„Bound" Chlorine in Casein and in Tissue Proteins

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Lindahl [1] recently analyzed casein and found that it contained about one per cent chlorine in bound form. In the analytical method used by him the casein was treated with a boiling mixture of chromic and sulphuric acids and the gases evolved passed through a known quantity of silver nitrate containing As_2O_3. The consumption of silver nitrate, assumed to be due to the chlorine in the casein was determined by the Volhard method. As the presence of chlorine in casein does not agree with the generally accepted opinion on the composition of this protein it seemed necessary to check Lindahls results. At the suggestion of Professor E. Jorpes I undertook to control the analytical procedure. Dr. Ragnar Berg who performed the analyses for Lindahl kindly demonstrated his technique in our laboratory, thereby greatly facilitating our work.

Casein, prepared by isoelectric precipitation with acetic acid was analyzed for chlorine by the Carius method. No trace of chlorine could be detected. When the analysis was performed with the technique used by Lindahl a precipitate insoluble in nitric acid was actually formed and the Volhard titration showed a consumption of silver nitrate corresponding to 0.25 per cent chlorine in the casein. It was however observed that the precipitate differed from silver chloride in that it did not darken when exposed to sunlight. When fused with Na_2CO_3 no chloride was obtained in the alkaline filtrate whereas a similar quantity of silver chloride gave a quantitative precipitate with $AgNO_3$ on acidification with HNO_3. Evidently the precipitate formed in the trap with silver nitrate was not silver chloride. Of the different silver salts that could come in question the formiate, carbonate and acetate are readily soluble in dilute nitric acid. The oxalate darkens easily in sunlight. The silver cyanide, however, is practically insoluble in dilute and only slowly soluble in concentrated nitric acid. Moreover it does not darken when exposed to sunlight. Consequently the precipitate was assumed to be silver cyanide. This assumption was confirmed by analyses of the sample. A qualitative test for the cyanide ion with ammonium polysulphide and ferric chloride was positive. On ignition of 31.265 mg the silver residue weighed 25.065 mg or 80.17 per cent. The calculated amount of silver in silver cyanide is 80.57 per cent. In another sample the ignition residue was dissolved in nitric acid, precipitated with hydrochloric acid

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ammonia assimilation (Table 1). The selective inhibitory effect of sodium fluoride on ammonia assimilation largely depends on the initial pH of the solution (Fig. 2).

The experiments show that the pH and the inhibitors have a different influence on ammonia and nitrate assimilation. In some cases the assimilation of ammonia is inhibited in a very high degree while that of nitrate is not disturbed. It seems therefore that even in the case of azotobacter the assimilation of nitrate does not necessarily proceed through ammonia but it can have other ways too.

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A Note on the Amino Acid Content of Bence-Jones Protein

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Dent and Rose [1] recently published a chemical study of Bence-Jones protein with special reference to its methionine content. We have for some time been engaged in a study of the amino acid content of this protein and the following short report may be given.

From a patient with multiple myelomatosis Bence-Jones protein was isolated by means of the classical heat reaction. A comparison was made of the amino acid content of two samples of protein, one prepared from urine, the other from a tumor extirpated for diagnostic purposes. Analysis of the hydrolyzed materials were carried out by paper chromatography (Consden, Gordon and Martin [2]), with some slight modifications [3]. Typical photographs of the chromatograms are given in Figs. 1 and 2.

A comparison of the spots in Figs. 1 and 2 shows that the protein isolated from

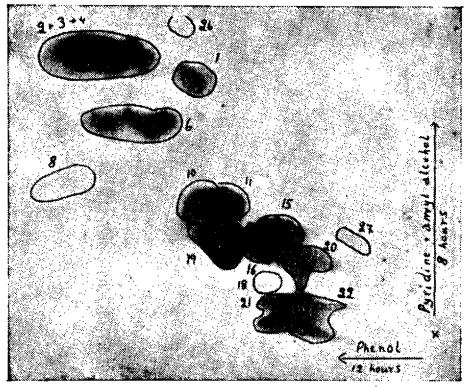

Fig. 1. Phenol-cupron (1 %)/pyridine-amyl alcohol chromatogram, showing the positions of the amino acids from 0.66 mg of hydrolyzed Bence-Jones protein prepared from a tumor.

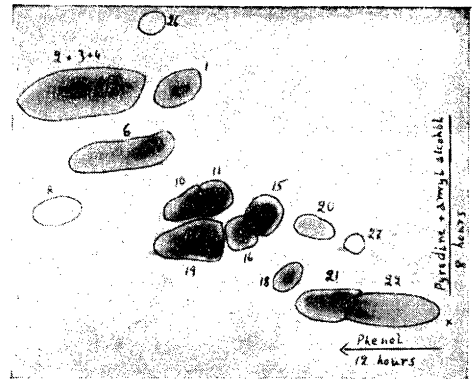

Fig. 2. Phenol-cupron (1 %)/pyridine-amyl alcohol chromatogram, showing the positions of the amino acids from 0.33 mg of hydrolyzed Bence-Jones protein prepared from urine.

1 = Tyr	*6 = Val*	*15 = Ser*	*20 = Asp*	*Abbreviations*
2 = Phe	*8 = Pro*	*16 = Gly*	*21 = Arg*	*according to*
3 = Ileu	*10 = Ala*	*18 = His*	*22 = Lys*	*Brand and Edsall* [7]
4 = Leu	*11 = Thr*	*19 = Glu*	*27 = (Cys-)$_2$*	

and the precipitate weighed. The silver content of the chloride corresponded to 80.2 per cent of the original sample.

Evidently this source of error in the chlorine determination escaped the attention of Lindahl. His conclusions about the protein bound chlorine in the body are consequently erroneous.

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