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FACULTY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOMEDICAL LABORATORY SCIENCES

CLINICAL CHEMISTRY II (CCH 2512)

MODULE HANDOUT 2018/2019

Year II, Semester I

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COURSE CONTENTS OUTLINE

- Principles and procedures of analysis of blood constituents: Blood electrolytes: sodium, potassium, chloride, bicarbonate, lithium
- Glucose, Glucose tolerance test,
- Proteins: Albumin, total protein , ,
- Renal function tests: creatinine, uric acid, urea
- Liver function tests: bilirubin, alkaline phosphatase, alanine transferase, aspartate transferase, Gama glutamate transferase
- Cardiac function tests, ,
- pancreas function tests: Amylase, lipase
- mminerals: calcium, phospherous,
- lipid profiles: trigylcerides, total cholesterol, LDL cholesterol, HDL cholesterol.
- Apply quality control tests and procedures

CHAPTER I

BLOOD ELECTROLYTES

ELECTROLYTES

Definition:

An electrolytes is a substance that produces an electrically conducting solution when dissolved in water.Electrolytes carry a charge and are essential for life. Electrolytes regulates our nerve and muscle function,our body's hydration ,blood pressure and the rebuilding of tissue damage.

Fruit and vegetables are good sources of electrolytes.

Electrolytes Include Sodium (Na), Potassium (K), Chloride (Cl^-) and Bicarbonate (HCO^{3-})

- Collectively these have a great effect on hydration, acid-base balance , osmotic pressure as well as pH and muscle contraction.

The organs which are constantly regulating the electrolyte levels are the:

- **Intestine**
- **Kidneys**

1.SODIUM

- This element is the major component of the cations of the
- extracellular fluid .It is largely associated with chloride and bicarbonate in regulation of acid base balance
- Water and Na balance are closely interdependent.
- Total body water (TBW) is about 60% of body weight (ranging from about 50% in obese people to 70% in lean people). Almost 2/3 of TBW is in the intracellular compartment (intracellular fluid, or ICF); the other 1/3 is extracellular (extracellular fluid, or ECF
- Normally, about 25% of the ECF is in the intravascular compartment; the other 75% is interstitial fluid. Fig. 1: Fluid and Electrolyte Metabolism: Fluid compartments in an average 70-kg man.
- Total body water = 70 kg × 0.60 = 42 L.
- The major intracellular cation is K+, with an average concentration of 140 mmol/L.
- The extracellular K+ concentration is 3.5 to 5 mmol/L.
- The major extracellular cation is Na+, with an average concentration of 140 mmol/L and an intracellular Na concentration of 12 mmol/L.

OSMOLALITY AND VOLUME REGULATION

- All osmotically active substances in plasma- virtually all ions contribute to osmolality.

- The concentration of combined solutes in water is osmolarity, which, in body fluids, is similar to osmolality.
- Serum osmolality can be measured in the laboratory or estimated according to the formula
- where serum Na+ is expressed in mmol/L and glucose and BUN are expressed in mg/dL.
- Osmolality of body fluids is normally between 275 and 290 mOsm/kg.
- Na+ is the major determinant of serum osmolality.

- **Main source**

- The main source of sodium is the sodium chloride used in cooking or seasoning .In addition to salted foods the contents of sodium is high in cheese ,wheat germ ,bread,carrots,eggs,milk,nuts,radishes,etc.About 4gms
- of sodium is ingested every day and about 95% of the sodium
- is excreted in urine.

Distribution of sodium in body fluids and cells

- Whole blood 160 mg/dl
- Plasma 330mg/dl

- Cells 85 mg/dl
- Nerve tissue 60-160mg/dl
- Muscle tissue 312 mg/dl

- <u>METABOLISM</u>

- The metabolism of sodium is influenced by the adrenocortical
- steroids .With the exception of the androgens,all the active corticosteroids increase the absorption of sodium and chloride by the renal tubules and decrease their excretion by the sweat gland ,salivaly gland and the gastrointestinal tract .
- Accompanying the retention of sodium by the kidney ,there is increased excretion of potassium.

Clinical significance of Sodium depletion

- Hyponatremia
- The syndrome of sodium depletion occurs in Greatly diminished intake of sodium.

- Burns,severe exudative skin lesions and massive sweating.
- Addison's disease (deficiency of mineralocoticoids)
- Chronic nephritis –Diabetes ketoacidosis
- Loss in alimentary secretions due to prolonged vomiting
- and diarrhea.

- **Symptoms**

- There is raised central venous pressure ,peripheral
- edema and pulmonary edema with eventual respiratory
- failure .

2.POTASSIUM

- Potassium is the principal cation of the intracellular fluid.
- Within the cells it plays important role in maintenance of
- acid base balance ,osmotic pressure and water retention.

- Intracellular potassium is essential for several important metabolic reactions catalysed by enzymes.
- It influences muscle activity notably the cardiac muscle
- Major intracellular cation, 98% in ICF and 2% in ECF.
- K+ is in rbcs is about 105mmol/L.
- The active Na-K-ATPse pump located in the cell membrane pumps Na out of the cell and K+ into the cell to maintain the high extercellular [Na+] and high intracellular [K+].
- K+ has 2 major physiologic functions.
- Important role in cell metabolism by participating in the regulation of many cellular processes.
- When K+ imbalance occurs a variety of cell functions become impaired.
- Important in neuromuscular excitation.
- Ratio of intracellular to extracellular conc is the major determinant of the resting potential across cell membranes.
- The resting potential permits the generation of action potential necessary for normal neural and muscular function.
- Thus either increase or decrease in plasma [K+] can upset the ratio and lead to cardiac arrhythmias and muscle paralysis.
- When plasma K is high the heart rate slows because of the decreased resting membrane potential of the cell relative to the threshold potential.
- A decrease in plasma [K] increases cardiac muscle excitability and often leads to arrhythmias.
- During exercise K is released from cells which may increase plasma [K] by 0.3 to 1.2mmol/L in mild to moderate exercise and by > 2.0 in rigorous excercise.
- Hyper-osmolality causes diffusion of water out of cells, in the process K moves out with it causing gradual loss of K from intracellular fluid.

- Cellular breakdowns releases K into the ECF, eg in severe trauma, tumor lysis syndrome, and massive blood transfusions.

- **Requirements and source**

- The normal intake of potassium in food is about 4g/day.
- A high content of potasssium is found in the foods such as chicken, apricots, peaches,bananas,oranges
- ,pineapples,potatoes,etc...

Distribution of potassium in body fluids and cells

- Whole blood 200 mg/dl
- Plasma 20mg/dl
- Cells 440 mg/dl
- Nerve tissue 250-400mg/dl
- Muscle tissue 530 mg/dl

Metabolism

Metabolism of potassium is controlled by the

- mineralocorticoids .The kidney is the principal organ of excretion for potassium .
- Variation in the extracellular potassium influences
- the activity of striated muscles(muscles tissue) so that paralysis of skeletal muscle and abnormalities in conduction and activity of cardiac muscle occurs.

Clinical significance of serum potassium

- Hyperkalemia
- Toxic elevation of serum potassium is observed
- in the case of patients with Renal failure
- Advanced dehydration shock and in addisons disease.
- Hyperkalemia may also occur if potassium is administered intravenously at an excessive rate.

The symptoms of hyperkalemia are mainly

- Cardiac and central nervous system depression
- Mental confunsion
- Weakness
- Numbness
- Weakness of respiratory muscles

Low potassium:hypokalemia

- Potassium deficiency is likely to develop in
- Gastrointestinal losses

- Chronic wasting disease with malnutrition
- Metabolic alkalosis
- Prolonged intravenous administration of solutions
- which do not contain potassium and in Cushing's syndrome
- In most of the above mentioned cases ,intracellular
- potassium is transferred to the extracellular fluid and this potassium is quickly removed by the kidneys.
- A prolonged deficiency of potassium may produce severe
- damage to the kidneys.
- During heart failure ,the potassium content of the myocardium becomes depleted.

Symptoms

- The symptoms of hypokalemia include :
- Muscle weakness with irritability
- Paralysis tachycardia and
- Dilation of the heart

CLINICAL SIGNIFICANCE

HYPONATREMIA:low serum sodium are observed in the condition such as:

- Severe prolonged diarrhea and vomiting

- Nephritis
- Addison's disease

- **HYPERNATREMIA**: Increased serum sodium values are observed in the conditions such as :

- Severe dehydration
- Diabetes insipidus(loss of dilute urine)
- Salt poisoning
- Cushing's syndrome
- Certain post renal condition (e.g enlarged prostate)

HYPOKALEMIA:it is observed in the conditions such as:

- Cushiing's syndrome
- Renal tubular damage
- Metabolic alkalosis
- Malnutrition

HYPERKALEMIA:high potassium values are observed in the condition such as:

- Addison's disease
- Renal glomerular disease
- Anuria and oliguria

DETERMINATION OF SODIUM AND POTASSIUM IN SERUM

NORMAL RANGE

- Serum sodium: 133-148mEq/l

- Serum potassium: 3.8-5.6mEq/l

Principle

The solution under test is passed carefully ,under controlled conditions as a every fine spray in the air supply to non luminous flame .In the flame, the solution evaporates and the salts dissociates to give neutral ions, which emit light of the characteristic wavelength.

The flame is simultaneously monitored by the channels.

Each channel consists of a dector which views the flame through a narrow band optical filter .

Initial calibration is done by using at least three standards of different concentrations.

Preparation of Standards

Mixed standards are prepared by using following

two stock standards.

1.Stock standard for sodium : it is prepared

by dissolving 5.85g of sodium chloride

in glass distilled water and diluted to 100ml by using a volumetric flask.

2.Stock standard for potassium : it is prepared

by dissolving 0.740g of potassium chloride

in glass distilled water and diluted to 100ml by using

a volumetric flask.

Working standards are prepared as follows:

1.sodium/potassium: it is prepared by mixing 12ml of stock standard 1 and 2 ml of stock standard 2 in 86ml of glass distilled water

2.sodium/potassium:it is prepared by mixing 14ml of stock standard 1 and 4.0ml of stock standard 2 in 82ml of glass distilled water.

3.sodium/potassium : it is prepared by mixing

16ml of stock standard 1 and 6 ml of stock standard 2 in 78ml of distilled water.

Procedure

	Test	std 1	std 2	std3
Distilled water,ml	10	10	10	10
Serum or heparinized Plasma,ml	0.1	-	-	-
Std 1,ml	-	0.1	-	-
Std 2 ,ml	-	-	0.1	-
Std 3, ml	-	-	-	0.1

Mix well and then go for the flame photometric determination

DETERMINATION OF URINARY SODIUM

METHOD

Flame photometry

Procedure

Perform the test by using exactly same procedure as for serum sodium determination .Use undiluted urine sample instead of serum.

Calculations

Urine sodium mEq/l=reading*10

24hrs excretion of urine sodium=

Sodium mEq/l * 24 hrs urine volume/1000

DETERMINATION OF URINARY POTASSIUM

PROCEDURE

Dilute urine (1:10)in distilled water and perform the test on flame photometer(exactly same way as for serum potassium)

Calculations

Urine potassium mEq/l=reading*10

24hrs excretion of urine potassium=

Urine potassium mEq/l*24hrs urine volume/1000

3. CHLORIDE

DEFINITION

As a component of sodium chloride ,the element chloride as chloride ion is essential in water balance ,osmotic pressure regulation and in acid base balance .

In gastric juice ,chloride also plays important role in the production of hydrochloric acid

Major extracellular anion (conc ranges from 99-109 mmol/L)

Metabolism closely linked to that of Na+.

ie maintenance of osmotic pressure.

Important in maintenance of normal anion-cation balance as it exchange with bicarbonate (HCO_3^-) in a process called chloride shift.

CO_2 from tissues diffuses into plasma where a small fraction is dissolved.

Majority of it diffuses down a conc gradient into rbcs, where it combines with H2O to form H2CO3(carbonic acid) in the presents of carbonic anhydrase.

Carbonic acid dissociates into H+ which is buffered by Hb and HCO3-.

As the conc of HCO3- builds up in the rbc, its conc becomes greater than the extracellular conc and it diffuses out of the cell.

To maintain electro neutrality, Cl^- flows into the cell in exchange of, $HCO3^-$ in a process called chloride shift.

Normal diet contains 70 to 200 mmol of chloride as sodium or potassium salts.

Chloride shift is Chloride ions exchange with bicarbonate as carbon dioxide is transported and buffered in the red blood cell.

Requirements and metabolism

The intake of chloride is satisfactory as long as sodium intake is adequate since chloride occurs almost entirely as sodium chloride.

Abnormalities of sodium metabolism are generally accompanied by abnormalities in chloride metabolism .

chloride deficit is hence observed in the conditions such

as diarrhea,profuse sweating and addisons disease when losses of sodium are excessive.

However ,in prolonged vomiting ,the loss of hydrochloric acid

and hence that of chloride occur .This leads to compensatory increase in serum bicarbonate.

Distribution of chloride in body fluids and cells

Whole blood	250 mg/dl
Plasma or serum	365mg/dl
Cells	90 mg/dl
Nerve tissue	171mg/dl
Muscle tissue	40 mg/dl
CSF	440mg/dl

CLINICAL SIGNIFICANCE

Low chloride values are observed in the conditions such as

- Prolonged vomiting
- Burns
- Renal disease
- Overhydration

High chloride values are observed in the conditions such as

- Dehydration
- Renal tubular disease
- In conditions causing decreased renal blood flow

  e.g congestive heart failure

DETERMINATION OF SERUM CHLORIDE

Normal range

1.Serum chloride: 95-106 mEq/l

2.CSF chloride: 700-750mg/dl

3.Urinary chloride:(24hrs excretion)average

excretion range from 120-250mEq/L

Principle

The protein free filtrate of the specimen is titrated with mercuric nitrate solution in the presence of diphenylcarbazone as the indicator . The free mercuric ions

combine with chloride ions to form soluble but nonionized

mercuric chloride .

After all chloride ions have reacted with mercuric ions ,any excess mercuric ions combine with the indicator diphenylcarbazone to form blue violet colored complex .

Color change of the reaction mixture is considered as the end point of the titration.

REAGENTS

1.Mercuric nitrate reagent

2.Diphenylcarbazone indicator

3.Chloride standard : it is prepared

by dissolving 5.85g of sodium chloride

in one liter of glass distilled water.

4.2/3N sulphuric acid

5.Sodium tungstate.

Specimen

Serum

Procedure

1.Prepare protein free filtrate of the serum sample as follows;

In a centrifuge tube,pipette

a.4.0ml distilled water

b.0.5ml serum

c.0.25ml 2/3N Sulfuric acid

d.0.25ml sodium tungstate

Mix thoroughly and centrifuge at 3000rpm for 10mins.

Next procedure is as follows:

2.Pipette in a titration tube(burette) 2.0ml of protein free filtrate .

3.Titrate against mercuric nitrate reagent.

4.Then add one drop of the indicator (0.05ml)

End point :

colorless to violet –blue color

5.Note the titration reading ; x ml.

6.Dilute standard 1:10 by using glass distilled water

7.Pipette 2.0ml of diluted standard in a titration tube(burette),

and titrate it against mercuric nitrate reagent .

(same as for the test)

By using diphenylcarbazone indicator.

8.Note the titration .reading :Y ml

Calculation

Serum chlorides,mEq/l= X/Y*100

4.BICARBONATE

DEFINITION

- The term "bicarbonate" was coined in 1814

The prefix "bi" in "bicarbonate" is based on the observation that there is twice as much carbonate (CO_{2-3})

Which is a salt of carbonic acid characherized by the carbonate ion.

Bicarbonate is tightly related to the carbon dioxide content of the plasma

As Blood gas analysis,it is of importance for the evaluation of lung function (oxygen saturation, CO_2), and the acid-base equilibrium (metabolic and respiratory acidosis and alkalosis).

Bicarbonate is a chemical that acts as a buffer. It keeps the pH of **blood** from becoming too **acidic** or too basic.

Your kidneys and lungs balance the levels of carbon dioxide, bicarbonate, and carbonic acid in the **blood**

When your cells burn nutrient molecules for energy, they generate carbon dioxide. This reacts with water in the bloodstream to make

carbonic acid, which further reacts in the bloodstream to produce **bicarbonate**. In this way, you continually produce a **pH**-stabilizing buffer as you respire

HCO_3^- is the second largest anion fraction of the ECF and is the major form of CO_2 in plasma.

Serum conc ranges from 22 to 28 mmol/L.

HCO_3^- functions as a major component of the carbonic acid buffer system , it acts promptly to buffer any sudden changes in blood pH.

Also serves as a transport form of CO_2 produced from tissues and delivered to the lungs for exhalation

HCO3- conc is regulated both in the kidneys through increased or decreased tubular reabsorption and in the lungs through exhalation of gaseous CO2 .

Decreased levels of HCO3- in plasma result in an acid base disorder known as metabolic acidosis as HCO3 combines with H+ to produce CO2 which is exhaled by lungs.

The typical response to metabolic acidosis is hyperventilation which lowers PCO2 and returns pH to normal

Elevated total conc of CO2 occurs in metabolic alkalosis as bicarbonate is retained, often with an increase in PCO2 as hypoventilation attempts to normalize pH.

Other causes of metabolic alkalosis include severe vomoting, hypokalaemia .

Bicarbonate is a chemical that acts as a buffer. It keeps the pH of **blood** from becoming too **acidic** or too basic.

Your kidneys and lungs balance the levels of carbon dioxide, bicarbonate, and carbonic acid in the **blood**

Determination of serum (or plasma)bicarbonate

Normal range

21-28mEq/l

Method

Titrimetric

Principle

Serum is added to standard 0.01N hydrochloric acid

and the loss of strength of the standard acid due to

Bicarbonate is determined by titrating the strength of acid against 0.01N sodium hydroxide.

Requirements

1.Glass distilled water

2.0.01N hydrochloric acid

3. Saline

4.0.01N sodium hydroxide

5.Phenol red indicator

Procedure

1.Pipette 5.0ml of Saline in a conical flask.

2.Add 0.1ml of the specimen and 2drops of the indicator.

3.Mix well .

This is considered as control and the test is titrated till same color develops as in the case of the control.

4.Pipette 4.0ml of Saline in another conical flask.

5.Add 1.0ml of 0.01N hydrochloric acid ,mix well .

6.Add 0.1ml of serum ,mix well.

7.Fill the mixture into the burette.

7.Titrate against 0.01N sodium hydroxide ,until color changes from yellow to red(same as in the case of the control).

8.Note the titration reading(R ml).

Calculation

Serum bicarbonate,mEq/l = (1-R)*100

CHAPTER II

Glucose, Glucose tolerance test

GLUCOSE TOLERANCE TEST

DEFINITION

The **glucose tolerance test** is a medical test in which glucose is given and blood samples taken afterward to determine how quickly it is cleared from the blood.

The test is usually used to test for diabetes, impaired beta cell function, and in diagnosis of intrahepatic diseases

General consideration

Glucose tolerance means ability of the body to utilize glucose in blood circulation .Glucose tolerance decreases in diabetes mellitus and in certain endocrine disorders like hyperthyroidism,hyperpituitarism and hypoadrenalism.

Blood sugar in the case of a normal individual remains fairly

constant through the day.

Following food intake ,there is a temporary rise in blood sugar,the extent and duration of which depends on the type of

food taken.Blood sugar level returns to normal within two to three hours after taking food.

In decreased glucose tolerance ,however,blood glucose level does not return to normal within 2 to3 hrs after food intake.

This effect of ingested carbohydrate can be studied under reasonably standard conditions by means of the glucose tolerance test.

Instructions given to the patient

1.The patient should be on balanced diet (containing normal daily requirement of carbohydrates)at least for 2 to 3 days prior to the test.

2.Patient should report to the laboratory after fasting for

12-16hrs.He can drink water.

3.He should bring fasting midstream urine sample collected in a clean and dry bottle.

4.Patient should be in a position to wait at the laboratory for at least 2-3hrs,since five blood samples are collected at the

interval of 30mins.

Instructions to the technician

Collect fasting blood sample ,2-3ml in a fluoride bulb .

If glucose is absent in the fasting serum ,then follow the

instructions as given below.

3.Give 75gm 0r 100gm of glucose (1.75g/kg weight)dissolved

in water to the patient .

Addition of lemon juice lessens the risk of the patient vomiting .Note the time.

4.Collect four more samples at the half hourly intervals for two hours,after the glucose has been taken.

5.Four urine samples are collected after the collection of each blood sample .If the patient is unable to give four urine samples,collect at least two urine samples at the one hour interval.

6.Determine blood and urine sugar by the specific methods used in the laboratory.

7.Prepare a glucose tolerance curve by plotting time on X axis and plasma glucose values on Y axis

Plasma or serum glucose determination

The Glucose Oxidase Method

- Glucose oxidase catalyzes oxidation of glucose to gluconic acid and H_2O_2
- Peroxidase catalyzes the reduction of H_2O_2 to water and a reduced chromogenic dye (colorless) is oxidized
- Production of oxidized colored dye is proportionate to H_2O_2 produced
- Increased colored dye → increased absorbance

Two Step Glucose Oxidase – Peroxidase Reaction

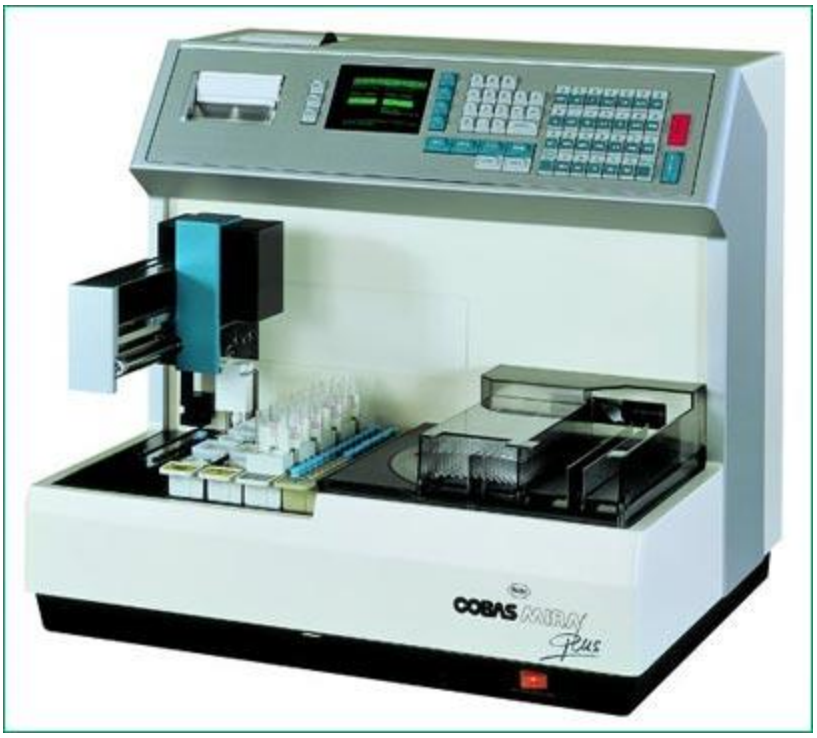
- $$D-Glucose + H_2O + O_{2(\,from air)} \xrightarrow[oxidase]{glucose} Gluconic\,acid + H_2O_2$$

$$H_2O_2 + \underset{(colorless)}{reduced\,dye} \xrightarrow{peroxidase} \underset{(chromogen)}{oxidized\,dye} + H_2O$$

Dyes used in various systems: *o* – dianisidine, 4-aminoantipyrine, or tetramethylbenzidine

- Advantages:
  - Visible wavelength used
  - Oxygen consumption can also be measured using a polarographic oxygen probe.
  - May be used with CSF
  - A.k.a.- *Trinder* method
- Limitations:
  - Glucose oxidase very specific to ß-D-glucose
  - Mutarotase must be included or incubation increased to detect α-D-glucose
  - Peroxidase reaction susceptible to reducing substances - uric acid, bilirubin, ascorbic acid, and strong oxidizers (i.e., bleach)

The Glucose Hexokinase Method



The Roche Cobas Mira analyzer

- Hexokinase catalyzes the phosphorylation of glucose from ATP which yields G6P –
  (glucose-6-phosphate)
- The second enzyme G6P-dehydrogenase oxidizes G6P and reduces NAD+ to NADH
- NADH causes an increase in absorbance at 340 nm, proportionate to the glucose conc

CLINICAL SIGNIFICANCE

Type 1 diabetes, once known as juvenile diabetes or insulin-dependent diabetes, is a chronic condition in which the pancreas produces little or no insulin. Insulin is a hormone needed to allow sugar (glucose) to enter cells to produce energy.

Different factors, including genetics and some viruses, may contribute to type 1 diabetes. Although type 1 diabetes usually appears during childhood or adolescence, it can develop in adults.

Despite active research, type 1 diabetes has no cure. Treatment focuses on managing blood sugar levels with insulin, diet and lifestyle to prevent complications.

Symptoms

Type 1 diabetes signs and symptoms can appear relatively suddenly and may include:

- Increased thirst
- Frequent urination
- Bed-wetting in children who previously didn't wet the bed during the night
- Extreme hunger
- Unintended weight loss
- Irritability and other mood changes

▶ Fatigue and weakness

▶ Blurred vision

▶ Unintended weight loss

Causes

The exact cause of type 1 diabetes is unknown. Usually, the body's own immune system — which normally fights harmful bacteria and viruses — mistakenly destroys the insulin-producing (islet, or islets of Langerhans) cells in the pancreas. Other possible causes include:

Genetics

Exposure to viruses and other environmental factors.

The role of insulin

Once a significant number of islet cells are destroyed, you'll produce little or no insulin. Insulin is a hormone that comes from a gland situated behind and below the stomach (pancreas).

The pancreas secretes insulin into the bloodstream.

Insulin circulates, allowing sugar to enter your cells.

Insulin lowers the amount of sugar in your bloodstream.

The role of glucose

Glucose — a sugar — is a main source of energy for the cells that make up muscles and other tissues.

Glucose comes from two major sources: food and your liver.

Sugar is absorbed into the bloodstream, where it enters cells with the help of insulin.

Your liver stores glucose as glycogen.

When your glucose levels are low, such as when you haven't eaten in a while, the liver breaks down the stored glycogen into glucose to keep your glucose levels within a normal range.

> In type 1 diabetes, there's no insulin to let glucose into the cells, so sugar builds up in your bloodstream. This can cause life-threatening complications

Risk factors

Some known risk factors for type 1 diabetes include:

Family history. Anyone with a parent or sibling with type 1 diabetes has a slightly increased risk of developing the condition.

Genetics. The presence of certain genes indicates an increased risk of developing type 1 diabetes.

Complications

Over time, type 1 diabetes complications can affect major organs in your body, including heart, blood vessels, nerves, eyes and kidneys. Maintaining a normal blood sugar level can dramatically reduce the risk of many complications.

Eventually, diabetes complications may be disabling or even life-threatening.

Heart and blood vessel disease. Diabetes dramatically increases your risk of various cardiovascular problems, including coronary artery disease with chest pain (angina), heart attack, stroke, narrowing of the arteries (atherosclerosis) and high blood pressure.

Nerve damage (neuropathy). Excess sugar can injure the walls of the tiny blood vessels (capillaries) that nourish your nerves, especially in the legs. This can cause tingling, numbness, burning or pain that usually begins at the tips of the toes or fingers and gradually spreads upward. Poorly controlled blood sugar could cause you to eventually lose all sense of feeling in the affected limbs.

Damage to the nerves that affect the gastrointestinal tract can cause problems with nausea, vomiting, diarrhea or constipation. For men, erectile dysfunction may be an issue.

Kidney damage (nephropathy). The kidneys contain millions of tiny blood vessel clusters that filter waste from your blood. Diabetes can damage this delicate filtering system. Severe damage can lead to kidney failure or irreversible end-stage kidney disease, which requires dialysis or a kidney transplant

Eye damage. Diabetes can damage the blood vessels of the retina (diabetic retinopathy), potentially causing blindness. Diabetes

also increases the risk of other serious vision conditions, such as cataracts and glaucoma.

Foot damage. Nerve damage in the feet or poor blood flow to the feet increases the risk of various foot complications. Left untreated, can become serious infections that may ultimately require toe, foot or leg amputation.

Skin and mouth conditions. Diabetes may leave you more susceptible to infections of the skin and mouth, including bacterial and fungal infections. Gum disease and dry mouth also are more likely.

Pregnancy complications. High blood sugar levels can be dangerous for both the mother and the baby. The risk of miscarriage, stillbirth and birth defects increases when diabetes isn't well-controlled. For the mother, diabetes increases the risk of diabetic eye problems (retinopathy), pregnancy-induced high blood pressure .

Prevention

There's no known way to prevent type 1 diabetes. But researchers are working on preventing the disease or further destruction of the islet cells in people who are newly diagnosed.

Ask your doctor if you might be eligible for one of these clinical trials.

Treatment

Treatment for type 1 diabetes includes:

Taking insulin

Carbohydrate, fat and protein counting

Frequent blood sugar monitoring

Eating healthy foods

Exercising regularly and maintaining a healthy weight

Anyone who has type 1 diabetes needs lifelong insulin therapy.

Types of insulin are many and include:

Short-acting (regular) insulin

Rapid-acting insulin

Intermediate-acting (NPH) insulin

Long-acting insulin

TYPE 2 DIABETES MELLITUS

INSULIN RESISTANCE

Insulin resistance is the name given to when cells of the body don't respond properly to the hormone insulin.

Insulin resistance is the driving factor that leads to type 2 diabetes, gestational diabetes and pre diabetes.

Insulin resistance is closely associated with obesity; however, it is possible to be insulin resistant without being overweight or obese.

Modern research has shown that insulin resistance can be combatted by treatment methods that reduce how much insulin the body is producing.

Reducing insulin resistance can be achieved by following low-carbohydrate and ketogenic diets.

We known that the role of insulin is to allow cells of the body to take in glucose to be used as fuel .

It also means that glucose is more likely to build up in the blood and this can lead to too high blood sugar levels.

When the body becomes resistant to insulin, it tries to cope by producing more insulin. People with insulin resistance are often producing too more insulin than healthy people.

Producing too much insulin is known as hyperinsulinemia.

Symptoms of insulin resistance

Initially, insulin resistance presents no symptoms. The symptoms only start to appear once it leads to secondary effects such as higher blood sugar levels. When this happens, the symptoms may include:

Lethargy (tiredness)

Hunger

Difficulty concentrating (brain fog=brain fatigue)

Other signs that often appear in people with insulin resistance include:

Weight gain around the middle (belly fat)

High blood pressure

High cholesterol levels

Causes of insulin resistance

Whilst the exact cause of insulin resistance is still not fully understood, it is well-known which factors can lead to insulin resistance developing.

Insulin resistance can commonly develop if one or more of the following factors apply:

If you are overweight or obese

Sedentary lifestyle – taking little physical activity

Taking high doses of steroids over an extended period of time

Having chronic stress

Having Cushing's disease or polycystic ovary disease

In terms of what is happening inside the body that causes insulin resistance, researchers have observed that insulin resistance occurs in people that have:

High levels of insulin circulating in their blood

Excessive fat stored in the liver and pancreas

High levels of inflammation

Can insulin resistance be reduced ?

It is certainly possible to reduce the effects of insulin resistance and there are a number of effective ways to do this.

Effective methods include:

- Low-carbohydrate and ketogenic diets
- Very-low-calorie diets
- Taking a lot of exercise in combination with a healthy diet

HYPERGLYCEMIA AND HYPOGLYCEMIA

- Increase in blood glucose level above normal is called **hyperglycemia**.
- Decrease in blood glucose level below normal is called **hypoglycemia**.

HYPERGLYCEMIA

Causes

- **Hyperactivity of the thyroids, pituitary, and adrenal glands.** Here, fasting blood glucose rarely exceeds 200 mg%.
- Emotional **'stress'** can increase the blood glucose level.
- **In diffuse diseases of pancreas**, e.g. in pancreatitis and carcinoma of pancreas, increase in fasting blood glucose may occur.
- Number of infectious diseases.
- **Intracranial diseases** such as meningitis, encephalitis and intracranial tumors .
- **Asphyxia** may also increase blood sugar level.

HYPOGLYCEMIA

Causes

- Some medications, like those used in adults and children with kidney failure.
- Excess amounts of alcohol , which can stop your liver from producing glucose.
- Any disorder that affects the liver,or kidneys.
- Some eating disorders, such as anorexia

Fasting blood glucose may be reduced in:

- **Hypoactivity of thyroids** (Myxoedema, and cretinism)
- **Hypopituitarism** (Simmond's disease) and
- **Hypoadrenalism** (Addison's disease).
- **Severe liver diseases**: Low blood glucose levels are often found.
- **Severe exercise** may produce hypoglycemia due to depletion of liver glycogen.
- Hypoglycemia is also found in some of the **Glycogen storage diseases** (GSDs), e.g. in Von Gierke's disease

CHAPTER III

1. PROTEINS

DEFINITION

Proteins are high molecular weight polymers of a group of low molecular weight monomers called amino acids. Proteins differ from one another primarily in their sequence of amino acids.

A linear chain of amino acid residues is called a polypeptide. A protein contains at least one long polypeptide. Short polypeptides, containing less than 20–30 residues, are rarely considered to be proteins and are commonly called peptides, or sometimes oligopeptides.

Protein synthesis

All the plasma proteins are synthesized in liver and excreted into circulation by hepatocytes; except gamma globulins which is synthesized by Plasma cells.

Almost all the plasma proteins are glycoproteins.

Some of the plasma proteins exhibit polymorphism(exist in different phenotypes) e.g, α1 antitrypsin, transferrin and hepatoglobin

The aa of a polypeptide are placed in a sequence determined by the corresponding sequence of bases G, C, A & T in the DNA that consistitute the appropriate coding gene

The normal serum protein level is 63-83 g/L.

Plasma proteins make up around 7% of the total blood volume, with levels which can fluctuate at times.

The type of proteins in serum include:

- Albumin
- Globulins
    - α1 & α 2–globulins
    - β1 & β2- globulins
    - γ– globulins
- Fibrinogen

Under different pathological conditions the protein levels depart from the normal range.

Plasma proteins fractions

Trace amounts of other proteins can also be found in the blood plasma, usually in concentrations of less than one percent of the total plasma, which can make them difficult to identify.

The concentration of certain plasma proteins (acute phase proteins) increases in disease states such as inflammation and tissue damage.

They include C-reactive proteins, hepatoglobin, fibrinogen and α1 antitrypsin

Electrophoresis is the most commonly employed technique for the separation plasma proteins.

It is used for the diagnosis of certain diseases e.g multiple myeloma, acute infections, nephrotic syndrome etc.

Functions of Plasma proteins

Plasma helps to regulate the body's osmotic pressure, which keeps the body's systems working properly.

It transports various compounds needed by the body

It playing a role in immune system function and blood clotting.

An imbalance of plasma proteins can lead a patient to experience symptoms ranging from abnormally dilated blood vessels to a weakened immune system.

TRANSPORT

Transferrin transports iron.

Ceruloplasmin transports copper.

Albumin transports fatty acids, bilirubin ,calcium, many drugs etc.

Transcortin transports cortisol and corticosterone

Retinol binding protein transports retinol.

Lipoproteins transport lipids.

Haptoglobin transports free haemoglobin.

Thyroxin binding globulin transports thyroxin

Decrease in albumin level results in loss of water from blood and its entry into interstitial fluids causes edema

Protein metabolism

- **Protein metabolism** denotes the various biochemical processes responsible for the synthesis of proteins , and the breakdown of proteins by catabolism.

- Dietary proteins are first broken down to individual amino acids by various enzymes and hydrochloric acid present in the gastro-intestinal tract.
- **Protein anabolism** is the process by which protein are formed from amino acids(anabolic amino acids synthesis).
- **Protein catabolism** is the process by which proteins are broken down to their amino acids.This is also called proteolysis.
  This can be followed by further amino acid degradation

Importance of proteins

- Your body uses it to build and repair tissue. You need it to make enzymes, hormones, and other body chemicals. It is an **important** building block of bones, muscles, skin, and blood.
- They do most of the work in cells and are required for the structure, function, and regulation of the body's **tissues** and organs. Proteins are made up of hundreds or thousands of smaller units called **amino acids**, which are attached to one another in long chains

- Proteins also serve as major components of the blood, epithelial tissues and connective tissue .Excess proteins serve as a source of energy.

Clinical information

- Plasma proteins are synthesized predominantly in the liver; immunoglobulins are synthesized by mononuclear cells of lymph nodes, spleen and bone marrow.
- The general cause of alteration of serum total protein is a change in the concentration of one or more of the specific proteins in the plasma Of the individual serum proteins, albumin is present in such high concentrations that low levels of this protein alone may cause hypoproteinemia

- Hemoconcentration (decrease in the volume of plasma water) results in relative hyperproteinemia.
- Hemodilution(decreased concentration (as after hemorrhage) of cells and solids results in relative hypoproteinemia.
- Hyperproteinemia may be seen in dehydration due to inadequate water intake or to excessive water loss (eg, severe vomiting, diarrhea, Addison disease, and diabetic acidosis) or as a result of increased production of proteins.

CLINICAL SIGNIFICANCE

- An increase in total proteins may occur in dehydration .
- Both albumin and globulin are increased due to hemoconcentration.

A decrease in total proteins is always due to a low albumin level,accompanied either by no increase in globulin.

A low serum albumin may be due to:

1.Heavy loss of albumin in urine (as in nephritis)

2.Malabsorption of amino acids from the alimentary tract (as in steatorrea).

3.Decreased formation in the liver (as in cirrhosis of liver)

4.Increased catabolism of proteins (as in fever)

5.Insufficient intake of proteins in the food(malnutrition)

A reduction in the total proteins is one of the causes of edema.

It may take place when total proteins fall below about 5.0g/dl and albumin below about 2.5g/dl.

Increased total protein values may be found in multiple myeloma.

DETERMINATION OF TOTAL SERUM PROTEINS

Normal range

Serum proteins: 6-8 g/dl

Name of the method

Biuret method

Test principle

Proteins react with cupric ions in alkaline medium to form a violet colored complex .The intensity of the color produced is directly proportional to proteins present in the specimen and can be measured on a photometer at 530nm.

Specimen

Serum

Reagents

-Proteins reagent(ready to use)

-Protein standard

-Distilled water

Procedure

Pipette in three tubes labelled as follows:

	Test	std	blank
Protein reagent,ml	5.0	5.0	5.0

Serum,ml	0.05	-	-
Protein std ,ml	-	0.05	-
Distilled water,ml	-	-	0.05

Mix thoroughly and keep at room temperature for exactly

10mins.

Measure the intensities of the test and standard against blank by using 530nm.

Calculations

Serum proteins,g/dl=OD OF TEST/OD OF STD*6

DETERMINATION OF SERUM ALBUMIN

Name of the method

Bromocresol green method

Principle

Albumin present in serum binds specifically with bromocresol green at ph 4.1 to form green colored complex ,intensity of which can be measured calorimetrically by using 640nm.

Normal range

3.3-4.8 g/dl

Reagents

1.Albumin reagent (ready to use)

2.Albumin std

3.Distilled water

Procedure

Pipette in three tubes labelled as follows:

	Test	std	blank

Albumin reagent,ml	5.0	5.0	5.0
Serum,ml	0.05	-	-
Albumin standard,ml	-	0.05	-
Distilled water,ml	-	-	0.05

Mix thoroughly and keep at room temperature for exactly

10mins.

Measure the intensity of the test and standard against blank by using 640nm.

Calculations

Serum albumin,g/dl= OD OF TEST/OD OF STD*4

CHAPTER 4

RENAL FUNCTION TESTS

1.Overview of Kidney Function

You have two kidneys on either side of your spine that are each approximately the size of a human fist. They're located posterior to your abdomen and below your rib cage.

Your kidneys play several vital roles in maintaining your health. One of their most important jobs is to filter waste materials from the blood and expel them from the body as urine. The kidneys also help control the levels of water and various essential minerals in the body.

If your doctor thinks your kidneys may not be working properly, you may need kidney function tests. These are simple blood and urine tests that can identify problems with your kidneys.

You may also need kidney function testing done if you have other conditions that can harm the kidneys, such as diabetes or high blood pressure. They can help doctors monitor these conditions.

Symptoms of Kidney Problems

▶ High blood pressure

▶ blood in the urine

▶ frequent urges to urinate

- ▶ difficulty beginning urination
- ▶ painful urination
- ▶ swelling of the hands and feet due to a buildup of fluids in the body
- ▶ A single symptom may not mean something serious. However, when occurring simultaneously, these symptoms suggest that your kidneys aren't working properly. Kidney function tests can help determine the reason.

The main functions of kidney

1. Regulation of water balance, electrolyte balance and osmotic pressure of body fluids
2. Regulation of acid-base balance
3. Removal of metabolic waste products and toxic substances from the body.

In order to maintain the fluid of constant composition in the body, kidney eliminates urine, which may vary in composition.

Urine serves as a channel for excretion of waste products formed in the body.

Excess water either consumed or formed during metabolism is also eliminated.

If the **kidneys**' ability to filter the blood is seriously damaged by disease, wastes and excess fluid may build up in the body. ...

A creatinine and Blood Urea Nitrogen (BUN) blood test, outside the normal range.Uric acid, BUN and creatinine are

waste that build up in your blood when your **kidney function** is reduced.

Blood Urea Nitrogen (BUN)

The blood urea nitrogen (BUN) test also checks for waste products in your blood. BUN tests measure the amount of urea nitrogen in the blood.

Urea nitrogen is a breakdown product of protein. However, not all elevated BUN tests are due to kidney damage. Common medications, including large doses of aspirin and some types of antibiotics, can also increase your BUN.

It's important to tell your doctor about any medications or supplements that you take regularly. You may need to stop certain drugs for a few days before the test.

A normal BUN level is between 7 and 20. A higher value could suggest several different health problems.

UREA METABOLISM

The first step towards the metabolic breakdown of amino acids which is deamination.The liver is the chief site of deamination although the kidney and perhaps,other organs may also accomplish it.

The enzyme amino acid oxidase carries out the reaction diamination.

Amino acids----------→ ketoacids+NH3

amino acid oxidase

The ammonia produced in deamination is toxic.

Deamination of amino acids results in the production of **ammonia** (NH_3).**Ammonia** is an extremely toxic base and its accumulation in the body would quickly be fatal. However, the liver contains a system of carrier molecules and enzymes which quickly converts the **ammonia** into **urea**.

CLINICAL SIGNIFICANCE

Elevated levels of urea are observed **in pre renal ,renal and post renal conditions.**

Pre renal conditions:

Diabetes mellitus,dehydration,cardiac

failure,severe burns,high fever etc

Renal conditions:diseases of kidney

Post renal conditions :Enlargements of prostate,stones in the urinary tract ,tumor of the bladder.

Decreased values have been reported in severe liver disease ,protein malnutrition and pregnancy.

DETERMINATION OF SERUM UREA NITROGEN

Name of the method

Diacetyl monoxine method

Normal range

Birth to 1year: 4-16mg/dl

- 1 -40years: 7-21mg/dl
- Gradual slight increase occur over 40years of age

Test principle

Urea reacts with diacetyl monoxime in hot acidic medium

in the presence of thiosemicarbazide and ferric ions to form a pink colored compound which can be measured at 520nm.

Sample material

Serum,heparinised plasma or fluoride plasma

Reagent required

1.Reagent 1(diacetylmonoxime reagent)

2.Reagent 2(thiocemicarbazide reagent)

3.Reagent 3: mixture of 60ml of conc.sulfuric acid ,10ml of orthophosphoric acid and 10ml 0f ferric chloride

in one liter of distilled water.

4.Urea nitrogen standard

Preparation of working reagent

It is prepared fresh by mixing one part of reagent 1,one part of reagent 2 and two parts of reagent 3.

Procedure

Pipette in the tubes labelled as follows

	Test	standard	blank
Working reagent ,ml	5.0	5.0	5.0
Serum/plasma,ml	0.05	-	-
Standard ,ml	-	0.05	-

Distilled water,ml	-	-	0.05

Mix the contents of the tubes thoroughly and place them

in a boiling water bath for exactly 15mins.

Cool immediately by using tap water and after 5mins measure the intensities of the test and standard against blank at 520nm(green filter)

Calculations

Plasma or serum urea nitrogen,mg/dl=

OD OF TEST /OD OF STD*20

DETERMINATION OF URINE UREA NITROGEN

METHOD

Diacetyl monoxime

Requirement

Preparation of a working reagent

Specimen

urine

Procedure

a.Dilute urine 1:10 in distilled water (9.0ml of distilled water and 1 ml of urine)

Mix well in the tubes labelled as follows

	Test	std	Blank
Working reagent,ml	5.0	5.0	5.0
Diluted urine,ml	0.05	-	-
Urea nitrogen std,ml	-	0.05	-
Distilled water,ml	-	-	0.05

Mix the contents of the respective tubes thoroughly and keep in boiling water bath for 20mins.

Cool the tubes to room temperature and read absorbance at 530nm.

Calculations

Urine urea nitrogen,mg/dl=OD OF TEST/OD OF STD*20*10

SERUM CREATININE

This blood test examines whether creatinine is building up in your blood. The kidneys usually completely filter creatinine from the blood. A high level of creatinine in blood suggests a kidney problem.

According to the National Kidney Foundation (NKF), a creatinine level higher than 1.2 for women and 1.4 for men is a sign of a kidney problem.

CREATININE METABOLISM

Creatinine: A chemical waste molecule that is generated from muscle metabolism.Creatinine is produced from creatine, a molecule of major importance for energy production in muscles. ... Creatinine is transported through the bloodstream to the kidneys

Formation of creatinine is a preliminary step required for the excretion of most of the creatine.

Three amino acids glycine,arginine and methionine are directly involved in the synthesis of creatine .

CLINICAL SIGNIFICANCE

Serum creatinine is increased in renal failure .Increased serum

creatinine concentration above 1.5 to 2.0 mg/dl is virtually diagnostic of renal disease .

Elevated values are also observed in certain other conditions like congestive heart failure ,shock and mechanical obstruction of the urinary tract.

DETERMINATION OF SERUM CREATININE

Name of the method

Alkaline picrate method

Test principle

Creatinine reacts with picric acid in alkaline medium to form

a reddish yellow complex ,intensity of which is directly proportional to the concentration of creatinine in the

specimen and can be measured at 520nm.

Specimen

Serum or plasma

Normal range

0.7-1.7mg/dl

Reagents

1.Picric acid reagent

2. Sodium hydroxide.

3.Working creatinine standards

4.2/3 N sulfuric acid

5.Sodium tungstate

Preparation of alkaline picrate reagent

It is prepared fresh by mixing 4ml of reagent 1 and 1ml of reagent 2.

Procedure

Pipette in the tubes labelled as follows

	Test	std
Distilled water,ml	3.0	4.0
Serum,ml	1.0	-

Standard ,ml	-	1.0
2/3 N sulfuric acid,ml	0.5	-
Sodium tungstate	0.5	-

Centrifuge the contents in the test and get clear filtrate .

Pipette in the tubes labelled as follows:

	Test	std1	blank
Distilled water	3.0	3.0	3.0
Filtrate,ml	2.0	-	-
Diluted std ,ml	-	2.0	-
Alkaline picrate reagent,ml	1.0	1.0	1.0

Mix and keep at room temperature for 20mins.

Read intensities of test and standard against blank at 520nm .

CALCULATIONS

SERUM CREATININE,mg/dl=OD OF TEST/OD OF STD*1.0

CLINICAL SIGNIFICANCE

Normal urinary excretion of creatinine is 1.5 -3.0g per 24hrs.

Excretion rate decreases in all kinds of renal diseases and also in the post renal conditions.

DETERMINATION OF URINE CREATININE

Procedure

1.Pipette about 5ml of urine in a test tube.

2.Add 2-3drops of 3gm/dl,sulfosalicylic acid.

Observations

a.Appearance of turbidity:urine contains proteins .

b.No turbidity: Urinary proteins absent

A.If urine contains proteins,deproteinize it by using following method.

Pipette in a centrifuge tube ,the following requirements

-Distilled water 8ml

-Urine 1ml

-2/3 sulfuric acid 0.5ml

- sodium tungstate 0.5ml

Mix well and centrifuge at 1500 rpm for 10mins.

B.If urine proteins are absent,dilute urine 1:10 by using distilled water(9ml of distilled water and 1ml of urine)

Mix thoroughly.

Now pipette in the tubes labelled as follows:

	Test	std	blank
Distilled water,ml	4.8	4.8	5.0
Deproteinized or diluted urine	0.2	-	-
Standard (undiluted)	-	0.2	-
Alkaline picrate reagent,ml	1.0	1.0	1.0

Mix and keep at room temperature for 20mins.Take OD readings against blank at 520 nm.

Calculations

Urine creatinine,mg/dl=OD OF TEST/OD OF STD*100

URIC ACID

A uric acid blood test, also known as a serum uric acid measurement, determines how much uric acid is present in your blood. The test can help determine how well your body produces and removes uric acid.

Uric acid is a chemical produced when your body breaks down foods that contain organic compounds called purines

Foods and beverages with a high purine content include:

- liver
- anchovies
- mackerel
- dried beans

- beer
- wine

Most uric acid is dissolved in the blood, filtered through the kidneys, and expelled in the urine. Sometimes the body produces too much uric acid or doesn't filter out enough of it. Hyperuricemia is the name of the disorder that occurs when you have too much uric acid in your body.

High levels of uric acid are associated with a condition called gout. Gout is a form of arthritis that causes swelling of the joints, especially in the feet and big toes. Another cause of hyperuricemia is increased cell death, due to cancer or cancer treatments. This can lead to an accumulation of uric acid in the body.

It's also possible to have too little uric acid in your blood, which is a symptom of liver or kidney disease. It's also a symptom of Fanconi syndrome, a disorder of the kidney tubules that prevents the absorption of substances such as glucose and uric acid. These substances are then passed in the urine instead.

Purposes of a uric acid blood test

Most commonly, the test is used to:

- diagnose and monitor people with gout

- monitor people who are undergoing chemotherapy or radiation treatment
- check kidney function after an injury
- find the cause of kidney stones

You may need a uric acid test if:

- you have joint pain or swelling that may be related to gout
- you're currently undergoing chemotherapy
- you have frequent kidney stones
- you've been diagnosed with gout in the past

Preparing for a uric acid blood test

The following may interfere with your uric acid test results:

- alcohol
- certain medications, such as aspirin (Bufferin) and ibuprofen (Motrin IB)
- high levels of vitamin C
- dyes used in X-ray tests

CLINICAL SIGNIFICANCE

The serum uric acid level is often raised in gout.

The determination has diagnostic value in differentiating gout from non gouty arthritis .Uric acid levels are also increased in renal failure ,uremia .

SERUM URIC ACID DETERMINATION

Name of the method

Henry-caraway's method

Principle

Uric acid in the protein free filtrate reacts with phosphotungstic acid reagent in the presence of sodium carbonate (alkaline solution) to form a blue colored complex.

The intensity of the color is measured at 660nm.

Reagent required

1. Deprotenizing reagent
2. Sodium carbonate

3.Stock phosphotungstic acid reagent

4.Stock uric acid standard

Procedure

1.Dilute the reagent 3,stock phosphotungstic acid 1:10

i.e by mixing 1.0ml of the reagent and 9 ml of distilled water ,mix well .

2.Dilute the stock uric acid standard(1:20): 1ml of standard and 19 ml of distilled water ,mix well.

3.Take a centrifuge tube labelled as Test and pipette as follows

	Test
Deproteinizing reagent,ml	5.0
Serum,ml	0.6

Mix thoroughly and centrifuge at 3000rpm for 10 mins

4. Again pipette in the tubes labelled as follows

	Test	std	blank
Filtrate,ml	3.0	-	-
Diluted standard	-	3.0	-
Distilled water,ml	-	-	3.0
Sodium carbonate reagent,ml	1.0	1.0	1.0

Diluted phosphotungstic acid,ml	1.0	1.0	1.0

Mix ,keep in the dark for exactly 10mins .Read OD of test and standard at 660nm(red filter) against blank.

Calculations

Serum uric acid mg/dl= OD OF TEST/OD OF STD*5

THE METHOD is linear up to 10mg/dl uric acid.

CLINICAL SIGNIFICANCE

The urine uric acid is made up of an exogenous part(formed from rich diet) and an endogenous part(formed from the breakdown of nucleoproteins).

The normal urine uric acid excretion for 24hrs is 0.6-1.0g. A Consistently high uric acid excretion is found in gout .

DETERMINATION OF URINE URIC ACID

Specimen

24hrs urine sample (use few thymol crystals as preservative)

PROCEDURE

A.Dilute urine 1:20 .pipette 19ml of distilled water in a test tube and add 1ml of urine .Mix well.

B.Dilute stock uric acid standard 1:20 .Pipette

19ml of distilled water in a test tube and add 1ml of cstock standard.

Mix well.

Now pipette in the tubes,labelled as follows

	Test	std	blank
Dilute urine,ml	3.0	-	-
Dilute standard,ml	-	3.0	-
Distilled water,ml	-	-	3.0
sodium carbonate,ml	1.0	1.0	1.0
Diluted phosphotungstic acid ,ml	1.0	1.0	1.0

Mix thoroughly,keep in the dark for exactly 10mins and read intensities at 660nm.

Calculations

Urine uric acid ,mg/dl=OD OF TEST/OD OF STD*100

CHAPTER V

LIVER FUNCTION TESTS

FUNCTION OF LIVER

INTRODUCTION

Liver cells contain several enzymes which may be released into the circulation in liver damage.

- Measurement of selected enzymes in serum is often used to assess the liver function.

Transaminases or Amino transferases

- SGPT(serum glutamate pyruvate transaminase:(recently called as alanine transaminase (ALT)
- SGOT(serum glutamate oxaloacetate transaminase :(recently called aspartate transaminase (AST))

- The ALT and AST belong to the group of enzymes known as transaminases or aminotransferases; they catalyze the transamination reaction, which is very important in amino acid metabolism.

1.ALANINE TRANSAMINASE

This enzyme catalyzes the transfer of the amino group from alanine to α –ketoglutarate .As a result , alanine is converted to pyruvate and α -ketoglutarate to glutamate .

ALT

L-Alanine+α-Ketoglutarate <----------> Pyruvate +L- glutamate

PLP(pyridoxal phosphate)

SOURCE OF ALT

Liver is the major source

Reference Range

- ALT, 6-37 IU/L (37°C).

2. ASPARTATE TRANSAMINASE (AST)

The AST transfer the amino group from aspartate to α-ketoglutarate .As a result ,aspartate is converted to

oxaloacetate and α-ketoglutarate is converted to glutamate. This is a reversible reaction,it requires a coenzyme plp (pyridoxal phosphate).

$$\text{Aspartate} + \alpha\text{-ketoglutarate} \xleftarrow[\text{PLP}]{\text{AST}} \text{oxaloacetate} + \text{glutamate}$$

SOURCE OF AST

- Liver
- Heart
- Skeletal muscle

Reference Range :4-17 IU/L (37°C).

3. ALKALINE PHOSPHATASE (ALP)

Alkaline phosphatase (**ALP**) or basic phosphatase is a protein enzyme of 86 kilodaltons and is optimally active at alkaline pH · As its name indicates, ALP functions best under alkaline pH environments and has the physiological role of dephosphorylating compounds.

- Catalyses the removal of phosphate groups at alkaline pH.

- Widely distributed in tissues, high concentrations in Intestines, Liver, Bone, Spleen, Placenta and Kidney.

- Useful in diagnosis of Hepatobiliary (Cholestatic liver disease)and Bone disorders.
- In Liver disease, increase plasma ALP is due to increased synthesis by cells lining the Bile , usually in response to Cholestasis, which may be either Intra-hepatic or Extra-hepatic.

NORMAL VALUE:

40 -140 IU/L

4.GAMMA-GLUTAMYL TRANSPEPTIDASE

Gamma-glutamyltransferase (also known as **γ-glutamyltransferase**, **GGT**, **gamma-GT)** is a transferase (a type of enzyme) that catalyzes the transfer of gamma-glutamyl functional groups from molecules such as glutathione to an acceptor that may be an amino acid, a peptide .

The enzyme is found in liver, kidney, pancreas and prostate

Normal value:

Normal serum activity has been shown to be:

- **Men:** 10 to 47 IU/L
- **Women:**7 to 30 IU/L

5.BILIRUBIN

Bilirubin is a bile pigment and is the excretory end product of heme degradation .It is conjugated in the liver to form bilirubin diglucuronide ,and excreted in bile.

Bilirubin is a yellowish breakdown product of the heme. It is a part of the hemoglobin molecule that is in the red blood cells. It is thrown out of our body by means of bile or urine. Hence an increase in the level of bilirubin indicates the person could be suffering from certain diseases like jaundice

It is lipid soluble as it is a four ring structure known as tetrapyrrole. Bilirubin when high is brown however when the level of bilirubin is slightly higher than normal it is yellowish. In some cases depending on the level of bilirubin that is elevated it may show even on our skin and sclera.

There are differences between unconjugated versus conjugated bilirubin where unconjugated bilirubin is not soluble with water and conjugated bilirubin is soluble with water.

Total bilirubin and direct bilirubin levels are measured directly in the blood, whereas indirect bilirubin levels are derived from the total and direct bilirubin measurements.

When bilirubin levels are high, the skin and whites of the eyes may appear yellow (jaundice). Jaundice may be caused by liver disease (hepatitis), blood disorders (hemolytic anemia), or blockage of the tubes (bile ducts) that allow bile to pass from the liver to the small intestine

Mild jaundice in newborns usually does not cause problems. But too much bilirubin (hyperbilirubinemia) in a newborn baby can cause brain damage (kernicterus) and other serious problems. So some babies who develop jaundice may need treatment to lower their bilirubin levels.

DETERMINATION OF SERUM BILIRUBIN

Method

Malloy and evelyn

Principle

When biririubin reacts with diazo reagent ,purple colored azobilirubin is formed .Methanol is used as reaction accelerator since total bilirubin is soluble in it .

The optical densities of total test and direct test are measured against respective blanks at 540nm

Normal range

Total bilirubin	up to 1.0mg/dl
Direct bilirubin	up to 0.5mg/dl
Indirect bilirubin	up to 0.5mg/dl

Sample material

Serum or heparinized plasma

Reagents

-Diazo A

-Diazo B

-Diazo blank reagent

-Methanol

- Artificial bilirubin standard:-stock standard

-working standard

Procedure

Prepare fresh diazo mixture by mixing 5.0ml of diazo A and 0.15ml of diazo B .

Pipette in the tubes labeled as follows:

	Total test	Total blank	Direct test	Direct blank
D.water,ml	1.8	1.8	1.8	1.8
Serum,ml	0.2	0.2	0.2	0.2
Diazo mixture,ml	0.5	-	0.5	-
Diazo blank reagent	-	0.5	-	0.5

Methanol	2.5	2.5	-	-

Keep in dark for 30mins.Calibrate with distilled water and then read the intensities at 540nm.

Read OD of the artificial bilirubin standard (undiluted)by transferring the standard solution in a dry cuvette at 540nm.

Calculations

OD of total bilirubin=OD of total test-OD of total blank

OD of direct bilirubin =OD of direct test-OD of direct blank

Total bilirubin mg/dl=OD of total bilirubin/OD of std*10

Direct bilirubin,mg/dl=OD of total bilirubin /OD of std*10

Indirect bilirubin,mg/dl=Total bilirubin,mg/dl –direct bilirubin ,mg/dl

Sources of error

Exposure to light decreases bilirubin in the sample.

DETERMINATION OF SGPT

METHOD

UV kinetic

Principle

The serum alanine transaminase catalyzes the conversion of alanine to pyruvate in the presence of α ketoglutarate liberating glutamic acid.

$$\alpha\text{-oxaloglutarate} + \text{alanine} \xleftarrow{\text{SGPT}} \text{L-glutamate} + \text{pyruvate}$$

Normal value

Men	up to 40 IU
WOMEN	up to 31 IU

Reagents

1.Bufferd substrate

2.α-oxoglutarate

3.LDH

4.NADH

Procedure

Pipette into cuvette

Buffered substrate,ml	1.0
NADH,ml	0.02
LDH,ml	0.02
Sample,ml	0.2
α-oxoglutarate,ml	0.04

Mix and read initial absorbance after 1min at 340nm (delay time).

Note readings after exactly 1,2and 3mins.Determine the mean absorbance change per min(mean abs/min).

Calculate the change in absorbance per minute by subtracting INITIAL ABSORBANCE from FINAL ABSORBANCE and dividing by 2.

calculations

SGPT:IU=1015*mean abs/min

DETERMINATION OF SGOT

Method

UV KINETIC

TEST Principle

The serum aspartate transaminase catalyses the conversion of aspartic acid to oxaloacetate in the presence of α-ketoglutarate liberating glutamic acid

sgot

Aspartate+ α- ketoglutarate ← ……. → Oxaloacetic acid+glutamic acid

Normal value: Men up to 37 IU

Women up to 31 IU

Sample material

Serum

Reagents

1.Buffered substrate :it is preparing by taking 41.62g 0f aspartic acid in one liter of Tris buffer

2. α -ketoglutarate

3.LDH

4.NADH

PROCEDURE

Buffered substrate 1.0ml

NADH 0.02ml

LDH 0.01ml

Mix and add sample 0.2ml

α-ketoglutarate 0.04ml

Mix and read initial absorbance after 1min.Afterwards note reading after exactly 1,2 and 3mins .Determine the mean absorbancies change per min(AA/min).

Calculations

SGOT,IU=1015*AA/min

ALKALINE PHOSPHATE DETERMINATION

METHOD

Visible –kinetic

Principle ALP

P-nitrophenyl phosphate+H20←----------→Phosphate+

P-nitrophenol

Increase in OD is measured after every one min at 405nm

Normal values

Adults :20-80 IU

Chilidren:93-221 IU

Sample material

Serum,heparinised plasma

Reagents

1.AMP Buffer(ph:10.3)

2.Magnesium chloride reagent

3.P-nitrophenyl phosphate:-42.5mg of PNP

-0.5ml of Magnesium chloride

Procedure

AMP Buffer,ml	2.7ml
PNP,ml	0.2ml
Serum or plasma,ml	0.1ml

Mix ,read initial absorbance and start stop watch at the same time.

Repeat readings after exactly 1 ,2 and 3 mins.Determine mean absorbance change per min.

Calculate the change in absorbance per minute by subtracting INITIAL ABSORBANCE from FINAL ABSORBANCE and dividing by 2.

Calculations

Serum alkaline phosphate:IU=1595*mean abs /min

Mix ,read initial absorbance and start stop watch at the same time.

Repeat readings after exactly 1 ,2 and 3 mins.Determine mean absorbance change per min.

Calculate the change in absorbance per minute by subtracting INITIAL ABSORBANCE from FINAL ABSORBANCE and dividing by 2

DETERMINATION OF GAMMA GT

Principle

gamma gt

L-gamma-glutamyl-p-nitroanilide + glycyl glycine←----------→

L-gammaglutamyl-glycyl glycine + p-nitroaniline

Normal values

Male: 4-23 IU

Female: 3.5-13 IU

Sample material

Serum

Reagents

1.substrate:-250mg of gamma-glutamyl paranitroanilide

-872mg of glycyl-glcine

-672mg of magnesium chloride

-300ml of AMP buffer

2.sodium hydroxide reagent

3.P-nitroaniline standard

Procedure

	Test	blank
Substrate	1.0	1.0
Serum,ml	0.2	-
Incubate at 37 degree c for 45mins		
Sodium hydroxide reagent,ml	5.0	5.0

Mix ,read initial absorbance and start stop watch at the same time.

Repeat readings after exactly 1 ,2 and 3 mins.Determine

mean absorbance change per min.

Calculate the change in absorbance per minute by subtracting INITIAL ABSORBANCE from FINAL ABSORBANCE and dividing by 2

Calculation

Gamma GT=1616*AA 405 per min

CHAPTER VI

CARDIAC FUNCTION TEST

INTRODUCTION

The **heart** is a hollow, muscular organ, which **functions** as a pump for the movement of blood through the body.

The **diagnostic tests in cardiology** are methods of identifying heart conditions associated with healthy and unhealthy, heart function.

For example, high levels of "bad" cholesterol in your blood can be a sign that you're at increased risk of having a heart attack. And other substances in your blood can help your doctor determine if you have heart failure or are at risk of developing plaques in your arteries (atherosclerosis).

DIFFERENT TESTS TO ACCESS HEART FUNCTION

- **Creatine Kinase** (CK) also called creatine phosphokinase(CPK)- Widely used to diagnosis and monitor heart attacks
- LDH(Lactose dehydrogenase)

CREATININE KINASE

Creatine kinase (CK) activity is greatest in striated muscle, heart tissue, and brain. The determination of CK activity is a proven tool in the investigation of skeletal muscle disease (muscular dystrophy) and is also useful in the diagnosis of myocardial infarction (MI) and cerebrovascular accidents. Increased levels of CK also can be found in viral myositis, polymyositis, and hypothyroidism.

When is it ordered?

A CK test may be ordered when muscle damage is suspected and at regular intervals to monitor for continued damage. It may be ordered when a muscle disease (myopathy) such as muscular dystrophy is suspected or when someone has experienced physical trauma, such as crushing injuries or extensive burns.

The test may be ordered when a person has symptoms associated with muscle injury such as:

- Muscle pain
- Muscle weakness
- Dark urine (The urine may be dark because of the presence of myoglobin, another substance released by damaged muscles that can be harmful to the kidneys.)

Testing may be ordered when a person has nonspecific symptoms, especially when taking a drug or after an exposure to a substance that has been linked with potential muscle damage.

Clinical significance

CPK(creatine phosphokinase)activity is highest in brain ,heart muscle and skeletal muscle.

Elevated serum CPK activity is of diagnosis importance in myocardial infarction and in muscular dystrophy .Increased levels may also be found in polymyositis,motor-neuron disorders and in acute cerebrovascular accidents(blood vessels in the brain)

1.DETERMINATION OF SERUM CK(CPK)

Normal range

Men : 20-50 IU

WOMEN : 10-37 IU

Principle

CPK catalyses the following reaction

CPK

Creatine phosphate+ADP----------→ creatine +ATP.

The creatine formed in the reaction,reacts with diacetyl and α-naphthol in alkaline medium to give colored complex.

The intensity of color is proportional to CPK activity and it is measured at 520nm.

Sample material

Serum

Precautions

Patient should not have undergone strenuous exercise before blood collection.

Reagents

1.Creatine phosphate

2.ADP

3.Tris buffer(substrate)

4.P-chloromercuribenzoic acid

5.Alkaline EDTA

6.α-Naphthol

7.Diacetyl stock

8.Working standard

Procedure

Pipette in the tubes labelled as follows

	Test	std	blank
1.Substrate,ml	0.4	0.4	0.4
2.Distilled water	-	0.2	0.2
3.Serum,ml	0.05	-	0.05
4.Alkaline ADTA,ml	0.2	-	-

Mix well and incubate at 37 degree c for 30mins

5.Working standard,ml	-	0.05	-
6.Reagent 4,ml	0.5	0.5	0.5
7.Reagent 5,ml	3.75	3.75	3.75
8.Reagent6,ml	1.0	1.0	1.0
9.Reagent7,ml	0.5	0.5	0.5

Mix well and keep in the dark at room temperature for 30mins.

Measure OD of test ,std and blank against distilled water at 520nm.

Calcutions

Serum activity,IU=

OD OF TEST-OD OF BLANK/OD OF STD - OD OF BLANK*66.7

LACTATE DEHYDROGENASE(LDH)

DEFINITION

Lactate dehydrogenase (LDH) is an enzyme required during the process of turning sugar into energy for your cells. LDH is present in many kinds of organs and tissues throughout the body, including the liver, heart, pancreas, kidneys, skeletal muscles, lymph tissue, and blood cells.

When illness or injury damages your cells, LDH may be released into the bloodstream, causing the level of LDH in your blood to rise. High levels of LDH in the blood point to acute or chronic cell damage, but additional tests are necessary to discover its cause. Abnormally low LDH levels only rarely occur and usually aren't considered harmful

Causes of high LDH levels

Because LDH is present in so many types of cells, high levels of LDH may indicate a number of conditions.

Elevated levels of LDH can include:

- blood flow deficiency
- cerebrovascular accident, also known as a stroke
- certain cancers
- heart attack
- hemolytic anemia
- liver disease, such as hepatitis
- muscle injury
- muscular dystrophy
- pancreatitis
- tissue death

- use of alcohol or certain drugs
- sepsis and septic shock

Precautions

Certain medications and drugs may interfere with an accurate LDH test. Large amounts of vitamin C (ascorbic acid) may lower LDH levels. Alcohol, anesthetics, aspirin, narcotics, and procainamide may raise LDH levels. Strenuous exercise may also raise LDH levels. Ask your doctor about any medications you should avoid before the test.

DETERMINATION OF LDH

Test principle

LDH

Lactate+NAD----------→Pyruvate + NADH+H

Increased in OD is measured after 45 seconds by the

interval of 1min.

Normal range

70-240 IU

Sample material

Serum or heparinised plasma

Reagent

1.Buffered substrate

2.NAD^+ solution

Procedure

1.Buffered substrate,ml 1.0

2.NAD^+solution,ml 0.2

3.Serum/plasma,ml 0.02

Mix ,take reading after 1min by the interval

of 1,2 and 3min.

Determine the mean absorbance change per min

Calculations

LDH,IU=9807*Mean abs/min.

If the absorbance change mean abs /min exceeds 0.100 at 340nm,dilute serum 1:10 by using normal saline .

CHAPTER VII

PANCREAS FUNCTION TESTS

FUNCTION OF PANCREAS

Definition

The pancreas is a gland having both exocrine and endocrine secretions. The exocrine secretion consists of enzymes and other components required for digestion.

The daily volume of pancreatic secretion is 500-800ml and the pH is alkaline.

The pancreas has a role in the body's production of insulin, and problems affecting the pancreas include pancreatic cancer and acute pancreatitis.

A number of tests are used to diagnose problems with the pancreas.

Blood tests can evaluate the function of pancreas. Levels of the pancreatic enzymes amylase and lipase can be measured.

Function

Function testing seeks to determine whether or not the pancreas is working normally. The three functions of the pancreas are to produce

- ▶ enzymes for digestion
- ▶ bicarbonate to neutralize gastric acid
- ▶ insulin to signal cells in the body .

Enzymatic component of the pancreas

- It contains enzymes and proenzymes involved in digestion.

- These include enzymes involved in digestion of

Proteins -trypsinogen

-chymotrypsinogen

-preocarboxypeptidase

-proelastas

Carbohydrates (α-amylase)

Lipids (prophospholipase, lipase, colipase) and

Nucleic acid (ribonuclease and deoxyribonuclease

1.<u>AMYLASE</u>

Amylase is an enzyme that helps digest carbohydrates. It is made in the pancreas and the glands that make saliva. When the pancreas is diseased or inflamed,**amylase** releases into the blood.

A test can be done to measure the level of this enzyme in your blood. An **amylase** is an **enzyme** that catalyses the hydrolysis of starch into sugars. **Amylase** is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion

The pancreas and salivary gland make amylase (alpha amylase) to hydrolyse dietary starch
into disaccharides and trisaccharides which are converted by other enzymes to glucose to supply the body with energy.

Plants and some bacteria also produce amylase. As *diastase*, amylase was the first enzyme to be discovered and isolated.

Two types of amylases are recognised:

-beta amylase

-alpha amylase.

Bacteria contains beta amylase

DETERMINATION OF SERUM AMYLASE

METHOD

Colorimetric:Amyloclastic,Lodometric

Principle

Amylase in the specimen acts on the substrate starch.

The products formed are dextrins and maltose.

After the incubation,when the end products are treated with the color reagent(iodine reagent),decrease in the blue color is observed

The disappearance of blue color is directly proportional to the amylase concentration in the specimen and gives the measure of amylase present in the specimen.

Reagent

- Buffered substrate
- Stock color reagent
- Working color reagent

Procedure

	Test	blank
1.Buffered substrate,ml	2.5	2.5
Keep at 37 degree c for 5mins		

2.Serum	0.1	-
Mix ,incubate at 37 degree c for 7.5 mins.		
3.Working color reagent,ml	2.5	2.5
4.Serum,ml	-	0.1
5.Distilled water,ml	2	2

Mix thoroughly,and red intensities of test and blank against distilled water at 660nm

Calculations

Serum amylse=

OD TEST/OD OF BLANK*400

LIPASE

A lipase test measures the amount of this enzyme in a blood sample.

High amounts of lipase may be found in the blood when the pancreas is damaged or when the tube leading from the pancreas (pancreatic duct) to the beginning of the small intestine is blocked

Your pancreas makes an enzyme called lipase. When you eat, lipase is released into your digestive tract to help your intestines break down the fats in the food you're eating. Lipase also allows cell nutrients and cell waste to move through the walls of the cells in your body.

Certain levels of lipase are needed to maintain normal digestive and cell function. Abnormally high levels of the enzyme in your blood can be a sign of a health problem.

The serum lipase test is used to measure the amount of lipase in the body. The lipase test is often ordered at the same time as the amylase test. An amylase test is used to diagnose diseases of the pancreas.

The results from these tests are typically used to diagnose and monitor specific health conditions, including:

acute pancreatitis, which is a sudden swelling of the pancreas

chronic pancreatitis, which is a chronic or recurrent swelling of the pancreas

- Crohn's disease
- cystic fibrosis
- pancreatic cancer

A lipase test is done to:

Check for pancreatitis and other diseases of the pancreas.

See if the treatment for pancreatitis is working.

Help check for cystic fibrosis or see if treatment for it is working

After an attack of acute pancreatitis ,the serum lipase activity increases within 4-8hrs,peaks at about 24hr,and decreases within 8-14days .Serum lipase levels remain elevated longer than those of amylase.

Lipase elevations usually parallel those of amylase;but increases in lipase activity may occur sooner or later than increases in amylase activity.

Clinical significance

Serum lipase assays may also be of value in diagnosis of chronic pancreatitis and in obstruction of the pancreatic duct by a carcinoma of the pancreas.

DETERMINATION OF SERUM LIPASE

Method

Rate of reaction

Lipase catalyzes the reaction

Principle

Lipase

Triolein+2H2O----------→monoglyceride+oleic acid

Test results are compared with a lipase standard

(containing known concentration of lipase activity in

terms of IU).

REAGENTS

1.Lipase reagent

2.Standard :contains lipase.

Concentration of the standard is specified on the label of the container.

Specimen

Serum

Reference range

Up to 200 IU

PROCEDURE

1.By using distilled water ,calibrate the calorimeter machine.

2.Keep the reconstituted 1.0ml of lipase reagent at 37 degree c for 5mins.

3.Add 0.1ml serum and mix well,and read absorbance immediately (A1) at 340nm.

4.Read absorbance again after 5mins at 340nm(A2).

5.Calculate TA1-TA2

Perform same procedure as test for lipase standard.

6.Calculate SA1-SA2

7.Serum lipase activity can be calculated by using following formula:

Serum lipase,IU=TA1-TA2/SA1-SA2*Conc of standard(IU)

CHAPTER VIII

MINERALS

INTRODUCTION

Definition

Minerals are chemical element required as an essential nutrient by organisms to perform functions necessary for life.Minerals originate in the earth and cannot be made by living organisms. Plants get minerals from soil. Most of the minerals in a human diet come from eating plants and animals

or from drinking water.As a group, *minerals* are one of the four groups of essential nutrients, the others of which
are vitamins, essential fatty acids, and essential amino acids.

Minerals are important for your body to stay healthy. Your body uses minerals for many different jobs, including keeping your bones, muscles, heart, and brain working properly. Minerals are also important for making enzymes and hormones.

There are two kinds of minerals: macrominerals and trace minerals. You need larger amounts of macrominerals. They include calcium, phosphorus, magnesium, sodium, potassium, chloride and sulfur. You only need small amounts of trace minerals. They include iron, manganese, copper, iodine, zinc, cobalt, fluoride and selenium.

1.CALCIUM

Calcium is present in the body in larger amounts than any other mineral element .It is mainly present in teeth and bone.

about 99% of the body calcium is in the skeleton .

It is present in the bones as deposits of calcium phosphate in a soft.

It is also present in small concentration in body fluids.The ionized calcium in the body fluids plays important role in blood coagulation and in maintaining the normal excitability of the heart,muscles and nerves

Sources

Milk and cheese are the richest sources of calcium .

Other foods such as egg yolk ,beans ,lentils,nuts,figs,cabbage also contain calcium.

The normal daily intake of calcium in adults is 0.5-1.0g.

Distribution of calcium in body fluids and tissues

Serum	4.5-5.5mEq/l or 9-11mg/dl
Nerve tissue	15mEq/l
Muscle tissue	70 mEq/l
CSF	2.0-2.5mEq/l or 9-11mg/dl

METABOLISM OF CALCIUM

Calcium metabolism refers to the movements and regulation of calcium ions (Ca^{2+}) into and out of various body compartments, such as the gastrointestinal tract, the blood plasma, the extracellular and the intracellular fluid, and bone tissue. An important aspect of calcium metabolism is plasma calcium homeostasis, the regulation of calcium ions in the blood plasma within narrow limits.

In this process, bone tissue acts as a calcium storage center for deposits and withdrawals as needed by the blood, via

continual bone remodeling.Derangements of this mechanism lead to hypercalcemia or hypocalcemia, both of which can have important consequences for health.

In humans, when the plasma calcium level rises above its set point, the thyroid gland releases calcitonin, causing the plasma calcium level to return to normal. When it falls below that set point, the parathyroid glands release parathyroid hormone (PTH), causing the plasma calcium level to rise.

DETERMINATION OF CALCIUM

PRINCIPLE

Calcium reacts directly with cresolphthalein complexon(CPC) reagent containing dimethyl sulfoxide and 8-hydroxyquinoline.

Since magnesium also reacts with CPC ,the addition of 8

hydroxyquinonoline virtually eliminates the interference from magnesium.

Clinical significance

Decreased serum calcium values are found in hypoparathyroidism , rickets ,osteomalacia and steatorrea.A fall in serum calcium can occur in acute pancreatitis and in those forms of renal diseases in which excessive proteinuria is observed .

Increased serum calcium values are observed in hyperparathyroidism ,hypervitaminosis D &multiple myeloma

Reagents

1.Calcium reagent 1

2.Calcium reagent 2

3.Calcium standard

PROCEDURE

1.Prepare fresh working reagent by mixing equal quantities of reagent 1 and 2 in ml (10ml into 10ml).

Note:This working reagent is stable only for a day at room temperature.

Pipette in the tubes labelled as follows:

	Test	std	blank
Working reagent,ml	6.o	6.0	6.0

Serum or(heparinised) Plasma,ml	0.05	-	-
Standard ,ml	-	0.05	-
Distilled water	-	-	0.05

Mix thoroughly and keep at room temperature for exactly 10mins.Read intensities of test and standard against blank (yellow filter).

Calculations

Serum calcium ,mg/dl=OD OF TEST/OD OF STD*10

PHOSPHORUS

Phosphorus is the second most plentiful mineral in your body. The first is calcium. Your body needs phosphorus for many functions, such as filtering waste and repairing tissue and cells.

Most people get the amount of phosphorus that they need through their daily diets. In fact, it's more common to have too much phosphorus in your body than too little. Kidney disease or eating too much phosphorus and not enough calcium can lead to an excess of phosphorous.

However, certain health conditions (such as diabetes and alcoholism) or medications (such as some antacids) can cause phosphorus levels in your body to drop too low.

Phosphorus levels that are too high or too low can cause medical complications, such as heart disease, joint pain, or fatigue.

Roles of Phosphorus

You need phosphorus to keep your bones strong and healthy, to help make energy, and to move your muscles.

In addition, phosphorus helps to:

- build strong bones and teeth
- filter out waste in your kidneys
- grow, maintain, and repair tissue and cells
- assist in muscle contraction
- maintain a regular heartbeat
- facilitate nerve conduction
- reduce muscle pain after exercise

Distribution of phosphorus in our body

About 80% of the total phosphorus is combined with calcium in bones and teeth .It is found in every cell of the body.

About 10% is combined with proteins ,lipids and carbohydrates and other compounds in blood and muscle .

The remaining 10% widely distributed in various chemical compounds.

Requirements and sources

Since the distribution of phosphorus in foods is very similar to calcium ,an adequate intake of calcium generally ensures an adequate intake of phosphorus.The daily intake of phosphorus in adults is 1.5-3.0g.

When there is defective absorption of calcium ,defective absorption of phosphorus usually results.

Metabolism

The metabolism of phosphorus in large part related to that of calcium as described there before.

An increase in carbohydrate metabolism is accompanied by a temporary decrease in serum phosphate.

Clinical significance

Decreased serum phosphorus values are observed in

Preliminary hyperparathyroidism ,rickets(vitamin D deficiency) and in the fanconi's syndrome (a disease associated with a defect in reabsorption of phosphorus).

Increased serum phosphorus levels may be found in hypervitaminosis D, hypoparathyroidism and in renal failure

Determination of serum phosphorus

Method

Gomorri's method

Principle

The Protein free filtrate obtained by the action of TCA(Trichloroacetic acid)reagent is treated with an acid molybdate reagent which reacts with inorganic phosphate to form phosphomolybdic acid.

The color reagent metol,reduces phosphomolybdic acid to give a blue compound which is estimated calorimetrically.

Normal value

Serum Adults 2.5-4.5mg/dl

Children 4.0-7.0mg/dl

SAMPLE

The specimen need not be a fasting one

Reagents

-Trichloroacetic acid

-Molybdate reagent

-Metol(color reagent)

-Phosphorus standard

Procedure

	Test	Diluted strd
TCA reagent,ml	4.5	4.5
Serum,ml	0.5	-

Standard ml	-	0.5

Mix ,centrifuge test and get clear filtrate.

Pipette in the tubes labelled as follows:

	Test	standard	blank
Filtrate ml	2.5	-	-
Diluted standard	-	2.5	-
Distilled water ml	-	-	2.5
Molybdate reagent	0.5	0.5	0.5

Color reagent	0.5	0.5	0.5

Mix thoroughly and keep in the dark for 10mins .Read the intensities at 660 nm(red filter)

Calculations

Serum phosphorus,mg/dl=

OD OF TEST/OD OF STD*5

CHAPTER IX

LIPID PROFILES

LIPIDS

Introduction

Lipids are important constituent of of the diet because they are a source of high energy value. Lipids are also important

because of the fat-soluble vitamins, and essential fatty acids found in the fat of the natural food stuffs. Body fat serves as a very good source of energy, it is stored in adipose tissues.

They also act as insulating material in the subcutaneous tissues and are also seen around certain organs. Lipids combined with proteins are important constituents of the cell membranes and mitochondria of the cell.

Lipids are naturally occurring organic compounds, commonly known as oils and fats. Lipids occur through out the living world in microorganisms, higher plants and animals and also in all cell types. Lipids contribute to cell structure, provide stored fuel and also take part in many biological processes.

Importance of lipids

Lipids have several important biological functions.

1.They serve as the reservoir of high energy value.

Its calorific value is 9kilo calories/g as compared to

carbohydrates which have calorific value of 4kilo calories/g.

2.They can be stored in concentrated form in water free state (as compared to carbohydrates)in the adipose tissue.

3.They are important components of cell membranes.

4.They form important constituent of nervous tissue.

5.They form insulating and protective coating in the subcutaneous tissues and around certain organs(e.g:kidneys)

6.In the form of oil soluble vitamins(A,D,E,K) and essential fatty acids (linoleic and linolenic acid),they are important dietary constituents.

7.Lipoproteins (combinations of lipids and proteins)are important constituents of cell membrane and mitochondria.

Classification of lipids

The lipids are classified into three main groups:

- ❑ Simple lipids
- ❑ Compound or conjugate lipids
- ❑ Deriveds lipids

These main group can be divided as follows:

SIMPLE LIPIDS

- **FATS**:Examples are

  tripalmitin,tristearin,animal fats,coconut oil,butter fat etc..

- -**WAXES**:Examples are

  cholesterols esters,vitamin D,vitamin A.

COMPOUNDS LIPIDS

Phospolipids:examples are

lecitins,cephalins,phospatidyl serine,phospatidyl inositol,cardiolipin,plasmologens

-**Cerebrosides**(glycolipids):Examples are

kerasin,cerebron,nervon,and oxynervon

-**Sulfolipids**:Examples are

sphingosine,cerebronic acid,sulfuric acid

Lipoproteins: lipids such as triglycerides,phospholipids and cholesterol are water insoluble and are transported in the body in blood in combination with various specific proteins.These occur in following four major forms:

-Chylomicrons

-Very low density lipoproteins(VLDL)

-Low density lipoproteins(LDL)

-High density lipoproteins (HDL)

DERIVED LIPIDS

- **Saturated fatty acid:**Examples are butyric,caproic,lauric,palmitic.

▶ **Unsaturated fatty acids:**oleic series,linoleic series,linolenic series.

Functions

Lipids have many dual roles:

Lipids are easily stored in the body where they serve as a source of fuel and are an important constituent of the structure of cells.

1. Fats provide more energy than carbohydrates and proteins
2. They are an integral part of the cell membranes-structural role
3. Fat is used in our bodies to cushion vital organs like the kidneys and also serve as insulation, especially just beneath the skin.
4. Lipoproteins constitute the body's petroleum industry
5. The large chylomicrons carry dietary triglycerides throughout the circulatory system to the cells

- The VLDL carry triglycerides assembled in the liver out to the cells for energy needs or storage as fat.
- The LDL, rich in cholesterol carry cholesterol to the peripheral cells
- The HDL are the clean up crew, gather excess cholesterol back to the liver
- Cholesterol, which in excess contributes to heart disease, is used in the body for useful functions such as facilitating transport of triglycerides for fuel needs of the body and

maintaining cell membranes and as precursor for hormone synthesis

Important lipids profile tests

- ▶ Serum total cholesterol
- ▶ Serum HDL cholesterol
- ▶ Serum triglycerides
- ▶ Serum phospholipids

TRIGLYCERIDES

- Contain 3 fatty acid molecules attached to 1 molecule of glycerol by an ester bond
- Triglycerides containing saturated fatty acids, which do not have kinks in their structure pack together more closely and tend to be solid at room temperature.
- The configuration of the double bonds in most unsaturated FA is cis;
- Triglycerides containing ***cis*** unsaturated fatty acids , with bends in their structure typically form oils at room temp. (S**o called because the 2 H atoms on the carbon atoms either side of the double bond are on the same side of the molecule) latin: Cis= this side of**

During the degradation of FA some *trans*-isomers are formed where the H on the carbon atoms on either side of the double bond are on opposite sides of molecule

- Thus most triglycerides from plants are unsaturated –oils
- Triglycerides from animal sources are saturated –fats / solid

Cholesterol

- Cholesterol is a sterol (a combination steroid and alcohol) and a lipid found in the cell membranes of all body tissues
- It is transported in the blood plasma of all animals.
- The name originates from the Greek *chole-* (bile) and *stereos* (solid), and the chemical suffix *-ol* for an alcohol.
- Most cholesterol is not dietary in origin; it is synthesized internally.
- Cholesterol plays a central role in many biochemical processes, but is best known for the association of cardiovascular disease with various lipoprotein for cholesterol transport patterns
- When doctors talk to their patients about the health concerns of cholesterol, they are often referring to "bad cholesterol", or low-density lipoprotein (LDL).
- "Good cholesterol" is high-density lipoprotein (HDL
- **Cholesterol is** 27 –carbon steroid structure
- The general structure of **cholesterol** consists of two six-membered rings side-by-side and sharing one side in common,

a third six-membered ring off the top corner of the right ring, and a five-membered ring attached to the right side of that.

- The central core of this molecule, consisting of four fused rings, is shared by all **steroids**, including:
    - estrogen (estradiol)
    - progesterone,
    - corticosteroids such as cortisol (cortisone)
    - aldosterone,
    - Testosterone,
    - Vitamin D.

CLINICAL SIGNIFICANCE

Elevated levels of serum cholesterol are associated with atherosclerosis,nephrosis,diabetes mellitus,obstructive jaundice and myxedema.

Decreased levels are observed in hyperthyroidism,

malabsorption and anemia.

1.DETERMINATION OF SERUM TOTAL CHOLESTEROL

Method

Calorimetric waston

Normal range

150-250mg/dl

Sample material

Serum(fasting):hemolysis interferes with the test

Test principle

Cholesterol reacts with acetic anhydride in the presence of glacial acetic acid and conc.sulfuric acid to form green colored complex.

Intensity of the color is proportional to the cholesterol concentration and can be measured at 580nm.

Reagents

1.Cholesterol reagent 1

2.Cholesterol reagent 2

3.Cholesterol standard

Procedure

Dispense in the tubes labelled as follows

	Test	std	blank
1.Cholesterol reagent1,ml	2.5	2.5	2.5
2.Serum,ml	0.1	-	-
3.Cholesterol std,ml	-	0.1	-
4.Distilled water	-	-	0.1
Mix well and cool to room temperature by placing in water bath (at room temperature).Add following reagent			
5.Cholesterol reagent 2,ml	0.5	0.5	0.5

Mix thoroughly,keep in the water-bath at room temperature for 10mins.

Read the absorbance of test and standard against blank at 580nm.

Calculations

Serum total cholesterol,mg/gl=OD OF TEST/OD OF STD*200

2.DETERMINATION OF SERUM HDL CHOLESTEROL

Method

Calorimetric waston

Sample material

Serum (fasting)

Test principle

In the presence of phosphotungstic acid and magnesium chloride,LDL,VLDL AND CHYLOMICONS are precipitated.

Centrifugation leaves only the HDL in the supernatant.

Cholesterol in the HDL fraction can be tested by the usual methods

Reagents

1.Cholesterol reagent 1

2.Cholesterol reagent 2

3.Phosphotungstic acid reagent(PTA)

4.Magnesium chloride reagent

5.Cholesterol standard

PROCEDURE

Pipette in the centrifuge tubes labelled as follows

	Test
1.Serum,ml	0.5
2.PTA reagent,ml	0.05
3.Mgcl2 reagent,ml	0.02

Mix well,centrifuge at 3000rpm for 20mins.

Separate the supernatant by using a Pasteur pipette

Now pipette in the tubes labelled as follows:

	Test	std	blank
1.Cholesterol reagent1,ml	2.5	2.5	2.5
2.Supernatant,ml	0.1	-	-
3.Cholesterol std,ml	-	0.1	-
4.Distilled water,ml	-	-	0.1
Mix well ,keep in water bath at room temperature for 5mins . Afterwards add,			
5.Cholesterol reagent 2,ml	0.5	0.5	0.5

Mix thoroughly,cool the tubes to room temperature by dipping in a water bath at 37^0C.

Read absorbance of test and standard against blank at 580nm.

Calculations

Serum HDL cholesterol,mg/dl=OD OF TEST/OD OF STD*114

3.ENZYMATIC DETERMINATION OF SERUM TRIGLYCERIDES

Principle of test

Lipoprotein lipase

Triglyceride+H20----------→glycerol+fatty acids

Glycerol kinase

Glycerol +ATP----------→Glycerol-3-phosphate+ADP

Glycerol phophate oxidase

Glycerol-3-phosphate+02----------→

dihydroxy acetone phosphate+H2O2

Peroxidase

H202+p-chlorophenol----------→

colored complex ,

it is measured at 520nm(green filter)

Reagents

1.Buffer chromogen (contain different enzymes)

2.P-chlorophenol reagent

Preparation of working reagent

It is prepared fresh by mixing 4ml of reagent 1 and 2ml of reagent 2.

Procedure

Pipette in the tubes labelled as follows

	Test	std	blank
1.Working reagent,ml	1.0	1.0	1.0
2.Serum,ml	0.1	-	-
3.Standard,ml	-	0.1	-
4.Distilled water	-	-	0.1

Mix well ,keep at 37^0C for 15mins. Read absorbance of test and standard against blank.

Calculations

Serum trigycerides,mg/dl=OD OF TEST/OD OF STD*100

What is Quality Control

Quality control in the medical laboratory is a statistical process used to monitor and evaluate the analytical process that produces patient results.

When a diagnostic test is performed in the medical laboratory, the outcome of the test is a result. The result may be a patient result or it may be a quality control (QC) result. The result may be quantitative (a number) or qualitative (positive or negative) or semi-quantitative (limited to a few different values).1 QC results are used to validate whether the instrument is operating within pre-defined specifications, inferring that patient test results are reliable.

Once the test system is validated, patient results can then be used for diagnosis, prognosis, or treatment planning. For example, when a patient's serum is assayed (tested) for potassium, the test result tells us how much potassium (concentration) is present in the blood. This result is then used by the physician to determine whether the patient has a low, normal or high potassium. Let's assume the measured value of potassium in a patient's serum is 2.8 mmol/L (a unit of measure, millimoles per liter).2 This result is abnormally low and indicates an inappropriate loss of potassium. But how does the person performing the test know that this result is truly reliable? It could be possible that the instrument is out of calibration and the patient's true potassium value is 4.2 mmol/L – a normal result. The question of reliability for most testing can be resolved by regular use of quality control materials and statistical process control.

Calculation and Use of QC Statistics

QC statistics for each test performed in the laboratory are calculated from the QC database collected by regular testing of control products. The data collected is specific for each level of control. Consequently, the statistics and ranges calculated from this data are also specific for each level of control and reflect the behavior of the test at specific concentrations. The most fundamental statistics used by the laboratory are the mean [x] and standard deviation [s].

The mean (or average) is the laboratory's best estimate of the analyte's true value for a specific level of control.
To calculate a mean for a specific level of control, first, add all the values collected for that control. Then divide the sum of these values by the total number of values. For instance, to calculate the mean for the normal control (Level I) in Table 1, find the sum of the data {4.0, 4.1, 4.0, 4.2, 4.1, 4.1, 4.2}. The sum [□] is 28.7 mmol/L. The number of values is 7 (n = 7). Therefore, the mean for the normal potassium control in Table 1 from November 1–7 is 4.1 mmol/L (or 28.7 mmol/L divided by 7).

Where:
□□= sum
xn = each value in the data set
n = the number of values in the data set

Calculating a Standard Deviation [s]

Standard deviation is a statistic that quantifies how close numerical values (i.e., QC values) are in relation to each other. The term precision is often used interchangeably with standard deviation. Another term, imprecision, is used to express how far apart numerical values are from each other. Standard deviation is calculated for control products from the same data used to calculate the mean. It provides the laboratory an estimate of test consistency at specific concentrations.

The repeatability of a test may be consistent (low standard deviation, low imprecision) or inconsistent (high standard deviation, high imprecision). Inconsistent repeatability may be due to the chemistry involved or to a malfunction. If it is a malfunction, the laboratory must correct the problem. It is desirable to get repeated measurements of the same specimen as close as possible. Good precision is especially needed for tests that are repeated regularly on the same patient to track treatment or disease progress.

For example, a diabetic patient in a critical care situation may have glucose levels run every 2 to 4 hours. In this case, it is important for the glucose test to be precise because lack of precision can cause loss of test reliability. If there is a lot of variability in the test performance (high imprecision, high standard deviation), the glucose result at different times may not be true.

Standard deviation may also be used to monitor on-going day-to-day performance. For instance,

if during the next week of testing, the standard deviation calculated in the example for the normal potassium control increases from .08 to 0.16 mmol/L, this indicates a serious loss of precision. This instability may be due to a malfunction of the analytical process.

Investigation of the test system is necessary and the following questions should be asked:

- Has the reagent or reagent lot changed recently?
- Has maintenance been performed routinely and on schedule?
- Does the potassium electrode require cleaning or replacement?
- Are the reagent and sample pipettes operating correctly?
- Has the test operator changed recently?

Where:
s = standard deviation
x = mean (average) of the QC values
□(xn - x)2 = the sum of the squares of differences between individual QC values and the mean
n = the number of values in the data set

Although most calculators and spreadsheet programs automatically calculate standard deviation, it is important to understand the underlying mathematics.

To calculate the standard deviation for the normal level of control (Level I) in Table 1,
begin by calculating the mean [x]:
x = 4.0 + 4.1 + 4.0 + 4.2 + 4.1 + 4.1 + 4.2 mmol/L ÷ 7
x = 28.7 mmol/L ÷ 7
x = 4.1 mmol/L
Calculate the standard deviation [s] as follows:
n - 1
s =
(4 - 4.1)2 + (4.1 - 4.1)2 + (4 - 4.1)2 + (4.2 - 4.1)2 + (4.1 - 4.1)2 + (4.2 - 4.1)2
6
s =
(-0.1)2 + (0.0)2 + (-0.1)2 + (+0.1)2 + (0.0)2 + (0.0)2 + (+0.1)2
6
s =
0.01 + 0.0 + 0.01 + 0.01 + 0.0 + 0.0 + 0.01
6
s =
0.04
6
s =
s = 0.082 OR 0.1 (Rounded)
The standard deviation for one week of testing of the normal potassium control level is 0.082 mmol/L.7 Now
that the amount of precision is known, some assumptions can be made about how well this test is performing.

Creating a Levey-Jennings Chart

Standard deviation is commonly used for preparing Levey-Jennings (L-J or LJ) charts. The Levey-Jennings chart is used to graph successive (run-to-run or day-to-day) quality control values. A chart is created for each test and level of control. The first step is to calculate decision limits. These limits are ±1s, ±2s and ±3s from the mean. The mean for the Level I potassium control in Table 1 is 4.1 mmol/L and the standard deviation is 0.1 mmol/L.8 Formula 3 provides examples on how ±1s, ±2s and ±3s quality control limits are calculated.

These ranges are used with the mean to construct the Levey-Jennings chart as shown in Figure 3.

±1s range is 4.0 to 4.2 mmol/L

4.1 – (0.1)(1) = 4.0

4.1 + (0.1)(1) = 4.2

±2s range is 3.9 to 4.3 mmol/L

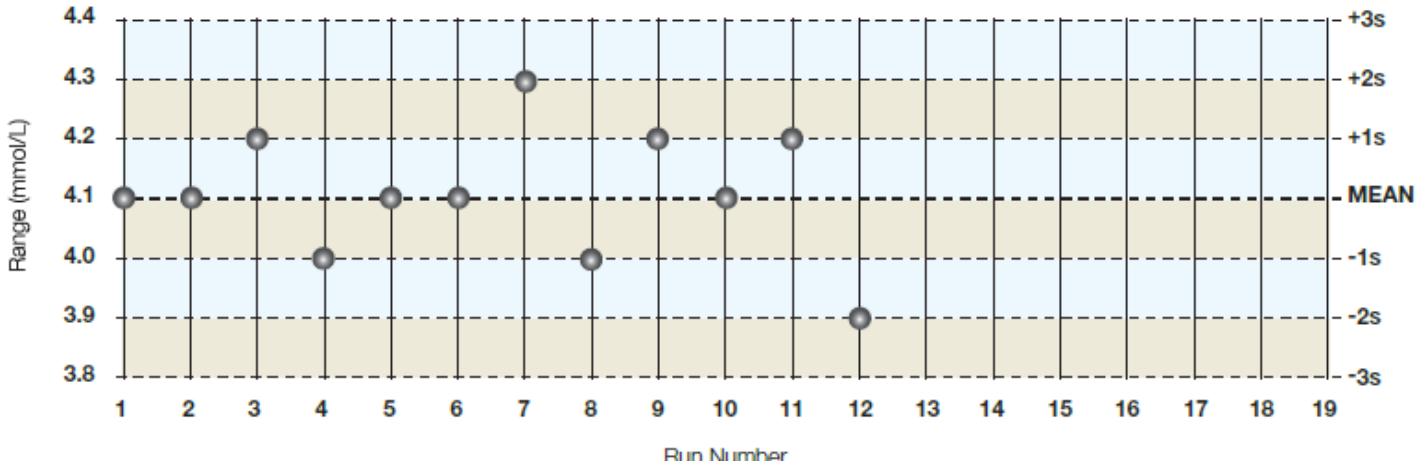
4.1 – (0.1)(2) = 3.9

4.1 + (0.1)(2) = 4.3

±3s range is 3.8 to 4.4 mmol/L

4.1 – (0.1)(3) = 3.8

4.1 + (0.1)(3) = 4.4


4.4
4.3
4.2
4.1
4.0
3.9
3.8
Range (mmol/L)
+3s
+2s
+1s
MEAN
-1s
-2s
-3s
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
Run Number

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