Glycogen Storage Disorders

The role of Biochemistry in Diagnosis

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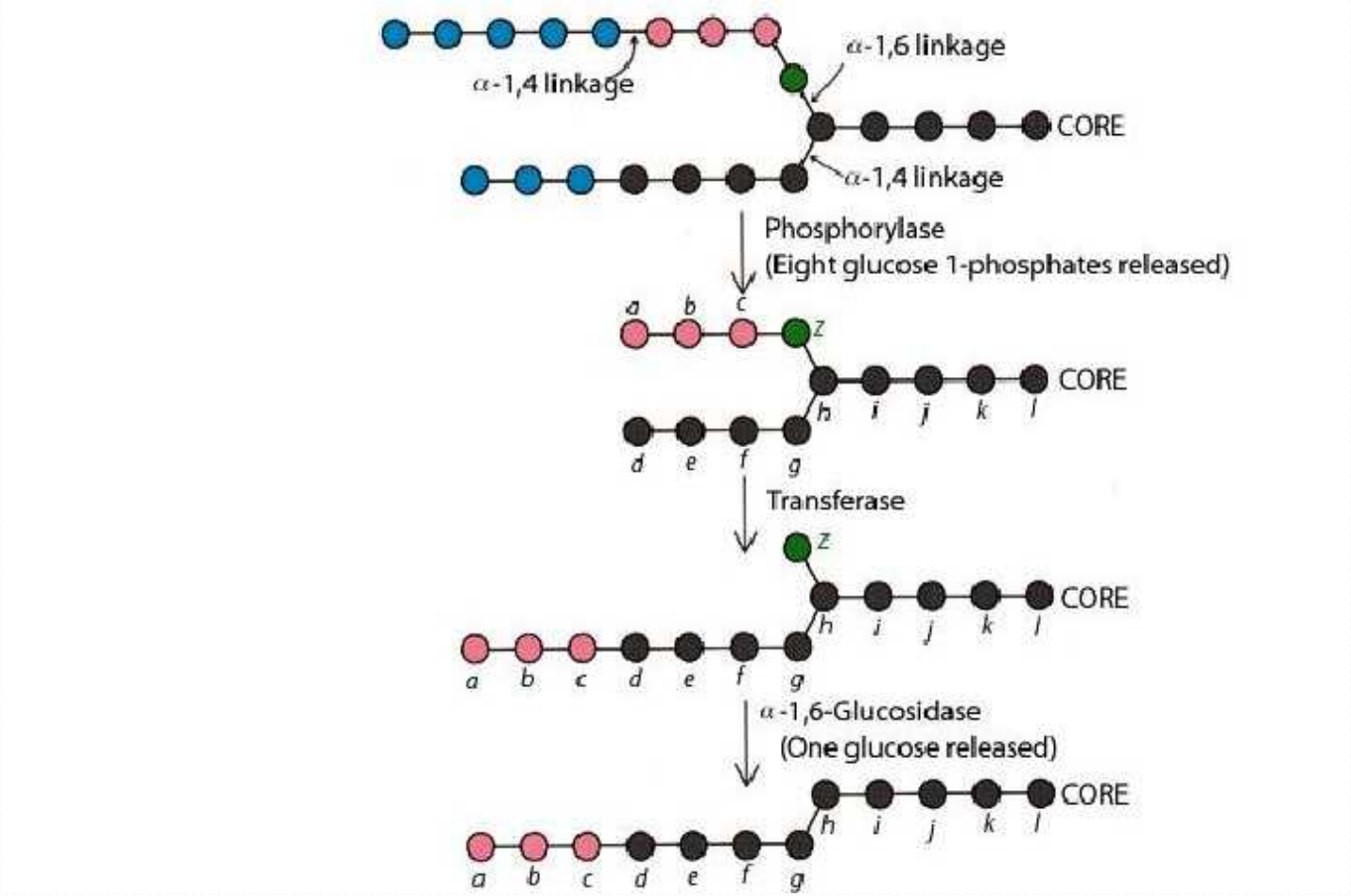
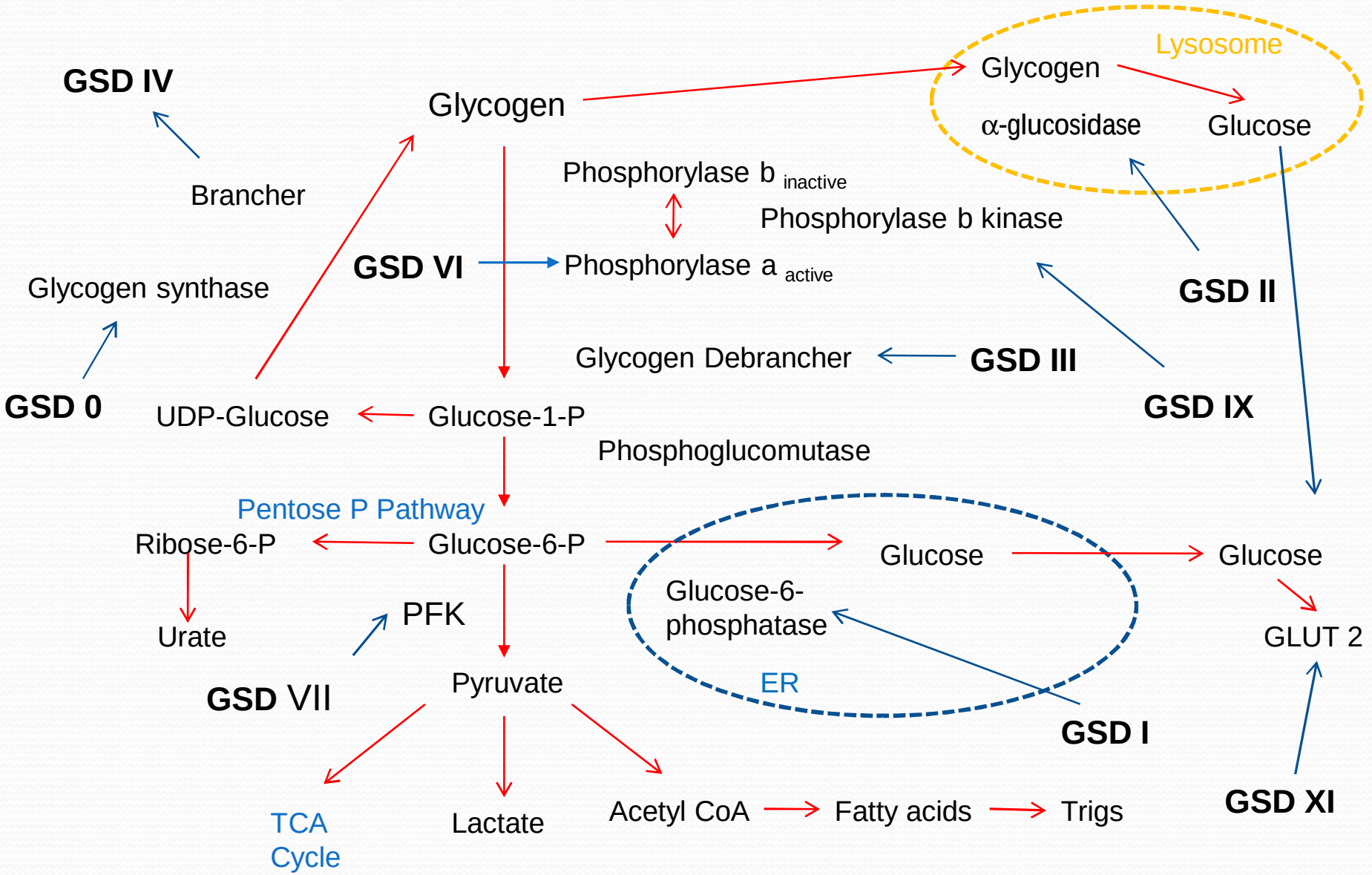
Glycogen degradation



Glycogen Metabolism & Glycogen Storage Disorders



Glycogen Storage Diseases

Predominately Hepatic GSDs:

GSD I – glucose-6-phosphatase or transport systems in ER

GSD III – debranching enzyme

GSD IV – branching enzyme

GSD VI – liver phosphorylase

GSD IX – liver phosphorylase b kinase

GSD 0 – glycogen synthase

Predominately Muscle GSDs:

GSD II – acid a-glucosidase

GSD V – muscle phosphorylase

GSD VII - muscle phosphofructokinase

GSD	Hepato-megaly	Muscle symptoms	Glucose homeostasis	Other Biochemistry
GSD 0	No	None	Fasting ketotic hypoglycaemia	
GSD I	Yes	None	Severe (ketotic) hypoglycaemia	Raised lipids, urate, lactate, AST/ALT, Abnormal renal biochemistry including proteinuria
GSD II	No	Truncal & proximal muscle weakness. More severe infantile form.	No overt effect	Raised CK,vacuolated lymphocytes
GSD III	Yes	Myopathy can occur	Fasting ketotic hypoglycaemia	Raised lipids, AST/ALT, CK may be raised
GSD IV Hepatic	Yes	Myopathy can occur	Normal until end stage liver disease	Raised AST/ALT, CK can be raised
GSD V	No	Exertional muscle weakness with risk of rhabdomyolysis	No effect	Raised CK
GSD VI	Yes	None	Fasting ketotic hypoglycaemia	Raised AST/ALT
GSD VII	No	Exertional muscle weakness with risk of rhabdomyolysis	No effect	Raised CK
GSD IX liver form	Yes	Myopathy can occur	Fasting ketotic hypoglycaemia can occur	CK can be raised
GSD XI	Yes	None	Ketotic hypoglycaemia	Raise AST/ALT, Abnormal renal biochemistry including tubular markers.

Initial Laboratory Tests for the Investigation of Suspected GSD

- Blood glucose
  - If hypoglycaemia include insulin, FFA, ketones
- Blood lactate
- Urate
- LFTs
- Lipids
- CK
- U&E, tubular proteins, protein/albumin

- GSD Screen

- Muscular symptoms only:
  - CK
  - Vacuolated lymphocytes
  - Renal function

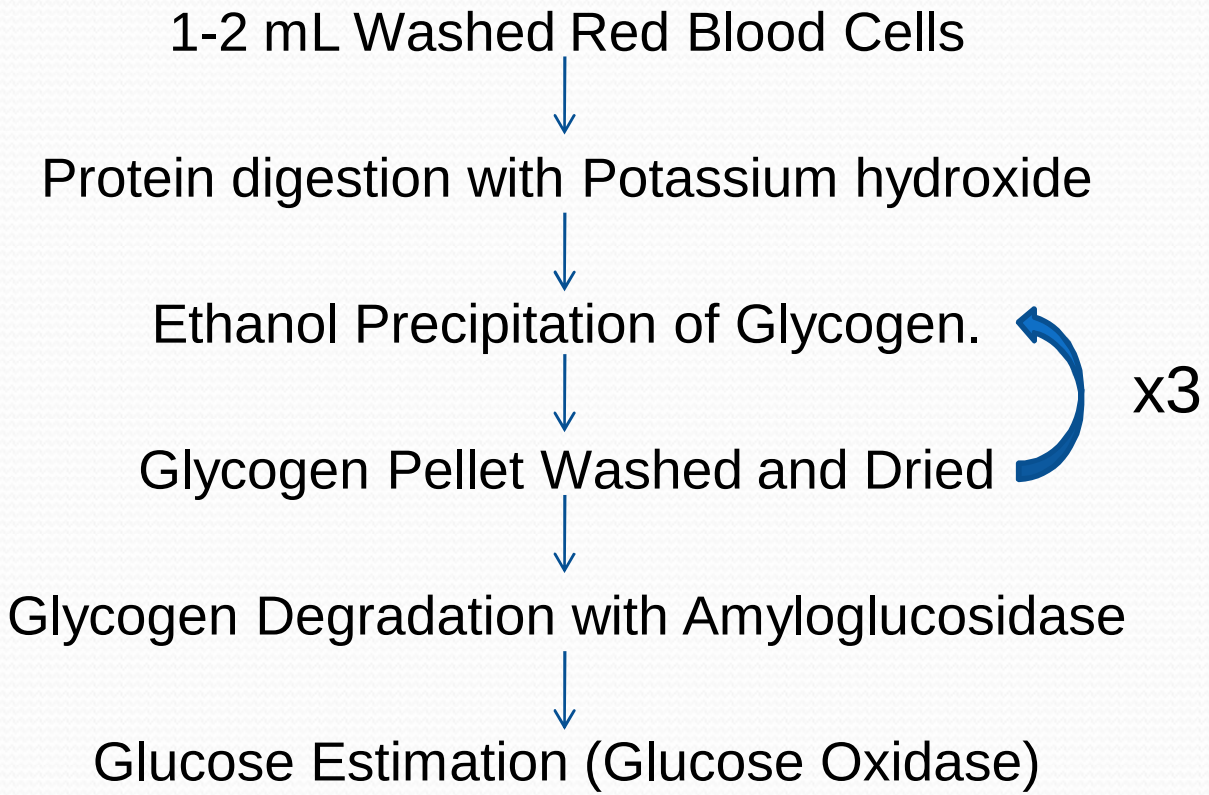
Glycogen storage disease screen:

- Minimum 5ml blood in lithium heparin
- Red cells – glycogen and phosphorylase b kinase
- White cells – debrancher and phosphorylase
  - (brancher)
- Batch consists of 8 samples (manageable no. of assay tubes)
- Screen takes operator one a week to complete

RBC glycogen

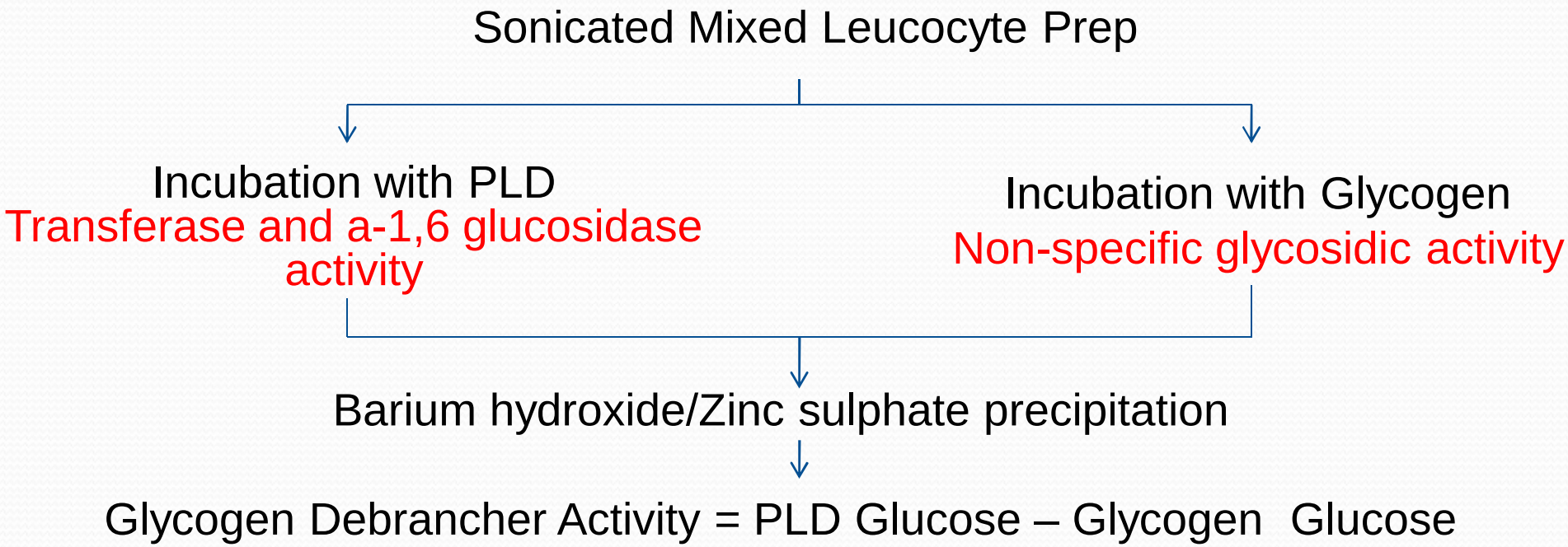
- Relatively non invasive assessment of glycogen storage
- Not elevated in GSD I, II or IV
- Most useful for confirmation of GSD III
- GSD IX – may be elevated to a lesser degree.

Glycogen Assay



- Available in liver and muscle
- This assay takes three days to complete

Total Glycogen Debrancher Activity



PLD = Phosphorylase Limit Dextran Substrate

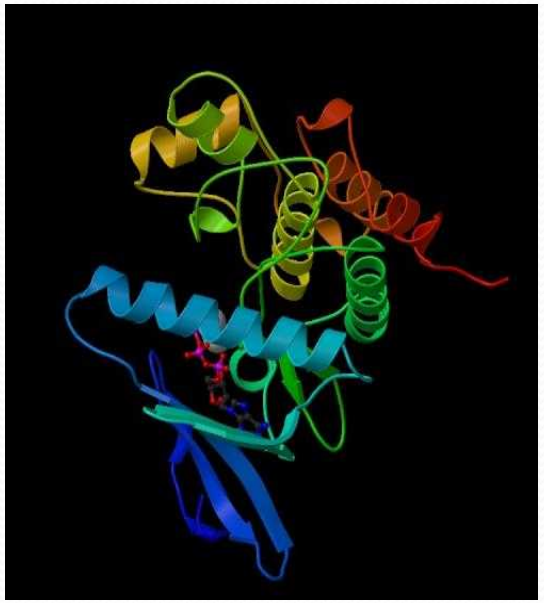
Glycogen digested with phosphorylase – leaving chains with four glucose units after each branch point.

NOT COMMERCIALLY AVAILABLE

- Assay available in fibroblasts and liver

Phosphorylase b Kinase Deficiency (GSD IX)

- Four Subunit
  - α subunit: regulatory, X allele , muscle & liver forms
  - β subunit: regulatory
  - γ subunit: catalytic
  - δ subunit: Calcium binding
- PBK Deficiency
  - PHKA Deficiency (aka GSD VIII, XLG)
    - Def α subunit
    - Low activity in liver & RBCs
    - Varient form (XLG2) normal activity in liver & RBCs
  - PHKB Deficiency
    - Def β subunit, low activity in liver & RBCs
  - Muscle PBK Def
    - X-linked & AR forms, normal PBK kinase activity in liver and RBCs



Phosphorylase b Kinase Activity

Washed Prepared RBCs

↓

Incubation of the sample with phophorylase b to generate phosphorylase a

↓

Samples collected at 0, 7 and 14 mins

↓

Incubation with glucose-1-phosphate and glycogen to generate free phosphate

↓

Precipitate proteins

↓

Quantify phosphate using an acid molybdate reaction

- Assay available in liver, fibroblasts and muscle

Problems with Enzymatic Diagnosis of Phosphorylase b Kinase Deficiency

- Even in confirmed cases total enzyme deficiency may not be seen in vitro.
  - Some cases have phosphorylase b kinase deficiency in liver but normal activity in red cells
  - Muscle forms will not be detected in RBCs
  - Mutations have been found that cause a deficiency in vivo but not in vitro

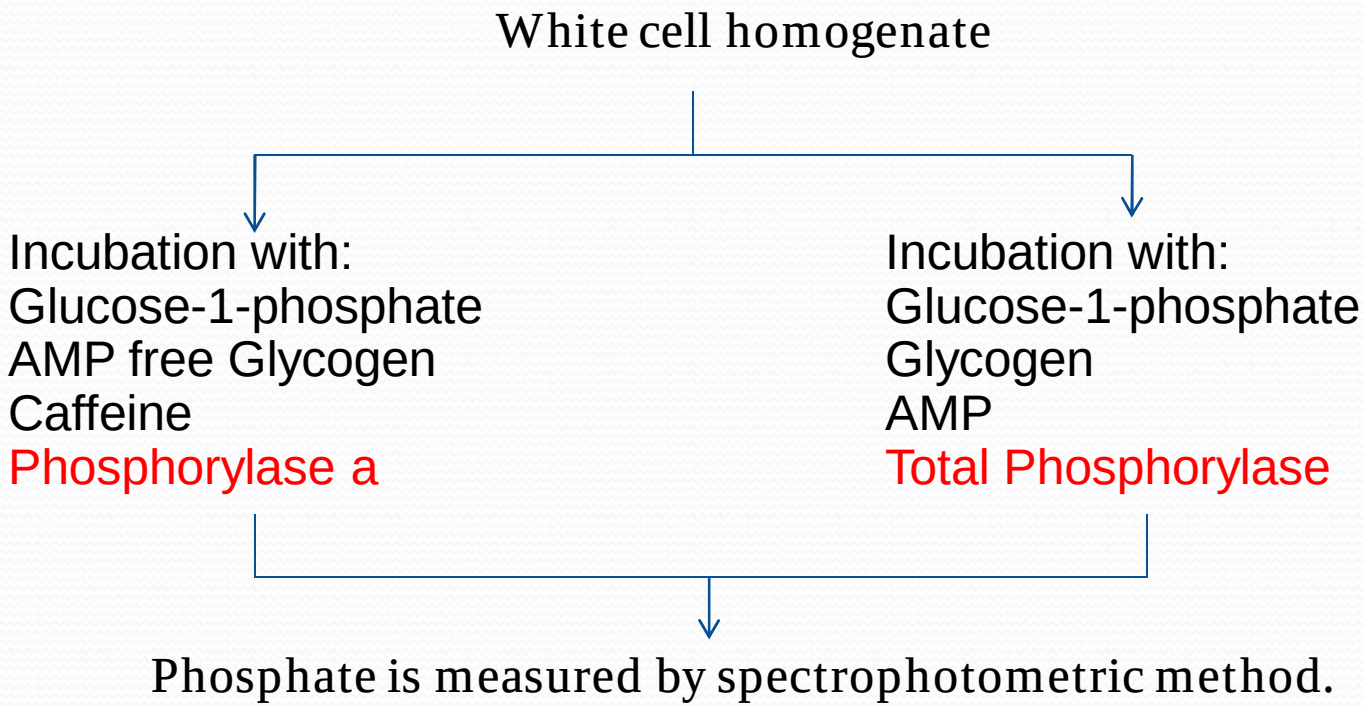
- Phosphorylase in leucocytes:

  Ratio of the active form to total – low in cases of phosphorylase b kinase deficiency. In some cases of phosphorylase b kinase deficiency the red cell glycogen may be raised BUT not always.

Results which may suggest a defect in the phosphorylase activating system

	1	2	3	Control ranges
Red cells:				
glycogen:	17	29	681*	(10 – 120 mg/gHb)
Phos b kinase	15.7	9*	ND*	(10 – 90 mg/g Hb)
White cell enzymes:				
Phosphorylase a (-AMP)	0.70	0.12*	0.48	(0.3 – 3.7 ug/hr/mg ptn)
Total phosphorylase (+AMP)	4.2	2.4	4.6	(2.4 – 10.4 ug/hr/mg ptn)
Phos a/total ratio	0.17*	0.05*	0.10*	(0.42 – 0.78)

Phosphorylase Activity



- Assay available in liver (and muscle: GSD V)
- Confirmed cases described with very high residual enzyme activity in leucocytes
- Very labile enzyme

Glycogen Brancher Activity

White cell homogenate

Incubation with:
Phosphorylase a
Glucose-1-phosphate
Background linear glycogenolysis + Brancher activity

Blank:
Phosphorylase a
Glucose-1-phosphate
Inefficient glycogenolysis of linear glycogen

Phosphate is measured by spectrophotometric method.

- Assay available in liver , muscle and fibroblasts

GSD I: Enzymatic Diagnosis

GSD Ia: Deficiency of glucose-6-phosphatase

GSD Ib: Deficiency glucose-6-phosphate ER transport protein (T1 transport protein)

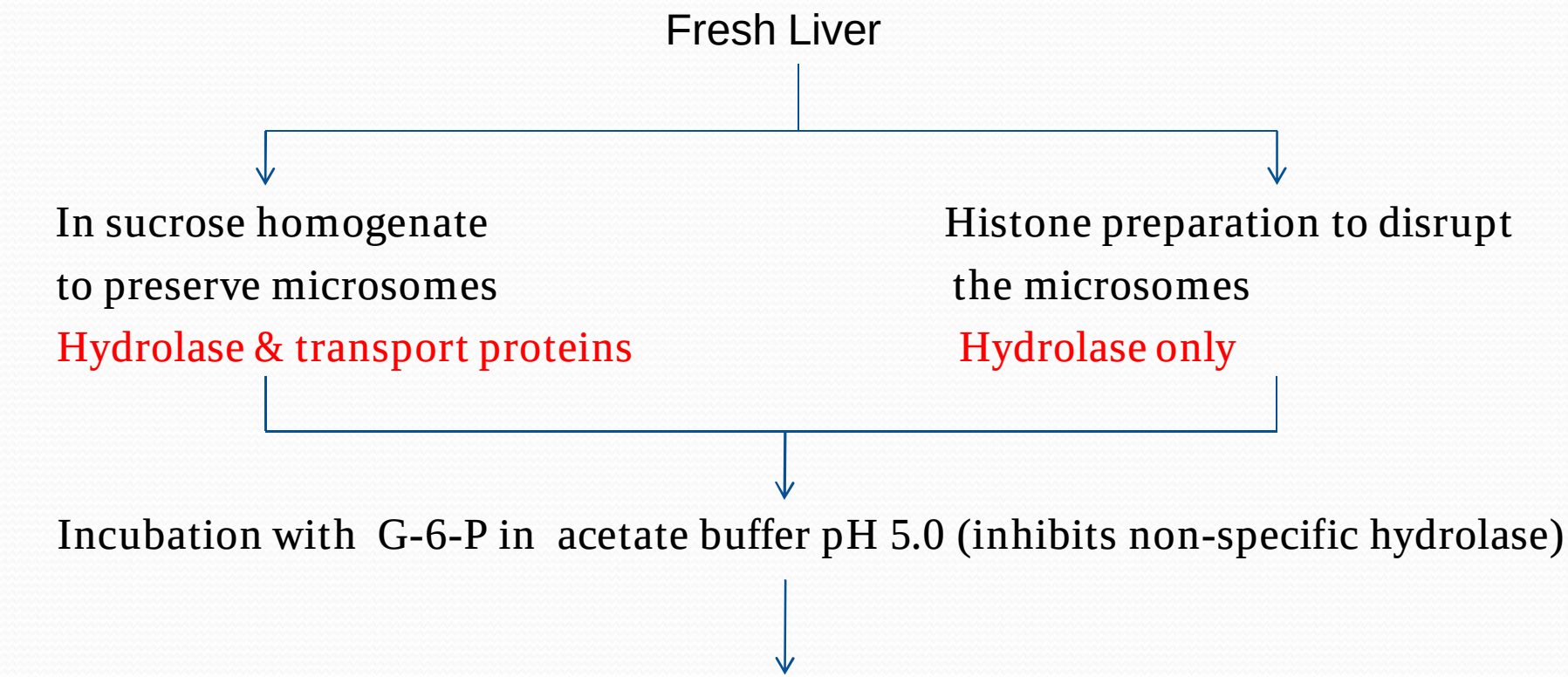
GSD Ic: Deficiency of phosphate translocator (T2β transport protein)

GSD Id: Deficiency of glucose translocator (GLUT 7 transport protein)

Glucose-6-phosphatase activity in frozen liver can only detect GSD Ia

Whole microsomes from fresh liver provide intact system testing the transport proteins and the hydrolase system.

Glucose-6-phosphatase Assay



- Requires in-patient at GOSH
- Problem with controls

Glycogen levels in GSDs

GSD	RBC Glycogen	Tissue glycogen	Histology
GSD 1	Normal	Raised liver glycogen	PAS pos cyoplasmic glycogen, significant lipid accumulation
GSD II	Normal	Raised muscle glycogen	PAS pos lysosomal glycogen
GSD III	Significantly raised	Significantly raised liver glycogen	PAS pos cyoplasmic glycogen, some lipid accumulation
GSD IV	Normal	Muscle glycogen conc may be normal	PAS positive amylopectin like cytoplasmic glycogen
GSD V	Normal	Muscle glycogen may be normal	PAS pos cyoplasmic glycogen
GSD VI	Normal	Raised liver glycogen	PAS pos cyoplasmic glycogen,
GSD VII	Normal	Muscle glycogen may be normal	PAS pos cyoplasmic glycogen,
GSD IX	Often mild/mod raised	Usually raised liver glycogen	PAS pos cyoplasmic glycogen,

Glycogen Storage Disorders affecting Predominately the Muscle

GSD V

- Deficiency of myophosphorylase
- 1: 100,000
- Exercise intolerance: rapid fatigue, myalgia and cramps precipitated by isometric excercise and sustained aerobic excercise.
- ‘Second wind’ phenonomen with relief of myalgia after a few minutes of rest.
- Presentation typically in the second and third decade.
- ~50% patients have episodes of myoglobinuria with risk of acute renal failure
- Heterozygotes at increased risk of statin induced myopathy
- Management: Avoidance of isometric excercise, caution with anaesthasia. Improved exercise tolerance with aerobic training and possibly creatine monohydrate and sucrose.

GSD V: Diagnosis

- CK
- Ischaemic forearm test
- Nonischaemic forearm test
- Cycle Test: Monitors heart rate to detect ‘second wind’ effect.
- Muscle biopsy: histopathology, enzymology
- Genetics

Ischeamic forearm Test

- Patient Preparation:
  - Overnight fast
  - Venous access obtained
- Baseline sample (-2 min): Ammonia and lactate
- Procedure:
  - Sphygomanometer cuff on upper arm inflated to above systolic blood pressure (200 mmHg)
  - Squeezing bulb at 1s intervals for 1 min (amount of effort noted)
  - Cuff remains inflated for further 1 min
- Samples collected at 0, 2 and 12 min for ammonia and lactate

Normal: lactate: $\geq$ 1.9 mmol/L over baseline in males

$\geq$ 0.6 mmol/L over baseline in females

Ammonia: $\geq$36 mmol/L over baseline males

$\geq$24 mmol/L over base;inefemales

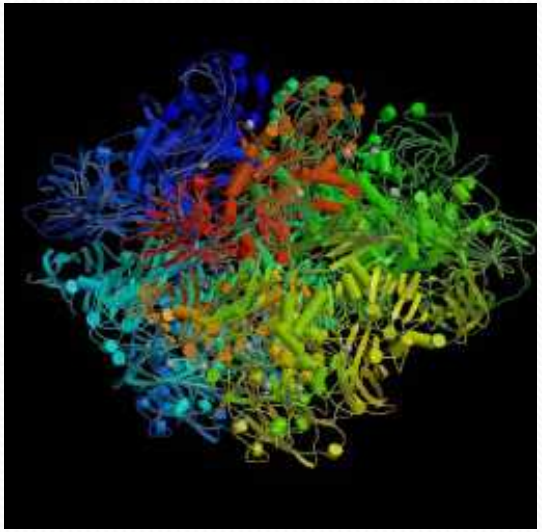
Ischeamic forearm test: interpretation

	Lactate Response	Ammonia Response
Poor Muscle Exertion	Flat/suboptimal/normal	Flat/suboptimal
Impaired muscle glycogenolysis or glycolysis eg GSD V, GSD III	Flat/suboptimal	Exaggerated
Myoadenylate deaminase	Normal	Flat/suboptimal

Problems

- Lack of exertional effort
- Variable protocols
- Poor specificity

GSD II: Pompe Disease



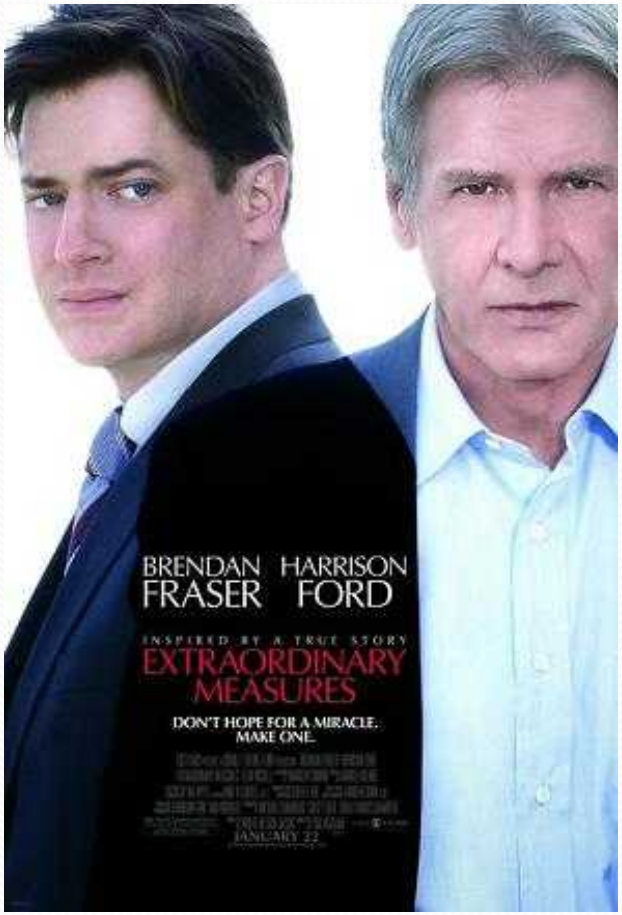
- Deficiency of lysosomal acid α-glucosidase (GAA)
- AR
- Rare, 1:40,000
- Characterised by the accumulation of glycogen in lysosomes of several cell types, particularly cardiac, skeletal and smooth muscle cells.

Pompe Disease

- 2 main forms:
- Infantile:
  - Presentation in the first few months of life
  - Feeding difficulties
  - Failure to thrive
  - Respiratory infections
  - Hypotonia
  - Hypertrophic cardiomyopathy
  - Almost invariably fatal by 12 months of age (without treatment)
- Late-onset Pompe disease
  - Presentation from infancy to late adulthood
  - Predominately skeletal muscle dysfunction
  - Muscle weakness (mobility problems)
  - Respiratory problems

Pompe Disease Management

- Respiratory therapy
- Physiotherapy
- Enzyme replacement therapy
  - IV administration of synthetic enzyme
  - Some patients respond better than others
  - Some patients develop inhibitory antibodies against ERT



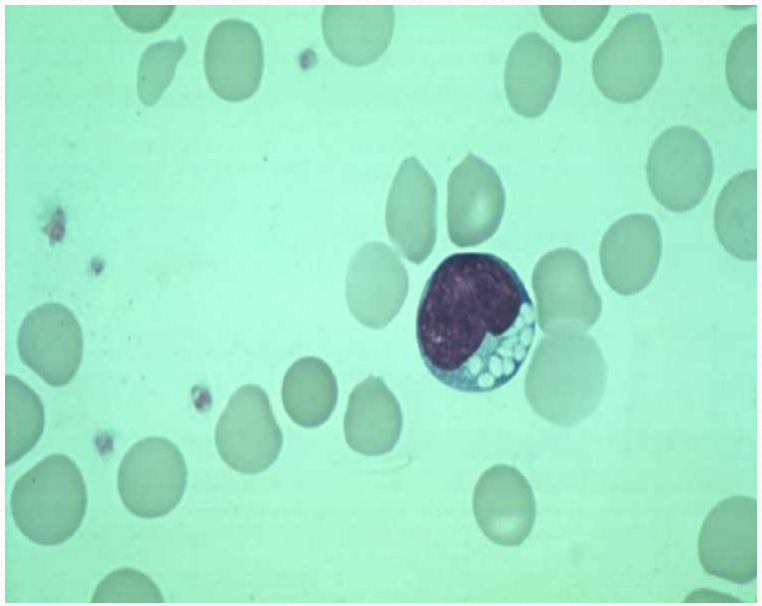
Pompe disease Diagnosis

- Muscle
  - Electromyography (EMG)/nerve conduction studies
  - Muscle strength testing
- Labs
  - Serum creatine kinase (CK)
  - Alanine and aspartate aminotransferase (ALT/AST) and lactate dehydrogenase (LDH)
  - Histopathology

Diagnosis of Glycogen Storage Disease Type II

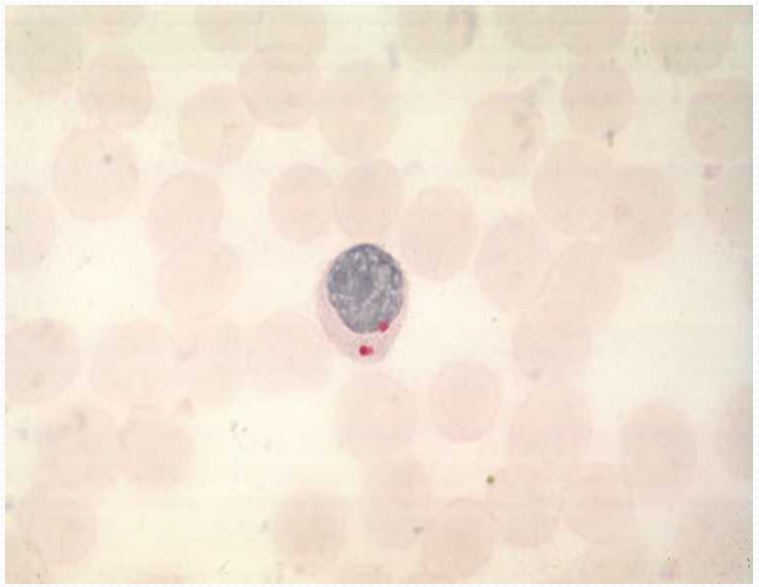
Blood film analysis: vacuolation of lymphocytes

May-Grunewald-Giemsa



Child

PAS periodic acid / Schiff



Adult

Courtesy of Hannah Lake and Glenn Anderson

Anderson et al. (2005) J Clin Pathol 58, 1305.

Confirmatory Diagnosis of Glycogen Storage Disease Type II

Demonstration of a deficiency of lysosomal a-glucosidase

- Direct: Muscle, fibroblasts
- With acarbose: To inhibit interference from Maltase-glucoamylase (MGA)
  - Leucocytes
  - Dried blood spots

Less invasive –heel prick, finger stick or blood draw
Small sample requirement
Convenient
Little specimen preparation
Can be sent in post (cheaper)
Stabile at RT during shipping and frozen for long term storage
Can be used for newborn screening
Less infectious

Other Myopathic GSDs

- GSD VII
  - Deficiency of phosphofructokinase
  - Severe infantile form: Respiratory failure
  - Mild adult form: Exercise intolerance
- GSD IV Muscle Form
  - Infantile neuromuscular form: Presentation at birth with severe hypotonia, muscular atrophy and neuronal involvement. Death in neonatal period.
  - Juvenile muscular form: Myopathy +/- cardiomyopathy
  - Mild adult muscular form: Exercise intolerance
- GSD IX Muscle form
  - Deficiency of muscle a subunit (x-linked) or AR forms (possibly γ subunit)

New Biomarkers

- Serum biotinidase:
  - Consistently mild/moderately elevated in GSD Ia & Ib
  - Also variably elevated in some cases of GSD III, VI and IX
  - Mechanism unknown

Paesold-Burda et al 2007

- Urine Tetrasaccharides:
  - Level of Glc_4, is elevated in urine and plasma of GSD II patients by HPLC & electrospray ionisation TMS

An et al. 2000 Analyt Biochem 287, 136, Young et al 2003

  - Good correlation between plasma and urine levels of Glc_4 and clinical response to treatment

An et al. (2005) Molec Genet Metab 85, 247.

Summary

- Variable presentation of glycogen storage disorders
- Initial biochemical investigation can provide diagnostic clues
- Enzymatic diagnosis is not always definitive particularly in blood
- Sometimes biopsy and/or genetic testing is required to confirm diagnosis