# 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

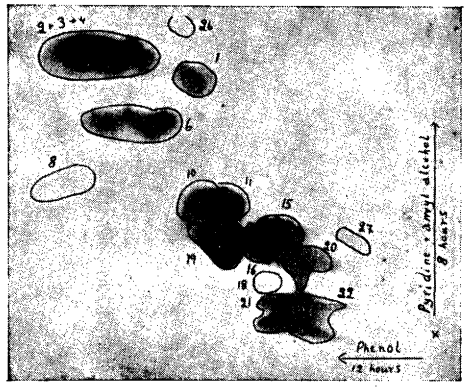


*Fig. 1. Phenol-cupron (1 %)/pyridine-amyl alcohol chromatogram, showing the positions of the amino acids from 0.66 mg of hydrolyzed Bence-Jones protein prepared from a tumor.*

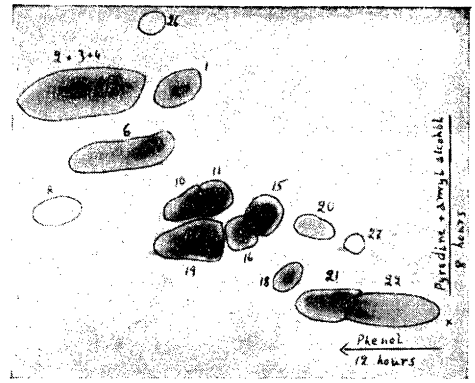


*Fig. 2. Phenol-cupron (1 %)/pyridine-amyl alcohol chromatogram, showing the positions of the amino acids from 0.33 mg of hydrolyzed Bence-Jones protein prepared from urine.*

| | | | | |
|---|---|---|---|---|
| *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.

1. Lindahl, O., *Acta Orthoped. Scand.* **18** (1948—1949) 28, 346.

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the two sources contain most of the common amino acids with the exception of methionine and hydroproline. From previous experience [3, 4] we know that the spots of these two amino acids would appear on the two-dimentional chromatograms with the amounts of hydrolyzed material used in the present investigation if they were present in amounts, normally occuring in proteins and plasma filtrates [3, 5]. Our results thus would seem to corroborate those of Dent and Rose [1]. However, it may be pointed out that Bence-Jones protein according to Devine [6] contains 0.6 per cent of methionine. This means that even on the chromatograms where 0.66 mg of hydrolyzed protein was analyzed (Fig. 1) the smallest amount of methionine (8 $\mu$g), which would give a spot on the paper, was not present. When larger amounts of material were analyzed clearly separated spots were not obtained. Devine [6] also reported that Bence-Jones protein did not contain hydroxyproline.

No attempt has been made to compare the amino acid content of the two samples of protein from a quantitative point of view but it is obvious from an inspection of the two series of chromatograms with different amounts of total nitrogen applied to the paper that the sizes and colour intensities of the spots are very similar. Microbiological determinations of the amino acids are at present carried out and will definitively decide on this point.

1. Dent, C. E., and Rose, G. *Biochem. J.* **43** (1948 liv.
2. Consden, R., Gordon, A. H., and Martin, A. J. P. *Biochem. J.* **38** (1944) 224.
3. Ågren, G. In press.
4. de Verdier, C. H., and Ågren, G. *Acta Chem. Scand.* **2** (1948) 783.
5. Block, R. J., and Bolling, D. *The amino acid composition of proteins and foods.* (1945).
6. Devine, J. *Biochem. J.* **35** (1941) 433.
7. Brand, E., and Edsall, J. T. *Ann. Rev. Biochem.* **16** (1947) 223.

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## Some New Phenothiazine Derivatives of Pharmacological Interest

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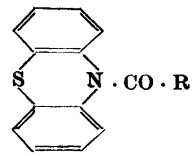
If phenothiazine (1 mole), dissolved in boiling benzene, is allowed to react with a halogeneacylhalogenide (1.5 mole), hydrogene halogenide is evolved and the resulting 10-halogeneacylphenothiazine separates from the cooled reaction mixture.

*Table 1. Halogeneacylphenothiazines.*



| R | M. p. °C |
|---|---|
| $-CH_2 \cdot Cl$ | 115—116.5 |
| $-\overset{CH_3}{\underset{H}{C}}-Br$ | 147.5—148.5 |
| $-CH_2 \cdot CH_2 \cdot Cl$ | 142—143 |
| $-\overset{C_2H_5}{\underset{H}{C}}-Br$ | 120—121 |

The halogene compounds react easily with primary, secundary and cyclic amines (cyclohexyl-, dimethyl-, diethylamine and piperidine) when heated with the amine (2.6 mole) to 70° in benzene solution (sealed tube). The reaction mixture is filtered and the filtrate evaporated. The residue is recrystallised or, when oily, transferred to hydrochloride.