

Amino Acid Disorders

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Amino Acid Physiology

Amino acids are small molecules with the general structure of a primary carbon atom attached to a carbonic acid group, an amino group, a hydrogen atom and a variable group known as the 'R' group (Fig 1).

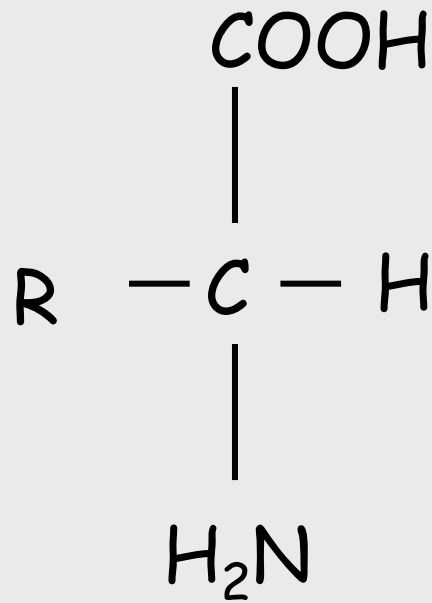
Most amino acids can be synthesised *de novo*. However there are some that have to be supplied in the diet and these are termed the essential amino acids. These are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine. In addition some amino acids are conditionally essential for neonates, this is because of delayed maturity of some enzymes. These are cysteine, tyrosine, taurine, arginine and glycine.

Amino acids have a wide variety of functions within the body. They are the building blocks of protein molecules. Although not all amino acids contribute to protein structure, ornithine, for example, has no tRNA carrier.

Amino acids are the precursors of many compounds. For example dihydroxyphenylalanine (DOPA) and dopamine are synthesised from tyrosine. Haem is synthesised from glycine and succinylCoA.

Amino acids are catabolised by pathways that are unique due to differences in the R groups. They follow common principles. There is a deamination, the amino groups are then passed by transamination reactions to the urea cycle. There is a decarboxylation followed by a series of conversions via organic acid intermediates to tricarboxylic acid cycle or ketotic metabolites. Thus amino acid catabolism generates energy. Amino acids and protein, are a reserve energy source in fasting and starvation.

Fig 1



Inborn Errors of Amino Acid Metabolism

Amino acids accumulate in body fluids when there are genetic defects i.e. inborn errors of metabolism (IEM), that affect their metabolism or transport. Sometimes the opposite can happen and an IEM can result in the deficiency of an amino acid, for example some disorders of the urea cycle result in arginine deficiency.

Amino acids are more likely to accumulate the closer they are to the metabolic block.

Metabolic blocks may result in the accumulation of novel compounds that are not normally observed-for example succinylacetone accumulates in tyrosinaemia type 1.

Inborn errors of amino acid metabolism are associated with clinical disease in most cases. For some disorders the toxic agent is readily apparent. For example high plasma concentrations of leucine cause damage to the brain-encephalopathy. For other conditions the nature of the toxicity is less well characterised. For example in phenylketonuria there remains debate as to how the high plasma phenylalanine concentrations cause damage to the developing brain and to what extent the novel compounds, the phenylketones and the deficiency of neurotransmitters contribute to the pathophysiology.

Methods of analysis

There are many methods for analysing amino acids. Listed below are those in common use in clinical laboratories in the UK. For illustrations of these methods see Fig 2.

Thin layer chromatography, TLC

Qualitative; detection is usually by ninhydrin staining

High pressure liquid chromatography, HPLC

Quantitative; there are a variety of derivitisation techniques in use and it is fast. Ultra performance liquid chromatography (UPLC) is even faster but does not detect all clinically relevant amino acids.

Automated ion exchange chromatography, AAA

Quantitative, relatively slow but generally considered to be the reference method

Tandem mass spectrometry, TMS

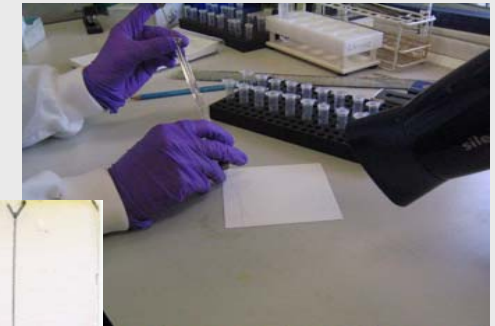
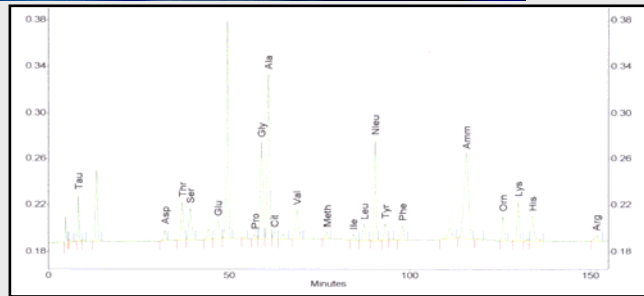
Quantitative and very fast with direct injection. The ideal neonatal screening technology. A limited range of amino acids are detectable unless there is prior chromatography which then increases the analytical time.

Gas chromatography: mass spectrometry, GCMS

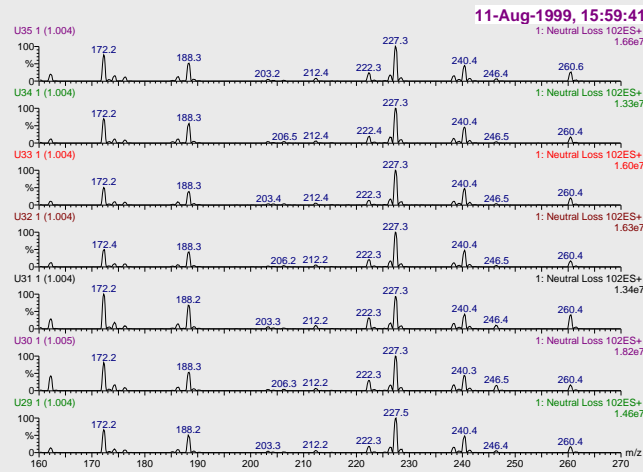
Rarely used for amino acid analysis but in widespread use for urinary organic acid analysis which can be critical in characterising amino acid disorders

Fig 2 Example chromatograms

Automated ion exchange amino acid analyser and plasma chromatogram



TLC urine amino acids, normal pattern



Tandem mass spectrometer and bloodspot chromatograms

Phenylketonuria

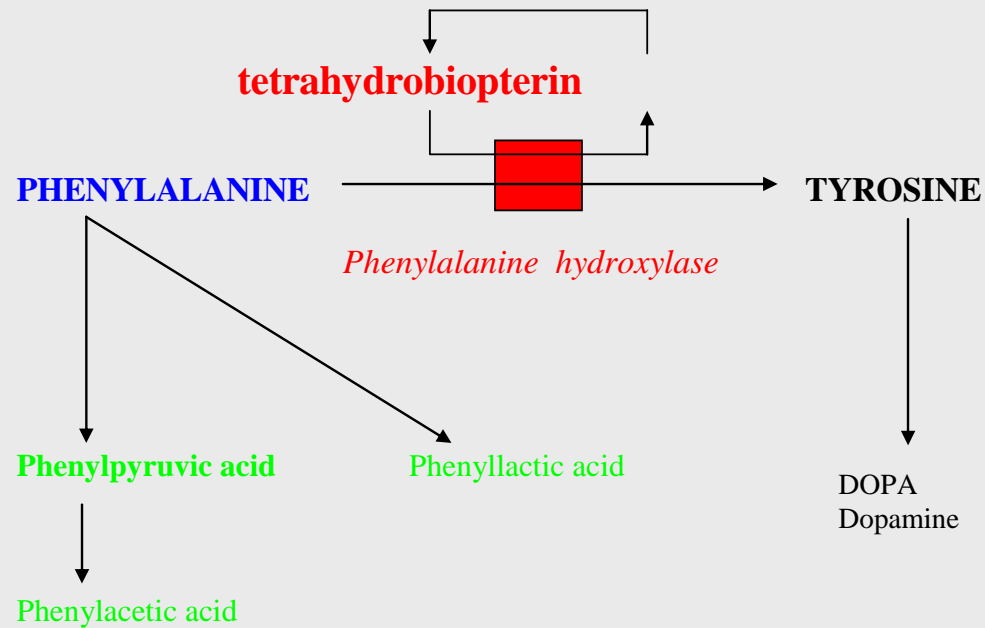
The natural history of phenylketonuria, PKU is severe and sustained damage to the developing infantile brain resulting in profound mental retardation, seizures and spasticity. However, the foetal brain is protected by maternal metabolism. Early detection, through neonatal screening, and the institution of dietary therapy in the first two or three weeks of life results in near normal development.

PKU is caused by genetic defects in the hepatic enzyme phenylalanine hydroxylase (fig 3). Phenylalanine accumulates and is further metabolised to the phenylketones.

PKU can also be caused by defects in the metabolism of tetrahydrobiopterin (BH4), an essential cofactor for phenylalanine hydroxylase. It is vital to identify these biopterin deficient patients when a new case of hyperphenylalaninaemia is found because the treatment is quite different.

The treatment of patients with 'classical' PKU is through dietary restriction of phenylalanine. The success of the diet is determined by monitoring plasma phenylalanine concentrations. Patients with BH4 deficient disorders have a more variable clinical course. They require biopterin supplements but don't benefit from a phenylalanine restricted diet.

Fig 3 Phenylalanine Metabolism



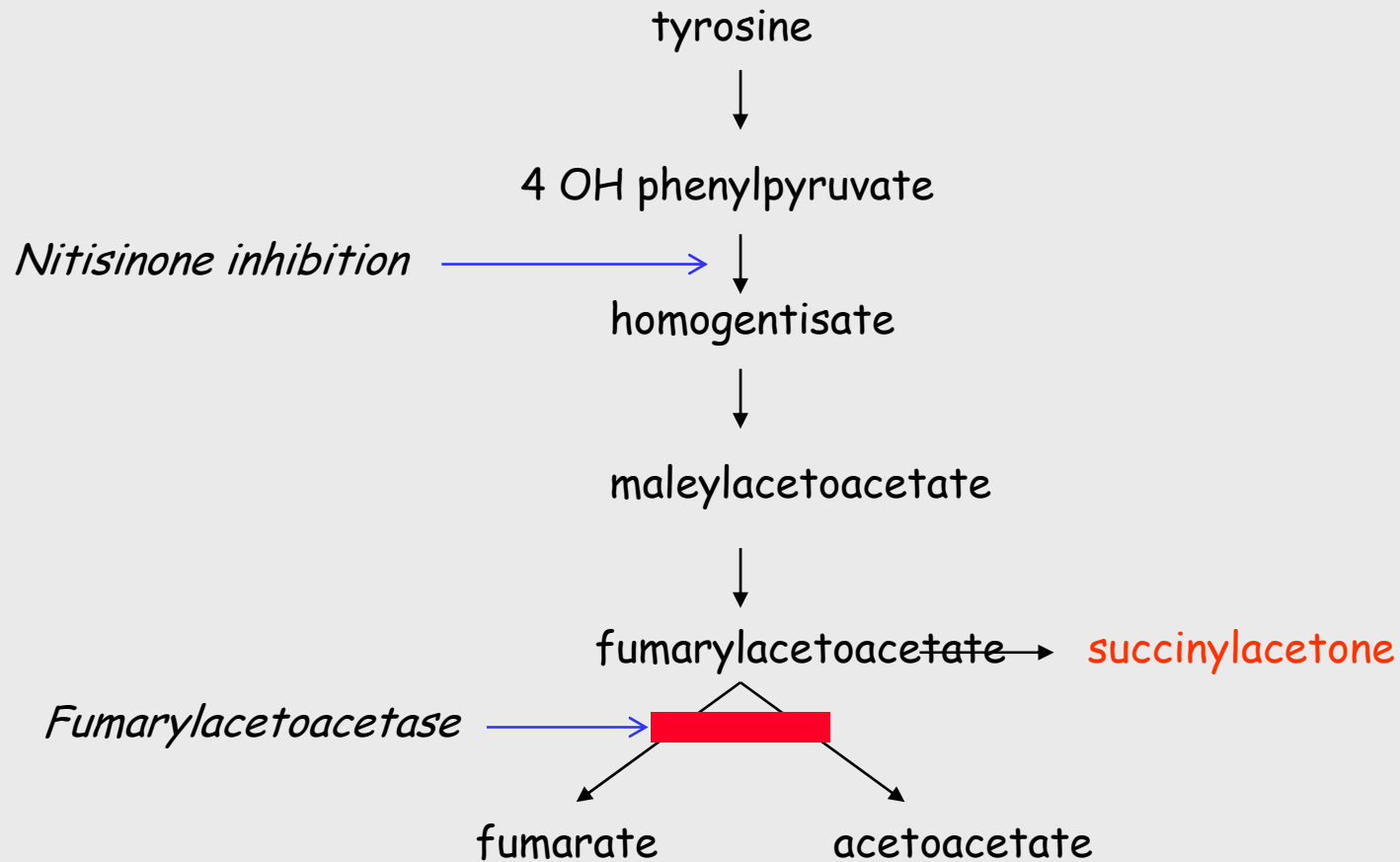
Tyrosinaemia type 1

A deficiency of the enzyme fumarylacetoacetase leads to an accumulation of the novel and extremely toxic compound succinylacetone (fig 4). This is detected by urine organic acid analysis. Tyrosine concentrations in body fluids are also elevated but can be variable.

Affected children typically present with severe liver disease and renal Fanconi syndrome.

The drug nitisinone inhibits an enzyme higher in the pathway, 4 hydroxyphenylpyruvate dioxygenase. This prevents the synthesis of succinylacetone and dramatically improves prognosis. Patients continue to need dietary therapy to prevent tyrosine accumulation.

Fig 4 Tyrosine Metabolism



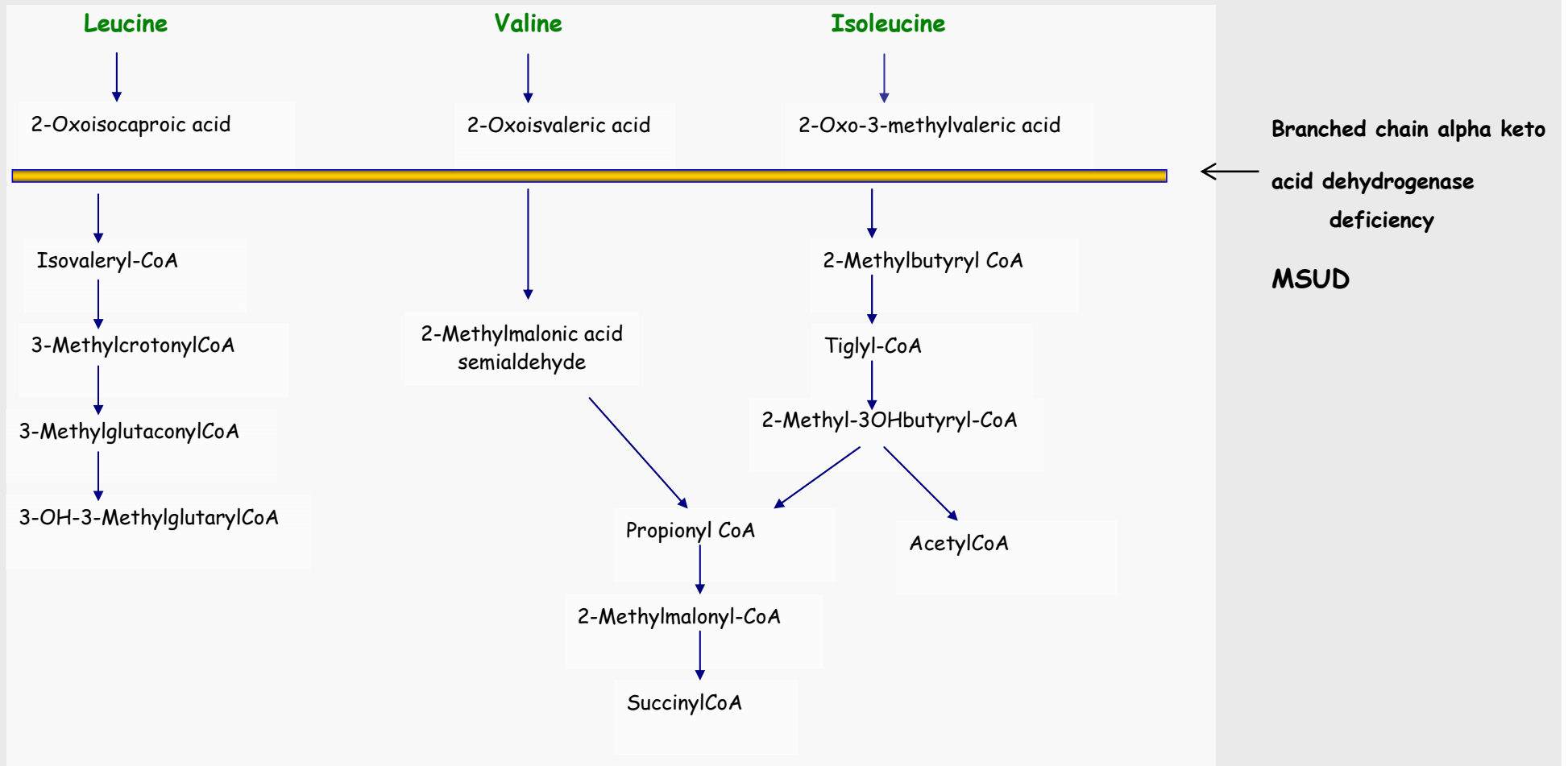
Maple syrup urine disease

A common enzyme catalyses the oxidative decarboxylation of the ketoacids derived from the deamination of leucine, isoleucine and valine. Deficiency of this enzyme causes maple syrup urine disease. The urine has a heavy, sickly odour but more importantly the affected children suffer from fluctuating but progressive neurological disease. The severity of the encephalopathy is directly related to the plasma concentration of leucine which needs to be carefully monitored. Children with MSUD are also at risk of metabolic crises. These often follow a catabolic event and can present with metabolic acidosis and sometimes hypoglycaemia.

Diagnosis is made by finding elevated concentrations of all branched chain amino acids in plasma and less reliably in urine together with the presence of the unusual amino acid alloisoleucine. Finding characteristic keto acids in urine by organic acid GCMS analysis can also be helpful.

Treatment consists of a diet low in branched chain amino acids. Emergency procedures to stimulate anabolism, and even haemodialysis, are necessary during acute episodes.

Fig 5
Branched chain amino acid catabolism



Homocystinuria

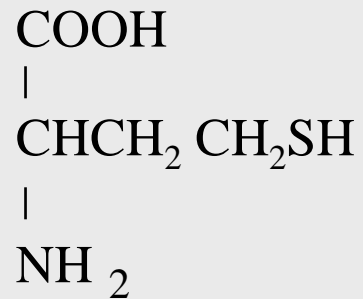
Homocysteine is an intermediate in the metabolic pathway between methionine and cysteine. In addition to the enzyme, cystathionine synthase, in the main pathway, there are two additional enzymes which can remethylate homocysteine back to methionine. All three enzymes have vitamin cofactors. Homocysteine is present in body fluids in several forms. It is a sulphhydryl compound that dimerises (oxidises) readily to form homocystine. Traditionally it was the 'free' homocystine (dimer) in plasma and urine that was measured by amino acid analysis to detect and monitor homocystinuria. But homocysteine can exist in plasma covalently bound to protein and to other sulphhydryl compounds. Therefore the most consistent and sensitive way to measure plasma homocysteine is to start by reducing all forms back to the monomer homocysteine initially. In this way total homocysteine is assayed.

Homocystinuria causes chronic progressive disease characterised by Marfan-like skeletal abnormalities, mental retardation and severe thrombotic tendencies.

Treatment is a combination of vitamin supplements, when effective, and a low methionine diet.

Moderate hyperhomocysteinaemia is more common and is associated with increased risk of cardiovascular disease. It usually responds to folate supplements, however controversy remains as to the clinical benefit of identifying and treating the milder forms of homocysteine accumulation.

Molecular Forms

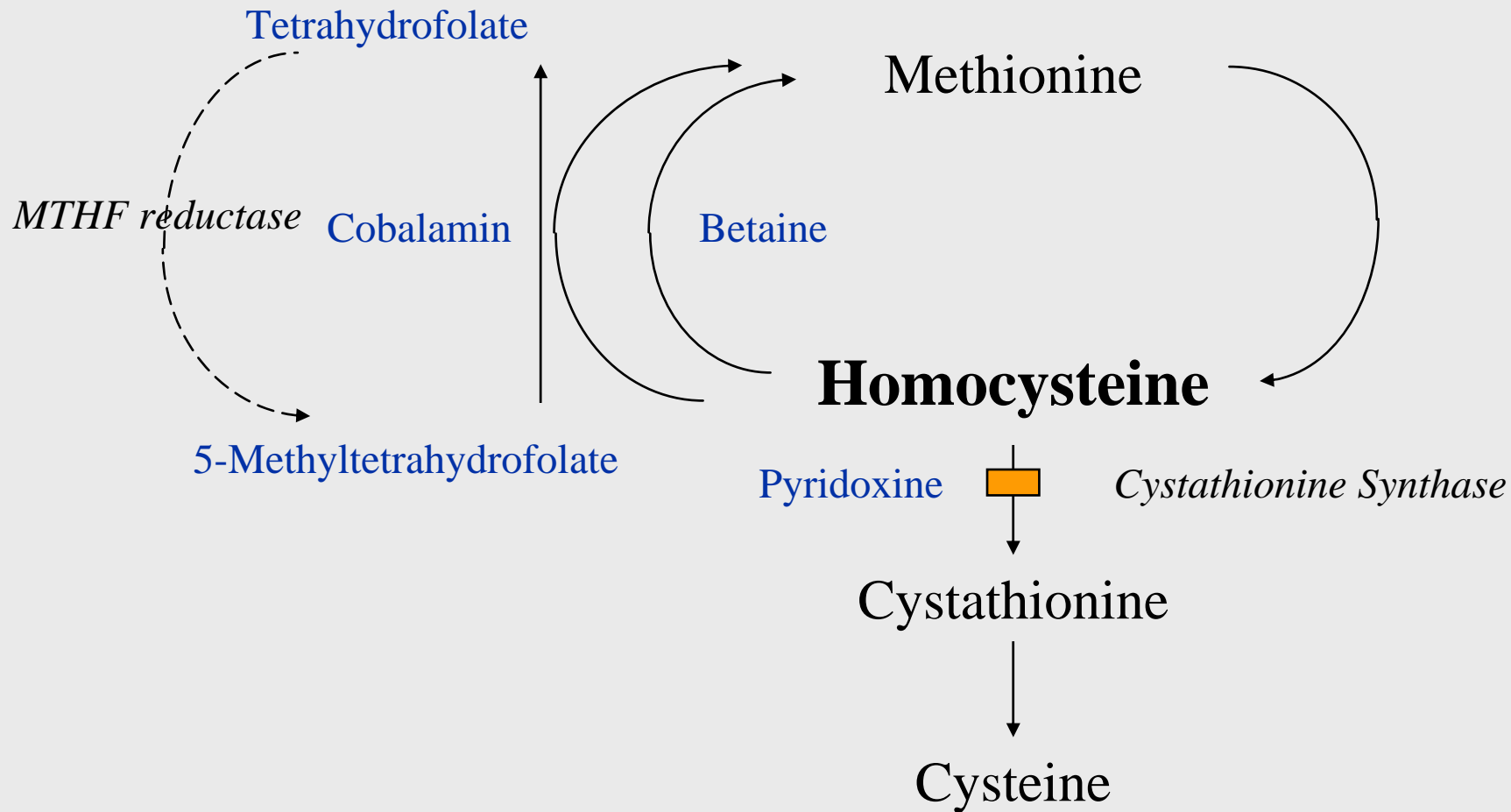


Homocysteine



Homocystine

Fig 6
Homocystine metabolism



Cystinuria

Cystine, arginine, ornithine and lysine, the dibasic amino acids, share a common transport protein in the luminal cells of the proximal renal tubule. Defects in this protein result in markedly elevated urinary excretion of all four amino acids. Cystine has a relatively low solubility and this is exceeded in patients with the defect. Cystine crystals can thus precipitate or aggregate around urinary debris, e.g. bacteria, blood cells or other solid matter. In this way urinary stones can aggregate. This can cause obstruction to the urinary tract, haematuria, pain and renal failure.

Treatment consists of promoting diuresis through increasing the volumes of liquid consumed. Chelation therapy may be necessary in refractory cases.

Hyperglycinaemia

Inborn errors of the glycine cleavage enzyme system lead to an accumulation of glycine in body fluids and particularly in cerebrospinal fluid. Affected children usually present with severe seizures in the neonatal period. There is no concurrent metabolic disturbance, i.e. an acidosis or hypoglycaemia. This disorder is commonly known as non ketotic hyperglycinaemia. This name arose in the early history of the discipline to distinguish this condition from the 'ketotic hyperglycinaemias' which were found to be disorders of organic acid metabolism which are often associated with glycine accumulation as a secondary effect.

The diagnosis is made by measuring glycine in paired CSF and plasma samples and comparing the ratio to reference data.

There is no effective treatment. The prognosis is very poor.

Self assessment questions

1. Why are some amino acid essential for neonates but not older children?
2. Which amino acid is a precursor of haem?
3. What is the enzyme defect in phenylketonuria?
4. How are children affected by phenylketonuria treated?
5. Why do patients with tyrosinaemia benefit from the drug nitisinone (NTBC)?
6. Do children affected with non ketotic hyperglycinaemia present with an acidosis?
7. Which amino acids are excreted in excess when there is a defect of the renal carrier for dibasic amino acids?
8. Name two of the vitamins involved in homocysteine metabolism
9. Which amino acid needs to be careful controlled in patients with maple syrup urine disease?
10. Which body fluid is most useful to diagnose non ketotic hyperglycinaemia?

Self assessment answers

1. Immaturity of synthetic enzymes
2. glycine
3. Phenylalanine hydroxylase
4. Phenylalanine restricted diet
5. It inhibits succinylacetone synthesis
6. No, they have severe seizures
7. cystine ornithine arginine and lysine (COAL)
8. Pyridoxine (B6), cobalamin (B12), folate and betaine
9. Leucine
10. CSF