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The Determination of Free Amino Acids in Blood Plasma

By D. K. J. TOMMEL, J. F. G. VLIEGENTHART and J. F. ARENS. (*Laboratory of Organic Chemistry, State University, Utrecht, The Netherlands*)

The importance of the determination of free amino acids and amines in small quantities of blood plasma is evident, e.g. in cases of inborn errors of metabolism. In several routine methods the proteins of the blood plasma are precipitated and subsequently the free amino acids are determined in the remaining solution. The disadvantages of precipitation techniques may be losses of amino acids due to inclusion or absorption or both. In an attempt to avoid these complications we decided to apply a method (Tommel, Vliegenthart, Penders & Arens, 1968) recently described for the separation of α-amino acids and peptides. The latter separation procedure utilizes the difference in charge of the cupric chelates of peptides and α-amino acids respectively.

In anion-exchange chromatography at pH 8·0 the peptide chelates are retarded on the column; the amino acid chelates, however, are eluted. The experiment was performed as follows. To 1ml. of human blood 0·2mg. of heparin was added. (Citrate cannot be applied, as it interferes during the anion-exchange chromatography.) The blood plasma was obtained by centrifugation. Plasma (250μl.) was diluted with an equal volume of 0·01M-2,4,6-collidine. Thereafter 50mg. of cupric carbonate was added and the mixture was magnetically stirred for 2hr. at room temperature. After removal of the excess of cupric carbonate by centrifugation the blue supernatant was applied to a column (10cm. × 0·6cm.) of TEAE-cellulose in the acetate form, which was equilibrated with 0·01M-2,4,6-collidine–acetate buffer, pH 8·0. The column was eluted with the 2,4,6-collidine–acetate buffer. The first 7ml. of eluate, containing the amino acid chelates, was collected in bulk. Cu^{2+} was removed from the eluate by extraction with a solution of 8-hydroxyquinoline in chloroform (concentration 6mg./ml.). Subsequently, the eluate was washed with chloroform to avoid a possible contamination with 8-hydroxyquinoline. In the remaining solution the amino acids can be quantitatively determined, e.g. by paper chromatography or by means of an automatic amino acid analyser. It is noteworthy that aspartic acid and glutamic acid remained partially on the column with the proteins. The content of these amino acids in plasma could not be determined. The amino acid fraction turned out to be completely free of proteins and of peptides.

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