Anesthesia Department
Biochemistry / First Stage
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Analytical chemistry

is the science of the characterization and measurement of chemicals and also involve separating ,identifying and determining the relative amounts of the components in a sample of matter. Chemical analysis is divided into two types Quantitative analysis and Qualitative analysis . techniques commonly used are titration, precipitation, spectroscopy, chromatography, etc.

Analytical chemistry studies and uses instruments and methods used to separate, identify, and quantify matter .

Analytical chemistry is the chemistry discipline that studies the chemical composition of materials and develops the tools used to examine chemical compositions. Analytical chemistry is important in science, engineering, medicine, and industry .

Analytical chemistry : is examining materials by separating them into their components and identifying each one and how much there is of each one .

study of methods for determining the composition of substances

-qualitative (what ?)

-quantitative (how much ?)

Qualitative analysis characterizes the identity of a specimen, while quantitative analysis examines its mass or concentration .

Concentration of solutions

A solution is a mixture of one substance dissolved in another so the properties are the same throughout. A solution is composed of a solute and the solvent .

Types of solutions

1- unsaturated solutions: containing less amount of solute in the solvent .

2- Saturated solution : containing equivalent amount of solute in the solvent .

3- Super Saturated solution : containing more amount of solute in the solvent .

Standard solutions : are solutions with a known concentration of a substance. They're used in chemistry, particularly analytical chemistry, to help identify or determine the concentration of unknown substances .

Concentration : is the ratio of solute in a solution to either solvent or total solution. Concentration usually is expressed in terms of mass per unit volume .

The methods of expressing the concentration of a solution

The word "Concentration" is frequently used as a general term referring to a quantity of substance in a defined volume of solution .

The concentration of solute in a solution may be expressed in many ways, depending upon the convenience to those concerned with its use. Chemists

more frequently prefer to work with the number of moles or equivalents of a particular solute. These quantities are also of importance to pharmacists .

1- Molarity

Number of moles of solute (Substance) dissolved in one liter (1000 ml) of Solution .

M= Wt * 1000/M.wt *V ml

2- Normality

Number of gram equivalent of solute (Substance) dissolved in one liter of solution .

Normality = Wt *1000 /eq. Wt*V ml

3- Molality

A molal solution contains 1 mole of solute per one kilogram of solvent (1 liter of solvent) .

$$\text{Molality} = \frac{\text{Moles of solute}}{\text{Mass of solvent in Kg}}$$

4- Formality

It is the number of formula mass in grams present per liter of solution . In case formula mass is equal to molecular mass .

Formality (F)= Wt *1000 /f. Wt*V ml

5- Mass by Mass Percentage W/W (W/W%)

It is defined as the amount of solute in grams present in 100 grams of the solution .

6- Volume by Volume Percentage V/V (V/V%)

It is defined as the volume of solute in ml present in 100 ml solution

7- Mass by Volume Percentage W/V (W/V%)

It is defined as the mass of solute present in 100 ml of solution .

Percentage Concentration

- $\%\ (w/w) = \frac{mass\ solute(g)}{mass\ solution(g)} \times 100$
- W is weight same as mass

- $\%\ (w/v) = \frac{mass\ solute(g)}{volume\ solution(mL)} \times 100$

- $\%\ (v/v) = \frac{volume\ solute(mL)}{volume\ solution(mL)} \times 100$

8- Parts-per-million (ppm) and parts-per billion (ppb)

For dilute aqueous solutions, we can make the assumption that the density of the solution is 1.00 g/ml .

Expressing Concentration with ppm, ppb & ppt

$$\frac{\textit{Amount of solu}}{\textit{Amount of solut}}$$

Concept of ppm: 1 ppm = 1 part of substance in one million parts of water solution, or 1/1,000,000

Concept of ppb: 1 ppb = 1 part of substance in one billion parts of water solution, or 1/1,000,000,000 .

Concept of ppt: 1 ppt = 1 part in one trillion parts of water solution,

or 1/1,000,000,000,000.

$$\text{ppm} = \frac{\text{Mass of Solute}}{\text{Mass of Solution}} \times 10^6$$

$$\text{ppb} = \frac{\text{Mass of Solute}}{\text{Mass of Solution}} \times 10^9$$

ppm and ppb express the mass of a specific solute relative to the mass of the solvent .

parts per million also can be expressed as milligrams per liter (mg/l). This measurement is the mass of a chemical or contaminate per unit volume of water .

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DILUTION EQUATION

M 1 V 1 = M 2 V 2

- M 1 and V 1 are the molarity and volume of the initial concentrated solution .

- M 2 and V 2 are the molarity and volume of the final diluted solution .

Chemical equilibrium: is the state reach by a reaction mixture when the rates of forward and reverse reactions become equal .

Equilibrium constant and rate constants

equilibrium constant : also known as K eq .

$$aA + bB \rightleftharpoons cC + dD$$

where A and B are reactants, C and D are products

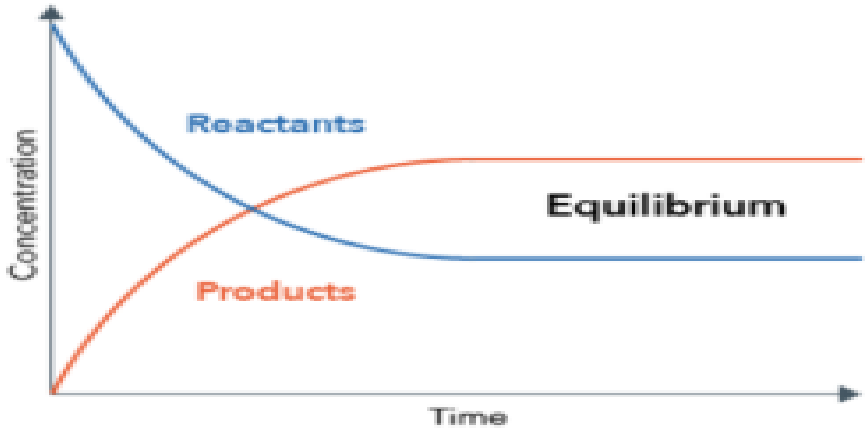
Equilibrium constant is defined by the concentration of each reactant and product in a chemical reaction at equilibrium is related; the concentrations cannot be random values, but they depend on each other. Calculating the equilibrium constant requires knowledge of the concentrations of the products and the reactants in the reaction when it's at equilibrium. the equilibrium constant doesn't have a unit .

For the reaction:

$$aA + bB \rightleftharpoons cC + dD$$

the equilibrium constant expression is

$$\frac{[C]_{eqm}^{c}[D]_{eqm}^{d}}{[A]_{eqm}^{a}[B]_{eqm}^{b}} = K_c$$



A weak acid is an acid that partially dissociates into its ions in an aqueous solution or water. **In contrast, a strong acid** fully dissociates into its ions in water .

A weak base is a base that partially dissociates into its ions in an aqueous solution or water . **In contrast, a strong base** fully dissociates into its ions in water .

pH + pOH = 14

pH = - log [H^+]

pOH = - log [OH^-]

Buffer System: is a solution of a weak acid and its salt to form acidic buffer solution or weak base and its salt to form basic buffer solution. A buffer solution has the ability to resist changes in pH upon the addition of small amounts of either acid or base. Biological systems use buffers to maintain pH.

Buffer solutions are used in a wide variety of chemical applications.

One example of a buffer solution found in nature is blood. The normal pH of human blood is 7.4. Some people suffer from alkalosis when experiencing severe anxiety. Alkalosis is a condition in which the pH of the blood is too high. The opposite condition - a blood pH lower than 7.4 is called acidosis .

Acidic Buffer Solution

-An acidic buffer solution is simply one which has a pH less than 7 .

- Can be prepared from a weak acid and its conjugate base .

- Example; an aqueous mixture of ethanoic acid and sodium ethanoate .

$CH_3COONa\ (aq) \longrightarrow CH_3COO^-\ (aq) + Na^+(aq)$

Ethanoic acid dissociates partially in water :

$CH_3COOH\ (aq) \longrightarrow CH_3COO^-\ (aq) + H^+(aq\ )$

When a little acid is added, the above equilibrium will shift to the left to mop up the added of H^+ ion (Added H^+ is mopped up by the conjugate base) .

$$CH_3COO^- + H^+ \longrightarrow CH_3COOH$$

When a little base is added, it is removed by the following reaction (Added OH^+ is mopped up by the acid).

$$CH_3COOH + OH^- \longrightarrow CH_3COO^- + H_2O$$

Basic (alkaline)Buffer Solution

• A basic buffer solution has a pH greater than 7 .

• Can be prepared from a weak base and its conjugate acid .

• Example ; an aqueous mixture of ammonia and ammonium chloride

$$NH_4Cl\ (aq) \longrightarrow NH_4^+(aq) + Cl^-\ (aq)$$

$$NH_3\ (aq) + H_2O(l) \longrightarrow NH_4^+(aq) + OH^-\ (aq)$$

When a little base is added, the above equilibrium will shift to the left to mop up the added of OH^- ion (Added OH^- is mopped up by the conjugate acid) .

$$NH_4^+(aq) + OH^- \longrightarrow NH_3 + H_2O$$

When a little acid is added, it is removed by the following reaction (Added H^+ is mopped up by the base) .

$$NH_3\ (aq) + H^+ \longrightarrow NH_4^+$$

Henderson Hasselbalch Equation

$$pH = pK_a + \log \frac{[conjugate\ base]}{[weak\ acid]} \text{ (for weak acid)}$$

$$pOH = pK_b + \log \frac{[conjugate\ acid]}{[weak\ base]} \text{ (for weak base)}$$

Maintaining the pH of blood

1- Are important in the proper functioning of cells and blood.

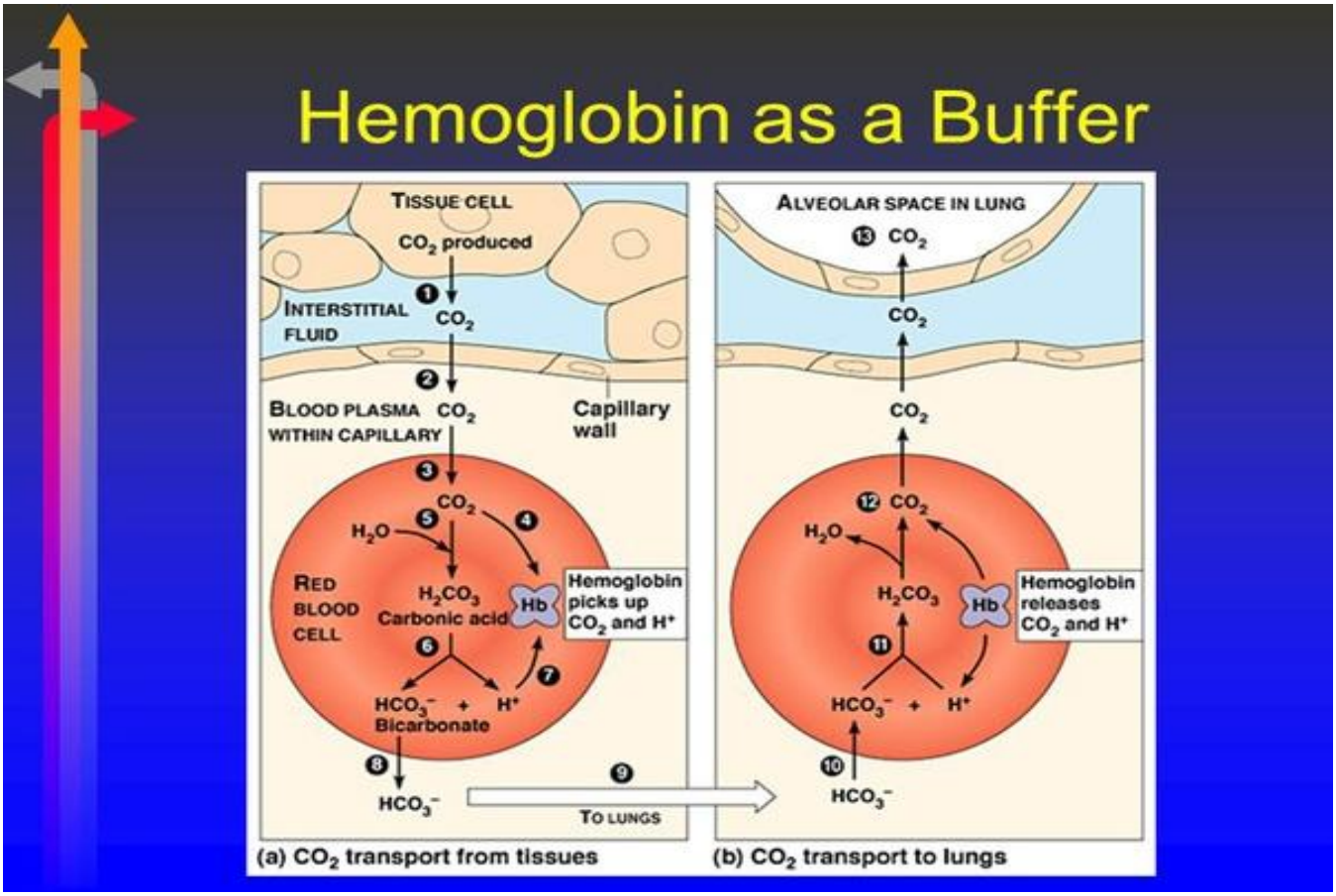
2- Help maintain the pH of blood close to 7.4.

3-A change in the pH of the blood affects the uptake of oxygen and cellular processes.

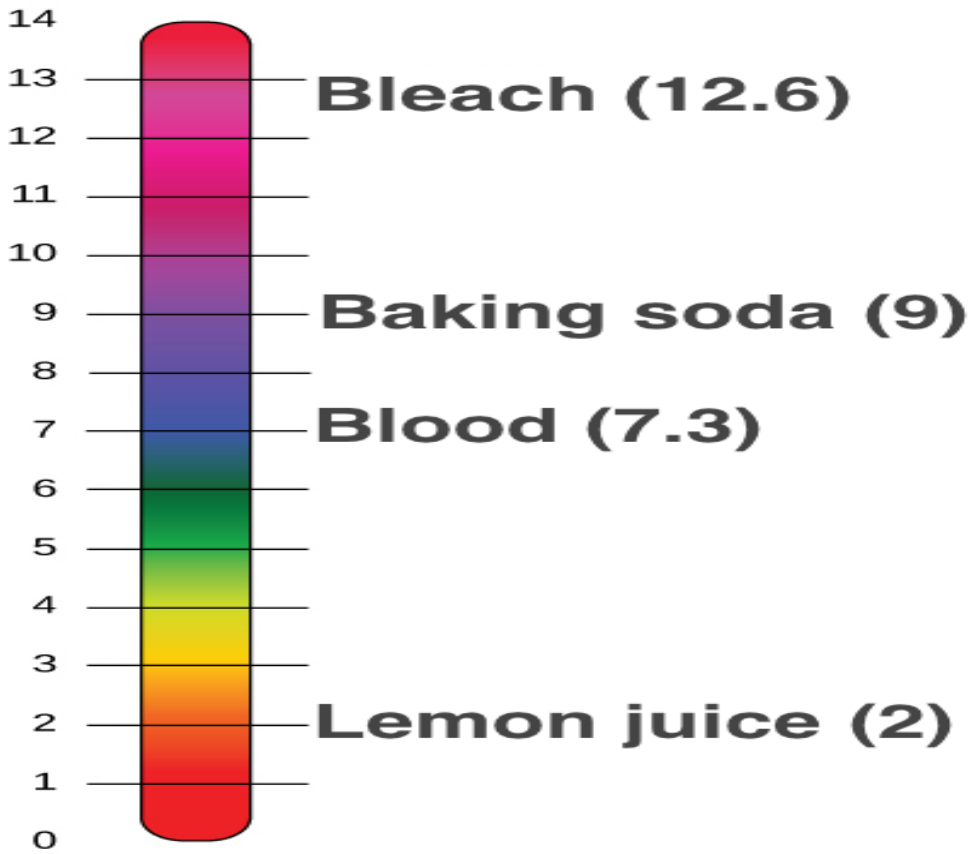
Human blood must be maintained at a pH of 7.40 ± 0.05 for the rest of the body biochemistry to function. This is achieved with a buffer made from CO2 :

$$CO_2\ (aq) + H_2O\ (l) \Leftrightarrow H_2CO_3\ (aq)$$

Maintaining a constant blood pH is critical to a person's well-being. The buffer that maintains the pH of human blood involves carbonic acid (H_2CO_3), bicarbonate ion (HCO_3^-), and carbon dioxide (CO_2). When bicarbonate ions combine with free hydrogen ions and become carbonic acid, hydrogen ions are removed, moderating pH changes. Similarly, excess carbonic acid can be converted into carbon dioxide gas and exhaled through the lungs; this prevents too many free hydrogen ions from building up in the blood and dangerously reducing its pH; likewise, if too much OH^- is introduced into the system, carbonic acid will combine with it to create bicarbonate, lowering the pH .



Antacids, which combat excess stomach acid, are another example of buffers. Many over-the-counter medications work similarly to blood buffers, often with at least one ion (usually carbonate) capable of absorbing hydrogen and moderating pH, bringing relief to those that suffer “heartburn” from stomach acid after eating .



Volumetric analysis

Volumetric analysis is a general term for a method in quantitative chemical analysis in which the amount of a substance is determined by the measurement of the volume that the substance occupies. It is commonly used to determine the unknown concentration of a known reactant. Volumetric analysis is often referred to as titration .

Arrhenius Acid: A substance that yields hydrogen ions (H^+) when dissolved in water .

Arrhenius Base: A substance that yields hydroxide ions (OH^-) when dissolved in water .

Bronsted acid : A substance capable of donationg a proton .

Bronsted base: A substance capable of accepting a proton .

What is the meaning of Titration ?

Titration is a common laboratory method of quantitative chemical analysis that is used to determine the unknown concentration of a known reactant. Because volume measurements play a key role in titration, it is also known as volumetric analysis .

A reagent, called the titrant or titrator, of a known concentration (a standard solution) and volume is used to react with a solution of the analyte or titrand, whose concentration is not known .

Standard solution: is one, which contains a known weight of the reagent in a definite volume of the solution.

A primary standard solution is a highly purified compound that serve as a reference material in all volumetric titrimetric methods .

Important requirements for a primary standard are :

1- High purify .

2- Stability toward air.

3- Absence of hydrate water .

4- Ready availability at modest cost .

5-Reasonable solubility in the titration medium .

6- Reasonable large molar mass so that the relative error associated with weighing the standard is minimized .

It is possible to determine the exact amount that has been consumed when the endpoint is reached .

The endpoint is the point at which the titration is complete, as determined by an indicator.

Types of titrations

1- Acid-base titration

2- Precipitation titration

3-Redox titration

4- Complexometric titration

Oxidation-reduction (redox) reaction

A redox reaction occurs when the oxidation number of atoms involved in the reaction are changed. Oxidation is the process by which an atom's oxidation number is increased, and reduction is the process by which an atom's oxidation number is decreased. If the oxidation states of any elements in a reaction change, the reaction is an oxidation-reduction reaction. An atom that undergoes oxidation is called the reducing agent, and the atom that undergoes reduction is called the oxidizing agent. An example of a redox reaction is the reaction between hydrogen gas and fluorine gas:

$$H_2(g)+F_2(g)\rightarrow 2HF(g)$$

In this reaction, hydrogen is oxidized from an oxidation state of 0 to +1, and is thus the reducing agent. Fluorine is reduced from 0 to -1, and is thus the oxidizing agent.

Acid-base (Neutralization) Reaction

A neutralization reaction occurs when an acid and base are mixed together. An acid is a substance that produces H+ ions in solution, whereas a base is a substance that that produces OH- ions in solution. A typical acid-base reaction will produce an ionic compound called a salt and water. A typical acid-base reaction is the reaction between hydrochloric acid and sodium hydroxide. This reaction is represented by the equation:

$$HCl(aq)+NaOH(aq)\rightarrow NaCl(aq)+H_2O(l)$$

In this reaction, HCl is the acid, NaOH is the base, and NaCl is the salt.

Clinical chemistry

refers to the biochemical analysis of body fluids. It uses chemical reactions to determine the levels of various chemical compounds in bodily fluids. Several simple chemical tests are used to detect and quantify different compounds in blood and urine, the most commonly tested specimens in clinical chemistry.

Techniques such as spectrophotometry, immunoassays, and electrophoresis are also used in clinical chemistry to measure the concentration of substances such as glucose, lipids, enzymes, electrolytes, hormones, proteins, and other metabolic products present in human blood and urine .

Specimens tested in clinical analysis

Whole blood

contains the liquid fraction of blood (i.e., plasma) as well as the cellular elements that lead to clotting under certain circumstances. These include red blood cells (RBCs), white blood cells, and other components.

Serum

Serum is the most common specimen tested - it is obtained by centrifugation of coagulated blood. Serum contains no blood cells or clotting factors but has electrolytes, hormones, antigens, antibodies, and other substances such as drugs, microbes, and proteins not used in coagulation .

Plasma

Plasma is obtained by centrifugation of uncoagualted blood. It contains blood cells, clotting factors, glucose, electrolytes (such as sodium, magnesium, calcium, chloride), hormones, and proteins (such as albumins, fibrinogen, and globulins).

Urine

Clinical tests usually require a 24-hour urine collection. The collection container usually contains a preservative.

Cerebrospinal spinal fluid (CSF)

CSF is a clear fluid present in the brain and spine which is largely similar to blood plasma though it differs by containing nearly no protein. It is usually analyzed in clinical chemistry to identify or rule out meningitis.

Blood : is a liquid tissue. Suspended in the watery plasma are seven types of cells and cell fragments. -Red blood cells(RBCs) -White blood cells(WBCs) –Platelets .

Blood serum :

Serum is the same as plasma except that clotting factors (such as fibrin) have been removed .

- For many biochemical laboratory tests, plasma and blood serum can be used interchangeably. Serum resembles plasma in composition but lacks the coagulation factors .

- It is obtained by letting a blood specimen clot prior to centrifugation .

Blood plasma :

- Plasma is the liquid component of blood .

- It is mainly composed of water, blood proteins and inorganic electrolytes.

- Roughly 92% water, mixed with organic and inorganic-substances .

- The most abundant plasma solute is the plasma protein, of which there are three groups: albumin, globulins, and fibrinogen .

Blood clot :

- When a blood sample is left standing without anticoagulant, it forms a coagulum or blood clot .

- One of the normal components of plasma is a soluble plasma protein called fibrinogen .

- On standing, this protein will be converted to insoluble substance called fibrin , this occurrence is referred to as blood coagulation or clotting

- The clot contains coagulation proteins, platelets, and entrapped red and white blood cells .

Anticoagulants

1- Heparin:

α-mucoitin polysulphuric acid inhibits the formation of thrombin from prothrombin. It is usually available as the Na, K, NH4 and Li salts.

2- EDTA (Ethylene diamine tetra acetate):

It chelates calcium ions which are essential for clotting mechanism. Its dipotassium and dilithium salts are most often used.

3- Oxalate and citrate:

Oxalate acts by precipitating the calcium. Potassium oxalate is the most soluble and so it is most commonly used. Sodium citrate does not precipitate calcium but converts it into a non-ionized form.

4- Sodium flouride:

It is also considered as an anticoagulant, but since larger amounts are needed, it is rather used as a preservative for glucose determination by inhibiting red cell metabolism, glycolysis and bacterial action .

Urine :

A liquid containing multiple waste products of metabolism, especially urea and other nitrogenous compounds, that are filtered from the blood by the kidneys.

Urinalysis :

The routine urine examination. Most useful tool for the clinicians as an indicator health or disease. Particularly, used in renal metabolic disorders. Often done for patients admitted to the hospital.

The purposes of performing a routine urine analysis are :

1- To aid in the diagnosis of disease .

2- To screen for asymptomatic, congenital, or hereditary diseases .

3- To monitor disease progression .

4- To monitor therapy effectiveness or complications .

PRINCIPLE: Through chemical and microscopic analysis of the urine specimen, information about the body's metabolic functions may be obtained. This aids in the evaluation of renal, urinary, and metabolic disorders.

The routine urine analysis is divided into four main groups:

A – Physic -Chemical properties.

1- Color.

Normal urine color ranges from pale yellow to deep. it is the result of a pigment called urochrome.

Most changes in urine color are harmless and temporary and may be due to :– Certain foods – Dyes in foods/drinks – Supplements

– vitamins –Prescription drugs •Unusual urine color can indicate an infection or serious illness.

2- Appearance Volume.

3- Specific gravity.

4- Odor.

- Chemical examination.

1- pH. 2-Sugar. 3-Protein.

4-Ketone bodies. 5- Bilirubin. 6- Urobilinogen.

7- Nitrite. 8- Ascorbic acid.

** Presence of glucose (glycosuria) indicates that the blood glucose level has exceeded the renal threshold. •Useful to screen for diabetes.

** Bilirubin is a byproduct of the breakdown of hemoglobin. •Normally contains no bilirubin. Presence may be an indication of liver disease, bile duct obstruction or hepatitis. Since the bilirubin in samples is sensitive to light, exposure of the urine samples to light for a long period of time may result in a false negative test result.

** Ketones are excreted when the body metabolizes fats in completely (ketonuria) .

** Presence of protein (proteinuria) is an important indicator of renal disease.

** Urobilinogen is a degradation product of bilirubin formed by intestinal bacteria . It may be increased in hepatic disease or hemolytic disease.

C- Microscopic examination.

1- WBCs 2-Epithelial cells 3-RBCs 4-Casts Crystals

5-Bacteria Yeasts 6-Nonbacterial organisms.

D- Bacterial screening.

Colony Count .

Urine collection :

Urine must be collected in a sterile bottle. The routine urine examination must be conducted within 30 min. In case of delay, urine must be refrigerated. Delay in sample examination will result in:

1-Decreased pH by the utilization of glucose by bacteria.

2- Increased pH by the conversion of urea to ammonia by bacteria. 3- In increased pH, the tendency of phosphates to precipitate will increase.

4- Oxidation of urobilinogen to urobilin. It may give false negative result for jaundice.

clinical significance

A urinalysis is used to detect and manage a wide range of disorders, such as urinary tract infections, kidney disease and diabetes. A urinalysis involves checking the appearance, concentration and content of urine. Abnormal urinalysis results may point to a disease or illness .

Electrolytes

are minerals that carry an electric charge when they are dissolved in a liquid such as blood. The blood electrolytes—sodium, potassium, chloride, and bicarbonate , help regulate nerve and muscle function and maintain acid-base balance and water balance.

Minerals are present in body tissues and body fluids .

MEDICAL AND BIOLOGICAL IMPORTANCE

1- Minerals are structural components of body soft tissues like liver, muscle etc. For example phospholipids are components of membrane structure .

2- Minerals participate in acid-base balance. Several minerals are components of blood buffers .

3- Minerals are integral parts of several physiologically important compounds like hemoglobin , hormones ,vitamins, enzymes, bile salts, nucleic acids .

4- Minerals participates in transport of gases in the body .

5- Minerals are required for several enzymatic reactions .

6- Deficient or Excess intake of minerals leads to diseases.

Sodium :

Sodium is the major cation of extracellular fluid(+ charge) and with its associated anions (-ve charge) accounts for the osmotic activity of the ECF. It plays a central role in the maintenance of the normal distribution of water and the osmotic pressure in the various fluid compartments. Hyponatremia (low serum sodium level) is associated with a variety of conditions, including metabolic acidosis, Addison's disease, diarrhea, and renal tubular disease .

Hypernatremia (increased serum sodium level) is associated with Cushing's syndrome, severe dehydration due to primary water loss, certain types of brain injury, diabetic coma after therapy with insulin, and excess treatment with sodium salts.

Control of Na balance :

1- The major factors controlling Na balance are renal blood flow .

2- aldosterone .

Normal serum sodium level is 140meq/L .

Functions

1. It is the major cation of extracellular fluid.
2. It is involved in the maintenance of plasma volume and acid-base balance.
3. It is essential for nerve and muscle function.
4. It is required for the absorption of glucose and amino acids in the intestine and kidney.
5. It is required for the formation of bile salts.
6. It is required for the activity of Na^+ K^+-ATPase.

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Potassium

Potassium is the principle cation of the intracellular fluid. The proper level of potassium is essential for normal cell function. Among the many functions of potassium in the body are regulation of the heartbeat and the function of the muscles. A seriously abnormal increase in potassium (hyperkalemia) or decrease in potassium (hypokalemia) can profoundly affect the nervous system and increases the chance of irregular heartbeats (arrhythmias), which, when extreme, can be fatal. Its intracellular function parallels that of its extracellular function, namely influencing acid-base balance and osmotic pressure, including water retention . It also helps move nutrients into cells and waste products out of cells. A diet rich in potassium helps to offset some of sodium's harmful effects on blood pressure .

Elevated potassium levels, hyperkalemia, are often associated with renal failure, dehydration shock or adrenal insufficiency. Decreased potassium levels, hypokalemia, are associated with malnutrition, negative nitrogen balance, gastrointestinal fluid losses and hyperactivity of the adrenal cortex .

- Increased potassium is known as **Hyperkalemia** . Potassium is normally excreted by the kidneys, so disorders that decrease the function of the kidneys can result in hyperkalemia. Certain medications may also predispose an individual to Hyperkalemia .

- **Hypokalemia** , or decreased potassium, can arise due to kidney diseases; excessive losses due to heavy sweating, vomiting, diarrhea, eating disorders, certain medications, or other causes .

The normal blood potassium level is 3.5 - 5.0 millimoles/liter (mmol/L) .

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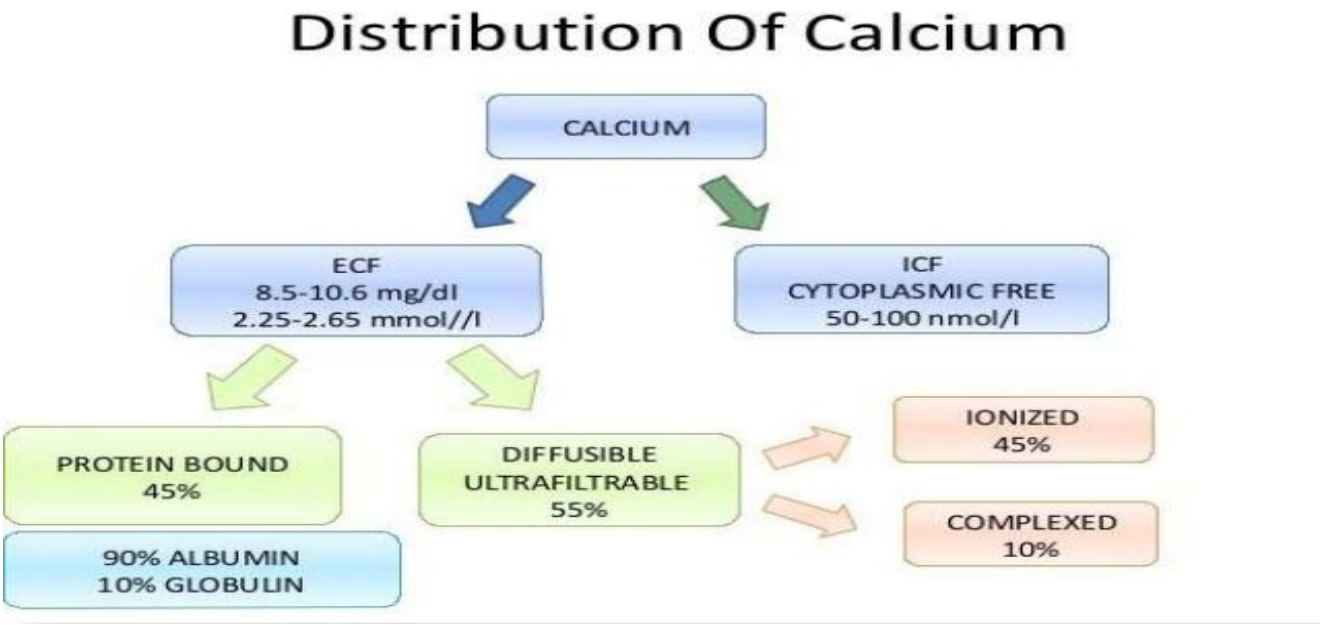
Calcium

is an essential mineral. In the plasma, it is present in three forms: free ionized (Ca^{++}) , protein-bound (principally to albumin) , complexed (primarily with phosphate). Only the free ionized fraction (approximately 50% of the total) is physiologically active , most of the physiological functions of calcium depend upon the ionized fraction .



Biochemical functions

- It is required for activation of several enzymes such as succinate dehydrogenase, ATPase and certain proteolytic enzymes(Calmodulin is a calcium binding regulatory protein, Calmodulin can bind with 4 calcium ions • Calcium binding leads to activation of enzymes) .

- Calcium is required for the Muscle contraction , Nerve conduction, Hormone release, Blood coagulation , In addition, specific Calcium concentration is required

for various other metabolic processes , maintain and development of bones and teeth - Blood coagulation: • Calcium is known as factor IV in blood coagulation process .

• Regulation of plasma calcium level by Calcitriol

Role of calcitriol on bone

Calcitriol stimulates calcium uptake for deposition as calcium phosphate , Calcitriol is essential for bone formation .

Role of calcitriol on kidneys

Calcitriol minimizing the excretion of Ca^{2+} & phosphate by decreasing their excretion & enhancing reabsorption .

Role of calcitriol on intestine :

Calcitriol increases the intestinal absorption of Ca^{2+} & phosphate .

Regulation by parathyroid hormone (PTH)

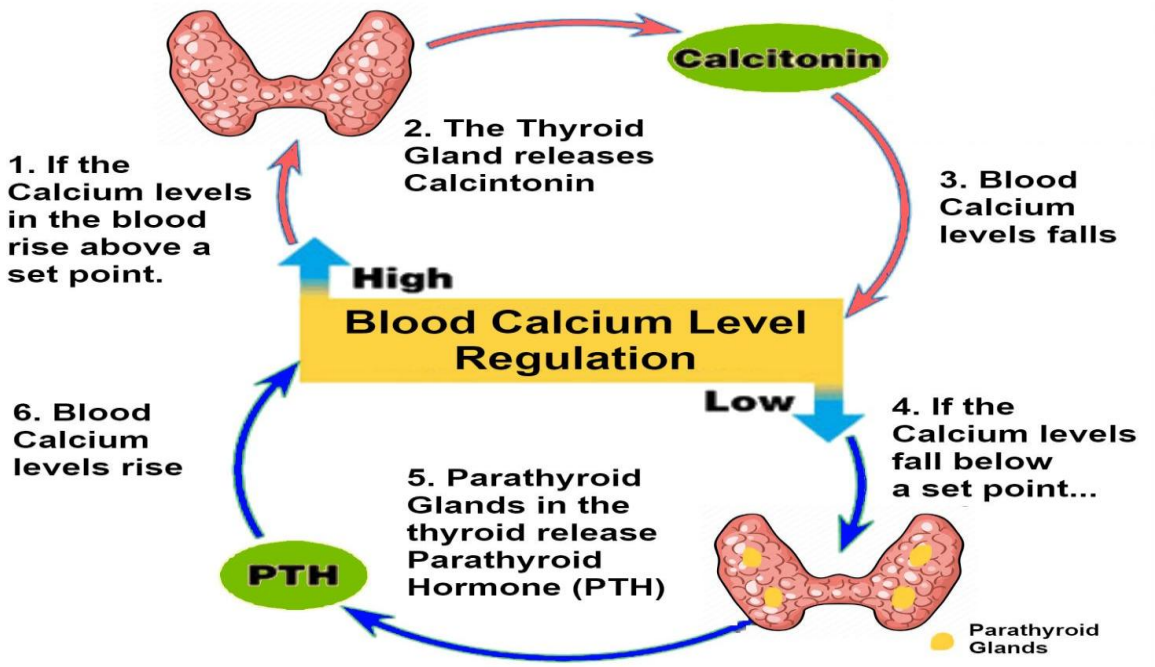
PTH causes decalcification or demineralization of bone, Demineralization ultimately leads to an increase in the blood Ca^{2+} level .

Action on the kidney

PTH increases the Ca^{2+} reabsorption by kidney tubules ,It most rapid action of PTH to elevate blood Ca^{2+} levels .

Action on the intestine

It increases the intestinal absorption of Ca^{2+} by promoting the synthesis of calcitriol .



What causes hypocalcemia and hypercalcemia ?

Both hypocalcemia and hypercalcemia are most commonly caused by conditions affecting parathyroid gland. thyroid gland produces a hormone called parathyroid hormone (PTH) that is responsible for regulating calcium levels in body. When thyroid produces too much (hypercalcemia) or too little (hypocalcemia) of PTH, the result is one of these two conditions .

Other conditions that can lead to calcium level abnormalities include :

- Severe dehydration .
- Certain medications .
- Some supplements .
- Certain types of cancer .
- Other medical conditions, such as kidney disease .

Chloride

Chloride is the major anion (negatively charged ion) found in the fluid outside of cells and in the blood. An anion is the negatively charged part of certain substances such as table salt (sodium chloride or NaCl) when dissolved in liquid. Chloride plays a role in helping the body maintain a normal balance of fluids.

Functions of Chloride :

1.maintenance osmotic pressure ,acid base balance .

2. formation of HCl (provides optimum pH for action of pepsin ,activation of pepsinogen, kills bacteria).

3. Maintains Homeostasis of Na^+ ,K^+, Cl^-.

4. Activation of Salivary Amylase by Chloride .

The balance of chloride ion (Cl^-) is closely regulated by the body. Significant increases or decreases in chloride can have deleterious or even fatal consequences:

- **Increased chloride (hyperchloremia):** Elevations in chloride may be seen in diarrhea, certain kidney diseases, and sometimes in over activity of the parathyroid glands.
- **Decreased chloride (hypochloremia):** Chloride is normally lost in the urine, sweat, and stomach secretions. Excessive loss can occur from heavy sweating, vomiting, and adrenal gland and kidney disease.

The normal serum range for chloride is 98 - 108 mmol/L .

Carbohydrates

Carbohydrates in general are polyhydroxy aldehydes or ketons.

The general molecular formula of carbohydrate is $C_nH_{2n}O_n$ or $(CH_2O)_n$, where n > 3.

Chemically, they contain the elements Carbon, hydrogen and oxygen. Thus they are Carbon compounds that contain large quantities of Hydroxyl groups.

Carbohydrates have functions within the body:

1- Carbohydrates provides energy and regulation of blood glucose.

2- Carbohydrates form a part of genetic material like DNA and RNA in the form of deoxyribose and ribose sugars.

3-They help make up the body mass by being included in all the parts of the cell and tissues.

4- Carbohydrates is basically the main fiber of the diet or provide the bulk fiber for better digestion.

Classification of Carbohydrates:

• Monosaccharides contain a single polyhydroxy aldehyde or ketone unit (e.g., glucose, fructose).

• Disaccharides consist of two monosaccharide units linked together by a covalent bond (e.g. ,Maltose ,Lactose , sucrose).

• Oligosaccharides contain from 3 to 10 monosaccharide units (e.g., raffinose).

• Polysaccharides contain very long chains of hundreds or thousands of monosaccharide units, which may be either in straight or branched chains such as :

1- Starch : consists of two polymeric units made of glucose called Amylose and Amylopectin but they differ in molecular architecture.

2- Cellulose: Cellulose is the most abundant structural polysaccharide in plants. It is fibrous ,tough, water insoluble. Cellulose is a linear un branched homo polysaccharide of 10,000 or more D- glucose units connected by β-(1, 4) glycosidic bonds. Humans cannot use cellulose because they lack of enzyme (cellulase) to hydrolyze the β-(1-4) linkages.

3- Glycogen:

Glycogen is the main storage polysaccharide of animal cells (Animal starch) , It is present in liver and in skeletal muscle, liver cell can store glycogen within a small space. Multiple terminals of branch points release many glucose units in short time .

A **glycosidic bond** or **glycosidic linkage** is a type of covalent bond that joins a carbohydrate (sugar) molecule to another group, which may or may not be another carbohydrate.

Metabolism

is a term that is used to describe all chemical reactions involved in maintaining the living state of the cells and the organism. **Metabolism** can be conveniently divided into two categories:

Anabolic:

is the sum of metabolic processes leading to the synthesis of complex molecules from simpler ones (consumption of ATP).

Catabolic:

is the sum of degradation processes leading to the cleavage of large molecules into smaller ones (release of energy).

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Carbohydrate Metabolism

Usually carbohydrate metabolism consist of several pathways. Some of them are catabolic, few are anabolic . Further, most of the metabolic pathways of carbohydrate metabolism either start with glucose or end with glucose. Hence, carbohydrate metabolism means it is the metabolism of glucose.

Medical and Biological Importance of Carbohydrate Metabolism :

1- Glucose is the major fuel for all types of cells in the body.

2- Glucose is used for the formation of glycogen, pentose and polysaccharides

3- brain is totally dependent on glucose for energy.

4- Most common metabolic disease diabetes mellitus is due to defective glucose metabolism .

GLYCOLYSIS

Glycolysis pathway describes the oxidation of glucose to pyruvate with the generation of ATP and NADH in cells and is present in all cell types. The central role of glycolysis in fuel metabolism is related to its ability to generate ATP with and without oxygen , Glycolysis is anaerobic; it does not require oxygen , In the presence of O_2 , pyruvate is further oxidized to CO_2 , In the absence of O_2 , pyruvate can be fermented to lactate or ethanol.

Site of Glycolysis : Enzymes of glycolysis are present in the cytosol of most of the cells present in the body.

Net Reaction :

Glucose + 2ADP +2NAD$^+$ +2Pi ⟶ 2Pyruvate + 2ATP +2NADH+2H$^+$+2H_2O

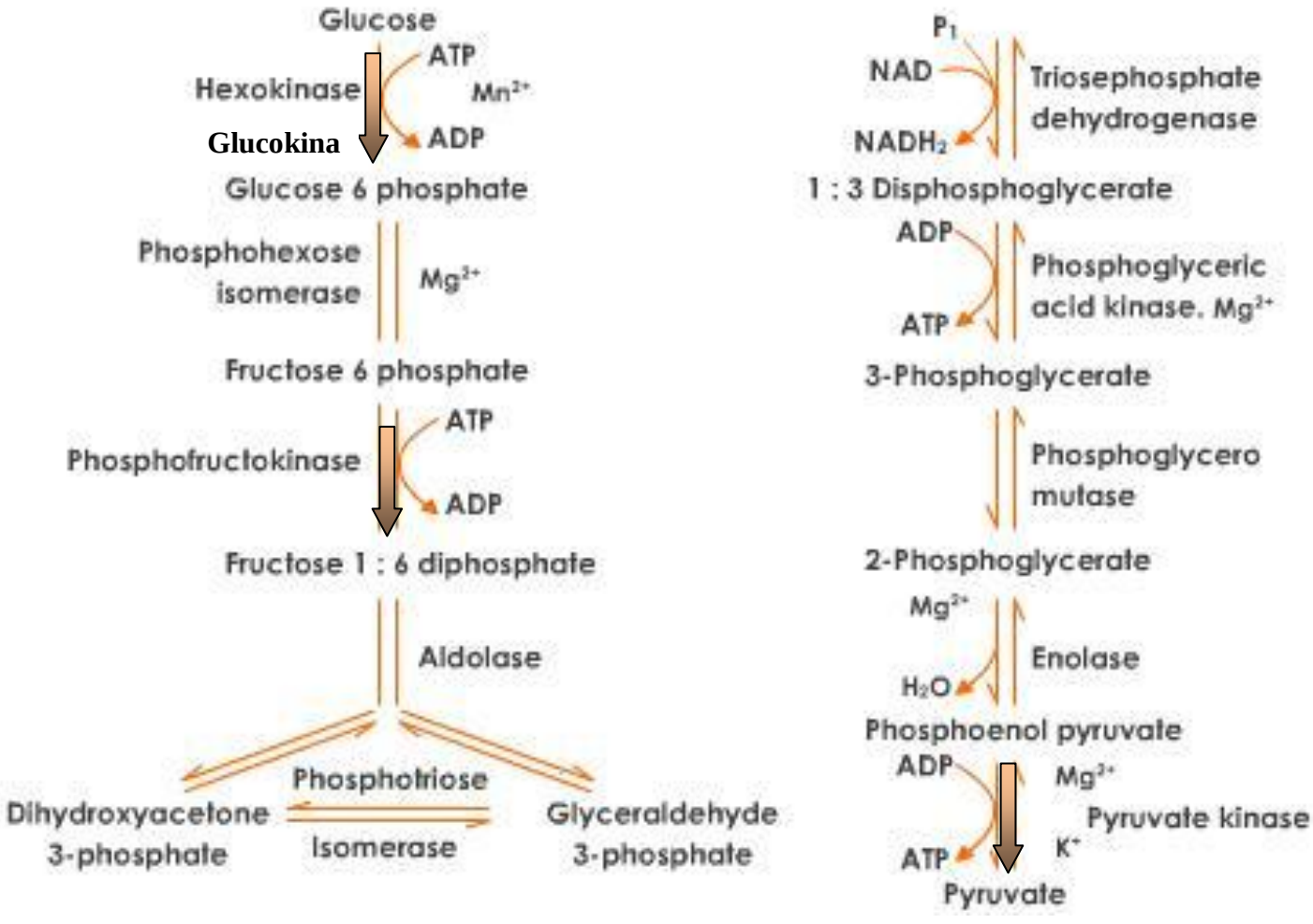
Significance of the Glycolysis Pathway :

1-Glycolysis is the only pathway that is taking place in all the cells of the body.

2-Glycolysis is the only source of energy in erythrocytes.

3-In exercises, when muscle tissue lacks enough oxygen, anaerobic glycolysis forms the major source of energy for muscles.

Steps of glycolysis



Regulation of glycolysis :

After carbohydrate meal :

- Blood glucose level increase , stimulates insulin secretion , increases synthesis three irreversible enzymes (regulatory enzyme):

- glucokinase or hexokinase : inhibited by glucose -6-phosphate .
- Phosphofructokinase: inhibited by ATP and citrate , fatty acid . activated by ADP and AMP .
- Pyruvate kinase: inhibited by ATP , AMP and citrate .activated phosphenol pyruvate and fructose 1,6 diphosphate .

During Fasting :

-Blood glucose level decrease , inhibits insulin secretion , stimulates glucagon secretion , increases the synthesis of the four enzymes that reverse glycolysis (stimulate gluconeogenesis) .

Diabetes mellitus

commonly known as diabetes, is a metabolic disease that causes high blood sugar. Untreated high blood sugar from diabetes can damage nerves, eyes, kidneys, and other organs .

Cause :

1- Lack of insulin action .

2- Missing or impaired insulin production .

3- Impaired insulin effect .

Symptoms of diabetes

1- increased amount of urine .

2-intense thirst .

3- loss of body weight .

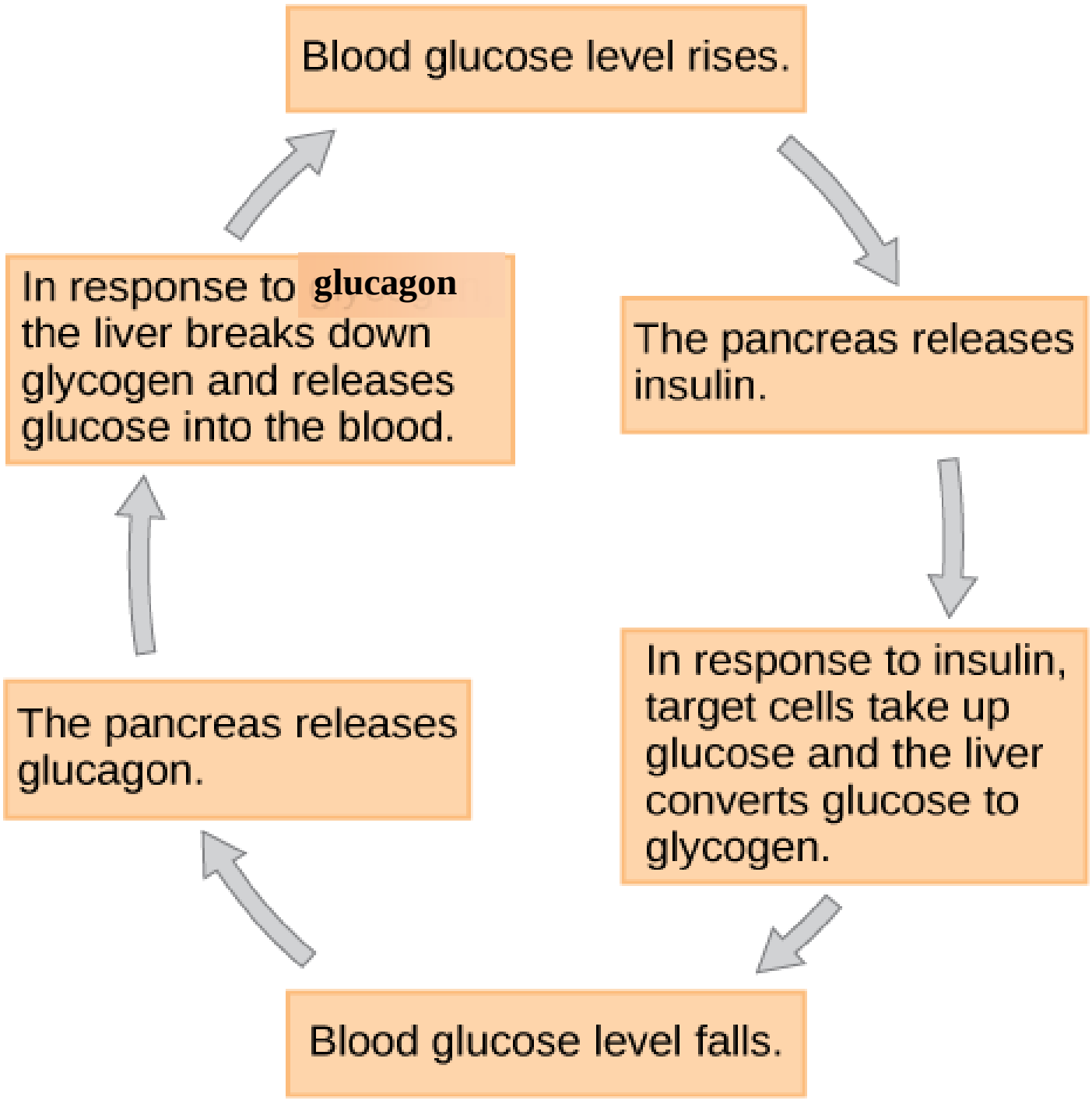
4- fatigue, skin and mucosal infections, blurred vision .

There are two main types of diabetes

Type 1 diabetes: occurs because the insulin-producing cells of the pancreas (beta cells) are damaged. In type 1 diabetes, the pancreas makes little or no insulin, so sugar cannot get into the body's cells for use as energy. People with type 1 diabetes must use insulin injections to control their blood glucose. Type 1 is the most common form of diabetes in people who are under age 30, but it can occur at any age.

Type 2 diabetes: (adult onset diabetes), the pancreas makes insulin, but it either doesn't produce enough, or the insulin does not work properly . This type occurs most often in people who are over 40 years old but can occur even in childhood if there are risk factors present. Type 2 diabetes may sometimes be controlled with a combination of diet, weight management and exercise. However, treatment also may include oral glucose-lowering medications (taken by mouth) or insulin injections .

Regulation of blood glucose:



Proteins

Amino Acid

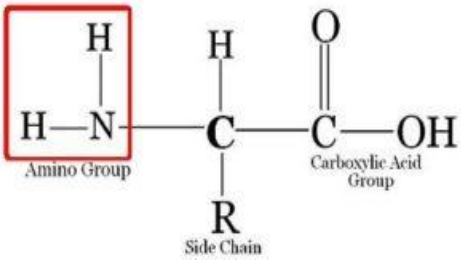
Building blocks of peptide and proteins, amino acids are carboxylic acids containing an amino group . 20 common amino acids used by all organisms .

-amino acid has a primary amino group, a carboxyl group, a hydrogen atom and a side-chain (R group) attached to a central α-carbon atom (Cα).

-Only differ at R group (side chain).

- Most of the amino acids found in human body are L-isomers .

Structure of Amino Acids



Peptides

-Peptides consist of 2 or more amino acid residues linked by peptide bond.

-A peptide bond is formed when carboxyl group of an amino acid react with α-amino group of another amino acid.

-A peptide or protein is named starting with N-terminal amino acid and usually the N-terminal is located on the left hand side.

Proteins

are biochemical molecules consisting of polypeptides joined by peptide bonds between the amino and carboxyl groups of amino acid residues.

- Optical Activity

All protein solutions rotate the plane of polarized light to the left, i.e., these are levoratotory.

Classification of Protein

1 - Classification by Structural Shape

Proteins can be classified on the basis of their structural shapes:

1-Fibrous proteins

-insoluble in water .

- major components of connective tissue, elastic tissue, hair, and skin.

- e.g., collagen, elastin, and keratin

2-Globular proteins

- dissolve in water or form stable suspensions .

- not found in structural tissue but are transport proteins .

- e.g., hemoglobin and transferrin .

2- Classification by Composition Proteins

1- Simple proteins : contain only amino acid residues.

2- Conjugated proteins : also contain other organic or inorganic components, called prosthetic groups. e.g. – nucleoproteins – lipoproteins - glycoproteins — phosphoproteins — hemoproteins – metalloproteins .

Protein Function

1- Catalytic function: are catalyzed by proteins functioning as enzymes.

2- Structural function: such as collagen (mechanical strength of skin and bone) and keratin (hair, skin, fingernails) .

3- Storage function: e.g., casein (a milk protein) and ferritin (a liver protein which complexes with iron ions) .

4- Protective function: Antibodies are proteins that protect the body from disease by combining with and destroying viruses, bacteria.

5- Regulatory function: Body processes regulated by proteins include growth (growth hormone) .

6- Nerve impulse transmission: Some proteins act as receptors for small molecules that transmit impulses across the synapses that separate nerve cells .

7- Movement function: The proteins actin and myosin are important in muscle activity, regulating the contraction of muscle fibers .

8-Transport function: Some proteins bind small molecules or ions and transport them through the body , such as Hemoglobin carries oxygen from the lungs to other body tissues.

Lipids

Lipids comprise very heterogeneous group of compounds which are insoluble in water but soluble in non-polar organic solvents such as benzene, chloroform, and ether. They are present in all living organisms. The group includes fats, oils, waxes and related compounds.

General Functions of Lipids

1. They are efficient energy sources.
2. They are structural components of the cell membrane.
3. Serve as precursors for hormones (steroid hormones).
4. They also dissolve the vitamins, which are fat-soluble and assist their digestion .
5- Bile acid synthesis .

Classification based on lipid composition

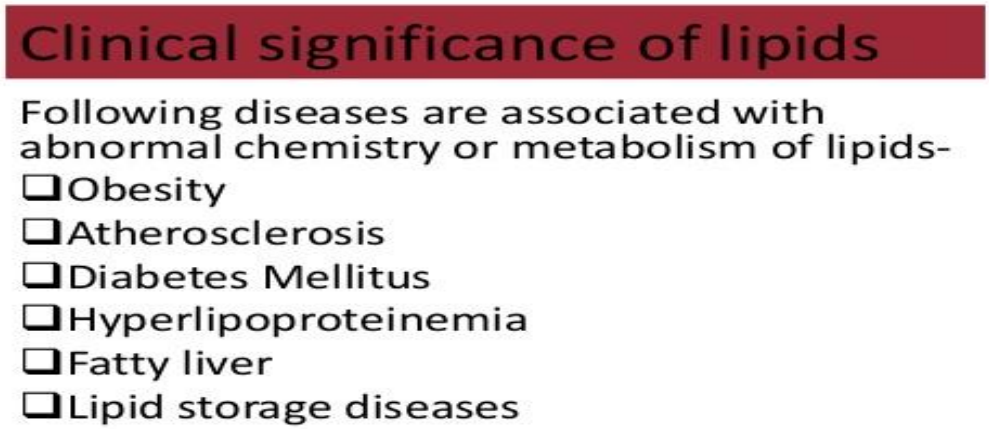
1. Simple lipids:- esters of fatty acids with different alcohols.

Fats and oils:- These are esters of fatty acids with glycerol.

Waxes:- Esters of fatty acids with high molecular weight monohydric alcohols

2. Complex lipids:- Esters of fatty acids and alcohols together with some other head groups. such as Phospholipids , Glycerophospholipids , Sphingophospholipids(The alcohol is shingosine) , Glycolipids(Lipids containing fatty acid, sphingosine and carbohydrate residues) .

3. Derived lipids:- include the hydrolytic products of the simple and complex lipids.
Eg. Fatty acids, cholesterol etc.



Major Roles of Biological Lipids

Biological molecules that are insoluble in aqueous solutions and soluble in organic solvents are classified as *lipids*. The lipids of physiological importance for humans serve as structural components of biological membranes; provide energy reserves, predominantly in the form of triglycerides, serve as biologically active molecules exerting a wide range of regulatory functions, and the lipophilic bile acids aid in lipid emulsification during digestion of fats .

Enzymes

(The biological catalysts) are protein catalysts for chemical reaction in biological systems. They increase the rate of chemical reactions taking place within living cells without changing themselves .

All the enzymes are proteins except ribozymes .

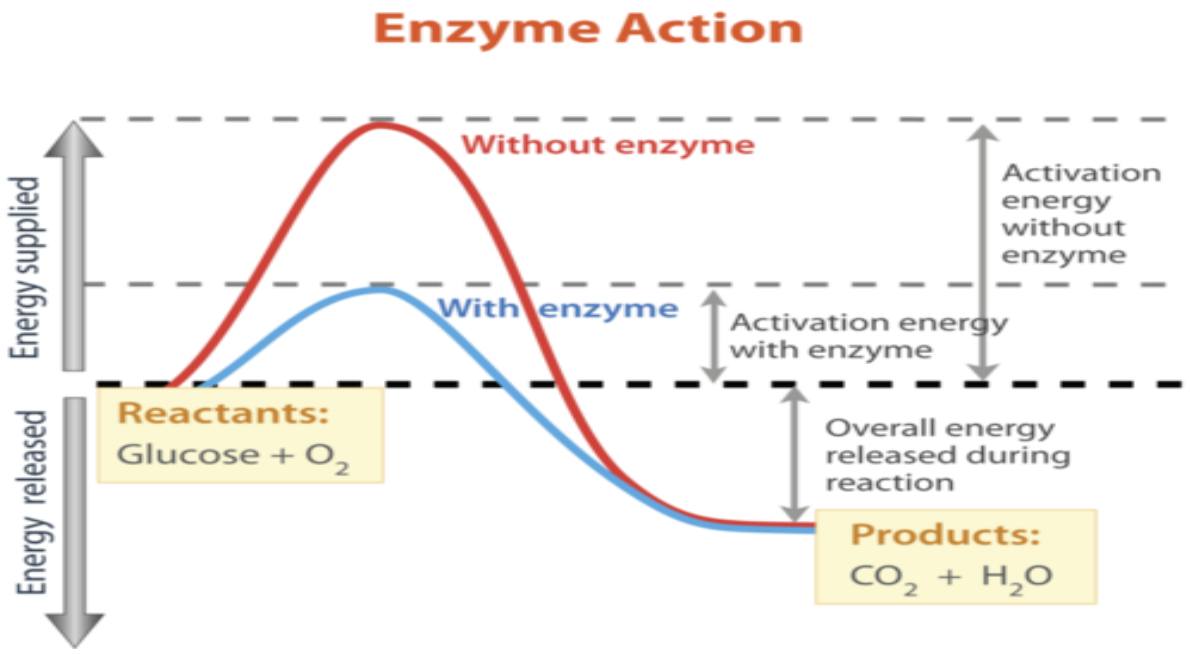
Enzymes cut big molecules apart and join small molecules to form big molecules.

Enzymes are not consumed in the overall reaction .

Enzyme action

1- Enzymes increase the rate of reaction by decreasing the activation energy of reaction.

2- The activation energy is the energy barrier between reactants and product .



Enzyme Nomenclature and Classification

International Union of Biochemistry classified all enzymes into six major classes based on the type of reaction they catalyze and reaction mechanism .

Nomenclature

The name of an enzyme has two parts. The first part indicates name of its substrate and second part ending in ‘ase’ indicates the type of reaction it catalyzes. Further, each enzyme has code (EC) number. It is a four-digit number .

- The first digit indicates major class .

- Second digit indicates sub class .

- Third digit denotes sub sub class .

- Fourth digit indicates specific enzyme .

Classification

The six major classes of enzymes with some example are:

1. Oxidoreductases

They catalyze oxidation and reduction reactions. Most often called dehydrogenases.

2. Tranferases

They catalyze transfer of groups. This group includes kinases which transfer a phosphoryl group from ATP.

3. Hydrolases

They catalyze hydrolysis of peptide , ester, glycosyl etc. bonds.

4. Lyases

They catalyze removal of groups from substrates by mechanisms other than hydrolysis forming double bonds.

5. Isomerases

They catalyze interconversion of optical, functional and geometrical isomers.

6. Ligases

They catalyze linking together of two compounds. The linking is coupled to the breaking of phosphate from ATP. Formation of C-C, C-S, C-O, and C-N bonds .

For example : 1.1.1.1 enzyme, 1 means oxidoreductase,1.1 means that the functional group is hydroxyl group (-OH) , 1.1.1 means NAD is the coenzyme and 1.1.1.1 means alcohol. So, 1.1.1.1 means alcohol dehydrogenase enzyme.

Factors Affecting Enzyme Action

Rates of enzyme catalyzed reactions are affected by:

1. Substrate concentration.
2. Enzyme concentration.
3. Temperature.
4. pH
5. Inhibitors .

clinical Significance of enzyme study:

1. Normal enzyme function is required for life maintenance .
2. Medical treatment and diagnostic .
3. Drug development .

Medical importance of enzymes of blood (cont.)

- Many diseases that cause tissue damage results in increased release of intracellular enzymes into plasma (blood)
  So, the **enzyme levels in blood** are measured for diagnosis of these diseases
- Diseases of the heart, liver, skeletal muscles and other tissues are diagnosed by an elevation of a blood enzymes.
- The **level of elevation of an enzyme** correlates with the extent of tissue damage in any of these organs.
- Some enzymes may be **available in high amount in only one organ**
  So, the elevation of blood levels of these enzymes are diagnostic for diseases of this organ only. (specific)
  Example: Alanine aminotransferase (**ALT**) enzyme elevation in blood indicates disease of the **liver** (specific for liver cells).

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Liver function test

Functions of liver

1- Detoxification

The liver takes compounds which are harmful and not very soluble and makes them less harmful and more soluble like ammonia, or in the ones we eat or drink .

2- Synthesis

liver assures the metabolism of the carbohydrates, the fat and the proteins while producing bile, essential element for our digestion. liver also avoids hemorrhages via the coagulation process .

3- Storage

The liver stores vitamins (A, D, E, and K) and glycogens (carbohydrates) meaning it stores energy like sugar and makes it available to organism when needed .

Liver function tests based on each functions are done routinely in laboratory .

In addition measurement of serum enzymes specific to liver is helpful in assessing liver damage .

**** Tests based on Secretory or Excretory function**

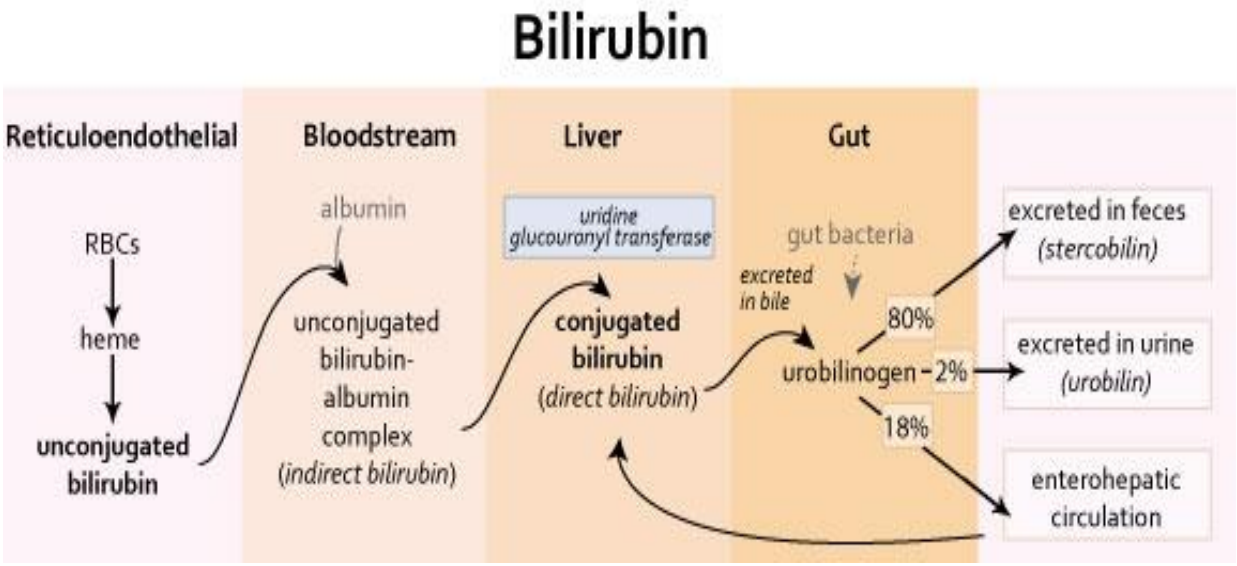
1- Serum bilirubin

Bilirubin is a yellow breakdown product of normal heme catabolism.

2- Urine bilirubin

3- Urine Urobilinogen

4- Serum bile acids



**** Tests based on Synthetic function**

1- Serum albumin

In several liver diseases hypoalbuminemia occurs. Since half life of albumin is 20 days decrease in albumin level occurs in chronic liver diseases.

2- Serum globulins

1. In chronic liver diseases globulins increase due to decreased clearance by hepatocytes.
2. IgA level increases in all types of cirrhosis.
3. IgG level increases in auto immune hepatitis and cirrhosis.
4. IgM is increased in biliary cirrhosis .

3- Prothrombin Time (PT)

is a blood test that measures how long it takes blood to clot. A prothrombin time test can be used to check for bleeding problems .

- Since prothrombin is one of the blood coagulation factor synthesized by liver its synthesis is decreased in liver disease .

4- Blood Urea

Since liver is the only organ involved in the production of urea its level decreases in liver failure cases .

** Tests based on metabolic function

1- Galactose tolerance test

1- Liver is the only organ involved in disposal of galactose. So, measurement of galactose clearance by liver is useful in assessing hepatic function .

2- After an intravenous galactose injection blood samples are collected for every 10 minutes until one hour. Galactose in measured in blood samples .

3- Normally liver clears galactose within 10-15 minutes. Delay of clearance indicates cirrhosis and hepatitis .

2- Blood Ammonia

1- Since liver converts ammonia to urea through urea cycle reactions ammonia level is elevated in liver diseases .

2- When ammonia accumulation reaches toxic level hepatic coma develops .

3- Enzyme Tests

- Aspartate aminotransferase.
- Alanine aminotransferase.
- Alkaline phosphatase.
- Lactate dehydrogenase.
- Glutamyltransferase.
- Glutamate dehydrogenase.
- Nucleotidase
- Sorbitol dehydrogenase.

Jaundice: is unnatural yellow coloration of the skin or sclera which is due to the presence of bilirubin in plasma in concentrations greater than 40 mmol / L .

The normal concentration of bilirubin in the plasma is under 22 mmol / L.

Types of jaundice

1- Hemolytic jaundice

Most often hemolytic jaundice is caused by an increased destruction of red blood cells .

2- Hepatocellular jaundice

Congenital disturbances of bilirubin transport lead to jaundice due to imperfect absorption, decreased conjugation or impaired excretion of bilirubin .

3- Cholestatic jaundice

Cholestatic jaundice may be the effect of obstruction of an outflow of bile from the hepatocytes in the duodenum .

Kidneys Function Test

The Kidneys Function

1- Regulation of electrolytes, maintenance of acid–base balance, and the regulation of blood pressure (by maintaining salt and water balance) .

2-They serve the body as a natural filter of the blood and remove wastes that are excreted through the urine. three major compounds that the kidneys remove are :

- Urea, which results from the breakdown of proteins .
- Uric acid from the breakdown of nucleic acids .
- Creatinine is a waste product that forms when creatine .

3- Reabsorption of water, glucose, and amino acids, and will maintain the balance of these molecules in the body .

The kidneys produce hormones including calcitriol, erythropoietin, and the enzyme renin , which are involved in renal and hematological physiological processes .

The more important and commonly employed tests are :

1- Blood Urea .

2- Uric acid .

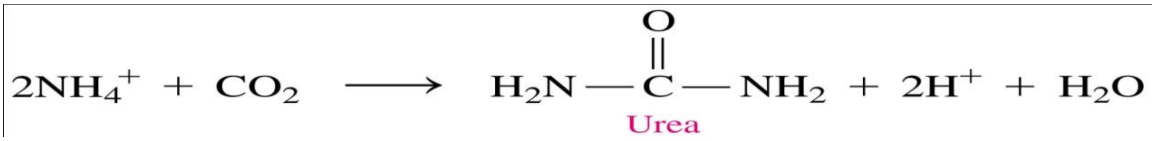
3- Serum Creatinine .

In many renal diseases, urea , uric acid and creatinine accumulate in the blood because they are not excreted properly.

1- Urea

an end product of protein metabolism is produced in the liver , The ammonium ion, the end product of amino acid degradation, is toxic if it is allowed to accumulate . The **urea cycle** converts ammonium ions to urea in the mitochondria and cytosol . which is transported to the kidneys and is excreted .

Decreased urea levels occurred with significant liver disease . Increased urea levels may indicate renal disease.



2- Uric Acid

Uric Acid is the end product of purine metabolism in man formed by oxidation of Purine bases. it are produced for Mainly in liver . Normally, uric acid dissolves in the blood. It passes through the kidneys and out of the body in urine.

3- Serum Creatinine

Creatinine is a waste product formed in muscle by creatine metabolism. Creatine is synthesized in the liver which then passes into circulation where it is taken up by skeletal muscle for conversion to creatine phosphate which serves as storage form of energy in skeletal muscles . Creatine phosphate utilized in the contraction of muscle fibers.

Creatine and creatine phosphate are spontaneously converted to creatinine at a rate of about 2% the total per day. This is related to muscle mass and body weight .Creatinine formed is excreted in the urine. Creatinine is not reabsorbed by the renal tubules. so tests for its concentration in the blood and urine are widely used to assess renal function .

Pancreatic Function tests

The pancreas has an endocrine function because it releases juices directly into the bloodstream, and it has an exocrine function because it releases juices into ducts .

Enzymes, or digestive juices, are secreted by the pancreas into the small intestine. There, it continues breaking down food that has left the stomach .

Tests that measure pancreatic function are most commonly performed to diagnose chronic pancreatitis, although they can also be used to determine the severity of the disease. The diagnosis of chronic pancreatitis is most surely made by histological examination .

Function

A healthy pancreas produces chemicals to digest the food eat. The exocrine tissues secrete a clear, watery, alkaline juice that contains several enzymes.

These break down food into small molecules that can be absorbed by the intestines .

The enzymes include :

- Trypsin and Chymotrypsin to digest proteins

- Amylase to break down carbohydrates

- Lipase, to break down fats into fatty acids and cholesterol

The endocrine portion, or islets of Langerhans, secrete hormone

they include

1-Insulin (Pancreatic beta cells release insulin) : moves glucose from the blood into muscles and other tissues, for use as energy , helps the liver absorb glucose, storing it as glycogen in case the body needs energy during stress or exercise .

2-Glucagone (pancreatic alpha cells release glucagon) : helps glycogen to be broken down into glucose in the liver. The glucose then enters the bloodstream, restoring blood sugar levels to normal .

3-Somatostatin :Somatostatin blocks the production of insulin and glucagon to help regulate blood sugar levels. Somatostatin increases when either glucagon or insulin levels get too high . Somatostatin also regulates the release of hormones in the gastrointestinal (digestive) system.

4-Vasoactive intestinal peptide (VIP) (pancreatic delta cells release glucagon): It has many functions, including vasodilation , controlling water absorption in the intestines and helping break down stores of glycogen into glucose in the liver and muscles.