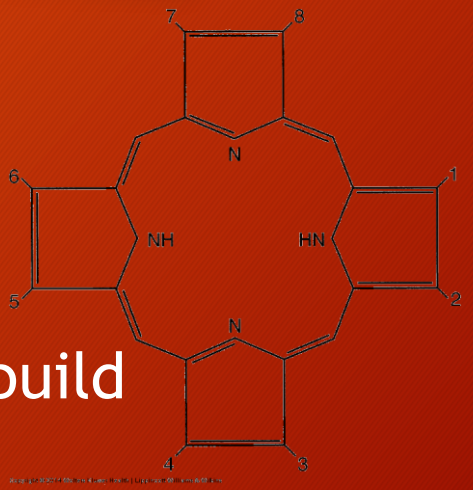
Porphyrins & Porphyrias

Ryan Collison MLS (ASCP)^{CM}

Porphyrins

- Chemical intermediates of heme synthesis
  - Hemoglobin, Myoglobin, Cytochromes
  - Iron captured to form heme
  - Heme + Proteins = hemoprotins
- If this process is disturbed, porphyrins can build up
  - The 8 side chain attachment points leads to a variety of porphyrins



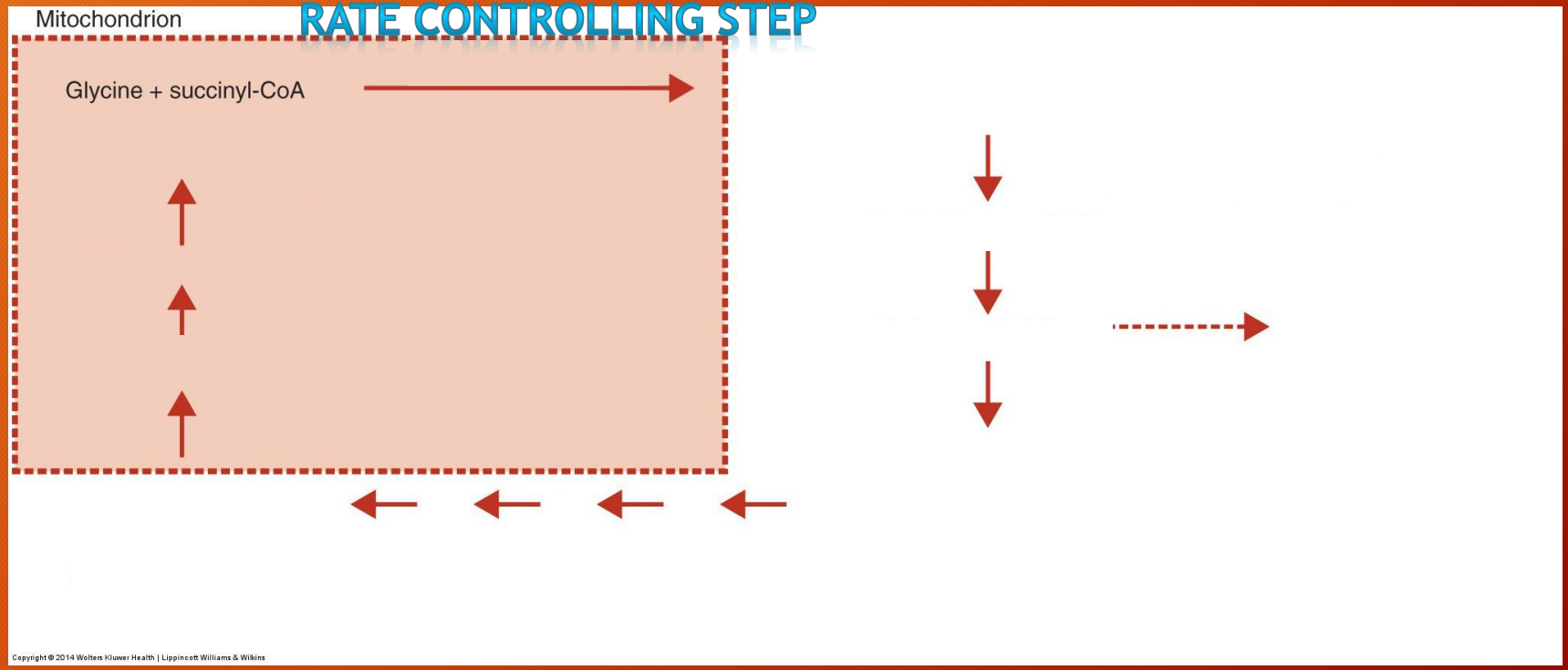
Porphyrins

- Stable compounds
- Color
  - Red-violet, red-brown
  - Fluoresce when stimulated @ 400 nm
- 3 clinically important compounds
  - Protoporphyrin- excreted in feces
  - Uroporphyrin-excreted in urine
  - Coproporphyrin- excreted in either!

Porphyrins

- Reduced porphyrins: Porphyrinogens (the actual building blocks)
- Porphyrinogens
  - Unstable
  - Colorless
  - No flourescence
  - Readily oxidized to porphyrins by light, O_2 or oxidizing agents
- Result: We generally look for porphyrins instead!

Porphyrin Synthesis



Porphyrias

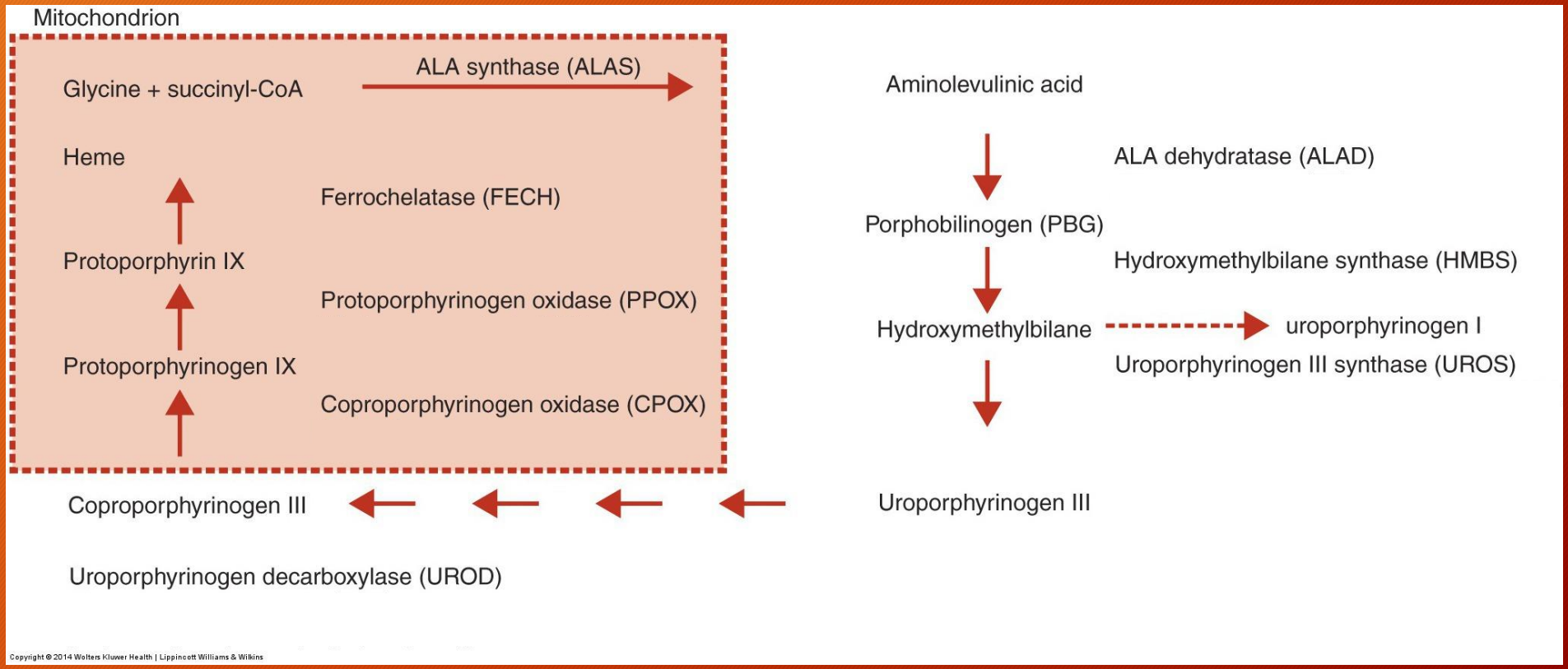
- Acquired or inherited enzyme deficiency
  - In Bone Marrow: Erythropoeitic
  - In Liver: Hepatic
- Symptoms Varry:
  - Early precurors
    - Abdominal pain, neuro-psych, vomiting, constipation, tachycardia, fever, leukocytosis, parastheia
  - Late precursors
    - Skin manifestations: blisters, facial hair, photosensitivity, hyperpigmentation

- The following image is graphic in nature but reflects the consequences of unmanaged cutaneous porphyria, if you are squeamish or simply do not wish to see this image, please look away now

Porphyrias

- Inherited
  - Autosomal dominant inheritance
    - ADP and CEP are autosomal recessive
  - Decrease in enzyme activity leads to build-up of precursor molecule
    - The level of activity left is enough to make the heme needed to prevent anemia

Porphyrias



Specific Porphyrias

- Inherited ALA Dehyratase Deficency Porphyria
  - Inherited ADP
  - 7 cases in the entire world, the ultimate zebra
  - Urinary ALA↑↑↑
  - PBG Norm
  - Coprophorphyrin III ↑-urine
    - Lead also decreases ALAD funtion
      - Adding dithiothreitol restores their ALA function

Specific Porphyrias

- Acute Intermittent Porphyria (AIP)
  - HMBS deficincy
  - Crisis often precipitated by drugs
  - Urine ALA ↑
  - Urine PBG↑
  - Urine turns red-brown on standing
    - Delayed by refrigeration, protection from light, pH preservation.

Specific Porphyrias

- Congenital Erythropoetic Porphyria (CEP)
  - Uroporphyrinogen III cosynthase deficiency
  - Appears shortly after birth
    - Red-brown urine staining diaper
    - Teeth fluoresce red under UV (stained red-brown)
    - Photosensitivity

Specific Porphyrias

- Porphyria cutanea tarda (PCT)
  - Deficiency of Uroporphyrinogen decarboxylase (UROD)
    - Most common porphyria
      - Type I: Limited to liver no family history
      - Type II: In all tissue, autosomal dominant
    - Blistering and fragility in light-exposed areas
    - Urine Uroporphyrin↑ hepatocarboxylic porphyrin↑ isocoproporphyrin↑
  - Will be resolved with low dose chloroquine and iron depletion
- Hepatoerythropoeitic porphyria more or less same
  - Has increased ZPP

Specific Porphyrias

- Hereditary Coproporphyria (HCP)
  - Deficencey of coproporphyrinogen oxidase (CPOX)
    - Urine & Feces ↑↑Copro III
  - Can be precipitated by drugs, hormones, nutritional changes
  - Mild neurological and photosensitive symptoms

Specific Porphyrias

- Variegate Porphyria (VP)
  - Prevalent in South Africa thanks to two Dutch people
  - Protoporphyrinogen oxidase activity decreased
    - Neurologic dysfuntion

    And/Or
    - Photodermatitis
    - Fecal Copro↑ Proto↑↑

Specific Porphyrias

- Erythropoeitic Porphyria (EP/EPP)
  - Ferochelatase the enzyme that puts the iron in heme
  - Photosensitivity present from infancy
    - Burning, itching, pain on exposure
    - Liver concequences
  - RBCs Proto↑
  - Greatly varied presentation

2° Porphyrias

- Heme synthesis interfered with
- Similar symptoms
  - Anemias, liver disease, lead, alcohol can cause
  - In 2° Porphyrias Urinary ALA↑ BUT PGB Normal
    - Lead poisoning will also have RBC ZPP ↑
      - Assay for lead is still better way to detect

Porphyrins in the Lab

- Individual assay for defective enzymes
  - Add substrate, flourometrically identify and quantify products
  - Quantitative assays for uro, proto, copro + ALA and PBG will identify most porphyrias
    - Performed on urine, plasma, stool

Porphyrins in the Lab

- Watson-Schwartz & Hoesch screening tests
  - PGB forms red-orange when mixed with Ehrlich's reagent
    - P-dimethylaminobenzaledhyde
  - Watson-Schwartz uses chloraform or butanol extraction
    - If cherry-red remains in aqueous phase + for PBG
  - In Hoesch test there is no reaction with urobilinogen

Porphyrins in the Lab

- Porphyrins fluoresce better in acidic solutions
  - After being extracted, ultraviolet light reveals pink or red fluorescence
  - They may be read quantitatively due to fluorescence peaks
    - 400-405nm and 594-598nm
    - Standards are used to calibrate curve
  - Each solvent has its own wavelengths in different solvents

Porphyrins in the Lab

- Zinc Protoporphyrin
  - Metabolite formed when Zn not Fe gets into protoporphyin
    - Why/When?
      - If Iron cannot get into the ring, the zinc is used instead
      - Will also increase in iron-deficiency anemia
    - Whole amount not usually reported, usually in ratio to normal heme
- Now molecular tests becoming more popular

ONE MORE TIME!