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Note

Effect of the presence of glucose on the determination of amino acids by gas–liquid chromatography

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The speed, sensitivity and low initial capital outlay makes gas–liquid chromatography (GLC) an ideal tool in the analysis of amino acids in biological fluids and tissues. Despite the advantages GLC has to offer, however, its acceptance as a routine tool in amino acid analysis is significantly hampered by the laborious and time consuming steps necessary in sample purification prior to derivatisation. The main approach in plasma sample purification over the past 10 years has been one of deproteinisation followed by ion exchange "clean up" of the protein-free extract[1–5]. Although this approach yields a product pure enough for derivatisation, the recovery and reproducibility of results for certain amino acids are variable and often unacceptable. The ion-exchange resins that have been used seem to bind certain amino acids irreversibly[4,6,7] or to reduce[8] their elution by on-column reactions.

MATERIALS AND METHODS

Crystalline amino acids and heptafluorobutyric anhydride were obtained from Sigma, St. Louis, MO, U.S.A. All other chemicals were obtained from Merck, Darmstadt, F.R.G. Venous blood was obtained from laboratory personnel in the fasting state following their expressed consent. Plasma (0.5 ml) was deproteinised using 2 ml methanol–conc. hydrochloric acid (4:1), centrifuged at 2000 *g* at 4°C for 15 min and the supernatant was filtered through a Millex 0.22-μm filter (Millipore). N-Heptafluorobutyryl isobutyl ester (HBB) derivatives of the amino acids were prepared as described previously[9], but modified for derivatisation of 25-μg quantities of each amino acid[10]. GLC was performed using conditions described elsewhere[10]. Glucose when derivatised was present in approximately the concentration found in the plasma of normal people.

RESULTS AND DISCUSSION

The GLC analysis of derivatised crystalline amino acid standards is shown in Fig. 1. Glucose when taken through the same derivatisation procedure, yielded three products whose chromatogram is shown in Fig. 2. The interference caused by glucose in the analysis of amino acid standards is shown in Fig. 3 and the identification, by

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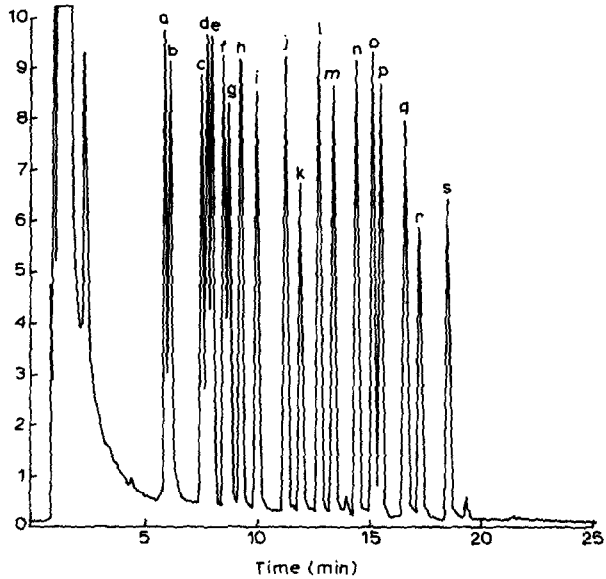

Fig. 1. GLC separation of HBB derivatives of amino acid standards. Peaks: a = alanine; b = glycine; c = valine; d = threonine; e = serine; f = leucine; g = isoleucine; h = norleucine (internal standard); i = proline; j = hydroxyproline; k = methionine; l = aspartic acid; m = phenylalanine; n = glutamic acid; o = lysine; p = tyrosine; q = arginine; r = histidine; s = tryptophan.

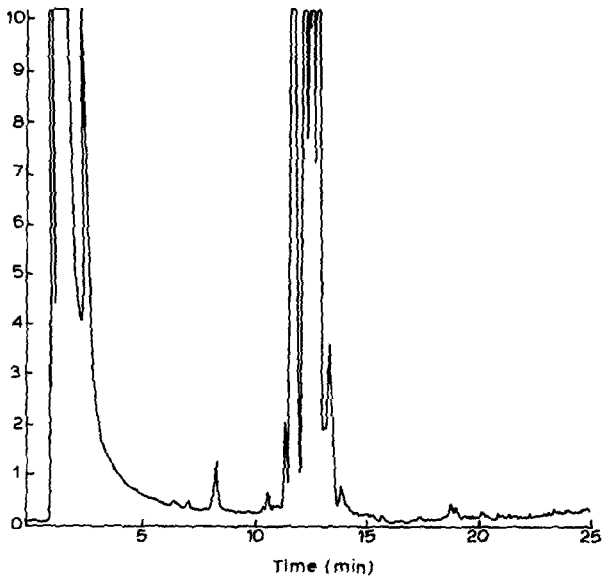

Fig. 2. Gas chromatogram of the product from glucose subjected to the derivatisation procedure.

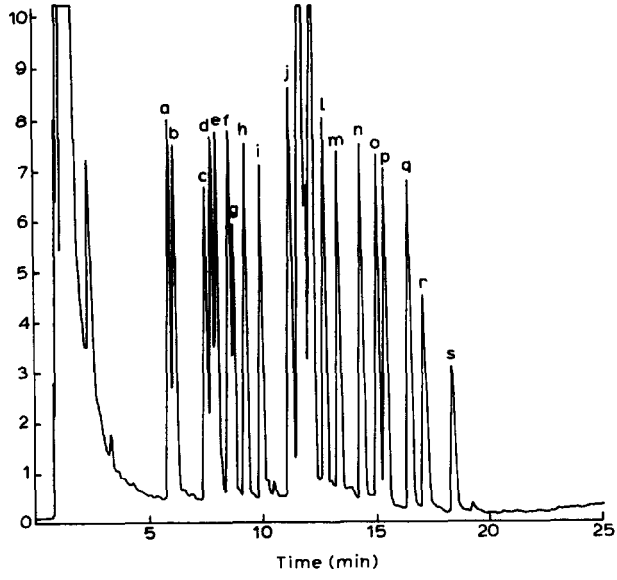

Fig. 3. GLC separation of HBB derivatives of amino acid standards in the presence of derivatised glucose. Peaks as in Fig. 1.

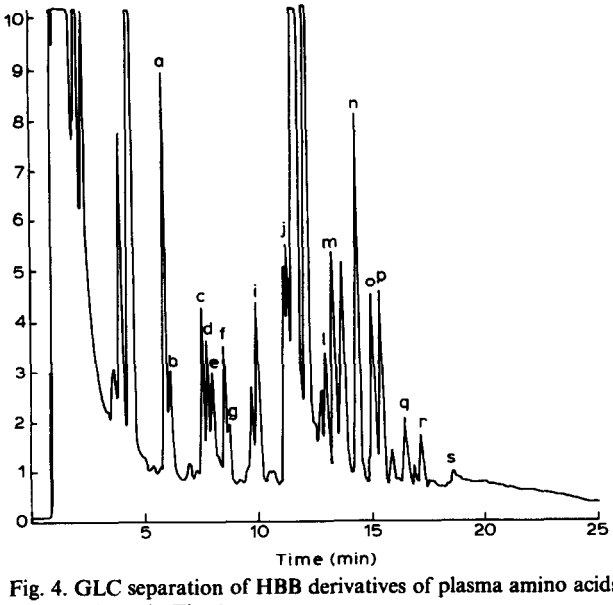

Fig. 4. GLC separation of HBB derivatives of plasma amino acids showing the glucose-induced interference. Peaks as in Fig. 1.

retention times, of glucose as the interfering compound in the analysis of plasma amino acids in Fig. 4.

There are two possible mechanisms which may explain the interference caused by glucose, a reducing sugar, during amino acid derivatisation. Firstly, the hydroxyl groups of glucose may react with heptafluorobutyric anhydride-forming derivatives[11] which co-elute with hydroxyproline, proline, methionine and aspartic acid, thus making the quantitation of these amino acids unattainable. Secondly, and in view of the high temperatures employed during the derivatisation, glucose may react with free amino acids forming the so-called Maillard compounds[12]. These latter compounds may themselves contribute directly to the spurious peaks observed or indirectly following their esterification and acylation during derivatisation. Under mild conditions of temperature (37°C) lysine will be the main contributor to the Maillard reactions[13]. However, with increasing severity of temperature conditions (>90°C), as employed in the derivatisation of amino acids, glycine, arginine, aspartic acid and glutamic acid will also participate in the Maillard reactions[14]. It would therefore appear that elimination of the glucose induced interference, by means such as enzymic degradation, will improve the qualitative and quantitative analysis of plasma amino acids. This approach to plasma purification is currently under investigation.

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