A fine inheritance, but it's not all in the bag

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Willink Biochemical Genetics Unit,
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Manchester M27 4HA, UK.

The Willink Unit, Manchester - a fine inheritance

June 1979

Brian Fowler

- Joined George Komrower's group in 1965
- Mental Retardation Unit, Royal Manchester Children's Hospital. Later renamed Willink Biochemical Genetics Unit.
- Working with Michael Griffiths and Ann Lambert to set up screening tests for amino acidaemias. Cath Bridge joined group in 1967
- Gained Higher National Certificate in 1967 and MIBiol in 1970 and subsequently PhD

Brian Fowler

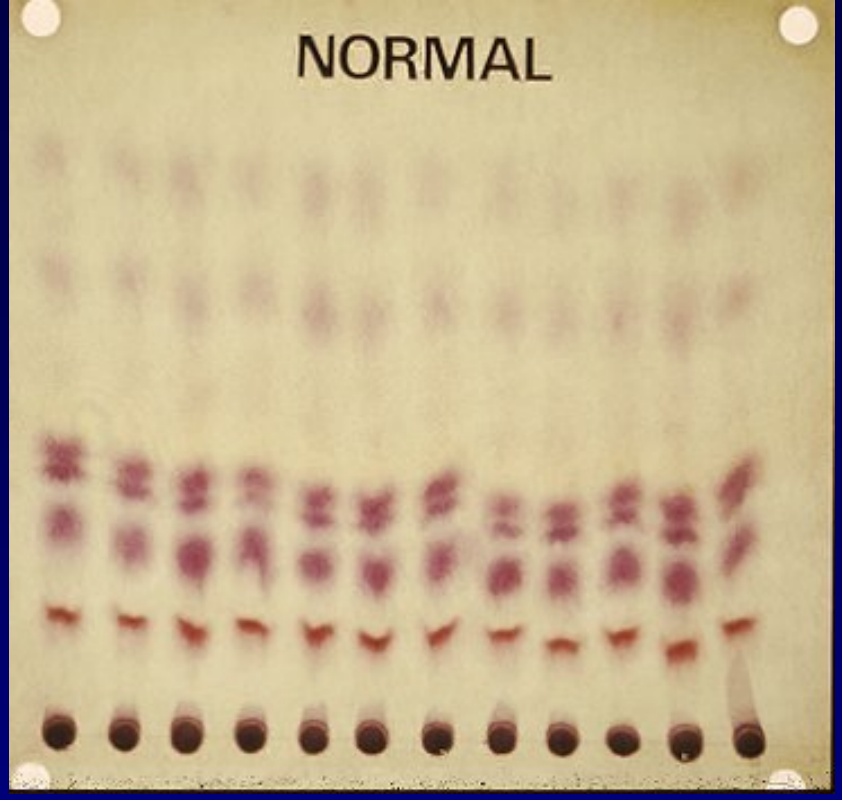
- On MRC travel scholarship
- Research at Yale with Rosenberg, Packman and Kraus on CBS
- Returned to Manchester in 1977
- Continued interest in CBS and MMA
- Willink Unit moved to a purpose-built establishment in 1984
- Left the 'new' Willink Unit in 1990

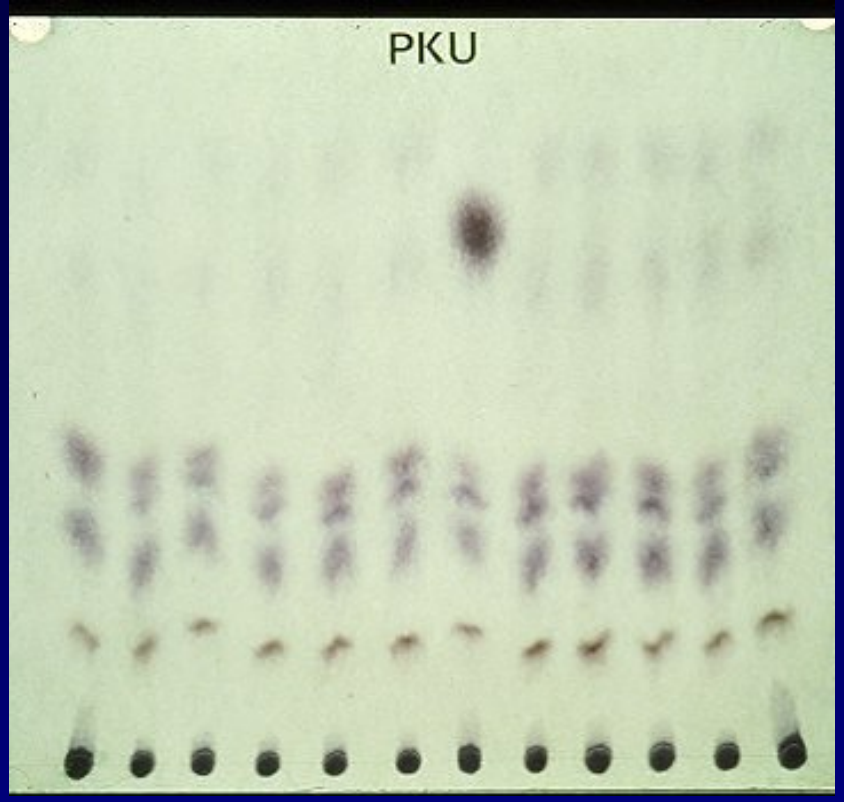
Phenylketonuria

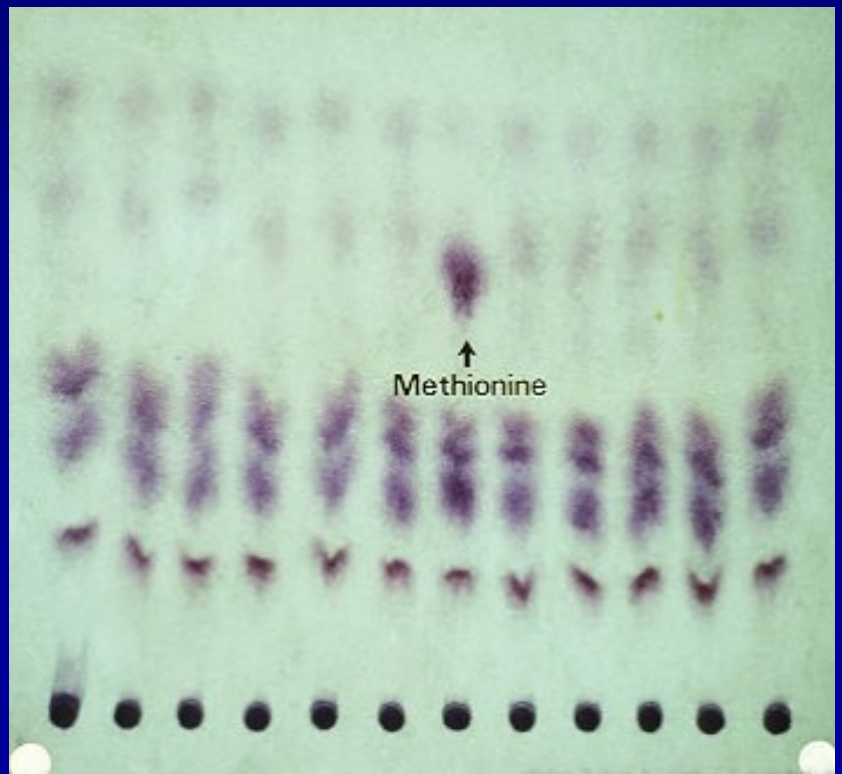
Patient Images removed

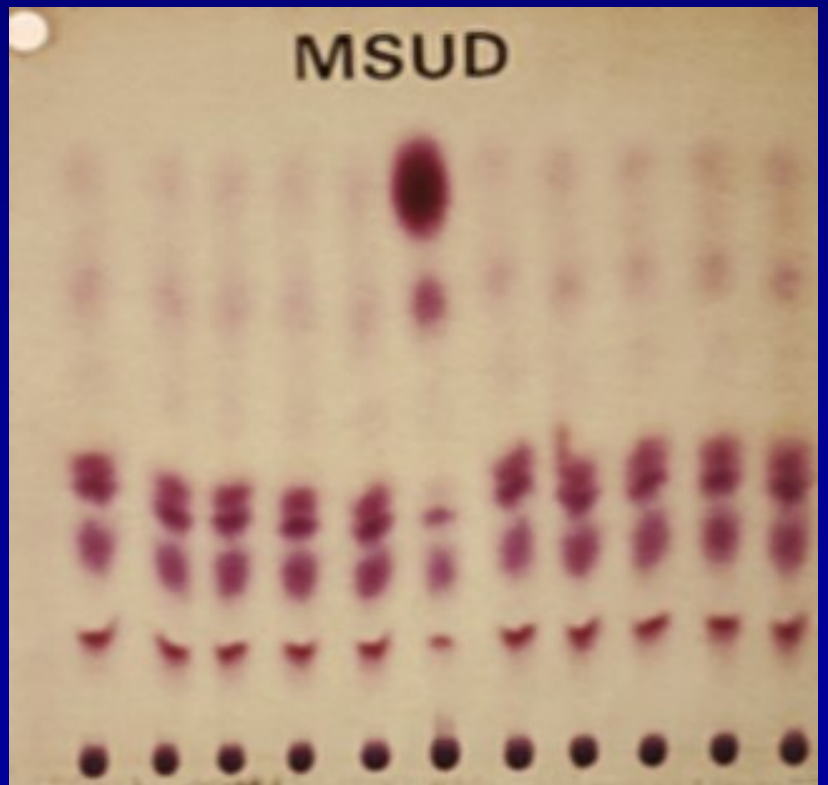
Newborn Screening

- Mental Retardation Unit (RMCH)
- Guthrie test felt to be too limited
- Scriver chromatography would identify other than PKU
- *A* community pilot survey for amino acidopathies (1967)
- Would this be workable using liquid blood?

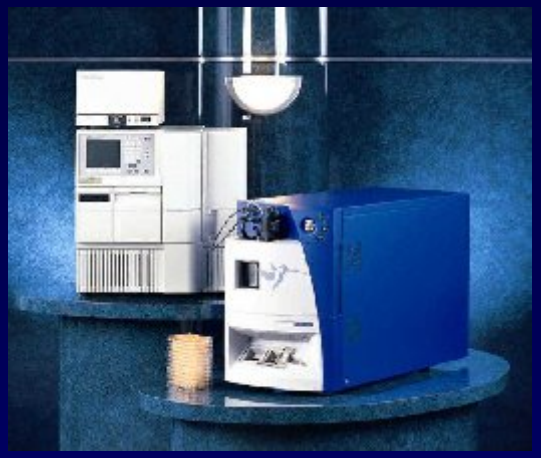

NORMAL


PKU


Methionine


MSUD

Newborn Screening

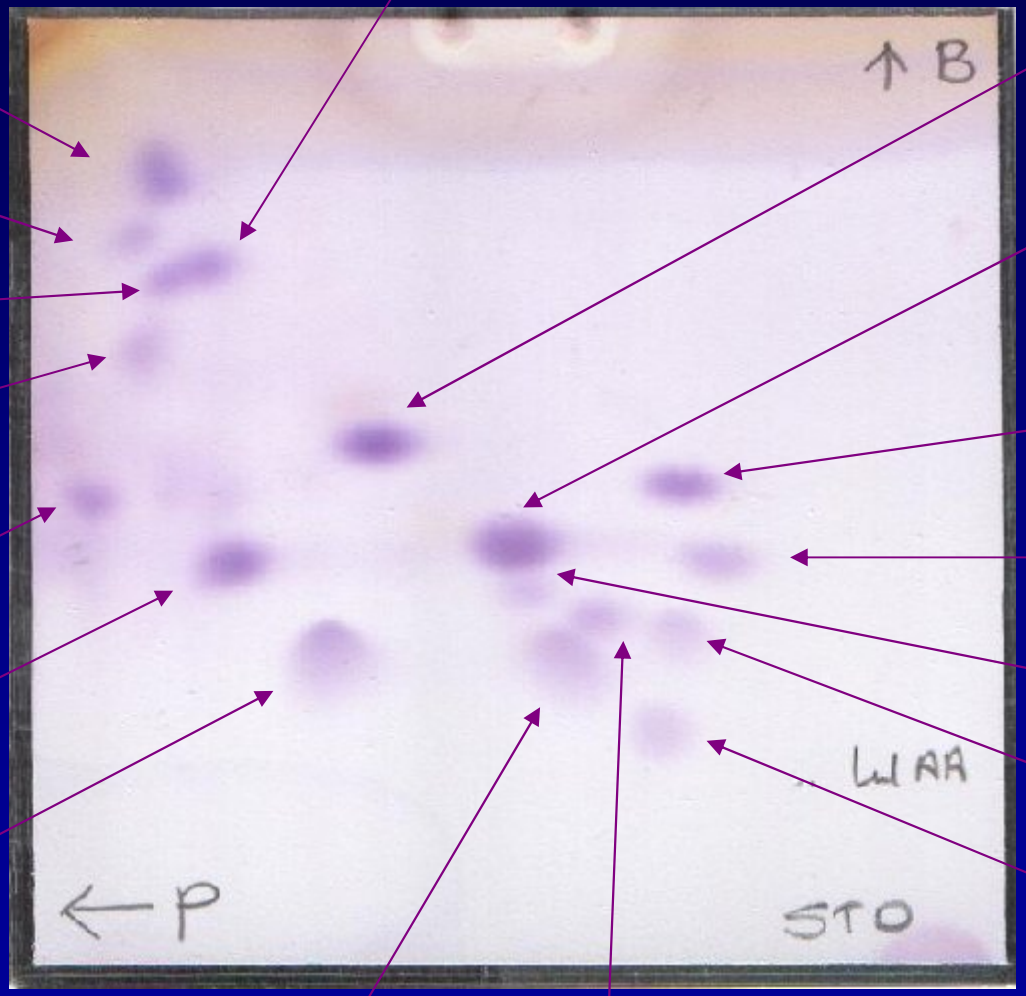


- Tandem MS purchased in 1998
- Initially used butylated samples for acylcarnitines
- PKU screening in 2001 and MCAD roll out on non-butylated in 2004
- However, continued to look at methionine and branched-chain amino acids
- UK still trying to expand newborn screening

Amino acid Screening

- 2D TLC
- Paper chromatography (iodoplatinate, Pauly and ninhydrin/Ehrlich stains)
- Spot tests
- Labour-intensive but excellent method

Interpretation of amino acid TLC patterns



Leucine/ Isoleucine

Phenylalanine

Valine

Tyrosine

Threonine

Serine

Histidine

Methionine

Alanine

Glycine

Glutamic acid

Aspartic acid

Glutamine

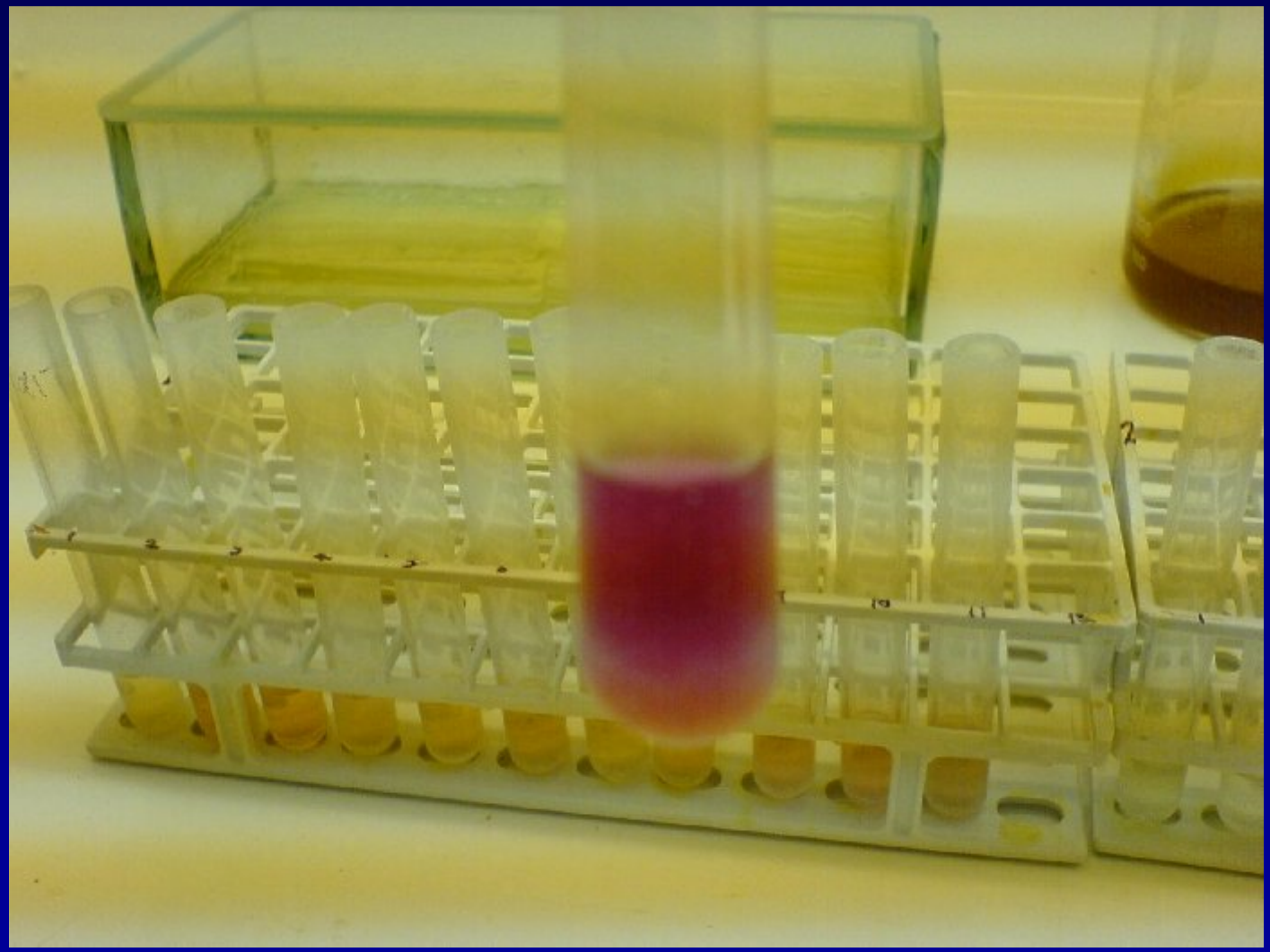
Asparagine

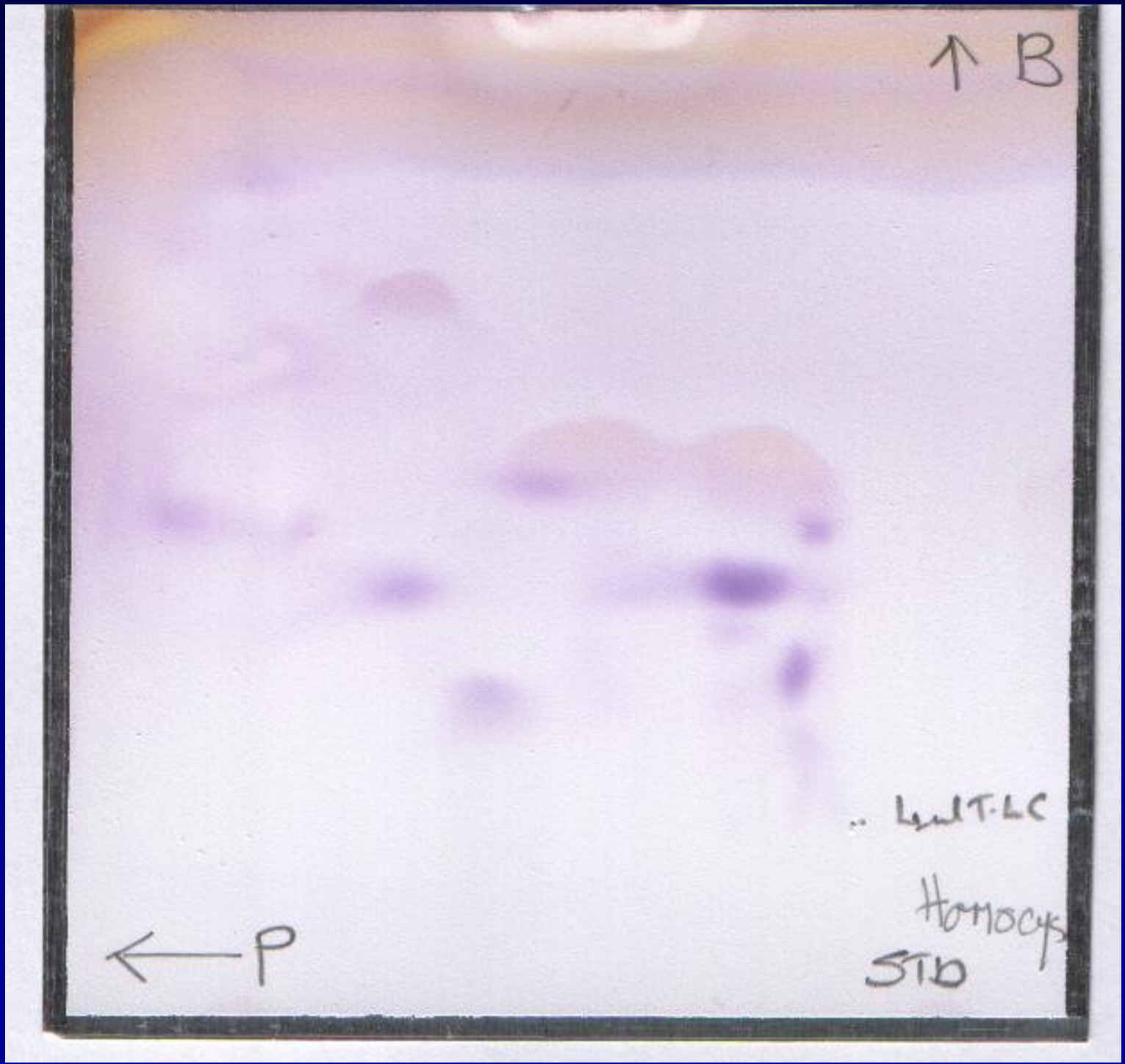
Cystine

Basics

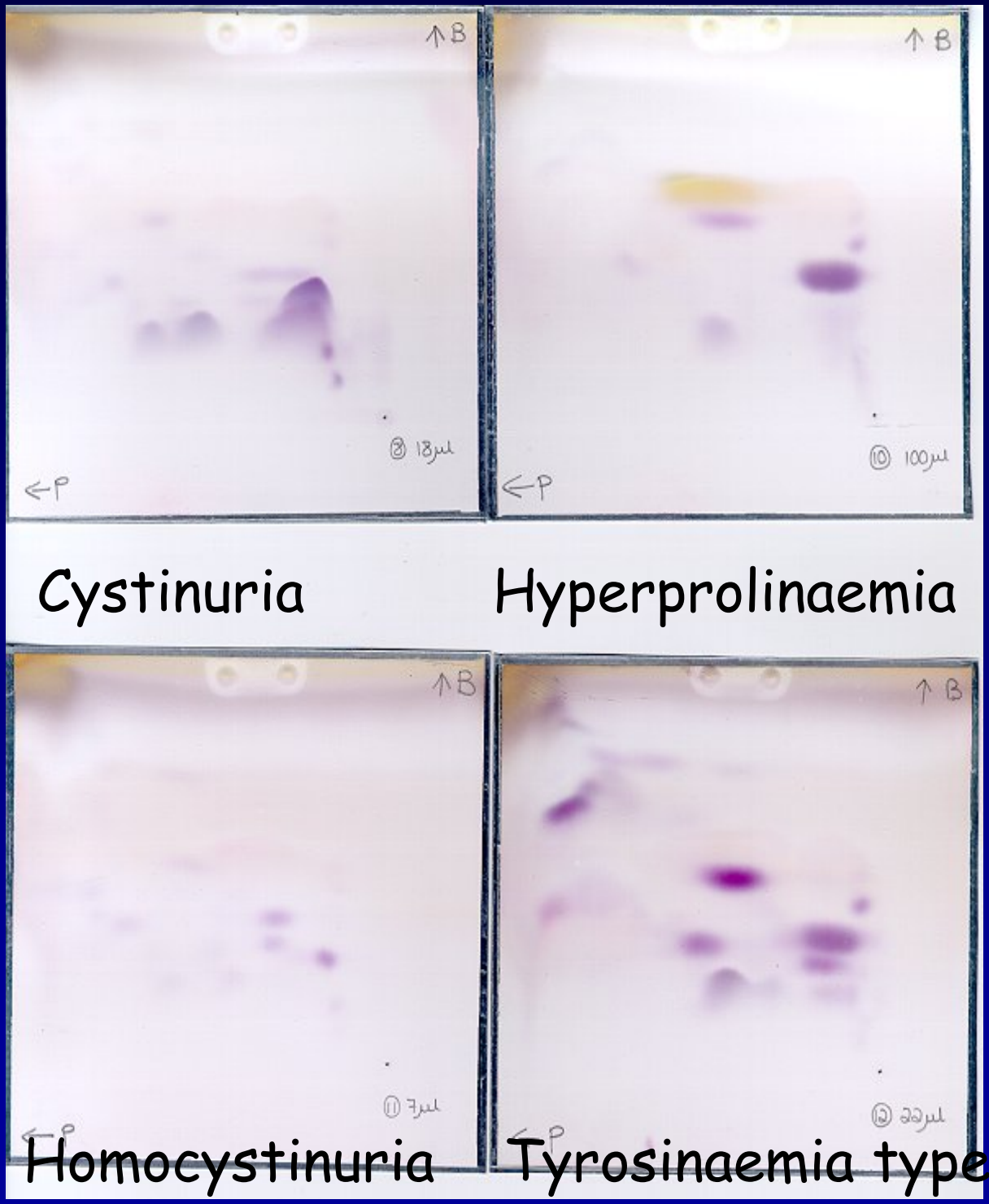
Homocystine

+ve CN/NP

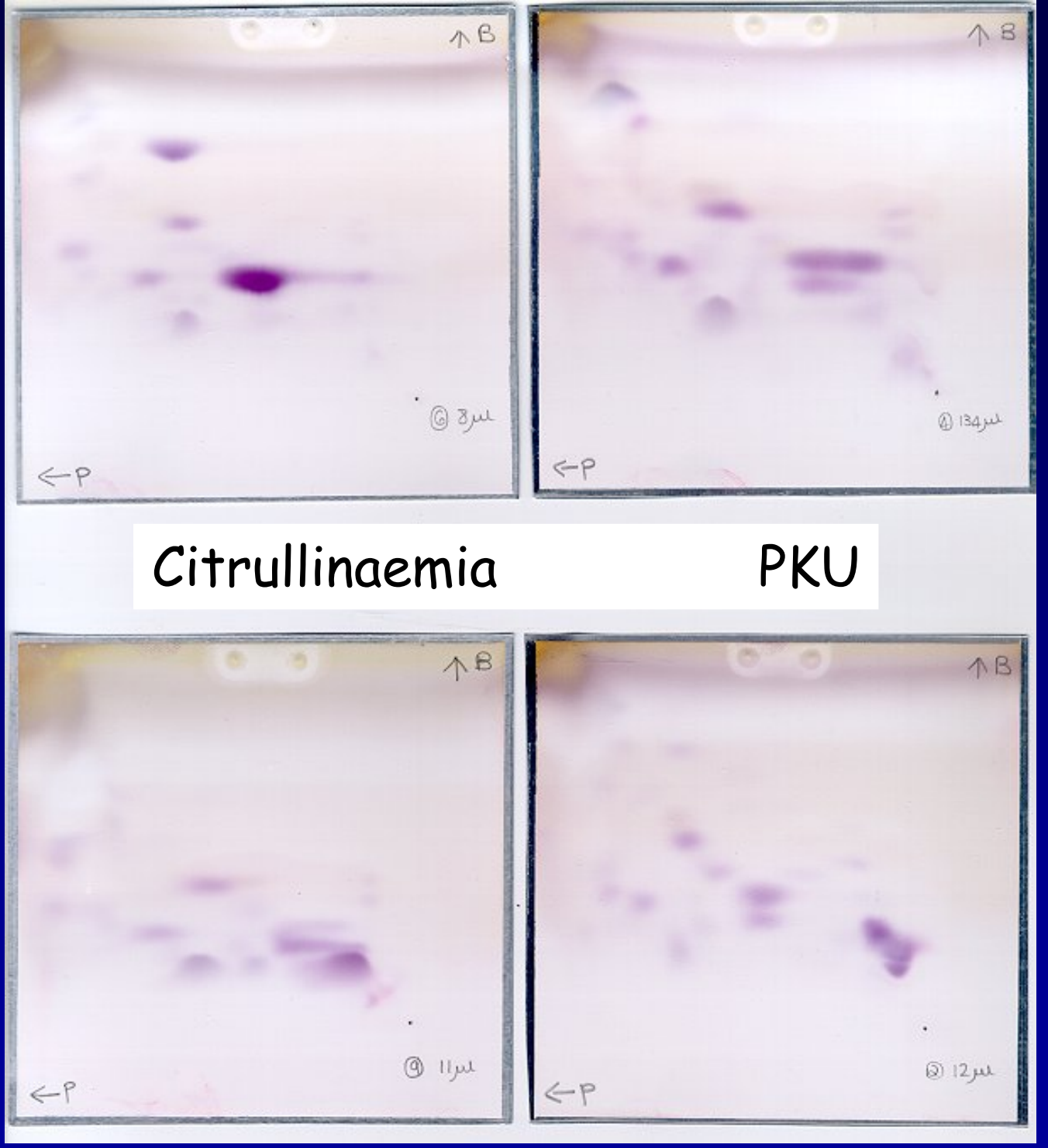



↑ B
2nd T-LC
Homocys
STD
← P

2D-urine AA


↑B
⑧ 18μl
←P
↑B
⑩ 100μl
←P
Cystinuria
Hyperprolinaemia
↑B
⑪ 7μl
←P
↑B
⑫ 22μl
←P
Homocystinuria
Tyrosinaemia type I

2D-urine AA


↑B
←P
Citrullinaemia
PKU
Ornithinaema
Argininosuccinic aciduria

Edinburgh SSIEM 1976

SSIEM Council hard at work June 1989

At ease with Royalty

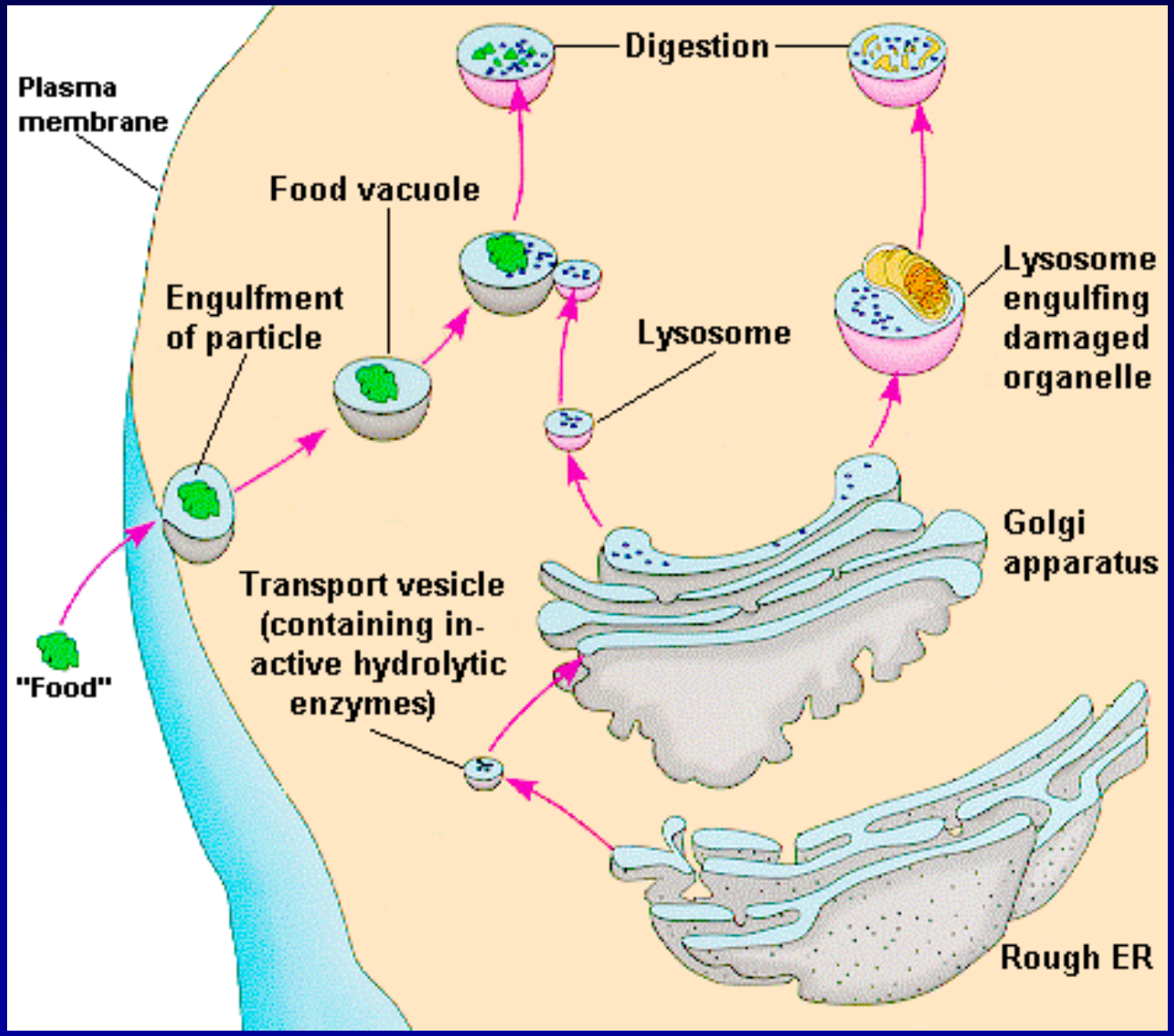
But a European at heart…

Expanding European interests

ERNDIM and lysosomal EQA

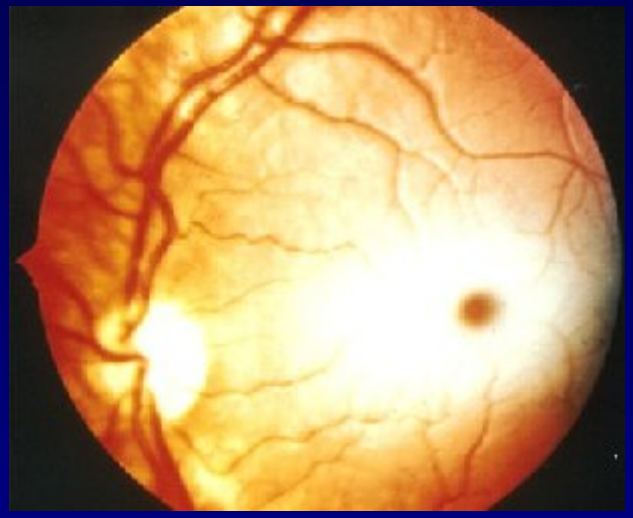
- But it's not all in the bag
- Developing an EQA for lysosomal enzyme assays has proved something of a challenge
- Activities are both cell and substrate-specific

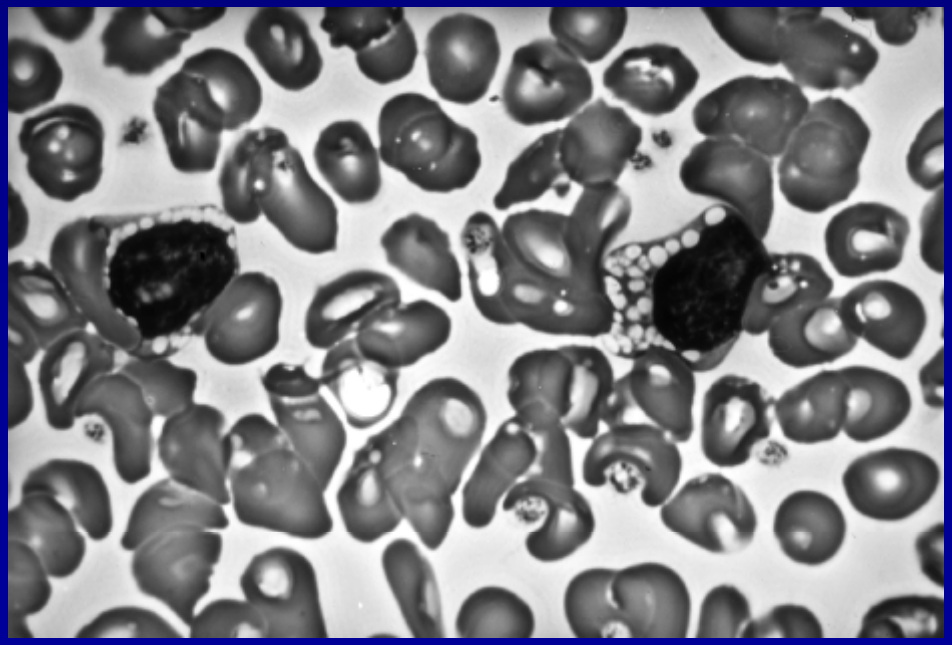
Lysosomal function

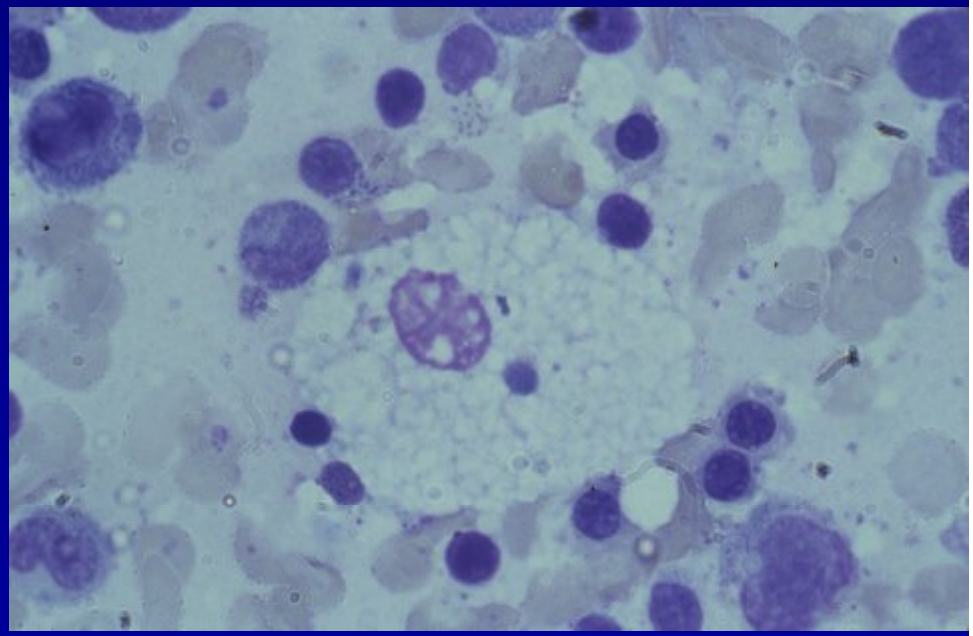


Evidence of lysosomal storage

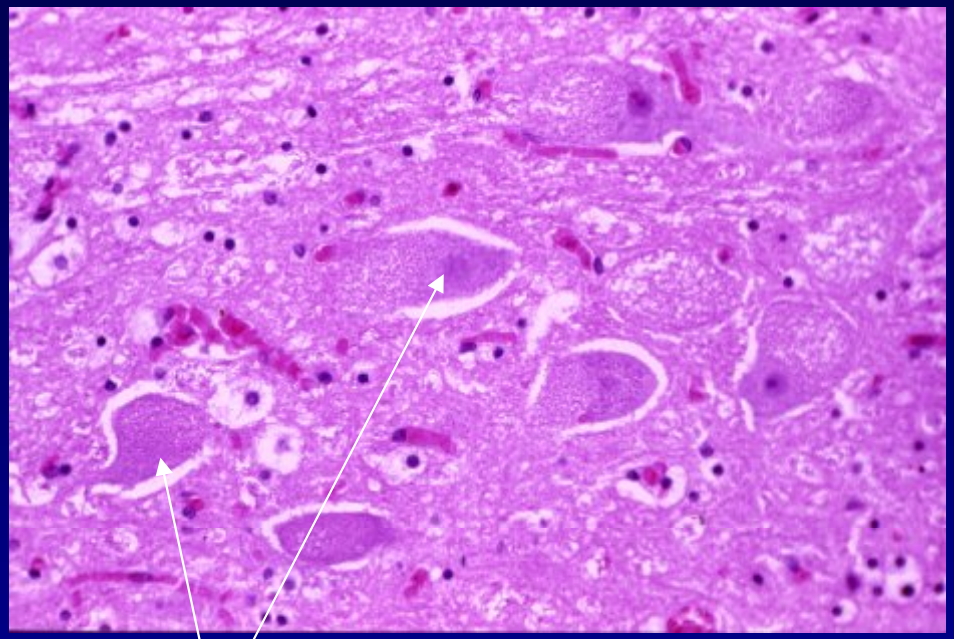
Vacuolated cells in blood
and bone marrow
Cherry-red spot in eye





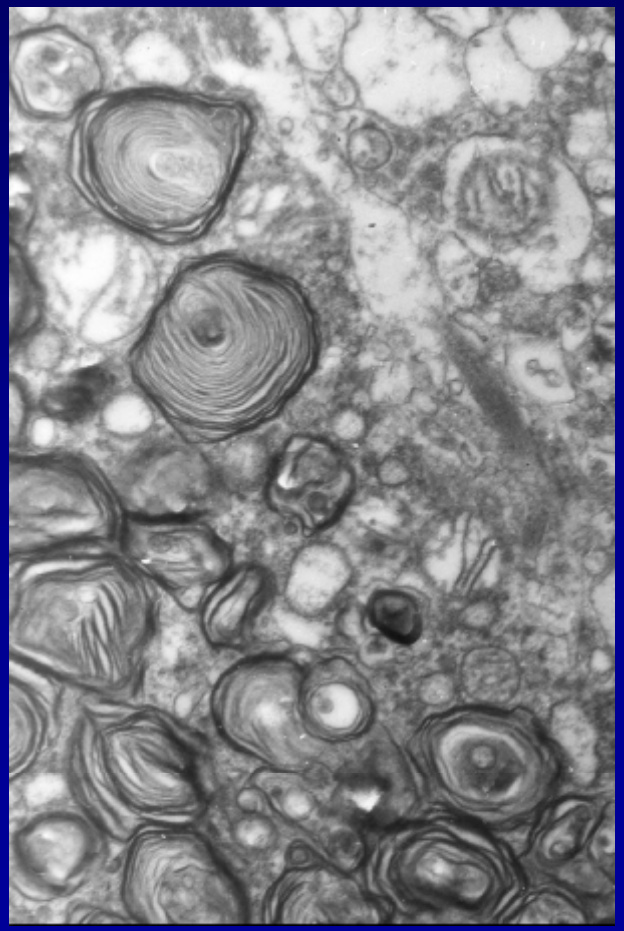


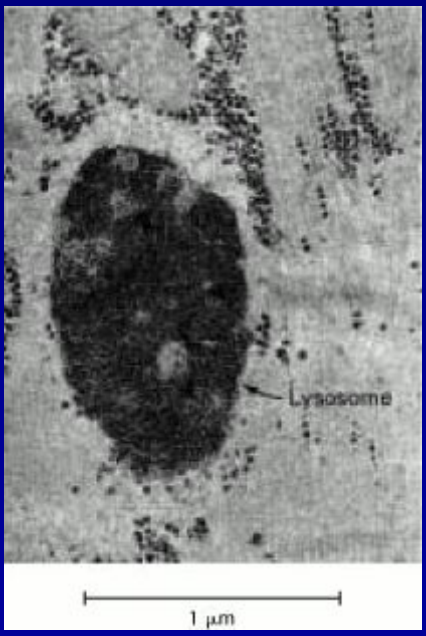
Lysosomal storage



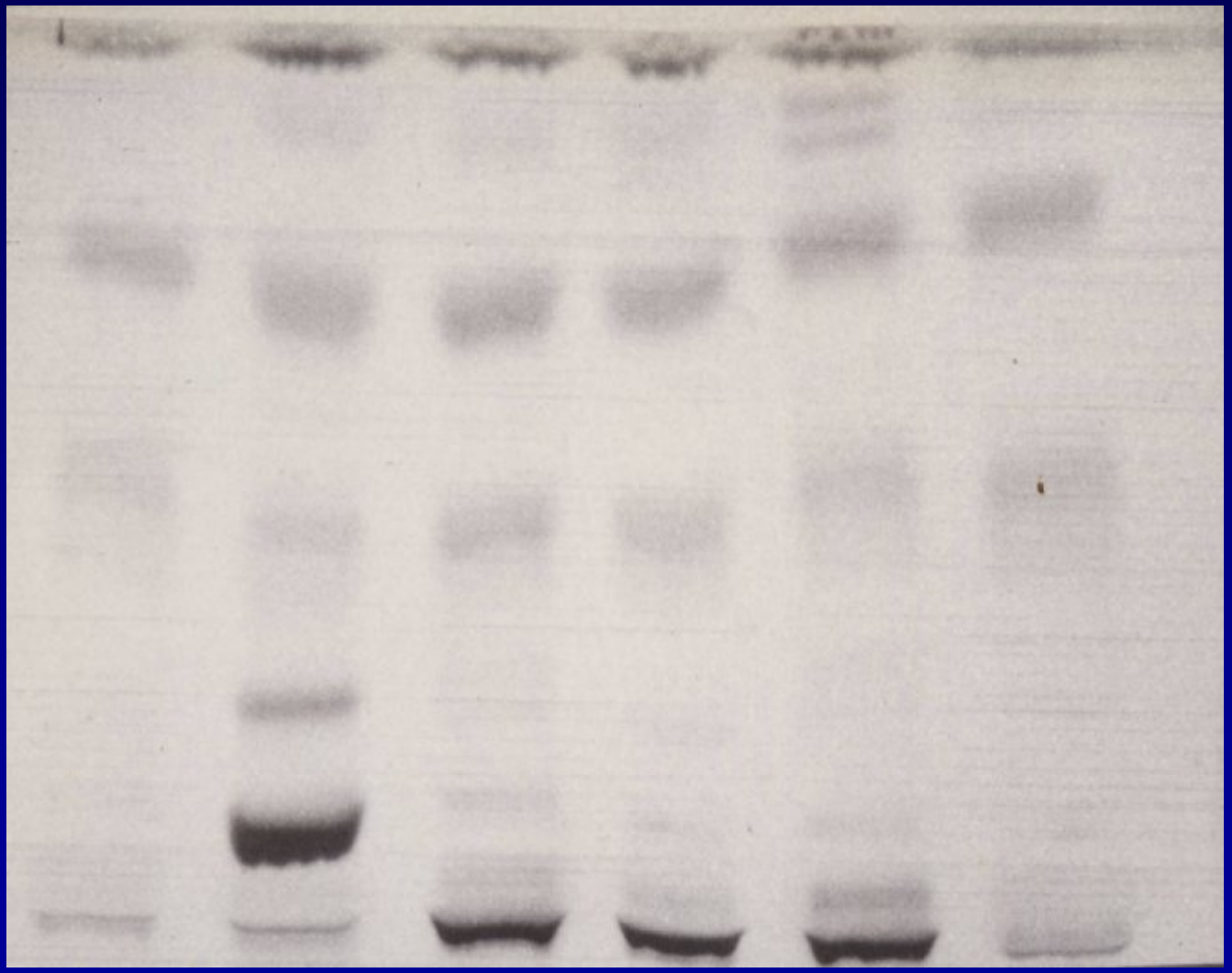
PAS staining showing ballooned neurones

EM showing MCBs





TLC of brain lipids

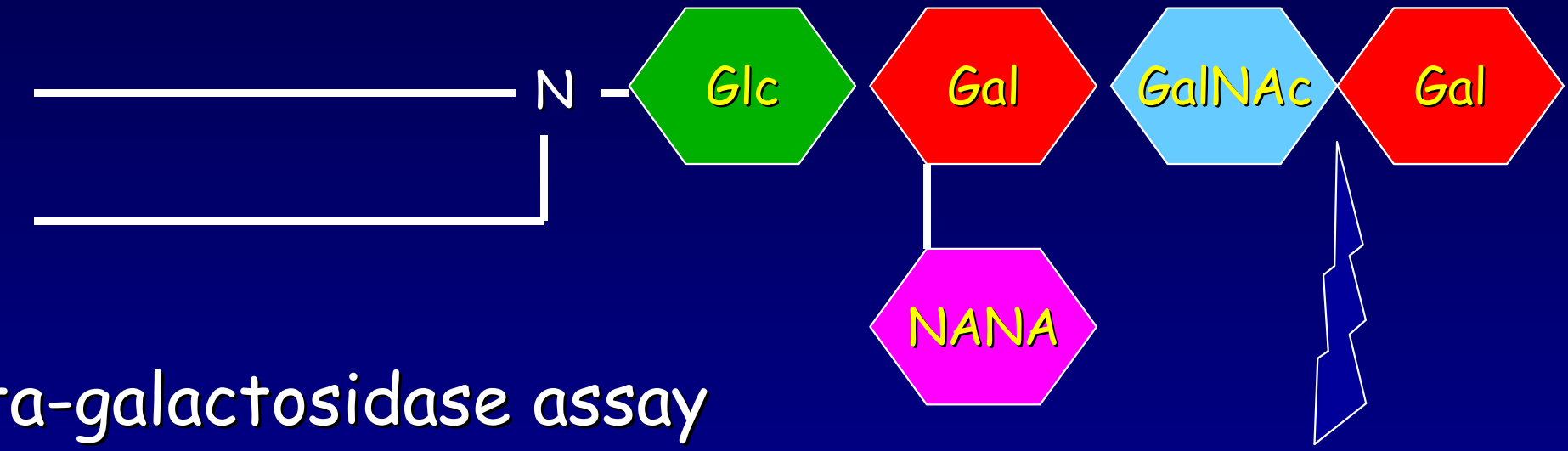


GM3

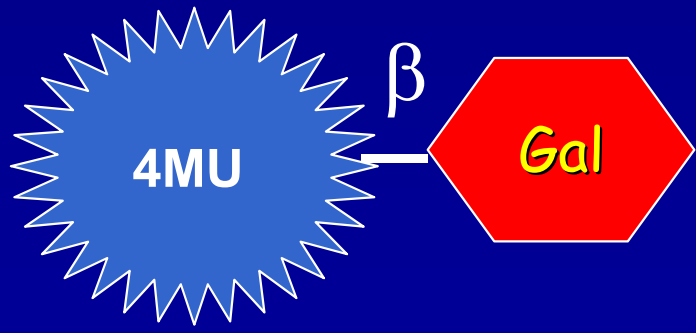
GM2

GM1

GM1-ganglioside and β-galactosidase



beta-galactosidase assay
GM1-gangliosidosis



4MU beta-galactoside

Enzyme activities in tissues
(nmol/min per mg protein)

Tissue	GM1-β-galactosidase	4MU-β-galactosidase
GM1 brain	0.009	0.33
Control brain	0.49	1.68
GM1 liver	0.007	0.26
Control liver	1.77	3.09

Enzyme activities in fibroblasts (nmol/min per mg protein)

Cells	4MU-β-galactosidase
GM1-gangliosidosis	0.12 and 0.03
Carriers	1.78 and 2.28
Controls	2.5 - 7.5

4MU β-galactosidase assay

- Diagnostic assays normally on leukocytes
- Very simple assay
- β-gal usually used as reference enzyme when other lysosomal assays performed
- But the enzyme is a complex containing a protective protein and is stabilised by chloride ions

4MU β-glucosidase assay

- For diagnosis of Gaucher disease
- Hydrophobic membrane-bound enzyme
- Requires activator *in vivo*
- And *in vitro*, the detergent taurocholate is required for leukocyte assays
- The assay conditions are critical for diagnostic testing

Leukocyte beta-glucosidase

Raghavan et al (1980)

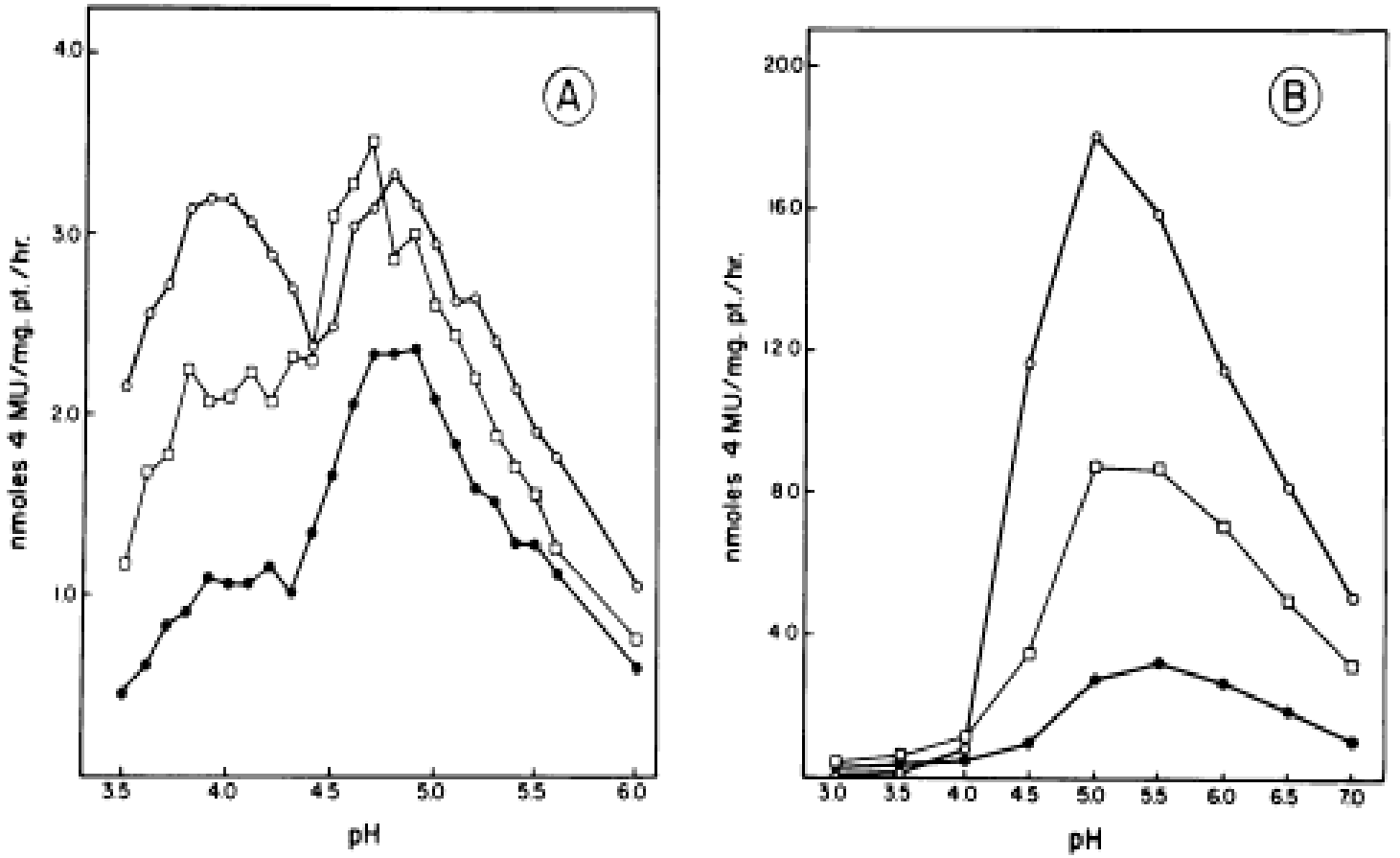


Fig. 2.—Comparison of pH activity curves for leukocyte β-glucosidase in control (○——○), obligate heterozygote (□——□), and patient with Gaucher disease (●——●) assayed in absence of detergent (*A*) and presence of 0.2% TDC (*B*).

4MU acid α-glucosidase assay

- Diagnostic assay for Pompe disease
- Problems using leukocytes due to interference from neutral/renal enzyme
- Can be overcome using lymphocytes or specific inhibitor

Diagnostic lysosomal enzyme assays

- Usually performed on mixed leukocytes and plasma
- A group of enzymes are assayed in parallel
- But a full understanding of specificities and optimum conditions for each are required
- Different methods of enzyme extraction and optimisations used

EQA requirement

- Ideally real samples should be used
- Activities in fibroblasts very different to leukocytes
- Activities in transformed lymphocytes also very different and not normally used
- This is a big challenge!
- Maybe plasma and DBS in the future
- To Kees Schoonderwoerd: Good luck

Munich SSIEM 1989 - Herzogstand

Good-Bye and Good Luck, Brian