Amino Acids Metabolism Part II

Conversion of amino acids to specialized products

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Nitrogen metabolism

N2

Atmospheric nitrogen N2 is most abundant but is too inert for use in most biochemical processes.

Dietary proteins

Atmospheric nitrogen is acted upon by bacteria (nitrogen fixation) and plants to nitrogen containing compounds. We assimilate these compounds as proteins (amino acids) in our diets.

Amino acids

Body proteins

α-amino groups

NH4+

Urea

excreted

Disposal of Nitrogen
Lecture I

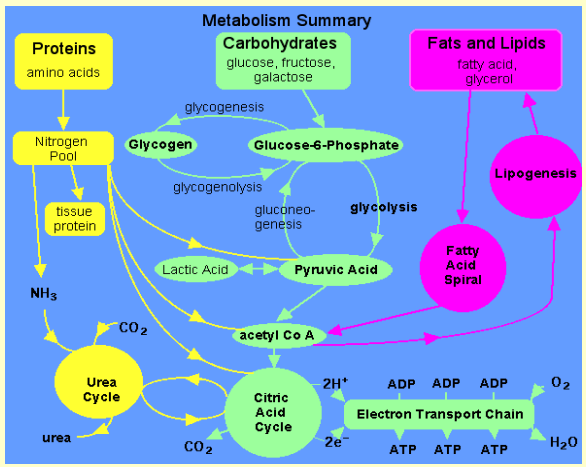
Other nitrogen containing compounds

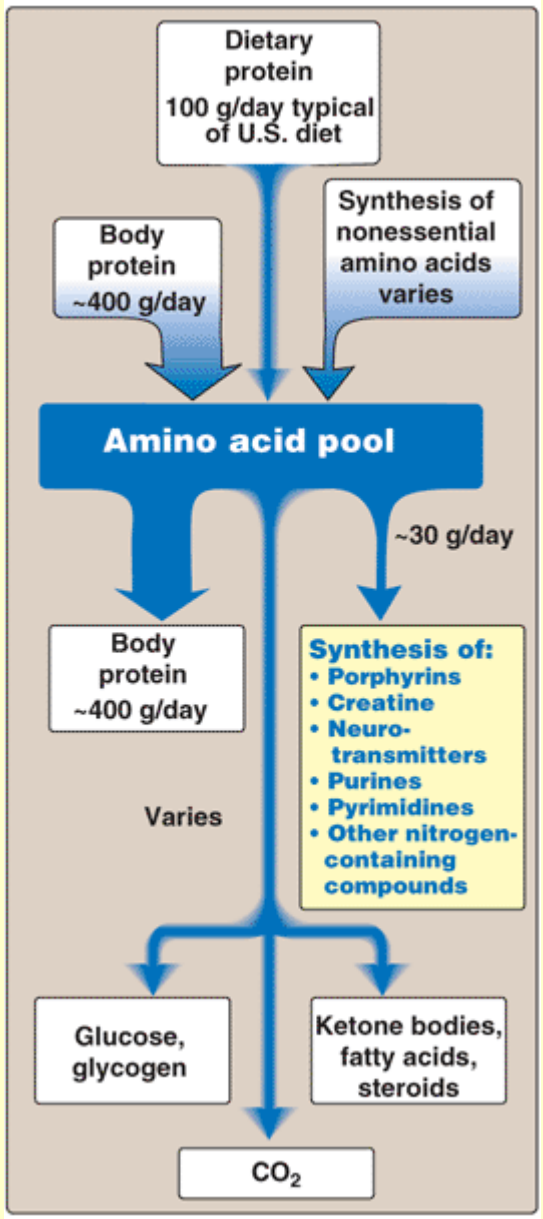
Conversion of nitrogen into specialized products
Lecture III

Carbon skeletons

Amino acids synthesis & degradation
Lecture II

Enters various metabolic pathways



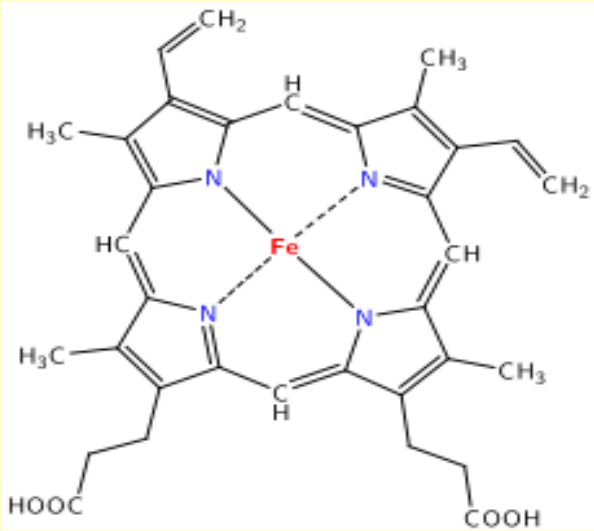


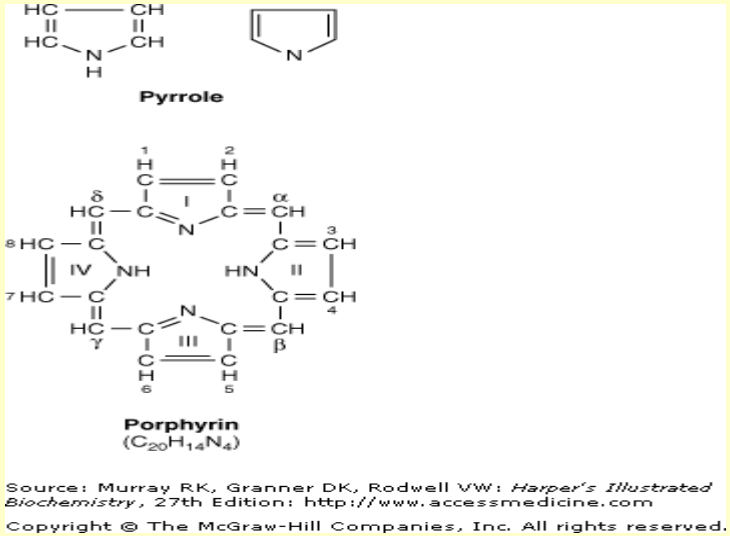
Amino Acids as precursors of nitrogen-containing compounds

Porphyrin metabolism

Porphyrins are cyclic compounds that bind metal ions, usually Fe^{2+} or Fe^{3+}

The most common metalloporphyrin is **heme**



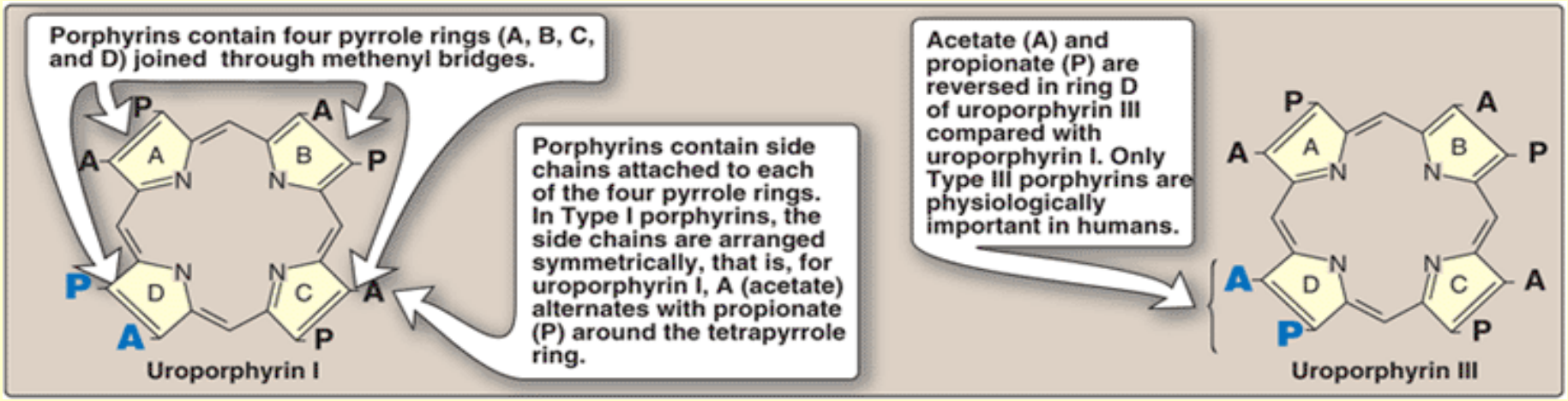


A heme group consists of an iron (Fe) ion (charged atom) held in a heterocyclic ring, known as a porphyrin

Protein	Function
Hemoglobin	Transport of oxygen in blood
Myoglobin	Storage of oxygen in muscle
Cytochrome *c*	Involvement in electron transport chain
Cytochrome P450	Hydroxylation of xenobiotics
Catalase	Degradation of hydrogen peroxide
Tryptophan pyrrolase	Oxidation of tryptophan

Example of some human and animal heme proteins

Structure of porphyrins



1)Porphyrins contain four pyrrole rings joined through methylene bridges

2) Side chains differs in different porphyrine.
Uroporphyrins contains acetate($-CH_2-COO^-$) and propionate ($-CH_2-CH_2-COO^-$) side chains
Coproporphyrins contains methyl and propionate groups.
Protoporphyrins IX (and heme) contains vinyl, methyl and propionate groups.

3) Side chains are ordered around porphyrine tetrapyrole nucleus in four different ways designated as roman letters I-IV.

4) These side chains are either symmetrically or asymetrically ordered on pyrrole rings
e.g. Type I uroporphyrins I, A acetate alternates with P (propionate) around the tetrapyrrole ring.

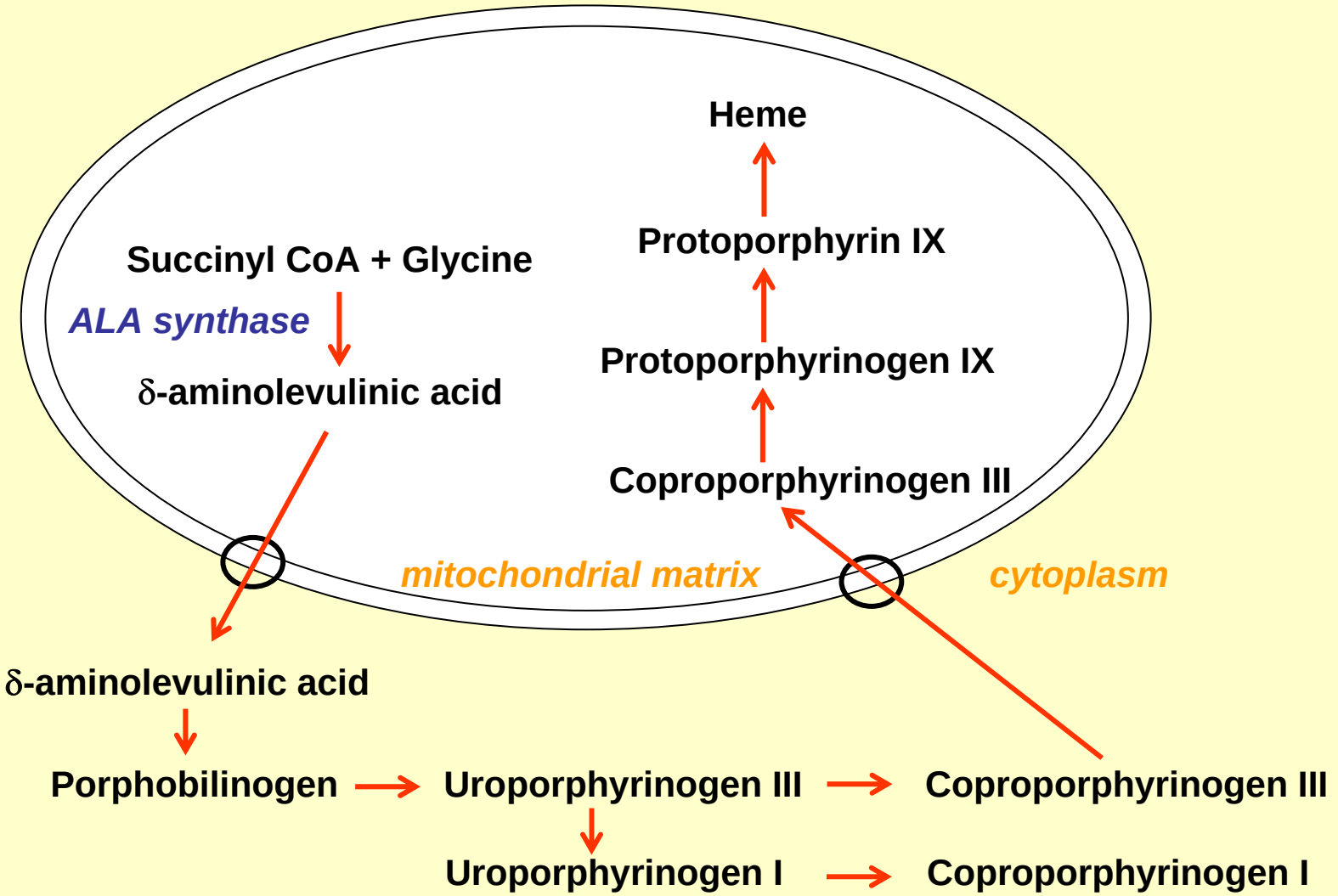
5) Type III porphyrines (e.g. uroporphyrin III) which contain an asymmetric substitution on ring D are physiologically important in humans.

Porphyrinogens: porphyrin precursors, intermediate between porphobilinogen and the oxidized colored protoporphyrins in heme biosyhthesis.

Boisynthesis of heme

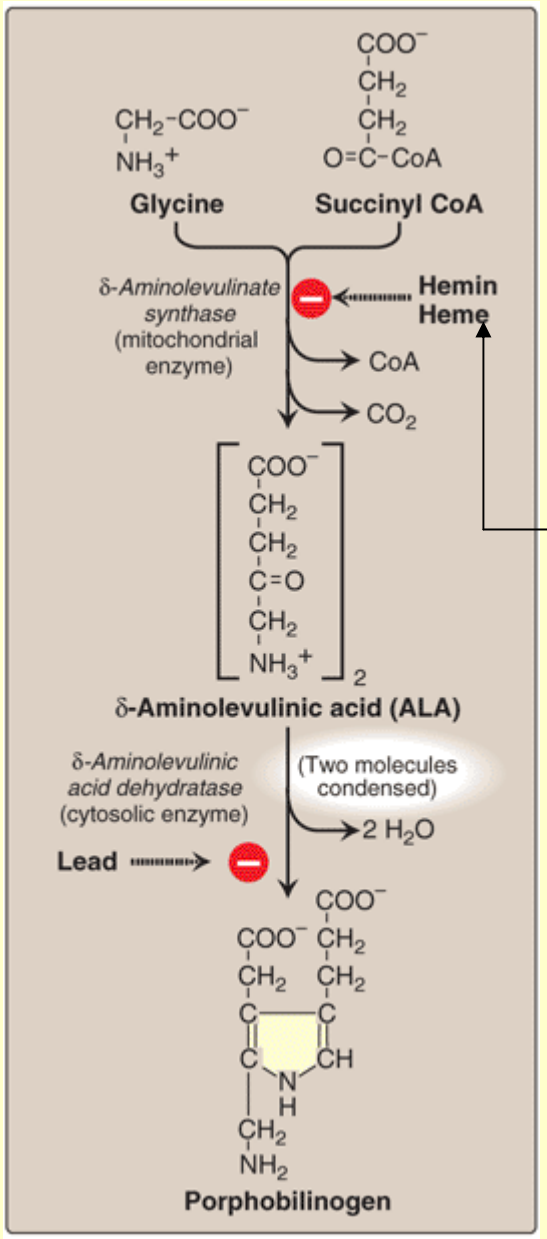
Heme synthesis occurs in all cells due to the requirement for heme as a prosthetic group on enzymes and electron transport chain proteins. By weight, the major locations of heme synthesis are the liver (cytochrome p450) and the erythroid progenitor cells (Hemoglobin) of the bone marrow.

Overview of Heme Synthesis



Mature red blood cells lack mitochondria and are unable to synthesize heme

Biosynthesis of heme



1) Formation of δ-aminolevulinic acid (ALA) (In mitochondria)

All the carbon and nitrogen atoms of porphyrin molecules are provided by Glycine (non essential aa) and Succinyl CoA (an intermediate in the citric acid cycle).

Glycine and succinyl CoA condense to form ALA, a reaction catalyzed by ALA synthatase. This reaction requires pyridoxal phosphate as a coenzyme.

When porphyrin production exceeds the availability of globin, heme accumulates and is converted to hemin by oxidation of Fe2+ to Fe3+.

Hemin negatively regulates ALA by decreasing synthesis of hepatic ALA synthase enzyme

Many drugs (e.g. antifungal, anticonvulsants) increase ALA synthesis. Because these drugs are metabolized in liver by Cyt. P450, a heme containing enzyme. This results in increase synthesis of Cyt. P450, leading to consumption of heme.

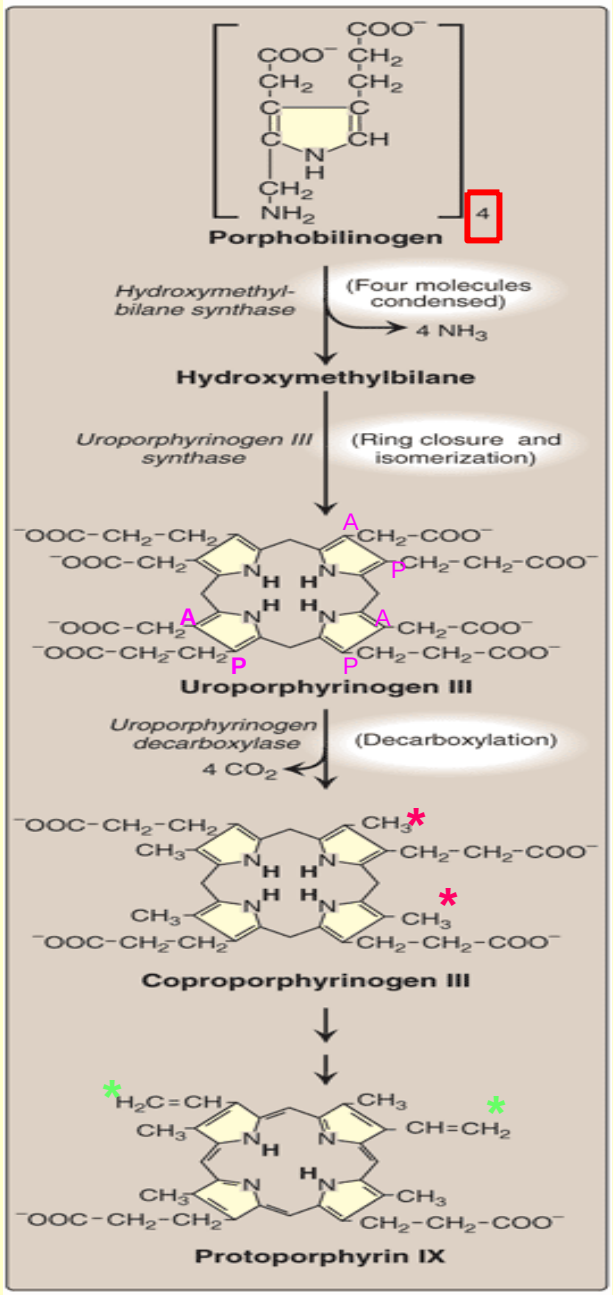
Heme ↓ → ALA ↑

In erythroid cells heme synthesis is under the control of erythropoietin and the availability of iron

2) Formation of porphobilinogen (In cytosol)

Two molecules of ALA condenses to form porphobilinogens by ALA dehydratase, the reaction sensitive to heavy metal ions.

Biosynthesis of heme



3) Formation of uroporphyrinogen (In cytosol)

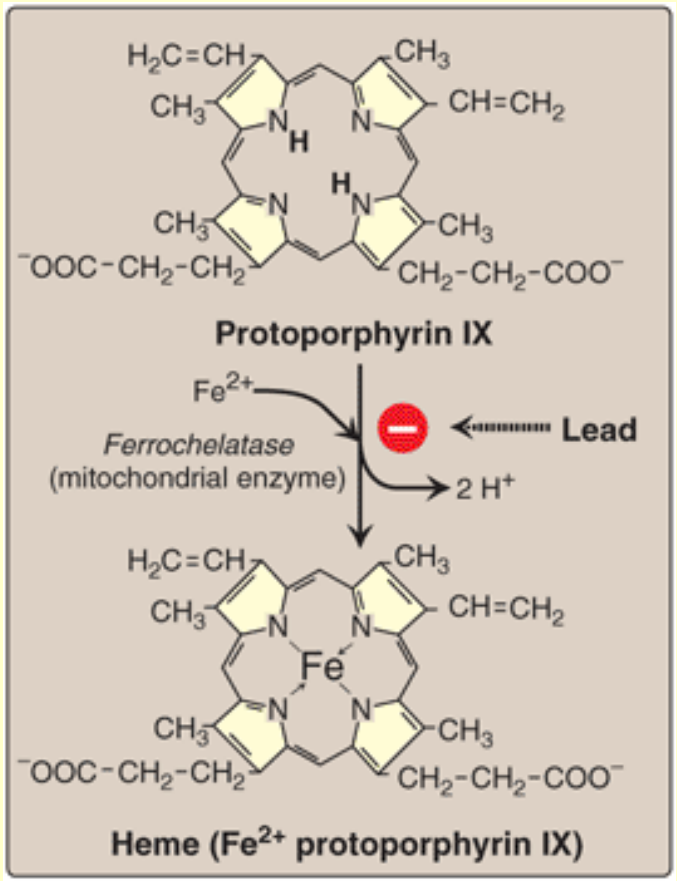
The condensation of four molecules of porphobillinogens results in the formation of tetrapyrrrole, hydroxymethylbilane, a reaction catalyzed by hydroxymethylbilane synthase

Isomerization and cyclization by uroporphyinogen III synthase leads to the formation of Uroporphyrinogen III

Uropprphyrinogen III undergoes decarboxylation at its acetate groups, generating coproporphyrinogen III, a reaction carried out by uroporphyrinogen decarboxylase

Two propionate side chains are decarboxylated to vinyl groups generating protoporphyrinogen IX, which is then oxidized to protoporphyrin IX.

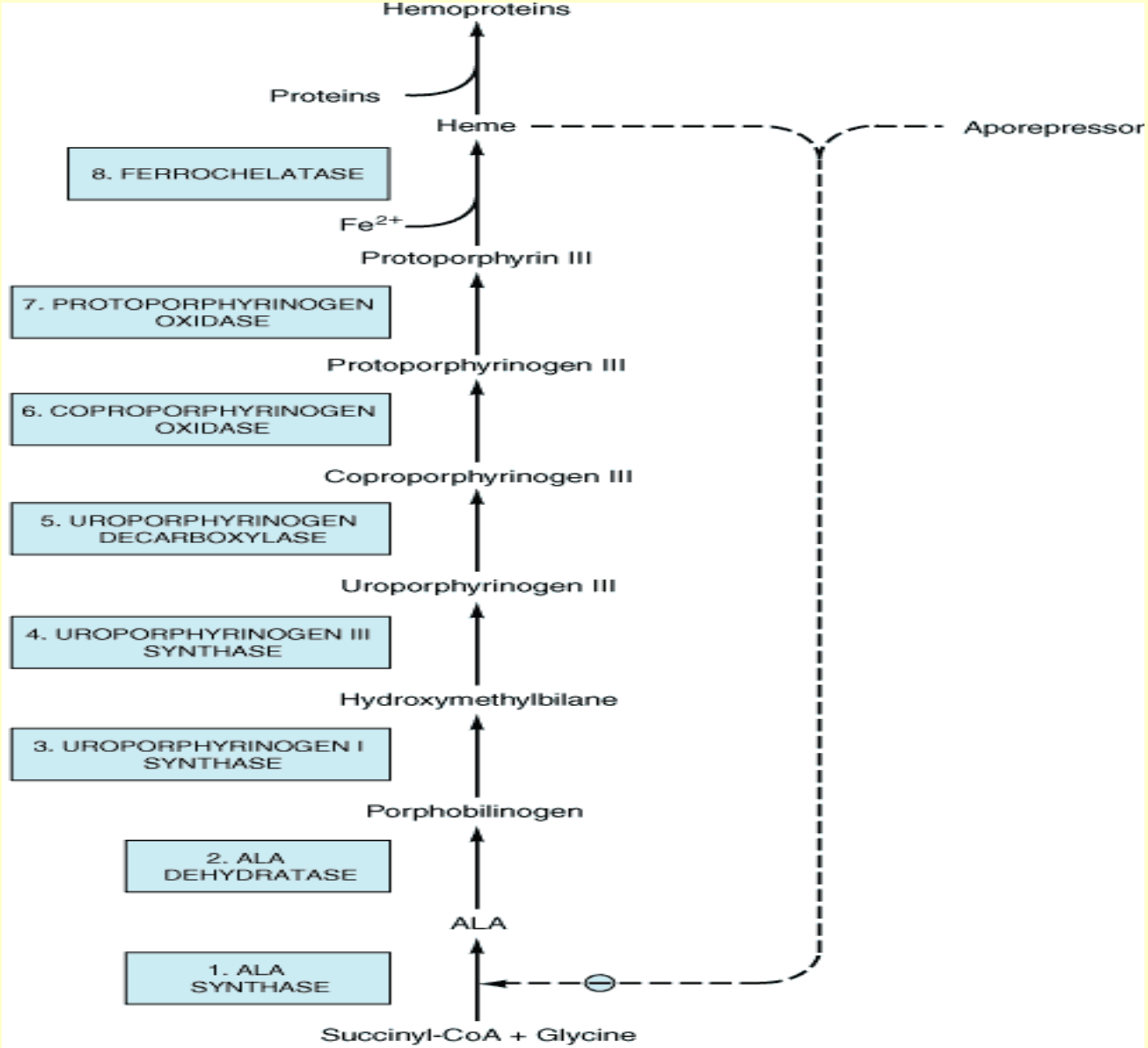
Biosynthesis of heme



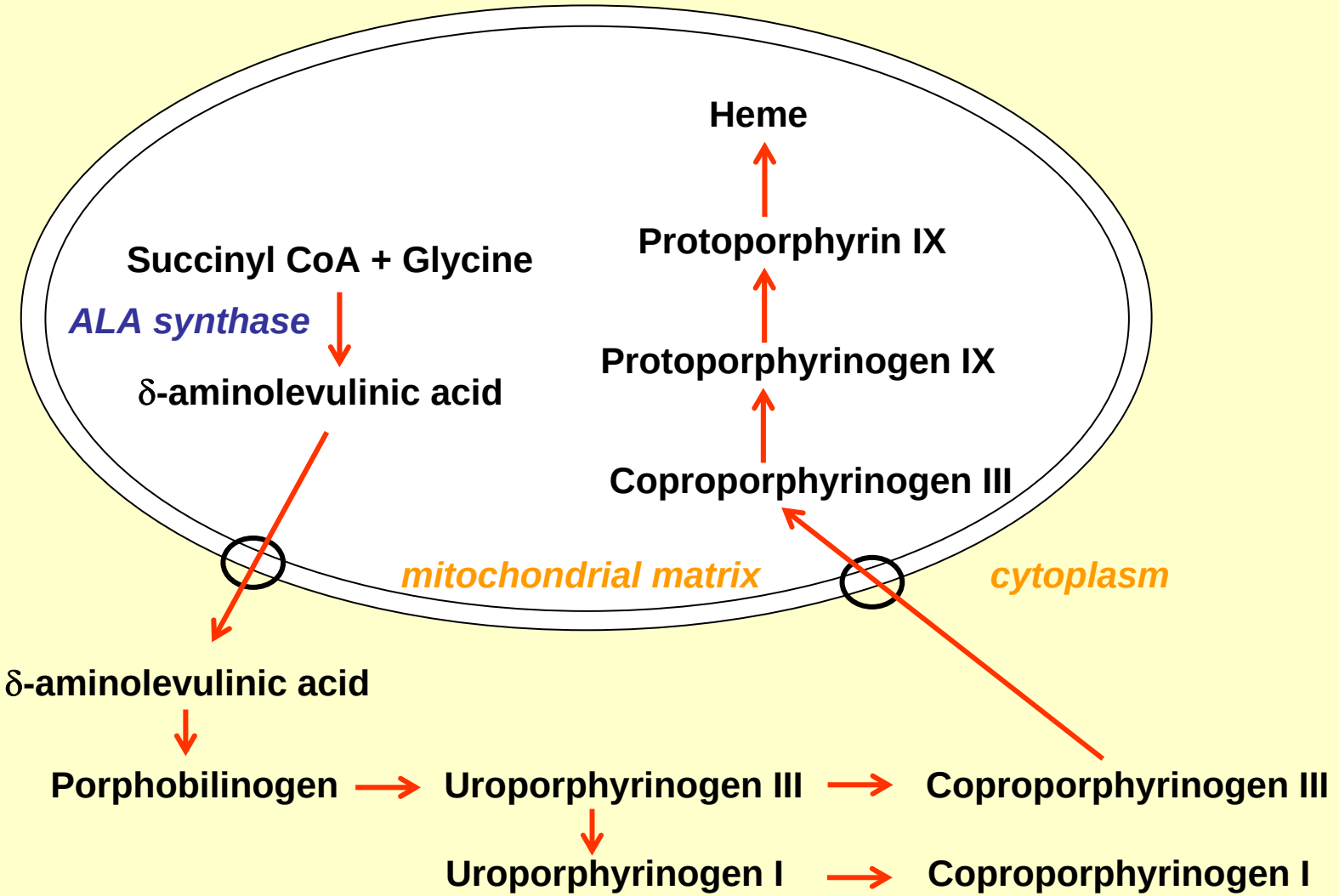
4) Formation of heme (In mitochondria)

Introduction of iron (as Fe2+) occurs spontaneously but the rate is enhanced by ferrochelatase. This enzyme like ALA is also inhibited by lead.

Heme Biosynthesis



Overview of Heme Synthesis



Mature red blood cells lack mitochondria and are unable to synthesize heme

Porphyrias

Purple color caused by pigment-like porphyrins in the urine

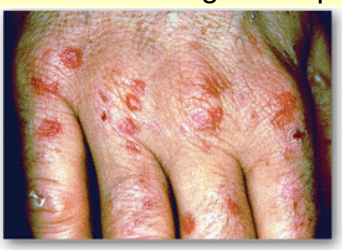
Porphyrias is caused due to the inherited (or occasionally acquired) defects in heme synthesis.

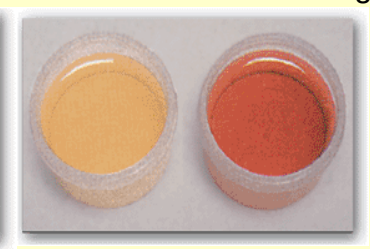
- Leads to the accumulation and increased excretion of porphyrins and porphyrins precurssors.
- Mutations that cause porphyria are heterogenous (not all the same DNA locus).
- Each porphyria leads to accumulation of a unique pattern of intermediates.
- Porphyrias are classified as erythropoeitic (enzyme deficiency is in the erythropoitic cell) or hepatic (enzyme deficiency is in the liver).

Hepatic

Chronic

Porphyrin accumulation leads to cutaneous symptoms and urine that is red to brown in natural light and pink to red in fluorescent light.





Acute

Neulological, cardivascular, symptoms
Abdominal pain

Porphyrias

Hepatic Porphyrias

Name	Deficient enzyme	Accumulated Intermediates	Photosensitivity
Acute intermittent porphria (Acute)	Hydroxymethylbiilane synthtase	Protoporphyrin and ALA in the urine	-
Variegate porphyria (Acute)	Protoporphyrinogen oxidase	Protoporphyrinogen IX and other intermedites prior to the block in the urine	+
Heriditary Coproporphyria (Acute)	Coproporphyrinogen oxidase	Coproporphyrinogen III other intermedites prior to the block in the urine	+

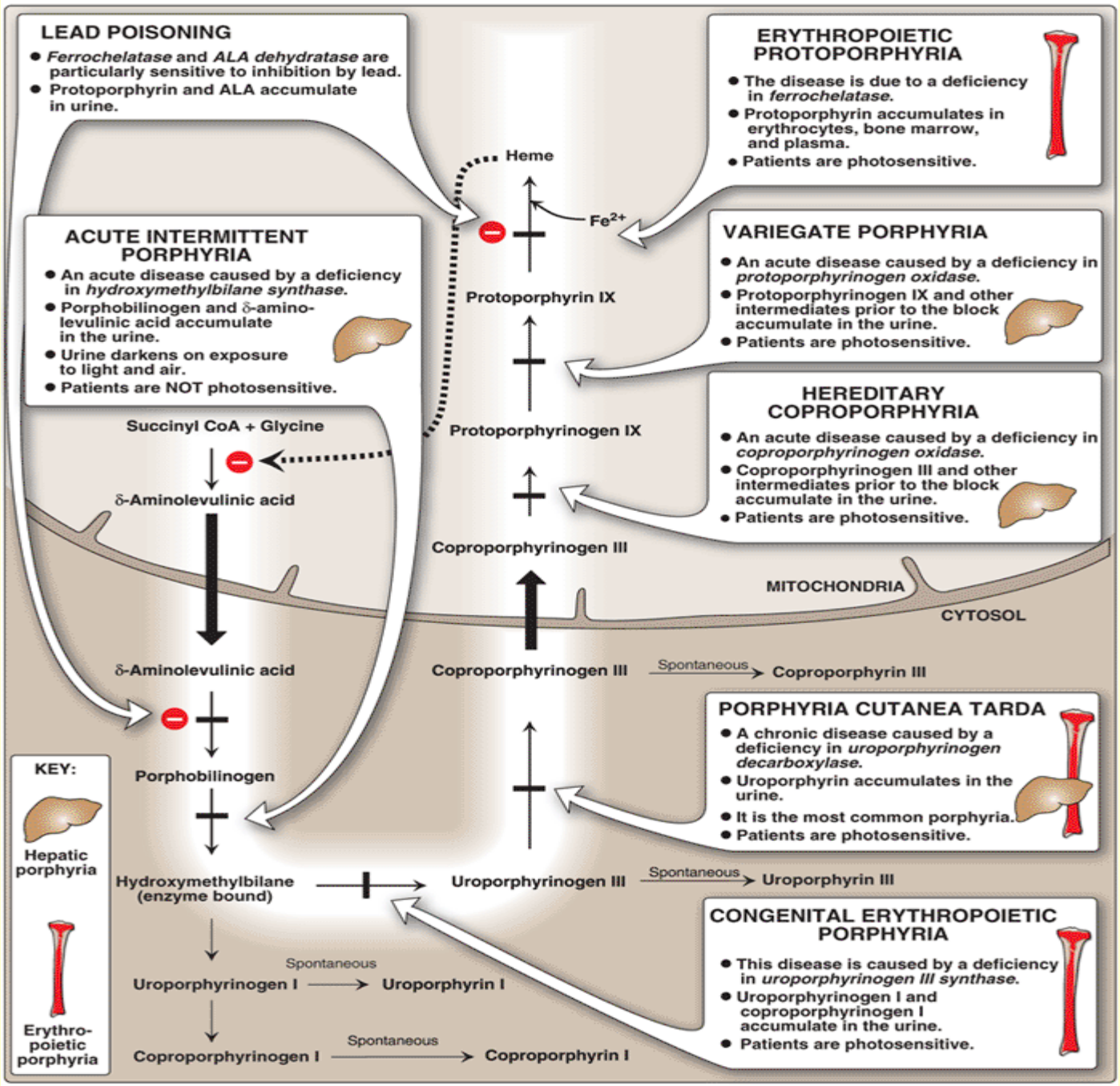
Erythropoietic porphyria

Erythropoietic protoporphyria	Ferrochelatase	Protoporphyrins accumulate in the Bone marrow, erythrocytes and plasma	+
Congenital Erythropoietic porphyria	Uroporphyrinogen III synthatase	Uroporphyrinogen I and coporphyrinogen I urine	+

Hepatic and Erythropoietic porphyria

Porphyria Cutanea Tarda (Chronic)	Uroporphyrinogen decarboxylase	Uroporphyrinogen I and coporphyrinogen Iin urine	+

Summary of heme synthesis and porphyrias



Summary of Major Findings in the Porphyrias

Enzyme Involved[2]	Type, Class, and MIM Number	Major Signs and Symptoms	Results of Laboratory Tests
1. ALA synthase (erythroid form)	X-linked sideroblastic anemia[3] (erythropoietic) (MIM 301300)	Anemia	Red cell counts and hemoglobin decreased
2. ALA dehydratase	ALA dehydratase deficiency (hepatic) (MIM 125270)	Abdominal pain, neuropsychiatric symptoms	Urinary ALA and coproporphyrin III increased
3. Uroporphyrinogen I synthase[4]	Acute intermittent porphyria (hepatic) (MIM 176000)	Abdominal pain, neuropsychiatric symptoms	Urinary ALA and PBG increased
4. Uroporphyrinogen III synthase	Congenital erythropoietic (erythropoietic) (MIM 263700)	No photosensitivity	Urinary, fecal, and red cell uroporphyrin I increased
5. Uroporphyrinogen decarboxylase	Porphyria cutanea tarda (hepatic) (MIM 176100)	Photosensitivity	Urinary uroporphyrin I increased
6. Coproporphyrinogen oxidase	Hereditary coproporphyria (hepatic) (MIM 121300)	Photosensitivity, abdominal pain, neuropsychiatric symptoms	Urinary ALA, PBG, and coproporphyrin III and fecal coproporphyrin III increased
7. Protoporphyrinogen oxidase	Variegate porphyria (hepatic) (MIM 176200)	Photosensitivity, abdominal pain, neuropsychiatric symptoms	Urinary ALA, PBG, and coproporphyrin III and fecal protoporphyrin IX increased
8. Ferrochelatase	Protoporphyria (erythropoietic) (MIM 177000)	Photosensitivity	Fecal and red cell protoporphyrin IX increased

Porphyrias Contd-----

Lead poisoning

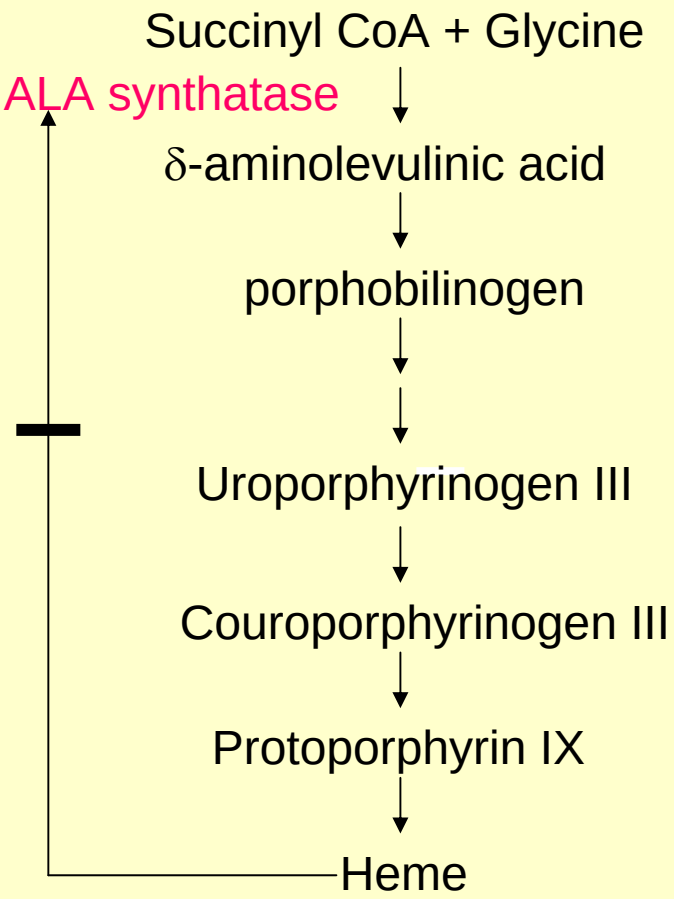
***Ferrochelatase and ALA synthase are inhibited**

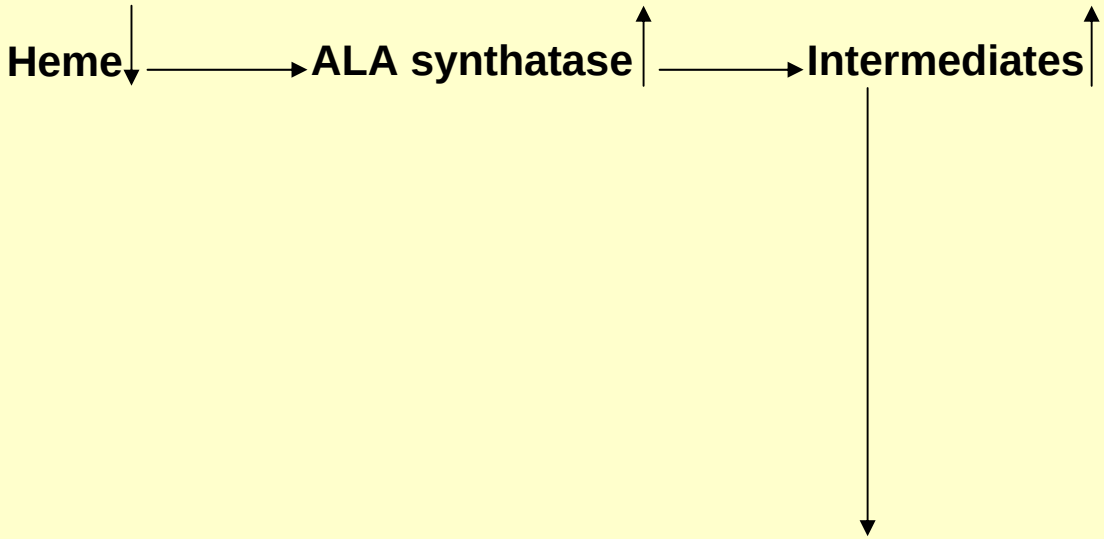
***Protoporphyrin and ALA accumulate in urine**

Photosensitivity

It is due to the porphyrin-mediated formation of superoxide radicals from oxygen. These reactive species can oxidatively damage membranes, and cause the release of lysosomal enzymes. Destruction of cellular components cause photosensitivity.

One common feature of porphyria is decrease synthesis of heme causing increase in ALA synthatase activity





Major pathophysiology of Porphyrias

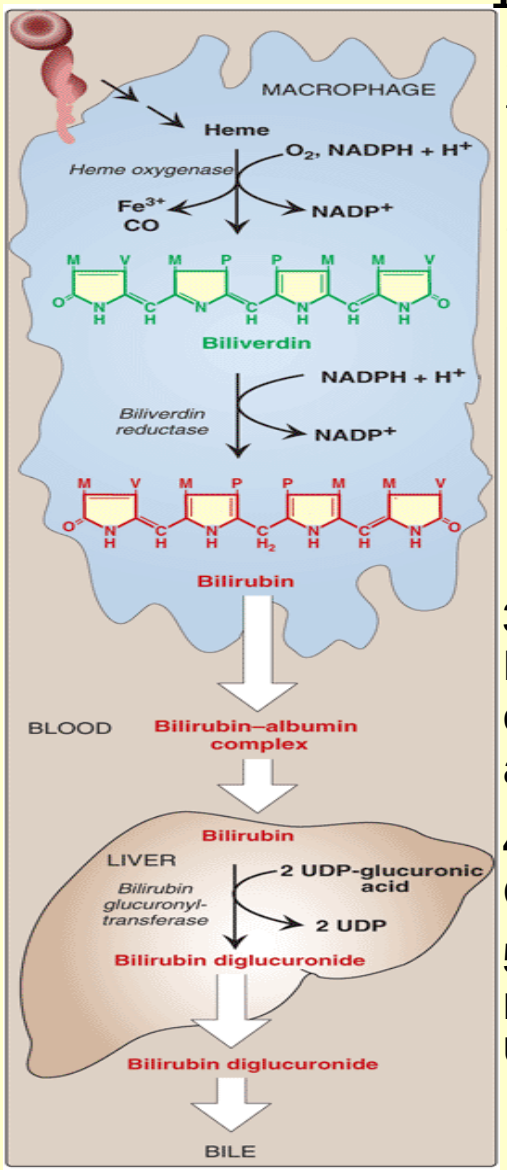
Treatment:

Intravenous injection of hemin to decrease the synthesis of ALA synthatase.

Degradation of heme

RBCs last for 120 days and are degraded by reticuloendothelial (RE) system [liver and spleen].

About 85% of heme destined for degradation comes from RBCs and 15% from cytochromes, and immature RBCs.



1) Formation of bilirubin

a) Microsomal heme oxygenase hydroxylates methenyl bridge between two pyrrole rings with concomitant oxidation of Fe^{2+} to Fe^{3+}.

b) A second oxidation by the same enzyme results in the cleavage of the porphyrin ring resulting in **biliverdin** (green color).

c) Biliverdin is then reduced by biliverdin reductase, forming the **bilirubin** (red-orange).

2) Uptake of bilirubin by liver

Bilirubin then binds to serum albumin and is transported to the liver.

3) Formation of bilirubin diglucuronide

Bilirubin is then conjugated to two molecules of glucuronic acid by the enzyme bilirubin glucuronyl-transferase using UDP-glucuronic acid as a glucuronate donor (to increase the solubility of bilirubin)

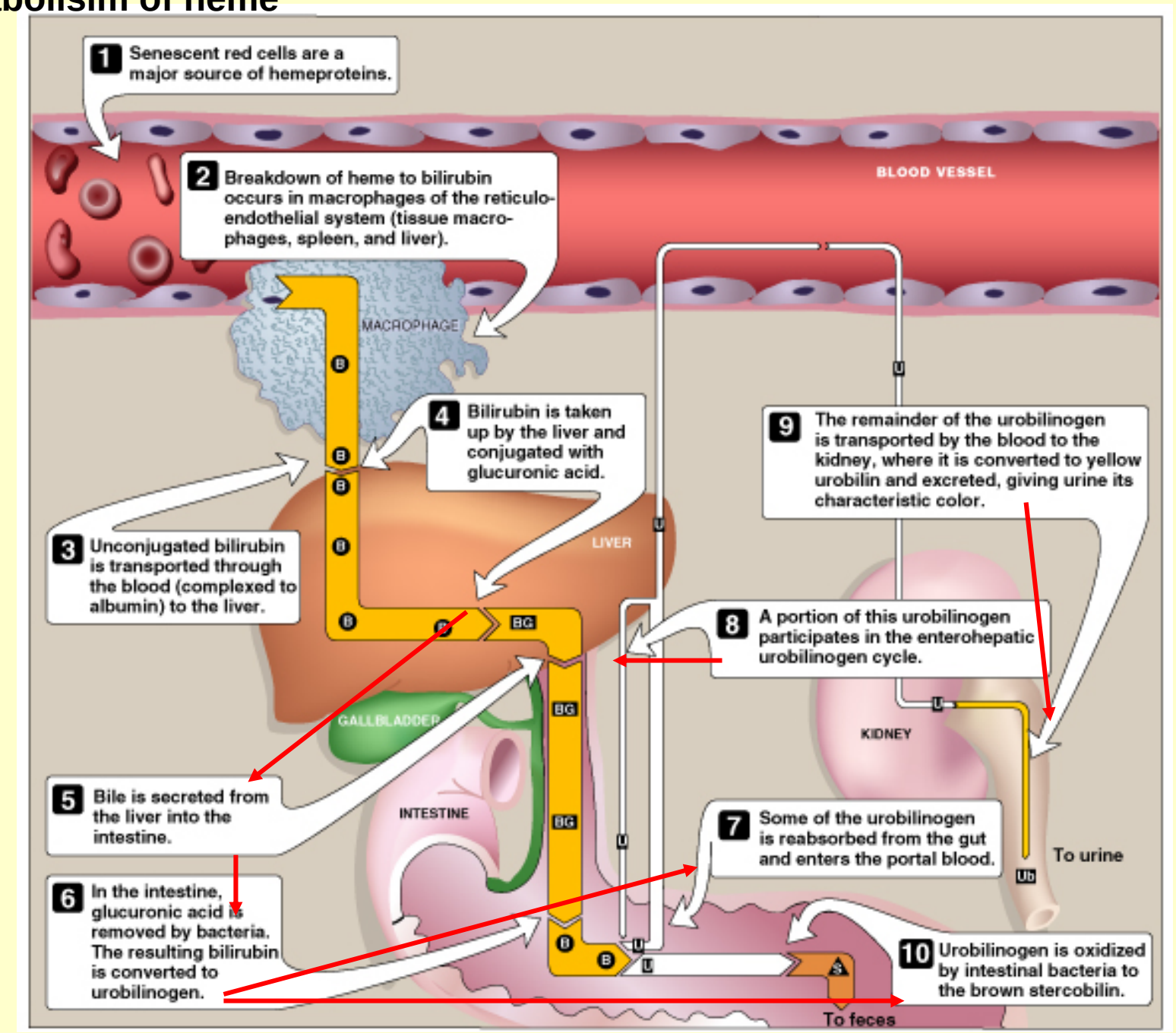
4) Secretion of bilirubin into bile

Conjugated form of bilirubin is the secreted into the bile.

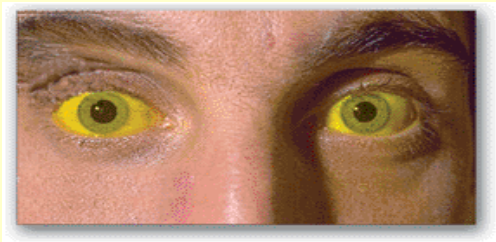
5) Formation of urobilins

Bilirubin diglucuronide is hydrolyzed and reduced by bacteria in the gut to yield Urobilinogen------oxidized to stercobilin.

Catabolisim of heme

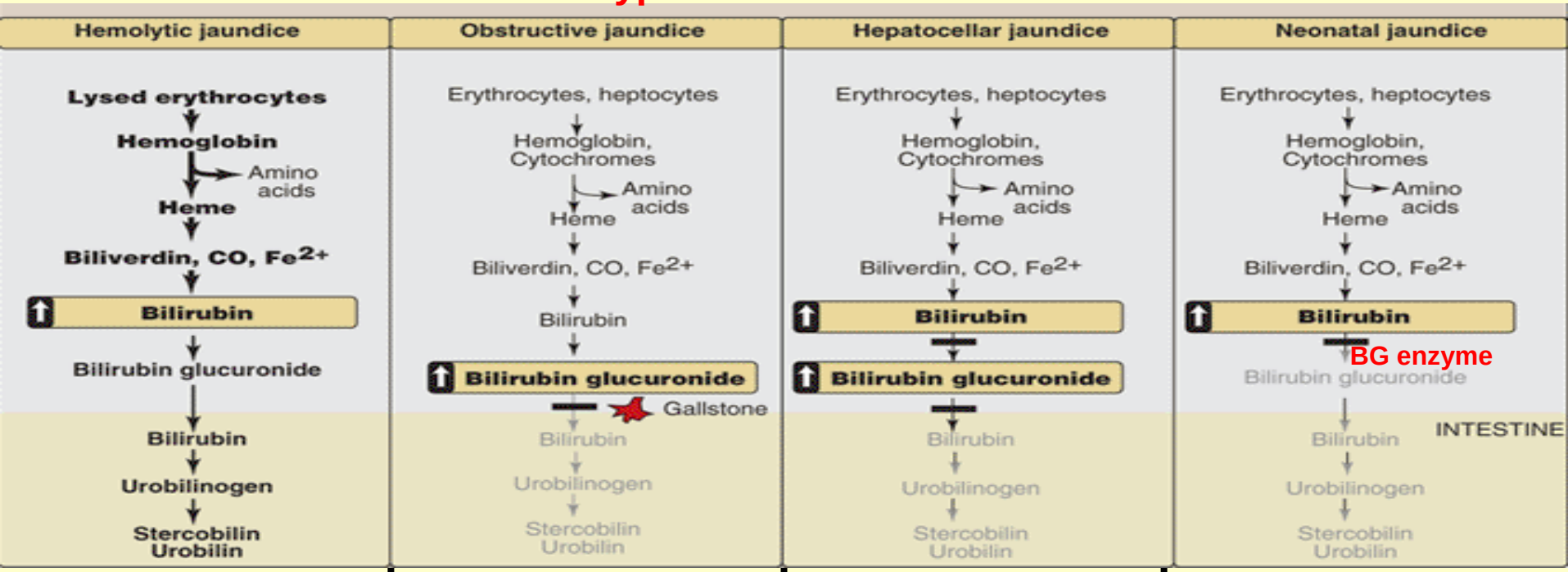


Jaundice



Yellow color of the skin, nailbeds, and sclerae (whites of the eyes) caused due to deposition of Bilirubin.

Types of Jaundice



*Liver can handle 3000 mg bilirubin/day – normal production is 300 mg/day in liver.
*Massive hemolysis leads to increase degradation of heme, and therefore production of bilirubin
*Bilirubin therefore cannot be conjugated.
* Increased bilirubin is excreted into bile, urobilinogen is increased in blood, urine. Unconjugated bilirubin in blood increases = jaundice

Obstruction of the bile duct (due to the hepatic tumor, or bile stones) prevents passage of bilrubin into intestine.

Prolonged obstruction of the bile duct can lead to liver damage and a subsequent increase in unconjugated Bilirubin

Damage to liver cells leads to decrease in glucuronidin Conjugation and increase in unconjugated bilirubin.

Premature babies often accumulate bilirubin due to late onset of expression of hepatic bilirubin Glucuronyltransferase (BG). This enzyme is normally low at birth and reaches adult levels in about four weeks.

Newborns are treated with blue Fluorescent light, which converts bilirubin to water soluble isomers.These photoisomers can be excreted into the bile without conjugation to gllucuronic acid.

Determination of Bilirubin concentration

Van der Bargh reaction

Diazotized sulfanilic acid + Bilirubin → **Diazopyrroles (red color)**

↓

Measured Calorimetrically

Other nitrogen containing compounds

Catecholamines

Dopamine, norepinephrine (noradrenaline)and epinephrin (adrenaline) are biologically active amines and are collectively called as Catecholeamines.

* Dopamine and norepinephrine functions as a neurotransmitters.

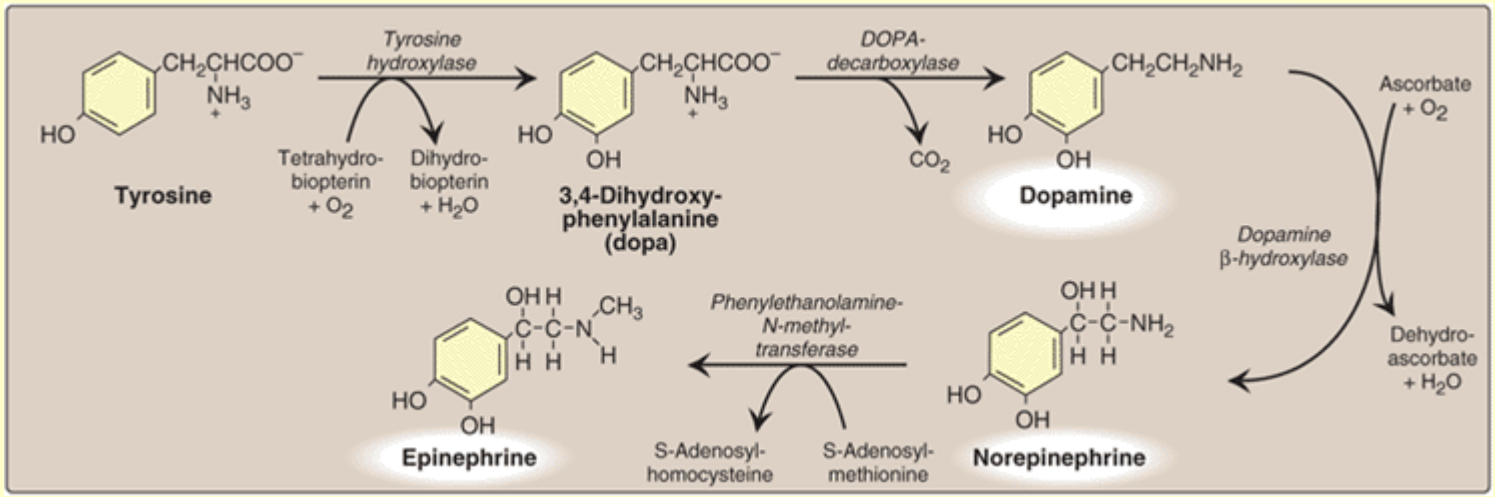
Outside the nervous system, norepinephrine and its methylated derivative, epinephrine regulates carbohydrate and lipid metabolism.

They are released from storage vehicles in the adrenal medulla in response to stress (fright, exercise, cold, and low levels of blood glucose).

They increase the degradation of glycogen,
and triglycerides, as well as increase blood pressure and the output of heart.

Synthesis of catecholamine

Catecholamines are synthesized from Tyrosine



- **Tyrosine is hydroxylated by tyrosine hydroxylase (rate limiting step in the pathway) to form DOPA.**
- **DOPA is decarboxylated by DOPA decarboxylase (pyridoxal phosphate requiring enzyme) to form dopamine.**
- **Dopamine is then hydroxylated by Dopamine β-hydroxylase to give norepinephrine.**
- **Epinephrine is formed by N-methylation reaction using S-adenosylmethionine as a methyl donor.**

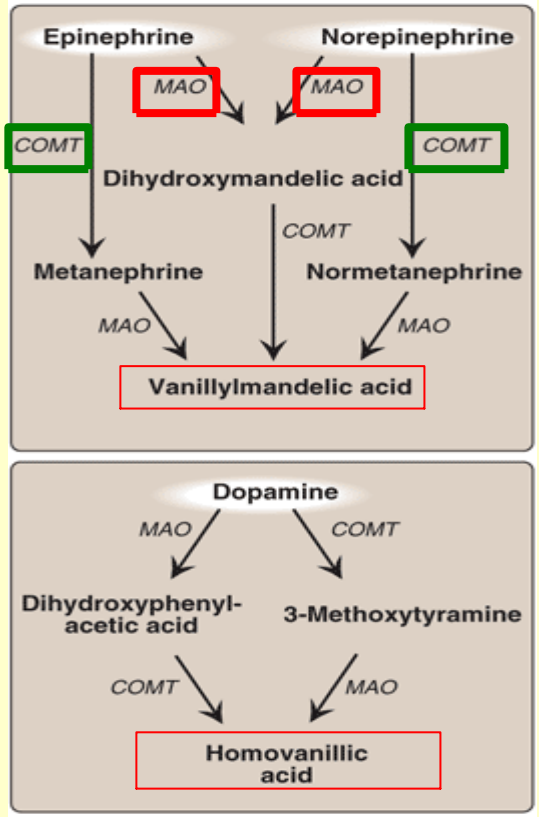
Parkinson's disease is caused due to the production of insufficient dopamine synthesis in brain

Degradation of catecholamines

The catecholamines are inactivated by oxidative deamination by **monoamine Oxiadase (MAO)** and by O-methylation carried out by **catechol-O-methyl-transferase (COMT)** as the one-carbon donor.

-two reactions can occur in either direction

-The aldehyde products of the MAO reaction are oxidized to the corresponding acids



MAO: inactivates catecholamines by oxidative deamination to yield the corresponding aldehyde

COMT: inactivates catecholamines by methylation using S-adenosylmethionine (SAM)

MAO inhibitors:

-found in neural tissue, gut and liver

-Antidepressant

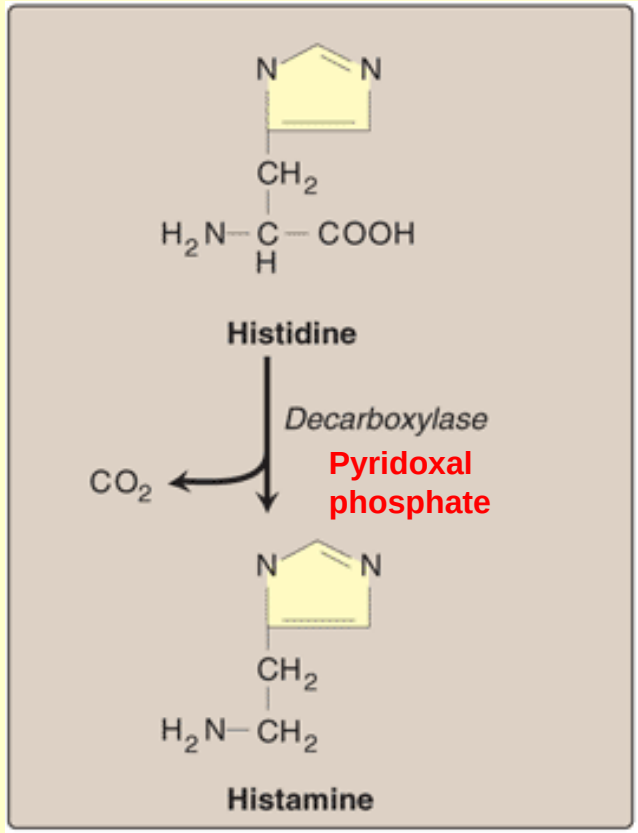
-Act by inhibiting MAOs

-Resulting in increase availability of neurotransmitters allowing their accumulation in the presynaptic neuron and subsequent leakage into circulation, providing an antidepressant action.

-metabolic products of the reaction are excreted in the urine.

Histamines

-A chemical messenger that mediates a wide range of cellular responses, including allergic and inflammatory reactions, gastric acid secretion, and possibly neurotransmission in the brain.

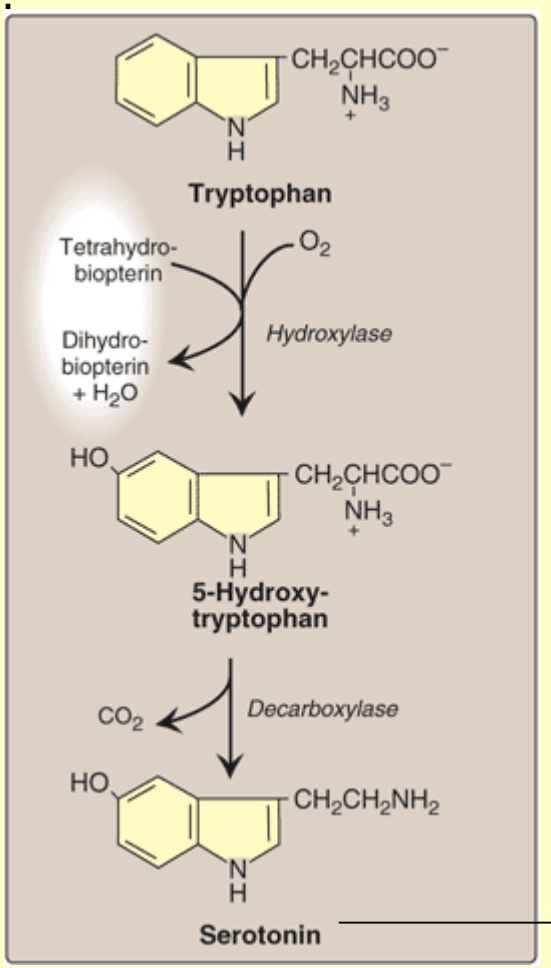


They are secreted by mast cells as a result of allergic reactions or trauma

Antihistamines are used to block histamine production during allergic reactions

Serotonin (5-hydroxytrptamine)

-mostly found in the cells of intestinal mucosa
-smaller amounts occurs in CNS were it functions as a neurotransmitter
-also found in platelet
-has roles in pain perception, affective disorders, regulation of sleep, temperature, and blood pressure.



Also degraded by MAO.

Creatine (phosphocreatine)

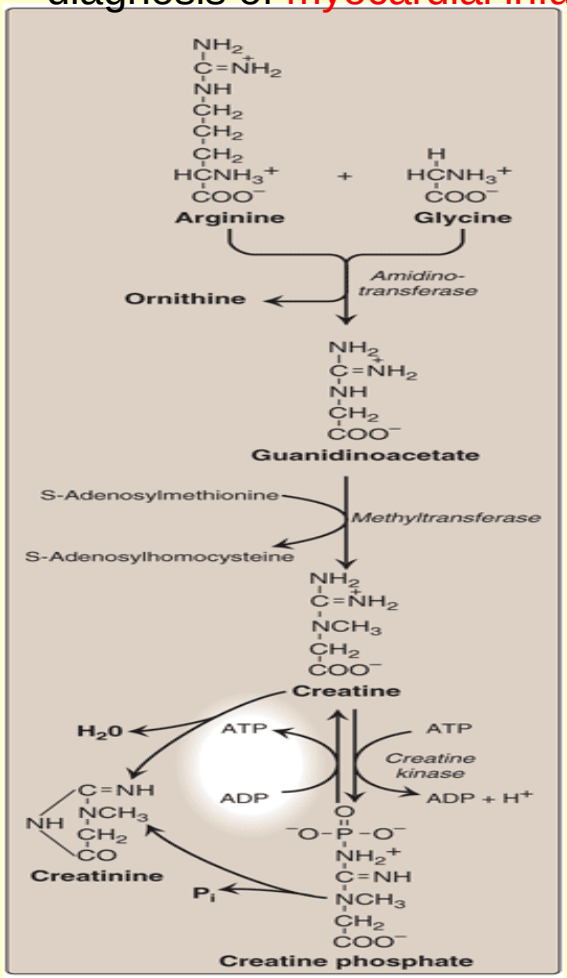
-Found in muscle

-High energy compound that can donate phosphate group to ADP to form ATP

-Creatine is reversibly phosphorylated to creatine phosphate by creatine kinase.

-Creatine phosphate serves as a reserve of high-energy phosphates that can be used to maintain ATP levels

-Levels of creatine kinase in plasma is an indicator of tissue damage and is used in the diagnosis of myocardial infarction



Synthesis

-Synthesized from glycine, and the guanidino group of arginine, plus a methyl group from S-adenosylmethionine.

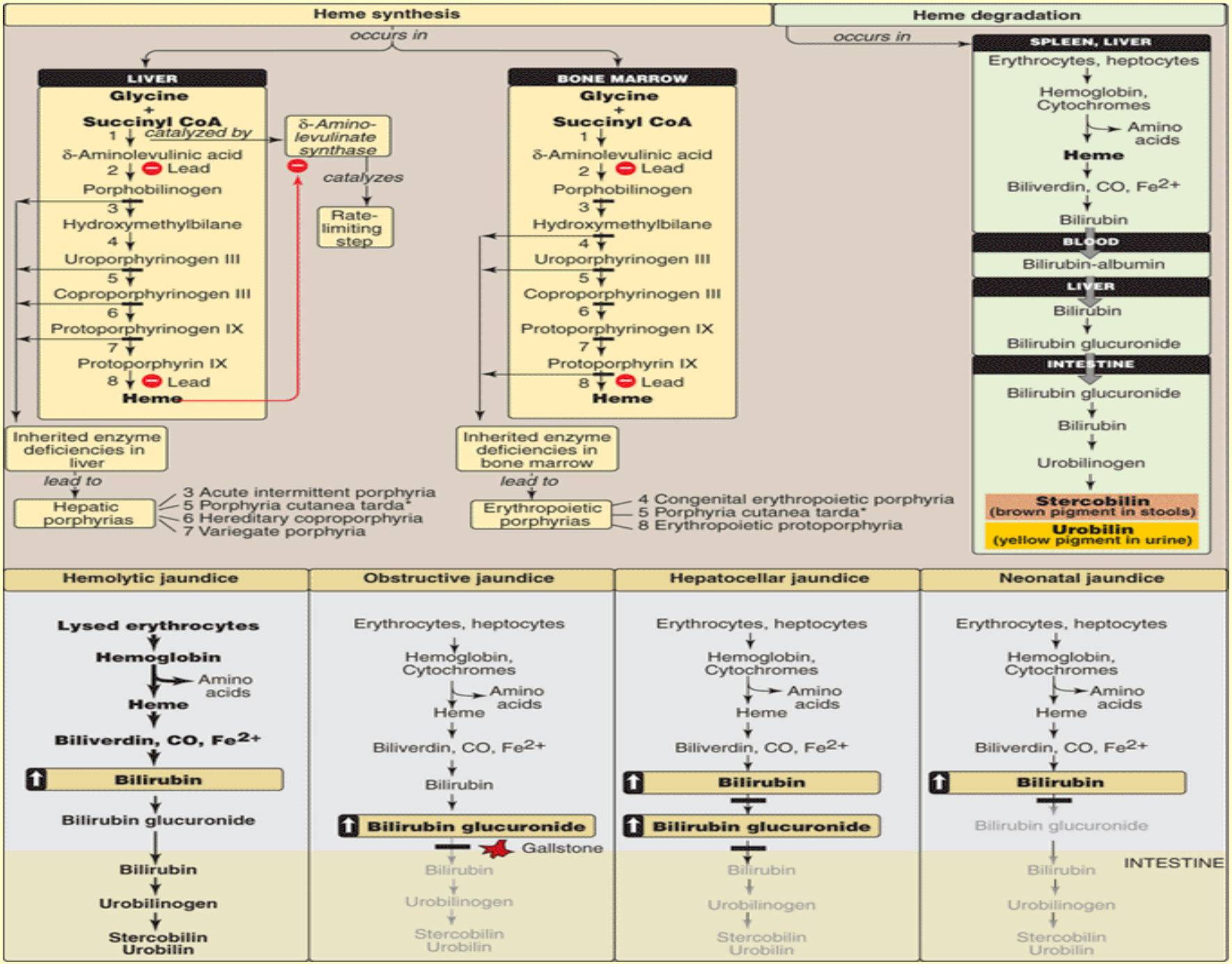
-reversibly phosphorylated to creatine phosphate by creatine kinase using ATP as a phosphate donor.

Degradation

Both creatine and creatine phosphate cyclize to form creatinine which is then excreted in the urine

Melanin

-Pigment that occurs in several tissues, e.g. in eye, skin, and hair.
-Synthesized from tyrosine in the epidermis by melaocytes
-Function is to protect tissues from sun-light
-Defect in melanin formation occurs in albinism due to the defective copper-containing enzyme tyrosinase.


Heme synthesis
Heme degradation
occurs in
occurs in
LIVER
Glycine
+
Succinyl CoA
1
catalyzed by
δ-Aminolevulinic acid
δ-Amino-levulinate synthase
2
Lead
Porphobilinogen
catalyzes
Rate-limiting step
3
Hydroxymethylbilane
4
Uroporphyrinogen III
5
Coproporphyrinogen III
6
Protoporphyrinogen IX
7
Protoporphyrin IX
8
Lead
Heme
BONE MARROW
Glycine
+
Succinyl CoA
1
δ-Aminolevulinic acid
2
Lead
Porphobilinogen
3
Hydroxymethylbilane
4
Uroporphyrinogen III
5
Coproporphyrinogen III
6
Protoporphyrinogen IX
7
Protoporphyrin IX
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lead to
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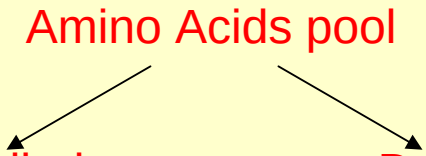
Erythropoietic porphyrias
4 Congenital erythropoietic porphyria
5 Porphyria cutanea tarda*
8 Erythropoietic protoporphyria
SPLEEN, LIVER
Erythrocytes, heptocytes
Hemoglobin, Cytochromes
Amino acids
Heme
Biliverdin, CO, Fe2+
Bilirubin
BLOOD
Bilirubin-albumin
LIVER
Bilirubin
Bilirubin glucuronide
INTESTINE
Bilirubin glucuronide
Bilirubin
Urobilinogen
Stercobilin
(brown pigment in stools)
Urobilin
(yellow pigment in urine)
Hemolytic jaundice
Lysed erythrocytes
Hemoglobin
Amino acids
Heme
Biliverdin, CO, Fe2+
Bilirubin
Bilirubin glucuronide
Bilirubin
Urobilinogen
Stercobilin
Urobilin
Obstructive jaundice
Erythrocytes, heptocytes
Hemoglobin, Cytochromes
Amino acids
Heme
Biliverdin, CO, Fe2+
Bilirubin
Bilirubin glucuronide
Gallstone
Bilirubin
Urobilinogen
Stercobilin
Urobilin
Hepatocellar jaundice
Erythrocytes, heptocytes
Hemoglobin, Cytochromes
Amino acids
Heme
Biliverdin, CO, Fe2+
Bilirubin
Bilirubin glucuronide
Bilirubin
Urobilinogen
Stercobilin
Urobilin
Neonatal jaundice
Erythrocytes, heptocytes
Hemoglobin, Cytochromes
Amino acids
Heme
Biliverdin, CO, Fe2+
Bilirubin
Bilirubin glucuronide
INTESTINE
Bilirubin
Urobilinogen
Stercobilin
Urobilin

I

Lecture I



Supplied

a) Degradation (Lysosomal and proteasome)
b) Dietary protein
c) Do novo synthesis

Depleted

a) Synthesis of body proteins
b) Precurssors for essential N-containing molecules

II Digestion of dietary proteins

a) Gastric enzymes
b) Pancreatic enzymes
c) Small Intestinal enzymes (proteases cascade)
d) Amino acid specificity for proteolytic enzymes

III) How amino acids are transported in to cells

Transport systems

IV) Removal of nitrogen from amino acids

a) Transamination (aminotransferases)
b) Oxidative deamination (Glutamine dehyrogenase)

V) Urea cycle

Reactions of urea cycle: a) locations b) sequence b) enzymes for each reaction c) end products for each reactions d) ATP requirements e) sources of nitrogens in urea

VI) Metabolism of ammonia

a) Sources of ammonia,
b) transport of ammonia,
c) Urea cycle defects in humans

Lecture II

I) Essential and non essential amino acids

Names of the essential and non essential aa

II) Glucogenic and ketogenic amino acids

a) Why amino acids are classified as glucogenic and ketogenic or both?
b) Seven intermediates of carbon skeleton
c) Amino acids that form those intermediates

III) Catabolism of the branched-chain amino acids

IV) Biosynthesis of nonessential amino acids

a) Syhthesis from α-keto acids
b) Synthesis by amidation
c) Synthesis of proline, serine, glycine, cysteine, tyrosine

V) Metabolic defects in amino acid metabolism

a) Phenylketourea
b) Maple Syrup urine disease
c) Albinism
d) Homocystinuria
e) Alkaptouria

Defective enzyme
Amino acid involved
Accumulated intermediate
Characteristics

Lecture III

I) Amino acids as a precursors for:

Porphyrines

Heme:

a) Synthesis
b) Degradation
c) Diseases caused due to the defective heme synthesis & degradation (Jaundice)

Catecholamines (Dopamine, epinephrine, Norepinephrine)

a) Synthesis
b) Degradation

Histamine

Serotonine

Creatine

Melanine