" Acid Base Balance"

Acid-base balance

Blood hydrogen ion concentration lies within the range pH 7.36-7.44. The terms acidosis and alkalosis in clinical practice indicate a change or a tendency to a change in the pH of the blood in a particular direction. In acidosis, there is an accumulation of acid or a loss of a base causing a fall or a tendency to a fall in the pH. The converse occurs in alkalosis.

Buffering systems

The pH of the blood is regulated and controlled by various buffering systems essentially consisting of:

1- Weak acids and bases, of which the most important is the bicarbonate:carbonic acid ratio HCO_3:H2CO_3.

2- The removal of carbon dioxide by the lungs and

3- The excretion of both acids and bases by the kidneys.

The ratio of bicarbonate to carbonic acid is normally 20:1. Alteration in this ratio alters the pH. A decrease in the ratio leads to increased acidity and vice versa.

The bicarbonate level can be altered by metabolic factors, while the carbonic acid level is subject to alteration by respiratory factors. Alteration of one is followed automatically by a compensatory alteration in the other, so that the ratio (HCO_3:H_2CO_3) and therefore the pH of the blood remains constant.

Terms and normal values

PCO_2 is a measurement of the tension or partial pressure of carbon dioxide in the blood. The normal arterial PCO_2 is 31-42 mmHg. PO_2 is a measurement of the tension or partial pressure of oxygen in the blood. The normal arterial PO_2 is 80-110 mmHg.

Standard bicarbonate is the concentration of the serum bicarbonate .Normal levels are 22-25 mmol/litre.

Alkalosis

Metabolic alkalosis

Metabolic alkalosis, a condition of base excess or a deficit of any acid other than H_2CO_3, can be caused by:

1) Excessive ingestion of absorbable alkali. This is common in patients who take proprietary indigestion remedies without medical supervision;

2) Loss of acid from the stomach by repeated vomiting or aspiration;

3) Cortisone excess, usually the result of over-administration of adrenal corticoids, but occasionally due to Cushing's syndrome.

Compensation is effected by:

(a) Retention of carbon dioxide by the lungs; and

(b) Excretion of bicarbonate base by the kidneys (alkaline urine).

Clinical features:

Alkalosis due to loss of acid from the stomach is the most common and most important. In its most typical form, it is seen in patients with pyloric stenosis in whom the loss of acid by repeated vomiting is often increased by the taking of medicines containing sodium bicarbonate. The most striking feature of severe alkalosis is apnea lasting from 5 to 30 seconds. Subclinical degrees of alkalosis are recognizable only by a raised standard bicarbonate concentration. Severe alkalosis may result in renal epithelial damage and consequent renal insufficiency.

Treatment:

Metabolic alkalosis without hypokalaemia seldom requires direct treatment. The cause of the alkalosis should be removed where possible and a high urinary output encouraged.

Hypokalaemic alkalosis

Hypokalaemic alkalosis is seen in patients who have lost potassium and acid owing to repeated vomiting from pyloric stenosis. The low serum potassium causes potassium to leave the cell and be replaced by Na^+ and H^+ ions. The shift of H^+ ion into the cell causes intracellular acidosis and increases the cellular acidosis of the kidney cells.

Treatment: When hypokalaemia is sufficient to cause a metabolic alkalosis, the losses can be massive (> 1000 mmol). Replacement can be achieved gradually and relatively safely by supplementing intravenous fluids with 40 mmol/litre of KCl if the urine output is adequate.

Respiratory alkalosis

Respiratory alkalosis, a condition where the arterial PCO_2 is below the normal range of 31-42 mmHg, is caused most commonly in surgical practice by excessive pulmonary ventilation carried out upon an anesthetised patient. Other causes are hyperventilation, hyperpyrexia. Compensation, which depends on increased renal excretion of bicarbonate, usually is inadequate. During anaesthesia alkalosis is accompanied by pallor and a fall in blood pressure. In severe cases respiratory arrest follows.

Treatment: Respiratory suppression due to alkalosis is treated by insufflation of carbon dioxide.

Acidosis

Metabolic acidosis

Metabolic acidosis, a condition where there is a deficit of base or an excess of any acid other than H_2CO_3, occurs as a result of:

1-Increase in fixed acids due to the formation of ketone bodies as in diabetes or starvation, the retention of metabolites in renal insufficiency, and the rapid increase of lactic and pyruvic acids by anaerobic tissue metabolism, following cardiac arrest.

2-Loss of bases such as occurs in sustained diarrhea, ulcerative colitis, gastrocolic fistula, a high intestinal fistula or prolonged intestinal aspiration.

Clinical features:

In severe acidosis, the leading sign is rapid, deep, noisy breathing. The hyperpnea is due to over stimulation of the respiratory center by the reduction in pH of the blood, and the physiological purpose of overbreathing is to eliminate as much as possible of the acid substance H_2CO_3.The urine is strongly acidic.

Treatment: The commonest cause of an acute peroperative metabolic acidosis is tissue hypoxia and the correct treatment is restoration of adequate tissue perfusion. Treatment with bicarbonate solutions will correct the measured metabolic acidosis but not treat the problem. Indeed, as bicarbonate is rapidly converted into carbon dioxide intracellular acidosis may, in fact, get worse. The acute acidosis seen in prolonged cardiac arrest may require the infusion of 50 mmol of 8.4 per cent sodium bicarbonate solution.

Respiratory acidosis

Respiratory acidosis, a condition where the PCO_2 is above the normal range, is caused by impaired alveolar ventilation. This problem most commonly occurs when there is inadequate ventilation of the anesthetised patient. There is also a risk of respiratory acidosis when the patient undergoing surgery already has pre-existing pulmonary disease (e.g. chronic bronchitis or emphysema).

The anion gap

The anion gap measures the difference or gap between the negatively and positively charged electrolytes in the blood. If the anion gap is too high, the blood is more acidic than normal. If the anion gap is too low, the blood is not acidic enough. Therefore, it is used to establish the cause of a

metabolic acidosis. Anion gap = (Na + K) ¬ (HCO3 + Cl). The normal anion gap is 10-16 mmol/litre. An increased anion gap acidosis is seen in metabolic acidosis due to ketoacidosis, lactic acidosis, poisoning (salicylates) and renal failure. A normal anion gap acidosis is seen in gastrointestinal or renal bicarbonate losses.

Liver Function Tests

(Seventh Lab)

The liver is a large ‘metabolic factory’. It has a significant role in metabolism, digestion, detoxification, and elimination of substances from the body, therefore it like the kidneys, excretes the end products of metabolism.

Liver function tests are blood tests used to help diagnose and monitor liver disease or damage. The tests measure the levels of certain enzymes and proteins in your blood.

- **Albumin**, a protein made in the liver.
- **Total protein.** This test measures the total amount of protein in the blood.
- **ALP** (alkaline phosphatase), **ALT** (alanine transaminase) , **AST** (aspartate aminotransferase), and gamma-glutamyl tansferase (GGT). These are different enzymes made by the liver.
- **Bilirubin**, a waste product made by the liver.
- **Prothrombin time (PT)**, a protein involved in blood clotting.

What are they used for?

Liver function tests are most often used to:

- Help diagnose liver diseases, such as hepatitis
- Monitor treatment of liver disease. These tests can show how well the treatment is working.
- Check how badly a liver has been damaged by disease, such as cirrhosis

- Monitor side effects of certain medicines

Why do I need liver function testing?

You may need liver function testing if you have symptoms of liver disease. These include:

- Jaundice, a condition that causes your skin and eyes to turn yellow
- Nausea and vomiting
- Diarrhea
- Abdominal pain
- Dark-colored urine
- Light-colored stool
- Fatigue

You may also need these tests if you have certain risk factors. You may be at higher risk for liver disease if you:

- Have a family history of liver disease
- Have alcohol use disorder, a condition in which you have difficulty controlling how much you drink
- Think you have been exposed to a hepatitis virus
- Take medicines that may cause liver damage

What is the normal range for liver function tests?

Normal ranges vary between different sexes and body sizes, as well as between different laboratories. On average, normal ranges are:

- Alanine transaminase (ALT): 0 to 45 IU/L.

- Aspartate transaminase (AST): 0 to 35 IU/L.
- Alkaline phosphatase (ALP): 30 to 120 IU/L.
- Gamma-glutamyltransferase (GGT): 0 to 30 IU/L.
- Bilirubin: 0.3 to 1.3 mg/dL.
- Prothrombin time (PT): 10.9 to 12.5 seconds.
- Albumin: 4.0 to 6.0 g/dL.
- Total proteins: 3 to 8.0 g/dL.

Bilirubin test

Bilirubin is a yellowish substance made during your body's normal process of breaking down old red blood cells. Bilirubin is found in bile, a fluid your liver makes that helps you digest food.

Bilirubin testing is usually one of a group of tests to check the health of your liver. Bilirubin testing may be done to: Investigate jaundice — a yellowing of the skin and eyes caused by high levels of bilirubin. This test is commonly used to measure bilirubin levels in newborns with infant jaundice.

jaundice occurs when bilirubin production exceeds the hepatic capacity to excrete it. This may be because:

_ An increased rate of bilirubin production exceeds normal excretory capacity of the liver (**prehepatic jaundice**).

_ The normal load of bilirubin cannot be conjugated and/or excreted by damaged liver cells (**hepatic jaundice**).

_ The biliary flow is obstructed, so that conjugated bilirubin cannot be excreted into the intestine and is regurgitated into the systemic circulation (**posthepatic jaundice**).

Many healthy babies get jaundice because Red cell destruction, together with immature hepatic processing of bilirubin. Newborn jaundice is usually not harmful and clears up within a few weeks. But in some cases, high bilirubin levels can lead to brain damage.

Why do I need a bilirubin blood test?

Your provider may order a bilirubin blood test:

- If you have symptoms such as jaundice, dark urine, or stomach pain. These could be symptoms of hepatitis, cirrhosis, or other liver diseases. They may also be signs of gallbladder disease.
- To find out if there is a blockage in the bile ducts, the tubes that carry bile from your liver.
- To check on an existing liver disease or disorder.
- To diagnose disorders related to problems with breaking down red blood cells. High bilirubin levels in the bloodstream may be a sign of a condition called hemolytic anemia. In this condition, the body destroys red blood cells faster than it makes them.

A bilirubin test measures total bilirubin. It can also give levels of two different types of bilirubin: unconjugated and conjugated.

Unconjugated ("indirect") bilirubin. This is the bilirubin created from red blood cell breakdown. It travels in the blood to the liver.

Conjugated (“direct”) bilirubin. This is the bilirubin once it reaches the liver and undergoes a chemical change. It moves to the intestines before being removed through your stool.

When severe jaundice goes untreated for too long, it can cause a condition called kernicterus. **Kernicterus** is a type of brain damage that can result from high levels of bilirubin in a baby’s blood. It can cause athetoid cerebral palsy and hearing loss. Kernicterus also causes problems with vision and teeth and sometimes can cause intellectual disabilities. Early detection and management of jaundice can prevent kernicterus.

Quantitative Determination of Glucose

Glucose is a monosaccharide. It is central molecule in carbohydrate metabolism. Stored as glycogen in liver and skeletal muscle.

For all practical purposes, glucose is the only sugar that is present in the blood.

Glucose is absorbed by the body cells and is the major source of cellular energy.

Clinical significance for glucose estimation:

Oxidation of glucose present in the peripheral blood represents the major source of cellular energy in the body. Dietary glucose is stored in the liver in the form of glycogen or converted to fatty acids and stored in the adipose tissues. The accurate estimation of glucose is important in the diagnosis and management of hyperglycemia and hypoglycemia. The most frequent cause of hyperglycemia is diabetes mellitus resulting from a deficiency in insulin secretion or action.

Hypoglycemia may be the result of an insulinoma, insulin administration, inborn error of carbohydrate metabolism or fasting.

The concentration of glucose in the blood is controlled within narrow limits by many hormones, the most important of which are produced by the pancreas (insulin and glucagon).

Glucose measurement in urine is used as diabetes screening procedure and to aid in the evaluation of glucosuria to detect renal tubular defect and in the management of diabetes mellitus.

Glucose measurement in cerebrospinal fluid (CSF) is used for evaluation of meningitis, neoplastic involvement of meninges and other neurological disorders.

• For blood glucose estimation, blood is collect in fluoride containing vial (if not centrifuged immediately). Fluoride inhibit glycolysis by inhibiting enolase enzyme.

• In CSF, bacteria & other cells are also present so analyzed immediately.

• For glucose estimation from urine, add 5ml glacial acetic acid as preservative to inhibit bacterial growth.

Methods used to measure blood glucose level

- Although a number of methods are used for glucose determination, commonly used two methods are discussed here.
- These can be grouped into two categories- chemical and enzymatic.
- **Chemical method**
    - Folin-Vui method
    - Ortho-Toluidine method
- **Enzymatic method**
    - Hixokinase method.
    - Glucose dehydrogenase method.
    - GOD-POD method. (Glucose oxidase method)

- Enzymatic methods provide maximum degree of glucose specificity, hence are very good in estimating true blood glucose.
- For this method, only blood plasma or serum is used.
- The glucose remains stable for 24 hours at 2-8^{o}C if serum or plasma is prepared within 30 minutes after collection.
- Glucose is determined after enzymatic oxidation in the presence of glucose oxidase (GOD).

- The enzyme peroxidase (POD) catalyzes the reaction of hydrogen peroxide with phenol and 4 aminoantipyrine to a red-violet dye as indicator.
- The intensity of the color formed is measured spectrophotometrically which is directly proportional to the blood glucose level.

Glucose + $2H_2O$ + O_2 ----^{GOD}-----→ Gluconic acid + H_2O_2

$2H_2O_2$ + phenol + 4-aminoantipyrine ----^{PAP}-----→ Quinoneimine + $4H_2O$

Procedure:

	Blank	Standard	Sample
Reagent(Working solution)	1 ml	1 ml	1 ml
Standard		10 µl	
Sample			10 µl

Mix and incubate for15 minutes at 37°C or 25 minutes at room temperature (15-25 °C).

Read the absorbance of sample and standard against blank at 500 nm.

Calculation: Result= $\frac{Absorption\ of\ Sample}{Absorption\ of\ Standard} \times Standard\ Conc..$

The normal value is 70-110 mg/dl (fasting), 110-180 mg/dl (random).

MEASUREMENT OF GLUCOSE IN URINE

Methods:

1.Qualitative

2.Quantitative

3.Semi-quantitative

1) QUALITATIVE METHOD:

• It is determination by Benedict test

2) QUANTITATIVE MATHOD:

• It Is Determination By Hexokinase & Glucose Dehydrogenase

3)SEMI QUANTITATIVE MATHOD:

• It is determination by Glucose Oxidase strip test (Urine strip).

Normal range of glucose in urine up to 15 mg/dl.

Hemoglobin A1C (HbA1c) Test

A hemoglobin A1C (HbA1C) test is a blood test that shows what your average blood sugar (glucose) level was over the past two to three months. It is useful as an aid in management and monitoring of the long term-glycemic status in patients with diabetes mellitus and it give the indication of controlled and uncontrolled diabetes.

Glucose in your blood sticks to hemoglobin, a protein in your red blood cells by a non-enzymatic reaction. As your blood glucose levels increase, more of your hemoglobin will be coated with glucose. An A1C test measures the percentage of your red blood cells that have glucose-coated hemoglobin.

- Glucose sticks to hemoglobin for as long as the red blood cells are alive.
- Red blood cells live about three months.

Uses:

An A1C test may be used to screen for or diagnose:

- **Type 2 diabetes.** With type 2 diabetes your blood glucose gets too high because your body doesn't make enough insulin to move blood sugar from your bloodstream into your cells, or because your cells stop responding to insulin.
- **Prediabetes.** Prediabetes means that your blood glucose levels are higher than normal, but not high enough to diagnosed as diabetes. Lifestyle changes, such as healthy eating and exercise, may help delay or prevent prediabetes from becoming type 2 diabetes.

The higher the HbA1c, the greater the risk of developing complications such as problems with your eyes and kidneys.

To diagnose diabetes or prediabetes, the percentages commonly used are:

- **Normal:** A1C below 5.7%
- **Prediabetes:** A1C between 5.7% and 6.4%
- **Diabetes:** A1C of 6.5% or higher

Blood sample collection

Blood sampling or blood collection method is an essential procedure in modern medicine. The blood can be taken from a vein where it is called venous blood collection or arteries where it is called arterial blood collection. Minute quantities of blood can be taken from various sites by pricking the skin.

TECHNIQUE OF BLOOD COLLECTION :

Blood is collected from the vein for various hematological investigations. In order to obtain accurate and precise results in the laboratory which will help the clinician to make a correct diagnosis of the patient's disease, it is of paramount importance to collect the blood sample in a correct manner. Each sample is sent to the laboratory accompanied by a laboratory requisition form filled in by the clinician. Brief clinical details and any other relevant information must be mentioned on the form. Prior to blood sample collection it is essential to check the patient's identity and make sure that it corresponds to the name and other details mentioned on the requisition form. Blood can be withdrawn from the vein, usually the antecubital vein on the forearm (Venous blood) or from the finger or heel (Capillary blood). Venous blood is preferred. It can be collected using a syringe and needle or a vacuum tube. Both these methods will be described seperately.

COLLECTION OF VENOUS BLOOD :

Blood is usually withdrawn from the antecubital vein or any other vein which is well identified on the forearm. The vein selected should be large, readily accessible, and sufficiently close to the surface to be seen and palpated.

Preparation of venipuncture site Clean the skin of the area around the identified vein with 70% isopropyl alcohol in a circular fashion beginning at the

site and moving outward. Allow to dry spontaneously. Do not touch the venipuncture site after it has been cleaned. Apply a tourniquet 3-4 inches above the venipuncture site. Ask the patient to make a fist a few times. Veins suitable for puncture will then become more apparent. Veins can become distended and easier to enter by allowing the arm to hang down for 2 or 3 minutes or by gently slapping the site of puncture.

COLLECTION OF VENOUS BLOOD USING A SYRINGE

1. Clean hands thoroughly with soap and water.

2. Write the name and hospital number of the patient on the tube in which blood is to be collected. A printed label with these particulars can also be used for patient identification.

3. Place the needle into the syringe. Keep the cap over the needle capped till it is used. Check that the syringe works smoothly.

4. With the needle bevel up and parallel to the surface of the skin insert it into the vein. Appearance of blood in the hub of the needle indicates that the needle has successfully entered the vein. Release the tourniquet as soon as blood enters the syringe.

5.Withdraw the piston slowly to avoid frothing.

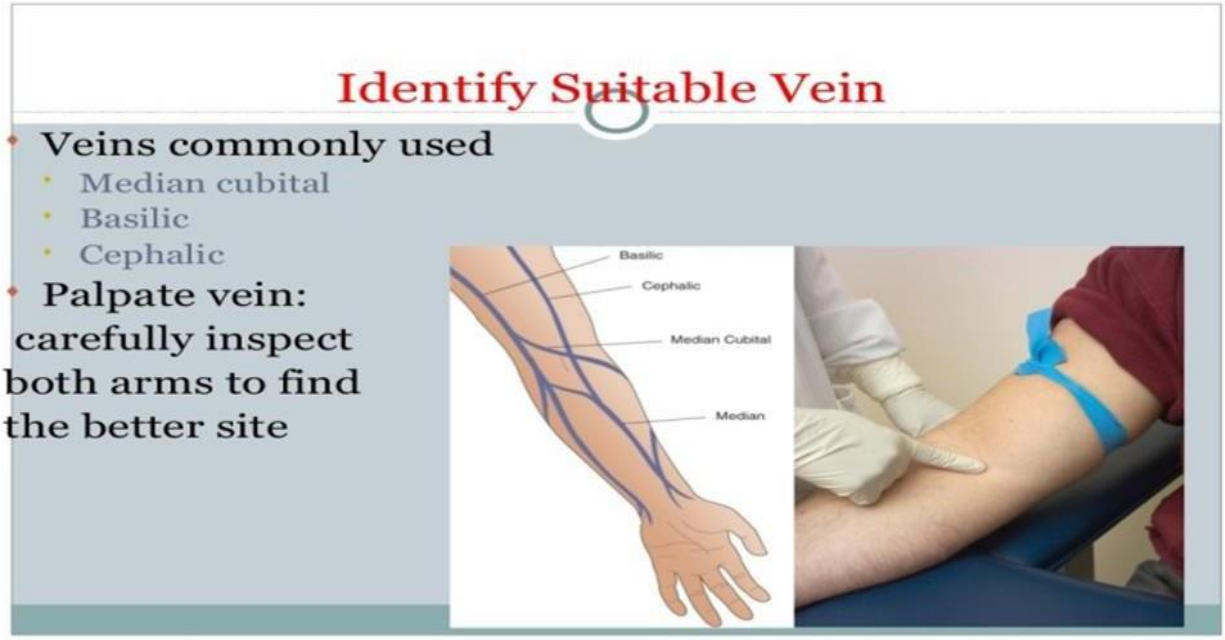
6. After obtaining the requisite amount of blood, place a sterile gauze pad over the point where the needle entered the skin and deftly withdraw the needle simultaneously while applying pressure over the site.

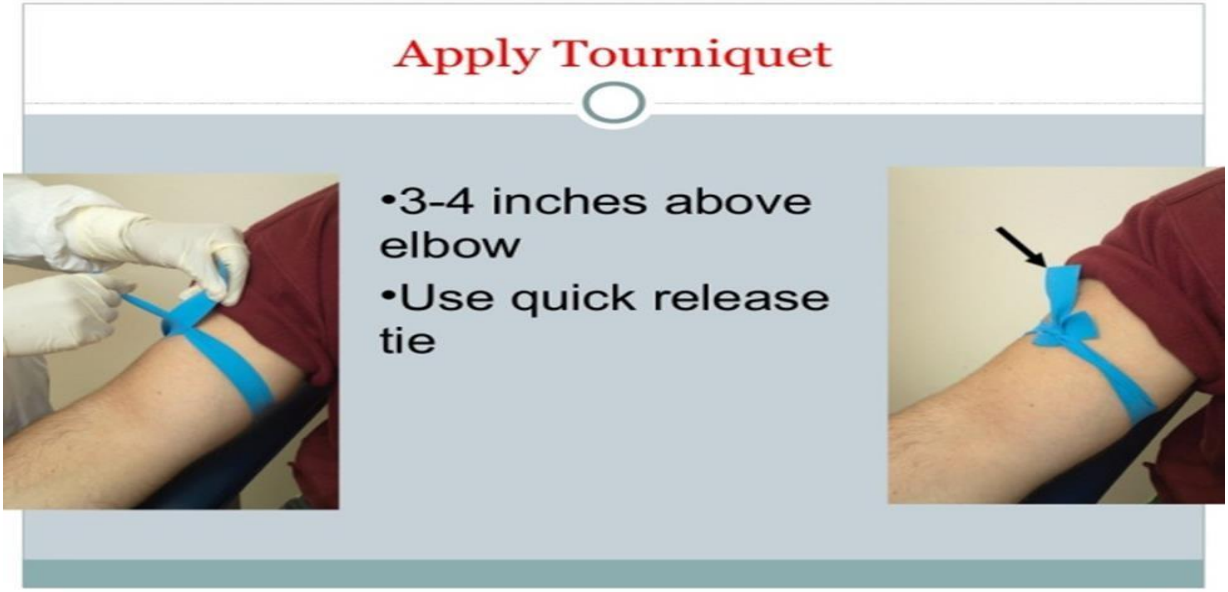
7. Deliver the blood gently into the specified receiver. Cap it firmly to prevent leakage.

8. Maintain light pressure on the gauze pad over venipuncture site till the bleeding stops and then cover the puncture site with a small adhesive dressing.

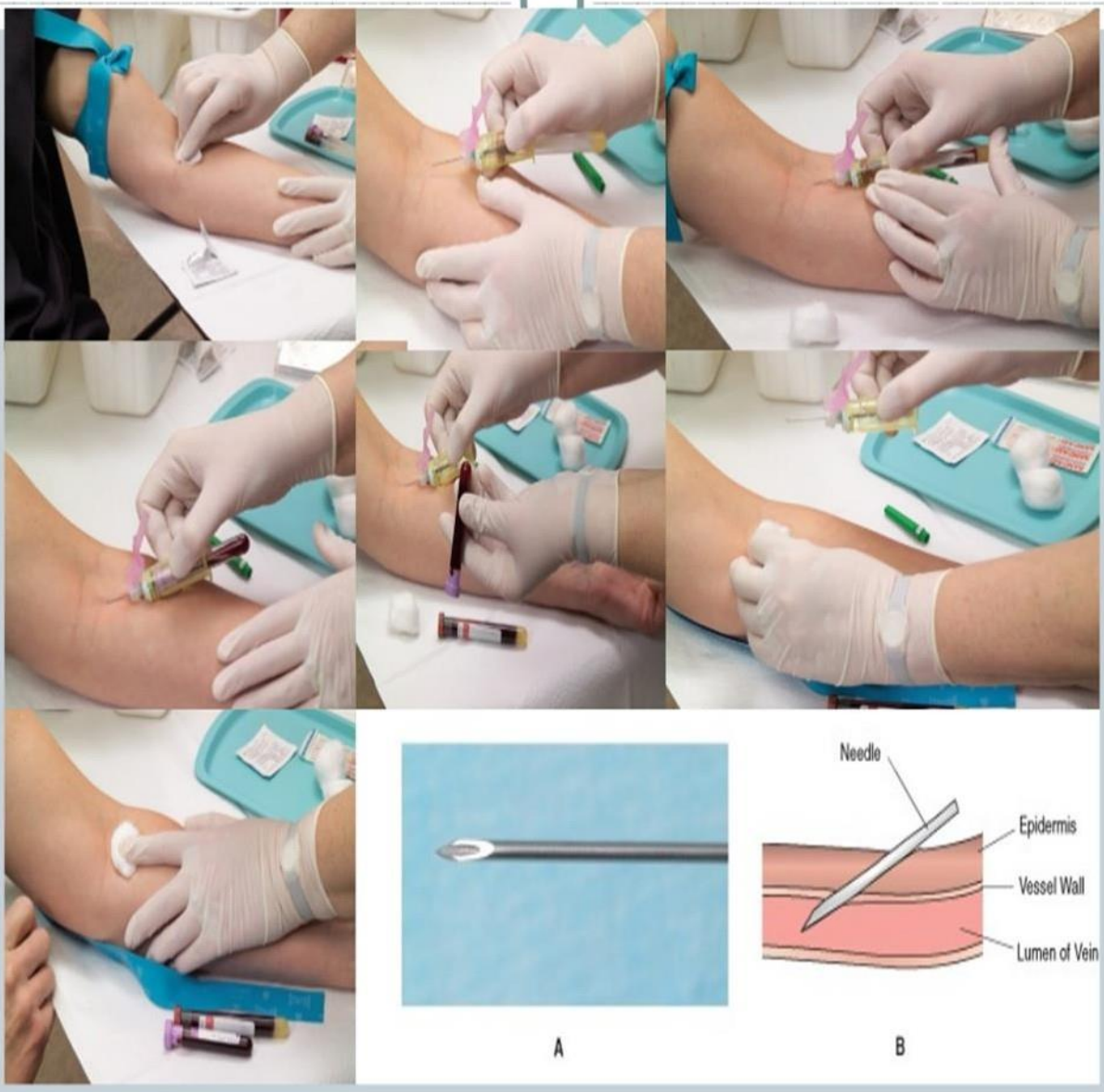
9. Destroy the needle in a special device (needle destroyer) immediately after use. DONOT break, bend or recap needles after use.

10. Place the used swab, syringe and any other contaminated material in a puncture resistant container for adequate disposal.





Perform Venipuncture



TYPES OF VACUUM TUBES

Vacuum tubes with different colored caps are available. Each contains a different anticoagulant and is used for various hematological tests as described below

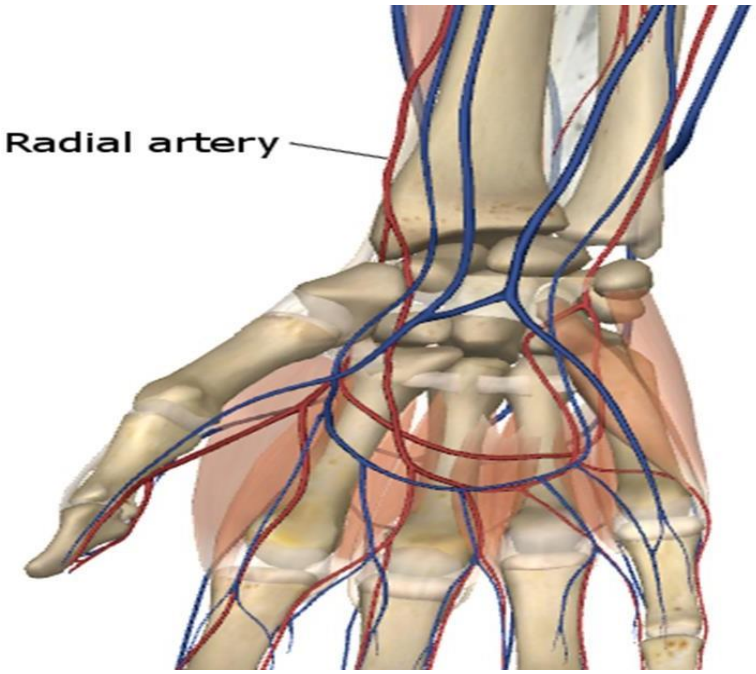
Color of cap	Anticoagulant	Test
Purple	EDTA	counts complete blood
Red	_	for tests which need serum
Blue	Sodium citrate	coagulation tests
Gray	fluoride	blood sugar

ARTERIAL BLOOD SAMPLE

The artery is a less common site for blood collection. The most common reason for taking blood from the artery is arterial blood gas (ABG) analysis.

The most common puncture site is the radial artery at the wrist and the femoral artery in the groin. Blood can also be drawn from an arterial catheter.

In contrast to venous blood sampling, the withdrawal of a sample from an artery requires training.



COLLECTION OF CAPILLARY BLOOD

This can be obtained by skin puncture with a needle or lancet and is specially used in small children or very obese adults in whom venepuncture fails. Samples can be used for making peripheral blood films, performing hematocrit/Hb and point of care testing. In adults capillary blood sample can be obtained from the lateral side of tip of the 3rd or 4th finger while in infants the sample can be obtained by a deep puncture of the plantar surface of the heel.

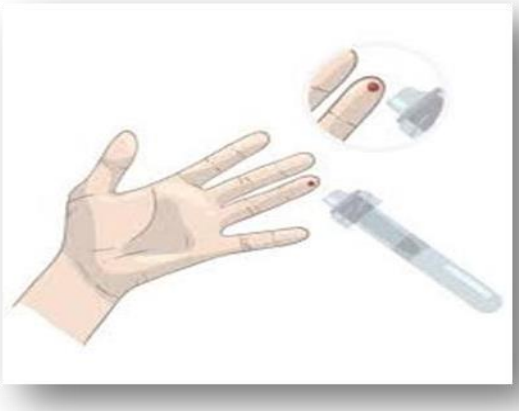
PROCEDURE

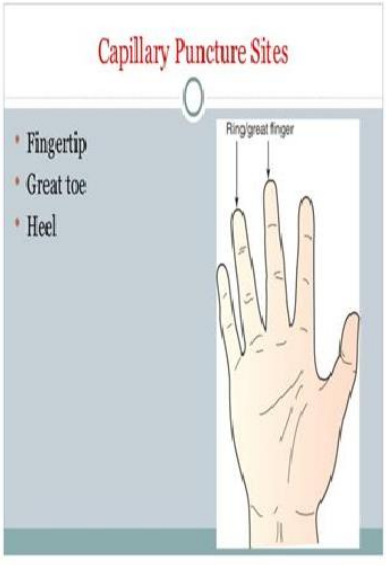
1. Clean the area with 70% alcohol and allow it to dry spontaneously. Puncture the skin to a depth of 2-3 mm with a sterile disposable lancet/needle.

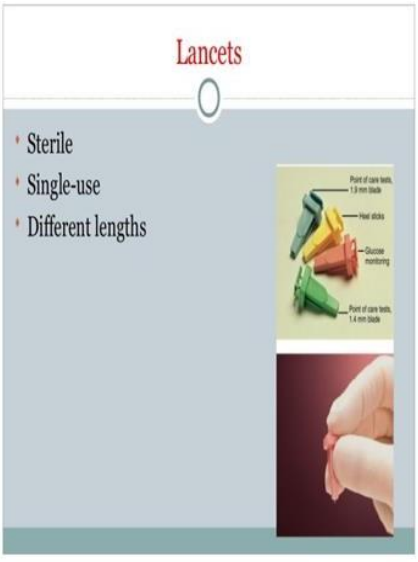
2. Wipe the first drop of blood and squeeze gently to allow free flow of blood and collect the sample. In an adequate puncture, large drops of blood should exude spontaneously. Do not squeeze firmly as this gives unreliable results.

FINGER STICK SAMPLING

The best locations for fingersticks are the center of the finger pads of the 3rd (middle) and 4th (ring) fingers of the non-dominant hand. The tip of the finger or the center of the finger should not be used. The sides of the finger where there is less soft tissue should also be avoided.







ANTICOAGULANTS USED IN HEMATOLOGY

The anticoagulants used commonly in the hematology laboratory are

EDTA: Ethylene Diamine Tetra Acetic acid

Sodium citrate

Heparin

EDTA: This is used for complete blood counts. The sodium and potassium salts can both be used but the dipotassium salt is preferred as it is more soluble. The dilithium salt can also be used but like the disodium salt is less soluble. The dipotassium salt is used in solid form. The International Council for Standardization in Hematology recommends use of the dipotassium salt at a concentration of 1.50±0.25mg/ml of blood. Coating the inside of a blood collection vial with a thin layer of EDTA improves the speed of uptake by blood. It exerts it's action by removing calcium which is essential for coagulation.

If EDTA is used in excess, it causes shrinkage of red cells and leucocytes. A significant decrease in hematocrit and increase in MCHC also occur. Platelets swell and disintegrate causing a false high count. It is thus important to add the correct amount of blood to the vial.

Trisodium citrate: This is used in coagulation studies in a ratio of 9 volumes of blood to one volume of anticoagulant (32g/L) (0.5ml citrate+4.5ml blood). It binds calcium thus preventing coagulation. It can also be used for estimation of ESR by Westergren method in a ratio of 4 volumes of blood to 1 volume of sodium citrate.

Heparin: The sodium or lithium salt of heparin is used at a concentration of 10-20IU/ml of blood for osmotic fragility and for red cell enzyme studies like glucose-6-phosphate dehydrogenase. It does not change red cell size and is the best anticoagulant for osmotic fragility. It can also be used for immunophenotyping.

Heparin is not suitable for complete blood counts as it induces platelet and leucocyte clumping. It should also not be used for making peripheral smears as it gives a faint blue background color after staining smears by Romanowsky dyes.

TYPES OF SAMPLES COLLECTED FOR HEMATOLOGICAL INVESTIGATIONS

Whole blood: This is used for performing complete blood counts including reticulocyte count and for making peripheral blood films.

Serum: If blood is allowed to clot at room temperature, a straw colored fluid appears which is called serum. This may be admixed with red cells which can be removed by centrifugation at 1200g for 10 minutes. It is used for various biochemical tests and also serum protein electrophoresis to diagnose plasma cell disorders such as multiple myeloma. Serum lacks coagulation factors.

Plasma: This is obtained by centrifugation of anticoagulated blood and is used for coagulation studies.

" Calcium Metabolism "

Calcium, phosphorus, and magnesium are important constituents of bone and their metabolism is interrelated. The adult body contains 1-1.5 kg calcium, over 98% of this being found in the skeleton, where it has an important structural function.

In bones, calcium occurs mainly as hydroxyapatite crystals composed of calcium and phosphate with small amounts of hydroxide and carbonate. Only a minor proportion of skeletal calcium(0.1%) is rapidly exchangeable with plasma, although remodeling of bone results in the turnover of nearly 20% of skeletal calcium each year. Approximately 1% of body calcium is present in the extracellular fluid, where functions include the regulation of neuromuscular excitability, and acting as a cofactor for clotting enzymes.

Regulation of calcium metabolism:

Calcium in the gastrointestinal tract(GIT) originates from the diet and also from the secretions. Approximately half is absorbed, mainly in the upper small intestine, by active transport. Up to 250 mmol calcium is filtered daily by the kidney, the majority being reabsorbed in the proximal tubule and loop of henle, urinary excretion is normally 2.5-7.5 mmol/L, depending on intake. Small amounts are lost in sweat, these being insignificant unless profuse sweating occurs for a prolonged period.

Parathyroid hormone(PTH), is a key regulatory hormone of calcium metabolism whose secretion is stimulated by low plasma calcium concentration and by low plasma magnesium concentration. The secretion of PTH is inhibited by increased calcium levels.

The main effect of PTH is to raise plasma calcium concentrations through actions on bone, the kidney and indirectly the gastrointestinal tract. In the bones, PTH stimulates osteoclast activity, while in the kidney it increases the reabsorption of calcium, also PTH stimulates the formation of calcitriol, which then acts on the gut to increase calcium and phosphate absorption.

Calcitonin lowers plasma calcium concentrations by inhibiting bone resorption and renal tubular calcium reabsorption.

Plasma calcium:

Three forms of calcium occur in the circulation, almost 50% as free ions (Ca^{2+}),40% bound to plasma proteins, particularly albumin, and the remainder complexed to other ions (phosphate, citrate and bicarbonate).

Protein-bound calcium increases if plasma protein concentrations, particularly albumin, are high.

Ionized calcium levels are affected by blood pH, particularly if changes in hydrogen ion concentrations are acute. Hydrogen ions compete with calcium for binding sites on albumin.

Hypercalcaemia:

Hypercalcaemia is relatively common, occurring in up to 3% of hospital patients, although it is found in less than 0.5% of a healthy ambulatory population.

Plasma total calcium levels above 3.0 mmol/l (12.0 mg/dl) are usually associated with symptoms, these including fatigue, depression, anorexia, vomiting and constipation. Defects of renal tubular function, particularly polyuria, occur and Electrocardiograph (ECG) abnormalities are also seen. If hypercalcaemia is chronic, soft tissue calcification and renal tract stones develop.

The commonest cause of hypercalcaemia is primery hyperparathy-roidism, the second most frequent being malignant disease; together, these are responsible for over 90% of cases of hypercalcaemiain adults. Other causes of hypercalcaemia are excess vitamin D, and high bone turnover.

Hypocalcaemia:

Hypocalcaemia may be accompanied by increased neuromuscular excitability with tetany, muscle cramps occurring. Prolonged hypocalcaemia is

also associated with cataracts, mental retardation, and increased intracranial pressure.

The main causes of hypocalcaemia are hypoalbuminaemia, reduced parathyroid hormone action, and defect in active vitamin D.

Metabolic bone diseases:

In healthy adult remodeling of bone is a continuous process, the constituent parts of which, resorption and new bone formation, are in balance. New bone formation involves the production of bone matrix (osteoid) by osteoblasts, followed by mineralization. Osteoclasts are responsible for bone resorption. Imbalances in these processes lead to metabolic bone diseases, osteoporosis, osteomalacia and rickets.

Osteoporosis is the most common metabolic bone disease, characterized by a reduction of bone mass which is not accompanied by changes in the ratio of mineral to osteoid. Biochemical tests are generally unhelpful in osteoporosis, calcium, phosphate and alkaline phosphatase usually being normal.

Osteomalacia and rickets are characterized by defective mineralization of the organic matrix of bone, most often resulting from vitamin D deficiency.

Carbohydrate Metabolism

Objectives

- Study utilization of glucose and other carbohydrates in the body
- Study the various mechanisms and fate of glucose in the body
- Study the energetics of the various mechanisms

*Fates of dietary glucose

The major source of dietary carbohydrate for humans is starch from consumed plant materials. This is supplemented with a small amount of glycogen from animal tissue, disaccharides such as sucrose from products containing refined sugar and lactose in milk.

Digestion in the gut converts all carbohydrate to monosaccharides which are transported to the liver and converted to glucose. The liver has a central role in the storage and distribution within the body of all fuels, including glucose.

Glucose in the body undergoes one of three metabolic fates :

1- It is catabolised to produce ATP

This occurs in all peripheral tissues, particularly in brain, muscle and kidney.

2- It is stored as glycogen

This storage occurs in liver and muscle.

3- It is converted to fatty acids

Once converted to fatty acids, these are stored in adipose tissue as triglycerides.

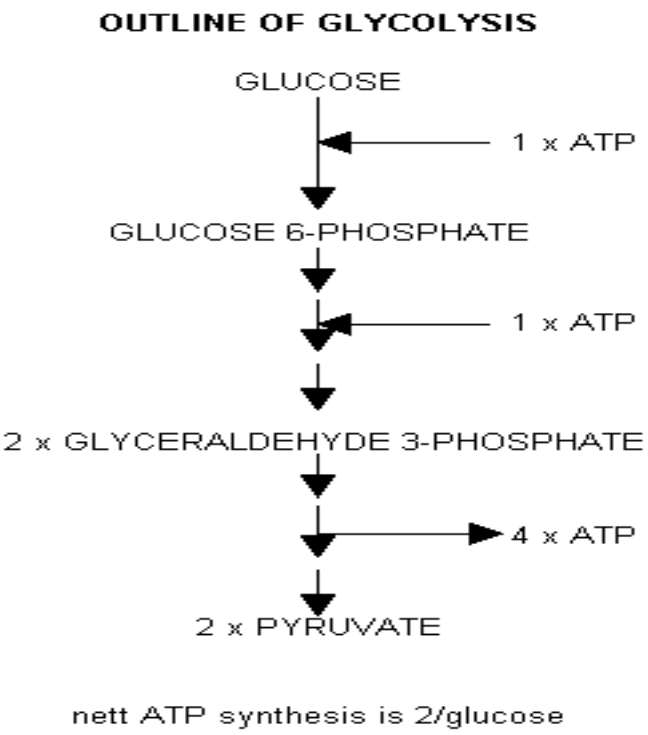
Glucose Metabolism

Glucose will be oxidized by all tissues to synthesis ATP. The first pathway which begins the complete oxidation of glucose is called **glycolysis.**

Glycolysis:

This pathway cleaves the six carbon glucose molecule ($C_6H_{12}O_6$) into two molecules of the three carbon compound pyruvate ($C_3H_3O_3^-$). This oxidation is coupled to the net production of two molecules of ATP/glucose.

The diagram below shows an outline of glycolysis.



One oxidation reaction occurs in the latter part of the pathway. It uses NAD as the electron acceptor. This cofactor is present only in limited amounts and once reduced to NADH, as in this reaction, it must be re-oxidized to NAD to permit continuation of the pathway.

This re-oxidation occurs by one of two methods :

Anaerobic glycolysis

pyruvate which resulted from glycolysis is reduced to a compound called lactate

This single reaction occurs in the absence of oxygen (anaerobically) and is ideally suited to utilization in heavily exercising muscle where oxygen supply is often insufficient to meet the demands of aerobic metabolism. The reduction of pyruvate to lactate is coupled to the oxidation of NADH to NAD.

The formation of lactate as an end product from glucose extracts only a relatively small amount of the bond energy contained in glucose. Accumulation of lactate (actually lactic acid) also causes a reduction in intracellular pH.

The lactate formed is removed to other tissues and dealt with by one of two mechanisms :

1- It is converted back to pyruvate

2- It is converted back to glucose in the liver.

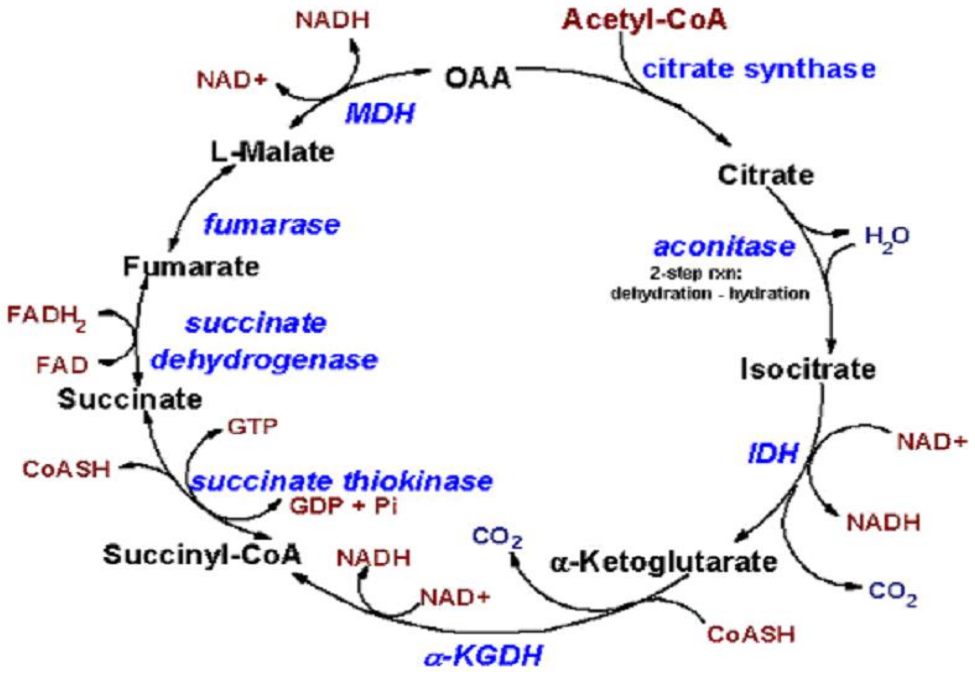
* Aerobic metabolism of glucose

pyruvate is transported inside **mitochondria** and oxidized to a compound called **acetyl coenzyme A** (abbreviated to "acetyl CoA"). This is an oxidation reaction and uses NAD as an electron acceptor.

By a further series of reactions collectively called the **citric acid cycle (CAC)** or also known tricarboxylic acid cycle (TCA), acetyl CoA is oxidized ultimately to CO_2.

Citric acid cycle is a chain of reactions occurring in the mitochondria, through which almost all living cells produce energy in aerobic respiration. It uses oxygen and gives out water and carbon dioxide as products.

These reactions are coupled to a process known as the **electron transport chain** which has the role of harnessing chemical bond energy from a series of oxidation/reduction reactions to the synthesis of ATP and simultaneously re-oxidizing NADH to NAD.



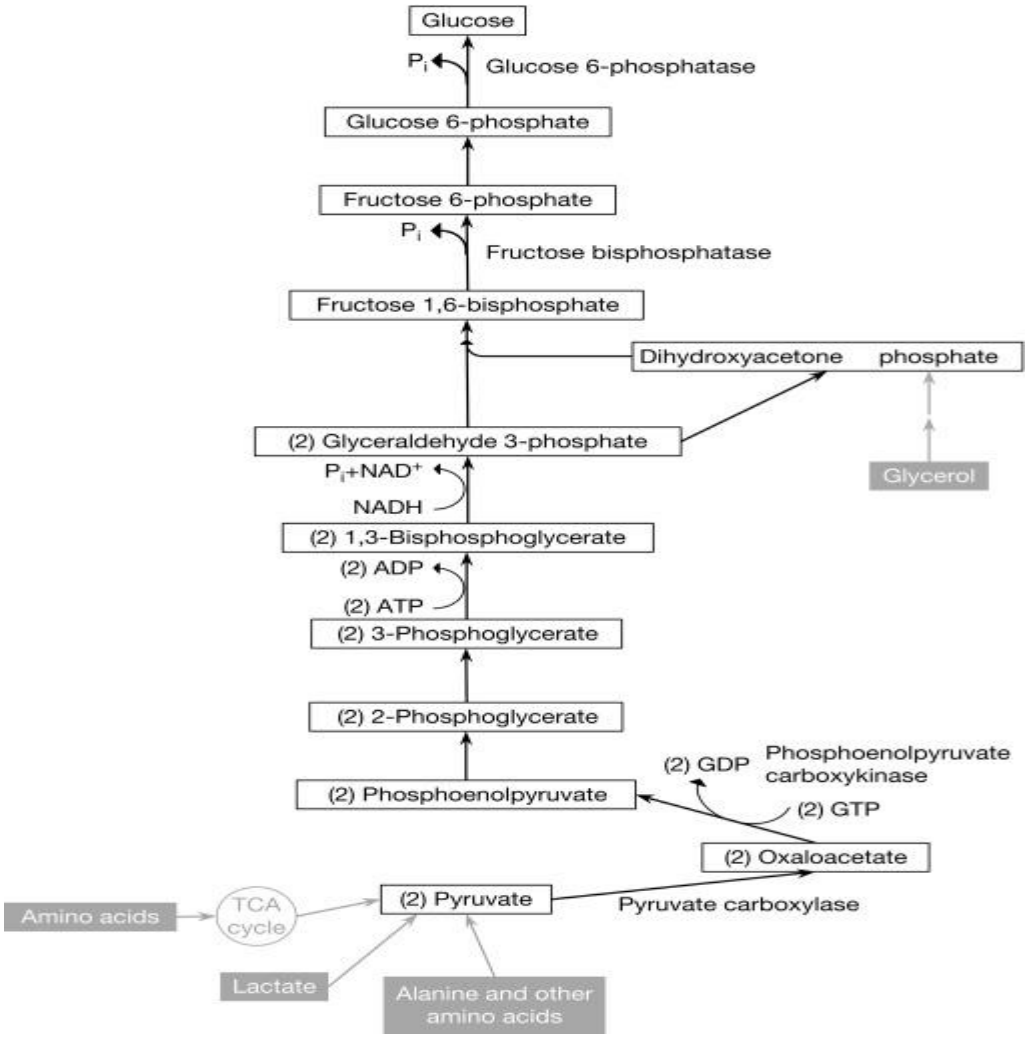
Gluconeogenesis

Gluconeogenesis refers to **synthesis of new glucose from noncarbohydrate precursors, provides glucose when dietary intake is insufficient or absent**. It also is essential in the regulation of acid-base balance, amino acid metabolism, and synthesis of carbohydrate derived structural components.

During a prolonged fast or vigorous exercise, glycogen stores become depleted, and glucose must be synthesized by gluconeogenesis principally in the liver and kidneys in order to maintain blood glucose levels from precursors such as glycerol, lactate, pyruvate, and glucogenic

amino acids. Gluconeogenesis is stimulated by the diabetogenic hormones (glucagon, growth hormone, epinephrine, and cortisol).

Insulin and glucagon are the most important hormones regulating hepatic gluconeogenesis. Hepatic glucose production is a sum of gluconeogenesis, which is the formation of glucose from pyruvate or other 3- or 4-carbon compounds, and glycogenolysis, which is the breakdown of glycogen to glucose.

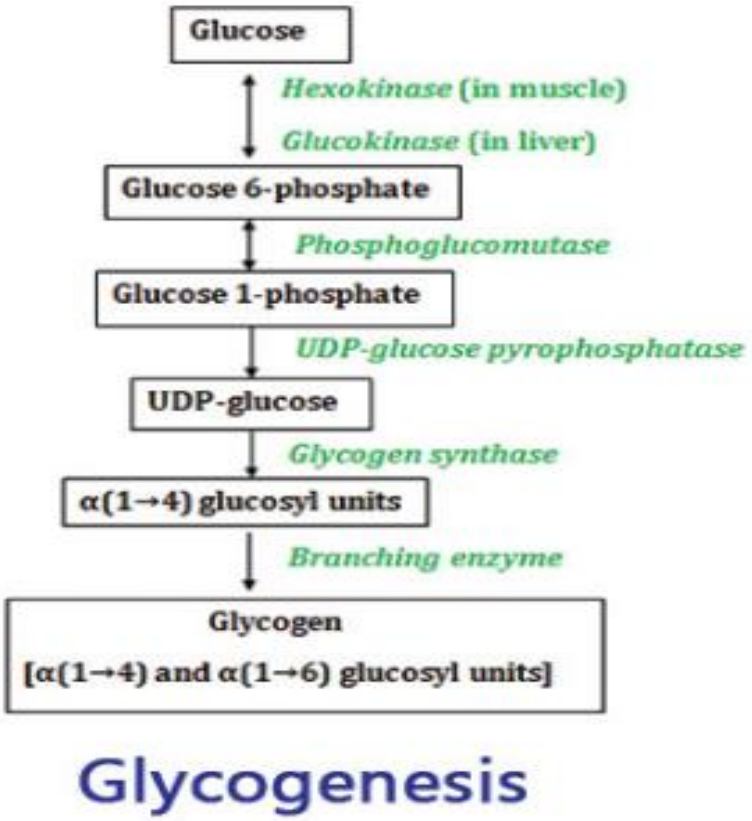


glycogenesis

glycogenesis, the formation of glycogen, the primary carbohydrate stored in the liver and muscle cells of animals, from glucose. Glycogenesis takes place when blood glucose levels are sufficiently high to allow excess glucose to be stored in liver and muscle cells.

When the cells have depleted all their glucose and are not receiving more from the body, they can turn to their stores of glycogen. Muscle cells, for example, commonly use glycogenesis to provide energy while exercising, because the blood glucose concentrations are not sufficient.

The process usually occurs in the liver. It is important to note that the process of glycogenesis can also be activated by the peptide hormone insulin in order to respond to relatively high glucose levels in the body.

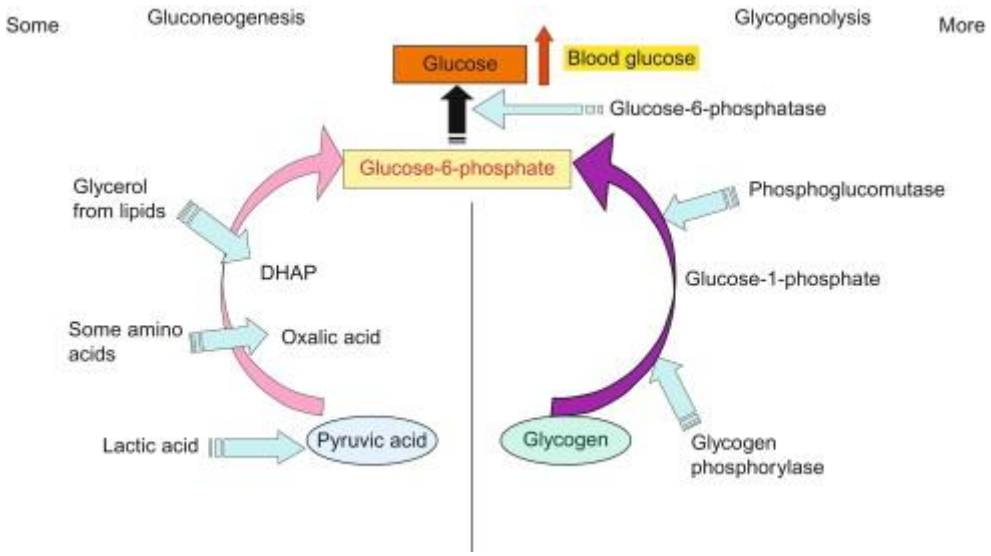


Glycogenesis

Glycogenolysis

Glycogenolysis is the biochemical pathway in which glycogen breaks down into glucose-1-phosphate and glucose. The reaction takes place in the hepatocytes and the myocytes. The process is under the regulation of two key enzymes: phosphorylase kinase and glycogen phosphorylase.

Low levels of ATP within live cells trigger the glycogenolysis process. When the cells detect a low level of ATP, the liver and muscles liberate glycogen and break it down into glucose or simple sugars, which are then used to produce ATP.



Dr. Salim.J.Kh.

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CARBOHYDRATES

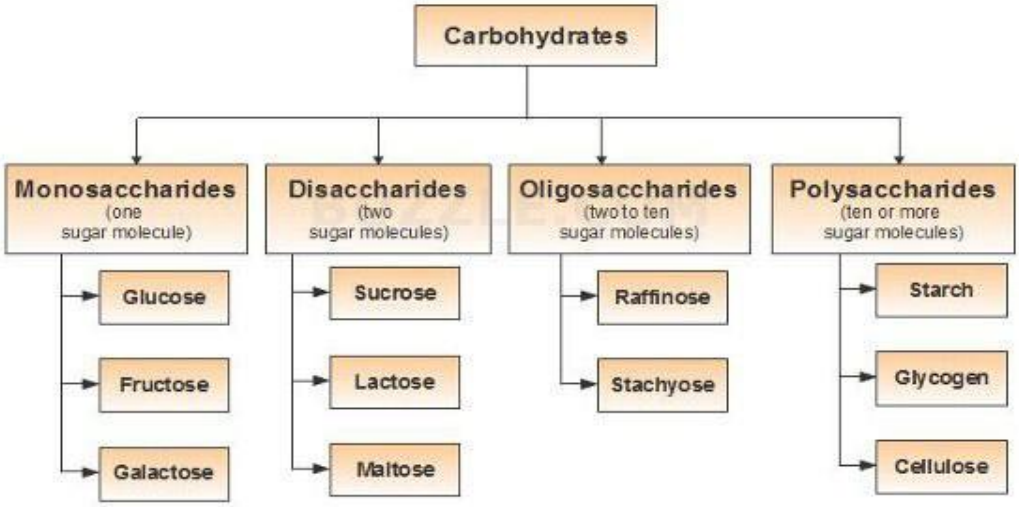
Carbohydrates are the most abundant biomolecules on earth. Oxidation of carbohydrates is the central energy-yielding pathway in most non-photosynthetic cells.

Definition: Carbohydrates are polyhydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis. carbohydrates have the formula $(CH_2O)_n$.

Biological Importance of carbohydrates

- Carbohydrates act as energy reserves and metabolic intermediates.
- Ribose and deoxyribose sugars forms the structural frame of the genetic material, RNA and DNA.
- Polysaccharides like cellulose are the structural elements in the cell walls of bacteria and plants.
- Carbohydrates are linked to proteins and lipids that play important roles in cell interactions.
- Carbohydrates are intermediates in biosynthesis of fats and proteins.
- In animals they are important constituent of connective tissues.
- They participate in biological transport, cell-cell communication and activation of growth factors.
- Carbohydrates that are rich in fiber content help to prevent constipation.

There are four major classes of carbohydrates:

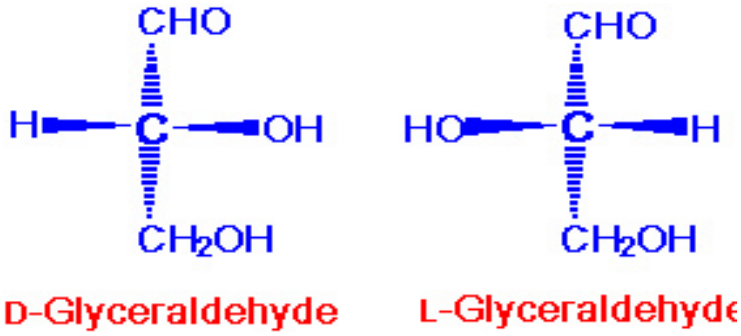


1. Monosaccharides

Monosaccharides, or simple sugars, consist of a single polyhydroxy aldehyde or ketone unit. The most monosaccharide in nature is the six-carbon sugar D- glucose, sometimes referred to as dextrose. The word “Monosaccharides” derived from the Greek word “Mono” means Single and “saccharide” means sugar. They contain 3 to 7 carbon atoms, 2 or more hydroxyl (OH) groups and one aldehyde (CHO) or one ketone (CO) group.

Physical Properties of monosaccharides

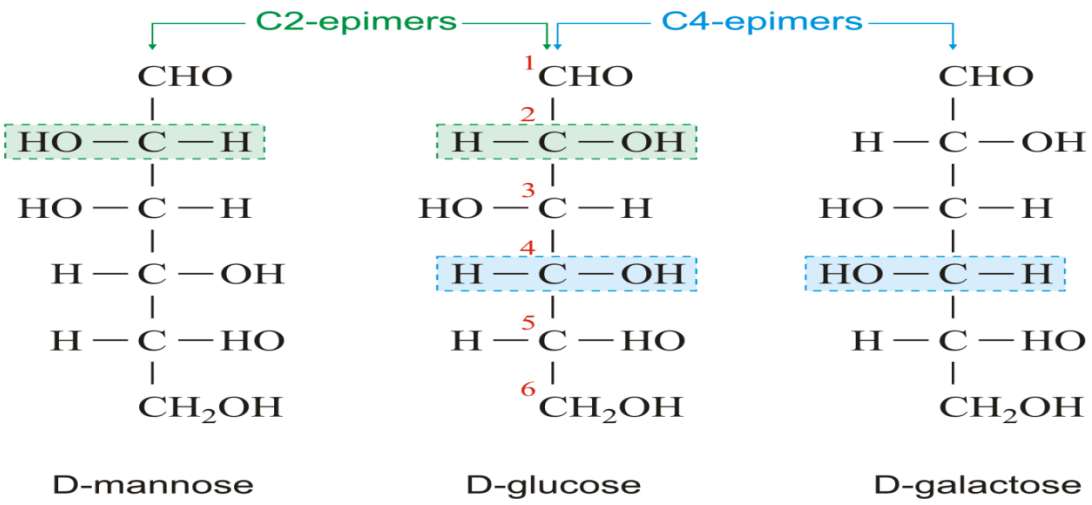
They are colorless, crystalline compounds, readily soluble in water. Their solutions are optically active. Carbohydrates spontaneously change between the α and β configuration.



When the - OH group around the carbon atom adjacent to the terminal primary alcohol carbon is on the right, the sugar is a member of the D-series, when it is on the left, it is a member of the L-series. These D and L configuration.

Optical Activity - The presence of asymmetric carbon atom causes optical activity. When a beam of plane polarized light is passed through a solution of carbohydrate it will rotate the light either to right or to left. Depending on the rotation, molecules are called dextrorotatory (+) (d) or levorotatory (-) (l).

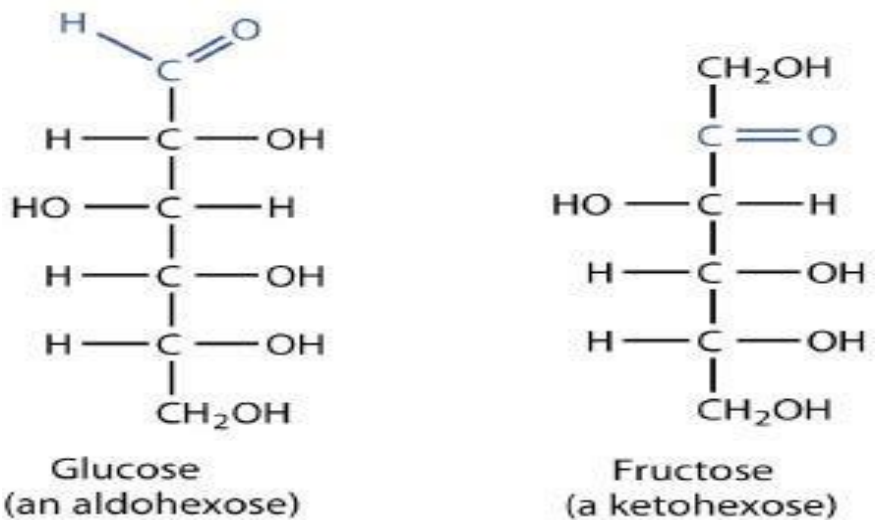
Epimers- When sugars are different from one another, only in a single carbon atom (around one carbon atom) they are called **epimers** of each other. For example glucose and mannose are epimers. They differ only in configuration around C2. Mannose and Galactose are epimers of Glucose.



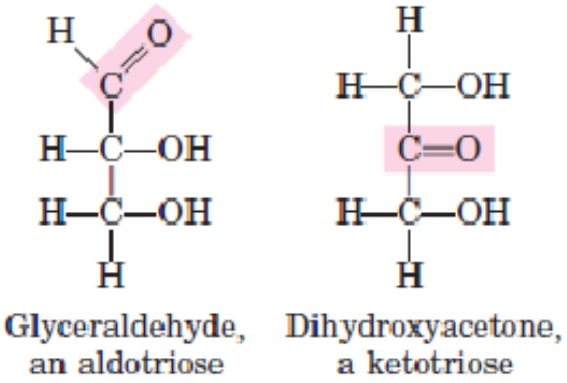
Classification of Monosaccharides

Monosaccharides are classified in two ways. (a) First of all, based on the number of carbon atoms present in them and (b) secondly based on the presence of carbonyl group.

The naturally occurring monosaccharides contain three to seven carbon atoms per molecule. For example, the terms *triose*, *tetrose*, *pentose*, and *hexose* signify monosaccharides with, respectively, three, four, five, and six carbon atoms. Monosaccharides are also classified as aldoses or ketoses. Those monosaccharides that contain an aldehyde functional group are called aldoses; those containing a ketone functional group on the second carbon atom are ketoses.



Name	Formula	Aldose	Ketose
Triose	$C_3H_6O_3$	Glycerose	Dihydroxy acetone
Tetrose	$C_4H_8O_4$	Erythrose	Erythrulose
Pentose	$C_5H_{10}O_5$	Ribose	Ribulose
Hexose	$C_6H_{12}O_6$	Glucose	Fructose
Heptose	$C_7H_{14}O_7$	Glucoheptose	Sedo heptulose



Glyceraldehyde, an aldotriose — Dihydroxyacetone, a ketotriose

Hexoses

Hexoses are “Monosaccharides” containing 6 carbon atoms. The molecular formula of Hexose is C6H12O6

Characteristics

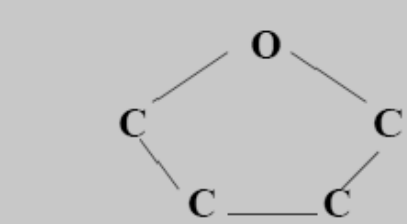
- Hexoses are simple sugars
- Hexoses are soluble in water
- They are sweet in taste .
- They are *crystalline* forms.
- The pentoses may contain an aldehyde group (aldohexose) or a ketone group (ketohexose).

Structure of Monosaccharides

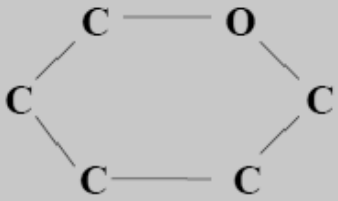
1. **Straight or Open Chain Structure**: Here 6 carbon atoms of glucose are arranged in a straight line. It is also called open chain structure because the two ends remain separate and they are not linked.

2. **Cyclic or Ring Structure:** Here the atoms are arranged in the form of a ring. Haworth (1929) proposed this formula and hence the name Haworth's Projection Formula. The sugar molecules exist in two type of rings which are as follows – (a) Furanose Ring – 5 membered ring

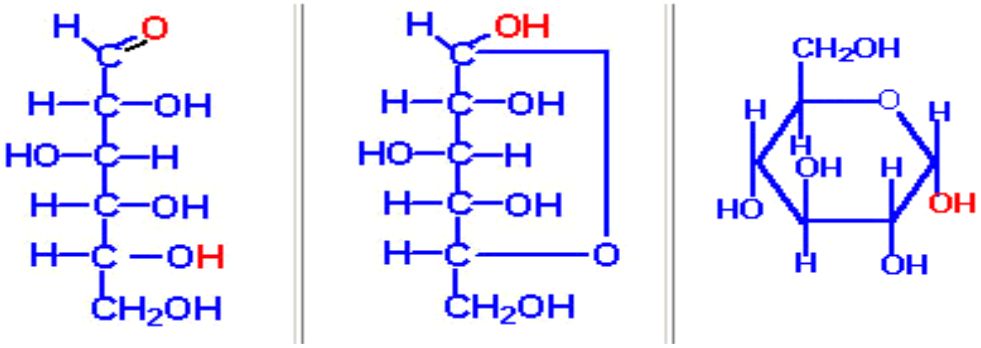
(b) Pyranose Ring- 6 membered ring



Furanose Ring



Pyranose Ring



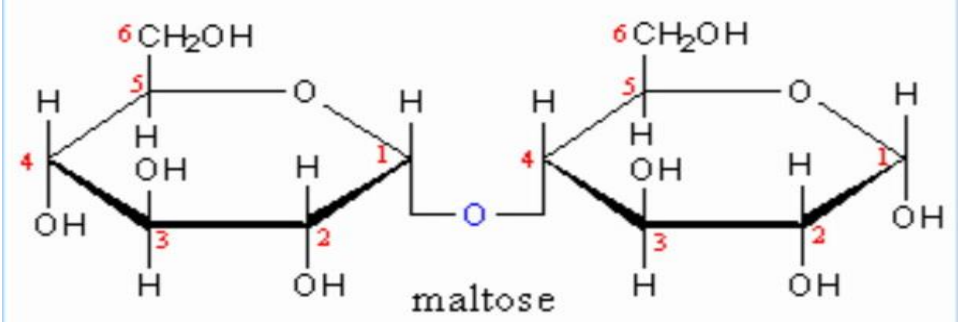
Open chain D-glucose α-D –glucose α-D –glucose
(Fisher formula) (Haworth formula)

2- Disaccharides

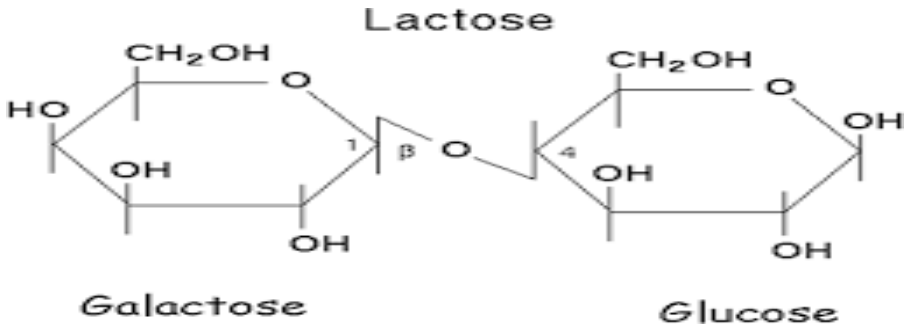
Disaccharides consist of two sugars joined by an glycosidic bond, like sucrose, lactose and maltose.

The disaccharides can be classified into:

1. Homodisaccharides



2. Heterodisaccharides: are formed of 2 different monosaccharide units like sucrose composed of glucose and fructose , lactose composed of glucose and galactose.



3- Oligosaccharides

Oligosaccharides consist of short chains of monosaccharide units (3-9), or residues, joined by characteristic linkages called glycosidic bonds. Common oligosaccharides include raffinose, and stachyose. These oligosaccharides can be found in relatively abundant levels in legumes, whole grains, some cruciferous vegetables, and some fruits.

4- Polysaccharides

Polysaccharides contain hundreds or thousands of carbohydrate units. The anomeric carbons are connected through glycosidic linkages.

Polysaccharides are of two types based on their function and composition.

A. Storage polysaccharide - starch.
B. Structural polysaccharide - cellulose.

Homopolysaccharide: a polysaccharide is made up of **one type** of monosaccharide unit.

Starch

- Starch is a polymer consisting of D-glucose units.
  Starches (and other glucose polymers) are usually insoluble in water because of the high molecular weight, but they can form thick colloidal suspensions with water.
- Starch is a **storage** compound in plants, and made of glucose units
- It is a homopolysaccharide made up of two components: **amylose** and **amylopectin**.
- Most starch is 10-30% amylose and 70-90% amylopectin.
- **Amylose** – a straight chain structure formed by **1,4 glycosidic bonds** between **α-D-glucose** molecules.

Glycogen

- Glycogen is the main storage polysaccharide of animal cells (Animal starch).
- - It is present in liver and in skeletal muscle.
- - Like amylopectin glycogen is a branched polysaccharide of D-glucose units in α - (1, 4) linkages, but it is highly branched.

- - The branches are formed by α -(1,6) glycosidic linkage that occurs after every 8 - 12 residues. Therefore liver cell can store glycogen within a small space. Multiple terminals of branch points release many glucose units in short time.
- Like amylopectin, glycogen gives a red-violet color with iodine.

Cellulose

- Cellulose is the most abundant structural polysaccharide in plants. It is fibrous, tough, water insoluble. Cellulose is a linear unbranched homopolysaccharide 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.
- Cellulose is an important structural polysaccharide, and is the single most abundant organic compound on earth. It is the material in plant cell walls that provides strength and rigidity; wood is 50% cellulose.
- Cellulose is also important industrially, from its presence in wood, paper, cotton, nitrocellulose, photographic films (cellulose acetate), etc.

" Chemistry of Proteins and Amino Acids "

Proteins are complex macromolecules with molecular weights ranging from approximately (5000 daltons) to many millions. Each is polymer with α-amino acids as the repeating units.

The molecular weights of amino acid polymers, often referred to as polypeptides, those with low molecular weights consisting of fewer than 50 amino acids, are called peptides. The term protein describes molecules with more than 50 amino acids, each protein consists of one or more polypeptide chains.

Some biochemists define oligopeptides as polymers consisting of two to ten amino acids and polypeptides as having more than ten residues. Proteins in this view, have molecular weights greater than 10,000 daltons.

* Amino Acids:

Introduction

There are approximately 300 amino acids present in various animals, plants, and microbial systems, but only 20 amino acids are coded by DNA to appear in proteins. Cells produce proteins with strikingly different properties and activities by joining the same 20 amino acids in many different combinations and sequences. This indicates that the properties of proteins are determined by the physical and chemical properties of their monomer units, the amino acids.

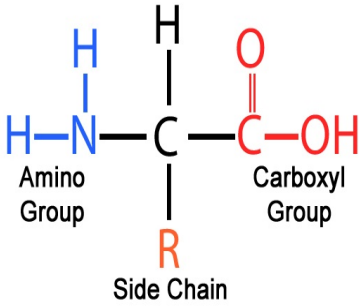
Definition:

Amino acids are the basic structural units of proteins consisting of an amino group, (-NH2) a carboxyl (-COOH) group a hydrogen (H) atom and a variable (R) group. All of the substituents in amino acid are

attached (bonded) to a central α carbon atom. This carbon atom is called (α) because it is bonded to the carboxyl (acidic) group.

Several of the amino acids found in proteins also serve functions distinct from the formation of peptides and proteins, e.g., tyrosine in the formation of thyroid hormones or glutamate acting as a neurotransmitter.

The α-amino acids in peptides and proteins (excluding proline) consist of a carboxylic acid (-COOH) and an amino ($-NH_2$) functional groups attached to the same tetrahedral carbon atom. This carbon is the α-carbon. Distinct R-groups, that distinguish one amino acid from another, also are attached to the α-carbon (except in the case of glycine where the R-group is hydrogen). The fourth substitution on the tetrahedral α-carbon of amino acid is hydrogen.



(General structure of amino acids)

In dipolar (zwitterion) form the amino group is protonated ($-NH3^+$) and the carboxyl group is dissociated (deprotonated) ($-COO^-$) leading to a net charge zero.

COO-

|α

NH3+ – C – H

|

R

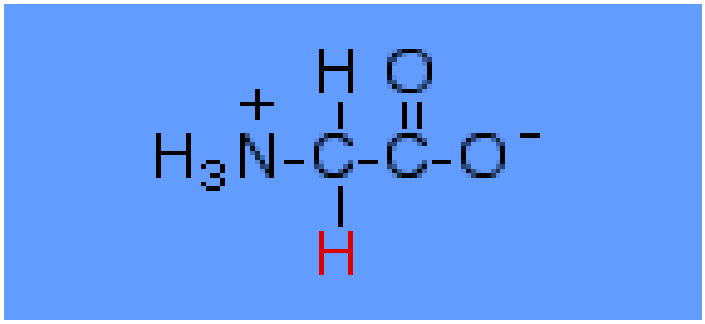
* Types of Amino Acids:

Amino acids found in proteins are classified into many types according to the R-group present in amino acid (side chain group):

A- Hydrophobic Amino Acids:

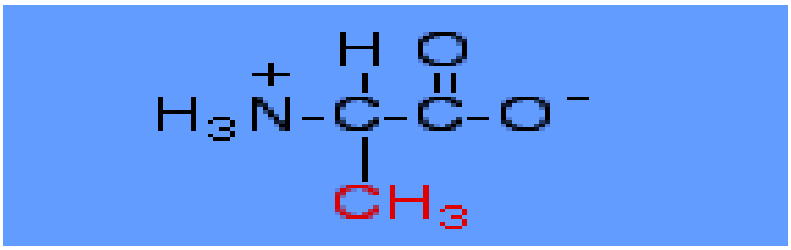
1- Aliphatic amino acids: are hydrophobic, they don't like to be in contact with water molecules in an aqueous solution, for this reason, they are often located in the core of the protein surrounded by the rest of the protein.

Glycine(Gly; G): is the simplest of all amino acids, and the only one which is not optically active, since it has a single hydrogen atom as its side chain.



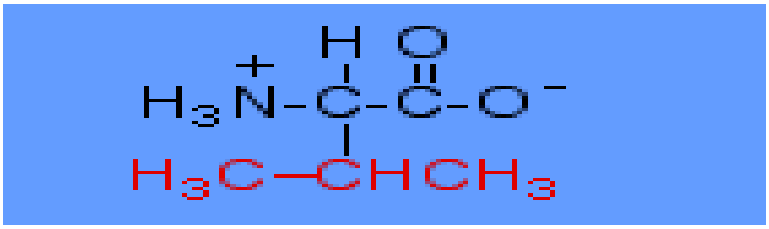
Glycine

Alanine(Ala; A): has a methyl group as its side chain



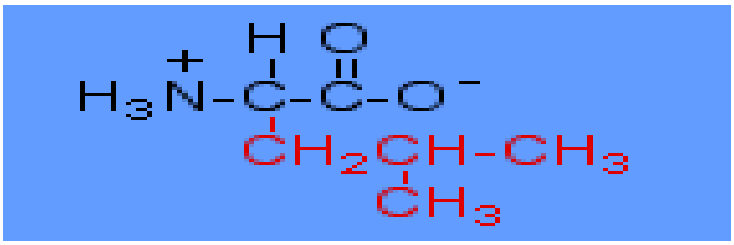
Alanine

Valine(Val; V): has a slightly longer side chain, and there is a branch , therefore, its more hydrophobic.



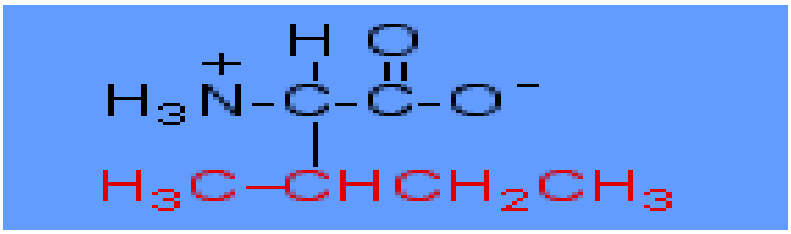
Valine

Leucine(Leu; L): is very similar to valine except it has another methyl group attached to the side chain.



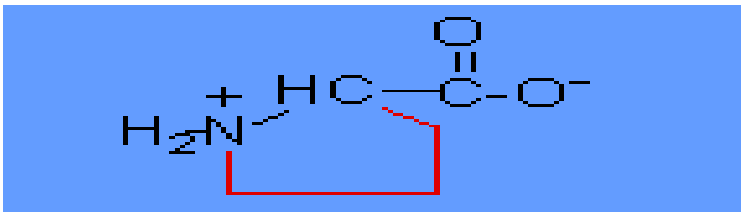
Leucine

Isoleucine(Ile; I): is again similar to leucine and valine except that the orientation of the atoms in the side chain is slightly different.



Isoleucine

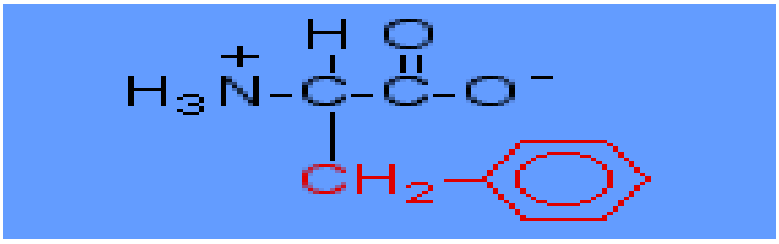
Proline(Pro; P): is different from all the other amino acids in that the side chain is bonded to both the α-carbon but also to the amino group. This has marked effects on the architecture of the proteins, therefore, its called imino acid.



Proline

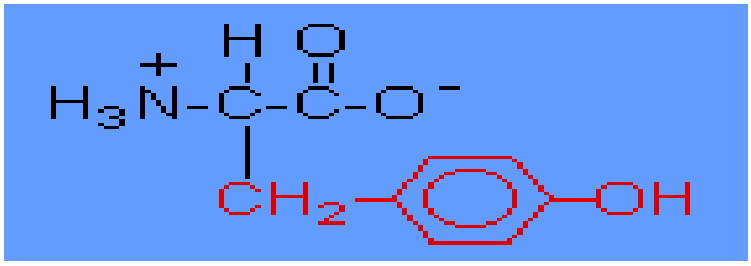
2- <u>Aromatic amino acids</u>: these are the amino acids which contain an aromatic ring as part of their side chains. These amino acids are highly hydrophobic.

<u>Phenylalanine(Phe; F)</u>: is the first of all the aromatic amino acids. It contains a phenyl ring attached to a methylene group. Due to a phenyl ring, it is a hydrophobic amino acid.



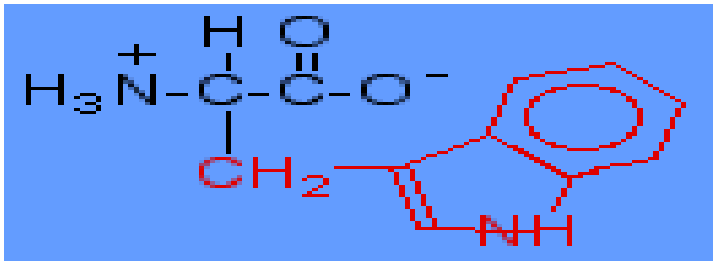
Phenylalanine

<u>Tyrosine(Tyr; Y)</u>: contains a hydroxyl group at the end of the phenyl ring. This makes tyrosine less hydrophobic than phenylalanine. It is also a reactive group, whereas the side chains before have all been unreactive.



Tyrosine

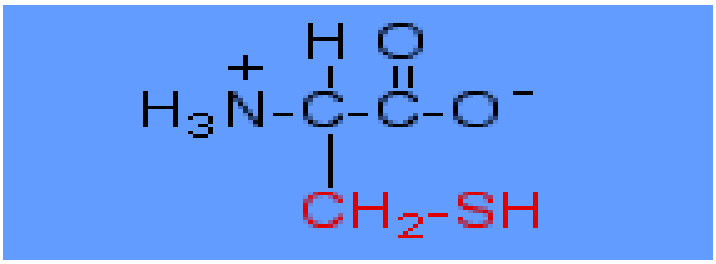
<u>Tryptophan(Trp; W)</u>: has a slightly different ring attached to the methylene group. This is an indole ring and it is highly hydrophobic.



Tryptophan

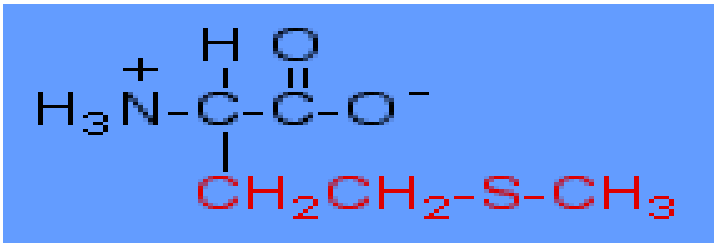
3- <u>Sulphur containing amino acids</u>: there are two amino acids which contain a sulphur atom.

Cysteine(Cys; C): contains a sulfhydryl group(-SH),this is extremely reactive, and can form hydrogen bonds. Cysteine is very important because it can also form disulfide bridges which help stabilize many polypeptides and proteins. The sulfhydryl group of cysteine is hydrophobic.



Cysteine

Methionine(Met; M): is a very special amino acid in that it is the "start" amino acid in the process of translation (protein synthesis), methionine has a highly hydrophobic side chain.

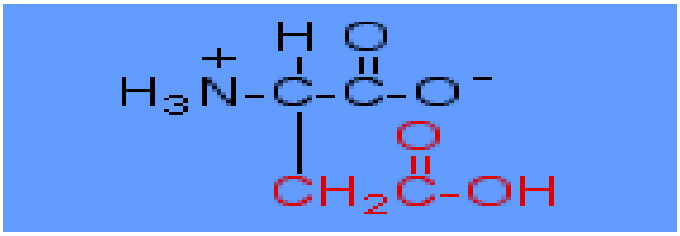


Methionine

B- Hydrophilic Amino Acids:

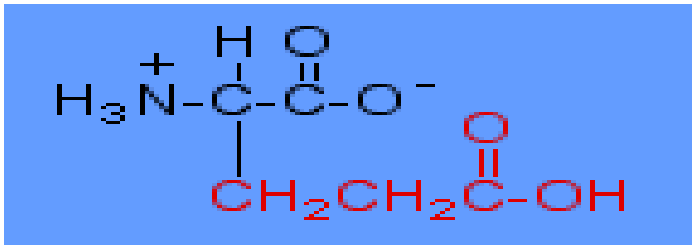
1- Acidic amino acids: these amino acids are highly polar, and are nearly always negatively charged at physiological pH.

Aspartate(Asp; D): is really aspartic acid, it is called aspartate because it is usually negatively charged at physiological pH and so it is named for the carboxylate anion, (compare acetic acid and acetate).



Aspartic acid

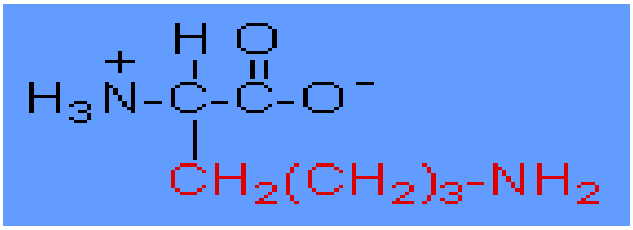
Glutamate(Glu; E): is also called glutamic acid. The side chain of glutamate also has a carboxylate group which has a negative charge at physiological pH.



Glutamic acid

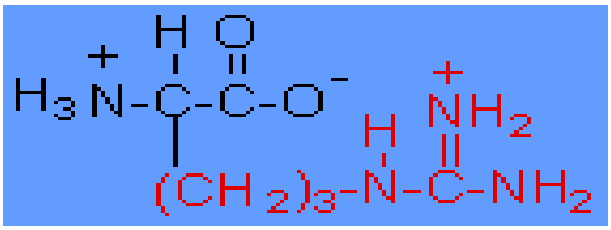
2- Basic amino acids: these amino acids contain side chains which are positively charged at physiological pH.

Lysine(Lys; K): has one of the longest side chains of the amino acids. Hydrocarbon chain is a very polar because of the terminal amino group and is classified as a hydrophilic amino acid.



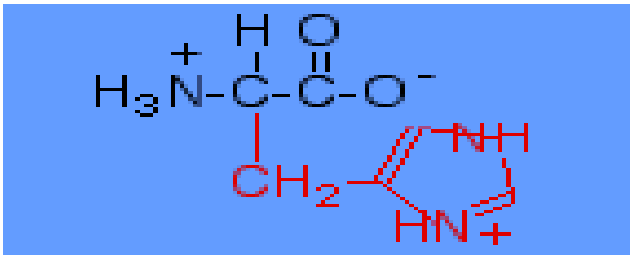
Lysine

Arginine(Arg; R): has in fact the longest of all side chains because of the guanidine group attached to the side chain and is positively charged at physiological pH.



Arginine

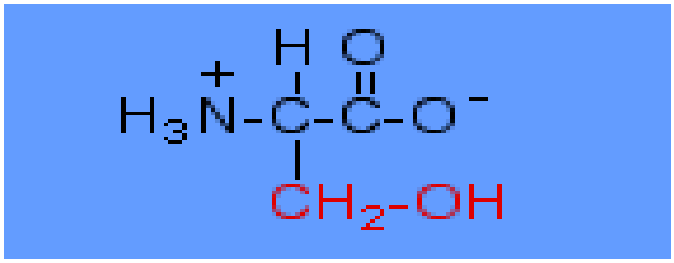
Histidine(His; H): has an imidazole ring which often be present inside the active site of an enzyme and helps bonds to be broken or made, therefore; it can exist in two states; uncharged, or positively charged.



Histidine

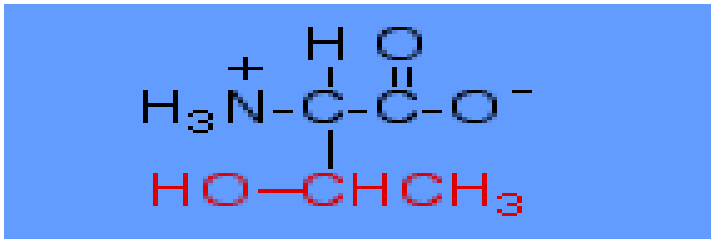
3- Neutral polar amino acids: these amino acids are not charged at physiological pH. However they all have groups on their side chains which are polar and can form hydrogen bonds, for this reason the amino acids are classified as hydrophilic.

Serine(Ser; S): contain an aliphatic chain with a hydroxyl group.The hydroxyl group make the amino acid highly reactive and hydrophilic as it readily forms hydrogen bonds.



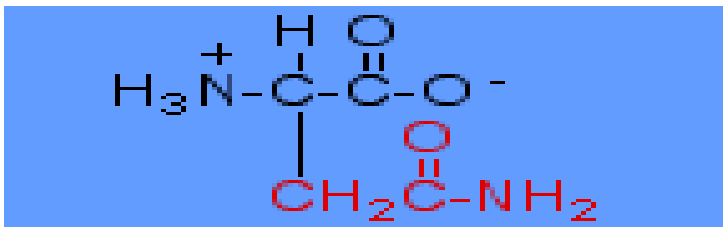
Serine

Threonine(Thr; T): is another neutral amino acid which has a highly reactive and highly hydrophilic hydroxyl group.



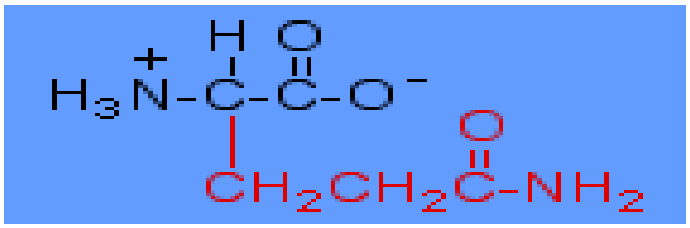
Threonine

Asparagine(Asn; N): is the amide derivative of aspartic acid, when the carboxylate side chain is amidated the resulting amide is uncharged.



Asparagine

Glutamine(Gln; Q): the terminal amide group instead of a carboxyl group as in glutamate and it is similar to asparagine. Therefore, these two are called the amide derivatives of their parent amino acids.



Glutamine

* Functions of Amino Acids:

In addition to their primary function as components of proteins, amino acids have several other biological roles:

1- Several α-amino acids or their derivatives act as chemical messengers, for example γ-amino butyric acid(GABA, derivative of glutamate), and serotonin and melatonin (derivatives of tryptophan) are neurotransmitters.

Thyroxine (a tyrosine derivative produced in the thyroid gland) and indole acetic acid (a tryptophan derivative found in plants) are hormones (chemical molecules produced in one cell that regulate the function of other cells).

2- Amino acids are precursors of a variety of complex nitrogen-containing molecules, examples include the nitrogenous base components of nucleotides and nucleic acids, heme (the iron-containing organic group required for the biological activity of several important proteins), and chlorophyll (a pigment important in photosynthesis).

3- Several standard and nonstandard amino acids act as metabolic intermediates, for example, arginine, ornithine, and citrulline are components of the urea cycle (synthesis of urea, a molecule formed in vertebrate livers, is the principal mechanism for the disposal of nitrogen-ous wastes).

* Amino Acid Stereoisomers:

Because the α-carbons of 19 of the 20 standard amino acids are attached to four different groups (a hydrogen, a carboxyl group, an amino group, and an R-group). They are referred to as asymmetric or chiral carbons. The amino acid, glycine, is a symmetrical molecule because its α-carbon is attached to two hydrogens).

These asymmetrical amino acids have the ability to rotate the plane of polarized light either to the right (dextrorotatory, D-isomers) or to the left (levorotatory, L-isomers). All of the amino acids in proteins are L-α- amino acids. D-amino acids are never found in proteins, although they exist in nature, D-amino acids are often found in polypeptide antibiotics.

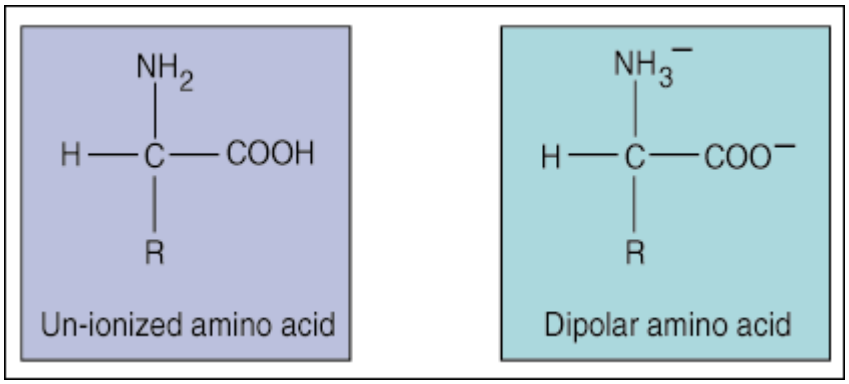
* Acid- Base properties of amino acids:

Charged and uncharged forms of the ionizable –COOH and $–NH_3^+$ weak acid groups exist in solution in protonic equilibrium:

$$R\text{-}COOH \leftrightarrow R\text{-}COO^- + H^+$$

$$R\text{-}NH_3 \leftrightarrow R\text{-}NH_2 + H^+$$

While both R-COOH and $R\text{-}NH_3^+$ are weak acids, R-COOH is far stronger acid than $R\text{-}NH_3^+$.



The NH_2 group is very basic and accepts protons (H+) from solution, while the carboxylic acid group is acidic and donates protons to the solution.

Amino acids can be polar or non-polar. Polar amino acids have R groups that do not ionize in solution but are quite soluble in water due to their polar character. They are also known as hydrophilic, or "water loving" amino acids. These include serine, threonine, asparagine, glutamine, tyrosine, and cysteine. The nonpolar amino acids include glycine, alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine and tryptophan. Nonpolar amino acids are soluble in nonpolar environments such as cell membranes and are called hydrophobic molecules because of their "water fearing" properties.

At physiologic pH (pH 7.4), carboxyl groups exist almost as R-COO^- and amino groups predominantly as R-NH_3^+. Molecules that contain an equal number of ionizable groups of opposite charge and that therefore give no net charge are termed zwitterions. The pH at which this occurs is called the isoelectric point(pI).

* The Essential Amino Acids:

An essential amino acids or indispensable amino acids is an amino acids that cannot be synthesized by the organism (usually reffered to human) and therefore must be supplied in the diet.

Nine amino acids are generally regarded as essential for human. They are(histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine).

In addition, the amino acids arginine, cysteine, and tyrosine are considered conditionally essential, meaning they are not normally required in the diet, but must be supplied exogenously to specific populations that do not synthesize it in adequate amounts. Phenylketonuria (PKU) is an example of diseased associated with deficiency of essential amino acid (phenylalanine).

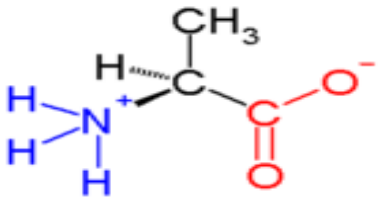
*Glucogenic amino acid

A glucogenic amino acid is an amino acid that can be converted into glucose through gluconeogenesis. This is in contrast to the ketogenic amino acids that are converted into ketone bodies. In humans, the glucogenic amino acids are: glycine, serine, threonine, valine, histidine, arginine, cysteine, proline, alanine, glutamate, glutamine, aspartate, asparagine, methionine.

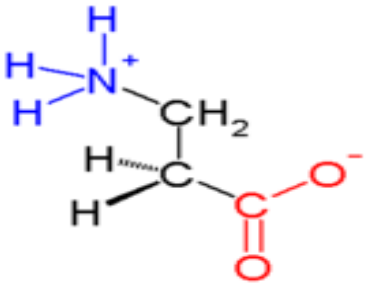
Amino acids which can be either glucogenic or ketogenic: isoleucine, phenylalanine, tyrosine, tryptophan.

* Beta-amino acids:

β amino acids, which have their amino group bonded to the β carbon rather than the α carbon as in the 20 standard biological amino acids. The only commonly naturally occurring β amino acid is β-alanine;



L-α-alanine

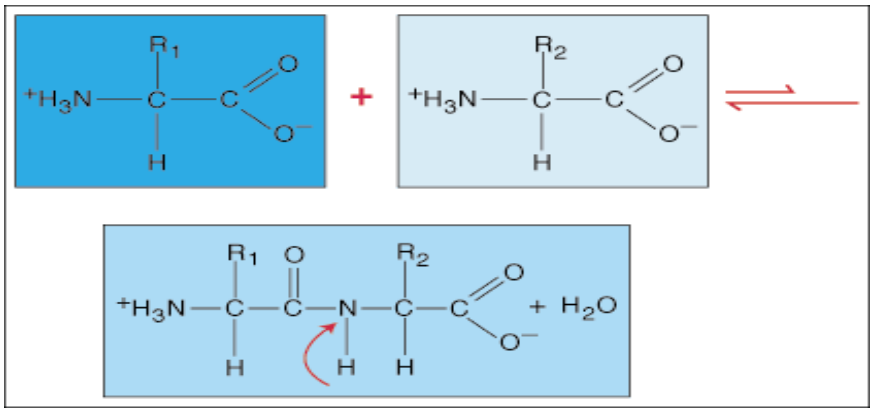


β-alanine

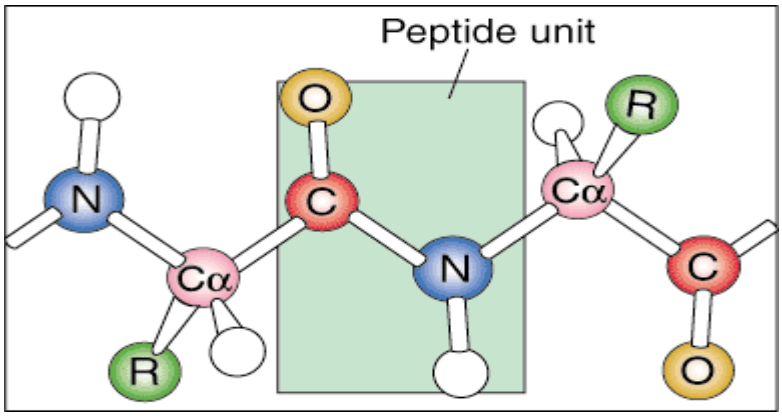
*The peptide bond:

Amino acids can be joined together to form a peptide or poly-peptide. They are called peptides because when the carboxyl group of one amino acid joins to the amino group of another, a peptide bond is formed.

Chemically, this is an amide bond but when it occurs in proteins it is given the name peptide bond. Because this reaction is a dehydration, that is, a water molecule is removed, the linked amino acids are referred to as amino acid residues.



(Amino acid bonding)



(Peptide bond)

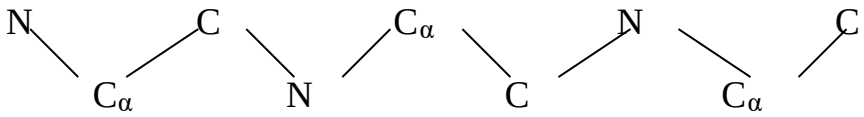
* Peptides:

The polymerization of L-α-amino acids by peptide bonds forms the structural framework for proteins.

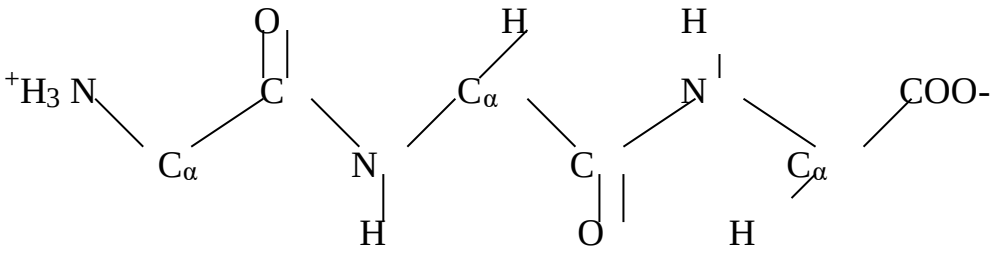
Peptides have a high biomedical importance, due to, many major hormones are peptides (insulin), some peptides act in the nervous system, either as neurotransmitters or as neuromodulators, certain antibiotics are peptides. When amino acids are in a polypeptides chain, they refered to as residues.

* Peptide Structure:

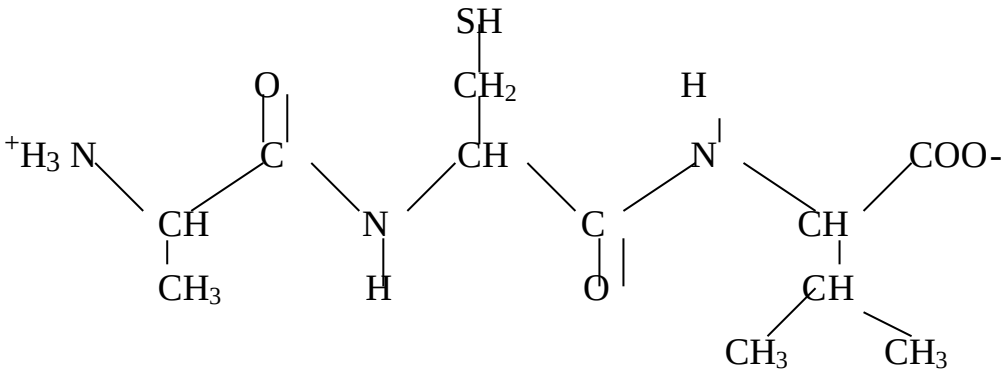
Peptides are drawn with their amino terminal on the left and carboxyl terminal on the right. For drawn peptide structures, first draw the main chain or backbone, then add appropriate R-groups. Write a zig-zag formed from the repeating sequence of main chain, or "backbone" atoms; amino nitrogen, α-carbon, and carboxyl carbon.



Complete the amino and carboxyl terminals, add a hydrogen to each α-carbon and to each peptide nitrogen and add oxygen to the carboxyl carbon.

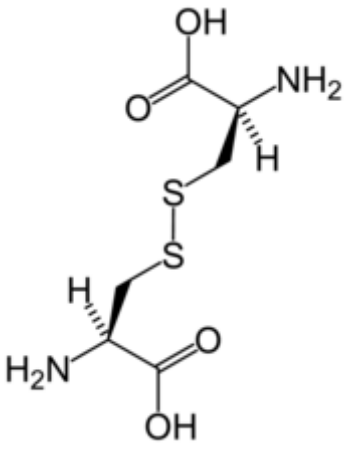


Add appropriate R-groups to each α- carbon atom according to amino acids present in a peptide. Peptides are then named as derivatives of the carboxyl terminal aminoacyl residue. For example(alanyl-cysteinyl-valine).



* Cysteine Oxidation:

The sulfhydryl group of cysteine is highly reactive. The most common reaction of this group is a reversible oxidation that forms a disulfide. Oxidation of two molecules of cysteine forms cystine, a molecule that contains a disulfide bond. When two cysteine residues form such a bond, it is referred to as a disulfide bridge.



(Skeletal formula of cystine)

This bond can occur in a single chain to form a ring or between two separate chains to form an intermolecular bridge. Disulfide bridges help stabilize many polypeptides and proteins.

* Proteins:

Types of Chemical Bonds in Proteins
1-Peptide Bonds
2-Hydrogen Bonds
3-Ionic Bonds,
4- Disulfide Bridges**.**
5-Hydrophobic
6- Hydrophilic Interactions

Proteins are the most functionally group of all biological compounds encountered in living organisms, proteins have the most functions such as:

1- **Catalysis**. Enzymes are proteins that accelerate thousands of biochemical reactions in such processes as digestion, energy production, and biosynthesis.

2- **Structure**. Some proteins provide protection and support. Struc- tural proteins often have very specialized properties, for example, collagen (the major component of connective tissues), fibrin, elastin, and others.

3- **Movement**. Proteins are involved in all cell movements, like, actin, tubulin, and other proteins compose the cytoskeleton.

4- **Defense**. A wide variety of proteins are protective, like keratin, the protein found in skin cells that aids in protecting the organism against mechanical and chemical injury, the blood clotting proteins fibrinogen and thrombin prevent blood loss when blood vessels are damaged, and the immunoglobulins (or antibodies) are produced by lymphocytes when foreign organisms such as bacteria invade an organism.

5- **Regulation**. Binding a hormone molecule to its target cell change cellular function, like peptide hormones include insulin and glucagon, which both regulate blood glucose levels.

6- **Transport**. Many proteins function as carriers of molecules or ions across membranes or between cells, like the Na^+-K^+ ATPase and the glucose transporter, others include hemoglobin which carries O_2 to the tissues from lungs, and the lipoproteins LDL and HDL, which transport lipids in the body.

* Classification of proteins:

Because of their diversity, proteins are often classified in two additional ways: (1) shape and (2) composition. Proteins are classified into two major groups based on their shape: **a- fibrous proteins** are long, rod-shaped molecules that are insoluble in water and physically tough. Fibrous proteins, such as the keratins found in skin, hair, and nails, have structural and protective functions. **b- globular proteins** are compact spherical molecules that are usually water-soluble. Typically, globular proteins have dynamic functions, for example, nearly all enzymes have globular structures. Other examples include the immunoglobulins and the transport proteins hemoglobin and albumin (a carrier of fatty acids in blood).

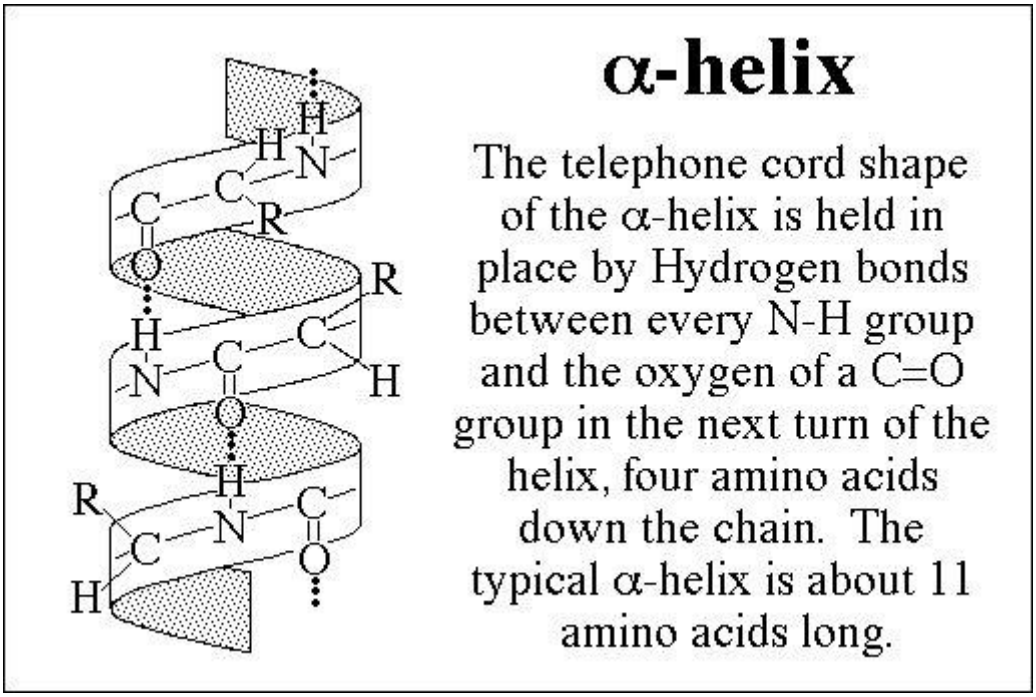
* Protein Structures:

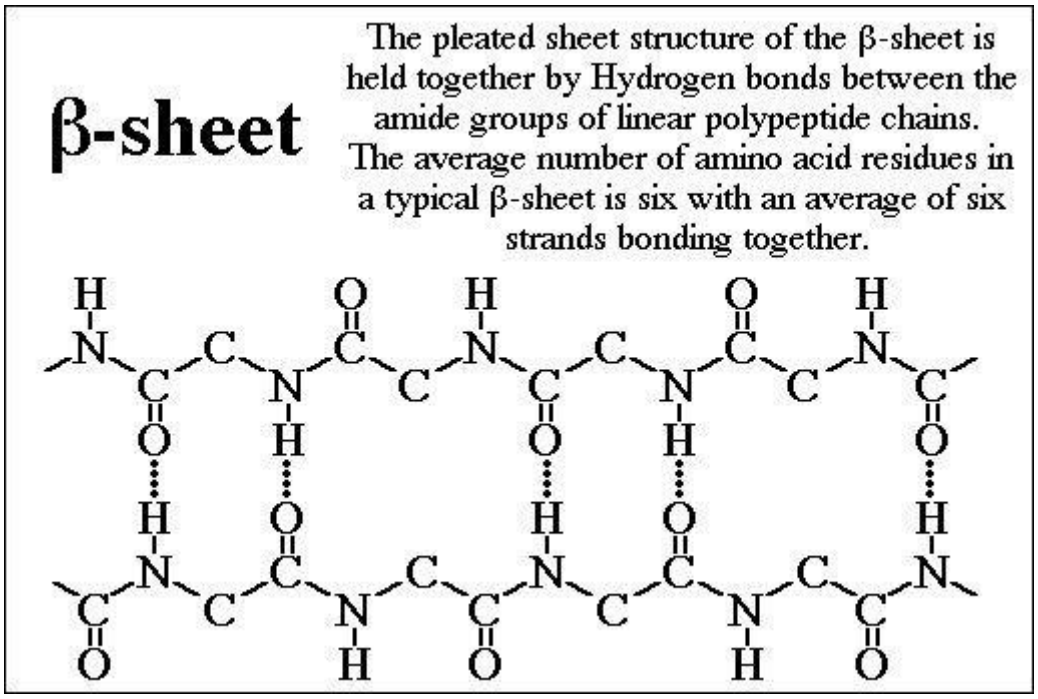
1- Primary structure

The sequence of the different amino acids is called the primary structure of the peptide or protein. Counting of residues always starts at the N-terminal end (NH_2-group), which is the end where the amino group is not involved in a peptide bond.

2- Secondary structure

By building models of peptides using known information about bond lengths and angles, the first elements of secondary structure, the alpha helix and the beta sheet. Both the alpha helix and the beta-sheet represent a way of saturating all the hydrogen bonds.



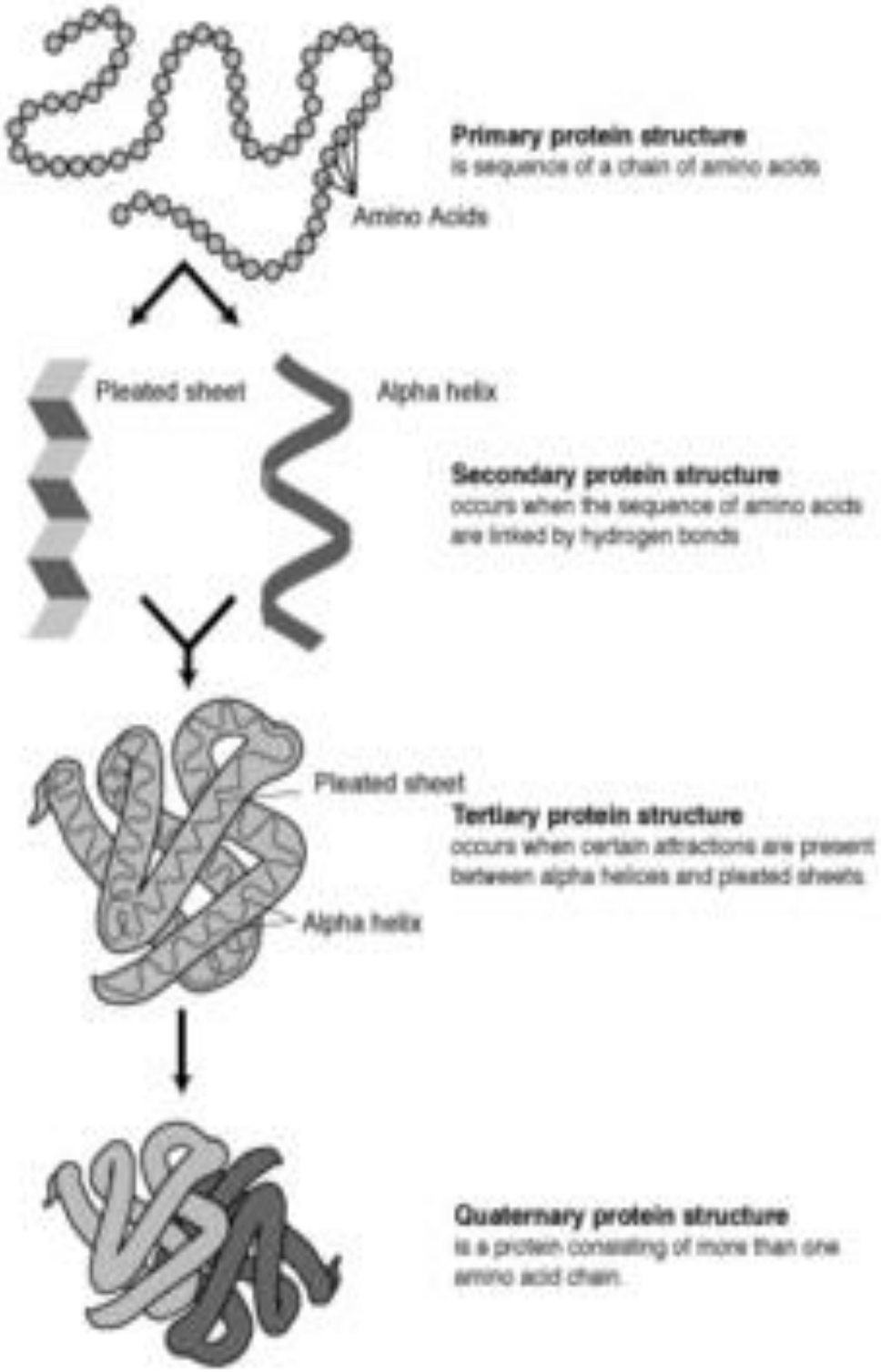


3-Tertiary structure

The elements of secondary structure are usually folded into a compact shape using a variety of loops and turns. The formation of tertiary structure is usually driven by the burial of hydrophobic residues, but other interactions such as hydrogen bonding, ionic interactions and disulfide bonds can also stabilize the tertiary structure.

4- Quaternary structure

The quaternary structure is the interaction between several chains of peptide bonds. The individual chains are called subunits. The individual subunits are not necessarily covalently connected, but might be connected by a disulfide bond. Not all proteins have quaternary structure, since they might be functional.


Primary protein structure
is sequence of a chain of amino acids
Amino Acids
Pleated sheet
Alpha helix
Secondary protein structure
occurs when the sequence of amino acids are linked by hydrogen bonds
Pleated sheet
Alpha helix
Tertiary protein structure
occurs when certain attractions are present between alpha helices and pleated sheets
Quaternary protein structure
is a protein consisting of more than one amino acid chain

Digestion and absorption

Digestion is the process by which food is broken down into simple chemical compounds that can be absorbed and used as nutrient substances the body can use for energy, tissue growth, and repair or eliminated by the body.

Absorption is the process by which the products of digestion are absorbed by the blood to be supplied to the rest of the body. During absorption, the digested products are transported into the blood or lymph through the mucous membrane.

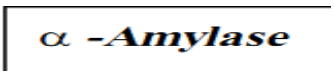
Digestion and absorption occur in the digestive tract. After the nutrients are absorbed, they are available to all cells in the body and are utilized by the body cells in metabolism.

Digestion and absorption of carbohydrates

Dietary carbohydrates principally consist of the polysaccharides: starch and glycogen. It also contains disaccharides: sucrose, lactose, maltose and in small amounts monosaccharides like fructose and pentoses. Liquid food materials like milk, soup, fruit juice escape digestion in mouth as they are swallowed, but solid foodstuffs are masticated thoroughly before they are swallowed.

1. Digestion in mouth

Digestion of carbohydrates starts at the mouth, where they come in contact with saliva during mastication. Saliva contains a carbohydrate splitting enzyme called salivary amylase (ptyalin).



Starch or glycogen ⟶ Glucose, Maltose and Maltotriose

2. Digestion in Stomach

No carbohydrate splitting enzymes are available in gastric juice. HCl may hydrolyze some dietary sucrose to equal amounts of glucose and fructose.

3. Digestion in small intestine

Food reaches the small intestine from stomach where it meets the pancreatic juice. Pancreatic juice contains a carbohydrate-splitting enzyme pancreatic amylase. Also action of other enzymes like Lactase, Maltase, Sucrase.

Absorption of carbohydrates

Products of digestion of dietary carbohydrates are practically completely absorbed almost entirely from the small intestine.

Absorption from proximal jejunum is three times greater than that of distal ileum. It is also proved that some disaccharides, which escape digestion, may enter the cells of the intestinal lumen by “pinocytosis” and are hydrolyzed within these cells. No carbohydrates higher than the monosaccharides can be absorbed directly into the blood stream.

Mechanism of absorption: Two mechanisms are involved:

1. Simple Diffusion

This is dependent on sugar concentration gradients between the intestinal lumen, mucosal cells and blood plasma. All the monosaccharides are probably absorbed to some extent by simple 'passive' diffusion.

2. "Active "Transport Mechanisms

Glucose and galactose are absorbed very rapidly and hence it has been suggested that they are absorbed actively and it requires energy. Fructose absorption is also rapid but not so much as compared to glucose and galactose.

Digestion of lipids

Lipid digestion and absorption pose some special challenges. Triglycerides are large molecules, and unlike carbohydrates and proteins, they're not water-soluble. Because of this, they like to cluster together in large droplets when they're in a watery environment like the digestive tract. The digestive process has to break those large droplets of fat into smaller droplets and then enzymatically digest lipid molecules using enzymes called lipases.

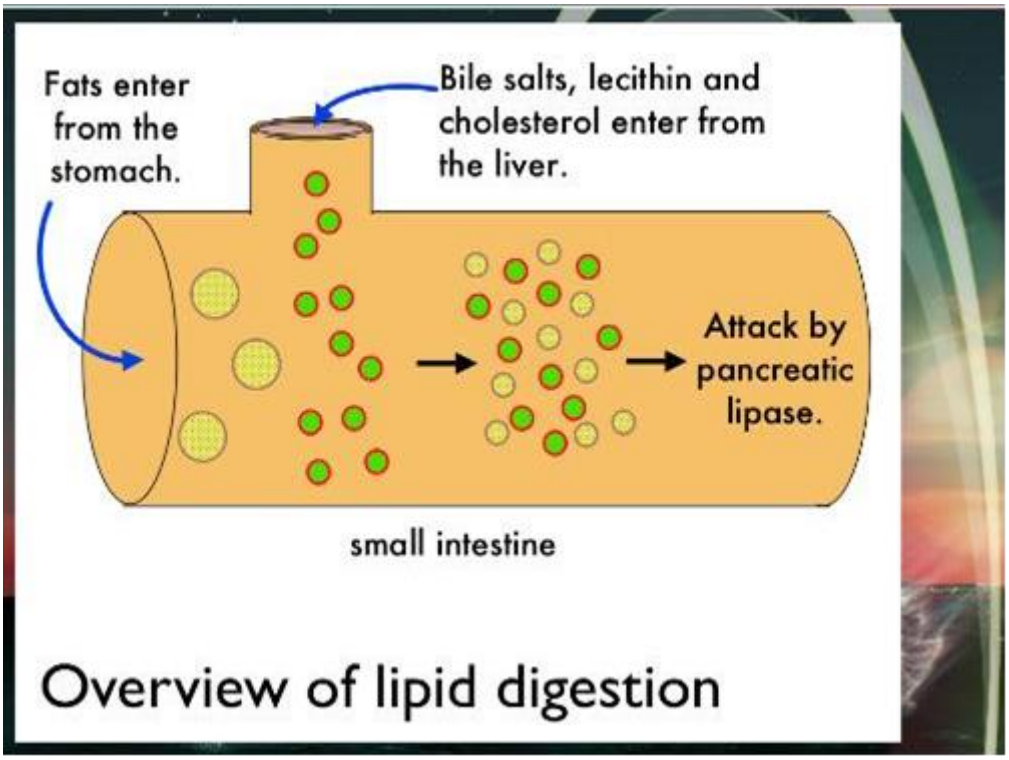
1- Lipid digestion in mouth and stomach

The digestion of lipids begins in the oral cavity through exposure to lingual lipases, which are secreted by glands in the tongue to begin the process of digesting triglycerides. Digestion continues in the stomach through the effects of both lingual and gastric enzymes (gastric lipase), these two lipases play only a minor role in fat digestion.

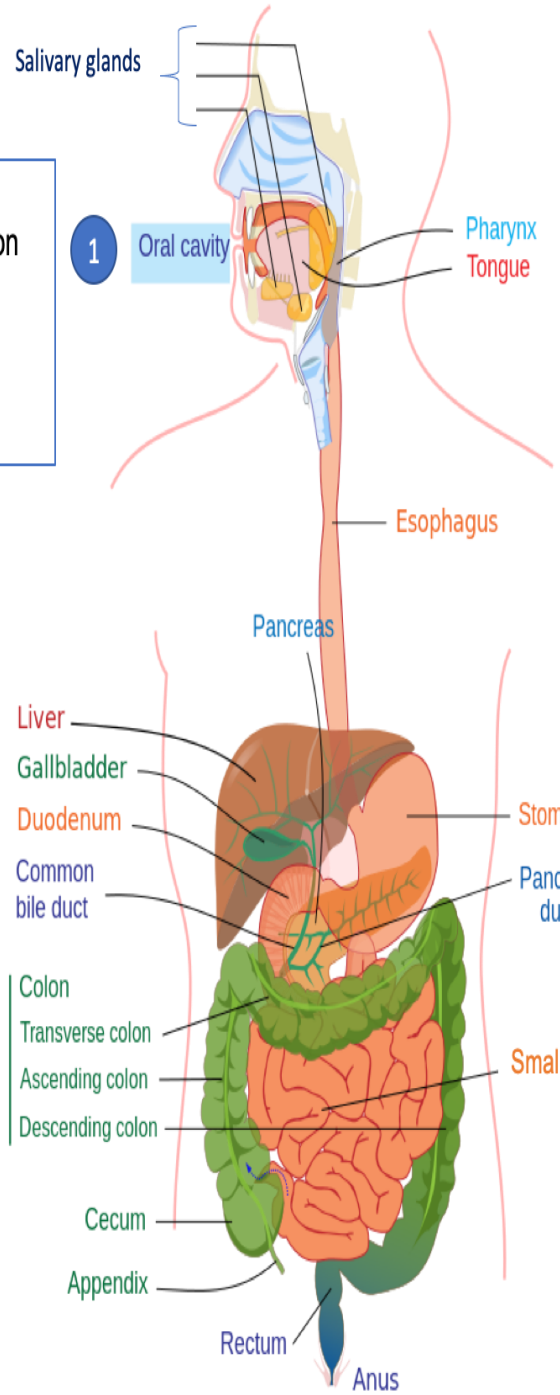
2- Lipid digestion in small intestine

As the stomach contents enter the small intestine, most of the dietary lipids are undigested and clustered in large droplets. **Bile**, which is made in the liver and stored in the gallbladder, is released into the duodenum, the first section of the small intestine. Bile salts have both a hydrophobic and a hydrophilic side, so they are attracted to both fats and water. This makes them effective emulsifiers, meaning that they break large fat globules into smaller droplets. Emulsification makes lipids more accessible to digestive enzymes by increasing the surface area for them to act.

The pancreas secretes ***pancreatic lipases*** into the small intestine to enzymatically digest triglycerides. Triglycerides are broken down to fatty acids, monoglycerides (glycerol backbone with one fatty acid still attached), and some free glycerol. Cholesterol and fat-soluble vitamins do not need to be enzymatically digested.



1. MOUTH
- mechanical digestion
- mixing with saliva
- limited enzymatic digestion (*lingual lipase*)



2. STOMACH
- mixing/churning
- limited enzymatic digestion (*gastric lipase*)

3. SMALL INTESTINE
- emulsification (bile)
- enzymatic digestion (*pancreatic lipases*)
- micelles help with absorption

Absorption of lipids

Next, those products of fat digestion (fatty acids, monoglycerides, glycerol, cholesterol, and fat-soluble vitamins) need to enter into the circulation so that they can be used by cells around the body. Again, bile helps with this process. Bile salts cluster around the products of fat digestion to form structures called **micelles**, which help the fats get close enough to the microvilli of intestinal cells so that they can be absorbed. The products of fat digestion diffuse across the membrane of the intestinal cells, and bile salts are recycled back to do more work emulsifying fat and forming micelles.

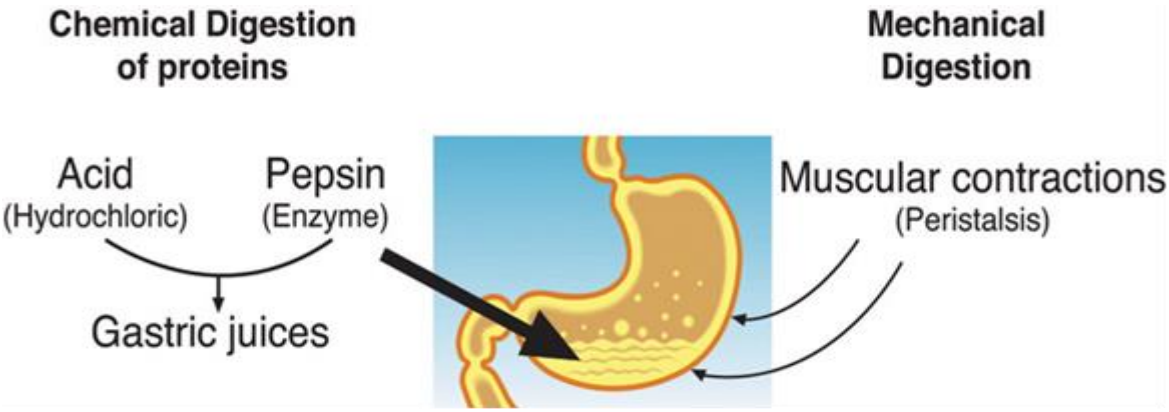
Digestion and absorption of proteins

Both mechanical and chemical digestion take place in the stomach. The stomach releases gastric juices containing **hydrochloric acid** and the enzyme, **pepsin**, which initiate the chemical digestion of protein. Muscular contractions, called peristalsis, also aid in digestion. The powerful stomach contractions churn the partially digested protein into a more uniform mixture, which is called **chyme**.

Because of the hydrochloric acid in the stomach, it has a very low pH of 1.5-3.5. The acidity of the stomach causes food proteins to denature, unfolding their three-dimensional structure to reveal just the polypeptide chain. This is the first step of chemical digestion of proteins. Recall that the three-dimensional structure of a protein is essential to its function, so denaturation in the stomach also destroys protein function. (This is why a protein such as insulin can’t be taken as an oral medication).

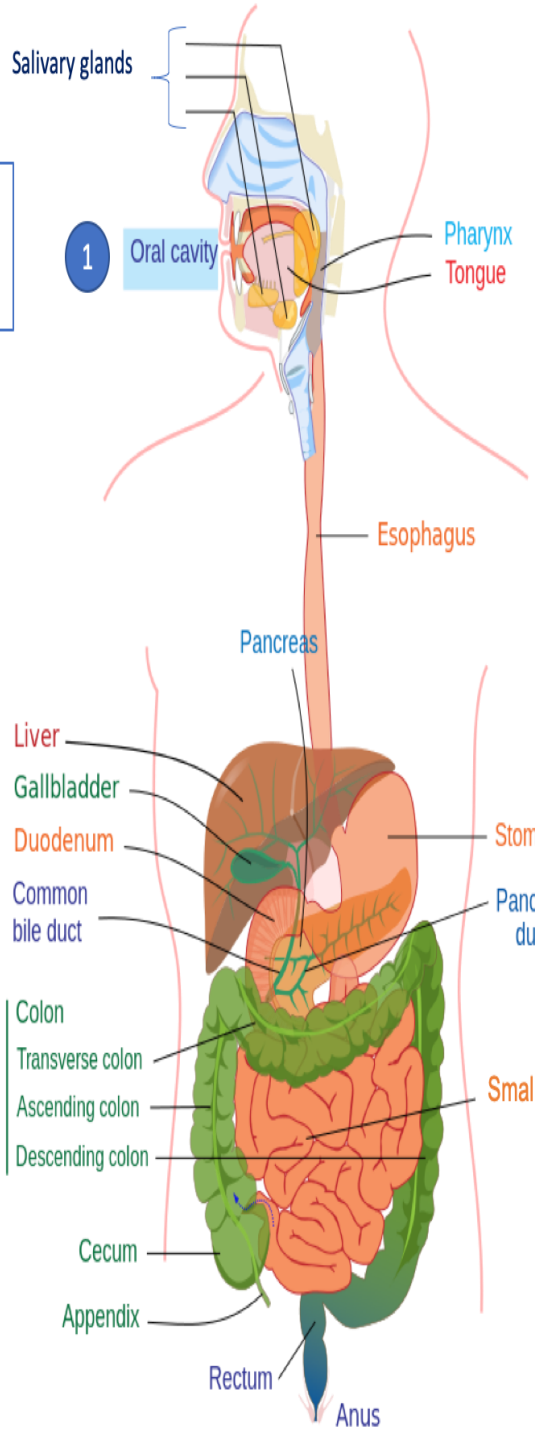
Once proteins are denatured in the stomach, the peptide bonds linking amino acids together are more accessible for enzymatic digestion. That

process is started by pepsin, an enzyme that is secreted by the cells that line the stomach and is activated by hydrochloric acid.



The two major pancreatic enzymes that digest proteins in the small intestine are ***chymotrypsin*** and ***trypsin***. Trypsin activates other protein-digesting enzymes called ***proteases***, and together, these enzymes break proteins down to tripeptides, dipeptides, and individual amino acids. The cells that line the small intestine release additional enzymes that also contribute to the enzymatic digestion of polypeptides.

1. MOUTH
- mechanical digestion (chewing)



2. STOMACH
- denaturation (HCl)
- enzymatic digestion (pepsin)

3. SMALL INTESTINE
- enzymatic digestion (chymotrypsin, trypsin, proteases)
- absorption of amino acids, di- and tripeptides

ENZYMES

General Properties

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

Nature of Enzymes

Most enzymes are protein in nature. Depending on the presence and absence of a non-protein component with the enzyme, enzymes can exist as; simple enzyme or holoenzyme:

1. Simple enzyme: It is made up of only protein molecules not bound to any non-proteins. Example: Pancreatic Ribonuclease.

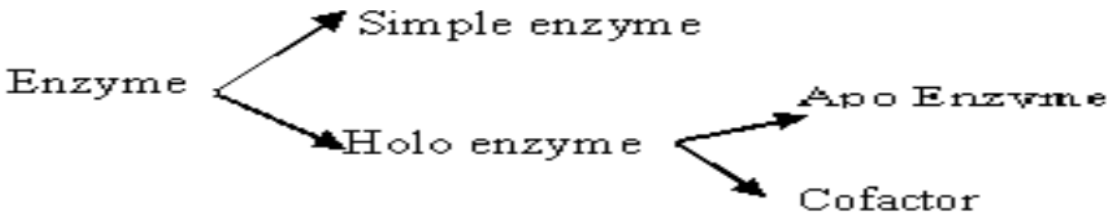
2. Holo enzyme is made up of protein groups and non-protein component.

-The protein component of this holo enzymes is called apoenzyme

-The non-protein component of the holo enzyme is called a cofactor.

If this cofactor is an organic compound like vitamins it is called a coenzyme and if it is an inorganic groups it is called activator (Fe^{2+}, Mn^{2+}, or Zn^{2+} ions).

If the cofactor is bound so tightly to the apoenzyme and is difficult to remove without damaging the enzyme it is sometimes called a **prosthetic group**



COENZYMES

Coenzymes are derivatives of vitamins without which the enzyme cannot exhibit any reaction. One molecule of coenzyme is able to convert a large number of substrate molecules with the help of enzyme. (Substrates are molecules that enzyme act on and converted it to product).

-Coenzyme accepts a particular group removed from the substrate or donates a particular group to the substrate.

-Coenzymes are called cosubstrate because the changes that take place in substrates are complimentary to the changes in coenzymes.

-The coenzyme may participate in forming an intermediate enzyme-substrate complex

Example: NAD, FAD, Coenzyme A

Metal ions in enzymes

Many enzymes require metal ions like Ca^{2+}, K^{+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+} and Co^{2+} for their activity.

Metal-activated enzymes form only loose and easily dissociable complexes with the metal and can easily release the metal without denaturation. Metalloenzymes hold the metal tightly on the molecule and do not release it even during extensive purification.

Metal ions promote enzyme action by

a. Maintaining or producing the active structural conformation of the enzyme (e.g. glutamine synthase).

b. Promoting the formation of the enzyme-substrate complex (example: enolase and carboxypeptidase A).

c. Acting as electron donors or acceptors (example: Fe-S proteins and cytochromes).

d. Causing distortions in the substrate or the enzyme, example: phosphotransferases).

General terminology

Activation energy: It is usually considered to be the energy required for a molecule to form an activated complex which is in the transition of making or breaking a chemical bond. In an enzyme-catalyzed reaction, this corresponds to the formation of the activated enzyme-substrate complex.

Activator: An effector molecule that increases the catalytic activity of an enzyme when it binds to a specific site.

Active site: The part of enzyme at which the initial binding of substrate and enzyme occurs to form the intermediate enzyme-substrate complex and at which location further chemical change characteristic of the catalyzed reaction takes place.

Allostery: A phenomenon whereby the conformation of an enzyme is altered by combination, at a site other than the substrate-binding site, with a small molecule, referred to as an effector, which results in either increased or decreased activity by the enzyme.

Apoenzyme: The protein part of an enzyme without the cofactor necessary for catalysis. The cofactor can be a metal ion, an organic molecule (coenzyme), or a combination of both.

Catalyst: A catalyst is a substance that speed up or increases the rate of a reaction and its unchanged at the end of the process.

Denaturation: The partial or total alteration of the structure of a protein without change in covalent structure by the action of certain physical procedures (heating) or chemical agents. Denaturation is the result of the disruption of tertiary bonding, which causes the opening of the folded structure of a protein and the loss of characteristic physiologic, enzymatic, or physicochemical properties; it can be either reversible or irreversible.

Holoenzyme: An active enzyme consisting of the apoenzyme and coenzyme.

Induction: In enzymology, induction is a biological process which results in an increased biosynthesis of an enzyme thereby increasing its apparent activity.

Inhibitor: An inhibitor is a substance that diminishes the rate of a chemical reaction; the process is called inhibition.

Isoenzyme: One of a group of related enzymes catalyzing the same reaction but having different molecular structures and characterized by varying physical, and biochemical properties. Like creatine Kinase(CK) (CK-BB, CK-BM, CK-MM)

- **Enzyme unit:** the amount of enzyme required for transformation one micromole of substrate (reactant molecules) in one minute.
- **Turnover number:** the number of substrate molecules transformed per one minute by a single enzyme molecule.
- **Specific activity:** number of units of enzyme present in one mg of protein.

Properties of Enzyme

A. Active site

Enzyme molecules contain a special pocket or cleft called the active site. The active site contains amino acid chains that create a three-dimensional surface complementary to the substrate. The active site binds the substrate, forming an enzyme-substrate (ES) complex.

ES is converted to enzyme-product (EP); which subsequently dissociates to enzyme and product. For the combination with substrate, each enzyme is said to possess one or more active sites where the substrate can be taken up.

It is also possible that the active site (Catalytic site) is different from the binding site in which case they are situated closely together in the enzyme molecule.

B. Catalytic efficiency/ Enzyme turnover number

Most enzyme- catalyzed reactions are highly efficient proceeding from 10^3 to 10^8 times faster than uncatalyzed reactions. Typically each enzyme molecule is capable of transforming 100 to 1000 substrate molecule in to product each second.

Enzyme turn over number refers to the amount of substrate converted per unit time (carbonic anhydrase is the fastest enzyme).

C. Specificity

Enzymes are specific for their substrate. Specificity of enzymes are divided into:

1. **Absolute specificity:**- this means one enzyme catalyzes or acts on only one substrate. For example: Urease catalyzes hydrolysis of urea but not thiourea.

2. **Stereo specificity**- some enzymes are specific to only one isomer even if the compound is one type of molecule:

For example: glucose oxidase catalyzes the oxidation of β-D-glucose but not α-D-glucose, and arginase catalyzes the hydrolysis of L-arginine but not D-arginine.

*Maltase catalyzes the hydrolysis of α- but not β –glycosides.

3- Bond Specificity : enzymes that are specific for a bond or linkage such as ester, peptide or glycosidic belong to this group

Examples:

a. Esterases- acts on ester bonds

b. Peptidases-acts on peptide bonds

c. Glycosidases- acts on glycosidic bonds.

D. Regulation

Enzyme activity can be regulated that is, enzyme can be, activated or inhibited so that the rate of product formation responds to the needs of the cell.

E. Zymogens (- inactive form of enzyme)

Some enzymes are produced in nature in an inactive form which can be activated when they are required. Such type of enzymes are called Zymogens (Proenzymes).

Many of the digestive enzymes and enzymes concerned with blood coagulation are in this group

Examples: Pepsinogen - This zymogen is from gastric juice. When required Pepsinogen converts to Pepsin

Trypsinogen - This zymogen is found in the pancreatic juice, and when it is required gets converted to trypsin.

* The activation is brought about by specific ions or by other enzymes that are proteolytic.

Pepsinogen + H^+ ⟶ Pepsin

Trypsinogen $\xrightarrow{\text{Enteropeptidase}}$ Trypsin

Zymogen forms of enzymes a protective mechanism to prevent auto digestion of tissue producing the digestive enzymes and to prevent intravascular coagulation of blood.

*** Classification of Enzymes**

Enzymes are usually named in terms of the reactions they catalyze, by adding the suffix " ase " to a major part of the substrate acted upon like urease act on urea, and tyrosinase on tyrosine. There are some trivial names like pepsin and trypsin which are proteases.

Enzymes are classified on the basis of the reactions they catalyze. Each enzyme is assigned a four-digit classification number and a systematic name, which identifies the reaction catalyzed.

The international union of Biochemistry and Molecular Biology developed a system of nomenclature on which enzymes are divided into six major classes, each with numerous sub groups. Enzymes are classified based on the reactions they catalyze. Each enzyme is characterized by a code number comprising four digits separated by points. The four digits characterize class, sub-class, sub-sub-class, and serial number of a particular enzyme.

Class I. Oxidoreducatases- Enzymes catalyzing oxidation reduction reactions.

Example: Lactate-dehydrogenase

1- Lactic acid + NAD^+ ⟶ Pyruvic acid + $NADH+H^+$

Class II. Transferases:

Enzymes catalyzing a transfer of a group other than hydrogen (methyl, acyl, amino or phosphate groups)

Example: Enzymes catalyzing transfer of phosphorus containing groups.

ATP: D-hexose-6 phosphotransferase (Hexokinase)

ATP+D-Hexose ⟶ ADP+D-hexose-6-phosphate

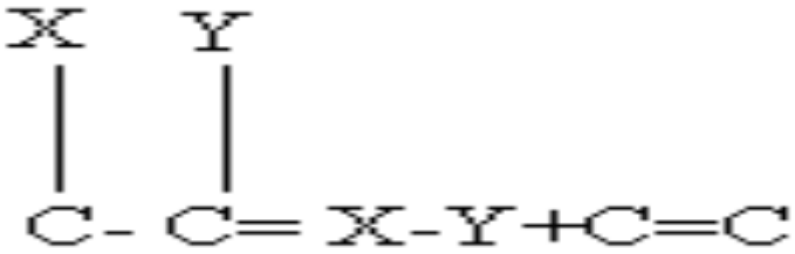
Class III. Hydrolases: Enzymes catalyzing hydrolysis of ester, ether, peptide, glycosyl, acid-anhydride, or C-C bonds by utilizing water.

Example: Enzymes action on glycosyl compounds

β-D- Galactoside galactohydrolase (β -Galactosidase)

β -D- Galactoside+H_2O ⟶ alcohol +D-Galactose

Class IV. Lyases: Enzymes that catalyze removal of groups from substances by mechanisms other than hydrolysis, leaving double bonds.



Enzymes acting on C-C, C-O, C-N, and C-S bonds.

Example : Carbon-Oxygen lyases

Malate hydrolyase (Fumarase)

Class V. Isomerases:

Includes all enzymes catalyzing interconversion of optical, geometric, or positional isomers.

Example: Enzymes catalyzing interconversion of aldose and ketoses

Glyceraldehyde-3- phosphate ketoisomerase (triosephosphate isomerase)

Glyceraldehyde-3phosphate ⟶ Dihydroxyacetone phosphate.

Class VI. Ligases or synthetases.

Enzymes catalyzing the linking together of 2 compounds coupled to the breaking of a pyrophosphate bond in ATP or similar trinucleotides: GTP, UTP etc. included are enzymes catalyzing reactions forming C-O, C-S, C-N, and C-C bonds.

Example: Enzymes catalyzing formation of C-C bonds

Acetyl-CoA: CO_2 ligase (ADP) [acetyl-CoA carboxylase] ATP+ Acetyl-COA+CO_2 ⟶ Malonyl-CoA + ADP + phosphate.

MECHANISM OF ACTION OF ENZYMES

Emil Fischer's model **lock and key model** 1890. Lock: Key model of enzyme action implies that the active site of the enzyme is complementary in shape to that of its substrate, i.e. the shape of the enzyme molecule and the substrate molecule should fit each other like a lock and Key

In 1958, Daniel Koshland, postulated another model, **induced-fit model**; which implies that the shapes & the active sites of enzymes are complementary to that of the substrate only after the substrate is bound.

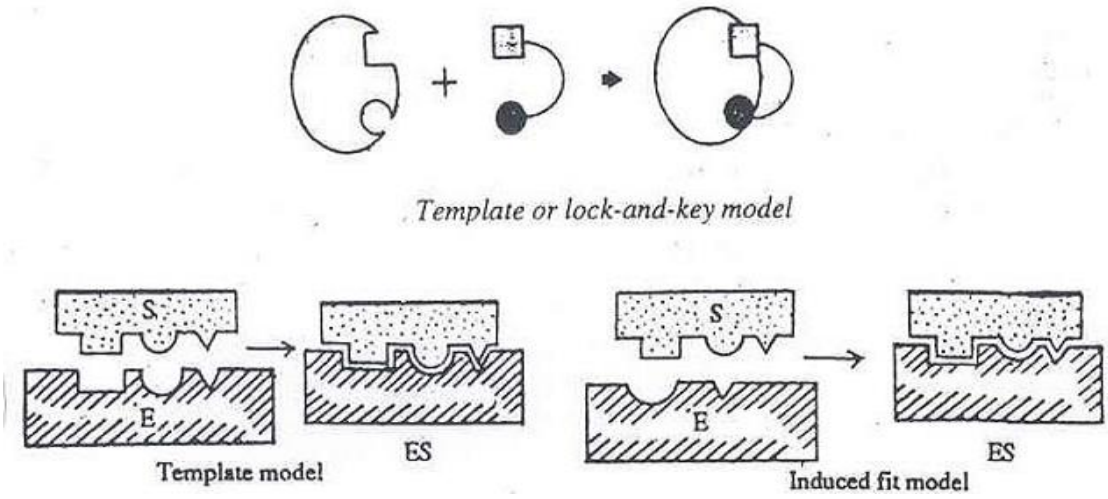


Figure: Models of enzyme- substrate interactions

Michaels and Menten have proposed a hypothesis for enzyme action, which is most acceptable. According to their hypothesis, the enzyme molecule (E) first combines with a substrate molecule (S) to form an enzyme substrate (ES) complex which further dissociates to form product (P) and enzyme (E) back. Enzyme once dissociated from the complex is free to combine with another molecule of substrate and form product in a similar way.

Enzymes Lowering Free Energy of Activation

Enzymes bind temporarily to one or more of the reactants of the reaction they catalyze, and this lead to lower the amount of activation energy needed and thus speed up the reaction.

A chemical reaction $S \longrightarrow P$ (where S is the substrate and P is the product or products) will take place when a certain number of S molecules at any given instant possess enough energy to attain an activated condition called the **"transition state"**, in which the probability of making or breaking a chemical bond to form the product is very high.

The transition state is the top of the energy barrier separating the reactants and products.

A rise in temperature, by increasing thermal motion and energy, causes an increase in the number of molecules on the transition state and thus accelerates a chemical reaction. The enzyme combines transiently with the substrate to produce a transient state having a lower energy of activation than that of substrate alone. This results in acceleration of the reaction.

Activation energy is defined as the energy required to convert all molecules in one mole of reacting substance from the ground state to the transition state.

Enzyme are said to reduce the magnitude of this activation energy.

* During the formation of an ES complex, the substrate attaches itself to the specific active sites on the enzyme molecule by Reversible interactions formed by Electrostatic bonds, Hydrogen bonds, Vanderwaals forces, Hydrophobic interactions.

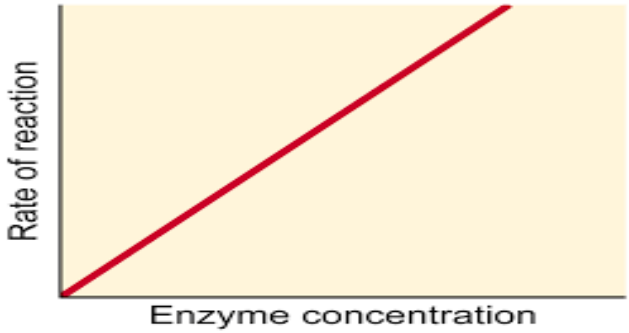
*** Factors Affecting Enzyme Activity**

Physical and chemical factors are affecting the enzyme activity. These include

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

1- **Enzyme concentration**

the enzyme activity increased with the increase of enzyme concentration, until reach all the reactant substances (substrates) will convert to products.



2.Temperature

Starting from low temperature as the temperature increases to certain degree the activity of the enzyme increases because the temperature increase the total energy of the chemical system. There is an optimal temperature at which the reaction is most rapid (maximum).

Above this the reaction rate decreases sharply, mainly due to denaturation of the enzyme by heat.

The temperature at which an enzyme shows maximum activity is known as the optimum temperature for the enzyme. For most body enzymes the optimum temperature is around 37^0C, which is body temperature.

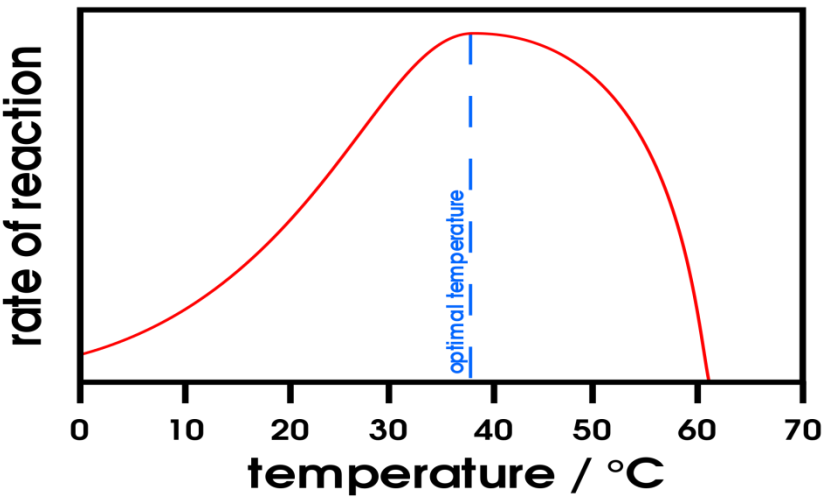


Figure. Effect of temperature on enzymatic reaction

3. Effect of pH

The concentration of H^+ affects reaction velocity in which the enzyme and substrate usually requires specific chemical groups in an ionized or unionized state in order to interact.

Extreme pH can lead to denaturation of the enzyme, because the structure of the catalytically active protein molecule depends on the ionic character of the amino acid chains.

The pH at which maximum enzyme activity is achieved is different for different enzymes.

For example, pepsin, a digestive enzyme in the stomach, has maximum action at pH 2, where as other enzymes, designed to work at neutral pH, are denatured by such an acidic environment like carbonic anhydrase, and others need alkaline medium like alkaline phosphatase and trypsin.

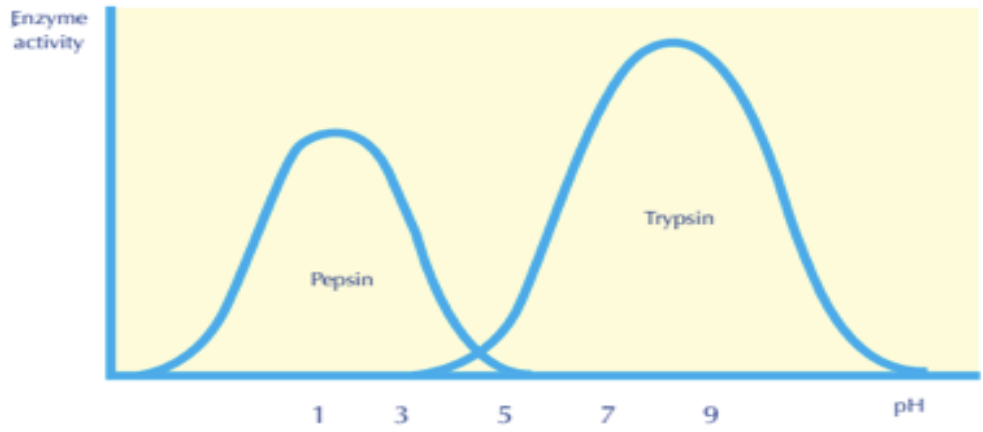


Figure. Effect of pH on enzymatic reaction

4. Concentration of substrate

At fixed enzyme concentration, pH and temperature, the activity of enzymes is influenced by increase in substrate concentration.

An increase in the substrate concentration increases the enzyme activity till a maximum is reached. Further increase in substrate concentration does not increase rate of reaction.

This condition shows that as concentration of substrate is increased, the substrate molecule combine with all available enzyme molecules at their active site till not more active sites are available (the active sites become saturated). At this state the enzymes obtained it maximum rate (V max).

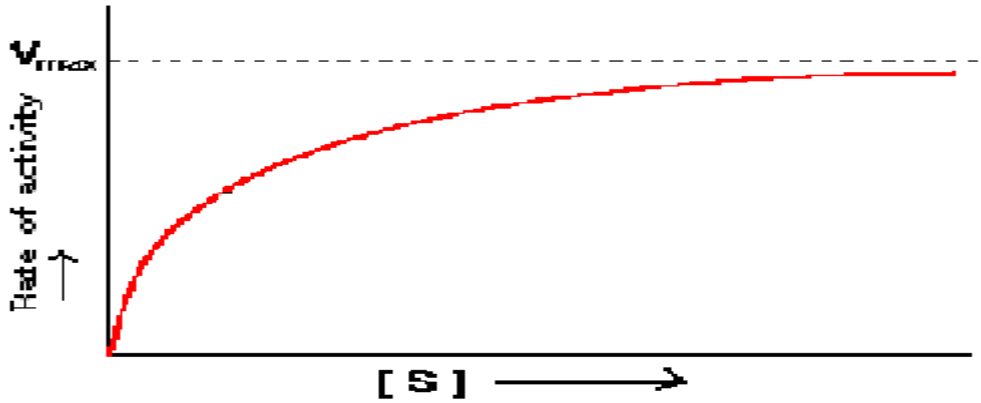


Figure. Effect of Concentration of substrate on enzyme activity

The characteristic shape of the substrate saturation curve for an enzyme can be expressed mathematically by the Michaelis Menten equation:

$$V = \frac{V\max[S]}{Km + [S]}$$

Where: V= Velocity at a given concentration of substrate (initial reaction velocity)

Vmax = Maximal velocity possible with excess of substrate

[S] = concentration of the substrate at velocity V

Km = michaelis-constant of the enzyme for particular substrate.

- Below the relationship between [S] and Km:

a- [S] << Km

$$V = \frac{Vmax\ [S]}{Km} \quad \text{--------- } V\alpha\ [S]$$

b- [S] = Km

$$V = \frac{Vmax\ [S]}{[S] + [S]} = \frac{Vmax\ [S]}{2[S]} = 1/2Vmax$$

c- [S] >> Km

$$V = \frac{Vmax\ [S]}{[S]} \quad \text{-------} \quad V = Vmax$$

When [S] is much less than Km, the velocity of the reaction is roughly proportional to the substrate concentration. The rate of reaction is then said to be first order configuration with respect to substrate. When [S] is much greater than Km, the velocity is constant and equal to Vmax. The rate of reaction is then independent of substrate concentration and said to be zero order with respect to substrate concentration.

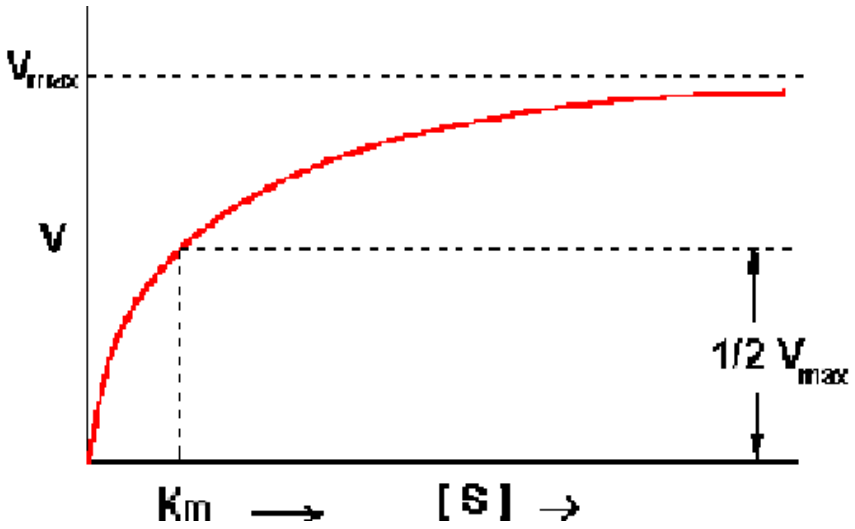


Figure: Relationship between [S] and Km

*** Characteristics of Km**

Km- can defined as the concentration of the substrate at which a reaction velocity equal to ½ Vmax.

Km- is characteristic of an enzyme and a particular substrate, and reflects the affinity of the enzyme for that substrate.

Km- values varies from enzyme to enzyme and used to characterized different enzymes.

Km- values of an enzyme helps to understand the nature and speed of the enzyme catalysis.

High Km value of an enzyme means the catalysis of that enzyme is slow compared to low Km.

Km does not vary with the concentration of enzyme.

*** Enzyme Inhibition**

Any substance that can diminish the velocity of an enzyme-catalyzed reaction is called an inhibitor and the process is known as inhibition.

There are two major types of enzyme inhibition, Irreversible and Reversible.

1- Irreversible Inhibition

The type of inhibition that cannot be reversed by increasing substrate concentration or removing the remaining free inhibitor is called Irreversible inhibition Eg. Diisopropyl fluorophosphate (DFP) Inhibits the enzyme acetyl cholinesterase, important in the transmission of nerve impulses. Acetyl cholinesterase catalyzes the hydrolysis of acetylcholine to acetic acid and choline, which acetylcholine is a neurotransmitter substance functioning in certain portions of the nervous system.

- DFP inhibits also trypsin, chymotrypsin, elastase, and phosphglucomutase. Organo-phosphorus compounds like malathion, parathron pesticides-inhibits acetyl cholinesterase by the same way as DFP.

2- Reversible inhibition

This type of inhibition can be Competitive, Non-competitive and uncompetitive.

a- Competitive Inhibition: This type of inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy, therefore, competes with the substrate for that site.

In competitive inhibition the inhibitor and substrate compete for the same active site on the enzyme as a result of similarity in structure. The enzyme substrate complex will be broken dawn to products (E+S $\longrightarrow$ ES $\longrightarrow$ E+P) where as enzyme inhibitor complex; (EI) will not be broken down to products.

A classical example is Malonate that competes with succinate and inhibits the action of succinate dehydrogenase to produce fumarate in the Krebs cycle.

The enzyme can be also inhibited by oxalate and glutarate because of the similarity of these substances with succinate

Eg.2 Allopurinol used for the treatment of Gout

Allopurinol inhibits xanthine oxidase by competing with uric acid precursors for the active site on the enzyme. This competition blocks the conversion of these precursors, and of hypoxanthine and xanthine, to uric acid and result in lower serum urate levels.

Since most clinical drug therapy is based on inhibiting the activity of enzymes.

Effect of competitive inhibitors

The effect of a competitive inhibitor is reversed by increasing [s]. at a sufficiently high substrate concentration, the reaction velocity reaches the Vmax. observed in the absence of inhibitor.

Also a competitive inhibitor increases the apparent Km for a given substrate. This means that in the presence of a competitive inhibitor more substrate is needed to achieve ½ Vmax.

b- Non-Competitive Inhibition

In non-competitive inhibition the inhibitor binds at different site rather than the substrate-binding site. When the inhibitor binds at this site there will be a change in conformation of the enzyme molecules, which leads to the reversible inactivation of the catalytic site.

Non-competitive inhibitors bind reversibly either to the free-enzyme or the ES complex to form the inactive complexes EI and ESI (Enzyme substrate Inhibitor).

The most important non-competitive inhibitors are naturally occurring metabolic intermediates that can combine reversibly with specific sites on certain regulatory enzymes, that changes the activity of their catalytic sites.

An Example: is the inhibition of threonine dehydratase by isoleucine.

*Such type of enzyme is called **Allosteric Enzyme**, which has a specific sites or allosteric site other than the substrate-binding site.

Non-Competitive inhibition cannot be overcome by increasing the concentration of substrate. Thus, non-competitive inhibitors decrease the Vmax of the reaction. Also Non-competitive inhibitors do not interfere with the binding of substrate to enzyme. Thus, the

enzyme shows the same Km in the presence or absence of the non-competitive inhibitor.

c- Uncompetitive Inhibition

Uncompetitive Inhibitor binds only to ES complex at locations other than the catalytic site. Substrate binding modifies enzyme structure, making inhibitor-binding site available. Inhibition cannot be reversed by substrate.

In this case apparent Vmax. and Km decreased.

*** Regulation of enzyme activity**

There are several means by which the activity of a particular enzyme is specifically regulated.

1. Irreversible covalent activation / zymogen activation

Some enzymes are secreted in an inactive form called Proenzymes or zymogens. At the site of action specific peptide bonds are hydrolysed either enzymatically or by pH changes to convert it into active form, e.g. Pepsinogen to pepsin, Trypsinogen to trypsin, plasminogen to plasmin. After hydrolysis when it is activated, it cannot be reconverted into proenzyme form.

2. Reversible Covalent Modification

By addition of or removal of phosphate or adenylate, certain enzymes are reversibly activated and inactivated as per the requirement. Protein kinase of muscle phosphorylate phosphorylase kinase, glycogen synthetase by making use of ATP.

3. Allosteric Modulation

In addition to simple enzymes that interact only with substrates and inhibitors, there is a class of enzymes that bind small, physiologically important molecules and modulate activity in ways other than those described above. These are known as **allosteric enzymes**; the small regulatory molecules to which they bind are known as **effectors**.

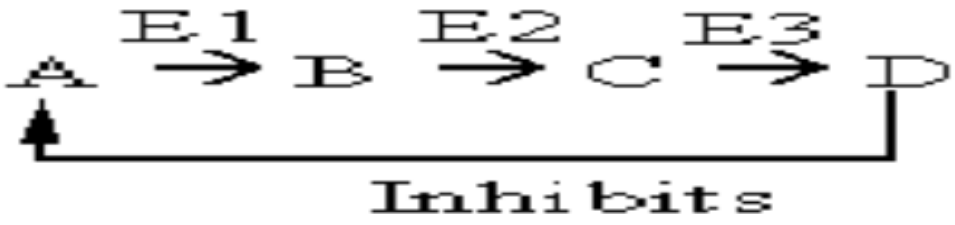
Allosteric effectors bring about catalytic modification by binding to the enzyme at distinct allosteric sites, and causing conformational changes that are transmitted through the bulk of the protein to the catalytically active site.

The hallmark of effectors is that when they bind to enzymes, they alter the catalytic properties of an enzyme's active site. Those that increase catalytic activity are known as positive effectors. Effectors that reduce or inhibit catalytic activity are negative effectors.

There are two ways that enzymatic activity can be altered by effectors: the Vmax can be increased or decreased, or the Km can be raised or lowered.

4. Feedback inhibition

In allosteric regulation in which end products inhibit the activity of the enzyme is called" feedback inhibition".



A high conc. D typically inhibits conversion of A to B.

This involves not simple backing up of intermediates but the activity of D to bind to and inhibit E1. D thus acts as negative allosteric affector or feedback inhibitor of E1.

The kinetics of feedback inhibition can be competitive or mixed. It is the commonest way of regulation of a biosynthetic pathway.

ENZYMES IN CLINICAL DIAGNOSIS

Plasma enzymes can be classified into two major groups

1. Those, relatively, small group of enzymes secreted into the plasma by certain organs (i.e. enzymes those have function in plasma) For example: - the liver secretes zymogens of the enzymes involved in blood coagulation.
2. Those large enzyme species released from cells during normal cell turnover. These enzymes are normally intracellular and have no physiologic function in the plasma. In healthy individuals the levels of these enzymes are fairly constant and represent steady state in which the rate of release from cells into the plasma is balanced by an equal rate or removal from the plasma.

Many diseases that cause tissue damage result in an increased release of intracellular enzymes into the plasma. The activities of many of these enzymes are routinely determined for diagnostic purposes in diseases of the heart, liver, skeletal muscle, and other tissues.

The level of specific enzyme activity in the plasma frequently correlates with the extent of tissue damage. Thus, the degree of elevation of a particular enzyme activity in plasma is often useful in evaluating the diagnosis and prognosis for the patient.

1. Lipase:

It is an enzyme catalyzing the hydrolysis of fats. It is secreted by tongue, pancreas and Liver.

The plasma lipase level may be low in liver disease, vitamin A deficiency, some malignancies, and diabetes mellitus. It may be elevated in acute pancreatitis and pancreatic carcinoma.

2. α- Amylase

α- amylase is the enzyme concerned with the breakdown of dietary starch and glycogen to maltose. It is present in pancreatic juice and saliva as well as in liver and muscles. The enzyme is excreted in the Urine. The main use of amylase estimations is in the diagnosis of acute pancreatitis.

The plasma amylase level may be low in liver disease and increased in high intestinal obstruction, mumps, acute pancreatitis and diabetes.

3. Acid Phosphatase (ACP)

Acid phosphatases catalyzing the hydrolysis of various phosphate esters at acidic pH is found in the prostate, liver, red cells, platelets and bone. It may be elevated in metastatic prostatic carcinoma.

4. Transaminases

Two transaminases are of clinical interest.

1. Aspartate Transaminase, AST (Glutamate oxaloacetate transaminase, GOT)

catalyzes the transfer of the amino group of aspartic acid to α-ketoglutarate forming glutamate and oxaloacetate.

AST or GOT is widely distributed, with high concentration, in the heart, liver, skeletal muscle, kidney and erythrocytes, and damage to any of these tissues may cause raised levels.

2. Alanine transaminase, ALT (Glutamate pyruvate transaminase, GPT)

Transfer the amino group of alanine to α- ketoglutarate, forming glutamate and pyruvate. It is present in high concentration in liver and to a lesser extent in skeletal muscle, kidney and heart.

- Serum levels of glutamate- pyruvate transaminase (SGPT) and Glutamate-oxaloacetate- transaminase (SGOT) are useful in the diagnosis of liver parenchymal damage and myocardial damage respectively. In liver damage, both enzymes are increased, but SGPT increases more. In myocardial infarction SGOT is increased with little or no increase in SGPT.

5. Lactate Dehydrogenase (LDH)

It catalyzes the reversible interconversion of lactate and pyruvate. It is widely distributed with high concentrations in the heart, skeletal muscle, liver, kidney, brain and erythrocytes.

The enzyme is increased in plasma in myocardial infarction, acute leukemia, generalized carcinomatosis and in acute hepatitis. Estimation of its isoenzymes is more useful in clinical diagnosis to differentiate hepatic disease and myocardial infarction.

6. Creatine kinase (CK) or creatine phosphokinase (CPK)

CK (CPK) is found in heart muscle, brain and skeletal muscle. Measurement of serum creatine phosphokinase activity is of value in the diagnosis of disorders affecting skeletal and cardiac muscle. The level of CPK in plasma highly increased in myocardial infarction.

Dr. Salim.J.Kh.

Tenth Lab

Kidney Function Tests

Quantitative Determination of Blood Urea Nitrogen(BUN)

Clinical significance:

Urea is the major end product of protein nitrogen metabolism. It is synthesized by the urea cycle in the liver and excreted through the kidneys. The circulating levels of urea depend upon protein intake, protein catabolism and kidney function.

Elevated urea levels can occur due to renal impairment or in some diseases such as diabetes, infection, congestive heart failure and during different liver diseases. Determination of blood urea nitrogen is the most widely used screening test for renal function together with serum creatinine.

Principle of action:

Urease-colorimetric method is the most widely used in determination of BUN.

The reaction involved in the assay system is as follows:

Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide.

$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$$

The free ammonia in an alkaline pH and in the presence of indicator forms coloured complex proportional to the urea concentration in the specimen.

Procedure:

	Blank	Standard	Sample
Reagent(R1)	1 ml	1 ml	1 ml
Standard		10 µl	
Sample			10 µl

Mix and incubate for 3 minutes at 37°C or 5 minutes at room temperature (15-25 °C).

Reagent(R2)	200µl	200µl	200µl

Mix and incubate for 5 minutes at 37°C or 10 minutes at room temperature (15-25 °C).

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

Calculation: Result= absorb. of assay/ absorb. of stand.× stand. conc.

The normal value is 10-40 mg/dl.

The BUN is stable in the sample(serum) for 1 days at 15-25°C, 7 days at 2-8°C, 1 year at -20°C.

Quantitative Determination of Serum Creatinine

Clinical significance:

Creatinine is derived from creatine and creatine phosphate in muscle tissue and may be defined as a nitrogenous waste product. Creatinine is not reutelized but is excreted from the body in the urine via the kidney. It is produced and excreted at a constant rate which is proportional to the body muscle mass.

As a consequence of the way in which creatinine is excreted by the kidney, creatinine measurement is used almost exclusively in the assessment of kidney function. Creatinine is regarded as the most useful endogenous marker in the diagnosis and treatment of kidney disease.

Creatinine is measured primerily to assess kidney function and has certain advantages over the measurement of urea. The plasma level of creatinine is relatively independent of protein ingestion, water intake, rate of urine production and exercise. Since its rate of production is constant, elevation of plasma creatinine is indicative of under-excretion, suggesting kidney impairment. Depressed levels of plasma creatinine are rare and not clinically significant.

Principle of action:

Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Creatinine in serum, heparinized or EDTA plasma stable for 7 days at +2 to +8°C. Norma value in serum about 0.6-1.3 mg/dl for males and 0.5-1.1 mg/dl for females.

Practical Biochemistry

(First Lab)

Laboratory Safety

Laboratory safety involves the development of skills and must be an integral part of every chemistry curriculum. It is involve implementation of a chemical hygiene plan that is in agreement with chemical safety efforts and must address the safe handling, storage, and disposal of chemicals.

Behavior in the laboratory

- Eye wash and showers must be in operating condition.

- Fume hoods are essential.

- Anyone working or visiting in the lab must be wearing goggles and labcoat .

- wear appropriate gloves when manipulating dangerous substances.

- Consumption of food or drinks must not be permitted.

- A clean, uncluttered laboratory is more likely to encourage careful work.

Development of safety skills may be divided into four emphasis areas:

• Recognize Hazards

• Assess Risks

• Minimize Risks

• Prepare for Emergencies.

Chemical Hazards

A hazard is a potential source of danger or harm and can result from working with chemicals, equipment, and instrumentation. Introduction to the terms describing chemical hazards, such as “toxic”, “flammable”, or “corrosive”, and how to obtain information from chemical labels, Safety Data Sheets (SDS), and other reference sources. Chemical hazards examples, acids, bases, flammables, and toxic compounds.

Minimize potential risks of chemicals by carrying out experiments in a fume hood with a protective shield and wearing protective gloves and goggles. The handling and storage of wastes is a critical component. It is often useful to consider case histories of incidents that have resulted in injury or damage.

Laboratorial should learn what to do in various emergencies and be prepared to act accordingly – for example, fires and injuries. Safety devices such as showers, eye washes, and fire extinguishers, must be clearly labeled and their use and location known to all those working in a laboratory. Emergency phone numbers, alarms, and escape routes should be clear to everyone.

Handling and Transportation of Chemicals

Many laboratory accidents occur by carrying chemicals from one place to another or transferring them from one container to another. The chemicals used in a laboratory are often corrosive, toxic or flammable and any accident involving these has the potential for personal injury.

Precautions:

- be familiar with the task at hand.

- carefully read the directions

- understand the chemistry

- carefully and calmly plan delicate manipulations

- think before acting.

Make sure you know

• telephone (and what are the emergency numbers!)

• safety door

• fire extinguisher and fire blanket

• sandbox (to extinguish burning metals)

• first aid box

• person to ask/alert in case of problems

• safety shower

• eye washing station

• gaz mask

• safety instruction poster.

Recommendations

- do not obstruct walkways and safety exits

- work in a stable position

- do not run in the lab

- use clean and functional lab material

- wash your hands before and after manipulations

- label all recipients clearly and readably

- run and clean the eye showers every month

- do not keep food in lab fridges containing chemicals.

Order- and cleanliness

- keep your lab equipment clean and in good shape

- clean used glassware

- keep order in your lab

- dispose regularly of your chemical waste.

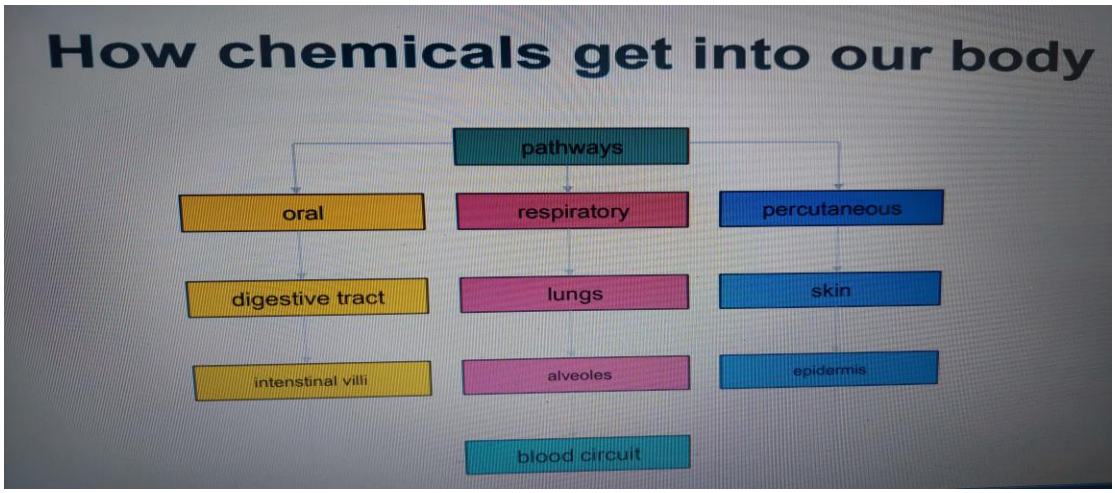
Protective measures

- read and respect the safety labels and instructions on chemical bottles.

- wear your personal safety equipment (goggles, lab coats, gloves etc.) correctly and keep it in shape.

- respect the safety directives.

How chemicals get into our body



When to change gloves

- as soon as a glove is soiled or it leaks

- at the end of a manipulation

- at least every work hour (because after some time gloves become porous).

Protective creams

- protective creams help the skin to heal little lesions and seal the skin from chemicals (close pores)

- apply the cream before you begin to work and after breaks.

- apply it also on the back of all fingers, around the fingernails, between the fingers, and on the wrist.

Hoods

the primary purpose of the hoods is to protect your health and during manipulation with chemical and vapor materials.

Before you leave the lab

- switch off

  • the ventilation in hoods that are not used

  • all apparatus (like heaters)

  • all lights

- close

  • the solvent cabinets

  • all windows

- all supplies of water, vacuum, gas, nitrogen
- covers of the used solvent containers

Security data sheets

1. identification of the substance composition and information on the components.

2. identification of dangers (first aid in case of exposure, how to fight a fire involving this product, and what to do if the products is accidentally spilled).

3. how to manipulate and store the product and how to control and how to protect oneself against exposure

4. physicochemical properties (stability / reactivity, toxicological and ecological informations.

5. how to eliminate / dispose and transport of the product.

Chemical Storage

Proper storage of chemicals is necessary to provide security, identification, and provide a "user friendly" system.

- Chemicals must be stored in ventilated cabinets (where such cabinets exist).

- Storage in the laboratory should be a « buffer storage » for the daily use, especially for solvents.

- Pay attention to the packing of the chemicals (form and material).

Endocrine System

Second Lecture

Hormones affect the following important functions:

The various functions performed by hormones may, in general, be discussed under following heads :

1. **Regulatory or homeostatic function**: The hormones have regulatory effects on the composition of the body fluids, the rate of gaseous exchange and the activity of the vascular system and the central nervous system (CNS). **Homeostasis** can be defined as the tendency to maintain uniformity or stability in the internal environment of the organism and to maintain the normal composition of the body fluids. In other words, homoeostasis is the maintenance of a constant internal environment in the face of changes in the external environment.

2. **Permissive function**: Not only does each endocrine gland affect a number of processes, but these glands also affect the functioning of one another. Thus certain hormones require the presence (or 'permission') of another hormone for the expression of their activity. This helps in maintaining a perfect hormonal balance. Derangements of this balance, either clinical or experimental, lead to a variety of metabolic disorders.

3. **Integrative function**: The integrative function of the hormones is reflected in the fact that they support the role of nervous system. However, the integrative properties of the endocrine system are slow and steady whereas those of the nervous system are rapid. This close tie between the two systems has led to the emergence of a new discipline of science called *neuroendocrinology.*

Classification according to function

The major hormones can be divided into 6 general types, based on their role in regulating homeostasis:

1-Master regulators control overall body processes . Most of these hormones come from the pituitary gland, or their production is controlled by the pituitary gland. e.g. thyroid hormones regulate processes like metabolic rate, which is critical for all cells and systems.

2- Several hormones manage energy balance & metabolism. These include insulin & glucagon , cortisol and epinephrine.

3- Several hormones work as a group to control ion balance e.g. PTH and calcitonin that control calcium balance, and aldosterone that controls sodium balance .

4- Hormones controlling growth , development, and reproduction are another group e.g. GH, LH, FSH, estrogen, progesterone & testosterone.

5- A major group of hormones that regulate routine body cycles, like wakefulness, hunger, variations in blood pressure, body composition, seasonal cell turnover , etc. These hormones like melatonin, thyroid stimulating hormone (TSH) and prolactin.

6- The last functional group are hormones that regulate responses to stress like epinephrine & cortisol.

Classification according to structure

Most commonly, hormones are categorized into four structural groups, with members of each group having many properties in common:

1- Peptides and proteins: like LH, FSH, prolactin, ACTH, GH, ADH, and oytocin.

2- Amino acid derivatives: tyrosine give thyroid hormones (T3 and T4) and Catecholamines (epinephrine and norepinephrine).

☼ Tryptophan is the precursor to serotonin and melatonin.

3- Cholesterol Derivatives: examples include the sex steroids such as testosterone and adrenal steroids such as cortisol.

4. **Fatty Acid Derivatives – Eicosanoids**: the principal groups of hormones of this class are prostaglandins and prostacyclins.

Hormone receptor is defines as a molecule or complex of molecules, in or on a cell, that binds its hormone with great selectivity and in so doing is changed in such a manner that a characteristic response or group of responses is initiated.

Characteristics of Receptors: hormone receptors are proteins or glycoproteins that are able to function as follows:

1-They distinguish their hormone from other molecules that may have very similar structures.

2-They bind to the hormone (sometimes called a ligand) even when its concentration is exceedingly low (10^{-8}–10^{-12}M).

3. They undergo a conformational change when bound to the hormone.

4. They catalyze biochemical events to produce a biochemical change.

#Two important terms are used to refer to molecules that bind to the

hormone-binding sites of receptors:

► **Agonists** are molecules that bind the receptor and induce all the post-receptor events that lead to a biologic effect. In other words, they act like the "normal" hormone, although perhaps more or less potently.

► **Antagonists** are molecules that bind the receptor and block binding of the agonist, but fail to trigger intracellular signaling events. Hormone antagonists are widely used as drugs.

Modulation of Hormone Levels

Hormone concentration is influenced by many factors:

a-Rate of hormone synthesis.

b-Release from the endocrine organ.

c- Transport in the circulation.

d- Efficiency of delivery into target cell.

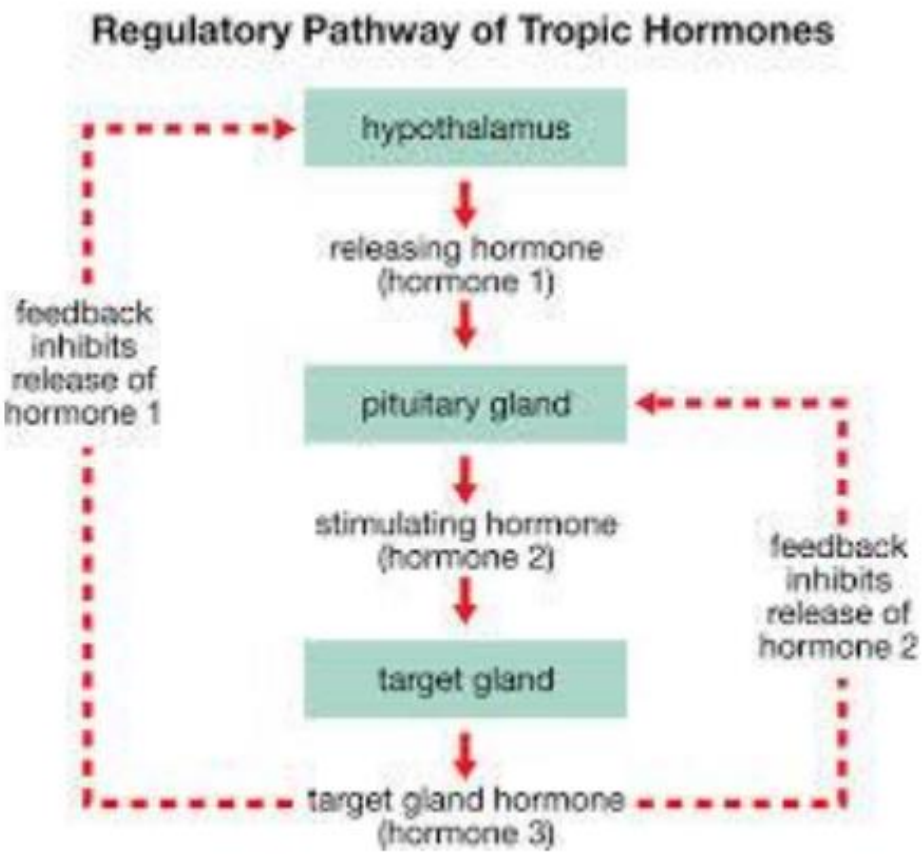
Regulation of Hormone Production

Hormones are produced by endocrine organs in response to:

1-Diverse signals , including other hormones.

2- innervations of the endocrine organ .

3- Environmental signals (including diet).



The hypothalamus-pituitary-target gland system

Hormones half-life: Most hormones are destroyed rapidly after secretion and have a half-life in blood of less than 10 minutes. The half-life of a hormone in blood is defined as that period of time needed for its concentration to be reduced by half and depends on its rate of degradation and on the rapidity with which it can escape from the circulation and equilibrate with fluids in extravascular compartments.

This process is sometimes called the metabolic clearance rate. Some hormones, e.g., epinephrine, have half-lives measured in seconds; others, e.g., thyroid hormones, have half-lives of the order of days.

Pharmacology

Many hormones and their analogues are used as medication. The most commonly prescribed hormones are estrogens and progestagens (as methods of hormonal contraception), thyroxine (as levothyroxine, for hypothyroidism) and steroids (for autoimmune diseases and several respiratory disorders). Insulin is used by many diabetics. Local preparations for use in otolaryngology often contain pharmacologic equivalents of adrenaline, while steroid and vitamin D creams are used extensively in dermatological practice.

A "pharmacologic dose" of a hormone is a medical usage referring to an amount of a hormone far greater than naturally occurs in a healthy body. The effects of pharmacologic doses of hormones may be different from responses to naturally-occurring amounts and may be therapeutically useful. An example is the ability of pharmacologic doses of glucocorticoid to suppress inflammation.

Endocrine System and Hormones

Endocrinology is the study of hormones, their receptors and the intracellular signaling pathways. In addition to the classical endocrine organs, many other cells in the body secrete hormones. Myocytes in the atria of the heart and scattered epithelial cells in the stomach and small intestine are examples of what is sometimes called the "diffuse" endocrine system.

The general function of the endocrine system is to integrate body systems, in conjunction with the nervous system, this by regulating cellular and organ function throughout life and maintaining homeostasis.

The endocrine system is one of the two coordinating and integrating systems of the body. It acts through chemical messengers - hormones – carried in the circulation.

Two systems control all physiologic processes:

☼The **nervous system** exerts point-to-point control through nerves, similar to sending messages by conventional telephone. Nervous control is electrical in nature and fast.

☼The **endocrine system** broadcasts its hormonal messages to essentially all cells by secretion into blood and extracellular fluid. Like a radio broadcast, it requires a receiver to get the message - in the case of endocrine messages, cells must bear a receptor for the hormone being broadcast in order to respond.

There are four types of chemical messengers:

1) Autocrine / Paracrine (prostaglandins / histamine)

• Local chemical messengers.

• Exert effect on neighboring cells (paracrine) or on same cell (autocrine).

2) Neurotransmitters (epinephrine and norepinephrine)

• Short-range chemical messengers.

• Diffuse across narrow space (synapse) to act on adjoining target cell (another neuron, a muscle, or a gland).

3) Neurohormones (oxytocin and antidiuretic hormones)

• Hormones released into blood by neurosecretory neurons.

• Distributed through blood to distant target cells.

4) Hormones (like insulin, parathyroid hormone, etc)

• Long-range messengers.

• Secreted into blood by endocrine glands in response to appropriate signal and act on distant target cells.

Nervous and Endocrine Systems

Property	Nervous System	Endocrine System
Structure	Wired system of neurons	Wireless system of glands
Chemical Messenger	Neurotransmitter	Hormones
Target site	Very close	Far away
Distance of Action	Across synaptic cleft	Carried by blood
Speed of Response	milliseconds	mins to hours
Duration of Action	milliseconds	mins to days

Important definitions:

A. **Endocrine gland**: a gland that secretes hormones directly into the bloodstream; a ductless gland.

B. **Exocrine gland** : a gland that secretes substances into ducts which then leave the body (i.e. sweat/sebaceous glands) or into an internal space or lumen (i.e. digestive glands). Exocrine glands are not part of the endocrine system.

C. **Hormone**: a very powerful substance secreted by an endocrine gland into the bloodstream, that affects the function of another cell or "target cell".

Like all molecules, hormones are synthesized, exist in a biologically active state for a time, and then degrade or are destroyed. Having an appreciation for the "half-life" and mode of elimination of a hormone aids in understanding its role in physiology and is critical when using hormones as drugs.

General characteristics of hormones:

1. needed in very small amounts;

2. produce long-lasting effects in the cells they target;

3. regulate metabolic processes (maintain homeostasis);

4. are regulated by negative-feedback mechanisms;

5. may be steroid (produced from cholesterol = fat-soluble) or non-steroid (water-soluble).

Below is a list of the main glands, some of the hormones they produce and what effects they have on the body.

Hypothalamus: an area in the base of the brain that links the brain to the hormonal system.

- major hormones — anti-diuretic hormone (ADH), oxytocin, dopamine, corticotrophin releasing hormone (CRH), thyrotrophin releasing hormone (TRH), gonadotrophin releasing hormone (GnRH), growth hormone releasing hormone (GHRH) and somatostatin.
- influences — they hypothalamus links the hormonal and nervous systems. Its hormones keep the body stable. They influence sleep rhythms, alertness, appetite, body weight, thirst, blood pressure, heart rate, sex drive, learning, memory, mood and how the body responds to being sick.

Pituitary gland: a kidney bean-shaped gland in the base of the brain.

- major hormones — luteinising hormone (LH), follicle-stimulating hormone (FSH), prolactin, growth hormone, thyroid stimulating hormone (TSH), adrenocorticotrophic hormone (ACTH).
- influences — the pituitary gland helps control other glands and makes hormones that control blood pressure, blood sugar levels, response to stress, menstruation, sperm production, bone growth, muscle mass, contractions during childbirth, making breastmilk and bonding between mother and baby.

Other glands

Pineal gland: a small gland near the center of the brain.

- major hormones — melatonin
- influences — sleep cycle

Thyroid gland: a small gland in the front of the neck, wrapping around the windpipe.

- major hormones — tri-iodothyronine (T3), thyroxine (T4), calcitonin
- influences — metabolism, bone growth, energy levels, body temperature, how the cells use oxygen, heart rate, blood flow, calcium levels, vitamin metabolism, brain development in babies and children, and reproduction

Parathyroid glands: four small glands in the neck behind the thyroid gland.

- major hormones — parathyroid hormone (PTH)

- influences — regulating calcium levels in the blood

Adrenal glands: 2 glands that sit above the kidneys on each side of the body.

- major hormones — adrenaline, cortisol, aldosterone, DHEA, testosterone
- influences — stress response, blood pressure, salt and water control, blood sugar levels, energy, development of sex organs, heart rate, attention, inflammation, development of the fetus.

Pancreas: a long gland behind the stomach, under the liver.

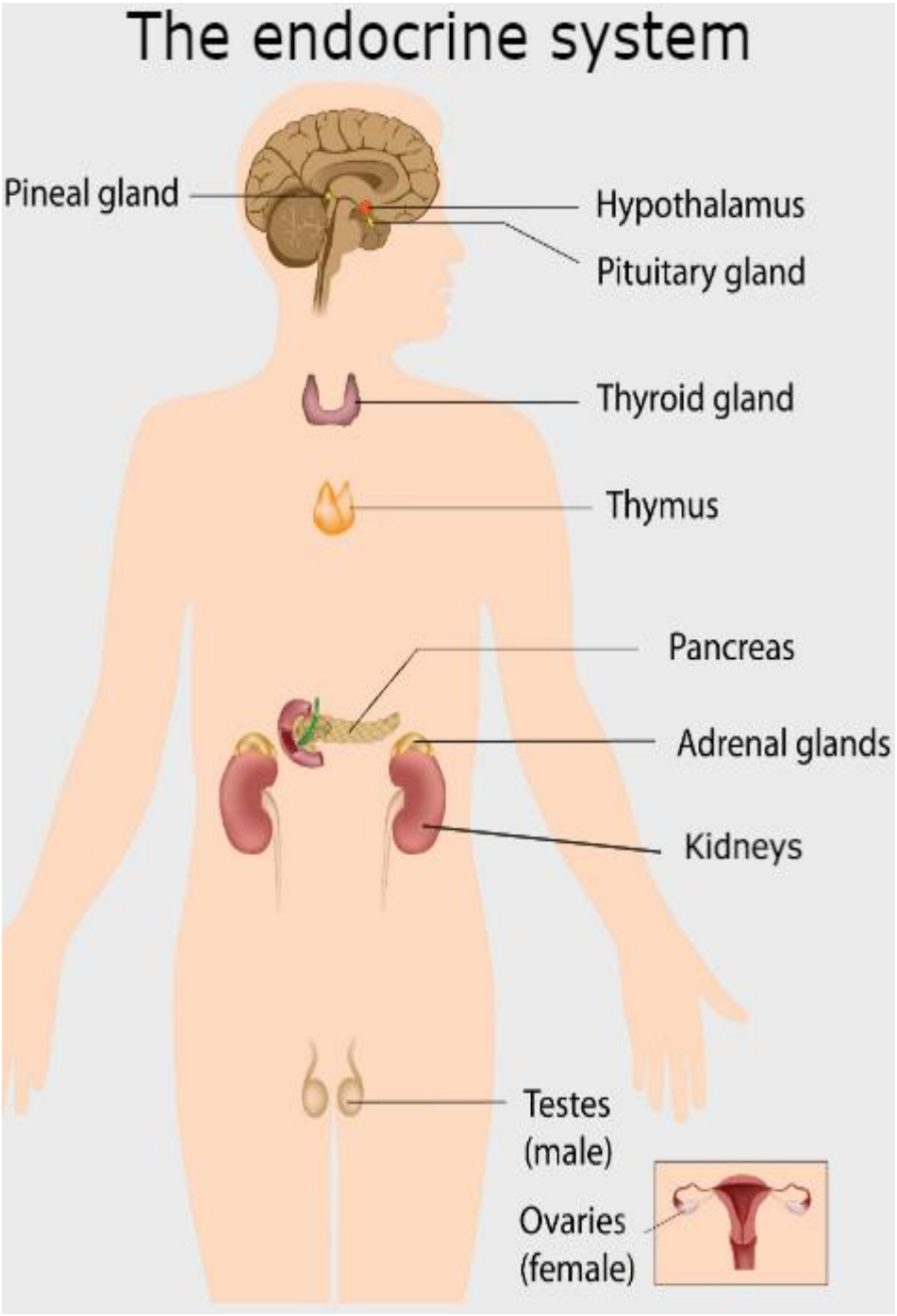
- major hormones — insulin, glucagon, somatostatin, vasoactive intestinal peptide (VIP)
- influences — blood sugar control.

Ovaries (females only): 2 glands found on each side of the uterus in the pelvis.

- major hormones — oestrogen, progesterone, testosterone, anti-mullerian hormone (AMH), Inhibin A and Inhibin B
- influences — female characteristics, storing and releasing eggs.

Testes (males only): 2 glands in the scrotum, behind the penis.

- major hormones — testosterone, anti-mullerian hormone (AMH), estradiol, inhibin B
- influences — male characteristics, sperm production.


The endocrine system
Pineal gland
Hypothalamus
Pituitary gland
Thyroid gland
Thymus
Pancreas
Adrenal glands
Kidneys
Testes
(male)
Ovaries
(female)

" Lipid Metabolism "

Fatty Acid Synthesis

There are three basic sources of fatty acids in animals that can be used for energy conversion processes, 1) fatty acids present in triacylglycerols obtained from the diet, 2) fatty acids stored as triacylglycerols in adipose tissue that are released by hydrolysis following hormone stimulation (glucagon or epinephrine signaling), and 3) fatty acids synthesized in the liver from excess carbohydrates and exported as triacylglycerols.

For F.A. synthesis which occur in endoplasmic reticulum membrane, acetyl-CoA is transported out of the mitochondria as citrate, Which is cleaved in the cytosol to regenerate acetyl-CoA and oxaloacetate.

The process takes place in three major steps: **the citrate shuttle, acetyl-CoA carboxylase (the rate-limiting step), and fatty acid synthase complex**.

Prior to its utilization for F.A. synthesis, acetyl-CoA is converted by carboxylation reaction into malonyl-CoA, this reaction is catalyzed by the enzyme acetyl-CoA carboxylase(ACC).

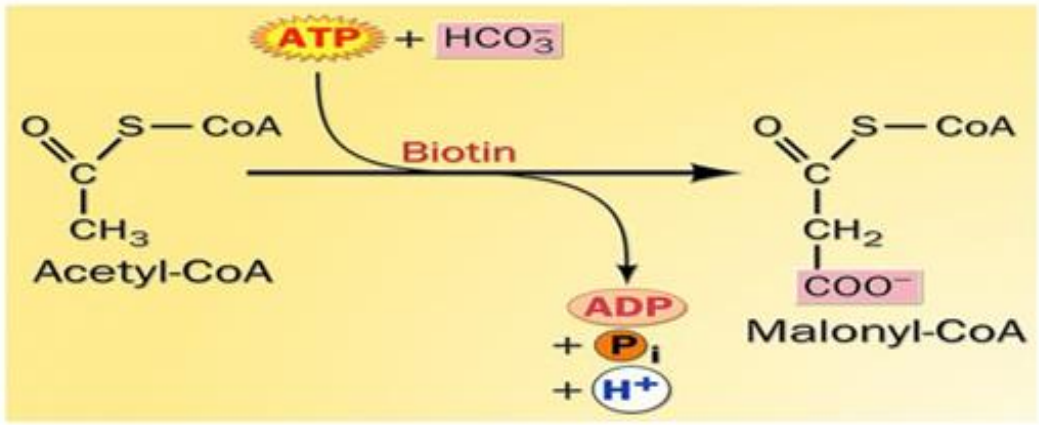
The synthesis of F.A. from acetyl-CoA and malonyl-CoA is carried out by fatty acid synthase(FAS).

Fatty acid synthesis involves four enzymatic activities, these are β-ketoacyl-ACP synthase, β-ketoacyl-ACP reductase, 3-OH acyl-ACP dehydratase, and enoyl-CoA reductase; where ACP is the acyl carrier protein (a carrier portion in synthetic complex).

The pathway for fatty acid synthesis occurs in the cytoplasm, whereas, oxidation occurs in the mitochondria. The other major difference is the use of nucleotide cofactors. Oxidation of fats involves the reduction of FAD^+ and NAD^+. Synthesis of fats involves the oxidation of NADPH. However,

the essential chemistry of the two processes are reversals of each other. Both oxidation and synthesis of fats utilize an activated two carbon intermediate, acetyl-CoA. However, the acetyl-CoA in fat synthesis exists temporarily bound to the enzyme complex as malonyl-CoA.

The synthesis of malonyl-CoA is the first committed step of fatty acid synthesis and the enzyme that catalyzes this reaction, acetyl-CoA carboxylase (ACC), is the major site of regulation of fatty acid synthesis. Like other enzymes that transfer CO_2 to substrates, ACC requires a biotin cofactor.



Activation of acetate : Acetyl-CoA to malonyl CoA

The acetyl-CoA and malonyl-CoA are transferred to acyl carrier protein (ACP) by the action of acetyl-CoA transacylase and malonyl-CoA transacylase, respectively. The attachment of these carbon atoms to ACP allows them to enter the fatty acid synthesis cycle.

The primary fatty acid synthesized by FAS is palmitate. Palmitate is then released from the enzyme and can then undergo separate elongation and/or unsaturation to yield other fatty acid molecules.

Characteristic	Degradation	Synthesis
Location	Mitochondrial Matrix	Cytosol
Activated intermediates	Thioesters of CoA	Thioesters of ACP
Enzymes	4 distinct, nonassociated enzymes	FAS is a multienzyme complex
Process	2-Carbon fragments removed as acetyl CoA	2-Carbon elongation using malonyl CoA
Direction	Starts at carboxyl end	Starts at methyl end
Fatty acid size	All sizes are degraded	Only Palmitate is made
Redox reaction cofactors	FAD/FADH2 and NAD+/NADH	NADP+/NADPH
Major tissue site	Muscle and liver	Liver
Nutritional status	In starvation	After carbohydrate-rich meal
Hormonal regulation	Low insulin / glucagon ratio	High insulin/glucagon ratio
Activator	FFA generated by hormone-sensitive lipase	Citrate
nhibitor	Malonyl CoA	Fatty acyl CoA

Elongation and Desaturation

The fatty acid product released from fatty acid synthesis is palmitate (via the action of palmitoyl thioesterase) which is a 16:0 fatty acid, i.e. 16 carbons and no sites of unsaturation. Elongation and unsaturation of fatty acids occurs in both the mitochondria and endoplasmic reticulum (microsomal

membranes). The predominant site of these processes is in the endoplasmic reticulum (ER) membranes. Elongation involves condensation of acyl-CoA groups with malonyl-CoA. The resultant product is two carbons longer. The reduction reactions of elongation require NADPH as cofactor.

Desaturation occurs in the ER membranes as well and in mammalian cells involves 4 broad specificity fatty acyl-CoA desaturases. These enzymes introduce unsaturation at C4, C5, C6 or C9. Since these enzymes cannot introduce sites of unsaturation beyond C9 they cannot synthesize either linoleate ($18:2^{\Delta 9,12}$) or linolenate ($18:3^{\Delta 9,12,15}$). These fatty acids must be acquired from the diet and are, therefore, referred to as **essential fatty acids**.

Dr.Salim.J.Kh.

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" Lipids And Lipoproteins "

* Lipids:

Are heterogeneous group of compounds related to the fatty acids. Lipids are biological molecules that are insoluble in aqueous solutions and soluble in organic solvents(ether, chloroform, and benzene), therefore, physical properties reflect the hydrophobic nature of their structures.

-Lipid functions:

1- They serve as structural components of biological membranes.

2- They provide energy reserves, predominantly in the form of triacylglycerides.

3- Both lipids and lipid derivatives serve as hormones.

4- Interactions with vitamins, assist in the regulation of biological processes.

- Classification of lipids:

1- Simple lipids: is ester of fatty acids(F.A) with alcohol include:

a- Fats: ester of fatty acids with trihydric alcohol(glycerol).

- Ester of F.A.(saturated) with glycerol called fats (sold).
- Ester of F.A.(unsaturated) with glycerol called oils (liquid).

They are also known as glycerides (triglycerides). Each molecule of glycerol bind to three molecules of fatty acids (may be the same or different F.A.).

b- Waxes: ester of F.A. with higher molecular weight monohydric alcohol (e.g.: insect secretions, protective coating on animal furs and leaves, Beeswax).

2- Complex lipids : ester of fatty acid -containing groups in addition to fatty acid and alcohol , include:

a- Phospholipids: ester of fatty acid containing phosphoric acid . They frequently have nitrogen containing base. If alcohol is glycerol called

glycerophospholipid, but if alcohol is sphingosine is called sphingophospholipids.

b- Glycolipid: lipid containing fatty acid, sphingosine and carbohydrate e.g.: glycosphingolipid.

c- Other complex lipids like sulfolipids, aminolipids, and lipoproteins may be placed in this group.

3- Precursor and derived lipids : these include fatty acids , glycerol, steroids, fatty aldehydes, ketone bodies, cholesterol and glycerides.

- Fatty acids(F.A.):

Are aliphatic carboxylic acids mostly obtained from the hydrolysis of natural fats and oils. Chemically fatty acids have a general structure of : $CH_3(CH_2)_nCOOH$

Fatty acids that occur in natural fats usually contain an even number of carbon atoms, because they are synthesized from 2 carbon units and are straight-chain derivatives. The chain may be saturated (containing no double bonds), or unsaturated (containing one or more double bonds).

* F.A. have two major roles in the body:

1- As the components of more complex membrane lipids.

2- As the major components of stored fat in the form of triacylglycerols.

The numbering of carbons in fatty acids begins with the carbon of the carboxylate group. At physiological pH, the carboxyl group is readily ionized, rendering a negative charge onto fatty acids in bodily fluids, therefore F.A. are weak acids.

Saturated and unsaturated fatty acids

Saturated Fats	Unsaturated Fats
Contains a single bond.	Contains at least one double bond.
Not to be consumed more than 10 percent of total calories per day.	Not to be consumed more than 30 percent of total calories per day.
Excessive consumption leads to heart diseases.	Good for consumption, but excessive may increase cholesterol.
Increases low- density lipoproteins (LDL), which is called as bad cholesterol.	Increases High-density lipoprotein (HDL), which is commonly known as good cholesterol and also reduce low-density lipoproteins (LDL).
Foods sources of saturated fats are whole milk, butter, cheese, meat, etc.	Foods sources of unsaturated fats are avocado, sunflower oil, soybean oil, fish oil, etc.
High melting point.	Low melting point.
Solid state in room temperature.	Liquid state in room temperature.

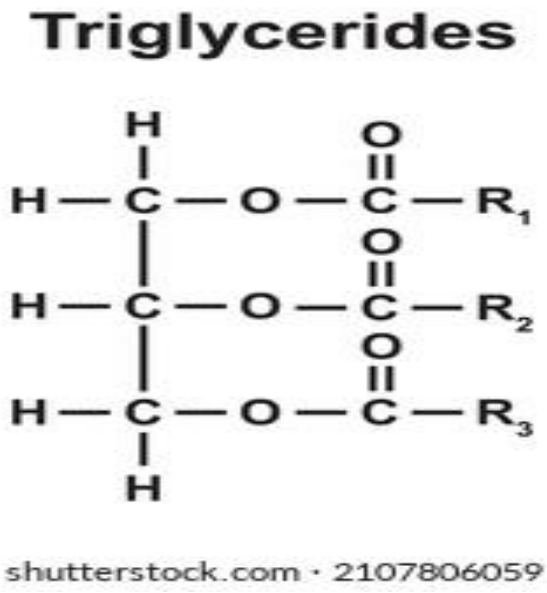
Table of major fatty acids found in plasma

Group	Name	Carbone-chain length	Source
Monounsaturated	Palmitoleic	C16	Plant oil
	Oleic	C18	Olive oil
Polyunsaturated	Linoleic	C18	Plant oil
	Linolenic	C18	Plant oil
	Arachidonic	C20	Plant oil
	Eicosapentaenoic	C20	Fish oil
Saturated	Myristic	C14	Coconut oil
	Palmitic	C16	Animal/plant oil
	Stearic	C18	Animal/plant oil

Triglycerides

Triglycerides are a type of fat that circulates in your blood. Your body makes triglycerides or gets them from the foods you eat. Your body needs some triglycerides for good health. However, high triglycerides in your blood can raise your risk of heart disease and stroke, including obesity and metabolic syndrome — a cluster of conditions that includes too much fat around the waist, high blood pressure, high triglycerides, high blood sugar and abnormal cholesterol levels.

The general structure of a triglyceride molecule is a glycerol unit attached to three fatty acids. Triglycerides are formed through a condensation reaction between a glycerol molecule and the fatty acids. Triglycerides will differ based on the different fatty acids attached to the glycerol.



Cholesterol

Cholesterol is a waxy, fat-like substance that's found in all the cells in your body. Your body needs some cholesterol to make hormones, vitamin D, and substances that help you digest foods. Your body makes all the cholesterol it needs. Cholesterol is also found in foods from animal sources, such as egg yolks, meat, and cheese.

If you have too much cholesterol in your blood, it can combine with other substances in the blood to form plaque. Plaque sticks to the walls of your arteries. This buildup of plaque is known as atherosclerosis. It can lead to coronary artery disease, where your coronary arteries become narrow or even blocked.

Lipoproteins

High-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) are lipoproteins. They are a combination of fat (lipid) and protein. The lipids need to be attached to the proteins so they can move through the blood. Different types of lipoproteins have different purposes:

- HDL : It is sometimes called "good" cholesterol because it carries cholesterol from other parts of your body back to your liver. Your liver then removes the cholesterol from your body.
- LDL : It is sometimes called "bad" cholesterol because a high LDL level leads to the buildup of plaque in your arteries.
- VLDL : Some people also call VLDL a "bad" cholesterol because it too contributes to the buildup of plaque in your arteries. But VLDL and LDL are different; VLDL mainly carries triglycerides and LDL mainly carries cholesterol.

Dr.Salim.J.Kh.

/ /

" Nucleic Acids "

Nucleic acids:

Nucleic acids are large biomolecules that play essential roles in all cells and viruses. A major function of nucleic acids involves the storage and expression of genomic information. Its naturally occurring chemical compound that is capable of being broken down to yield phosphoric acid, sugars, and a mixture of organic bases (purines and pyrimidines).

The two main classes of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is the master blueprint for life and constitutes the genetic material in all free-living organisms and most viruses. RNA is the genetic material of certain viruses, but it is also found in all living cells, where it plays an important role in certain processes such as the making of proteins.

Basic structure of nucleic acids:

Nucleic acids are polynucleotides—that is, long chainlike molecules composed of a series of nearly identical building blocks called nucleotides. Each nucleotide consists of a nitrogen-containing aromatic base attached to a pentose (five-carbon) sugar, which is in turn attached to a phosphate group.

Each nucleic acid contains four of five possible nitrogen-containing bases: adenine (A), guanine (G), cytosine (C), thymine (T), and uracil (U). A and G are categorized as purines, and C, T, and U are collectively called pyrimidines.

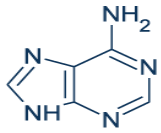
All nucleic acids contain the bases A, C, and G; T, however, is found only in DNA, while U is found in RNA. The pentose sugar in DNA (2′-deoxyribose) differs from the sugar in RNA (ribose) by the absence of a hydroxyl group (—OH) on the 2′ carbon of the sugar ring. Without an

attached phosphate group, the sugar attached to one of the bases is known as a nucleoside.

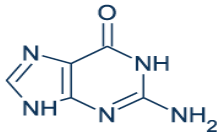
The phosphate group connects successive sugar residues by bridging the 5′-hydroxyl group on one sugar to the 3′-hydroxyl group of the next sugar in the chain. These nucleoside linkages are called phosphodiester bonds and are the same in RNA and DNA.

Purines are larger than pyrimidines because they have a two-ring structure while pyrimidines only have a single ring.

Purines

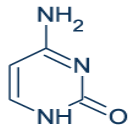


Adenine

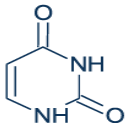


Guanine

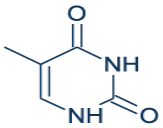
Pyrimidines



Cytosine



Uracil



Thymine

Purines	Pyrimidines
Purine is a heterocyclic aromatic organic compound composed of a pyrimidine ring fused with imidazole ring.	Pyrimidine is a heterocyclic aromatic organic compound that is composed of carbon and hydrogen.
It comprises adenine and guanine as nucleobases.	It comprises cytosine, thymine, and uracil as nucleobases
It consists of two hydrogen-carbon rings and four nitrogen atoms	It consists of one hydrogen-carbon ring and two nitrogen atoms
The melting point of purine is 214 °C	The melting point of pyrimidine is 20-22 °C

Deoxyribonucleic acid (DNA)

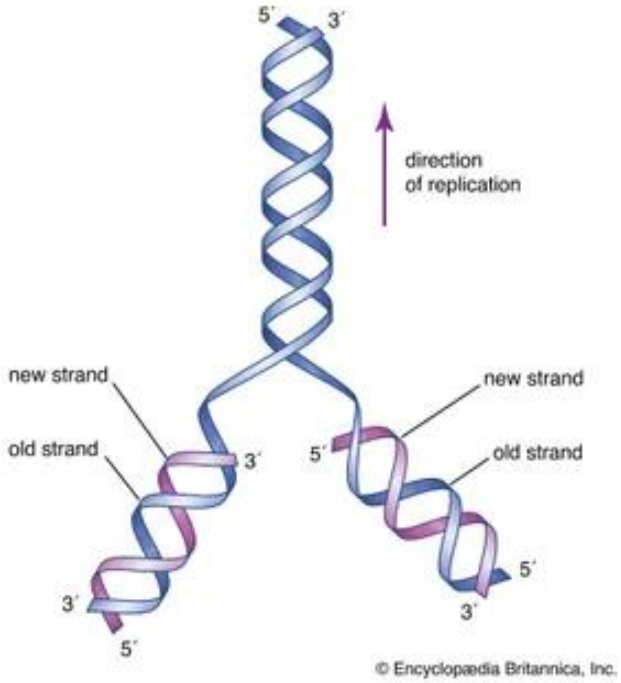
DNA is a polymer of the four nucleotides A, C, G, and T, which are joined through a backbone of alternating phosphate and deoxyribose sugar residues. These nitrogen-containing bases occur in complementary pairs as determined by their ability to form hydrogen bonds between them. A always pairs with T through two hydrogen bonds, and G always pairs with C through three hydrogen bonds. This structure, along with the molecule's chemical stability, makes DNA the ideal genetic material. The bonding between complementary bases also provides a mechanism for the replication of DNA and the transmission of genetic information.

Chemical structure

In 1953 James D. Watson and Francis H.C. Crick proposed a three-dimensional structure for DNA based on low-resolution X-ray crystallographic data which is, in naturally occurring DNA, the amount of T equals the amount of A and the amount of G equals the amount of C. Watson and Crick, who shared a Nobel Prize in 1962 for their efforts, postulated that two strands of polynucleotides coil around each other, forming a double helix.

The two strands, though identical, run in opposite directions as determined by the orientation of the 5′ to 3′ phosphodiester bond. The sugar-phosphate chains run along the outside of the helix, and the bases lie on the inside, where they are linked to complementary bases on the other strand through hydrogen bonds.

Human DNA, consists of 23 pairs of linear chromosomes containing three billion base pairs. In all cells, DNA does not exist free in solution but rather as a protein-coated complex called chromatin. Chromatin contains proteins that control gene expression and determine the characteristic shapes of chromosomes.



Ribonucleic acid (RNA)

RNA is a single-stranded nucleic acid polymer of the four nucleotides A, C, G, and U joined through a backbone of alternating phosphate and ribose sugar residues. It is the first intermediate in converting the information from DNA into proteins essential for the working of a cell. RNA is made by copying the base sequence of a section of double-stranded DNA, called a gene, into a piece of single-stranded nucleic acid. This process, called transcription, is catalyzed by an enzyme called RNA polymerase. There are three types of RNA [Messenger RNA (mRNA), Transfer RNA (tRNA), and Ribosomal RNA (rRNA)].

Chemical structure

Whereas DNA provides the genetic information for the cell and is inherently quite stable, RNA has many roles and is much more reactive chemically. RNA is sensitive to oxidizing agents such as periodate that lead to opening of the 3′-terminal ribose ring. The 2′-hydroxyl group on the ribose ring is a major cause of instability in RNA, because the presence

of alkali leads to rapid cleavage of the phosphodiester bond linking ribose and phosphate groups. In general, this instability is not a significant problem for the cell, because RNA is constantly being synthesized and degraded.

BASIS FOR COMPARISON	mRNA	tRNA	rRNA
Meaning	mRNA or messenger RNA is the connection between gene and protein, and it is the result of the transcribed gene by RNA polymerase.	tRNA or transfer RNA is a cloverleaf shaped RNA molecule and provides specific amino acids to the ribosomes.	rRNA or ribosomal RNA is used for the formation of the ribosomes.
Role	mRNA carries genetic information from the nucleus to ribosomes for the synthesis of proteins.	tRNA carries specific amino acids to the ribosomes to assist the protein biosynthesis.	rRNA these provide the structural framework for the formation of ribosomes.
Synthesized in	Nucleus.	Cytoplasm.	Ribosome.
Size	In mammals, the size of the molecules is around 400 to 12, 000 nucleotides (nt).	The size of the molecule of tRNA is 76 to 90 nucleotides (nt).	The size of the molecule of rRNA may vary from the 30S, 40S, 50S and 60S.
Shape	mRNA is linear in shape.	tRNA is a cloverleaf shape.	rRNA is a sphere shape (complex structure).

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Quantitative Determination of Serum Calcium

Clinical significance:

Total calcium exists in three physiochemical states in plasma, of which approximately 50% is free ionized calcium, 40% is bound to plasma proteins, and 10% is complexed with small anions.

The level of serum calcium may be affected by intestinal malabsorption, by alterations in plasma proteins level, especially albumin, which should be measured concurrently with calcium.

Hypercalcemia is found in hyperparathyroidism, multiple myeloma, bone parathyroidal neoplasms and in states with bone demineralization.

Hypocalcemia is encountered in hypoparathyroidism and in several cases of necrosis and acute pancreatitis.

Principle of action:

CPC(O-cresol Phtalein Complexone) method allows to determinate total calcium concentration in serum, plasma or urine.

In alkaline solution CPC reacts with calcium to form a dark-red coloured complex which absorbance measured at 570 nm is proportional to the amount of calcium in the specimen.

Procedure:

	Blank	Standard	Sample
Reagent	1 ml(R1+ R2)	1 ml(R1+ R2)	1 ml(R1+ R2)
Standard		25 µl	
Sample			25 µl

Mix and incubate for 10 min. at room temperature (15-25 C°). Read the absorbance of sample and standard against blank at 570 nm.

The color is stable for 1 h. at room temperature.

Calculation:

Result= absorb. of assay/ absorb. of stand.× stand. conc.

The normal value is 8.5-10.5 mg/dl.

Phosphorus

An adult human body contains approximately 600 g of phosphates expressed as phosphorus, of which about 85% is bound to calcium in bones and the rest principally in other tissue cells. Elevation of phosphorus in serum/plasma is often associated with bone diseases, renal failure, hyperparathyroidism, hypervitaminosis D. Decreased of phosphorus in serum/plasma are found in case of osteomalacia, vitamin D deficiency.

Phosphorus and calcium metabolism are interwined. In healthy persons, as serum calcium levels rise, those of phosphorus fall. Control of phosphorus levels is in part accomplished by regulation of renal excretion. However, fairly rapid fluctuations in serum inorganic phosphate can occur because the serum inorganic phosphate concentration is influenced by carbohydrate metabolism.

In *diabetes*, severe loss of phosphate is possible, since carbohydrate metabolism is deranged and phosphate tends to pass from the cell into extracellular fluid and then into plasma. It is then extracted and excreted by the Kidney.

Increased levels are associated with *hypoparathyroidism*, during insulin treatment of *diabetic coma*, and with chronic *nephritis* rising as renal failure progresses.

Normal range: Children 4.0 - 7.0 mg/dL , Men 2.5 - 4.5 mg/dL,
Women 1.50 - 6.8 mg/dL.

Sixth Lab

Determination of protein Concentration

Determination of protein concentration is necessary and widely used in protein biology, molecular biology, and other research applications. The concentration of protein samples have to be estimated before proceeding to isolation, purification, and analysis.

Three main factors should be considered before choosing a method.

1. **Sensitivity**- A method is known to be sensitive if it can detect protein at very low concentrations.
2. **Specificity**- Specificity of a method is determined by its efficiency to detect protein specifically from all other interfering substances.
3. **Time**- Duration of time for assay completion and interpretation of results.

Different methods of estimation of protein concentration are mentioned below:

Biuret method: Sensitivity of this method is very low. It requires high protein levels from 1-20 mg of protein. This method does not rely on amino acid composition and hence can measure all protein samples with accuracy. The main disadvantage of this method is that buffers with ammonia interferes with the reaction.

Smith assay: This method is highly sensitive and detects proteins at a low concentration of 1 µg. This method takes the longest time to get the end result approximately an hour. Common interfering substances are lipids and carbohydrates.

Bradford assay: This is a very sensitive method. This method takes very less time within about 10-15 min. The main disadvantage of this method is the less specificity.

***Quantitative Determination of Serum Total Protein:**

Clinical Significance: Its important to determine total protein concentration for evaluate some body functions and problems.

Decrease in the volume of plasma water (hemoconcentration), noted in dehydration (sever vomiting, diarrhea, Addisons disease, or diabetic acidosis), is reflected as relative hyperproteinemia.

Hemodilution (increase in plasma water volume) occurring with water intoxication or salt retention syndromes, during massive intravenous infusions, and physiologically when a recumbent position is assumed, is reflected as relative hypoproteinemia. Hypoproteinemia due to low levels of albumin in plasma is also common and has many causes.

Mild hyperproteinemia may be caused by an increased in the concentration of specific proteins(infection). Marked hyperproteinemia may be caused by high levels of monoclonal immunoglobulins produced in multiple myeloma and other malignant paraproteinemias.

Principle of action: the peptide bonds of proteins react with Cu^{2+} in alkaline solution to form a colored complex which absorbance proportional to the concentration of total protein in the specimen, is measured at 550 nm.

Serum or plasma analyze fresh or store at 2-8 °C less than 72 h. Total protein in serum is stable for 6 months at -20°C or indefinitely at -70°C .

Procedure:

	Blank	Standard	Assay
Reagent 1	1 ml	1 ml	1 ml
Reagent 2	1 ml	1 ml	1 ml
Standard		0.02 ml	
Specimen			0.02 ml

Mix well, let stand for 10 min. at room temperature, record the absorbance at 550 nm against reagent blank.

Calculation: Result= absorb. of assay/ absorb. of stand.×stand. conc.

The normal value is 6.5-8.5 g/dl.

***Quantitative Determination of Serum Albumin:**

Clinical Significance: one of the most important serum proteins produced in the liver is albumin. This molecule has an extraordinarily wide range of functions including nutrition, maintenance of oncotic pressure and transport of Ca^{2+} , bilirubin, free fatty acids, drugs and steroids.

Variation in albumin levels indicate liver diseases, malnutrition, skin lesions (dermatitis and burns) or dehydration.

Principle of action: albumin in the presence of bromocresol green at a slightly acid pH, produces a color change of the indicator from yellow-green to green-blue. The intensity of the color formed is proportional to the albumin concentration in the sample.

Albumin in the serum or plasma, free of hemolysis, is stable for 1 month at 2-8 °C or 1 week at 15-25°C.

Procedure:

	Blank	Standard	Sample
Reagent	1 ml	1 ml	1 ml
Standard		5 µl	
Sample			5 µl

Mix and incubate for 10 min. at room temperature (15-25 °C). Read the absorbance of sample and standard against blank at 630 nm.

The color is stable for 1 h. at room temperature.

Calculation: Result= absorb. of assay/ absorb. of stand.× stand. conc.

The normal value is 3.5-5.0 g/dl.

globulin

The globulin fraction includes hundreds of serum proteins including carrier proteins, enzymes, complement, and immunoglobulins. It have higher molecular weight than albumins and are insoluble in pure water but dissolve in dilute salt solutions. Most of these are synthesized in the liver, although the immunoglobulins are synthesized by plasma cells. Globulins are divided into four groups which are α1, α2, β and γ.

Increases in the globulin fraction usually result from an increase in immunoglobulins in chronic inflammation, infection, autoimmune disease, and liver disease. Malnutrition and congenital immune deficiency can cause a decrease in total globulins due to decreased synthesis, and nephrotic syndrome can cause a decrease due to protein loss through the kidney.

Globulin calculated by: Globulin = Total protein - Albumin

Normal range: Neonate 1.2-3.6 g/dl, Adults 2.5-3.5 g/dl .

Protein Metabolism

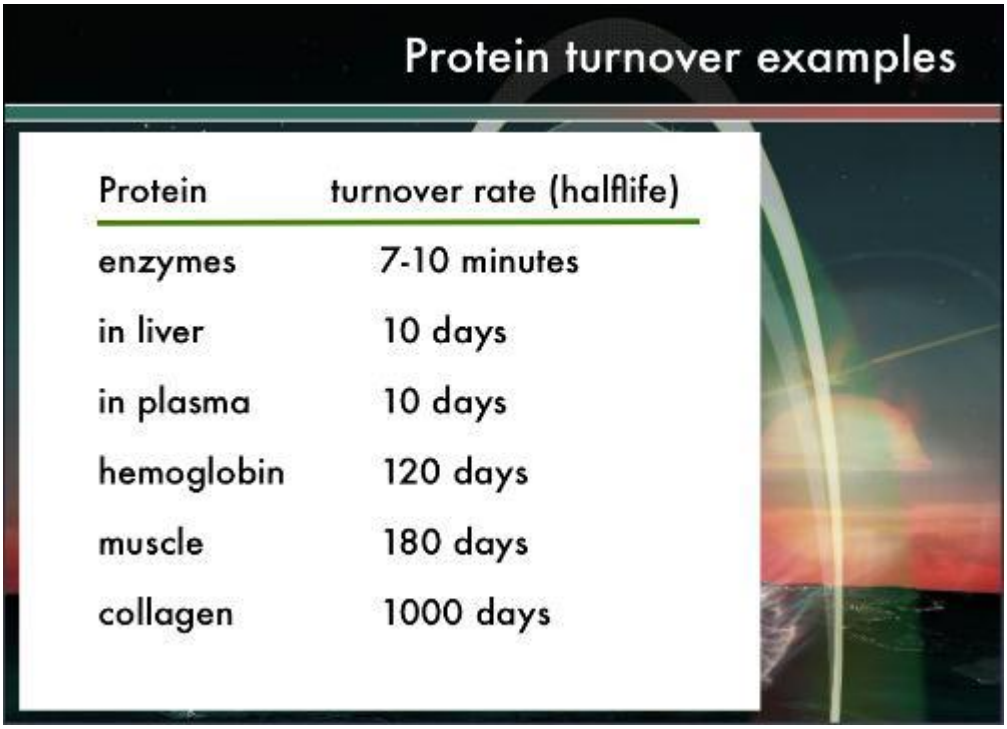
Most of the foods and drinks people ingest are complex materials that the body must break down into simpler substances. This process may involve several steps. The simpler substances are then used as building blocks, which are assembled into the materials the body needs to sustain life. This complicated process of breaking down and converting the substances ingested is called **metabolism**.

The metabolic pathways fall into two categories: (1) **Anabolic pathways** are those involved in the synthesis of compounds. Protein synthesis is such a pathway, as is the synthesis of fuel reserves of triacylglycerol and glycogen.(2) **Catabolic pathways** are involved in the breakdown of larger molecules, commonly involving oxidative reactions.

Metabolism is carried out by chemical substances called enzymes, which are made by the body. If a genetic abnormality affects the function of an enzyme or causes it to be deficient or missing altogether, various disorders can occur.

Protein metabolism is no less important than carbohydrate and lipid metabolism. **(1)** Proteins make up the structural tissue for muscles and tendons, **(2)** transport oxygen as hemoglobin, **(3)** catalyze all biochemical reactions as enzymes, and **(4)** regulate reactions as hormones. Our bodies must be able to synthesize the many proteins, amino acids, and other non-protein nitrogen containing compounds needed for growth, replacement, and repair. Proteins in excess are used to supply energy or build reserves of glucose, glycogen, or lipids.

About 75% of all amino acids are used for the production of protein. Amino acids can come from the protein we eat or from degraded proteins in the body. This degradation is a continuous process as proteins in body are constantly being replace (protein turnover).



Amino acids serve as a source of nitrogen for other compounds in the body:

1- Nitrogen bases of DNA and RNA.

2- Heme and similar structures in myoglobin, hemoglobin, cytochromes, enzymes…..

3- Acetylcholine and other neurotransmetters.

4- Hormones and phospholipids.

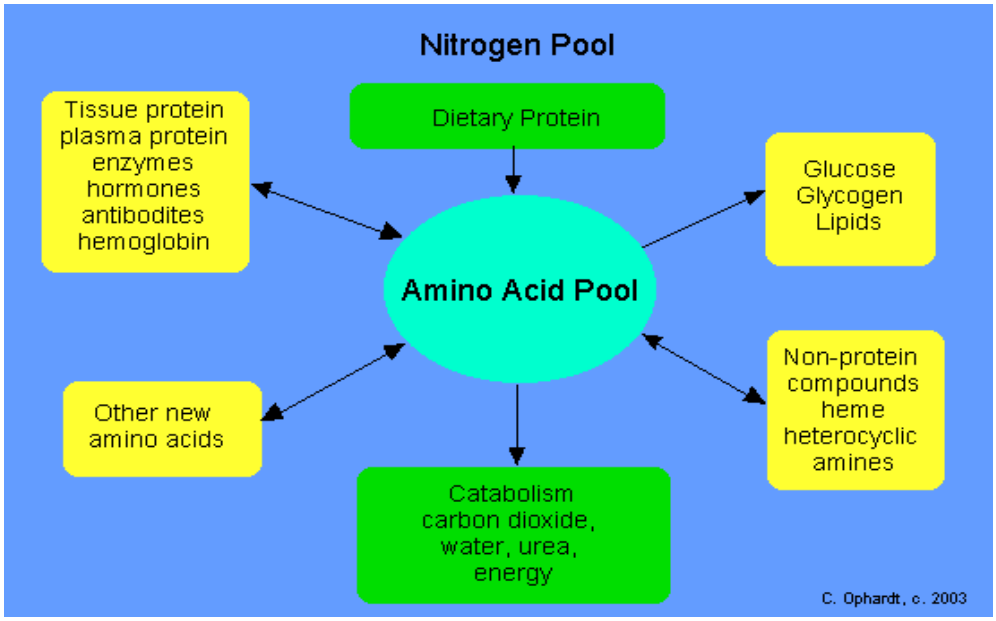
* **Nitrogen Cycle**:

Nitrogen is recycled just as carbon and oxygen are recycled in nature. Various microorganisms have the appropriate enzymes (**nitrogenase**) to convert elemental nitrogen from the air into ammonia. Green plants use the ammonia or nitrate as raw materials for the synthesis of amino acids and proteins. Animals and humans in turn use the plants to supply nitrogen to make amino acids and proteins. We humans are not as versatile as plants since we are unable to synthesize eight amino acids which must be included in the diet. Finally, the nitrogen cycle is completed when plant and animal residues are decayed by microorganisms back to nitrogen gas for the air.

The "nitrogen or amino acid pool" is a grand mixture of amino acids available in the cell derived from dietary sources or the degradation of protein. Since proteins and amino acids are not stored in the body, there is a constant turnover of protein. Some protein is constantly being synthesized while other protein is being degraded. For example, liver and plasma proteins have a half-life of 10 days or more, while enzymes and hormones may be recycled in minutes or hours.

Each day, some of the amino acids are catabolized producing energy and ammonia. The ammonia is converted to urea and excreted from the body and represents a drain on the nitrogen pool.

A nitrogen balance is achieved by a healthy person when the dietary intake is balanced by the excretion of urea wastes. If nitrogen excretion is greater than the nitrogen content of the diet, the person is said to be in negative nitrogen balance. This is usually interpreted as an indication of tissue destruction. If the nitrogen excretion is less than the content of the diet, a positive nitrogen balance indicates the formation of protein.



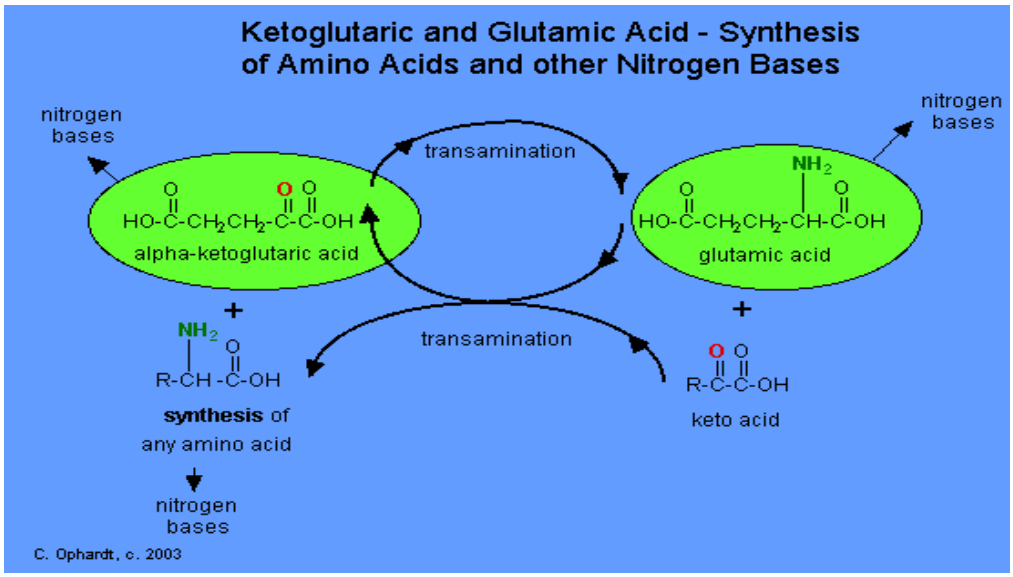
*** Protein biosynthesis:**

Protein synthesis is the process in which cells build proteins. The term is sometimes used to refer only to protein translation but more often it refers to a multi-step process, beginning with amino acid synthesis and transcription which are then used for translation.

* **Amino Acid Synthesis**:

Amino acids are the monomers which are polymerized to produce proteins. Amino acid synthesis is the set of biochemical processes (metabolic pathways) which build the amino acids from carbon sources like glucose. Not all amino acids may be synthesized by every organism, for example adult humans have to obtain 8 of the 20 amino acids from their diet.

For the synthesis of amino acids, the alpha-ketoglutaric acid first uses transamination of a different amino acid to make glutamic acid, which then reacts with a keto acid to make a new amino acid. In effect, the interconversion of alpha-ketoglutaric acid and glutamic acid lies at the very heart of nitrogen metabolism, therefore, glutamate is a key intermediate in amino acid metabolism. These molecules serve as the "collection and receiving agent" for nitrogen. The subsequent fate of the amino group is in new amino acids, any nitrogen bases, or any other nitrogen containing compounds. Carbon skeletons come from intermediates of glycolysis, pentose phosphate pathway(PPP), or citric acid cycle.



*** Transcription:**

Transcription is the process by which an mRNA template, encoding the sequence of the protein in the form of a trinucleotide code, is transcribed from the genome to provide a template for translation. Transcription copies the template from one strand of the DNA double helix, called the template strand. Transcription can be divided into 3 stages: Initiation, Elongation and Termination, each regulated by a large number of proteins such as transcription factors and coactivators that ensure the correct gene is transcribed in response to appropriate signals.

*** Translation:**

The synthesis of proteins is known as translation. Translation occurs in the cytoplasm where the ribosomes are located. Ribosomes are made of a small and large subunit which surrounds the mRNA. In translation, messenger RNA (mRNA) is decoded to produce a specific polypeptide according to the rules specified by the genetic code. This uses an mRNA sequence as a template to guide the synthesis of a chain of amino acids that form a protein. Translation is necessarily preceded by transcription. Translation proceeds in four phases: activation, initiation, elongation and termination (all describing the growth of the amino acid chain, or polypeptide that is the product of translation).

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* Catabolism of amino acids:

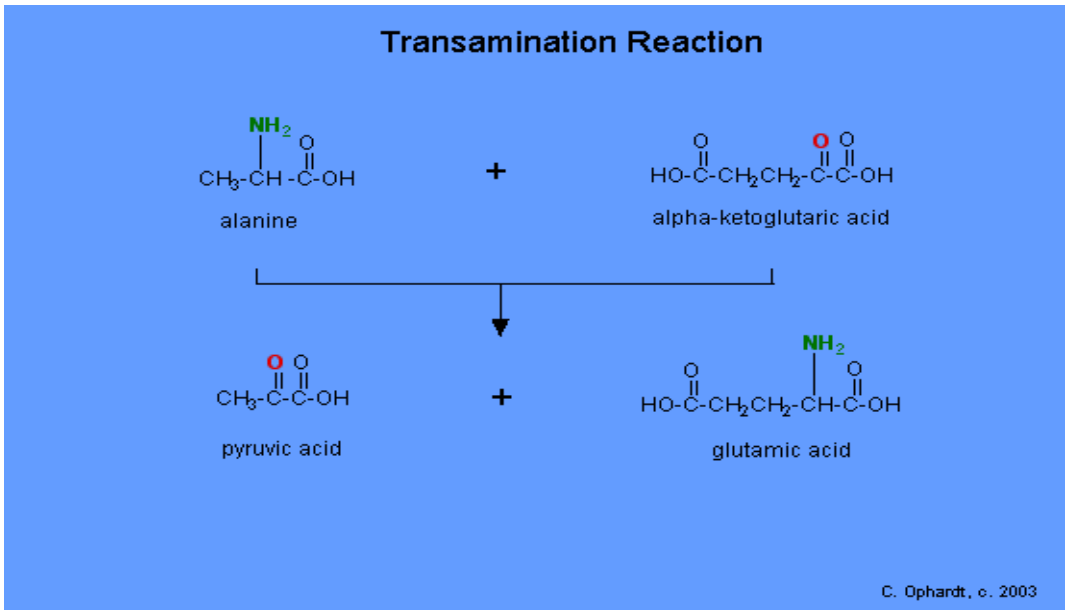
Amino acids cannot be stored by the body, if there is an excess of amino acids or a lack of other energy sources, the body will use them for energy production. Unlike fats and carbohydrates, amino acids require the removal of amine group, it must then be disposed of as it is toxic to the body.

Removal of α-amino group require two step process:

1- Transamination Reaction:

Transamination as the name implies, refers to the transfer of an amine group from one molecule to another. This reaction is catalyzed by a family of enzymes called transaminases. Actually, the transamination reaction results in the exchange of an amine group on one acid with a ketone group on another acid. The most usual and major keto acid involved with transamination reactions

is alpha-ketoglutaric acid, an intermediate in the citric acid cycle. A specific example is the transamination of alanine to make pyruvic acid and glutamic acid.

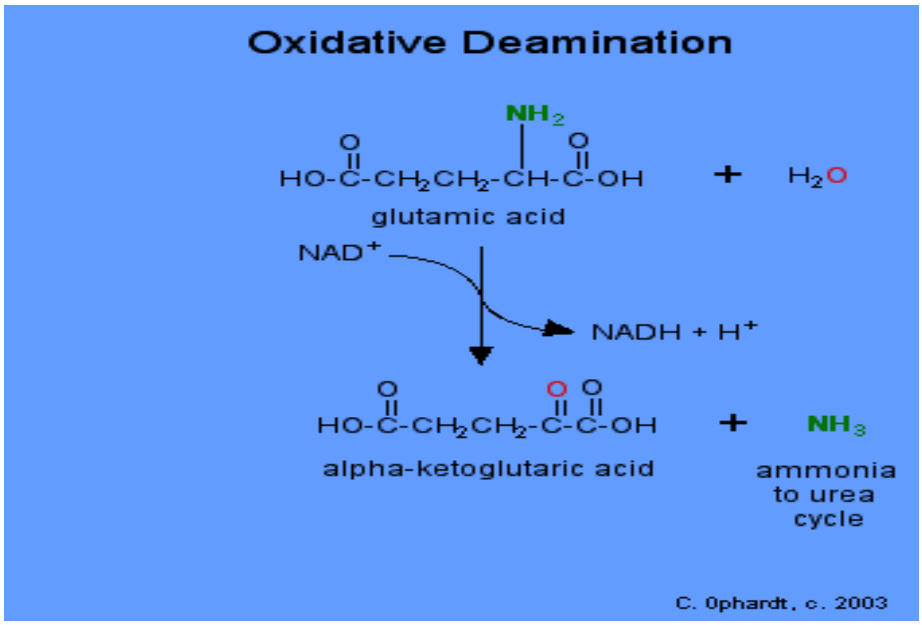


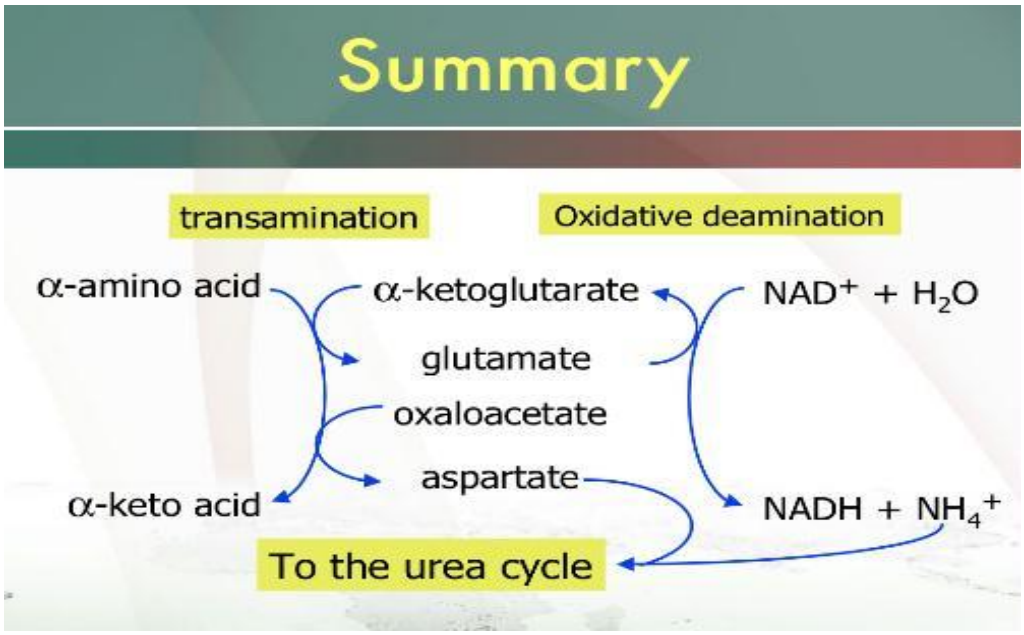
All of the amino acids can be converted through a variety of reactions and transamination into a keto acid which is a part of or feeds into the citric acid cycle.

*** Oxidative Deamination Reaction:**

Deamination is also an oxidative reaction that occurs under aerobic conditions in all tissues but especially the liver. During oxidative deamination, an amino acid is converted into the corresponding keto acid by the removal of the amine functional group as ammonia and the amine functional group is replaced by the ketone group.

Oxidative deamination occurs primarily on glutamic acid because glutamic acid was the end product of many transamination reactions.



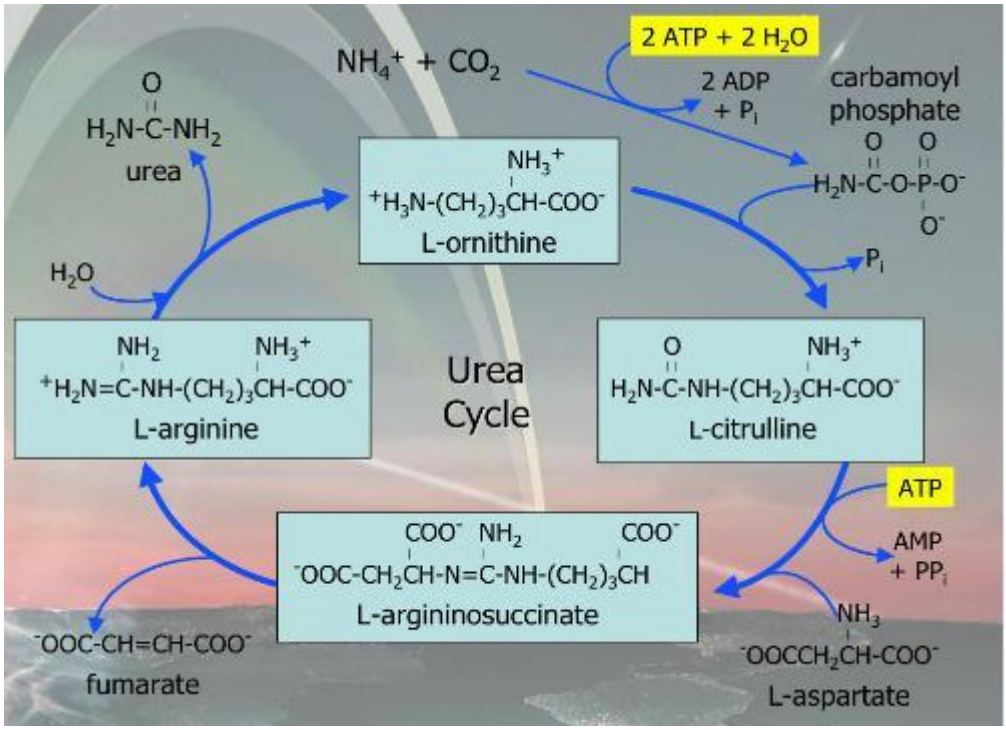


*** <u>Urea Cycle</u> :**

Urea is the major end product of nitrogen metabolism in humans and mammals. Ammonia, the product of oxidative deamination reactions, is toxic in even small amounts and must be removed from the body. The urea cycle or the ornithine cycle describes the conversion reactions of ammonia into urea. Since these reactions occur in the liver, the urea is then transported to the kidneys where it is excreted.

Ammonia + carbon dioxide + 3ATP ---> urea + water + 3 ADP

Urea is routinely measured in the blood as: Blood Urea Nitrogen (BUN). BUN levels may be elevated (a condition called uremia) in both acute and chronic renal (kidney) failure. Various diseases damage the kidney and cause faulty urine formation and excretion. Congestive heart failure leads to a low blood pressure and consequent reduced filtration rates through the kidneys, therefore, BUN may be elevated. Urinary tract obstructions can also lead to an increased BUN. In severe cases, hemodialysis is used to remove the soluble urea and other waste products from the blood. Waste products diffuse through the dialyzing membrane because their concentration is lower in the dialyzing solution. Ions, such as Na+ and Cl- which are to remain in the blood, are maintained at the same concentration in the dialyzing solution - no net diffusion occurs.



High ammonia levels are toxic to humans. A complete block of any step in the urea cycle is fatal since there is no known alternative pathway for the synthesis of urea. Inherited disorders from defective enzymes may cause a partial block in some of the reactions and results in hyperammonemia which can lead to mental retardation. Extensive ammonia accumulation leads to extensive liver damage and death.

*** Disorders of Amino Acid Metabolism:**

*** Phenylketonuria:** Phenylketonuria (PKU) is a disorder that causes a buildup of the amino acid phenylalanine, which is an essential amino acid that cannot be synthesized in the body but is present in food. Excess phenylalanine is normally converted to tyrosine, another amino acid, and eliminated from the body. Without the enzyme that converts it to tyrosine, phenylalanine builds up in the blood and is toxic to the brain, causing mental retardation.

Symptoms include seizures, nausea and vomiting, an eczema-like rash, lighter skin and hair than their family members, aggressive or self-injurious behavior, hyperactivity, and sometimes psychiatric symptoms. Untreated children often give off a "mousy" body and urine odor as a result of a by-product of phenylalanine (phenylacetic acid) in their urine and sweat.

To prevent mental retardation, phenylalanine intake must be restricted, beginning in the first few weeks of life. Because all natural sources of protein contain too much phenylalanine for children with PKU, affected children cannot have meat, milk, or other common foods that contain protein. Instead, they must eat a variety of phenylalanine-free processed foods, which are low-protein natural foods, such as fruits, vegetables.

*** Maple Syrup Urine Disease:** Children with maple syrup urine disease are unable to metabolize certain amino acids. By-products of these amino acids build up, causing neurologic changes, including seizures and mental retardation. These by-products also cause body fluids, such as urine and sweat, to smell like maple syrup.

Infants with severe disease are treated with dialysis. Some children with mild disease benefit from injections of the vitamin B_1 (thiamin). After the disease has been brought under control, children must always consume a special artificial diet that is low in the particular amino acids that are affected by the missing enzyme.

*** Homocystinuria:** Children with homocystinuria are unable to metabolize the amino acid homocysteine, which, along with certain toxic by-products, builds up to cause a variety of symptoms. Symptoms may be mild or severe, depending on the particular enzyme defect.

Infants with this disorder are normal at birth. The first symptoms, including dislocation of the lens of the eye, causing severely decreased vision, usually begin after 3 years of age. Most children have skeletal abnormalities, including osteoporosis; the child is usually tall and thin with a curved spine, elongated limbs, and long, spiderlike fingers. Psychiatric and behavioral disorders and mental retardation are common. Homocystinuria makes the blood more likely to spontaneously clot, resulting in strokes, high blood pressure, and many other serious problems.

In a few states, children are screened for homocystinuria at birth with a blood test. The diagnosis is confirmed by a test measuring enzyme function in liver or skin cells. Some children with homocystinuria improve when given vitamin B_6 (pyridoxine) or vitamin B_{12} (cobalamin).

* **Tyrosinemia:** Children with tyrosinemia are unable to completely metabolize the amino acid tyrosine. By-products of this amino acid build up, causing a variety of symptoms. In some states, the disorder is detected on the newborn screening tests.

There are two main types of tyrosinemia: I and II. Type I tyrosinemia is most common in children of French-Canadian or Scandinavian descent. Children with this disorder typically become ill sometime within the first year of life with dysfunction of the liver, kidneys, and nerves, resulting in irritability, rickets, or even liver failure and death. Restriction of tyrosine in the diet is of little help. An experimental drug, which blocks production of toxic metabolites, may help children with type I tyrosinemia. Often, children with type I tyrosinemia require a liver transplant.

Type II tyrosinemia is less common. Affected children sometimes have mental retardation and frequently develop sores on the skin and eyes. Unlike type I tyrosinemia, restriction of tyrosine in the diet can prevent problems from developing.

(Fourth Lab)

Standard Curve

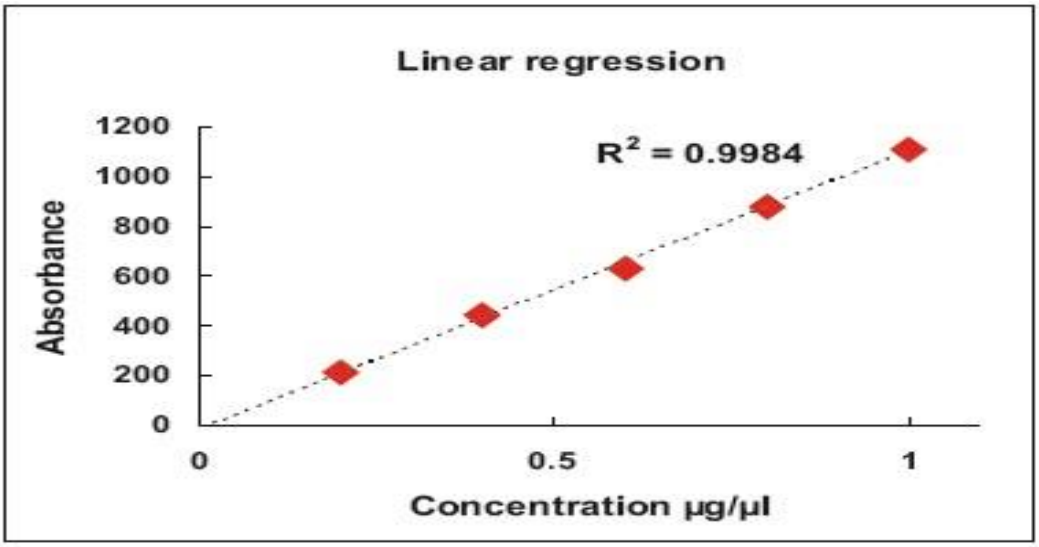
In analytical chemistry, a calibration curve, also known as a standard curve, is used to accurately determine the concentration of your sample from the signal generated by an assay.

Many laboratory tests require the measurement of concentration be evaluated or read in a photometer (colorimeter or spectrophotometer). Since these instruments are capable of only measuring the amount of light being allowed to pass through the cuvette.

One method of obtaining concentration from % transmittance or absorbance is through the use of a standard curve. For our purposes, standard curves are defined as a graphs with absorption or % transmission plotted on the Y axis, and increasing concentrations of standard along the X axis.

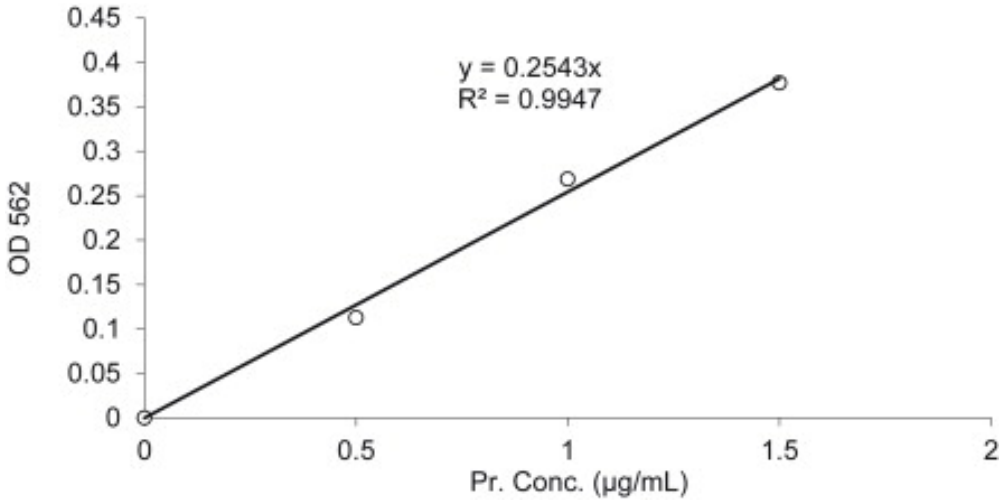
Generally, a standard curve is 5 points or more. Sometimes in a tight sample range it can be less but should not be less than 3.

A standard curve is prepared by making serial dilutions of the protein standard within a range of concentrations. If Beer's Law is followed, the resulting line representing absorbance vs concentration will be straight.



For spectrophotometry, require the determination of an instrumental constant by analysis reference standards; a calibration curve is used for this approach. The calibration curve for a particular analyte in a particular sample provides the exact relationship needed for those particular measurements.

The standard curve method is the most widely used for real-time Q-PCR quantification analysis. It is necessary to generate a standard curve for both the target gene and the reference gene each time the assay is run.



This figure of standard curve showing determination of protein concentration

To calculate the sample concentration based on the standard curve, first you find the concentration for each sample absorbance on the standard curve; then you multiply the concentration by the dilution factor for each sample.

Advantages

There are a number of advantages to the analytical techniques like:

- Method Proficiency. ...
- Quality Control. ...
- Reduces Chances of Error. ...
- Decrease Cost ...

Disadvantages

(1) The standards require a supply of the analyte material in high purity and in known concentration.

(2) The standards and the unknown are in the same matrix.

Applications

- Analysis of concentration
- Verifying the proper functioning of an analytical instrument or a sensor device such as an ion selective electrode
- Determining the basic effects of a control treatment (such as a dose-survival curve in clonogenic assay)

Quantitative Lipid Estimation

Ninth Lab

* Total Serum Cholesterol (TC) Concentration

Clinical Significance

Cholesterol measurements are used in the diagnosis and treatments of lipid and lipoprotein metabolism disorders. Lipids play an important role in the body; they serve as hormones or hormone precursors, aid in digestion, provide energy, storage and metabolic fuels, act as functional and structural components in biomembranes and form insulation to allow nerve conduction and prevent heat loss.

In clinical chemistry, over the last decade however, lipids have become associated with lipoprotein metabolism and atherosclerosis.

A patient will be offered a blood cholesterol level test if they:

•have been diagnosed with coronary heart disease, stroke or mini-stroke

•are over 40

•have a family history of early cardiovascular disease

•have a close family member who has an inherited cholesterol-related condition

•are overweight or obese

•have high blood pressure or diabetes

•Have another medical condition such as a kidney condition, an underactive thyroid gland or pancreatitis

Principle

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine (PAP) in the presence of phenol and peroxidase.

$$\text{Cholesterol ester} \xrightarrow[\text{esterase}]{\text{Cholesterol}} \text{Cholesterol + Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{oxidase}]{\text{Cholesterol}} \text{Cholestene-4-one-3} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{PAP} \xrightarrow{\text{peroxidase}} \text{quinoneimine (pink)} + 4H_2O$$

Procedure

Pipette into test tubes	Blank	Standard	Assay
Working reagent	1 ml	1 ml	1 ml
Demineralized water	10 µl	--------	--------
Standard	--------	10 µl	--------
Specimen	--------	--------	10 µl

The solutions were mixed well and incubated at 37˚C for 5 minutes. Then the absorbances of the standard ($A_{standard}$) and sample (A_{sample}) were recorded against the reagent blank at a wave length of 500 nm within 60 minutes.

Calculations

$$\text{TC conc.} = \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{standard}}} \times \text{standard conc.(5.17 mmol/L)(200 mg/dl)}$$

The normal value of serum TC is (<200 mg/dl), (< 5.18 mmol/L).

*** Estimation of Serum Triglyceride**

Clinical Significance

The measurement of the triglycerides concentration in blood is important for the diagnosis and the follow-up of hyperlipidemia. Its increase can be of genetic origin or secondary to other metabolic disorders such as: diabetes mellitus, hyper and hypothyroidisim, hepatic diseases, acute and chronic pancreatitis, nephrosis. A rise in triglycerides also represents an atherogenic risk factor. Its responsible for the opalescence, or even the cloudiness of the serum.

Principle

The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

$$\text{Triglyceride} \xrightarrow{\text{lipase}} \text{Cholesterol + Fatty acids}$$

$$\text{Glycerol + ATP} \xrightleftharpoons{\text{glycerokinase}} \text{glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightleftharpoons[\text{oxidase}]{\text{glycerol-3-phosphate}} \text{dihydroxyacetone + phosphate} + H_2O_2$$

$$H_2O_2 + \text{PAP} + \text{4-chlorophenol} \xrightarrow{\text{peroxidase}} \text{quinoneimine (pink)} + H_2O$$

Procedure

Pipette into test tubes	Blank	Standard	Assay
Working reagent	1 ml	1 ml	1 ml
Demineralized water	10 µl	--------	--------
Standard	--------	10 µl	--------
Specimen	--------	--------	10 µl

The solutions were mixed well and incubated at 37˚C for 5 minutes. Then the absorbance of the standard ($A_{standard}$) and sample (A_{sample}) were recorded against the reagent blank at a wave length of 500 nm within 60 minutes.

Calculations

$$\text{TG conc.} = \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{standard}}} \times \text{standard conc. (2.28 mmol/L)(200 mg/dl)}$$

The normal value or serum TG is (35-160 mg/dl), (0.40-1.82 mmol/L).

Lipoproteins

Lipoproteins are complex particles that have a central hydrophobic core surrounded by a hydrophilic membrane. Lipoproteins play a key role in the absorption and transport of dietary lipids by the small intestine, in the transport of lipids from the liver to peripheral tissues, and the transport of lipids from peripheral tissues to the liver and intestine (reverse cholesterol transport). A secondary function is to transport toxic foreign hydrophobic and amphipathic compounds, such as bacterial endotoxin, from areas of invasion and infection.

Determination of serum HDL-cholesterol is a useful tool in identifying high-risk patients. Increased total cholesterol/HDL-C ratio is significant of an increased risk of atherosclerosis.

Atherogenic risk factor = total cholesterol / HDL-C

LDL-C transport cholesterol from liver to arteries, therefore; increased it associated with increased risk of atherosclerosis and coronary artery disease.

LDL-C = Total cholesterol – (HDL-C – VLDL-C)

VLDL-C transport triglycerides in blood stream an is calculated by:

VLDL-C = Triglyceride / 5

" Vitamins "

Vitamins are organic nutrients (molecules), that are required in small quantities for a variety of biochemical functions,(the most prominent function is as cofactors for enzymatic reactions), also they are essential for the normal processes of metabolism, including growth and maintenance of health.

The distinguishing feature of the vitamins is that they generally cannot be synthesized by mammalian cells and, therefore, must be supplied in the diet, (except, the body is able to produce part or even all of its requirements for some of the vitamins, Example: Vitamin D from cholesterol and niacin from Tryptophan).

The vitamins are classified into two major groups:

1- Water-soluble vitamins: (thiamin (B_1) , riboflavin (B_2) , niacin(B_3) , pantothenic acid (B_5) , pyridoxal (pyridoxine, pyridoxamine (B_6) , biotin , cobalamin (B_{12}) , folic acid and ascorbic acid).

2- Fat-soluble vitamins: (vitamin A , vitamin D , vitamin E ,vitamin K).

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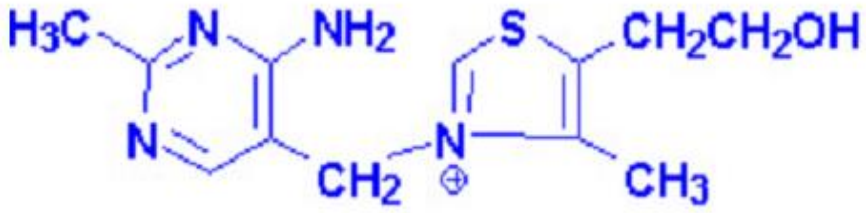
* Water-soluble vitamins: it's a polar hydrophilic molecules and, therefore, are soluble in water.

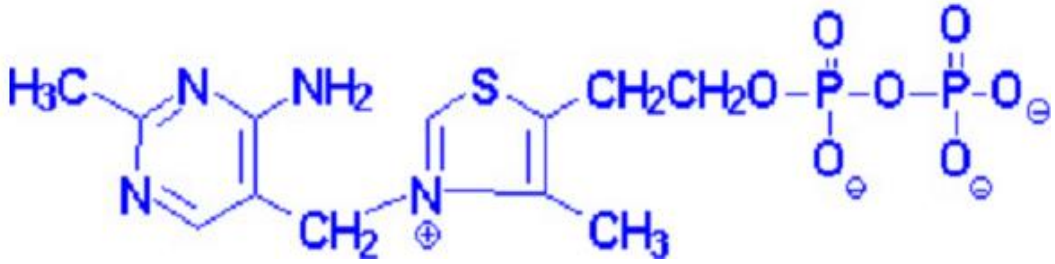
Because of their water solubility, excesses of these vitamins are excreted in urine and so rarely accumulate in toxic concentrations, for this reason their storage is limited.

1-Thiamin (B_1) :

Thiamin is also known as vitamin B_1, is derived from a substituted pyrimidine and a thiazole which are coupled by a methylene bridge.



Thiamin is rapidly converted to its active form, thiamin pyrophosphate (TPP), in the brain and liver by specific enzyme, thiamin diphosphotrans-ferase.



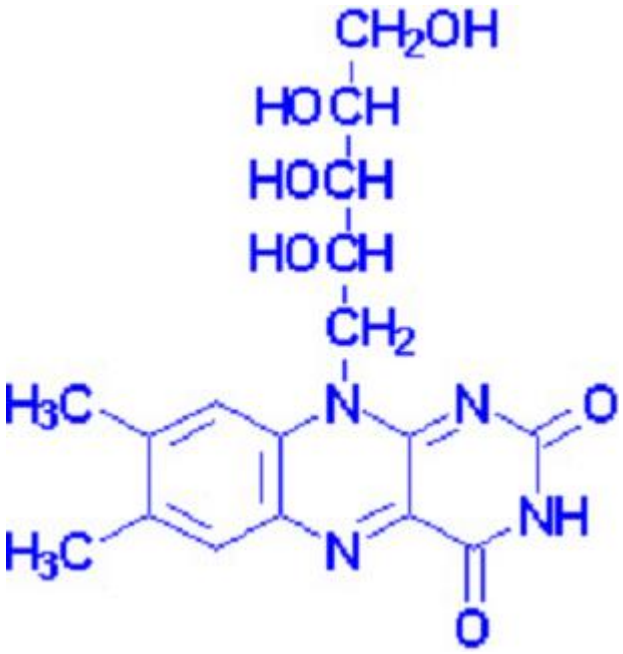
TPP is necessary as a cofactor for the pyruvate and α-ketoglutarate dehydrogenase catalyzed reactions like in pentose phosphate pathway, therefore, a deficiency in thiamin intake leads to a severely reduced capacity of cells to generate energy as a result of its role in these reactions.

The dietary requirement for thiamin is proportional to the caloric intake of the diet and ranges from 1.0-1.5 mg/day for normal adults. If the carbohydrate content of the diet is excessive, then an excess thiamin intake will be required.

The severe thiamin deficiency disease known as beriberi is the result of a diet that is carbohydrate rich and thiamin deficient, in such individuals TPP dependent reactions are prevented, leading to accumulation of substrates like Pyruvate, Pentose sugars etc .

Thiamin is present in almost all plant and animal tissues commonly used as foods like seeds, nuts, wheat, & lean meat.

2- Riboflavin (B_2) :



Riboflavin is also known as vitamin B_2, is the precursor for the coenzyme, flavin mononucleotide(FMN) and flavin adenine dinucleotide (FAD). The enzymes that require FMN or FAD as cofactors are termed flavoproteins.

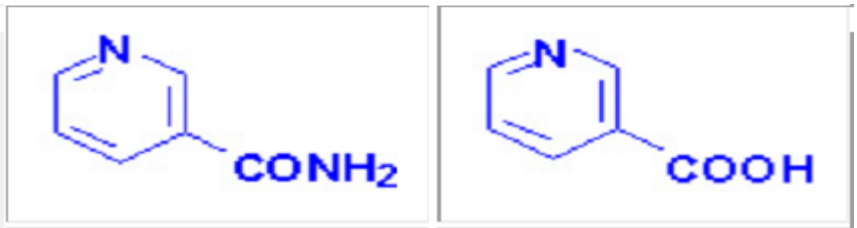
Riboflavin deficiencies are rare due to the presence of adequate amounts of the vitamin in eggs, milk, meat, and cereals.

Requirement's about 1.5-2.5mg for adults, infants 0.6mg, children 1.0-1.8mg.

Deficiency: Lack of riboflavin in the diet causes a generally non fatal syndrome of inflammation of the corner of mouth (angular stomatitis), painful glossitis of tongue (Purple) and Scaly dermatitis.

Riboflavin decomposes when exposed to visible light, this characteristic can lead to riboflavin deficiencies in newborns treated for hyperbilirubinemia by phototherapy.

3-Niacin (B_3) :



Nicotinamide	Nicotinic Acid

Niacin (nicotinic acid and nicotinamide) is also known as vitamin B_3. Both nicotinic acid and nicotinamide can serve as dietary sources of vit.B_3.

Niacin is required for the synthesis of the active forms of vit.B_3, which are nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). Both NAD^+ and $NADP^+$ function as cofactors for numerous dehydrogenases, e.g.: lactate and malate dehydrogenases.

Niacin is not a true vitamin, since it can be derived from the amino acid tryptophan.

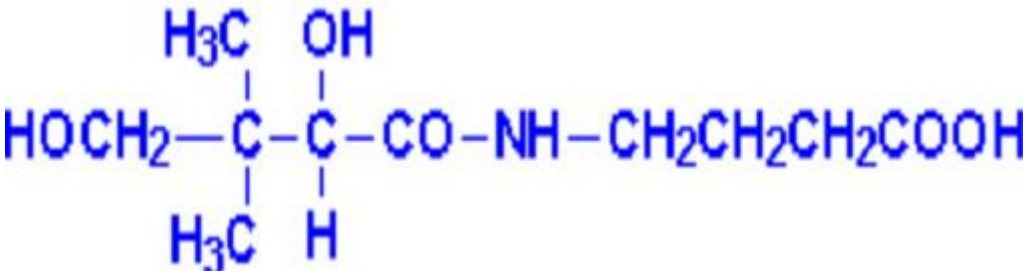
The ability to utilize tryptophan for niacin synthesis is inefficient, due to (60) mg of tryptophan are required to synthesize (1) mg of niacin, also, synthesis of niacin from tryptophan requires vitamins B_1, B_2, and B_6 which may be limited in the diet.

The daily requirement for niacin is 17-21mg for adults, infants 6mg.The requirement increases with increased intake of calories, illness, severe injury ,infection ,burns, high corn (maize) diet, pregnancy and lactation.

Lack of niacin causes of the deficiency syndrome which called "pellagra" in peoples depend on corn as diet, a disease involving gastrointestinal tract (GIT) and central nervous system (CNS).

The major action of nicotinic acid is reduction the fatty acid mobilization from adipose tissue, therefore, it used in treatment of hypercholesterolemia.

4- Pantothenic acid (B_5) :



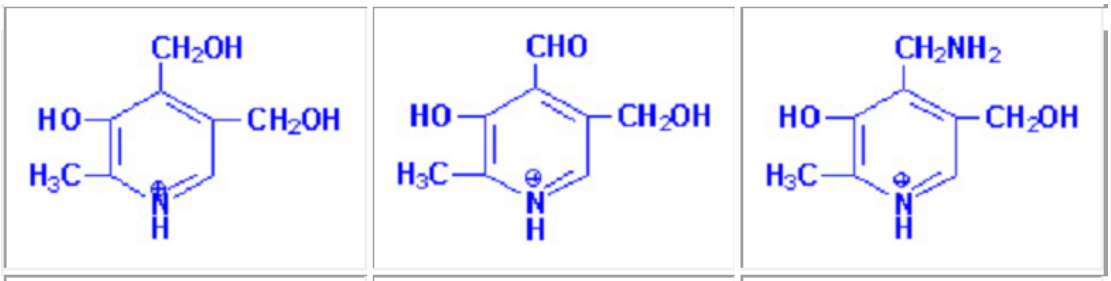
Pantothenic acid is also known as vitamin B_5. Its formed from β-alanine and pantoic acid. Pantothenate is required for synthesis of coenzyme A (CoA) and is a component of the acyl carrier protein(ACP), which is (ACP) used in fatty acid synthesis.

Pantothenate is, therefore, required for the metabolism of carbohydrate and all fats and proteins. At least (70) enzyme have been identified as requiring CoA or ACP derivatives for their function.

Daily requirements about 4-7mg and deficiency is rare due to its widespread distribution in whole grain cereals, legumes and meat.

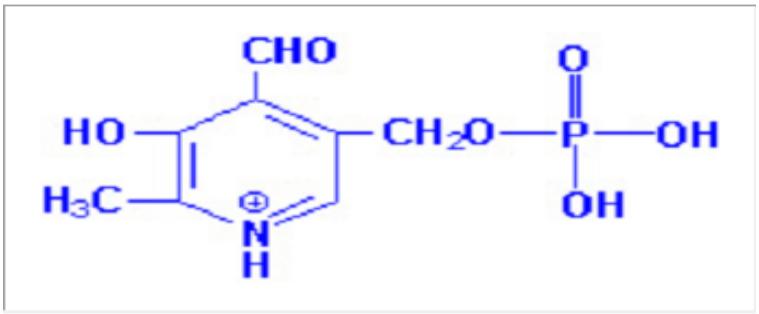
The burning foot syndrome in prisoners which is associated with reduced capacity for acetylation is ascribed to pantothenic acid deficiency.

5- Vitamin B_6 :



Pyridoxine	Pyridoxal	Pyridoxamine

Pyridoxal, pyridoxine, and pyridoxamine are collectively known as vitamin B_6. All three compounds are efficiently converted to the biologically active form of vit.B_6, pyridoxal phosphate, this conversion is catalyzed by the ATP requiring enzyme, pyridoxal kinase.



Pyridoxal Phosphate

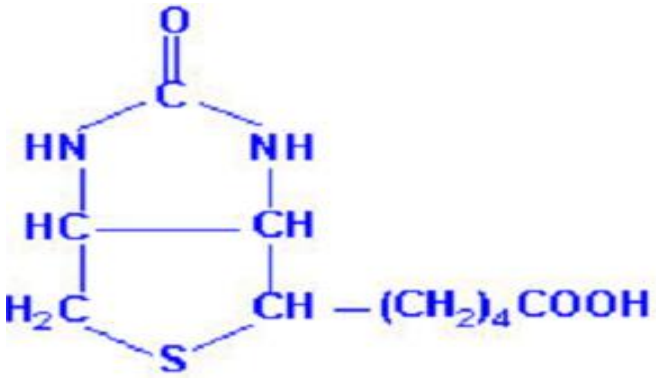
Pyridoxal phosphate functions as a cofactor in enzymes involved in transamination reactions required for the synthesis and catabolism of the amino acids as well as in glycogenolysis as a cofactor for glycogen phosphorylase.

The requirement for vitamin B6 in the diet is proportional to the level of protein consumption ranging from 1.4-2.0 mg/day for a normal adult.

During pregnancy and lactation the requirement for vit.B_6 increases approximately 0.6 mg/day.

Deficiencies of vit.B_6 are rare and usually are related to an overall deficiency of all the B-complex vitamins and if happened may lead to hypochromic microcytic anemia since it is required for heme synthesis .

6- Biotin :



It is the cofactor required for enzymes that are involved in carboxylation reactions (e.g.: acetyl-CoA carboxylase and pyruvate carboxylase).

Biotin is found in numerous foods and also is synthesized by intestinal bacteria and as such deficiencies of the vitamin are rare. Deficiencies are generally seen after long antibiotic therapies which deplete the intestinal fauna, also deficiency may happen after excessive consumption of raw eggs, due to the egg white protein (avidin) prevent intestinal absorption of the biotin.

Daily requirements about 100-200µg/day. Requirement increase in pregnancy and lactation. Patients on oral antibiotics for a long period of time require more of this vitamin.

7- Cobalamin :

It is more commonly known as vit.B_{12}. It is composed of a complex tetrapyrol ring structure (corrin ring) and a cobalt ion in the center.

There are two reactions in the body that require this vitamin as a cofactor :

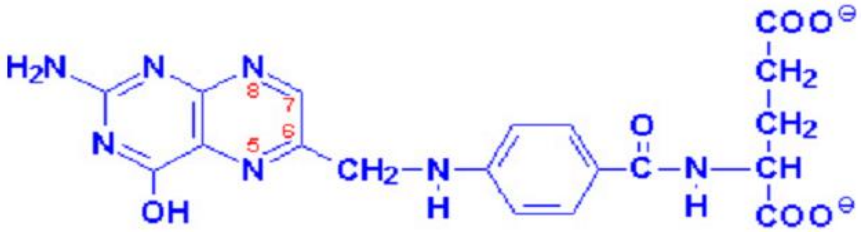
1- During the catabolism of fatty acids, and the amino acids valine, isoleucine and threonine, the enzyme required is methylmalonyl-CoA mutase.

2- During the conversion of homocysteine to methionine and is catalyzed by methionine synthase.

Deficiencies of vit.B_{12} is rare due to the liver can store it up to six years, but if happened, deficiencies lead to pernicious anemia (due to impaired DNA synthesis) and also lead to neurological complications (due to progressive demyelination of nerve cells).

Daily requirements about 3mg, and its synthesized in small quantities by microorganisms.

8- Folic acid :



Folic acid is a conjugated molecule consisting of a pteridine ring structure linked to para-aminobenzoic acid (PABA). Folic acid is obtained primarily from yeasts and leafy vegetables as well as animal liver.

The active form of folic acid is Tetra hydro folate (THF). Animals cannot synthesize PABA, thus, requiring folate intake in the diet. Folate deficiency is rare, but if happened, leads to impairment of DNA synthesis and this lead to abnormally large erythrocytes (macrocytic anemia).

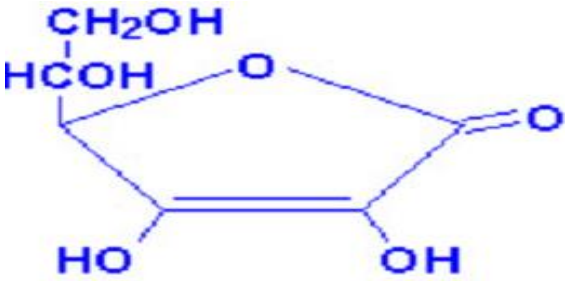
Daily requirements about 100µg (during Lactation & pregnancy are 500 - 800µ g/day).

Deficiency: The causes of folate deficiency are inadequate intake, impaired absorption, increased demand during pregnancy, lactation and impaired metabolism that leads to megaloblastic anemia. In this condition

production of erythrocytes slows down, macrocytic erythrocytes with fragile membrane are formed.

Inadequate folate levels during the early stages of pregnancy increases the risk of neural tube defects (a type of birth defect) and spontaneous abortions.

9- Ascorbic acid :



Ascorbic acid is more commonly known as vitamin C. Ascorbic acid is derived from glucose via the uronic acid pathway.

The active form of vitamin C is ascorbate itself. The main functions of ascorbate in: collagen biosynthesis, degradation of tyrosine, absorption of iron, steroidogenesis, adrenaline synthesis, bile acid formation, bone mineral metabolism and as potent antioxidant.

Deficiency of vit.C leads to the disease Scurvy due to the role of the vitamin in modification of collagen.

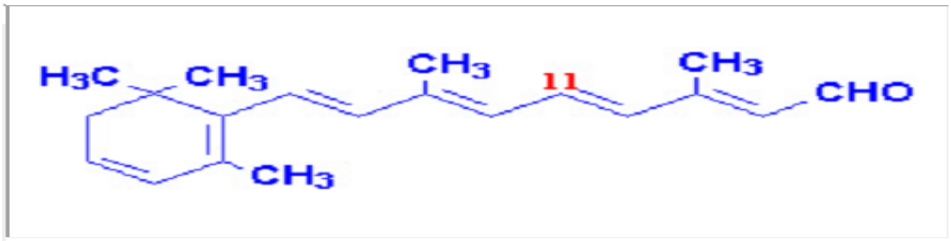
WBC's are rich in vit C and plays an important role in immunity.

Source: citrus fruits, potato, tomato & green vegetables, and the daily requirements about 60mg.

* Fat-soluble vitamins :

Ample reserves of fat soluble vitamins are stored in the tissues as they are not readily absorbed from the food. With the exception of Vit. K, they do not serve as coenzymes. Indeed Vit D act more like hormone.

1-Vitamin A:



All-*trans*-retinal

Vitamin A consists of three biologically active molecules, retinol, retinal and retinoic acid. Each of these compounds are derived from the plant precursor molecule, β-carotene.

Functions: β-carotene has an antioxidant role and prevents the development of diseases in which the action of free radicals is implicated .

It plays a protective role against cancer and cardiovascular disease, as the normal proliferation of epithelial cell growth and differentiation depends on retinoids.

Vitamin A is necessary for vision, and deficiency cause night blindness due to the visual pigment, rhodopsin is found in the rod-cells of the retina and is formed by the binding of retinal to the apoprotein opsin.

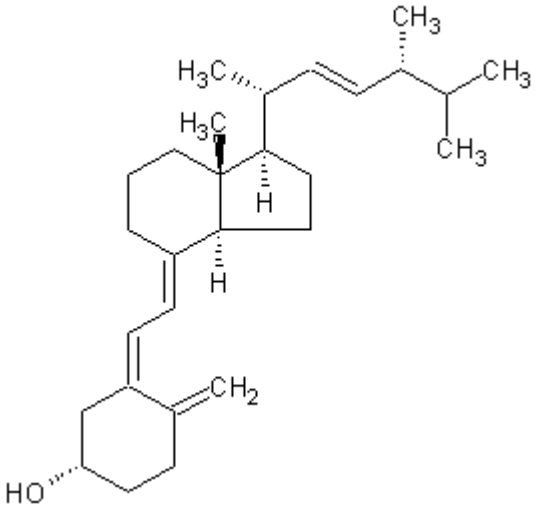
Source: a rich source is liver, but leafy vegetables and some fruits provide the largest amount of β-carotene. Liver, egg yolk, butter and milk are good sources of β-carotene.

Vitamin A is stored in the liver and deficiency of the vitamin occurs only after prolonged lack of dietary intake. The earliest symptoms of vitamin A deficiency are night blindness. Severe Vit A deficiency leads to progressive keratinization of the cornea and possibly permanent blindness.

The deficiency causes dryness and roughness of skin developing keratosis of hair follicles. Bone growth is markedly impaired.

Excessive intake of vitamin A (hypervitaminosis), in humans cause head ache, nausea, vomiting and dizziness. This might be related to increased spinal fluid pressure.

2-Vitamin D :



Vitamin D is the only vitamin that is usually not required in the diet, for this reason it is rather classified as a hormone since under conditions of inadequate exposure to sunlight that dietary intake is required.

Vitamin D is a steroid hormone that functions to regulate specific gene expression following interaction with its intracellular receptor.

The biologically active form of the hormone is 1,25-dihydroxy vitamin D_3 (1,25-$(OH)_2$ D_3), also termed calcitriol.

Function: Calcitriol functions in concert with parathyroid hormone(PTH) and calcitonin to regulate serum calcium and phosphorous levels.

- Intestine: This vitamin promotes absorption of calcium, phosphates.

- Kidney: Reabsorption of calcium, phosphate are enhanced.

- Bone: It promotes synthesis of osteocalcin which is needed for bone mineralization. It also promotes bone collagen synthesis.

Sources: Fish oils, egg yolk are naturally rich sources of Vit D.

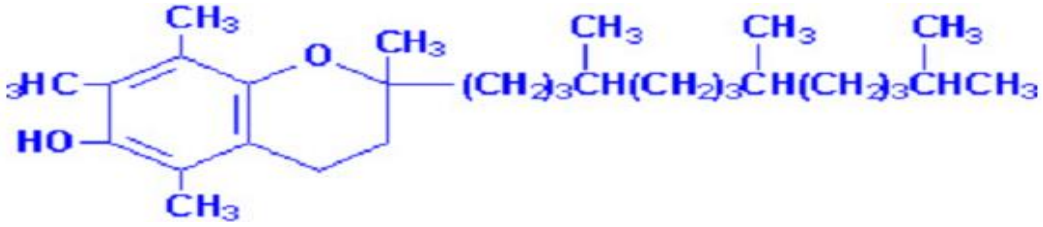
Deficiency: usually deficiency of Vit D are due to insufficient exposure to sunlight, inadequate dietary intake, gastrointestinal disorder, obstructive jaundice and Partial gastrectomy.

The main symptom of vitamin D deficiency in children is rickets and in adults is osteomalacia.

Vit D toxicity :

Excess Vit. D level enhances calcium absorption leading to hypercalcemia and metastatic calcium deposits. There is a tendency to develop kidney stones from the hypercalciuria, secondary to hypercalcemia.

3- Vitamin E :



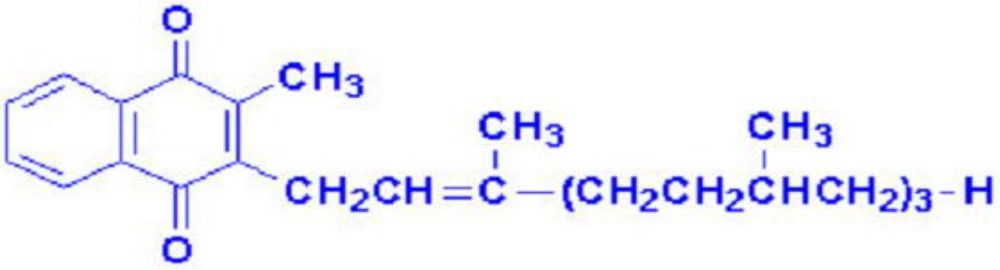
Vitamin E is a mixture of several related compounds known as tocopherols. The α-tocopherol molecule is the most potent of the tocopherols.

The major function of vitamin E is to act as a natural antioxidant, therefore, it's important for preventing peroxidation of polyunsaturated membrane fatty acids, this protective phenomenon is very much evident in the prevention of hemolysis of RBCs by H_2O_2.

Source: The reachest source is vegetable oils and nuts.

The major symptom of vitamin E deficiency in humans is an increase in red blood cell fragility. Neurological disorders have been associated with vitamin E deficiencies.

4- Vitamin K :



The major function of the K vitamin is in the maintenance of normal levels of the blood clotting proteins.

Deficiency of the vitamin in adults is rare, but the long time antibiotic treatment can lead to deficiency in adults. The intestine of newborn infants don't contain bacteria, therefore , deficiency is possible if lacking from the early diet. The primary symptom of a deficiency in infants is a hemorrhagic syndrome.

Dr.Salim.J.Kh.

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