt to synthesize the selenium glutathione compound, which may be formed in tissues poisoned by selenium, was indicated.

The role of glutathione, which is widely distributed (1) in animal tissues, is not clear but it has been shown to be an important detoxifying agent in selenium poisoning (2). It is a tripeptide containing three amino acids which are glutamic acid, cysteine, and glycine. The amino acid, cysteine, has a free sulfhydryl group which we believed to be the point where selenium was bound to GSH by an oxidation—reduction reaction. In previous experiments by titration of GSH in the presence of varying amounts of Na_2SeO_3 it was found that one mole of Na_2SeO_3 completely oxidized four moles of GSH.

The compound was prepared by mixing aqueous solutions of 0.01 molar GSH and 0.0025 molar selenious acid, and ethanol was added until a white precipitate formed. The product was cooled overnight on ice, filtered, washed with ethanol, and dried in a vacuum dessicator. Elemental analysis of the compound showed a sulfur to selenium ratio of 4:1.

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The elemental analyses are shown in Table I.

Table I
Elemental Analyses of Selenium Tetraglutathione

Element	% Found	% Calculated
S	10.5	10.2
Se	5.7	6.0
N	12.1	12.9

Calculated molecular weight of the compound=1303.0

The crystalline compound is white, slightly soluble in cold water, moderately soluble in hot water, and is stable in an acid solution. Decomposition occurs in an alkaline solution with liberation of elemental selenium, and upon heating the compound to 140° C.

Tests were carried out to show that a pure compound was formed and that the substance precipitated was not a mechanical mixture of the two reactants. Iodimetric titration of an aqueous solution of the compound showed no free SH groups present. No free selenious acid could be titrated with $Na_2S_2O_3$ showing complete recation between H_2SeO_3 and GSH had taken place. Further tests showed only a trace of disulphide sulfur, indicating that practically all the sulfur was bound to selenium.

The results of these tests indicate that the compound formed is selenium tetraglutathione with one Se atom bound to four S atoms in the molecule.

Injection of 65 mg/Kg (equivalent to a toxic dose of 4 mg Se/Kg) of the compound into rats produced symptoms of selenium intoxication for about a week following the injection, during which time the animals consumed little or no food, indicating a definite metabolic block. The rats gradually regained their appetites and health with no apparent after-effects.

It is very possible that this compound is formed within the animal's body when selenium is ingested. This may explain how GSH serves as a detoxifying agent to convert

selenium into a less toxic form which can be eliminated from the body.

Selenium tetra-cysteine has been prepared by Stekol (3) and numerous compounds in which selenium has replaced sulphur in sulfhydryl groups have been reported. (4)

Attempts to form selenium derivatives of thiourea, BAL (2, 3 Dimercaptopropanol) and thioglycollic acid met with no success. Each of these compounds rapidly reduced selenite completely to elemental selenium.

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