ISOLATION OF GLUTATHIONE AND THIONEINE FROM THE BLOOD OF SELENIZED STEERS[1]

H. D. Anderson and A. L. Moxon
South Dakota State College

With the recognition of selenium as the principal etiological factor in the syndrome of alkali disease, most investigators turned their attention to a closely related element, sulfur, as a guide in their study of selenium. Such a step was necessitated by the fact that selenium in minute traces (0.1 to 3 p. p. m.) in animal tissues is sufficient to produce toxic effects. This concentration offers little hope to the chemist who would like to isolate and study these selenium compounds separately.

Much greater possibilities lie in the isolation of the organic selenium from plants where the selenium runs as high as 10,000 p. p. m. Some such work has already been done by Horn and Jones from the U. S. Department of Agriculture who have reported the isolation of a crystalline amino acid containing selenium from **Astragalus pectinatus.**

With animals, however, most investigators have been forced to turn to the sulfur metabolism of the animal and attempted to draw conclusion from comparative data, or they have synthesized organic selenium compounds and compared their effects with those of the natural compounds.

From earlier work in our own laboratory it was known that all but a small fraction (transitory, depending upon the level of intake) of the selenium in the blood occurred in the red blood cells. Further work showed that most of this selenium was bound to the protein of the red blood cells but that a certain definite amount was not removed by the usual protein precipitants.

Turning to the sulfur compounds of mammalian blood it was known that thioneine (also known as ergothioneine and sympectothion), and glutathione occur in the red blood cells and similarly were not precipitated by usual protein precipitants. At an early date Potter and Franke (unpublished

[1] South Dakota Agricultural Experiment Station—Journal Series No. 152.

data) determined the concentration of thioneine in normal and selenized animals but could find no significant difference. This may have been due to the fact that the level of thioneine in the blood is directly dependent upon other factors that may have outweighed the selenium in their influence on the blood concentration of thioneine. During the past year there have been at our disposal approximately 25 gallons of steer blood taken from selenized animals. Thioneine and glutathione have been isolated following the procedure devised by Hunter and Eagles[2] and modified[3] to eliminate the use of H_2S in the separation of thioneine (sympectothion).

On the basis of melting point determinations, and the color reactions[4] [5] applied to the recrystallized materials it was concluded that the products were sufficiently pure for the purposes and selenium determinations were then made on each of the fractions.

The greater portion of the selenium remained in the protein precipitates. The precipitate from the treatment with dilute acid and heat contained 10 p. p. m. of selenium. The uranium acetate precipitate contained 0.8 p. p. m. The only glutathione fraction which contained selenium was the HgS precipitate. Previous experience has indicated that H_2S will remove the selenium from solution but it is not established whether the H_2S breaks down the selenium compound, substituting sulfur for selenium, or whether the sulfide precipitate absorbs the selenium compounds. Further work will be necessary to establish this point.

The data do indicate that some of the organic selenium does follow the fractionation procedure for glutathione and lends support to the view that some selenium may occur in the blood as the selenium homologue of cysteine in glutathione.

From the thioneine fractions 0.5 p. p. m. was found in

[2] Hunter, G., and Eagles, B. A. J. Biol. Chem. 72, 133, (1927).
[3] Hunter, G., and Eagles, B. A. J. Biol. Chem. 72, 130, (1927).
[4] Diazo Test for Thioneine: Diazotized sulfanilic acid + 0.5 cc carbonate-acetate buffer + 2 cc test soln., after 30 seconds add 2 cc of 10% NaOH.
[5] Nitroprusside Test for Glutathione: 5 cc test soln. + 3 gms $(NH_4)_2SO_4$ + 2.5 cc 30% NaCN + 0.2 cc sat. $Na_2Fe(CN)_5NO.2H_2O$ (sodium nitroprusside).

the filtrate from the preciptation with phosphotungstic acid. Since this selenium occurred in the filtrate it is doubtful if it was present as the selenium homologue of thioneine which would have been precipitated by phosphotungstic acid.

In what form the selenium is bound to the protein fraction is not known. Some selenium is liberated from the protein (not precipitated by protein precipitants) by tryptic hydrolysis. Beyond this there is little evidence at present. Work is in progress which may offer some additional information. At present there is little hope of isolating these compounds since they occur in such small amounts.