University of Mosul

College of Nursing

Undergraduate Studies

Biochemistry/ practical part

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Exp. No. : (1)

Name of Exp. : Handling of laboratory equipment

The important equipment can you use in laboratory:

1- Centrifuge

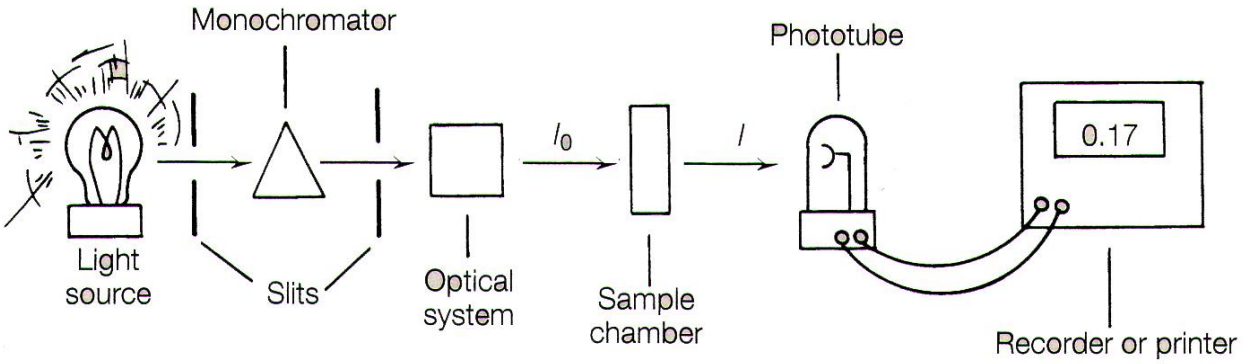
Centrifuges have many applications , but they are used primarily for the preparation of biological samples and for the analysis of the physical properties of biomolecules , organelles , and cells . centrifugation is carried out by spinning a biological sample at a high rate of speed , thus subjecting it to an intense force (artificial gravitational field).

Most centrifuge techniques fit into one of two categories – preparative centrifugation or analytical centrifugation . A preparative procedure is one that can be applied to the separation or purification of biological samples (cells , organelles , macromolecules ,etc.) by sedmentation. Analytical procedures are used to measure physical characteristics of biological samples .for example , the purity , size shape ,and density of macromolecules may be defined by centrifugation .

2- Spectrophotometer

Instrumentation :

The srectrophotometer is used to measure absorbance experimentally . this instrument preduces light of a preselected wavelength , directs it through the sample (usually dissolved in a solvent and placed in a cuvette) , and measures the intensity of light transmitted by the sample . the major components are shown in figure (1) these consist of a light source , a monochromator (including various filters , slits , and mirrors) , a sample chamber , a detector , and a meter or recorder . All of these components are usually under the control of a computer.



Fig(1): Spectrophotometer

- Light Source :

For absorption measurements in the ultraviolet region , a high – pressure hydrogen or deuterium lamp is used . these lamps produce radiation in the 200 to 340 nm range. The light source for the visible region is the tungsten – halogen lamp, with a wavelength range of 340 to 800 nm . Instruments with both lamps have greater flexibility and can be used for the study of most biologically significant molecules .

- Monochromator:

Both lamps discussed above produce continuous emissions of all wavelengths within their range . therefore , a spectrophotometer must have an optical system to select monochromatic light (light of a specific wavelength). It should be noted that light emitted from the monochromator is not entirely of a single wavelength , but is enhanced in that wavelength.

Before the monochromatic light impinges on the sample , it passes through a series of slits , lenses , filters , and mirrors . This optical system concentrates the light , increases the spectral purity and focuses it toward the sample .

- Sample Chamber :

The processed monochromatic light is then directed into a sample chamber, which can accommodate a wide variety of sample holders . Most UV- VIS measurements on biomolecules are taken on solution of the molecules . The sample is placed in a tube or cuvette made of glass, quartz , or other transparent material.

Glass cuvettes are inexpensive , but , because they absorb UV light , they can be used only above 340nm . Quartz or fused silica cuvettes may be used throughout the UVand visible regions (200 – 800 nm) .

- Detector :

The intensity of the light that passes through the sample depende on the amount of light absorbed by the sample . Intensity is measured by a light – sensitive detector , usually a photomultiplier tube (PMT).

- Water bath

Water bath is used for boiling of different chemical solution in different temperature .

Composition of the blood :

1- water 75 - 80%
2- Total protein 20% (albumin , globulin , fibrinogen)
3-Carbohydrates 0.1% (glucose)
4-Total lipid 1-2% (cholesterol, triacylglycerides, phospholipids
5- Inorganic salts 2%(sodium , potassium , calcium , magnesium ,ferric , zinc)
6- Other products 0.5%(CO2 ,uric acid , bilirubin , creatinine)
7- Enzymes (amylase , glutamate oxaloacetate transaminase (GOT),glutamate pyruvate transaminase (GPT)

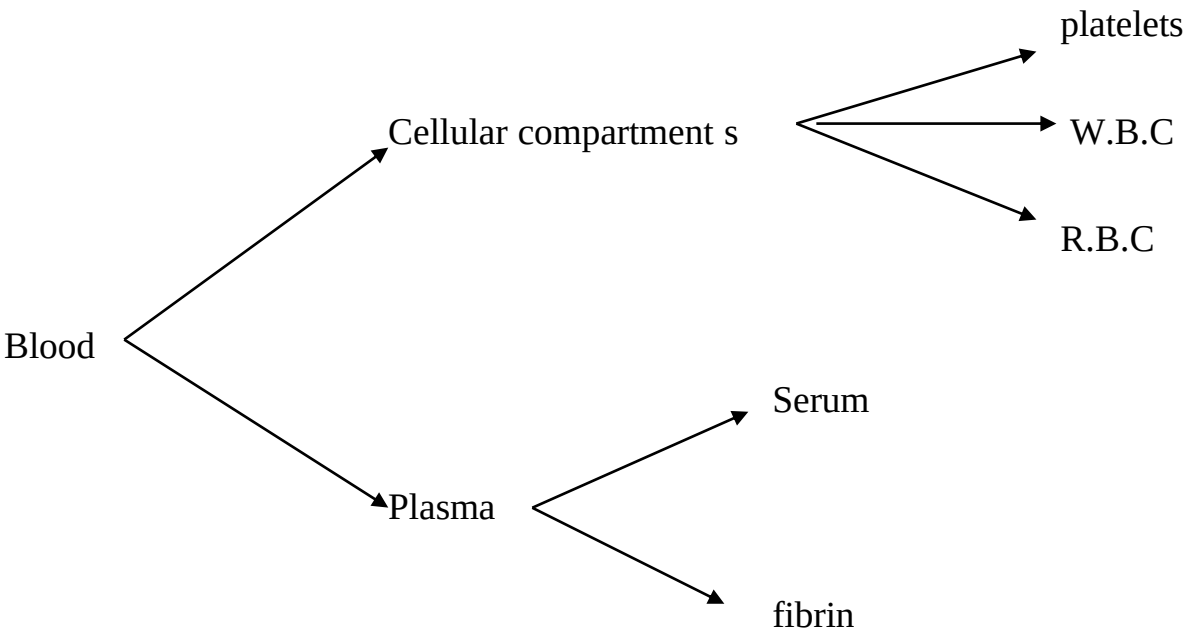
Collection of blood :

Blood may be obtained from veins or arteries , most tests in the clinical chemistry laboratory are performed on venous blood , while arterial blood is primarily used for blood gas determination .

- What are difference between serum and plasma ?

If the blood is allowed to clot at least 10 to 15 minutes at room temperature , the tube is centrifuged and the supernatant serum removed ,the serum shoud be removed from the clot soon after collection ,serum did not contain on fibrin because the fibrin is used to clot the blood and it mix with the celluler compartments .

If whole blood or plasma is desired , an anti- coagulant must be added to the specimen immediately after it is placed into the tube in which the blood is collected ,plasma is contain on fibrin because the fibrin is didn't use to clot the blood and didn't mix with the celluler compartement .



Coagulation of the blood:

1- prothrombin $\xrightarrow{\text{thromboplastin}}$ thrombin

2- fibrinogen $\xrightarrow{\text{thrombin}}$ fibrin

Anticoagulant:

1- Heparin :

Heparin is present in most of the tissues of the body , it er exists in the highest concentrations in the liver and the lungs ,it is available as the sodium,potassium and ammonium salt .

It is believed to act as an antithrombin , preventing the transformation of prothrombin into thrombin , and thus preventing the formation of fibrin from

fibrinogen , heparin has also been shown to posses antithromboplastin activity and to inhibit the lysis of platelets .

2- Oxalates:

Such as sodium , ammonium , or lithium , inhibit blood coagulation by forming insoluble complexes with calcium ions , which are necessary for coagulation . potassium oxalate at a concentration of about 2mg/ml of blood is the most widely used .

3- Sodium fluoride :

Sodium fluoride usually considered as preservative for blood glucose determination , also acts as a weak anticoagulant . when used as a preservative along with an anticoagulant such as potassium oxalate , it is effective in a concentration of about 2mg/ml of blood .

It is action by inhibiting the enzyme system involved in glycolysis , when sodium fluoride is used as an anticoagulant alone , the concentration must be greater, (6 to 10 mg /ml of blood) .

4- Ethylenediaminetetra acetic acid (EDTA):

It is a chelating agent that is particularly useful for hematological examination since it preserves the cellular constituents of the blood .it is used as the disodium or dipotassium salt, it is effective in concentration of 1to 2mg/ml of blood , anticoagulant activity derives to essentially binds calcium which is needed for the clotting mechanism .

EXP. NO. :(2)

Name of EXP. : Estimation of blood glucose .

Theory :

The glucose belongs to the carbohydrates that are considered the major food supply and energy source for the people of the world .

Despite the major utilization of carbohydrates for energy , only a small amount is stored in the body . the average adult reserve is about 370 g , stored chiefly as liver and muscle ,Since 1g carbohydrate supplies 4 calories. Glucose is monosaccharide (hexoses and aldoses sugar), has reducing power.

Insulin hormone plays central role in regulation of the blood glucose concentration, it is produced by the B-cells of the islets of langerhans in the pancreas and is secreted into the blood as a direct response to hyperglycemia.

There are number of hormones effect of blood glucose concentration except insulin as : (glucagon , growth hormone , thyroxin , epinephrine , and hydrocortisone), the blood glucose concentration increase after a meal , then return to normal level by effect of hormones, thus the sample used in blood glucose test take after fast , (fasting blood sugar).

Clinical significance :

1-Hyperglycemia (increase of glucose level)

a- Diabetes mellitus b- Hyperthyroidism c- Nephritics

d- Hyperpitutarism e- pregnancy f- Uremia

2- Hypoglycemia (decrease of glucose)

a- Hyperinsulinism b- Hypothyroidism c- Hypopitatarism

d- Addisons disease e- Hepatic disease f- Starvation

g- Pernicious vomiting

* Normal rang of blood glucose :

80 - 120 mg / 100ml (in serum)

60 - 100 mg / 100ml (in blood)

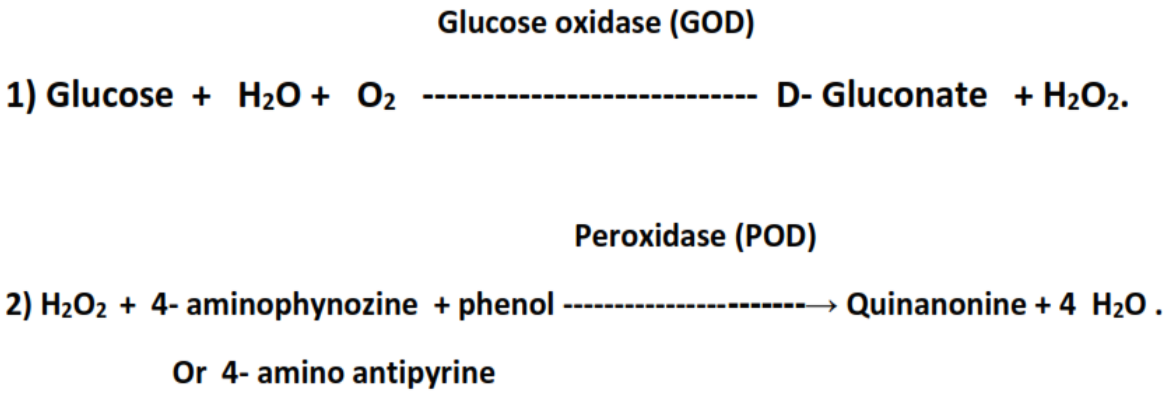
Metabolism of glucose :

Apsoption of monosaccharides is fairly complete and appears to occur by an active enzymatic transfer process .

Following absorption into the portal vein , the glucose (hexoses) are transported to the liver , depending on the needs of the body , the glucose may be converted to and stored as liver glycogen ,metabolized to carbon dioxide and water to provid energy , converted to keto acids , amino acids, and proteins , or converted to fat and stored as adiposetissue .

Enzymatic Method For Blood Glucose Estimation:

Principle:



Procedure:

1) Drow 2-3 ml of blood.

2) Centrifugation.

3) Prepare the test tubes, & classified them as: Blank, standard and Samp

4) Addition as follow: 10 Ml

Tubes	Blank	Sample	Standard
Work solution	1.0 ml	1.0 ml	1.0 ml
Sample		10 Ml	
Standard			10 Ml

5) Mixing, then put the test tubes in a water path for 10 minutes at 37 C .

6) Put these tubes in spectrophotometer & arrangement of it as:

Blank, Standard & Standard.

7) Calculations:

$$\text{Glucose conc.(mg/dl)} = \frac{\text{Ab. Sample}}{\text{Ab. Standard}} \times \text{Standard concentration.}$$

NOTES:

1) The concentration of standard is 100 mg/dl.

2) The wave length is 500 nm.

3) Intensity of colour depends upon glucose concentration.

4) 10 Ml = 10/ 1000 =0.01

EXP. No. :(3)

Name of EXP. : Determination of Albumin , Globulin and Total protein

Theory :

Prpteins are nitrogenous compounds , have high molecular weight , consist of long chains of amino acids (there are 20 L-α- amino acids commonly found in proteins) linked by peptide bonds formed between the carboxyl group of one amino acid and the amino group of the other $\left(-\overset{\overset{O}{\|}}{C}-\overset{\overset{H}{|}}{N}-\right)$.

Proteins may be classified as simple and conjugated proteins according to their structure , conjugated protein such as glycoprotein and lipoproteins,

and they also classified as fibrous and globular according to their physical properties .

Human body contains soluble and nonsoluble proteins , clinical chemistry deals with soluble proteins which found in plasma , urine and cerebrosбpinal fluids , plasma is (97%)water , (the remaining (3)occupied by solids , over (95) of it , is occupied by protein) , plasma proteins comprise major part of the solid of plasma .

There are three major types of plasma proteins , they are ;(albumin , globulin , fibrinogen),globulin have four types and they are " (α1- glob. , α2- glob. ,β- glob. γ - glob.).

Functions of plasma proteins :

1- Transport ' e.g hormones , metals.

2-Colloid osmotic pressure . (mainly albumin)

3- Active enzymes " e.g clotting factors .

4- Enzyme inhibitors e.g antiproteases .

5-Endogenous source of amino acids .

Clinical significance :

Hyperproteinemia (increase of concentration of plasma proteins) :

1- Dehydration: may result either from '

a- decrease in water intake about 10 to 15%

b- loss of water as in increase vomiting diarrhea , addisons disease or diabetic acidosis .

2- Multiple myeloma: may reach to 10 g/ 100 .

Hypoproteinemia (decrease of concentration of plasma proteins) :

1- Nephrotic syndrome.

2- Malnutrition .

3-Chronic liver diseases.

4- Burns and extensive bleeding .

5- Gastro – intestinal tract diseases .

Normal range of plasma proteins :

Albumin = 4.0 - 5.5 g / 100 ml serum

Globulin = 2.2 - 2.7 g / 100 ml serum

Total protein = 6.2 - 8.2 g / 100 ml serum

Principle:

Total protein + Cu^{+2} ----------⟶ Violet- blue complex.

Procedure:

1) Drow 2-3 ml of blood.

2) Centrifugation.

3) Addition as follow:

Tubes	Blank	Sample	Standard
Work solution (R1)	1.0 ml	1.0 ml	1.0 ml
Sample		25 Ml	
Standard			25 Ml

4) Mixing, then put the test tubes in a water path for 10 minutes at 37 C .

5) Put these tubes in spectrophotometer, The wave length is 540 nm.

6) Calculations:

$$\text{Total proteins conc.(mg/dL)} = \frac{\text{Ab. Sample}}{\text{Ab. Standard}} \times \text{Standard concentration.}$$

NOTE:

The concentration of standard is 7 mg/dL .

Principle:

Albumin + Bromcresol Green ----------→ Green Albumin (BCG) complex.

Procedure:

1) Drow 2-3 ml of blood.

2) Centrifugation.

3) Addition as follow:

Tubes	Blank	Sample	Standard
Work solution	1.0 ml	1.0 ml	1.0 ml
Sample		50 Ml	
Standard			50 Ml

4) Mixing, then put the test tubes in a water path for 10 minutes at 37 C .

5) Put these tubes in spectrophotometer, the wave length is 540 nm.

6) Calculations:

$$\text{Albumin conc.(mg/dl)} = \frac{\text{Ab. Sample}}{\text{Ab. Standard}} \times \text{Standard concentration.}$$

NOTE:

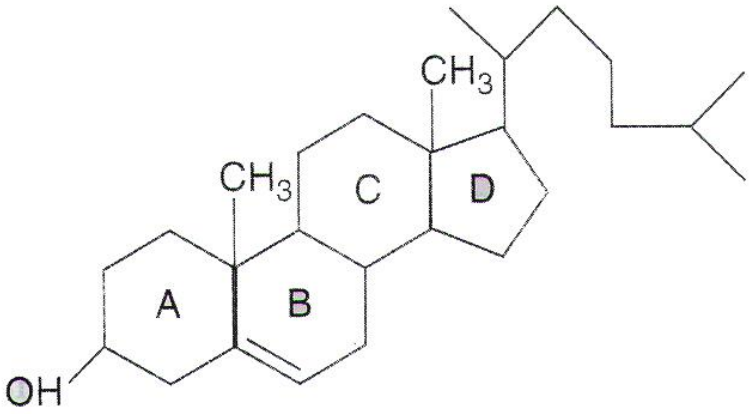
The concentration of standard is 5 mg/dl.

Normal value in adults: 35-52 mg/dl.

Exp. No.: (4)

Name of Exp. : Cholesterol

Formula :



Cholesterol

Category:

The cholesterol is a steroid compound containing a steroid nucleus (the ring A, B, C , D) chole : means Bile , sterol : means solid Alcohol , cholesterol means Bile solid Alcohol .

The molecular structure of cholesterol is ($C_{27}H_{45}OH$)

Occurrence :

The is present in human tissues , the largest amount of it present in :

1- Brain 2- Nerve tissue 3- Bile 4- Blood 5- Liver

14% of the white matter of the brain is cholesterol

10% of the spinal cord is cholesterol .

Cholesterol has been found in blood in two forms :

1- Free cholesterol (1/3 of total cholesterol)

2- Cholesterol esters (2/3 of total cholesterol)

Cholesterol is present in many dietary food such as :

1- Egg yolk

2- Meats

3- Animal fats
4- Dairy products .

There are two sources of cholesterol in the body :
1- Absorption of cholesterol in small intestine .
2- Produce the cholesterol from A cetyl COA (carbohydrates , amino acids, fats) mainly in the liver .

*Normal range of cholesterol in human body is : (140 - 250 mg / 100 ml)

* Clinical significance :
1- Hypercholesterolemia (increase of cholesterol in blood)
a- Atherosclerosis
b- Diabetes mellitus
c- Obstractive jaundice and bile calculi
d- Hypothyroidism
e- Nephrotic syndrome

2- Hypocholesterolemia (decrease of cholesterol in blood)
a- Sever hepatitis
b- Malnutration
c- Hyperthyroidism
d- Sever anemia

Principle

(CE)

1) Cholesterol esters ----------→ Cholesterol + Fatty acids .

(CO)

2) Cholesterol + O_2 ----------→ Cholestenone + H_2O_2 .

H_2O_2

3) 4- amino antipyrine + phenol ----------→ Quinanonine + 4 H_2O .

Peroxidase (POD)

Procedure:

1) Drow 2-3 ml of blood.

2) Centrifugation.

3) Addition as follow:

Tubes	Blank	Sample	Standard
Work solution (R1)	1.0 ml	1.0 ml	1.0 ml
Sample		10 Ml	
Standard			10 Ml

4) Mixing, then put the test tubes in a water path for 10 minutes at 37 C .

5) Put these tubes in spectrophotometer, The wave length is 500 nm.

6) Calculations:

$$\text{Cholesterol conc. (mg/dL)} = \frac{\text{Ab. Sample}}{\text{Ab. Standard}} \times \text{Standard concentration.}$$

NOTE:

The concentration of standard is 200 mg/dl.

Exp. No.:(5)

Name of Exp.: Determination of serum Bilirubin

Theory :

There are small amounts of bilirubin in serum , it cause the light yellow colour of serum .

Bilirubin , the predominant pigment in bile , is the major product of hemoglobin catabolism . After being formed in the reticuloendothelial cells of Liver , Spleen and Bonemarrow , bilirubin is bound to Alb. (Indirect bilirubin or unconjugated bilirubin) and is transported to the Liver , where it is conjugated with two or one molecules of glucuronic acid to form bilirubin diglucuronide and bilirubin monoglucuronide compounds (Direct bilirubin or conjugated bilirubin) that are excreted in bile . In bile the bilirubin diglucuronide is the main pigment with smaller amounts of bilirubin monoglucuronide .

The increase of bilirubin level in serum called Hyperbilirubinemia when the bile pigment (bilirubin) in the blood is excessive , it escapes into the tissues (chiefly Sclera and Skin) , which then become yellow .This condition is known as Jaundice .

According to bilirubin mode of production , Jaundice is sometimes subdivided into three major groups : (Hepatic Jaundice , Hemolytic Jaundice , Obstructive Jaundice).

1- Hepatic jaundice :

Due to conjugation failure or Neonatal physiologic Jaundice or viral hepatitis toxic or cirrhosis .

2- Hemolytic jaundice :

Due to acut hemolytic anemia or chronic hemolytic anemia or Neonatal physiologic jaundice .

3- Obstructive jaundice :

Due to obstruction of common bile duct by stones or neoplasm or spasm or stricture .

Clinical significance :

Determination of serum bilirubin help in :

1- Evaluate Liver function .

2- Aid differential diagnosis of jaundice .

3- Aid diagnosis if biliary obstruction and hemolytic anemia .

4- Determine whether aneonate requires an exchange transfusion or phototherapy .

Normal levels of bilirubin in serum :

Conj. - Bil . = 0 - 0.2 mg / 100 ml

Unconj. - Bil . = 0.2 - 0. 8 mg / 100 ml

Total Bil. = 0 - 1.0 mg / 100 ml

Principle:

Bilirubin reacts with diazotized sulphanilic acid to form a coloured azobiliru compound. The unconjucated Bilirubin couples with the sulphanilic acid in t presence of a caffeine benzoate accelerator. The intensity of the colour forı is directly proportional to the amount of bilirubin present in the sample.

Bilirubin + Diazotized Sulphanilic acid → Azobilirubin compound.

Procedure:

1) Drow 2-3 ml of blood.

2) Centrifugation.

3) Addition as follow:

Addition sequence	Blank	Test
Total bilirubin reagent	1.0 ml	1.0 ml
Total nitrite reagent		0.05 ml
Sample	1.0 ml	1.0 ml

4) Mixing, then put the test tubes in a water path for 10 minutes.

5) Put these tubes in spectrophotometer, the wave length is 546 nm.

6) Calculations:

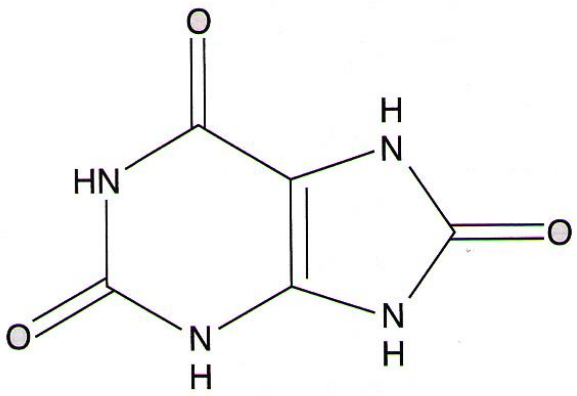
Total or direct bilirubin in mg/dl = Abs. T × 13 .

(13 being the factor)

Exp. No. : (6)

Name of Exp. : Determination of Uric acid in serum

Formula :



Uric acid

Category :

Uric acid belongs to the non - protein nitrogen compounds

Biosynthesis :

Uric acid is the end product of purines metabolism in human , in most other mammals it is further broken down to the soluble compound , Allantoin . The synthesis of uric acid takes place in the Liver by two major pathways :

1- Endogenously :

When it is synthesized from the metabolism purines present in nucleic acids of the molecule of the nucleoproteins .

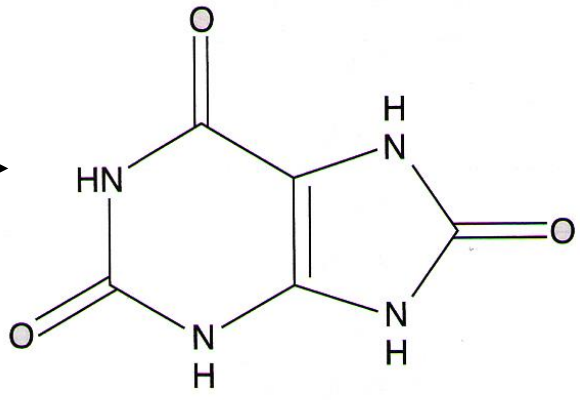
2- Exogenously :

When uric acid is synthesized from purines taken in the food (mainly in meat which is rich in cells) .

Adenine → Hypoxanthine

↓ xanthine

Guanine → Xanthine ⟶ (oxidase)



Uric acid

Renal excretion of 75% plasma uric acid , the 25% of uric acid go to the intesten , uric acid is filtered by the glomeruli and is subsequently reabsorbed to about 90% by the tubules .

Clinical significance:

Determination of serum uric acid levels are most helpful in the diagnosis of Gout , in which serum levels are freguently between 6.5 and 10 mg / 100 ml.

Causes of Hyperuricaemia

1- Increased synthesis of purines .

2- Increased intake of purines .

3- Reduced rate of excretion of urate / Renal failure .

All these cases lead to the formation of uric acid precipitation in Joints , especially those of the foot , produces the classical picture of gout .

4- Increased in Malignancy , infection , psoriasis .

Principle:

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{----------}} \text{Allantoin} + H_2O_2.$$

$$4\text{- amino antipyrine} + \text{DCPS} \xrightarrow[\text{Peroxidase (POD)}]{H_2O_2} \text{Quinoneimine} + 4\,H_2O\,.$$

Procedure:

1) Drow 2-3 ml of blood.

2) Centrifugation.

3) Addition as follow:

Tubes	Blank	Sample	Standard
Work solution	1.0 ml	1.0 ml	1.0 ml
Sample		25 Ml	
Standard			25 Ml

4) Mixing, then put the test tubes in a water path for 10 minutes at 37 C .

5) Put these tubes in spectrophotometer, the wave length is 520 nm.

6) Calculations:

$$\text{Uric acid conc.(mg/dl)} = \frac{\text{Ab. Sample}}{\text{Ab. Standard}} \times \text{Standard concentration.}$$

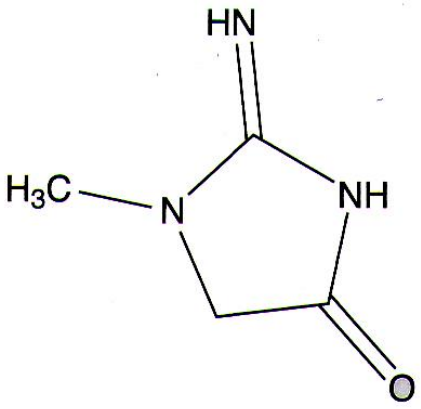
NOTE:

The concentration of standard is 8 mg/dl.

Exp. No. : (7)

Name of Exp . : Determination of Creatinine in Serum

Formula :



Creatinine

Category :

Creatinine and creatine are non – protein nitrogen compounds existing in the blood in relatively low concentrations in healthy individuals .

Biosynthesis :

Creatine is synthesized endogenously in the liver and pancreas from three amino acids : Arginine , Glycine and Methionine .

After synthesis of creatine , it diffuses into the vascular system and is then supplied to many kinds of cells , particularly those of muscle , where it becomes phosphorylated , it form phosphocreatine - high energy compound.

Creatine and creatine phosphate total about 400 mg / 100 g of fresh muscle .Both compound are spontaneously converted into Creatinine at the rate of about 2% perday . Creatinine is a waste product derived from Creatine and is excreted by the kidney .

Principle:

Picric acid in an alkaline medium reacts with creatinine to form orange coloured complex with the alkaline picrate . Intensity of the colour formed is directly proportional to the amount of creatinine present in the sample.

Creatinine + Alkaline picric acid → Orange coloured complex.

Procedure:

1) Drow 2-3 ml of blood. Creatinine is stable in serum for 1 day at 2-8 C.

Urine of 24 hours collection is preferred. Dilute the specimen 1:50 with Distilled / deionized water before the assay.

2) Centrifugation.

3) Mix well 1 ml of picric acid reagent with 0.2 ml of sample in a clean dry tes tube & centrifuge at 2500-3000 rpm for 10 min. to obtain a clear supernatar

4) Addition as follow:

Addition sequence	Blank(ml)	Sample (ml)	Test(ml)
Supernatant			1.1
Picric acid reagent	1.0	1.0	
Distilled water	0.1		
Creatinine standard		0.1	
Buffer reagent	0.1	0.1	0.1

4) Mixing, then put the test tubes in a water path for 20 minutes.

5) Put these tubes in spectrophotometer, the wave length is 520 nm.

6) Calculations:

$$\text{Serum Creatinine in mg \%} = \frac{\text{Abs T}}{\text{Abs S}} \times 2$$

$$\text{Urine Creatinine in mg / Lit.} = \frac{\text{Abs T}}{\text{Abs S}} \times 1$$

Urine Creatinine in gm/24 Hrs=Urine Creatinine (gm/L) X Urine vol. (24h/L).

Normal values:

Serum creatinine in male 0.6 - 1.2 mg%

Serum creatinine in female 0.5 – 1.1 mg%

Elevated creatinine levels:

1) Renal dysfunction.

2) Reduced renal blood flow :

a) Shock .

b) Dehydration.

c) Congestive heart failure.

3) Diabetes acromegaly.

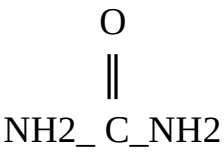
Decreased creatinine levels:

1) Muscular dystrophy.

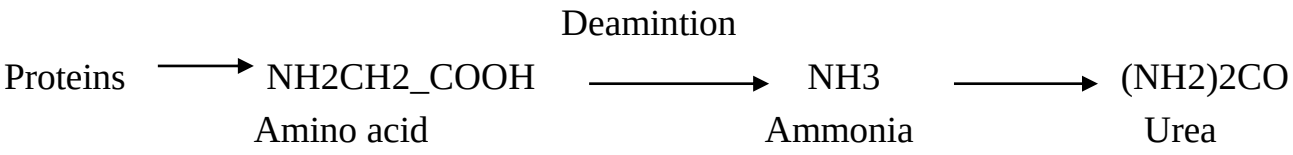
Exp.No. : (8)

Name of Exp.: Determination of Urea

Formula :



Synthesis : Urea is synthesized in the liver from Ammonia produsedas a result of deamination of Amino acids :



It is customary to express urea as urea Nitrogen in order to distinguish between the quantity of Nitrogen in urea with that of other components included in the non-protein nitrogen compound (NPN) category .

Mol.wt. of urea =60 and it contains 2 nitrogen atoms with a combined wt. of 28, a urea nitrogen value can be converted to urea by multiplying by 60/ 28 or (2.14).

$$\text{Urea Nitrogen Value} = \frac{\text{Urea value}}{2.14}$$

Classification:

Urea belong to the non – protein nitrogen compound which include : creatine and creatinine , uric acid , Ammonia , Amino acids .

All the mentioned blood constituents are studied under the Renal Function Study .

Normal values:

Serum , plasma 14 - 40 mg / d

Hyperuremia:

1) Burns.
2) Renal diseases.
3) Shock .
4) Urinary obstructions.
5) Congestive heart failure.

Hypouremia:

1) Liver failure.
2) Pregnancy.

Principle:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} \text{Ammonia} + CO_2 .$$

2) Ammonia + Phenolic chromagen + Hypochlorite ---→ Green coloured complex.

Procedure:

1) Drow 2-3 ml of blood. Urea is stable in serum for 5 days at 2-8 C.

2) Centrifugation.

3) Addition as follow:

Tubes	Blank	Sample	Standard
Work solution (R1)	1 ml	1 ml	1 ml
Sample		10 Ml	
Standard			10 Ml

4) Mixing, and then put the test tubes in a water path for 5 minutes at 37 C.

5) Put these tubes in spectrophotometer, the wave length is 570 nm.

6) Calculations:

$$\text{Serum urea (mg / dl)} = \frac{\text{Abs T}}{\text{Abs S}} \times 40$$

Normal values:

Serum , plasma 14 - 40 mg / dl.

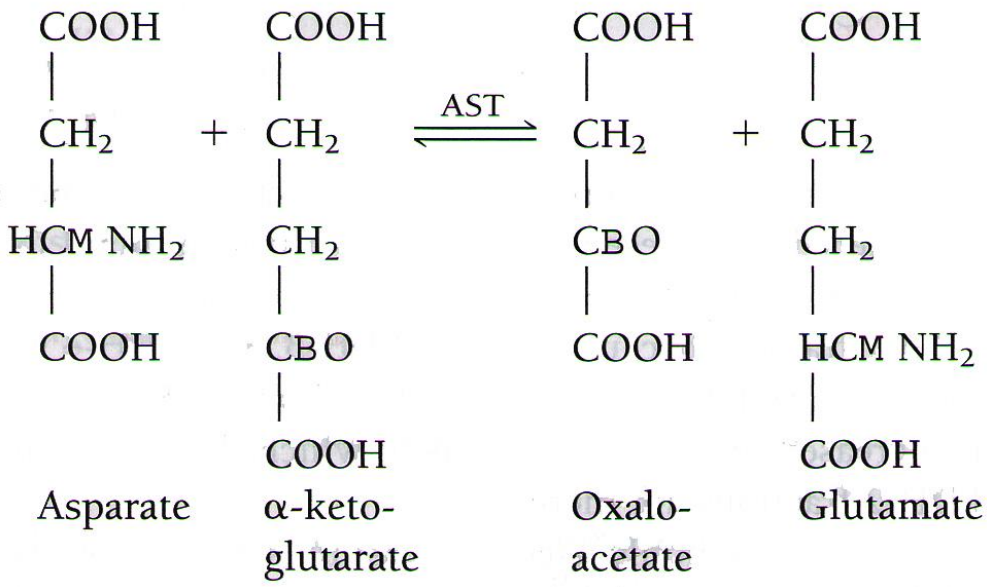
Exp. No.: (9)

Name of Exp. : Determination of the transaminases GOT

GOT , Glutamate Oxaloacetate Transaminase also called Aspartaic Transaminase (AST)

Classification :

GOT and GPT belong to aclass of enzymes called Transaminases or Transferases .the transaminases area group of enzyme which catalyze the interconversion of amino acid and α- keto acids by the transfer of amino group (-NH2) from an amino acid to an α – keto position of an α- keto acid . the reaction is reversible .



Sources and Synthesis :

Both GOT and GPT are present in human plasma , Bile , Cerebrospinal fluid (CSF) .

Got is normally present in blood in relatively low concentration .

GOT is present in greatest concentration in cardiac , muscle , Liver , skeletal muscle and kidney .

Clinical significance of GOT:

GOT estimation is of great interest in three groups of diseases :

1-Heart diseases - Cardiac damage - Myocardial infarction , an increase level of serum GOT becomes apparent in 4 to 6 hours after onset pain .peak values of GOT activity are reached after 24 to 36 hours then activity values fall to normal range by the fourth or fifth day .

2- Liver disease - liver cancer - obstructive jaundice .

3- Muscular diseases

Procedure (GOT) :

Normal values : 2- 20 I . U / L or 2 - 23 U mole / min / L

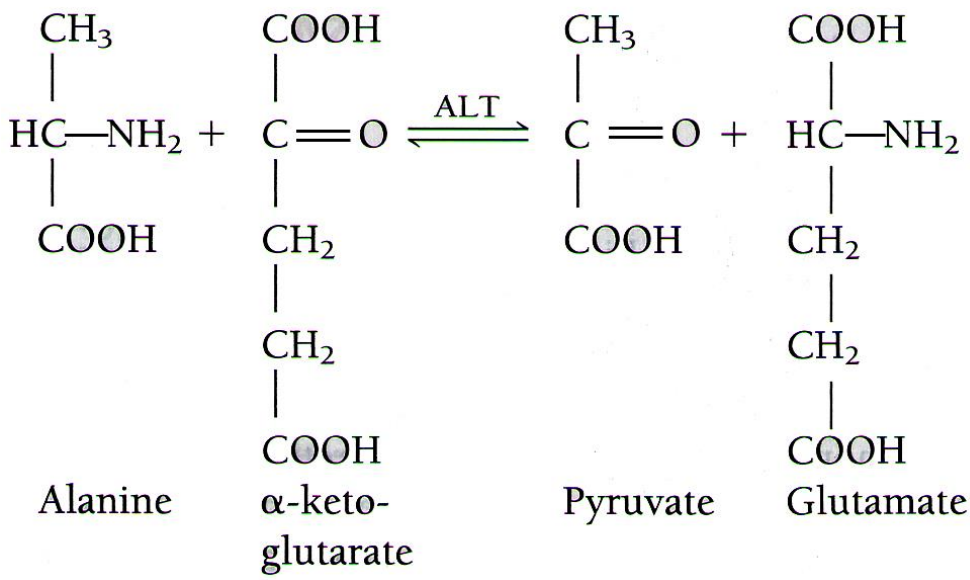
Exp. No. : (10)

Name of Exp. : Determination of Transaminase GPT

GPT , Glutamate pyruvate Transaminase also called Alanine Transaminase (ALT)

Classification :

GOT and GPT belong to aclass of enzymes called Transaminases or Transferases .the transaminases area group of enzyme which catalyze the interconversion of amino acid and α- keto acids by the transfer of amino group (-NH2) from an amino acid to an α – keto position of an α- keto acid . the reaction is reversible .



Sources and Synthesis :

Both GOT and GPT are present in human plasma , Bile , Cerebrospinal fluid (CSF) .

GPT is present in greatest concentration in Liver but other tissues such as kidney , heart ,skeletal muscle , also have abound concentration .

Clinical Significance of GPT :

The Liver is especially rich in GPT ,thus , this enzyme measurement is used primarily as a test for Hepatitis .

In infection hepatitis the GPT level of activity in serum is greatest than GOT but both activities usually are increased .

Procedure GPT :

PROCEDURE

A) 30 or 37°C

I- MACROTECHNIQUE

In a cuvette at 30-37°C place:

Reconstituted Reagent A	2 ml
Sample	200 ul

Mix immediately and simultaneously start the stopwatch. After 1 minute record the initial absorbance and then at 1, 2 and 3 minutes from the first reading. Determine average change in Absorbance/min (ΔA/min) subtracting each reading from the previous one and averaging these values. Use this means for the calculations.

CALCULATIONS

GPT (U/l) = ΔA/min x factor

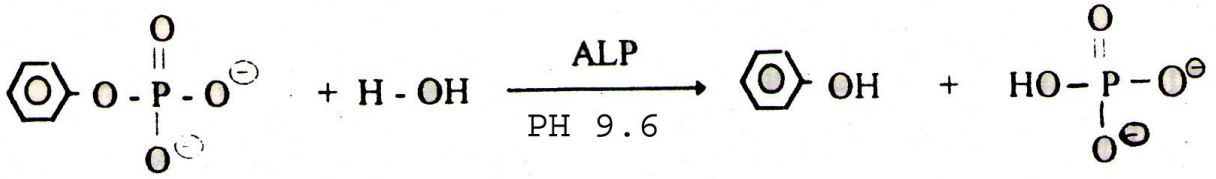
Factor =1780

Normal values : 2 - 15 I . U / L or 2 - 38 U mole / min. / L

Exp. No. :(11)

Name of Exp.: Determination of Alkaline Phosphatase (ALP)

The ALP belong to the class of enzymes called " Hydrolases " Hydrolytic enzymes catalyze the scission of compounds containing acyl or phosphate bond , While this bond is being split , concurrent splitting of an O_H bond in water molecule also takes place , As a result , a phenol compound (alcohol – like) is formed .



Phenyl phosphate Phenol phosphate ion

Biosynthesis of ALP :

The ALP enzyme is present in many tissues of the body , especially in the cell membranes , and it occurs particularly high levels in intestinal epithelium kidney tubules , bone (osteoblasts) and liver .

Function of ALP :

ALP enzyme is facilitates transfer of metabolites across cell membranes , and it is associated with lipid transport , and with the calcification process in bone synthesis.

Clinical Significance :

Serum ALP estimation are of interest in the diagnosis of :

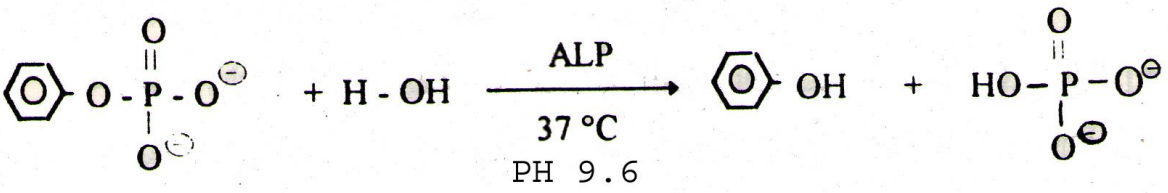
1- Hepatobiliary disease: ALP level is elevated in hepatobiliary disease was a result of failure to excrete the through the bile, raises serum ALP in obstructive jaundice .

2- Bone disease is associated with high serum ALP levels such as :

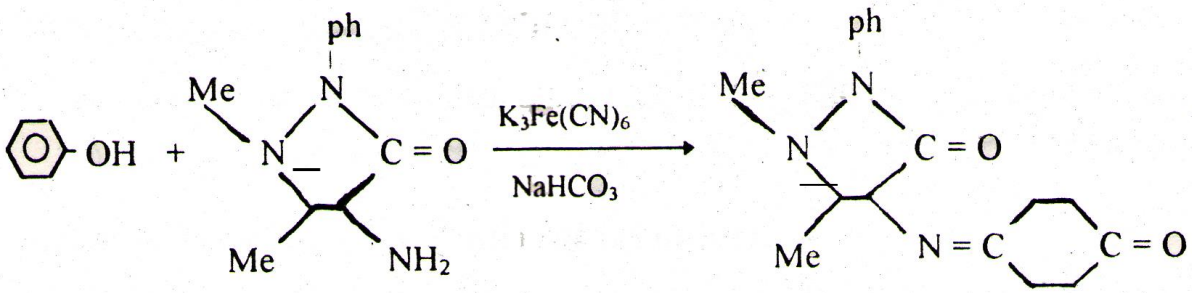
- Pagets disease (Osteitis defomans) .
- Rickets disease - levels are high 2 to 4 times of normal .

Principle of the method :

In 1934 King and Armstrong proposed the use of phenyl phosphate as substrate , the enzyme splits off the phosphate group to form free phenol and phosphate ion , under alkaline conditions , the phenol is converted to red Quinone with the additions of 4- amino – antipyrine .



Phenyl phosphate Phenol phosphate ion



Phenol 4-amino antipyrine red Quinone

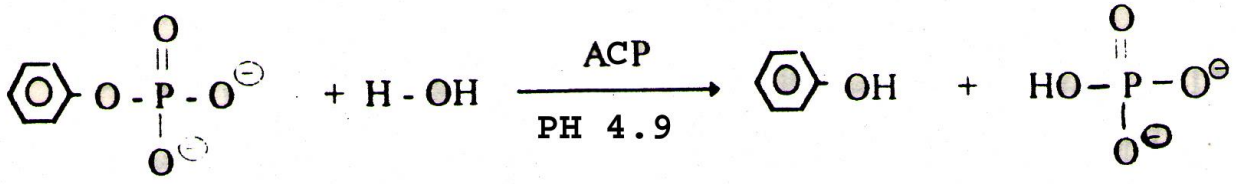
Normal values : 3 - 13 K.A.U / 100ml serum

Exp.No. :(12)

Name of Exp . : Determination of Acid Phosphates (ACP)

Acid phosphates as alkaline phosphates belong to the class of enzyme called "Hydrolasess" Hydrolytic enzyme catalyze the scission of compounds containing phosphate ester bonds . while these bonds are being split , a concurrent splitting of an O-H bond in a water molecule also take place .

The name of acid phosphatase (ACP) are included all phosphates with optimal activity below a PH of 7.0 .



Phenyl phosphate Phenol phosphate ion

Biosynthesis of ACP :

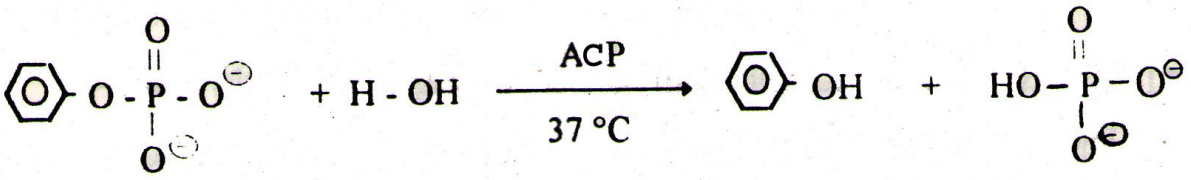
The greatest concentration of acid phosphates activity are present in liver, Spleen , milk , erythrocytes , platelets , bone marrow , and the prostate gland , the prostate gland is the richest source , and contributes about one – third to one – half of the enzyme present in serum of healthy males .

Clinical Significance :

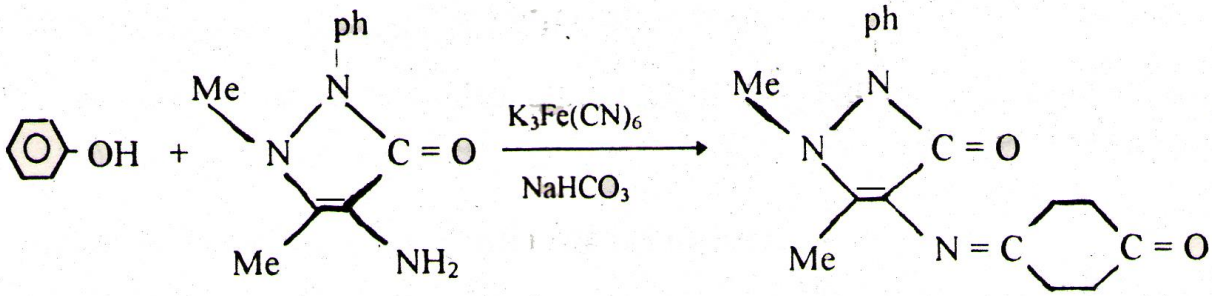
1- The main use of ACP is in the diagnosis of prostatic carcinoma ,elevations of the prostatic ACP in the sera of males with prostatic cancer associated with metastasis, total activities may reach 40 to 50 times the upper limit of normal values .
2- Raised plasma ACP is also found in Pagets disease, and in hyperparathyroidism.
3- Elevations in total ACP actively occur in Female breast cancer .
4-Elevation of ACP are also observed in Myelocytic Leukemia.

Principle of the method :

The enzyme ACP splits off the phosphate group to form free phenol and phosphate ion , under alkaline condition , the phenol is converted to red Quinone with the addition of 4-amino- antipyrine .



Phenyl phosphate Phenol phosphate ion



Phenol 4-amino antipyrine red Quinone Color Complex

Normal values : 1.0 - 3.5 K.A .U/ 100 ml serum

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