The Determination of Free Amino Acids in Blood Plasma

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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 1 ml. of human blood 0.2 mg. of heparin was added. (Citrate cannot be applied, as it interferes during the anion-exchange chromatography.) The blood plasma was obtained by centrifugation. Plasma (250 μ l.) was diluted with an equal volume of 0.01 M-2,4,6-collidine. Thereafter 50 mg. of cupric carbonate was added and the mixture was magnetically stirred for 2 hr. at room temperature. After removal of the excess of cupric carbonate by centrifugation the blue supernatant was applied to a column (10 cm. $\times$ 0.6 cm.) of TEAE-cellulose in the acetate form, which was equilibrated with 0.01 M-2,4,6-collidine-acetate buffer, pH 8.0. The column was eluted with the 2,4,6-collidine-acetate buffer. The first 7 ml. 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 6 mg./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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The Biological Half-life of Pulmonary Surface-Active Agent

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Pulmonary surface-active agent is remarkable for the large amount of highly saturated (mainly dipalmitoyl) phosphatidylcholine that it contains (Abrams, 1966; Tierney, Clements & Trahan, 1967). To try and measure its turnover rate and to determine the possible synthetic pathway of this phosphatidylcholine, the incorporation of [^{14}C]-glucose into pulmonary surface-active agent has been studied. [^{14}C]Glucose was injected intravenously into New Zealand White rabbits (wt. 2.5–3 kg.), and the animals were killed with intravenous pentobarbital at various times up to 116 hr. The lungs were removed rapidly and used (a) for extraction of surface-active agent (Abrams, 1966) and (b) after homogenization in chloroform-methanol (2:1, v/v), for extraction of total lipid (Folch, Lees & Sloane-Stanley, 1957). The lipid extracts were further fractionated by column chromatography on silicic acid and thin-layer chromatography (silica gel G or H). The ^{14}C content and weight of the various fractions were determined and specific radioactivities were calculated. The results indicated a biological half-life for the phosphatidylcholine fraction of about 28 hr. in surface-active lipid and about 35 hr. in total lung lipid. Some of the non-phospholipid constituents of the total lung lipid were also examined and the following biological half-lives found: 1,2-diglycerides, 40 hr.; 1,3-diglycerides, 70 hr.; triglycerides, 35 hr.; free fatty acids, 40 hr. A phospholipid present in surface-active agent in small amount (approx. 3% of the total phospholipid) was found to have a high specific radioactivity and a half-life of about 15 hr. This phospholipid has been tentatively identified as one of the lysobisphosphatidic acid compounds described by Body & Gray (1967) and thus lends support to their suggestion that 'these phospholipids are metabolically "highly active" and are acting as "carriers" for the palmitic acid at some stage in a reaction sequence involving lung lecithin, lipolytic enzymes and acyl CoA-acyltransferases, the end product being dipalmityllecithin.'

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