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Note

Simple gas chromatographic screening procedure for lactic and pyruvic acids in human plasma

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The presence of increased amounts of various organic acids in human blood plasma has been identified in a variety of diseases. Although some of these are rare and hereditary there are a number of more widespread conditions in which this occurs. One of these is lactic acidosis, a condition which results in the presence of increased amounts of lactic acid in plasma and urine, generally accompanied by elevated pyruvic acid levels.

The determination of organic acids in biological fluids is a difficult analytical problem because of the polar and hydrophilic nature of these compounds. The use of solvents such as diethyl ether and ethyl acetate for the extraction of acidic metabolites from acidified plasma or urine is not only non-quantitative because of the unfavourable partition coefficients for these acids between the solvents and water, but also results in the extraction of non-acidic components [1]. Steam distillation has been used [2—4] to extract organic acids but often causes an intolerable dilution of the relevant compounds.

The determination of organic acids by gas chromatography usually requires a suitable derivatization procedure because of the polar nature and low thermal stability of the parent compounds [5, 6]. However, some reports of the direct determination of organic acids on conventional gas—liquid chromatographic

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columns have appeared [7, 8]. Pyruvic and lactic acids may be assayed by an enzymatic method

$$\text{pyruvate} \xrightleftharpoons{\text{(lactate dehydrogenase)}} \text{lactate}$$
$$\text{NADH} + \text{H}^+ \longleftrightarrow \text{NAD}$$

but the procedure is rather complex as each acid has to be determined separately. Paper and thin-layer chromatographic methods have also been used to detect the presence of organic acids in biological fluids but these procedures are only semi-quantitative.

In this preliminary report we describe a simple gas chromatographic assay for lactic and pyruvic acids in human plasma. Plasma, rather than urine, was chosen because it contains fewer constituents than urine, and because lactic acid is present in negligible amounts in the urine of normal subjects.

MATERIALS AND METHODS

Chromatography was carried out on a Pye-Unicam GC-V gas chromatograph equipped with dual flame ionisation detectors, and a 90 cm × 0.3 cm coiled glass column packed with Tenax G.C. (35—60 mesh) (Applied Science Labs., State College, Pa., U.S.A.) conditioned according to the manufacturer's recommendations (Applied Science Laboratories Technical Bulletin No. 24). A nitrogen carrier gas flow-rate of 15 ml min^{-1} and a column temperature of 155° were employed. Standard acids were obtained from BDH (Poole, Great Britain).

Blood samples were obtained 10—14 h after the last meal. Deproteinisation was accomplished by adding blood to an equal volume of 5% perchloric acid. After standing for 15 min the sample was centrifuged at 2600 *g* for 20 min. Excess perchloric acid was removed prior to analysis by neutralisation with a buffer solution containing 20% (w/v) potassium hydroxide and 1 *M* in triethanolamine. Re-acidification with concentrated HCl to give an approximately 0.1 *M* acid solution was followed by injection onto the gas chromatographic column.

Gas chromatography—mass spectrometry was performed on a V.G. Micromass 16B2 mass spectrometer interfaced to a Pye 104 gas chromatograph via a single-stage jet separator. Helium was used as the carrier gas.

RESULTS AND DISCUSSION

Using these conditions the retention times of pyruvic and lactic acids were 2.6 and 6.5 min, respectively. For batch analyses the clear supernatant after centrifugation may be decanted and stored until required.

Standard solutions of lactic and pyruvic acids corresponding to, and above, the normal values found in human plasma gave a linear response for the required ranges of 0—50 $\mu g\ ml^{-1}$ (regression equation $y = 4.379x + 5.69$, correlation coefficient 0.9926) for pyruvic acid, and 0—350 $\mu g\ ml^{-1}$ (regression equation $y = 2.212x - 25.27$, correlation coefficient 0.9971) for lactic acid. The levels in the plasma of the subjects studied ranged over 90—160 $\mu g\ ml^{-1}$ for lactic acid and 3.5— 7.0 $\mu g\ ml^{-1}$ for pyruvic acid.

As no suitable cases of lactic acidosis were available for study, blood samples from subjects undergoing fructose loading tests were analysed. It has been reported [9] that about 35% of the fructose load is converted to lactate and pyruvate, the remainder being converted to glucose. Subjects were in the fasting state prior to the first sampling. Subsequent to the first sampling 1 g of fructose per kg of body weight was administered. Further blood samples were taken after 30 min and 45 min. Gas chromatographic analysis (duplicate injection) was performed after the sample preparation procedure described above. Typical results obtained for one of the subjects studied are displayed in Table I, and Fig. 1b–d. Fig. 1a is a chromatogram of standard pyruvic, lactic and hydroxybutyric acids.

TABLE I

LACTIC AND PYRUVIC ACID LEVELS IN A FRUCTOSE-LOADED SUBJECT

Sample No.	Time (min)	Pyruvic acid (μg/ml)	Lactic acid (μg/ml)
1	0	6.0	120
2	30	8.2	290
3	45	8.2	330

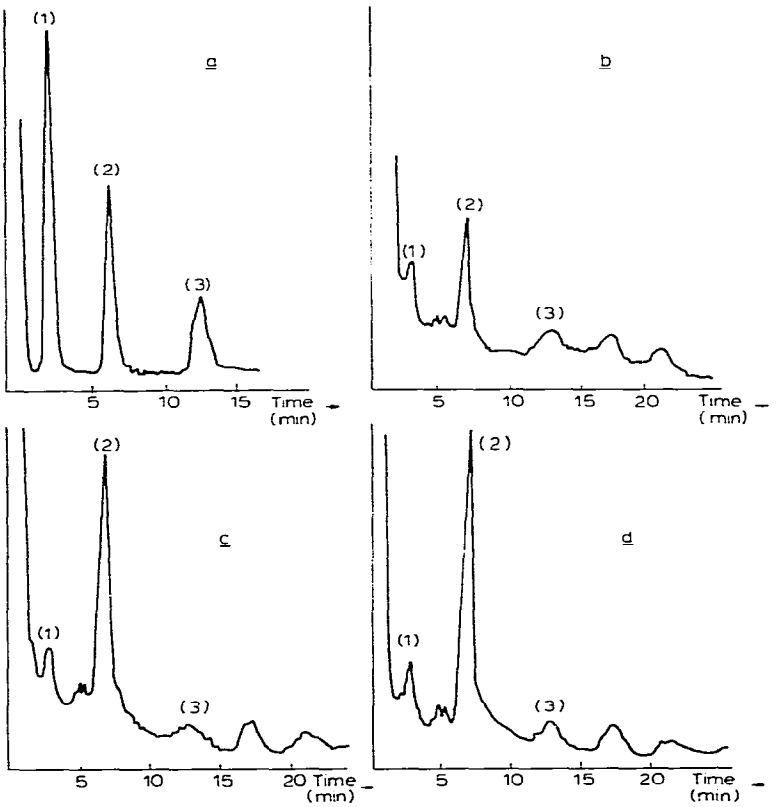

Fig. 1. Chromatograms of (a) pyruvic (1) lactic (2) and hydroxybutyric (3) acid standards; (b) normal plasma; (c) plasma 30 min after fructose loading; (d) plasma 45 min after fructose loading.

It was apparent from the chromatograms obtained that there were other constituents present in the sample, but no interference by any biologically significant organic acids from any major metabolic pathway has been observed. Co-injection with standards suggested that one of these constituents was hydroxybutyric acid (t_R = 12.8 min). The co-determination of hydroxybutyric acid is particularly desirable since there is a close relationship between hydroxybutyric acid levels and the occurrence of lactic acidosis [10, 11]. Confirmation of column eluates by gas chromatography—mass spectrometry proved unsuccessful. Presumably the underivatized acids do not survive transfer from the column to the mass spectrometer source via an interface maintained at 220°.

After injection of about ten samples of treated plasma solution the top 1—2 cm of the column became visibly contaminated by the build-up of non-volatile material from the injected sample. Although this was observed not to affect column performance the top of the column was renewed periodically (approximately every 25 injections). Frequent injections of solvent (0.1 *M* HCl) were carried out to preserve column performance. Ghosting (i.e. the observation of spurious peaks) was caused by the presence of a glass-wool plug (silanized or unsilanized) at the front end of the column. Glass wool should not be used to contain the column packing for this assay.

Quantitation for this work was accomplished by the measurement of peak areas. However, use of an internal standard should provide improved accuracy. Pivalic acid has been recommended [8] for this purpose but was found to be unsuitable as it eluted as a broad band at the temperature employed for the analysis. Its hygroscopicity and unpleasant smell are further disadvantages. *n*-Butanoic, *n*-pentanoic and *n*-hexanoic acids were also evaluated. Of these three *n*-hexanoic has an ideal retention time, being eluted after lactic acid and before hydroxybutyric acid. Unfortunately the low solubility of *n*-hexanoic acid in an aqueous medium precludes its use as an internal standard.

CONCLUSIONS

The simultaneous determination of pyruvic, lactic and possibly of hydroxybutyric acids at the levels found in human plasma may be achieved using the porous packing Tenax G.C. Small sample volumes (ca. 250 μl) are needed, little sample preparation is necessary and automation to cope with routine analysis should be possible. The development of a suitable internal standard would be an added advantage, although, due to the gross nature of many metabolic disorders, it can be argued that the proposed method using peak areas and calibration curves is sufficiently accurate as it stands.

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