Amino acid metabolism

Unlike fats and carbohydrates, amino acids are not stored by the body.

No protein exists whose sole function is to maintain a supply of amino acids for future use. Therefore, amino acids must be:

- Obtained from the diet,

- Synthesized *de novo*,

- Produced from the degradation of body protein.

Amino acid pool

Free amino acids are present throughout the body, such as in cells, blood, and the extracellular fluids

This pool is supplied by three sources:

1) Amino acids provided by the degradation of endogenous (body) proteins, most of which are reutilized.

2) Amino acids derived from exogenous (dietary) protein

3) Nonessential amino acids synthesized from simple intermediates of metabolism.

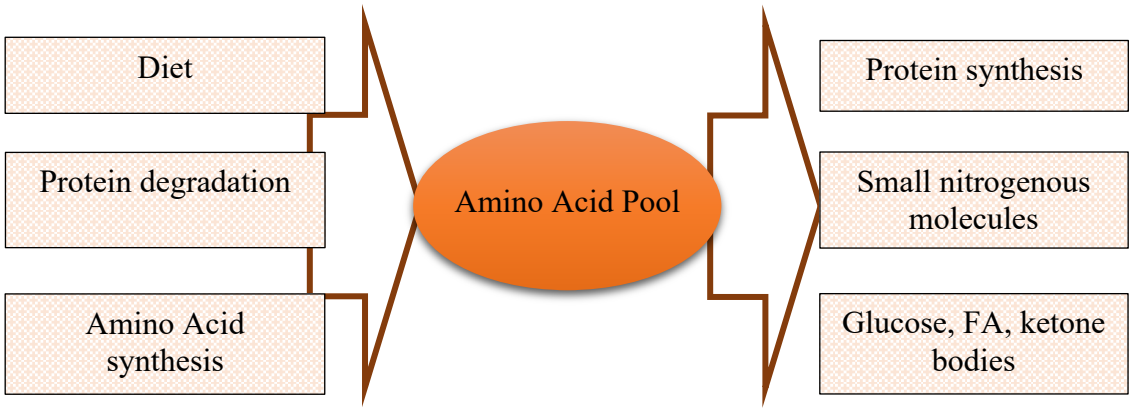
The pool is depleted by three routes:

1) Synthesis of body protein.

2) Consumption of amino acids as precursors of essential nitrogen-containing small molecules

3) Conversion of amino acids to glucose, glycogen, fatty acids, and ketone bodies or oxidation to $CO_2 + H_2O$.

The amino acid pool is small (90-100 g) vs body protein (12 kg)



Amino Acid Catabolism:

The first phase: involves the removal of the α-amino groups, usually by transamination (in most tissues) and subsequent oxidative deamination (in the liver), forming ammonia and the corresponding α-keto acids (the carbon skeletons of amino acids).

In the second phase: the carbon skeletons of the α-keto acids are converted to common intermediates of energy-producing metabolic pathways. These compounds can be metabolized to CO_2 and H_2O, glucose, fatty acids, or ketone bodies

The degradation pathways of the various amino acids converge to form seven intermediate products:

1. Oxaloacetate
2. Pyruvate
3. α-ketoglutarate
4. Fumarate
5. Succinyl CoA
6. Acetyl CoA
7. Acetoacetate or acetoacetyl CoA

The products enter the pathways of intermediary metabolism, resulting either in the synthesis of glucose, ketone bodies, lipids or in the production of energy through their oxidation to CO_2 by the CAC.

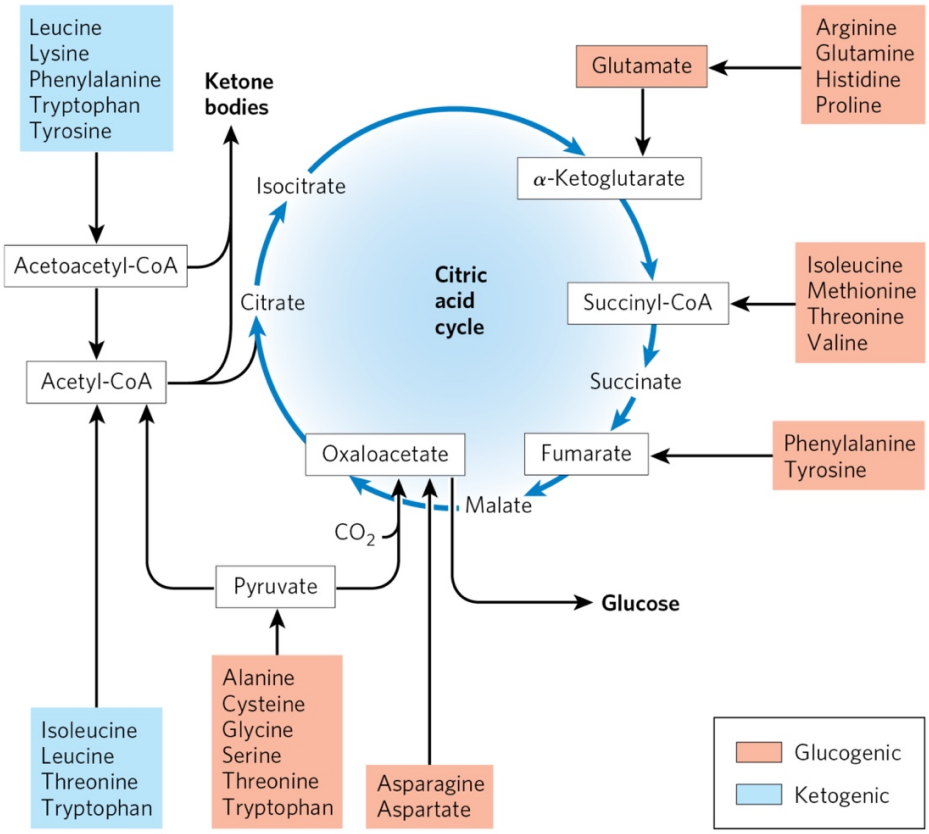
A. Glucogenic amino acids

Amino acids whose catabolism yields pyruvate or one of the intermediates of the CAC are termed glucogenic amino acids because these intermediates are substrates for gluconeogenesis.

B. Ketogenic amino acids

Amino acids whose catabolism yields either acetyl CoA (directly, without pyruvate serving as an intermediate) or acetoacetate (or its precursor acetoacetyl CoA)

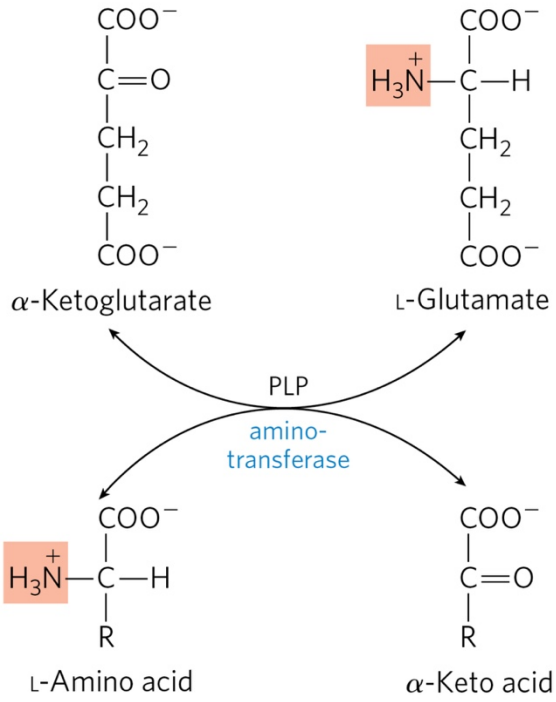
	Glucogenic	Glucogenic and Ketogenic	Ketogenic
Nonessential	Alanine Arginine Asparagine Aspartate Cysteine Glutamate Glutamine Glycine Proline Serine	Tyrosine	
Essential	Histidine Methionine Valine	Threonine Isoleucine Phenyl-alanine Tryptophan	Leucine Lysine



Transamination:

The first step in the catabolism of most L-amino acids. It occurs in most tissues. It is promoted by enzymes called **aminotransferases** or **transaminases**.

The α-amino group is transferred to the α-carbon atom of α-ketoglutarate, leaving behind the corresponding α-keto acid analog of the amino acid.

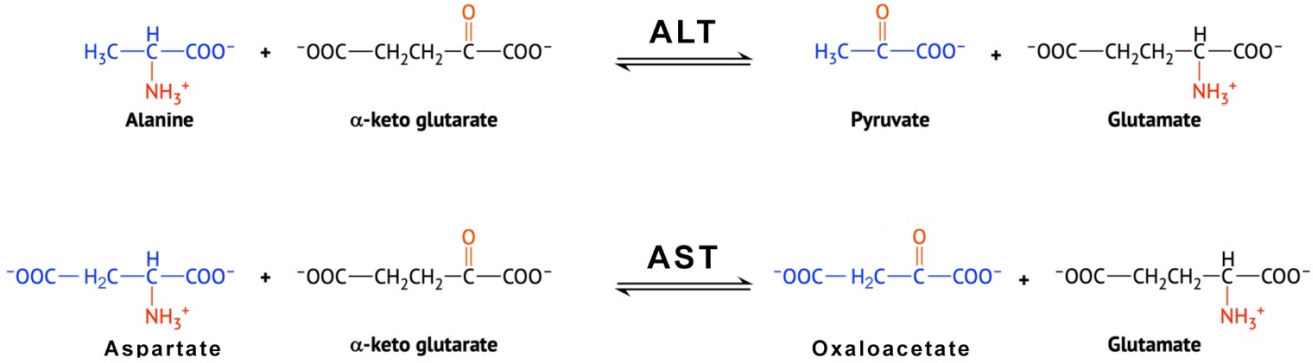


Many are specific for α-ketoglutarate as the amino group acceptor but differ in their specificity for the L-amino acid.

These reactions are freely reversible so they can be used to resynthesize amino acids.

All aminotransferases have **pyridoxal phosphate (PLP)**, the coenzyme form of pyridoxine (vitamin B_6). Pyridoxal phosphate functions as an intermediate carrier of amino groups at the active site of aminotransferases.

The 2 most clinically important aminotransferases are ALT (GPT) and AST (GOT):



All amino acids undergo transamination except lysine, threonine, proline and hydroxyproline which they bypass transamination and undergo direct oxidative deamination.

Oxidative deamination:

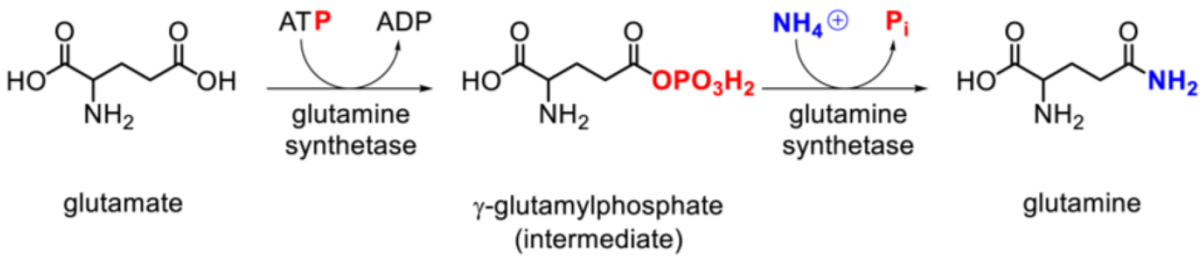
Occurs in hepatocytes. Glutamate is transported from the cytosol into mitochondria, where it undergoes **oxidative deamination** catalyzed by **L-glutamate dehydrogenase** to produce NH_4^+ and α-ketoglutarate.

The combined action of an aminotransferase and glutamate dehydrogenase is referred to as **transdeamination**.

How can glutamate be delivered to the liver for oxidative deamination?

1. Glutamine synthesis: In many tissues, including the brain, some processes such as nucleotide degradation and termination of neurotransmitter signals generate free ammonia.

The free ammonia produced in tissues is combined with glutamate to yield glutamine by the action of **glutamine synthetase**. This reaction requires ATP and occurs in two steps:



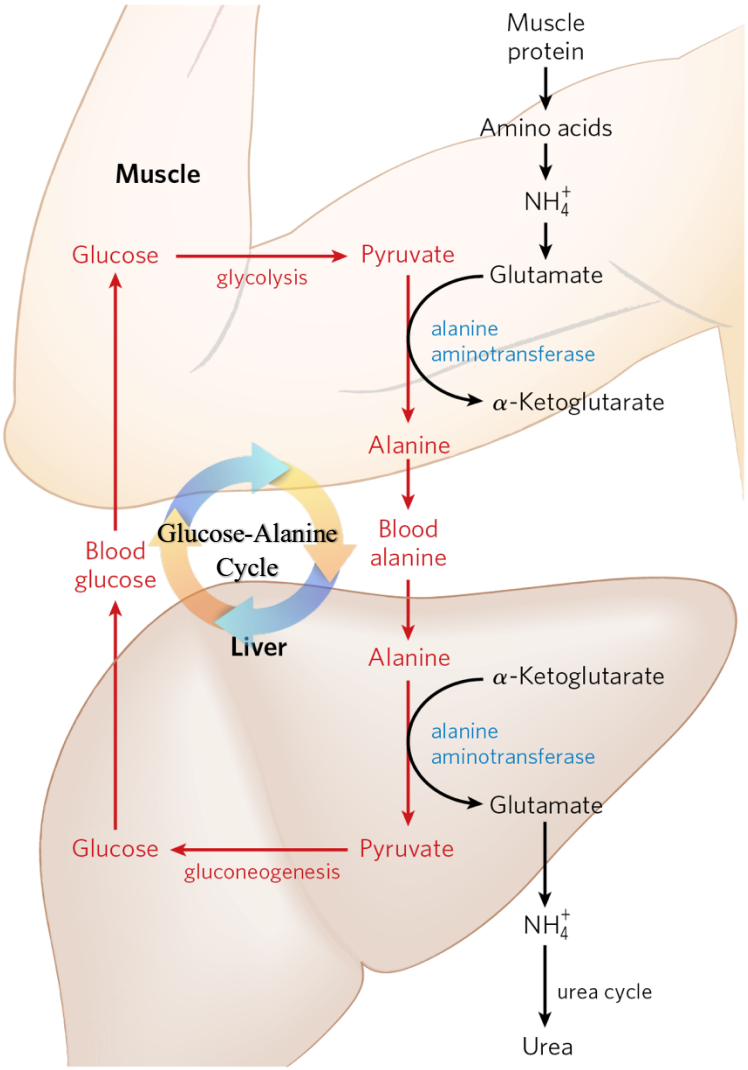
Excess glutamine is transported in the blood to the intestine, liver, and kidneys where the enzyme **glutaminase** converts glutamine to glutamate and NH_4^+. The NH_4^+ from intestine and kidney is transported in the blood to the liver.

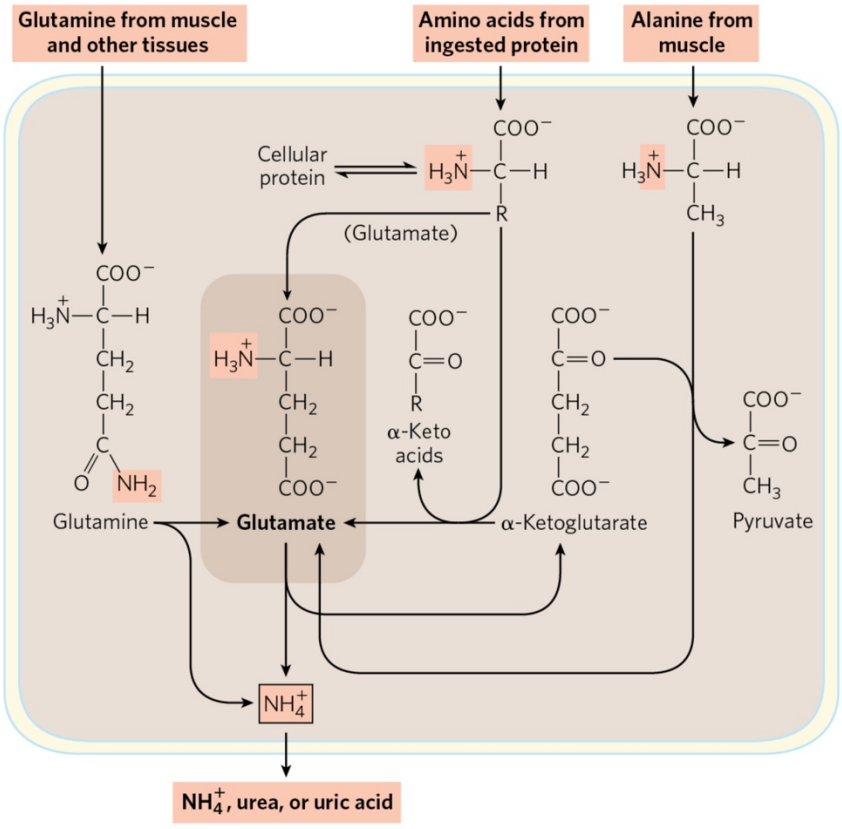
2. Alanine synthesis: Vigorously contracting skeletal muscles operate anaerobically, producing large amounts of pyruvate and lactate from glycolysis as well as ammonia from protein breakdown. These products must find their way to the liver.

Pyruvate is converted to alanine via transamination with glutamate by the action of **alanine aminotransferase**.

Thus, alanine transports amino groups from muscle to the liver in a nontoxic form, ultimately delivering the free ammonia to hepatocyte mitochondria via glutamate in a pathway called the **glucose-alanine cycle**

In the cytosol of hepatocytes, alanine aminotransferase acts in reverse to transfer the amino group from alanine to α-ketoglutarate, forming pyruvate and glutamate. Glutamate can then enter mitochondria, where the glutamate dehydrogenase reaction releases NH_4^+ while pyruvate is used to synthesize glucose, which is returned to the muscles.

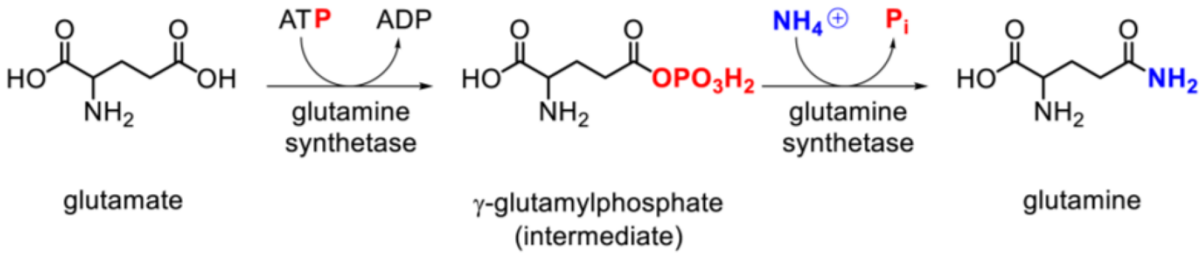

Muscle protein
Amino acids
NH_4^+
Muscle
Glucose
glycolysis
Pyruvate
Glutamate
alanine aminotransferase
α-Ketoglutarate
Alanine
Blood glucose
Glucose-Alanine Cycle
Blood alanine
Liver
Alanine
α-Ketoglutarate
alanine aminotransferase
Glutamate
Glucose
gluconeogenesis
Pyruvate
NH_4^+
urea cycle
Urea


Glutamine from muscle and other tissues
Amino acids from ingested protein
Alanine from muscle
Cellular protein
(Glutamate)
α-Keto acids
Glutamine
Glutamate
α-Ketoglutarate
Pyruvate
NH_4^+
NH_4^+, urea, or uric acid

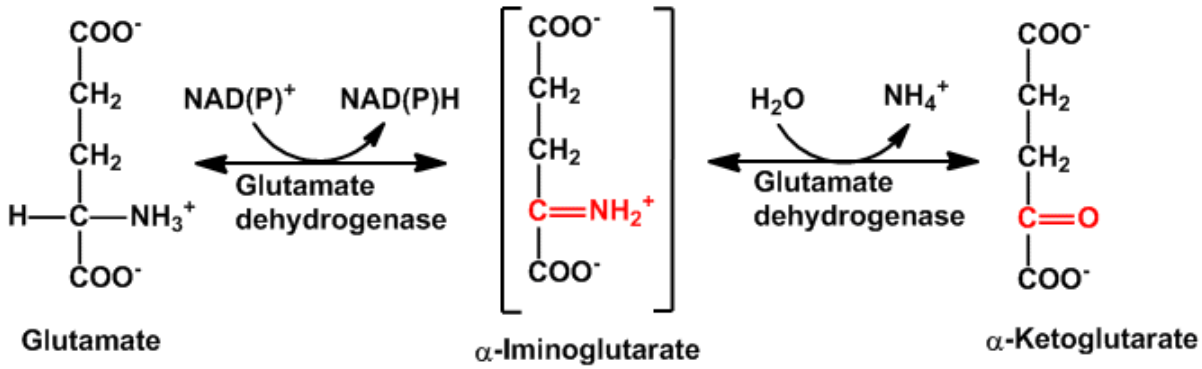
Fate of ammonia

There are 3 mechanisms for disposal of toxic NH_3:

1. Synthesis of glutamine from glutamate by the action of glutamine synthetase.



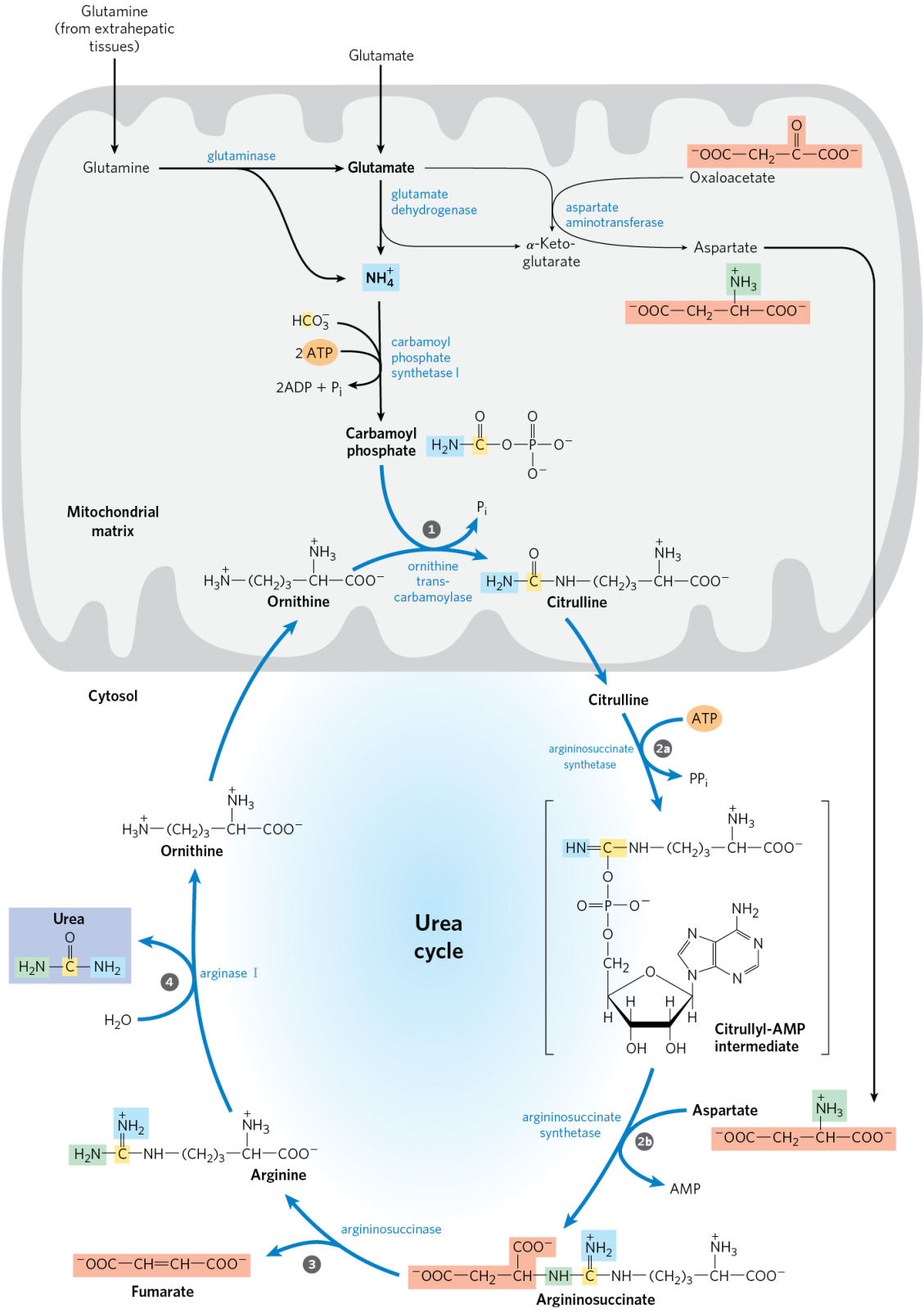
2. The interaction of NH_3 with α-ketoglutarate to form glutamate by the action of glutamate dehydrogenase.



3. Synthesis of carbamoyl phosphate (urea cycle).

Urea cycle

- Ammonia is neurotoxic.
- Urea production occurs almost exclusively in the liver.
- The rate of production of urea is dependent on the rate of protein catabolism from both dietary sources and endogenous protein (largely derived from muscle tissue).
- The urea cycle is irreversible.
- Urea cycle consumes 4 ATP:
    - Two ATP are utilized for the synthesis of carbamoyl phosphate.
    - One ATP is converted to AMP and PPi to produce arginosuccinate which equals to 2 ATP.
- Urea is Produced from Ammonia in Five Enzymatic Steps:
    - The first 2 take place in the mitochondria.
    - The last 3 take place in the cytoplasm.
- Urea cycle occurs exclusively in the liver because the enzyme **Arginase I** is only present in the liver (cytoplasm of hepatocytes) (Arginase II is located in the mitochondrial matrix, and is involved primarily in the production of ornithine as a precursor of proline and glutamate).
- The rate limiting step in the synthesis of urea is the reaction that is catalyzed by Carbamoyl Phosphate Synthetase I (Carbamoyl Phosphate Synthetase II is exclusively cytosolic and is an important enzyme in de novo synthesis of pyrimidine nucleotides).


Glutamine (from extrahepatic tissues)
Glutamate
Glutamine
glutaminase
Glutamate
Oxaloacetate
glutamate dehydrogenase
aspartate aminotransferase
α-Keto-glutarate
Aspartate
NH4+
HCO3−
2 ATP
2ADP + Pi
carbamoyl phosphate synthetase I
Carbamoyl phosphate
Mitochondrial matrix
Pi
1
Ornithine
ornithine trans-carbamoylase
Citrulline
Cytosol
Citrulline
ATP
argininosuccinate synthetase
2a
PPi
Ornithine
Urea cycle
Urea
arginase I
4
H2O
Citrullyl-AMP intermediate
Aspartate
argininosuccinate synthetase
2b
AMP
Arginine
argininosuccinase
3
Fumarate
Argininosuccinate

Biosynthesis of non-essential amino acids:

A. Synthesis from α-keto acids:

Alanine, from pyruvate

Aspartate, from oxaloacetate

Glutamate, from α-ketoglutarate

B. Synthesis by amidation:

Glutamine, from glutamate by glutamine synthetase

Asparagine, from aspartate by asparagine synthetase

C. Proline: from glutamate by cyclization and reduction reactions

D. Serine, glycine, and cysteine:

Serine, from 3-phosphoglycerate

Glycine, from serine

Cysteine, from combining homocysteine with serine

E. From essential amino acids:

Cysteine, from methionine

Tyrosine, from phenylalanine by the action of phenylalanine hydroxylase. The reaction requires molecular oxygen and the coenzyme tetrahydrobiopterin.

Note: Tyrosine is nonessential only in the presence of adequate dietary phenylalanine.

Conversion of amino acids to specialized products

1. Porphyrin:

glycine is a major precursor

Glycine + Succinyl CoA ⟶ δ-Aminolevulinic acid → → → Heme

2. Creatine:

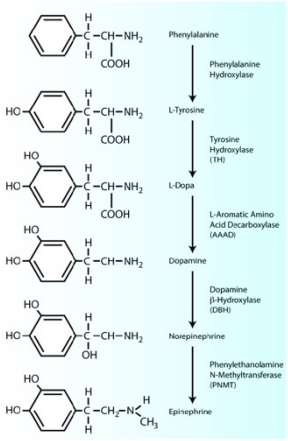
Creatine phosphate, the phosphorylated derivative of creatine found in muscle, is a high energy compound that provides a small but rapidly mobilized reserve of high-energy phosphates that can be reversibly transferred to ADP to maintain the intracellular level of ATP during the first few minutes of intense muscular contraction.

Creatine is synthesized in the liver and kidneys from glycine, arginine and methionine (S-adenosylmethionine). Creatine is reversibly phosphorylated to creatine phosphate by creatine kinase using ATP.

Creatine and creatine phosphate spontaneously cyclize at a slow but constant rate to form creatinine, which is excreted in the urine.

3. Glutathione (GSH): It is derived from glutamate, cysteine, and glycine.

4. Neurotransmitters



A. Dopamine, norepinephrine, and epinephrine are biologically active amines that are collectively called catecholamines. They are synthesized from Tyrosine.

B. Histamine: is formed by decarboxylation of histidine

C. Serotonin: is synthesized from tryptophan.

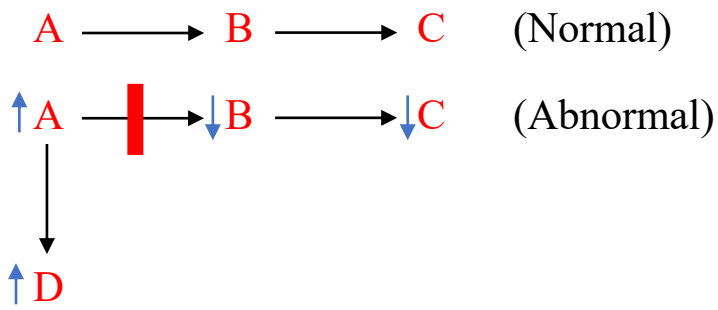
5. Melanin:

It is a pigment that is found in several tissues, particularly the eye, hair, and skin. It is synthesized from tyrosine.

Inborn error of metabolism

A heterogeneous group of inherited disorders. These diseases involve failure of the metabolic pathways involved in either the break-down or storage of carbohydrates, fatty acids, and proteins.

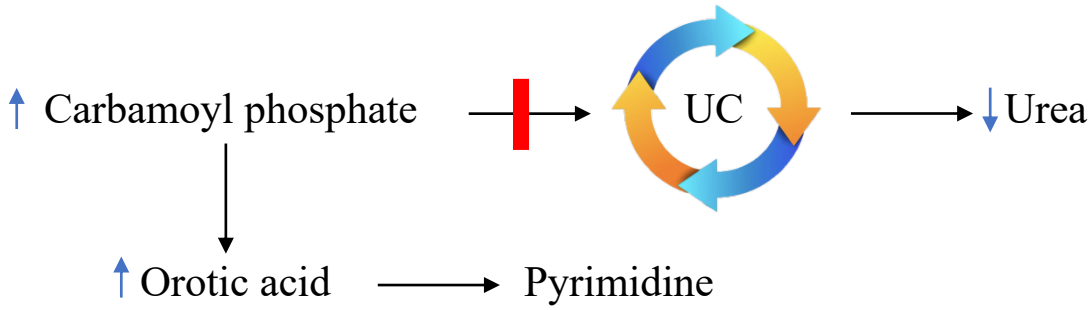
Inherited inborn disorders may involve any peptide or protein, and are usually most obvious if there is an enzyme abnormality.



1. Urea cycle disorders:

Urea cycle defects are important causes of hyperammonaemia and low plasma urea concentration.

There may also be raised urinary orotic acid concentration, an intermediate metabolite of pyrimidine synthesis derived from carbamoyl phosphate.



Ornithine transcarbamoylase deficiency is probably the most common urea cycle defect.

Carbamoyl phosphate synthetase deficiency is a urea cycle disorder in which urinary orotic acid is not raised.

2. Cystinuria:

It is an autosomal recessive inherited abnormality due to defect in the tubular reabsorption of the dibasic amino acids cystine, ornithine, arginine and lysine. This leads to excessive urinary excretion of these amino acids.

Deficiencies of these amino acids do not occur because they can be synthesized in the body.

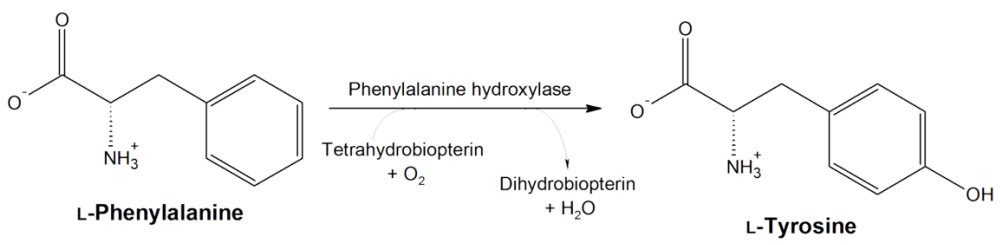
Cystine is relatively insoluble and because of the high urinary concentrations may it may precipitate and form calculi.

3. Homocystinuria:

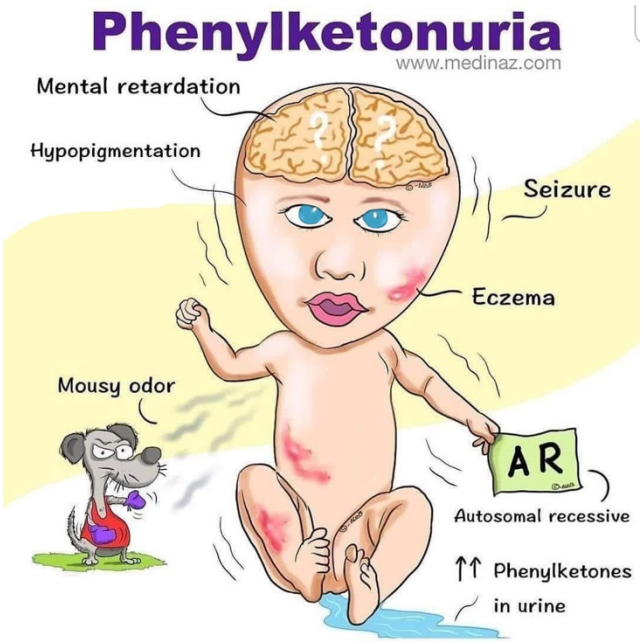
The homocystinurias are a group of disorders involving defects in the metabolism of homocysteine. They are characterized by high plasma levels of homocysteine and methionine, and low plasma levels of cysteine. (Why?)

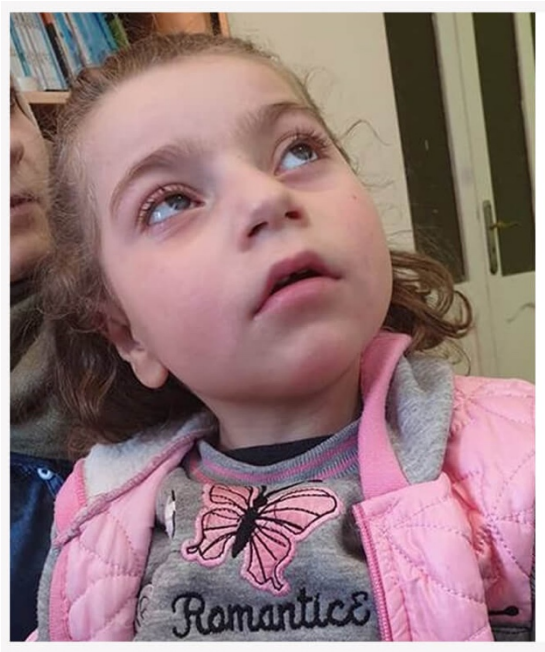
4. Phenylketonuria:

Phenylketonuria is an autosomal recessive disorder caused by deficiency of phenylalanine hydroxylase (97%) or enzymes responsible for the synthesis of the cofactor tetrahydrobiopterin (3%).



Phenylalanine cannot be converted to tyrosine, and accumulates in plasma and is excreted in the urine with its metabolites (phenylpyruvate and phenyl lactate).





Many patients are pale skinned, fair haired and blue eyed. (WHY?)

Diagnosis may involve measuring the phenylalanine concentration in blood taken from a heel prick.

5. Albinism:

It is an autosomal recessive disorder.

It is caused by deficiency of tyrosinase in melanocytes which catalyzes the first two steps in the synthesis of melanin.

Pigmentation of the skin, hair and iris is reduced and the eyes may appear pink. Reduced pigmentation of the iris affect vision and causes photophobia. There is increase in risk of skin cancer.

6. Alkaptonuria:

Alkaptonuria is an autosomal recessive disorder associated with a deficiency of homogentisic acid oxidase.

Homogentisic acid accumulates in tissues and blood, and is passed in the urine. Oxidation and polymerization of homogentisic acid produce the pigment alkapton. The deposition of alkapton in cartilages results in visible darkening of the cartilages of the ears.

Diagnosis: darkening of the urine on standing (over period of time) as urine becomes more alkaline on standing. The conversion of homogentisic acid to alkapton is accelerated in alkaline conditions.

7. Maple syrup urine disease:

It is inherited as an autosomal recessive condition.

There is deficient decarboxylation of the α-ketoacids resulting from deamination of the branched-chain amino acids (leucine, isoleucine and valine.)

These amino acids accumulate in the plasma and are excreted in the urine with their corresponding α-ketoacids. The sweet smell of the urine is like that of maple syrup

Diagnosis: raised concentrations of branched-chain amino acids in plasma and urine and low plasma alanine concentration. (Why?)

8. Histidinaemia:

Histidinaemia is associated with deficiency of histidinase.

9. Hartnup's disease:

There are reduced intestinal absorption and increased urinary loss of tryptophan. This amino acid is normally partly converted to nicotinamide, the conversion being especially important if the dietary intake of nicotinamide is low.

The clinical features of Hartnup's disease are intermittent and resemble those of pellagra (DDD).

Case 1:

A 2-year-old male was referred to the metabolism service after his mother noticed dark-colored staining on his crib sheets on several occasions. She also noted that the child's urine was normal when he urinated, but turned a dark color over a period of time.

What is the most appropriate diagnosis?

Case 2:

A 3-month-old boy was seen in the paediatric out- patient department because of failure to thrive and hypotonia. The family had previously lost a male child, who had died at the age of 9 months. Some of the presenting child's abnormal biochemistry results were as follows:

Investigation: Plasma

Sodium 142 mmol/L (135–145)

Potassium 3.8 mmol/L (3.5–5.0)

Urea 0.5 mmol/L (2.5–7.5)

Creatinine 44 μmol/L (40–80)

Ammonia 654 μmol/L (< 20)

Plasma amino acid analysis revealed elevated alanine, glutamine and orotic acid concentrations.

What is the most appropriate diagnosis?