X. THE ANALYSIS OF PROTEINS. I. THE ESTIMATION OF ARGININE BY DECOMPOSITION WITH ALKALI.

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In the procedure suggested by van Slyke [1911] for the analysis of proteins, the arginine, which is precipitated together with the histidine, lysine and cystine by phosphotungstic acid, is estimated by boiling the solution containing these four compounds with 50 % caustic potash for six hours. Under these conditions the guanidine grouping of the arginine is decomposed with the formation of ammonia, which is collected in excess of standard acid and determined by titration. Twice the amount of the nitrogen as ammonia corresponds to the amount of arginine according to the equation:

In carrying out analyses of proteins by this method difficulty has been experienced in obtaining data for the amount of arginine. Numerous determinations have been lost through the action of the strong caustic alkali upon the glass vessels and duplicate analyses have not agreed with one another. Further, results obtained by the substitution of a copper vessel in which the alkaline decomposition was carried out were generally higher than those obtained with glass vessels. It became necessary therefore to enquire more closely into the decomposition of arginine and the other compounds by caustic alkali.

Previous workers have not described any detailed experiments upon the action of alkali upon arginine. Schulze and Likiernik [1891] described the

formation of urea by boiling arginine with saturated baryta water and of ammonia and carbon dioxide by heating arginine under pressure with baryta water. Osborne, Leavenworth and Brautlecht [1908] found that arginine was slowly decomposed by distillation with 0.1 N sodium hydroxide, and van Slyke [1911] makes no other statement than that arginine is completely decomposed by boiling with 50 % caustic potash (12.5 g. KOH to 25 cc. solution) for six hours.

Cystine, as is well known and as was pointed out by van Slyke, is decomposed by boiling with alkali, but its amount in proteins other than the keratins is usually so small that the amount of ammonia evolved from it is negligible: in the case of the keratins van Slyke gives a correction.

Lysine is a diamino acid and is stable to alkali.

Histidine contains a glyoxaline ring in its molecule and it is possible that this ring is broken down with liberation of ammonia by the drastic treatment with caustic alkali.

(1) *Action of Caustic Alkali upon Arginine.*

The arginine used in these experiments was prepared by the usual method from gelatin and isolated as carbonate. This salt, instead of the nitrate, was chosen so as to avoid any possibility of the action of nitrates upon the decomposition, but it is more difficult to obtain in a pure state. The recrystallised preparation consisted partly of carbonate and partly of free base as shown by its nitrogen content of 26.2 %; the free base contains 32.0 % and the carbonate 23.7 %.

Solutions of this preparation of about 0.1 % concentration were gently boiled with caustic soda of various strengths for periods of 6 to 11 hours either in a glass flask of about 200 cc. capacity or in a copper flask of about 400 cc. capacity; to the flask was attached a condenser and to the condenser a U-tube of the pattern devised by Folin. The experiments were carried out exactly as described by van Slyke.

The results were as follows:

(a) *With the copper flask.*

Concentration of NaOH %	Time of boiling hours	NH ₃ evolved in cc. 0.1 N	One-half total nitrogen in solution in cc. 0.1 N
10	6	9.2	11.4
50	6	27.4	28.5
20	6	10.7	11.4
20	8	8.3	8.7
20	8	7.6	8.0

(b) With a glass flask.

	Concentration of NaOH %	Time of boiling hours	NH ₃ evolved in cc. 0.1 N	One-half total nitrogen in solution in cc. 0.1 N
I	4	6	17.2	28.5
	10	6	8.8	11.4
	20	6	10.6	11.4
II	20	8	7.8	8.0
	50	6	7.3	8.0
	20	6	7.3	8.0
	20	6	7.3	8.0
	20	6	7.3	8.0
III	20	7	3.2	2.8
	20	7	2.5	2.8
	20	6.5	3.2	2.8
	20	6.5	2.7	2.8

The experiments were performed in three groups with different quantities of total nitrogen in solution. In the first group it was found at once that 4 and 10 % caustic soda did not give an amount of ammonia corresponding to half the amount of arginine nitrogen in solution, and that 20 % gave nearly the correct amount. In the second group confirmation of the previous result was obtained; the same result was given when 50 % caustic soda was used. Further confirmation is given by the experiments in the third group. In all experiments an amount of ammonia corresponding to half the total was never obtained. This was probably due to impurity in the preparation of arginine, which was not purified by conversion into the nitrate or the copper nitrate double salt. The experiments show that the estimation of arginine can be effected by boiling with 20 % caustic soda in place of 50 %.

In practice it is more convenient to add an equal volume of 40 % caustic soda to the solution than to weigh out the correct quantity of soda; in this way also a larger volume of liquid is contained in the flask. After boiling for six hours it is necessary according to van Slyke's directions to disconnect the parts of the apparatus and distil off the remaining ammonia which does not diffuse into the U-tube. Loss of ammonia may thus occur; it is sometimes noticeable by its smell. By using an equal volume of 40 % sodium hydroxide and consequently more liquid in the flask, it is not necessary to disconnect the parts of the apparatus; the ammonia remaining in the flask and condenser can be distilled directly into the U-tube if the water be run out of the condenser and the boiling continued for another 15 to 20 minutes.

The same results are obtained when the decomposition is carried out in a copper flask, but as will be seen from the data given by histidine under the same conditions it is not possible to use a copper flask.

(2) *Estimation of the Total Nitrogen of the Bases in the Solution.*

In van Slyke's procedure the total nitrogen of the arginine and of the other compounds in the solution is determined in the residue remaining after the arginine estimation. Owing to the presence of silica in the solution, dissolved by the alkali from the glass, bumping occurs and nitrogen is lost. This is lessened by using 20 % sodium hydroxide in the arginine estimation but is not eliminated; it is avoided by using a copper flask, but as previously stated a copper flask cannot be used.

It is advisable to estimate the total nitrogen in a separate portion (5 cc.) of the solution of the bases rather than in the residue.

(3) *Action of Caustic Alkali upon Histidine.*

The histidine used in these experiments was prepared by the usual method from defibrinated blood and isolated as hydrochloride. The preparation gave the following figures on analysis:

$$\text{H}_2\text{O} = 8.55; \text{N} = 19.8; \text{Cl} = 16.7 \%$$

Calculated for

20 or 25 cc. of approximately 0.5 % histidine hydrochloride solution were boiled with caustic soda of varying concentration in a glass or copper flask in the same way as described above in the case of arginine. The results were as follows:

(a) *With the copper flask.*

Concentration of NaOH %	Time of boiling hours	NH ₃ evolved in cc. 0.1 N	Total nitrogen of histidine solution in cc. 0.1 N
50	6	3.3	14
50	6	4.6	23
25	6	1.9	13
25	11	2.1	13
20	8	1.9	13

(b) *With a glass flask.*

Concentration of NaOH %	Time of boiling hours	NH ₃ evolved in cc. 0.1 N	Total nitrogen of histidine solution in cc. 0.1 N
50	2*	0.3	14.0
50	6*	0.3	14.0
50	6	0.4	23.0
20	6	0.5	13.0

* In these experiments the glass flask was destroyed by the alkali and the ammonia given in the table had diffused into the standard acid.

Histidine is thus decomposed by boiling with caustic alkali. The amount of decomposition in the cases in which a copper flask was used corresponds to about one-fourth of the total nitrogen of the histidine but the figures are not exact multiples and vary considerably. The amount of decomposition in the experiments in which a glass flask was used is very much less and corresponds to from 1.5 to 3 % of the total nitrogen in the solution.

This slight decomposition of histidine may however be neglected in the estimation of arginine; to some extent it makes up for the small loss which occurs in the determination of the arginine.

SUMMARY.

The estimation of arginine can be effected by boiling with 20 % sodium hydroxide, instead of 50 %, for six hours. The small loss of ammonia which occurs is counterbalanced by the slight decomposition of histidine which takes place when a solution of bases as obtained by van Slyke's method is being analysed.

The estimation cannot be effected in a copper flask if histidine is present, as under these conditions histidine undergoes considerable decomposition. It is advisable, on account of bumping with consequent loss of nitrogen, to estimate the total nitrogen of the arginine and other bases in a fresh portion of the solution rather than in the residue from the arginine estimation.

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