CLIV. THE CHEMICAL DETERMINATION OF VITAMIN C WITH REMOVAL OF INTERFERING REDUCING AND COLOURED SUBSTANCES.

BY ADRIANUS EMMERIE AND MARIE VAN EEKELEN.

From the Laboratory of Hygiene, The University of Utrecht.

(*Received May 11th, 1934.*)

BIRCH *et al.* [1933] have demonstrated that the titration of ascorbic acid with 2:6-dichlorophenolindophenol must take place in acid solution, because substances like glutathione and ferrous salts, which reduce the indicator in neutral medium, do not show this property in acid solution.

Tillmans *et al.* [1932] have pointed out the fact that it is necessary to reduce with H_2S, because ascorbic acid can also be present in the reversibly oxidised state.

We have found that ascorbic acid is not stable in dilute trichloroacetic acid solutions, even in the presence of H_2S.

Table I.

Ascorbic acid solution 2 ml. = 0·25 ml. indicator. In different concentrations of trichloroacetic acid under H_2S for 16 hours.

Trichloroacetic acid concentration %	ml. indicator per 2 ml. solution
3	0·15
1·5	0·23
0·75	0·24

However, in acetic acid solutions, ascorbic acid in the presence of H_2S is very stable. In different concentrations of acetic acid up to 10 % under H_2S after 18 hours no difference in titration value was found.

In a previous communication one of us [Emmerie, 1934] has demonstrated that cysteine and probably other unknown substances, which reduce the indicator in acid solution, can be precipitated by mercuric acetate. The ascorbic acid is not precipitated but remains in solution in the reversibly oxidised state. After centrifuging, the solution is treated with H_2S to reduce the oxidised form and to remove the excess of mercury which is precipitated as HgS.

Precipitation with mercuric acetate has also proved to be very important in relation to red-coloured plant juices or extracts (*e.g.* red cabbage and tomato), because it is impossible to titrate these coloured solutions with the indicator, whereas the colour is removed by precipitation with mercuric acetate.

We give below a description of our technique for determining the ascorbic acid content of different biological fluids and tissue or plant extracts.

Experimental.

Blood. To 10 ml. newly shed oxalated blood (in an Erlenmeyer flask of 100 ml.) 10 ml. 10 % trichloroacetic acid are added. After thorough mixing and precipitation of the proteins 5 ml. 20 % mercuric acetate solution are added and well mixed by stirring the contents of the flask. After some minutes 0·5 g. of pure $CaCO_3$ is added to neutralise the trichloroacetic acid. The mixture must be shaken till a drop gives a faint violet colour with a strip of Congo red paper. The mixture is centrifuged, and the supernatant liquid is decanted off and treated with H_2S. After precipitation of the HgS, the solution is filtered and left overnight with H_2S. The H_2S is removed by a stream of pure nitrogen (controlled by lead acetate paper). This removal is complete after about 10–30 minutes. For titration 5 ml. of the solution are taken, to which is added 1 ml. 10 % trichloroacetic acid.

5 ml. filtrate from human blood use about 0·1–0·25 ml. indicator (1 mg. ascorbic acid = 12 ml. indicator).

When a definite quantity of pure ascorbic acid was added to the blood, this quantity was completely recovered by this procedure.

Urine. To 20 ml. fresh urine diluted 1 : 6 are added 10 ml. 20 % mercuric acetate solution. After centrifuging, the supernatant liquid is decanted off and treated with H_2S. The further procedure is the same as for blood. In normal cases 5 ml. filtrate are titrated to which 1 ml. 10 % trichloroacetic acid is added.

Milk. 25 ml. milk are mixed with 15 ml. 20 % mercuric acetate solution and the same procedure as given for urine is followed. 5 ml. filtrate are titrated.

Tissue and plant extracts. The material is ground up with iron-free pure sand in a mortar with 3 % trichloroacetic acid solution. The quantity of trichloroacetic acid solution is about 5–10 times the weight of the tissue or plant material. After centrifuging, the extract is neutralised with $CaCO_3$ and filtered. To a known volume of this extract mercuric acetate solution is added till no further precipitation takes place. After centrifuging the procedure as given above is followed.

Summary.

Methods are given for determining the ascorbic acid content of blood, urine, milk, tissue and plant extracts with removal of interfering reducing and coloured substances by means of mercuric acetate.

REFERENCES.

Birch, Harris and Ray (1933). *Biochem. J.* **27**, 580, 590.
Emmerie (1934). *Biochem. J.* **28**, 268.
Tillmans, Hirsch and Dick (1932). *Z. Unters. Lebensm.* **63**, 267.