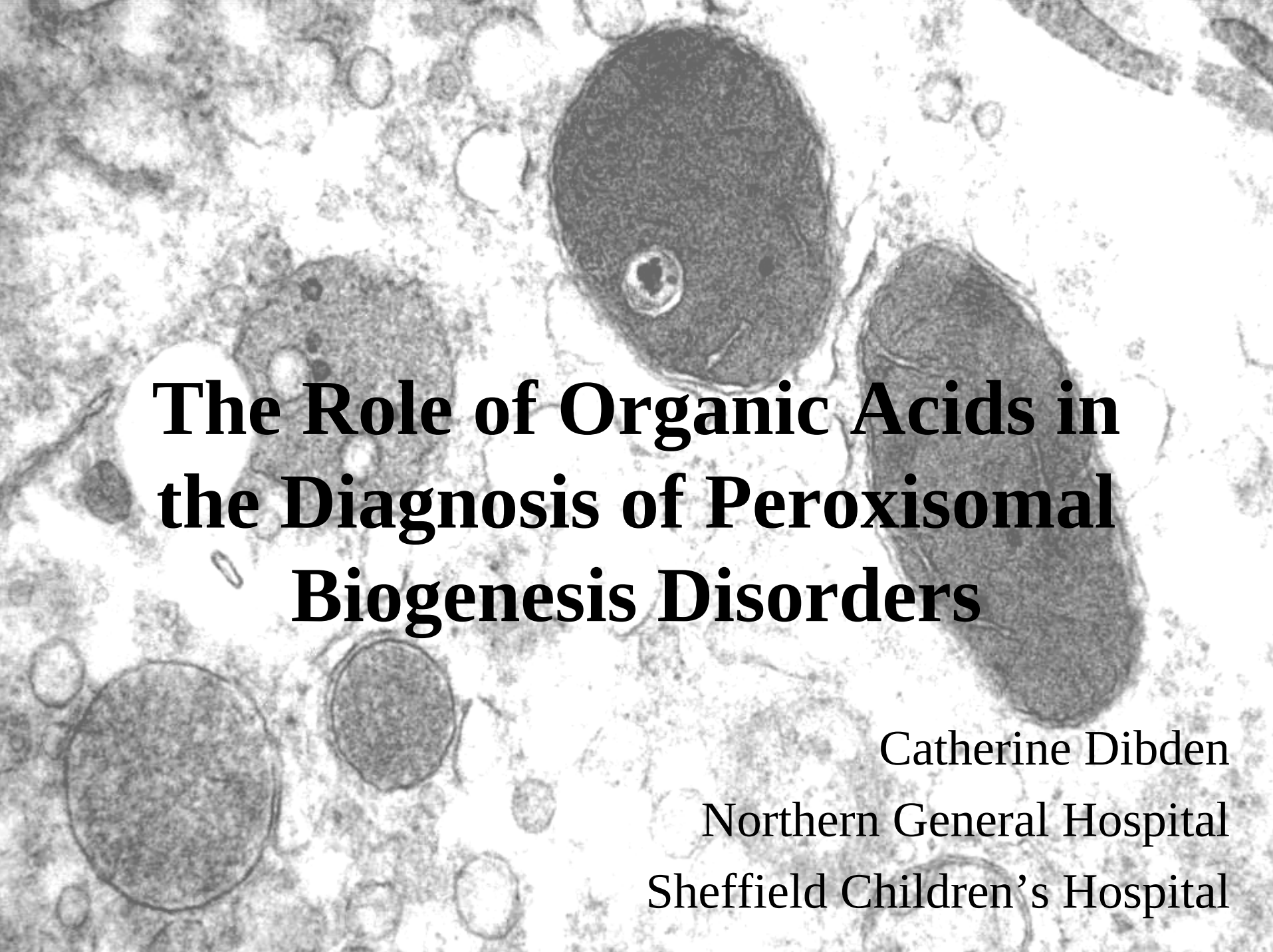


The Role of Organic Acids in the Diagnosis of Peroxisomal Biogenesis Disorders

Catherine Dibden

Northern General Hospital

Sheffield Children's Hospital

Peroxisomes

- Small sub-cellular organelles
- Present in all eukaryotic cells
- Abundant in tissues actively involved in lipid metabolism
  - Liver
  - Kidney
  - Nervous tissue

Functions of Peroxisomes

- Fatty acid β-oxidation
- Fatty acid α-oxidation
- Ether-phospholipid biosynthesis
- H_2O_2 metabolism
- L-pipecolate degradation
- Glutaryl-CoA metabolism
- Glyoxylate detoxification
- Isoprenoid biosynthesis

Peroxisomal Biogenesis

- Three steps:
  - Formation of lipid bilayer
  - Incorporation of membrane-bound peroxisomal proteins
  - Import of matrix proteins into the peroxisome

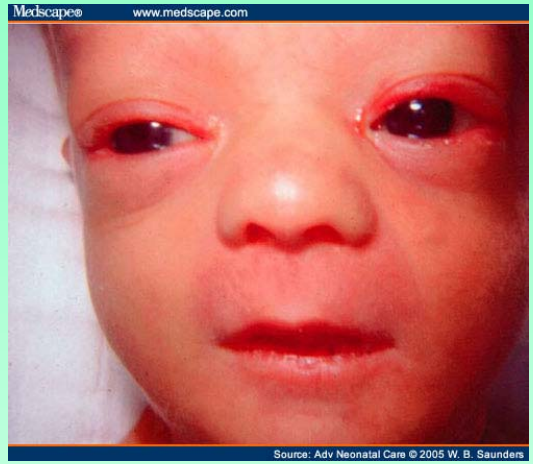
- *PEX* genes encode proteins required for assembly of the peroxisomal membrane and support the import of matrix proteins
- Protein products known as peroxins

Peroxisomal Disorders

- Single peroxisomal protein deficiencies
- Peroxisomal biogenesis disorders:
  - Rhizomelic Chondrodysplasia Punctata (RCDP) phenotype
  - Zellweger Spectrum:
    - Zellweger syndrome (ZS)
    - Neonatal adrenoleukodystrophy (NALD)
    - Infantile Refsum disease (IRD)

Gould S.J., Raymond G.V., Valle D., 2001. The Peroxisome Biogenesis Disorders, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), The Metabolic & Molecular Bases of Inherited Disease. McGraw-Hill, New York

Zellweger syndrome



- Presents at birth
- Reduction or absence of peroxisomes
- Clinical phenotype:
  - Craniofacial dysmorphism
  - Hypotonia
  - Impaired hearing/eye abnormalities
  - Psychomotor retardation, neonatal seizures
  - Liver disease
  - Calcific stippling of epiphyses
  - Renal cysts
- Death usually occurs within 6 months

Zellweger syndrome (2)

- Biochemical phenotype:
  - Plasma: increased very long chain fatty acids (VLCFA), phytanic acid, pipecolic acid, and bile acid intermediates DHCA and THCA
  - Erythrocytes: reduced plasmalogen synthesis
  - Fibroblast cultures: reduced dihydroxyacetone phosphate acyltransferase (DHAPAT) activity
  - Urine: increased pipecolic acid and bile acid intermediates
- Diagnosis: abnormal plasma VLCFA levels, confirmed by DHAPAT activity

Problems with Diagnosis of PBD

- Plasma VLCFA are not part of routine ‘metabolic screen’ in most metabolic laboratories
- Clinicians unfamiliar with rare disorders may not request VLCFA examination
- Urine most commonly submitted specimen type for metabolic screening

Therefore patients with an undiagnosed PBD may be missed when being screened for a metabolic disease

Role of Organic Acids

- GC-MS analysis of urinary organic acids commonly included in the routine ‘metabolic screen’
- Characteristic organic aciduria of PBDs has been reported, showing increased excretion of:
  - 3,6-epoxydicarboxylic acids (C10, C12, C13, C14)
  - Odd-chain C7 – C15 dicarboxylic acids
  - 2-hydroxydecanedioate
  - Saturated and unsaturated C6 - C10 dicarboxylic acids
  - C10:C6 and C8:C6 dicarboxylic acid ratios >1
  - 4-hydroxyphenyllactic and 4-hydroxyphenylacetic acid

Korman *et al*, 2000. *J.Inherit. Metab. Dis.* 23: 425 – 428

Aim of Study

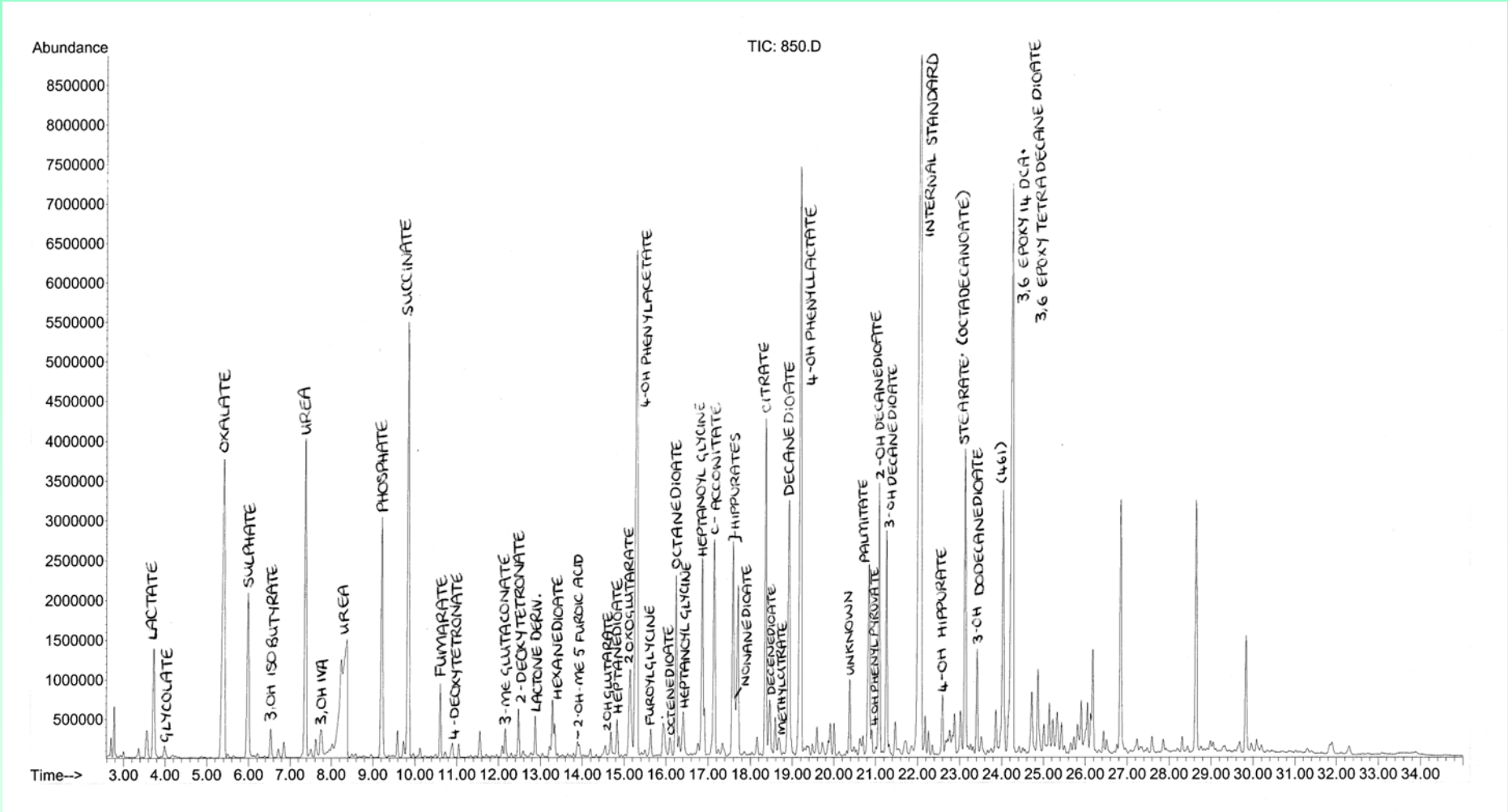
- To look for the presence of characteristic metabolites and other features of an organic acid profile in patients with a PBD as previously reported

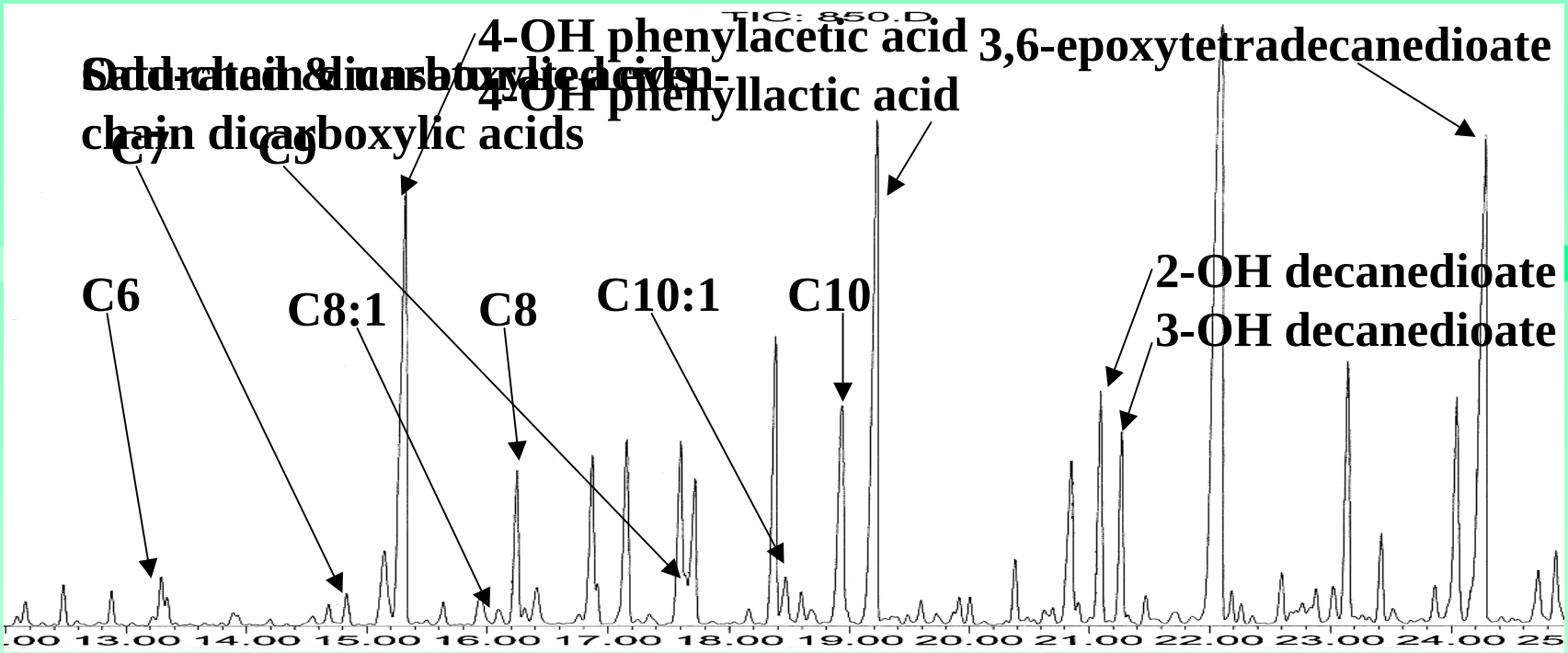
- To identify the mass spectra of relevant metabolites for addition to the GC-MS searchable library, to allow routine identification of these metabolites in patient samples.

Methods

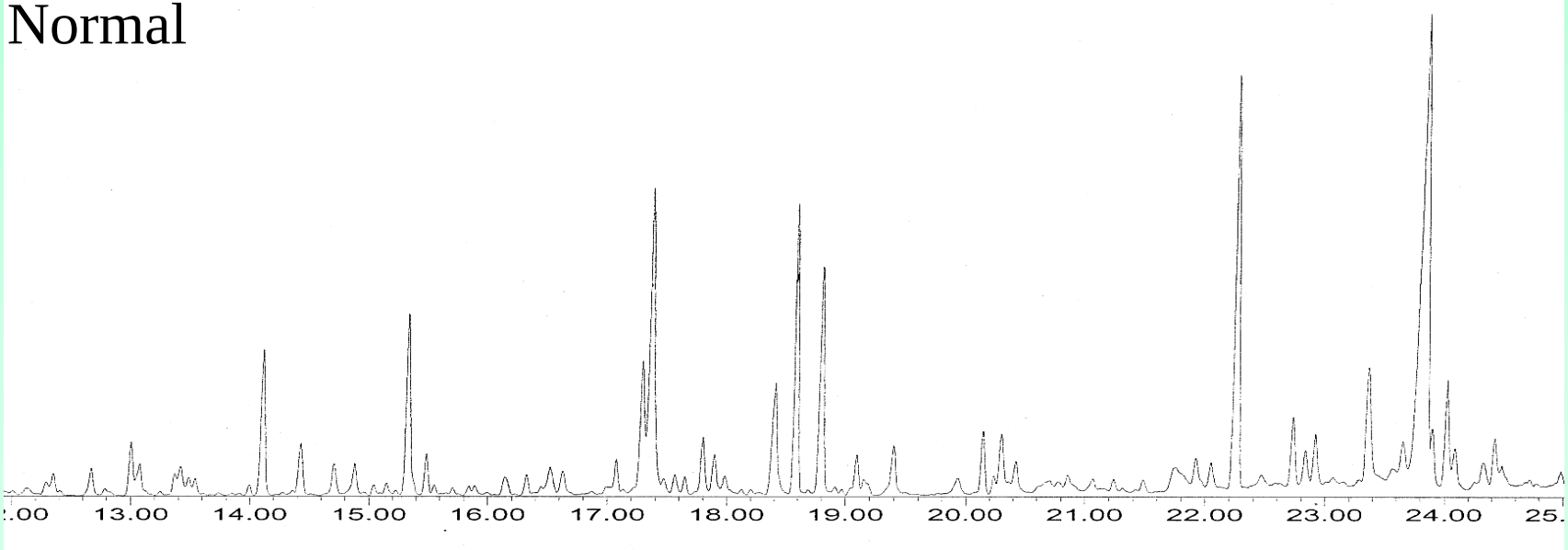
- Urine from 14 patients with various peroxisomal disorders was examined:
  - 8 Zellweger Syndrome
  - 2 Infantile Refsum Disease
  - 2 X-linked Adrenoleukodystrophy
  - 1 Pseudo-Zellwegers
  - 1 Refsum's Disease
- Urine from 20 patients with no specific abnormality on urinary organic acids analysis was also examined
- GC-MS analysis of urine organic acids was carried out on all samples

Results:
Organic Aciduria of PBD

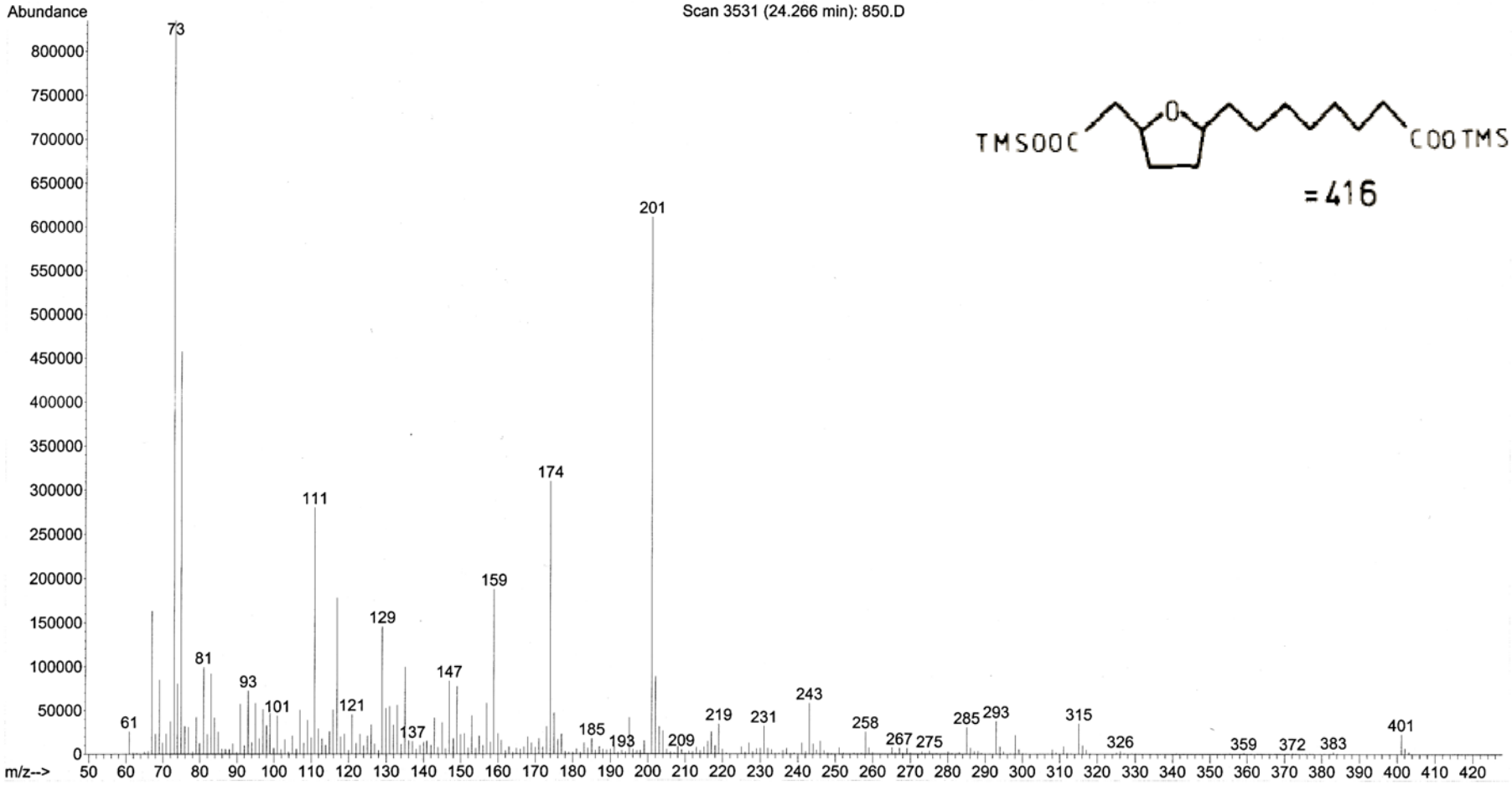



TIC: 850.D
4-OH phenylacetic acid
3,6-epoxytetradecanedioate
4-OH phenyllactic acid
C7
C9
C6
C8:1
C8
C10:1
C10
2-OH decanedioate
3-OH decanedioate
13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00 22.00 23.00 24.00

Normal


13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00 22.00 23.00 24.00

Mass Spectrum of 3,6-epoxytetradecanedioate



Results

- 8/10 patients with PBDs showed increased excretion of:
  - 3,6-epoxydicarboxylic acids (mostly C14)
  - 2-hydroxydecanedioate
- 3,6-epoxytetradecanedioate:
  - Specificity: 100%
  - Sensitivity: 80%
- All types of peroxisomal disorders showed elevated levels of odd-chain dicarboxylic acids (mostly C7 and C9)

Results

- 7/10 patients with PBDs showed C10:C6 and C8:C6 dicarboxylic acid ratios of >1
  - Specificity: 100%
  - Sensitivity: 70%
- Increased levels of 4-hydroxyphenyllactic acid and 4-hydroxyphenylacetic acid were present in patients with a PBD or pseudo-Zellwegers

A few weeks later…

- Urine from a 1 month old baby analysed
- Clinical details “failure to thrive”
- Urine organic acids:
  - Increased 2-hydroxydecanedioate
  - Increased 3,6-E14DA
- Peroxisomal disorder suspected
- Plasma for VLCFA already received, which confirmed diagnosis of a PBD

Conclusions

- Urinary organic acids can be a useful indicator to the diagnosis of a PBD
- This particular organic acid profile should alert the laboratory to the possibility of a PBD and prompt appropriate investigations, including VLCFAs.

- Awareness of the characteristic organic aciduria of a PBD may improve detection of these conditions in an initial metabolic screen

References

Gould S.J., Raymond G.V., Valle D., in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), 2001. The Metabolic & Molecular Bases of Inherited Disease. McGraw-Hill, New York, pp. 3181-3219

Pitt J.J., Poulos A., 1993. *Clin Chim Acta.* 223: 23-29

Rizzo C., Bertucci P., Federici G., Wanders R.J.A., Sabetta G., Dionisi-Vici C., 2000. *J Inherit Metab Dis.* 23 (S1): 241.

Yamaguchi S., Iga M., Kimura M., Suzuki Y., Shimozawa N., Fukao T., Kondo N., Tazawa Y., Orii T., 2001. *J Chrom B.* 758: 81-86

www.humpath.com/IMG/jpg/mitochondria_peroxisome_hepatocyte_04-2.jpg

Acknowledgements

Nigel Manning	Sheffield Children’s Hospital
Claire Hart	Sheffield Children’s Hospital