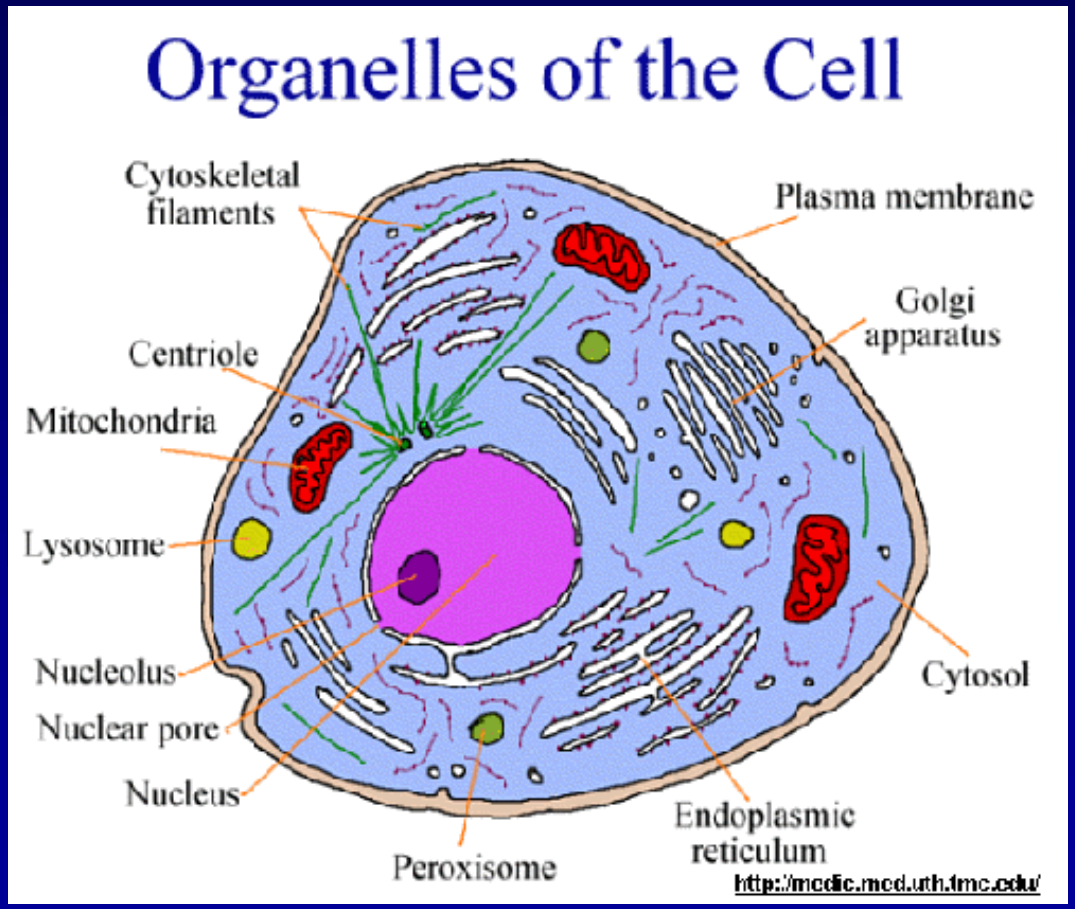
The role of the laboratory in diagnosing lysosomal disorders

Dr Guy Besley,
formerly
Willink Biochemical Genetics Unit,
Manchester Children's Hospital,
Manchester M27 4HA, UK.

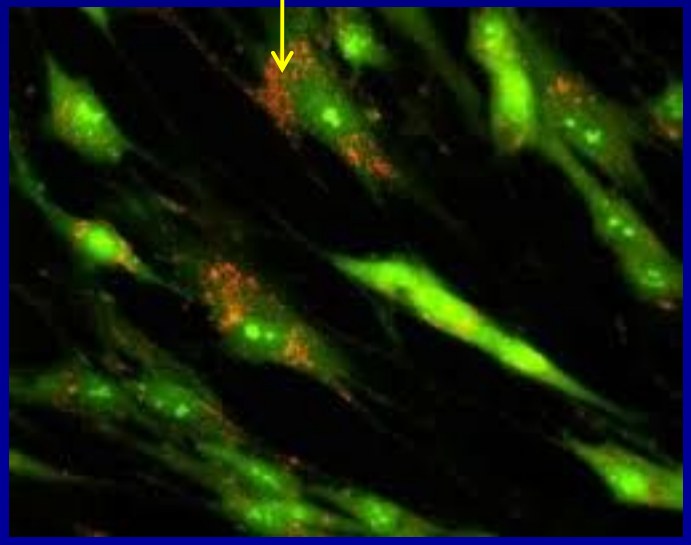
Lysosomal disorders

- What are lysosomes?
- What do lysosomes normally do?
- What are lysosomal disorders?
- How do we diagnose these in the laboratory?

What are lysosomes?

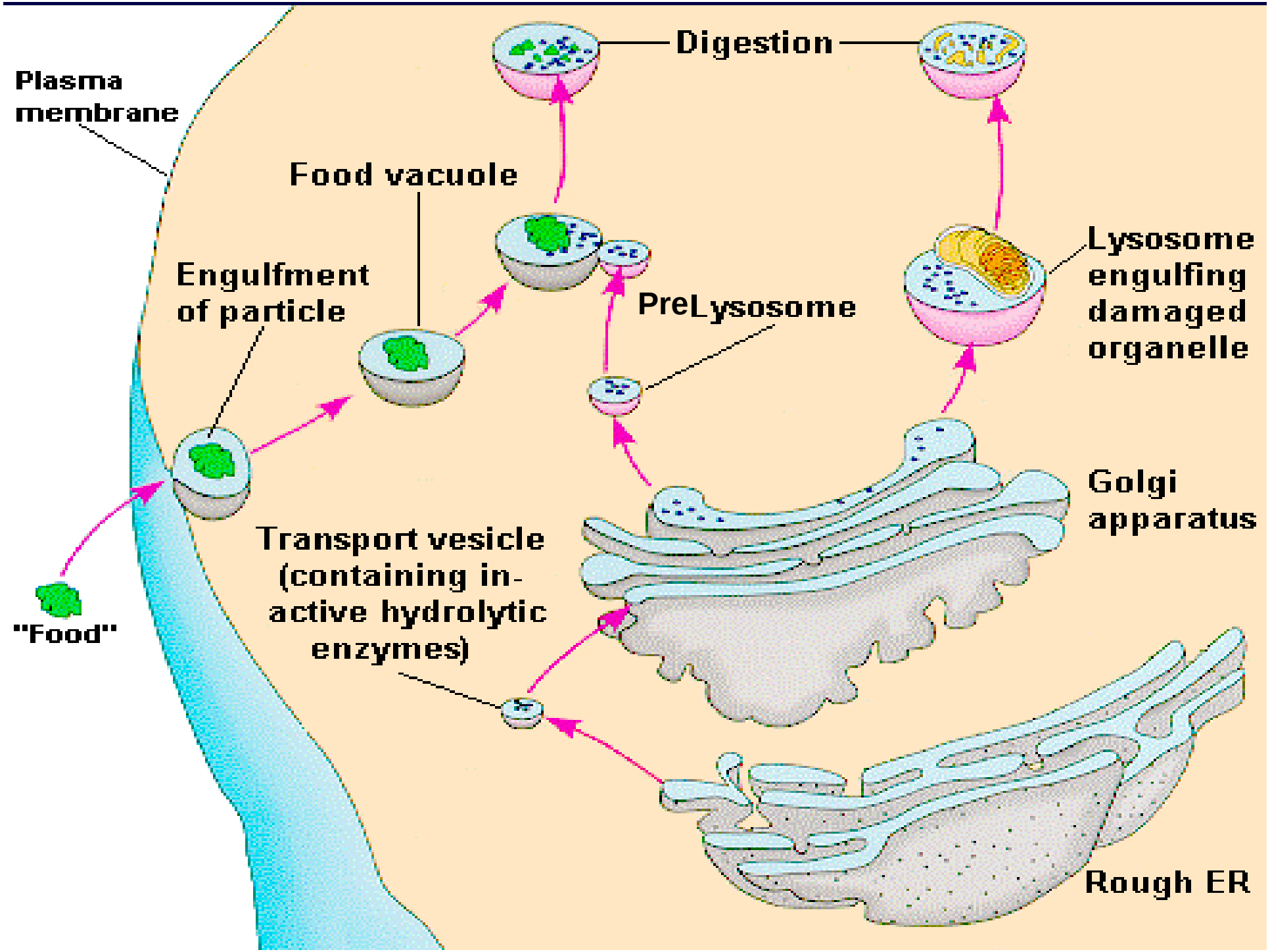


Fibroblasts with lysosomal storage material



What do lysosomes do?

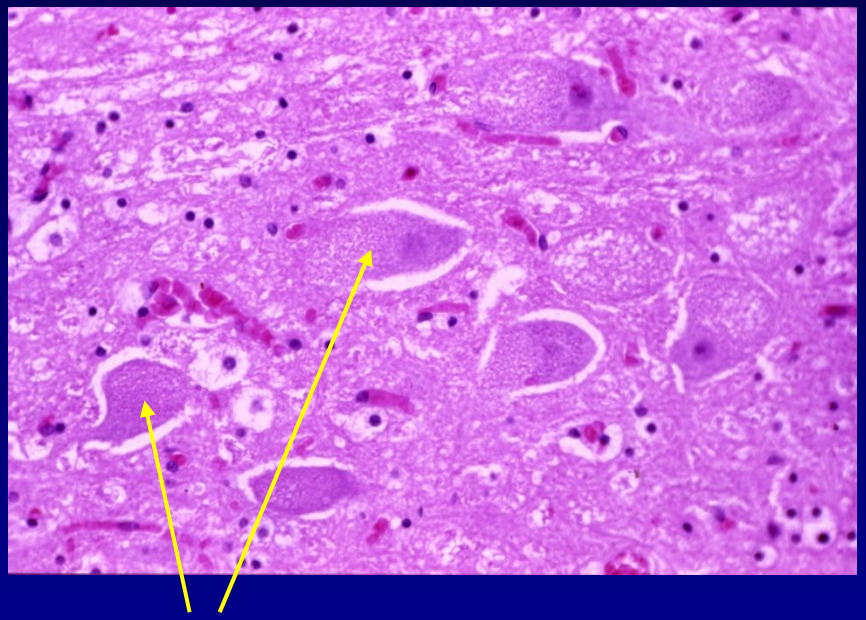
- An intracellular digestive system
- Responsible for recycling complex molecules and cell constituents
- They contain a number of enzymes that degrade complex molecules in a sequential manner
- The resulting small units can then be exported out of the lysosome for reuse


Plasma membrane
Digestion
Food vacuole
Engulfment of particle
PreLysosome
Lysosome engulfing damaged organelle
Golgi apparatus
Transport vesicle (containing in-active hydrolytic enzymes)
"Food"
Rough ER

What are lysosomal disorders?

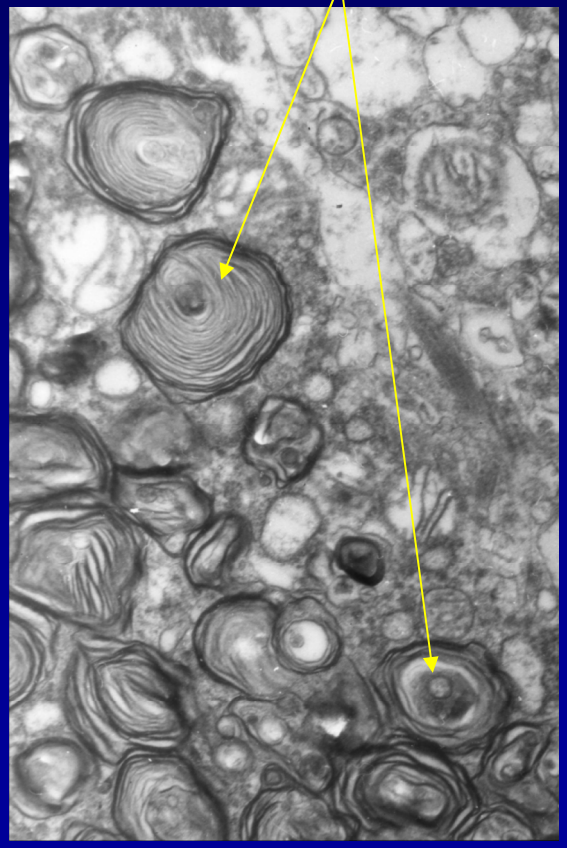
- Lysosomal disorders arise when there is a failure of a lysosomal function
- Usually this is because a DNA mutation has resulted in a defective enzyme
- This leads to a progressive accumulation of partially degraded material
- Resulting in a lysosomal storage disorder

Examples of Lysosomal storage

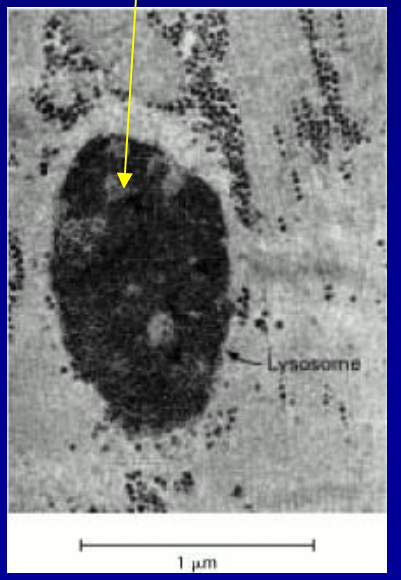


PAS staining showing ballooned neurones in GM1-gangliosidosis

Electron Microscopy showing Multiple Curvilinear Bodies in gangliosidosis



Glycogen in Pompe disease



Lysosomal disorders

- Some 500 different inherited metabolic disorders are known
- Approx 50 are lysosomal disorders
- Individually very rare
- But overall incidence approx 1 in 5000 but based on newborn screening data maybe more common.
- Mostly autosomal recessive inheritance

Lysosomal disorders

- Four main groups
  - Lipid/sphingolipidoses (Gaucher, Tay-Sachs Diseases)
  - Glycoproteinoses/mucolipidoses (mannosidosis, I-cell disease)
  - Mucopolysaccharidoses (MPS disorders, Hurler, Hunter Diseases)
  - Others (Pompe, Cystinosis, Batten Diseases)

Lysosomal disorders

- Because the storage metabolites are trapped in the lysosome, there are in most cases no simple screening tests on urine or blood. However in the case of the mucopolysaccharidoses and some oligosaccharidoses the correct urine test can give the first clue to a diagnosis.
- Specific enzyme tests are needed
- Diagnosis will rely on GP referral to a specialist centre/paediatrician
- But some typical clinical signs should alert referral

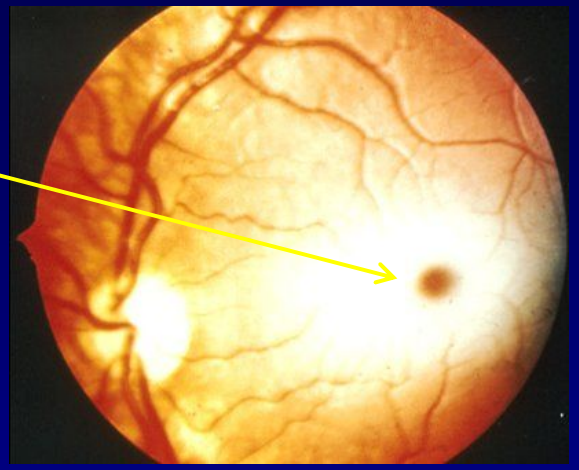
Clinical Symptoms in Lysosomal disorders

Any combination of:-

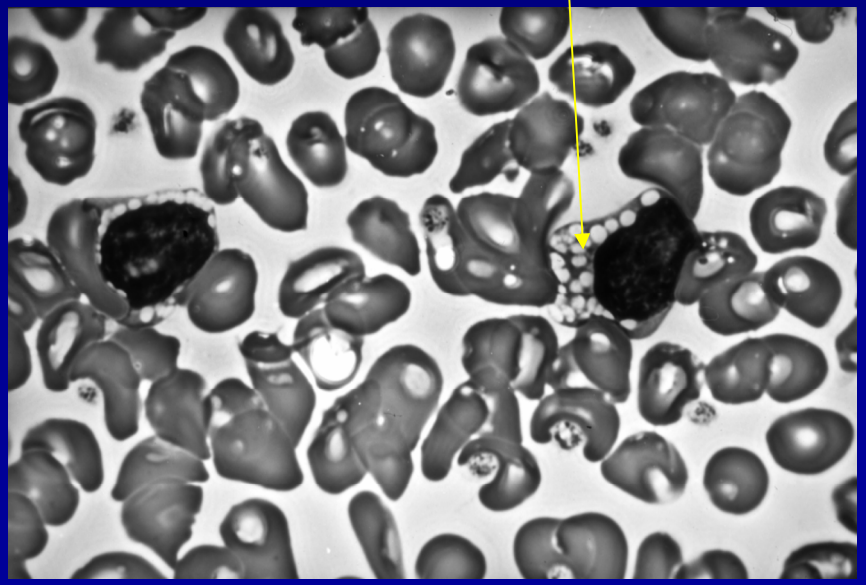
- Progressive neurological or developmental regression
- Enlarged liver or spleen
- Bone deformities and coarse facial features
- Many of these changes may take several months appear

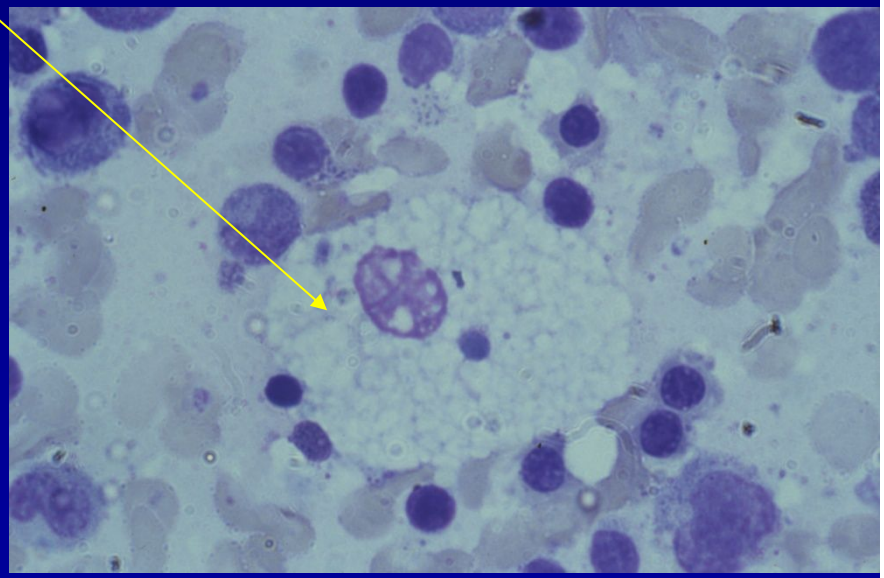
Evidence of lysosomal storage

Cherry-red spot in eye
(Gangliosidosis)



Vacuolated cells in
blood and bone marrow
(GM1-gangliosidosis and Gaucher)



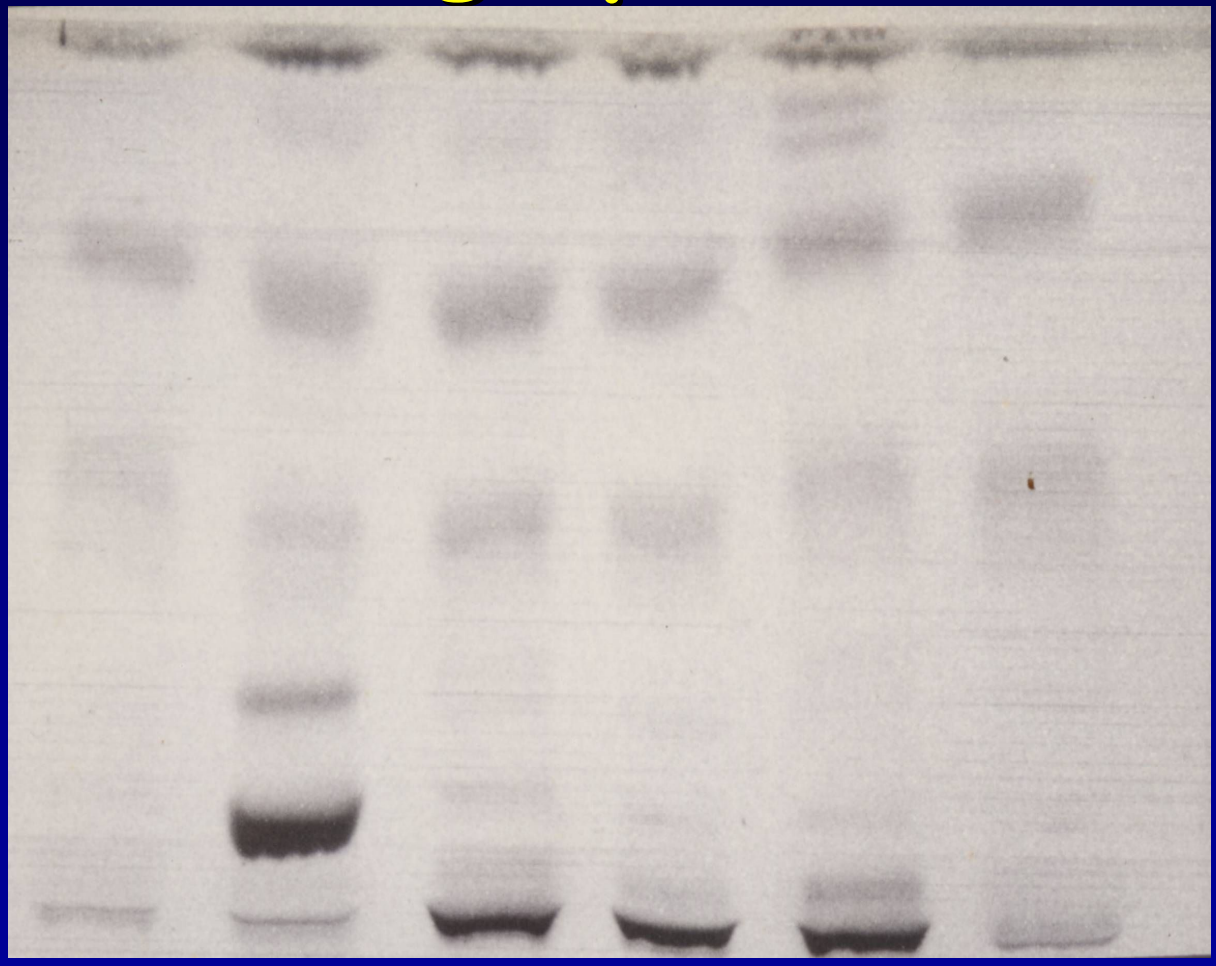


Defining the Primary Defect

In the early days analysis of lipids by thin layer chromatography revealed the nature of the accumulating metabolites giving clues to the primary enzyme defect.

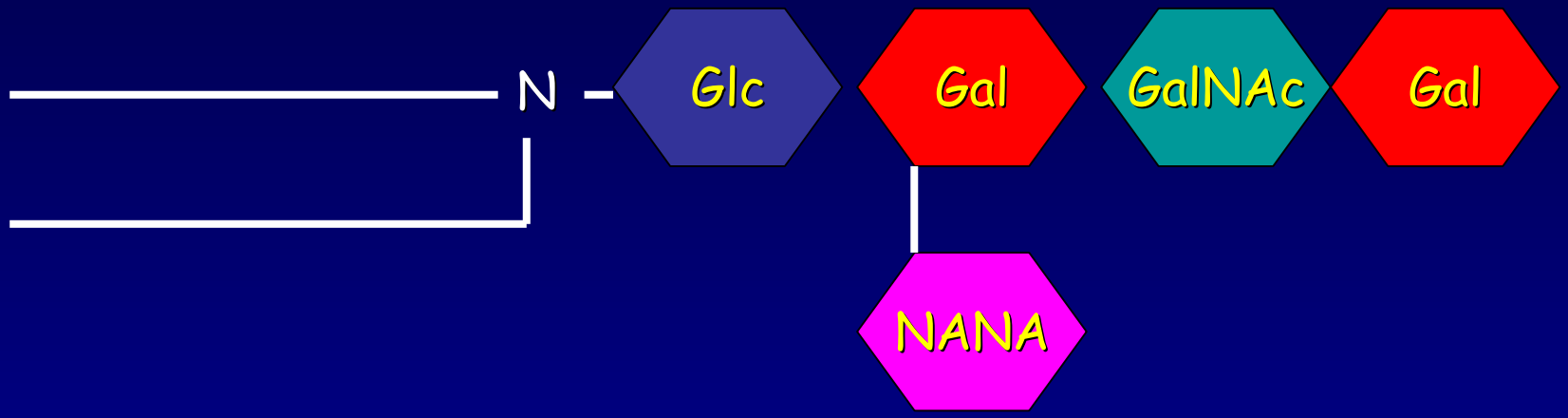
TLC of brain lipids identified the accumulating lipids

Gangliosides indicating the degradative pathway



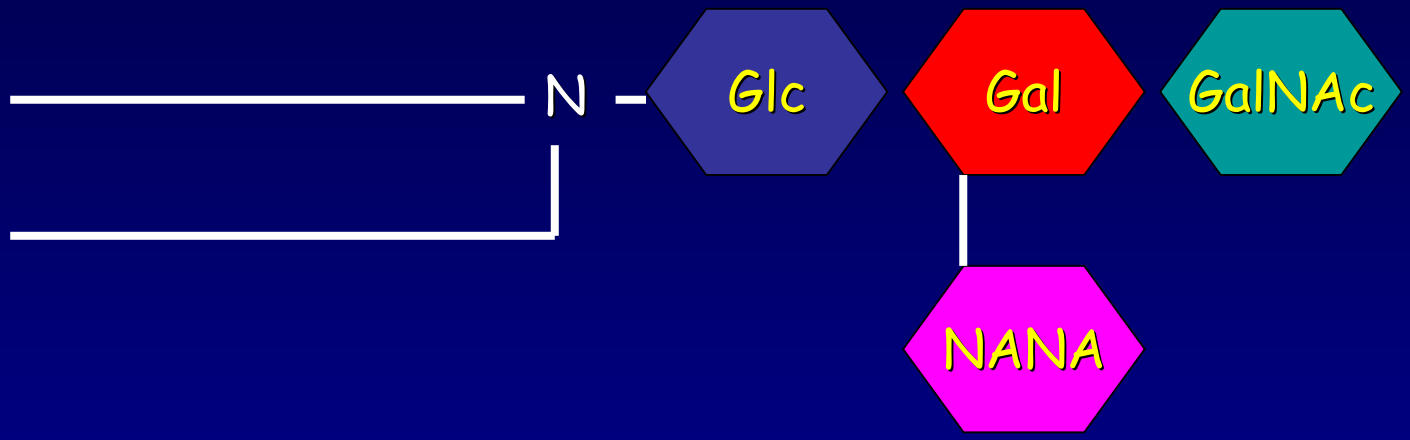
Control | Sandhoff Disease | GM1-Gangliosidosis | GM1- | GM1- | Control

Glycosphingolipid – GM1 ganglioside



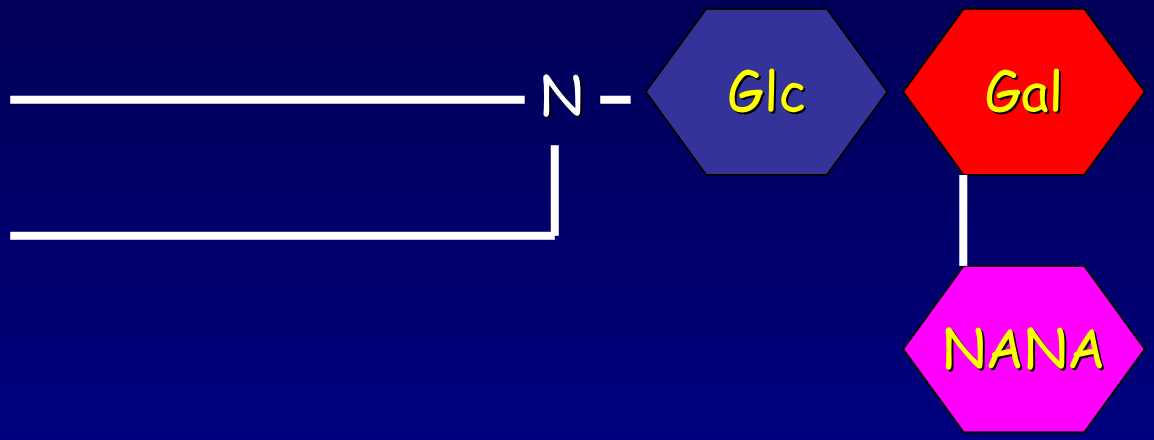
Accumulates in beta-galactosidase deficiency: (GM1-gangliosidosis)

Glycosphingolipid – GM2 ganglioside



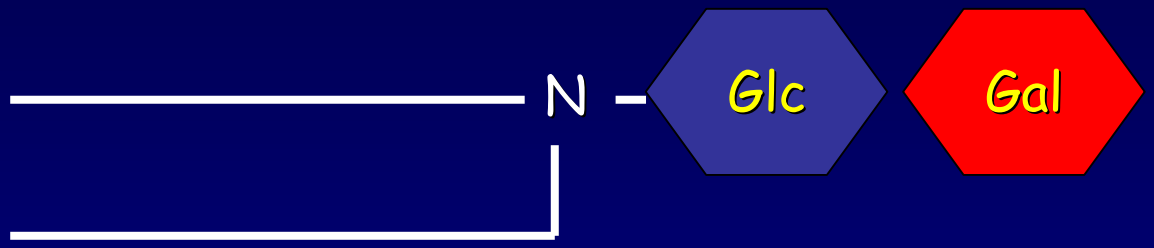
Accumulates in beta-N-acetylgalactosaminidase deficiencies (hexosaminidase deficiency) (Sandhoff and Tay-Sachs diseases)

Glycosphingolipid – GM3 ganglioside



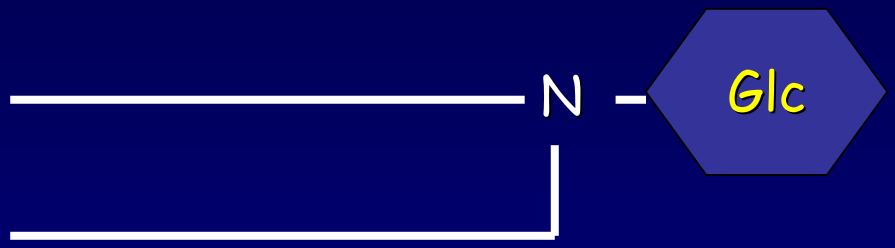
Accumulates in alpha-neuraminidase deficiency: (Sialidosis)

Glycosphingolipid – lactosylceramide



Hydrolysed by two beta-galactosidases:
GM1-gangliosidosis and
Krabbe enzyme (beta-galactocerebrosidase)
but does not accumulate in either disorder

Glycosphingolipid – glucosylceramide



Accumulates in beta-glucosidase deficiency:
(Gaucher disease)

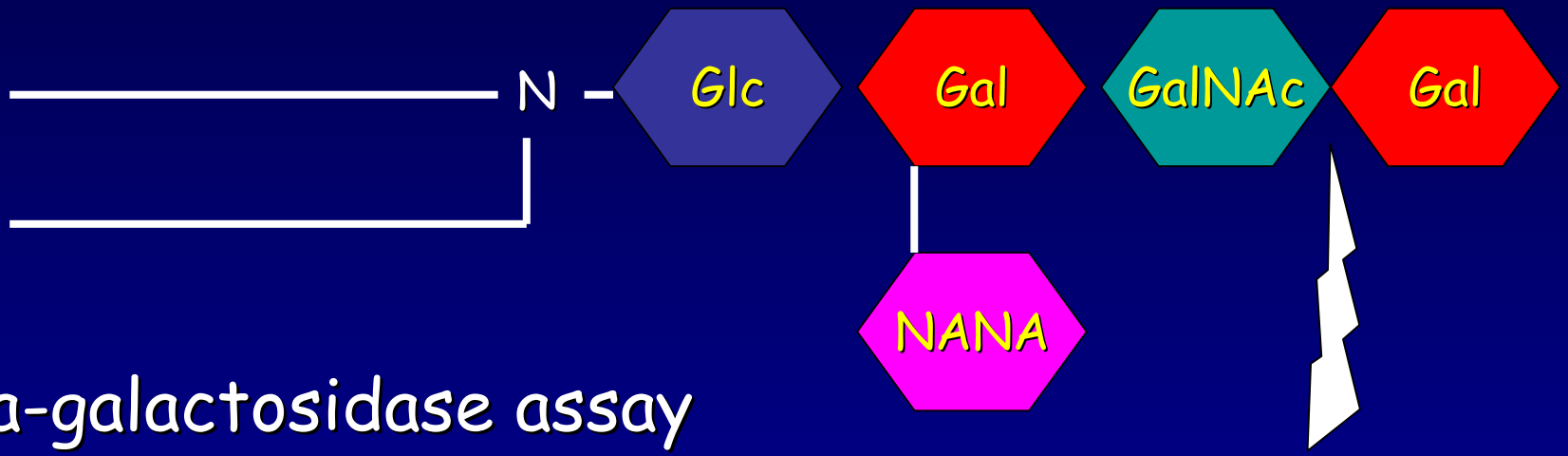
Glycosphingolipid – ceramide

Accumulates in ceramidase deficiency:
(Farber disease)

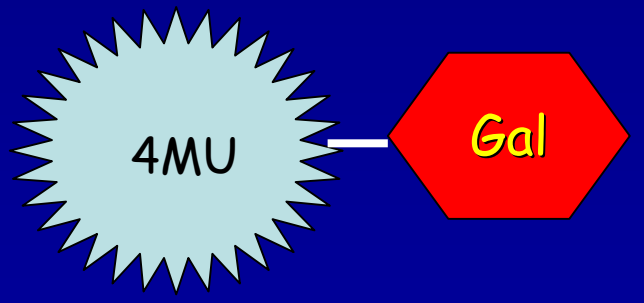
4MU enzyme assays

- Because lysosomal enzymes are often specific towards the terminal unit and linkage, artificial substrates can be often used
- 4MU enzyme assays are simple and use commercially available water soluble substrates
- However, there maybe a lack of sensitivity/specificity necessitating strict assay conditions

GM1-ganglioside and β-galactosidase



beta-galactosidase assay
GM1-gangliosidosis



4MU beta-galactoside

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

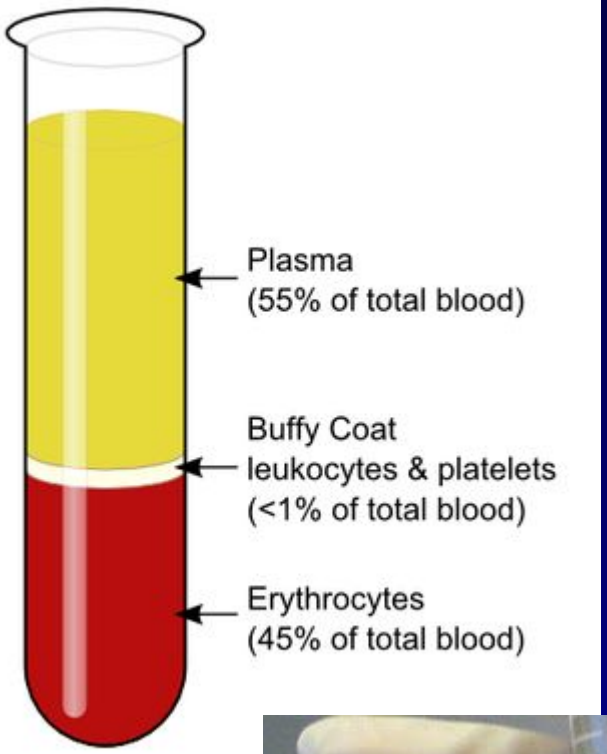
Tissue	GM1-β-galactosidase	4MU-β-galactosidase
GM1 brain	0.009	0.33
Control brain	0.49	1.68
GM1 liver	0.007	0.26
Control liver	1.77	3.09

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

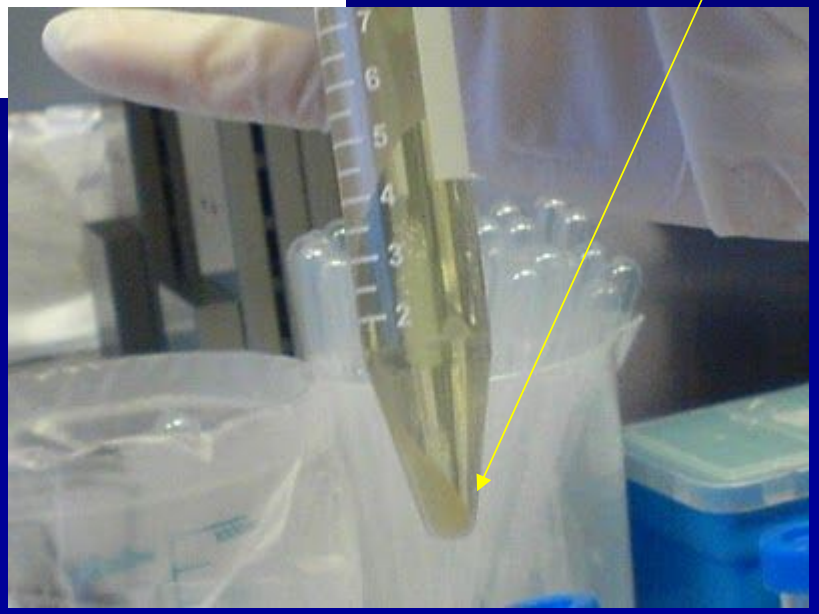
Cells	4MU-β-galactosidase
GM1-gangliosidosis	0.12 and 0.03
Carriers	1.78 and 2.28
Controls	2.5 - 7.5

Prenatal diagnosis of GM1-gangliosidosis
(nmol/min per mg protein)

Sample	β-galactosidase	β-glucosidase
Test CVS	0.05	4.86
Control CVS	2.93	3.51
Control CVS	2.85	3.33
GM1 fibroblasts	0.10	5.17
Control fibroblasts	7.14	4.74



Leukocyte pellet



Diagnostic enzyme assays

Diagnostic enzyme assays

- Screening for most sphingolipidoses and oligosaccharidoses can conveniently be carried out on 5ml fresh blood
- Most enzymes are assayed on separated leukocytes
- Some enzymes can also be assayed on plasma, especially for I-cell disease where many activities are increased
- Plasma assay also of the phagocyte-marker enzyme, chitotriosidase, is often included
- This enzyme may be increased x1000 fold in Gaucher disease as well as moderate increases in Niemann-Pick type C and others

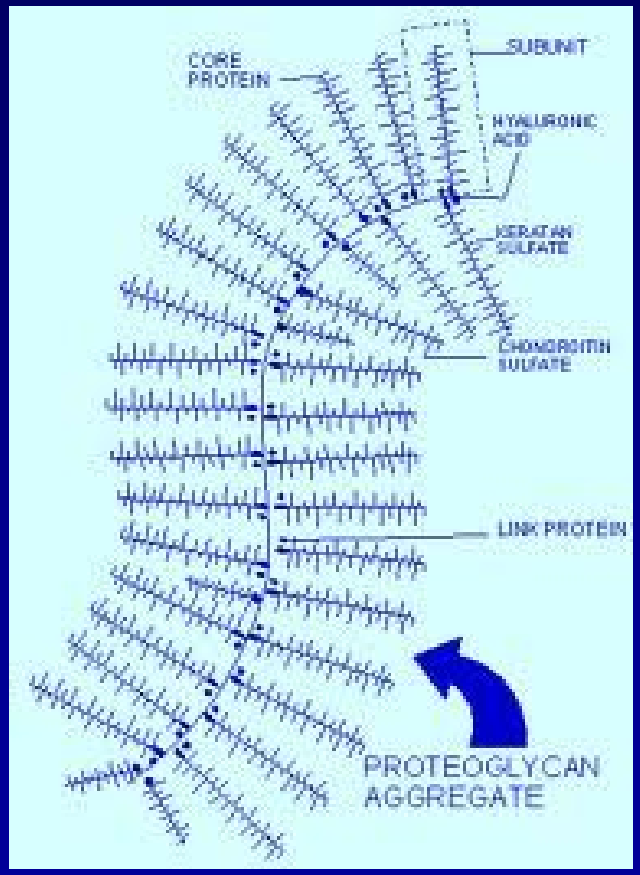
Diagnostic enzyme assays

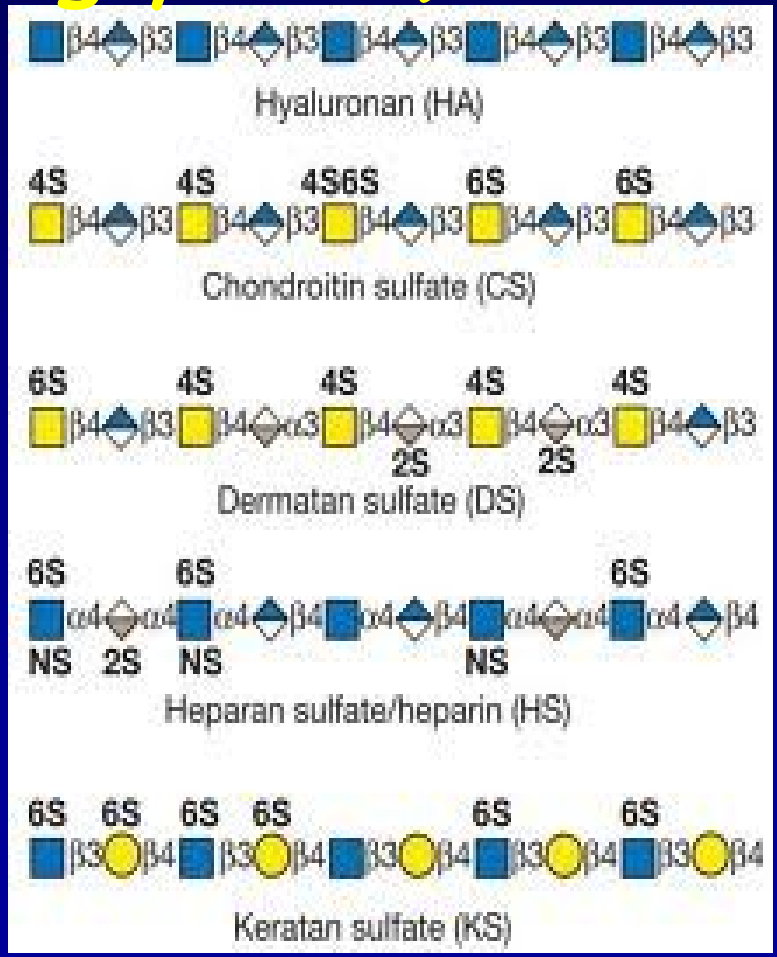
- Routinely in the Willink Unit (Manchester) white blood cells are isolated from a blood sample and are tested for a wide range of lysosomal enzymes are analysed.
- A full lysosomal screen for sphingolipid and oligosaccharide disorders is performed
- Some 16 different disorders can be diagnosed on 5ml blood
- Most assays are based on fluorescent substrates

Diagnosis of MPS disorders

- The storage material in MPS disorders is largely water-soluble (glycosaminoglycans)
- GAGs are made up of repeating disaccharide units with varying degrees of sulphation
- Small amounts leak out and can be measured in urine
- Quantitative analysis is useful but limited
- Qualitative analysis will help to point to a diagnosis and confirmatory enzyme assay

Storage material in MPS disorders (Glycosaminoglycans)

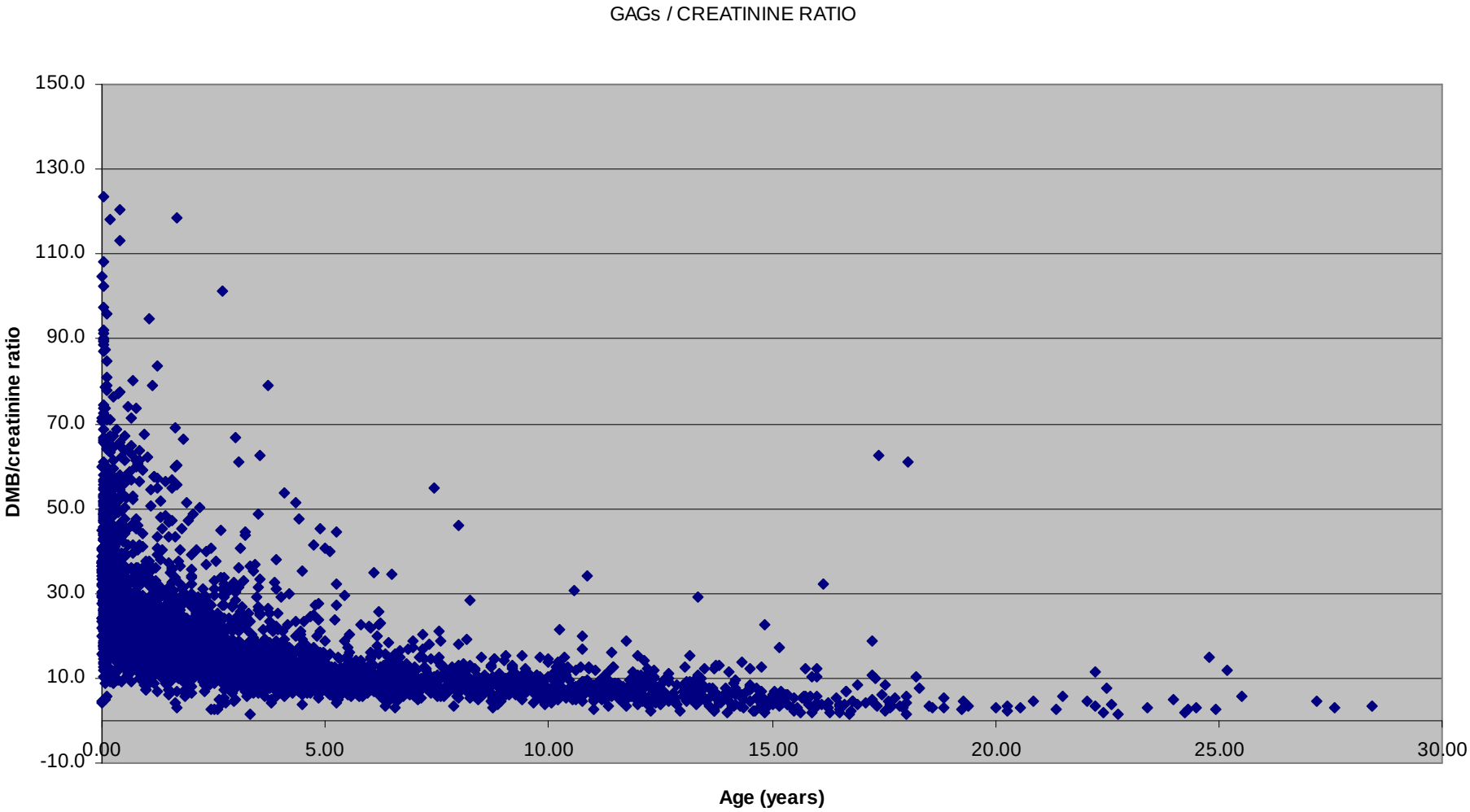




Quantitation of Glycosaminoglycans (GAGs)

- Glycosaminoglycans are acidic macromolecules that can be quantified by a variety of techniques
- The spectrophotometric method based on binding to the dye DMB is the most useful
- Values are related to creatinine but are age specific
- Care is needed to avoid false positives and false negatives

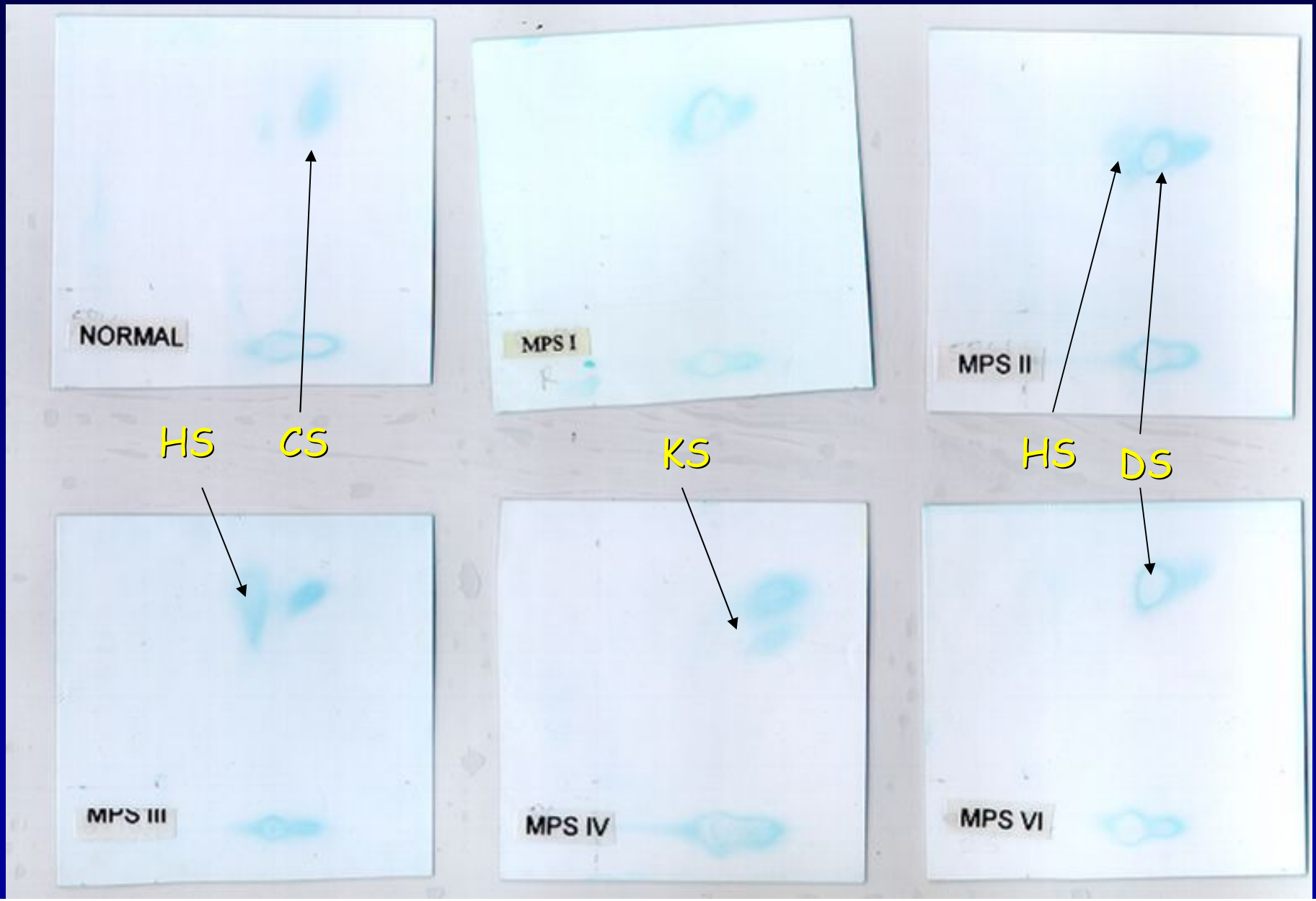
Normal distribution by age of urinary glycosaminoglycans by DMB dye-binding assay



Glycosaminoglycan analysis

- Increased urinary GAGs are usually found in all types of MPS disorder
- But is some cases (MPS III or IV) this may be less marked, especially in older patients
- Qualitative analysis is therefore also necessary
- 2-dimensional electrophoresis provides a useful diagnostic test

Urinary MPS two dimensional electrophoresis



Thin-layer chromatography of oligosaccharides

- Some oligosaccharide disorders may present similar to MPS disorders
- Therefore TLC of urinary oligosaccharides may also be useful
- It is particularly useful for sialic acid storage disorders
- However for other conditions, patterns are not always easy to interpret and specific diagnostic enzyme assays are also needed

Enzyme assays on dried blood spots (DBS)

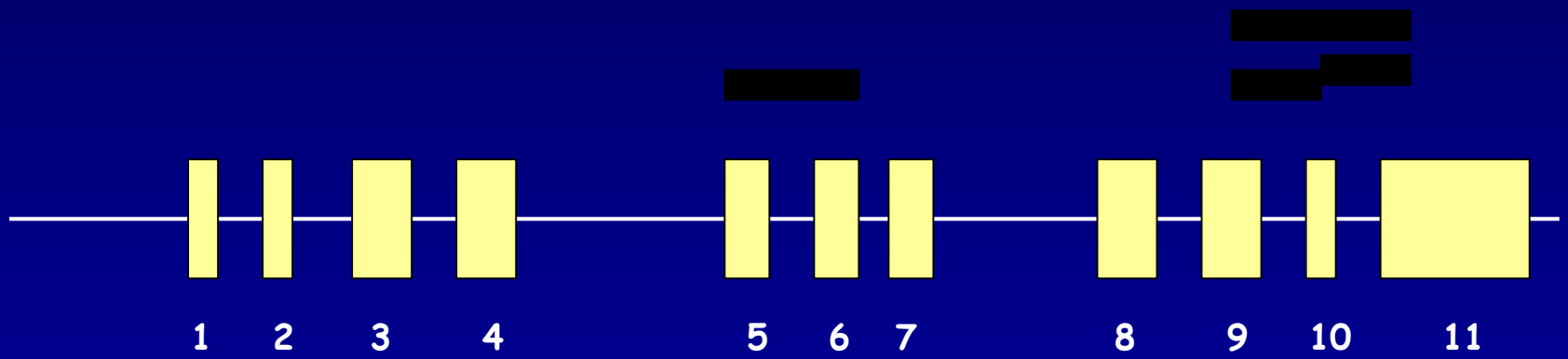
- In recent years DBS have been used to screen for lysosomal enzyme disorders
- Although this approach may prove useful for newborn screening, it currently lacks the specificity and sensitivity required for diagnostic testing
- Nevertheless, new technologies based on multiplex assays using tandem mass-spectrometry are improving all the time

GLUCOCEREBROSIDASE GENE
Gaucher disease

Situated on chromosome 1 - 1q 21 with 11 exons

N370S mutation mild (common Jewish)

L444P mutation severe neuronopathic



Intron - non coding sequence
exon - coding sequence
recombinant allele - pseudogene derived

N370S
1226A>G
mild

L444P
1448T>G
Severe

Other lysosomal disorders

- Many other lysosomal disorders will need specific enzyme or other diagnostic tests
- Pompe disease is usually not included in leukocyte enzyme screening
- Ceroid Lipofuscinoses (Batten disease) require special tests
- As too do transport disorders, such as Cystinosis and Sialic Acid Storage Disease
- Information on these and other tests will be found on the MetBioNet website

DNA testing

- Will help to confirm a diagnosis especially when screening on DBS
- But for most disorders there are many different mutations
- May help to predict phenotype especially important when targeting treatment strategies
- Particularly useful for carrier testing

The future

- Over the last decade or so, we have seen major advances in the diagnosis and treatment of lysosomal disorders
- Much of this has been fuelled by new and commercial interests in treatment (enzyme replacement, chaperone therapy, stem cells etc)
- The need for early diagnosis has stimulated interest in newborn screening and this too will lead to a wider recognition of these disorders and their range of phenotypes