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ORGANIC ACID PROFILES OF HUMAN TISSUE BIOPSIES BY CAPILLARY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

A method is described to determine the organic acid content of tissue specimens comparable in size to those obtainable by closed biopsy. The method involves solvent extraction of tissue homogenates followed by analysis of trimethylsilyl derivatives of the organic acids by combined capillary gas chromatography–mass spectrometry. Organic acid profiles of human liver, pancreas, kidney and muscle are shown.

INTRODUCTION

The profiling approach to the study of body fluids has become a valuable method in biomedicine[1–3]. Most published work has dealt with the analysis of urine and serum, and to a lesser extent with other extracellular fluids such as cerebrospinal, amniotic and seminal fluids[1]. Little information exists, however, on the organic acid content of tissues, which might contain substantial information on the intracellular space, and the purpose of this paper is to present a gas chromatographic–mass spectrometric (GC–MS) method whereby such information can be acquired.

EXPERIMENTAL

Chemicals

Solvents were re-distilled before use. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from Supelco (Bellefonte, Pa., U.S.A.). All other reagents were commercially available products of analytical grade.

Sample preparation

Tissue specimens weighing *ca.* 100 mg were obtained from normal organs during surgery under nitrous oxide and halothane anaesthesia and were cut into small pieces which weighed about 5 mg; these pieces were then weighed accurately and stored at −20° until homogenization.

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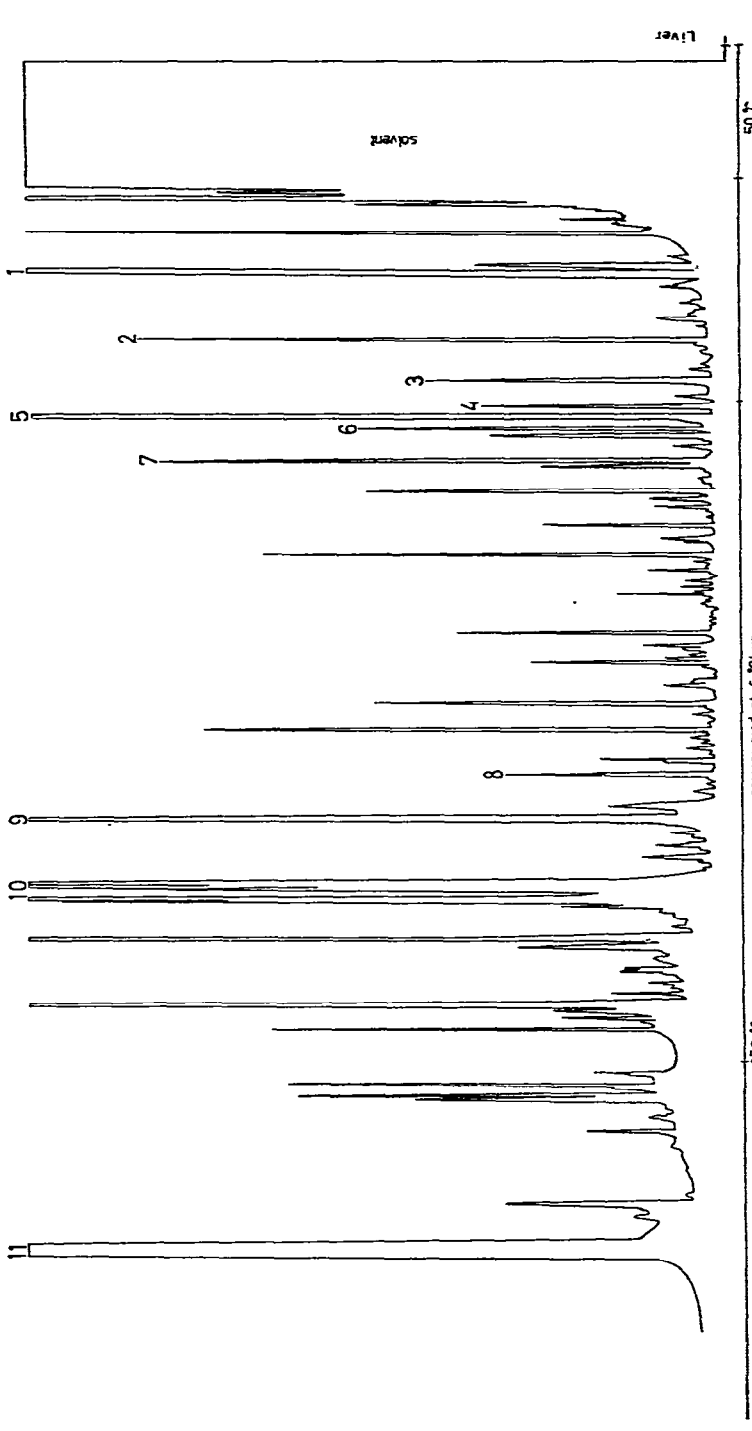

Fig. 1. Organic acid profile of human liver. The biopsies were taken from a patient undergoing surgery in connection with a kidney transplant. Sample work-up and separation on an SE-30 glass open-tubular capillary column (25 m) in a combined GC–MS–computer instrument are described in the text. Peaks: 1 = lactic acid; 2 = 3-hydroxybutyric acid; 3 = benzoic acid; 4 = glyoxylic acid; 5 = phosphoric acid; 6 = glycerol; 7 = fumaric acid; 8 = phthalate plasticiser; 9 = palmitic acid; 10 = C_{18} fatty acids (stearic, linolic); 11 = cholesterol.

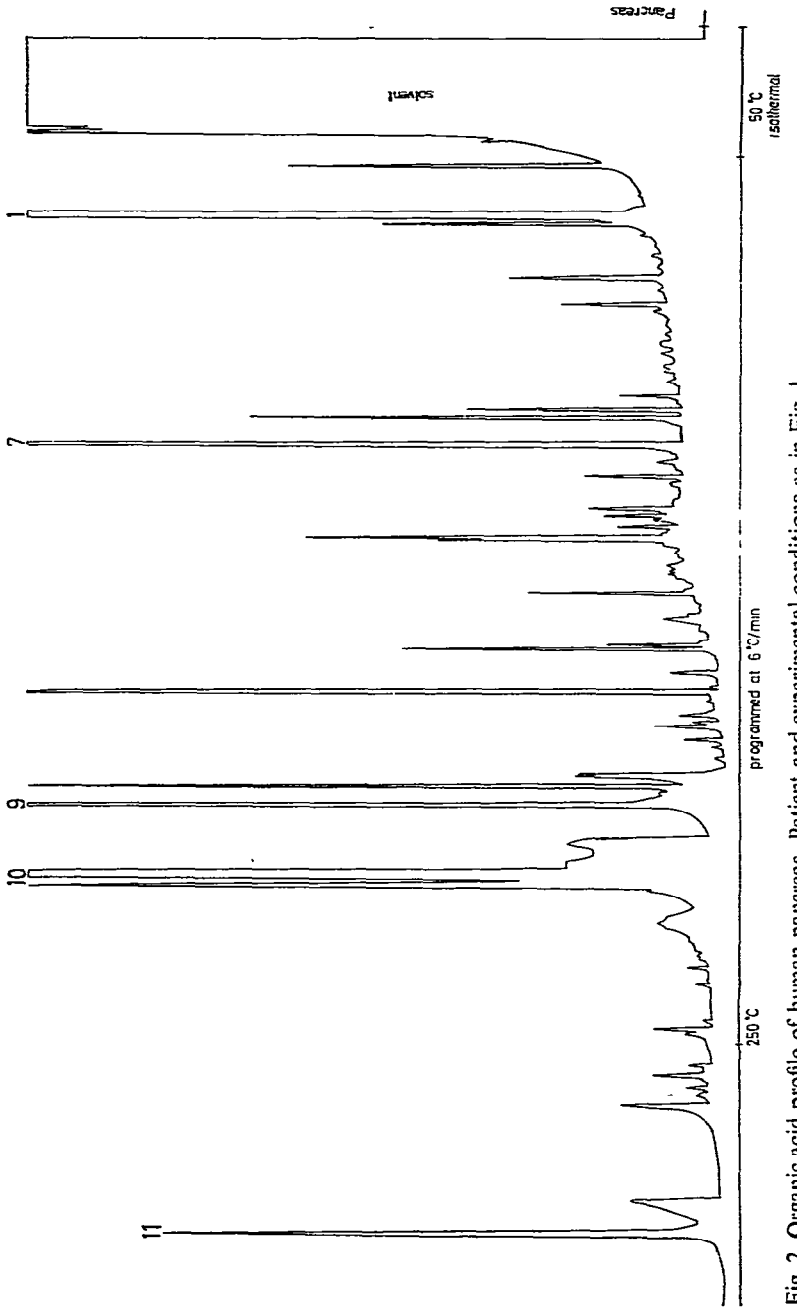

Fig. 2. Organic acid profile of human pancreas. Patient and experimental conditions as in Fig. 1.

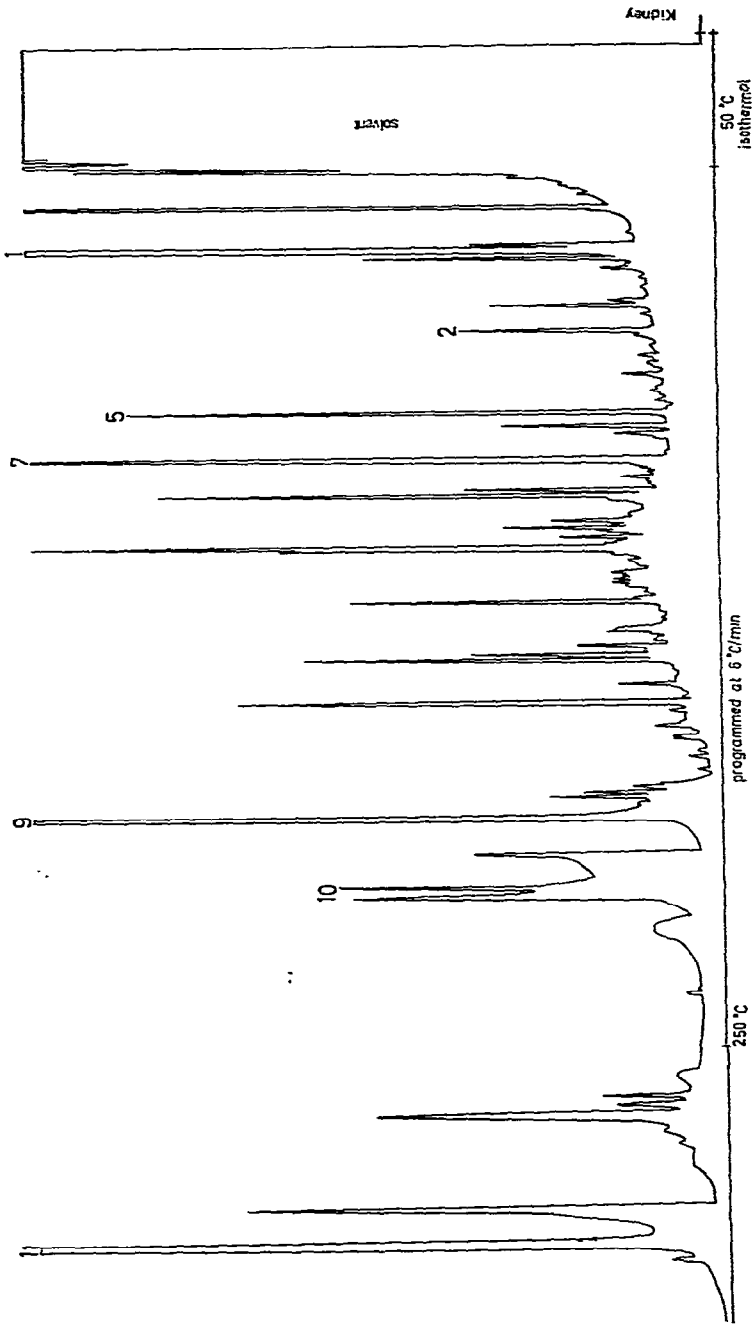

Fig. 3. Organic acid profile of human kidney. The biopsy was taken from a patient undergoing abdominal surgery. Experimental conditions and peak identifications as in Fig. 1.

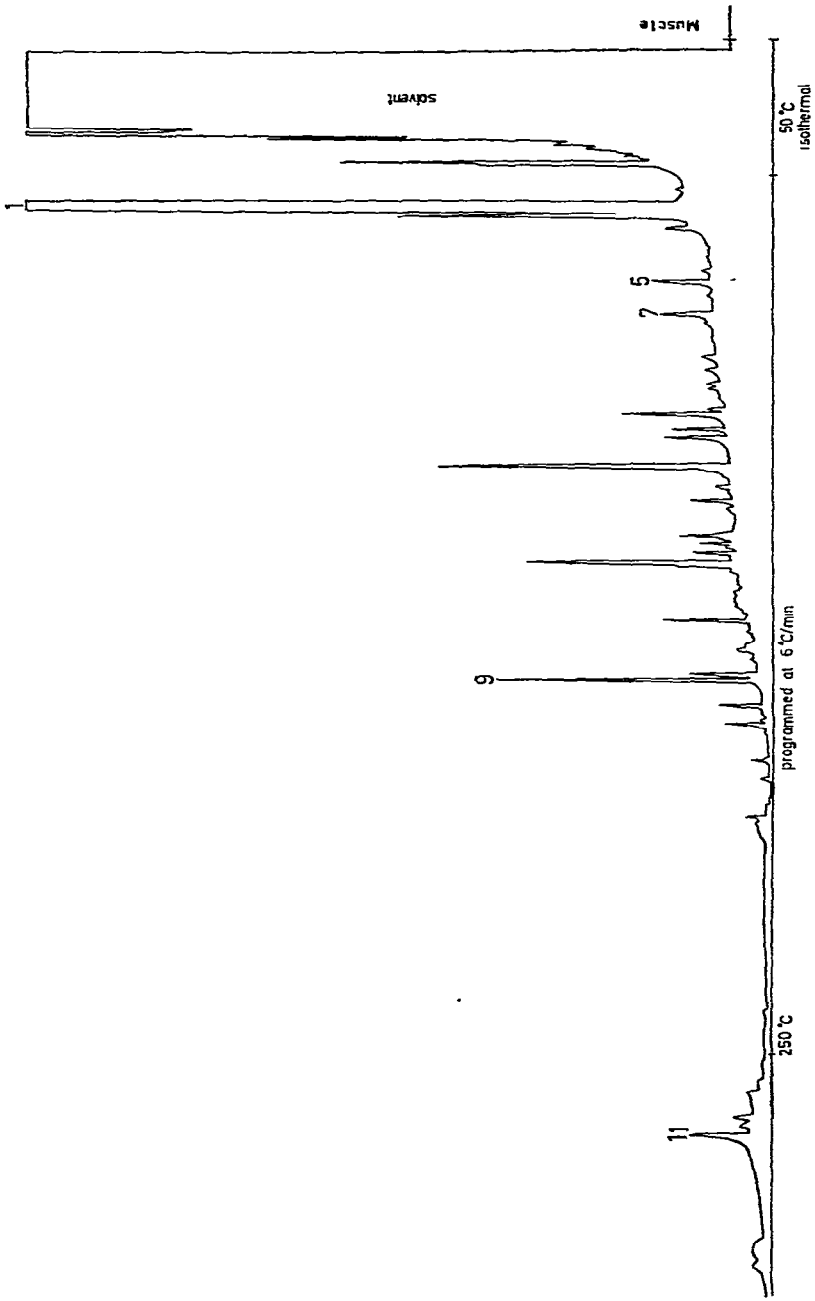

Fig. 4. Organic acid profile of human muscle. Experimental conditions and peak identifications as in Fig. 1.

Tissue samples weighing *ca.* 5 mg were homogenized in 1 ml water using a Kontes 7-ml ground-glass homogenizer (Kontes, Vineland, N.J., U.S.A.), 100 μl of 2 *M* potassium hydroxide solution were added, and the coenzyme A esters were hydrolyzed at room temperature for 20 min. The alkaline solution was extracted three times with triple volumes of diethyl ether and the organic phases were discarded. The remaining aqueous phase was acidified with 6 *N* hydrochloric acid and extracted three times with triple volumes of diethyl ether; extraction with light petroleum (b.p. 40–60°) was sometimes applied prior to this stage in order to remove most free fatty acids. The combined diethyl ether extract was dried over anhydrous sodium sulphate and most of the solvent was then evaporated under nitrogen. When the volume had been reduced to about 0.2 ml, the sample was transferred into a 0.3-ml Kontes Microflex tube and carefully evaporated to dryness. Then 25 μl of BSTFA were added to the residue and the mixture was allowed to react for 30 min in a sand-bath at 110°. A 2-μl aliquot was subsequently injected into the GC–MS instrument.

Gas chromatography–mass spectrometry

GC–MS was performed on a Varian Model 112 combined gas chromatograph–mass spectrometer (Varian-MAT, Bremen, G.F.R.) to which an on-line data system (Spectrosystem 100, Varian-MAT) with dual discs and a magnetic tape unit were attached. The gas chromatograph was a Varian Model 1400 (Varian, Walnut Creek, Calif., U.S.A.) and the glass capillary column (LKB, Stockholm, Sweden) was 25 m in length, 0.28 mm O.D. and wall-coated with SE-30. The number of theoretical plates was $80–85 \cdot 10^3$. The capillary column led directly into the ion source of the mass spectrometer, which was equipped with dual turbomolecular pumps. A splitting ratio of 1:30 was normally used and the injection temperature was 230°. The column temperature was maintained at 50° for 5 min after injection, and was then increased at 6°/min to 250°. The flow-rate of helium carrier gas was 1 ml/min.

Peak identification

Some peaks were identified by comparison with the GC retention times and MS data for authentic compounds. Other peaks were tentatively identified by computer matching of their mass spectra against a comprehensive file of reference spectra containing 77,889 entries[4].

RESULTS AND DISCUSSION

Figs. 1–4 show organic acid profiles of small biopsy specimens of human liver, pancreas, kidney and muscle. Fatty acids and cholesterol, 3-hydroxybutyric acid, lactic acid, glycerol, glyoxylic acid and a phthalate plasticizer have been identified. As shown, there are both differences and similarities between the organs. Muscle regularly contained lower concentrations of nearly all components than the other tissues examined.

The results demonstrate that the analysis of tissue fluids for organic acids by combined capillary GC–MS–computer techniques is sufficiently sensitive to be applied to samples of a size obtainable by percutaneous biopsy. The usefulness of studying such profiles, however, remains to be established.

ACKNOWLEDGEMENTS

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REFERENCES

1 E. Jellum, *J. Chromatogr.*, 143 (1977) 427.
2 A. M. Lawson, *Clin. Chem.*, 21 (1975) 803.
3 L. R. Politzer, B. J. Dowty and J. L. Laseter, *Clin. Chem.*, 22 (1976) 1775.
4 E. Jellum, P. Størseth, J. Alexander, P. Helland, O. Stokke and E. Teig, *J. Chromatogr.*, 126 (1976) 487.