CXLV. ANALYSIS OF PROTEINS.

VII. DIRECT ESTIMATION OF ARGININE.

BY ROBERT HENRY ADERS PLIMMER

AND JOHN LEWIS ROSEDALE (*Beit Memorial Research Fellow*).

From the Chemical Department, St Thomas's Hospital Medical School, London.

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ARGININE is estimated in the Van Slyke method of determining the nitrogen distribution in proteins by decomposition of the di-amino fraction by boiling with sodium hydroxide. As far as is known, no other amino-acid, with the possible exception of cystine, yields ammonia under these conditions. Histidine was found by Plimmer [1916] to give a very small amount. Consequently, it should be possible to determine arginine directly in the solution of hydrolysed protein without precipitation by phosphotungstic acid. On making experiments in this way, it was found that higher results were obtained. On further examination, the mono-amino filtrate from the phosphotungstate precipitate was found to give amounts of ammonia corresponding to the difference. In Part V [1925] it was shown that the errors of the Van Slyke method arose from incomplete precipitation by phosphotungstic acid. If no other amino-acid gives ammonia under these conditions, the arginine content of a protein is the sum of the values obtained from the di-amino and mono-amino fractions, or the value obtained by direct estimation without precipitation by phosphotungstic acid.

EXPERIMENTAL.

The same solutions of hydrolysed proteins were used as described in the previous communications.

Separate portions of 100 cc. were treated with lime to remove amide and humin nitrogen. An equal volume of 40 % sodium hydroxide was added and the solution boiled under a reflux condenser for 6 hours [Plimmer, 1916]. The values for the di-amino fractions were given in the previous papers. The mono-amino fractions were also tested by heating 50 cc. quantities from the experiments in a similar way.

The results, all calculated per 100 cc. of original solution, in terms of cc. $N/10$ were as follows:

	Direct	Di-amino fraction	Mono-amino fraction	Sum of di-amino and mono-amino	N % of total N
Haemoglobin	13.8	10.3	6.0	16.3	13.4
Caseinogen I	12.5	9.0	3.5	12.5	10.4
Caseinogen II	12.0	8.8	3.7	12.5	10.6
Gelatin III	27.8	23.5	3.0	26.5	17.8
Egg-yolk proteins	17.5	14.0	4.5	18.5	19.2
Egg-white proteins	17.4	12.5	4.5	17.0	16.0
Egg-membrane	24.8	19.0	6.0	25.0	22.2
Ovomucoid	21.4	11.8	8.5	20.3	18.6

In all cases, the amount of arginine by direct estimation was higher than by determination in the di-amino fraction. The amount in the mono-amino filtrate in most cases corresponds with the difference. A few values are not concordant. The agreement is better in the experiments in which our direct procedure for the whole series of manipulations was used. The error is greatest in the case of haemoglobin; it was not possible to repeat the experiment as no more of the same solution was available.

The presence of arginine, assuming the absence of an unknown amino-acid, in the mono-amino fraction is further evidence of incomplete precipitation by phosphotungstic acid. This was shown in the previous communication to be a cause of the irregularities in the results by the Van Slyke method. The behaviour of cystine under these conditions has still to be ascertained, but as this compound is not present in gelatin, caseinogen and haemoglobin the only conclusion at present is that arginine as estimated is too low, and that its real value is that obtained by direct estimation or the sum of the figures from the di-amino and mono-amino fractions.

SUMMARY.

Arginine, estimated directly in solutions of hydrolysed proteins by boiling with sodium hydroxide, has a higher value than if estimated in the di-amino fraction precipitated by phosphotungstic acid. On treating the mono-amino fraction in the same way, a value is obtained representing the difference. The arginine value of a protein is the sum of the figures obtained from the di-amino and mono-amino fractions, or the figure obtained directly, assuming that no other amino-acid behaving like arginine is present in proteins.

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REFERENCES.

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