V. SOME CRITICAL REMARKS ON THE DETERMINATION OF ASCORBIC ACID.

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TILLMANS, HIRSCH AND HIRSCH [1932] were the first to use 2:6-dichlorophenol-indophenol to determine the antiscorbutic value of vegetables and fruit-juices. Their estimations were carried out in a neutral medium, but this offers difficulties because other substances such as cysteine, glutathione and ferrous salts interfere under these circumstances. Svrbely and Szent-Györgyi [1933], Birch *et al.* [1933] and Wolff *et al.* [1933] carried out the titration in acid medium; the interference of glutathione and ferrous salts is avoided in this way. Cysteine and ergothioneine however reduce the indicator in acid solution. We have indicated a method of removing by the aid of mercuric acetate, cysteine, ergothioneine, glutathione and tannins [Emmerie, 1934, 1; Emmerie and Van Eekelen, 1934].

These substances are precipitated by mercuric acetate whereas the ascorbic acid remains in solution in the reversibly oxidised state. By this method interfering pigments, which sometimes make the estimation impossible, are also removed. We also have found that mercuric acetate precipitates thiosulphate in urine [Van Eekelen, 1934].

To remove the excess of mercuric acetate after the precipitation and to reduce the reversibly oxidised ascorbic acid H_2S is used. Reduction with H_2S is always necessary, because the oxidases which are present in some vegetables are able to change the reduced ascorbic acid into the reversibly oxidised state when the vegetables are ground up [Van Eekelen, 1935, 1].

Before the introduction of the mercuric acetate method it was thoroughly tested on pure ascorbic acid solutions and on blood, urine and vegetables or plant-juices, to which definite quantities of ascorbic acid had been added. The ascorbic acid was always recovered to the extent of at least 94 %. Tauber and Kleiner [1935] and Plaut *et al.* [1935] have also shown that after the mercuric acetate treatment the ascorbic acid was quantitatively recovered.

According to a recent publication of McHenry and Graham [1935] these authors were not able to recover the ascorbic acid quantitatively after mercuric acetate precipitation. They used the method of Tillmans, Hirsch and Jackisch [1932] to remove interfering pigments. The utility of this method is limited and interfering reducing substances are not removed. For this reason it seemed necessary to us once more to direct attention to the precautions which must be taken to obtain satisfactory results with the mercuric acetate method.

1. The solution or extract to which mercuric acetate is added must be slightly acid (p_H about 5). The precipitation of small quantities of cysteine and ergothioneine is more complete in trichloroacetic acid solution than in acetic acid solution; for this reason we always prepare the extract with trichloroacetic acid.

2. Excess of mercuric acetate must be avoided, for this reason the amount of reagent necessary must be determined on a sample.

3. A very important factor is rapid working after addition of the mercuric acetate to avoid irreversible oxidation. It is necessary to centrifuge off the precipitate and the time between adding the mercuric acetate and passing H_2S into the filtrate must not exceed 5–10 min. (see Table III).

4. When working up urine which contains little ascorbic acid 10 ml. urine + 20 ml. mercuric acetate solution (20 %) can be taken.

In the following tables we give some results obtained by the mercuric acetate method with pure ascorbic acid solutions and with urine, blood and potatoes to which ascorbic acid has been added.

Table I. *Pure ascorbic acid solutions with different quantities of mercuric acetate. 10 min. after the addition of the mercuric acetate H_2S was passed through to precipitate HgS .*

	Found mg. ascorbic acid	% recovery
10 ml. of ascorbic acid solution	0.48	100
„ „ + 0.5 ml. 20 % mercuric acetate	0.46	96
„ „ + 1 ml. „ „	0.46	96
„ „ + 6 ml. „ „	0.45	94
„ „ + 10 ml. „ „	0.45	94

Table II. *Addition of pure ascorbic acid to blood, urine and potato and regeneration by H_2S after mercuric acetate precipitation.*

	Found mg. ascorbic acid	% recovery
20 ml. blood	0.13	—
„ „ + 1.09 mg. ascorbic acid	1.18	97
10 ml. blood	0.04	—
„ „ + 4.92 mg. ascorbic acid	4.91	99
10 ml. urine	0.13	—
„ „ + 1.66 mg. ascorbic acid	1.70	95
10 ml. urine	0.06	—
„ „ + 2 mg. ascorbic acid	2.02	98
10 gm. potato	3.11	—
„ „ + 2.25 mg. ascorbic acid	5.35	100

Table III. *Relation between recovered ascorbic acid and time during which the ascorbic acid has been in contact with mercuric acetate.*

Time in min.				Found mg. ascorbic acid	% recovery
	10 ml. ascorbic acid solution without mercuric acetate			0.43	100
1	”	”	+ 1 ml. 20 % mercuric acetate	0.42	98
5	”	”	”	0.42	98
10	”	”	”	0.41	95
20	”	”	”	0.40	93
30	”	”	”	0.37	86
60	”	”	”	0.35	81

Table IV. *Precipitation of thiosulphate with mercuric acetate.*

	(9 ml. indicator = 1 mg. ascorbic acid)	ml. indicator
10 ml. urine after mercuric acetate precipitation		1.4
„ + thiosulphate without mercuric acetate precipitation		13
„ + thiosulphate after mercuric acetate precipitation		1.4

Dewjatnin and Doroschenko [1935] used lead acetate to remove interfering substances.

We have the following objections against this method:

1. The authors do not use H_2S , so that reversibly oxidised ascorbic acid is not estimated.

2. Cysteine is not precipitated by lead acetate.

3. We have found [Emmerie, 1934, 2] that, though pure ascorbic acid solutions are not precipitated by lead acetate in slightly acid or neutral medium, addition of ascorbic acid to urine and precipitation with lead acetate in slightly acid and neutral solution causes great losses of the ascorbic acid.

The reduction of silver nitrate by organs and extracts as a criterion for their ascorbic acid content has proved to be very doubtful, because substances such as cysteine, glutathione and others are able to inhibit the reduction [Huszak, 1933; Emmerie, 1934, 2; Svirbely, 1935]. The quantitative determination of ascorbic acid by the tungstic acid method of Fujita *et al.* [1935] cannot be used in the presence of adrenaline (suprarenal extracts), because adrenaline inhibits the reaction and the values obtained are too low [Van Eekelen, 1935, 2].

SUMMARY.

1. Directions are given for obtaining satisfactory results with the mercuric acetate method of determining the ascorbic acid content of different substances and fluids.

2. Some critical remarks on other methods are made.

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