XXXVI. SEPARATION OF CYSTEINE FROM ASCORBIC ACID BY MERCURIC ACETATE.

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DIFFERENT investigators have demonstrated that the reducing capacity of plant juices, determined by means of 2 : 6-dichlorophenolindophenol solution, accounts accurately for its observed antiscorbutic potency [Tillmans *et al.*, 1928; 1932; Birch *et al.*, 1933; Harris and Ray, 1933]. This agreement also has been demonstrated with animal tissues [Harris and Ray, 1932; 1933; Birch *et al.*, 1933]. However, biological tests on Jensen rat sarcoma, carried out by Harris [1933], indicate that other reducing substances must be present, because the antiscorbutic activity is much less than would be calculated from the reduction value.

When the titration is carried out in acid medium as indicated by Svirbely and Szent-Györgyi [1933], Birch *et al.* [1933] and Wolff *et al.* [1933], glutathione does not interfere. The presence of cysteine however will be a source of error in the titration with the indicator, because cysteine also reduces the indicator in acid solution [Birch *et al.*, 1933], though not as rapidly as does ascorbic acid. The quantity of cysteine can be determined [Birch *et al.*, 1933] by means of Sullivan's test [Sullivan, 1929, 1, 2], whereas the reduction of iodine solution in acid medium is a measure of the total amount of reducing substances which are present; but we cannot assume that the difference between the total iodine reduction and the cysteine iodine value (calculated from Sullivan's reaction) is due to ascorbic acid. The indicator solution (0·02 % in water) is added from a burette to the solution to be tested (acidified by dilute trichloroacetic acid) till a permanent faint pink colour is obtained. The titration is finished within a minute, whereas the colour generally does not fade after about half a minute. Sometimes this end-point is not sharp, owing to the presence of slowly-reducing substances, because pure ascorbic acid solutions do not show this tendency. Titrating a solution of cysteine-HCl in this way (containing 4 mg. per ml.), 1 ml. solution (acidified by 2 drops trichloroacetic acid) decolorises about 0·4–0·5 ml. indicator after 1 minute. When however 2 ml. indicator solution at once are added to 1 ml. cysteine solution, decoloration takes place in about half a minute. This different behaviour is mentioned because the titration has also been carried out by others by adding the unknown solution to a definite volume of the indicator [Birch *et al.*, 1933]. The two different methods of titration are without influence upon the amount of indicator used by ascorbic acid solutions.

In a previous communication [Van Eekelen *et al.*, 1933] it was stated that cysteine is precipitated by mercuric acetate, whereas ascorbic acid is reversibly oxidised and can be quantitatively regenerated by hydrogen sulphide [Tillmans, Hirsch and Dick, 1932; confirmed by Johnson, 1933].

The ascorbic acid content of solutions of pure ascorbic acid, lemon juice and orange juice was quantitatively recovered by the mercuric acetate precipitation. The reduction with hydrogen sulphide must be effected in a slightly acid

medium, because otherwise, and especially in alkaline solution, reducing sulphur compounds can be formed. It is also important to mention that mercuric acetate solution removes many impurities, a fact which has proved to be very useful in our spectrographic investigations.

Experimental.

Mercuric acetate solution. A 20 % solution in water was prepared and filtered after one day (some hydrolysis takes place).

Precipitation with mercuric acetate solution. To the solution to be tested (which must be slightly acid, excess of acid being removed by $CaCO_3$ and filtration) the mercuric acetate solution is added drop by drop till the precipitation is finished, the reaction taking place in a graduated centrifuge-tube. Care must be taken to avoid a large excess of mercuric acetate, which dissolves the precipitate more or less. After centrifuging, the solution is treated with H_2S. After filtration the solution is left standing overnight, the H_2S removed by nitrogen (controlled by lead acetate paper) and the titration is then carried out.

Example. To 20 ml. of a dilute orange juice solution (titration value 0·5 ml. indicator per ml. solution) 80 mg. crystalline cysteine-HCl were added (4 mg. per ml.). This solution of ascorbic acid and cysteine decolorises after 1 minute about 0·9 ml. indicator per ml. solution (adding the indicator drop by drop to the solution). 18 ml. from this solution (after $CaCO_3$ treatment) required 3 ml. mercuric acetate solution and the procedure given above was followed.

1 ml. solution required 0·43 ml. indicator, calculated $\frac{18}{21} \times 0{\cdot}5 = 0{\cdot}43$ ml. The sodium nitroprusside test was completely negative.

Summary.

Cysteine, being a source of error in the determination of ascorbic acid by means of 2 : 6-dichlorophenolindophenol solution, is removed quantitatively by mercuric acetate solution.

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Note added February 6th, 1934. Ergothioneine, which reduces the indicator in acid solution, is also precipitated by mercuric acetate in acid medium.