CVIII. A SENSITIVE REACTION FOR CYSTEINE.

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SULLIVAN [1926] found that cystine reduced with sodium cyanide and sodium sulphite gives a colour with sodium β -naphthoquinone-4-sulphonate. This substance is used by Folin [1922] for the estimation of amino-acids and so would appear not to be specific for cysteine. According to Hunter and Eagles [1927], however, the colour given by other amino-acids is not the same as that given by cysteine. The importance of cysteine in biological chemistry and the labour involved in the preparation of the naphthoquinone reagent led us to investigate the possibility of finding a more simple and specific test. We found that cysteine hydrochloride reacts readily with dimethyl-*p*-phenylenediamine hydrochloride when heated in presence of a small quantity of FeCl_3 giving a deep blue colour. The colour is stable. It resembles that of methylene blue but does not match it exactly. Like the latter it can be precipitated in presence of ZnCl_2 and NaCl at high concentrations. The cysteine employed by us was supplied by the British Drug Houses. We also used other samples prepared from a sample of Merck's cystine by reduction with zinc in hydrochloric acid solution. The solution of cysteine hydrochloride was made by dissolving 0.04 g. in 100 cc. of water, and the solution of dimethyl-*p*-phenylenediamine hydrochloride by dissolving 0.2 g. in 100 cc. of water. It is preferable though not absolutely necessary to use a freshly prepared solution of the latter, as on standing for some time it develops a purple colour. If, however, the purple colour is not too pronounced the addition of the cysteine solution usually renders it colourless again. The concentration of the FeCl_3 solution was 5 %. In testing for very small quantities of cysteine, however, it is preferable to use a more dilute solution of FeCl_3 . The most suitable proportions in which the reagents should be mixed are as follows: to 0.5 cc. of the *p*-phenylenediamine reagent should be added 1 cc. of the cysteine solution followed by one drop of the FeCl_3 solution. On heating the mixture almost to boiling point and allowing it to stand the deep blue colour is produced. The mixture of cysteine and *p*-phenylenediamine reagent may first be heated together and the drop of FeCl_3 then added to the hot solution when the blue colour develops as before. The test is sensitive with 0.05 mg. of cysteine hydrochloride.

Under similar conditions soluble sulphides do not give methylene blue but require a much more acid medium. The methylene blue reaction for sulphides is given in the cold.

Cystine under the conditions above mentioned gives no colour with these reagents but of course reacts on reduction. Thiolactic and thioglycollic acids do not react with these reagents. Using yeast extracts as a source of glutathione we found that neither oxidised nor reduced glutathione reacted with our reagents. On hydrolysis, extracts of fresh yeast give a positive reaction indicating the presence of cysteine in the decomposition products of glutathione. We hope soon to be able to publish a method, based on this reaction, for the estimation of cysteine and glutathione in tissue extracts.

REFERENCES.

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