CVII. THE PRECIPITATION OF CYSTINE BY PHOSPHOTUNGSTIC ACID.

By MANAYATH DAMODARAN
AND TARAKAD GANAPATHY SIVASWAMY.

From the University Biochemical Laboratory, Chepauk, Madras.

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ATTENTION was drawn in the previous communication [Damodaran and Sivaswamy, 1936] to the fact that in analysing the globulin anacardein according to the Van Slyke nitrogen distribution method the precipitate obtained with phosphotungstic acid was completely free from sulphur, though the presence of cystine in the protein was established both qualitatively and quantitatively. The existing literature on the subject (for a historical summary see Hoffman and Gortner [1922]) would give the impression that the precipitation of cystine by phosphotungstic acid is nearly quantitative except when the former has been subjected to prolonged boiling with acid. Thus Van Slyke [1911] records a recovery of 88·8%, Hoffman and Gortner [1922] of 89·7%, and Plimmer and Lowndes [1927] of 97% of cystine in the phosphotungstic acid precipitate. According to the first two authors, the precipitability of cystine is reduced by boiling with concentrated hydrochloric acid for 24 hours to 50 and 56·78% respectively of the cystine originally present. Plimmer found that after similar treatment for 36 hours the phosphotungstic acid precipitate represented 40% of the cystine-S. These observations are however incapable of explaining the complete absence of sulphur from the phosphotungstic acid precipitate observed in the case cited above. Such an observation could only be understood if the precipitability of cystine were influenced by its concentration and there existed a certain minimum value of the latter below which cystine failed to give a precipitate with phosphotungstic acid. No systematic investigation seems to have been carried out from this point of view and it was considered advisable to study this question, especially as cystine-N values based on the sulphur content of the phosphotungstic acid precipitate obtained in the Van Slyke method continue to be widely quoted and the point is of importance in view of the occurrence of more than one sulphur-containing amino-acid in proteins.

Solutions of pure cystine in varying concentrations were treated with phosphotungstic acid under exactly the same conditions as in the usual nitrogen distribution method and the amount of precipitation taking place was determined. Experiments were carried out at three different temperatures, *viz.* at room temperature, which during the period varied from 29 to 30°, in a thermostat at 20° and at the temperature of the refrigerator (5–8°).

The results are represented graphically in Fig. 1.

It is obvious that precipitability is considerably influenced both by the temperature and by the concentration of cystine, and that within certain ranges of the latter no precipitation takes place. From the shape of the curves it can be inferred that at no concentration is the precipitation likely to be complete. By extrapolation it is possible to estimate the limiting concentrations at the various temperatures below which no precipitation would take place. These are approximately at 5–8° 3·1 mg., at 20° 16·9 mg. and at 29–30° 50 mg. of cystine per 100 ml. of solution. Translated into terms of protein, assuming the standard conditions

of the Van Slyke method, where 3 g. of protein are hydrolysed and the "bases" precipitated in a volume of 200 ml. at about 20° the results obtained show that a protein containing less than 1.13 % by weight of cystine would give no precipitate of cystine phosphotungstate, whilst from one containing as much as 5 % of this amino-acid only about 81.4 % would be precipitated. In the experiments of the authors already mentioned only one concentration of cystine was tried in

Fig. 1. Precipitation curves of cystine at different temperatures.

each case. Van Slyke [1911] as well as Hoffman and Gortner [1922] (with whose results the values now obtained are in close agreement at the particular concentration tried by them) used a solution containing 0.1 g. cystine per 100 ml.; the experiments of Plimmer and Lowndes [1927] were carried out on a solution containing 0.25 g. in 200 ml. These figures correspond to 3 g. of protein with cystine contents of 6.66 % and 8.33 % respectively. Since no protein, apart from some keratins, contains such large proportions of cystine, the results obtained by the above authors at the high concentrations investigated by them have no bearing on the applicability of the process to the analysis of protein hydrolysates. It also follows that the solubility correction of 0.0026 g. to be added to the cystine-N, recommended by Van Slyke on the basis of his experiments on the cystine solution of the strength mentioned above, is purely arbitrary and of little significance. Further the extent of the alteration or decomposition of cystine during treatment with acid as determined by Van Slyke and by Plimmer and Lowndes is subject to correction, inasmuch as these authors assumed that precipitability by phosphotungstic acid was a measure of unaltered cystine.

It will be seen that at the temperature of the refrigerator almost quantitative precipitation can be obtained. This is in conformity with the results recently published by Toennies and Elliott [1935] who carried out the precipitation at 0°. But advantage cannot be taken of this fact in the analysis of proteins, as at such low temperatures the nitrogen content of the phosphotungstic acid precipitate is out of all proportion to the basic amino-acids present.

EXPERIMENTAL.

Cystine was prepared from human hair without the use of sodium acetate for precipitation. After two recrystallisations, using sodium hydroxide for neutralisation, the preparation was analysed. (Found: N, 11.61; S, 26.58%. $C_6H_{12}O_4N_2S_2$ requires: N, 11.66; S, 26.67%.) For precipitation, solutions of the cystine were made up in varying concentrations in $N/5$ hydrochloric acid and the exact quantity of the amino-acid present ascertained by micro-Kjeldahl determinations on aliquots. Known volumes of the solution were pipetted out into stoppered pyrex bottles calibrated to contain 40 ml., 20 ml. of a solution containing 3 g. of 24-phosphotungstic acid (Kahlbaum) and 3.6 g. concentrated hydrochloric acid were added and the volume was made up with water to exactly 40 ml. After redissolving the precipitate by placing the bottles in a boiling water-bath they were allowed to stand for 48 hours either at room temperature, in a thermostat at 20° or in the refrigerator according to the temperature at which the precipitability was to be determined. The contents of the bottles were then decanted through a dry filter and the nitrogen in the filtrate determined in aliquots (referred to the original volume of 40 ml.) by the micro-Kjeldahl method. The amount of cystine-N in the precipitate was then calculated by difference.

SUMMARY.

The precipitation of cystine by 24-phosphotungstic acid at varying concentrations of the former has been studied under the usual conditions of protein analysis with the following results.

1. The extent of the precipitation at any particular temperature is dependent on the concentration of cystine present.
2. Below a certain minimum concentration of cystine no precipitation takes place, so that a protein may contain cystine and still show no cystine-S in the phosphotungstic acid precipitate.
3. Precipitation does not appear to be complete at any concentration. At the concentrations of cystine usually present in protein hydrolysates only a small fraction is precipitated. Cystine-N values based upon this method are therefore of no significance.
4. Nearly quantitative precipitation is obtained only at such concentrations or at such low temperatures as are inapplicable in the analysis of proteins.

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