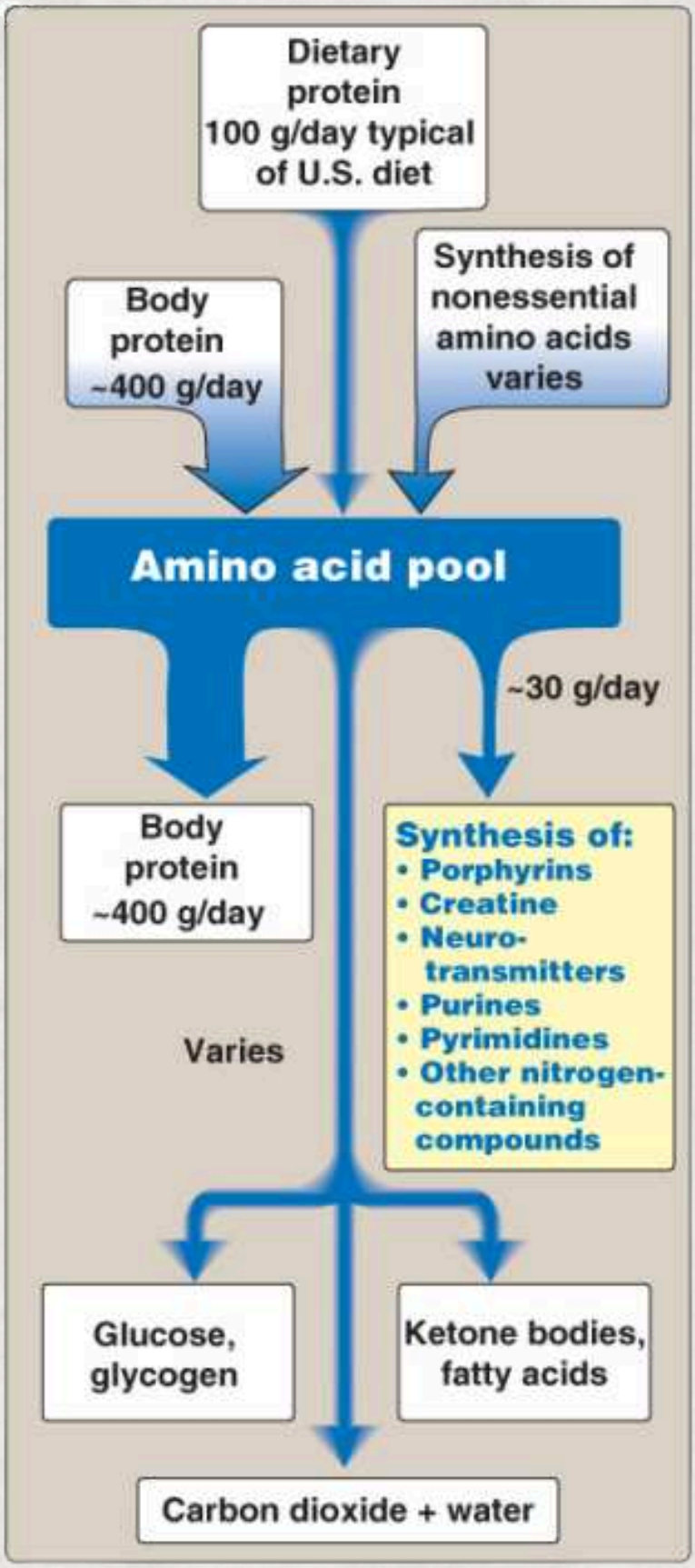
"Amino Acids: Conversion to Specialized Products"

I. Overview

- Amino acids are not only building blocks for proteins.
- They are also precursors of many nitrogen (N)-containing compounds with important physiologic functions.
- These N-containing molecules include:
    - Porphyrins
    - Neurotransmitters
    - Hormones
    - Purines
    - Pyrimidines

Amino acids as precursors of nitrogen-containing compounds.



II. Porphyrin Metabolism

A. General Characteristics

- Porphyrins = cyclic compounds that readily bind metal ions, typically:
    - Ferrous (Fe^{2+})
    - Ferric (Fe^{3+})
- Most prevalent metalloporphyrin in humans:
    - Heme
        - Composed of one Fe^{2+} centrally coordinated in the tetrapyrrole ring of protoporphyrin IX

B. Functional Role of Heme

- Heme functions as a prosthetic group in:
    - Hemoglobin (Hb)
    - Myoglobin
    - Cytochromes, including the cytochrome P450 (CYP) monooxygenase system
    - Catalase
    - Nitric oxide synthase
    - Peroxidase

- These heme proteins undergo rapid synthesis and degradation:
    - Example: 6 to 7 g of Hb synthesized daily to replace heme lost via normal erythrocyte turnover

C. Coordination of Processes

- The processes of:
    - Synthesis and degradation of porphyrins
    - Iron recycling
- Are coordinated with heme protein turnover

Structure of Porphyrins

- Porphyrins are cyclic, planar molecules formed by:
    - Linkage of four pyrrole rings
    - Connected via methenyl bridges

Three Structural Features of Medical Relevance:

1. Side Chains on Pyrrole Rings

Porphyrin Type	Side Chains Present
Uroporphyrin	Acetate ($-CH_2-COO^-$), Propionate ($-CH_2-CH_2-COO^-$)
Coproporphyrin	Methyl ($-CH_3$), Propionate
Protoporphyrin IX (and heme b)	Vinyl ($-CH=CH_2$), Methyl, Propionate

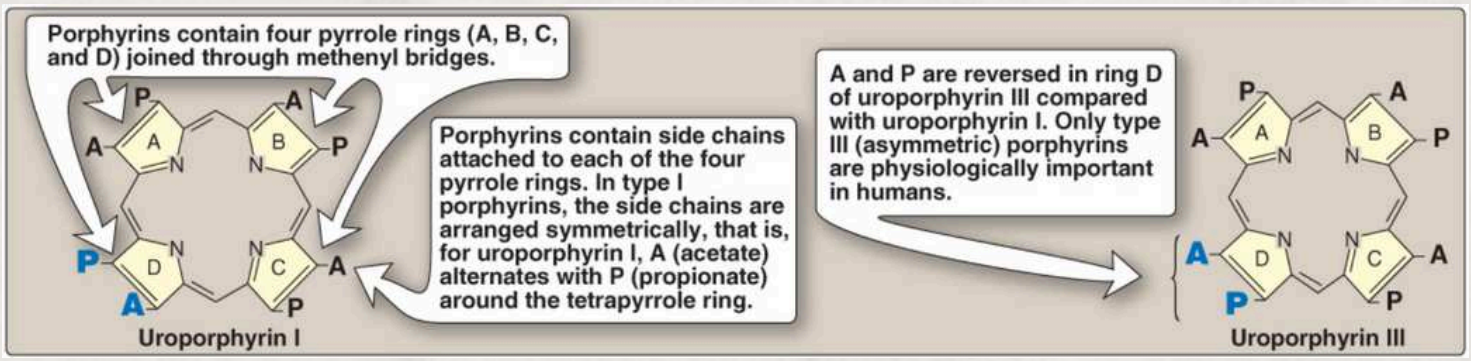
- (Note: Methyl and vinyl groups are produced by decarboxylation of acetate and propionate side chains, respectively.)

2. Side Chain Distribution

- Side chains around tetrapyrrole nucleus can be arranged in four types, labeled:
    - Type I to IV (Roman numerals)

- Only Type III porphyrins are physiologically important in humans
    - Feature: Asymmetric substitution on ring D
    - Example: Protoporphyrin IX is a Type III porphyrin

Structures of Uroporphyrin I and Uroporphyrin III.



3. Porphyrinogens

- Porphyrinogens = chemically reduced, colorless precursors of porphyrins
    - Example: Uroporphyrinogen
- Function as intermediates between:
    - Porphobilinogen (PBG) and
    - Oxidized, colored protoporphyrins

- Role: Intermediates in heme biosynthesis

B. Heme Biosynthesis

Sites of Heme Biosynthesis

- Major sites:
    - Liver
    - Erythrocyte-producing cells of the bone marrow

Liver

- Synthesizes many heme proteins, especially:
    - Cytochrome P450 (CYP) proteins
- Rate of heme synthesis is:
    - Highly variable
    - Responsive to alterations in the cellular heme pool
    - Fluctuations due to changing demands for heme proteins

Erythroid Cells

- Active in hemoglobin (Hb) synthesis
- Heme synthesis is:
    - Relatively constant
    - Matched to the rate of globin synthesis
- Over 85% of all heme synthesis occurs in erythroid tissue
- Mature red blood cells (RBCs):
    - Lack mitochondria
    - Therefore unable to synthesize heme

Subcellular Localization of Heme Synthesis

Step Location	Process
Mitochondria	Initial reaction & last three steps
Cytosol	Intermediate steps of the biosynthetic pathway

1. δ-Aminolevulinic Acid (ALA) Formation

Precursors

- Glycine (nonessential amino acid)
- Succinyl coenzyme A (succinyl CoA) (a tricarboxylic acid cycle intermediate)

Reaction

- Glycine + Succinyl CoA → δ-Aminolevulinic acid (ALA)
- Catalyzed by:
    - ALA synthase (ALAS)

Coenzyme Required

- Pyridoxal phosphate (PLP)

Significance of This Reaction

- Committed step in porphyrin biosynthesis
- Rate-limiting step in porphyrin biosynthesis

Isoforms of ALA Synthase (ALAS)

Isoform	Tissue Distribution	Control Mechanism	Clinical Relevance
ALAS1	Found in all tissues	Controlled by general mechanisms	—
ALAS2	Erythroid-specific	Controlled by erythroid mechanisms	Loss-of-function mutations in ALAS2 → X-linked sideroblastic anemia and iron overload

Regulation of ALA Synthase (ALAS1 and ALAS2)

a. Heme (Hemin) Effects

- When porphyrin production exceeds availability of required apoproteins:
    - Heme accumulates
    - Heme is converted to hemin via:
        - Oxidation of Fe^{2+} to Fe^{3+}

Regulatory Effects of Hemin on ALAS1

- Decreases amount and activity of ALAS1 through:
    - Repression of transcription of the ALAS1 gene
    - Increased degradation of ALAS1 messenger RNA
    - Decreased import of the ALAS1 enzyme into mitochondria

Note: In erythroid cells, ALAS2 is regulated by the availability of intracellular iron

b. Drug Effects on ALAS1 Activity

- Administration of drugs or exposure to environmental xenobiotic chemicals can:
    - Significantly increase hepatic ALAS1 activity

Sources of These Compounds

- Certain:
    - Drugs
    - Foods
    - Cosmetics
    - Commercial products

Mechanism Involving CYP Monooxygenase System

- These compounds are metabolized by the microsomal CYP monooxygenase system
    - A heme protein oxidase system located in the liver

Response to Drug Exposure

- CYP protein synthesis increases
- Leads to increased heme consumption
    - Heme is a component of CYP proteins

Consequences of Increased Heme Utilization

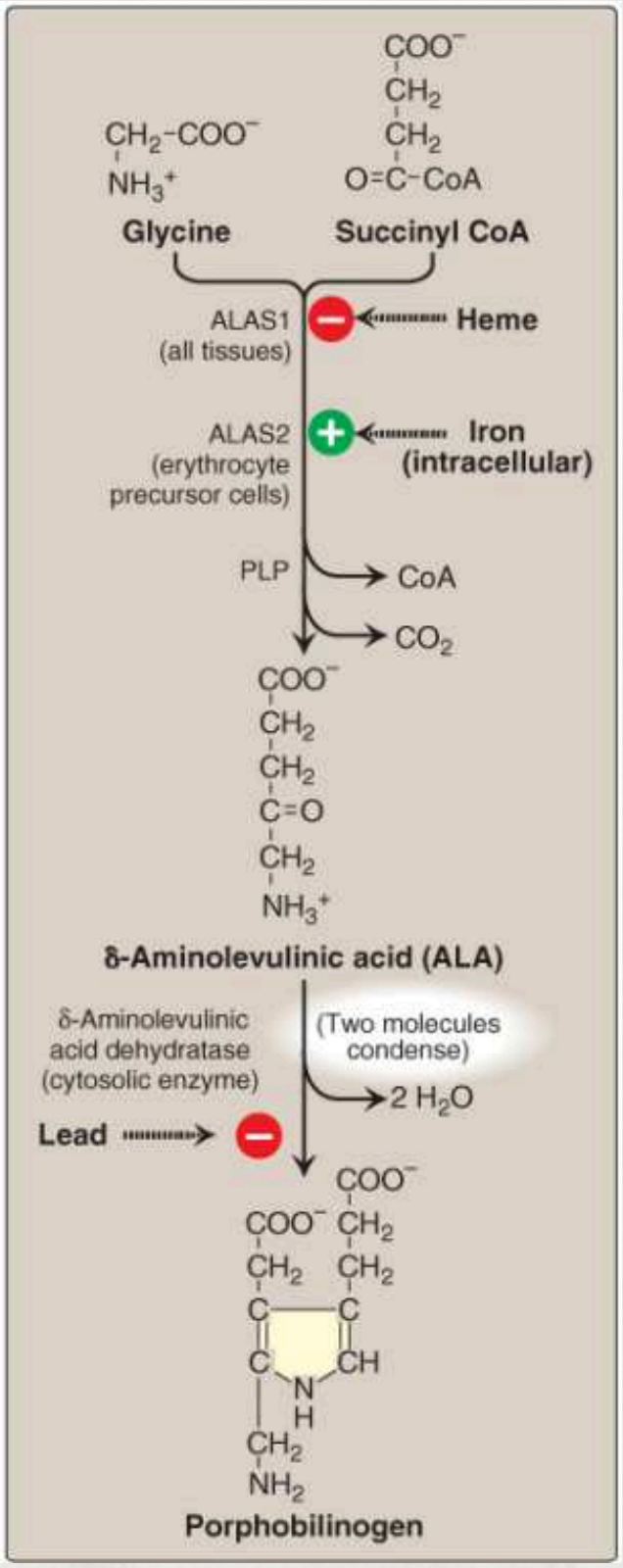
- Results in decreased concentration of free (unbound) heme in liver cells
- Lower free heme triggers:
    - Increased ALAS1 synthesis
    - Corresponding increase in ALA synthesis

2. Porphobilinogen (PBG) Formation

- Location: Cytosol
- Reaction: Condensation of two ALA molecules to form:
    - Porphobilinogen (PBG)
- Enzyme: Zinc-containing ALA dehydratase
    - Also known as: PBG synthase
- Sensitivity:
    - Extremely sensitive to inhibition by heavy metal ions (e.g., lead)
    - Mechanism: Heavy metals replace zinc
- Clinical consequence:
    - Lead poisoning causes:
        - Elevation in ALA
        - Anemia

Pathway of Porphyrin Synthesis: Formation of Porphobilinogen.

(Note: ALAS1 is regulated by heme; ALAS2 is regulated by iron.)



3. Uroporphyrinogen Formation

Step 1: Hydroxymethylbilane Formation

- Condensation of 4 PBG molecules
- Enzyme: Hydroxymethylbilane synthase
- Product: Linear tetrapyrrole called hydroxymethylbilane
- Enzyme deficiency causes:
    - Acute intermittent porphyria (AIP)

Step 2: Uroporphyrinogen III Formation

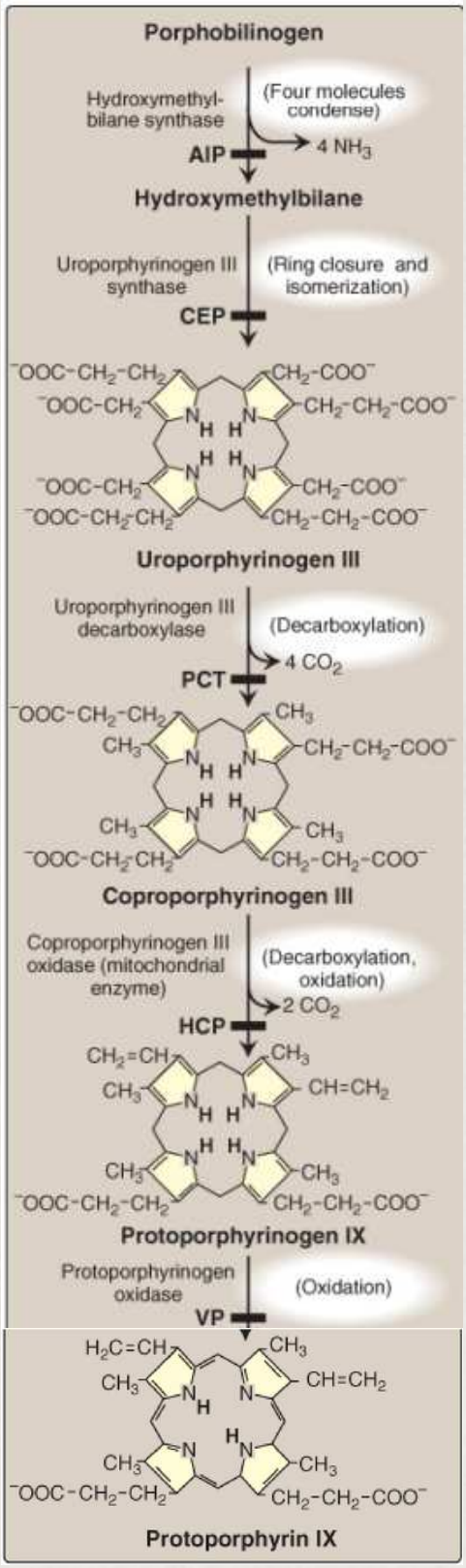
- Enzyme: Uroporphyrinogen III synthase
- Function:
    - Cyclizes and isomerizes hydroxymethylbilane
- Product: Asymmetric uroporphyrinogen III
- Enzyme deficiency causes:
    - Congenital erythropoietic porphyria (CEP)

Step 3: Coproporphyrinogen III Formation

- Enzyme: Uroporphyrinogen III decarboxylase (UROD)
- Reaction: Decarboxylation of acetate groups on uroporphyrinogen III
- Product: Coproporphyrinogen III
- Enzyme deficiency causes:
    - Porphyria cutanea tarda (PCT)

All three steps occur in the cytosol

Pathway of Porphyrin Synthesis: Formation of Protoporphyrin IX.



4. Heme Formation

Step 1: Protoporphyrinogen IX Formation

- Location: Mitochondria
- Substrate: Coproporphyrinogen III
- Enzyme: Coproporphyrinogen III oxidase
- Reaction:
    - Decarboxylation of two propionate side chains to vinyl groups
- Product: Protoporphyrinogen IX
- Enzyme deficiency causes:
    - Hereditary coproporphyria (HCP)

Step 2: Protoporphyrin IX Formation

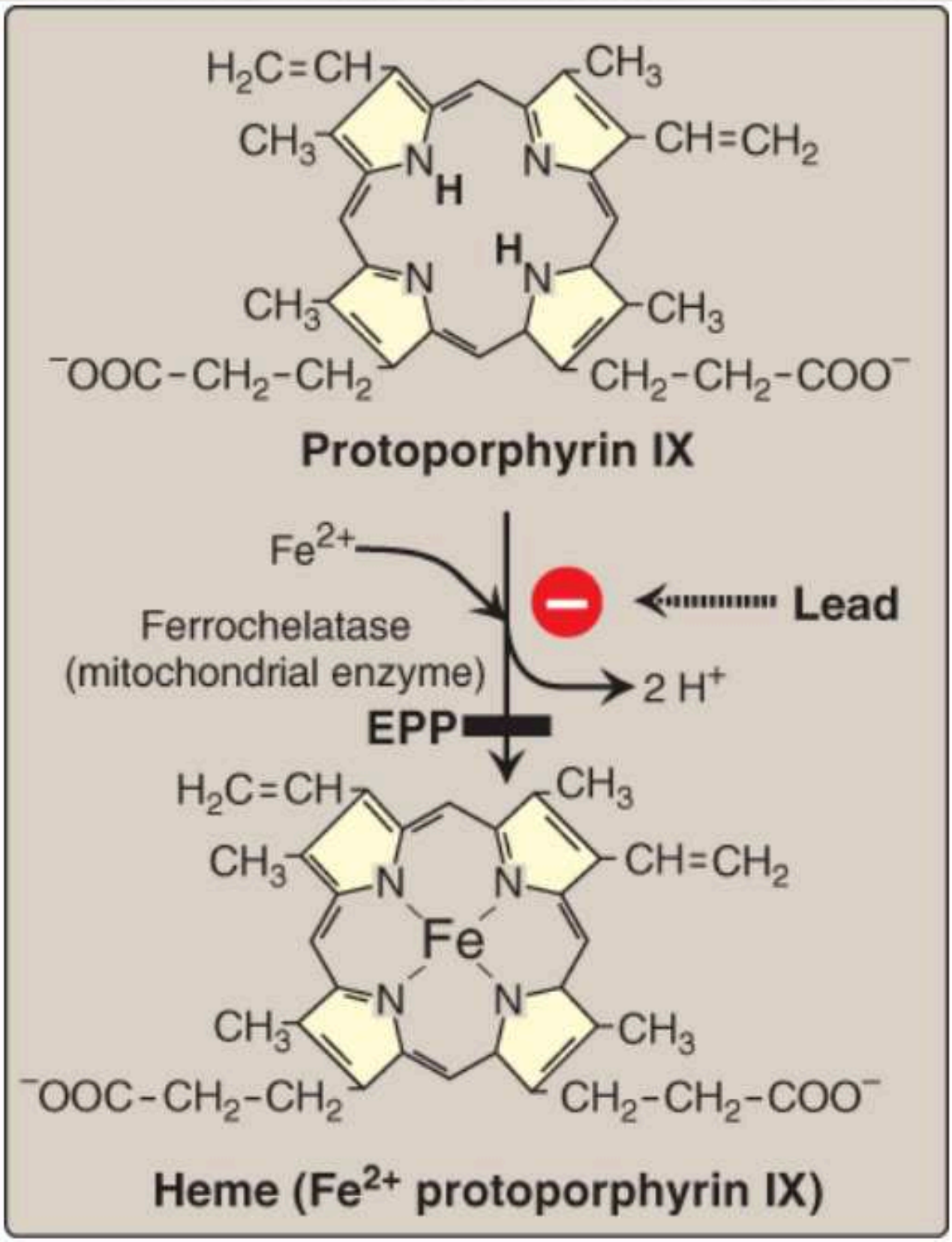
- Enzyme: Protoporphyrinogen oxidase
- Reaction: Oxidation of protoporphyrinogen IX

- Product: Protoporphyrin IX
- Enzyme deficiency causes:
    - Variegate porphyria (VP)

Step 3: Heme Formation

- Reaction: Iron (Fe^{2+}) is introduced into protoporphyrin IX
- Enzyme: Ferrochelatase
    - Enhances insertion of Fe^{2+}
    - Can occur spontaneously but rate is enhanced by enzyme
- Inhibited by: Lead
    - (Like ALA dehydratase)
- Enzyme deficiency causes:
    - Erythropoietic protoporphyria (EPP)

Pathway of Porphyrin Synthesis: Formation of Heme



Flowchart: Summary of Key Steps & Enzymes in Heme Biosynthesis

Glycine + Succinyl-CoA

|

(ALA synthase)

↓

ALA

|

(ALA dehydratase / PBG synthase)

↓

PBG

|

(Hydroxymethylbilane synthase)

↓

Hydroxymethylbilane

|

(Uroporphyrinogen III synthase)

↓

Uroporphyrinogen III

|

(UROD)

↓

Coproporphyrinogen III

|

(Coproporphyrinogen III oxidase)

↓

Protoporphyrinogen IX

|

(Protoporphyrinogen oxidase)

↓

Protoporphyrin IX

|

(Ferrochelatase + Fe^{2+})

↓

Heme

Clinical Applications of Heme Biosynthesis: Lead Poisoning

A. Definition and Sources

- Lead poisoning = buildup of lead in the body over months to years

- Common sources:

    - Lead-based paints and paint dust/flakes (especially in older buildings)
    - Lead-contaminated drinking water from household plumbing

- Routes of exposure:
    - Inhalation
    - Skin or mucous membrane contact
    - Ingestion
- Lead has a sweet taste:
    - Infants and toddlers are at higher risk of ingestion exposure

B. Symptoms

- Developmental delays
- Learning disabilities
- Low IQ
- Abdominal pain
- Constipation
- Neurologic changes
- Irritability
- Very high lead levels can be fatal

C. Biochemical Mechanism

Target Enzyme	Effect of Lead Inhibition
ALA dehydratase	Inhibited → ↑ ALA accumulation
Ferrochelatase	Inhibited → ↓ Heme synthesis; ↑ Zinc protoporphyrin

- Impaired iron utilization leads to:
    - ↑ Zinc substitution for iron by ferrochelatase
- Clinical result:
    - Anemia
    - ↑ Zinc protoporphyrin levels

D. Neurological Toxicity

- ↑ ALA is neurotoxic
- Lead can cross the blood-brain barrier
- Lead is neurotoxic

E. Treatment

- Primary step: Remove the lead source
- Severe cases (serum lead > 45 µg/dL): use divalent chelators
    - Succimer (DMSA) — 2,3-dimercaptosuccinic acid
    - Calcium disodium EDTA — Ethylenediaminetetraacetic acid
    - Other chelating agents may be used to remove lead ions from blood

C. Porphyrias

A. Definition and Cause

- Porphyrias = rare, mostly inherited (occasionally acquired) defects in heme synthesis
- Lead to:
    - Accumulation and increased excretion of porphyrins or porphyrin precursors

- Genetic inheritance:
    - Autosomal Dominant (AD) or
    - Autosomal Recessive (AR)

Note: "Porphyria" comes from Greek porphyra (purple) — refers to red-blue urine color due to pigment-like porphyrins

B. Enzyme Deficiencies and Accumulated Intermediates

- Each type of porphyria is associated with:
    - Deficiency in a specific enzyme
    - Accumulation of a unique pattern of intermediates in the heme biosynthetic pathway

I. Clinical Manifestations

Classification Based on Site of Enzyme Defect

Type	Defect Location
Erythropoietic	Erythropoietic cells of bone marrow
Hepatic	Liver

- Hepatic porphyrias further classified into:
    - Chronic hepatic porphyrias
    - Acute hepatic porphyrias

Symptoms Based on Step Affected

Step Affected	Clinical Manifestation
Before tetrapyrrole synthesis	Abdominal and neuropsychiatric signs
During tetrapyrrole accumulation	Photosensitivity (itching, burning skin in sunlight)

Photosensitivity Mechanism:

- Caused by oxidation of porphyrinogens (colorless) to porphyrins (colored)
- Porphyrins are photosensitizing molecules:
    - Generate superoxide radicals from oxygen
    - Radicals cause:
        - Oxidative damage to membranes
        - Release of destructive enzymes from lysosomes

Types of Porphyrias (Detailed Classification)

a. Chronic Hepatic Porphyria

Porphyria Cutanea Tarda (PCT)

- Most common porphyria
- Chronic liver disease
- Associated with severe deficiency of UROD
    - (Uroporphyrinogen III decarboxylase)

Influencing Factors on Clinical Expression:

- Hepatic iron overload
- Exposure to sunlight
- Alcohol ingestion
- Estrogen therapy
- Hepatitis B or C infection
- HIV infection

Genetics:

- Mutations in UROD found in only 20% of affected individuals
- Inheritance: Autosomal Dominant (AD)

Clinical Features:

- Onset: typically during 4th or 5th decade
- Porphyrin accumulation leads to:
    - Cutaneous symptoms
    - Urine color changes:
        - Red to brown in natural light
        - Pink to red in fluorescent light

b. Acute Hepatic Porphyrias

Types Included:

- ALA dehydratase-deficiency porphyria
- Acute intermittent porphyria (AIP)
- Hereditary coproporphyria (HCP)

- Variegate porphyria (VP)

Clinical Characteristics:

- Characterized by acute attacks:
    - Gastrointestinal (GI) symptoms
    - Neuropsychiatric symptoms
    - Motor symptoms
    - May include photosensitivity

Specific Manifestations in AIP:

- Accumulation of:
    - ALA (δ-aminolevulinic acid)
    - PBG (Porphobilinogen)
- Symptoms:
    - Abdominal pain
    - Neuropsychiatric disturbances
        - Range: Anxiety → Delirium

Triggers of Acute Attacks:

- Drug use, such as:
    - Barbiturates
    - Ethanol

Mechanism:

- These agents induce CYP microsomal drug-oxidation system (a heme-containing enzyme system)
- This causes:
    - Decreased available heme
    - Which upregulates ALAS1 synthesis
    - Leading to further accumulation of toxic intermediates

c. Erythropoietic Porphyrias

Types Included:

- Congenital erythropoietic porphyria (CEP)
- Erythropoietic protoporphyria (EPP)

Clinical Characteristics:

- Chronic in nature
- Symptoms appear in early childhood
- Characterized by:
    - Photosensitivity
        - Skin rashes
        - Blisters

2. Increased δ-Aminolevulinic Acid Synthase (ALAS1) Activity

A. Common Feature in Hepatic Porphyrias

- Decreased synthesis of heme is a shared feature of hepatic porphyrias

B. Role of Heme in Regulation

- In the liver, heme normally acts as a:
    - Repressor of the ALAS1 gene

C. Consequence of Heme Deficiency

- Absence of heme → derepression (activation) of ALAS1 gene

- Result:

    - ↑ ALAS1 synthesis
    - ↑ Synthesis of upstream intermediates (those formed before the genetic enzyme block)
    - These toxic intermediates accumulate

This accumulation is the major pathophysiological mechanism of porphyrias

3. Treatment of Porphyrias

A. Acute Porphyria Attacks

Supportive Care

- Patients require:

    - Medical support
    - Treatment for pain
    - Treatment for vomiting

B. Pharmacologic Interventions

1. Hemin Therapy

- Composition:
    - Protoporphyrin structure with ferric iron (Fe^{3+}) coordinated with a chloride ion
- Administration: Intravenous
- Effects:
    - Reduces porphyrin deficit
    - Decreases ALAS1 synthesis
    - Minimizes production of toxic intermediates

2. Glucose Therapy

- High doses of glucose can:
    - Suppress porphyrin biosynthesis in the liver

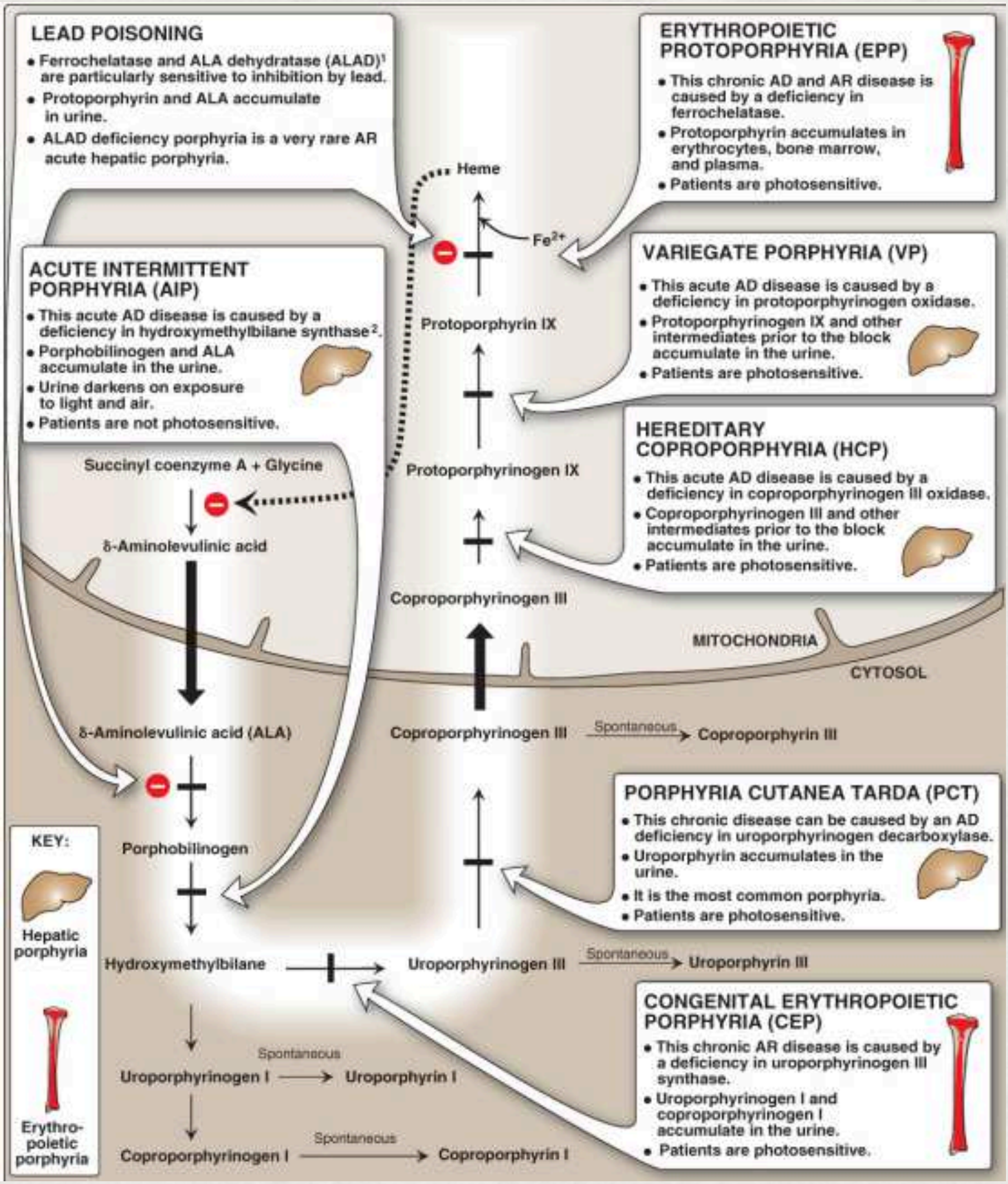
Both hemin and glucose treatments are particularly effective for:

- Acute intermittent porphyria (AIP)
- Other acute hepatic porphyrias

C. Management of Photosensitive Porphyrias

Management Strategy	Mechanism / Benefit
Protection from sunlight	Prevents UV-triggered oxidative damage
β-Carotene (provitamin A)	Scavenges free radicals
Phlebotomy	Removes excess porphyrins from circulation

Summary of Heme Synthesis



D. Heme Degradation

Overview

- After ~120 days in circulation, RBCs are:
    - Taken up and degraded by the mononuclear phagocyte system (MPS)
    - MPS is active particularly in the liver and spleen
- ~85% of heme targeted for degradation originates from:
    - Senescent RBCs
- The remaining ~15% comes from:
    - Degradation of heme proteins other than hemoglobin (Hb)

1. Bilirubin Formation

A. Enzymatic Process

- Enzyme: Microsomal heme oxygenase

- Location: Macrophages of the mononuclear phagocyte system (MPS)

- Cofactors required:

    - NADPH (Nicotinamide adenine dinucleotide phosphate)
    - Oxygen (O_2)

B. Reaction

- Three successive oxygenations

- Results in:

    a. Opening of the porphyrin ring
    b. → Converts cyclic heme → linear biliverdin
    c. Production of carbon monoxide (CO)
    d. Release of Fe^{2+}

Notes:

- CO has biological functions:

    - Acts as a signaling molecule
    - Has anti-inflammatory properties

C. Conversion to Bilirubin

- Biliverdin (a green pigment) is:
    - Reduced to form bilirubin (a red-orange pigment)
- Bilirubin and derivatives = collectively known as bile pigments

Note: Changing colors of a bruise reflect different intermediates during heme degradation

D. Antioxidant Role of Bilirubin

- Bilirubin is unique to mammals
- Functions at low levels as an antioxidant
- Mechanism:
    - Oxidized to biliverdin
    - Biliverdin is reduced back to bilirubin by:
        - Biliverdin reductase

2. Bilirubin Uptake by the Liver

A. Plasma Transport

- Bilirubin is only slightly soluble in plasma
- Transported via noncovalent binding to albumin

Note:

- Certain anionic drugs (e.g. salicylates, sulfonamides) can:
    - Displace bilirubin from albumin
    - Allow free bilirubin to enter the CNS
    - Potential consequence: Neural damage in infants

B. Hepatic Uptake

- At the liver:
    - Bilirubin dissociates from albumin
    - Enters hepatocyte via facilitated diffusion
    - Inside hepatocyte:
        - Binds intracellular proteins, especially:
            - Ligandin

3. Bilirubin Diglucuronide Formation

A. Site and Purpose

- Occurs in the hepatocyte
- Purpose: Increase bilirubin solubility via conjugation

B. Reaction Details

- Two molecules of glucuronic acid are sequentially added to bilirubin
- Enzyme: Microsomal bilirubin UDP-glucuronosyltransferase (bilirubin UGT)
- Donor molecule: UDP-glucuronic acid (uridine diphosphate-glucuronic acid)
- Product: Bilirubin diglucuronide
- → Also called conjugated bilirubin (CB)

Clinical Note:

- Deficiencies in bilirubin UGT lead to:
    - Crigler-Najjar syndrome type I (most severe)
    - Crigler-Najjar syndrome type II
    - Gilbert syndrome

4. Bilirubin Secretion into Bile

A. Transport Mechanism

- CB is actively transported:
    - Against a concentration gradient
    - Into the bile canaliculi
    - Then into bile

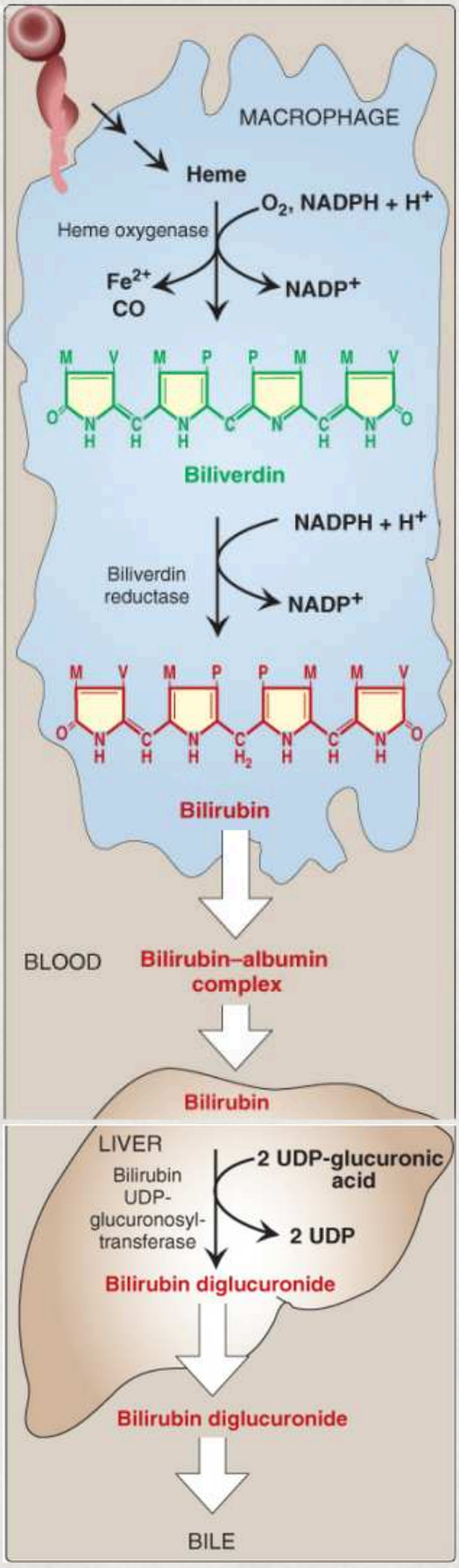
B. Characteristics of the Step

- Energy-dependent
- Rate-limiting
- Susceptible to impairment in liver disease

Clinical Note:

- Dubin-Johnson syndrome results from:
    - Rare deficiency in the protein required for CB transport out of the liver
- Unconjugated bilirubin (UCB):
    - Not normally secreted into bile

Formation of Bilirubin from Heme and its Conversion to Bilirubin Diglucuronide



5. Urobilin Formation in the Intestine

A. Intestinal Conversion

- CB is hydrolyzed and reduced by gut bacteria
- Product: Urobilinogen (colorless)

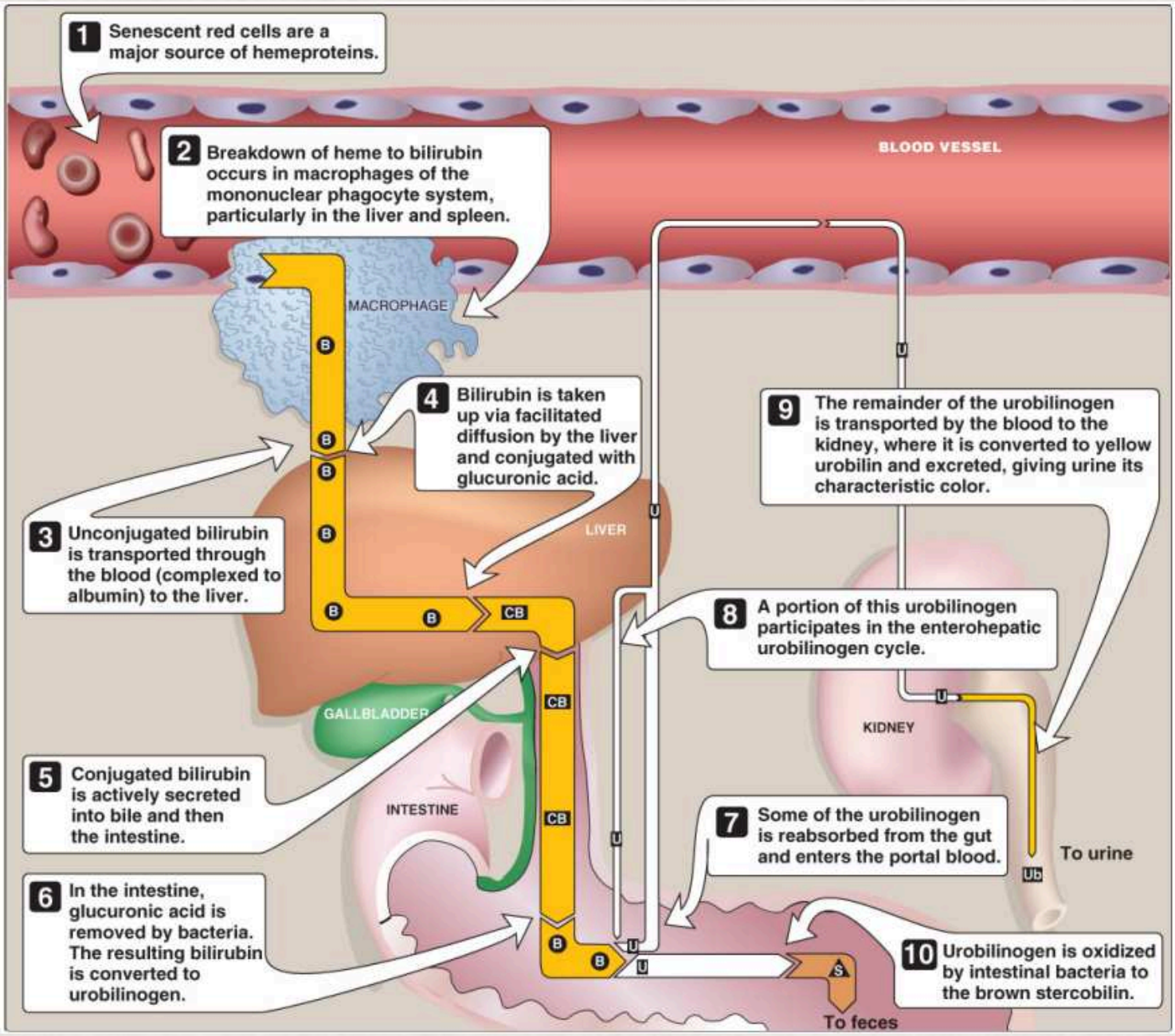
B. Fate of Urobilinogen

Pathway	Outcome
Majority	Further oxidized by bacteria to stercobilin → gives feces brown color
Some reabsorbed into portal blood	Enters enterohepatic urobilinogen cycle Taken up again by liver → resecreted into bile
Some enters systemic circulation	Transported to kidney → converted to yellow urobilin → excreted in urine

Summary of Physiological Effects

- Stercobilin → gives feces brown color
- Urobilin → gives urine yellow color

Catabolism of heme



E. Jaundice

Definition & Overview

- Jaundice (icterus):
- Yellow discoloration of:
  - Skin
  - Nail beds
  - Sclerae (whites of the eyes)
- Cause:
  - Deposition of bilirubin due to elevated levels in blood
  - → Hyperbilirubinemia

Normal blood bilirubin: ⩽ 1 mg/dL

Jaundice visible at: 2–3 mg/dL

- Jaundice is not a disease itself but a symptom of an underlying disorder

1. Types of Jaundice

a. Hemolytic (Prehepatic) Jaundice

- Cause: Extensive hemolysis, e.g.:
    - Sickle cell anemia
    - Pyruvate kinase deficiency
    - Glucose-6-phosphate dehydrogenase (G6PD) deficiency

Liver's Capacity

Parameter	Amount
Normal bilirubin production	~300 mg/day
Liver's conjugation capacity	>3,000 mg/day

- Effect: Liver can initially keep up with increased bilirubin
- But in excessive hemolysis, bilirubin production > conjugation capacity

Clinical Findings:

- ↑ Unconjugated bilirubin (UCB) in blood
- → Unconjugated hyperbilirubinemia → Jaundice
- Conjugated bilirubin (CB) may:
    - Increase to high-normal range
    - Be excreted into bile

Urobilinogen Pathway:

- ↑ Urobilinogen enters:
    - Enterohepatic circulation
    - Blood → filtered into urine

Substance	Level
UCB (blood)	↑ ↑ (abnormally high)
CB, urobilinogen, stercobilin, urobilin	High side of normal

b. Hepatocellular (Hepatic) Jaundice

- Cause: Liver cell damage, e.g.:
    - Cirrhosis
    - Hepatitis

Mechanism:

- ↓ Conjugation of bilirubin → ↑ UCB in blood
- → Unconjugated hyperbilirubinemia
- Impaired enterohepatic circulation:
    - ↑ Urobilinogen in blood
    - → Filtered into urine

Urine and Stool Findings:

- Urine: Darkens due to urobilinogen
- Stool: May appear pale/clay-colored

Liver Enzymes:

- ↑ ALT (Alanine transaminase)
- ↑ AST (Aspartate transaminase)

Cholestasis Scenario (Intrahepatic):

- If CB is formed but not secreted into bile:
  - Regurgitates into blood
  - → Conjugated hyperbilirubinemia

Clinical Summary:

Substance in Blood	Level in Hepatic Jaundice
UCB	↑ (abnormal)
CB	↑ (abnormal)

c. Obstructive (Posthepatic) Jaundice

Cause:

- Not due to overproduction or impaired conjugation
- Results from obstruction of the common bile duct → extrahepatic cholestasis
- Common causes:
    - Tumors
    - Bile stones

Effect:

- Prevents CB (conjugated bilirubin) from entering the intestine

Clinical Features:

-> GI pain
-> Nausea
-> Stools: Pale, clay-colored

Biochemical Changes:

- CB regurgitates into blood → Conjugated hyperbilirubinemia
- CB is excreted in urine:
    - Urine darkens
    - Called urinary bilirubin
- Urinary urobilinogen is absent

2. Jaundice in Newborns

Prevalence:

- Seen in:
    - 60% of full-term infants
    - 80% of preterm infants
- Occurs in the first postnatal week
- Called transient, physiologic jaundice

Cause:

- Low activity of hepatic bilirubin UGT at birth
    - Reaches adult levels in ~4 weeks

Risk of Kernicterus (Toxic Encephalopathy):

- Excess UCB > albumin binding capacity (20–25 mg/dL) → Diffuses into basal ganglia
- Leads to:
    - Toxic encephalopathy
    - Pathologic jaundice

Treatment:

- Phototherapy with blue fluorescent light
    - Converts bilirubin → polar, water-soluble photoisomers
    - These isomers:
        - Can be excreted into bile
        - Do not require conjugation with glucuronic acid

Important Notes:

- Only UCB crosses blood-brain barrier
- Only CB appears in urine

3. Bilirubin Measurement

Method:

- Van den Bergh reaction:
  - Uses diazotized sulfanilic acid
  - Reacts with bilirubin → red azodipyrroles
  - Measured colorimetrically

A. Direct-Reacting Bilirubin (CB)

- Water-soluble
- Reacts rapidly (within 1 min) in aqueous solution
- Called direct-reacting bilirubin

B. Indirect-Reacting Bilirubin (UCB)

- Less soluble in aqueous solution
- Reacts slowly
- In methanol, both CB and UCB become soluble and react
    - Gives the total bilirubin value

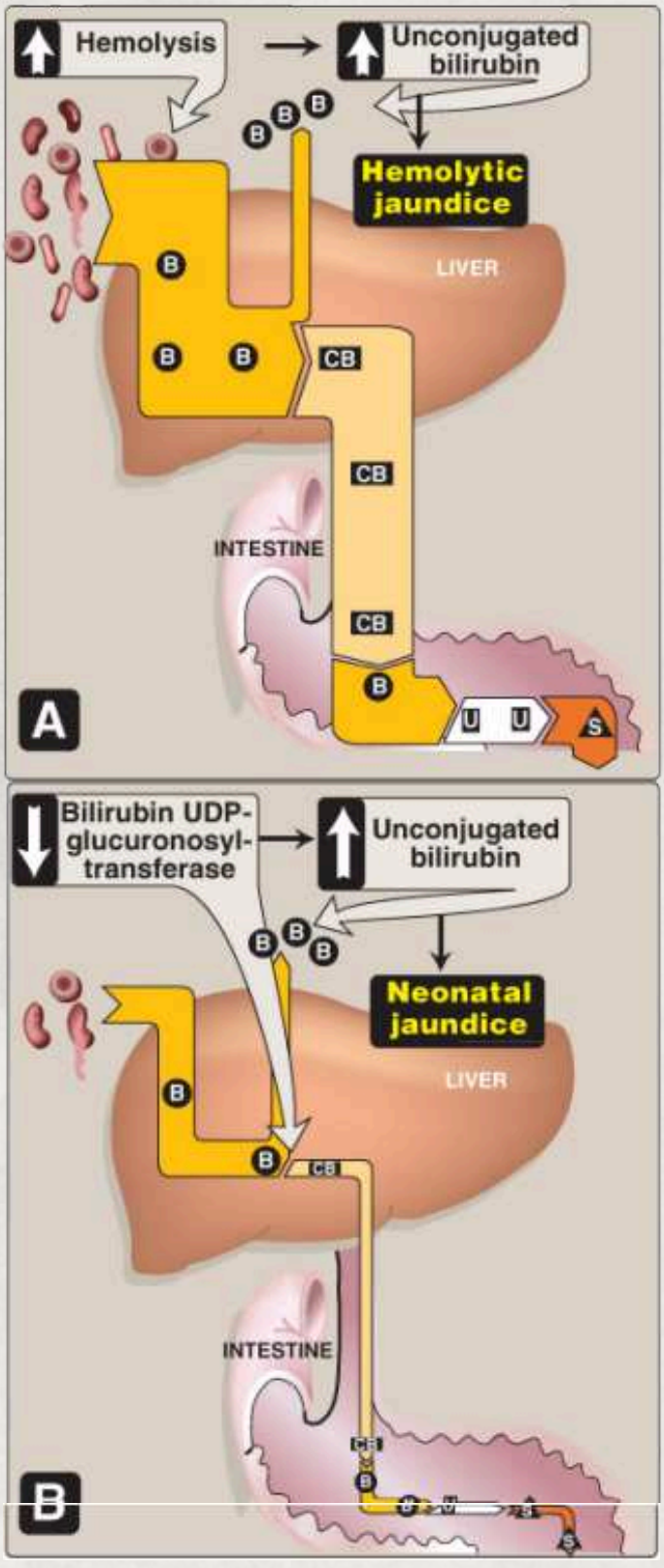
C. Calculation of Indirect (UCB):

Indirect-reacting bilirubin = Total bilirubin - Direct - reacting bilirubin

Note: In normal plasma,

- Only ~4% of total bilirubin is conjugated (direct-reacting)
- Most CB is secreted into bile, not found in plasma

Alterations in the Metabolism of Heme



III. Other Nitrogen-Containing Compounds

A. Catecholamines

Overview:

- Dopamine, norepinephrine (NE), and epinephrine (adrenaline) are:
    - Biologically active (biogenic) amines
    - Collectively called catecholamines
- Dopamine and NE:
    - Synthesized in the brain
    - Function as neurotransmitters
- Epinephrine:
    - Synthesized from NE
    - Occurs in the adrenal medulla

1. Function

- Outside CNS, NE and epinephrine act as:
    - Hormone regulators of:
        - Carbohydrate metabolism
        - Lipid metabolism
- Released from storage vesicles in adrenal medulla in response to:
    - Fright
    - Exercise
    - Cold
    - Low blood glucose

Physiologic Effects (Fight-or-Flight Reaction):

- ↑ Glycogen degradation
- ↑ Triacylglycerol degradation
- ↑ Blood pressure
- ↑ Cardiac output

All effects are part of a coordinated stress response
→ Known as "fight-or-flight" reactions

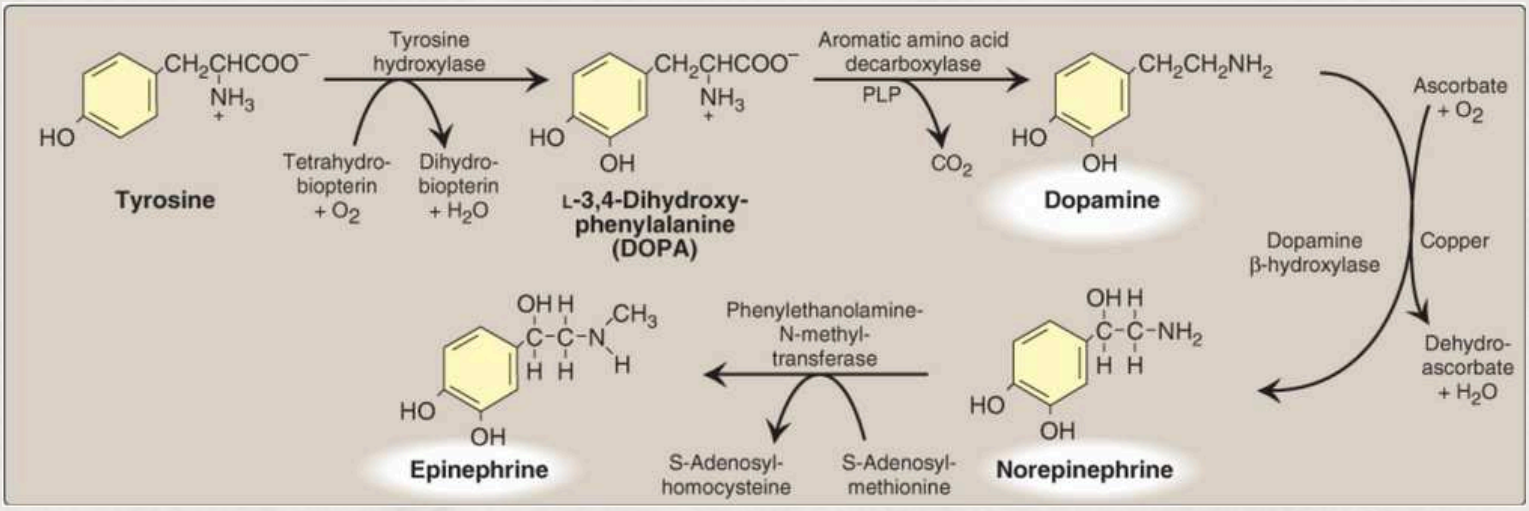
2. Synthesis of Catecholamines

- Precursor: Tyrosine

Stepwise Pathway

Step	Reaction	Enzyme	Cofactors/Notes
1	Tyrosine → L-DOPA (L-3,4-dihydroxyphenylalanine)	Tyrosine hydroxylase	Requires BH_4 (tetrahydrobiopterin) Rate-limiting step Occurs in CNS, sympathetic ganglia, adrenal medulla
2	DOPA → Dopamine	DOPA decarboxylase (DDC)	Requires PLP (pyridoxal phosphate)
3	Dopamine → Norepinephrine (NE)	Dopamine β-hydroxylase	Requires Ascorbic acid (Vitamin C) and Copper
4	Norepinephrine → Epinephrine	N-methyltransferase	S-adenosylmethionine (SAM) is the methyl donor

Synthesis of Catecholamines
(Note: Catechols have two adjacent hydroxyl groups.)



3. Degradation of Catecholamines

Enzymatic Inactivation:

- Catecholamines are inactivated by:
    - Oxidative deamination:
    - → Catalyzed by monoamine oxidase (MAO)
    - O-methylation:
    - → Catalyzed by catechol-O-methyltransferase (COMT)
    - → Uses S-adenosylmethionine (SAM) as methyl donor

Reactions can occur in either order

Fate of Aldehyde Products (MAO reaction):

- Aldehydes are oxidized to corresponding acids

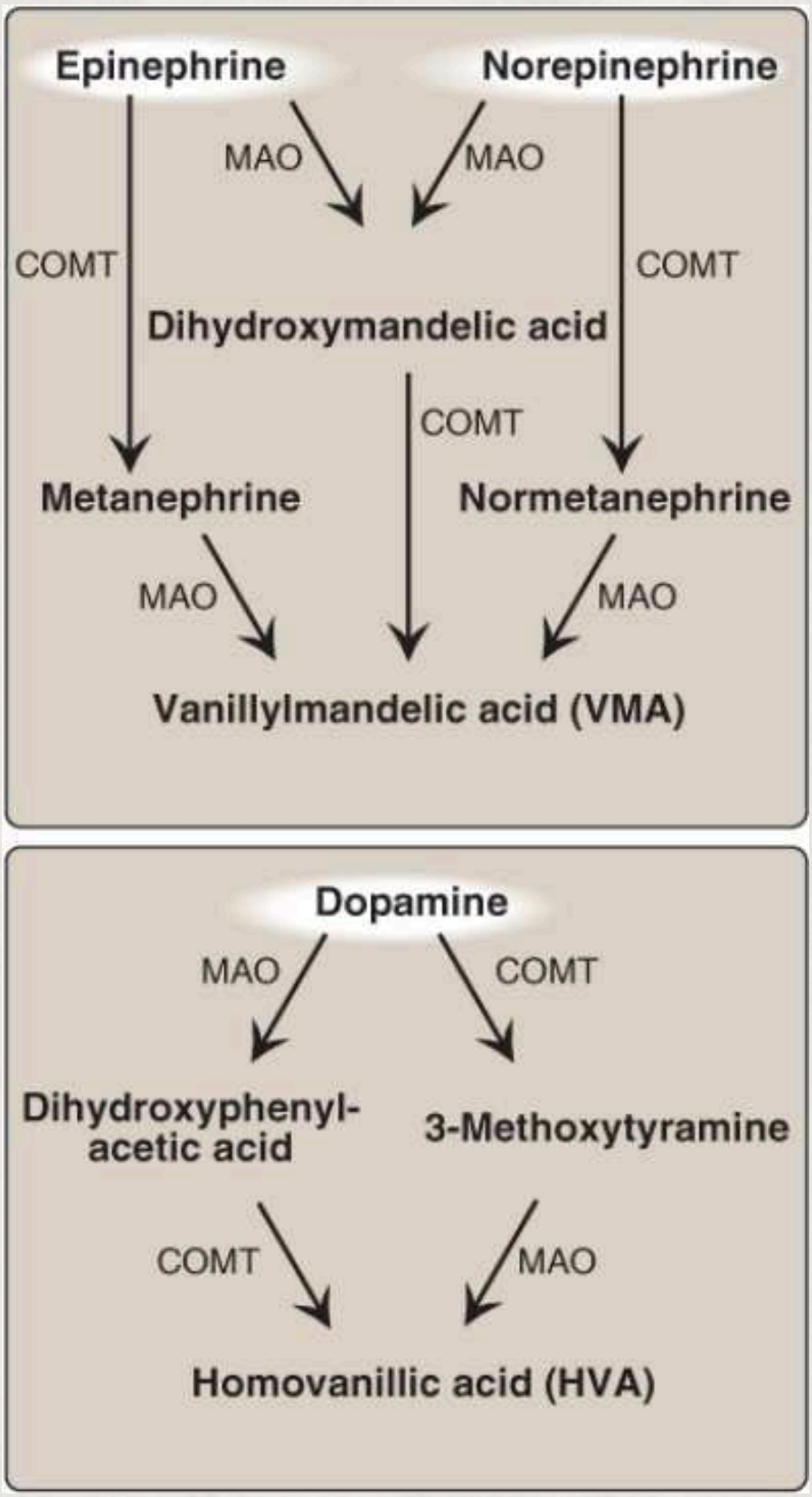
Catecholamine	Final Degradation Product	Excreted In
Epinephrine & NE	Vanillylmandelic acid (VMA)	Urine
Dopamine	Homovanillic acid (HVA)	Urine

VMA and metanephrines are increased in:

- Pheochromocytomas
- → Rare adrenal gland tumors with excess catecholamine production

Metabolism of the Catecholamines by Catechol-O-Methyltranferase (COMT) and Monoamine Oxidase (MAO)

(Note: COMT requires Sadenosylmethionine.)



Clinical Application: Parkinson Disease

Cause:

- Neurodegenerative movement disorder
- Due to insufficient dopamine production
- Caused by idiopathic loss of dopamine-producing cells in the brain

Treatment:

- Levodopa (L-DOPA):
    - Most common treatment
    - Crosses blood-brain barrier
    - Converted to dopamine in CNS
- Carbidopa:
    - Inhibits DOPA decarboxylase (DDC) in peripheral nervous system
    - Prevents peripheral conversion of L-DOPA → dopamine
    - Cannot cross the blood-brain barrier

- Used in combination with L-DOPA:
  - Increases L-DOPA availability to CNS
  - Reaches therapeutic range

BH_4 Deficiency Case:

- L-DOPA may be given as a neurotransmitter supplement

- To produce:

  - Dopamine
  - Norepinephrine
  - Epinephrine

4. Monoamine Oxidase Inhibitors (MAOI)

MAO Enzyme:

- Found in:

  - Neural tissue
  - Intestine
  - Liver

- Role in neurons:
    - Oxidatively deaminates and inactivates any excess neurotransmitters (NE, dopamine, serotonin)
    - Prevents leakage from resting synaptic vesicles

MAOI Drugs:

- Inactivate MAO (reversibly or irreversibly)

- Effects:
    - Neurotransmitters escape degradation
    - Accumulate in presynaptic neuron
    - Leak into synaptic cleft
    - → Activate NE and serotonin receptors

Clinical Use:

- Believed to be the basis for antidepressant action of MAOIs

B. Histamine

Function:

- Histamine is a chemical messenger mediating:
    - Allergic reactions
    - Inflammatory responses
    - Gastric acid secretion
- Acts as a powerful vasodilator

Synthesis:

Substrate	Product	Enzyme	Cofactor
Histidine	Histamine	Histidine decarboxylase	PLP

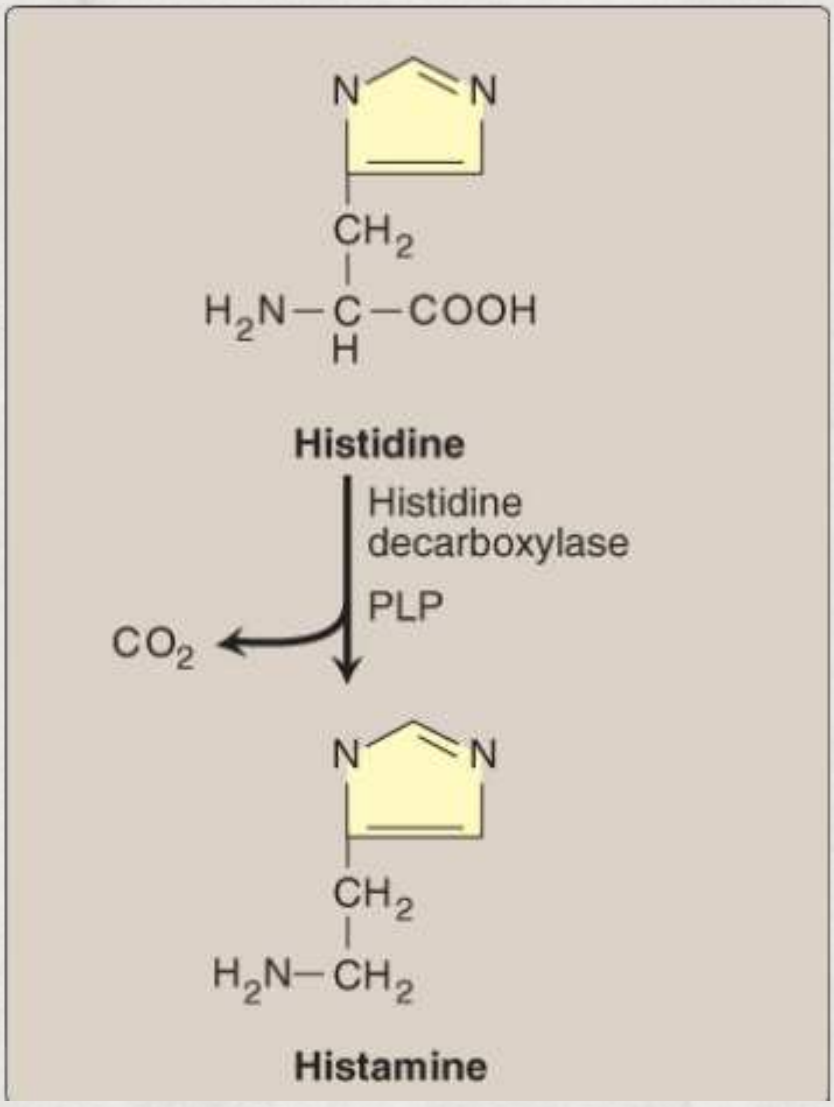
Release:

- Secreted by mast cells
- Triggered by:
    - Allergic reactions
    - Trauma

Clinical Note:

- Histamine itself has no clinical applications
- Antihistamines:
  - Interfere with histamine action
  - Are histamine analogs
  - Block histamine binding to receptors
  - Used to reduce histamine responses

Biosynthesis of Histamine



C. Serotonin (5-hydroxytryptamine, 5-HT)

Location & Storage:

- Synthesized and/or stored in multiple body sites:
    - Intestinal mucosa (largest amount)
    - Central nervous system (CNS)
        - Functions as neurotransmitter
    - Platelets

Synthesis Pathway:

Step	Substrate	Product	Enzyme	Cofactor
1	Tryptophan	5-Hydroxytryptophan	Tryptophan hydroxylase	BH_4
2	5-Hydroxytryptophan	Serotonin (5-HT)	Aromatic L-amino acid decarboxylase	PLP

- Reaction is analogous to phenylalanine hydroxylation
- In BH_4 deficiency, 5-hydroxytryptophan can be given as supplement to produce serotonin

Physiologic Roles of Serotonin:

- Pain perception
- Regulation of:
    - Sleep
    - Appetite
    - Temperature
    - Blood pressure
    - Cognitive functions
    - Mood (causes a feeling of well-being)

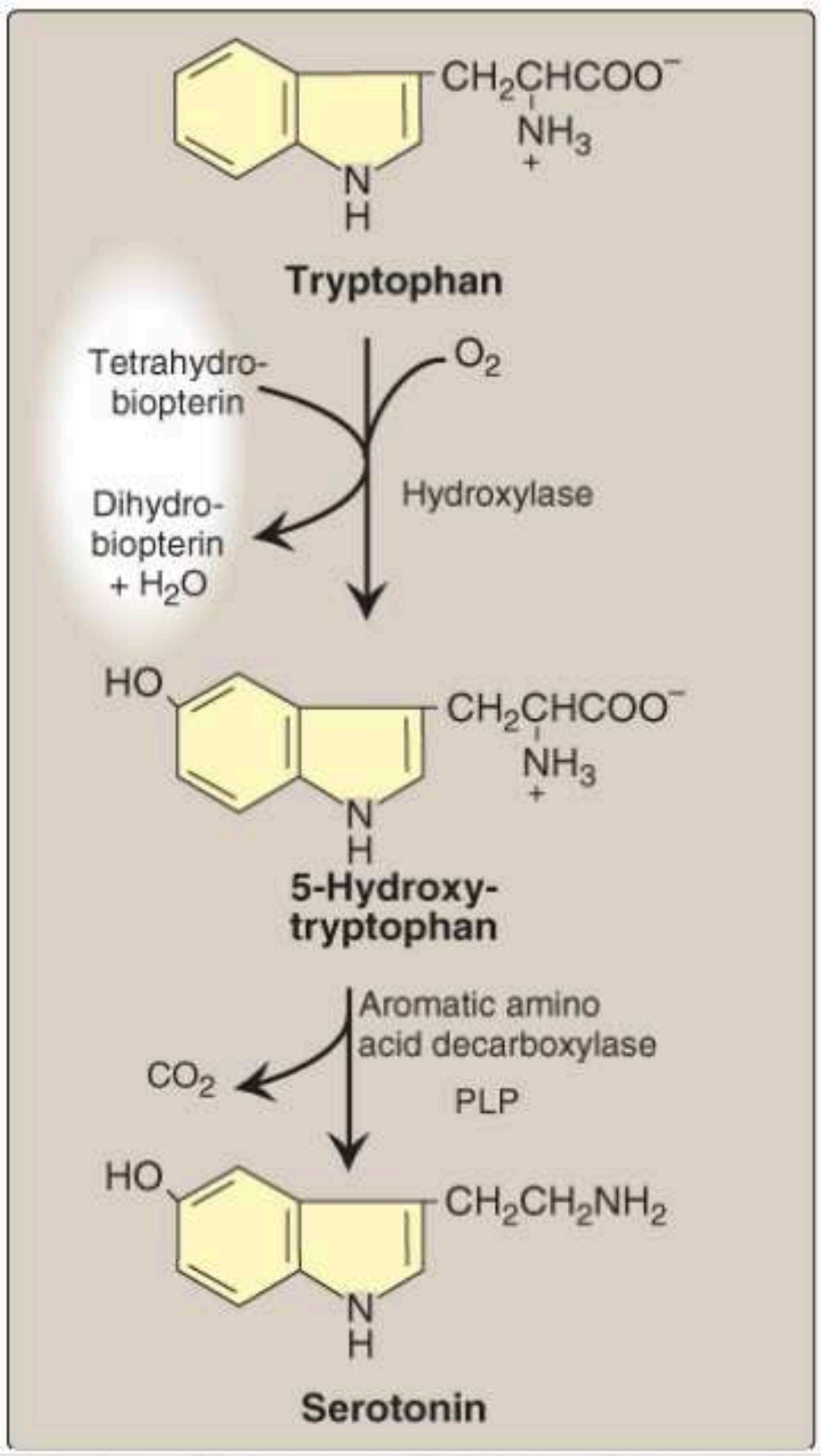
Selective serotonin reuptake inhibitors (SSRIs):

- Maintain serotonin levels
- Function as antidepressants

Degradation:

Substrate	Enzyme	Product
Serotonin	MAO	5-hydroxy-3-indoleacetic acid (5-HIAA)

Synthesis of Serotonin.
(Note: Serotonin is converted to melatonin, a regulator of circadian rhythm, in the pineal gland.)



D. Creatine

Role of Creatine Phosphate (Phosphocreatine):

- High-energy compound in muscle
- Serves as a small but rapidly mobilized reserve of high-energy phosphates
- Energy is reversibly transferred to ADP to maintain intracellular ATP levels
- Functions during the first few minutes of intense muscular contraction
- Amount in body is proportional to muscle mass

1. Synthesis of Creatine:

Site	Precursors	Additional Groups	Methyl Donor
Liver & Kidneys	Glycine + Guanidino group of Arginine	Methyl group	SAM

- Dietary source: Animal products

- Creatine is reversibly phosphorylated to creatine phosphate by:

    - Enzyme: Creatine kinase
    - Phosphate donor: ATP

- Creatine kinase (MB isozyme) in plasma is a marker of heart damage, used to diagnose myocardial infarction

2. Degradation of Creatine:

- Spontaneous, nonenzymatic cyclization:

    - Creatine ↔ Creatine phosphate → Creatinine

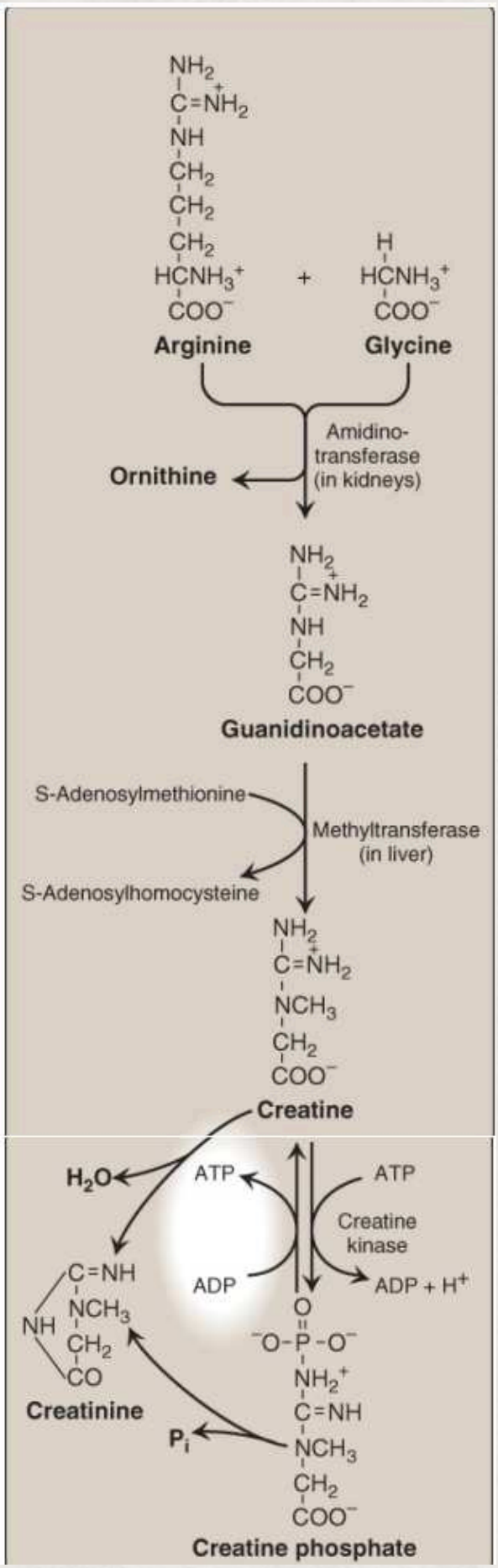
- Excretion:

    - Creatinine is excreted in urine
    - Rate of creatinine excretion is proportional to total creatine phosphate content of the body
    - Used to estimate muscle mass

Clinical Relevance:

- Muscle wasting (e.g., paralysis, muscular dystrophy): ↓ urinary creatinine
- Kidney malfunction: ↑ blood creatinine
    - Because creatinine is normally rapidly cleared by the kidney
- Typical adult male excretes 1 to 2 g/day of creatinine

Synthesis of Creatine



E. Melanin

Overview:

- Pigment found in:
    - Eye
    - Hair
    - Skin

Synthesis

Precursor	Location	Cell Type
Tyrosine	Epidermis	Melanocytes (pigment-forming cells)

Function:

- Protects underlying cells from harmful effects of sunlight

Clinical Note:

- Defect in melanin production → Oculocutaneous albinism

- Most common type caused by defects in copper-containing tyrosinase

Quick Review: Types of Jaundice

Feature	Hemolytic Jaundice (Prehepatic)	Hepatocellular Jaundice (Hepatic)	Obstructive Jaundice (Posthepatic)
Cause	Excessive RBC breakdown (e.g., sickle cell anemia, G6PD or pyruvate kinase deficiency)	Hepatocellular damage (e.g., hepatitis, cirrhosis)	Bile duct obstruction (e.g., stones, tumor)
Type of Hyperbilirubinemia	Unconjugated (UCB)	Mixed (UCB + CB)	Conjugated (CB)
Bilirubin in Blood	↑ ↑ UCB (CB may be high-normal)	↑ UCB and ↑ CB	↑ ↑ CB
Bilirubin in Urine	Absent (UCB not water-soluble)	CB may be present	CB present (called urinary bilirubin)
Urobilinogen in Urine	↑ ↑	↑ (due to impaired enterohepatic circulation)	Absent (no CB in intestine → no urobilinogen)

Continued...

Feature	Hemolytic Jaundice (Prehepatic)	Hepatocellular Jaundice (Hepatic)	Obstructive Jaundice (Posthepatic)
Stool Color	Normal or darker (↑ stercobilin)	Pale/clay-colored (if intrahepatic cholestasis)	Clay-colored (no stercobilin formation)
Urine Color	Normal	Dark (↑ CB and ↑ urobilinogen)	Dark (CB excreted in urine)
Liver Enzymes (ALT/AST)	Normal	↑ ↑ (due to hepatocellular injury)	Usually normal or mildly elevated
Clinical Features	Anemia, splenomegaly	Jaundice, fatigue, liver tenderness	Jaundice, GI pain, nausea
Plasma Bilirubin Levels	↑ UCB only	↑ UCB and ↑ CB	↑ CB only

Quick Review: Jaundice Diagnostic Cheat Sheet

1. 🩸 Hemolytic (Prehepatic) Jaundice

- 🔍 Clues from History:
  - Hemolytic anemia (e.g., G6PD deficiency, sickle cell disease)
  - Recent infection or oxidative drug exposure (in G6PD)
- 🧪 Lab Findings:
  - ↑ Unconjugated bilirubin (UCB)
  - Normal/↑ reticulocyte count
  - ↑ Lactate dehydrogenase (LDH)
  - ↓ Haptoglobin
  - No bilirubin in urine
  - ↑ Urinary urobilinogen
- 💡 Pearls:
  - Liver is healthy; the problem is excess RBC breakdown
  - Stools and urine may be darker due to ↑ urobilin and stercobilin
  - Only UCB is elevated in plasma

Continued...

2. 🏥 Hepatocellular (Hepatic) Jaundice

- 🔍 Clues from History:
  - Hepatitis (viral or alcoholic), cirrhosis, drug toxicity
  - May present with fatigue, ascites, or liver tenderness
- 🧪 Lab Findings:
  - ↑ Both UCB and CB
  - ↑ ALT/AST
  - ↑ Urinary urobilinogen
  - Bilirubin present in urine (CB)
  - Albumin ↓ if chronic
- 💡 Pearls:
  - Damage to hepatocytes impairs conjugation and secretion
  - Stools may be pale due to intrahepatic cholestasis
  - Urine is dark due to both CB and urobilinogen

Continued...

3. 🚫 Obstructive (Posthepatic) Jaundice

- 🔍 Clues from History:
  - Gallstones, pancreatic tumor, biliary tract obstruction
  - RUQ pain, nausea, pruritus (itching)
- 🧪 Lab Findings:
  - ↑ Conjugated bilirubin (CB)
  - ↑ Alkaline phosphatase (ALP) and γGT
  - No urobilinogen in urine
  - Bilirubin present in urine (CB) — darkens urine
  - Pale/clay-colored stools
- 💡 Pearls:
  - Bile can't reach gut → ↓ stercobilin & urobilin
  - Urine turns tea-colored
  - CB backs up into blood (regurgitation)

Continued...

🔑 One-Liner Clues for Diagnosis:

- 🩸 Unconjugated ↑ only → Hemolytic
- 🏥 Mixed ↑ (UCB + CB) → Hepatocellular
- 🚫 Conjugated ↑ only + pale stools, dark urine → Obstructive