MINISTRY OF HEALTH OF REPUBLIC OF BELARUS
VITEBSK STATE MEDICAL UNIVERSITY

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# BIOCHEMISTRY

## In schemes and tables

The manual
For students of higher medical educational institution

Vitebsk, 2014

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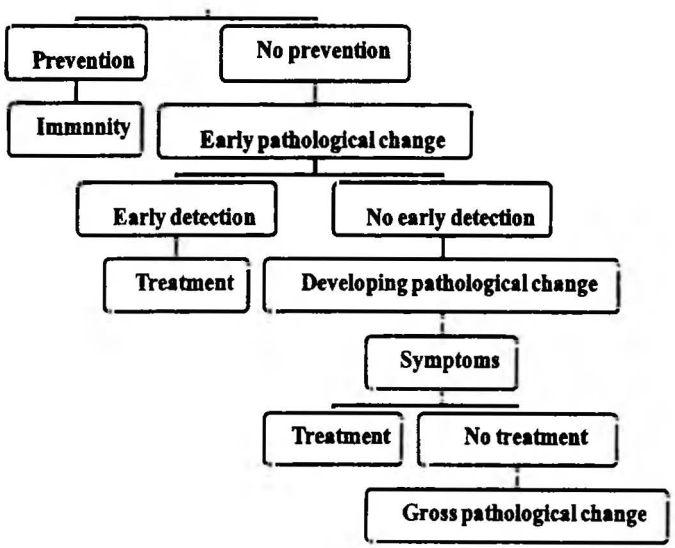
# Principles and sources of laboratory information in the diagnostics of diseases

1

## Stages in early detection of disease




2

## Nonpathological factors of variability of laboratory researches results

| Considered factors of a biological variation | |
|---|---|
| **Physiological factors** | **Factors of environment** |
| Ethnic group | Geographical factors |
| Gender (pregnancy, childbirth, menstrual cycle. menopause) | Temperature, humidity |
| Age | Season |
| Constitution | Time of day |
| Physical activity | Diet (water composition, soil composition) |
| Nutrition | Social and household environment |
| **Results of laboratory research** | |
| Ethanol, caffeine, nicotine, contraceptives, sedative substance, psychotropic drugs, professional and household toxic substance, diagnostic and medical factors | Food intake time, physical activity, the body position, previous rest, stress during a test taking, stasis of blood during a test taking, preservatives, ware, temperature, time keeping |
| Toxic and therapeutic factors | Conditions of test taking |

3

**Some uses of biochemical investigations and laboratory tests in relation to diseases**

| Use | Example |
|---|---|
| 1. To reveal the fundamental causes and mechanisms of diseases | Demonstration of the nature of the genetic defects in cystic fibrosis |
| 2. To suggest rational treatments of diseases based on item 1 above | A diet low in phenylalanine for treatment of phenylketonuria |
| 3. To assist in the diagnosis of specific diseases | Use of the plasma levels of troponin I or T in the diagnosis of myocardial infarction |
| 4. To act as screening tests for the early diagnosis of certain diseases | Use of measurement of blood thyroxine or thyroid-stimulating hormone (TSH) in the neonatal diagnosis of congenital hypothyroidism |
| 5. To assist in monitoring the progress (ie, recovery, worsening, remission, or relapse) of certain diseases | Use of the plasma enzyme alanine aminotransferase (ALT) in monitoring the progress of infectious hepatitis |
| 6. To assist in assessing the response of diseases to therapy | Use of measurement of blood carcinoembryonic antigen (CEA) in certain patients who have been treated for cancer of the colon |

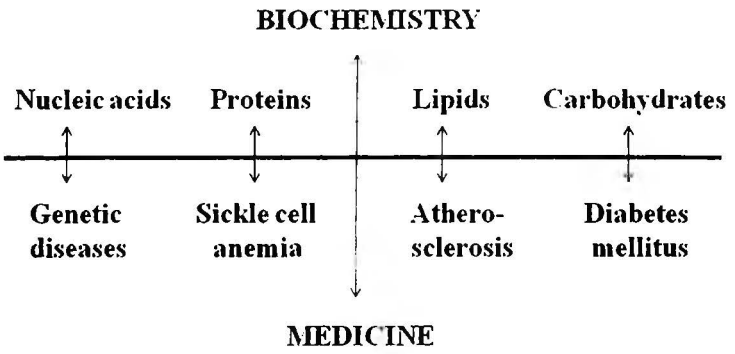
4

## Why analytical results vary

1. Inter-individual variation.
    a. Age.
    b. Sex.
    c. Race.
    d. Genetics.
    e. Long term health status.
2. Pre-analytical variation.
    a. Transport.
    b. Exposure to UV light.
    c. Standing time before separation of cells.
    d. Centrifugation time.
    e. Storage conditions.
3. Intra-individual variation.
    a. Diet.
    b. Exercise.
    c. Drugs.
    d. Sleep pattern.
    e. Posture.
    f. Time of venipucture.
    g. Length of time tourniquet is applied.
4. Analytical variation.
    a. Random errors.
    b. Systematic errors.
5. Post-analytical.
    a. Transcriptions errors.
    b. Results reported to wrong patient.

5

## Medicine and Biochemistry

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## Steps in the investigation of a patient

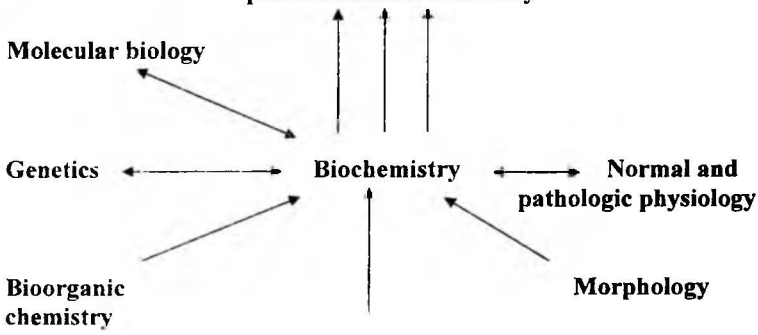
1. Patient history.
2. Physical examination.
3. Laboratory tests.
4. Imaging techniques.
5. Diagnosis.
6. Therapy.
7. Evaluation.

7

# Introduction to Biochemistry

8

## Biochemistry relations with other sciences

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## Objects of biochemical researches

1. Organism.
2. Isolated perfused organs.
3. Slices of organs.
4. Homogenates of organs.
5. Extracts from organs.
6. Cells.
7. Yeast, bacteria.
8. Subcellular components:
   a. membranes;
   b. organoids;
   c. cytosol.
9. Enzymes.
10. Biological fluid.

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## Content of biochemistry subject

2. Composition, structure и chemical description of substances in living organism.
3. Bioenergetics.
4. Metabolism: catabolism- anabolism.
5. Regulation of metabolism.
6. Molecular basic of heredity and morphogenesis.
7. Molecular mechanisms of specific physiological processes.
8. Features of biochemical processes in organs and tissues.

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## Methods

1. Analytical procedures.
2. Isotopic immunoassay.
3. Colorimetry.
4. Spectrometry.
5. Chromatography.
6. Electrophoresis.
7. Centrifugation:
   a) differential velocity centrifugation,
   b) ultracentrifugation,
   c) equilibrium density-gradient centrifugation.
8. Analysis of enzymatic activity.
9. Photometry.
10. Fluorometry.
11. Nephelometry.

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## Aim of biochemistry

Describe and explain all the chemical processes in living cells in terms of molecular, nature of the chemical constituents of living matter, their transformations in biological systems and the energy changes associated with these transformations. Such studies effort to understand how life originated.

## Aim of biochemistry is decided by:

1. Descriptive biochemistry that concerns with the qualitative and quantitative characterization of the various cell components and
2. Dynamic biochemistry that deals with the elucidation of the nature and the mechanism of the reactions involving these cell components.

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## Major causes of disease

1. Physical agents: mechanical trauma, temperature extremes, radiation, electric shock.
2. Chemical agents: toxic compounds, drugs.
3. Biologic agents: viruses, bacteria, fungi, parasites.
4. Genetic disease: congenital, molecular.
5. Oxygen lack: loss of blood, decreased oxygen-carrying capacity of blood, mitochondrial poisoning.
6. Immunologic reactions: anaphylaxis, autoimmune disorders, hypersensitivity.
7. Nutritional imbalances: deficiencies, excesses.
8. Endocrine imbalances: hormonal deficiencies/excesses.

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## Biochemistry makes possible to:

1. Determine the etiology, diagnosis, and prognosis of disease.
2. Explain the causes of disease and to design appropriate therapy.
3. Establish the diagnosis and monitoring of therapy results.

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# Structure and functions of proteins

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## Classification of proteins based on the functions

1. Enzymes (ribonuclease, trypsin, DNA- and RNA-polymerases)
2. Protective proteins (immunoglobulin, complement, interferon)
3. Contractile proteins (actin, myosin)
4. Structural proteins (collagen, silk fibroin, keratin)
5. Reserve proteins (casein, albumin)
6. Antibiotics
7. Toxins (botulinum toxin, diphtherial toxins)
8. Transport proteins (hemoglobin, myoglobin, cytochrome c, membrane ATP-ase)
9. Regulatory proteins (histones, initiation factors)
10. Receptor proteins (rhodopsin, choline receptor)
11. Hormones (insulin, growth hormone)

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## Biological functions of peptides:

1. Hormones (oxytocin, andiuretic hormone, bradykinin, gastrin).
2. Neuropeptides of brain (enkephalin, endorphin).
3. Alkaloid (ergotamine).
4. Antibiotic (gramicidins A, B, C).
5. Toxins and antitoxins.
6. Regulatory peptides (releasing factors, carnosine, anserine).

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## Molecular mass of proteins

| | | |
|---|---|---|
| 1. | Insulin | 6 000 |
| 2. | Growth hormone | 21 000 |
| 3. | Pepsin | 35 000 |
| 4. | Albumin (serum) | 65 000 |
| 5. | Hemoglobin | 68 000 |
| 6. | γ- Globulin (serum) | 160 000 |
| 7. | Fibrinogen | 330 000 |
| 8. | Thyroglobulin | 660 000 |
| 9. | Pyruvate dehydrogenase | 4 000 000 |

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## Degree of protein α-spiralization

| | | |
|---|---|---|
| 1. | Hemoglobin | 80% |
| 2. | Insulin | 46-60% |
| 3. | Albumin | 30-45% |
| 4. | Pepsin | 20-30% |
| 5. | Casein | 10% |

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## Functions of organs and protein metabolism

| *Organs* | *Function in protein metabolism* | *Malfunction* | *Change in protein metabolism* |
|---|---|---|---|
| Gastrointestinal tract | Splitting of exogenous proteins | Insufficient synthesis of digestive enzymes | Protein starvation |
| | Absorption of amino acids | Impaired absorption | Protein deficiency |
| | | Impairment of intestinal wall permeability | Deficiency due to loss of protein |
| Endocrine glands | Regulation of synthesis and degradation | Hyper- and hypofunction | Increased anabolism or catabolism |
| Liver | Synthesis of albumins and some of globulins | Hepatocyte dysfunction | Hypoalbuminemia. Decreased formation of blood clotting factors |
| | Detoxication of products of nitrogenous metabolism | | Accumulation of nitrogenous products, decreased urea formation |
| | Metabolism of amino acids | Defects of enzymes | Metabolic disorders |
| | Synthesis of fibrinogen | Reaction on inflammation | Hyperfibrinogenemia |
| | | Hepatoma | Presence of α-fetoprotein |
| Kidneys | Regulation of protein excretion | Nephrosis | Loss of albumin |
| | Excretion of products of nitrous metabolism | Impaired nitrogen excretory function of glomerules | Accumulation of nitrogenous products in blood |
| | | Impaired reabsorption in tubules | Loss of amino acids |
| Connective tissue | Synthesis of γ-globulins | Immune reactions | Hypergammaglobulinemia |
| Immune system | | Immunodeficiency syndrome | Hypogammaglobulinemia |
| | | Reaction on decreased formation of albumin | Hypergammaglobulinemia |
| | | Myeloma | Para proteinemia |

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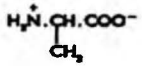
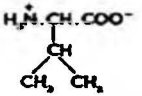
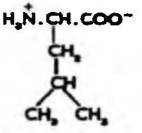
## The structure of the twenty amino acids found in proteins



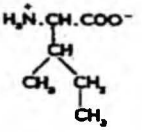
Alanine (Ala)



Valine (Val)

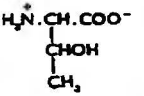


Leucine (Leu)

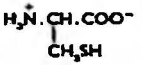


Isoleucine (Ile)

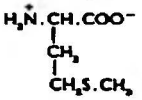
Serine (Ser)



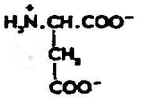
Threonine (Thr)



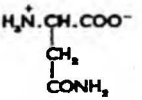
Cysteine (Cys)



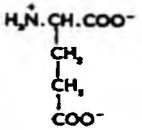
Methionine (Met)



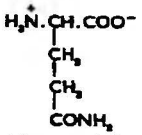
Aspartic acid (Asp)



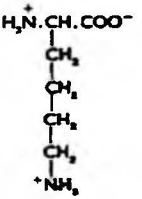
Asparagine (Asn)



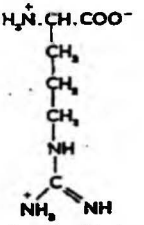
Glutamic acid (Glu)



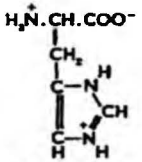
Glutamine (Gln)



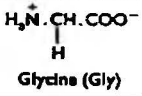
Lysine (Lys)



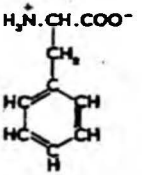
Arginine (Arg)



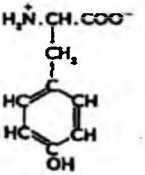
Histidine (His)



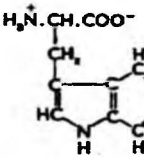
Glycine (Gly)



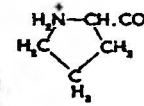
Phenylalanine (Phe)



Tyrosine (Tyr)

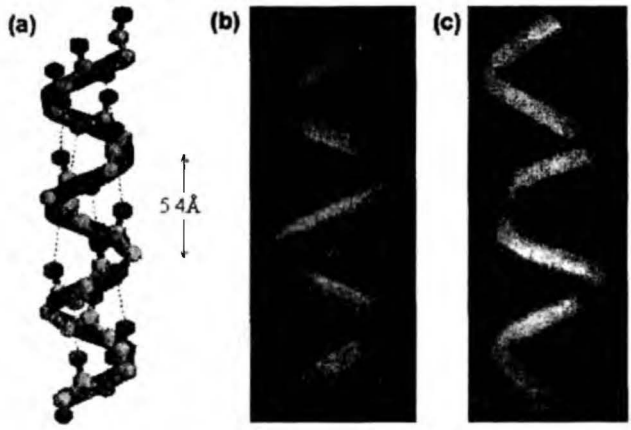


Tryptophan (Trp)

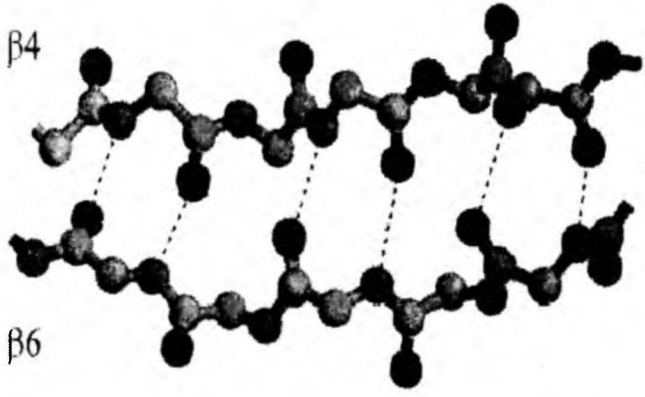


Proline (Pro)

22

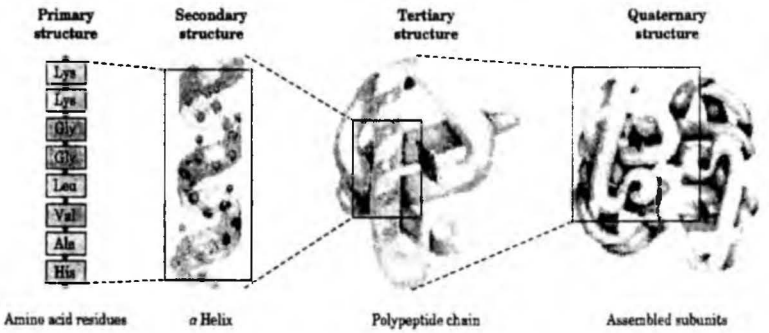



**Secondary structure – α helix** 23




**Secondary structure – β-pleated sheet** 24

## The structural hierarchy in proteins

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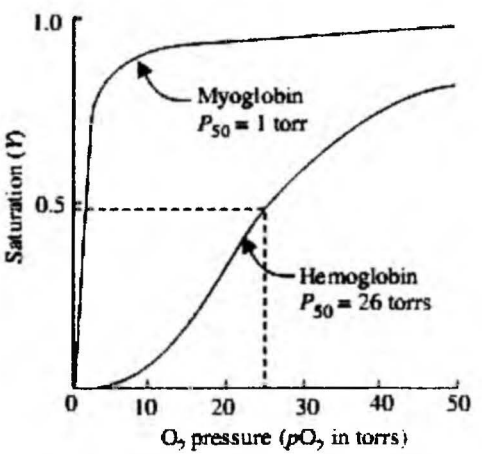
## Oxygen dissociation curves of myoglobin and hemoglobin in whole blood

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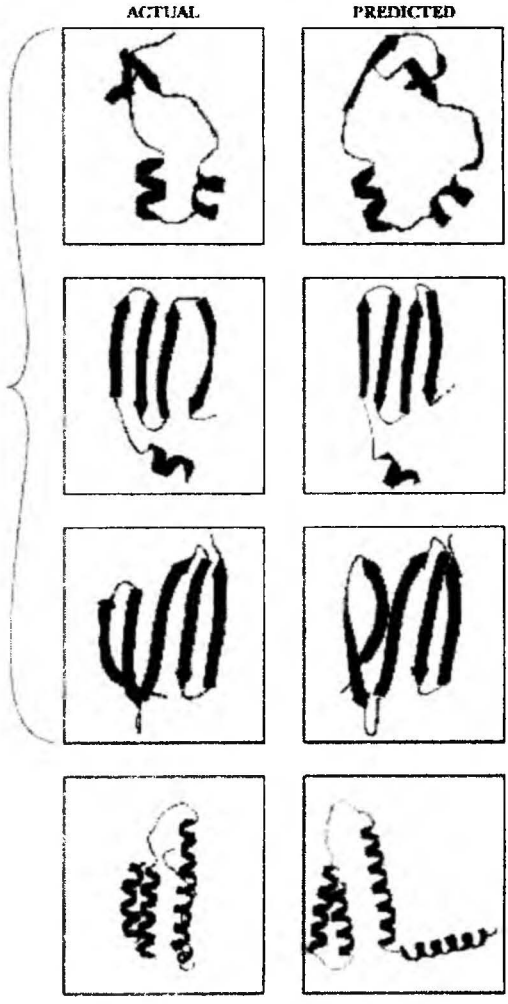
## Modeling protein folding with Linus (George Rose)




Actual and predicted structures of three domains of intestinal fatty acid binding protein

Actual and predicted structures of an α-helical domain of cytochrome $b_{562}$

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## Structural and functional advantages driving quaternary association

1. Stability: reduction of surface to volume ratio.
2. Genetic economy and efficiency.
3. Bringing catalytic sites together.
4. Cooperativity.

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## Chaperones

- Most proteins fold spontaneously into their native conformation.
- In the unfolded state, the apolar regions of the peptide chain tend to aggregate with other proteins or with each other to form insoluble products. In addition, unfolded proteins are susceptible to proteinases. To protect partly folded proteins, there are proteins called chaperones.
- Chaperones are formed increasingly during temperature stress and are therefore also known as heat-shock proteins (hsp60 and hsp70).
- Chaperones of the hsp70 type are common, as are type hsp60 chaperonins.
- While small proteins can often reach their native conformation without any help (1), larger molecules require hsp70 proteins for protection against aggregation (2) which bind as monomers and can dissociate again, (3). By contrast, type hsp60 chaperonins form large, barrel-shaped complexes with 14 subunits in which proteins can fold independently while shielded from their environment (4).

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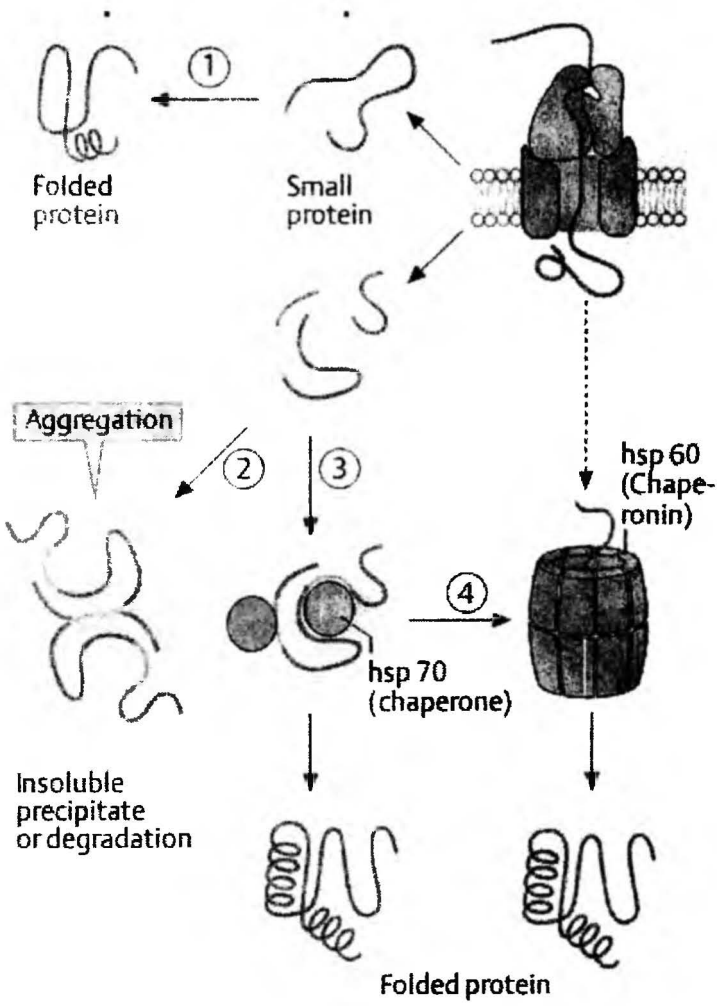
## Protein maturation by chaperones and chaperonins

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## Proteinpathies

| Protein (Enzyme) | Disease | Disease characteristics |
|---|---|---|
| **Nonenzymatic proteinpathies** | | |
| Hemoglobin | Sickle cell anemia | Mutation results in the b chain of HbA contains Val rather than Glu at position 6. RBC that contains large complexes of HbS molecules can assume a sickle shape. These cells undergo hemolysis, and an anemia results |
| **Enzymatic proteinpathies** | | |
| Deficiency of enzymes of glycogen metabolism | Glycogenosises | See p 125 |
| Deficiency of enzymes of fructose metabolism | Defects in fructose metabolism | See p 126 |
| Galactose-1-phosphate uridyl transferase | Galactosemia | See p 127 |
| Deficiency of enzymes of tyrosine (phenylalanine) metabolism | Disorders of tyrosine (phenylalanine) metabolism | See p 176 |

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## Polymorphism of proteins

- Many proteins exist as **polymorphisms** (genetically determined variations in primary structure due to mutations in the genetic code).
- Within the same individual, the primary structure of these proteins varies with the stage of development and is present in fetal and adult isoforms, such as *fetal* and *adult hemoglobin*. The primary structure of some proteins, such as *creatine kinase*, can also vary between tissues (tissue-specific isozymes) or between intracellular locations in the same tissue.

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# Enzymes. Structure and properties. Kinetics of enzymatic reactions

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**Similarities between enzymes and chemical catalysts**

1. Do not alter the nature and quantity of the end products.
2. Do not alter the chemical equilibrium point of a reversible reaction but only the speed of the reaction is changed.
3. Catalyse the forward and reverse reactions proportionately.
4. Increase the rate of a chemical reaction without being used up in the reaction and can be recovered chemically unchanged at the end of the reaction.
5. Do not accelerate reaction that is not thermodynamically favorable.

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## Differences between enzymes and chemical catalysts

1. Enzymes are almost all proteins and, like proteins, they undergo denaturation.
2. Enzyme catalyzed reactions usually take place under relatively mild conditions (temperatures well below 100°C, atmospheric pressure, neutral pH).
3. Enzymes are highly efficient, being formed in the living system as per their requirements.
4. The activity of an enzyme is maximum, activity of enzymes is regulated.
5. Activity of enzymes is regulated on genetic level, and by low molecular weight compounds – *effectors (inhibitors and activators)*.
6. Enzymes are very specific in that they act either on a single or at the most on some structurally-related substrates.

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## Substrate specificity of enzymes

1. **Absolute substrate specificity** - enzymes are capable of acting on only one substrate. For example, urease acts only on urea to produce ammonia and carbon dioxide.
2. **Relative substrate specificity** – one enzyme acts on more than one substrate, having one type of bond. For example, lipase hydrolyzes fat to 3 fatty acids and glycerol acting on ester bond.
3. **Relative group substrate specificity** – one enzyme acts on more than one substrate, having one type of bond, but appointed atomic groups forming this bond are required. For example, proteolytic enzymes (pepsin, trypsin, chymotrypsin) catalyze a different but related reaction, namely the hydrolysis of a peptide bond.
4. **Optical substrate specificity** – enzyme reacts with only one of the two optical isomers. For example, L-oxidase acts only on L- amino acids and not on its D-isomer.

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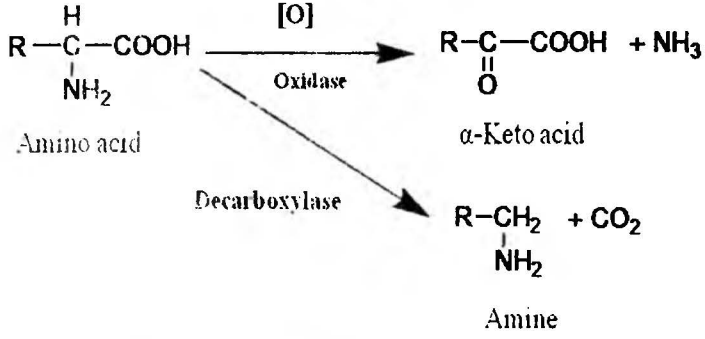
## Reaction specificity of enzymes

**- same substrate can undergo different types of reactions, each catalyzed by a separate enzyme**

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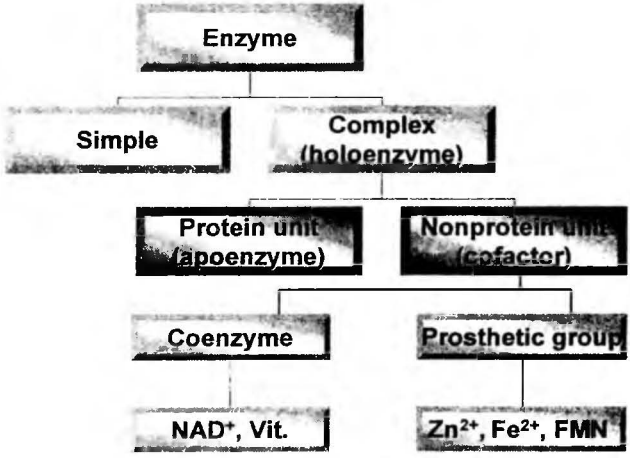
## Structure of enzyme

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## Active site of enzymes is formed:

- ***Simple-protein enzymes:*** active site is a three-dimensional entity formed by functional groups of amino acid residues that can lie far apart in the linear polypeptide chain.
- ***Complex-protein enzymes:*** active site of simple-protein enzymes + cofactor.

Examples of functional groups of amino acid residues in active sites:

**$-NH_2$ – Lys** **-OH – Ser**
**-COOH – Glu, Asp** **-SH – Cys**

**Parts of active sites:**

1. ***substrate binding site*** – for substrate fixation
2. ***catalytic site*** – for catalysis of the specific reaction

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## Features of active site:

1. It is the region that binds the substrate and converts it into product.
2. It is a three-dimensional entity formed by side-chains of amino acid residues that can lie far apart in the primary structure.
3. It is a relatively small part of the whole enzyme molecule and is often a cleft or crevice on the surface of the enzyme.
4. The substrate(s) is bound in the active site by multiple weak forces (electrostatic bonds, hydrogen bonds, van der Waals' forces and hydrophobic interactions) and in some cases by reversible covalent bonds.
5. Substrate specificity applies to the nature of the substrates that are involved. The properties and spatial arrangement of the amino acid residues forming the active site of an enzyme will determine which molecules can bind and be substrates for that enzyme.

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**Classification of enzymes based on the type of reaction catalyzed:**

1. Oxidoreductases: Enzymes involved in oxidation-reduction reactions between two substrates.
2. Transferases: Enzymes catalyze the transfer of functional groups between two substrates.
3. Hydrolases: Enzymes catalyze the hydrolysis of their substrates by adding constituents of water across the bond they split.
4. Lyases: Enzymes catalyze the removal of groups from substrates by mechanisms other than hydrolysis, leaving double bonds.
5. Isomerases: Enzymes catalyze interconversions of optical, geometric or positional isomers by intramolecular rearrangement of atoms or groups (isomerization reactions).
6. Ligases: Enzymes catalyzing the linking together of two compounds, utilizing the energy of ATP or a similar compound, forming C-O, C-S, C-N and C-C bonds.

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## Units of enzyme activity

- **Katal (kat)**: One kat denotes the conversion of 1 mole substrate per second (mol/sec).

- **Enzyme unit (U)**: One enzyme unit is that amount of enzyme, which will catalyze the transformation of 1 µmol of substrate per minute at 25°C under optimal conditions for that enzyme.

  1µmol min = 1 U = 16.67 nanokat.

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## Ways to increase rates of chemical reactions:

1. Increase average energy of molecules.
2. Decrease energy barrier of reactions.

**Energy barrier** is the energy needed to transform the substrate molecules into the transition state.

**The Gibbs free energy of activation ($\Delta G^{\ddagger}$)** is equal to the difference in free energy between the transition state and the substrate.

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## Stages of enzyme catalysis

$$E + S \rightleftarrows ES \rightarrow EZ \rightarrow EP \rightarrow E + P$$

**I. $E + S \rightleftarrows ES$ – formation of enzyme-substrate complex**

**Two models have been proposed to explain how an enzyme binds its substrate:**

1. In the lock-and-key model proposed by Fischer, the shape of the substrate and the active site of the enzyme are thought to fit together like a key into its lock. Thus the active site of an enzyme is a rigid and pre-shaped template where only a specific substrate can bind.
2. In the induced-fit model proposed by Koshland, the active site of the enzyme is not rigid and pre-shaped. The interaction of the substrate with the enzyme induces a fit or a conformation change in the active site. Due to induced fit, the appropriate amino acids of the enzyme are repositioned to form the active site and bring about the catalysis.

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## Reasons of the rate acceleration on the I stage:

1. Substrate binding induces changes that bring reactive groups into proximity with one another at the active site of an enzyme. The particular proper orientation of the substrate sorbed at the active site, which favors interaction with the site's catalytic segment;

2. This increases the effective concentration of the substrate.

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## Stages of enzyme catalysis

**II. ES → EZ → EP** – ***formation of intermediate state***

**Enzyme works by lowering the activation energy of a reaction.**

**Enzymes decrease the activation energy in 2 ways:**

1. The induced structural changes upon formation of the enzyme-substrate complex introduce strain in the substrate bonds, which allow it to attain the transition state more easily («rack» hypothesis);
2. The reduction of activation energy increases the number of reactant molecules with enough energy to reach the activation energy and form the product.

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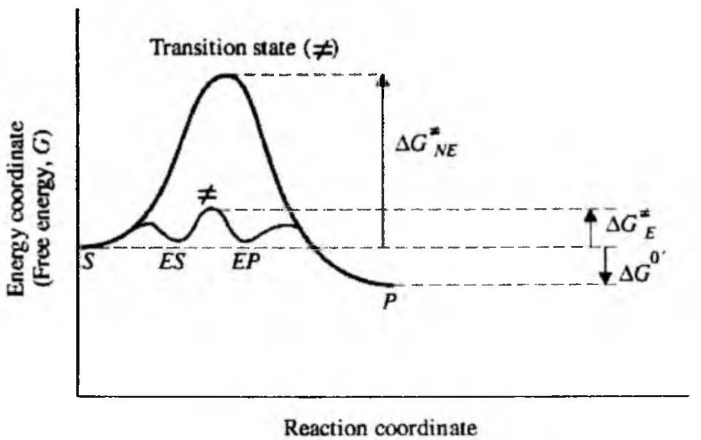
## Energy diagram, comparing the nonenzymatic and enzymatic reactions, S → P

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## Stages of enzyme catalysis

**III. EP ⟶ E + P** – ***product is released***

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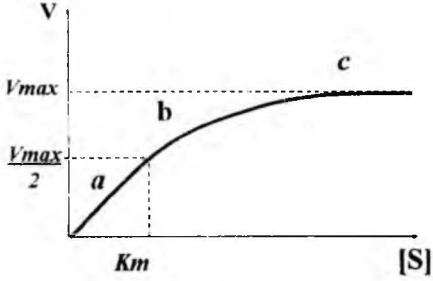
## Factors affecting reaction velocity

### *1. Substrate concentration*




When S is low, the equation for rate is 1st order with respect to substrate (a);

When S is high the rate of reaction is independent of S, the equation for rate is 0-order (c).

**For part «a» - Michaelis-Menten equation:**

$$v = \frac{v_{max} \cdot [s]}{k_s + [s]} \qquad v = \frac{v_{max}}{1 + \frac{k_s}{[s]}}$$

V - measured velocity,
Vm - maximum velocity,
[S] - substrate concentration,
Ks – dissociation constant of enzyme-substrate complex.

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## Brig and Haldane modify Michaelis-Menten equation for curve, entering Michaelis constant $k_m$

$$v = \frac{v_{max}}{1 + \frac{k_m}{[s]}}$$

**Km** is equivalent to the substrate concentration at which the velocity is equal to half of Vmax.

$$k_m = [S] \qquad v = \frac{v_{max}}{1+1} = \frac{v_{max}}{2}$$

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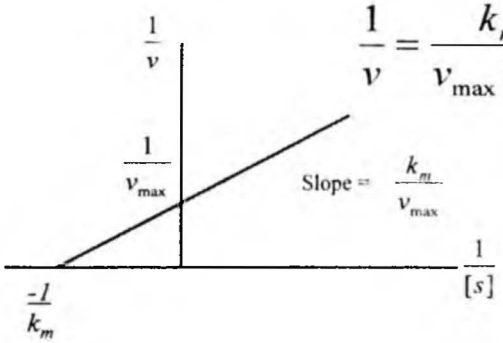
## Lineweaver-Burk double reciprocal plot

$$\frac{1}{v} = \frac{k_m}{v_{max} \cdot [s]} + \frac{1}{v_{max}}$$

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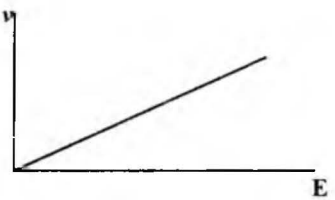
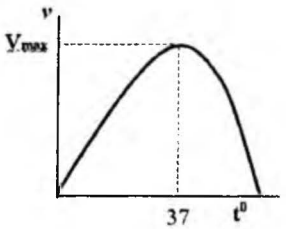
## Factors affecting reaction velocity

### *2. Enzyme concentration*

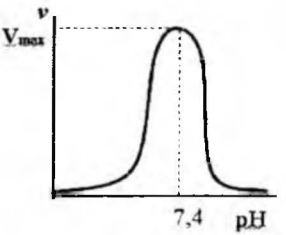



In situations where the substrate concentration is saturating, a doubling of the enzyme will lead to a doubling of $V_0$: **V =k [E].**

### *3. Temperature*




### *4. pH*

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## General mechanisms that affect enzyme activity:

1. Control of the overall quantities of enzyme.
2. Factors that affect the rate of enzyme-catalyzed reactions (pH, t. p), concentration of substrate and enzymes, availability of activators, inhibitors.
3. Alteration of the catalytic efficiency of the enzyme.

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## Regulation of the enzyme levels

The amount of enzyme present is a balance between the rates of its synthesis and degradation.

There are two types of enzymes in a cell:

1. **Constitutive enzymes** (house-keeping enzymes) – one produced constantly, irrespective of environmental conditions or demand, the levels of which are not controlled and remain fairly constant (e.g., lactate dehydrogenase, transaminases, acid and alkaline phosphatases, creatine phosphokinase).
2. **Adaptive enzymes** – their concentrations increase or decrease as per body needs and are well-regulated (inducible and repressible enzymes).
   - **Inducible enzyme** one whose rate of production can be stimulated or accelerated by substrate of that enzyme. The inducible enzyme is used for the catabolism (process of degradation) in the cell.
   - **Repressible enzyme** one whose rate of production is decreased as the concentration of certain substances, often a final product is increased. The repressible enzyme is used for the anabolism (process of synthesis) in the cell.

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## Activators of enzymes

**- any molecules which acts directly on an enzyme to increase its activity.**

### Features of activators:

1. Form the active site of enzyme ($Co^{2+}$, $Mg^{2+}$, $Zn^{2+}$, $Fe^{2+}$, $Cu^{2+}$).
2. Facilitate formation of enzyme substrate complex ($Mg^{2+}$, $Mn^{2+}$).
3. Stabilize native structure of enzyme.
4. Protect functional groups of the active site from damage, for example, restore SH-groups of the active site (glutathione, cysteine).
5. Influence subunits of enzyme molecules (cAMP regulate enzyme protein kinase).

Activators are usually cations, more rarely are anions ($Cl^-$ activates pepsin, amylase).

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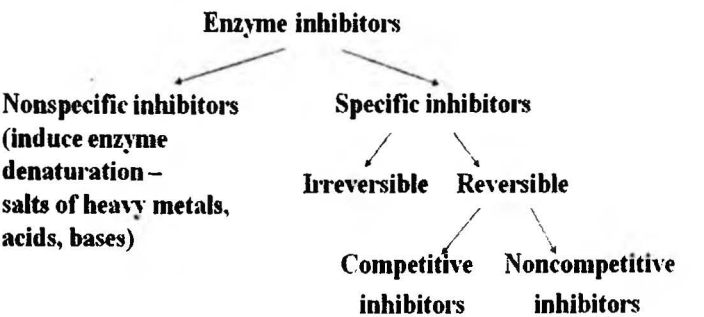
## Inhibitors of enzymes

**- any molecules which acts directly on an enzyme to decrease its activity.**

### Classification of inhibitors

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## Irreversible inhibitors

**- inhibitors bind irreversibly to active site of enzyme often form a covalent bond to an amino acid residue and permanently inactivate the enzyme**

1. Inhibitors that strongly change valency of Fe and Cu of metall-containing enzymes of respiratory chain (e.g., cytochrome oxidase), preventing electron transfer to $O_2$ – HCN, KCN, CO, $NaN_3$ (respiratory poisons).
2. Alkylating reagents that combine with sulfhydryl (-SH) groups of cysteine at the active site of enzyme – Iodoacetate, substances of mercury and arsenic.
3. Organophosphorus compounds that bind with enzymes containing OH-groups of serine at the active site (e.g., serine proteases, acetylcholine esterase) – Diisopropylphosphofluoridate (nerve gas).

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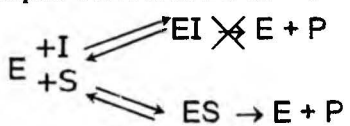
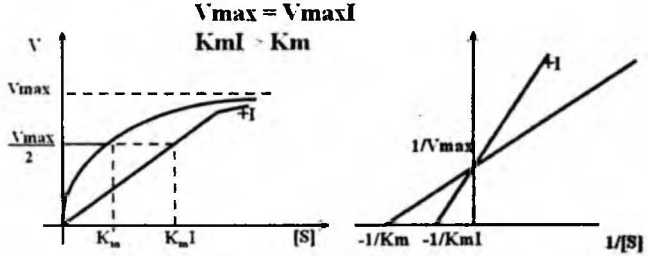
## Reversible competitive inhibitors

**- inhibitors have close structural similarities to the substrate therefore compete with the substrate to bind with the active site of enzyme**




**there is no change in the Vmax and Km increases**

**Vmax = VmaxI**
**KmI > Km**

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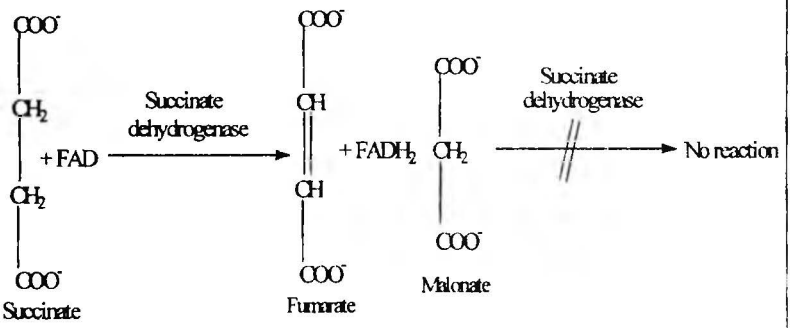
## Example of competitive inhibition




**Malonate is competitive inhibitor**

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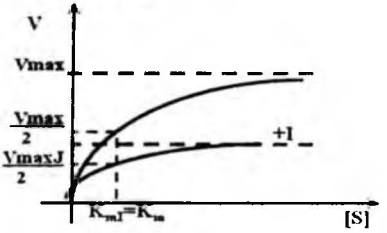
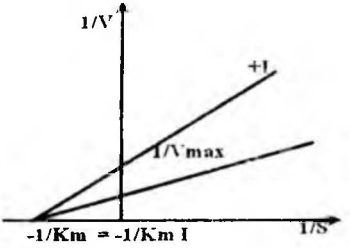
## Reversible noncompetitive inhibitors

**- inhibitors bind reversibly at a site other than the active site (i.e., at the allosteric site) and causes a change in the overall three-dimensional shape of the enzyme**

$$E + S + I \rightarrow ESI \xrightarrow{slowly} E + P + I$$

**VmaxI<Vmax**
**KmI=Km**




**Vmax is decreased**
**Km remains constant**

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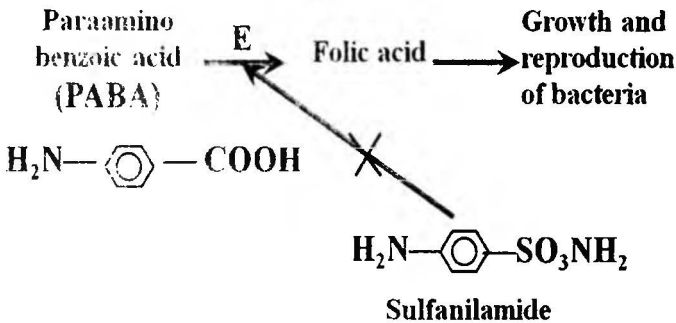
## Antimetabolites

**- competitive inhibitors of enzymes, mimicking the structure of the native substrates.**

***For example,* sulfanilamides**

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## Regulation of enzyme activity

**- all alterations of enzyme activity at its constant quantity.**

Some methods to regulate enzyme activity are:

1. Feedback regulation.
2. Allosteric regulation.
3. Reversible covalent modification.
4. Formation of multienzyme systems.
5. Proteolytic activation.

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## Allosteric regulation

### Models for allosteric behavior

1. Concerted or symmetry model (Monod, Wyman, Changeux)
2. Simple sequential model (Koshland, Nemethy, Filmer).

**Concerted or Symmetry model:** allosteric enzyme can exist in still two conformations, active and inactive. All subunits are in the active form or all are inactive. Binding of substrate to one of the subunits increases the probability that both switch from the T to the R form. Allosteric inhibitor shifts the R ⟶ T conformational equilibrium toward T, whereas an allosteric activator shifts it toward R. The result is that an allosteric activator increases the binding of substrate to the enzyme, whereas an allosteric inhibitor decreases substrate binding.

**Quaternary structure: TT, RR,**

$$S+TT \rightarrow RR$$

**Simple sequential model:** allosteric enzyme can exist in only two conformational changes individually. Consider an allosteric enzyme consisting of two identical subunits, each containing an active site. The T (tense) form has low affinity and the R (relaxed) form has high affinity for substrate. Binding of substrate to one of the subunits induces a T ⟶ R transition in that subunit but not in the other. The affinity of the other subunit for substrate is increased because the subunit interface has been altered by the binding of the first substrate molecule.

**Quaternary structure: TT, RR, TR**

$$S + TT \rightarrow TR \rightarrow RR(+)$$

$$S + RR \rightarrow TR \rightarrow TT(-)$$

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## Formation of multienzyme systems

- Complex enzyme systems are not independent molecules but occur as aggregates involving several different enzymes.
- Fatty acid synthase is one such example. Each component of this complex enzyme is so arranged as to provide an efficient coupling of the individual reactions catalyzed by these enzymes. The product of the first enzyme becomes the substrate of the second and so on.

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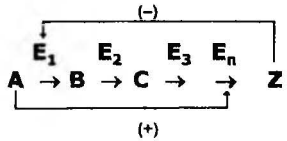
## Feedback regulation

Control mechanism that uses the consequences of a process to regulate the rate at which the process occurs.

**Feedback inhibition**




**Feed-forward activation**

**Feedback inhibition** – negative feedback regulation.

The enzyme catalyzing the first step ($E_1$) is inhibited by the end product of the reaction (Z). Increased levels of Z will result in $E_1$ decreased synthesis. Control of the enzyme which carries out the committed step of a metabolic pathway conserves the metabolic energy supply of the organism, and prevents the build-up of large quantities of unwanted metabolic intermediates further along the pathway.

**Feed-forward activation** – the activation of an enzyme ($E_n$) by a precursor of the substrate (A).

High concentration of an earlier reactant not involved in the continual chemical reactions of the pathway will help to catalyze final reactions down the pathway. The reactant which serves as the feed-forward activator is intrinsically linked to the final product it helps to catalyze.

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## Proteolytic activation

- Some enzymes are synthesized as larger inactive precursors called proenzymes or zymogens. These are activated by the irreversible hydrolysis of one or more peptide bonds (proteolysis).
- The pancreatic proteases trypsin, chymotrypsin and elastase are all derived from zymogen precursors (trypsinogen, chymotrypsinogen and proelastase, respectively) by proteolytic activation.

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## Reversible covalent modification

- Reversible covalent modification is the making and breaking of a covalent bond between a nonprotein group and an enzyme molecule.
- The most common modification is the addition and removal of a phosphate group (phosphorylation and dephosphorylation, respectively). A phosphorylated enzyme may be either more or less active than its dephosphorylated form.
- For example, glycogen phosphorylase, an enzyme involved in glycogen breakdown, is active in its phosphorylated form, and glycogen synthase, involved in glycogen synthesis, is most active in its unphosphorylated form.

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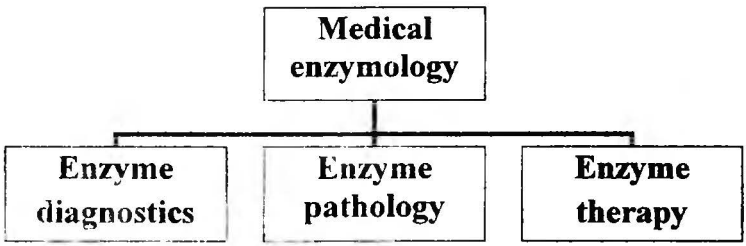


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## Enzyme diagnostics

**– research of enzymes in biological medium of an organism with the diagnostic purpose.**

**There are 4 groups of enzymes:**

1. Enzymes widely distributed in various organs and tissues. These are enzymes of the basic metabolic processes without which cell life is impossible (metabolism of proteins, fats, carbohydrates). Estimation of raised enzyme levels in blood due to cellular membrane damage is not used for the diagnosis of diseases.

2. Tissue-specific enzymes are localized in certain organs. These enzymes are either totally absent or present at a low concentration in the cells of other tissues. Tissue-specific enzymes are conveniently used as markers to detect the cellular damage which ultimately helps in the diagnosis of diseases.

3. Isoenzymes.

4. Enzymes are localized in organelles of the cells (oxidative-reductive enzymes in mitochondria, acid hydrolases in lysosomes). The enzymes when elevated in serum are markers of deep cellular damage.

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## Mechanisms of plasma enzyme activities alteration

| | | |
|---|---|---|
| **Increased plasma enzyme activities** | | |
| 1. | Increased enzyme synthesis | Alkaline phosphatase (rickets) |
| 2. | Increase of membrane-permeability | Creatine kinase (muscular dystrophy)<br>Alanine transaminase. Aspartate transaminase (viral hepatitis) |
| 3. | Necrosis of cells | Aspartate transaminase, Aldolase, Creatine kinase |
| 4. | Decreased enzyme excretion | Alkaline phosphatase |
| **Decreased plasma enzyme activities** | | |
| 1. | Reduction of number of cells, that secrete enzyme | Cholinesterase (cirrhosis of liver)<br>Pepsinogen (gastroectomy) |
| 2. | Decreased enzyme synthesis | Ceruloplasmin (Wilson's disease) |
| 3. | Increased enzyme secretion | Ceruloplasmin (nephrosis) |
| 4. | Inhibition of enzyme activity | Trypsin (under influence of antitrypsin) |

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## Isoenzymes

**– are different forms of an enzyme, which catalyze the same reaction, but which exhibit different chemical, physical or kinetic properties (structure, isoelectric point, pH optimum, substrate affinity or effect of inhibitors).**

### Creatine kinase (CK) or creatine phosphokinase (CPK)

**Creatine + ATP ↔ Creatine phosphate + ADP**

Enzyme Creatine kinase is dimer of 2 subunits, called B (''brain'') and M (''muscle'').

### Isoenzymes of Creatine kinase:

1. BB ($CK_1$) is found in brain, prostate, stomach, lungs, placenta, thyroid gland.
2. MB ($CK_2$) is found in cardiac muscle (25-46% of total CK) and in skeletal muscle (5%).
3. MM ($CK_3$) is found in skeletal muscle and cardiac muscle.

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### Lactate dehydrogenase (LDH)

**Lactate + $NAD^+$ ↔ Pyruvate + NADH + $H^+$**

Enzyme Lactate dehydrogenase is a tetramer of two different types of 4 subunits, called H (''heart'') and M (''muscle'').

### Isoenzymes of Lactate dehydrogenase:

1. $H_4$ ($LDH_1$) is found in heart and RBC.
2. $H_3M$ ($LDH_2$) is found in heart and RBC.
3. $H_2M_2$ ($LDH_3$) is found in lungs, brain and kidney.
4. $HM_3$ ($LDH_4$) is found in liver and skeletal muscle.
5. $M_4$ ($LDH_5$) is found in skeletal muscle and liver.

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## Applications of tissue-specific enzymes (isoenzymes) in the diagnosis

| Enzyme (isoenzyme) | Organ damaged from which enzymes arise |
|---|---|
| LDH 1, 2 | Cardiac muscle |
| LDH 3 | Lungs |
| LDH 4, 5 | Liver, muscle |
| Amylase | Pancreas |
| Alanine transaminase (ALT) | Liver |
| Aspartate transaminase (AST) | Cardiac muscle |
| Acid phosphatase | Prostate |
| Alkaline phosphatase | Bone |

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## Enzymopathology

***Hereditary enzymopathies*** - diseases related to inherited defects in enzymes. Changes can be of two types:

1. associated with the formation of a substrate excess or its precursors in a reaction. For example, the accumulation and excretion of galactose due to the deficiency of the enzyme converted galactose to fructose - galactosemia;

2. associated with the inadequate formation of products of altered chemical reaction. This type is accompanied by clinical manifestations. In this step, usually already too late to apply therapeutically effective measures.

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## Immobilized enzymes

**- are physically immobilized by binding them to a solid, insoluble matrix which will not affect the enzyme stability or its catalytic activity**

The bound enzymes can be preserved for long periods without loss of activity.

**Benefits of immobilizing an enzyme:**

- **Convenience:** Minuscule amounts of protein dissolve in the reaction, so workup can be much easier. Upon completion, reaction mixtures typically contain only solvent and reaction products.
- **Economy:** The immobilized enzyme is easily removed from the reaction making it easy to recycle the biocatalyst.
- **Stability:** Immobilized enzymes typically have greater thermal and operational stability than the soluble form of the enzyme. This can provide increased resistance to changes in conditions such as pH or temperature.

75

## Application of enzymes as analytical reagents (for concentration estimation in blood)

| Enzyme | Application |
|---|---|
| Glucose oxidase | Glucose |
| Cholesterol oxidase | Cholesterol |
| Lipase | Triacylglycerols |
| Urease | Urea |

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# Enzyme therapy

**- is application of enzymes and regulators of their activity as medicines**

| *Medicine* | *Characteristic* | *Indications* | *The purpose of applications* |
|---|---|---|---|
| Pepsin | Proteoytic enzyme of stomach | Insufficiency of gastric digestion (gastritis) | Replacement therapy |
| Trypsin | Proteoytic enzyme of pancreas | a) Festering wound; b) inflammatory disorders of respiratory tract | Splitting of necrotic tissues and clots of blood |
| Aprotinin (trasylol) | Inhibitor, of trypsin and related proteolytic enzymes | Pancreatitis | Prevention of pancreas autodigestion by the trypsin, which is already activated in the gland ducts at pancreatitis |
| Fibrinolysin | Proteinase that dissolves the fibrin of blood clots | Thrombosis of vessels | Resorption of formed trombuses |
| Iprazide | Inhibitor of monoamine oxidases, that inhibit catecholamines | States of depression | Stimulates the mental sphere. Eliminates states of depression |
| Hyaluronidase | Glycosidase, an enzyme that catalyzes the breakdown of hyaluronic acid in the body | Scars after burns and operations, commissures | Breaking down the redundant connective tissue |
| Penicilline, cycloserine | Inhibitors of the enzymes that form the components of the bacterial cell wall | Bacterial infections | Bacteriostatic and bactericidal action |

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# Biological membrane

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## Distribution of membrane components (structural asymmetry)

- carbohydrates are located in the outer leaflet of membrane only;
- sphingomyelin and phosphatidylcholine are entially located in the outer leaflet of membrane;
- phosphatidylethanolamine and phosphatidylserine are mainly located in the inner leaflet of membrane;
- cholesterol is mainly located in the outer leaflet of membrane.

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## Lipids of membrane

| *Lipids* | *Hydrophobic part* | *Hydrophilic part* |
|---|---|---|
| Glycerophospholipids | two hydrocarbon fatty acid chains | phosphorylated alcohols (choline, ethanolamine, glycerol, inositol or serine). |
| Sphingomyelins | hydrocarbon fatty acid chain and sphingosine backbone | phosphorylated choline |
| Glycolipids | hydrocarbon fatty acid chain and sphingosine backbone | carbohydrate |
| Cholesterol | fused ring system | OH at C-3 |

80

Membrane fluidity is regulated by degree of saturation of fatty acids in phospholipids and cholesterol content.

Increasing the length of the fatty acid chains or decreasing the number of unsaturated double bonds in the fatty acid chains and increasing the amount of cholesterol leads to a decrease in the fluidity of the membrane.

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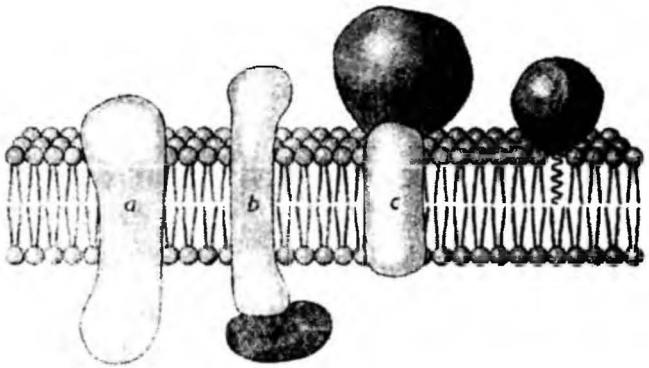
## Membrane proteins




**a**, **b**, and **c** are integral membrane proteins
**d**, **e** and **f** are peripheral membrane proteins

82

## Enzymatic markers of different membranes

| *Membrane* | *Enzyme* |
|---|---|
| Plasma membrane | 5'-nucleotidase, adenylate cyclase, γ-glutamyl transferase |
| Endoplasmic reticulum | Glucose 6-phosphatase |
| Golgi apparatus | Galactosyl-transferase |
| Inner mitochondrial membrane | ATP- synthase |

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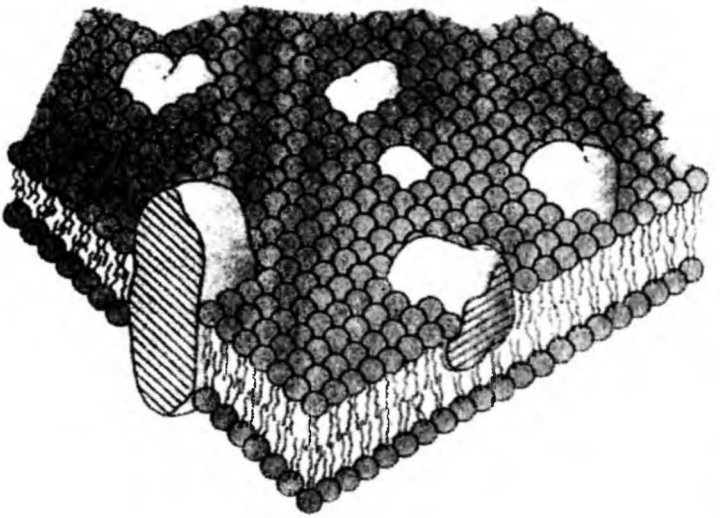
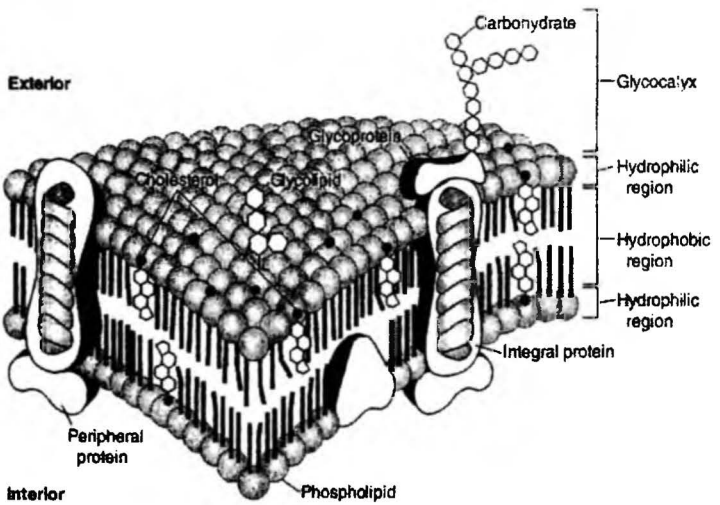


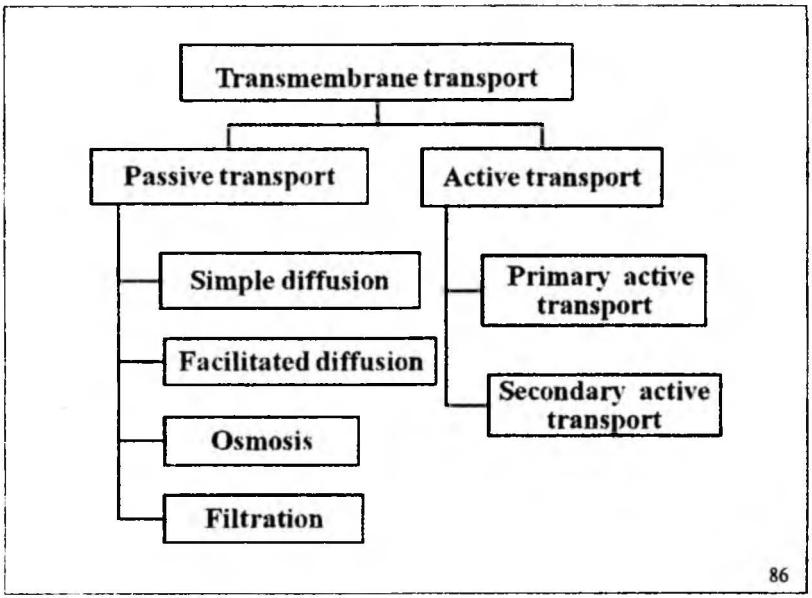
**Fluid mosaic model of membrane structure according to Jonathan Singer and Garth Nicholson**

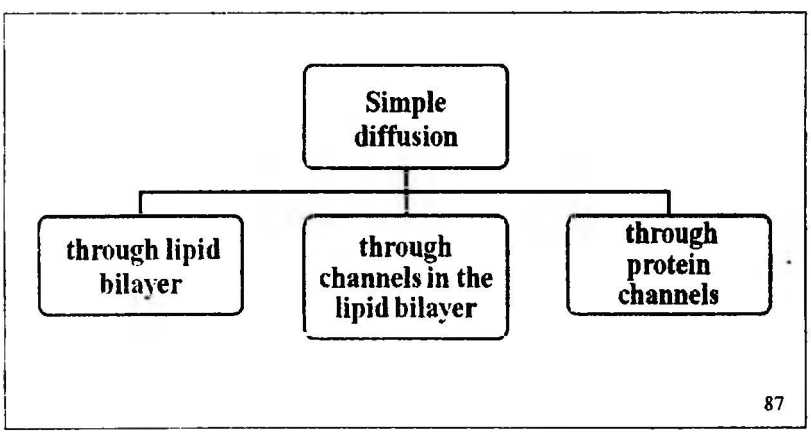
84




**Structure of plasma membrane**

85


Transmembrane transport
Passive transport
Active transport
Simple diffusion
Facilitated diffusion
Osmosis
Filtration
Primary active transport
Secondary active transport
86


Simple diffusion
through lipid bilayer
through channels in the lipid bilayer
through protein channels
87

**Facilitated diffusion**

**with mobile carrier ionophores**

Example, **Valinomycin**
Forms complex with $K^+$ and diffuses freely and rapid across lipid membrane

**with channel-forming ionophores**

Example, **Gramicidin**
Forms channels adopted a fixed orientation in a membrane, creating a hole that permits the transmembrane movement of ions

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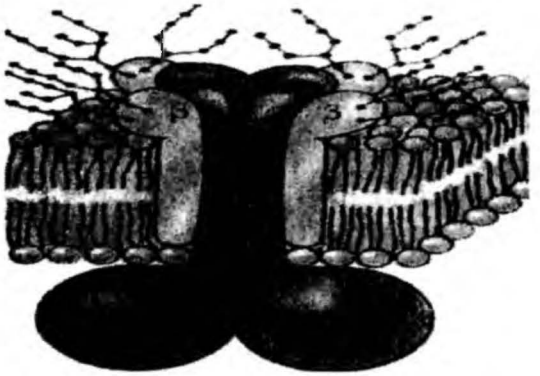
## Structure of the $Na^+/K^+$ - ATPase



The $Na^+/K^+$-ATPase is an integral membrane protein, heterotetramer *($a_2\beta_2$)*, consisting of 110 kDa α and 55 kDa β subunits.

Binding sites on α subunits for:

- ATP, $Na^+$ (in the inner side of membrane)
- $K^+$, steroid inhibitors (in the outer side of membrane)

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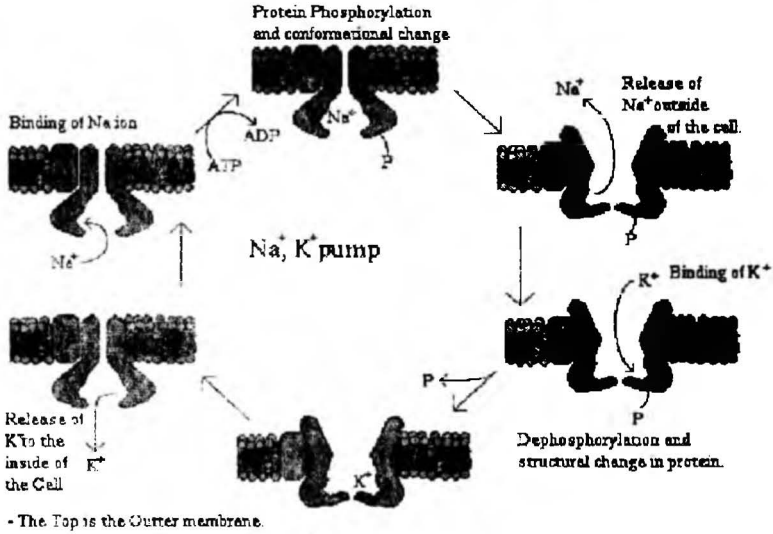
## Mechanism of $Na^+$ and $K^+$ transport

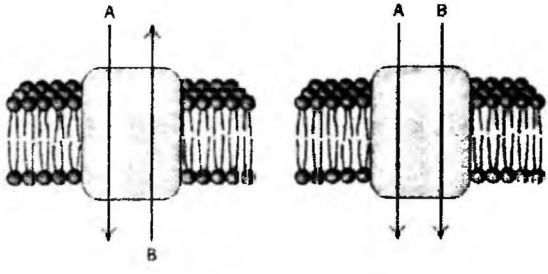



- The Top is the Outter membrane.
- The Bottom is the inner membrane (inside of the Cell)

90

## Secondary active transport system

**Cotransport** - transport of a substance through the membrane is coupled to the spontaneous movement of another substance




**Antiport** **Symport**

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# Metabolism. Biochemistry of nutrition and digestion

92

- **Metabolism** – entire spectrum of chemical enzymatic reactions that convert nutrients into energy and the chemically complex products of cells, occurring in the living system.
- **Metabolic pathway** – enzymatic chain of chemical reactions.
- **Map of metabolism** – set of metabolic pathways of all substances related to each other by common metabolites.

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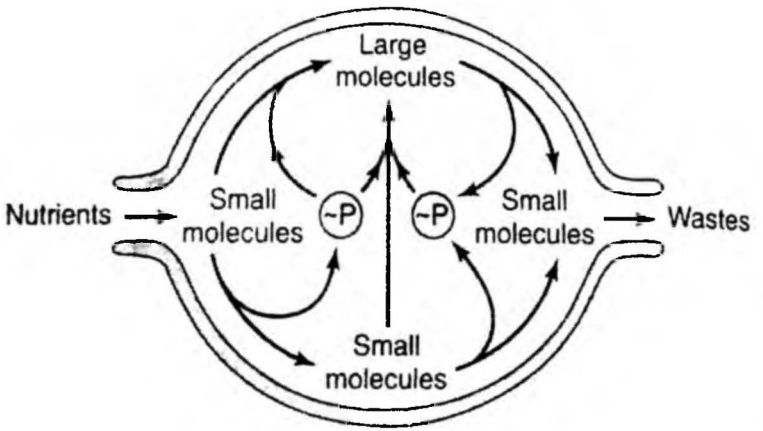
**Metabolic homeostasis**

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# Catabolism

The enzymatic degradative processes concerned with the breakdown of complex large organic nutrient molecules to smaller simpler compounds (e.g., $CO_2$, $H_2O$, $NH_3$) with a concomitant release of chemical free energy, much of which is conserved in the form of energy-carrying molecule adenosine triphosphate (ATP).

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## Catabolism occurs in three stages:

I. Degradation of polymers to monomers:

- starch, glycogen → glucose;
- proteins → amino acids;
- triacylglycerols → glycerol + free fatty acids.

II. Specific pathways of catabolism of monomers with formation of simple intermediates – pyruvate and acetyl CoA.

Examples of specific pathways of catabolism:

- oxidation of glucose to pyruvate, 2 ATP;
- oxidative deamination of amino acids to ketoacids (pyruvate), acetyl CoA and $NH_3$, 2-3 ATP;
- β-oxidation of fatty acids to acetyl CoA, 5 ATP.

III. Common pathways of catabolism with formation of end products:

- oxidative decarboxylation of pyruvate to acetyl CoA and $CO_2$, 3 ATP;
- oxidation of acetyl CoA via Krebs cycle (or citric acid cycle) to $CO_2$, $H_2O$, 12 ATP.

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## Anabolism

The enzymatic biosynthetic reactions involving the formation of complex organic molecules from simple precursors and require an input of chemical free energy which is furnished by the breakdown of ATP to ADP and phosphate.

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## Stages of anabolism

I. Third stage of catabolism (amphibolic).

II. Formation of monomers.

***The anabolic and catabolic pathways are not reversible and must be controlled by different regulatory signals independently.***

III. Synthesis of polymers from monomers.

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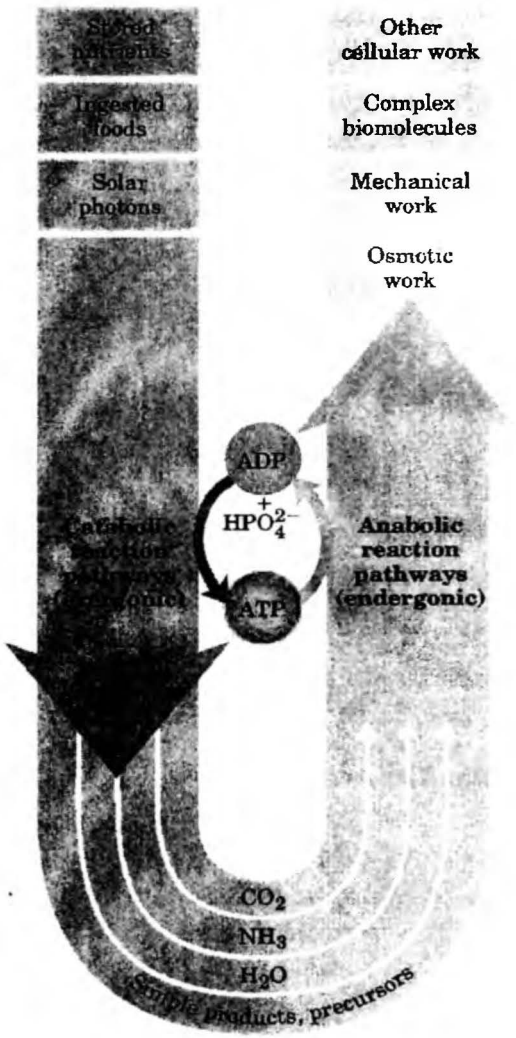
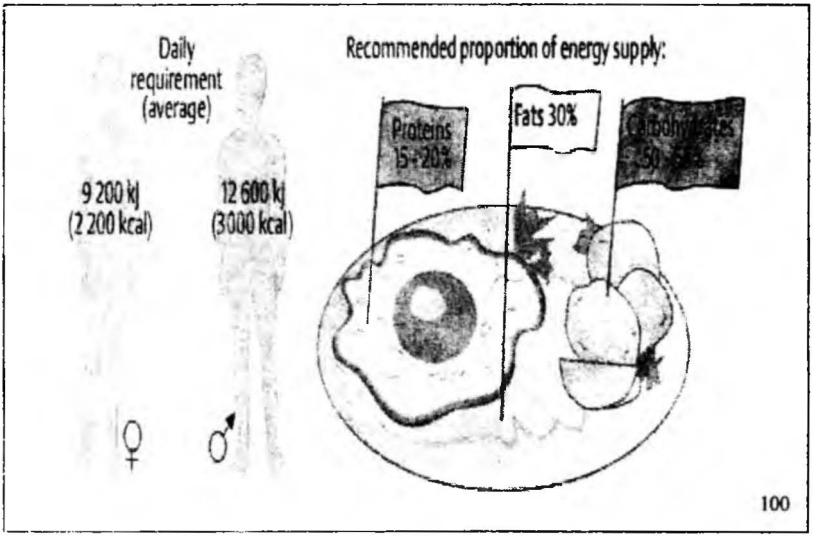
## The central role of ATP in metabolism




99




100

## Essential components of diet

**Amino acids**

- Leu-Ile
- Trp-Met
- Thr-Val
- Lys-Phe
- Arg-His

**Fatty acids**

- Linoleic acid
- Linolenic acid
- Arachidonic acid

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## Regulation of digestion by the hormones of the gastrointestinal tract

| *Hormone* | *Site of synthesis* | *Stimulus of secretion* | *Effect* |
|---|---|---|---|
| Gastrin, histamine | gastric mucosa | proteins (derived from food) or by HCl | stimulate secretion of gastric HCI and pepsin by fundic cells of stomach |
| Enterogastrone | duodenal mucosa | fat (derived from food) | inhibits gastric HCl and pepsin secretion possibly by blocking the activity of gastrin |
| Secretin | mucosa of the upper small intestine | HCl present in the acid chyme | stimulates the flow of pancreatic juice rich in bicarbonate, water, and the flow of bile by the pancreas |
| Cholecystokinin (pancreozymin) | --//-- | products of protein and lipid digestion | stimulates the contraction of the gall bladder and release of bile into the intestine, and the release of pancreatic juice, which is rich in digestive enzymes and $HCO_3^-$ by the pancreas |
| Himodenin | --//-- | | stimulates synthesis and secretion of chymotrypsinogen by the pancreas |
| Enterocrinin | --//-- | | stimulates secretion of the digestive glands of the small intestine |
| Villikinin | --//-- | | stimulates movements of the intestinal villi and intestinal absorption of digested food materials |

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## Failures of nutrition today

1. High fat intake.
2. Deficiency of polyunsaturated fatty acids.
3. Deficiency of (animal) proteins.
4. Deficiency of majority of vitamins.
5. Deficiency of macro- and microminerals – Ca, Fe, I, F, Se.
6. Deficiency of dietary fibers.

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## Nutraceuticals

– natural ingredients of food, such as vitamins or their precursors (e.g., β-carotins), polyunsaturated fatty acids of omega-3 family, some mono- and disaccharides, dietary fibres (cellulose, pectins) and so on.

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## Nutraceuticals with reported medicinal value:

- **Antioxidants:** resveratrol from red grape products; flavonoids inside citrus, tea, wine, and dark chocolate foods; anthocyanins found in berries.
- **Reducing hypercholesterolemia:** soluble dietary fiber products, such as psyllium seed husk.
- **Cancer prevention:** broccoli (sulforaphane) fiddleheads (Matteuccia Struthiopteus).
- **Improved arterial health:** soy or clover (isoflavonoids).
- **Lowered risk of cardiovascular disease:** alpha-linolenic acid from flax or Chia seeds.

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# Bioenergetics. Biological oxidation. Cellular respiration

106

**Laws of thermodynamics provide the underlying principles to:**

1. Predict the direction of a reaction, whether from left to right or vice versa.
2. Explain why some reactions may occur while others do not.
3. Understand the accomplishment of work, whether useful or not, and whether the energy for driving a reaction must be delivered from an external source.

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## Thermodynamic principles

**Enthalpy (H)** is a measure of the change in heat content of the reactants compared to products.

**Change in free energy (ΔG)** is that portion of the total energy change in a system that is available for doing work; i.e., it is the useful energy.

**Entropy (S)** represents the extent of disorder or randomness of the system and becomes maximum in a system as it approaches true equilibrium.

$$\Delta G = \Delta H - T\Delta S$$

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## Reaction:

$$A \rightarrow B \quad \Delta G = G_B - G_A$$

1) $G_B > G_A$ $\Delta G$ (+)

if ΔG is positive, the reaction proceeds only if free energy can be gained; i.e., **it is endergonic**.

2) $G_B < G_A$ $\Delta G$ (-)

If ΔG is negative, the reaction proceeds spontaneously with loss of free energy; i.e., **it is exergonic**.

3) $G_B = G_A$ $\Delta G = 0$

If ΔG is zero, the system **is at equilibrium** and no net change takes place.

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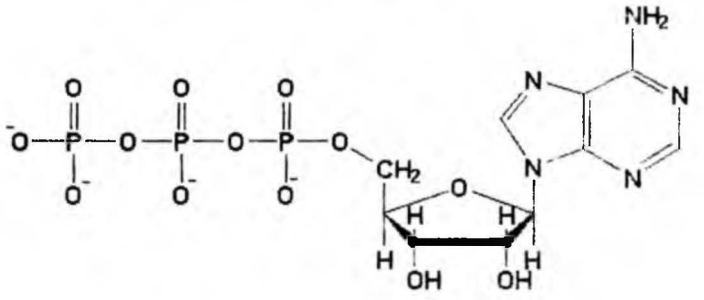
## Adenosine triphosphate (ATP)



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## High-energy (or energy rich) compounds

**- are substances when hydrolyzed liberate more energy than that of ATP (30,5 kJ/mol)**

### There are 3 groups of high-energy compounds:

1). **High-energy phosphates**:

1. Anhydrides (e.g., 1,3-Bisphosphoglycerate, ATP, CTP, GTP);
2. Enolphosphates (e.g., Phosphoenolpyruvate);
3. Phosphoguanidines (e.g., Creatine phosphate).

2). **Energy-riched thiol esters** involving coenzyme A (e.g., acetyl-CoA), acyl carrier protein (ACP), S-adenosylmethionine (S-AM), UDP- glucose, 5-phosphoribosyl-1-pyrophosphate.

3). **$NADPH_2$** as energy accumulator of electrons in cytosol provides with electrons and protons the processes of reductive biosynthesis.

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## Standard free energy of hydrolysis of some organophosphates of biochemical importance

| *Compound* | $\Delta G^{0'}$ | |
|---|---|---|
| | *KJ/ mol* | *Kcal/mol* |
| Phosphoenolpyruvate | -61.9 | -14.8 |
| Carbamoyl phosphate | -51.4 | -12.3 |
| 1,3-Bisphosphoglycerate | -49.3 | -11.8 |
| Creatine phosphate | -43.1 | -10.3 |
| ATP | -30.5 | -7.3 |
| ADP | -27.6 | -6.6 |
| Pyrophosphate | -27.6 | -7.4 |
| Glucose-1-phosphate | -20.9 | -5.0 |
| Glucose-6-phosphate | -13.8 | -3.3 |

The intermediate position of ATP allows it to play an important role in energy transfer.

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## Cycle ATP/ADP

**Endergonic reaction:**

Glucose —(*Hexokinase*)→ Glucose-6-phosphate

**Exergonic reaction:**

ATP → ADP + Pi

**Exergonic reaction:**

1,3-Bisphosphoglycerate —(*Phosphoglycerate kinase*)→ 3-phosphoglycerate

**Endergonic reaction:**

ADP + Pi → ATP

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## ATP can be synthesized in 3 ways:

1. **Oxidative phosphorylation** - ATP synthesis via transport of electrons produced by substrate oxidation through a respiratory chain and proton gradient in the inner mitochondrial membrane.

2. **Substrate level phosphorylation** - reactions, in which ATP is synthesized from the high-energy phosphates.

3. **Photosynthetic phosphorylation** - ATP synthesis via light energy during photosynthesis in the thylakoid membrane.

**Conclusion: ATP is universal accumulator of energy.**

$$ADP + Pi \longrightarrow ATP$$

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## ATP is universal source of energy.
## ATP is required mainly for the following 4 purposes:

1. To synthesize macromolecules from simpler and smaller precursors.

2. To transport molecules and ions across membranes against gradients (active transport).

3. To perform mechanical work, as in the muscle contraction.

4. To ensure fidelity of information transfer (nerve impulse conduction).

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## Adenylate kinase

**ATP + AMP → 2 ADP**

This reaction performs 3 main functions:

1. Permits the high energy phosphate of ADP to be used in the formation of ATP;
2. It is a means by which AMP can be rephosphorylated to form ADP;
3. Under stress of ATP depletion, AMP concentration increases which acts as a metabolic signal to increase the rate of catabolic reactions so that more ATP molecules are regenerated.

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## Energy $\triangle\mu H^+$ generated during electron transport is used for following biological purposes:

1. To provide energy for the synthesis of ATP during oxidative phosphorylation (chemical work);
2. For secondary active transport of substances through the membrane against concentration gradient (transport $Ca^{2+}$ from the cytosol into the matrix of mitochondria) (osmotic work);
3. To generate heat in order to maintain the body temperature;
4. Rotation of bacterial flagella is also controlled by the proton gradient generated across the membrane (mechanical work).

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## Biological oxidation

**- is the whole set of all oxidative processes proceeding in an organism with participation of $O_2$**

**Ways of substrate oxidation:**

**I. By dehydrogenation ($O_2$ is acceptor of $H_2$).**
1). $H_2$ removed from substrate is transferred on $O_2$ through a number of carriers to produce $H_2O$ in mitochondria. Energy is released as ATP.

$$SH_2 + \ldots \tfrac{1}{2}O_2 \rightarrow S + H_2O + ATP \text{ (cellular respiration)}$$

2). $H_2$ removed from substrate is transferred at once on $O_2$, to form hydrogen peroxide in peroxisomes. Energy is released as heat.

$$SH_2 + O_2 \rightarrow S + H_2O_2 + Q \text{ (peroxidase oxidation)}$$

**II. By incorporation of $O_2$ (oxygenase oxidation).**
1). Incorporation only one atom of oxygen into the substrate (endoplasmic reticulum).

$$S + \tfrac{1}{2}O_2 \rightarrow S\text{-}OH \text{ (microsomal or hydroxylase oxidation)}$$

2. Incorporation both oxygen atoms into the substrate (endoplasmic reticulum).

$$S + O_2 \rightarrow SO_2 \text{ (dioxygenase oxidation)}$$

3. Oxidation with participation of oxygen free radicals (active forms of oxygen) **(free radical oxidation).**

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## Purpose of biological oxidation:

1) Recover energy from various compounds (cellular respiration);

2) Destruction or detoxication of xenobiotics (peroxidase, oxygenase oxidation);

3) Biosynthesis (hydroxylase oxidation);

4) Change in membrane permeability, oxidative modification of molecule (free radical oxidation).

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## Characteristic features of cellular respiration

1) It is a part of biological oxidation where substrate is oxidized by dehydrogenation and $O_2$ is acceptor of hydrogen to produce $H_2O$ in mitochondria.

2) Reducing equivalents ($H^+$ or $e^-$) are funneled into the respiratory chain, where they are passed down a redox gradient of carriers to their final reaction with oxygen.

3) The flow of electrons through the respiratory chain generates ATP by the process of oxidative phosphorylation.

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## Respiratory chains:

**Complete respiratory chain**

$S_{II, III} \rightarrow NAD \rightarrow FMN \rightarrow$ co-enzyme Q $\rightarrow$ Cyt b $\rightarrow$ Cyt $c_1 \rightarrow$ Cyt c $\rightarrow$ Cyt $aa_3 \rightarrow \frac{1}{2} O_2$

**Incomplete respiratory chain**

$S_I \rightarrow FAD$

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## Types of oxygenated substrates ($SH_2$)

1. $S_I$ Hydrocarbons (succinate, acyl-CoA) are oxidized by FAD-linked dehydrogenases (incomplete respiratory chain). $\Delta G$ = 150 kJ/mol.

2. $S_{II}$ Alcohols (isocitrate, malate, lactate) are oxidized by NAD-linked dehydrogenases (complete respiratory chain). $\Delta G$ = 200 kJ/mol.

3. $S_{III}$ Aldehydes (glyceraldehyde-3-phosphate) are oxidized by NAD-linked dehydrogenases (complete respiratory chain). $\Delta G$ = 250 kJ/mol.

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## Components of the complete respiratory chain:

1. NAD-linked dehydrogenases (coenzyme is NAD);
2. NADH dehydrogenases (or FMN-linked dehydrogenases) (prosthetic groups are FMN and FeS-centers);
3. Ubiquinone (Q, co-enzyme Q);
4. Cytochromes (prosthetic group is heme).

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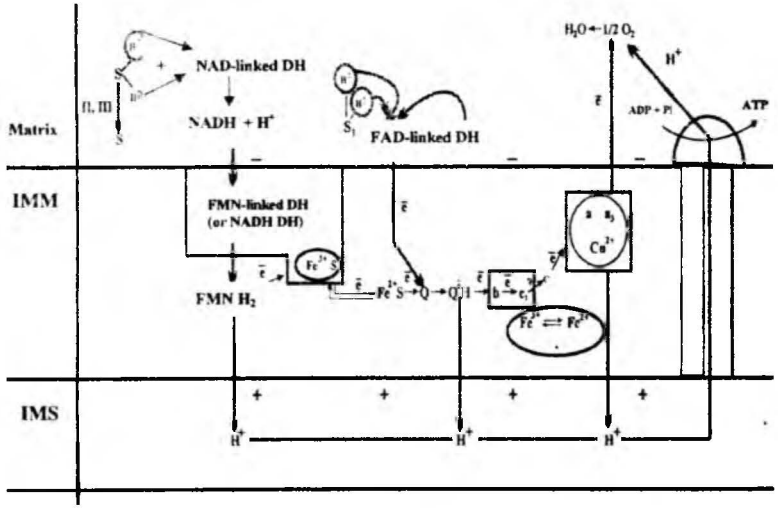
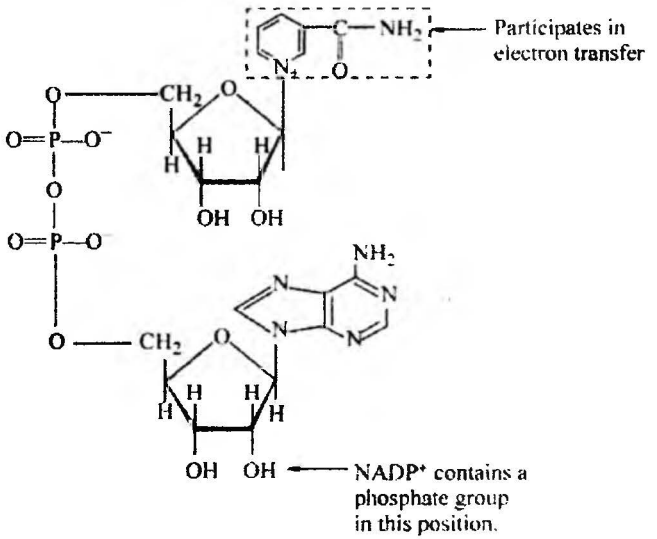
## Mechanism of cellular respiration



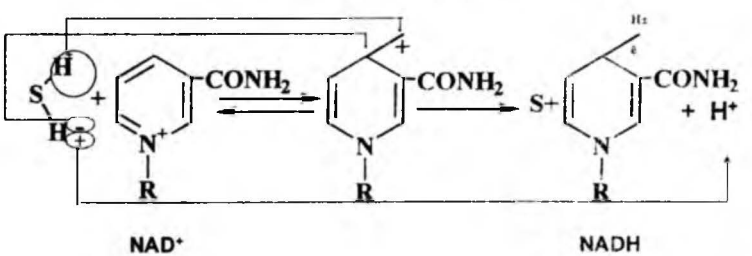

124

## Nicotinamide adenine dinucleotide ($NAD^+$)



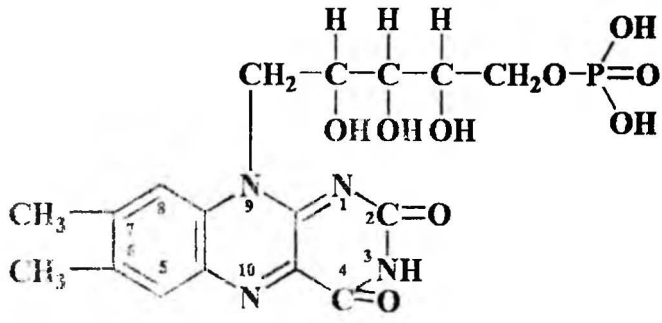

125

## Mechanism of substrate oxidation by NAD-linked dehydrogenases



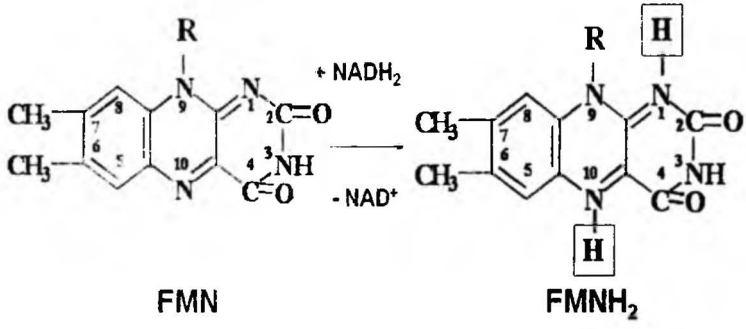

126

## Flavin mononucleotide (FMN)



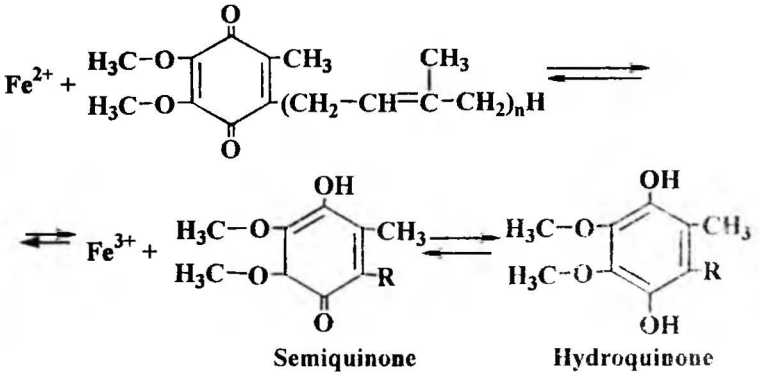
127

## Mechanism of NADH oxidation by FMN-linked dehydrogenases



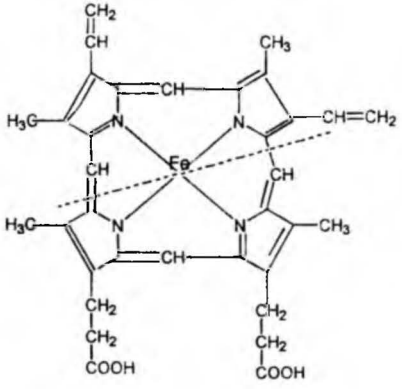

128

## Ubiquinone structure and mechanism of reduction




129

## Heme in cytochrome b




Iron protoporphyrin IX

130

## Heme is attached to apoprotein part of cytochromes by 5 and 6 coordination bonds of Fe

| *Cytochrome* | *5* | *6* |
|---|---|---|
| «b» | His | His |
| «c» | His | Met, covalent bond of vinyl group of heme with Cys |
| «a» | $NH_2$-group of amino sugar | $O_2$, HCN, CO |

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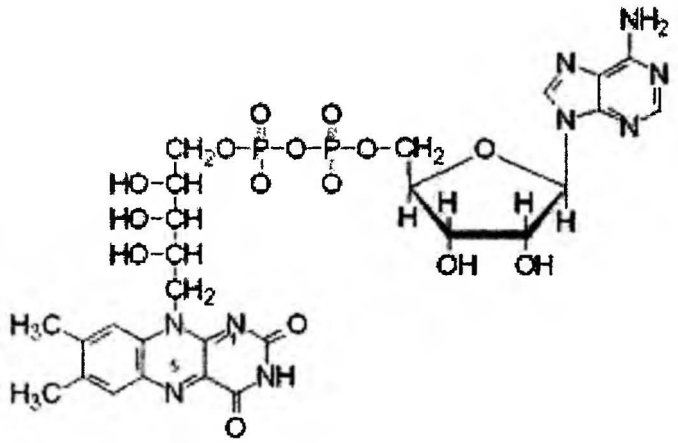
## Flavin adenine dinucleotide (FAD)



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## Structural organization of electron transport chain

| *Complex* | *Composition* |
|---|---|
| I<br>NADH dehydrogenase | FMN, FeS - centers |
| II<br>Succinate dehydrogenase | FAD, FeS - centers |
| III<br>$QH_2$- dehydrogenase | Cytochromes b, $c_1$,<br>FeS - centers |
| IV<br>Cytochrome oxidase | Cytochromes a, $a_3$,<br>$Cu^{2+}$ |

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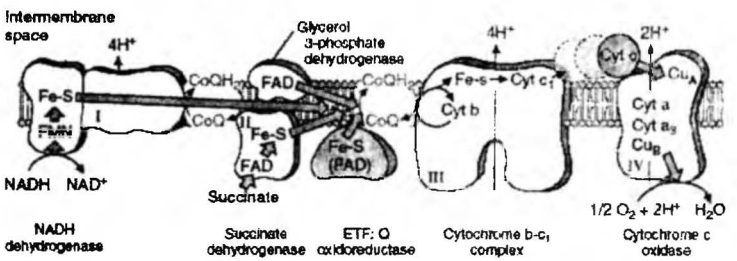
## The electron transport chain, showing the respiratory complexes

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## ADP + Pi → ATP

The process of synthesizing ATP from ADP and Pi coupled with the transport of electrons produced by substrate oxidation through a respiratory chain and creation of the proton gradient in the inner mitochondrial membrane is known as **oxidative phosphorylation**.

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## P/O ratio

- P/O ratio refers to the number of inorganic phosphate molecules utilized for ATP generation for every atom of oxygen consumed.
- P/O ratio represents the number of molecules of ATP synthesized per pair of electrons carried through electron transport chain.

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## Sites of oxidative phosphorylation in electron transport chain

1. Oxidation of $FMNH_2$ by coenzyme Q (complex I);
2. Oxidation of cytochrome b by cytochrome $c_1$ (complex III);
3. Cytochrome oxidase reaction (complex IV).

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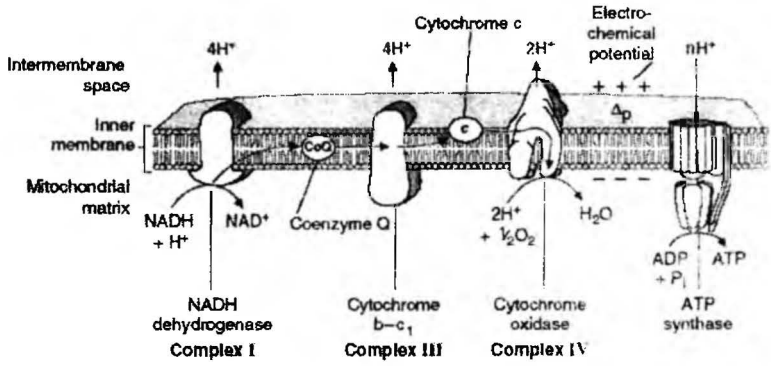
## Coupling $e^-$ transport and oxidative phosphorylation

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## Efficiency coefficient of complete respiratory chain

Substrate oxidation energy – 220 kJ/mol

ATP synthesis: 30,5 * 3 = 91,5 kJ/mol

*30,5 kJ/mol – synthesis of 1 ATP;*
*3 – number of synthesized ATP via complete respiratory chain*

$$\text{Efficiency coefficient} = \frac{91{,}5 \times 100}{220} \approx 41\%$$

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## Hypotheses of mechanisms of oxidative phosphorylation

1. **Chemical Coupling Hypothesis** (E. Slater, A.L. Lehninger) proposes that electron transport is coupled to ATP synthesis by a sequence of consecutive reactions in which a high-energy covalent intermediate is formed by electron transport and subsequently is cleaved and donates its energy to make ATP. These reactions are believed to be analogous to the substrate level phosphorylation.

$$NADH + FMN \rightarrow FMN\,H_2 + NAD^+$$
$$NADH + X \rightarrow NAD + XH_2$$
$$XH_2 + H_3PO_4 \rightarrow X{\cdot}H_2{\cdot}H_3PO_4$$
$$X{\cdot}H_2{\cdot}H_3PO_4 + FMN \rightarrow FMN\,H_2 + X{\sim}H_3PO_4$$
$$X{\sim}H_3PO_4 + ADP \rightarrow X + ATP$$

2. **Mechanochemical or conformational hypothesis** (P. Boyer) is envisaged that the energy released in the transport of electrons along the respiratory chain causes the conformational changes in the inner mitochondrial membrane and that this energy-rich condensed structure, in turn, is utilized for ATP synthesis.

3. **Chemiosmotic theory according to Peter Mitchell.**
   1. The theory postulates that oxidation of substrates in the respiratory chain generates protons ($H^+$) driven by the respiratory chain complexes I, III, and IV, each of which acts as a proton pump, which are ejected from the inside to the intermembrane space of membrane in the mitochondrion.
   2. Inner membrane is impermeable to protons, which accumulate intermembrane space, creating an electrochemical potential difference ($\Delta\mu H^+$) across the membrane composed of the membrane potential and the proton gradient.
   3. The transmembrane flow of protons to the matrix down their concentration gradient through specific protein channels provides the free energy for synthesis of ATP. This is performed by a membrane ATP synthase that couples proton flow to phosphorylation of ADP.

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## Chemiosmotic theory

**Transport of electrons through electron transport chain from substrate to $O_2$ (cellular respiration)**

↓

**Protons are pumped from the mitochondrial matrix to the intermembrane space and generation an electrochemical gradient**

↓

**Proton influx from the intermembrane space to the mitochondrial matrix through the ATP synthase pore generates ATP**

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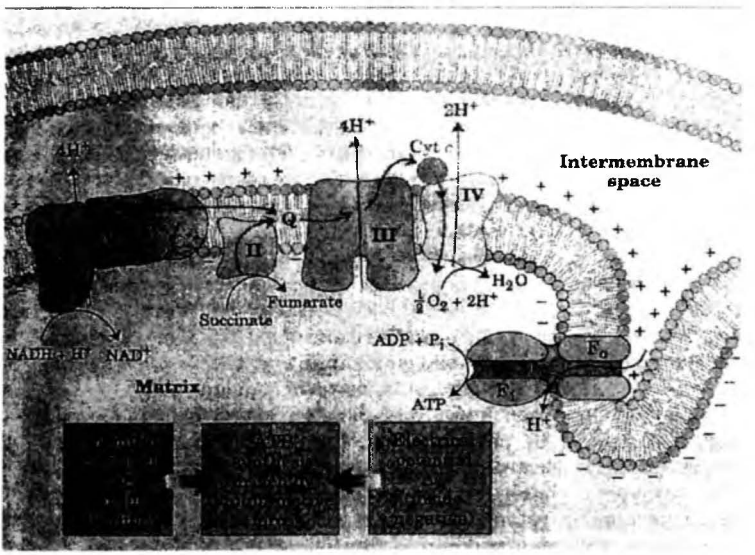
## Chemiosmotic theory

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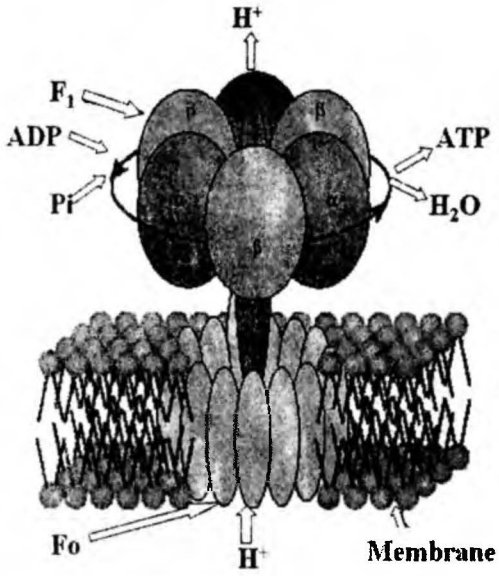


**$H^+$/ATP synthase**

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## Role of $H^+$/ATP synthase:

1. $F_0$ serves as a channel for protons flowing back into the matrix from intermembrane space.
2. $F_1$ is an enzyme that hydrolyses ATP to ADP and Pi.
3. $F_0$ - $F_1$ complex generates ATP.

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**For the synthesis of ATP during cellular respiration following are required:**

1. Substrates of oxidation;
2. $O_2$;
3. Substrates of phosphorylation (ADP and Pi).

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**Respiratory control**

**- alteration of respiration rate in mitochondria with the alteration of ADP concentration**

**States of respiratory control**

| | *Conditions limiting the rate of respiration* |
|---|---|
| State 1 | Availability of ADP and substrate |
| State 2 | Availability of substrate only |
| State 3 | The capacity of the respiratory chain itself, when all substrates and components are present in saturating amounts. Availability of ADP only |
| State 4 | Availability of ADP only |
| State 5 | Availability of oxygen only |

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## Uncouplers

Some chemicals valinomycin, 2,4-dinitrophenol, rotenone, $H_2S$, cyanide, azide and CO, oligomycin, barbiturates (amobarbital), dimercaprol, antimycin A, carboxin, malonate uncouple mitochondria by carrying H ions across the inner mitochondrial membrane and hence dissipate the proton gradient. More than 60% of oxidation energy derived from uncoupled electron transport is released as heat → hyperthermia.

There are endogenous uncouplers phenols, unsaturated fatty acids, and their peroxides.

Uncoupling of oxidation with phosphorylation is at extreme temperature, radiation.

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## Hypoenergetic conditions:

1) No substrates of oxidation (starvation);

2) Low levels of oxygen (hypoxia):
- lack of oxygen in the air;
- failure of oxygen supply to cardiovascular and respiratory systems due to their damage;
- anemias of different origin.

3) Mitochondrial damage (or uncouplers).

4) Different types of hypovitaminosis – deficiency of vitamins PP, $B_2$, $B_1$.

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## Primary uncoupling respiratory systems

***Peroxidase oxidation:***

Oxidases catalyze the removal of hydrogen from a substrate using oxygen as a hydrogen acceptor. They form hydrogen peroxide ($H_2O_2$) as a reaction product. Substrates are aldehydes. amines, D- and L- amino acids, purines.

$$SH_2 + O_2 \rightarrow S + H_2O_2 + Q$$

***Oxygenase oxidation:***

Oxygenases may be divided into 2 subgroups:

**1. Dioxygenases (Oxygen transferases, True oxygenases)** incorporate both oxygen atoms ($O_2$) into the substrate.

$$S + O_2 \rightarrow SO_2$$

Examples of this type include enzymes that contain iron such as homogentisate dioxygenase and 3-hydroxyanthranilate dioxygenase from the supernatant fraction of the liver, and enzymes utilizing heme such as L-tryptophan dioxygenase from the liver.

**2. Monooxygenases (Mixed-function oxidases, Hydroxylases)** incorporate only one atom of oxygen into the substrate. The other oxygen atom is reduced to water, an additional electron donor or cosubstrate being necessary for this purpose. Substrates are benzpyrene, aminopyrine, aniline, morphine, and benzphetamine and endogenous substances such as steroid hormones.

$$S\text{-}H + O_2 + ZH_2 \rightarrow S\text{-}OH + H_2O + Z,$$

**Z is cosubstrate**

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**Main components of microsomal oxidation are:**

1) Apolar compounds containing aliphatic or aromatic rings;

2) Cytochrome P-450;

3) $O_2$;

4) $NADPH_2$.

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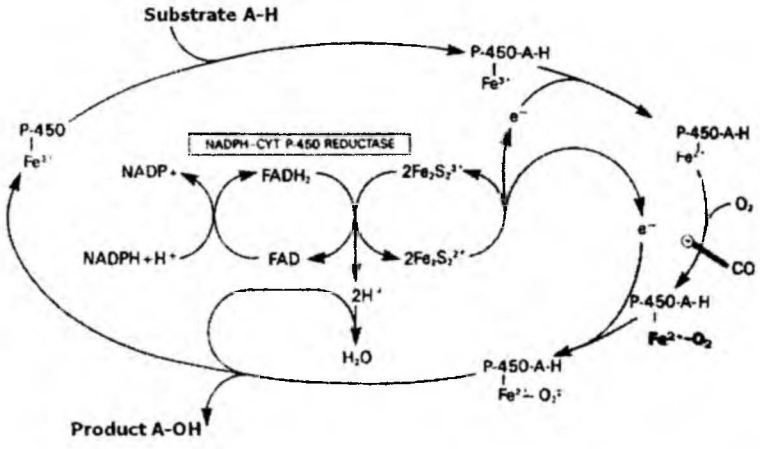
## Microsomal oxidation

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## Reaction mechanism of microsomal oxidation

1. In the resting state, in cytochrome P-450 the heme has $Fe^{3+}$. The substrate binds near the heme group.
2. Transfer of a first ē from $NADPH_2$ reduces $Fe^{3+}$ to $Fe^{2+}$ that is able to bind an $O_2$.
3. Transfer of a second ē reduces the bound $O_2$ to the superoxide anion $O_2^{\cdot-}$.
4. Uptake of $H^+$ gives rise to $H_2O$.
5. The activated oxygen atom inserts itself into a C–H bond in the substrate, thereby forming an OH group.
6. Dissociation of the product returns the enzyme to its initial state.

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## Functions of microsomal oxidation:

1. Hydroxylation of many drugs as the 1-st step of xenobiotic detoxication.
2. Synthesis of bile acids and steroid hormones from cholesterol and eicosanoids as well as the formation of unsaturated fatty acids.

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## Free radical oxidation

Oxygen is a potentially toxic substance is due to the involvement of oxygen free radicals:

$$O_2 + \bar{e} \rightarrow O_2^{-\bullet} \text{ (superoxide)}$$

$$O_2^{-\bullet} + H^+ \rightarrow HO_2^{\bullet} \text{ (peroxide)}$$

$$O_2^{-\bullet} + HO_2^{\bullet} + H^+ \rightarrow O_2 + H_2O_2 \text{ (hydrogen peroxide)}$$

$O_2^{-\bullet}$ is formed during oxidations in the respiratory chain:

$$EnzH_2 + O_2 \rightarrow EnzH + O_2^{-\bullet} + H^+$$

$O_2^{-\bullet}$ is especially dangerous. It does not itself react readily with cellular constituents, but it will combine with peroxides to form hydroxyl radicals ($OH^{\bullet}$), which are reactive:

$$O_2^{-\bullet} + H_2O_2 \xrightarrow[Fe^{3+}\ Fe^{2+}]{} O_2 + OH^- + OH^{\bullet} \text{ (hydroxyl radical)}$$

$H_2O_2$ and $O_2^{-\bullet}$ are toxic to cells as they attack the unsaturated fatty acid components of membrane lipids, thus damaging the membrane structure.

$$H_2O_2 + Fe^{2+} \rightarrow OH^- + OH^{\bullet} + Fe^{3+}$$

$O_2^{-\bullet}$ can reduce oxidized cyt c:

$$O_2^{-\bullet} + \text{Cyt C } (Fe^{3+}) \rightarrow O_2 + \text{Cyt C } (Fe^{2+})$$

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## Free radicals and diseases

- Atherosclerosis.
- Diabetes mellitus and diabetic angiopathy.
- Respiratory diseases (chronic obstructive bronchitis, bronchial asthma, emphysema, lung edema).
- Rheumatico- inflammatory and moronic diseases of joints (rheumatoid arthritis).
- Cataract.
- Cancer.
- Aging.
- Intoxications.
- Inflammatory disorders – chronic glomerulonephritis and ulcerative colitis.
- Other diseases - Parkinson's disease, Alzheimer's disease, multiple sclerosis, liver cirrhosis, muscular dystrophy, toxemia of pregnancy.

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## Antioxidative system (AOS)

**- system of organism protection against toxic action of oxygen. AOS includes enzymatic and non-enzymatic antioxidants**

**Enzymatic antioxidants:** enzymes *superoxide dismutase, peroxidase, catalase.*

### *Superoxide dismutase*

*Superoxide dismutase* is a high active specific enzyme; protect aerobic organisms against the potential deleterious effects of superoxide. The *dismutase* is present in all major aerobic tissues.

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$$

*Hydroperoxidases (peroxidases* and *catalase)* protect the body against harmful hydrogen peroxides.

### *Glutathione peroxidase*

*Peroxidases* are found in leukocytes, erythrocytes, and other tissues involved in eicosanoid metabolism.

$$H_2O_2 + 2HS\text{-Glutathione} \rightarrow H_2O + G\text{-}SS\text{-}G$$

The pentose phosphate pathway supplies $NADPH+H^+$, which is needed to regenerate glutathione. The oxidized glutathione can be reduced by the enzyme *glutathione reductase*:

$$NADPH_2 + G\text{-}SS\text{-}G \rightarrow 2\ GSH + NADP^+$$

### *Catalase*

*Catalase* is a hemoprotein containing 4 heme groups. It is found in blood, bone marrow, mucous membranes, kidney, and liver.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

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## Non-enzymatic antioxidants

1) Vitamin E (Tocopherol) is fat soluble vitamin. It is an antioxidant present in all cellular membranes, and protects against lipid peroxidation. α-Tocopherol can directly act on oxyradicals (e.g., $O_2^{\cdot}$, $OH^{\cdot}$), and thus serves as an important chain breaking antioxidant.

2) Vitamin C (Ascorbic acid) is water-soluble antioxidant. It is efficiently scavanges free radicals, and inhibits lipid peroxidation. It also promotes the regeneration of α-Tocopherol (from α-tocopheroxyl radical produced during scavenging of free radicals); Uric acid is powerful scavenger of singlet oxygen ($^1O_2$) and $OH^{\cdot}$ radicals.

3) Bioregulators - thyroxin, steroid hormones.

4) Compounds containing SH-group - glutathione, cysteine.

5) Fe binding complexons.

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## Antioxidants according to lipid peroxidation:

*1)* Preventive antioxidants that will block the initial production of free radicals, e.g. *catalase, glutathione peroxidase.*

2) Chain breaking antioxidants that inhibit the propagative phase of lipid peroxidation. e.g. *superoxide dismutase,* vitamins E, C, uric acid.

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## Lipid peroxidation in membrane can have effects

1. Increased membrane rigidity.
2. Decreased activity of membrane-bound enzymes (e.g. Sodium pumps).
3. Altered activity of membrane receptors.
4. Altered permeability.

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## Nutrient antioxidants and their dietary sources

| *Antioxidant* | *Dietary source* |
|---|---|
| Vitamin C (ascorbic acid) | Cherry, papaya, orange, grapes, watermelons, melons, grapefruit, kiwi fruit, strawberry, gooseberry (amla), guava, cauliflower, cabbage, spinach |
| Vitamin E (α-tocopherol) | Cotton seed oil, sunflower seed oil, peanut oil, whole grains, legumes, almonds, hazelnut, leafy vegetables mayonnaise, egg yolk, butter |
| β-carotene | Dark green and yellow-orange vegetables and fruits: carrots, insipid potato, tomato, spinach, pumpkin, watermelon, papaya, apricot, spinach, turnip |

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# Common pathways of catabolism

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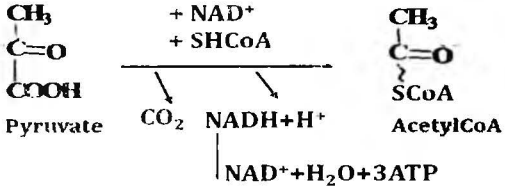
## Oxidative decarboxylation of pyruvate




The NADH produced by the pyruvate decarboxylation is reoxidized and the energy released is used to synthesize 3 ATP by oxidative phosphorylation.

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## Pyruvate dehydrogenase complex consists of the 3 enzymes:

1. Pyruvate dehydrogenase (coenzyme is thiamin diphosphate);
2. Dihydrolipoyl transacetylase (cofactors are coenzyme A and lipoic acid);
3. Dihydrolipoyl dehydrogenase (cofactors are FAD and $NAD^+$).

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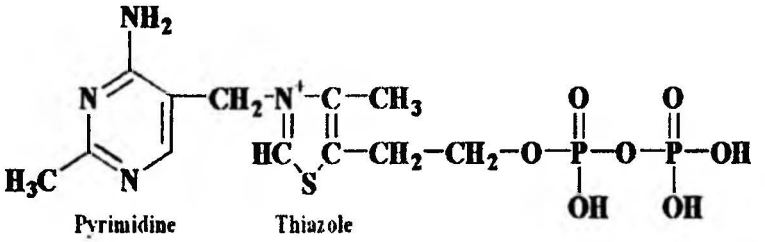
## Thiamin diphosphate (TDP)

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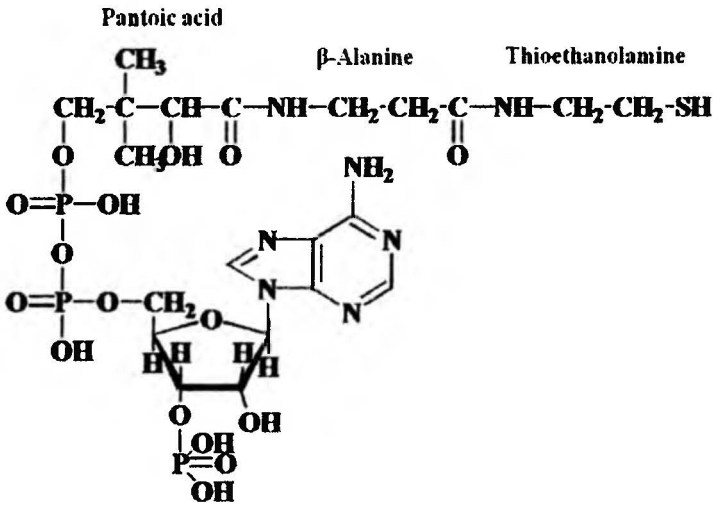
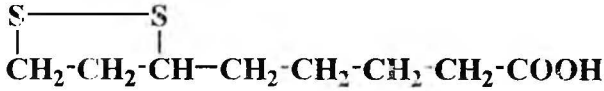
## Coenzyme A (HSCoA)




165

## Lipoic acid (LA)



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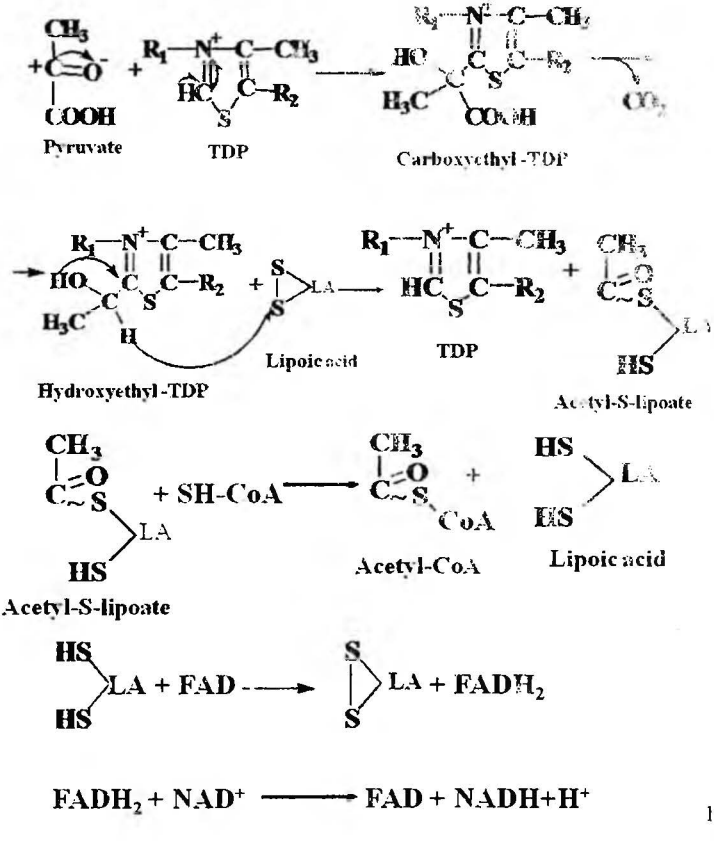
## Mechanism of the pyruvate decarboxylation

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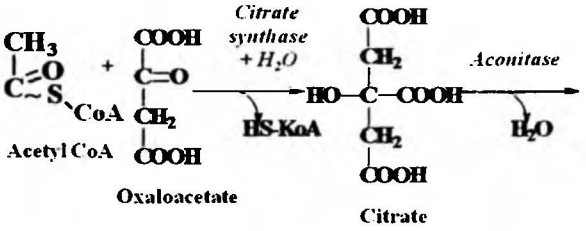
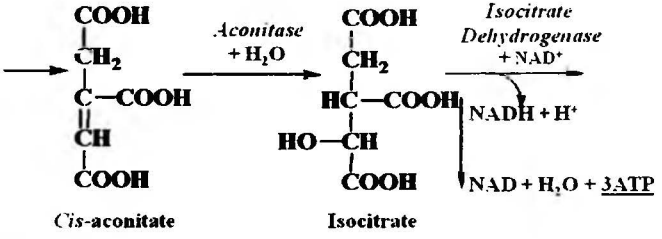
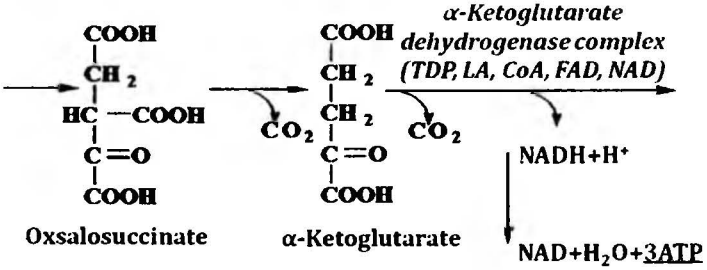
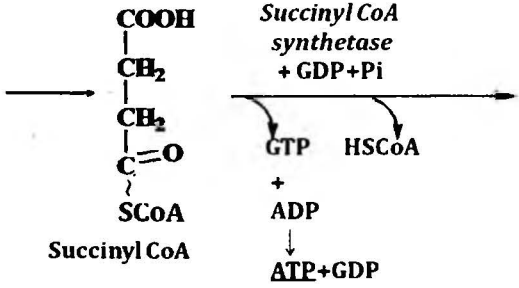
# Citric acid cycle

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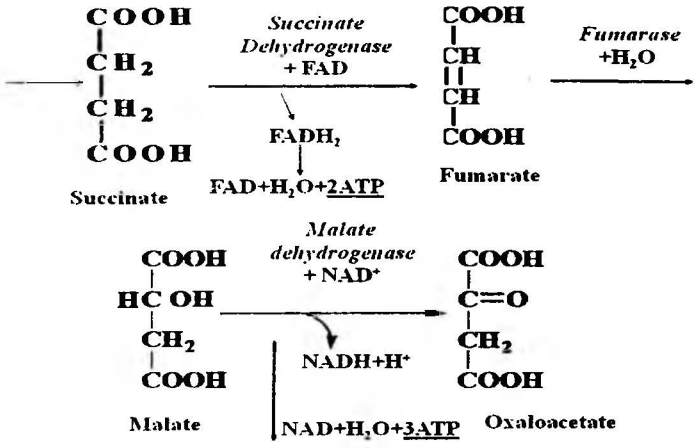
## Citric acid cycle
*(continuation)*

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## Regulation of citric acid cycle

Three enzymes regulate citric acid cycle:

1. *Citrate synthase* is activated by substrate, and inhibited by ATP and NADH.
2. *Isocitrate dehydrogenase* is activated by ADP, and inhibited by ATP and NADH.
3. *Succinate dehydrogenase* is activated by substrate (succinate), phosphate, and inhibited by oxaloacetate.

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## Functions of citric acid cycle (CAC)

1. CAC is the common pathway of acetylCoA oxidation that is the product of carbohydrate, lipid and protein degradation by successive decarboxylations and dehydrogenations.

2. CAC is the generator of protons and electrons to respiratory chains: 11 ATP molecules produced from the reoxidation of the 3 NADH (3 ATP×3 NADH) and 1 $FADH_2$ (2 ATP×1 $FADH_2$) by oxidative phosphorylation.

3. Energetic function: 1 ATP molecule is produced directly from the cycle by substrate-level phosphorylation.

4. Amphibolic function: CAC is the third stage of catabolism and is the first stage of anabolism.

5. Integrative function: CAC unifies the catabolic pathways of carbohydrates, lipids and proteins.

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# Metabolism of carbohydrates

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## Biological role of fiber

1. Helps to maintain the normal motility of gastrointestinal tract and prevents constipation.
2. Adsorbs large quantities of water and also the toxic compounds produced by intestinal bacteria that lead to increased fecal mass and its easier expulsion.
3. The lower incidence of cancers of gastrointestinal tract (e.g., colon and rectum).
4. Improves glucose tolerance by the body. This is mainly done by a diminished rate of glucose absorption from the intestine.
5. Decreases the absorption of dietary cholesterol from the intestine. Fiber binds with the bile salts and reduces their enterohepatic circulation. This causes increased degradation of cholesterol to bile salts and its disposal from the body.
6. Adds to the weight of the foodstuff ingested and gives a sensation of stomachfullness.

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## Glucose transport through biological membranes

1. Transport of glucose through the apical membrane of intestinal, choroid plexus and kidney epithelial cells depends on the presence of secondary active $Na^+$/glucose symporters, which concentrate glucose inside the cells, using the energy provided by cotransport of $Na^+$ ions down their electrochemical gradient.
2. Facilitated diffusion of glucose through the cellular membrane is otherwise catalyzed by glucose carriers (GLUT). Molecule movement by such transporter proteins occurs by facilitated diffusion.

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## Digestion of carbohydrates

| *Source of secretion* | *Enzyme* | *Substrate* | *End product* |
|---|---|---|---|
| Salivary glands | Salivary amylase | Starch, glycogen | Maltose plus 1:6 glycosides (oligosac-charides) plus malto-triose |
| Pancreas | Pancreatic amylase | Starch, glycogen | Maltose plus 1:6 glycosides (oligosac-charides) plus malto-triose |
| Small intestine | Sucrase | Sucrose | Fructose, glucose |
| | Maltase | Maltose | Glucose |
| | Lactase | Lactose | Glucose, galactose |
| | Trehalase | Trehalose | Glucose |
| | Isomaltase or 1:6 glucosidase | 1:6 glucosides | Glucose |

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## Types of GLUT

Five glucose transporters in the cell membranes have been identified. They exhibit tissue specificity.

**GLUT-1** is ubiquitously expressed and transport glucose in most cells. Responsible for the low-level of basal glucose uptake required to sustain respiration in all cells. It is found at highest levels in erythrocytes and also in the protective membrane of the blood vessels in the brain (blood-brain barrier) and in fetal tissues.

**GLUT-2** is expressed by renal tubular cells and small intestinal epithelial cells that transport glucose, liver cells and pancreatic β cells. GLUT-2 has a lower affinity for glucose than GLUT-1. GLUT-2 is most active when high levels of glucose are present, such as after food. Bidirectionality is required in liver cells to uptake glucose for glycolysis, and release of glucose during gluconeogenesis. In pancreatic β-cells, free flowing glucose is required so that the intracellular environment of these cells can accurately gauge the serum glucose levels. Gucose is transported from the intestinal mucosal cell into the portal circulation by GLUT-2.

**GLUT-3** is the primary glucose carrier for neurons. It is also the main transporter in the placenta and testes.

**GLUT-4** is abundant in striated muscle (skeletal muscle and cardiac muscle) and adipose tissue. GLUT-4 is an insulin-regulated glucose transporter that has a high affinity for glucose. Adipocyte cells and the skeletal system both require insulin as well as a glucose transporter to absorb glucose molecules from the bloodstream. Insulin is released from the pancreas which then attaches to receptors on the adipocyte and skeletal cell membranes. As GLUT-4 is an insulin responsive protein, it is alerted to the presence of the insulin bound to the receptors on the cell membrane. The GLUT-4 molecule is then able to transport the glucose molecule across the cell membrane and into the cell.

**GLUT-5** transports fructose in intestine and testis.

**All carbohydrates of tissues are divided into 2 groups:**

1). Carbohydrates with mainly energetic function:
   1. 1 molecule of glucose oxidation gives 38 molecules of ATP. From glucose all other carbohydrates of organism are formed, except ascorbic acid.
   2. Polysaccharides (starch in plants, glycogen in animal cells). Glycogen, a large polymer of glucose residues, provides an important energy reserve for the body.

2). Carbohydrates with mainly structural function:
   1. Glycolipids and glycoproteins are abundant in the plasma membrane of eukaryotic cells but are also involved in specific interactions (for example, they are receptors).
   2. Glycosaminoglycans are a part of a connective tissue. Some of them (e.g., heparin) perform regulatory function.

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## Sources and utilization of blood glucose

**Sources of blood glucose:**

1. Dietary carbohydrates.
2. Degradation of glycogen in liver (glycogenolysis).
3. The synthesis of glucose from noncarbohydrate precursors such as glucogenic amino acids, lactate, glycerol in liver and kidney (gluconeogenesis).

**Utilization of blood glucose:**

1. Generation of energy at aerobic and anaerobic glucose oxidation.
2. Synthesis of other monosaccharides.
3. Synthesis of glycogen and heteropolysaccharides.
4. Synthesis of fat, some amino acids and other compounds.

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## Catabolism of carbohydrates

**I stage** – degradation of polymers to monomers, including digestion of carbohydrates in gastrointestinal tract and breakdown of glycogen in the cells of organs.

**II stage** – specific pathway of glucose catabolism to pyruvate under aerobic condition and to lactate under anaerobic condition.

**III stage** – common pathways of catabolism:
– oxidative decarboxylation of pyruvate;
– citric acid cycle.

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## Glucose catabolism under aerobic conditions

1. Specific pathway of glucose catabolism to pyruvate (glycolysis).

2. Common pathways of catabolism:
- oxidative decarboxylation of pyruvate to acetyl-CoA;
- oxidation of acetyl-CoA via citric acid cycle to $CO_2$ and $H_2O$.

3. Malate-oxaloacetate shuttle of NADH from cytoplasm to mitochondria.

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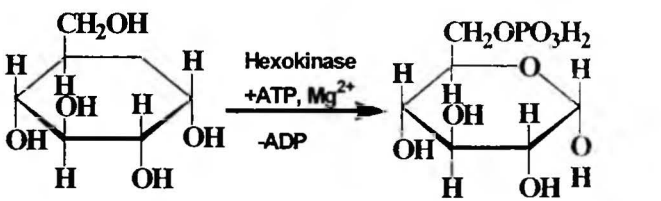
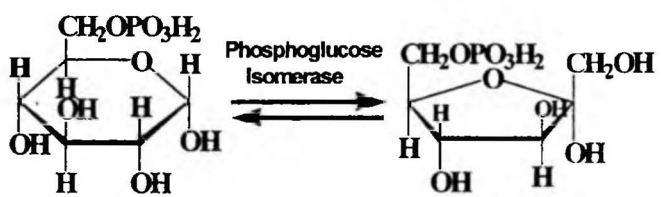
## Specific pathway of glucose catabolism

Glycolysis takes place in all cells of the body in the cytoplasm.
1. Glucose is phosphorylated to glucose 6-phosphate by *hexokinase* (all the tissues) or *glucokinase* (in liver). This is an irreversible reaction, dependent on ATP and $Mg^{2+}$ or $Mn^{2+}$. *Hexokinase* has low Km = $10^{-5}$M (high substrate affinity) therefore acts even at low concentration of glucose and is inhibited by glucose 6-phosphate. *Glucokinase* has high Km = $10^{-3}$M (low substrate affinity) therefore acts only at higher levels of glucose i.e., after a meal when blood glucose concentration is above 100 mg/dl and is not inhibited by glucose 6-phosphate. Glucose-6-phosphate is impermeable to the cell membrane; therefore it is a central molecule with a variety of metabolic pathways.




**Glucose** **Glucose-6-phosphate**

2. Glucose-6-phosphate is converted to fructose-6-phosphate by *phosphoglucose isomerase*. This is a reversible reaction.




**Glucose -6-phosphate** **Fructose-6-phosphate**

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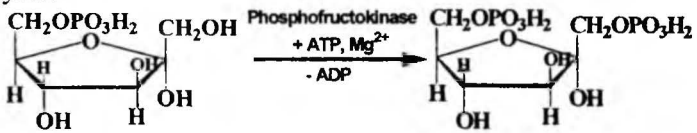
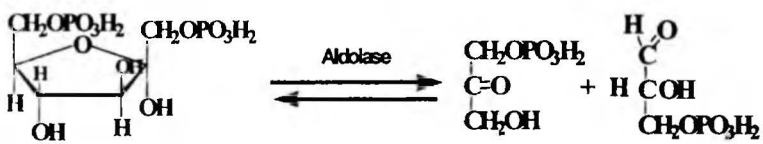
3. Fructose-6-phosphate is phosphorylated to fructose-1,6-bisphosphate by *phosphofructokinase*. This is an irreversible reaction, dependent on ATP and $Mg^{2+}$ and a regulatory step in glycolysis.



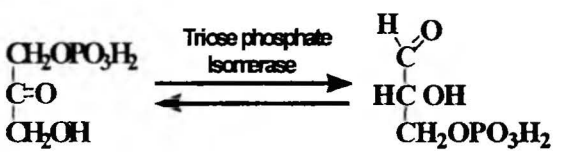

**Fructose-6-phosphate** **Fructose-1,6-bisphosphate**

4. *Aldolase* splits fructose-1,6-bisphosphate glycolysis to two three-carbon compounds, glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.




**Fructose-1,6-bisphosphate -** **Dihydroxyacetone-phosphate** **Glyceraldehyde--3- phosphate**

5. Dihydroxyacetone phosphate formed can rapidly be converted to glyceraldehyde-3-phosphate by *triose phosphate isomerase* as glyceraldehyde-3-phosphate is used by the rest of glycolysis. Thus, for each molecule of fructose-1,6-bisphosphate that is cleaved, two molecules of glyceraldehyde 3-phosphate continue down the pathway.




**Dihydroxyacetone-phosphate** **Glyceraldehyde --3-phosphate**

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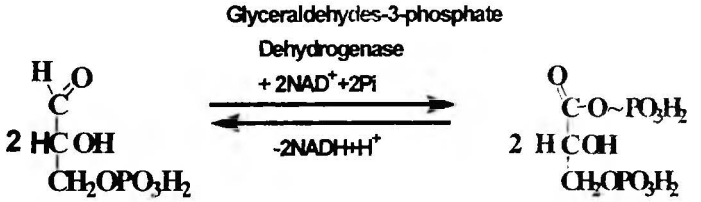
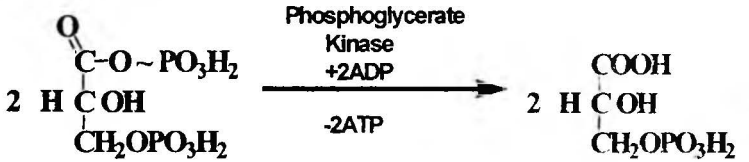
6. Glyceraldehyde-3-phosphate is converted to 1,3-bisphosphoglycerate. The reaction is catalyzed by *glyceraldehyde-3-phosphate dehydrogenase* and uses Pi and $NAD^+$. The other product is $NADH_2$. The energy for creating this high-energy phosphate bond comes from oxidation of the aldehyde group of the glyceraldehyde-3-phosphate.




**Glyceraldehyde-3-phosphate** **1,3-Bisphosphoglycerate**

7. The newly created high-energy phosphate bond of 1,3-bisphosphoglycerate is now used to synthesize ATP. *Phosphoglycerate kinase* catalyzes the transfer of the phosphoryl group from the 1,3-bisphosphoglycerate to ADP, generating ATP and 3-phosphoglycerate. This step is example of substrate level phosphorylation.




**1,3-Bisphosphoglycerate** **3-Phosphoglycerate**

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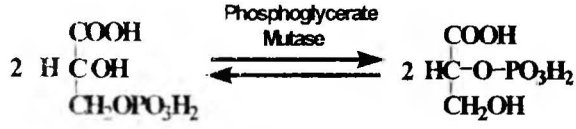
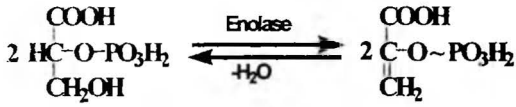
8. 3-Phosphoglycerate is converted to 2-phosphoglycerate by *phosphoglycerate mutase*. This is an isomerization reaction. Thus the reaction is a movement of the phosphate group to a different carbon atom within the same molecule.



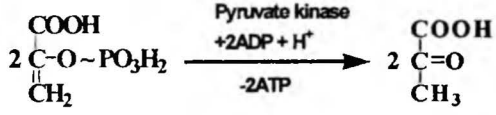

**3-Phosphoglycerate** **2-phosphoglycerate**

9. *Enolase* catalyzes the dehydration of 2-phosphoglycerate to form phosphoenolpyruvate (PEP). This reaction converts the low-energy phosphate ester bond of 2-phosphoglycerate into the high-energy phosphate bond of PEP, dependent on $Mn^{2+}$ or $Mg^{2+}$.




**2-Phosphoglycerate** **Phosphoenolpyruvate**

10. *Pyruvate kinase* catalyzes the transfer of the phosphoryl group from PEP to ADP to form ATP and pyruvate. This step also is a substrate level phosphorylation. *Pyruvate kinase* requires $K^+$ and either $Mn^{2+}$ or $Mg^{2+}$. Reaction is irreversible.




**Phosphoenolpyruvate** **Pyruvate**

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## Energy yield of glucose catabolism under aerobic conditions

1) Specific pathway of glucose catabolism to pyruvate (glycolysis) in cytoplasm.

Glucose ⟶ 2 Pyruvate + 2 $NADH_2$ + 2 ATP

(4 ATP (by substrate-level-phosphorylation) - 2 ATP (used in 1-st and 3-rd reactions).

2) Common pathways of catabolism in mitochondria.

- Oxidative decarboxylation of pyruvate:

2 Pyruvate ⟶ 2AcetylCoA+2$CO_2$+2$NADH_2$ (2×3 = 6 ATP)

- Citric acid cycle: 2 Acetyl-CoA ⟶ 4 $CO_2$ + 2×3 $NADH_2$ (6×3 = 18 ATP) + 2×1 $FADH_2$ (2×2 = 4 ATP) + 2×1 ATP.

3) Malate-oxaloacetate shuttle of $NADH_2$ from cytoplasm to mitochondria: 2 $NADH_2$ (2×3 = 6 ATP).

In summary = 38 ATP

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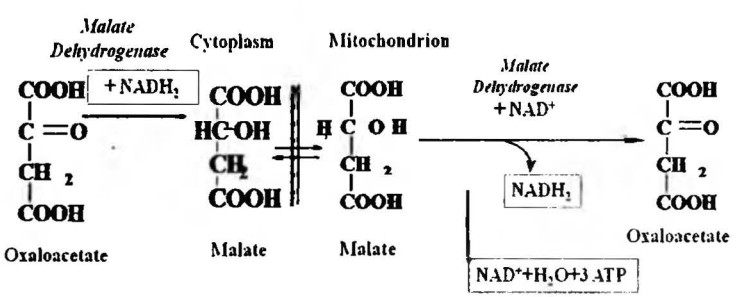
## Malate-oxaloacetate shuttle of $NADH_2$ from cytoplasm to mitochondria




The NADH produced is reoxidized and the energy released is used to synthesize 3 ATP by oxidative phosphorylation.

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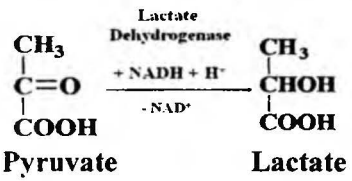
## Anaerobic glycolysis

1) Specific pathway of glucose catabolism to pyruvate (glycolysis).
2) Pyruvate is converted to lactate by lactate dehydrogenase. Reoxidation of NADH formed from oxidation of the glyceraldehyde-3-phosphate.




**Pyruvate** **Lactate**

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## Energy yield of glucose catabolism under anaerobic conditions

Specific pathway of glucose catabolism to pyruvate (glycolysis) in cytoplasm.

Glucose ⟶ 2 Pyruvate + 2 NADH
+ 4 ATP (by substrate-level-phosphorylation)
- 2 ATP (for phosphorylation in 1 and 3 reactions)
2 Pyruvate ⟶ 2 Lactate

In summary = 2 ATP

At glycogenolysis (anaerobic degradation of glycogen) one more ATP is generated. This is because no ATP is consumed for the activation of glucose (glycogen directly produces glucose-1-phosphate which forms glucose-6-phosphate). Thus, 3 ATP are produced from glycogen degradation.

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## All tissues may be divided into 3 groups according to a role of glucose oxidation:

1. Anaerobic glycolysis is a major pathway for ATP synthesis in tissues with few or no mitochondria, such as the medulla of the kidney, mature erythrocytes, leukocytes, and cells of the lens, cornea, and testes. The occurrence of uninterrupted glycolysis is very essential in skeletal muscle during strenuous intensive short-term exercise where oxygen supply is very limited.
2. Aerobic glycolysis is very essential for brain, retina, skin, renal medulla and gastrointestinal tract which are dependent on glucose for energy.
3. Neuroglial cells, hepatocytes, lipocytes. These cells synthesize lipids if necessary. Therefore products of glucose oxidation usually are used to synthesize fatty acids and glycerol.

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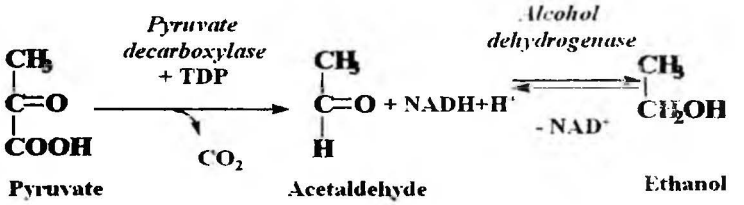
## Alcoholic fermentation

In yeast and some other microorganisms under anaerobic conditions, pyruvate formed from glucose is transformed into ethanol and $CO_2$, a process called **alcoholic fermentation**.

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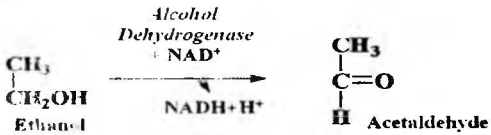
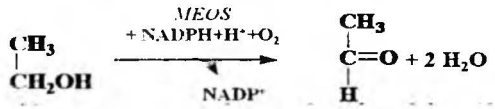
## Metabolism of ethanol

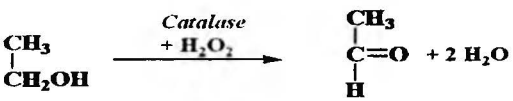
1. Ethanol is detoxified by the cytoplasmic ***alcohol dehydrogenase (ADH)***. The normal function of ADH is the oxidation of the small amounts of various alcohols produced by microbial fermentation processes that occur within the intestine.



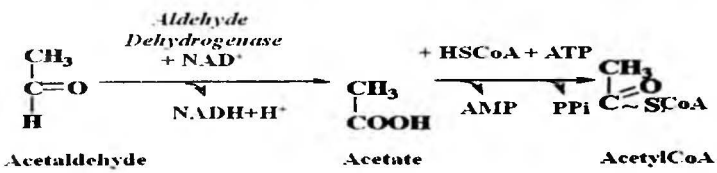

2. When the concentration of ethanol in hepatocytes becomes high, it is also detoxified by the ***microsomal ethanol-oxidizing system (MEOS)***.




3. Less important mechanism for ethanol detoxification involves ***catalase***, an enzyme found within peroxisomes.




Acetaldehyde is converted to acetate by ***aldehyde dehydrogenase*** which is located within the mitochondrial matrix. Acetate is metabolized in other tissues, such as cardiac and skeletal muscles.

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## Gluconeogenesis

**Gluconeogenesis is glucose biosynthesis from noncarbohydrate precursors in the liver and kidneys.**

1. During starvation the formation of glucose particularly uses **amino acids** produced by the protein breakdown and **glycerol** by fat breakdown.
2. During exercise the blood glucose levels required for brain and skeletal muscle function are maintained by gluconeogenesis using **lactate** produced by the glycolysis and **glycerol** by fat breakdown.

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## Glucose biosynthesis from lactate

Gluconeogenesis resembles the reversed pathway of glycolysis. The 7 (out of 10) reactions are common for both glycolysis and gluconeogenesis. Essentially, 3 reactions of glycolysis are irreversible catalyzed by the enzymes, namely *hexokinase*, *phosphofructokinase* and *pyruvate kinase*. These three steps have to be reversed in gluconeogenesis by using other enzymes:

**1. Conversion of pyruvate to phosphoenolpyruvate:** Lactate is converted to pyruvate by the *lactate dehydrogenase* in cytoplasm. Pyruvate formed is transported into the mitochondria and converted to oxaloacetate using the enzyme *pyruvate carboxylase* that is located in the mitochondrial matrix. Since the inner mitochondrial membrane is impermeable to oxaloacetate, it is converted to malate inside the mitochondria by *mitochondrial malate dehydrogenase*. The malate is transported through the mitochondrial membrane and then converted back to oxaloacetate in the cytosol by *cytoplasmic malate dehydrogenase*. The oxaloacetate is acted on by *phosphoenolpyruvate carboxykinase* which converts it to phosphoenolpyruvate.

**2. Conversion of fructose 1,6-bisphosphate to fructose-6-phosphate:** Phosphoenolpyruvate undergoes the reversal of glycolysis until fructose-1,6-bisphosphate is produced. The enzyme *fructose-1,6-bisphosphatase* converts fructose-1,6-bisphosphate to fructose-6-phosphate.

**3. Conversion of glucose-6-phosphate to glucose:** *Glucose-6-phosphatase* catalyses the conversion of glucose-6-phosphate to glucose.

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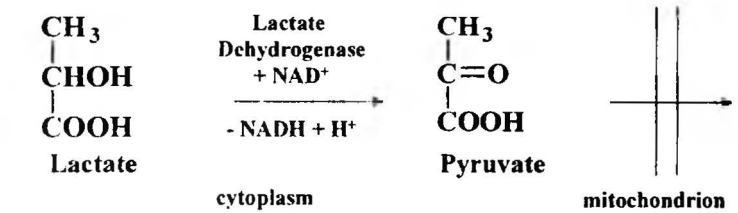
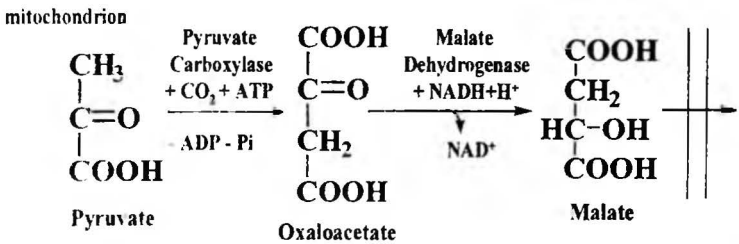
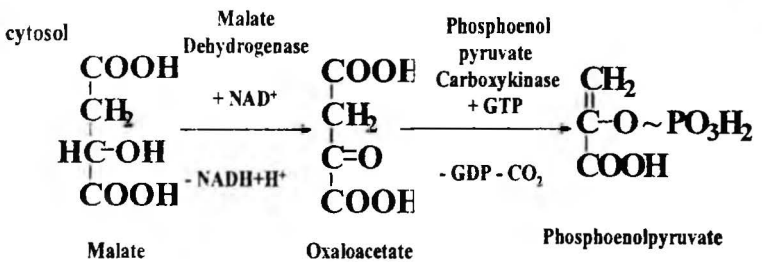
## Conversion of pyruvate to phosphoenolpyruvate

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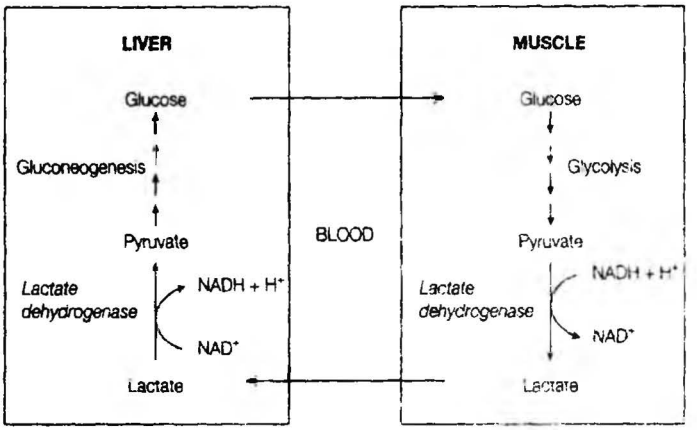
## Cori cycle

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## Pasteur effect - inhibition of anaerobic glycolysis by oxygen (aerobic condition)

Mechanism of effect:

1. In the aerobic condition, the levels of glycolytic intermediates from fructose-1,6-bisphosphate onwards decrease while the earlier intermediates accumulate. This clearly indicates that Pasteur effect is due to the inhibition of the enzyme *phosphofructokinase*.
2. The inhibitory effect of citrate and ATP (produced in the presence of oxygen) on *phosphofructokinase* explains the Pasteur effect.

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## Reciprocal regulation of glycolysis and gluconeogenesis

If glycolysis and gluconeogenesis operated simultaneously, the net effect would be a futile cycle resulting in the hydrolysis of two ATP and two GTP molecules.

This is prevented by reciprocal regulation at the enzyme steps that are distinct in each pathway. AMP activates *phosphofructokinase* (glycolysis) but inhibits *fructose-1,6-bisphosphatase* (gluconeogenesis). ATP and citrate inhibit *phosphofructokinase* but citrate stimulates *fructose-1,6-bisphosphatase*.

Thus glycolysis is inhibited in times when ATP and biosynthetic intermediates are in excess whilst gluconeogenesis is inhibited in times when the ATP level is low.

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## Pentose phosphate pathway

Pathway takes place in the cytosol and is particularly important in tissues such as liver, adipose tissue, adrenal gland, erythrocytes, testes and lactating mammary gland. Most of these tissues are involved in the biosynthesis of fatty acids and steroids which are dependent on the supply of NADPH.

The sequence of reactions of pentose phosphate pathway is divided into two phases-oxidative and non-oxidative.

**A. Oxidative phase:** Pentoses formation includes 2 dehydrogenation reactions. The coenzyme of dehydrogenases is $NADP^+$, which is reduced to $NADPH_2$. Pentoses are formed as a result of oxidative decarboxylation reactions.

**B. Non-oxidative phase:** The non-oxidative reactions are concerned with the interconversion of three, four, five and seven carbon monosaccharides. Hexoses are converted into pentoses, the most important being ribose-5-phosphate.

The overall reaction may be represented as:

6 Glucose-6-phosphate + 12 $NADP^+$ + 6 $H_2O$ ⟶ 5 Glucose-6-phosphate + 6 $CO_2$ +12 NADPH + 12 $H^+$.

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## Pentose phosphate pathway

| *Basic stages of process* | *Enzymes* |
|---|---|
| **Oxidative phase of pentoses formation:** Overall reaction: Glucose-6-phosphate + $2NADP^+$ + $H_2O$ → Ribulose-5-phosphate + $2NADPH$ + $+2H^+ + CO_2$ Oxidative phase includes 2 dehydrogenation reactions and oxidative decarboxylation reaction. | 1. *Glucose-6-phosphate dehydrogenase*<br>2. *Lactonase*<br>3. *6-phosphogluconate dehydrogenase* |
| **Non-oxidative phase of pentoses formation:** Overall reaction: 5 Fructose-6-phosphate ↔ 5 Ribose-5-phosphate Non-oxidative phase includes interconversion of three, four, five and seven carbon monosaccharides. | 1. *Transketolase*<br>2. *Transaldolase*<br>3. *Transketolase*<br>4. *Phosphopentose isomerase* |

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## Functions of pentose phosphate pathway

The two major products of the pathway are reduced nicotinamide adenine dinucleotide phosphate ($NADPH_2$) and ribose-5-phosphate.

1. NADPH is required for the reductive biosynthesis of fatty acids, cholesterol and certain amino acids.
2. Ribose-5-phosphate or its derivatives are useful for the synthesis of nucleic acids (RNA and DNA) and many nucleotides such as ATP, $NAD^+$, FAD and CoA.
3. Microsomal cytochrome P450 system (in liver) brings about the detoxification of drugs and xenobiotics by reactions involving NADPH.
4. Interconversions of monosaccharides from $C_3$ to $C_7$.

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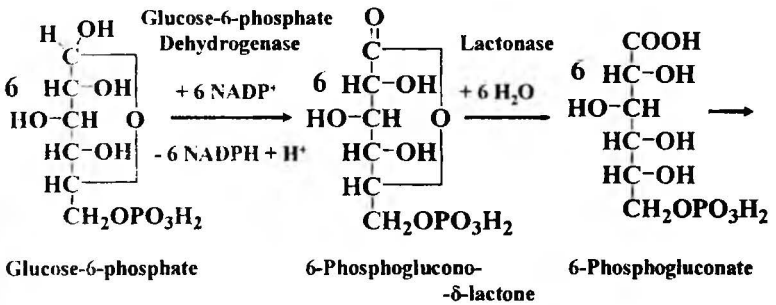
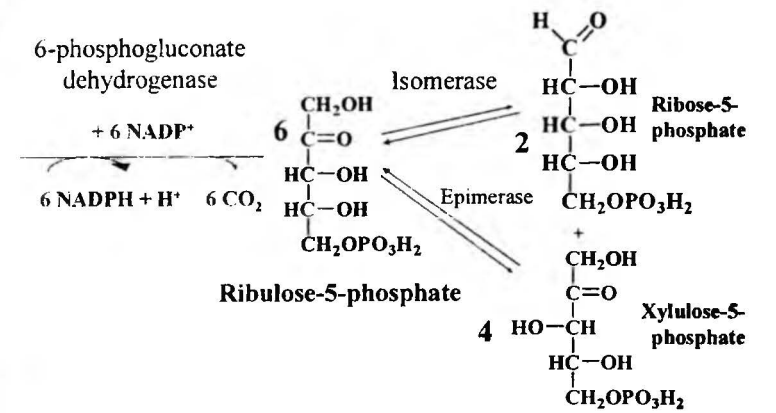
## Oxidative reactions of pentose phosphate pathway

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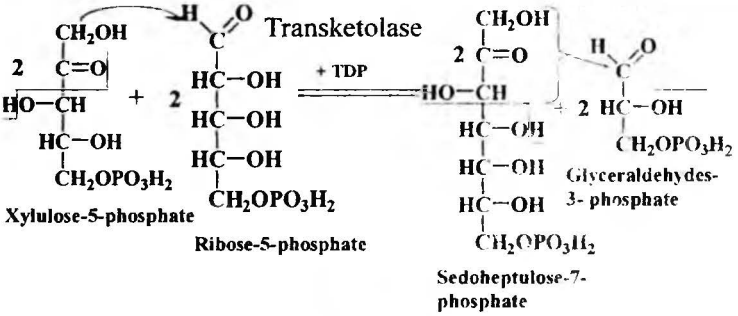
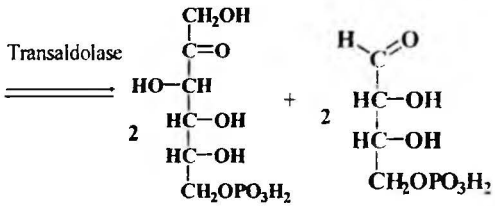
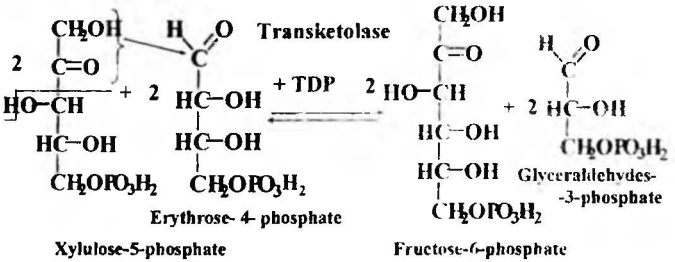
## Non-oxidative reactions of pentose phosphate pathway

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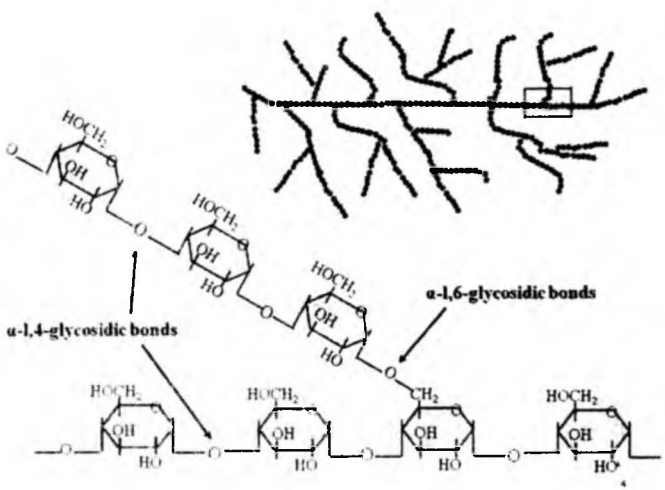
## Metabolism of glycogen

### Importance of glycogen «branching»

1. The glycogen molecule branches like a tree and has many nonreducing ends at which addition and release of glucose residues occur during synthesis and degradation, respectively.
2. Branching improves solubility of glycogen.

### The cell is not capable to deposit free glucose for following reasons:

1. Glycogen is high-molecular substance and, unlike glucose, influences osmotic pressure in a cell not very much. Accumulation of free glucose would lead to increase of osmotic pressure and, as result, to edema of a cell.
2. Glycogen is more stable molecule than glucose.

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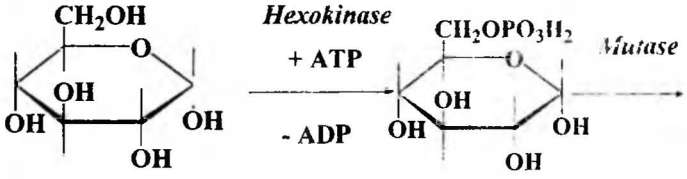
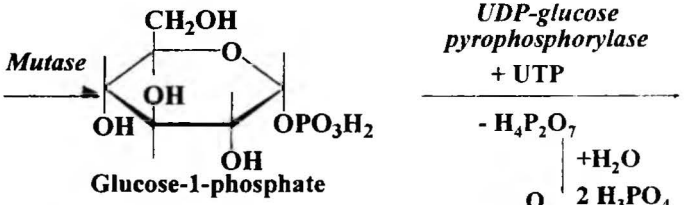
## Glycogen synthesis

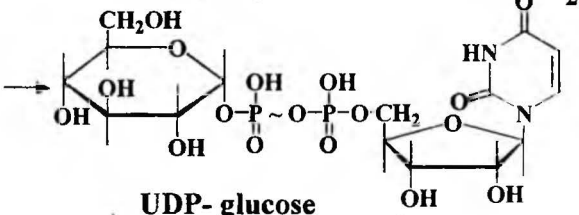
The two main storage sites of glycogen are the liver and skeletal muscle.

Glycogen synthesis consists of 3 stages:

1. **Formation of the UDP-glucose.**



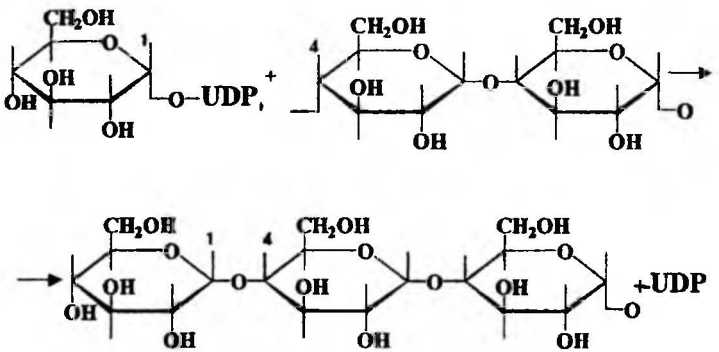



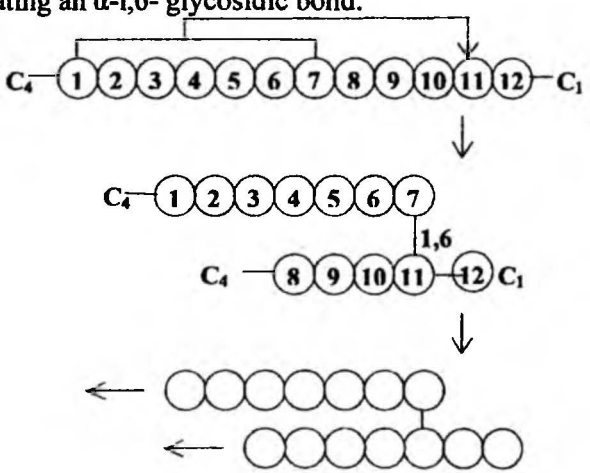
**UDP- glucose**
**(active form of glucose)**

## Glycogen synthesis

**2. Glycogen synthesis by *glycogen synthase*:** *Glycogen synthase* now transfers the glucosyl-residue from UDP-glucose to the C4-OH group at the nonreducing end of a glycogen molecule, forming an α-1,4-glycosidic bond.




**3. Formation of branches in glycogen:** After a number of glucose units (usually about 11) have been joined as a chain with α-1,4-linkages, ***branching enzyme*** breaks one of the α-1,4- glycosidic bonds and transfers a block of residues (usually about 7) to a more interior site in the glycogen molecule, reattaching these by creating an α-1,6- glycosidic bond.

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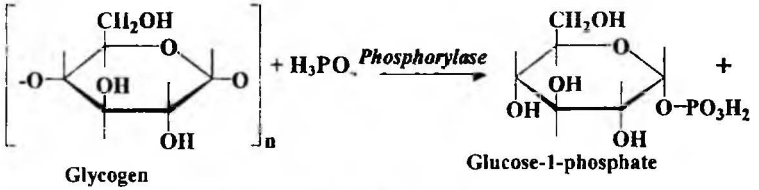
## Glycogen degradation is in 2 ways:

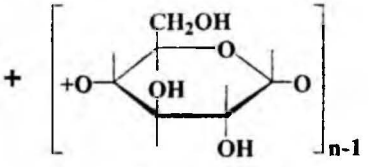
1. Degradation of glycogen occurs by ***acid maltase*** **or** ***α-1,4-glucosidase*** that is a lysosomal enzyme. This enzyme continuously degrades a small quantity of glycogen to free glucose molecules.

2. Phosphorolysis: The α-1,4-glycosidic bonds (from the non-reducing ends) are cleaved sequentially by the enzyme ***glycogen phosphorylase*** to yield glucose-1-phosphate.

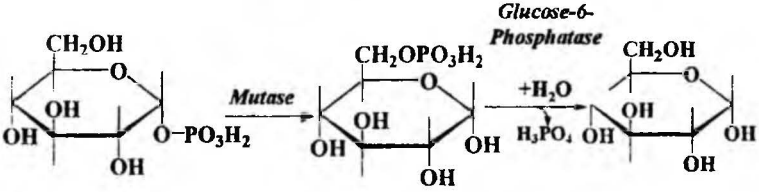



Glycogen

Glucose-1-phosphate




Glycogen (n-1)




Glucose-1-phosphate Glucose-6-phosphate Glucose

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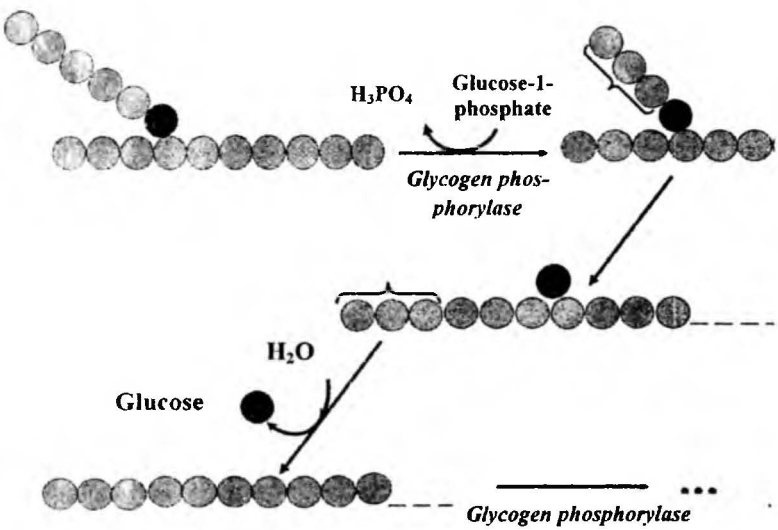
**Mechanism of glycogen degradation**

1. *Glycogen phosphorylase* removes glucose units (that are more than five residues from a branchpoint) sequentially from the nonreducing ends of a glycogen molecule, forming glucose-1-phosphate and continues until four glucose residues remain on either side of branching point (α-1,6-glycosidic bond).

2. *Oligo-α-1,4→1,6-glucantransferase* removes a fragment of three glucose residues attached at a branch and transfers them to another chain.

3. *Debranching enzyme* or *amylo-α-1,6-glucosidase* breaks the α-1.6-glycosidic bond at the branch with a single glucose residue and releases a free glucose.

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## The characteristics of the enzymes participating in regulation of glycogen metabolism

1. ***Protein kinase A*** consists of two regulatory subunits (R) and two catalytic subunits (C), making a complex $R_2C_2$ that is normally inactive. The binding of two molecules of cAMP to each of the R subunits leads to dissociation of the complex into a 2R complex and 2C subunits that are now catalytically active. $Ca^{2+}$ ions activate protein kinase in muscle.
2. ***Phosphorylase*** splits glycogen. Phosphorylase exists in phosphorylated active ''a'' form (tetramer) and dephosphorylated inactive ''b'' form (dimer). Phosphorylase ''b'' is converted into phosphorylase ''a'' by phosphorylation by the enzyme ***phosphorylase kinase***. Phosphorylase kinase in turn is activated by protein kinase A. The process can be reversed and phosphorylase ''a'' inactivated by removal of the phosphate group by ***protein phosphatase***.
3. ***Glycogen synthase*** synthesizes glycogen, forming an α-l,4-glycosidic bond. Glycogen synthase exists as dephosphorylated active glycogen synthase ''a'' (tetramer) and phosphorylated inactive glycogen synthase ''b'' (tetramer). Glycogen synthase ''a'' is converted into glycogen synthase ''b'' by phosphorylation by the enzyme ***protein kinase A***. The process can be reversed and glycogen synthase ''a'' activated by removal of the phosphate group by ***protein phosphatase***.
4. ***Adenylate cyclase*** converts ATP to 3'5' cyclic AMP (cAMP) (activated by epinephrine and glucagon, inhibited by insulin).
5. ***Phosphodiesterase*** acts on cAMP which is converted to 5'AMP (activated by insulin).

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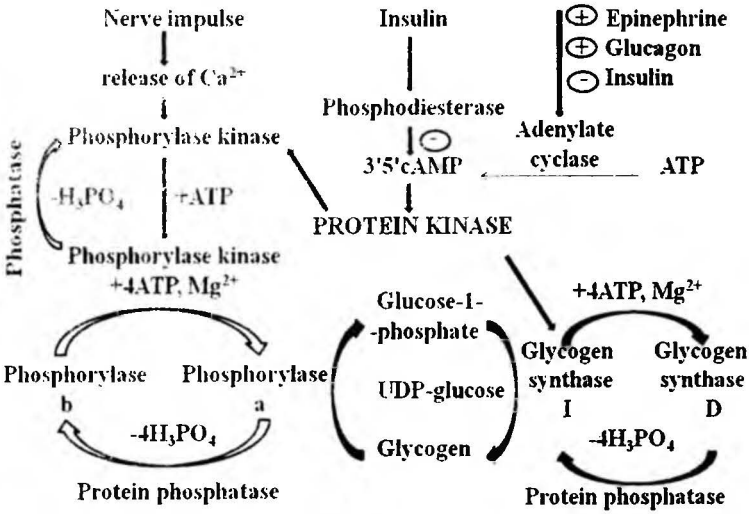
## Scheme of regulation of glycogen synthesis and degradation in muscle and liver

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**Features of glycogen degradation in liver and muscle**

| | Liver | Muscle |
|---|---|---|
| **The process scheme** | Glycogen<br>↓<br>Glucose-1-phosphate<br>↓<br>Glucose-6-phosphate<br>↓$H_3PO_4$<br>Glucose<br>↓<br>into the bloodstream | Glycogen<br>↓<br>Glucose-1-phosphate<br>↓<br>Glucose-6-phosphate<br>↓<br>Aerobic or anaerobic glycolysis |
| **Features of process** | Liver contains the enzyme glucose-6-phosphatase, which converts the glucose-6-phosphate to glucose | Muscle does not contain glucose-6-phosphatase |
| **Physiological** | Glucose-6-phosphate converts to glucose, which then diffuses out into the bloodstream and so maintains the blood glucose concentration. It enables the blood glucose level to be maintained between meals | In muscle the main aim is to produce energy quickly and so the glucose-6-phosphate is metabolized via glycolysis and is oxidized to yield energy for muscle contraction |

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**Glycogenosises - glycogen storage diseases caused by inherited defects of one or more enzymes involved in glycogen synthesis or degradation**

| *Type* | *Organs involved* | *Deficiency of enzyme* | *Symptoms* |
|---|---|---|---|
| I (E. von Gierke's disease) | Liver | Glucose-6-phosphatase | Hypoglycemia, lactoacidosis, hepatomegaly |
| II (I.C. Pompe's disease) | Heart, Liver, Muscle | Lysosomal(1,4) glycosidase | Muscular weakness, heart hypertrophy |
| III (Cori's disease) | Liver, Muscle | Debranching enzyme | Hypoglycemia, hepatomegaly |
| IV (Anderson's disease) | Liver | Branching enzyme | Hypoglycemia, hepatomegaly |
| V (B. Mac Ardle's disease) | Muscle | Phosphorylase | Cirrhosis, muscular exhaustion after loading myoglobinuria |
| VI (Her's disease) | Liver | Phosphorylase | Hepatomegaly |

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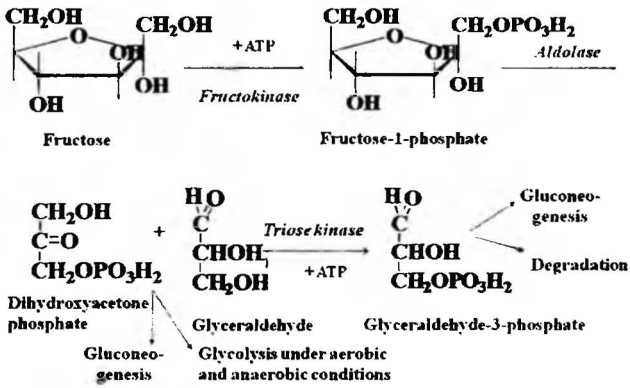
## Metabolism of fructose

1. In liver fructose is metabolized by the fructose-1-phosphate pathway.




2. In muscle and adipose tissue, fructose can be phosphorylated by *hexokinase* to form fructose-6-phosphate, which then enters glycolysis.

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## Defects in fructose metabolism

Fructose —(*Fructokinase*, 1)→ Fructose-1-phosphate —(*Aldolase*, 2)→

**1. Essential fructosuria.** Due to the deficiency of the enzyme hepatic ***fructokinase***, fructose is not converted to fructose-1-phosphate. This is an asymptomatic condition with excretion of fructose in urine. Treatment involves the restriction of dietary fructose.

**2. Hereditary fructose intolerance.** This is due to the absence of the enzyme ***aldolase***. Hereditary fructose intolerance causes intracellular accumulation of fructose-l-phosphate, severe hypoglycemia, vomiting, hepatic failure and jaundice. Fructose-1-phosphate inhibits liver *glycogen phosphorylase* and blocks glycogen degradation leading to hypoglycemia.

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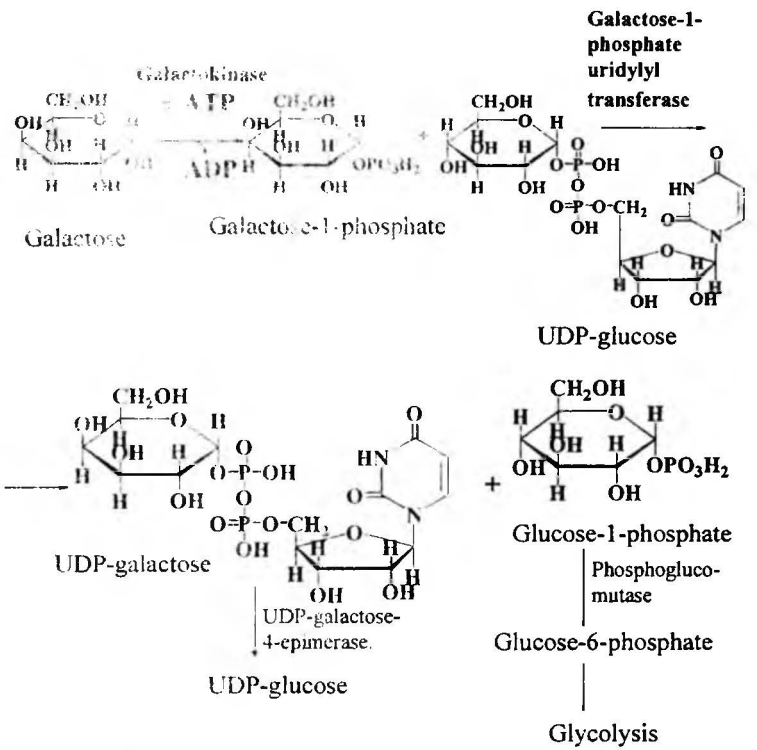
## Metabolism of galactose




Galactose metabolism is impaired leading to increased galactose levels in circulation (galactosemia) and urine (galactosuria). Galactosemia is due to the deficiency of the enzyme ***galactose-1-phosphate uridyltransferase***. It is a rare congenital disease in infants, inherited as an autosomal recessive disorder. The clinical symptoms of galactosemia are loss of weight (in infants), mental retardation, cataract, hepatosplenomegaly etc. The accumulation of galactose-1-phosphate in various tissues like liver, nervous tissue, lens and kidney leads to impairment in their function. The therapy includes the supply of diet deprived of galactose and lactose.

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# Metabolism of lipids. Eicosanoids

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**Lipids are biological substances that are insoluble in water but soluble in organic solvents such as chloroform and methanol.**

**Functions of lipids:**

1. **Food material.** Lipids are source of energy which is stored in the adipose tissues. 1g of lipid produces 9.3 kcal of heat.
2. **Structural component.** Lipids are an important constituent of the cell membrane.
3. **Heat insulation.** Lipids are characterized for their high insulating capacity.
4. **Hormone synthesis.** The sex hormones, adrenocorticoids, vitamin D are all synthesized from cholesterol.
5. **Vitamin carriers.** Lipids act as carriers of fat-soluble vitamins such as vitamin A, D and E.

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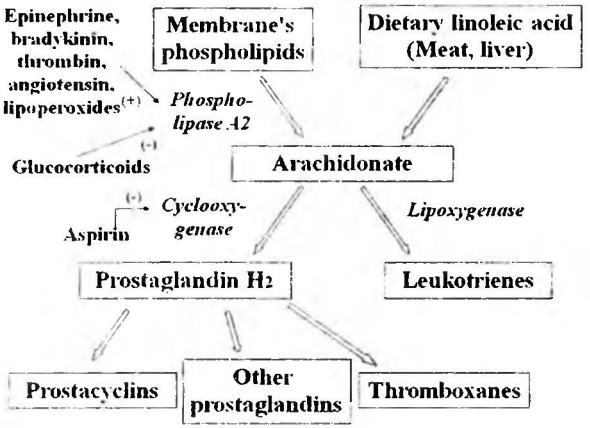
## Biosynthesis of eicosanoids

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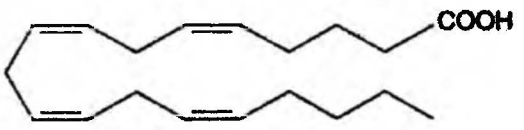
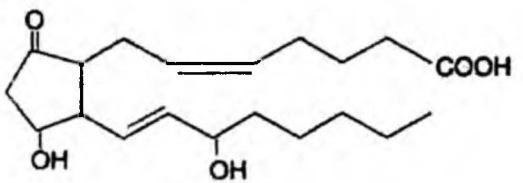



Arachidonic acid




$PGE_2$

**The structures of arachidonic acid and prostaglandin $PGE_2$**

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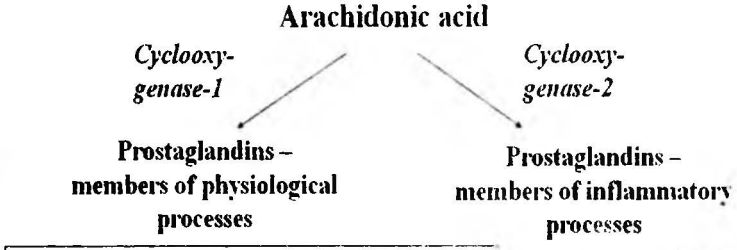
## Biochemical actions of prostaglandins




- lowering of blood pressure (PG-E, PG-A);
- inhibition of gastric HCl secretion (PG-E);
- decrease in immunological response (PG-E);
- induction of labor (PG-E2 and PG-F2);
- platelet aggregation: promotion by PG-E1 and thromboxan A2, inhibition by prostacyclins I2;
- promotion of fever and pain (PG-E2);
- PG-E is a bronchodilator, PG-F is a constrictor of bronchial smooth muscles;
- increasing of glomerular filtration rate (PG-E)

PG-E1 and PG-E2

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| | |
|---|---|
| **Leukotrienes** | 1. Stimulants of allergic reactions.<br>2. Implicated in asthma, inflammatory reactions, hypersensitivity (allergy) and heart attacks.<br>3. Cause contraction of smooth muscles, bronchoconstriction, and vasoconstriction, adhesion of white blood cells and release of lysosomal enzymes. |

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## Dietary lipids and digestion

| Food lipids | Hydrolase enzymes | Products of digestion |
|---|---|---|
| Triacylglycerols | Pancreatic lipase, lingual and gastric lipases | Glycerol, fatty acids, mono- and diacylglycerols |
| Phospholipids | Phospholipases $A_1$, $A_2$, C, D. | Fatty acids, glycerol, mono- and diacylglycerols, $H_3PO_4$, alcohols (choline, ethanolamine, glycerol, inositol or serine) |
| Cholesterol esters | Cholesterol esterase | Fatty acids, cholesterol |

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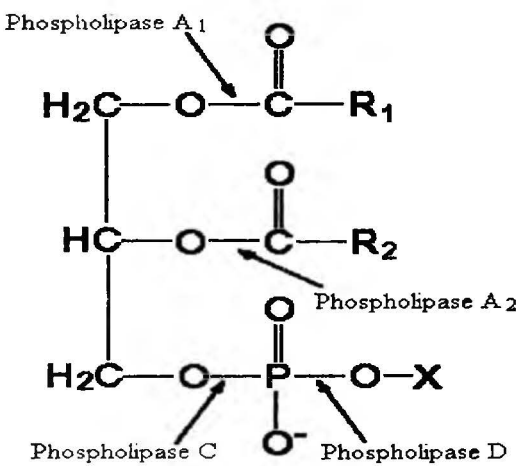
## Hydrolysis of phospholipids

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## Absorption of lipid digestion products

| *Hydrophilic products:* | *Hydrophobic products:* |
|---|---|
| ➢ Glycerol<br>➢ Fatty acids (C<12)<br>➢ $H_3PO_4$<br>➢ Choline<br>➢ Serine<br>➢ Ethanolamine | Cholesterol<br>Fatty acids (C>12)<br>Mono - and diacylglycerols<br>Bile acids<br>Micelles |

⇩ ⇩

**Enterocytes**

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## Role of bile acids in lipid digestion

1. Emulsification of dietary lipids.
2. Increase the effectiveness of pancreatic lipases.
3. Make PH optimum for lipase action.
4. Promote micelle formation for absorption of hydrophobic products of lipid digestion.

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## Lipoproteins

Lipoproteins are globular particles consisting of:

1. Hydrophobic core of triacylglycerols and cholesterol esters.
2. Amphipathic coat of protein, phospholipid and free cholesterol.

The protein components of lipoproteins are called apolipoproteins (or apoproteins).

Lipoproteins function as transport vehicles for lipids in blood plasma.

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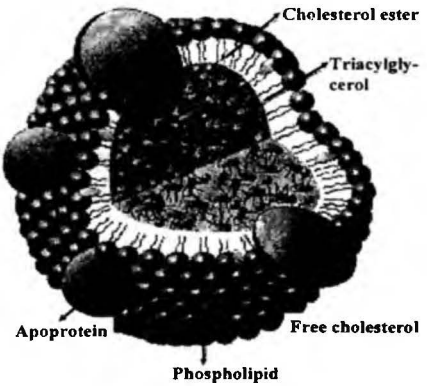
## Structure of lipoprotein

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## Characteristics of lipoproteins (LP)

| *LP names based on* | | *Function* | *Apo-proteins* |
|---|---|---|---|
| *Density* | *Electrophoretic mobility* | | |
| Chylomicrons (CM) | CM | Largest LP. Synthesized by gut after a meal. Main carrier of dietary triacylglycerols | A-I, B-48, C-II, E |
| VLDLs | pre-β-LP | Synthesized in the liver. Main carrier of endogenously produced triacylglycerols | B-100, C-II, E |
| LDLs | β-LP | Formed from VLDL in the circulation. Main carrier of cholesterol | B-100 |
| HDLs | α-LP | Smallest LP. Takes cholesterol from extrahepatic tissues to the liver for excretion | A-I, A-II, D, E |

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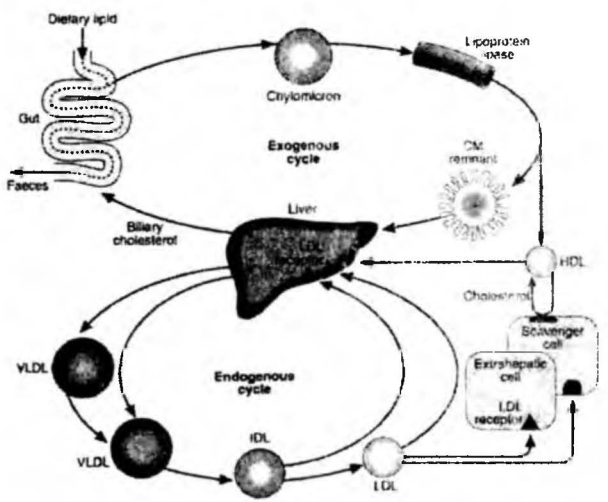
## Lipid transport

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## Fatty acid oxidation consists of 3 stages:

1. Activation of fatty acids and transport into mitochondria;
2. β-Oxidation pathway;
3. Acetyl-CoA oxidation via the citric acid cycle.

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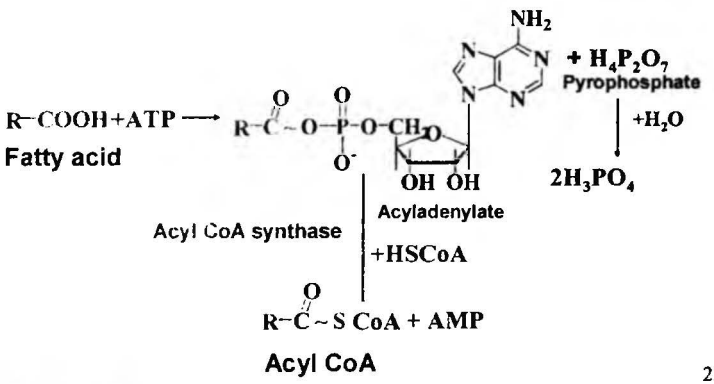
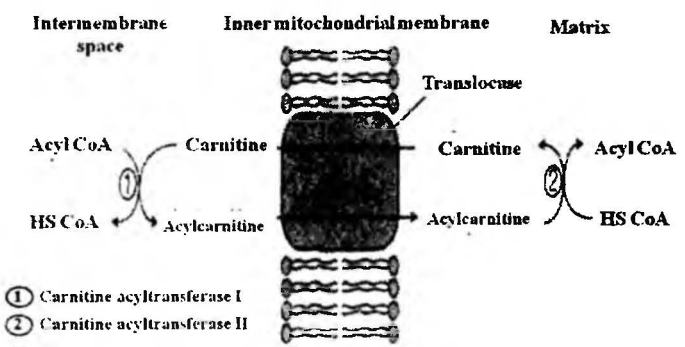
## Activation of fatty acid in the cytosol



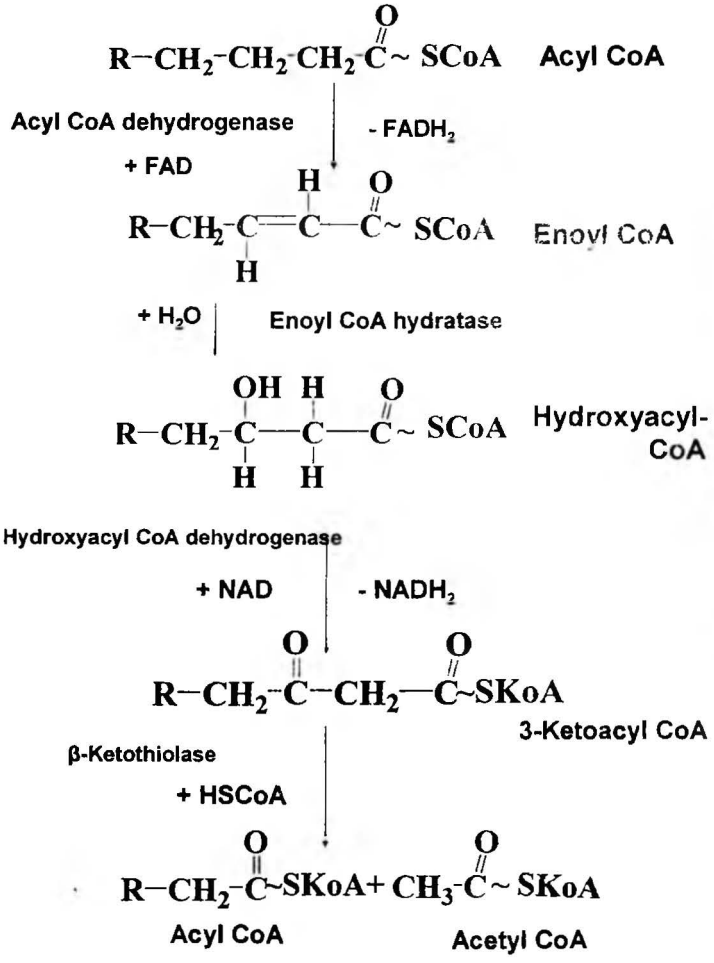

230

## Transport of acyl CoA into mitochondria




231

## β-Oxidation pathway




232

## Calculation of the ATP yield of fatty acid oxidation:

**$[5 \cdot (n/2\text{-}1) + n/2 \cdot 12] - 1$,** where

- 5 - number of ATP produced by oxidation of NADH (3 ATP) and $FADH_2$ (2 ATP) in each round of oxidation;
- (n/2-1) - number of oxidation rounds;
- n/2 - number of acetyl CoA;
- 12 - number of ATP produced by acetyl CoA oxidation in the citric acid cycle;
- number of ATP used for the fatty acid activation.

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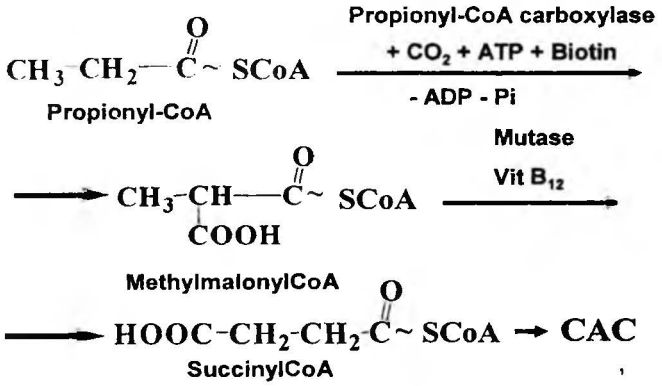
## Fatty acids oxidation having an odd number of carbon atoms

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## Glycerol oxidation

### Sources of glycerol:

1) Dietary triacylglycerols (Tg) hydrolysis by *pancreatic lipase.*

2) Tg hydrolysis in chylomicrons and VLDL by *lipoprotein lipase* in capillaries of adipose tissue and muscle.

3) Tg hydrolysis in IDL by *hepatic TG lipase* in blood stream.

4) Tg hydrolysis in adipose tissue by *intracellular lipases.*

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## Energy yield of glycerol oxidation to $CO_2$ and $H_2O$

| | |
|---|---|
| 1. Glycerol activation | - 1 ATP |
| 2. *Glycerolphosphate gehydrogenase* | + 2 ATP |
| 3. Catabolism of glyceraldehyde-3-phosphate to pyruvate: | |
| Substrate-level-phosphorylation | + 2 ATP |
| NADH + $H^+$ malate-oxaloacetate shuttle | + 3 ATP |
| 4. Oxidative decarboxylation of pyruvate | + 3 ATP |
| 5. AcetylCoA oxidation via CAC | + 12 ATP |
| In summary: | 21 ATP |

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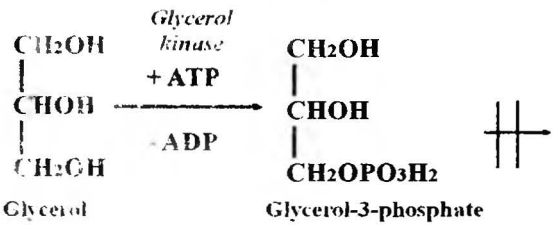
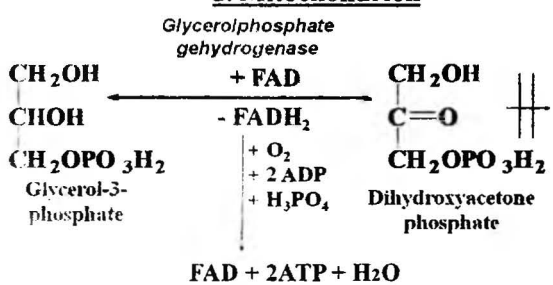
## Glycerol oxidation

**Intestine, kidney, liver**



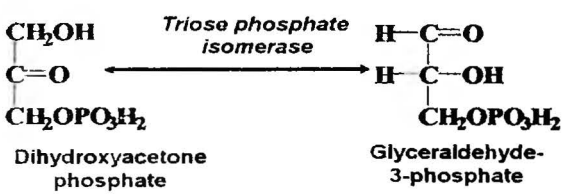

**1. Cytosol**

**2. Mitochondrion**



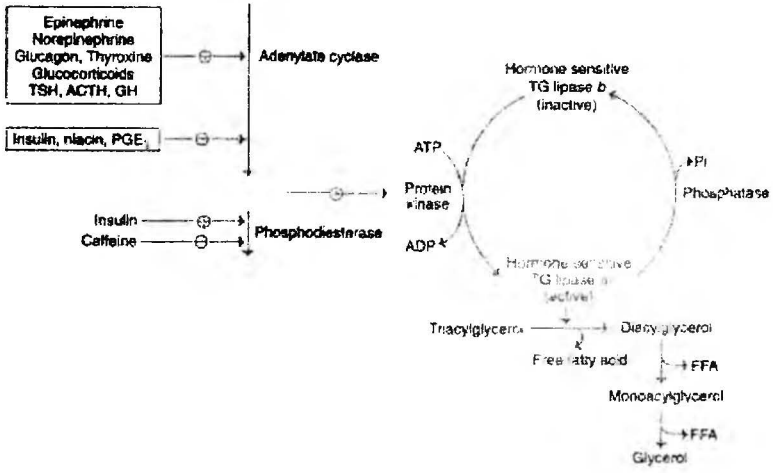

FAD + 2ATP + $H_2O$

**3. Cytosol**




237

## Control of lipolysis in adipose tissue

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## Acetyl-CoA sources

1. β-Oxidation of fatty acids.
2. Catabolism of ketogenic amino acids.
3. Oxidative decarboxylation of pyruvate.

## Use of Acetyl-CoA

1. Oxidation via CAC to $CO_2$ and $H_2O$ with loss of energy.
2. Ketone bodies synthesis.
3. Cholesterol synthesis.
4. Fatty acids synthesis.

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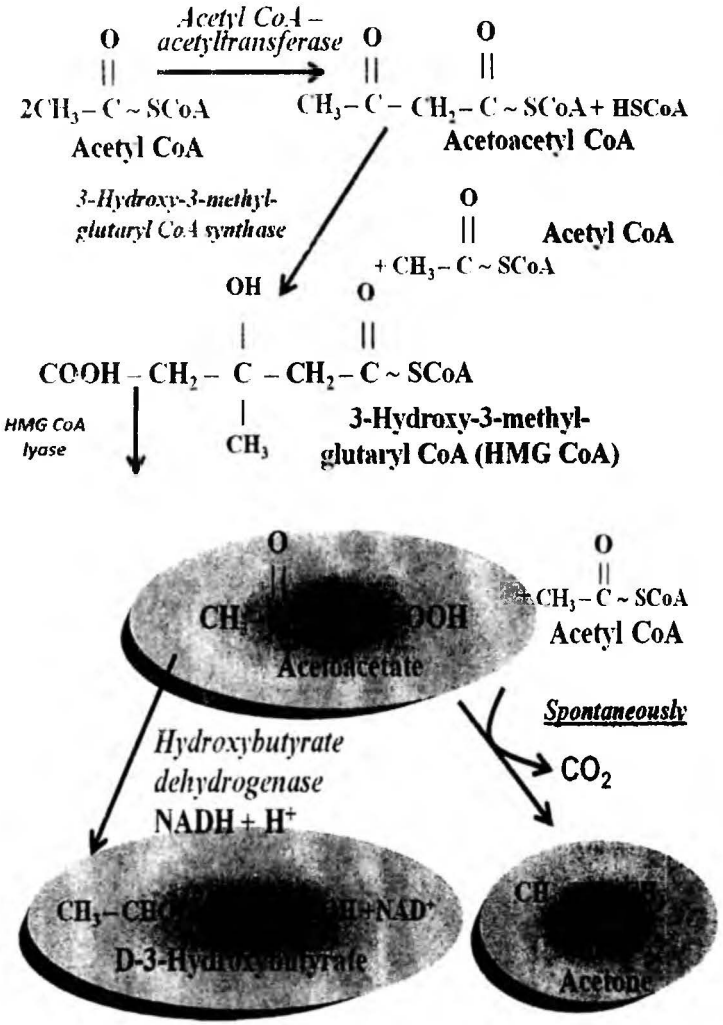
## Ketone bodies synthesis (mitochondria of liver)

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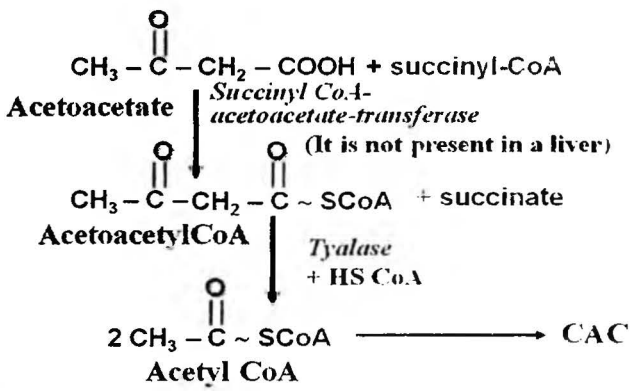
## Use of ketone bodies

**Heart muscle, kidney cortex, sceletal muscle, brain**

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## Biosynthesis of cholesterol

Liver - 80 %

Intestine - 10 %

Skin - 5 %

Other tissues - 5 %

800 mg daily.

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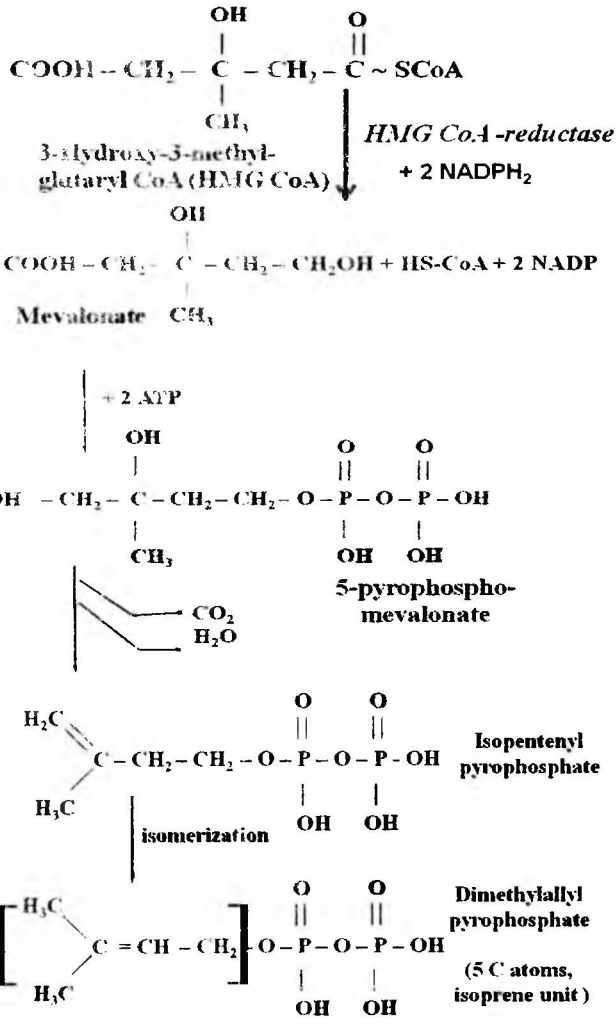
# Biosynthesis of cholesterol

**1- st stage– formation of isopentenyl pyrophosphate**

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## Biosynthesis of cholesterol *(continuation)*

**2-nd stage. The C5 isoprene units in isopentenyl pyrophosphate are then condensed to form the C30 compound squalene**

3 C 5 units —*Condensation*→ Farnesyl (15 C atoms)

2 Farnesyl —*Condensation*→ Squalene (30 C atoms)

Squalene → Lanosterol (30 C atoms)

Lanosterol → Cholesterol (27 C atoms)

**Regulation of HMG CoA reductase activity**

| Activation: | Inhibition: |
|---|---|
| • Radiation action | • Starvation |
| • Insulin introduction | • Thyroidectomy |
| • Introduction of thyroid hormones | • Introduction of glucagon |
| • Hypophysectomy | • Introduction of glucocorticoids |
| | • Introduction of the nicotinic acid large doses |
| ↓ | ↓ |
| Increasing of cholesterol synthesis | Inhibition of cholesterol synthesis |

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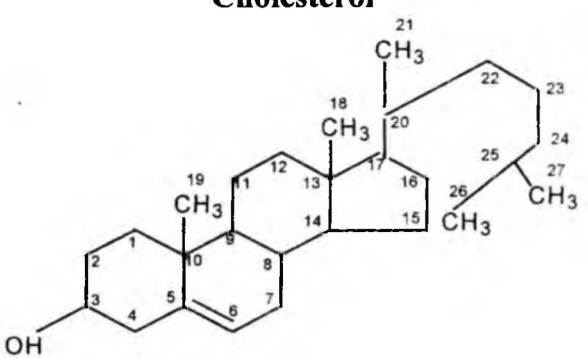
## Cholesterol

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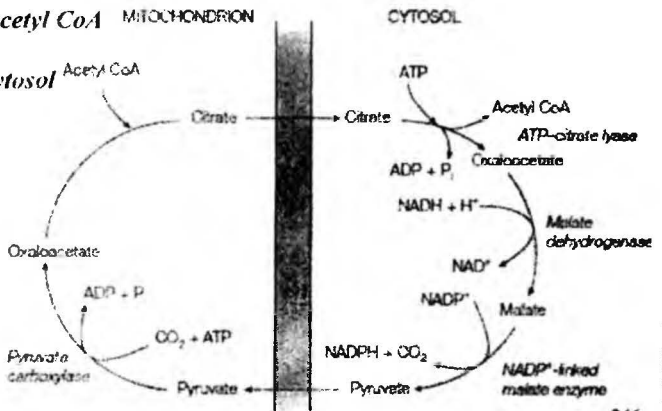
## Fatty acid synthesis

***Consists of 3 stages:***

1. Acetyl CoA transport into the cytosol;
2. Formation of malonyl CoA;
3. The elongation steps of fatty acid synthesis.

***1 stage: Acetyl CoA transport into the cytosol***

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## *2-nd stage: Formation of malonylCoA*

$$CH_3 - \underset{\underset{O}{\|}}{C} \sim SCoA + CO_2 + ATP \xrightarrow[\text{Biotin}]{\text{Acetyl CoA carboxylase}}$$

**Acetyl CoA**

$$COOH - CH_2 - \underset{\underset{O}{\|}}{C} \sim SCoA + ADP + Pi$$

**Malonyl CoA**

***Committed step***

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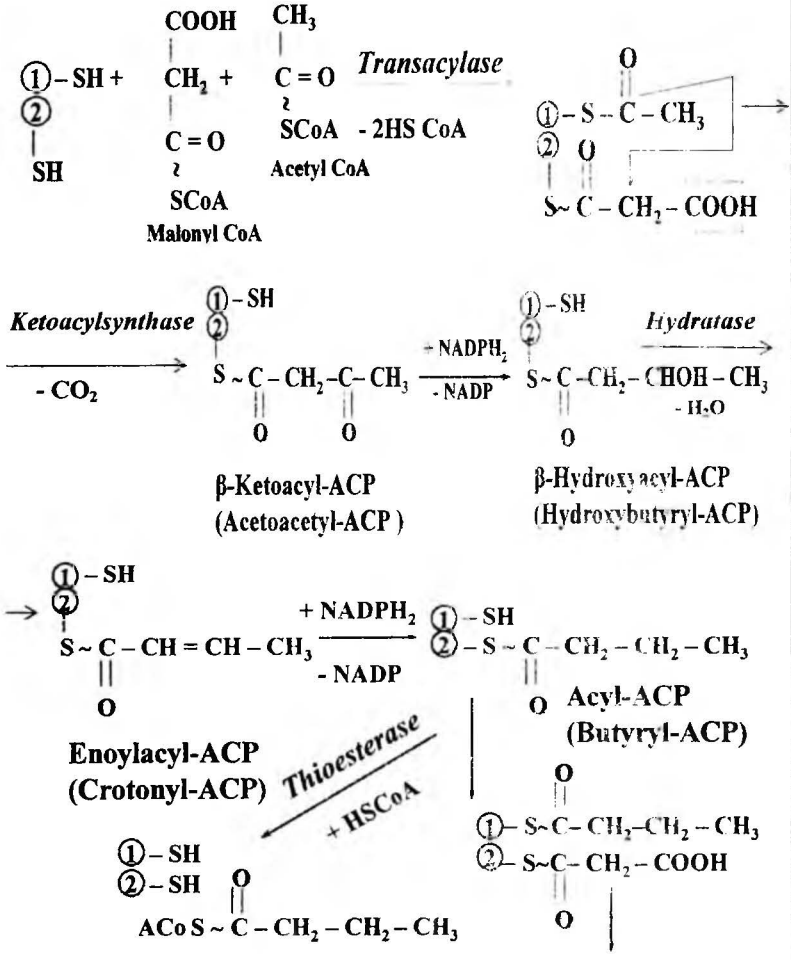
## *Reactions of 3-d stage*




**Designations:**
① - SH– Peripheral SH-group
② - SH – Central SH-group

The cycle now repeats with malonyl-ACP adding two-carbon units in each cycle to the lengthening acyl-ACP chain. This continues until the 16-carbon palmitoyl-ACP is formed.

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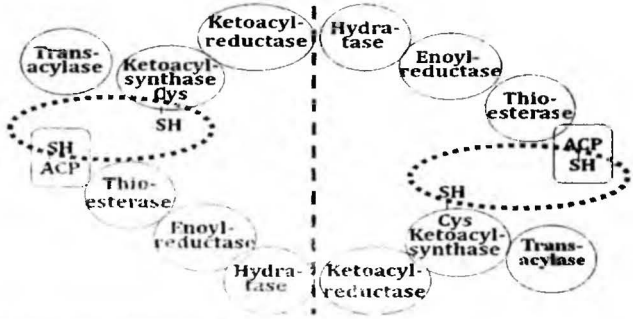
**Palmitate synthase complex**

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## Synthesis of long chain fatty acids from palmitate

Further, chain elongation can take place by separate mechanisms:

1. **Mitochondrial.** Palmitoyl CoA is carried by carnitine into mitochondria matrix where the fatty acid chain elongation is almost a reversal of β-oxidation of fatty acids. Acetyl CoA molecules are successively added to fatty acid to lengthen the chain. The reducing equivalents are derived from NADPH.

2. **Microsomal.** The microsomal chain elongation is more predominant and involves successive additions of malonyl CoA with the participation of NADPH in endoplasmic reticulum (microsomes). These reactions are similar to that catalyzed by palmitate synthase complex. A specific group of enzymes, namely elongases, bring about fatty acid chain elongation.

## Synthesis of unsaturated fatty acids

Reaction is catalyzed by a membrane-bound complex of three enzymes: NADPH-cytochrome b5 reductase, cytochrome b5 and desaturase in the SER. The reaction may be repeated to introduce more than one double bond into a fatty acid.

**Saturated fatty acyl CoA + $NADPH_2$ + $O_2$ ⟶**
**⟶ Mono-unsaturated acylCoA + $2H_2O$ + NADP**

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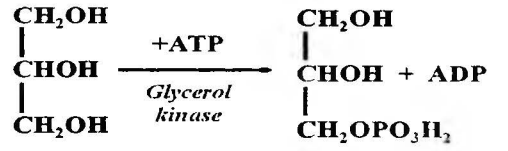
# Synthesis of triacylglycerols

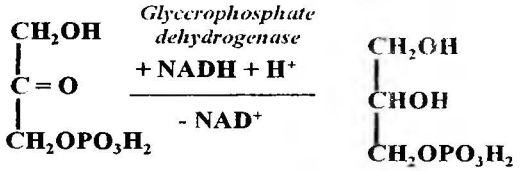
*Glycerol activation*

**1. Intestine, kidney, liver.**

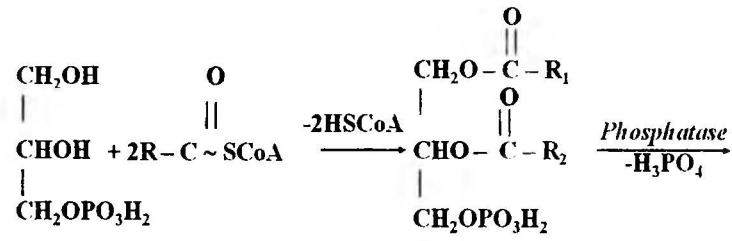



Glycerol — Glycerol -3-phosphate

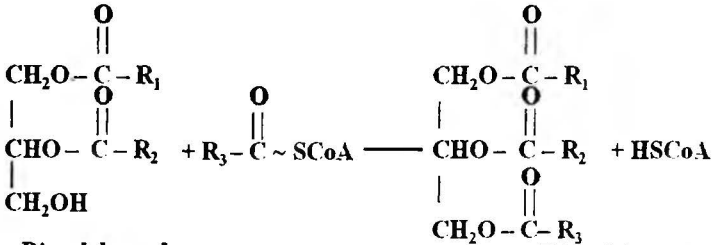
**2. Adipose tissue, muscle, liver:**




Dihydroxyacetone phosphate — Glycerol 3-phosphate



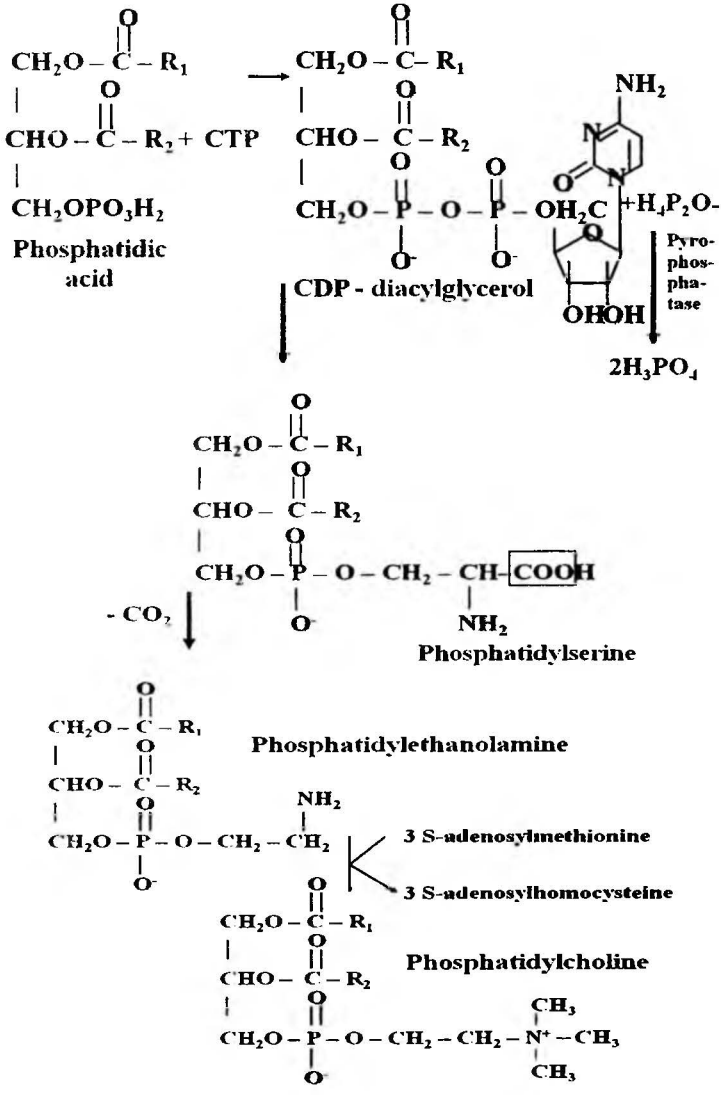

Glycerol 3-phosphate — Phosphatidic acid




Diacylglycerol — Triacylglycerol

# Glycerophospholipids synthesis

**Liver, intestine, spermaries, mammary gland**

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# Cholesterol metabolism. Biochemistry of atherosclerosis

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## Cholesterol functions

1. It is a major component of animal plasma membranes;

2. It is a metabolic precursor for the synthesis of:

   1. Bile acids
   2. Corticosteroids
   3. Sex hormones
   4. Vitamin D

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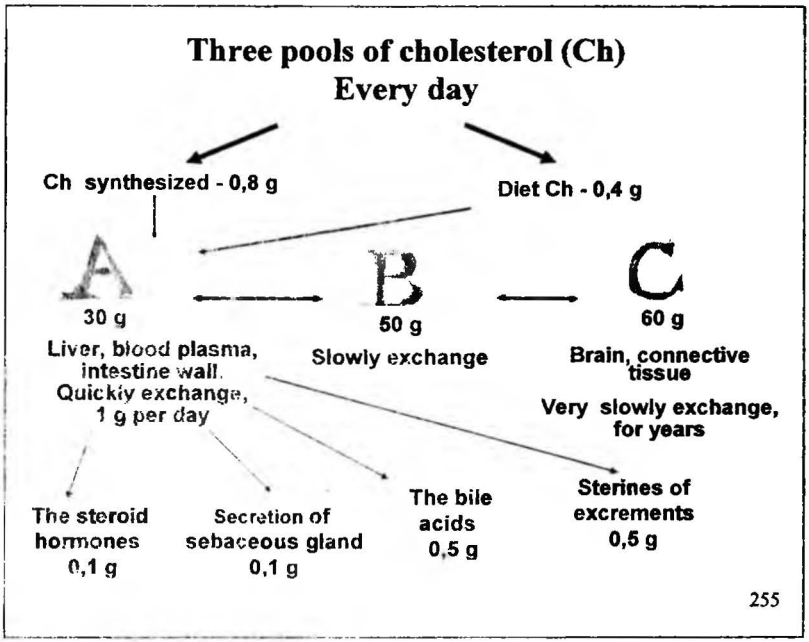
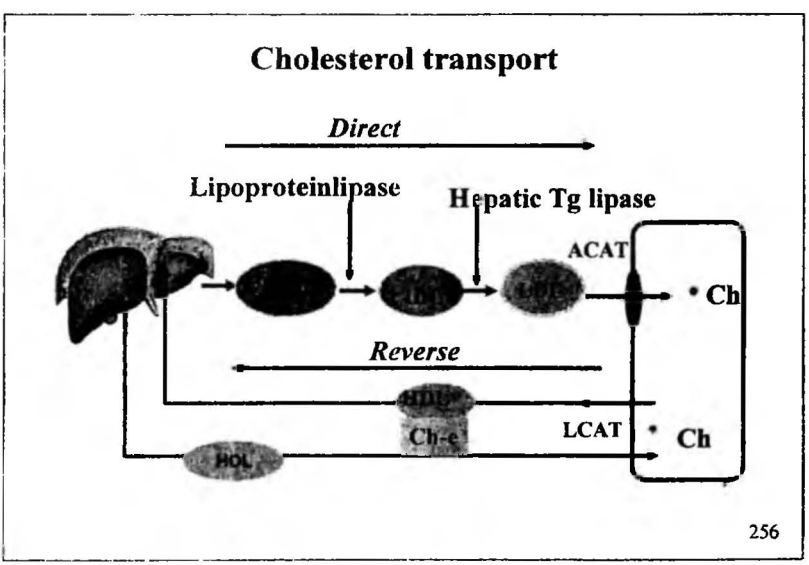
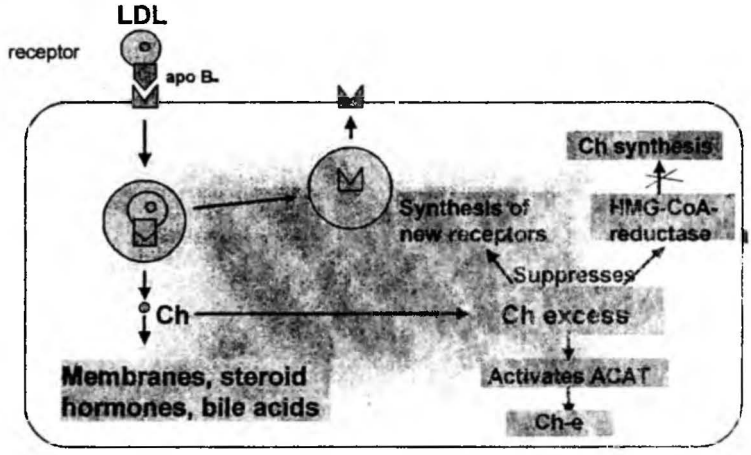

Three pools of cholesterol (Ch)
Every day
Ch synthesized - 0,8 g
Diet Ch - 0,4 g
A
B
C
30 g
50 g
60 g
Liver, blood plasma, intestine wall. Quickly exchange, 1 g per day
Slowly exchange
Brain, connective tissue
Very slowly exchange, for years
The steroid hormones 0,1 g
Secretion of sebaceous gland 0,1 g
The bile acids 0,5 g
Sterines of excrements 0,5 g
255


Cholesterol transport
Direct
Lipoproteinlipase
Hepatic Tg lipase
ACAT
Ch
Reverse
Ch-e
LCAT
Ch
HDL
256

## Receptor-mediated endocytosis of LDL

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## Stages of LDL receptor-mediated endocytosis

1. Apoprotein B-100 on the surface of an LDL particle binds to a specific receptor protein on the plasma membrane of nonliver cells.
2. LDL - receptor complex is taken up by cell through endocytosis.
3. Once in the lysosomes, the LDLs are digested by lysosomal enzymes, with the cholesterol esters being hydrolyzed by a lysosomal lipase to release the free cholesterol and protein component being hydrolyzed to free amino acids.
4. The LDL receptor itself returns unscathed to the plasma membrane.

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## Receptor-mediated endocytosis protects cell from excess of cholesterol:

1) Any excess of cholesterol is re-esterified for storage by *acyl CoA cholesterol acyltransferase (ACAT).*

2) To prevent the build up of cholesterol and its ester derivatives in the cell, high levels of cholesterol:

1. decrease the synthesis of the LDL receptor, thereby reducing the rate of uptake of cholesterol by receptor-mediated endocytosis;

2. inhibit the cellular biosynthesis of cholesterol through inhibition of *HMG CoA reductase*.

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## Unregulated pathways of cellular cholesterol uptake

1. Nonspecific endocytosis of LDL by "scavenger" receptors on macrophages.
2. Receptor-mediated pathway (through receptors not having high specificity to certain apoproteins).
3. Pathway of physico-chemical exchange of cholesterol between cellular membrane and LDL.

These pathways of cholesterol transport can lead to cholesterol accumulation in a cell.

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## Reverse cholesterol transport mediated by HDL

1. The plasma enzyme *lecithin-cholesterol acyl-transferase (LCAT)* catalyses the transfer of fatty acid from the second position of phosphatidylcholine (lecithin) to the hydroxyl group of cholesterol present in the extrahepatic tissues and transfers to the HDL.
2. Apoprotein A promotes the activity of *LCAT.*
3. Due to the addition of cholesterol, HDL particles become spherical.
4. The HDLs are then either taken up directly by the liver.
5. The liver is the only organ that can dispose of significant quantities of cholesterol, primarily in the form of bile salts.

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## Frederickson's classification of hyperlipidemias

| *Type* | *Increased plasma lipoprotein(s)* | *Triacylglycerols* | *Cholesterol* |
|---|---|---|---|
| I | Chylomicrons | ↑↑ | N (↑) |
| IIa | LDL | N | ↑ |
| IIb | LDL<br>VLDL | ↑ | ↑ |
| III | IDL | ↑ | ↑ |
| IV | VLDL | ↑ | N - ↑ |
| V | VLDL<br>Chylomicrons | ↑↑ | ↑ |

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## The factors influencing HDL level

| *Increased HDL* | *Decreased HDL* |
|---|---|
| Fish (protein) | Vegetarianism, carbohydrate diet |
| Ethanol | No ethanol |
| The physical activity | Physical inactivity, obesity |
| Estrogens | Progestins, androgens |
| Hypothyroidism | Effective treatment |
| Effective treatment | Diabetes mellitus |
| | Smoking |

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## The factors influencing LDL level

| *Increased LDL* | *Decreased LDL* |
|---|---|
| Aging | Newborns |
| Dietary intake of saturated fatty acids | Dietary intake of polyunsaturated fatty acids |
| High cholesterol fat diet | Low cholesterol fat diet |
| Low fiber diet | High fiber diet |
| Alcohol abuse | Moderate alcohol cosumption |
| Pregnancy | Labor |
| Obesity | Weight loss |
| Diabetes mellitus | Effective treatment |
| Hypothyroidism | |
| Cushing's syndrome | |
| Uraemia | |
| Nephrosis | |
| Familial hyperlipidaemias | |

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## Lipid atherogenity index

The **index of atherogenity (IA)** is the ratio between cholesterol of atherogenic lipoprotein classes to cholesterol of antiatherogenic lipoprotein classes:

**$IA = (Ch_{(total)} - Ch\text{-}HDL) / Ch\text{-}HDL$ (absolute units)**

The **index of atherogenity** is used for estimation of atherosclerosis risk degree.

Norm – up to 3
Low risk – from 3 to 4
Moderate risk – from 4 to 5
High risk – greater than 5.

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## Blood lipid levels at atherogenic types of hyperlipidaemias

| *Phenotype* | *LDL-Ch, mmol/l* | *Tg, mmol/l* |
|---|---|---|
| IIa | > 5,05 | < 2,25 |
| IIb | > 5,05 | > 2,25 |
| IV | < 5,05 | > 2,25 |

**VLDL-Ch = (Tg • 2,29)/5 mmol/l**

**VLDL-Ch = Tg/5 mg/dl**

**$LDL\text{-}Ch = Ch_{total} - (HDL\text{-}Ch + Ch - VLDL)$**

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## Primary hyperlipidaemias

| *Phenotype* | *Probable metabolic defect* |
|---|---|
| 1 | Familial lipoprotein lipase deficiency,<br>Familial apo-C-II deficiency |
| 2a, 2b | Familial hypercholesterolaemia |
| 3 | Familial abetalipoproteinaemia |
| 4 | Familial hypertriglyceridaemia,<br>Familial combined hyperlipidaemia |
| 5 | Familial apo-C-II deficiency,<br>Familial hypertriglyceridaemia,<br>Familial combined hyperlipidaemia |

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## Secondary hyperlipidaemia

| *Phenotype* | *Disease, syndrome* |
|---|---|
| 2a | Hypothyroidism, Cushing's syndrome, porphyria, glucocorticoids, nonselective β-blockers, nephrotic syndrome |
| 2b | Cushing's syndrome, glucocorticoids, nonselective β-blockers, thiazide diuretics, nephrotic syndrome |
| 4 | Diabetes mellitus, acromegaly, gout, oral contraceptives, alcohol abuse, thiazide diuretics, uraemia, acute hepatitis, stress |

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## Classification of serum lipid levels

| *Indication* | *Values, mmol/l* | | |
|---|---|---|---|
| | *Total Ch* | *LDL-Ch* | *Tg* |
| Optimal | 3.56-5.2 | 1.91-2.6 | 0.5-1.8 |
| Desirable | > 6.5 | > 3.4 | > 2.25 |
| Borderline high | > 7.3 | > 5.05 | > 5.6 |
| High | > 7.3 | > 5.05 | > 5.6 |

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## Optimal and desirable serum lipid levels

| *Classification of level* | *Total cholesterol* | | *LDL-cholesterol* | |
|---|---|---|---|---|
| | *mg/dL* | *mmol/L* | *mg/dL* | *mmol/L* |
| Optimal | <150 | <3.9 | <100 | <2.6 |
| Desirable | 150–199 | 3.9–5.1 | 100–129 | 2.6–3.3 |
| Borderline high | 200–239 | 5.2–6.2 | 130–159 | 3.4–4.1 |
| High | >240 | >6.2 | >160 | >4.1 |

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**Risk factors for development of atherosclerosis and coronary heart disease**

| *Traditional risk factors* | *Novel risk factors* |
|---|---|
| Increasing age | Antioxidants, low intake |
| Family history of hypertension | Fibrinogen, high serum level |
| Smoking tobacco | Homocysteine, high plasma level |
| Total cholesterol, high plasma level | Dimethylarginine, high plasma level |
| LDL cholesterol, high serum level | Lipoprotein (a), high serum level |
| HDL cholesterol low serum level | Physical inactivity |
| Family history of myocardial infarction | Trans fatty acids, high intake |
| Triacylglycerol high serum level | |

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**Risk factors for development of coronary heart disease**

| | |
|---|---|
| Smoking | Regardless of the cigarettes number |
| Abdominal obesity | Waist circumference:<br>males > 102 cm,<br>females > 88 см |
| Overweight | Body mass index (BMI) = weight (in kilograms) / height (in meters$^2$) = 30 and > |
| Arterial hypertension | > 140/90 mmHg<br>Antihypertensive drugs<br>Arterial hypertension oscillation for young |
| Hypodynamia | Less than 10 hours per week of physical activity during leisure time |
| Diabetes mellitus | Fasting glucose > 6,5 mmol/l (125 mg/dl) |
| Age | males > 45 years old<br>females > 55 years old or early menopause |
| Early onset of coronary heart disease in the immediate family | Myocardial infarction<br>males < 55 years old<br>females < 65 years old |
| Hypercholesterolemia | Total cholesterol > 5 mmol/l (150 mg/dl)<br>LDL-Ch ≥ 3,0 mmol/l (115 mg/dl) |
| Hypertriacylglycerolemia, low HDL-Ch | TG ≥ 1,7 mmol/l (150 mg/dl)<br>HDL-Ch < 1 mmol/l (40 mg/dl) |

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# Metabolism of proteins

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## Digestion of proteins

| *Secretion* | *Content of secretion* | *Functions* |
|---|---|---|
| Gastric juice | Hydrochloric acid (HCl) | Denaturation of proteins and killing of certain microorganisms |
| | Pepsin | Formation of peptides and a few amino acids |
| | Rennin (chymosin) | Coagulation of milk (protein casein conversion to calcium paracaseinate) |
| Pancreatic juice | Trypsin | Formation of free amino acids and small peptides (2-8 amino acids) |
| | Chymotrypsin | |
| | Elastase | |
| | Carboxypeptidase | |
| Intestinal juice | Aminopeptidase | Formation of free amino acids and smaller peptide |
| | Dipeptidase | Formation of free amino acids |

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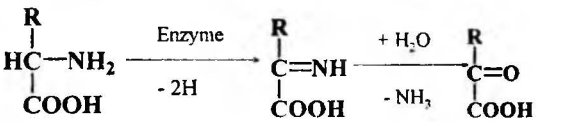
## Oxidative deamination

**- removal of amino group from the amino acids as $NH_3$ coupled with oxidation**

| Enzymes | L-amino acid oxidase | D-amino acid oxidase | Glutamate dehydrogenase |
|---|---|---|---|
| Specificity | Optical | Optical | Absolute |
| Substrates | L-aminoacids | D-aminoacids | Glutamate |
| Cofactors | FMN | FAD | $NAD^+$ |
| Localization | Peroxisomes | Microsomes | Mitochondria |
| Activity | Low (10%) | High | High |




Amino acid — Imine — α-Keto acid

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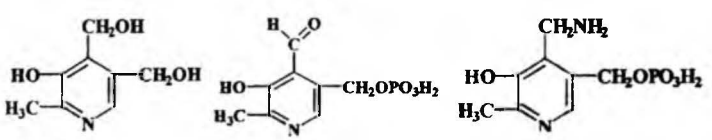
## Transamination

**- transfer of amino group from amino acid to α-keto acid (pyruvate, oxaloacetate, α-ketoglutarate) to form corresponding amino acid and α-keto acid**

The enzymes that catalyze these reactions are called *transaminases (or aminotransferases)*.
The coenzyme of transaminases is pyridoxal phosphate derived from pyridoxine (vitamin B6).




Pyridoxine ($B_6$) — Pyridoxal phosphate — Pyridoxamine phosphate

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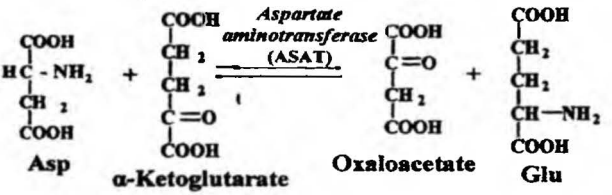
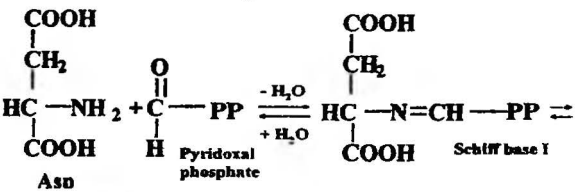
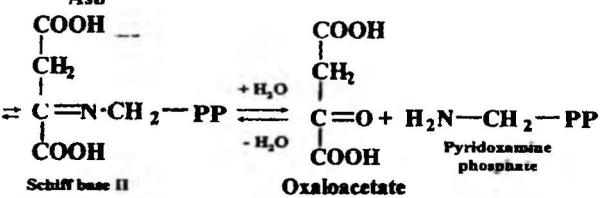
# Transamination



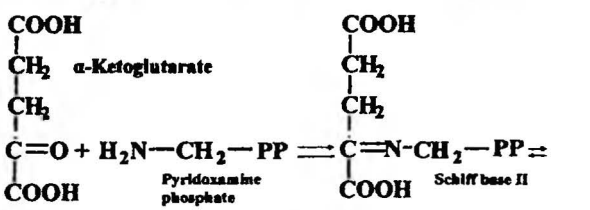

## *Mechanism of transamination:*

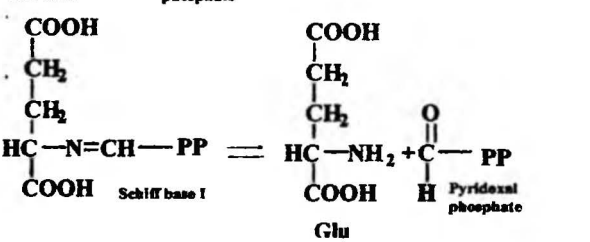
***Stage 1***




***Stage 2***

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## Role of the transamination

1. Synthesis of the nonessential amino acids.
2. The first stage of the indirect deamination of amino acids.
3. Unifies the catabolic pathways of carbohydrates, lipids and proteins.

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## Indirect deamination of amino acids

1. Deamination of the most amino acids proceeds by indirect pathway.
2. Glutamate serves as a collection centre for amino groups from amino acids.

$$\text{Aminoacid} + \text{Pyruvate} \rightarrow \alpha\text{-Ketoacid} + \text{Ala} \xrightarrow{+\ \alpha\text{-Ketoglutarate}} \text{Pyruvate} + \text{Glu}$$

$$\text{Aminoacid} + \text{Oxaloacetate} \rightarrow \alpha\text{-Ketoacid} + \text{Asp} \xrightarrow{+\ \alpha\text{-Ketoglutarate}} \text{Oxaloacetate} + \text{Glu}$$

$$\text{Aminoacid} + \alpha\text{-Ketoglutarate} \rightarrow \alpha\text{-Ketoacid} + \text{Glu}$$

$$\text{Glu} \xrightarrow[\textit{dehydrogenase}]{\textit{Glutamate}} \alpha\text{-Ketoglutarate} + NH_3$$

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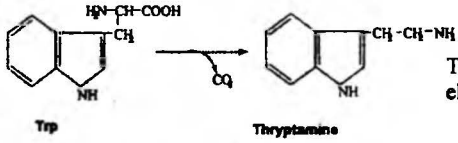
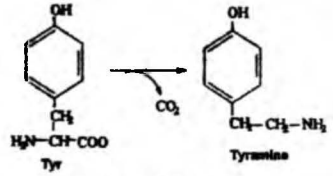
## Decarboxylation of amino acids

**-removal of carboxylic group from the amino acids as $CO_2$ to form the biogenic amines**

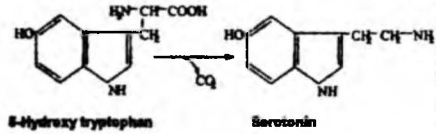
The enzymes that catalyze these reactions are called ***decarboxylases*** which are dependent on pyridoxal phosphate.



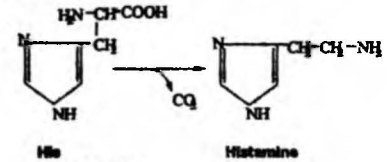

Thryptamine has blood pressure elevating property



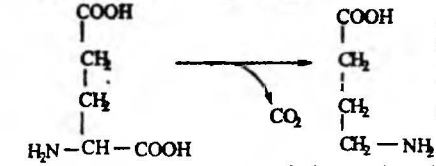

Tyramine has blood pressure elevating property



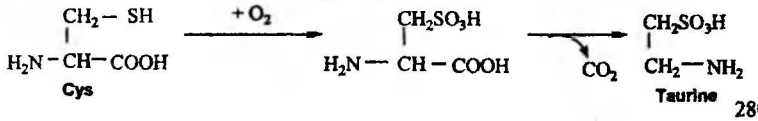

Serotonin is important in nerve impulse transmission (neurotransmitter). It regulates sleep, behaviour, temperature, blood pressure, pain perception etc




Histamine is a vasodilator and lowers blood pressure. It stimulates gastric HCl secretion and is involved in inflammation and allergic reaction




GABA acts as an inhibitory neurotransmitter in the nervous system




Taurine conjugates with bile acids to increase their hydrophilism

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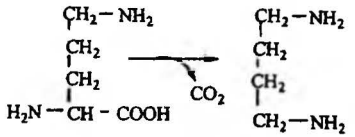
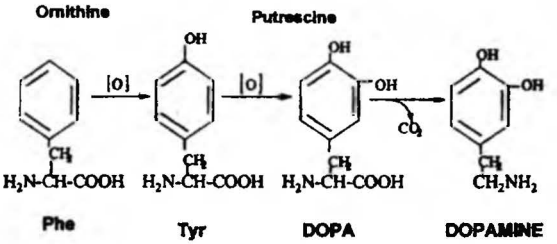
## Decarboxylation of amino acids



Putrescine is converted to spermine and spermidine




Dopamine is utilized in the brain as excitatory neurotransmitter

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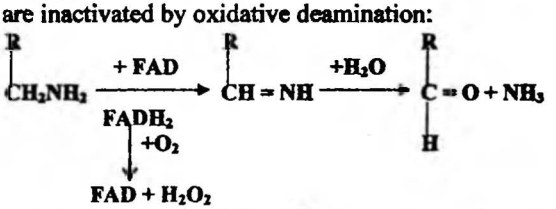
## Detoxification of biogenic amines

Biogenic amines are metabolized to less active degradation products. This detoxification system includes specific enzymes: *monoamine oxidases (MAO)* and *diamine oxidase (DAO)* are complex enzymes localized in mitochondria. Prosthetic group of MAO is FAD; prosthetic groups of DAO are pyridoxal phosphate and $Cu^{2+}$.

Amines are inactivated by oxidative deamination:




The formed aldehydes are oxidized further to fatty acids which are oxidized to end products.

Certain drugs (e.g., MAO-inhibitors) decrease the activity of MAO, for example iproniazid.

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## Detoxication of ammonia

### Sources of ammonia:

1. Deamination of the amino acids.
2. Deamination of the glutamate.
3. Deamination of biogenic amines.
4. Deamination of purines and pyrimidines.
5. Ammonia generated by enteric bacteria.

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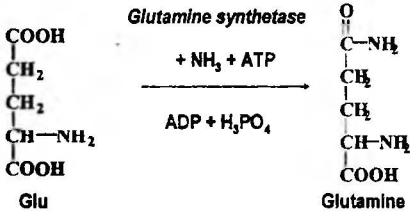
## Local detoxication of ammonia

***Mechanisms:***

1. Human tissues (brain, muscle, retina, kidney, liver) initially detoxify $NH_3$ formed by conversion to glutamine from glutamate for transport to the liver.




2. Detoxication of $NH_3$ in tissues by amidation of glutamate residues in proteins.

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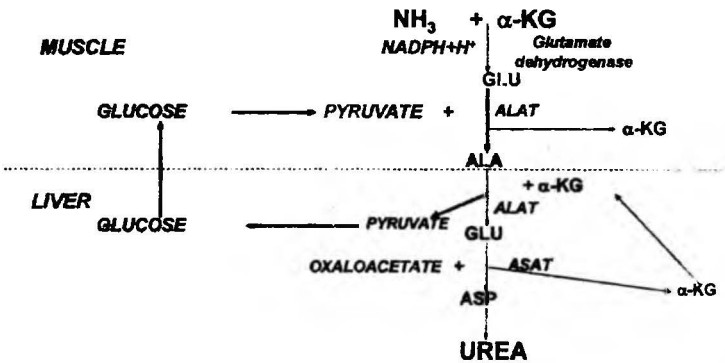
## Local detoxication of ammonia

3. Reductive amination of α-ketoglutarate in muscle (glucose-alanine cycle).




*ALAT - alanine aminotransferase*
*ASAT - aspartate aminotransferase*

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## General detoxication of ammonia

***Mechanisms:***

1. Ammonium salts are synthesized in the kidneys from ammonia derived from hydrolysis of glutamine by *glutaminase*.

$$NH_3 + H^+ \rightarrow NH_4^+$$
$$NH_4^+ + PO_4^{3-}, SO_4^{2-}, Cl^-, CO_3^{2-}$$

Ammonium salts are normally excreted in 1 – 1.2 g per day by urine.
Formation and secretion of ammonium salts maintain acid-base balance.

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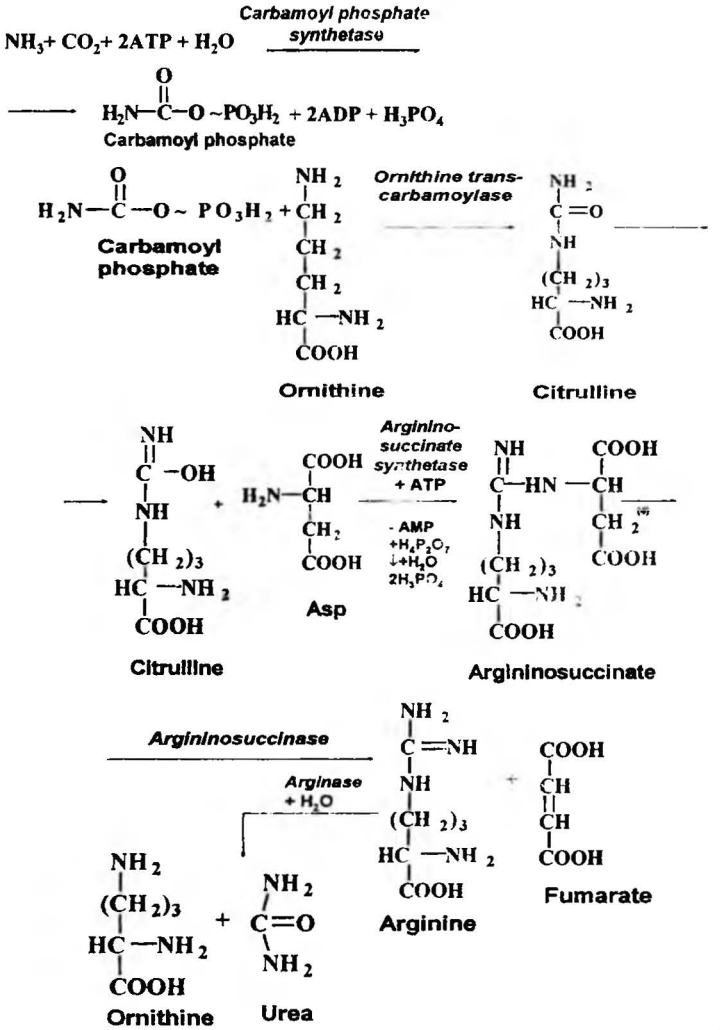
## General detoxication of ammonia

2. Urea is synthesized in liver from ammonia derived from hydrolysis of glutamine by *glutaminase*.

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# Metabolism and functions of individual amino acids

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## Methylation

**- is introduction of methyl groups into substrate molecule**

***Functions of methylation:***

1. Formation of low-molecular compounds – choline, adrenaline, creatine, thymidine nucleotides.
2. Inactivation of biologically active substances – catecholamines, hormones.
3. Neutralization of xenobiotics.
4. Maturation of DNA, RNA.

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## Transmethylation

**- is transport of methyl groups from a source of methyl groups to a substrate of methylation with aid of donors (or carriers) of methyl groups**

**Sources of methyl groups** are serine, choline, betaine.

**Donors (or carriers) of methyl groups** for substrates are active form of folic acid – tetrahydrofolic acid (THFA) and active form of methionine – S-adenosyl methionine (S-AM).

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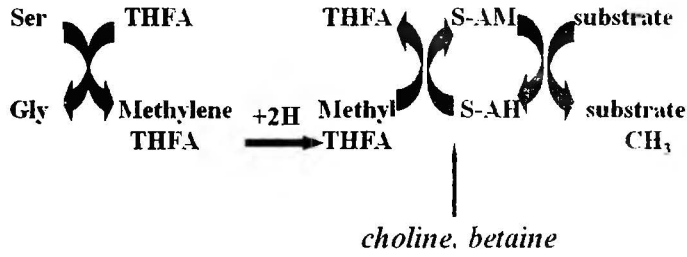
## Scheme of transmethylation

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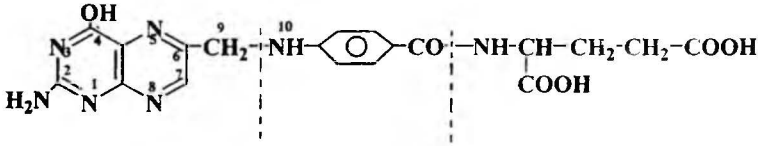
## Folic acid




*Pteridine* *Para-aminobenzoic acid, PAB(A)* *L- Glutamate*

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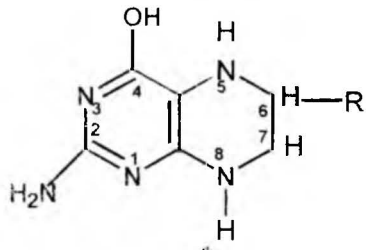
## 5,6,7,8 - Tetrahydrofolic acid (THFA)




The methyl group is transferred in 5$^{th}$-position – 5-methyl THFA.
But THFA can form other derivatives:
$N^5N^{10}$ methylene THFA (–$CH_2$–);
$N^5N^{10}$ methenyl THFA (–CH=), etc.

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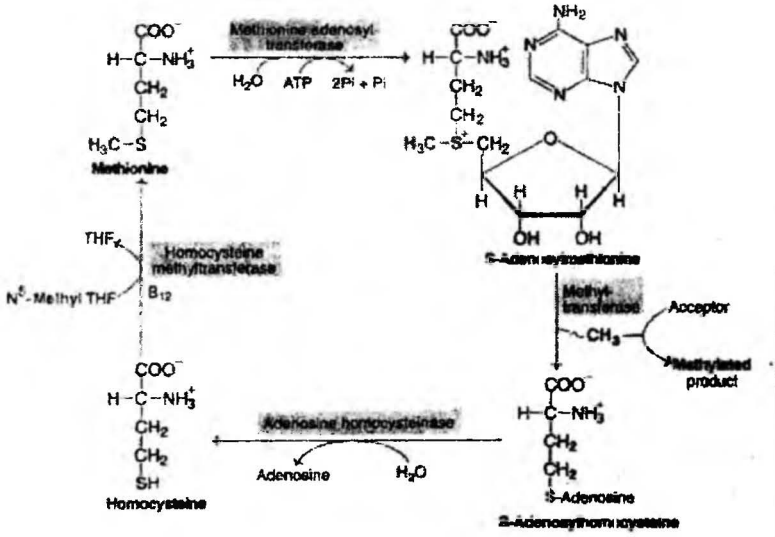
## The activated methyl cycle

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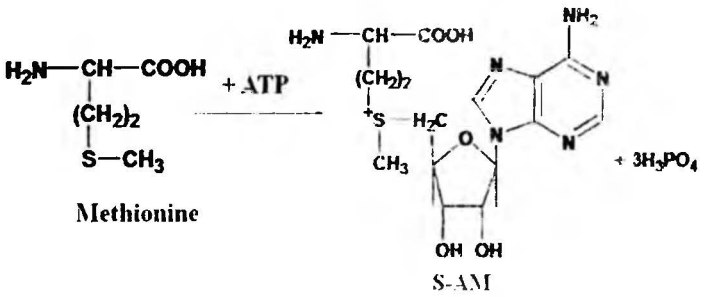
## Formation of S-adenosyl methionine




S-AM + substrate $\xrightarrow[\text{Transferase}]{\text{Methyl}}$ S-AH + substrate-$CH_3$

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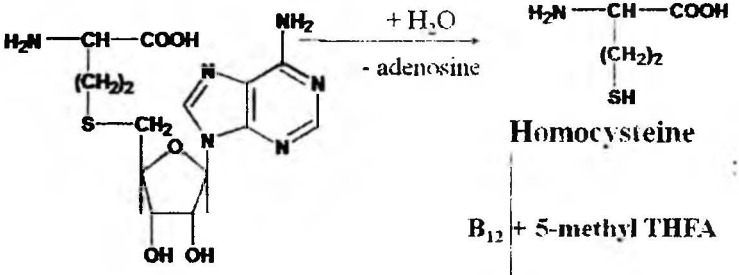
## Regeneration of methionine

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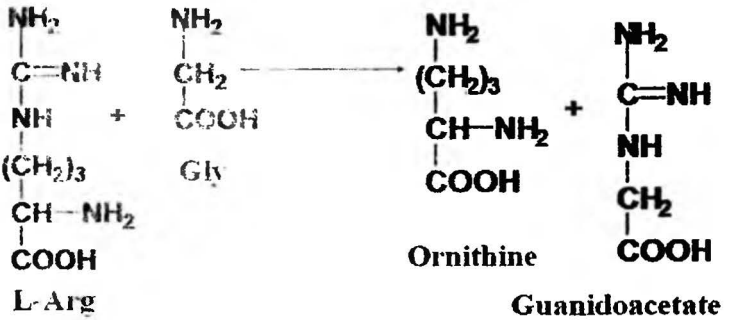
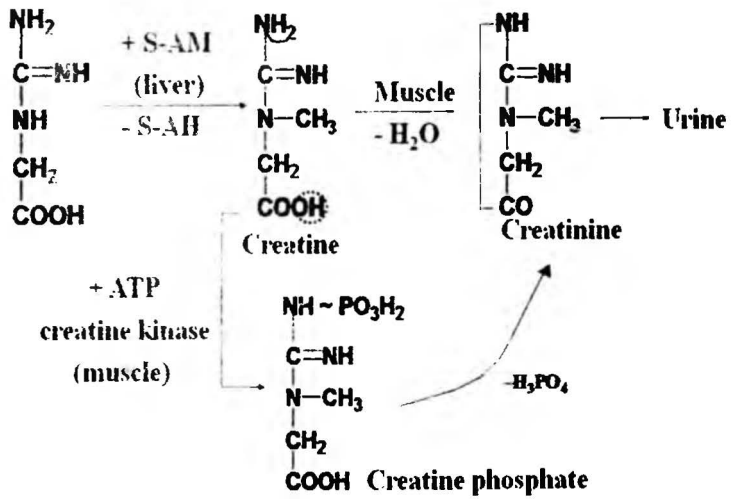
## Examples of methylation reactions

### I. There are 2 stages of creatine synthesis:

**1) Guanidoacetate is formed in kidneys and pancreas**




**2) Synthesis of creatine is completed by methylation of guanidoacetate in the liver:**

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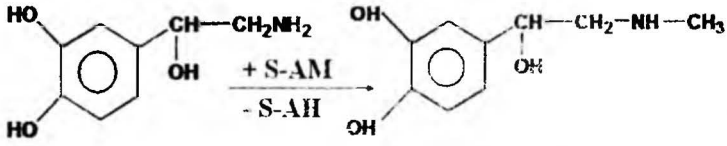
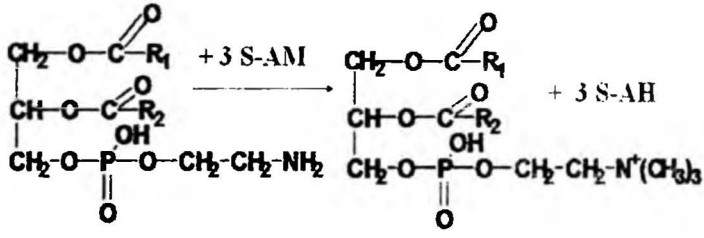
## Examples of methylation reactions

**II. Epinephrine** is synthesized by methylation of norepinephrine in the adrenal medulla.




**Norepinephrine** **Epinephrine**

### III. Synthesis of phosphatidylcholine




**Phosphatidylethanolamine** **Phosphatidylcholine**

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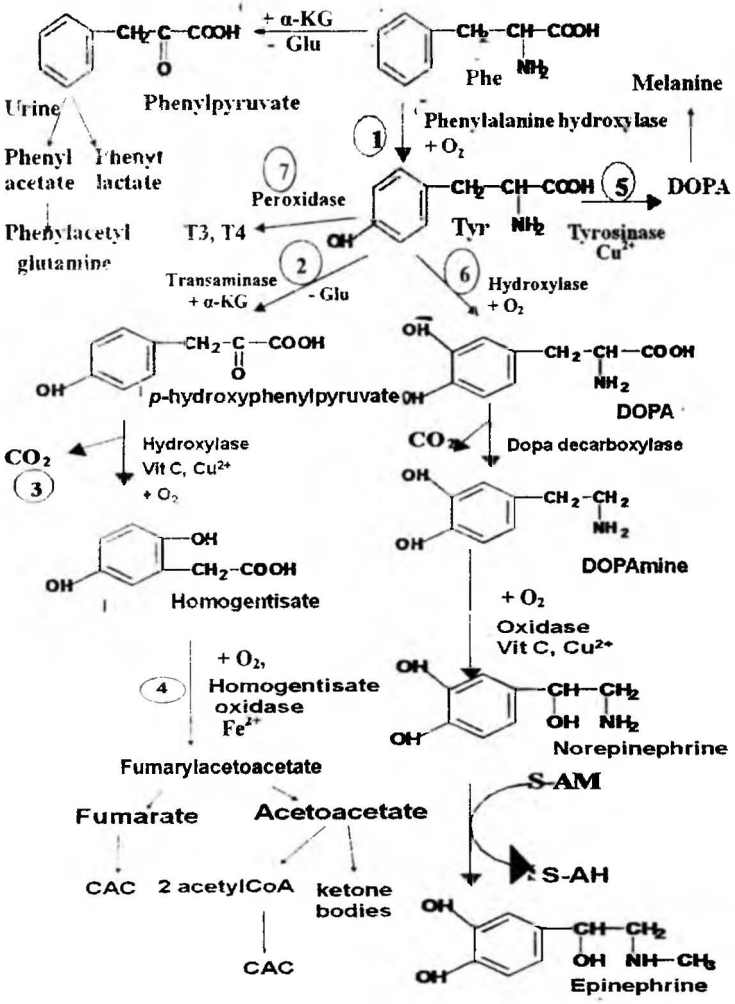
## Metabolism of phenylalanine and tyrosine

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## Disorders of tyrosine (phenylalanine) metabolism

| *Medical condition* | *Defective process* | *Defective enzyme* | *Symptoms and effects* |
|---|---|---|---|
| Albinism | Melanin synthesis from tyrosine | Tyrosinase | Hypopigmentation that causes white hair, pink skin, blue eyes etc. |
| Alkaptonuria | Tyrosine degradation | Homogentisate oxidase | Dark pigment in urine, late developing arthritis, alkronosis. |
| Phenylketonuria | Conversion of phenylalanine to tyrosine | Phenylalanine hydroxylase | Neonatal vomiting, mental retardation, failure to walk or talk, failure of growth, seizures and tremor |
| Richner-Hanhart syndrome (Tyrosinemia type II) | Tyrosine degradation | Tyrosine transaminase | Skin (dermatitis) and eye lesions and, mental retardation. |
| Neonatal tyrosinemia | Tyrosine degradation | p-hydroxyphenylpyruvate dioxygenase | Chronic degenerative changes in liver and kidney. |
| Tyrosinosis or tyrosinemia type I | Tyrosine degradation | Fumarylacetoacetatehydroxylase and/or maleylacetoacetate isomerase | Liver failure, rickets, renal tubular dysfunction and polyneuropathy. |
| Parkinson's disease | Dopamine synthesis from tyrosine | Tyrosine hydroxylase, DOPA-decarboxylase | Muscular rigidity, tremors, akinesia, expressionless face, lethargy, involuntary movements. |

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# Metabolism of nucleic acids

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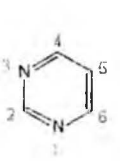
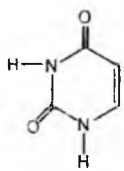
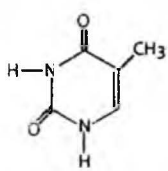
## Nitrogenous bases



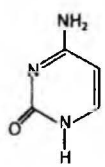
Pyrimidine



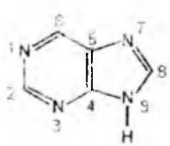
Uracil (U)
RNA only



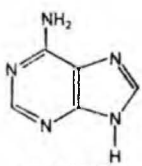
Thymine (T)
DNA only



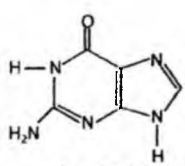
Cytosine (C)
both DNA and RNA



Purine



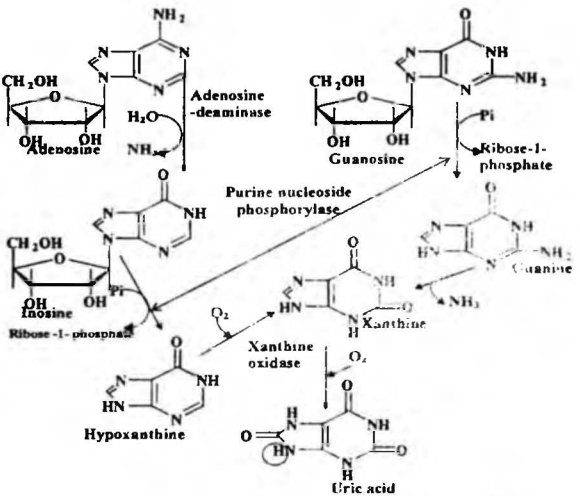
Adenine (A)



Guanine (G)

302

## Degradation of purine nucleotides

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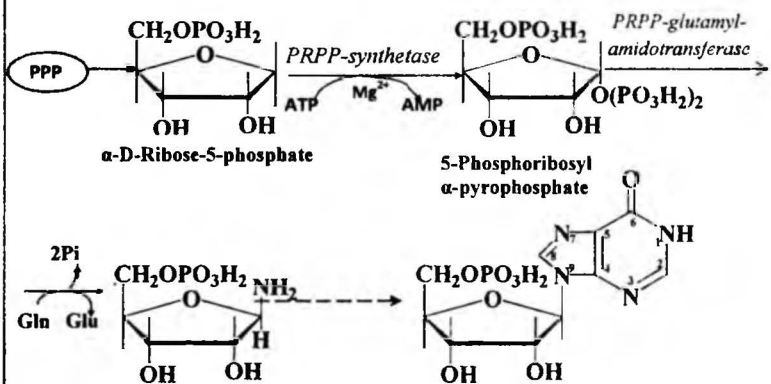
## Biosynthesis of purine nucleotide




*PPP – pentose phosphate pathway*
*PRPP- Phosphoribosyl pyrophosphate*

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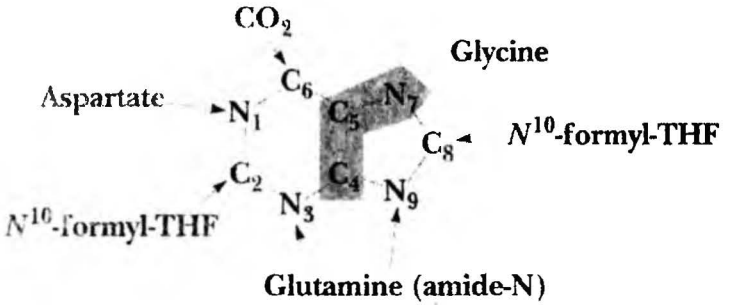
## Sources of individual atoms in purine ring

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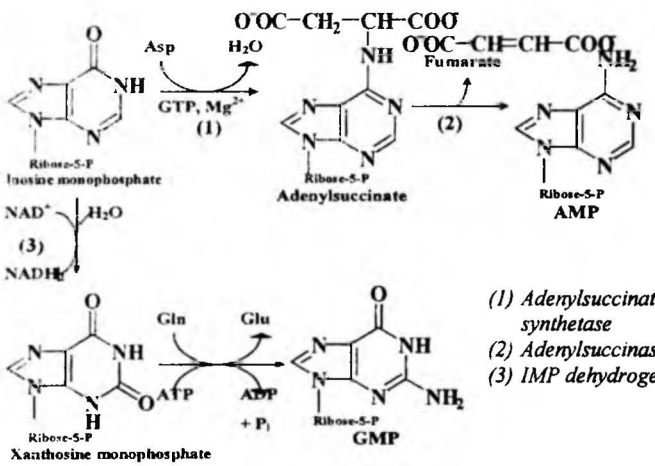
## Synthesis of AMP and GMP




*(1) Adenylsuccinate synthetase*
*(2) Adenylsuccinase*
*(3) IMP dehydrogenase*

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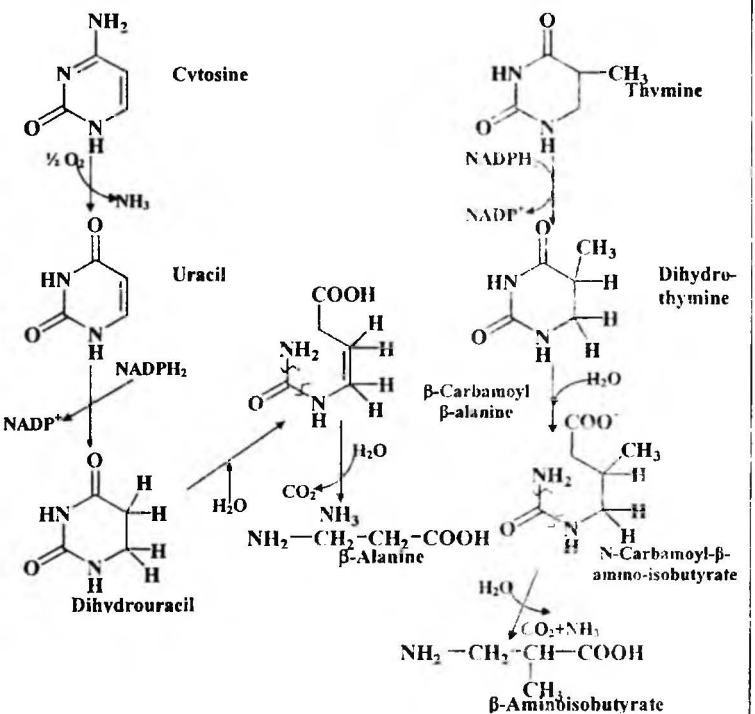
## Degradation of pyrimidine nucleotide

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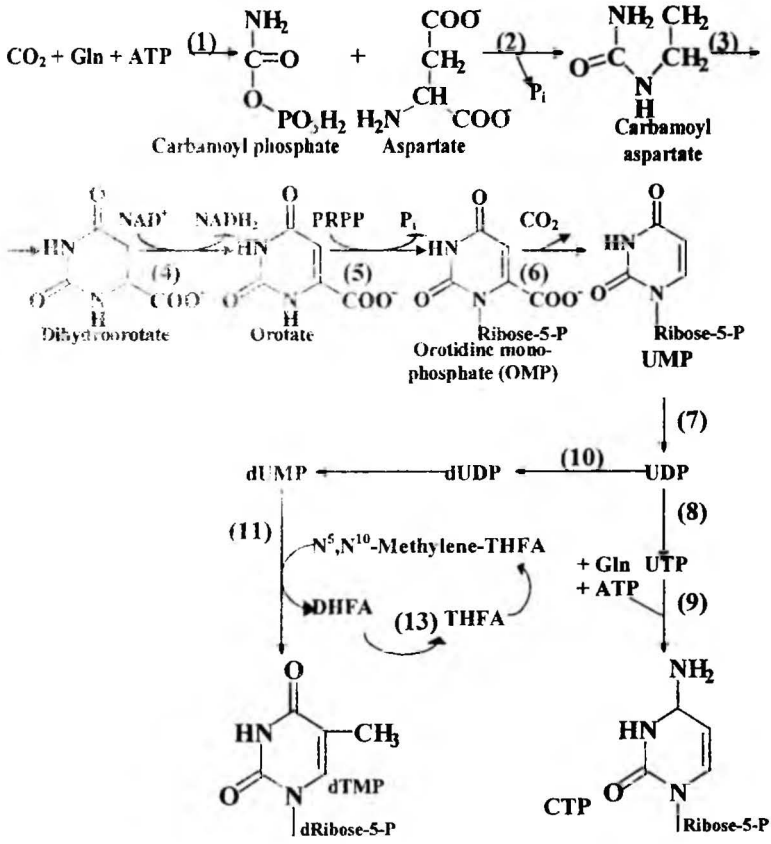
## Biosynthesis of pyrimidine nucleotide




*(1) Carbamoyl phosphate synthetase*
*(2) Aspartate transcarbamoylase*
*(3) Dihydroorotase*
*(4) Dihydroorotate dehydrogenase*
*(5) Orotate phosphoribosyltransrerase*
*(6) OMP decarboxylase*
*(7) Nucleoside phosphate kinase*
*(8) Nucleoside diphosphate kinase*
*(9) CTP synthetase*
*(10) Ribonucleoside diphosphate reductase*
*(11) Thymidylate synthetase*

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## Disorders of nucleotide metabolism

| *Medical condition* | *Enzymes deficiency* | *Symptoms and effects* |
|---|---|---|
| **Disorders of purine metabolism** | | |
| Gout | PRPP synthetase<br>PRPP glutamylamidotransferase | Hyperuricemia, deposition of sodium urate crystals in the joints (tophi), inflammation in the joints resulting in a painful gouty arthritis, precipitation of sodium urate and/or uric acid in kidneys and ureters that results in renal damage and stone formation |
| Xanthinuria | Xanthine oxidase | High concentrations of xanthine in blood and urine, which can lead to health problems such as renal failure and xanthine kidney stones, one of the rarest types of kidney stones. |
| Lesch-Nyhan syndrome | Hypoxanthine-guanine phosphoribosyltransferase | Hyperuricemia, neurological abnormalities such as mental retardation, aggressive behavior, learning disability etc. The patients have an irresistible urge to bite their fingers and lips, causing self-mutilation |
| **Disorders of pyrimidine metabolism** | | |
| Orotic aciduria | Orotate phosphoribosyl transferase<br>OMP decarboxylase | Excretion of orotic acid in urine, severe anemia and retarded growth |
| Reye's syndrome (secondary orotic aciduria) | Ornithine transcarbamoylase | Accumulation of carbamoyl phosphate. Increased synthesis and excretion of orotic acid |

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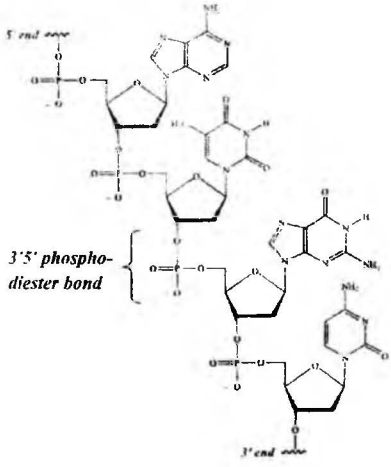
## Primary structure of DNA

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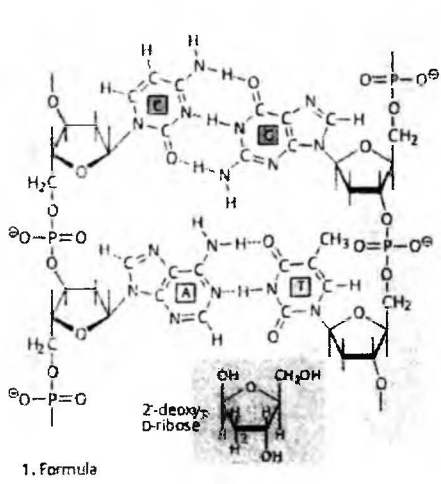
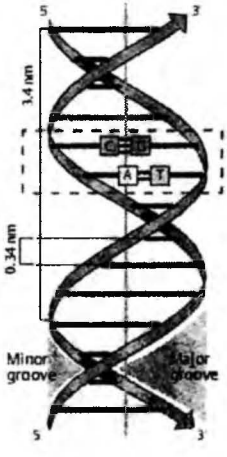
## Secondary structure of DNA




1. Formula




2. Double strand

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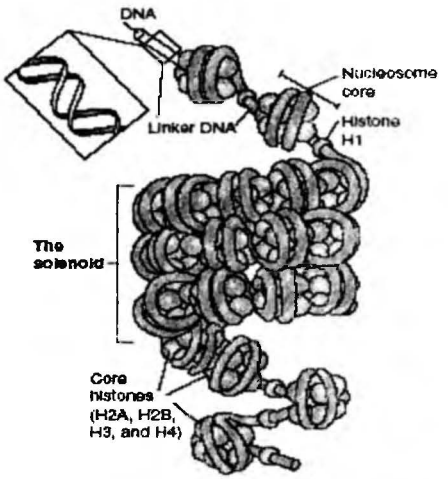
## Tertiary structure of DNA

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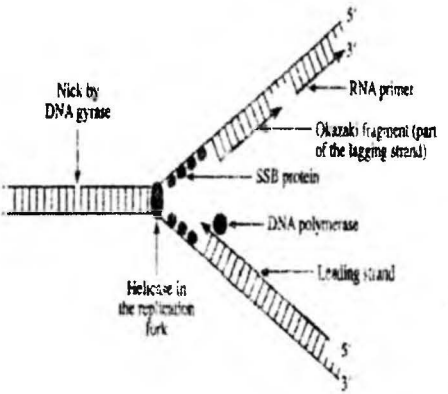
## Replication

**- is a process in which DNA copies itself to produce identical daughter molecules of DNA**

Components required for the stages of replication:
1. **Initiation step:** initiation proteins, single-strand binding protein (SSB), *helicase, gyrase, primase*, nucleoside triphosphates;
2. **Elongation step:** deoxynucleoside triphosphates, *DNA polymerase III*, primer, *DNA polymerase I*;
3. **Termination step:** *DNA ligase*.



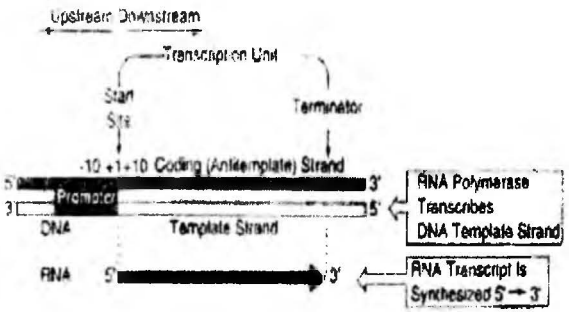

313

# Transcription

**- is a process of RNA synthesis directed by a DNA template**

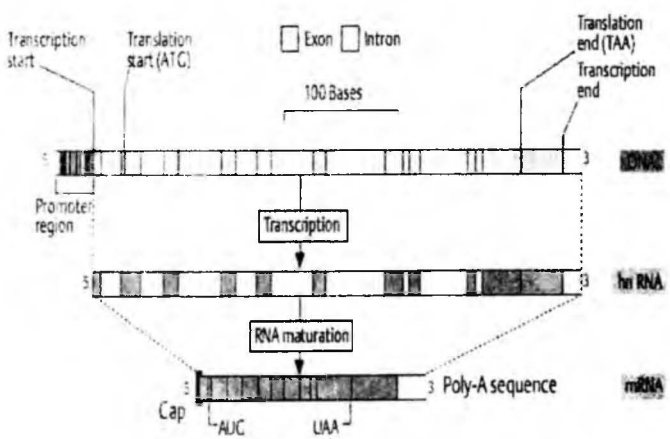
Components required for the stages of transcription:

1. **Initiation step:** *RNA polymerase*, template strand of DNA.
2. **Elongation step:** nucleoside triphosphates.
3. **Termination step:** termination sequence.




314

# Processing of mRNA

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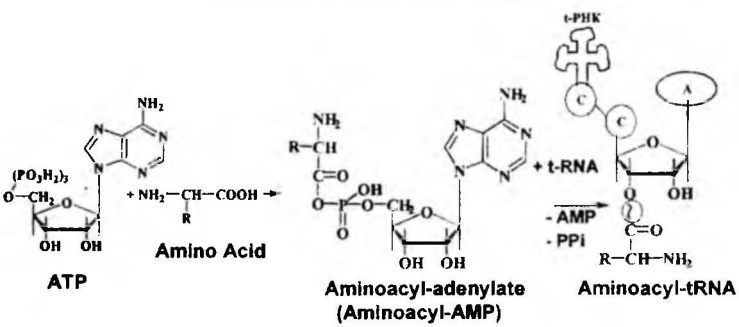
## Protein synthesis (or translation)

Cellular components required for the stages of protein synthesis:

1. **Activation of amino acids:** 20 proteinogenous amino acids (L type), tRNAs, *aminoacyl-tRNA synthetases*, ATP, $H_2O$, $Mg^{2+}$;
2. **Initiation step:** Initiation factors (IF-1, IF-2, IF-3), small (30S) and large (50S) ribosomal subunits, N-formylmethionyl-tRNA (= initiator tRNA), mRNA, initiation codon in mRNA (AUG, GUG), GTP, $Mg^{2+}$;
3. **Elongation step:** Functional 70S ribosome (initiation complex), aminoacyl-tRNAs specified by codons, elongation factors (EF-Tu, EF-Ts, EF-G), *peptidyl transferase*, GTP, $Mg^{2+}$;
4. **Termination step:** 70S ribosome, termination codons in mRNA (UAA, UAG, UGA), polypeptide release factors (RF1, RF2, RF3), ATP;
5. **Post-translational modifications:** Specific enzymes and cofactors for removal of initiating residues and signal sequences; additional proteolytic processing: modification of terminal residues; attachment of phosphate, methyl, carboxyl, carbohydrate, or prosthetic groups.

**Activation of amino acid:**

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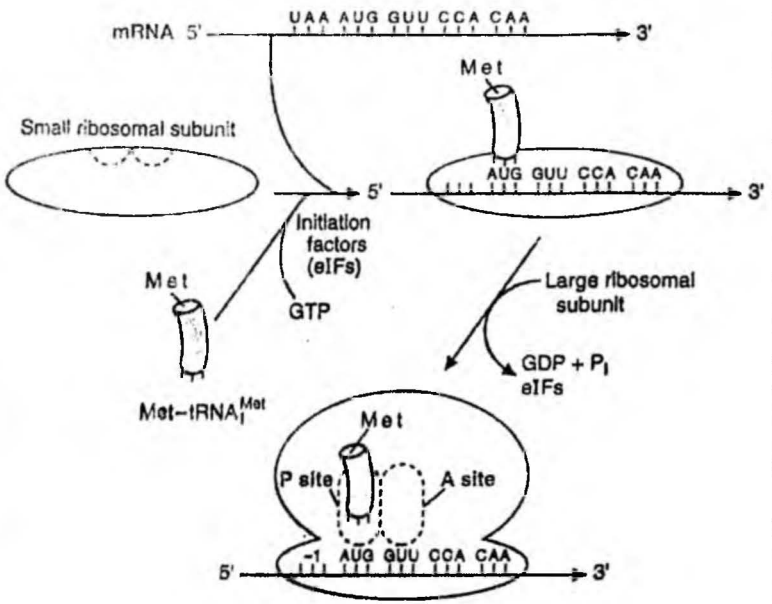
## Initiation step

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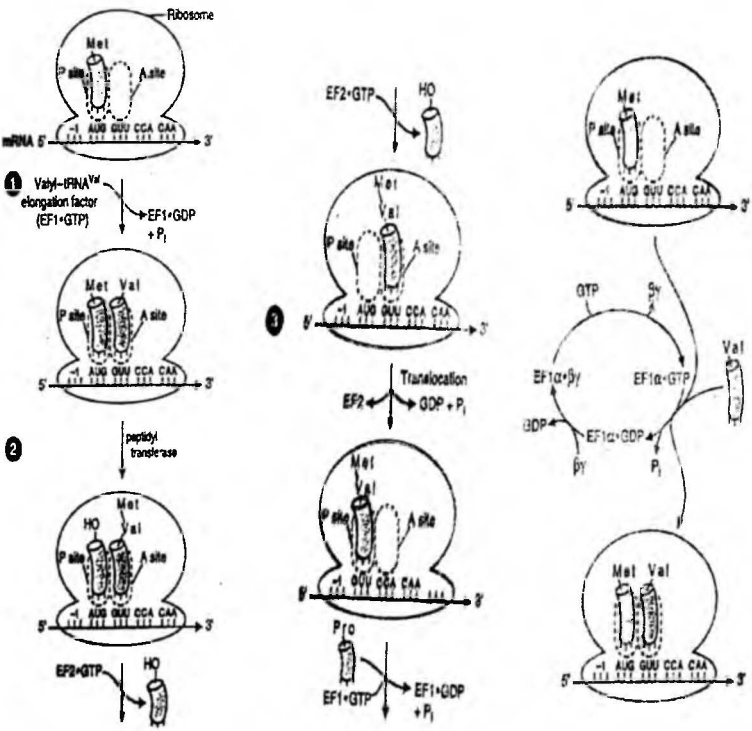
# Elongation step




**Elongation steps**
**1-3 repeated**

1. Binding of aminoacyl tRNA to the A site.
2. Formation of a peptide bond.
3. Translocation.

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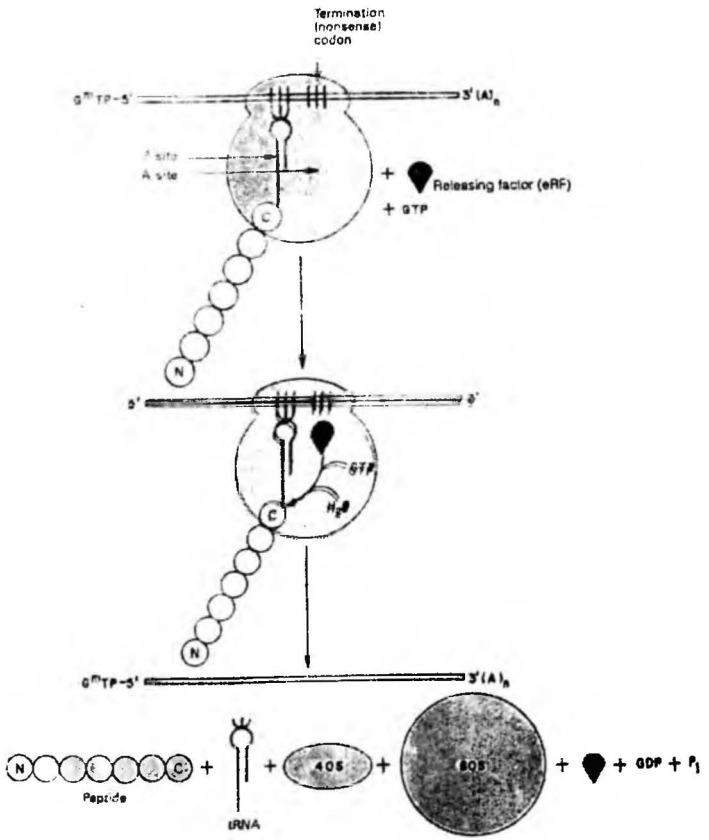
The two steps in **3** occur simultaneously

# Termination step

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# Vitamins

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**Antivitamins are antagonistic to the action of vitamins**

1. Antivitamins are a structural analogue of the vitamins and a competitive inhibitors of the vitamin's action, causing deficiencies, e.g., dicumarol, 4`-hydroxythiamine.
2. Some antivitamins are enzymes, destroying vitamins, e.g., *thiaminase* found in raw fish cleaves thiamine.
3. Some antivitamins form with vitamins strong inactive complexes, e.g., avidin combines tightly with biotin, preventing its absorption.
4. Some antivitamins have ability to induce chemical modification of vitamins.

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## Exogenous causes of hypovitaminosis

1. One-sided nutrition (insufficient intake).
2. Inadequate amounts of or absence of vitamins in food.
3. Improper storage of food products and gross violations in preparing and cooking food, which result in the destruction of most of the vitamins.

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## Endogenous causes of hypovitaminosis

The need of the human organism for vitamins is greatly increased during severe physical stress, pregnancy, and breast feeding.

1. Increased destruction of vitamins at febrile states.
2. Diseases affecting the gastrointestinal tract – impaired absorption of vitamins.
3. Diseases affecting the liver, pancreas – impaired intake of lipid soluble vitamins.
4. Drug therapy:
   - antibiotics, sulfanilamides administration suppress the intestinal microflora;
   - suppression of endogenous synthesis of vitamin K, biotin, vitamins of B group in the intestines;
   - tranquilizers suppress synthesis of coenzyme form of vitamin $B_2$;
   - aspirin suppresses utilization of folic acid;
   - nitrous oxide used in surgery inactivates $B_{12}$;
   - some medicines are antivitamins.
5. Administration of antivitamins.

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## Features of lipid soluble vitamins

1. Apolar hydrophobic molecules, which are all isoprene derivatives.
2. Cannot be synthesized by the body and must be supplied by the diet.
3. Require normal fat absorption in order to be absorbed.
4. Must be transported in the blood in lipoproteins or specific binding proteins.
5. Can be stored in liver and adipose tissue.
6. Excess consumption of these vitamins (particularly A and D) leads to their accumulation and toxic effects (hypervitaminosis).

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## Provision rate of organism with vitamins

| *Vitamin* | *Rate* | *Parameter* | *Diagnostic value* |
|---|---|---|---|
| Retinol | In plasma μmol/L | >0.7<br>0.35-0.7<br><0.35 | Norma<br>Moderate deficit<br>Extreme deficit |
| Calciferol | In plasma nmol/L | >25<br>12.5-25.0<br><12.5 | Norma<br>Moderate deficit<br>Extreme deficit |
| Tocopherol | In plasma α-Tocopherol / cholesterol μmol /mmol | >2.22<br><2.22 | Norma<br>Deficit |

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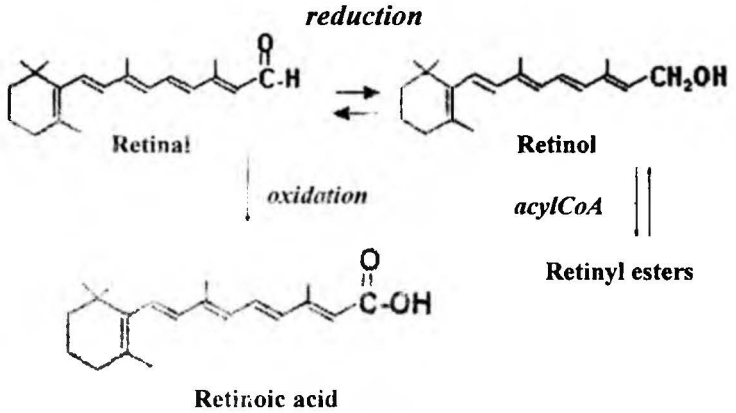
## Vitamin A (retinol, antixerophthalmic vitamin)

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## Biological role of vitamin A

1. **Retinol** supports the normal function of the reproductive system in males and females.
2. **Retinol** and **Retinoic acid** are involved in the control of the expression and transcription of certain genes. Thus they regulate the protein synthesis and are involved in the cell growth and differentiation.
3. **Vitamin A** is essential to maintain epithelial tissue. This is due to the fact that retinol and retinoic acid are required to prevent keratin synthesis (responsible for horny surface).
4. **Retinoic acid** is necessary for the synthesis of certain glycoproteins, which are required for growth and mucus secretion.
5. **β-Carotene** is an antioxidant and may play a role in trapping peroxy free radicals in tissues at low partial pressures of oxygen.
6. **Vitamin A** and **β-carotene** antioxidant properties may well account for their possible anticancer activity.
7. **Vitamin A** is essential for the maintenance of proper immune system to fight against various infections.
8. **Retinal** participates in the visual cycle.

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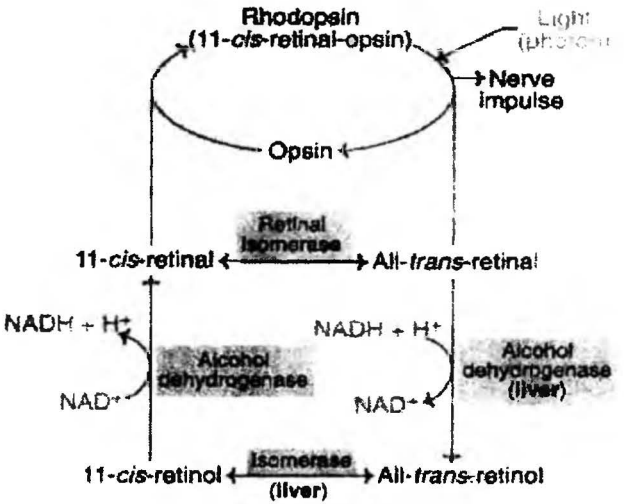
## Visual cycle

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## The daily requirement

**1.5-2.5 mg of vitamin A (5.000-7.000 IU) or 2-5 mg of carotin.**

***Sources:*** liver, kidney, egg yolk, milk, cheese, butter, cod-liver oil.

Vegetable sources – β-carotenes (A provitamins) – carrots, spinach, tomato, pumpkins, mango, papaya etc.

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## Hypovitaminosis A

1. Night-blindness (nyctalopia) is defective night vision.
2. Dryness and deterioration in conjunctiva and cornea of the eye (xerophthalmia).
3. Keratinization of epithelial tissues of the eye, lungs, gastrointestinal, and genitourinary tracts, coupled with reduction in mucous secretion.
4. Destruction of the cornea (keratomalacia) causing total blindness
5. Drying of skin and atrophy of sebaceous glands, appearance of pustules around hair follicles.
6. Degeneration of germinal epithelium, thus affecting reproduction.
7. Defective formation of the enamel of teeth.
8. Growth retardation due to impairment in skeletal formation.

## Hypervitaminosis A

This is possible in children by ingestion of large dose of vitamin A. Manifestations are headache, lack of appetite, dermatitis (drying and redness of skin), enlargement of liver and spleen, skeletal decalcification, thickening of long bones, loss of weight, irritability, loss of hair, joint pains etc.

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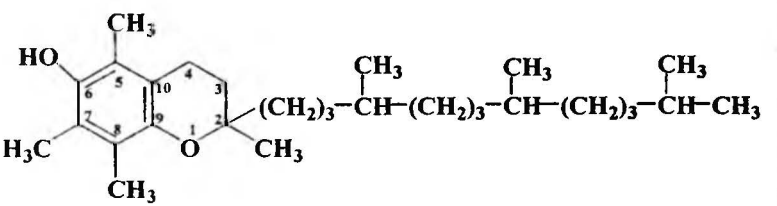
## Vitamin E
## (tocopherol, antisterile vitamin, vitamin of reproduction)




**α - Tocopherol**

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## Biological role of vitamin E

1. Vitamin E is essential for the membrane structure and integrity of the cell.
2. Vitamin E is a most potent fat soluble antioxidant:

- Vitamin E prevents the peroxidation of polyunsaturated fatty acids contained in cellular and subcellular membrane phospholipids of mitochondria, ER, and plasma membranes by $O_2$ and free radicals such as $O_2^{\cdot-}$, $OH^{\cdot}$, $HO_2^{\cdot}$ and $H_2O_2$.

$$\text{Toc-OH} + O_2^{\cdot-} \rightarrow \text{Toc-O}^{\cdot} + HO_2^{\cdot}$$

- Vitamin E breaks free-radical chain reactions as a result of it ability to transfer a phenolic hydrogen to a peroxyl free radical of a peroxidized polyunsaturated fatty acid. The phenoxy free radical formed then reacts with a further peroxyl free radical.

$$\text{Toc-OH} + R^{\cdot} \rightarrow \text{Toc-O}^{\cdot} + RH$$
$$\text{Toc-O}^{\cdot} + R^{\cdot} \rightarrow \text{Toc-O-R}$$

- Selenium as prosthetic group of *glutathione peroxidase* offers protection against the peroxidation of membrane lipids. Selenium and vitamin E spare each other by cooperative functioning.

$$\text{ROOH} + 2\text{G-SH} \rightarrow \text{ROH} + \text{G-S-S-G} + H_2O$$

3. Vitamin E prevents the oxidation of vitamin A and carotenes from oxidative destruction.
4. Vitamin E is required for cellular respiration through electron transport chain (believed to stabilize coenzyme Q).

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## The daily requirement

### 20-30 mg of vitamin E

Requirement of vitamin E increases with increased intake of polyunsaturated fatty acids (PUFA): 1g of PUFA is corresponding to1mg of vitamin E.

***Sources:*** Wheat germ oil, cotton seed oil, sunflower seed oils, corn, peanut and soya bean oil, grain crops, hips, salad, cabbage, eggs, meat, fish, liver, milk and butter.

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## Hypovitaminosis E

1. Severe symptoms of vitamin E deficiency are not seen in humans except increased fragility of erythrocytes, muscular weakness, creatinuria and minor neurological symptoms.
2. In many animals like rabbit, rat, guinea pig, the deficiency is associated with sterility, degenerative changes in muscle, megaloblastic anemia and changes in central nervous system.

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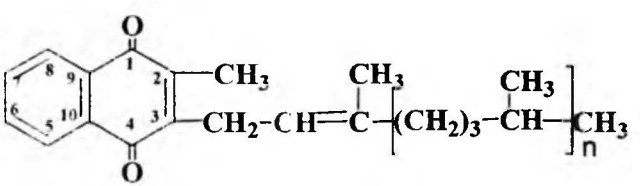
## Vitamin K
## (phylloquinone, antihemorrhagic vitamin)




**Phylloquinone ($K_1$)**

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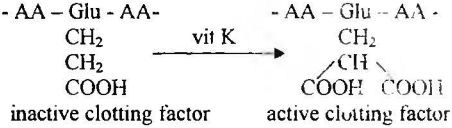
## Biological role of vitamin K

1. The functions of vitamin K are concerned with blood clotting process. It brings about the posttranslational modification of clotting factors II (prothrombin), VII, IX and X, synthesized as inactive precursors in the liver. For their modification into the active forms they require vitamin K as coenzyme for the *γ-glutamate carboxylase* enzyme that forms γ-carboxy glutamate from glutamate residues present in the proteins. γ-carboxy glutamate residues are high affinity calcium chelators.




inactive clotting factor — active clotting factor

2. Vitamin K is also required for the carboxylation of glutamate residues of osteocalcin, a calcium binding protein present in the bone.
3. Vitamin K as a cofactor in oxidative phosphorylation being associated with mitochondrial lipids.

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## The daily requirement

### 0.2-0.3 mg of vitamin K

***Sources:*** Vitamin K can be adequately synthesized in the gut by the microflora, cabbage, cauliflower, tomatoes, alfa alfa, spinach, egg yolk, meat, liver, cheese and dairy products.

Vitamin K is widely distributed in plant and animal tissues used as food, and production of the vitamin in the intestine ensures that dietary deficiency does not occur in adults.

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## Hypovitaminosis K

1. Vitamin K deficiency can be caused by fat malabsorption, which may be associated with pancreatic dysfunction, biliary disease, atrophy of the intestinal mucosa, or any cause of steatorrhea. In addition, sterilization of the large intestine by antibiotics can result in deficiency when dietary intake is limited.
2. At vitamin K deficiency blood coagulation is adversely affected. The individual bleeds profusely even for minor injuries. The blood clotting time is increased.
3. Vitamin K deficiency leads to bleeding and hemorrhage of the newborn.

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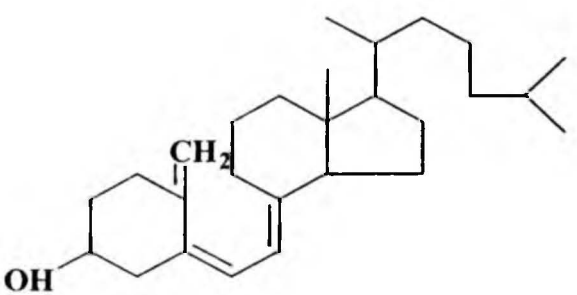
## Vitamin D (cholecalciferol, calciol, antirachitic vitamin)




**Cholecalciferol (vitamin $D_3$)**

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## Biological role of calcitriol (or 1,25-dihydroxycolecalciferol)

1. Action of calcitriol on the intestine.

- Calcitriol stimulate the intestinal reabsorption of Ca and phosphate by increasing the synthesis of the calcium-binding protein which increases the Ca uptake by the intestine.

2. Action of calcitriol on the bone.

- Promotes bone formation by stimulating osteoblast differentiation. In the osteoblasts calcitriol stimulates Ca uptake for deposition as calcium phosphate thereby influencing the mineralization of young bone tissues.
- Increases blood Ca levels through increased Ca and phosphate mobilization from old bone.

3. Action of calcitriol on the kidney.

- Calcitriol is involved in minimizing the excretion of Ca and phosphate by decreasing their excretion and enhancing reabsorption by inducing transporters.

Thus calcitriol causes elevation in the plasma Ca and phosphate levels.

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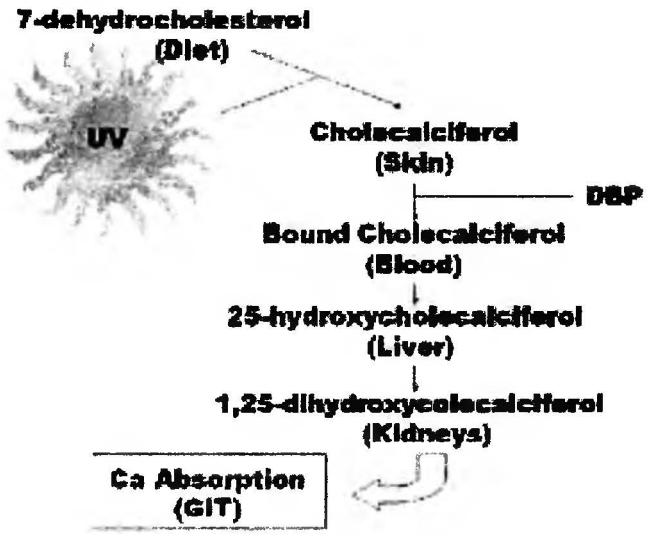
## Metabolism of vitamin D

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## The daily requirement

**12-25 μg (500-1000 IU) of vitamin D**

*Sources:* fish liver oils, liver, butter, milk, fatty fish, egg yolk etc.

Vitamin D can be provided to the bodv in three ways:

1. Exposure of skin to sunlight for synthesis of vitamin D;
2. Consumption of natural foods;
3. By irradiating foods (like yeast) that contain precursors of vitamin D and fortification of foods (milk, butter etc.).

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## Hypovitaminosis D

Deficiency of vitamin D causes rickets in children and osteomalacia in adults.

1. Rickets is characterized by defective ossification leading to soft and pliable bones, bow-legs, knock-knees, bead-like swellings at the rib junctions (rachitic rosary), enlargement of the epiphyses and contracted pelvis.
2. In case of osteomalacia (adult rickets) demineralization of the bones occurs (bones become softer). increasing their susceptibility to fractures. The ionic serum calcium is reduced to low levels resulting in neuro-muscular irritability and tetany.

## Hypervitaminosis D

1. Excess of vitamin D causes demineralization of bone (resorption) and increased calcium absorption from the intestine, leading to elevated calcium in plasma (hypercalcemia).
2. Prolonged hypercalcemia is associated with metastatic calcification of soft tissues such as kidney and arteries, calculi formation.
3. High consumption of vitamin D is associated with loss of appetite, nausea, increased thirst, loss of weight, digestive disturbances etc.

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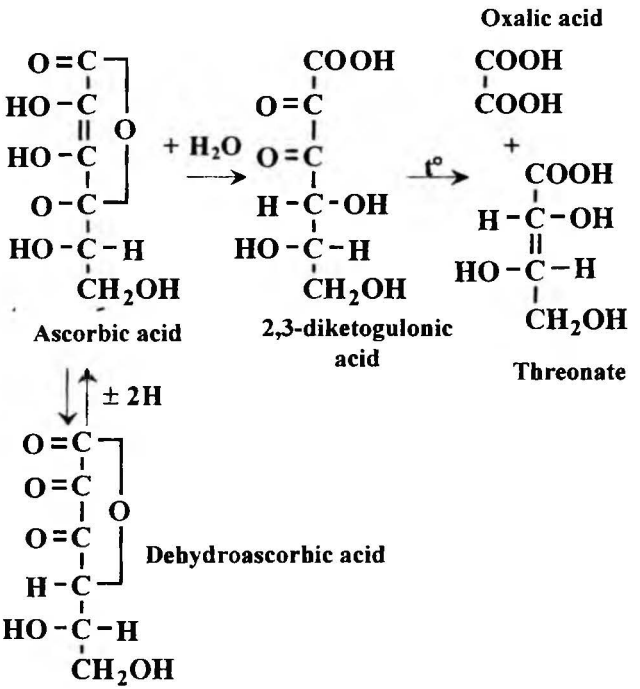
## Vitamin C
## (ascorbic acid, anti-scorbutic vitamin)

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## Biological role of vitamin C

Vitamin C participates in several types of reactions:

**I-st type of reactions:** Ascorbic acid + NADP → Dehydroascorbic acid + $NADPH_2$; $NADPH_2$ is used **in hydroxylation reactions:**

Examples:

1. Vitamin C as coenzyme of *lysyl hydroxylase* and *prolyl hydroxylase* in hydroxylation of proline to hydroxy proline and lysine to hydroxy lysine. *Thus vitamin C is involved in collagen synthesis.*
2. Hydroxylation of tryptophan (enzyme *hydroxylase*) to hydroxytryptophan in the synthesis of serotonin.
3. Synthesis of norepinephrine from dopamine.
4. Neutralization of toxic substances by microsomal oxidation.
5. Role in the production of the steroid hormones from cholesterol, by activating certain enzyme systems involved in the process in the gonads and adrenal cortex.
6. Oxidation of p-hydroxyphenylpyruvate (enzyme *hydroxylase*) to homogentisate in tyrosine metabolism. The subsequent step is catalyzed by *homogentisate dioxygenase*, which also requires ascorbic acid.
7. The bile acid formation at the initial *7α-hydroxylase* step.

**II-nd type of reactions: reactions of reduction:**

1. Ascorbic acid is involved in the redox mechanisms.
2. Iron absorption by conversion of the ferric form ($Fe^{3+}$) to the ferrous form ($Fe^{2+}$).
3. Reconversion of methemoglobin to hemoglobin by conversion of the ferric form ($Fe^{3+}$) to the ferrous form ($Fe^{2+}$).
4. The degradation of hemoglobin to bile pigments.
5. Formation of deoxyribose from ribose.
6. Formation of tetrahydrofolate from folic acid (enzyme *folic acid reductase*). *Thus vitamin C is involved in the maturation of erythrocytes.*

**III-d type of reactions: as antioxidant:**

1. Sparing of vitamins A and E from oxidation and may inhibit the formation of nitrosamines during digestion. Act as a general water-soluble antioxidant.
2. Synthesis of immunoglobulins (antibodies) and increasing of the phagocytic action of leucocytes. *Immunological function of vitamin C.*

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## Eventual effects of vitamin C

1. Increasing of organism resistance to the action of adverse factors. Participation of vitamin C in I type of reactions, (examples 2-5) and in III type of reactions.
2. Maintenance of normal connective tissue – synthesis of collagen.
3. Participation in hematopoiesis (II type of reactions).

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## Hypovitaminosis C

- The deficiency of ascorbic acid results in scurvy. This disease is characterized by spongy and sore gums, loose teeth, anemia, pains at the swollen joints, decreased immunocompetence, delayed wound healing, union of fractures, sluggish hormonal function of adrenal cortex and gonads, reduction in the amount of inter-cellular substance and weakens the endothelial wall of the capillaries, fragile blood vessels, hemorrhages inside the muscles, from the mucous membranes of the mouth and gastrointestinal tract and under the skin (petechial hemorrhages), osteoporosis, swelling and bleeding of gums with ulceration and even gangrene, etc. Subcutaneous bleeding results in the formation of red patches under the skin.
- Most of these symptoms are related to impairment in the synthesis of collagen and/or the antioxidant property of vitamin C.

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## Medicines of vitamin C

| | |
|---|---|
| **Ca ascorbate** | Treatment of diabetes, cardiac insufficiency |
| **Fe ascorbate** | Treatment of anemia, antiulcerant, tonic effects |
| **Mg ascorbate** | Treatment of psychical disorders, asthenia, physical and mental retardation |

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## Provision rate of organism with vitamin C

| *Rate* | *Parameter* | *Diagnostic value* |
|---|---|---|
| In plasma μmole /L | > 17.0 | Norma |
| | 6.0 – 17.0 | Moderate deficit |
| | < 6.0 | Extreme deficit |

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## The daily requirement

**70-120 mg of vitamin C**

***Sources:*** Citrus fruits, gooseberry, guava/ green vegetables (cabbage, spinach), tomatoes, potatoes (particularly skin), green peppers, hips, currant, sorrel. High content of vitamin C is found in adrenal gland and gonads.

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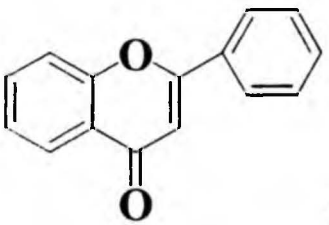
## Vitamin P (vitamin of permeability)

The colouring pigments in plants like rutin and hesperidin are referred to as vitamin P. They are bioflavonoids.



**Rutin**

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## Biological role of vitamin P

1. Bioflavonoids act as antioxidants.
2. Bioflavonoids protect vitamin C from being destroyed.
3. Bioflavonoid`s antioxidant property responsible for maintenance of capillary permeability for correct the vascular abnormality.

## The daily requirement

**30-50 mg of vitamin P**

***Sources:*** Tea, peels and pulp of citrus fruits (oranges, lemons), tobacco leaves, hips, currants, sorrel and many vegetables.

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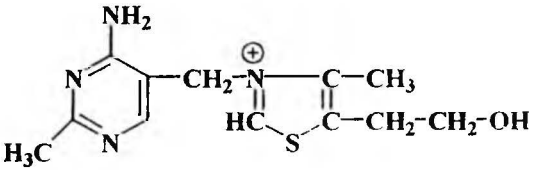
## Vitamin $B_1$ (thiamine, anti-beri-beri vitamin, antineuritic vitamin)




Thiamine

Active form is coenzyme, **Thiamine diphosphate (TDP).** The pyrophosphate moiety is donated by ATP and the reaction is catalyzed by the *thiamine pyrophosphate transferase* mainly in brain and liver.

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## Biological role of vitamin $B_1$

Enzymes are dependent on TDP:

1. *Pyruvate dehydrogenase* catalyses oxidative decarboxylation of pyruvate to acetylCoA.
2. *α-Ketoglutarate dehydrogenase* catalyses oxidative decarboxylation of α-ketoglutarate to succinyl CoA (citric acid cycle).
3. *α-Keto acid dehydrogenase* (decarboxylase) catalyses the oxidative decarboxylation of the α-ketoanalogs of Leu, Ile, and Val.
4. *Transketolase* (pentose phosphate pathway of glucose metabolism).
5. TDP is required for acetylcholine synthesis by inhibition of *choline esterase*.
6. *Pyruvate decarboxylase* catalyses decarboxylation of pyruvate to acetaldehyde (alcoholic fermentation).

Thus $B_1$ is involved in carbohydrate metabolism.

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## The daily requirement

**2-3 mg of vitamin $B_1$**

***Sources:*** Whole grains, rice bran, wheat bran, cereals (oat, buckwheat, millet), meal, pulses, oil seeds nuts, hazelnut, walnut, yeast, liver, eggs, fish, pork, heart, kidney, milk etc.

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## Hypovitaminosis $B_1$

The deficiency of vitamin $B_1$ results in a condition called **beri-beri**. The early symptoms of Beri-beri are loss of appetite (anorexia), weakness, constipation, nausea, mental depression, peripheral neuropathy, irritability, fatigue, headache, insomnia, gastro-intestinal disorders and tachycardia etc. Numbness in the legs complaints of "pins and needles sensations" is reported.

## Manifestations of hypovitaminosis $B_1$ in European countries

1. Wernicke's encephalopathy – neurological disorder caused by thiamine deficiency, typically from chronic alcoholism or persistent vomiting, and marked by mental confusion, abnormal eye movements, and unsteady gait.
2. Wernicke-Korsakoff syndrome – disorder mostly seen in chronic alcoholics. It is characterized by loss of memory; apathy and a rhythmical to and fro motion of the eye balls.

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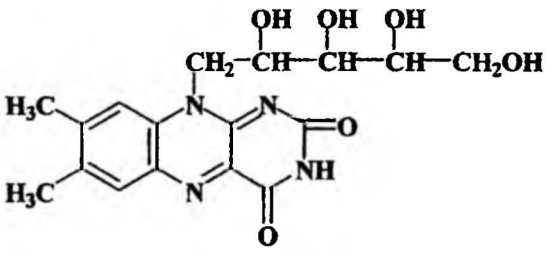
## Vitamin $B_2$ (riboflavin, growth factor)



Riboflavin

**Flavin mononucleotide (FMN)** and **flavin adenine dinucleotide (FAD)** are the two coenzyme forms of riboflavin.

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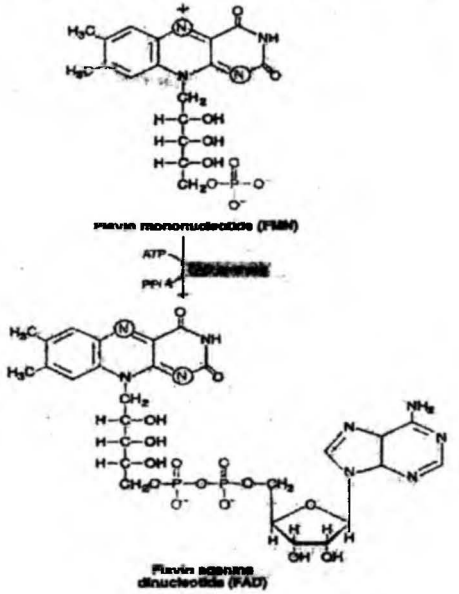
## Synthesis of FAD from FMN

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## The daily requirement

**2-4 mg of vitamin $B_2$**

***Sources***: It is synthesized by intestinal microflora, milk and milk products, meat, liver, kidney, heart, eggs, yeast, cereals, fruits, vegetables, fish, roots, grain, seeds.

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## Biological role of vitamin $B_2$

Enzymes that use flavin coenzymes (FMN or FAD) are called *flavoproteins* and participate in redox reactions responsible for energy production. The functional unit of FMN or FAD is isoalloxazine ring which serves as an acceptor of two hydrogen forming $FMNH_2$ or $FADH_2$. FAD and FMN are associated with enzymes involved in various metabolisms:

1). Cellular respiration, where FAD and FMN act as components of the respiratory chain (RC):

1. FMN is a component of the complete RC *(FMN-linked-dehydrogenase or NADH dehydrogenase).*
2. FAD is a component of the incomplete RC:
   - *Succinate dehydrogenase – citric acid cycle,*
   - *Acyl CoA dehydrogenase* – β-oxidation of fatty acids).

2). FAD is a prosthetic group of pyruvate and α-ketoglutarate dehydrogenase complexes (oxidative decarboxylation).

3). FAD is the prosthetic group of:
   - *D-amino acid oxidase* (amino acid deamination),
   - *Xanthine oxidase* (purine degradation),
   - *Aldehyde dehydrogenase* (degradation of aldehydes).

4). FMN is a prosthetic group of *L-amino acid oxidase* (amino acid deamination).

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## Hypovitaminosis of vitamin $B_2$

Riboflavin deficiency symptoms include:

1. Cheilosis (fissures at the angles of the mouth or angular stomatitis).
2. Glossitis (tongue smooth and purplish).
3. Dermatitis.
4. Inflammation of cornea, blood-shot eyes, dimness of vision, photophobia, burning and dryness of eyes and redness of the conjunctiva.
5. Itching.

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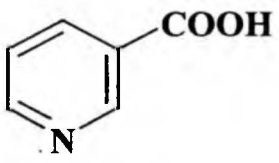
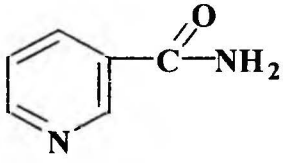
## Vitamin PP (niacin, pellagra-preventing factor, $B_5$)




**Nicotinic acid or niacin** **Nicotinamide or niacinamide**

Niacin in the form of niacinamide is a part of the coenzymes **nicotinamide adenine dinucleotide ($NAD^+$)** and **nicotinamide adenine dinucleotide phosphate ($NADP^+$).**

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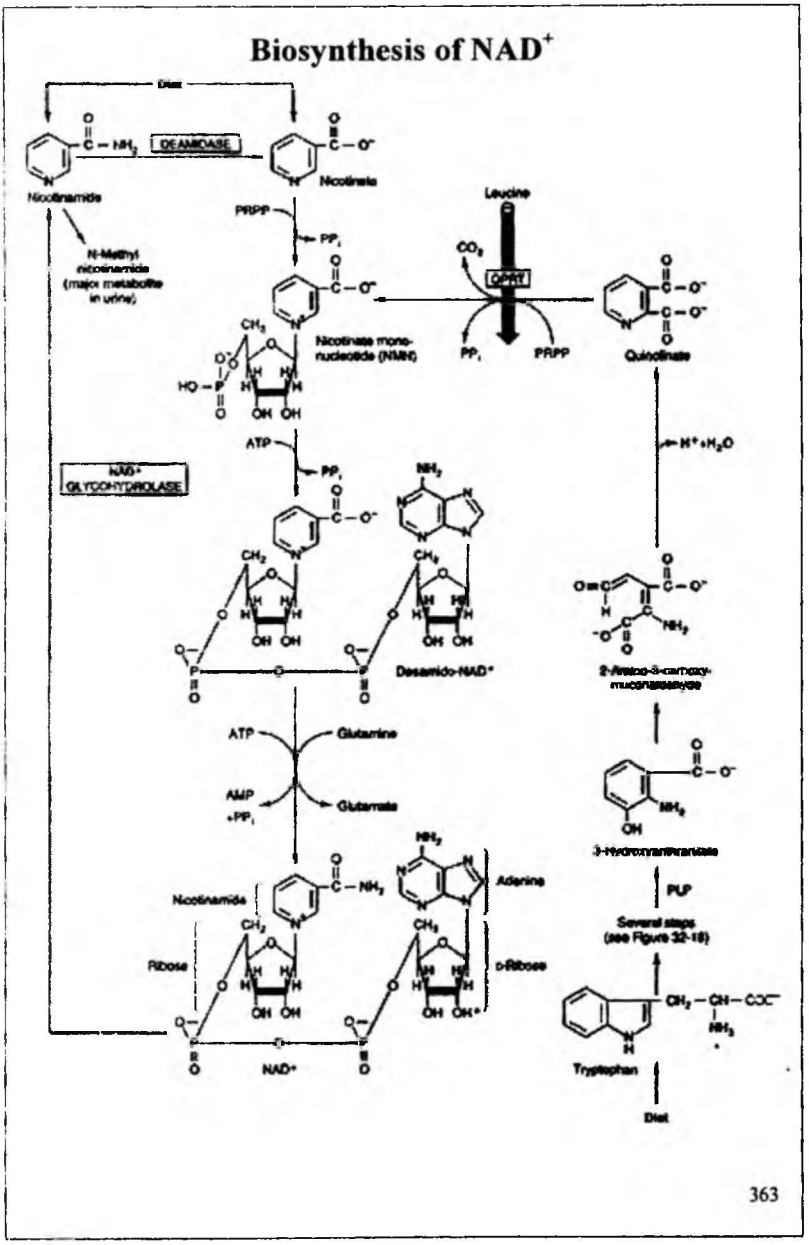
Biosynthesis of NAD+
Diet
DEAMIDASE
Nicotinamide
Nicotinate
N-Methyl nicotinamide (major metabolite in urine)
PRPP
PPi
Leucine
CO2
QPRT
PPi
PRPP
Quinolinate
Nicotinate mono-nucleotide (NMN)
ATP
PPi
NAD+ GLYCOHYDROLASE
H+ + H2O
Desamido-NAD+
2-Amino-3-carboxy-muconatesemialdehyde
ATP
Glutamine
AMP + PPi
Glutamate
3-Hydroxyanthranilate
PLP
Several steps (see Figure 32-18)
Nicotinamide
Adenine
Ribose
D-Ribose
NAD+
Tryptophan
Diet

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## Biological role of vitamin PP

The $NAD^+$ and $NADP^+$ coenzymes of *oxidoreductases* are involved in a variety of oxidation-reduction reactions. They accept hydride ion and undergo reduction in the pyridine ring.

1. $NAD^+$ is concerned with cellular respiration as a component of the complete respiratory chain *(NAD-linked dehydrogenase)*. NADH produced is oxidized in the electron transport chain to generate ATP.
   - *Malate dehydrogenase, isocitrate dehydrogenase* – CAC,
   - *Lactate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase* – anaerobic glycolysis,
   - *β-Hydroxyacyl CoA dehydrogenase* – β-oxidation of fatty acids,
   - *Alcohol dehydrogenase* – oxidation of alcohol,
   - *Glutamate dehydrogenase* – deamination of Glu.
2. NAD is a coenzyme of pyruvate and α-ketoglutarate dehydrogenase complexes (oxidative decarboxylation).
3. NADPH is a component of microsomal oxidation for neutralization of xenobiotics.
4. NADPH takes part in biosynthesis of fatty acids, cholesterol, bile acids, steroid hormones, vitamin D.
5. $NADP^+$ is a coenzyme of *glucose-6-phosphate dehydrogenase* and *6-phosphogluconate dehydrogenase* – pentose phosphate pathway.

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## The daily requirement

**15-25 mg of vitamin PP**

***Sources:*** Liver, kidney, heart, meat, fish, yeast, beans and peanuts, wheat and rice bran, whole grains, cereals, eggs, vegetables, milk. Tryptophan can be converted to niacin in the liver. 1 g of a protein containing 60 mg of tryptophan is equivalent to 1 mg of niacin.

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## Hypovitaminosis PP

Niacin deficiency results in a condition called pellagra (rough skin). This disease involves skin, gastrointestinal tract and central nervous system.

The symptoms of pellagra are referred to as 3 ''D''s:

1. **Dermatitis** (inflammation of skin) is found in the areas of the skin exposed to sunlight (neck, dorsal part of feet, ankle and parts of face). Stages are erythema, desquamation, pigmentation,
2. **Diarrhea** may be in the form of loose stools, often with blood and mucus. Prolonged diarrhea leads to weight loss;
3. **Dementia** is associated with degeneration of nervous tissue. The symptoms of dementia include delusion, hallucinations, anxiety, irritability, poor memory, insomnia (sleeplessness) etc.

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## Provision rate of organism with vitamin PP

| *Rate* | *Parameter* | *Diagnostic value* |
|---|---|---|
| in urine N-methyl-nicotinamide / creatinine, mlmole/L | 1.3-3.9 | Norma |
| | 0.4-1.3 | Moderate deficit |
| | <0.4 | Extreme deficit |

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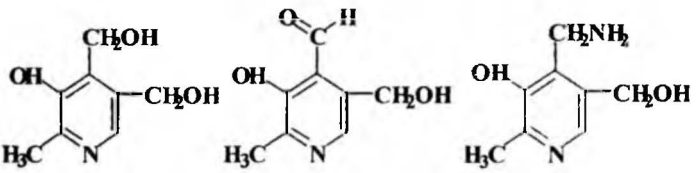
## Vitamin $B_6$ (pyridoxine, anti-dermatitis vitamin)




**Pyridoxine** **Pyridoxal** **Pyridoxamine**

The active forms of vitamin $B_6$ are the coenzymes, **pyridoxal phosphate** and **pyridoxamine phosphate.**

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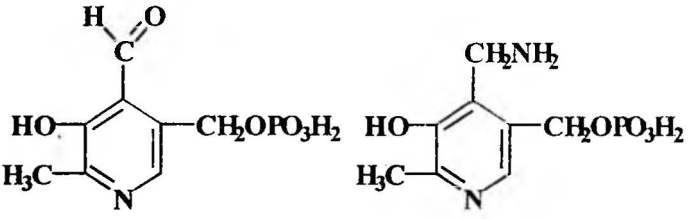
## Coenzymes of vitamin $B_6$




**Pyridoxal phosphate** **Pyridoxamine phosphate**

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## Biological role of vitamin $B_6$

**Pyridoxal phosphate** as coenzyme is used for certain enzymes:

1. *Aminotrasferase* – transamination reactions (synthesis of nonessential amino acids, indirect deamination).
2. *Decarboxylase* – decarboxylation of certain amino acids (synthesis of biogenic amines).
3. *Kynureninase* – biosynthesis of NAD and NADP from Trp.
4. *Cystathionine synthase* – transsulfuration reactions in Cys metabolism.
5. *Dehydratases* – deamination of Ser and Thr.
6. *Hydroxymethyltransferase* – Ser biosynthesis from Gly.
7. *Amino levulinic acid synthase* – biosynthesis of δ-amino levulinic acid (precursor for heme synthesis) from succinyl CoA and Gly.
8. *Glycogen phosphorylase* (glycogen degradation).

Thus $B_6$ is closely associated with the metabolism of amino acids.

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## The daily requirement

**2-3 mg, pregnant 6-7 mg**

***Sources:*** synthesized by intestinal microflora, yeast, liver, heart, kidney, meat, egg yolk, fish, milk, whole grains, wheat, legumes, peas, crude molasses, green pepper, cabbage, roots and tubers.

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## Hypovitaminosis $B_6$

- Pyridoxine deficiency is associated with neurological symptoms such as depression, irritability, lethargy, nervousness and mental confusion. Convulsions, peripheral neuropathy, vomiting, diarrhoea and decrease in hemoglobin levels, associated with hypochromic microcytic anemia are observed in deficiency.
- In children, $B_6$ deficiency results in convulsions (epilepsy).
- Vitamin $B_6$ more than 200 mg/day may cause neurological damage.

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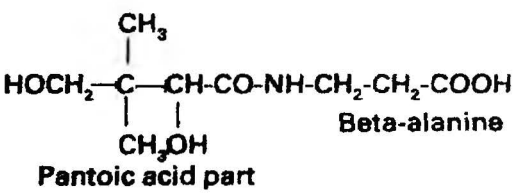
## Vitamin $B_3$ (pantothenic acid, chick anti-dermatitis factor or filtrate factor)




**Pantothenic acid**

Coenzyme forms of pantothenic acid:

1. **4`-phosphopantetheine** – non-protein group of Acyl carrier protein in the enzyme *fatty acid synthase*;
2. **Dephospho-CoA** – coenzyme of *citrate lyase* (fatty acid synthesis) and *N-acetyltransferase* (amino acid metabolism);
3. **HSCoA**.

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## Biological role of vitamin $B_3$

I. Acyl carrier protein containing pantothenic acid moiety - 4`-phosphopantetheine and has an important role in the biosynthesis of fatty acids.

II. Reactions involving Coenzyme A (HSCoA):
   1. Metabolism of carbohydrates – oxidative decarboxylation of pyruvate to acetyl CoA and α-ketoglutarate to succinyl CoA. Succinyl CoA is required for the synthesis of porphyrins.
   2. Metabolism of lipids – acylCoA and acetylCoA during the oxidation of fatty acids are produced.
   3. Acetyl CoA is required for the synthesis of acetyl choline, cholesterol, steroid hormones, fatty acids, ketone bodies.
   4. Acetyl CoA is a central molecule involved in all the metabolisms (carbohydrates, lipids and proteins).

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## Hypovitaminosis $B_3$

Manifistations of pantothenic acid deficiency are:

1. Dermatitis.
2. Ulcers of the mucous of gastrointestinal tract.
3. Burning feet syndrome (pain and numbness in the toes, sleeplessness, and fatigue).
4. Depigmentation.

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## The daily requirement

**0-15 mg of vitamin $B_3$**

***Sources:*** liver, kidney, eggs, beef, milk, yeast, cabbage, cauliflower, potatoes, peanuts and peas.

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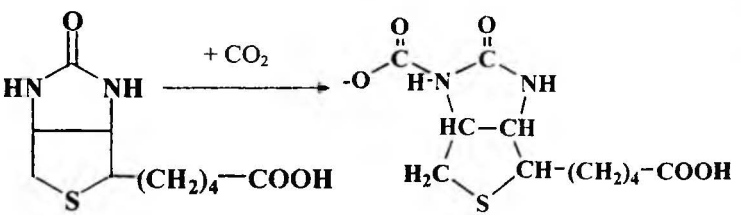
## Vitamin H (biotin, antiseborrheic vitamin, anti-egg white injury factor, vitamin $B_7$)




**Biotin** **Carboxybiotin**

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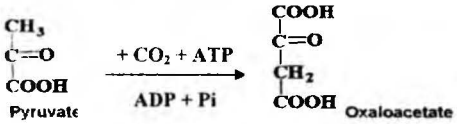
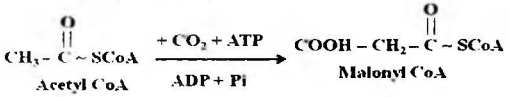
## Biological role of vitamin H

Biotin functions as cofactor of the multi-subunit enzymes which are involved in carboxylation reactions:

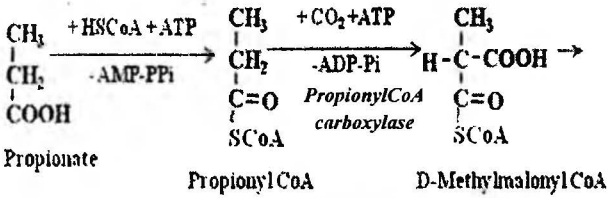
1. *Pyruvate carboxylase* (gluconeogenesis).

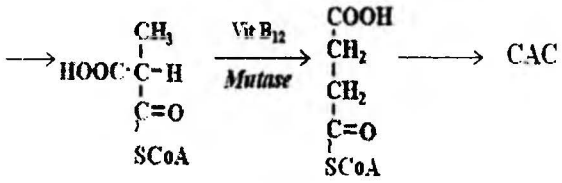



2. *AcetylCoA carboxylase* (synthesis of fatty acids).




3. *PropionylCoA carboxylase* (oxidation of fatty acids with odd numbers of carbon atoms).




4. *β-MethylcrotonylCoA carboxylase* (catabolism of leucine and certain isoprenoid compounds).

**β-MethylcrotonylCoA ⟶ β-MethylglutaconylCoA**

5. *Methylmalonyl-oxaloacetate transcarboxylase* (reaction of transcarboxylation).

**MethylmalonylCoA + Pyruvate ⟶ PropionylCoA + oxaloacetate**

## Hypovitaminosis H

- The symptoms of biotin deficiency include seborrhea, alopecia, paralysis, anemia, loss of appetite, nausea, dermatitis, glossitis, depression, hallucinations, muscle pain, scaly desquamation of skin, lethargy.
- Children develop dermatitis, alopecia, and loss of muscular control, and have retarded growth.

The deficiency may be associated with two causes:

1. Destruction of intestinal flora due to prolonged use of drugs such as antibiotics, sulfonamides.
2. High consumption of raw eggs containing avidin, which tightly combines with biotin and hampers its absorption from the intestine.

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## The daily requirement

**120-200 μg of vitamin H**

***Sources***: supplied by intestinal bacteria, liver, kidney, heart, milk, yeast, egg yolk, tomatoes, grains, brans, beans, soya beans, cauliflower.

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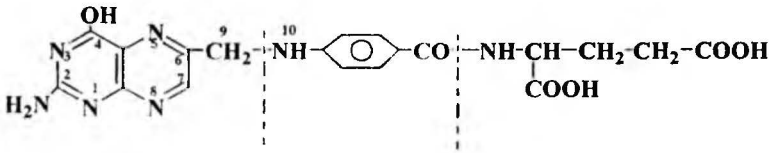
## Folic acid (folacin, vitamin $B_9$, pteroyl glutamic acid, growth factor, $B_c$, antianemic vitamin)




*Pteridine* *Para-aminobenzoic acid,* *L- Glutamate*

*PAB(A)*

**Folic acid**

Coenzyme form of Folic acid is **Tetrahydrofolic acid (THFA)**.

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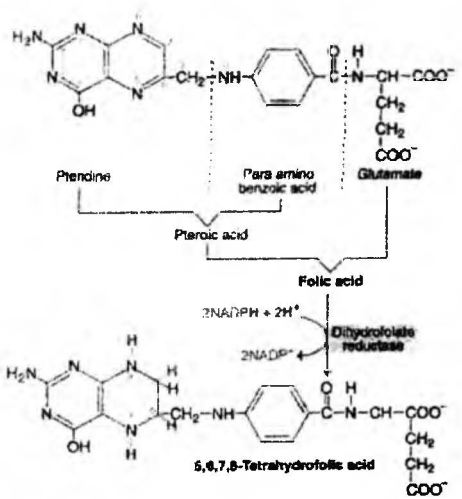
## Synthesis of tetrahydrofolic acid

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## Biological role of folic acid

The THFA acts as the one-carbon acceptor and forms derivatives:

1. $N^5(N^{10})$-Formyl (-CHO),
2. $N^5$-Hydroxy methyl ($-CH_2OH$),
3. $N^5$-Formate ($HCOO^-$),
4. $N^5$-Formimino (-HC = NH),
5. $N^5,N^{10}$-Methylene ($-CH_2-$),
6. $N^5,N^{10}$-Methenyl (-CH=),
7. $N^5$-Methyl ($-CH_3$).

1. **Folic acid** plays biochemical role in the metabolism of glycine, serine, glutamic acid, histidine, ethanolamine, betaine and choline.
2. **$N^5,N^{10}$-methenyl-THFA** and **$N^{10}$-formyl-THFA** are involved in the incorporation of formyl carbon into the purine skeleton (carbon 2, 8) which is incorporated into DNA and RNA.
3. **$N^5,N^{10}$-methylene-THFA** is involved for the synthesis of pyrimidine nucleotide - deoxythymidine (dTMP) involved in the synthesis of DNA.
4. **$N^5$-methyl-THFA** (the major form of folate derivative) is required for the formation of methionine.

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## Hypovitaminosis folic acid

The deficiency of vitamin results in a **macrocytic anemia** that may be attributed to the decreased formation of new red blood cells due to interference with purine and thymine synthesis. Due to synthesis, the maturation block in DNA erythrocytes is slowed down leading to macrocytic red blood cells.

**Manifestations of macrocytic anemia:**

1. Low hemoglobin levels.
2. Decreased number of erythrocytes.
3. Presence of megaloblasts.
4. Leukopenia.

**Treatment**

**200 of folic acid per os or 10-20 mg intravenously**

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## The daily requirement

**50-200 μg of folic acid**

***Sources:*** synthesized by intestinal microflora, green leafy vegetables, cauliflower, cabbage, salad, carrots, tomatoes, onion, whole grains, cereals, liver, kidney, yeast, eggs, milk.

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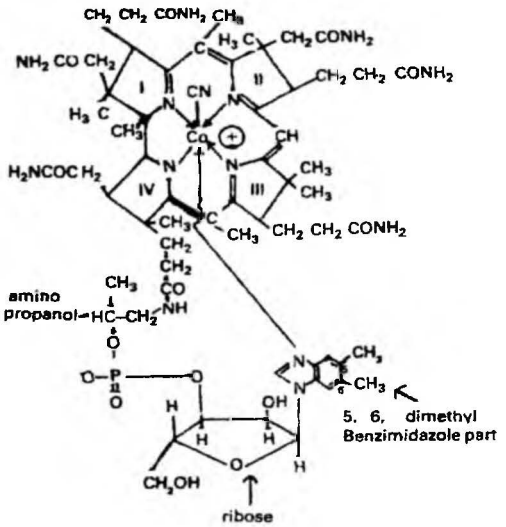
## Vitamin $B_{12}$ (cobalamin, antianemic vitamin, anti-pernicious anemia vitamin)




**Cyanocobalamin**

1. The structure of vitamin $B_{12}$ consists of a **corrin** ring having 4 pyrrole rings with a central cobalt atom.
2. Cobalt also holds dimethylbenzimidazole containing ribose-5-phosphate and aminoisopropanol.

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## There are two coenzyme forms of vitamin $B_{12}$

1. **Deoxyadenosyl cobalamin** - cyanide is replaced by 5' deoxyadenosine.
2. **Methylcobalamin** - cyanide is replaced by methyl group.

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## Absorption of vitamin $B_{12}$

1. Cobalamin binding proteins, known as 'R-proteins', are secreted by the salivary glands and the stomach and bind the $B_{12}$.
2. In the stomach 'R-proteins under action of HCL are released.
3. Cobalamins bind to the intrinsic factor of Castle. This binding protects $B_{12}$ against its uptake and use by bacteria.
   *Intrinsic factor of Castle is secreted by the parietal cells of the gastric mucosa of the Cardia and the fundus of the stomach. It is resistant to proteolytic digestive enzymes.*
4. The complex crosses the ilea mucosa.
5. In the mucosal cells, intrinsic factor is released and $B_{12}$ is converted to methylcobalamin.
6. Methylcobalamin is then transported in the circulation in a bound form to a plasma transport protein, transcobalamin II.
7. Methylcobalamin is taken up by the liver, converted to deoxyadenosyl $B_{12}$ and stored in this form in a bound form to a protein, transcobalamin I.

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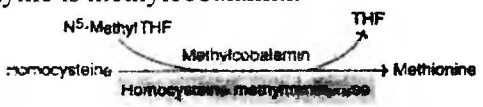
## Biological role of vitamin $B_{12}$

There are only two reactions that are dependent on vitamin $B_{12}$.

1. Conversion of methyl malonyl CoA to succinyl CoA in the mitochondria (see biotin above). The enzyme is *L-methyl-malonyl CoA-mutase* and the coenzyme is 5`-deoxyadenosyl cobalamin.
2. Synthesis of methionine from homocysteine in the cytosol. $B_{12}$ converts $N^5$-methyl-THFA to THFA. The enzyme is *homocysteine methyltransferase* (or *methionine synthase)* and the coenzyme is methylcobalamin.

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## Hypovitaminosis $B_{12}$

The disease associated with vitamin $B_{12}$ deficiency is **pernicious (malignant) anemia.**

**Manifestations of pernicious anemia are:**

1. Macrocytic megaloblastic anemia (see folic acid above): folate is trapped as $N^5$-methyl THFA (Folate-trap) results in decreased folate coenzymes that lead to reduced nucleotide and DNA synthesis.
2. Neurological manifestations (neuronal degeneration of the cord and demyelination of nervous system) due to the accumulation of methylmalonyl CoA and deficiency of methionine that interferes in myelin sheath formation. The symptoms include paresthesia (numbness and tingling) of fingers and toes, confusion, loss of memory and psychosis.
3. Loss of appetite and failure of growth.

The deficiency of vitamin $B_{12}$ may be associated with following causes:

1. Autoimmune destruction of gastric parietal cells, partial or total gastrectomy - intrinsic factor of Castle deficient.
2. Hereditary malabsorption of vitamin $B_{12}$.

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## The daily requirement

## 2-5 μg of vitamin $B_{12}$

***Sources*:**

1. Vitamin $B_{12}$ synthesized by only microorganisms and not by animals and plants.
2. Liver can store about 4-5 mg of vitamin $B_{12}$.
3. Vitamin $B_{12}$ is abundant in liver, meat, eggs, and milk, but not in plant products.

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# Hormones

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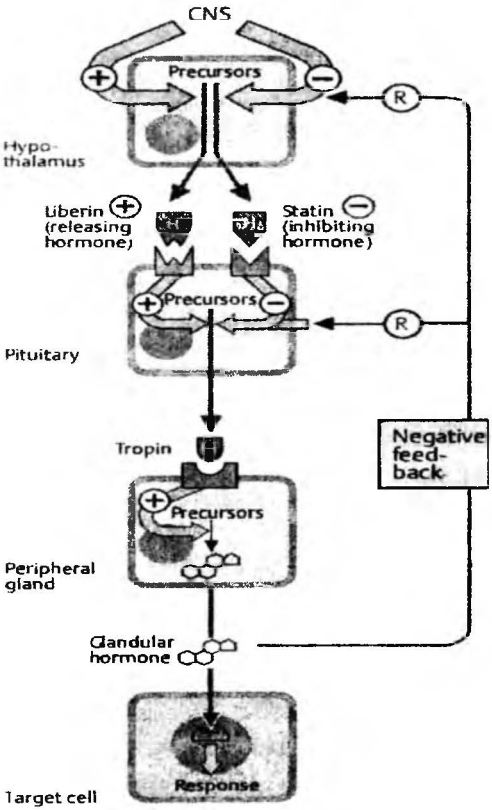
## Hormone hierarchy

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## Major mechanisms of hormones on metabolic regulation

1. Regulation of alterations in enzyme activity (hydrophilic hormones with cell-surface receptors).
2. Regulation of amounts of enzymes in the cell (lipophilic hormones with intracellular receptors).
3. Regulation of membrane permeability (insulin).

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## Classification of hormones according to chemical structure

1. Peptides or proteins (insulin, calcitonin, ACTH, ADH, glucagon).
2. Amino acid derivatives (epinephrine, norepinephrine, thyroxine (T4), triiodothyronine (T3)).
3. Steroids (sex hormones, glucocorticoids, mineralocorticoids).

## Classification of hormones according to mechanism of action

| Lipophilic hormones with intracellular receptors | Hydrophilic hormones with cell-surface receptors | | | |
|---|---|---|---|---|
| | G protein-linked receptors | | | Enzyme-linked receptors |
| Steroid-thyroid mechanism | Adenylate cyclase mechanism | Guanylate cyclase mechanism | $Ca^{2+}$/phosphotidylinositol mechanism | Kinase mechanism |
| Gluco- and mineralocorticoids, sex hormones, T3 and T4, calcitriol | $\alpha_2$- and $\beta$-adrenergic catecholamines, PTH, ACTH, ADH, glucagon, calcitonin, TSH | Atrionatriuretic factor (ANF). | $\alpha_1$-adrenergic catecholamines, oxytocin, ADH, TRH | Insulin, GH, PRL |

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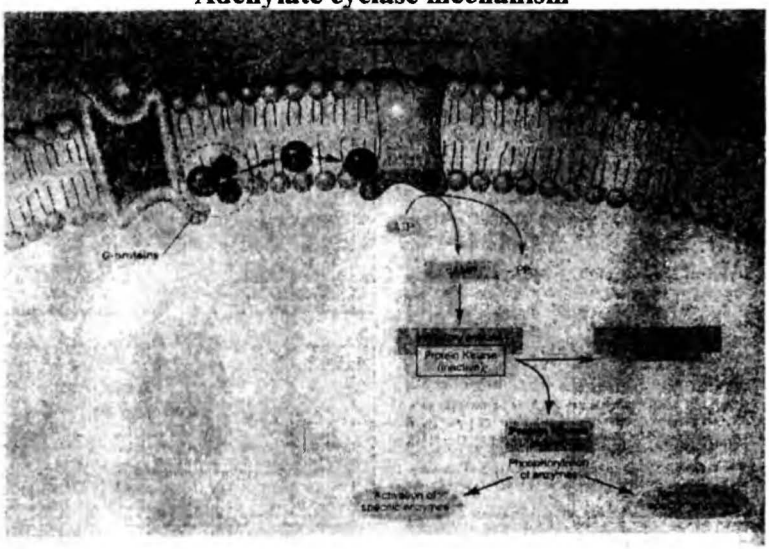
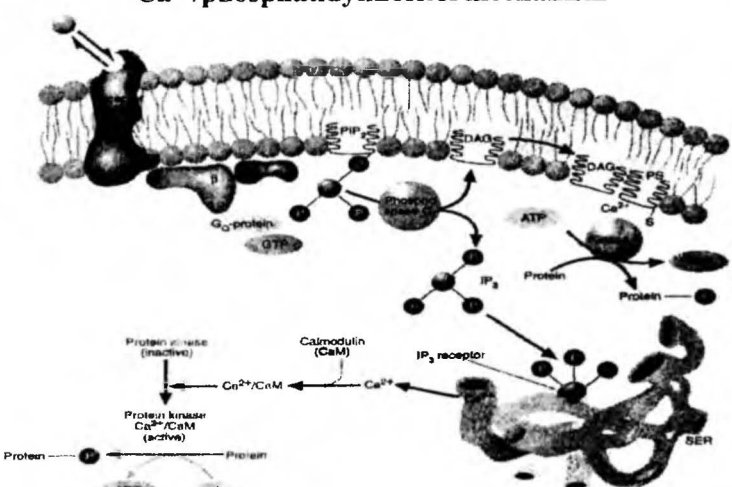
## Mechanisms of hormone action

### Adenylate cyclase mechanism



### $Ca^{2+}$/phosphatidylinositol mechanism

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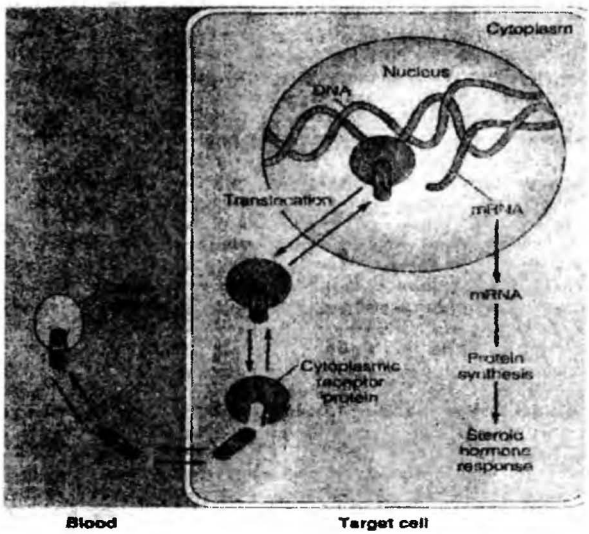
**Mechanism of hormone action**

**Steroid-thyroid mechanism**

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# Hypothalamic and pituitary hormones

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## Hypothalamic hormones

| *Hypothalamic hormone* | *Structure* | *Anterior pituitary hormone affected* |
|---|---|---|
| Corticotropin releasing hormone (CRH or corticoliberin) | 41 amino acids | Adrenocorticotropic hormone (ACTH or corticotropin) |
| Thyrotropin releasing hormone (TRH or thyroliberin) | tripeptide | Thyroid stimulating hormone (TSH or thyrotropin) |
| Gonadotropin releasing hormone (GnRH or gonadoliberin) | decapeptide | 1. Luteinizing hormone (LH or lutropin);<br>2. Follicle-stimulating hormone (FSH or follitropin) |
| Growth hormone-releasing hormone (GRH or somatotropin-releasing hormone, somatoliberin) | 44 amino acids | Growth hormone (GH or somatotropin) |
| Growth hormone-release-inhibiting hormone (GRIH or somatostatin) | 14 amino acids | — |
| Prolactin releasing hormone (PRH or prolactoliberin) | tripeptide | Prolactin (PRL or mammatropin, lactotropin) |
| Prolactin release-inhibiting hormone (PRIH or prolactostatin) | dopamine and/or peptide (56 amino acids) | — |
| Melanotropin releasing hormone (MRH or melanoliberin) | hexapeptide | Melanocyte stimulating hormone (MSH or melanotropin) |
| Melanotropin release-inhibiting hormone (MRIH or melanostatin) | tripeptide | — |

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## Pituitary hormones

| *Anterior pituitary hormone* | *Structure* | *Target tissue* | *Hormone affected* | *Pathophysiology* |
|---|---|---|---|---|
| ACTH | Polypeptide (39 amino acids) | Adrenal cortex | Adrenal cortisol, aldosterone | *Excessive production:* Cushing's syndrome – hyperpigmentation, increased production of adrenocorticosteroids, negative nitrogen balance, impaired glucose tolerance, hypertension, edema, muscle atrophy |
| TSH | Dimer (αβ) Glycoprotein (Mm 30.000) | Thyroid gland | T3,T4 | — |
| LH | Glycoprotein (Mm 25.000) | Corpus luteum cells, Leydig cells in the testis | Estradiol, progesterone, testosterone | Desensitization |
| FSH | Glycoprotein (Mm 25.000) | Follicular cells in the ovary, Sertoli cells in the testis | Testosterone, estradiol | — |

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## Pituitary hormones *(continuation)*

| *Anterior pituitary hormone* | *Structure* | *Target tissue* | *Hormone affected* | *Pathophysiology* |
|---|---|---|---|---|
| GH | 191 amino acids (Mm 22.000) | Muscle, liver, adipose tissue, bone, mammary glands | Insulin like growth factor I (ICF-I or somatomedin C) and Insulin like growth factor II (ICF-II or somatomedin A. | *Deficiency:* in the growing age causes dwarfism. *Excessive production:* gigantism in children and acromegaly in adults. Gigantism is characterized by increased growth of long bones. Acromegaly is characterized by increase in the size of hands, facial changes enlarged nose, protruding jaw), excessive hair, thickening of skin etc |
| PRL | Protein (Mm 23.000) | Mammary gland | Progesterone | Amenorrhea, galactorrhea in women; in men gynecomastia, impotence |
| MSH | Polypeptide | Melanocytes in the skin | — | Hyperpigmentation |
| Endorphins and enkephalins | Peptides | Central nervous system | — | — |

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## Anterior pituitary hormones. Biological role

| | |
|---|---|
| ACTH | Promotes the conversion of cholesterol to pregnenolone, enhances RNA and protein synthesis, adrenocortical growth, increases lipolysis by activating lipase of adipose tissue. |
| TSH | Promotes the uptake of iodide (iodide pump) by thyroid gland. Enhances the conversion of $I^-$ to $I^+$. Increases the proteolysis of thyroglobulin to secrete T3 and T4. Increases the synthesis of proteins, nucleic acids and phospholipids in thyroid gland. |
| LH | Stimulates the ripe follicle to ovulate, leaving the residual follicle, which forms the corpus luteum. Causes ovulation. |
| FSH | In females, stimulates ovulation, maturation of the ovum, stimulates follicular growth, and increases the weight of the ovaries. In males, stimulates spermatogenesis, growth of seminiferous tubule. |
| GH | Promotes the uptake of amino acids into the tissues and increases the protein synthesis, positive nitrogen balance, hyperglycemia, increases hepatic gluconeogenesis, hepatic glycogen synthesis, decreases peripheral glucose utilization, impairs glycolysis in muscle and reduces the tissue uptake of glucose. Promotes lipolysis in the adipose tissue and increases the levels of free fatty acids and their oxidation in the liver, increases ketogenesis. Promotes bone mineralization and its growth, cell growth, synthesis of RNA and DNA, positive Ca, Mg, $PO_4^{3-}$ balance, the retention of $Na^+$, $K^+$, and $Cl^-$, lactogenesis. |
| PRL | Growth of mammary gland, initiation and maintenance of lactation. Synthesis of milk proteins (casein, lactalbumin), lactose during lactation. Increases the levels of several enzymes involved in carbohydrate and lipid metabolism. Promotes HMP shunt, increases lipid biosynthesis. Promotes the growth of corpus luteum. |
| MSH | Promotes the synthesis of skin pigment melanin (melanogenesis) and disperses melanin granules that ultimately lead to darkening of the skin. In humans, MSH does not appear to play any role in melanin synthesis. |
| Endorphins and enkephalins | Produce analgesic opiate-like effects, hence they are also known as opioid-peptides. They bind to the receptors and control the endogenous pain perception. |

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**Pituitary hormones *(continuation)***

| ***Posterior pituitary hormones*** | Vasopressin (antidiuretic hormone, ADH) | Oxytocin |
|---|---|---|
| ***Site of synthesis*** | Supraoptic nucleus of hypothalamus | Paraventricular nucleus of hypothalamus |
| ***Stimulus of secretion*** | Increased osmolarity of plasma, hypovolemia, hypotension, emotional and physical stress, pain | Stimulation of the nipples, vaginal and uterine distention |
| ***Target tissue*** | Distal convoluted and collecting tubules of the kidney | Mammary gland, uterus |
| ***Structure*** | Nanopeptide | Nanopeptide |
| ***Pathophysiology*** | *Deficiency:* Diabetes insipidus: excretion of large volumes of dilute urine (polyuria) | |
| ***Process affected*** | Acts on renal collecting ducts via V2 receptors-aquaporins to increase water reabsorption, which leads to decreased urine formation. This increases blood volume, cardiac output and arterial pressure. Binding to V1 receptors on vascular smooth muscle cause vasoconstriction, which increases arterial pressure | Contraction of pregnant uterus (smooth muscles) and induces labor. Contraction of myoepithelial cells (look like smooth muscle cells) of breast. This causes milk ejection. Oxytocin synthesized in the ovary appears to inhibit steroidogenesis |

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# Hormones of the adrenal cortex

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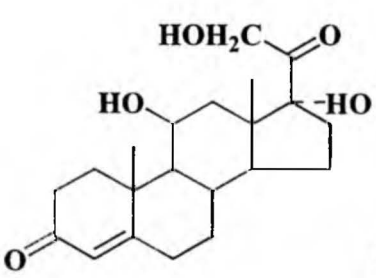
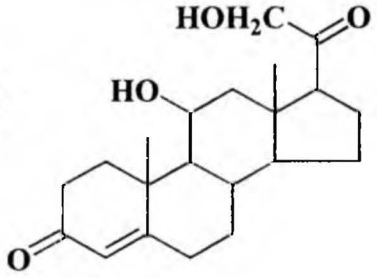
## Glucocorticoids




Cortisol




Corticosterone

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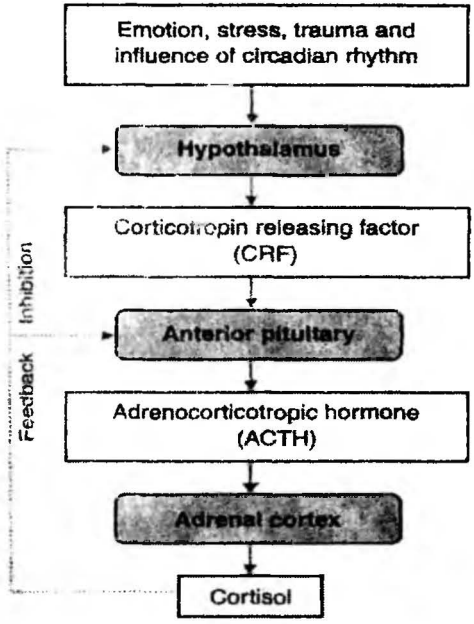
## Regulation of cortisol secretion

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## Transport of cortisol

- The main plasma binding protein is a α-globulin called **transcortin** or **corticosteroid-binding globulin**.
- Much smaller amounts of cortisol are bound to **albumin**.
- The unbound, or free, fraction constitutes about 8% of the total plasma cortisol and represents the biologically active fraction.

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## Biochemical functions of glucocorticoids

### I. Effects on intermediary metabolism

1). Effects on carbohydrate metabolism is to increase blood glucose concentration. Increase glucose production in liver by:

- increasing the delivery of amino acids from peripheral tissues (amino acids are released from proteins of muscle, lymphoid and connective tissue).
- increasing the rate of gluconeogenesis by inducing synthesis and activity of the enzyme *phosphoenolpyruvate carboxykinase*. Glucose produced is stored as glycogen in the liver by the activation of *glycogen synthase*.

2). Effects on lipid metabolism is to increase the circulating free fatty acids by two mechanisms:

- increasing the breakdown of triacylglycerol (lipolysis) in adipose tissue but can cause lipogenesis in other sites (face and trunk).
- reducing utilization of plasma free fatty acids for the synthesis of triacylglycerols.

3). Effects on protein and nucleic acid metabolism:

- anabolic effects in liver - promote transcription and synthesis of proteins by the stimulation of specific genes.
- catabolic effects (particularly at high concentration) in extrahepatic tissues (e.g., muscle, adipose tissue, bone, lymphoid and connective tissue) - promote degradation of proteins.

### II. Effects on host defense mechanisms

1). Suppress the immune response by:

- species- and cell-type-specific lysis of lymphocytes.
- impairment of antibody synthesis.

2). Suppress the inflammatory response by:

- decreasing the number of circulating leukocytes and the migration of tissue leukocytes.
- inhibiting fibroblast proliferation.
- inducing the synthesis of lipocortins, which, by inhibiting phospholipase A2, blunt the production of the inflammatory prostaglandins, thromboxanes and leukotrienes.

408

### III. Other effects of glucocorticoids

1) Necessary for maintenance of normal blood pressure and cardiac output.
2) Required for maintenance of normal water and electrolyte balance.
3) Necessary in allowing the organism to respond to stress. Stimulate the fight and flight response (to face sudden emergencies) of catecholamines.
4) Increase the production of gastric HCl and pepsinogen.
5) Inhibit the bone formation, hence the subjects are at a risk for osteoporosis.

409

### Disorders of glucocorticoid hormone deficiency

1) ***Primary adrenal insufficiency (Addison's disease)*** results in:

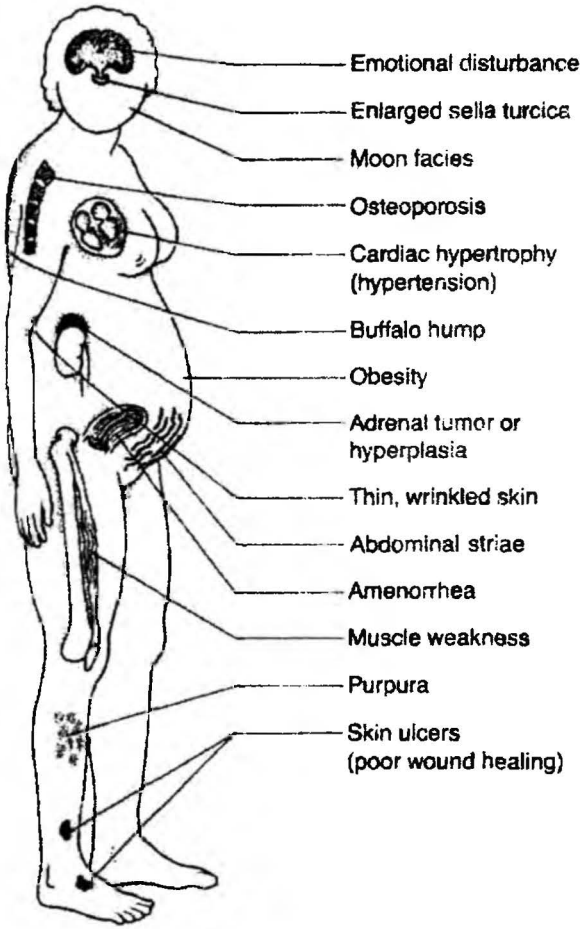
- decreased blood glucose level (hypoglycemia), extreme sensitivity to insulin.
- increased susceptibility to stress.
- loss of appetite (anorexia), weight loss, nausea.
- muscle weakness.
- low blood pressure.
- decreased ability to excrete a water load.
- impaired cardiac function.
- decreased $Na^+$ and increased $K^+$ level in serum.

2) ***Secondary adrenal insufficiency*** is due to a deficiency of ACTH resulting from tumor, infarction, or infection.

410

## Disorders of glucocorticoid hormone excess

**Cushing's syndrome** is due to long term pharmacological use of steroids, tumor of adrenal cortex or tumor of pituitary.

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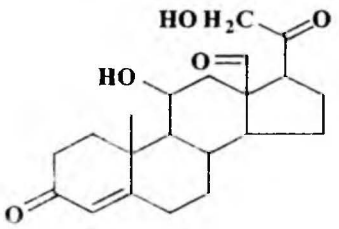
## Mineralocorticoids



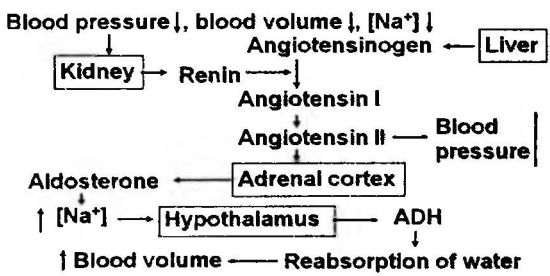

**Aldosterone**

Aldosterone does not have a specific plasma transport protein, but it forms a very weak association with albumin.

412

## Regulation of aldosterone production

### The Renin-Angiotensin System:




Aldosterone secretion is sensitive to changes in plasma potassium (K) level:

- increased level of K stimulates production of aldosterone.
- decreased level of K reduces production of aldosterone.

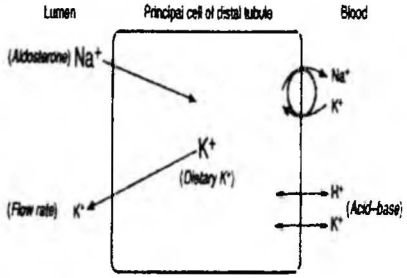
413

## Biochemical functions of mineralocorticoids

1). Aldosterone acts in the kidney to stimulate active $Na^+$ transport by the distal convoluted and collecting tubules, the net result being $Na^+$ reabsorption.

- Aldosterone causes the production of proteins in cells of the kidney. *Permease* produced allows $Na^+$ to enter cells from the lumen.
- Aldosterone increases the number of apical membrane $Na^+$ channels, and this increases intracellular $Na^+$.
- Aldosterone also increases the activity of several mitochondrial enzymes. *Citrate synthase* is induced, which stimulates citric acid cycle activity and results in the generation of ATP. Energy is thus provided to drive the *$Na^+/K^+$ ATPase*, which also may be induced.

2). Aldosterone promotes the excretion of $K^+$, $H^+$, and $NH_4^+$ in the kidney.




3). Aldosterone affects ion transport in other epithelial tissues including sweat glands, intestinal mucosa, and salivary glands.

414

## Disorders of mineralocorticoid hormone deficiency

- A deficiency of aldosterone secretion is accompanied by a reduction in the secretion of other adrenal steroid hormones as well. The loss of adrenocortical steroids is known as **Addison's disease** *(see disorders of glucocorticoid hormone deficiency)*.
- The mineralocorticoid deficiency leads to a net loss of $Na^+$ and water into the urine with a reciprocal retention of $K^+$ (hyperkalemia) and $H^+$ (mild metabolic acidosis). The subsequent contraction of the effective plasma volume may lead to a reduction in blood pressure.

415

## Disorders of mineralocorticoid hormone excess

- Adenomas of the glomerulosa cells result in ***primary aldosteronism (Conn's syndrome)***, the manifestations of which include hypertension, hypokalemia, hypernatremia, and alkalosis, low levels of renin and angiotensin II.
- Renal artery stenosis, with the decrease in perfusion pressure, can lead to hyperplasia and hyperfunction of the juxtaglomerular cells and cause elevated levels of renin and angiotensin II. This action results in ***secondary aldosteronism***.

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# Hormones of the adrenal medulla

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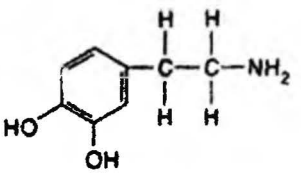
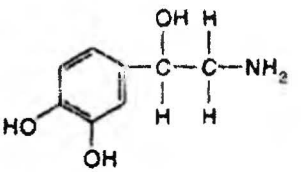
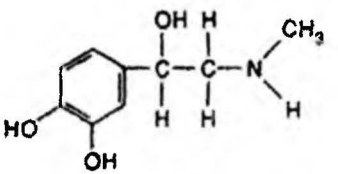
## Catecholamine hormones




**Dopamine**




**Norepinephrine**




**Epinephrine**

- *Synthesis of catecholamine hormones see in metabolism of phenylalanine and tyrosine.*
- Catecholamines circulate in plasma in a loose association with albumin.
- Catecholamines have short biologic half-life (10-30 seconds).

418

## Regulation of catecholamine hormones production

- Catecholamines are produced in response to stress (fight, fright and flight). These include the emergencies like shock, cold, fatigue, emotional conditions like anger, etc.
- Response involves many processes in the organs vital to the response (brain, muscles, cardiopulmonary system, and liver) at the expense of other organs that are less immediately involved (skin, gastrointestinal system, lymphoid tissue).

419

## Biochemical functions of catecholamines

1). Effects on carbohydrate metabolism is to elevate blood glucose level:
1. Increase the degradation of glycogen and decrease glycogen formation in the liver and muscles.
2. Increase the hepatic gluconeogenesis.
3. Inhibit insulin secretion.
2). Effects on lipid metabolism is to increase the free fatty acids in the circulation by enhance the breakdown of triacylglycerols (lipolysis) in adipose tissue.
3). Effects on physiological functions:
1. Increase cardiac function (myocardial contraction) and oxygen consumption, constrict the blood vessels and thereby increase blood pressure. They cause smooth muscle relaxation in bronchi, gastro-intestinal tract, genitourinary tract and the blood vessels supplying skeletal muscle.
2. On the other hand, catecholamines stimulate smooth muscle contraction of the blood vessels supplying skin and kidney.

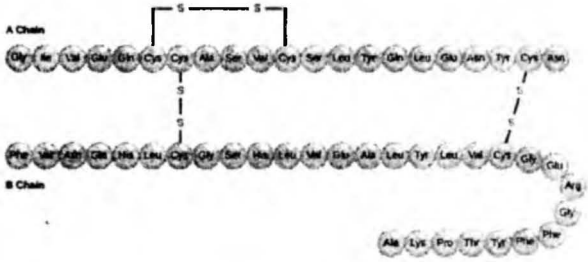
420

# Hormones of the pancreas

421

## Insulin

- Insulin is a polypeptide hormone (MW=5.73) containing 51 amino acids, arranged in two polypeptide chains A and B.




- Insulin has no plasma carrier protein.
- Plasma half-life is less than 3-5 minutes under normal conditions.

422

## Regulation of insulin secretion

- Increase in plasma glucose concentration is the most important regulator of insulin secretion.
- The threshold concentration for secretion is the fasting plasma glucose level (80-100 mg/dl), and the maximal response is obtained at glucose levels between 300 and 500 mg/dl.
- α-Adrenergic agonists, epinephrine inhibit insulin release.
- β-Adrenergic agonists, growth hormone, cortisol, placental lactogen, estrogens, and progestins stimulate insulin release.

423

## Biosynthesis of insulin

- Insulin is produced by the β-cells of pancreatic islets of Langerhans.
- It is synthesized as a preprohormone with 108 amino acids (MW 11.500) which undergoes proteolytic processing to give proinsulin with 86 amino acids (MW 9.000). The arrangement of proinsulin is B chain-connecting (C) peptide-A chain. The proinsulin molecule undergoes cleavages that result in the formation of mature insulin and C- peptide.
- C-peptide has no biological activity; however its estimation in the plasma serves as a useful index for the endogenous production of insulin.
- In the β-cells, insulin (and also proinsulin) combines with zinc to form complexes. In this form, insulin is stored in the granules of the cytosol which is released by exocytosis.

424

## Biochemical functions of insulin

1) Effects on carbohydrate metabolism is to decrease the blood glucose level:

1. Enhances the glycolysis in muscle and liver by increasing the activity and the quantities of key enzymes *glucokinase (or hexokinase), phosphofructokinase,* and *pyruvate kinase.*

2. Enhances the glycogen synthesis by activation of *glycogen synthetase* whereas glycogen degradation is decreased by inhibition of *phosphorylase.*

3. Inhibits the gluconeogenesis by suppressing the key enzymes *pyruvate carboxylase, phosphoenolpyruvate carboxykinase* and *glucose-6-phosphatase.*

4. Enhances the pentose phosphate pathway by increasing the activity of key enzyme *glucose-6-phosphate dehydrogenase* to provide NADPH required for fatty acid synthesis.

5. Enhances the uptake of glucose by muscle (skeletal, cardiac and smooth) and adipose tissue by increasing the quantities of GLUT-4.

2) Effects on lipid metabolism is to reduce the release of fatty acids from the stored fat:

1. Stimulates the synthesis of triacylglycerols (lipogenesis) and fatty acid synthesis by:

- increasing the uptake of glucose by adipose tissue and providing glycerol-3-phosphate from glycolysis.
- increasing the activity of key enzyme of pentose phosphate pathway to provide NADPH required for fatty acid synthesis.
- increasing the activity of a key enzyme *acetyl CoA carboxylase* of fatty acid synthesis.

2. Inhibits lipolysis in liver and adipose tissue by decreasing the activity of *hormone-sensitive lipase.*

3. Decreases circulating free fatty acids and mobilization of fatty acids from liver.

4. Inhibits ketogenesis by decreasing the activity of *HMG CoA reductase.*

5. Insulin affects the formation or clearance of VLDL and LDL.

3) Effects on protein metabolism and cell replication:

1. Stimulates the entry of amino acids into the cells, including muscle, liver, bone and lymphocytes, enhances protein synthesis and reduces protein degradation.

2. Promotes cell growth and replication.

## Comparison of two types of diabetes mellitus

| *Character* | *Insulin-dependent diabetes mellitus Type 1 diabetes* | *Non-insulin dependent diabetes mellitus Type 2 diabetes* |
|---|---|---|
| Prevalence | 10-20% of diabetic population | 80-90% of diabetic population |
| Body weight | Normal or low | Obese |
| Genetic predisposition | Mild or moderate | Very strong |
| Defect | Insulin deficiency due to destruction of β-cells | impairment in the production of insulin by β-cells and/or resistance of target cells to insulin |
| Plasma insulin | Decreased or absent | Normal or increased |
| Acute complications | Ketoacidosis | Hyperosmolar coma |
| Administration of insulin | Always required | Usually not necessary |

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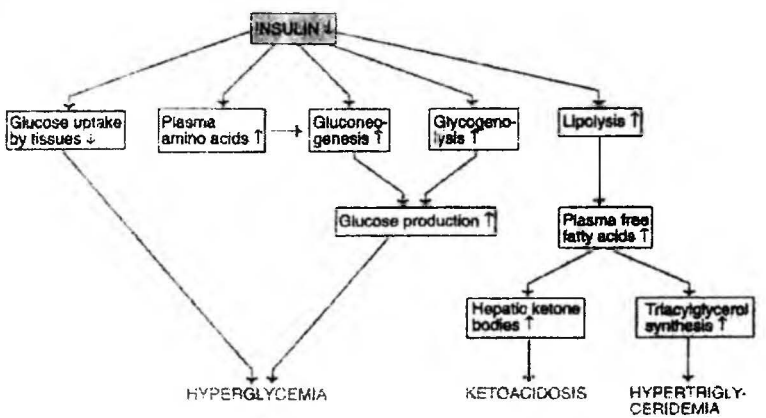
## Major metabolic alterations in diabetes mellitus




427

## Glucagon

- Glucagon is secreted by α-cells of the pancreas.
- Glucagon is a polypeptide hormone composed of 29 amino acids in a single chain (MW=3.500).
- Glucagon is actually synthesized as proglucagon (MW= 9.000).
- Glucagon has no plasma carrier protein.
- Plasma half-life is less than 5-10 minutes.

428

## Regulation of glucagon secretion

- The secretion of glucagon is stimulated by low blood glucose concentration, amino acids derived from dietary protein and low levels of epinephrine.
- Increased blood glucose level markedly inhibits glucagon secretion.

429

## Biochemical functions of glucagon

1). Effects on carbohydrate metabolism is to enhance the blood glucose level:

- Acts on liver to cause increased gluconeogenesis by activation of gene transcription of *phosphoenolpyruvate carboxykinase* and activation of *fructose-1,6-bisphosphatase*.
- Enhances glycogen degradation in liver.

The actions of glucagon are mediated through cyclic AMP.

2). Effects on lipid metabolism:

- Stimulates lipolysis in adipose tissue by increasing the activity of *hormone-sensitive lipase*.
- Promotes fatty acid oxidation resulting in energy production for muscle and myocardium.
- Enhances ketone body synthesis (ketogenesis).

3). Effects on protein metabolism:

- Enhances protein degradation and reduces protein synthesis.
- Increases the amino acid uptake by liver which, in turn, promotes gluconeogenesis. Thus glucagon lowers plasma amino acids.

430

# Hormones that regulate calcium metabolism

431

## Calcium metabolism

$Ca^{2+}$ regulates following processes:

1. Neuromuscular excitability.
2. Blood coagulation.
3. Activation of muscle phosphorylase.
4. Secretory processes.
5. Membrane integrity and plasma membrane transport.
6. Enzyme reactions.
7. The release of hormones and neurotransmitters.
8. The intracellular action of hormones.
9. Bone mineralization.

432

## Parathyroid hormone (PTH)

- Parathyroid hormone is secreted by parathyroid glands.
- PTH is a single chain polypeptide (MW= 95.000), containing 84 amino acids.
- PTH is synthesized as preproPTH which is degraded to proPTH and, finally, to active PTH.
- Low plasma calcium enhances the production of PTH.
- Phosphate has no effect on PTH secretion.

433

## Biochemical functions of parathyroid hormone

**1. Action on the bone:**

PTH binds to osteoblasts, the cells responsible for creating bone. Binding stimulates osteoblasts to release cytokines. In turn, the cytokines stimulate the development of mature osteoclasts. This is brought out by PTH stimulated increased activity of the enzymes *pyrophosphatase* and *collagenase*. These enzymes result in bone resorption Demineralization (decalcification) leads to an increase the blood Ca level. PTH also promotes phosphate mobilization from bone.

**2. Action on the kidney:**

PTH increases the Ca active reabsorption or reduces Ca excretion from kidney distal tubules and the thick ascending limb by promoting the production of calcitriol. PTH reduces the reabsorption of phosphate from the proximal tubule of the kidney which means phosphate is excreted through the urine.

**3. Action on the intestine:**

The action of PTH on the intestine is indirect. It increases the intestinal absorption of Ca by stimulating the biosynthesis of calcitriol in the kidney. PTH activates 1,α-hydroxylase, the enzyme responsible for hydroxylation of 25-hydroxy vitamin D (calcidiol) to 1,25-dihydroxy vitamin D (calcitriol).

Thus the net effect of PTH is to elevate calcium and decrease the phosphate concentrations in the blood.

434

## Hypoparathyroidism

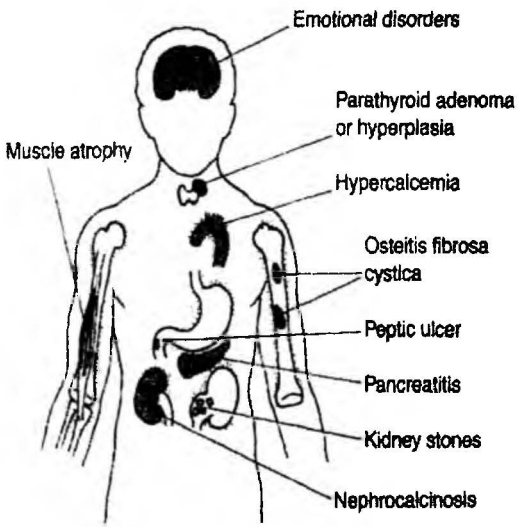
- A low level of PTH in the blood is known as hypoparathyroidism. The usual cause of hypoparathyroidism is accidental removal or damage of the glands during neck surgery ***(secondary hypoparathyroidism)***, but the disorder occasionally results from autoimmune destruction of the glands and inborn errors of metabolism ***(primary hypoparathyroidism)***.
- The biochemical hallmarks are decreased serum ionized calcium (hypocalcemia) and elevated serum phosphate levels. Symptoms include neuromuscular irritability, spasms and convulsions which, when mild, cause muscle cramps and tetany.
- Severe, acute hypocalcemia results in tetanic paralysis of the respiratory muscles, laryngospasm, severe convulsions, and death.
- Long standing hypocalcemia results in cutaneous changes, cataracts, and calcification of the basal ganglia of the brain.

435

## Hyperparathyroidism

A high level of PTH in the blood is known as hyperparathyroidism. If the cause is in the parathyroid gland it is called ***primary hyperparathyroidism*** due to the parathyroid adenoma, parathyroid hyperplasia or to ectopic production of PTH in a malignant tumor. If the cause is outside the gland, it is known as ***secondary hyperparathyroidism***. This can occur in chronic renal failure.

The biochemical hallmarks are elevated serum ionized calcium and PTH and depressed serum phosphate levels (due to increased renal losses) and increase in *alkaline phosphatase* activity. Elevation in the urinary excretion of Ca and P, often resulting in the formation of urinary calculi.




The symptoms include lethargy, loss of appetite, constipation, nausea, increased myocardial contractility and susceptibility to fractures.

436

## Calcitriol

- Calcitriol or 1,25-dihydroxycholecalciferol is the physiologically active form of vitamin D3 and is a steroid hormone.
- The synthesis of calcitriol, biochemical actions, hypo- and hypervitaminosises are described under vitamins.

437

## Calcitonin

- Calcitonin is secreted by the parafollicular C cells of the human thyroid.
- Calcitonin is a 32-amino-acid peptide cleaved from a larger prohormone.
- Elevated blood Ca levels strongly stimulate secretion, and secretion is suppressed when Ca concentration falls below normal.

438

## Biochemical functions of calcitonin

**1. Action on bone:**
Calcitonin suppresses resorption of bone by inhibiting the activity of osteoclasts therefore directly reduces the amount of Ca and phosphate released into the blood. However, this inhibition has been shown to be short-lived. Calcitonin also prevents Ca release from bone by inhibiting the release of cytokines by osteoblasts. At the same time, it promotes the development of osteoblasts.
**2. Action on kidney:**
Calcitonin inhibits tubular reabsorption of Ca and phosphate. leading to increased rates of their excretion in urine.

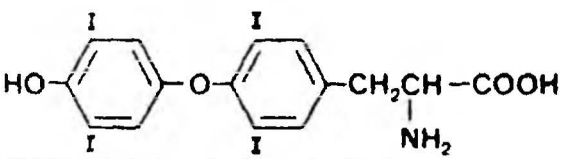
Thus the net effect of calcitonin on bone and kidney is to decrease the calcium and the phosphate concentrations in the blood.

439

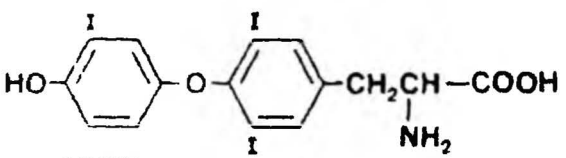
# Thyroid hormones

440

## Thyroid hormones



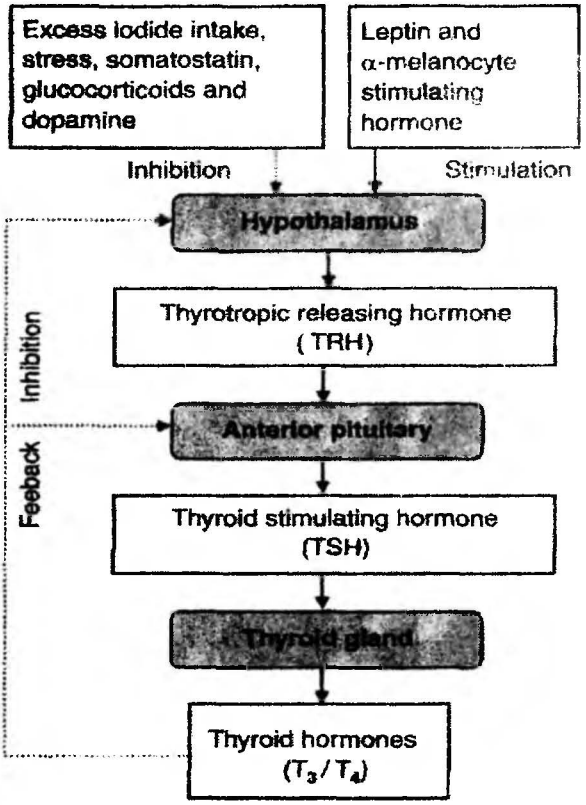

3,5,3',5'-Tetraiodothyronine (thyroxine [$T_4$])




3,5,3'-Triiodothyronine ($T_3$)

441

## Regulation of thyroid hormones secretion




442

## Transport of thyroid hormones

- T4 and T3 circulate in bound form, ie, bound to 2 specific binding proteins, **thyroxine-binding globulin** and **thyroxine-binding prealbumin**.
- The small, unbound fraction is responsible for the biologic activity.
- T4 has a half-life of 4-7 days while T3 has about one day.

443

## Synthesis of thyroid hormones

T4 and T3 are synthesized from the tyrosine residues of the glycoprotein thyroglobulin and activated iodine.

1. The uptake of iodide by the thyroid gland is linked to the *$Na^+/K^+$ ATPase*.
2. The conversion of iodide ($I^-$) to active iodine ($I^+$), concentrated in the follicular cells, is catalyzed by the enzyme *thyroperoxidase*.
3. The follicular cells of the thyroid gland produce the protein ***thyroglobulin***, which is secreted into the colloid. Iodination of tyrosine residues (organification) in thyroglobulin produces monoiodotyrosine (MIT) and diiodotyrosine (DIT) (inactive precursors).
4. The coupling of two DIT molecules to form T4 or of MIT and DIT to form T3.
5. Thyroglobulin is digested by lysosomal proteolytic enzymes in the thyroid gland to release the free hormones, T3 and T4.
6. T4 (90%) and T3 (10%) are released into the blood, a process stimulated by TSH.

*Thyroglobulin containing T4 and T3 can be stored for 1-3 months in the thyroid gland.*

444

## Biochemical functions of thyroid hormones

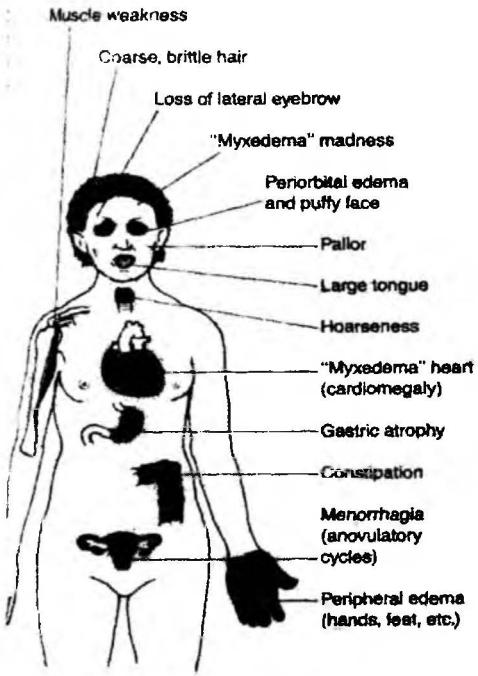
T3 is about four times more active in its biological functions than T4.

1. Influence on the basal metabolic rate by regulating mitochondrial ATP synthesis:
   - Stimulate the metabolic activities and increases the oxygen consumption via oxidative phosphorylation in most of the tissues of the body (exception-brain, lungs, testes and retina).
   - Enhance the function of the *$Na^+/K^+$ ATPase* pump with ATP utilization by increasing the number of pump units.
2. Effect on protein synthesis:
   - Enhance protein synthesis and cause positive nitrogen balance; while very high concentrations of T3 inhibit protein synthesis and cause negative nitrogen balance.
   - T3 and glucocorticoids enhance transcription of the growth hormone gene, so that more GH is produced.
   - Promote embryonic development.
   - Regulate gene expression, tissue differentiation, growth, general development and maintenance of almost all tissues of the body.
3. Effect on carbohydrate metabolism is to enhance blood glucose level:
   - Promote intestinal absorption of glucose and its utilization.
   - Increase gluconeogenesis and glycogen degradation.
4. Effect on lipid metabolism:
   - Stimulate lipolysis in adipose tissue by increasing the activity of *hormone-sensitive lipase*.

445

## Hypothyroidism

- Hypothyroidism is due to impairment in the function of thyroid gland that causes decreased levels of T3 and T4. Disorders of pituitary or hypothalamus also contribute to hypothyroidism. Women are more susceptible than men.
- Hypothyroidism is characterized by reduced basal metabolic rate, slow heart rate, weight gain, sluggish behavior, constipation sensitivity to cold, dry skin etc.
- Hypothyroidism in adult causes **myxedema.**




- Intrauterine or neonatal hypothyroidism in children is associated with physical multiple congenital defects and severe, irreversible mental retardation, collectively known as **cretinism.**
- Hypothyroidism later in childhood results in short stature but no mental retardation.

446

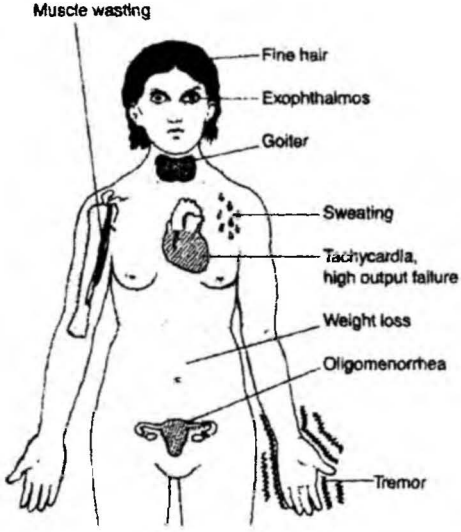
## Simple endemic goiter

- Any enlargement of the thyroid is referred to as a **goiter**.
- Enlargement of thyroid gland is mostly to compensate the decreased synthesis of thyroid hormones and is associated with elevated TSH.
- Endemic goiter is due to iodine deficiency in the diet. It is mostly found in the geographical regions away from sea coast where the water and soil are low in iodine content.
- Consumption of iodized salt is advocated to overcome the problem of endemic goiter.

447

## Hyperthyroidism

Hyperthyroidism is caused by **Grave's disease**. It is also known as thyrotoxicosis and causes a diffuse enlargement of the thyroid and excessive, uncontrolled production of T3 and T4.

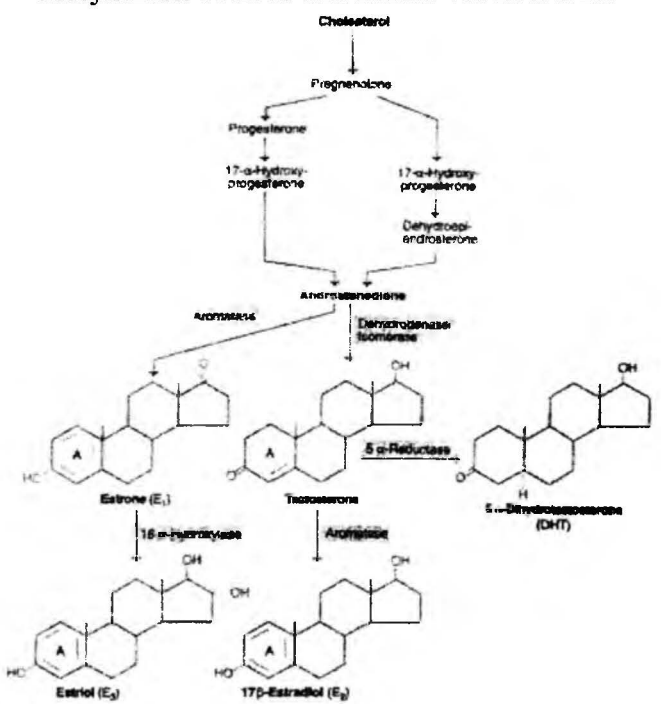



448

# Hormones of the gonads

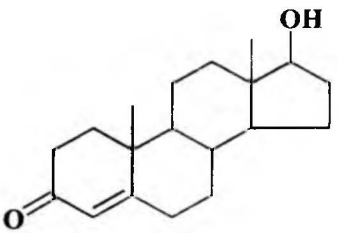
449

## Biosynthesis of male and female sex hormones




450

## Androgens (male sex hormones)

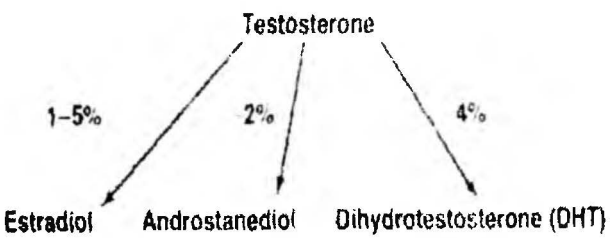



**Testosterone**

- Androgens are produced by the Leydig cells of the testes and to a minor extent by the adrenal glands in both the sexes. Ovaries also produce small amounts of androgens.
- Testosterone binds with plasma β-globulin which is called **sex hormone-binding globulin** (SHBG or **testosterone-estrogen-binding globulin**). Since SHBG and albumin bind 97-99% of testosterone, only a small fraction of the hormone is in the free (biologically active) form.
- The production of androgens is under the control of LH and FSH.

451

## Metabolic pathways of testosterone




452

## Physiological and biochemical functions of androgens

**1). Sex-related physiological functions:**

- growth, development and maintenance of male reproductive organs.
- sexual differentiation.
- spermatogenesis.
- male pattern of aggressive behavior.
- development of secondary sexual organs and ornamental structures (muscles, hair, etc.).

**2). Biochemical functions:**

1. Effects on protein metabolism:
   - promote RNA synthesis (transcription) and protein synthesis.
   - cause positive nitrogen balance.
   - increase the muscle mass.
2. Effects on carbohydrate and fat metabolisms:
   - increase glycolysis.
   - increase fatty acid synthesis.
   - increase citric acid cycle.
3. Effects on mineral metabolism:
   - promote mineral deposition.
   - promote bone growth before the closure of epiphyseal cartilage.

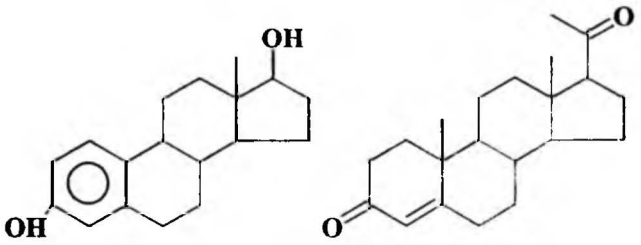
453

## The pathophysiology of the male reproductive system

- The lack of testosterone synthesis is called hypogonadism. If this occurs before puberty, secondary sex characteristics fail to develop, and if it occurs in adults, many of these features regress.
- ***Primary hypogonadism*** is due to processes that affect the testes directly and cause testicular failure.
- ***Secondary hypogonadism*** is due to defective secretion of the gonadotropins.

454

## Estrogens (female sex hormones)




17β-estradiol (E2) Progesterone

- Estrogens are systhesized by the follicles and corpus luteum of ovary, progesterone - by corpus luteum and placenta.
- Estrogens are bound to **sex hormone-binding globulin** and progestins to **cortisol-binding globulin**.
- The synthesis of estrogens is under the control of LH and FSH.

455

## Physiological and biochemical functions of estrogens

**1). Sex-related physiological functions:**

- Growth, development and maintenance of female reproductive organs.
- Maintenance of menstrual cycles.
- Development of female sexual characteristics.

**2). Biochemical functions:**

1. Lipogenic effect: Estrogens increase lipogenesis in adipose tissue.
2. Hypocholesterolemic effect: Estrogens lower the plasma total cholesterol. The LDL fraction of lipoproteins is decreased while the HDL fraction is increased.
3. Anabolic effect: Estrogens promote the synthesis of many proteins in liver.
4. Effect on bone growth: Estrogens promote calcification and bone growth.

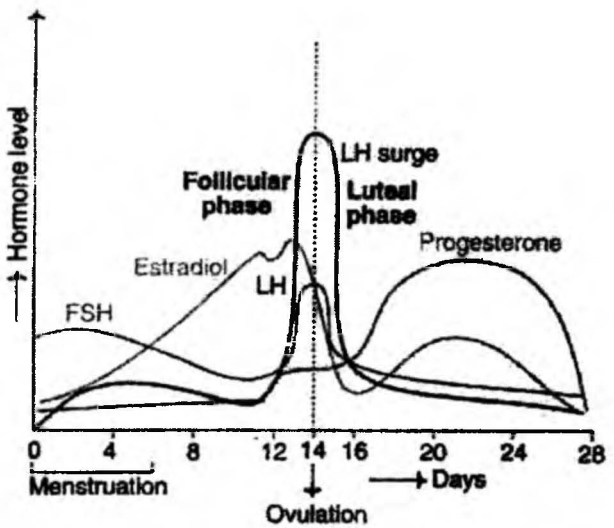
456

## Biochemical functions of progesterone

1. Progesterone is essentially required for the implantation of fertilized ovum and maintenance of pregnancy.
2. Progesterone promotes the growth of glandular tissue in uterus and mammary gland.

457

## Hormonal pattern during mestrual cycle

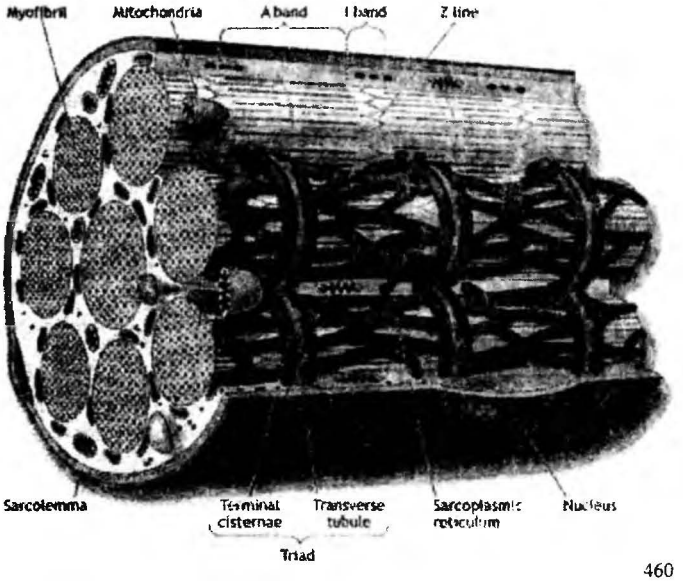



458

# Biochemistry of muscle

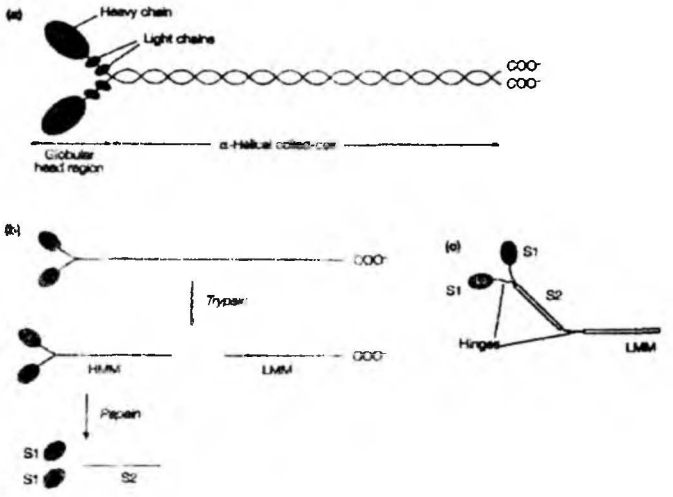
459

## Structure of muscle



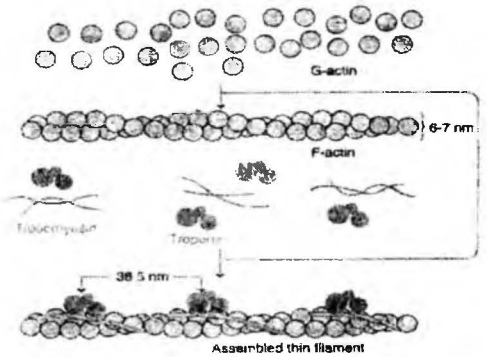

460

## Structure of myosin along with its digested products by trypsin and papain



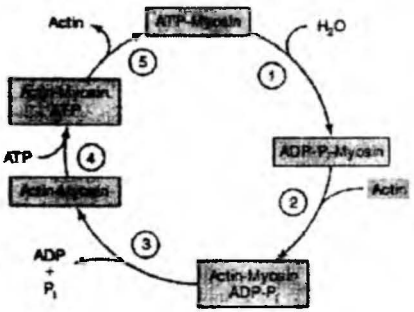

461

## A diagrammatic representation of the thin filament




462

## Mechanism of muscle contraction




**1.** During the relaxation phase of muscle contraction, the S-1 head of myosin hydrolyses the bound ATP to ADP and Pi. This results in the formation high energy **ADP-Pi-myosin complex**.

**2.** On contraction, when action potential reaches the end of motor neuron (nerve terminal) in the neuromuscular junction. The neurotransmitter released and will elicit action potential in muscle fiber. Action potential spread all muscle cell membrane and cause release of $Ca^{2+}$ from sarcoplasmic reticulum in cytosol to contractile myosin and actin. $Ca^{2+}$ binds Troponin C and will result in conformational changes in Troponin molecule that result in pulling of Tropomyosin away from active sites of actin (myosin binding sites on actin). When active site of actin is exposed, it will react with active site of myosin and they will attach to each other to finally form **actin-myosin-ADP-Pi complex**.

**3.** Myosin releases the ADP and Pi and ATP energy released [by hydrolyzed ATP to ADP and Pi] will make the cross bridges of myosin bend forward, toward the center of sarcomere, by this, driving movement of actin filaments over myosin filaments. *This movement of cross bridges, pulling actin filaments and movement of the head of myosin that caused by energy released from hydrolyzed ATP is called (power stroke).* The **actin-myosin complex** is in a low energy state.

**4.** Up to now movement is achieved (shortening), second ATP will bind to myosin head (ATP site), to form **actin-myosin-ATP complex**. This binding will result in detachment of cross bridge from actin active site and cross bridge will return back to its position, facing another actin active site.

**5.** Actin is released, as **myosin-ATP** has low affinity for actin.

A fresh cycle of muscle contraction and relaxation now commences with the hydrolysis of ATP and the formation of **ADP-Pi-myosin complex**.

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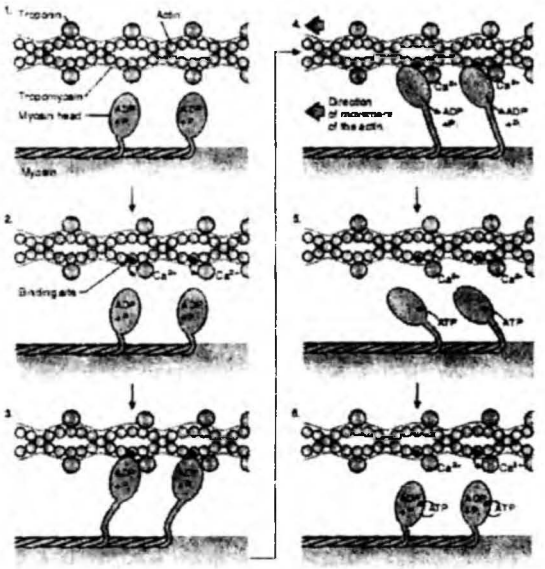
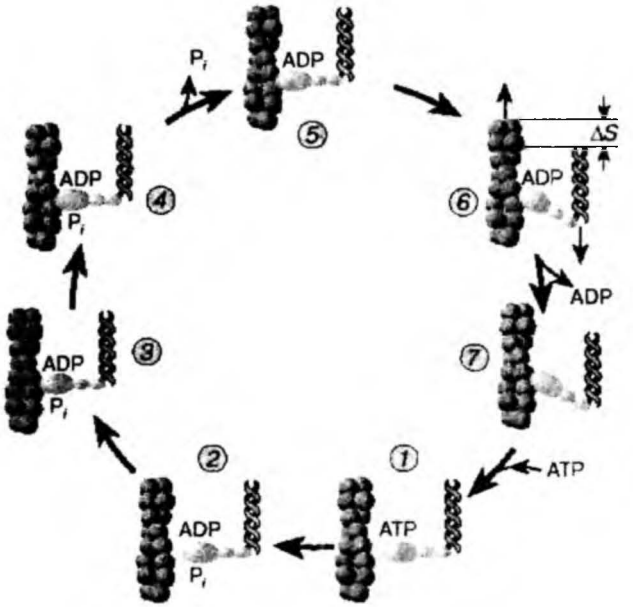
## An overview of muscle contraction



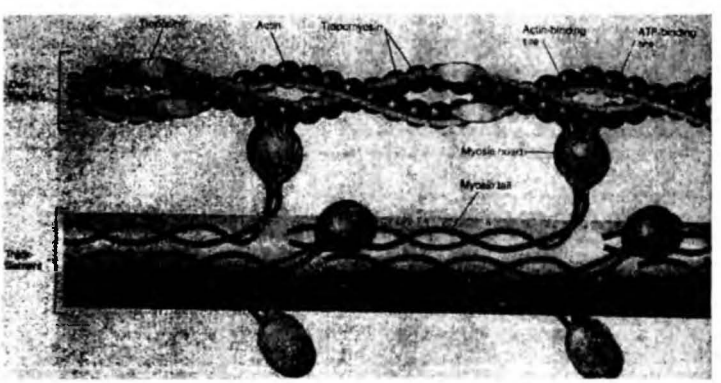
**1.** The troponin–tropomyosin complex is blocking the myosin-binding sites on the actin. The myosin head is already energized to power a contraction. **2.** After Ca binding to troponin, a conformational change in the troponin molecule pulls the troponin away from the binding site.
**3.** Once the binding sites on the actin are exposed, the myosin head binds to it. **4.** After cross-bridge attachment, the energy stored in the myosin head is released, and the myosin head pivots toward the center of the sarcomere (power stroke). Now the ADP and phosphate bound to the myosin head are released. **5.** Now a molecule of ATP binds to the myosin head with simultaneous detachment of the myosin head from the binding site on the actin molecule. **6.** The ATPase activity of the myosin head hydrolyzes the ATP into ADP and phosphate. The energy released from the hydrolysis of this high-energy bond is used to re-energize the myosin head, and the entire cycle can be repeated as long as calcium is present and there are sufficient ATP reserves.

**Cycle of the structured conversions of the actin-myosin complex, leading to the movement of the myosin along the actin**



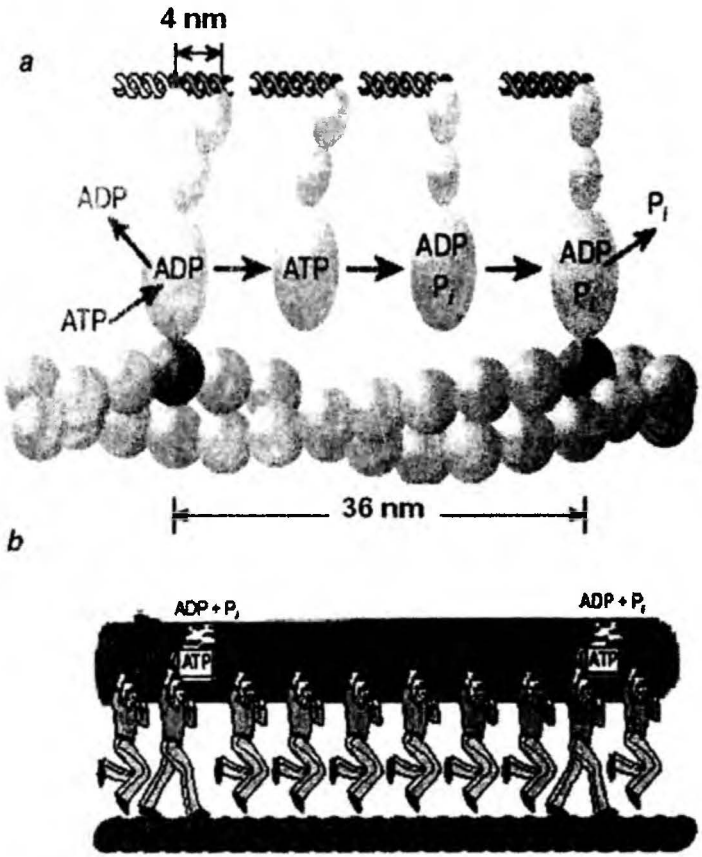

465

**Actin-myosin orientation**




466

**The scheme of the myosin movement along the actin**

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## Characteristics of white and red fibers of skeletal muscle

| | *White (fast) muscle fibers* | *Red (slow) muscle fibers* |
|---|---|---|
| Color | White | Red |
| Myoglobin | No | Yes |
| Myosin ATPase activity | High | Low |
| Energy utilization | High | Low |
| Contraction rate | Fast | Slow |
| Duration | Short | Prolonged |
| Mitochondria | Few | Many |

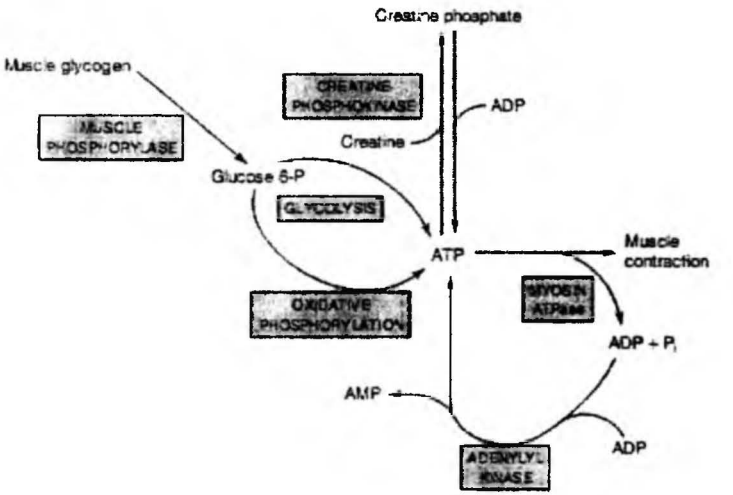
468

## There are 3 ATP- dependent mechanisms in the muscles:

1. *$Na^+/K^+$ ATP-ase* pump in the cellular membrane.
2. Intracellular calcium pump.
3. Actin-myosin interaction (contraction - relaxation).

469

## The multiple sources of ATP in muscle

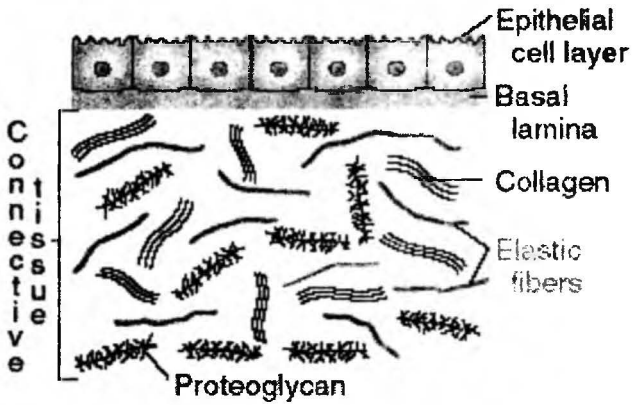



470

# Biochemistry of connective tissue

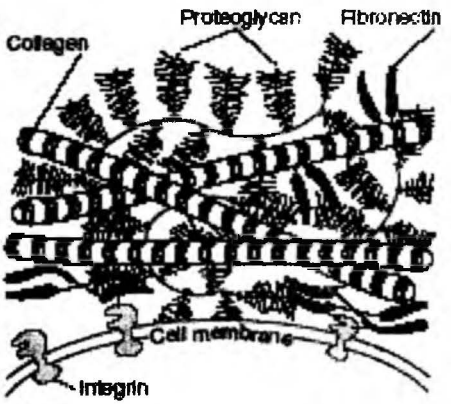
471

## An overview of connective tissue extracellular matrix



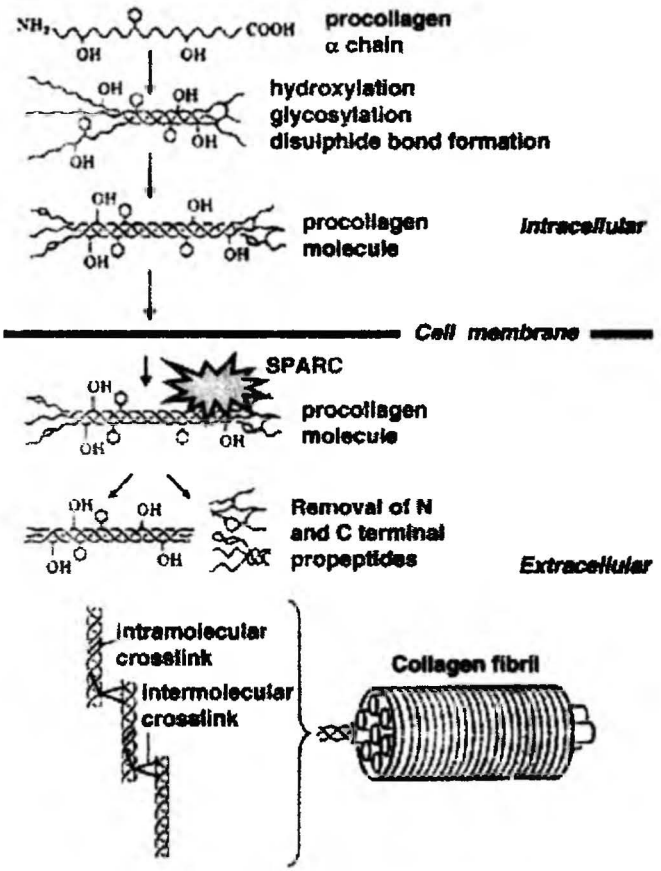

472

## Interactions between the cell membrane and the components of the extracellular matrix



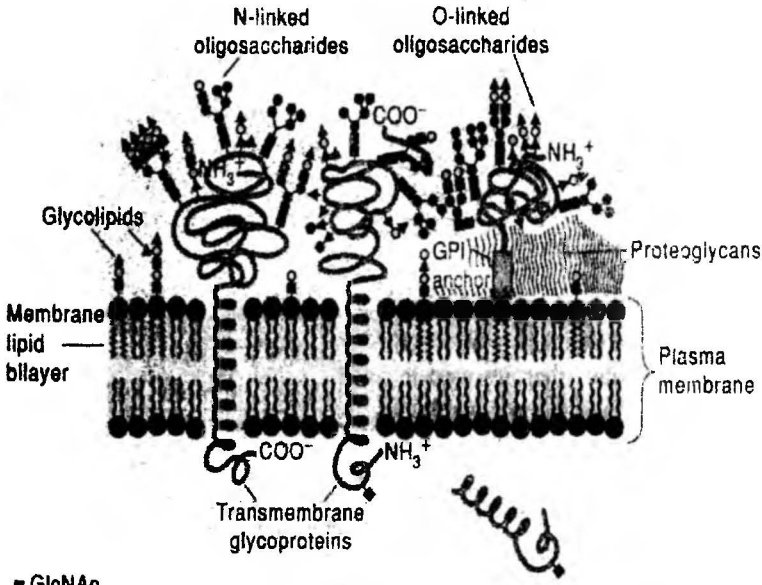

473

## Steps in the formation of mature collagen fibrils



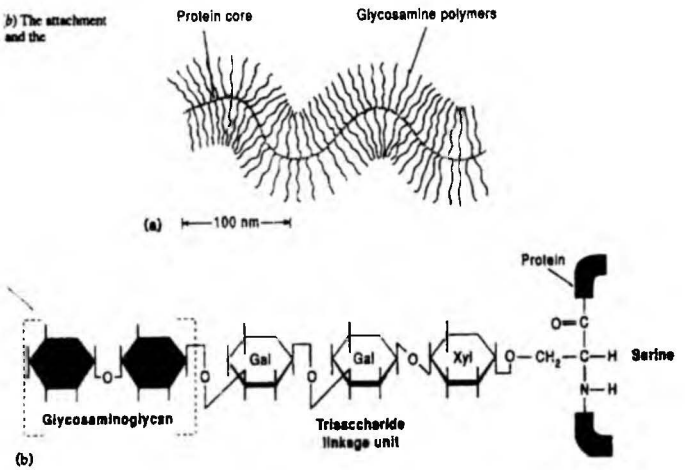

474

## Plasmatic membrane of the eukaryotic cell



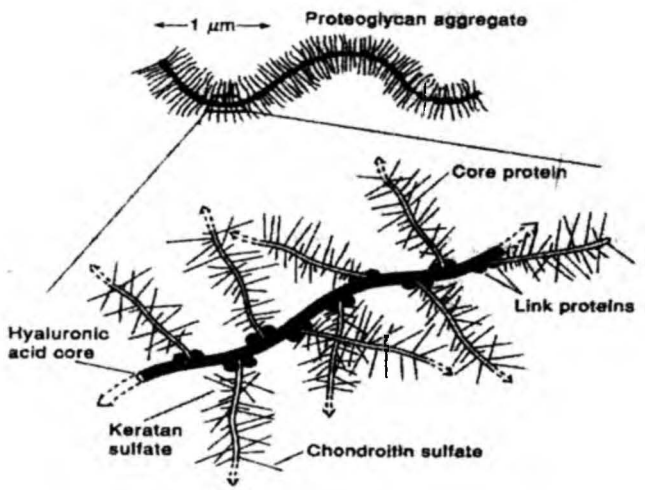

475

## Structure of proteoglycan




476

## Hyaluronic acid – proteoglycan complex




477

# Biochemistry of nervous system

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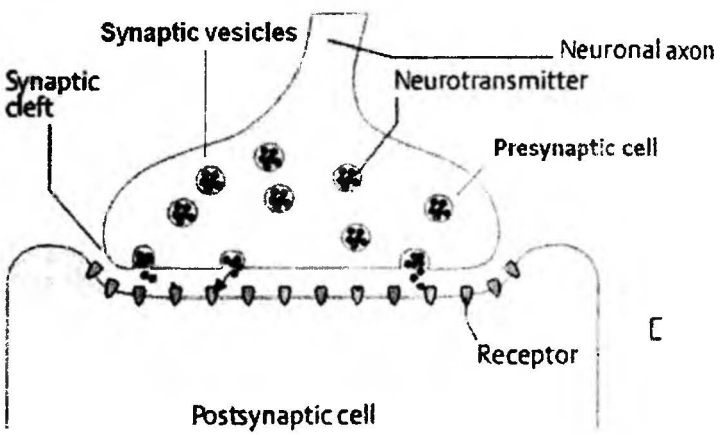
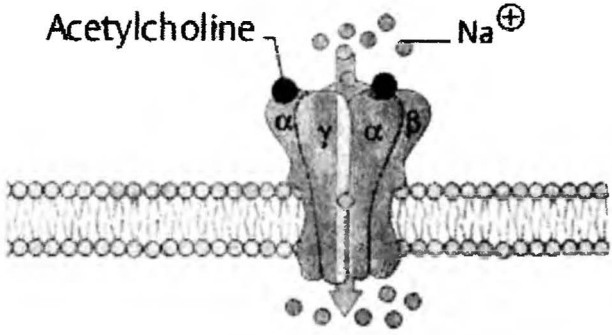
## Structure of synapse




479

## Acetylcholine receptor




480

## Compounds effecting synaptic transmission
## Cholinergic synapses

| | |
|---|---|
| **Botulinum toxin** | Protein of rod-shaped bacterium Clostridium botulinum. Blocks neuromuscular transmission through preventing vesicles from anchoring to the membrane to release acetylcholine and causes poisoning by growing in badly handled or prepared meat products. |
| **Nicotine** | Alkaloid contained in tobacco. Acts as a nicotinic acetylcholine receptor agonist. Blocks the action of autonomic nerve and skeletal muscle cells. |
| **Muscarine** | Alkaloid contained in the bright red fly agaric mushroom. Acts as a muscarinic acetylcholine receptor agonist. Muscarine bound at the receptor is not broken down and therefore causes permanent stimulation of muscle. |
| **Tubocurarin** | Alkaloid, active ingredient of curare, an extract from the bark and stems of the South American vine (Chondodendron tomentosum). Blocks acetylcholine receptor sites at neuromuscular junctions, causing relaxation (neuromuscular relaxant) and paralysis of muscles, including respiratory organs and the heart. |
| **Atropine** | Alkaloid poisonous compound found in deadly nightshade and related plants. Blocks muscarinic receptors. It is used in medicine as a muscle relaxant, e.g., in dilating the pupil of the eye. |
| **Physostigmine** | Alkaloid which is the active ingredient of the Calabar bean and is used medicinally in eye drops (treatment of glaucoma) on account of its anticholinesterase activity. |

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## Adrenergic synapses

| | |
|---|---|
| **Dihydroergotamine** | Derivative of ergot ergotamine. It is α-adrenergic receptors blocker and is used mainly in the treatment of migraine. |
| **Propranolol** | Synthetic compound which acts as a β-adrenergic receptors blocker and is used mainly in the treatment of cardiac arrhythmia, stenocardia. |
| **Imipramine** | Moderate to strong reuptake inhibition of catecholamines. Synthetic compound, antidepressant is mainly used in the treatment of major depression such as depression associated with agitation or anxiety and enuresis. Imipramine has a significant analgesic effect and, thus, is very useful in some pain conditions. |
| **Reserpine** | Alkaloid obtained from Indian snakeroot (rauwolfia). Depletes catecholamines in the synapses from peripheral sympathetic nerve endings. As drug is used in the treatment of hypertension and as antidepressant for the relief of psychotic symptoms (schizophrenia). |

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## Glycine synapses

| | |
|---|---|
| **Strychnine** | Highly poisonous compound alkaloid obtained from the seeds of the nux vomica and related plants. Acts as a blocker or antagonist at the inhibitory or strychnine-sensitive glycine receptor. It is used as a stimulant in small doses. |
| **Apamin** | Apamin is an element in bee venom. Effect is similar to strychnine. |

## Peptide synapses

| | |
|---|---|
| **Morphine** | It acts as an enkephalin receptor agonist. Analgesic and narcotic drug obtained from opium and used medicinally to relieve pain. |
| **Naloxone** | Synthetic drug, similar to morphine. Blocks opiate receptors in the nervous system. It is used to counter the effects of opiate overdose, for example heroin or morphine overdose and to counteract life-threatening depression of the central nervous system and respiratory system. |

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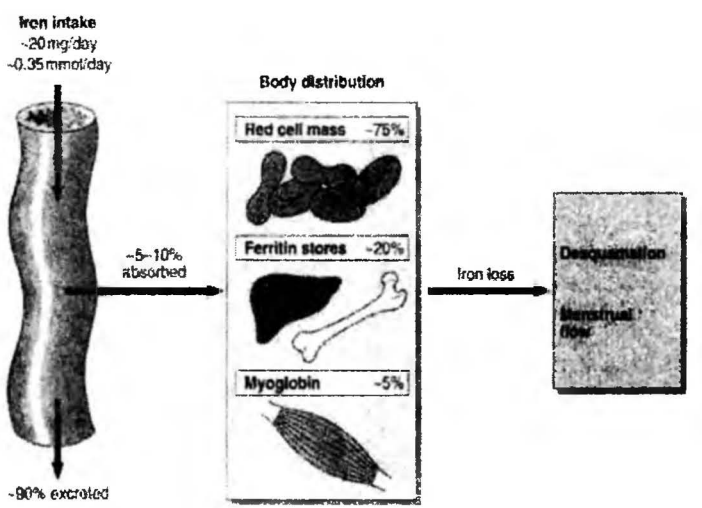
# Biochemistry of blood

484

## Iron balance




485

## The major functions of the blood

1. ***Respiration*** - transport of oxygen from the lungs to the tissues and of $CO_2$ from the tissues to the lungs.
2. ***Nutrition*** - transport of absorbed food materials.
3. ***Excretion*** - transport of metabolic waste to the kidneys, lungs, skin, and intestines for removal.
4. Maintenance of normal ***acid-base balance*** in the body.
5. Regulation of ***water balance*** through the effects of blood on the exchange of water between the circulating fluid and the tissue fluid.
6. Regulation of body ***temperature*** by the distribution of body heat.
7. ***Defense*** against infection by the white blood cells and circulating antibodies.
8. ***Transport*** of hormones and regulation of metabolism.
9. ***Transport*** of metabolites.
10. ***Coagulation***.

486

## Albumin

1. Albumin is the major protein of human plasma (35-52 g/l), has a MW of approximately 69.000, and comprises some 60% of the total plasma protein.
2. Some 40% of albumin is present in the plasma, and the other 60% is present in the extracellular space.
3. The liver produces about 12g of albumin per day, representing about 25% of total hepatic protein synthesis and half of all of its secreted protein.
4. Their half life is 17-20 days.
5. Albumins are soluble in pure water.

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## Functions of albumin

1. ***Oncotic function*** - albumin is responsible for 75-80% of the oncotic pressure of human plasma (25 mm Hg) because of its relatively low MW and high concentration.
   Decrease in plasma albumin level results in a fall in osmotic pressure, leading to enhanced fluid retention in tissue spaces, causing edema.
2. ***Transport function*** - albumin binds various biochemically important compounds and transports them in the circulation (free fatty acids, Ca, Zn, I, 10% of the plasma Cu, steroid hormones, vitamin D, bilirubin, variety of drugs - sulfonamides, penicillin, dicoumarol, aspirin).
3. ***Nutritive function*** - albumin serves as a source of amino acids for tissue protein synthesis to a limited extent, particularly in nutritional deprivation of amino acids.
4. ***Buffering function*** - albumin has the maximum buffering capacity among the plasma proteins.

488

## Globulins

1. Concentration of globulins in blood – 20-30 g/L.
2. Globulins dissolve in the presence of salts.
3. Globulins constitute several proteins that are separated into 4 distinct bands ($\alpha 1$, $\alpha 2$, $\beta$ and $\gamma$) on electrophoresis.
4. Globulins have a MW of approximately 75.000.

489

## Functions of globulins

1. ***Oncotic function*** - globulins are bigger in size molecules when compared with albumin. Hence, the contribution of globulins to oncotic pressure is less than that of albumin.

2. ***Transport function*** (globulins as specific transporters):
   - Lipoproteins transfer lipids;
   - Cortisol-binding globulin transfers steroid hormones;
   - Testosterone-estradiol-binding globulin transfers sex hormones;
   - Transcobalamin transfers vitamin $B_{12}$;
   - Thyroxine-binding globulin transfers thyroid hormones;
   - Transferrin transfers Fe;
   - Ceruloplasmin transfers Cu.

3. ***Maintenance of PH.***

4. ***Protective function*** – immunoglobulins.

5. ***Coagulation*** - fibrinogen.

Almost all globulins are synthesized by the liver, γ-globulins are synthesized by reticuloendothelial cells.

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## Acute phase proteins

*C-reactive Protein (CRP)* is a major component of acute phase proteins. It is produced in the liver and is present in the circulation in minute concentration. C-reactive protein (C strands for carbohydrate to which it binds on the capsule of pneumococci) is involved in the promotion of immune system through the activation of complement cascade. Estimation of CRP in serum is important for the evaluation of acute phase response and to make a diagnosis of inflammatory and oncological diseases.

*α1-Antitrypsin* is a protease inhibitor with a normal concentration of about 200 mg/dl. It inhibits *trypsin*, *elastase*, and certain other proteases by forming complexes with them. It is synthesized in the liver. A deficiency of this protein has a role in certain cases (approximately 5%) of emphysema and in one type of cirrhosis.

*Haptoglobin (Hp)* plasma concentration is increased in several inflammatory conditions. The levels of Hp in human plasma vary and are of some diagnostic use. Low levels of Hp are found in patients with hemolytic anemias. Haptoglobin binds with the free hemoglobin that spills into the plasma due to hemolysis. The haptoglobin-hemoglobin complex cannot pass through glomeruli of kidney. Haptoglobin, therefore, prevents the loss of free Hb into urine.

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# Xenobiotics.
# Biochemistry of liver

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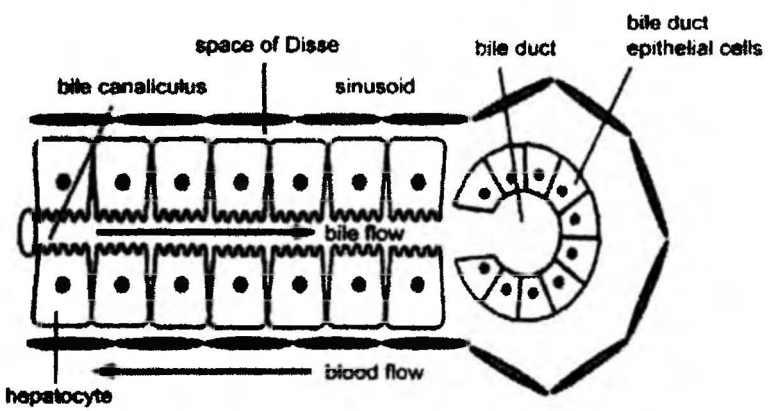
## Cross section of liver lobule

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## Functions of the liver

1. ***Biosynthesis*** of endogenous substances (plasma proteins) and ***storage*** (glycogen, lipid-soluble vitamins, $B_6$, $B_{12}$, Cu and Fe).
2. ***Transformation*** and ***degradation*** of endogenous substances to excretable molecules (metabolism).
3. ***Synthesis, storage, interconversion,*** and ***degradation*** of metabolites (metabolism).
4. ***Regulated supply*** of energy-rich intermediates and building blocks for biosynthetic reactions.
5. ***Detoxification*** of xenobiotics by biotransformation.
6. ***Excretion*** of bile pigments, bile salts and cholesterol in the bile into intestine.
7. ***Protective functions*** (Kupffer cells perform phagocytosis to eliminate foreign compounds).

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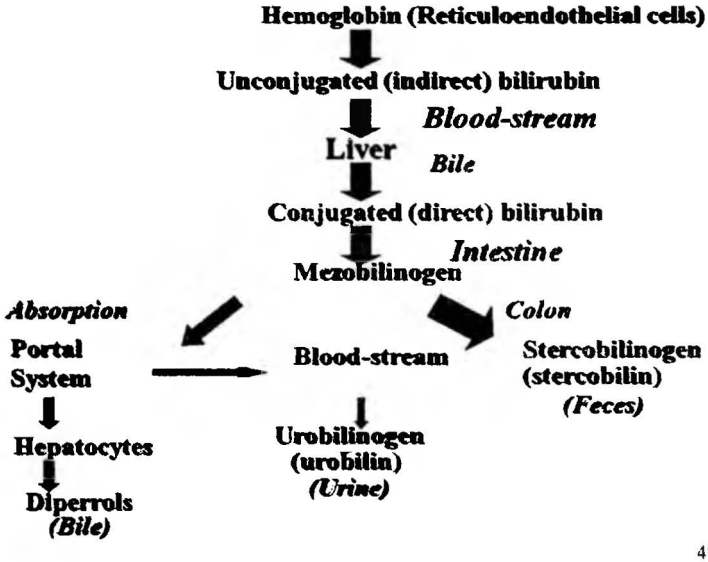
## Hemoglobin degradation

1. Enzyme *heme oxygenase* cleaves the methenyl bridges between the two pyrrole rings (A and B) of porphyrin to form **verdoglobin** (green pigment). Simultaneously, ferrous iron ($Fe^{2+}$) is oxidized to ferric form ($Fe^{3+}$).
2. Iron and globin are released. Porphyrin is unwound to chain to form **biliverdin** (green-yellow pigment).
3. Biliverdin's methenyl bridges (between the pyrrole rings C and D) are reduced to methylene group to form **bilirubin** (yellow pigment).

495

## Bilirubin metabolism and excretion

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## Distribution of bile pigments

| | | |
|---|---|---|
| Blood | Total bilirubin | 3.2-20.52 *μmol/l* |
| | Indirect bilirubin | 1.7-17.1 *μmol/l* |
| | Direct bilirubin | 2.2-5.1 *μmol/l* |
| Bile | Direct bilirubin | |
| Feces | Stercobilinogen (stercobilin) | |
| Urine | Urobilinogen (urobilin) | Traces |

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## *Hemolytic (choluric) jaundice*

| | |
|---|---|
| Blood | ↑ Total bilirubin at the expense of ↑ Indirect bilirubin |
| Feces | ↑ Stercobilinogen, dark color |
| Urine | Bilirubin (-), ↑ Urobilinogen |

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## *Hepatocellular (parenchymal) jaundice*

| | |
|---|---|
| Blood | ↑ Total bilirubin at the expense of ↑ Indirect and Direct bilirubin |
| Feces | ↓ Stercobilinogen |
| Urine | Bilirubin (+), ↑ Urobilinogen |

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## *Obstructive (cholestatic) jaundice*

| | |
|---|---|
| Blood | ↑ Total bilirubin at the expense of ↑↑ Direct and ↑ Indirect bilirubin, ↑ alkaline phasphatase activity, skin itch (↑ bile acids) |
| Feces | ↓ ↓ Stercobilinogen (light-colored feces) |
| Urine | Bilirubin (+), Urobilinogen (-) |

500

## Serum-biochemical hepatic syndromes

**1. Syndrome of hepatocyte integrity impairment (cytolytic syndrome, syndrome of hyperpermeability):**

1. Increased activity of serum hepaticospecific enzymes: *alanine transaminase (ALT)*, decreased de Ritis coefficient – AST/ALT, *aldolase*, isoforms of *lactate dehydrogenase* $LDH_1$ and $LDH_5$, *glutamate dehydrogenase, ornithine carbamoyl transferase, fructose-1-phosphate aldolase* and so on;
2. Hyperbilirubinemia (mainly at the expense of direct bilirubin);
3. Increased serum vitamin $B_{12}$, iron.

**2. Cholestasis syndrome (excretory-biliary syndrome):**

1. Increased activity of serum *γ-glutamyltranspeptidase*;
2. Increased activity of serum *alkaline phosphatase*;
3. Hyperbilirubinemia;
4. Hypercholesterolemia, increased serum LDL and decreased serum HDL.

**3. Hepatocellular insufficiency syndrome:**

1. Decreased activity of serum *choline esterase*;
2. Hypoproteinemia and disproteinemia with decreased albumins;
3. Decreased serum prothrombin and another clotting factors, impaired coagulation;
4. Hypocholesterolemia, decreased cholesterol esterification ratio;
5. Hyperbilirubinemia.

**4. Hepatic reticuloendothelium stimulation syndrome («inflammatory» syndrome):**

1. Increased serum globulins (sometimes with hyperproteinemia);
2. Alteration of protein- sedimentary tests (corrosive sublimate test, thymol test, zinc - sulfate test, heparin test).

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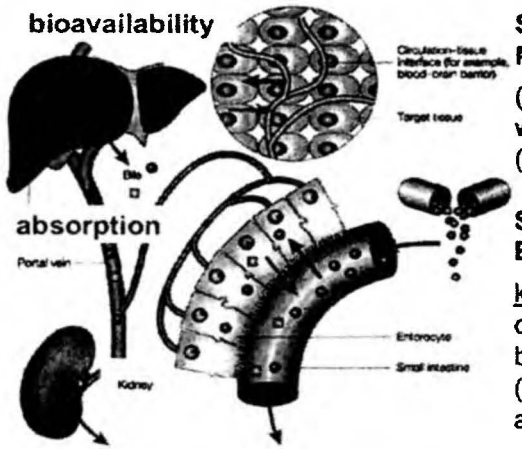


**Sites of Drug Metabolism:**

(intestinal wall), liver, (organs)

**Sites of Drug Elimination:**

kidneys (polar compounds), bile, feces (lipophilic analogs), lung

502

## Factors affecting drug metabolism

1. Physiological conditions:
   - age (drug metabolism slows down with old age).
   - diet (level of albumins, presence of cofactors).
   - hormone balance.
   - pregnancy.
2. Pathological conditions (impaired liver or kidney function: hepatitis, cirrhosis, hepatic cancer, nephritis, etc., general decrease with acute or chronic liver disease).
3. Genetic factors (gender, ethnic polymorphism, individual differences).
4. Drug-drug interactions (enzyme induction > more metabolism > larger k of elimination; enzyme inhibition > less metabolism > smaller k of elimination).
5. Chemistry of drug.

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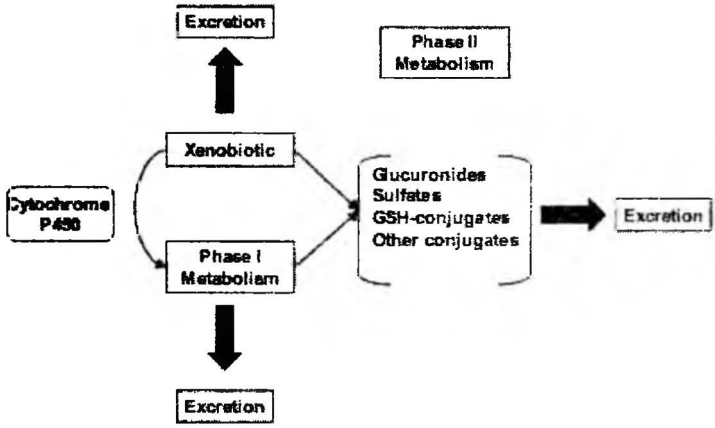
## Purpose of phase I and phase II drug metabolism

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# Functional biochemistry of kidney

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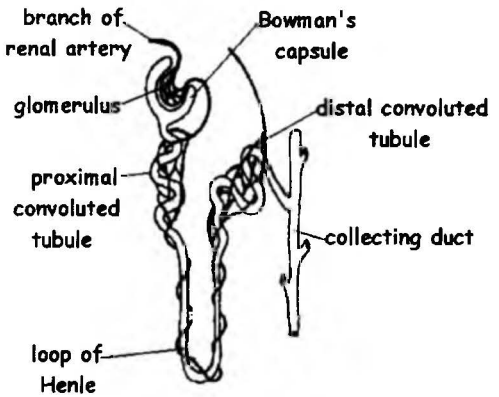
## Renal tubules




506

## Functions of the kidney

1. ***Maintenance of homeostasis*** (regulation of water, electrolyte, acid-base balance).
2. ***Excretion*** of metabolic waste products (urea, creatinine, creatine, uric acid, sulfate and phosphate), water soluble toxic substances and drugs.
3. ***Retention*** of substances vital to body (reabsorption of several substances, e.g. glucose, amino acids).
4. ***Endocrine functions*** (synthesis of hormones):
   - Erythropoietin - peptide hormone, stimulating hemoglobin synthesis and formation of erythrocytes.
   - 1,25-Dihydroxycholecalciferol (calcitriol) - the biochemically active form of vitamin D, regulating calcium absorption from the gut.
   - Renin - proteolytic enzyme, stimulating the formation of angiotensin II which leads to aldosterone production for the regulation of electrolyte balance.

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## Kidneys metabolism

1. Concentrating urine and transporting it through membranes are processes that require large amounts of energy. The kidneys therefore have very high energy demands. Although the kidneys only represents about 0.5% of the body's mass, it consumes around 10% of the oxygen. ATP needed is obtained from oxidative metabolism of glucose, fatty acids, ketone bodies, and several amino acids, lactate, glycerol, and citric acid.
2. Transport of substances through the apical membrane of kidney epithelial cells at urine formation depends on the cellular $Na^+$ gradient, which is maintained by the *$Na^+K^+$-ATPase* in secondary active transport.
3. Kidneys are capable of glucose synthesis from amino acids and lactate (gluconeogenesis) but a major precursor, in acidotic conditions, is glutamine.
4. The kidney takes up glutamine, and by the action of *renal glutaminase*, initially release $NH_3$ and glutamate. $NH_3$ formed is released into the tubules to buffer protons that are secreted into the glomerular filtrate by the kidney, to reduce the hydrogen ion concentration in the blood. This helps to maintain the normal pH of the blood.
5. In the kidneys from the glutamate formed, a second molecule of $NH_3$ can be obtained by oxidative deamination with the help of *glutamate dehydrogenase*. The resulting α-ketoglutarate is further metabolized in the citric acid cycle. Several other amino acids - alanine in particular, as well as serine, glycine, and aspartate - can also serve as suppliers of ammonia.

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## The formation of urine

Urine is formed as a result of a three phase process – glomerular filtration, selective (active) and passive reabsorption, secretion.

**Glomerular filtration.** This is a passive process that results in the formation of ultrafiltrate of blood. Filtration takes place through the semipermeable walls of the glomerular capillaries. About 20% of renal plasma flow is filtered each minute (~125 ml/min). All the (unbound) constituents of plasma, with a molecular weight less than about 70.000, are passed into the filtrate. Therefore, the glomerular filtrate (primary urine) (~150L) is almost similar in composition to plasma. The driving hydrostatic pressure is provided by arterial pressure.

**Selective (active) and passive reabsorption.** In the proximal and distal tubule, the glomerular filtrate becomes highly concentrated as the result of the removal of water. The renal tubules retain water and most of the soluble constituents of the glomerular filtrate by reabsorption. This may occur either by passive or active process.

***Proximal tubule.*** Many low molecular weight constituents are rebsorbed by active transport – proteins, peptide, amino acid, uric acid, glucose, $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, phosphate and sulphate; by passive transport – water, $HCO_3^-$, $Cl^-$, urea. Reabsorbs 60% of all solute (100% of glucose and amino acids, 90% of bicarbonate, 80-90% of inorganic phosphate and water. Parathormone stimulates the absorption of calcium and inhibits the resorption of phosphate.

***Loop of Henle.*** Next reabsorption of NaCl. In descending loop of Henle: resorption of water by osmosis and increasing of osmolarity; in ascending loop of Henle: active transport of NaCl out of the tubule and decreasing of osmolarity.

***Distal tubule and collecting ducts***. Reabsorption of $Na^+$, $Ca^{2+}$, $Mg^{2+}$ by active transport and by passive transport - water, chlorides and urea. Aldosterone promotes $Na^+$ ions and water reabsorption and excretion of $H^+$ ions. Antidiuretic increases the water permeability thus increasing the concentration of urine. The tubule cells absorb $CO_2$ from the blood and then hydrate it to carbonic acid (enzyme carbonate dehydratase). Carbonic acid then dissociates to $HCO_3^-$ and $H^+$. $H^+$ is exported to the urine by an ATP-driven transport system, while $HCO_3^-$ returns to the blood.

**Secretion**. Some of the substances are released into urine by active transport – $H^+$ and $K^+$ ions, urea, creatinine and drugs (antibiotics); by passive transport – ammonia.

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**Substances according to their entering into urine may be divided into the following groups:**

1. Substances filtered by the glomerulus (inulin, creatinine, urea).
2. Substances, concentration of which in urine is determined by ratio between the processes of secretion and reabsorption in the tubules (electrolytes).
3. Substances mainly secreted from plasma into lumen of proximal tubules (organic acids and basis).
4. Substances, which are practically absent in plasma and enter into urine from the renal tubules ($NH_3$, some enzymes).
5. Substances reabsorbed in the proximal tubule and do not appear in urine until the plasma level reaches a certain concentration known as **the renal threshold** (glucose, amino acids, ketone bodies, $Na^+$, $Ca^{2+}$, $Cl^-$, $HCO_3^-$, $HPO_4^{2-}$, $SO_4^{2-}$).

Substances whose excretion in urine is dependent on their concentration are referred to as **renal threshold substances**. At the normal concentration in the blood, they are completely reabsorbed by the kidneys, with a result that their excretion in urine is almost negligible. While calculating the renal threshold of a particular compound, it is assumed that both the kidneys are optimally functioning, without any abnormality. But this is not always true-in which case the renal threshold is altered. For instance, renal glycosuria is associated with reduced threshold for glucose due to its diminished tubular reabsorption. The renal threshold for glucose is 10 mmol/l or 180 mg/dl.

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## Functions of the kidney in the acid-base balance

Renal regulation of the acid-base balance which occurs by the following mechanisms:

1) Reabsorption of bicarbonate $HCO_3^-$;
2) Regeneration of $HCO_3^-$;
3) $H^+$ secretion.

**1. Reabsorption of bicarbonate $HCO_3^-$.** This mechanism is primarily responsible to conserve the blood $HCO_3^-$, with a simultaneous excretion of $H^+$ ions. The normal urine is almost free from $HCO_3^-$. Bicarbonate freely diffuses from the plasma into the tubular lumen. Here $HCO_3^-$ combines with $H^+$, secreted by tubular cells, to form $H_2CO_3$. $H_2CO_3$ is then cleaved by *carbonic anhydrase* (of tubular cell membrane) to form $CO_2$ and $H_2O$. As the $CO_2$ concentration builds up in the lumen, it diffuses into the proximal tubular cells along the concentration gradient. In the tubular cell, $CO_2$ again combines with $H_2O$ to form $H_2CO_3$ which then dissociates into $H^+$ and $HCO_3^-$. The $H^+$ is secreted into the tubular lumen in exchange for $Na^+$. The $HCO_3^-$ is reabsorbed into plasma in association with $Na^+$.

$$H^+ + HCO_3^- \xrightleftharpoons{\text{Carbonic anhydrase}} H_2CO_3 \rightleftharpoons CO_2 + H_2O$$

$$CO_2 + H_2O \xrightleftharpoons{\text{Carbonic anhydrase}} H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

**2. Regeneration of $HCO_3^-$.**

$CO_2$ diffuses from the urine into the cells of the distal tubules and forms $H_2CO_3$. $H_2CO_3$ dissociates into $H^+$ and $HCO_3^-$. The $H^+$ is secreted into the urine and $HCO_3^-$ is reabsorbed into plasma.

**3. $H^+$ secretion.**

$H^+$ ions are secreted into the urine in the free form, in the form of $H_2PO_4^-$ and $NH_4^+$.

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## Disturbances of urine formation

- Urine formation depends on **glomerular filtration rate (GFR)**. Decrease in GFR leads to oliguria, uremia, metabolic acidosis, hyperkalemia and delay of the urea and other final products of the metabolism of the nitrogen substances in organism, decrease in bicarbonate level in plasma. Low GFR is due to damaged kidneys, and due to decreased hydrostatic pressure between capillaries of renal corpuscle and contents of tubules. This is usually related to renal blood flow insufficiency or can be caused by obstruction for urine outflow.
- Polyuria, low level of bicarbonate in plasma and metabolic acidosis, hypokalemia, hypophosphatemia, hypouricaemia are due to **damaged tubules**.

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## Renal failure (or renal insufficiency)

Renal failure is caused by impaired processes of renal blood flow, glomerular filtration, renal tubular reabsorption and secretion.

Causes of renal failure:

1. Decreased volume of extracellular space due to cardiovascular insufficiency, shock, and decrease in arterial pressure. This leads to decrease in renal blood flow and as result to decrease in renal filtration.
2. Decreased volume of functional renal parenchyma at nephritis, neoplasms, intoxications, traumatic loss of kidneys.
3. Obstruction in the urinary tract below the kidneys. This can occur from urinary tract stones, tumors, and anatomic obstruction due to benign prostatic hypertrophy that lead to the increase in renal pressure and decrease in glomerular filtration.

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## Signs of renal failure

1. Azotemia - waste accumulates in the blood and the body, when kidneys fail to filter properly.
2. Proteinuria, hypo- and proteinemia as a result of increased catabolism of proteins, secondary hyperlipidemia of IV type; decrease in urine concentration; metabolic acidosis.
3. Anemia a decreased red blood cell count, because lower levels of erythropoietin produced by failing kidneys. It's also important that degree hemolysis is increased, which shortens erythrocytes life duration.
4. Qualitative changes of thrombocytes (thrombocylopathy). It appears as bleeding duration increase.

514

## Kidney stones

Kidney stones are produced whenever the body exceeds its normal levels of minerals such as oxalate, uric acid and calcium. When these minerals exceed normal quantities, they crystallize among the tissues of the kidney along with the other elements that comprise urine. Sometimes, these kinds of kidney stones are small enough to be excreted along with normal urine, but often, they are too big and remain inside the kidney indefinitely.

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## Causes of kidney stones

1. Supersaturation. The urine carries salts, including calcium oxalate, uric acid, cystine, or xanthine. These salts can become extremely concentrated if there is not enough urine, or if unusually high amounts of crystal-forming salts are present. When salt concentration levels reach the point at which they no longer dissolve, these salts form crystals.
2. Changes in the acidity of the urine.
3. Congestion of urine due to impairment of urination.
4. Low levels of stone-blocking compounds. Normally, urine contains substances that may protect against stone formation, including magnesium, citrate, pyrophosphate, enzymes.

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## Kinds of kidney stones

1. **Calcium stones**, which comprise 70-90% of all kinds of kidney stones, are made of calcium, usually combined with oxalate, or oxalic acid. About 6% of calcium stones are made of calcium phosphate. Calcium phosphate increases in alkaline urine. Hypercalciuria is responsible for as much as 70% of calcium-containing stones. The following can lead to hypercalciuria and calcium stones: 1) too much calcium absorption in the intestines; 2) excessive chloride; 3) renal calcium leak; 4) excessive sodium. Hyperoxaluria is responsible for up to 60% of calcium stones and is usually caused by too much dietary oxalates (found in a number of common vegetables, fruits, and grains) or by hypervitaminosis C.
2. **Uric acid stones**. Excess uric acid is responsible for close to 10% of kidney stones. It is the breakdown product of purines, found in certain foods (dried beans, peas, and liver). Uric acid mainly forms in acidic urine.
3. **Cystine stones** are caused by a rare congenital defect that can cause stones composed of the amino acid cystine to form in the kidney called cystinuria.
4. **Xanthine stones** are composed of xanthine. These stones are extremely uncommon and usually occur as a result of a rare genetic disorder.

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## Components of urine and change of its composition

- Rate of urine formation, volume and composition of urine are subject to wide variation and depend on diuresis, food intake, digestion, body weight, age, sex, and living conditions such as temperature, humidity, physical activity, health status, and many other factors. It is rather thereby to analyze a daily portion of urine.

- As there is a marked circadian rhythm in urine excretion, the amount of urine and its composition are usually given relative to a 24-hour period. The urine volume varies from 600 to 2500 ml for 24 h and depends mainly on amount of the liquid absorbed.

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## Color of urine

Urine is a transparent solution that can range from colorless to amber but is usually a pale yellow. In the urine of a healthy individual, the color comes primarily from the presence of urobilinogen which is related to the bile pigments produced by hemoglobin degradation. If urine is left to stand long enough, oxidation of the urobilinogen may lead to a darkening in color. Unusual dark color is the excess presence of bilirubin, urobilin, porphyrins and homogentisate. Many things affect urine color, including fluid balance, diet, medicines, and diseases.

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## Precipitates of urine

Precipitates of urine are formed due to urine standing and are the following:

1) Flaky precipitates formed from nucleoproteins or muco-proteins and epithelial cells of genitourinary tract.
2) Precipitates formed from the mixture of calcium phosphate and ammonium phosphate, if urine is alkaline.
3) Precipitates formed from oxalates and urates, dissolving during acidification.
4) Precipitates formed from uric acid, if urine is acid.

520

## Specific gravity and PH of urine

Specific gravity checks the concentration of solutes in the urine and ranges between 1.008 and 1.012. The specific gravity varies depending on the time of day, amount of food and liquids consumed, and the amount of recent exercise.

The pH of urine is close to neutral (7) but can normally vary between 4.6 and 8. Acidity of urine is due to presence of phosphoric acid as the product of breakdown of tissue, foods rich in phosphoproteins, phospholipids and nucleoproteins. A diet high in citrus, vegetables or dairy can increase urine pH (more basic). A diet high in meat can decrease urine pH (more acidic). Acidity of urine increased in acidosis, fevers, and diet with excess proteins; alkaline - increased in chronic cystitis and urine retention due to decomposition of urine in bladder.

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## Protein in urine

Protein is normally not found in the urine. Quantities of protein (0,03g/24 h) may be present in urine which cannot be detectable by chemical methods. When chemically detectable quantities of proteins are present in urine, the condition is called as proteinuria. Proteins are filtered through glomeruli in kidney diseases due to altered glomerular permeability. Fever, hard exercise, pregnancy may also cause protein to be in the urine.

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## Reasons of proteinuria:

*1. Proteinuria due to hyperproteinemia:*
a) normal proteins
- hemoglobin (hemolysis).
- myoglobin (rhabdomyolysis).

b) abnormal proteins
- fragments of immunoglobulin (Bence Jones protein).

*2. Proteinuria due to glomerular impairment:*
a) renal haemodynamics failure
- orthostatic proteinuria, physical activities.

b) increased permeability of glomerules

*3. Tubular proteinuria - the presence of proteins normally filtered, which are not reabsorbed in tubules:*
a) tubular impairment
- heavy metal and medicine intoxication.

b) interstitial diseases
- nephritis, pyelonephritis.

*4. Secreted proteinuria:*
a) Tamm-Horsfall proteinuria

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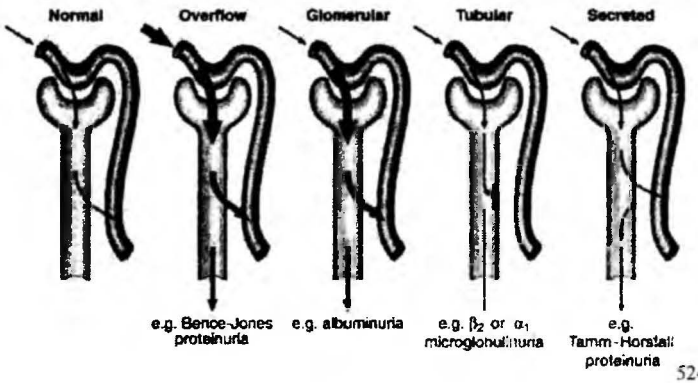
## The classification of proteinuria

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## Nonprotein nitrogen-containing compounds excretion

**1. Urea** is the product of protein metabolism and constitutes about 80%-90% total nitrogen excretion. About 15-30 g of urea (7-15 g nitrogen) or 333-583 mmol/24h is excreted in urine per day. This is associated with increased protein breakdown, leading to a negative nitrogen balance, as observed during starvation, burns, traumas, after major surgery, prolonged fever, diabetic coma, thyrotoxicosis etc. Urea excretion is decreased in pre renal (diabetes mellitus, dehydration, cardiac failure, burns, high fever etc.), renal (kidney diseases) and post renal (prostate enlargement etc.) conditions.

**2. Uric acid** is the end product of purine metabolism. The daily excretion of uric acid is about 500-700 mg. Elevation in the serum uric acid concentration is associated with increased uric acid excretion (uricosuria). Hyperuricaemia may be caused by an increased rate of purine synthesis, an increased rate of turnover of nucleic acids, as in malignancies, tissue damage or starvation, a reduced renal excretion. High uric acid is excreted in gout and leukaemia. Lesch-Nyhan syndrome and some cases of glycogen storage disease are associated with uricosuria. Salicylates in doses of less than 2-3 g/d may produce renal retention of uric acid.

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## Nonprotein nitrogen-containing compounds excretion

**3. Creatinine** is the anhydride of creatine as a product from metabolism in the muscle tissues. Normal urinary excretion of creatinine is 1.5 to 3.0 g per 24 hours. Excretion rate decreases in all kinds of renal diseases and post renal conditions. Creatinine bears a direct relation to the muscle mass of the individual expressed as creatinine coefficient (amount of creatinine in mg excreted in 24 hours per kg of body weight); average adult male coefficient is 18-32; female is 14-22. Decrease value indicates muscle wasting due to prolonged negative nitrogen balance; seen in starvation, diabetes, muscle dystrophy.

**4. Creatine** is mostly found in the muscle as creatine phosphate, a high energy compound. Normally, small amounts of creatine are excreted in the urine. Increased output of creatine in urine is referred to as creatinuria. Elevated in states of elevated catabolism, in muscular dystrophies such as progressive muscular dystrophy, myotonia atrophica, and myasthenia gravis; muscle wasting, as in acute poliomyelitis, amyotrophic lateral sclerosis, and myositis manifested by muscle wasting; starvation and cachectic states; hyperthyroidism; and febrile diseases, diabetes mellitus. Decreased in hypothyroidism, amyotonia congenita, and renal insufficiency.

**5. Amino acids** are normally excreted in 0.29-5.35 mmol per 24 hours (nitrogen of amino acids). Glycine, histidine and alanine are the most abundant amino acids excreted into urine. They may be present in urine in excessive amount due to defect in protein targeting, defective renal reabsorption, increased catabolism of proteins, because the plasma concentration exceeds the renal threshold, or because there is specific failure of normal tubular reabsorptive mechanisms, such as in the inherited metabolic disorder, cystinuria or more commonly because of acquired renal tubular damage.

**6. Ammonium salts** are normally excreted in 30-60 mmol /24 h (ammonia of ammonium salts). Increased in acidosis, decreased [illegible] to damage of distal tubules, where ammonium salts [illegible].

**7. Hippuric acid** is excreted into urine in amounts that are proportional to the amounts of the taken food. Usually excretion is about 5,5 mmol /24 h.

**8. Indican** presents in urine in trace amounts and derived from indole which in turn arises from the action of putrefying bacteria [illegible]. Increased in lavish meat products or due to putrefactive processes in intestine.

**9. Nitrogen pigments**, in particular urobilinogen, and also glucuronides of bile acids, bilirubin are excreted into urine in small amounts.

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## Nonnitrogen-containing compounds excretion

1. **Glucose** is normally not present in urine. Small amount of glucose (2-20 mg or 0.3-1.1 mmol) may be present in fasting urine which can not be detectable by chemical methods. When glucose is present, the condition is called glucosuria. It results from either: diabetes mellitus or diabetes insipidus; a reduction in the "renal threshold (renal glycosuria); hormonal disorders, liver disease, medications, pregnancy, hyperthyroidism and peptic ulcer (alimentary glycosuria).
2. **Lactate** and **pyruvate** normal urinary excretion are 1.1 to 0.11 mmol/24 h. Large amounts may be present during intensive muscular exercises or hypoxia, and at pathologic conditions such as diabetes mellitus, starvation;
3. **Ketone bodies** are not normally found in the urine. Small amount of ketone bodies (20-50 mg/24 h) may be present in urine which can not be detectable by chemical methods. Large amounts of ketones in the urine (ketonuria) may mean a diabetes mellitus is present. A diet low in carbohydrates, starvation or high-protein diets also cause ketones to be in the urine.
4. **Mineral salts:**

- Urine sodium (Na) excretion per 24 hours is 174-222 and urine potassium (K) excretion per 24 hours is 69-71. Potassium and chloride excretion of potassium in urine is found in fever, acidosis. In Addison's disease, potassium is retained and sodium chloride is excreted. In Cushing's syndrome, sodium chloride is retained and potassium is excreted. Sodium chloride is excessively lost in salt losing nephritis;
- Urine calcium (Ca) excretion per 24 hours is 4.02-4.99. Excretion of calcium in urine is low in rickets. It is high in hyperparathyroidism and hyper thyroidism. In multiple myeloma, it is high. It is frequent in renal stones,
- Urine inorganic phosphorous (P) average value excreted in 24 hours urine is 33 mmol. Excretion is increased in hyperparathyroidism. Excretion is decreased in hypoparathyroidism and rickets.

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# Biochemistry of carcinogenesis

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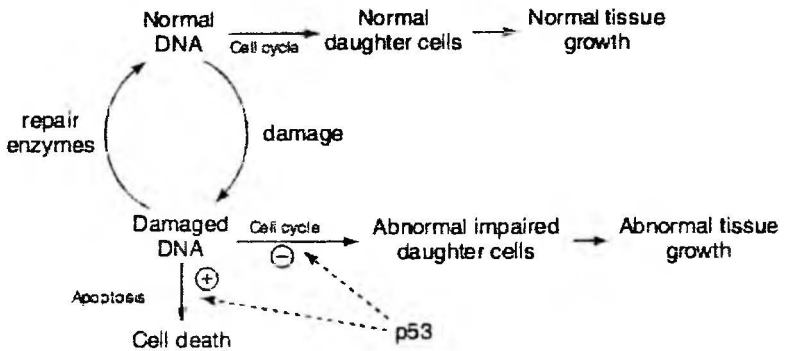


**Role of p53 as a tumour suppressor gene. p53 induces a cell with damaged DNA either to initiate apoptosis, or arrest the cell cycle, to give time for damaged DNA to be repaired. Damage can be, for example, a mutation, DNA strand breakage or chromosomal rearrangement.**

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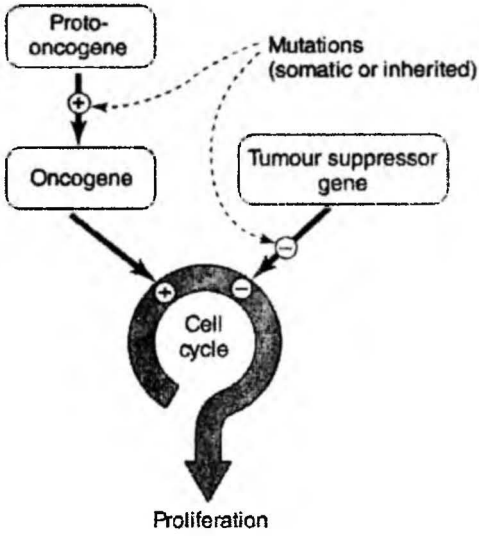


**A diagram representing a simplified account of how oncogenes and tumour suppressor genes affect progrssion through the cell cycle and the possible effects of mutations. An oncogene increases progression through the cell cycle (+) and a tumoursuppressor gene decreases or arrests progression (−). The dotted line from mutations to the conversion of a proto-oncogene to an oncogene indicates activation of the process, whereas that to the tumour suppressor gene indicates inactivation of the gene.**

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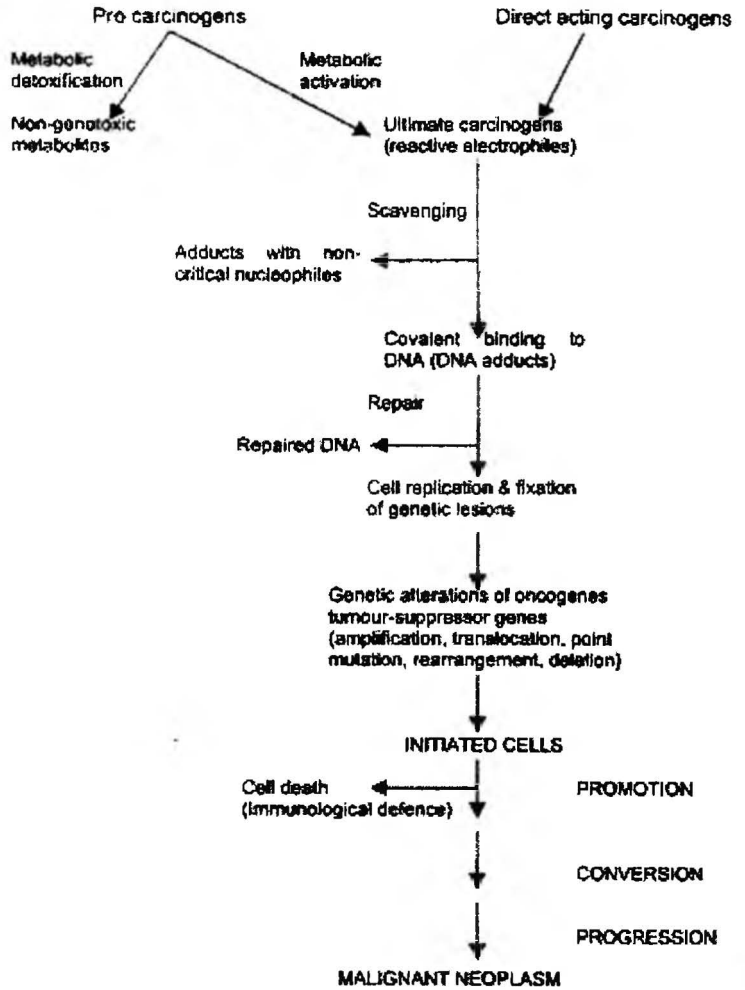
## Summary of events in the carcinogenic process

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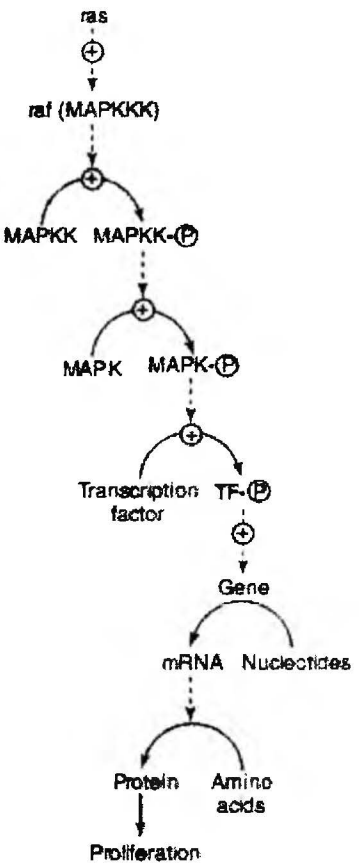


**The mitogen-activated protein kinase cascade (MAP kinase cascade). The active protein Ras activates Raf by promoting its recruitment to a cell membrane. Through a series of phosphorylations MAP kinase is activated as follows: MAP kinase kinase kinase (Raf) phosphorylates MAP kinase kinase which, in turn, phosphorylates MAP kinase, the final target enzyme. MAP kinase phosphorylates transcription factors for genes that express proteins involved in proliferation.**

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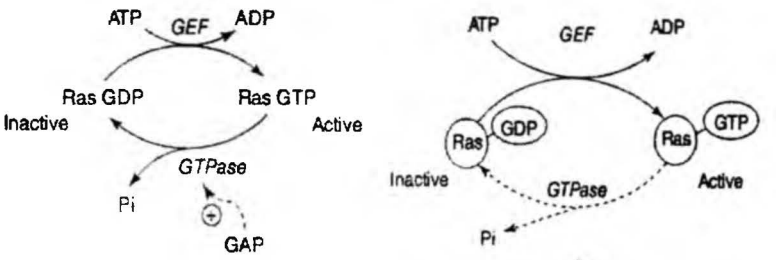


**a - control of the activity of Ras by a balance of the activities of guanine nucleotide exchange factor and GTPase. GAP is the abbreviation for GTPase-activating factor and GEF for guanine nucleotide exchange factor. Ras oncogenes are present in about 30% of all human tumours.**

**b - maintenance of Ras in the activated from by loss of GTPase activity. A point mutation in the Ras protooncogene leads to a very low activity of the GTPase so that Ras protein remains in the active form. The broken line indicates low activity of GTPase.**

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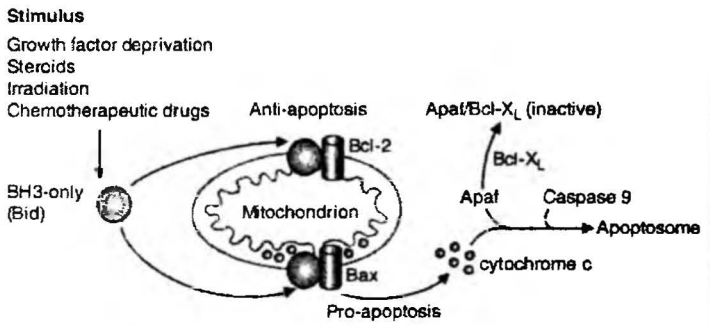
## Pathways in the process of apoptosis




Apaf - apoptotic protease activating factor

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## Selected tumor markers and associated cancers

| *Tumor marker* | *Associated cancer(s)* |
|---|---|
| **Oncofetal antigens** | |
| α-Fetoprotein (AFP) | Cancer of liver and germ cells of testis |
| Carcinoembryonic antigen (CEA) | Cancers of colon, stomach, lung, pancreas and breast |
| Cancer antigen-125 (CA-125) | Ovarian cancer |
| **Hormones** | |
| Human chorionic gonadotropin (hCG) | Choriocarcinoma |
| Calcitonin | Carcinoma of medullary thyroid |
| Catecholamines and their metabolites (mainly vanillyl mandelic acid) | Pheochromocytoma and neuroblastoma |
| **Enzymes** | |
| Prostatic acid phosphatase | Prostate cancer |
| Neuron specific enolase | Neuroblastoma |
| **Specific protein** | |
| Prostate specific antigen (PSA) | Prostate cancer |
| Immunoglobulins | Multiple myeloma |

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## Etiology of common tumors

| *Cancer site* | *Major hypothesized cause* |
|---|---|
| Lung | Smoking, asbestos, chemical substances, uv radiation, radon gas and occupation |
| Large intestine and rectum | Familial predisposition, high fat and low fiber intake, intestinal flora, smoking |
| Skin (melanoma) | Endogenous hormones, solar radiation, light skin, coal pitch, creosote, arsenic, radium |
| Liver | High fat intake, alcohol, vinyl chloride, nitrosoamines, chlorinated hydrocarbons |
| Pancreas | Age, smoking, high fat intake, smoking and occupation |
| Lymphatic system | Slackening of immunity, herbicides, solvents, vinyl chloride, viruses, benzene, and irradiation |
| Blood | Genetic disturbances, ionize radiation, chemical substances, viruses |
| Windpipe | Smoking, alcohol abuse, nutrition |
| Kidney | Smoking, heavy metals, dyes, drugs |
| Bladder | Smoking, heavy metals, dyes, drugs |
| Body of uterus | Reproductive history, early sexual life, , smoking, no pregnancy |
| Ovary | Age, familial predisposition, genetic disturbances, no pregnancy |
| Female breast | Age, familial predisposition, no pregnancy, early menarche, late menopause, late first birth, high fat intake, viruses |
| Prostate | Age, familial predisposition, high fat intake, occupation, and smoking |

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## Agents causing cancer (carcinogens)

**I. Compounds and the products made and used by the industry. Natural carcinogens.**

1. Asbestos and similar fibers
2. Aflatoxins (B, G, $G_2$)
3. Benzidine
4. Benzene
5. Beryllium and its compounds
6. Vinyl chloride
7. Cadmium and its compounds
8. Polycyclic hydrocarbons in soot, tar, oil and resultant fumes and products of combustion
9. Arsenic and its compounds
10. Nickel and its compounds
11. Chromium (VI)

**II. Therapeutic drugs**

1. Phenacetin
2. Azathioprine
3. Melphalan
4. Methoxsalen
5. Mileran
6. Thiophosphamide
7. Treosulfan
8. Chlorambucil
9. Ciclosporin
10. Cyclophosphamide
11. Steroid estrogens (estradiol -17 and its ethers, estriol, estrone, ethinyl estradiol, mestranol, estrogens conjugated)
12. Nonsteroid estrogens (diethylstilbestrol, dienestrol, hexestrol)
13. Oral contraceptives

**III. Household and natural factors**

1. Alcohol
2. Radon gas
3. Ultraviolet radiation from the sun
4. Tobacco

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## Selected oncoproteins, protooncogenes and associated cancers

| *Oncoproteins* | *Protoonco-genes* | *Associated human cancers* |
|---|---|---|
| **Growth factors** Platelet derived growth factor (PDGF) | sis | Osteosarcoma |
| Epidermal growth factor (EGF) | hst-1 | Cancers of stomach, breast and bladder |
| **Growth factor receptors** | erb-B1 | Lung cancer |
| | erb-B2 | Stomach cancer |
| | erb-B3 | Breast cancer |
| **Signal-transducing proteins** GTP-binding proteins | ras | Leukemia, cancers of lung, pancreas and colon |
| Non-receptor tyrosine kinase | abl | Leukemia |

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# Biochemistry of tooth tissues and saliva

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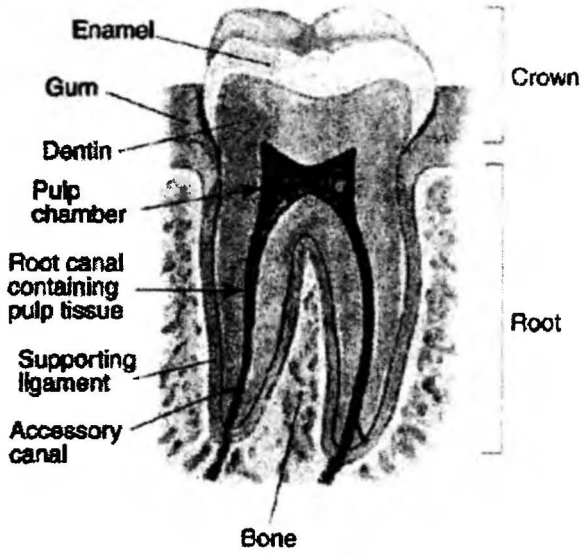
**Tooth is a complex system of specialized tissues**

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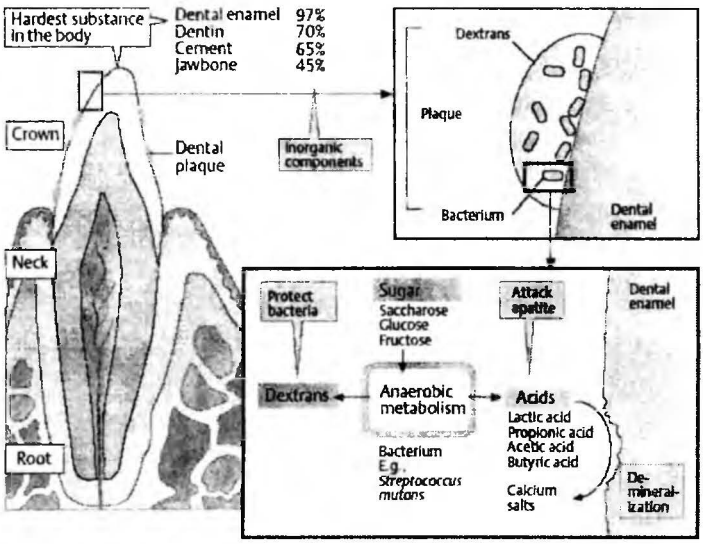
## Biochemistry of teeth

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## Biochemical composition of teeth tissues

| *Compounds* | *Pulp, %* | *Dentin, %* | *Enamel, %* | *Cement, %* |
|---|---|---|---|---|
| Water | 30-40 | 13 | 2,5 | 10 |
| Organic compounds | 40 | 17 | 4 | 20 |
| Inorganic compounds | 20-30 | 70 | 96 | 70 |
| Ca | 30 | 35 | 36 | 35.5 |
| Mg | 0.8 | 1.2 | 0.5 | 0.9 |
| Na | 0.2 | 1,2 | 0.2 | 1.1 |
| K | 0.1 | 0.1 | 0.3 | 0.1 |
| P | 17.0 | 17.4 | 17.3 | 17.1 |
| F | 0.02 | 0.02 | 0.02 | 0.02 |
| Citrate | - | 1.0 | 0.3 | - |

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## Macromolecules associated with calcification

| *Components* | *Cartilage* | *Bone* | *Dentine* | *Enamel* |
|---|---|---|---|---|
| Collagen I | + | + | + | - |
| GLA Proteins (γ-carboxyglutamate) (osteocalcin, osteopontin, osteonectin, osteogenin | + | + | + | - |
| Glycoproteins | + | + | + | + |
| Phosphoproteins | + | + | + | + |
| Proteoglycans | + | + | + | ND |

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## Components of calcified oral tissues

| *Tissue* | *Inorganic components* | *Protein components* | *Other components* |
|---|---|---|---|
| Cement | 45 % | 33 % (protein mainly collagen type I and III) | Water 22 % |
| Dentin | 70 % | 20 % (collagen I, b-FGF, osteocalcin, osteonectin, osteogenin, osteopontin phosphoproteins, dentin specific proteins) | 10 % (glycoproteins, proteoglycans), phospholipids, water |
| Enamel | ~95 % | 5% (ameloblastin, amelogenin, enamelins, tuftelins) | <1 % |

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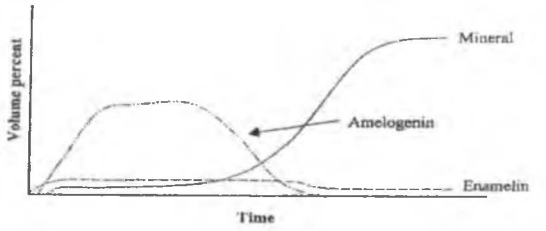


**Loss of proteins and increase in mineral content with time during enamel formation**

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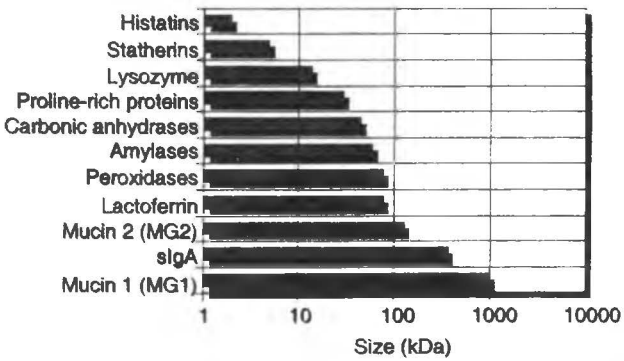
## Major salivary components

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**The most important functions of saliva related to the each salivary component**

| *Functions* | *Components in saliva* |
|---|---|
| **Protection of teeth and oral, pharyngeal and oesophageal mucosa** | |
| Mechanical cleansing of teeth and mucosa | Water |
| Lubrication of teeth and mucosa | Water, mucins |
| Keep oral mucosa intact, soft and moistened | Water, mucins, salts, epidermal growth factor, fibroblast growth factor, nerve growth factor |
| Prevent tooth demineralization | Proline-rich proteins, statherins cystatins, histatins, calcium and phosphate |
| Buffer capacity | Bicarbonate, phosphate and protein |
| **Antimicrobial activities** | |
| Antibacterial functions | Amylases, cystatins, histatins, mucins, peroxidase, lysozyme, lactoferrin, calprotectin, immunoglobulins, chromogranin A. |
| Fungicidal functions | Histatins, immunoglobulins, chromogranin A |
| Antiviral functions | Cystatins, mucins, immunoglobulins |
| **Digestive properties** | |
| Formation of food bolus | Water, mucins |
| Facilitation of mastication and swallowing | Water, mucins |
| Initial digestion | α-amylases, lipases, ribonucleases, proteases, water, mucins |
| Dissolution of taste compounds | Gustin (carbonanhydrase), zinc, water |

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## The most important inorganic and organic components of saliva

| *Inorganic components* | *Content* | *Organic components* | *Content* |
|---|---|---|---|
| Water | 98 % | Proteins | 0.95-2.32 g/L |
| pH | 6.5-7.5 | Glucose | 0.05 mM/L |
| $HCO_3$ | 5-10 mM/L | Lactate | 0.03-0.05 g/L |
| P | 1.9-7.7 mM/L | Pyruvate | 22.7-45.4 μM/L |
| Na | 1-5 mM/L | Cholesterol | 0.065-0.233 mM/L |
| Cl | 5 mM/L | Urea | 1.83 mM/L |
| K | 15 mM/L | Uric acid | 0.088 mM/L |
| Ca | 1.5-5 mM/L | $NH_3$ | 1.2-1.6 μM/L |

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## Chemical composition of dental plaque and dental calculus

| | *Dental plaque* | *Dental calculus* |
|---|---|---|
| **Water** | 80 % | 4-10 % |
| **Organic components** | 60 %<br>Glycosaminoglycans, glycoproteins, polysaccharides, heteropolysaccharides | 13-25 %<br>Amino acids, monosaccharides (glucose, galactose, galactosamine, glucuronic acid), phospholipids, cholesterol, di- and triacylglycerols, fatty acids, enzymes, bacterias, leukocytes |
| **Inorganic components** | 40 %<br>P, Na, K, Ca, F, Zn, Fe | 72-82 %<br>Ca, P, Mg, Na, Si, Zn, Pb, Cd |

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## Stages in the development of caries

1. The enamel becomes decalcified.
2. A small white spot appear.
3. Discoloration becomes pronounced.
4. The tooth surface softens and decay penetrates through the enamel into the dentine.
5. Caries spreads laterally and in depth.
6. Cavitation occurs.
7. The lesion deepens, and pulp becomes affected, first reacting to stimuli (e.g. sweets, temp.), then damaged and dead.
8. Bacteria travel down the root canal, out through the apex causing abscesses.

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## Factors determining the incidence of caries

1. Microorganisms: Acid producing bacteria, especially if they produce extracellular polysaccharides, will increase risk.
2. Host factors:

a. Decreased saliva secretion increase incidence.
b. Buffering power of saliva to raise pH decrease incidence.
c. The morphology of the teeth: well spaced teeth decrease incidence, while fissures & pits increase it.
d. The composition of the teeth: certain trace elements decrease incidence (e.g. F, Mb and B), while others (e.g. Cu & Mn) increase it.

3. Time: frequency of consumption of carbohydrates and length of time in the mouth are related to incidence. Good oral hygiene can counter act this.
4. Substrates for acid production provided in diet.

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## Factors in unrefined food lead to decreased incidence of caries

1. Substances that decrease solubility of calcium phosphate.
2. More phytate and/or calcium.
3. Antibacterial substances.
4. Substances that inhibit aggregation of bacteria: decrease plaque formation.
5. Absence of free sugar (most important).

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