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Biochemistry

Doctor 2017 | Medicine | JU

Sheet ○ Slides

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DIALA

NOTE : ask OMAR about the first 5 pages and SAIF about last 5

Amino Acid Metabolism Disorders

Phenylketonuria: (PKU)

Phenylketonuria is a disorder involving the **Metabolism** of **Phenylalanine**, normally it is **hydroxylated** into **Tyrosine** by the Phenylalanine **Hydroxylase** and its co-enzyme Tetrahydrobiopterin (**BH4**).

During the reaction, BH4 is **Oxidized** into **BH2** (Dihydropteridine) and is recycled back to BH4 by an enzyme called Dihydropteridine **Reductase**. Deficiencies in BH2 Reductase may cause PKU as well as deficiencies in Phenylalanine Hydroxylase.

Other than Phenylalanine metabolism into Tyrosine, BH4 is also involved in:

1) **Catecholamine** synthesis from Tyrosine.

2) **Serotonin** Synthesis from Tryptophan.

So any deficiencies involved in BH2 Reductase will affect these reactions as well.

PKU Main Symptoms: (HMM)

Hypopigmentation/ Musky Urine/ Mental Retardation.

Maple Syrup Urine Disease (MSUD)

A rare disease that is caused by **Branched Chain α- ketoacid Dehydrogenase** Deficiencies, which is the main enzyme responsible for the **Metabolism** of Branched Chain Amino Acids (**Leucine**/**Isoleucine**/**Valine**). This leads to accumulation of these BCAAs, having toxic effects on brain functions, mainly Mental Retardation or Physical Diseases.

Deficiency of the enzyme is classified according to its **severity** into 2 types:

1) **Classical** (*MORE SEVERE*): Almost complete deficiency of the enzyme, could be life threatening.

2) **Intermediate**: Less severe form.

(**Signs and Symptoms**):

1) Urine that smells like maple syrup.

2) Neonate feeding problems: (Refuses feeding/ vomiting/ dehydration)

3) Mental retardation or physical disabilities.

Symptomatic Treatment is usually the best solution, mainly by controlling the diet by decreasing the consumption of BCAAs.

Albinism

A disorder that is caused by the **inability to metabolize Tyrosine** as a precursor of **Melanin** (Pigment).

It involves a wide spectrum of genes and enzymes, and therefore it has many types of inheritance: Autosomal **Recessive** (Primarily) / Autosomal Dominant / X- linked, Depending on the gene and enzyme affected.

In the **most severe** case of Albinism, the copper- requiring enzyme **Tyrosinase** is deficient.

Signs and Symptoms include:

1) Absence of pigment (Melanin) in hair/ eyes/ skin.

2) Photophobia (Sensitivity of light) + Vision problems

3) Higher risk of skin cancer.

Homocystinuria

A disorder involving the **inability to metabolize Homocysteine** (RECALL: The branching point in Methionine metabolism), because of a deficiency in one of the enzymes involved in cysteine production (one of 2 pathways homocysteine can undergo in its metabolism).

This enzyme is **Cystathionine β synthetase**, responsible for the joining of **Serine** with **HCys** with the depletion of ATP to form Cystathionine, using **vitamin B6** as a co-enzyme.

Increased **HCys** + **Met** concentrations in the plasma + urine.

It has an autosomal **Recessive** mode of inheritance.

Treatment is symptomatic, usually by giving the patient:

1) Vitamin B6: Increase cysteine synth. From HCys. (*MAY HELP IF DISORDER IS NOT SEVERE*)

2) Vitamin B12 + Folic Acid: Increase Methionine Synthesis from HCys.

Signs and symptoms usually include:

Neurological Problems/ Skeletal Problems (**Osteoporosis**) / Ectopia Lentis / Thrombi.

Alkaptonuria

A *rare non-life threatening* disorder, caused by the deficiency of an enzyme called **Homogentisic Acid Oxidase**, which is involved in the **metabolism of Phenylalanine and Tyrosine**, causing the accumulation of **Homogentisic Acid** (**Black**) in the plasma and urine (common in all cases) as well as other variable locations (cartilage) in different cases like certain joints: (Treatment is usually by joint replacement):

a) Intervertebral Discs: Back pain, easily misdiagnosed as a case of a slipped disc.

b) Earlobe: Black pigmentation, usually thought of as racial pigmentation.

Recent cases have been found in Jordan and multiple researches and tests are being done to treat it.

Synthesis of Nitrogen Containing Compounds from AAs.

Causes the depletion of the AA pool in the body.

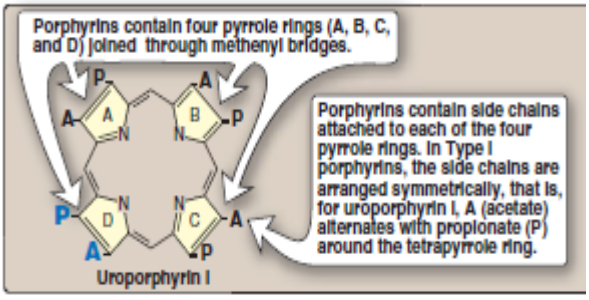
Porphyrin:

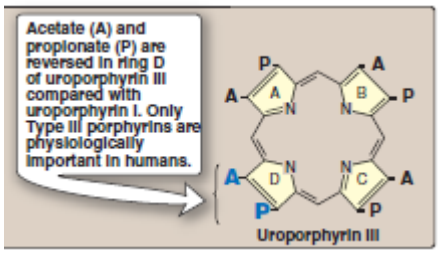
Ring Molecule made of **4 pyrrole rings** and a central **Fe** atom bound to all 4.

Each pyrrole ring contains **2** side chains.

Heme is an example of a Porphyrin Structure.

Porphyrins differ from each other by their side chain variability:





1) **Uro**porphyrin: Contains **Acetate** and **Propionate**, as their sequence is important as well: (A stands for Acetate and P for Propionate)

a) Uroporphyrin I: AP AP AP **AP (look at the picture above)**

b) Uroporphyrin III: AP AP AP **PA**

2) **Copro**porphyrin: Contains **Methyl** and **Propionate**.

3) **Proto**porphyrin IX + Heme: Contains **Vinyl**, **Methyl** and **Propionate**.

Porphyrin Synthesis:

Heme synthesis occurs <u>at all times</u> to supply the renewal of RBCs, as heme (Hemoglobin) is destroyed along with the RBCs and synthesized from the beginning.

Other heme containing proteins are **Myoglobin** and **Cytochromes** (P450).

85% of heme synthesis occurs in the **bone marrow**, specifically in cells called <u>Erythrocyte Producing cells</u> (not mature RBCs because they <u>lack a nucleus and the organelles (like mitochondria) required</u> for the process), while **15**% occurs at the **liver** to produce CYP450.

The Steps: (*PICTURES OF THE STEPS ARE INCLUDED IN THE NEXT PAGE*)

1) **Glycine** (AA) and **succinyl CoA** (AA derivative) are joined together by: ALAS 1 in the liver and ALAS 2 in the bone marrow, to form **ALA**. (They are inhibited by both Hemin and Iron, respectively). (NOTE: HEMIN is almost identical to HEME except the Fe is in the **<u>FERRIC</u>** state (Fe+3))

(***<u>**RATE LIMITING STEP**</u>***)

*ALAS: γ- **AminoLaevulinic Acid** Synthase and its co-enzyme is pyridoxal phosphate (PLP)

2) The **Condensation** of **2 ALA molecules** together by an enzyme called Porphobilinogen Synthase, producing **Porphobilinogen**.

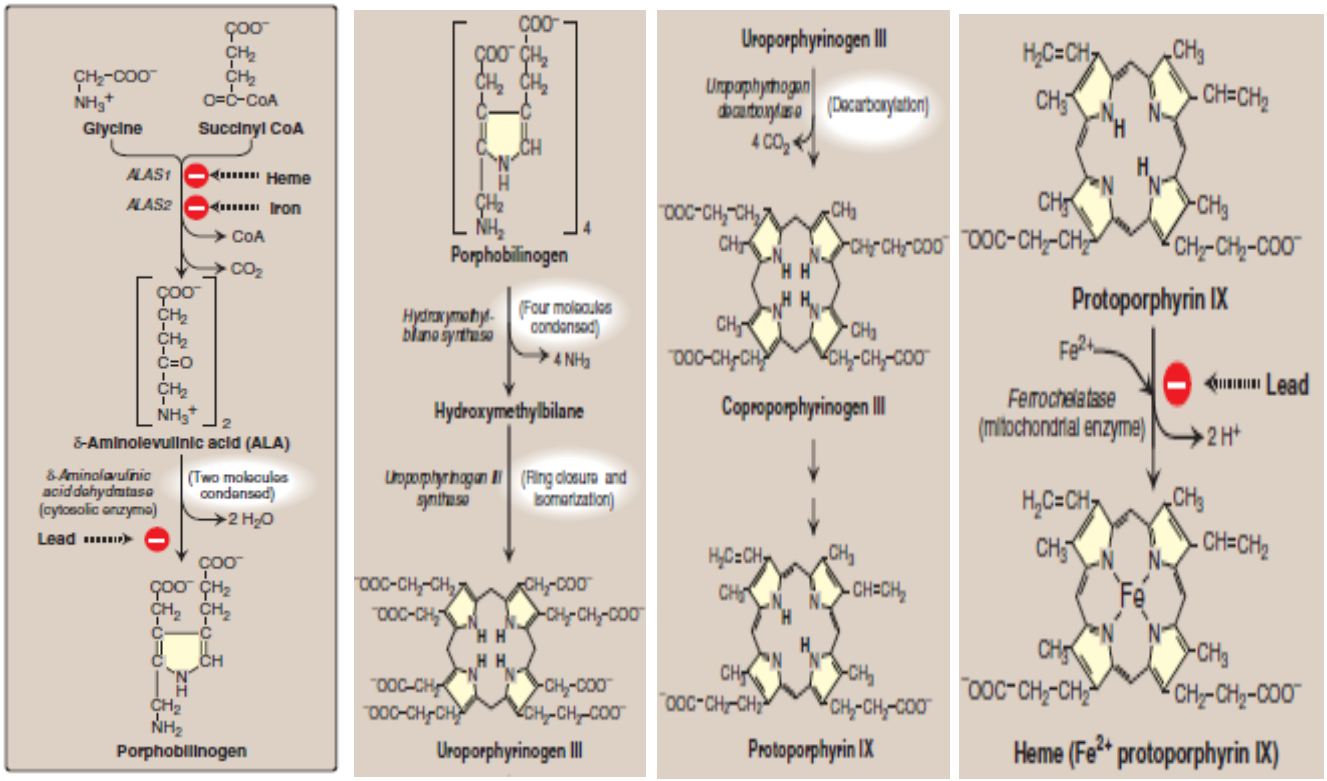
3) The formation of **Uro**porphyrinogen **III** by the **Condensation**, **Isomerization** and **Cyclization** of **4 molecules of Porphobilinogen** by the action of Uroporphyrinogen III Synthase enzyme.

4) Uroporphyrinogen III undergoes **Decarboxylation** reactions (from its **4 acetate** groups yielding **4 methyl** groups instead) and **Copro**porphyrinogen III is produced.

(**UP UNTIL NOW EVERYTHING DISCUSSED OCCURS IN THE CYTOSOL**)

5) Coproporphyrinogen III enters the **Mitochondria** and undergoes **Decarboxylation** reactions (from **2 of its propionate** groups yielding **2 vinyl groups** instead) and **Proto**porphyrinogen IX, this process also involves the **Removal of 2 H atoms (Oxidation)** from the middle of the ring structure.

6) The final step involves the activation of **Proto**porphyrinogen IX by **Oxidation (the removal of the remaining 2 H atoms)** to produce **Proto**porphyrin IX, and the introduction of iron in its FERROUS (Fe+2) state occurs spontaneously (Sped up by the action of **ferrochelatase**) to generate the completed **heme** molecule.

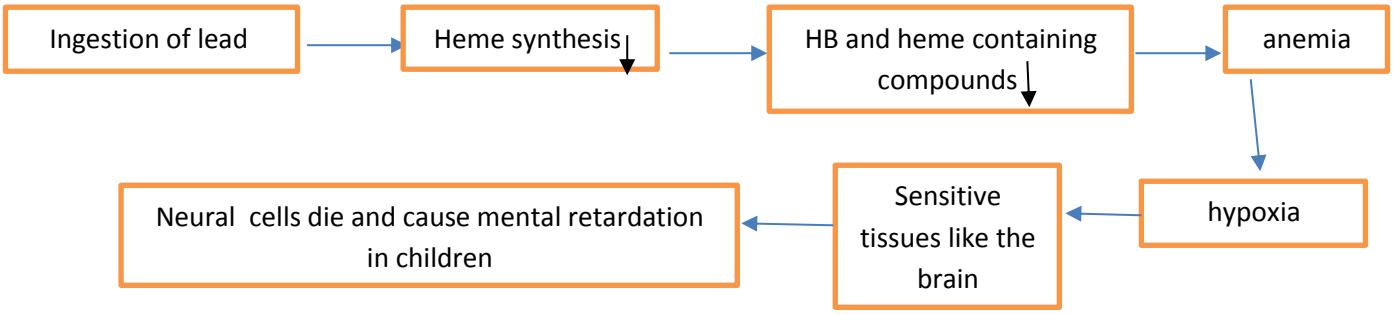


Let's talk about how **lead affect heme synthesis "inhibition"**

1-It inhibits the formation of **porphobilinogen** "the first ring structure that has a pyrrole ring" by inhibiting ALA dehydratase, so ALA accumulates.

2- Inhibiting the introduction of iron to the **protoporphyrin IX** by Inhibiting ferrochelatese so protoporphyrin IX accumulates.

Susceptible patients :children are most susceptible because cheap toys have lead mixed with plastic and metals AND people who work in paint factories because it contains lead.



Now we will talk briefly about **drug metabolism** :

Ingestion→absorption→detoxification by liver (specifically cytochrome P450 monooxygenase system)→ excretion.

How drugs affect heme synthesis ?

a lot of drugs activate ALAS1, therefore increasing heme synthesis which is used in building cytochrome P450 to degrade these drugs "cytP450 contains heme for electron transporting" so heme concentration decreases, and this activates ALAS1 in hepatocytes to resynthesize heme. **SO DRUGS INCREASE HEME SYNTHESIS.**

***Porphyrias:**

refers to the purple color caused by pigment-like porphyrins in the urine of patients so the disease has pigmentation effect.

Rare. inherited (or occasionally acquired) defects in heme synthesis.

Accumulation and increased excretion of porphyrins or porphyrin precursors .

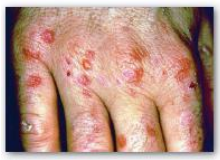


Figure 21.6
Skin eruptions in a patient with porphyria cutanea tarda.

Figure 21.7
Urine from a patient with porphyria cutanea tarda (right) and from a patient with normal porphyrin excretion (left).

Mutations are heterogenous (not all are at the same DNA locus), and nearly every affected family has its own mutation.

Each porphyria results from a different enzyme deficiency ”by genetic mutation” and results in the accumulation of different intermediates of heme synthesis

So reduction of heme synthesis → heme level is decreased→activating ALAS1→ but we already have enzyme deficiency in the heme synthetic pathway so no heme will be synthesized resulting in exhaustion of the liver by trying to synthesize heme→intermediate accumulation → pigmentation of urine , skin…

How to treat this condition ?

Simply by inhibiting ALAS1 through IV injections of hemin “heme that has ferric ion” or glucose.

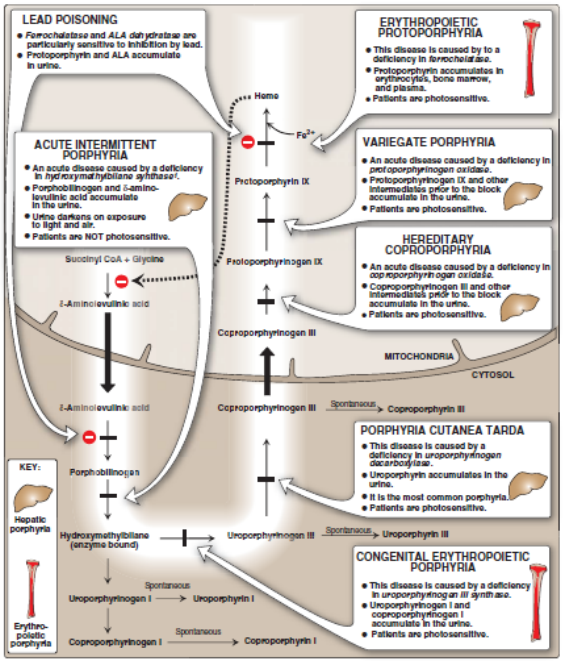
NOTE : heme conc.↑ → Repress genes coding for ALAS1

Heme conc.↓ → Activate genes coding for ALAS1

Also **Treatment of symptoms** like **:** pain and vomiting during acute attacks.

Ingestion of β-carotene (a free-radical scavenger) will help.

*the following picture is not for memorizing read it for extra info.



INTRODUCTION for heme degradation :

Rbc’s are degraded by reticuloendothelial system(RES) every 120 day so heme is degraded and **we resynthesize it again from scratch** “ not recycling”…this is waste of energy but it has functions that we will discuss later.

RES: it’s not a system actually it’s a group of macrophages scattered in different organs mainly liver and spleen **macrophages of spleen are mainly involved in heme degradation but removal of the spleen due to energy is not lethal, while spleen is important, RES can compensate for the missing degradation function of the spleen **

HEME DEGRADATION

FIRST step of heme degradation is: removal of heme from hemoglobin or other heme containing compound "cyt,myoglobin.." then the ***protein part*** will be degraded either by lysosomal or proteasomal **mainly proteasomal inside cells**

And ***heme*** will enter macrophages of RES for degradation .

~85% of degraded heme comes from senescent RBCs AND **~15%** of degraded heme comes from immature RBCs turnover and cytochromes of nonerythroid tissues.

SECOND STEP: is this reaction in the macrophages ""heme→biliverdin→bilirubin""

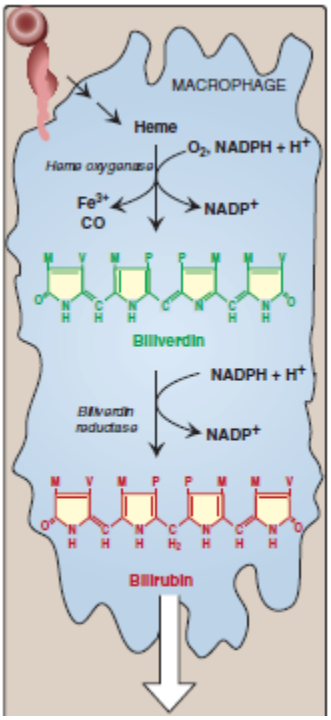
MECHANISM:(1) heme oxygenase will introduce O2 and reduce it using NADPH to make **biliverdin** , ferric ion (+3) , CO and NADP+

Biliverdin formation by the addition of an ***OH*** to the methenyl bridge between two **pyrrole** rings, and then a **second oxidation** by the same enzyme system to cleave the porphyrin ring.SO **biliverdin** is linear porphyrin (opened) and it has a **green** color.

(2): biliverdin is reduced by **biliverdin** reductase to **bilirubin (colored red-orange)** using one NADPH…SO bilirubin is mostly made by non-polar bonds and it's a non-polar molecule (hydrophobic).

Bilirubin and its derivatives are called bile pigments. Bilirubin functions as an antioxidant (oxidized to biliverdin)

SUMMARY



INTRODUCTION to the **3rd step:**
We said bilirubin is hydrophobic so we need to transport it to the liver to solubilize it there "metabolism always tend to solubilize molecules"

THIRD STEP: uptake of bilirubin by the liver :
1-bilirubin exits the macrophages into the blood where it binds to **albumin non-covalently** " so it can unbind again"
2- when it reaches the liver it will be detached from albumin and **enter hepatocytes by facilitated diffusion.**

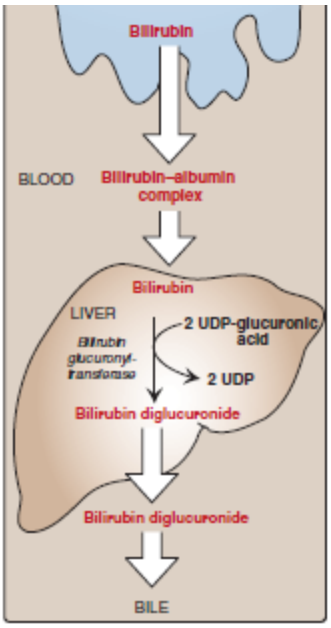
Note: certain anionic drugs, such as salicylates and sulfonamides, can displace bilirubin from albumin, allowing bilirubin to enter the CNS causing neural damage in infants.

3-in hepatocytes, bilirubin binds to intracellular proteins, such as, ligandin as a carrier to ER where it will detach to be conjugated with ***two glucuronate molecules "***which is highly oxidized having 2 carboxyl groups so highly soluble"

** UDP is the active carrier of glucoronate and the conjugation reaction is carried by **microsomal bilirubin glucuronyl transferase** **forming** **bilirubin diglucuronide**

Deficiency of this enzyme results in Crigler-Najjar I and II (more severe) and Gilbert syndrome(mild and common **, A**symptomatic, get tired when fasting , live normally unlike crigler syndrome).

INTRODUCTION TO STEP 4: now the complex has to leave the liver to perform its function before being excreted



STEP FOUR: (*****RATE LIMITING STEP**)*** conjugated bilirubin is **actively** transported into the **canaliculi of the biliary system** then into the bile in the common bile duct with the excretion of gall bladder changing to yellow color.

REMEMBER :1-rate limiting step is the one which needs energy always.

2- the bile contains bile acids and salts which help in lipid metabolism as emulsifiers.

Dubin-Johnson syndrome results from a deficiency in the transport protein of conjugated bilirubin so the complex accumulates . Unconjugated bilirubin is normally not secreted.

STEP FIVE: once the complex reaches the small intestines it will be exposed to the normal flora of the intestines which hydrolyze bilirubin from glucuronate and reduce bilirubin to colorless molecule called urobilinogen

Urobilinogen fates:
Oxidation by intestinal bacteria to stercobilin (gives feces the characteristic brown color) OR Reabsorption from the gut and entrance to the portal blood then back to the liver to either get 1- transported by the blood to the kidney, where it is converted to yellow urobilin and excreted, giving urine its characteristic color.2- Some urobilinogen participates in the enterohepatic urobilinogen cycle where it is taken up by the liver, and then resecreted into the bile.

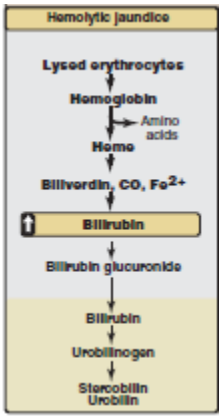
*abnormal color of the urine indication for metabolism problems and indication about what food patient consumes as some foods contain pigments.

TAKEAWAYS: senescent RBC's are major source of heme proteins→breakdown of heme to bilirubin occurs in macrophages of RES→ bilirubin attached to albumin in the blood plasma and transported to the liver → bilirubin is taken up by facilitated diffusion to the liver and conjugated to glucuronate → the complex actively transported to the bile coloring it yellow then to the intestines → the complex is hydrolyzed and bilirubin is reduced by intestinal flora into urobilinogen(colorless) →some of urobilinogen enters portal blood either to participate in enterohepatic urobilinogen cycle or to get transported to the kidney where its converted to urobilin(yellow pigmentation of urine)→other urobilinogen oxidized by intestinal flora to brown stercobilin.

Jaundice

Jaundice (or icterus) is the yellow color of skin, nail beds, and sclera(white part of eye) primarily **due to bilirubin deposition,** secondarily to **hyperbilirubinemia** …Jaundice is a symptom not a disease which is related to heme degradation.

First type is **hemolytic jaundice** : due to increased hemolysis. RBC's turnover is controlled (every 120 days) because the amount of heme degraded is compatible with the liver's capacity to conjugate bilirubin BUT in a patient with **sickle cell anemia, pyruvate kinase or glucose-6-phosphate dehydrogenase deficiency** which suffers favism when consuming fava beans or other nutrients.



HB degradation↑→heme↑→ Bilirubin conjugation and excretion capacity of the liver is >3,000 mg/day and normally 300 mg/day of bilirubin produced so increase in heme production beyond liver's capacity leads to accumulation of bilirubin.

Second type is **Hepatocellular jaundice** due to damage of liver cells.SO heme production is normal but liver is damaged due to disease such as fibrosis, cirrhosis.. which prevent it from conjugating bilirubin and bilirubin accumulates according to the severity of the damage SO if 25% of the liver is damaged→ 25% of bilirubin accumulates (300x25%) ..More unconjugated bilirubin levels in the blood
Urobilinogen is increased in the urine cuz its production is not related to the liver.(the enterohepatic circulation is reduced) resulting in dark urine.
Stools may have a pale, clay color.

TEST YOURSELF

1-How many NADPH molecules are used in heme degradation:

a)7 b)4 c)8 d) 2 e)5

2-bilirubin is red-orange , urobilin is yellow , stercobilin is colorless and urobilinogen is brown …choose the correct answer regarding the previous statement :

a) True b) correct c)a+b c) all of the above e) false

3 – the rate limiting step in heme degradation is :

a) Bilirubin conjugation to glucuronate
b) Bilirubin uptake by the liver
c) Heme attachment to albumin
d) Bilirubin diglucuronide secretion into the bile

4-regarding heme synthesis which of the following is inhibited by lead :

a) uroporphyrinogen III synthase
b) elastase
c) decarboxylase
d) catalase
e) ferrochelatase

5- Which of the following is the correct sequence of Acetate and Propionate in Uroporphyrin III:

a) PP AP PP AP
b) AP PA PA AP
c) AP AP AP PA
d) AP AP AP PA

6- Regarding heme synthesis, the first reaction that takes place in the mitochondria is:

a) Decarboxylation
b) Hydrolysis
c) Conjugation
d) Reduction

FLIP UR SHEET

DEDECA