Liver function test

Bilirubin

5th stage

Bilirubin

Bilirubin is an orange-yellow pigment, a waste product primarily produced by the normal breakdown of heme. Heme is a component of hemoglobin, which is found in red blood cells (RBCs). Bilirubin is ultimately processed by the liver to allow its elimination from the body.

Normal Value:

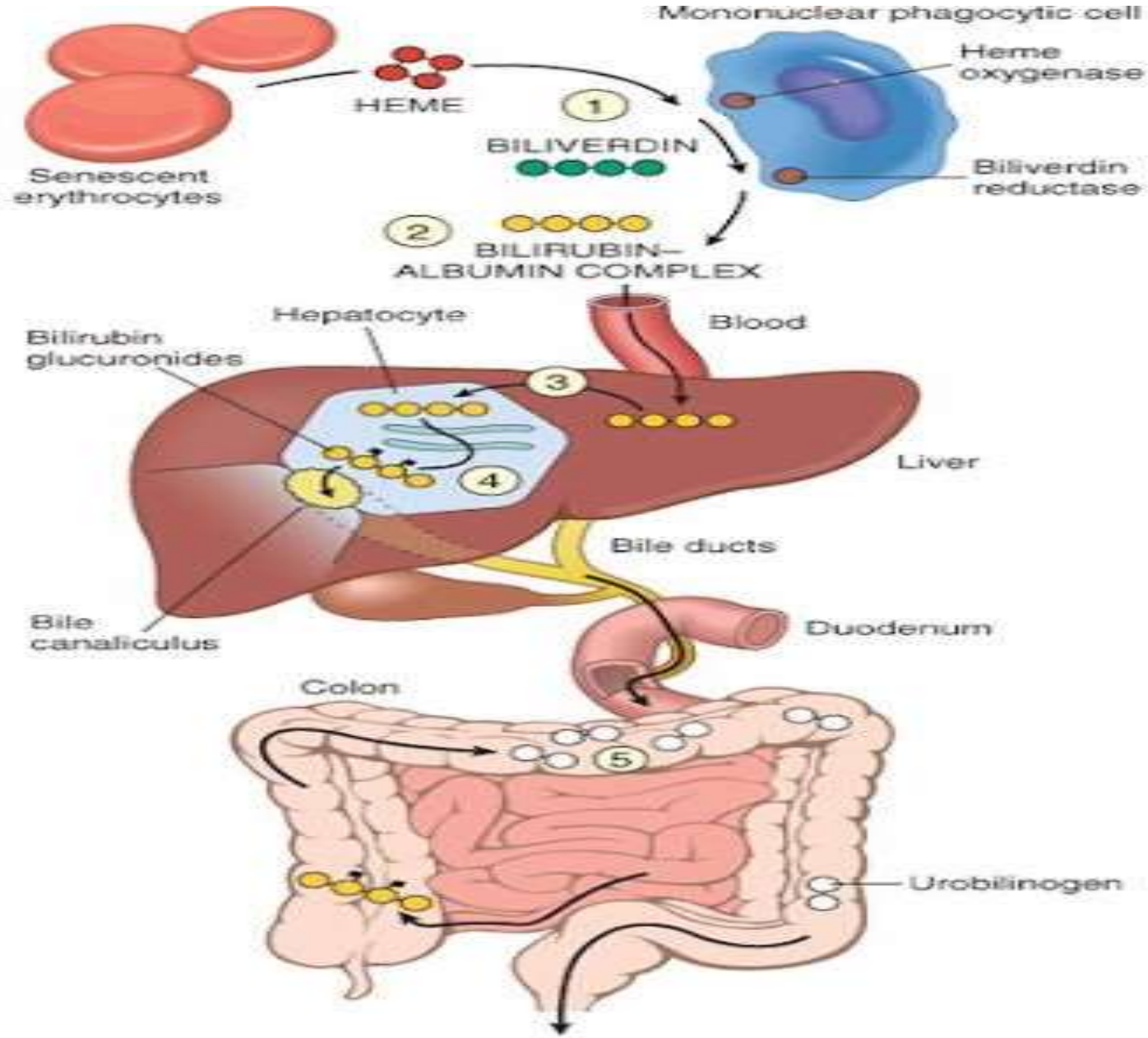
Total : 0.3 – 1 mg/dl

Indirect (unconjugated) : 0.2 – 0.8 mg/dl

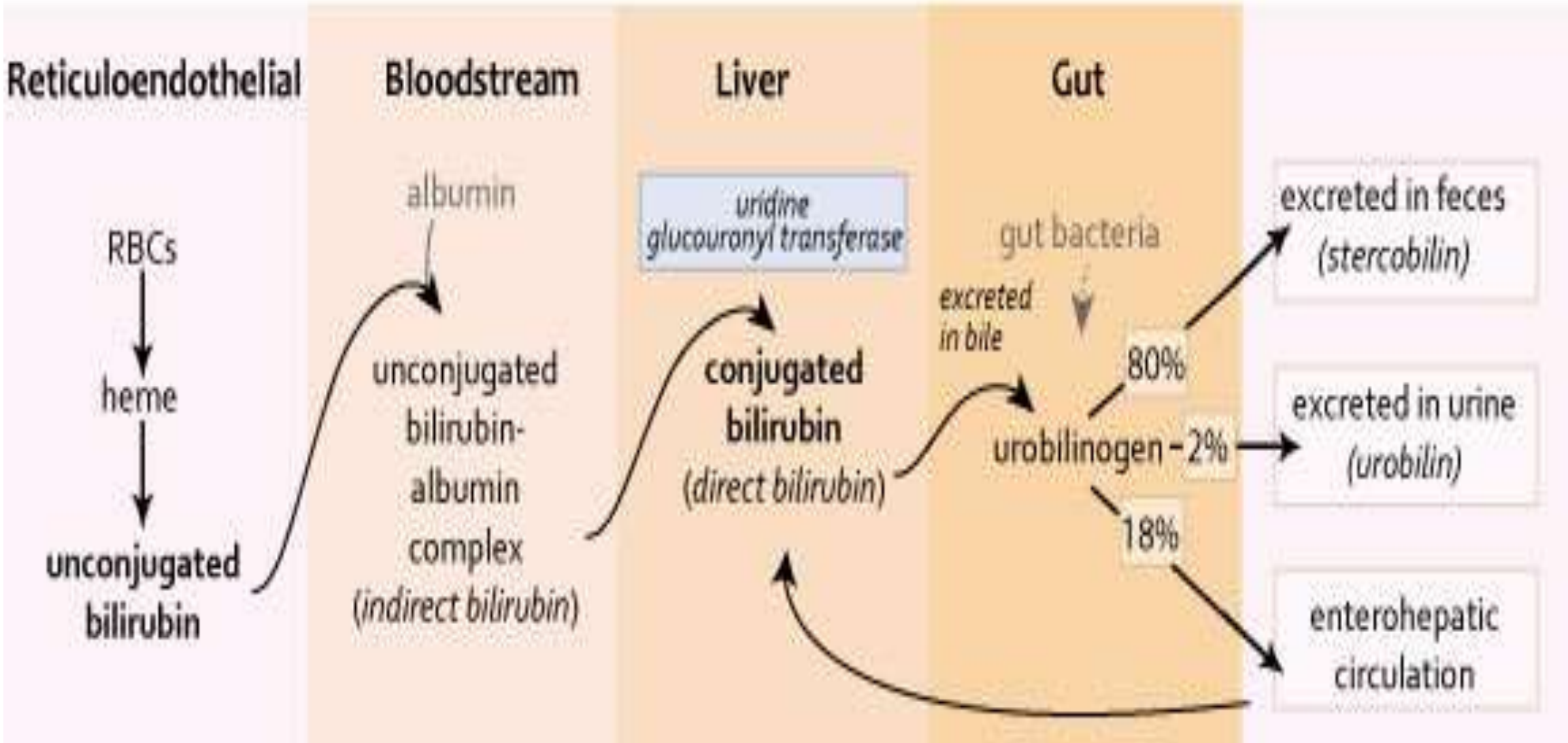
Direct (conjugated) : o.1 – 0.3 mg/dl

Panic volume for neonatal bilirubin : > 15 mg/dl (mental retardation)

Metabolism


Mononuclear phagocytic cell
Heme oxygenase
HEME
1
BILIVERDIN
Biliverdin reductase
Senescent erythrocytes
2
BILIRUBIN–ALBUMIN COMPLEX
Hepatocyte
Blood
Bilirubin glucuronides
3
4
Liver
Bile ducts
Bile canaliculus
Duodenum
Colon
5
Urobilinogen

Bilirubin


Reticuloendothelial
Bloodstream
Liver
Gut
RBCs
heme
unconjugated bilirubin
albumin
unconjugated bilirubin-albumin complex (indirect bilirubin)
uridine glucouronyl transferase
conjugated bilirubin (direct bilirubin)
gut bacteria
excreted in bile
urobilinogen
80%
2%
18%
excreted in feces (stercobilin)
excreted in urine (urobilin)
enterohepatic circulation

Jaundice

- It is yellow discoloration of body tissues by abnormally high blood levels of bilirubin. It is recognized when the total serum bilirubin exceeds 2.5 mg/dl. Bilirubin is the major bile pigment in humans, and is produced as an end-product of heme catabolism.
- physiologic jaundice (increase unconjugated) of the newborn occurs if the newborns liver is immature and does not have enough conjugating enzymes. This results in a high circulating blood levels of unconjugated bilirubin which can pass through the blood brain- barrier and deposit in the brain cells of the newborn. This can cause encephalopathy (kernicterus)

Types of jaundice

1. Hemolytic or pre-hepatic

in which there is increased breakdown of hemoglobin so that the liver cells are unable to conjugate all the increased bilirubin formed and excessive breakdown of RBC may be due to internal factor (abnormalities within the cells) or external factors as (an incompatible blood transfusion, malaria and some drugs such as sulfonamide.

2. Hepatic or hepatocellular

In which there is a disease of the parenchymal cell of the liver and is essentially of 2 groups (defective conjugation , infective and toxic jaundice).

It is result in elevated of unconjugated bilirubin (as in hepatitis) which cause unconjugated hyperbilirubinemia

3. Obstructive or post-hepatic

In which there is obstruction in flow of bile in the extrahepatic ducts , due to gallstone, infection or carcinoma of head of pancreas, scaring of extrahepatic duct which result conjugated hyperbilirubinemia . This type can be resolved surgically or endoscopically .

Normally unconjugated bilirubin make up 70%-80% of the total bilirubin , conjugated bilirubin 15%-20% of total bilirubin.

Specimen

- collect 5-7 ml venous blood
- serum is used for analysis. Fasting is preferred . Avoid hemolysis during sample collection.
- use a heel puncture for blood collection in infants.

Interfering factors

- blood hemolysis and lipemia can produce false results.
- avoid exposure of sample to sunlight or high intensity of artificial light at room temperature. Because this will decrease bilirubin content.
- air bubbles and shaking of sample may cause decrease of bilirubin levels
- Certain food (like carrots , yams) may increase the yellow hue in serum thus causing falsely increased bilirubin levels
- prolonged fasting raises bilirubin level as does anorexia
- drugs : increase (antibiotics , diuretics …….) decrease (barbiturate, caffeine , penicillin and high doses of salicylate)

Method of estimation

Van den Bergh reaction

Principle

Bilirubin (both direct and indirect) reacts with diazotized sulphanilic acid (DSA) to form a red azo bilirubin. The absorbance of this dye is directly proportional to the bilirubin concentration in the sample.

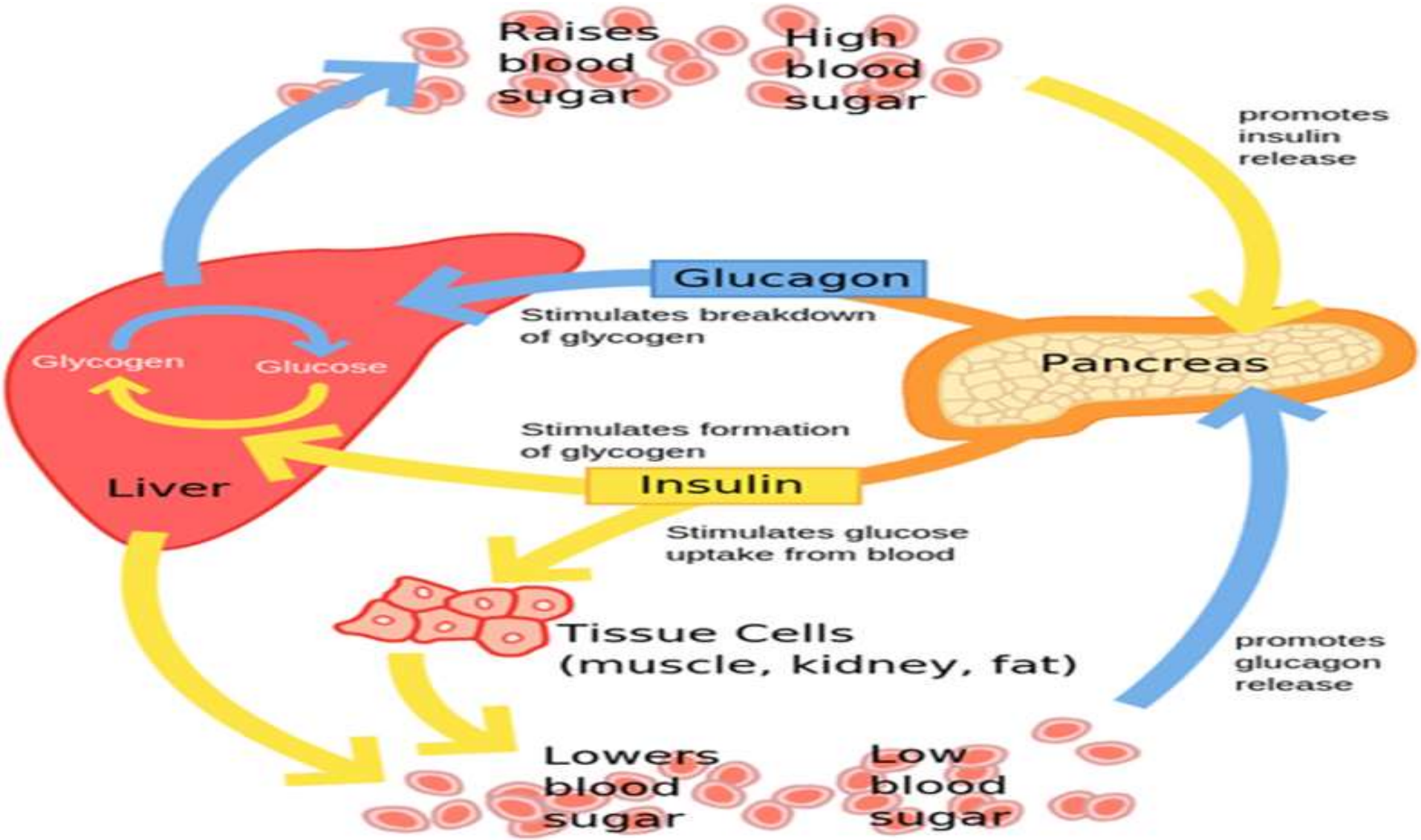
Conjugate react directly (direct reaction) with aqueous solution but the unconjugated bilirubin required accelerator or solubilize (indirect reaction)

Blood Glucose Measurement

Clinical biochemistry

5th stage

Regulation Of Blood Glucose



hormone	tissue of origin	effect on blood glucose
insulin	pancreatic beta cells	lowers
somatostatin	pancreatic gamma cells	lowers
glucagon	pancreatic alpha cells	raises
epinephrine	adrenal medulla	raises
cortisol	adrenal cortex	raises
ACTH+Growth hormone	anterior pituitary	raises
Thyroxine	thyriod	raises

Blood Glucose Tests

- Blood glucose estimation is a common test done in all laboratories because it helps in diagnosis, management of diabetes mellitus and is a common prerequisite for any surgery.
- It is used to diagnose hyperglycemic conditions like diabetes mellitus and hypoglycemic situations.
- The time of day effect on blood glucose level.
- The fasting blood glucose is 3.6-6.1 mmol/L (65-110 mg/dl)

- Fasting whole blood glucose concentration is approximately 10 to 12% lower than plasma or serum glucose!!!

Random blood glucose : In young or adult even after meals, the blood glucose rarely exceed 150 mg/dl .**Diabetes is diagnosed at blood glucose of greater than or equal to 200 mg/dl**

- The precautions require when collect blood sample:

a) Blood static should be avoided
b) Blood should not be taken when or while I.V solution are being administered
c) Chemically clean and dry syringes should be used
d) Blood kept in proper container

Abnormal glucose metabolism may be caused by

1. Inability of pancreatic islet B-cells to produce insulin.
2. Reduced numbers of insulin receptors.
3. Defect of glucose absorption
4. Inability of the liver to metabolize glycogen
5. Altered level of hormones that play a role in glucose metabolism.

- **Hyperglycemia** is occur when the fasting blood sugar levels 7 mmol/L (>125mg/dl) which are usually diagnostic for diabetes mellitus .
- **Glucose Renal Threshold** When the blood glucose level exceeds about 160 – 180 mg/dl, the proximal tubule becomes overwhelmed and begins to excrete glucose in the urine. The proximal tubule can only reabsorb a limited amount of glucose. this point is called the renal threshold for glucose (RTG) .

  Normal urine is nearly glucose free even after carbohydrate meal.

Methods of estimation

1- **Commercial strips** : which give color that can be determined visually or calorimetrically. Estimate glucose in urine and blood .

2- **Enzymetic method:** by use of glucose specific enzyme (glucose oxidase) which catalyze the oxidation of glucose to gluconoic acid and H_2O_2 and by the action of peroxidase enzyme can convert a colorless chromogen in to a colored substance (Quinonimine) which can be estimated by spectrophotometer.

3- **Reductive method :** depend upon the ability of glucose to reduce the cupric ion(Cu^{+2}) to cuprous ion (Cu^{+1}) due to property of aldehyde group of sugar. The cuprous ion is precipitate as Cu_2O which in turn treated to reduce arsenomolybedic acid or phsphomolybedic acid solution and give blue coloration which is estimated photometerically, the intensity of blue color depend upon the initial reducing ability of glucose .it is not specific for glucose because they measure other reducing sugar like galactose and other reducing substance (creatinine, uric acid, and ascorbic acid)so the result tend to be higher than the normal limit of glucose.

Hyperglycemia

1- diabetes mellitus

2- In conditions of increased insulin antagonized hormones like:

a. Cushing syndrome

b. Acromegaly

c. Pheocromocytoma

3- Chronic renal failure

4- Acute stress response

5- Acute pancreatitis

Hypoglycemia

1- insulin excess

2- insulinoma

3- Addisons disease

4- Deficiency oh insulin antagonizing hormone like

hypopituitarism and hypothyroidism

5- starvation

Oral Glucose Tolerance Test(OGTT)

- Test measure the ability of body to remove an added glucose load from the circulation. This is accomplished at such a rate that the blood glucose level not exceed the renal threshold and no glucose appear in urine.
- Following the ingestion of glucose the blood level of glucose alter depending on

  1- the dose of glucose

  2- the rate of absorption from intestine

  3- the rate at which the glucose leave circulation
- The main value of GTT is that it may help to establish the diagnosis of DM or impaired glucose tolerance at time when the metabolic abnormality is mild.

Oral Glucose ToleranceTest

This test suggested in the following conditions :

1- When the glucose appear in urine

2-When fasting blood level or 2- hour postprandial blood sugar is significantly elevated.

3- Patients with a family history of diabetes.

4- Patients massively obese

5- Patients with recurrent infections

6- Patients with delayed healing of wounds

7- hyperglycemia during pregnancy

Test performance

- The patients ability to tolerate standard oral glucose load is evaluated by obtain serum and urine specimen. For glucose level determination the samples obtained before glucose load (zero time), 30 min after load, 1 hour, 2 hour, 3 hour and sometime 4 hour.
- Normally there is rapid insulin response to the large oral glucose load. Peak response occur in 30-60 min and return to normal in about 3 hour.
- Subject with appropriate normal insulin response are able to tolerate the glucose load quite easily with only minimal and transient rise in serum glucose level within 1-2 hour after ingestion and glucose dose not spill over in urine.
- Normal value:

  *fasting (zero time): adult 3.9-6.1 mmol/L or 70-110 mg/dl

  * 30 min : adult 6.1 -9.4 mmol/L or 110-170 mg/dl

  *60 min : adult 6.7-9.4 mmol/L or 120-170 mg/dl

  * 120 min :adult 3.9-6.7 mmol/L or 70-120 mg/dl

  *3 hour : adult 3.9-6.7 mmol/L or 70 -120 mg/dl

Specimen

- Collect fasting blood and urine specimen.
- Collect 5ml of venous blood at 30 min and hourly periods.
- Collect urine specimen at hourly period
- Mark the tube with the time that the specimens are collected

Potential complication

1- dizziness, tremor, anxiety, sweating, fainting during test.

2- if these symptoms occur measure the blood glucose level if too high then stop the test and give insulin

3- patients with concurrent infection or have endocrine disorders bec. Glucose intolerance will be observe even though these patients may not have diabetes.

Interfering factor

1. Smoking
2. Stress
3. Exercise during test
4. Reduced caloric intake befor test
5. Drugs
6. If the payients vomit the glucose solution, the test is declared invalid it can be repeated in 3 days.

Patient preparation

1. Patient should have high carbohydrate diet for 3 days preceding the test
2. Patient should fast 12 hour but not more than 16 hour before test
3. Patient should rest during the test
4. Collect blood specimens (at 30 min interval)and urine specimen hourly
5. Record patient weight

Glucose load

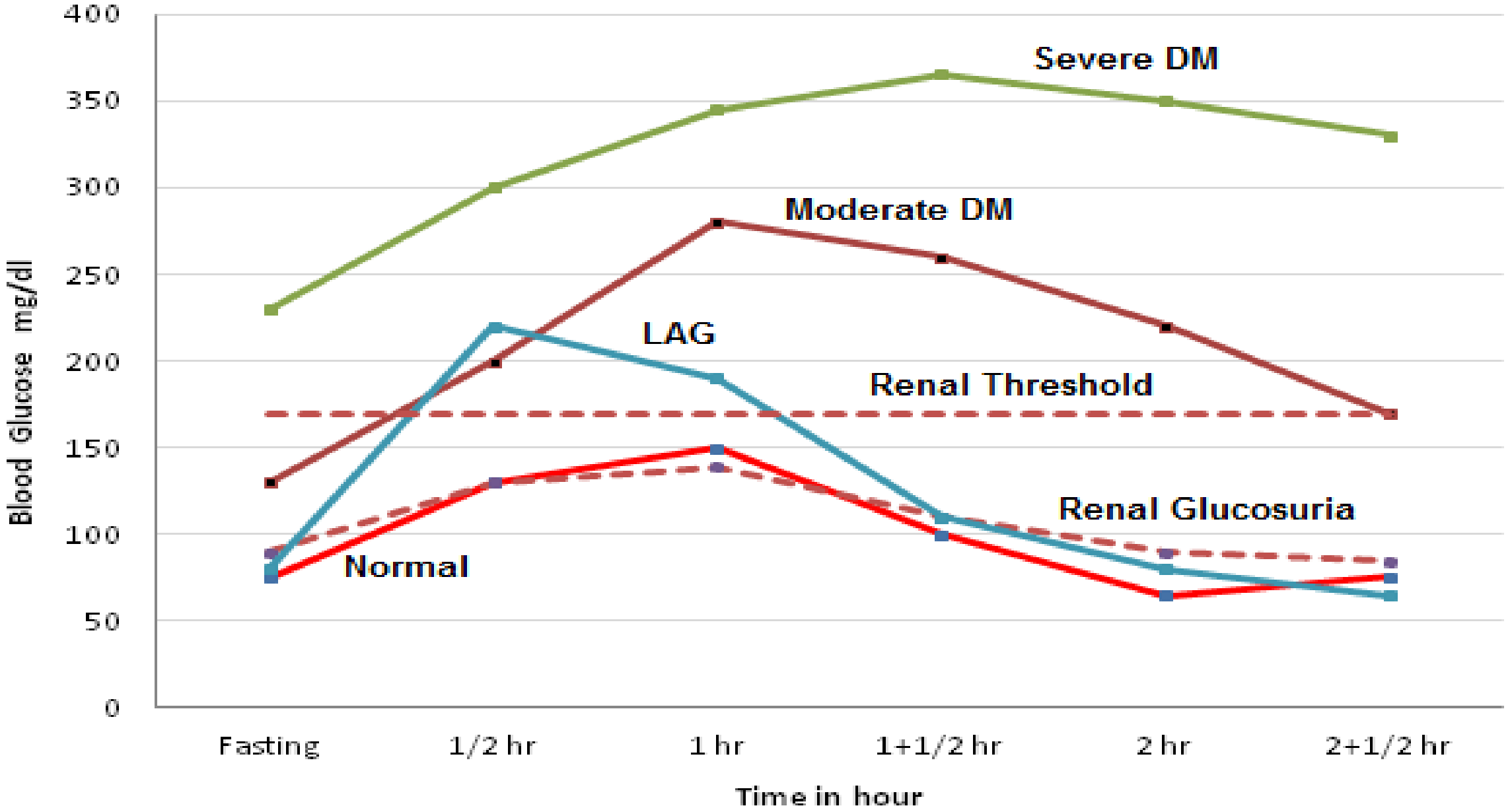
1- based on body weight calculated as 1.75gm/kg of body weight up to 75 gm

2- pregnant women 100gm glucose

3- non-pregnant adult 75gm

Glucose dissolved in 250-300ml of water and flavored with vit C

Oral Glucose Tolerance Test (OGTT)



Estimation Of Glucose By Enzymatic Colorimetric Method

Principle

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{GOD}} \text{Gluconate} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{POD}} H2O + \text{Quinoneimine dye}$$

Reagent

R1: phosphate buffer ,Glucose oxidase, peroxidase, aminoantipyrine and phenol

R2: standard (100mg/dl or 5.55mmol/L)

Procedure :

Tubes	Blank	standard	sample
Standard	-	10 µl	-
Sample	-	-	10 µl
Reagent	1ml	1ml	1 ml

Calculation :

$$\text{Glucose con.} = \frac{\text{A. sample}}{\text{A. standard}} \times n$$

n= con. Of STD

mg/dl= n= 100

mmol/L=n= 5.55

Lipid profile

Clinical laboratory science

5th stage

Lipid

Lipids are defined as organic compounds that are poorly soluble in water but miscible in organic solvents like chloroform and ether.

They are ester of fatty acids and utilized by living organisim.

Lipid are classified to:

1- Simple lipid (neutral fats and waxes)

2- Compound lipids (phospholipid , glycolipids, lipoprotein)

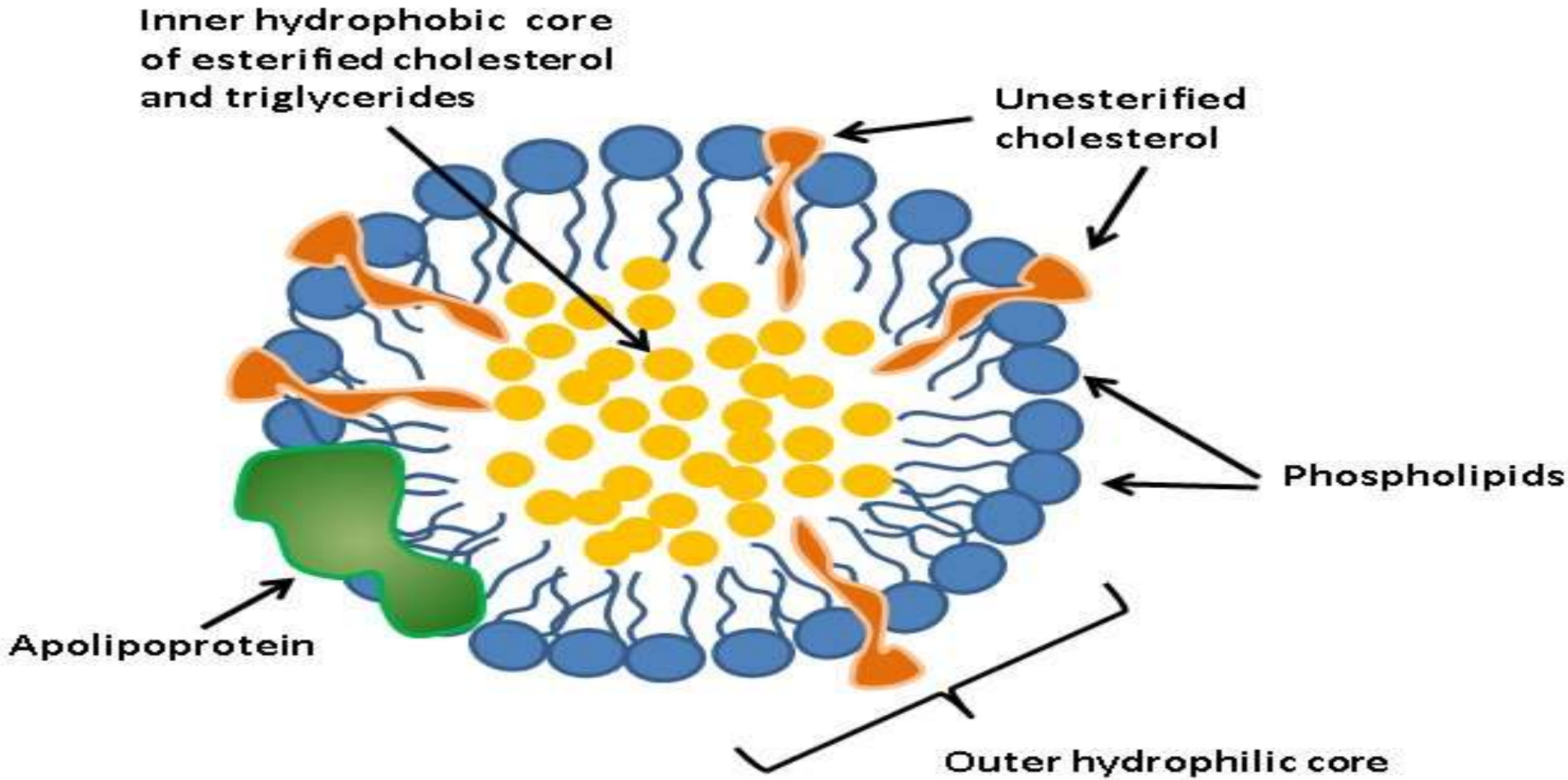
3- Derived lipids [fatty acid, glycerol ester(triglyceride), sterols (cholesterol) and others]

Lipoproteins

Because lipids , such as cholesterol and triglyceride, are relatively insoluble in water, they are transported in body fluids as, soluble protein complexes called lipoproteins.

Lipids can be derived from food (exogenous) or synthesized in the body (endogenous).

Lipoprotein



Classification of lipoprotein

Lipoproteins can be classified into five main groups according to density which inversely reflects their size.

1- Chylomicrons are the largest and least dense lipoproteins and transport exogenous lipid from the intestine to all cells.

2-Very low-density lipoproteins (VLDLs) transport endogenous lipid from the liver to cells.

3-Intermediate-density lipoproteins (IDLs), which are transient and formed during the conversion of VLDL to low-density lipoprotein (LDL), are not normally present in plasma.

4- Low-density lipoproteins (LDL) are formed from VLDLs and carry cholesterol to cells.

5- High-density lipoproteins (HDLs) are the most dense lipoproteins and are involved in the transport of cholesterol from cells back to the liver (reverse cholesterol transport)

Lipid profile test

Lipid profile : it is a group of tests are often ordered together to determine the risk of coronary heart disease and these tests are good indicator if someone likely to have hear attack or stroke that cause by blocking of blood vessels.

It is include:

1- cholesterol

2- triglyceride

3- HDL cholesterol

4- LDL cholesterol

5- VLDL cholesterol

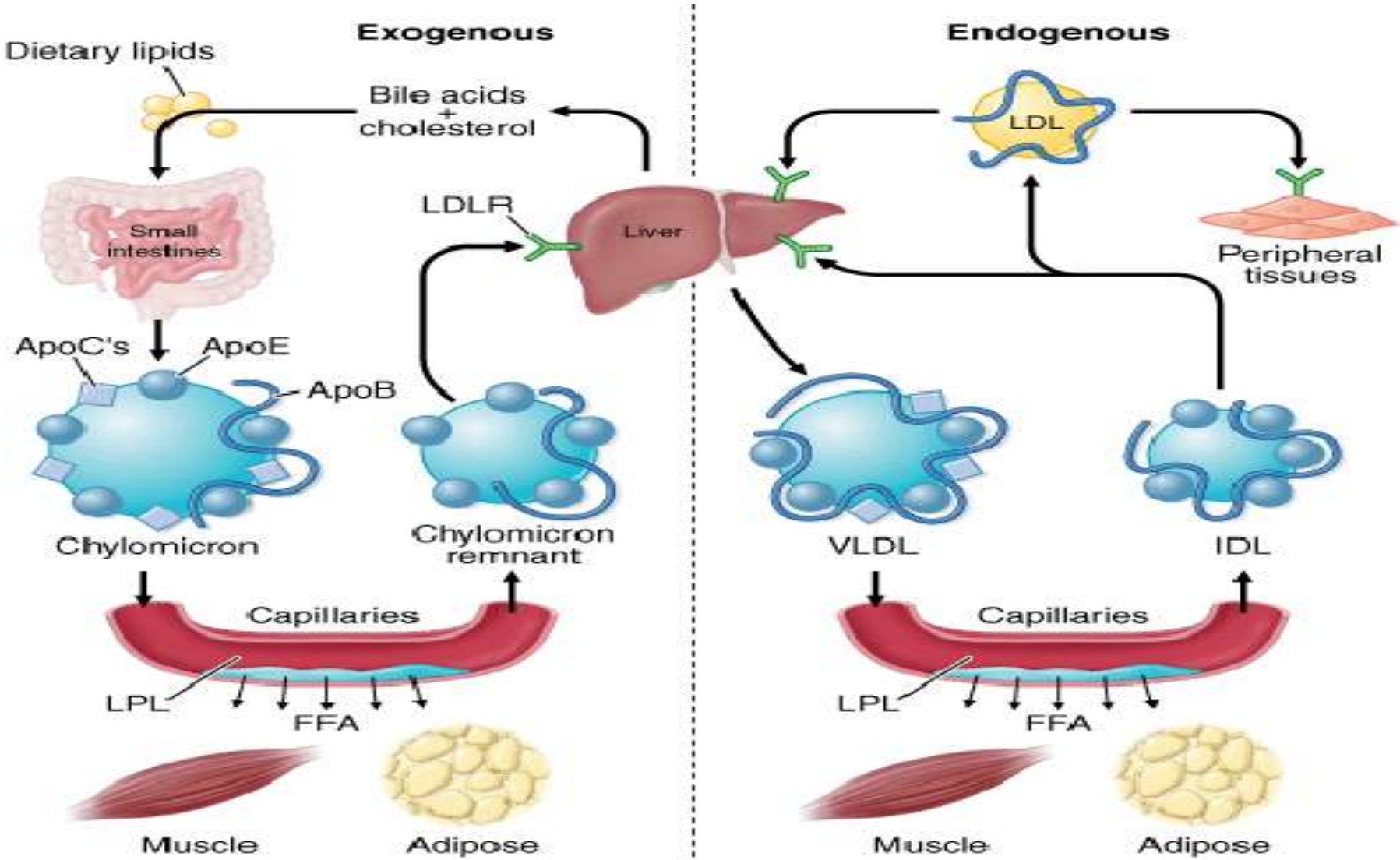
Cholesterol

- Its steroid alcohol formed in animal fats , widely distributed in the body like in blood, brain, liver, kidney and nerve fibers.
- The normal value is 200 mg/dl or < 5.20 mmol/L , usually normal value raises with age , diet and geographic region.
- The cholesterol in the blood are found as esterified form and free form.

Source of cholesterol

- Cholesterol in the body is derived from exogenous (diet) and endogenous source. Several physiologically important compounds are derived from it, e.g. vitamin D, bile acids, steroid hormones.
- Most of cholesterol we eat comes from animal origin of the foods. Average diet supplies about 0.3 gm of cholesterol per day , but over 1 gm of it is synthesis in the body as endogenous.
- Rich source : brain , nerve tissue , adrenal gland and egg yolk

Metabolism


Exogenous
Endogenous
Dietary lipids
Bile acids
+
cholesterol
LDL
LDLR
Liver
Small intestines
Peripheral tissues
ApoC's
ApoE
ApoB
Chylomicron
Chylomicron remnant
VLDL
IDL
Capillaries
Capillaries
LPL
FFA
LPL
FFA
Muscle
Adipose
Muscle
Adipose

Excretion

1-the cholesterol (80%) is converted in the liver in to salts of bile acids which excreted through the bile in the intestine and serves as important function in the absorption of lipids by intestinal mucosa.

2- some of cholesterol is incorporated in to the cell membrane and the rest is re-enter the plasma where it is taken up by HDL and returned to the liver.

3- the free cholesterol taken up by HDL is esterified to cholesterol ester by the enzyme lecithin cholesterol acyltransferase (LCAT).

Function

1- it occurs as a major constituent of the plasma membrane .

2- cholesterol is a precursor of bile acids, which are essential for fat digestion.

3- cholesterol is the precursor of all steroid hormones(androgen, estrogens, glucocorticoid , steroid , mineralocorticoid.

4- vitamin D

Hypercholesterolemia

- hypolipoproteinemia
- Nephrotic syndrome
- myxedema
- Obstructive jaundice
- Diabetes mellitus

Hypocholesterolemia

- Hyperthyroidism
- pernicious anemia
- Hemolytic jaundice
- malabsorption
- liver diseases

Blood Creatinine

Laboratory of clinical biochemistry

5th stage

Creatinine

- It is a byproduct in the breakdown of muscle creatine phosphate from energy metabolism .
- It is produced at constant rate depending on the muscle mass of the person and its removed from the body by the kidney.
- Creatinine production is constant as long as muscle mass remains constant.
- Endogenous creatinine is a result of some special process of normal metabolism (byproduct in breakdown of muscle creatine phosphate from energy metabolism.

- Serum creatinine test aid in the diagnosis of impaired renal function. It is more specific and sensitive indicator of kidney disease than BUN, although in chronic renal disease both BUN and creatinine are ordered to evaluate renal problem , because the BUN/creatinine provide more information
- Normal value :

- adult : 62-125µmol/l or 0.6 – 1.5 mg/dl
- child(3- 18 yr): 44-88µmol/l or 0.5-1 mg/dl
- BUN/creatinine ration : 10:1 to 20:1

Serum is preferred, but heparinized blood can be used

Interfering factor

- high levels of vit C and cephalosporin antibiotics can cause a falsely increased Cr level. the reagents also interfere with BUN/Creatinine ratio.
- A diet rich in meat can cause increase of Cr level.
- Creatinine is falsely decreased by bilirubin, glucose, histidine and quinidine compounds.
- ketoacidosis may increase serum creatinine substantially.
- drugs that may cause increase creatinine : aminoglycoside , chemotherapeutic agents (cisplastine)

Increase level

- glomerulonephritis
- pyelonephritis
- urinary tract infection
- Nephritis
- Shock, dehydration
- Starvation
- Fever
- DM
- Hyperthyroidism
- Muscle disorder

Decrease level

- debilitation
- decreased muscle mass(like muscular dystrophy , myasthenia gravis)

Creatinine Clearance (CCr)

Adult (> 40 yr) :

Male : 107- 139 ml/min.

Female: 87- 107 ml/min.

CCr test: it is a specific measurement of kidney function primarily for glomerular filtration. It measure the rate at which the kidneys clear creatinine from the blood .

Creatinine Clearance *: the volume (milliliters) of plasma from which the substance would have to be completely cleared by kidney in 1 min.*

This test is used to evaluate renal function in patients , to monitor the progression of renal disease.

Interfering factors

1- Exercise may cause increase Cr clearance levels

2- pregnancy increase Cr level

3- A diet high in meat content contain significant amount of creatinine , particularly after cooking ,so creatinine excretion decrease some what in starvation.

4- Incomplete urine collection may give a falsely lowered level .

Lipid profile

HDL , LDL

(5TH stage)

Lipoprotein are proteins in blood whose main purpose is to transport cholesterol , triglycerides and other insoluble fats. With the use of electrophoresis these lipoprotein can be grouped into :

1- Chylomicrons (origin)

2- LDL (beta- lipoproteins)

3- VLDL (prebeta- lipoproteins)

4- HDL (alpha-lipoproteins)

- *High Density Lipoprotein(HDL)* are carries of cholesterol , that are produced in the liver .
- HDL values are age and sex dependent.
- The main function of HDL is to remove cholesterol for excretion. Also HDLs prevent cellular uptake of cholesterol and lipids. These potential actions may be the cause of their protective cardiovascular characteristics associated with HDLs(good cholesterol) within the blood.

HDL :

Male > 0.75 mmol/l OR > 45mg/dl

Female > 0.91 mmol/l OR > 55mg/dl

Low density lipoproteins(LDL) are cholesterol rich. Cholesterol carried by LDLs can be deposited in the peripheral tissue and is associated with increased risk of arteriosclerotic and vascular diseases. Therefore , high level of LDLs (bad cholesterol) are atherogenic.

LDL (mg/dl) = Total Cholesterol – (HDL + TG/5).....Friedwald Formula

- LDL : > 3.3 mmol/l OR 60-180 mg/dl

Interfering factors

- Smoking and alcohol ingestion decrease HDL
- Oral estrogen therapy show increase HDL and decrease LDL

  While in pregnancy LDL increased
- Steroid , diuretics, beta- blocker decreased HDL levels
- HDL level is elevated in hypothyroidism and diminished in hyperthyroidism.

- ***Specimen***

  Collect 5- 10 ml venous blood

1- Patient should fast for 12-14 hr

2- No alcohol should be consumed for at least 24 hr

3- If possible, stop all medication for at least 24 hr

Increase level of HDL

- Familial lipoproteinemia
- Excessive exercise

Decrease level of LDL

- Familial low HDL
- Hepatocellular disease (hepatitis or cirrhosis)
- Hypoproteinemia (nephrotic syndrome or malnutrition)

Estimation of HDL- cholesterol

- Principle

The chylomicrons, VLDL and LDL that are contained in the sample are precipitated by addition of phosphotungestic acid in the presence of Mg. the supernatant obtained after centrifugation contains HDL from which the cholesterol can be determined by using the cholesterol enzymatic kit.

Procedure

R1 : phosphotungestic acid and magnesium chloride.

R2 : standard

- Mix 200 µl of sample and 400 µl of R1 , stand 10 min at room temperature , centrifuge at 5000 rpm for 10 min, collect the supernatant and proceed it as a sample in the total cholesterol.

	blank	Std	sample
Std – 50mg/dl	-	50 µl	-
supernatant	-	-	50 µl
cholesterol reagent	1 ml	1ml	1ml

Collecting and Transporting of Specimens

Clinical biochemistry laboratory

5th stage

Laboratory tests are tools give additional information about the patient .

These tests used in conjunction with patients history and physical examination and provide valuable information about the patient response to therapy that not appear for the history of patient and his physical examination.

These tools involve blood, urine, stool, x-ray, nuclear scanning, ultrasound and endoscopy .

Factors effect testing out come

1. Old age
2. History of illness
3. History of allergies
4. Infection
5. Uncontrolled pain
6. Neuromuscular conditions
7. weakness.
8. Addiction , hearing and visual impairment

Major phases of diagnostic test

a) Pre test phase
b) Intra test phase
c) Post test phase

Interfering factors

- Sampling errors

1. Incorrect specimen collection, handling, storage, or labeling
2. Wrong preservation or lack preservation
3. Delayed specimen delivery
4. Incomplete patient preparation
5. Hemolysis blood samples
6. Old or deteriorating specimens

- Patients factors

1. Incorrect pretest diet
2. Current drug therapy
3. Dehydration
4. Position at time of specimen collection
5. Time of day
6. Pregnancy
7. Age
8. Stress
9. Alcohol use

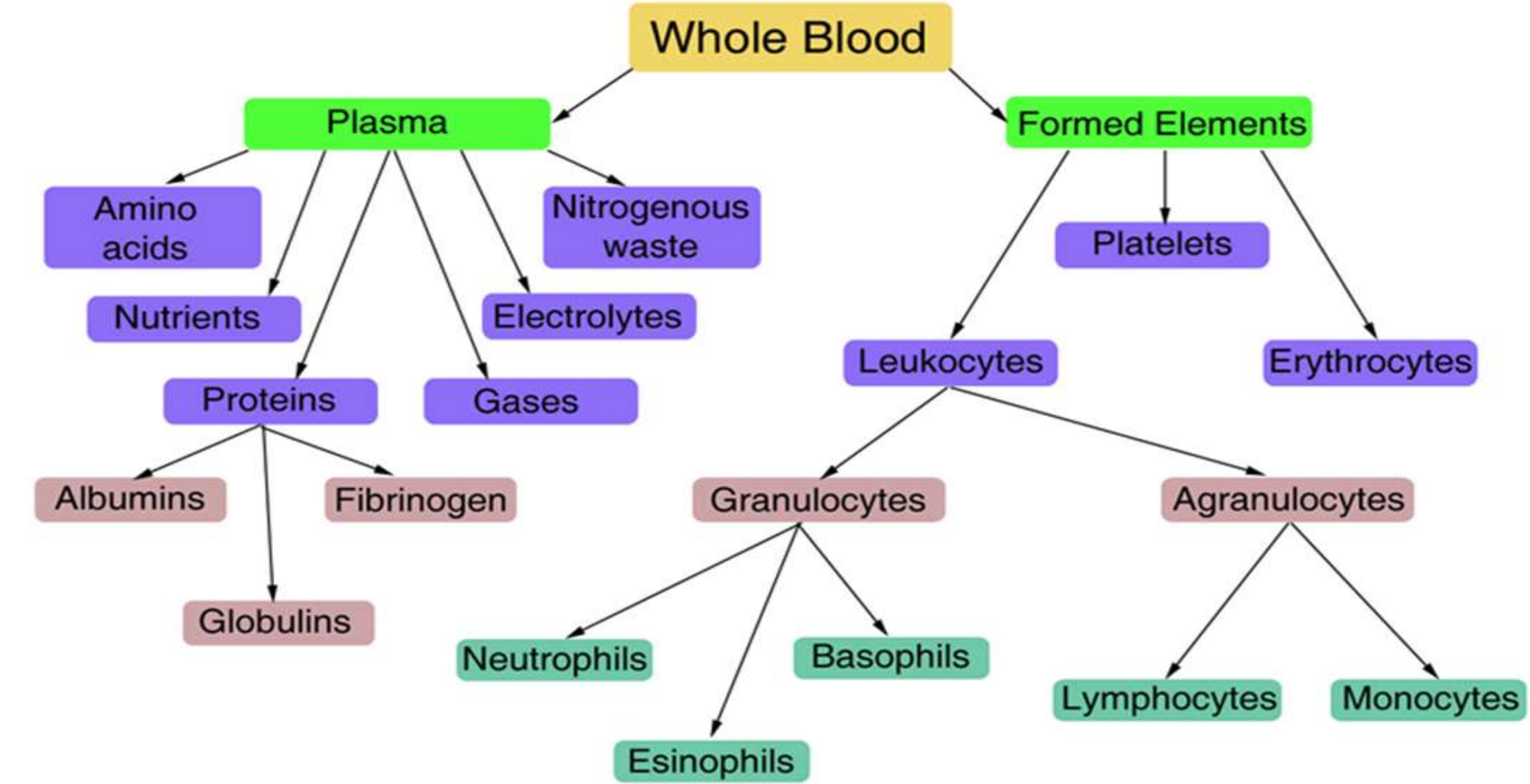
Types of biological specimens

- Blood ((whole, plasma, serum)
- Urine
- stool
- Sputum
- Biological fluids
- Bone marrow

Collection and Preparation of Blood Specimen

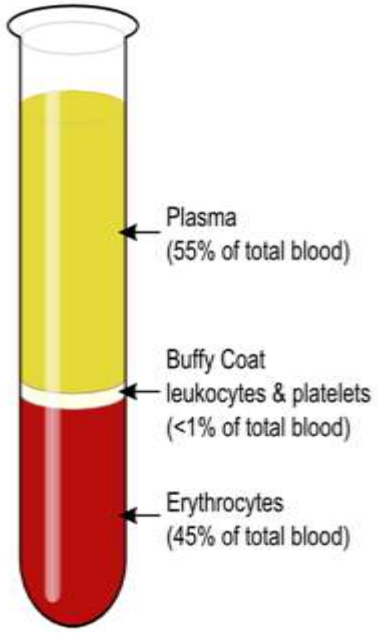
- Blood is a body fluid that runs throughout the body. It is the medium through which all necessary elements like nutrients and oxygen are transferred to cells and all metabolic wastes are transferred from cells. Blood consists of almost 8% of human body's weight.
- Functions of blood include transportation of necessary elements, protection from the foreign materials like bacteria, fungus etc., and regulation of pH by interacting with acids and bases.

Composition of Blood



Plasma (Fluid portion of non-clotted blood) is obtained from the anticoagulated blood.

Serum (Fluid portion of clotted blood) is obtained from clotted blood. Usually, blood is obtained by vein puncture with the help of a sterilized dry syringe. For clinical biochemistry, mostly serum/plasma and somtimes whole blood is required.



Types of test tubes require for blood sample collection

Tube cap color	Additive	Function of Additive	Common laboratory tests
Light-blue 	3.2% Sodium citrate	Prevents blood from clotting by binding calcium	Coagulation studies (PT,PTT)
Red or gold 	Serum tube with or without clot activator or gel	Clot activator promotes blood clotting with glass or silica particles. Gel separates serum from cells.	Chemistry, serology, immunology
Green 	Sodium or lithium heparin with or without gel	Prevents clotting by inhibiting thrombin and thromboplastin	Hematological studies
Lavender or pink 	Potassium EDTA *(Ethylene Diamine Tetraacetate)*	Prevents clotting by binding calcium	Hematology (CBC) and blood bank
Gray 	Sodium fluoride, and sodium or potassium oxalate	Fluoride inhibits glycolysis, and oxalate prevents clotting by precipitating calcium.	Glucose (especially when testing will be delayed), blood alcohol, lactic acid
 **Dark Blue-Top**	There are 2 type One with K2 EDTA and one with no anti-coagulant		This tube is used primarily for trace metal analysis

➢ESR tube

It contain sodium citrate which bind with calcium

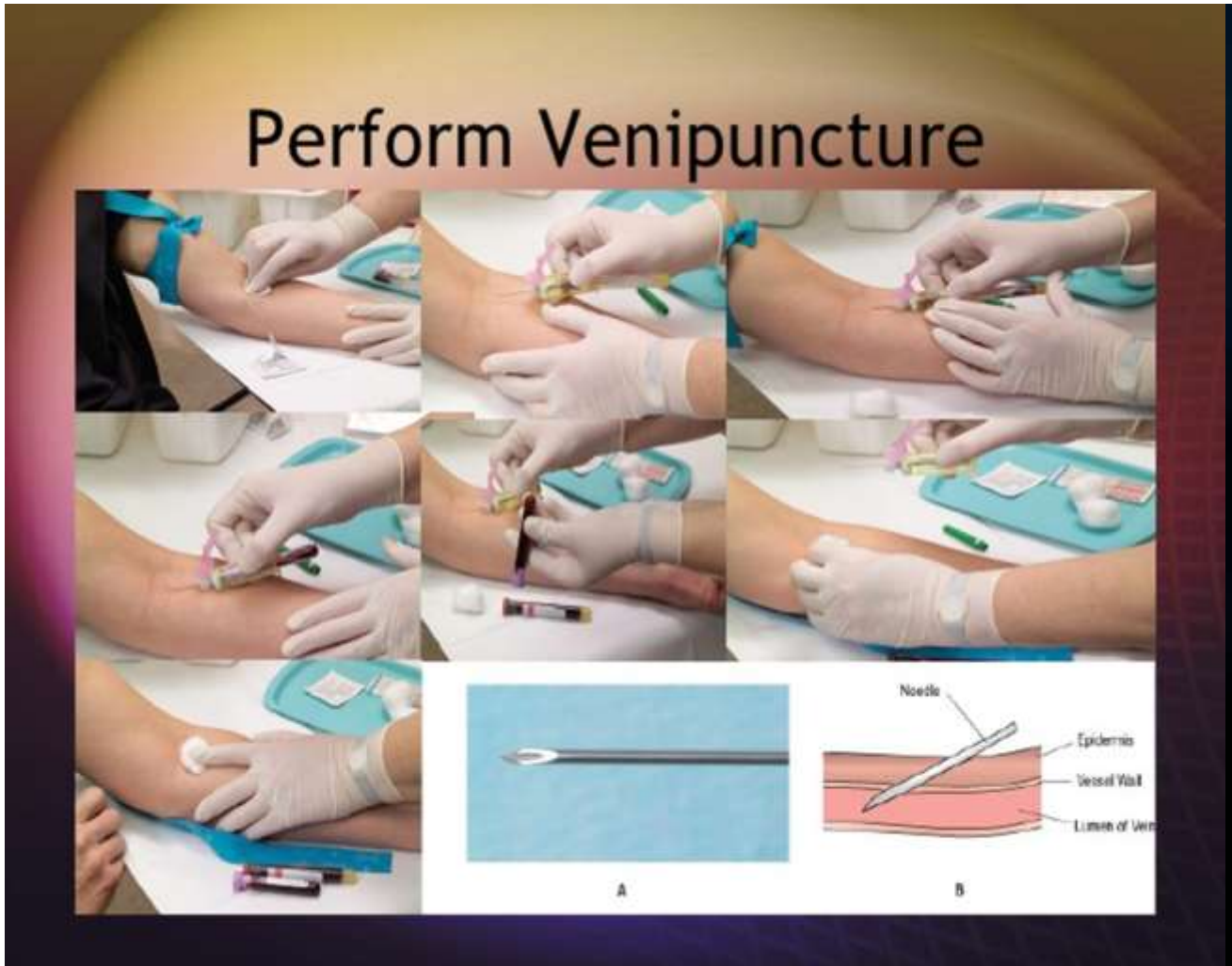
to remove calcium

➢Blood culture tube

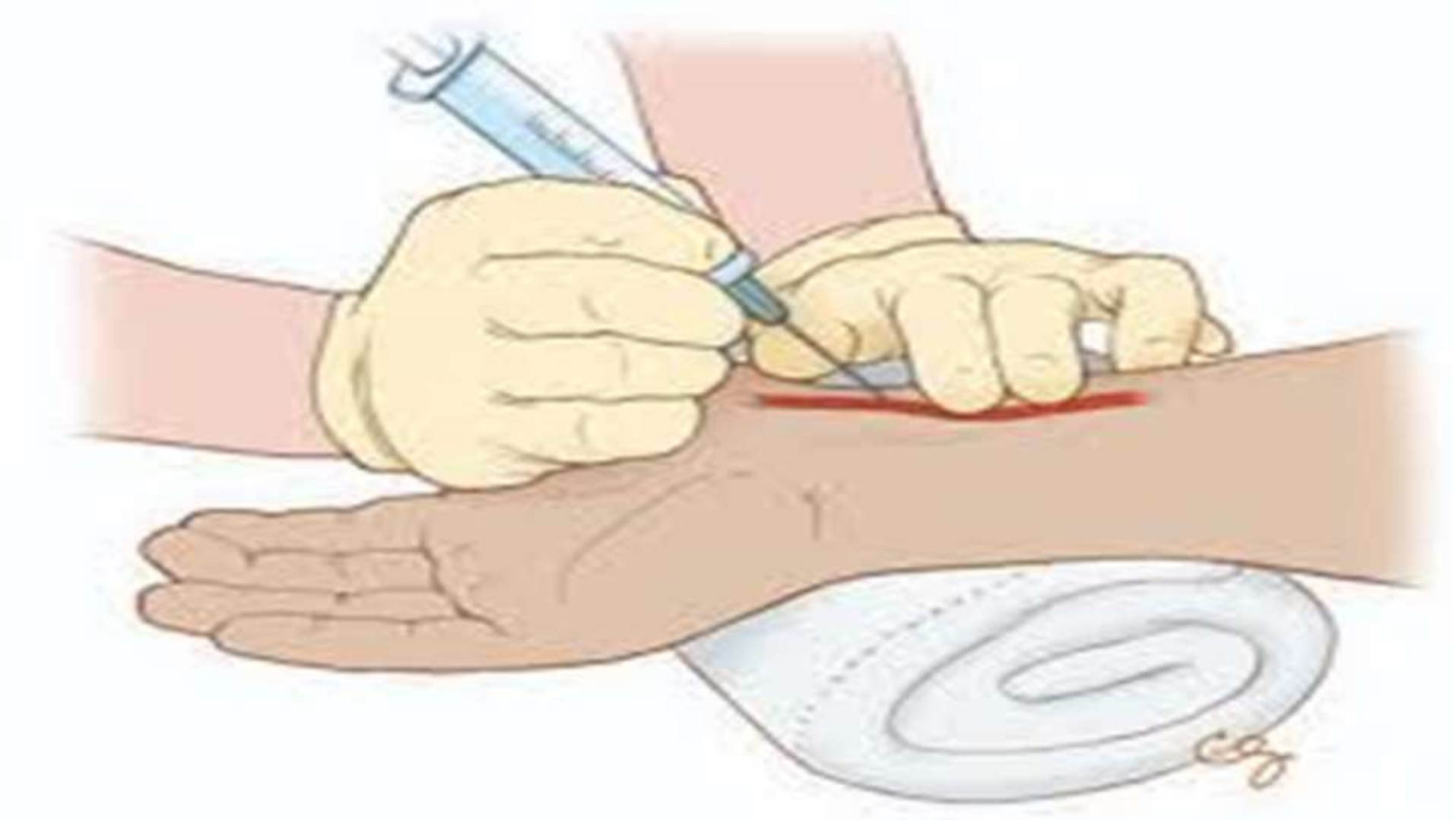
COLLECTION OF BLOOD

- Blood sample is collected by:

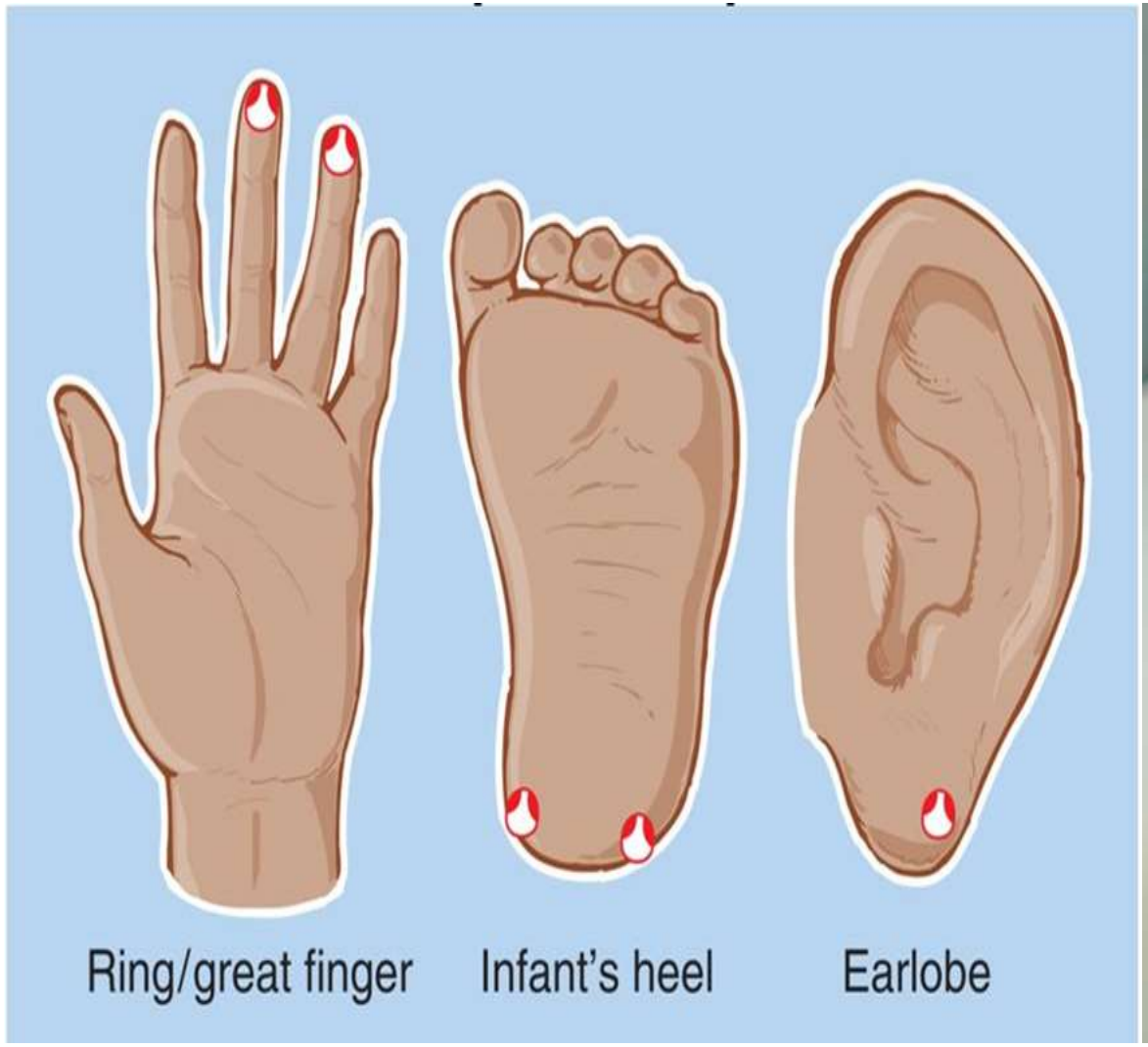
a- Venipuncture



B-Arterial puncture



C- Capillary puncture or skin puncture



Precautions of Sample drawing

1- Exclusion criteria (history of bleeding disorder, take anticoagulant drug)

2- Fasting before the sample collection

3- Position of subject (The position of the subject can influence the cholesterol values. Standardization of the position is necessary. It is recommended that all blood samples should be drawn in a sitting position and that the participant remain in sitting position for 15 minutes prior to blood collection.)

4- Use of tourniquet

Prolonged venous occlusion can cause stasis and hemoconcentrations. Therefore, the use of a tourniquet should be minimized. If a tourniquet is used to search for a vein, it should be released before withdrawal of blood begins. In any case, the use of a tourniquet should be limited to less than one minute.

5-Vigorous shacking of blood specimen may result hemolysis of blood specimen.

6- Site of blood sample drawing: blood sample should not be drawing when an intravenous line is infusing proximal to the intended puncture site because of the potential for dilution when the specimen and the IV solution combine in the collection container, falsely decreasing the result.

7- Previous puncture sites should be avoided when accessing a blood vessel by any means to reduce the potential for infection.

8-Collection times for therapeutic drug (peak and trough) or other specific monitoring (e.g., chemotherapy, glucose, insulin, or potassium) should be documented carefully in relation to the time of medication administration. It is essential that this information be communicated clearly and accurately to avoid misunderstanding of the dose time in relation to the collection time.

Storage and transfer of blood samples

Samples should be refrigerated to 4°C immediately after collection. At that temperature they are stable for 7 days. If it is anticipated that analysis cannot occur within 7 days, samples should be frozen immediately at -70°C (-20°C is not sufficient).

Collection of Urine specimens

- Urine test are easy to obtain and provide valuable information about many body system functions such as kidney function, glucose metabolism and varies hormone levels.
- Standard urine specimen can be collected at any time, whereas first morning, fasting and timed specimen require collection at specific times of day. Assess for presence of interfering factors: failure to follow collection instruction, inadequate fluid intake, certain medications or food may affect the result.

1- Single, Random urine specimen

- This is the most commonly request specimen. Because the composition of urine changes over the course of the day, the time of day when the specimen is collected may influence the finding.
- The fist voided morning specimen is particularly valuable because it is usually more concentrated and therefore more likely to reveal abnormalities as well as the presence of formed substance.it is also free of dietary influence and of changes caused by physical activity because the specimen is collected after a period of fasting or rest.

2- long-term, timed urine specimen (2-hour, 24-hour)

- Substance excreted by the kidney are not excreted at the same rate or in the same amount during different period of time so random urine specimen might not give an accurate picture of the processes taking place over 24-hour period.
- This method used for measurement of total urine protein, creatinine and electrolytes.

Preservation of Urine Samples

The urine specimen should be analysis within one hour otherwise store in the refrigerator, if it is not store in the refrigerator the following changes in the composition:

1- increase PH from breakdown of urea to ammonia by urease-producing bacteria

2- decreased glucose from glycolysis and bacterial utilization

3- decrease ketones because of volatilization

4- decreased bilirubin from exposure to light

5- decreased urobilinogen as it is oxidation to urobilin

6- increase turbidity caused by bacterial growth and possible precipitation of amorpous material

7- disintegration of RBC and casts, particularly alkaline urine

8- changes in color caused by oxidation or reduction of metabolites

Triglyceride

- Triglycerides are the most abundant glycerol esters & encompass 95% of all fat stored in the body adipose tissue.
- Triglyceride produced in the liver by using glycerol and other fatty acid as building blocks.
- Triglyceride act as a storage store source for energy.

Interfering factors

- 1-ingestion of fatty meals may cause elevated triglycerides.
- 2-Drining alcohol may cause elevated triglyceride.
- 3-Pregnancy may cause high level of triglyceride
- 4-Estrogen & oral contraceptives cause high level of triglyceride

- 5-Ascorbic acid ,clofibrate &colestipol causing low level of triglyceride.

Triglyceride rises in the following cases:-

1-Cardiovascular disease
2-Hypothyrodisim
3-Hyperlipedemia
4-Nephrotic syndrom
5-Alcoholic crrihosis
6-Diabetes
7-Pregnancy

- Low Triglycerides occurs in the following cases:-
- 1-Malabsorption
- 2-Malnutrition
- 3-Hyperthyrodisim
- 4-Liver disease

Metabolism

- Triglycerides are removed by lipoprotein lipase & transfer in to LDL which contain a large amount of cholesterol and it is ester.
- LDL is then transport in to the cells
- In adipose tissue triglycerides formation and breakdown both.

- Adipose tissue is mobile to re_use glycerol formed as a result of hydrolysis of triglyceride.
- Most of free faty acid in plasma derived from triglyceride of adipose tissue.

Estimation

- Patient preparation:-
- 12 hours fasting or more
- Specimen collection:-
- Serum,plasma (heparinzed plasma used only)
- Specimen storage:-
- 4 C° for 7 days 20 C°for 90 days thawed samples should be mixed well and brought to room temprature before analysis

- Reference range:-
- 40-160 mg/dl male
- 39-135 mg/dl emale
- Methods:-
- Methods measure free glycerol by a variety of coupled enzymes

- Reagents:-
- Reagent 1:- stander glycerol
- Reagent 2-Triglyceride bufer PH 7.6 ,100 mmol/L parachlorophenol ,2.7 mmol/L magnesium
- Reagent3:- Amino antipyrine>0.4 mmol/L,lipase1000 u/L,glycerokinase > 200u/L,glycerol 3 phosphate oxodase>2000u/L,peroxidase>200 u/L,ATP 0.8 mmol/L

Blood urea

- Urea is the main product of protein metabolism which occurs in liver by the break down of amino acids and by the removal of amino group (NH_3) from amino acids these are catabolized in the liver to form ammonia.

- Urea is formed from NH_3 and CO_2 then transport by blood to kidneys for excretion.

- So,urea level is controlling by two organs liver and kidneys.

- Normal values:-
- Adult:-2.5-6.4 mmo/L or 7.0-18.0 mg/dl .
- Elderly >60 y:-2.9-7.5 mmol/L or 8-20 mg/dl .

- Usually the normal range of BUN in persons on a full ordinary diet is about 2.5 -6.4 mmol/L or 7.0-18.0 mg/dl.
- Urea some what higher in men then women.
- There is slow raise with age.
- Serum urea is lower in pregnancy due to heamodilution (15-20%).
- After 3rd trimester of pregnancy urea rises.

Azotemia

- Elevated level of blood urea nitrogen is referred to as Azotemia.
- If urea > 36 mmol/l or >100 mg/dl indicates serious impairment of renal function.
- Azotemia markedly increase in BUN give evidence of severe impairment of glomerular function

Specimen

- Serum is preferred then whole blood !! Why?
- No fasting is required.
- Avoid hemolysis.
- Interfering factors:-
- 1-Changes in protein intake may effect BUN levels.
- 2-Over hydration & un hydration.
- 3-Drugs my cause high or low level of BUN

Causes of high urea level

- 1-Pre-Renal causes.
- 2-Renal causes.
- 3-Post renal disease.

Pre-Renal causes

- Most common type (60-70%) the most important cause is **hypovolemia** which is due to water & salt depletion because of:-
- Sever vomiting
- Sever and prolong diarrhea.
- Ulcerative colitis
- Shock
- Fever
- Starvation

Renal causes

- The most complicated cause of renal failure(25-40%) renal causes are :-
- **Renal disease such as:-** (glomerulonephritis,polynephritis,acute tubular necrosis).
- **Renal failure** :-The cause of renal failure is either
- 1-Affecting the filtering function of the kidney.
- 2-Affecting blood supply within the kidney.
- 3-Damage of kidney tissue.
- **Nephrotoxic drugs.**

Post renal cause

- This due to the existing of obstruction to the flow of urine which is leads to retention of urine and this reduces the filtration of glomeruli and among these are:-
- 1-Ureteral obstruction of one or both ureter
- 2-Bladder outlet obstruction or tumor of bladder.
- 3-Enlargment of prostate.
- 4-Stones in urinary tract.

Causes of low urea level

- Liver failure, liver disease such as viral hepatitis
- Over hydration caused by fluid overload.
- Malnutrition or malabsorption.
- Pregnancy.
- Nephritic syndrome.

How we do estimation of urea?

- We do it by:-
- 1-Nessler's method.
- 2-Urea kit.
- 3-Diacetyl monoxime method.

Nessler method

- Nesslerization method has been most widely used.

- Its include conversion of urea to Ammonium carbonate by urease enzyme (found in soya , jack beans & in water melon seeds).
- The ammonia which is formed has been detected by reaction in alkaline media(with salicylate and hypochlorite and determined colorimetrically with comparison with standard urea solution.

Difficulties with Nesslerization method

- 1-Turbidity:-RBC contain glutathione & ergothionine which produce turbidity because it forms mercuric salt which is insoluble so, we use zinc hydroxide that come from addition of zinc sulfate &sodium hydroxide as deproteinizing agent to eliminate a small amount of turbidity.

- 2-Ammonia from air or from reagent must be strictly avoided so, use rubber plug.
- 3-Aceton avoided to be used for drying the pipettes or other glassware.
- 4- Iced water:- Put the tubes in iced water for few minutes before adding Nessler's reagent to prevent turbidity.

Diacetyl monoxie method

- It is a direct colorimetric method

urea react with diacetyl monoxime give a colored condensation product (diazine) in the presence of strong acid medium. Upon initial hydrolysis diacetylmonoxime release that react with Urea. The color of the condensation product diazine is pink measure by spectrophotometer at 520 .

Berthelot reaction

- Berthelot's reagent is an alkaline solu.of phenol & hypochlorite .the enzyme urease used to catalyze the hydrolysis of urea in to CO_2 and NH_3 , ammonia is formed which is in alkaline media react with salicylate and hypochlorite to form green colored indophenol-Berthelot method.
- The reaction of ammonia with phenol-hypochlorite to give indophenols (the principle is used to analyze ammonia concentration in body fluids)

Urea kit

- Berthelot reaction

- Reagent 1 std urea
- Reagent 2 enzyme urease
- Reagent 3 color reagent :- Phosphate buffer PH 8,sodium salicylate ,sodiume nitroprusside &EDTA.
- Reagent 4 :-Sodium carbonate ,sodium hypochlorite.


Uric acid

- Uric acid is a waste product of purine metabolism in human.

- The purine basis is adenine and guanina formed in course of nucleic acid catabolism &free nucleotide undergo oxidation to uric acid .

- In animals degrade uric acid to allantoin by means of uricase enzyme which is missing in human.
-

Purine Degradation to Uric Acid

- **Xanthine oxidase** catalyzes the final conversions to uric acid

Uric acid sources

- 1-Exogenous sources:-
- Red meat,liver,stimulants in coffee and tea.
- 2-Endogenous sources:-
- Nucliec acid catabolism.
- Liver is the main site of uric acid formation
- Plasma uric acid is filtered by the glomeruli and about 90% reabsorbed by the tubules.

REFERENCE VALUE

- Male : 3.4_7 mg/dl
- Female :2.4_5 mg/dl
- Urine :250_750 mg/dl

- Clinical significance:-
- Determination of serum uric acid is most helpful in the diagnoses gout where sodium urate are deposited in solid form in and around the joints.

Gout

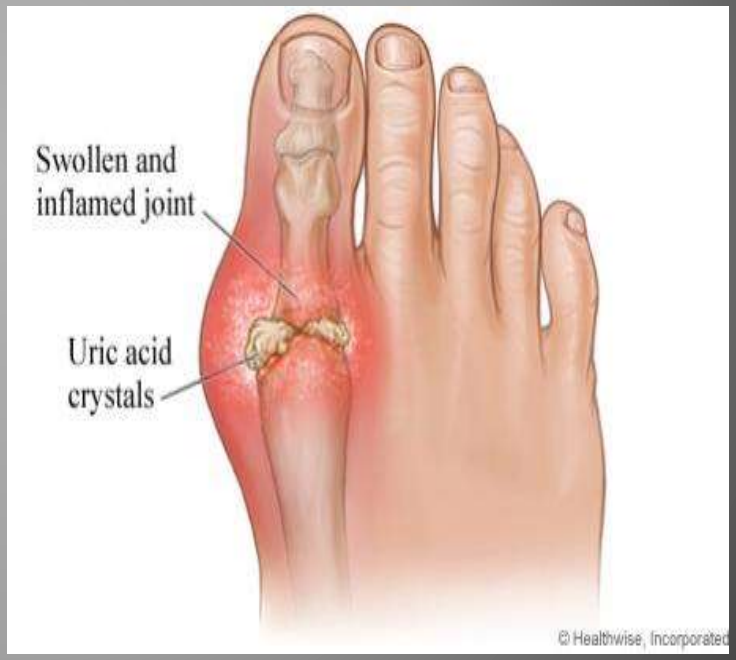
- Is disease characterized by high level of uric acid which deposited in solid form in joins causing *arthritis .*
- *The concentration of uric acid reaches a certain level it cannot stay dissolved in the blood (crystals)*

There are several factors that can make a person more susceptible to gout

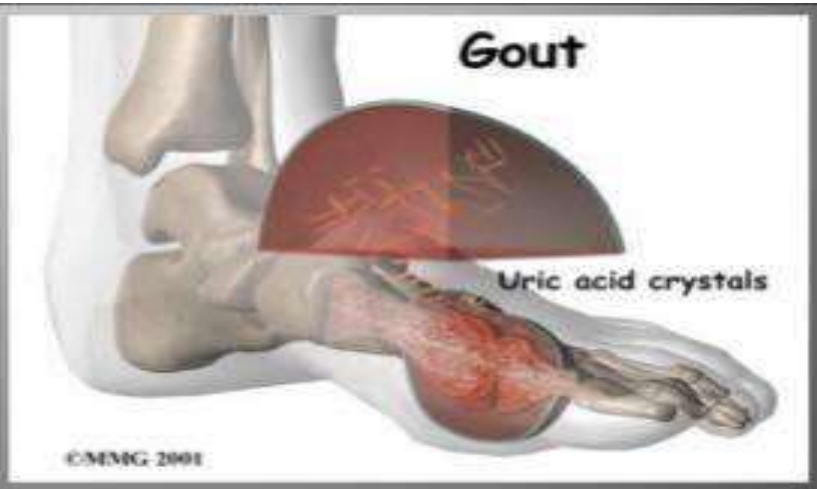
- Family history of gout .
- Being overweight .
- Having kidney problems .
- Lead exposure .
- Drinking too much alcohol .
- Taking certain medications like diuretic .

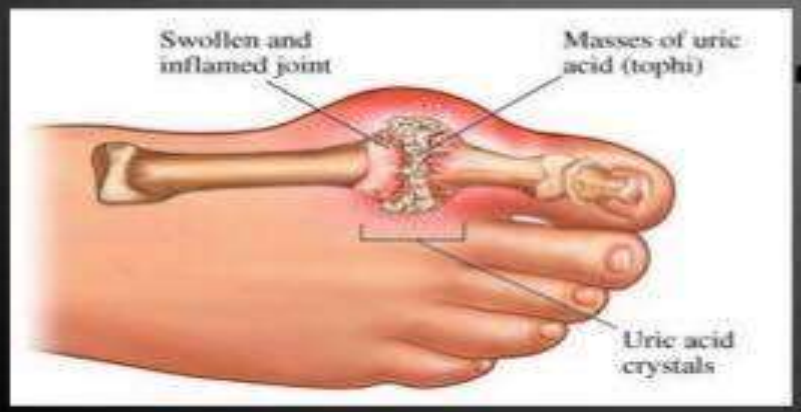
Gout

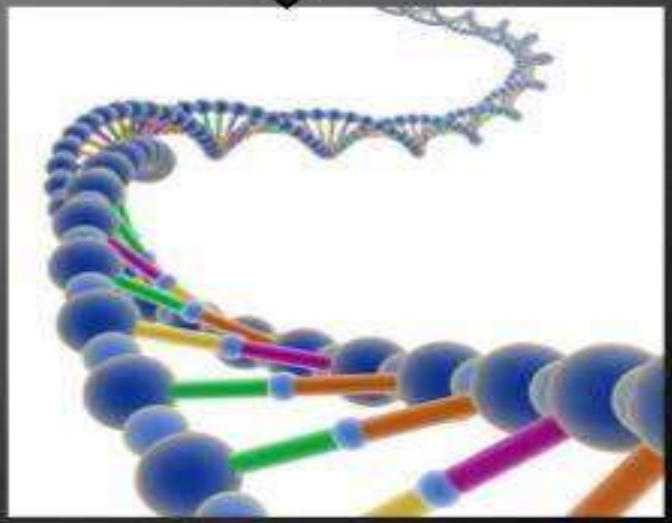

Uric Acid Crystals


Swollen and
inflamed joint
Uric acid
crystals
© Healthwise, Incorporated

- Uric acid crystals are tiny needle shaped crystals , between 5 _25 microns in length .







Types of goat

- Primary gout :-
- Is a metabolic disorder in which the kinetic enzyme phosphoribosyl pyrophosphate (PRPP)Synthetase are altered leading to overproduction pf (prpp) & more purine synthesis.

- Secondary gout:-
- Due to elevated of purine catabolism such as in case of leukemia or in renal failure.

Increase Uric Acid

Hyperuricemia (high levels of uric acid), Causes an elevated in blood uric acid level,Include genetics, obesity, Certain medications such as diurtics and chronic decrease in kidney function.. which induces gout, that has various causes :

- Diet may be a factor. High intake of dietary purine, high-fructose corn syrup, and table sugar can increase levels of uric acid.
- Serum uric acid can be elevated by reduced excretion via the kidneys.
- Fasting or rapid weight Loss.
- Certain drugs, such as thiazide diuretic
- Tumor lysis syndrome, a metabolic complication of certain cancers or chemotherapy, due to nucleobase and potassium release into the plasma.

- Starvation
- Alcoholism
- Leukemia (increased turnover of cells)
- Gout
- DM

Hypourecemia

- Proximal renal damage where urate reabsorbtion will reduced.
- Xanthine oxidase deficiency
- Deficiency of purine nucleotide phosphorylase will result in excretion of purine nucleoside in urine so, uric acid decrease in blood.

Allopurinol

- It’s structure is similar to hypoxanthine competatively inhibits the xanthine oxidase enzyme &decrease the production of uric acid

PROCEDURE:

Pippete in cuvete	Blank	Standard	sample
Reagnt	1 ml	1ml	1 ml
Standard	----	0.02 ml	----
Sample	----	-------	0.02 ml

M ix and incubate 10 min and measure abs at 546 nm

CALCULATION:

URIC ACID CONC = Abs sample

---------- x 6 == _____ mg /dl

Abs of standard

We should get light pink