

Lactic Acidaemias

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Lactic acid metabolism

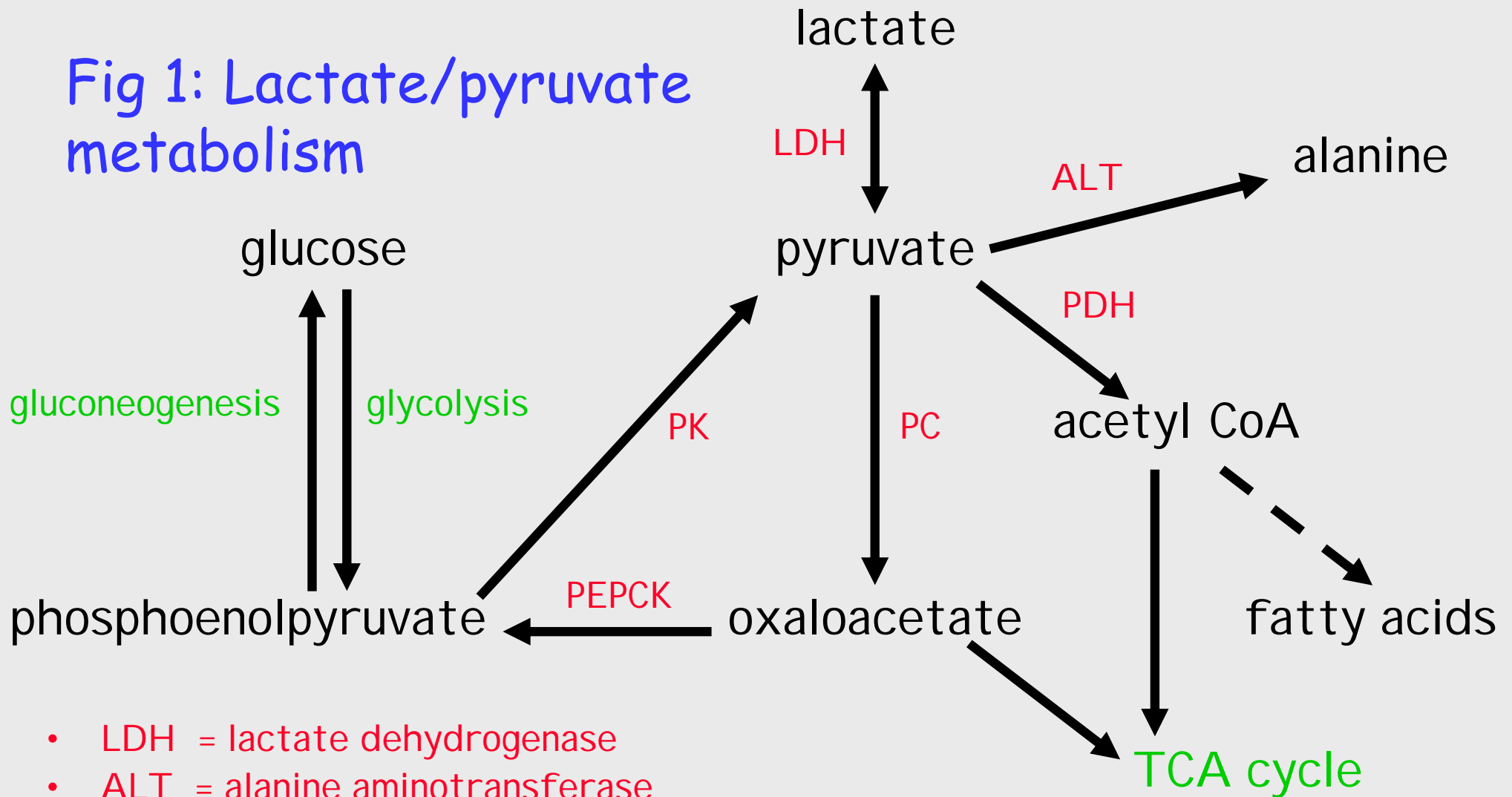
Lactate is produced as the fate of anaerobic metabolism of pyruvate. It is an important intermediary metabolite especially with regards to production of ATP (see Fig 1) and is intrinsically linked with pyruvate in many pathways of energy production.

Since lactate is an intermediary metabolite and a substrate for gluconeogenesis some is usually present in the circulation. There is usually a balance between lactate production and utilisation and subsequently plasma lactate concentrations are generally stable. Excess lactate produced by peripheral tissues is transported to the liver for conversion to pyruvate and subsequently glucose or utilised in fatty acid synthesis (The Cori Cycle).

Increased concentrations are often attributed to non-specific (acquired) causes – in particular decreased tissue perfusion and subsequent hypoxia. Many drugs/toxic substances can result in elevated lactate either due to direct effects on lactate metabolism (as in ethylene glycol and methanol poisoning) or by causing hepatotoxicity which affects the metabolism of peripheral lactate production (e.g. paracetamol poisoning.) Common acquired causes are listed in Fig 2.

As a consequence minor elevations of lactate may be difficult to interpret and a metabolic acidosis does not usually arise until lactate levels are greater than 5mmol/L. Levels >3mmol/L can be considered as significant levels above this warrant further investigation.

Fig 1: Lactate/pyruvate metabolism



- LDH = lactate dehydrogenase
- ALT = alanine aminotransferase
- PDH = pyruvate dehydrogenase
- PC = pyruvate carboxylase
- PEPCK = phosphoenol pyruvate carboxykinase
- PK = pyruvate kinase

Fig 2: Non-specific (acquired) causes lactic acidosis

Hypoxia/hypoperfusion:	hypovolaemia septic shock cardiogenic shock asphyxia severe anaemia
Systemic disease	liver disease renal failure diabetes mellitus seizures
Other causes of muscle activity	exercise struggling infant etc
Immaturity	
Drugs/toxins	carbon monoxide salicylates/ paracetamol methanol, ethylene glycol, ethanol, etc.

Secondary lactic acidosis due to inborn errors of metabolism

An increased lactate is commonly seen as a secondary consequence of some inherited (i.e. non-acquired) metabolic disorders such as organic acidurias and fatty acid oxidation defects. In these disorders the raised lactate is not due to a primary defect in lactate metabolism but believed to be due to a secondary affect on coenzyme A metabolism.

These disorders are usually readily identified by urine organic acid analysis, blood spot or plasma acyl carnitine analysis or plasma and urinary amino acid analysis. See training modules in these areas for more details.

Other inborn errors can also cause secondary acquired causes of raised lactates such as those which present with severe seizures or liver disease.

Primary lactic acidaemias

The conditions that produce a “primary” lactic acidosis are those that directly affect pyruvate metabolism which is subsequently converted to lactate. These disorders can be categorised as follows:

1. disorders of **glycogen** metabolism
 - Glycogen Storage Disease (GSD) Types 1 and 0
2. disorders of **gluconeogenesis**
 - fructose 1,6 bisphosphatase deficiency, phosphoenolpyruvate carboxykinase deficiency, pyruvate carboxylase deficiency, multiple carboxylase deficiency
3. Disorders of **pyruvate metabolism**
 - pyruvate dehydrogenase complex deficiency, pyruvate carboxylase deficiency, multiple carboxylase deficiency
4. **TCA cycle** defects
 - fumarase deficiency, isolated α -ketoglutarate dehydrogenase deficiency
5. **Mitochondrial respiratory chain** defects
 - including specific syndromes e.g. MELAS, MERRF

Disorders of pyruvate metabolism

The pyruvate dehydrogenase complex:

This is situated in the mitochondrial matrix and is a multi-enzyme complex involved in decarboxylation of pyruvate and its activation to acetyl CoA. It is important in providing the link between the end product of glycolysis (pyruvate) and the TCA cycle.

It is a large complex primarily made up of three subunits (E_1 , E_2 and E_3) and also requires a number of cofactors including thiamine. Defects in any of the subunits (and indeed thiamine deficiency) result in accumulation of pyruvate and subsequent conversion to lactate. It is one of the most commonly identified causes of inherited paediatric lactic acidosis.

Clinically these patients have a wide spectrum of presentation besides the metabolic acidosis. These usually range from a severe neurological abnormalities and early death to more chronic neurological problems. Treatment with a ketogenic (i.e. low carbohydrate) diet and thiamine to stimulate residual enzyme activity is often ineffective and the prognosis is poor. Plasma lactates can be grossly increased but in partial defects it may be normal or only moderately elevated. However in these cases patients often have a significantly raised CSF lactate. The enzyme deficiency can be demonstrated by measuring its activity in cultured fibroblasts.

E_3 deficiency

Defects in the E_3 subunit also affect other enzyme systems. This subunit is also part of 2-ketoglutarate dehydrogenase (see TCA cycle defects) and the branch-chain oxoacid decarboxylase. This defect can be primarily identified by organic acid analysis as it produces a pattern similar to MSUD together with a profound lactic acidosis.

Pyruvate carboxylase deficiency

Pyruvate carboxylase is the first step in gluconeogenesis converting pyruvate to oxaloacetate. The primary defect is covered in the carbohydrate disorders module.

Pyruvate carboxylase deficiency is also seen in **multiple carboxylase deficiency**. In this disorder all biotin dependent carboxylases are deficient with a lactic acidosis resulting from the secondary pyruvate carboxylase deficiency. Other biochemical abnormalities include hyperammonaemia and characteristic organic acid patterns. Some forms can be treated by replacing biotin.

Glycogen storage diseases

Glycogen is the storage form of glucose which can be readily mobilised at times when there is a sudden need for glucose (e.g. in extended periods of fasting). Its metabolism is shown in Fig 3.

Glycogen storage disorders (GSDs) are caused by defects in the breakdown and synthesis of glycogen in muscle or liver, the tissues in which are predominately involved in glycogen metabolism.

They characteristically present with either hypoglycaemia and/or symptoms of organ dysfunction. The organs affected depending on the enzyme defect but are most commonly liver (often with hepatomegaly), heart and skeletal muscle tissue.

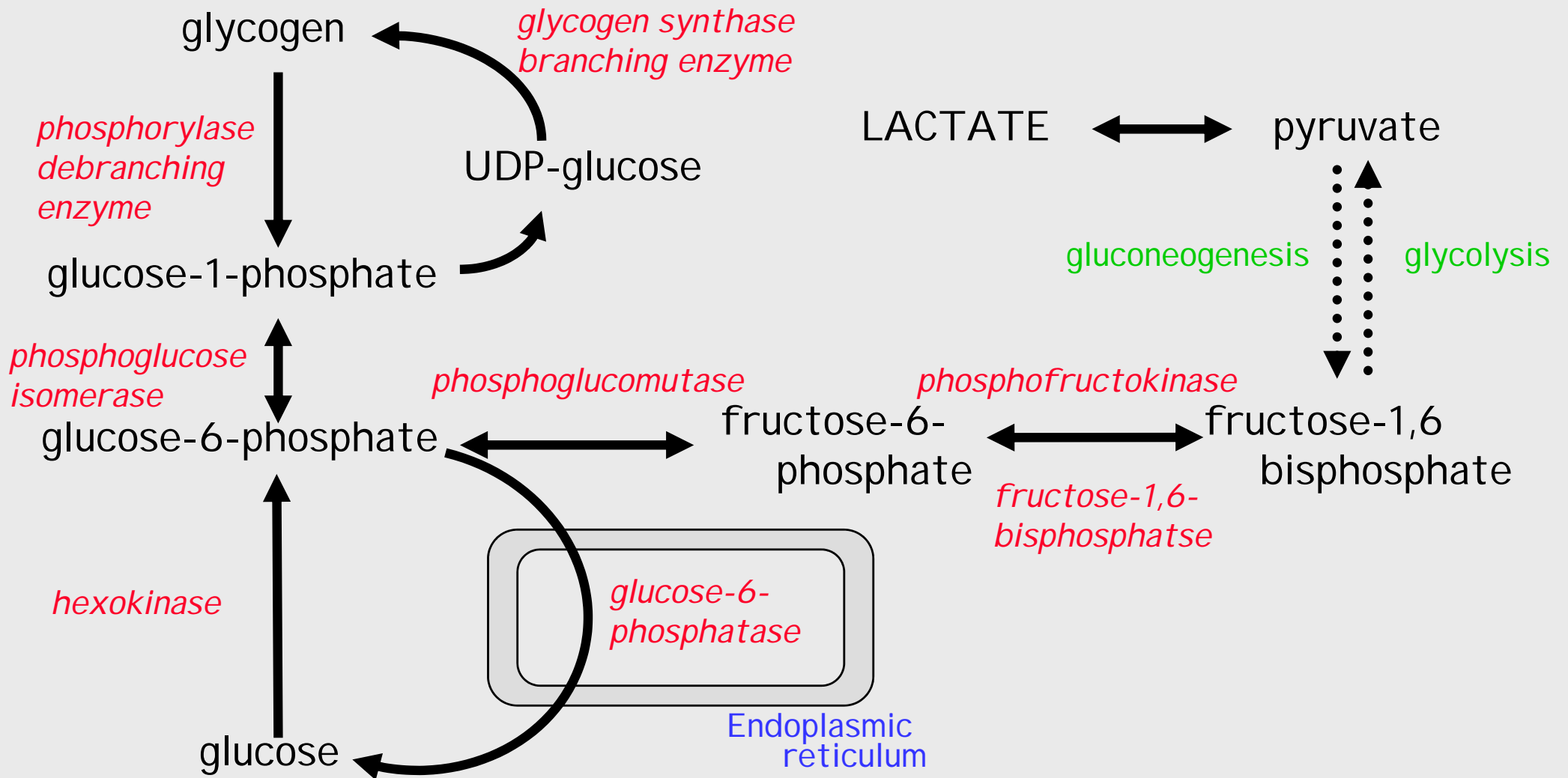
Lactic acidosis does not occur in all GSDs. The two which are commonly associated with an increased lactate are as follows:

- **Type 1** (von Gierke) = glucose-6-phosphatase deficiency
- **Type 0** = liver glycogen synthase deficiency, a glycogen synthesis defect. This presents with recurrent fasting ketotic hypoglycaemia and on feeding with hyperglycaemia and lactic acidemia. Unlike the other GSDs there are decreased amounts of glycogen and subsequently no hepatomegaly.

In these disorders lactic acidosis occurs in the fed state and is usually of moderate severity (not $>7\text{mmol/L}$). It is believed to be due to the increased flux of glucose metabolites down the glycolytic pathway as they cannot be converted to glucose or glycogen. The lactate concentration is variable and there may be periods where no elevation of lactate is observed.

See module on carbohydrate disorders for more information on GSDs.

Fig 3: Glycogen Metabolism



Gluconeogenesis disorders

Gluconeogenesis is the production of glucose from non-carbohydrate sources and occurs primarily in the liver. It is important in maintaining blood glucose levels during fasting.

Gluconeogenesis defects that cause a **lactic acidosis** tend to be **defects close to pyruvate** (namely pyruvate carboxylase deficiency and phosphoenolpyruvate carboxykinase deficiency) whereas those closer to glucose tend to be more prone to hypoglycaemia.

The disorders are discussed in detail in the carbohydrate disorders module.

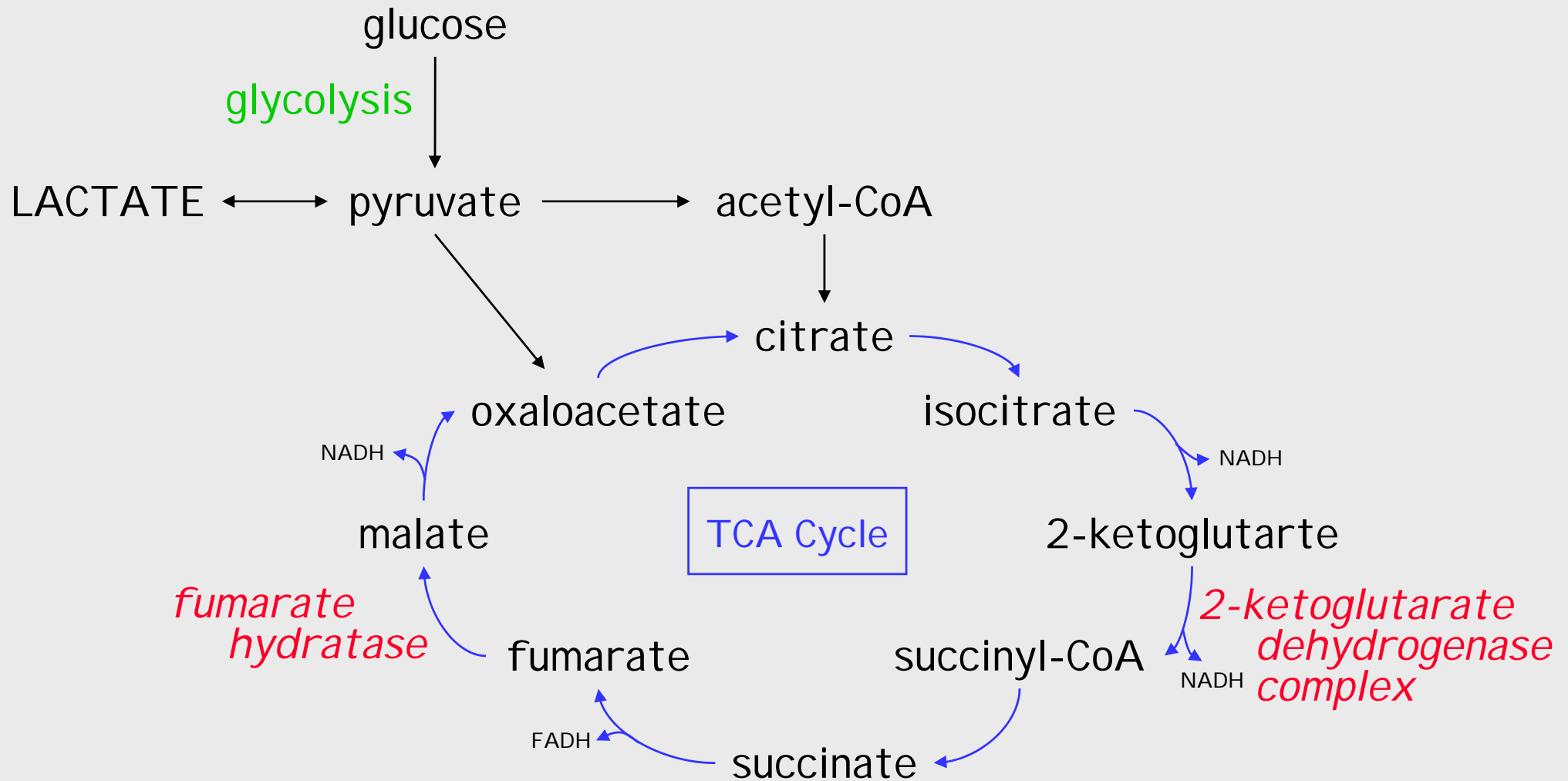
TCA cycle defects

The TCA (Krebs) cycle (Fig 4) utilises Acetyl CoA formed from the oxidation of pyruvate and as a product of fatty acid and amino acid degradation. It is key to producing the reduced forms of electron carriers (NADH and FADH) utilised in the electron transport chain, a number of intermediary metabolites and a small amount of ATP. Such as its central importance in metabolism there are few known defects in the TCA cycle that are compatible with life. The two defects described below are very rare. Lactate is produced in these disorders from the accumulation of pyruvate which is unable to be used efficiently in the cycle.

Fumarate hydratase catalyses the conversion of fumarate to malate consuming water in the process. Deficiency in this enzyme results in severe neurological abnormalities including encephalopathy, hypotonia, psychomotor retardation and progressive brain atrophy. Large amounts of fumarate are excreted in the urine which are detected by urine organic acid analysis. Large increases in lactate are observed in plasma, urine and CSF. The enzyme can be measured in fibroblasts.

2-ketoglutarate dehydrogenase complex deficiency can be due to either a primary defect in the enzyme or secondary to E_3 deficiency. There are only a few cases of the primary disorder which has a poor clinical outcome. In the latter the E_3 subunit is shared by a number of different enzyme complex systems including that of 2-ketoglutarate dehydrogenase. A lactic acidosis and abnormal organic acid pattern are observed.

Fig 4: Tricarboxylic acid (TCA) cycle



Mitochondrial Respiratory Chain Disorders

These are disorders that effect ATP production of the respiratory chain by oxidative phosphorylation. The respiratory transport chain is divided into five complexes (see Fig 6) which reside on the inner mitochondrial membrane. The redox reactions transfer electrons along the chain (via two mobile carriers) until they reach the terminal electron acceptor, oxygen. The process produces a gradient of H⁺ ions which then pass down the concentration gradient through complex V (ATP synthase) generating ATP as they do so.

Two separate genomes encode for the enzymes and enzyme complexes of the respiratory chain:

i. **Nuclear DNA** – follows Mendelian inheritance and encodes the majority of the proteins in the respiratory chain which must then be transported into the mitochondrion.

ii. **Mitochondrial DNA (mtDNA) (Fig 5)**

There are multiple copies of the mtDNA per mitochondrion. It encodes 37 genes involved in the synthesis and function of the respiratory chain including 13 which code for polypeptide chains used in the complexes of the respiratory chain.

The genome has a high mutation rate (about 10-20x greater than that of nuclear DNA). There are also no introns and so mutations generally affect the coding sequence.

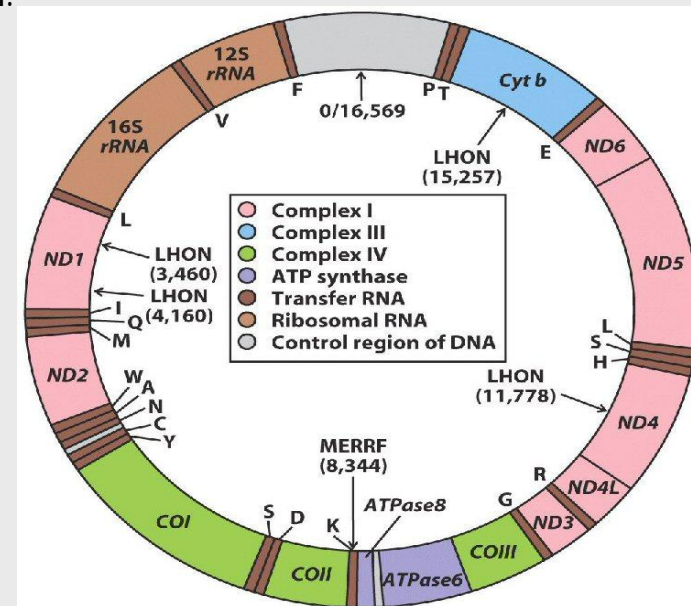
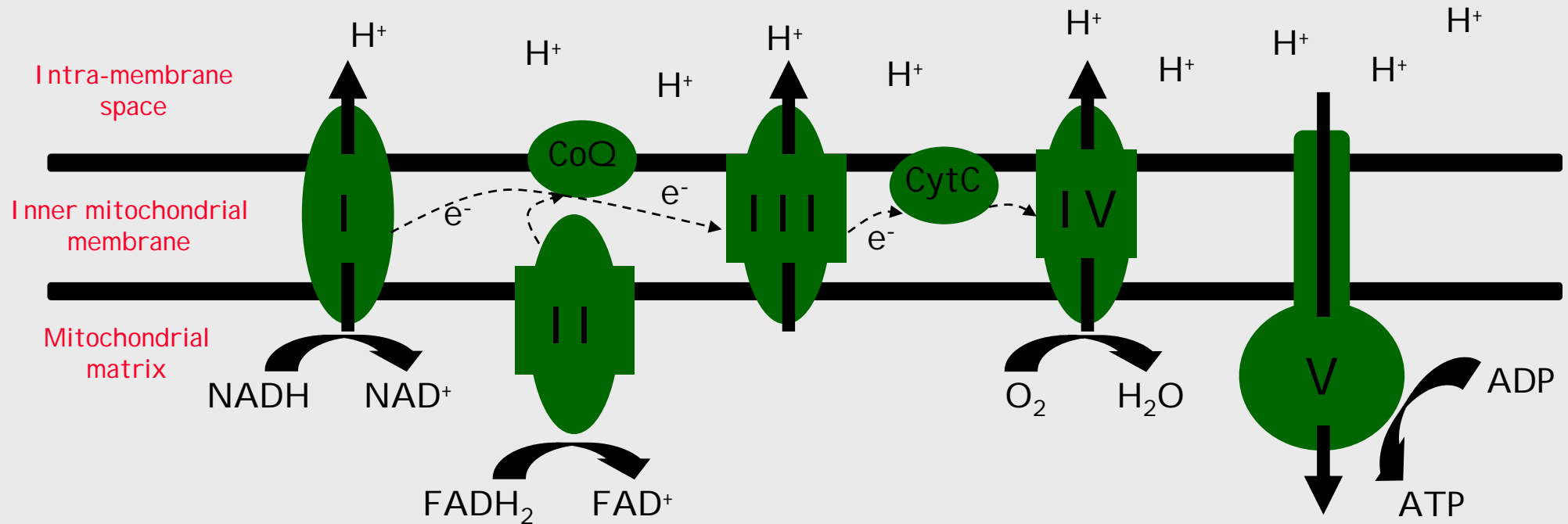


Fig 5: The mitochondrial genome

Fig 6: Mitochondrial electron transport chain



Complex I = NADH reductase
Complex II = Succinate reductase
Complex III = Cytochrome-C reductase
Complex IV = Cytochrome-C oxidase
Complex V = ATP synthase
CoQ = Ubiquinone
CytC = Cytochrome-C

Respiratory Chain Disorders- Inheritance

- Inheritance of mitochondrial disorders caused by mutations in nuclear DNA can be recessive, dominant or X-linked with variable expression and penetrance. Inheritance in of mitochondrial DNA is different (Fig 7.)

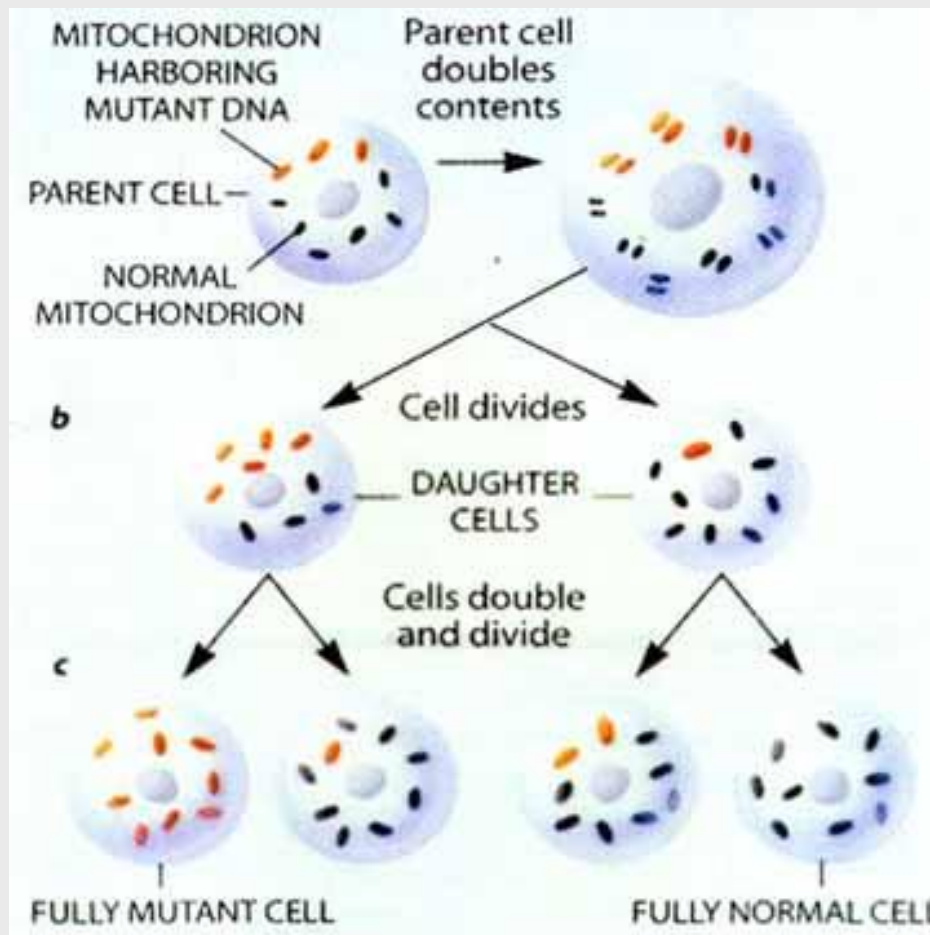


Fig 7: Mitochondrial DNA inheritance

Mitochondrial DNA follows maternal inheritance. No mitochondria are transferred from the sperm during fertilisation and hence only females can transmit the condition to both male and female offspring.

A cell containing all wild type or all mutant mtDNA is known as **homoplasmic**.

Often a mix of wild type DNA and mutant DNA coexist (= **heteroplasmic**.) The degree of heteroplasmy is dependent on how many of each type of mitochondria are passed on during cell division. There is a **threshold** of the number of mutant mtDNA that can be tolerated beyond which the biochemical abnormality will start to be expressed. This means that the level of the mutation can vary from tissue to tissue within the same person and even siblings may have different levels of the mutation - which greatly complicates diagnosis!

Clinical features in respiratory chain disorders

Respiratory chain dysfunction is usually most symptomatic in tissues with a high aerobic demand (in red below) - although disorders can present at any age and in any organ with a very diverse spectrum of clinical features and severity.

Examples of the main affected tissues and clinical features include:

- **muscle**: fatigue, myopathy, exercise intolerance
- **cardiac**: often hypertrophy
- **CNS**: motor delay, hypotonia, seizures, developmental delay, apnoea, encephalopathy, strokes, psychomotor retardation etc
- renal: renal tubulopathy
- hepatic: fulminant liver failure
- haematological: pancytopenia
- ophthalmic: retinitis pigmentosa, optic atrophy, ptosis
- endocrine: diabetes mellitus

Mitochondrial Respiratory Chain Disorders – Investigations

In disorders of the respiratory chain, metabolites prior to the block accumulate. Urinary organic acid analysis sometimes shows elevated excretion of TCA cycle intermediates. Prior to the TCA cycle **lactate accumulates** and is often raised in the plasma. Plasma alanine is often increased secondary to the raised lactate. CSF lactate is raised either secondary to increased plasma lactate or is disproportionately elevated if the lesion is confined to the CNS indicating lactate formation in that tissue. Respiratory Chain Disorders also affects other pathways and a distinctive pattern can sometimes be seen on acyl carnitine analysis with many medium chain acyl carnitines elevated.

Lactate:pyruvate ratio's can be abnormal however collection of samples for the measurement of pyruvate is difficult and the this analysis is not often performed.

Blood mtDNA analysis can be used to diagnose specific mtDNA syndromes , however because of the tissue variability caused by heteroplasmy they may not reveal the mutation unless the leucocytes are affected.

Definitive testing often requires a tissue biopsy. Choosing a tissue type is critical as mitochondrial disorders are expressed in such a wide spectrum of tissues. Most commonly a muscle biopsy is performed as this provides the best diagnostic yield.

A variety of techniques can be used to identify the exact site of the defect in the electron transport chain including:

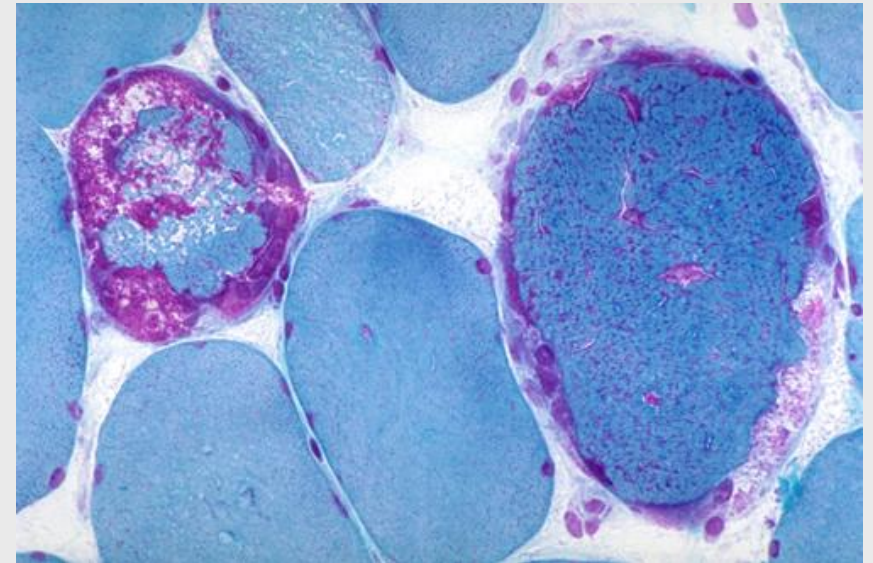
- Respiratory chain enzyme analysis
- Histochemistry
- Polarography of isolated mitochondria
- mtDNA analysis can also be performed on the affected tissue

Mitochondrial Syndromes

- There are a number of specific syndromes caused predominantly by mitochondrial DNA mutations. Unlike many mitochondrial disorders these are characterised by specific clinical features. Many of these have common mutations and therefore DNA mutation analysis is key in their investigation.

Examples include:

- MELAS = mitochondrial encephalomyopathy, lactic acidosis and stroke like symptoms. Caused by mutations in the tRNA^{leu} gene.
- MERRF = myoclonic epilepsy with “ragged red fibres” (MERRF). Caused by mutations in tRNA^{lys} gene.
- LHON = Leber’s hereditary optic neuroretinopathy – causes visual loss, men affected more often than women (although this syndrome is NOT associated with a raised lactate.)
- KSS = Kearns-Sayer Syndrome – causes chronic progressive external ophthalmoplegia (CPEO), ptosis, bilateral deafness. High CSF protein present. Caused by defects in mtDNA, usually deletions +/- duplications.



- **Fig 8: Ragged red fibres**
- caused by accumulation of affected mitochondria which are often abnormal in shape and architecture. The red colour appears when affected muscle tissue is stained with Gömöri Trichrom stain.

Self assessment questions

Statement:	True / False?
Inborn errors of metabolism are the most common causes of a raised lactate in paediatrics.	
The liver is the main organ responsible for metabolising excess lactate.	
Seizures result in a raised plasma lactate.	
Excess glycogen accumulates in Glycogen storage disease type 0.	
All respiratory chain defects follow maternal inheritance.	
Ragged red fibres are caused by an accumulation of abnormal mitochondria in muscle tissue.	
Mitochondrial DNA codes for most genes in the respiratory chain.	

Self assessment questions

Statement:	True / False?
Inborn errors of metabolism are the most common causes of a raised lactate in paediatrics. <i>As with adults acquired causes such as hypoxia are the most common causes of a raised lactate.</i>	False
The liver is the main organ responsible for metabolising excess lactate.	True
Seizures result in a raised plasma lactate.	True
Excess glycogen accumulates in Glycogen storage disease type 0. <i>GSD0 is the glycogen synthesis defect where there is a lack of glycogen.</i>	False
Respiratory chain defects follow maternal inheritance. <i>Only those that are coded for by mitochondrial DNA. Therefore some but not all of the respiratory chain defects follow maternal inheritance.</i>	False
Ragged red fibres are caused by an accumulation of abnormal mitochondria in muscle tissue.	True
Mitochondrial DNA codes for most genes in the respiratory chain. <i>The majority are coded by nuclear DNA.</i>	False

Further Reading

Clinical Biochemistry Metabolic and Clinical Aspects. Marshall WJ and Bangert SK, 2nd Ed 2008 , Churchill Livingstone (Elsevier) I SBN:978-0-443-10186-1

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