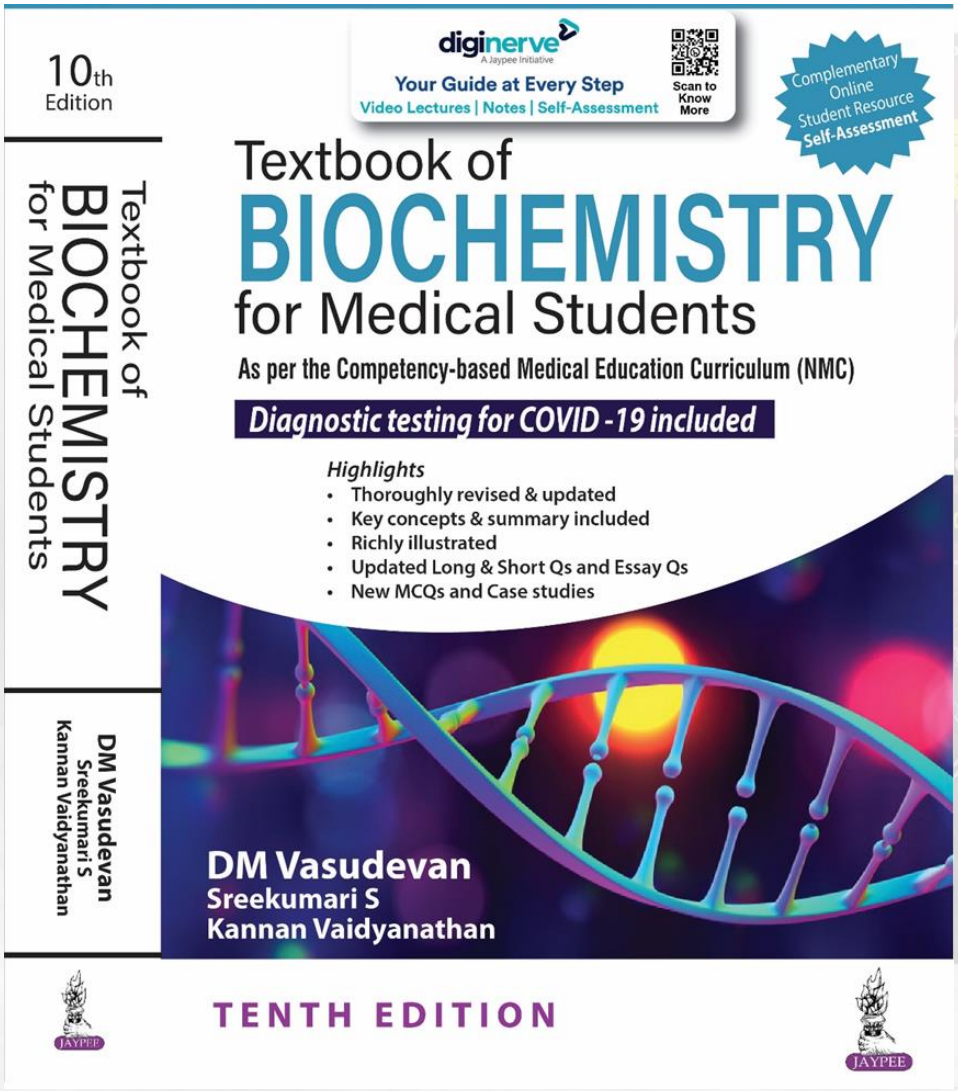


Chapter 20A:

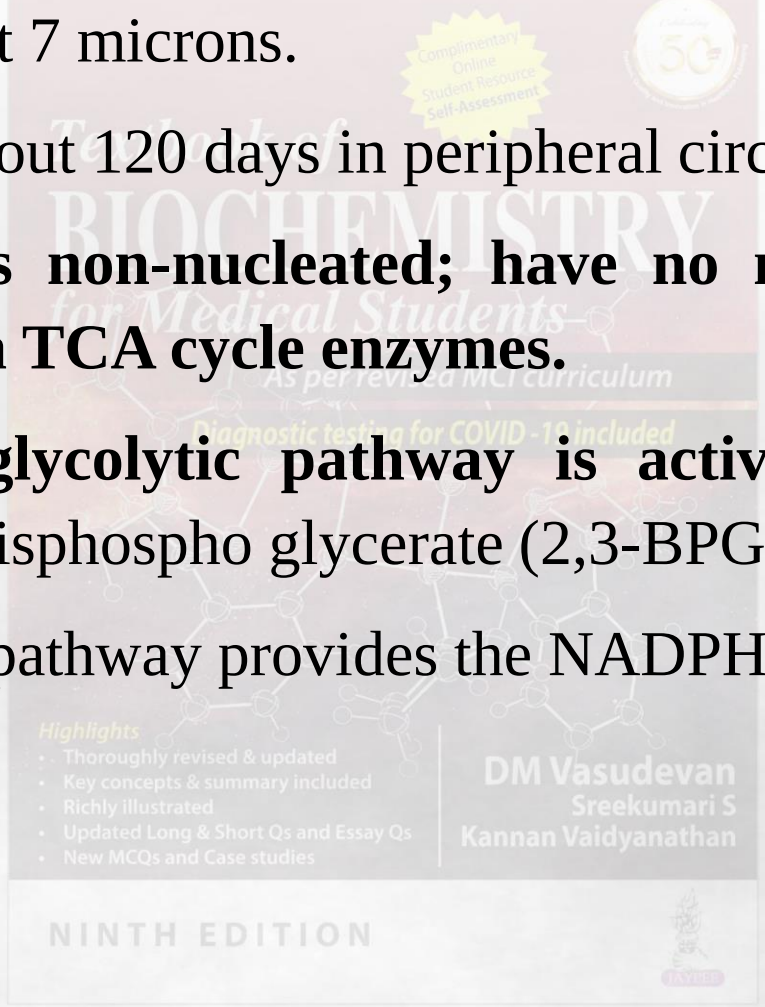
Heme, Synthesis and Breakdown

Textbook of
BIOCHEMISTRY
for Medical Students

By DM Vasudevan, *et al.*

TENTH EDITION

- Red blood cells (RBC) are biconcave discs, with a diameter of about 7 microns.
- RBCs live for about 120 days in peripheral circulation
- **Mature RBC is non-nucleated; have no mitochondria and does not contain TCA cycle enzymes.**
- **However, the glycolytic pathway is active which** provides energy and 2,3-bisphospho glycerate (2,3-BPG).
- The HMP shunt pathway provides the NADPH.



Structure of Hemoglobin

- RBC formation in the bone marrow requires amino acids, iron, copper, folic acid, vitamin B12, vitamin C, pyridoxal phosphate, and pantothenic acid; they are used as hematinics in clinical practice.
- Hemoglobin is a **conjugated protein having heme** as the prosthetic group and the protein, the globin.
- It is a tetrameric protein with 4 subunits, each subunit having a prosthetic heme group and the globin polypeptide.
- The polypeptide chains are usually **two alpha and two beta chains.**
- **Hemoglobin has a molecular** weight of about 67,000 Daltons.
- Each gram of Hb contains **3.4 mg of iron.**

- **Heme is present in**

  a. Hemoglobin

  b. Myoglobin

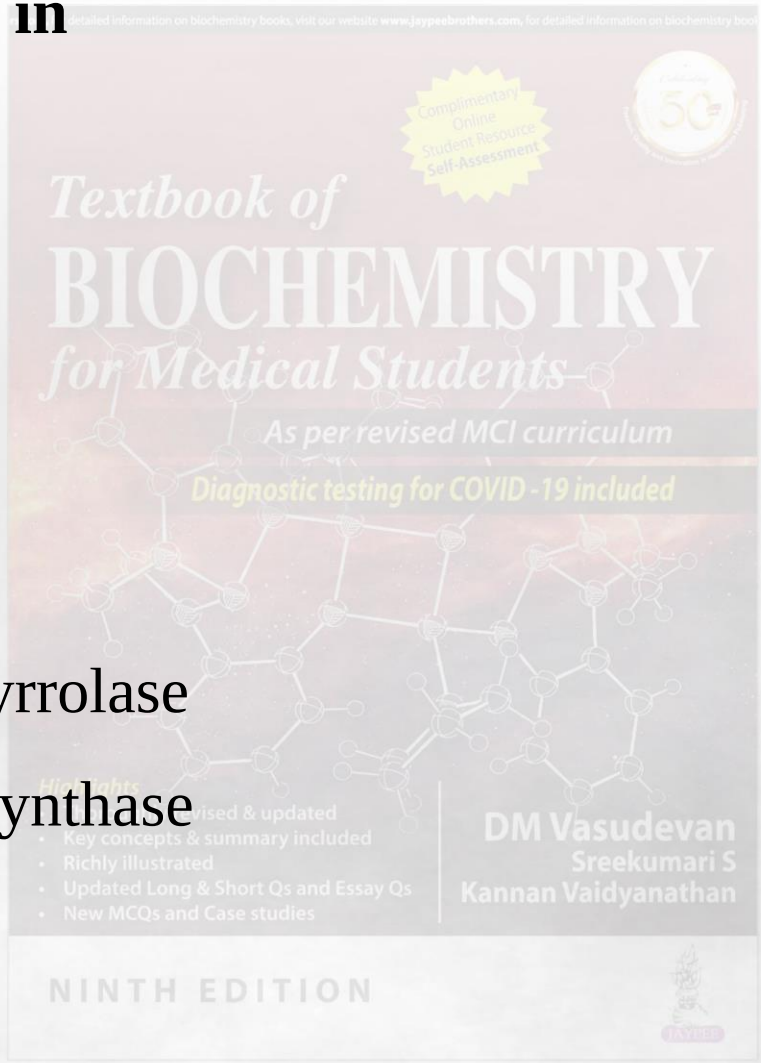
  c. Cytochromes

  d. Peroxidase

  e. Catalase

  f. Tryptophan pyrrolase

  g. Nitric oxide synthase



- Heme is produced by the combination of iron with a porphyrin ring.
- Chlorophyll, the photosynthetic green pigment in plants is magnesium-porphyrin complex.

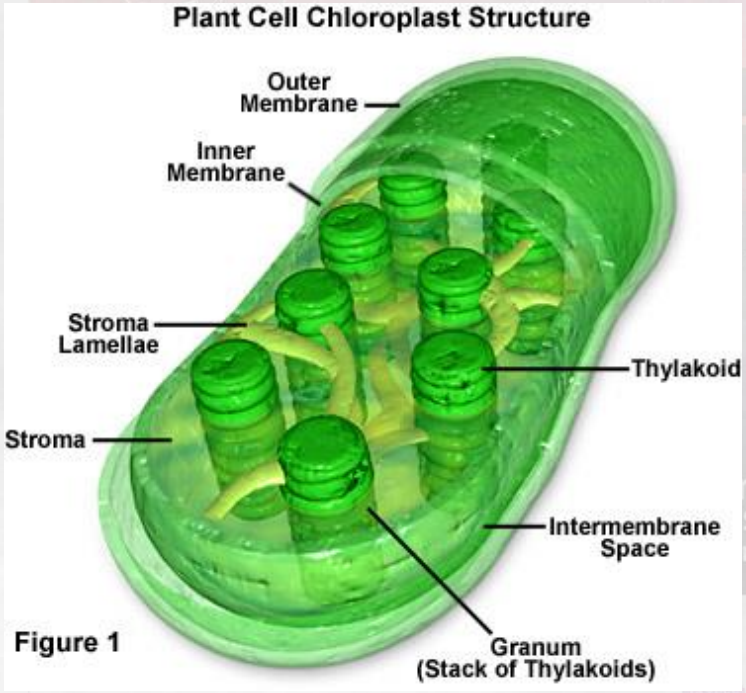
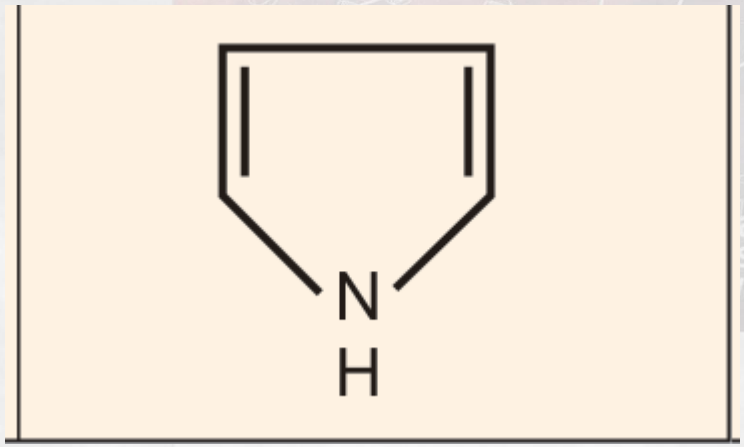


Figure 1

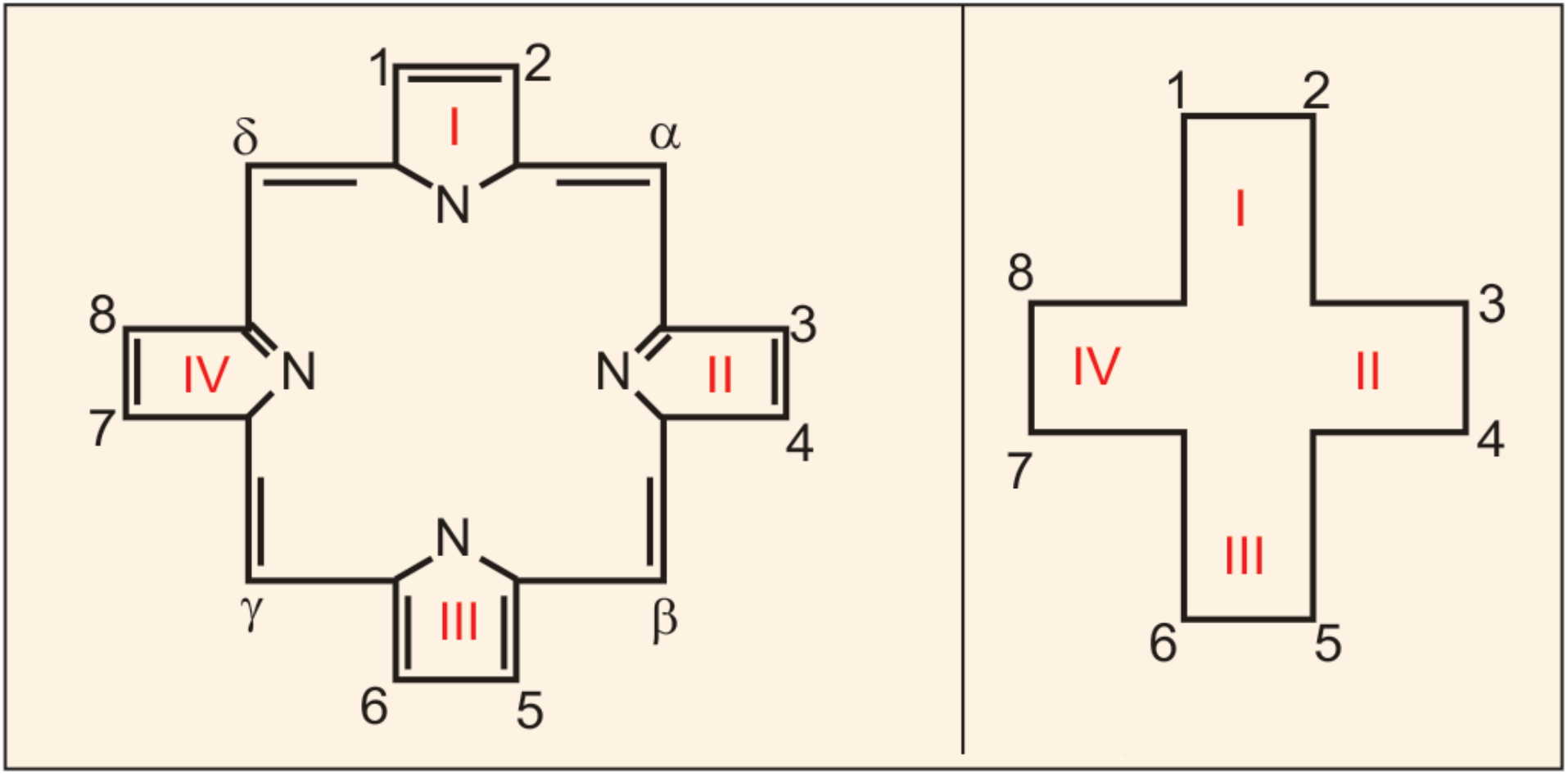
Structure of Heme

- Heme is a derivative of the porphyrin.
- **Porphyrins are** cyclic compounds formed by fusion of **4 pyrrole rings linked by methenyl (=CH-)** bridges.
- Since an atom of iron is present, heme is a **ferroprotoporphyrin.**
- **The pyrrole rings are** named as I, II, III, IV and the bridges as alpha, beta, gamma and delta.



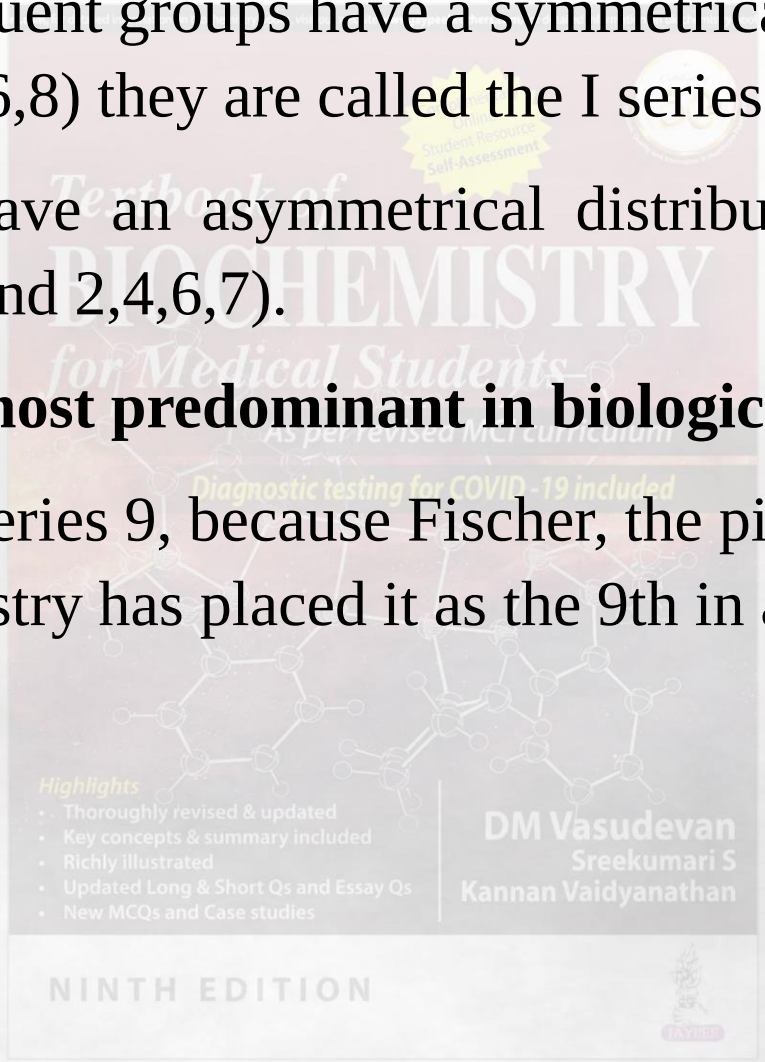
Pyrrole ring

Porphyrin Ring



Porphyrin ring. The pyrrole rings are numbered I to IV; the bridges are named as alpha to delta. The possible sites of substitutions are denoted from 1 to 8. For brevity, the bridges and double bonds are sometimes omitted, as shown on the right.

- When the substituent groups have a symmetrical arrangement (1,3,5,7 and 2,4,6,8) they are called the I series.
- The III series have an asymmetrical distribution of substituent groups (1,3,5,8 and 2,4,6,7).
- **Type III is the most predominant in biological** systems.
- It is also called series 9, because Fischer, the pioneer in porphyrin chemistry has placed it as the 9th in a series of 15 possible isomers.



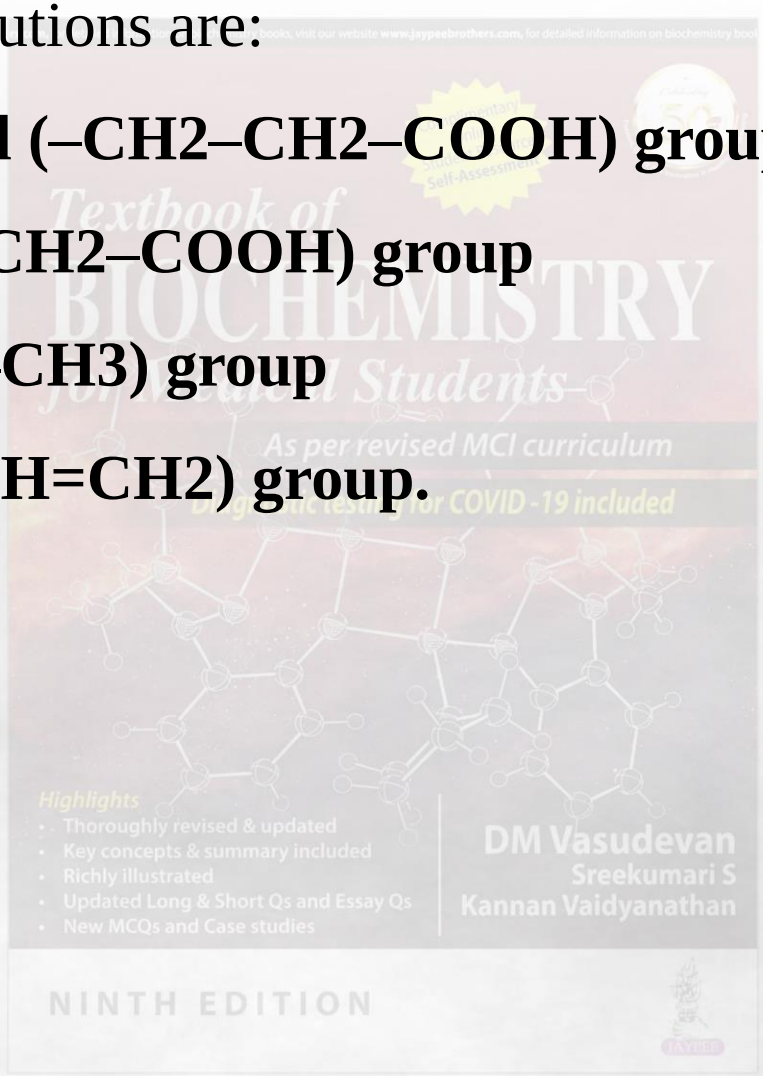
- The usual substitutions are:

a. propionyl (–CH2–CH2–COOH) group

b. acetyl (–CH2–COOH) group

c. methyl (–CH3) group

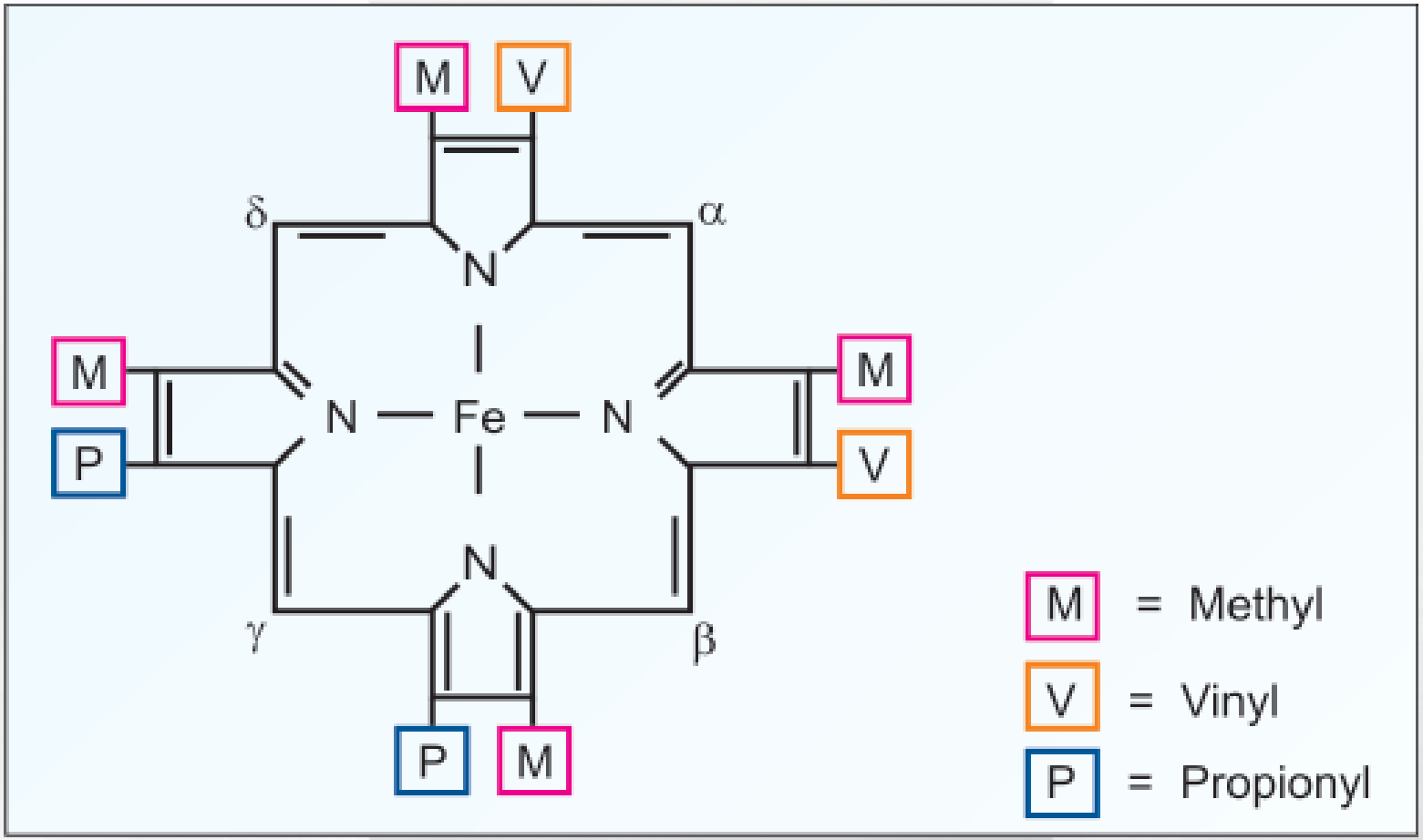
d. vinyl (–CH=CH2) group.



Porphyrins of Biological Importance

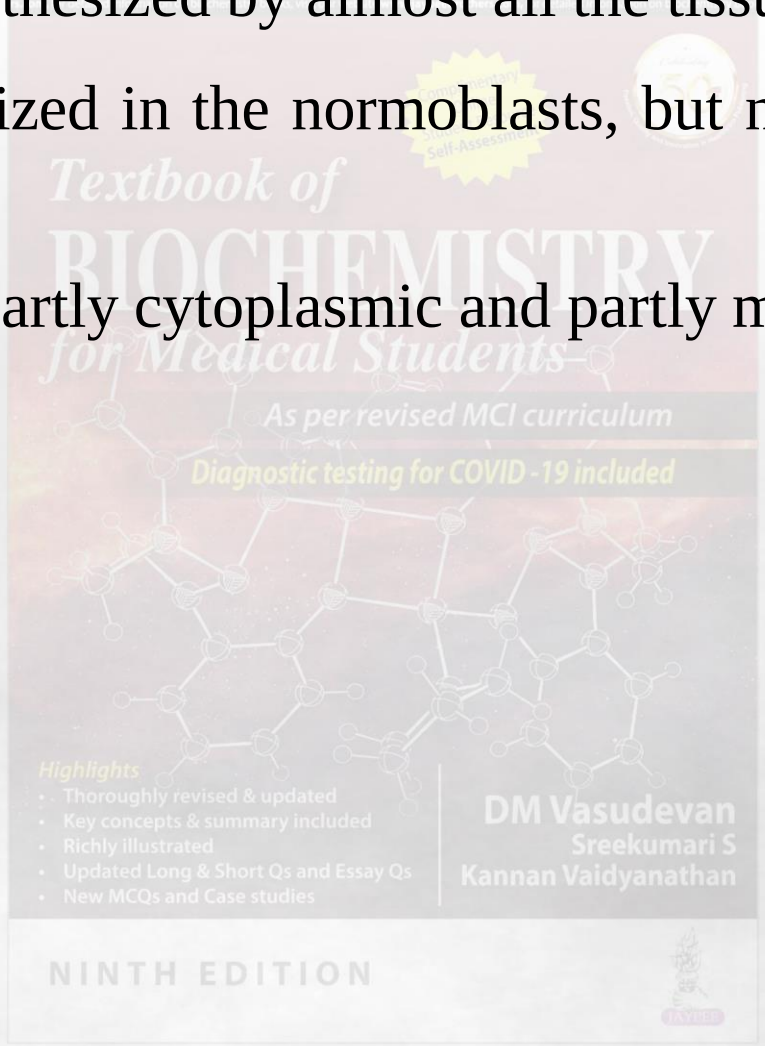
Name of Porphyrin	Order of substituents from 1st to 8th positions
Uroporphyrin I	A,P, A,P, A,P, A,P
Uroporphyrin III	A,P, A,P, A,P, P,A
Coproporphyrin I	M,P, M,P, M,P, M,P
Coproporphyrin III	M,P, M,P, M,P, P,M
Protoporphyrin III	M,V, M,V, M,P, P,M
(A = acetyl; P = propionyl; M = methyl; V = vinyl)	

Structure of Heme



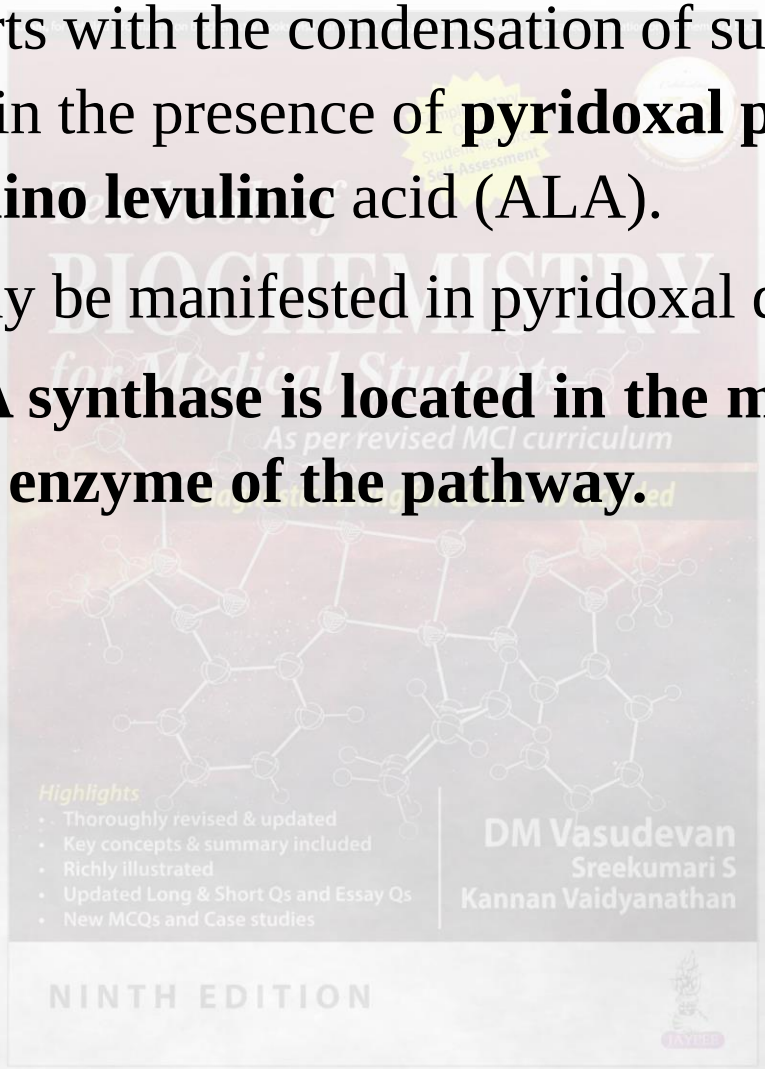
Biosynthesis of Heme

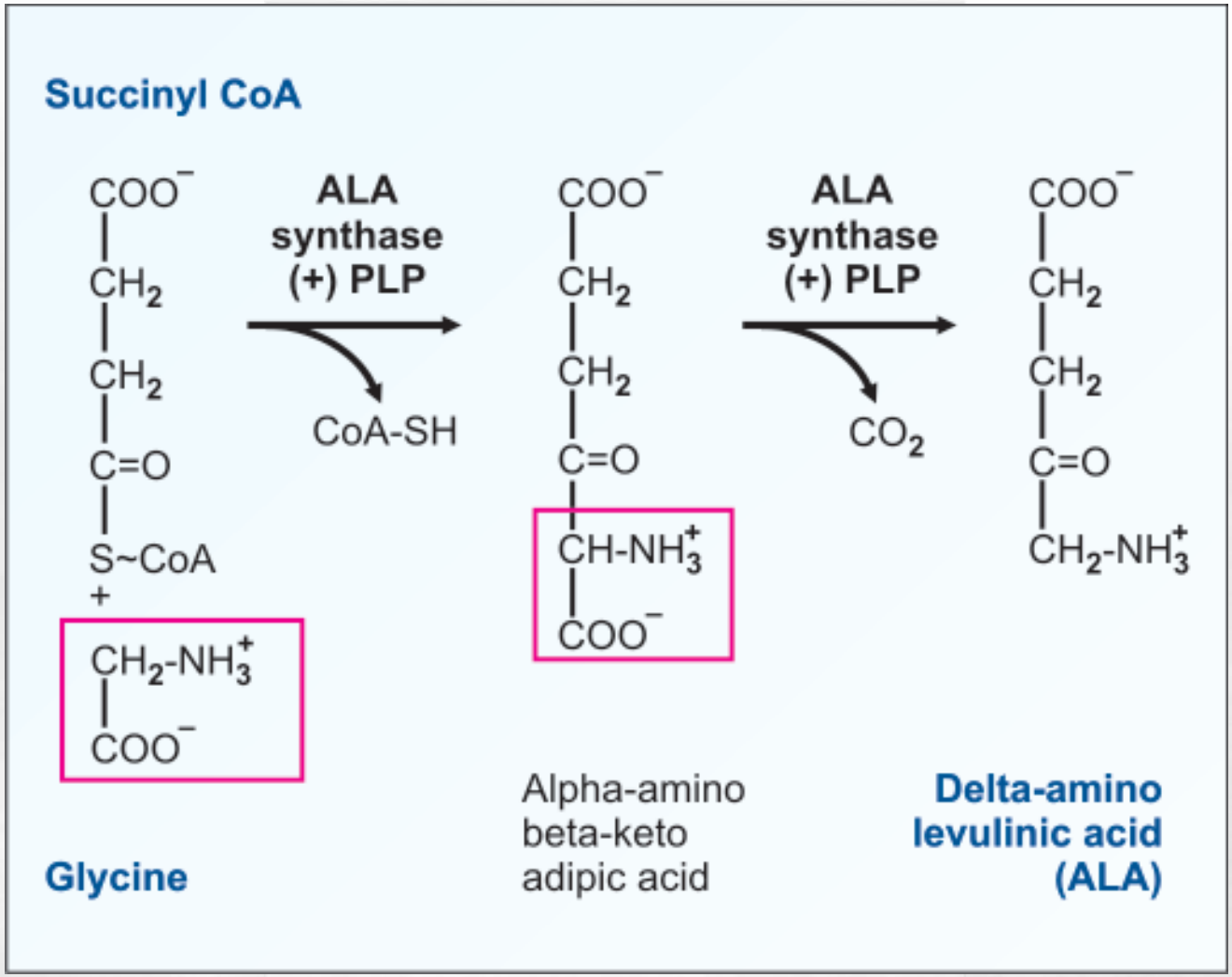
- Heme can be synthesized by almost all the tissues in the body.
- Heme is synthesized in the normoblasts, but not in the matured ones.
- The pathway is partly cytoplasmic and partly mitochondrial.



Step 1: ALA Synthesis

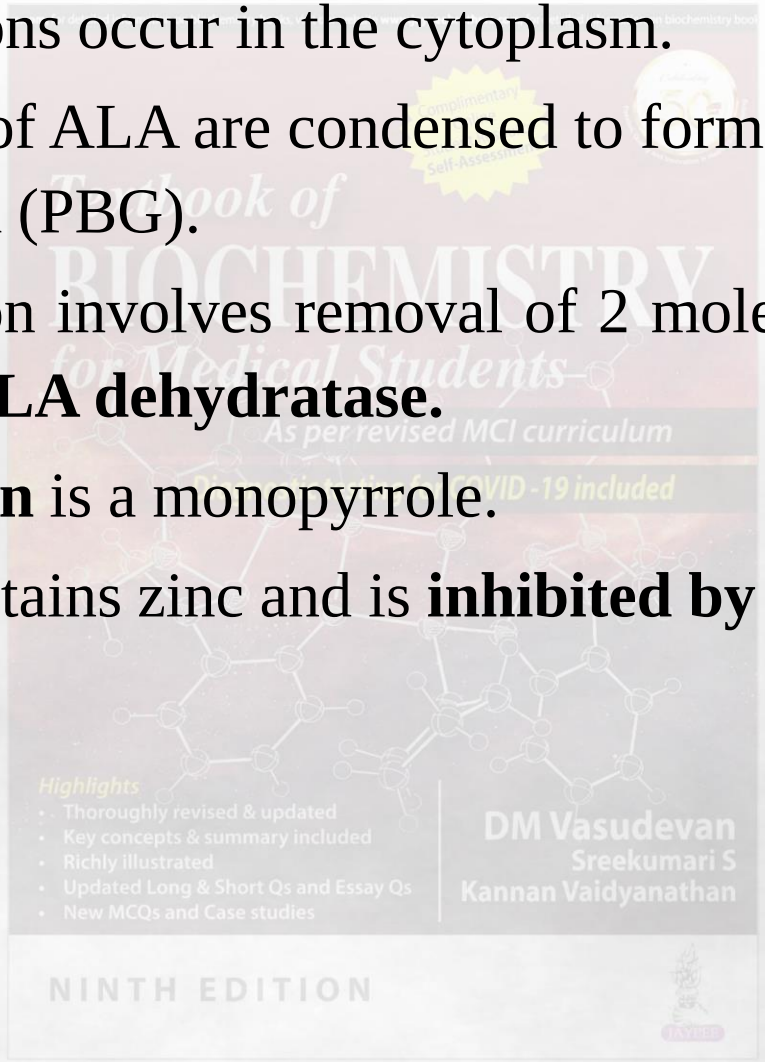
- The synthesis starts with the condensation of succinyl CoA and glycine in the presence of **pyridoxal phosphate to form delta amino levulinic** acid (ALA).
- Hence anemia may be manifested in pyridoxal deficiency.
- The enzyme **ALA synthase is located in the mitochondria and is the rate-limiting enzyme of the pathway.**

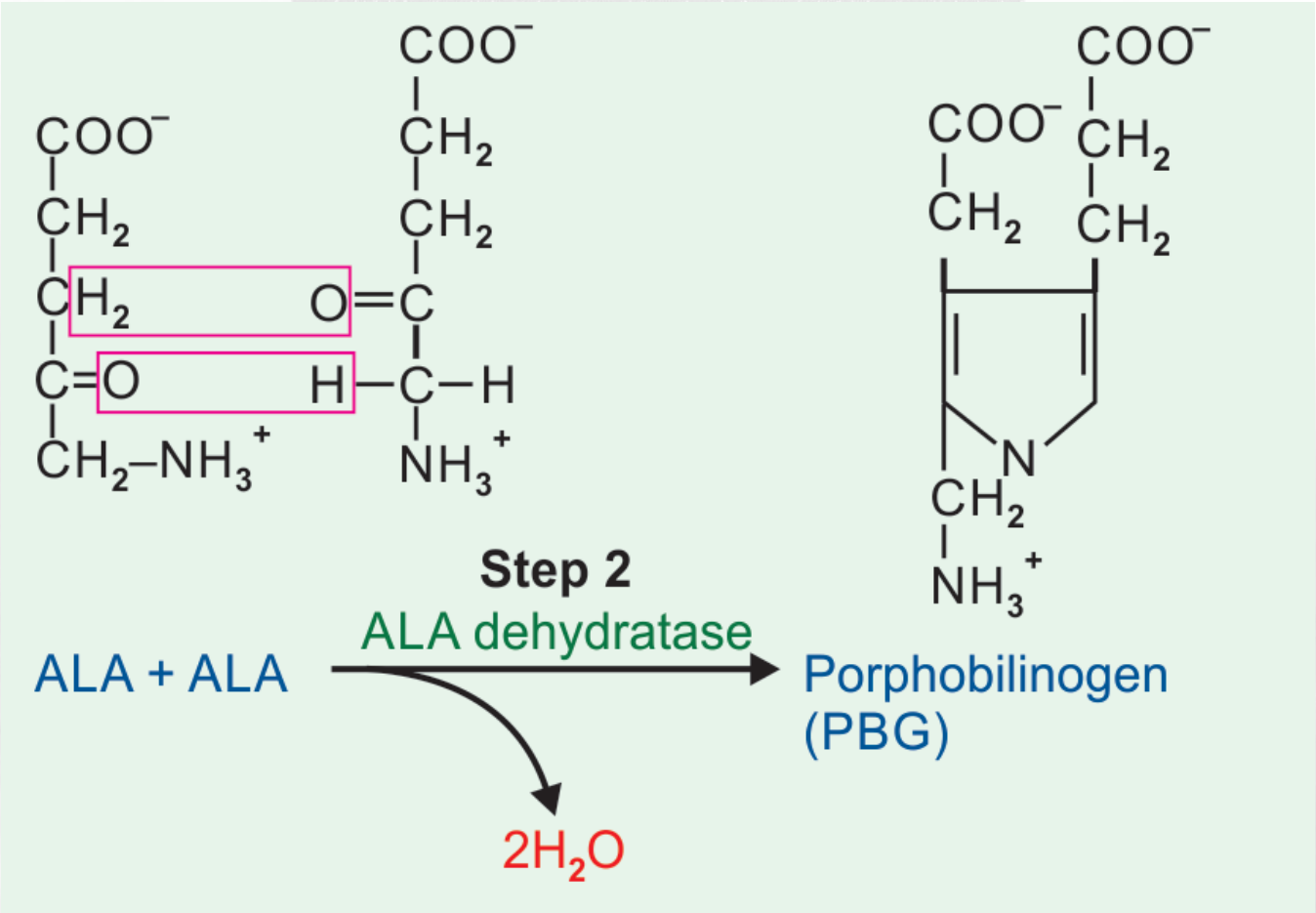



Succinyl CoA
ALA synthase (+) PLP
CoA-SH
ALA synthase (+) PLP
CO_2
S~CoA
+
Glycine
Alpha-amino beta-keto adipic acid
Delta-amino levulinic acid (ALA)

Step 2: Formation of PBG

- Next few reactions occur in the cytoplasm.
- Two molecules of ALA are condensed to form porphobilinogen (PBG).
- The condensation involves removal of 2 molecules of water and the enzyme is **ALA dehydratase.**
- **Porphobilinogen** is a monopyrrole.
- The enzyme contains zinc and is **inhibited by lead.**

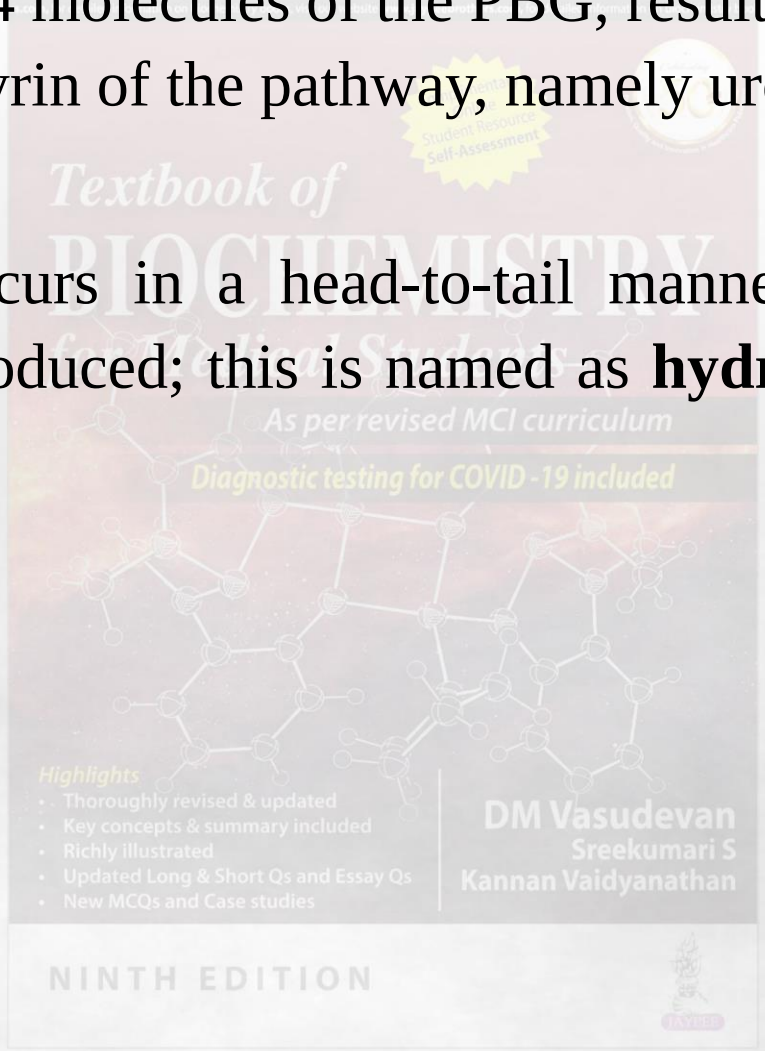




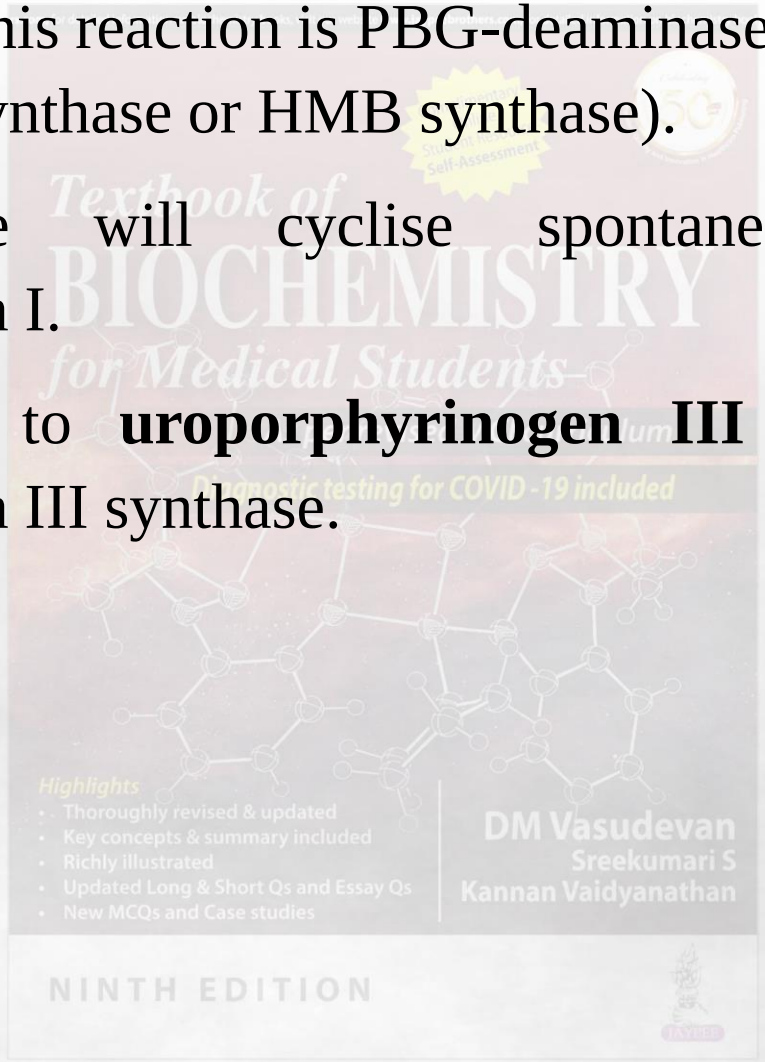
Step 2 of Heme Synthesis

Step 3: Formation of UPG

- Condensation of 4 molecules of the PBG, results in the formation of the first porphyrin of the pathway, namely uroporphyrinogen (UPG).
- Condensation occurs in a head-to-tail manner, so that a linear tetrapyrrole is produced; this is named as **hydroxy methyl bilane (HMB).**

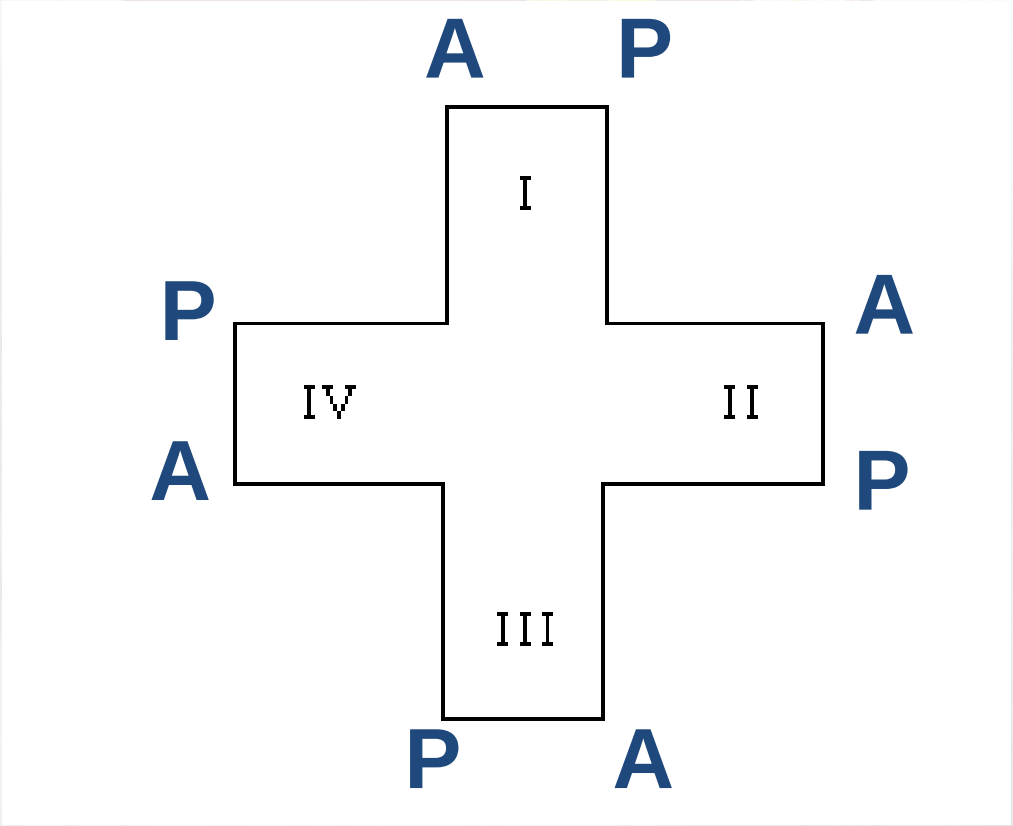


- The enzyme for this reaction is PBG-deaminase (otherwise called Uroporphyrin I synthase or HMB synthase).
- HMB molecule will cyclise spontaneously to form uroporphyrinogen I.
- It is converted to **uroporphyrinogen III** by the enzyme, uroporphyrinogen III synthase.



Uroporphyrinogen Type I

UPG I (Symmetric isomer)

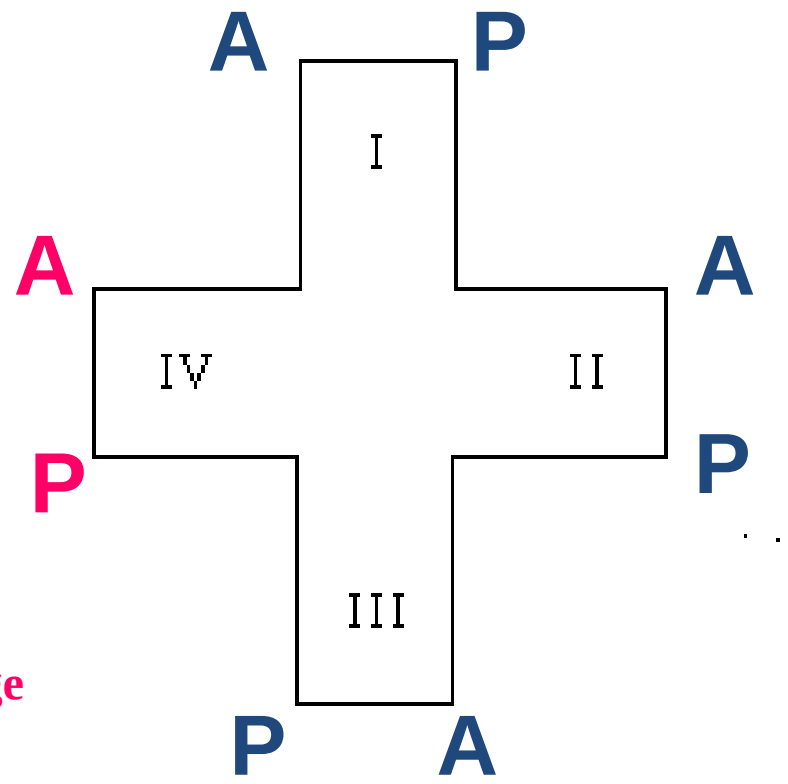


Bridge joining pyrrole ring -CH2-(Methylene bridge)

Uroporphyrinogen type III

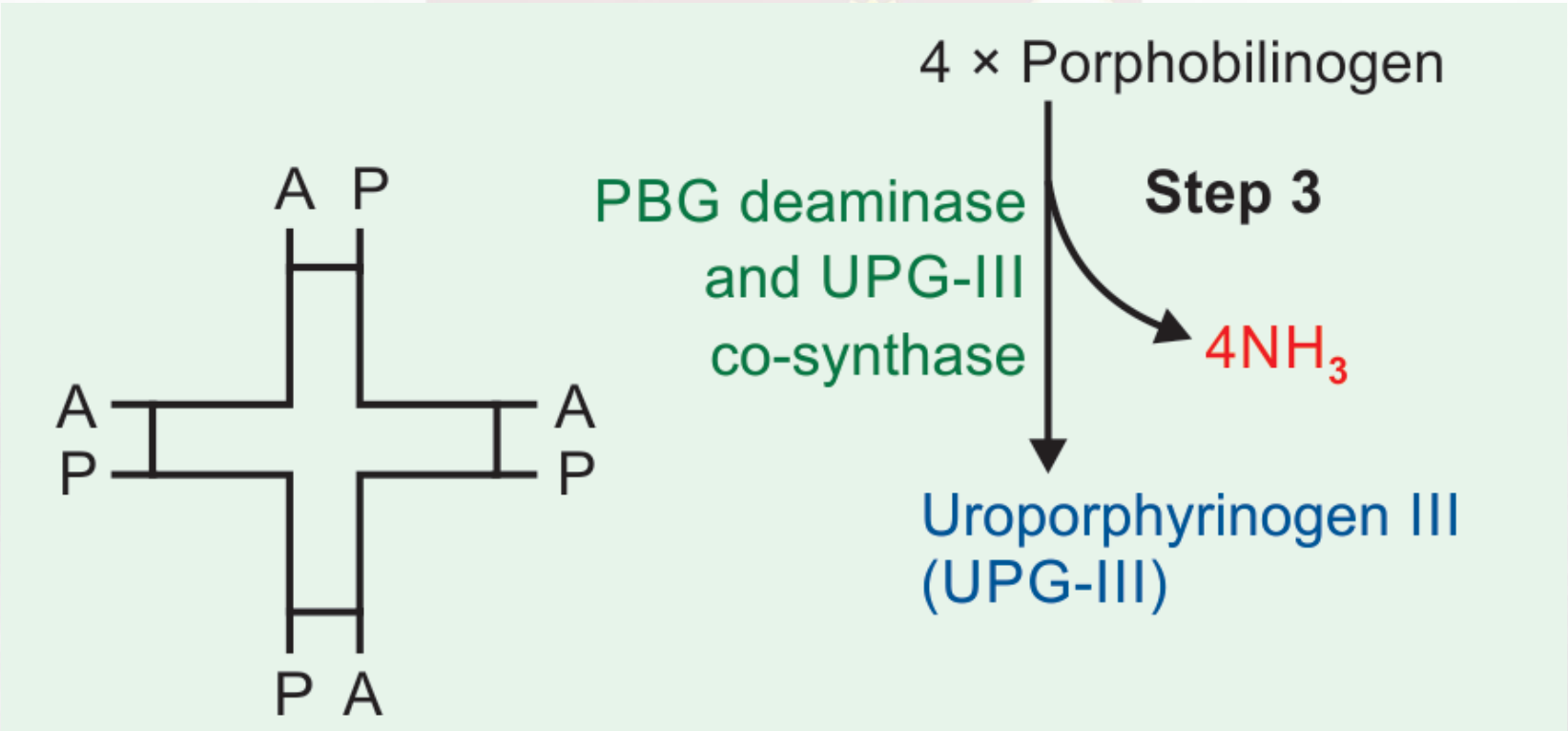
UPG III (Asymmetric isomer)

Only type III
Isomers are
used for heme synthesis



(Precursor of heme-bridge joining -CH_2-)

- When the fusion occurs, the III series of isomers are predominantly formed; and only the **III series are further utilized.**
- **The pyrrole rings** are joined together by methylene bridges (-CH2-), which are derived from the alpha carbon of glycine.
- During this deamination reaction 4 molecules of ammonia are removed.
- Porphyrinogens are colorless, but are readily oxidized to porphyrins, which are colored compounds.

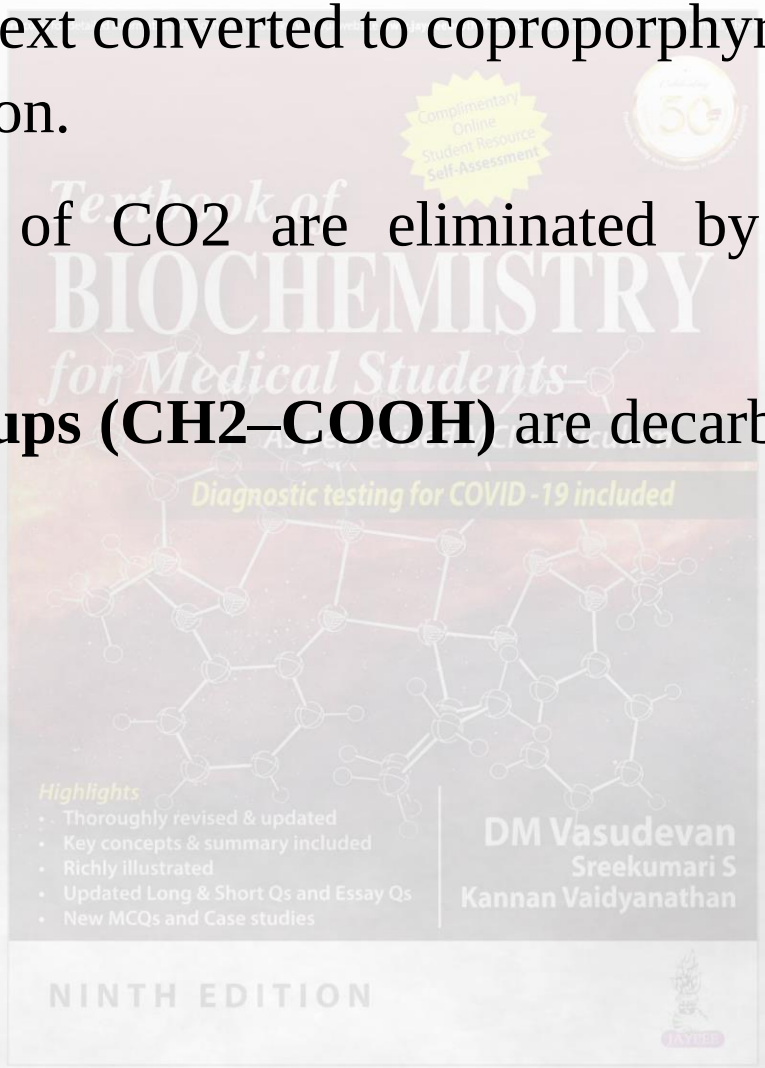


Step 3 of heme synthesis

PBG deaminase and UPG 3 co-synthase

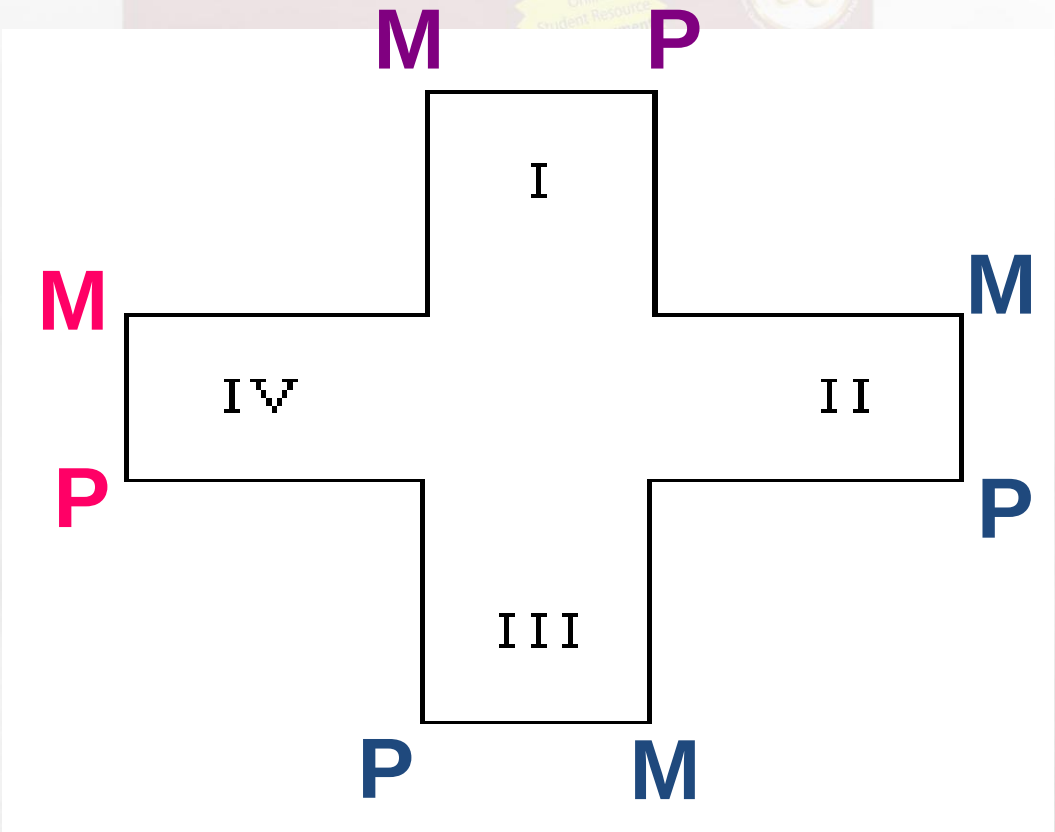
Step 4: Synthesis of CPG

- The UPG-III is next converted to coproporphyrinogen (CPG-III) by decarboxylation.
- Four molecules of CO2 are eliminated by uroporphyrinogen **decarboxylase.**
- **The acetate groups (CH2–COOH)** are decarboxylated to methyl (CH3) groups.



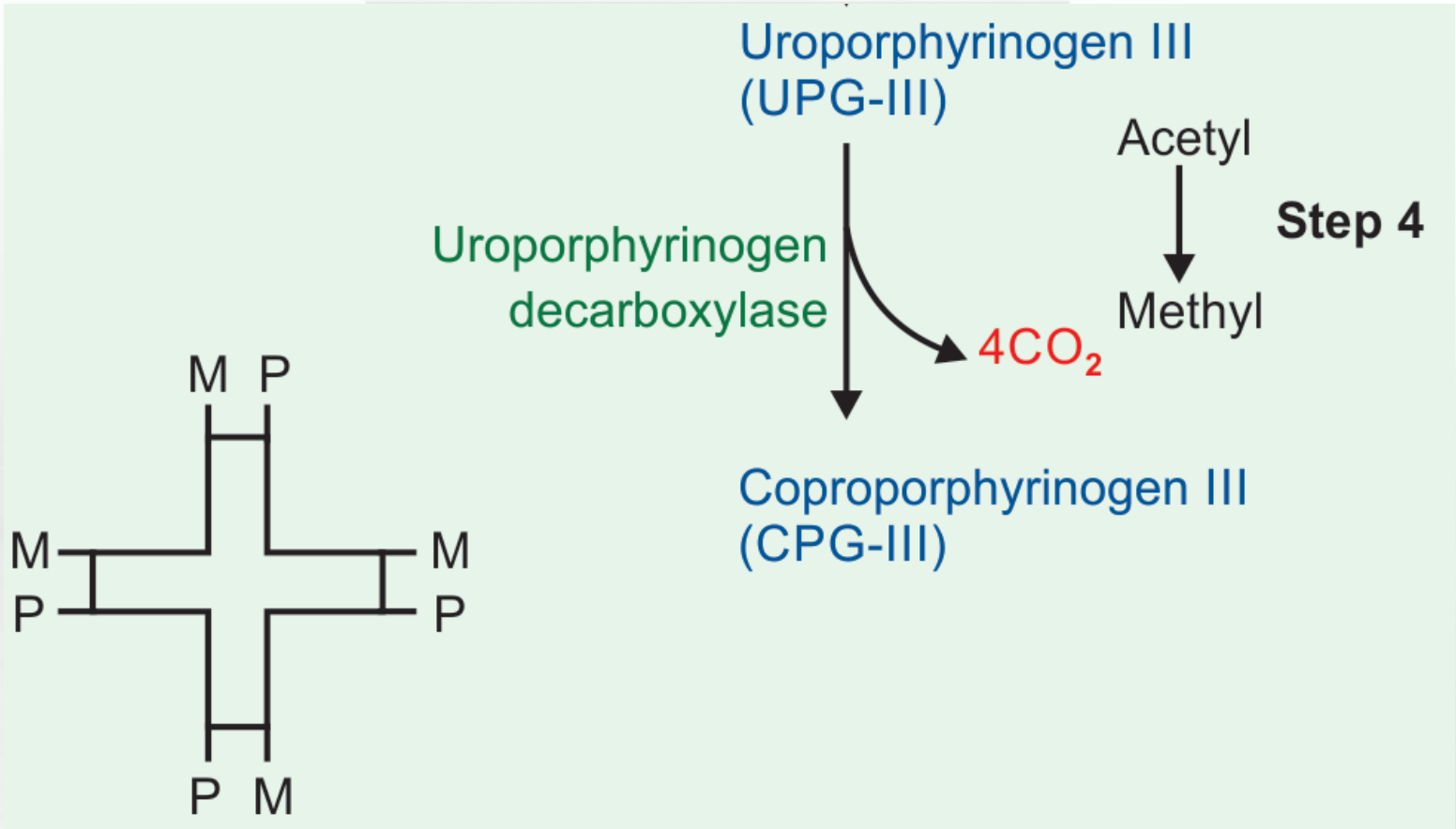
Uroporphyrinogen Decarboxylase reaction (cytosol)

- **UPG type III**



CPG type III

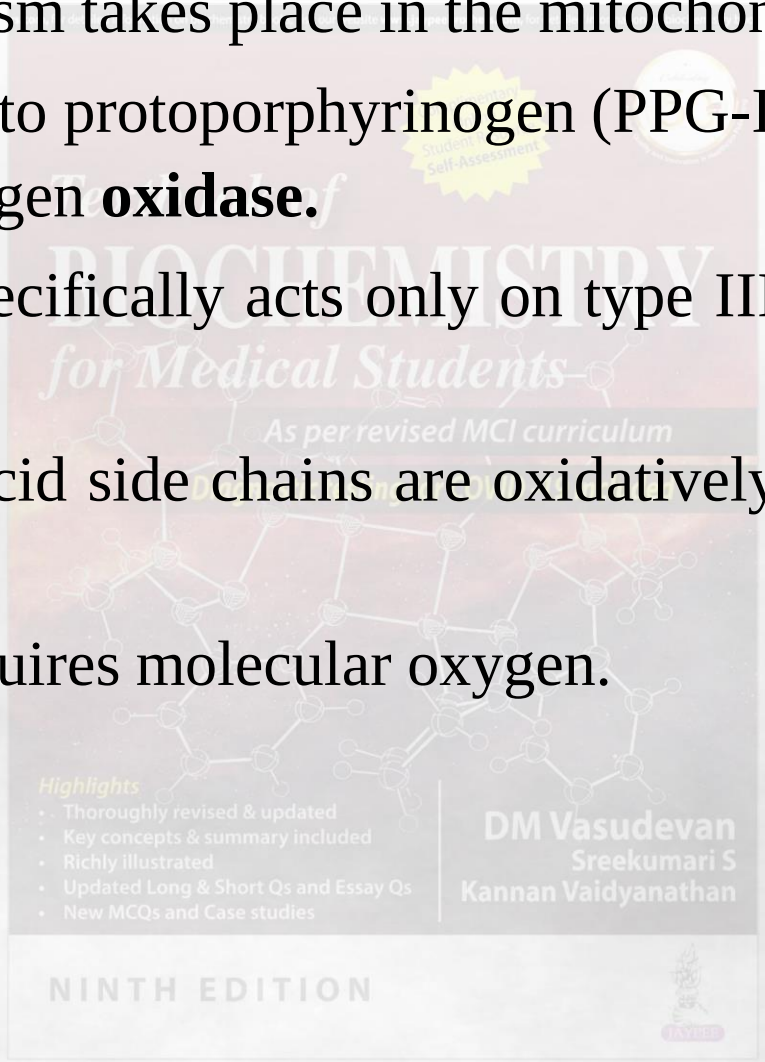
(Bridge joining Pyrrole ring is -CH2-)



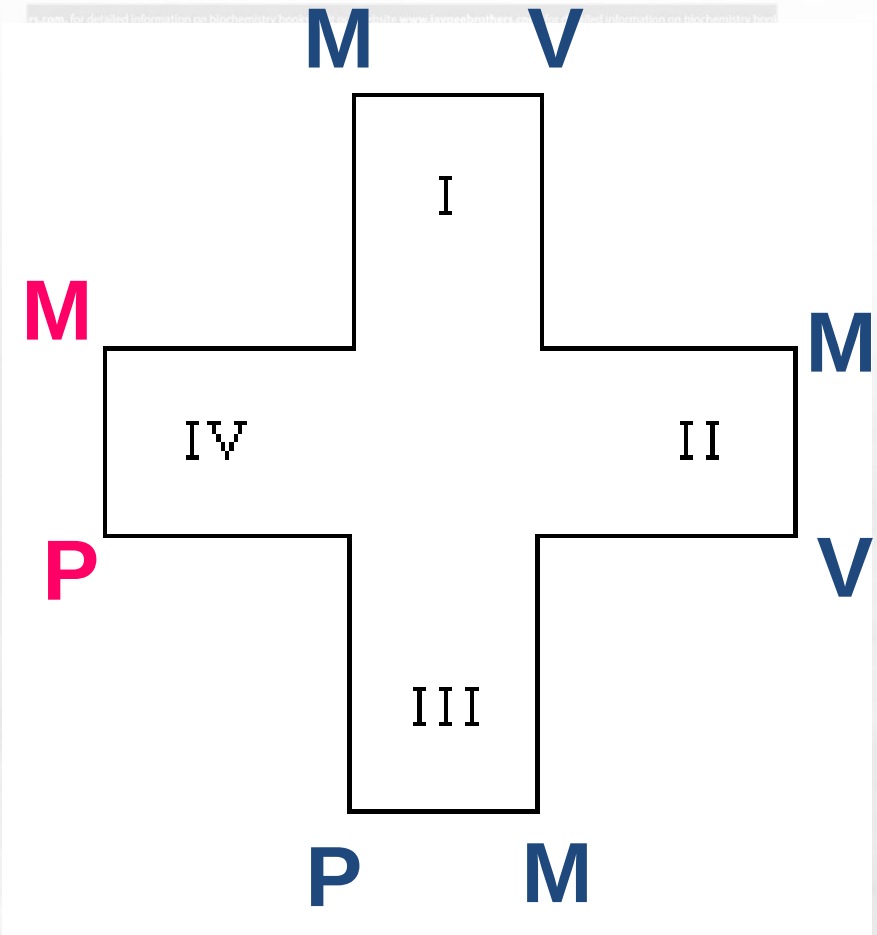
Step 4 – Uroporphyrinogen decarboxylase

Step 5: Synthesis of PPG

- Further metabolism takes place in the mitochondria.
- CPG is oxidized to protoporphyrinogen (PPG-III) by coproporphyrinogen **oxidase.**
- **This enzyme** specifically acts only on type III series, and not on type I series.
- Two propionic acid side chains are oxidatively decarboxylated to vinyl groups.
- This reaction requires molecular oxygen.

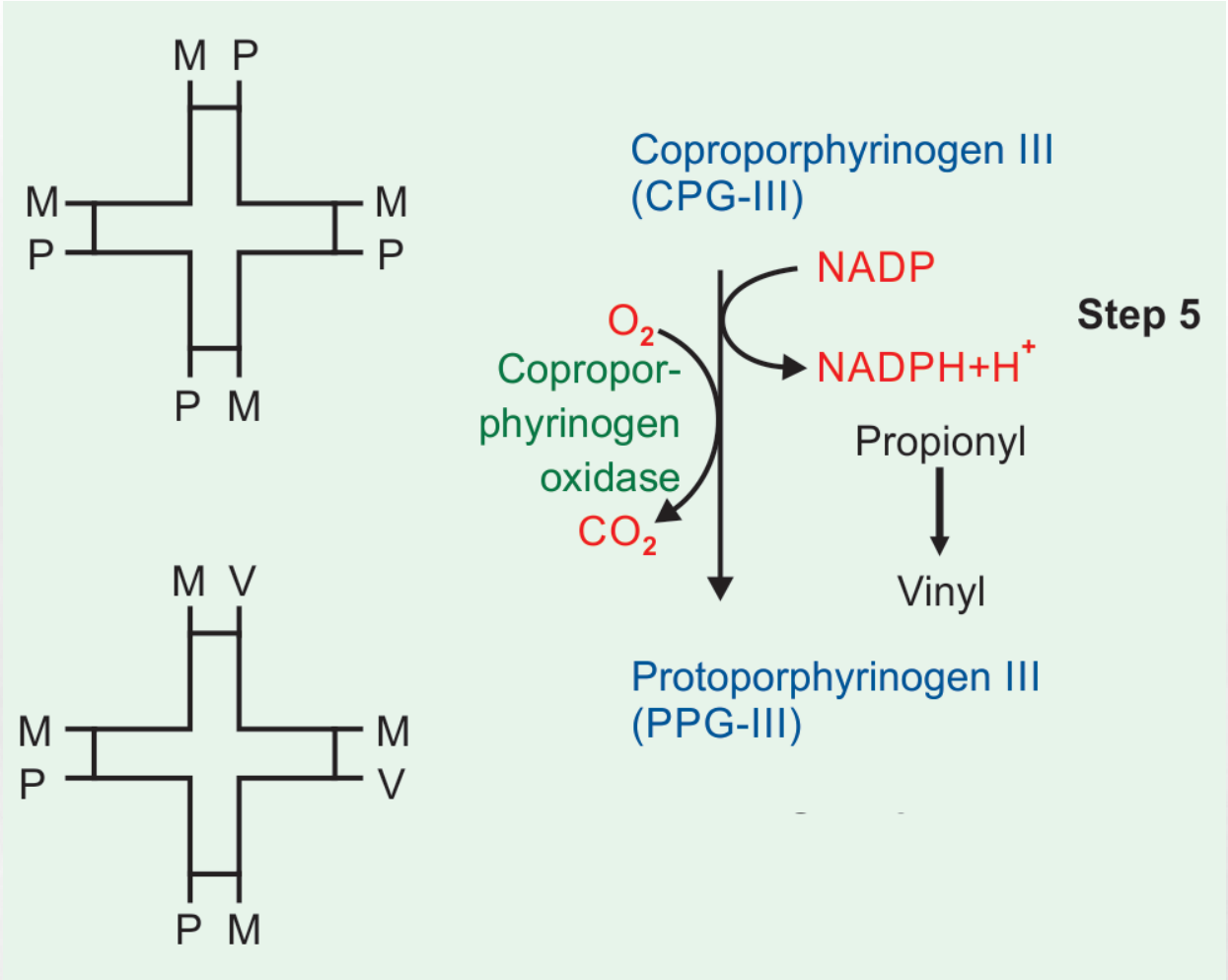


CPG –III Oxidase Reaction – Mitochondria



PPG type III

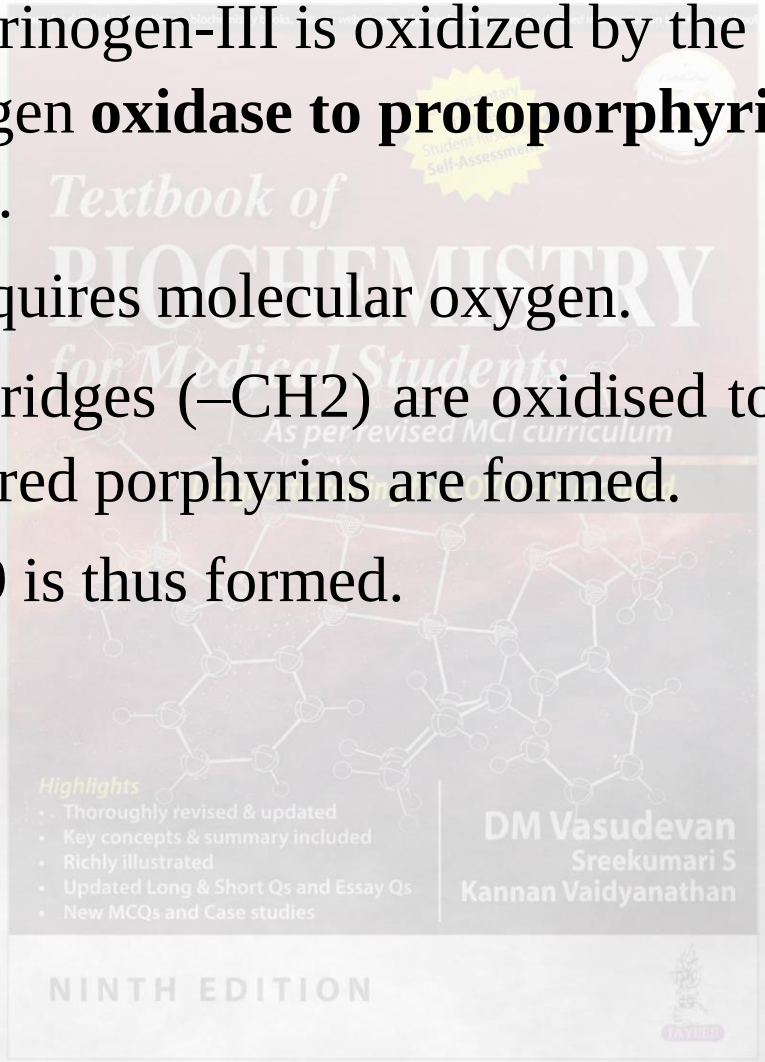
(Bridge joining pyrrole ring is -CH2-)

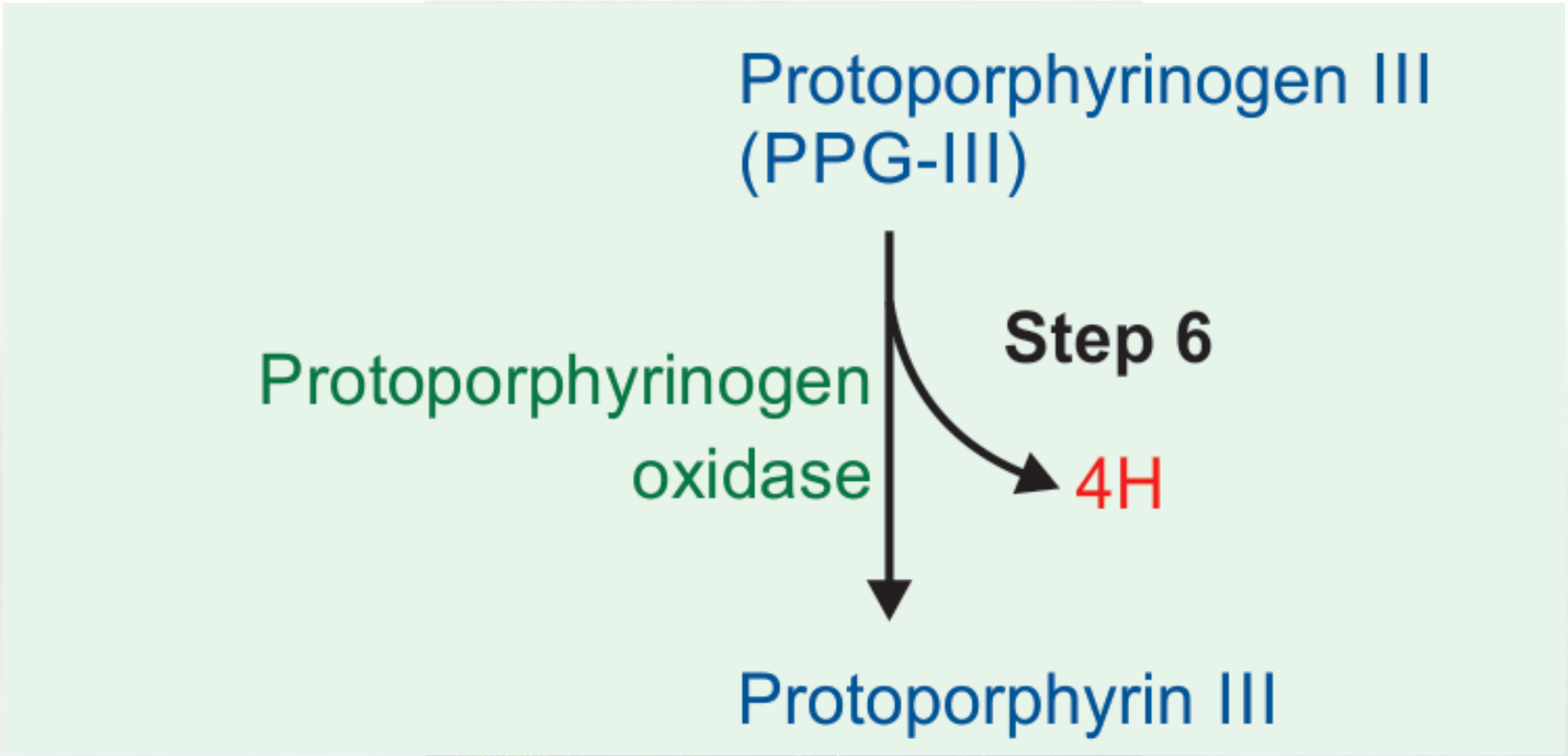


Step 5 – Coproporphyrinogen Oxidase

Step 6: Generation of PP

- The Protoporphyrinogen-III is oxidized by the enzyme protoporphyrinogen **oxidase to protoporphyrin-III** (PP-III) in the mitochondria.
- The oxidation requires molecular oxygen.
- The methylene bridges (–CH2) are oxidised to methenyl bridges (–CH=) and colored porphyrins are formed.
- Protoporphyrin-9 is thus formed.

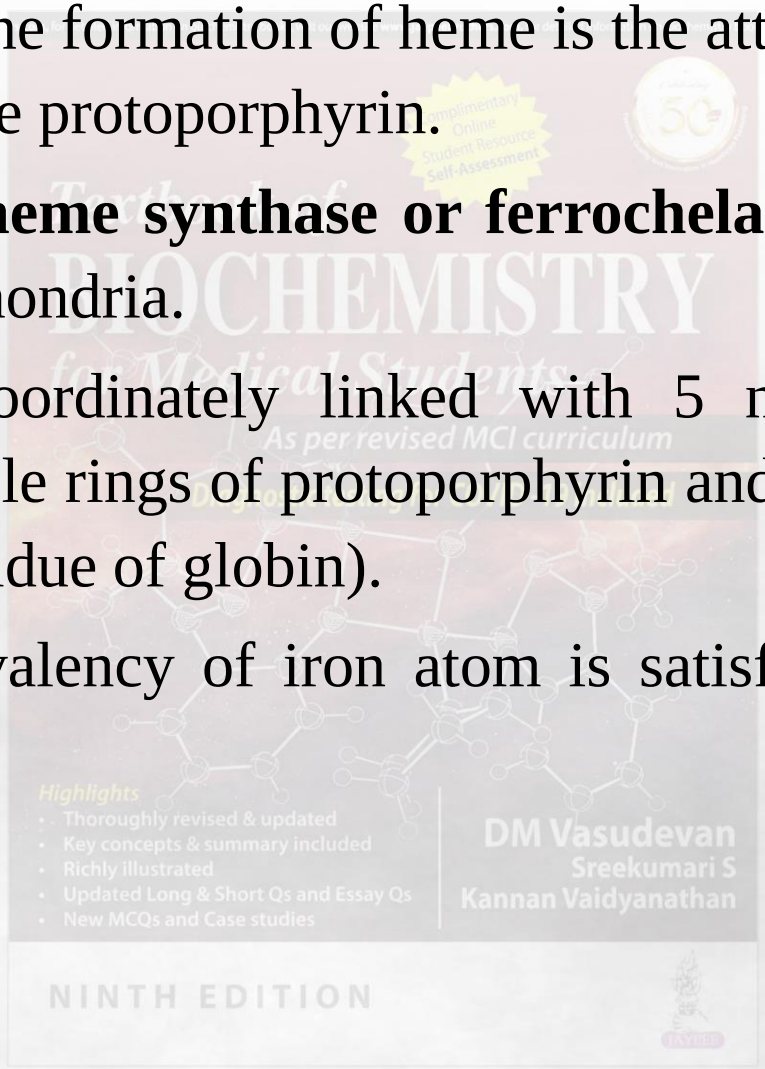


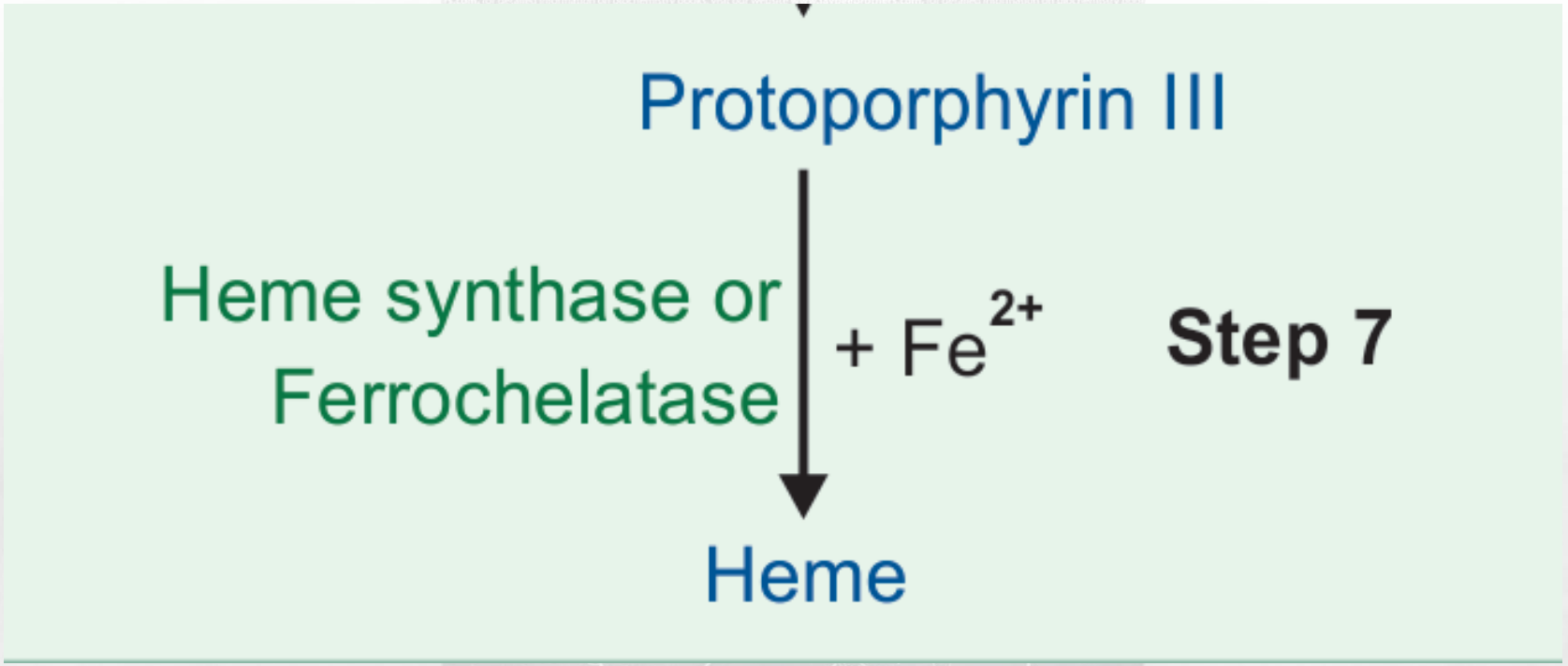


Step 6 – Protoporphyrinogen Oxidase

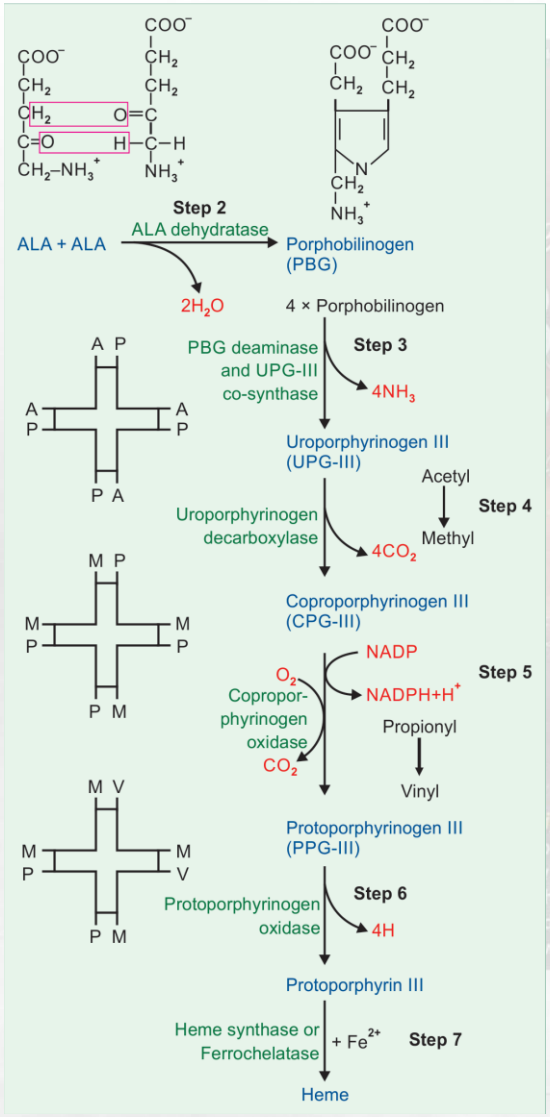
Step 7: Generation of Heme

- The last step in the formation of heme is the attachment of ferrous iron to the protoporphyrin.
- The enzyme is **heme synthase or ferrochelatase** which is also located in mitochondria.
- Iron atom is coordinately linked with 5 nitrogen atoms (4 nitrogen of pyrrole rings of protoporphyrin and 1st nitrogen atom of a histidine residue of globin).
- The remaining valency of iron atom is satisfied with water or oxygen atom

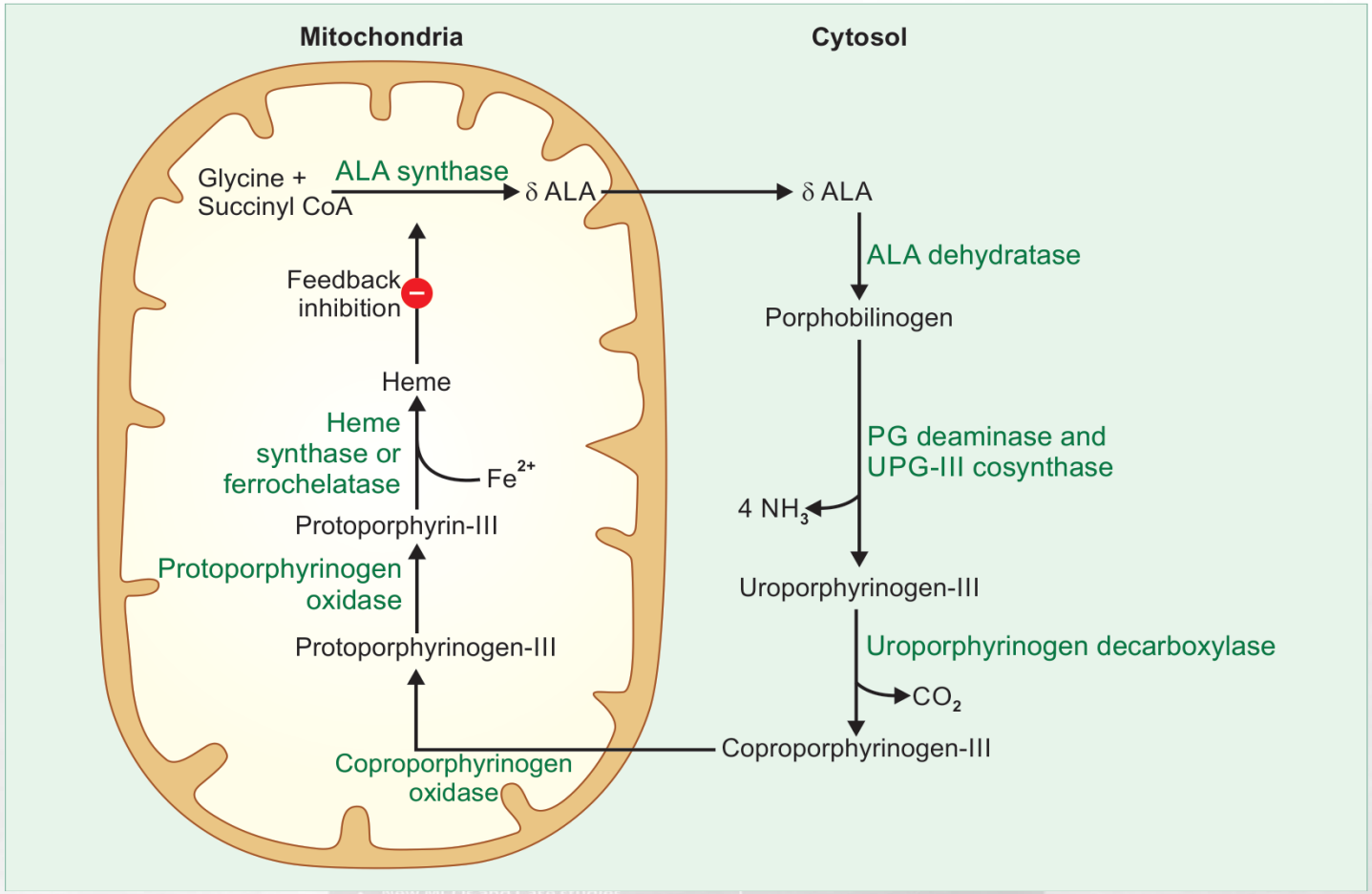




Step 7 – Heme synthase (Ferrochelatase)

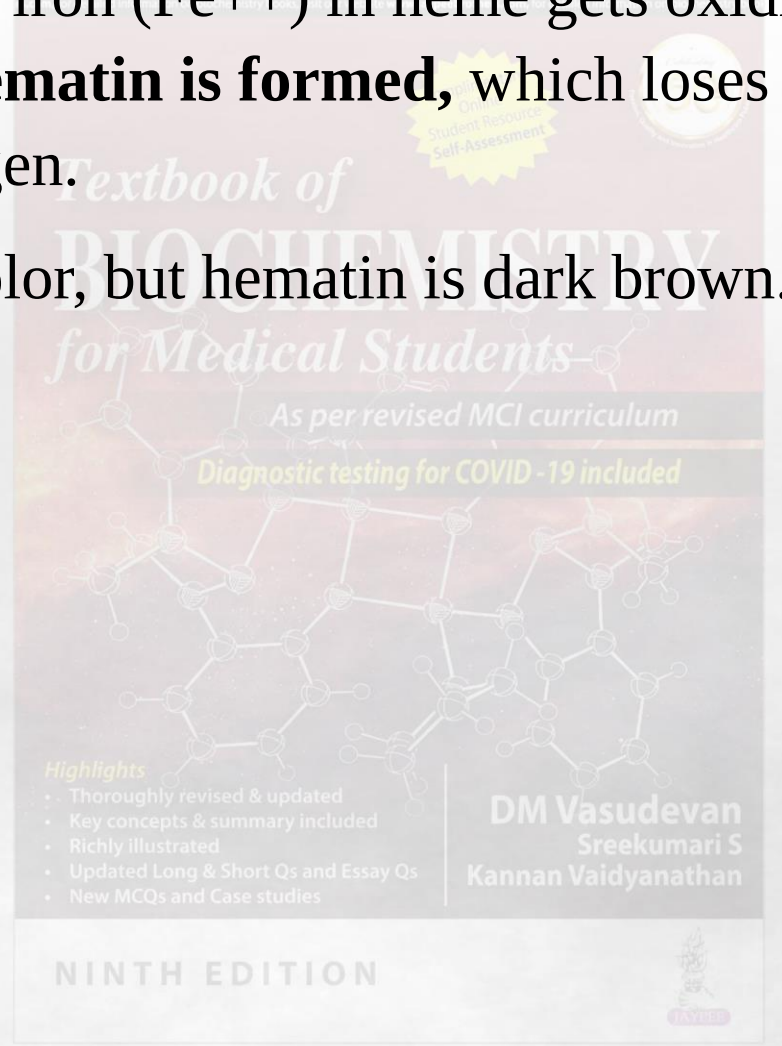


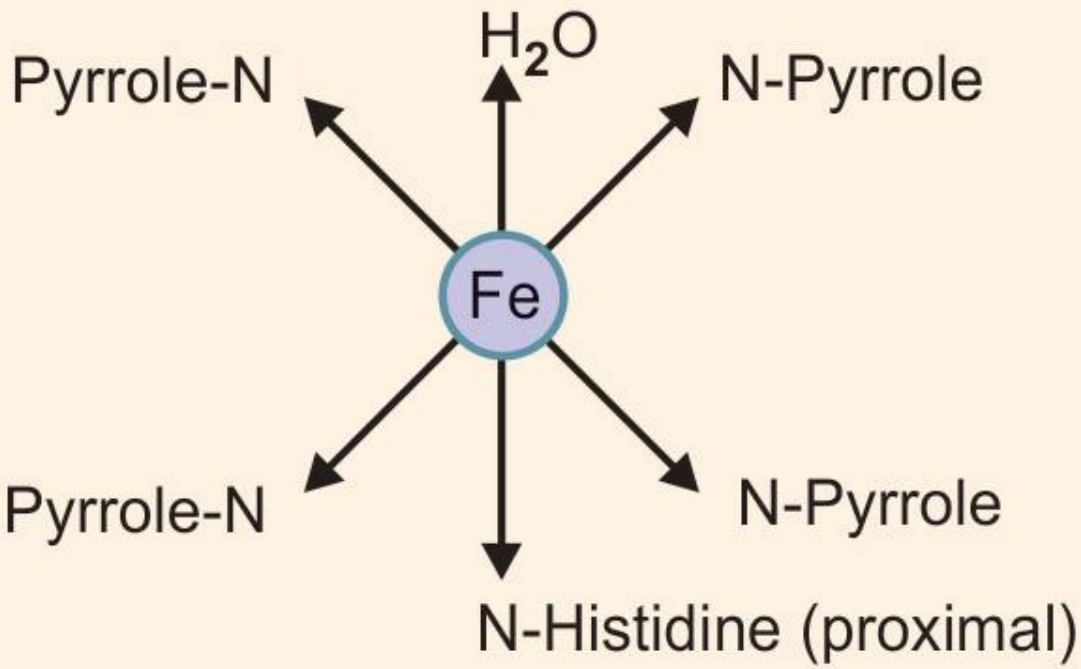
Steps of Heme Synthesis.



Compartmentalization of enzymes of heme synthesis.
Part of synthesis is in mitochondria, and the rest in cytoplasm.

- When the ferrous iron (Fe++) in heme gets oxidized to ferric (Fe+++) form, **hematin is formed,** which loses the property of carrying the oxygen.
- Heme is red in color, but hematin is dark brown.





In the Heme Molecule, Iron Atom is coordinately linked with Nitrogen Atoms

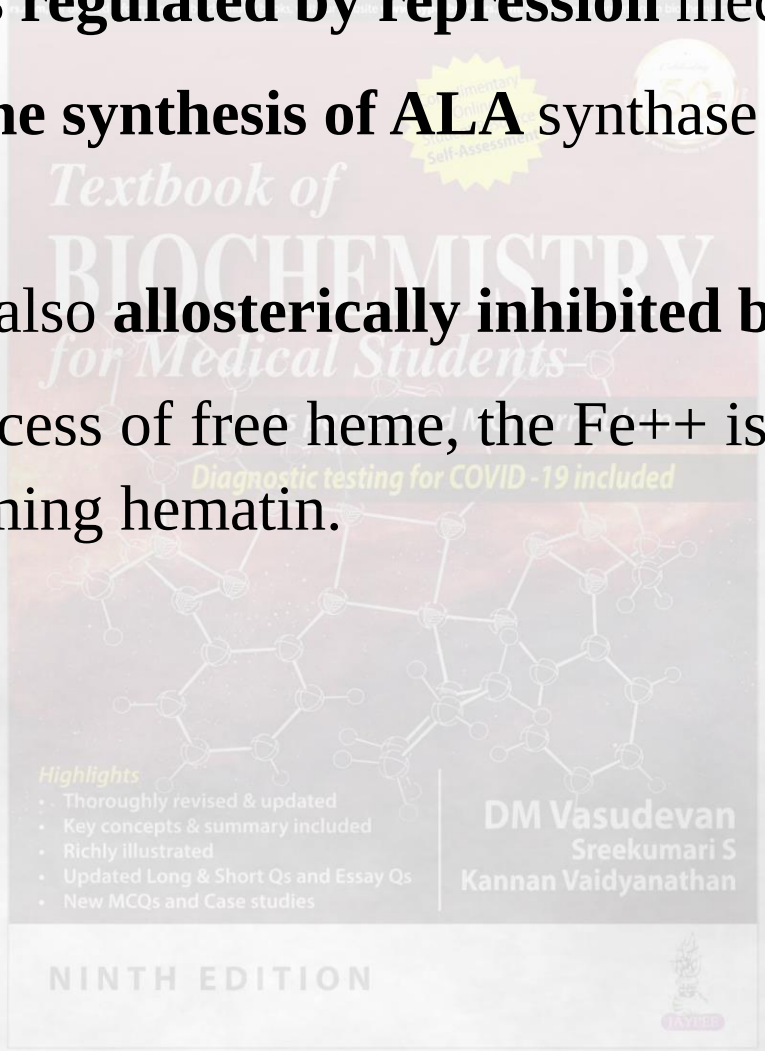
Regulation of Heme Synthesis

1. **ALA synthase is regulated by repression** mechanism.

- **Heme inhibits the synthesis of ALA** synthase by acting as a co-repressor.

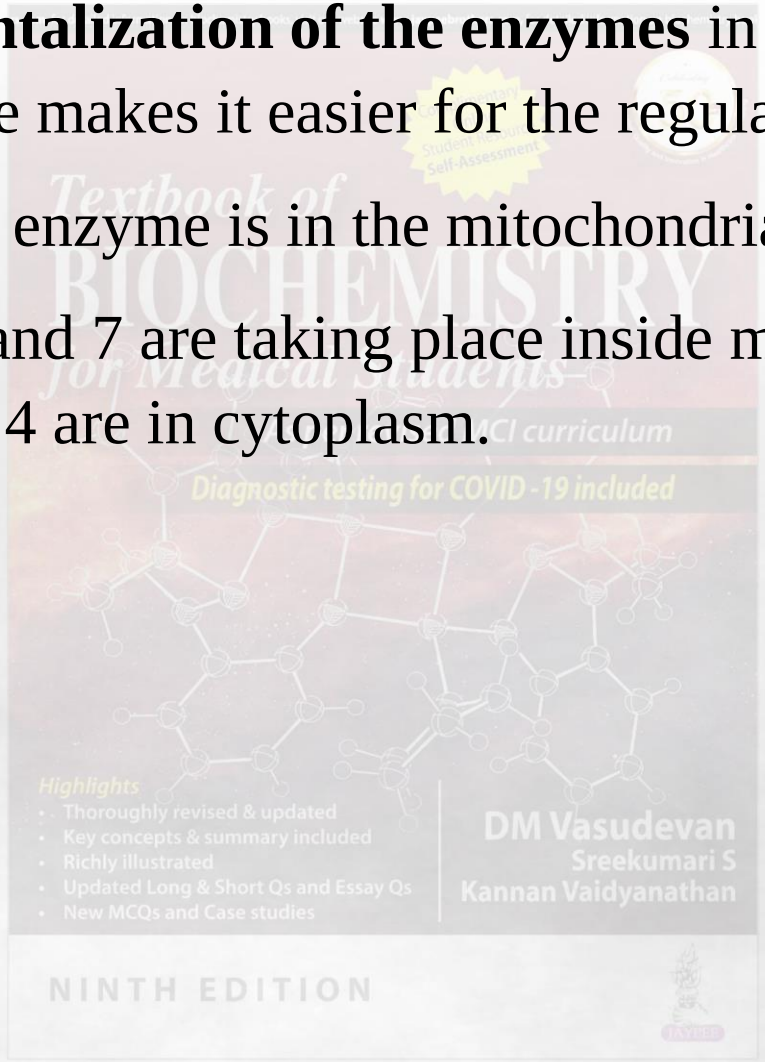
2. ALA synthase is also **allosterically inhibited by** hematin.

- When there is excess of free heme, the Fe++ is oxidized to Fe+++ (ferric), thus forming hematin.



3. The **compartmentalization of the enzymes** in the synthesis of heme makes it easier for the regulation.

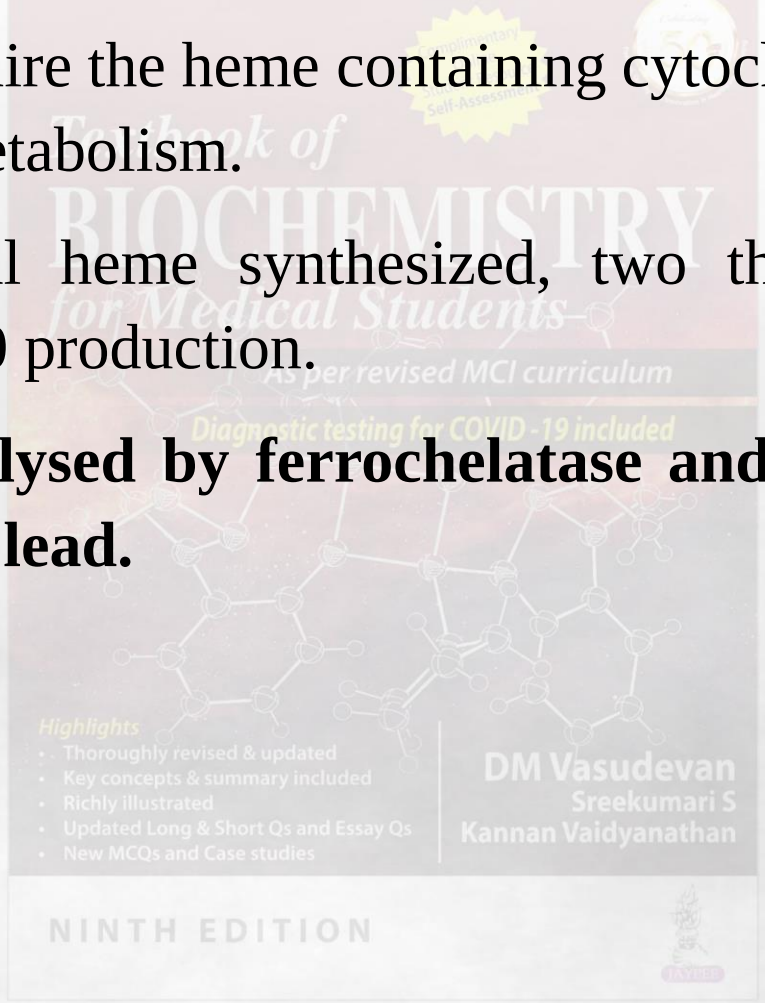
- The rate-limiting enzyme is in the mitochondria.
- The steps 1,5,6, and 7 are taking place inside mitochondria, while the steps 2,3 and 4 are in cytoplasm.



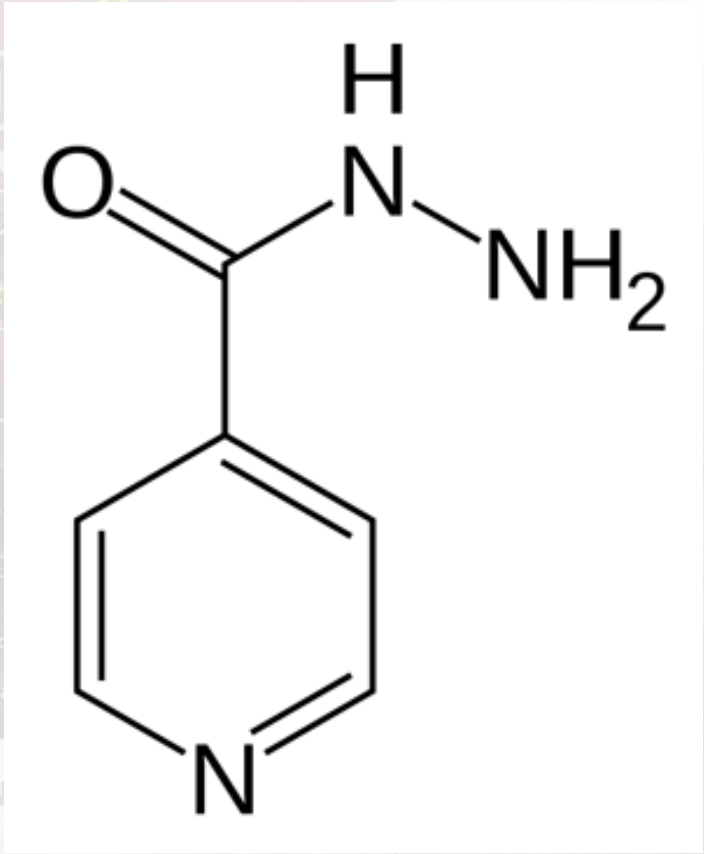
4. **Drugs like barbiturates induce heme synthesis.**

- Barbiturates require the heme containing cytochrome p450 for their metabolism.
- Out of the total heme synthesized, two thirds are used for cytochrome p450 production.

5. **The steps catalysed by ferrochelatase and ALA** dehydratase are inhibited by **lead.**

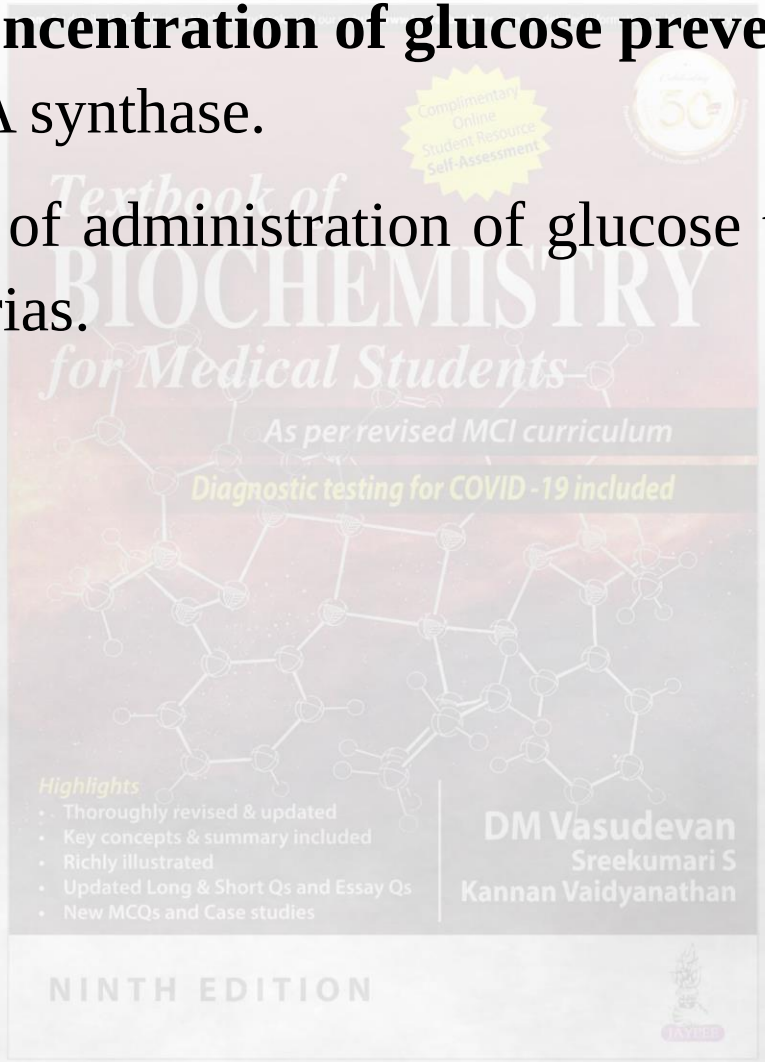


6. **INH (Isonicotinic acid hydrazide) that decreases** the availability of pyridoxal phosphate may also affect heme synthesis.



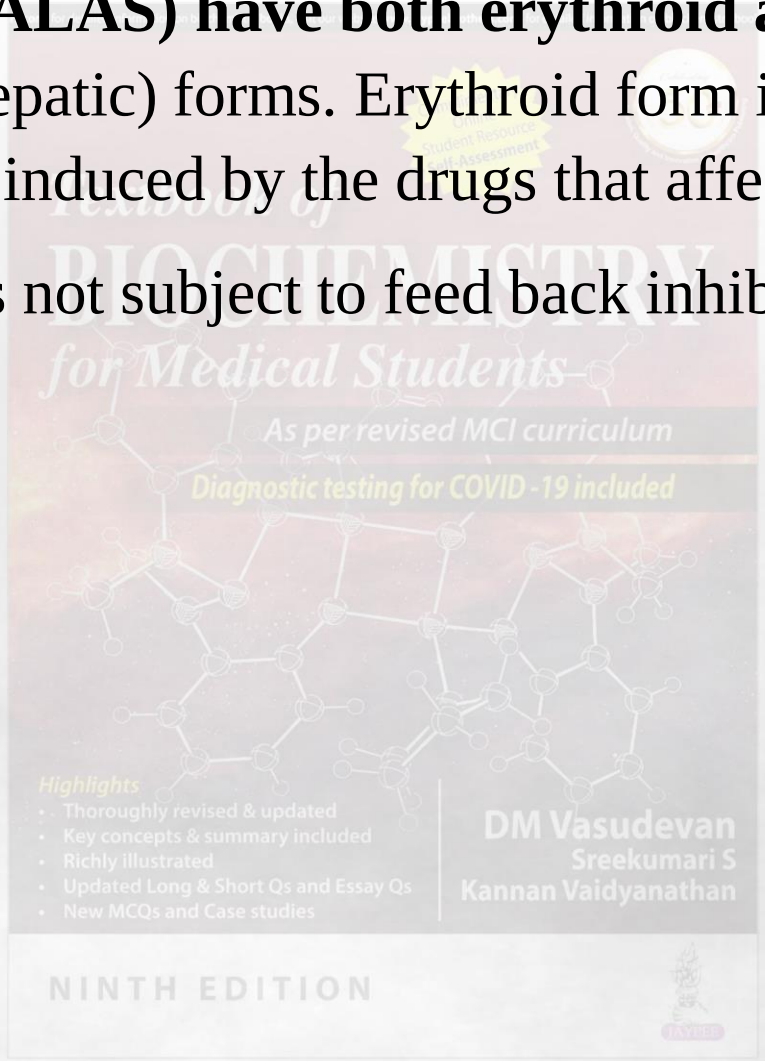
7. **High cellular concentration of glucose prevents** induction of ALA synthase.

- This is the basis of administration of glucose to relieve the acute attack of porphyrias.



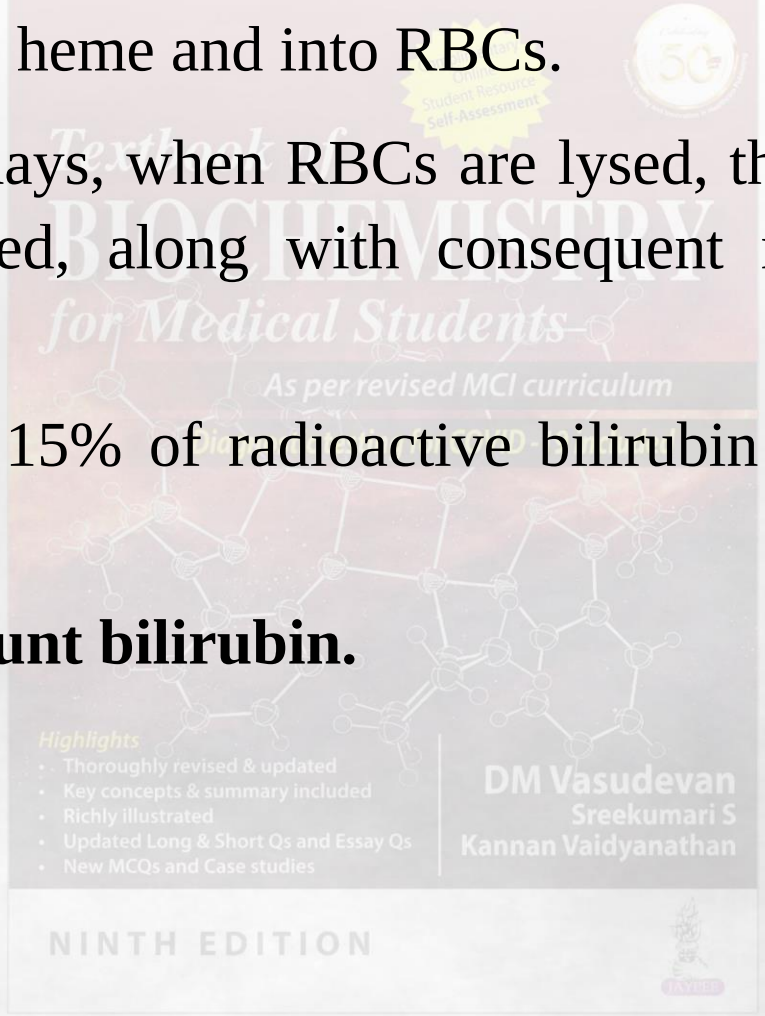
8. **ALA synthase (ALAS) have both erythroid and** non-erythroid (hepatic) forms. Erythroid form is called ALAS2; it is not induced by the drugs that affect ALAS1.

- Erythroid form is not subject to feed back inhibition by heme.

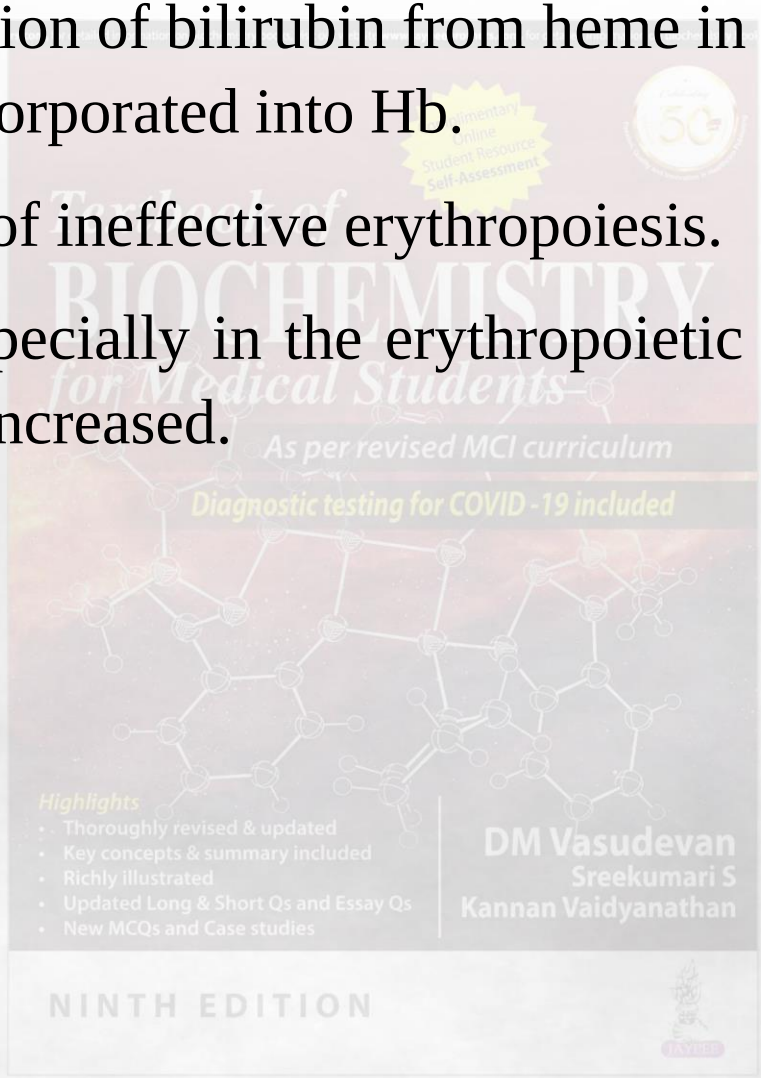


Shunt Bilirubin

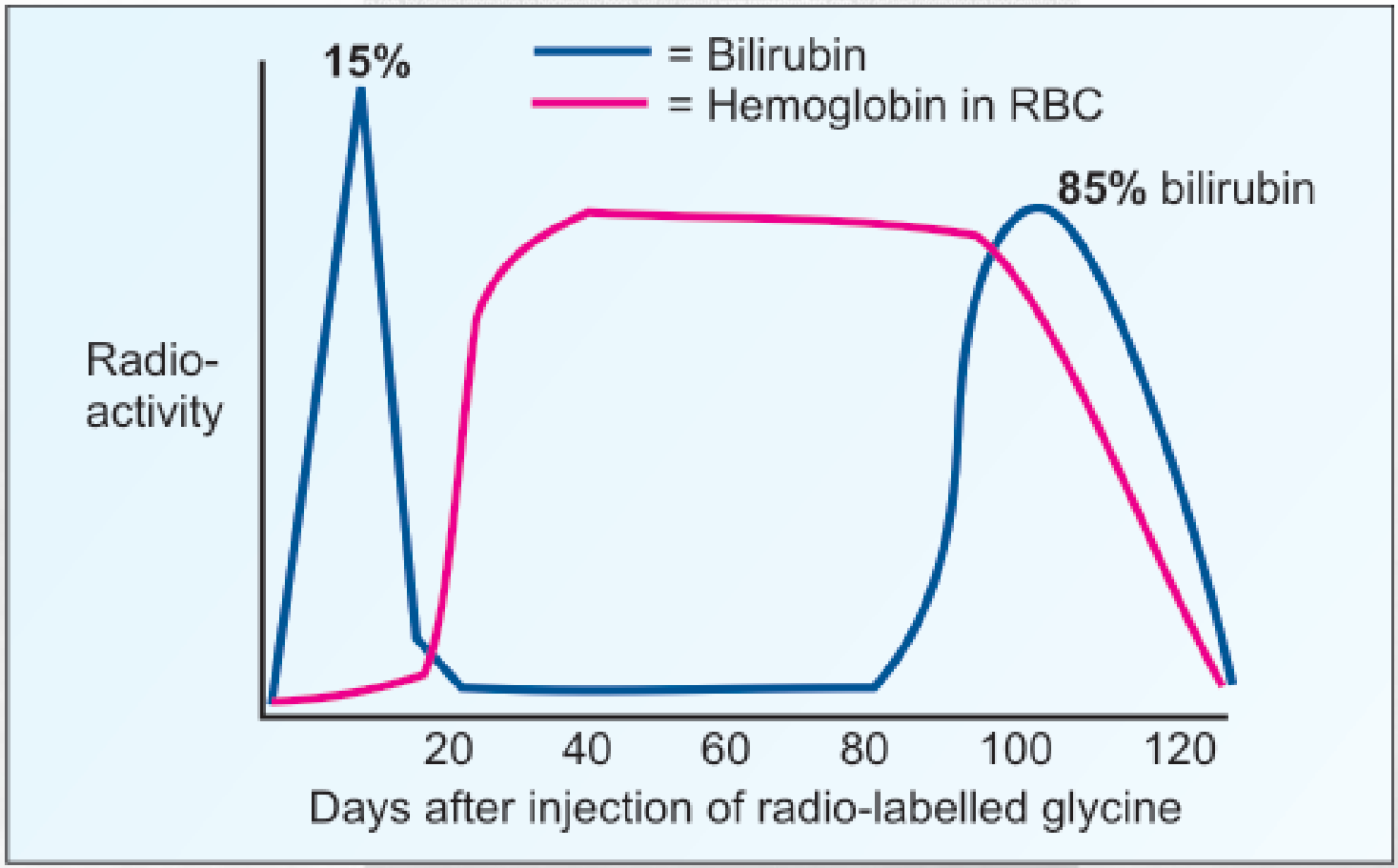
- When 15N or 14C labelled glycine is injected, this is incorporated into heme and into RBCs.
- After 100- 120 days, when RBCs are lysed, the radiolabelled Hb level is decreased, along with consequent rise in radioactive bilirubin.
- However, about 15% of radioactive bilirubin is excreted within about 10 days.
- This is called **Shunt bilirubin.**



- This is the formation of bilirubin from heme in bone marrow, without being incorporated into Hb.
- This is the result of ineffective erythropoiesis.
- In porphyrias, especially in the erythropoietic varieties, the shunt bilirubin will be increased.

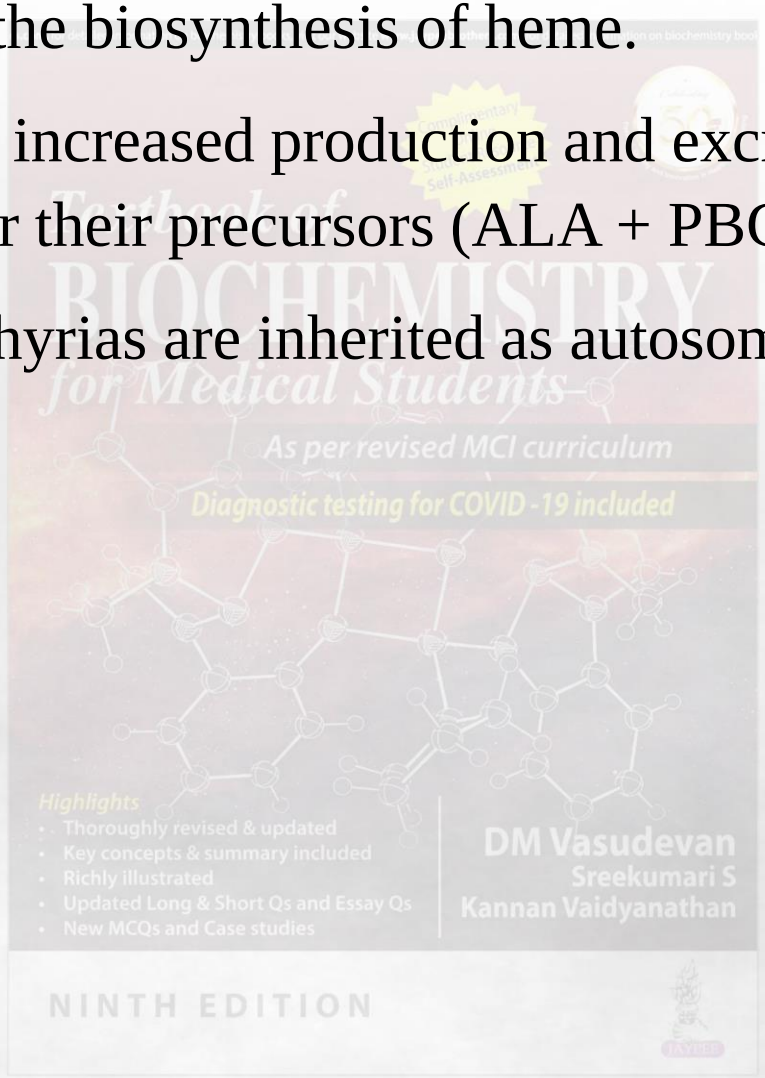


Shunt Bilirubin



Porphyrias

- Associated with the biosynthesis of heme.
- Characterized by increased production and excretion of porphyrins and/or their precursors (ALA + PBG).
- Most of the porphyrias are inherited as autosomal dominant traits.



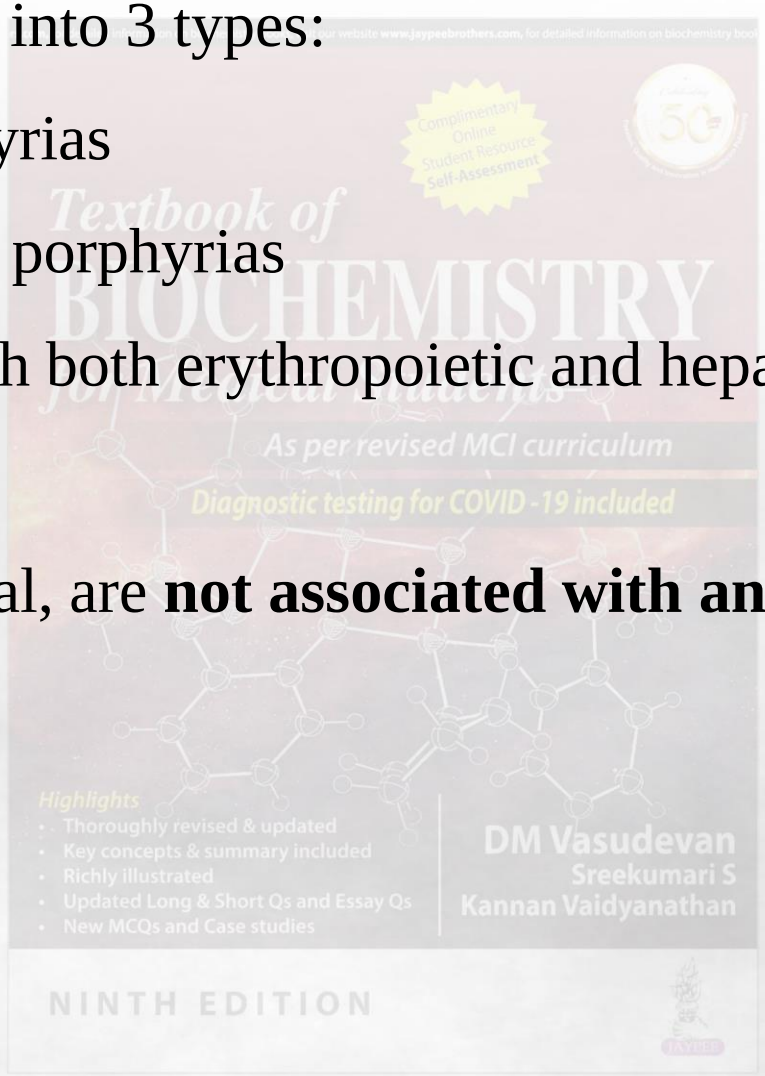
- Broadly grouped into 3 types:

  a. Hepatic porphyrias

  b. Erythropoietic porphyrias

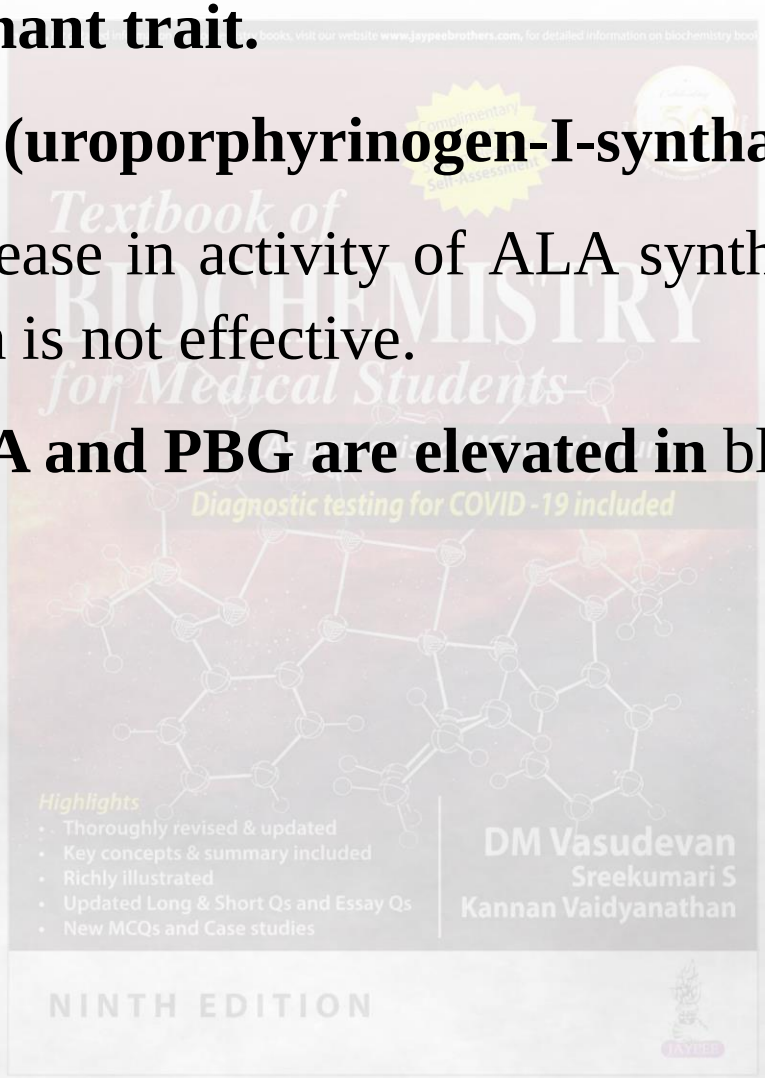
  c. Porphyrias with both erythropoietic and hepatic abnormalities.

Porphyrias in general, are **not associated with anemia.**

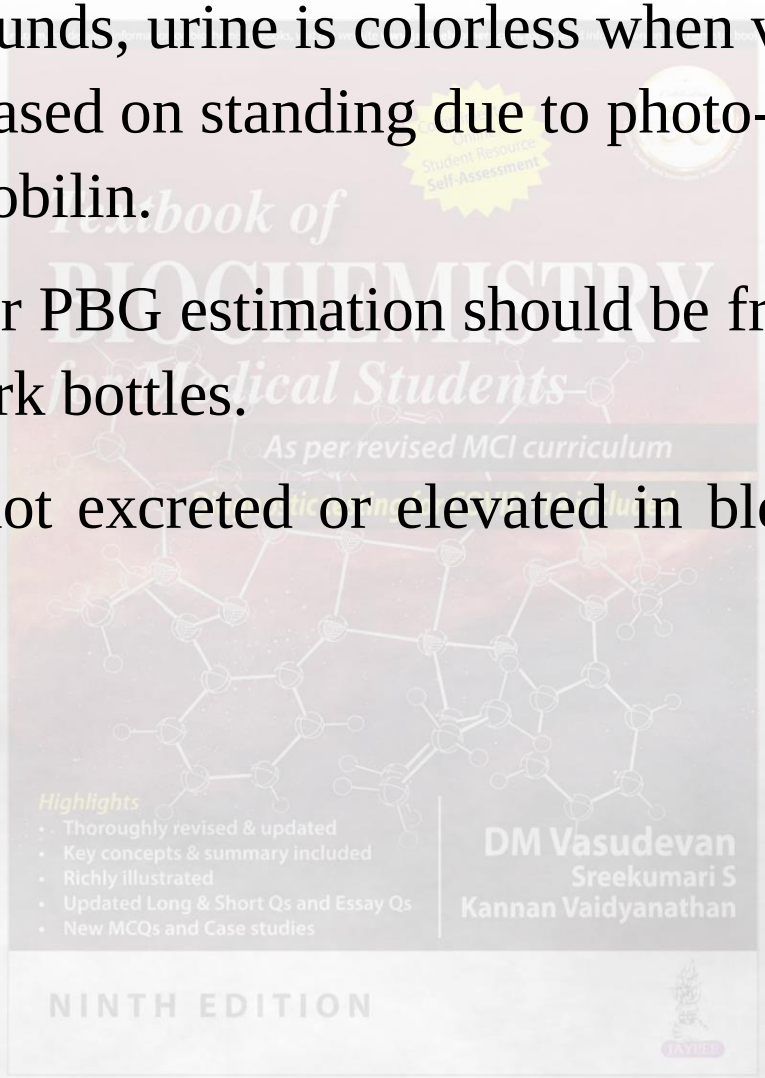


Acute Intermittent Porphyria (AIP)

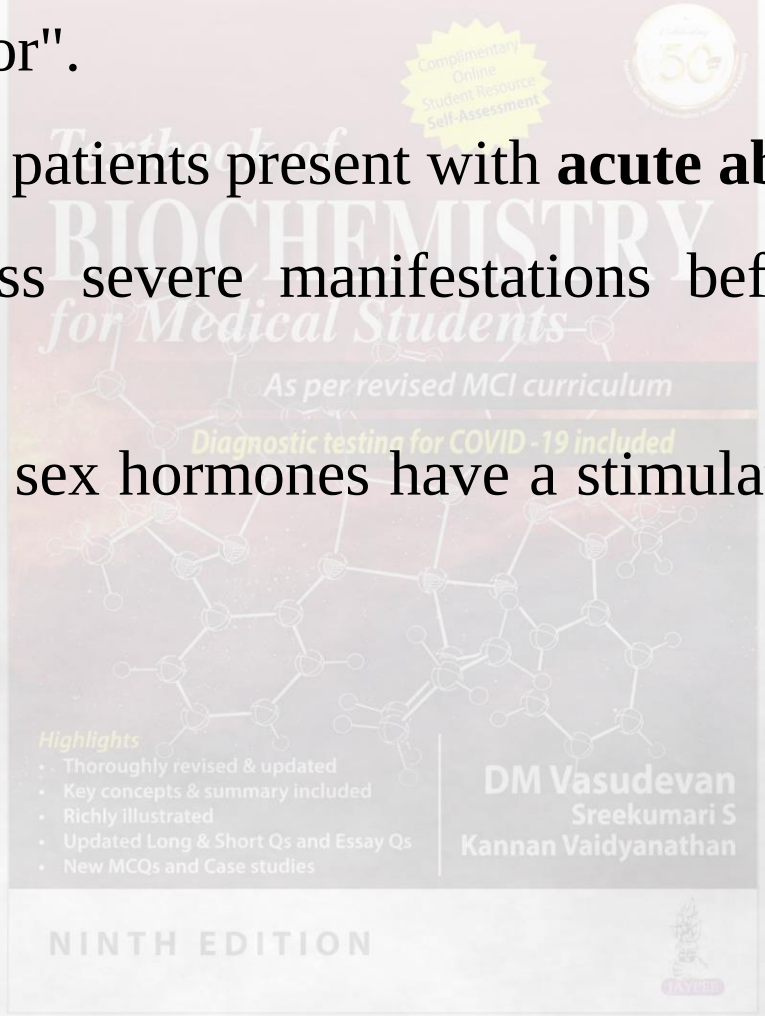
- Autosomal **dominant trait.**
- **PBG-deaminase (uroporphyrinogen-I-synthase)** deficient.
- A secondary increase in activity of ALA synthase, because end-product inhibition is not effective.
- The levels of **ALA and PBG are elevated in** blood and urine.



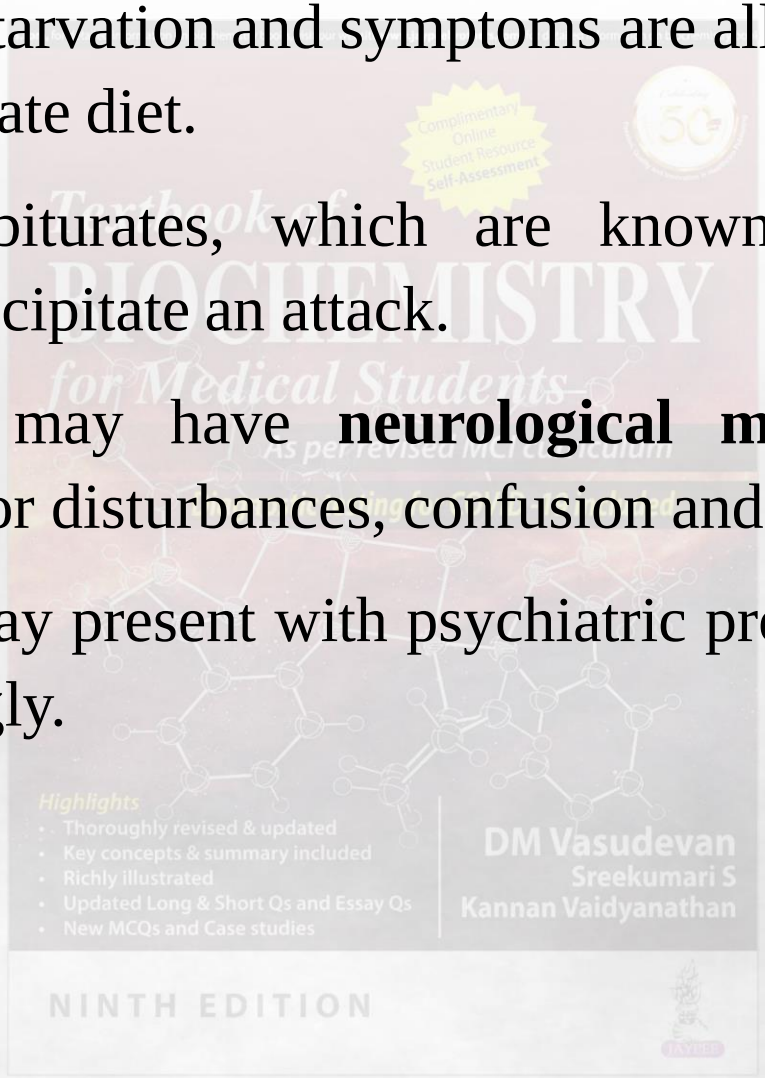
- Colorless compounds, urine is colorless when voided, but the color is increased on standing due to photo-oxidation of PBG to porphobilin.
- Urine samples for PBG estimation should be freshly collected and transported in dark bottles.
- Porphyrins are not excreted or elevated in blood; so there is no photosensitivity.



- Symptoms appear intermittently and they are quite vague – The "little imitator".
- Most commonly, patients present with **acute abdominal pain.**
- Women have less severe manifestations before menarche and after menopause.
- Thus, the female sex hormones have a stimulatory effect on ALA synthase.

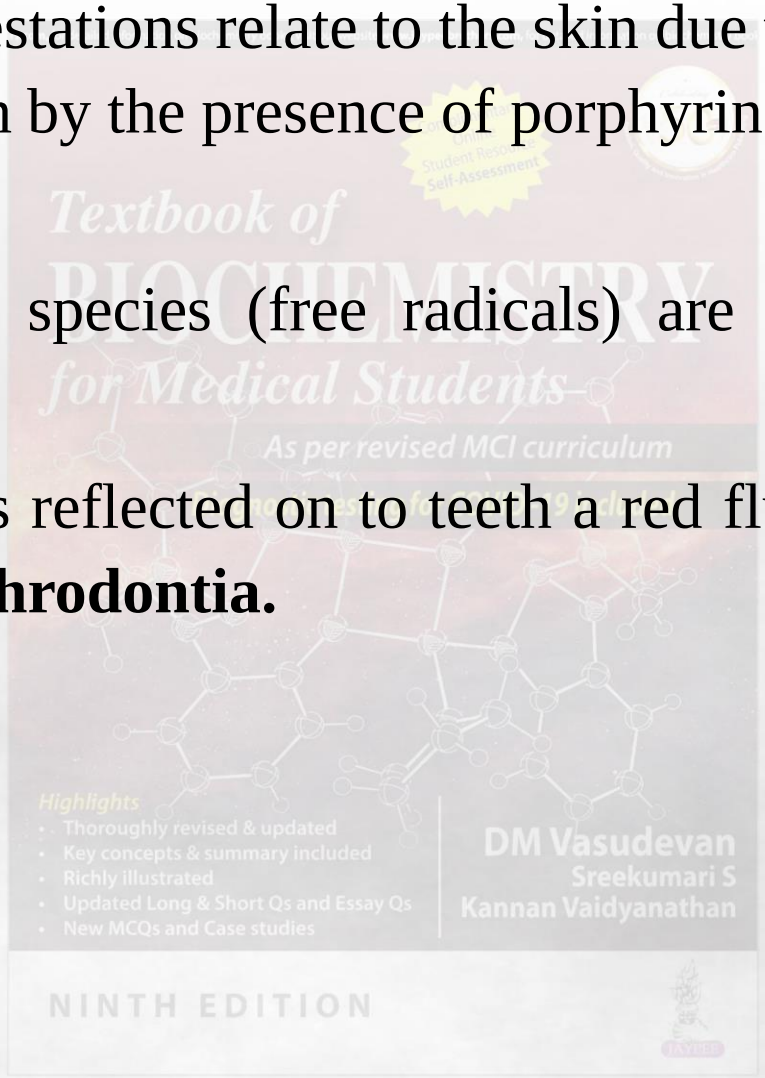


- Precipitated by starvation and symptoms are alleviated by a high carbohydrate diet.
- Drugs like barbiturates, which are known to induce ALA synthase, can precipitate an attack.
- Another group may have **neurological manifestations** like sensory and motor disturbances, confusion and agitation.
- Some patients may present with psychiatric problems and may be treated accordingly.

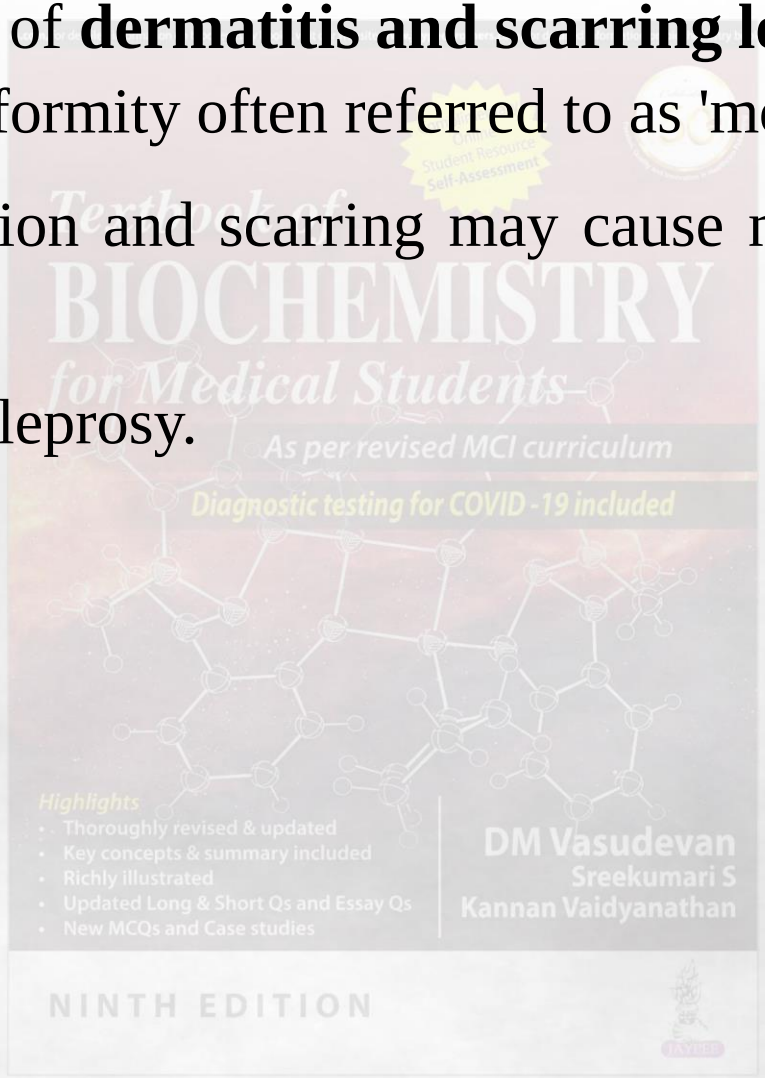


Congenital Erythropoietic Porphyria

- The major manifestations relate to the skin due to the photosensitization by the presence of porphyrins in the capillaries.
- Reactive oxygen species (free radicals) are the cause for cell destruction.
- When UV light is reflected on to teeth a red fluorescence is seen; this is called **erythrodontia.**



- Repeated attacks of **dermatitis and scarring lead to a typical facial** deformity often referred to as 'monkey face'.
- Repeated ulceration and scarring may cause mutilation of nose, ear and cartilage.
- This may mimic leprosy.



Glycine + Succinyl-CoA

↓ (Enzyme 1) ALA-synthase

Delta amino levulinic acid (ALA)

↓ (Enzyme 2) ALA-dehydratase

Porphobilinogen (PBG)

↓ (Enzyme 3) PBG-deaminase → Acute intermittent porphyria

↓ (Enzyme 3b) UPG-III-co-synthase → Congenital erythropoietic porphyria

Uroporphyrinogen (UPG)

↓ (Enzyme 4) UPG-decarboxylase → Porphyria cutanea tarda

Coproporphyrinogen (CPG)

↓ (Enzyme 5) CPG-oxidase → Hereditary coproporphyria

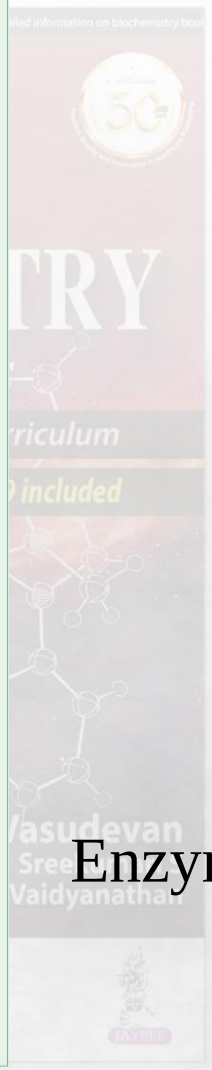
Protoporphyrinogen (PPG)

↓ (Enzyme 6) PPG-oxidase → Porphyria variegata

Protoporphyrin

↓ (Enzyme 7) Heme synthase → Hereditary protoporphyria

Heme



Enzyme deficiencies in porphyrias

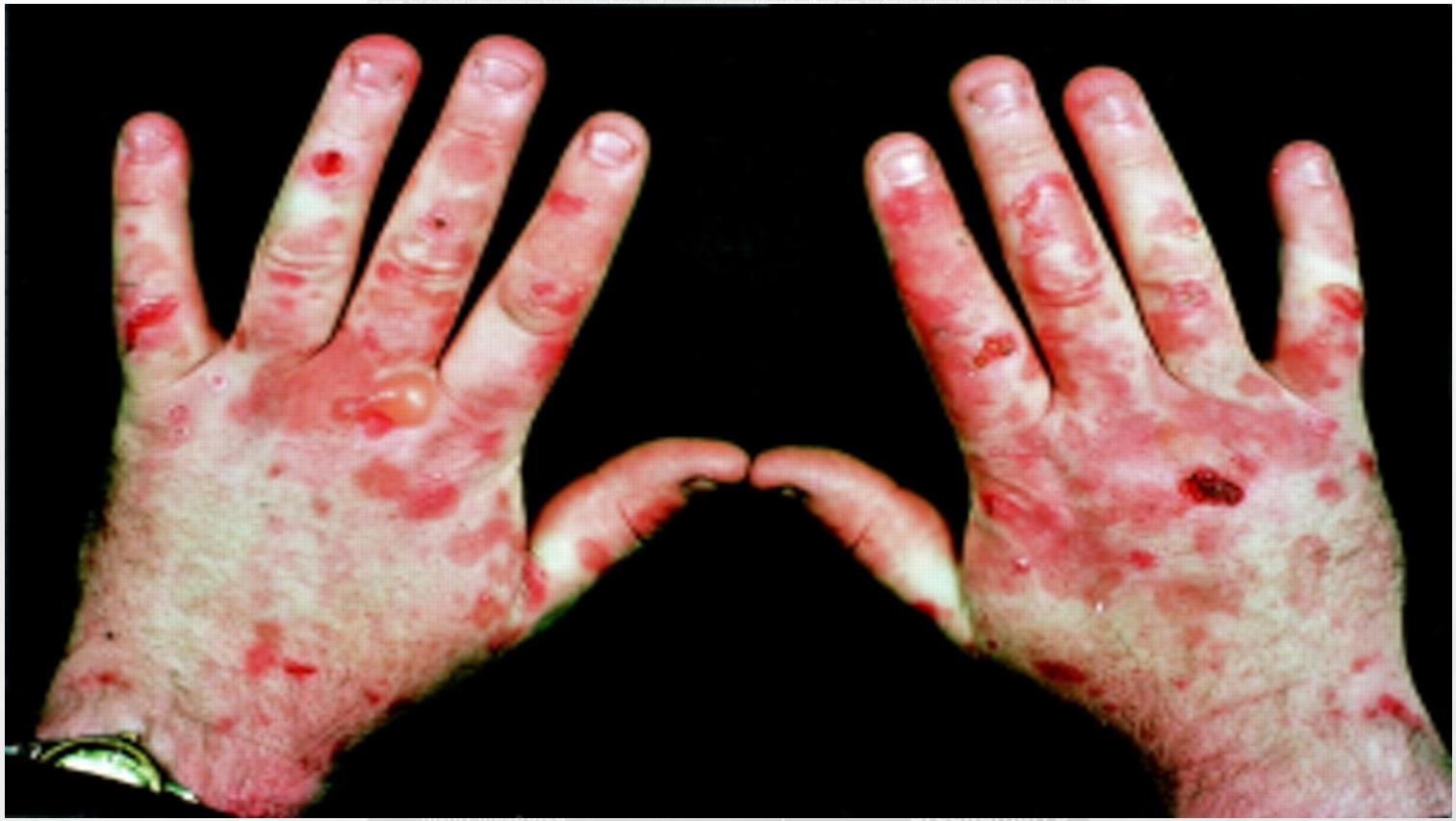
Features of Important Types of Porphyrias

Type	Enzyme defect	Inheri-tance	Excretion in urine	Other salient features
Acute inter-mittent porphyria (AIP)	PBG-deaminase (UPG-1 synthase) (enzyme 3)	Auto-somal domi-nant	Precursors, ALA and PBG. No color on voiding	Most common porphyria (1 in 10,000). Hepatic porphyria. Abdominal and neurological manifestations. No photosensitivity.
Congenital erythro-poietic porphyria	UPG-cosynthase (enzyme 3b)	Auto-somal rece-ssive	UP and CP; Port-wine appearance	Marked photosensitivity. Erythrodontia. Incidence, rare.
Porphyria cutanea tarda	UPG-decarboxy-lase (enz 4)	Auto-somal domi-nant	Uroporphy-rins. Urine colored.	Second most common; incidence 1 in 25,000. Photosensitivity.

Features of important types of porphyrias

Type	Enzyme defect	Inheri-tance	Excretion in urine	Other salient features
Hereditary copro-porphyria	CPG-III-oxidase (enzyme 5)	Auto-somal domin ant	UP and CP excreted in urine and feces. Colored urine.	Symptoms similar to AIP; but milder. Photosensitivity is also seen.
Hereditary proto-porphyria	Heme synthase or Ferro-chelatase (enzyme 7)	Auto-somal domi-nant	Neither porphyrins nor precursors are excreted in urine.	Protoporphyrin increased in plasma, RBCs and feces. RBCs show fluorescence.

Porphyria Cutanea Tarda



Note the skin lesions due to photosensitivity

Diagnosis of Porphyrias with Neurovisceral Manifestations

Disease and enzyme defect	Laboratory findings
ALAD-porphyria; (ALA dehydratase deficiency)(Enz 2)	Urine ALA ++ Urine PBG normal RBC HMBS normal
AIP (HMBS or PBG Deaminase (Enzyme 3)	Urine ALA ++ Urine PBG ++ Plasma porphyrins normal
HCP; Coproporphy- rinogen oxidase deficiency,Enz 5	Urine ALA normal Urine PBG ++ Plasma porphyrins normal
VP; Protoporphyrinogen oxidase deficiency (Enzyme 6)	Urine ALA normal Urine PBG +++ Urine porphyrins ++ Plasma porphyrins ++
ALA = amino levulinic acid; ALAD = amino levulinic acid dehydratase; PBG = porphobilinogen; HMBS = hydroxymethyl bilane synthase (PBG deaminase); AIP = acute intermittent porphyria; HCP = hereditary coproporphyria; VP = variegate porphyria.	

Diagnosis of Porphyrias with Cutaneous Manifestations

Disease and enzyme defect	Laboratory findings
PCT (UPG decarboxylase deficiency) (Enzyme 4)	RBC uroporphyrin ++ Plasma uroporphyrins ++ Urine uroporphyrin ++
CEP (UPG III synthase deficiency) (Enzyme 3b)	RBC UP and CP ++ Plasma UP and CP ++ Urine UP and CP ++
EPP (Ferrochelatase deficiency) (Enzyme 7)	RBC protoporphyrin + Plasma porphyrin + Urine protoporphyrin +
PCT = Porphyria cutanea tarda; UPG = uroporphyrinogen; CEP = congenital erythropoietic porphyria; UP= uroporphyrin; CP = coproporphyrin; EPP = erythropoietic protoporphyria	

Acquired Porphyrias

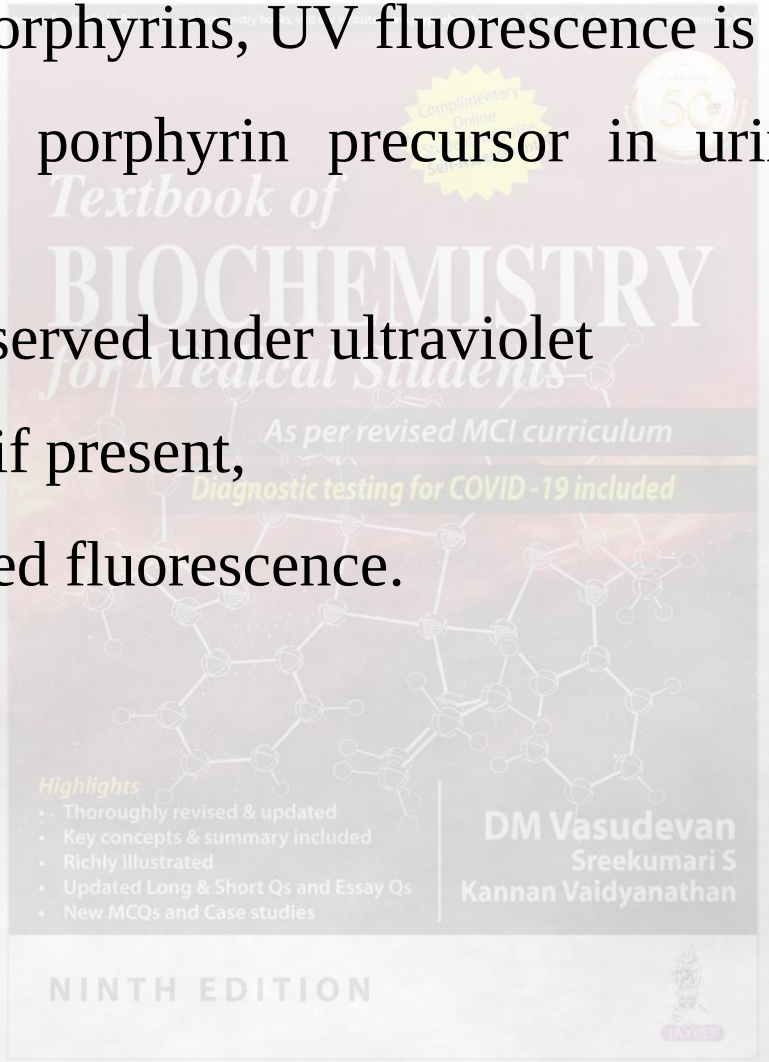
Ethanol and lead poisoning, some malignancies	PBG synthase inhibited, ALA accumulates
Some malignancies	HMB synthase; PBG and ALA
Chronic renal failure, some malignancies	UPG decarboxylase; UP
Diet, liver diseases, chronic renal failure, some cancers, hexachloro benzene, lead, mercury and arsenic poisoning	CPG oxidase; CP
Iron deficiency anemia, lead and aluminum poisoning	Ferrochelatase; PP

Diagnosis of Porphyrias

- To demonstrate porphyrins, UV fluorescence is the best technique.
- The presence of porphyrin precursor in urine is detected by Ehrlich's reagent.
- When urine is observed under ultraviolet

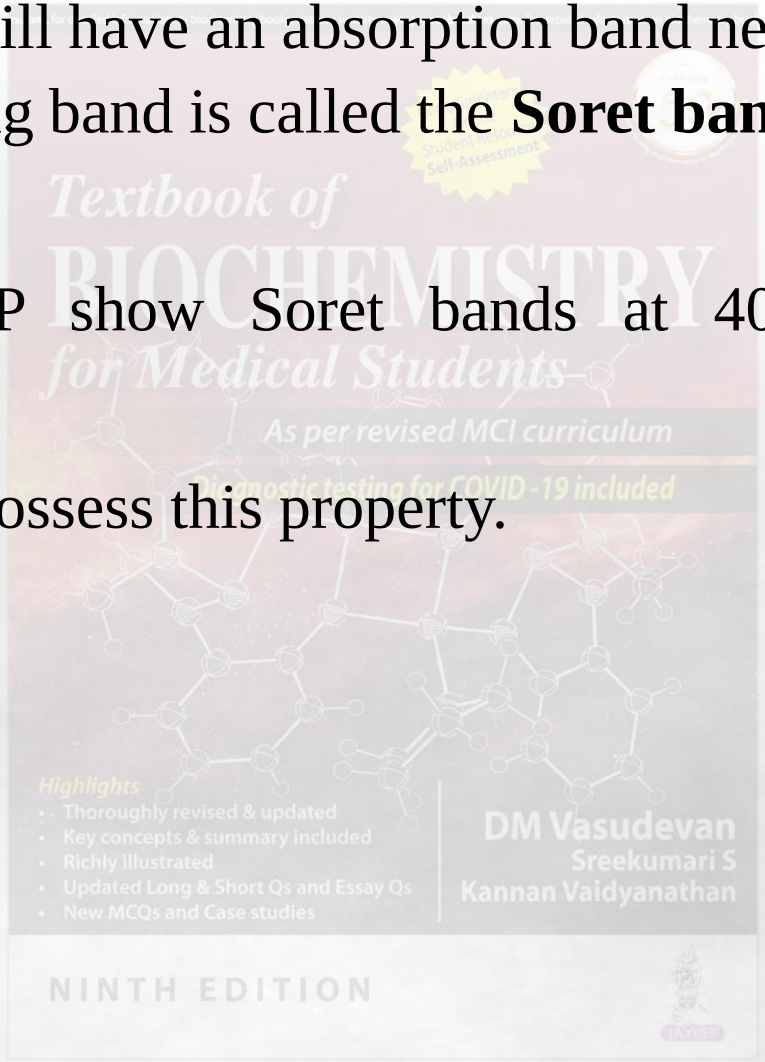
  light; porphyrins if present,

  will emit strong red fluorescence.



Soret Band

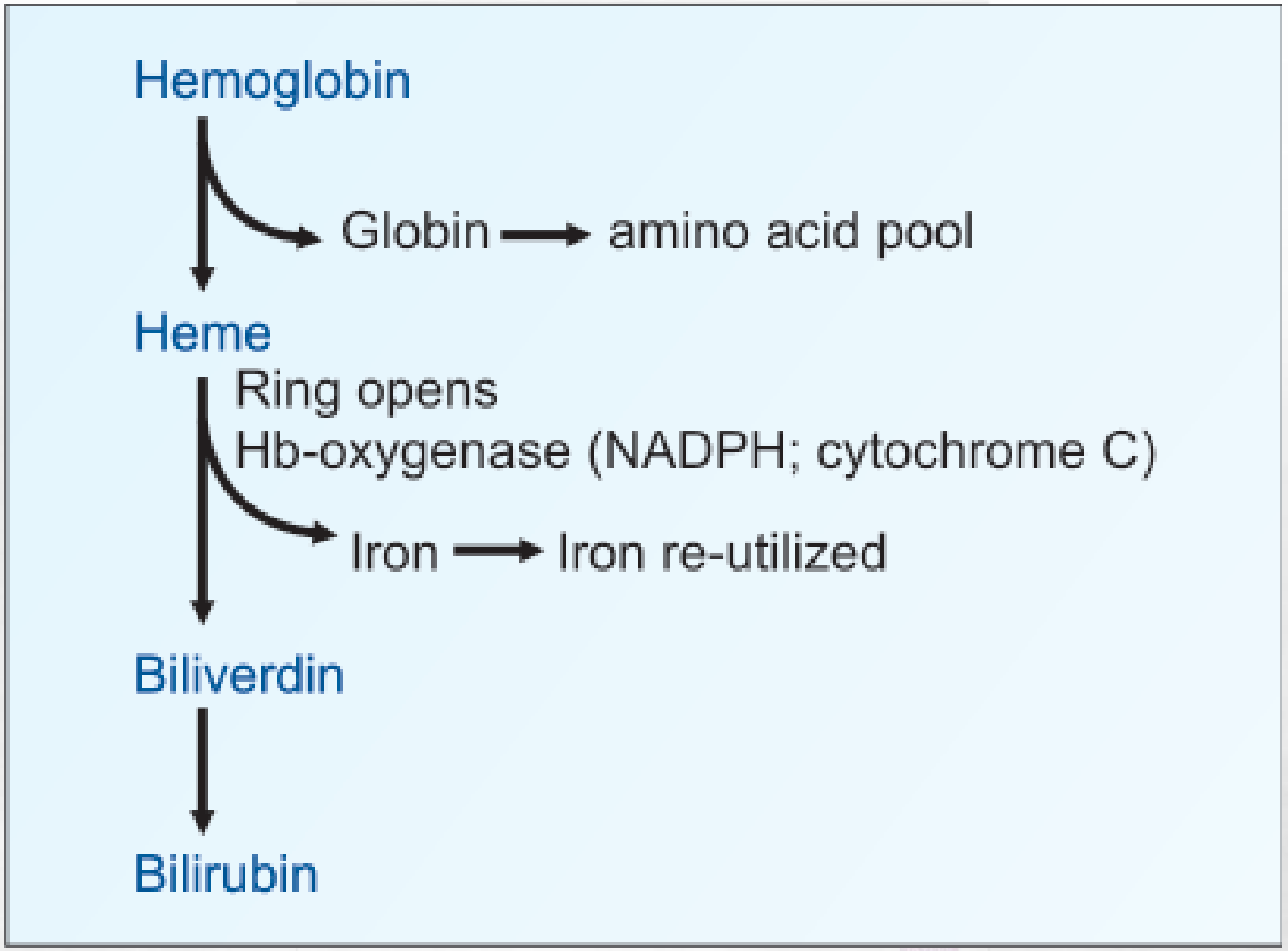
- All porphyrins will have an absorption band near 400 nm; this distinguishing band is called the **Soret band, after its** discoverer.
- UP, CP and PP show Soret bands at 406, 400 and 408 respectively.
- Heme does not possess this property.



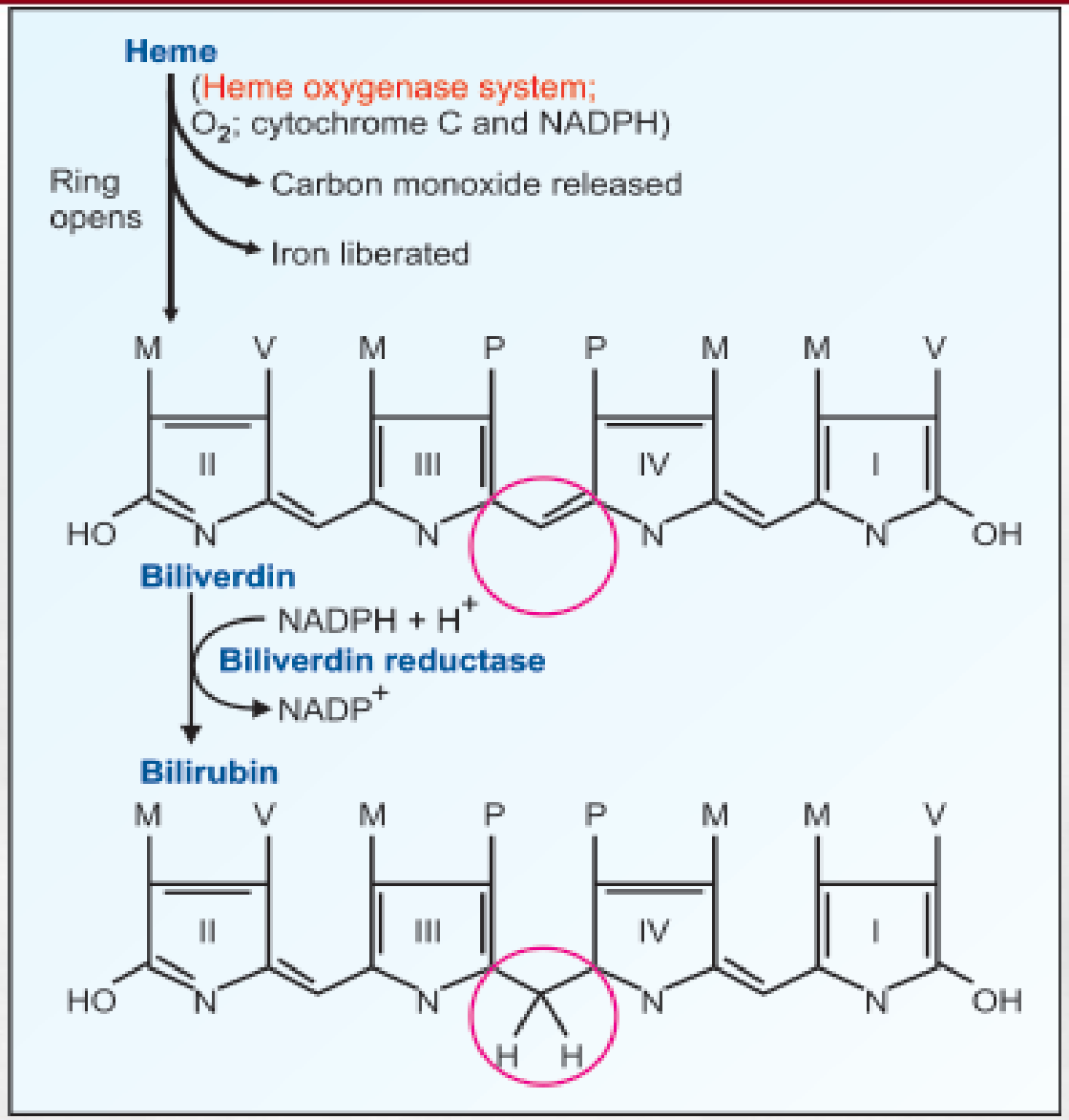
Urinary Excretion of Prophyrins

ALA	Acute intermittent porphyria (AIP)
PBG	AIP
CP	Erythropoeitic porphyria
UP	Acquired porphyrias
PP	Acquired porphyrias

Breakdown of Hemoglobin

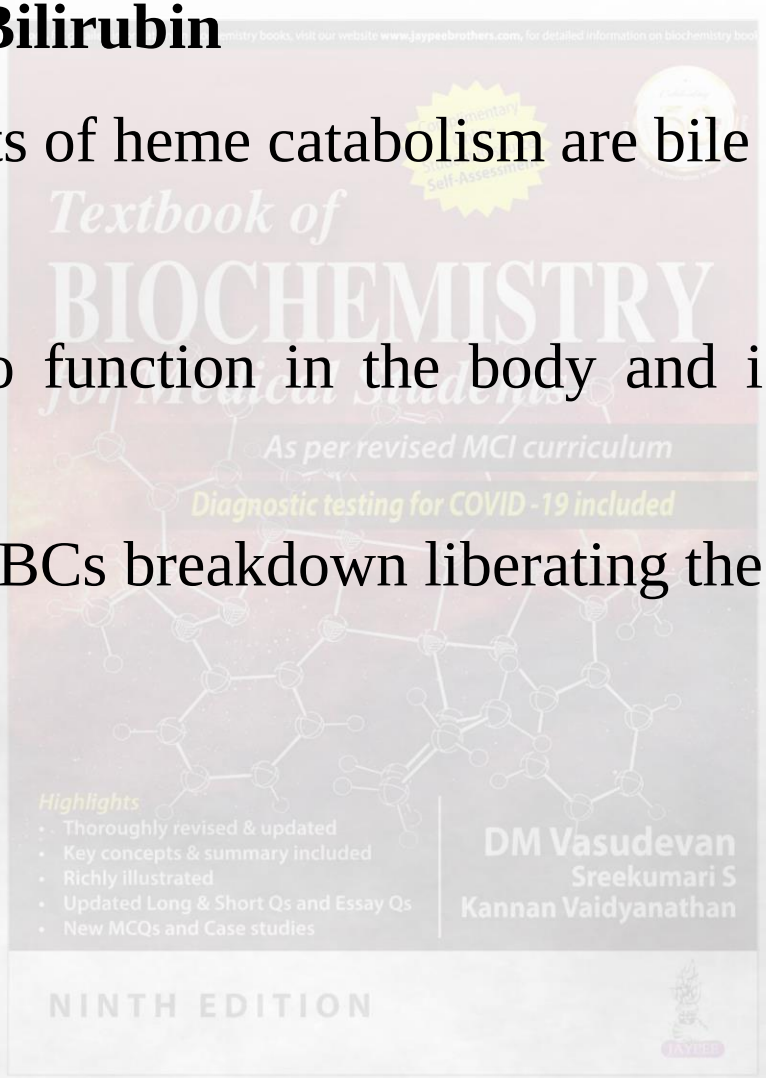


Breakdown of Heme

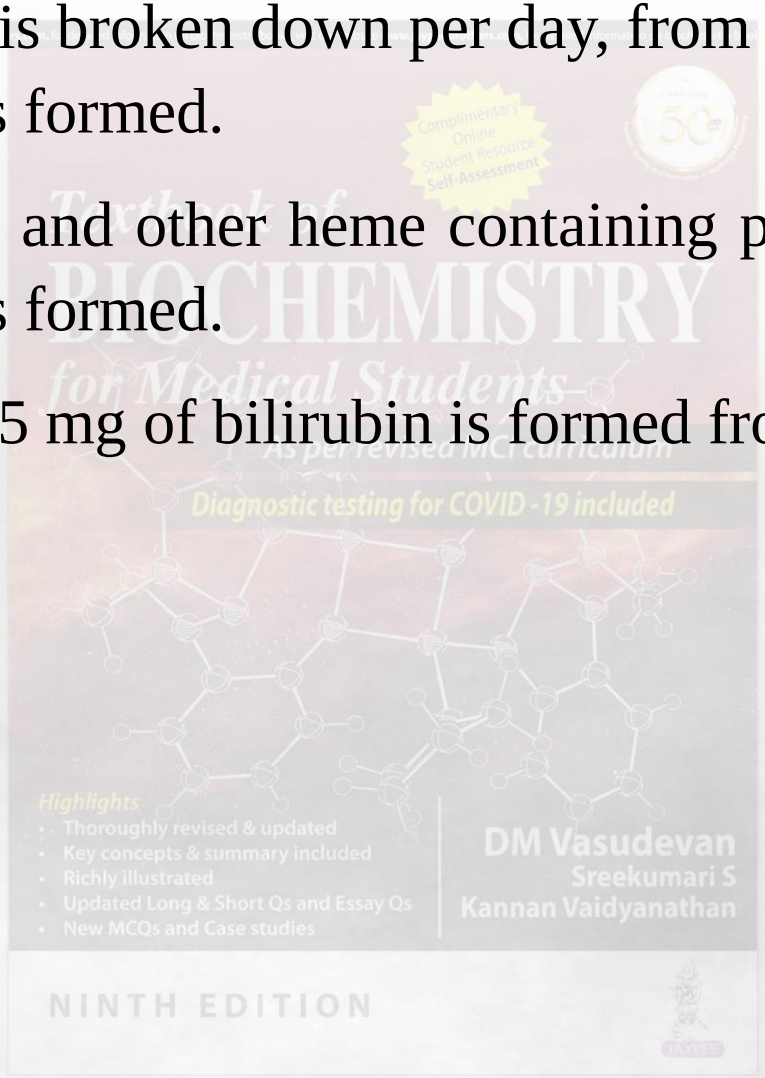


Catabolism of Heme

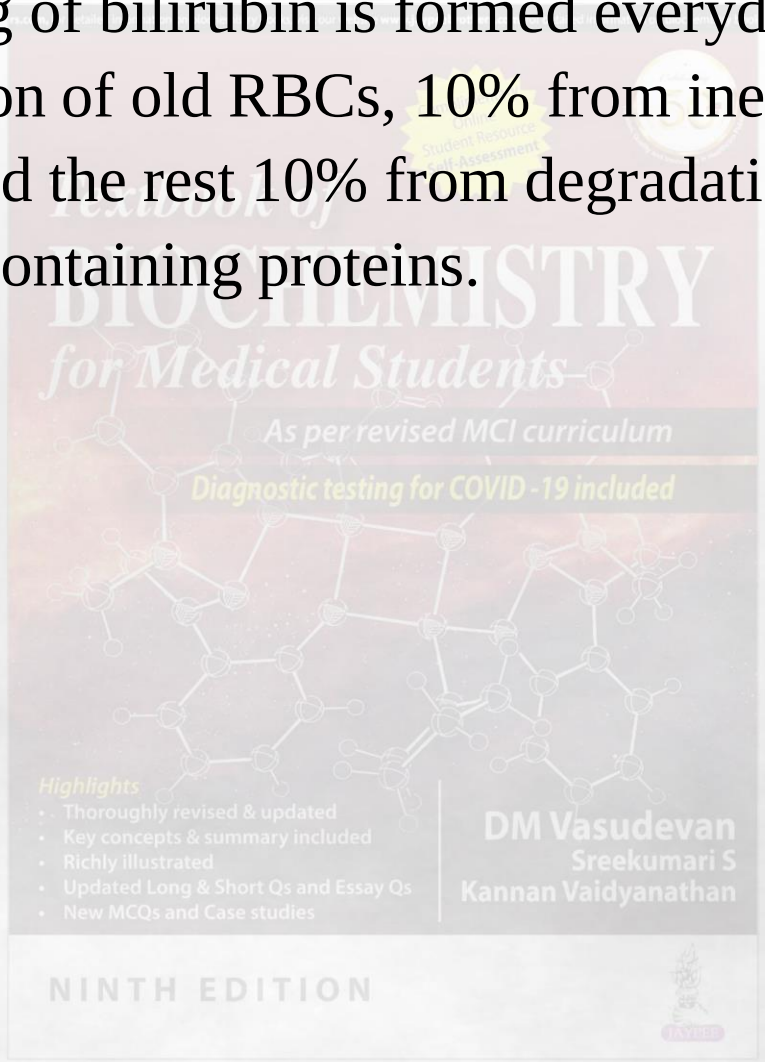
- **Generation of Bilirubin**
- The end-products of heme catabolism are bile
- pigments.
- Bilirubin has no function in the body and is excreted through bile.
- The senescent RBCs breakdown liberating the hemoglobin.



- About 6 g of Hb is broken down per day, from which about 250 mg of bilirubin is formed.
- From myoglobin and other heme containing proteins, another 50 mg of bilirubin is formed.
- Approximately 35 mg of bilirubin is formed from 1 g of Hb.



- A total of 300 mg of bilirubin is formed everyday; of which 80% is from destruction of old RBCs, 10% from ineffective erythropoiesis and the rest 10% from degradation of myoglobin and other heme containing proteins.

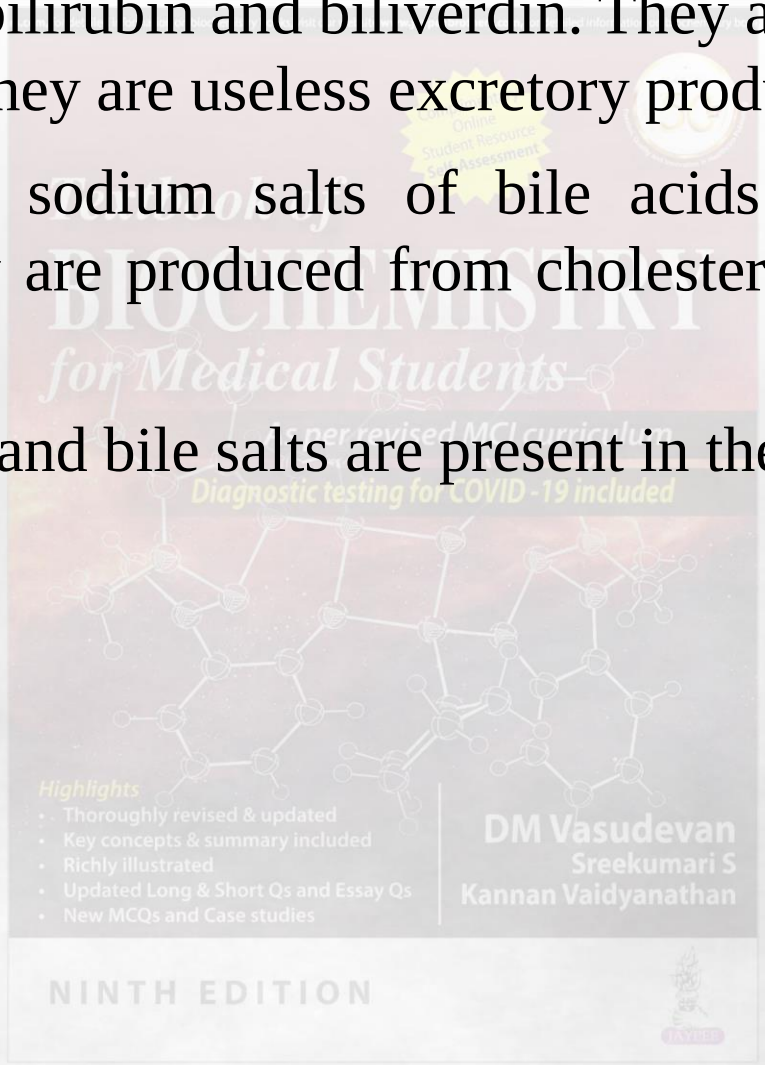


Bile Pigments and Bile Salts are Different

Bile pigments are bilirubin and biliverdin. They are the breakdown products of heme; they are useless excretory products.

Bile salts are the sodium salts of bile acids (glycocholate and taurocholate). They are produced from cholesterol; they help in the absorption of fat.

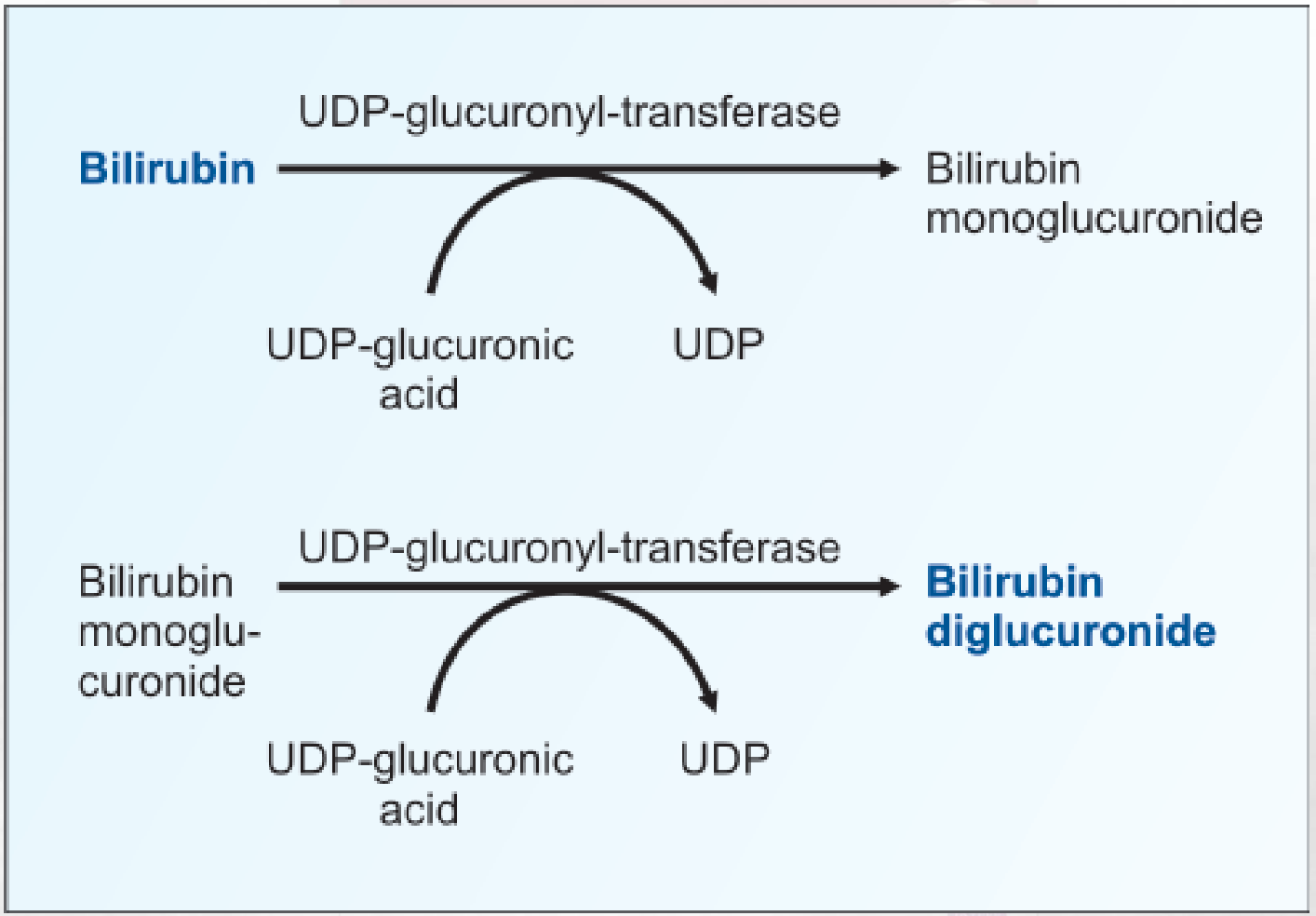
Both bile pigments and bile salts are present in the bile.

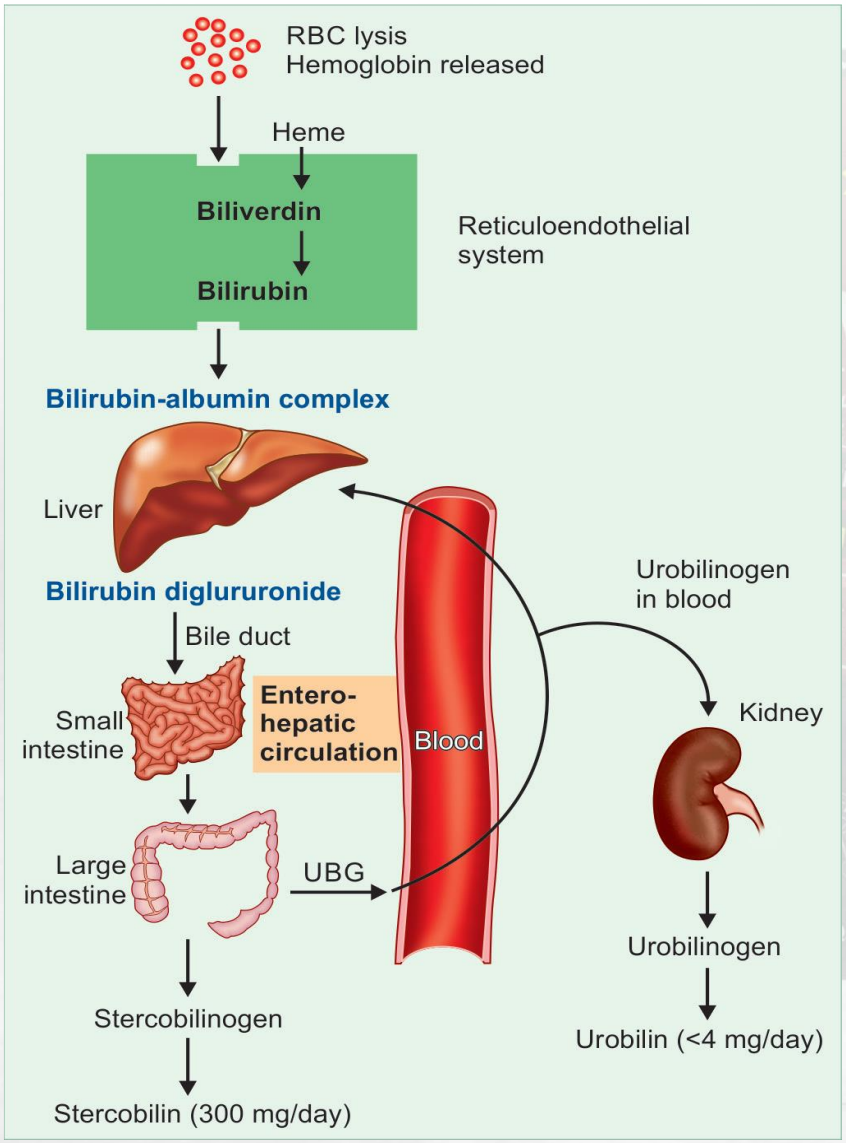


Transport to Liver

- The liver plays the central role in the further disposal of the bilirubin.
- The bilirubin formed in the reticuloendothelial cells is insoluble in water.
- The lipophilic bilirubin is therefore transported in plasma bound to **albumin.**
- One molecule of albumin can bind 2 molecules of bilirubin.
- 100 ml of plasma can transport upto 25 mg of bilirubin.

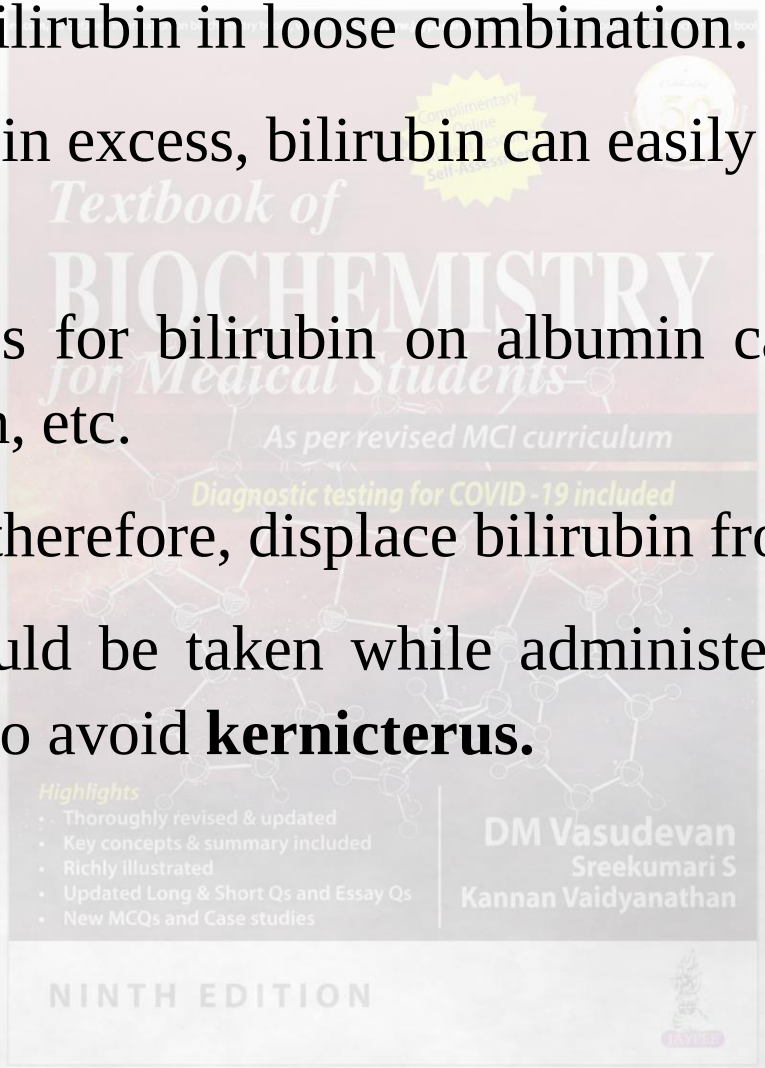
Production of Bilirubin Diglucuronide





Production and excretion of bilirubin

- Albumin binds bilirubin in loose combination.
- So when present in excess, bilirubin can easily dissociate from albumin.
- The binding sites for bilirubin on albumin can be occupied by aspirin, penicillin, etc.
- Such drugs can, therefore, displace bilirubin from albumin.
- Hence, care should be taken while administering such drugs to newborn babies to avoid **kernicterus.**



Conjugation in Liver

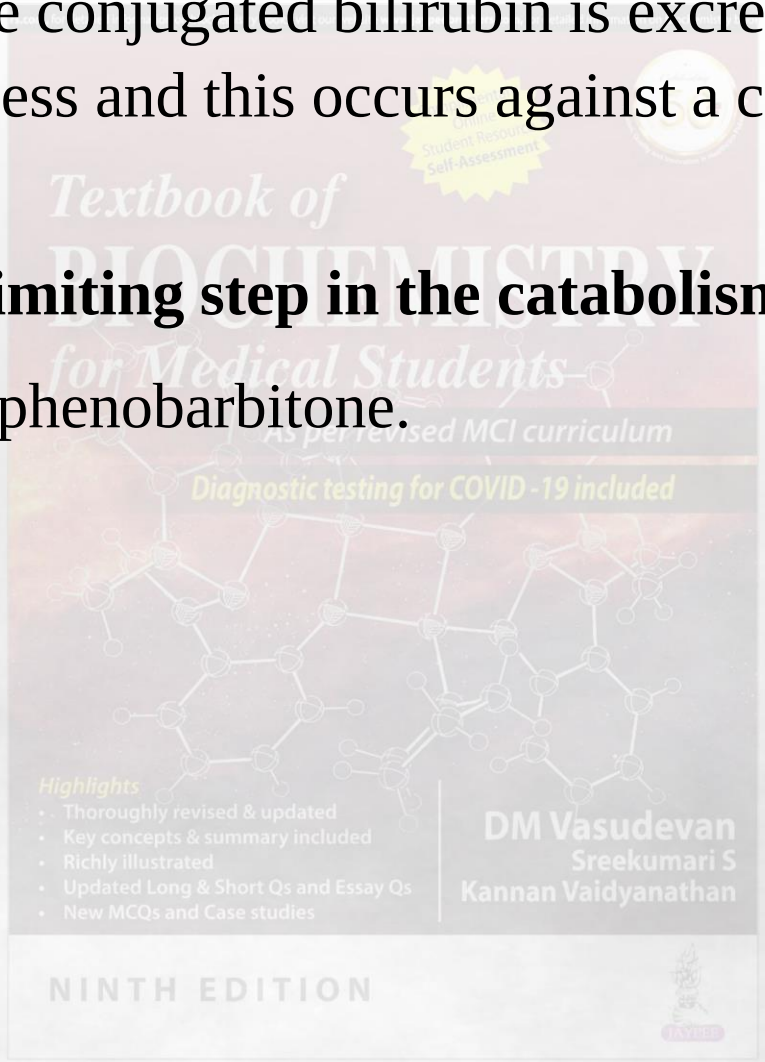
- Inside the liver cell, the bilirubin is conjugated with glucuronic acid, to make it **water soluble**.
- The first carbon of glucuronic acid is combined with the carboxyl group of the propionic acid side chains of the bilirubin molecule.
- About 80% molecules are in the **diglucuronide form, while 20% are monoglucuronides.**
- Drugs like primaquine, novobiocin, chloramphenicol, androgens and pregnanediol may interfere in this conjugation process and may cause jaundice.

Properties of Conjugated and Free Bilirubin

	Free bilirubin bilirubin	Conjugated
In water	insoluble	soluble
In alcohol	soluble	soluble
Normal plasma level	0.2 – 0.6 mg/dl	0 – 0.2 mg/dl
In bile	absent	present
In urine	always absent	normally absent
Absorption from GIT	absorbed	not absorbed
Diffusion into tissue	diffuses	does not diffuse
van den Bergh's test	indirect positive	direct positive

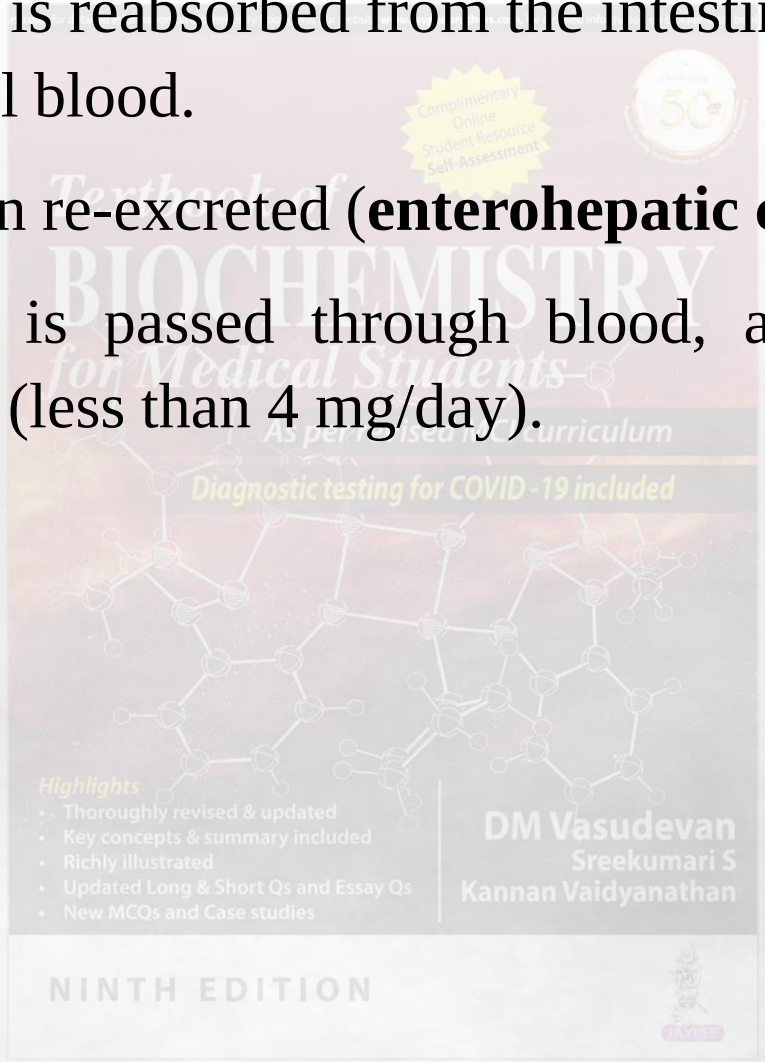
Excretion of Bilirubin to Bile

- The water soluble conjugated bilirubin is excreted into the bile by an active process and this occurs against a concentration gradient.
- This is the **rate limiting step in the catabolism of heme.**
- **It is induced** by phenobarbitone.



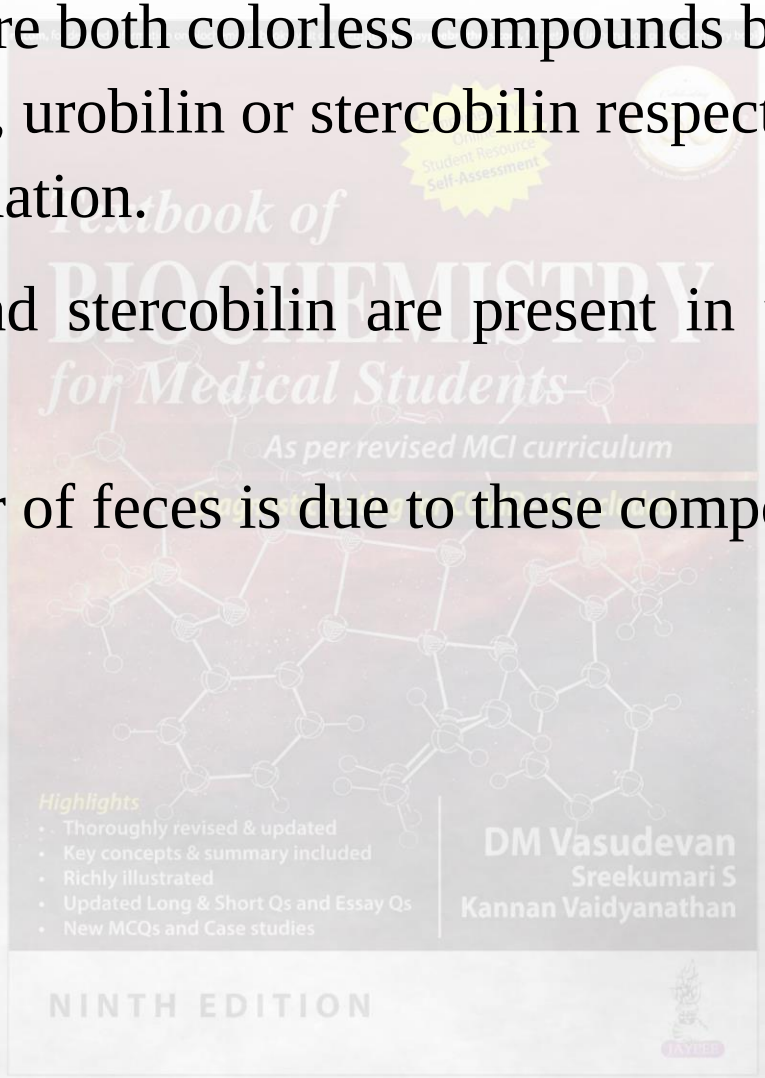
Enterohepatic Circulation

- 20% of the UBG is reabsorbed from the intestine and returned to the liver by portal blood.
- The UBG is again re-excreted (**enterohepatic circulation)**.
- Since the UBG is passed through blood, a small fraction is excreted in urine (less than 4 mg/day).



Final Excretion

- UBG and SBG are both colorless compounds but are oxidized to colored products, urobilin or stercobilin respectively by atmospheric oxidation.
- Both urobilin and stercobilin are present in urine as well as in feces.
- The normal color of feces is due to these compounds.



Hyperbilirubinemia

- Normal plasma bilirubin level ranges from **0.2– 0.8 mg/dl.**
- **The unconjugated bilirubin is about** 0.2–0.6 mg/dl, while conjugated bilirubin is only 0–0.2 mg/dl.
- If the plasma bilirubin level exceeds 1 mg/dl, the condition is called **hyperbilirubinemia.**
- Levels between 1 and 2 mg/dl are indicative of **latent jaundice.**
- When the bilirubin level exceeds 2 mg/dl, it diffuses into tissues producing yellowish discoloration of sclera, conjunctiva, skin and mucous membrane resulting in **jaundice.**
- Icterus is the Greek term for jaundice.

Tests for Bile Pigmentsa

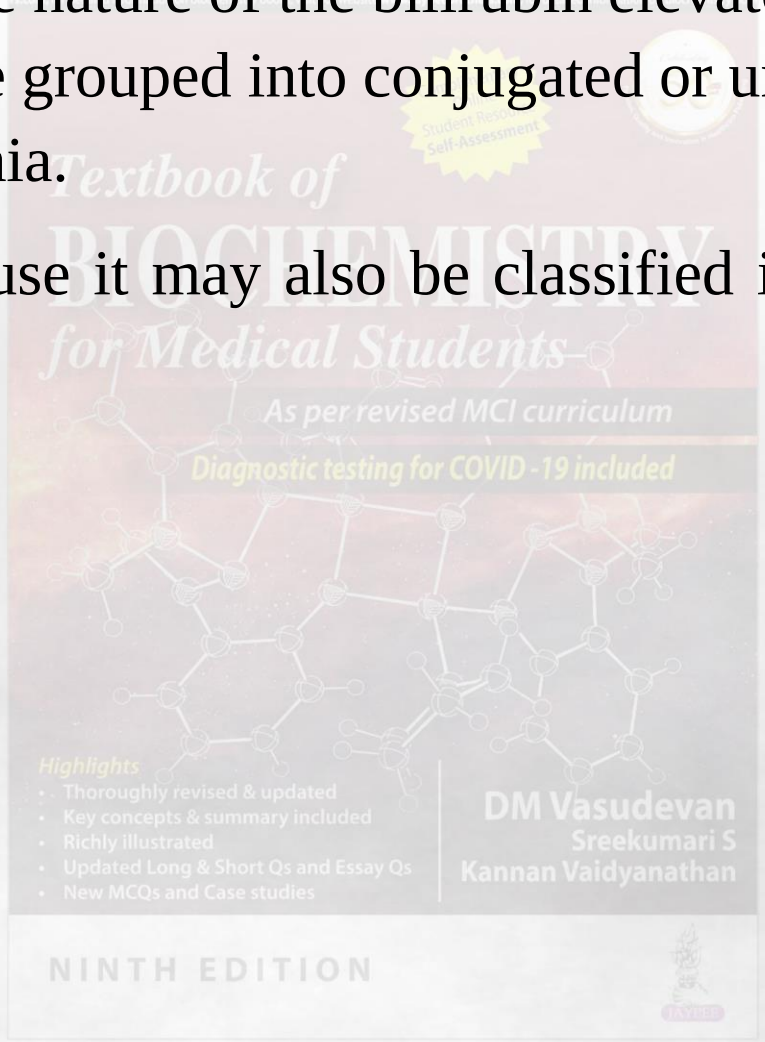
Bile Pigments	Fouchet's; Gmelin's; van den Bergh	Ehrlich's test	Schlesinger's test
Bilirubin	+ve	–ve	–ve
Bilinogens (UBG)	–ve	+ve	–ve
Bilins (UB + SB)	–ve	–ve	+ve

Van Den Bergh Test for Bilirubin

- Bilirubin reacts with *diazo reagent (diazotized* sulphanilic acid) to produce colored azo pigment.
- At pH 5, the pigment is purple in color.
- Conjugated bilirubin, being water soluble gives the color immediately; hence called **direct reaction.**
- Free bilirubin is water insoluble.
- It has to be extracted first with alcohol, when the reaction becomes positive; hence called **indirect reaction.**

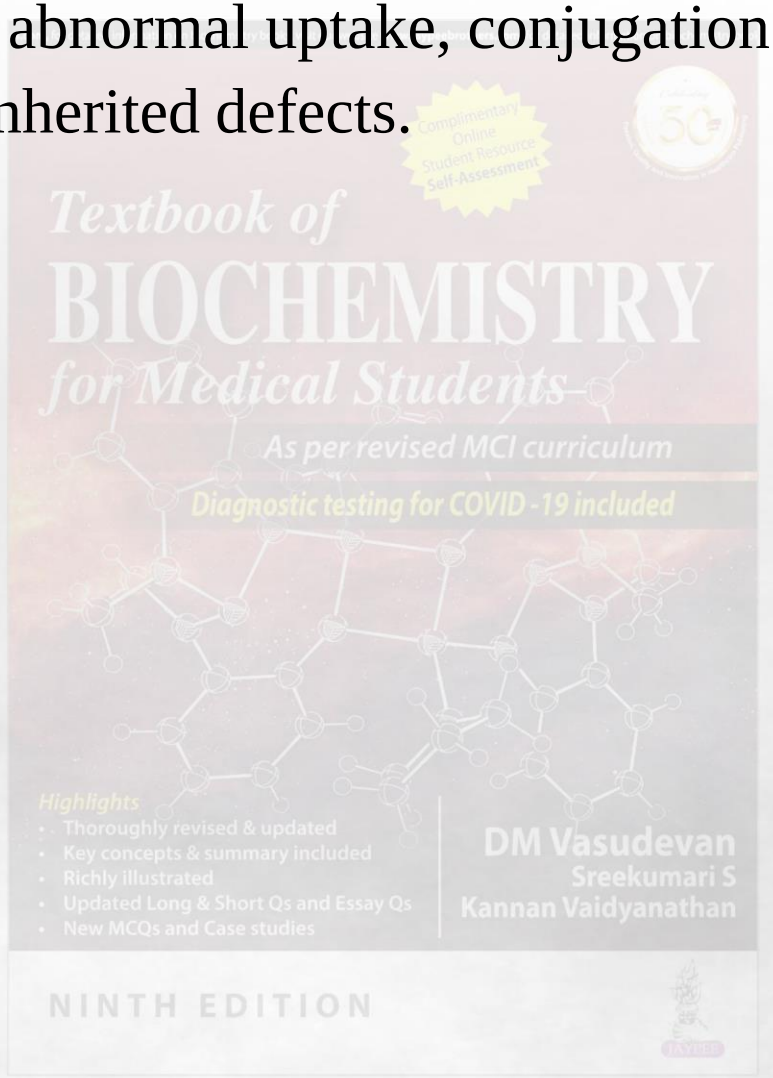
Hyperbilirubinemias

- Depending on the nature of the bilirubin elevated, the condition may be grouped into conjugated or unconjugated hyperbilirubinemia.
- Based on the cause it may also be classified into congenital and acquired.



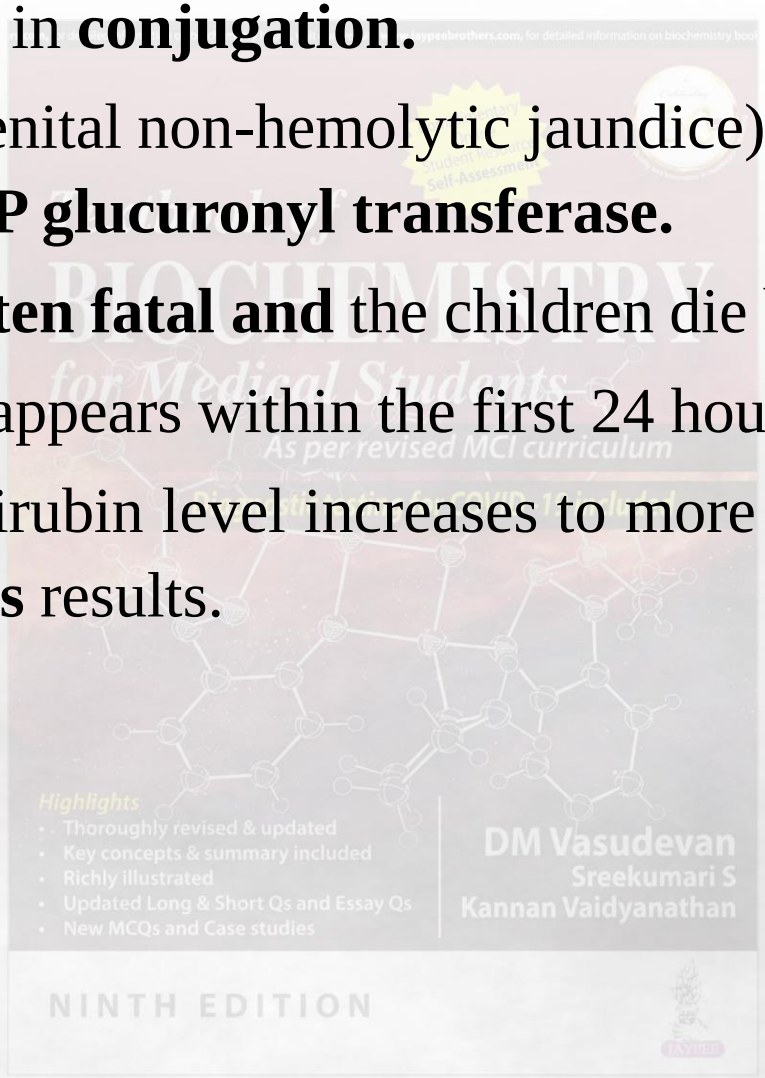
Congenital Hyperbilirubinemias

- They result from abnormal uptake, conjugation or excretion of bilirubin due to inherited defects.

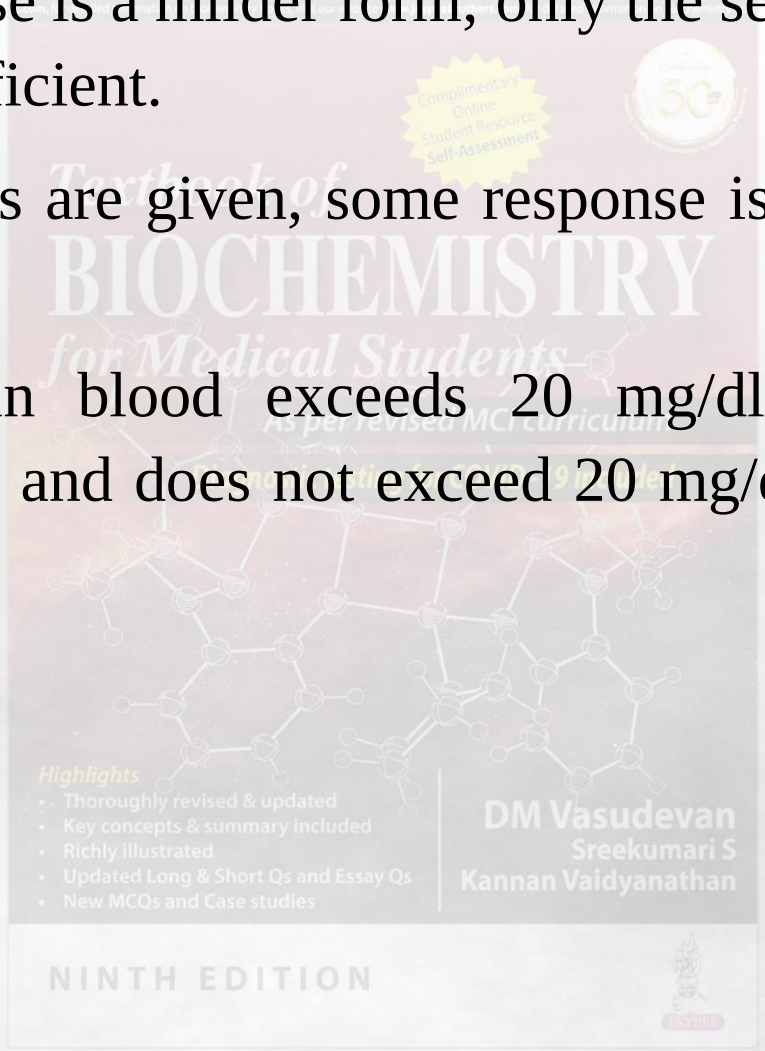


Crigler-Najjar Syndrome

- Here the defect is in **conjugation.**
- **In Type 1** (Congenital non-hemolytic jaundice), there is severe deficiency of **UDP glucuronyl transferase.**
- **The disease is often fatal and** the children die before the age of 2.
- Jaundice usually appears within the first 24 hours of life.
- Unconjugated bilirubin level increases to more than 20 mg/dl, and hence **kernicterus** results.

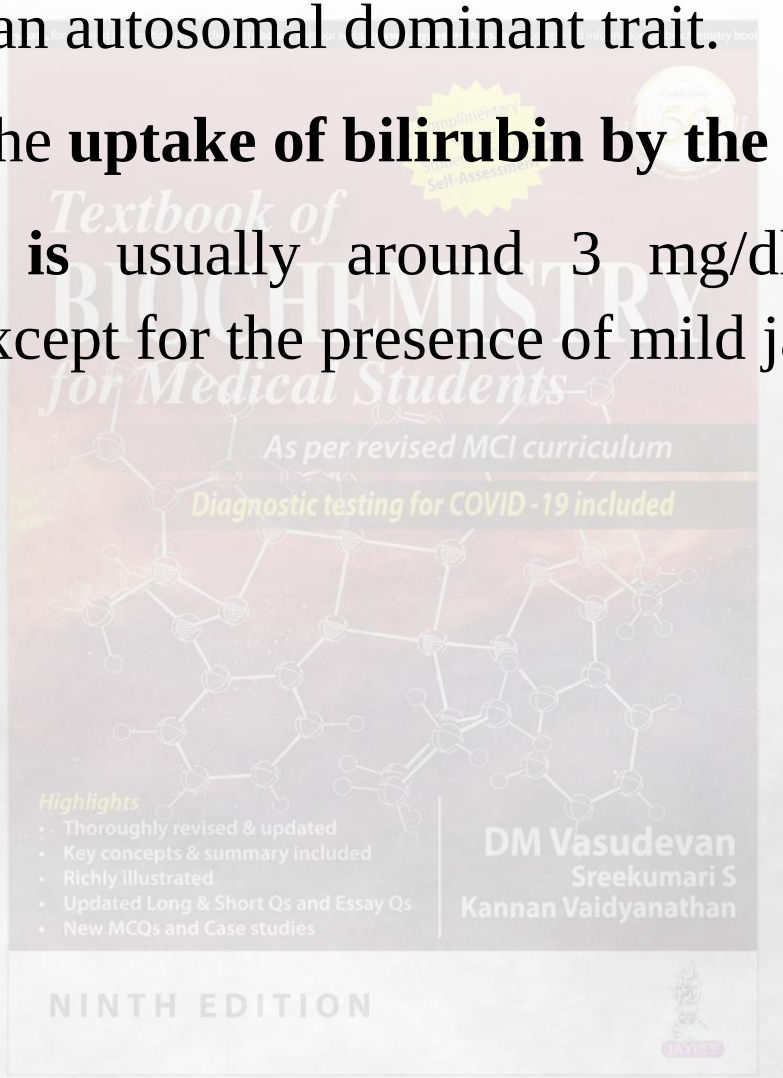


- The Type 2 disease is a milder form; only the second stage of conjugation is deficient.
- When barbiturates are given, some response is seen and jaundice improves.
- Bilirubin level in blood exceeds 20 mg/dl in Crigler-Najjar syndrome Type 1 and does not exceed 20 mg/dl in Crigler-Najjar syndrome Type 2.



Gilbert's Disease

- It is inherited as an autosomal dominant trait.
- The defect is in the **uptake of bilirubin by the liver.**
- **Bilirubin level is** usually around 3 mg/dl, and patient is asymptomatic, except for the presence of mild jaundice.

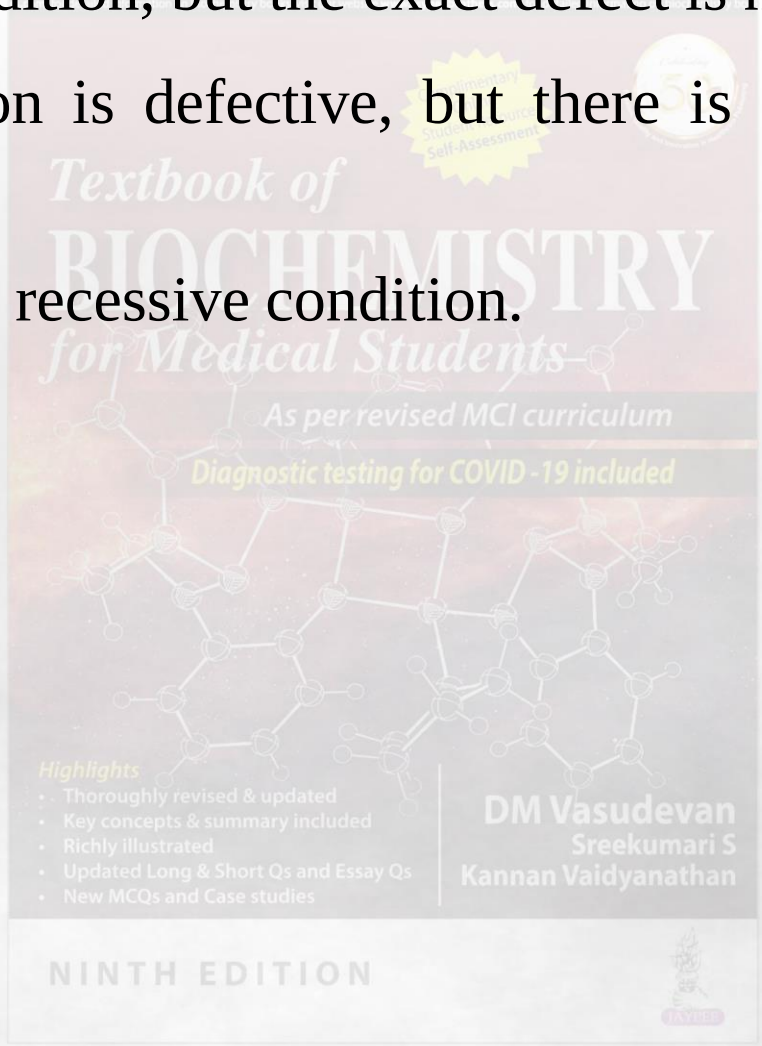


Dubin-Johnson Syndrome

- It is an autosomal **recessive trait leading to** defective excretion of conjugated bilirubin; so conjugated bilirubin in blood is increased.
- The disease results from the defective ATP dependent organic **anion transport in bile** canaliculi.
- There is a mutation in the MRP-2 protein which is responsible for transport of conjugated bilirubin into bile.
- The bilirubin gets deposited in the liver and the liver appears black.
- The condition is referred to as **Black liver jaundice.**

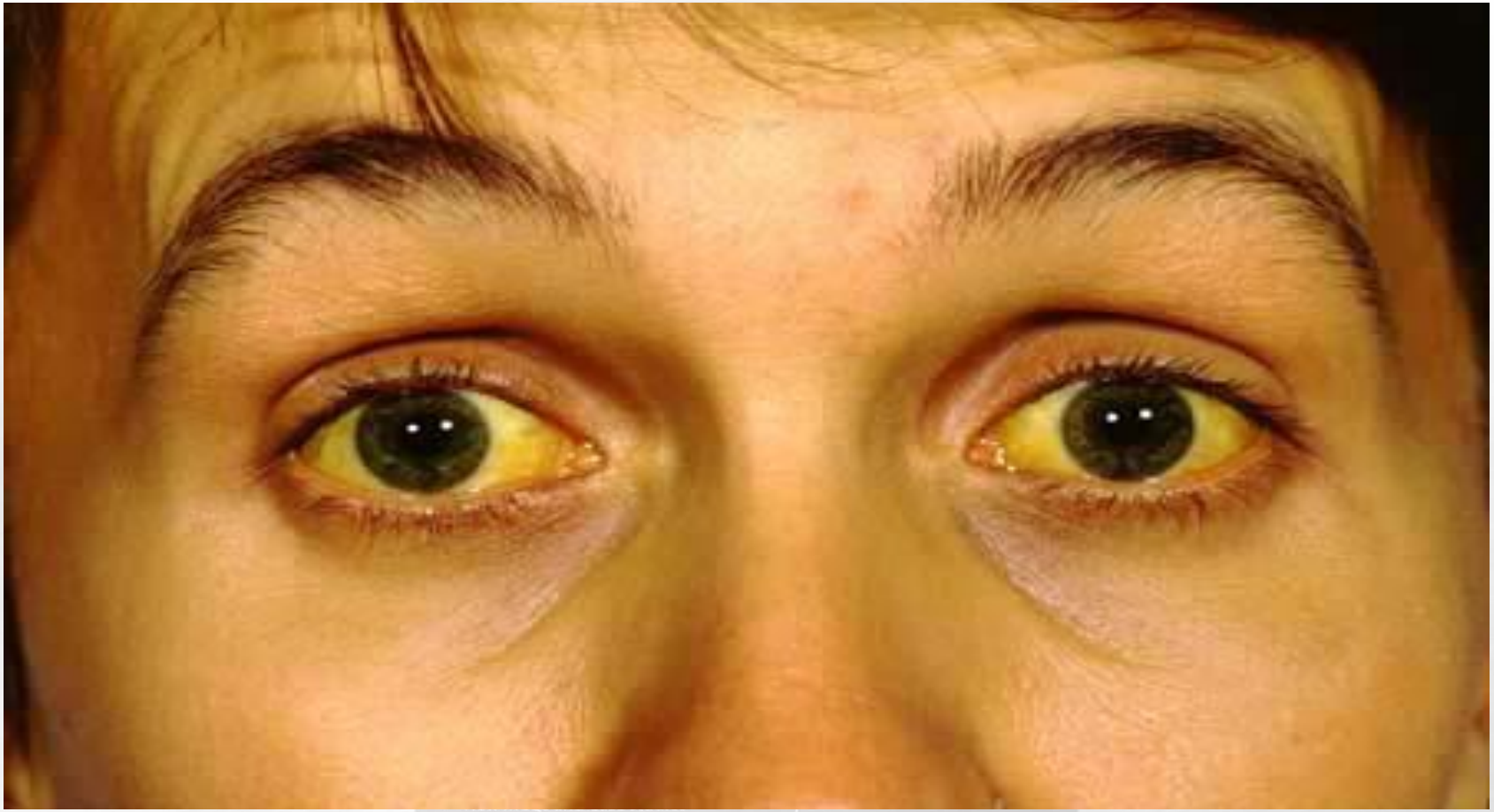
Rotor Syndrome

- It is a similar condition, but the exact defect is not identified.
- Bilirubin excretion is defective, but there is no staining of the liver.
- It is an autosomal recessive condition.



Acquired Hyperbilirubinemias

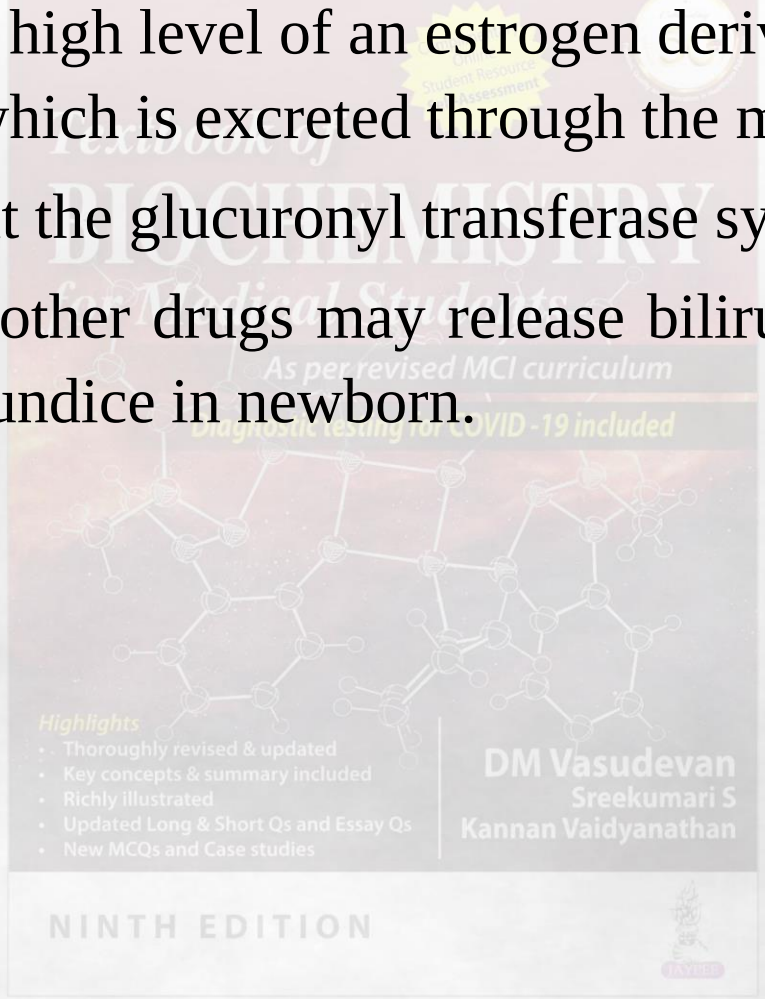
- **Physiological Jaundice**
- It is also called as neonatal hyperbilirubinemia.
- In all newborn infants after the 2nd day of life, mild jaundice appears.
- This transient hyperbilirubinemia is due to an accelerated rate of destruction of RBCs and also because of the immature hepatic system of conjugation of bilirubin.
- In such cases, bilirubin does not increase above 5 mg/dl.
- It disappears by the second week of life.



NINTH EDITION

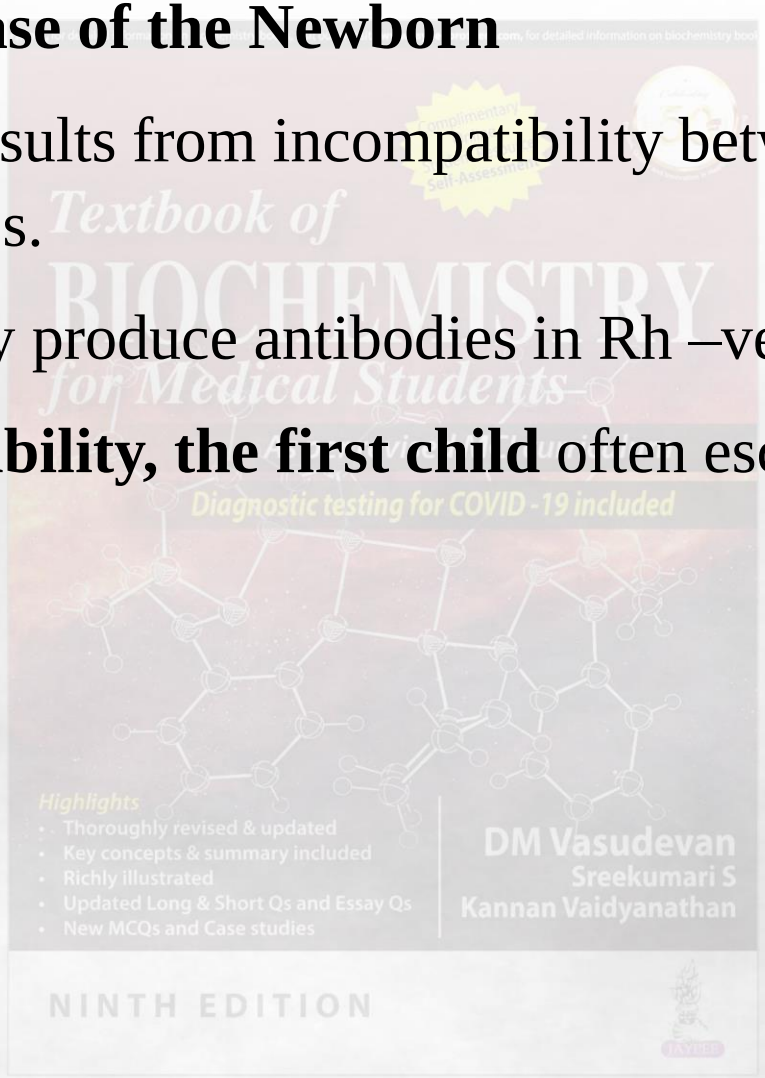
Breast Milk Jaundice

- In some breast-fed infants, prolongation of the jaundice has been attributed to high level of an estrogen derivative in maternal blood, which is excreted through the milk.
- This would inhibit the glucuronyl transferase system.
- Sulpha and such other drugs may release bilirubin from albumin, and may cause jaundice in newborn.

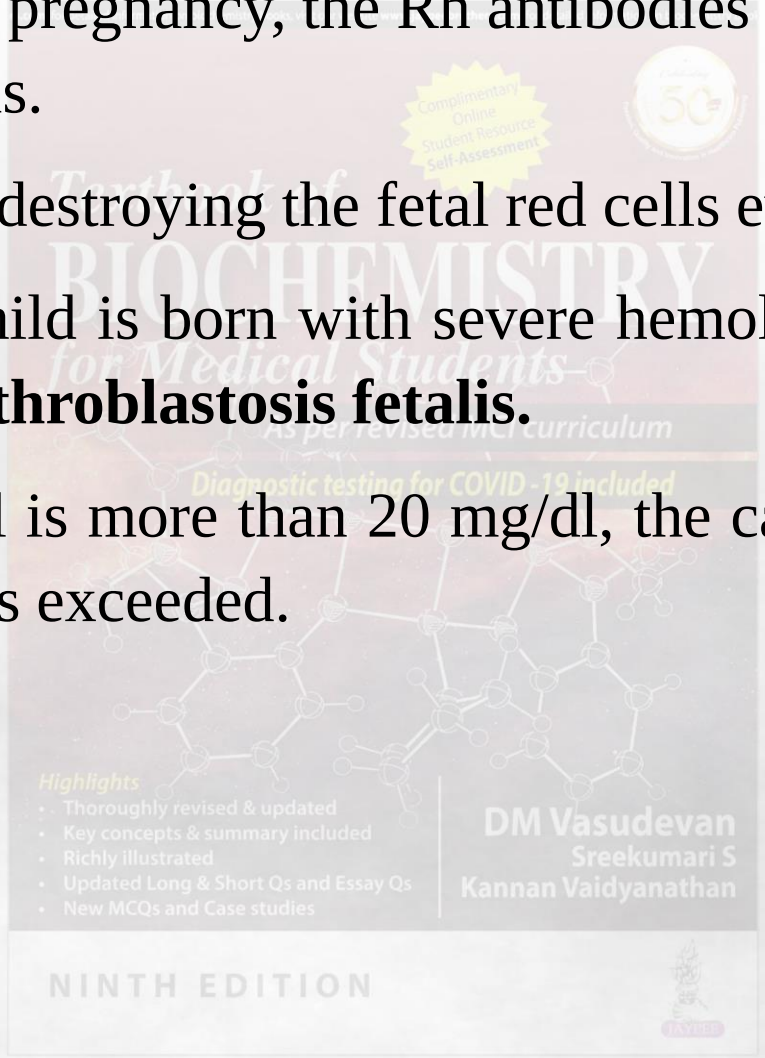


Hemolytic Jaundice

- **Hemolytic Disease of the Newborn**
- This condition results from incompatibility between maternal and fetal blood groups.
- Rh +ve fetus may produce antibodies in Rh –ve mother.
- In **Rh incompatibility, the first child** often escapes.



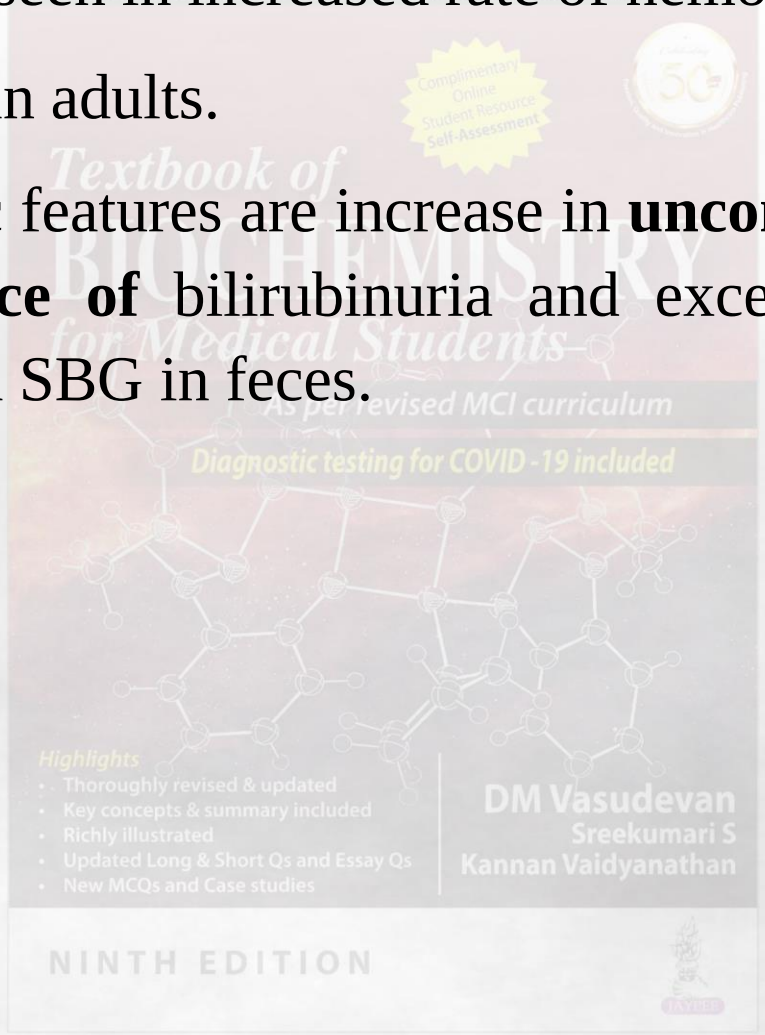
- But in the second pregnancy, the Rh antibodies will pass from mother to the fetus.
- They would start destroying the fetal red cells even before birth.
- Sometimes the child is born with severe hemolytic disease, often referred to as **erythroblastosis fetalis.**
- When blood level is more than 20 mg/dl, the capacity of albumin to bind bilirubin is exceeded.



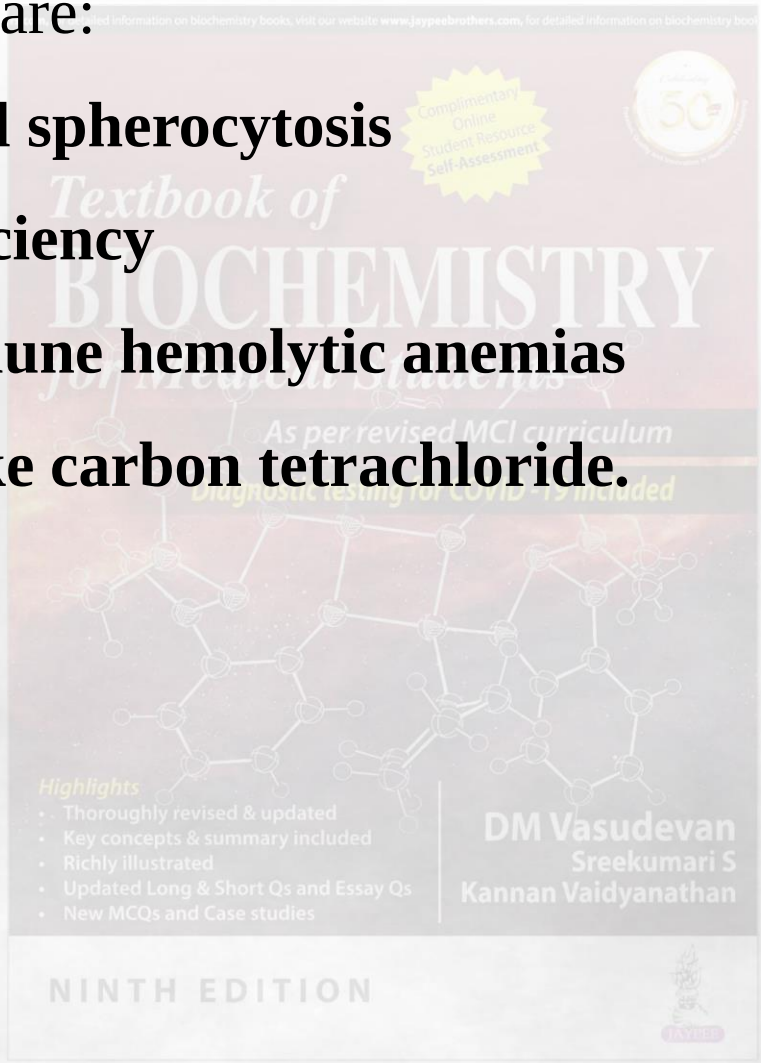
- In young children before the age of 1 year, the blood-brain barrier is not fully matured, and therefore free bilirubin enters in the brain **(Kernicterus).**
- ***It is deposited in*** brain, leading to mental retardation, fits, toxic encephalitis and spasticity.
- If the child develops hemolytic disease, child may be given exchange transfusion along with phototherapy and barbiturates.
- **Phototherapy** with blue light (440 nm wave length) isomerizes the insoluble bilirubin to more soluble isomers.
- These can be excreted through urine without conjugation.

Hemolytic Diseases of Adults

- This condition is seen in increased rate of hemolysis.
- It usually occurs in adults.
- The characteristic features are increase in **unconjugated bilirubin in blood, absence of** bilirubinuria and excessive excretion of UBG in urine and SBG in feces.

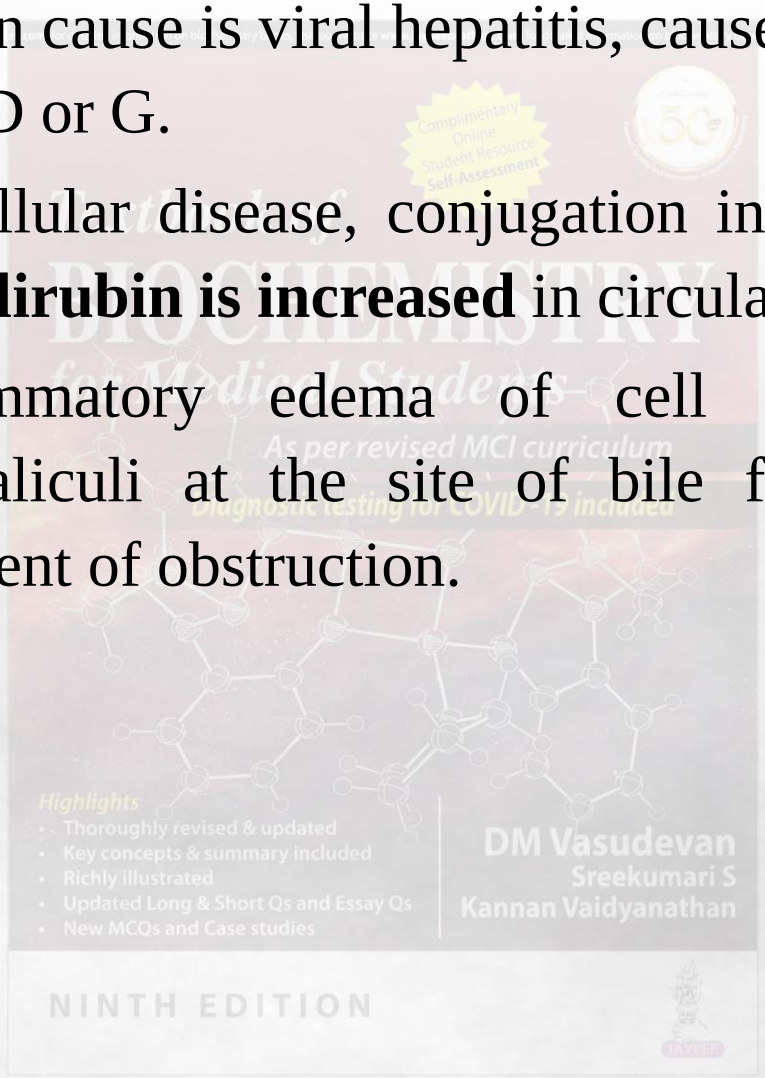


- Common causes are:
  - **i. Congenital spherocytosis**
  - **ii. GPD deficiency**
  - **iii. Autoimmune hemolytic anemias**
  - **iv. Toxins like carbon tetrachloride.**

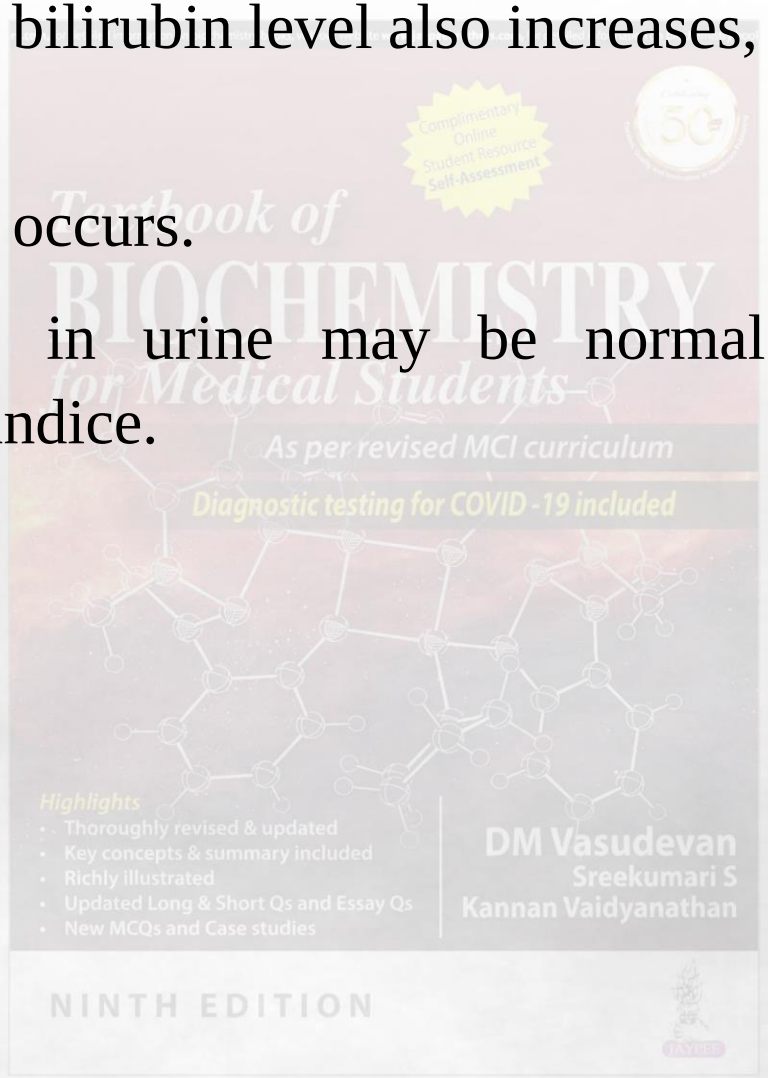


Hepatocellular Jaundice

- The most common cause is viral hepatitis, caused by Hepatitis Viruses A, B, C, D or G.
- In pure hepatocellular disease, conjugation in liver is decreased and hence **free bilirubin is increased** in circulation.
- However, inflammatory edema of cell often compresses intracellular canaliculi at the site of bile formation and this produces an element of obstruction.

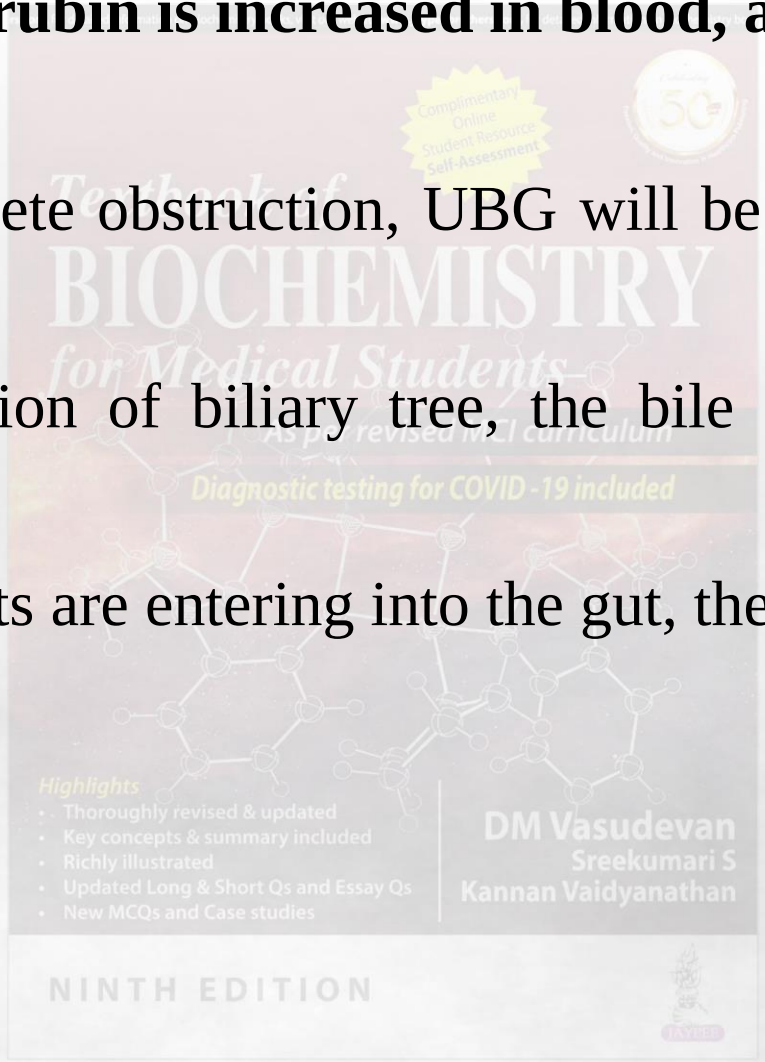


- When conjugated bilirubin level also increases, mixed type of jaundice results.
- Bilirubinuria also occurs.
- The UBG level in urine may be normal or decreased in hepatocellular jaundice.

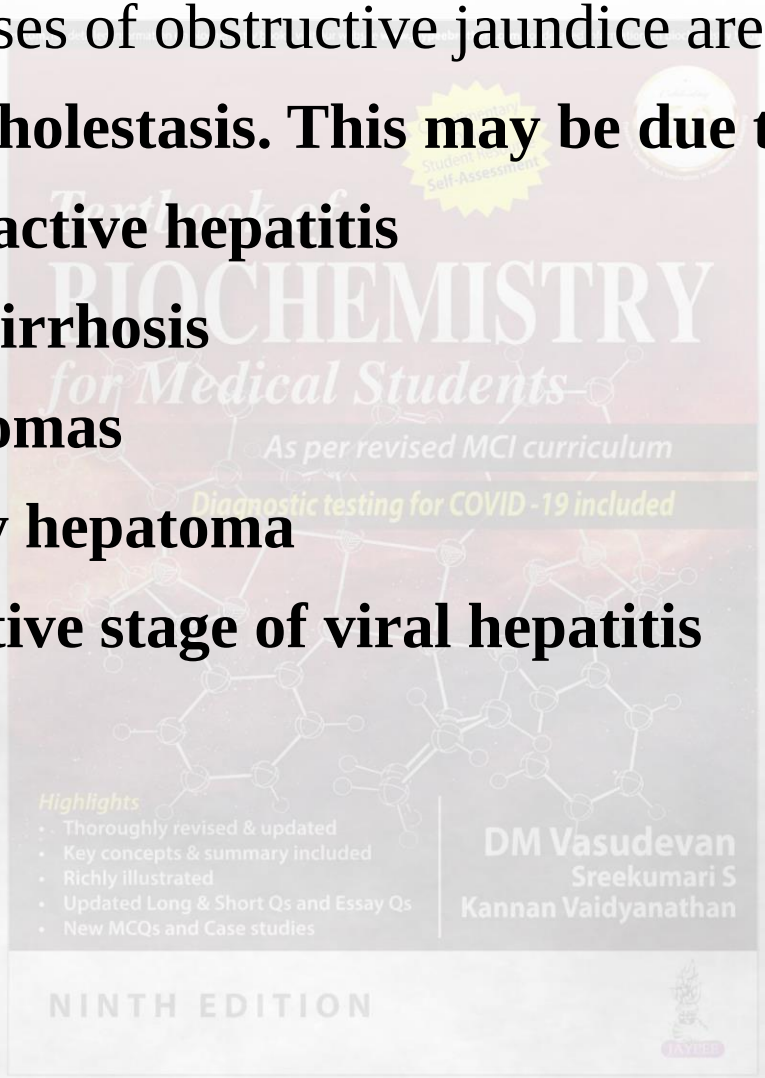


Obstructive Jaundice

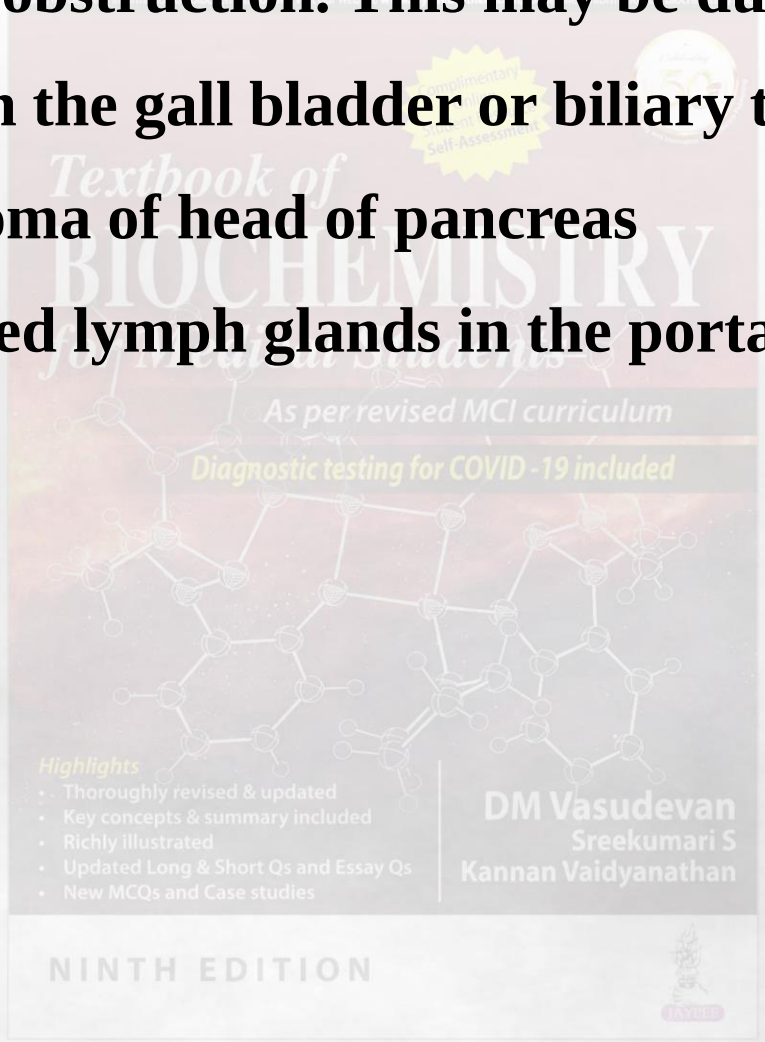
- **Conjugated bilirubin is increased in blood, and it** is excreted in urine.
- If there is complete obstruction, UBG will be decreased in urine or even absent.
- In total obstruction of biliary tree, the bile does not enter the intestine.
- Since no pigments are entering into the gut, the feces become **clay colored.**



- The common causes of obstructive jaundice are:
- **a. Intrahepatic cholestasis. This may be due to**
  - **a-i. Chronic active hepatitis**
  - **a-ii. Biliary cirrhosis**
  - **a-iii. Lymphomas**
  - **a-iv. Primary hepatoma**
  - **a-v. Obstructive stage of viral hepatitis**

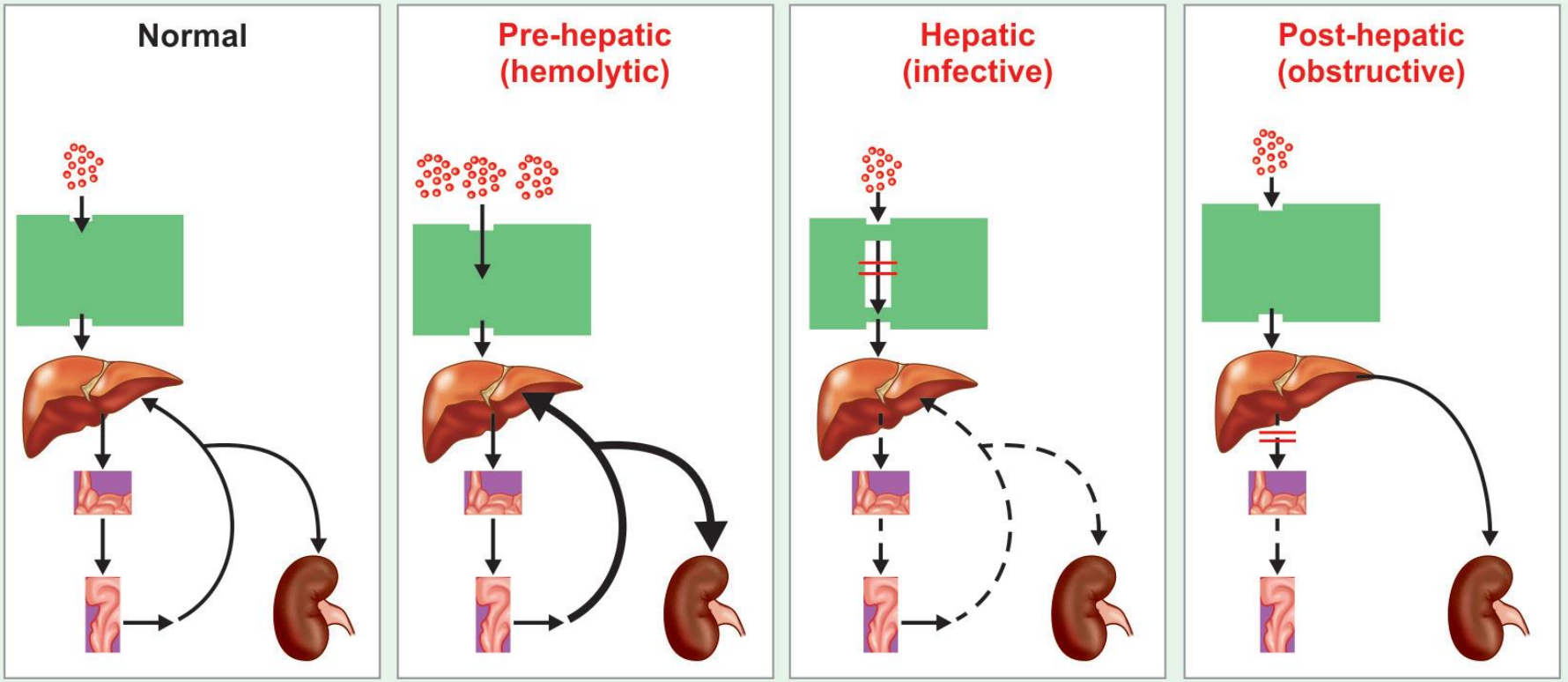


- **b. Extrahepatic obstruction. This may be due to**
  - **b-i. Stones in the gall bladder or biliary tract**
  - **b-ii. Carcinoma of head of pancreas**
  - **b-iii. Enlarged lymph glands in the porta hepatis.**



Differential Diagnosis of Jaundice

	Hemolytic jaundice	Hepatocellular jaundice	Obstructive jaundice
Blood, free bilirubin	Increased	Increased	Normal
Blood, conjugated bilirubin	Normal	Increased	Increased
Blood, ALP	Normal	Increased	Very high
Urine, bile salts	Nil	Nil	Present
Urine, conjugated bilirubin	Nil	Nil	Present
Urine, Bilinogens	Increased	Nil	Nil
Fecal urobilinogen	Increased	Decreased	Absent


Normal
Pre-hepatic (hemolytic)
Hepatic (infective)
Post-hepatic (obstructive)
↑ RBC lysis
↑ Bilirubin
↑ Bilirubin glucuronide
↑ EH circulation
↑ Urobilinogen
↓ Conjugation
↓ Enterohepatic circulation
↓ Urobilinogen
Obstruction
Regurgitation
↑ Urine
Bilirubin glucuronide
↑ Urine bile salts

Delta Bilirubin

Delta bilirubin is albumin-bound conjugated bilirubin. This reaction with albumin is nonenzymatic, but irreversible. It is found in the serum of most of jaundiced patients. Since albumin is not filtered through glomeruli, the delta bilirubin is not seen in the urine. Delta bilirubin is nontoxic and excreted neither in urine nor in bile. In hepatocellular jaundice, it may persist in the blood for a week or more after urine clears.

Bilirubin Toxicity

Bilirubin will uncouple oxidative phosphorylation, and inhibit ATPase activity in brain mitochondria. All of these toxic effects of bilirubin are reversed by binding to albumin. In fact, albumin transports bilirubin from its sites of production (bone marrow and spleen) to its site of excretion which is the liver.