GCMS in diagnosis of metabolic disorders at NTC ..........Bruce Bettany
This is an acronym for inborn errors of metabolism

There are many types involving the absence of a key enzyme in a pathway

The effects can be dramatic and catastrophic or slow, hard to define, inexorable progress

Sometimes benign
Metabolic disorders

- Small molecule disorders … often acute
- Storage disorders … late onset of effects
- Structural disorders … skeletal, muscular
Small Molecule Disorders: Intoxication

Protein → Amino Acids → Organic acids → Ammonia → ATP, CO₂, H₂O → Urea

PKU, MSUD, Tyrosinaemia

MMA, PA, IVA

Urea Cycle Disorders
### Disorders of Intermediary Metabolism Diagnosed in New Zealand: 2004-06.

*n*=175000

<table>
<thead>
<tr>
<th>Disease</th>
<th>Method of Initial Diagnosis</th>
<th>Age of Diagnosis</th>
<th>Outcome</th>
<th>Ability to diagnose on Expanded Newborn Screening</th>
<th>Early Diagnosis likely to improve outcome</th>
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### Disorders of Intermediary Metabolism Diagnosed in New South Wales: 2004-06
N=255,000

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Sample needs

• see Labplus handbook

• Listed as “organic acids”

• Done by Biochemical Genetics (NTC)

• (Urgently if required)

• Contact 309 4949  x6678
• ORGANIC ACIDS  Urine

• Test performed by Biochemical Genetics (NTC)
• For results, phone Lablink: 5995 or (09) 307-8995 or 0800 522 758.

• SPECIMEN:  10 mL random urine.

• Keep cool and freeze (-20°) if not sent to the laboratory within 4 hours.

• A brief clinical history is essential.

• Turnaround time 3 weeks, or urgently if required (contact laboratory: ext 6678 or via Lablink.)

• DIAGNOSTIC USE:

• This is a test for patients with symptoms suggestive of metabolic disorders.

• Urine organic acids are one of the key screening tests for patients with possible metabolic disease. It is the test of choice for investigating children with suspected organic acidemias. These conditions frequently present in the neonatal period with a sepsis like illness, severe acidosis and hyperammonaemia.

• They can also present at a later age with unexplained recurrent illness, developmental delay and/or hypoglycaemia/acidosis.

• The 'cerebral organic acidemias' can present with just developmental delay and thus organic acids is an important screening test in children with these problems. As ketones and lactate are organic acids, the test can be helpful in assessing conditions where ketosis or lactic acidosis are a feature. This test includes orotic acid.

• For further information contact laboratory: ext 6678 or via Lablink.

• or, Dr Callum Wilson, Metabolic Physician: Ph  021 555 392
Urine

- Often difficult because the patients are frequently babies

- We need a clean random urine

- 10mL, but ...sometimes we can get a result on a lot less...phone!

- If delay we need a frozen sample
GCMS.....the instrument

- Gas chromatograph linked to a mass spectrometer
GCMS trace

- The mass spectrometer turns out a constant stream of “flash cards” or mass spectra
- There are 5 per second; 300 per minute; 9000 in a 30 minute GLC trace
- The GCMS trace we look at is a graph of the total ions per “flash card” versus time
GCMS capabilities

- Compounds pop out of the column in order of their volatility.
- GCMS runs mass scans on the effluent from a GLC column.
- Each peak on the GCMS contains pictures of what it is, like flash-card movies; a stack of 30 flash cards.
- In GCMS you can look at the pictures and match each peak against a library of contenders.
Obtaining a mass spectrum (.....the picture)
Spectral library search
(...rogues gallery)
Again (..... the picture)
Now to extract ions from interfering peaks
Many examples of disorder produce simple carboxylic acids

We extract them from urine using EtAc

Carboxylic acids often decompose on heating before they boil so we TMS them to make them volatile and stable
ORGANIC ACIDS in IEM

- Malonic
- Glutaric
- Methylmalonic
- Glycines
- 2-OH-i-valeric
- Etc, ... all mixed up together
Extraction of urine acids

- Many examples of disorder produce simple carboxylic acids
  - We add “Internal Standard” & extract them from urine using ethyl acetate
  - Carboxylic acids often decompose on heating before they boil so we TMS them to make them volatile and stable
Internal standards

We add a substance to the sample before analysis to escort the things we are after through the assay to give a scale
Extraction of urine acids

Types of internal standard

A compound which is chemically similar to the analyte

Isotopic standards using C-13, N-15, deuterium etc.
Extraction of urine acids

we extract acids from urines with ethyl acetate

add IS according to Cr value

also get out "neutrals" such as: glycots, sugars, some protein and glycines

urine sample

ethyl acetate

solid NaCl
Derivatives of urine acids

• Many examples of disorder produce simple carboxylic acids

• We extract them from urine using EtAc

• Carboxylic acids often decompose on heating before they boil so we TMS them to make them volatile and stable
2-ketoglutaric acid....

hovers between two forms....enol/keto
2-ketoglututaric .TMS

After single TMS esterification
2-ketoglututaric .TMS

M/Z=218
2-ketoglutaric . TMS3 can take TMS in three positions
MS of 2-ketoglututaric.tms3

M/Z=362
GCMS of 2-ketoglutaric.tms

pt urine using direct extraction
(no oximation)
Oxime formation

Simple & rapid conversion by adding hydroxylamine solution before extraction
various oximes we know

Perillaldehyde oxime ..... a.k.a. pellartine

Pralidoxime
Oxime formation
Oxime formation

3 peaks from 2-ketoglutaric

pt urine using direct extraction
(no oximation)

same pt urine
oximated
Oxime formation

M/Z=377

2-ketoglutaric oxime. tms3
Another useful example is conversion of pyruvic acid to pyruvic oxime. Helps to confirm that the lactic acid we see is due to a “lactic acidosis”.
oximation......showing pyruvic
Application of GCMS to IEM

• Identify the acids present ……match them against known disorders
• Or look for large increases above normal
• Our clinicians will often have a family history or have a differential diagnosis
• The GCMS trace can confirm that option as in the following slides:
Fumarase deficiency
Fumarase deficiency

- rare IEM of the citric acid cycle
- Fumaric increased
- Some elevation of succinic
Glutaric aciduria type 1

- produces toxic glutaric acid in the brain
- and also 3-OH-glutaric
Glutaric aciduria type 2 (a.k.a. MADD)

- reveals much higher urine concentrations of glutaric acid
- with much less neural damage likely
Disorder of fatty acid metabolism – patients can’t burn fat so after using blood glucose and liver glycogen brain runs out of glucose and patient becomes comatose.

The distinctive acid patterns are the increases in the dioic acids adipic, suberic and sebacic, hydroxyacids and

The diagnostic feature is the presence of certain acyl glycines e.g. hexanoyl, suberyl

Emergency treatment – glucose and later regular feeding.
Chirality……yes, even in IEM!
Summary

- There are many disorders caused by missing enzymes interrupting metabolic cycles
- Many of these can be diagnosed from examination of carboxylic acids excreted in the urine
- GCMS is a powerful tool to identify and measure these