

INTERMEDIARY METABOLISM

Porphyrin Metabolism

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31 – Mar – 2007 (Revised 15 – Oct – 2007)

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Keywords

Porphyrias; heme; bilirubin; conjugation; urobilinogen; jaundice; glucuronide; bile; bile pigments; hemolysis; biliverdin; reticuloendothelial

Porphyrins

Porphyrins are made up of four pyrrole rings, joined by methenyl ($-\text{CH}=\text{}$) bridges (Fig. 1). A characteristic property of the porphyrins is the formation of complexes with metal ions bound to the nitrogen atom of the pyrrole rings.

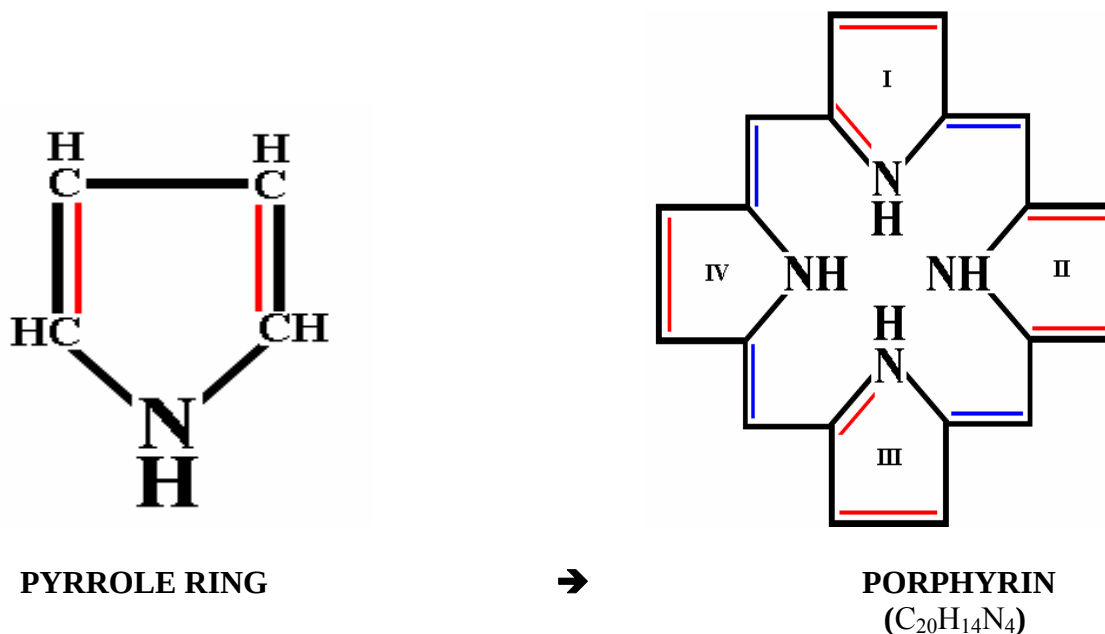


Fig. 1: Porphyrin molecule

The nitrogen atom of four pyrrole rings forms complex with metallic ions such as Fe^{2+} and Mg^{2+} . They form the prosthetic groups of conjugated proteins as shown in Table 1.

Fe^{2+} – porphyrins	Mg^{2+} – porphyrins
Hemoglobin of mammalian RBC	Chlorophyll - in plants
Myoglobin of muscle	
Erythrocruorins of some invertebrates	
Cytochromes - respiratory enzymes of electron transport chain (ETC).	
Oxidative enzyme like tryptophan pyrrolose	
Enzymes like catalase and peroxidase	

Table 1: Important haemoproteins

The porphyrins found in nature are compounds in which various side chains are substituted for the eight hydrogen atoms numbered in the porphyrin nucleus shown above. A porphyrin with a completely symmetric arrangement of the substituents (acetate- A and propionate- P) is classified as a type I porphyrin (Fig. 2) and one with asymmetric substitution is classified as type III (by far more abundant and includes heme and protoporphyrin IX). Uroporphyrins were first found in the

urine, but are not restricted to urine and coproporphyrins were first isolated from faeces, but they are also found in urine.

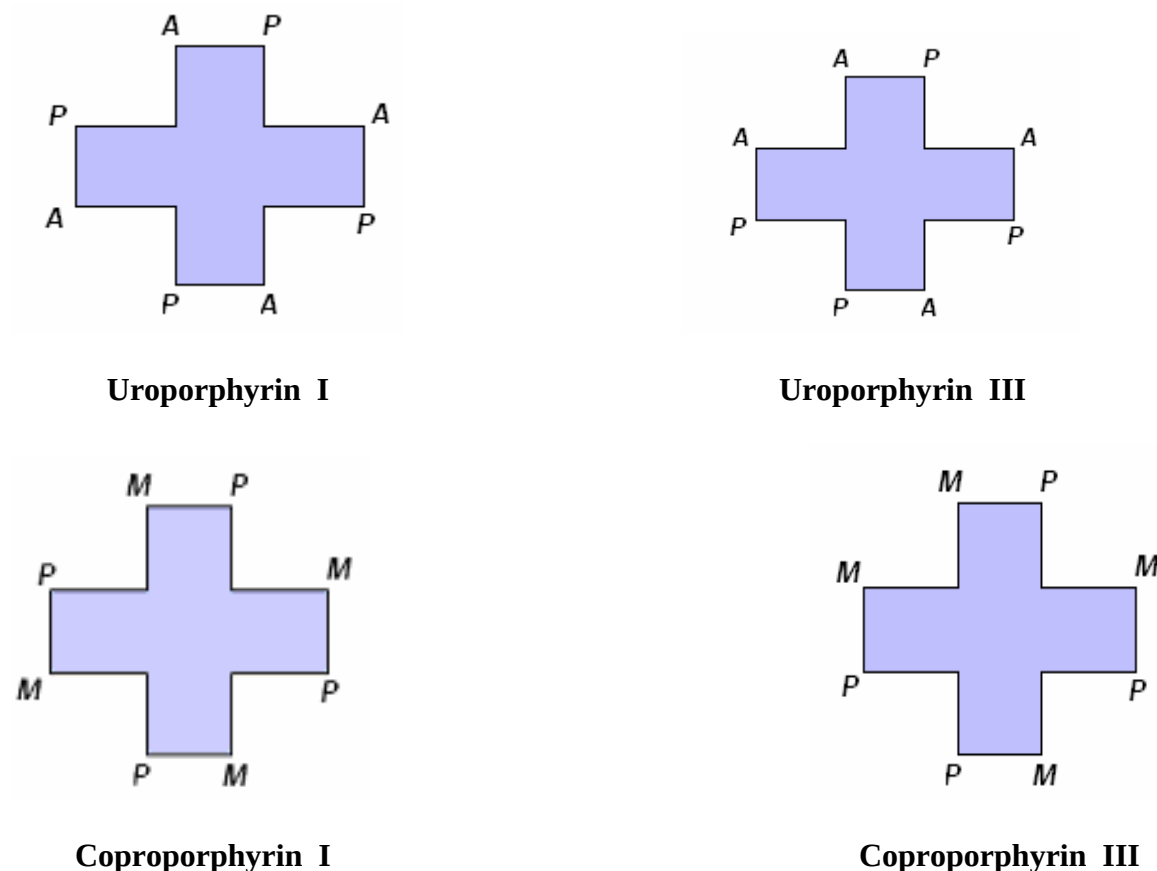


Fig. 2: Uroporphyrins and coproporphyrins
[A (acetate) ; P (propionate) ; M (methyl)
= -CH₃ ; V (vinyl) = -CH=CH₂]

Chemistry

Heme, a chemical compound that contains iron and gives blood its red color, is the key component of several important proteins in the body. The essential function of heme as a carrier of oxygen depends on its ability to bind oxygen. Heme is incorporated into hemoglobin, a protein that enables red blood cells to carry oxygen from the lungs to all parts of the body. Heme is also a component of cytochromes. Some cytochromes in the liver (cyt P450) process (metabolize) chemicals—including drugs and hormones—so that they are more easily removed from the body.

Heme is the nonprotein functional component of hemoproteins, which are found in all tissues. Heme is produced in the bone marrow and liver through a complex process regulated by eight different enzymes. As this production process progresses, several different intermediate compounds (heme precursors) are created and modified. If there is a deficiency in one of the enzymes that are essential for heme production, certain heme precursors may accumulate in tissues (especially in the bone marrow or liver), appear in excess amounts in the blood, and get

excreted in the urine or stool. The specific precursors that accumulate depend on which enzyme is deficient. One group of heme precursors is called the porphyrins.

Biosynthesis of Heme

Heme synthesis begins with Succinyl-CoA (derived from citric acid cycle in mitochondria) and the amino acid glycine. The heme biosynthetic pathway is illustrated below. The eight different enzymes that drive the sequential steps in this pathway are numbered 1 through 8 and are briefly described below (Fig. 3). Heme is partly synthesized in mitochondria and partly in cytosol of aerobic cells like developing erythrocytes and hepatic cells. The first enzyme and the last three are found in mitochondria; the intermediate enzymes occur in the cytosol.

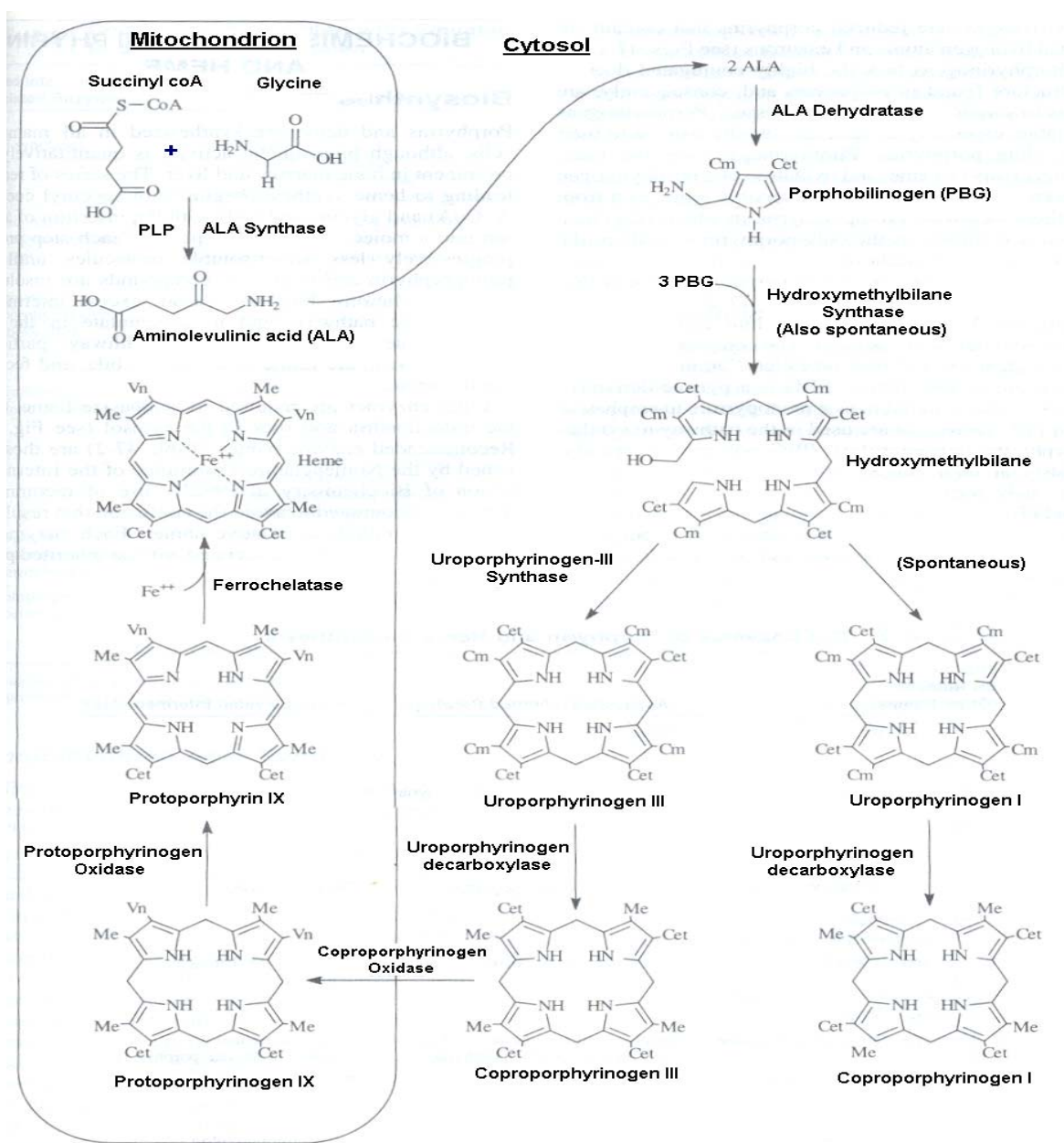


Fig. 3: Heme Biosynthetic Pathway

1. ALA synthase, the first enzyme of the heme biosynthetic pathway and the rate-controlling enzyme in porphyrin biosynthesis in mammalian liver, catalyzes the condensation of glycine and succinyl coenzyme A to form α -amino- β -ketoadipic acid, which is rapidly decarboxylated to δ -aminolevulinate (ALA). The enzyme is localized in the inner membrane of mitochondria and requires pyridoxal 5'-phosphate as a cofactor (to "activate" glycine). Separate genes encode erythroid and nonerythroid ALA synthases.
2. ALA dehydratase, a cytosolic enzyme, converts two molecules of ALA into a monopyrrole, porphobilinogen (PBG), with the removal of two molecules of water. Lead inhibits ALA dehydratase activity by displacing zinc (the metal essential for enzyme activity) from the enzyme. The most potent inhibitor of the enzyme is succinylacetone, a structural analog of ALA, which is found in urine and blood of patients with hereditary tyrosinemia.
3. Porphobilinogen (PBG) deaminase (also called uroporphyrinogen I synthase) catalyzes the condensation of four molecules of PBG (head to tail) to yield a linear tetrapyrrole, hydroxymethylbilane (HMB). There are two isozymes of PBG deaminase; one is present exclusively in erythroid cells, whereas the other is in nonerythroid cells. The two isoforms of PBG deaminase are encoded by distinct messenger RNAs (mRNAs) that are transcribed from a single gene by alternate transcription and splicing.
4. Uroporphyrinogen III cosynthase catalyzes the formation of uroporphyrinogen III from HMB. This involves an intramolecular rearrangement that reverses the orientation of ring D, followed by closure of the macrocycle to form uroporphyrinogen III. When this enzyme is deficient, HMB can undergo spontaneous closure of the macrocycle without reversal of ring D, leading to formation of uroporphyrinogen I. Under normal conditions, the uroporphyrinogen formed is almost exclusively the III isomer, but in some porphyrias type I isomer may be formed in excess. Both uroporphyrinogen I and III have pyrrole rings connected by methylene bridges, which do not form a conjugated ring structure. Thus, these compounds are colourless (as are all porphyrinogens), but are readily auto-oxidized to their respective coloured porphyrins catalyzed by light and by the porphyrins that are formed.
5. Uroporphyrinogen decarboxylase, a cytosolic enzyme, catalyzes four sequential decarboxylations of the carboxymethyl side chains (acetate groups change to methyl substituents) in uroporphyrinogen III (an octacarboxyl porphyrin) to yield heptacarboxyl porphyrin, hexacarboxyl porphyrin, pentacarboxyl porphyrin, and, finally, coproporphyrinogen III (a tetracarboxyl porphyrin). This enzyme can also metabolize uroporphyrinogen I to coproporphyrinogen I. Coproporphyrinogen III enters mitochondria for next reaction.
6. Coproporphyrinogen oxidase, a mitochondrial enzyme in mammalian cells, catalyzes the removal of the carboxyl group and two hydrogens from the prop ionic groups of pyrrole rings A and B of coproporphyrinogen III to form vinyl groups at these positions, forming protoporphyrinogen III. This enzyme is unable to metabolize coproporphyrinogen I.

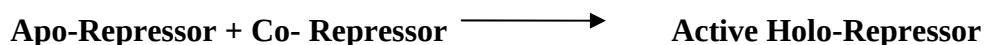
7. Protoporphyrinogen oxidase mediates the oxidation of protoporphyrinogen III (IX) to protoporphyrin IX, catalyzing the removal of six hydrogen atoms from the porphyrinogen nucleus. In mammalian liver the conversion of coproporphyrinogen to protoporphyrin requires molecular oxygen.
8. Ferrochelatase catalyzes the insertion of iron into protoporphyrin, which represents the final step in the heme biosynthetic pathway. The enzyme is not specific for iron and can catalyze the insertion of some other metals such as zinc.

The intermediates of the pathway are conserved within cells and therefore are normally excreted only in small amounts. They differ markedly from each other in molecular size, solubility, and other properties. ALA, PBG, and porphyrinogens (hexahydroporphyrins, i.e., porphyrins in the chemically reduced state) are colorless and non-fluorescent. Protoporphyrin, the final intermediate in the pathway, is the only intermediate that is an oxidized porphyrin. In the study of porphyrins and their derivatives the absorption spectrum of each in both the visible and the ultraviolet regions of the spectrum are of great value. An example is the absorption curve of a porphyrin solution in 5% hydrochloric acid. A sharp absorption band near 400nm is distinguishing feature of porphyrin ring and is characteristic of all porphyrins regardless of the side chains present. This band is called Soret band, after its discoverer. Porphyrins in the oxidized state are reddish and fluoresce (also when dissolved in strong mineral acids or in strong organic solvents) when exposed to long-wave ultraviolet light. This property is used to detect small amounts of free porphyrins. The double bonds joining the pyrrole rings in the porphyrins (absent in porphyrinogens) are responsible for the characteristic absorption and fluorescence of these compounds. This property is used in cancer phototherapy. Tumours often take up more porphyrins than do normal tissues. Thus, heptoporphyrin or other related compounds are administered to a cancer patient with an appropriate tumour. The tumour is then exposed to an argon laser, which excites the porphyrins, producing cytotoxic effects.

Porphyrinogens that leak into extracellular fluid undergo auto-oxidation and are excreted primarily as porphyrins. However, appreciable amounts of unoxidized coproporphyrinogen may be excreted in urine. ALA, PBG, uroporphyrin, hepta-, hexa-, and pentacarboxyl porphyrins are water-soluble and are excreted mostly in urine. Coproporphyrin (a tetracarboxyl porphyrin) is excreted in urine and bile. Harderoporphylin (a tricarboxyl porphyrin) and protoporphyrin (a dicarboxyl porphyrin) are poorly soluble in water and thus cannot be excreted by the kidneys. If they accumulate in bone marrow or liver they appear in plasma, are taken up by the liver, and are excreted in bile and feces.

Regulation of Heme Synthesis

1. ALA synthase (regulatory enzymes), the first enzymes of the heme biosynthetic pathways, is regulated by repression mechanism. Heme the end product of the synthetic pathway inhibits the activity of ALA synthase.



Active Holo-Repressor binds to operator and blocks the transcription of structural genes (Fig. 4).

ALA synthase is localized in the inner membrane of mitochondria and requires PLP as a cofactor. Separate genes encode erythroid and nonerythroid ALA synthase, thus it exists in two isoforms.

Note: Erythroid ALA synthase is not repressed by heme.

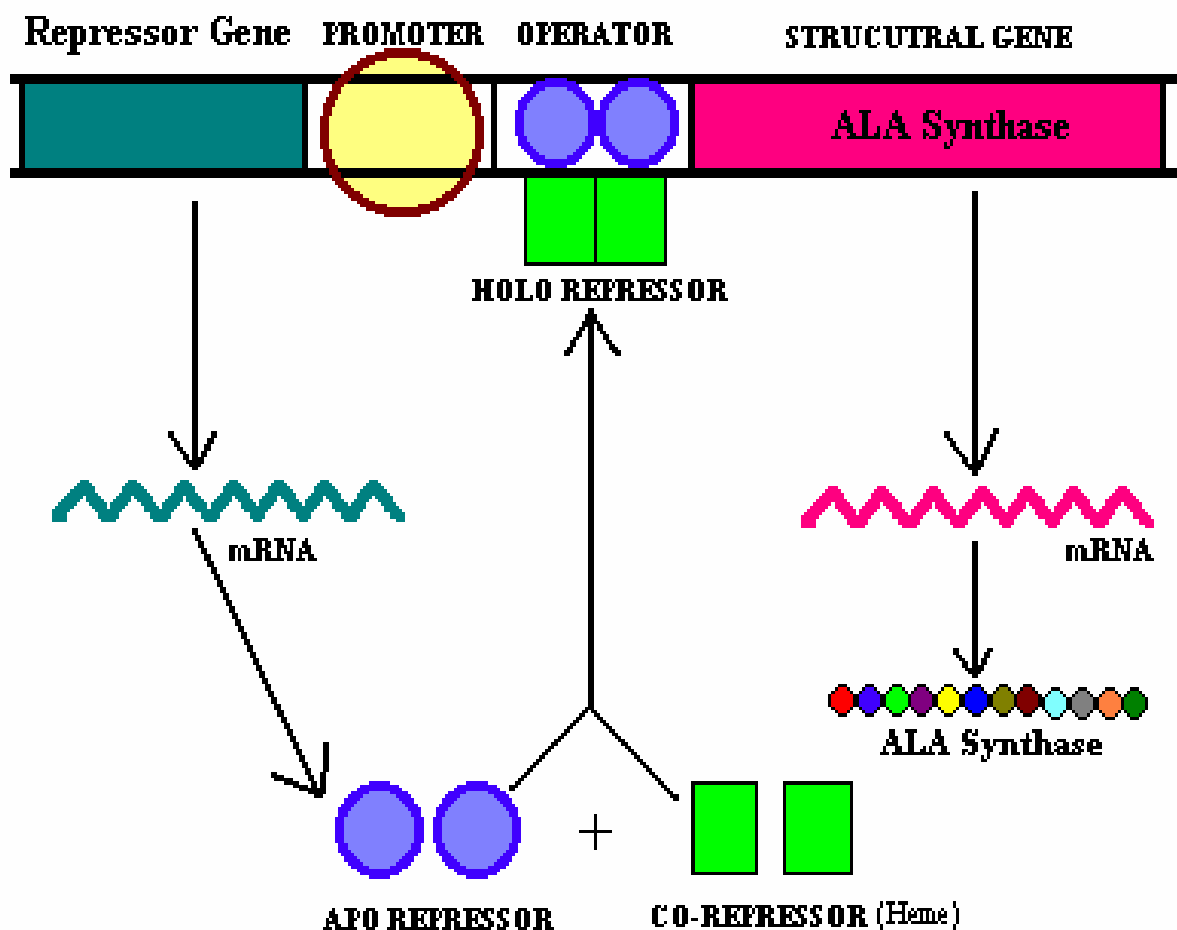


Fig. 4: Regulation of Heme Synthesis

- High cellular concentration of glucose prevents induction of ALA synthase. This is due to an increased level of catabolite repressor protein (CRP) within the cell. This is the basis of administration of glucose to relieve the acute attack of porphyrias.

Certain insecticides, carcinogens and drugs like griseofulvin, barbiturates induce ALA by inducing cytochrome P450 which uses up heme and thus derepresses (induces) ALA synthase. Thus taking these drugs can precipitate attacks of porphyria. Steroids play a permissive role in drug mediated derepression of ALA synthase *in vivo*.

- Feed back inhibition: ALA synthase is allosterically inhibited by hematin (administered as a drug), when there is excess of free heme (Fe^{2+} is oxidized to Fe^{3+} forming hematin). Compartmentalization of the enzyme in heme synthesis makes it easier for regulation:

Step 1, 5, 6 and 7 in Mitochondria
Step 2, 3, 4 in Cytoplasm

4. *In vivo* heme synthesis is stimulated by low O₂ tension (e.g. living at high altitudes, hypoxic states), whereas *in vitro* conversion of PBG to uroporphyrinogen and protoporphyrin to heme are both inhibited by O₂. But oxygen is required for decarboxylation of coproporphyrinogen and oxidation of protoporphyrinogen.
5. ALA synthase is also controlled by other factors like
 - Rate of protein (globin) synthesis
 - Step catalyzed by ALA dehydratase or ferrochelatase is inhibited by lead.
 - Isonicotinic acid hydrazide (INH) decreases the availability of PLP may also affect heme synthesis.

Porphyrias

Porphyrias are a group of inherited (inborn errors of metabolism) or acquired disorders of heme biosynthesis pathway (Greek: porphyria means purple). The main causes are enzyme deficiencies due to mutations in genes directing synthesis of enzymes involved in heme biosynthesis, that lead to accumulation of heme pathway intermediates. In general, porphyrias are inherited in an autosomal dominant manner, with the exception of congenital erythropoietic porphyria, which is inherited in a recessive manner. Porphyrias are characterized by increased production and excretion of porphyrins or their precursors. Only one gene is present to produce a key functional enzyme in heme synthesis. This results in at least a 50% of the normal enzyme activity. This reduced level of enzyme results in build up of precursors above the deficient enzyme that then accumulate in body fluids and tissues (Flow Chart 1). When the blood levels of coproporphyrins and uroporphyrins are increased above the normal level and excreted in urine or faeces, the condition is called porphyrias.

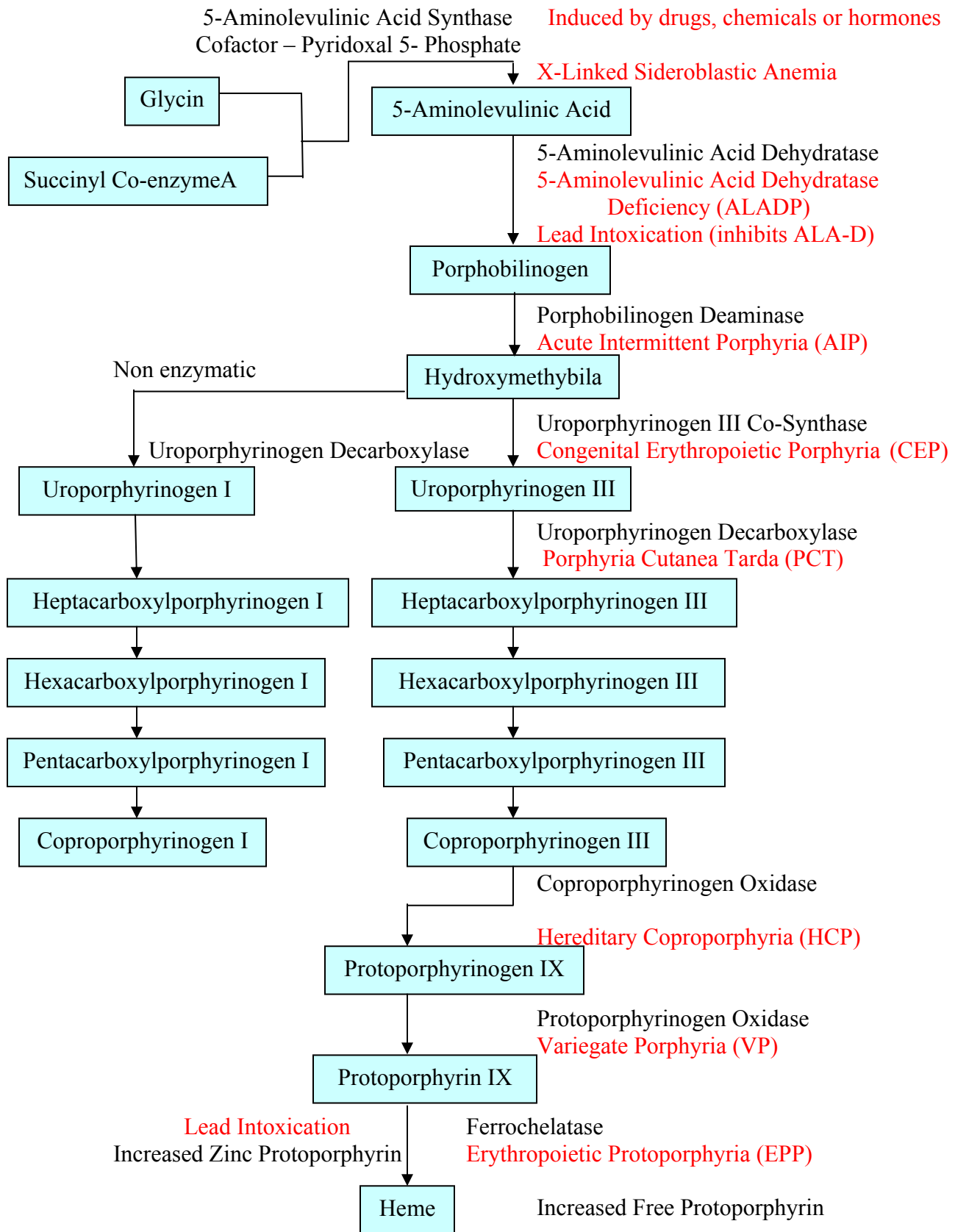
Classification of Porphyrias

Porphyrias can be classified in several ways. Classification according to the specific enzyme deficiency is considered most accurate. One classification distinguishes porphyrias that cause neurologic symptoms (acute porphyrias- precipitated by certain factors) from those that cause photosensitivity (cutaneous porphyrias):

- Acute porphyrias- Acute intermittent porphyria, variegate porphyria, hereditary coproporphyria, ALA dehydratase deficiency
- Cutaneous porphyrias- Porphyria cutanea tarda, hepatoerythropoietic porphyria, erythropoietic protoporphyria, congenital erythropoietic porphyria.

A third classification system is based on whether the excess precursors originate primarily in the liver (hepatic porphyrias) or primarily in the bone marrow (erythropoietic porphyrias): Some porphyrias are classified into more than one of these categories (Fig. 5).

- Erythropoietic porphyria is a defect of porphyrin metabolism of blood-producing tissues.
- Hepatic porphyria is a defect in porphyrin metabolism in the liver.



Flow Chart 1: Metabolic Defects in Heme Biosynthesis

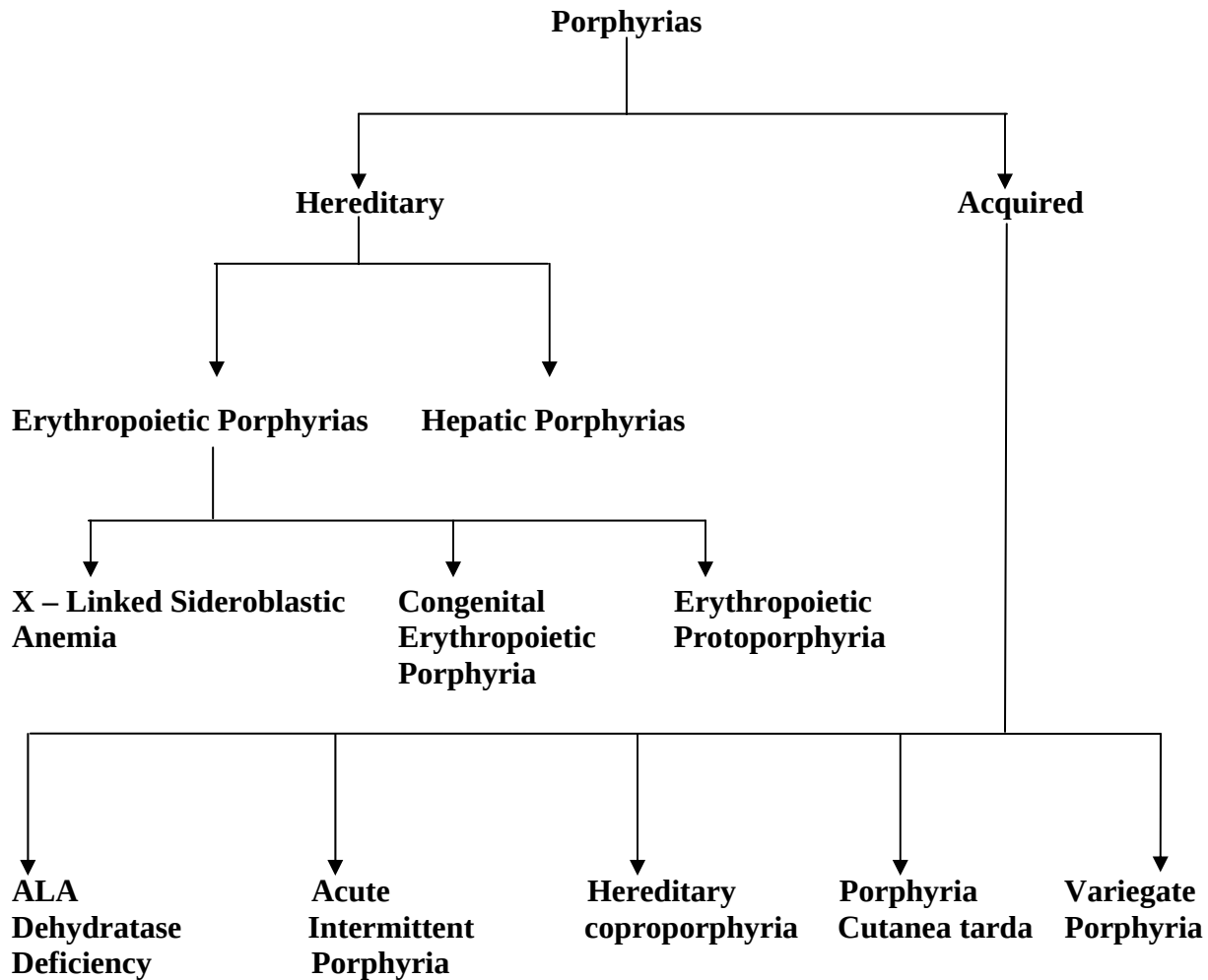


Fig. 5: Classification of Porphyrias

Either type may be hereditary (caused by a gene defect) or acquired (e.g. poisoning).

Hereditary Porphyria

Erythropoietic Porphyrias

A. Erythropoietic Protoporphyria (EPP)

Erythropoietic protoporphyria is a condition characterized by photosensitivity. It is the third most common porphyria. It occurs most often in whites but can also occur in people of any origin. Erythropoietic protoporphyria occurs equally in men and women. It is inherited as an autosomal dominant trait with reduced penetrance. Only about 10% of individuals with an enzyme deficiency develop clinical symptoms. Onset usually occurs before 10 years of age, clinical presentation may occur during childhood or adulthood.

- **Causes:** In erythropoietic protoporphyria, a deficiency of the enzyme ferrochelatase leads to accumulation of protoporphyrin in plasma, erythrocytes, skin, and liver. The enzyme deficiency is usually inherited from one parent.
- **Accumulation Product:** Accumulation of protoporphyrin IX in the skin results in extreme sensitivity to sunlight. The sunlight activates the protoporphyrin molecules, which damage the surrounding tissue. Accumulation of protoporphyrins in the liver can cause liver damage. Protoporphyrins in the bile can lead to bile stones.
- **Symptoms and Diagnosis:** Painful erythema, burning and oedema develop soon after the skin is exposed to sunlight. Symptoms are worst in spring and summer and occur at few minutes to 1 hour of exposure to sunlight in light-exposed areas with burning and tingling rather than itching. Because blistering and scarring seldom occur, doctors do not always recognize the disorder. Chronic exposure may result in a waxy and thickened skin with faint linear scars. Gallstones formation is common and cause characteristic abdominal pain. Liver damage may lead to increasing liver failure (in up to 5% cases), with jaundice and enlargement of the spleen. Some individuals show mild hypertriglyceridemia.
- Protoporphyrin is not soluble in urine hence porphyrin levels in urine are not increased. The diagnosis is therefore made when increased levels of protoporphyrin are detected in the plasma and red blood cells by measuring total porphyrins and protoporphyrin fractionation. Fecal protoporphyrin is positive (Table 2).
- **Prevention and Treatment:** Extreme care should be taken to avoid exposure to sunlight. Accidental sun exposure is given the same treatment, as is sunburn. Beta-carotene (in a dose of 50-200mg daily) when taken in sufficient amounts to cause slight yellowing of the skin, makes many people more tolerant of sunlight; however, sunlight should still be avoided. People who develop gallstones that contain protoporphyrin may need to have them surgically removed. Liver damage, if severe, may necessitate liver transplantation. Cholestyramine has been shown to reduce photosensitivity and to decrease hepatic protoporphyrin content.

B. *X – Linked Sideroblastic Anemia*

It is a type of erythropoietic porphyria and is inherited as an autosomal recessive trait.

- **Cause:** It is due to ALA dehydratase deficiency.
- **Symptoms:** Anemia
- **Diagnosis:** Red cell count and hemoglobin decreased.

Sideroblastic anemia is a group of iron-loading disorders of unknown cause. In a hereditary type of this disorder, there is deficiency of δ -aminolevulinic acid synthetase in RBC precursors. Iron accumulates in mitochondria because of this metabolic bottleneck.

C. *Congenital Erythropoietic Porphyria*

It is a type of erythropoietic porphyria and is inherited as an autosomal recessive trait. It is also known as Gunther disease. This is a very rare and severe type of porphyria. Although the

disorder manifests typically in infancy, variability in age of onset and severity are related to the level of residual enzyme activity. Prenatal manifestation of this condition presents as nonimmune hydrops foetalis due to severe hemolytic anemia, whereas only cutaneous lesions are observed in the mildest cases in adults.

Type of Porphyria	Deficient Enzyme	Enzyme Assay	Biochemical Findings – Increased Levels Seen in Affected Patients			
			Urine	Feces	Erythrocytes	Plasma
Acute Intermittent Porphyria (AIP)	Porphobilinogen deaminase	Yes	ALA, PBG, Uroporphyrin, Coproporphyrin*			Increased porphyrins during acute episodes
ALA Dehydratase Deficiency (ALAD)	ALA Dehydrogenase	Yes	ALA			
Congenital Erythropoietic Porphyria (CEP)	Uroporphyrinogen cosynthase	Yes	Uroporphyrin, Coproporphyrin	Uroporphyrin I, Coproporphyrin I	Uroporphyrin, Coproporphyrin, Zinc protoporphyrin	Uroporphyrin, Coproporphyrin I
Erythropoietic Protoporphyrin (EPP)	Ferrochelatase	No		Protoporphyrin	Free protoporphyrin	Protoporphyrin
Hepatoerythrocytic Porphyria (HEP)	Uroporphyrinogen decarboxylase	Yes	Uroporphyrin, Heptacarboxyl porphyrin	Isocoporphyrin, Heptacarboxylporphyrin III	Zinc protoporphyrin	Uroporphyrin
Hereditary Coproporphyrin (HCP)	Coproporphyrinogen oxidase	No	Coproporphyrin, ALA, PBG	Coproporphyrin, Coproporphyrin III/ Coproporphyrin I ratio		Coproporphyrin
Porphyria Cutanea Tarda (PCT)	Uroporphyrinogen decarboxylase	Yes	Uroporphyrin, Heptacarboxyl porphyrin	Isocoporphyrin, Heptacarboxylporphyrin III		Uroporphyrin
Variegate Porphyrin (VP)	Protoporphyrinogen oxidase	No	Coproporphyrin, PBG, ALA	Protoporphyrin > Coproporphyrin Coproporphyrin III/ Coproporphyrin I ratio		

Table 2: Summary of Porphyrins

- **Cause:** It is due to deficiency of uroporphyrinogen III cosynthase activity.
- **Accumulation Product:** Uroporphyrin I is accumulated in skin and gets excreted in urine and coproporphyrin and uroporphyrin I in stool.
- **Symptoms and Diagnosis:** Photosensitivity is in some cases almost immediate and so severe that the infant may scream when put in sunlight. Erythema, swelling and blistering occur on exposed sites. Ulceration may follow and healing is slow with scar formation. Repeated episodes of blistering results in mutilation of the ears, nose and fingers. Hypertrichosis, seen in areas of mild involvement, takes the form of lanugo hair over the limbs and coarser hair on the face, but the most severely affected areas show a scarring alopecia often involving the scalp. Irregular hyperpigmentation follows in areas of scarring $\pm$ hypopigmentation.

Extracutaneous manifestations include brown teeth that fluoresce reddish-pink under Wood's light, hemolytic anemia, pathologic fractures, vertebral compression and splenomegaly. Many patients develop porphyrin rich gallstones. Photophobia is commonly present and patients may develop ectropion (related to facial scarring) or keratoconjunctivitis, up to loss of vision. Severely affected individuals exhibit growth and cognitive developmental delays and a decreased lifespan.

Pink or reddish brown urine is often observed as a result of the increased excretion of urinary porphyrins. In urine uroporphyrin and coproporphyrin I are positive and porphobilinogen is negative. The diagnosis is confirmed by erythrocyte uroporphyrinogen III cosynthase enzyme analysis.

- **Prevention and Treatment:** Rigid protection from ultraviolet light and sunlight is essential; topical sunscreens are relatively ineffective. β -Carotene can improve light tolerance, but it is not as effective as in EPP. Splenectomy may be performed for intractable hemolytic anemia. Hypertransfusion with packed erythrocytes may be helpful as it suppresses erythropoiesis and depresses the production of porphyrins. Iron overload may be avoided by the concurrent administration of desferrioxamine. Secondary infections must be treated immediately.

Hepatic Porphyrrias

A. Acute Intermittent Porphyria (AIP)

It is the second most common porphyria. Acute intermittent porphyria occurs more commonly in Northern Europe. People first experience acute intermittent porphyria with acute onset of neurological symptoms. Attacks are more common in women than in men. These acute episodes are potentially life threatening, highly variable and although short in duration, may last from a few days to several months.

This is inherited in an autosomal dominant manner with reduced penetrance. The disorder is inherited due to a single abnormal gene from one parent. The normal gene from the other parent keeps the deficient enzyme at half-normal levels, which is sufficient to produce normal amounts of heme. Only 10-20% of individuals with the enzyme deficiency will become symptomatic during their lifetime. Very rarely, the disease is inherited from both parents (and therefore two abnormal genes are present); symptoms may then appear in childhood and include developmental abnormalities.

- **Causes:** Acute intermittent porphyria is due to a deficiency of the enzyme porphobilinogen deaminase (also known as hydroxymethylbilane synthase or uroporphyrinogen I synthase). Most people with a deficiency of porphobilinogen deaminase never develop symptoms. In some people it may be precipitated by certain factors, as mentioned below:
Drugs: barbiturates, anticonvulsants and sulfonamide antibiotics.
Hormones: progesterone and related steroids can precipitate symptoms.
Stress: that results from an infection, another illness, surgery, or a psychological upset is also sometimes implicated.
 Usually a combination of factors is involved. Sometimes the factors that cause an attack cannot be identified.
- **Accumulation Product:** Due to deficiency of above enzyme, the heme precursors δ -ALA and porphobilinogen accumulate initially in the liver.
- **Symptoms and Diagnosis:** Symptoms occur as attacks lasting several days or weeks, and sometimes even longer. Such attacks usually first appear after puberty. In some women, attacks develop during the second half of the menstrual cycle.

Abdominal pain is the most common symptom. The pain can be so severe that the doctor may mistakenly think that abdominal surgery is needed. Gastrointestinal symptoms include nausea, vomiting, constipation or diarrhea, and abdominal bloating. The bladder may be affected, making urination difficult and sometimes resulting in an overfull bladder. A rapid heart rate, high blood pressure, sweating, and restlessness are also common during attacks; interference with sleep is typical. High blood pressure can continue after the attack.

All of these symptoms, including the gastrointestinal ones, result from effects on the nervous system. Nerves that control muscles can be damaged, leading to weakness, usually beginning in the shoulders and arms. The weakness can progress to virtually all the muscles, including those involved in breathing. Tremors and seizures may develop. Recovery from symptoms may occur within a few days, although complete recovery from severe muscle weakness may take several months or years. Attacks are rarely fatal; however, in a few people, attacks are disabling. Patients frequently display psychiatric symptoms presenting in the form of psychotic episodes, depression and anxiety.

The severe gastrointestinal and neurological symptoms resemble those of many more common disorders. Laboratory tests performed on samples of urine show increased levels of two heme precursors (δ -ALA acid and porphobilinogen). Levels of these precursors are very high during attacks and remain high in people who have repeated attacks. The precursors can form porphyrins, which are reddish in color, and other substances that are brownish. These turn the urine dark, especially after exposure to light.

Relatives without symptoms can be identified as carriers of the disorder by measuring porphobilinogen deaminase in red blood cells or sometimes by DNA testing. Diagnosis before birth is also possible but usually is not needed because most affected people never get symptoms.

- **Prevention and Treatment:** Attacks of acute intermittent porphyria can be prevented by maintaining good nutrition and avoiding the drugs that can provoke them. Crash diets to lose weight rapidly should be avoided. Heme can be given to prevent attacks. Premenstrual attacks in women can be prevented with one of the gonadotropin-releasing hormone agonists

used to treat endometriosis (see Endometriosis), although this treatment is still investigational.

People who have attacks of acute intermittent porphyria are often hospitalized for treatment of severe symptoms. People with severe attacks are treated with heme given intravenously. Blood and urine levels of δ -aminolevulinic acid and porphobilinogen are promptly lowered and symptoms improve, usually within several days. If treatment is delayed, recovery takes longer, and some nerve damage may be permanent.

Glucose given intravenously or a diet high in carbohydrates can also be beneficial, particularly in people whose attacks are brought on by a low-calorie or low-carbohydrate diet, but these measures are less effective than heme. Pain can be controlled with drugs (such as opioids) until the person responds to heme or glucose. Nausea, vomiting, anxiety, and restlessness are treated with a phenothiazine for a short time. Insomnia may be treated with chloral hydrate or low doses of a benzodiazepine but not a barbiturate. An overfull bladder may be treated by draining the urine with a catheter.

The doctor ensures that the person does not take any of the drugs known to precipitate an attack, and if possible addresses other factors that may have contributed to the attack. Treatment of seizures is problematic, because almost any anticonvulsant would worsen an attack. Beta-blockers may be used to treat rapid heart rate and high blood pressure but are not used in people who are dehydrated, in whom a rapid heart rate is needed to maintain the blood circulation.

B. Porphyria Cutanea Tarda (PCT)

Porphyria cutanea tarda is the most common porphyria and causes blistering of skin exposed to sunlight. Porphyria cutanea tarda occurs throughout the world (more common in males) and is amenable to treatment. As far as is known, this porphyria is the only one that can occur in someone without an inherited deficiency of an enzyme involved in heme production (sporadic, type I) besides the inherited forms (familial, types II and III). About 75% of patients have type I variety. Familial type is inherited as an autosomal dominant condition.

Several common factors (precipitating factors) are associated with porphyria cutanea tarda; these include excess iron in body tissues, moderate or heavy alcohol use, taking estrogens, liver diseases, infection with hepatitis C virus, smoking and occupational exposure to polychlorinated cyclic hydrocarbons. Historically outbreaks have been caused by toxic exposure to certain organic chemicals. Infection with the human immunodeficiency virus (HIV) is a less common precipitating factor. These factors are thought to interact with iron and oxygen in the liver and thereby inhibit or damage the enzyme uroporphyrinogen decarboxylase.

- **Causes:** Porphyria cutanea tarda results from underactivity of the enzyme uroporphyrinogen decarboxylase, which leads to accumulation of porphyrins in the liver. Skin damage occurs because porphyrins produced in the liver are transported by the blood plasma to the skin.
- **Symptoms and Diagnosis:** PCT is characterized by photosensitivity and skin fragility. People with porphyria cutanea tarda experience chronic, recurring blisters of various sizes on sun-exposed areas such as the arms, face, and especially the backs of the hands. Crusting and

scarring follow the blisters and take a long time to heal. The skin, especially on the hands, is also sensitive to minor injury. Hair growth on the face and other sun-exposed area may increase. Liver damage usually occurs; cirrhosis and even liver cancer (hepatocellular carcinoma) may eventually develop.

For diagnosis, tests are performed on the blood, plasma, urine, and stool for increased levels of porphyrins. Uroporphyrin is elevated by 4-250 times normal value and hepatocaryxylporphyrin elevated by 25%. Elevated uroporphyrin without a concomitant elevation of hepatocaryxylporphyrin is often observed in acute porphyrias, rather than PCT. The specific porphyrins that are increased provide a pattern that distinguishes porphyria cutanea tarda from other porphyrias.

- **Prevention and Treatment:** Porphyria cutanea tarda is the most readily treated porphyria. Avoiding alcohol and other precipitating factors is beneficial. A procedure called phlebotomy, in which a pint of blood is removed, is the most widely recommended treatment. With phlebotomy, the excess iron is gradually removed, the activity of uroporphyrinogen decarboxylase in the liver returns toward normal, and porphyrin levels in the liver and blood plasma fall gradually. The skin symptoms improve, and the skin eventually returns to normal. Phlebotomy is discontinued when the person becomes slightly iron deficient. Anemia develops if phlebotomy is performed too frequently.

Very low doses of chloroquine or hydroxychloroquine are also effective in treating porphyria cutanea tarda. These drugs remove excess porphyrins from the liver. However, doses those are too high cause porphyrins to be removed too rapidly, resulting in a temporary worsening of the disorder and damage to the liver.

For women taking estrogen, doctors discontinue the estrogen therapy (because it is a precipitating factor of the porphyria) until phlebotomy has been completed and porphyrin levels are normal. The estrogen is then restarted and seldom causes a recurrence of the porphyria.

C. Variegate Porphyria (Mixed Or Combined)

Variegate porphyria (VP) is an autosomal dominant acute porphyria and is a potentially life-threatening disease affecting mainly inhabitants of South Africans and Finland. The disease has a low penetrance, usually manifests clinically or biochemically after puberty. This means most children of porphyrics require repeated and expensive testing of stool, urine and blood-from their early teens until the disease becomes apparent, or until they reach the age of about 23 years.

- **Cause:** This is due to deficiency of protoporphyrinogen oxidase and rarely ferrochelatase.
- **Symptoms and Diagnosis:** Neurologic symptoms are similar to seen in acute intermittent porphyria and cutaneous manifestations are similar to porphyria cutanea tarda. Photosensitivity, propensity to develop acute neuropsychiatric attacks with abdominal pain, vomiting, constipation, tachycardia, hypertension and quadriplegia are the different symptoms usually seen.

- Depending upon whether the patient is experiencing an acute crisis or is symptomatic, urine coproporphyrin, ALA and PBG may be elevated to varying degrees (up to 20 times normal values). During crises, faecal porphyrin analysis shows coproporphyrin levels about double the normal levels and ratio of coproporphyrin III to coproporphyrin I in 3-10 range (normal ratio <1.2). Also faecal protoporphyrin IX is elevated to a greater extent than coproporphyrin. In asymptomatic patients, faecal porphyrins typically remain elevated.
- **Prevention and Treatment:** The treatment of a patient with an acute attack of VP is identical to that described for AIP. The skin lesions are managed in a similar way to those of PCT. Besides sun avoidance and sunscreens, canthaxanthine, a β -carotene analogue, is reported to be some benefit although it may cause retinopathy. Glucose administration and hematin infusion may also be used.

D. Hereditary Coproporphyrria (HCP)

This is inherited as an autosomal dominant condition and is one of the least common of the acute porphyrias. Clinical penetrance is low and symptoms very rarely present before puberty.

- **Cause:** A reduction of coproporphyrinogen oxidase activity.
- **Symptoms and Diagnosis:** Similar to “acute intermittent porphyria,” except for cutaneous photosensitivity that occurs in one third of cases. During acute attacks, it is impossible to differentiate clinically and by laboratory tests HCP and IAP. Harderoporphyria, a very rare variant of HCP, is inherited in a homozygous manner and has a very low residual enzyme activity. Features include neonatal hemolytic anemia and cutaneous involvement. The biochemical hallmark of HCP is heperexcretion of coproporphyrin in the urine and faeces at levels similar to that observed in VP. However, in HCP the faecal coproporphyrin III to coproporphyrin I ratio is in the 10-20 range (normal ratio <1.2). Urinary porphobilinogen, urinary uroporphyrin, and fecal protoporphyrin are positive.
- **Prevention and Treatment:** Similar to acute intermittent porphyria.

E. ALA Dehydratase Deficiency (ALAD)

ALAD is a rare autosomal recessive variant of the acute porphyria (hepatic type). The age of onset and degree of severity are highly variable among the few reported cases.

- **Cause:** Results from homozygous or compound heterozygous deficiency of 5-ALA dehydratase.
- **Symptoms and Diagnosis:** Clinical manifestations are predominantly neuropathic and include abdominal pain, vomiting, pain in the extremities and neuropsychiatric symptoms. Zn-protoporphyrins accumulates in tissues. ALA, coproporphyrin III is excreted in urine.
- ALAD can be differentiated from other acute porphyrias by normal urinary PBG in the presence of significantly elevated ALA and markedly decreased ALA dehydratase activity.

- **Prevention and Treatment:** The current management approach is similar to that for other acute porphyrias, as emphasis is placed on the prevention of acute attacks.

Heme Catabolism

Bilirubin, a bile pigment produced principally by heme catabolism, is a waste product which requires elimination. Under physiologic conditions in an adult, $1-2 \times 10^8$ red blood cells are destroyed every hour. In a 70kg human about 6gm of haemoglobin is turned over per day producing globin which is degraded to its constituent amino acids (which are reused) and the iron of heme enters the iron pool (reused or stored as ferritin). The iron free porphyrin is degraded in microsomes of reticuloendothelial cells of liver, spleen and bone marrow (most important site) by a complex enzyme system called heme oxygenase to bile pigments, biliverdin and bilirubin (Fig. 6). Partial or complete failure at any point in this sequence can result in clinical condition Jaundice. By the time heme portion reaches this system the iron is usually oxidized to the ferric form constituting hemin. Approximately 85% of bilirubin is derived from senescent erythrocytes by conversion of 'heme' of hemoglobin to biliverdin within R.E. cells. Approximately 250-350 mg of bilirubin is formed daily. One gram of Hb yields approx. 35 mg bilirubin. The chemical conversion of heme to bilirubin by reticuloendothelial cells can be observed *in vivo* as the purple colour of heme in a haematoma slowly gets converted to yellow pigment of bilirubin.

Bile pigments

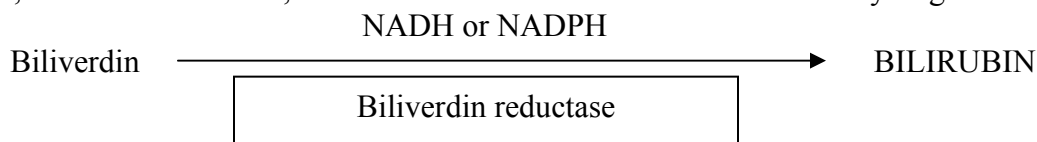
Principal bile pigments are "biliverdin" and "bilirubin." The colour of the bile is due primarily to these and their derivatives. Normally, there are only slight traces of biliverdin in human bile, bilirubin is the principal bile pigment. Biliverdin is the chief pigment of the bile in birds.

Formation of Biliverdin from "Heme"

The heme oxygenase is substrate inducible and is located in close proximity to microsomal electron transport chain (ETC). The hemin is reduced with NADPH and with help of more NADPH, oxygen is added to the α -methenyl bridge, between pyrrole rings I and II of the porphyrin. The ferrous iron is again oxidized to ferric form. With further addition of oxygen, ferric ion is released, producing carbon dioxide and equimolar amount of biliverdin IX- α with the splitting of the tetrapyrrole ring. Whether the globin chain is separated first or after ring opening is still not clear. Rate of elimination of CO in expired air has been used clinically as an index of rate of heme catabolism. In birds and amphibian, the green biliverdin is excreted; in mammals it is further metabolized.

Formation of Bilirubin from Biliverdin

Biliverdin is converted to bilirubin in RE cells. Conversion occurs in presence of a specific enzyme, biliverdin reductase, which utilizes either NADH or NADPH as hydrogen donor.



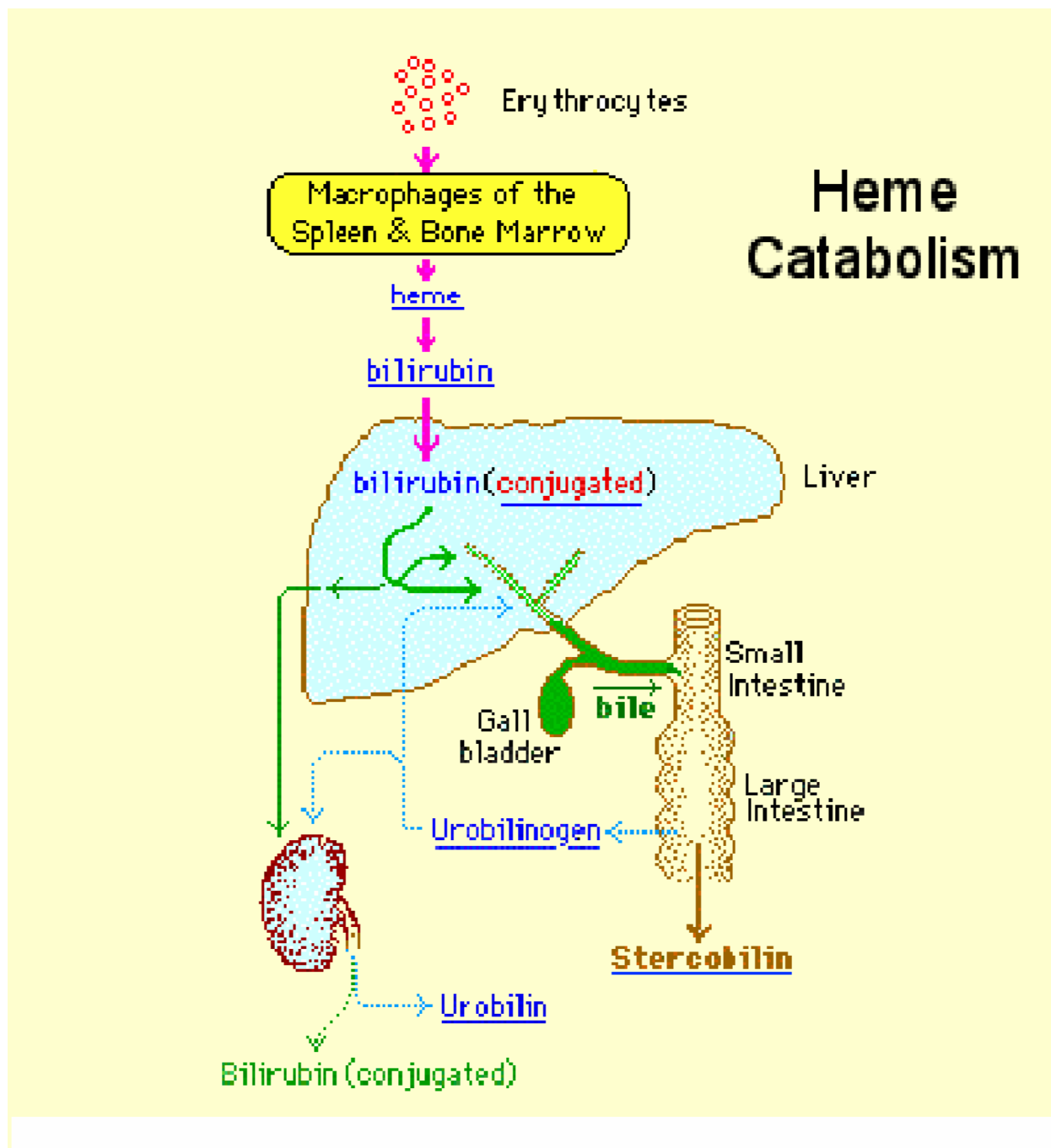


Fig. 6: Heme Catabolism

Biliverdin reductase reduces the methenyl bridge between pyrrole III and pyrrole IV to a methylene group to produce bilirubin IX-a, a yellow pigment (Fig. 7). Fifteen percent of newly synthesized bilirubin is derived from sources other than circulating erythrocytes. These sources are as follows:

- Heme formed from Hb-synthesis

- Destruction of immature erythrocytes in the bone marrow
- Degradation of Hb, within erythrocyte precursors
- Breakdown of other heme pigments such as cytochromes, myoglobin and catalase

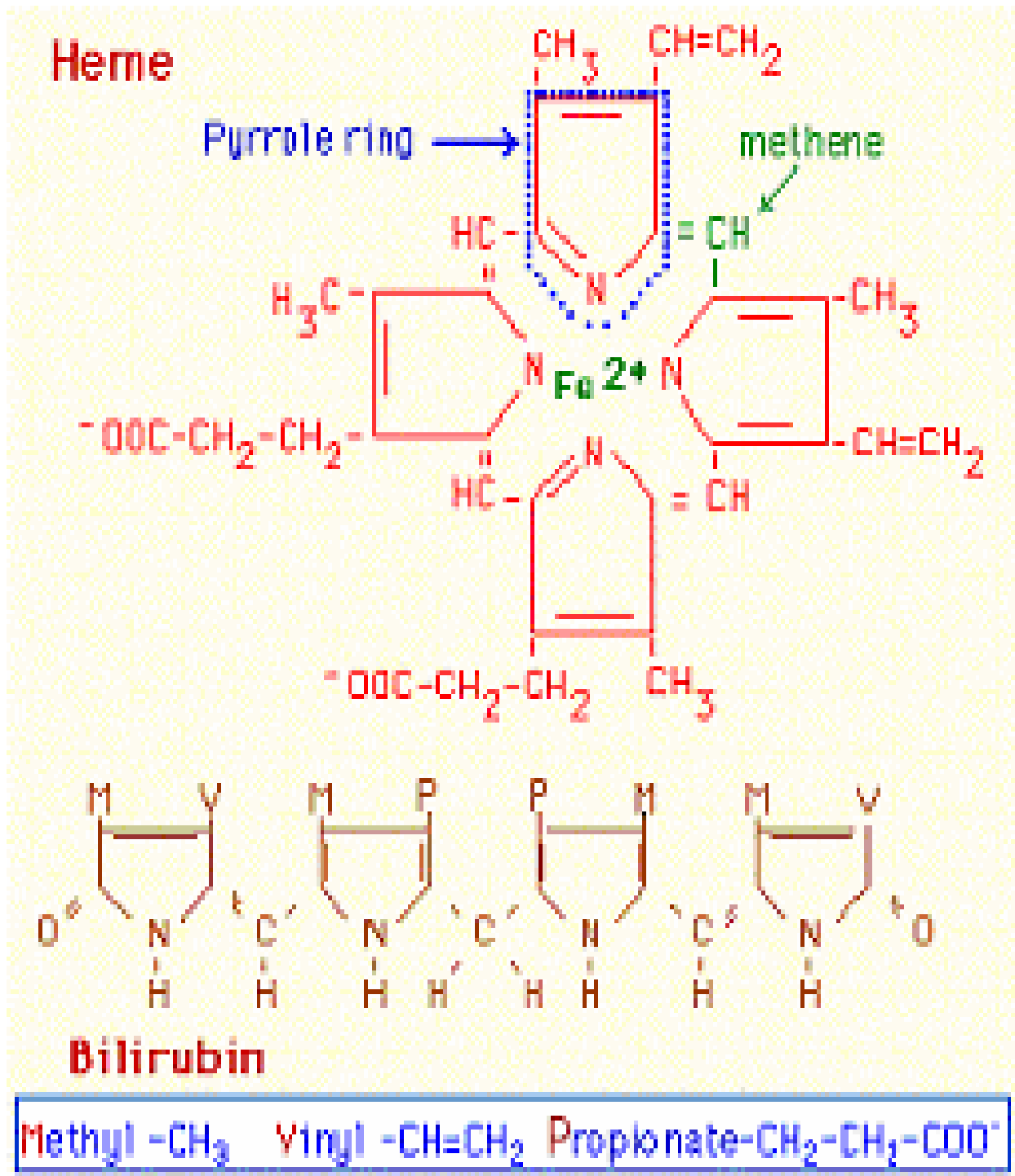


Fig. 7: Structural Comparison of Heme with Bilirubin

Excessive production of bilirubin from heme or erythrocyte precursors in bone marrow, or direct synthesis in marrow, gives rise to increased bilirubin level in blood, producing jaundice. Such a condition is called as “shunt hyperbilirubinemia.”

Transport of bilirubin

The bilirubin formed in RE cells from breakdown of Hb is called “unconjugated bilirubin,” which is highly lipid soluble, has limited aqueous solubility from 0.1 to 5 mg/100 ml at physiologic pH and toxicity. Bilirubin formed in peripheral tissues is transported to the liver by plasma albumin. Binding of bilirubin by albumin increases its solubility in plasma. Each molecule of albumin has one “high-affinity” site and one “low-affinity” site for bilirubin. Normally in 100 ml of plasma, approx. 25 mg of bilirubin can be tightly bound to albumin to its high affinity site. Bilirubin in excess of this quantity can be bound only loosely and can thus be easily detached and can diffuse into the tissues. A number of compounds such as antibiotics and other drugs compete with bilirubin for the high affinity binding site on albumin. Thus, these compounds can displace bilirubin from albumin and have significant clinical effects. Following are some examples:

- Several “anionic drugs” such as sulphonamides. Administration of sulphonamides to pregnant women and neonates increases the risk of kernicterus* in the jaundiced infants.
- Increased free fatty acids behave similarly
- Asphyxia, hypoxia and acidosis are also associated with increased risks:
 - by interfering with bilirubin-albumin binding, and
 - may increase the permeability of brain for unconjugated bilirubin.

*In “kernicterus,” a condition seen rarely outside the newborn period, unconjugated bilirubin enters the neurons of the basal ganglia, hippocampus, cerebellum, and medulla, causing necrosis of nerve cells, probably by interfering with cellular respiration.

Transfer of bilirubin in to liver cells

Liver appears to have a selective affinity to remove unconjugated bilirubin. In the liver, the bilirubin is removed from albumin and taken up at the sinusoidal surface of the hepatocytes by a carrier (ligandins) mediated saturable system with very high capacity so that even under pathologic conditions the system does not appear to be rate-limiting in metabolism of bilirubin. The net uptake of bilirubin is dependent upon the removal of bilirubin by subsequent metabolic pathways.

Conjugation of bilirubin

Bilirubin being water insoluble will persist in cells (e.g. bound to lipids) if not made water-soluble. Mammalian liver cells contains an enzyme known as glucuronyl transferase (two isoforms) which converts bilirubin to a polar form by adding glucuronic acid molecules to it, by a process called conjugation. Other polar groups can also be added to bilirubin like sulfates, besides glucuronic acid (Figs. 8 and 9.). Glucuronyl transferase is located in the smooth endoplasmic reticulum. It uses UDP-glucuronic acid (“active-glucuronide”) as the glucuronosyl donor forming bilirubin monoglucuronide (intermediate in which propionic acid carboxyl group of bilirubin is linked with glucuronide with ester linkage). Two bilirubin monoglucuronide

molecules form one molecule of bilirubin diglucuronide and one molecule of free 'bilirubin' by the action of the enzyme dismutase. Bilirubin diglucuronide is the major form excreted in mammalian bile. Conjugated bilirubins, unlike unconjugated bilirubins are water-soluble and are smaller in molecular size as they are not bound to albumin. Hence, conjugated bilirubin can pass through glomerular filter and can appear in urine (bilirubinuria). Unconjugated bilirubin cannot pass through glomerular filter and does not appear in urine.

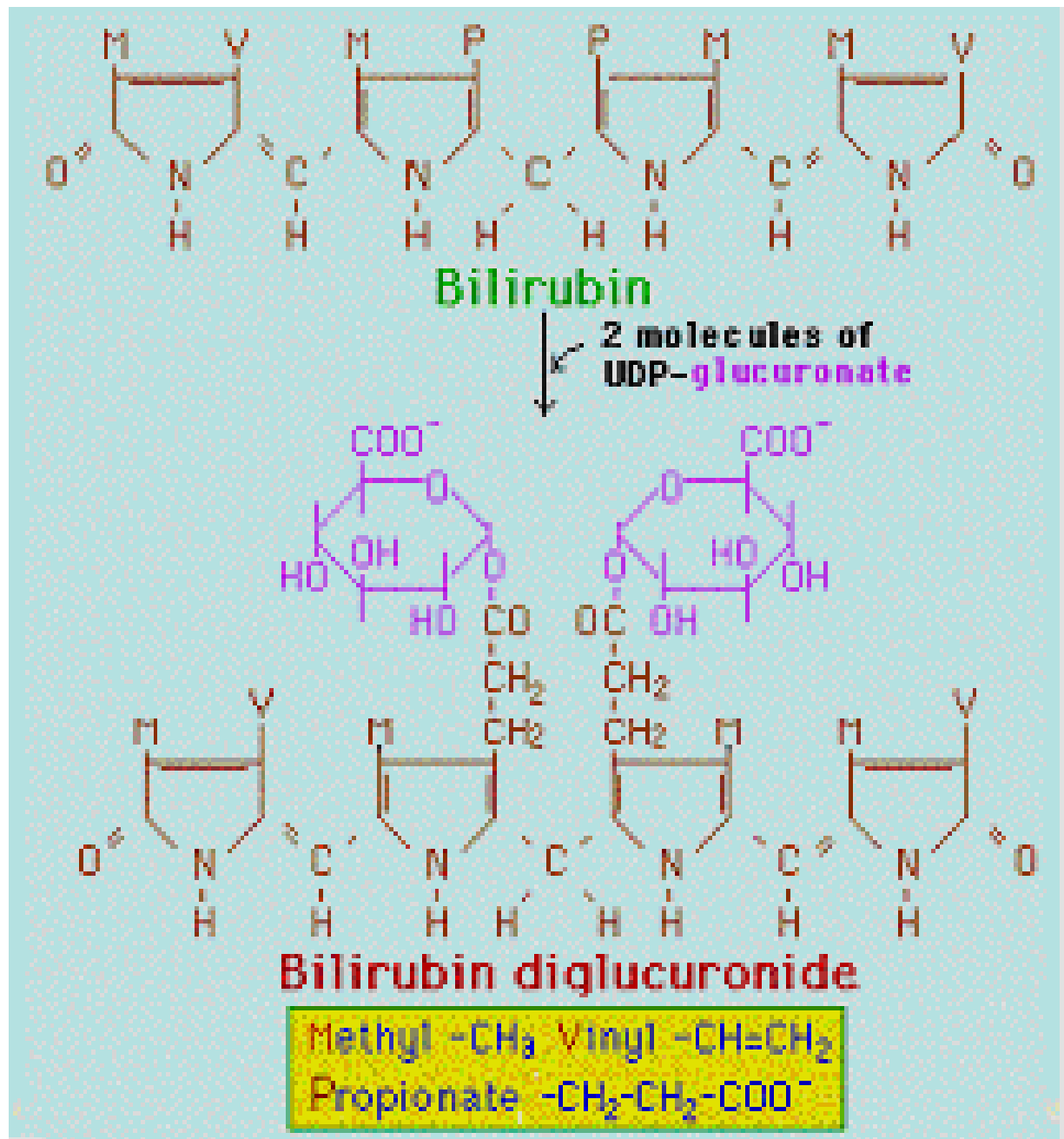


Figure 8. Conjugation of Bilirubin with Glucuronide

When conjugated bilirubin levels remain high for sustained periods, a fraction of it binds covalently to albumin (delta bilirubin). This fraction has a longer half-life (since bound to albumin). This fraction remains elevated even during the recovery phase of obstructive jaundice even after the conjugated bilirubin levels have normalized.

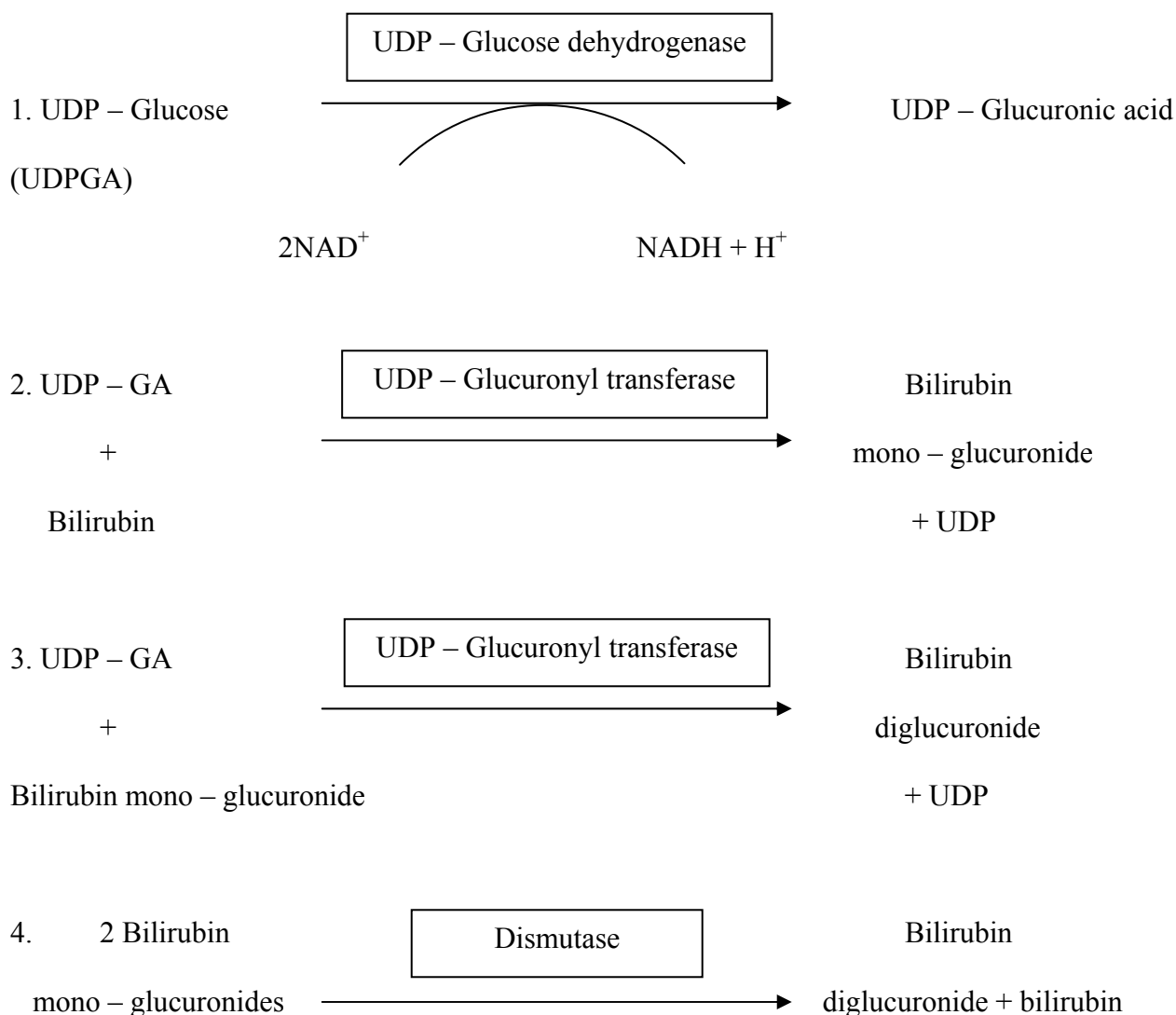


Fig.9: Conjugation of Bilirubin in Hepatocytes

Recently glucuronyl transferase activity has also been detected in certain extrahepatic tissues, viz., skin, kidneys, adrenal glands, and ovary, testes, intestinal mucosa and synovial membrane. However, the role of the enzyme in these extra-hepatic tissues is uncertain. Probably bilirubin monoglucuronide may be formed but not the diglucuronide in these tissues.

(a) Decreased activity of Glucuronyl Transferase

(1) Glucuronyl transferase activity

This may be inhibited by certain drugs, viz., novobiocin, dyes and steroidal derivatives e.g., pregnane-3 α -20 β -diol. The latter is an unusual isomer of pregnanediol which can form due to inherited defect in steroid metabolism. This isomer may be excreted in the breast milk by a small proportion of nursing mothers. The isomer can inhibit the glucuronyl transferase activity and produce prolonged non-haemolytic unconjugated hyperbilirubinemia leading to jaundice in infants. On stopping breast milk feeding jaundice disappears.

(2) Lucey-Driscoll syndrome

This is a transient familial neonatal nonhemolytic unconjugated hyperbilirubinemia in which healthy looking women give birth to infants with severe nonhemolytic unconjugated hyperbilirubinemia with risk of kernicterus. An unidentified factor, probably progestational steroid, has been isolated from serum of the mother, which inhibits the glucuronyl transferase activity producing this condition.

(3) Transient neonatal “physiological” jaundice

This is the most common cause of neonatal unconjugated hyperbilirubinemia. It results from an accelerated hemolysis and due to an immature hepatic system for uptake, conjugation and secretion of bilirubin. Glucuronyl transferase activity is delayed and reduced. There is probably also reduced synthesis of substrate i.e., UDP-glucuronic acid. The increased unconjugated bilirubin is capable of crossing the blood brain barrier when its concentration in plasma exceeds the level beyond which albumin is fully saturated (20-25mg/dL). This can result in hyperbilirubinemic toxic encephalopathy or kernicterus. Administration of phenobarbital, which stimulates the enzyme activity, is usually effective as a therapy. So is exposure to visible light (phototherapy). It promotes hepatic excretion of unconjugated bilirubin by converting some of bilirubin to other derivatives as maleimide fragments, and geometric isomers, which are excreted in bile.

(4) Crigler-Najjar syndrome

(a) Type 1 (Congenital nonhemolytic jaundice)

A rare autosomal recessive disorder in humans in which primary metabolic defect is inherited absence of glucuronyl transferase activity. It is characterized by severe congenital nonhemolytic unconjugated hyperbilirubinemia and jaundice. This condition is usually fatal within the first fifteen months of life (some cases have been reported to have reached teens without developing any complications until puberty) and if untreated, serum bilirubin may exceed 20 mg/dl leading to risk of kernicterus. Phototherapy has been found to be useful but phenobarbital has not been found effective.

(b) Type II

A rare inherited disorder due to a milder defect in the bilirubin conjugating system. It follows a more benign course with unconjugated hyperbilirubinemia. Serum bilirubin usually does not exceed 20 mg/dl. No risk of kernicterus. Bile of these subjects contains “bilirubin monoglucuronide” only, suggesting genetic

defect involving hepatic UDP-glucuronyl transferase that adds the second glucuronyl group to bilirubin monoglucuronide. Patients respond well to treatment with large doses of phenobarbitone.

(5) Gilbert's syndrome

A heterogeneous group of diseases due to following causes:

- due to compensated hemolysis associated with unconjugated hyperbilirubinemia
- due to a defect in hepatic clearance of bilirubin, possibly due to defect in uptake of bilirubin by liver parenchymal cells
- due to reduced glucuronyl transferase activity

Gilbert and his colleagues described the syndrome to be characterized by low-grade chronic unconjugated hyperbilirubinemia and jaundice. Bilirubin level in 85% cases is usually less < 3 mg/dL and is seen in age group of 18 to 25 years, detected suddenly during examination— a mild icterus of sclera of eye. Patient usually complains of fatigue, weakness and abdominal pain.

(6) Dubin-Johnson Syndrome (chronic idiopathic jaundice)

An autosomal recessive disorder characterized by noncholestatic conjugated hyperbilirubinemia and jaundice in childhood and during adult life. Defect is in hepatic secretion of conjugated bilirubin in bile and in secretion of conjugated oestrogens and test compounds such as the dye sulfobromophthalein. Another interesting feature is that 80 to 90% of coproporphyrins excreted in urine are of type I (reasons not known), but no abnormalities in porphyrin synthesis are seen. Hepatocytes in centrilobular area have been found to contain an abnormal pigment in this disease that has not been identified. Aminotransferase and alkaline phosphatase levels are usually normal. For unknown reasons, a typical derangement in urinary coproporphyrin excretion with reversal of the normal isomer I: III ratio accompanies this syndrome.

BSP Test

Shows a secondary rise in plasma concentration due to reflux of the conjugated BSP (pathognomonic). Dyes viz., Indocyanine Green and Rose Bengal, do not require conjugation hence secondary rise does not occur.

(7) Rotor's syndrome

This rare disorder is similar to Dubin-Johnson syndrome (i.e. chronic noncholestatic conjugated hyperbilirubinemia), but the liver is not pigmented and other subtle metabolic differences are present. The precise cause is not known but may be due to defect in transport by hepatocytes of organic ions, including bilirubin.

(b) Increased activity of Glucuronyl Transferase

Hepatic glucuronyl transferase activity is increased after administration of certain drugs viz., benzpyrene, aminoquinolones, chlorcyclizine and phenobarbitones to normal adults and neonates. Administration of these drugs results in proliferation of smooth endoplasmic reticulum and increases the synthesis of the enzyme.

Excretion of bile pigments

Conjugated bilirubins are secreted into the bile by active transport system and this appears to be the rate-limiting step for the entire process of hepatic bilirubin metabolism. The hepatic transport of conjugated bilirubin into the bile is inducible by same drugs that can induce conjugation of bilirubin indicating that the conjugation and excretion systems behave as a coordinated functional unit. Only after phototherapy can unconjugated bilirubin be detected in bile, otherwise all the bilirubin secreted into bile is conjugated.

The liver secretes many naturally occurring compounds and drugs into the bile after their metabolism by multiple systems (some shared by bilirubin diglucuronide). In the lower portion of the intestinal tract, especially in caecum and colon, the bilirubin is released with the help of the enzyme β -glucuronidase produced by bacteria, and then the released bilirubin is subjected to a series of enzyme systems present in the intestinal tract, mainly derived from the anaerobic bacteria in the caecum.

Faecal flora as well as a pure strain of clostridium derived from the rat colon has been demonstrated *in vitro* to be able to complete the reduction of bilirubin to L-stercobilinogen, the normal end product of bilirubin metabolism in the colon.

In the intestine, progressive hydrogenation (reduction) occurs to produce a series of intermediary compounds which beginning with “meso-bilirubinogen” comprise a number of colourless urobilinoids, which may be oxidized, with loss of hydrogen, to colored compounds (Fig. 10). The end product is colorless tetrapyrrolic compounds called urobilinogens. Auto-oxidation in the presence of air produces “L-stercobilin” (L-urobilin), an orange-yellow pigment which contributes to the normal color of the faeces and urine. Stercobilin is strongly laevo-rotatory.

Urobilin IX-D (1) urobilin or inactive (1) urobilin, is an optically inactive urobilinoid that has been identified in the faeces. It is less stable than stercobilin and is oxidized in air to form violet or blue-green pigments.

Entero-hepatic circulation of Bile Pigments

The various products derived from the progressive reduction of bilirubin may in part be absorbed from the intestine and returned to the liver for its re-excretion, called as “entero-hepatic circulation” of bile pigments. A small part escapes entero-hepatic circulation and is excreted in urine, which normally contains traces of “urobilinogen” and urobilin as well as meso-bilirubinogen and perhaps other intermediary products. The great majority of the metabolites of bilirubin are however, excreted in faeces.

If the intestinal flora is modified or diminished, as by the administration of orally effective broad spectrum antibiotics, which are capable of producing partial sterilization of the intestinal tract, bilirubin may not be further reduced or may later be auto-oxidized, in contact with air, to “biliverdin.” Thus, the faeces acquire a greenish tinge under the above circumstances. Similar condition may develop in premature babies/ or in infants where the bacterial flora develop late.

In the patients whose intestinal flora is altered by oral administration of oxy-tetracyclines/or chlortetracyclines a dextro-rotatory urobilinoid “D-urobilin” has been identified.

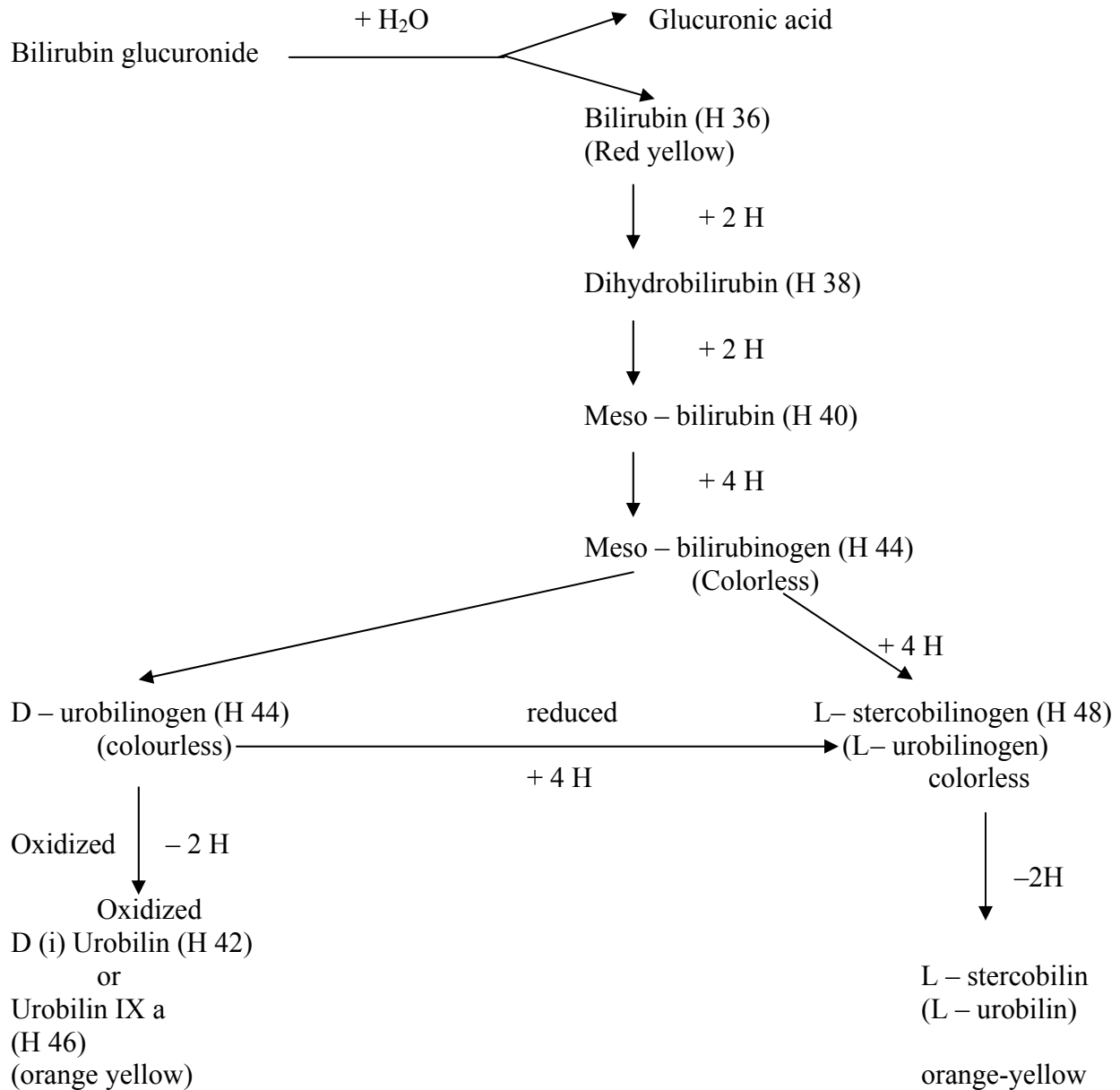


Fig. 10: Bilirubin Metabolism

Jaundice

When bilirubin levels exceed 1mg/dL, hyperbilirubinemia is present. This results from either excess production compared to what liver can excrete, may be due to compromised liver function or due to obstruction to outflow of bile. In all these conditions accumulation of bilirubin in the blood produces jaundice. When bilirubin levels in blood are 2-2.5mg/dL or more it diffuses into tissues (e.g. sclera, skin) producing yellow discolouration (icterus).

Clinical and laboratory assessment with a detailed history and physical examination are crucial, because diagnostic errors usually result from inadequate clinical judgment and over reliance on laboratory data.

Symptoms and Signs

Mild jaundice without dark urine suggests unconjugated hyperbilirubinemia caused by hemolysis or Gilbert's syndrome rather than hepatobiliary disease. More severe jaundice or dark urine clearly indicates a liver or biliary disorder. Signs of portal hypertension, ascites, or skin and endocrine changes usually imply a chronic rather than an acute process. Patients often notice dark urine before skin discoloration; thus, the onset of dark urine better indicates the duration of jaundice. Nausea and vomiting preceding jaundice most often indicate acute hepatitis or common duct obstruction by a stone; abdominal pain or rigors favor the latter. More insidious anorexia and malaise occur in many conditions but particularly suggest alcoholic liver disease or chronic hepatitis.

A systemic disorder should also be considered (e.g., distended jugular veins suggest heart failure or constrictive pericarditis in a patient with hepatomegaly and ascites). Cachexia and an unusually hard or lumpy liver are more often caused by metastases than by cirrhosis. Diffuse lymphadenopathy suggests infectious mononucleosis in acute jaundice and lymphoma or leukemia in a chronic illness. Hepatosplenomegaly without other signs of chronic liver disease may be caused by an infiltrative disorder (e.g., lymphoma, amyloidosis), although jaundice is usually minimal or absent in such disorders; schistosomiasis and malaria commonly give this picture in endemic areas.

The severity of jaundice and bilirubin fractionation does not help differentiate hepatocellular from cholestatic jaundice. Disproportionate increases of alkaline phosphatase suggest a cholestatic or infiltrative disorder (Table 3). In the latter, bilirubin is typically normal or only slightly increased. Low albumin and high globulin levels indicate chronic rather than acute liver disease. Imaging is most valuable for diagnosing infiltrative and cholestatic disorders. Abdominal ultrasound, CT, and MRI often detect metastatic and other focal liver lesions and have replaced radionuclide scans for this purpose.

Table 3: Enzyme levels in liver diseases

Serum Enzymes	Active Viral Hepatitis (no. of times of normal)	Complete Biliary Obstruction (no. of times of normal)
AST	14	3
ALT	17	4
ALP	1 – 2	4 – 14
Leucine aminopeptidase	1 – 2	3
Isocitrate dehydrogenase	6	1
5'-Nucleotidase	1 – 2	6

Hemolytic Jaundice

Excessive hemolysis or breakdown of red blood cells causes the formation of higher than normal amounts of bilirubin. Hemolytic jaundice can occur due to mismatched blood transfusion, glucose-6-phosphate dehydrogenase deficiency, due to infections, as side effect of drugs, due to autoimmunity, structural abnormalities of RBC etc. (Table 4.)

Table 4: Differentiation of three types of Jaundice

	Hemolytic	Hepatic	Obstructive
Causes	Excessive breakdown of RBC	Infections, toxins, liver failure, cirrhosis etc.	Gallstones, tumours, enlarged lymph nodes etc.
Icterus	Mild	Moderate to severe	Moderate to severe
Stool	Dark coloured	Variable, usually pale	Clay coloured
Van den Bergh reaction	Indirect, may be delayed positive	Biphasic	Direct
Bile pigment in circulation	Unconjugated bilirubin	Mixed	Conjugated bilirubin
Serum levels of bilirubin	3-5mg%	<20mg%	Up to 50mg%
Urinary bilirubin	Not detected	Present	Strongly present
Urobilinogen in urine	Strongly raised	Normal or increase	Decreased or absent
Faecal stercolilinogen	Strongly increased	Decreased	Decreased or absent
Steatorrhoea	Not present	Present	Strongly present
Prothrombin time	Normal	Increased	Increased, normal after parental Vit K
Amino transferases (ALT/AST)	Usually normal	Marked increase	Increased
Alkaline phosphatase (ALP)	Normal	Slightly increased	Marked increase

Hepatic Jaundice

Hepatic jaundice is caused by damage or disease in the liver. This can also occur due to various causes like infections (especially viral), worm infestations, toxic effects of drugs and chemical poisons, cancer metastasis, etc. The amount of urobilinogen in the urine will be either normal or low if not enough bilirubin is being removed by the liver into bile and the intestines.

Obstructive Jaundice

The most common intrahepatic causes are hepatitis, drug toxicity, and alcoholic liver disease. Less common causes include primary biliary cirrhosis, cholestasis of pregnancy, metastatic carcinoma, and numerous uncommon disorders.

The most common extrahepatic causes are a common duct stone and pancreatic cancer. Less common causes include benign stricture of the common duct (usually related to prior surgery),

ductal carcinoma, pancreatitis or pancreatic pseudocyst, and sclerosing cholangitis. Jaundice, dark urine, pale stools, and generalized pruritus are the clinical hallmarks of cholestasis.

In clinical diagnosis of jaundice, measurement of bilirubin is of utmost importance. Van den Berg first devised a method to quantitatively assay the bilirubin content by applying Ehrlich's test in urine (based on coupling of diazotized sulfanilic acid and bilirubin to produce a reddish-purple azo compound). That form of bilirubin which can be measured only after the addition of methanol (unconjugated bilirubin) is termed "indirect-reacting" and the form (conjugated bilirubin) that can be measured without the addition of methanol is called "direct-reacting."

Depending on the type of bilirubin present in plasma hyperbilirubinemia may be classified as retention hyperbilirubinemia, due to overproduction or regurgitation hyperbilirubinemia, due to reflux into the blood stream because of biliary obstruction. Choluric jaundice (presence of biliary derivatives in urine) occurs only in regurgitation hyperbilirubinemia and acholuric jaundice occurs only in the presence of an excess of unconjugated bilirubin.

Suggested Readings

1. Harper's Biochemistry 25th edition. By Murray, Robert K., Granner, Darryl K., Mayes, Peter A., and Rodwell, Victor W. McGraw-Hill Medical.
2. Tietz Textbook of Clinical Chemistry 3rd edition. By Carl. A Burtis, Edward R., M.D. Ashwood, Norbert W. Tietz. W.B. Saunders Company.
3. Principles of Biochemistry 3rd edition. By Albert L. Lehninger, David L. Nelson, Michael M. Cox. W H Freeman and Company.
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5. Harrison's Principles of Internal Medicine 16th edition. By Dennis L. Kasper, Eugene Braunwald, Stephen Hauser, Don Longo, J. Larry Jameson, Anthony S. Fauci. McGraw-Hill Medical.