CCXVIII. A NOTE ON THE DETERMINATION OF CYSTINE IN PROTEINS BY THE METHOD OF FOLIN AND MARENZI.

By SIDNEY LIONEL TOMPSETT.

From the Biochemical Laboratory of the Institute of Pathology of the Royal Infirmary and University of Glasgow.

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THERE have been many modifications of the original method of Folin and Looney [1922] for the determination of cystine in proteins. Folin and Trimble [1924] made the method more specific for cystine by removing most of the molybdenum compounds from the phosphotungstic acid reagent thereby decreasing its sensitivity towards tryptophan, tyrosine and phenols. Folin and Marenzi [1929, 1] have recently stated that it is necessary to add much more of the reagent than employed by Folin and Looney and also recommend adding the sulphite before the alkali since a smaller amount is required. Folin and Marenzi [1929, 2] have published the details for the preparation of the phosphotungstic reagent free from all traces of molybdenum. Rimington [1930] examining this method found that a turbidity often formed in the standard and the test solutions, and that the intensity of the colour varied appreciably with slight changes in the concentration of sodium carbonate. Folin [1930] recommended the use of urea for the prevention of this turbidity. Rimington [1930] found this to be quite satisfactory for concentrations of sodium carbonate up to 3*N*. Hunter and Eagles [1927] have recommended the use of sodium hydroxide in place of sodium carbonate.

The present writer found that even when urea was used as recommended by Folin [1930] turbidities often occurred in the test and standard solutions in the Folin-Marenzi method. It was found however that a blue colour developed when sodium bicarbonate solutions were used in place of sodium carbonate. The colour obtained was stronger than that with sodium carbonate and turbidity did not occur even after 30 minutes. It was therefore decided to test the possibility of replacing the carbonate by bicarbonate.

The following solutions were used:

- (1) phosphotungstic reagent of Folin and Marenzi [1929, 2];
- (2) 20 % sodium sulphite;
- (3) saturated sodium bicarbonate solution;
- (4) standard cystine solution, containing 1 mg. in 1-cc. *N* H₂SO₄.

Into each of three 100 cc. volumetric flasks were measured 2 cc. of standard cystine solution followed by 2 cc. of sulphite solution. After allowing to stand 1 minute to ensure reduction of cystine to cysteine, bicarbonate solution was added as follows: to the first flask 5 cc., to the second 10 cc., and to the third 20 cc. Then 8 cc. of phosphotungstic reagent were added to each, and after 8 minutes they were diluted to 100 cc. and compared in a colorimeter. These three solutions were identical. Similar results were obtained using 1 cc. and 4 cc. of standard respectively, the colour being proportional to the concentration of the cystine in the range tested, *i.e.* 1–4 mg. Turbidities were never encountered so that it was unnecessary to use lithium sulphate or urea. The colours obtained remained stable for at least 30 minutes.

A blank determination was carried out and found to be inappreciable. Solutions of tyrosine, tryptophan, glycine, alanine, glutamic acid, aspartic acid and histidine in concentrations greater than those present in protein hydrolysates were tested by this method. No colour developed apart from the slight colour which develops during a blank determination. No colour was developed by uric acid.

From the results shown in Table I it will be seen that there was no appreciable loss when known amounts of cystine were boiled with 20 cc. of 6*N* H₂SO₄ for 18 hours under the same conditions as used in the hydrolysis of the proteins.

Table I.

Exp.	Initial amount of cystine mg.	Amount of cystine deter- mined after hydrolysis mg.
1	10	10.4
2	20	19.3
3	40	39.4

For the determination of cystine in proteins, 1–5 g. of protein were boiled with 20 cc. of 6*N* H₂SO₄ in a 300 cc. Kjeldahl flask under a reflux condenser for 18 hours. After cooling, the hydrolysate was filtered, washed into a 100 cc. volumetric flask and made up to 100 cc. A volume of 5–10 cc. containing about 1–4 mg. cystine was measured into a 100 cc. volumetric flask, followed by 2 cc. of sulphite solution. After 1 minute 20 cc. of saturated sodium bicarbonate solution were added and then 8 cc. of phosphotungstic reagent. After 8 minutes the mixture was diluted to 100 cc. and, after mixing, compared with a suitable standard prepared in the same way.

The cystine contents of a number of proteins were determined and the results are shown in Table II.

Table II.

Protein	Cystine (%)
Gelatin (B.D.H.)	Trace
Caseinogen (B.D.H.)	0.38, 0.41, 0.34 (average 0.38)
Edestin (B.D.H.)	1.46, 1.42, 1.48 (average 1.45)
Egg-albumin (Kahlbaum)	2.29, 2.29, 2.35 (average 2.31)
Serum-albumin (Kahlbaum)	2.29, 2.26, 2.34 (average 2.30)

SUMMARY.

A modification of the Folin-Marenzi method for the determination of cystine in proteins is described in which a solution of sodium bicarbonate takes the place of the carbonate used in the original method.

The colour produced in the presence of bicarbonate is uninfluenced by the concentration of this substance and turbidity has never been experienced.

The cystine contents of certain proteins have been determined.

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