PORPHYRINS
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I. LECTURE OUTLINE (all illustrations are available from the MGB web site as PowerPoint files)
   A. Heme function
   B. Heme synthesis and regulation
   C. Iron metabolism and disorders of iron metabolism
   D. Porphyrias and other disorders of heme synthesis
   E. Heme degradation and disorders of heme degradation

II. PORPHYRINS: Cyclic compounds that bind metal ions
     A. Chlorophylls: Mg$^{2+}$ porphyrin that is central to solar energy utilization
     B. Heme:
         1. Most prevalent metalloporphyrin in humans.
         2. Central to oxygen sensing and utilization.
         3. One ferrous (Fe$^{2+}$) atom in the center of the tetrapyrrole ring of Protoporphyrin IX.
         4. Prosthetic group for
            a. Hemoglobin
            b. Myoglobin
            c. The cytochromes
            d. Catalase
            e. Nitric Oxide Synthase
            f. Tryptophan pyrrolase, etc
         5. Hemeprotein turnover is coordinated with synthesis and degradation of porphyrins
         6. Bound iron is recycled

III. ROLES OF HEME
     A. Oxygen sensing (heme and hemoproteins)
     B. Oxygen transport (hemoglobin)
     C. Oxygen storage (myoglobin)
     D. Electron transport (cytochromes)
     E. Effector of Apoptosis (Mitochondrial cytochrome c)
     F. Involvement in the Nitric Oxide system
     G. Oxidation (cytochrome p450, tryptophan pyrrolase, guanylate cyclase, etc)
     H. Decomposition and activation of H$_2$O$_2$ (catalase and peroxidase)
     I. Regulation of cellular processes
        a. Transcription of globin and cytochrome genes
        b. Translation
        c. Translocation
        d. Assembly and degradation of cytochromes and hemoglobin

IV. BIOSYNTHESIS OF HEME
     A. General considerations
        1. Occurs in association with mitochondria (some steps inside of the matrix, some steps in the cytoplasm)
2. Liver (15%):
   a. 65% cytochrome P450
   b. Synthesis fluctuates greatly
   c. Alterations in cellular heme pool
3. Bone Marrow (80%)
   a. Erythrocyte precursors: hemoglobin
   b. Synthesis relatively constant
   c. Matched to rate of globin synthesis
   d. Developmental signals
   e. Largely unaffected by other factors

B. Synthetic steps:
   1. Initial step
      a. **This is the rate-limiting step. It is highly regulated.**
      b. Glycine and succinyl CoA condense to form \( \delta \text{aminolevulinic acid} \) (\( \delta \text{ALA} \)) in the mitochondria.
      c. Reaction is catalyzed by \( \delta \text{aminolevulinic acid synthase} \) (\( \delta \text{ALAS} \)).
      d. \( \delta \text{ALA} \) moves out of the mitochondrion for subsequent steps.

   2. **Pyrrrole ring:** porphobilinogen

   3. **Tetrapyrrrole rings (porphyrins and porphyrinogens):**
      a. Differ according to side chain modifications

b. Porphyrinogens: chemically reduced, colorless intermediates
   c. Porphyrins: intensely colored, fluorescent
4. **Uroporphyrinogen III** → **protoporphyrin IX**: formed through a series of decarboxylations and oxidations:
a. **Uroporphyrinogen III** goes to:
b. **Coproporphyrinogen III** which then:
c. Moves back into mitochondrion where it is converted to:
d. **Protoporphyrinogen IX** which goes to:
e. **Protoporphyrin IX**

V. **REGULATION OF HEME SYNTHESIS**
A. δ-aminolevulinic acid synthase: **Rate-Controlling Step in Heme Synthesis**
   1. Role of δALAS in regulating hepatic heme synthesis (the red blood cell ALAS is unregulated; see below for heme synthesis in the RBC):
      a. Heme accumulates and is converted to hemin (Fe^{3+})
      b. Hemin decreases hepatic isoform of δALAS
      c. By causing decreased synthesis of this enzyme (transcription and translation)
      d. By inhibiting translocation to mitochondria
      e. By direct feedback inhibition of δALAS activity
      f. Hemin is used therapeutically in Porphyrias
   2. Effects of drugs on heme synthesis in the liver:
      a. A large number of drugs (phenobarbital, etc) cause an increase in hepatic δALAS activity. These drugs are metabolized by cytochrome P450 system - a heme protein oxidase system in the liver. In response to these drugs P450 synthesis increases, leading to enhanced heme consumption. This in turn causes a decrease in heme concentration in the liver cell. Lower intracellular heme leads to increase in synthesis of δALAS (derepression) and δALA.
      b. Similar effects with sex steroids, glucose, metals.
   3. Regulation of red blood cell heme synthesis
      a. Heme synthesis occurs in red blood cell precursors located primarily in the bone marrow (mature red cells do not have mitochondria). Amplification of the red blood cell precursors is under the control of the hormone erythropoietin, which is released by the kidney.
      b. The availabilities of intracellular iron and oxygen also regulate the rate of heme synthesis.
      c. There is an erythroid specific isoform of δALAS. While this isoform is the rate-limiting step in red cell heme synthesis, it is not regulated by feedback inhibition from
heme.

d. In red cell precursors the requirement for heme synthesis is generally constant for the production of hemoglobin.
e. Deficiency state is X-linked Sideroblastic Anemia

VI. IRON METABOLISM

A. Iron is a reactive transition metal that is normally present in the body complexed with proteins that limit its chemical reactivity.
B. Both iron deficiency and iron overload cause cellular defects and disease.
C. Iron content within the body is controlled only at the level of iron absorption through the duodenal enterocyte.
D. There is no known mechanism for regulation of iron egress from the body. Small amounts of iron (1 – 2 mg per day) are lost with skin and bleeding, but this is not a regulated process.
E. Most iron available for use in the body is generated by macrophages that recycle iron from red cells.
F. Dietary ferric (Fe$^{3+}$) iron in the duodenal lumen is converted to ferrous (Fe$^{2+}$), absorbed by the duodenal enterocyte and subsequently transported to the blood.

G. Iron is transported in the blood as two atoms of ferric iron bound to transferrin.
H. Transferrin is internalized in heme-producing cells after binding to a surface transferrin receptor.
I. Transferrin ferric iron is converted to ferrous iron within the endosome mitochondrion.

J. Ferritin is the main iron storage protein in the body.
K. Hemosiderin is a form of Ferritin complexed to additional iron that accumulates in the body as a result of iron overload.
L. Iron metabolism is an area of intense investigation, and our understanding is dramatically expanding. Within the last year the central roles of Hepcidin and Ferroportin have been
proposed.

1. Ferroportin is the only known protein known to be involved in exporting iron from cells (macrophages, enterocytes, etc.)

2. Hepcidin regulates the availability of cell iron by binding to Ferroportin. Hepcidin causes the internalization and degradation of Ferroportin; shutting off the egress of iron from the cell. (Nemeth et al, Science, December 2004)

3. Hepcidin is a liver-synthesized protein (also known as Hepcidin Antimicrobial Protein: HAMP). It goes up in inflammatory states, decreasing the amount of iron available for heme synthesis. It is therefore responsible for the **Anemia of Inflammation** (Anemia of Chronic Disease).

4. Dysregulation of the Hepcidin – Ferroportin interaction is the most likely final pathway for the known forms of **Hemochromatosis**.

M. Other molecules which are essential for normal iron homeostasis include:

1. DMT (divalent metal transporter) transports iron into the enterocyte, and out of endosomes

2. Ceruloplasmin, and hephaestin convert Ferrous (Fe$^{2+}$) to Ferric (Fe$^{3+}$) iron.

3. Haptoglobin binds hemoglobin in the plasma and delivers it macrophages.

4. Hemojuvelin, a protein in the hepcidin pathway, was identified by identifying a molecular basis for juvenile hemochromatosis.

5. Iron regulatory proteins interact with mRNA iron responsive elements (sequences that are present in either the 5’ or 3’ untranslated region of certain mRNAs) to regulate many of these processes.

N. Mutations in the HFE gene are the most common cause of **hereditary hemochromatosis**

1. Hemochromatosis is a very common condition in people of northern European extraction.
It has an autosomal recessive pattern of inheritance.
2. The HFE gene product is involved in iron transport via effects on Hepcidin levels.
3. The extremely variable penetrance of homozygous HFE mutation implicates other factors in clinical disease.

VII. DISORDERS OF HEME SYNTHESIS
A. X-linked sideroblastic anemia
B. Lead poisoning
C. Iron deficiency anemia
D. The porphyries: Inherited defects in heme synthesis.
   1. Defects in seven of the eight enzymatic steps are associated with porphyries
   2. Accumulation and excretion of porphyrins
   3. Pattern of porphyrin excretion and clinical manifestations depends on which enzyme affected
   4. 5 out of 7 Porphyrias are Autosomal dominant, with “low penetrance (CEP and ALAD porphyria are recessive)
   5. Multiple alleles with some genotype/phenotype correlations
   6. The molecular basis of the observed “low penetrance” is in the process of being elucidated
      a. Haploinsufficiency (no dominant negative mutations have been observed)
      b. 50% residual activity is usually sufficient
      c. Increased demand, such as fasting or drugs can precipitate attack
      d. Association with a low expression allele in trans
      e. Iron / HFE hemochromatosis can directly inhibit the enzyme
      f. Other epigenetic phenomena
   7. May be erythropoietic, hepatic or mixed depending on main site of synthesis / accumulation
   8. May be acute or chronic
      a. Acute Attacks in the “Neurovisceral Porphyrias” are clinically indistinguishable
      b. Due to neuro-toxicity of metabolic byproducts, particularly PBG
   9. Decreased heme synthesis derepresses (increases) δALAS activity.
 10. Porphyrin accumulation: photosensitivity
 11. Formation of reactive oxygen species; damage to tissues, release of lysosomal enzymes

VIII. DEGRADATION OF HEME
A. At end of their 120-day lifespan, red blood cells are taken up and degraded by macrophages in the reticuloendothelial system (RES) mainly in the liver and spleen.
   1. 85% heme for degradation comes from dying RBC
   2. 15% comes from immature RBC, cytochromes and from other extraerythroid tissues
B. Degradative pathway

1. Heme oxygenase in the macrophages converts heme to the green pigment **biliverdin** in a two-step process. (Heme oxygenase also has cytoprotective effects against oxidative injury and cellular stresses, particularly in the lung)

2. Biliverdin reductase converts biliverdin to the yellow pigment **bilirubin**.

3. Bilirubin is released into the blood where it is bound to albumin. Decreased levels of albumin, or drugs that interfere with this binding can allow toxic, unbound bilirubin to leak into the tissues.

4. Albumin bound bilirubin is delivered to the liver where it is bound to ligandin.

5. Bilirubin in the liver is conjugated with two glucuronides by bilirubin glucuronyl transferase. Conjugated bilirubin is called **direct bilirubin**, because this water-soluble molecule reacts very rapidly in the colorimetric van den Bergh reaction for bilirubin determination (see below).

6. Conjugated bilirubin is actively transported into the bile canaliculi, and eventually into the bile ducts (with some storage in the gall bladder). Conjugated bilirubin in the bile is released into the intestines, where the bacterial flora converts it to urobilinogen (which is colorless) and then to stercobilin (which gives the brown color to feces).

7. Most urobilinogen diffuses from the intestine into the blood supply, where it is delivered to the kidney. The kidney converts urobilinogen to urobilin (the yellow color of urine).

8. Some urobilinogen goes back to the liver in the enterohepatic circulation. It is then
secreted in the bile.

C. Bilirubin determination

1. Unconjugated bilirubin, which is less soluble and reacts more slowly, is called **indirect bilirubin**. Indirect bilirubin is usually measured by subtracting the direct bilirubin from total bilirubin (measured by the van den Bergh reaction done in methanol, which solubilizes both forms of bilirubin).

2. Conjugated (direct) bilirubin is actively transported into the bile canaliculi, and eventually into the bile ducts (with some storage in the gall bladder). Conjugated bilirubin in the bile is released into the intestines, where the bacterial flora converts it to urobilinogen (which is colorless) and then to stercobilin (which gives the brown color to feces).

3. Medical significance:
   a. **Hemolysis** causes an elevation of indirect bilirubin because the amount of bilirubin produced exceeds the liver’s conjugating capacity.
   b. **Liver hepatocellular disease** can also cause an elevation of indirect bilirubin.
   c. **Obstruction of the bile duct** causes backup and elevation of direct bilirubin in the plasma.

D. Disorders of heme degradation:

1. **Jaundice** is the symptom which is observed when there is accumulation of bilirubin in the skin and sclera.

2. Kernicterus is toxic bilirubin encephalopathy that is observed in neonates with high levels of unbound bilirubin. All neonates have a tendency toward elevated bilirubin levels because **bilirubin glucuronyl transferase** is low at birth. Hyperbilirubinemia in the neonate can be treated with blue fluorescent light phototherapy which converts bilirubin to water soluble isomers.

3. Crigler-Najar syndrome is the genetic disease caused by deficiency of bilirubin glucuronyl transferase.