CXL. CHARACTERISATION OF VERY SMALL QUANTITIES OF PROTEINS BY VAN SLYKE'S METHOD.

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In the course of our studies on animal and vegetable proteins, it became necessary to deal with very small quantities of substances, about 100 to 150 mg., a twentieth of the quantity usually employed for a macro-analysis. Thimann [1926] has described a method for the determination of the Hausmann numbers in small quantities of proteins and we have extended the method for a more comprehensive characterisation of proteins by Van Slyke's method.

Experimental.

Digestion. 100 to 150 mg. of the protein were weighed out into a small Kjeldahl flask (4 × 30 cm.) fitted with a ground glass air-condenser and digested for 36 hours with 10 cc. of 20 % hydrochloric acid. The acid was then removed by distillation *in vacuo* (20–25 mm.) at 40–45°. Soda-lime was placed in the receiver and a slow current of ammonia-free air was passed during the distillation to facilitate removal of the acid. A fresh quantity of ammonia-free water was added and the distillation repeated under similar conditions. The titratable acidity of the digest after distillation in the successive duplicates lay between very narrow limits. This fact is of great importance in connection with the subsequent precipitation of the hexone bases, which has been shown by Knaggs [1923] to depend on the concentration of the salts present in the digest of the amino-acids.

The syrupy mass was dissolved in ammonia-free water and made up to about 40 cc. Two portions of 2 cc. each were taken for total nitrogen, two of 10 cc. each for the series of analyses and two of 5 cc. each for the estimation of arginine.

Amide nitrogen. Each of the 10 cc. portions was neutralised with $N/2$ alkali till just pink to phenolphthalein and, after adding a drop of the alkali in excess, the mixture was aerated for 2 hours at room temperature, air free from ammonia and carbon dioxide being passed. A few drops of amyl alcohol were added to prevent foaming. The ammonia was absorbed in 10 cc. of $N/70$ alkali.

Melanin nitrogen. The solution, after aeration, was carefully neutralised with $N/5$ hydrochloric acid and the melanin filtered through a small Jena glass filter covered with a thin layer of asbestos. The nitrogen in the precipitate was determined by the micro-Kjeldahl process.

Hexone bases. The filtrate was evaporated on a water-bath to about 5 cc., washed into a 20 cc. stoppered centrifuge tube and made up to nearly 10 cc. About 1 cc. of concentrated hydrochloric acid was then added. The tube was plugged with cotton wool and autoclaved for 30 mins. at 25 lbs. pressure [Knaggs, 1923]. 3 cc. of 25 % phosphotungstic acid solution were then added and the tube was placed in a boiling water-bath until the precipitate had almost redissolved. After cooling, the tube was placed in the ice-chest for 36 hours. The precipitate was then centrifuged, and washed in the centrifuge thrice with ice-cold hydrochloric acid (1–10), using 3 cc. each time. The clear centrifugate and the washings were passed through a small filter to recover any suspended particles. The combined precipitates were dissolved by the gradual addition of $N/2$ sodium hydroxide until the pink colour formed with phenolphthalein remained just permanent. The solution was made up to 25 cc. and 5 cc. were employed for total nitrogen and another 5 cc. for amino-nitrogen and 10 cc. for a micro-estimation of sulphur according to Pregl.

From the sulphur content the amount of cystine in the precipitate of the bases was calculated.

Arginine. Arginine was estimated by arginase which was prepared from the liver of a ram. The enzyme preparation was found to be free from aminases and amidases which liberate ammonia from amino-acids and amides and its activity on a solution of arginine was determined. 97 to 98 % of the arginine was found to be hydrolysed under the conditions which obtain in the accompanying experiments.

5 cc. portions of the original amino-acid digest were neutralised with $N/2$ sodium hydroxide, ammonia was removed by aeration as before and the p_H adjusted to 9·7 by adding a phosphate buffer. After addition of 0·5 cc. of the enzyme extract and 1 cc. of toluene, the reaction was allowed to proceed for 36 hours at 37°. Controls were run with 5 cc. of water and 0·5 cc. of the fresh enzyme extract. Urease solution, corresponding to half a pellet of Dunning's preparation, was then added to the reaction mixture, the p_H being readjusted to 7. After 12 hours' reaction at 37° a saturated solution of potassium carbonate was added in slight excess and the ammonia estimated by aeration.

From a knowledge of the content of cystine, arginine and the non-amino-nitrogen in the basic fraction, the histidine and lysine contents were calculated.

Mono-amino-fraction. The combined filtrates from the precipitation of the hexone bases were made up to 50 cc., of which two 10 cc. portions were used for total nitrogen and two 5 cc. portions for amino-nitrogen.

All ammonia distillations were carried out in the Parnas-Wagner modification of Pregl's micro-apparatus, and the amino-nitrogen determinations made with Van Slyke's micro-apparatus.

Table I. *Caseinogen.*

Results expressed as percentages of total nitrogen.

	Micro Narayana and Sreenivasaya	Macro Narayana and Sreenivasaya	Hoffman and Gortner [1924]	Dunn and Lewis [1921]	Van Slyke [1914]
Ammonia-N	9·90	10·12	10·20	10·49	10·27
Humin-N	1·16	1·86	1·51	2·13	1·28
Basic N:					
Arginine-N	9·89	9·01	9·20	7·42	7·41
Histidine-N	3·88	4·16	6·26	6·01	6·21
Cystine-N	0·96	0·68	1·05	0·48	0·21
Lysine-N	7·67	8·12	8·49	9·09	10·30
N in filtrate from bases:					
Amino-N	59·20	57·30	54·12	58·78	55·81
Non-amino-N	9·02	9·10	8·76	5·93	7·13
Total	101·68	100·35	99·59	100·33	98·61

Table II. *Gelatin.*

Results expressed as percentages of total nitrogen.

	Micro Narayana and Sreenivasaya	Macro Narayana and Sreenivasaya	Micro Thimann [1926]
Ammonia-N	1·84	1·70	2·60
Humin-N	Trace	0·56	0·00
Basic N	22·61	25·04	17·60
Arginine-N	15·87	—	—
Histidine-N	2·74	—	—
Lysine-N	4·00	—	—
N in filtrate from bases	76·2	71·06	79·60
Amino-N	57·40	—	—
Non-amino-N	18·80	—	—
Total	100·65	98·36	99·8

SUMMARY.

A method for the characterisation of very small quantities of proteins by Van Slyke's method has been described.

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