XXIV. THE FREE AMINO NITROGEN OF THE DIFFERENT PROTEINS OF OX AND HORSE SERUM.

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In a recent communication van Slyke and Birchard [1913] have shown that when certain native proteins are treated with nitrous acid the amount of nitrogen formed as a result of the reaction is equal to one-half the lysine nitrogen of the protein. They suggest that one of the amino groups of lysine, the ω -group, exists free in the intact protein molecule, and, moreover, that this ω -amino nitrogen of the lysine represents practically the entire amount of free amino nitrogen which can be demonstrated by the nitrous acid method of van Slyke. All the amino acids, except lysine, react quantitatively with nitrous acid in three to four minutes; lysine reacts more slowly, thirty minutes being required for complete reaction, and van Slyke and Birchard show that reaction between nitrous acid and the native proteins also requires about thirty minutes to attain completion. The authors quote observations of other workers which support their view that the free amino nitrogen of native proteins is due to the ω -amino group of the lysine which they contain. Gelatin, caseinogen and serum globulin after treatment with nitrous acid yield no lysine on subsequent hydrolysis with acid. Clupeine, which contains no lysine, yields no nitrogen on treatment with nitrous acid, while sturine and cyprinine, which contain lysine, do. It has also been shown that certain protamines and proteins which contain lysine reveal free amino groups by the formal titration method of Sørensen, while those which contain no lysine do not.

The analysis of the different proteins of ox and horse serum, an account of which has recently appeared [Hartley, 1914], shows that albumin differs in several important respects from the globulins. In particular it has been found that albumin contains a much larger amount of lysine than the globulins. The experiments described in this paper have been carried out in order to study the relationship between the free amino nitrogen of the different serum proteins and their lysine content.

The preparation of the different serum proteins has been described in the earlier paper. For the determination of the amino nitrogen the following procedure was adopted in each case. Two weighed quantities of the air-dried preparations were taken: one of these was used for the determination of the total nitrogen of the protein by Kjeldahl's method and the other was dissolved in water or very dilute alkali in a standard 15 cc. flask. Ten cc. of this solution were treated with nitrous acid in van Slyke's apparatus and the free amino nitrogen of the protein determined. The reaction was conducted in the standard size apparatus described by van Slyke [1912], 1 cc. of octyl alcohol being added in each case to prevent frothing. The mixture of nitrous acid and protein was shaken at intervals for twenty-five minutes and finally shaken continuously for five minutes.

The results of the determination of the total nitrogen and the free amino nitrogen are collected in the following tables.

Total globulin is the name given to the proteins precipitated from the diluted serum by half saturation with ammonium sulphate. It consists of pseudoglobulin and euglobulin. Euglobulin *A* was prepared from the total globulin by salting out with ammonium sulphate, and euglobulin *P* was prepared by the dilution and acidification method of Panum.

TABLE I.

Total Nitrogen Determinations.

No.	Protein	Quantity taken g.	N/10 H ₂ SO ₄ neutralised cc.	Nitrogen mgm.
1.	Albumin (ox)	0.2065	18.8	26.32
2.	Total globulin (ox)	0.2110	19.9	27.86
3.	Albumin (horse)	0.2562	23.7	33.18
4.	Total globulin (horse)	0.2604	24.9	34.86
5.	Pseudoglobulin (horse) ...	0.2882	27.1	37.94
6.	Euglobulin <i>A</i> (horse)	0.2894	26.4	36.96
7.	Euglobulin <i>P</i> (horse)	0.3047	25.2	35.28
8.	Whole proteins (horse) ...	0.2761	23.3	32.62

TABLE II.
Amino Nitrogen Determinations.

No.	Protein	Quantity taken gm.	N gas cc.	Temp. °C.	Pressure mm.	Amino N mgm.	Per cent. of total N as free NH ₂
1.	Albumin (ox)	0.5157	9.9	16	755	5.70	8.7
2.	Total globulin (ox) ..	0.5013	4.8	16	758	2.80	4.2
3.	Albumin (horse)	0.3875	7.0	17	762	4.05	8.0
4.	Total globulin (horse)	0.3932	5.2	19	762	2.98	5.6
5.	Pseudoglobulin (horse)	0.2806	3.1	15	760	1.81	4.6
6.	Euglobulin A (horse)	0.4096	5.4	17	749	3.07	5.8
7.	Euglobulin P (horse)	0.4050	4.3	17	749	2.44	5.2
8.	Whole protein (horse)	0.3963	5.1	19	762	2.92	6.2

In Table III the results of the determination of the free amino nitrogen are given and compared with the lysine content of the different proteins. The figures for the lysine are taken from the earlier paper [1914] dealing with the analysis of the different proteins of ox and horse serum.

TABLE III.

No.	Protein	Per cent. of total nitrogen as	
		Free amino N	Half lysine N
1.	Albumin (ox)	8.7	8.15
2.	Total globulin (ox)	4.2	4.50
3.	Albumin (horse)	8.0	7.95
4.	Total globulin (horse)	5.6	5.40
5.	Pseudoglobulin (horse)	4.6	4.90
6.	Euglobulin A (horse)	5.8	4.97
7.	Euglobulin P (horse)	5.2	5.05
8.	Whole proteins (horse)	6.2	6.40

Except in the case of Euglobulin A (horse) in which the result is a little high, the free amino nitrogen of the proteins of ox and horse serum is equal to one-half the lysine nitrogen. The results are in agreement with those obtained by van Slyke and Birchard for other native proteins and lend support to the suggestion advanced by them that in the intact protein molecule the ω -amino group of the lysine exists free, and, further, that this group represents practically the whole of the free amino nitrogen determinable by means of nitrous acid.

The results given above, taken in conjunction with those of van Slyke and Birchard, indicate that the lysine content of a native protein may be rapidly estimated by determining the amino nitrogen and the total nitrogen of the protein.

REFERENCES.

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