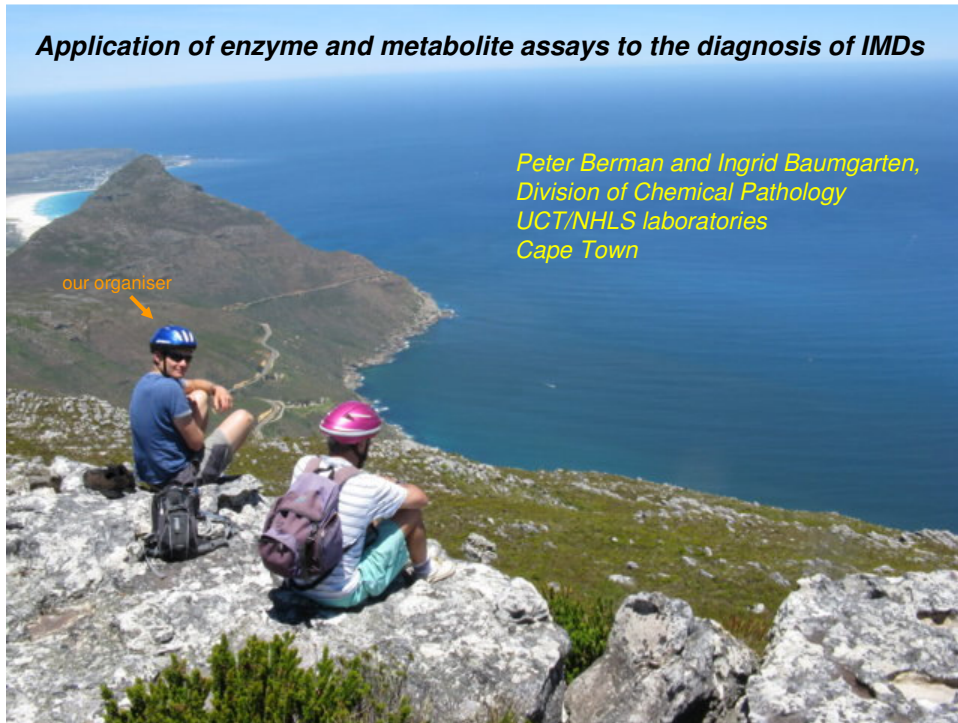


Application of enzyme and metabolite assays to the diagnosis of IMDs

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Division of Chemical Pathology
UCT/NHLS laboratories
Cape Town



Primary hyperoxaluria

Defect: alanine-glyoxalate transaminase

Location: hepatic peroxisomes

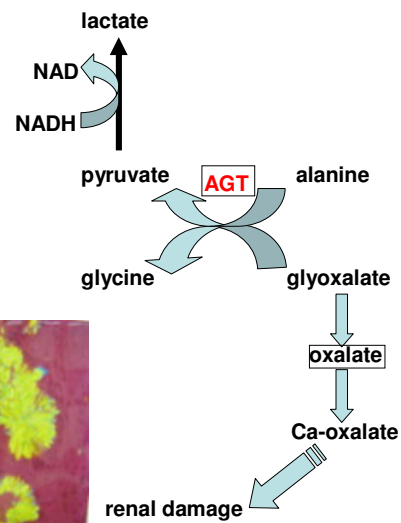
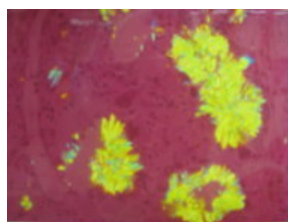
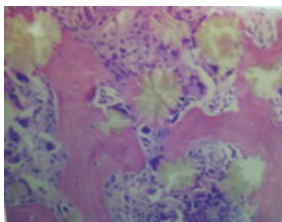
Assays offered:

- urine oxalate
- AGT in liver biopsy (*Gill Rumsby, UK*)

Diagnostic importance:

The definitive treatment to avoid permanent renal damage is liver transplant.

Surgeons like to be absolutely sure of diagnosis before transplanting a healthy (looking) liver



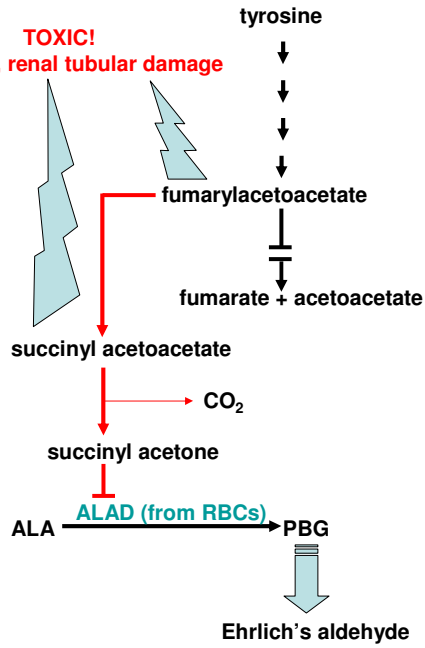
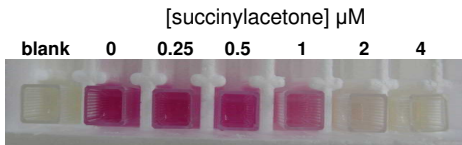
Tyrosinosis type 1

Defect: fumaryl acetoacetate hydrolase

Diagnostic marker: succinyl-acetone

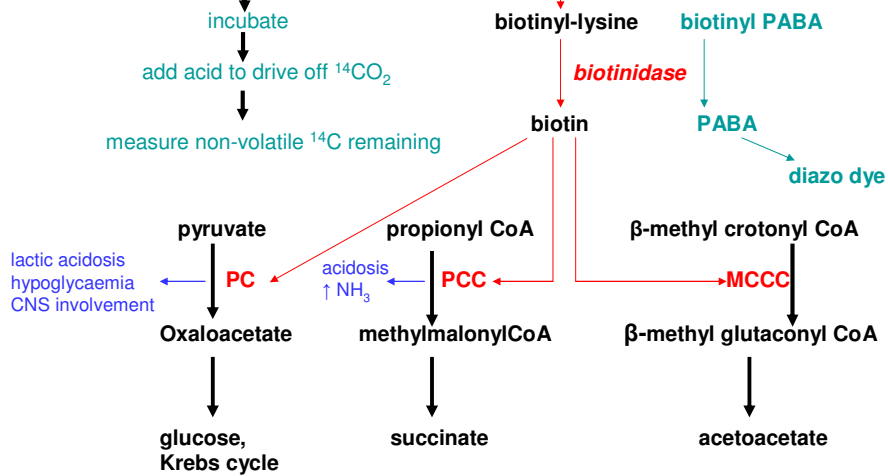
Basis of diagnostic test:
inhibition of ALA dehydratase

Can also be used to assay for lead (Pb)



Carboxylase defects

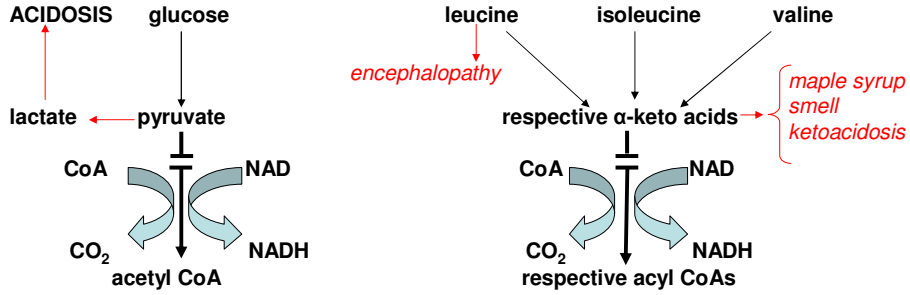
Assay: fibroblast* extract + substrate + $^{14}\text{C}[\text{HCO}_3^-]$



* or amniocyte

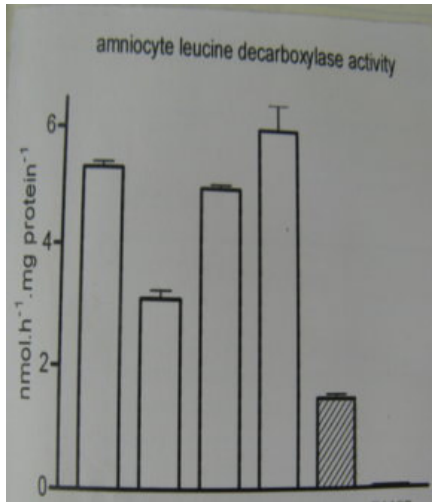
Decarboxylase defects

- Pyruvate dehydrogenase → congenital lactic acidosis
- Branch chain α -keto acid dehydrogenase → maple syrup urine disease



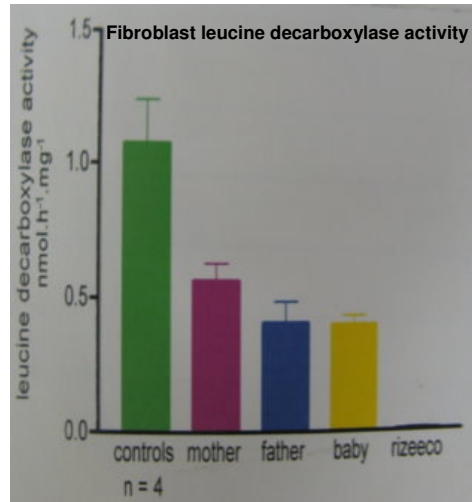
Assay: intact fibroblasts* + 1-¹⁴COOH-labelled substrate (pyruvate or leucine)
 ↓ incubate
 *or amniocytes ↓ add acid → trap ¹⁴CO₂ released → count

Prenatal diagnosis of Maple Syrup Urine Disease



Control 1 2 3 4 fetus rizeeco

at 16 weeks



n = 4

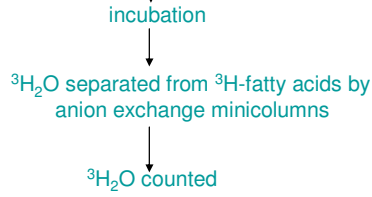
after birth

Fatty acid oxidation defects

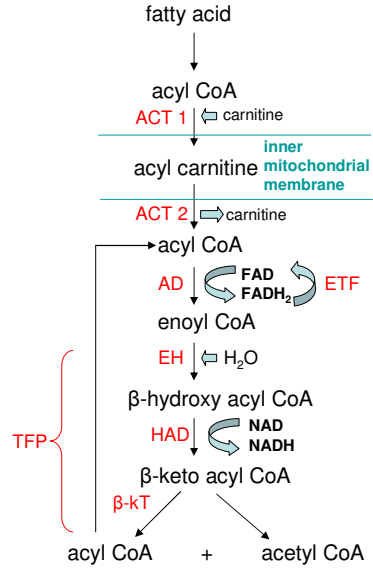
Screening test for transport and oxidation of fatty acids

Principle: *from Simon Olpin, Sheffield*

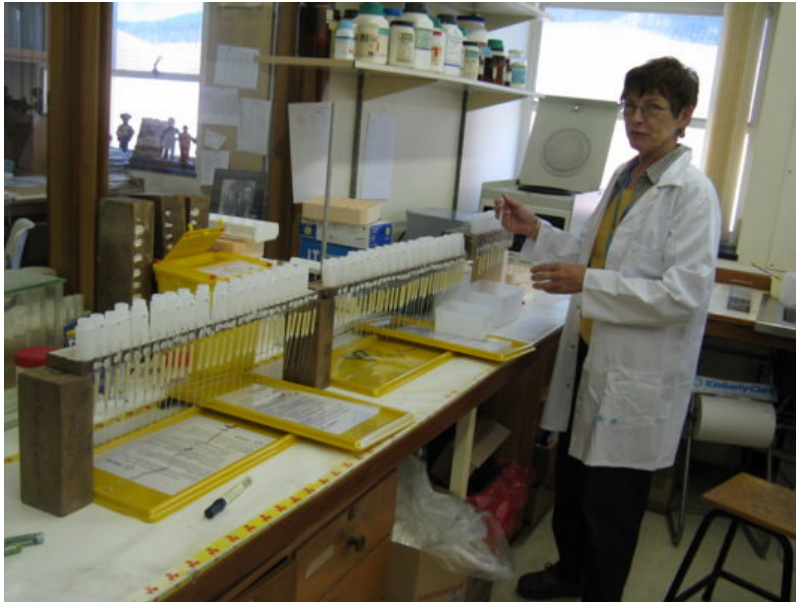
³H-labelled fatty acids of varying chain length (C14:0, C16:0, C18:1) + intact fibroblasts

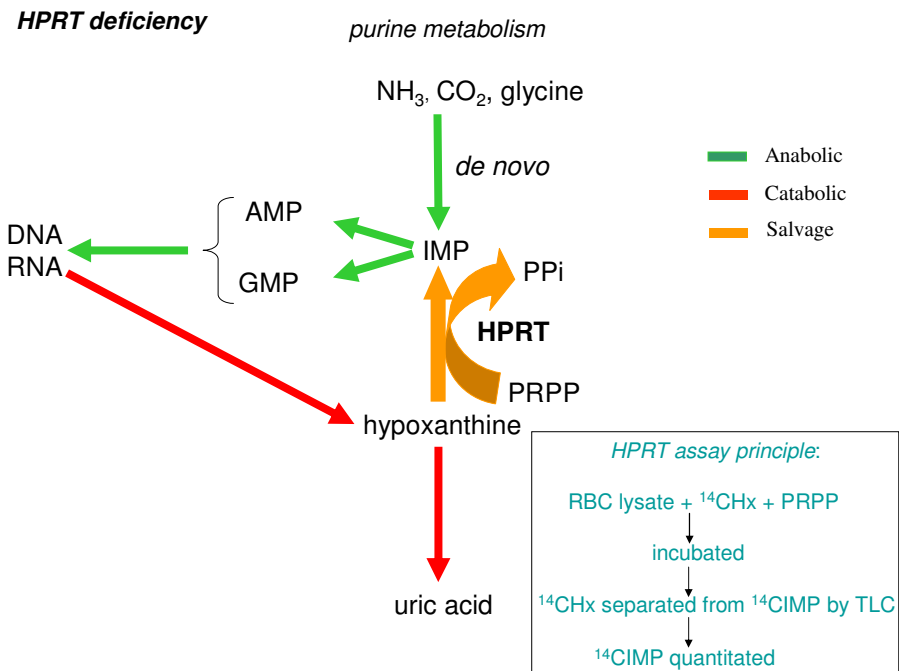


FAO in nmol.h ⁻¹ .mg ⁻¹	myr	palm	oleate
Control	4.0	9.2	2.2
Glut aciduria II	0.1	0.1	0.1



Ingrid (alias DB) hard at work with 72 anion exchange minicolumns doing a fatty acid oxidation assay

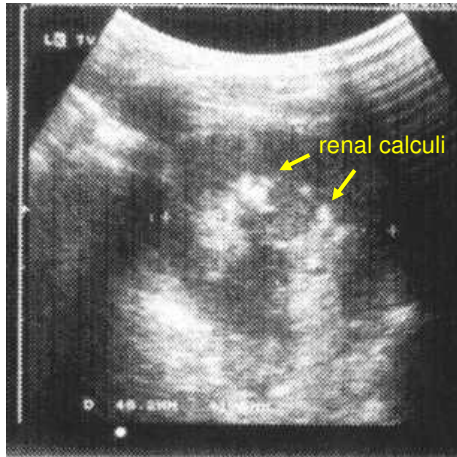




- N vW, 21y, spent most of his life in Alexandra Institute
- grossly physically and mentally handicapped
 - choreoathetoid movements
 - tendency to hurt himself (or anyone else he can reach!)



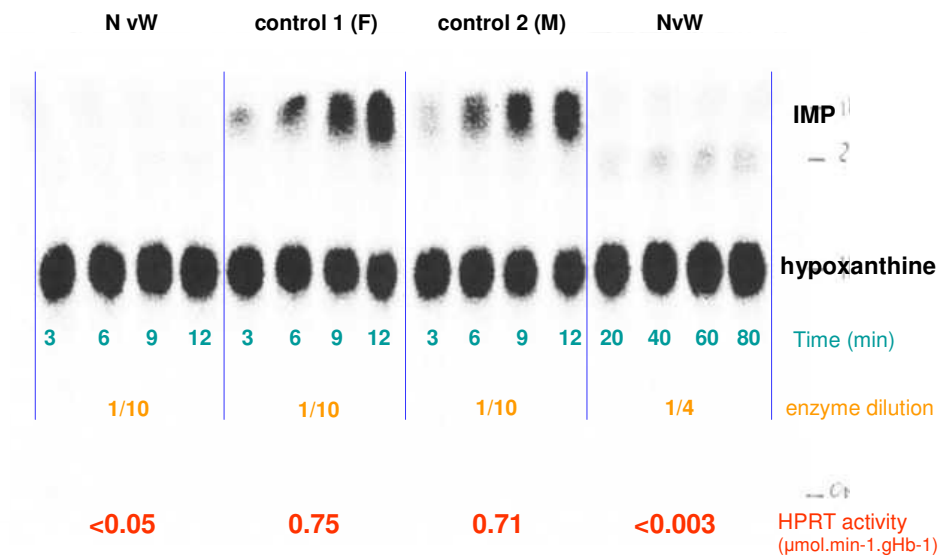
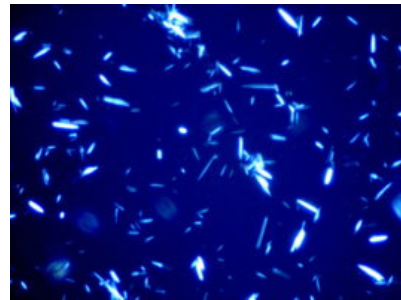
abdominal ultrasound



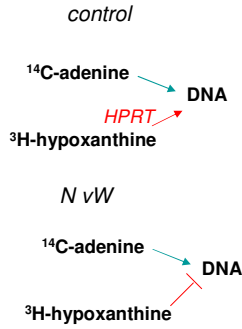
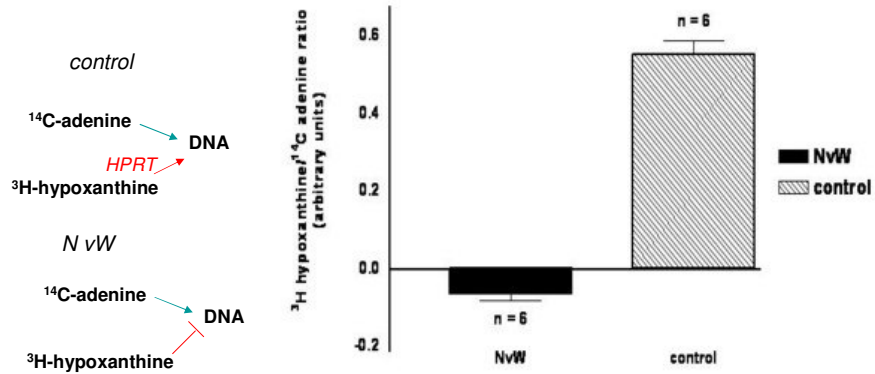
Gouty tophus



urate crystals



Incorporation of ^3H hypoxanthine into acid precipitable material by cultured fibroblasts* (mean \pm SD)

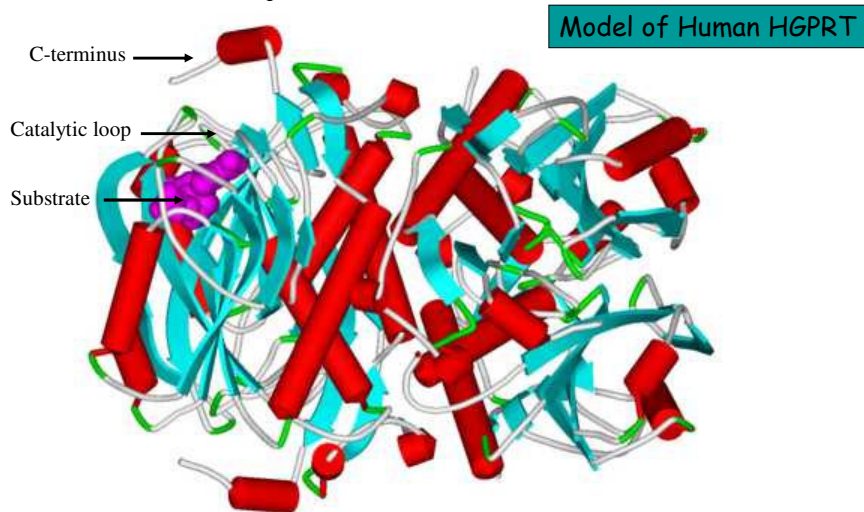


*Relevant if diagnosis ever needs to be made prenatally on amniocytes

Mutation analysis of *N vW*:

10 9 8 7 6 5 4 3 2 1
 AGT-GAA-ACT-GGA-AAA-GCA-AAA-TAC-AAA-GCC-TAA
 ↓
 T glu → stop; 9 amino acids from C-terminal

Note C-terminus acting as 'lid' to cover the substrate binding site

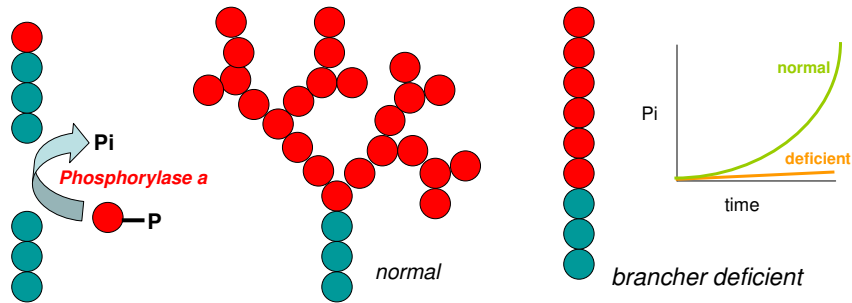


Brancher deficiency (for glycogen storage deficiency type IV)

While biochemical diagnosis of many GSDs requires liver biopsy, glycogen brancher enzyme is expressed in RBCs as well.

Assay: haemolysate + glycogen + glucose-1-P + phosphorylase a
 ↓
 incubate
 ↓
 measure Pi released from incorporation of glucose into growing oligosaccharide chains

The more branch points, the the more sites for addition of new glucose molecules by phosphorylase – effectively increasing 'substrate' concentration



Other assays we have done (but don't have time to talk about):

ASSAY	DISORDER
β-keto-thiolase and succinyl CoA 3-oxoacid CoA transferase (SCOT) (fibroblasts)	sporadic ketoacidosis
Citrulline (plasma) and orotate (urine)	urea cycle disorders
Liver copper	Wilson's disease
Glucose-6-phosphatase (liver)	glycogen storage disease type I
Cytochrome c oxidase/citrate synthase (fibroblasts)	mitochondrial myopathy, lactic acidosis
Pyruvate kinase (RBCs)	} haemolysis
Pyrimidine 5' nucleotidase (RBCs)	
Purine base quantitation by HPLC (urine)	molybdenum co-factor deficiency
Glutamate dehydrogenase (lymphoblasts)	hyperinsulinism of infancy
Ethanol (blood, CSF, eye fluid post mortem)	?death by alcohol



We hope through our efforts to create some light at the end of the tunnel for affected children, their parents and their siblings (both born and unborn)

Acknowledgements

- UK collaborators, including Gill Rumsby, Garry Brown and *especially* Simon Olpin for their methodology and advice
- Patients and their parents who have allowed us to take samples, and to present their data and photographs
- Paediatricians and pathologists (eg George van der Watt, Helen Wainwright, Liz Goddard) for providing us with a steady demand for assays to establish, and a steady supply of patients on whom to perform them