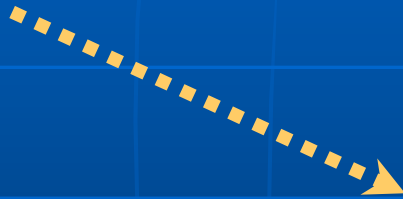


# Organic acids

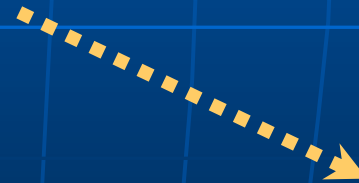
*Dr J R Bonham, Sheffield Children's Hospital, UK*

# Where do difficulties arise?

**Pre analytical**

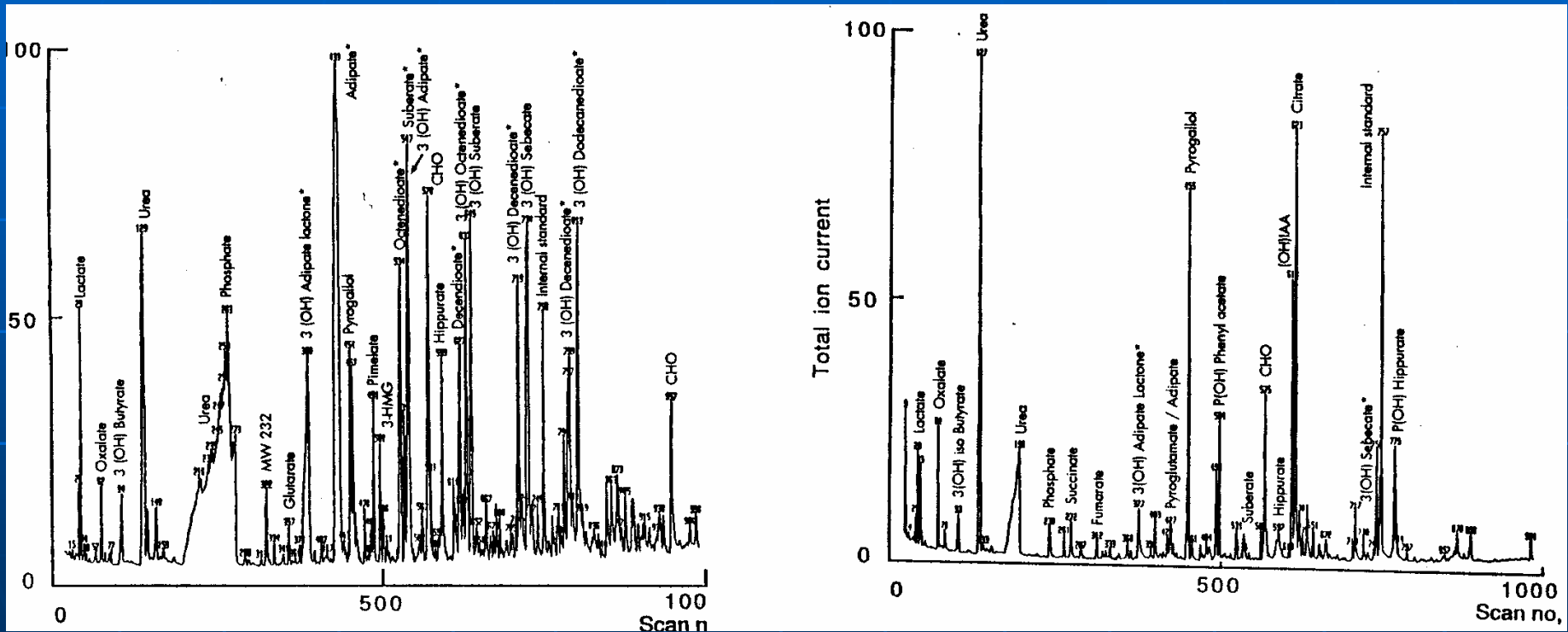


**Analytical**



**Post analytical**

# Pre – analytical Samples taken at the right time



Analogous to the poisoned patient

# Analytical

## The challenges

- Biochemically heterogeneous
- Small amounts of key compounds are important
- Small sample size
- Episodic excretion
- Often performed only once
- Very often on Friday afternoon

# Analytical

So how do we perform?

MMA	100%
MCAD	100%
MMA	100%
Ornithine aminotransferase deficiency	100%
Hunter disease	100%
Ethylene glycol intoxication	96%
Glutaric aciduria type 1	94%
Cystinuria	93%
D-glyceric aciduria	93%
2-OH glutaric aciduria	92%
Malonic aciduria	92%
4-hydroxybutyric aciduria	91%
Hurler disease	87%

# Analytical

So how do we perform?

<b>Biotinidase deficiency</b>	<b>82%</b>
<b>Morquio disease</b>	<b>82%</b>
<b>Hypophosphatasia</b>	<b>69%*</b>
<b>Homocystinuria</b>	<b>68%*</b>
<b>Fumarase deficiency</b>	<b>53%*</b>
<b>Peroxisomal disorder</b>	<b>46%*</b>
<b>Prolidase deficiency</b>	<b>38%*</b>
<b>Sialidosis</b>	<b>27%*</b>

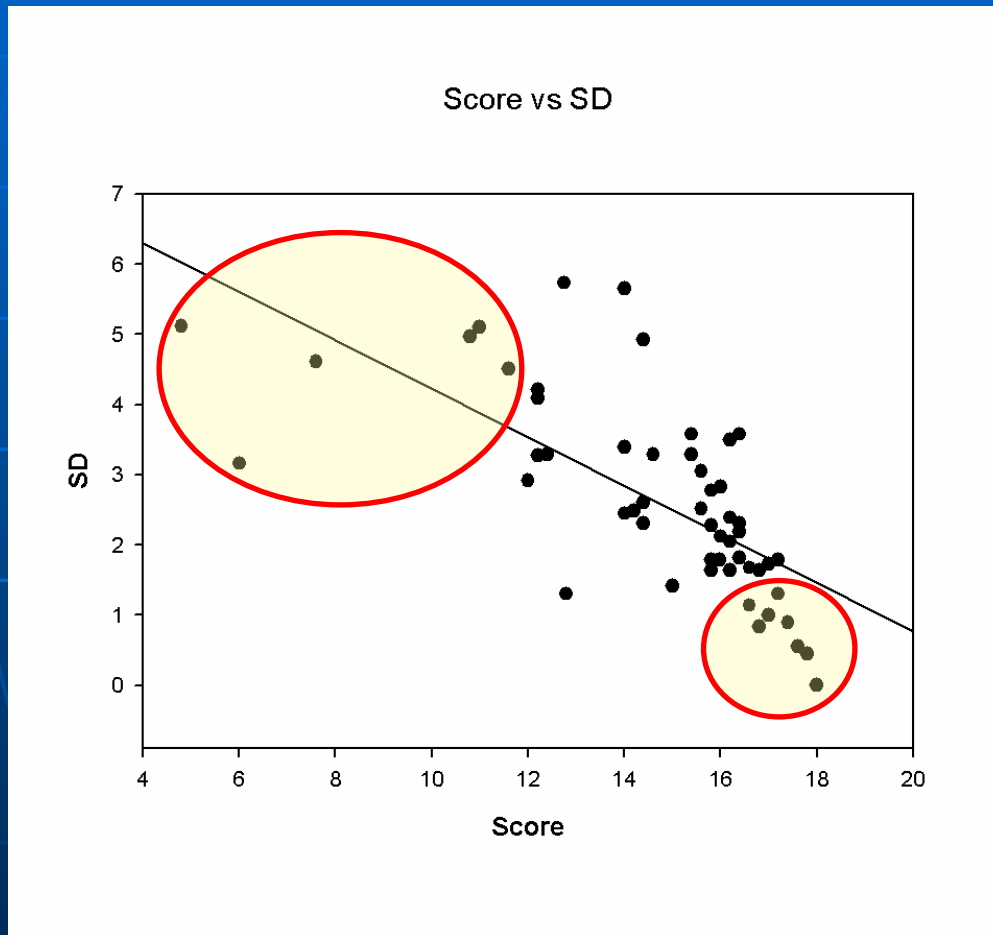
# Analytical

So how do we perform?

- **In optimal conditions with specialist laboratories in straightforward samples**
  - **93% of laboratories identify disorders**
  - **1 in 14 are missed**
- **There are particular problems with less common or unusual biochemical presentations BUT we know that in practice heterogeneity is marked and QA samples are treated with extra caution**

# Analytical

Some laboratories do well and others do not



## ERNDIM urinary organic acid scheme

- 3 urine samples sent 3 times pa
- Scored as 2,1,0 or -2
- Maximum score 18

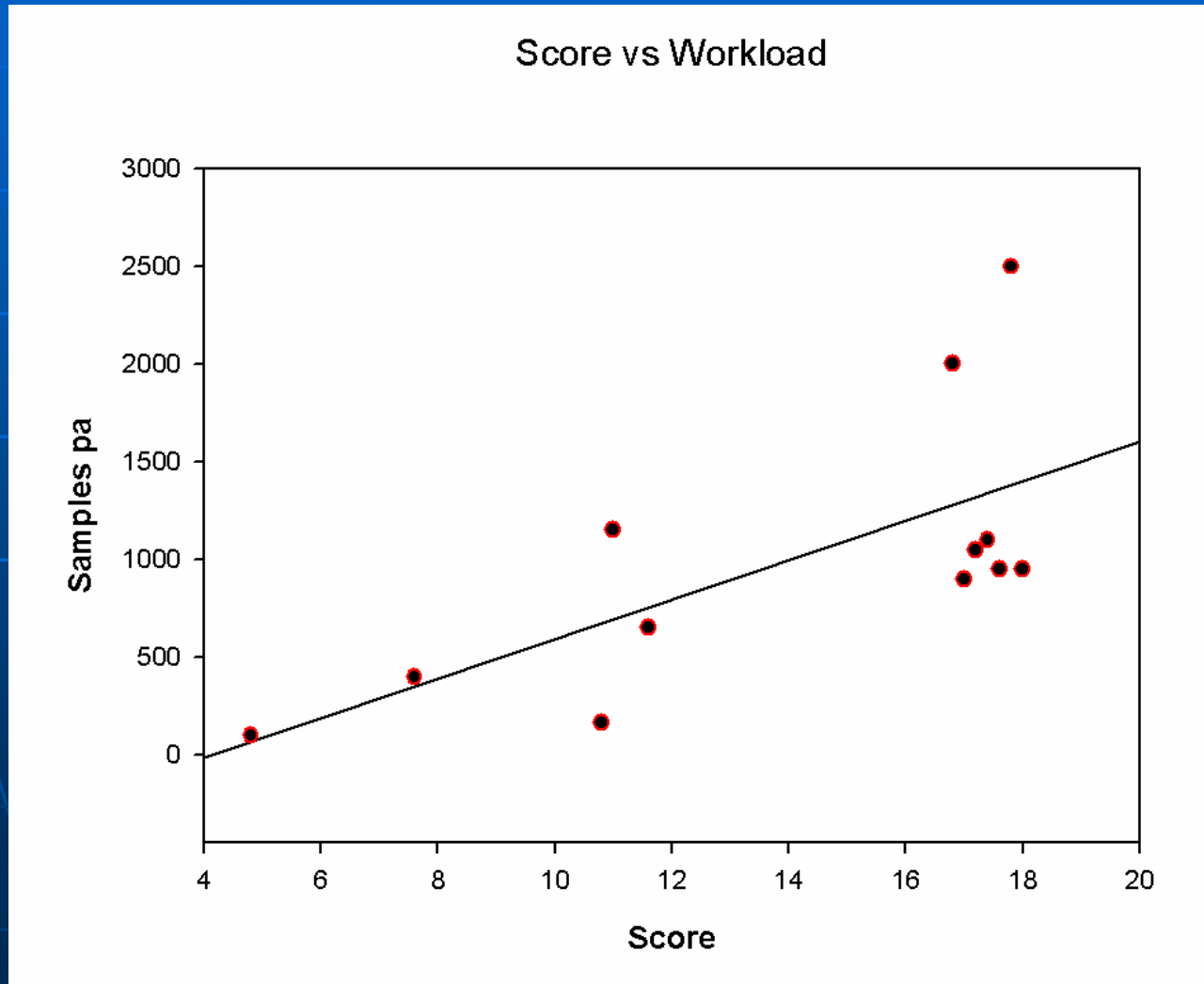


# Analytical

## Technology does not solve the problem

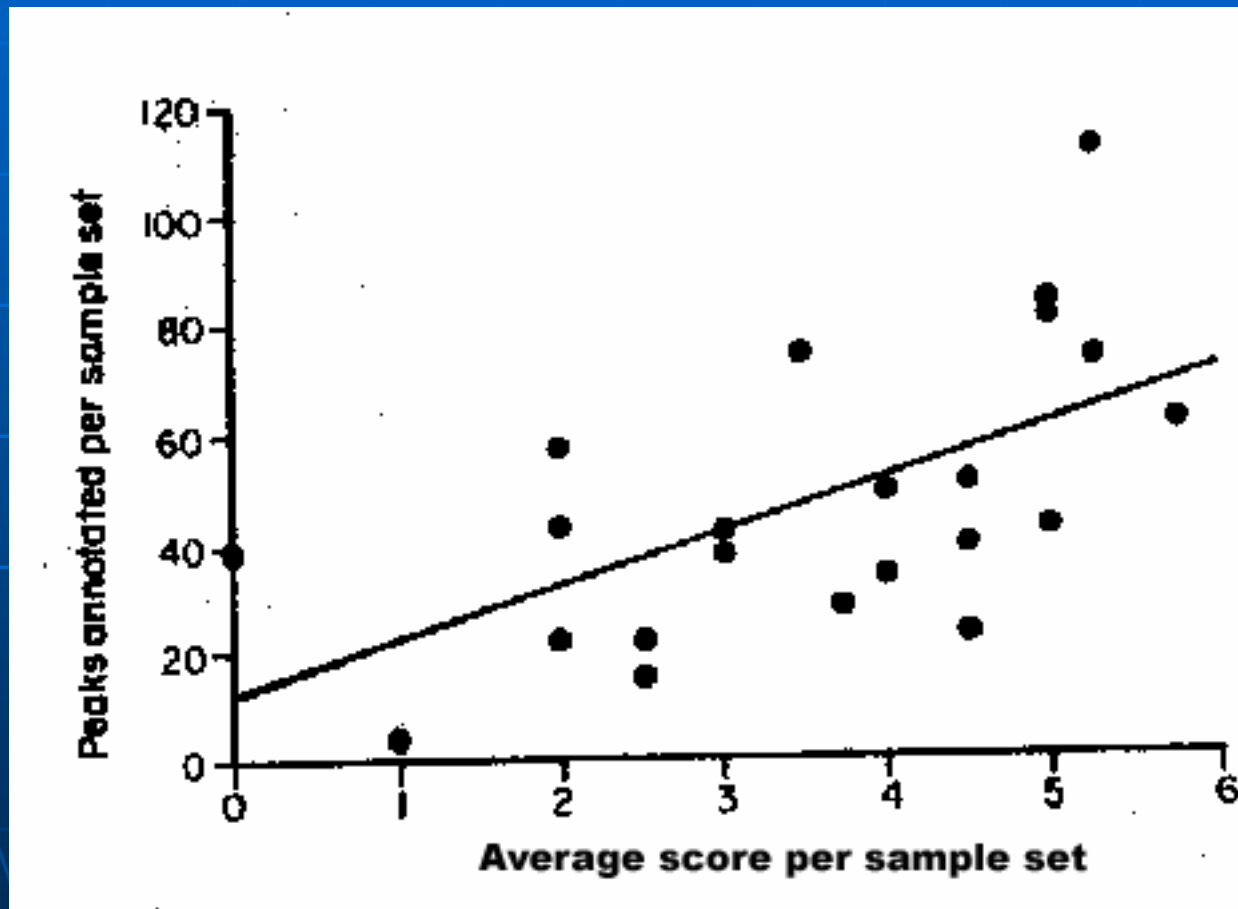
- **No correlation with equipment**
  - Type of GCMS
  - Type of column
  - Method of extraction
  - Software
- **No correlation with analytical method**
  - Type of extraction
  - Oximation
  - Use of extracted ion chromatograms
  - Use of internal standards
- **No correlation with the organisation of staff**
  - Rotation or not
  - Type of staffing
  - Group or individual interpretation
  - Turn around time

# Analytical Experience is important



# Analytical

Attention to detail is important



# Analytical

Education and awareness are important

- **Attendance at meetings**
  - mean score non-attendees 3.1
  - mean score attendees 4.4
  - $P = 0.08$

**The ERNDIM  
proficiency scheme  
2005**

# Samples in 2005

- Patient 05.1

A 20 year old patient, who was born to non consanguineous parents. He is slightly retarded (stopped school at 12) but is working as a gardener. From 17 years old, he presented with ophthalmological symptoms ascribed to allergy and from 18, palmer keratosis ascribed to verucca

*This sample was obtained from a patient with tyrosinaemia type 2*

- Patient 05.2

A male aged 3 years, unexplained recurrent hypoglycaemia

*This sample was from a healthy child of one of the laboratory staff*

- Patient 05.3

Male aged 6 years, rickets, ? Cause

*This sample was obtained from a patient with tyrosinaemia type 1*

# Samples in 2005

- **Patient 05.4**

**A male aged 13 years with dorsal kyphosis**

*This sample was obtained from a patient with MPS type 4 aged 13 yrs*

- **Patient 05.5**

**A female aged 27 years with osteoarthritis**

*This sample was from a patient with alkaptonuria*

- **Patient 05.6**

**A female, aged 30 years, severe osteoporosis**

*This sample was obtained from a 30 yr old woman with classical homocystinuria*

# Scoring

<b>Analytical results :</b>	<b>2 points</b>
<b>Interpretative conclusions:</b>	<b>2 points</b>
<b>Further testing advice:</b>	<b>1 point</b>
<b>No return or incorrect findings</b>	<b>0 points</b>
<b><i>Maximum obtainable</i></b>	<b><i>30 points</i></b>

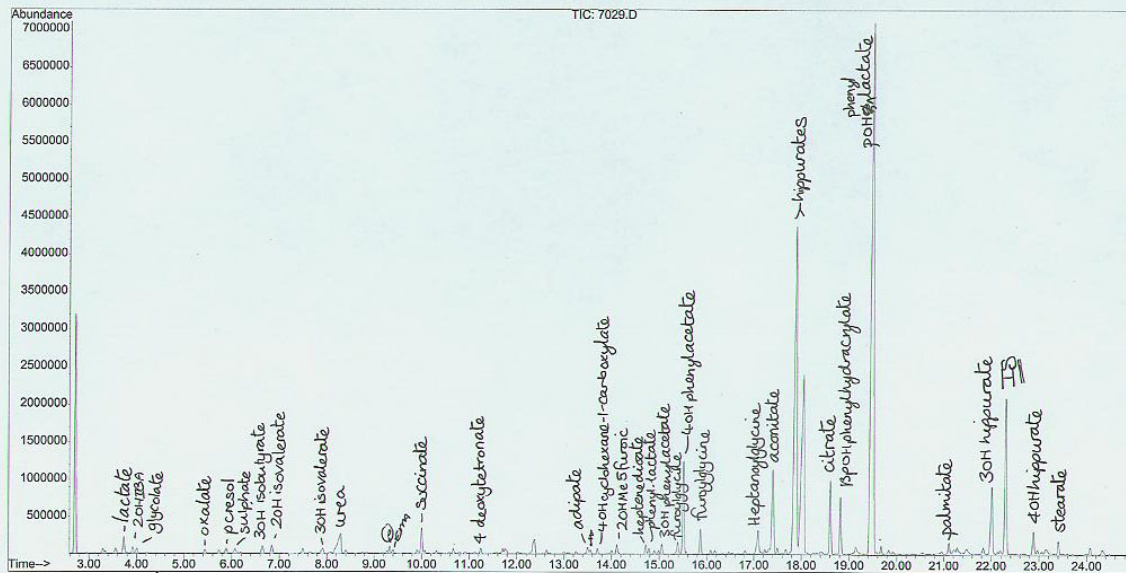


# Sample 05.1

File : C:\MSDCHEM\1\DATA\ORGACIDS\7029.D  
 Operator :  
 Acquired : 23 Mar 2005 1:59 using AcqMethod OA  
 Instrument : Instrumen  
 Sample Name: PA 05/01 70576.K (NA)  
 Misc Info :  
 Vial Number: 29

Hept gly 102-44K

No hex /swb /PPG  
 No IVG /MCG /ISG  
 No ondate /4OHBA  
 No succate /Acte  
 No 3OH glut



# Results

## *Sample 05.1*

*Returns were received from all of the 26 participants*

- All 26 participants noted an increased excretion of tyrosine
- 16/26 participants quantitated the excretion, mean tyrosine 87  $\mu\text{mol/L}$ , SD 8.0  $\mu\text{mol/L}$
- 16/26 reported succinyl acetone not present or not detected
- 25/26 participants concluded that the most likely diagnosis was tyrosinaemia type 2, the remaining lab suggesting liver dysfunction
- 25/26 recommended quantitative plasma aminoacid analysis
- 3/26 advised enzyme assay on liver biopsy material, 13/26 commented that this may not be indicated
- THIS WAS THE COMMON SAMPLE

# Results

## *Sample 05.2*

*Returns were received from all of the 26 participants*

- 22/26 laboratories clearly reported “no abnormality detected”
- Given the history of hypoglycaemia 25/26 laboratories indicated that further laboratory or clinical investigations were warranted
- 20/26 would have advocated blood/plasma acyl carnitine profile
- 9/26 indicating the need to obtain a urine sample during or shortly after a period of documented hypoglycaemia
- 5/26 laboratories would have recommended a controlled fast

# Sample 05.3

Hept gly 102 = 48K

No hex (sub) PPG

No IVG/MCG/IBG

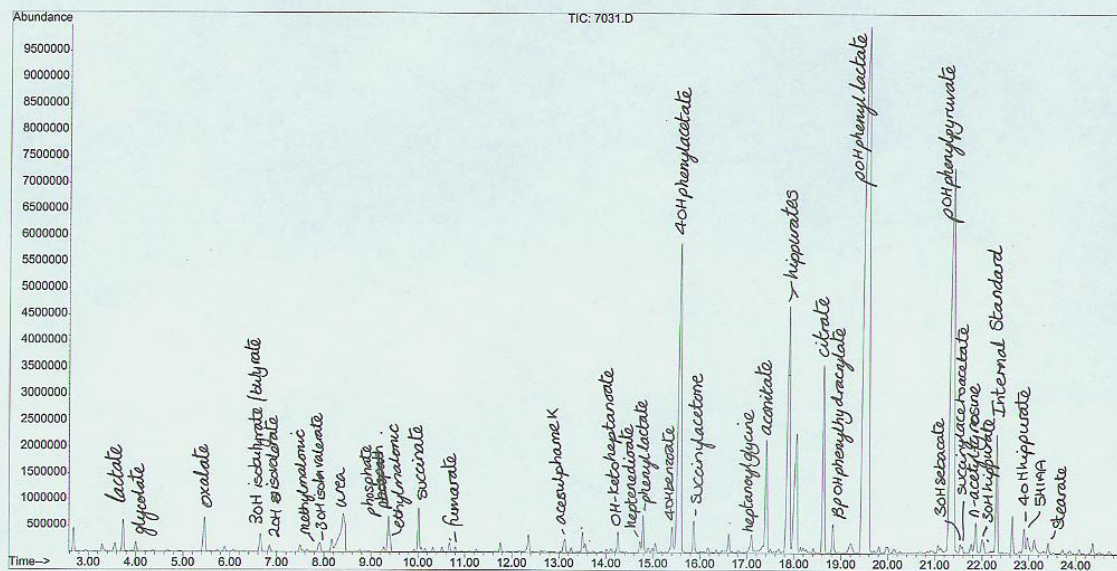
No onitate /4OHBA

✓ Succinylacetone 169 = 40K

✓ Succinylacetoacetate 345 = 5K

No 3OH glutarate

File : C:\MSDCHEM\1\DATA\ORGACIDS\7031.D  
 Operator :  
 Acquired : 23 Mar 2005 3:23 using AcqMethod OA  
 Instrument : Instrumen  
 Sample Name : PA 05/03 70578.B (NA)  
 Misc Info :  
 Vial Number: 31



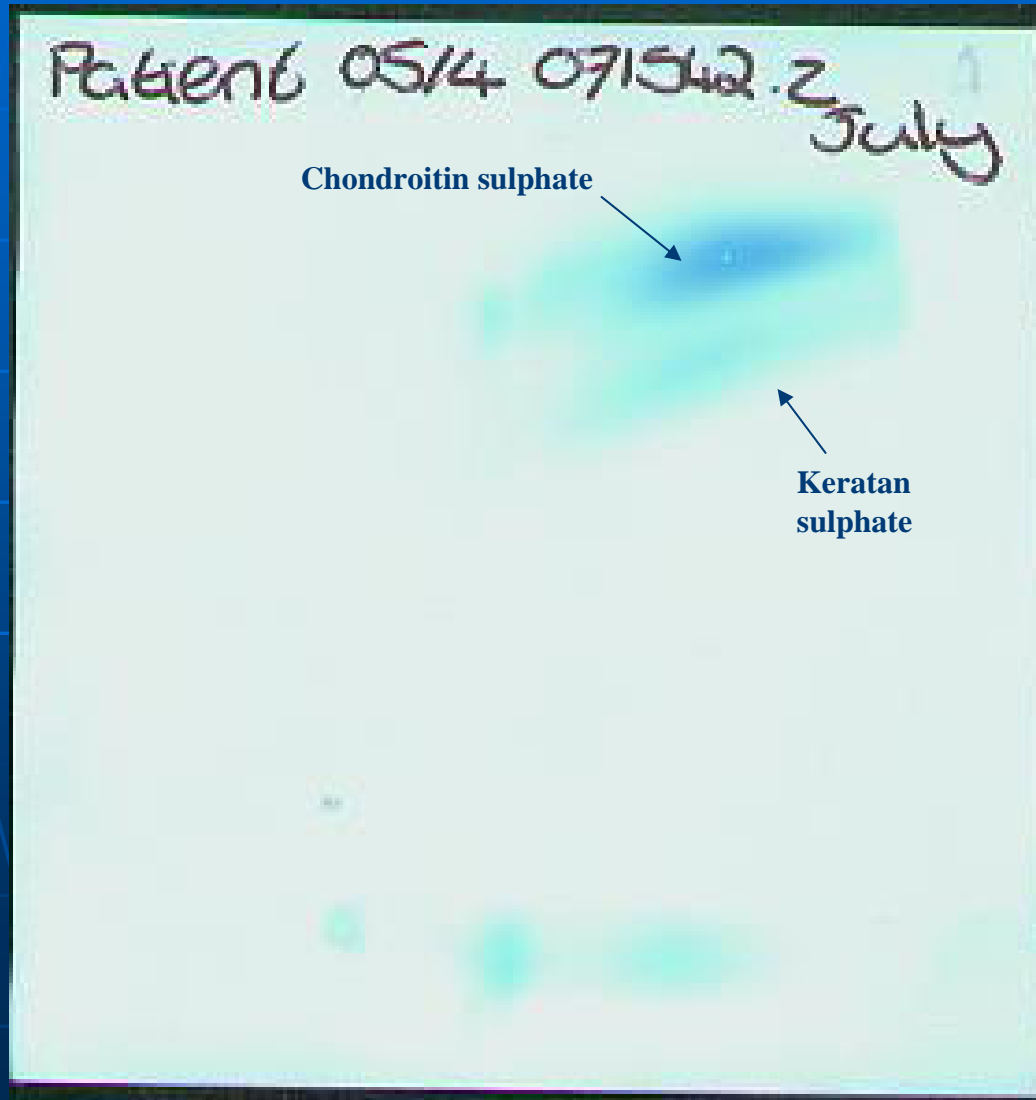
# Results

## *Sample 05.3*

*Results were received from all of the 26 participants.*

- 24/26 commented upon an increased excretion of tyrosine
- 2/26 reporting a generalised aminoaciduria
- 15/26 quantitated tyrosine, mean= 331  $\mu\text{mol/L}$ , SD 65
- All participants noted an increased excretion of tyrosine metabolites on urinary organic acid analysis
- 23/26 commented on a significant excretion of succinyl acetone or derivatives, 3/26 did not comment on succinyl acetone, one of these specifically indicating that this was "not detected"
- 23/26 participants concluded that the patient suffered from tyrosinaemia type 1. 2/26 (both had not detected succinyl acetone) felt that tyrosinaemia type 1 was possible

# Sample 05.4



# Results

## *Sample 05.4*

*Results were received from all 26 participants*

- All 14 participants who made quantitative measurement of GAGS noted an increase
- 9/26 commented specifically on the excretion of keratan sulphate
- 24/26 participants, on the basis of laboratory findings or clinical description, considered that an MPS disorder was likely or possible
- 13/26 specifically considered MPSIV (Morquio disease) as a possibility
- 16/26 laboratories would have recommended enzyme analysis



# Sample 05.5

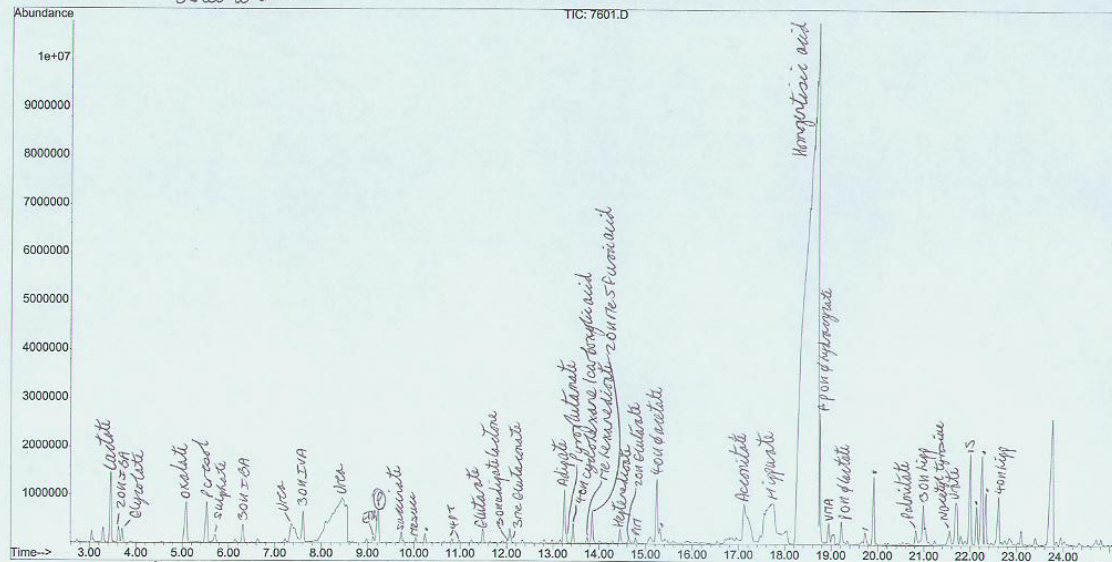
File : C:\MSDCHEM\1\DATA\ORGACIDS\7601.D  
 Operator :  
 Acquired : 19 Jul 2005 17:11 using AcqMethod 3M  
 Instrument : Instrumen  
 Sample Name: PATIENT 5.5 071543.Q (NA)/(1 day)  
 Misc Info :  
 Vial Number: 1 27 ♀

HeptGly 102 = 12K  
 158 = -

No Hex/Sub/PPG  
 No NE/TCE/IBC  
 No ORO/40MBA  
 No succ AC/ACAC  
 No 30M elut

Allopterin

Osteoarthritis





# Results

## *Sample 05.5*

*Results were received from all 26 participants*

- All 26 participants reported an increased excretion of homogentisic acid
- All concluded that the patient suffered from alkaptonuria
- 10/26 participants reported a generalised increase in aminoacid excretion
- All 6 participants who reported quantitative MPS noted an increased excretion, possibly due to interference
- 5/26 would have recommended that other family members should be investigated

# Results

## *Sample 05.6*

*Results were received from all 26 participants*

- 25/26 reported an increased excretion of homocystine, mean concentration 59  $\mu\text{mol}/\text{mmol cr}$
- All participants who noted an increased excretion of homocystine concluded that CBS deficiency was the most likely diagnosis
- 24/25 participants asked for plasma aminoacid analysis and 20/25 would have requested a sample for total plasma homocysteine
- Only 9/25 commented directly on or the need to assess MMA excretion
- 16/25 would have recommended a therapeutic trial with pyridoxine.

# Scores

ERN Lab No	05.1	05.2	05.3	05.4	05.5	05.6	Total Score
004	5	5	5	3	5	5	28
010	5	5	5	5	5	5	30
011	5	3	4	3	2	5	22
021	5	4	5	4	5	5	28
029	5	5	5	2	5	5	27
032	5	4	5	2	5	5	26
042	3	3	3	3	5	5	22
060	5	5	5	3	5	5	28
066	5	4	5	5	5	5	29
099	4	4	5	2	5	5	25
100	5	4	5	5	5	5	29
104	5	4	4	5	5	5	28
107	5	4	4	5	5	5	28

# Scores

ERN Lab No	05.1	05.2	05.3	05.4	05.5	05.6	Total Score
110	5	4	5	2	5	5	26
114	5	5	5	5	5	5	30
117	5	4	5	5	5	5	29
142	5	4	5	5	5	5	29
149	5	4	5	3	5	5	27
158	5	4	5	0	5	5	24
175	5	5	5	2	5	5	27
194	5	4	5	5	5	5	29
240	5	3	5	3	5	5	26
251	5	4	5	2	5	0	21
284	5	5	3	0	5	5	23
285	5	4	5	2	5	5	26
293	5	4	1	2	3	5	20

# **The NEQAS Orotic acid scheme**

# Performance

Distribution	Mean $\mu\text{mol}/\text{mmol cr}$	Range $\mu\text{mol}/\text{mmol cr}$	Normal	Equivocal	High
<b>Sept 05</b>	1.7	0.5-5.0	11	0	1
	2.1	1.5-4.5	12	0	0
	3.0	2.0-6.0	12	0	0
<b>Aug 05</b>	51.0	36.0-68.0	0	0	11
	50.0	40.0-60.0	0	0	11
	50.0	40.0-64.0	0	0	11
<b>May 05</b>	2.0	0.5-4.5	10	1	0
	4.8	3.5-6.5	3	6	2
	5.5	3.5->8.0	3	5	3
<b>Mar 05</b>	1.2	0.5-3.0	9	0	0
	2.8	2.0->6.0	8	0	1
	9.8	8.0->16.0	0	4	5

# Performance

Distribution	Mean μmol/mmol cr	Range μmol/mmol cr	Normal	Equivocal	High
<b>Dec 04</b>	2.0				
	4.9				
	5.8				
<b>Oct 04</b>	1.6				
	3.2				
	9.6				
<b>Aug 04</b>	48.9				
	48.7				
	50.9				
<b>Jun 04</b>	100.9				
	8.1				
	37.9				
<b>Apr 04</b>	73.8				
	101.0				
	51.7				
<b>Feb 2004</b>	47.6				
	48.6				
	49.0				

# Performance

Distribution	Mean μmol/mmol cr	Range μmol/mmol cr	Normal	Equivocal	High
<b>Dec 04</b>	2.0	1.0-6.0	11	0	0
	4.9	3.0-7.0	4	7	0
	5.8	3.0-7.0	4	6	1
<b>Oct 04</b>	1.6	0->6.0	9	1	0
	3.2	1.5->6.0	9	1	0
	9.6	5.0->16.0	1	2	7
<b>Aug 04</b>	48.9	36.0-64.0	11	0	0
	48.7	36.0-64.0	11	0	0
	50.9	32.0-60.0	11	0	0
<b>Jun 04</b>	100.9	40.0-130.0	11	0	0
	8.1	0-12.0	1	4	6
	37.9	5.0-60.0	1	0	10
<b>Apr 04</b>	73.8	41.0-91.0	0	0	9
	101.0	58.0-116.0	0	0	9
	51.7	25.0-62.0	0	0	9
<b>Feb 2004</b>	47.6	8.0-68.0	0	0	7
	48.6	16.0-66.0	0	0	8
	49.0	16.0-58.0	0	0	8



# Conclusions

- We know that there is a problem in the range 4.0-10.0  $\mu\text{mol}/\text{mmol}$  creat
- We don't know but could find out whether this is analytical or interpretative
- Could things be improved with a calibrant ?
- Could things be improved by adopting clear guidance for interpretation ?