1. **Acquired Porphyrias (lead poisoning)**
   - inactivates ALA DHD
   - ALA appears in urine
   - inhibits protoporphyrinogen oxidase
   - free protoporphyrinogen III and Zn-protoporphyrinogen III accumulate
   - blocks ferrochelatase

   Overall effect: interferes with iron transport into the mitochondria

2. **Causes and characteristics of jaundice**  
   (use examples from class)
   - You can tell jaundice because high levels of bilirubin in blood → so much so that it will start to diffuse into the tissues and give skin yellow color

   Causes:
   1. increased production of bilirubin eg. during hemolytic anemia when heme and iron is being recycled
   2. decreased hepatic metabolism of bilirubin
      - decreased hepatic uptake
      - decreased conjugation (to bilirubin glucuronide)
      - decreased transport of conjugates into bile
   3. bile flow obstruction

3. **Characteristics of senescent RBCs**
   - Nascent cells labeled with biotin
   - Nascent cells isolated with avidin linked to a solid support
   - Nascent cells cleared as a cohort (grouped together), not randomly

   RBCs in spleen enriched for senescent cells

   Signs of nascent RBCs:
   - Decreased glycolytic enzymes
   - Membrane damage - oxidation
   - Increased Hb concentration
   - Decreased pliability
   - Phosphatidylserine in outer membrane leaflet
   - Cross-linking of Hb to band 3 and spectrin (forms patches of Hb on the RBC that can be recognized)
   - Progressive reduction in size and increased density
   - More spherical, less likely to clear splenic capillary bed
   - Heinz bodies (denatured Hb)
   - Siderocytes (iron aggregates)

4. **Congenital erythropoietic porphyria**
   - Defect in Uroporphyrinogen III synthase in heme synthesis

   Happens in RBC

   Build-up of ALA, hydroxymethylbilane and uroporphyrin I

   Symptoms: neurovisceral photosensitivity

5. **Formation of carbon monoxide**
   - Product of heme oxygenase reaction

   CO complexes with Hb heme and transported to lungs for exhalation

   Expired CO is an indirect measure of heme turnover

   CO activates guanylate cyclase and is also a neurotransmitter
| 6. **Heme functions in humans** | - transport and retention of O2 (hemoglobin and myoglobin)
- electron transport to cytochromes in mitochondria
- O2 activation (cytochrome c oxidase, tryptophan pyrrolase, P450 mixed-function oxidases)
- Reduction of H2O2 using peroxidases (reduce to H2O)
- Destruction of peroxide using a catalase |
| 7. **Hepatic Jaundice** | damage to liver causes ↓ uptake of bilirubin
unconjugated bilirubin in blood elevated due to reduced uptake
conjugated bilirubin may also be elevated since liver damage causes release of bilirubin glucuronide
occurs in people with hepatitis and cirrhosis
liver enzymes in serum elevated (since damage to liver) |
| 8. **How are erythrocyte recognized and cleared from circulation?** | Classical pathway:
- senescence epitopes recognized by antibodies:
  Band 3 - oligmerization or cleavage
  Glycophorin - desiliated glycophorin

  Antibodies recognize the patches of membrane proteins and binds to them, then
  macrophages recognize Ab in spleen and phagocytose them

  Alternate pathway:
  - macrophages see phosphatidylserine and destroy

  macrophages come from reticuloendothelial sys of spleen, liver, bone marrow

  remember: splenectomy ↑ # damaged red cells in circulation → reflects role of spleen
  clearing senescent RBCs |
| 9. **How are RBCs degraded?** | 1. senescent RBC → reticuloendothelial system (macrophages in spleen, liver and bone marrow)

  Hb released and picked up by heme

  Heme metabolized to bilirubin → Fe3+ released to blood where it binds to transferrin

  2. RBC → hemolysis → Hb released and dissociates into dimers → Hb dimers picked up by Haptoglobin (Hp) → Hb-Hp complex delivered to liver

  3. RBC → hemolysis → Hb → Heme → Heme oxidized to hemin (Fe3+) with Cl- attached → Hemin-Hemopexin → liver for degradation

  4. RBC → Hb → Heme → Heme oxidized to hemin → hemin-albumin → liver for degradation

  5. RBC → Hb → Heme → heme-albumin → liver for degradation (not all heme oxidized to hemin) |
| 10. **how can you tell if biliary drainage is effective?** | measure amount of conjugated bilirubin (bilirubin glucuronide) linked to albumin in the blood

tells you if there is biliary obstruction because levels should be low unless something is wrong |
<table>
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<tr>
<th>Section</th>
<th>Content</th>
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| **Main features of the structure and nomenclature of heme precursors** | porphyrin:  
pyrrole rings I to IV  
Methyne bridges - alpha to delta  
Ring substituents 1-8 |
| **Metabolism of Bilirubin in Intestinal lumen** | -glucuronidase cleaves bilirubin diglucuronides  
-unconjugated bilirubin becomes colorless urobilinogens  
-urobilinogens are oxidized to urobilins in colon (dark color)  
-some urobilinogens reabsorbed by colon, taken up by liver, excreted into bile, secreted into blood and excreted in urine |
| **Nature and function of haptoglobin (Hp)** | if Hb gets outside the RBC (maybe senescent cells release it) it dissociates into 2 dimers  
Hp binds Hb dimers and saves them from being filtered out of body by kidney via urine  
thus Hp prevents iron loss & oxidative damage to kidney by Fe  
Hp-Hb complex taken up by liver hepatocytes → Hb goes to amino acids → iron released from Hb to make porphyrin → porphyrin then metabolized to bilirubin |
| **Neonatal physiologic jaundice** | common in newborns because:  
-heme production elevated due to degradation of fetal Hb  
-low bilirubin conjugation (activity of UDP glucuronosyl transferase in liver is only 0.1% of adults)  
-reabsorption of unconjugated bilirubin from intestine  
-exacerbated by conditions that increase heme degradation (eg. blood group incompatibility when mom’s antibodies present in fetal blood)  
-bilirubin penetrates BBB when its concentration exceeds that which can be tightly bound by plasma albumin |
| **Obstructive Jaundice** | blockage in delivery of conjugated, bilirubin glucuronide to gut  
increased total bilirubin and urinary bilirubin glucuronide  
fecal urobilinogen reduced or absent PALE POOP!!!!!!  
liver enzymes slightly elevated  
can be caused by gallstones or carcinoma, or inflammation/scarring |
16. **Pathway for synthesis of heme starting with succinyl-CoA and glycine**

WRITE OUT: p. 18

1. ALA synthase
   Succinyl-CoA + Glycine $\rightarrow$ Aminolevulinate (ALA)

2. ALA dehydratase
   2 ALA $\rightarrow$ Prophobilinogen

3. Uroporphyrinogen I Synthase
   4 Prophobilinogen $\rightarrow$ Hydroxymethylbilane

4a. Spontaneous cyclization
   Hydroxymethylbilane $\rightarrow$ Type I uroporphyrinogen

4b. Uroporphyrinogen III Synthase
   Hydroxymethylbilane $\rightarrow$ Type III uroporphyrinogen

5. Uroporphyrinogen Decarboxylase
   Uroporphyrinogen III $\rightarrow$ Coporphyrinogen III

6. IN MITOCHONDRIA
   Coproporphyrinogen III $\rightarrow$ Protoporphyrinogen III $\rightarrow$ Protoporhyin III $\rightarrow$ Heme (when Fe$^{2+}$ added)

Last step requires ferrochelatase, where ferrous (Fe$^{2+}$) iron inserted

17. **Porphyria Cutanea Tarde**

defect in uroporphyrinogen decarboxylase in heme synthesis
(think C from Cutanea like C from deCarboxylase)

happens in liver (hepatic)

build-up of uroporphyrin I and III

symptoms: blistering photosensitivity

18. **Prehepatic Hemolytic Jaundice**

$\uparrow$ destruction of red cells with release of Hb
$\uparrow$ unconjugated bilirubin

serum liver enzymes are normal since no damage to liver, but urobilinogen in urine

Extravascular blood hematoma
Hemolytic anemia (eg. hereditary spherocytosis or sickle cell)

19. **Regulation of Globin synthesis by heme**
synthesis of globins are correlated to iron and heme levels

when iron low $\downarrow$ $\rightarrow$ IRE-BP inhibits translation of ALAS2 mRNA $\rightarrow$ inhibition of heme synthesis $\rightarrow$ decreased heme levels $\rightarrow$ activation of protein kinase (HRTI)

HRTI protein kinase $\rightarrow$ phosphorylates translation initiation factor eIF2 $\rightarrow$ inhibits translation of globins
20. **Regulation of heme synthesis and its role in globin synthesis**

1. Hepatic ALA synthase 1 "housekeeping" isoform
2. Erythroid ALA synthase 2

**1. Hepatic ALA synthase 1 "housekeeping" isoform**

- Rate limiting step!
- Heme inhibits expression of ALAS1 because it doesn’t need to make more (product inhibits enzyme that made it)
- Short half life of the enzyme because needs to respond to rapid changes in translation and transcription
- P450s and phenobarbital stimulate its expression
- Carbohydrates (glucose) repress expression of ALA synthase I

**2. Erythroid ALA synthase 2**

- On different gene than hepatic ALA synthase 1
- Expression is NOT regulated by heme, but by factors that regulate RBC synthesis and by iron
- Translation regulated by IRE-BP (iron response element binding protein)
- If iron is high, IRE-BP doesn’t bind to BP → ALAS2 gets translated! → production of heme

---

21. **Role of albumin in recycling of Fe from heme**

- Albumin in plasma is secreted by liver
- Binds to heme and hemin (oxidized heme) and transfers it to hemopexin (Hx) or directly to the liver
- In liver, hemin metabolized to bilirubin and iron reused

---

22. **Role of bilirubin and why it is green/yellow**

- Nonpolar, sparingly soluble in water
- Toxic to many biochemical and neurological functions (bad news)
- Scavenges peroxyl radicals - anti-oxidant (good news!)
- Prevents deleterious effect of iron in hemolytic crisis (rescues it from heme released from senescent RBC after hemolysis)
- Transports to liver by plasma albumin
- Binds to ligandin and protein Y on hepatocytes
- Conjugated to glucuronic acid in liver
- Glucuronides secreted into bile (gives you green/yellow color of bile)
- Note: bilirubin is insoluble in water but diglucuronides that it forms are soluble in water

---

23. **Role of hemopexin (Hx)**

- Hemopexin (Hx) binds to heme or hemin to prevent heme-coupled peroxidation and thus oxidative damage to kidneys
- Hx binds to heme or hemin and takes it to the liver where it’s metabolized to bilirubin → iron reused
### Synthesis of bilirubin

1. Heme oxygenase
   - Hemin reduced to ferrous iron (Fe\(^{2+}\)) by NADPH
   - Heme hydroxylated and oxidized back to ferric (Fe\(^{3+}\)) state
   - Respiration (inhalation of O\(_2\)) further oxidizes and causes Fe\(^{3+}\) to be released and CO exhaled to form keto group \(\rightarrow\) biliverdin

   Note: Heme oxygenase produced 85% of CO in body

   Heme oxygenase complex with NADPH cytochrom P450 reductase

2. Biliverdin reductase
   Biliverdin reduced by NADPH \(\rightarrow\) bilirubin

cytoplasmic (occurs in cytoplasm)
forms REVERSIBLE complex with HO (hemooxygenase) and NADPH cytochrome P450 reductase

### Transport of heme and hemin

- Heme: porphyrin with ferrous Fe attached (can bind oxygen)
- Hemin: porphyrin with oxidized Fe (ferric Fe\(^{3+}\)) attached to a Cl- molecule (cannot bind oxygen!)

### Treatment of neonatal physiologic jaundice

- Phototherapy (isomerizes bilirubin into water soluble isomers)
- Exchange transfusion
- Phenobarbital \(\rightarrow\) induces UDP glucuronosyl transferase \(\rightarrow\) stimulates bilirubin conjugation to water soluble form
- Heme oxygenase inhibitors \(\rightarrow\) decreases synthesis of bilirubin

### Variegate Porphyria

defect in protoporphyrinogen oxidase in heme synthesis

happens in liver

buildup of protoporphyrinogen III, coproporphyrin III, porphobilogen, ALA

symptoms: both neurovisceral and blistering and photosensitivity (red urine like King George)

### Vitamin B\(_12\) (Cobalamin) is a cofactor for what?

Cofactor for internal rearrangement reactions:

1. Methylmalonyl CoA isomerase
   methylmalonyl CoA \(\rightarrow\) succinyl-CoA

2. Methionine synthase
   - makes methionine from homocysteine

   Important bc if you don’t make it, you can’t make RBCs \(\rightarrow\) anemia \(\rightarrow\) degeneration of spinal cord

### What is bilirubin conjugation?

Formation of bilirubin glucuronide from bilirubin

- Bilirubin: nonpolar and non-soluble
  - can’t pass through membrane
  - can’t be excreted in urine

- Conjugated bilirubin (bilirubin diglucuronide):
  - polar and soluble
  - can pass through membrane
  - excreted in urine
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
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<tbody>
<tr>
<td><strong>30. What is Hereditary Porphyrias?</strong></td>
<td>defects in enzymes of heme synthesis</td>
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<tr>
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<td>substrate before enzyme action accumulates</td>
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<td>heme levels decrease</td>
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<td>symptoms can include:</td>
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<td>- photosensitivity (accumulation of precursor in skin)</td>
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<td>- anemia</td>
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<td>- porphyrins in urine (red urine)</td>
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<td><strong>31. What is the role of multiple drug resistance protein 2 (MRP2) in handling of bilirubin?</strong></td>
<td>bilirubin diglucuronide gets taken up into bile duct (canaliculus) by MRP2 protein transporter</td>
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<td>canaliculus eventually becomes hepatic duct and ends in liver</td>
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<td>this transport is ATP-dependent (active) and is the rate limiting step for bile excretion</td>
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