Biosynthesis of heme

Amino acids are precursors of many nitrogen-containing compounds as porphyrins, neurotransmitters, hormones, purines, and pyrimidines.

Heme consists of one ferrous (Fe^{2+}) iron ion coordinated in the center of the tetrapyrrole ring of protoporphyrin IX. Heme is the prosthetic group for hemoglobin, myoglobin, the cytochromes, catalase and peroxidase. Porphyrins are cyclic molecules formed by the linkage of four pyrrole rings through methenyl bridges.

Over 85% of all heme synthesis occurs in bone marrow, which are active in hemoglobin synthesis. Mature red blood cells lack mitochondria and are unable to synthesize heme The next site of heme biosynthesis is the liver, which synthesizes a number of heme proteins (particularly cytochrome P450 proteins).

The initial reaction and the last three steps in the formation of porphyrins occur in mitochondria, whereas the intermediate steps of the biosynthetic pathway occur in the cytosol.

1. **Formation of delta-aminolevulinic acid (ALA):** All the carbon and nitrogen atoms of the porphyrin molecule are provided by glycine and succinyl coenzyme A, that condense to form amino levulinic acid (ALA) in a reaction catalyzed by *ALA synthase.*This reaction requires pyridoxal phosphate (PALP) as a coenzyme, and is the rate-limiting step in porphyrin biosynthesis. When porphyrin production exceeds the availability require it, heme accumulates and is converted to hemin by the oxidation of Fe^{2+} to Fe^{3+}. Hemin decreases the activity of hepatic ALA synthase.

2. Formation of porphobilinogen: The condensation of two molecules of ALA to form porphobilinogen is driven by *porphobilinogen synthase*.

3. Formation of uroporphyrinogen: The condensation of four porphobilinogens produces the linear tetrapyrrole, which is isomerized and cyclized by

uroporphyrinogen III synthase to produce the uroporphyrinogen III. After decarboxylation uroporphyrinogen III is converted to coproporphyrinogen III reactions occur in the cytosol.

4. Formation of heme: Coproporphyrinogen III enters again the mitochondrion, and turns firstly into protoporphyrinogen IX, which then is oxidized to protoporphyrin IX. The incorporation of iron (as Fe^{2+}) into protoporphyrin IX occurs by ferrochelatase, an enzyme that, like ALA dehydratase, is inhibited by lead.

Porphyrias

Porphyrias are rare defects in heme synthesis, resulting in the accumulation and increased excretion of porphyrins. The porphyrias are classified as *erythropoietic* or *hepatic*, depending on whether the enzyme deficiency occurs in the erythropoietic cells of the bone marrow or in the liver. In general, individuals with an enzyme defect prior to the synthesis of the tetrapyrroles manifest abdominal and neuro- psychiatric signs, whereas those with enzyme defects leading to the accumulation of tetrapyrrole intermediates show photosensitivity—that is, they have pruritus: their skin itches and burns when exposed to visible light.

Porphyria *cutanea tarda* is the most common porphyria. The disease is associated with a deficiency in *uroporphyrinogen decarboxylase*. Clinical onset is typically during the fourth or fifth decade of life. Porphyrin accumulation leads to cutaneous symptoms and urine that is red, pink or brown.

Acute hepatic porphyrias (*ALA dehydratase deficiency)* are characterized by acute attacks of gastrointestinal, neuropsychiatric, and motor symptoms that may be accompanied by photosensitivity. Porphyrias leading to accumulation of ALA and porphobilinogen, such as acute intermittent porphyria, cause abdominal pain and neuropsychiatric disturbances, ranging from anxiety to delirium.

The *erythropoietic porphyrias* are characterized by skin rashes and blisters that appear in early childhood. The diseases are complicated by cholestatic liver

cirrhosis and progressive hepatic failure.

2. Increased ALA synthase activity: One common feature of the porphyrias is a decreased synthesis of heme. In the liver, heme normally functions as a repressor of the gene for ALA synthase. Therefore, the absence of this end product results in an increase in the synthesis of ALA synthase. This causes an increased synthesis and accumulation of these toxic intermediates.

During acute porphyria attacks, patients require medical support, particularly treatment for pain and vomiting. The severity of symptoms of the porphyrias can be diminished by intravenous injection of hemin and glucose, which decreases the synthesis of ALA synthase. Avoidance of sunlight and ingestion of ®-carotene (a free-radical scavenger) are helpful in porphyrias.

Degradation of heme

After approximately 120 days in the circulation, red blood cells are taken up and degraded by the reticuloendothelial system, particularly in the liver and spleen.

1. Formation of bilirubin: The first step in the degradation of heme is catalyzed by the microsomal *heme oxygenase* system of the reticuloendothelial cells. In the presence of NADPH and O_2 , the enzyme adds a hydroxyl group to the methenyl bridge between two pyrrole rings, with a concomitant oxidation of ferrous iron to Fe^{3+}, obtained compound is called verdoglobin. A second oxidation by the same enzyme system results in cleavage of the porphyrin ring. The *green pigment biliverdin* is produced as ferric iron and CO are released. The CO has biologic function, acting as a signaling molecule and vasodilator. Biliverdin is reduced, forming the *red-orange bilirubin*. Bilirubin and its derivatives are collectively termed *bile pigments.* The changing colors of a bruise reflect the varying pattern of intermediates that occurs during heme degradation.

Bilirubin is only slightly soluble in plasma and, therefore, is transported to the liver by binding non- covalently to albumin. Certain anionic drugs, such as

salicylates and sulfonamides, can displace bilirubin from albumin, permitting bilirubin to enter the central nervous system. This causes the potential for neural damage in infants. Bilirubin dissociates from the carrier albumin molecule, enters a hepatocyte via facilitated diffusion, and binds to intracellular proteins, particularly the protein ligandin.

Congenital jundice types.

Conjugation of bilirubin in microsoms: In the hepatocyte, the solubility of bilirubin is increased by the addition of two molecules of glucuronic acid. The reaction is catalyzed by *bilirubin glucuronyltransferase* using uridine diphosphate-glucuronic acid as the glucuronate donor. *Deficiency* of this enzyme result in **Crigler-Najjar II** and **absent** of this enzyme leads to **Crigler-Najjar I.** Crigler-Najjar I is the most severe disease**. Gilbert** syndrome is result of absence of bilirubin –binding protein in hepatocytes.

Bilirubin diglucuronide (conjugated bilirubin) is actively transported against a concentration gradient into the bile canaliculi and then into the bile. This energy-dependent, rate-limiting step is susceptible to impairment in liver disease. A deficiency in the protein required for transport of conjugated bilirubin out of the liver results in **Dubin-Johnson** syndrome.

Bilirubin diglucuronide is hydrolyzed and reduced by bacteria in the gut to yield *urobilinogen*, a colorless compound. Most of the urobilinogen is oxidized by intestinal bacteria to *stercobilin,* which gives feces the characteristic brown color. However, some of the urobilinogen is reabsorbed from the gut and enters the portal blood. A portion of this urobilinogen participates in the enterohepatic urobilinogen cycle in which it is taken up by the liver, and then resecreted into the bile. The remainder of the urobilinogen is transported by the blood to the kidney, where it is converted to *yellow urobilin* and excreted, giving urine its characteristic color.

Jaundice

Jaundice (also called icterus) refers to the yellow color of skin, nail beds, and sclerae (whites of the eyes) caused by deposiotion of bilirubin, secondary to increased bilirubin levels in the blood called hyperbilirubinemia.

Types of jaundice: The presence of a tumor or bile stones may block the bile ducts, preventing passage of bilirubin into the intestine. This caises *obstructive jaundice*, or extrahepatic cholestasis, which results from obstruction of the bile duct. Stercobilinogen is poorly formed in their intestines and absent in the urine. Therefore patients produce stools that are a pale, clay color, and urine that darkens only upon standing. The liver *conjugated bilirubin* is washed out into and elevated in the blood.

Massive lysis of red blood cells (for example, in patients with sickle cell anemia, pyruvate kinase or glucose 6-phosphate dehydrogenase deficiency) causes overproduction of bilirubin, that may produce bilirubin faster than it can be conjugated in the liver. Unconjugated*, free bilirubin* levels in the blood become *elevated,* causing **hemolytic** jaundice Simultaneously, more conjugated bilirubin is excreted into the bile, the amount of urobilinogen entering the enterohepatic circulation is increased, and urinary urobilinogen is increased. Therefore urine and stools darkens with increased amount of *stercobilinogen.*

***Hepatocellular* jaundice** occurs as a result of decreased bilirubin conjugation in damage liver cells. In intrahepatic cholestasis conjugated bilirubin is not efficiently secreted from the liver into bile, it can diffuse (“leak”) into the blood, therefore causing a *conjugated hyperbilirubinemia*. Plasma levels of AST and ALT are elevated. The urine darkens, whereas stools may be a pale, clay color because *stercobilinogen is decreased* in the feces.

Jaundice in newborns: Newborn infants, particularly if premature, often accumulate bilirubin, because the activity of hepatic *bilirubin glucuronyltransferase* is low at birth—it reaches adult levels in about 4 weeks. Elevated bilirubin, in excess of the binding capacity of albumin, can diffuse into the basal ganglia and cause toxic

encephalopathy. Thus, newborns with significantly elevated bilirubin levels are treated with blue fluorescent light.

SYNTHESIS OF PURINE NUCLEOTIDES

The atoms of the purine ring are contributed by a number of compounds, including amino acids (The purine ring is constructed primarily in the liver by a series of reactions that add the donated carbons and nitrogens to a preformed ribose 5-phosphate.

A. Synthesis of 5-phosphoribosyl-1-pyrophosphate (PRPP)

PRPP is an “activated pentose” that participates in the synthesis and salvage of purines and pyrimidines. Synthesis of PRPP from ATP and ribose 5-phosphate is catalyzed by *PRPP synthetase* ,

B. Synthesis of 5'-phosphoribosylamine

In the first step of synthesis of 5'-phosphoribosylamine from PRPP and glutamine, the amide group of glutamine replaces the pyrophosphate group attached to carbon 1 of PRPP. This is the committed step in purine nucleotide biosynthesis.

Synthesis of inosine monophosphate, the “parent” purine nucleotide

The next nine steps in purine nucleotide biosynthesis leading to the synthesis of inosine monophosphate (IMP, whose base is hypoxanthine) require aspartic acid, glycine, and glutamine) and CO_2 , ATP as an energy source. Two steps in the pathway require: N^{10}-formyl THFA and methyl-THFA.

D. Synthetic inhibitors of purine synthesis

Some synthetic inhibitors of purine synthesis, for example the sulfonamides are

designed to inhibit the growth of rapidly dividing microorganisms without interfering with human cell functions. Other purine synthesis inhibitors, such as structural analogs of folic acid (for example, methotrexate, are used pharmacologically to control the spread of cancer by interfering with the synthesis of nucleotides and, therefore, of DNA and RNA.

Conversion of IMP to AMP and GMP. The conversion of IMP to either AMP or GMP uses a two-step, energy-requiring pathway. Note that the synthesis of AMP requires guanosine triphosphate (GTP) as an energy source, whereas the synthesis of GMP requires ATP. Also, the first reaction in each pathway is inhibited by the end product of that pathway.

Nucleoside diphosphates are synthesized from the corresponding nucleoside monophosphates by base-specific nucleoside mono-phosphate kinases. These kinases do not discriminate between ribose or deoxyribose in the substrate. *Adenylate kinase* is particularly active in liver and muscle, where the turnover of energy from ATP is high. Its function is to maintain an equilibrium among AMP, ADP, and ATP. *Nucleoside diphosphate kinase* interconverts nucleoside diphosphates and triphosphates.

Salvage pathway for purines

Purines obtained from the diet and not degraded, can be converted to nucleoside triphosphates and used by the body. This is referred to as the “salvage pathway” for purines.

Two enzymes are involved in this process: *adenine phosphoribosyl-transferase* (APRT) and *hypoxanthine-guanine phosphoribosyltransferase* (HGPRT). Both enzymes use PRPP as the source of the ribose 5-phosphate group. In this process purine bases are converted to nucleotides.

Also *adenosine kinase* can convert adenosine, the only purine nucleoside to be salvaged by phosphorylation, to AMP.

Lesch-Nyhan syndrome: This disorder is associated with a virtually complete deficiency of hypoxanthine-guanine phosphoribosyltransferase. This deficiency results in an inability to salvage hypoxanthine or guanine. As a result, they enter the purine degradation, pathway with production of uric acid. In addition, the lack of this salvage pathway causes increased PRPP levels and decreased IMP and GMP levels. As an enzyme glutamine:phosphoribosylpyrophosphate amidotransferase catalysing the committed step in purine synthesis has excess substrate and decreased inhibitors available, *de novo* purine synthesis is increased. The combination of increased purine synthesis and decreased purine reutilization results in increased degradation of purines and the production of large amounts of uric acid, making Lesch-Nyhan a heritable cause of hyperuricemia. In patients with Lesch-Nyhan syndrome, the hyperuricemia frequently results in the formation of uric acid stones in the kidneys (urolithiasis) and the deposition of urate crystals in the joints (gouty arthritis) and soft tissues. In addition, the syndrome is characterized by motor dysfunction, cognitive deficits, and behavioral disturbances that include self-mutilation (biting of lips and fingers,

SYNTHESIS OF DEOXYRIBONUCLEOTIDES

The nucleotides required for DNA synthesisare 2 '-deoxy- ribonucleotides, which are produced from ribonucleoside diphosphates by the enzyme *ribonucleotide reductase*. The immediate donors of the hydrogen atoms needed for the reduction of the 2 '-hydroxyl group are two sulfhydryl groups on the enzyme itself, which, during the reaction, form a disulfide bond. The disulfide bond created during the production of the 2 '-deoxy carbon must be reduced. The source of the reducing equivalents for this purpose is thioredoxin—a peptide coenzyme of ribonucleotide reductase. Thioredoxin contains two cysteine residues separated by two amino acids in the peptide chain. The two sulfhydryl groups of thioredoxin donate their hydrogen atoms to ribonucleotide reductase, Then thioredoxin must be converted back to its reduced form in order to continue its function. The necessary reducing

equivalents are provided by NADPH + H^+, and the reaction is catalyzed by *thioredoxin reductase*

DEGRADATION OF PURINE NUCLEOTIDES

Degradation of dietary nucleic acids occurs in the small intestine, where a family of pancreatic enzymes hydrolyzes the nucleic acids to nucleotides. Inside the intestinal mucosal cells, purine nucleotides are sequentially degraded by specific enzymes to nucleosides and free bases, with uric acid as the end product of this pathway. [Note: Purine nucleotides from de novo synthesis are degraded in the liver primarily. The free bases are sent out from liver and salvaged by peripheral tissues.]

A. Degradation of dietary nucleic acids in the small intestine

Ribonucleases and deoxyribonucleases, secreted by the pancreas, hydrolyze dietary RNA and DNA primarily to oligonucleotides. Oligonucleotides are further hydrolyzed by pancreatic phosphodiesterases, producing a mixture of mononucleotides. In the intestinal mucosal cells, a family of nucleotidases removes the phosphate groups hydrolytically, releasing nucleosides that are further degraded to free bases. Dietary purine bases are generally converted to uric acid in intestinal mucosal cells. Most of the uric acid enters the blood, and is eventually excreted in the urine.

Uric acid formation.

1. Firstly *AMP deaminase* removes an amino group from AMP to produce IMP, or *adenosine deaminase* removes an amino group from adenosine to produce inosine (hypoxanthine- ribose).

2. *5'-nucleotidase* convertes IMP and GMP into their nucleoside forms—inosine and guanosine by removal of phosphates of these mono-nucleotides.

3. *Purine nucleoside phosphorylase* converts inosine and guanosine into their respective purine bases, hypoxanthine and guanine by removal of ribose 5-phosphate. Then *mutase* interconverts ribose 5-phosphate and ribose 1- phosphate.

4. Guanine is deaminated by *guanine deaminase* to form xanthine.

5. Hypoxanthine is oxidized by *xanthine oxidase* to xanthine, which is further oxidized by *xanthine oxidase* to uric acid, the final product of human purine degradation. Uric acid is excreted primarily in the urine. Mammals other than primates express *urate oxidase* which cleaves the purine ring, generating *allantoin.* Use of recombinant ***urate oxidase*** is a potential therapeutic strategy to lower urate levels.

Diseases of purine degradation

1. Gout: Gout is a disorder characterized by high levels of uric acid— the end product of purine catabolism in blood (hyperuricemia). The hyperuricemia can lead to the deposition of urate crystals in the joints, and an inflammatory response to the crystals, causing first acute and then progressing to chronic gouty arthritis. Nodular urate crystals (tophi) may be deposited in the soft tissues, resulting in chronic tophaceous gout. Formation of uric acid stones in the kidney (urolithiasis) may also be seen.

Increased availability of *PRPP synthetase* result in the the increased purine production, resulting in elevated levels of plasma uric acid. Secondary hyperuricemia is typically the consequence of increased availability of purines, for example, in patients with myeloproliferative disorders or who are undergoing chemotherapy and so have a high rate of cell turnover.

PYRIMIDINE SYNTHESIS AND DEGRADATION

Unlike the synthesis of the purine ring, which is constructed on a ribose 5-phosphate, the pyrimidine ring is synthesized before being attached to ribose 5-phosphate. The sources of the atoms in the pyrimidine ring are: *glutamine, CO_2, and aspartic acid*. Glutamine and aspartic acid are thus required for both purine and pyrimidine synthesis.

1. Synthesis of carbamoyl phosphate. The regulated step of this pathway in mammalian cells is the synthesis of carbamoyl phosphate from glutamine and CO_2, catalyzed by *carbamoyl phosphate synthetase* II. Reaction is inhibited by the end product of this pathway, UTP. Defects in *ornithine transcarbamylase* of the urea cycle promote pyrimidine synthesis due to increased availability of *carbamoyl phosphate*.

2. Synthesis of orotic acid.

a. The next step in pyrimidine synthesis is the formation of carbamoylaspartate, catalyzed by *aspartate transcarbamoylase*.

b. Then pyrimidine ring (dihydroorotate) is formed by dihydroorotase.

c. The dihydroorotate is oxidized by *dihydroorotate dehydrogenase* to produce orotic acid. The enzyme dihydroorotate dehydrogenase is associated with the mitochondrial membrane, while all other enzymes in pyrimidine biosynthesis are cytosolic.

Formation of a nucleotide

The pyrimidine ring is converted to the nucleotide orotidine 5'-monophosphate (OMP) in the last stage. PRPP is again the ribose 5-phosphate donor. The enzyme *orotate phosphoribosyltransferase* produces OMP. Therefore both purine and pyrimidine nucleotide biosynthesis require glutamine, aspartic acid, and PRPP as essential precursors. *Orotidylate decarboxylase* removes the carboxyl group from the parent pyrimidine mononucleotide, OMP, converting it to uridine monophosphate (UMP). Orotate phosphoribosyltransferase and orotidylate

decarboxylase are also catalytic domains of a single polypeptide chain called *UMP synthase.* Orotic aciduria—a rare genetic defect—may be caused by a deficiency of one or both activities of this bifunctional enzyme, resulting in orotic acid in the urine. UMP is sequentially phosphorylated to UDP and UTP.

The UDP is a substrate for ribonucleotide reductase, which generates dUDP. Then dUDP is phosphorylated to dUTP. dUMP is converted to dTMP by *thymidylate synthase*, which uses N^5,N^{10}-methylene tetrahydrofolate as the source of the methyl group. CTP is also produced from UTP in amination by assistance of *CTP synthetase*, with glutamine providing the nitrogen.

Degradation of pyrimidine nucleotides

Unlike the purine ring, which is not cleaved in human cells, the pyrimidine ring is opened and degraded to simple products, such as β-alanine and β-amino isobutyrate, with the production of NH_3 and CO_2 .

First, cytidine deaminase converts cytidine to uridine. At the next stage, uridine and cytidine lose ribose, turning them into uracil and thymine respectively. Then, using the $NADPH_2$ molecule, *dihydrouracil dehydrogenase* forms from them dihyduracil and dihydthymine. Using a water molecule, dihydropyrimidinase breaks up the pyrimidine ring to form carbamyl propionic acid - from uracil and carbamyl isoobutyric acid - from thymine. Both being decarboxylated and deaminated, they form β-alanine and β-aminoisobutirate respectively. β-alanine in reaction of transamination with pyruvate forms β-alanine. β-aminoisobutirate forms in reaction of transamination with pyruvate β-alanine and methyl malonyl-CoA, which then with assistance of vitamin B_{12} is converted to one of Krebs cycle methabolits – succinyl-CoA.