MLU 3242 - Cell Biology & Basic Biochemistry

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DISORDERS OF PURINE AND PYRIMIDINE METABOLISM
Lesch-Nyhan syndrome

• Rare, X-linked, recessive disorder caused by deficiency of hypoxanthine-guanine phosphoribosyl transferase (HPRT)
• Degree of deficiency (and hence manifestations) vary with the specific mutation.
• HPRT deficiency results in failure of the salvage pathway for hypoxanthine and guanine.
Figure 1 Inter relationships in the process of purine metabolism
• These purines are instead degraded to uric acid.
• Hyperuricemia predisposes to gout and its complications.
• The disease usually manifests between 3 months and 12 months of age with the appearance of orange sandy precipitate (xanthine) in the urine
• Serum uric acid levels are usually elevated, but confirmation by HPRT enzyme assay is usually done.
Figure 2: The catabolic pathways of purine bases
Adenine phosphoribosyltransferase deficiency

- Rare autosomal recessive disorder that results in the inability to salvage adenine for purine synthesis
- Accumulated adenine is oxidized to 2,8-dihydroxyadenine, which precipitates in the urinary tract, causing problems similar to those of uric acid nephropathy
- Onset can occur at any age.
- Diagnosis is by demonstrating elevated levels of 2,8-dihydroxyadenine, 8-hyroxoyadenine, and adenine in urine and confirmed by enzyme assay; serum uric acid is normal.
Phosphoribosylation of adenine, hypoxanthine, and guanine to form AMP, IMP, and GMP.
Disorders of Purine Nucleotide Synthesis

Phosphoribosylpyrophosphate synthetase superactivity

• X-linked, recessive disorder that causes purine overproduction

• Excess purine is degraded, resulting in hyperuricemia and gout, and neurologic and developmental abnormalities.

• Diagnosis is by enzyme studies on RBCs and cultured skin fibroblasts.
Adenylosuccinase deficiency

• Autosomal recessive disorder causing profound mental retardation, autistic behavior, and seizures.
• Diagnosis is by identifying elevated levels of succinylaminoimidazole carboxamide riboside and succinyladenosine in cerebro-spinal fluid (CSF) and urine
Purine biosynthesis from ribose 5-phosphate and ATP
Disorders of Purine Catabolism

Myoadenylate deaminase deficiency (or muscle adenosine monophosphate deaminase deficiency)

• Enzyme converts AMP to inosine and ammonia

• Deficiency may be asymptomatic or it may cause exercise-induced cramping;
Adenosine deaminase (ADA) deficiency

- Adenosine deaminase converts adenosine and deoxyadenosine to inosine and deoxyinosine, which are further broken down and excreted.

- Enzyme deficiency results in accumulation of adenosine, which is converted to its ribonucleotide and deoxyribonucleotide (dATP) forms by cellular kinases.
• The dATP increase results in inhibition of ribonucleotide reductase and underproduction of other deoxyribonucleotides.

• DNA replication is compromised as a result.
• Immune cells are especially sensitive to this defect; adenosine deaminase deficiency causes one form of severe combined.

• Diagnosis is by low RBC and WBC enzyme activity.
Purine nucleoside phosphorylase deficiency

• Rare, autosomal recessive deficiency is characterized by immunodeficiency with severe T-cell dysfunction and often neurologic symptoms.

• Diagnosis is by low enzyme activity in RBCs.
Xanthine oxidase deficiency

- **Xanthine oxidase** catalyzes uric acid production from xanthine and hypoxanthine.
- Deficiency causes build up of xanthine, which may precipitate in the urine, causing symptomatic stones with hematuria, urinary colic, and UTIs.
- Diagnosis is by low plasma uric acid and high urine and plasma hypoxanthine and xanthine.
- Enzyme determination requires liver or intestinal mucosal biopsy and is rarely indicated.
Adenosine monophosphate (AMP) → Adenosine → Adenosine Deaminase → Inosine → Hypoxanthine → Xanthine Oxidase → Xanthine → Xanthine Oxidase → Uric Acid

Guanosine monophosphate (GMP) → Guanosine → Guanine → Uric Acid
Disorders of Pyrimidine Metabolism

• Since the end products of pyrimidine catabolism are highly water-soluble, pyrimidine overproduction results in few clinical signs or symptoms.
Phosphoribosylation of adenine, hypoxanthine, and guanine to form AMP, IMP, and GMP, respectively.
Bile acid metabolism and role of bile acids in the body
Biochemistry of bile acids and the role of bile acids

• Most abundant bile acids in human bile are chenodeoxycholic acid (45%) and cholic acid (31%).
• Referred to as the primary bile acids.
• Within the intestines the primary bile acids are acted upon by bacteria and converted to the secondary bile acids, identified as deoxycholate and lithocholate.
• Bile acids are derivatives of cholesterol synthesized in the hepatocyte.

• Cholesterol, ingested as part of the diet or derived from hepatic synthesis is converted into the bile acids; cholic and chenodeoxycholic acids, which are then conjugated to an amino acid (glycine or taurine) to yield the conjugated form that is actively secreted into canaliculi.
SYNTHESIS OF BILE ACIDS

Cholesterol

NADPH+H+O2------NADP
7-α-Hydroxylase
7-Hydroxycholesterol

Cholic acid
Chenodeoxycholic acid

Glycine
Glycocholic acid
Taurine
Taurocholic acid

INTESTINAL BACTERIA

Glycocholic acid
Deoxycholic acid

Tauro-or Glycochenodeoxycholic acid
Lithocholic acid

Taurine or Glycine
Synthesis of bile acids

• Precursor cholesterol undergoes a series of steps in the hepatic bile acid synthesis.

• Major difference of the products: chenodeoxycholic acid from cholic acid is the absence of a OH group at 12th position.

• 7th Hydroxylation is regulated by a rare enzyme 7-α-hydroxylase and it’s a feed back inhibition by bile acids returning to the liver from the entero-hepatic circulation.
• Primary bile acids are conjugated at the carboxylic acid carbon with either taurine or glycine which increases the property of water solubility by reduction of pKa value.

• This conjugation results major 4 bile acids. They include

  • Cholyltaurine
  • Cholylglycine
  • Chenodeoxycholyltaurine
  • Chenodeoxycholylglycine
Major bile acids in the human body

Glyco-(Tauro-)cholate

Cholic acid

Deoxycholic acid

Glyco-(Tauro-)chenodeoxycholate

Chenodeoxycholic acid

Lithocholic acid
• Un-conjugated bile acids are never excreted under normal conditions, and therefore it is not found in bile juice.

• Bile acids are converted to \( \text{II}^{ry} \) bile acids, due to 7-\( \alpha \) dehydroxylation by bacterial flora of the colon.
• The enterohepatic circulation is important in the regulation of bile acids in the body. Alteration of bile acid metabolism is usually a reflection of liver dysfunction.

• This could be due to
  • alteration in hepatic bile acid synthesis
  • defective intracellular metabolism
  • impaired excretion
  • defective intestinal absorption.
Enterohepatic circulation of bile acids

• Bile acids are absorbed by the terminal ileum.

• Enterohepatic circulation refers to the circulation of biliary acids from the liver, where they are produced and secreted in the bile, to the small intestine, where it aids in digestion of fats and other substances, back to the liver.

• These bile acids travel to the gall bladder during the interdigestive phase for storage and to the descending part of the duodenum via the common bile duct through the major duodenal papilla during digestion.
Enterohepatic Circulation of Bile Salts

Liver
- Synthesis: 0.4 g/d
- Secretion: 24 g/d

Biliary Transport and Storage

Duodenum

Jejunum

Ileum

Colon
- Portal Venous Return (>95% of Biliary Secretion)
- Fecal excretion: 0.4 g/d
Almost 95% of the bile acids which are delivered to the duodenum will be recycled by the enterohepatic circulation.

The canaliculi, which have a blind end at the pericentral region of the hepatic lobule, are surrounded by a spiral of actin microfilaments. The contraction of these drives canalicular bile toward the biliary ductules initiating bile flow.
• Normally, bile acid synthesis is down regulated.

• With interruption of the enterohepatic circulation, bile acid biosynthesis increases up to 15-fold.

• Because bile acids are derived from cholesterol, increased bile acid biosynthesis must be accompanied by an equivalent amount of cholesterol catabolism.
Role of Bile Acids in Fat Digestion and Absorption

• Bile acids are amphipathic, that is, they contain both hydrophobic (lipid soluble) and polar (hydrophilic) groups.

• The cholesterol-derived portion of a bile acid is hydrophobic

• The amino acid conjugate is polar and hydrophilic
• Their amphipathic nature enables bile acids to carry out important functions:
• Emulsification of lipid aggregates: Bile acids have detergent action on particles of dietary fat which causes fat globules to break down or be emulsified into minute, microscopic droplets.
• Emulsification is not digestion, but is of importance because it greatly increases the surface area of fat, making it available for digestion by lipases.
Fat globule

Emulsification

Nonpolar region

Bile salt

Polar (charged) regions

Fat droplets coated with bile salts are suspended in water

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Solubilization and transport of lipids in an aqueous environment

• Bile acids are lipid carriers and are able to solubilize many lipids by forming micelles.

• Bile acids are also critical for transport and absorption of the fat-soluble vitamins.

• In micelles the polar lipids are arranged radially with their hydrophilic heads facing outward towards the aqueous phase.
• The bile acid molecules are arranged perpendicularly between their polar heads.

• The hydrophobic face of the bile acid molecule rests like a wedge between the heads of the alkyl chains of the PC (or fatty acid) molecules.

• The hydrophilic face of the bile acid molecule faces the aqueous environment.
Role of Bile Acids in Cholesterol Homeostasis

• Hepatic synthesis of bile acids accounts for the majority of cholesterol breakdown in the body.

• In humans, roughly 500 mg of cholesterol are converted to bile acids and eliminated in bile every day.

• This is the only route for the excretion of cholesterol.
Regulation of Hepatic bile acid synthesis

• Bile acids are end products of cholesterol breakdown.

• Bile acids are synthesized in the liver in a process that is regulated by many factors and pathways.

• Factors include nutrients, hormones and bile acids.

• After their absorption from the intestine, bile acids return to the liver and inhibit their own synthesis in a feedback regulatory loop
Laboratory analysis of bile acids in blood and urine

• Urine bile salts and Hay’s test
  • Normally bile salts are not present in the urine, but they may appear due to bilary obstruction which results in an increase of bile acids in circulation which are excreted in urine.
  • Hay’s test is used to detect bile salts in urine.

![Test for Bile salts](image)
• The principle of surface tension is used to check the presence of bile salts in urine.

• When fine sulphur powder is sprinkled on urine containing bile salts (as in jaundice), it sinks due to the surface tension lowering effect of bile salts.

• If there are no bile salts in urine as in normal individuals, it floats
Serum bile acid estimation

- Bile acid level in different body fluids can be measured quantitatively either as of total bile acid as well as individual bile acids.
- Bile acid level in the serum can be quantified using gas chromatography (GLC) and high performance liquid chromatography.
- Immuno assay like ELISA and fast atom bombardment mass spectrometry techniques are also used.
- Enzyme assay is the simplest and most widely used spectrophotometric assay.
- Serum deoxycholic acid: 0.09--0.35μg/ml
- Serum chenodeoxycholic acid: 0.0--0.63μg/ml
- Serum cholic acid: 0.03—0.37
• Enzyme, 3-α hydroxyl steroid hydrogenase, that is produced by *pseudomonas testosterone* catalyze the oxidation by NAD$^+$ of the 3-α hydroxyl group of all bile acids, producing a 3-keto group.

• Analysis is carried by measuring the absorbance change at 340 nm as NADH is formed.

• The same technique modified using luciferase produce chemiluminance that increases the detection limit of the assay.
Defects of bile acid metabolism

• Bile salts increase the elimination of water, cholesterol, lecithin and conjugated bilirubin in the bile.

• In liver diseases bile acid synthesis and turnover are decreased, therefore micelle formation and emulsification of fats is impaired, favouring the development of gall stones and steatorrhoea.

• Some defects of bile acid metabolism due to deficiency of enzymes have been identified.
• These are characterized by jaundice, hepatomegaly, pale stools and dark urine.

• When hepatic function is altered by disease, the bile-acid metabolism reflects this change.

• Disturbances of bile acid metabolism in hepatocellular disease may lead to an increase in fasting serum bile acid concentrations and a larger than normal increase in postprandial serum bile acid concentrations.
• Abnormalities of bile acid delivery to the bowel, including intrahepatic cholestasis and extrahepatic bile duct obstruction, may increase the concentration of cholic acid.

• Interruption of enterohepatic circulation of bile acids may lead to a decrease in serum bile acid concentrations.
Porphyrin and bilirubin metabolism
Porphyрин and bilirubin metabolism

• Porphyrrins are intermediates of the biosynthetic pathway of haem.
• Secondary metabolic defects producing intermediate products are common rather than inherited defects in porphyrin metabolism.
• Laboratory diagnosis is mainly based on the detection of intermediate metabolic products in urine, blood and faeces.
• Many porphyrin compounds are known, but only a limited number is clinically important.
Structure of porphyrin and porphyrinogen
• A porphyrin is an organic compound that contains four pyrrole rings.

• A pyrrole is a ring of four carbon atoms with a nitrogen atom at one corner ($C_4H_5N$)

• Four nitrogen atoms in the centre of the porphyrin ring enable it to chelate with various metal ions.
• Porphyrinogen is a related porphyrin with six additional hydrogen atoms and electrons.

• They are colourless, partially soluble in water and dark coloured in the solution.

• The δ Amino Laevulinic Acid (δ ALA) and porphobilinogen (PBG) are porphyrin precursors which are highly soluble in water.
Haem synthesis

- There are four major steps in haem synthesis:
- These include:
  1) Synthesis of δ-amino laevulinic acid (ALA).
  2) Synthesis of porphobilinogen (PBG).
  3) Synthesis of the tetrapyrrole.
  4) Addition of Fe$^{2+}$ to form haem.
- The "committed step" for porphyrin biosynthesis is the formation of δ-aminolevulinic acid (δ ALA) by the reaction of the amino acid glycine and succinyl-CoA.
- Two molecules of δ ALA combine to give porphobilinogen (PBG), which contains a pyrrole ring.
Haem synthesis

Fe²⁺-binding elements → mRNA transcription → Glycine

ALA synthetase → d-Amino-levulinic acid (dALA)

ALAD dehydrogenase → Porphobilinogen

4x → PB deaminase → Hydroxymethyl bilane

Uporphyrinogen III synthase → Uroporphyrinogen III

Uporphyrinogen III decarboxylase → Coproporphyrinogen III

Protoporphyrinogen III → Protoporphyrin-III oxidase

Protoporphyrin IX → Hemoglobin

Heme → Ferrochelatase

Mitochondrion → Cytoplasm

Globin chains
• Intermediates are used in different species to form particular substances, but, in humans, the main end-product protoporphyrin IX is combined with iron to form haem.

• Eight enzymes are requested to synthesize haem; four located inside the mitochondria and others in the cytosol.
Structures of the intermediates of the haem synthetic pathway

1. \[\text{Glycine} \rightarrow \text{CH}_2\text{C}-\text{CH}_2\text{-COOH} \]
2. \[\text{HOOCC-CH}_2\text{-CH}_2\text{COOH} \]
3. \[\text{CH}_2\text{NH}_2\text{H}_2 \]
4. \[\text{HOOC-CH}_2\text{-CH}_2\text{-COOH} \]
5. \[\text{Porphobilinogen} \]
6. \[\text{Uroporphyrinogen} \]
7. \[\text{Heptacarboxyporphyrinogen} \]
8. \[\text{Hexacarboxyporphyrinogen} \]

Mitochondrion

Cytosol

- Succinyl CoA
- δ-Aminolevulinic acid
- Heme
- Protoporphyrin IX
- Protoporphyrinogen IX
- Coproporphyrinogen
- Pentacarboxyporphyrinogen

A=acetic acid, Me=methyl, P=proionic acid, V=vinyl
Porphyrin metabolic disorders and porphobilinogen

• Term porphyria refers specifically to a group of diseases in which cells in the liver and/or bone marrow are unable to properly carry out all the steps involved in making haem, due to inherited defects.

• When the haem-making pathway is disrupted in this way, the excess porphyrins start to accumulate in certain body organs where they can have toxic effects.

• There are two categories of porphyria,
  1. Primary porphyria or inherited porphyria
  2. Secondary porphyria or acquired porphyria (Porphyrinopathy)
Primary porphyria

- Metabolic abnormalities of porphyrin metabolism pathway resulted due to inherited enzyme deficiencies.

- They can be divided into two main groups based on clinical manifestation.
  1. Neurological form of porphyria (Acute)
  2. Cutaneous porphyria

- The first group is associated with elevation of the porphyrin precursors, primarily porphobilinogen and amino laevulnic acid.
• There are three of these types but an estimated 90% of those with the inherited enzyme deficiency have no symptoms until their disease is triggered.

• The symptoms of group two is associated with an accumulation of porphyrins.

• The porphyrins deposited in the skin and exposed to sunlight cause considerable skin damage.
Acute and chronic porphyria of both liver as well as erythropoietic origin

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<th>Classification of the porphyrias</th>
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<tr>
<td>hereditary coproporphyria</td>
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<tr>
<td>variegate porphyria</td>
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<td>cutaneous hepatic porphyria</td>
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<tr>
<td>chronic</td>
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<tr>
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<tr>
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<tr>
<td>hepatic</td>
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<td>erythropoietic</td>
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</table>
Secondary porphyria

• Disturbance of porphyrin metabolism not due to enzyme defects are discussed under acquired porphyria.

• Instead of enzyme deficiency a secondary toxin or drug is the cause of disease.

• The most common disorder is lead poisoning
• Lead inhibits the enzymes ALA dehydrase and ferrochelatase leading to accumulation of ALA and coproporphyrin III in urine.

• Mild alterations of porphyrin metabolism are seen in chronic renal failure with altered levels of porphyrin metabolites ratios.
### The features of porphyrias

#### Classification and characteristics of the porphyrias

<table>
<thead>
<tr>
<th>condition</th>
<th>deficient enzyme</th>
<th>inheritance</th>
<th>course</th>
<th>erythroid/hepatic</th>
<th>symptomatology</th>
<th>abnormal porphyrin concentrations</th>
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<tbody>
<tr>
<td>ALA dehydratase deficiency porphyria</td>
<td>ALA dehydratase</td>
<td>AR</td>
<td>acute</td>
<td>E</td>
<td>N</td>
<td>proto ALA</td>
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<td>acute intermittent porphyria</td>
<td>PBG deaminase</td>
<td>AD</td>
<td>acute</td>
<td>H</td>
<td>N</td>
<td>ALA, PBG</td>
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<td>chronic</td>
<td>E</td>
<td>P</td>
<td>Zn-proto proto</td>
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Watson-Schwartz test for urine porphobilinogen

- A qualitative screening test for diagnosis of acute intermittent porphyria, in which Ehrlich diazo agent and saturated sodium acetate are added to the urine is available.
- A pink or red colour indicates the presence of porphobilinogen or urobilinogen.
• This is a useful test because it removes most common interfering substances.

• The presence of thiols, urea, and certain indole compounds can inhibit colour formation and cause a false negative test.
Sensitive method of screening for urinary porphobilinogen

• This screening method is for urinary porphobilinogen (PBG).

• Urine is added to Dowex 2 resin under alkaline conditions in a test tube and mixed.

• The supernatant is removed and the adsorbed PBG is eluted with acid and reacted with Ehrlich's reagent.

• However the HPLC methods are the most accurate test procedure.
Haem compounds, bilirubin and metabolic products

• Haem - a prosthetic group that consists of an iron atom contained in the center of a large heterocyclic organic ring called a porphyrin.

• Not all porphyrins contain iron, but a substantial fraction of porphyrin-containing metalloproteins have haem as their prosthetic group; these are known as haemoproteins.

• Ferrous ion (Fe$^{2+}$) associated with protoporphyrin is referred as haem.
• Haem acts as the prosthetic group of many proteins and haem containing proteins play a variety of role in the body. They include
1. Oxygen transport and storage (Haemoglobin, Myoglobin)
2. Mitochondrial respiration (Cytochrome b, c₁, c₁a, a₃)
3. Enzymic destruction of peroxidase (Catalase, peroxidases)
4. Drug Metabolism (Cytochrome P₄₅₀ mono oxygenase)
5. Tryptophan catabolism (tryptophan oxygenase)
6. The de-saturation of fatty acid (Mitochondrial b₅)
Structure of haem

Heme
(Fe-protoporphyrin IX)
Catabolism of haem

- Haem is degraded to produce biliverdin and bilirubin.

**Haem oxygenase**

Haem → Biliverdin + Fe$^{3+}$

**Biliverdin reductase**

Biliverdin → Bilirubin
• Bilirubin is the orange yellow pigment derived from haem catabolism.

• Bilirubin is produced from protoporphyrin IX by microsomal haem oxygenase.

• The tetrapyrrolic structure is open at the $\alpha$ methane bridge to yield a green biliverdin.
• Biliverdin is converted to bilirubin by biliverdin reductase.

• Daily bilirubin production is approximately 250-300 mg.
  85 % from haemoglobin.
  15 % from myoglobin, cytochrome and peroxidase
Conjugated bilirubin
Serum bilirubin

• Normally, a small amount of bilirubin circulates in the blood.

• Serum bilirubin is considered a true test of liver function, as it reflects the liver's ability to take up, process, and secrete bilirubin into the bile.

• “Free bilirubin," is in a lipid-soluble form that must be made water-soluble to be excreted.
• Free/ unconjugated bilirubin is carried by albumin to the liver, where it is conjugated and made water soluble by forming bilirubin diglucuronide.

• Once it is conjugated into a water-soluble form, bilirubin can be excreted in the urine.
• Bilirubin is chemically different after it goes through the conjugation process in the liver and laboratory tests can differentiate between the unconjugated/indirect bilirubin and conjugated/direct bilirubin.

UDP-glucuronide transferase
Bilirubin + 2 UDP-glucuronate → bilirubin diglucuronide

• Terms "direct" and "indirect" reflect the way the two types of bilirubin react to certain dyes.
• Conjugated bilirubin is water-soluble and reacts directly when dyes are added to the blood specimen.
• Non-water soluble, free bilirubin does not react to the reagents until alcohol or an accelerator is added to the solution.

• Therefore, the measurement of this type of bilirubin is indirect.
• Bilirubin concentrations are elevated in the blood either by increased production, decreased conjugation and decreased secretion by the liver or blockage of the bile ducts.

• In cases of increased production, or decreased conjugation, the unconjugated or indirect form of bilirubin will be elevated.
Bilirubin Estimation

• Most widely used methods for the assay of total bilirubin in serum are based on a coupling reaction with various diazo dyes in the presence of an accelerating agent.

• ‘Direct' reaction, without an accelerator, gives a good estimate of the conjugated and protein bound species of bilirubin only if carefully standardized reaction conditions are used.
Chemical reaction between bilirubin and diazotized sulphanilic acid to yield azo bilirubin
Jendrassik-Jrof method for serum total and conjugated Bilirubin

• **Principle**: Total bilirubin in serum is measured by adding caffeine benzoate reagent to the sample, followed by addition of diazotized sulphanilic acid.

• Both conjugated and unconjugated bilirubin reacts with diazo reagent to produce azo-bilirubin.
Bile pigments in urine

• Bilirubin is found in urine in obstructive jaundice and infective and toxic hepatitis.
• This is due to the fact that only water soluble conjugated bilirubin is filtered at the glomeruli and excreted in urine when present in plasma above a small threshold amount.
• Bilirubin may be present in urine in the early stages of infective hepatitis.
• Bile pigments in urine are tested by Fouche's test; a bluish green colour is developed.
### Urine analysis – Chemical Characteristics

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<th>Associated Clinical Conditions</th>
<th>Characteristics</th>
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<td>Hay’s test</td>
<td>Viral hepatitis</td>
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<td>Alcoholic hepatitis</td>
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<td>Toxic hepatitis</td>
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<td>Drug induced hepatitis</td>
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<td></td>
<td></td>
<td>Obstructive jaundice</td>
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<tr>
<td>Bile pigments</td>
<td>Fouchet’s test</td>
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Urobilinogen

• Urobilinogens are formed in the intestine by bacterial action on conjugated bilirubin.

• The major part is excreted in the faeces where it exists as a mixture of urobilinogen and urobilin formed by partial oxidation of urobilinogens on exposure to air.

• Enterohepatic circulation makes the reabsorption of urobilinogen.
Small fraction of urobilinogen is reabsorbed in terminal ileum.

Some urobilinogen excreted in urine. Converted to **Urobilin** in urine. Gives yellow color to urine.
• A very small part enters general circulation and is excreted in urine.

• Urobilinogen in urine is tested by Ehrlich`s reagent; where a solution of para dimethyl aminobenzaldehyde is used.

• If urobilinogen is present in increased amounts a distinct red colour is obtained. Urobilinogen in faeces is tested by modified Ehrlich`s reagent.

• Faecal urobilinogen is increased in haemolytic jaundice and pernicious anaemia.
The amount of urobilinogen present in urine depends both on the amount of urobilinogen entering the intestine and on the ability of the liver to excrete the urobilinogen coming to it from intestine.

In obstructive jaundice, urobilinogen is very low in urine due to almost complete absence of urobilinogen in intestine while test for bilirubin is positive.
Bilirubin metabolism and related disorders

• The largest repository of haem in the human body is in red blood cells, which have a life span of about 120 days.

• There is a turnover of about 6 g/day of haemoglobin, which presents two problems.

• First, the porphyrin ring is hydrophobic and must be solubilized to be excreted.
• Second, iron must be conserved for new haem synthesis.

• Red blood cells and haem from other sources are engulfed by cells of the reticulo endothelial system.

• Haem is oxidized, with the haem ring being opened by the endoplasmic reticulum enzyme, haem oxygenase.
Defects of bilirubin metabolism at any step of the cycle results in jaundice.
Inherited disorders of bilirubin metabolism

• Common five types of syndromes have been identified.

• Some of them are due to enzyme deficiencies and others are due to autosomal dominant disorders.

• Three grades of inherited unconjugated hyperbilirubinaemia are recognised in humans.

• This spectrum of disorders is distinguished primarily on the basis of the plasma bilirubin level.
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<th>Syndrome</th>
<th>Defect</th>
<th>Clinical features</th>
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<tr>
<td>Gilbert’s</td>
<td>decreased conjugation of bilirubin and decreased uptake in some cases (autosomal dominant)</td>
<td>mild, fluctuant unconjugated hyperbilirubinaemia which increases on fasting</td>
</tr>
<tr>
<td>Crigler–Najjar</td>
<td>Type 1 (autosomal recessive) absence of conjugating enzyme</td>
<td>severe unconjugated hyperbilirubinaemia, early death due to kernicterus</td>
</tr>
<tr>
<td></td>
<td>Type 2 (?autosomal recessive) partial defect of conjugating enzyme</td>
<td>partial response to phototherapy, none to phenobarbitone</td>
</tr>
<tr>
<td></td>
<td>decreased hepatic excretion of bilirubin (autosomal recessive)</td>
<td>severe unconjugated hyperbilirubinaemia, but good response to phenobarbitone and phototherapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>often survive to adulthood</td>
</tr>
<tr>
<td>Dubin–Johnson</td>
<td></td>
<td>mild, fluctuant conjugated hyperbilirubinaemia</td>
</tr>
<tr>
<td></td>
<td>hepatic pigment disposition (melanin)</td>
<td>increased coproporphyrin I/III ratio in urine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bilirubinuria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>normal lifespan</td>
</tr>
<tr>
<td>Rotor</td>
<td>unknown (autosomal recessive)</td>
<td>similar to Dubin–Johnson but no hepatic pigmentation</td>
</tr>
</tbody>
</table>
Jaundice of newborn

• Newborn jaundice is a condition marked by high levels of bilirubin in the blood.
• Increased bilirubin cause the infant's skin and sclera of the eyes to look yellow.
• Placenta removes the bilirubin from the infant so that it can be processed by the mother's liver.
• Immediately after birth, the baby's liver begins to take over the job, but this can take time.
• Therefore, bilirubin levels in an infant are normally a little higher after birth.
Higher levels of bilirubin can be due to

• Conditions that increase the number of red blood cells that need to be processed
• Anything that interferes with the body’s ability to process and remove bilirubin
• The following increase the number of red blood cells that need to be processed:
  – abnormal blood cell shapes,
  – congenital spherocytic anemia,
  – elliptocytosis,
  – blood type incompatibilities such as ABO incompatibility and Rh incompatibility,
  – cephalohaematoma or other birth injury and
  – glucose-6-phosphate dehydrogenase deficiency.
• Jaundice in newborns most commonly occurs because their livers are not mature enough to remove bilirubin from the blood.

• Jaundice may also be caused by a number of other medical conditions.

• There are two main types of newborn jaundice
  
  physiologic and
  
  hyperbilirubinemia.
Physiological jaundice

• Caused by a mild elevation of bilirubin and is not usually harmful to infants.
• Affects nearly all newborns, develops between 72 and 96 hours after birth, and usually goes away by one to two weeks after birth.
• Infants who are born at 35 to 37 weeks of gestation, or who are Asians, may require more time to resolve.
Jaundice

Yellowing of eyes

Yellowing of skin

Excess bilirubin in blood

Kernicterus

Bilirubin moves from bloodstream into brain tissue

Before phototherapy

After phototherapy
Hyperbilirubinaemia

• More serious than physiological jaundice.
• Infants with hyperbilirubinaemia may develop it within the first 24 hours of life, have higher blood bilirubin levels, or have a rapid rise in bilirubin levels.
Neonatal unconjugated hyperbilirubinaemia

- Increased production — The factors that increase red blood cell breakdown beyond what is normally seen in the newborn period, include: bruising and birth trauma
- Blood incompatibility between the mother and infant's blood, which results in the mother's immune system attacking and destroying the infant's red blood cells
- Inherited causes of red blood cell breakdown (such as glucose-6-phosphate dehydrogenase [G6PD] deficiency)
Breastfeeding

There are two types of jaundice associated with breastfeeding,
1. Breast milk jaundice and
2. Breastfeeding failure jaundice.

Breast milk jaundice

- Breast milk jaundice typically begins after three to five days of life, peaks within two weeks after birth, and declines over three to 12 weeks.

- Breast milk jaundice occurs in one to two percent of breastfed babies due to mother’s milk.
Breastfeeding failure jaundice

• This is distinct from breast milk jaundice, as it is not caused by mother's milk.
• This occurs if a newborn is not getting enough breast milk.
• Occurs in 5 to 10 % of infants, particularly in babies who have difficulty with breastfeeding due to physical problems (prematurity, cleft lip or palate, tongue-tie) or insufficient mother's milk supply.
Conjugated hyperbilirubinaemia

• Defined as a conjugated bilirubin concentration > than 2 mg/dL (34.2 mmol/L) or more than 20% of total bilirubin.

• Conjugated hyperbilirubinemia in the newborn is a sign of cholestasis and always requires further investigation.
• Because cholestasis implies impairment of bile flow at any point from its formation in the hepatocyte to its excretion from the common bile duct, the causes of neonatal cholestasis are many.

• Causes for this may be due to infections, metabolic defects, extrahepatic causes and intrahepatic lesions.
Metabolism of creatine, creatinine and the estimation in blood and urine
Metabolism of creatine, creatinine and the estimation in blood and urine

• Creatine is a natural substance that is found in the muscles of humans and animals.

• It is composed of three amino acids (arginine, glycine and methionine).

• The more creatine phosphate that is stored in the muscle the more rapidly the ADP converts to ATP.
• When the ATP supply in the body has been depleted, fatigue sets in.

• Estimation of serum creatinine is a useful and inexpensive method of evaluating renal dysfunction.

• Creatinine is a non-protein waste product of creatine phosphate metabolism by skeletal muscle tissue.

• Its production is continuous and proportional to the muscle mass.
• Creatinine is freely filtered from the glomeruli and therefore the serum creatinine level depends on the Glomerular Filtration Rate (GFR).

• Renal dysfunction diminishes the ability to filter creatinine and the serum creatinine rises.

• A threefold increase is considered to reflect a 75 % loss of kidney function.
Biochemistry of creatine and creatinine

• In humans, half of the daily creatine is biosynthesized and the rest is taken in from diet (fish and meat).

• 95% of creatine is later stored in the skeletal muscles

• Creatine supplementation has been, and continues to be, investigated as a possible therapeutic approach for the treatment of muscular, neuromuscular, neurological and neurodegenerative diseases
Action of creatine phosphokinase (creatine kinase)
Phosphocreatine/ creatine phosphate or PCr (Pcr), serves as a rapidly mobilizable reserve of high-energy phosphates in skeletal muscle and brain.

Phosphocreatine can anaerobically donate a phosphate group to ADP to form ATP during the first 2 to 7 seconds following an intense muscular or neuronal effort.

Conversely, excess ATP can be used during a period of low effort to convert creatine to phosphocreatine.

The reversible phosphorylation of creatine (i.e., both the forward and backward reaction) is catalyzed by several creatine kinases (CK).
• Creatinine production is proportional to muscle mass and varies little from day to day.

• Approximately 2 % (15 to 30 mg creatinine per kg body weight) of the body's creatine is converted to creatinine every day.

• Creatinine is transported through the bloodstream to the kidneys.
• Creatinine is small (mw 113 daltons) and it does not bind to plasma proteins.

• The kidneys filter out most of the creatinine and dispose of it in the urine.

• There is little-to-no tubular reabsorption of creatinine.

• If the filtering of the kidney is deficient, blood levels rise.
• Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which reflects the glomerular filtration rate (GFR).

• GFR is clinically important - is a measurement of renal function.
Serum creatinine

• **Plasma creatinine (PCr)**
  • Measuring serum creatinine is a simple test and it is the most commonly used indicator of renal function.
  • A rise in blood creatinine levels is observed only with marked damage to functioning nephrons.
  • Therefore, this test is not suitable for detecting early stage kidney disease.
  • A better estimation of kidney function is given by the creatinine clearance test.
• Serum creatinine will vary a little from one test to another and it should not be interpreted as an "improvements" or "worsening" of kidney function.

• In addition to an error margin of about 10 %, serum creatinine results may vary depending on which lab method was used, and being dehydrated will increase it.

• It may also rise during fevers which cause an acute renal insufficiency
• Serum creatinine level will not be significantly affected by exercise or the protein intake.

• Serum creatinine does not rise above normal until creatinine clearance has already declined to half of normal (50% kidney function).

• By the time a blood test shows an elevated serum creatinine, almost half of the renal function would be lost.
Estimation of serum creatinine

• Measurement of serum creatinine is the most widely used measure of renal function.
• The creatinine blood test may be ordered, along with blood urea nitrogen (BUN) test and microalbumin, at regular intervals when you have a known kidney disorder or have a disease that may affect kidney function
• Both BUN and creatinine may be ordered when a CT scan is planned, prior to and during certain drug therapies, before and after dialysis to monitor the effectiveness of treatments.
Jaffe Reaction

- Most commonly used methods for measuring creatinine
- Creatinine is treated with an alkaline picrate solution to yield a yellowish red complex
- This reaction is nonspecific and subject to interference from many non creatinine chromogens, (including acetone, acetoacetate, pyruvate, ascorbic acid, glucose)
- It is also sensitive to pH and temperature changes.
• Modifications done to reduce these sources of error

• Kinetic-rate modification, which isolates the brief time interval during which only true creatinine contributes to total colour formation, is the basis of recent kit base assays.

• Numerous methods have been developed which remove the interfering substance by some type of sample pretreatment, the most commonly performed pretreatment methods being protein precipitation and removal by filtration, treatment with Lloyd's reagent, or removal of protein by dialysis through a membrane.
• Creatinine must be determined in plasma or serum and not in whole blood because erythrocytes contain considerable amounts of non creatinine chromogens.

• To minimize the conversion of creatine to creatinine, specimens must be as fresh as possible and maintained at pH 7 during storage.
Test principle

• Creatinine + alkaline picrate → creatinine-picrate complex

• The rate of increase in absorbance at 510 nm due to the formation of the creatinine picrate complex is directly proportional to the concentration of creatinine in the sample.

• Enzymatic method is non-caustic, non-staining and easy to handle.

• Unlike the Jaffe method the enzymatic assay shows virtually no interference from ascorbic acid (50 mg/dL), bilirubin (16mg/dL), haemoglobin (200 mg/dL) and triacyl glycerol (1000 mg/L).
Enzymic reactions of the enzymic end point estimation of serum creatinine
Excretion of urinary creatinine and creatinine clearance (CCr)

- Urine creatinine value may also be used with a variety of other urine tests as a correction factor.

- Since it is produced and removed at a relatively constant rate, the amount of urine creatinine can be compared to the amount of another substance being measured. Examples of this are when creatinine is measured with protein to calculate a urine protein/creatinine ratio (UP/CR) and when it is measured with microalbumin to calculate microalbumin/creatinine ratio (ACR).
• These tests are used to evaluate kidney function as well as to detect other urinary tract disorders.

• Creatinine clearance can be accurately calculated using the serum creatinine concentration and some or all of the following variables: sex, age, weight, and race

• A combination of blood and urine creatinine levels may be used to calculate the creatinine clearance.
• A creatinine clearance test compares serum creatinine to the amount of creatinine eliminated in urine during a given time period, usually 24 hours.

• To start this test, the bladder is emptied and urine samples are collected for the next 24 hours.

• One blood sample will be taken to measure serum creatinine.
Calculation of CrCl

Creatinine clearance (CrCl) can be calculated if values for
– creatinine's urine concentration (UCr),
– urine flow rate (V), and
– creatinine's plasma concentration (PCr) are known.
\[ GFR = \frac{\text{Urine Concentration} \times \text{Urine Flow}}{\text{Plasma Concentration}} \]

\[ C_{Cr} = \frac{U_{Cr} \times V}{P_{Cr}} \]
• Commonly a 24 hour urine collection is undertaken with a comparative blood test then taken.

• The urinary flow rate is still calculated per minute, hence:

\[ C_{Cr} = \frac{U_{Cr} \times 24\text{-hour volume}}{P_{Cr} \times 24 \times 60\text{mins}} \]

\[ C_{Cr-\text{corrected}} = \frac{C_{Cr} \times 1.73}{BSA} \]
• To allow comparison of results between people of different sizes, the CrCl is often corrected for the body surface area (BSA) and expressed compared to the average sized man as mL/min/1.73 m².

• While most adults have a BSA that approaches 1.7 (1.6-1.9), extremely obese or slim patients should have their CrCl corrected for their actual BSA.

• **Urine volume** - Measured using a 1000 ml measuring cylinder
• **Serum creatinine** – As described above in 35.03
• **Urine creatinine** - Conduct from diluted urine
Creatinine clearance test and clinical interpretation

• **Why the test is performed?**
• Done to evaluate kidney function.
• Creatinine is removed from the body entirely by the kidneys.
• If kidney function is abnormal, creatinine levels will increase in the blood.
• Females usually have a lower creatinine level than males, because they usually have less muscle volume.
The abnormal findings include

- Higher-than-normal levels may indicate:
  - Acute tubular necrosis
  - Dehydration
  - Diabetic nephropathy
  - Eclampsia (a condition of pregnancy that includes seizures)
  - Glomerulonephritis
  - Kidney failure
  - Muscular dystrophy
  - Preeclampsia (pregnancy-induced hypertension)
  - Pyelonephritis
  - Reduced kidney blood flow (shock, congestive heart failure)
  - Rhabdomyolysis
  - Urinary tract obstruction
Lower-than-normal levels may indicate

- Muscular dystrophy (late stage)
- Myasthenia gravis
Additional conditions under which the test may be performed

• Cushing syndrome
• Diabetes mellitus
• Digitalis toxicity
• Haemolytic-uraemic syndrome (HUS)
• Lupus nephritis
• Malignant hypertension (arteriolar nephrosclerosis)
• Thrombotic thrombocytopenic purpura
• **Reference values for serum creatinine:**

• Adult males: 0.4 - 1.4 mg/dl: (values are slightly higher in males due to larger muscle mass)

• Adult females: 0.4 - 1.1 mg/dl: (creatinine clearance is increased in pregnancy, resulting in lower serum levels)

• Children: 0.2 - 1.0 mg/dl: (slight increases with age because values are proportional to body mass)