## What is Clinical chemistry

Clinical chemistry : refers to the biochemical analysis of body fluids. It uses chemical reactions to determine the levels of various chemical compounds in body fluids.

Or clinical chemistry: involves the use of biochemical measurements to support the diagnosis, treatment, prevention and monitoring of disease. Measurements are made in blood, urine, cerebrospinal fluid and other body fluids.

## Why are Clinical biochemistry tests ordered.

- Diagnosis
- Monitor progression of disease
- Monitor effectiveness of treatment
- To identify complications of treatment
- To check the accuracy of an unexpected data
- To prevent malpractice
- To conduct research: response to new drugs

### What the specimens used in clinical laboratory

Serum, Plasma, Whole blood, urine, CSF…… etc.

Blood: is a fluid connective tissue that consists of plasma, blood cells and platelets. It circulates throughout our body delivering oxygen and nutrients to various cells and tissues. It makes up 7% of our body weight. (about 5.6 liters in a 72 Kg man). This proportion is less in women, while in children is greater (gradually decreasing until the adult level is reached).

## Function of the blood.

Provides oxygen to the cells

-Blood absorbs oxygen from the lungs and transports it to different cells of the body. The waste carbon dioxide moves from the blood to the lungs and is exhaled.

-Transports Hormones and Nutrients

The digested nutrients such as glucose, vitamins, minerals, and proteins are absorbed into the blood through the capillaries in the villi lining the small intestine.

The hormones secreted by the endocrine glands are also transported by the blood to different organs and tissues.

-Homeostasis

Blood helps to maintain the internal body temperature by absorbing or releasing heat.

-Blood Clotting at Site of Injury

The platelets help in the clotting of blood at the site of injury. Platelets along with the fibrin form clot at the wound site

-Transport of waste to the Kidney and Liver

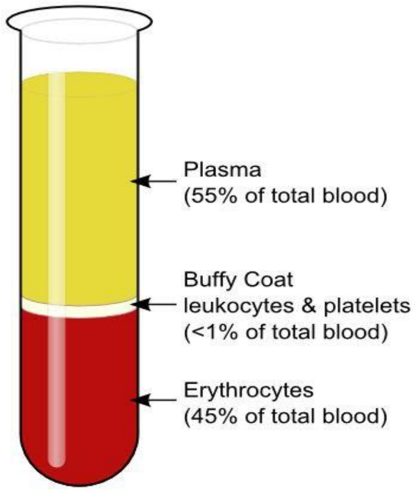
Blood enters the kidney where it is filtered to remove nitrogenous waste out of the blood plasma. The toxins from the blood are also removed by the liver.

-Protection of the body against pathogens

The White Blood Cells fight against infections. They multiply rapidly during infections.

## Components of Blood

The blood classified as a connective tissue, There are many cellular structures in the composition of blood. When a sample of blood is spun in a centrifuge machine, they separate into the following constituents: Plasma, buffy coat and erythrocytes. Thus blood contains RBC, WBC, platelets and plasma.




## Blood Plasma

Plasma is the liquid component that make up 55% of the blood it composed from

1. Mainly water 90% from the plasma
2. Nutrient including fats, amino acid, sugar, fatty acid, vitamin the nutrient absorbed from the gut.
3. Dissolved oxygen and carbon dioxide in small amount as well as a significant amount of nitrogen.
4. Amino acids These are formed from the breakdown of tissue or plasma proteins.
5. Nitrogenous waste compounds such as urea are produced by the breakdown of various substance in the body. These are carried in the plasma to the kidney to be excreted.

6. Electrolytes The most abundant of these are sodium ions and contributes most of the plasma osmolarlity.
7. Proteins: These are the most abundant substance in plasma by weight and play a part in a variety of roles including clotting, defense and transport.

Kind of plasma protein:

- ***Albumins*** which are the smallest size among plasma proteins but makes up the largest percentage . Reductions in plasma albumin content can result in a loss of fluid in the plasma seeps out into the space around the blood vessels and may result in interstitial edema, a feature of liver disorders, kidney disease and malnutrition. Albumin also helps many substances dissolve in the plasma by binding to them, hence playing an important role in plasma transport of substances such as drugs, hormones and fatty acids.
- ***Globulins*** which can be subdivided into three classes from smallest to largest in molecular weight into alpha, beta and gamma globulins. The globulins include high density lipoproteins (HDL), an alpha-1 globulin, and low density lipoproteins (LDL), a beta-1 globulin. HDL functions in lipid transport carrying fats to cells for use in energy metabolism, membrane reconstruction and hormone function. HDLs also appear to prevent cholesterol from invading and settling in the walls of arteries. LDL carries cholesterol and fats to tissues for use in manufacturing steroid hormones and building cell membranes, but it also favors the deposition of cholesterol in arterial walls and thus appears to play a role in disease of the blood vessels and heart. HDL and LDL therefore play important parts in the regulation of

cholesterol and hence have a large impact on cardiovascular disease.

- *__Fibrinogen__* which is a soluble precursor of a sticky protein called fibrin, which forms the framework of blood clot. Fibrin plays a key role in coagulation of blood, which is discussed later in this article under Platelets.

## White Blood Cells (WBC)

Leucocytes are colorless blood cells. They are colorless because it is devoid of hemoglobin. They are further classified as granulocytes and a granulocytes.

Granulocytes include

- Neutrophils
- Basophils
- Eosinophils

Agranulocytes include

- Monocytes
- lymphocytes

White blood cells are responsible for fighting foreign pathogens (such as bacteria, viruses, and fungi) that enter our body. They circulate throughout our body and originate from the bone marrow.

## Red Blood Cells (RBC)

RBCs: are biconcave cells without nucleus in humans; also known as erythrocytes. RBCs contain the iron-rich protein called hemoglobin;  give blood its red colour. RBCs are the most copious blood cells produced in bone marrows. Hemoglobin carries most of the oxygen and some of the carbon dioxide transported by the blood. Circulating erythrocytes live for about 120 days.

hemolysis

Hemolysis refers to the rupture of RBCs, where hemoglobin is released leaving empty plasma membranes which are easily digested by cells known as macrophages in the liver and spleen. The Hb is then further broken down into its different components and either recycled in the body for further use or disposed of.

## Platelets:

Platelets are small fragments of bone marrow cells and are therefore not really classified as cells themselves. Platelets have the following functions:

1- Secrete vasoconstrictors which constrict blood vessels, causing vascular spasms in broken blood vessels
2- Form temporary platelet plugs to stop bleeding
3- Secrete (clotting factors) to promote blood clotting.
4- Dissolve blood clots when they are no longer needed .
5- Digest and destroy bacteria.
6- Secrete chemicals that attract neutrophils and monocytes to sites of inflammation.
7- Secrete growth factors to maintain the linings of blood vessels.

The first three functions listed above refer to important hemostatic mechanisms in which platelets play a role in during bleeding: vascular spasms, platelet plug formation and blood clotting (coagulation).

# Coagulation (blood clotting)

Coagulation: is an important process that stop bleeding when a bloods vessel is injured.

The process of coagulation is complex and involves many steps and many factors (most of which is proteins). The factors are identified by roman numerals (I – XIII).

Stages of biochemical pathway of blood clotting

Stage1. Formation of prothrombin activator

Stage2. Conversion of prothrombin into thrombin by prothrombin activator.

Stage3. Conversion of soluble fibrinogen into insoluble fibrin by thrombin.

In the last fibers of fibrin form a meshwork in which blood get entangled to form a sold clot.

**Anticoagulants** are medications that help prevent blood clots. Although people call them “blood thinners,” these medications do not actually thin the blood. Instead, they prevent the blood from thickening, or clotting.

Blood clots can block the blood vessels and stop the blood from flowing to important organs, such as the lungs, brain, and heart. This can increase a person’s risk of heart attack or stroke. **Anticoagulants,** such as heparin or warfarin.

# Urine

Urine: is a liquid waste product of the body secreted by the kidneys by process of filtration from blood. The average amount of urine excreted in 24 hours is about 1200 cubes cm and normally, contain about 960 part of water to 40 part of solid matter. There are three main step of urine formation glomerular filtration, reabsorption, and secretion.

Collection of a sample of urine Over the course of a 24-hour period, the composition and concentration of urine changes continuously. For this reason, various types of specimens may be collected, including:

| Sample type | sampling | purpose |
|---|---|---|
| Morning sample | First urine in the morning, most concentrated | Pregnancy test, microscopic test. |
| Random specimen | No specific time | Routing screening |
| postprandial | 2 hours after meal | Determine glucose in diabetic monitoring |
| Clean catch midstream | Discard first few ml, collect the rest | culture |
| 24 hours | All the urine passed during the day and night and next day 1$^{st}$sample is collected | Used for quantitative and qualitative analysis of substance |
| Supra-pubic aspired | Needle aspiration | Obtaining sterile urine |

culture and cytological analyses Urine specimens need to be examined within 2 hours. Urine that is left to standing too long becomes alkaline because bacteria begin to split the urea contained in urine into ammonia.

A urine specimen should be refrigerated if it cannot be sent to the laboratory within 2 hours.

## Urine samples are usually examined for the main items:

< Physical examination.

< Biochemical examination.

< Microscopic examination.

# Physical examination

## Urine volume

Urine volume measurements are part of the assessment for fluid balance and kidney function. The normal volume of urine voided by the average adult in a 24-hour

period ranges from 600 to 2500 ml; the typical amount is about 1200 ml .

Urine volume has many condition :

Polyuria : is a condition characterized by the passage of large volumes of urine at least 2500 ml over 24 hours in adults and is seen in Diabetes Mellitus ، Diabetes Insipidus ، Chronic Renal Failure.

Oliguria : are the decreased production of urine < 400 ml as seen in Acute renal failure, Severe dehydration, Acute glomerulonephritis.

Anuria : It non passage of urine as seen in urinary tract obstruction, Acute renal failure.

## Urine color

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

Very pale yellow or colorless urine

a) Large fluid intake
b) Diabetes Mellitus
c) Diabetes Insipidus
d) Alcohol ingestion (inhibit ADH release )
e) Caffeine ingestion (increase GFR by dilating afferent arterioles).

Deep yellow urine

a) Dehydration or drinking too few fluids can concentrate urochrome (or Urobilin is the chemical primarily responsible for the yellow color) of urine
b) Concentrated urine caused by fever, sweating, reduced fluid intake

Orange urine

a) Certain medications such as the antibiotic Rifampicin
b) Large amounts of carotene .

Red or pink urine

a) The presence of red blood cells is the main reason
b) Hemoglobinuria turns urine translucent red
c) Beets, and blackberries can turn urine red

Greenish-yellow urine

a) May indicate bilirubin in the urine

b) Greenish urine may be caused by dietary supplemental vitamins, especially the B vitamins .

## Smell (Odor)

a. Uriniferous odor: normal odor of fresh voided urine (due to presence of aromatic acids).
b. Fruity odor: is due to acetone (diabetic ketoacidosis).
c. Ammoniacal odor: is due to release of ammonia as a result of the bacterial urease enzyme in the contaminated and long-standing exposed urine sample.
d. Mousy odor: is due to PKU (Phenylketonuria).
e. Burnt sugar odor: is due to maple syrup urine disease.

## Urine specific gravity

is defined as the ratio of the density of a given solid on liquid substance to the density of water at a specific temperature and pressure

normal: the range of urine SG depends on the state of hydration and usually between 1.005 and 1.030.

# chemical examination

urine reaction(PH): defined as the measure of the acidity or basicity of solution. The kidneys maintain normal acid-base balance primarily through reabsorption of sodium and tubular secretion of hydrogen and ammonium ions .

Normal:- The pH of normal urine can vary widely, from 4.6 to 7.0 (The average pH value is about 6.0)

Acidic urine ( PH < 7.0 ) occurs in :

a) Prolonged diarrhea
b) Starvation
c) UTI caused by Escherichia coli .
d) Gout
e) A diet high in meat and protein keeps the urine acid

Alkaline urine (pH >7.0) occurs in :

a) UTIs caused by urea-splitting bacteria
b) Renal tubular acidosis
c) Chronic renal failure
d) Metabolic alkalosis (as in prolonged vomiting due to lose of hydrogen ion ).

**Proteins of urine:** A little quantity, of protein are found normally in urine (150 mg/day) any access in protein called proteinuria which is an indication for many diseases like kidney diseases, fever and pregnancy. Types of protein in urine:

1- Albumin: is the first protein appearing in urine due to its low molecular weight and size (albuminuria), this protein appears in Diabetes and hypertension.
2- Immunoglobulin's : appear in urine due to inflammations and microbial infections

3-Hemoglobin: found in urine due to blood hemolysis.

## Urine Glucose

No glucose is present in the urine normally which passes glomerular filter, because it is completely absorbed in the tubules. It present when the blood glucose level elevated to(180mg/ml) which is called **renal threshold**, when blood glucose elevated the glucose present in urine as in diabetes.

## Acetone urine

(Ketone body) The ketone bodies include acetone, acetoacetic acid (diacetic acid) and beta hydroxybutric acid that are produced when fatty acids are broken down for energy in the liver and kidney.
A state in which these substances are present in increased amount in the blood and urine called ketosis

Ketonuria: (Presence of ketones "Acetone, acetoacetic acid and β- Hydroxybutyric acid" in urine).

Metabolic conditions

- Diabetes mellitus (diabetic acidosis)
- Glycogen storage disease (von Gierke's disease)

Dietary conditions

- Starvation, fasting.
- High-fat diets.
- Prolonged vomiting, diarrhea (cause dehydration).
- Anorexia (poor appetite whatever the cause).
- Low-carbohydrate diet.

### Urine 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.

## Microscopic examination

Classification of Sediment :

A - cell

- Erythrocytes
- Leucocytes
- Epithelial cells

B- casts: presence of cast is frequently associated with proteinuria

- Hyaline cast
- Red cell cast

- Granular cast
- Epithelial cast
- waxy cast
- Fatty cast

C-Crystals : Some are common in Acidic urine ,Some are common in Alkaline urine.

- Uric acid crystal
- Calcium oxalates crystal
- Amorphous urates
- cystine

D-Bacteria: Normal urine is free from bacteria .

E-Yeast .

F-Ova

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## Electrolytes:

Electrolytes are minerals that give off an electrical charge when they dissolve in fluids like blood and urine. Your body makes electrolytes. You also get these minerals from foods, drinks and supplements. Electrolytes in blood, tissue, urine and other body fluids play a critical role in balancing body fluids, regulating your heart rhythm and supporting nerve and muscle function.

An electrolyte imbalance occurs when you have too much or not enough of certain minerals in your body. This imbalance may be a sign of a problem like kidney disease.

## What do electrolytes do?

Electrolytes perform different functions in your body:

- Sodium controls fluid levels and aids nerve and muscle function.
- Potassium supports heart, nerve and muscle functions. It also moves nutrients into cells and waste products out of them while supporting your metabolism.
- Calcium helps blood vessels contract and expand to stabilize blood pressure. It also secretes hormones and enzymes (proteins) that help the nervous system send messages.
- Chloride helps maintain healthy blood levels, blood pressure and body fluids.
- Magnesium aids nerve and muscle function. It also promotes the growth of healthy bones and teeth.

- Phosphate supports the skeletal system, as well as nerve and muscle function.
- Bicarbonate helps balance acids and basic alkaline compounds (bases) in blood (pH balance). Bicarbonate also helps move carbon dioxide (a waste product) through your bloodstream.

**What are the types of high electrolyte imbalances?**

High electrolyte imbalances include:

- **Sodium:** Hypernatremia.
- **Potassium:** Hyperkalemia.
- **Calcium:** Hypercalcemia.
- **Chloride:** Hyperchloremia.
- **Magnesium:** Hypermagnesemia.
- **Phosphate:** Hyperphosphatemia.
- **Bicarbonate:** Alkalosis (low alkaline base).

**What are the types of low electrolytes or electrolyte deficiencies?**

Low electrolytes or electrolyte deficiencies include:

- **Sodium:** Hyponatremia.
- **Potassium:** Hypokalemia.
- **Calcium:** Hypocalcemia.
- **Chloride:** Hypochloremia.
- **Magnesium:** Hypomagnesemia.
- **Phosphate:** Hypophosphatemia.
- **Bicarbonate:** Acidosis (high acid levels).

## What are kidney function tests?

Kidney function tests are a group of blood tests that provide information on how well your kidneys are working.

Most people have 2 kidneys. They're part of your urinary system. Kidneys have several important functions:

- filtering and cleaning your blood to remove waste products into urine (wee)
- balancing the fluids and salts or minerals in your body
- making vitamin D
- making hormones that help to control your blood pressure

Kidney function tests can help your doctor check your kidney function and to monitor it over time.

Kidney function tests are also known as renal function tests and include: urea and electrolytes tests. These are often shortened to: U&E, EUC or UEC.

The tests measure levels of various substances in your blood, including:

- electrolytes: sodium, potassium, chloride, bicarbonate
- minerals: phosphorus, calcium
- protein: albumin
- waste products: urea, creatinine
- glucose (sugar)

### What is a Renal Function Profile?

A Renal Profile includes testing several parameters: Urea, Blood Urea Nitrogen, Creatinine, Uric Acid, Calcium, Phosphorus, Sodium, Potassium, Chloride, BUN/Creatinine Ratio, Urea/Creatinine Ratio. Some laboratories calculate glomerular filtration rate/GFR (which is an estimation of how much blood is filtered through kidneys every minute).

## What are the parameters tested under RFP?

Albumin

Albumin is a protein found in the blood. If the kidneys are healthy, there should be very little protein in your urine – if any. However, if the kidneys are damaged, albumin may leak out of the kidneys and into your urine.

BUN (Blood Urea Nitrogen)

Urea nitrogen is a byproduct from the breakdown of food proteins. A normal BUN level is between 7 and 20. As kidney function decreases, the BUN level rises.

BUN-to-Creatinine Ratio (calculated)

The ratio of BUN to creatinine (BUN: creatinine) is usually between 10:1 and 20:1. An increased ratio may be due to a condition that causes a decrease in the flow of blood to the kidneys.

Calcium

Measuring calcium can help determine whether the kidneys are excreting the proper amount of calcium. Too much calcium can also help indicate kidney stones.

Carbon Dioxide

Your kidneys and lungs balance the levels of carbon dioxide, bicarbonate, and carbonic acid in the blood. High carbon dioxide levels can be used to help diagnose kidney disease.

Chloride

Chloride is an important electrolyte used by the body to maintain the proper blood volume, blood pressure, blood acidity, and balance of fluid in cells. An increased level of blood chloride may indicate kidney diseases like tubular acidosis, which is when the kidneys do not remove enough acid.

Creatinine

A waste product that comes from normal wear and tear on the body's muscles. Creatinine levels in the blood vary depending on age, race, and body size, but a creatinine level greater than 1.2 for women or greater than 1.4 for men may be an indicator that the kidneys are not working properly. The level of creatinine in the blood rises as kidney disease progresses.

Estimated Glomerular Filtration Rate (calculated)

This is a calculated measurement of how well the kidneys are removing waste and excess fluid from the blood. Your age, race, sex, and creatinine levels are all considerations when this calculation is made. The normal value for eGFR is 90 or above but this can decrease with age. An eGFR below 60 is a sign that the kidneys are not working properly. An eGFR below 15 indicates kidney failure.

Glucose

Abnormally high levels of glucose in your blood (hyperglycemia) can indicate glycosuria. Renal glycosuria occurs when the renal tubules fail to reabsorb all glucose at a level that is normal.

Phosphorus

Functioning kidneys can remove extra phosphorus from your blood. Individuals with chronic kidney disease often cannot remove phosphorus from the blood very well. High phosphorus levels can cause damage to your body.

Potassium

Potassium is an electrolyte that assists in many bodily functions like water balance, digestion, and nerve impulses. Kidney disease can cause both high and low potassium levels

Sodium

Sodium is one of the three major electrolytes your body utilizes to control fluid balance inside and out of cells, among other functions. High sodium levels could indicate kidney disease because the body is unable to effectively remove the correct amount.

## What are the healthy ranges of each test?

Results higher or lower than the normal range may indicate issues.

| | |
|---|---|
| **Albumin** | 3.4 -5.4g/dL |
| **BUN (Blood Urea Nitrogen)** | 10 – 20 mg/dL |
| **BUN-to-Creatinine Ratio (calculated)** | 5 – 18mg/dL |
| **Calcium** | 8.5 – 10.3mg/dL |
| **Carbon Dioxide** | Given in percentage of total blood composition, an adult's blood should be made of less than 2.3% (or 0.023) of carbon dioxide. Smokers should have between 2.1% and 4.2% carbon dioxide. |
| **Chloride** | 96 – 106MEq/L |
| **Creatinine** | 0.9 to 1.5mg/dL for men and 0.6 to 1.1mg/dL for women |
| **Estimated Glomerular Filtration Rate (calculated)** | 90 – 120mL/minute |
| **Glucose** | <140mg/dL is ideal. Over 200mg/dl can indicate diabetes |
| **Phosphorus** | 2.5 to 4.5 mg/dL |

## What could cause an abnormal Renal Profile report?

Tests of renal function can be used to assess overall renal function by direct measurement or to determine the presence of renal impairment. If reduced over a specified period, it can identify the presence of chronic kidney disease as well as its staging. Additionally, tests of renal function can be utilized to determine if the renal disease is acute or chronic. In the case of urine albumin, it can be used to detect kidney failure in at-risk patients, for example, in patients with diabetes. Other causes of abnormal RFT is Serum creatinine/urea is elevated when at least 50% of kidney function is lost.

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Proteins: They are organic compounds that contain C, H, O, N and in addition to sulfur, phosphorus, and iodine, as well as other elemental components present in some proteins.

_ Proteins are the vital material necessary is present in all living material

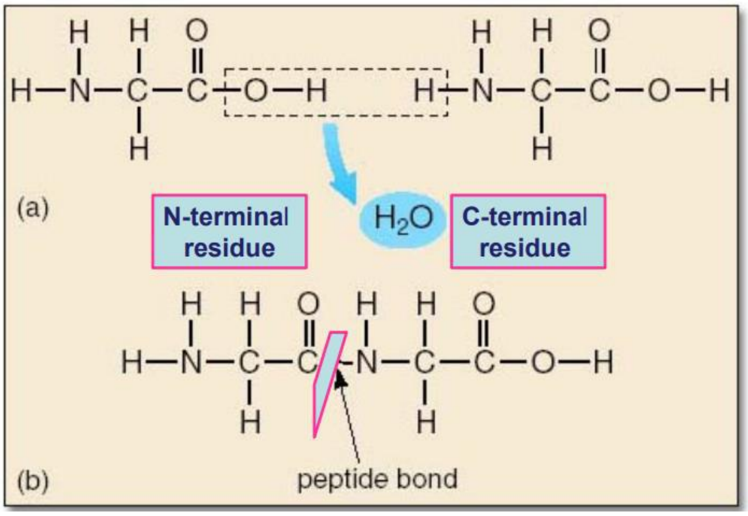
_ It is the only source that provides the body with the nitrogen necessary for the formation and repairment of body tissues.

_are made up of hundreds or thousands of smaller units called amino acid.

Amino acids: are the Building blocks of peptide and proteins, 20 common amino acids used by all organisms.

## Amino acids are organic acids consist from

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




Peptides consist from two or more amino acid residues linked by peptide bond.

Peptide bond: is the covalent chemical bond linkge between carboxyl group of an amino acid and with α-amino group of another amino acid.

Essential amino acids : Amino acids are not synthesized in the body and are essential as constituents of tissue proteins , therefore it must be supplied in food. ( valine, phenylalanine, lysine, tryptophan, leucine, isoleucine, therionine , methionine).

Non-Essential Amino Acids (N.E.A.A): are 14 amino acids, and they are those amino acids that are made in the human body and are called non-essential because the human body is able to adequately manufacture them and does not need to obtain them from food sources such as: Alanine, Serine, Cysteine Cystine, Tyrosine, Arginine, Aspartic Acid, Glutamine and Glycine.

## CLASSIFICATION OF PROTEINS

### Classification based on composition and solubility:

1. Simple protein: contain only amino acids, Examples Albumin and globulins.
2. Conjugated protein: They are combinations of protein with a non-protein part, called prosthetic group Conjugated proteins ex. Glycoproteins, Lipoproteins, Nucleoproteins, Phosphoproteins.

### Classification Depending on the Shape

| Fibrous | Globular |
|---|---|
| 1) polypeptides arranged in long strands or sheets<br>2) water insoluble (lots of hydrophobic AA's)<br>3) strong but flexible<br>4) Structural (keratin, collagen) | 1) polypeptide chains folded into spherical or globular form<br>2) water soluble<br>3) contain several types of secondary structure<br>4) diverse functions (enzymes, regulatory proteins |

## Classification Based on function:

1. Catalytic proteins, e.g. enzymes
2. Structural proteins: such as collagen (mechanical strength of skin and bone) and keratin (hair, skin, fingernails ) . e.g. collagen, elastin
3. Contractile proteins: The proteins actin and myosin are important in muscle activity, regulating the contraction of muscle fibers
4. Transport proteins, e.g. hemoglobin, myoglobin, albumin, transferrin
5. Regulatory proteins or hormones, e.g. ACTH, insulin, growth hormone
6. Genetic proteins, e.g. histones
7. Protective proteins, e.g. immunoglobulins, interferons, clotting factors.

## Clinical significance of proteins

Hyperproteinemia occurs in the following cases:

**1.** Dehydration: where the total protein level reaches 10 or 15% of its normal level and dehydration can occur in the event of a lack of water intake or in the case of water loss resulting from severe vomiting, severe diarrhea or as a result of the condition advanced diabetic acidosis, and Addison's disease.

**2.** Cancer diseases, such as multiple myeloma, up to 10 g / 100 ml of blood serum.

Hypoproteinemia occurs in the following cases:

**1.** Nephrotic Syndrome, which leads to the loss and leakage of albumin in large quantities through the damaged tissue of the kidney, and therefore the protein level is lower than its normal level.

**2.** Burns and cases of severe bleeding, as protein decreases in the case of intestinal diseases in which absorption is disrupted as a result of inflammation of the intestine.

## Diseases caused by changes in protein structure:

- Sickle Cell Anemia: it cause when single amino acid change in hemoglobin.
- Osteoarthritis: it cause when single amino acid change in collagen protein.

## Methods of plasma protein separation:

methods of protein separation into: albumin, globulins (alpha, beta & gamma) and fibrinogen by:

- Electrophoresis
- Salting out
- Ultracentrifugation
- chromatography.

## Non protein nitrogenous (NPN) substances:

are end products of metabolism that contains nitrogen

These are compounds that contain nitrogen, but are not proteins called NPN compounds Include end products of metabolism, Kidneys act to excrete metabolic waste into urine, measurement of NPN compounds in plasma is useful for assessment of kidney function.

1. Amino acids
2. Ammonia
3. Blood urea nitrogen (BUN)

Protein → aminoacids → ammonia → urea

4. Creatinine(muscle break down products)
5. Uric acid(nucleic acid catabolism)
6. Urea:

Synthesized in the liver: ammonia → urea

Protein → amino acids → ammonia →[LIVER] → urea

## Clinical Significance of urea:

Plasma levels are dependent upon

- ☐ Diet

- ☐ Liver function
- ☐ Kidney function
- ☐ State of hydration

## ■ Clinical Significance:

Increased urea (BUN):

- ❑ Azotemia or uremia
  - ☐ Increased protein intake (leads to increased urea formation)
  - ☐ Decreased kidney function (decreased excretion into urine results in elevated plasma levels)
  - ☐ Dehydration (lack of body water results in increased levels)

Decreased urea (BUN):

- ☐ Decreased protein intake (leads to decreased urea formation)
- ☐ Decreased liver function (decreased conversion of ammonia to urea)

**Creatinine**

derived from creatine in muscle, High energy ATP storage and use in muscle, Produced at a constant rate day to day ,Excreted into urine.

## ■ Clinical Significance

- ☐ Endogenous substance
- ☐ Amount produced is constant day to day: levels vary <10% per day
- ☐ Amount produced is proportional to muscle mass
- ☐ Filtered by glomerulus; not handled by tubules

Good test for GFR

Increased serum creatinine:

- ☐ Renal disease = impaired renal function
- ☐ 50-60% renal function lost before serum creatinine increased (renal reserve)

### Uric Acid

End product of purine metabolism (by liver)

Purines are precursors to nucleic acids ATP and GTP

Readily filtered by glomerulus.

- Clinical Significance
  - Increased uric acid
    - Gout
    - Increased breakdown of nucleic acids
    - Renal disease
  - Decreased uric acid
    - Severe liver disease
    - Fanconi's syndrome

## Urea Cycle Disorders

Urea Cycle Disorders: are a group of genetic disorders caused by a deficiency of enzymes in the urea cycle that is responsible for the removal of ammonia from the blood stream.

## Symptoms

- feeling tired.
- Nausea or vomiting.
- Can't eat or feed.
- Breathing too fast or too slow.
- Confusion
- coma

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## Enzymology:

**Definition :** (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 .

. produced by the living organism in small amounts.

. Functions: digestion, breathing, synthesis and break down of carbohydrates, proteins, fats .

- enzymes acts upon substance called substrate.

. enzymes convert substrate into product. Ex: lactose **lactase** galactose + glucose .

### General properties of enzymes:

1. all enzymes are proteins.
2. enzymes accelerate the reaction but:

a. do not alter the reaction equilibrium

b. not consumed in overall reaction

c. required in very small quantities.

3. enzymes are highly specific for their substrate.
4. enzymes possess active site, at which interaction with substrate take place.

### Sources of enzymes:

**Endoenzymes:** enzymes that function within the cells, most of enzymes are these types.

Ex: metabolic oxidase.

**Exoenzymes:** enzymes that are liberated by cells and catalyze reactions outside the cell.

Ex: digestive enzymes (amylase, lipase, protease).

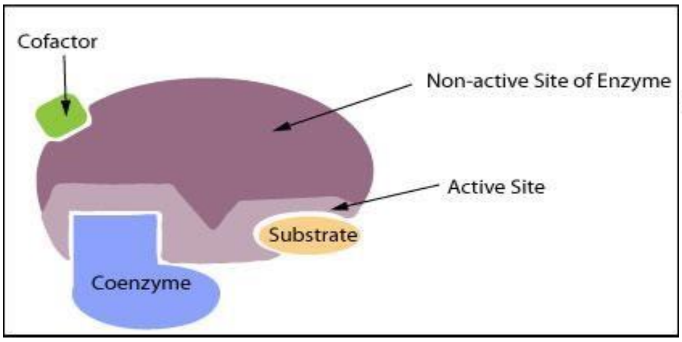
**Chemical composition of enzymes:**

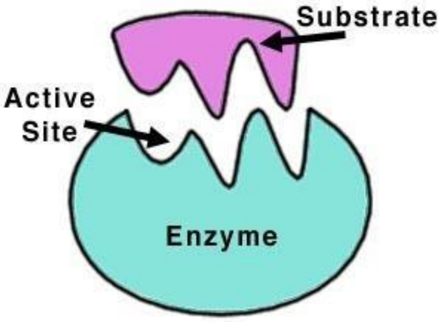
Enzymes classified according to their chemical composition into.

1. **Enzyme consist of only protein.**

Ex: pepsin, trypsin(amino acid sbinding peptide bonds).

2. **Enzyme consist of** :protein (enzyme) + Co - Enzyme = **Holoenzyme**




**Holoenzyme :** enzyme consist of Apoenzyme + prosthetic group

**Apoenzyme :** term refers to the protein part of enzyme.

**Active site of enzyme:** the point in the enzyme which interaction with substrate, co-enzyme, inhibitor take place.

**Zymogen:** the active form of enzyme.

**The difference between Co-enzymes and Co- factors:**

| **Co-enzymes** | **Co - factors** |
|---|---|
| 1-binds loosely and can easily separated from enzyme<br><br>2-organic compounds (ex: water soluble vitamins.<br><br>3-non protein. | **1**.conjugated with protein(enzyme)<br><br>2-. metallic ions (Fe, Mn, Cu,Mg)<br><br>3-has low molecular weight |

| | |
|---|---|
| 4-heat resistance.<br><br>5-their function as co-substrate. | |

## Classification of enzymes:

1. **Oxidoreductases:** one compound oxidized, another reduced. Ex: lactate dehydrogenase, tyrosinase,

2. **Transferase**

Enzyme transfer group containing C, N or S, from one substrate to another substrate.

**Ex**: **Transaminase** ( glutamate oxaloacetate transaminase(**GOT**) or Aspartate transaminase (**AST**). and glutamate pyruvate transaminase(**GPT**), alanine transaminase(**ALT**) ( transfer of amine group(

**3-Hydrolyase:**

Catalyse hydrolysis of ester, peptide or glycoside bound by addition of $H_2O$ across the bond.

**4-Lyasis:**

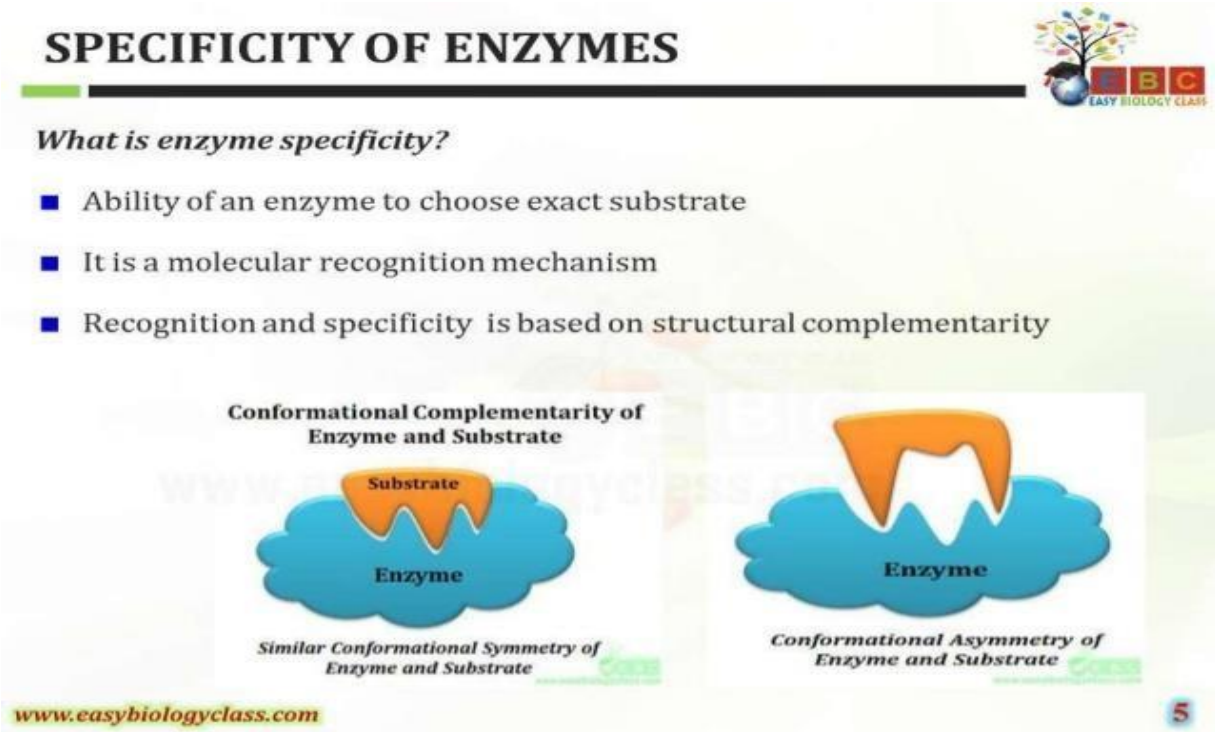
Additional or removal of group without hydrolysis,oxidation, reduction producing double Bond.

**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 .




## Factors affecting enzyme activity:

### 1. Enzyme concentration.

a. The rate of reaction depends directly on the amount of enzyme present. b. At a specific time.

c. Unlimited substrate concentration.

### 2. Substrate concentration.

a) The rate of reaction is directly proportional to the substrate avalible.

b) If the enzyme concentration is kept constant, and the amount of substrate is Increased

### 3. Temperature

a) **The rate of enzyme may increase with increase in temperature** but up to a certain limit.
b) All enzymes can work at their maximum rate at optimum temperature.
c) For enzymes of human body 37°C is the optimum temperature.
d) Enzymes denature at high temperatures.

## 4. Value of PH.

a) Enzymes have specific range of PH at which will work.
b) loose activity in low or high PH.
c) Enzyme denature (change shape and become ineffective). (in temperature and PH).

## Enzyme inhibition:

**Inhibitors :** a chemical substance, can react in place of substrate with the enzyme but is not transformed into product(s). the process called enzyme inhibition.

**The Inhibitors** : poisons, like cyanide, antibiotics, anti-metabolites and some drugs.

## Classification of inhibitors:

Inhibitors can be divided into two types: **(i) Irreversible (ii) Reversible .**

## All these enzymes are seen in blood:

1. **Normal level of (GOT) in blood. (5 to 40 units / liter)**

Found in high concentrations in liver, heart, skeletal muscle and kidney, in both cytoplasm and mitochondria.

2. **Normal level of (GPT) ( 7 to 56 units / liter )**

3. **Enzyme phosphatase:** is agroup of enzyme , hydrolysis the monophosohate ester under acidic or alkaline condition.

Type of phosphatase are: **1. Acid phosphatase ACP**

**2. Alkaline phosphatase ALP**

4. **Enzyme amylase.** **Normal value: 100 – 330 IU/L**

**Disease elevated** in: **1. Acute pancreatitis 2. Severe diabetic ( ketosis and acidosis) 3. salivary gland disorder ( mumps, parotitis)**

5. **Lactate dehydrogenase LDH: Normal level ( 70 – 240IU/L )**

**Disease elevated** in: **1myocardial infarction (MI)**

## Liver Function Tests

The liver is the second largest organ in your body and is located under on the right side.

The liver performs many jobs in your body. It processes what you eat and drink into energy and nutrients your body can use. The liver also removes harmful substances from your blood.

What are the commonly used liver function tests?

### A- **Liver Enzyme Tests**

1- Alanine Transaminase GPT (ALT) is an enzyme mainly found in your liver. high levels of ALT in your blood can be a sign of liver damage.

2- Aspartate Transaminase GOT (AST) is an enzyme found in large amounts in your liver and other parts of your body. High levels of AST can be a sign of liver damage.

3- Alkaline Phosphatase (ALP) is an enzyme found in large amounts in your liver, bile ducts, and other parts of your body. High levels of ALP can be a sign of liver or bile duct damage.

4- Gamma-glutamyl transpeptidase (GGT) is an enzyme found in large amounts in your liver, bile ducts, and pancreas. High levels of GGT can be a sign of liver or bile duct damage.

### B-**Liver Protein Tests**

Total Protein measures the amount of protein in your blood.

1-Globulin is a protein made in your liver and helps the immune system fight infections. Low globulin levels can be a sign of liver damage or other conditions.

1- Albumin is a protein made in your liver. An albumin test measures how well your liver is making the proteins that your body needs. Low albumin levels can be a sign of liver damage.

2- Prothrombin is a protein made in your liver and helps with clotting blood.

**C-A prothrombin time test** measures how much time it takes for your blood to clot. A high prothrombin time can be a sign of liver damage.

D- **Bilirubin Tests**:

Bilirubin is a yellow fluid made in your body when red blood cells break down. A bilirubin test measures the level of bilirubin in your blood. If your liver is damaged, bilirubin can leak out of your liver into your blood and can cause jaundice (yellowing of skin and eyes). It also can come out in the urine making it look very dark

*Department of Emergency Medicine and First Aid Techniques*
*Clinical chemistry / Theoretical*
*The first stage*

## Carbohydrates:

Definition:-

**Carbohydrates**: may be defined chemically as aldehyde or ketone derivatives of polyhydroxy alcohols or as compounds that yield these derivatives on hydrolysis Carbohydrates are

- A major source of energy from our diet.
- Composed of the elements C, H, and O.
- Also called saccharides, which means "sugars."
- Carbohydrates are produced by photosynthesis in plants. glucose is synthesized in plants from $CO_2$, $H_2O$, and energy from the sun then oxidized in living cells (respiration) to produce $CO_2$, $H_2O$, and energy.

## Functions of Carbohydrates:

1 - Source of energy for living beings, e.g. glucose

2 - Storage form of energy, e.g. glycogen in animal tissue and starch in plants

3 - Serve as structural component, e.g. glycosaminoglycans in humans, cellulose in plants and chitin in insects

4 - Non-digestible carbohydrates like cellulose, serve as dietary fibers

5 - Constituent of nucleic acids RNA and DNA, e.g. ribose and deoxyribose sugar

## Classification of Carbohydrates

Carbohydrates are classified into three groups:

1. Monosaccharides=single unit

2. Oligosaccharides =2-10 units .

3- Polysaccharides >10 units The suffix ose indicates that a molecule is a carbohydrate .e.g maltose, glucose, lactose, fructose ,ribose

Monosaccharides (Greek: Mono = one)

• Monosaccharides are also called simple sugars. The term sugar is applied to carbohydrates that are soluble in water and sweet to taste

• They consist of a single unit

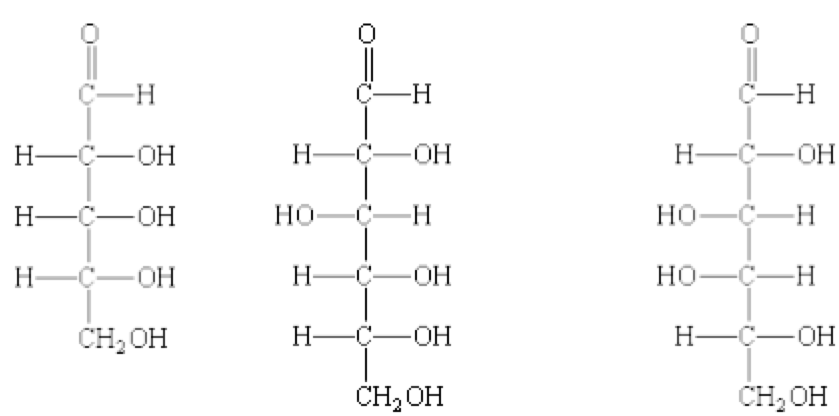
• polyhydroxy aldehyde or ketone unit, and thus cannot be hydrolyzed into a simpler form.

Monosaccharides may be subdivided into two groups as follows:

1. Depending upon the number of carbon atoms they possess, e.g. • Trioses 3 carbon Glyceraldehyde
2. • Tetroses 4 carbon Erythrose
3. • Pentoses 5 carbon Ribose, Xylose
4. • Hexoses 6 carbon Glucose, Galactose, fructose
5. • Heptoses. 7 carbon Glucoheptos

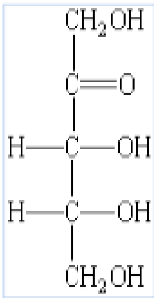
2. Depending upon the functional aldehyde (CHO) or ketone (C=O) group present: • Aldoses CHO Glucose, Galactose • Ketoses C=O Fructose

**ALDOSES:**

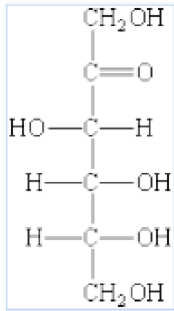


D-ribose D-glucose D-galactose

KETOSES:




D-ribulose




D-fructose

Fructose, glucose and galactose are all hexoses

Ribose and deoxyribose are pentoses. The ribose unit forms part of a nucleotide of RNA. The deoxyribose unit forms part of the nucleotide of DNA

## GLUCOSE

- Physiologically and biomedically , glucose is the most important monosaccharide
- It is called blood sugar
- C6H12O6
- It is monosaccharide (aldose)
- It is source of energy
- It is produced by hydrolysis of glycogen

Note: • The monosaccharides are joined by glycosidic bonds to form disaccharides.

**Disaccharides**

• Disaccharides consist of two monosaccharide units.

• They are crystalline, water soluble and sweet to taste.

sucrose(glucose and fructose ) , maltose (2 glucose) and lactose(glucose and galactose).

**Polysaccharide**

These are complex carbohydrates made up of repeating units of monosaccharides that are attached together by Glucosidic linkage.
Ex: 1-Starch : It is the storage form of glucose in plants, e.g. in potato.
2-Glycogen (Animal Starch) : Glycogen is the major storage form of carbohydrate(glucose) in animals, found mostly in liver and muscle.
Functions of glycogen :
1- The function of muscle glycogen is to act as a readily available source of glucose for energy within muscle itself.
2- Liver glycogen is concerned with storage and maintenance of the blood glucose.
**A plasma glucose test** is a **measure of how much sugar/glucose you have circulating in your blood**.
Glucose is transported from the intestines or liver to body cells via the bloodstream, and is made available for cell absorption via the hormone insulin, produced by the body primarily in the pancreas

Diabetes Mellitus (DM)

1-Insulin dependent(DM) 2-Non insuline dependent (DM).

| Profile | Insulin Dependent | Non Insulin Dependent |
|---|---|---|
| Insulin production | Body is not in condition to make sufficient Insulin | Body can make sufficient Insulin |
| Status of Beta cell | Destruction of Beta cell | No loss or Destruction of Beta cell |
| Cause | Autoimmune disorder | (1) Abnormality of gluco-receptor<br>(2) Reduced sensitivity of peripheral tissue for Insulin<br>(3) Excess of hyperglycemic hormones |
| Treatment | Insulin from external sources | Oral hypo glycaemic Drugs |

Hormonal regulation of glucose in the body:
Insulin
Tissue of Origin: Pancreatic β Cells
**1. Insulin** is a peptide hormone produced by beta cells in the pancreas,It regulates the metabolism of carbohydrates and fats by

promoting the absorption of glucose from the blood to skeletal muscles and fat tissue and by causing fat to be stored rather than used for energy.

Effect on Blood Glucose:- Lowers

**2-Glucagon**

1.It is a peptide hormone, produced by alpha cells of the pancreas that raises the concentration of glucose in the bloodstream.

2. Its effect is opposite that of insulin, which lowers the glucose concentration.

**3-Epinephrine**

Tissue of Origin: Adrenal medulla

Metabolic Effect

1. Enhances release of glucose from glycogen;
2. Enhances release of fatty acids from adipose tissue.

Effect on Blood Glucose- Raises

**4-Cortisol**

Tissue of Origin: Adrenal cortex

Effect on Blood Glucose- Raises

**5-ACTH**

Tissue of Origin:Anterior pituitary

Metabolic Effect

1. Enhances release of cortisol;
2. Enhances release of fatty acids from adipose tissue.

Effect on Blood Glucose- Raises

**6-Growth Hormone**

Tissue of Origin: Anterior pituitary

Metabolic Effect: Antagonizes Insulin

Effect on Blood Glucose- Raises

**7-Thyroxine**

Tissue of Origin: Thyroid

Metabolic Effect

1. Enhances release of glucose from glycogen
2. Enhances absorption of sugars from intestine

Effect on Blood Glucose- Raise

**What tests are used to diagnose diabetes?**

**1-Fasting plasma glucose test**

2-HbA1C test

3-Random plasma glucose test

**Lipids:** are organic compounds, found in living organisms that are soluble in nonpolar organic solvents.

Unlike the polysaccharides, proteins, and nucleic acids, lipids are not polymers. They are mostly small molecules.

**General properties of lipids:**

1- Lipids are relatively soluble in organic solvents .
2- Lipids are insoluble in water.
3- The hydrophobic (water-hating) nature of lipids

Function of lipids :

1- Lipids are a source of high energy value:
   i. Fat: 1 gram = 9 calories
   ii. Protein: 1 gram = 4 calories
   iii. Carbohydrate: 1 gram = 4 calories
2- Lipids are the constituents of membrane structure and regulate the membrane permeability
3- Lipids serve as a source of fat soluble vitamins (A, D, K and E)
4- 4-Lipids are important as cellular metabolic regulators (steroid hormones and prostaglandins).

5- Lipids protect the internal organs, serve as insulating materials and give shape and smooth appearance to the body

**Fatty acids**: are long-chain hydrocarbon molecules that terminate with carboxylic acid groups. The numbering of carbons in fatty acids begins with the carbon of the carboxylate group (COOH) .

The fatty acid chains in membranes usually contain between 14 and 24 carbon atoms; they may be saturated or unsaturated.

Essential fatty acids :The fatty acids cannot be synthesized by the body and, therefore, should be supplied in the diet are known as essential fatty acids (EFA). Chemically, they are unsaturated fatty acids, namely linoleic acid and linolenic acid . Arachidonic acid .

Functions of EFA

- Membrane structure and functions.
- Transport of cholesterol .
- Formation of lipoproteins .
- Prevention of fatty liver .

Triacylglycerols :

Triacylglycerols (triglycerides) are the esters of glycerol with fatty acids. They are insoluble in water and nonpolar solvents and known as neutral fats. TG are the most abundant group of lipids that primarily functions as fuel reserves of animals.

**Classification of Lipids:**

1- Simple Lipids
2- Simple lipids: are Esters of fatty acids with various alcohols.
3- Compound (complex) Lipids: : Esters of fatty acids with alcohols containing additional groups such as phosphate, nitrogenous base, carbohydrate, protein(lipoprotein).
4- . Derived Lipids: These are the derivatives obtained by the hydrolysis of simple and compound lipids. These include fatty acids, alcohols, mono-and diacylglycerol, lipid soluble vitamins and Steroids. The most common derived lipids are

steroids(cholesterol, bile acids, vitamin D, sex hormones, adrenocortical hormone).

Lipoproteins: Macromolecular complex of lipids and proteins .
They are the transport vehicles for lipids in the circulation

There are five types of lipoproteins :

1- **Chylomicrons**, transport dietary lipids from intestine to peripheral tissues.

2- **Very low density lipoproteins (VLDL),** transport the lipids (endogenously synthesized) mainly TG from liver to peripheral tissues).

3- **Low density lipoproteins (LDL)** ("bad" cholesterol) , transport cholesterol from liver to peripheral tissues.

4- **High density lipoproteins (HDL)** ("good" cholesterol) , carry cholesterol from peripheral tissues to liver.

5- **Intermediate density lipoproteins (IDL)**.

**Cholesterol**:

A waxy, fat-like substance made in the liver, and found in the blood and in all cells of the body. Cholesterol is important for good health and is needed for making cell walls, tissues, hormones, vitamin D, and bile acid.

Cholesterol is exclusively found in animals and is the most abundant animal sterol.

It is a major component of cell membranes and lipoproteins.

Precursor of steroid hormones, bile salts and Vitamin D

Major site of cholesterol synthesis is the liver.

**What is the most common lipid disorder?**

The most common lipid disorder is high blood cholesterol (hyperlipidemia or dyslipidemia). We also treat other less common lipid disorders such as hypertriglyceridemia, which can lead to pancreatitis, and familial hypercholesterolemia, a genetic tendency toward high blood pressure.