Tishk International University
Faculty of Science
Department of Medical Analysis

ADVANCED CLNICAL BIOCHEMISTRY

Metabolic disorders of proteins and amino acids

Grade 4-Spring 2020-2021

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1-**Protein-calorie malnutrition**

Inadequate intake of protein and/or energy may be observed. Affected individuals show a variety of symptoms, including a depressed immune system with reduced ability to resist infection. Death from secondary infection is common.

There are two extreme forms of malnutrition are;

Kwashiorkor and marasmus

A. Kwshiorkor:

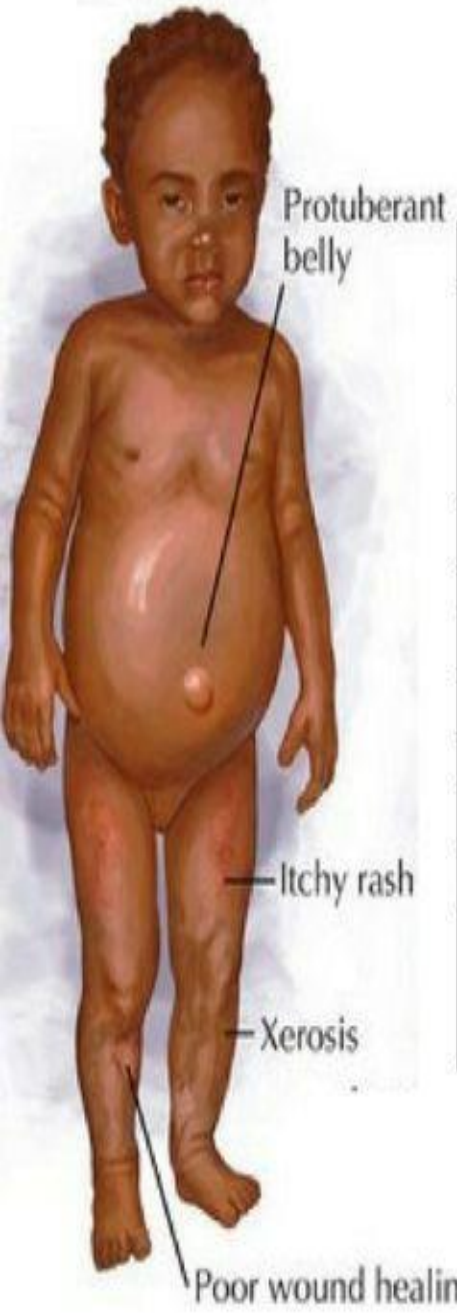
Occurs when there is deficiency of protein only, is frequently seen in children after **weaning** at about one **year of age**, when their diet consists predominantly of carbohydrates. Typical symptoms include **stunted growth**, **edema**, **skin lesions**, **depigmented hair**, **anorexia**, **enlarged fatty liver**, and decreased plasma **albumin** concentration.

B. Marasmus:

Occurs when there is deficiency of dietary **protein** and **calories**, usually occurs in children younger than one year of age when the mothers breast milk is supplemented with thin watery gruels of native cereals, which are usually deficient in protein and calories. Typical symptoms include **arrested growth**, **extreme muscle wasting**, **weakness, and anemia**. Victims of marasmus do not show the edema or change in plasma proteins observed in kwashiorkor.

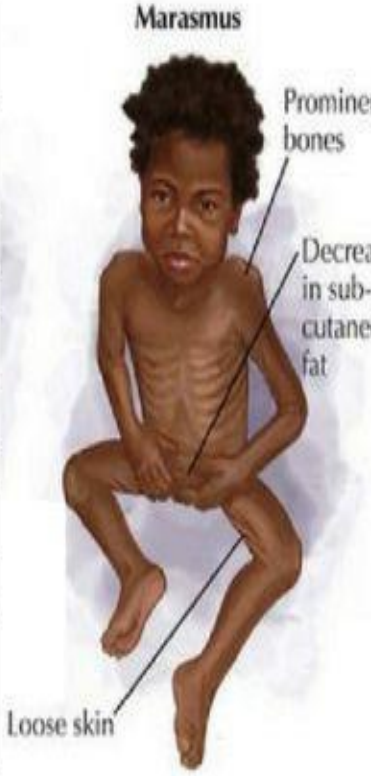
10 Differences between Kwashiorkor and Marasmus

www.majordifferences.com



Comparison Table

Kwashiorkor	Marasmus
It develops in children whose diets are deficient of protein.	It is due to deficiency of proteins and calories.
It occurs in children between 6 months and 3 years of age.	It is common in infants under 1 year of age.
Subcutaneous fat is preserved.	Subcutaneous fat is not preserved.
Oedema is present.	Oedema is absent
Enlarged fatty liver.	No fatty liver.
Ribs are not very prominent.	Ribs become very prominent.
Lethargic	Alert and irritable.
Muscle wasting mild or absent.	Severe muscle wasting
Poor appetite.	Voracious feeder.
The person suffering from Kwashiorkor needs adequate amounts of proteins.	The person suffering from Marasmus needs adequate amount of protein, fats and carbohydrates.



Kwashiorkor vs Marasmus

2-Metabolic disorders of amino acid metabolism

Abnormal metabolism of amino acids results in a number of metabolic disorders which must be early diagnosed and treated otherwise they produce **irreversible brain damage**.

Biochemical investigations include **urinary** and **serum** estimation of amino acids by different methods such as **chromatography**.

The principal disorders are:

1-Glycine

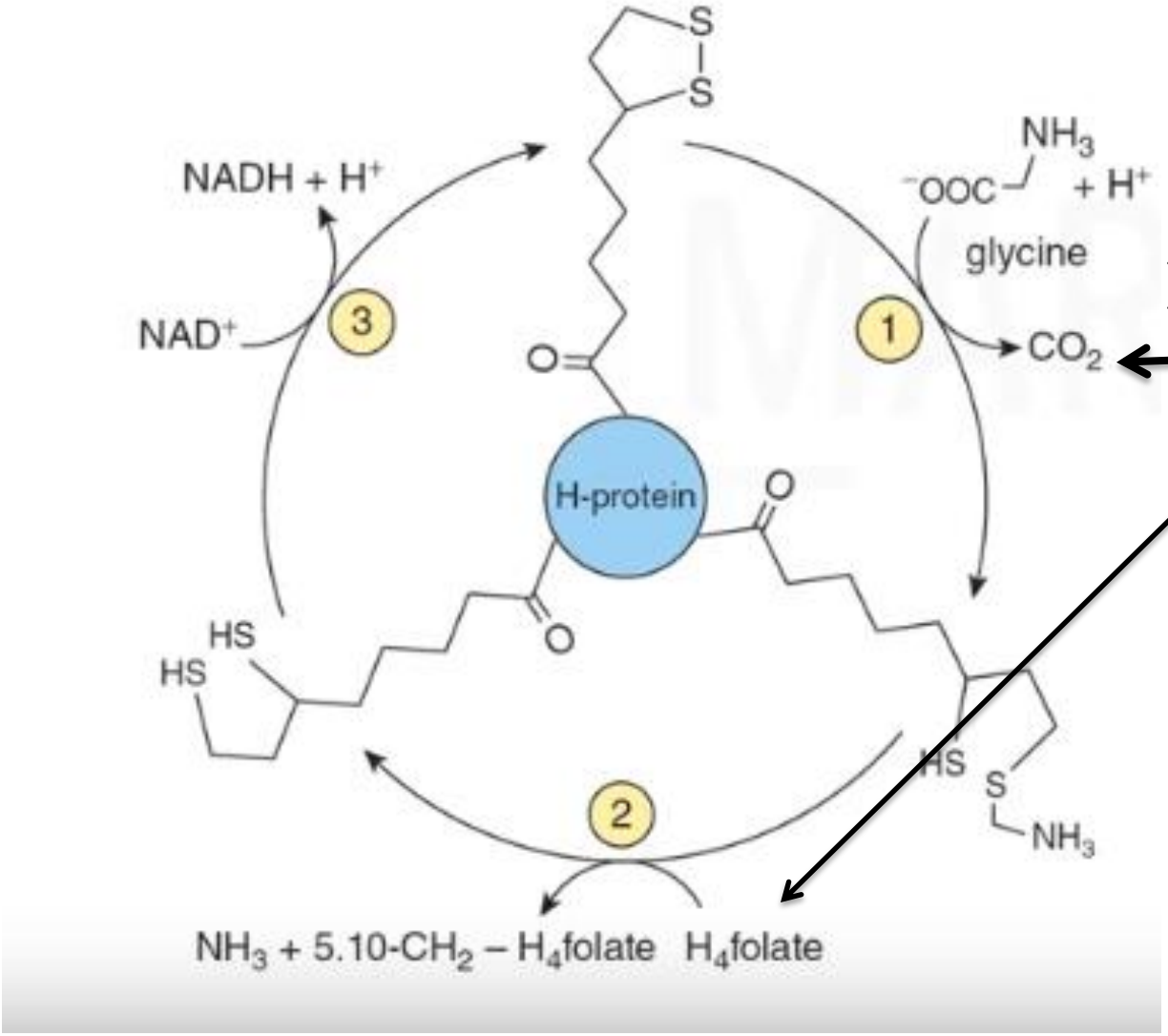
a-Glycinuria :

This is a rare metabolic disorder of glycine metabolism.

Serum glycine is normal, but there is an excessive urinary excretion of glycine which is due to a defect in **tubular transport and reabsorption of glycine**.

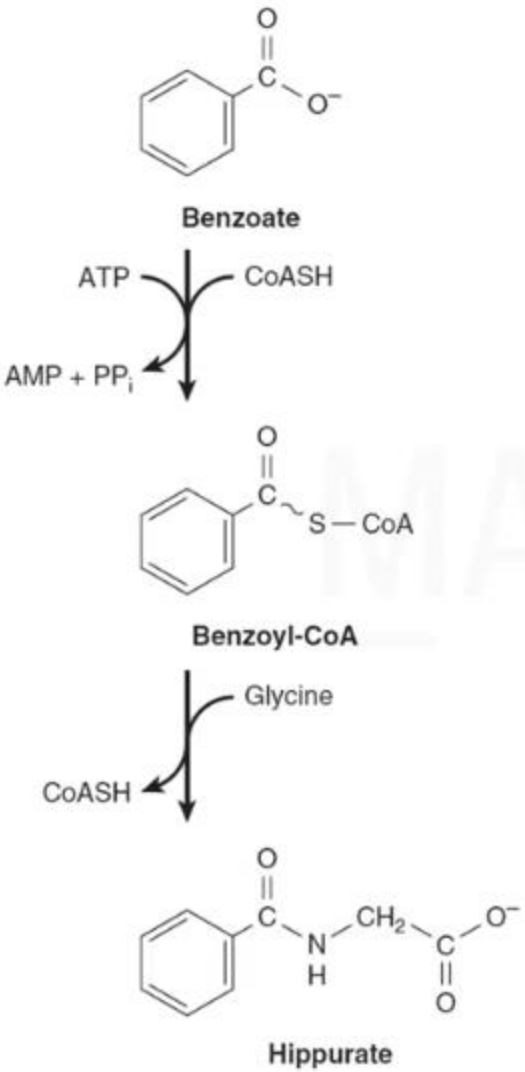
Hyperglycinemia

- ✓ non-ketotic hyperglycinemia
- ✓ Glycine is a neurotransmitter, excitatory in central nervous system and inhibitory in peripheral nervous system.
- ✓ Excess glycine results in mental retardation and other neuronal symptoms.



NH2-CH2-COOH

If there is a defect in this complex, glycine will accumulate and lead to non-ketotic hyperglycinemia.

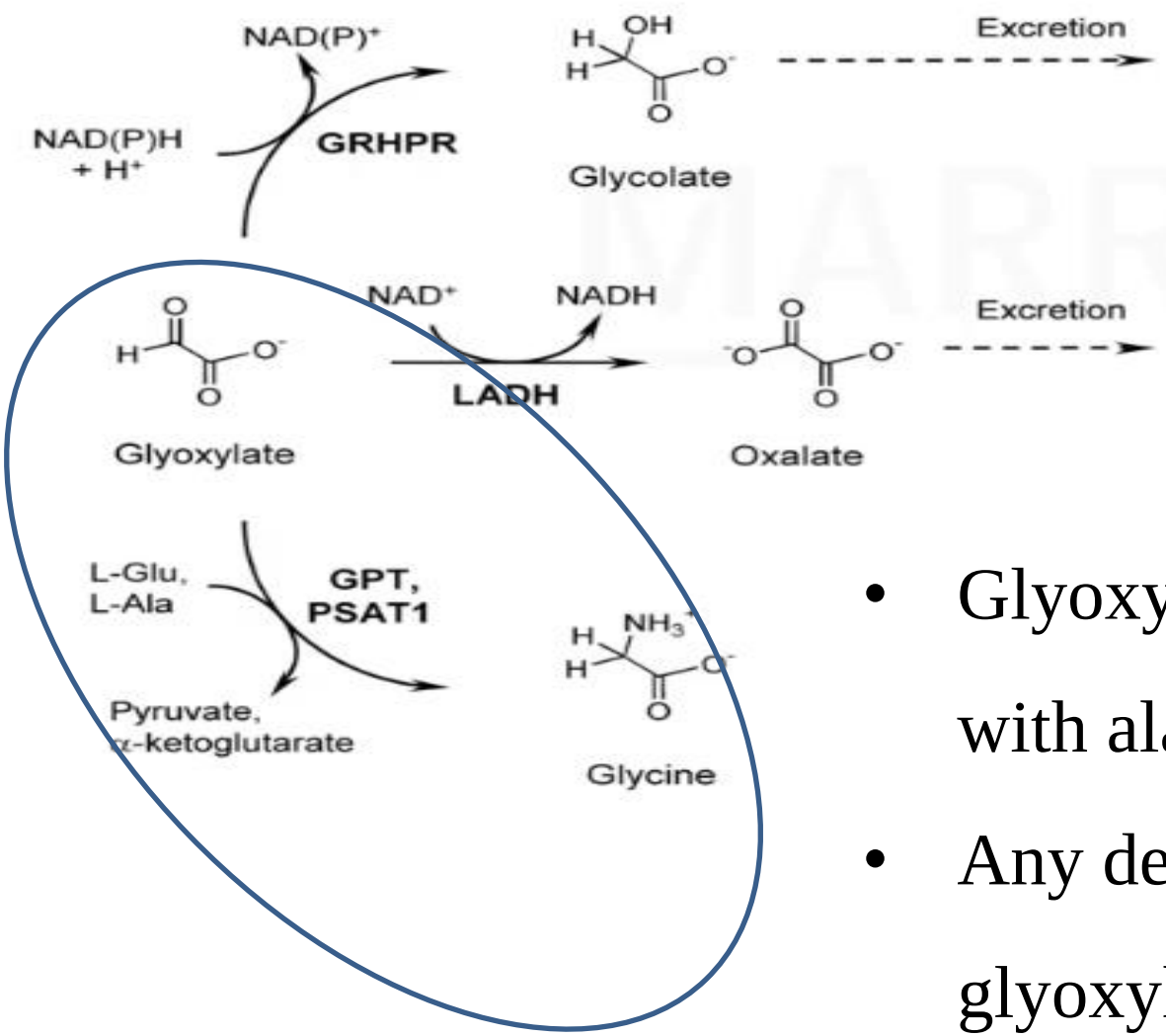


Sodium benzoate

Used in treatment

b-Primary hyperoxaluria:

Another metabolic disorder of glycine metabolism, characterized chemically by a continuous high urinary excretion of oxalate which is unrelated to the dietary intake of oxalate (exogenous), but its of endogenous origin and comes from glycine after its deamination and conversion into glyoxylate which oxidizes to oxalate.



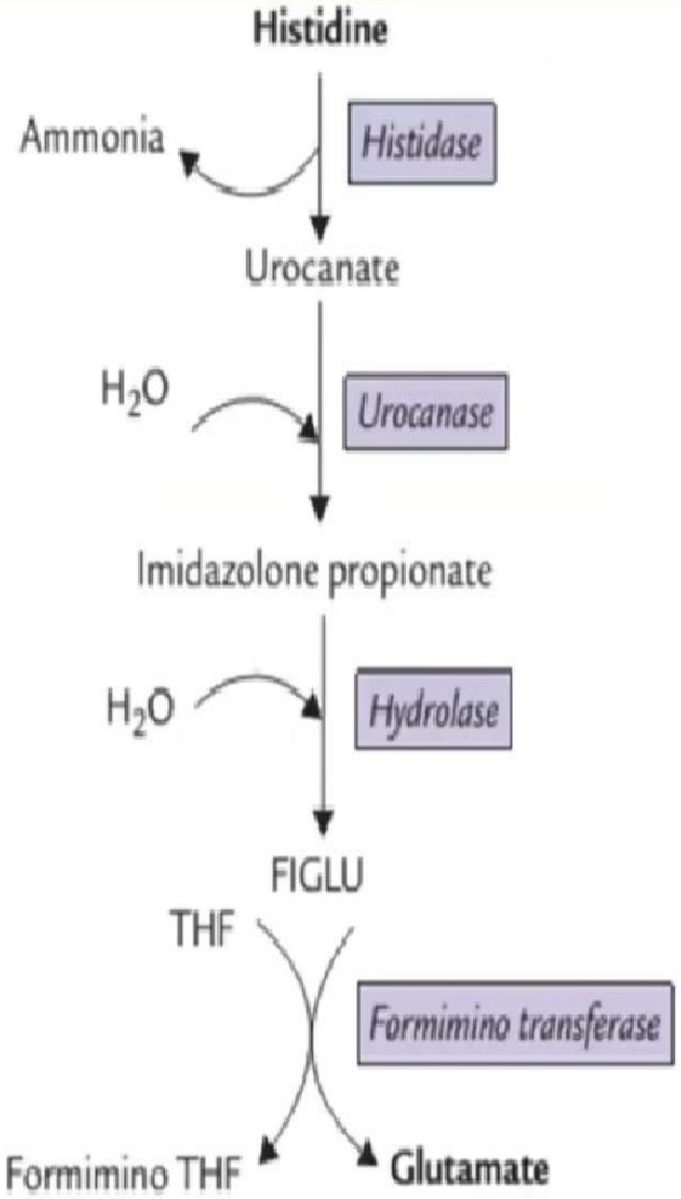
- Glyoxylate transamination with alanine produces glycine.
- Any defect in alanin glyoxylate aminotransferase there will be primary hyperoxaluria.

2-Histidine

Histidinemia:

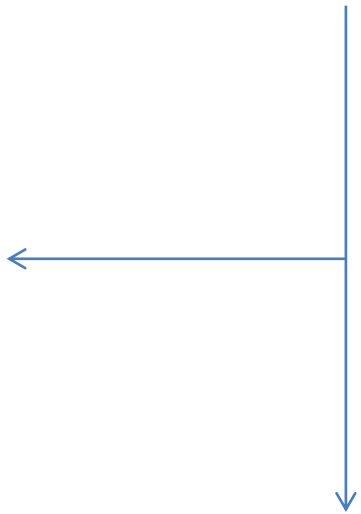
An inherited metabolic disorder of histidine metabolism due to the deficiency of histidase and characterized by elevated serum and urine levels of histidine and clinically there is retardation of speech .

HISTIDINE METABOLISM



→ Alpha keto glutarate → TCA

Histadine



CO2
Decarboxylation
B6

Histamine

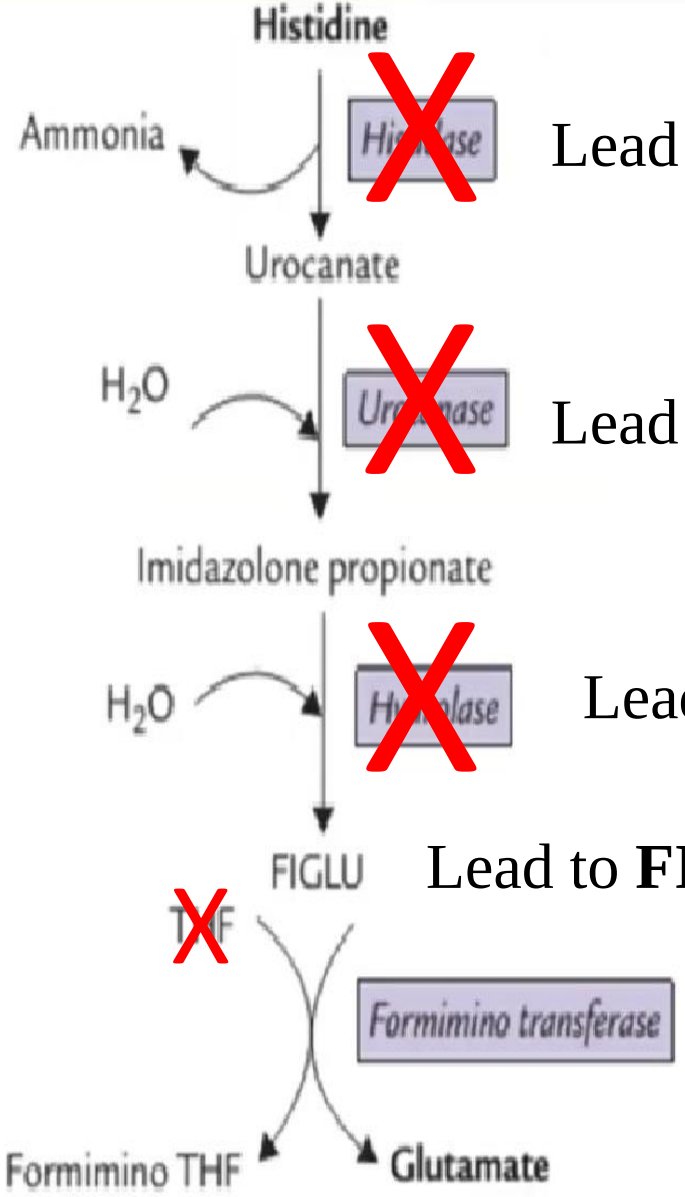
Histamine produced by platelet, mast cell, and basophil Action:

- Vasodilator effect
- Decrease BP
- Increase the vascular permeability

Histamine

- Has receptor on smooth muscle called H1, cause dilatation
- Has receptor on gastric gland called H2, secrete HCL
- Has receptor on brain called H3, histamine release.

HISTIDINE METABOLISM



Lead to **Histidinemia** → Mental retardation

Lead to **urocanic aciduria** → Urocanic acid and histadine excrete in the urine

Lead to **Imidazole aciduria** → Cause blindness

Lead to **FIGLU excretion in urine**

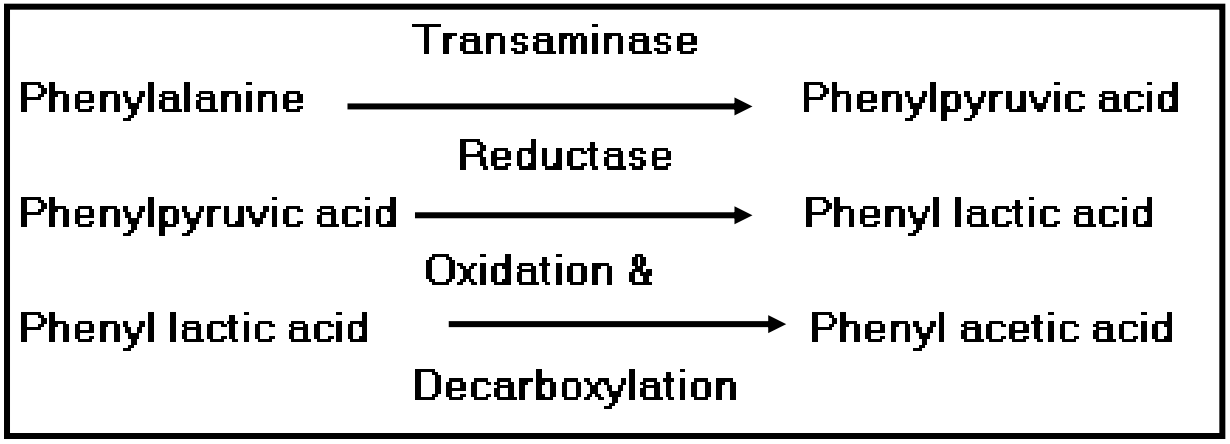
FIGLU excretion test

- 5gm histadine given 3 times at 4 hourly intervals
- Urine collected for 24 h
- Normally FIGLU excretion is less than 30mg/day
- Increased value suggestive for FA deficiency.

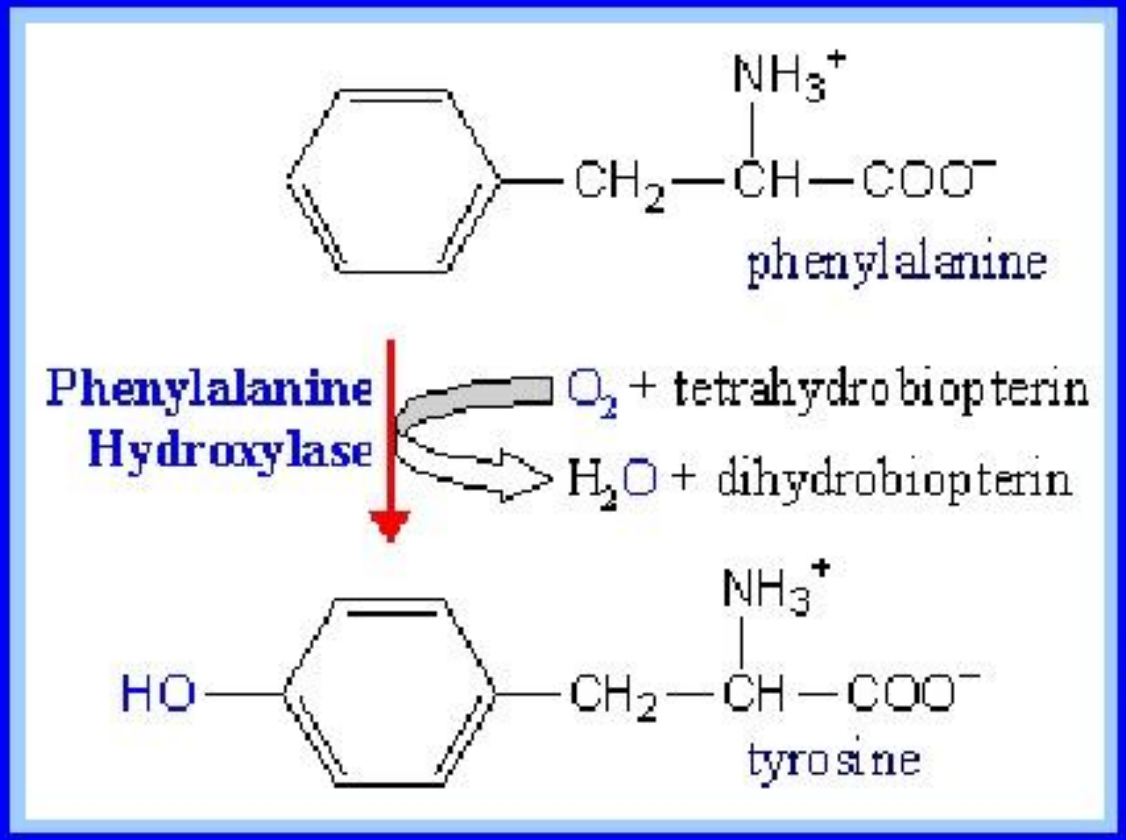
3-Phenylalanine

Phenylketonuria (PKU) :

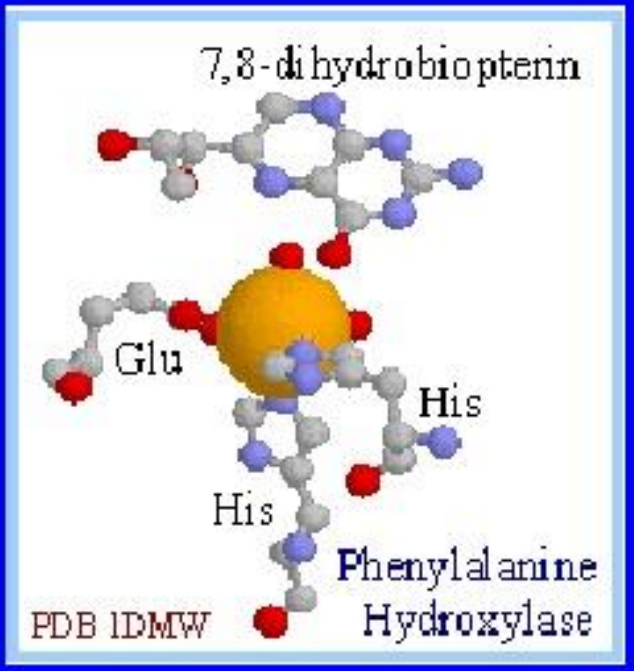
An inherited metabolic disorder of phenyalanine metabolism. Its due to the deficiency of the enzyme phenylalanine mono-oxygenase (phenylalanine hydroxylase), so phenylalanine can not converted into tyrosine and its metabolism takes an alternative pathway as follows:

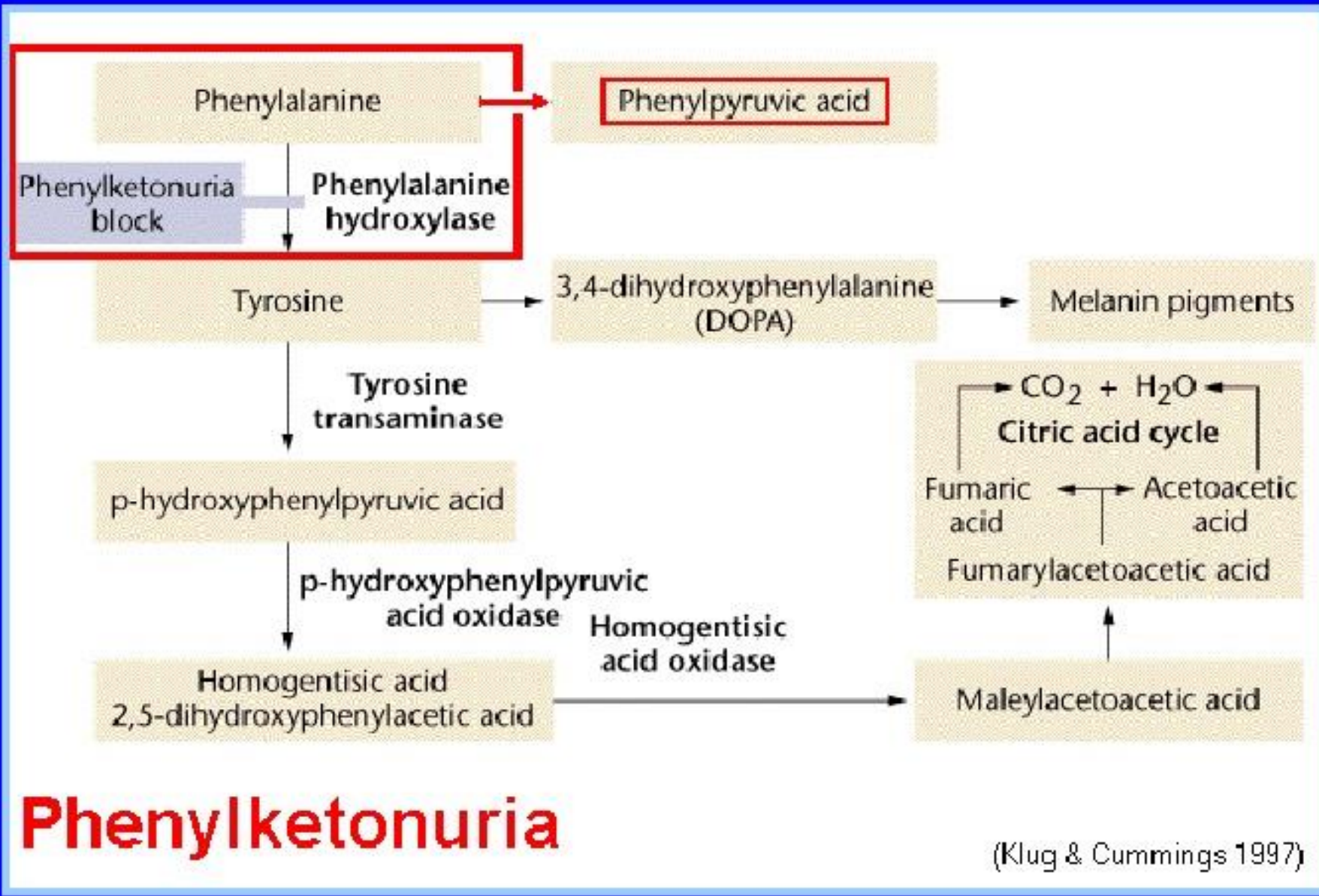


So there is urinary excretion of phenyl pyruvic, phenyl lactic and phenyl acetic acids and clinically there are mental retardation, vomiting, convulsion and special odor of urine (odor of rats).



Phenylketonuria





Phenylketonuria

(Klug & Cummings 1997)

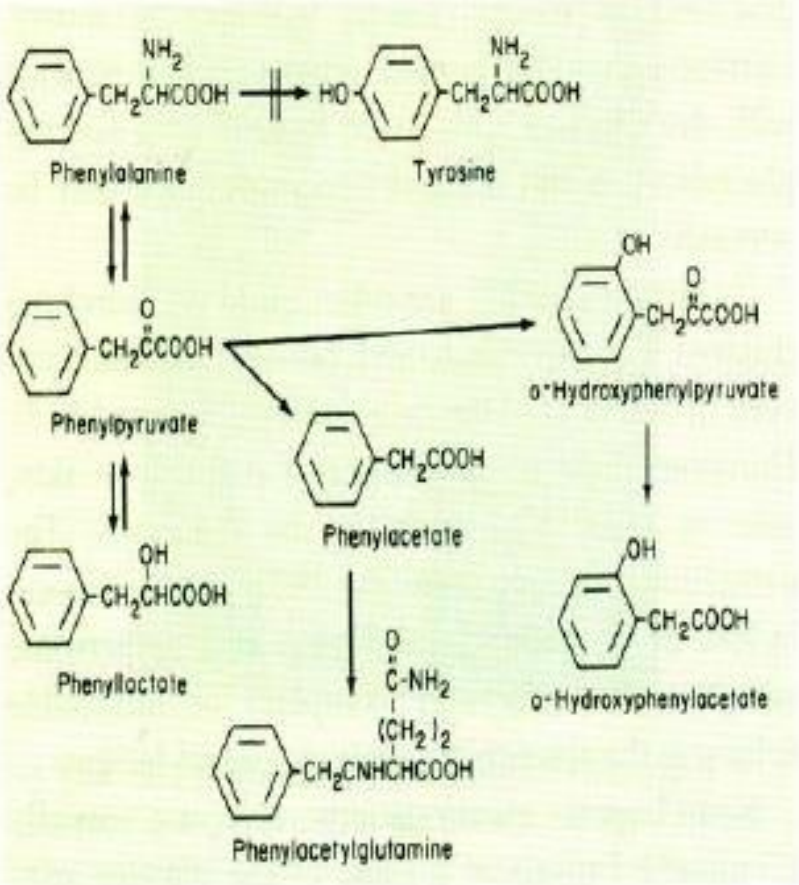


Fig. 19.1 Metabolism of phenylalanine. The site of the defect in PKU is in phenylalanine hydroxylase. The compounds which accumulate as a consequence of the block are shown below.

Fig. 19.2 A positive ferric chloride test in a patient with untreated PKU.

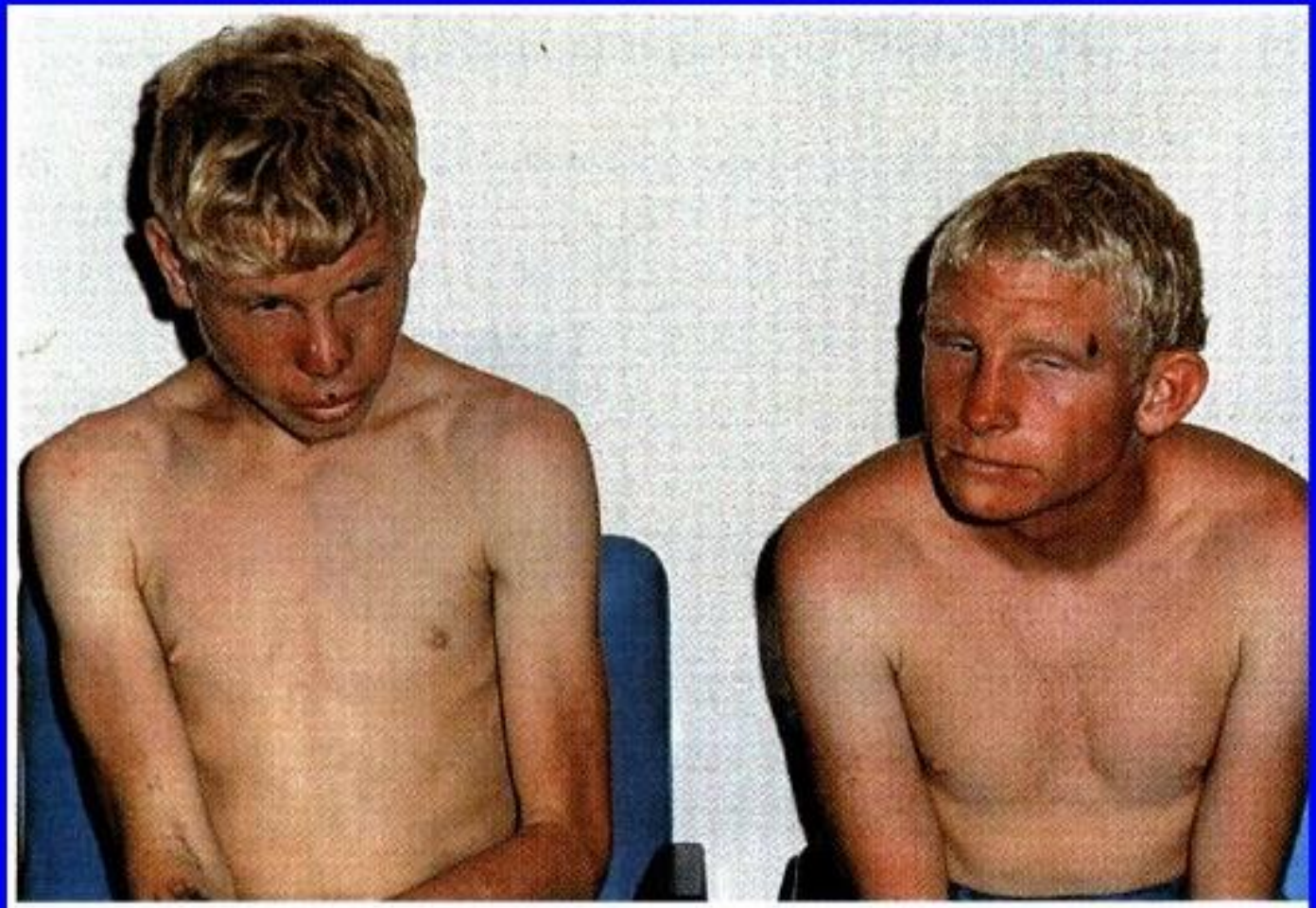


Fig. 19.5 B.A. and L.A. Severely retarded, institutionalized brothers with untreated PKU. They were quite fair of hair and skin.

4-Proline

Hyperprolinemia:

Characterized by increased serum proline, caused by defective enzyme proline oxidase.

5-Tyrosine

a-Tyrosinosis:

Metabolic disorder of tyrosine metabolism due to the deficiency of tyrosine transaminase, characterized by excessive urinary excretion of tyrosine.

b-Tyrosinemia (Richner –Hanhard syndrome):

Another metabolic disorder of tyrosine metabolism, there is an increase in the serum level of tyrosine, however the exact nature of the enzyme responsible of this disorder is yet not known.

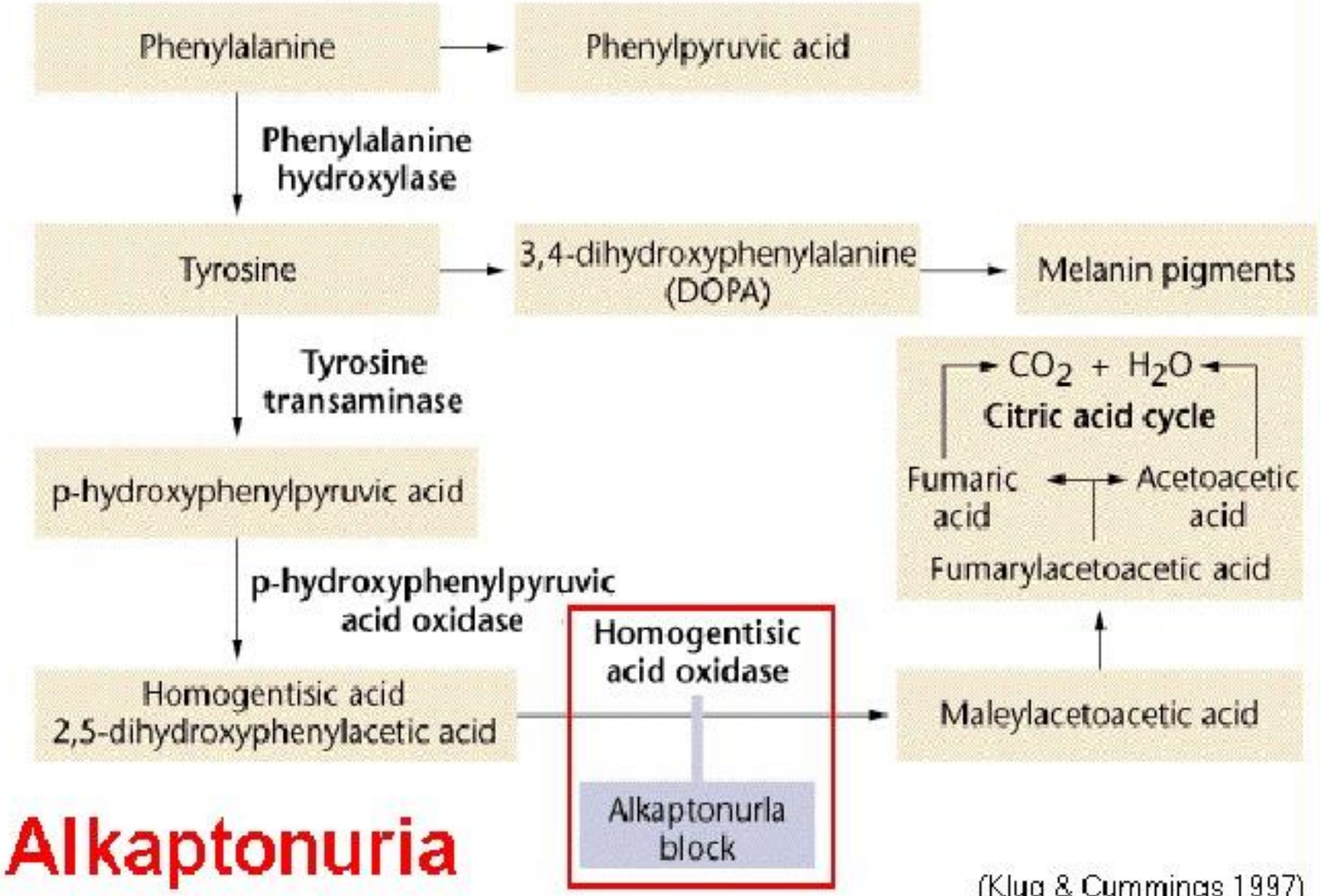
The clinical symptoms are:

Hepatosplenomegally, liver cirrhosis, rickets, hyperphosphaturia and aminoaciduria .

c-Alkaptonuria:

Inherited metabolic disorder of tyrosine metabolism due to the deficiency of homogentisate oxidase a specific enzyme acts on homogentisic acid an intermediate compound formed during metabolism of tyrosine . Characterized by an excess urinary excretion of homogentisic acid, there are also darkening of urine when left in air, due to the oxidation of homogentisic acid into brownish –black pigment.

In addition there is pigmentation of connective tissues, termed as Orchronosis-associated with characteristic arthritic changes that affect the large joints and spine in later half.



Alkaptonuria

(Klug & Cummings 1997)

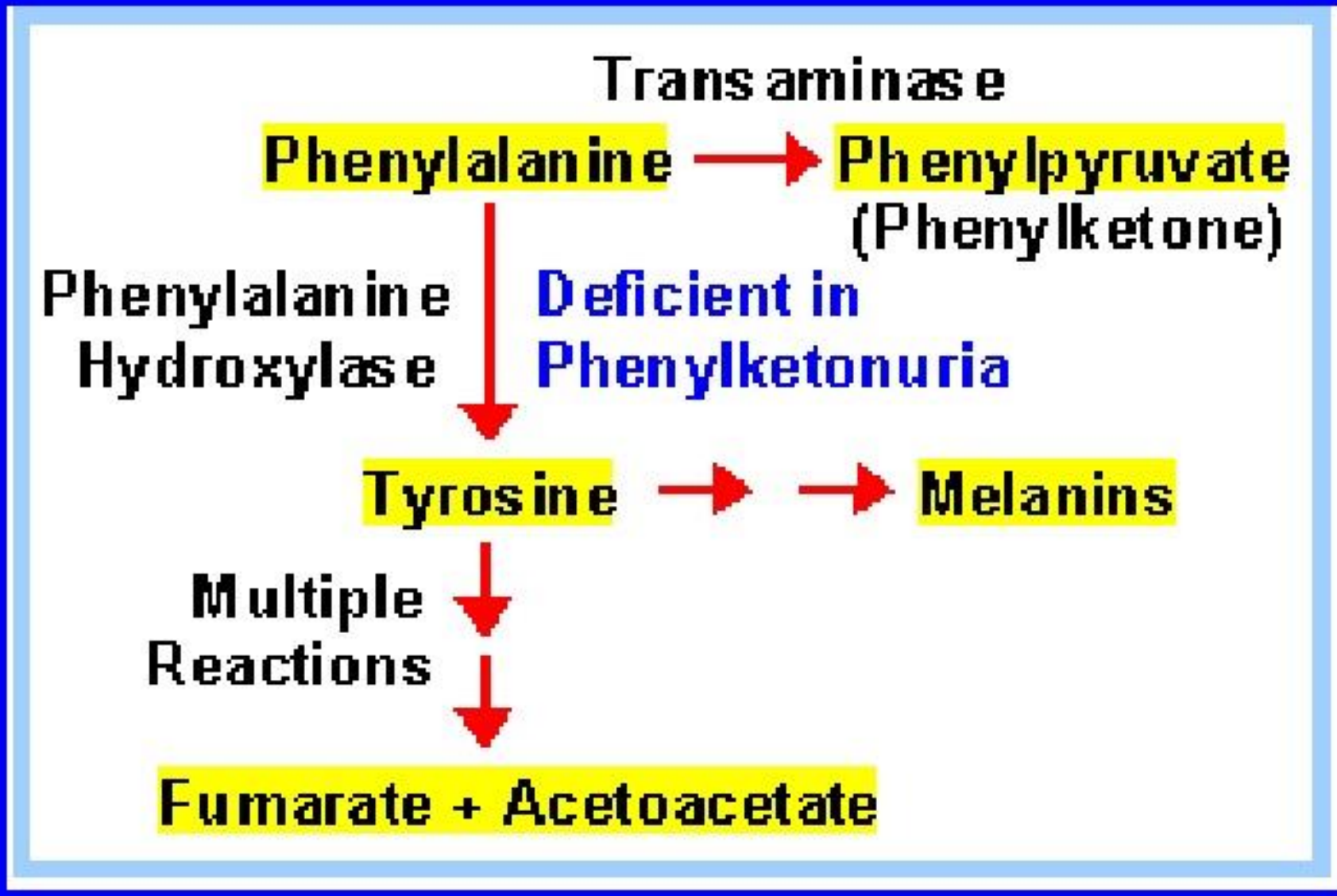
d-Albinism:

Another metabolic disorder of tyrosine metabolism due to the deficiency of melanocyte tyrosine hydroxylase resulting in hypomelanosis.

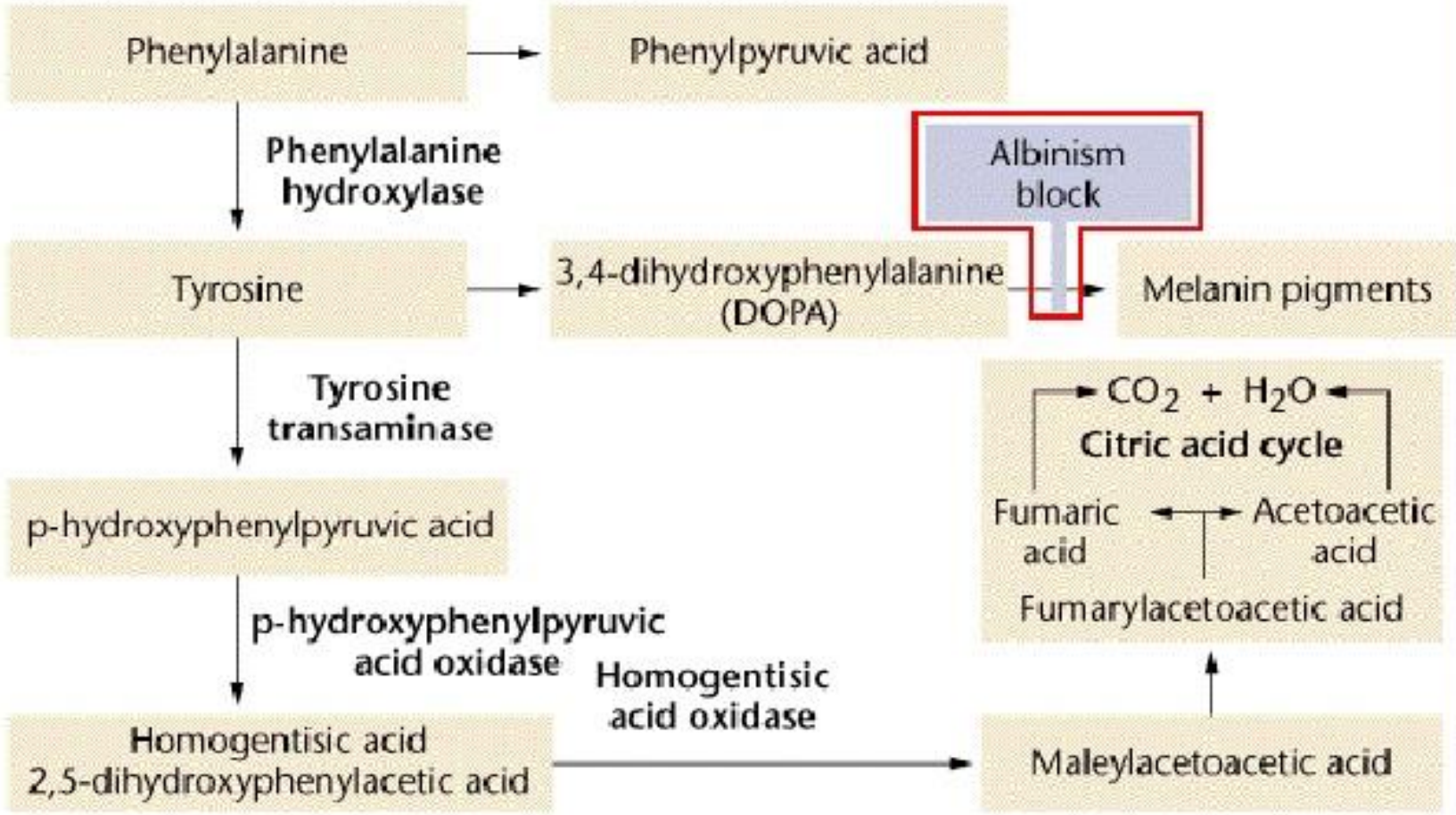
There are two principal types of albinism:

1-Oculocutaneous albinism (OCA) : melanin pigment (photoprotective pigment) is missing in the skin ,hair and eyes .

2-Ocular albinism (OA) : melanin pigment is missing mainly in the eyes .


Transaminase
Phenylalanine
Phenylpyruvate
(Phenylketone)
Phenylalanine
Hydroxylase
Deficient in
Phenylketonuria
Tyrosine
Melanins
Multiple
Reactions
Fumarate + Acetoacetate

Albinism



Albinism

(Klug & Cummings 1997)

6-Branched chain amino acids (valine ,leucine and iso-leucine):

Maple syrup urine disease (branched chain ketonuria):

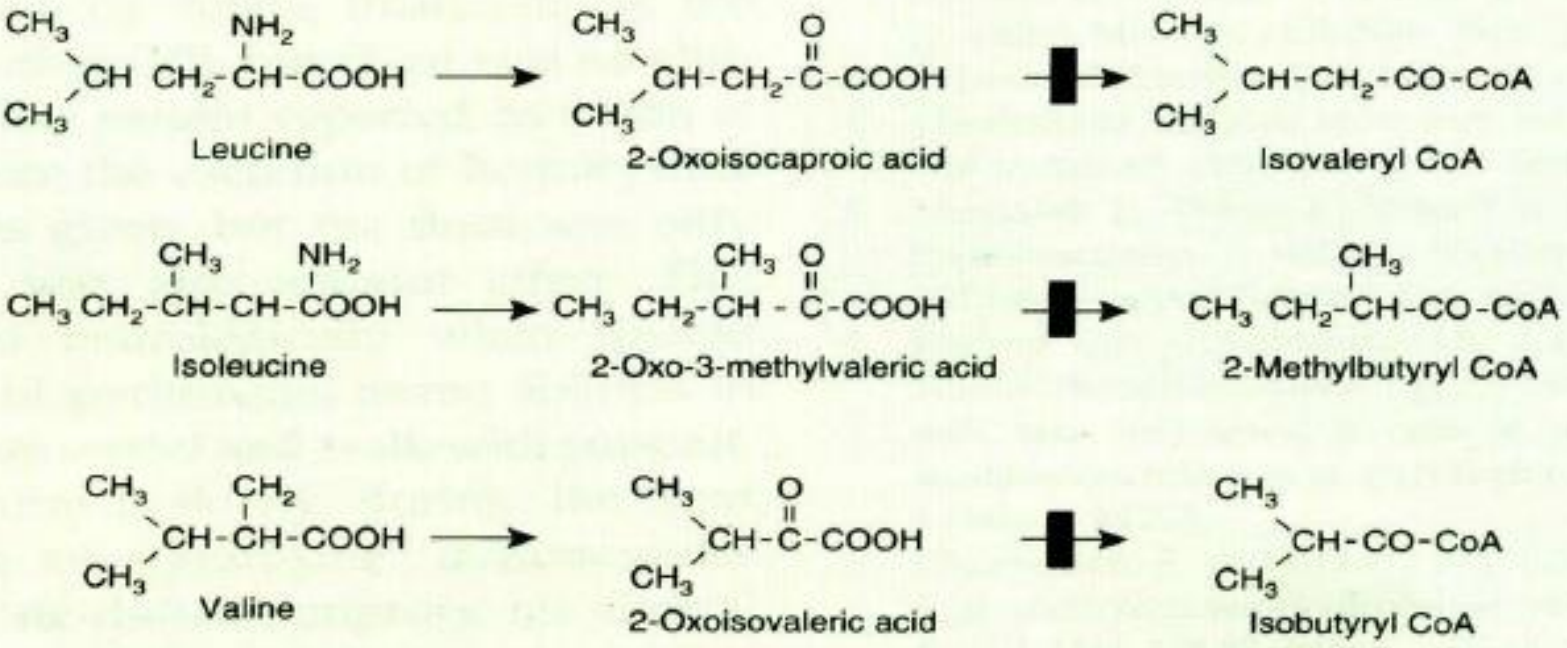
An inherited metabolic disorder in which the odor of urine resembles that of maple syrup or burnt sugar.

Characterized by elevated serum levels of valine, leucine, iso-leucine and their keto acids, its due to the deficiency of the enzyme alpha keto acid decarboxylase which converts these amino acids into CO2 and acylcoA.

Maple syrup urine disease

MA

Overwhelming illness in the first days of life with lethargy progressive to coma, opisthotonus, and convulsions; recurrent episodes leading to developmental delay; characteristic maple syrup odor, branched-chain aminoacidemia and aminoaciduria; branched-chain oxoaciduria; deficiency of branched-chain ketoacid dehydrogenase



7-Tryptophan:

Hartnups disease:

Inherited metabolic disorder of tryptophan metabolism due to the deficiency of tryptophan di-oxygenase. Characterized by excessive urinary excretion of tryptophan, mental retardation and pellagra-like skin conditions.

The treatment supplementing the diet with additional amount of niacin, to alleviate the dermatological and neurological lesions.

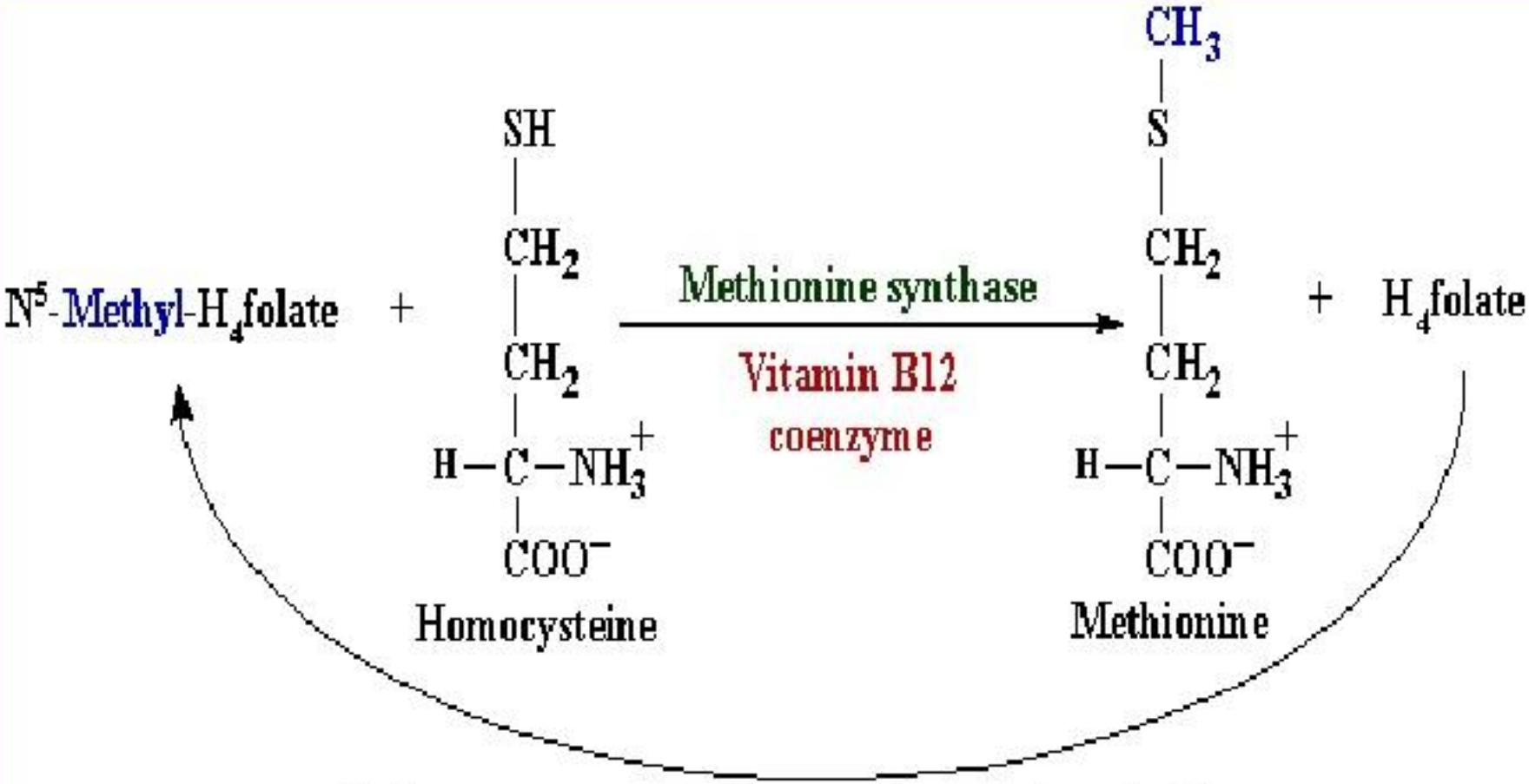
8-Cysteine and methionine:

Homocysteinuria:- characterized by high plasma homocysteine and methionine and low levels of cysteine, the most common cause of homocysteinuria is defect in the enzyme cystathionine synthase which converts homocysteine to cystathionine.

Symptoms:

Are ectopia (displacement of the lens of the eye), skeletal abnormalities, premature arterial disease, osteoporosis and mental retardation.

Methionine synthesis

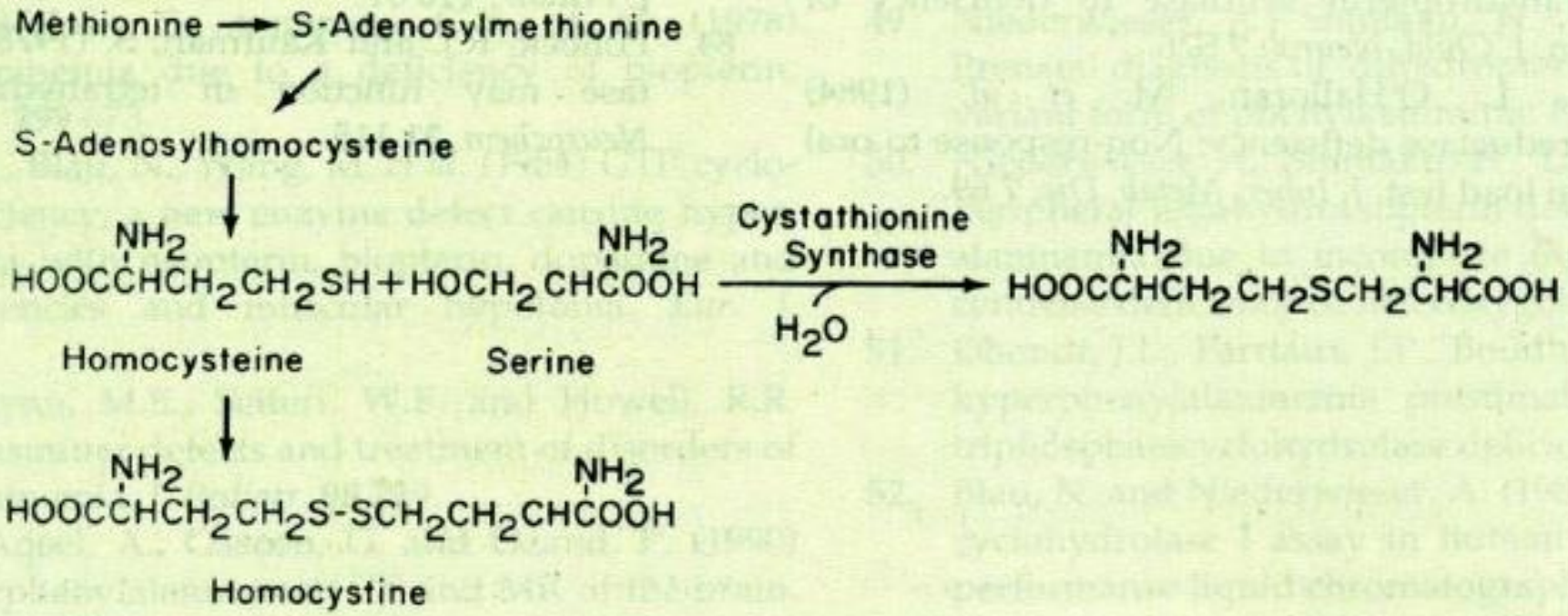


H_4folate accepts methyl groups in a number of different reactions an is converted back to N^5-Methyl-H_4folate

Homocystinuria

MAJOR PHENOTYPIC EXPRESSION

Ectopia lentis, vascular occlusive disease, malar flush, osteoporosis, accumulation of homocystine and methionine and defective activity of cystathionine synthase.



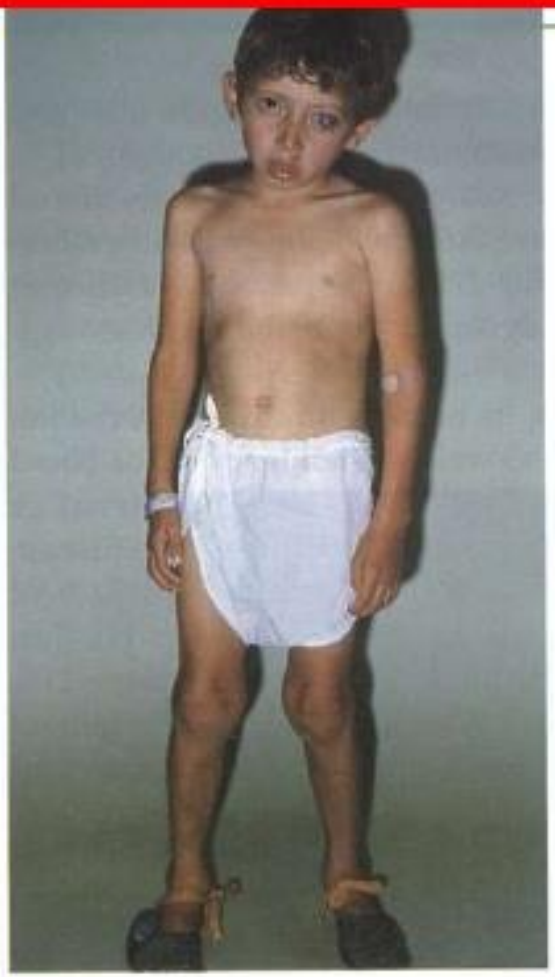


Fig. 21.2 M.G., a 6-year-old boy with homocystinuria. He had short stature and genu valgum.

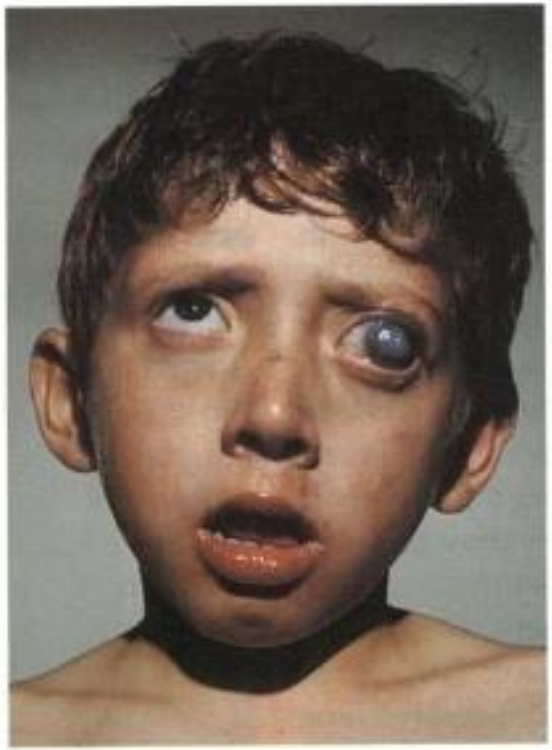


Fig. 21.3 Closer view illustrates M.G.'s eyes. Subluxed lenses had previously been removed bilaterally, after which he developed glaucoma in the left eye. He had fair skin and hair and a pronounced malar flush.

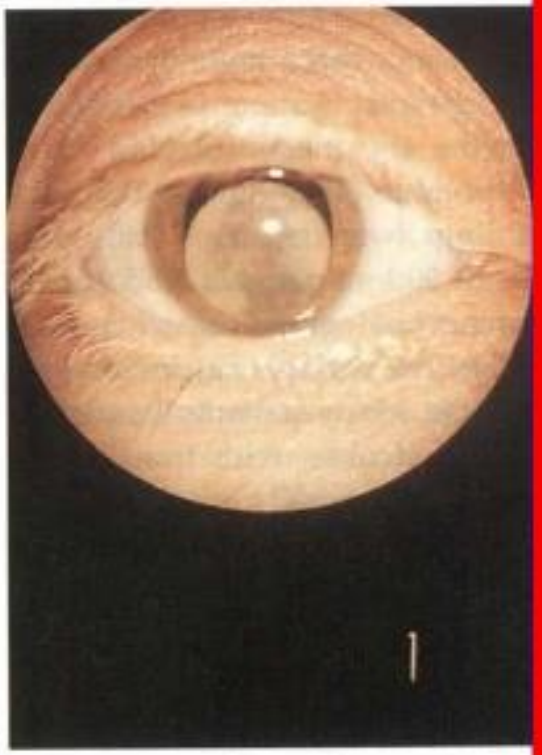


Fig. 21.4 The dislocated lens in homocystinuria is usually downward, while in Marfan syndrome it is upward.

3-Urea cycle and its disorders

In man ammonia is formed as a result of transamination and deamination of amino acids.

The formed ammonia may be excreted as such, but only in small amounts.

Certain amount of ammonia may be used for the biosynthesis of new amino acids by amination of ketoacids, mostly alpha ketoglutarate .

The majority of ammonia is converted to urea in a process known as urea cycle.

Urea synthesis through urea cycle

Under normal condition an adult excretes about (16.5) gm of nitrogen daily, about (95%) is excreted in urine by the kidneys and the remaining (5%) in the stool.

The principal pathway of nitrogen excretion in human is as urea, synthesized in the liver, releases into the blood and cleared by the kidneys . Urea cycle includes (5) reactions :

1-Synthesis of carbamoyl-p-:

Condensation of one mole of CO_2, ammonia and phosphate derived from ATP to form carbamoyl phosphate.

This reaction is catalyzed by the enzyme carbamoyl phosphate synthetase of liver mitochondria .Mg and a dicarboxylic acid mainly N-acetyl glutamate is required in this reaction as enzyme activator.

2-Synthesis of citrulline:

This is done by transfer of carbamoyl moiety (portion) from carbamoyl phosphate to ornithine in the presence of ornithine transcarbamoylase of liver mitochondria.

3-Synthesis of argininosuccinic acid :

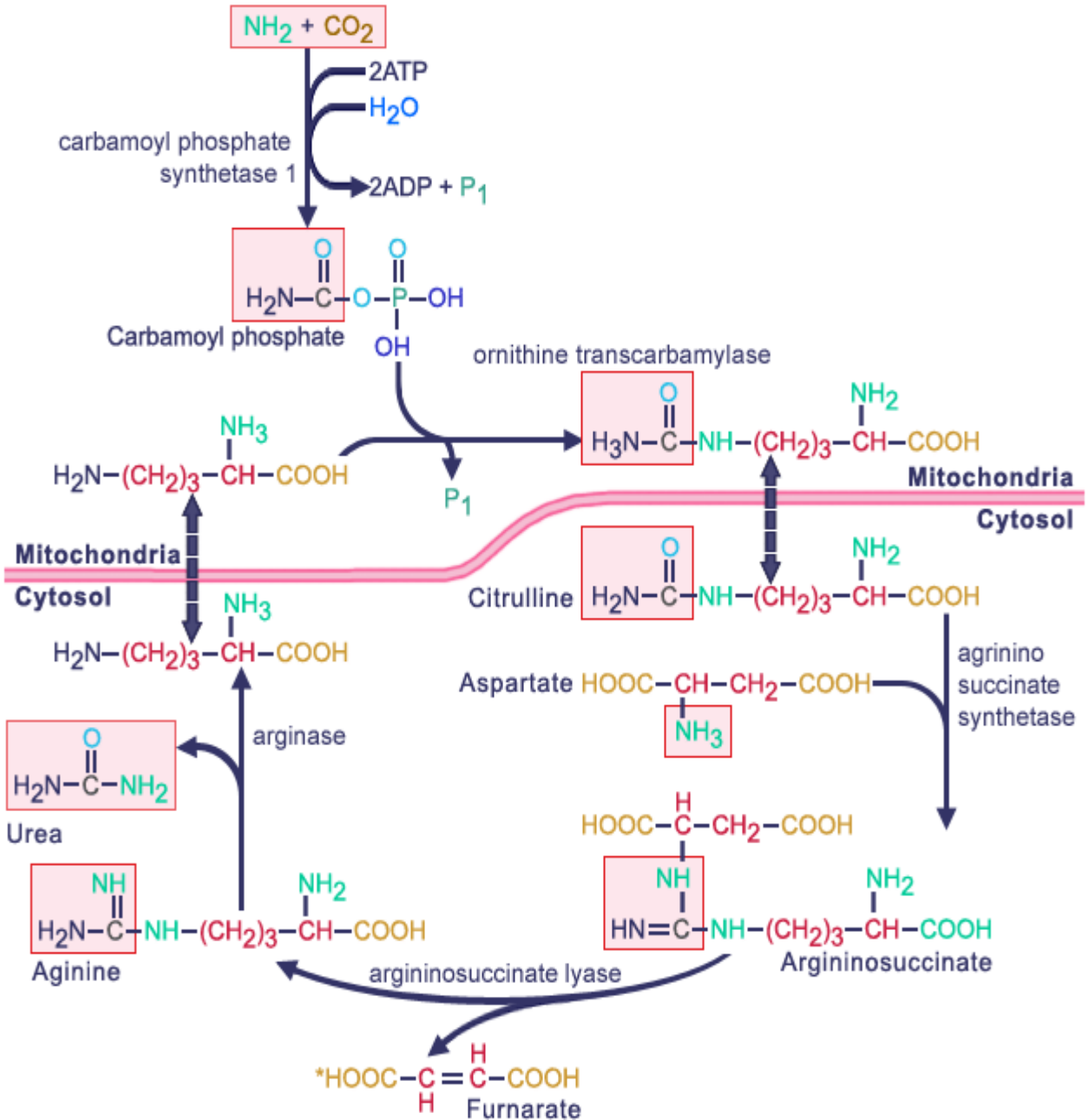
This is done by linking of aspartic acid and citrulline in the presence of argininosuccinate synthetase .

4-Cleavage of argininosuccinate into arginine and fumarate in the presence of argininosuccinase .

5-Cleavage of arginine into ornithine and urea in the presence of arginase.

This reaction completes urea cycle and regenerates ornithine as a substrate for reaction number(2) of urea cycle .

These reactions can be illustrated by the following diagram



C-CLINICAL CORRELATIONS

1-Metabolic disorders of urea cycle

Urea cycle in liver involves (5) reactions catalyzed by (5) different enzymes, metabolic disorders associated with the deficiency of each of these (5) enzymes are known.

The only function of urea cycle is to convert the toxic substance ammonia into non toxic substance urea . All disorders of urea cycle cause ammonia toxicities which is more severe when the metabolic block occurs at reactions(1) or (2).

Clinical symptoms associated with all disorders of urea cycle are :

a-Intolerance to high protein diet .
b-Mental disorders.
c-Retard development of CNS.
d-Excessive urinary excretion of ammonia.

The principal disorders are :

1-Hyperammonemia type 1
There is deficiency of carbamoyl-p- synthetase.

2-Hyperammonemia type 2
There is deficiency of ornithine transcarbamoylase .

3-Citrullinemia

There is deficiency of argininosuccinate synthetase and characterized by: excessive urinary excretion of citrulline, high plasma and C.S.F. levels of citrulline .

4-Argininosuccinic aciduria

There is deficiency of argininosuccinase, characterized by elevated blood and C.S.F. levels of argininosuccinic acid.

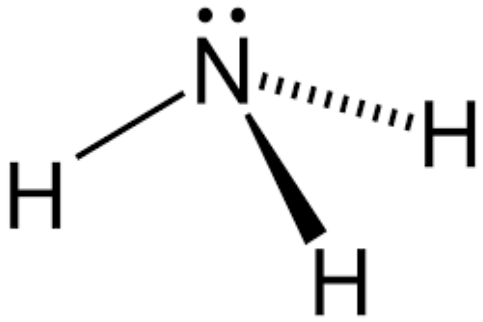
5-Hyperargeninemia

There is deficiency of arginase.

“Ammonia toxicity”
Why ammonia is toxic?

Ammonia

- **Is a chemical compound of nitrogen and hydrogen atoms with formula of NH3, always dissolve in water found as NH4OH**



Some facts about ammonia

- Ammonia is normally present in all body fluids including CSF in CNS
- The normal blood ammonium is normally lesser than 50 micro mol\L
- When ammonia is accumulated in abnormal quantities leads to hyperammonemia
- The major source of ammonia is amino acid metabolism.
- High ammonia level maybe a sign of underlying cause.

So HOW body regulate the normal amount of ammonia in order to preserve homeostasis and avoid toxicity?

Ammonia is a toxic product of nitrogen metabolism which should be removed from our body. The **urea** cycle or ornithine cycle converts excess **ammonia into urea** in the mitochondria of liver cells. The **urea** forms, then enters the blood stream, is filtered by the kidneys and is ultimately excreted in the urine.

The causes of hyperammonemia:

- Primary
  (hereditary urea cycle enzyme defects)

- Secondary
  (acquired: liver damage)

Acquired cause: LIVER DAMAGE

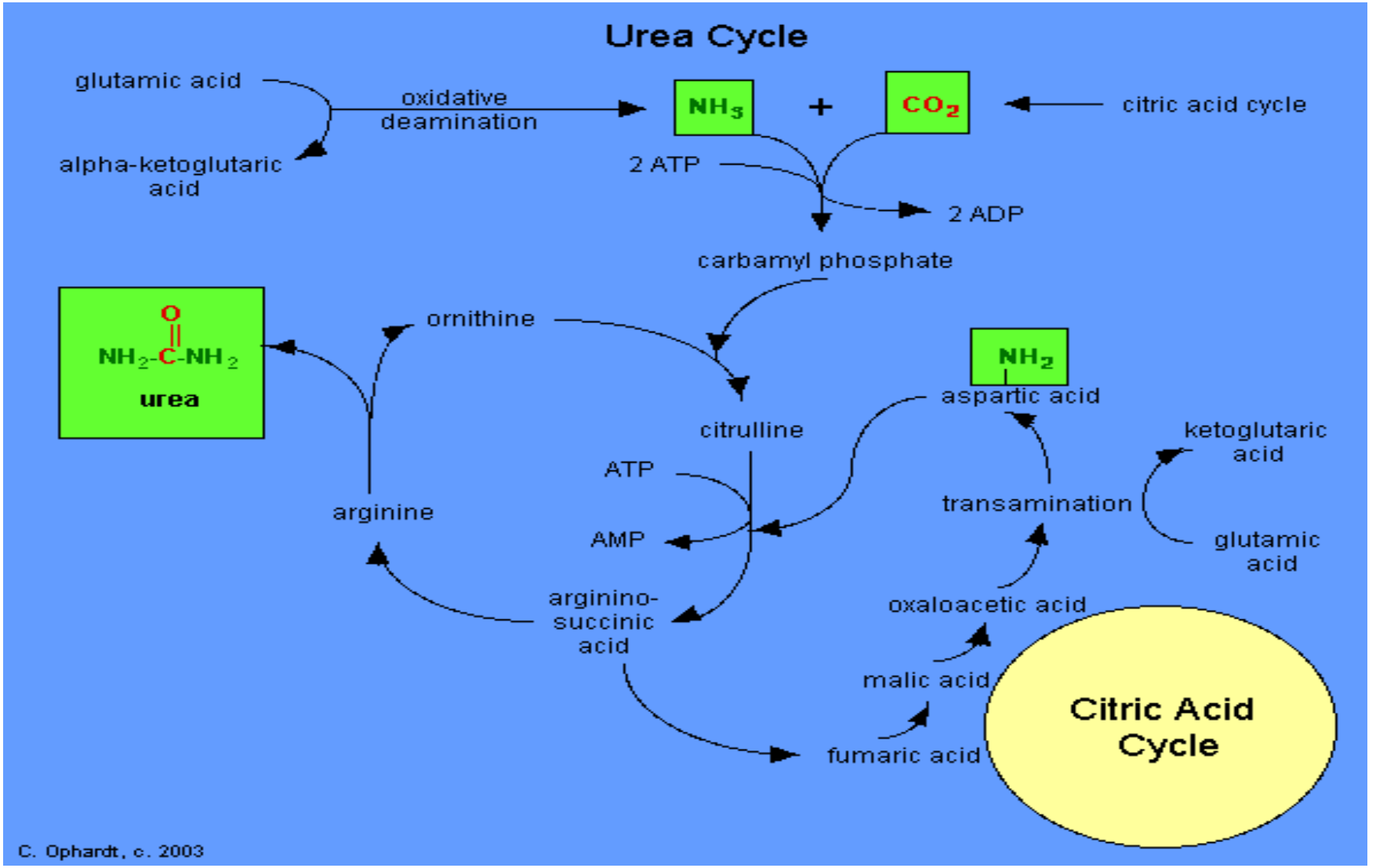
- High protein diet
- Liver cirrhosis
- Infections (hepatitis)
- Liver injury
- Medications
- Alcohol
- Processed food
- High insulin

METABOLISM OF GLUTAMATE

- In body, normally alpha-ketoglutarate binds with ammonium under the catalysis of glutamate dehydrogenase forming glutamate.
- Glutamate bind with ammonium under catalysis of glutamine synthetase forming glutamine.
- Alpha-ketoglutarate is an important compound in krebs cycle, hence ATP production.

The toxic effects of hyperammonemia on body (especially BRAIN):

- Inhibits ATP production by inhibiting Krebs cycle
- In glutamate metabolism, ammonia makes the production of glutamine dominant
- SO, low levels of alpha-ketoglutarate that inhibits Krebs cycle
- And low levels of glutamate which is the precursor of GABA (inhibitory neurotransmitter).


Urea Cycle
glutamic acid
oxidative deamination
alpha-ketoglutaric acid
NH3
+
CO2
citric acid cycle
2 ATP
2 ADP
carbamyl phosphate
ornithine
NH2-C-NH2
urea
citrulline
NH2
aspartic acid
ketoglutaric acid
ATP
AMP
arginine
transamination
glutamic acid
arginino-succinic acid
oxaloacetic acid
malic acid
fumaric acid
Citric Acid Cycle
C. Ophardt, c. 2003

Normal Range Of Blood Ammonia

The normal amount of ammonia found in the blood is about 50µmol/L. If the level of ammonia reaches 100µmol/L, the patient may experience alterations in their level of consciousness. An ammonia level of 200µmol/L may lead to convulsions and coma.

Clinical features of hyperammonemia:

- Confusion
- Memory loss
- Swelling in the brain (edema)
- Musty sweet breath
- Vomiting (>24 hrs)
- Overly sleeping
- encephalopathy
- Problems in thinking
- Problems with balance
- Tremor
- Slurring of speech
- Blurry vision
- Convulsions and seizure

So how we will protect ourselves from hyperammonemia or what are the treatments ?

Protect your LIVER

- Avoid toxins (alcohol, drugs…)
- Discuss all your medications or supplements with your doctor
- Limit protein in diet
- Healthy weight (diet, exercise)
- Regular water drinking
- Fruits (banana)
- Vegetables
- Fish
- Garlic (it activates the enzymes that have role in intoxification).


Chiquita
Chiquita
Chiquita

Treatment:

- Enzymatic defeciency→Sodium Banzoate and sodium phenylbutyrate (adjunctive, help ammonia excretion)
- Hepatic encephalopathy→Lactulose (synthetic ,by gut flora fragmentation convert into lactose and producing H+ which binds with ammonia forming ammonium ions NH4+ that can not be absorbed due to changes in PH or acidification in a process called “ammonium trapping”.
- Severe hyperammonemia (>1000 micro mol/L)→ begin with hemodialysis (purifying the blood).

Acute phase proteins (APPs)

Acute phase proteins(APPs):-

The acute phase proteins are a group of proteins whose plasma concentration changes in response to a variety of inflammatory states including infection, surgery, trauma, myocardial infarction, malignancy, the birth process, and any condition associated with tissue necrosis.

Some of these proteins are called positive acute phase proteins, meaning that their plasma levels increase, whereas others are designated negative acute phase proteins to indicate that there levels decrease.

Interleukins(IL), especially interleukin-1(IL-1), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and interferon-γ (IFN-γ), released by macrophages and lymphocytes, are primary agents which cause induction and release of these acute phase proteins will synthesize in the liver.

These acute phase proteins may be used to monitor progress the condition or its treatment.

Table (1): Major acute phase proteins(APPs):-

Positive acute phase proteins (+APPs)	Negative acute phase proteins (-APPs)
•Alpha-antitrypsin (AAT) •Alpha-anti chemotrypsin •Serum amyloid A protein •Haptoglobin •Ceruloplasmin •Fibrinogen •C_3 •C-Reactive protein •Hemopexin	•Albumin •Prealbumin •Retinol-binding protein •Transferrin (increase in late acute phase).

Alpha anti trypsin (AAT):-

Is otherwise called alpha-anti protease or protease inhibitor(Pi). It inhibits all serine proteases (proteolytic enzymes having a serine in their active centre). Such as plasmin, thrombin, trypsin, chemotrypsin, elastase, and cathepsin.

Binding of this inhibitor to protease is very tight; once bound it is not released.

Normally, about 95% of the anti protease activity in plasma is due to AAT.

Clinical correlation

Excessive cigarette smoking leads to emphysema. As cigarette smoke inhibits the activity of AAT. Because the methionine residue at 385 position of AAT is important in the enzyme binding. This methionine may be oxidized to methionine sulphoxide by smoking. So emphysema is very common in smokers with normal AAT level and smoking will worsen the situation in AAT deficiency.

Cigarette smoke increases the number of neutrophils in the lung, and, therefore, increases the amount of elastase (elastase is released from neutrophils in lungs). Elastase then causes the tissue breakdown and loss of elasticity in the lungs which called emphysema.

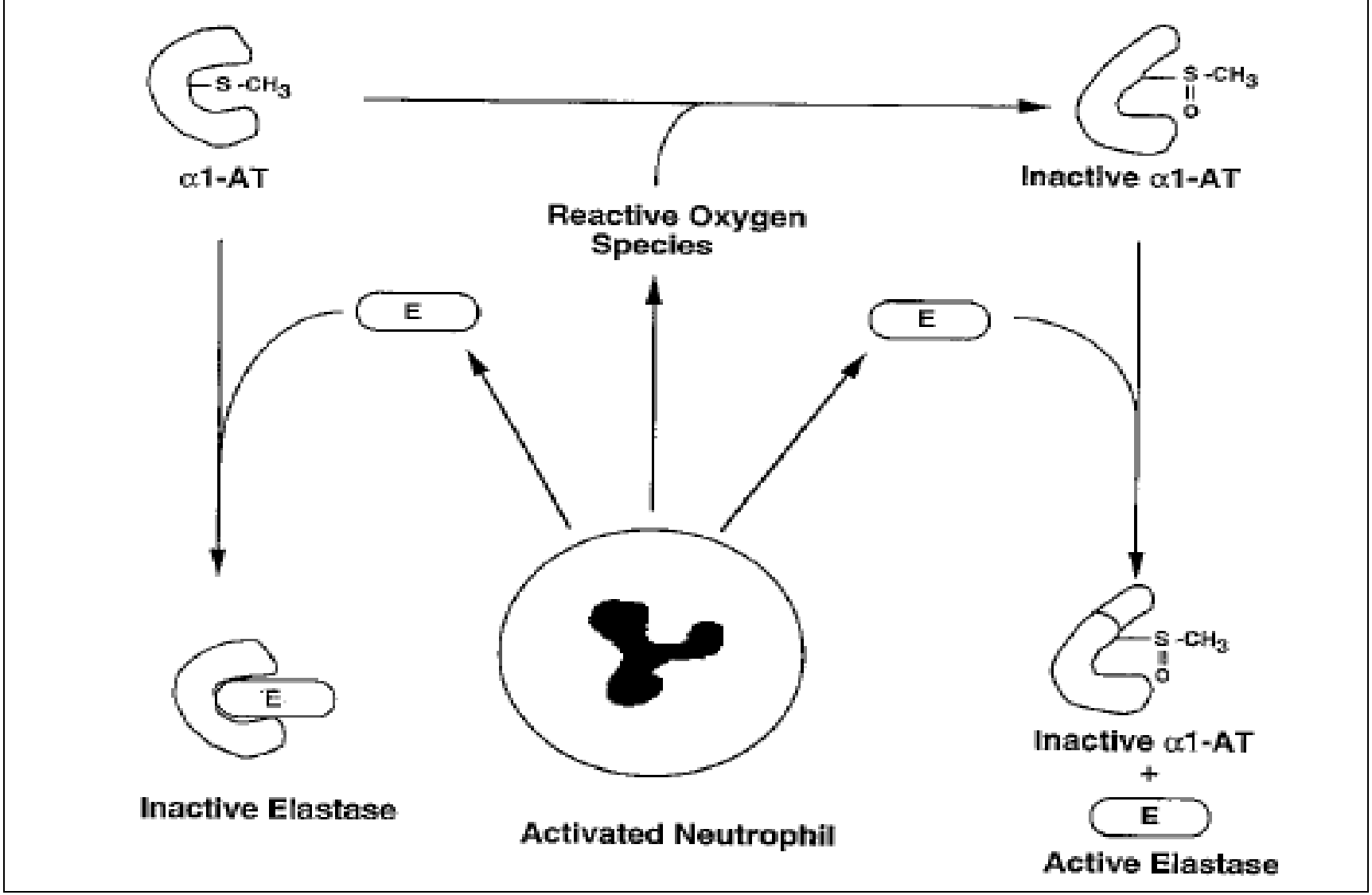


Figure1- Proposed mechanism of action of a1-AT when activated neutrophils release elastase.