Ministry of Public Health of Ukraine
Poltava State Medical University

Amino acid metabolism - 2.
Specialized pathways of amino acids transformation.

ASSOC.

PROF.

BILETS M.V.

Lecture plan

Biosynthesis and biological role of creatine and creatine phosphate.

Glutathione: structure, biosynthesis and biological functions of glutathione.

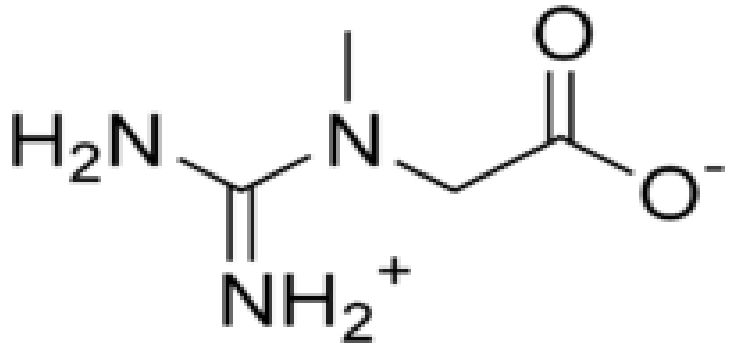
Hereditary enzymopathies of cyclic amino acids metabolism – phenylalanine and tyrosine.

Metabolism of porphyrins: structure of heme; the scheme of reactions of protoporphyrins IX and heme biosynthesis.

The specialized pathways of cyclic amino acids metabolism – phenylalanine and tyrosine.

Creatine

Creatine, or methyl guanidine-acetic acid molecule that is stored largely in skeletal muscle, in both free and phosphorylated forms. The phosphorylated form of creatine is appropriately termed *phosphocreatine* or *creatine phosphate*.



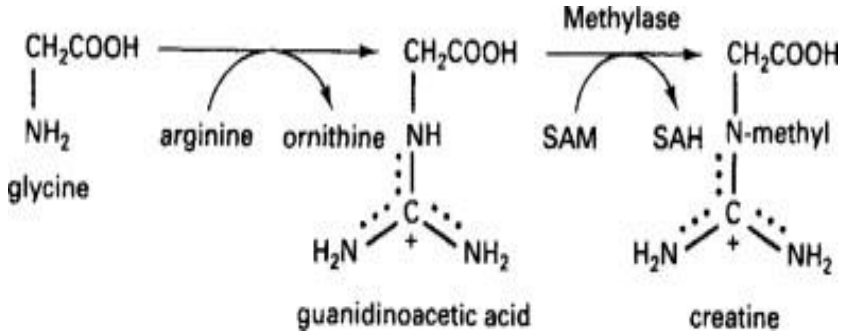
https://en.wikipedia.org/wiki/Creatine

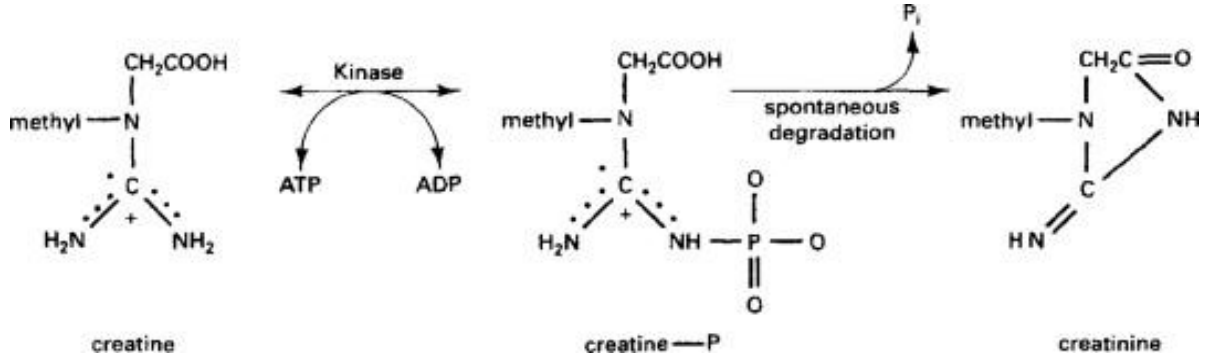
Creatine synthesis

The **first step (takes place in the kidney)** involves transfer of an amidine group from arginine to glycine by the enzyme glycine transaminidase. The result is guanidinoacetic acid.

The second step **(takes place in the liver) -** guanidinoacetic acid is methylated by guanidinoacetate methyltransferase (with the methyl group coming from S-adenosylmethionine) to form creatine.

The creatine is then transported via the bloodstream to storage sites in skeletal muscle (95%), where it can be phosphorylated by ATP to form creatine phosphate. About 60%-70% of the creatine in skeletal muscle is phosphorylated, thereby preventing migration across the plasma membrane, and essentially trapping the molecule within the muscle cell.



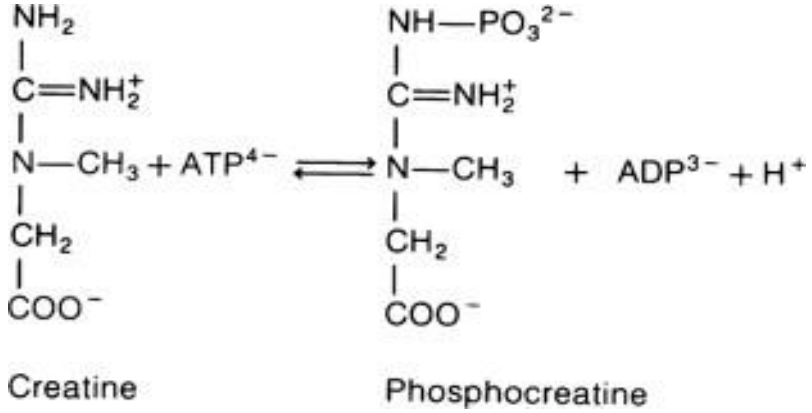


https://www.sciencedirect.com/topics/nursing-and-health-professions/creatine-phosphate

Creatine phosphate is a phosphorylated creatine molecule that serves as a rapidly mobilizable reserve of high-energy phosphates in skeletal muscle, myocardium and the brain to recycle adenosine triphosphate, the energy currency of the cell.
Phosphocreatine can anaerobically donate a phosphate group to ADP to form ATP Reaction of substrate level phosphorylation) during the first five to eight seconds of a maximal muscular effort. Conversely, excess ATP can be used during a period of low effort to convert creatine back to phosphocreatine.

The reversible phosphorylation of creatine (i.e., both the forward and backward reaction) is catalyzed by creatine kinase.

Creatine kinase is a dimer of M and B (M = muscle, B = brain) subunits produced by different structural genes. Three isozymes are possible: **BB (CK-1), MB (CK-2), and MM (CK-3).** Tissues rich in CK-l are brain, prostate, gut, lung, bladder, uterus, placenta, and thyroid; those rich in CK-3 are skeletal and cardiac muscle. Cardiac muscle contains significant amounts of CK-2 (25–46% of total CK activity, as opposed to less than 5% in skeletal muscle), so that in myocardial infarction the rise in serum total CK activity is accompanied by a parallel rise in that of CK-2. CK-3 – present in skeletal muscles.



https://www.sciencedirect.com/topics/nursing-and-health-professions/creatine-phosphate

Creatinine

- **Creatinine** is the waste product formed in muscle from a high energy storage compound ,creatine phosphate (**phosphocreatine**).
- **ATP** is the immediate source of energy for muscular contraction as it hydrolyzed to ADP.
- **Creatine phosphate** can be stored in muscle at **4** times the concentration ATP.
- **Small amount** of creatinine is ingested as constituent of meat.
- **Creatinine is the best indicator** of renal function than others. The amount of creatinine excreted daily is a function of the muscle mass and is **not affected** by diet, age and sex.

https://slideplayer.com/slide/10620870/

Blood creatine concentration: 0/08-0/11 mmol/l

An increase in the concentration of creatine in the blood (hypercreatinemia) is observed in muscular dystrophy of skeletal muscle diseases (myasthenia gravis, myositis), avitaminosis C and E, after childbirth.

Creatine is absent in the urine of an adult healthy person.

Physiological creatinuria is possible in children and the elderly persons.

Creatinuria is characteristic of muscle tissue atrophy or damage.

Blood creatinine concentration: 0/06-0.076 mmol/l

An increase in the concentration of creatinine in the blood (hypercreatininemia) is observed with severe physical activity, increased protein catabolism, fever, prolonged pressure sydrome, renal and cardiovascular failure.

Decreased concentration - with muscular dystophia or muscle diseases, hypodynamia.

Creatinine urea excretion: 0.8-1.8 g/day (female), 1-2 g/day (male)

Increased excretion: High protein diet, increased catabolism of proteins, fever, severe physical activity .

Decreased excretion: renal and cardiovascular failure.

Creatinine clearance (CrCl) is the volume of blood plasma cleared of creatinine per unit time. It is a rapid and cost-effective method for the measurement of renal function. Creatinine clearance is a good estimation of the glomerular filtration rate because in the process of urine formation, reatinine is filtered, but almost not reabsorbed. This makes it possible to assess precisely the filtration capacity of the kidneys.

Normal creatinine clearance – 80-120 ml/min.

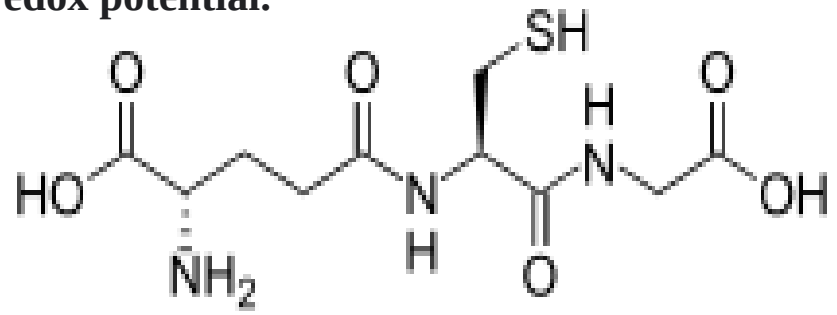
Glutathione

Glutathione consists of of three amino acids – **glutamate, cysteine, glycine** (gamma-glutamyl-cysteinyl-glycine). Glutathione can be found in virtually every cell of the human body.

SH-groups of cysteine residues are very important for the functioning of enzymes and processes, underlying responses to environmental factors and intracellular information transmission - cellular signaling. Basic mechanism of the central role of thiol-mediated oxidation-reduction (redox) control in cellular metabolism is the ability of thiol groups to reversibly change their redox state with subsequent change in conformational, catalytic or regulatory functions of the protein.

The redox state of the thiol groups of proteins is maintained by the ratio of reduced (GSH) and oxidized (GSSG) glutathione.

Reduced Glutathione (GSH) is less susceptible oxidation unlike cysteine, which makes it most suitable for maintenance of intracellular redox potential.



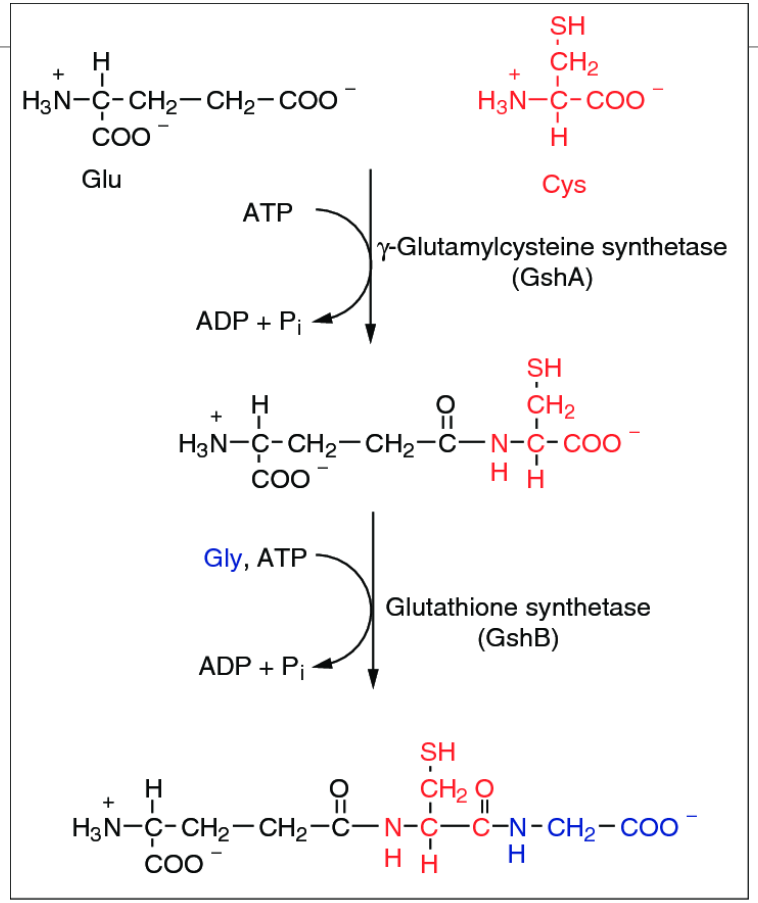
https://en.wikipedia.org/wiki/Glutathione

Glutathione synthesis

GSH synthesis involves two closely linked, enzymatically-controlled reactions that utilize ATP.
First reaction: cysteine and glutamate are combined by gamma-glutamyl cysteinyl synthetase.
Second reaction: GSH-synthetase combines gamma-glutamylcysteine with glycine to generate GSH.

As GSH levels rise, they self-limit further GSH synthesis; otherwise, cysteine availability is usually rate-limiting. Fasting, protein-energy malnutrition, or other dietary amino acid deficiencies limit GSH synthesis.

GSH recycling is catalyzed by glutathione disulfide reductase, which uses reducing equivalents from NADPH to reconvert GSSG to 2GSH. The reducing power of ascorbate helps conserve systemic GSH.



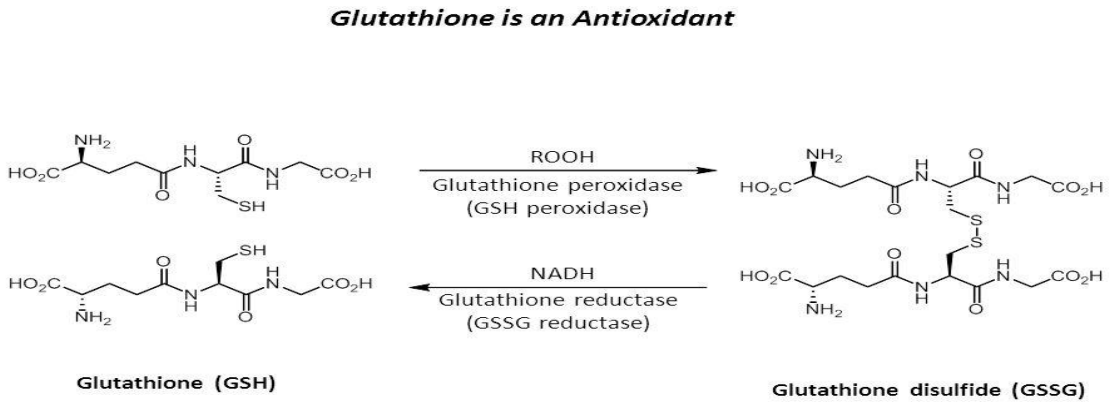
https://www.researchgate.net/figure/Pathway-for-the-biosynthesis-of-glutathione_fig9_11323304

Glutathione biological role

Glutathione is one of the inmportant antioxidant:

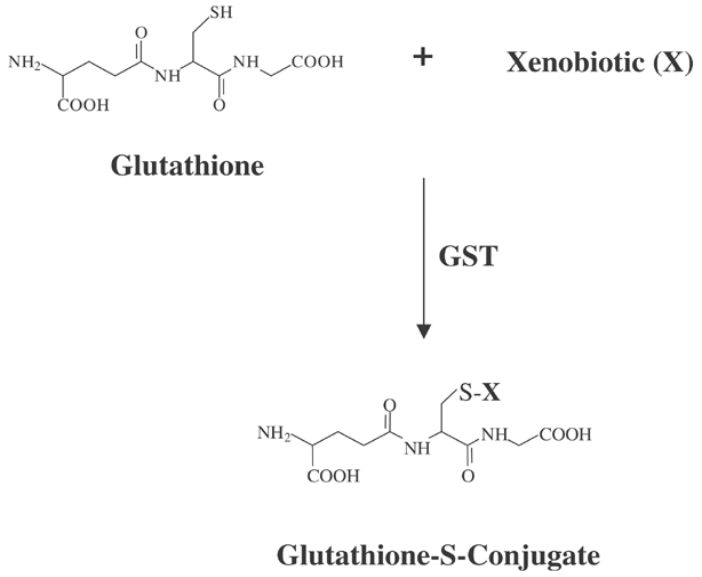
Hydrogen peroxide is reduced to water by **glutathione peroxidase** using GSH as a cosubstrate.The reduction of organic hydroperoxides to the corresponding alcohols may not be carried out only due to the catalytic activity of glutathione peroxidase, but also peroxidases activity of Se-independent **glutathione S-transferase**, also using GSH as a cosubstrate:

$$ROOH + 2GSH \rightarrow ROH + GSSG$$



https://slideplayer.com/slide/5217798/

Glutathione takes part in the reactions of biotransformation of xenobitics in the liver (conjugation reactions) – the enzyme is glutathione-S-transferase

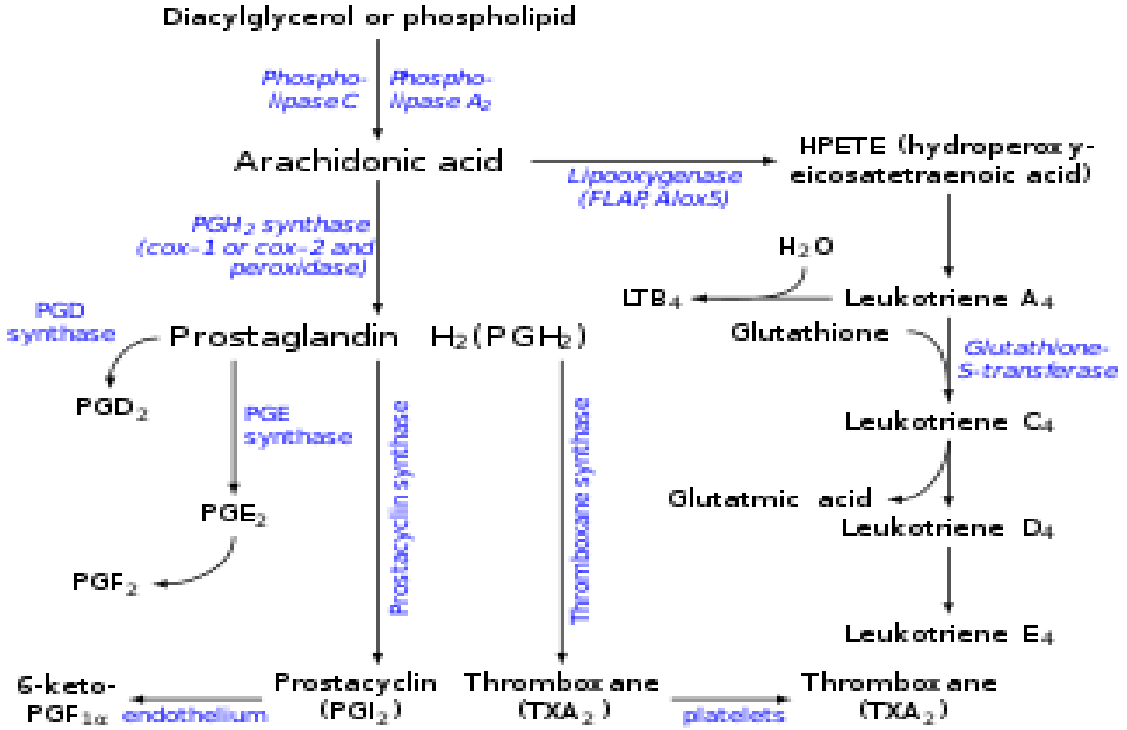


Glutathione is important in the apoptosis mechanism. Decreased GSH levels leads to the appearance of a signal for the development of apoptosis, which is initiated by activation of the death receptor or mitochondrial apoptoptotic signaling. On the contrary, an increase in the GSH content provides cellular protection against Fas-induced apoptosis.

Glutathione also is involved in the regulation of gene expression and DNA repair

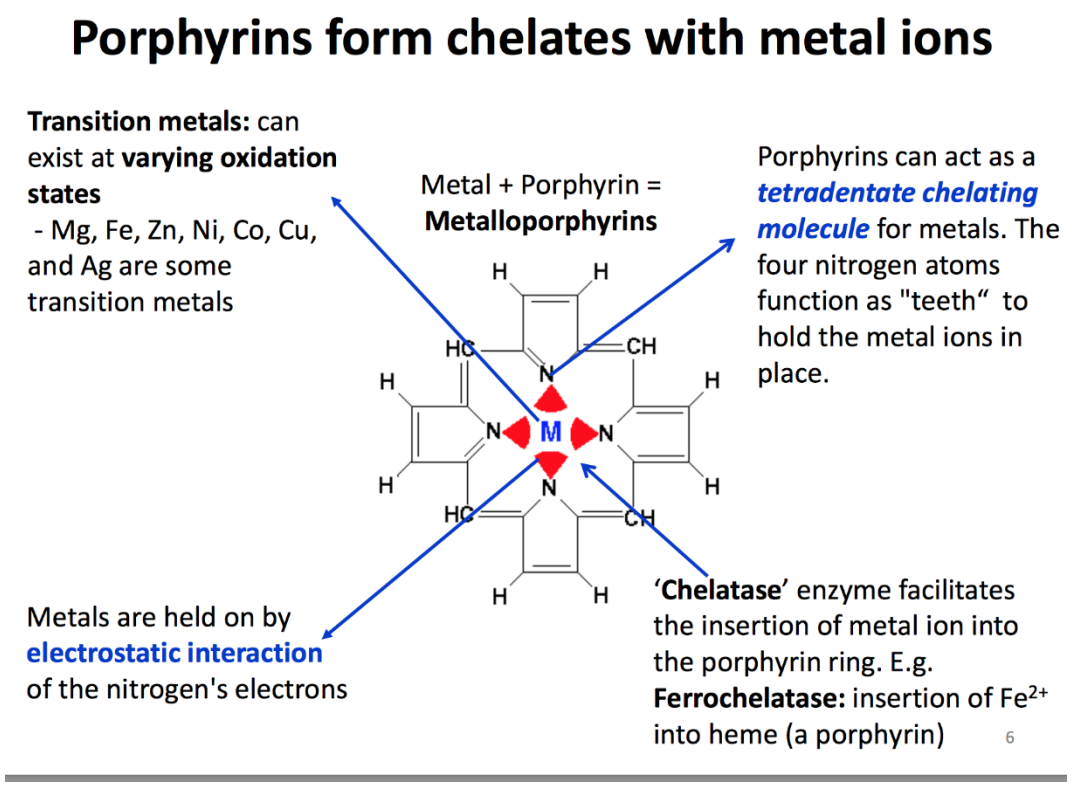
https://www.nature.com/articles/1206940

Glutathione is involved in the synthesis of leukotrienes and is a cofactor for the enzyme glutathione peroxidase.



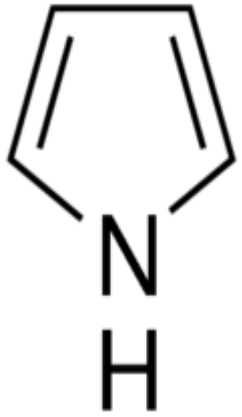
https://en.wikipedia.org/wiki/Leukotriene

Porphyrins



Porphyrins are a class of macrocyclic aromatic compounds composed of four pyrrole rings connected by methine bridges Porphyrins are ubiquitous in nature, as a heme cofactor of hemoglobin, cytochromes, and other redox active enzymes, and, as more saturated analogs.

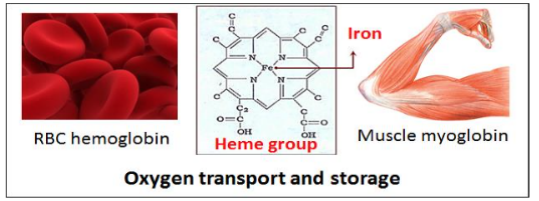
Pyrrole:



https://www.memorangapp.com/flashcards/68286/GS+L51+Porphyrin+Biosynthesis

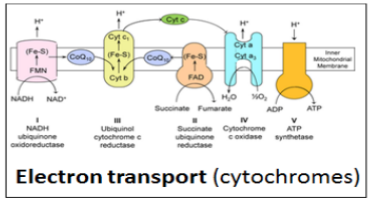
- Metalloporphyrins can exist at **varying oxidation levels** and this allows them to perform **diverse functions** in nature such as:
  - **Oxygen storage** and **transport**

    Heme is a metalloporphyrin where porphyrin forms a complex with **iron**.
    - The heme group is an important component of **hemoglobin** – a protein found in red blood cells that is important for the transport of oxygen in blood.
    - The heme group is also found in **myoglobin** – a protein responsible for storage of oxygen in muscle.

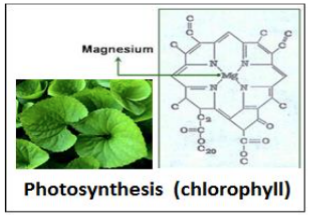


Oxygen transport and storage

    - The heme group is also found in **Cytochrome c** where it is involved in the electron transport chain (ETC) for energy metabolism.
    - The heme group is also a part of **Cytochrome P450** family of enzymes responsible for the metabolism of xenobiotics.
    - The heme group is found in the enzyme **Catalase** that is responsible for the degradation of hydrogen peroxide for protection from cellular oxidative stress.
    - Other enzymes such as the **trypotophan pyrrolase** that is responsible for tryptophan oxidation also contain heme.

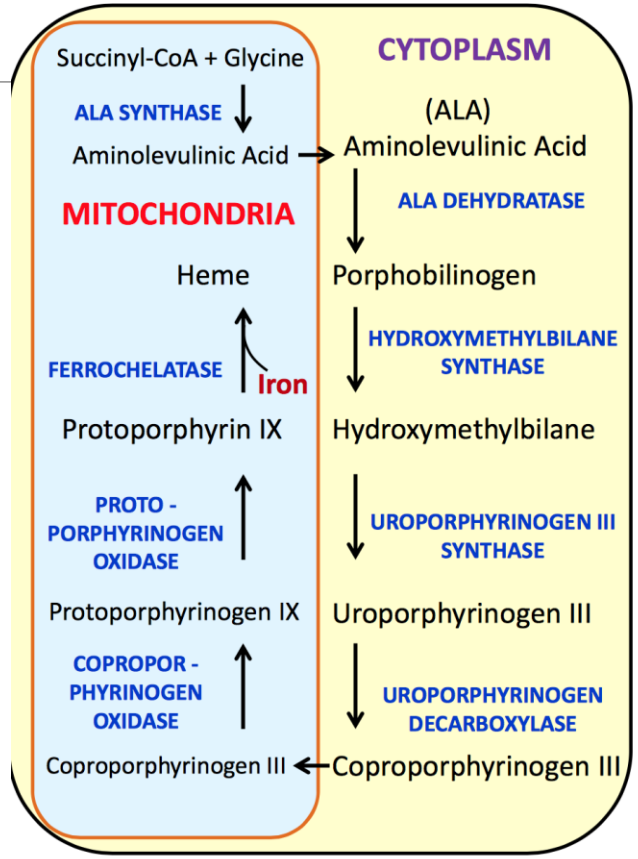

Electron transport (cytochromes)

  - **Photosynthesis:** **Chlorophyll** pigments that are found in photosynthetic plants have **magnesium**-containing porphyrins.



Photosynthesis (chlorophyll)

https://www.memorangapp.com/flashcards/68286/GS+L51+Porphyrin+Biosynthesis/



Heme biosynthesis

Major **sites** of synthesis in the body:

- **Bone marrow erythroid cells** : about **85%** of heme produced in the body. Synthesis rate is **relatively constant** at all times
- **Liver (~10%)**: where **cytochrome P450** is synthesized. Synthesis is up-regulated in response to drug/alcohol metabolism

Cellular location:

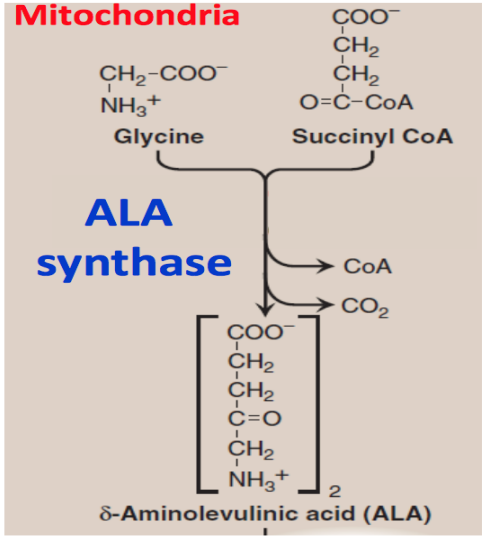
- **Mitochondria**: the initial reaction and the last three steps
- **Cytosol**: the intermediate 4 steps

Note: mature RBCs don't have a mitochondria and cannot make heme

https://www.memorangapp.com/flashcards/68286/GS+L51+Porphyrin+Biosynthesis/

Heme biosynthesis: formation of ALA

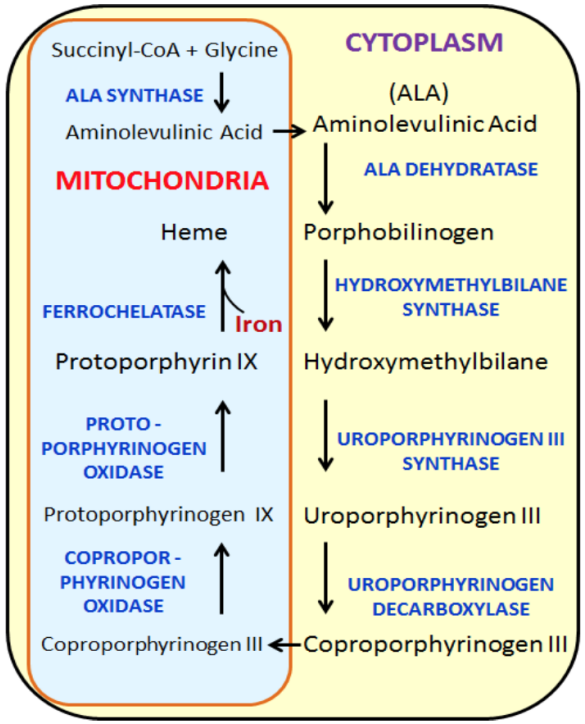
- ALA = δ-aminolevulinic acid
- The **committed** and **rate-limiting step** of porphyrin biosynthesis.
- Occurs in the **mitochondria**
- Glycine and Succinyl CoA condense to form ALA
- All the C and N atoms of the porphyrin molecule are provided by **Glycine** (a nonessential amino acid) and **Succinyl Coenzyme A** (an intermediate of the TCA cycle)
- Reaction is catalyzed by **ALA synthase**
- **Pyridoxal phosphate** is a required **coenzyme** for this reaction



https://www.memorangapp.com/flashcards/68286/GS+L51+Porphyrin+Biosynthesis/

Defects in Heme Biosynthesis: Porphyrias

- Porphyrias result from **defects in heme biosynthesis pathway**
- **Genetic disorders** *(mostly)* with mutations leading to the **deficiency** of an **enzyme** in the heme biosynthetic pathway
- Extent of enzyme deficiency depends on the mutation. **Complete absence** of a heme pathway enzyme is **incompatible with life**.



- **Accumulation** and **increased excretion** of porphyrins or porphyrin precursors in patients
- **Urine color** is altered in some patients. In some cases it can turn **purple** (***Porphyria = purple color***)

https://www.memorangapp.com/flashcards/68286/GS+L51+Porphyrin+Biosynthesis/

Classification of Porphyrias

Based on the **affected step** in the heme biosynthesis pathway:

- Enzyme defect **prior to the synthesis of the tetrapyrroles**: Affected individuals, in general, manifest abdominal and neuro-psychiatric signs
- Enzyme defects leading to the **accumulation of tetrapyrrole intermediates**: Affected individuals show photosensitivity. Exposure of skin to visible light causes skin itches and burns. **Light exposure → Tetrapyrrole photo oxidation → reactive oxygen species formation → oxidative damage to skin cells**

Based on **body cells/tissues** where heme biosynthesis is affected:

- **Erythropoietic porphyrias**: heme biosynthesis in the erythropoietic cells of the bone marrow is affected
- **Hepatic porphyrias:** heme biosynthesis in the liver is affected.

Tissue-based porphyrias can be **acute or chronic**

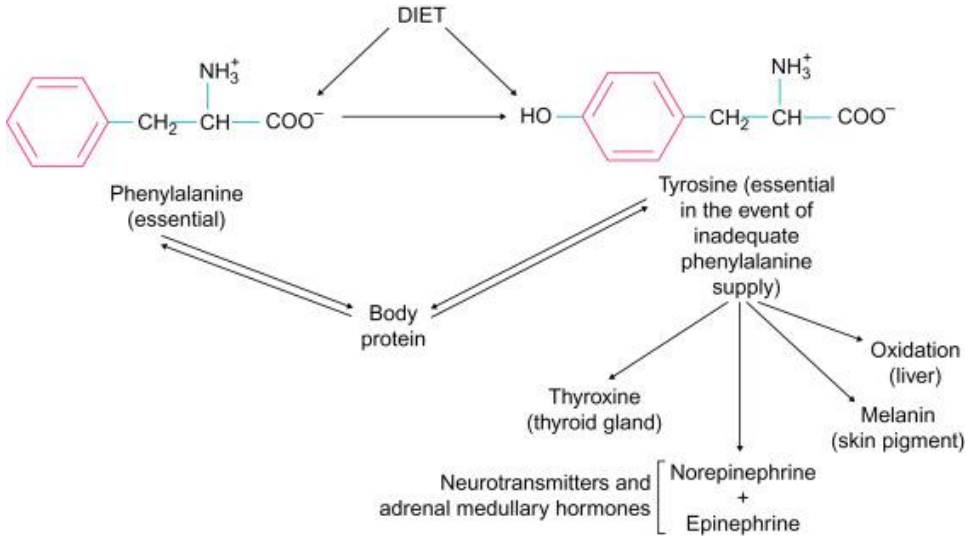
https://www.memorangapp.com/flashcards/68286/GS+L51+Porphyrin+Biosynthesis/

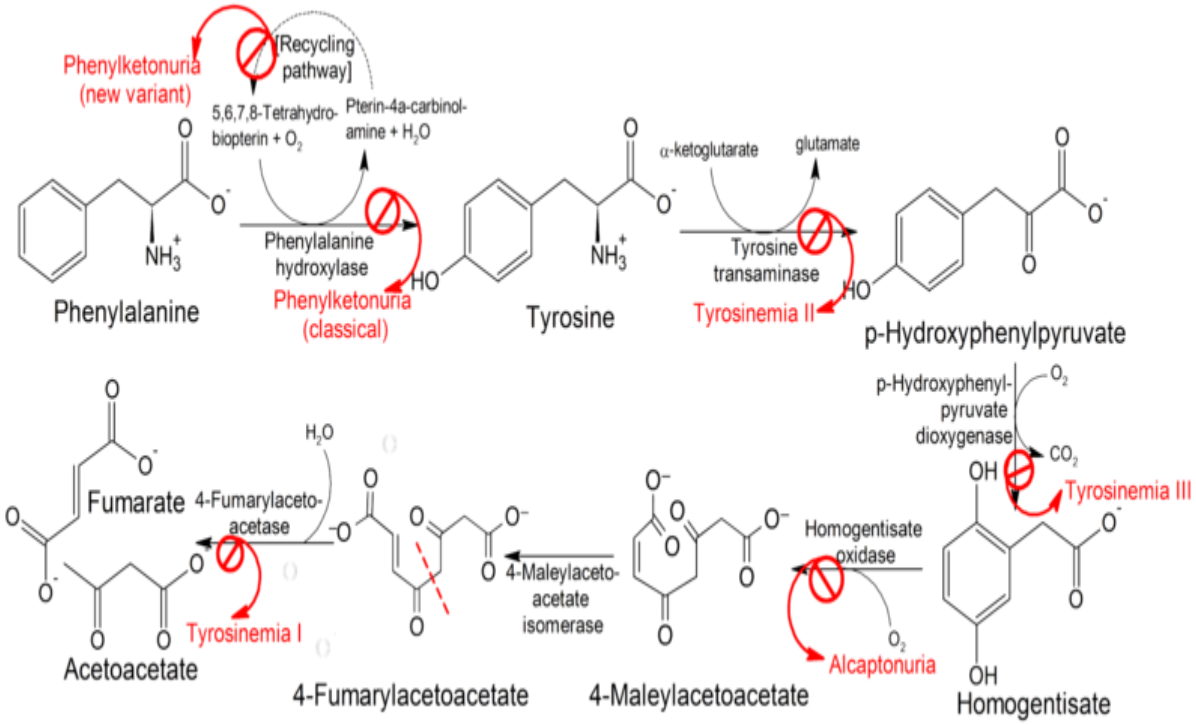
Different types of porphyrias

Type of porphyria	Enzyme defect	Characteristics
HEPATIC		
Acute intermittent porphyria	Uroporphyrinogen I synthase	Abdominal pain, neuropsychiatric symptoms
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase	Photosensitivity
Hereditary coproporphyria	Coproporphyrinogen oxidase	Abdominal pain, Photosensitivity , neuropsychiatric symptoms
Variegate porphyria	Protoporphyrinogen oxidase	Abdominal pain, Photosensitivity , neuropsychiatric symptoms
ERYTHROPOIETIC PORPHYRIA		
Congenital erythropoietic porphyria	Uroporphyrinogen III cosynthase	Photosensitivity , increased hemolysis
Protoporphyria	Ferrochelatase	Photosensitivity

https://www.slideshare.net/YESANNA/4porphyrias

Metabolism of tyrosine and phenylalanine





https://www.sciencedirect.com/topics/neuroscience/phenylalanine#:~:text=Phenylalanine%20and%20Tyrosine,in%20Parkinson%20disease%20and%20schizophrenia

https://commons.wikimedia.org/wiki/File:Pathophysiology_of_metabolic_disorders_of_phenylalanine_and_tyrosine.png

Phenylketonuria is a genetic disorder – deficiency of the enzyme phenylalanine hydroxylase

Jerky movements of the arms and legs

Skin and eyes lighter color. Infants with PKU cannot adequately produce melanin, the pigment responsible for skin color and hair.

Body odor similar to Rust

Seizures

Skin Rashes

Small head size

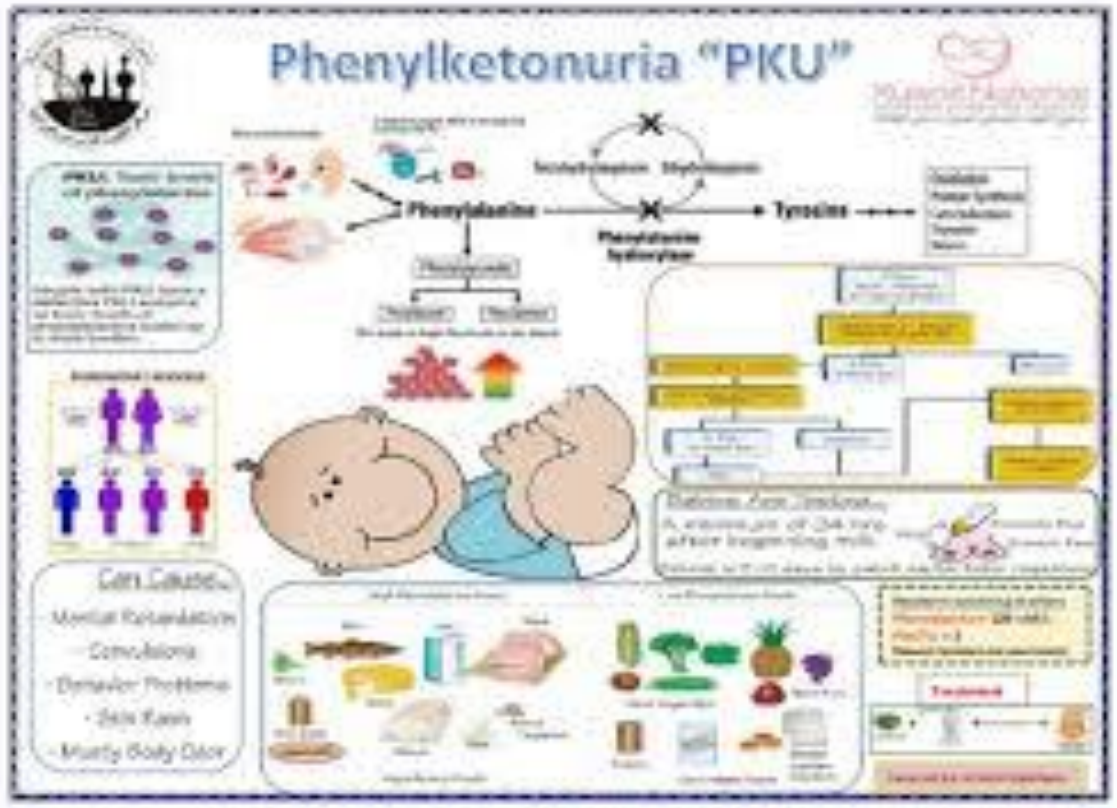
Learn to sit, crawl or walk later than planned

Loses interest in the surrounding environment

Delays in mental and social skills

Intellectual disabilities

Behavioral problems such as hyperactivity

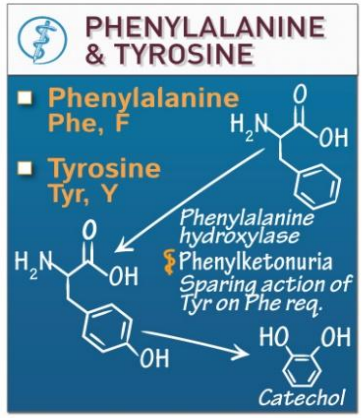


https://phenylketonuria101.wordpress.com

Alkaptonuria is a rare genetic metabolic disorder - deficiency of the enzyme homogentisate oxidase, characterized by the accumulation of homogentisic acid in the body. Affected individuals lack enough functional levels of an enzyme required to breakdown homogentisic acid.

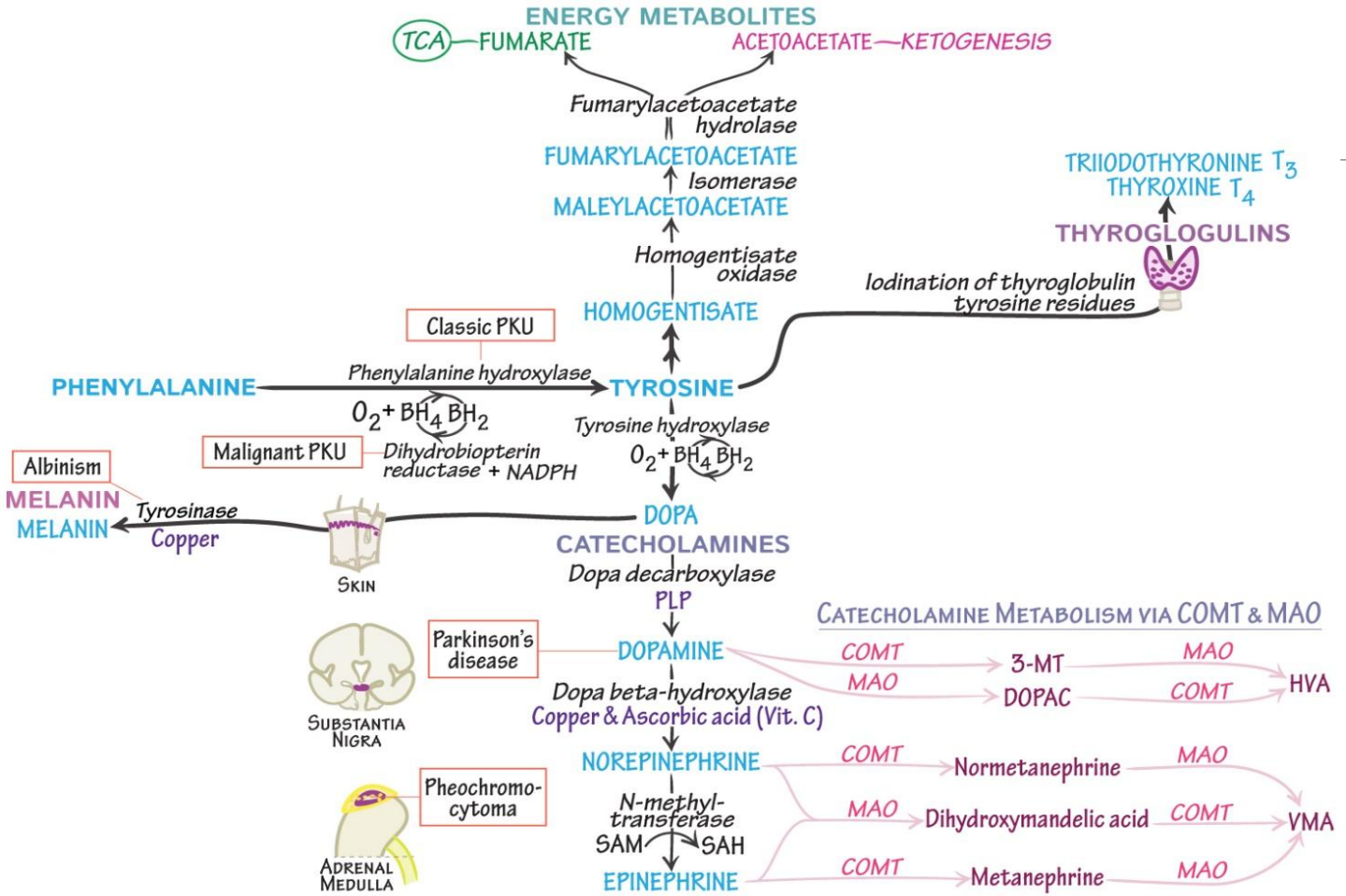
Albinism - is a genetic disorder – deficiency of the enzyme tyrosine
•an absence of color in the hair, skin, or eyes
•lighter than normal coloring of the hair, skin, or eyes
patches of skin that have avision problems, which may include:
•strabismus (crossed eyes)
•photophobia (sensitivity to light)
•n absence of color
Albinism occurs with nystagmus (involuntary rapid eye movements)
•impaired vision or blindness
•astigmatism

https://www.slideshare.net/ashokktt/metabolic-disorders-of-phenylalanine-and-tyrosine



ESSENTIAL	NONESSENTIAL
Methionine	Alanine
Threonine	Arginine*
Histidine*	Asparagine
Valine	Aspartate
Phenylalanine	Cysteine*
Isoleucine	Glutamate
Tryptophan	Glutamine
Lysine	Glycine
Leucine	Proline
KETOGENIC	Serine
GLUCOGENIC	Tyrosine*
GLUCOGENIC & KETOGENIC	*CONDITIONALLY ESSENTIAL

Maple Syrup Urine Disease — BCKDH



https://drawittoknowit.com/course/biochemistry-fundamentals/nitrogen-metabolism/phenylalanine-tyrosine-metabolism/1462/phenylalanine-tyrosine-metabolism

Sources of information

Halkerston I.D.K. Biochemistry: 2nd edition. The National medical series for independent study / Halkerston I.D.K. - 1988. - 522 p.

Harper`s Biochemistry. R.K.Murray, D.K.Granner, P.A.Mayes, V.W.Rodwell. Prentice-Hall International Inc., 2010. – 1134 p.

Koolman J. Color Atlas of Biochemistry / J.Koolman, K.-H. Rom. – Stuttgart. New York. – Thieme Verlag. — 1996. – 435 p.

Lehninger A. Principles of Biochemistry / Lehninger A. – New York. – W.H.Freeman and Company. – 2005. – 1010 p.

Pamela C.Champe Lippincott's Illustrated Reviews: Biochemistry, 3rd Edition / Pamela C.Champe and Richard A.Harvey. – Baltimore, Lippincott Williams & Wilkins, MD ©, 2005. – 534p.

https://www.sciencedirect.com/topics/nursing-and-health-professions/creatine-phosphate

https://www.sciencedirect.com/topics/nursing-and-health-professions/creatine-phosphate

https://slideplayer.com/slide/10620870/

---

https://en.wikipedia.org/wiki/Creatine

https://en.wikipedia.org/wiki/Glutathione

https://www.researchgate.net/figure/Pathway-for-the-biosynthesis-of-glutathione_fig9_11323304

https://www.nature.com/articles/1206940

https://en.wikipedia.org/wiki/Leukotriene

https://www.memorangapp.com/flashcards/68286/GS+L51+Porphyrin+Biosynthesis

https://www.memorangapp.com/flashcards/68286/GS+L51+Porphyrin+Biosynthesis/

https://www.slideshare.net/YESANNA/4porphyrias

https://www.sciencedirect.com/topics/neuroscience/phenylalanine#:~:text=Phenylalanine%20and%20Tyrosine,in%20Parkinson%20disease%20and%20schizophrenia

https://commons.wikimedia.org/wiki/File:Pathophysiology_of_metabolic_disorders_of_phenylalanine_and_tyrosine.png

https://phenylketonuria101.wordpress.com

https://www.slideshare.net/ashokktt/metabolic-disorders-of-phenylalanine-and-tyrosine

https://drawittoknowit.com/course/biochemistry-fundamentals/nitrogen-metabolism/phenylalanine-tyrosine-metabolism/1462/phenylalanine-tyrosine-metabolism