

# Analytical chemistry

The analytical chemistry: is deals with methods for the identification of one or more components in a sample of matter , and **can be classified it in to three types :**

1. Qualitative analysis: is concerned with involve identification of the components as elements, ions, compounds found in the unknown sample.
2. Quantitative analysis: determination of the quantity one or more of the component present in the sample.
3. Instrumental analysis.

**The quantitative analysis can be classified in to:**

1. Volumetric analysis: type of the quantitative analysis depending on measurement the volume of the standard solution to find the quantity of components.

The titration is type of it , its include react the analyte with the solution known concentration called (standard solution).



Titrant: is the solution which is in the burette.

Titrand: is the solution which is in the conical flask.

The requirement of titration:-

- a. Stoichiometric.
- b. Fast.
- c. End point sharp.

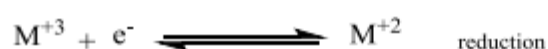
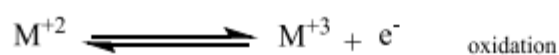
end point: the point at which the indicator changes its color.

**The properties of standard solution is:-**

- a. React fast and complete.
- b. Stay the concentration constant after much time.
- c. Can be referred to its reaction by the balanced chemical equation.
- d. Give the equivalent point sharp.

**Volumetric analysis type:-**

1. Acid-Base reaction.
2. Precipitation and complex formation reaction.
3. Oxidation-Reduction reaction(Redox reaction):



2. Gravimetric analysis: type of the quantitative analysis depending on measurement of the weight of the analyte to find its quantity.

## Methods of expressing of analytical concentration:-

### 1. Chemical units: (M, m, N, F).

a. Molarity (M): number of molecular per 1 liter of solution.

$$M = \frac{Wt}{M.Wt} \times \frac{1000}{V(mL)}$$

b. Normality (N): number of equivalents per 1 liter of solution.

$$N = \frac{Wt}{eq.Wt} \times \frac{1000}{V(mL)}$$

### 2. Physical units: (w/w%, w/v%, v/v%, ppm)

## Preparation of solutions:-

### 1. The solid:

$$N = \frac{N \cdot eq.wt \cdot V(mL)}{1000}$$

$$eq.wt = \frac{M.Wt}{n}$$

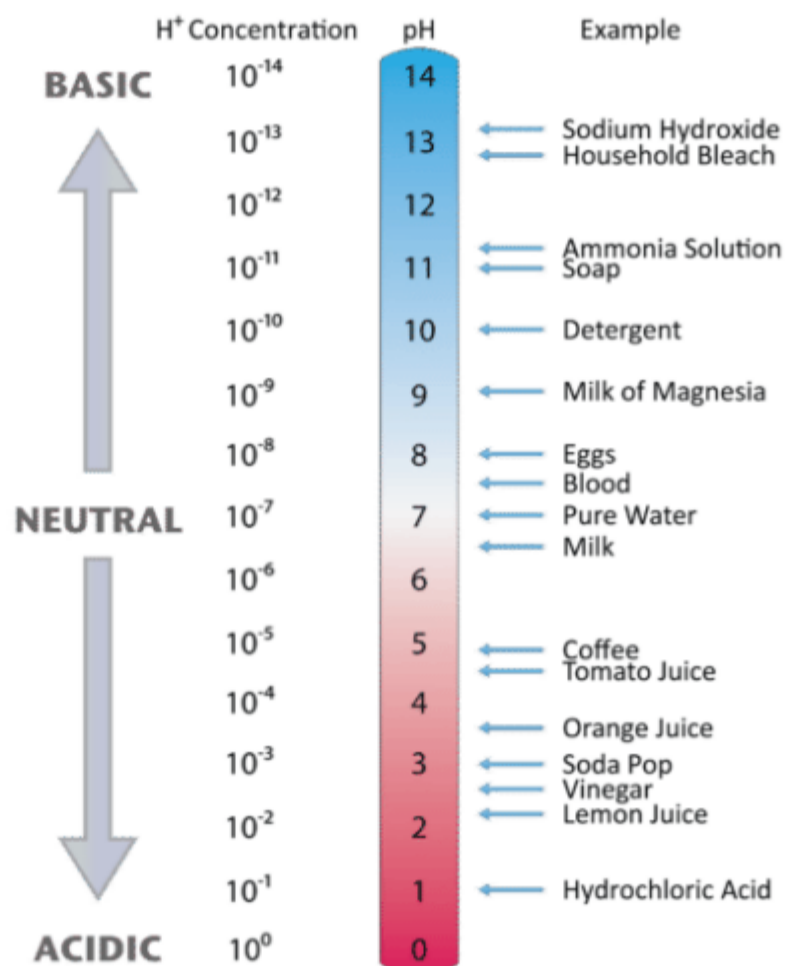
### 2. The liquid:

$$N_{conc.} = \frac{Sp.Gr \cdot \% \cdot 1000}{eq.wt}$$

$$(N_1 V_1)_{conc.} = (N_2 V_2)_{dilute}$$

## Hydrogen concentration (PH):

Hydrogen ion concentration is the logarithm of the reciprocal of the hydrogen ion concentration in gram moles per liter. Thus, in a neutral solution the hydrogen ion ( $H^+$ ) and the hydroxyl ion ( $OH^-$ ) concentrations are equal, and each is equal to  $10^{-7}$ . A PH of 7 is neutral.



Hydrogen ions are by-products of the metabolism of substances such as proteins, fats, and carbohydrates. These by-products create extra hydrogen ions ( $H^+$ ) in the blood that need to be balanced and kept within normal

range as described earlier. The body has several mechanisms for maintaining blood PH.

### **Buffer solutions:**

Is define as a solution the resists the change in PH value, when a small amount of an acid or base is added or when the solution is diluted (PH approximately constant).

### **Type of buffers:**

1. weak acid + conjugated base
2. weak base + conjugated acid

### **Effect of adding HCl to the buffer solution:**



-When HCl will be added that's meaning added  $\text{H}^+$ , so the concentration of  $\text{H}_3\text{O}^+$  was increased in the solution (the equilibrium is disturbed).

-According to the Le chatelier's principle the reaction will shift to the left by the conjugated base and  $\text{H}_3\text{O}^+$  reaction to form an extra  $\text{CH}_3\text{COOH}$ .

-As a result of the reaction shifting towards the left, the effect of the increase in the concentration of  $\text{H}_3\text{O}^+$  resulting from the addition of the strong acid HCl will almost disappear, and thus the PH value of the solution will remain approximately constant.

### Effect of adding NaOH to the buffer solution:



-Adding a NaOH means adding  $\text{OH}^-$ , which will react with  $\text{H}_3\text{O}^+$  forming water, which has a neutralizing effect in the solution, thus disturbing the equilibrium.

-According to Le chatelier's principle, the reaction will shift to the right with the dissociation of more  $\text{CH}_3\text{COOH}$  so the decrease in  $\text{H}_3\text{O}^+$  will be compensated, so its concentration will remain approximately constant, and thus, instead of increasing the PH value of the solution, it will be remain approximately constant (acidic).

-Even if the amount of acid  $\text{CH}_3\text{COOH}$  in the solution, the PH value will rise.

### Making buffer solutions:

The action of the buffer solution is summarized as follows:

- Carbonic acid, which is a weak acid, reacts with the hydroxide ion, which represents the increase in basicity that occurs in the blood, that is, it neutralizes the excess amount of hydroxide ion.
- The bicarbonate ion, which represents the salt of a weak acid or the conjugated base of a weak carbonic acid, neutralizes the excess hydrogen ion that causes high blood acidity.
- Carbonic acid is a good material for catching excess protons because it is a weak acid and its degree of ionization is very low.

- Contains fluids inside or outside the cell in organisms have conjugate acid-base pairs that behave like buffer solutions.
- The main buffer in blood plasma is the solution “regulated bicarbonate”.

### **The importance of the buffer solution:**

Many chemical and biological processes require that the PH of the reaction medium not change much, but rather remain close to a certain value.

An example of this:

1.The blood in the human body cannot perform the function of transporting oxygen to the cells unless the PH value is = 7.4

2.Enzymes need a medium in which the PH value is approximately constant in order to work activity. If the PH of the solution changes, these enzymes will lose or their shape will change, and they may lose their function vetality.

## Qualitative analysis:-

**Chromatography:** is an analytical technique that can be used for qualitative analysis, it can both identify components in a mixture and determine how much of each component is present.

The word chromatography is derived from the Greek words for colour and write. It quite literally means “to write with colour”.

### Stationary and mobile phases:-

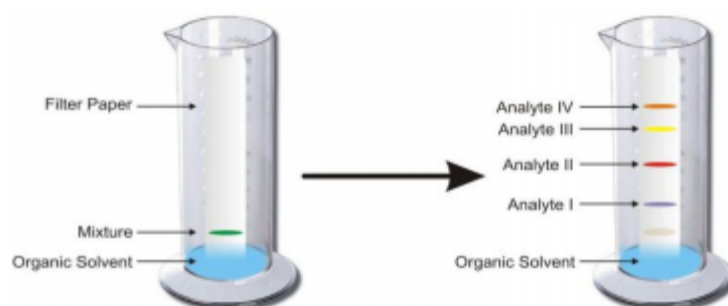
All chromatography uses two phases to separate a mixture, these are the:

Mobile phase, which is a solvent, that moves over or through the stationary phase.

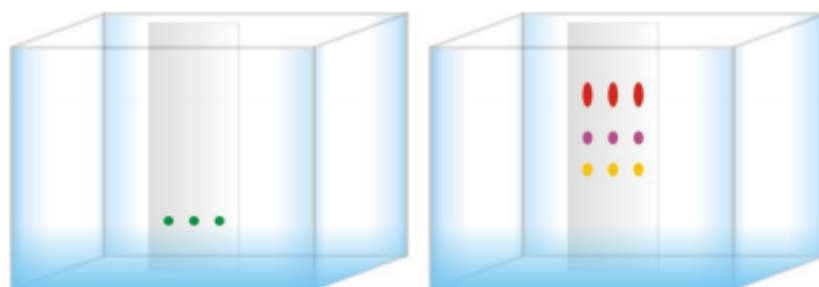
Stationary phase, which is either a solid support. It always stays still.

### Types of chromatography:-

**1. paper chromatography:** The most common type of chromatography, the paper is the stationary phase. This uses capillary action to pull the solutes up through the paper and separate the solutes.

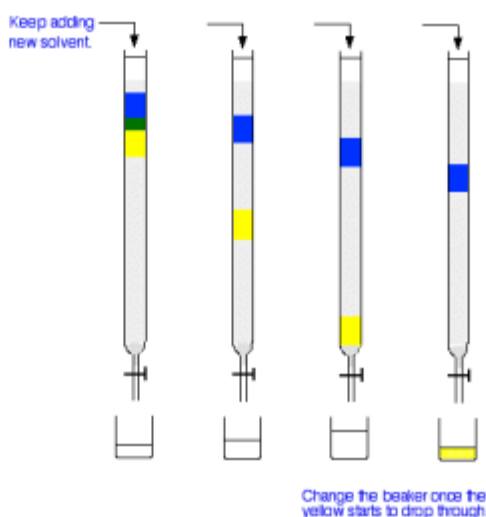


**2. Thin layer Chromatography:** It is a commonly used chromatography technique in synthetic chemistry for determining the purity of small molecules and also for identifying the progress of a reaction. This technique is much faster and requires only small quantities of the compound.

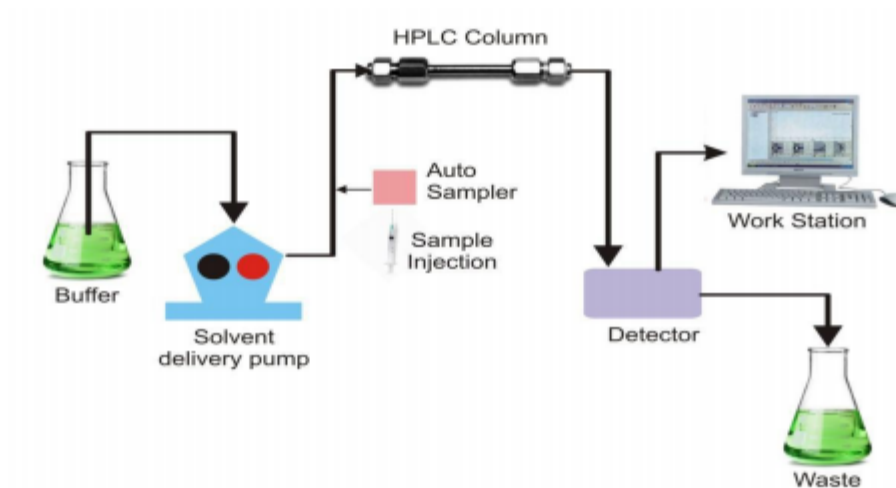


**3. Column chromatography:** is a preparative technique used to purify compounds depending on their polarity or hydrophobicity.

In column chromatography, a mixture of molecules is separated based on their differential partitioning between a mobile phase and a stationary phase.



**4. High Performance Liquid Chromatography:** HPLC is advanced form of column chromatography in which elution of analytes is carried down by applying high pressure.

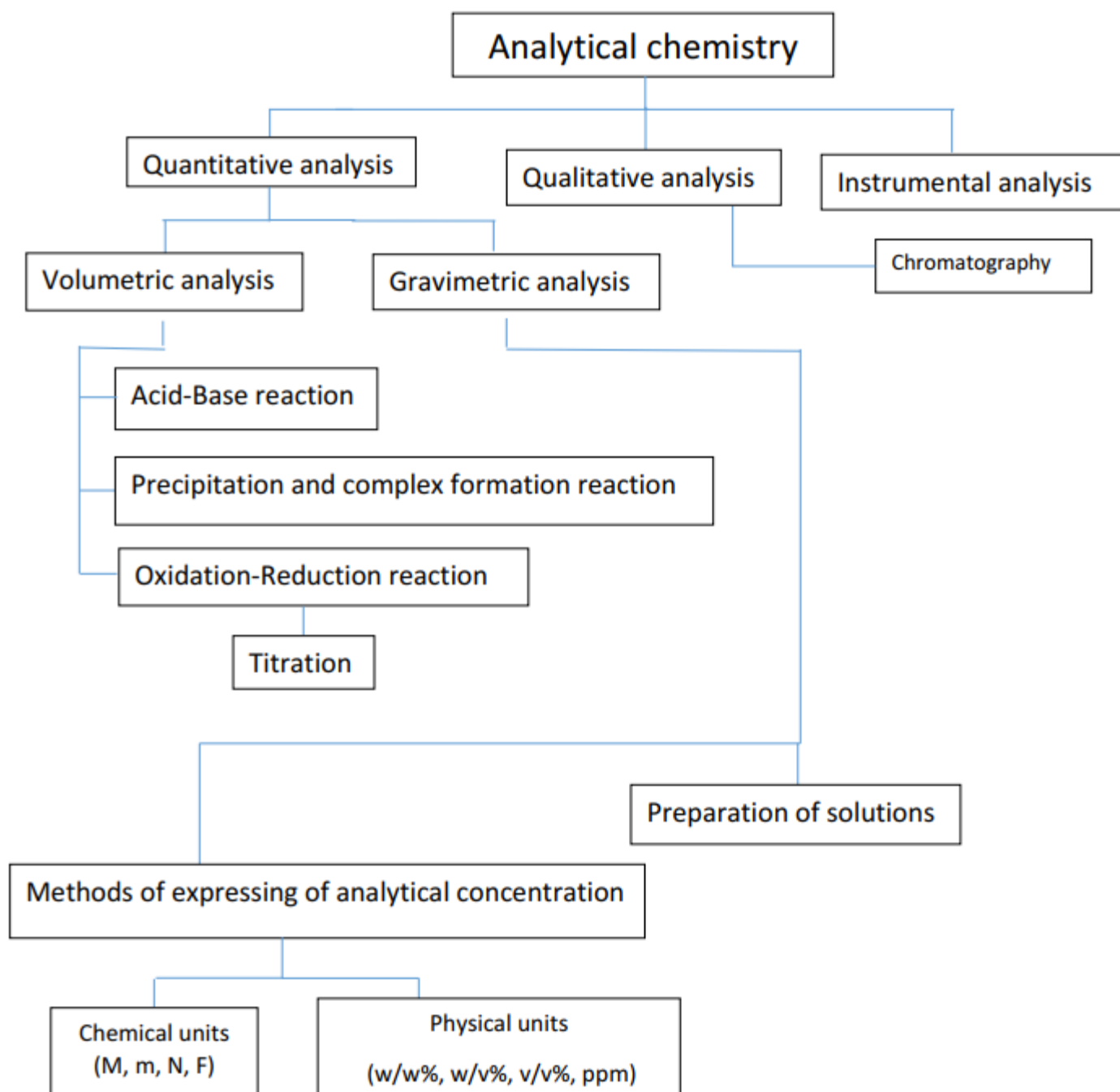


**5. Ion Exchange Chromatography.**

**6. Gel Filtration Chromatography (molecular exclusion chromatography).**

**7. Affinity Chromatography.**

## The summary



## **Biochemistry and clinical biochemistry**

- **Definition of biochemistry.**
- **Definition of clinical biochemistry.**
- **Carbohydrate compounds:**
  - **classification.**
  - **glucose metabolism.**
  - **Factors that maintain blood glucose level.**
  - **Glucose level in the blood.**
  - **clinical significance.**

**Biochemistry:** it is the science that studies processes related to organisms.

**Clinical biochemistry:** one of the branches of diseases science, are concerned with the analysis of different body fluids.

**Carbohydrate compounds:** Carbohydrates are the most abundant macromolecules in nature. They are the main source and storage of energy in the body. They serve also as structural component of cell membrane. The general molecular formula of carbohydrate is  $C_nH_{2n}O_n$  or  $(CH_2O)_n$ , where  $n > 3$ . Chemically, they contain the elements Carbon, hydrogen and oxygen. Thus, they are Carbon compounds that contain large quantities of Hydroxyl groups. Carbohydrates in general are polyhydroxy aldehydes or ketones or compounds which give these substances on hydrolysis.

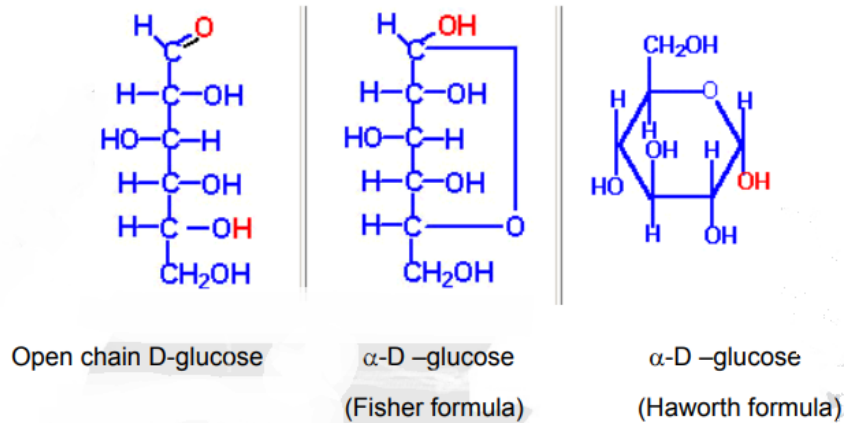
## Classification and Structure Classification

There are three major classes of carbohydrates

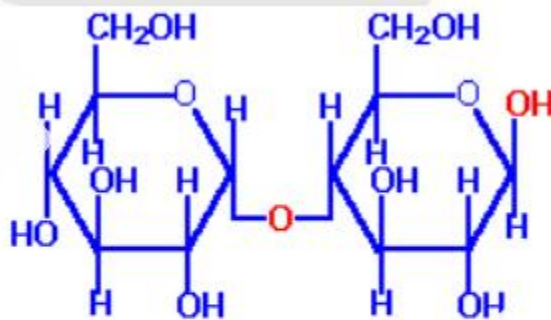
- Monosaccharides (Greek, mono = one)
- Oligosaccharides (Greek, oligo= few) 2-10 monosaccharide units.
- Polysaccharides (Greek, Poly = many) >10 monosaccharide units.

**Monosaccharides:** also called simple sugars, they consist of a single polyhydroxy aldehyde or ketone units.

The most abundant monosaccharides in nature are the 6-carbon sugars like D-glucose and fructose.



**Oligosaccharides:** contain 2 to 10 monosaccharide units. The most abundant oligosaccharides found in nature are the Disaccharides:



**Structure of Maltose**

**Polysaccharides:** Most of the carbohydrates found in nature occur in the form of high molecular polymers called polysaccharides.

There are two types of polysaccharides.

- Homopolysaccharides that contain only one type of monosaccharide building blocks.

- Heteropolysaccharides, which contain two or more different kinds monosaccharide building blocks.

## Glucose metabolism

**Metabolism:** The chemical processes occurring within a living cell or organism that are necessary for the maintenance of life. All these are called anabolism and catabolism.

**-Anabolic reaction: -**

1. synthesis of complex molecules from simple compound.
2. energy is needed for synthesis (endergonic reaction)

**-catabolic reaction: -**

1. break down of large molecules Such as polysaccharides, proteins into small molecules like,  $\text{CO}_2$ ,  $\text{NH}_3$ ,  $\text{H}_2\text{O}$ .
2. liberated energy. (exergonic reaction)

## Digestion and absorption:

**1. mouth:** salivary glands secrete saliva

Saliva contains:  $\alpha$ - amylase (ptyalin), water 99.5% and glycoprotein as food lubricant.

$\alpha$ - amylase, hydrolysis starch to dextrin and maltose.

PH of  $\alpha$ - amylase = 5.8 – 7.1 less than 4.0 is in active

**2. stomach** ----- no digestion is seen in stomach; amylase is in active Because the PH of stomach (1 - 2) very acidic.

**3. small intestine:** it is the major site of digestion of CHO, pancreatic amylase hydrolyzes dextrin into maltose.

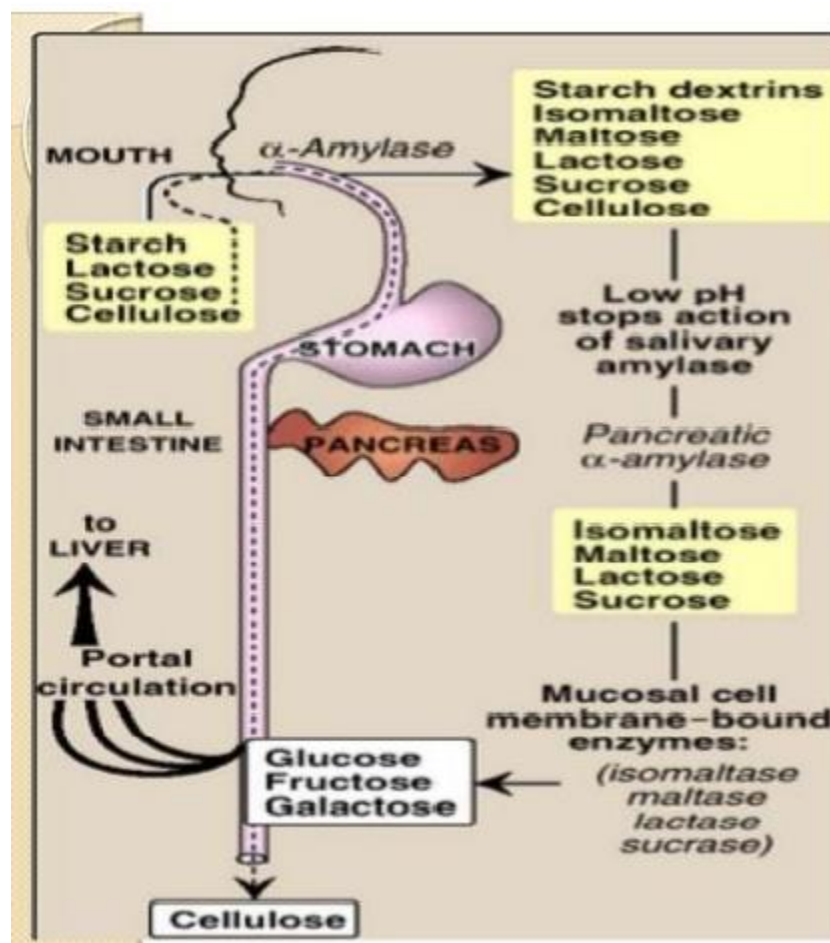
The optimum PH of amylase = 7.1

**4. intestinal mucosal:** mucosal cell membrane – bound enzymes, the site where disaccharides hydrolyze.

Maltose  $\xrightarrow{\text{maltase}}$  glucose + glucose

Sucrose  $\xrightarrow{\text{sucrase}}$  glucose + fructose

Lactose  $\xrightarrow{\text{lactase}}$  glucose + galactose



## Fate of glucose after absorption

**In the liver**, glucose undergoes variety of chemical changes depending upon the physiological need of the body.

1. **Body need for energy**: glucose oxidized completely to  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  and energy by (glycolysis and citric acid cycle).
2. **Excess glucose may be converted to glycogen**, deposit in liver, muscle tissues

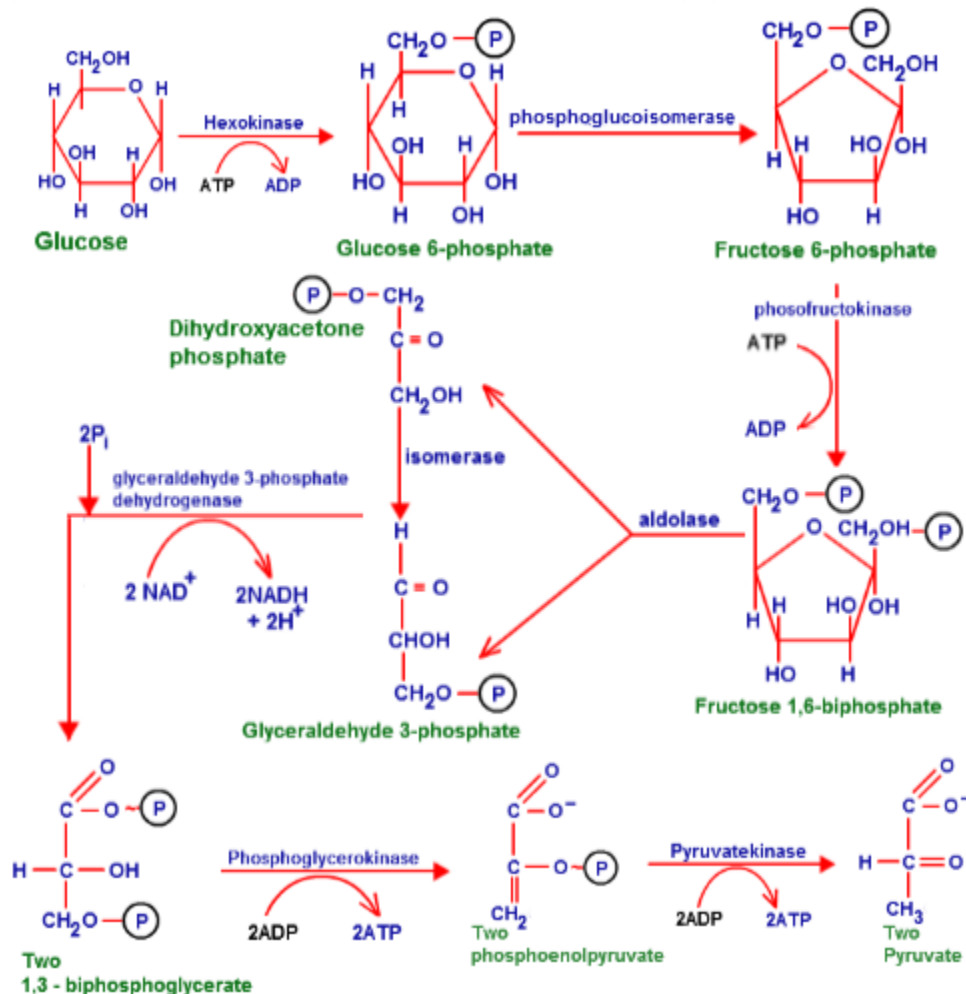
By (glycogenesis).

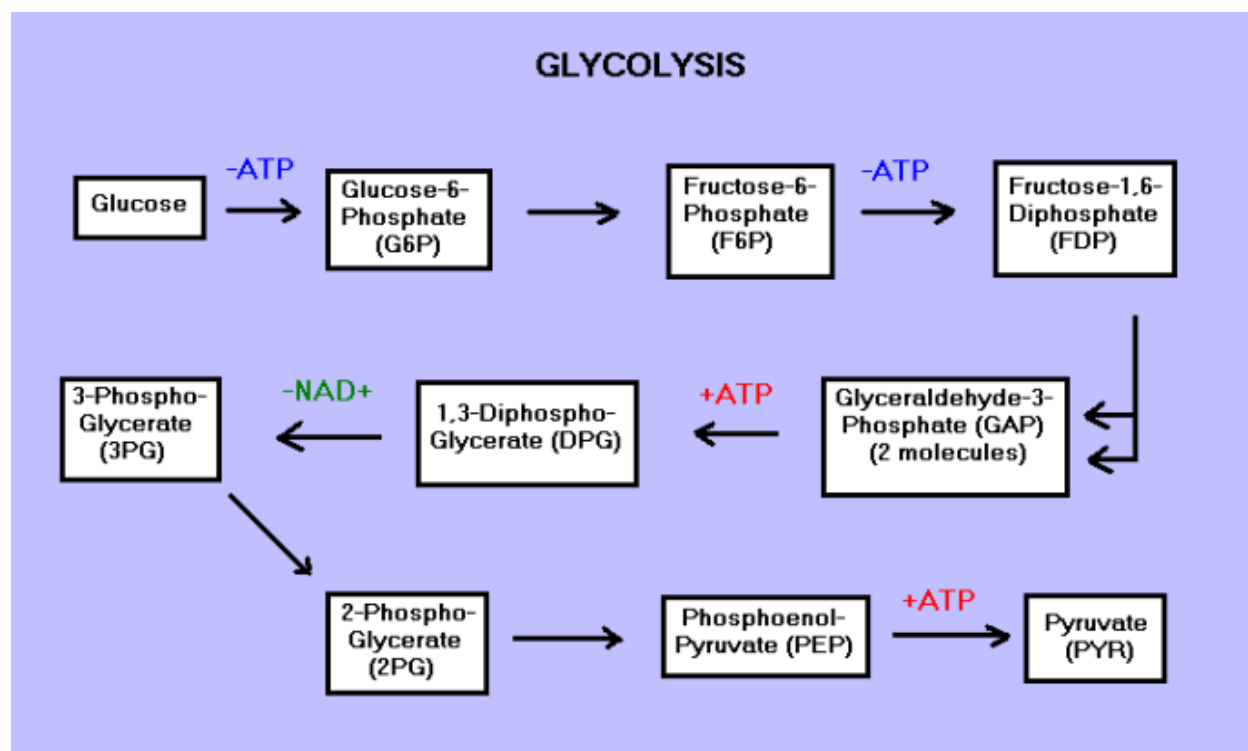
3. To maintain glucose blood level, liver glycogen reconverted to glucose enters blood By (glycogenolysis).
4. **excess glucose after conversion to glycogen**, convert to fatty acids stored in adipose tissue as triglycerides (lipogenesis).
5. small amounts of glucose may be utilized for the synthesis of ribose and deoxyribose for synthesis of nucleic acids.
6. **in muscle contraction**, only partial degradation of glucose may take place, resulting in formation of lactic acid disposed of by the liver.

**Glycolysis**: (from glycolose, a term for glucose + -lysis degradation)

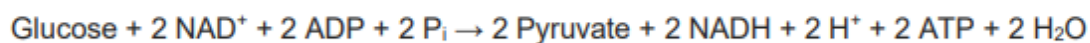
1. It is the metabolic pathway that converts glucose  $\text{C}_6\text{H}_{12}\text{O}_6$ , into pyruvate.
2. The free energy released in this process is used to form the high-energy molecules ATP and NADH.
3. Glycolysis is an oxygen independent metabolic pathway, said to be anaerobic.
4. Glycolysis occurs in the cytosol (cytoplasm) of the cell.

5. The glucose in the blood circulation, when enter the cell become phosphorylated given by ATP (Activation by phosphate group).
6. This phosphorylation occurs on the cell membrane by the action of two enzymes:
  1. specific enzyme (glucokinase) in the liver.
  2. nonspecific enzyme (hexokinase), Present in liver and other extra hepatic cell
7. Glu-6- p is an important compound for several metabolic pathways, the reaction is irreversible.





The overall process of glycolysis is:



**Energy production:** The net of ATP molecules during glycolysis is equal to (8).

|                        |                                                 |       |
|------------------------|-------------------------------------------------|-------|
| <b><u>ATP used</u></b> | 1. ( glu $\longrightarrow$ glu-6-pho )          | 1 ATP |
|                        | 2. ( F-6-pho $\longrightarrow$ F-1,6 - di pho ) | 1 ATP |

|                        |                                           |                  |
|------------------------|-------------------------------------------|------------------|
| <b><u>ATP gain</u></b> | Gly-3- pho $\longrightarrow$ 1,3-diphoGly | NADH $\times 2$  |
|                        | 1,3 -diphoGly $\longrightarrow$ 3phoGly   | 1 ATP $\times 2$ |
|                        | PEP $\longrightarrow$ pyruvate            | 1 ATP $\times 2$ |

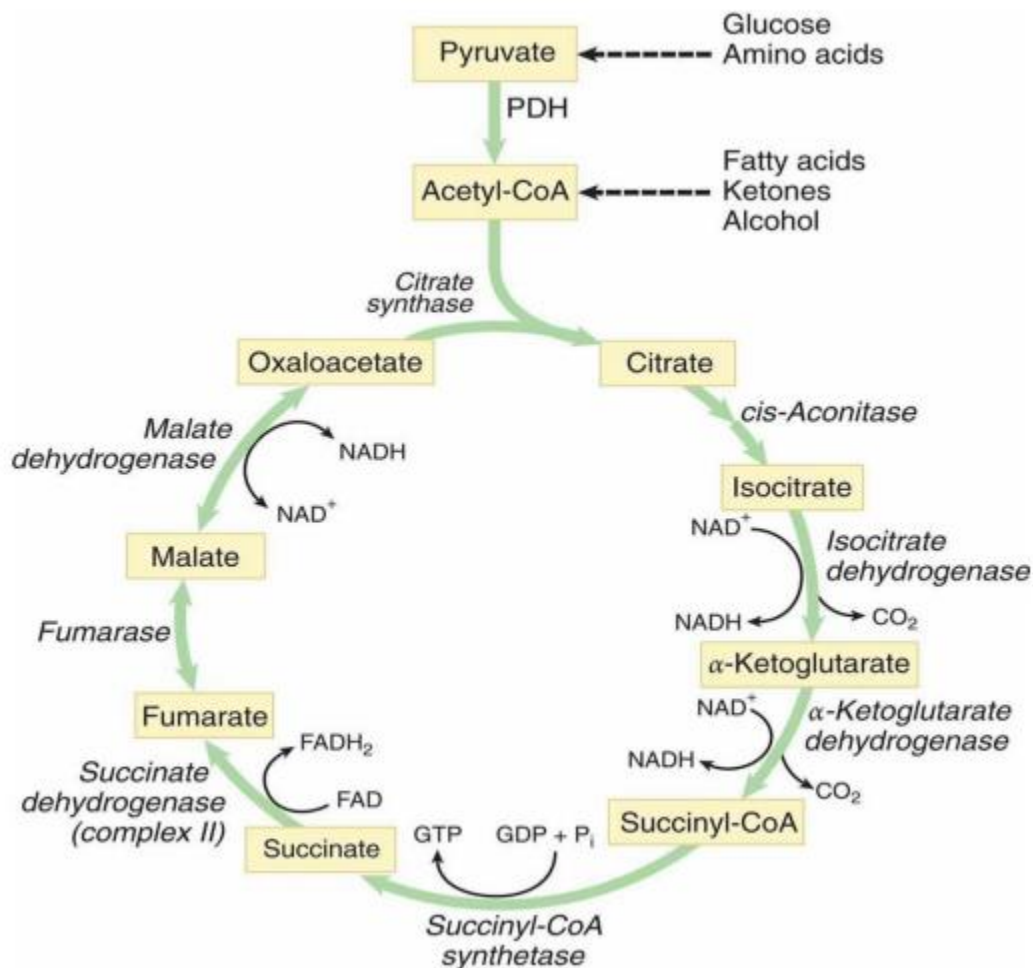
---

Net of ATP from anaerobic glycolysis

$$10 - 2 = 8 \text{ ATP}$$

Formation of lactate from pyruvate is the major steps in RBCs, lens and cornea, kidney, medulla, and leukocytes.

## Tricarboxylic Acid Cycle (TCA) OR Krebs Cycle:



### ATP generated in TCA cycle

Conversion of :

pyruvic acid to acetyl CoA

1 NADH

= 3ATP

|                                           |       |              |
|-------------------------------------------|-------|--------------|
| Isocitric acid to $\alpha$ -ketoglutarate | 1NADH | =3ATP        |
| $\alpha$ -ketoglutarate to succinyl CoA   | 1NADH | =3ATP        |
| Succinyl CoA to succinic acid             | 1GTP  | =1ATP        |
| Succinic acid to fumaric acid             | 1FAD  | =2 ATP       |
| Malic acid to oxaloacetic acid            | 1NADH | <u>=3ATP</u> |
|                                           | total | 15 ATP       |

Net ATP produced per glucose molecule =  $15 \times 2 = 30$  ATP

Total ATP per glucose (aerobic oxidation + anaerobic)  $30 + 8 = 38$  ATP

## Factors that maintain blood glucose level:

The blood sugar level is maintained by two factors:

**a. factors adding glucose to blood** (increase blood glucose level).

- from diet (intestinal absorption).
- glycogenolysis (liver).
- gluconeogenesis.
- lipolysis
- conversion of fructose and galactose into glucose

**b. factors remove glucose from blood** (decrease blood glucose level).

- glycogen formation in liver and muscle (glycogenesis).
- glycolysis in liver (oxidation of glucose).
- converting of glucose to fat in adipose tissue (lipogenesis).
- $\beta$ -oxidation (supply energy).
- synthesis of glycoprotein.
- excretion in urine (diabetes).

## Glucose level in the blood:

| BLOOD SUGAR LEVEL CHART |         |          |                      |
|-------------------------|---------|----------|----------------------|
|                         | FASTING | JUST ATE | 3 HOURS AFTER EATING |
| NORMAL                  | 80-100  | 170-200  | 120-140              |
| PRE-DIABETIC            | 101-125 | 190-230  | 140-160              |
| DIABETIC                | 126+    | 220-300  | 200+                 |

### Clinical significance of glucose:

**1. Hyperglycemia:** is an abnormally high blood glucose (blood sugar) level.

- Hyperglycemia is a hallmark sign of diabetes (both type 1 diabetes and type 2 diabetes) and prediabetes.
- Diabetes is the most common cause of hyperglycemia.

**2. Hypoglycemia:** is an abnormally low level of sugar (glucose) in the blood, hypoglycemia is not a disease in itself.

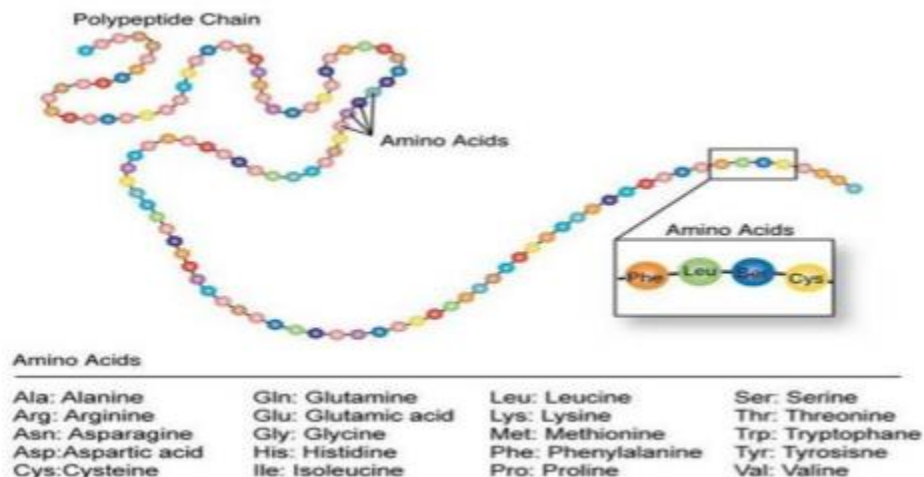
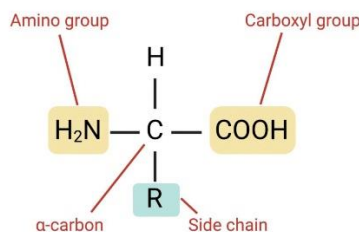
- The brain needs a continuous supply of glucose to function because it can neither store nor manufacture glucose.
- Hypoglycemia is not a disease, it is commonly linked with diabetes or caused by other conditions.

# Proteins

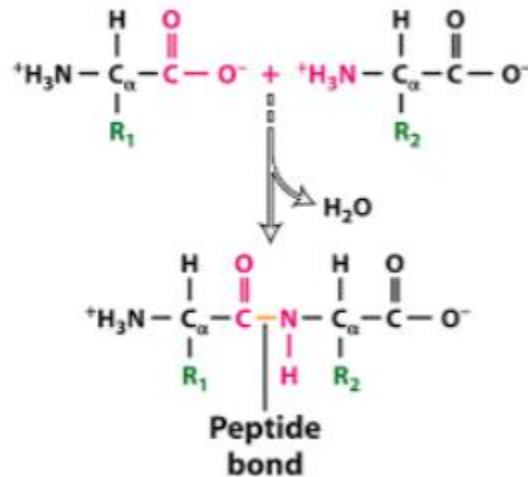
**Protein:** are complex nitrogenous polymers present in all living matter, contain C,H,O and nitrogen, also contain sulfur, phosphorous, zinc, copper and iron.

are made up of hundreds or thousands of smaller units called amino acids which are attached to one another in long chains, there are 20 different types of amino acids that can be combined to make a protein.

**Amino acids:** are organic acids containing an amino group (NH<sub>2</sub>) and a carboxylic acid (COOH) group. The side chain can be, aliphatic, aromatic, heterocyclic.



Peptide bond: is a covalent bond that formed between the carboxyl group of amino acid 1 and the amino group of amino acid 2.



**Blood Proteins:** includes

1. Albumin(A) 2. Globulin(G) 3. Fibrinogen

**Normal value:**

A = 3.5 - 5.0 mg/dl (53%)

G = 2.3 - 3.5 mg/dl (47%)

Normal ratio A/ G = 1.2 / 1

**Hyperproteinemia:** increase level of plasma protein from their normal value (increase both A and G). is shown in:

1. dehydration
2. multiple myeloma.
3. after a stressful exercise. It is a physiological process without any disease.

4. Amyloidosis: in this condition there is an abnormal production of protein in the bone marrow. The condition is called amyloidosis. Liver, spleen and kidney are generally affected.

5. Inflammation in the tissues as it occurs in bacterial infection, or in burns.

6. Kidney failure.

**hypoproteinemia:** decrease level of plasma protein from their normal value (decrease both A and G).

A/G also decrease. Is shown in:

1. malnutrition
2. malabsorption
3. hemorrhage
4. kidney disorder
5. liver disease.

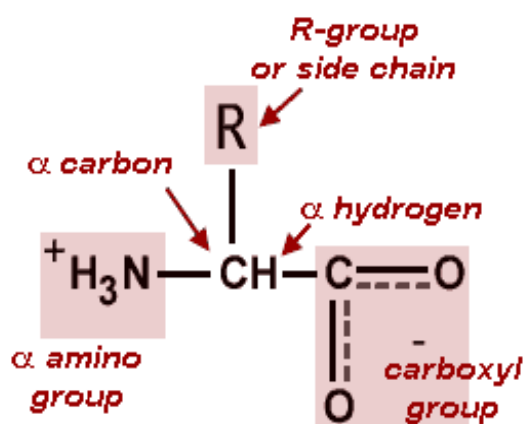
## AMINO ACID: STRUCTURE AND CLASSIFICATION.

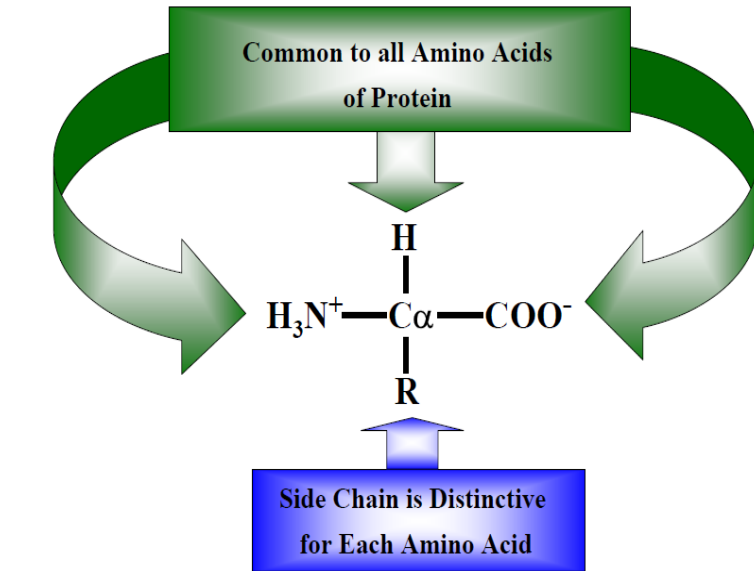
Amino Acids are the building units of proteins. There are about 300 amino acids occur in nature. Only 20 of them enter in proteins synthesis.

### Structure of amino acids:

Each amino acid has 4 different groups attached to  $\alpha$ - carbon (which is C-atom next to COOH). These 4 groups are: amino group, COOH group, Hydrogen atom and side Chain (R). At physiological pH (7.4), -COOH group is dissociated forming a negatively charged carboxylate ion ( $\text{COO}^-$ ) and amino group is protonated forming positively charged ion ( $\text{NH}_3^+$ ) forming Zwitter ion .

- Proline is an imino acid not amino acid .





### Classification of Amino Acids:

- I. Classification by R group
- II. Chemical Classification
- III. Nutritional Classification
- IV. Metabolic Classification

### Classification according to polarity of side chain (R):

**A- Polar amino acids:** in which R contains polar hydrophilic group so can forms hydrogen bond with H<sub>2</sub>O. In those amino acids, R may contain:

- 1- OH group : as in serine, threonine and tyrosine
- 2- - SH group : as in cysteine
- 3- amide group: as in glutamine and asparagine
- 4- NH<sub>2</sub> group or nitrogen act as a base (basic amino acids ): as lysine, arginine and histidine
- 5- COOH group (acidic amino acids): as aspartic and glutamic.

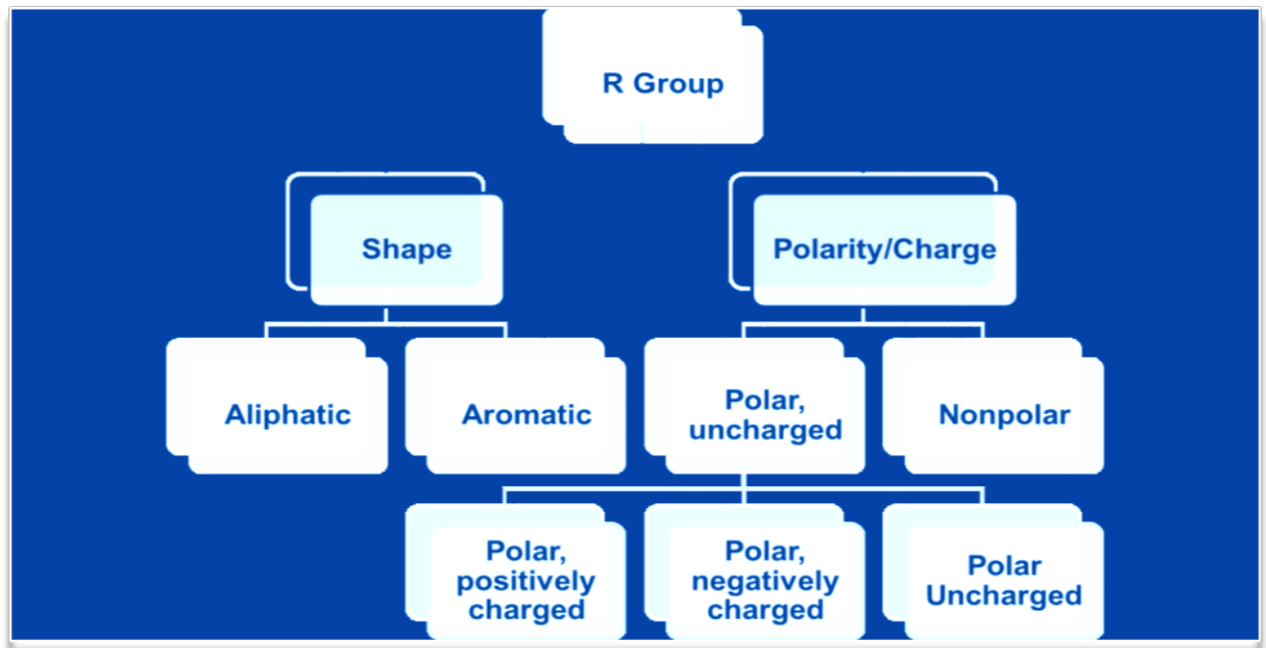


Figure: Classification by R group

**B- Non polar amino acids:** R is alkyl hydrophobic group which can't enter in hydrogen bond formation. 9 amino acids are non-polar (glycine, alanine, valine, leucine, isoleucine, phenyl alanine, tryptophan, proline and methionine).

The *twenty common amino acids* are often referred to using three-letter abbreviations. The structures, names, and abbreviations for the twenty common amino acids are shown below. Note that they are all  $\alpha$ -amino acids.

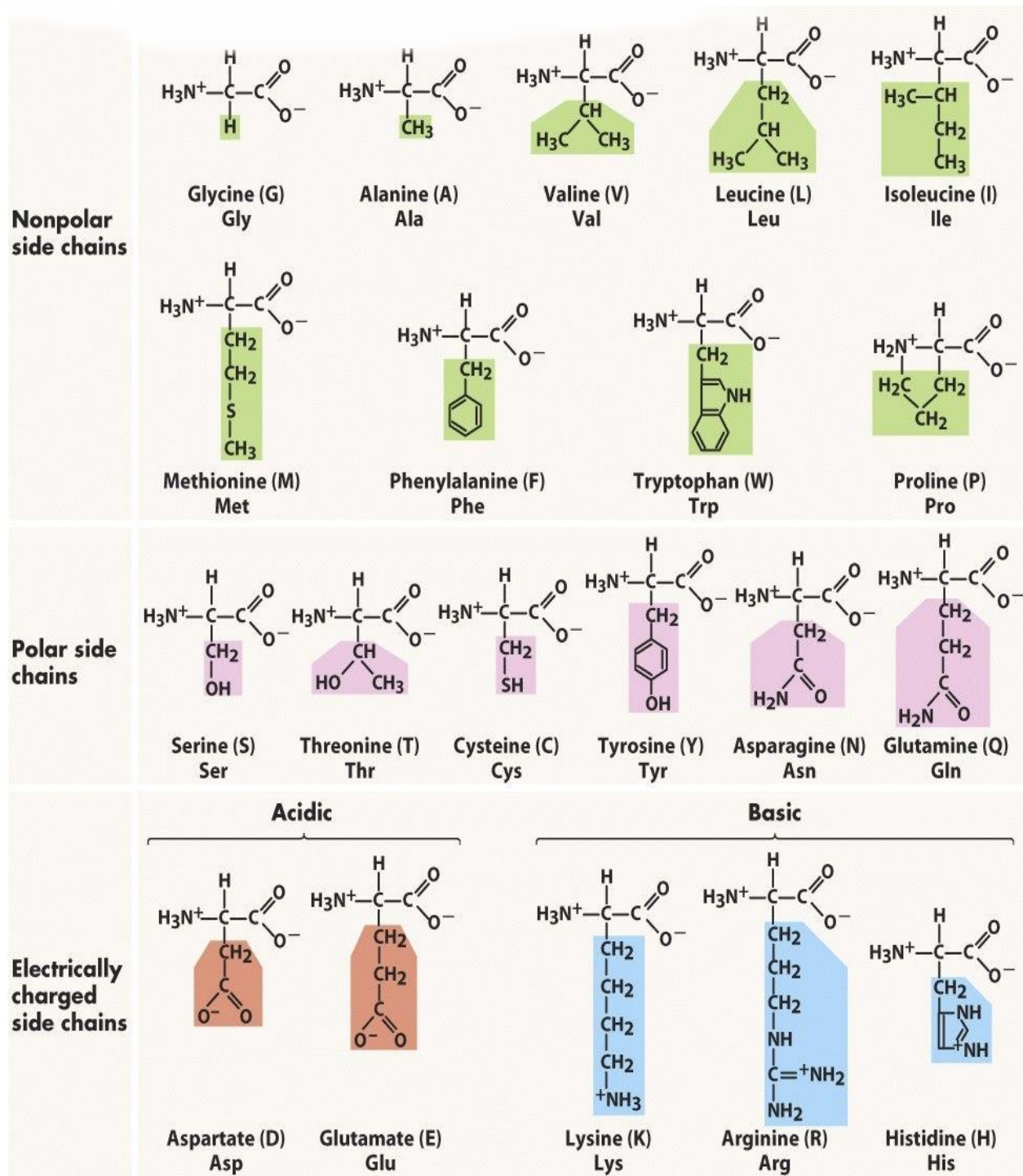


Figure 3-5 Biological Science, 2/e

© 2005 Pearson Prentice Hall, Inc.

Each amino acid, aside from its name, has a three letter abbreviation and a one letter code.

## Nomenclature

| Amino Acid     | 3 letter code | 1 letter code | Amino Acid    | 3 letter code | 1 letter code |
|----------------|---------------|---------------|---------------|---------------|---------------|
| Glycine        | <b>Gly</b>    | <b>G</b>      | Threonine     | <b>Thr</b>    | <b>T</b>      |
| Alanine        | <b>Ala</b>    | <b>A</b>      | Cysteine      | <b>Cys</b>    | <b>C</b>      |
| Valine         | <b>Val</b>    | <b>V</b>      | Tyrosine      | <b>Tyr</b>    | <b>Y</b>      |
| Leucine        | <b>Leu</b>    | <b>L</b>      | Asparagine    | <b>Asn</b>    | <b>N</b>      |
| Isoleucine     | <b>Ile</b>    | <b>I</b>      | Glutamine     | <b>Gln</b>    | <b>Q</b>      |
| Methionine     | <b>Met</b>    | <b>M</b>      | Aspartic Acid | <b>Asp</b>    | <b>D</b>      |
| Proline        | <b>Pro</b>    | <b>P</b>      | Glutamic Acid | <b>Glu</b>    | <b>E</b>      |
| Phenyl alanine | <b>Phe</b>    | <b>F</b>      | Lysine        | <b>Lys</b>    | <b>K</b>      |
| Tryptophan     | <b>Trp</b>    | <b>W</b>      | Arginine      | <b>Arg</b>    | <b>R</b>      |
| Serine         | <b>Ser</b>    | <b>S</b>      | Histidine     | <b>His</b>    | <b>H</b>      |

## Nutritional Classification

1- Essential Amino Acids 10 in number can't be synthesized in the body, essential to be taken in diet. Their deficiency affects growth, health and protein synthesis.

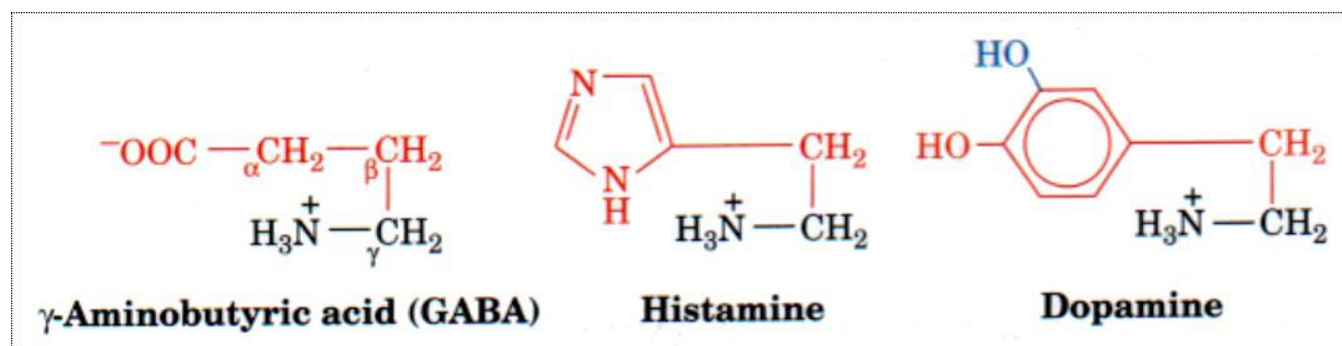
- 2- Semi-essential formed in the body but not in sufficient amount for body requirements especially in children. Arginine and histidine are semi-essential
- 3- Non-essential can be synthesized in the body.

### Non Standard Amino Acids

A nonstandard amino acid is an amino acid that occurs naturally in cells but do not participate in peptide synthesis. Many are metabolic intermediates *eg.* ornithine and citrulline are intermediates in urea biosynthesis

### Amino Acid Derivatives

Chemical derivatives of amino acids also have important biological functions, *eg.* Catecholamines (below) lack the carboxylate of amino acids



GABA & Dopamine are neurotransmitters. Histamine mediates parts of the immune response.

### Functions of Amino Acids

Apart from being the monomeric constituents of proteins and peptides, amino acids serve variety of functions.

- (a) Some amino acids are converted to carbohydrates and are called as **glucogenic amino acids**.

(b) Specific amino acids give rise to specialized products, e.g.

- **Tyrosine** forms hormones such as **thyroid hormones**, (T3, T4), **epinephrine** and **norepinephrine** and a pigment called **melanin**.
- **Tryptophan** can synthesize a vitamin called **niacin**.
- Glycine, arginine and methionine synthesis **creatine**.
- Glycine and cysteine help in **synthesize of Bile salts**.
- Glutamate, cysteine and glycine synthesis **glutathione**.
- **Histidine** changes to **histamine** on decarboxylation.
- **Serotonin** is formed from tryptophan.
- Glycine is used for the synthesis of **haem**.
- Pyrimidines and purines use several amino acids for their synthesis such as aspartate and glutamine for pyrimidines and glycine, aspartic acid, Glutamine and serine for purine synthesis.

(c) Some amino acids such as glycine and cysteine are used as detoxicants of specific substances.

(d) Methionine acts as “active” methionine (S-adenosylmethionine) and transfers methyl group to various substances by transmethylation.

(e) Cystine and methionine are sources of sulphur

# Lipids

compounds that are relatively insoluble in water, but freely soluble in nonpolar organic solvents like benzene, chloroform, ether, hot alcohol, acetone, etc.

## Functions of Lipids:

1. Storage form of energy (triglycerides)
2. Structural components of biomembranes (phospholipids and cholesterol)
3. Metabolic regulators (steroid hormones and prostaglandins)
4. Act as surfactants, detergents and emulsifying agents (amphipathic lipids)
5. Act as electric insulators in neurons
6. Provide insulation against changes in external temperature (subcutaneous fat)
7. Give shape and contour to the body
8. Protect internal organs by providing a cushioning effect (pads of fat)
9. Help in the absorption of fat-soluble vitamins (A, D, E, and K)
10. Improve the taste and palatability of food.

## **Clinical Applications:**

1. Excessive fat deposits cause obesity.
2. Abnormality in cholesterol and lipoprotein metabolism leads to atherosclerosis and cardiovascular diseases.
3. In diabetes mellitus, the metabolisms of fatty acids and lipoproteins are deranged, leading to ketosis.

## **Classification of lipids:**

Based on the chemical nature, lipids are classified:

**I. Simple Lipids:** They are esters of fatty acids with glycerol or other higher alcohols. They are subclassified as:

- a) Triacylglycerol or Triglycerides or neutral fat.
- b) Wax

**II. Compound Lipids:** They are fatty acids esterified with alcohol, but in addition, they contain other groups. Depending on these extra groups, they are subclassified as:

- A) Phospholipids, Containing Phosphoric Acid
- B) Non-phosphorylated Lipids such as Glycosphingolipids (carbohydrate)

**III. Derived Lipids:** They are compounds that are derived from lipids or precursors of lipids, e.g. fatty acids, steroids, prostaglandins, leukotrienes, terpenes, dolichols, etc. For details of cholesterol and steroids.

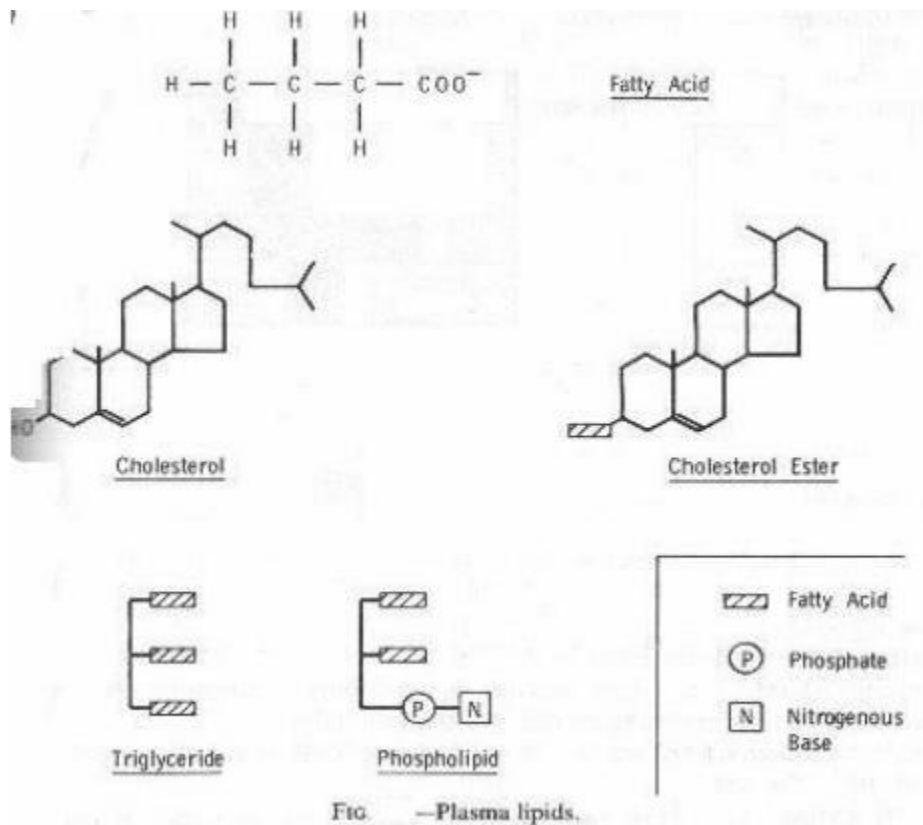
**IV. Lipids Complexed to Other Compounds** Proteolipids and lipoproteins.

### III. Derived lipids: Such as

**1. Fatty acids:** the body breaks down the fats into parts, called fatty acids, that can enter the blood.

It is straight-chain hydrocarbons with a terminal carboxyl group, such as ester and some phospholipids C18 to C22

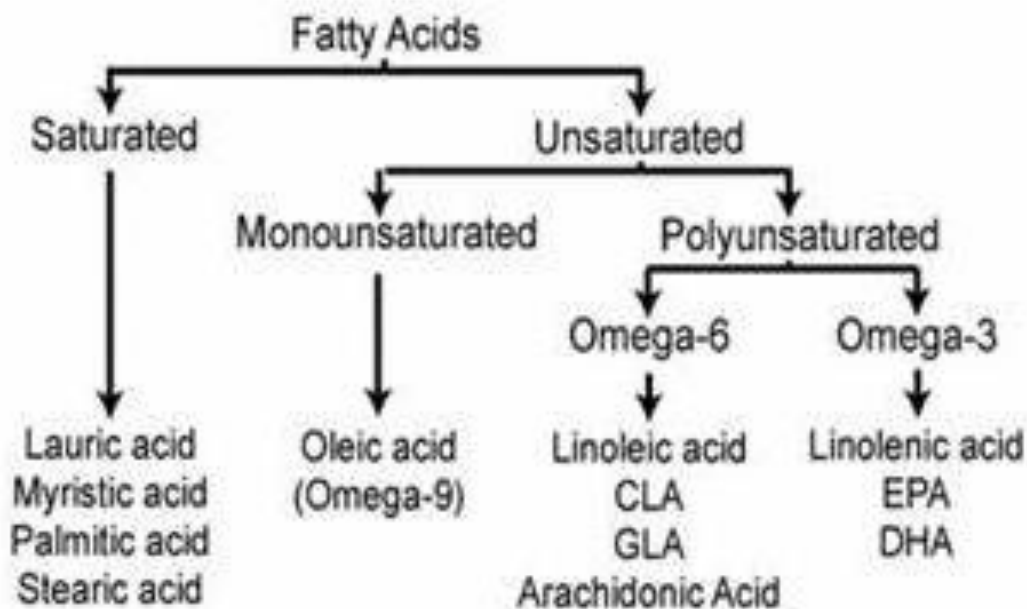
triglycerides C16 or C18.

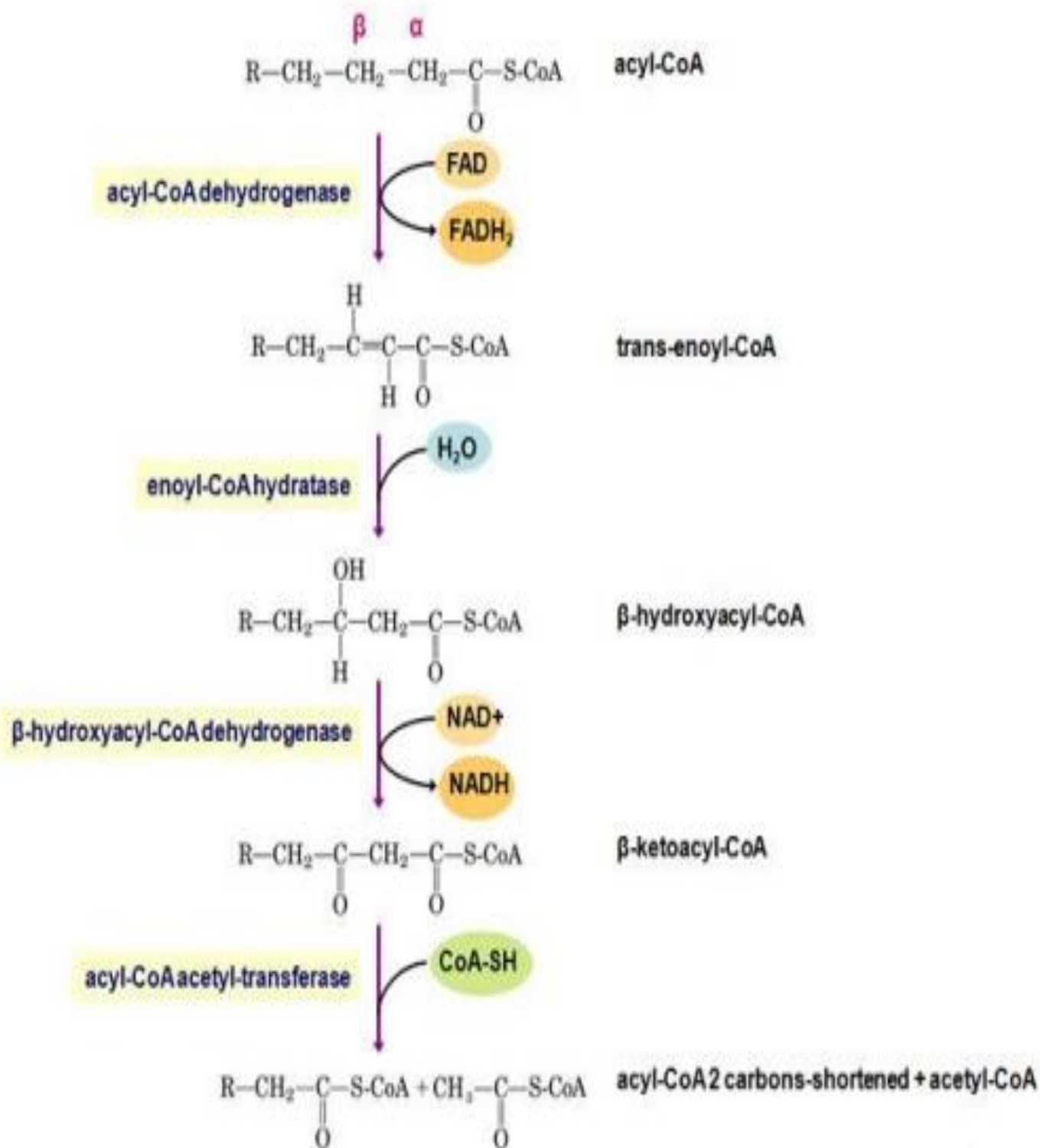


## $\beta$ oxidation:

The major pathway for the catabolism of saturated fatty acid is a mitochondrial pathway called  $\beta$ -oxidation. In this process, oxidation of fatty acids occurs at the  $\beta$ -carbon atom, and two carbon fragments are successively removed from the carboxyl end of fatty acetyl-CoA. This results in the elimination of two terminal carbon atoms as acetyl CoA, thereby leaving fatty acyl CoA that has two carbons less than the original fatty acid.

### BETA-OXIDATION OF FATTY ACID





## **Energetics of Beta-oxidation (ATP Yield)**

Palmitic acid (16 C) needs 7 cycles of beta-oxidation, which give rise to 8 molecules of acetyl CoA.

Every molecule of acetyl CoA when oxidized in the TCA cycle gives 12 molecules of ATP.

Each molecule of FADH<sub>2</sub> produces 2 molecules of ATP and each NADH generates 3 molecules of ATP when oxidized in the electron transport chain.

Hence, the energy yield from one molecule of palmitate may be calculated as:

$$8 \text{ acetyl CoA} \times 12 = 96 \text{ ATP}$$

$$7 \text{ FADH}_2 \times 2 = 14 \text{ ATP}$$

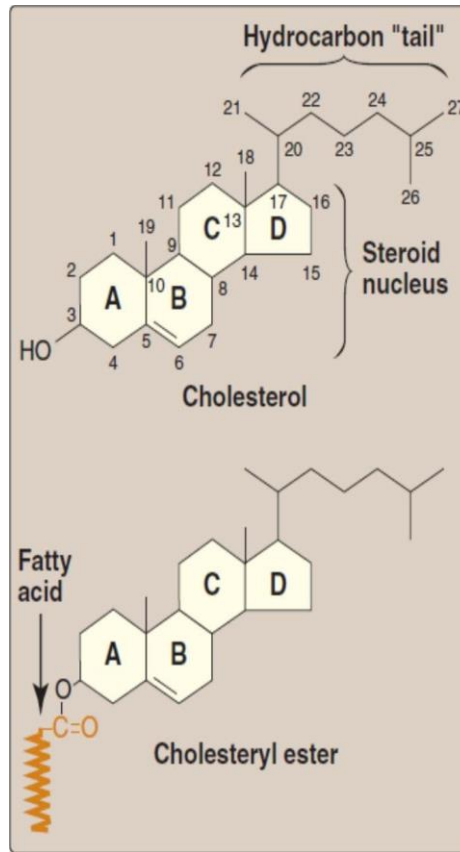
$$7 \text{ NADH} \times 3 = 21 \text{ ATP}$$

$$\text{Gross total} = 131 \text{ ATP}$$

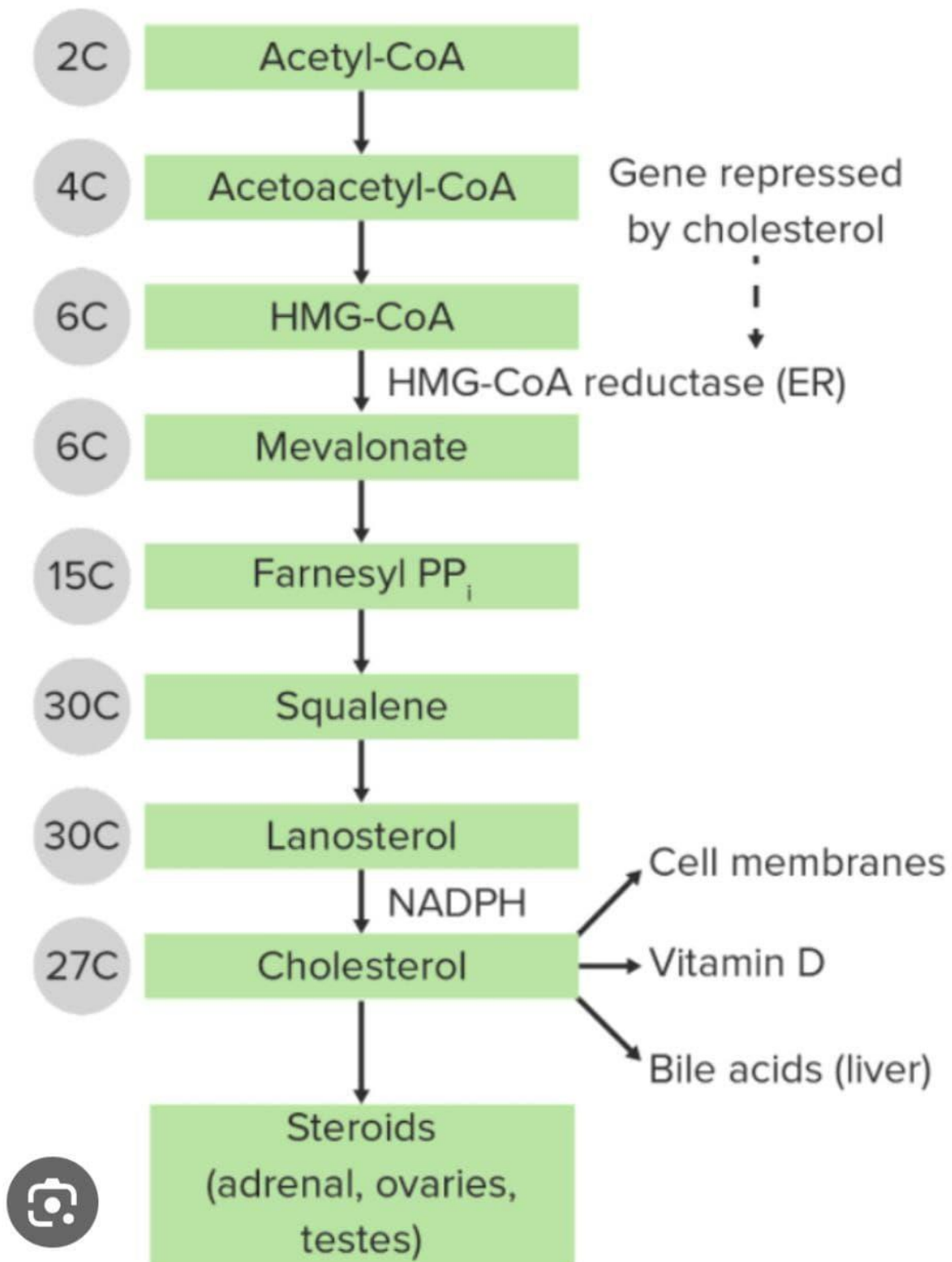
$$\text{Net yield} = 131 \text{ minus } 2 = 129 \text{ ATP}$$

(In the initial activation reaction, the equivalent of 2 high-energy bonds are utilized). The efficiency of beta-oxidation is about 40 percent.

**2. cholesterol:** is a fatty substance (lipid) that has a waxy appearance and is found in the cells of humans and animals, particularly the brain, kidneys, and liver.



**Cholesterol biosynthesis:** from 2 acetyl coA can be synthesized 3-hydroxy-3-methylglutaryl coenzyme A, then synthesis the mevalonate compound, and then cholesterol.



## **Functions of Cholesterol:**

### **1. Regulated of body fluids**

Cholesterol is esterified with essential fatty acids to form ester cholesterol, tend to lower the plasma bad cholesterol level.

### **2. Formation of Vit D3 , bile acid and steroid hormones (testosterone, estradiol, progesterone).**

### **3. Neutralize the hemolytic action of number of agents like snake venoms and bacterial toxins.**

## **Elimination of cholesterol:**

Cholesterol is eliminated from the liver by converted to: 1. bile acid and bile salts, secreted into the intestinal lumen(duodenum).

### **2. Serve as a component of lipoproteins sent to the peripheral tissues.**

Plasma Lipoproteins:

Lipoproteins: spherical macromolecular complex lipids and specific proteins apoproteins

or apolipoproteins transport cholesterol and triglycerides in the blood stream.

Types of lipoproteins:

### **1. Chylomicrons.**

### **2. Very low density lipoproteins (VLDL).**

3. Low density lipoproteins (LDL).
4. High density lipoproteins (HDL).

## Blood Urea {(NH<sub>2</sub>)<sub>2</sub>CO}

is considered one of the most commonly performed procedures in clinical biochemistry laboratory.

- Urea is the main non-protein nitrogen compound in the blood.
- - it is synthesized in the liver, produced as a by-product of the deamination reactions of amino acids after proteins break down and elimination in the urine is the major route for nitrogen excretion.
- it is filtered from the blood at the glomerulus, but passive tubular reabsorption occurs in low rate of urine flow.
- the normal value of blood urea is between 15 and 40 mg per 100 ml.

## Clinical Implications

- Increased BUN levels (azotemia) occur in the following conditions:
  - a. Impaired renal function caused by the following conditions:
    - (1) Congestive heart failure
    - (2) Salt and water depletion
    - (3) Shock
    - (4) Stress
    - (5) Acute MI

- b. Chronic renal disease such as glomerulonephritis and pyelonephritis
- c. Urinary tract obstruction
- d. Hemorrhage into GI tract
- e. Diabetes mellitus with ketoacidosis
- f. Excessive protein intake or protein catabolism as occurs in burns or cancer
- g. Anabolic steroid use

- **Decreased BUN levels** are associated with the following conditions, although Clinically of little significance.

- a. Liver failure (severe liver disease), such as that resulting from hepatitis, drugs, or poisoning
- b. Acromegaly
- c. Malnutrition, low-protein diets
- d. Impaired absorption (celiac disease)
- e. Nephrotic syndrome (occasional)
- f. Syndrome of inappropriate antidiuretic hormone (SIADH).

Azotemia (an abnormal increase in blood urea level)

## Serum Creatinine

Serum creatinine affected partly by:

1. **The amount of muscle tissue you have.** Men tend to have higher levels of blood creatinine because they have more skeletal muscle tissues than women.
2. **Protein in diet.** Vegetarians have been shown to have lower creatinine levels in blood.

This test diagnoses impaired renal function. It is a more specific and sensitive indicator of kidney disease than BUN, although in chronic renal disease, both BUN and creatinine are ordered to evaluate renal problems because the BUN-to-creatinine ratio provides more information.

## Reference Values Normal

- Adult men: 0.9–1.3 mg/dL
- Adult women: 0.6–1.1 mg/dL
- BUN-to-creatinine ratio: 10:1 to 20:1

blood urea nitrogen (BUN).

## Clinical Implications

1. **Increased blood creatinine levels** occur in the following conditions:

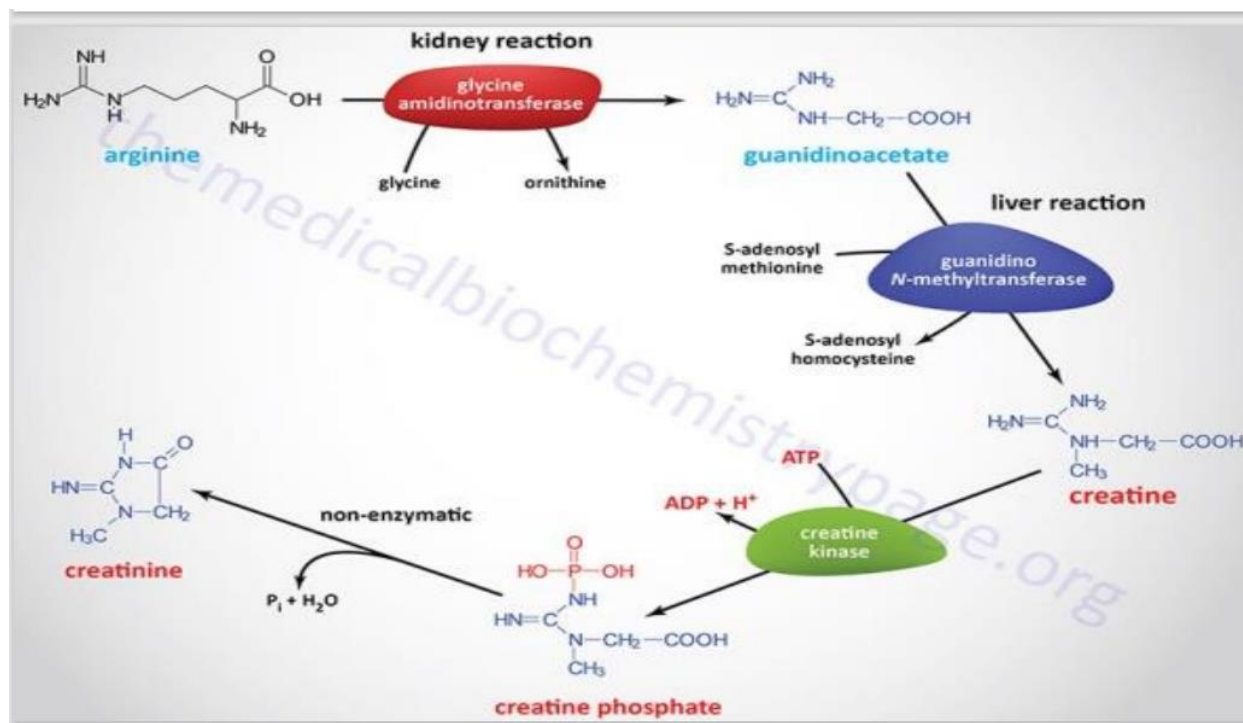
a. Impaired renal function

- b. Chronic nephritis
- c. Obstruction of urinary tract
- d. Muscle disease
  - (1) Gigantism
  - (2) Acromegaly
  - (3) Myasthenia gravis
  - (4) Muscular dystrophy
  - (5) Poliomyelitis
- e. Congestive heart failure
- f. Shock
- g. Dehydration
- h. Rhabdomyolysis (skeletal muscle tissue breakdown)
- i. Hyperthyroidism

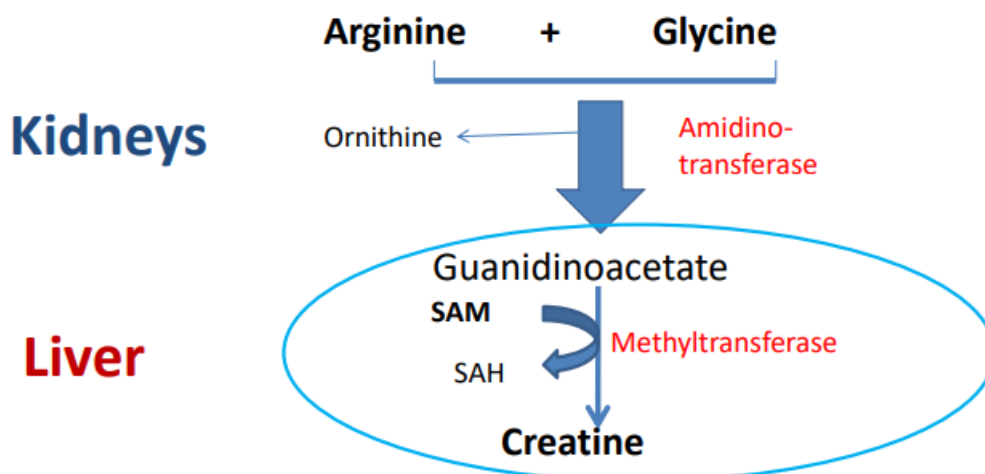
2. Decreased creatinine levels occur in the following conditions:

- a. Small stature
- b. Decreased muscle mass
- c. Advanced and severe liver disease
- d. Inadequate dietary protein
- e. Pregnancy (0.4–0.6 mg/dL is normal; 0.8 mg/dL is abnormal and should be noted)

## BIOSYNTHESIS OF CREATINE AND CREATININE

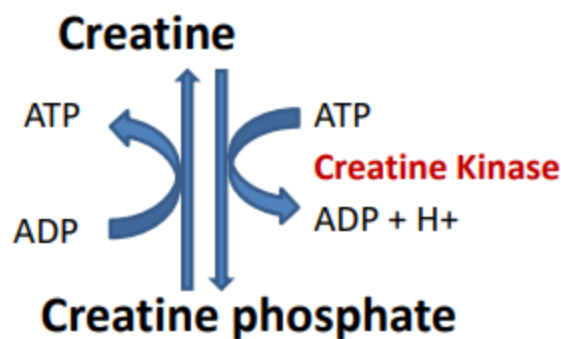


## Creatine Biosynthesis



## Distribution of body creatine

- From liver, transported to other tissues
- 98% are present in skeletal and heart muscles
- In Muscle, gets converted to the high energy source creatine phosphate (phosphocreatine)

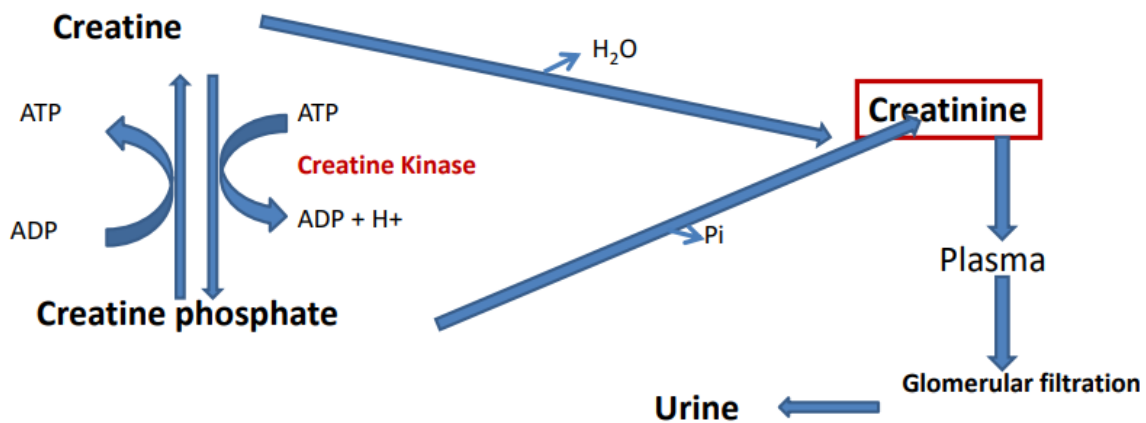


## Creatine Phosphate:

- Is a high-energy phosphate compound
- Acts as a storage form of energy in the muscle
- Provides a small but, ready source of energy during first few minutes of intense muscular contraction The amount of creatine phosphate in the body is proportional to the muscle mass.
- Creatine and creatine phosphate spontaneously form creatinine as an end product
- Creatinine is excreted in the urine

- Serum creatinine is a sensitive indicator of kidney disease (Kidney function test)
- Serum creatinine increases with the impairment of kidney function

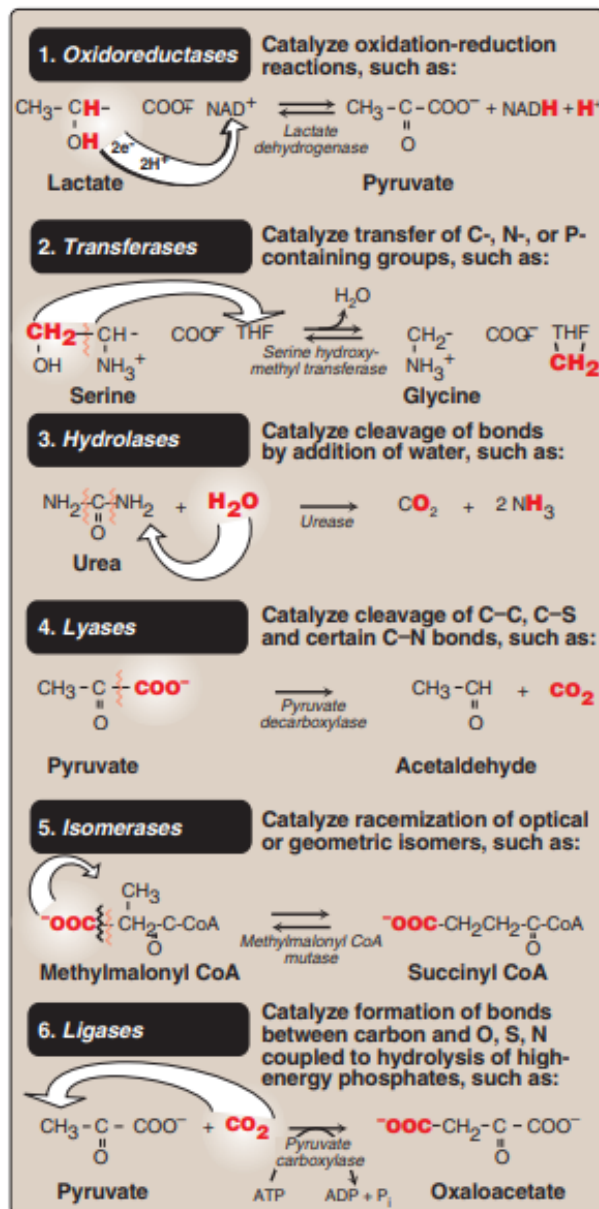
## Creatine Degradation



# Enzymes

all reactions in the body are mediated by enzymes, which are protein catalysts that increase the rate of reactions without being changed in the overall process.

## Classification of enzymes



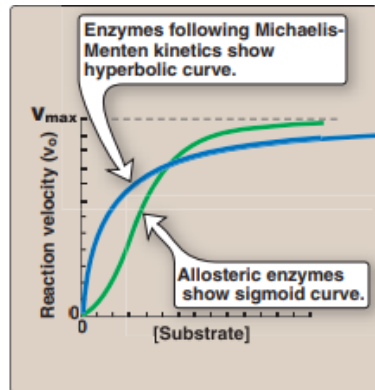
# Factors affecting the work of enzymes

## 1. Substrate concentration:

. **Maximal velocity:** The rate or velocity of a reaction ( $v$ ) is the number of substrate molecules converted to product per unit time; velocity is usually expressed as  $\mu\text{mol}$  of product formed per minute. The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity ( $V_{\text{max}}$ ) is reached. The leveling off of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme molecules present.

. **Hyperbolic shape of the enzyme kinetics curve:** Most enzymes show Michaelis-Menten kinetics, in which the plot of initial reaction velocity ( $v_o$ ) against substrate concentration ( $[S]$ ), is hyperbolic (similar in shape to that of the oxygen-dissociation curve of myoglobin).

In contrast, allosteric enzymes do not follow Michaelis-Menton kinetics and show a sigmoidal curve that is similar in shape to the oxygen dissociation curve of hemoglobin.

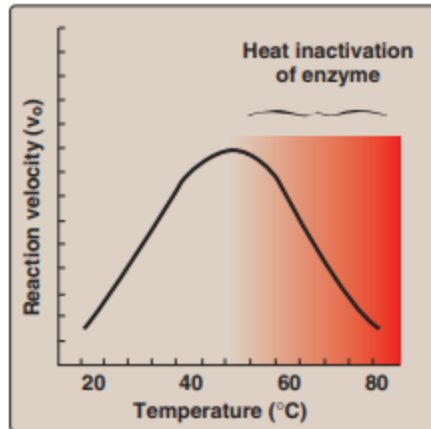


Effect of substrate concentration on reaction velocity.

## 2. Temperature:

- . **Increase of velocity with temperature:** The reaction velocity increases with temperature until a peak velocity is reached. This increase is the result of the increased number of molecules having sufficient energy to pass over the energy barrier and form the products of the reaction.
- . **Decrease of velocity with higher temperature:** Further elevation of the temperature results in a decrease in reaction velocity as a result of temperature-induced denaturation of the enzyme.

**Note:** The optimum temperature for most human enzymes is between 35 and 40°C. Human enzymes start to denature at temperatures above 40°C, but thermophilic bacteria found in the hot springs have optimum temperatures of 70°C.



Effect of temperature on an enzyme-catalyzed re

### 3. pH:

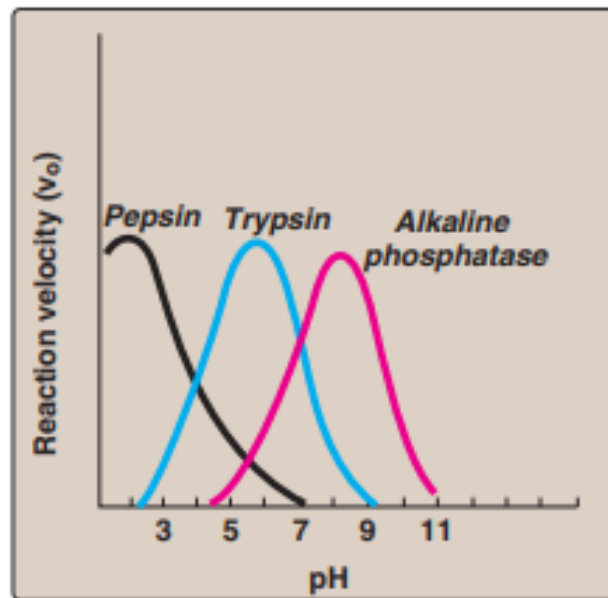
**. Effect of pH on the ionization of the active site:** The concentration of  $H^+$  affects reaction velocity in several ways. First, the catalytic process usually requires that the enzyme and substrate have specific chemical groups in either an ionized or un-ionized state in order to interact. For example, catalytic activity may require that an amino group of the enzyme be in the protonated form ( $-NH_3^+$ ). At alkaline pH, this group is deprotonated, and the rate of the reaction, therefore, declines.

**. Effect of pH on enzyme denaturation:** Extremes of pH can also lead to denaturation of the enzyme, because the structure of the catalytically active protein molecule depends on the ionic character of the amino acid side chains.

**. The pH optimum varies for different enzymes:** The pH at which maximal enzyme activity is achieved is different for

different enzymes, and often reflects the  $[H^+]$  at which the enzyme functions in the body.

For example, pepsin, a digestive enzyme in the stomach, is maximally active at pH 2, whereas other enzymes, designed to work at neutral pH, are denatured by such an acidic environment.



Effect of pH on enzyme-catalyzed reactions.

## Diagnostic value of plasma enzyme

When a tissue is injured some cells of that tissue are destroyed and their contents including enzymes are released into the bloodstream. The increasing of enzyme in blood will indicate the disease:-

| <b>Enzymes</b>                                    | <b>Increase in disease</b>                                           |
|---------------------------------------------------|----------------------------------------------------------------------|
| 1. Aspartate transaminase (GOT) (previously)(AST) | myocardial infarction                                                |
| 2. Alanine transaminase (GPT)(Previously) (ALT)   | liver disease especially with liver cell damage                      |
| 3. Amylase                                        | Acute pancreatitis                                                   |
| 4. Acid phosphatase (ACP)                         | Prostatic carcinoma                                                  |
| 5. Alkaline phosphatase (ALP)                     | Liver disease, bone disease (rickets)                                |
| 6. Lactate Dehydrogenase (LDH)                    | myocardial infarction, liver disease, Blood disease                  |
| 7. Creatine Kinase (CK)                           | myocardial infarction, skeletal muscle disease<br>(Muscle dystrophy) |
| 8. Glutamyl transferase (GT)                      | liver disease, biliary obstruction                                   |

## Non-protein compounds of nitrogen

Blood serum contains compounds of nitrogen other than proteins and peptides. **Urea, creatinine, uric acid, ammonia and amino acids** are the most important of them and have implications in clinical biochemistry. These nitrogen compounds remain dissolved even after precipitating the serum proteins with deproteinizing agents.

### uric acid

- It is the end product of metabolism of purines which are present in nucleic acids of nucleoproteins.
- It is formed endogenously from nucleoproteins and exogenously from metabolism of purines taken in food.
- The enzyme xanthine oxidase makes uric acid from xanthine and hypoxanthine.
- It is formed in liver and filtered by kidneys to be excreted in urine with little amount passes in stool.

High uric acid levels in blood occur when its formation is too much or its excretion is low.

### Uric acid test done to:

- Help diagnose gout
- Check for kidney stone

- Check if medicines that decrease uric acid level are working -
- Check for uric acid level in patients on chemotherapy or radiation.

### The normal value:

- Child : 2 - 5.5 mg/dl
- Men : 3.5 - 7.2 mg/dl
- Women : 2.6 - 6 mg/dl

**Hyperuricemia:** It means high level of uric acid in the blood.

### Causes :

- ♣ Hereditary reasons
- ♣ High intake of dietary purines
- ♣ Reduced excretion by the kidneys
- ♣ Fasting or rapid weight loss can temporarily elevate uric acid levels.
- ♣ Certain drugs such as thiazide diuretics.

### Examples of purines rich foods:

- Meat
- Meat organs (liver, kidney, brain)
- Poultry

- Sea food (scallops, sardines)
- Beans (moderate)
- Green peas (moderate)
- Spinach (moderate)

high uric acid level in blood lead to:

-It will form solid crystals within joints which is painful and called gout if remain untreated it will lead to hard lumpy deposits called tophi

-It can also cause kidney stones or kidney failure.



**Hypouricemia:** It means low level of uric acid in the blood, It is seldom.

**Causes:**

- ♣ Some liver diseases
- ♣ Some kidney diseases
- ♣ Diet low in proteins
- ♣ Some toxic compounds