Estimation of Serum Bilirubin

Background

- **Bilirubin** (formerly referred to as **haematoidin**) is the yellow breakdown product of normal haeme catabolism.
- Having molecular formula $C_{33}H_{36}N_4O_6$.
- Bilirubin is excreted in bile and urine, and elevated levels may indicate certain diseases.
- It is responsible for the yellow color of bruises, the background straw-yellow color of urine.
- the brown color of faeces (via its conversion to stercobilin), and the yellow discoloration in jaundice.
- Bilirubin is the end product of hemoglobin and serve as a diagnostic marker of liver and blood disorders.

Formation of Bilirubin

- The breakdown of haeme produces bilirubin (an insoluble waste product) and other bile pigments. Bilirubin must be made water soluble to be excreted. This transformation occurs in 5 steps;-

1. **Formation**... Macrophages converts heme to Biliverdin
2. **Plasma transport**... via albumin and
3. **Liver uptake**... bilirubin is taken by hepatocytes
4. **Conjugation**... to glucoronic acid via UDP-glucoranyle transferase
5. **Biliary excretion**... in bile→ intestine→ stercobilin/urobilin formed and excreted

- **One gram of hemoglobin yields 35 mg of bilirubin. The daily bilirubin formation in adult human is about 250 mg.**

Difference Between Conjugated and Unconjugated Bilirubin

Conjugated Bilirubin

- Present Normally in Bile
- Conjugated to glucoronic acid
- Can be filtered by kidney
- Cannot cross BBB

Unconjugated Bilirubin

- Present Normally in Plasma
- Conjugated to Albumin
- Cannot be filtered by kidney
- Can cross BBB

Functions of Bilirubin

- Levels of serum bilirubin are inversely related to risk of certain heart diseases
- Acts as uncoupler in Neonates and thus maintain body heat
- Bile pigments such as Biliverdin naturally possess significant anti-mutagenic and antioxidant properties
- Biliverdin and bilirubin have been shown to be potent scavengers of peroxyl radicals
- inhibit the effects of polycyclic aromatic hydrocarbons, heterocyclic amines, and oxidants—all of which are mutagens.

Diagnostic Importance of Bilirubin

- Clinically hyperbilirubinemia appears as jaundice or icterus
- Jaundice can usually be detected when the serum bilirubin level exceeds 2.0 to 2.5 mg/dl
- When the level of bilirubin is between 1 to 2 mg/dl ,it is known as latent jaundice
- In neonates, unconjugated bilirubin can cross BBB and thus lead to accumulation in Brain of neonates. This interfere with nervous system development and permanent Nervous impairment.

Normal Range

Bilirubin Form	Normal Value
Total (elderly/Adult)	0.2 — 0.8 mg/dl
Newborn	0.8 — 12 mg/dl
Critical Value(adult)	>12 mg/dl
Critical Value (Newborn)	>15 mg/dl
Fecal urobilinogen	40 – 280 mg/dl
Urine	0.0 – 0.02 mg/dl

Clinical Correlates

Hypo Bilirubinemia

- No clinical significance of low bilirubin level.
- Some researches suggest that low bilirubin may contribute to Cerebrovascular or cardiovascular events.

Hyper Bilirubinemia

- Two Types
  - Unconjugated Hyperbilirubinemia
  - Conjugated Hyperbilirubinemia

Unconjugated Hyperbilirubinemia

- **Overproduction**
  - Hemolysis (intra and extravascular)
- **Decreased hepatic uptake**
- **Decreased bilirubin conjugation (Transferase deficiency)**
  - Gilbert's Syndrome
  - Crigler-Najjar Syndrome
  - Neonatal Jaundice
- **Acquired Transferase Deficiency**
  - Drug inhibition (chloramphenicol and pregnanediol)
  - Breast milk jaundice (Transferase inhibition by pregnanediol and fatty acids in breast milk)
  - Hepatocellular disease (hepatitis, cirrhosis)
  - Sepsis

Conjugated Hyper Bilirubinemia

- **Impaired hepatic excretion**
  - Dubin-Johnson syndrome
  - Recurrent (benign) intrahepatic cholestasis
  - Cholestatic jaundice of pregnancy
- **Acquired disorders**
  - Hepatocellular disease (e.g. Viral or drug induced hepatitis, cirrhosis)
  - Drug induced cholestasis (e.g. Oral contraceptives, androgens, chlorpromazine)
  - Alcoholic liver disease.
  - Sepsis
  - Biliary cirrhosis
- **Extra-hepatic Biliary obstruction**
  - Gallstones
  - Biliary malformation .
  - Infection
  - Malignancy
  - Hemophilia (trauma, tumor)
  - Sclerosing cholangitis
  - Malignancy
  - Inflammation (pancreatitis)

Practical / Procedure

Requirements

- Working Reagent
  - R1 → Sulphanilic Acid + HCl
  - R2 → Sodium Nitrite
  - R3 → Caffeine + Sodium Benzoate
  - R4 → Tartarate + Sodium Hydroxide

- Reaction/ Method is called "Jandrassik-groff Method"

Principle

- Total bilirubin concentration is determined in presence of caffeine by the reaction with diazotized sulphanilic acid to produce colored diazo dye.
- Intensity of the color of the dye, measured at 560-600nm, is proportionate to the concentration of the total bilirubin.
- Direct Bilirubin is determined in absence of caffeine by direct reaction with diazotized sulphanilic acid to form Red Colored azobilirubin.
- Intensity of the color of the dye, measured at 546 nm, is proportionate to the concentration of the direct bilirubin.

- Sulphanilic Acid + $NaNO_2$----------→ Diazotized sulphanilic acid
- Bilirubin + Diazotized Sulphanilic Acid----------→ Azobilirubin

Procedure for Total Bilirubin

- Take Two test tubes marked as Sample Blank(B) and Unknown(U)
- Add R1 reagent about 200 µl to both test tubes.
- Add R2 reagent about one drop to the Test tube marked as unknown
- Add R3 reagent about 1.0 ml to both Test Tubes
- Now add Serum about 200 µl to both test tubes.
- Mix and incubate for 10 minutes at 20—25 degree Celsius
- Now add R4 1.0 ml to both Test tubes
- Mix and incubate for 5 minutes at 20—25 degree Celsius
- Check color intensity at 578 nm

- **Calculation: Unknown Intensity x 10.8**

Procedure for Direct Bilirubin

- Take Two test tubes marked as Sample Blank(B) and Unknown(U)
- Add R1 reagent about 200 µl to both test tubes.
- Add R2 reagent about one drop to the Test tube marked as unknown
- Add Normal Saline about 2.0 ml to both Test Tubes
- Now add Serum about 200 µl to both test tubes.
- Mix and incubate for 10 minutes at 20—25 degree Celsius
- Check color intensity at 546 nm

- **Calculations: Unknown intensity x 14.4**

Precautions

- The only acceptable anticoagulants are heparin and oxalate.
- Fresh Serum to be obtained for Experiment.
- Ideally, after Centrifugation, serum should be immediately separated from blood cells.
- Do not try to ingest or inhale the reagent Solution. In case of contact, wash thoroughly and seek medical help.

Thanks A Lot

Liver Function Tests (LFTs)

ALT&AST

The liver is in the upper right part of the abdomen.

The functions of the liver include:

1 - storing glycogen (fuel for the body) which is made from sugars.

2- helping to process fats and proteins from digested food.

3- making proteins that are essential for blood to clot (clotting factors).

4- processing many medicines which you may take.

5- helping to remove poisons and toxins from the body.

Liver function tests: ***-LFTs are group of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver.***

-As the liver performs it's various functions it makes a number of chemicals that pass into the bloodstream and bile. Various liver disorders alter the blood level of these chemicals. Some of these chemicals can be measured in a blood sample. Some tests that are commonly done on a blood sample are called 'LFTs' (liver function tests).

Liver function tests can be classified as:

a. Tests of excretion by the liver.
b. Evaluation of synthesis in liver.
C. Evaluation of enzyme activity.

Liver function tests are most often employed to determine:

i. The presence of liver disease.
ii. The type of liver disease.
iii. The extent and progression of liver disease.

Note: The diagnosis of liver disease depends upon a complete history, complete physical examination, and evaluation of liver function tests and further invasive and noninvasive tests.

These usually measure the following:

- Alanine Aminotransferase (ALT) = SGPT:

  -ALT is the enzyme produced within the cells of the liver. The level of ALT abnormality is increased in conditions where cells of the liver have been inflamed or undergone cell death. As the cells are damaged, the ALT leaks into the bloodstream leading to a rise in the serum levels. Any form of hepatic cell damage can result in an elevation in the ALT. ALT is the most sensitive marker for liver cell damage.

- Clinical applications of ALT assays are confined mainly to evaluation of hepatic disorders.
- Higher elevations are found in hepatocellular disorders than in extrahepatic or intrahepatic obstructive disorders.
- In acute inflammatory conditions of the liver, ALT elevated higher than AST. (More specific than AST).

Aspartate Aminotransferase (AST) = SGOT: -This enzyme also reflects damage to the hepatic cell. It is less specific for liver disease. It may be elevated and other conditions such as a myocardial infarct (heart attack). Although AST is not a specific for liver as the ALT, ratios between ALT and AST are useful to physicians in assessing the etiology of liver enzyme abnormalities.

• Alkaline Phosphatase (ALP):

-Alkaline phosphatase is an enzyme, which is associated with the biliary tract. It is not specific to the biliary tract. It is also found in bone and the placenta.

-If the alkaline phosphatase is elevated, biliary tract damage and inflammation should be considered. One of the more common methods to assess the etiology of the elevated alkaline phosphatase is to determine whether the GGT is elevated or whether other function tests are abnormal (such as bilirubin)

-Alkaline phosphatase may be elevated in primary biliary cirrhosis, alcoholic

1 - Immunological studies

2- Prothrombin time (PT)

3- Liver biopsy, ultrasound scan, other types of scan, etc, may be needed to clarify the cause of a liver disorder, and/or to monitor its progress.

Lab practices: ***-Collect blood and prepare serum in an appropriate tube.***

-Measure ALT and AST levels.

-Compare the results to the normal values.

Assay for Enzyme Activity:

- Alanine + a-Ketoglutarate ALT > Glutamate + Pyruvate
- Pyruvate + NADH + H LDH Lactate + NAD

- The rate of decrease in conc. of NADH, measured photometrically, is proportional to the catalytic con. of ALT present in the sample.

  Reference Range:

  Normal: 6 - 37 U/L.

  Interfering Factors:

  - Many drugs may cause falsely increased and decreased ALT levels.
  - Therapeutic heparin increases ALT.
  - Hemolysed blood increases ALT.

CALCIUM

- 5th most abundant element of body.
- The MOST PREVALENT cation.
- Skeleton of body contains 99% of total Ca.

Tissue	Calcium	Phosphate	Magnesium
Skeleton	99%	85%	55%
Soft tissue	1%	15%	45%
Extracellular fluid	<0.2%	<0.1%	1%
Total	1000 g (25 mol)	600 g (19.4 mol)	25 g (1.0 mol)

Modified from Auerbach GD, Marx SJ, Spiegel AM. Parathyroid hormone, calcitonin, and the calciferols. In: Wilson JD, Foster DW, eds. Williams textbook of endocrinology, 8th ed. Philadelphia: WB Saunders, 1992:1397-476

Distribution of Ca, P, Mg in the body

The conc. of Ca, P, Mg depends on net effect of

- Bone mineral deposition and resorption
- Intestinal absorption
- Renal excretion

Principle hormones regulating these three processes are

- PTH
- 1,25 Dihydroxy Vit-D

- Virtually all Ca is present in plasma with an average concentration of 9.5mg/dL.
- Ca exists in plasma in three phyiological form

- 50% free (ionized)
- 40% bound to proteins (primarily albumin)
- 10% complexed to small anions

Ca is redistributed among these pools by

- Alteration in conc of proteins or small ions
- Change in pH
- Change in free and total calcium in serum

- Free Ca is the BIOLOGICALLY ACTIVE form, with tight regulation by PTH and 1,25 DHCC
- Key role in

- Muscle contraction
- Hormone secretion
- Glycogen metabolism
- Cell division

Extracellular Ca is needed for bone mineralization, blood coagulation and other function.

A decreased FREE Ca level in serum causes increased neuromuscular excitability and TETANY.

Reference Intervals

The reference interval for total calcium in adults is approximately 8.6 to 10.3 mg/dL (2.15 to 2.57 mmol/L). The reference interval for free calcium in adults is about 4.6 to 5.3 mg/dL (1.15 to 1.33 mmol/L).

Total calcium declines in parallel with serum albumin during pregnancy, whereas free calcium is unchanged.

Causes of Hypocalcemia

Hypoalbuminemia
Chronic renal failure
Magnesium deficiency
Hypoparathyroidism
Pseudohypoparathyroidism
Osteomalacia and rickets due to vitamin D deficiency or resistance
Acute hemorrhagic and edematous pancreatitis
Healing phase of bone disease of treated hyperparathyroidism, hyperthyroidism, and hematological malignancies (hungry bone syndrome)

Cause of Hypercalcemia

- Primary hyperparathyroidism
  - Adenoma, hyperplasia, carcinoma
  - Familial
  - Multiple endocrine neoplasia type I
  - Multiple endocrine neoplasia type II
- Malignancy
  - Skeletal metastases
  - Humoral hypercalcemia
    - Parathyroid hormone–related protein
    - Growth factor(s) (e.g., epidermal and platelet-derive
  - Hematological malignancy
    - Cytokines (interleukin-1, tumor necrosis factor, etc.
    - 1,25-Dihydroxyvitamin D (lymphoma)
  - Coexistent primary hyperparathyroidism
- Other endocrine disorders
  - Hyperthyroidism
  - Hypothyroidism
  - Acromegaly
  - Acute adrenal insufficiency
  - Pheochromocytoma
- Familial hypocalciuric hypercalcemia
- Idiopathic hypercalcemia of infancy
- Vitamin overdose, vitamin D or A
- Granulomatous diseases, e.g., Sarcoid, tuberculosis
- Renal failure
  - Chronic, acute (diuretic phase) or after transplant
- Chlorothiazide diuretics
- Lithium therapy
- Milk-alkali syndrome
- Hyperalimentation regimens
- Immobilization
- Increased serum proteins
  - Hemoconcentration
  - Paraprotein

Measurement of Total Calcium

- **METHODS:**
  - Chelation with o-Cresolphthalein Complexone(Colorimetric)
  - Atomic absorption Spectrophotometry (AAS)
  - Flame photometer
  - ISE (used to measure FREE Ca also)

PRINCIPLE OF THE METHOD

The measurement of calcium in the sample is based on formation of color complex between calcium and *o*-cresolphtalein in alkaline medium:

$$Ca^{++} + o\text{-Cresolphtalein OH} \longrightarrow \text{Colored complex}$$

O-Cresolphthalein Complex one gives violet color in alkaline medium.

The intensity of the colour formed is proportional to the calcium concentration in the sample

SAMPLES -

- Serum or plasma: Separated from cells as rapidly as possible. Blood anticoagulants with oxalate or EDTA are not acceptable since these chemicals will strongly chelate calcium.

- Urine: Collect 24 hour urine specimen in calcium free containers. The collecting bottles should contain 10 ml of diluted Nitric acid (50% v/v). Record the volume.

PROCEDURE

1. Assay conditions:

- Wavelength: . . . . . 570 nm (550-590)
- Cuvette: . . . . . .1 cm. light path
- Temperature . . . . . 37ºC / 15-25ºC

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

	Blank	Standard	Sample
R1 (mL)	0.5	0.5	0.5
R2 (mL)	0.5	0.5	0.5
Standard (µL)	-----	10	-----
Sample (µL)	-----	------	10

4. Mix and incubate for 5 min at 37°C / 15-25°C. 5. Read the absorbance (A) of the samples and calibrator, against the Blank.
The color is stable for at least 40 minutes.

PRECAUTIONS:

- Avoid venous stasis (Increase protein & calcium)
- Do not use contaminated glass ware (Increase calcium)
- Lipemic Samples (Prepare blank 0.05 ml sample + 2.5 D.W)

Corrected Calcium (mg/dL) = Total Calcium (mg/dL) + 0.8(4 – Albumin [g/dL])

ESTIMATION OF PHOSPHORUS

By

WHAT IS A SERUM PHOSPHOROUS TEST?

- Phosphorus is an important part of several of your body's processes. It helps with bone growth, energy storage, and nerve and muscle production. Many foods, especially meats and dairy products, contain phosphorus, so it's usually easy to get enough of this mineral in your diet.

- Most of your body's phosphorus is contained in your bones and teeth. However, some is in your blood. Your doctor can assess your blood phosphorus levels using a serum phosphorus test.

- **Hyperphosphatemia** is when you have too much phosphorus in your blood. **Hypophosphatemia** is the opposite: having too little phosphorus. Various conditions, including liver disease and vitamin D deficiency, can cause your blood phosphorus level to become too high or too low.
- A serum phosphorus test can be used to determine whether you have high or low phosphorus levels, but it cannot help your doctor diagnose the cause of your condition. The doctor will need to perform more tests to determine what is causing your abnormal serum phosphorus test results.

WHY DOCTORS PERFORM A SERUM PHOSPHORUS TEST

Your doctor may order a serum phosphorus test if he or she suspects that your phosphorus level is too low or too high. Either extreme can lead to health problems.

Symptoms that may indicate your phosphorus level is too low include:

- anxiety or irritability
- bone issues, such as: pain, fragility, and poor development (in children)
- breathing irregularity
- fatigue
- loss of appetite
- muscle weakness
- weight gain or loss

If the level of phosphorus in your blood is too high, you may have deposits of phosphorus (combined with calcium) in your muscles. This is rare and only occurs in people with severe calcium absorption or kidney problems. More commonly, excess phosphorus leads to cardiovascular disease or osteoporosis (weakening of your bones).

Your doctor may also order a serum phosphorus test if you received abnormal results from a blood calcium test. Your body needs to maintain a delicate balance between levels of calcium and phosphorus, so an abnormal result on a calcium test may indicate that your phosphorus levels are also atypical.

CLINICAL SIGNIFICANCE

High Levels

If your kidney function is impaired, excess phosphorus will likely build up in your bloodstream. Avoiding high-phosphorus foods, such as milk, nuts, beans, and liver can help you lower your phosphorus levels.

In other cases, high phosphorus levels may be caused by:

- certain medications, such as laxatives that contain phosphates
- dietary problems, such as consuming too much phosphate and/or vitamin D
- diabetic ketoacidosis (when your body runs out of insulin and begins to burn fatty acids instead)
- hypocalcemia (low serum calcium levels)
- hypoparathyroidism (impaired parathyroid glands, leading to low levels of parathyroid hormone)
- liver disease

CLINICAL SIGNIFICANCE

- **Low Levels**

Low phosphorus levels may be caused by a range of nutritional problems and medical conditions, including:

- lack of vitamin D
- not getting enough phosphorus in your diet
- malnutrition
- alcoholism
- hypercalcemia (high serum calcium levels)

- hyperparathyroidism (overactive parathyroid glands, leading to high levels of parathyroid hormone)
- severe burns

ESTIMATION OF PHOSPHORUS

Specimen

Serum.

Plasma must not be used. Anticoagulants may cause false low results.

Stability in serum: 7 days at +4°C
2 days at 20...25°C

Assay

Wavelength: 340 nm, Hg 334 nm
Optical path: 1 cm
Temperature: 20...25°C
Measurement: against reagent blank; one reagent blank per series is required

Pipetting Scheme

Pipette into cuvettes	Reagent blank	Sample or STD
Sample/STD	---	10 µl
RGT	1000 µl	1000 µl
Mix, incubate at least 1 minute at room temperature. Measure the absorbance of the sample and the STD against the reagent blank within 60 minutes (ΔA).		

Calculation of phosphorus concentration

$$C = 10 \times \frac{\Delta A_{Sample}}{\Delta A_{STD}} \quad (mg/dl)$$

or

$$C = 3.2 \times \frac{\Delta A_{Sample}}{\Delta A_{STD}} \quad (mmol/l)$$

Normal Values [3]

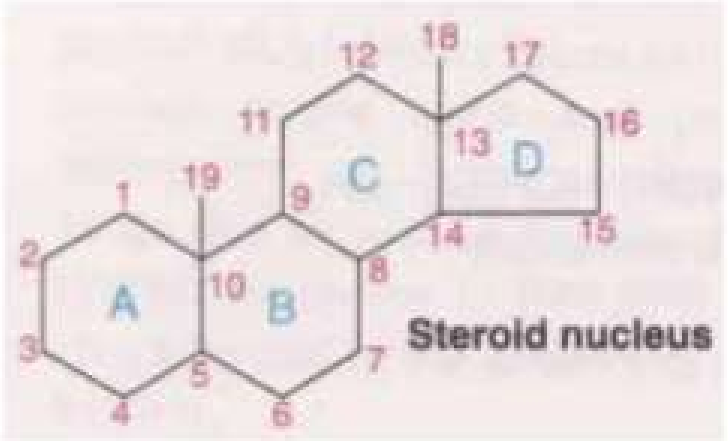
Inorganic phosphorus

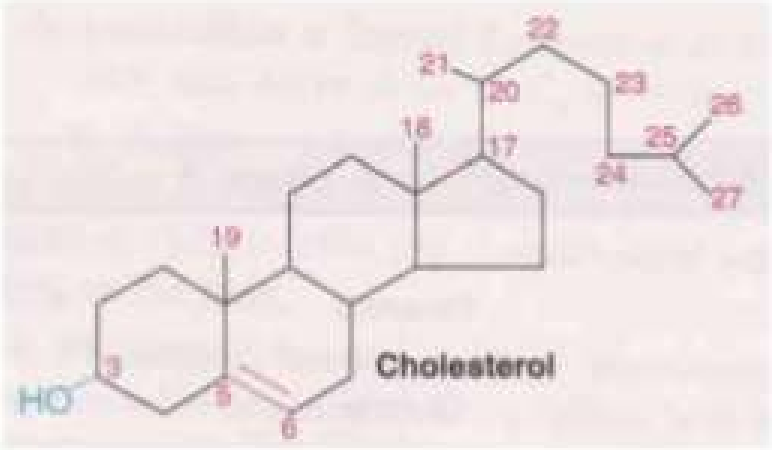
Adults:	2.5-5.0 mg/dl	0.81-1.62 mmol/l
Children:	4.0-7.0 mg/dl	1.30-2.26 mmol/l

Estimation of Serum Total Cholesterol by CHOD-PAP method

- **Cholesterol:**
  - Most abundant animal sterol.
  - Crystalline yellow solid.
  - Distributed in all animal cells.
  - Major component of cell memb.
    - Maintain memb. Fluidity
    - Determinant of membrane permeability.
  - Solid alcohol from bile

- Occur in two form:
  - Free cholesterol (~ 30 %)
  - Cholesterol ester (~ 70%)
    - Cholesterol esterified with FA at C_3.

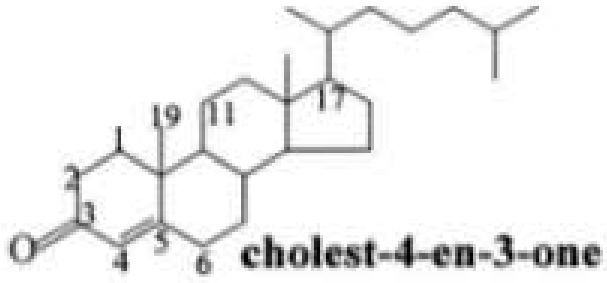




- **<u>Structure</u>**:
- cppp (phenanthrene nucleus and cyclopentano)
  - One –OH group at C_3. [esterifies with FA]
  - One double bond between C_5 & C_6.
  - An 8-carbon aliphatic side chain attached to C_{17}.
  - Contain 5 methyl groups. [C_{20},C_{13},C_{10},C_{25}]

- **FUNCTION**
  - Cell membrane: modulating effect on fluid state of the membrane
  - Nerve conduction: insulate nerve tissue
  - Bile salts and acids
  - Steroid hormones
  - Vitamin D

- **Principle**
  - Cholesterol esterase hydrolyzes Cholesterol esters in serum to give Free cholesterol & Fatty acid.
  - Cholesterol oxidase oxidises 3-OH group of Free cholesterol to liberate Cholest-4-ene-3-one & H_2O_2.
  - H_2O_2 is then converted to H_2O & [O] by Peroxidase.
  - 4-Amino Antipyrine takes up the [O] & together with phenol forms a pink coloured quinoneimine dye, which is measured at 520nm.
  - Absorbance ∞ Total Cholesterol in sample.



cholest-4-en-3-one

Cholesterol ester $\xrightarrow{\text{Cholesterol Esterase}}$ Cholesterol + Fatty Acid

Cholesterol $\xrightarrow{\text{Cholesterol Oxidase}}$ Cholest-4-en-3-one + H_2O_2

$H_2O_2 \xrightarrow{\text{POD}} H_2O + [0]$

[0] + 4 aminoophenazone + Phenol $\longrightarrow$ Chromogen (pink)

quinoneimine

- **Specimen**
  - Serum sample is used.
  - Fasting blood sample is preferred.

- **Procedure**

	Blank (B)	Standard (S)	Test (T)
Working cholesterol reagent	1.0 ml	1.0 ml	1.0 ml
Serum	-	-	10 µml
Standard (200 mg/dl)	-	10 µml	-

- Mix well. Incubate at 37°C in a water bath for 10 minutes or at RT (25- 35°C) for 15 minutes.
- Remove from water-bath & cool to RT.
- Set colorimeter to zero using blank at 520 nm & measure the absorbance of standard, test.

- **Hazardous materials**
  - This procedure uses phenol, which is caustic.
    - Avoid mouth pipetting.
    - Avoid contact with skin & mucous memb.

- **HDL cholesterol estimation**
  - LDL,VLDL, Chylomicron (ApoB containing lipoprotein) are removed by precipitating them using polyanion-divalent cation.
  - Example of polyanion-divalent cation:
    - Heparin-Mn^{2+}
    - Dextran sulphate-Mg^{2+}
    - Sodium phosphotungstate-Mg^{2+}

- Polyanions react with +vely charged groups on lipoproteins (facilitated in +nce of divalent cations) causing aggregation & a cloudy precipitate.
- Precipitation is usually complete within 10-15 min at RT.

- Precipitate is then sedimented by centrifugation.
- Centrifugation at higher forces, accelerates sedimentation & improve complete precipitation of apo-B containing particles.
- HDl-cholesterol in clear supernatant is estimated by CHOD- PAP method.

- **<u>LDL cholesterol estimation</u>**
  - Estimated by indirect method using friedwald equation

$$\text{Total cholesterol} = \text{HDLc} + \text{LDLc} + \text{VLDL}$$

$$\text{LDL} = \text{T. Cholesterol} - (\text{HDLc} + \text{VLDL})$$

$$[\text{VLDL} = \text{TG}/5]$$

- Thus equation becomes,

$$\text{LDL} = \text{T. Cholesterol} - (\text{HDLc} + \text{TG}/5)$$

Clinical significance

Tests included in Lipid profile test

1. Serum Total cholesterol
2. Serum Triglyceride
3. VLDL
4. LDLc
5. HDLc

- Normal range:
  - **Total cholesterol**
    - Desirable: <200 mg/dl
    - Borderline: 200-239 mg/dl
    - High risk: ≥ 240 mg/dl
  - **HDLc**
    - Low risk: ≥ 60 mg/dl
    - High risk: ≤ 40 mg/dl
  - **LDLc**
    - Desirable: < 130 mg/dl
    - Borderline: 130-159 mg/dl
    - High risk: ≥ 160 mg/dl

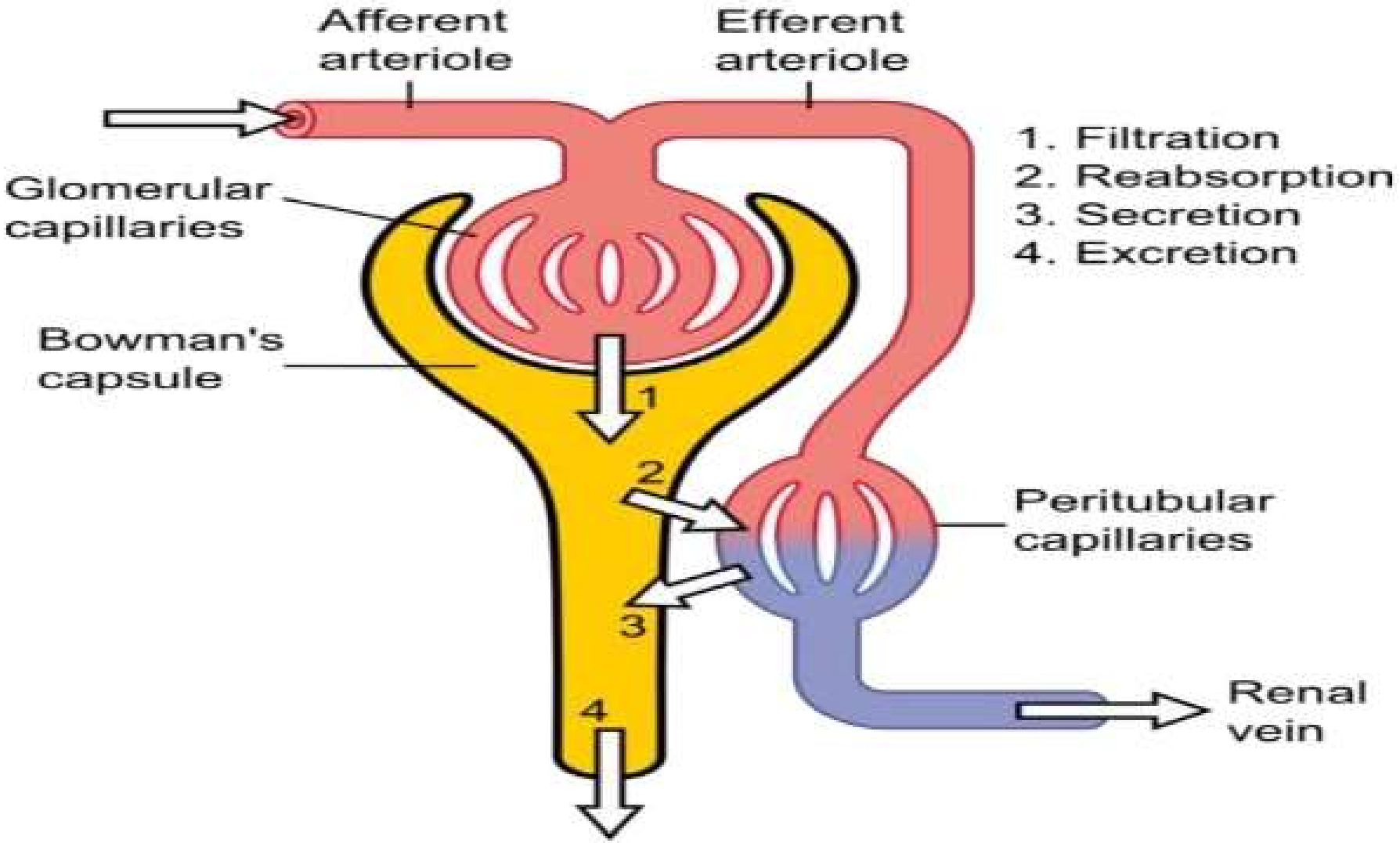
- **Causes of Hypercholesterolemia**
  - » Nephrotic syndrome
  - » Diabetes Mellitus
  - » Obstructive Jaundice
  - » Hypothyroidism
  - » Chronic alcoholism
  - » Type IIa Hyperlipoproteinemia

- **Causes of Hypocholesterolemia**
  I. Malabsorption
  II. Pernicious anemia
  III. Hyperthyroidism
  IV. Drugs like Nicotinamide, Clofibrate

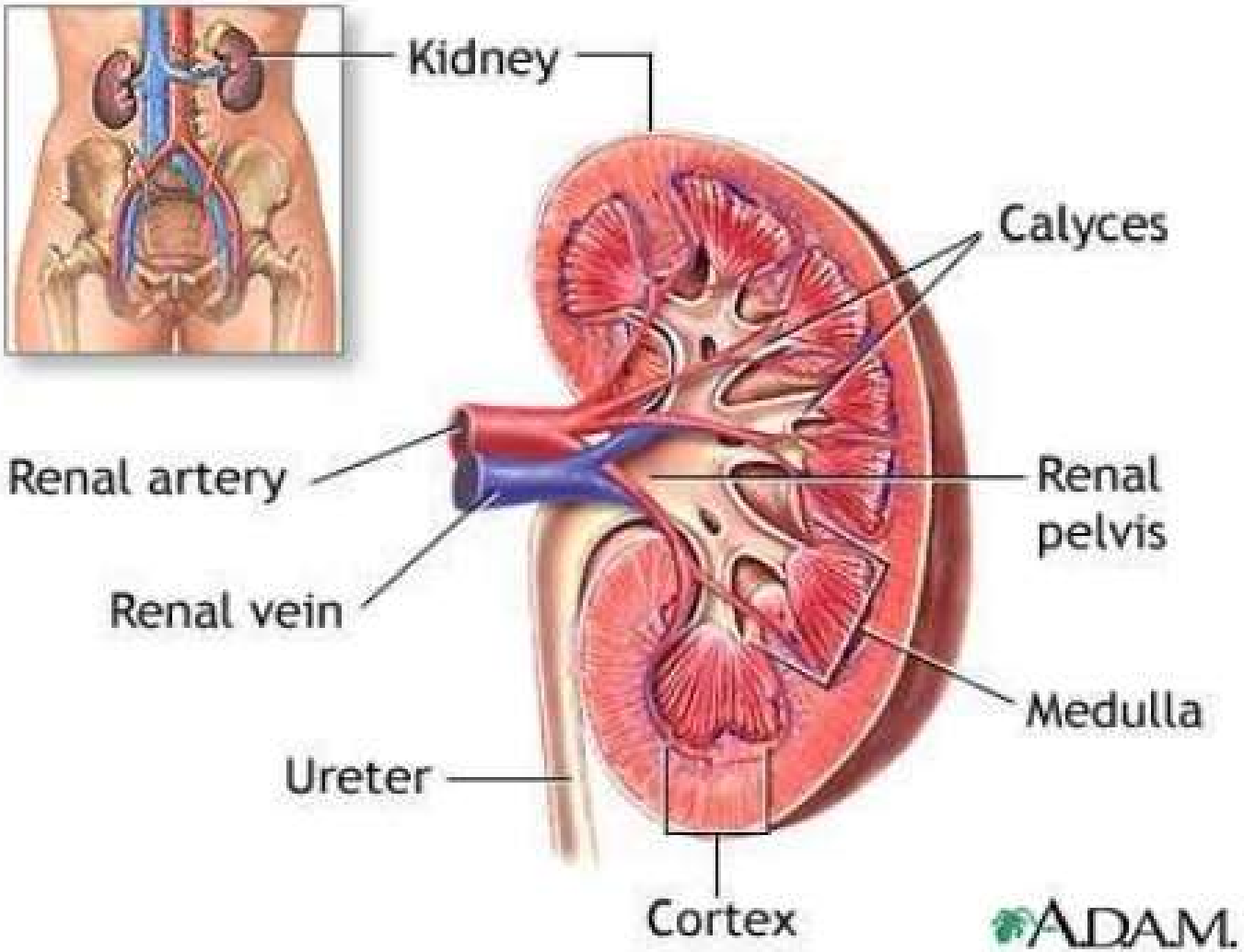
CLEARANCE TESTS AND SIGNIFICANCE

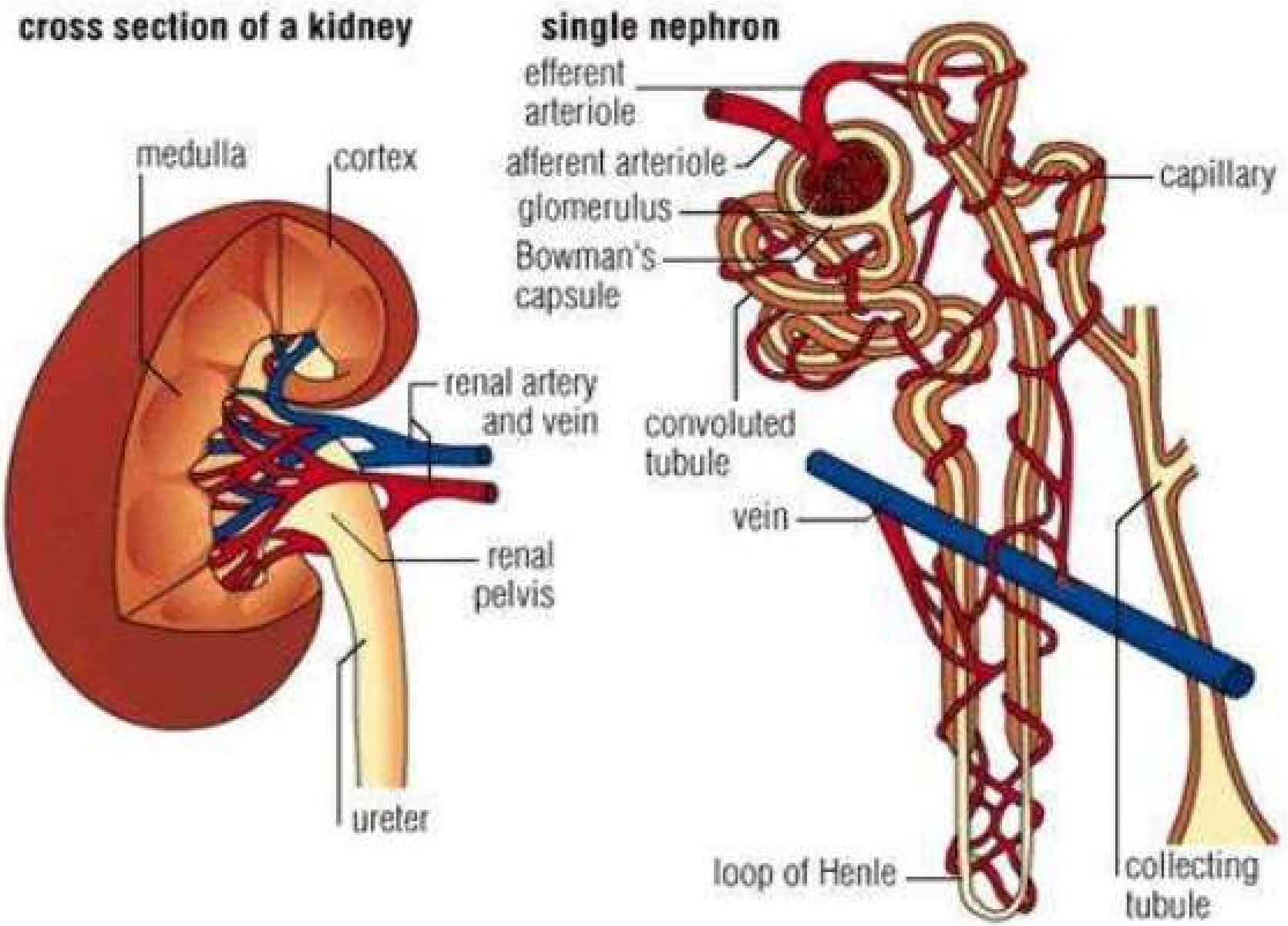
FUNCTIONS OF KIDNEY

- ✓ REMOVES WASTE PRODUCTS
- ✓ REMOVES FOREIGN AND NON ENDOGENOUS SUBSTANCES
- ✓ MAINTAINS ELECTROLYTES AND WATER
- ✓ MAINTAINS ACIDBASE BALANCE
- ✓ HORMONAL FUNCTIONS



Excretion = Filtration – Reabsorption + Secretion


Kidney
Calyces
Renal artery
Renal pelvis
Renal vein
Medulla
Ureter
Cortex
A.D.A.M.


cross section of a kidney
medulla
cortex
renal artery
and vein
renal
pelvis
ureter
single nephron
efferent
arteriole
afferent arteriole
glomerulus
Bowman's
capsule
capillary
convoluted
tubule
vein
loop of Henle
collecting
tubule

FORMATION OF URINE

- NEPHRON----FUNCTIONAL UNIT OF KIDNEY
- NEPHRON CONTAINS
    a) GLOMERULUS
    b) TUBULES

- 1000 to 2000 ml of urine is excreted/day

1)*STEPS IN URINE FORMATION*

Glomerular filtration tubular reabsorption tubular secretion

GLOMERULAR FILTRATION

- ✓ 700ml of plasma passes through kidneys per minute.
- ✓ From this 120 to125 ml is filtered per/minute by kidneys.
- ✓ THIS IS GFR.

GFR DECREASES

GFR DECREASES IN

1. OCCLUSION OF AFFERENT ARTERIOLES

2. DECREASED PERMEABILITY OF THE MEMBRANE.
3. INCREASED INTRA CAPSULAR PRESSURE

GFR INCREASES IN

1. INCRESED BP
2. DECREASED BLOOD PROTEIN OSMATIC PRESSURE
3. INCRESED GLOMERULAR PERMEABILITY

RENAL FUNCTION TESTS

1.TEST BASED ON GLOMERULAR FILTRATION:

- Urea clearance test
- Endogenous creatinine clearance test
- Inulin clearance test
- Cr51-EDTA clearance test

CLEARANCE TESTS

- CLEARANCE;DEFINED AS A VOLUME OF BLOOD OR PLASMA WHICH CONTAINS THEAMOUNT OF THE SUBSTANCE WHICH IS EXCRETED IN THE URINE OR
- *THE CLEARANCE OF SUBSTANCE MAY BE DEFINED AS THE VOLUME OF BLOOD OR PLASMA CLEARED OF THE SUBSTANCE FOUND IN ONE MINUTE EXCRETION OF URINE*

CREATININE CLEARANCE

- CREATININE CLEARANCE: Useful in the diagnosis and prognosis of the kidney disease.
- CREATININE CLEARANCE=

$$\frac{\text{Urine creatinine in mg/dl x Total volume of urine}}{\text{Serum creatinine in mg/dl x 1440}}$$

REF.RANGE: Male:125ml/mt

Female:115ml/mt

A decresed creatinine clearance is very sensitive indicator of a reduced glomerular filtration rate, due to acute or chronic damage.

CREATININE

- The kidney reserve is such that only when about 50% kidney function must be lost before creatinine level in blood is raised.
- But serum creatinine minor change in concentration may be of significance, and the serum level usually parallel to the severity of the disease.

CREATININE

- CREATININE:REF.RANGE:800---2000mg/day.
- Creatinine is a waste product formed from

  Creatinine phosphate which is stored form of the energy in muscle. this conversion is spontaneous, non enzymatic process and is dependant on total muscle mass and this mass is not affected by diet,age,sex or exercise.
- Since the production is continuous and it is not affected by other factors that's why Creatinine is an ideal substance for clearance test. This test is mainly to assess the glomerular filtration. GFR provides the most useful index for the assessment of the severity of renal disease.

24 hour urine collection

A 24 hour urine collection is a simple diagnostic procedure that measures the components of urine. The test is cheap, sensitive and is used to assess kidney function, hormones other substances can also be estimated

Assessment of Renal Function

- Assessment of the extent of renal damage
- Monitoring the progression of renal disease
- Monitoring & adjusting the dose of renal toxic drugs

RFT devised to give information regarding following parameters

- Renal blood flow
- Glomerular Filtration Rate
- Urine output

Renal tubular function

Renal Glomeruli Function

RFT

- a) those which measure GFR
- b) those which study tubular function

RFT Classification

1. Urine analysis
    - Physical examination
    - Chemical examination
    - Microscopic examination
2. Assessment of Glomerular function
    - Renal Clearance tests
    - Blood analaysis of Urea & Creatinine
    - Proteinuria
    - Hematuria

RFT - Tests for Glomerular Function

Renal Clearance Tests

To assess the rate of glomerular filtration & renal blood flow.

"The renal clearance of a on substance is defined as the volume of plasma from which the substance is completely cleared by the kidneys per minute."

This	**- plasma conc. Of the substance & it's excretary rate**
Depend	**- GFR**
On	**- Renal plasma flow**

Renal Clearance Tests

- The GFR (Normal = 120 ml/minute)
- Usually equal to clearance of that substance and is calculated by the following equation

$$C = \frac{U \times V}{P}$$

where,

C = clearance of the substance (ml/mt)

U = Conc.of substance in urine (mg/L)

P = Conc.of substance in plasma(mg/L)

V = Vol.of the urine passed per sminute

Renal Clearance Tests

- GFR – Normal 120 ml/minute
- Lower than normal GFR indicate
  - Acute tubular necrosis
  - Glomerulonephritis
  - Shock
  - Acute Nephrotic syndrome
  - Ac. & Ch. Renal failure

- In order to determine the GFR, the sub.should be slected in such a way that which is
  - freely filtered by glomerulus
  - should not be reabsorbed or secreted
  - should not be metabolized in the kidney
  - should not be toxic
  - should not be affected by dietary intake

- **The substances which are used for Clearance tests include :**

Endogenous	**-**	**Creatinine**
	-	**Urea**
Exogenous	**-**	**Inulin**

Creatinine Clearance Test

- Based on the rate of excretion by the kidneys of metabolocally produced creatinine
- Creatinine freely filtered in the glomerulus
- Not reabsorbed by the tubules

(a small amount of creatinine is produced by the tubules)

Creatinine Clearance Test

- **Creatinine clearance is determined by**
  - **collecting urine over 24hrs. Period**
  - **a sample of blood is drawn during the urine collection period.**

$$\text{Creatinine Clearance} = \frac{U \times V}{P}$$

U = Urinary creatinine(mg/L)

P = Plasma creatinine (mg/L)

V = Volume of urine per minute

Creatinine Clearance Test

- **Creatinine Clearance Normal range 90-120 ml/mt**
- **↓ Creat. Clearance is very sensitive indicator of decreased GFR**
- **↓ GFR may be caused by**

  Acute or Chronic damage to glomerulus or any of its components
- **↓ Blood flow to glomerulus may also produce decreased creat.clearance**

Urea Clearance Test

- The sensitivity of urea clearance is much less than the creatinine clearance because—

  -plasma conc. Of urea is affected by number of factors

  e.g : dietary protein

  fluid intake

  infection

  surgery, etc…

  - Approximately 40 % of filtered urea is normally reabsorbed by the tubules.

- Normal value of Urea clearance : 75 ml/mt.