PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Simple Urinary Acylcarnitine Profiling by Gas Chromatography Mass Spectrometry

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Phil. Trans. R. Soc. Lond. A 1990 **333**, 169-170
doi: 10.1098/rsta.1990.0155

References

Aas, N., Sakakini, B. H., West, R. H. & Vickerman, J. C. 1990 *Surface Interface Analysis* **16**, 359.
Moretti, G. 1990 *Surface Interface Analysis* **16**, 359.
Wagner, C. D. 1975 *Analyt. Chem.* **47**, 1201.
West, R. H. & Castle, J. E. 1982 *Surface Interface Analysis* **4**, 68.

Simple urinary acylcarnitine profiling by gas chromatography mass spectrometry

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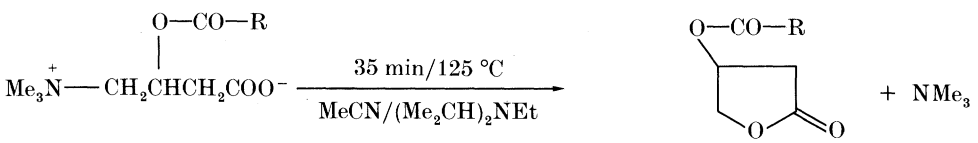
In many cases of sudden infant death, victims have been shown to be deficient in medium-chain acyl-CoA dehydrogenase (MCAD), a key enzyme for β-oxidation of fatty acids. This disease, and several other inborn errors of metabolism leading to organic aciduria, are characterized by concentrations of certain acylcarnitines **1** in urine of the order of 1 μmol ml^{-1}. Normal levels of acylcarnitines are of the order of 1 nmol ml^{-1}. An affordable, routine analytical procedure for traces of urinary acylcarnitines would facilitate diagnosis of acidurias and acidemias. Acylcarnitines are essential to β-oxidation because they carry the fatty acyl units (RCO-) across the mitochondrial membrane. Carnitine detoxifies mitochondria of excesses of acyl groups by carrying them, as acylcarnitines, into urine. Thus profiling of urinary acylcarnitines would also allow the biochemistry of some currently ill-defined diseases, and the metabolic routes of acidic drugs, to be elucidated.

Several methods exist for detecting urinary acylcarnitines but none is ideal for the clinical laboratory (Lowes & Rose 1989). We are developing a simple method based on gas chromatography mass spectrometry (GCMS), following an ion-exchange work-up and a very simple derivatization to volatile lactones **2** (Lowes & Rose 1990):



1 **2**

For example, a standard aqueous mixture of 12 acylcarnitines from R = CH_3 to R = $C_{15}H_{31}$ at the level of 1 nmol ml^{-1} can be easily examined by temperature-programmed GCMS (BP-5 column) following the cyclization shown. All 12 components were resolved, including isomers (e.g. octanoyl, R = $CH_3(CH_2)_6$, and valproyl, R = $(CH_3CH_2CH_2)_2CH$). Diagnostic mass spectra were obtained in electron ionization and ammonia chemical ionization modes.

The detection limits for individual acylcarnitines in the complex matrix of urine are of the order of 1 nmol ml^{-1} but dependent on the chemical background. Many

clinical samples have now been examined successfully by this GCMS approach. It has confirmed that acetyl-, hexanoyl- and octanoylcarnitine were present in elevated amounts in five cases of infants with a deficiency of MCAD. We have identified isovalerylcarnitine in the urine of a patient with isovaleric acidemia. In all these cases the mass spectra of lactones **2** derived from the various acylcarnitines match those of authentic samples. The method should also be useful for drug metabolism studies. In two instances where infants were known to have had a 3-phenylpropanoic acid load, GCMS showed a component with retention time and mass spectrum consistent with the lactone from the metabolite, 3-phenylpropanoylcarnitine. In the urine of an infant on valproic acid (2-propylvaleric acid) therapy, GCMS revealed a compound consistent with the lactone from 2-propyl-3-oxovalerylcarnitine. 2-Propyl-3-oxovaleric acid is known to be the major metabolite of valproate in man.

We thank the following for sponsorship, medical advice and/or clinical samples: RSC Analytical Chemistry Trust, The Foundation for the Study of Infant Deaths, Dr R. J. Pollitt (Sheffield) and Dr G. S. Mills (Southampton).

References

Lowes, S. & Rose, M. E. 1989 *Trends analyt. Chem.* **8**, 184–188.
Lowes, S. & Rose, M. E. 1990 *Analyst* **115**, 511–516.

Atomizer, source, inductively coupled plasmas in atomic fluorescence spectrometry (ASIA)

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Atomic fluorescence spectrometry has been an active research area for several years. Interest in the analytical capabilities of the technique has increased recently with the introduction of a commercial instrument based on the hollow cathode lamp excitation of atoms generated in an argon inductively coupled plasma (ICP). An alternative approach has been pioneered at Loughborough involving the use of a second ICP as a light source.

The use of an inductively coupled plasma in atomic emission spectrometry (ICPAES) imparts a number of figures of merit to that technique, namely low detection limits, long linear dynamic range and relative freedom from chemical and ionization effects. However, ICPAES is prone to spectral interference, whereas atomic fluorescence spectrometry (AFS) is not. The ASIA system was assembled to demonstrate that the ICP could be used as both a line source and as an atomizer in AFS and also to demonstrate freedom from spectral interference; in addition, it was hoped to show that the other figures of merit exhibited by ICPAES would be retained.

The poster presentation demonstrated that these desirable aims have been largely achieved. Atomic emission and fluorescence spectra were presented showing that well-known spectral interferences which occur in the emission technique, such as background shifts due to radiative recombination, collisional broadening and direct spectral overlap are absent in the fluorescence technique.