Amino Acid Conjugates in Blood and Urine. I. A Simple Method for N-Substituted Amino Acid Determination

There are two sources of amino acids liberated during the hydrolysis of deproteinized serum and urine: peptides and ninhydrin-negative conjugates[1,2]. Up to now, the authors have determined amino acids bound in these conjugates after separating them from peptides and free amino acids on the cationic ion-exchangers[3-5] in the support of a non-ampholitic character of the conjugates. The same feature may be helpful in electrophoresis separation, as was suggested recently by YOUNG[6].

The purpose of the present investigation was to elaborate a simple method for separation of free amino acids and peptides from ninhydrin-negative conjugates. In consequence, the possibility arose of determination of N-substituted amino acids.

Materials and methods. Material for the examinations was blood and urine of healthy people. Serum and urine were deproteinized using 95% ethanol, after CAWLEY et al.[7].

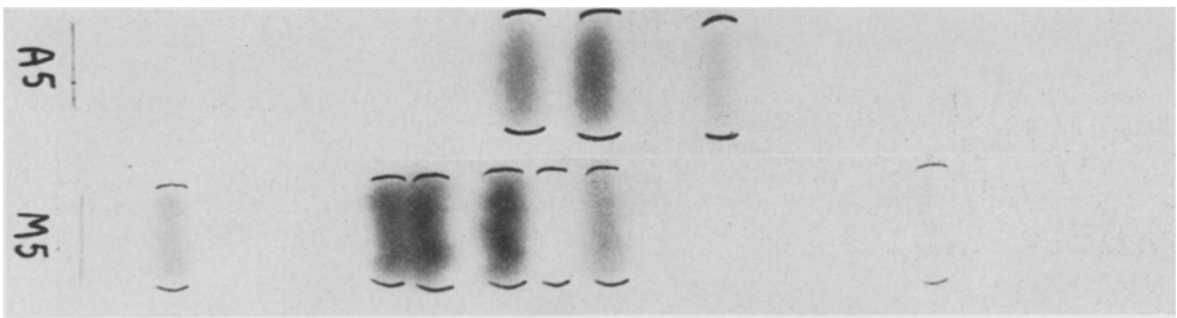
The solution examined was dropped 9 cm from the shorter side of the plate (18 × 25 cm) covered with silica gel – Kieselgel G (Merck). The plates were sprayed with buffer pH 2.0 (0.6 *N* formic acid and 2.0 *N* acetic acid 1:1 v/v) and were brought to the electrophoretic apparatus for 1 h (250 V, 8.4 mA). The electrophoregrams were next dried and developed with the ninhydrin reagent. The most slowly moving ninhydrin-positive spot was 1.4 cm from the starting line in the cathode direction. On the plates the ninhydrin-negative zone (2 × 6 cm) was designed with the starting line inside 1 cm from the zone cathodic border.

Gel in the zone region was taken off the plate and the ninhydrin was inactivated by heating the gel together with 4 ml 3% H_2O_2. Reaction lasted 2 min in the temperature of the ebullition of the solution, which was then evaporated to dryness. The dry rest was next hydrolysed with the aid of 6 *N* HCl for 16 h at a temperature of 105 °C. The hydrolysates after removal of the hydrochloric acid were examined chromatographically. One directional ascending paper chromatography with the eluent *n*-butanol–glacial acetic acid–water 4:1:1 v/v was used. For the determinations of glycine and serine, the solvent methyl ethyl ketone–piridine–water–acetic acid 70:15:15:2 v/v was also used. Chromatograms were developed with 0.15% acetic solution of ninhydrin. The manner of chromatographic separation was given in another work[8].

The Figure demonstrates chromatograms of the amino acids liberated after the hydrolysis of the ninhydrin-negative gel zone from the electrophoregrams of blood and urine. The quantitative determinations were made by the method of photometry of the negative printed chromatograms[9] with modification[10].

Discussion. The method demonstrated permitted a better knowledge of the ninhydrin-negative amino acid conjugates. In consequence of using electrophoresis on silica gel, the duration of analysis was shortened to 1 h, while the column method requires many hours[5,11,12]. The accuracy of quantitative determinations was a difficult problem. In the present work, the standard mixture of amino acids used for calculations passed all analysis (after electrophoresis) which was helpful in the elimination of the eventual loss. Thus the method presented makes possible a serial examination of amino acids bound in ninhydrin-negative conjugates.

The problem of the character and the role of these compounds in human physiology and pathology will be the subject of future work.



Chromatogram of amino acids bound in the ninhydrin-negative conjugates. A 5, blood; M 5, urine.

Zusammenfassung. Eine neue, vereinfachte, elektrophoretische Trennungstechnik zur quantitativen Bestimmung von Aminosäuren in Körperflüssigkeiten in konjugierter Form oder als ninhydrin-negative Polypeptide wird beschrieben.

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