

SLIDE NOTES FOR “PORPHYRINS and PORPHYRIAS”
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TITLE AND CONTACT INFORMATION

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OBJECTIVES

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OUTLINE

Porphyrins are intermediates in the biosynthetic pathway for the formation of heme, the non-protein, iron-containing prosthetic group of hemoglobin. Porphyrins are a group of diseases that result from decreased activities of specific enzymes in the pathway.

The word porphyria comes from the Greek word *porphura* meaning purple. The Greeks borrowed the word from the Phoenicians who extracted a purple pigment from mollusks to dye garments for the royal family.

This presentation examines heme biosynthesis as a means of understanding the properties of porphyrins that contribute to their toxicity (and produce the symptoms of disease) and allow for their detection in body fluids by the laboratory.

That is, the biochemical pathway provides a logical guide for laboratory investigation of disease.

In addition, disorders of porphyrin metabolism (diseases), and outline laboratory testing for the evaluation of porphyrias will be described.

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Heme biosynthesis is accomplished in a sequence of reactions catalyzed by eight different enzymes in which one molecule of heme is produced from eight molecules of aminolevulinic acid.

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Heme biosynthesis is typically diagrammed in this way to show that the first and last two reactions require a significant amount of energy and so take place in the mitochondrion, and the other reactions, which don't require as much energy, take place in the cytosol.

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To consider a bit of vocabulary:

The biologically active compounds are termed porphyrinogens. These are reduced compounds that are oxidized on exposure to air to porphyrins – the compounds we detect and measure in the clinical laboratory.

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The small precursor compounds -- aminolevulinic acid (ALA) and porphobilinogen (PBG) -- are water soluble and are excreted and measured in urine. Porphyrins are larger compounds whose aqueous solubility varies in part based on the number of carboxylic acid side chains in the molecule. The octacarboxylate compound, uroporphyrin, has eight carboxylic acid groups and is the most soluble; the dicarboxylate porphyrins are the least soluble in water. This affects the specimen type selected for the measurement of particular porphyrin intermediates.

In this slide, the number of carboxylate groups (COO^-) present in each porphyrinogen is shown in a yellow box.

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Each reaction in the pathway is catalyzed by a particular enzyme – shown in this slide in color.

The first step in the pathway is the condensation of glycine and succinyl CoA to form aminolevulinic acid – which is used to synthesize heme.

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This is a line-angle or zigzag diagram of aminolevulinic acid (ALA). Recall from organic chemistry that each bend in the chain represents a carbon atom and hydrogen atoms bound to carbon are not shown. The common name for the molecule is *delta*-aminolevulinic acid – *delta* (δ) indicates the position of the amino group in the chain. The compound sometimes is termed “5” ALA using a different numbering system that labels the carbonyl carbon atom number 1.

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Two molecules of ALA are enzymatically condensed to the monopyrrole porphobilinogen (PBG). *PBG synthase* is the enzyme that catalyzes the reaction.

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PBG is a five-membered heterocycle called a pyrrole. Note the carboxylic acid side chains.

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PBG is easily polymerized to a dimer,

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a trimer,

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... and a linear tetrapyrrole, which is termed hydroxymethylbilane. Polymerization of PBG can be catalyzed by the enzyme *hydroxymethylbilane synthase* (often called *PBG deaminase*).

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The enzyme *uroporphyrinogen III synthase* catalyzes an intramolecular rearrangement and ring closure. Note the re-orientation of the longer three-carbon carboxylic acid side chain in the D ring (lower left of the drawing). The product of this reaction is uroporphyrinogen, and the compound is designated as the Roman numeral III isomer to distinguish it from other structural isomers. Structural isomers are molecules with the same molecular formula but whose atoms are connected differently. This is important since biological molecules can perform an intended function only if their structure is correct.

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The enzyme *uroporphyrinogen decarboxylase* catalyzes the sequential removal of one carboxyl group from each pyrrole ring in the cyclic tetrapyrrole. Following removal of the first carboxyl group, the molecule is termed heptacarboxylate porphyrinogen.

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After removal of two carboxyl groups, the intermediate has six carboxyl groups and is termed hexacarboxylate porphyrinogen.

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After removal of a carboxyl group from each of three rings, the intermediate has five carboxyl groups and is termed pentacarboxylate porphyrinogen.

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After a carboxyl group has been removed from each of the four pyrrole rings, the intermediate has four carboxyl groups remaining and is termed coproporphyrinogen. The prefix “copro” refers to feces. This intermediate is much less water-soluble and is excreted in the feces as well as in the urine, so it is aptly named.

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The enzyme *coproporphyrinogen oxidase* catalyzes the removal of two additional carboxylate groups to form the compound protoporphyrinogen IX.

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Protoporphyrinogen oxidase catalyzes the oxidation of the dicarboxylate compound, protoporphyrinogen IX, to protoporphyrin.

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Oxidation to the porphyrin compound creates an extended conjugation system that allows the molecules to absorb visible light. This property results in clinical manifestations of disease because release of the absorbed energy produces reactive oxygen species that damage tissue. In the lab the spectrophotometric and fluorescent properties are used to detect the compounds in body fluid specimens.

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In the final step in the pathway, *ferrochelatase* catalyzes the insertion of ferrous iron into the ring.

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This is an image of heme in hemoglobin. The Fe^{2+} is bound to the nitrogen atom in each of the four pyrrole groups of heme and to a histidine residue in the globin chain. The sixth ligand is dioxygen.

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There is more to the story. PBG can polymerize without enzymatic catalysis if its concentration is sufficiently high.

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If *Hydroxymethylbilane synthase* is deficient, PBG accumulates and polymerizes to the linear tetrapyrrole, hydroxymethylbilane, which spontaneously cyclizes. Polymerization is head-to-tail, there is no intramolecular rearrangement, and the isomer formed is designated Roman numeral I. This compound, uroporphyrinogen I, is a substrate for *Uroporphyrinogen decarboxylase*, and coproporphyrinogen isomer I is produced. The next enzyme in the pathway, *Coproporphyrinogen oxidase*, is stereospecific. Coproporphyrinogen I is not a substrate for the enzyme; the compound accumulates and is excreted.

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If *Uroporphyrinogen III synthase* is the deficient enzyme, the linear tetrapyrrole, hydroxymethylbilane, accumulates in excess and spontaneously cyclizes to uroporphyrinogen I, which is decarboxylated to coproporphyrinogen I. In this case, too, coproporphyrinogen I accumulates and is excreted.

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The yellow dashed line highlights the differences in the structures of the Roman numeral I isomers, uroporphyrinogen I and coproporphyrinogen I, from the III isomers. Determination of the type of porphyrin isomers present in a sample provides information on the deficient enzyme in the pathway and aids in the diagnosis of disease.

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The porphyrias are a group of rare inherited or acquired metabolic disorders caused by loss of function mutations in any of seven enzymes in the heme biosynthetic pathway. The defective enzyme results in overproduction, accumulation and excretion of toxic precursor compounds and/or porphyrins. Most of the diseases have an autosomal dominant inheritance pattern and there is a 50% reduction in activity of the affected enzyme. Compensatory overproduction of intermediates usually maintains an adequate rate of heme synthesis.

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Enzymes of heme biosynthesis are shown on this slide.

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The disease associated with each enzyme is listed on this slide. Deficient activity of the first enzyme in the pathway, *ALA synthase*, is associated with an X-linked recessive disorder and sideroblastic anemia. Deficient activity of any of the other enzymes in the pathway produces a particular porphyria that is characterized by excessive accumulation and excretion of intermediate compounds that produce particular symptoms.

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Porphyrias can be grouped by the clinical manifestations of disease. The small molecules PBG and ALA produce characteristic symptoms associated with acute attacks that can be life threatening. The signs and symptoms are thought to result from neurologic dysfunction, but the mechanism of neural damage is not understood.

The acute porphyrias are ALA dehydratase deficiency porphyria (ADP), Acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), and variegate porphyria (VP). The location of the block in the pathway associated with each disease is shown on the slide. The substrate compound that accumulates in each disease appears in bright red, bold font.

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HCP and VP both produce porphyrins that inhibit *Hydroxymethylbilane synthase* and are associated with increased PBG concentrations. For this reason HCP and VP are classified as acute porphyrias, although photosensitivity may occur as well.

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Signs and symptoms of acute porphyria include:

- Abdominal pain
- Nausea, vomiting, constipation
- Muscle weakness
- Psychiatric effects (agitation, anxiety, apathy, depression, hallucinations, psychosis; affect – delirium, hysteria)
- Hypertension, tachycardia
- Convulsions, possible paralysis
- Fever, leukocytosis

Acute intermittent porphyria (AIP) is the most common acute porphyria. Most (~80%) individuals with a genetic defect are asymptomatic. If attacks occur, they typically begin in early adulthood, occur more often in women, and may be precipitated by a variety of endogenous and exogenous factors. Severe neurological attacks can be life-threatening.

The photograph is of King George III of England, who has been rumored to have had an acute porphyria. It is more likely that the monarch suffered from mania, not porphyria; however, his great-great-great-grandson, Prince William of Gloucester, was diagnosed with variegate porphyria.

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The non-acute porphyrias present as chronic conditions. Exposure to sunlight results in photosensitization of the skin due to accumulation of excess porphyrins in tissues. The porphyrins absorb light and react, producing blistering skin lesions on sun-exposed areas that characterize the disease.

Non-acute porphyrias are congenital erythropoietic porphyria (CEP), porphyria cutanea tarda (PCT), and erythropoietic protoporphyria (EPP). The location of the block in the pathway associated with each disease is shown on the slide. The substrate compound that accumulates in each disease appears in bright red, bold font.

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Porphyria cutanea tarda (PCT) is the most common non-acute porphyria and occurs more often as a sporadic condition. If genetic, the defect is inherited in an autosomal dominant manner. The disease usually occurs in association with exogenous factors including alcohol, drugs, smoking and hepatitis C infection, and with factors that increase hepatic iron content such as mutations of the *HFE* gene. Hepatoerythropoietic porphyria (HEP) is a homozygous form of PCT characterized by severe clinical symptoms. Deficiency of *Ferrochelatase* results in massive accumulation of protoporphyrin in red blood cells, plasma, and feces, and symptoms of erythropoietic protoporphyria (EPP). Redness, edema, and painful burning sensations occur within a short time of light exposure. Disease onset occurs in childhood. The autosomal recessive disease, congenital erythropoietic porphyria (CEP), is associated with extreme photosensitivity. Exposure to sunlight ravages skin and may cause disfiguring changes to facial features, fingers, and toes, and corneal scarring. Affected individuals must strictly avoid sunlight.

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Although some enzyme assays and molecular tests are available, laboratory testing for porphyrin disorders is most often based on identification of characteristic patterns of excess porphyrin precursor and intermediate compounds in body fluids. The specimen type (urine, blood, or feces) depends on the solubility of the metabolic intermediates suspected to be in excess. Acute symptoms indicate testing for the porphyrin precursors PBG and ALA in urine. Patients with cutaneous photosensitivity should be evaluated for excess porphyrin intermediates.

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When acute neurologic disease is suspected, initial testing should be detection of PBG in a urine specimen. If symptoms are present, a random urine specimen will be positive for PBG. A positive PBG can be followed by testing for the deficient enzyme, *Hydroxymethylbilane synthase (PBG deaminase)*, and analysis of fecal porphyrins to differentiate among the acute porphyrias (AIP, VP, and HCP). Negative results do not exclude porphyria completely. Testing may be repeated on a specimen collected over 24 hours or when symptoms are present.

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Testing for the precursor compounds, PBG and ALA, utilizes ion exchange chromatography to remove interferences and isolate each compound. Detection is accomplished by the addition of 4-dimethylaminobenzaldehyde (Ehrlich's reagent) to produce a characteristic cherry red color that is detected spectrophotometrically. The spectrum has an absorbance maximum at approximately 555 nm and a shoulder at 525 nm.

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Initial testing for non-acute cutaneous disease requires separation and quantitation of porphyrins in a random or timed urine collection. Increased excretion of porphyrins in a characteristic pattern particular for each disease is diagnostic for PCT and CEP. Testing for PBG is negative in both of these diseases. Suspected erythropoietic protoporphyria (EPP) can be evaluated by assessing porphyrins in plasma or red blood cells. Plasma testing can be used to monitor PCT.

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This is a chromatogram of a negative urine specimen. There are small peaks of uroporphyrin I and III, heptacarboxylate porphyrin, and coproporphyrin I and III. Note that there is more coproporphyrin than uroporphyrin. Deuteroporphyrin IX is the internal standard.

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This is a chromatogram of a urine specimen positive for porphyria cutanea tarda (PCT). Uroporphyrin I and III, and heptacarboxylate, hexacarboxylate, and pentacarboxylate porphyrin are elevated. Isocoproporphyrin, an alternate metabolic product that is produced when *uroporphyrinogen decarboxylase* is deficient, is present in the specimen. There is much more uroporphyrin than coproporphyrin.

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This chromatogram is characteristic of congenital erythropoietic porphyria (CEP). As is expected from review of the metabolic pathway, both uroporphyrin I and coproporphyrin I are massively increased.

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This is a simplified algorithm for the diagnosis of porphyrias. It is based on the separation of disease symptoms into acute (neurological) and non-acute (cutaneous) categories and allows rapid diagnosis of the more common porphyrias – AIP and PCT – associated with each type, and provides guidance for investigation of other porphyrin disorders.

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SUMMARY

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Sources for additional information on porphyrins and porphyrias