DANYLO HALYTSKYI LVIV NATIONAL MEDICAL UNIVERSITY
Department of biological chemistry

TEXTBOOK

MEDICAL BIOCHEMISTRY

Edited by Iryna FOMENKO

PART I

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Medical Biochemistry. Part 1.
by Iryna Fomenko

The textbook was prepared for the students of the medical faculty, who study according to the specialty “General Medicine” in English language. The book may also be helpful for the students of Dentistry and Pharmacy faculties as well as for students of biological specialities.

The textbook was approved and recommended for the publication by the Scientific Council of the Danylo Halytsky Lviv National Medical University dated 28.062023.

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PREFACE

This book is aimed at studying the fundamentals of biochemical processes in the human body under normal conditions and in pathology. It can be used by students of medical universities and colleges, as well as students in biological specialties. However, the particular feature of this book is that it aligns with the curriculum for the study of biological chemistry in medical institutions in Ukraine. In a concise and succinct manner, I have tried to describe the main issues. To achieve this, I have analyzed a considerable amount of educational and scientific literature, with references provided at the end of each chapter. It is challenging to present biochemistry in a completely new way when the basic metabolic processes have been described multiple times by well-known authors. My goal was not to compete with them but rather to simplify the presentation of information, considering the difficulties that students face when studying the subject.

The first part of the book consists of 16 chapters. The first chapter introduces the objectives of biochemistry as a science and briefly describes its history and research methods. Chapters 2-3 focus on enzymology, providing essential information about enzymes. Chapter 4 deals with vitamins, with particular emphasis on the biological functions of water-soluble and fat-soluble vitamins and vitamin-like substances. Chapters 5-6 discuss the production and utilization of energy in various metabolic pathways of carbohydrates, lipids, and amino acids. Chapters 7-10 describe the main carbohydrate metabolism pathways under normal and pathological conditions. Chapters 11-13 cover lipid metabolism, while chapters 14-16 delve into amino acid metabolism. The continuation of the book will be available in Part 2 soon.

I sincerely hope that this book will be useful to you.

Profesor of biological chemistry department
of LNMU Iryna Fomenko

CONTENTS

1. BIOCHEMISTRY AS A SUBJECT. METHODS OF BIOCHEMICAL INVESTIGATIONS

OBJECTIVES after studying this chapter, you should be able to:

- *Know principal stages and regularities in the origin and development of biochemistry as fundamental medical and biological science and educational discipline.*
- *Appreciate the scope of biochemistry and its central role in the life sciences, and that biochemistry and medicine are intimately related disciplines.*
- *Appreciate that biochemistry integrates the knowledge of the chemical processes in living cells with strategies to maintain health, understand the disease, identify potential therapies, and enhance our understanding of the origins of life on earth.*
- *Recognize principles of methods of investigation of functional status in the human body in health and disease.*
- *Interpret data of biochemical investigations and evaluate the status of selected metabolic pathways*

1.1. Objectives and assignments of biochemistry.

Biochemistry is a scientific discipline that investigates the fundamental chemical principles underlying vital processes, focusing on the chemical structure and transformations of molecules within living organisms.

The objectives of biochemistry encompass the study of:

- The structure and functions of living organisms, including supramolecular formations.
- The mechanisms involved in the transport of plastic and biologically active substances within organisms.
- Enzymatic reactions, their mechanisms, and regulation.
- The mechanisms of energy release, accumulation, and utilization.
- The mechanisms of reproduction.

Biochemistry as a science is concerned with the chemical structure of various organisms, including humans, animals, plants, microorganisms, and viruses, and the chemical reactions that occur between their bioorganic and bioinorganic compounds.

The scope of biochemistry extends to the study of viruses, bacteria, plants, animals, and human organisms.

Biochemistry is comprised of major branches, including:

- **Static biochemistry**, which investigates the chemical structure of living organisms and the structure of biomolecules.
- **Dynamic biochemistry**, which explores biochemical reactions as the foundation for substance exchange and metabolic processes in living organisms.
- **Functional biochemistry**, which examines biochemical reactions and their roles in specific physiological functions.

Biochemistry encompasses a broad range of fields and sub-disciplines that delve into various aspects of chemical processes and molecules within living organisms. **Key fields within biochemistry include:**

- **Protein Biochemistry:** This field focuses on the structure, function, and properties of proteins, including their folding, interactions, and enzymatic activities.
- **Carbohydrate Biochemistry:** Carbohydrate biochemistry involves the study of carbohydrates, including their structures, synthesis, and metabolism. It investigates processes such as glycolysis, gluconeogenesis, and carbohydrate-protein interactions.
- **Lipid Biochemistry:** This field focuses on the structure, function, and metabolism of lipids, including fatty acids, triglycerides, phospholipids, and cholesterol. It explores their roles in cellular membranes, signaling, and energy storage.
- **Enzymology:** Enzymology is the study of enzymes, which are biological catalysts that facilitate biochemical reactions in cells. This field investigates enzyme kinetics, mechanisms of action, and regulation.
- **Metabolism:** Metabolism refers to the set of chemical reactions that occur in living organisms to maintain life. Biochemists studying metabolism examine processes such as glycolysis, the citric acid cycle, and oxidative phosphorylation.
- **Molecular Genetics:** Molecular genetics investigates the structure and function of genes at the molecular level. It encompasses the study of DNA, RNA, gene expression, and the regulation of genetic information.
- **Biophysical Chemistry:** Biophysical chemistry combines principles of physics and chemistry to study the physical properties and behaviors of biological molecules. It includes areas such as spectroscopy, thermodynamics, and kinetics applied to biological systems.
- **Bioinformatics:** Bioinformatics involves the development and application of computational tools and algorithms to analyze and interpret biological data, such as DNA sequences, protein structures, and genomic information.
- **Medical Biochemistry:** Medical biochemistry focuses on the biochemical mechanisms underlying human diseases and their diagnosis and treatment. It plays a vital role in clinical laboratories and in understanding the biochemical basis of various disorders.

These are only a few examples of the diverse fields within biochemistry. The interdisciplinary nature of biochemistry allows for collaboration and integration with other scientific disciplines, contributing to a deeper understanding of life's molecular processes.

Medical biochemistry primarily focuses on the study of the human body and its metabolic processes, both in normal and pathological conditions, as well as the impact of various damaging factors. Biochemistry and medicine have a symbiotic relationship, where the study of health and disease sheds light on new areas of biochemistry, while biochemistry contributes significantly to fields like physiology, pharmacology, toxicology, and more. This highlights the vital role played by biochemical reactions and processes in sustaining life.

Many diseases result from genetic, protein, or biochemical abnormalities, leading to disruptions in critical bodily functions. These disturbances in human biochemistry can arise due to factors such as electrolyte imbalance, hormonal imbalances, defective nutrient absorption, exposure to toxic chemicals or biological agents, and genetic disorders that affect DNA. Consequently, biochemical research continues to work alongside disciplines such as genetics, immunology, nutrition, pathology, and pharmacology to tackle these challenges.

1.2. A short history of biochemistry.

The study of biochemistry has a rich history that extends over several centuries. While the term "biochemistry" was indeed coined by **Carl Neuberg** in 1903, the roots of biochemistry can be traced back even further.

The foundations of biochemistry can be traced back to notable scientists such as **Robert Boyle and Antoine Lavoisier** in the 17th and 18th centuries. Their experiments on the chemical processes occurring in living organisms laid the groundwork for the study of the chemical basis of life. However, it was in the 19th century that significant advancements were made in this field.

A key breakthrough came in 1828 when **Friedrich Wöhler** synthesized urea from inorganic materials, challenging the prevailing belief of vitalism and demonstrating that organic compounds could be created through chemical processes. This discovery paved the way for the understanding that the chemistry of life could be studied using principles of organic and inorganic chemistry.

The late 19th and early 20th centuries saw the development of techniques such as spectroscopy and chromatography, enabling scientists to analyze the chemical composition of biological molecules. This period witnessed the isolation and characterization of important compounds like proteins, carbohydrates, and lipids.

The emergence of enzymology as a distinct field in the early 20th century, with contributions from scientists such as **Eduard Buchner and James Sumner**, further deepened our understanding of biochemical reactions and the catalytic role of enzymes.

The discovery of DNA's structure by **James Watson and Francis Crick** in 1953 opened new avenues for biochemistry. It led to the expansion of the field to include molecular genetics and the exploration of gene expression mechanisms.

Today, biochemistry is a multidisciplinary field that integrates principles from biology, chemistry, and medicine to investigate the chemical processes and molecules essential for life. It plays a critical role in understanding diseases, developing new drugs, and unraveling the complexities of biological systems.

The main discoveries in biochemistry are represented in the table 1.1.

Table 1.1. History of biochemistry

No	Period	Name of Scientist	Contribution
1	1742-1786	Karl Wilhelm Scheele	Isolated citric acid, lactic acid, malic acid
2	1743-1794	Antonie Lavoisier	“Father” of biochemistry, developed the concept of oxidation of organic meterials
3	1838	Berzelius	Suggested the name proteins
4	1822-1895	Loius Paster	10dentified organizes responsible for fermentation
5	1852-1919	Emil Fisher	Studied structure of charbohydratets, amino acids and fats
6	1852-1919	F.G. Hopkins	Concept of deficiency diseases
7	1912	K. Funk	Isolated and characterized the curative agents for scurvy, beri-beri and rickets

8	1925	G. Embden, O. Meyerhof, O. Parnas	Revealed the glyclolytic pathway
9	1937	Hans Krebs	Elucidated the citric acid cycle.
10	1944	O. Avery, C. MacLeod, and M. McCarty	Showed DNA to be the agent of genetic transformation.
11	1950	E. Chargaff	Published observation that A=T, G=C (Chargaff's rules)
12	1952	L. Pauling and R. Corey	Proposed the **α**-helix and the **β**-pleated sheet structures for proteins
13	1953	J.Watson and F.Crick	Proposed the double helix for DNA
14	1959	M. Perutz	Determined 3-D structure of hemoglobin
15	1961	F.Jacob and J. Monod	Propound the operon model
16	1965	M.Nirenberg, H. G. Khorana, and S. Ochoa	Complete the elucidation of the genetic code
17	1970	H. Temin and D. Baltimore	Discovered reverse transcriptase
18	1983	K. Mullis	Discovered polymerase chain reaction (PCR)
19	1998	R. F. Furchgott, L.J. Ignarro, F. Murad	Studied the role of nitric oxide as a signalling molecule in the cardiovascular system
20	2006	A. Z. Fire, C.C. Mello	Discovered the RNA interference - gene silencing by double-stranded RNA
21	2011	C.W. Greide, J. W. Szostak	Proved how chromosomes are protected by telomeres and the enzyme telomerase
22	2015	T. Lindahl, P. Modrich, A. Sancar	Studied mechanisms of DNA reparation

1.2.1. Contribution of scientists of biochemistry department of LNMU to development of biochemistry.

The Unit of Biological Chemistry was established in 1894 at the Medical Department of Lviv University under the leadership of Professor **V. Niemilovych**. Prof. Niemilovych, invited from Vienna in 1891, served as an associate professor in Pharmacognosy and a chemistry lecturer.

From 1904 to 1919, the unit was headed by Prof. **S. Bondzinski**, who was also the founder and first president of the Academy of the Medical Sciences of Poland. During this period, the faculty conducted research on oxyproteins, which are the products of protein metabolism found in small quantities in urine.

Prof. **W. Morachewsky** supervised the unit from 1919 to 1922. He was also appointed as the rector of the Academy of Veterinary Medicine in Lviv. Prof. Morachewsky, certified to teach medical chemistry in 1918, began his professorship at Lviv Academy of Veterinary Medicine in 1921.

From 1922 until 1941, the renowned scientist **Jakub Oscar Parnas** led the unit. His significant contributions included studying enzymatic products related to muscular activity and alcohol fermentation. His investigations on ammonium content in blood and muscular tissues, as well as his discovery of ATP and ADP as phosphorylated derivatives of adenylic acid, became classic works in biochemistry. He also pioneered the isotopic methods in biochemical research and made notable contributions to the study of anaerobic degradation of carbohydrates (glycolysis).

From 1944 to 1973, the unit was headed by Prof. **B.A. Sobchuk**, who began his scientific career under the guidance of J. Parnas. Prof. Sobchuk focused on carbohydrate metabolism in muscular tissue and yeast. His research on pyruvate's significance in glycogenolysis earned him a Doctor of Medicine degree in 1937. He later received a Doctor of Biological Sciences degree for his work on xanthopterin synthesis and its effect on experimental tumors. The department's investigations also explored carbohydrate metabolism in tumor cells and the toxicology of carbon monoxide.

From 1974 to 1995, Prof. **M.P. Shlemkevych** led the unit, collaborating closely with the oncology department to study the mechanisms of sensitivity and resistance of stomach cancer tumor cells to chemotherapeutic agents.

Prof. M.F. Tymochko became the head of the Biochemistry unit from 1995 to 1998. His research focused on adaptive-compensatory processes and changes in oxygen-dependent reactions under various experimental conditions. He investigated oxygen homeostasis in different functional states and its relevance to medical practice, including the impact of harmful environmental factors on the digestive system and the role of energy exchange in chronic liver and cardiovascular diseases. Prof. Tymochko also studied the risk level in abdominal, endocrine, and cardiovascular surgeries, as well as endogenous intoxication in oncological diseases.

From 1998 to 2021, the Biochemistry unit has been headed by Prof. **O.Ja. Sklyarov**, who initially worked in the unit of normal physiology. His scientific work concentrated on studying the regulatory mechanisms of gastric secretion under the influence of combined neurohormonal substances. Prof. Sklyarov also dedicates significant attention to educational activities. He investigated the systemic influence of malignant tumors and endogenous intoxication in patients and experimental animals. They are also studying the effects of stress on cytoprotective and ulcerogenic mechanisms of gastric mucosa, the ion-transport and metabolic processes in it and the action of exogenous factors on endoecological conditions.

Since 2021, the Department of Biochemistry has been headed by **L.I. Kobylinska**, Candidate of Medical Sciences, Doctor of Biological Sciences, and Professor.

The subject of her research is the delivery of antitumor drugs using nanoparticles and polymeric nanoscale carriers, as well as their toxicity and metabolic impact. Under the guidance of Prof. L. Kobylinska, the Biochemistry department conducts studies on systemic biochemical changes induced by the influence of Covid-19 on the human body in patients at

high risk who have the most common metabolic disorders such as type 2 diabetes, cardiovascular diseases, obesity, etc., during SARS-CoV-2 infection.

Prof. L. Kobylinska's priority is the educational process. The department focuses on medical biochemistry and the role of biochemical processes in various metabolic states of the human body to explain complex metabolic disorders associated with cardiovascular and neurodegenerative diseases, which are currently global public health problems. In addition to her scientific activities, the department head pays significant attention to scientific and educational work with students.

1.3. Chemical constitution of living organisms.

Living matter is primarily composed of six elements: **carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur.** These elements make up approximately 90% of the dry weight of the human body. In addition to these elements, cells also contain several other elements that play important functional roles. Some of these elements include calcium (Ca), potassium (K), sodium (Na), chlorine (Cl), magnesium (Mg), iron (Fe), copper (Cu), cobalt (Co), iodine (I), zinc (Zn), fluorine (F), molybdenum (Mo), and selenium (Se).

Organic compounds such as amino acids, nucleotides, and monosaccharides play a crucial role as the building blocks or monomeric units of complex biomolecules. Proteins, nucleic acids (DNA and RNA), and polysaccharides are composed of these organic compounds (as shown in table 1.2). It is important to note that lipids, although not considered biopolymers in a strict sense, commonly contain fatty acids.

Table 1.2. The characteristics of main biomolecules

The major biomolecules (macromolecules) of cell		
Biomolecule	Building blocks	Major functions
Protein	Amino acids	Fundamental basis of structure and function of cell (static and dynamic functions)
Deoxyribonucleic acid (DNA)	Deoxyribonucleotides	Resposible fot genetic information
Ribonucleic acid (RNA)	Ribonucleotides	Essentially required lor protein biosynth
Polysaccharide(glycogen)	Monosaccharides (glucose)	Storage form of energy to meet short term demand
Lipids	Fatty acids, glycerol	Storage form of energy to meet long term demand

The **macromolecules** (proteins, lipids, nucleic acids and polysaccharides) form supramolecular assemblies (e.g. membranes) which in turn organize into organelles, cells, tissues, organs and finally the whole organism.

1.4. The structural components of the prokaryotic and eukaryotic cells.

The cell is recognized as both the structural and functional unit of life and can be considered the fundamental unit of biological activity. Cells within the living kingdom can be classified into two categories:

- **Prokaryotes:** These cells lack a distinct nucleus and exhibit a relatively simple structure. Examples of prokaryotes include various types of bacteria.
- **Eukaryotes:** These cells possess a well-defined nucleus and are more complex in both their structure and function. Eukaryotic cells constitute the building blocks of higher organisms such as animals and plants.

All prokaryotic and eukaryotic cells possess plasma membranes, which are the outermost surfaces of the cells separating them from the external environment (fig. 1.1). **The plasma membrane** is primarily composed of proteins and lipids, particularly phospholipids. The lipids are arranged in a bilayer, while proteins embedded within the bilayer contribute to the membrane's functions. This dynamic nature of the membrane, with proteins floating within the lipid bilayer, gives rise to the term **"fluid mosaic structure"** (see chapter 11).

Both prokaryotic and eukaryotic cells also contain **cytoplasm**, a semi-liquid substance that fills the cell's volume. The cytoplasm is the gel-like material enclosed by the plasma membrane.

Eukaryotic cells have additional membrane-bound organelles within their cytoplasm, which serve specialized functions. One such organelle is the **endoplasmic reticulum (ER)**, consisting of a series of membranes that extend throughout the cytoplasm. The ER can be **rough or smooth**, depending on the presence or absence of ribosomes. Rough ER, with ribosomes attached to its surface, is involved in protein synthesis, while smooth ER, lacking ribosomes, participates in lipid production.

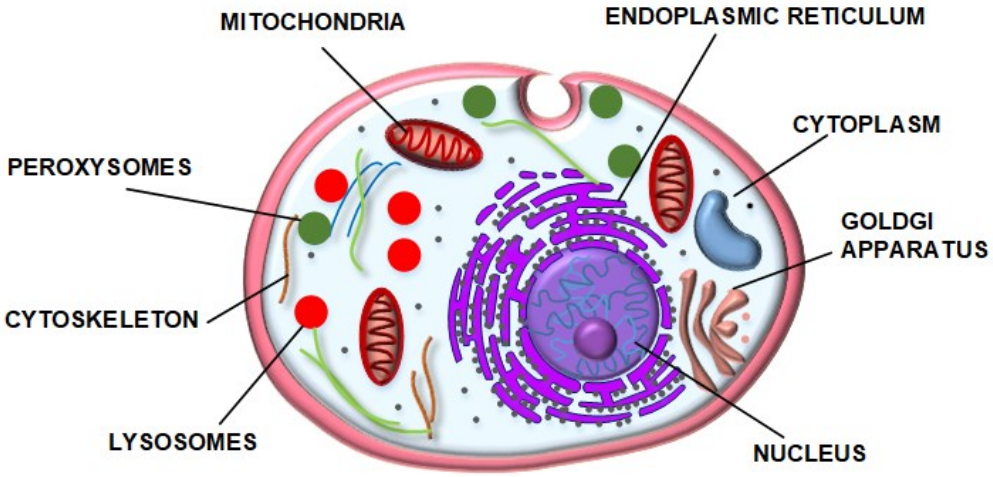

Fig.1.1. Cell organelles are specialized structures within cells that perform specific functions, contributing to the overall organization and functioning of the cell

The Golgi apparatus, another organelle in eukaryotic cells, consists of flattened sacs and plays a role in processing and packaging proteins and lipids before sending them to their final destinations. The outermost sac of the Golgi body often forms vesicles called secretory vesicles, which contain the processed substances.

Lysosomes, derived from the Golgi body, are organelles resembling droplike sacks filled with enzymes. They are responsible for intracellular digestion, breaking down food particles and recycling old cell organelles. Another cytoplasmic body called the peroxisome also contains enzymes for various metabolic processes.

Mitochondria, often referred to as the "powerhouses of the cells," are organelles involved in energy production. They release energy to generate adenosine triphosphate (ATP), which serves as a cellular energy source.

The cytoskeleton is an interconnected system of fibers, threads, and interwoven molecules that give structure to the cell. The main components of the cytoskeleton are **microtubules, microfilaments, and intermediate filaments**. All are assembled from subunits of protein.

The centriole organelle is a cylinder-like structure that occurs in pairs. Centrioles function in cell division.

Prokaryotic cells lack a nucleus. **The nucleus** of eukaryotic cells is composed primarily of protein and deoxyribonucleic acid, or DNA. The DNA is tightly wound around special proteins called histones; the mixture of DNA and histone proteins is called chromatin. The chromatin is folded even further into distinct threads called chromosomes. Functional segments of the chromosomes are referred to as genes. Approximately 21,000 genes are located in the nucleus of all human cells.

Although prokaryotic cells have no nucleus, they do have DNA. The DNA exists freely in the cytoplasm as a closed loop. It has no protein to support it and no membrane covering it. A bacterium typically has a single looped chromosome.

Many kinds of prokaryotes and eukaryotes contain a structure outside the cell membrane called the **cell wall.** With only a few exceptions, all prokaryotes have thick, rigid cell walls that give them their shape. Among the eukaryotes, some protists, and all fungi and plants have cell walls. Cell walls are not identical in these organisms, however. In fungi, the cell wall contains a polysaccharide called chitin. Plant cells, in contrast, have no chitin; their cell walls are composed exclusively of the polysaccharide cellulose.

Cell walls provide support and help cells resist mechanical pressures, but they are not solid, so materials can pass through rather easily. Cell walls are not selective devices, as plasma membranes are.

1.5. Biological fluids used in biochemical laboratories.

The clinical biochemistry laboratory utilizes various biological fluids for diagnostic investigations. The primary specimens employed in these laboratories are **blood** (including whole blood, plasma, serum, and red blood cells**), urine, and cerebrospinal fluid** (CSF). Each specimen type serves different purposes based on the parameters to be estimated.

- **Whole Blood:** Whole blood mixed with an anticoagulant is used for the estimation of several parameters, including: hemoglobin, carboxyhemoglobin, pH, glucose, urea, non-protein nitrogen, pyruvate, lactate, ammonia, etc.
- **Plasma:** Plasma is obtained by centrifuging whole blood collected with an anticoagulant. It is employed for the estimation of various parameters, such as: fibrinogen, glucose, bicarbonate, chloride, ascorbic acid, among others.
- **Serum:** Serum is the supernatant fluid obtained after centrifuging clotted blood. It is the most commonly used specimen in clinical biochemistry laboratories. Parameters estimated in serum include: proteins (albumin/globulins), creatinine, bilirubin, cholesterol, uric acid, electrolytes (sodium, potassium, chloride), enzymes (ALT, AST, LDH, CK, ALP, ACP, amylase, lipase), vitamins, and more.

- **Red Blood Cells:** Red blood cells are utilized for the determination of specific parameters, including: abnormal hemoglobins, glucose 6-phosphate dehydrogenase, pyruvate kinase, and others.

Urine is a crucial excretory fluid that contains metabolic waste products dissolved in water. It is collected as either a single specimen or a 24-hour collection for biochemical investigations. Single specimens of urine, typically collected in the morning, are useful for qualitative tests like the detection of sugar and proteins. On the other hand, 24-hour urine collections, conducted from 8 a.m. to 8 a.m., are employed for quantitative estimation of certain urinary constituents such as proteins, hormones, and metabolites.

Cerebrospinal fluid (CSF) is a fluid of the nervous system. It is produced through selective dialysis of plasma by the choroid plexuses of the brain's ventricles. CSF is collected by puncturing the interspace between the 3rd and 5th lumbar vertebrae under aseptic conditions and local anesthesia. CSF analysis can provide valuable information about various neurological conditions and infections.

1.6. Cell fractionation by differential centrifugation.

Principles of centrifugation. A centrifuge is a device for separating particles from a solution according to their **size, shape, density, viscosity, a viscosity of the medium and rotor speed.**

Centrifugation types:

- **Analytical Centrifugation.** Measure the shape or mass of supermolecular molecules.
- **Preparative Centrifugation.** Separation of cell subcellular structure, membrane vesicles.
- **Differential centrifugation** is based on the differences in the sedimentation rate of the biological particles of different size, shape, and density.

Differential centrifugation is a technique used to separate and isolate cellular components based on their size, shape, and density using centrifugal force. It is a widely employed method in cell biology, biochemistry, and molecular biology to obtain purified subcellular fractions or isolate specific organelles.

The principle behind differential centrifugation is that when a heterogeneous mixture containing cells or cellular components is subjected to centrifugal force, the components will sediment at different rates based on their mass and density. By adjusting the speed and duration of centrifugation, it is possible to sequentially separate different cellular components based on their sedimentation properties (fig. 1.2).

For the separation of cellular organnels the following steps are recommended:

- **Homogenization:** The starting material, such as a tissue sample or cell culture, is first homogenized to disrupt cells and release their contents. This can be done using a variety of methods, including mechanical disruption, sonication, or enzymatic digestion.
- **Centrifugation at low speed:** The homogenized mixture is subjected to low-speed centrifugation (typically around 500-2,000 × g) to remove large debris, unbroken cells, and nuclei. This step is called the "pelleting" step, where heavier components sediment to the bottom of the centrifuge tube, forming a pellet, while lighter components remain in the supernatant.

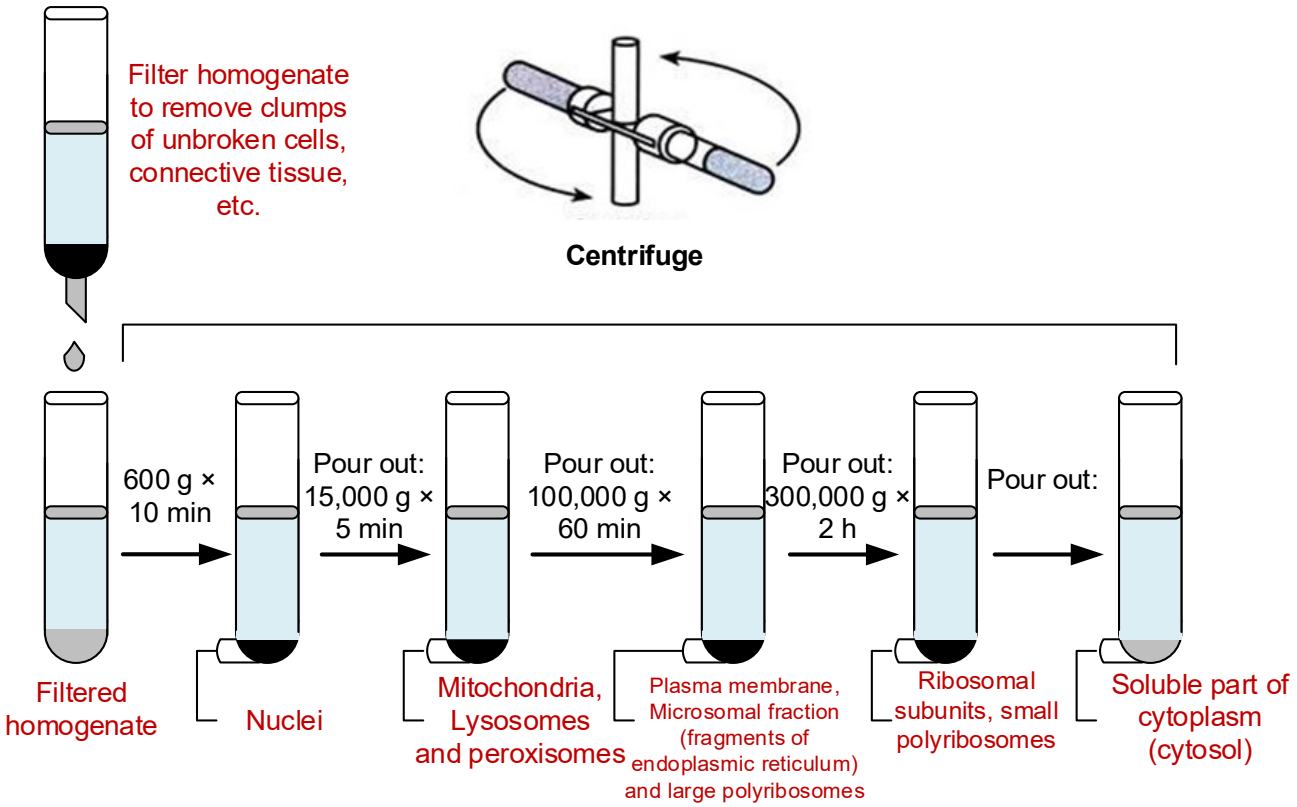

Fig. 1.2. Differential centrifugation is a versatile and widely used technique for fractionating and isolating cellular components based on their sedimentation properties, allowing researchers to study the structure and function of organelles and analyze their biochemical composition.

- **Supernatant collection:** The supernatant, containing the desired cellular fraction, is carefully collected and transferred to a new tube for further processing.
- **Centrifugation at higher speeds:** The collected supernatant is subjected to subsequent rounds of centrifugation at progressively higher speeds to isolate specific organelles or cellular components. Each centrifugation step is performed to pellet the desired fraction while leaving unwanted components in the supernatant.
- **Fraction collection:** After each centrifugation step, the pellet containing the desired fraction is carefully collected, while the supernatant is discarded or used for subsequent fractionation steps. This process can be repeated multiple times with increasing centrifugal forces to obtain highly purified fractions.

The choice of centrifugation speed, time, and temperature depends on the specific cellular components being targeted for isolation. Differential centrifugation can be combined with other techniques, such as density gradient centrifugation, to further purify and separate components based on their density.

1.7. Fractionation of material using differences in solubility.

Solubility of different classes bioorganic moleculs differs very much, for example proteins are water soluble, whereas lipids are not. Diiferent solubility of molecules is widely used for their separation.

Proteins exist in colloidal solution due to hydration of polar groups (-COO^-, -NH_3^+, -OH). They can be precipitated by dehydration or neutralization of polar groups.

Several methods are in use to achieve **protein precipitation**:

1**. Precipitation by neutral salts**: The process of protein precipitation by the addition of neutral salts such as ammonium sulfate or sodium sulfate is referred to as **salting out**. This phenomenon is explained on the basis of dehydration of protein molecules by salts. This causes increased proteinprotein interaction, resulting in molecular aggregation and precipitation.

2. **Precipitation by salts of heavy metals**: Heavy metal ions f ike Pb^{2+}, Hg^{2+}, Fe^{2+}, Zn^{2+}, Cd^{2+} cause precipitation of proteins. These metals being positively charged, when added to protein solution (negatively charged) in alkaline medium result in precipitate formation.

3. **Precipitation by anionic or alkaloid reagents**: Proteins can be precipitated by trichloroacetic acid, sulphosalicylic acid, phosphotungstic acid, picric acid, tannic acid, phosphomolybdic acid etc.

4**. Precipitation br organic solvents:** Organic solvents such as alcohol are good protein precipitating agents. They dehydrate the protein molecule by removing that water envelope and cause precipitation.

1.8. Optical methods in biochemical investigations.

Optical methods allow determining **absorbance (A) or optical density (OD)** of solutions, which are very commonly used in laboratories.

Photometry is the most common analytical technique used in clinical biochemistry. The principle of photometry: when light at a particular wavelength is passed through a solution (incident light), some amount of it is absorbed and, therefore, the light that comes out (transmitted light) is diminished. The nature of light absorption in a solution is governed by **Lambert-Beer law**.

Photometric principles are applied in several kinds of analytical measurements.

1. Measurement of absorbed or transmitted light: **colorimetry, spectrophotometry, atomic absorption, turbidometry.**

2. Measurement of emitted light: **flame emission photometry, fluorometry**.

Colorimetry is used for the measurement of colored substances. This technic is operative in the **visible range (400-800 nm)** of the electromagnetic spectrum of light.

The setup of a colorimeter typically includes a light source, a filter or monochromator, a sample holder, and a detector with a display (fig. 1.2).

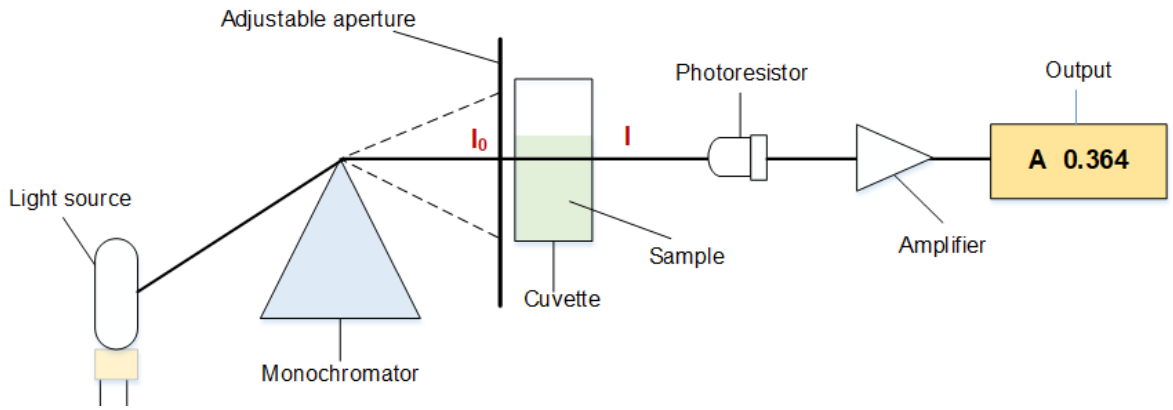

Fig. 1.2. The general scheme of photocolorymetry

A filament lamp is commonly used as the light source in colorimeters. It emits broad-spectrum white light that covers a wide range of wavelengths within the visible spectrum. Filters or monochromators are employed in colorimeters to select a specific range of wavelengths for measurement. These devices allow only a narrow band of wavelengths to pass through, ensuring that the incident light is of a specific wavelength or range of wavelengths. The sample holder, typically a glass cuvette with a fixed thickness, holds the sample to be measured. It ensures that the light passes through a consistent path length, which is important for accurate colorimetric measurements.

As for the detector, colorimeters often use photoelectric selenium cells or other photodetectors. These detectors convert the incident light into an electrical signal proportional to its intensity. The electrical signal is then processed and displayed on a meter or a digital display, allowing the user to read the colorimetric measurement.

Spectrophotometry primarily differs from colorimetry by covering **the ultraviolet region (200-400 nm)** of the electromagnetic spectrum. Further, the spectrophotometer is more sophisticated with several additional devices that ultimately increase the sensitivity of its operation severalfold when compared to a colorimeter. A precisely selected wavelength (say 234 nm or 610 nm) in both ultraviolet and visible range can be used for measurements. In place of glass cuvettes (in colorimeter), quartz cells are used in a spectrophotometer.

Fluorometry. When certain compounds are subjected to the light of a particular wavelength, some of the molecules get excited. These molecules, while they return to the ground state, emit light in the form of fluorescence which is proportional to the concentration of the compound. This is the principle in the operation of the instrument fluorometer.

1.9. Electrophoresis

Electrophoresis is a method whereby charged molecules in solution, chiefly proteins, and nucleic acids, migrate in response to an electrical field. Their rate of migration through the electrical field, depends on the strength of the field, on the **net charge, size, and shape** of the molecules, and also on the ionic strength, viscosity, and temperature of the medium in which the molecules are moving.

Zone electrophoresis: A simple and modified method of moving boundary electrophoresis is the zone electrophoresis. Inert supporting material such as paper or gel is used.

Isoelectric focusing. This technique is primarily based on the immobilization of the molecules at isoelectric pH during electrophoresis.

Electrophoresis is usually done with gels (**gel electrophoresis**) formed in tubes, slabs, or on a flatbed. The gels commonly used in gel electrophoresis are **agarose** and **polyacrylamide, sodium dodecyl sulfate (SDS).** Biological molecules, like amino acids, peptides, proteins, nucleic acids, and nucleotides, possess ionizable groups. These molecules exist in solution as electrically charged species, cations (+), or anions (-) at any given pH. Thus, the electric field allows the migration of the negatively charged molecule towards the anode (a positive terminal). In contrast, the positively charged molecule migrates towards the cathode (a negative terminal) (fig. 1.3).

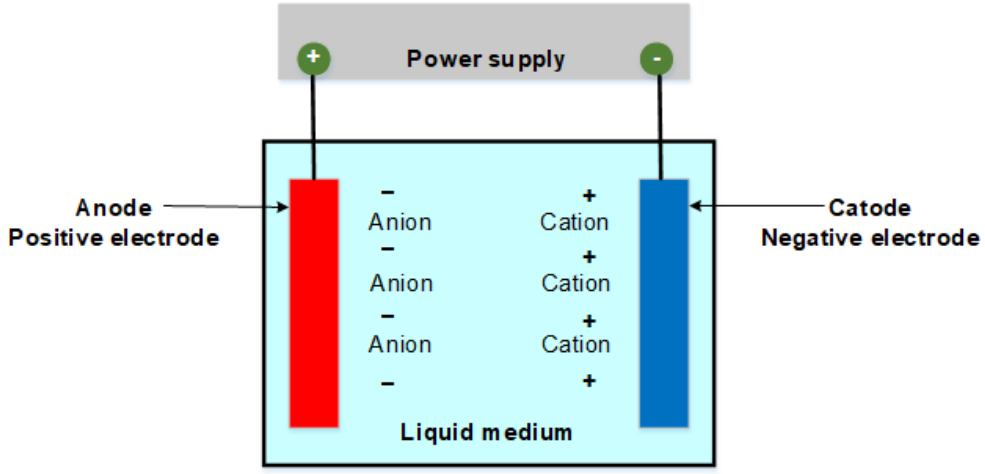

Fig. 1.3. General principle of gel lectrophoresis

Proteins are amphoteric compounds, that is, they contain both acidic and basic residues. Each protein has its characteristic charge properties depending on the number and kinds of amino acids carrying amino or carboxyl groups. Nucleic acids, unlike proteins, are not amphoteric. They remain negative at any pH used for electrophoresis.

Blotting is used to transfer proteins or nucleic acids from a slab gel to a membrane such as nitrocellulose, nylon, or CM paper.

Blotting types:

1. **Southern blot** is a method used for the detection of a specific DNA sequence in DNA samples. It was is named after the British biologist Edwin Southern, who first published it in 1975. Southern blot has the following stages:
   - DNA sample is enzymatically cleaved into smaller pieces, which are separated on a gel by electrophoresis, and then transferred to a filter.
   - Filter is exposed to radiolabeled DNA probe that recognizes and anneals to its complementary strand.
   - Resulting double-stranded, labeled piece of DNA is visualized when filter is exposed to film.
2. **Nothern blot** is a technique to study gene expression by detection of RNA (or isolated mRNA) in a sample. Similar to Southern blot, except that an RNA sample is electrophoresed.
3. **Western blot** (sometimes called the **protein immunoblot**) is a method to detect specific proteins in a sample of tissue homogenate or extract. Sample protein is separated via gel electrophoresis and transferred to a membrane. Labeled antibody is used to bind to relevant protein.
4. **Southwestern blot** is a technique which involves identifying and characterizing DNA-binding proteins (proteins that bind to DNA)[by their ability to bind to specific oligonucleotide probes. The proteins are separated by gel electrophoresis and are

subsequently transferred to nitrocellulose membranes, similar to other types of blotting.

1.10. Chromatography

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases one of which is stationary (**stationary phase**) while the other (the **mobile phase**) moves through it in a definite direction. Is a technique used to separate and identify the components of a mixture.

Classification of chromatography according to **mobile phase:**

1. Liquid chromatography: mobile phase is a liquid.

2. Gas chromatography: mobile phase is a gas.

Classification according to **the packing of the stationary phase:**

1. **Thin layer chromatography (TLC):** the stationary phase is a thin layer supported on glass, plastic or aluminium plates. TLC is a method for identifying substances and testing the purity of compounds. TLC is a useful technique because it is relatively quick and requires small quantities of material. Separations in TLC involve distributing a mixture of two or more substances between a stationary phase and a mobile phase.
2. **Paper chromatography (PC):** the stationary phase is a thin film of liquid supported on an inert support. A method of partition chromatography using filter paper strips as carrier or inert support. The factor governing separation of mixtures of solutes on filter paper is the partition between two immiscible phases. One is usually water adsorbed on cellulose fibres in the paper (stationary phase). The second is the organic solvent flows past the sample on the paper (stationary phase).
3. **Column chromatography (CC):** stationary phase is packed in a glass column. This includes chromatographic methods in which: the stationary phase is packed into a column, The mobile phase is a moving liquid or gas. According to the mechanism of separation of solutes, five major types of CC are ditinguished. Usually, one mechanism predominates but does not exclude the others

Classification according to **the force of separation:**

- **Adsorption chromatography:** The adsorbents such as silica gel, alumina, charcoal powder and calcium hydroxyapatite are packed into a column in a glass tube. This serves as the stationary phase. The sample mixture in a solvent is loaded on this column. The individual components get differentially adsorbed on to the adsorbent. The elution is carried out by a buffer system (mobile phase). The individual compounds come out of the column at different rates which may be separately collected and identifie.
- **Partition chromatography:** The molecules of a mixure get partitioned between the stationary phase and mobile phase depending on their relative affinity to each one of the phases.

- **Ion exchange chromatography:** Involves the separation of molecules on the basis of their electrical charges. lon-exchange resins-cation exchangers and anion exchangers-are used for this purpose. An anion exchanger exchanges its anion with another anion in solution.
- **Gel filtration chromatography:** The separation of molecules is based on their size, shape and molecular weight. This technique is also referred to as molecular sieve or molecular exclusion chromatography. The apparatus consists of a column packed with spongelike gel beads (usual ly cross-l inked polysaccharides) contai n i ng pores. The gels serve as molecular sieves, for the separation of smaller and bigger molecule.
- **Affinity chromatography:** The principle of affinity chromatography is based on the property of specific and non-covalent binding of proteins to other molecules, referred to as ligands, for instance, enzymes bind specifically to ligands such as substrates or cofactors.

1.11. Radioisotopic methods

Radioimmunoassay (RIA) is a technique that combines radioactivity and immunological reactions to measure the concentration of specific substances in a sample. It involves the use of a radioactive (labelled) antigen and an antibody that specifically binds to the antigen of interest.

The principle of RIA is based on competition between the labelled antigen and the unlabelled antigen present in the sample. The unlabelled antigen is the substance to be determined, such as insulin. Antibodies against the antigen are produced by injecting the antigen into an animal, typically a goat or a rabbit.

In the RIA procedure, the specific antibody is mixed with the sample containing the unlabelled antigen. At the same time, a known amount of the labelled antigen (which has a known radioactivity) is also added to the mixture. The labelled and unlabelled antigens compete for binding to the antibody. The more unlabelled antigen present in the sample, the less labelled antigen will bind to the antibody.

After allowing sufficient time for the reaction to occur, the mixture is separated into bound and unbound fractions. The bound fraction contains the antigen-antibody complexes, while the unbound fraction contains the free (unbound) labelled antigen.

The radioactivity of either the bound or unbound fraction is then measured using a radiation detector. The amount of radioactivity detected is inversely proportional to the concentration of unlabelled antigen in the sample. By comparing the measured radioactivity to a standard curve generated using known concentrations of the antigen, the concentration of the antigen in the sample can be determined.

RIA was initially developed for the estimation of hormones and proteins that exhibit antigenic properties. However, it has been expanded to include a wide range of substances by making them antigenic using haptens (small molecules that are not antigenic by themselves). This has allowed RIA to be used for the estimation of peptides, steroid hormones, vitamins, drugs, antibiotics, nucleic acids, structural proteins, and hormone receptor proteins.

The applications of RIA are extensive. It is widely used in the diagnosis of hormonal disorders, such as thyroid or adrenal gland dysfunction, as well as in the detection and monitoring of certain cancers. RIA is also valuable in therapeutic drug monitoring, where it helps to determine the concentration of drugs in a patient's blood to ensure proper dosing. Additionally, RIA finds utility in biomedical research for studying the levels of various substances in biological samples.

1.12. Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) is a non-isotopic immunoassay. An enzyme is used as a label in ELISA in place of radioactive isotope employed in RlA. ELISA is as sensitive as or even more sensitive than RlA. ln addition, there is no risk of radiation hazards (as is the case with RIA) in ELISA. ELISA is based on the immunochemical principles of antigen-antibody reaction.

Sandwich ELISA is used when we are looking for antigens, in this case, a primary, the unlabeled antibody is bound to the wells, if there are antigens they will bind to these antibodies, the second set of labeled antibodies is then introduced. These antibodies bind to the antigen bound to the primary antibody. Again a substrate for the enzyme is added and this produces an optically measurable signal.

ELISA is widely used for the determination of small quantities of proteins (hormones, antigens, antibodies) and other biological substances. The most commonly used pregnancy test for the detection of human chorionic gonadotropin in urine is based on ELISA. By this test, pregnancy can be detected within a few days after conception. ELISA is also been used for the diagnosis of AIDS and COVID-19.

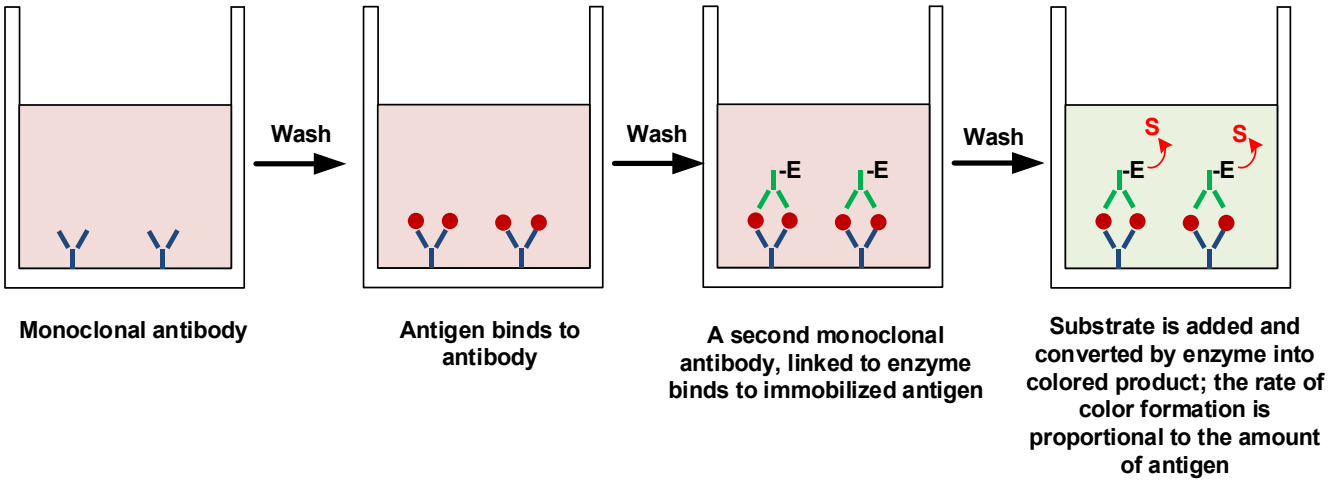

Fig. 1.4. The stages of a sandwich ELISA

1.13. Methods of targeted drug delivery using nanoparticles

Nanotechnology is the utilization of nano-sized structures and devices to track, correct, design, and control biological systems in humans at the molecular level. A nanoparticle is an isolated solid object with a clearly defined boundary from the surrounding environment, ranging in size from 1 to 100 nm.

The idea of using nanoparticles to enhance the effectiveness of pharmacological diagnostics and therapies is based on the fact that substances in nanoform have properties distinct from those in macrodispersed form. Specifically, the high specific surface area of nanomaterials leads to surface phenomena (adsorption-desorption, adhesion) dominating their interactions with macromolecules and biological objects. As a result, even low concentrations of nanoparticles, which do not have significant toxic effects, can have a substantial impact on living organisms.

Some nanostructures, both of biogenic (viral particles, capsids) and non-biogenic origin, have a container-like shape, which enables their use as delivery vehicles for therapeutic or diagnostic components (including other nanoparticles) to target cells or organs. The specificity of delivering nanosystems to target cells is determined by the use of specific antibodies, receptors, or ligands. Nanomedicine has the potential to make significant advancements in disease treatment.

Based on their chemical origin, nanoparticles can be classified as follows:

- **Inorganic:** ceramics, metals (Fe, Mg, Zn, Ti, Cu, Ag), alloys (Cu-Ta, Cu-V, Cu-W). Metallic nanoparticles hold a special place among nanomaterials, such as gold, silver, copper, as well as magnetic materials like iron, nickel, cobalt, and their alloys.
- Organic: polymers (chitosan), biological nanostructures (vesicles), carbon nanomaterials (fullerenes, nanotubes, nanofibers, nanosprings).
- **Inorganic-organic:** metal-organic (PbS, CdS, ZnS), metal-polymer nanostructures.

Nanoparticles are also classified based on the substance, cluster form, and type of bond. This classification includes the following groups of nanoparticles: liposomes - small unilamellar vesicles, large unilamellar vesicles, multilamellar vesicles, structural components of cells; nanoemulsions; polymeric nanoparticles - nanospheres, nanocapsules, dendrimers, polymer-protein conjugates; ceramic nanoparticles - silicon compounds; metallic nanoparticles - iron, magnesium, copper, titanium, zinc, silver; nano-shells - gold; carbon nanoparticles - fullerenes, nanotubes, nanodiamonds, nanofibers, nanosprings; quantum dots - CdSe, ZnS; nanocapsules.

Carbon nanotubes are artificially created structures that consist of atoms arranged in the form of hollow tubes with a length of up to 100 nm and a diameter of 1-2 nm. Carbon nanotubes provide space for the accommodation of other substances, such as drugs, and their open ends can serve as gateways for the entry and exit of other medications. Due to this property, they can serve as ideal drug carriers. Carbon nanotubes can easily penetrate into the vascular bed through the respiratory organs, which is utilized in the treatment of cardiac rhythm disorders and vascular diseases.

Nanotubes are particularly valuable as carriers of DNA and RNA - molecular biosensors. These biosensors enable rapid diagnosis of genetic diseases, early-stage cancer, autoimmune diseases, and more. Due to their strength, nanotubes can replace microcapillaries, and it is promising to create a combination of nanotubes with various polymers that exhibit properties similar to soft human tissues, allowing tissue transplantation without the risk of rejection.

1.14. Errors that occur during laboratory research.

Mistakes can happen during laboratory research. The final result of each determination includes both the true value (actual quantity) and certain errors. Assessing the reliability of the result and its clinical evaluation require knowledge of the types of errors. In general, the following types of errors can occur during diagnostic research:

Pre-analytical error: This group of factors can affect the final research result until the material is analyzed in the laboratory. These factors are related to the preparation of the patient for the research, the collection and storage of the material prior to analysis.

The influence of medications taken should always be taken into account when interpreting the result. When addressing the impact of drugs on the research result, it is necessary to first determine the existence of two main mechanisms of interference.

The first mechanism involves the interference of drugs or their products with identifiers or methods of determination. This includes physical, chemical, and biochemical effects. Examples of physical-chemical interaction include the possibility of changing the relative density of urine when using a larger amount of dextran or the effect of tetracycline on the measurement of glucose concentration. An example of biochemical influence could be the effect of drugs with reducing properties on the elevated results of creatinine determination. These influences, namely physical-chemical and biochemical interference, are mainly caused by a lack of specific methodologies.

The second mechanism is based on the pharmacological action of drugs, regardless of the planned treatment, on components or processes of the system that lead to changes unrelated to the disease.

Interference caused by the pharmacological action of drugs is of great importance and should not be overlooked. The number of medications used, individual sensitivity of the organism, and the method of their administration also play a significant role.

Excluding errors related to the influence of drugs requires conducting research before treatment or during treatment with the selection of the best method of material collection. It is believed that the interference of drugs will be least pronounced at the lowest concentrations in the body.

Regardless of whether there are limitations on the influence of drugs, this factor should be taken into account when interpreting the result, especially in cases of unexpected outcomes.

Analytical (laboratory) error: This error is associated with the process of analyzing biological material in the laboratory. Systematic error (accuracy) reproduces the difference between the obtained and true values. The properties of the method or the ways of its implementation that cause the determined value to deviate from the true value are the source of systematic error, which can be attributed to the method or the laboratory itself (the researcher).

An example of a method's systematic error could be the determination of glucose concentration using the Hagedorn-Jensen method, which is based on the reducing properties of glucose. Other substances in the blood with reducing properties also participate in the reaction. Therefore, the result of glucose determination by this method is overestimated.

Systematic error in the laboratory refers to the constant inaccuracy identified during the research process. It can relate to every stage of the process (production of identifiers, preparation and determination of the standard) or be associated with laboratory equipment.

Random error (reproducibility) is ensured by the difference between the results of measuring the same sample with the same laboratory equipment and the same method, caused by changes in conditions during measurements. The source of random error is associated with the interaction of many unpredictable factors and those cases that change the conditions of determination. It is this variation that results in differences between the results of multiple determinations of the same sample in the same material.

The measure of random error is precision (accuracy). Often its deviation is considered a standard or determined as a coefficient of variation.

REVIEW TEST:

№	MCQs	Answers and explanations
1.	**A patient has been hospitalized with a provisional diagnosis of virus B hepatitis. Serological reaction based on the complementation of antigen with antibody chemically bound to peroxidase or alkaline phosphatase has been used for disease diagnostics. What is the name of the applied serological reaction?** A. Enzyme-linked immunosorbent assay B. Radioimmuno assay technique C. Immunofluorescence test D. Complementfixation test E. Immobilizationtest	**The answer is A.** Enzyme-linked immunosorbent assay (El.lSA) is a non-isotopic immunoassay, based on the immunochemical principles of antigen-antibody reaction. The biological sample containing the protein (hepatitis B virus surface antigen (HBsAg)) to be estimated is applied to the antibody-coated surface. The protein-antibody complex is then reacted with a second protein specific antibody to which an enzyme is covalently linked. Peroxidase and alkaline phosphatase are commonly used. After washing the unbound antibody linked enzyme, the enzyme bound to the second antibody complex is assayed. The enzyme activity is determined by its action on a substrate to form a product (usually colored). This is related to the concentration of the protein being estimated.
2.	**In the investigation of serum proteins various physical and physicochemical methods can be used. In particular, serum albumins and globulins can be separated by the method of:** A. Dialysis B. Polarography C. Electrophoresis D. Spectrography E. Refractometry	**The answer is C.** Electrophoresis is a method whereby charged proteins in the solution migrate in response to an electrical field. Their rate of migration through the electrical field, depends on the strength of the field, on the net charge, size, and shape of the proteins, and also on the ionic strength, viscosity, and temperature of the medium in which the molecules are moving. Serum proteins (albumins and globulins) have different phisico-chemical properties (a net charge, size, and shape) that is why they can be separated by electrophoresis method.
3.	**Labeled amino acids alanine and tryptophane were injected to a mouse to study the localization of protein synthesis in its cells. The labeled amino acids will be accumulated near the following organelles:** A. Golgi apparatus B. Smooth endoplasmic reticulum C. Cell centre	**The answer is E.** Protein is assembled inside cells by an organelle called a ribosome. Ribosomes are found in every major cell type and are the site of protein synthesis.

	D. Lysosomes E. Ribosomes	
4.	**In course of practical training students studied a stained blood smear of a mouse with bacteria phagocyted by leukocytes. What cell organelles do complete digestion of these bacteria?** A. Lisosomes B. Mytochondrion C. Granular endoplasmic reticulum D. Golgi apparatus E. Ribosomes	**The answer is A.** A lysosome is a membrane-bound organelle found in nearly all animal cells. They are spherical vesicles that contain hydrolytic enzymes that can break down (digest) many kinds of biomolecules including components of bacterial cells.
5.	**Healthy parents have got a fair-haired, blue-eyed girl. Irritability, anxiety, sleep and feeding disturbance developed in the first months of the infant's life. Neurological examination revealed a developmental lag. What method of genetic investigation should be used for the exact diagnosis?** A. Cytological B. Biochemical C. Gemellary D. Genealogical E. Population-statistical	**The answer is B.** Biochemical analisis in performed with blood plasma or other biological fluids (blood, urine, ascitic fluids, CSF) to detect a wide variety of substances—substrates, enzymes, hormones, etc—and their use in diagnosis and monitoring of disease. One test is very seldom specific to one clinical condition, and basic checklists of factors affecting the most commonly requested analytes are given below. Thus, rather than six tests that merely confirm or deny six possibilities, a well-chosen group of six tests can provide information pointing to a wide variety of different conditions by a process of pattern recognition.
6.	**Protein preparations from human blood plasma are frequently used in clinical medicine for the treatment of many diseases. Fractionation of blood plasma and preparation of distinct protein fractions is achieved by the next method:** A. Fractional precipitation with ethanol by Cohn VI method B. Precipitation with salts of heavy metals C. Fractional precipitation with ammonium sulfate D. Electrophoresis in agarose gel E. Ultracentrifugation	**The answer is C.** Ammonium sulfate is highly hydrated. Concentrated ammonium sulfate solution reduces the available water vary considerably. This allows separating proteins from a mixture based on their relative hydrophilicity by gradually increasing the concentration of ammonium sulfate. The two main fractions of proteins in serum are globulin (and albumin. Globulin is insoluble in distilled water and slightly soluble in dilute salt solutions and can be precipitated using 50%-saturated ammonium sulfate. Albumin is readily soluble in water and dilutes salt solutions. Albumin precipitates first when using saturated ammonium sulfate concentrations. The difference in solubility allows separating these proteins from each other, using salting-out.
7.	**Determination of proportion between protein fractions in blood plasma or serum has an important clinical and diagnostic significance. The following routine method for obtaining results of this sort is most frequently used in clinical laboratories:** A. Absorption chromatography B. Precipitation with strong acids C. Electrophoresis in agar gel or on acetyl-cellulose films	**The answer is D.** Proteins exist in colloidal solution due to hydration of polar groups ($-COO^-$, $-NH_3^+$, -OH). They can be precipitated by dehydration or neutralization of polar groups. Precipitation by neutral salts is the process of protein precipitation by the addition of neutral salts such as ammonium sulfate or sodium sulfate is referred to as salting out. This phenomenon is explained on the basis of dehydration of protein molecules by salts. This causes increased protein interaction, resulting in molecular aggregation and precipitation. Identification of certain

	D. Salting out with neutral salts E. Immunoprecipitation	fractions of serum proteins is important for clinical diagnosis. Normally blood albumin content is 40 - 45 g / l, globulin - 20 - 30 g / l. In various diseases of the liver (cirrhosis, hepatitis), in nephrosis, chronic diseases of the stomach, digestive tract tumors of blood, albumin concentration decreases. In acute infectious diseases, rheumatism concentration of α_2-globulins is increased. The concentration of β-globulins increases in hepatitis, multiple myeloma, and γ –globulins in chronic diseases, chronic polyarthritis.
8.	**For determination of DNA synthesis in the cell usually is used the measurement of the incorporation of H3-thymidine into cellular biopolymers. The next type of analysis is used in this specific case:** A. Radioisotope method B. Polymerase chain reaction (PCR) C. Electrophoresis D. Radioimmunoassay E. Affinity chromatography	**The answer is A.** Isotopes of a given element have nuclei with the same number of protons but different numbers of neutrons. Radiolabeled nucleotides are commonly used for detection of specific nucleic acid sequences. They are typically incorporated enzymatically into DNA and RNA sequences for detection and analysis. Labeled nucleotides may be incorporated by a variety of methods including *in vitro* transcription with SP6, T3 or T7 RNA polymerase, 3' end labeling with terminal deoxynucleotidyl transferase (TdT),
9.	**A patient has a disease that leads to the hyperexcretion of a protein in the urine. Which methodology is the easiest, and least expensive to determine the molecular weight of the native protein the urine?** A. Ion exchange chromatography B. Size exclusion chromatography C. X-ray crystallography D. NMR E. SDS-PAGE	**The answer is B.** Size exclusion (gel) chromatography separates proteins by mass. Ion exchange chromatography involves either anion or cation exchange for separating proteins with a netpositive or negative charge. No information concerning the mass of a protein results from ion exchange chromatography. NMR spectra and X-ray crystallographic diffraction techniques require laborious effort and are inexpensive. SDS-PAGE does not yield the mass of a native protein because the proteins are denatured before being separated by size.
10	**Which is the best technique to separate oxygenated normal hemoglobin A (HbA) from oxygenated sickle cell hemoglobin (HbS), assuming no protein aggregation?** A. Native gel electrophoresis B. SDS-PAGE C. Gel filtration D. Affinity chromatography with a C-terminal antibody E. Ultracentrifugation	**The answer is A** Because the difference between HbA and HbS is the sixth amino acid of the Hb β-chain, the difference in mass is very small, and separation based on charge will be able to differentiate between the two forms of hemoglobin. Native gel electrophoresis can accomplish this. SDS-PAGE blankets the protein with a uniform negative charge that will mask the inherent difference. Gel filtration and ultracentrifugation are not sensitive enough, and a C-terminal antibody will detect both forms because the difference between the two forms is manifested in the amino-terminal end of the protein.

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2. ENZYMES: MECHANISM OF ACTION

OBJECTIVES after studying this chapter, you should be able to:

- *Realize the role of enzymes for a human body.*
- *Interpret biochemical principles of structure and functioning of different classes of enzymes.*
- *Appreciate and describe the structural properties of enzymes, their active and allosteric sites.*
- *On the basis of physical and chemical properties of enzymes as proteins to explain the dependence of enzymatic activity from pH of medium, temperature and other factors.*
- *Analyze values of the activity of enzymes in blood plasma in dependence from their localization in the cell, tissue or organ.*
- *Realise methods of determination of enzymatic activity for an optimal application in clinical practice.*

2.1. Enzymes: definition, properties, differences between enzymes and inorganic catalysts.

Enzymes are higly efficient organic biocatalyst that regulate the rates of chemical reactions in living organisms without itself being altered in the catalitical process. Each cell in the human body contains numerous (around thousand) different enzymes. Enzymes speed up cellular reactions to make tham million times faster than corresponding uncatalized reactions. Thus enzymes direct all metabolic events. The substance transformed by an enzyme (E) is called **a substrate** (S). The substance formed by an enzyme is a **product** (P) (fig. 2.1).

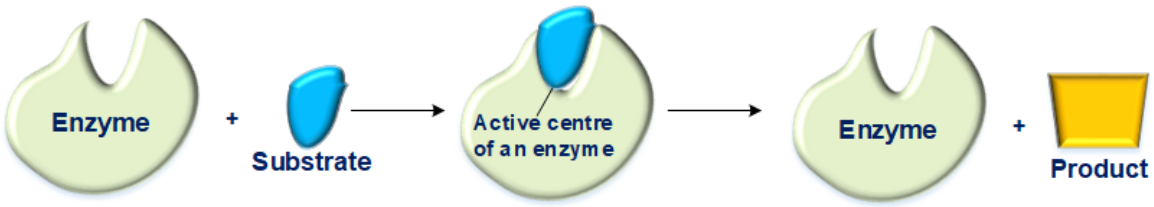

Fig. 2.1. A simplified representation of an enzyme-catalyzed reaction. The enzyme interacts with its substrate to form an enzyme-substrate complex. This interaction is facilitated by the complementary shape of the enzyme's active site and the substrate. Once the substrate is bound to the enzyme, the enzyme can catalyze the conversion of the substrate into a product. Finally, the enzyme releases the product and returns to its original state, ready to catalyze another reaction.

Enzymes are indeed **biocatalysts** that **increase the rates of chemical reactions.** While they share some properties with inorganic catalysts, such as the ability to increase reaction rates and lower activation energy, enzymes have several unique characteristics that distinguish them from inorganic catalysts. One key difference is that most of enzymes are proteins, while most inorganic catalysts are not. Being proteins, enzymes are composed of amino acids that are folded into specific three-dimensional shapes, which determine their catalytic activity. This means that enzymes have a specific active site that is complementary in shape to the reactant molecules they catalyze, allowing them to selectively catalyze specific reactions. Enzymes also have the ability to be regulated, either

by activators or inhibitors, allowing them to be turned on or off as needed. Furthermore, enzymes can be highly specific and efficient in catalyzing reactions, often surpassing inorganic catalysts in both selectivity and reaction rates.

While enzymes and inorganic catalysts share some common properties, the fact that enzymes are proteins provides them with unique properties and characteristics that make them essential for life processes (table 2.1).

Table 2.1. **Common and different properties of enzymes and inorganic catalysts**

Common properties of enzymes and inorganic catalysts:	**Difference between enzymes and inorganic catalysts:**
• Both enzymes and inorganic catalysts enhance only energy grounded reactions; • they never change the direction of the reaction; • they do not change the status of equilibrium in a reverse reaction, they only enhance its establishment; • they are not involved as components or parts in the reaction end products, they release unchanged; • they are not consumed in the overall reaction	• The efficiency of enzymes is higher than that of non-protein catalysts, they increase rate of reaction to 10^3 to 10^8 folds (for example, *carbonic anhydrase* can hydrate to 10^6 molecules of CO_2 per second, in the absence of enzyme hydration of CO_2 is 10^{-1} per second); • enzymes possess a high specificity action; • the ability of enzyme activity to be regulated; • enzymes catalyze chemical processes in "mild" conditions (the temperature is not high (about +37 - +40°C), pH of the medium 6-8, and normal pressure); • during enzymatic reactions in contrast to non-enzymatic ones, only insignificant adverse effects have been noticed; • the enzymes are big particles, their molecular weight ranges from few thousands to millions.

MEDICAL IMPORTANCE

1. *Enzymes play a crucial role in regulating various physiological processes in the body. Any genetic defects or mutations in enzymes can lead to a range of diseases.*
2. *In case of cell injury, enzymes are released into the bloodstream, making the determination of enzymatic activity an important diagnostic tool in medicine.*
3. *Enzymes can be used as medications or as targets for pharmacotherapeutic agents, owing to their ability to catalyze specific chemical reactions.*
4. *Immobilized enzymes, attached to inert insoluble materials, are widely used in clinical laboratories and industrial processes.*
5. *Enzymes can also be used as biosensors, where they react with the target substrate, generating a chemical signal that is transferred to a transducer. The transducer then produces a physical signal, which is amplified by an electronic amplifier, resulting in an amplified signal.*

2.2. Nomenclature and classification of enzymes

The suffix **-ase** is commonly used to identify a substance as an enzyme, such as *lipase* and *peptidase*, although the suffix **-in** is still used in some names like *pepsin* and *trypsin* for digestive enzymes.

Enzymes can be named in several ways:

- **Trivial nomenclature** involves attaching the **-ase** suffix to the name of the substrate, resulting in names like *maltase* for the enzyme that breaks down maltose and *lactase* for the enzyme that breaks down lactose.

- Enzymes can also be named based on the **type of reaction they catalyze**, with a prefix indicating the type of reaction. For example, **aminotransferase** transfers an amino group, while oxidase catalyzes oxidation reactions.
- To standardize enzyme nomenclature, the International Union of Biochemistry (IUB) has created a list of **systematic names** for all known enzymes. This name can indicate the substrate acted upon, the coenzyme involved in the reaction, and the type of reaction catalyzed. For example, *lactate dehydrogenase* is the trivial name, but according to IUB systematic nomenclature, it is called *lactate-NAD^+-oxidoreductase.*

In addition to naming enzymes, the (IUB) classifies enzymes by giving each enzyme a number. This number is called **enzyme commission numerical code (EC)**, it provides a unique numerical code for each enzyme based on the type of reaction it catalyzes. The first digit represents the general type of reaction, such as oxidoreductase (1), transferase (2), hydrolase (3), lyase (4), isomerase (5), or ligase (6). The second digit specifies the more specific type of reaction within that class (a sub class). The third digit further refines the classification based on the substrate or type of reaction involved (a sub-sub class). The fourth digit is an individual number assigned to each enzyme within that sub-sub class. This system allows for a standardized naming and classification system for all known enzymes.

As mentioned above according **to IUB classification** enzymes are divided into six major classes (table 2.2).

Table 2.2. Classification of enzymes

N	Class	Type of reaction catalyzed	Examples	Major subclasses
1	Oxido-reductases	Enzymes involved in oxidation-reduction reactions **S(oxidized) + Y(reduced)→ S(reduced) +Y(oxidized)**	*Alcohol dehydrogenase* (E.C. 1.1.1.1), *L- and D-amino acid oxidases, cytochrome oxidase*	• Dehydrogenases • Oxidases • Oxigenases • Cytochromes • Peroxydases
2	Transferases	Enzymes that catalyse the transfer of functional groups **SX + Y →S + YX**	*Hexokinase* (EC. 2.7.1.1), *transaminases, transmethylases, phosphorylase*	• Kinases • Transaminases • Methyltransferases • Acetyltransferases
3	Hydrolases	Enzymes catalyse the hydrolytic cleavage of C-O, C-N, C-C and some other bonds: **A-B + H_2O→AH + BOH**	*Lipase* (E.C. 3.1.1.3), *choline esterase, acid and alkaline phosphatases, pepsin, urease*	• Proteases • Glycosidases • Lipases • Esterases
4	Lyases	Enzymes cleaving C-C, C-O, C-N, and other bonds by elimination, leaving double bonds or rings, or conversely adding groups to double bonds: **A-B+Y-X→AX-BY**	*Aldolase* (E.C. 4.1.2.7), *fumarase, histidase*	• Carbon-carbon lyases • Carbon-oxygen lyases • Carbon-nitrogen lyases • Carbon-sulfur lyases • Phosphorus-oxygen lyases

5	Isomerases	Enzymes catalyse geometric or structural changes within one molecule. **A→A'**	*Triose phosphate isomerase* (E.C. 5.3.1.1), *phosphohexose isomerase*	• Racemases, • Epimerases, • Isomerases, • Tautomerases, • Mutases
6	Ligases	Enzymes catalysing the synthetic reactions **A + B + ATP → A-B+ADP**	*Glutamine synthetase* (E.C. 6.3.1.2), *acetyl CoA carboxylase, succinate thiokinase*	• Forming carbon-oxygen bonds • Forming carbon-sulfur bonds • Forming carbon-nitrogen bonds • Forming carbon-carbon bonds • Forming phosphoric-ester bonds

Potentially confusing questions concerning enzyme nomenclature. *It is important to pay attention to the specific suffixes used in the names of enzymes, as they often indicate important characteristics of the enzyme's function. For example, the suffix "-synthase" typically indicates that an enzyme synthesizes a molecule without the need for ATP, while the suffix "-synthetase" indicates that an enzyme synthesizes a molecule using ATP. Similarly, the suffix "-phosphatase" indicates that an enzyme removes a phosphate group using water, while the suffix "-phosphorylase" indicates that an enzyme splits a bond and releases a phosphorylated product using inorganic phosphate. Finally, the suffix "-oxidase" typically indicates that an enzyme uses oxygen as an acceptor but does not incorporate its atoms into the substrate, while the suffix "-oxygenase" indicates that an enzyme incorporates one or two oxygen atoms into* the substrate.

2.3. Structure of enzymes (primary, secondary, tertiary and quaternary structure).

Exception for several of catalytic RNA molecules, all enzymes are **proteins**. Their catalytic activity is determined by their native protein conformation. If an enzyme is denatured its catalytic activity is lost, moreover if an enzyme is broken down into its component amino acids, its does'n exist as a catalyst any more. The amino acid sequence determines the three-dimensional structure of the enzyme neseserly for its functioning. This shape is a thermodynamically most favorable one among the kinetically possible ways of folding. Thus **levels of structural organization** such as the **primary, secondary, tertiary, and quaternary structures** of protein enzymes are essential to their catalytic activity:

1. **Primary structure.** The linear sequence of amino acids forming the backbone of polypeptide chain of enzymes. The amino acids are held together in by covalent peptide bonds or linkages (fig.2.2).

The primary structure of an enzyme can be determined by sequencing the DNA of the gene that encodes the enzyme, and then using that information to deduce the sequence of amino acids in the polypeptide chain. This information can then be used to predict the three-dimensional structure of the protein using computational methods, such as molecular modeling. The primary structure of an enzyme is a critical determinant of its function, and understanding the amino acid sequence of an enzyme is an important first step in understanding its catalytic activity and biological function.

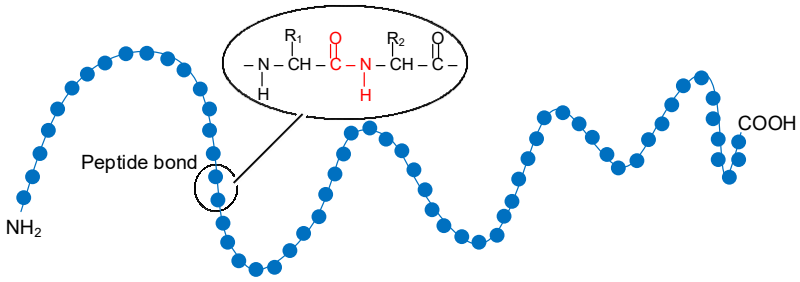

Fig. 2.2 Primary structure of an enzyme. The primary structure of an enzyme is critical to its function, as it determines the three-dimensional shape of the protein, which in turn determines the enzyme's ability to catalyze specific chemical reactions.

1. **Secondary structure.** The regular arrangement of a polypeptide chain of an enzyme in space by its twisting around its long axis. The term secondary structure refers to the fixed arrangement of the polypeptide chain stabilized by hydrogen bonds. Two types of secondary structure of protein, **α-helix, and β-sheet**, are mainly identified.

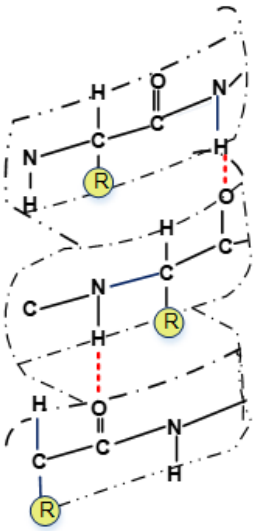

Fig.2.3. Secondary structure of proteins (α-helix), stabilized by hydrogen bonds

In α-helix type of secondary structure (fig. 2.3) the distance between two amino acid residues is 1.5 Å, each turn contain 3.6 amino acid residues per turn. The R-group of amino acids project outwards from the coiled. An α-helix is formed spontaneously since it is the most stable conformation of polypeptide chain.

In β-pleated sheet, the polypeptide chain is fully extended, it lines up side by side to form sheet. The side chains are above or below the plane of the sheet.The β-pleated sheet is stabilized by inter chain hydrogen bonds. Both parallel and anti-parallel β-pleated sheet occur in enzymes.

2. **Tertiary structure.** The three dimensional arrangement of polypeptide structure in space defines the tertiary structure of enzymes. The enzyme molecule arranges itself in space to achieve low energy and maximum stability. Enzymes are proteins that function as biological catalysts, and their catalytic activity is dependent on their specific three-dimensional structure, known as the tertiary structure. The tertiary structure of enzymes is stabilized by various types of bonds (fig. 2.4), including:

- **Hydrogen bonds:** These are weak, electrostatic interactions between polar groups in the protein, such as between the nitrogen and oxygen atoms in

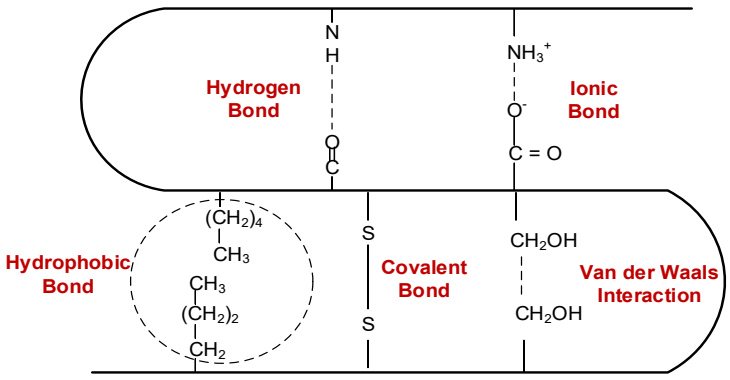

Fig. 2.4 Various types bonds, stabilizing tertiary structure of enzyme

the peptide backbone. Hydrogen bonds help to stabilize the overall structure of the protein and play a key role in determining the specificity of the enzyme.

- **Ionic bonds:** These are electrostatic interactions between charged groups in the protein, such as between the positively charged amino acid lysine and the negatively charged amino acid aspartate. Ionic bonds can be quite strong and contribute significantly to the stability of the tertiary structure.
- **Disulfide bonds:** These are covalent bonds between two cysteine residues, formed by the oxidation of the thiol (-SH) groups on each cysteine. Disulfide bonds are extremely strong and can provide significant stability to the tertiary structure of enzymes.
- **Van der Waals interactions:** These are weak, non-covalent interactions between nonpolar groups in the protein, such as between the hydrophobic side chains of amino acids. Van der Waals interactions help to stabilize the overall structure of the protein and are critical for maintaining the shape of the active site.
- **Hydrophobic interactions:** These are interactions between nonpolar groups in the protein that are driven by the tendency of hydrophobic groups to avoid contact with water. Hydrophobic interactions are critical for maintaining the overall stability of the protein, as they help to bury hydrophobic amino acids in the interior of the protein where they are shielded from the aqueous environment.

Under physiologic conditions (favorable pH for an enzyme), the side chains of amino acids which are hydrophobic in nature such as valine or isoleucine, are hiden within the enzyme core, due to their low affinity for the aqueous medium. The alkyl groups of Ala, Val, Leu, Ileu often form hydrophobic interactions between one-another. Acidic, basic or OH-groups containing amino acid side chains are polar in nature, and therefore remain exposed on the enzyme surface, to allow enzymes to be water soluble.

4. Quaternary structure. Some of the enzymes are composed of two or more polypeptide chains referred to as subunits. The arrangement of these subunits in space is known as a quaternary structure. The monomeric subunits are held together by noncovalent hydrogen bonds or hydrophobic interactions. Fig. 2.5 ilustrates the three-dimensional structure of a tetrameric enzyme *lactate dehydrogenase (LDH)*.

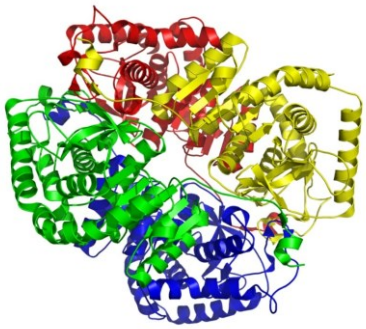

Fig. 2.5. Lactate dehydrogenase (LDH) is an oligomeric enzyme, composed of 4 subunits

Quatenary structure can contribute to fast and effective transfer of the substrate from one active center of enzymes to the next. For example, in *fatty acid synthase*.

Enzymes are proteins in nature, so they have all chemical and physical properties as proteins: high molecular weight, electrochemical properties and solubility.

2.3. Physical and chemical properties of enzymes.

Enzymes have similar physical and chemical properties to other proteins, which include

- **Hight molecular weights:** ranging from about 12,000 to more than 1 million Daltones (table 2.3).

Table 2.3 Data on Some Enzymes Molecular weight

Enzyme	Molecular weight, Da	Number of residues	Number of polypeptide chains
Cytochrome c (human)	13,000	104	1
Ribonuclease A (bovine pancreas)	13,700	124	1
Lysozyme (chicken egg white)	13,930	129	1
Chymotrypsin (bovine pancreas)	21,600	241	3
Hexokinase (yeast)	102,000	972	2
RNA polymerase (E. coli)	450,000	4,158	5

- **Electrochemical properties:** They depend on their amino acid composition. Each enzyme, having a polypeptide nature has its charge. The charge is a result of charges on the side chains of acidic amino acids aspartic (Asp) and glutamic (Glu) as well as basic amino acids histidine (His), lysine (Lys) and arginine (Arg). The charge of an enzyme depends mostly on amino acids, located on the surface of enzyme molecule. **Isoelectric point (pI)** is defined as the pH at which the enzyme has a zero net of charge because the number of positive charges are equal tonumber of negative charges. At isoelectric point enzymes are insoluble or have minimum solubility. The acidic amino acids (Asp, Glu) and basic amino acids (His, Lys, Arg) strongly influence the pl. At isoelectric pH, the enzymes exist as zwitterions so they are electrically neutral and do not migrate in the electric field. By changing the pH of surrounding medium the charge of an enzyme can be altered, it can be used to separate enzymes from mixtures.
- **Solubility of enzymes:** can be influenced intrinsic and extrinsic factors. The intrinsic factors as desctibed before are determined by the amino acids composition. Extrinsic factors influence enzyme solubility includeing pH, ionic strength, temperature, and the presence of various solvent additives. Varying these extrinsic factors can lead to increased solubility. Having protein nature, enzymes form **colloidal solutions** instead of true solutions in water. This is due to their large size.
- **Denaturation.** The phenomenon of disorganization of native protein structure is known as denaturation. Denaturation of enzymes can occur due to various physical and chemical factors, such as heat, pH, organic solvents, detergents, and heavy metal ions, which can break the weak interactions that stabilize the protein structure, leading to the loss of its native conformation and activity. Denatured enzymes are often inactive and sometimes even insoluble, as they may form aggregates or precipitates. Denaturation is a reversible process in some cases, but irreversible in others, depending on the extent and nature of the perturbation. A wide variety of reagents and conditions, such as heat, organic compounds, pH changes, and heavy metal ions can cause enzyme denaturation (table 2.4).

Table 2.4. Enzyme denaturation factors

Factor	Effect on Protein Structure
Heat above 50°C or ultraviolet (UV) radiation	Heat or UV radiation supplies kinetic energy to protein molecules, leading to the disruption of the weak non-covalent bonds that maintain the protein's native conformation. This can cause the protein to unravel and lose its activity, leading to denaturation.
Use of organic compounds, such as ethyl alcohol.	These compounds are capable of engaging in intermolecular hydrogen bonding with protein molecules, disrupting intramolecular hydrogen bonding within the protein.
Salts of heavy metal ions, such as mercury, silver.	These ions form strong bonds with the carboxylate anions of the acidic amino acids or SH groups of cysteine, disrupting ionic bonds and disulfide linkages.
Alkaloid reagents, such as tannic acid (used in tanning leather)	These reagents combine with positively charged amino groups in proteins to disrupt ionic bonds.

- **Optical properties:** Enzymes do not exhibit significant **optical properties** themselves, but they can be used in enzymatic assays to measure changes in optical properties that occur as a result of enzyme-catalyzed reactions. One common example is the use of enzymes in colorimetric assays, which involve the formation of a colored product that can be detected spectrophotometrically. Enzymes can also be used in **fluorescence assays**, which involve the use of fluorescent molecules to detect changes in enzyme activity. For example, the enzyme beta-galactosidase can be used in a fluorescent assay that involves the hydrolysis of a non-fluorescent substrate to produce a fluorescent product.
- **Sedimentation rate:** Enzymes can be separated and purified based on their **sedimentation rate**, which is the rate at which they settle to the bottom of a solution under the influence of gravity. This process is known as sedimentation and is commonly used in biochemistry and molecular biology research to isolate and purify enzymes. **Sedimentation** is typically performed using a technique known as ultracentrifugation, which involves spinning a sample containing the enzyme at high speeds in a specialized centrifuge. The rate at which the enzyme sediment to the bottom of the sample depends on its size, shape, and density, and can be used to separate it from other components in the sample. One common method of ultracentrifugation is **analytical ultracentrifugation (AUC)**, which involves spinning a sample at a constant speed and measuring the rate at which the enzyme sediment over time. This information can be used to determine the molecular weight and shape of the enzyme, as well as its purity and concentration.

2.5. Simple and conjugated enzymes. Classification of coenzymes.

Some enzymes require no chemical groups for activity other than their amino acid residues, they are reffered to as **simple enzymes.** For example, *pepsin, trypsin, papain, urease, lysozymase, ribonuclease, and phosphatase.* Others enzymes called **conjugated** require an additional chemical component (nonprotein part), which can by represented by inorganic ions or complex organic or metalloorganic molecules.

The functional unit of the conjugated enzyme is known as **holoenzyme** which is often made up of the protein part (**apoenzyme** or apoprotein) and a non-protein organic part (**coenzyme, prosthetic group or cofactor**):

Protein part (apoenzyme)+nonprotein part (coenzyme/prosthetic group)=Holoenzyme

The term **prosthetic group** is used when the non-protein moiety tightly (covalently) binds with the apoenzyme. The **coenzyme** can be separated by dialysis from the enzyme while the prosthetic group cannot be. Most of coenzymes and prosthetic groups are derived from vitamins (table 2.5), organic nutrients required in small amounts in the diet.

Table 2.5. Some coenzymes and prosthetic groups that serve as carriers of specific atoms or functional groups

Coenzyme/prosthetic group	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5'-Deoxy-adenosylcobalamin	H atoms and alkyl groups	Vitamin B_{12} (coenzyme B_{12})
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

Metal ions constitute the most common type of prosthetic group. The roughly one-third of all enzymes that contain tightly bound Fe, Co, Cu, Mg, Mn, and Zn are termed **metalloenzymes** (table 2.6).

Table 2.6. Metaloenzymes

Examples of metal ions in different metalloenzymes	
Cu^{2+}	Cytochrome oxidase, superoxidedismutase, lysyloxidase, ceruloplasmin.
Fe^{2+}	Cytochrome oxidase, catalase, xanthine oxidase, succinate dehydrogenase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, Glucose-6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonuclease, reductase
Mo^{2+}	Dinitrogenase
Se	Glutathione peroxidase
Ni^{2+}	Urease
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B, alkaline phosphatase

Potentially confusing questions concerning cofactors. *The term cofactors can used as a collective name of nonprotein parts of enzymes (both coenzymes and prosthetic groups). In other literature, coenzymes are refered to as metal ions in the structure of enzymes.*

Cofactors serve functions similar to those of prosthetic groups, hovewer they can dissociate from an enzyme similarly as coenzymes do. The main difference with coenzymes that cofactors can associate with the enzyme either directly or in the form of a cofactor-substrate complex. They must be present in the medium surrounding the enzyme for catalysis to occur. The most common cofactors are metal ions.

Numerous enzymes require metal ions, neded for maintenance of enzyme conformation and catalysis. Metal ions participate in enzymatic reactions in three main ways:

- **Metalloenzymes:** metal is tightly bound to enzyme molecule and it is an integral part of enzyme molecule. Metals are attached to enzyme through coordinate bonds. They participate in catalysis (table 2.6).
- **Metal-dependent enzymes:** metal is loosely associated with enzyme molecule or it may be required for enzyme substrate complex formation. In the absence of metal, enzyme may not interact with substrate molecule or with co-enzyme molecule. For example, Mg^{2+} is needed by enzymes using ATP (*hexokinase, galactokinase, pyruvate kinase* etc); Ca^{2+} is required for the activity of *calpain*, a calcium-dependent protease.
- **Metal-activated enzymes:** in presence of metals, some enzymes get activated i.e., their activity increases many folds. E.g. Ca^{2+} activates *trypsin*.

A diverse metal are observed at the active-site of enzymes arranged as:

- Single metal sites
  - mostly structural sites Ca^{2+}, Zn^{2+}
  - exceptions Cu^{2+}
- Metal clusters
  - Fe,S (Fe_4S_4, Fe_2)
  - FeMoCo
  - Mn_4, Mn_2, Cu_2, mixed metal clusters
- Organometallic cofactors
  - Porphyrins
  - Cobalamin

Coenzymes are classified due to the following properties:

I. Due to their **chemical nature** coenzymes (prosthetic groups) are classified into following groups:

1. Vitamin derivatives:

- B_1-thiamine pyrophosphate,
- B_2- FAD, FMN,
- B_3 -NAD, B_5 - HS-CoA,
- B_6 - pyridoxal phosphate

1. **Nucleotides** (ATP, ADP, CTP, UTP, UDT).
2. **Dinucleotides** (NADH, NADPH, FADH).
3. **Metalloporphyrins** (cytochromes).
4. **Vitamin-like compounds** (lipoic acid, coenzyme Q).
5. **Peptides** (glutathione).

II. Due to **the type of catalytic reaction** coenzymes (prosthetic groups) are classified into 3 classes:

1. **Coenzymes as transporters of hydrogen atoms and electrons:**

- NAD^+, $NADP^+$ coenzymes – derivatives of vitamin PP;
- FAD, FMN coenzymes – derivatives of vitamin B_2 – riboflavin;
- Vitamin C (ascorbic acid);
- Metalloporphyrins.

2. **Coenzymes as transporters of chemical groups:**

- Pyridoxal phosphate;
- HS-CoA – coenzyme of acylation;
- Lipoic acid;
- THF – derivatives of folic acid

3. **Coenzymes of isomerisation, synthesis and cleavage of C-C bonds :**

- Thiamine pyrophosphate – coenzyme form of vitamin B_1;
- Biocytin – coenzyme form of vitamin H – biotin;
- Methylcobalamin and deoxyadenosylcobalamin – coenzyme forms of vitamin B_{12}

2.6. Active centres and allosteric sites of enzymes. Multi-enzyme complexes

The unique combination of amino acid residues within an enzyme molecule that enables its direct interaction with substrate molecules is known as the **enzyme's active center.** This active site is three-dimensional and is formed by amino acid residues that are widely separated within the enzyme molecule. However, during catalysis, these residues are brought together to form the active site. The precise arrangement of amino acids within the active site allows only specific substrates to bind to it.

The following areas are found in the active center (fig. 2.6):

•**substrate-binding site** is responsible for recognizing and binding with the substrate resulting in the formation of the enzyme-substrate complex (ES);

•**the catalytic site** directly performs the catalysis.

In conjugated enzymes also need to bind with some non-protein part to fulfill their jobs. The active site is usually a groove or pocket of the enzyme which can be located in a deep tunnel within the enzyme, or between the interfaces of multimeric enzymes.

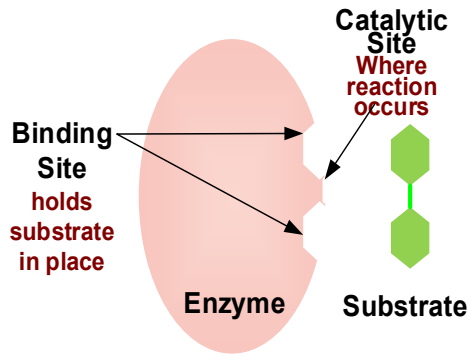

Fig. 2.6. The active center of an enzyme is a specific region within the enzyme molecule where catalysis occurs.

The functional groups of the enzymes active centers:

• COOH – of dicarboxylic amino acids;
• NH_2 of lysine;
• OH-groups of tyrosine, serine and threonine;
• SH-groups of cysteine and disulfide bridges of cystine;
• indole groups of tryptophan;
• guanidine – of arginine;
• imidazole – of histidine;
• thioether – of methionine;
• hydrophobic – of aliphatic amino acids
• aromatic ring – of phenylalanine.

Allosteric sites are places where molecules that regulate enzyme activity can bind. Proteins are not machines. They are firm and forceful, yet slightly fluid, meaning that they can undergo shape changes. The binding of an inhibitor or an activator to an allosteric site indirectly changes the shape of the active site.

Certain enzymes are components of **supramolecular complexes, or multienzyme complexes.** Within cells, enzymes often catalyze multistep metabolic pathways, where the product of one enzyme becomes the substrate for the next. While some enzymes in these pathways are free-floating in the cytosol without any physical association, allowing substrates to diffuse between them, there are also more complex forms of organization in multi-enzyme systems:

- **Multifunctional enzymes** are proteins that have at least two (or more) enzymatic activities ans two (or more) distinct active sites. It is a group of several associated polypeptide chains, wich may have different functions. This type of enzyme organization increases the efficiency of catalyzed reactions. For example, *DNA polymerase* is an enzyme of DNA synthesys but it is also involved in the proof reading.
- Several enzymes of same pathway can be associated non covalently or sometimes covalently to create a **multienzyme complex**, such as the **pyruvate and the *α-ketoglutarate dehydrogenase* complexes**. The ***fatty acid synthase*** of mammals is a homodimer of two cooperating multifunctional enzyme chains.
- The most highly organized enzyme systems are associated with **supramolecular structures,** such as ribosomes, the proteasome or the components of the respiratory chain which spans the inner membrane *of mitochondria.*

2.7. Specificity of enzymes.

Enzymes are distinguished from inorganic catalysts by their **specificity** of action, which is an important property. Most enzymes are capable of recognizing only a single compound as a substrate, whereas others, such as proteases that break down proteins into peptides, are less specific, particularly those involved in digestion. There are several general types of enzyme specificity, including:

- **Substrate Specificity:** Enzymes are specific towards their substrates. For example, *glucokinase* is an enzyme that specifically catalyzes the transfer of phosphate from ATP to glucose:

Glucose+ATP → glucose-6-phosphate +AMP
Glucokinase

Galactokinase catalyzes transfer of phosphate from ATP to galactose.

Galactose+ATP→galactose-1-phosphate +AMP
Galavtokinase

Even though both enzymes catalyze the transfer of phosphate from ATP, they are specific to particular substrates. Similarly, *transaminases* that facilitate the transfer of amino groups are specific to their substrates. For instance, *aspartate transaminase* catalyzes the transfer of an amino group from aspartate, while *alanine transaminase* catalyzes the transfer of an amino group from alanine only.

- **Reaction apecificity:** An enzyme catalyzes only **one** specific reaction. This type of specificity. For instance, *lipases* specifically catalyze the hydrolysis of lipids, while *urease* specifically catalyzes the hydrolysis of urea, and they are not capable of catalyzing any other type of reaction. Similarly, *amino acid oxidase* specifically catalyzes the oxidation of amino acids, while *decarboxylase* catalyzes only the decarboxylation of amino acids.

$$Urea + H_2O \leftrightarrow CO_2 + H_2O$$
Urease

- **Group (relative) specificity:** Some hydrolytic (hydrolases) enzymes act on specific groups. *Proteases* are specific for peptide groups, *glycosidases* are specific to glycosidic bonds.
- **Absolute group specificity:** Some hydrolytic enzymes demonstrate high-order group specificity, meaning they exhibit a preference for specific functional groups or amino acid residues within the substrate molecule. *Chymotrypsin* is an example of such an enzyme. It is a protein-splitting enzyme that hydrolyzes peptide bonds. However, it displays a higher preference for peptide bonds in which the carboxyl group is contributed by aromatic amino acids such as phenylalanine, tyrosine, and tryptophan(fig. 2.7). Likewise, *trypsin* another peptide bond hydrolyzing enzyme preferentially hydrolyzes peptide bonds on the carboxyl side of positively charged amino acids such as arginine and lysine. *Carboxypeptidases A and B* are exopeptidases that cleave single amino acids from the carboxyl terminus of peptide chains. *Aminopeptidases* are also exopeptidases that cleave single amino acids from the amino terminus of peptide chains. *Thrombin* is a protease involved in blood clotting and is highly specific for peptide bonds that involve the amino acid sequence Arg-Gly. These examples illustrate how enzymes exhibit specificity towards particular substrates or types of substrates based on their unique active center and catalytic properties.

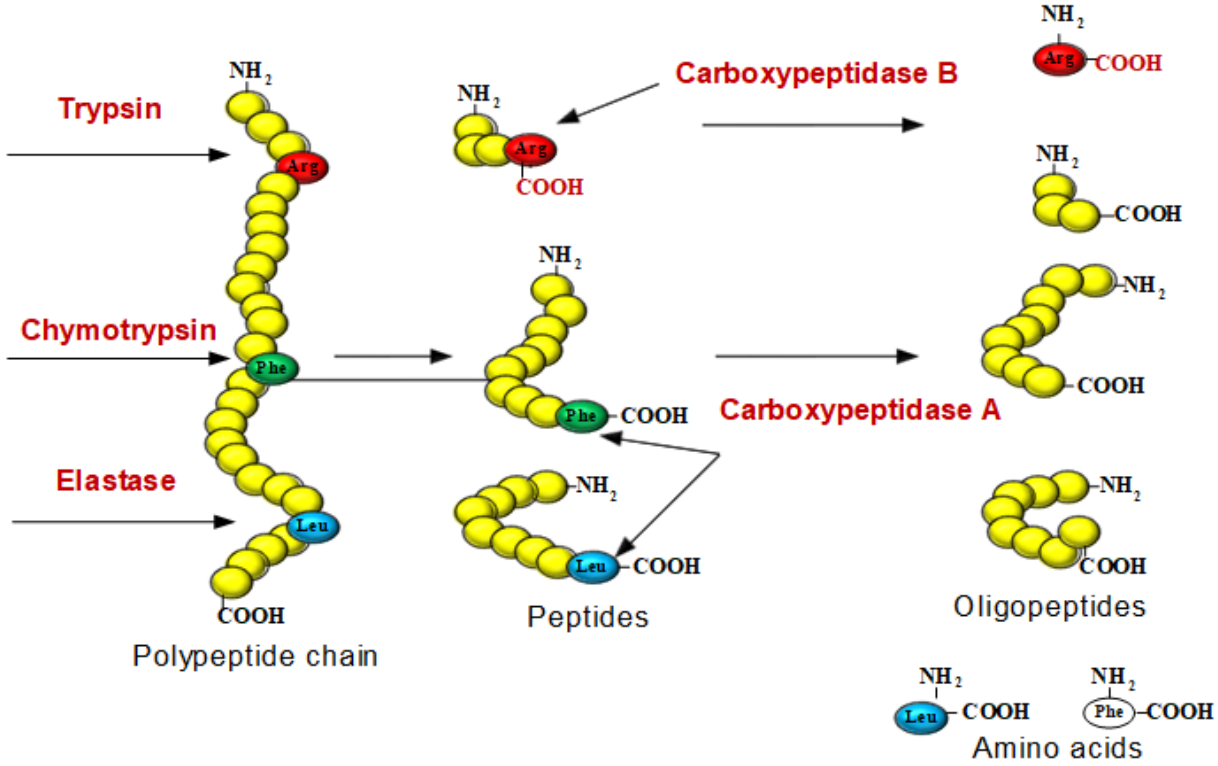

Fig. 2.6. Pancreatic proteolytic enzymes exhibit group specificity, meaning they display a preference for specific amino acid residues within the substrate molecule.

- **Optical (stereo-) specificity**: Enzymes can exhibit optical specificity towards substrates that have chiral centers, such as amino acids and sugars. Chiral centers are carbon atoms that have four different groups attached to them, resulting in two possible stereoisomers, known as enantiomers. Enzymes that act on chiral substrates can exhibit selectivity towards one enantiomer over the other. For example, enzymes involved in amino acid metabolism, such as *amino acid oxidases*, are typically specific for L-amino acids, while enzymes involved in carbohydrate metabolism, such as *hexokinase*, are specific for D-sugars. This is because the stereochemistry of the active site of the enzyme is complementary to the stereochemistry of the substrate, allowing for the recognition and binding of the specific enantiomer.

2.8. The localization of enzymes in cells (compartmentation) and organs.

Compartmentation (the location of the prosses in a particular subcellular organelle called compartment) is an important component of metabolic regulation. Compartmentation of metabolism is a fundamental feature of eukaryotic cells, and it plays an important role in regulating metabolic pathways. Each organelle within the cell has a specific set of enzymes and metabolites, which allows for the localization and concentration of specific reactions within a confined space. This spatial organization of metabolic pathways allows for greater efficiency and regulation of biochemical reactions, as well as the prevention of undesirable interactions between different pathways. For example, the mitochondria are specialized organelles that are responsible for oxidative metabolism and energy production through the electron transport chain, while the endoplasmic reticulum is involved in protein synthesis and modification. The

compartmentalization of metabolism also enables cells to respond to different physiological and environmental cues by regulating the activity and localization of enzymes within organelles (table 2.7).

Table 2.7. Compartmentation of enzymes

Organelle	**Enzyme/Enzymatic pathway**
Cytoplasm	Enzymes of glycolysis; fatty acid synthesis; hexose monophosphate shunt; purine and pyrimidine catabolism; aminotransferases; peptidases
Mitochondria	Fatty acid oxidation; amino acid oxidation; Krebs cycle; urea synthesis; electron transport chain and oxidative phosphorylation
Nucleus	Biosynthesis of DNA and RNA.
Endoplasmic reticulum (microsomes)	Protein biosynthesis; triacylglycerol and phospholipid synthesis; steroid synthesis and reduction; cytochrome P450; esterase
Lysosomes	Lysozyme; phosphatases; phospholipases; hydrolases; proteases; lipases; nucleases
Golgi apparatus	Glucose 6-phosphatase; 5'-nucleotidase; glucosyltransferase
Peroxisomes	Catalase; urate oxidase; D-amino acid oxidase

Enzymes can be classified based on their location and function as **intracellular or extracellular enzymes.** Intracellular enzymes are synthesized and function within cells, and they are involved in various metabolic pathways and cellular processes. For example, enzymes involved in the TCA cycle and oxidative phosphorylation are intracellular enzymes that generate ATP within the mitochondria. On the other hand, extracellular enzymes are secreted by cells and function outside the cell. They are involved in various physiological processes such as digestion, blood coagulation, and immune response. For example, digestive enzymes such as pepsin and trypsin are secreted by the stomach and pancreas, respectively, to break down food proteins into smaller peptides and amino acids that can be absorbed by the body.

Localzation of enzymes in organs and tissues:

- **Liver enzymes:** Liver is a central metabolic organ of metabolism in the body. It detoxifies almost any substance and helps in their excretion from the body through urine. Cytochrome P-450 enzymes, also known as microsomal monooxygenases, are a large family of heme-containing enzymes located in the liver and other tissues. They are involved in the oxidative metabolism of various endogenous and exogenous compounds, including drugs, toxins, and environmental pollutants. These enzymes play a crucial role in drug metabolism and can affect the pharmacokinetics and toxicity of many medications. Additionally, the liver is also responsible for producing enzymes involved in the breakdown of carbohydrates, fats, and proteins, such as amylase, lipase, and protease.There are number of enzymes in the liver and some of them are used in clinical diagnostics indicating

hepatocytes damage. For example, *aspartate aminotransferase (AST)* and *alanine transaminase (ALT).* Both the enzymes catalyze transamination of amino acids.

- **Enzymes of the nervous system: There are numerous enzy**mes invilved in metabolism of neurotransmitters in cells of nervous system. For example, *acetyl cholinesterase* breaks down acetylcholine, *monoamine oxidase* breaks down catecholamines like dopamine, adrenalin and noradrenalin, *dopadecaboxylase* generates dopamine. In addition to the enzymes mentioned, there are several other important enzymes involved in neurotransmitter metabolism in the nervous system. For example, *tyrosine hydroxylase* is responsible for the synthesis of catecholamines, including dopamine, from the amino acid tyrosine. *Aromatic L-amino acid decarboxylase* converts L-DOPA into dopamine, and serotonin is synthesized from tryptophan by the enzyme *tryptophan hydroxylase*. Other enzymes involved in the metabolism of neurotransmitters include acetyltransferase, which catalyzes the synthesis of acetylcholine, and glutamic acid decarboxylase, which is involved in the synthesis of the neurotransmitter gamma-aminobutyric acid (GABA).
- **Pancreatic enzymes**: Trypsinogen, which is an inactive (zymogenic) protease that, once activated in the duodenum into *trypsin,* breaks down proteins; chymotrypsinogen, which turns into *chymotrypsin* and breaks down proteins; *carboxypeptidase*, which is a protease that takes off the terminal amino acid group from a protein; *pancreatic lipase* that degrades triglycerides into fatty acids and glycerol; several *nucleases* that degrade nucleic acids, like *DNAase* and *RNAase*; *pancreatic amylase* that breaks down starch. Additionally, the pancreas also produces enzymes such as *elastase, collagenase, and phospholipase* which aid in the digestion of elastin, collagen, and phospholipids, respectively. These enzymes are secreted into the small intestine to aid in the digestion and absorption of nutrient
- **Kidney enzymes:** the kidneys produce and secrete several enzymes involved in the regulation of blood pressure and fluid balance, such as *erythropoietin (EPO)*, which stimulates the production of red blood cells, and the enzyme *renin*, which is involved in the renin-angiotensin-aldosterone system (RAAS) and helps regulate blood pressure. The kidneys also produce enzymes involved in the metabolism of drugs and other xenobiotics, such as *cytochrome P450* enzymes. Vitamin D is activated in the kidney to $1,25(OH)_2D_3$ by the renal enzyme 1-*alpha hydroxylase*.
- **Enzymes of muscle tissue**: *creatine phosphokinase* transfers phosphate groups from creatine phosphate to ADP, producing ATP and creatine; myosin *ATPase* catalases the hydrolysis of terminal phosphate group of ATP by headgroup; actomyosin *ATPase* – hydrolysis of the terminal phosphate group of ATP when head-group is in interaction with actin. Additionally, there are other enzymes present in muscle tissue such as *lactate dehydrogenase (LDH),* which catalyzes the interconversion of pyruvate and lactate during anaerobic metabolism, and *glycogen phosphorylase*, which catalyzes the breakdown of glycogen into glucose-1-phosphate to provide energy for muscle contraction. *Myoglobin*, a protein found in muscle tissue, also has enzymatic activity and facilitates the diffusion of oxygen from the blood to the muscle cells.

2.9. Mechanisms of enzymes action

Many reactions in the human body are thermodynamically favorable (ΔG<0) and therefore spontaneous. However, without enzymatic catalysis, the rate of these reactions is very low. For instance, the oxidation of glucose to carbon dioxide and water has a negative ΔG°' of -2870 kJ/mol, but a jar of sugar, even in water, is remarkably stable. Biochemical reactions are typically controlled by enzymes, which require a certain amount of energy input (activation energy) to release energy. The highest point on the reaction path is known as the **transition state** for the reaction, where the activated complex can either break down to form products or revert to reactants. The rate of a reaction is exponentially related to the activation energy.

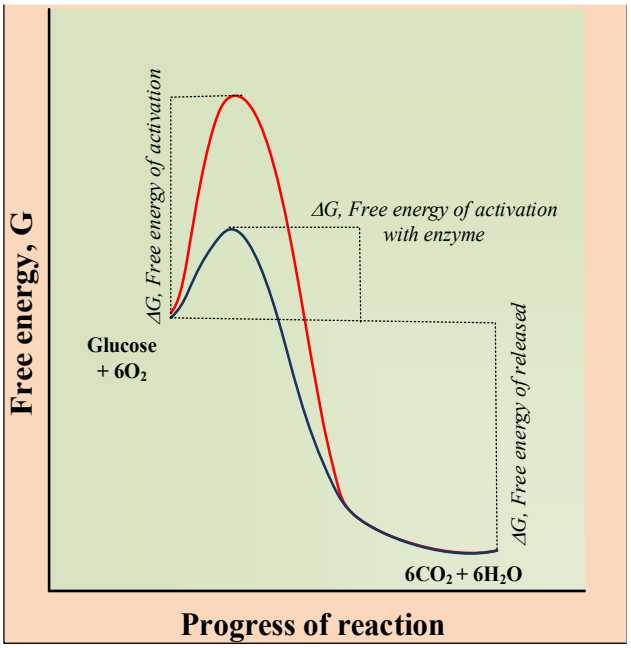

Fig. 2.8. An enzyme can significantly lower the activation energy of a chemical reaction, making it easier for the reaction to occur.

There are two modes to speed up the reaction. Raise the free energy of the substrates, or decrease the energy of activation for the reaction. Enzymes catalyze **reactions by lowering the activation energy barrier** (fig.2.8). A chemical reaction without an enzyme is like a climb over a mountain. The enzyme bores a tunnel through it so that passage is far quicker and takes much less energy.

Theories of enzyme action:

The initial concept of enzyme function was introduced by **E. Fischer**, who proposed that enzymes and substrates fit together like a lock and key. The amino acids located at the active site of the enzyme are arranged in a specific way to allow only a particular substrate to bind to the enzyme (see Figure 2.9).

This model was unable to explain the possibility of rigid active site combining with the product to form substrate in reversible reaction.

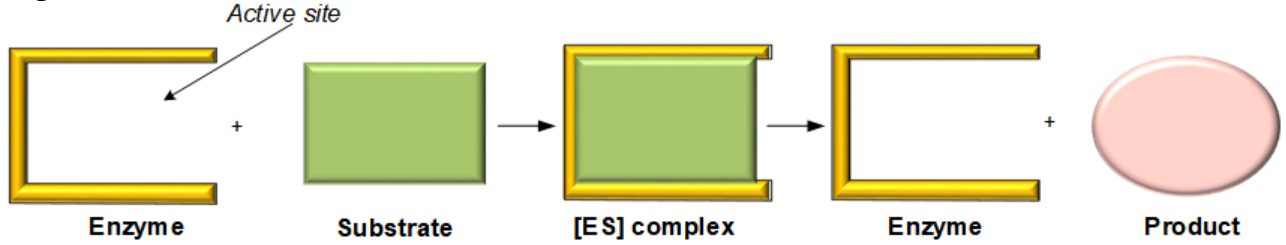

Fig. 2.9. Lock and key model of enzyme action. According to this model, enzymes and substrates have a rigid, complementary shape that fits together like a lock and key.

Later **D. Koshland** proposed the theory of **induced fit**, which suggests that the active site of the enzyme is flexible, with alternating rigid and flexible areas in the protein

molecule. When the substrate binds to the enzyme, it induces a conformational change in the enzyme molecule, causing a shift in the amino acids that make up the active site. This change favors the formation of a tightly bound enzyme-substrate complex, followed by catalysis. The induced conformation is unstable and the enzyme molecule returns to its native conformation in the absence of substrate. (See figure 2.10 for illustration.)

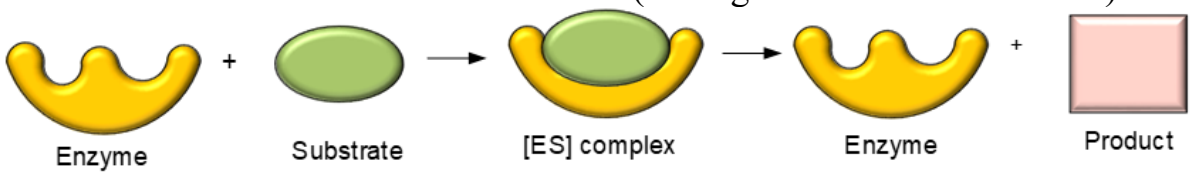

Fig. 2.10. Koshland model of enzyme action. This model explains that the binding of substrate to the active site of an enzyme induces a conformational change in the enzyme that leads to the formation of an enzyme-substrate complex. In other words, the active site is not a rigid structure, but rather a flexible one that changes its shape to fit the substrate.

The concept of the enzyme-substrate complex was first proposed by **V. Heneri** in 1903, suggesting that the enzyme (E) and substrate (S) must bind to each other to form an **intermediate complex (ES)** in order for catalysis to occur. This idea was further developed by **L. Michaelis and M. Menten** in 1913, who proposed a general theory of enzyme action. According to their theory, the enzyme first binds reversibly with the substrate to form an **enzyme-substrate complex**, which then undergoes a slower breakdown to form the product and enzyme.

The overall enzymatic reaction used in Michaelis-Menten model is:

$$E + S \underset{K_2}{\overset{K_1}{\rightleftarrows}} ES \overset{K_3}{\rightarrow} E + P$$

Here K_1, K_2 and K_3 symbolize the rate constants.

MEDICAL IMPORTANCE

Understanding the mechanism of enzyme action can provide insights for the design of drugs that can target specific enzymes involved in diseases. For example, the mechanism of action of carboxypeptidase has been used to design specific inhibitors for angiotensin converting enzyme, which is involved in regulating blood pressure. These inhibitors, such as captopril, can be used to treat hypertension.

In addition, knowledge of enzyme mechanisms can also lead to the design of enzymes with specific properties for therapeutic purposes. For example, enzymes can be designed to correct specific abnormalities associated with genetic disorders by introducing them into the patient's body. This approach is still in its early stages, but it has the potential to revolutionize the treatment of many diseases.

2.9. Enzyme kinetics. Factors affecting enzymatic activity.

Enzyme kinetics is the branch of biochemistry that studies the chemical reaction catalyzed by an enzyme, including the rate (velocity) of the reaction and the factors that affect it. **Main factors influencing enzyme activity:**

- enzyme concentration;
- substrate concentration;
- temperature;
- pH;

• presence of activators or inhibitors.

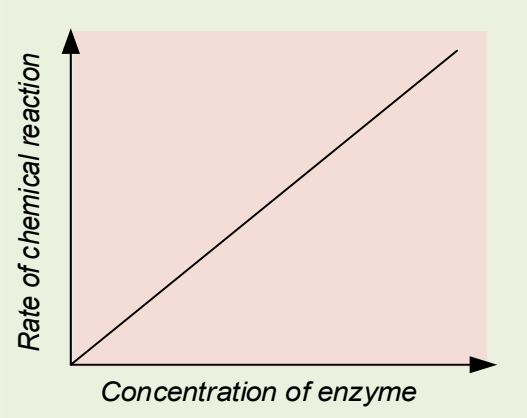

Fig. 2.11. The velocity of an enzyme-catalyzed reaction is dependent on the concentration of the enzyme. As the concentration of the enzyme increases, the velocity of the reaction also increases proportionally.

When the concentration of the enzyme is increased, the velocity of the reaction also increases proportionately, as shown in figure 2.11. This is because at low enzyme concentrations, the substrate molecules may not encounter the enzyme frequently enough to be converted into products. As the enzyme concentration increases, more substrate molecules are bound by the enzyme, leading to an increase in product formation.

This property of enzymes is used to determine the serum enzyme levels for diagnosing diseases. In a biochemical laboratory, a known volume of serum can be used, and all other factors such as substrate, pH, and temperature can be kept at their optimum levels, allowing the measurement of the enzyme activity.

When the velocity is plotted **versus concentration of substrate [S]**, a hyperbolic curve is drowen. At low substrate concentration (left part of the curve), the reaction rate increases sharply with increasing substrate concentration because there is abundant free enzyme available to bind added substrate. At high substrate concentration, the reaction rate reaches a plateau (V_{max}) the point at which all enzyme active sites are fully occupied by substrate (en effect of saturation), and any further increase in substrate concentration does not affect the reaction rate. The hyperbolic plot of the dependence of velocisity of reaction on [S] is called as **Michaelis plot** (fig. 2.12).

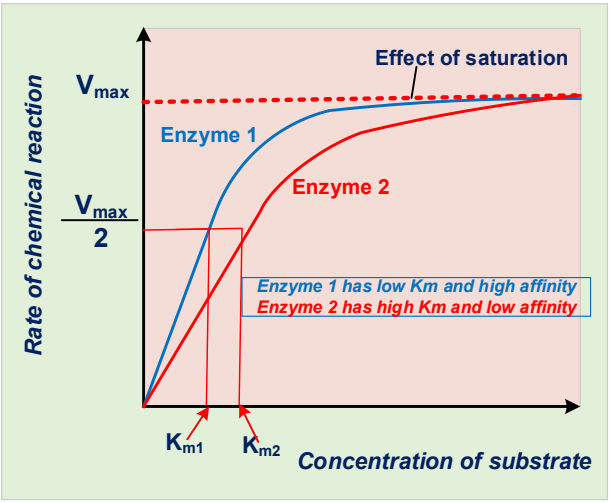

Fig. 2.12. The Michaelis plot is a common way to graphically represent the relationship between substrate concentration and reaction velocity for an enzyme-catalyzed reaction.

Potentially confusing question concerning kinetics for allosteric enzymes. *Allosteric enzymes have a different kinetic behavior, and their substrate binding and reaction rate can be regulated by modulators that bind to specific regulatory sites on the enzyme.*

Michaelis equation describes the dependence of the enzymatic reaction rate on the substrate concentration:

$$v = \frac{v_{max}[S]}{[S] + K_m}$$

Here, **K_m - Michaelis-Menten** constant is defined as the substrate concentration (expressed in moles/l) to produce half-maximum velocity in an enzyme catalysed reaction. It indicates that half of the enzyme molecules are bound with the substrate molecules when the substrate concentration equals the K_m value.

MEDICAL IMPORTANCE

Enzymes can be designed with specific Km values to suit different applications, such as in industrial processes or in the production of pharmaceuticals. Additionally, Km values can be useful in optimizing the conditions for enzyme reactions in various applications. Use of enzymes in immunodiagnostics (ELISA) require Km of the enzyme.

K_m value is a constant and a characteristic feature of a given enzyme (comparable to a thumb impression or signature). lt is a representative for measuring the strength of ES complex. A **low K_m** value indicates a **strong affinity** between enzyme and substrate, whereas a **high K_m** value reflects a **weak affinity** between them. For majority of enzymes, the K_m values are in the range of 10^{-5} to 10^{-2} moles/l. It may however, be noted that K_m is not dependent on the concentration of enzvme. For example, *hexokinase* and *glucokinase* both phosphorylates glucose. However, *hexokinase* can phosphorelate glucose 2000 times more efficiently than *glucokinase* because Km of *hexokinase* is low (1×10^{-5} M) whereas Km of *glucokinase* is high (2.0×10^{-2} M).

The Lineweaver-Burk plot (fig.2.13) is a double reciprocal plot of the Michaelis-Menten equation, which can be used to determine Km and Vmax values more accurately than the Michaelis plot. By taking the reciprocal of both sides of the Michaelis-Menten equation and rearranging, a straight line equation can be obtained that allows for the determination of Km and Vmax from the slope and y-intercept, respectively, of the resulting graph.

Lineweaver-Burk plot is obtained by taking the reciprocals of the Michaelis equation:

$$\frac{1}{V_o} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}}\frac{1}{[S]}$$

The straight line intersects y-axis, which corresponds to Vmax value. A line extended from point of intersection to x-axis of second quadrant provides K_m. The Lineweaver-Burk plot is also useful in comparing the kinetics of different enzymes or different conditions affecting enzyme activity.

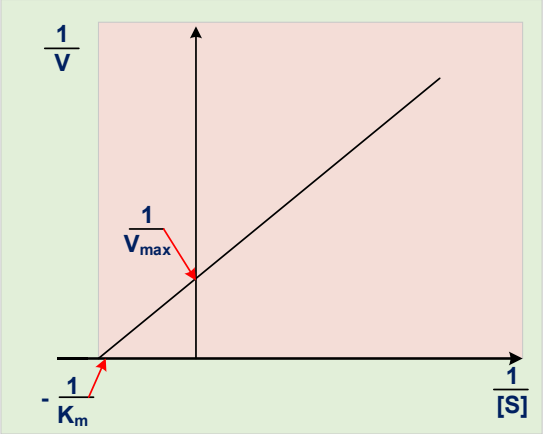

Fig. 2.13. The Lineweaver-Burk plot, also known as the double reciprocal plot, is a graphical representation of enzyme kinetics data that is used to determine the kinetic parameters of an enzyme-catalyzed reaction, including the maximum reaction rate (Vmax) and the Michaelis constant (Km).

Enzymes have an optimal temperature at which they exhibit maximum activity, but beyond that temperature, they become denatured and lose their activity. The optimal temperature of enzymes varies depending on the source organism and their habitat. 37°C is **the optimal temperature** for humans (fig 2.14). Enzymes from organisms that live in extreme environments, such as thermophilic bacteria or archaea living in hot springs, have evolved to function at high temperatures, while enzymes from psychrophilic organisms, such as those living in Arctic waters, have evolved to function at low temperatures. It is important to note that temperature can also affect the stability of enzymes, and prolonged exposure to high temperatures can lead to irreversible denaturation.

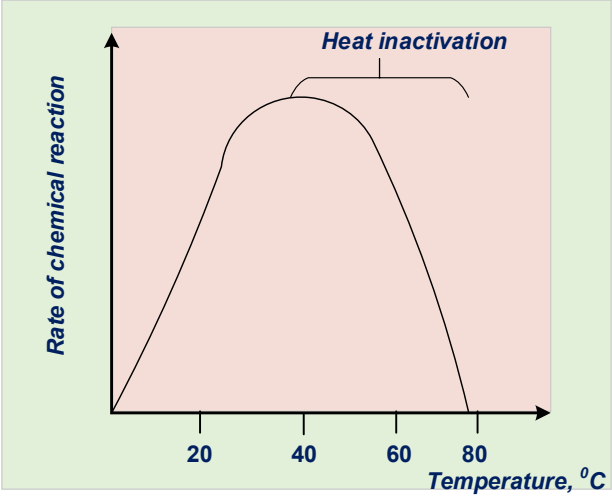

Fig. 2.14. The velocity of an enzymatic reaction is dependent on temperature. The rate of reaction generally increases with increasing temperature until it reaches an optimal temperature at which the enzyme catalyzes the reaction at the maximum rate.

Each enzyme-catalyzed reaction has an optimal pH (not always physiologic pH) (figure 2.15). The reason for the dependence on of enzymatic reaction on pH is that enzymes contain amino acid residues with ionizable groups, such as carboxylic acids, amino groups, and histidine residues. These groups can be protonated or deprotonated depending on the pH of the environment, which can affect the enzyme's activity by altering its shape, its ability to bind to substrate molecules, or its catalytic activity.

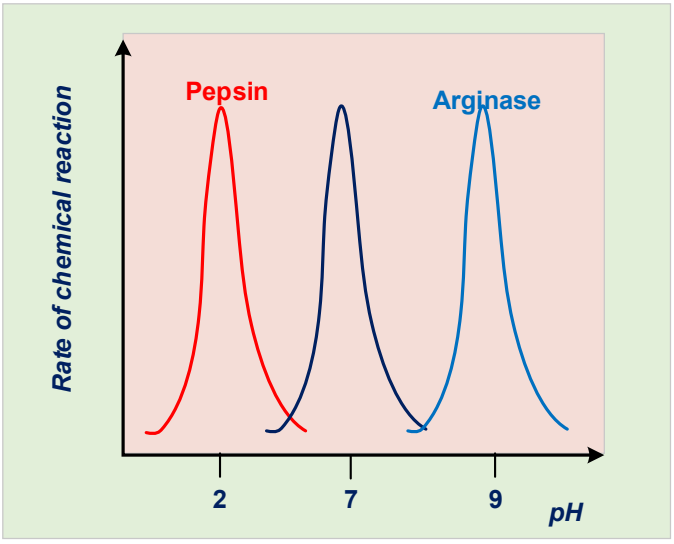

Fig. 2.15. The velocity of an enzymatic reaction can be strongly influenced by pH, and the optimal pH for an enzyme depends on its specific structure and function.

The unraveling of the tertiary structure of a protein occurs at extremes of pH. This is called denaturation, and is due in part to the disturbance of normal hydrogen bonding patterns. However, the velocity of an enzymatic reaction within that pH range might vary drastically. pH could also affect the concentration of appropriately ionized groups on the enzyme that participate in substrate binding. Examples. of pH optimum: *pepsin* – 1,5, *catalase* – 7,6, *trypsin* – 7,7, *fumarase* – 7,8, *ribonuclease* – 7,8, *arginase* – 9,7. In the case of oligomeric enzymes, optimum pH is required for the association of protomers. When the pH is altered, the protomers dissociate with loss of biological activity.

MEDICAL IMPORTANCE

Enzymatic reactions are essential for normal functioning of the body, and alterations in their rates can disrupt tissue homeostasis. Changes in pH can also affect enzymatic reactions, as the activity of many enzymes is pH-dependent. Preservation of organs for transplantation, blood, and serum at low temperatures is important to slow down enzymatic reactions and prevent degradation of biological material. Temperature can also affect rates of enzymatic reactions, which is why fever and hypothermia can alter enzymatic activity. Drugs can be designed to target specific enzymes and alter their activity, as is the case with AZT, Lovastatin, and Captopril. Finally, some poisons work by affecting essential enzymatic reactions, leading to toxic effects on the body.

2.10. Mechanisms of enzyme catalysis.

Enzymes use a combination of the molecular effects to achieve their remarkable catalytic activity:

- **Proximity and Orientation:** Enzymes bring the substrate molecules into close proximity and in a specific orientation within the active site. This arrangement allows the reactive groups of the substrate and the enzyme to interact optimally, facilitating the formation of the transition state and increasing the rate of the reaction.
- **Acid-Base Catalysis:** Enzymes can contain acidic and basic groups in their active sites, which can donate or accept protons. This acid-base catalysis helps in the breaking and rearrangement of chemical bonds within the substrate. The proton transfer can stabilize charged intermediates or activate nucleophiles or electrophiles, promoting the reaction.
- **Covalent Catalysis:** Enzymes can form a covalent bond with the substrate, creating a covalent intermediate. This intermediate lowers the energy of subsequent transition states, making the reaction more favorable. Covalent catalysis can occur through the use of residues in the active site or with the help of cofactors.
- **Entropy Effect:** Enzymes can reduce the entropy (extent of disorder) of the reactants, allowing them to come closer to the enzyme. By decreasing the entropy, enzymes increase the effective concentration of the reactants, leading to more frequent collisions and enhancing the rate of the reaction.

Enzymes often employ multiple mechanisms simultaneously to enhance their catalytic efficiency. The combination of proximity and orientation, acid-base catalysis, covalent catalysis, and the entropy effect allows enzymes to accelerate reactions to levels that would be otherwise unattainable under normal physiological conditions.

A good example of enzyme action is the mechanism of ***acetylcholinesterase (AChE)*** reaction. This enzyme that plays a crucial role in terminating the action of the neurotransmitter acetylcholine (ACh) at cholinergic synapses. Its primary function is to hydrolyze acetylcholine into choline and acetate, thereby preventing prolonged stimulation of cholinergic receptors. The mechanism of acetylcholinesterase action is as follows:

- **Substrate Binding:** Acetylcholine (ACh) binds to the active site of acetylcholinesterase through non-covalent interactions, specifically hydrogen bonding and electrostatic interactions (fig. 2.16).

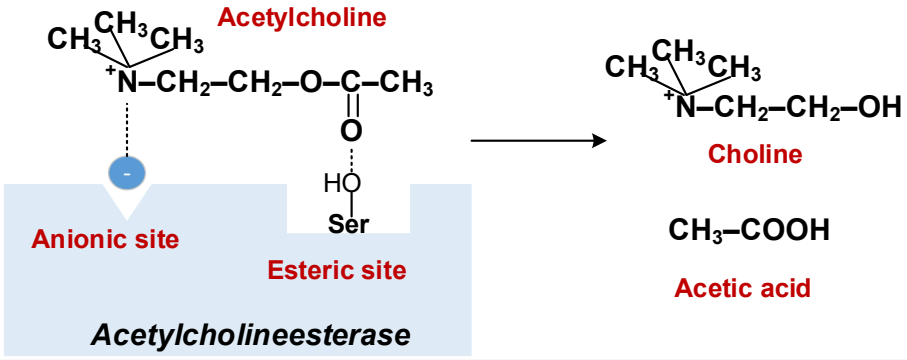

Fig. 2.16. Acetylcholinesterase catalyses the reaction of hydrolysis of acetylcholine, terminating its action at cholinergic synapses.

- **Transition State Stabilization:** As the ACh molecule binds to the active site, the serine residue in the catalytic site of acetylcholinesterase undergoes a nucleophilic attack on the acetyl group of ACh. This forms a covalent enzyme-substrate intermediate called an acyl-enzyme complex.
- **Acylation:** The nucleophilic attack of the serine residue on the acetyl group of ACh results in the transfer of the acetyl group to the serine residue of acetylcholinesterase. This acyl-enzyme complex is short-lived.
- **Transition State Destabilization:** The acyl-enzyme complex undergoes a transition state destabilization, which is facilitated by the active site of acetylcholinesterase. This helps in the rapid breakdown of the acyl-enzyme complex.
- **Choline Release:** The acyl-enzyme complex undergoes a rapid hydrolysis reaction, resulting in the release of choline and acetate from the active site of acetylcholinesterase.
- **Regeneration of the Enzyme:** After choline is released, acetylcholinesterase is regenerated and ready to bind to and hydrolyze more acetylcholine molecules.

2.11. Activity of enzymes. Units of enzymatic activity. Methods of enzymatic activity assays.

The enzyme activity is detined as the quantity ot substrate converted by ot a given amount of an enzyme per time unit in the presence enzyme under specified assay conditions.

The specific activity of an enzyme preparation is defined as the number of enzyme units per milligram of protein *(µmol/min of protein or U/mg of protein*). The specific activity is frequently used to characterize the degree of purification oi isolated enzymes

The Commission on Enzyme Nomenclature of the International Union of Biochemistry is recommended unit – **katal**, is proposed where 1 katal denotes the conversion of 1 mol substrate per second.

For practical reasons usually the activity is expressed as [umol/mm] This term is named **international unit (U)** if the measurement is performed under standard conditions (with isolated enzymes at conditions which are optimized is much as possible).

The turnover number of an enzyme is defined as the number of molecules converted by one molecule of enzyme per unit of time if the enzyme is saturated with

substrate ([E]= [ES]) It is identical to the rate constant k and can be calculated as k = Vm /[E] Most turnover numbers are in the range of 10^4.

Methods for the determination of enzymatic activity can be divided into: **direct, indirect and coupled** assays. The **direct method** is based on measurement of the substrate or product concentration as a function of time. For example, the enzyme *cytochrome c oxidase* catalyzes the oxidation of the heme-containing protein *cytochrome c.* In its reduced (ferrous iron) form, *cytochrome c* displays a strong absorption band at 550 nm, which is significantly diminished in intensity when the heme iron is oxidized (ferric form) by the oxidase. One can thus measure the change in light absorption at 550 nm for a solution of ferrous *cytochrome c* as a function of time after addition of *cytochrome c oxidase*; the diminution of absorption at 550 nm that is observed is a direct measure of the loss of substrate (ferrous *cytochrome c*) concentration.

In some cases the substrate and product of an enzymatic reaction do not provide a distinct signal for convenient measurement of their concentrations. Often, however, product generation can be coupled to another, nonenzymatic, reaction that does produce a convenient signal; such a strategy is referred to as an **indirect** assay. *Dihydroorotate dehydrogenase (DHODase)* provides an example of the use of an indirect assay. This enzyme catalyzes the conversion of dihydroorotate to orotic acid in the presence of the exogenous cofactor ubiquinone. During enzyme turnover, electrons generated by the conversion of dihydroorotate to orotic acid are transferred by the enzyme to a ubiquinone cofactor to form ubiquinol. It is difficult to measure this reaction directly, but the reduction of ubiquinone can be coupled to other nonenzymatic redox reactions.

A third way of following the course of an enzyme-catalyzed reaction is referred to as the **coupled** assays method. Here the enzymatic reaction of interest is paired with a second enzymatic reaction, which can be conveniently measured. In a typically coupled assay, the product of the enzyme reaction of interest is the substrate for the enzyme reaction to which it is coupled for convenient measurement. An example of this strategy is the measurement of activity for *hexokinase*, the enzyme that catalyzes the formation of glucose 6-phosphate and ADP from glucose and ATP. None of these products or substrates provide a particularly convenient means of measuring enzymatic activity. However, the product glucose 6-phosphate is the substrate for the enzyme glucose 6-phosphate dehydrogenase, which, in the presence of $NADP^+$, converts this molecule to 6-phosphogluconolactone. In the course of the second enzymatic reaction, $NADP^+$ is reduced to NADPH, and this cofactor reduction can be monitored easily by light absorption at 340 nm.

REVIEW TEST:

№	MCQs	Answers and explanations
1.	**When investigating human saliva it is necessary to assess its hydrolytic properties. What substance should be used as a substrate in the process?** A. Starch B. Proteins C. Fats D. Fiber E. Amino acids	**The answer is A** The main hydrolytic enzyme of saliva is amylase, it catalyses the hydrolysis of starch. The salivary gland makes amylase to hydrolyse α-1,4-glycosidic bonds of starch into disaccharides (maltose) which are converted by other enzymes to glucose to supply the body with energy. As *diastase*, amylase was the first enzyme to be discovered and isolated (by A. Payen in 1833).

2.	**There are several groups of molecular mechanisms playing an important part in the pathogenesis of insult to cells which contributes to the pathology development. What processes are stimulated by proteinic damage mechanisms?** A. Osmotic membrane distension B. Lipid peroxidation C. Phospholipase activation D. Enzyme inhibition E. Acidosis	**The answer is D** Enzyme is a substance of protein structure that acts as a catalyst in living organisms, regulating the rate at which chemical reactions proceed without itself being altered in the process. Proteolitic damage leads to the break down of enzymes and inhibition of their functions that may cause the pathochemical changes in many pathologies.
3.	**In the cell, enzymes are located in subsequent organelles, providing their specific functioning. Note enzymes located in lysosomes:** A. Fatty acid synthesis enzyme complex B. Cathepsins and glucosaminidase C. Enzymes of protein biosynthesis D. Enzymes of urea synthesis E. Glycogen synthetase and branching enzyme	**The answer is B** Lysosomes are the key degradative compartments of the cell. Lysosomal cathepsins and glucosaminidase, which are enclosed in the lysosomes, help to maintain the homeostasis of the cell's metabolism by participating in the degradation of heterophagic and autophagic material. Ezymes of fatty acid, protein, urea and glycogen synthesis are mainly localised in the cytoplasm of living cells.
4.	**Cytochrome c participates in the transport of electrons in the respiratory chain of the cell and is located in the next cellular compartment:** A. Golgi vesicles B. Cytoplasm C. Nucleus D. Lysosomes E. Mitochondria	**The answer is E** Cytochrome c is an essential component of the electron transport chain, where it carries one electron. In eukaryotes, an electron transport or respiratory chain is found in the inner mitochondrial membrane where it serves as the site of oxidative phosphorylation through the action of ATP synthase.
5.	**Michaelis-Menten constants of two enzymes are 1.3×10^{-5} M/l and 2.3×10^{-3} M/l subsequently. Indicate true statement about the affinity of these enzymes to substrate.** A. The first enzyme has the higher affinity to a substrate B. Enzymes possess the equal affinity to a substrate C. The second enzyme has the higher affinity to a substrate D. For decision, information on the concentration of enzyme is needed E. Data are incomplete and it is impossible to draw a conclusion	**The answer is A** In an enzyme catalysed reaction when there is large excess of the substrate and the enzyme concentration is held constant, if substrate concentration (S) is plotted against velocity (V) or reaction rate, a hyperbolic curve is obtained. The concentration of substrate required to half saturate the enzyme or in other words to cause half the maximal reaction rate (1/2 V_{max}) called as Michaelis Constant or Michaelis-Menten Constant and is denoted by Km. Michaelis constant is a reflection of the affinity of enzyme for its substrate and is characteristic of a particular enzyme-substrate system. The smaller the value of Km, the more strongly the enzyme binds the substrate. That is why the first enzyme with Km 1.3×10^{-5} M/l has higher affinity to substrate than the second enazyme which Km is 2.3×10^{-3} M/l.
6.	**A patient with hypochromic anemia has splitting and loss of hair, increased nail**	**The answer is B** Hypochromic anemia is also called iron-deficiency

	brittling and taste alteration. What is the mechanism of the development of the symptoms? A. Deficiency of vitamin B_{12} B. Deficiency of iron-containing enzymes C. Decreased production of parathyrin D. Decreased production of thyroid hormones E. Deficiency of vitamin A	anemia. Iron deficiency is the state in which a body has not enough iron to supply its eventual needs. Most of the iron in the body is present in the RBC as hemoglobin, a molecule composed of four units, each containing one heme group and one protein chain. The iron-containing oxygen storage protein in the muscles, myoglobin, is similar in structure to hemoglobin but has only one heme unit and one globin chain. Several iron-containing enzymes, the cytochromes, also have one heme group and one globin protein chain. These enzymes act as electron carriers within the cell and their structures do not permit reversible loading and unloading of oxygen. Their role in the oxidative metabolism is to transfer energy within the cell and specifically in the mitochondria. Other key functions for the iron-containing enzymes (e.g., cytochrome P450) include the synthesis of steroid hormones and bile acids; detoxification of foreign substances in the liver; and signal controlling in some neurotransmitters, such as the dopamine and serotonin systems in the brain. Iron is reversibly stored within the liver as ferritin and hemosiderin whereas it is transported between different compartments in the body by the protein transferrin.
7.	**The cytochemical investigation has revealed the high content of hydrolytic enzymes in cytoplasm. This phenomenon indicates high activity of the following organelles:** A. Cytocentrum B. Endoplasmic reticulum C. Polysomes D. Lysosomes E. Mitochondria	**The answer is D** Lysosomes are membrane-enclosed compartments filled with hydrolytic enzymes that are used for the controlled intracellular digestion of macromolecules. They contain about 40 types of hydrolytic enzymes, including proteases, nucleases, glycosidases, lipases, phospholipases, phosphatases, and sulfatases. The presence of hydrolytic enzymes in cytosol indicates the increased activity of lysosomal enzymes or impaired lysosomal functions.
8.	**Which one of the following ailments, seen by an emergency room physician, is most likely caused by enzyme denaturation?** A. A 34-year-old man diagnosed with a gastrinoma complaining of diarrhea for 2 weeks B. A 58-year-old man with chest pain and shortness of breath with increased activity C. An 18-year-old boy presenting with a sore throat and fever of $38,5^0$C; he has small minimally tender anterior cervical lymph nodes and a red pharynx D. A 48-year-old woman complaining of knee pain after twisting her leg playing tennis	**The answer is A** The excessive gastric acid secretion caused by the gastrinoma tumor results in a paradoxical acidic environment in the duodenum, which can denature the pancreatic digestive enzymes and prevent them from properly digesting nutrients. This can lead to diarrhea due to the undigested nutrients being unable to be absorbed in the gut. Although a fever (choice C) included an increase in temperature, most proteins are denatured above 50^0C, a temperature well above the normal body temperature of 38^0C. The other choices (potential heart attack, choice B; an ear infection, choice D; and a sore knee, choice E) are not initially a result of enzyme denaturation.

	E. An 18-month-old boy with a 4-day history of symptoms of an upper respiratory infection presenting with fever, irritability, and pulling at his left ear for the past 24 hours	
9.	**A 3-year-old boy in good health began having generalized seizures consisting of a sudden turning of the head to the left, tonic posturing of the left arm, and loss of awareness for1to 2 minutes. The patient was successfully treated with the anticonvulsant phenytoin (dilantin). Dilantin is a substrate that binds to and is metabolized by an enzyme in the liver. Which one of the following statements best describes the relationship between an enzyme, substrate, and product?** A. All the active sites of the enzyme are saturated with substrate at high substrate concentrations B. Enzyme–product complexes enhance substrate binding C. At high substrate concentrations, substrate–substrate interactions interfere with enzyme activity D. At low substrate concentrations, none of the enzyme is found in the ES complex. E. Significant product formation results in activation of the reaction	**The answer is A** The rate of an enzyme-catalyzed reaction will generally increase exponentially with respect to substrate concentration until the substrate concentration exhausts the catalytic sites of the enzyme population. Once this occurs, the rate of reaction remains the same regardless of an increase of substrate because all enzymes are saturated (Vmax has been achieved). Substrate cannot bind to enzyme–product complexes because the substrate binding sites are occupied by product. Substrate–substrate interactions are the same regardless of concentration of substrate, and such interactions do not affect enzyme activity. An ES complex can form at low substrate concentration as well as at high substrate concentration. Product formation does not stimulate enzyme activityand can slow down the reaction rate.
10	**Active centers in non conjugated (simple) enzymes, e.g. trypsin, are formed by the next constituents of enzyme molecule:** A. Peptide bonds between selected amino acids B. Amino acid side chains only C. Nucleotides D. Carbohydrates **E.** Phospholipids	**The answer is B** Active centers of simple enzymes are composed of amino acids residues only. The most frequently used amino acids in active centers of many enzymes are: Serine, Aspartic acid, Histidine, Lysine, Glutamic acid, Cysteine. Trypsin is a medium size globular protein that functions as a pancreatic serine protease. Trypsin is composed of 220 amino acid residues. Three amino acid His57, Asp102, and Cys195 are vital to the proteolytic function of the trypsin.

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3. REGULATION OF ENZYMATIC ACTIVITY. MEDICAL ENZYMOLOGY

OBJECTIVES after studying this chapter, you should be able to:

- *Appreciate pathways and mechanisms of regulation of enzymatic reactions as a background of metabolism in health and disease.*
- *Explain the benefits of enzymes being produced as proenzymes and discuss the characteristic structural modifications that occur during the conversion of a proenzyme into its active form.Outline the application of activators and inhibitors of enzymes as medicines and pharmaceuticals for correction of metabolic disorders in pathology.*
- *Describe changes in metabolic pathways and accumulation of distinct metabolic intermediates in the inborn (hereditary) and acquired disorders of metabolism – enzymopathias.*
- *Analyze changes in activity of indicatory enzymes in blood plasma in pathology of distinct organs and tissues.*

3.1 Regulation of enzyme synthesis and degradation.

The quantity of enzyme (as a protein) in a cell, tissue or body is regulated by:

I. Enzyme induction and repression.
II. Enzyme degradation.

According to the type of the **control of enzyme synthesis** (induction or repression) there are two types of enzymes:

- **Constitutive enzymes** (house-keeping enzymes) – the levels of which are not controlled and remain fairly constant. They are present in fixed quantities.
- **Inducible enzymes** are enzymes whose synthesis is increased in response to the presence of a specific molecule or substrate called an inducer. The synthesis of these enzymes is regulated at the level of gene expression, meaning that the genes that code for these enzymes are only turned on and transcribed into messenger RNA when the inducer is present. This allows for the cell to quickly respond to changing environmental conditions and adjust its metabolism accordingly. Examples of inducible enzymes include *lactase*, which is induced by lactose in bacteria and yeast, and cytochrome P450 enzymes, which are induced by foreign chemicals and drugs in the liver. Increased synthesis of an inducible enzyme in response to inducer is known as **induction.**

Induction and repression are both regulatory mechanisms that control the synthesis of enzymes in response to changes in the body's needs. Induction refers to the increased synthesis of enzymes in response to certain signals or inducers, while repression refers to the decreased synthesis of enzymes in response to other signals or repressors. These regulatory mechanisms are essential for maintaining a balance between the production and utilization of metabolic intermediates in the body. For example, *arginase,* an enzyme of urea-cycle formation is more in starvation and on high protein diet, *pyruvate carboxylase*

an enzyme of gluconeogenesis is induced by glucocorticoids and repressed by insulin, phenobarbitol and anti-convulsive drug induces *alkaline phosphatase*.

MEDICAL IMPORTANCE

Cyclooxygenase (COX) exists in form of 2 main isoforms. COX-1 (constitutive) is responsible for the production of prostaglandins that are important for the normal functioning of various physiological systems such as the stomach lining and blood clotting. On the other hand, COX-2 is induced in response to inflammatory stimuli and is involved in the biosynthesis of prostaglandins that promote inflammation, pain, and fever. Inhibition of COX-2 by drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) can help in relieving inflammation and pain, but it can also lead to side effects such as gastrointestinal bleeding and cardiovascular events.

Regulation of enzyme activity by the degradation. Enzyme activity is regulated through degradation, as enzymes produced during development or in response to environmental conditions or toxins are no longer necessary and can become harmful if they persist in the body. Immortal enzymes would lead to unwanted side effects, so turnover is necessary, with individual enzymes having varying lifespans ranging from seconds to days. Mechanisms exist for specific enzyme degradation, with key metabolic enzymes degraded quickly and defective enzymes rapidly degraded as they are no longer useful to the body.

3.2. Enzyme inhibition.

An enzyme inhibitor is a substance that binds to an enzyme and results in a reduction of its catalytic activity. The inhibitor may be of organic or inorganic origin. Enzyme inhibitors can be broadly classified into two main categories (reversible and irreversible) (fig. 3.1).

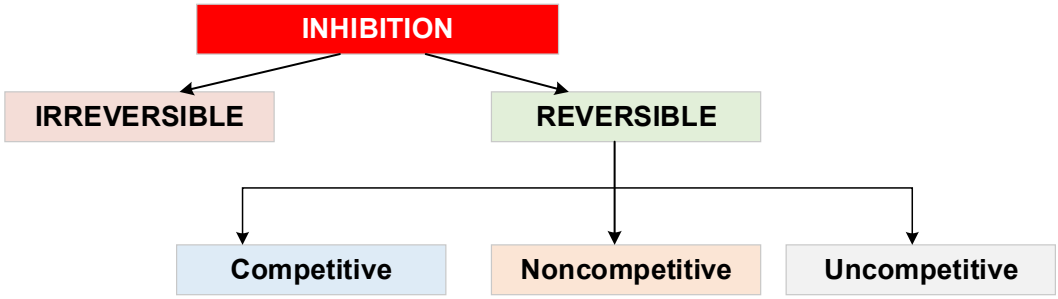


Fig. 3.1. Types of enzyme inhibitors

Irreversible inhibitors irreversibly bind to the functional groups of amino acids in the active site of an enzyme. This can occur through covalent modification of the active site if the inhibitor reacts at or near the active site. In other cases, the inhibitor may not structurally resemble the enzyme's substrate and can bind to a different site than the substrate-binding site. This type of inhibition is similar to non-competitive inhibition but involves the formation of covalent bonds.

For example, **iodoacetate** is a common example of an **irreversible inhibitor** that can bind to and inactivate *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)*, an important enzyme involved in glycolysis. Iodoacetate functions as an alkylating agent, meaning it can form a covalent bond with a nucleophilic group on the enzyme, rendering

it inactive. Specifically, iodoacetate can react with the active site cysteine residue of *GAPDH* (fig. 3.2), which is essential for the enzyme's catalytic activity. Once iodoacetate forms a covalent bond with this cysteine residue, it can no longer participate in the enzyme's catalytic mechanism, effectively rendering the enzyme inactive. Since iodoacetate is an irreversible inhibitor, the inhibition of *GAPDH* activity is long-lasting and requires the synthesis of new enzyme to restore the glycolytic pathway. This makes iodoacetate a useful tool for studying the regulation of glycolysis and the role of *GAPDH* in cellular metabolism.

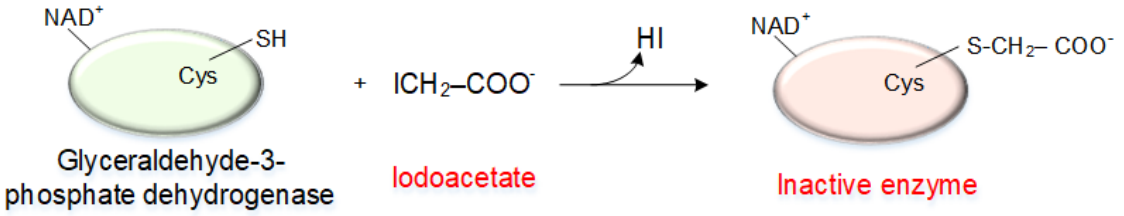


Fig. 3.1. Irreversible inhibition of glyceraldehyde-3-phosphate dehydrogenase by iodoacetate, wich provides an important example of how enzymes can be inactivated by covalent modification, and how such modifications can be used to study enzyme function and regulation

Diisopropyl fluorophosphate (DFP) is an example of an irreversible inhibitor of enzymes. DFP is an organophosphate compound that can covalently modify the active site serine residue of enzymes that contain a serine protease catalytic triad, including *acetylcholinesterase and trypsin.* DFP is highly reactive and can form a stable covalent bond with the serine residue in the active site of these enzymes. Once this bond is formed, the enzyme is permanently inactivated and cannot be restored to its original activity. DFP has been used extensively in biochemical research to study the mechanisms of enzymes that contain a serine protease catalytic triad, as well as to develop new drugs that target these enzymes. For example, DFP-based inhibitors have been developed to treat diseases such as Alzheimer's, in which the activity of *acetylcholinesterase* is implicated.

Disulfiram is a drug used in the treatment of alcoholism it irreversibly inhibits the enzyme *aldehyde dehydrogenase. Aldehyde dehydrogenase* is an enzyme involved in the metabolism of alcohol in the liver. It converts acetaldehyde, a toxic intermediate produced during the breakdown of alcohol, into acetic acid, which is further metabolized and eliminated from the body. Disulfiram binds covalently to the sulfhydryl group of the active site cysteine residue of ALDH, rendering the enzyme permanently inactive. By inhibiting ALDH, disulfiram causes a buildup of acetaldehyde in the blood, leading to unpleasant symptoms such as nausea, headache, flushing, and palpitations when alcohol is consumed. This serves as a deterrent for individuals seeking to abstain from alcohol, as the unpleasant symptoms make drinking alcohol less desirable. Additionally, disulfiram has been studied as a potential treatment for other conditions, such as cocaine addiction and cancer, due to its ability to inhibit ALDH activity.

Suicide inhibition is irreversible because the inhibitor becomes covalently bound to the enzyme during the inhibition and thus cannot be removed. Suicide inhibition rather closely resembles competitive inhibition because the inhibitor generally resembles the substrate and binds to the active site of the enzyme. The primary difference is that the suicide inhibitor is chemically reactive in the active site and makes a bond with it that

precludes its removal. For example, penicillin covalently links to the bacterial enzyme, *D-D transpeptidase* and stops it from functioning. Since the normal function of the enzyme is to make a bond necessary for the peptidoglycan complex of the bacterial cell wall, the cell wall cannot properly form and bacteria cannot reproduce.

Reversible inhibitors: act by forming a loose, dissociable complex with the corresponding enzyme. Inhibitors can be washed out (removed) of the solution of enzyme by dialysis. Is divided into two categories:

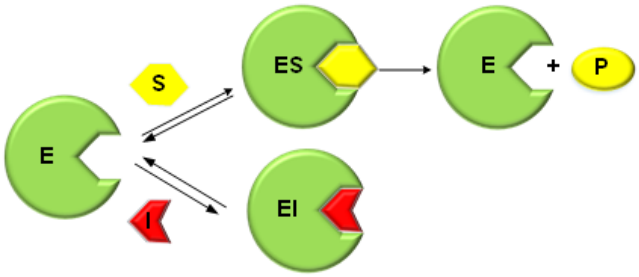


Figure 3.3. Competitive inhibition

Competitive inhibitors are substances that share a structural similarity with the substrate molecules and thus compete with them for the active site. As a result, the inhibitor's effectiveness is directly proportional to its concentration (as shown in figure 3.3). For example, **malonate** is a structural analogue of succinate it inhibits *succinate dehydrogenase* (fig.3.4). Malonate's molecular structure is similar to that of succinate, the natural substrate of the enzyme. Therefore, malonate can bind to the active site of the enzyme and prevent succinate from binding, thereby reducing the enzyme's activity.

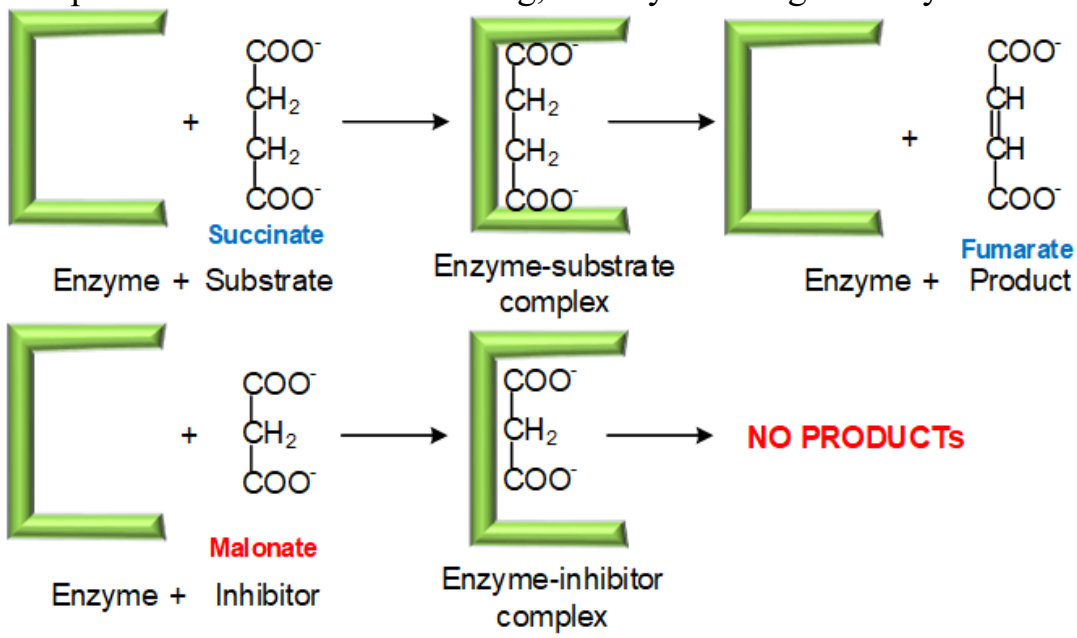


Fig. 3.4. Malonate competitively inhibits succinate dehydrogenase, which is an enzyme involved in the citric acid cycle.

Sulfonamide drugs have a similar chemical structure to para-aminobenzoic acid (PABA), which is a necessary precursor for the synthesis of folates. Sulfonamides can competitively inhibit the enzyme *dihydropteroate synthase*, which is involved in the production of folates. Because of the structural similarity between sulfonamides and PABA, the sulfonamide drug molecules can bind to the active site of the enzyme and block the entry of PABA, leading to a reduced production of folates. This can be detrimental to bacterial growth, as folates are important cofactors for many metabolic pathways.

The degree of competitive inhibition is determined by the relative concentrations of the substrate and inhibitor, as well as their respective affinities for the enzyme. **A high substrate concentration can overcome the inhibition** by effectively outcompeting the inhibitor for the enzyme's active site.

In competitive inhibition, the inhibitor binds to the active site of the enzyme, thereby preventing the substrate from binding. This results in an increase in the apparent Michaelis constant (Km) because the enzyme requires a higher concentration of substrate to achieve the same reaction rate as in the absence of the inhibitor. However, the maximum reaction rate (Vmax) remains unchanged because the inhibitor does not affect the enzyme's catalytic activity once it has bound to the active site (fig. 3.5).

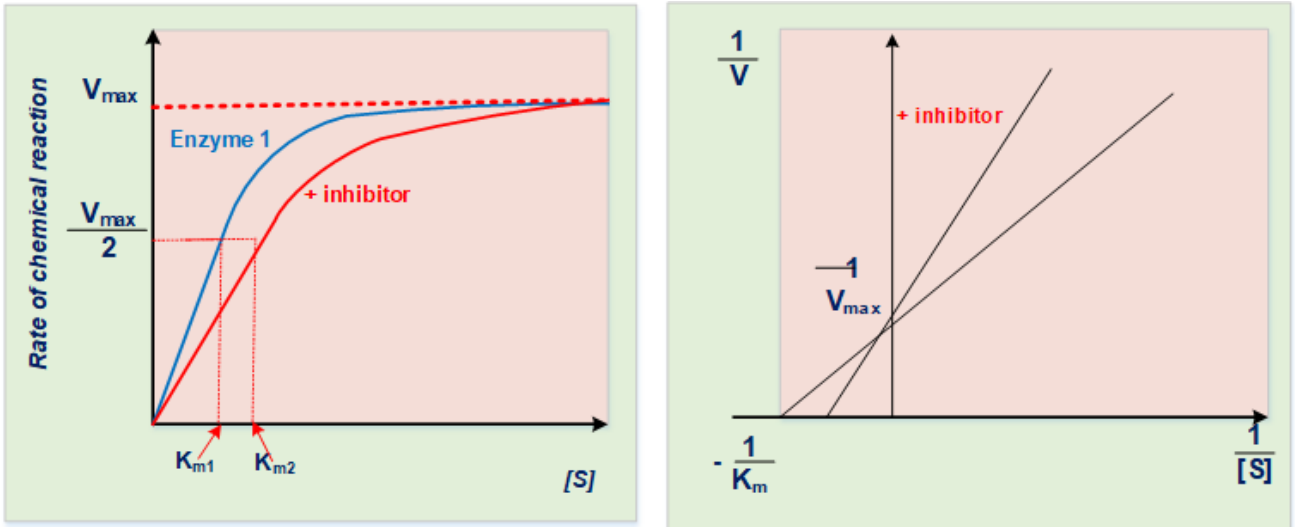


Fig. 3.5. Kinetic parameters in competitive inhibition. Competitive inhibition increases the apparent Km value and does not affect the maximum reaction rate of the enzyme.

Non-competitive inhibitors bind to a different site on the enzyme than the active (substrate-binding) site (as illustrated in figure 3.6), resulting in enzyme inhibition. Unlike competitive inhibitors, non-competitive inhibitors do not have a similar structure to the substrate and cannot be overcome by increasing the substrate concentration. Non-competitive inhibitors can act on both the free enzyme and the enzyme-substrate complex, which prevents the reaction from progressing to the product formation.

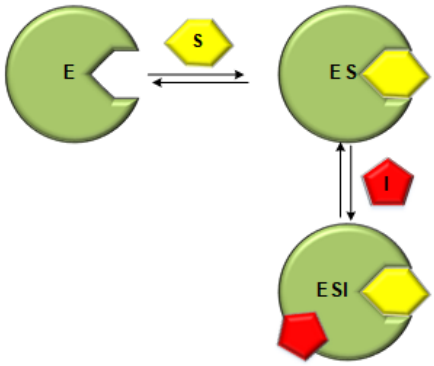


Figure 3.6. Non-competitive inhibition

Fluoride is a reversible non-competitive inhibitor of ***enolase***, which is an enzyme involved in glycolysis. Enolase catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate in the second-to-last step of glycolysis. Fluoride is known to bind to the active site of enolase, inhibiting its activity by preventing the formation of the enzyme-substrate complex. This binding is reversible, meaning that once the fluoride is removed, enolase activity can be restored. The inhibition of enolase by fluoride can have significant effects on cellular metabolism and energy production, as

glycolysis is a central pathway for the production of ATP, the primary energy currency of the cell.

Heavy metals such as Hg^{2+}, Ag^{+}, Pb^{2+}, and arsenite are known to act as enzyme poisons or **non-competitive inhibitors**. These metals can bind to enzymes and disrupt their structure and function, leading to enzyme inhibition and potentially harmful effects on cellular metabolism and physiology.

For example, mercury (Hg^{2+}) is a potent inhibitor of enzymes involved in cellular respiration and ATP production, such as *cytochrome oxidase* and *ATP synthase*. This can lead to a decrease in ATP production and energy levels, which can be harmful to the cell.

Silver (Ag^{+}) has been shown to inhibit a wide range of enzymes, including those involved in DNA replication and protein synthesis. This inhibition can disrupt cellular processes and lead to decreased cell viability.

Lead (Pb^{2+}) can inhibit enzymes involved in heme synthesis and the nervous system, leading to toxic effects on brain function and development.

Arsenite can inhibit enzymes involved in glycolysis and the citric acid cycle, leading to decreased ATP production and cellular energy levels.

For non-competitive inhibition, the K_m value is unchanged while Vmax is lowered (fig. 3.7).

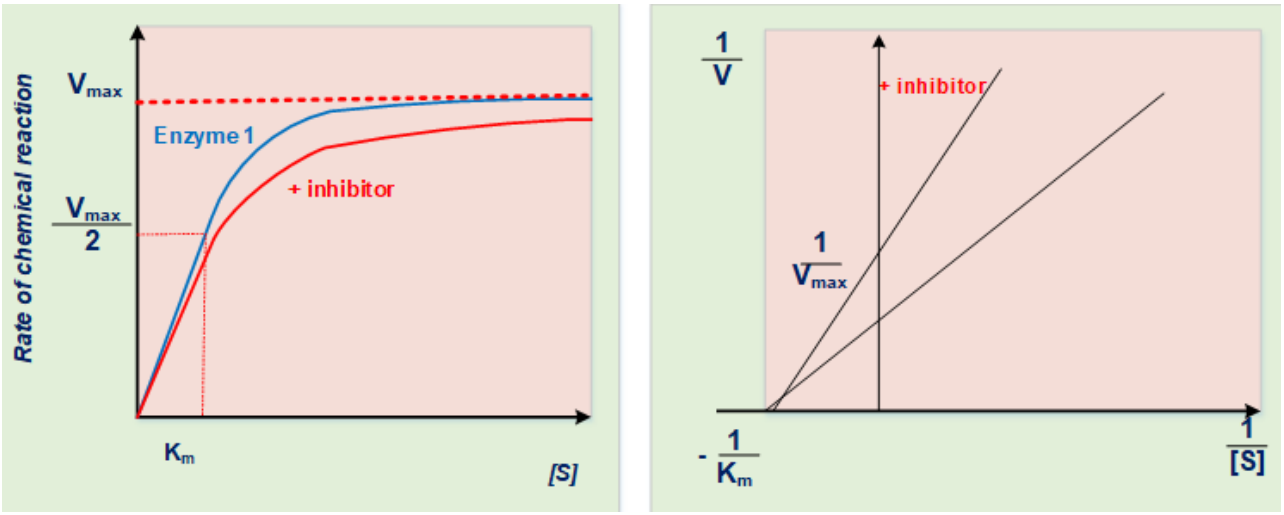


Fig. 3.7. Kinetic parameters in non-competitive inhibition. In non-competitive inhibition, the inhibitor binds to a site on the enzyme that is distinct from the active site, leading to a change in the enzyme's conformation and activity. This binding results in a decrease in the maximum velocity (Vmax) of the enzyme-catalyzed reaction and does not affect the affinity of the enzyme for the substrate (Km).

The comparison of competitive and non-competitive inhibition is given in table 3.1

Uncompetitive inhibitors: bind only to the enzyme-substrate (ES) complex The inhibitor-bound complex forms mostly under concentrations of high substrate and the ES-I complex cannot release product while the inhibitor is bound, thus result in reduced V*max.*

Uncompetitive inhibitors are a type of enzyme inhibitor that binds specifically to the enzyme-substrate (ES) complex. The binding of the inhibitor to the ES complex stabilizes the complex and prevents the release of the product, leading to a decrease in the rate of the reaction and a reduction in the maximum velocity (Vmax) of the reaction. The binding of the uncompetitive inhibitor to the ES complex is dependent on the concentration

of substrate, with the formation of the ES-I complex occurring predominantly at high substrate concentrations. This is because the inhibitor can only bind to the ES complex,

Potentially confusing questions concerning noncompetitive and uncompetitive uinhibition. *It's important to note that the terminology used to describe enzyme inhibition can vary in different texts or scientific literature. In some cases, noncompetitive inhibitors may be referred to as mixed inhibitors, especially if the focus is on their ability to bind to both the free enzyme and the enzyme-substrate complex. This can lead to confusion, as the specific definitions and mechanisms of uncompetitive and noncompetitive inhibition are distinct.*

and at low substrate concentrations, there is less ES complex available for binding.

Table 3.1. The comparative characteristics of competitive and noncompetitive types of revercible inhibition

Inhibitor type	Binding site on enzyme	Kinetic effect
COMPETITIVE	Specifically at the catalytic site, where it competes with substrate for binding in a dynamic equilibrium-like process. Inhibition is reversible by substrate.	Vmax is unchanged. Km, as defined by [S] required for 1/2 maximal activity, is increased.
NONCOMPETITIVE	Binds E or ES complex other than at the catalytic site. Substrate binding unaltered, but ESI complex cannot form products, inhibition cannot be reversed by substrate.	Vmax is decreased proportionately to inhibitor concentration. Km appears unaltered.

Nocompetitive inhibitors are less common than other types of inhibitors, but there are still several examples of uncompetitive inhibitors known in biochemistry. Here are a few examples:

- **Amino acids**: Some amino acids, such as phenylalanine, can act as uncompetitive inhibitors of certain enzymes. For example, **phenylalanine** can inhibit the enzyme *phenylalanine hydroxylase*, which is responsible for converting phenylalanine to tyrosine.
- **Metal ions:** Certain metal ions, such as mercury and silver, can act as uncompetitive inhibitors of enzymes. For example, **mercury** can inhibit the enzyme *urease*, which is involved in the breakdown of urea.
- **Some drugs**: Some drugs, such as the **antifungal drug ketoconazole** and the **anticancer drug imatinib**, can act as uncompetitive inhibitors of enzymes. For example, **imatinib** can inhibit the enzyme *tyrosine kinase*, which is involved in cell signaling pathways.

In terms of kinetic parameters, the presence of an uncompetitive inhibitor results in a decrease in both the Vmax and the Km values. This is because the inhibitor binds only to the ES complex and reduces the amount of active enzyme available to catalyze the reaction, while also reducing the affinity of the enzyme for the substrate.

Thus, uncompetitive inhibitors are unique in that they require the presence of both substrate and enzyme to exert their inhibitory effects.

3.3. Regulation of enzyme activity in the living system.

In biological system, regulation of enzyme activities occurs at different stages in one or more of the following ways:

- Allosteric regulation.
- Feedback inhibition.
- Covalent modification.
- Activation of latent enzymes.
- Action of second messengers.
- Control of enzyme synthesis.

Allosteric regulation is a type of enzyme regulation where certain substances, known as allosteric modulators, bind to a specific site on the enzyme, called the allosteric site, to regulate its activity. These modulators can either be **positive or negative**, and their binding can lead to an increase or decrease in enzyme activity, respectively.

When a **positive allosteric effector** binds to the allosteric site, also known as the activator site, the enzyme activity is increased. On the other hand, when **a negative allosteric effector** binds to the allosteric site, called the inhibitor site, the enzyme activity is inhibited.

Most allosteric enzymes are composed of multiple subunits, and the binding of allosteric modulators can cause a conformational change in the enzyme structure, which affects the active site's shape and, therefore, its catalytic activity (see fig. 3.7).

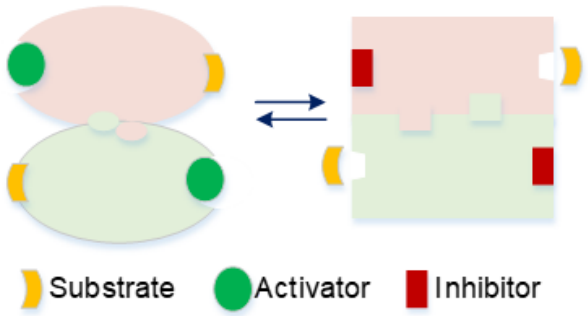


Fig. 3.7. Simplified illustration of allosteric regulation

In summary, allosteric regulation is a type of enzyme regulation that involves the binding of allosteric modulators to an enzyme's allosteric site, resulting in a change in enzyme activity through a conformational change in enzyme structure.

Allosteric enzymes differ from classical Michaelis-Menten enzymes in that their velocity-substrate concentration plot is **sigmoidal or S-shaped**, rather than hyperbolic. This sigmoidal curve suggests a rapid increase in velocity after a certain substrate concentration, indicating the phenomenon of cooperativity. To explain this cooperativity in allosteric enzymes, the **T and R model** was proposed.

According to the T and R model, allosteric enzymes exist in two states, a tense (T) state and a relaxed (R) state. The binding of a substrate (ligand) to the T form is initially slow and causes a conformational change in the subunits, resulting in the R form. Subsequent binding of ligands to the R form is rapid.

Allosteric inhibitors stabilize the enzyme in the T form, which is less active, resulting in decreased enzyme activity. On the other hand, allosteric activators stabilize the enzyme in the R form, which is highly active, leading to an increase in enzyme activity.

This model explains the sigmoidal curve seen in the velocity-substrate concentration plot of allosteric enzymes.

Feedback regulation is a process where the final product of a metabolic pathway inhibits the first or second step of the pathway, often by binding to the allosteric site of a regulatory enzyme. This inhibition leads to the cessation of the pathway, ensuring that the concentration of the end product does not exceed a certain threshold. Figure 3.8 illustrates how an end product binds to the allosteric site of a regulatory enzyme, causing inhibition of the pathway.

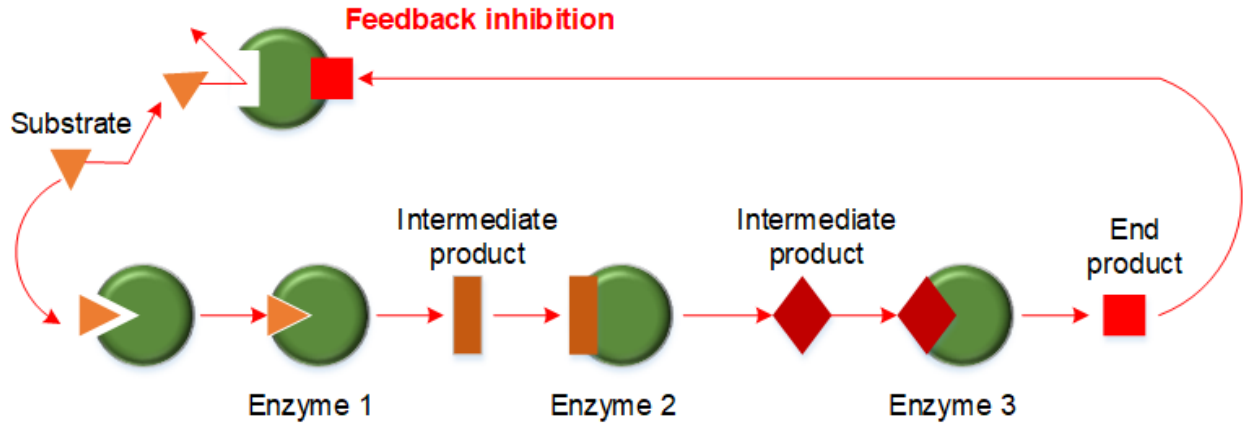


Fig. 3.8 Feedback regulation of enzymatic activity

Feedback inhibition is necessary to control metabolic pathways for efficient cellular function. For example, the inhibition of *aspartate transcarbamoylase* by CTP; the inhibition of *HMG-CoA reductase* by cholesterol; the inhibi *ALA-synthase* by heme; the inhibition of *anthranilate synthetase* by tryptophan.

Feedback inhibitors are typically structurally distinct from the substrates of the enzymes they inhibit. For example, serine, which is a feedback inhibitor of *3-phosphoglycerate dehydrogenase* (the enzyme that catalyzes the first committed step in serine biosynthesis), bears no resemblance to the substrates of the enzyme, namely NAD+ and 3-phosphoglycerate. This lack of structural similarity allows for specificity in the regulation of metabolic pathways.

Covalent modification of enzymes is a posttranslational modification process that involves the covalent alteration of enzyme structure, leading to changes in enzyme activity. This modification can occur by adding or removing a group from the enzyme structure. Several covalent modifications of enzymes have been identified, including **phosphorylation, methylation, acetylation, UDP-ribosylation, adenylation**, and others. Phosphorylation-dephosphorylation, which involves the addition or removal of a phosphate group, is the most common covalent modification of enzymes (fig. 3.9):

- phosphate group is often derived from an ATP molecule;
- addition of the phosphate group (phosphorylation) is catalysed by a *kinase* enzyme;
- removal of the phosphate group (dephosphorylation) is catalysed by a *phosphatase* enzyme;
- phosphate group is added to (or removed from) the R group of a serine tyrosine or threonine amino acid residue in the enzyme regulated.

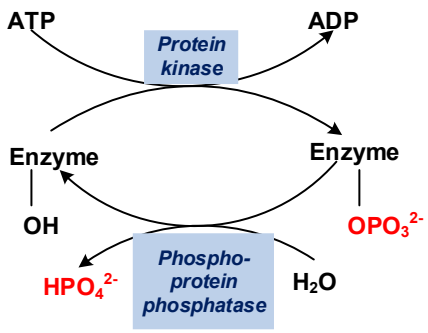


Fig. 3.9. Covalent modification by the addition and removal of phosphate groups.

For example, *pyruvate dehydrogenase and glycogen synthase* are inhibited by phosphorylation, whereas *glycogen phosphorylase* is activated.

Proenzymes. Some enzymes are synthesized as inactive **latent forms** called **zymogens or proenzymes**, for example, *trypsinogen and pepsinogen.* Zymogens are inactive because their catalytic sites are masked by a polypeptide chain. To activate zymogens, the polypeptide chain is cleaved to open the catalytic site for its substrate. This mechanism is called **limited proteolysis**.

In the stomach pepsin is synthesized in inactive pepsinogen form. At the proper acidic pH of gastric juice pepsinogen undergo limited proteolysis, which results in the formation of pepsin. 42 amino acids are cleave from pepsinogen under the action of HCl. When once pepsin is formed it catalyzes its own formation from pepsinogen. This process is called as **autocatalysis.** The protein degradative enzymes of pancreas are synthesized in inactive forms. They are *trypsinogen, chymotrypsinogen, procarboxypeptidase and proelastase.* A lipid digesting enzyme is also produced in pancreatic cells as a zymogen (fig. 3.10). It is *prophospholipase*. The conversion of these pro-enzymes to active enzymes is initiated by *enterokinase* produced by mucosal cells of duodenum. *Enterokinase* removes a hexapeptide from trypsinogen, converting trypsinogen to *trypsin*. When once few molecules of *trypsin* are formed it further catalyzes not only formation from trypsinogen but also the conversion of other proenzymes to active enzymes. Since single molecule of trypsin can trigger the formation of battery of protein digesting enzymes, pancreas has another selfprotecting mechanism. It contains trypsin inhibitor in small amounts.

The formation of blood clot involves activation of (zymogens) blood clotting factors. *Prothrombin* is converted to active *thrombin* by factor X and V. *Thrombin* in turn converts *fibrinogen to fibrin.*

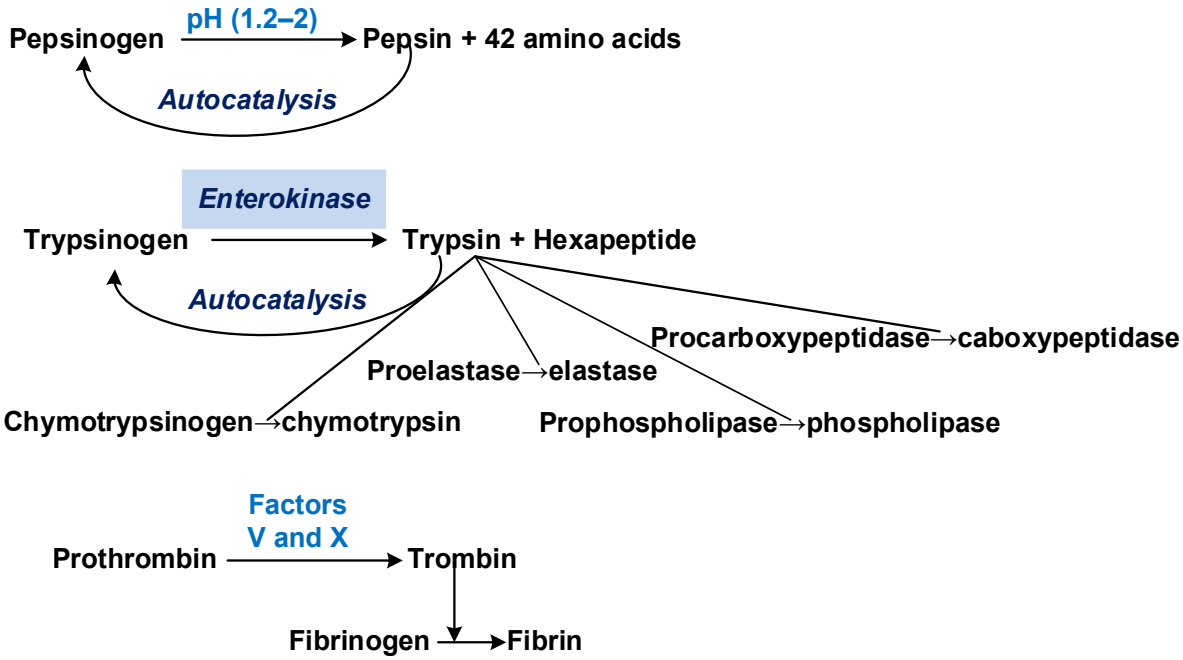


Fig. 3.10. Activation of zymogens by limited proteolysis mechanism

Medical Importance

In acute pancreatitis, trypsinogen can become inappropriately activated to trypsin, leading to a cascade of events that can cause damage to cells and tissues in the pancreas and other organs. Protease inhibitors can help to interrupt this cascade by inhibiting the activity of trypsin and other proteases that contribute to the disease process. By doing so, they can help to reduce inflammation and tissue damage and improve outcomes for patients with acute pancreatitis.

Action of secondary messengers. When a cell receives a signal, it often needs to activate certain enzymes to carry out specific functions. Secondary messengers play a crucial role in this process by relaying signals from the extracellular environment to intracellular targets.

Some of the most common secondary messengers involved in the activation of enzymes include **cyclic AMP (cAMP), calcium ions (Ca^{2+}), and diacylglycerol (DAG).**

cAMP is a molecule that is synthesized by the enzyme *adenylyl cyclase* in response to signals such as hormones or neurotransmitters. cAMP then activates protein *kinase A (PKA)*, which in turn can activate or inhibit a variety of target enzymes.

Calcium ions (Ca^{2+}) are another important secondary messenger that can activate enzymes. In response to a signal, calcium channels on the cell membrane or on internal organelles such as the endoplasmic reticulum or mitochondria can open, causing a rapid influx of calcium ions into the cell. These calcium ions can then activate a variety of enzymes, such as *calmodulin-dependent protein kinase (CaMK)*, which can modify the activity of other enzymes.

DAG is a lipid-derived secondary messenger that is produced when phospholipase C cleaves the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP2). DAG can then *activate protein kinase C (PKC),* which can modify the activity of a wide range of enzymes.

Other secondary messengers involved in enzyme activation include **inositol triphosphate (IP3), nitric oxide (NO), and cyclic GMP (cGMP).** Each of these

secondary messengers can activate specific enzymes or signaling pathways, allowing cells to respond to a wide range of signals and stimuli.

3.4. Application of enzymes.

Uses of enzymes in medicine include:

- **Enzymes as therapeutic agents:** Enzymes have therapeutic potential and have been employed as treatment options for various conditions. Some of the enzymes that are utilized for therapeutic purposes are listed in table 3.2.
-

Table 3.2. Therapeutic uses of enzymes

Name of the enzyme	Availability	Mechanism of action	Indications
A. Enzymes used systemically			
Streptokinase and urokinase	Streptokinase available enzyme 750,000 to 15,00,000 IU vial. Urokinase— 50,000 to 500,000 IU vial	Increases amounts of proteolytic "***plasmin***" by either: • Increasing the circulating level of its precursor ***"plasminogen"*** • Increasing the conversion of plasminogen to plasmin.	• Acute myocardial infarction • Acute thrombosis of arteries Pulmonary embolism. • Deep vein thrombosis (DVT)
L-Asparaginase	Available as ***"Leunase".*** 10,000 KU of L-Asparaginase per vial	Certain tumour cells require L-Asparagine for growth. L-Asparaginase hydrolyses L-Asparagine and growth of tumour cell suffer.	• Acute leukaemia • Malignant lymphomas
Digestive enzymes: amylase, lipase and protease	Available as tablets and syrup	Replacement therapy in pancreatic insufficiency.	• Cystic fibrosis • Chronic pancreatitis • Following pancreatectomy
α-Chymotrypsin	5.775 mg sublingual tablets	Mucolytic and proteolytic activity	**Used as adjunct therapy:** • In management of inflammatory oedema due to injury. • Postsurgical infections and dental procedures.
Serratopeptidase	5 mg tablet	Fibrinolytic activity, high bradykinin decomposing activity, and potent caseinolytic activity	Effective adjunct in inflammation after traumatic injury and after sergury
Enzymes used locally			
Hyaluronidase	Available as "Hyalase" 1500 IU per ml.	Brings about depolymerisation of ground substance and helps in absorption of fluids	• Promotes diffusion of fluids given subcutaneously (SC) • Intra-articular injection in joints to alleviate pain in osteoarthritis

Enzymes are sometimes prescribed as medication to replace deficient enzymes in patients. For instance, **blood clotting factors** can be used to treat **hemophilia**, while ***proteases*** can be employed to break down fibrin and **prevent the formation of harmful blood clots**. ***Proteases*** are also useful **in wound cleaning**, which can accelerate the healing process. ***Asparaginase*** is an enzyme that is used to treat **leukemias**. Tumor cells rely on *asparagine* in the host's plasma for their growth and proliferation. Administering *asparaginase* can drastically reduce the host's plasma levels of asparagine, resulting in a decline in the viability of tumor cells.

- **Enzymes as analytical reagents (table 3.3):** Enzymes can also be utilized as analytical reagents in the clinical laboratory. They can be used for measuring substrates, drugs, and the activities of other enzymes. Enzymatic procedures are often preferred over conventional chemical methods for the estimation of biochemical compounds such as glucose, urea, uric acid, and cholesterol, as they offer greater accuracy and specificity. An example of this is the estimation of plasma glucose using the ***glucose oxidase and peroxidase*** method.
- **Immobilized enzymes:** Immobilized enzymes are a method of restricting the mobility of enzymes through chemical or physical treatments. This approach was first attempted in the 1960s and is now an emerging approach to new drug therapies. Enzymes are typically immobilized by binding them onto carrier materials or loading them onto polymeric matrices. The industrial use of enzymes is often limited by their relative instability, high cost of purification, and cumbersome process of recovery of the active enzyme from reaction mixtures after the completion of the catalytic process. However, immobilized enzymes are more stable to pH and temperature stress and less susceptible to denaturing agents. In addition, an immobilized enzyme should have long-term stability and unaltered sensitivity and biological activity after attachment to the matrix when used for therapeutic purposes. Immobilization has been successfully utilized for studies with enzymes such as *cytochrome P-450, UDP-glucuronosyltransferases, glutathione S-transferases, S-methyltransferases, and N-acetyltransferases.*

Table 3.3. Application of enzymes as analitic agents

Analitic application reagents (for estimation)	
Glucose oxidase, peroxidase	Glucose
Urease	Urea
Cholesterol oxidase	Cholesterol
Uricase	Uric acid
Lipase	Triacylglycerols
Luciferase	To detect bacterial contamination of blood
Alcaline phosphatase	In ELISA

MEDICAL IMPORTANCE

ELISA, or enzyme-linked immunosorbent assay, is a technique that combines enzymology, immunology, and photometry to detect and estimate antigens or antibodies in biological fluids. ELISA is capable of detecting very small amounts (picograms) of these substances, making it a useful tool for clinical and diagnostic applications. For example, several hormones can be measured using ELISA. In addition, ELISA is widely used for detecting antibodies by fixing the antigen to a support material. This technique is also used for detecting highly infectious Furthermore, ELISA is used to detect and estimate tumor markers in biological fluids.

3.5. Diagnostical importance of enzymes.

Estimation of enzyme activities in biological fluids (particularly plasma/serum) is of great clinical importance. Enzymes in the circulation are divided into two groups - **plasma functional** and **plasma non-functional.**

Plasma specific or plasma functional enzymes are those that are synthesized in and secreted from organs and tissues and have a physiological role in the plasma. Certain enzymes are normally present in the plasma and they have specific functions to perform.

- Present in plasma at higher concentration than tissues.
- They function in plasma.
- Mostly synthesized by the liver.
- Usually decreased in disease conditions.

Examples of plasma functional enzymes include *lipoprotein lipase, plasmin, thrombin, choline esterase, ceruloplasmin* etc. Deficiency of *ceruloplasmin* in Wilson's disease.

Enzymes that are not specific to plasma **or non-functional** in plasma are those that are either absent or present at lower concentrations in plasma compared to their levels found in tissues. These enzymes are primarily synthesized by organs such as the liver, pancreas, skeletal muscle, heart, and brain. Usually, the levels of these enzymes increase in disease conditions, making their estimation crucial for the diagnosis and prognosis of several diseases. Examples of such enzymes include ***creatine kinase and alanine transaminase***. The plasma enzymes associated with the metabolism of the cell are collectively referred to as constitutive enzymes (for example, ***lactate dehydrogenase, transaminases, acid and alkaline phosphatases, creatine phosphokinase***), while the hydrolytic enzymes of the gastrointestinal tract present in plasma are called **secretory enzymes** (for example, **amylase, pepsin, trypsin, lipase**, etc.).

Plasma non-functional enzymes, on the other hand, are those that are released from damaged or necrotic cells as a result of tissue injury or disease. These enzymes are not synthesized in the plasma but instead leak into the circulation from various organs and tissues. Examples of plasma non-functional enzymes include enzymes released from the liver *(AST, ALT)*, heart (troponin), and skeletal muscle *(creatine kinase).*

Measurement of enzyme activities in biological fluids can provide important diagnostic information about organ and tissue damage or dysfunction. For example, measurement of liver enzyme activities in plasma (such as **AST and ALT**) can indicate liver damage, while measurement of troponin levels in plasma can indicate myocardial infarction (heart attack).

The normal level of an enzyme in the serum reflects the equilibrium between its production and release during regular cell turnover. Elevated levels of enzymes may result from cellular damage (as shown in figure 3.11), increased cell turnover rate, cell proliferation, and increased enzyme synthesis. Measurement of serum enzyme levels is a convenient method to detect cellular damage, aiding in the diagnosis of diseases.

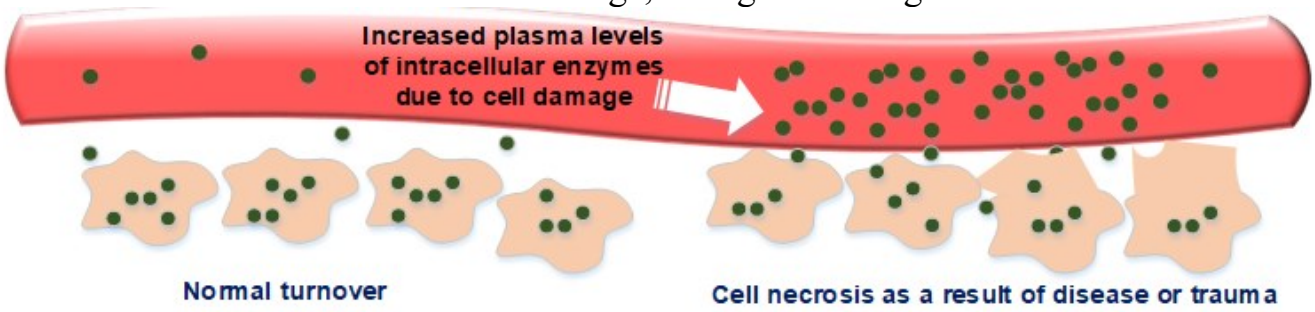


Fig 3.11. Release of enzymes from normal and diseased or traumatized cells.

3.6. Isoenzymes, their role in enzymodiagnostics

Isoenzymes or isozymes refer to the multiple forms of an enzyme that exist within the same organism, tissue, or cell, which catalyze the same reaction but have distinct kinetic properties (K_m and Vmax) and amino acid compositions. These variations allow the isoenzymes to perform different functions within the same cell or organism.

Isozymes are of widespread nature. Over a hundred enzymes have been identified as isozymes, and they occur in two or more molecular forms. This diversity of isozymes provides a means for cells to regulate metabolic pathways and respond to changes in the environment. It also has important implications for clinical diagnosis and genetic analysis.

Lactate dehydrogenase (LDH), for example, is an enzyme which exists in 5 possible forms in various organs. LDH catalyzes the reversible oxidation/reduction reaction :

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

LDH contains two types of subunits: **H** (heart) and **M** (muscle) in the structure of LDH (fig. 3.12). The synthesis of two subunits H and M is controlled by different genes. H is acidic while M is basic in nature. The molecule weight of each subunit is 35,000.

LDH has **five** distinct **isoenzymes: LDH1, LDH2, LDH3, LDH4** and **LDH5** each of them is composed of four polypeptide chains to form an oligomeric (tetrameric) enzyme:

LDH1 – H4 (heart, RBC):

LDH2 – H3M1 (heart, RBC);

LDH3 – H2M2 (brain, kidneys);

LDH4 – H1M3 (liver, skeletal muscles);

LDH5 – M4 (skeletal muscles, liver).

Most commonly used technique for the separation of isoenzymes is **electrophoresis** (cellulose or starch gel or agarose gel). Separation of isoenzymes of LDH can be achieved through techniques

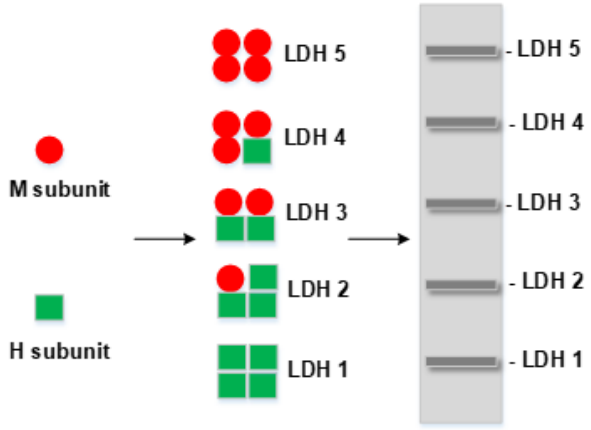


Fig. 3.12. Isoenzymes of lactate dehydrogenase

such as **electrophoresis,** and their presence or absence can be used as markers to identify specific tissue damage or disease states. On electrophoresis, isoenzymes *of lactate dehydrogenase* separates into five bands. LDH1 has more positive charge and fastest in electrophoretic mobility while LDH5 is the slowest. The proportions of LDH isoenzymes in normal serum are 25%, 35%, 27%, 8% and 5% for LDH1, LDH2, LDH3, LDH4 and LDH5, respectively.

Creatine kinase (CK) or creatine phosphokinase (CPK) catalyses the inter-conversion of phosphocreatine (or creatine phosphate) to creatine:

Phosphocreatine + ADP ↔ Creatine + ADP

Creatine kinase contains two subunits. Each subunit may be either of the muscle (**M**) or the brain (**B**) type. Three isozymes exist:

CK1 (BB) – brain;
CK2 (MB) –heart muscle;
CK3 (MM) - skeletal muscles.

MEDICAL IMPORTANCE

With regard to isozyme distribution, CK-MM makes up 99% of skeletal muscle and about 75% of myocardium. CK-MB makes up about 25% of myocardium, but it is not found in any other tissues, so CK-MB is a significant marker for a myocardial infarction (heart attack, MI). CK-MB levels begin to rise within a few hours of an MI and remain elevated for up to 3 days. This is used in conjunction with another protein marker, troponin.

***Alkaline phosphatase* isoenzymes**. Electrophoresis is used for the separation of isoenzymes of alkaline phosphatase in serum. On electrophoresis, isoenzymes of alkaline phosphatase separates into four bands, which are tissue specific. They differ in their carbohydrate content. The four isoenzymes of *alkaline phosphatase* that are separated by electrophoresis and have tissue-specific distribution are:

Intestinal alkaline phosphatase (IAP) - predominantly found in the small intestine

Placental alkaline phosphatase (PLAP) - produced by the placenta during pregnancy.

Germ cell alkaline phosphatase (GCAP) - found in the testes and ovaries.

Liver/bone/kidney alkaline phosphatase (L/B/K ALP) - found in the liver, bone, and kidneys.

Each of these isoenzymes has a slightly different amino acid sequence and carbohydrate content, resulting in differences in their electrophoretic mobility.

MEDICAL IMPORTANCE

Alkaline phosphatase catalyzes the hydrolysis of organic esters at alkaline pH 9.0, hence the name alkaline phosphatase. The normal level is 20-90 units/L. The level of the isoenzymes of alkaline phosphatase is elevated in rickets, obstructive jaundice, hyper para thyroidism, metastatic cancer, bone cancer and osteomalacia. In obstructive jaundice, its level is 10 times the normal level because its secretion is blocked due to obstruction. Its level also increases in some non-specific diseases like leukemia, lung and kidney damages and congestive heart failure, Hodgkin's disease and intestinal disorders.

Carbonic anhydrase isoenzymes. On electrophoresis *carbonic anhydrase* gives three bands. The three isoenzymes differ in amino acid composition.

Damages to tissues and cell death both result in a release of intracellular enzymes from damaged cells into the blood. Therefore, the concentrations of these enzymes increase in the blood serum and can serve as a valuable diagnostic aid in a number of diseases, including myocardial infarction, pancreatitis, liver disease and prostatic cancer.

3.7. Diagnostic markers of pathological processes in distinct organs.

The release of intracellular enzymes into the bloodstream is often observed in various diseases that lead to tissue damage. Such enzymes are commonly measured for diagnostic purposes in diseases affecting the heart, liver, skeletal muscle, and other tissues. The level of enzyme activity in the plasma is often indicative of the degree of tissue damage. Therefore, determining the degree of elevation of a particular enzyme activity in the plasma can aid in evaluating the prognosis for the patient. However, enzymes that are highly active in only one or a few tissues are more specific indicators of cellular injury at the corresponding site. In contrast, enzymes with broad tissue distribution are less specific and may limit their diagnostic value.

3.7.1. Pancreatic enzymes.

a-Amylase (EC 3.2.1.1) is a type of hydrolase enzyme that catalyzes the hydrolysis of 1,4-α-glycosidic linkages in polysaccharides. This enzyme has a low molecular weight, typically ranging from 54 to 62 kDa, and can pass through the glomeruli of the kidneys. Interestingly, it is the only plasma enzyme that is normally found in urine. The amylase activity that is present in both normal serum and urine is derived from two sources: pancreatic amylase and salivary gland amylase.

Causes of elevated plasma amylase activity include:

- Marked increase (5-10 times the upper reference limit): acute pancreatitis and severe glomerular impairment.
- Moderate increase (up to 5 times the upper reference limit): perforated peptic ulcer, acute cholecystitis, intestinal obstruction, and salivary gland disorders such as mumps and salivary calculi.

Lipase (EC 3.1.1.3) is a glycoprotein with a single chain and a molecular weight of 48 kDa. It belongs to the hydrolase class and catalyzes the hydrolysis of triacylglycerols to fatty acids and glycerol. Elevated levels of plasma lipase are specifically indicative of **acute pancreatitis and pancreatic carcinoma**. In contrast, serum amylase can also be increased due to other causes, such as mumps or pancreatic disease. Therefore, measuring both amylase and lipase levels together can help in the accurate diagnosis of **acute pancreatitis.**

Trypsin (EC 3.4.21.4) is a serine proteinase that specifically cleaves peptide bonds formed by the carboxyl groups of lysine and arginine with other amino acids. It is increased in pancreatic disease, but its estimation is not considered of significant clinical value in routine management of patients with acute pancreatitis.

3.7.2. Liver enzymes.

The analysis of serum enzymes is highly valuable in distinguishing and tracking various hepatobiliary disorders. There are three categories of enzymes that serve different functions:

1. Enzymes that are naturally found inside hepatocytes are released into the bloodstream when there is damage to the cells, and they serve as **markers of hepatocellular damage**.
2. Enzymes that are primarily bound to the hepatocyte's membrane, whether it's the plasma membrane or the side of hepatocytes, **are markers of cholestasis**.
3. Enzymes that are synthesized within the hepatocyte are **indicators of disruptions in hepatocellular synthesis**, which can affect other liver enzymes.

1. Markers of hepatocellular damage: ***aminotransferases*, also known as *transaminases***, are enzymes that facilitate the transfer of an amino group from an α-amino acid to a α-ketoacid. They are present throughout the body. The two main types of transaminases: ***AST (aspartate transaminase)*** and ***ALT (alanine transaminase).***

In the liver, the concentration of ALT is higher than that of AST. The AST and ALT enzymes are especially useful in determining the extent of liver cell inflammation and necrosis. Elevated plasma ALT levels are relatively specific to liver disease. AST levels may be elevated in other forms of tissue damage, such as myocardial infarction, muscle necrosis, and renal disorders.

In liver disease, the ALT level increases more significantly than the AST level. **In cases of acute viral hepatitis, both the ALT and AST levels can increase 100-1000 times, but the increase in ALT level is greater than that of AST.**

2. Markers of cholestasis:

Alkaline phosphatase (ALP) is a group of enzymes that breaks down organic phosphates at high pH levels. They are found in most tissues but are particularly concentrated in osteoblasts of bone, as well as cells in the hepatobiliary tract, intestinal wall, renal tubules, and placenta. Although the exact metabolic function of ALP is unknown, it is believed to play an important role in bone calcification. In adults, plasma ALP is derived equally from bone and liver, with the bone fraction increasing during periods of increased osteoblastic activity. Possible **causes of elevated plasma ALP** activity include:

- **Physiological factors:** As individuals age, there is a gradual increase in liver-derived ALP activity, and in the elderly, the plasma bone isoenzyme activity may slightly increase.
- Bone disorders: such as rickets and osteomalacia.
- Liver disease.
- Malignancy involving bone or liver, or direct tumor production.

Possible causes of **low plasma ALP activity** include:

- Stunted bone growth.
- Hypophosphatasia, an autosomal recessive disorder associated with rickets or osteomalacia.

Causes of raised plasma GGT activity are induction of enzyme synthesis, without cell damage, by drugs or alcohol, hepatocellular damage, such as that due to infectious hepatitis.

Gamma-glutamyl transferase (GGT) is an enzyme that transfers the gamma-glutamyl group from peptides and compounds containing it to an acceptor. It is mainly found in the cells of the liver, kidneys, pancreas, and prostate, and its plasma activity is higher in men than in women. Elevated plasma GGT activity can be caused by drugs or alcohol that induce enzyme synthesis without cell damage, as well as **hepatocellular damage, such as that caused by infectious hepatitis.**

3. Other liver enzymes:

Cholinesterase (CHE) is also an enzyme found in erythrocytes, lung and spleen, nerve endings, and the gray matter of the brain. It is also known as *true cholinesterase* or *cholineesterase I*. Increased plasma cholinesterase activity may be seen **during recovery from liver damage,** which is characterized by actively growing hepatocytes, or in cases of **nephrotic syndrome**.

Glutamate dehydrogenase (GDH) is a mitochondrial enzyme found mainly in the liver, heart muscle, and kidneys, but small amounts also occur in other tissues, including the brain, skeletal muscle tissue, and leukocytes. Elevated serum levels of GLH are seen in patients with **hepatocellular damage** and can provide differential diagnostic potential in the investigation of liver disease, especially when interpreted alongside other enzyme test results. As an exclusively mitochondrial enzyme, GLH is released from necrotic cells and can be useful in estimating the severity of liver cell damage.

3.7.3. Muscle enzymes.

Creatine Kinase (CK, creatine phosphokinase), is primarily found in cells of the heart, skeletal muscle, and brain, but can also be present in other tissues including smooth muscle. The levels of CK in serum can vary between different human tissues. In individuals with **muscular dystrophy**, there is a significant increase in serum CK activity. This is particularly true for those with progressive muscular dystrophy, including **Duchenne sex-linked muscular dystrophy**. In fact, CK activity in serum is highest in infancy and childhood (around 7-10 years of age) and may increase well before the disease is clinically evident. As the disease progresses and functional muscle mass diminishes, serum CK activity tends to decline. Asymptomatic female carriers of Duchenne dystrophy often have a 3-6-fold increase in CK activity. Elevated levels of CK can also be observed in other muscle diseases such as **viral myositis, polymyositis, and neurogenic muscle disease like myasthenia gravis, multiple sclerosis, polimyeltis, and Parkinson's disease**.

Lactate dehydrogenase (LDH) is an enzyme that catalyzes the reversible interconversion of lactate and pyruvate. LDH is widely distributed in the body, with high concentrations in cells of cardiac and skeletal muscle, liver, kidney, brain, and erythrocytes. Therefore, measuring plasma total LDH activity is a non-specific marker of cell damage.

Elevated plasma total LDH activity can have various causes. **A marked increase (more than 5 times the upper reference limit in adults)** can be seen in **myocardial**

infarction and some **haematological disorders** such as **megaloblastic anaemia, acute leukaemias, and lymphomas**, where very high levels (up to 20 times the upper reference limit in adults) may be found. **A moderate increase** can be seen in **viral hepatitis, malignancy of any tissue, skeletal muscle disease, pulmonary embolism, and infectious mononucleosis.**

Acid phosphatase (ACP) is an enzyme found in lysosomes and some other cells such as the prostate, bone, spleen, platelets, and erythrocytes. Elevated ACP levels can be indicative of **Paget's disease, hyperparathyroidism** with skeletal involvement, or **malignant invasion of bones by cancers**. ACP is commonly used to help diagnose and monitor treatment for **prostatic carcinoma**, although it is gradually being replaced by the more specific and sensitive plasma prostate-specific antigen (PSA) test. ACP may still be useful in monitoring the treatment of known cases of disseminated prostatic carcinoma, but is less effective for making the initial diagnosis.

3.7.4. Markers of myocardial infarction.

Myocardial infarction, also known as a heart attack, is a condition that occurs when blood flow to a part of the heart is blocked, causing damage to the cardiac muscle. Diagnosis of myocardial infarction is usually based on the WHO (World Health Organization) criteria, which include chest pain, ECG changes, and an increase in biochemical markers of myocardial injury. However, it should be noted that not all patients with typical symptoms have a myocardial infarction, while biochemical markers have excellent sensitivity in diagnosing this disease.

Biochemical markers in acute myocardial infarction are:

- *Creatine kinase (CK);*
- *Aspartate transaminase (AST);*
- *Lactate dehydrogenase (LDH);*
- Troponins;
- Myoglobin.

After myocardial infarction serum value of CK is found to increase within 3-6 hours, reaches a peak level in 24-30 hours and returns to normal level in 2-4 days (usually in 72 hours) (fig. 3.12). CK is a sensitive indicator in the early stages of myocardial ischemia. No increase in activity found in heart failure and coronary insufficiency.

MB isoform of CK is the main one in cardiac tissue. It exists in two forms: CK-MB 1 and CK-MB 2. CK-MB2 is more specific and elevated in the earliest stages of myocardial infarction.

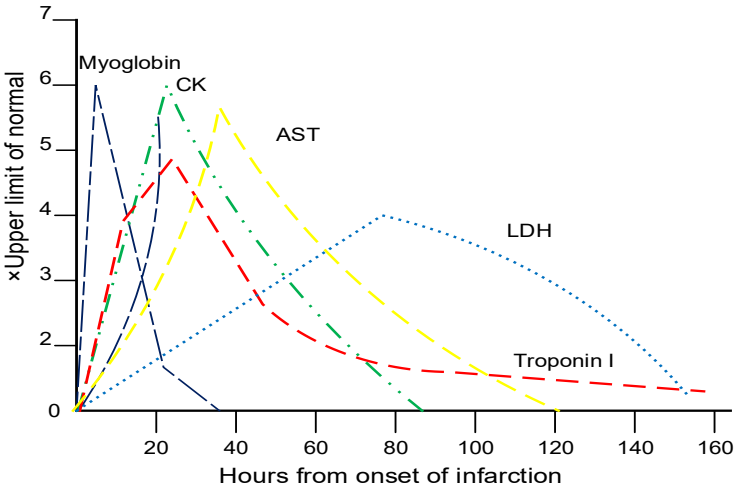


Fig. 3.13. Biochemical markers of myocardial infarction

Activity of AST sharply rises within first 12 hours, with a peak level at 24 hours or over and returns to normal within 3-5 days. The rise depends on the extent of infarction. However because of the abundance AST in liver, skeletal muscle, and other tissues this enzyme was recently superseded for cardiac diagnostics by two other enzymes CK and LDH.

The serum activity of LDH rises within 12 to 24 hours, attains a peak et 48 hours (2 to 4 days) and then returns gradually to normal from 8th to 14th day. The magnitude of rise is proportional to the extent of myocardial infarction. LDH 1 isoform of LDH is prevalent for heart tissue.

Troponins and Myoglobin are non-enzymatic markers of myocardial infarction. Troponin is a thin filament of striated muscle. Tree individual proteins of troponin are: tropomyosin binding subunit (TnT), inhibitory subunit (TnI) and Ca-binding subunit (TnC). Myoglobin is a heme containing protein that binds oxygen within cardiac and skeletal muscle. Its concentration rises about 6 hours after myocardial infarction and returns to baseline after 24 hours.

3.8. Inborn and acquired metabolic defects of metabolism.

Metabolism is a complex process in the body that is facilitated by enzymes. When an abnormality affects the function of an enzyme or causes it to be deficient or missing altogether, it can lead to various disorders. These defects can either be **genetic**, caused by gene mutations, or **acquired**, caused by lifestyle or environmental factors. These disorders typically result from the body's inability to break down a particular substance, leading to the buildup of toxic intermediate substances or the inability to produce an essential substance. Metabolic disorders are classified based on the specific building block that is affected.

Genetic disorders are commonly associated with enzyme synthesis damage or regulation infringements in tissues. These disorders are challenging to treat, and diagnosis is often made during prenatal or newborn screening by measuring the concentration of certain substrates or products for enzymes that are deficient.

3.8.1. Metabolic defects of amino acids metabolism.

Phenylketonuria (PKU) is a disorder in amino acid metabolism and is the most prevalent of its kind. It occurs in 1 in 10,000 births and is caused by a recessive autosomal gene, resulting in a deficiency of ***phenylalanine hydroxylase***, a hepatic enzyme. The

condition prevents the conversion of phenylalanine to tyrosine, leading to the accumulation of phenylalanine in tissues and blood, and its increased excretion in urine. This disturbed metabolism causes the production of phenylpyruvate, phenylacetate, and phenylactate, resulting in various clinical and biochemical manifestations. This can cause various clinical and biochemical manifestations, including intellectual disability, seizures, behavioral problems, skin rashes, and a musty odor in the breath and urine.

Alkaptonuria is a disorder of tyrosine metabolism, caused by a deficiency in ***homogentisate oxidase***, resulting in the accumulation of homogentisate in tissues and blood, and its excretion in urine. Homogentisate gets oxidized to quinones that polymerize and give urine a black or brown colour. The deposition of the pigment alkapton occurs in various organs, bones, and connective tissues, leading to ochronosis. This deposition is believed to cause arthritis in many alkaptonuric patients.

Albinism is an autosomal recessive disorder that results in the lack of synthesis of the pigment melanin. The most common cause of albinism is a defect in the enzyme ***tyrosinase***, responsible for melanin synthesis. Melanin plays a crucial role in protecting the body from sun radiation. The absence of melanin in albinos causes them to be highly sensitive to sunlight, and they are more susceptible to skin cancer.

3.8.2 Metabolic defects of carbohydrates metabolism.

Galactosemia is a rare genetic disorder that results from a deficiency of the enzyme **galactose-1-phosphate uridyltransferase**. This leads to impaired metabolism of galactose and increased levels of galactose in the blood and urine. Galactose metabolism is impaired leading to increased galactose levels in circulation (galactosemia) and urine (galactosuria). The accumulated galactose is diverted for the production of galactitol (dulcitol) by the enzyme aldose reductase (the same enzyme that converts glucose to sorbitol). The excess galactose is then converted to galactitol, which has been linked to the development of cataracts.

Fructose intolerance, on the other hand, is caused by a deficiency in the enzyme **aldolase B *(fructose-1-phpsphate aldolase)*,** leading to the accumulation of fructose-1-phosphate and severe symptoms such as hypoglycemia, vomiting, and liver failure.

Glycogen storage diseases refer to a group of inherited disorders characterized by abnormal glycogen deposition in tissues, such as von Gierke's disease (type I), which leads to fasting hypoglycemia due to a defect in the enzyme glucose 6-phosphatase. These metabolic defects can have various clinical and biochemical manifestations.

3.8.3. Metabolic defects of lipids metabolism.

Gaucher's disease develops due to a defect in the enzyme ***β-glucosidase***. As a result, tissue glucocerebroside levels increase. This disorder is commonly associated with enlargement of liver and spleen, osteoporosis, pigmentation of skin, anemia, mental retardation etc.

Krabbe's disease. Defect in the enzyme ***β-galactosidase*** *r*esults in the accumulation of galactocerebrosides. A total absence of myelin in the nervous tissue is a common feature. Severe mental retardation, convulsions, blindness, deafness etc. are seen.

Niemann-Pick disease and **Farber's** disease connected with sphingomylein

metabolism are already described.

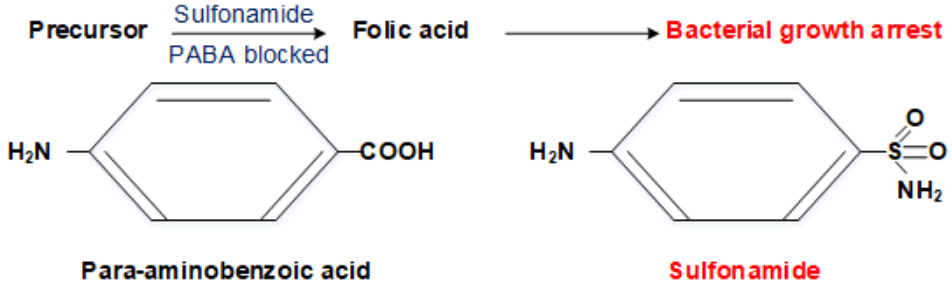
Fatty Acid Oxidation Disorders. Several enzymes help break fats down so that they may be turned into energy. An inherited defect or deficiency of one of these enzymes leaves the body short of energy and allows breakdown products, such as acyl-CoA, to accumulate. The enzyme most commonly deficient is **medium chain acyl-CoA dehydrogenase (MCAD).** MCAD deficiency is one of the most common inherited disorders of metabolism, particularly in people of Northern European descent. Symptoms usually develop between birth and age of three years old. Children are most likely to develop symptoms if they go without food for a period of time (which depletes other sources of energy) or have an increased need for calories because of exercise or illness. The level of sugar in the blood drops significantly, causing confusion or coma. The child becomes weak and may have vomiting or seizures. Over the long term, children have delayed mental and physical development, an enlarged liver, heart muscle weakness, and an irregular heartbeat. Sudden death may occur.

Some states screen newborns for MCAD deficiency with a blood test. Immediate treatment is with intravenous glucose. For long-term treatment, the child must eat often, never skipping meals, and consume a diet high in carbohydrates and low in fats. Supplements of the amino acid carnitine may be helpful.

3.9 Application of enzyme inhibitors as medicinal and drugs.

Enzyme inhibitors are molecules that bind to enzymes and lower their activity. Many drugs are enzyme inhibitors due to the correction of metabolic imbalance may occur, as a result of blocking an enzyme activity.

Sulfonamide antibiotics are used in the treatment of bacterial infections. **Sulfonamides** are the structural analogs of para-aminobenzoic acid (PABA) (fig. 3.13), wich is normally synthsised by bacteria. These sulfanilamide drugs can be used to inhibit (competitively) the synthesis of folic acid by microorganisms. This indirectlv reduces the synthesis of purines and, therefore, the nucleic acids (DNA and RNA). Sulfonamides have no influence on humans, since folic acid is not svnthesized and is supplied through diet.



Fig, 3.13. Para-aminobenzoic acid (PABA) is a naturally occurring compound that is used by bacteria in the synthesis of folate, a vitamin that is essential for the production of DNA and RNA. Sulfanilamide is a synthetic compound that is structurally similar to PABA and competitively inhibits the enzymatic conversion of PABA to folate.

Allopurinol is a drug used in the treatment of gout. Gout is due to excessive production of uric acid. *Xanthine oxidase* is an enzyme involved in the formation of uric acid from hypoxanthine. Allopurinol is a structural analog of hypoxanthine and hence it is

an antimetabolite of hypoxanthine (fig. 3.14). When it is used it blocks formation of uric acid by inhibiting the enzyme *xanthine oxidase*.

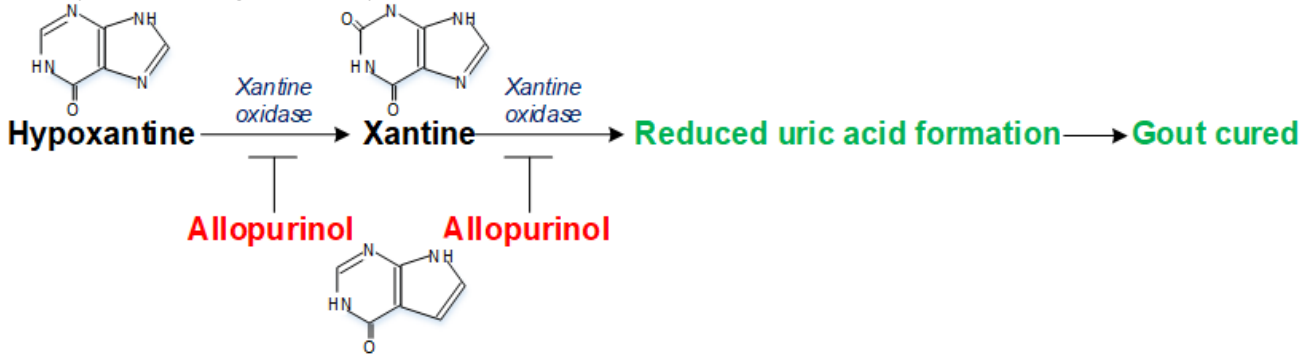


Fig. 3.14. Inhibition of xanthine oxidase by allopurinol. By reducing the production of uric acid, allopurinol helps to prevent gout attacks and other complications associated with hyperuricemia.

Further, allopurinol is oxidized to alloxanthine by *xanthine oxidase*. Alloxanthine, in turn, is a more effective inhibitor of xanthineoxidase

Inhibition of *xanthine oxidase* by allopurinol leads to the accumulation of hypoxanthine and xanthine. These two compounds are more soluble than uric acid, hence easily excreted.

Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the synthesis of prostaglandins, prostacyclins and thromboxanes. Prostaglandins are a class of eicosanoids, fatty acid derivatives with a variety of extremely potent actions on vertebrate tissues. They are responsible for producing fever and inflammation and its associated pain. NSADs are inhibitors of the enzyme ***cyclooxygenase (COX)***. For example, **aspirin** (acetyl salicylic acid) has been used since nineteenth century as an antipyretic (fever-reducing) and analgesic (pain relieving). The mechanism of action of aspirin however, was not known for a long period. lt was only in 1971, John Vane discovered that aspirin inhibits the synthesis of prostaglandines from arachidonic acid. **Aspirin** irreversibly inhibits the enzyme ***cyclooxygenase***. Other anti-inflammatory drugs, such as indomethacin and phenylbutazone act as reversible e inhibitors of the enzyme cyclooxygenase.

Lovastatin is an inhibitor (competitive) of enzyme ***HMG-CoA reductase***, when used it blocks production of cholesterol. In atherosclerosis, cholesterol is more. Lovastatin reduces cholesterol formation thus arrest the advancement of atherosclerosis.

In the treatment of cancer inhibitors **aminopterin and amethopterin (methotrexate)** are used. They are structural analog of folic acid. They are competitive inhibitors for the enzyme ***dihydrofolate reductase***. They are used in the treatment of leukaemia, a type of cancer. When used these drugs block the synthesis of nucleic acids (DNA and RNA). For cell proliferation, nucleic acid are needed. So, lack of nucleic acids lead to arrest of tumour growth and advancement of cancer is prevented.

Competitive inhibitors used in the treatment of hypertension are **captopril, lisinopril and enalapril**. They competitively inhibit ***angiotensin converting enzyme***. This enzyme catalyzes the proteolytic cleavage of angiotensin I to angiotensin II; the latter elevates the arterial blood pressure. ACE inhibitors decrease formation of angiotensin II, thereby lowering blood pressure. The ACE inhibitors contain a proline residue, which is an important substrate-binding determinant.

REVIEW TEST:

№	MCQs	Answers and explanations
1.	**Blood test of the patient revealed albumine content of 20 g/l and increased activity of lactate dehydrogenase isoenzyme 5 (LDH 5). These results indicate disorder of the following organ:** A. Lungs B. Kidneys C. Heart D. Liver E. Spleen	**The answer is D.** Lactate dehydrogenase, LDHis an enzyme which exists in 5 possible forms in various organs of most vertebrates. LDH catalyzes the reversible oxidationreduction reaction: $Lactate + NAD^+ \leftrightarrow Pyruvate + NADH+H^+$ LDH contains two types of subunits, produced by two separate genes namely **H** (heart) and **M** (muscle) in the structure of LDH. Subunits have the same molecular weight (35,000) but differ in amino acid composition, thus M-subunit is basic while H subunit is acidic. **LDH5** is an isoenzyme, located in liver or muscles.
2.	**A patient suffering from gout was prescribed allopurinol. What pharmacological property of allopurinol provides therapeutic effect in this case?** A. Acceleration of nitrogen-containing substances excretion B. Competitive inhibition of xanthine oxidase C. Acceleration of pyrimidine nucleotides catabolism D. Deceleration of pyrimidine nucleotides salvage E. Acceleration of nucleic acids synthesis	**The answer is B.** Allopurinol is a structural analog of hypoxanthine that competitively inhibits the enzyme xanthine oxidase. It is commonly used for the treatment of gout, a disease caused by overproduction and accomulation of uric acid. Inhibition of xanthine oxidase by allopurinol leads to the accumulation of hypoxanthine and xanthine. These two compounds are more soluble than uric acid, hence easily excreted with urine.
3.	**A 15-year-old boy has been diagnosed with acute viral hepatitis. What blood value should be determined to confirm acute affectionof hepatic cells?** A. Aminotransferase activity (AST, ALT) B. Unconjugated and conjugated bilirubin content C. Erythrocytes sedimentation rate (ESR) D. Cholesterol content E. Protein fraction content	**The answer is A.** There are two main transaminases: AST (aspartate transaminase) and ALT (alanine transaminase), located in liver, that is why these enzymes are more important in assessing and monitoring the degree of liver cell inflammation and necrosis. In acute viral hepatitis there is a 100-1000 times increase in both ALT and AST but ALT level is increased more than that of AST.
4.	**A 50-year-old woman diagnosed with cardiac infarction has been delivered into an intensive care ward. What enzyme will be the most active during the first two days?** A. Aspartate aminotransferase B. Alanine aminotransferase C. Alanine aminopeptidase D. LDH4 E. LDH5	**The answer is A.** Activity of AST sharply rises within first 12 hours after miocardial infarction, with a peak level at 24 hours or over and returns to normal within 3-5 days. The rise depends on the extent of infarction. However because of the abundance AST in liver, skeletal muscle, and other tissues this enzyme was recently superseded for cardiac diagnostics by two other enzymes CK and LDH.

5.	**A 46-year-old female patient has continuous history of progressive muscular (Duchenne's) dystrophy. Which blood enzyme changes will be of diagnostic value in this case?** A. Glutamate dehydrogenase B. Lactate dehydrogenase C. Pyruvate dehydrogenase D. Creatine phosphokinase E. Adenylate cyclase	**The answer is D.** Creatine phospokinase – CK is most abundant in cells of cardiac and skeletal muscle and in brain, but also occurs in other tissues such as smooth muscle. Serum CK activity is greatly elevated in all types of muscular dystrophy. In progressive muscular dystrophy (particularly Duchenne sex-linked muscular dystrophy), enzyme activity in serum is highest in infancy and childhood (7-10 years of age) and may increase long before the disease is clinically apparent. Serum CK activity characteristically falls as patients get older and as the mass functioning muscle diminishes with the progression of the disease. About 50%-80% of the asymptomatic female carriers of Duchenne dystrophy show threefold to six-fold increase of CK activity.
6.	**A patient is diagnosed with cardiac infarction. Blood test for cardio specific enzymes activity was performed. Which of the enzymes has three isoforms?** A. Creatine kinase B. Lactate dehydrogenase C. Aspartate transaminase D. Alanine transaminase E. Pyruvate kinase	**The answer is A.** Creatine kinase (CK) catalyses the inter-conversion of phosphocreatine (or creatine phosphate) to creatine.Creatine kinase contains two subunits. Each subunit may be either of the muscle (M) or the brain (B) type. MB isoform of CK is the main one in cardiac tissue. It exists in two forms : CK-MB 1 and CK-MB 2. CK-MB2 is more specific and elevated in the earliest stages of myocardial infarction.
7.	**For biochemical diagnostics of myocardial infarction, it is necessary to measure activity of a number of enzymes and their isoenzymes. What enzymatic test is considered to be the best to prove or disprove the diagnosis of infarction in the early period after the chest pain is detected?** A. Creatine kinase isoenzyme CK-MB B. Creatine kinase isoenzyme CK-MM C. LDH1 lactate dehydrogenase isoenzyme D. LDH2 lactate dehydrogenase isoenzyme E. Aspartate aminotransferase cytoplasmicisoenzyme	**The answer is A.** After myocardial infarction serum value of Creatine kinase CK is found to increase within 3-6 hours, reaches a peak level in 24-30 hours and returns to normal level in 2-4 days (usually in 72 hours). CK is a sensitive indicator in the early stages of myocardial ischemia. No increase in activity found in heart failure and coronary insufficiency. MB isoform of CK is the main one in cardiac tissue.
8.	**6 hours after the myocardial infarction a patient was found to have elevated level of lactate dehydrogenase in blood. What isoenzyme should be expected in this case?** A. LDH1 B. LDH2 C. LDH3 D. LDH4 E. LDH5	**The answer is A.** The serum activity of lactate dehydrogenase (LDH) rises within 12 to 24 hours, attains a peak et 48 hours (2 to 4 days) and then returns gradually to normal from 8^{th} to 14^{th} day. The magnitude of rise is proportional to the extent of myocardial infarction. LDH 1 isoform of LDH is prevalent for heart tissue.

9.	**A competitive reversible inhibitor such as physostigmine is used to treat glaucoma and myasthenia gravis and to reverse anticholinergic syndrome. Based on this, which one of the following statements is true concerning the clinical implications of using physostigmine?** A. Use of the drug will decrease the Km of the targeted enzyme B. Physostigmine will increase the Vmax of the targeted enzyme. C. An overdose of physostigmine can typically be reversed D. Physostigmine will decrease the Vmax of the targeted enzyme **E.** Physostigmine is unable to cross the blood brain barrier.	**The answer is C.** Physostigmine is both a naturally occurring substance (Calabarbean) and a chemically synthesized substance that is a competitive reversible inhibitor of acetylcholinesterase. The drug easily crosses the blood-brain barrier. By definition, a competitive reversible inhibitoracts at the catalytic site with the substrate and competes with substrate binding to the enzyme. Thus, the effects of the inhibitor can be overcome by addition of the substrate, leading toan effective reversal of drug overdose. This is a reversible inhibition, so the Vmax is unchanged becauseif sufficient substrate is added, the effects of the inhibitor can be overcome. With a competitive inhibitor, the Km is increased because more substrate is needed to reach 1/2 Vmax.
10	**A10-year-old boy presents with vomiting, sweating, drooling, and a decreased heart rate. His friends state that he was in a cornfield when it was sprayed by a crop duster. The chemical being sprayed was an organophosphate derivative that covalently binds to acetylcholine-sterase and inactivates the enzyme. What type of inhibition is being displayed?** A. Irreversible B. Competitive C. Noncompetitive D. Allosteric E. Feedback	**The answer is A.** This is an example of irreversible inhibition because a covalent bond has been formed between the inhibitor and the required serine at the active site of the enzyme. This enzyme can only be reactivated if that covalent bond is hydrolyzed, which is unlikely. Bothc ompetitive and noncompetitive binding are reversible because the inhibitor is not covalently linked to the enzyme. Allosteric inhibitors also bind to enzymes via noncovalent forces, and feedback inhibition refers to the normal regulation of a pathway by an end product of the pathway.

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4. VITAMINS

OBJECTIVES after studying this chapter, you should be able to:

- *Evaluate the role of water soluble and fat soluble vitamins in metabolism, development of hypo- and hyper- vitaminoses, their prevention and treatment.*
- *Understand the role of nonprotein groups (coenzymes and prosthetic groups) in mechanism of catalysis by enzymes of different classes.*
- *Interpret the role of water soluble and fat soluble vitamins and their precursors as nutritional components in metabolic and physiological processes.*
- *Explain application of antivitamins as enzyme inhibitors in contagious diseases and in disorders of homeostasis.*
- *Explain the role of vitaminoids in metabolic processes.*

4.1. Vitamins as essential biologically active components of nutrition.

Vitamins are organic molecules that are necessary in small quantities for a range of biochemical functions in the body. They cannot be synthesized by the body and therefore must be obtained through the diet. The primary function of vitamins, particularly water-soluble ones, is to act as coenzymes or prosthetic groups for enzymes.

Classification of vitamins is primarily based on their **solubility,** which can be divided into two categories: **water-soluble and fat-soluble**. **Water-soluble** vitamins generally contain more oxygen and nitrogen in their structure and have a significant hydrocarbon portion, whereas **fat-soluble** vitamins are hydrophobic in nature. Examples of water-soluble vitamins include vitamin C and B vitamins, which are typically involved in coenzyme synthesis or act as coenzymes themselves. Fat-soluble vitamins such as vitamins A, D, K, E, and F, have a variety of biochemical functions.

Another popular classification of vitamins is based on pharmacodynamic properties. Vitamis are devided into:

•**Coenzyme vitamins** are water soluble vitamins, used for the synthesis of coenzymes, composing the key enzymes of the metabolism.

•**Redox-vitamins** are natural antioxidants, inactivating active forms of oxygen and lipoperoxides, thus protecting biomembranes and cell strutures from the toxic products of lipid peroxidation (in oxidative stress).

•**Hormone-like vitamins** are the vitamins, who after some structural modifications enter karyoplasm and affect genes expression.

Insufficient intake of vitamins through the diet can lead to the development of various pathological conditions. While deficiency of a single B-complex vitamin is rare, poor diets are often associated with **multiple deficiency states**. Specific syndromes are characteristic of deficiencies of individual vitamins, such as beriberi (thiamin), cheilosis, glossitis, and seborrhea (riboflavin), pellagra (niacin), megaloblastic anemia, methylmalonic aciduria, and pernicious anemia (vitamin B12), megaloblastic anemia (folic acid), and scurvy (vitamin C).

There are two types of causes for the development of **hypovitaminosis**:

- **Exogenous factors** are related to the lack of vitamins in the diet or the presence of factors that hinder vitamin absorption, such as consuming large amounts of raw eggs containing avidin, which can result in vitamin H deficiency.
- **Endogenous factors** can be caused by increased vitamin needs, such as during pregnancy; long-term severe infectious diseases during the recovery period; disturbances of vitamin absorption, such as deficiency of Castle factor leading to the disturbance of vitamin B_{12} absorption; intestinal dysbiosis; or metabolic disorders related to the activation of vitamins to coenzymes or prosthetic groups.

4.2. Water-soluble vitamins.

4.2.1. Thiamine.

Thiamine – vitamin B_1 (anti-beri-beri, antineuritic vitamin) is water soluble. Its structure consists of pyrimidine and thiazole rings held by a methylene bridge, with a hydroxyethyl side chain at position 5 of the thiazole ring, which becomes phosphorylated in the cell (fig. 4.1).

Absorption and transport. Thiamine is absorbed in the small intestine through active transport mechanisms and simple diffusion. It then reaches the liver through circulation.

Active form. The active form of thiamine is **thiamine pyrophosphate (TPP)**, which is a coenzyme that is formed by the action of **thiamine kinase** (also known as thiamine diphosphotransferase or **cocarboxylase**) in the liver (fig. 4.1). Two additional forms of thiamine, thiamine monophosphate (TMP) and thiamine triphosphate (TTP), are less important compared to TPP.

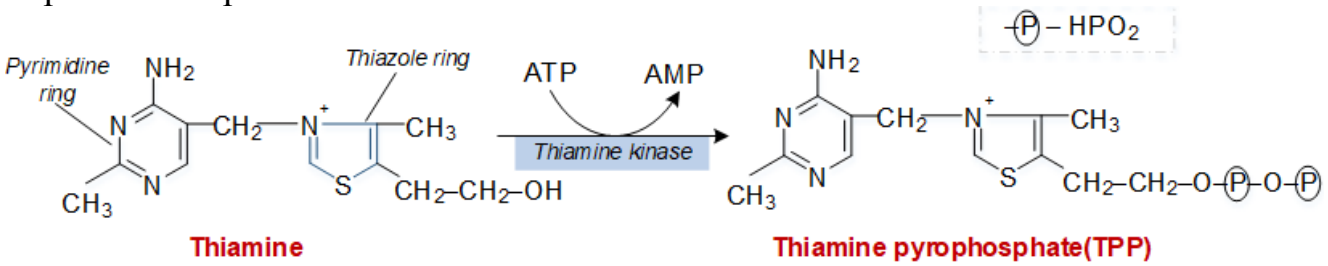


Fig. 4.1. Synthesis of thiamine pyrophosphate (TPP) from thiamine. The synthesis of TPP from thiamine involves enzymatic steps and requires the presence of ATP.

Biological role:

- TPP catalyzes **oxidative decarboxylation of α-keto acids** (pyruvic, α-ketoglutaric)
- ***Transketolase*** is dependent on TPP. This is an enzyme of pentose phosphate pathway (PPP).
- **Oxidative decarboxylation of branched chain amino acids** (valine, leucine, isoleucine) to α-keto acids.
- TPP plays an important role in the **transmission of nerve impulse**. It is believed that TPP is required for **acetylcholine synthesis** and the ion translocation of nervous system.

Examples of TPP-dependent enzymes: *pyruvate and a-ketoglutarate dehydrogenase complexes, transketolase, dehydrogenase of branched chain a-keto acids*.

Dietary sources: Thiamine can be found in a variety of dietary sources. In animal products, 95%–98% of the thiamine is present in the phosphorylated form, with around 80%–85% as the pyrophosphate. Thiamine is found in a variety of foods, including: whole grains, legumes (lentils, black beans, soybeans, and peas), nuts and seeds (sunflower seeds, macadamia nuts, and pecans are among the nuts and seeds that contain thiamine), meat and fish (pork, beef, chicken, fish, and shellfish), vegetables (spinach, asparagus, brussels sprouts, and green peas), fruits (oranges, grapefruit, and berries), dairy products (milk and cheese).

Some cooking methods, such as boiling and simmering, can lead to a loss of thiamine in foods. Therefore, it's important to include a variety of thiamine-rich foods in your diet to ensure you're meeting your daily requirements.

Deficiency of vitamin B_1 results in a condition called **beri-beri** (once associated with white polished rice diets and with highly milled wheat diets). There are two clinical types of bery-bery:

- **Dry beri beri** or neuritic beriberi, associated with polyneuropathy (depressed peripheral nerve function, sensory disturbance, loss of reflexes and motor control and muscle wasting.
- **Wet beri beri** or cardiovacular beriberi, associated with edema, congestive heart failure.

Other types of diseases, caused by tiamine severe deficiency include:

- Alcohol neuritis (peripheral neuropathy), with following symptoms: sharp burning pain in the feet, deep muscle tenderness with numbness coarse tremors, foot drop).
- Wernicke's encephalopathy, resulting from degeneration of basal ganglia due to chronic/heavy use of alcohol (rigidity of extremities, complete or partial ophthalmoplegia, sleep disturbances, nausea and vomiting).
- Korsakoff's syndrome or psychosis, which is also a complication of chronic/heavy use of alcohol (memory loss, delusions, disorientation, ocular palsies).
- Combined Wenicke-Korsakoff syndrome.
- Pregnancy neuritis.
- Certain gastrointestinal disorders.

Thiamine destroying food factors. It is important to note that thiamine in foodstuffs can be destroyed by **thiaminase**, an enzyme found in some fish and Japanese intestinal flora. Additionally, certain plant foods and seafoods may also contain thiamine-destroying factors.

MEDICAL IMPORTANCE

Thiamine is generally well tolerated by the body, even at high oral intakes, due in part to the brush border limit on excessive absorption. Commercial multivitamin supplements typically contain 1–5 mg of thiamine per daily dose for the prevention of deficiency, while supplements used for treatment of deficiency may provide 10–35 mg per day. Thiamine as a single nutrient is available at dosages of up to 300 mg per day.

4.2.2 Riboflavin.

Riboflavin – vitamin B_2 (6,7-dimethyl-(9-D-1-rybitol)-isoalloxazine) contains heterocyclic isoalloxazine ring and ribitol (a reduced form of ribose sugar) (fig. 4.2).

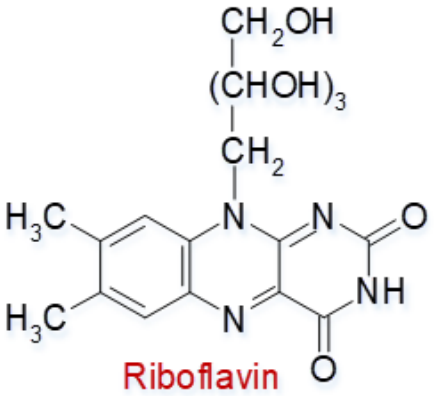


Fig. 4.2. Chemical structure of riboflavin - 6,7-dimethyl-(9-D-1-rybitol)-isoalloxazine

Absorption and transport. Riboflavin is absorbed in the small intestine and then distributed to all the tissues in the body through the bloodstream.

Active forms. There are two important active forms of riboflavin (fig. 4.3):

- **Flavin mononucleotide (FMN)**
- **Flavin adenine dinucleotide (FAD)**

The conversion of riboflavin into its active coenzyme forms, FMN and FAD, involves two phosphorylation steps that occur inside the cell. Firstly, riboflavin is converted into flavin mononucleotide (FMN) by the action of **flavokinase**, which uses ATP as a phosphate donor. Secondly, FMN is converted into flavin adenine dinucleotide (FAD) by the action of FAD synthase, which adds an AMP molecule from another ATP molecule to the phosphate group of FMN. The resulting FAD molecule is the active form of riboflavin that is used as a cofactor by a variety of enzymes in the body.

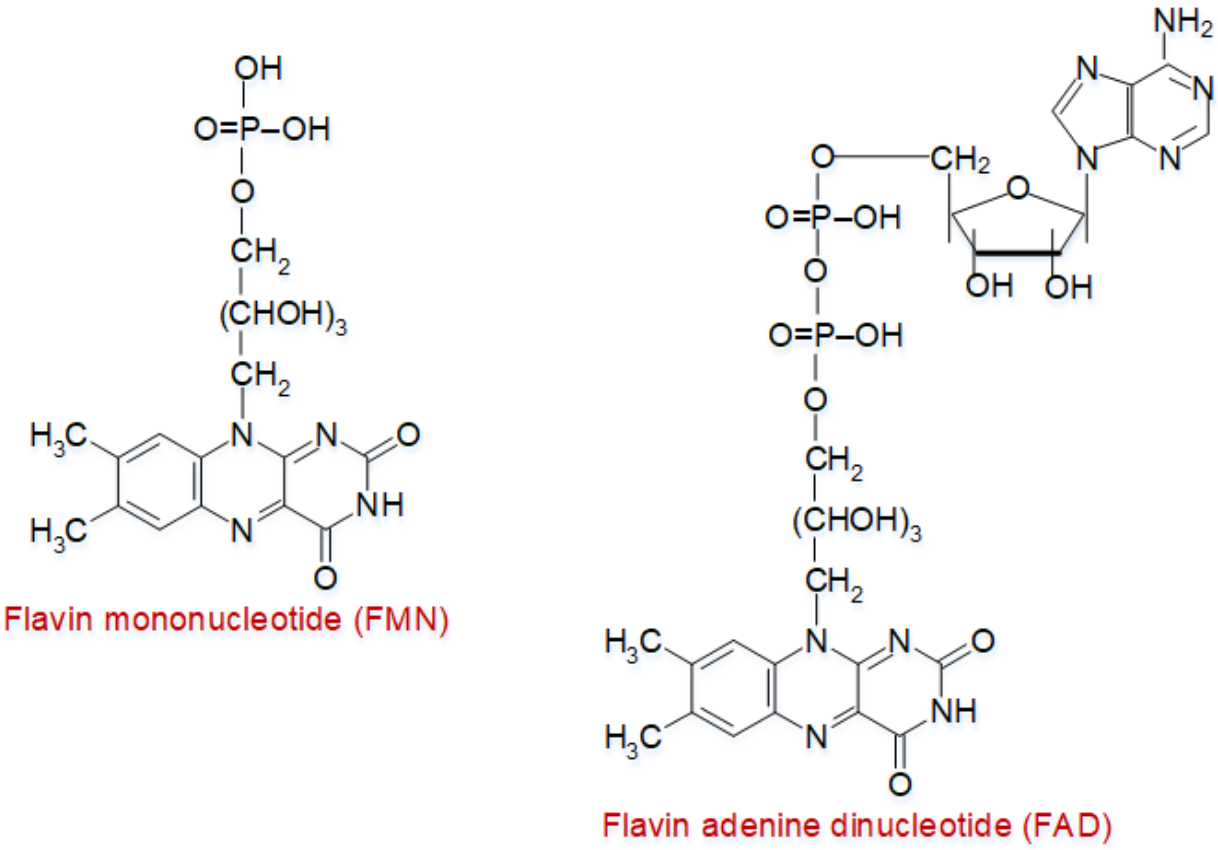


Fig. 4.3. The active forms of riboflavin are flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

Flavin coenzymes (mostly FAD and to a lesser extent FMN) participate in many reactions reduction and oxidation, often responsible for energy generation. The functional unit of both coenzymes is **isoalloxazine ring** which serves as an acceptor of two hydrogen atoms (with electrons). FMN or FAD undergo identical reversible reactions accepting two hydrogen atoms forming $FMNH_2$ or $FADH_2$ (fig. 4.4).

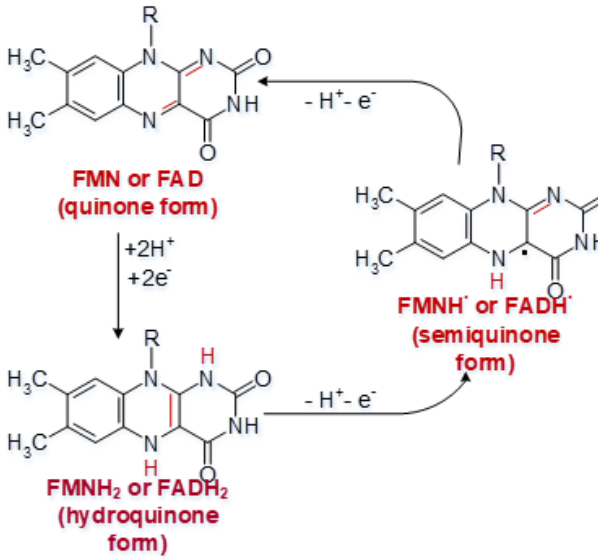


Fig. 4.4. Reduction of FMN or FAD

Enzymes that use flavin coenzymes (FMN or FAD) are called **flavoproteins.** The coenzymes (prosthetic groups) often bind rather tightly, to the protein (apoenzyme) either by non-covalent bonds (mostly) or covalent bonds in the holoenzyme. Many flavoproteins contain metal atoms (iron, molybdenum etc.).

Biological role of the coenzymes, FAD and FMN is connected to their Red/Ox properties, they are involved in carbohydrate, lipid, protein and purine metabolisms, besides the electron transport chain. Enzymes containing FMN or FAD as prosthetic group are called as flavoenzymes, some examples are represented in table 4.1.

Table 4.1. Flavoenzymes

Examples of FAD and FMN dependent enzymes		
Coenzyme	**Enzyme**	**Reaction**
FAD dependent	Succinate dehydrogenase	Succinate → Fumarate
	Xanthine oxidase	Xanthine → Uric acid
	Pyruvate dehydrogenase complex	Pyruvate → Acetyl CoA
	α-Ketoglutarate dehydrogenase complex	α-Ketoglutarate → Succinyl Coa
FMN dependent	L-Amino acid oxidase	L-Amino acid → α-Ketoacid + NH_3

Dietary sources: Some of the dietary sources of riboflavin are:

- Dairy products such as milk, cheese, and yogurt
- Eggs
- Meats such as liver, beef, pork, and chicken
- Leafy green vegetables such as spinach and broccoli
- Nuts and seeds, particularly almonds and sunflower seeds
- Whole grains, including fortified cereals and breads

It is important to note that riboflavin is a sensitive vitamin and can be destroyed by light, so it is recommended to store riboflavin-rich foods in a dark and cool place.

Deficiency of riboflavin. Although riboflavin is actively involved in lipid and carbohydrate metabolism, and deficiency occurs in many countries, it is not fatal, because there is very efficient conservation of tissue riboflavin. Riboflavin released by the catabolism of enzymes israpidly incorporated into newly synthesized enzymes. Symptoms of B_2 hypovitaminoses:

- cheilosis (vertical fissure in the lips);
- angular stomatitis (craks in the corner of the mouth);
- glossitis;
- photophobia;
- seborrheic dermatitis;
- normochromic normocytic anemia;
- usually encountered along with pellagra (niacin deficiency);
- newborns treated for hyperbilirubinemia by phototherapy (riboflavin is unstable to light).

Chronic alcoholics are susceptible to B_2 deficiency. Assay of the enzyme glutathione reductase in RBCs will be useful in assessing riboflavin deficiency.

Antimetabolite. Calactoflavin is an antimetabolite of vitamin B_2.

MEDICAL IMPORTANCE

Measurement of erythrocyte riboflavin level and urinary riboflavin are reliable indicators of riboflavin status. Erythrocyte riboflavin level reflects the amount of riboflavin that has been incorporated into the cells, and urinary riboflavin excretion reflects the amount of riboflavin that has not been absorbed and utilized by the body. Decreased levels of both erythrocyte riboflavin and urinary riboflavin are seen in riboflavin deficiency.

4.2.2 Niacine.

Niacine (vitamin PP - pellagra, vitamin B_3, nicotinamidemide) is water soluble vitamin. Niacin is a pyridine derivative. Structurally, it is **pyridine-3-carboxylic acid**. The amide form of niacin is known as niacinamide or **nicotinamide (fig. 4.5).**

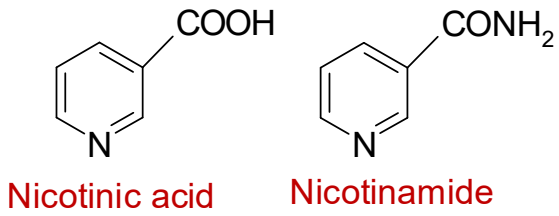


Fig. 4.5. Chemical structure of niacine

Potentially confusing names of niacin. *The terms vitamin B_3 and niacin, nicotinic acid are used interchangeably, but this isn't strictly accurate. Confusion can arise from the multiple names associated the vitamin and similar molecules. For example, even though one of the correct names for B3 names is nicotinic acid, it and nicotine could hardly be more different chemically or pharmacologically. They simply share part of the name and part of the chemical structure. Sometimes (especially in Ukranian biochemical literature) niacian is unexpectedly called vitamin B5.*

Absorption and transport. Nicotinic acid and nicotinamide are absorbed in small intestine and reach various tissues through circulation where they are converted to NAD and NADP. Niacin can be synthzised from essential amino acid **tryptophan**. 60 mg of tryptophan is equivalent to 1 mg of

dietary niacin. The conversion of tryptophan to niacin occurs through a pathway called the **"kynurenine pathway."** The conversion of tryptophan to niacin is influenced by a number of factors, including the presence of other nutrients (such as vitamin B_6). The niacin content of foods is expressed as:

mg niacin equivalents = mg preformed niacin + 1/60 × mg tryptophan

The liver plays a crucial role in the metabolism of niacin. In addition to its involvement in the conversion of tryptophan to niacin, the liver receives nicotinamide and some nicotinic acid through the portal circulation, as well as nicotinamide released from other tissues outside of the liver. Once in the liver, nicotinic acid and nicotinamide are metabolized to produce NAD^+ or to form compounds for excretion in the urine, depending on the niacin status of the organism. The liver also has the ability to store some amount of NAD^+.

Potentially confusing question concerning Nicotinic acid vs Nicotine – Nicotinic acid is a B-complex vitamin; nicotine is an alkaloid present in tobacco leaves.

Active forms. Nicotinamide is component of two coenzymes (fig. 4.6):

- **Nicotinamide adenine dinucleotide (NAD^+)**
- **Nicotinamide adenine dinucleotide phosphate ($NADP^+$)**

NAD^+ and $NADP^+$ are considered "loosely bound" coenzymes, meaning that they can detach from the enzyme after a reaction and be used again in subsequent reactions. This ability to be recycled makes them particularly important as coenzymes, as they can participate in multiple metabolic pathways within a cell.

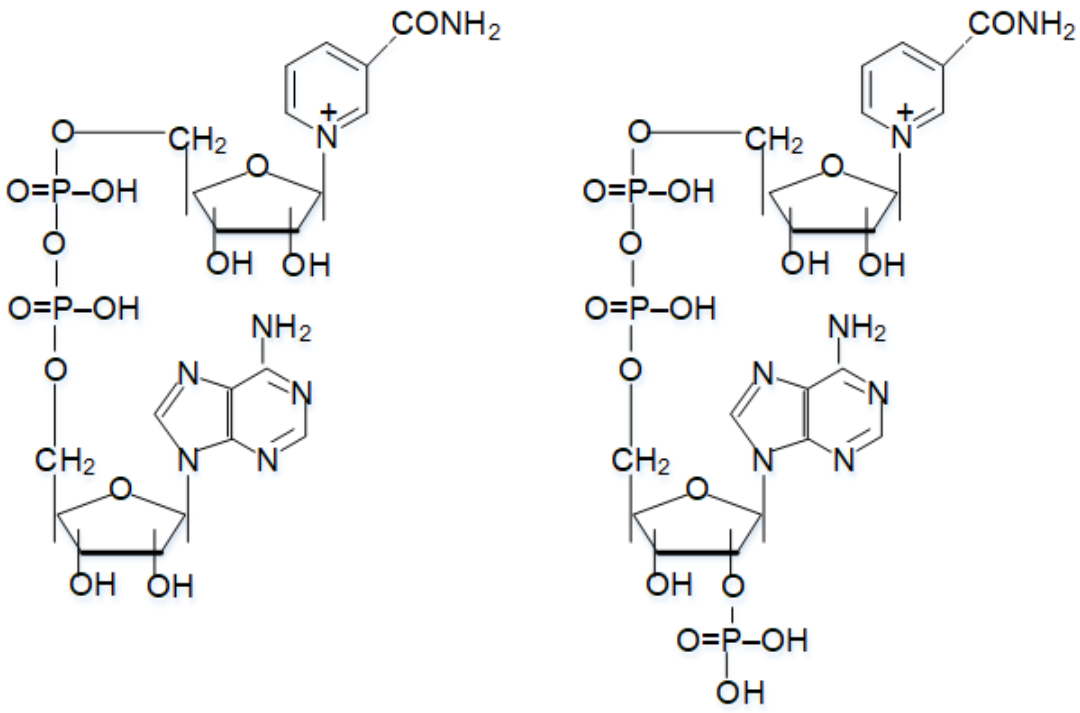


Fig. 4.6. NAD^+ and $NADP^+$ are the active forms of niacin, which serve as coenzymes for a wide range of enzymatic reactions in the body.

Biological role of the coenzymes NAD^+ and $NADP^+$ is to catalase a variety of Red/Ox reactions. NAD^+ and $NADP^+$ act as carriers of hydride anion (an anion of

hydrogen H^-) in such reactions (fig. 4.7). They are involved in both catabolic and anabolic processes, including cellular respiration, the breakdown of fatty acids and amino acids for energy, and the biosynthesis of macromolecules such as nucleotides, fatty acids, and cholesterol. The reduction of NAD^+ to NADH and $NADP^+$ to NADPH during these reactions allows for the transfer of electrons, which can be used to generate ATP or reduce other molecules. The resulting energy and reduction potential are essential for the proper functioning of metabolic pathways and cellular processes in the body.

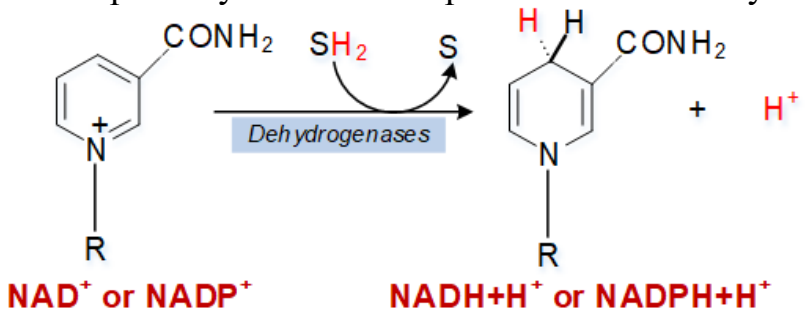


Fig. 4.7. Reduction of NAD^+ or $NADP^+$ involves the transfer of two electrons and a hydrogen ion (H^+) to the coenzyme, resulting in the formation of NADH or NADPH, respectively.

NAD^+ and $NADP^+$ are essential coenzymes for many oxidoreductase enzymes (table 4.2) involved in various metabolic pathways, including those of carbohydrates, lipids, and proteins. Some enzymes require only NAD^+ or $NADP^+$, while others can use either. Additionally, NAD^+ serves as a source of ADP-ribose for the ADP-ribosylation of proteins and polyADP-ribosylation of nucleoproteins involved in DNA repair. Also *NAD-dependent protein deacetylases*, also known as *sirtuins*, play a critical role in maintaining genomic stability through their role in chromatin structure and gene expression.

Table 4.2. Selected examples of NAD^+ or $NADP^+$ dependent enzyme

Coenzyme	Enzyme	Reaction
NAD^+ Dependent	Glyceraldehyde 3-phosphate dehydrogenase	Glyceraldehyde 3-phosphate → 1, 3-Bisphosphoglycerate
	Lactate dehydrogenase	Pyruvate → Lactate
	Alcohol dehydrogenase	Ethanol → Acetaldehyde
NAD^+ or $NADP^+$ dependent	Glutamate dehydrogenase	Glutamate → α-Ketoglutarate + NH_3
	lsocitrate dehydrogenase	lsocitrate → α-Ketoglutarate
$NADP^+$ dependent	Glucose 6-phosphate dehydrogenase	Glucose 6-phosphate → 6-Phosphogluconolactone
	Malic enzyme	Malate → Ptruvate
	Dihydrofolate reductase	Folic acid → Tetrahvdrofolic
	Phenylalanine hydroxylase	Phenylalanine → Tyrosine

Dietary sources. Niacin, also known as vitamin B_3, is found in a variety of dietary sources, including: meat (chicken, turkey, beef, pork, liver, and other organ meats), fish (tuna, salmon, and other fatty fish), whole grains (brown rice, wheat, barley), legumes (peanuts, lentils, beans, and peas), vegetables (avocado, broccoli, and potatoes), dairy

products (milk, cheese, and yogurt), nuts and seeds (sunflower seeds, almonds, and peanuts), eggs.

It is important to note that niacin is also synthesized in the body from the amino acid tryptophan, so consuming protein-rich foods can also contribute to niacin intake.

Niacin deficiency results in a condition called pellagra. This disease involves skin, gastrointestinal tract and central nervous system. The symptoms of pellagra are commonly referred to as fhree Ds:

- **D1 – Dermatitis.** It occurs in light exposed areas of skin due to photosensitivity. Initially exposed areas of skin develops sunburn which then progress to pigmentation and ulceration. The most affected areas are neck, forearms and fingers.
- **D2 – Diarrhoea.** It occurs due to inflammation of mucous membranes of gastrointestinal tract. If it prolongs death may occur.
- **D3 – Dementia.** It occurs in chronic cases. Neurological disturbances like depression, headache, delerium and memory loss are seen.

The disease also progresses in that order dermatitis, diarrhea, dementia, and if not treated may rarely lead to **death (4th D).**

Antagonists. Acetyl pyridine, pyridine sulfonamide, and aminonicotinamide are some examples of niacin antagonists. These compounds compete with niacin for binding to the enzyme responsible for niacin metabolism, leading to a decrease in NAD and NADP production. This, in turn, affects the energy production and other cellular processes that require NAD^+ and $NADP^+$. Long-term use of these antagonists can lead to niacin deficiency and related health problems.

MEDICAL IMPORTANCE

High doseges of niacin are used in attempts to treat Hartnup's disease, carcinoid syndrome, poor glucose tolerance, atherosclerosis, schizophrenia, hyperlipidemia, and a variety of skin disorders. High doses of niacin can be beneficial in treating certain conditions, but it can also be toxic in excess. Nicotinic acid has been used in the treatment of hyperlipidemia, but at high doses, it can cause side effects such as flushing, skin irritation, and liver damage. Similarly, intakes of both nicotinic acid and nicotinamide in excess of 500 mg/d can cause liver damage.

4.2.4 Pantothenic acid.

Pantothenic acid (vitamin B_5) is an amide of β-alanine and dihydroxy dimethyl butyric acid (fig. 4.8). It is widely distributed in nature.

Absorption and transport. Intestinal phosphatases can hydrolyze the phosphate group from various forms of pantothenic acid, including pantothenate, the most common form found in foods. Once released, free pantothenate is readily absorbed from the small intestine into the bloodstream and distributed to various tissues where it can be utilized for the biosynthesis of **coenzyme A (CoA-SH)**.

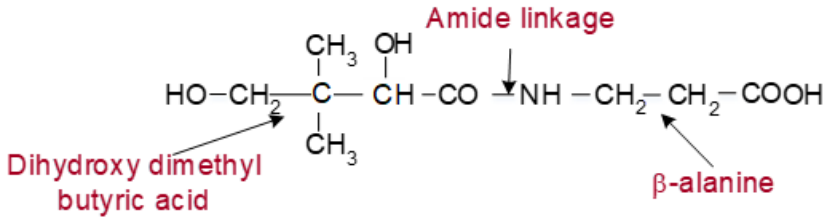


Fig. 4.8. Chemical structure of pantothenic acid

Active form. Coenzyme A (CoA-SH) is the active form of pantothenic acid (fig 4.9). First pantothenic acid is phosphorylated by ATP to **4'-phosphopantothenate**. Next **4'-phosphopantetheine** is formed by addition of cysteine and decarboxylation. Than adenylation by ATP happens to form **dephospho-CoA**. Finally, phosphorylation to the 3'-OH of the ribose generates CoA-SH (coenzyme A).

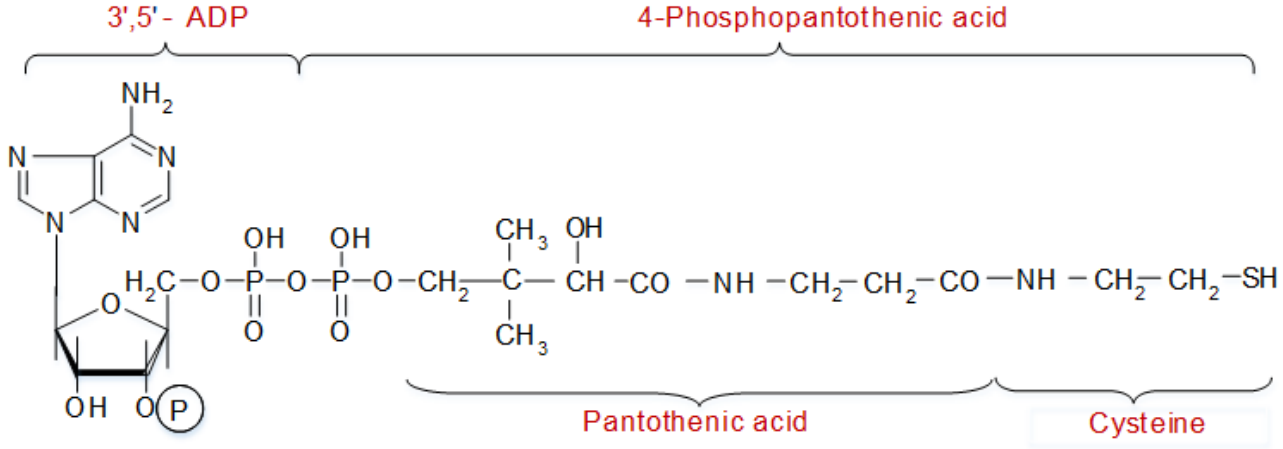


Fig. 4.9. Chemical structure of CoA-SH

Biological role. Coenzyme A (CoA-SH) plays a crucial role in the metabolism of carbohydrates, lipids, and amino acids by acting as a carrier of acetyl groups. CoA-SH binds to the acetyl group, forming acetyl-CoA, which is then used as a substrate in various enzymatic reactions, such as the citric acid cycle and fatty acid synthesis. The acetyl group can be transferred from acetyl-CoA to other moleculesto form new intermediates and facilitate further metabolic reactions:

- Pantothenic acid is required for the synthesis of **phosphopantotheine of acyl-carrier protein (ACP)** *of fatty acid synthase* complex. Phosphopantotheine of fatty acid synthase complex serve as carrier of acyl groups esetial for fatty acid biosynthesis.
- Thioesters, such as those formed by CoA and phosphopantetheine in ACP, are considered high-energy compounds because they are less thermodynamically stable than esters or amides. As a result, they have a relatively high-energy potential, which allows them to participate in many enzymatic reactions without requiring additional energy. These transfer reactions play important roles in carbohydrate, lipid, and amino acid metabolism by allowing acetyl groups to be transported from one substrate to another.
- CoA-SH is involved in a broad array of **acetyl and acyl transfer** reactions and processes related to primarily oxidative metabolism and catabolism, whereas ACP is involved in synthetic reactions.
- The addition of an acetyl group into an amino acid can markedly alter chemical properties. The same is true for biogenic amines, carbohydrates, complex lipids and hormones, xenobiotics.

Examples of CoA-SH-dependent enzymes: *pyruvate dehydrogenase and a-ketoglutarate dehydrogenase complexes, thiokinase.*

Dietary sources. Pantothenic acid is found in a wide variety of food sources, including: meat (beef, pork, and chicken liver, as well as turkey, duck, and chicken), fish (salmon, tuna), dairy products (milk, cheese, and yogurt), whole grains (brown rice, whole wheat bread, and other whole grain products), legumes (lentils, chickpeas, and other types of beans), vegetables (broccoli, potatoes, and mushrooms), nuts and seeds (peanuts, sunflower seeds, and others), eggs.

Pantothenic acid is found in small amounts in most foods, so it is generally easy to get enough of it in a balanced diet.

Deficiency of pantothenic acid. Pantothenic acid deficiency in humans can cause symptoms such as burning feet, abdominal cramps, restlessness, and fatigue. In experimental animals, deficiency of pantothenate can lead to dermatitis, graying of hair, fatty liver, growth failure, and neurological lesions. However, it is important to note that deficiency of pantothenic acid is rare in humans, as it is widely available in many foods.

Experimentally induced pantothenic acid deficiency produce parasthesia of extremities (burning feet), abdominal cramps, restlessness and fatigue in humans. In experimental animals pantothenate deficiency produce dermatitis, graying of hair, fatty liver, growth failure and neurological lesions.

MEDICAL IMPORTANCE

It is important to note that while pantothenic acid supplementation is generally safe, individuals should always consult with their healthcare provider before taking any dietary supplements, especially at high doses. High doses of pantothenic acid may also interfere with the absorption of biotin, another B-vitamin, and can lead to a biotin deficiency. Additionally, it is important to obtain nutrients through a well-balanced diet rather than relying solely on supplements.

4.2.5. Pyridoxine.

Pyridoxine (vitamins B_6) exists in form of three vitamers**: pyridoxine (pyridoxol), pyridoxal and pyridoxamine.** They differ from each other in the structure of a functional group attached to 4th carbon in the pyridine ring (fig 4.10).

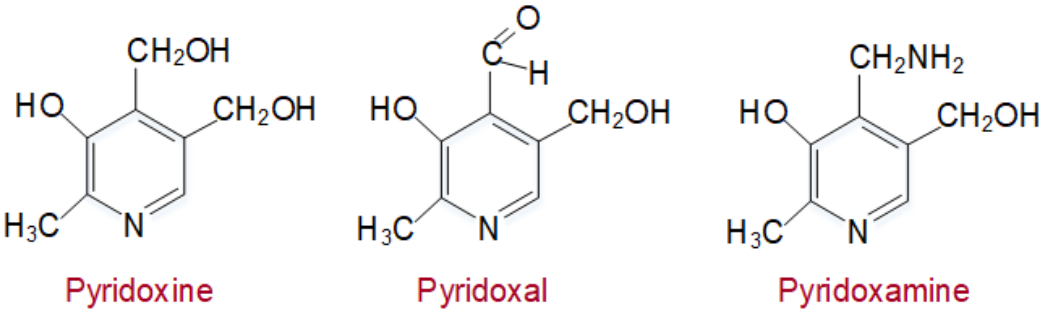


Fig. 4.10. The vitamer forms of pyridoxine, also known as vitamin B6, include pyridoxal, pyridoxamine

Absorption and transport. Pyridoxine is readily absorbed from the small intestine and transported to various tissues through the bloodstream. Once inside the cells, pyridoxine is converted to pyridoxal and pyridoxamine.

The active form. Pyridoxal phosphate (PLP) is the active form of vitamin B_6 and is formed from pyridoxal by phosphorylation catalyzed by *pyridoxal kinase*. **Pyridoxamine** is also found in tissues in small amounts, but it is mainly excreted in urine

as **4-pyridoxic acid**. Both PLP and pyridoxamine phosphate are involved in various enzymatic reactions, especially in amino acid metabolism.

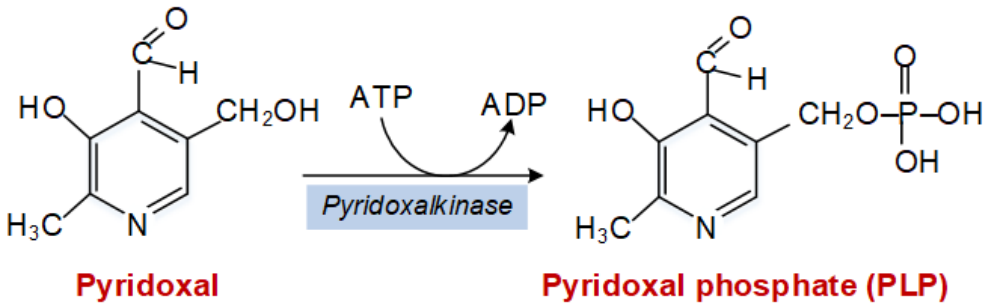


Fig. 4.11. Formation of pyridoxal phosphate from pyridoxal catalyzed by pyrydoxalkinase

Biological role of vitamin B_6:

- **Transamination**: PLP is involved in the transamination reaction (by *transaminase*) converting amino acids to keto acids. In the transamination reaction, an amino group is transferred from an amino acid to an α-keto acid, resulting in the formation of a new amino acid and a new α-keto acid. PLP serves as a coenzyme by binding to the amino acid substrate and facilitating the transfer of the amino group to the α-keto acid.
- **Decarboxylation**: Some of the amino acids undergo decarboxylation to form the respective amines. This is carried out by a group of enzymes called *decarboxylases* which are dependent on PLP. Many biogenic amines with important functions are synthesized by PLP decarboxylation.
- Pyridoxal phosphate is required for the **synthesis of δ-amino levulinic acid**, the precursor for heme synthesis.
- PLP plays an important role in metabolism of **sulfur containing amino acid**. **Transulfuration** from homocysteine to serine occurs in synthesis of cysteine.
- **Conversion of tryptophan to niacin**. Enzyme *kinureninase* needs PLP as a coenzyme.
- **Conversion of linoleic acid into arachidonic acid** (prostaglandins precursor)
- **Formation of sphingolipids.**

Examples of PLP-dependent enzymes: *aspartate transaminase (AST) and alanine transaminase (ALT), L-aromatic amino acid decarboxylase (AADC).*

Dietary sources. Good dietary sources of vitamin B_6 include: poultry, such as chicken and turkey, fish, such as salmon and tuna, beef and pork, beans and legumes, such as chickpeas and lentils, nuts and seeds, such as sunflower seeds and pistachios, whole grains, such as brown rice and oats, vegetables, such as potatoes and spinach.

Vitamin B_6 is sensitive to heat, so cooking these foods for long periods of time or at high temperatures can reduce their vitamin B_6 content.

Deficiency of vitamin B_6. Vitamin B_6 deficiency is rare in humans, but it can occur in certain populations such as pregnant women, elderly people, and alcoholics. Symptoms of vitamin B6 deficiency may include microcytic hypochromic anemia, skin lesions, depression, confusion, and convulsions. However, these symptoms are not specific to vitamin B_6 deficiency and may also occur due to other nutrient deficiencies or underlying

medical conditions. Microcyticypochromic anemia due to decreased heme synthesis, skin lesions that resemble those occur in niacin deficiency, depression and mental disturbances are observed in experimentally induced vitamin B_6 deficiency in humans.

Vitamin B_6 antagonists. Isoniazid is a drug frequently used for the treatment of tuherculosis. lt combines with pyridoxal phosphate to form inactive hydrazone derivatives which inhibit PLP dependent enzymes. Tuberculosis patients, on long term use of isoniazid develop peripheral neuropathy which responds to B_6 therapy. The drug **penicillamine** (B-dimethyl cysteine) is used in the treatment of patients with rheumatoid arthritis, Wilson's disease and cystinuria. This drug also reactsw ith PLP to form inactive thiazolidine derivative.

MEDICAL IMPORTANCE

High doses of pyridoxine have been used in the treatment of various clinical conditions. For example, pyridoxine supplementation has been shown to lower homocysteine levels in people with hyperhomocysteinemia. Pyridoxine-dependent convulsions are a rare genetic disorder that can be treated with high doses of pyridoxine. However, the use of high doses of pyridoxine for the treatment of autism and Down's syndrome is controversial and not supported by strong scientific evidence. It's important to note that high doses of pyridoxine can have adverse effects, including nerve damage, so it should only be taken under medical supervision.

4.2.6. Biotin

Biotin (vitamin B_7 or vitamin H) is a sulfur-containing biocyclyc water-soluble vitamin. It consists of imidozole ring fused to tetrahydrothiophene with valerie acid side chain (fig. 4.12).

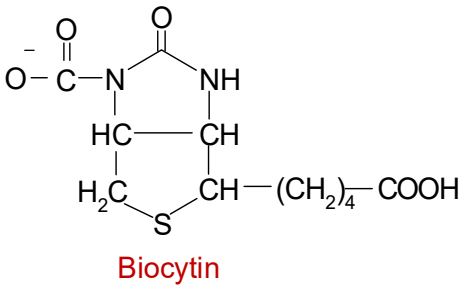


Fig. 4.12. Chemical structure of carboxybiotine

Absorption and transport. Biotin is a water-soluble vitamin that is absorbed in the small intestine through a process that involves both passive diffusion and active transport. Once absorbed, biotin enters the bloodstream and is transported to the liver, where it is converted into its active form, wich is then distributed to other tissues throughout the body, where it is used in a variety of important metabolic processes.

Active form of biotin is biocytin (caboxybiotine). Biotin is converted to its coenzyme form biocytin by formation of a covalent amide bond to the nitrogen of a lysine residue. Activation of biotin requires enzyme, CO_2, ATP and Mg^{2+}.

Biological role. Biotin plays a critical role in a wide variety of metabolic processes in the body, wich include:

- **Energy production:** Biotin is involved in the metabolism of carbohydrates, fats, and proteins, which are the primary sources of energy for the body. Biotin-dependent enzymes help to convert these macronutrients into energy that can be used by the body's cells.
- **Gluconeogenesis:** Biotin is required for the synthesis of glucose from non-carbohydrate sources, such as amino acids and fatty acids. This process, known as

gluconeogenesis, is important for maintaining normal blood glucose levels, particularly during periods of fasting or prolonged exercise.

- **Fatty acid synthesis:** Biotin is involved in the synthesis of fatty acids, which are essential building blocks for cell membranes and play a critical role in energy storage. Biotin-dependent enzymes are required for the elongation of fatty acid chains during fatty acid synthesis.
- **Amino acid metabolism:** Biotin is involved in the metabolism of certain amino acids, including the breakdown of branched-chain amino acids and the conversion of tryptophan to niacin.
- **Gene expression:** Biotin plays a role in gene expression by acting as a coenzyme for enzymes involved in DNA replication and repair.
- **Skin and hair health:** Biotin is important for the maintenance of healthy skin, hair, and nails. In fact, biotin is sometimes used as a supplement to improve the appearance of hair and nails.
- **Pregnancy and fetal development:** Biotin is essential for proper fetal development during pregnancy, particularly in the development of the central nervous system.

Examples of biotin-dependent enzymes: *pyruvate carboxylase* (synthesis of oxaloacetate for gluconeogenesis and replenishment of the citric acid cycle), *acetyl CoA carboxylase* (fatty acid biosynthesis), *propionyl-CoA carboxylase, β-methylcrotonyl-CoA carboxylase.*

Dietary sources of biotine include: egg yolk (it is one of the richest sources of biotin, with one large egg providing about 10 micrograms of biotin), meat and poultry (liver, kidney, and other organ meats), fish (salmon, tuna, and mackerel), dairy products (milk, cheese, and yogurt), whole grains (such as wheat, oats, and barley), legumes and nuts, vegetables and fruits (spinach, broccoli, bananas and avocados).

Deficiency of biotin is rare but can occur in certain circumstances. Causes of biotin deficiency include:

- **Dietary deficiency:** Biotin deficiency can occur if someone consumes a diet that is very low in biotin-rich foods.
- **Raw egg white consumption:** Avidin, a protein found in raw egg whites, binds to biotin and prevents its absorption in the body.
- Intestinal disorders: Some gastrointestinal disorders, such as Crohn's disease, can interfere with biotin absorption.
- **Long-term use of certain medications:** Antibiotics and anticonvulsants, such as sulfonamides, can destroy beneficial gut bacteria that produce biotin.

Symptoms of biotin deficiency:

- **Skin rash:** Dermatitis, a scaly red rash, is a common symptom of biotin deficiency.
- **Hair loss:** Biotin deficiency can lead to hair loss or thinning.
- **Neurological symptoms:** Biotin deficiency can cause neurological symptoms, such as depression, lethargy, and hallucinations.
- **Anemia:** Biotin deficiency can cause anemia, which is characterized by fatigue, weakness, and shortness of breath.

Consequences of biotin deficiency:

- **Impaired growth and development:** Biotin deficiency can cause growth and developmental problems in infants and children.
- **Impaired immune function:** Biotin deficiency can impair immune function and increase the risk of infections.
- **Skin and nail problems**: Biotin deficiency can cause skin and nail problems, such as brittle nails and skin infections.
- **Neurological problems:** Biotin deficiency can cause neurological problems, such as seizures, ataxia, and hypotonia.

Antagonist of biotin. Avidin is a glycoprotein present in raw egg whites that binds to biotin and forms a complex, which is not absorbed by the intestine. This can lead to biotin deficiency. Avidin binds to biotin with very high affinity, and one molecule of avidin can bind to up to four molecules of biotin. Consuming raw eggs can indeed increase the risk of biotin deficiency, especially if consumed in large quantities. However, cooking the egg denatures the avidin protein, which reduces its ability to bind to biotin. Therefore, consuming cooked eggs is a good way to avoid the risk of biotin deficiency from avidin.

MEDICAL IMPORTANCE

Biotin is generally considered safe and non-toxic, even at high doses. Daily doses of up to 200 mg orally and up to 10-20 mg intravenously have been given for extended periods of time (over 6 months) to treat biotin-responsive inborn errors of metabolism and acquired biotin deficiency, and no toxic effects have been reported.

Biotin is a water-soluble vitamin, which means that excess amounts are excreted in the urine rather than stored in the body. However, it is always important to follow recommended doses and consult a healthcare professional before taking any dietary supplements, including biotin. High doses of biotin can interfere with laboratory test results, particularly those involving thyroid function and troponin levels, so it is important to inform your healthcare provider if you are taking biotin supplements.

4.2.7. Folic acid

Folic acid (folacin vitamin B_c, B_9) is abundantly found in green leafy vegetables. Folic acid consist of pteridine ring, para-aminobenzoic acid and glutamate (fig. 4.13).

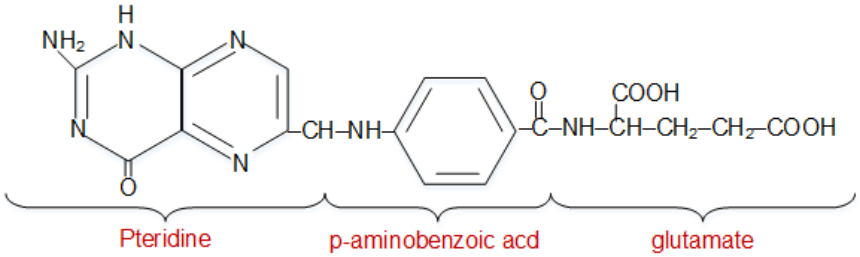


Fig. 4.13. Structure of folic acid. Folic acid is composed of a pteridine ring, para-aminobenzoic acid (PABA), and glutamic acid.

Folic acid is present in natural foods **as folyl polyglutamate**, which contains up to 7 glutamate residues. In the intestinal mucosal cells, a lysosomal enzyme called *folyl polyglutamate hydrolase* removes excess glutamate residues to form folic acid. Folic acid is then reduced to tetrahydrofolate (THF) and methylated to form N5-methyl THF. This is the major circulating form of folic acid, and it is usually bound to protein (fig. 4.14).

The liver and other tissues take up circulating methyl THF and convert it back to the polyglutamate form after the transfer of a methyl group. The polyglutamate form of folate is the active form that is used in the body.

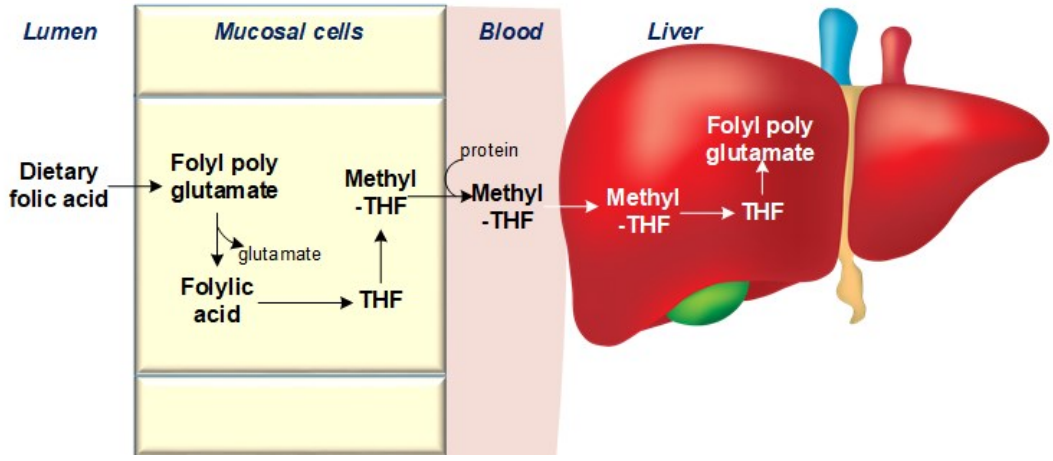


Fig. 4.14. Absorption, transport and fate of folic acid. Folic acid is absorbed in the small intestine through a process that requires enzymes and carriers. Once absorbed, folic acid is transported to the liver, where it is converted to its active form, tetrahydrofolate (THF).

Active form of folic acid is **tetrahydrofolic acid (THF or FH_4)** is synthesized from folic acid by the enzyme *dihydrofolate reductase*, wich uses NADPH as a hydrogen donor.

Biological role. THF is actively involved in the **one carbon metabolism.** THF serves as an acceptor or donor of one carbon units: methyl ($-CH_3$), methylene ($-CH_2-$), methenyl (=CH-), formyl (-CHO), formimino (-CHNH) and formate (-COOH) groups. Some of the key functions of folic acid include:

- DNA synthesis and repair: Folic acid is essential for the synthesis and repair of DNA, the genetic material in our cells. It is required for the synthesis of nucleotides, the building blocks of DNA, and is also involved in the methylation of DNA, which helps regulate gene expression.
- **Red blood cell formation**: Folic acid is necessary for the formation of red blood cells, which are responsible for carrying oxygen throughout the body. A deficiency in folic acid can lead to anemia, a condition characterized by a decrease in the number of red blood cells.
- **Homocysteine metabolism**: Folic acid is involved in the metabolism of homocysteine, an amino acid that is associated with an increased risk of heart disease when present in high levels in the blood.
- **Neural tube development**: Adequate folic acid intake is important during pregnancy to support the proper development of the neural tube, which eventually develops into the baby's brain and spinal cord. Folic acid supplementation before and during early pregnancy can help reduce the risk of neural tube defects such as spina bifida.
- **Cognitive function**: Folic acid may play a role in cognitive function and mood regulation. Some studies have suggested that low levels of folic acid may be associated with depression and cognitive decline in older adults.

Examples of THF-dependent enzymes: *methionine synthase, hydroxymethyltransferase*.

Dietary sources. Folic acid is found in a variety of natural food sources, including: leafy green vegetables (spinach, broccoli, asparagus), legumes (chickpeas, lentils, kidney beans), fruits (citrus fruits, bananas), fortified cereals and breads, liver and organ meats (beef liver, chicken liver), yeast extract.

It is important to note that cooking and processing of food can destroy folic acid, so it is recommended to eat these foods raw or minimally cooked to maximize folic acid intake. Additionally, folic acid supplements are widely available and commonly recommended for pregnant women and individuals at risk for folic acid deficiency.

Deficiency of folic acid can by caused by: defective absorption (most common): sprue, gastric resection and intestinal disorders, acute and chronic alcoholism, drugs (anticonvulsants and oral contraceptives), pellagra; abnormal metabolism of folates: folic acid antagonists (dihydrofolate reductase inhibibitors - methotrexate, pyrimethamine, trimethoprim), enzyme deficiency, vitamin B_{12} deficiency, oral contraceptives; increased requirement (pregnancy, infancy).

Folic acid deficiency can lead to a condition called megaloblastic anemia, in which the bone marrow produces large, immature red blood cells that cannot function properly. In addition to anemia, other symptoms of folic acid deficiency may include: fatigue, weakness, shortness of breath, irritability, mouth sores, poor growth in children, changes in skin, hair, or nails, cognitive impairment in older adults. Pregnant women with folic acid deficiency are at an increased risk of giving birth to infants with neural tube defects, such as spina bifida. It is recommended that all women of childbearing age consume adequate amounts of folic acid to prevent these birth defects.

Folic acid antagonists. Aminopterin and amethopterin are structural analogues of folic acid that competitively inhibit ***dihydrofolatereductase*** and stop the synthesis of THF. The biosynthesis of purines, thymine nucleotides and hence DNA is impaired. **Sulfonamides** are structural analogues of PABA. They competitively inhibit the enzyme (*dihydropteroate synthase*) responsible for the incorporation of PABA into pteridine to produce folic acid. For this reason, sulfonamides are used as antibacterial drugs. Sulfonamides, have no effect on human body, since folic acid is not synthesized and supplied through the diet.

Folic acid antagonists can also be found in some foods, such as legumes, leafy vegetables, and liver, but these are not typically associated with adverse effects and are usually considered beneficial for health.

MEDICAL IMPORTANCE

The neural tube develops from the neural plate during the first 28 days postconception and incomplete closure can result in birth defects known as NTDs. Folic acid supplements taken before and during pregnancy can significantly reduce the risk of NTDs. Poor folate status has been associated with an increased risk of cancer, particularly colorectal cancer and its precancerous lesion, adenoma, based on observational data from population-based studies.

4.2.8. Cobalamine.

Vitamin B_{12} or **cobalamine** is also known as **anti-pernicious anemia vitamin**. It is not produced by animals and plants but rather by microorganisms such as bacteria, fungi, and algae. The structure of vitamin B_{12} consists of a corrin ring with a central cobalt atom (fig.4.15). The corrin ring has four pyrrole units, similarly to porphyrin ring.

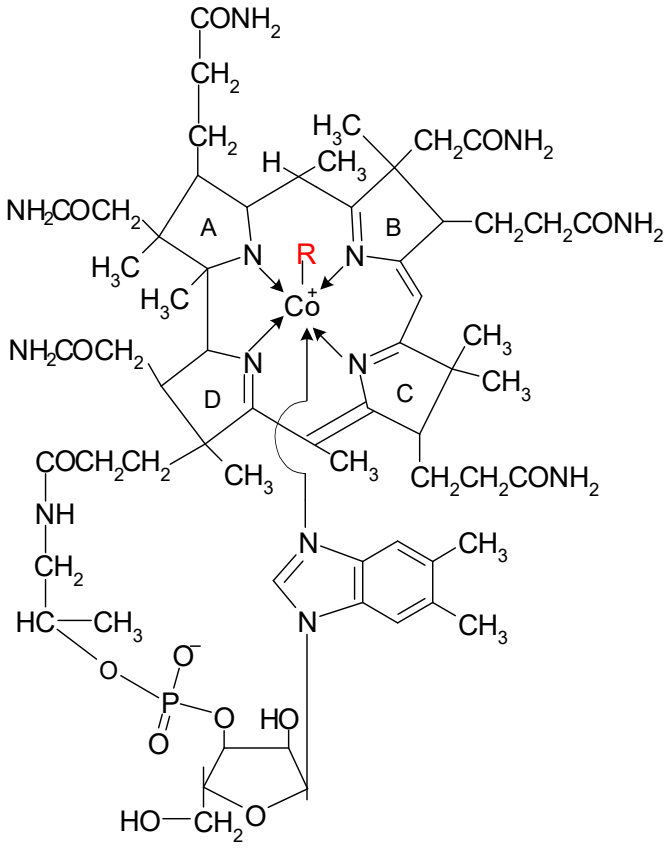


Fig. 4.15. Chemical structure of cobalamine

Vitamin B_{12} has cobalt atom in a coordination state of six. Cobalt present at the centre of the **corrin ring** is bonded to the four pyrrole nitrogens. Cobalt also holds (below the corrin plane) **dimethyl-benzimidazole (DMB)** containing ribose 5-phosphate and aminoisopropanol. A nitrogen atom of dimethylbenzimidazole is linked to cobalt. The amide group of aminoisopropanol binds with corrin. A "R" group is attached to central cobalt atom.

Absorption and transport. The absorption of vitamin B_{12} takes place in ileum. The dietary vitamin B_{12} which is bound to some substances dissociates at acidic pH of stomach.

.A special type of proteins known as **haptocorrins (cobalofilin)** present in stomach combines with the free vitamin B_{12}. Subsequently, the hahtocorrin is digested by pancreatic trypsin in the duodenum pancreatic and vitamin B_{12} is released. This vitamin B_{12} combines with **intrinsic factor (Castl factor)** – a glycoprotein secreted by parietal cells of the stomach to form vitamin B_{12} intrinsic factor complex. **One mg of intrinsic factor binds 3 mg of vitamin ·B_{12}.** Through a receptor mediated mechanism vitamin B_{12}-intrinsic factor complex is absorbed in the ileum. In the ileal cells the intrinsic factor is released and the vitamin B_{12} is transferred to a plasma transport protein **transcobalamin II**. The transcobalamin II delivers vitamin B_{12} to tissues. The transcobalamin II vitamin B_{12} complex enters the cells through a specific cell surface receptor. In the cytosol of the cell cobalamin is released from transcobalamin II as hydroxycobalamin. In the cells hydroxycobalamin is converted to methylcobalamin or deoxyadenosylcobalamin (Fig. 4.16). **Transcobalamin I** is another vitamin B_{12} transport protein present in plasma.

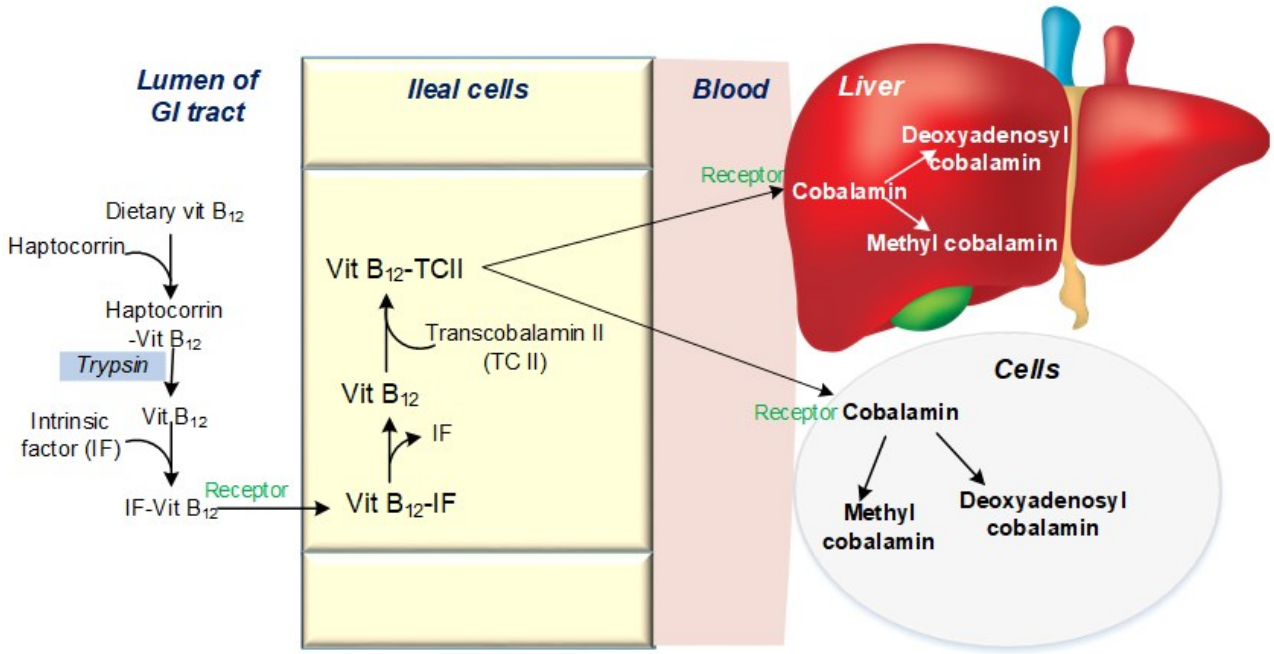


Fig. 4.16. The absorption, transport, and fate of vitamin B_{12} are highly regulated processes that are essential for its proper utilization by the body.

Unlike other water soluble vitamins vitamin B_{12} is stored in the liver and other tissues which is unique to vitamin B_{12}. The total body content of vitamin B_{12} is 3-4 mg. In the liver it is stored as deoxyadenosylcobalamin. Further liver cobalamins are secreted in the bile and undergo enterohepatic circulation.

Active forms. Various forms of vitamin B_{12} are named according to "R" group attached to central cobalt atom:

- If "R" group is **cyanide (CN)** then that form of vitamin B_{12} is called as **cyanocobalamin.**
- If "R" group is **hydroxyl (-OH)** then that form of vitamin B_{12} is called as **hydroxycobalamin.**
- If the "R" group is **methyl (-CH_3)** then that form of vitamin B_{12} is called as **methyl cobalamin.**
- If the "R" group is **deoxyadenosine** then that form of vitamin B_{12} is called as **deoxyadenosyl cobalamin**.

All the above forms exhibit vitamin B_{12} activity. However most of the therapeutic preparations contain cyanocobalamin.

Biological role:

1. Vitamin B_{12} act as prosthetic group or coenzyme. Vitamin B_{12} coenzymes are called as **cobarnide coenzymes**. Two forms of vitamin B_{12} that are required for activity of enzymes are **methylcobalamin** and **deoxyadenosylcobalamin**:
   - Methyl cobalamin is the coenzyme of ***methionine synthase.***
   - Deoxyadenosyl cobalamin is the prosthetic group of methyl ***malonyl-COA mutase***.

2. Vitamin B_{12} is effective in lowering concentration of plasma **homocysteine** of coronary artery disease patients.

Together with folic acid, vitamin B_{12} participates in the synthesis of creatine, nitrogenous bases, amino acids, proteins, nucleic acidsn. Deoxyadenosylcobalamin is

involved in the final stage of the oxidation of fatty acids with an odd number of carbon atoms, the side chain of cholesterol, thymine, branched-chain amino acids, and so on.

Examples of vitamin B_{12}-dependent enzymes: *methylmalonyl CoA mutase, homocysteine methyltransferase.*

Dietary sources. Some dietary sources of vitamin B_{12} include: meat (beef, pork, lamb), seafood (fish, shellfish, and other seafood, such as clams, salmon, tuna, and crab), dairy products (milk, cheese), eggs, fortified foods (some cereals, plant-based milks)

Vitamin B_{12} is not found in plant-based foods, so vegans and vegetarians may need to take supplements or consume fortified foods to ensure they are meeting their daily recommended intake.

Deficiency of vitamin B_{12} can by caused by:

- Autoimmune gastritis against parietal cells - loss of intrinsic factor).
- Rarely due dietary deficiency.
- Oral contaceptive drugs.
- Intestinal parasite.
- Gastrectomy.
- Chronic gastritis.

Deficiency of vitamin B_{12} is manifested by:

- **Macrocytic megaloblastic anemia:**
  - megaloblasts are abnormal erythroid precursors in bone marrow (most cells die in the bone marrow);
  - reticulocyte index is low;
  - hyperchromic macrocytes appear in blood;
  - anemia reflects impaired DNA synthesis;
  - other cells may be involved (leukopenia, thrombocytopenia).
- **Spinal cord degeneration (irreversible)**
  - swelling, demyelination, cell death;
  - neurological disease;
  - results from deficient methylmalonyl-CoA mutase;
  - this cannot be treated with folic acid!!

MEDICAL IMPORTANCE

Drugs like colchicine, neomycin and salicylates produce vit B_{12} deficiency by interacting with vitamin B_{12} intrinsic factor. Alcohol consumption for prolonged period produce vitamin B_{12} deficiency by interfering with vitamin B_{12} absorption. Repeated exposure to nitrous oxide an anaesthetic causes megaloblastic anemia. It inactivates methionine synthase.

4.2.8. Vitamin C

Vitamin C, ascorbic acid (2-oxo-L-theo-hexono-4-lactone-2,3-enediol). The chemical structures of ascorbic acid are shown in fig. 4.17. Ascorbic acid has two chiral centers, which contain four stereoisomers.

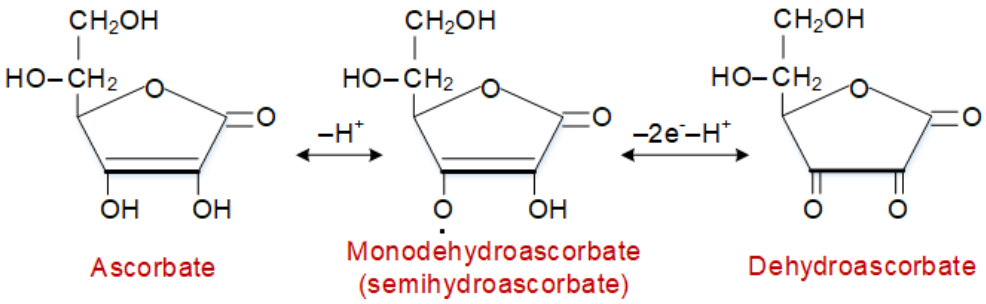


Fig. 4.17. Chemical structure of ascorbic acid. The molecule has a near planar fivemember ring.

Absorption and transport. Vitamin C is absorbed in the small intestine through both passive diffusion and active transport mechanisms. The active transport system is mediated by a specific sodium-dependent vitamin C transporter and glucose transporter (GLUT) family members. The absorption efficiency of vitamin C is dependent on the dose ingested and decreases with increasing dose. Once absorbed, vitamin C enters the bloodstream and is transported to tissues throughout the body. Vitamin C is water-soluble, and excess amounts are excreted in the urine, so the body does not store large amounts of this vitamin. As a result, it must be obtained regularly through the diet or supplements. Vitamin C can also be actively transported into cells through specific transporters in various tissues, including the liver, kidney, and brain. The active transport mechanism for vitamin C appears to be different among tissues, and the transporters in each tissue have different properties and regulation mechanisms.

Active forms of vitamin C are both **ascorbic acid** and **dehydroascorbic acid.**

Most of the functions of vitamin C are related to its property to undergo reversible oxidationreduction i.e., interconversion of ascorbic acid and dehydroascorbic acid. Thus the **biological role of vitamin C**:

- **Production and maintenance of collagen.** Vitamin C plays the role of a coenzyme in hydroxylation of proline and lysine while protocollagen is converted to collagen
  - Proline →**hydroxyproline**
  - Lysine → **hydroxylysine**
- **Metabolism of tyrosine.** Ascorbic acid is required for the oxidation of p-hydroxy phenylpyruvate (enzyme hydroxylase) to homogentisic acid in tyrosine metabolism.
- **Iron and hemoglobin metabolism.** Vitamin C enhances iron absorption by reducing ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), which is better absorbed in the intestine. Ascorbic acid also facilitates the uptake of iron into cells and helps in the formation of ferritin, which is the storage form of iron in the body. Furthermore, vitamin C plays a role in the mobilization of iron from ferritin, making it available for use in the body's metabolic processes.
- **Synthesis of corticosteroid hormones**. Adrenal gland possesses high levels of ascorbic acid, particularly in periods of stress. lt is believed that vitamin C is necessary for the hydroxylation reactions in the synthesis of corticosteroid hormones.
- **Antixidant action**. Normal metabolic processes in the cell lead to the generation of reactive oxidizing agents such as superoxide. Superoxide can react with and damage

protein and DNA, leading to cellular changes that can lead to premature aging and cancer. Vitamin C reacts with superoxide, thus preventing this damage.

- **Can stimulate the immune system.** Vitamin C enhances the synthesis of immunoglobulins (antibodies) and increases the phagocytic action of leucocytes.

Dietary sources of ascorbic acid :

- Citrus fruits, such as oranges, grapefruits, lemons, and limes
- Berries, such as strawberries, raspberries, blueberries, and cranberries
- Kiwi fruit, pineapple, mango, papaya
- Melons, such as cantaloupe and honeydew
- Tomatoes, bell peppers, particularly red and green peppers
- Broccoli, brussels sprouts, cauliflower, spinach

It's important to note that Vitamin C is sensitive to heat and light, so cooking and processing can decrease its concentration in foods. Therefore, it's recommended to eat these foods raw or lightly cooked to obtain the maximum amount of Vitamin C.

Ascorbic acid defiency leads to the development **of scurvy** with the following symtoms:

- Hemorrhage from mucous membranes, mouth and GIT, skin and muscles;
- Gingivitis: swelling, tenderness, redness and ulceration of gums;
- Loosening or loss of teeth;
- Sswelling of joints;
- Rarefaction of bones and dentine.

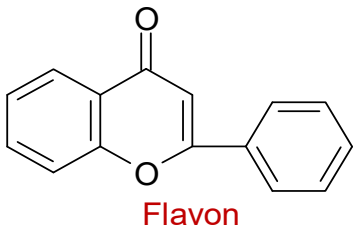
MEDICAL IMPORTANCE

The body's capacity to metabolize vitamin C becomes saturated at intakes above 100 mg/day, leading to increased excretion in the urine. However, some studies have suggested that higher intakes of vitamin C may provide additional health benefits, such as reducing the risk of chronic diseases like cancer and cardiovascular disease.

4.2.9. Flavonoids

Flavonoids (**vitamin P** or **bioflavonoids**) are a class of polyphenolic secondary metabolites found in plants, and thus commonly consumed in the diets of humans.

Chemically, flavonoids have the general structure of a 15-carbon skeleton (flavon), which consists of two phenyl rings (A and B) and a heterocyclic ring (C, the ring containing the embedded oxygen) (fig. 4.18). This carbon structure can be abbreviated C6-C3-C6. According to the IUPAC nomenclature, they can be classified into:



Flavon

Fig. 4.18. Cemical structure of flavon

- **flavonoids or bioflavonoids**
- **isoflavonoids**, derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure
- **neoflavonoids**, derived from 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure

The three flavonoid classes above are all ketone-containing compounds and as such, anthoxanthins (flavones and flavonols). This class was the first to be termed bioflavonoids. The terms flavonoid and bioflavonoid have also

been more loosely used to describe non-ketone polyhydroxy polyphenol compounds, which are more specifically termed flavanoids.

The biological role of flavonoids:

- Dietary flavonoids play an important role in the prevention of diseases related to **oxidative stress** in living systems.
- Flavonoids **regulate capillary permeability.**
- Several flavonoids such as catechin, apigenin, quercetin, naringenin, rutin, and venoruton are reported for **their hapatoprotective activities.**
- Flavonoids are known to be synthesized by plants in response to microbial infection; thus it should not be surprising that they have been found *in vitro* to be **effective antimicrobial and antiviral substances** against a wide array of microorganisms.
- Certain members of flavonoids significantly affect the **function of the immune system** and inflammatory cells.

The main **dietary sources of flavonoids** include tea, citrus fruit, citrus fruit juices, berries, red wine, apples, and legumes.

Frequent colds or infections, reflective of generally weakened immune function, can be a symptom of **flavonoid deficiency**. Conditions that reflect increased capillary permeability such as excessive bruising, swelling after injury, nose bleeds and hemorrhoids can also be a sign of inadequate dietary intake of flavonoids.

4.3. Fat-soluble vitamins

4.3.1. Vitamin A

Vitamin A is a fat-soluble vitamin that is essential for vision, growth, reproduction, immune function, and the maintenance of healthy skin and mucous membranes. Retinol is an alcohol containing **β-ionone ring.** The side chain has two isoprenoid units, four double bonds and one hydroxyl group (fig. 4.19).

Two groups of compounds have vitamin A activity: retinoids and carotinoids. Retinoids comprise **retinol, retinaldehyde,** and **retinoic acid** (preformed vitamin A, found only in foods of animal origin); carotenoids, found in plants, are composed of carotenes and related compounds; many are precursors of vitamin A, as they can be cleaved to yield retinaldehyde, then retinol and retinoic acid.

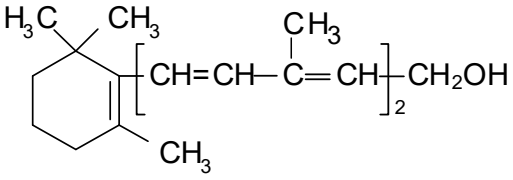


Fig. 4,19. Chemical structure of retinol

Absorption and transport of vitamin A. Vitamin A is absorbed and transported in the body through a complex process. Retinyl esters, which are present in the diet, are broken down into retinol and free fatty acids in the intestinal mucosa. This retinol is then converted into long-chain fatty acids and secreted into the lymphatic system as a component of chylomicrons. The chylomicron remnants containing retinyl esters are stored in the liver until needed. When required, the liver releases retinol, which is transported to other tissues by plasma **retinol-binding protein (RBP)**. The RBP-retinol complex attaches to specific receptors on the surface of peripheral tissue cells, allowing retinol to enter. Some tissues have a cellular retinol-binding protein that carries retinol to the nucleus where it acts like a steroid hormone (fig.4.20).

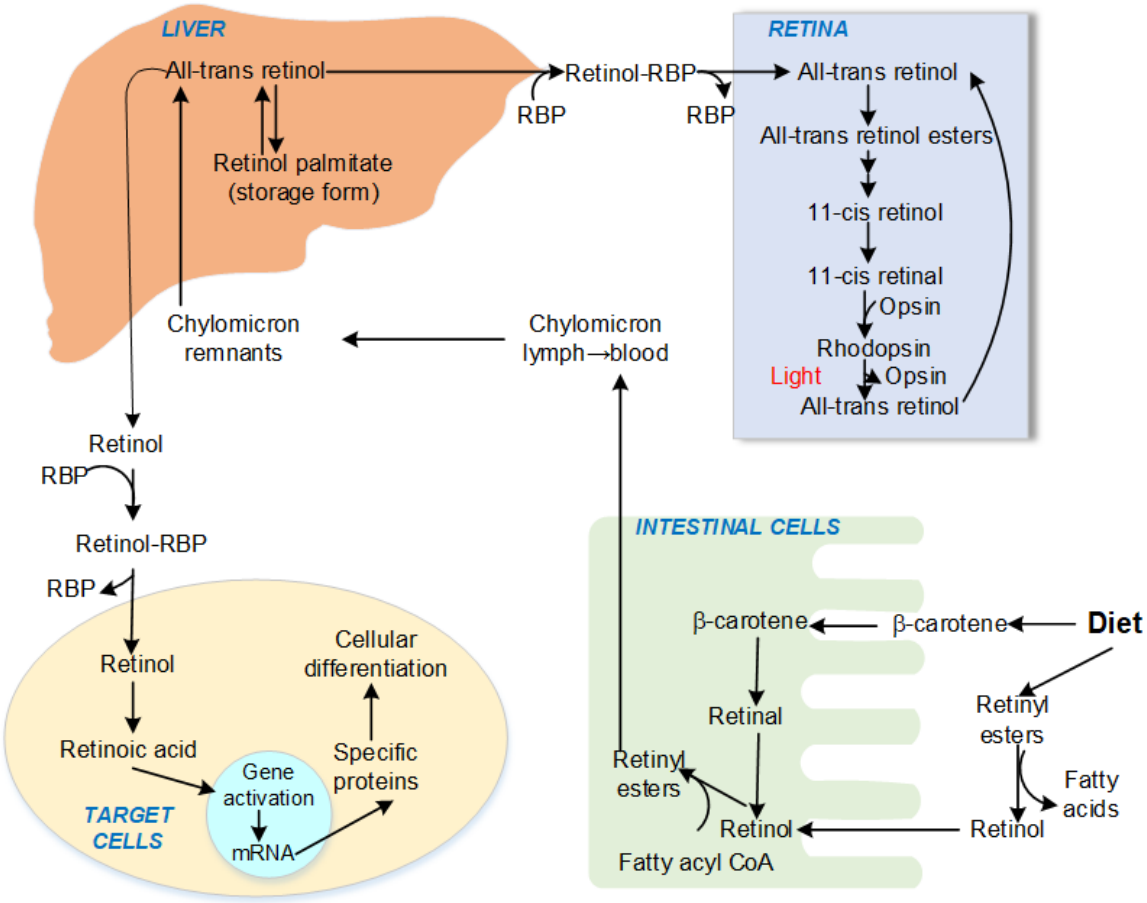


Fig. 4.20. Absorption, transport, and storage of vitamin A and its derivatives. RBP – retinol-binding protein

Mechanism of action. Retinol is converted to retinoic acid and binds with high affinity to receptor proteins located in the nucleus of specific target tissues, including epithelial cells (fig. 4.20). The activated retinoic acid-receptor complex interacts with nuclear chromatin to regulate the synthesis of retinoid-specific RNA, which controls the production of certain proteins involved in various physiological processes. One of the functions controlled by retinoids is the gene expression of keratin in most of the body's epithelial tissues.

Active forms. The various forms of retinoids found in the body are primarily produced by modifying the polar end group of the molecule. **Retinol and retinyl esters** are the most abundant types of retinoids in the body. Vitamin A is defined as **all-trans-retinol**. Retinyl esters, formed by esterifying a fatty acyl group to the hydroxyl end of retinol, serve as a storage form of retinol, with palmitic acid, oleic acid, stearic acid, and linoleic acid being the most common esters. Although retinyl esters have no known biological activity, they are necessary for the synthesis of 11-cis-retinal, which is involved in the formation of visual pigments. Retinol acts as a transport and precursor molecule that is enzymatically converted to **retinoic acid** via two oxidation steps. The primary function of **retinal** is in the eye, where it is required for the formation of visual pigments. In other tissues, retinal serves as an intermediary molecule in the synthesis of retinoic acid from retinol.

Biological role of vitamin A:

1. **Visual cycle:** Vitamin A plays a crucial role in the visual system by being a component of the visual pigments in both rod and cone cells. In rod cells, the visual pigment, rhodopsin, is composed of 11-cis retinal bound to the protein opsin. When rhodopsin absorbs light, a series of chemical reactions occur, resulting in the release of all-trans retinal and opsin, and the bleaching of the visual pigment. This process triggers a nerve impulse that is transmitted to the brain via the optic nerve. Regeneration of rhodopsin requires the conversion of all-trans retinal to 11-cis retinal through a series of reactions that involve reduction, esterification, isomerization, and oxidation. These reactions are also responsible for color vision in cone cells.
2. **Growth:** Vitamin A is essential for normal growth and development of the body. It plays a critical role in cell differentiation, proliferation, and maturation. Vitamin A is particularly important for the growth and differentiation of epithelial cells, which make up the skin, respiratory tract, gastrointestinal tract, and other organs. Vitamin A deficiency can result in impaired growth and development, particularly in children. It can also lead to weakened immune function, increased susceptibility to infections, and even blindness. Adequate intake of vitamin A, on the other hand, can promote healthy growth and development, particularly in children.
3. **Reproduction:** Retinol and retinal play essential roles in normal reproduction, supporting spermatogenesis in males and preventing fetal resorption in females. However, retinoic acid is not involved in maintaining reproduction or the visual cycle. Instead, it promotes growth and differentiation of epithelial cells. Animals given vitamin A only as retinoic acid from birth are blind and sterile, as retinoic acid is inactive in maintaining reproduction and the visual cycle.
4. **Maintenance of epithelial cells:** Vitamin A plays an important role in maintaining the health of epithelial tissues, such as those lining the respiratory, digestive, and urinary tracts, as well as the skin. Retinol and retinoic acid help to prevent the excessive accumulation of keratin, which is important for the normal functioning of these tissues. In addition, retinyl phosphate, which is synthesized from retinol, is necessary for the synthesis of certain glycoproteins that are involved in cell growth and mucus secretion.
5. **Immune system:** Vitamin A plays an important role in maintaining the health of the immune system. It helps in the development and differentiation of immune cells such as T cells, B cells, and natural killer cells. Vitamin A deficiency can impair the immune system and increase susceptibility to infections, particularly respiratory infections. Adequate intake of vitamin A is important for a healthy immune response.
6. **Antioxidant:** Carotenoids function as antioxidants. They function as antioxidants in the body, helping to neutralize harmful free radicals and other reactive oxygen species that can damage cells and DNA, leading to chronic diseases like cancer. Some carotenoids, such as beta-carotene, can also be converted to vitamin A in the body, providing additional health benefits.

Dietary sources of vitamin A. There are two main forms of vitamin A found in food: preformed vitamin A (also known as retinoids) and provitamin A carotenoids (also known as carotenes). Preformed vitamin A: liver (beef, pork, chicken, and fish), eggs, dairy products (milk, cheese, butter). Provitamin A carotenoids: dark green leafy

vegetables (spinach, kale, collards, turnip greens), orange and yellow vegetables (sweet potatoes, carrots, pumpkin, squash, red bell peppers), fruits (mangoes, papayas, apricots, cantaloupe), some fortified foods (such as orange juice and breakfast cereals).

The amount of vitamin A in plant sources varies depending on the type of plant and its growing conditions. Also, the absorption of carotenoids can be enhanced by cooking or processing the food, and consuming them with a source of fat may also improve their absorption.

Vitamin A deficiency is a serious health issue, and it can lead to various health problems, including blindness. The first sign of deficiency is difficulty seeing in low light, followed by night blindness. Prolonged deficiency can lead to **xerophthalmia,** which is a condition that causes the cornea to become dry and keratinized, leading to blindness. Vitamin A also plays an important role in the immune system by regulating the differentiation of immune cells. Even mild deficiency can increase the risk of infectious diseases. During infections, the synthesis of retinol binding protein, which is required to transport vitamin A in the bloodstream, is reduced, further impairing immune responses.

Hypervitaminosis There is only a limited capacity to metabolize vitamin A, and excessive intakes (more then 7.5 mg/day) lead to accumulation beyond the capacity of intracellular binding proteins; unbound vitamin A causes membrane lysis and tissue damage. Symptoms of toxicity affect the central nervous system (headache, nausea, ataxia, and anorexia, all associated with increased cerebrospinal fluid pressure); the liver (hepatomegaly with histological changes and hyperlipidemia); calcium homeostasis (thickening of the long bones, hypercalcemia, and calcification of soft tissues); and the skin (excessive dryness, desquamation, and alopecia).

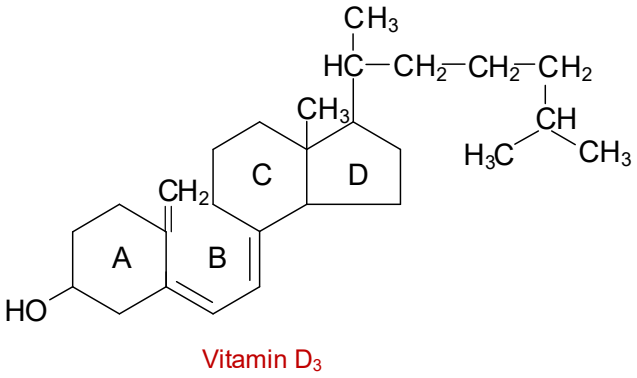
MEDICAL IMPORTANCE

Retinoic acid or its derivatives, including tretinoin and isotretinoin, are commonly used in dermatology to treat various skin conditions such as acne, psoriasis, and skin aging. Retinoic acid can help regulate the growth and differentiation of skin cells, leading to improvements in skin texture and appearance. Isotretinoin is a potent drug used to treat severe, cystic acne that is resistant to other treatments. Retinoic acid is also used in the treatment of acute promyelocytic leukemia, a type of cancer of the blood and bone marrow.

4.3.2. Vitamin D.

Vitamin D (calciferol) exists in two vitamers, **vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) (fig. 4.21),** which differ in the structure of their side chains. These forms of vitamin D are formed are synthesized for **sterols.** The provitamin of vitamin D_2 is **ergosterol** which is found in ergot and yeast. It is a derivative of **cholesterol**. The provitamin of vitamin D_3 is **7-dehydrocholesterol** which is found in animals. It is also a derivative of **cholesterol**. 7-Dehydrocholesterol (an intermediate in the synthesis of cholesterol that accumulates in the skin) undergoes a nonenzymic reaction on exposure to ultraviolet light, yielding previtamin D.

Absorption, transport and storage. Dietary vitamin D2 and vitamin D3 are absorbed in the small intestine with the help of bile salts. Once absorbed, they are incorporated into chylomicrons and enter circulation via the lymphatic system. Vitamin D then binds to **vitamin D binding protein (DBP)** in the blood, forming a complex that is transported to different tissues where it is taken up by cells. Vitamin D can also be stored in the liver and adipose tissue. Vitamin D binding protein has a high affinity for vitamin D3, but it can also bind to other forms of vitamin D.



Vitamin D_3

Fig. 4.21. Chemical structure of cholecalciferol

Active form of vitamin D is **1,25-dihydroxycholecalciferol** (calcitriol). Cholecalciferol is first hydroxylated at 25th position to **25-hydroxycholecalciferol** (25-OH D_3) bv a specific *hydroxylase* present **in liver**. 25-OH D_3 is the major storage and circulatory form of vitamin D. **Kidney** possesseas specific enzyme, *25-hydroxycholecalciferol (calciol) 1-hydroxylase* which hydroxylates 25-hydroxycholecalciferol at position 1 to produce **1,25-dihydroxycholecalciferol (1,25-DHCC).** 1,25-DHCC contains 3 hydroxyl groups (1,3 and 25 carbon) hence referred to as **calcitriol** (fig. 4.22)**.** Both the hydroxylase enzymes (of liver and kidney) require cytochrome P450, NADPH and molecular oxygen for the hydroxylation process.

Biological role of calcitriol which is the biologically active form of vitamin D is related to regulation of plasma levels of calcium and phosphates by following mechanisms:

- **Calcitriof increlses the intestinal absorption of calcium** and phosphate in the intestinal cells, calcitriol binds with a cytosolic receptor to form a calcitriol-receptor complex. This complex then approaches the nucleus and interacts with a specific DNA leading to the synthesis of a specific calcium binding protein (fig. 4.22). The calcitriol-receptor complex then translocates to the nucleus and acts as a transcription factor, binding to vitamin D response elements (VDREs) in the promoter regions of target genes. This leads to the activation of genes that are involved in calcium and phosphate absorption in the intestines, such as those encoding calcium-binding proteins, as well as genes involved in bone metabolism. By increasing the absorption of calcium and phosphate, calcitriol helps to maintain adequate levels of these minerals in the blood and support proper bone mineralization.

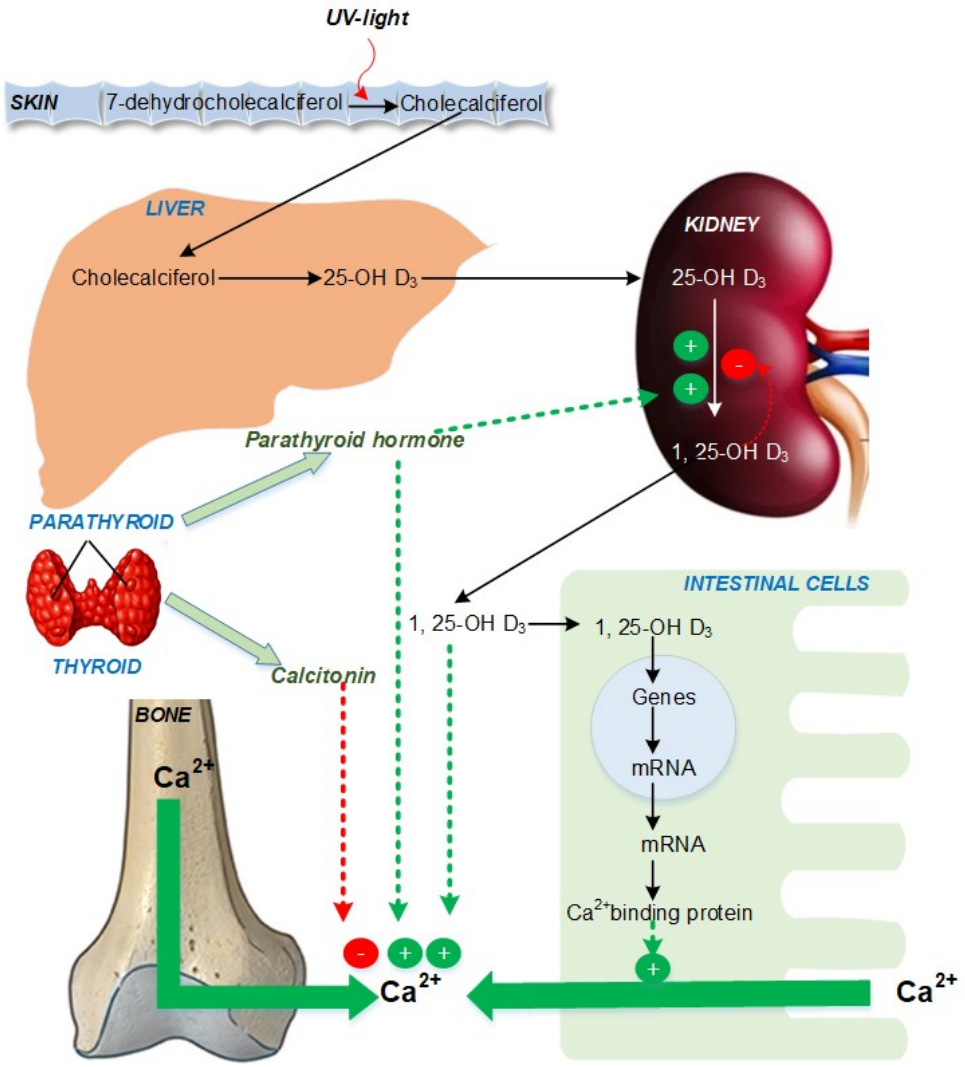


Fig. 4.22. Metabolism and actions of vitamin D

- **In the osteoblasts of bone, calcitriol stimulates calcium uptake** for deposition as calcium phosphe. Calcitriol plays an important role in bone metabolism by promoting calcium and phosphate uptake by osteoblasts, which are cells responsible for bone formation. This process is necessary for maintaining proper bone density and strength. Without sufficient levels of calcitriol, the body may not be able to absorb enough calcium and phosphate from the diet, which can lead to weakened bones and an increased risk of fractures.
- Calcitriol is also involved in **minimizing the excretion of calcium and phosphate through the kidney,** by decreasingt heir excretion and enhancing rdeabsorption.

Dietary sources of vitamin D include fany fish, fish liver oils, egg yolk etc. Milk is not a good source of vitamin D. Vitamin D can be provided to the body in three ways:

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

Deficiency of vitamin D. Vitamin D deficiency can lead to a number of health problems, including:

- **Rickets:** A disease that causes softening and weakening of bones in children, resulting in skeletal deformities.
- **Osteomalacia:** A similar condition that occurs in adults, characterized by bone pain and muscle weakness.
- **Increased risk of fractures:** Low vitamin D levels can lead to reduced bone density, increasing the risk of fractures, particularly in older adults.
- **Muscle weakness:** Vitamin D deficiency can cause muscle weakness and atrophy.
- **Increased risk of certain diseases**: Low vitamin D levels have been associated with an increased risk of several chronic diseases, including cancer, diabetes, and autoimmune disorders.
- **Increased risk of infections:** Vitamin D plays a critical role in immune function, and deficiency has been associated with an increased risk of infections, particularly respiratory infections.
- **Mood disorders:** Low vitamin D levels have been linked to depression and other mood disorders.
- **Impaired wound healing:** Vitamin D deficiency has been associated with impaired wound healing and increased risk of infections following surgery.

Hypervitaminosis D. Vitamin D is stored mostly in liver and slowly metabolised. Among the vitamins, vitamin D is the mosf toxic in overdoses. Toxic effects of hypervitaminosis D include demineralization of bone (resorption) and increased calcium absorptionf rom the intestine, Ieading to elevated calcium in plasma (hypercalcemia). Prolonged hypercalcemia is associated with deposition of calcium in many soft tissues such as kidney and arteries. Hypervitaminosis D may lead to formation of stones in kidneys (renal calculi).

MEDICAL IMPORTANCE

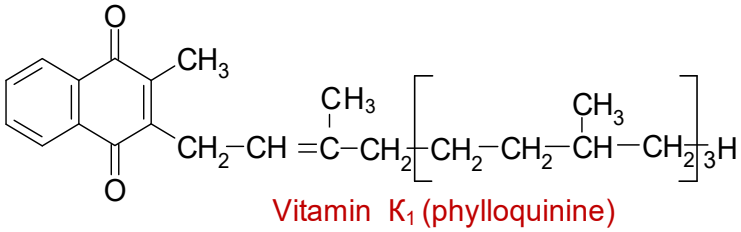
Persons suffering from chronic alcoholism exhibit a decrease in plasma 25(OH)D3 levels and bone mineral content. This is observed in patients with and without cirrhosis of the liver. However, how chronic alcoholism results in low 25(OH)D3 levels is at present not understood.

The fact that changes in the metabolism of vitamin D may occur with aging has been suggested by the observation that the ability to absorb dietary calcium decreases with age. In addition, loss of bone increases in the elderly along with age-related hypoplasia of bone cells. Further, 1a,25(OH)2D3 levels in the plasma and responsivity of the renal 25(OH)D3-1a-hydroxylase to PTH are both known to decrease with age.

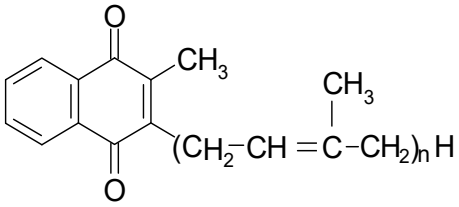
4.3.3. Vitamin K.

Vitamin K. The term vitamin K refers to group of compounds that exhibits vitamin K activity. There are following vitamers:

- **Vitamin K_1 also called as phylloquinone** (fig. 4.23) is the major form of vitamin found in plants particularly in green leafy vegetables.
- **Vitamin K_2 also known as menaquinone** is the vitamin K present in animals and synthesized by intestinal flora.
- **Vitamin K_3 (menadione)** is a synthetic form.



Vitamin K_1 (phylloquinine)



Vitamin K_2 (menaquinone)

Fig. 4.23. Chemical structure of vitamins K_1 and K_2

Vitamin K forms are **naphthoquinone** derivatives. lsoprenoid side chain is present in vitamins K_1 and K_2.

Absorption and Transport. Vitamin is absorbed in the small intestine along with dietary fat. It is incorporated into chylomicrons and transported to the liver, where it is either stored or incorporated into lipoproteins for transport to other tissues. Unlike some other fat-soluble vitamins, vitamin K is not stored extensively in the body. Instead, it is continuously recycled through a process called the vitamin K cycle, which allows it to be used repeatedly to activate clotting factors and other proteins that require vitamin K as a cofactor.

Biological role of vitamin K

1. **Formation of γ-carboxyglutamate (Gla):** Vitamin K is required in the hepatic synthesis of prothrombin and blood clotting factors II, VII, IX, and X. These proteins are synthesized as inactive precursor molecules. Formation of the clotting factors requires the vitamin K–dependent carboxylation of glutamic acid residues to Gla residues (fig. 4.24). This forms a mature clotting factor that contains Gla and is capable of subsequent activation. The reaction requires O_2, CO_2, and the hydroquinone form of vitamin K. The formation of Gla is sensitive to inhibition by **dicumarol**, an anticoagulant occurring naturally in spoiled sweet clover, and by **warfarin**, a synthetic analog of vitamin K.
2. **Interaction of prothrombin with platelets:** The Gla residues of prothrombin are good chelators of positively charged calcium ions, because of the two adjacent, negatively charged carboxylate groups. The prothrombin–calcium complex is then able to bind to phospholipids essential for blood clotting on the surface of platelets.

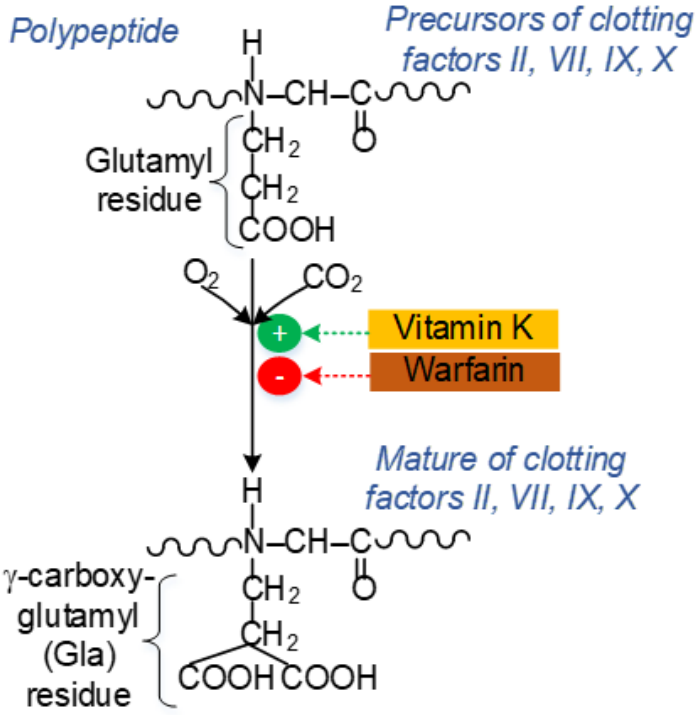


Fig. 4.24. Carboxylation of glutamate to form γ-carboxy-

Attachment to the platelet increases the rate at which the proteolytic conversion of prothrombin to thrombin can occur (fig.4.25).

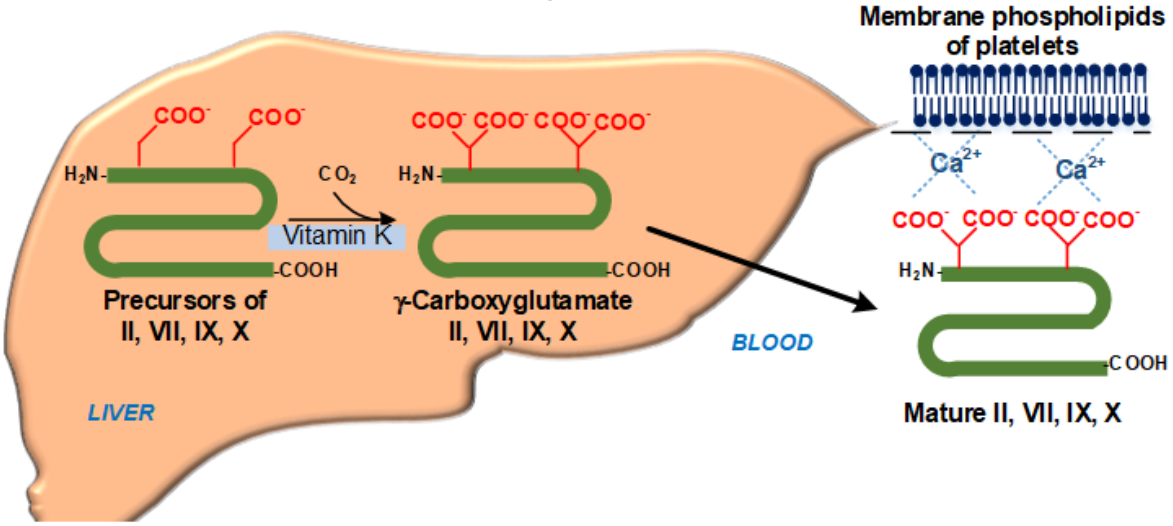


Fig. 4.25. Role of vitamin K in blood coagulation

Dietary sources. The main dietary sources of vitamin K include: green leafy vegetables (kale, spinach, broccoli, brussels sprouts, parsley, lettuce), vegetable oils (soybean, canola, and olive oils), fruits (avocado and kiwi), meat and dairy products (beef liver, egg yolk, and cheese).

Deflciency symptoms. The deficiency of vitamin K is uncommon, since it is present in the diet in sufficient quantity and/or is adequately synthesized by the intestinal bacteria. The most common symptom of vitamin K deficiency is **abnormal bleeding** or easy bruising, as vitamin K is essential for the production of clotting factors in the liver. In severe cases, deficiency can lead to hemorrhaging and internal bleeding. Infants are particularly vulnerable to vitamin K deficiency and are often given a vitamin K injection shortly after birth to prevent bleeding disorders.Vitamin K deficiency can also lead to weakened bones and an increased risk of fractures, as vitamin K is involved in the production of osteocalcin, a protein that helps maintain bone density. However, this effect is not as well established as the role of vitamin K in blood clotting.

Hypervitaminosis K. Administration of large doses of vitamin K produces hemolytic anaemia and jaundice, particularly in infants. The toxic effect is due to increased breakdown of RBC.

MEDICAL IMPORTANCE

Newborns are born with low levels of vitamin K and do not have the intestinal bacteria necessary for the synthesis of vitamin K. Breast milk also contains only a small amount of vitamin K, which may not be sufficient to meet the newborn's needs. This puts them at risk for a bleeding disorder known as hemorrhagic disease of the newborn (HDN). To prevent HDN, it is recommended that all newborns receive a single injection of vitamin K at birth. This is a safe and effective way to ensure that the newborn has adequate vitamin K levels.

4.3.4. Vitamin E (tocopherol).

The term **vitamin E** refers to group of four compounds that exhibit vitamin E activity. They are **α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol**. They are derivatives of **tocol or 6-hydroxy chromane ring** with **phytyl side chain**. They differ in methyl groups in positions 5, 7, and 8 of chromane ring. α-Tocopherol has three methyl groups in positions, 5, 7 and 8 of chromane ring(fig. 4.26). The chromane ring of β and γ tocopherols contain two methyl groups in positions 5, 8 and 7, 8 respectively. However δ-tocopherol has one methyl group in position 8 of chromane ring. Among vitamers, α-tocopherol is the most active.

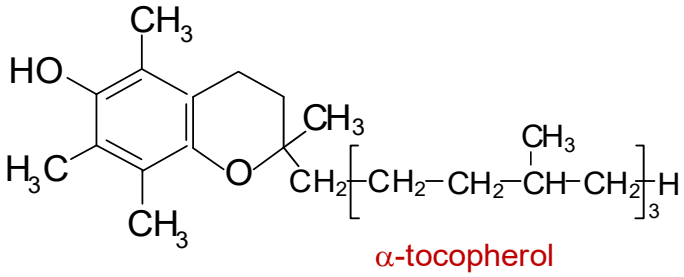


Fig. 4.26. Chemical structure of α-tocoferol

Absorption, transport and storage. Once in circulation, tocopherols are transported by lipoproteins to different tissues where they can act as antioxidants and protect cell membranes from oxidative damage. The liver plays a central role in the metabolism of vitamin E, by regulating its distribution to other tissues and excretion from the body. When tissue needs for vitamin E are met, excess vitamin E is excreted mainly via bile into the small intestine, and some of it may be reabsorbed.

Biological role. Most of the functions of vitamin E are related to its anfioxidant property. lt prevents the nonenzymatic oxidations of various cell components (for example, unsaturated fatty acids) by molecular oxygen and free radicals such as superoxide and hydrogen peroxide.

- Vitamin E is essential for the membrane structure and integrity of the cell, hence it is regarded as a membrane antioxidant.
- It prevents the peroxidation of polyunsaturated fatty acids in various tissues and membranes. lt protects RBC from hemolysis by oxidizing agents (e.g. H_2O_2).
- It is closely associatedw ith reproductive functions and prevents sterility. Vitamin E preserves a nd maintainsg erminal epithelium of gonads for proper reproductive function.
- lt increases the synthesis of heme by enhancing the activity of enzymes 6 - aminolevulinic acid (ALA) synthase and ALA dehydratase.
- lt is required for cellular respiration through electron transport chain (believed to stabilize coenzyme Q).
- Vitamin E prevents the oxidation of vitamin A and carotenes.
- lt is required for proper storage of creatine in skeletal muscle.
- Vitamin E is needed for optimal absorption of amino acids from the intestine.

Dietary sources. Vitamin E is found in a variety of foods, including: Nuts and seeds, such as almonds, sunflower seeds, and hazelnuts, vegetable oils, such as wheat germ, sunflower, and safflower oils, green leafy vegetables, such as spinach and broccoli, animal products, such as meat, poultry, and eggs, contain little vitamin E.

Vitamin E deficiency. Vitamin E deficiency is relatively rare, but it can occur in individuals with fat malabsorption disorders such as cystic fibrosis, cholestasis, and liver disease. A severe deficiency of vitamin E can lead to neurological symptoms such as ataxia, impaired vision, and muscle weakness, as well as immune dysfunction. In infants, vitamin E deficiency can lead to hemolytic anemia, a condition in which red blood cells are destroyed faster than they can be replaced. Vitamin E deficiency can also increase the risk of oxidative damage to cells and tissues, which may contribute to the development of chronic diseases such as cardiovascular disease, cancer, and Alzheimer's disease.

MEDICAL IMPORTANCE

Ataxia with isolated Vit. E deficiency (AVED) is a rare neurological disease characterized by defect in α-tocopherol transport protein (α-TTP). α-TTP is a cytosolic protein and function as sorting protein. α-TTP catalyzes transferof α-tocopherol taken up by liver into nascent VLDL. From VLDL α-tocopherol is released into circulation. Due to defective gene non functional α-TTP is produced.

4.4. Vitamin-like substances

Besides the vitamins, there are many other compounds present in foods as accessory factors. They are often referred to as "conditionally essential," as their synthesis within the body may be insufficient to meet the body's requirements under certain conditions, such as illness, stress, or increased metabolic demand. Some examples of such substances which may be regarded as **vitamin-like compounds** are given below.

4.4.1. Water-soluble vitamin-like substances

Choline (vitamin B_4). Choline is **trimethylhydroxy ethanolamine**. Choline is used in one of two pathways: it can be metabolized to betaine by oxidation to an aldehyde and a subsequent dehydrogenase-mediated step or it can be used as a substrate for the synthesis of phosphatidylcholine that is used to form cell membranes (fig. 4.27).

Biological role:

- Choline, as a component of **plrospholipids**;
- Choline prevents the accumulation of fat in liver (as **lipotropic factor**). It promotes the svnthesis of phospholipids and lipoproteins and the disposal of triacylglycerols from liver;
- Due to the presence of three methylgroups (one carbon fragments), choline is actively involved in one carbon metabolism.
- Choline is a precurso rf or the synthesis of **acetylcholine** which is required for transmission of nerve impulse.

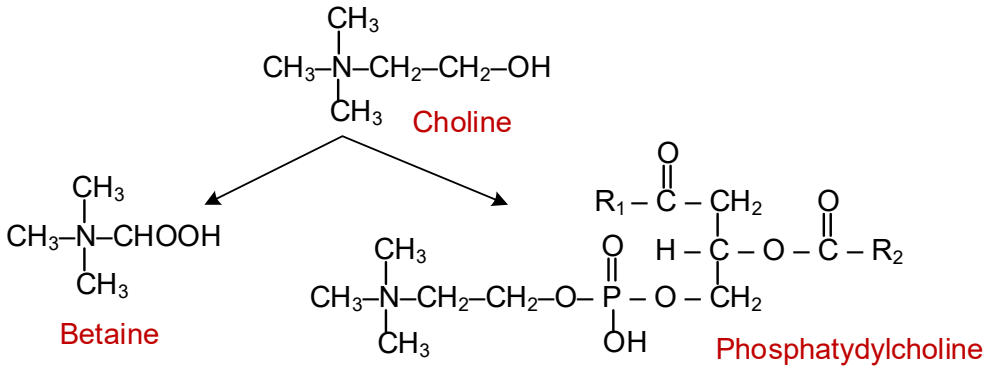


Fig. 4.27. Synthesis of betaine and phosphatidylcholine from choline

Dietary sourses of choline: whole eggs, meat, fish, shiitake mushrooms, soybeans, beef, wheat germ.

Choline deficiency symptoms:

- low energy levels of fatigue.
- memory loss.
- cognitive decline.
- learning disabilities.
- muscle aches.
- nerve damage.
- mood changes or disorders.

Lipoic acid. Lipoic acid exists in 2 forms: an open-chain reduced form of **dihydrolipoic acid** and a closed-ring disulfide form of **lipoic acid** (fig. 4.28). Oxidation-reduction cycles interconvert these 2 species. Lpoic acid exists covalently attached in an amide linkage with lysine residues on enzymes.

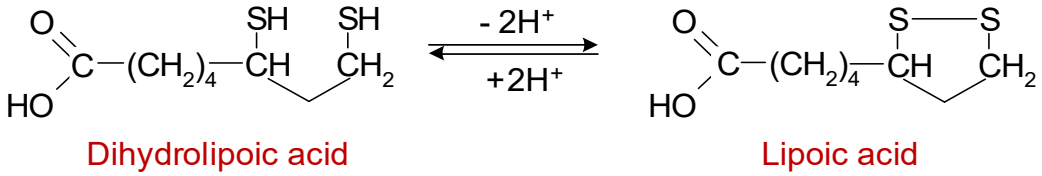


Fig. 4.28. Lipoic and dihydrolipoic acid

Lipoic acid function is to **couple acyl group transfer** and **electron transfer** during oxidation and decarboxylation of α-ketoacids.

Lipoic acid is a co-factor found in *pyruvate dehydrogenase* and *a-ketoglutarate dehydrogenase complexes*, two multienzymes involved in α-keto acid oxidation.

Detary sourses: lipoic acid can be eaten in foods, such as red meat, carrots, beets, spinach, broccoli, and potatoes.

Lipoic acid deficiency is rare in humans because it can be synthesized endogenously and is found in many food sources. However, some rare genetic disorders, such as defects in the synthesis or transport of lipoic acid, can lead to deficiency. In addition, chronic alcohol consumption can interfere with lipoic acid metabolism and contribute to deficiency. The symptoms of lipoic acid deficiency are not well-defined but may include muscle weakness, fatigue, and cardiovascular problems.

Para-amino benzoic (PABA). Para-amino benzoic acid is a growth factory for bacteria and lower animals. It is a component of **folic acid.**

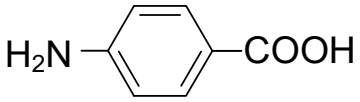


Fig. 4.29. Chemical structure of PABA

Dietary sources: grains, eggs, milk, and meat.

The deficiency of PABA was first found to be associatedw ith failure of lactation and greying of black hair in rats. The specific functions of PABA in humans, except that it is a component of folic acid, have not been identified.

Inositol. Inositol is synthesized after cyclization glucose-6-phosphate and is after cyclization and is of similar structure to glucose.

Biological role. As a component of phospholipids in membranes, it plays a key role in the cell replication. Inositol also plays an important role in phospholipid assembly, clearance of lipid and cellular signal transduction.

Detary sourses: Inositol is relatively abundant in cereal grains.

Similar to choline, **deficiency of inositol** can lead to fatty liver.

Taurine. Taurine is a sulfur-containing amino acid-like compound, but it is not incorporated into proteins like most amino acids.

Biological role. Taurine takes part in in a variety of physiological activities, including neuromodulation, osmotic regulation and the stabilization of cell membranes. It is essential for the metabolism of bile acids salts.

Detary sourses. Taurine is found in various animal-based foods such as meat (particularly dark meat), fish, and dairy products. It is also available in some energy drinks and supplements. However, taurine is not commonly found in plant-based foods.

Taurine deficiency is rare in humans, as the body can synthesize taurine from other amino acids. However, there are certain conditions that can lead to taurine deficiency, such as liver disease, cystic fibrosis, and some genetic disorders. Symptoms of taurine deficiency can include developmental delays, poor vision, and cardiovascular problems. Taurine supplementation may be recommended for individuals with these conditions or those with a restricted diet that is low in taurine-containing foods.

Carnitine. Carnitine is a quaternary ammonium compound synthesized in the liver, kidneys and brain from the amino acids lysine and methionine.

Biological role. Carnitine is required for the transport of long-chain fatty acids from the cytosol to the mitochondria, where they are oxidized to produce energy. It plays a critical role in the metabolism of fatty acids, particularly in tissues such as skeletal and cardiac muscle that rely on fatty acids as a primary source of energy.

Detary sourses: Carnitine can be obtained from dietary sources such as meat, fish, poultry, and dairy products. Vegetarian diets may provide lower levels of carnitine as it is found primarily in animal-based products.

Deficiencies in carnitine can be caused by genetic disorders or conditions that affect its biosynthesis, as well as malnutrition, gastrointestinal diseases, and medications

such as valproic acid. Symptoms of carnitine deficiency can include muscle weakness, fatigue, and hypoglycemia. Treatment typically involves supplementation with carnitine.

4.4.2 Fat-soluble vitamin-like substances

Vitamin F is a mixture of the only two essential **polyunsaturated fatty acids (PUFA)**, such as **linoleic acid (LA)**, and α **linolenic acid (ALA)** required by humans (fig.4,30). α-linolenic acid satisfies the need for an **omega-3** fatty acid back-bone structure and linoleic acid satisfies the need for an **omega-6** fatty acids back-bone structure. Although humans and other mammals can synthesize saturated fatty acids and some monounsaturated fatty acids from carbon groups in carbohydrates and proteins, they lack the delta (Δ) 12 and Δ15 desaturase enzymes necessary to insert a *cis* double bond at the n-6 or the n-3 position of a fatty acid. Consequently, omega-6 and omega-3 fatty acids are essential nutrients. The parent fatty acid of the omega-6 series is linoleic acid (LA; 18:2n-6), and the parent fatty acid of the omega-3 series is ALA. Humans can synthesize long-chain (20 carbons or more) omega-6 fatty acids, such as dihomo-γ-linolenic acid (DGLA; 20:3n-6) and arachidonic acid (AA; 20:4n-6), from LA and long-chain omega-3 fatty acids, such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), from ALA.

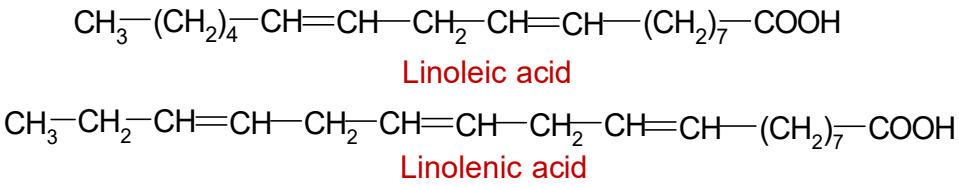


Fig. 4.30. Vitamin F

Biological role. Omega-6 and omega-3 PUFA are important structural components of **cell membranes.** When incorporated into phospholipids, they affect cell membrane properties, such as fluidity, flexibility, permeability, and the activity of membrane-bound enzymes and cell-signaling pathways . In addition to endogenous metabolism, dietary consumption of fatty acids can modify the composition and molecular structure of cellular membranes. Thus, increasing omega-3 fatty acid intake increases the omega-3 content of red blood cells, immune cells, atherosclerotic plaques, cardiac tissue, and other cell types throughout the body.

The phospholipids of the brain's gray matter contain high proportions of long-chain PUFA, suggesting they are important to **central nervous system function**.

PUFA are pleiotropic regulators of cell function. They can regulate gene expression directly by interacting with transcription factors or indirectly by influencing membrane lipid composition and cell signaling pathways.

Deficiency of essential fatty acid include a dry scaly rash, decreased growth in infants and children, increased susceptibility to infection, and poor wound healing. Essential fatty acid deficiency has also been found to occur in patients with chronic fat malabsorption and in patients with cystic fibrosis . It has been proposed that essential fatty acid deficiency may play a role in the pathology of protein-energy malnutrition.

REVIEW TEST:

№	MCQs	Answers and explanations
1.	**After an extended treatment with sulfanamides a patient has developed macrocytic anemia. Production of active forms of the following vitamin is disrupted in such a condition:** A. Riboflavin B. Thiamine C. Folic acid D. Pyridoxine E. Cyanocobalamin	**The answer is C.** Sulfonamides interfere with folic acid synthesis by preventing addition of para-aminobenzoic acid (PABA) into the folic acid molecule through competing for the enzyme dihydropteroate synthetase. Folate deficiency results in an impairment in dTMP synthesis which leads to cell cycle arrest in S-phase of rapidly proliferating cells, in particular hematopoietic cells. The inability to synthesize DNA during erythrocyte maturation leads to abnormally large erythrocytes termed macrocytic (megaloblastic) anemia.
2.	**Coenzyme A participates in numerous important metabolic reactions. It is a derivative of the following vitamin:** A. Thiamine B. Pantothenic acid C. Niacin D. Calciferol E. Ubiquinone	**The answer is B.** Pantothenic acid **(p**antothenate) is required for synthesis of coenzyme A (abbreviated CoA or CoASH). Pantothenate is, therefore, required for the metabolism of carbohydrate via the tricarboxylic acid cycle and all fats and proteins. At least 70 enzymes have been identified as requiring coenzyme A for their function.
3.	**Malaria is treated with structural analogs of vitamin B_2 (riboflavin). These drugs disrupt the synthesis of the following enzymes in plasmodium:** A. FAD-dependent dehydrogenase B. Cytochromeoxidase C. Peptidase D. NAD-dependent dehydrogenase E. Aminotransferase	**The answer is A.** Vitamin B_2 riboflavin is the precursor for the coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The enzymes that require FMN or FAD as cofactors are termed flavoproteins. Both FMN and FAD are crucial rate limiting factors in most cellular enzymatic processes. As an example, they are crucial for the synthesis, conversion and recycling of niacin, folate and vitamin B_6, and for the synthesis of all hemoproteins, including hemeglobin, nitric oxide synthases, P450 enzymes, and proteins involved in electron transfer and oxygen transport and storage. The flavoproteins are also cofactors in the metabolism of essential fatty acids in brain lipids the absorption and utilisation of iron and the regulation of thyroid hormones . Riboflavin deficiency prolongs recovery from malaria, despite preventing growth of plasmodium (the malaria parasite).
4.	**A 36-year-old female patient has a history of B_2-hypovitaminosis. The most likely cause of specific symptoms (epithelial, mucosal, cutaneous, corneal lesions) is the deficiency of:** A. Flavin coenzymes B. CytochromeA_1 C. Cytochromeoxidase D. CytochromeB E. CytochromeC	**The answer is A.** Riboflavin deficiencies are rare due to the presence of adequate amounts of the vitamin in eggs, milk, meat and cereals. Symptoms associated with riboflavin deficiency include itching and burning eyes, angular stomatitis and cheilosis (cracks and sores in the mouth and lips), bloodshot eyes, glossitis (inflammation of the tongue leading to purplish discoloration), seborrhea (dandruff, flaking skin on scalp and face), trembling, sluggishness, and photophobia (excessive light sensitivity).

5.	**A number of diseases can be diagnosed by evaluating activity of blood transaminases. What vitamin is one of cofactors of these enzymes?** A. B_8 B. B_2 C. B_1 D. B_6 E. B_5	**The answer is D.** Pyridoxal phosphate (PLP) functions as a cofactor in all of the enzymes that carry out the transamination reactions required for the synthesis and catabolism of the amino acids. `
6.	**Malignant hyperchrome anemia, or Birmer's disease, is a pathological state caused by the deficiency of vitamin B_{12}. What chemical element is a constituent of the structure of this vitamin?** A. Magnesium. B. Molybdenum. C. Zinc. D. Iron. E. Cobalt.	**The answer is E.** Vitamin B_{12} deficiency lead to an anemia secondary to a reduction in DNA synthesis. Biermer's disease, also called acquired pernicious anemia, is a condition in which the body is unable to properly utilize vitamin B_{12}. Vitamin B_{12} is composed of a complex tetrapyrrol ring structure (corrin ring) and a cobalt ion in the center.
7.	**Vitamin B_1 deficiency causes disturbance of oxidative decarboxylation of α-ketoglutaric acid. This leads to the impaired synthesis of the following coenzyme:** A. Thiamine pyrophosphate B. Nicotinamide adenine dinucleotide C. Flavine adenine dinucleotide D. Lipoic acid E. Coenzyme A	**The answer is A.** Thiamine pyrophosphate (TPP) is the active form of vitamin B_1, necessary as a cofactor for pyruvate dehydrogenase complex (PDHc) and 2-oxoglutarate (α-ketoglutarate) dehydrogenase both of which are associated with the TCA cycle. Thiamine is converted to its active form, TPP, by the enzyme thiamine pyrophosphokinase. Vitamin B_1 deficiency resulting lackin TPP leads to disturbance of oxidative decarboxylation of α-ketoglutaric acid.
8.	**A 45-year-old alcoholic man walks into the emergency room with a clumsy, wide-based gaitand appears confused. He has pronounced nystagmus, and laboratory tests are significant for a metabolic acidosis and a serum blood alcohollevel of 0.13. This patient should most probably be treated with IV fluids containing which of the following?** A. Thiamine B. Riboflavin C. Niacin D. Pantothenic acid E. Biotin	**The answer is A.** Wernicke encephalopathy, with the classic triad of ataxia, confusion, and ophthalmoplegia (and nystagmus), is due to thiamine deficiency. Thiamine is anessential coenzyme in carbohydrate metabolism, including the pentose-phosphate pathway (transketolase) and the TCAcycle (pyruvate dehydrogenase and α-ketoglutarate dehydrogenase). Riboflavin deficiency is possible in malnourished alcoholics, causing cheilosis, glossitis, and corneal changes. Niacin deficiency causes diarrhea, dementia, and dermatitis. Deficiencies of pantothenic acid and biotinarerare, although a biotin lead to hypoglycemia and mild ketosis/
9.	**A patient is diagnosed with seborrheic dermatitis caused by vitamin H (biotin) deficiency. Observed is activity disruption of the following enzyme:** A. Pyruvate decarboxylase B. Alcohol dehydrogenase C. Acetyl-CoA carboxylase D. Carbamoyl phosphate synthetase	**The answer is C.** Biotin is the cofactor required of enzymes that are involved in carboxylation, decarboxylation, or transcarboxylation reactions in prokaryotes and eukaryotes. Biotin is sometimes referred to as vitamin H. In humans, the biotin-requiring enzymes include acetyl-CoA carboxylase, pyruvate carboxylase, propionyl-CoA carboxylase, and 3-methylcrotonyl-CoA carboxylase.

	E. Aminotranspherases	
10	**In a patient with frequent intraorgan and mucosal bleeding in urine were detected proline and lysine. Deficiency of what vitamin cause a damage of their hydroxylation?** A. Vitamin C B. Vitamin A C. Vitamin D D. Vitamin B_1 E. Vitamin E	**The answer is A.** The most important reactions requiring vitamin c (ascorbic acid) as a cofactor are the hydroxylations of lysine and proline residues in collagen. Vitamin C is, therefore, required for the maintenance of normal connective tissue as well as for wound healing since synthesis of connective tissue is the first event in wound tissue remodeling. Vitamin C also is necessary for bone remodeling due to the presence of collagen in the organic matrix of bones.

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5. METABOLISM AND ENERGY. TRICARBOXYLIC ACID CYCLE

OBJECTIVES after studying this chapter, you should be able to:

- *Interprete biochemical principles of metabolic pathways: catabolic, anabolic, amphibolic pathways.*
- *Explain biochemical mechanisms of regulation of catabolic and anabolic reactions.*
- *Interprete biochemical principles of TCA cycle functioning and its anaplerotic reactions and their amphibolic sense.*
- *Explain biochemical regulatory mechanisms in TCA cycle and its principal position in turnover of matter and energy.*

5.1. Conception of turnover of material and energy (metabolism). Catabolic, anabolic and amphibolic reactions.

Metabolism is a complex and highly integrated system of thousands of chemical reactions that occur within living cells, serving to sustain and promote the overall health and survival of the body. These reactions are often organized into metabolic pathways and are facilitated by enzymes that help to catalyze and regulate them. Metabolism involves the transformation and interconversion of a diverse array of chemicals known as **metabolites**, which serve as the building blocks and energy sources for the cell.

Metabolic pathways are devided into three types:

- **Anabolic pathways**, which are those involved in the synthesis of larger and more complex compounds from smaller precursors (tabl.5.1). For example, the synthesis of protein from amino acids and the synthesis of reserves of triacylglycerol and glycogen. Anabolic pathways are endergonic.
- **Catabolic pathways**, which are involved in the breakdown of larger molecules, commonly involving oxidative reactions; they are exothermic, producing reducing equivalents (NADH, $FADH_2$) and, mainly via the respiratory chain, ATP. For example, glycolysis, β-oxydation of fatty acids.
- **Amphibolic pathways**, which occur at the "crossroads" of metabolism, acting as links between the anabolic and catabolic pathways, for example, the TCA cycle.

Table 5.1. Comparative charachteristics anabolic and catabolic pathways

Anabolic pathways	Catabolic pathways
Pathways that consume energy to build larger, complicated molecules from simpler ones	Pathways that release energy by breaking down complex molecules into simpler compounds
Biosynthetic	Degradative
Reductive	Oxidative
Energy Required	Energy Liberated
Diverging	Converging

Catabolic and anabolic pathways are interconnected in three ways, as illustrated in figure 5.1. Firstly, catabolic pathways provide precursor compounds for anabolism. Secondly, catabolic pathways provide the energy required to power anabolism. And thirdly, catabolic pathways provide reducing power (electrons) for anabolism.

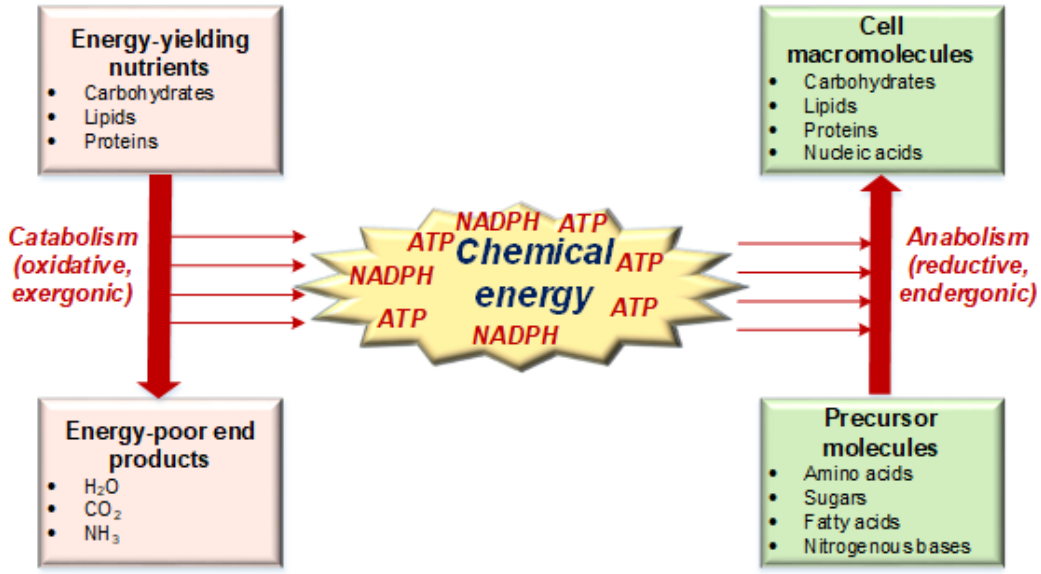


Fig.5.1. Integration of metabolism

The reaction of metabolism are organized into **metabolic pathways**. There are four ways that pathways can be organized "topologically" (fig. 5.2):

- In **linear pathways**, the starting molecule is converted through a series of intermediates to the final product. In the case of glycolysis, glucose is broken down into two molecules of pyruvate through a series of enzymatic reactions.
- **Branched metabolic pathways** can take two forms: divergent and convergent. In divergent pathways, an intermediate molecule can enter multiple linear pathways, leading to the production of different end products. In convergent pathways, multiple precursor molecules can be converted into a common intermediate. Examples of convergent pathways include the biosynthesis of purines and some amino acids. Usually, a regulatory mechanism is present at the branch point to control the flow of metabolites into each pathway.

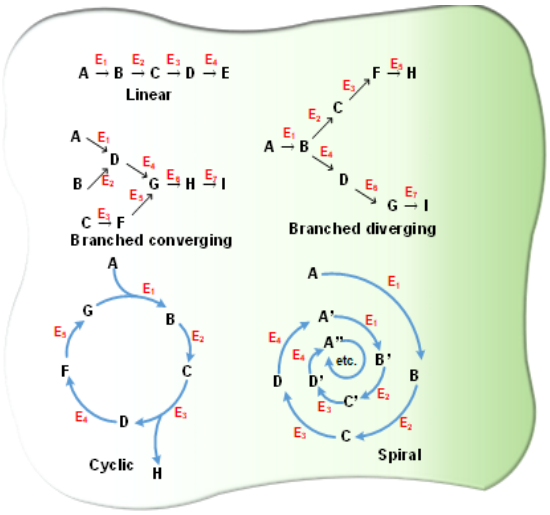


Fig. 5.2. Types of metabolic pathways

- **Cyclic pathways** are those where the intermediates are regenerated, meaning that some of the intermediates act as catalysts. The TCA (tricarboxylic acid) cycle is an example of a cyclic pathway. In this pathway, acetyl-CoA enters the cycle and is converted to citrate, which undergoes a series of reactions ultimately regenerating the starting compound, oxaloacetate.
- **Spiral pathways** are characterized by the repeated use of the same set of enzymes to gradually break down a substrate. An example of a spiral pathway is the β-oxidation of fatty acids, in which a series of reactions repeatedly shorten the fatty

acid chain by two carbons until it is completely broken down into acetyl-CoA. The enzymes involved in the pathway are used repeatedly in each cycle of the spiral, gradually breaking down the substrate until it is fully metabolized.

5.2. Exergonic and endergonic reactions, role of ATP and other macroergic compounds in their coupling.

ll living organisms need energy to survive. The direction and rate of chemical reactions are influenced by three key factors: **enthalpy,** which is the amount of heat released or absorbed during a reaction; **entropy**, which is a measure of the degree of disorder or randomness in a system and is related to the second law of thermodynamics; and **free energy**, which is a measure of the tendency of a reaction to occur and is determined by the balance between enthalpy and entropy.

The change in free energy during a reaction provides useful information about the reaction's energetics and spontaneity (whether it can happen without added energy). We can write out a simple definition of the change in free energy as:

$$\Delta G^o = G_{final} - G_{initial}$$

In other words, ΔG is the change in free energy of a system as it goes from an initial state, from reactants, to a final state, such as products. This value tells us the maximum usable energy released (or absorbed) in going from the initial to the final state. In addition, its sign (positive or negative) tells us whether a reaction will occur spontaneously (that is, without added energy).

Reactions that have a negative ΔG^o release free energy and are called **exergonic reactions** (fig. 5.3). A negative ΔG^o means that the reactants, or initial state, have more free energy than the products, or final state. Exergonic reactions are also called **spontaneous reactions**, because they can occur without the addition of energy.

On the other hand, reactions with a positive ΔG^o ($\Delta > 0$) require an input of energy and are called **endergonic reactions**. In this case, the products, or final state, have more free energy than the reactants, or initial state. Endergonic reactions are non-spontaneous, meaning that energy must be added before they can proceed.

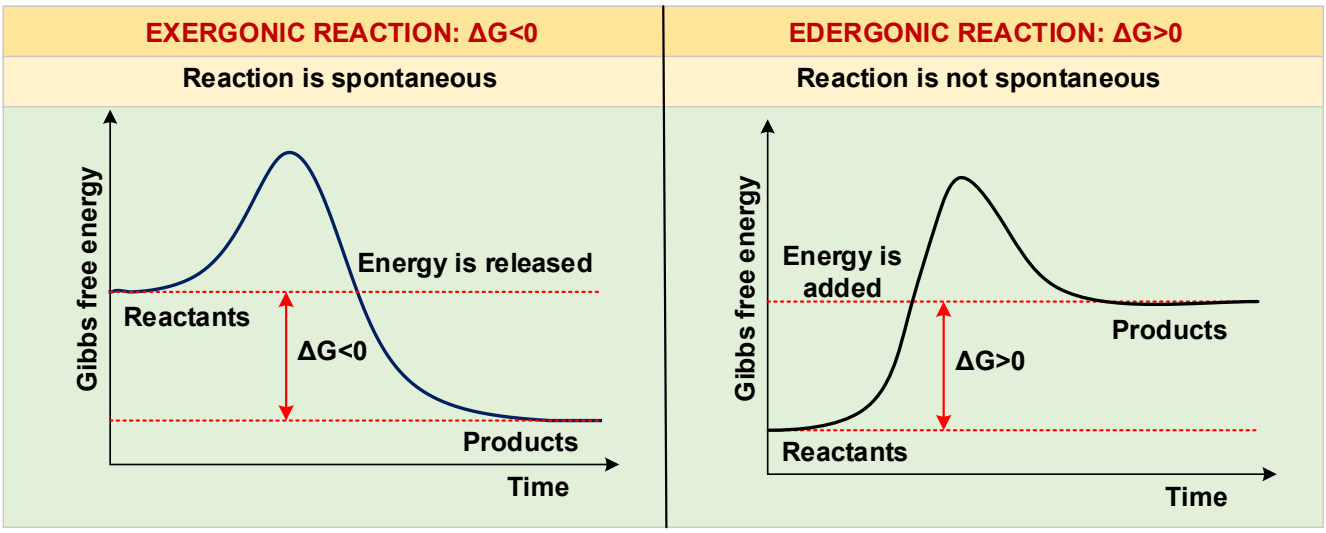


Fig. 5.3. Changes of free energy in exergonic and endergonic reactions

ATP powers cellular work by coupling exergonic to endergonic reactions. Adenosine triphosphate (ATP) is a crucial molecule in living cells, playing a vital role in many biological processes. ATP can be hydrolyzed to release energy, which can be used to drive a variety of endergonic biochemical reactions. It is synthesized from adenosine diphosphate (ADP) and inorganic phosphate (Pi) using the energy obtained from the breakdown of food molecules and the light reactions of photosynthesis. ATP is used to fuel biosynthesis of biomolecules, active transport of molecules across cell membranes, and mechanical work, such as muscle contraction.

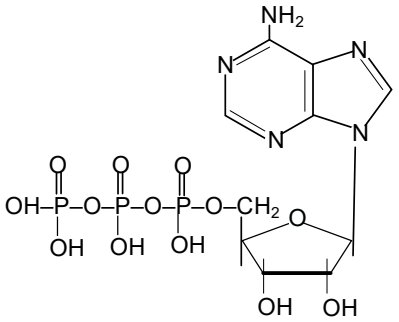


Fig. 5.4. Chemichal structure of ATP

ATP may be hydrolysed to form ADP and P_i (orthophosphate) or AMP and PP_i (pyrophosphate) (fig. 5.5). Pyrophosphate may be subsequently hydrolyzed to orthophosphate releasing additional free energy. The hydrolysis of ATP to form AMP and pyrophosphate is often utilized to drive reactions with high positive $\Delta G^{o\prime}$ values or to ensure that a reaction goes to completion.

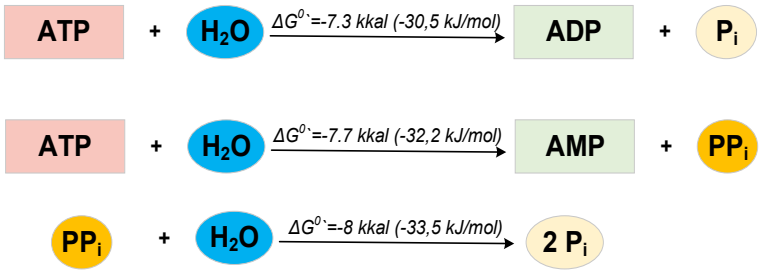


Fig. 5.5 Hydrolysis of ATP

ATP is uniquely suited to its role as the universal energy currency due to its structure (fig. 5.6). It is a nucleotide made up of adenine, ribose, and a triphosphate unit 9fig. 5.4). The two terminal phosphoryl groups are linked by phosphoanhydride bonds, which are relatively stable under intracellular conditions despite anhydrides typically being easily hydrolyzed. Enzymes specifically designed for the hydrolysis of ATP facilitate this process.

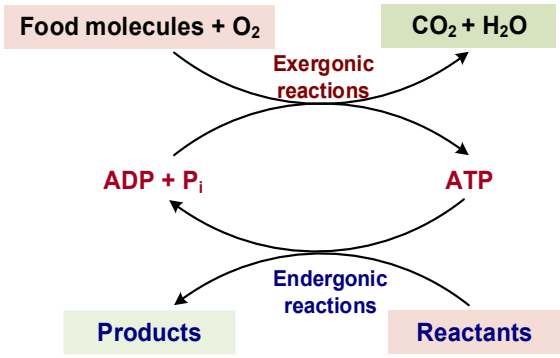


Fig. 5.6. The role of ATP**.** ATP is an intermediate in the flow of energy from food molecules to the energy released from reactions of metabolism.

The tendency of ATP to undergo hydrolysis, also referred to as its **phosphate group transfer potential**, is not unique. In addition to ATP, other biomolecules with high phosphate transfer potential tab. 5.2). These molecules play important roles in various cellular processes such as protein synthesis, signal transduction, and muscle contraction. However, ATP remains the primary energy currency in most living cells due to its high concentration and ubiquitous role in cellular metabolism.

Tab. 5.2. Standard Free Energy of Hydrolysis of Selected Phosphorylated Biomolecules

Molecule	$\Delta G^{o'}$	
	kcal/mol	kJ/mol
Phosphenolpyruvate	-14.8	-61.9
1,3-Bisphosphophoglycerate	-11.8	-49.4
Carbamoyl phosphate	-12.3	-51.4
Phosphocreatine	-10.3	-43.1
PP_i	-7.4	-31
$ATP \rightarrow ADP+P_i$	-7.3	-30.5
$ADP \rightarrow AMP+PP_i$	-7.7	-32.2
Glucose-1-phosphate	-5	-20.9
Fructose-6-phosphate	-3.8	-15.9
Glucose-6-phosphate	-3.3	-13.8

Phosphorylated compounds with high $\Delta G^{o'}$ values of hydrolysis have higher phosphate group transfer potentials than those compounds with lower values. ATP has an intermediate phosphate group transfer potential. This property makes it possible for ATP to serve as an intermediate carrier of phosphoryl groups from higher-energy compounds such as **phosphenolpyruvate** to low-energy compounds. ATP is therefore the "energy currency" for living systems, since cells have no mechanisms for direct transfer of phosphoryl groups from high-energy to low-energy compounds.

5.3. Compartmentalization of metabolic reactions in the cell. Methods of investigation of metabolism

Understanding the organization of metabolic pathways at both the tissue/organ and subcellular levels is important for understanding how different pathways are regulated and how they work together to maintain cellular function. At the tissue/organ level, techniques such as metabolic profiling and isotopic tracing can be used to identify the metabolic pathways that are active in specific tissues and organs, as well as the substrates and products of those pathways. At the subcellular level, studies using organelle isolation, imaging techniques, and biochemical assays can help identify the metabolic pathways that occur in specific organelles or compartments, such as the mitochondria or cytosol.

Compartmentation of pathways in separate subcellular compartments or organelles permits integration and regulation of metabolism (fig. 5.7). Not all pathways are of equal importance in all cells.

The central role of the **mitochondrion** is immediately apparent, since it acts as the focus of carbohydrate, lipid, and amino acid metabolism. It contains the enzymes of the citric acid cycle, β-oxidation of fatty acids and ketogenesis, as well as the respiratory chain and ATP synthase.

Glycolysis, the pentose phosphate pathway (HMP shunt) and fatty acid synthesis occur in the **cytosol**.

The membranes of the **endoplasmic reticulum** contain the enzyme system for triacylglycerol and phospholipid synthesis.

Ribosomes are responsible for protein synthesis.

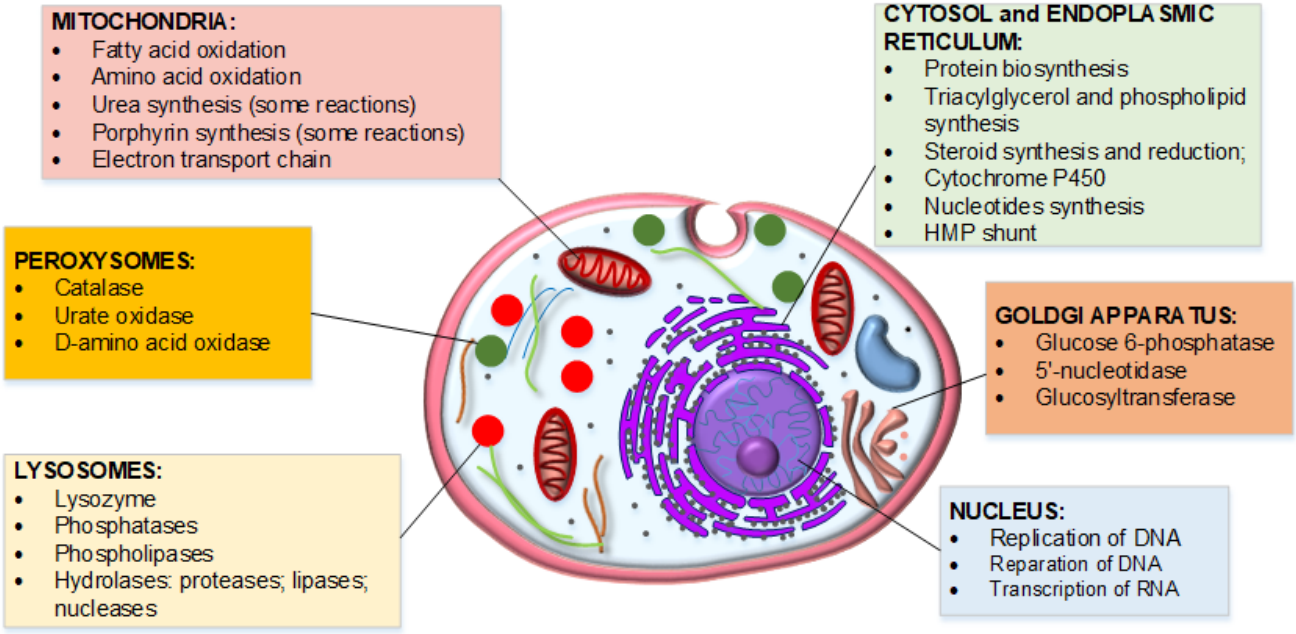


Fig. 5.7. Compartmentalisation of intracellular metabolic pathways

The metabolic reactions do not occur in isolation. They are interdependent and integrated into specific series that constitute metabolic pathways. lt is, therefore, not an easy task to study metabolisms.

In the process of studying metabolism, the main focus is on the transformations that substances (substrates) undergo and the enzymes that catalyze them, how chemical processes are energized, the nutrients that enter cells, and how end products are eliminated from the organism.

The study of metabolism is carried out at different levels of the structural organization of living organisms:

- **Study of metabolism at the level of the whole organism:** Laboratory animals are most often fed artificial diets that lack certain essential substances, which allows us to understand the concept of essential nutrients (vitamins, essential amino acids, polyunsaturated fatty acids, minerals, macro- and micronutrients). These substances must be obtained from food.
- **Balance observations**: These are conducted at the level of the entire organism. They allow us to determine the daily requirement and degree of absorption of specific substances from food. These studies are conducted on animals, taking into account the amount of consumed substances and the excretion of metabolites. Nitrogen, water, and mineral balances are most commonly investigated.
- **Experimental fistulas**: An example of this is the creation of artificial fistulas in experimental animals at different sections of the gastrointestinal tract, studying the content of individual substances and their exchange products.
- **Isotopic observations**: For this purpose, isotopes of elements such as H, N, C, S, P, O, Na, K, etc., are used. The isotopic indicator method is applied in vivo and in vitro. Labeled compounds are obtained by incorporating labeled atoms during synthesis or biosynthesis of these compounds. The isotopic indicator method allows monitoring the transformation of a given substance in an organism. In this case, a labeled isotope

substance is introduced, and the appearance of the isotopic label in the products of its transformation is studied.

- **Method of isolated organs:** Surgical removal of organs is one way to study metabolism. Nutrients (perfusion) are continuously supplied to the isolated organ to maintain its viability. Perfusion is performed using blood plasma, physiological solution, to which the substances under study are added. By examining the composition of fluids entering and exiting the organ, it is possible to determine the pathways of transformation of the introduced substances and the role of corresponding organs in metabolism.
- **Utilization of homogenates and subcellular fractions:** To obtain homogenates, the organ is cut into small pieces, which are then placed in a liquid isotonic medium (such as 0.25 M sucrose or physiological solution) and ground in a mortar with sand or a homogenizer. This process yields a homogenate that largely preserves its biological activity.

5.4. Catabolic transformation of biomolecules.

All the catabolic processes in the living system are divided into 3 stages (fig.5.8).

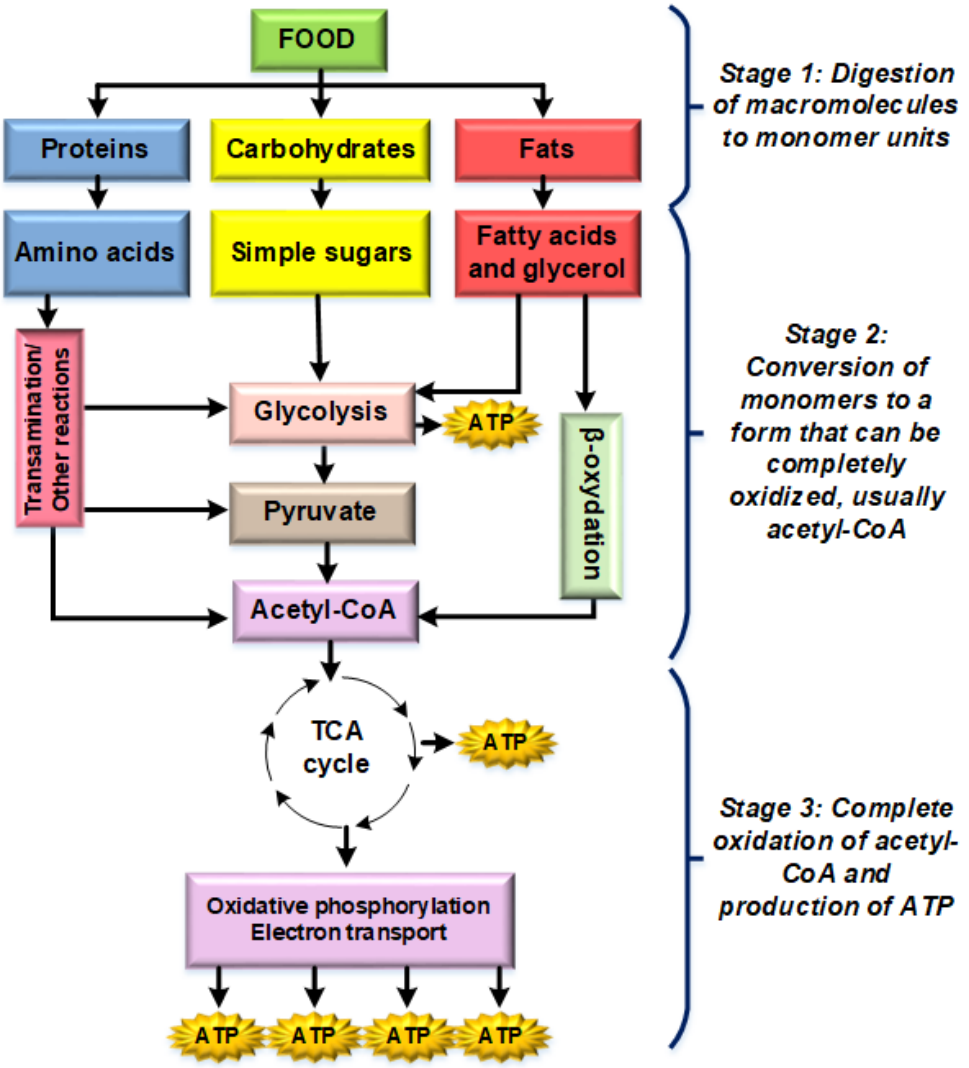


Fig. 5.8. Stages of catabolism

Stage 1 – biomolecules such as, carbohydrates, fats, and proteins are broken down into their individual monomer units: carbohydrates into simple sugars, fats into fatty acids and glycerol, and proteins into amino acids. One part of stage I of catabolism is the breakdown of food molecules by hydrolysis reactions into the individual monomer units—which occurs in the mouth, stomach, and small intestine and is referred to as digestion.

Stage 2 –these monomer units (or building blocks) are further broken down through different reaction pathways, one of which produces ATP, to form a common end product that can then be used in stage III to produce even more ATP.

Stage 3 – The acetyl group on the CoA is oxidised to water and carbon dioxide in the tricarboxilyc acid cycle (TCA) releasing the energy that is stored mainly by reducing the coenzyme nicotinamide adenine dinucleotide (NAD^+) into NADH and oxidizing it later in the electron transport chain produsing ATP by oxidative phosphorylation.

5.5. The most important metabolites of amphibolic pathways

There are several important metabolites that play a key role in both anabolic and catabolic pathways:

Acetyl-CoA is the key amphibolic metabolite. Its function is to deliver the acetyl group to the citric acid cycle (Krebs cycle, TCA) to be oxidized for energy production. Coenzyme A (CoASH or CoA) consists of a β-mercaptoethylamine group linked to the vitamin pantothenic acid through an amide linkage and 3'-phosphorylated ADP. The acetyl group of acetyl-CoA is linked to the sulfhydryl substituent of the β-mercaptoethylamine group. This thioester linkage is a "high energy" bond, which is particularly reactive.

Acetyl-CoA besides TCA partisipates in differen metabolic pathways such as:

✓ **Fatty acid metabolism:**

- Acetyl-CoA is produced by the breakdown of both carbohydrates (by glycolysis) and lipids (**by β-oxidation**). It then enters the citric acid cycle in the mitochondrion by combining with oxaloacetate to form citrate.
- Two acetyl-CoA molecules condense to form acetoacetyl-CoA, which gives rise to the formation of acetoacetate and β-hydroxybutyrate. Acetoacetate, β-hydroxybutyrate, and their spontaneous breakdown product acetone are known as **ketone bodies**.
- On the other hand, when the insulin concentration in the blood is high, and that of glucagon is low (i.e. after meals), the acetyl-CoA produced by glycolysis condenses as normal with oxaloacetate to form citrate in the mitochondrion. Citrate is transferred from the mitochondrion into the cytoplasm, where it is cleaved by ATP citrate lyase into acetyl-CoA and oxaloacetate. The oxaloacetate is then returned to the mitochondrion. This **cytosolic acetyl-CoA** can then **be used to synthesize fatty acids** through carboxylation by acetyl-CoA carboxylase into malonyl CoA.
- The cytosolic acetyl-CoA can also condense with acetoacetyl-CoA to form **3-hydroxy-3-methylglutaryl-CoA** (HMG-CoA) which is the rate-limiting step controlling the synthesis of **cholesterol**. Cholesterol can be used as is, as a

structural component of cellular membranes, or it can be used to synthesize steroid hormones, bile salts, and vitamin D.

- ✓ **Acetylcholine synthesis**:
  - Acetyl-CoA is also an important component in the biogenic synthesis of the **neurotransmitter acetylcholine**. Choline, in combination with acetyl-CoA, is catalyzed by the enzyme choline acetyltransferaseto produce acetylcholine and coenzyme A as abyproduct.
- ✓ **Melatonin synthesis**
- ✓ **Acetylation**
  - Acetyl-CoA is also the source of the acetyl group incorporated onto certain lysine residues of histone and nonhistone proteins in the posttranslational modification acetylation. This acetylation is catalyzed by acetyltransferases. This acetylation affects cell growth, mitosis, and apoptosis.

➢ **Pyruvate** also plays the role of an amphibolic metabolite. Pyruvic acid can be made from glucose through glycolysis, converted back to carbohydrates (such as glucose) via gluconeogenesis, or to fatty acids through a reaction with acetyl-CoA.[3] It can also be used to construct the amino acid alanine and can be converted into ethanol or lactic acid via fermentation. Pyruvic acid supplies energy to cells through the citric acid cycle when oxygen is present (aerobic respiration), and alternatively ferments to produce lactate when oxygen is absent (lactic acid fermentation).

➢ **Glucose-6-phosphate** is the main metabolite of carbohydrates metabolism. Glucose-6-phosphate acts as a branch point for a wide variety of pathways. It can be used:

1) as a glycolytic substrate,
2) as a substrate for other synthetic reactions,
3) as a substrate for glycogen synthesis,
4) as a source of biosynthetic reducing equivalents and intermediates via the hexose monophosphate shunt,
5) (in liver and kidney only) be converted back to glucose and released into the bloodstream.

5.6. Tricarboxylic acid (TCA) cycle

5.6.1 Cellular location of TCA cycle enzymes

TCA cycle (Krebs cycle, citric acid cycleor tricarboxylic acid-TCA cycle) is the most important metabolic pathway for the energy supply to the body. **TCA cycle can be defines as a cyclic arrangement of sequence of reactions that convert acetyl-CoA to two molecules of CO_2.** The TCA cycle is an aerobic pathway, because O_2 is required as the final electron acceptor. This cycle utilizes about twothirds of total oxygen consumed by the body. The name TCA cycle is used, since tricarboxylic acids (citrate, cisaconitate and isocitrate) participate.

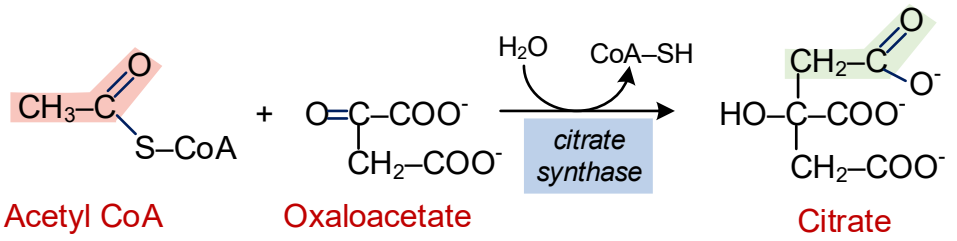
TCA cycle is the final common oxidative pathway for carbohydrates, fats and amino acids. This cycle not only supplies energy but also provides many intermediates required for the synthesis of amino acids, glucose, heme etc. Krebs cycle is the most

important central pathway connecting almost all the individual metabolic pathways (either directly or indirectly).

The enzymes of TCA cycle are located in **mitochondrial matrix**, in close proximity to the electron transport chain. This enables the synthesis of ATP by oxidative phosphorylation.

5.6.2. Sequence of TCA cycle reactions. • Characterization of enzymes and coenzymes participating TCA cycle

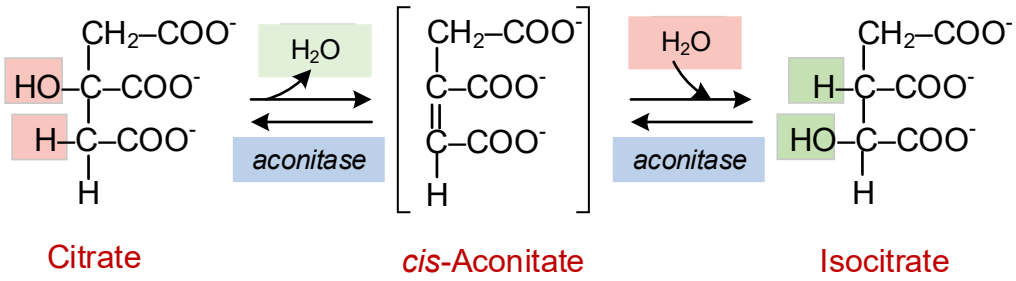
1. Citrate synthase: TCA cycle starts with the **condensation** of **acetyl CoA** and **oxaloacetate**, catalysed by the enzyme ***citrate synthase***. Binding of oxaloacetate to the enzyme results in conformational change which facilitates the binding of the next substrate, the acetyl Coenzyme A. There is a further conformational change which leads to formation of products.



Potentially confusing questions about differences between synTHASes and synTHETASes. They are both are responsible for new compounds formation, however synthases are lyases, they join two molecules together and don't need ATP, whereas synthetases are ligases, they join two molecules together and require a high energy molecule, like ATP or GTP.

Citrate synthase is an allosteric enzyme, it catalyses a rate limiting irreversible reaction. It is inhibited by its product, citrate. Substrate availability is another means of regulation for citrate synthase. The binding of oxaloacetate causes a conformational change in the enzyme that generates a binding site for acetyl CoA.

2. Aconitase reaction. Citrate is tertiary alcohol it cannot be oxidized directly. Hence, in reaction-2 it is isomerized to isocitrate a secondary alcohol and can be oxidized easily. ***Aconitase (***an iron-sulfur containing enzyme) catalyses the isomerization reaction by removing water and then adding it back (H and OH to ***cis*-aconitate** in at different positions. This reaction is reversible. **Isocitrate** is consumed rapidly by the next step thus driving the reaction forward.

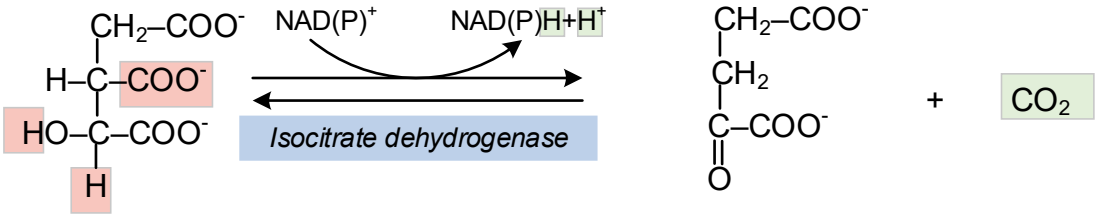


Citrate *cis*-Aconitate Isocitrate

MEDICAL IMPORTANCE

Fluoroacetate, a common rat poison, can react with oxaloacetate (OAA) in the body to form fluorocitrate, which inhibits a key enzyme called aconitase in the citric acid cycle (TCA cycle). This leads to an accumulation of citrate, disrupting the normal function of the cycle and causing a buildup of other intermediates. Ingesting fluoroacetate can result in a range of serious symptoms, including convulsions, cardiac arrhythmias, and ultimately, death.

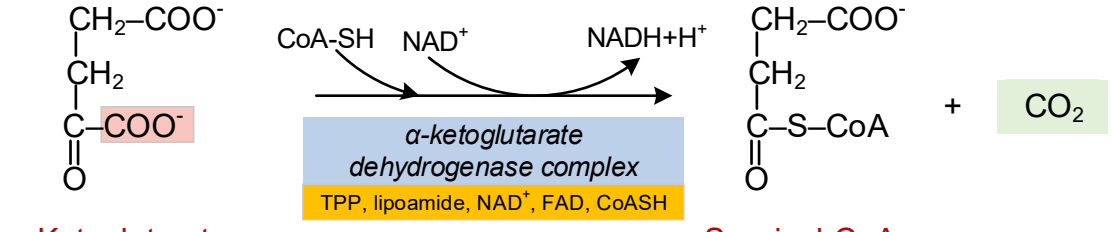
3. Isocitrate dehydrogenase: The enzyme ***isocitrate dehydrogenase*** catalyses the conversion **(oxidative decarboxylation)** of isocitrate to oxalosuccinate and then to α-ketoglutarate. The formation of NADH and the liberation of CO_2 occur at this stage. There are two isoforms of ICDH, one uses NAD^+ and the other uses $NADP^+$ as electron acceptor.



Isocitrate α-Ketoglutarate

Isocitrate dehydrogenase is one of the rate-limiting steps of the TCA cycle. The enzyme is allosterically activated by ADP (a low-energy signal) and Ca^{2+} and is inhibited by ATP and NADH, whose levels are elevated when the cell has abundant energy stores.

4. α-Ketoglutarate dehydrogenase: Conversion of α-ketoglutarate to succinyl CoA occurs through oxidative decarboxylation, catalysed by ***a-ketoglutarate dehydrogenase complex*** (a multimolecular aggregate of three enzymes). The mechanism of this oxidative decarboxylation is very similar to that used for the conversion of pyruvate to acetyl CoA by the pyruvate dehydrogenase complex This enzyme complex is dependent on five cofactors: **TPP, lipoamide, NAD^+, FAD and CoA.** At this stage of the TCA cycle, a second NADH is produced and the second CO_2 is liberated.



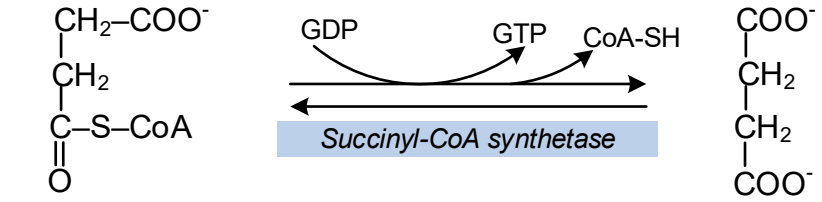
α-Ketoglutarate Succinyl-CoA

The equilibrium of the reaction is far in the direction of succinyl CoA – a high-energy thioester similar to acetyl CoA. α-Ketoglutarate dehydrogenase complex is inhibited by its products, NADH and succinyl CoA, and activated by Ca^{2+}.

MEDICAL IMPORTANCE

Arsenite can react with the sulfhydryl (-SH) groups of lipoic acid, forming a stable complex that inhibits its activity as a coenzyme for α-ketoglutarate dehydrogenase complex. As a result, the inhibition of lipoic acid can lead to a reduction in the activity of the citric acid cycle, as well as other processes that rely on lipoic acid, such as fatty acid metabolism and oxidative phosphorylation. This can result in a variety of toxic effects, including inhibition of energy production, oxidative stress, and cell death. Ingestion of arsenic or exposure to it in the environment can result in arsenic poisoning, which can lead to a range of symptoms, including gastrointestinal distress, skin lesions, neurological effects, and increased risk of cancer.

5. Succinyl CoA synthase (also called succinate thiokinase): Succinyl CoA, like acetyl CoA has a thioester bond with a very negative free energy of hydrolysis. In this reaction, the hydrolysis of the thioester bond leads to the formation of phosphoester bond with inorganic phosphate. This phosphate is transferred to a histidine residue of the enzyme and this high energy, unstable phosphate is finally transferred to GDP resulting in the generation of GTP.



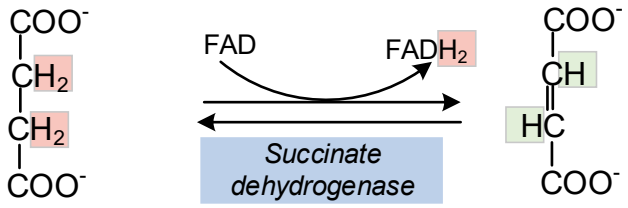
Succinyl-CoA Succinate

This is a **substrate level phosphorylation** reaction.

GTP is converted to ATP bv the enzvme ***nucleoside diphosphate kinase***.

GTP + ADP → ATP + GDP

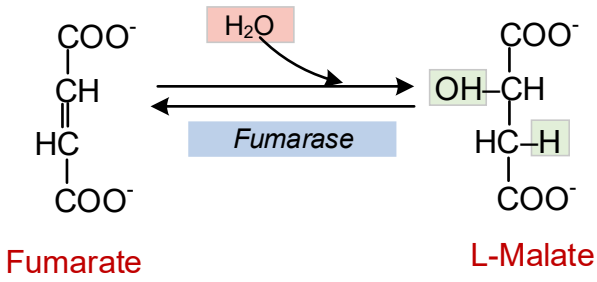
6. Succinate Dehydrogenase: Oxidation of succinate to fumarate. This is the only citric acid cycle enzyme that is tightly bound to the inner mitochondrial membrane. As such, it functions as Complex II of the electron transport chain. It is an FAD dependent enzyme. This enzyme also contains iron sulfur centres in addition to FAD.



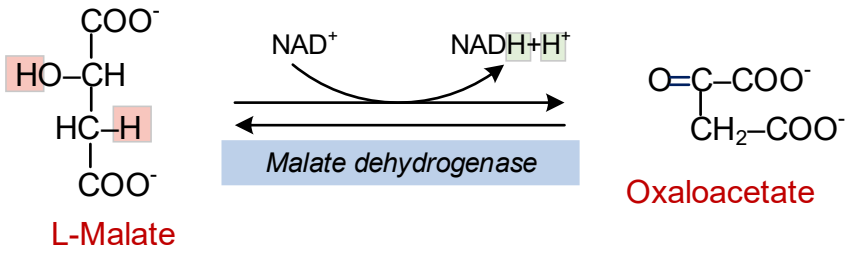
Succinate Fumarate

Malonate has similar structure to **Succinate,** and it **competitively** inhibits **SDH.**

7. Fumarase: Hydration of Fumarate to malate: It is a highly **stereospecific enzyme.** Cis-Maleate (the cis form of fumarate) is not recognized by this enzyme.



8. L-Malate dehydrogenase: Malate is oxidized to oxaloacetate by malate dehydrogenase. The third and final synthesis of NADH occurs at this stage. The oxaloacetate which is regenerated can combine with another molecule of acetyl CoA, and continue the cycle.



Summary of TCA cycle:

Acetyl CoA + 3 NAD^+ + FAD + GDP + P_i + $2H_2O$ → $2CO_2$ + 3NADH + $3H^+$ + $FADH_2$ + GTP + CoA

Thus, the two carbon acetyl group enter the TCA, and two molecules of CO_2 are released from cycle. Thus there is complete oxidation of two carbons during one cycle. Although the two carbons which enter the cycle become part of oxaloacetate, they are released as CO_2 only in the third round of the cycle. **The energy released due to this oxidation is conserved in the reduction of 3 NAD+, 1 FAD** molecule and synthesis of one **GTP** molecule which is converted to **ATP**.

5.6.3 Energetics and regulation of citric acid cycle

During the process of oxidation of acetyl CoA via citric acid cycle, **4 reducing equivalents** (3 as NADH and one as $FADH_2$) are produced. Oxidation of **3 NADH** by electron transport chain coupled with oxidative phosphorylation results in the synthesis of **9 ATP**, whereas **$FADH_2$** leads to the formation of **2 ATP**. In addition, there is one ATP produced by substrate level phosphorylation. Thus, a total of **twelve ATP** are produced from one acetyl CoA.

TCA cycle enzymes are **regulated** by:

- Substrate availability
- Product inhibition
- Allosteric inhibition or activation by other intermediates.

Three enzymes-namely **citrate synthase, isocitrate dehydrogenase and a-ketoglutarate dehydrogenase**-regulate citric acid cycle (fig 5.9).

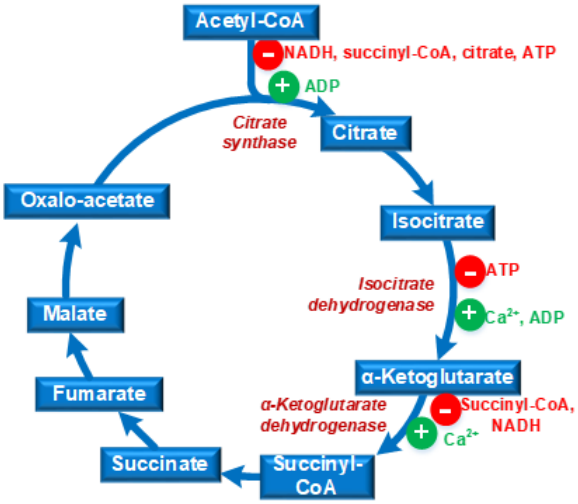


Fig.5.9. Regulatory steps of TCA cycle

1. **Citrate synthase** is inhibited by **ATP, NADH citrate and succinyl CoA**.
2. **Isocitrate dehydrogenase** is activated by **ADP**, and inhibited by **ATP** and **NADH.**
3. **α-Ketoglutarate dehydrogenase** is inhibited by **succinyl CoA** and **NADH.**

Availability of ADP is very important for the citric acid cycle to proceed. This cycle proceeds unless sufficient levels of ADP are available, oxidation (coupled with phosphorylation of ADP to ATP) of NADH and $FADH_2$ through electron transport chain stops. The accumulation of NADH and $FADH_2$ will lead to inhibition of the enzymes (as stated above) and also limits the supply of NAD^+ and FAD which are essential for TCA cycle to proceed.

Cofactors required for reactions of the TCA cycle.

- **NAD^+** accepts a hydride ion, which reacts with its nicotinamide ring. NAD^+ is reduced; the substrate (RH_2) is oxidized; and a proton is released. NAD^+ is frequently involved inoxidizing a hydroxyl group to a ketone. NAD^+ is used in the isocitrate dehydrogenase, a-ketoglutarate dehydrogenase, and malate dehydrogenase reactions.
- **FAD** accepts two hydrogen atoms (with their electrons). FAD is reduced, and the substrate is oxidized. FAD is frequently involved in reactions that produce a double bond. FAD is the prostethic group for succinate dehydrogenase. FAD is also required by α-ketoglutarate dehydrogenase.
- **Coenzyme A** contains a sulfhydryl group that reacts with carboxylic acids to form thioesters, such as acetyl CoA and succinyl CoA. CoA isused in the α -ketoglutarate dehydrogenase complex.
- **Thiamine pyrophosphate (TPP) and lipoic acid,** coenzymes for α-keto acid dehydrogenases The α-ketoacid dehydrogenases in TCA is α-ketoglutarate dehydrogenase, which

5.6.4 Amphibolic and anaplerotic reactions of TCA cycle

TCA cycle has amphibolic nature and is utilized for the both **catabolic reactions** to **generate energy** as well as for **anabolic reactions** to **generate metabolic intermediates** for biosynthesis.

TCA cycle is actively involved in gluconeogenesis, transamination and deamination.

The most important synthetic (anabolic) reactions connected with TCA cycle are as follows:

1. Oxaloacetate and α-ketoglutarate, respectively, serve as precursors for the synthesis of aspartate and glutamate which, in turn, are required for the synthesis of other non-essential amino acids, purines and pyrimidines.

2. Succinyl CoA is used for the synthesis of porphyrins and heme.

3. Mitochondrial citrate is transported to the cytosol, where it is cleaved to provide acetyl CoA for the biosynthesis of fatty acids, sterols etc.

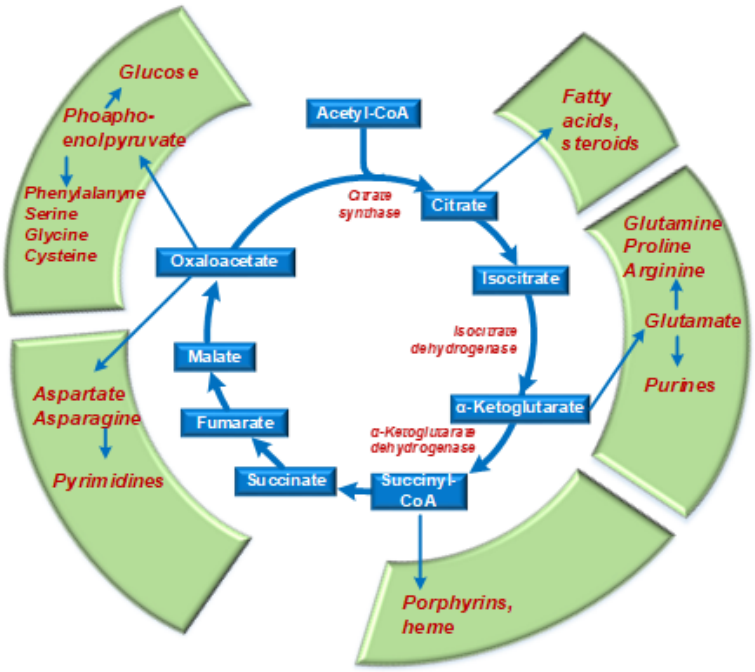


Fig. 5 Amphybolic role of TCA

Anaplerosis or anaplerotic reactions.

The synthetic reactions described above deplete the intermediates of citric acid cycle. The cycle will cease to operate unless the intermediates drawn out are replenished. Ihe reactions concerned to **replenish** or to **fill up** the intermediates of citric acid cycle are called **anaplerotic reactions** or anaplerosis.

The most important anaplerotic reactions:

1. **Pyruvate carboxylase** catalyses the conversion of pyruvate to oxaloacetate. This is an ATP dependent carboxylation reaction.

$$\text{Pyruvate} + CO_2 + ATP \rightarrow \text{Oxaloacetate} + ADP + P_i$$

It's a reaction of gluconeogenesis.

2. Pyruvate is converted to malate by $NADP^+$ dependent **malate dehydrogenase** (malic enzyme).

$$\text{Pyruvate} + CO_2 + NADPH \rightarrow \text{Malate} + NADP^+ + H_2O$$

3. **Transamination** is a process where an amino acid transfers its amino group to a keto acid and itself gets converted to a keto acid. The formation of α-ketoglutarate and oxaloacetate occurs by this mechanism.

4. α-Ketoglutarate can also be synthesized from glutamate by **glutamate dehydrogenase action.**

$$\text{Glutamate} + NAD(P)^+ + H_2O \rightarrow \alpha\text{-Ketoglutarate} + NAD(P)H + NH_3$$

REVIEW TEST:

№	MCQs	Answers and explanations
1.	**Examination of a patient revealed II grade obesity. It is known that he consumes a lot of sweets and rich food,**	**The answer is B.** The term amphibolic (Greek: amphi meaning "both sides") is used to describe a biochemical pathway that

	has sedentary way of life. That's why anabolic metabolism has the priority in his organism. Which of the following pathways is amphibolic? A. Glyconeogenesis B. Cycle of tricarboxylic acids C. Lipolysis D. Glycolysis E. Fatty acids oxidation	involves both catabolism and anabolism. The cycle of tricarboxylic acids is a good example of amphibolic pathway, because it function in both the degradative (carbohydrate, protein, and fatty acid) and biosynthetic processes. On one hand it generates ATP by providing electrons to the electron transport chain, on the other hand it produces specific intermediates, such as oxaloacetate, α-ketoglutarate, wich can act as precursors of amino acid e.g. glutamate and aspartate.
2.	**A patient was admitted into hospital with a diagnosis diabetes mellitus type I. In metabolic changes the decrease of oxaloacetate synthesis rate is detected. What metabolic pathway is damaged as a result?** A. Glycolysis B. Cholesterol biosynthesis C. Glycogen mobilization D. Tricarboxylic acid cycle E. Urea synthesis	**The answer is D.** Tricarboxylic acid cycle (TCA) to be continued needs to to be relpenished intermediates, so called analperosis. The main anaplerotic reaction for TCA functioning is catalysed by pyruvate carboxylase, an enzyme activated by acetyl-CoA, indicating a lack of oxaloacetate. In diabetes mellitus there is an increase in the utilization of oxaloacetate for gluconeogenesis this depletes the amount, required for TCA.
3.	**A biochemistry graduate student isolates all the enzymes of the TCA cycle and adds OAA and acetyl CoA, including the appropriate energy precursors, cofactors, and water. Which of the following will not be a direct product of his experiment?** A. ATP B. GTP C. NADH D. CO_2 E. $FADH_2$	**The answer is A.** The Krebs cycle does not directly produce ATP. The one substrate level phosphorylation reaction in the cycle generates GTP (the step catalyzed by succinate thiokinase). NADH is generated in three steps (catalyzed by isocitrate dehydrogenase, α-ketoglutaratedehydrogenase, and malatedehydrogenase) and $FADH_2$ in one step (catalyzed by succinate dehydrogenase). CO_2 is a product of the isocitrate and α-ketoglutarate dehydrogenase reactions.
4.	**A medicinal chemist working for a pharmaceutical company is synthesizing the barbiturate, barbital, for a clinical trial. In the following pathway for the synthesis of the barbital, which substrate is most likely to inhibit the only membrane-bound enzyme of the Krebs cycle?** A. Malonate B. Ethyl oxide C. Diethyl ethyl malonate D. Urea E. Barbital	**The answer is A.** Succinate dehydrogenase isthe only membrane-bound enzyme of the Krebs cycle. Malonate (choice A) resembles succinate (it is lacking a methylene group as compared to succinate) and binds to the active site of succinate dehydrogenase, yet it is not oxidized owing to the absence of an ethyl group between the carboxyl groups. Ethyl oxide (choice B) acts as nucleophiles but has no inhibitory effects on the Krebs cycle. Diethyl ethyl malonate (choice C), urea (choice D), and barbital (choice E) differ greatly from the endogenous substrate succinate and, therefore, would likely not inhibit the enzyme.
5.	**Most of the metabolic pathways are either anabolic or catabolic. Which of the following pathways is considered as "amphibolic" in nature?** A. Glycogenesis	**The answer is D.** In TCA Cycle energy is both consumed and produced. During the TCA cycle, acetyl CoA is converted to CO_2 and H_2O but during this process electrons are transfered to NADH and $FADH_2$. These electron go through the

	B. Glycolytic pathway C. Lipolysis D. TCA cycle E. Pentosophosphate pathway	electron transport chain to make ATP (the energy production part). In addition, during the conversion process ATP (GTP) is used to convert the intermediates (Succinyl CoA to Succinate). Hence TCA cycle is amphibolic pathway).
6.	**Mitochondria are subcellular organelles and are present in a cytoplasm of every cell except mature red blood cells, bacteria, blue-green algae. What method is used principally for their isolation?** A. Gel-filtration B. Chromatography C. Electrophoresis D. Spectrophotometry E. Differential centrifugation	**The answer is E.** Isolation of mitochondria involves cell disruption and centrifugation. The process of cell disruption involves breaking open of cell so as to spill out the contents within the cell. Centrifugation is the process by which mixtures of cell components are separated by centrifugal force. The more dense particles migrate away from the axis, while the less dense components of the mixture migrates towards the axis of centrifuge. The centrifugal technique which is used to separate the cell components from whole cell is called differential centrifugation.
7.	**In a patient are manifested symptoms of intoxication with arsenic compounds. What metabolic process is damaged taking into account that arsen containing substances inactivate lipoic acid.** A. Oxidative decarboxylation of α-ketoglutarate B. Fatty acids biosynthesis C. Neutralization of superoxide anions D. Coupling of oxidation and phopsphorylation E. Microsomal oxidation	The answer is A. Arsenic compounds have the ability to form covalent bonds with sulfhydryl groups present in various enzymes. This interaction leads to the inactivation of lipoamide-containing enzymes, specifically pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. The α-ketoglutarate dehydrogenase complex plays a crucial role in cellular energy production. When exposed to arsenic compounds, the sulfhydryl groups present in lipoamide-containing enzymes become covalently bound to arsenic, leading to their inactivation. As a result, the normal functioning of the α-ketoglutarate dehydrogenase complex is disrupted, impairing the conversion of α-ketoglutarate to succinyl CoA. The inhibition of α-ketoglutarate dehydrogenase by arsenic compounds interferes with the proper functioning of the TCA cycle and disrupts cellular energy metabolism. This can have detrimental effects on various physiological processes that rely on the efficient production of ATP, the primary energy currency of cells.
8.	**Substrate phosphorylation is a process of phosphate residue transfer from macroergic donor substance to ADP or some other nucleoside diphosphate. What enzyme of tricarboxylic acid cycle participates in reaction of substrate phosphorylation.** A. Succinyl CoA synthase (Succinyl thiokinase) B. Citrate synthase C. Succinate dehydrogenase D. Fumarase E. Alpha-ketoglutarate dehydrogenase complex	**The answer is A.** Inside the mitochondria ATP is synthesized by oxidative phosphorylation, but some ATP can be made in the cytoplasm through a process called substrate phosphorylation. This is a process of forming ATP by the physical addition of a phosphate group to ADP. Substrate-level phosphorylation reaction in TCA cycle is catalized by succinyl CoA synthase. In this reaction, the hydrolysis of the thioester bond leads to the formation of phosphoester bond with inorganic phosphate. This phosphate is transferred to Histidine residue of the enzyme and this high energy, unstable phosphate is finally transferred to GDP resulting in the generation of GTP

9.	**The number of molecules of ATP produced by the total oxidation of acetyl CoA in TCA cycle is:** A. 6 B. 8 C. 10 D. 12 E. 15	**The answer is D.** In one turn of TCA cycle, 4 reducing equivalents (3 as NADH and one as $FADH_2$) are produced. Oxidation of 3 NADH by electron transport chain coupled with oxidative phosphorylation results in the synthesis of 9 ATP, whereas $FADH_2$ leads to the formation of 2 ATP. Besides, there is one substrate level phosphorylation. Thus, a total of 12 ATP are produced from one acetyl CoA.
10	**Enzymes of tricarboxylic acids cycle oxidize acetyl-CoA and produce 3 molecules of reduced NAD and one molecule of reduced FAD. Where are localized these enzymes?** A. In mitochondrial matrix B. On plasma membrane C. On external mitochondrial membrane D. In cell cytoplasm E. On inner mitochondrial membrane	**The answer is A.** In the tricarboxylic acid cycle most of the enzymes are located within the mitochondrial matrix in eukaryotes. These enzymes work in close proximity to the electron transport chain, which is essential for the production of cellular energy in the form of ATP. However, there is one enzyme in the TCA cycle that has a unique location. Succinate dehydrogenase, also known as complex II of the electron transport chain, is an exception among the TCA cycle enzymes. In eukaryotic cells, succinate dehydrogenase is located in the inner mitochondrial membrane. This enzyme plays a dual role by participating in both the TCA cycle and the electron transport chain.

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6. BIOLOGICAL OXIDATION AND OXIDATIVE PHOSPHORYLATION

OBJECTIVES after studying this chapter, you should be able to:

- *Interprete processes of biological oxidation of different substrates in the cell and reservation of released energy in a form of macroergic bonds of ATP.*
- *Appreciate that energy from the oxidation of fuel substrates (fats, carbohydrates, amino acids) is almost all liberated in mitochondria as reducing equivalents, which are passed by a process termed electron transport through a series of redox carriers or complexes embedded in the inner mitochondrial membrane known as the respiratory chain, until they are finally reacted with oxygen to form water. To analyze reactions of biological oxidation and their role in providement of fundamental biochemical processes in tissues.*
- *Describe the four protein complexes involved in the transfer of electrons through the respiratory chain and explain the roles of flavoproteins, iron sulfur proteins, and coenzyme Q.*
- *Appreciate role of biological oxidation, tissue respiration and oxidative phosphorylation in generation of ATP in aerobic conditions.*
- *Understand how electron transport through the respiratory chain generates a proton gradient across the inner mitochondrial membrane, leading to the buildup of a proton motive force that generates ATP by the process of oxidative phosphorylation.*

6.1. Biological oxidation of substrates in cells.

We all know we have to eat to get the energy that sustains life and the materials for maintaining our bodies, but what does the body do with that food to get the energy out and put the matter into the right places?

Most organisms obtain energy from reduced compounds through the process of **cellular respiration**. These words have meant different things at different times. It is commonly refered as breathing – inhaling and exhaling – and a moment's reflection will show that breathing is intimately related to getting energy. But what does breathing have to do with getting energy?

The relationship between air and energy first started to emerge in 1755 when **Joseph Black** showed that respiring animals give off a gas then known as "fixed air", which we now call as carbon dioxide. **Antony Lavoisier** established that the gas he named oxygen is removed from the air during respiration or combustion and replaced by carbon dioxide. Initially he thought that carbon is carried through the blood to the lungs where it is slowly combined with oxygen, but he gradually developed a more realistic theory by moving the site of respiration from the lungs to the blood and finally to all of the body tissues. After

the rise of ezymology it was established, largely through the work of **Otto Warburg**, that biological oxidations are catalized by intracellular enzymes.

Chemically, oxidation is defined as the removal of electrons and reduction as the gain of electrons. Thus, **oxidation** of a molecule (the electron donor) is always accompanied by **reduction** of a second molecule (the electron acceptor). This principle of oxidation-reduction applies equally to biochemical systems and is an important concept underlying the understanding of the nature of biological oxidation.

Biological oxidation is oxidation which occurs in biological systems **to produce energy.** Energy is required to maintain the structure and function of the living cells. Energy is produced by oxidation of food e.g. glucose, fatty acids, amino acids.

Oxidation can occur by:

- Addition of oxygen (less common).
- Removal of hydrogen (common).
- Removal of electrons (most common).

Electrons are not stable in the free state. Electron removal (oxidation) should paired with electron acceptance (reduction) by another substance (fig. 6.1). Oxidation-reduction reactions or redox reactions are done by **oxido-reductases**.

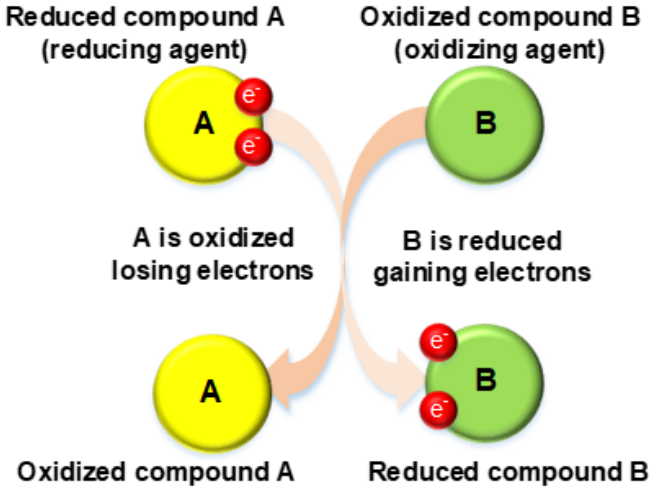


Fig. 6.1. Oxidation-reduction reactions

Redox potential is the ability of a substance to accept electrons (to be reduced) or to lose them (to be oxidized). Electrons are transferred from substances with **low** redox potential to substances with **higher** redox potential. Electrons transfer is an energy yielding process. The amount of energy liberated depends on the redox potential difference between the electron donor and acceptor (table 6.1).

The redox potential difference ΔE between the electron donor and acceptor is related to the associated free energy change ΔG of the reaction via **ΔG=nFΔE**, where n is the number of electrons transferred and F is Faraday's constant (96,485 J/mol/V or ≈100 kJ/mol/V). By inspecting tabulated values of these potentials, it is possible to presuppose the tendency for electron transfer and hence, of the direction of the reaction.

Table 6.1. Some Redox Potentials

System	E`₀ Volts
H^+/H_2	-0.42
NAD^+/NADH	-0.32
Lipoate ox/red	-0.29
Pyruvate/lactate	-0.19
Oxaloacetate/malate	-0.17
Fumarate/succinate	+0.03
Cytochrome b (Fe^{3+}/Fe^{2+})	+0.08
Ubiquinone ox/red	+0.10
Cytochrome c_1 (Fe^{3+}/Fe^{2+})	+0.22
Cytochrome a (Fe^{3+}/Fe^{2+})	+0.29
Oxygen/water	+0.82

6.1.1 Types of reactions of biological oxidation and their functional significance

Enzymes involved in oxidation and reduction are called **oxido-reductases** and are classified into four groups: **oxidases, dehydrogenases, hydroperoxidases, and oxygenases.**

I. Oxidases catalyze the removal of hydrogen from a substrate using oxygen as a hydrogen acceptor (fig. 6.2). They form water or hydrogen peroxide as a reaction product.

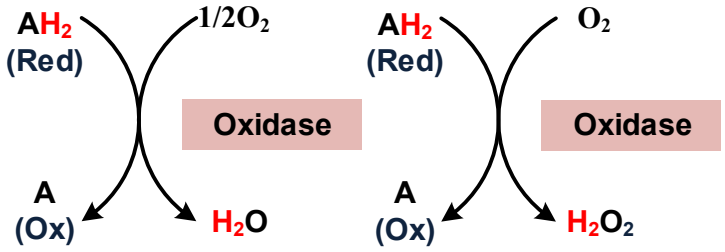


Fig. 6.2. Oxidase reaction

Cytochrome oxidase is an example of oxidases, it is a hemoprotein widely distributed in many tissues, having the typical heme prosthetic group present in myoglobin, hemoglobin, and other cytochromes. Other example – **flavoprotein enzymes** that contain flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as prosthetic groups. Examples of flavoprotein oxidases include ***L-amino acid oxidase***, an enzyme found in kidney with general specificity for the oxidative deamination of the naturally occurring L-amino acids; ***xanthine oxidase***, which contains molybdenum and plays an important role in the conversion of purine bases to uric acid; and ***aldehyde dehydrogenase***, an FAD-linked enzyme present in mammalian livers, which contains molybdenum and nonheme iron and acts upon aldehydes and N-heterocyclic substrates.

II. Dehydrogenases. There are a large number of enzymes in the **dehydrogenase** subclass (fig. 6.3). They perform the following two main functions:

- Transfer of hydrogen from one substrate to another in a coupled oxidation–reduction reaction. These enzymes are also called **anaerobic dehydrogenases**, they are specific for their substrates but often utilize common coenzymes or hydrogen carriers, for example, NAD^+. Since the reactions are reversible, these properties enable reducing equivalents to be freely transferred within the cell.

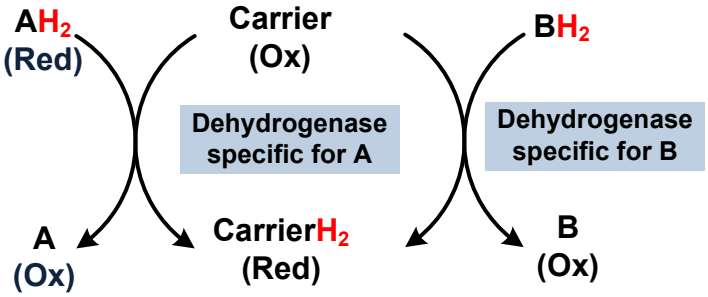


Fig. 6.3. Dehydrogese reaction

- Transfer of electrons in the respiratory chain of electron transport from substrate to oxygen **(aerobic dehydrogenases).**

Most of dehydrogenases use NAD^+ or $NADP^+$ (sometimes both) coenzymes. which are formed in the body from the vitamin PP (niacin). For example, ***lactate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase***. The flavin groups such as FMN and FAD are associated with dehydrogenases as well as with oxidases. For example, ***succinate dehydrogenase***, ***acyl-CoA dehydrogenase,*** and mitochondrial ***glycerol-3-phosphate dehydrogenase***.

III Hydroperoxidases. Two type of enzymes found fall into the hydroperoxidase category: ***peroxidases and catalase***. Hydroperoxidases play an important role in protecting the body against the harmful effects of **reactive oxygen species (ROS).** ROS are highly reactive oxygen-containing molecules such as peroxides which are formed during normal metabolism, but can be damaging if they accumulate.

In the reaction catalyzed by peroxidases, hydrogen peroxide is reduced at the expense of several substances that will act as electron acceptors, such as ascorbate (vitamin C), quinones, and cytochrome c. The reaction catalyzed by peroxidase is complex, but the overall reaction is as follows:

$$H_2O_2 + AH_2 \rightarrow 2H_2O + A$$

In RBCs and other tissues, the enzyme ***glutathione peroxidase***, containing selenium as a prosthetic group, catalyzes the destruction of H_2O_2 and lipid hydroperoxides through the conversion of reduced glutathione to its oxidized form hence protecting membrane lipids and hemoglobin against oxidation by peroxides. ***Catalase*** is a hemoprotein containing four heme groups. It can act as a peroxidase, catalyzing reactions of the type shown above, but it is also able to catalyze the breakdown of H_2O_2.

IV. Oxygenases are concerned with the synthesis or degradation of many different types of metabolites. They catalyze the incorporation of oxygen into a substrate molecule in two steps: (1) oxygen is bound to the enzyme at the active site and (2) the bound oxygen is reduced or transferred to the substrate. Oxygenases may be divided into two subgroups: **dioxygenases and monooxygenases**.

Dioxygenases incorporate both atoms of molecular oxygen into the substrate. The basic reaction catalyzed by dioxygenases is:

$$A + O_2 \rightarrow AO_2$$

Examples include the liver enzymes, *homogentisate dioxygenase* (oxidase) and *3-hydroxyanthranilate dioxygenase* (oxidase), which contain iron; and *L-tryptophan dioxygenase (tryptophan pyrolase)*

Monooxygenases incorporate only one atom of molecular oxygen into the substrate. The other oxygen atom is reduced to water an additional electron donor or cosubstrate is necessary for this purpose:

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

Thus, oxidoreductases have a variety of functions in metabolism; oxidases and dehydrogenases play major roles in respiration; hydroperoxidases protect the body against damage by free radicals; and oxygenases mediate the hydroxylation of drugs and steroids.

6.1.2. Pyridine dependent dehydrogenases

Two coenzymes **NAD^+ and $NADP^+$** derived from the vitamin niacin serve as coenzymes of so-called **pyridine dependent dehydrogenases** (because the niacin ring resembles pyridine). Of the two coenzymes, NAD^+ is more activelv involved in the electron transport chain. NAD^+ is reduced to $NADH+H^+$ by dehydrogenases with the removal of two hydrogen atoms from the substrate (AH_2).

Nicotinamide coenzymes consist of a nicotinamide ring linked to an adenosine via a ribose and a phosphate group, forming a dinucleotide. An oxidation reaction involves the transfer of two electrons and one H^+ from the substrate to the nicotinamide ring of NAD^+

forming NADH and the oxidized product (fig. 6.4). The remaining hydrogen of the hydrogen pair removed from the substrate remains free as a hydrogen ion. NADH is oxidized to NAD^+ by the reverse reaction.

Note that the reduced nucleotides absorb light at 340 nm; the oxidized forms do not.

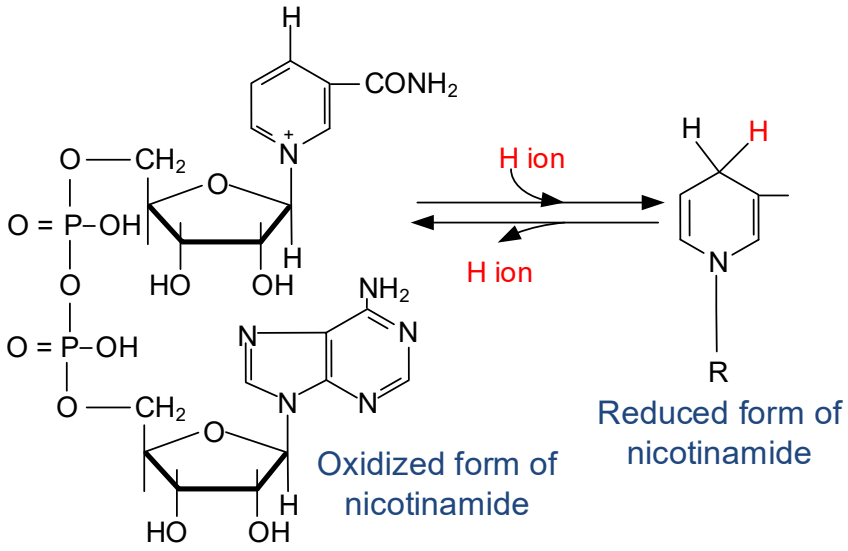


Fig. 6.4. Reduction of NAD^+

The total concentration of NAD^+ + NADH in most tissues is about 10^{-5} M; that of $NADP^+$ + NADPH is about 10^{-6} M. In many cells and tissues, the ratio of NAD^+ (oxidized) to NADH (reduced) is high, favoring hydride transfer from a substrate to NAD^+ to form NADH. In contrast, NADPH (reduced) is generally present in greater amounts then its oxidized form ($NADP^+$), favoring hydride transfer from NADPH to a substrate. This reflects the specialized metabolic roles of the two coenzymes: NAD^+ generally functions in oxidations – usually as part of a catabolic reaction; and NADPH is the usual coenzyme in reductions – nearly always as part of an anabolic reaction.

6.1.3. Flavine dependent dehydrogenases

Flavine dependent dehydrogenases (flavoproteins) are enzymes that catalyze oxidation-reduction reactions using either **flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD)** as prosthetic groups. These coenzymes are derived from the vitamin riboflavin. The fused ring structure of flavin nucleotides (the isoalloxazine ring) undergoes reversible reduction by accepting either one or two electrons in the form of (one or two) hydrogen atoms (each atom an electron plus a proton) from a reduced substrate. The fully reduced forms are abbreviated $FADH_2$ and $FMNH_2$ (fig. 6.5).

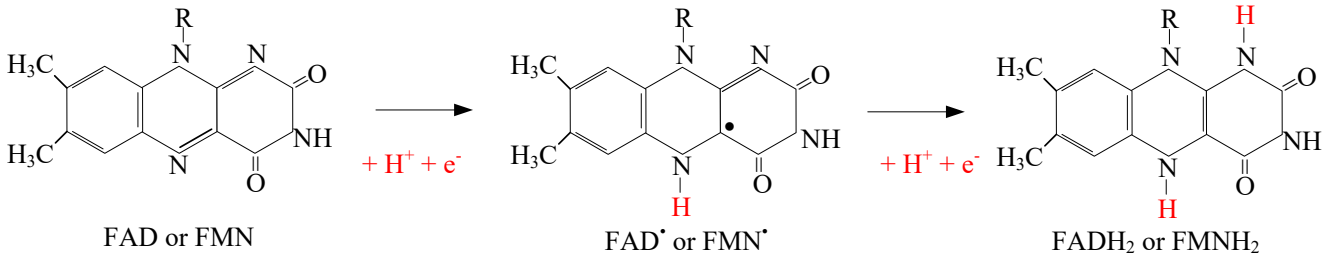


Fig. 6.5. Reduction of FAD (or FMN)

When a fully oxidized flavin nucleotide accepts only one electron (one hydrogen atom), the semiquinone form of the isoalloxazine ring is produced, abbreviated FADH• and FMNH•. Because flavoproteins can participate in either one- or two-electron transfers,

this class of proteins is involved in a greater diversity of reactions than the NAD(P)-linked dehydrogenases.

The flavin nucleotide in most flavoproteins is bound rather tightly to the protein, and in some enzymes, such as succinate dehydrogenase, it is bound covalently. Such tightly bound coenzymes are properly called **prosthetic groups**. They do not transfer electrons by diffusing from one enzyme to another; rather, they provide a means by which the flavoprotein can temporarily hold electrons while it catalyzes electron transfer from a reduced substrate to an electron acceptor.

6.1.4. Cytochromes and their role in tissue respiration

Cytochromes are electron carriers containing heme. Hemes in the 3 classes of cytochrome (**a**, **b**, **c**) differ in substituents on the porphyrin ring. Some cytochromes (b, c_1, c, a, a_3) are part of large **integral membrane protein complexes**.

Each group consists of four five-membered, nitrogen-containing rings in a cyclic structure called a **porphyrin**. The four nitrogen atoms are coordinated with a central Fe ion, either Fe^{2+} or Fe^{3+} (fig 6.6). Iron protoporphyrin IX is found in b-type cytochromes and in hemoglobin and myoglobin.

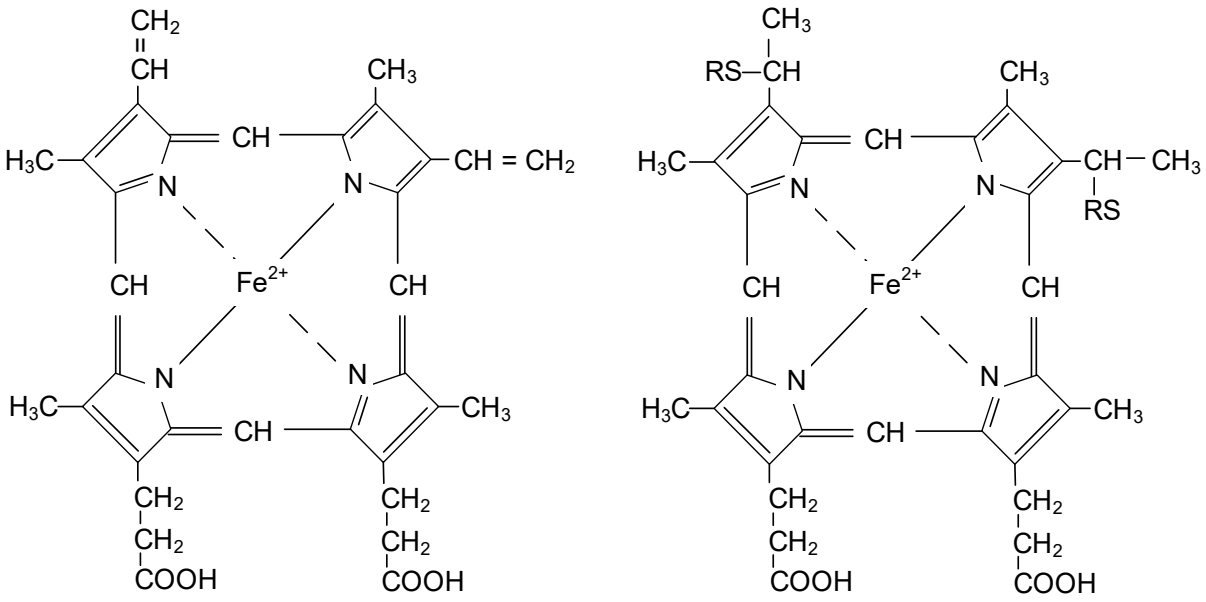


Fig. 6.6. Structure of prosthetic groups of cytochromes b and c

Cytochrome c is a small protein containing 104 amino acids and a heme group. lt is a central member of electron transport chain with an intermediate redox potential. lt is rather loosely bound to inner mitochondrial membrane and can be easily extracted. **Cytochrome oxidase** is the terminal component of the chain of respiratory carriers composed of cytochromes a and a_3 It contains two molecules of heme, each having one **Fe** atom that oscillates between Fe^{3+} and Fe^{2+} during oxidation and reduction. Furthermore, two atoms of **Cu** are present, each associated with a heme unit.

6.1.5. Coenzyme Q

Coenzyme Q is also known as **ubiquinone** since it is ubiquitous in living system. lt is a quinone derivative with a variable isoprenoid side chain. The mammalian tissues possess a quinone with 10 isoprenoid units which is known as coenzyme Q_{10} (CoQ_{10}). CoQ is lipid-soluble. It dissolves in the hydrocarbon core of a membrane. It is the only electron carrier not bound to a protein.

When bound to special sites in respiratory complexes, **CoQ** can accept **1** e^- to form a **semiquinone radical (Q•$^-$)** (fig. 6.7).

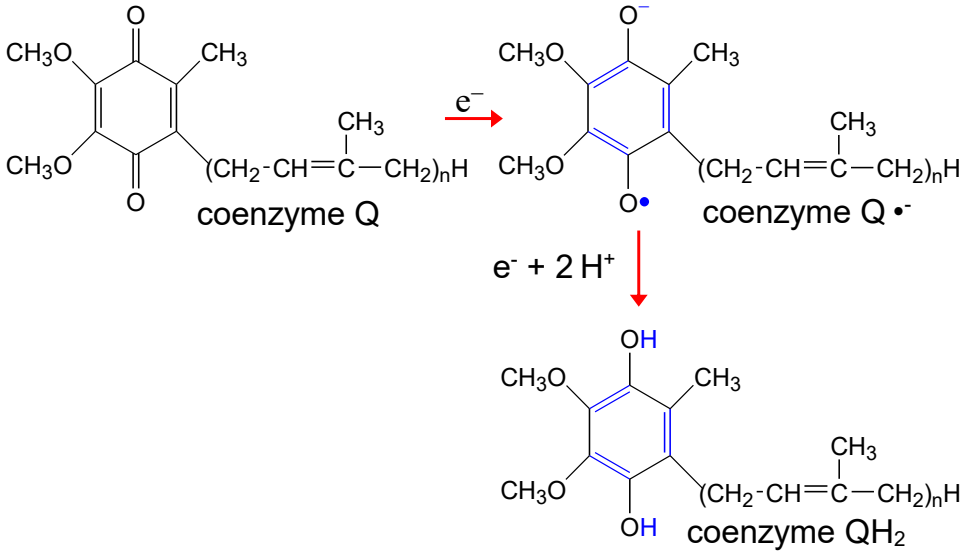


Fig. 6.7. Reduction of coenzyme Q

6.2. Molecular organization of electron the transport chain of mitochondria

The mitochondrion is the site of eukaryotic oxidative metabolism. It contains the enzymes that mediate this process, including TCA cycle enzymes and the enzymes catalyzing fatty acids phosphorylation. The mitochondrion has a smooth outer membrane and an extensively invaginated inner membrane. The number invagination, called **cristae,** varies with the respiratory activity of the particular type of cell. This is because the proteins mediating electron transport and oxidative phosphorilation are bound to the inner mitochondrial membrane so that the respiration rate varies with membrane surface area (fig. 6.8).

The outer mitochondrial membrane contains porin, a protein that forms nonspecific pores that permit free diffusion of up to 10kDa molecules. The inner membrane, which contains ~75 % proteins by weight, is considerably richer in proteins than the outer membrane. It is freely permeable only to O_2 CO_2, and H_2O and contains, in addition to respiratory chain, numerous transport proteins that control the passage of metabolites such as ATP, ADP, pyruvate, Ca^{2+}, and phosphate.

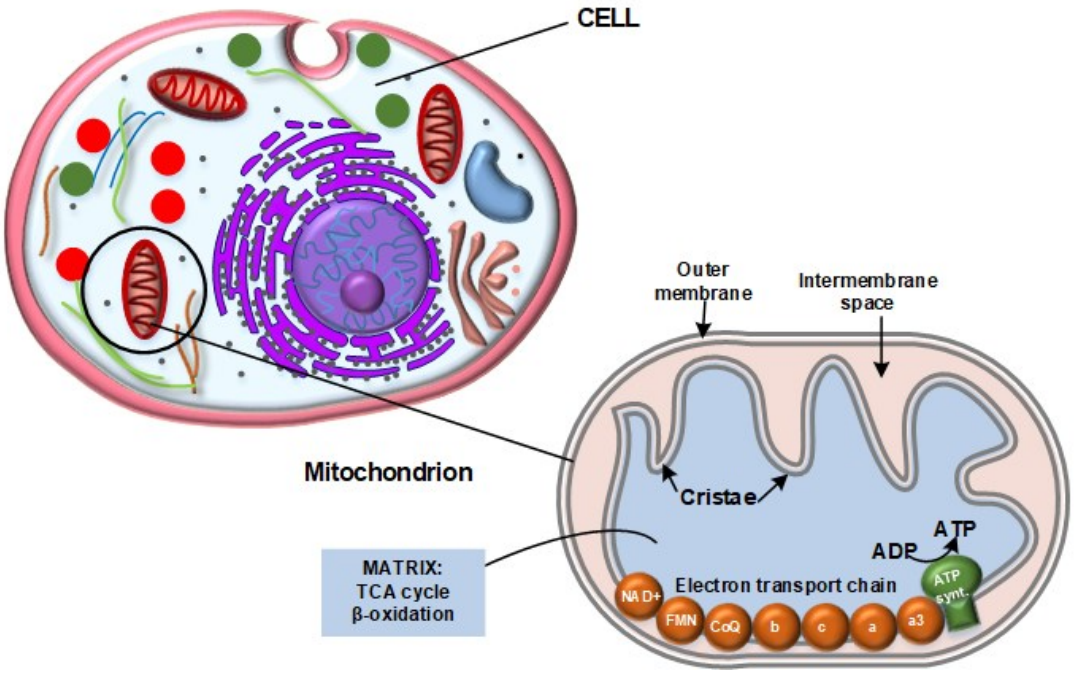


Fig. 6.8. Mitochondrion

The components of an **electron transport chain (ETC)** form four protein complexes that are embedded in the inner mitochondrial membrane like a mosaic, but are free to move laterally because membrane are so fluid. Electrons, donated primarily by NADH and secondarily by $FADH_2$, pass from one complex to another via mobile carriers (ubiquinone and cytochrome c) and finally to cytochrome oxidase.

In the overall reaction catalyzed by the ETC electrons move from NADH, succinate, or some other primary electron donor through flavoproteins, ubiquinone, iron-sulfur proteins, and cytochromes, and finally to O_2.

6.2.1. Supramolecular complexes of respiratory chain in inner membrane of mitochondria

Protein complexex of inner mitochondrial membrane form 4 supramolecular complexes (fig. 6.9).

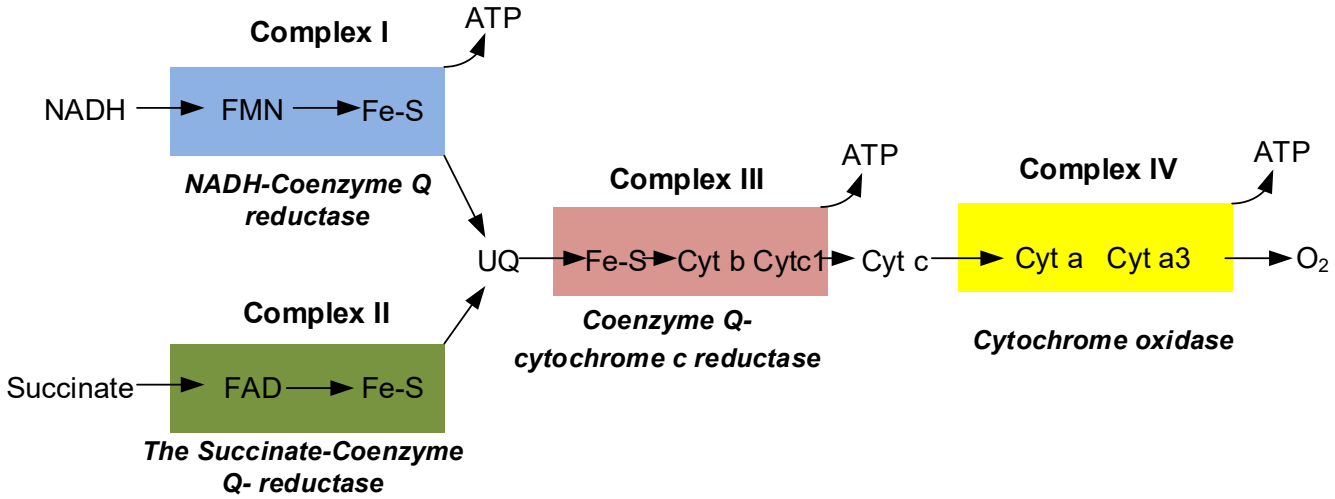


Fig. 6.9. IV complexes of electron transport chain

Complex I catalyzes oxidation of NADH by CoQ:

$$NADH + CoQ\ (oxidized) \rightarrow NAD^+ + CoQ\ (reduced)$$

Complex III catalyzes oxidation of CoQ (reduced) by cytochrome c.

$$CoQ\ (reduced) + cytochrome\ c\ (oxidized) \rightarrow CoQ\ (oxidized) + cytochrome\ C\ (reduced)$$

Complex IV catalyzes oxidation of cytochrome c (reduced) by O_2, the terminal electron acceptor of the electron transport process.

$$Cytochrome\ C\ (reduced) + 1/2O_2 \rightarrow cytochrome\ c\ (oxidized) + H_2O$$

The change in standard reduction potential of an electron pair as it successively traverses Complex I, III, and IV corresponds, at each stage, to sufficient free energy to power the synthesis of an ATP molecule.

Complex II catalyzes the oxidation of $FADH_2$ by CoQ.

$$FADH_2 + CoQ\ (oxidized) \rightarrow FAD + CoQ\ (reduced)$$

This redox reaction does not release sufficient free energy to synthesize ATP; it functions only to inject the electrons from $FADH_2$ into the electron-transport chain.

Complex I: NADH-Coenzyme Q reductase. The NADH dehydrogenase complex is the largest complex in mitochondrial inner membrane (fig. 6.10). It has moere than 44 different subunits, including approximately six to seven iron-sulfur center and a flavoprotein with bound flavine mononucleotite (FMN; redox-active prosthetic group).

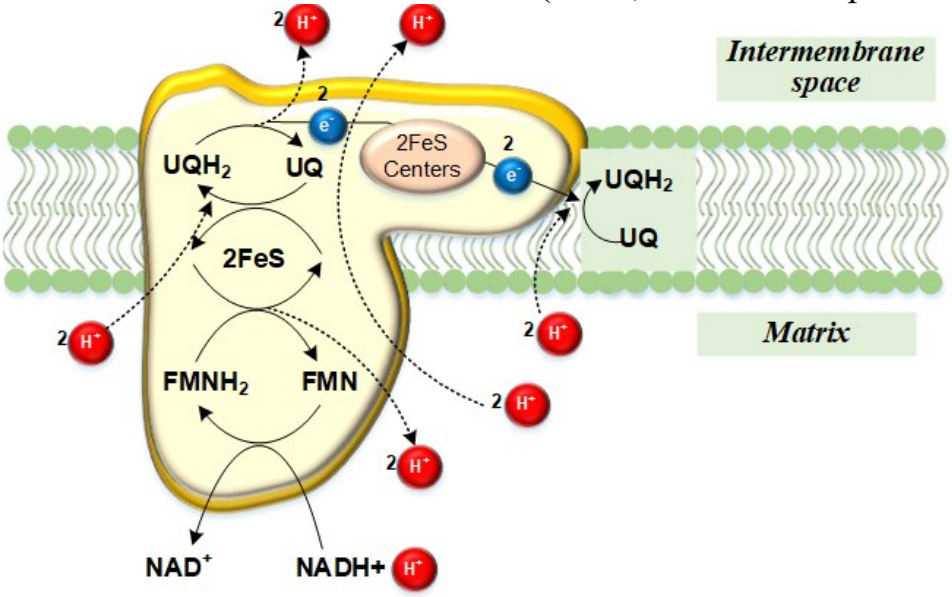


Fig. 6.10. NADH-Coenzyme Q reductase has a complex structure. It has three main modules: NADH-binding module, wich includes subunits that bind and oxidize NADH, transferring the electrons to the next module; ubiquinone-binding module, wich receives the electrons from the NADH-binding module and transfers them to coenzyme Q (ubiquinone, UQ). During this process, protons are pumped across the inner mitochondrial membrane, establishing an electrochemical gradient. Proton-pumping module responsible for the translocation of protons across the membrane.

The reduction of FMN to $FADH_2$ by NADH requires the uptake of one protone from the matrix. $FADH_2$ subsequently transfers electrons to a series of iron-sulfur centers and releases protons to the solution in the intermembrane space. Iron-sulfur (Fe-S) clusters are essential cofactors most commonly known for their role mediating electron transfer within

the mitochondrial respiratory chain (fig. 6.11). When the iron-sulfur centers reduce UQ to UQH_2, two more protons are taken up from the matrix.

The NADH dehydrogenase is oriented in the inner membrane so that its binding site for NADH faces inwardly toward the matrix space. This orientation is appropriate for the oxidation of **NADH generated in the matrix by TCA cycle**. The UQH_2 formed by complex I diffuses to complex III in the membrane's phospholipids bilayer, but the protons that are taken up when the quinine undergoes reduction come from the solution on the matrix side of the membrane. In the course of the electron-transfer reactions, protons also are released to the solution in the intermembrane space. These proton movements will have special significance when discuss the formation of ATP.

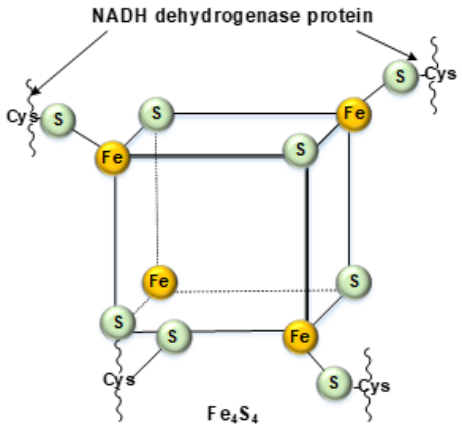


Fig. 6.11. Iron-sulfur clastrs of Complex I

Complex II: The Succinate-Coenzyme Q- reductase. Succinate dehydrogenase is the only enzyme of the TCA cycle that is embedded in inner membrane. Its four subunits include two iron-sulfur proteins, one of which also has a covalently attached FAD. As in NADH dehydrogenase, the substrate-oxidation site is on the matrix side of the membrane (fig. 6.12).

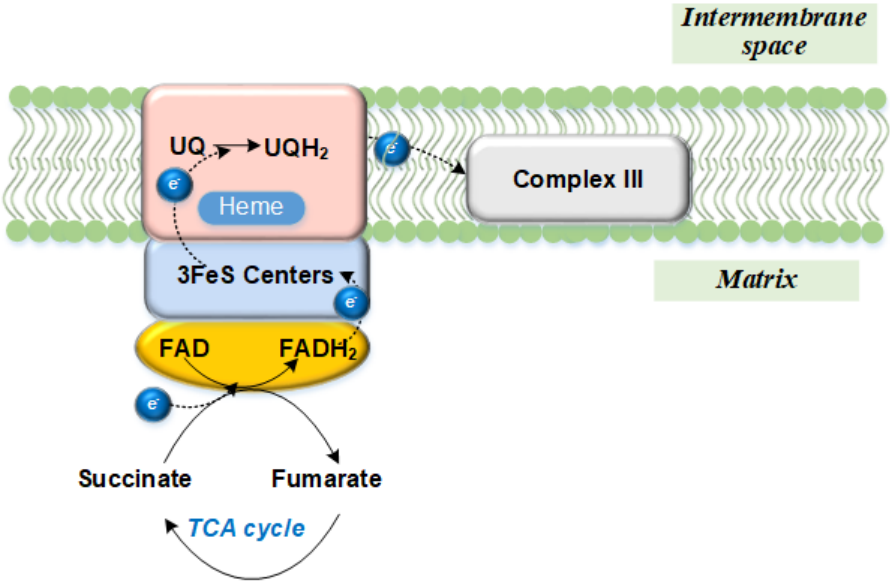


Fig. 6.12. The Succinate-Coenzyme Q- reductase – complex II consists of several subunits, including a flavoprotein subunit and an iron-sulfur protein subunit. These subunits work together to carry out the oxidation-reduction reactions and electron transfer. The flavoprotein subunit contains a covalently bound flavin adenine dinucleotide (FAD) cofactor, which accepts the electrons from succinate. The iron-sulfur protein subunit contains iron-sulfur clusters that mediate the transfer of electrons from the flavoprotein subunit to coenzyme Q (UQ).

The reduction of coenzyme Q (CoQ) to $CoQH_2$ is coupled with the transfer of electrons from succinate, but unlike Complex I, Complex II does not contribute to the pumping of protons across the membrane.

Complex II plays a critical role in both cellular respiration and the citric acid cycle. In addition to its involvement in the ETC, Complex II also catalyzes a step in the citric acid cycle, where it converts succinate to fumarate. This dual function connects the citric acid cycle with the electron transport chain, allowing for the efficient production of ATP.

MEDICAL IMPORTANCE

Mutations or dysfunction in Complex II can be associated with various mitochondrial disorders and have been linked to diseases such as hereditary paraganglioma-pheochromocytoma syndrome and Leigh syndrome. Studying Complex II helps deepen our understanding of mitochondrial function and its impact on cellular metabolism and human health.

Complex III: The Coenzyme Q-cytochrome c reductase Complex. Complex III transfers electrons from reduced coenzyme Q (UQH_2) to cytochrome c. The cytochrome bc_1 complex contains two b-type cytochromes (b_L and b_H), one cytochrome c_1, and one iron-sulfur center (fig. 6.13). The movement of electrons from UQH_2 to cytochrome c is a complex, multistep process, known as Q-cycle. In this sequence of reactions, coenzyme UQH_2 (reduced form) is oxidized to coenzyme Q, and cytochrome c (Fe3+) is reduced to cytochrome c (Fe^{2+}). The transfer of electrons from coenzyme Q to cytochrome c is coupled with the movement of protons from the matrix to the intermembrane space, contributing to the proton motive force.

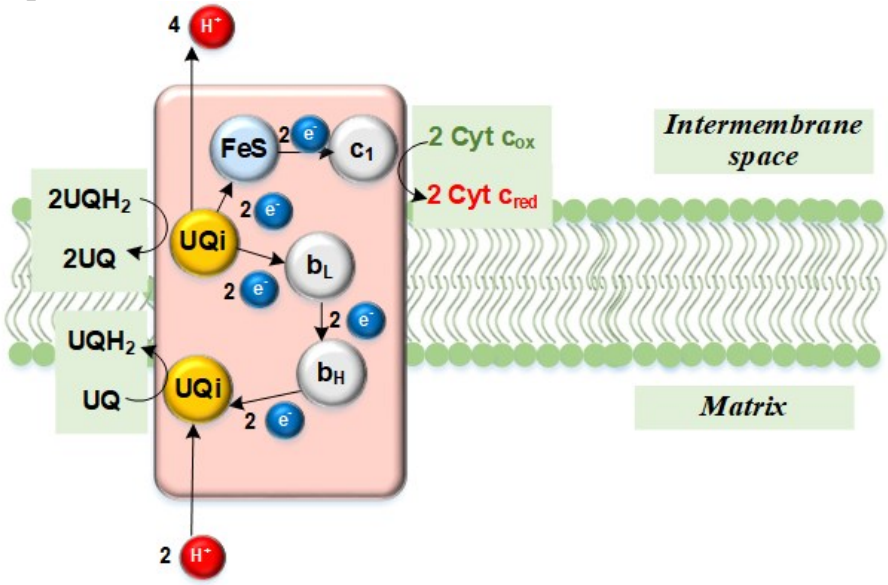


Fig. 8.13. The Coenzyme Q-cytochrome c reductase Complex consists of several subunits, including cytochromes and iron-sulfur proteins, which work together to facilitate electron transfer and proton pumping.

Complex III is a crucial component of the ETC, as it connects Complex I and Complex II to the final complex, Complex IV (cytochrome c oxidase).

Understanding the structure and function of Complex III helps to elucidate the mechanisms of electron transfer and energy conversion in aerobic respiration, contributing to our knowledge of cellular metabolism and mitochondrial diseases.

MEDICAL IMPORTANCE

Dysfunction or mutations in Complex III can lead to various mitochondrial disorders and are associated with diseases such as mitochondrial encephalopathy and myopathy with lactic acidosis and stroke-like episodes (MELAS syndrome).

Complex IV: Cytochrome Oxidase is composed of multiple subunits, including cytochromes, copper ions, and other cofactors. These components work together to catalyze the reduction of molecular oxygen, resulting in the formation of water (H_2O) (fig.8.14).

The primary function of Complex IV is to facilitate the transfer of electrons from cytochrome c to molecular oxygen, reducing it to water. The process occurs in multiple steps:

- Cytochrome c, which carries electrons from Complex III, binds to Complex IV.
- Electrons from cytochrome c are transferred through a series of redox reactions involving copper ions and cytochromes within Complex IV.
- Molecular oxygen (O_2) is reduced by accepting four electrons from four cytochrome c molecules and four protons (H^+) from the matrix.
- The protons (H^+) released from the matrix during the transfer of electrons are pumped across the mitochondrial membrane, contributing to the establishment of the proton motive force.
- Finally, the electrons and protons are used to reduce molecular oxygen completely, forming water (H_2O).

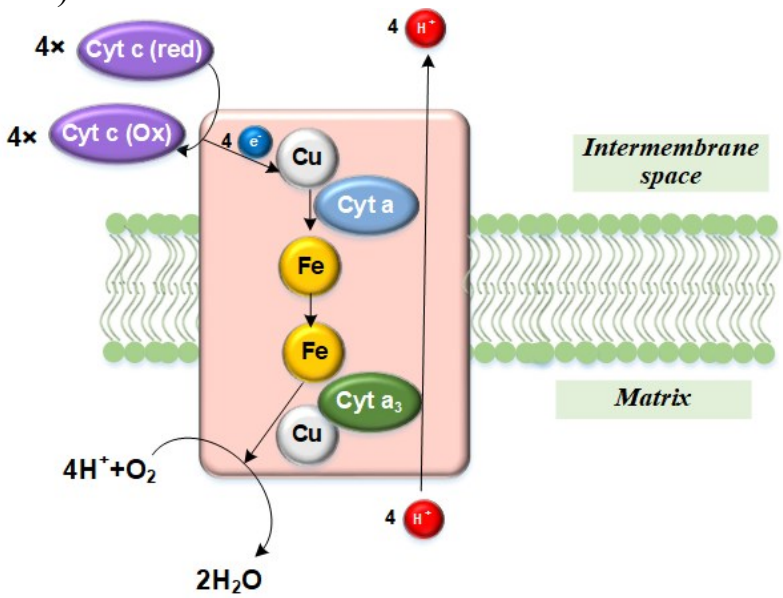


Fig. 8.14. The Complex IV is composed of multiple subunits, including cytochromes, copper ions, and other cofactors. These components work together to catalyze the reduction of molecular oxygen, resulting in the formation of water (H_2O).

MEDICAL IMPORTANCE

Complex IV plays a crucial role in maintaining the flow of electrons and the balance of redox reactions in the electron transport chain. Dysfunction or mutations in Complex IV can lead to mitochondrial disorders such as Leigh syndrome and cytochrome c oxidase deficiency.

6.3. Inhibitors of electron transport in a respiratory chain of mitochondria

The understanding of the sequence of events in electron transport is largely based on the use of specific inhibitors. This sequence has been collaborated by measurement of the standard reduction potentials of redox components on each of the complexes as well as by determining the stoichiometry of the electron transport and coupled ATP synthesis.

Several molecules are known to specifically inhibit the electron transport process (fig. 8,15). In addition to reduction potential measurements, inhibitors have been invaluable in the determination of the correct order of electron-transport chain components. In such experiments, electron transport is measured with oxygen electrode. Inhibition of electron transport by an inhibitors results in a reduction or elimination of oxygen consumption. ETC components on the oxygen side of blockage will become more oxidized because they are no longer able to accept electrons.

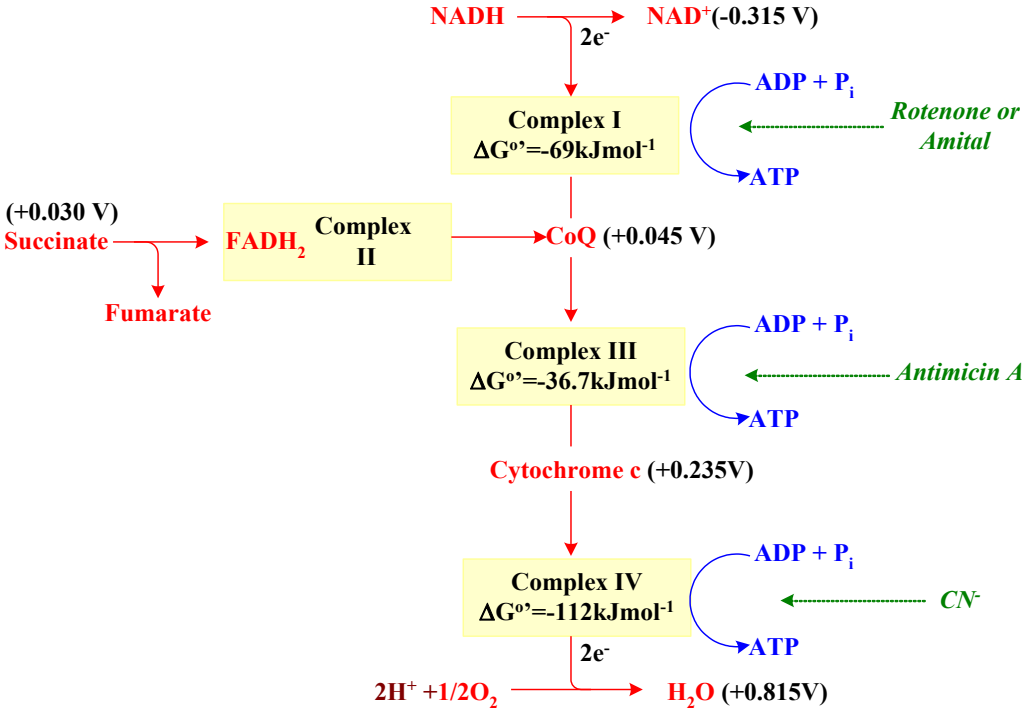


Fig. 8.15. The mitochondrial electron-transport chain. The standard potentials of its most mobile components (red) are indicated as are the points where sufficient free energy is harvested to synthesize ATP (blue) and the sites of action of several respiratory inhibitors (green).

Three possible sites of action for the inhibitors of ETC are identified:

1. **NADH and coenzyme Q:** Fish poison **rotenone, barbituate drug amytal** and antibiotic **piercidin A** inhibit this site (fig. 8.15).

2. **Between cytochrome b and c1:** Antimycin A - an antibiotic, **British antilewisite (BAL)-**an antidote used against war-gas are the two important inhibitors of the site between cytochrome b and c1.

3. **Inhibitors of cytochrome oxidase: carbon monoxide, cyanide, hydrogen sulphide and azide** effectively inhibit cytochrome oxidase. Carbon monoxide reacts with reduced form of the cytochrome while cyanide and azide react with oxidized form.

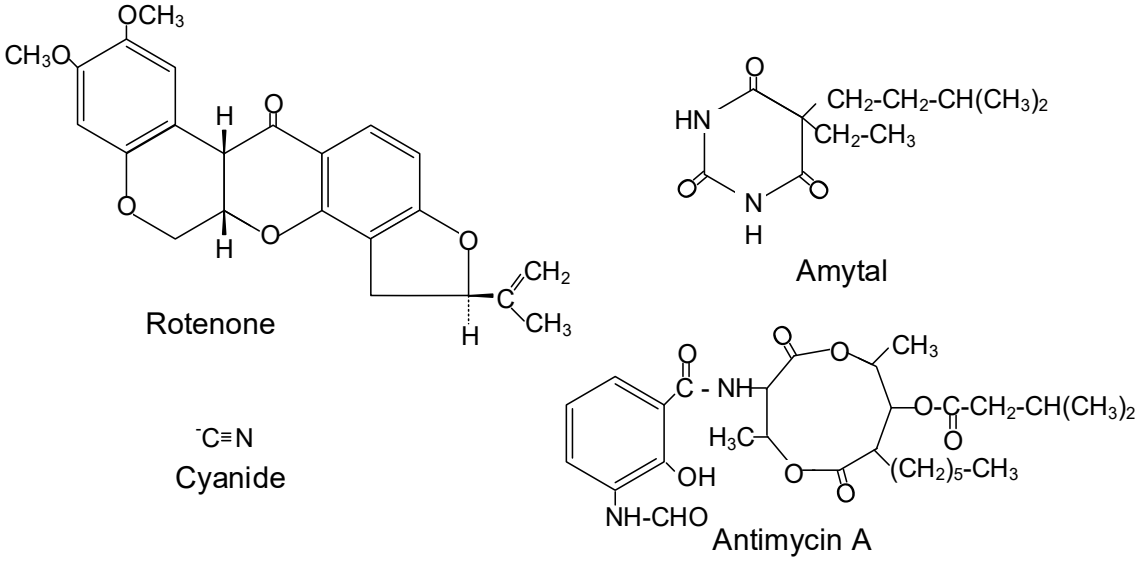


Fig. 6.16. Chemichal structure of the most common inhibitors of respiratory chain

6.4. Oxidative phosphorylation

The transport of electrons through the ETC is linked with the release of free energy. The process of synthesizing ATP from ADP and Pi coupled with the electron transport chain is known as **oxidative phosphorylation**.

The **P:O ratio** refers to the number of inorganic phosphate molecules utilized for ATP generation for every atom of oxygen consumed. More appropriately, P:O ratio represents the number of molecules of ATP synthesized per pair of electrons carried through ETC. When NADH is used as the reducing substrate, the measured P:O ratio is about 3; whereas the succinate it is about 2. Reductants that feed electrons directly to cyt c give a P:O of about 1.0, which conducts electrons from cytochrome c to O_2, has a site where the energy of the electron-transfer reactions is coupled to ATP synthesis. Because the electron-transport chain for NADH and succinate converge at UQ, the higher P:O ratio obtained with complex, between NADH and UQ. Finally, the intermediate P:O ratio obtained with succinate indicates that a coupling site occurs in complex III, but probably not in complex II. **Thus, the respiratory chain appears to have three distinct coupling sites for synthesis of ATP.**

The contribution of the individual coupling sites to the overall P:O ratios may be estimated from the differences in the P:O ratios observed when different reducing substrates are used. The difference in P:O ratios observed when NADH and succinate are used (3-2) suggests that **1 ATP is synthesized per electron pair at the first coupling site (complex I)**, while the difference observed when succinate and ascobate are used (2 – 1) suggests that **1 ATPs are synthesized per electron pair at the second coupling site (complex III)**. Finally the P:O ratio obtained when ascorbate is used suggests that **one ATP is synthesized per electron pair at the third coupling site (complex IV).**

In 1961, **Peter Mitchell** suggested a radically new theory to explain the **coupling mechanism of oxidative phosphorylation**. Mitchell proposed that the component generated at all three coupling sites is not a high-energy chemical species but rather **an electrochemical potential gradient for protons across the mitochondrial inner membrane**.

According to the **chemiosmotic theory**, flow of electrons through the electron-transport complexes pumps protons across the inner membrane from the matrix to the intermembrane space. This raises the pH in the matrix and leaves the matrix negatively charged with respect to the intermembrane space and cytosol. Protons flow passively back into the matrix through a channel in the ATP-synthase, and this flow drives the formation of ATP (fig. 6.17).

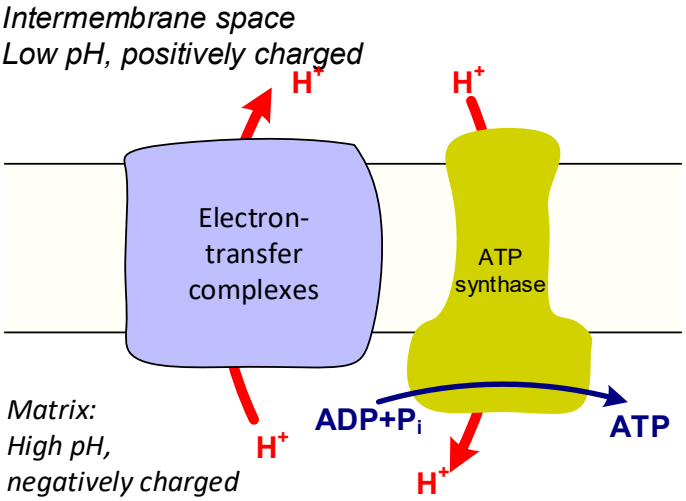


Fig. 6. 17. Proton gradient creates the driving force for ATP synthesis

Catalitic sites of the ATP-synthase reside in spherical complexes that line the matrix side of the mitochondrial inner membrane. This part of the ATP-synthase is referred to as F_1 and it consists of five different polypeptide subunits with a total molecular weight of about 360,000. F_1 is attached loosely toa base-piece of three to five polypeptides (F_0), which is embedded in the inner membrane (fig. 6,18, tab.6.2).

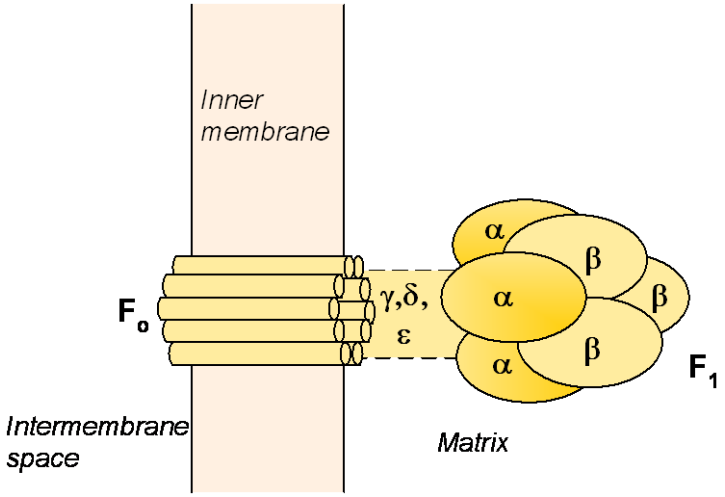


Fig. 6.18. Components of proton-conducting ATP-synthase. The F_1 headpiece includes three α and three β subunits one copy each of three other subunits (γ, δ and ε. F_0 includes a cluster of 9-12 copies of a small peptide, which appears to form a transmembrane channel for protons.

Table 6.2. Components of Mitochondrial Proton-Translocating synthase (F_0F_1-ATPase)

Component	Subunit composition	Functions
F_1	$\alpha_3\beta_3\gamma\delta\varepsilon$	β contains ATP synthase site; δ forms the gate coupling the F_0 proton channel with F_1
F_0	4-5 types of subunit including 6-10 copies of DCCD*-binding proteolipid	DCCD-binding proteolipid oligomer forms the proton chanel
Stalk	One copy each of OSCP** and F_6***	Required to bind F_0 to F_1
Associated polypeptides	IF_1 F_B	Inhibits ATP hydrolysis; bind to the F_1 β

*DCCD – dicycloheylcarbodiimide; **OSCP – oligomicyn-sensitivity-conferring protein; ***-F_6 – coupling factor 6

F_0 appears to contain the proton-conducting channel of the ATP-synthase. If F_0 alone is incorporated into liposomes, it makes the membranes leaky to protons. The multiple copies of one of the small subunits of F_0 may assemble to form a tubular channel across the membrane.

Proton movements do not have much effect on the equilibrium constant for the formation of ATP from bound ADP and P_i on synthase. Instead, they affect the release of ATP from the enzyme. The nucleotides evidently are bound in an environment that favors formation of ATP, and proton movements charge the enzyme's conformation in such a way that the ATP is released.

MEDICAL IMPORTANCE

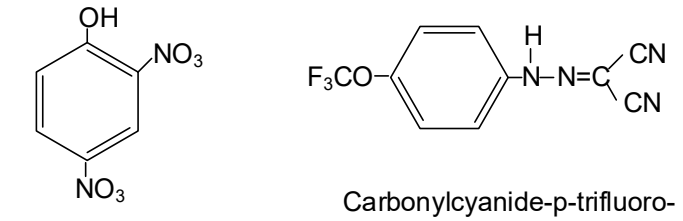
Thirteen of the approximately 120 polypeptides required for oxidative phosphorylation are coded for by mtDNA and synthesized in mitochondria, whereas the remaining mitochondrial proteins are synthesized in the cytosol and transported into mitochondria. Defects in oxidative phosphorylation are more likely a result of alterations in mtDNA, which has a mutation rate about ten times greater than that of nuclear DNA. Tissues with the greatest ATP requirement (for example, central nervous system, skeletal and heart muscle, kidney, and liver) are most affected by defects in oxidative phosphorylation. Mutations in mtDNA are responsible for several diseases, including some cases of ***mitochondrial myopathies****, and* ***Leber hereditary optic neuropathy****, a disease in which bilateral loss of central vision occurs as a result of neuroretinal degeneration, including damage to the optic nerve. The mtDNA is maternally inherited because mitochondria from the sperm cell do not enter the fertilized egg.*

There is a class of phosphorylation inhibitors functions by blocking the ATP-synthase enzyme directly. Inhibitors of this type include the antibiotic **oligomycin**. This drug binds to the F_o domain of ATP synthase, closing the H^+ channel, preventing reentry of protons into the mitochondrial matrix, and thus preventing phosphorylation of ADP to ATP. Because the pH and electrical gradients cannot be dissipated in the presence of this drug, electron transport stops because of the difficulty of pumping any more protons against the steep gradients. This dependency of cellular respiration on the ability to phosphorylate ADP to ATP is known as **respiratory control,** and is the consequence of the tight coupling of these processes. Electron transport and phosphorylation are, therefore, again shown to be tightly coupled processes. Inhibition of one process inhibits the other. If mitochondria are treated with oligomycin, ADP no longer is able to increase the rate of respiration. Oligomycin does not block the stimulation of respiration cause by an uncoupler, emonstrating that the two types of inhibitors of phosphorylation act by different mechanisms.

6.5. Uncouplers of electron transport and oxidative phosphorylation in a respiratory chain of mitochondria

Respiration and phosphorylation usually are tightly coupled process; if phosphorylation stops, so, does respiration and vice versa. As an example, respiration slows down greatly if mitochondria run out of ADP. Addition of a small amount of ADP in the presence of an oxidizable substrate and excess P_i causes a brief period of brisk respiration that can be repeated by adding more ADP. Experiments of this type provide one means for estimating P:O ratios. The P:O ratio is proportional to the amount of ADP consumed to reduce one oxygen of O_2 to one oxygen of water.

The tight coupling of respiration and phosphorylation can be disrupted by molecules known as **uncouplers**. Uncouplers include a diverse group of molecules structurally, but they are all lipophilic weak acids. Such uncouplers, like **2,4-dinitrophenol and carbonylcyanide-p-trifluorometho-xyphenylhydrazone (CCCP)** increases permeability of mitochondrial membrane to H^+. They are proton-transporting ionophores (fig.6.19).



2,4-Dinitrophenol — Carbonylcyanide-p-trifluoro-methoxyphenylhydrazone

Fig. 6.19. Chemichal structures of the most common ionoforic uncouplers

If an uncoupler is added to mitochondria in the presence of an oxidizable substrate, O_2 uptake commences immediately and continues until essentially all of the O_2 in the solution is used up. This happens even in the absence of ADP or P_i (the free energy released in the electron-transfer reactions).

If an uncoupler somehow caused the breakdown of an intermediate form of an electron carrier, the electron carrier would be set free and electron transport to O_2 could continue. However, something evidently is different about oxidative phosphorylation compared with the substrate-level phosphorylation catalyzed by 3-phosphoglyceraldehydedehydrogenase, hence uncouplers have no effect on the latter reaction, nor do they affect other soluble enzymes that make or use ATP. On the other hand, a molecule that acts as an uncoupler at any one of the three coupling sites of oxidative phosphorylation invariably has a similar effect at the other two sites. This suggests that uncouplers cause the breakdown of something that is generated at all three sites.

Physiological uncouplers: Certain physiological substances which act as uncouplers at higher concentration have been identified. These include **thermogenin, thyroxine and long chain free fatty acids.** Uncoupling of respiration from oxidative phosphorylation under natural conditions assumes biological significance. The maintenance of body temperature is particularly important in hairless animals, hibernating animals and the animals adapted to cold. These animals possess a specialized tissue called brown adipose tissue in the upper back and neck portions. The mitochondria of brown adipose tissue are rich in electron carriers and are specialized to carry out an oxidation uncoupled from phosphorylation. This causes liberation of heat when fat is oxidized in the brown adipose tissue. Brown adipose tissue may be considered as a site of non-shivering thermogenesis.

The presence of active brown adioose tissue in certain individuals is believed to protect them from becoming obese. The excess calories consumed by these people are burnt and Iibirated as heat, instead of being stored as fat. **Thermogenin (or uncoupling protein, UCP)** is a natural uncoupler located in the inner mitochondrial membrane of brown adipose tissue. lt acts like an uncoupler, blocks the formation of ATP, and liberates heat.

6.6. Free radicals and mechanisms of their production and inactivation

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals. Free radicals may have positive, negative or zero charge, i.e., they have an unpaired electron on an open shell configuration, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur until subsequent free radicals are deactivated. Thousands of free radical reactions can occur within seconds of the initial reaction.

The formation of radicals may involve breaking of covalent bonds homolytically. Radicals that require more energy to form are less stable than those requiring less energy. Homolytic bond cleavage most often happens between two atoms of similar electronegativity. In organic chemistry this is often the O-O bond or O-N bonds. Most species are electrically neutral but radical ions do exist. Radicals may also be formed by single electron oxidation or reduction of an atom or molecule. An example is the production of superoxide by the electron transport chain. Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline and brain dysfunction. Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases.

Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. These include oxygen in its triplet state ($^3O_2$) or singlet state ($^1O_2$), superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($^{\cdot}OH$), peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), alkoxyl radical ($RO^{\cdot}$) and the peroxyl radical ($RO^{\cdot}{}_2$).

All ROS are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, resulting in cellular damage. ROS form as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling. However, during times of environmental stress (e.g. UV or heat exposure) ROS levels can increase drastically, which can result in significant damage to cell structures. This accumulates into a situation known as **oxidative stress**. ROS are also generated by exogenous sources such as ionizing radiation. Exogenous ROS can be produced from pollutants, food additives, tobacco, smoke, drugs, xenobiotics and modern day lifestyles related stress is also a contributing factor to excess free radicals circulating in our body.

In ETC incomplete processing of oxygen and /or release of free electrons results in the production of oxygen free radicals (fig.6.20). Mitochondria constantly metabolize oxygen thereby producing ROS as a byproduct. These organelles have their own ROS

scavenging mechanisms that are required for cell survival. It has been shown, however, that mitochondria produce ROS at a rate higher than their scavenging capacity, resulting in the incomplete metabolism of approximately 1–3% of the consumed oxygen. The byproducts of incomplete oxygen metabolism are superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH). The formation of superoxide occurs via the transfer of a free electron to molecular oxygen. This reaction occurs at specific sites of the electron transport chain, which resides in the inner mitochondrial membran). Complexes I and III produce most of the superoxide, which is then scavenged by the mitochondrial enzyme manganese superoxide dismutase (MnSOD) to produce H_2O_2.

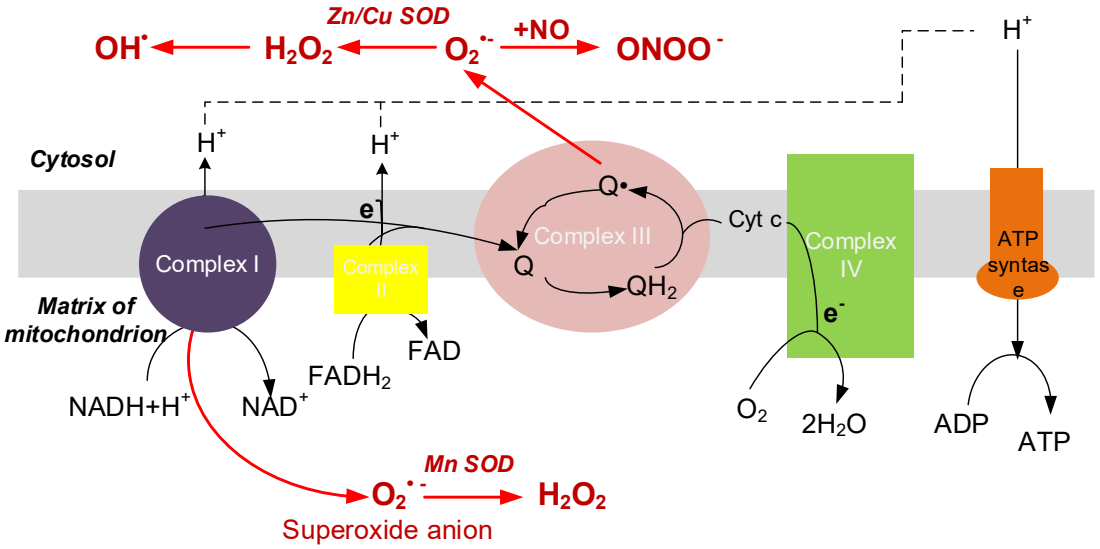


Fig. 6.20. Generation of reactive oxygen species in electron transport chain

Antioxidant means "against oxidation. Antioxidants are effective because they are willing to give up their own electrons to free radicals. When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken. After donating an electron an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive.

Antioxidants have been classified based on their occurrence, mode of action, kinetics and solubility.

I. Based on their occurrence. There are two types:

1. **Natural antioxidants.** They are the chain breaking antioxidants which react with radicals and convert them into more stable products. Antioxidants of this group are mainly phenolic in structures and include the following: **antioxidants minerals** - these are co factor of antioxidants enzymes, e.g. selenium, copper, iron, zinc and manganese; **antioxidants vitamins** – These are needed for most body metabolic functions includes - vitamin C, E; **phytochemicals** - phenolic compounds that are neither vitamins nor minerals e.g flavonoids, catechins, carotenoids, beta carotene, lycopene, garlic and curcumin and derivatives.

2. Synthetic antioxidants. These are phenolic compounds that perform the function of capturing free radicals and stopping the chain reactions.

II. Based on their mode of action. There are two types:

1. **Primary antioxidants.** These interrupt the primary oxidation cycle by removing the propagating radicals. Such compounds are also called chain breaking antioxidants. They have reactive OH or NH groups.

2. Secondary antioxidants. These compounds are also called preventative antioxidants as they interrupt the oxidative cycle by preventing or inhibiting the formation of free radicals. Phosphites or phosphonites, organic sulphur containing compounds and dithiophosphonates are widely used to achieve this, acting as peroxide decomposers.

REVIEW TEST:

№	MCQs	Answers and explanations
1.	**The production of thyroid hormones is stimulated under thyrotoxicosis. It leads to body weigh loss, tachycardia, and rise of psychic irritability. Choose the biochemical mechanism by which thyroid hormones affect the tissue bioenergetics from the listed below:** A. Blockage of mitochondrial respiratory chain B. Uncoupling of oxidation and oxidative phosphorylation C. Activation of substrate level phosphorylation D. Blockage of substrate level phosphorylation E. Activation of oxidation and oxidative phosphorylation	**The answer is B.** In the electron transpot chain some protons reenter the mitochondrial matrix through uncoupling proteins (UCP) without ATP synthesis. These protons are released as heat. Thyroid hormones regulate mitochondrial UCP causing the uncoupling of oxidation and ATP phosphorylation. That is why thyroid hormones are essential for thermogenesis.
2.	Barbiturates are used as soporifics. These substances, similar to rotenone, are tissue respiration inhibitors. What complex level do these compounds suppress respiratory chain at? A. Cytochrome oxidase B. Cytochrome C reductase C. Adenosine triphosphate synthetase D. NADH-coenzyme Q reductase E. Succinate dehydrogenase	**The answer is D.** The inhibitors of tissue respiration (electron transport chain) are substances that bind to some of the components of the ETC blocking its ability to change in a reversible form from an oxidized state to a reduced state. Barbiturates and **r**otenone block the electron transport chain between NADH dehydrogenase (Complex I) and CoQ. Consequently, they prevent the utilization of NADH as a substrate. On the contrary, electron flow resulting from the oxidation of Complex II is not affected, because these electrons enter through QH_2, beyond the block.
3.	**Cyanide is a poison that causes instant death of the organism. What enzymes found in mitochondria are affected by cyanide?** A. Cytochromeoxidase (aa_3) B. Flavin enzymes C. Cytochrome B_5 D. NAD^+-dependentdehydrogenase	**The answer is A.** Intoxication by cyanide can be seen relatively frequent in patients with smoke inhalation from residential or industrial fires. Also in persons related professionally with cyanide or derivatives in certain industries. Intentional poisoning can be seen in suicidal persons with access to cyanide compounds. Cyanide affects practically all metalloenzymes, but its principal toxicity derives from

	E. CytochromeP-450	the binding to the Fe^{3+} in the heme groups in cytochromeoxidase, inhibiting the functioning of the electron transport chain. As a consequence, redox reactions in the respiratory chain will stop, energy will not be released, proton pumps will not function, so they will not return through Complex V, and the production of ATP will cease.
4.	**An 8-year-old boy is seen by anophthalmologist for vision difficulties, and the physician notices a slowing of the boy's eye movements. The ophthalmologist finds ophthalmoplegia and pigmentary retinopathy and suspects the child has Kearns-Sayre syndrome. Assuming that the defect in this disorder is due to a mutationin complex II of the ETC, electron transfer from which substrate would be impaired?** A. Malate B. α-Ketoglutarate C. Isocitrate D. Succinate E. Pyruvate	**The answer is D.** Succinate feeds electrons into complex II, which, in this case, would be impaired. The other substrates listed, malate, α-ketoglutarate, isocitrate, and pyruvate, all feed their electrons via NADH through complex I. Malate dehydrogenase, α -ketoglutaratedehydrogenase, isocitratedehydrogenase, and pyruvate dehydrogenase all generate NADH during the course of the reactions that they catalyze.
5.	**Researches of the latest decades established that immediate "executors"of cell apoptosis are special enzymes called caspases. Generation of one of them proceeds with participation of cytochrome c. What is its function in a normal cell?** A. Enzyme of respiratory chain of electron transport B. Enzyme of tricarboxylic acid cycle C. Enzyme of beta-oxidation of fatty acids D. Component of H+ATP system E. Component of pyruvate-dehydrogenase system	**The answer is A.** Cytochrome c is a heme protein that is localized in the inner mitochondrial membrane where it functions to transfer electrons between complex III and complex IV of the respiratory chain. The heme group of cytochrome c is a highly-conjugated ring system surrounding an iron ion, which readily interconverts between its primary oxidation states. The iron ion interconverts between the Fe^{2+} (reduced) and Fe^{3+} (oxidized) states in electron-transfer processes or between the Fe^{2+} (reduced) and Fe^{3+} (formal, oxidized) states in oxidative processes. Cytochrome c is a key protein that initiates the intrinsic apoptosis pathway.
6.	**The process of metabolism in the human body produces active forms of oxygen, including superoxide anion radical. This anion is inactivated by the following enzyme:** A. Glutathioneperoxidase B. Catalase C. Peroxidase D. Superoxide dismutase E. Glutathionereductase	**The answer is D.** Superoxide dismutase (SOD) is an enzyme that catalyze the dismutation of superoxide radicals (O_2^-) to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2), providing cellular defense against reactive oxygen species (ROS).

7.	**Those organisms which in the process of evolution failed to develop protection from H_2O_2 can exist only in anaerobic conditions. Which of the following enzymes can break hydrogen peroxide down?** A. Oxygenase and hydroxylase B. Peroxidase and catalase C. Cytochrome oxidase, cytochrome b_5 D. Oxygenase and catalase E. Flavin-dependent oxidase	**The answer is B.** Peroxidase and catalase are enzymes able to use hydrogen peroxide (H_2O_2) as a substrate. Catalase generates water and Oxygen, while peroxidase generates water and activated donor molecule. Both enzymes are very important members of enzymatic antioxidant system.
8.	**A scientistis studying oxidative phosphorylation in intact, carefully isolated mitochondria. Upon adding an oxidizable substrate, such as pyruvate, a constant rate of oxygen utilization is noted. The scientist then adds a compound that greatly enhances the rate of oxygen consumption. This compound is most likely which one of the following?** A. Rotenone B. Carbonmonoxide C. Antimycin D. Cyanide E. Dinitrophenol	**The answer is E.** An uncoupler was added to the mitochondria to greatly increase the rate of oxygen consumption. Dinitrophenol will allow free proton diffusion across the inner mitochondrial membrane, thereby dissipating the proton gradient andpreventing ATP synthesis. Without an existing proton gradient to "push" against, electron flow through the electron transport chain is accelerated, resulting in enhanced oxygen consumption. Rotenone inhibits electron transfer from complex I to coenzymeQ. Carbon monoxide and cyanide block complex IV from accepting electrons. Antimycin blocks electron flow from complex III. Since electron flow is blocked using rotenone, carbonmonoxide, cyanide, orantimycin, oxygen uptake will cease.
9.	**A 53-year-old, previously successful man recently lost his job and is under investigation for racketeering. His wife returns home to find him slumped over the steering wheel of his idling carin the closed garage. He is unresponsive and has a cherry color to his lips and cheeks. Which of the following is inhibited by the carbon monoxide in the car's exhaust fumes?** A. Complex I of the ETC B. Cytochromeoxidase C. The ATP-ADPantiporter D. The F_0 componentof the F_0-F_1 ATPase E. The F_1 component of the the F_0-F_1 ATPase	**The answer is B.** Carbon monoxide intoxication causes impaired oxygen delivery and utilization at the cellular level. The affinity of Hb for CO is almost 300 times higher than for Oxygen. The affinity of respiratory chain components for CO is lower than for Oxygen, but since the clinical status does not correlate very well with the carboxyhemoglobin levels, it is considered that the inhibition of cytochromeoxidase by CO also plays a role in CO intoxication. CO binds to the reduced form of iron in heme groups (Fe^{++}) in cytochromeoxidase
10	**A patient who was previously ill with mastectomy as a result of breast cancer was prescribed radiation therapy. What vitamin preparation has marked radioprotective action caused by antioxidant activity?** A. Tocopherol acetate	**The answer is A.** Tocopherol (vitamin E) belongs to a class of natural phenolic antioxidants which can inhibit lipid autoxidation by scavenging free radicals and by reacting with singlet oxygen.

	B. Ergocalciferol C. Thiamine chloride D. Riboflavin E. Folic acid	

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7. DIGESTION OF CARBOHYDRATES. STUDY OF GLYCOLYSIS – ANAEROBIC OXIDATION OF CARBOHYDRATES

OBJECTIVES after studying this chapter, you should be able to:

- *Describe mechanism of carbohydrates digestion abn absobtion in norm and pathology*
- *Interpret biochemical pathways of intracellular oxidation of glucose in anaerobic conditions*
- *Analyze peculiarities of glycolytic reactions, which occur with involvement of ATP*
- *Analyze peculiarities of substrate phosphorylation and production of ATP in this way*
- *Interpret role of coenzymes and enzymes in glycolytic reactions*
- *Analyze regulatory mechanisms of glucose oxidation in anaerobic conditions*

1.1. Digestion of carbohydrates.

1.1.1. The principal dietary carbohydrates.

Carbohydrates wich include glucose are the most abundant biomolecules on Earth. **Carbohydrates are polyhydroxy- aldehydes or ketones**, or substances that yield such compounds on hydrolysis. Many, but not all, carbohydrates have the empirical formula $(CH_2O)n$; some also contain nitrogen, phosphorus, or sulfur.

There are three major classes of carbohydrates: **monosaccharides, oligosaccharides, and polysaccharides**. **Monosaccharides**, or simple sugars, consist of a single polyhydroxy aldehyde or ketone unit. The most abundant monosaccharide in nature is the six-carbon sugar **D-glucose (fig 7.1)**, sometimes referred to as dextrose. Monosaccharides of four or more carbons tend to have cyclic structures.

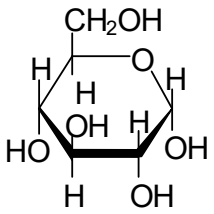
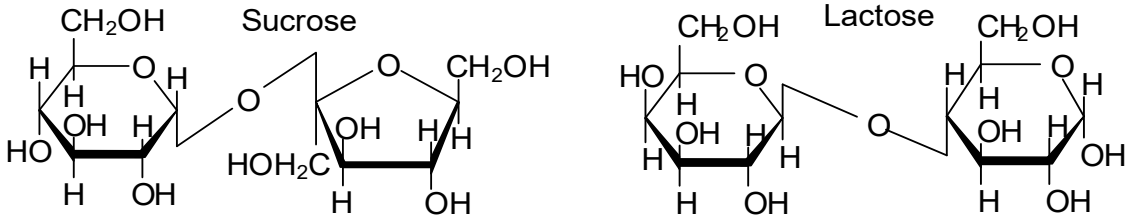


Fig. 7.1. D-glucose

Oligosaccharides consist of short chains of monosaccharide units, or residues, joined by characteristic linkages called glycosidic bonds. The most abundant are the disaccharides, with two monosaccharide units, e.g sucrose which consists of the six-carbon sugars D-glucose and D-fructose and lactose which is made of D-glucose and D-galactose (fig. 7.2).



Fig, 7.2. Predominant dietary oligosussurides: sucrose and lactose

The polysaccharides are sugar polyrmers containing more than 20 or so monosaccharide units; some have hundreds or thousands of units. Some polysaccharides, such as cellulose, are linear chains; others, such as glycogen, are branched. Both glycogen

and cellulose consist of recurring units of D-glucose, but they differ in the type of glycosidic linkage and consequently have strikingly different properties and biological roles.

More than 60 % of our foods are carbohydrates. They serve as a major energy source of human diet. The principal dietary carbohydrates are polysaccharides (starch, glycogen), disaccharides (lactose, sucrose) and, to a minor extent, monosaccharides (glucose, fructose). Mean intakes of total carbohydrate in adults and children aged 4 years and over are 200-240 g/day, and 150 g/day in children aged 1½- 3 years. Mean intakes of total carbohydrate as a percentage of total dietary energy are 51-52 % in children and 46 % in adults. Cereals and cereal products are the main source of total carbohydrate intake in all age groups, providing around 45 % of intake. The other food groups that made a substantial contribution to total carbohydrate intake were potatoes, fruit, drinks (soft and alcoholic), and table sugar, preserves and confectionery; each providing up to 10% of intake.

7.1.2. Mechanisms of carbohydrates digestion in digestive tube

The digestion of carbohydrates is by hydrolysis to liberate oligosaccharides, then free mono- and disaccharides. The increase in blood glucose after a test dose of a carbohydrate compared with that after an equivalent amount of glucose (as glucose or from a reference starchy food) is known as the **glycemic index.** Glucose and galactose have an index of 1 (or 100 %), as do lactose, maltose, isomaltose, and trehalose, which give rise to these monosaccharides on hydrolysis. Fructose and the sugar alcohols are absorbed less rapidly and have a lower glycemic index, as does sucrose. The glycemic index of starch varies between near 1 (or 100%) and near 0 as a result of variable rates of hydrolysis, and that of nonstarch polysaccharides is 0. Foods that have a low glycemic index are considered to be more beneficial since they cause less fluctuation in insulin secretion. Resistant starch and nonstarch polysaccharides provide substrates for bacterial fermentation in the large intestine, and the resultant butyrate and other short chain fatty acids provide a significant source of fuel for intestinal enterocytes. There is evidence that butyrate also has antiproliferative activity, and so provides protection against colorectal cancer.

Dietary carbohydrate are digested in the mouth and small intestinal lumen by enzymes known as ***glycoside hydrolases (glycosidases)*** that hydrolyze glycosidic bonds. For most humans, starch is the major source of carbohydrates in the diet. Dietary glycogen has almost the same structure as starch, and its digestion proceeds by the same pathway. During mastication, salivary ***α-amylase*** acts on dietary starch, hydrolyzing α(1→4) bonds of linear parts of starch called **amylose**. Branched parts of starch contain α(1→6) bonds and are called **amylopectin**. Salyvary α-amylase is it able to hydrolyze α(1→6) bonds. Thus the products of starch digestion a mixture of short, branched and unbranched oligosaccharides kown as **dextrins.** Disaccharides (sucrose and lactose) also present in food are resistant to salivary amylase action. In stomach, gastric juise because the its high acidity denatures salivary amylase and anables the carbohydrate digestion.

In the intestine bicarbonate, secreted by the pancreas, neutralizes the hydrochloric acid of gastric juice, creating the optimal range for the action of the intestinal enzymes. Pancerase also secreates into the small intestine a second form of ***a-amylase***, which continues the breakdown of dextrines. Pancreatic ***a-amylase*** yields mainly **maltose** and

maltotriose (the di- and trisaccharides composed of glucose) and oligosaccharides called **limit dextrins**, fragments of amylopectin containing branch points.

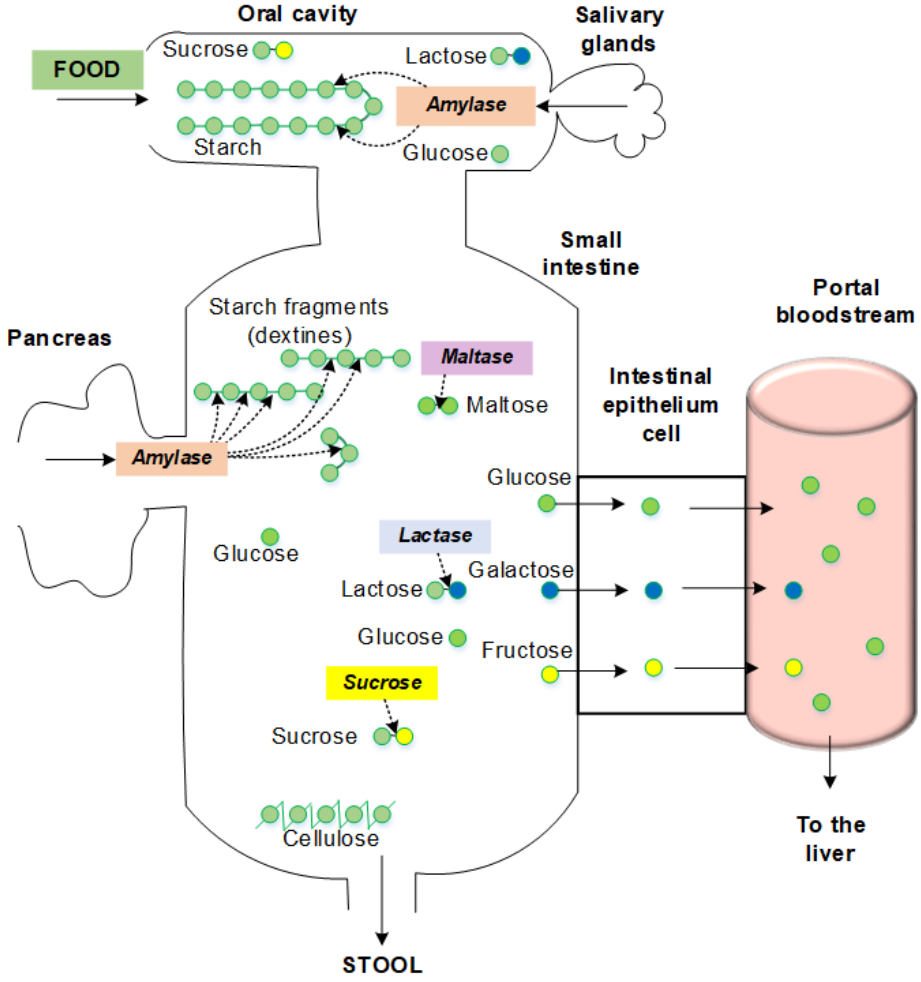


Fig. 7.3. Digestion of carbohydrates

Potentially confusing question **concerning** ***Dextrins; Dextrans; Dextrose*** – *The first two are polysaccharides composed of glucose. Dextrins are the breakdown products of starch; dextrans are gels produced by bacteria from glucose. Dextrose is glucose in solution (dextrorotatory) used in medical practice.*

Maltose and dextrins are degraded by enzymes of the intestinal brush border (the fingerlike microvilli of intestinal epithelial cells, which greatly increase the area of the intestinal surface).

Disaccharides must be hydrolyzed to monosaccharides before entering cells. Intestinal disaccharides and dextrins are hydrolyzed by enzymes attached to the outer surface of the intestinal epithelial cells:

$$\text{Dextrin} + n H_2O \xrightarrow[\textbf{dextrinase}]{} n \text{ D-glucose}$$

$$\text{Maltose} + H_2O \xrightarrow[\textbf{maltase}]{} 2 \text{ D-glucose}$$

$$\text{Lactose} + H_2O \xrightarrow[\textbf{lactase}]{} \text{D-galactose} + \text{D-glucose}$$

$$\text{Sucrose} + H_2O \xrightarrow[\text{sucrase}]{} \text{D-fructose} + \text{D-glucose}$$

$$\text{Trehalose} + H_2O \xrightarrow[\text{trehalase}]{} 2\ \text{D-glucose}$$

MEDICAL IMPORTANCE

Lactose intolerance, *common among adults of most human populations except those originating in Northern Europe and some parts of Africa, is due to the disappearance after childhood of most or all of the* ***lactase activity*** *of the intestinal cells. Lactose cannot be completely digested and absorbed in the small intestine and passes into the large intestine, where bacteria convert it to toxic products that cause abdominal cramps and diarrhea. The problem is further complicated because undigested lactose and its metabolites increase the osmolarity of the intestinal contents, favoring the retention of water in the intestine. In most parts of the world where lactose intolerance is prevalent, milk is not used as a food by adults, although milk products predigested with lactase are commercially available in some countries. In certain human disorders, several or all of the* ***intestinal disaccharidases*** *are missing. In these cases, the digestive disturbances triggered by dietary disaccharides can sometimes be minimized by a controlled diet.*

Isomaltase and sucrase deficiency *occurs in childhood. Isomaltase and sucrase are deficient.* ***Disaccharіduria*** *is characterized by excretion of disaccharides in urine. It is due to deficiency of disaccharidases.* ***Malabsorption syndromes*** *of monosaccharides are due to defective transporter. Due to defective transporter absorption of monosaccharides is impaired.*

Acarbose, *an a-glucosidase inhibitor, works in the intestine, slowing down digestion of carbohydrates and lengthening the time ittakes for carbohydrates to be converted to glucose, which facilitates better postdigestive blood glucose control.*

1.1.2. Mechanisms of dietary carbohydrates absorption

The duodenum and upper jejunum absorb the bulk of the dietary monosaccharides. Different sugars have different mechanisms of absorption. Glucose and galactose are absorbed by a **sodium-dependent process**. They are carried by the same transport protein (SGLT 1) and compete with each other for intestinal absorption. Fructose uptake requires a sodium-independent monosaccharide transporter (GLUT-5) for its absorption. All three monosaccharides are transported from the intestinal mucosal cell into the portal circulation by yet another transporter, GLUT-2. Major types of glucose transporters are show in table 7.1.

Table. 7.1. Major Glucose Transporters

	Tissue Location	**Functions**
Facilitative bidirectional transporters		
GLUT 1	Brain, kidney, colon, placenta, erythrocytes	Glucose uptake
GLUT 2	Liver, pancreatic β cell, small intestine, kidney	Rapid uptake or release of glucose
GLUT 3	Brain, kidney, placenta	Glucose uptake
GLUT 4	Heart and skeletal muscle, adipose tissue	Insulin-stimulated glucose uptake
GLUT 5	Small intestine	Absorption of fructose
Sodium-dependent unidirectional transporter		
SGLT 1	Small intestine and kidney	Active uptake of glucose against a concentration gradient

GLUT proteins are encoded by the SLC2 genes. These proteins are members of the major facilitator superfamily (MFS) of membrane transporters. These transporters are uniporters. The human GLUT proteins are comprised of about 500 amino acid residues. They are predicted to possess 12 transmembrane-spanning α-helices and a single N-linked oligosaccharide. The cytoplasmic domain contains a short N-terminal segment, a large intracellular loop between transmembrane domains 6 and 7, and a large C-terminal segment. The sequences among members of family are 14–63% identical and 30–79% conservative. The fact that the transmembrane domain primary structure is largely conserved suggests that the glucose channel is basically identical in structure among the members of this GLUT family.

The overall process of carbohydrate digestion and absorption is so efficient in healthy individuals that ordinarily all digestible dietary carbohydrate is absorbed by the time the ingested material reaches the lower jejunum. However, because it is mono saccharides that are absorbed, any defect in a specific disaccharidase activity of the intestinal mucosa causes the passage of undigested carbohydrate into the large intestine. As a consequence of the presence of this osmotically active material, water is drawn from the mucosa into the large intestine, causing osmotic diarrhea. This is reinforced by the bacterial fermentation of the remaining carbohydrate to two- and three-carbon compounds (which are also osmotically active) plus large volumes of CO_2 and H_2 gas, causing abdominal cramps, diarrhea, and flatulence.

1.1.3. Transport of glucose into the cell

Glucose cannot diffuse directly into cells, but enters by one of two transport mechanisms: a Na^+-independent, facilitated diffusion transport system or a Na^+-monosaccharide cotransporter system.

Na^+-independent facilitated diffusion transport. This system is mediated by a family of 14 glucose transporters **(GLUT-1 to GLUT-14)** in cell membranes. These transporters exist in the membrane in two conformational states (Fig. 7.4). Extra cellular glucose binds to the transporter, which then alters its conformation, transporting glucose across the cell membrane.

The glucose transporters display a tissue-specific pattern of expression. For example, **GLUT-3** is the primary glucose transporter in **neurons**. **GLUT-1** is abundant in **erythrocytes and blood brain barrier**, but is low in adult muscle, whereas **GLUT-4** is abundant in **adipose tissue and skeletal muscle**. The other GLUT isoforms also have tissue-specific distributions.

Specialized functions of GLUT isoforms: In facilitated diffusion, glucose movement follows a concentration gradient, that is, from a high glucose concentration to a lower one. For example, GLUT-1, GLUT -3, and GLUT-4 are primarily involved in glucose

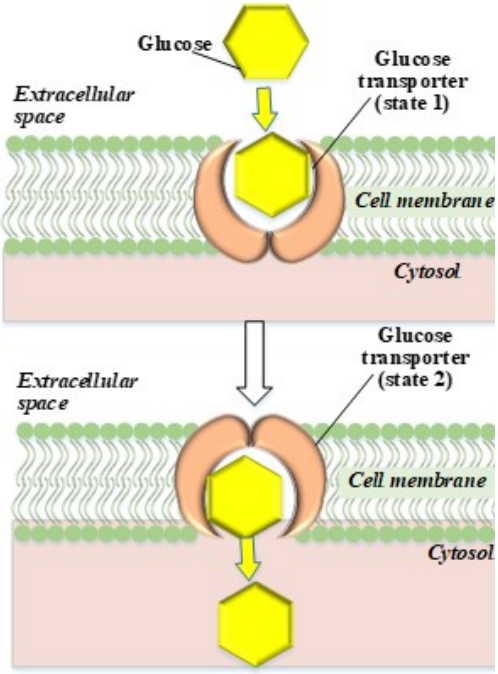


Fig. 7.4. Schematic representation of the facilitated transport of glucose

uptake from the blood. In contrast, GLUT-2, which is found in the liver and kidney, can either transport glucose into these cells when blood glucose levels are high, or transport glucose from these cells when blood glucose levels are low (for example, during fasting). **GLUT-5** is unusual in that it is the primary transporter for **fructose** (instead of glucose) in the small intestine and the testes.

Na^+-monosaccharide cotransporter system This is an energy-requiring process that transports glucose "against" a concentration gradient – that is, from low glucose concentrations outside the cell to higher concentrations within the cell. This system is a carrier-mediated process in which the movement of glucose is coupled to the concentration gradient of Na^+, which is transported into the cell at the same time. The carrier is a sodium-dependent glucose transporter or SGLT. This type of transport occurs in the epithelial cells of the intestine as described above and renal tubules.

7.2. Glucose as an important metabolite in carbohydrate metabolism: general scheme of sources and turnover of glucose in the organism.

Thus, **glucose is the central molecule in carbohydrate metabolism** since all the major pathways of carbohydrate metabolism are connected with it or its derivatives. It is relatively rich in potential energy, and thus a good fuel; the complete oxidation of glucose to carbon dioxide and water proceeds with a standard free-energy change of -2,840 kJ/mol. Glucose also can be oxidized in pentose phosphate pathway to producw ribose-5 phosphate and NADPH or in uronic acid pathway. By storing glucose as a high molecular weight polyrmer such as starch or glycogen, a cell can stockpile large quantities of hexose units while maintaining a relatively low cytosolic osmolarity.

Glucose is not only an excellent fuel, it is also a remarkably versatile precursor, capable of supplying a huge array of metabolic intermediates for biosynthetic reactions such as lipids nonessential fatty acids.

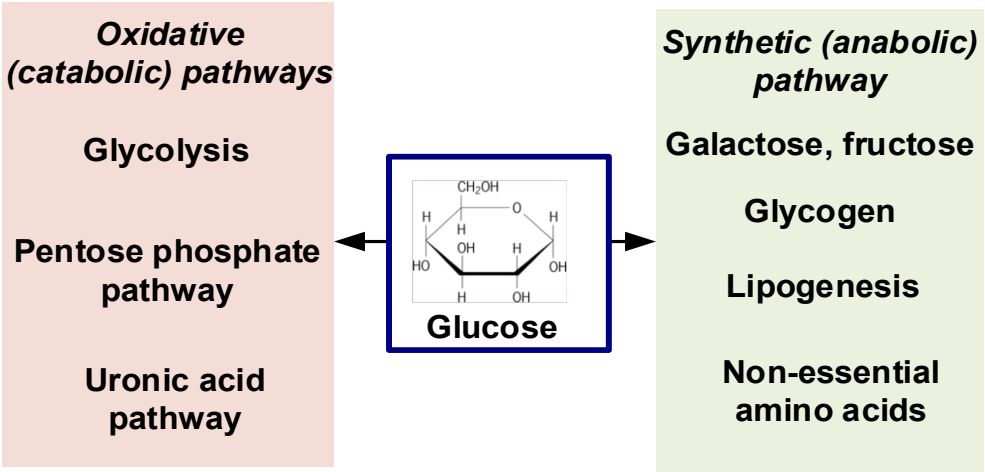


Fig. 7.5 The general scheme of sources and turnover of glucose

Glucose, a sugar molecule, serves as a vital energy source for organisms. Its metabolism involves various pathways and processes that contribute to the production and

utilization of glucose within the body. Here is a general scheme of the sources and turnover of glucose:

- **Dietary intake:** Glucose is obtained through the consumption of carbohydrates in the diet. Carbohydrate-rich foods, such as grains, fruits, and starchy vegetables, are broken down into glucose during digestion.
- **Glycogen breakdown:** Excess glucose from the diet is converted into glycogen and stored in the liver and muscles for future use. When glucose levels drop, glycogen is broken down into glucose through glycogenolysis, providing a quick source of glucose.
- **Gluconeogenesis:** In situations where glucose levels are low, the body can synthesize new glucose molecules through gluconeogenesis. This process occurs primarily in the liver and, to a lesser extent, in the kidneys, where precursors like lactate, amino acids, and glycerol are converted into glucose.
- **Glycolysis:** Glycolysis is a metabolic pathway that occurs in the cytoplasm of cells and breaks down glucose into pyruvate. This process generates a small amount of ATP and NADH.
- **Pyruvate fate:** Pyruvate can follow different pathways based on the metabolic needs of the cell. In the presence of oxygen, pyruvate enters the mitochondria and undergoes further oxidation through the citric acid cycle (also known as the Krebs cycle) and oxidative phosphorylation, producing a significant amount of ATP. If oxygen is limited, pyruvate can undergo fermentation, leading to the production of lactate or other fermentation byproducts.
- **Release of glucose into circulation:** Glucose is released into the bloodstream from the liver to maintain blood glucose levels within a narrow range. This process, called hepatic glucose output, is tightly regulated by hormones like insulin and glucagon.
- **Glucose uptake by cells:** Glucose in the bloodstream is taken up by various tissues, including muscle and adipose tissue, through the action of glucose transporters, such as GLUT4. Inside the cells, glucose can be further metabolized for energy production or stored as glycogen.
- **Glycogen synthesis:** Excess glucose can be converted into glycogen through glycogenesis. This occurs primarily in the liver and muscles and serves as a long-term energy storage form of glucose.

The turnover of glucose is a dynamic process, and its regulation is tightly controlled to meet the energy demands of the body. Hormones, such as insulin, glucagon, and adrenaline, play crucial roles in maintaining glucose homeostasis by regulating glucose uptake, release, and metabolism in different tissues.

7.3. Glycolysis – anaerobic oxidation of glucose

Glycolysis can be defined as the sequence of reactions for the breakdown of **glucose** (6-carbon molecule) to two molecules of **pyruvic acid** (3-carbon molecule) under **aerobic conditions**; or **lactate** under **anaerobic conditions** along with the production of small amount of energy.

All of the glycolytic enzymes are found in **the cytosol**. Glycolysis takes place in all tissues and organs, but it has the physiological importance in:

- tissues with no mitochondria: e.g. mature RBCs.

- tissues with few mitochondria: e.g. testes and leucocytes.
- tissues undergo frequent oxygen lack: skeletal muscles especially during exercise.

7.3.1. The contribution of works of Embden, Meyerhof and Parnas in detection of sequence of enzymatic glycolysis reactions

Many scientists contributed to the complete determination of glycolytic pathway. The elucidation of the glycolytic pathway, the process whereby glucose is converted into pyruvate and ATP, began in 1860 when Louis Pasteur observed that microorganisms were responsible for fermentation. Several years later, in 1897, Eduard Buchner made the significant discovery that cell-free extracts could carry out fermentation. The complete glycolytic pathway was elucidated by 1940 by the combined efforts of several scientists including **Otto Fritz Meyerhof** (1884-1951). Otto Meyerhof determined that glycogen is converted to lactic acid in the absence of oxygen and showed that in the presence of oxygen only a small portion of lactic acid is oxidized and the rest is converted back to glycogen. This discovery of the lactic acid cycle provided the first evidence of the cyclical nature of energy transformation in cells. **Gustav Embden** (1874-1933) conducted studies on carbohydrate metabolism and muscle contraction, and he was the first to discover and link together all the steps involved in the conversion o glycogen to lactic acid. **Jacub Oskar Parnas's** (1884-1949) major work was the study of the mechanisms of carbohydrate metabolism in muscle tissue. Together with Władysław Baranowski, he discovered the process of phosphorolysis. Parnas also made a major contribution to the theoretical analysis of glycolysis. Glycolysis is formally known as the **Embden-Meyerhof-Parnas (EMP) Pathway**

7.3.2. The sequence of reactions in glycolysis

The glycolytic patway can be divided into two phases:

I. Preparatory (an energy investment) phase. This phase is also called **glucose activation phase**. In the preparatory phase of glycolysis, two molecules of ATP are invested and the hexose chain is cleaved into two triose phosphates. During this, phosphorylation of glucose and it's conversion to glyceraldehyde-3-phosphate take place.

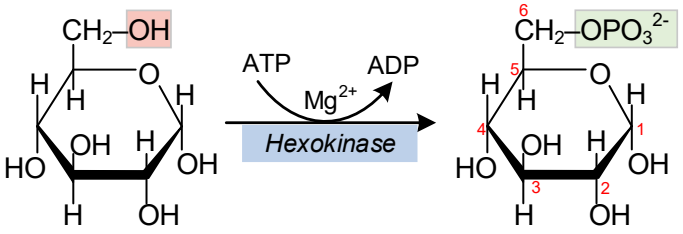
II. Energy payoff phase. This phase is also called **energy extraction phase**. During this phase, conversion of glyceraldehyde-3-phophate to pyruvate and the coupled formation of ATP take place.

I. Preparatory (an energy investment) phase:

1. Uptake and Phosphorylation of Glucose.

Glucose enters the glycolysis pathway by conversion to **glucose-6-phosphate**. The reaction is catalysed by the specific enzyme **glucokinase** in liver cells and by non specific enzyme **hexokinase** extrahepatic tissue. The enzyme splits the ATP into ADP, and the P_i is added onto the glucose. **Hexokinase is a key glycolytic enzyme,** because it catalyses a

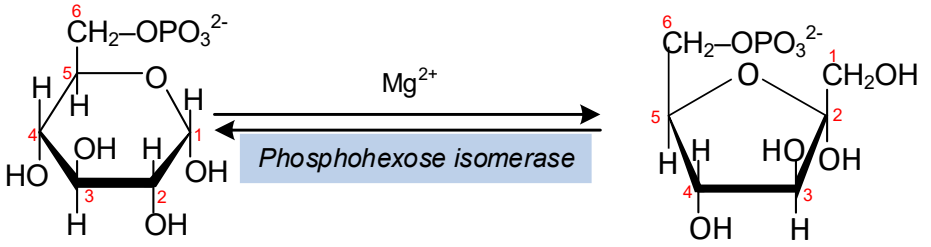
regulatory step in glycolysis that is **irreversible.** Hexokinase, like many other kinases, requires Mg^{2+} for its activity.



Glucose Glucose 6-phosphate

2. Isomerization of Glucose-6-Phsphate to Fructose-6-Phosphate.

Glucose-6-phosphate is isomerised to fructose-6-phosphate by **phosphohexose isomerase**.This reaction involves an aldose-ketose isomerisastion catalysed by phosphohexose isomerase. There is opening of the glucopyranose ring of glucose-6-phosphate to a linear structure which then changes to the furanose ring structure of fructose-6-phosphate.

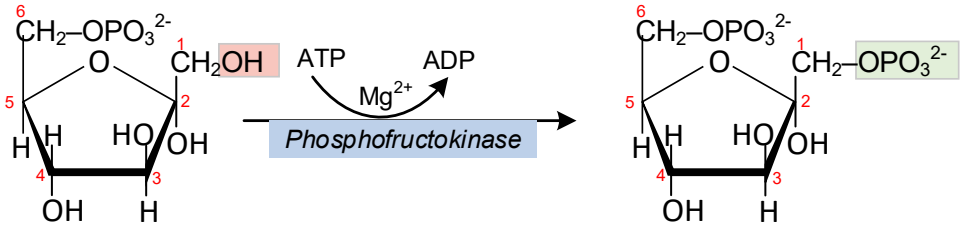


Glucose 6-phosphate Fructose 6-phosphate

3. Phosphorylation of F-6-P to Fructose 1,6-Biphosphate.

Fructose-6-phosphate is further phosphorylated to fructose 1,6-bisphosphate.

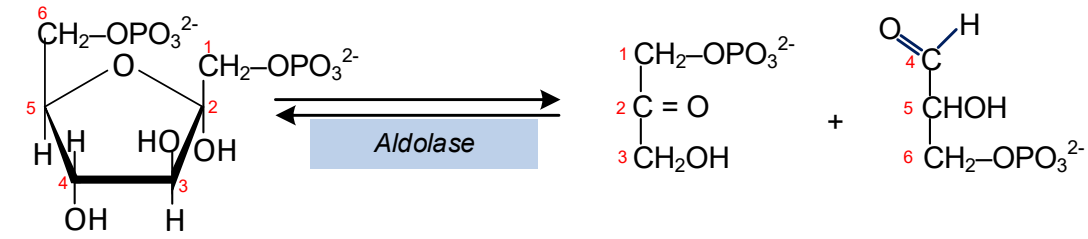
The enzyme is **phosphofructokinase**. It catalyses the transfer of a phosphate group from ATP to fructose-6-phosphate. **The reaction is irreversible.** One ATP is utilised for phosphorylation. Phosphofructokinase is the **key enzyme** in glycolysis which regulates breakdown of glucose.



Fructose 6-phosphate Fructose 1,6-bisphosphate

4. ***Cleavage of Fructose 1,6-Biphosphate.***

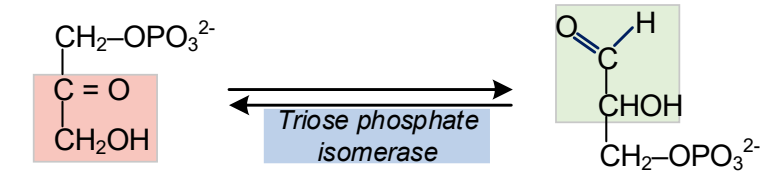
The 6 carbon fructose-1,6-bisphosphate is cleaved into two 3 carbon units; one **glyceraldehyde-3-phosphate (GAP)** and another molecule of **dihydroxy acetone phosphate (DHAP).** The enzyme which catalyses the reaction is **aldolase**. Since the backward reaction is an aldol condensation, the enzyme is called aldolase. The reaction is reversible.



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

1. **Interconversion of the Triose Phosphates.**

GAP is on the direct pathway of glycolysis, whereas DHAP is not. Hence **Triose-phosphate isomerase** converts DHAP into GAP useful for generating ATP. Thus net result is that glucose is now cleaved into 2 molecules of glyceraldehyde-3-phosphate. This reaction is rapid and reversible.



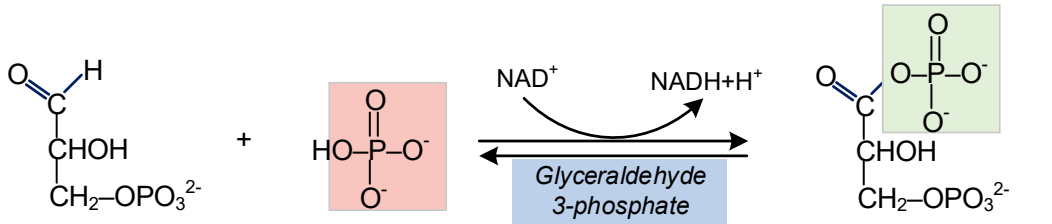
Dihydroxyacetone phosphate — Glyceraldehyde 3-phosphate

II. Energy payoff phase.

6. **Oxidative phosphorylation of GAP to 1,3-Bisphosphoglycerate**

The first step in the payoff phase is the oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate. This reaction is catalyzed by **glyceraldehyde 3-phosphate dehydrogenase**. It is the energy-yielding reaction. Reactions of this type in which an aldehyde group is oxidised to an acid are accompanied by liberation of large amounts of potentially useful energy. During this reaction, NAD^+ is reduced to NADH.

This is a reversible reaction.

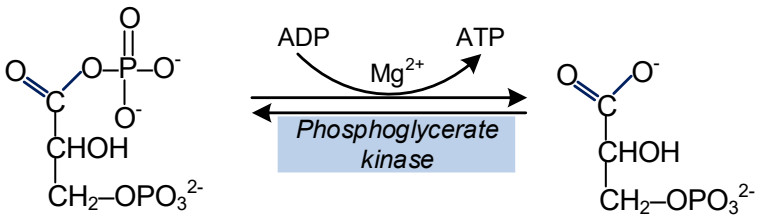


Glyceraldehyde 3-phosphate — Inorganic phosphate — 1,3-Bisphosphoglycerate

7. Conversion of 1,3-Biphosphoglycerate to 3-Phosphoglycerate.

The enzyme ***phosphoglycerate kinase*** transfers the high-energy phosphoryl group from the carboxyl group of 1,3-bisphosphoglycerate to ADP, forming ATP and 3-phosphoglycerate.

This is a unique example where ATP can be produced at substrate level without participating in electron transport chain. This type of reaction where ATP is formed at substrate level is called as **Substrate level phosphorylation**.

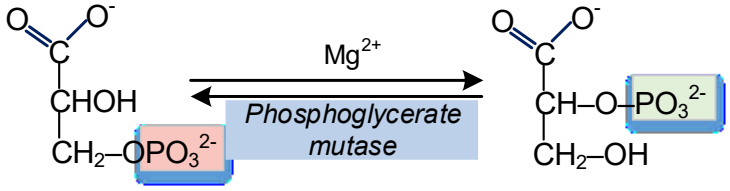


1,3-Bisphosphoglycerate 3-Phosphoglycerate

In red blood cells, the reaction catalysed by ***phosphoglycerate kinase*** can be partially bypassed by the action of ***bisphosphoglycerate mutase***. This enzyme converts 1,3-bisphosphoglycerate into **2,3-bisphosphoglycerate**, which is then hydrolyzed to 3-phosphoglycerate and Pi by *2,3-bisphosphoglycerate phosphatase*. Although this pathway does not generate any ATP during glycolysis, it produces **2,3-bisphosphoglycerate**, which binds to **hemoglobin** and decreases its affinity for oxygen. As a result, oxygen becomes more readily available to the tissues.

2. Conversion of 3-Phosphoglycerate to 2-Phosphoglycerate

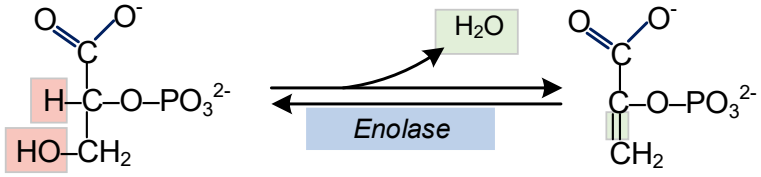
3-phosphoglycerate is isomerized to 2-phospho glycerate by shifting the phosphate group from 3rd to 2nd carbon atom. The enzyme is ***phosphoglucomutase***. This is a readily reversible reaction. Mg^{2+} is essential for this reaction.



3-Phosphoglycerate 2-Phosphoglycerate

3. *Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate*

2-phosphoglycerate is converted to phosphoenol pyruvate by the enzyme ***enolase***. One water molecule is removed. A high energy phosphate bond is produced. The reaction is reversible. Enolase requires Mg^{2+}.



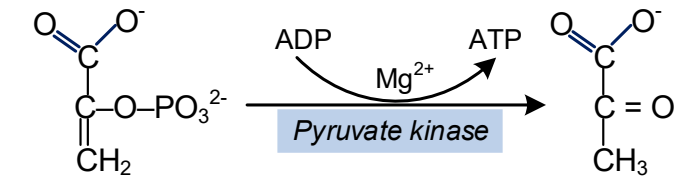
2-Phosphoglycerate Phosphoenolpyruvate

10. Conversion of Phosphoenol Pyruvate to Pyruvate

Phosphoenol pyruvate (PEP) is dephosphorylated to pyruvate, by ***pyruvate kinase***.

First PEP is made into a transient intermediary of enol pyruvate; which is spontaneously isomerized into keto pyruvate, the stable form of pyruvate. One mole of ATP is generated during this reaction. This is again an example of substrate level phosphorylation.

The pyruvate kinase is a **key glycolytic enzyme**. This step is irreversible.



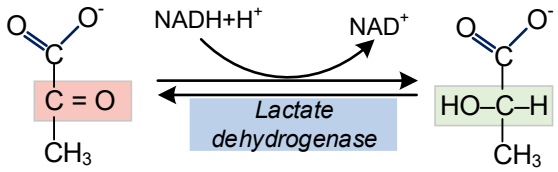
Phosphoenolpyruvate Pyruvate

MEDICAL IMPORTANCE

Pyruvate kinase deficiency *leads to excessive hemolysis of RBCs resulting in hemolytic anemia. Genetic deficiency of pyruvate kinase enzyme causes the decrease in the rate of glycolysis and decreased production of ATP.*

III Additional Step in Anaerobic Condition

When animal tissues cannot be supplied with sufficient oxygen to support aerobic oxidation of the pyruvate and NADH produced in glycolysis, NAD+ is regenerated from NADH by the reduction of pyruvate to lactate. Some tissues and cell types (such as erythrocytes, which have no mitochondria and thus cannot oxidize pyruvate to CO_2) produce lactate from glucose even under aerobic conditions. The reduction of pyruvate is catalyzed by ***lactate dehydrogenase***.



Pyruvate Lactate

✓ **Energetic effect of anaerobic oxidation of glucose and aerobic glycolysis**

The details of ATP generation in glycolysis (from glucose) are given in tables 7.2. Under anaerobic conditions, 2 ATP are synthesized while, under aerobic conditions, 8 or 6 ATP are synthesized-depending on the shuttle pathway that operates.

Table 7.2. Energy yield in aerobic glycolysis:

Step	Enzyme	Source	Number of ATP
1	Hexokinase	-	-1
3	Phosphofructokinase	-	-1
6	Glyceraldehyde-3- phosphate dehydrogenase	NADH	(+3) x 2 = +6
7	Phosphoglycerate kinase	ATP	(+1) x 2 = +2
10	Pyruvate kinase	ATP	(+1) x 2 = +2
	Net Yield		**8 ATPs**

Energy yield in anaerobic glycolysis:

Step	Enzyme	Source	Number of ATP
1	Hexokinase	-	-1
3	Phosphofructokinase	-	-1
6	Glyceraldehyde-3-phosphate dehydrogenase	NADH	(+3) x 2 = +6
7	Phosphoglycerate kinase	ATP	(+1) x 2 = +2
10	Pyruvate kinase	ATP	(+1) x 2 = +2
11	Lactate dehydrogenase		(-3) x 2 = -6
	Net Yield		**2 ATPs**

7.3.3. Significance of the Glycolysis Pathway:

- Glycolysis is the only pathway that is taking place in all the cells of the body. Glycolysis is the only source of energy in RBCs.
- In strenuous exercise, when muscle tissue lacks enough oxygen, anaerobic glycolysis forms the major source of energy for muscles.
- The glycolytic pathway may be considered as the preliminary step before complete oxidation.
- The glycolytic pathway provides carbon skeletons for synthesis of non-essential amino acids as well as glycerol part of triacylglycerols.
- Most of the reactions of the glycolytic pathway are reversible, which are also used for gluconeogenesis.

7.3.4. Regulation of glycolysis.

Flux through the glycolysis pathway is **regulated** by control of 3 enzymes that catalyze **irrevercible** reactions: ***hexokinase, phosphofructokinase and pyruvate kinase***. There are two main types of the control of glycolysis:

- **Global control** is for the benefit of the whole organism, & often involves **hormone-activated signal cascades**. The main hormones, able to regulate glycolysis are:
  - **Insulin:** stimulates synthesis of all key enzymes of glycolysis. It is secreted after meal (in response to high blood glucose level).
  - **Glucagon and Epinephrine:** inhibit the activity of all key enzymes of glycolysis. They are secreted in response to low blood glucose level**.**
- **Local control** of metabolism involves regulatory effects of varied concentrations of pathway **substrates** or **intermediates**, to benefit the cell.

Hexokinase is **inhibited** by **product glucose-6-phosphate**:

- by **competition** at the **active site**
- by **allosteric** interaction at a **separate** enzyme site.

Phosphofructokinase is usually the rate-limiting step of the glycolysis pathway. Phosphofructokinase is allosterically inhibited by ATP. At low concentration, the substrate ATP binds only at the active site. At high concentration, ATP binds also at a low-affinity regulatory site, promoting the tense conformation.

Pyruvate kinase, the last step Glycolysis, is **controlled** in **liver** partly by modulation of the amount of enzyme.

- **In vitro inhibition of glycolysis:**
  - **Arsenate** by the competing with inorganic phosphate in the reaction, catalyzed by ***glyceraldehyde 3-phosphate dehydrogenase.***
  - **Iodoacetate** by inhibiting ***glyceraldehyde 3-phosphate dehydrogenase.***
  - **Fluoride** be inhibiting ***enolase*** enzyme. Clinical laboratories use fluoride to inhibit glycolysis by adding it to the blood before measuring blood glucose.

7.3.5. The role of lactate dehydrogenase (LDH) in glycolysis, mechanism of reaction and its peculiarities. Isoenzymes of ldh and their clinical diagnostic significance

When animal tissues cannot be supplied wrth sufficient oxygen to support aerobic oxidation of the pyruvate and NADH produced in glycolysis, NAD^+ is regenerated from NADH by the reduction of pyruvate to lactate. Some tissues and cell types (such as RBCs, which have no mitochondria and thus cannot oxidize pyruvate to CO_2) produce lactate from glucose even under aerobic conditions. The reduction of pyruvate in this pathway is catalyzed by ***lactate dehydrogenase (LDH)***.

The lactate formed by active skeletal muscles (or by RBCs) can be recycled; it is carried in the blood to the liver, where it is converted to glucose during the recovery from strenuous muscular activity. When lactate is produced in large quantities during **vigorous muscle contraction** (during a sprint, for example), the acidification that results from ionization of lactic acid in muscle and blood limits the period of vigorous activity. The best-conditioned athletes can sprint at top speed for no more than a minute.

Although conversion of glucose to lactate includes two oxidation-reduction steps, there is no net change in the oxidation state of carbon; in glucose ($C_6H_{12}O_6$) and lactic acid ($C_3H_6O_3$), the H:C ratio is the same. Nevertheless, some of the energy of the glucose molecule has been extracted by its conversion to lactate-enough to give a net yield of **two molecules of ATP** for every glucose molecule consumed.

Lactate serves as a fuel source for **cardiac muscle** as well as **brain neurons**. **Astrocytes**, which surround and protect neurons in the brain, **convert glucose** to **lactate** and release it. Lactate taken up by adjacent neurons is converted to pyruvate that is oxidized via TCA cycle.

Increased formation of lactic acid or its decreased utilization maylead to **lactic acidosis**, it is the **lowered blood pH and bicarbonate levels** due to increased blood lactate above normal level.

Lactic acidosis may result from:

- increased formation of lactate as in severe muscular exercises;
- decreased utilization of lactate in tissues: it occurs in cases of anoxia or lack of oxygen e.g. myocardial infarction, respiratory disorders, and anemia;

- phenformin is oral hypoglycemic drug, causing excessive anaerobic oxidation of glucose and excess lactate production.

The conversion of puruvate to lactate is the mechqnism for regeneration of NAD^+. This helps continuity of glycolysis, as the generated NAD^+ will be used once more for oxidation of another glucose molecule.

Lactate dehydrogenase (LDH) enzyme is present in all cells, but it is concentrated in muscle, liver, and kidney. LDH exists **as five isozymes**, LDH-1 through LDH-5, each composed of four subunits. Differential LDH isozyme levels are used diagnostically.

- H_4, also called type 1, LDH_1, or A_4, a homopolymer of H subunits, is found in **cardiac muscle**, kidney, and red blood cells;
- H_3M_1, also called type 2, LDH_2, or A_3B, has a tissue distribution similar to that of LDH_1;
- H_2M_2, also called type 3, LDH_3, or A_2B_2, is found in the spleen, brain, white cells, kidney, and lung;
- H_1M_3, also called type 4, LDH_4, or AB_3, is found in the spleen, lung, skeletal muscle, lung, red blood cells, and kidney;
- M_4, also called type 5, LDH_5, or B_4, a homopolymer of M subunits, is found in the **liver**, **skeletal muscle**, and spleen.

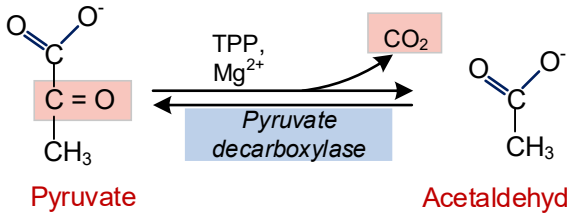
The H_4 isoenzyme has a higher substrate affinity than the M_4 isoenzyme. The H_4 isoenzyme is allosterically inhibited by high levels of pyruvate (its product), whereas the M_4 isoenzyme is not. The other LDH isoenzymes have intermediate properties, depending on the ratio between the two types of subunits. It is thought that the **H_4 isoenzyme** is the most suitable for catalyzing the oxidation of lactate to pyruvate that, in the heart, due to its exclusively aerobic metabolism, is then completely oxidized to CO_2 and H_2O. Instead, the **M_4 isoenzyme** is the main isoenzyme found in skeletal muscle, most suitable for catalyzing the reduction of pyruvate to lactate, thus allowing glycolysis to proceed in anaerobic conditions.

The activity of LDH is elevated in serum as a result of organ infarction and significant cell death that results in loss of cytoplasm. For example, elevations of LDH result from conditions such as hepatitis, shock, hypoxia, extreme hypothermia, and meningitis, among others. The LDH enzyme has often been used in laboratory animals, along with troponin levels, to detect myocardial damage.

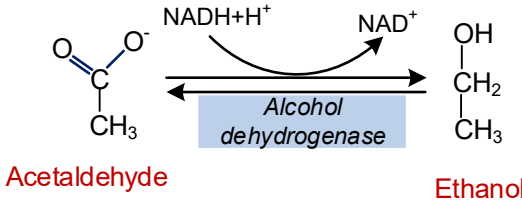
7.4. Ethanol fermentation, common and different reactions in glycolysis and fermentation.

Yeast and other microorganisms ferment glucose to **ethanol and CO_2**, rather than to lactate. **Ethanol fermentation is conversion of glucose into ethanol by yeast enzymes.** Glucose is converted to pyruvate by glycolysis, and the pyruvate thereafter is converted to ethanol and CO_2 in a two-step process.

1. In the first step, pyruvate is decarboxylated in an irreversible reaction catalyzed by ***pyruvate decarboxylase***. This reaction is a simple decarboxylation and does not involve the net oxidation of pyruvate. Pyruvate decarboxylase requires Mg^{2+} and has a tightly bound coenzyrme, **thiamine pyrophosphate (TPP).**



2. In the second step, acetaldehyde is reduced to ethanol through the action of ***alcohol dehydrogenase***, with the reducing power furnished by NADH derived from the dehydrogenation of glyceraldehyde 3-phosphate.



Ethanol and CO_2 are thus the end products of ethanol fermentation, and the overall equation is:

$$\text{Glucose} + 2\text{ADP} + 2\text{P}_i \rightarrow 2\ \text{ethanol} + 2\text{CO}_2 + 2\text{ATP} + 2\text{H}_2\text{O}$$

As in lactic acid fermentation, there is no net change in the ratio of hydrogen to carbon atoms when glucose (H:C ratio: 12/6 :2) is fermented to two ethanol and two CO_2 (combined H:C ratio: 12/6:2). In all fermentations, the H:C ratio of the reactants and products remains the same. Pyruvate decarboxylase is present in brewer's and baker's yeast and in all other organisms that ferment glucose to ethanol, including some plants. The CO_2 produced by pyruvate decarboxylation in brewer's yeast is responsible for the characteristic carbonation of champagne. In baking, CO_2 released by pyruvate decarboxylase when yeast is mixed with a fermentable sugar causes dough to rise.

Alcohol dehydrogenase is present in many organisms that metabolize ethanol, including humans. In the liver it catalyzes the oxidation of ethanol, either ingested or produced by intestinal microorganisms, with the concomitant reduction of NAD^+ to NADH. In this case, the reaction proceeds in the direction opposite to that involved in the production of ethanol by fermentation.

7.5. Pasteur and Crabtree effects regulation of glycolysis.

The Pasteur effect, also known as the Warburg effect, refers to a phenomenon observed in cellular metabolism where the rate of glycolysis is reduced in the presence of oxygen. It was named after the French scientist Louis Pasteur and the German scientist Otto Warburg, who made significant contributions to understanding this effect. In the anaerobic conditions, cells primarily rely on glycolysis to produce energy in the form of ATP. Glycolysis breaks down glucose into pyruvate, generating a small amount of ATP. However, when oxygen is available (aerobic conditions), cells have the option to undergo oxidative phosphorylation, a more efficient process that takes place in the mitochondria and generates a larger amount of ATP.

The Pasteur effect occurs when the presence of oxygen inhibits glycolysis. This is because oxidative phosphorylation becomes the preferred pathway for ATP production

when oxygen is abundant. The exact mechanism underlying the Pasteur effect is not fully understood, but it is believed to involve several factors:

- **ATP levels:** Oxygen-dependent oxidative phosphorylation produces more ATP per glucose molecule than glycolysis. As a result, when oxygen is available, cells can meet their energy demands more efficiently through oxidative phosphorylation. Higher ATP levels can inhibit key enzymes in the glycolytic pathway, slowing down glycolysis.
- **NADH/NAD$^+$ ratio:** Glycolysis generates NADH, which can be oxidized to NAD+ during the process of fermentation in the absence of oxygen. In the presence of oxygen, NADH is reoxidized through oxidative phosphorylation in the mitochondria, maintaining a lower NADH/NAD$^+$ ratio. The lower NADH/NAD$^+$ ratio inhibits the activity of certain enzymes in glycolysis, reducing its rate.
- **Oxygen-dependent regulation:** The presence of oxygen can trigger regulatory mechanisms that downregulate the enzymes involved in glycolysis and upregulate enzymes involved in oxidative phosphorylation. This shift in enzyme activity favors the use of the more efficient oxidative phosphorylation pathway.

MEDICAL IMPORTANCE

The Pasteur effect has been extensively studied in the context of cancer biology. Cancer cells often exhibit a preference for glycolysis even in the presence of oxygen, a phenomenon known as the "Warburg effect." This metabolic alteration allows cancer cells to rapidly produce ATP and biomolecules necessary for their high proliferation rate. The exact reasons why cancer cells exhibit this metabolic shift are still under investigation, and multiple factors likely contribute to the Warburg effect.

The Crabtree effect, also known as **glucose repression**, is a phenomenon observed in some microorganisms, particularly yeast, where the presence of high glucose concentrations leads to the repression of other carbohydrate utilization pathways, such as the metabolism of alternative sugars or non-fermentable carbon sources. This effect was named after the British scientist George Crabtree, who first described it in the early 20th century.

When yeast cells are exposed to high glucose concentrations, they preferentially utilize glucose as their energy source through the process of glycolysis and fermentation, even in the presence of oxygen. This preference for glucose metabolism leads to the repression or inhibition of other metabolic pathways involved in the utilization of alternative carbon sources. The Crabtree effect is thought to occur due to multiple factors:

- **Glucose signaling:** High levels of intracellular glucose can act as a signal to trigger specific signaling pathways that repress the expression of genes involved in alternative carbohydrate metabolism. The exact mechanisms of this signaling are still under investigation, but it is known that glucose signaling pathways involve various protein kinases and transcription factors.
- **Metabolic regulation:** The preference for glucose metabolism in yeast cells under high glucose conditions is believed to be an outcome of metabolic regulation. The rapid metabolism of glucose through glycolysis and fermentation allows for fast ATP production, which can meet the immediate energy demands of the cell. This rapid ATP production may outcompete oxidative phosphorylation, leading to the repression of mitochondrial respiration and the metabolism of other carbon sources.

- **Feedback inhibition:** Glycolysis, the primary pathway for glucose metabolism, involves multiple enzymatic reactions. Some of these enzymes are subject to feedback inhibition by high levels of glycolytic intermediates or ATP, which can be accumulated when glucose is abundant. The feedback inhibition acts as a regulatory mechanism to dampen the flux through alternative carbohydrate utilization pathways.

The Crabtree effect has practical implications in various biotechnological applications, including industrial fermentation processes. Understanding and manipulating the Crabtree effect can be useful for optimizing the production of desired products, such as biofuels or recombinant proteins, in microbial systems.

REVIEW TEST:

№	MCQs	Answers and explanations
1.	**Human red blood cells contain no mitochondria. What is the main pathway for ATP production in these cells?** A. Aerobic glycolysis B. Anaerobic glycolysis C. Oxidative phosphorylation D. Creatine kinase reaction E. Cyclase reaction	**The answer is B.** Anaerobic glycolysis – oxidation of glucose to lactate in the absence of oxygen, with the production of ATP. Glycolysis is the first step in the pathway of glucose metabolism and occurs in the cytosol of all cells. Due to red blood cells (RBCs) contain no mitochondria, anaerobic oxidation of glucose is the only source of energy for RBC.
2.	**Blood test of the patient revealed albumine content of 20 g/l and increased activity of lactate dehydrogenase isoenzyme 5 (LDH 5). These results indicate disorder of the following organ:** A. Lungs B. Kidneys C. Heart D. Liver E. Spleen	**The answer is D.** Lactate dehydrogenase (LDH) catalyses the interconversion of lactate and pyruvate. It has five isoenzyme. LDH_5 is mostly present in skeletal muscle and liver. The activity of LDH in healthy individual serum: $LDH_2 > LDH_1 > LDH_3 > LDH_4 > LDH_5$. Increased activity of LDH_5 in serum is an indicator of liver disease.
3.	**Untrained people often have muscle pain after sprints as a result of lactate accumulation. This can be caused by intensification of the following biochemical process:** A. Glycolysis B. Gluconeogenesis C. Pentose phosphate pathway D. Lipogenesis E. Glycogenesis	**The answer is A.** Different modes of energy coverage are used depending on intensity and duration of the workload put on the organism. The production of energy used in muscle contraction takes place through the anaerobic glycolysis.
4.	**When investigating human saliva it is necessary to assess its hydrolytic properties. What substance should be used as a substrate in the process?** A. Proteins B. Starch C. Fats D. Fiber E. Amino acids	**The answer is B.** α -amylase – enzyme catalyse hydrolysis of α-1,4-glycosidic bonds of polysaccharides. It is produced by salivary and pancreatic glands. Salivary amylase catalyse hydrolysis of starch to dextrin and maltose. Dextrins in presence of iodine form red-brown colour or no change in colour at all. Maltose also does not form a coloured complex with iodine. If solution of iodine and potassium

		iodine add to starch the light orange-brown color changes to a deep blue.
5.	**Diseases of respiratory system and circulatory disorders impair the transport of oxygen, thus causing hypoxia. Under these conditions the energy metabolism is carried out by anaerobic glycolysis. As a result, the following substance is generated and accumulated in blood:** A. Glutamic acid B. Pyruvic acid C. Lactic acid D. Citric acid E. Fumaric acid	**The answer is C.** Anaerobic glycolysis - breakdown of glucose into lactate with the production of 2 molecules of ATP.
6.	6 hours after the myocardial infarction a patient was found to have elevated level of lactate dehydrogenase in blood. What isoenzyme should be expected in this case? A. LDH1 B. LDH2 C. LDH3 D. LDH4 E. LDH5	**The answer is A.** LDH_1 is predominantly found in heart muscle. In the case of myocardial infarction, LDH_1 is much greater than LDH_2 and this happens within 12 to 24 hours after infarction: $LDH_1 > LDH_2 > LDH_3 > LDH_4 > LDH_5$.
7.	Some students developed myodynia after continuous physical activity during physical education. The reason for such condition was accumulation of lactic acid in the skeletal muscles. It was generated in the students' bodies after activation of the following process: A. Glycogenolysis B. Gluconeogenesis C. Pentose-phosphate cycle D. Lipolysis E. Glycolysis	**The answer is E.** The working muscles generate energy anaerobically. Exercise increases your muscles' oxygen demand and your muscles are producing energy by anaerobic glycolysis – converting glucose to lactic acid. Lactate may be accumulated in muscles causing muscle pain and muscle fatigue.
8.	**A 36-year-old woman ist raining for her first marathon, and her coach has her keeping a pace that allows her to stay below her anaerobic threshold. By avoiding anaerobic muscle glycolysis, the pyruvate produced in the muscle does not accumulate because it is converted towhich on eof the following?** Ethanol Lactic acid AcetylCoA Alanine OAA	**The answer is B.** Glycolysis is dependent on NAD^+ (for the glyceraldehyde 3-phosphatedehydrogenase reaction) as a substrate for the pathway to continue to metabolize glucose. Under aerobic conditions, NAD^+ is generated via the electron transport chain. Under anaerobic conditions,a n oxygen deficit limits the electron transport chain, and NAD^+ is generated by the conversion of pyruvate to lactate in mammals and to ethanol in yeast and some microorganisms. When oxygen is not limiting, the pyruvateis converted to acetyl CoA to generate energy via the TCA cycle and oxidative phosphorylation. Because acetyl CoA levels are low during exercise, pyruvate carboxylase is notactive, and OAA will not be

		formed.
9.	**A 33-year-old triathlete is admitted to the hospital after he spent the whole day training. He looks ill and complains of diffuse weakness, fatigue, and myalgia. Laboratory tests are sent for analysis, and his lactate level is elevated, creatinine is elevated (suggesting acute renal failure), creatine kinase is 76,000, and urine tests positive for myoglobin. You determine he has rhabdomyolysis and treat him with aggressive intravenous hydration. The basis for the elevated lactate is which one of the following?** An increase in ATP due to the lack of oxygen for the muscle An increase in NADH due to the lack of oxygen for the muscle A defectin the M form of lactate dehydrogenase A defectin the H form of lactate dehydrogenase A defect in the B form of muscl ealdolase	**The answer is B.** Because of the intensity of the patient's training, oxygen delivery to the muscle lagged behind the need to produce ATP, so anaerobic glycolysis was providing the majority of energy (ATP) formation. This leads to elevated NADH, which is converted back to NAD^+ by the lactate dehydrogenase reaction. Because the patient was exercising, ATP levels in the muscle are low, and ADP and AMP levels increase. There is no indication for a change either in the muscle (M) or in the heart (H) forms of lactate dehydrogenase. Muscle expresses the A form of aldolase, but not the B form (which is expressed in the liver).
10	**Which one of the following statements is correct concerning the formation of muscle lactate during exercise?** Lactate formation occurs when the NADH/ NAD^+ ratio is high. The liver preferentially converts lactate into carbon dioxide and water. The heart preferentially converts lactate into glucose. Lactate formation is less likely to bef ound in the eye, testes, and RBCs than in other tissues. The intracellular pH is typically increased whenlactate is produced.	**The answer is A.** Lactate formation occurs in a high NADH/NAD^+ state. The NADH has been generated by the glyceraldehyde 3-phosphate dehydrogenase reaction, and NAD^+ needs to be regenerated, under anaerobic conditions, for glycolysis to continue. The formation of lactate, an acid, results in a drop in pH. Lactate formation commonly occurs in poorly vascularized tissues (e.g., eye, renal medulla, testes) or tissues without mitochondria (e.g., RBCs). Lactate, once formed in muscle, diffuses into the blood stream and is used by other tissues. In the liver, lacate is converted to glucose, whereas in the heart, lactate is preferentially oxidized to provide energy.

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8. GLUCOSE OXIDATION UNDER AEROBIC CONDITIONS AND ALTERNATIVE METABOLIC PATHWAYS OF MONOSACCHARIDES METABOLISM

OBJECTIVES after studying this chapter, you should be able to:

- *Interpret mechanisms of monosaccharides transformation to final metabolic products and energetic effect in aerobic conditions.*
- *Analyze structural and functional peculiarities of pyruvate dehydrogenase complex.*
- *Describe the pentose phosphate pathway and its roles as a source of NADPH and in the synthesis of ribose for nucleotide synthesis.*
- *Analyze metabolic pathways of fructose and galactose transformations in human body.*
- *Explain the consequences of genetic defects of glucose-6-phosphate dehydrogenase deficiency (favism), the uronic acid pathway (essential pentosuria), and fructose and galactose metabolism.*

8.1. Aerobic metabolism of pyruvate.

Pyruvate, the end product of aerobic glycolysis, can undergo various metabolic fates depending on the cellular conditions and requirements. Three major pathways for pyruvate utilization are:

4. **Conversion to acetyl CoA:** Pyruvate can be oxidatively decarboxylated by *pyruvate dehydrogenase*, producing acetyl CoA, which then enters the TCA cycle for further oxidation and ATP generation.
5. **Carboxylation to oxaloacetate:** *Pyruvate carboxylase* can carboxylate pyruvate to form oxaloacetate, a TCA cycle intermediate. This pathway is important for anaplerosis (replenishment) of TCA cycle intermediates and gluconeogenesis (the synthesis of glucose from non-carbohydrate precursors).
6. **Conversion to lactate** by *lactate dehydrogenase* under anaerobic conditions.
7. **Reduction to ethanol:** In some microorganisms, pyruvate can be reduced to ethanol by the enzyme pyruvate decarboxylase, which generates NAD^+ from NADH and releases CO_2. This pathway is important for the fermentation of sugars to produce ethanol, a process used in the production of alcoholic beverages and biofuels.

8.1.1 Oxidative decarboxylation of pyruvate

Pyruvate, formed in the **cytosol**, is transported into the mitochondrial matrix by a **pyruvate/H^+ symporter** located in the inner mitochondrial membrane. Once inside the matrix, pyruvate is oxidatively decarboxylated by the **pyruvate dehydrogenase complex**, a multienzyme complex that converts pyruvate to acetyl-CoA, which then enters the TCA cycle. The pyruvate dehydrogenase complex consists of three enzymes: ***pyruvate dehydrogenase, dihydrolipoyl transacetylase, and dihydrolipoyl dehydrogenase***, and requires 5 coenzymes (table 8.1)

Table 8.1. Enzymes and coenzymes of PDH complex

Enzyme	Abbreviation	Coenzyme
Pyruvate dehydrogenase	E1	Thiamine pyrophosphate, (TPP)
Dihydrolipoyl transacetylase	E1	Lipoic acid (lipoamide), coenzyme A
Dihydrolipoyl dehydrogenase	E2	Flavine adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD^+)

In mammals, the PDH complex has a molecular weight of approximately 9 million daltons, and is composed of many copies of the individual enzymes arranged in a highly organized structure. The *dihydrolipoyltransacetylase* subunit is the most abundant, with around 60 copies per complex, while the *pyruvate dehydrogenase and dihydrolipoyl dehydrogenase* are present in smaller amounts, with 20-30 copies each.

The sceme of oxidative decarboxylation of pyruvate by PDH complex is represented on the fig. 8.1.

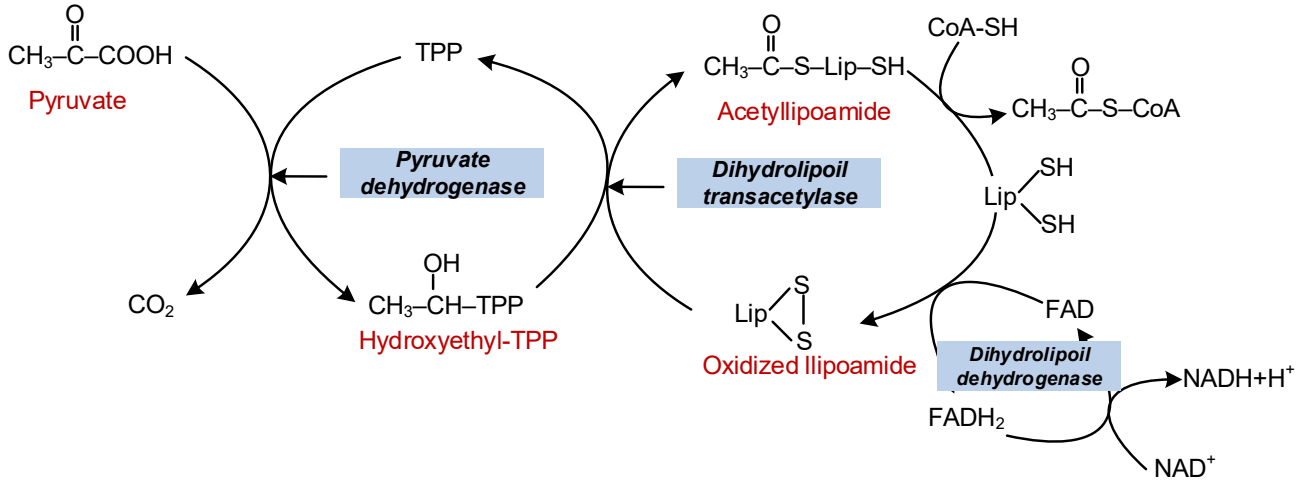
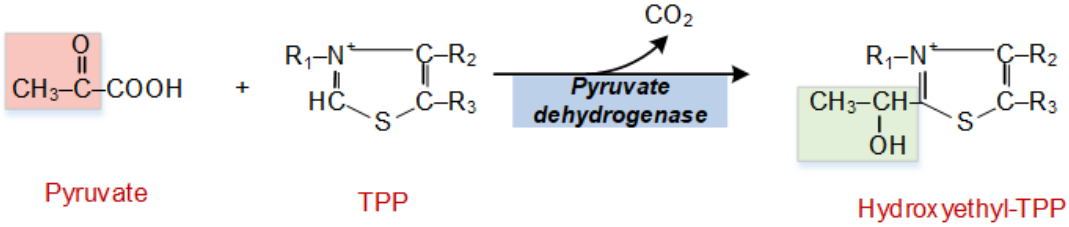


Fig. 8.1. Convertion of pyruvate to acetyl-CoA catalyzed by PDH complex

8.1.2. Mechanism of oxidative decarboxylation of pyruvate

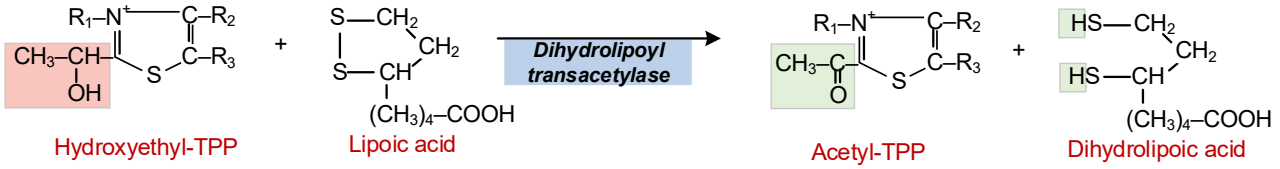
Sequense of chemichal reactions that demonstrate the mechanism of oxidative decarboxylation of pyruvate are as follows.

1. E1 - *pyruvate dehydrogenase*. This enzyme catalyzes the decarboxylation of pyruvate. This involves the prosthetic group **thiamine pyrophosphate (TPP)**.

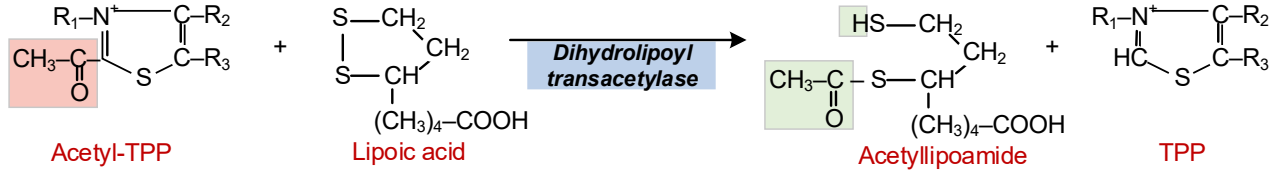


2. E2 - *dihydrolipoyl transacetylase*. Tree steps of the pathway are catalyzed by this enzyme:

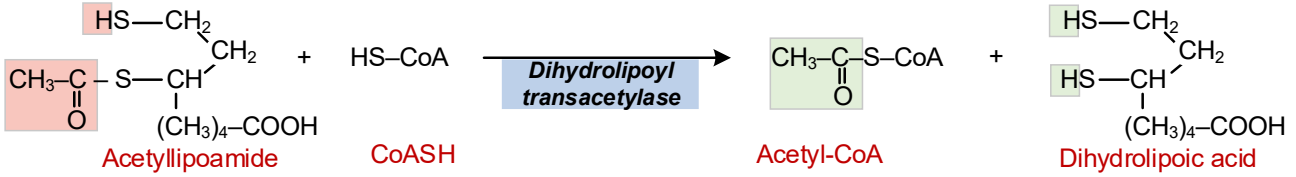
Oxidation of the hydroxyethyl-TPP to acetyl-TPP and reduction of lipoic acid.



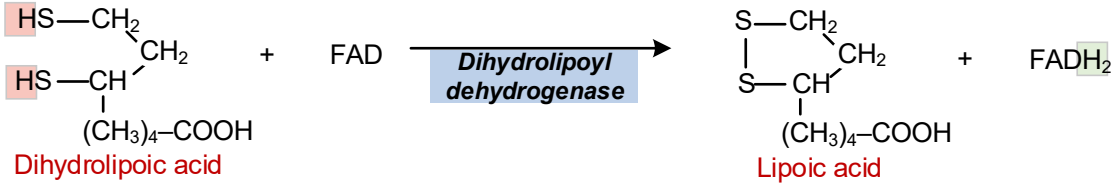
Transfer of acetyl-TPP to one more molecule of **lipoic acid** (the prosthetic group of the enzyme), giving an **acetyllipoamide** group.



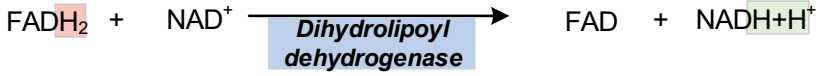
Transfer of the acetyl group from the lipoamide to **CoA,** giving **acetyl-CoA.**



3. **E3 - *dihydrolipoyl dehydrogenase***. This enzyme regenerates the oxidized form of lipoic acid. This involves the **FAD** as a prosthetic group.



Finally, ***dihydrolipoyl dehydrogenase*** **transfers electrons and protons to NAD^+.**



Thus, TPP, lipoamide and FAD are **catalytic cofactors** which remain unaltered by the net reaction, whereas coenzyme A and NAD^+ are **stoichiometric cofactors**; the overall reaction is:

Pyruvate + CoA + NAD^+ → acetyl CoA + CO_2 + $NADH+H^+$

MEDICAL IMPORTANCE

Thiamine pyrophosphate (TPP) is a cofactor required by the enzyme pyruvate dehydrogenase (PDH) to convert pyruvate to acetyl-CoA, a key step in the citric acid cycle. In the absence of TPP, PDH activity is inhibited, leading to the accumulation of pyruvate. In thiamine-deficient alcoholics, pyruvate is rapidly converted to lactate, resulting in lactic acidosis. This occurs because without TPP, PDH cannot convert pyruvate to acetyl-CoA, and thus, pyruvate is shunted to the anaerobic pathway, where it is converted to lactate. ***Wernicke-Korsakoff syndrome*** *is a neurological disorder caused by thiamine deficiency, often seen in alcoholics. It is characterized by a combination of encephalopathy (brain dysfunction) and psychosis (loss of contact with reality). In patients with inherited deficiency of PDH, lactic acidosis is observed, usually after a glucose load. This is because PDH is necessary for the metabolism of glucose, and its deficiency results in the accumulation of pyruvate and subsequent conversion to lactate.*

PDH activity can also be inhibited by certain heavy metal ions such as arsenic and mercuric ions, which bind to the SH groups of lipoic acid, an essential component of the enzyme complex.

8.1.3. Regulation of PDH complex.

Pyruvate dehydrogenase (PDH) is an enzyme that plays a critical role in the metabolism of carbohydrates. It converts pyruvate, which is the end product of glycolysis, into acetyl CoA, which can then be used in the citric acid cycle to produce ATP. However, the activity of PDH is regulated by several factors, including end product inhibition, phosphorylation, and dephosphorylation (fig.8.2).

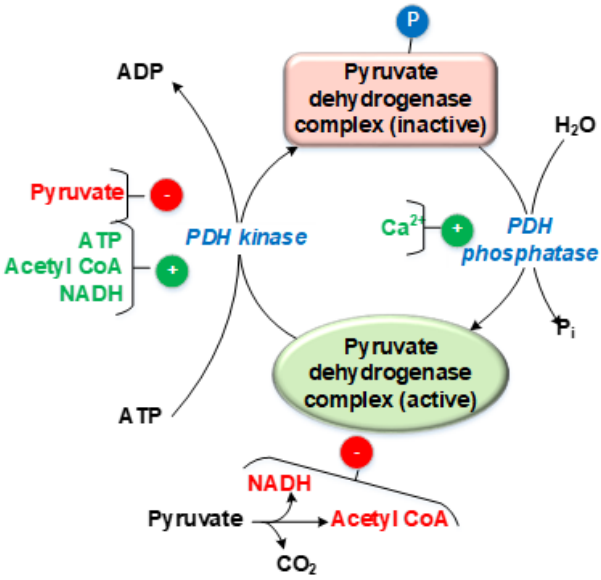


Fig. 8.2. Regulation of PDH complex

End product inhibition occurs when the end products of a metabolic pathway inhibit the activity of an earlier enzyme in that pathway. In the case of PDH, **acetyl CoA and NADH** act as inhibitors of the enzyme. This ensures that the production of acetyl CoA is regulated in response to the energy needs of the cell.

PDH is also regulated by phosphorylation and dephosphorylation. When PDH is **phosphorylated,** it is **inactive** as a phosphoenzyme. However, when it is **dephosphorylated**, it becomes **active** as a dephosphoenzyme. PDH phosphatase activity, which is responsible for dephosphorylating PDH, is promoted by Ca^{2+} and insulin in adipose tissue. Calcium released during muscle contraction also stimulates PDH by increasing phosphatase activity, which helps to produce energy.

PDH kinase is the enzyme responsible for phosphorylating PDH and making it inactive. PDH kinase is promoted by ATP, NADH, and acetyl CoA, while it is inhibited by pyruvate.

8.2. Stages and energetic balance of aerobic oxidation of glucose.

Glucose is the major fuel of most organisms and occupies a central position in metabolism. It is relatively rich in potential energy; its complete oxidation to carbon dioxide and water proceeds with a standard free-energy change of -2,840 kJ/mol.

Complete oxidation of glucose under aerobic conditions to CO_2 and H_2O is divided into **4 stages (fig. 8.3)**:

I stage – Glycolysis (see chapter 7).

Glycolysis is located **in cytosol** of every living cell. It can be divided into two phases: energy consuming (also called chemical priming) and energy yielding. The first phase is the **energy-consuming phase**, so it requires two ATP molecules to start the reaction for each molecule of glucose. However, the second phase of glycolysis (**pay-off phase**) produces four ATPs by the substrate level phosphorylation, resulting in a net gain of **two ATP** energy molecules.

Glycolysis can be expressed as the following equation:

$$\text{Glucose} + 2\text{ATP} + 2\text{NAD}^+ + 4\text{ADP} + 2\text{P}_i \rightarrow 2\ \text{Pyruvate} + 4\text{ATP} + 2\text{NADH} + \text{H}^+$$

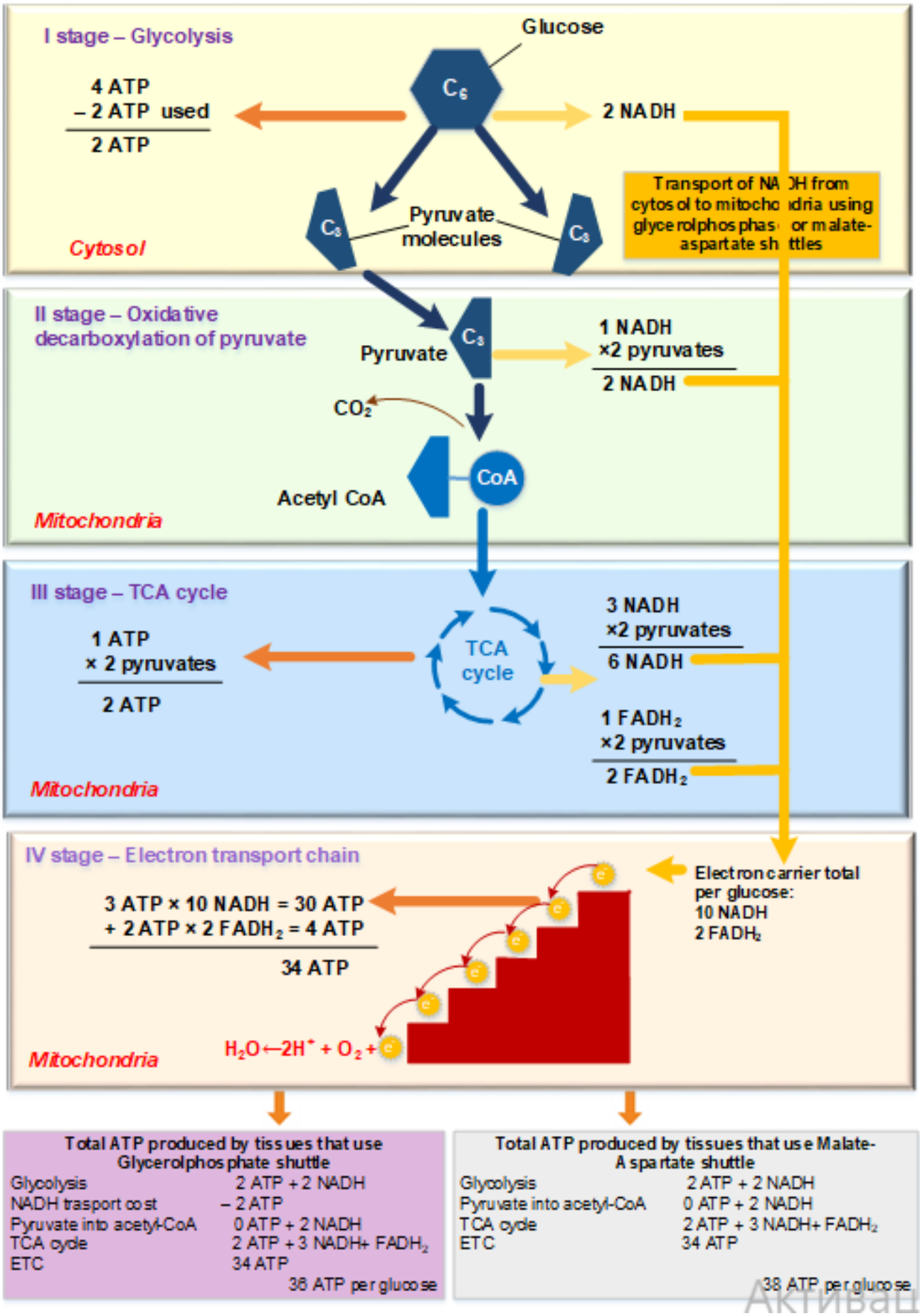


Fig. 8.3. Stages of glucose oxidation

Thus. one glucose molecule generates **two pyruvate molecules**, **two high-energy ATP molecules, and two electron-carrying NADH molecules.**

NADH, which is produced in the cytosol through glycolysis, cannot move across the mitochondrial membrane on its own. As a result, two shuttle systems are used to transfer the electrons to the mitochondrial electron transport chain:

- One of these shuttle systems is the **glycerophosphate shuttle (fig.8.4)**. This system involves the conversion of dihydroxyacetone phosphate, an intermediate in glycolysis, to glycerol-3-phosphate by the enzyme *glycerol-3-phosphate dehydrogenase*, which also converts **NADH to NAD**. Glycerol-3-phosphate is then transformed back to dihydroxyacetone phosphate by the *flavoprotein dehydrogenase*, a different form of *glycerol-3-phosphate dehydrogenase* that depends on **FAD** and is located in the inner membrane of the mitochondria. Similar to Complex II of the electron transport chain, flavoprotein dehydrogenase transfers electrons directly to Coenzyme Q without pumping protons. However, since electrons transferred through the glycerophosphate shuttle join the electron transport pathway at the level of Coenzyme Q, they can only produce a maximum of two ATP, as opposed to the maximum of three ATP produced by NADH generated inside the mitochondria. Glycerolphosphate shuttle mechanism is active in in skeletal muscle and brain.

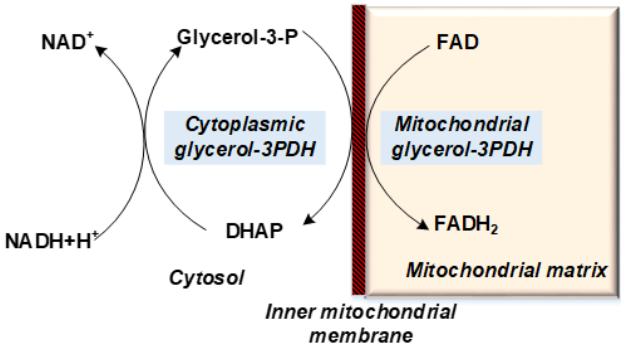


Fig. 8.4. Glycerophosphate shuttle: DHAP - dihydroxyacetone phosphate

- **The Malate-aspartate shuttle** (fig. 8.5) is a mechanism used by mammalian tissues to transfer electrons across the inner membrane of the mitochondria. The process involves the conversion of cytoplasmic oxaloacetate to malate by *malate dehydrogenase*, which results in the oxidation of **NADH to NAD**. The malate is then transported into the mitochondria by an exchange protein, which also transports α-ketoglutarate in the opposite direction. Inside the mitochondria, malate is oxidized back to oxaloacetate by mitochondrial *malate dehydrogenase*, leading to the formation of **NADH** that can be used in the electron transport chain. This shuttle only moves electrons from NADH from outside to inside the mitochondria, without any carbon or ni trogen movement. This enables the conservation of all the energy in the NADH electrons, and allows for the production of three ATP (under optimal conditions) from

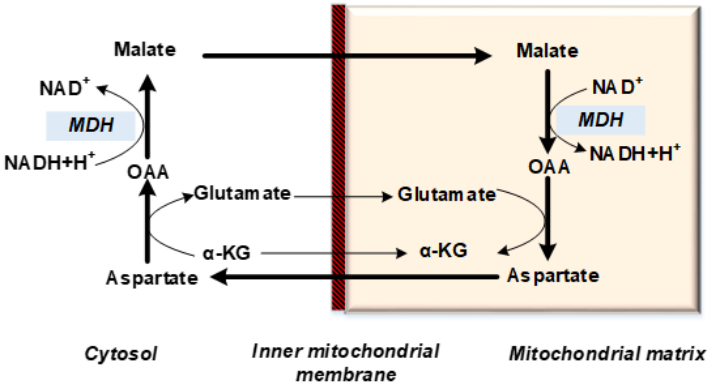


Fig. 8.5. Malate-aspartate shuttle. OAA – oxaloacetate, α-KG – α-Ketoglutarate, MDH – *malatedehydrogenase*

NADH generated in the cytoplasm. The malate-aspartate shuttle mechanism is active in liver, kidney and heart.

- **II stage – Oxidative decarboxylation of pyruvate.**

In the presence of oxygen, pyruvate is transferred to **mitochondria**, where it undergoes **oxidative decarboxylation**. Oxidation of **2 molecules of pyruvate**, generated in glycolysis produce **2 molecules of 2 acetyl CoA and 2 NADH:**

2 Pyruvate + 2 CoA + 2 NAD^+ → 2 acetyl CoA + 2 CO_2 + 2 NADH+H^+

NADH then enters the electron transport chain and produses tree ATP each, so for 2 molecules of NADH (per 1 glucose) maximum **six** molecules of **ATP** can be finally generated.

III stage – TCA cycle (see chapter 5).

The acetyl-CoA generated during the second stage of glucose aerobic oxidation is metabolized by enzymes TCA cycle. For each turn of the cycle, **three NADH**, **one ATP** (through GTP, syntesised in substrate phosphorylation reaction**) and one $FADH_2$** are created. 2 molecules of acetyl-CoA were produced per one glucose, so **two** turns of TCA are needed. Each carbon of acetyl-CoA is converted into CO_2, which is released as a byproduct of oxidative (aerobic) respiration.

IV stage – Electron transport chain.

The **electron transport chain (ETC)** uses the NADH and $FADH_2$ produced by previous stages to generate ATP. Electrons from NADH and $FADH_2$ are transferred through protein complexes embedded in the inner mitochondrial membrane by a series of enzymatic reactions.

Thus, total energetic balance of glucose aerobic oxidation to carbon dioxide and water is **36 moleculs of ATP** for tissues that use glycerophosphate shuttle and **38 mocules of ATP** for tissues that use malate-aspartate shuttle.

8.3. Pentose phosphate pathway of glucose utilization

The pentose phosphate pathway (PPP), hexose monophosphate shunt or the **phosphogluconate oxidative pathway** is an alternative pathway for glucose metabolism that does not result in the production of ATP. Its primary functions are twofold:

1. To produce **NADPH**, which is essential for synthesizing fatty acids and steroids, as well as maintaining reduced glutathione levels for antioxidant activity.
2. To produce **ribose**, which is required for the formation of nucleotides and nucleic acids.

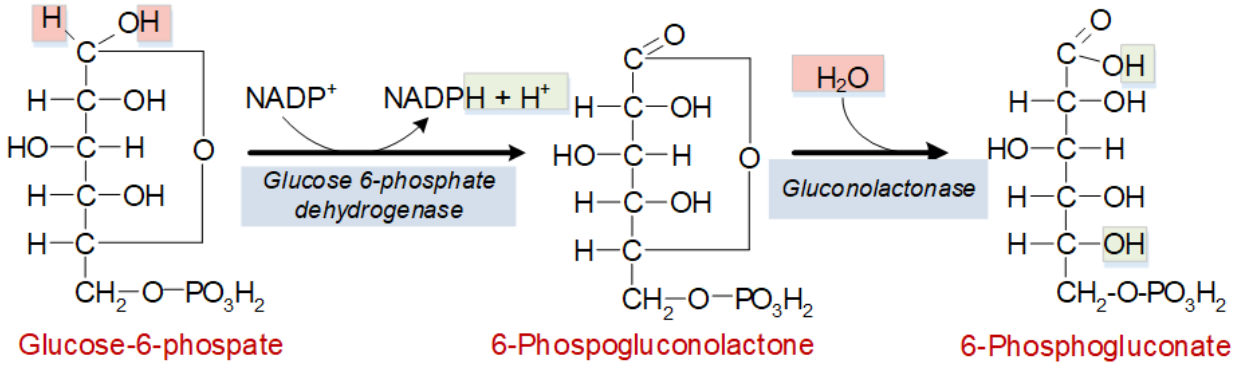
Similar to glycolysis, the enzymes involved in the pentose phosphate pathway are present in the **cytosol**. However, unlike glycolysis, oxidation occurs through dehydrogenation using **$NADP^+$** as the hydrogen acceptor instead of NAD^+. The cytosol of various organs and tissues such as the liver, adipose tissue, erythrocytes, neutrophils, adrenal cortex, thyroid, testis, ovaries, and lactating mammary gland contain enzymes of the pentose phosphate pathway. However, in skeletal muscle, this pathway is not as active.

The pentose phosphate pathway comprises two distinct phases: **an irreversible oxidative phase and a reversible nonoxidative phase.** During the oxidative phase, glucose-6-phosphate is dehydrogenated and decarboxylated to produce ribulose-5-

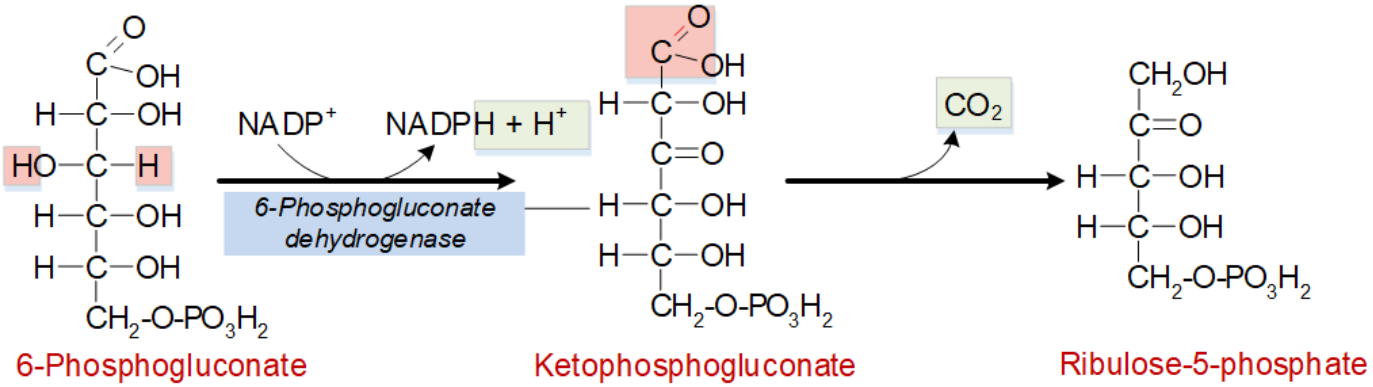
phosphate. This reaction is catalyzed by glucose 6-phosphate dehydrogenase, which is an NADP-dependent enzyme. The resulting product, 6-phosphogluconolactone, is then hydrolyzed by the enzyme gluconolactonase.

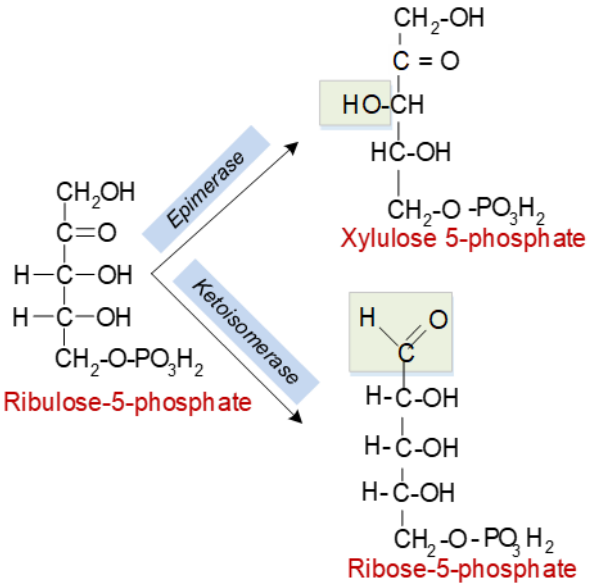
In the nonoxidative phase, ribulose-5-phosphate is converted back to glucose-6-phosphate through a series of reactions involving primarily two enzymes: transketolase and transaldolase.

During the **oxidative phase** of the pentose phosphate pathway, glucose-6-phosphate is first dehydrogenated to form 6-phosphogluconolactone via the action of *glucose 6-phosphate dehydrogenase*, which is an enzyme that depends on $NADP^+$ as a coenzyme. The next step involves the hydrolysis of 6-phosphogluconolactone, which is catalysed by the enzyme *gluconolactonase.* This reaction ultimately results in the formation of 6-phosphogluconate. This stage of PPP requires presence of metal ions like Mg^{2+} or Ca^{2+} or Mn^{2+}.



A second oxidative reaction of a pathway is catalyzed by *6-phosphogluconate dehydrogenase*, which is a second enzyme that uses $NADP^+$ as coenzyme an hydrogen acceptor. Decarboxylation follows with the formation of the ketopentose **ribulose-5-phosphate.** Mg^{2+} is essential for decarboxylation.





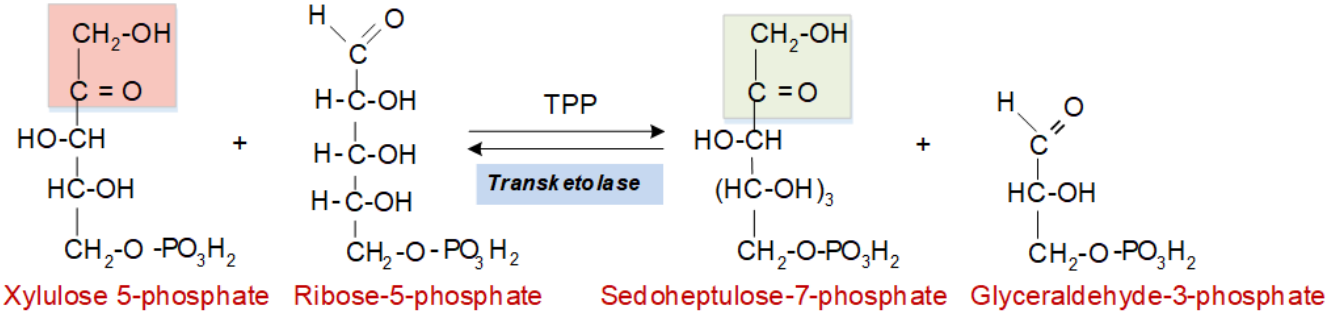
Nonoxidative phase. The non-oxidative reactions that occur during the pentose phosphate pathway involve the interconversion of monosaccharides with three, four, five, and seven carbon atoms. Ribulose-5-phosphate serves as a substrate for two enzymes that play important roles in this phase of the pathway.

The first enzyme, *ribulose-5-phosphate 3-epimerase*, catalyzes the conversion of ribulose-5-phosphate to xylulose-5-phosphate by altering the configuration of carbon 3. Xylulose-5-phosphate is also a ketopentose.

The second enzyme, *ribose-5-phosphate ketoisomerase*, converts ribulose-5-phosphate to ribose-5-phosphate, which is an aldopentose. Ribose-5-phosphate is utilized for the synthesis of nucleotides and nucleic acids.

Transketolase is an enzyme that plays a key role in the non-oxidative phase of the pentose phosphate pathway. It facilitates the transfer of a two-carbon unit consisting of carbons 1 and 2 of a ketose to the aldehyde carbon of an aldose sugar. This results in the conversion of a ketose sugar to an aldose with two fewer carbons and an aldose sugar to a ketose with two additional carbons.

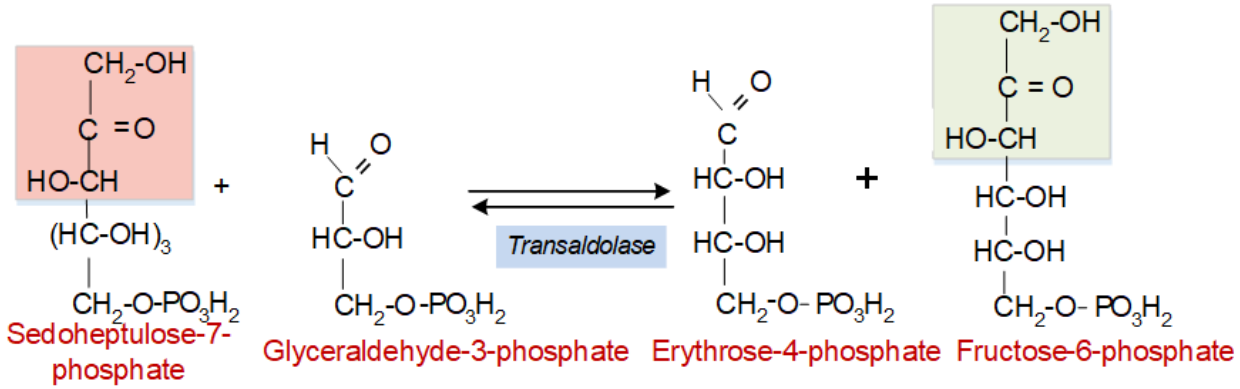
To catalyze this reaction, *transketolase* requires the presence of two coenzymes: Mg^{2+} and **thiamine pyrophosphate (TPP).** Specifically, the enzyme transfers the two-carbon unit from xylulose-5-phosphate to ribose-5-phosphate, resulting in the formation of two new molecules: sedoheptulose-7-phosphate, which is a seven-carbon ketose, and glyceraldehyde-3-phosphate, which is an aldose.



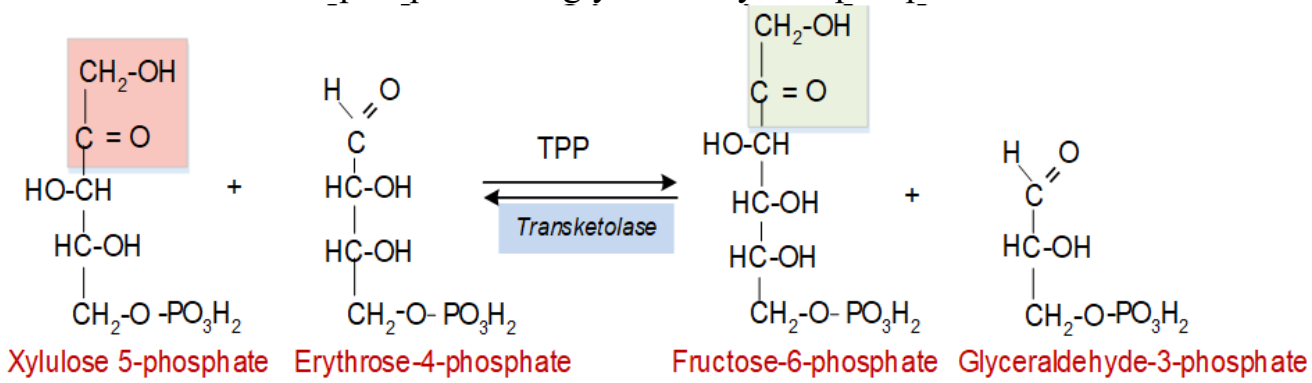
After transketolase transfers the two-carbon unit from xylulose-5-phosphate to ribose-5-phosphate, the resulting sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate undergo **transaldolation**, a reaction catalyzed by *transaldolase.*

Transaldolase catalyses the transfer of a three-carbon dihydroxyacetone moiety, comprising carbons 1-3, from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate. This results in the formation of two new molecules: fructose 6-phosphate, a ketose with six carbons, and erythrose 4-phosphate, an aldose with four carbons.

Unlike transketolase, transaldolase does not require any cofactor to catalyze the reaction.



The second ***transketolase*** **reaction,** xylulose-5-phosphate serves as a donor of glycolaldehyde. In this case, erythrose-4-phosphate is the acceptor, and the products of the reaction are fructose-6-phosphate and glyceraldehyde-3-phosphate.



It is important to note that the complete oxidation of glucose to CO_2 via the pentose phosphate pathway is not the primary function of this pathway. As mentioned earlier, the pathway serves mainly to generate NADPH for biosynthetic reactions and to produce ribose-5-phosphate for nucleotide synthesis.

However, in some tissues, such as adipose tissue and lactating mammary glands, the pentose phosphate pathway can contribute to a significant portion of glucose oxidation. In these tissues, the enzymes required for the reversal of glycolysis and the conversion of glyceraldehyde-3-phosphate to glucose-6-phosphate are present.

To fully oxidize one molecule of glucose via the pentose phosphate pathway in these tissues, six molecules of glucose-6-phosphate must enter the pathway, producing 12 molecules of NADPH and 6 molecules of CO_2. It is important to note that this pathway is not as efficient at producing ATP as glycolysis and the TCA cycle, which are the primary pathways for glucose oxidation in most tissues.

The overall reaction may be represented as:

6 Glucose 6-phosphate + 12 $NADP^+$ + 6 H_2O → 6 CO_2 +12 NADPH + 5 glucose 6-phosphate.

Although glucose-6-phosphate is common to both pathways, the PPP is markedly different from glycolysis (table 8.2). Oxidation utilizes $NADP^+$ rather than NAD^+, and CO_2, which is not produced in glycolysis, is a characteristic product. **No ATP** is generated in the PPP, whereas it is a product of glycolysis. The two pathways are, however, connected. The PPP and glycolysis are interconnected through the intermediate metabolite

glyceraldehyde-3-phosphate. Glyceraldehyde-3-phosphate can either be oxidized in the pentose phosphate pathway or it can be used to generate pyruvate and ATP via glycolysis. Additionally, the NADPH generated in the pentose phosphate pathway can be used in biosynthetic pathways, including fatty acid synthesis, which can then be broken down via beta-oxidation to generate ATP.

Table. 8.2. Differences between pentose phosphate pathway and glycolysis:

	PPP	Glycolysis
Location	In certain cells	In all cells
Oxidation of glucose	Oxidation occurs in the first reaction	Phosphorylation occurs first then oxidation
Coenzyme	$NADP^+$	NAD^+
Energy	No energy poduction	2 or 8 ATP produced
CO_2	Produced	Not produced
Pentoses	Produced	Not produced

8.3.1. Biological role of PPP

NADPH produced in the PPP is used for biosynthesis of several important compounds in various organs.

- In the **liver**, NADPH is used for fatty acid synthesis, cholesterol synthesis, bile acid synthesis, glutamate synthesis and cytochrome P450-hydroxylase system.
- In the **adrenal cortex and gonads**, NADPH is used for cholesterol and hormone synthesis.
- In the **adipose tissue**, NADPH is used for fatty acid synthesis.
- NADPH is used for formation of **deoxyribonucleotides and pyrimidine nucleotides**.
- **In RBC**, NADPH produced in PPP is used for the formation of reduced glutathione from oxidized glutathione. *Glutathione reductase* catalyzes this reaction. *Glutathione reductase* contains FAD. Electrons are transferred to FAD from NADPH. Reduced glutathione is required for the removal of H_2O_2 by glutathione peroxidase (fig. 8.6) for the conversion of methemoglobin to normal hemoglobin and for maintenance of –SH groups of erythrocyte proteins. So, reduced glutathione is essential for the integrity of normal red cell structure. Usually cells with reduced glutathione level are more prone to hemolysis.

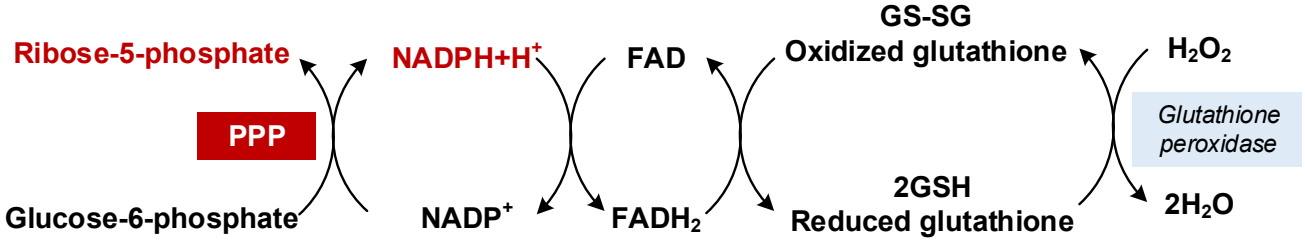


Fig. 8.6. The role of PPP in RBCs

- **In neutrophils**, NADPH is required for the formation of superoxide by NADPH oxidase. Respiratory burst of neutrophils during phagocytosis involves superoxide formation.

Pentoses produced in this pathway are used for nucleic acid synthesis and nucleotide coenzymes like **NAD^+, FAD and FMN synthesis**. Non-oxidative phase of the pathway converts pentoses of endogenous or dietary nucleic acids into intermediates of glycolysis where they are, further oxidized to generate energy.

The PPP converts **xylulose of uronic acid pathway** to either glucose or intermediates of glycolysis.

8.3.2. Regulation of of pentose phosphate pathway

According to cell needs PPP produces either **NADPH or pentoses**. When more **pentoses** are needed glucose-6-phosphate is converted to fructose-6-phosphate and glyceraldehyde-3-phosphate by glycolysis. Pentoses are formed from these molecules through non-oxidative phase of the pathway and there is no NADPH production (fig. 8.7). When cell needs more **NADPH** then glucose-6-phosphate is converted to pentose-5-phosphate, which is inturn converted to fructose-6-phosphate and glyceraldehyde-3-phosphate by non-oxidative branch. Glucose-6-phosphate is again formed from fructose-6-phosphate and glyceraldehyde-3-phosphate through the reversal of glycolysis. Ribose-5-phosphats is formed from regenerated glucose-6-phosphate through the oxidative phaseand thus there is no net pentose production (fig. 8.7).

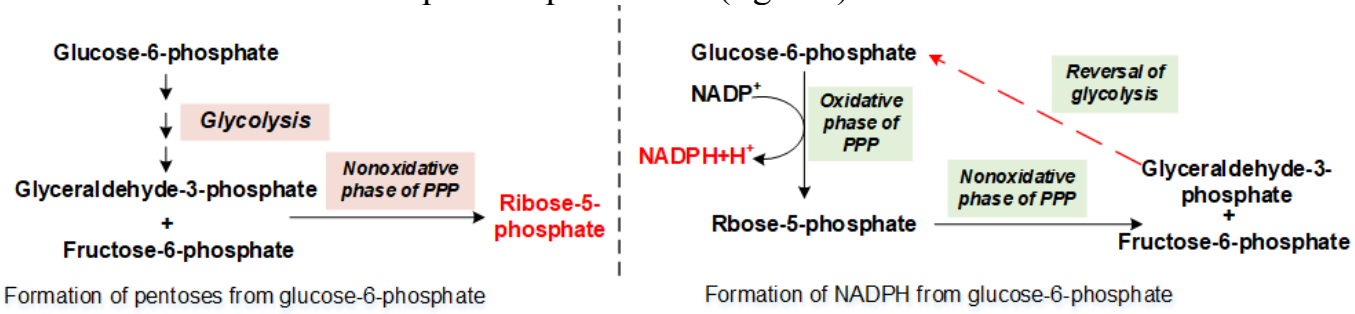


Fig. 8.7. Regulation of PPP according to cell needs

The pentose phosphate pathway is allostericlally regulated. The first reaction catalysed by *glucose-6-phosphate dehydrogenase* is most regulatory in the pathway. This enzyme catalyses an irreversible reaction. **NADPH** competitively inhibits enzyme. It is the ratio of NADPH/NAD^+ that ultimately determines the flux of PPP.

8.3.3. Disorders of pentose phosphate pathway

When individuals with genetic defects in ***glucose-6-phosphate dehydrogenase*** are exposed to oxidative stress from various sources, such as certain drugs or infections, their red blood cells may undergo **hemolysis**, a condition known as **hemolytic anemia**. This is due to the impairment of the generation of NADPH caused by the genetic defect. For example, drugs like the antimalarial primaquine and sulfonamides can trigger this condition in susceptible individuals. Additionally, consumption of **fava beans**, also known as Vicia faba, can cause hemolysis in affected individuals, hence the name of the disease,

favism. The gene for glucose-6-phosphate dehydrogenase can have many different mutations, which can lead to two main variants of **favism**.

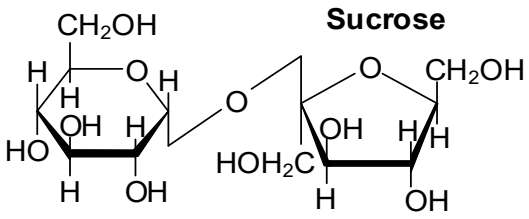
Drugs that can precipitate this reaction:

- antimalarial agents:
- sulfonamides (antibiotic):
- nonsteroidal antiinflammatory drugs (NSAIDs):
- nitrofurantoin:
- quinidine:
- quinine:
- exposure to certain chemicals – mothballs.

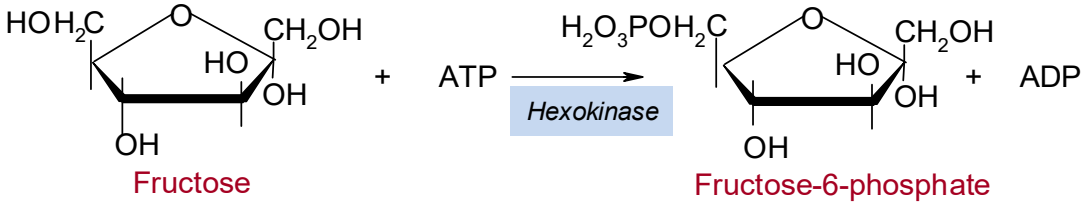
Signs and symptoms of favism: patients with enzyme deficiency show attacks of hemolytic anemia in the form of severe jaundice and decreased, hemoglobin concentration. Other symptoms of favism may include fatigue, weakness, shortness of breath, and dark urine due to the increased breakdown of red blood cells. In severe cases, kidney damage may occur. The onset of symptoms typically occurs within 24-48 hours of exposure to the triggering agent, such as fava beans or certain medications. In some cases, the symptoms may be mild and resolve on their own, while in others, hospitalization and blood transfusions may be necessary. It is important for individuals with glucose-6-phosphate dehydrogenase deficiency to avoid triggers that can cause oxidative stress and lead to hemolytic anemia.

8.4. Enzymatic reactions of fructose turnover in human body. Hereditary enzymopathias of fructose metabolism

The primary source of fructose in our diet is from **sucrose**, a type of disaccharide that contains equal amounts of fructose and glucose. Fructose can also be found in its free form in honey and numerous types of fruits. Unlike glucose, which is carefully regulated by insulin for entry into most tissues of the body, fructose can enter cells without insulin control.

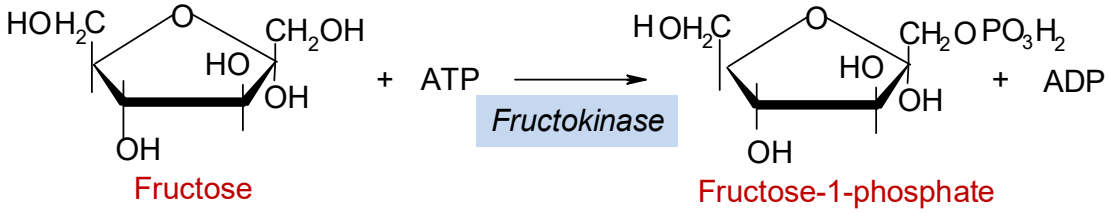


Fructose undergoes phosphorylation by ***hexokinase*** in muscle and adipose tissue, resulting in the formation of fructose-6-phosphate. Since fructose-6-phosphate is an intermediate of glycolysis, it continues to be metabolized through this pathway. However, hexokinase has low affinity (high K_m) for fructose, hence this is a minor pathway.



Fructose is metabolized in the liver, kidney, and intestine through a process that involves the enzyme ***fructokinase***, in presence of magnesium ions, and ATP. During this

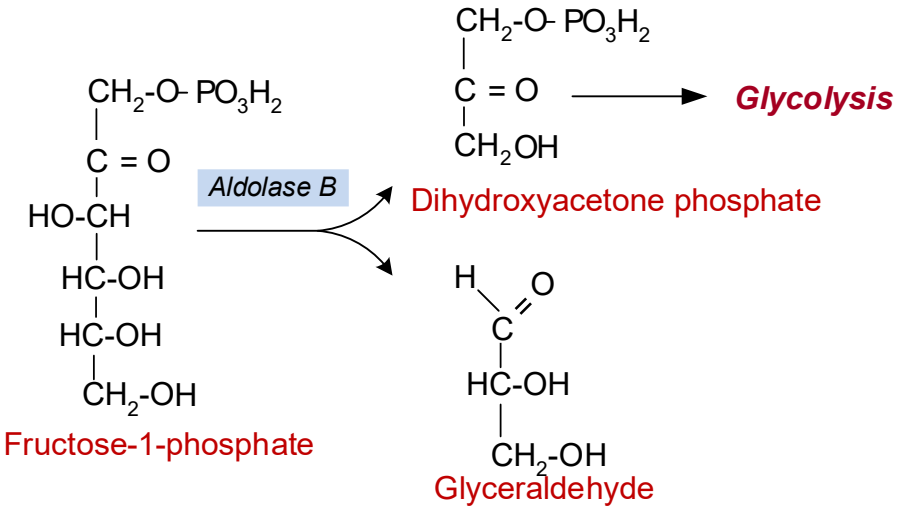
process, ATP donates a phosphate group to fructose at carbon-1, leading to the formation of **fructose-1-phosphate** as the end product.



MEDICAL IMPORTANCE

In fructose metabolism, the phosphofructokinase reaction of glycolysis is bypassed, leading to unregulated conversion of fructose to trioses as opposed to glucose. As a result, the rate of utilization of fructose in the liver is much higher than that of glucose, which explains the lipogenic effect of sucrose. The intermediates or trioses produced from fructose are further converted to acetyl-CoA through pyruvate, leading to increased fatty acid synthesis. However, the capacity of Aldolase B to metabolize fructose is limited, which is why fructose cannot be used as an alternative to glucose in diabetics. Additionally, excess consumption of fructose leads to the depletion of ATP due to the conversion of fructose to fructose-1-phosphate, leading to liver damage.

Aldolase B (fructose-1-phosphate aldolase) is responsible for breaking down fructose-1-phosphate in the liver into two trioses. This process results in the formation of dihydroxyacetone phosphate and glyceraldehyde as the reaction products. This is in contrast to fructose 6-phosphate which is converted to fructose 1, 6-bisphosphate and split by ***aldolase A***.



In the liver, ***aldehydekinase*** converts glyceraldehyde to glyceraldehyde-3-phosphate using ATP as the phosphate donor and requiring the presence of Mg^{2+}. Glycolysis and gluconeogenesis enzymes then facilitate the reversible reactions of glucose to and from glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. This process represents the major route of fructose metabolism in the liver. Alternatively, the triose phosphate may undergo glycolytic reactions to produce pyruvate and generate ATP. Additionally, the liver and other tissues utilize another pathway for glyceraldehyde metabolism, in which glyceraldehyde is converted to dihydroxyacetone phosphate through

glycerol-3-phosphate. Both dihydroxyacetone phosphate and glycerol-3-phosphate are used for triglyceride or phospholipid synthesis.

Alcohol dehydrogenase converts glyceraldehyde to glycerol in a NADH dependent reduction reaction. **Glycerokinase** phosphorylates glycerol to glycerol-3-phosphate. ATP is the phosphate donor and Mg^{2+} is also required. Glycerol-3-phosphate serve as precursor for triglyceride and phospholipid synthesis.

Dihydroxy acetone phosphate is generated from glycerol-3-phosphate by glycerol-***3- phosphate dehydrogenase*** in a NAD^+ dependent oxidation reaction. Dihydroxyacetone phosphate so formed can enter glycolysis or may be used for lipid biosynthesis (fig.8.8).

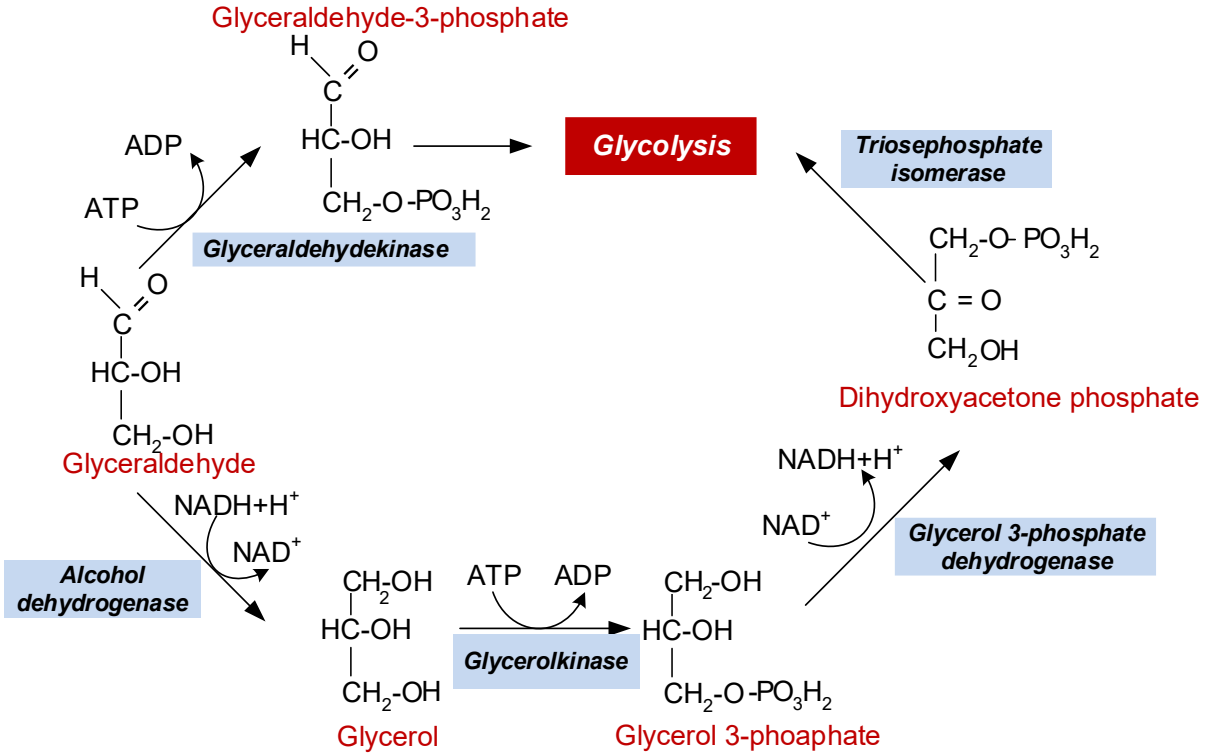


Fig.8.8. Metabolism of glyceraldehyde in liver.

Disorders of fructose metabolism:

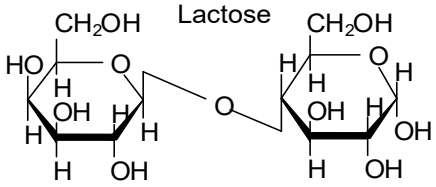
- **Essential fructosuria:** Due to the deficiency of the enzyme hepatic **fructokinase,** fructose is not converted to fructose 1-phosphate. This is an asymptomatic condition with excretion of fructose in urine. Treatment involves the restriction of dietary fructose.
- **Hereditary fructose intolerance**: This is due to the absence of the enzyme a**ldolase B**. Hereditary fructose intolerance causes intracellular accumulation of fructose .l -phosphate, severe hypoglycemia, vomiting, hepatic failure and jaundice. Fructose 1-phosphate allosterically inhibits liver phosphorylase and blocks glycogenolysis leading to hypoglycemia. Early detection and intake of diet free from fructose and sucrose, are advised to overcome fructose intolerance.

MEDICAL IMPORTANCE

Consuming diets high in sucrose or high-fructose syrups (HFS) found in many manufactured foods and beverages can result in large amounts of fructose (and glucose) entering the hepatic portal vein. Fructose undergoes more rapid glycolysis in the liver compared to glucose, which allows it to flood the pathways in the liver and lead to increased fatty acid synthesis, esterification of fatty acids, and secretion of VLDL. This may ultimately raise serum triacylglycerols and LDL cholesterol concentrations. Fructokinase in the liver, kidney, and intestine is responsible for catalyzing the phosphorylation of fructose to fructose-1-phosphate. Unlike glucokinase, fructokinase is not affected by fasting or insulin, and it does not act on glucose. This may explain why fructose is cleared from the blood of diabetic patients at a normal rate.

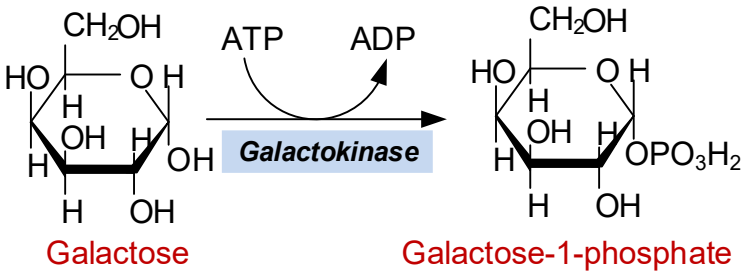
8.5. Enzymatic reactions of galactose metabolism in human body. Hereditary enzymopathias of galactose metabolism

The primary source of galactose in the diet is from lactose. However, galactose can also come from the lysosomal breakdown of glycolipids, glycoproteins, and normal cell turnover. It's important to note that insulin is not required for the entry of galactose into liver cells.



To convert galactose to glucose, a process called active galactose formation is involved. This process generates active galacto se, which acts as a donor of galactose for the synthesis of various compounds such as glycolipids, mucopolysaccharides, blood group substances, and lactose. The formation of active galactose from galactose follows a similar set of reactions to the formation of active glucose from glucose.

Galactokinase catalyzes the phosphorylation of galactose, using ATP as phosphate donor.



Galactose 1-phosphate reacts with **UDPGlc (Uridine Diphosphate Glucose)** to form uridine **diphosphate galactose (UDPGal)** and glucose 1-phosphate. ***Galactose-1-phosphate uridyl transferase*** catalyzes this reaction. It transfers galactose to UDPGlc by replacing glucose.The conversion of UDPGal to UDPGlc is catalyzed by ***UDPGal 4-epimerase.*** The reaction involves oxidation, and then reduction, at carbon 4, with NAD^+ as a coenzyme. The UDPGlc is then incorporated into glycogen. The **epimerase** reaction is freely reversible, so glucose can be converted to galactose, and galactose is not a dietary essential (fig. 8.9).

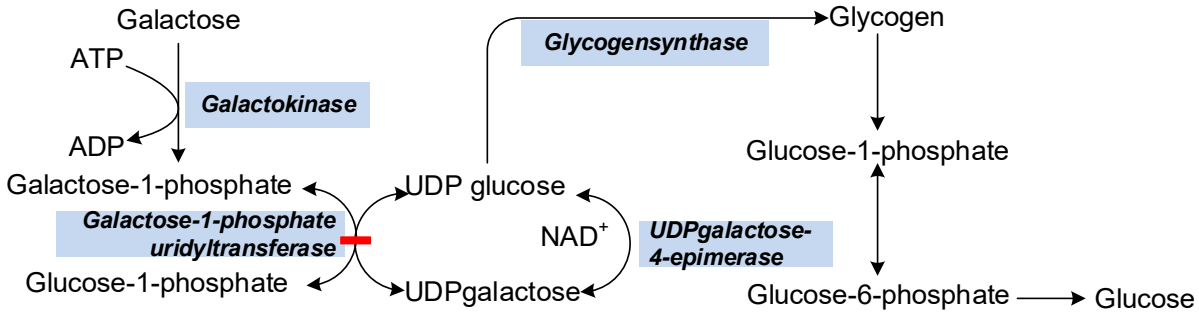


Fig. 8.9. Metabolism of galactose: The initial step involves the conversion of galactose to galactose-1-phosphate, which is catalyzed by the enzyme galactokinase. Galactose-1-phosphate is then converted to glucose-1-phosphate through a series of reactions involving the enzymes galactose-1-phosphate uridylyltransferase and phosphoglucomutase. Glucose-1-phosphate can then enter the glycolytic pathway and be further metabolized for energy production.

During lactation, galactose is an important component of milk and is metabolized in mammary gland cells to support lactose synthesis, which is the primary carbohydrate in milk. In the synthesis of lactose in the mammary gland, UDPGal condenses with glucose to yield lactose, catalyzed by lactose synthase (fig.8.10).

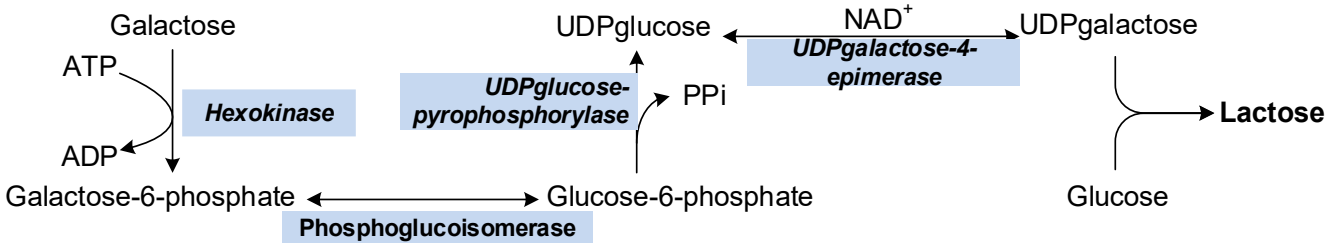


Fig. 8.10. Metabolism of galactose during lactation. UDP-galactose is used as a substrate for lactose synthase, which catalyzes the transfer of galactose from UDP-galactose to glucose to form lactose. Lactose is then transported to the mammary gland ducts for secretion into milk.

Disorders of galactose metabolism Inability to metabolize galactose occurs in the **galactosemias**, which may be caused by inherited defects of **galactokinase**, **uridyl transferase**, or **4-epimerase**, though deficiency of uridyl transferase is best known. Galactose is a substrate for aldose reductase, forming **galactitol**, which accumulates in the lens of the eye, causing **cataract**. The condition is more severe if it is the result of a defect in the uridyl transferase since galactose-1-phosphate accumulates and depletes the liver of inorganic phosphate.

Ultimately, **liver failure** and mental deterioration result. In uridyl transferase deficiency, the epimerase is present in adequate amounts, so that the galactosemic individual can still form UDPGal from glucose. This explains how it is possible for normal growth and development of affected children to occur despite the galactose-free diets used to control the symptoms of the disease.

REVIEW TEST:

№	MCQs	Answers and explanations
1.	**A patient, who has been subsisting exclusively on polished rice, has developed polyneuritis due to thiamine deficiency. What substance is an indicator of such avitaminosis, when it is excreted with urine?** A. Phenyl pyruvate B. Malate C. Methylmalonicacid D. Uric acid E. Pyruvic acid	**The answer is E.** Vitamin B_1 deficiency (beri-beri) is mostly seen in populations consuming exclusively polished rice as staple food. Thiamine pyrophosphate is a coenzyme of pyruvate dehydrogenase complex catalyses conversion of pyruvate to acetyl CoA. Thiamine deficiency results in inhibition of oxidative decarboxylation of pyruvate and accumulation of pyruvate in blood and urine.
2.	**It is known that pentose-phosphate pathway actively functions in the erythrocytes. What is the main function of this metabolic pathway in the erythrocytes?** A. Counteraction to lipid peroxidation B. Activation of microsomal oxidation C. Neutralization of xenobiotics D. Oxidation of glucose into lactate E. Increase of lipid peroxidation	**The answer is A.** Pentose-phosphate pathway is unique in generating two important products – penfoses and NADPH. NADPH produced in erythrocytes maintains the concentration of reduced glutathione which detoxifies H_2O_2 and prevent lipid peroxidation processes.
3.	**An infant, who was on synthetic formula feeding, developed signs of vitamin B_1 deficiency. What reactions does this vitamin take part in?** A. Amino acids transamination B. Keto acids oxidative decarboxylation C. Amino acids decarboxylation D. Proline hydroxylation E. Redox reactions	**The answer is B.** The coenzyme of vitamine B_1 - thiamine pyrophosphate or cocarboxylase - takes part in reactions of oxidative decarboxylation of α-keto acids (pyruvate, α-ketoglutarate).
4.	**It has been determined that one of a pesticide components is sodium arsenate that blocks lipoic acid. Enzyme activity can be impaired by this pesticide. Name this enzyme:** A. Glutathione peroxidase B. Microsomal oxidation C. Methemoglobin reductase D. Pyruvate dehydrogenase complex E. Glutathione reductase	**The answer is D.** The conversion of pyruvate to acetyl CoA (by pyruvate dehydrogenase) requires lipoic acid. Lipoic acid deficiency results in inhibition of pyruvate dehydrogenase complex activity.
5.	**Fructosuria is known to be connected with inherited deficiency of fructose-1-phosphate aldolase. What product of fructose metabolism will accumulate in the organism resultingin toxic action?** A. Fructose-1-phosphate B. Glucose-1-phosphate C. Glucose-6-phosphate D. Fructose-1,6-biphosphate	**The answer is D.** Fructosuria - disturbance of fructose metabolism due to the deficiency of the enzyme fructose-1-phosphate aldolase. Fructose 1-phosphate is cleaved to glyceraldehyde and dihydroxyacetone phosphate by fructose-1-phosphate aldolase. Hereditary fructose intolerance causes intracellular accumulation of fructose-l-phosphate, severe hypoglycemia, vomiting, hepatic failure and jaundice.

	E. Fructose-6-phosphate	
6.	**A young man was given primaquine as a prophylaxis before his trip to a malaria-endemic area in India. However, he developed a hemolytic condition shortly after taking the medication. The probable reason for the hemolysis is a deficiency in which of the following components?** A. Glucose 6-phosphate B. Oxidized form of NAD C. Reduced form of glutathione D. Ribose 5-phosphate E. Ribulose 5-phosphate	**The answer is C.** Glutathione is necessary for maintaining the integrity of red blood cells, and it relies on NADPH-dependent glutathione reductase to keep it functional. NADPH is produced by the oxidative part of the pentose phosphate pathway. However, people with a deficiency of the G6PD enzyme in this pathway cannot generate NADPH effectively, resulting in a reduced ability to maintain functional glutathione. Hemolytic anemia may occur in G6PD-deficient patients who receive oxidant drugs like primaquine. Primaquine does not affect glucose 6-phosphate levels, and neither does NAD+ play a role in glutathione reductase. Ribulose 5-phosphate can be converted to ribose 5-phosphate, but a deficiency in either does not cause hemolysis.
7.	**A 14-day-old breast-fed neonate fails to gain weight during infancy. Although concerned, the mother continues to breast-feed and wait. The infant subsequently develops cataracts, an enlarged liver, and mental retardation. Urin alysis is significant for high levels of galactose in the urine, as well as galactosemia. What food product inthe baby's diet is leading to these symptoms?** A. Fructose B. Lactose C. Phenylalanine D. Glucose E. Sorbitol	**The answer is B.** The patient has a condition called classic galactosemia, which means their body can't break down galactose after they consume lactose in breast milk. This is caused by a genetic mutation inherited in an autosomal recessive manner that affects the galactose1-phosphate uridylyl-transferase enzyme. To treat this condition, the patient needs to avoid consuming lactose and galactose. The enzyme deficiency results in the accumulation of galactose and galactose 1-phosphate, which can lead to cataracts due to galactitol buildup and hepatomegaly due to inhibition of phosphoglucomutase by high levels of galactose1-phosphate.
8.	**A 19-year-old, African American male militaryre cruit is about to besent to Iraqon his assignment. In preparation for his tour of duty, he is given aprophylactic dose of primaquine to prevent malaria. Several days after he begins takingthe drug, he develops fatigue and hemolytic anemia. Which of the following proteins is likely deficient?** A. Fructokinase B. Aldolase B C. Glucose6-phosphate dehydrogenase D. Galactokinase E. Galactosyl transferase	**The answer is C.** Drugs such as primaquine and sulfa-containing drugs can cause hemolytic disease in individuals with G6PDH deficiency by inducing oxidative stress. When strong oxidizing agents are present, individuals who lack G6PDH cannot effectively regenerate reduced glutathione in their red blood cells, which can result in damage to the cell membrane and ultimately lead to cell lysis. Fructokinase deficiency is a harmless condition, whereas aldolase B deficiency can cause hereditary fructose intolerance. Galactokinase deficiency results in a milder form of galactosemia compared to deficiency in galactose 1-phosphate uridylyl-transferase. Additionally, galactosyltransferase plays a crucial role in protein glycosylation and in the metabolism of substances like bilirubin.
9.	**A native of East Africa presents with jaundice and splenomegaly after eating fava beans. A blood smear reveals**	The answer is A. The patient's glucose6-phosphate dehydrogenase (G6PDH) is defective, which reduces their ability to produce NADPH. NADPH is essential for the production

	hemolysis. Wich of the following enzymes is deficient? A. Glucose6-phosphate dehydrogenase B. Galactose 1-phosphateuridylyl transferase C. N-acetylglucosamine 1-phosphate transferase D. Galactokinase E. Fructokinase	of the reduced form of glutathione, which provides protection against oxidative damage. This deficiency is especially noticeable in red blood cells (RBCs), as they do not have mitochondria and can only generate NADPH via G6PDH. When exposed to strong oxidizing agents, such as those found in fava beans, the RBCs are unable to produce enough reduced glutathione to protect their membrane from oxidative damage. This results in damage to the RBCs' membranes and their subsequent lysis. The jaundice is caused by the excess heme (from hemoglobin) released from the RBCs. This heme is converted into bilirubin, which overwhelms the liver's conjugation system and leads to the accumulation of bilirubin in the body.
10	**A 12-month-old, other wise healthy male has cataracts and galactosemia. Difficiency of which of the following enzymes may lead to the disease:** A. Glucose6-phosphate dehydrogenase B. Galactose 1-phosphateuridylyl transferase C. N-acetylglucosamine 1-phosphate transferase D. Galactokinase E. Fructokinase	**The answer is D.** The child has a galactokinase deficiency. Galactose can not be converted to galactose 1-phosphate, so galactose accumulates whenever lactose is present in the diet. The elevated galactose is converted tot he sugar alcohol galactitol int he lens oft he eye by aldosereductase, which leads to cataract formation. This is a less severe disorder than galactose 1-phosphateuridylyltransferase deficiency because the toxic metabolite galactose 1-phosphate does not accumulate. Galactokinase deficiency is considered the nonclassic form of galactosemia.

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9. BREAKDOWN AND BIOSYNTHESIS OF GLYCOGEN. REGULATION OF GLYCOGEN METABOLISM, BIOSYNTHESIS OF GLUCOSE – GLUCONEOGENESIS

OBJECTIVES after studying this chapter, you should be able to:

- *Explain characteristic features of glycogen breakdown and biosynthesis.*
- *Analyze mechanisms of humoral regulation of glycogen metabolism in liver and muscles.*
- *Explain hereditary disorders of glycogen metabolism.*
- *Know genetically determined disorders of glycoconjugate metabolism*
- *Analyze specific features of gluconeogenesis reactions and substrates of this process.*
- *Explain and interpret regulatory mechanisms of gluconeogenesis*

9.1. Glycogen biosynthesis – glycogenesis.

In humans, glycogen serves as the primary carbohydrate storage molecule, analogous to how starch functions in plants. This storage molecule is composed of α-D glucose units that are connected by **1→4 glycosidic bonds**, linking the first carbon atom of one glucose residue to the sixth carbon atom of an adjacent glucose molecule. When a glycogen fragment contains 8 to 10 glycosidic residues, branching occurs through **1→6 linkages (fig. 9.1).**

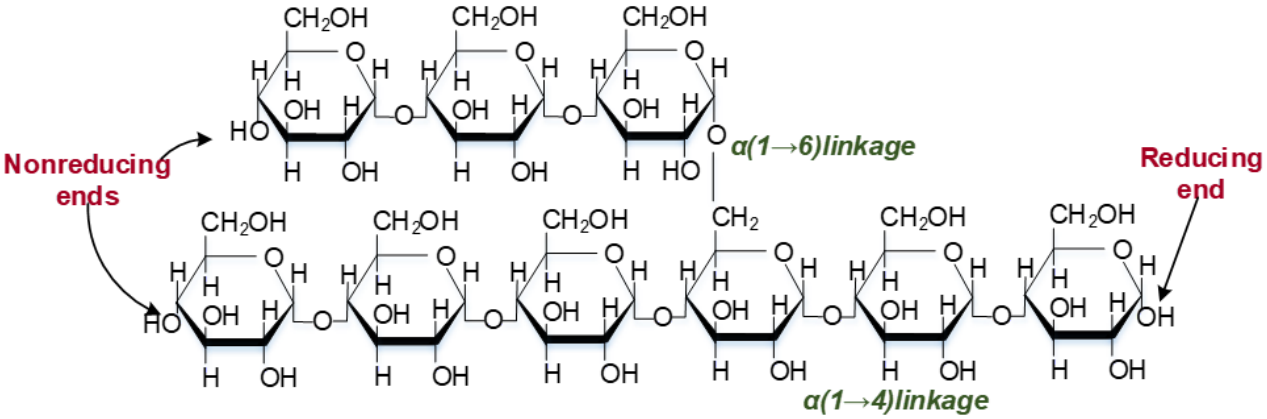


Fig. 9.1. Glycogen is a branched polymer consisting of repeated units of α-D-glucose. The glucose molecules are linked together by α-1,4-glycosidic bonds, forming linear chains. Once a chain contains approximately 8 to 10 glucose residues, branching occurs through α-1,6-glycosidic bonds, creating a three-dimensional network.

Glycogen is primarily present in the liver and muscles, and to a lesser extent in the brain. While the liver contains more glycogen than the muscles, the higher muscle mass in the body means that approximately 75% of the total glycogen in the body is stored in the muscles.

Functions of glycogen:

- Liver glycogen: It maintains normal blood glucose concentration especially during the early stage of fasting (between meals). After 12-18 hours fasting, liver glycogen is depleted.
- Muscle glycogen: It acts as a source of energy within the muscle itself especially during muscle contractions.

9.1.2. Reactions of glycogen synthesys

Glycogenesis – is the synthesis of glycogen in liver and muscles. Glycogen is synthesized from molecules of α-D-glucose. The process occurs in the cytosol, and requires energy supplied by ATP and the presemce of uridine triphosphate (UTP).

First, glucose is phosphorylated to glucose-6-phosphate, catalysed by ***hexokinase*** in muscle and ***glucokinase*** in liver. Then, glucose6-phosphate is isomerized to glucose 1-phosphate by ***phosphoglucomutase***. The enzyme itself is phosphorylated, and the phosphate group takes part in a reversible reaction (in this reaction glucose 1,6-bisphosphate serves as an intermediate).

Next, glucose-1-phosphate reacts with uridine triphosphate (UTP) to form the active nucleotide uridine diphosphate glucose (UDPGlc) and pyrophosphate, catalyzed by ***UDPGlc pyrophosphorylase*** (fig.9.2).

Glycogen synthesis cannot start from scratch. It needs a basic molecule (**a primer**) on which the glucose residues can be added so that the chain can get elongated. Thus, UDP-glucose reacts with glycogen **primer,** which may be:

- Few molecules of glucose linked together by α 1→4 linkage.
- A protein called **glycogenin**.

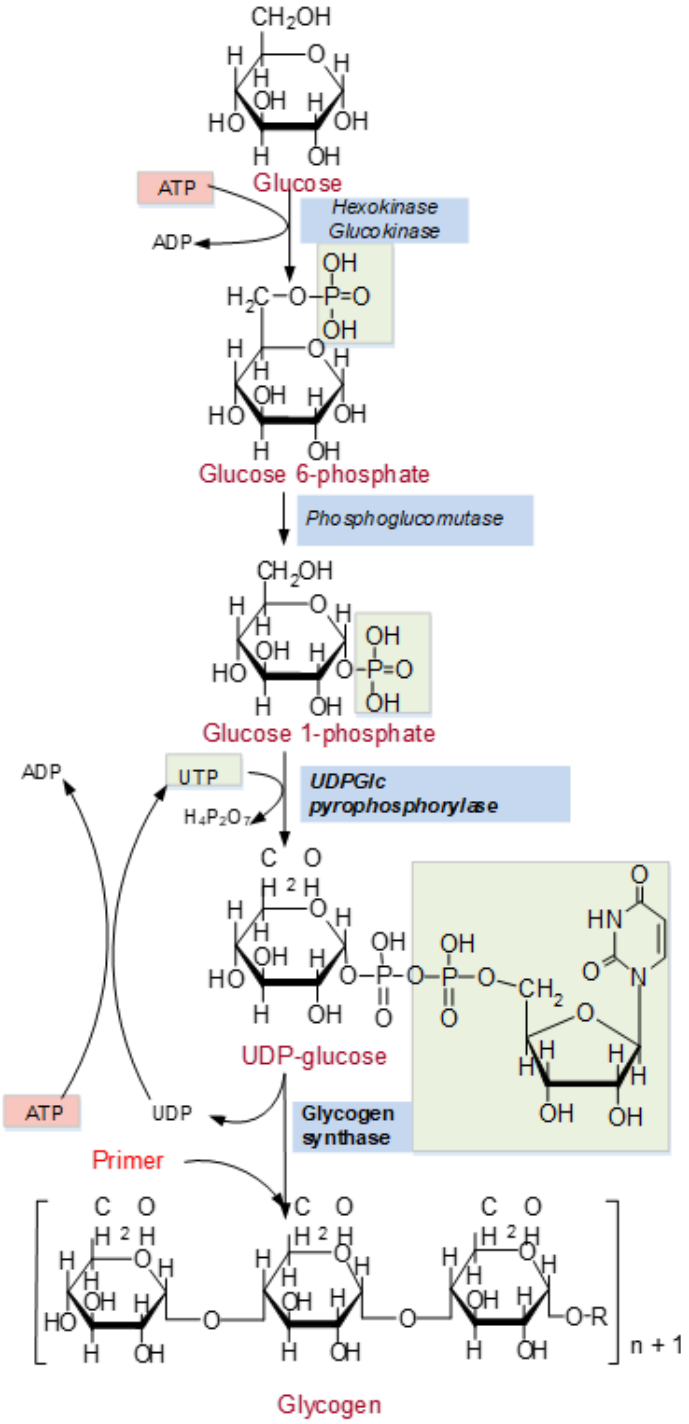


Fig. 9.2. Reactions og glycogen synthesis. Glycogenesis is the process by which glucose molecules are converted and stored as glycogen in liver and muscle cells. It occurs mainly in the liver and muscle tissues.

UDPGlc molecules react with -OH of tyrosine of that protein to initiate glycogen synthesis.

The next step is the actual synthesis of glycogen. By the action of ***glycogen synthase*** **(key enzyme of glycogenesis),** UDP-Glc molecules are added to glycogen primer causing elongation of the α 1→4 branches up to 12-14 glucose units.

Branching enzyme (amylo-α(1→4)→α(1→6)-transglucosidase) transfers parts of the elongated chains (5-8 glucose residues) to the next chain forming a new α 1→6 glycosidic bond. The new branches are elongated by the ***glycogen synthase*** and the process is repeated (fig. 9.3).

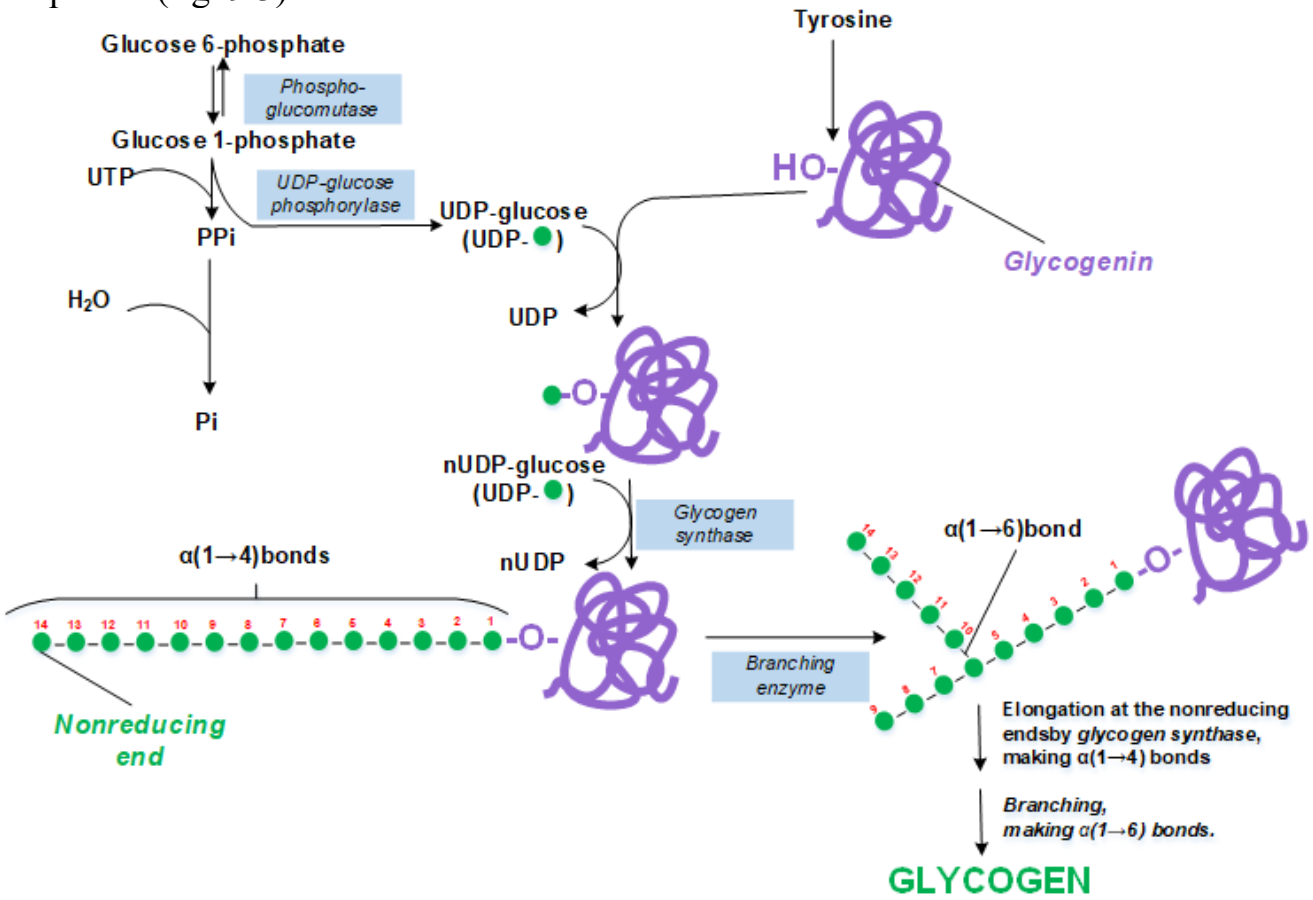


Fig.9.3. Glycogen synthesis. The first step in glycogen synthesis is the conversion of glucose 6-phosphate to glucose 1-phosphate by the enzyme phosphoglucomutase. Glucose 1-phosphate is then activated by the addition of uridine triphosphate (UTP) to form UDP-glucose. This reaction is catalyzed by the enzyme UDP-glucose pyrophosphorylase. Glycogen synthase is the key enzyme in glycogen synthesis, and it catalyzes the transfer of UDP-glucose to a growing glycogen chain. Branching enzyme then catalyzes the formation of branch points in the glycogen molecule.

The process of glycogen synthesis is crucially important in maintaining a balanced blood glucose level. This metabolic process is regulated by the body in response to the fed and fasting states. The availability of substrate is a key factor in the regulation of glycogenesis. When the body is well-fed, the blood glucose level is high, resulting in an increased availability of glucose 6-phosphate, which is the substrate for UDP glucose. This increase in substrate allosterically activates the process of glycogenesis. Conversely, in the fasting state, the availability of substrate is low, and the body needs glucose to sustain normal physiological processes. This results in the breakdown of glycogen, which is the

opposite of the glycogen synthesis process. Therefore, the regulation of glycogen synthesis is tightly monitored to ensure proper blood glucose homeostasis in the body.

9.2. Degradation of glycogen – glycogenolysis.

Glycogenolysis is the process of breack down of glycogen to glucose and glucose 1-phosphate in liver and muscle correspondently.

When there is more supply of glucose to our body, immediately after meals, it gets stored in the form of glycogen in liver and muscles. The stored glycogen comes to rescue when the blood glucose drops down, a situation which prevails between our daily meals.

The pathways for the synthesis and degradation of glycogen are not reversible. An independent set of enzymes present in the cytosol carry out glycogenolysis.

The key enzyme of glycogenolysis is ***glycogen phosphorylase***. This enzyme cleaves the glucose residues sequentially and yield glucose 1-phosphate. *Glycogen phosphorylase* contains a molecule of covalently bound **pyridoxal phosphate (PLP)** that is required as a coenzyme. This cleavage is known as phosphorolysis which continues until 4 residues are present on either side of a branching point. The resultant smaller and less branched glycogen molecule **limit dextrin**. It cannot be broken down further by glycogen phosphorylase (fig. 9.4).

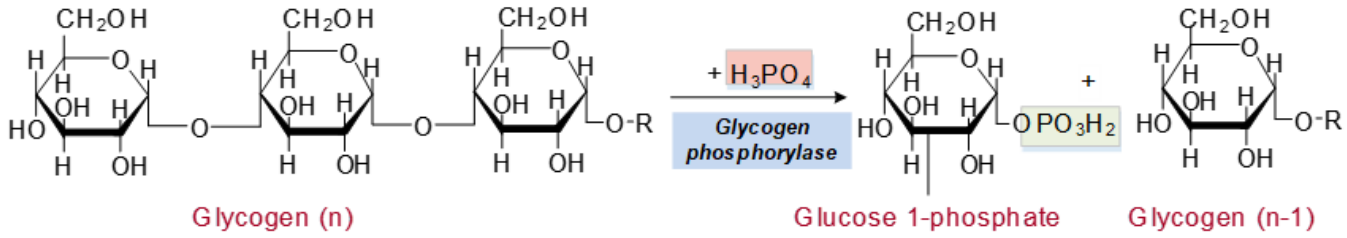


Fig. 9.4. Glycogen phosphorylase reaction.

Debranching enzyme is a single molecule consisting of 2 enzyme activities – α – [1→4]→α-[1→4] glucan transferase and glucosidase. The first enzyme activity removes the glycogen fragment containing 3 or 4 residues in a branch and move them to a nearby chain. This involves breaking up of 1→ 4 glycosidic link in one point and the formation of the same at another point on the molecule (fig. 9.5).

The second enzyme activity breaks the 1→ 6 glycosidic link at the branching point and release free glucose. Once the branching is lost, remaining linear fragment of glycogen is available for the action of phosphorylase and the process continues.

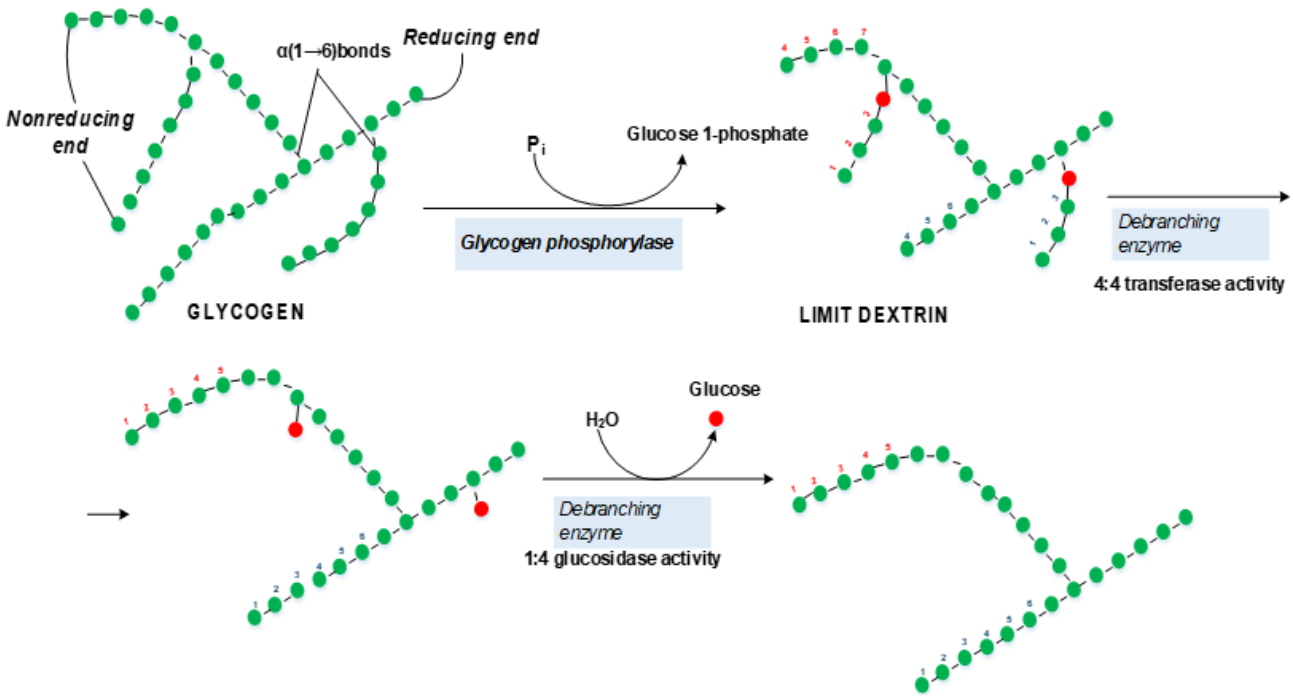
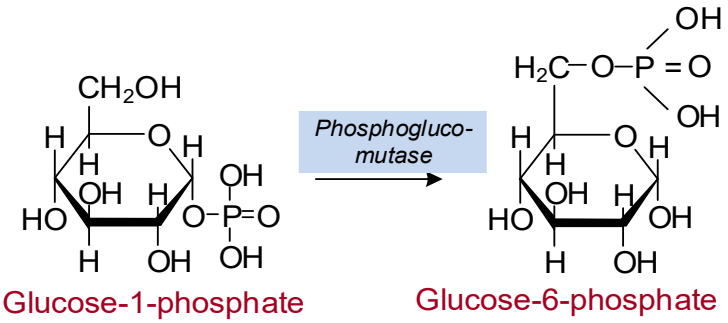
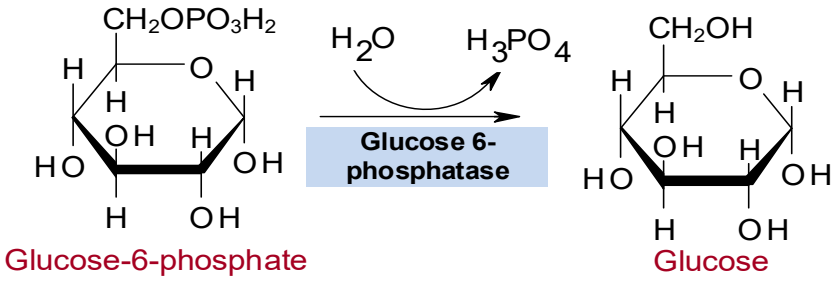


Fig.9.5. Glycogen degradation by glycogen phosphorylase and branching enzyme

The **glucose 1 phosphate** gets converted to glucose 6 phosphate by an enzyme ***phosphoglucomutase.***



The glucose 6-phosphate gets cleaved to glucose by ***glucose 6-phosphatase*** which is present in liver, kidney and intestine.As muscle lacks this enzyme, the glucose 6 phosphate is diverted to glycolysis, which is a process that provides energy to the cells.



Defect in any one of the steps in glycogenolysis results in accumulation of glycogen in the cells resulting in a group of disorders called **glycogen storage disorders** causing damage to liver and muscle. This occurs mainly due to deficiency of the enzymes.

A small amount (1–3%) of glycogen is continuously degraded by the lysosomal enzyme, ***α(1→4)-glucosidase (acid maltase)***. The purpose of this pathway is unknown.

However, a deficiency of this enzyme causes accumulation of glycogen in vacuoles in the lysosomes, resulting in the serious glycogen storage disease Pompe disease.

9.3. Regulation of glycogen metabolism in liver and muscles.

Glycogen synthesis and degradation are regulated to maintain blood glucose levels within a narrow range. The regulation of glycogen metabolism is tissue-specific and depends on the energy needs of the body. In the liver, glycogen is broken down and released as glucose into the bloodstream during fasting to maintain normal blood glucose levels. In the well-fed state, glucose is stored as glycogen in the liver. In skeletal muscle, glycogen is broken down to provide energy during exercise, and it is synthesized during periods of rest to replenish glycogen stores (fig. 9.6).

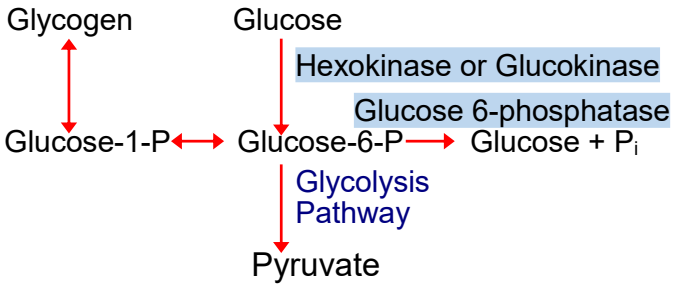


Fig. 9.6. Glycogen metabolism

There are certain metabolites that **allosterically regulate** the activities of *glycogen synthase and glycogen phosphorylase.* High cytosolic **glucose-6-phosphate**, which would result when blood glucose is high, turns off the signal with regard to glycogen synthesis. The conformation of *glycogen synthase* induced by the allosteric activator glucose-6-phosphate is susceptible to dephosphorylation by *protein phosphatase*.

The main enzymes controlling glycogen metabolism are ***glycogen phosphorylase*** *and* ***glycogen synthase*** — are regulated in opposite directions by allosteric mechanisms and covalent modification by reversible phosphorylation and dephosphorylation of enzyme protein in response to hormone action.

Glucagon, a peptide hormone, acts on liver cells, and **epinephrine (adrenaline)** acts on muscle cells to stimulate **glycogen degradation (glycogenolysis)**. Both glucagon and epinephrine activate G-protein coupled receptors to trigger **cAMP cascades (fig. 9.7)**.

The cAMP cascade results in **phosphorylation** of a serine hydroxyl of **glycogen phosphorylase**, which promotes transition to the **active** state. The phosphorylated enzyme is **less sensitive to allosteric inhibitors**. Thus, even if cellular ATP and glucose-6-phosphate are high, phosphorylase will be active. The glucose-1-phosphate produced from glycogen in liver may be converted to free **glucose** for release to the blood. With this hormone-activated regulation, the needs of the organism take precedence over needs of the cell.

Glycogen phosphorylase exists in two forms:

- "**a**" is the form of the enzyme that tends to be **active**, and **independent** of allosteric regulators (in the case of glycogen phosphorylase, when phosphorylated).
- "**b**" is the form of the enzyme that is **dependent** on local allosteric controls (in the case of glycogen phosphorylase when dephosphorylated).

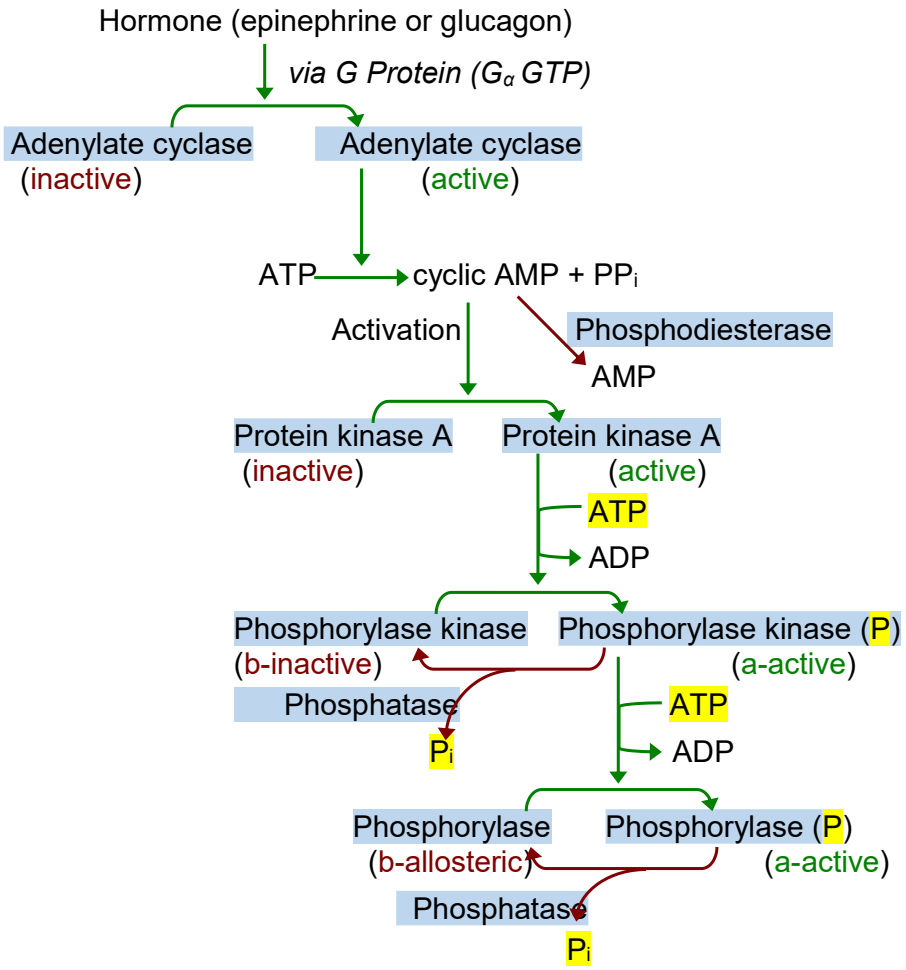


Fig. 9.7. cAMP-dependent regulation of glycogen degradation

The **cAMP cascade** induced in liver by glucagon or epinephrine has the **opposite effect on glycogen synthesis**. **Glycogen synthase** is phosphorylated by **protein kinase A**. **Phosphorylation** of glycogen synthase promotes the "**b**" (less active) conformation. The cAMP cascade thus **inhibits glycogen synthesis**. Instead of being converted to glycogen, glucose-1-phosphate in liver may be converted to glucose-6-phospate, and dephosphorylated for release to the blood.

After meal blood glucose level tends to be increased. This stimulates secretion of **insulin**. Insulin causes the following:

- Stimulation of **phosphodiesteras**e enzyme, which converts, cAMP into AMP i.e. abolishes the stimulatory effect of cAMP.
- Stimulation of **phosphatase** enzyme, which removes phosphate from phosphorylase (inhibiting it) and glycogen synthase (stimulating it). As a result glycogenesis will proceed and glycogenolysis will be inhibited.
- Stimulation of glucose transport into muscle cells, providing increased substrate for glycogen synthesis.

9.4. Glycogen storage diseases

The metabolic defects concerned with the glycogen synthesis and degradation are collectively referred to as **glycogen storage diseases (table 9. 1)**. These disorders are due

to defects in the enzymes which may be either generalized (affecting all tissues) or tissue-specific. The inherited disorders are characterized by deposition of normal or abnormal type of glycogen in one or more tissues.

The liver forms of glycogen storage diseases (type I, III, IV and VI) are marked by hepatomegaly due to increased liver glycogen and hypoglycemia caused by inability to convert glycogen to glucose. The muscle forms (type II, IIIA, V and VII) have mild symptoms appearing during sternous exercise owing to inability to provide energy for muscle contraction.

Type 0 (Glycogen synthase deficiency). There is hypoglycemia; hyperketonemia and early death.

Type I (Glucose-6-phosphatase deficiency)-Von Gierke's disease. This is the most common autosomal recessive disease. This disease is characterized by severe hypoglycemia that coincides with metabolic acidosis, ketonemia and elevated lactate (due to excess glycolysis) and alanine. Hypoglycemia occurs because glycogen cannot be converted back to glucose. A glycogen build up is found in liver causing hepatomegaly. The patients have severe hypoglycemia, hyperlipidemia (increased lipolysis caused by decreased glucose), uricemia (caused by competitive inhibition by lactate of renal tubular urate secretion and increased uric acid production) and growth retardation. Glucagon and epinephrine cannot produce hyperglycemia but result in increased lactate concentration and lipolysis. A variant of the disease type IB has been identified as a defect in endoplasmic reticulum glucose-6-phosphatase transport system. Other forms include a defect in microsomal phosphate or pyrophosphate transport (type IC) and defect in microsomal glucose transport (type ID).

Type II (Lysosomal α-1→4 and α-1→6 Glucosidase deficiency)- Pompes disease. It affects predominantly the heart and skeletal muscle, producing muscle weakness and cardiomegaly. Liver function is normal and patients do not have hypoglycemia. Two forms identified:

(1) infantile (pompes disease) that develop in first few months of life with weakness and respiratory difficulties;

(2) juvenile that is present in second or third decade of life with difficulty in walking.

Type III (Amylo-1,6-Glucosidase deficiency)-Forbe's or Cori's disease. Deficiency of glycogen debranching enzyme results in storage of an abnormal form of glycogen (limit dextrinosis). Both liver and muscle are affected (type IIIA), producing hepatomegaly and muscle weakness. About 15% have only liver involvement (Type IIIB). Differentiation from type I is by hyperglycemic response to galactose, low concentration of urate and lactate in blood, and elevated serum transaminases and creatinine kinase activities.

Type IV (Branching Enzyme deficiency)-Andersons disease of amylopectinosis. It is extremely rare disorder manifested by production of an abnormal form of unbranched glycogen in all tissue. Patients exhibit hepatospleenomegaly with ascites and liver failure. There is death from heart or liver failure before 5 years of age.

Table. 9.1. Glycogen storage diseases

Type	Defective enzyme	Organ affected	Glycogen in the affected organ	Clinical features
I Von Gierke	Glucose 6-phosphatase	Liver, kidney and intestine	Increased amount, normal structure	Glyogen accumulates in hepatocytes and renal cells, enhrged liver and kidney, tasting hypoglycemia, acidemia; hyperlipidemia; ketosis; gouty arthritis.
II Pompe	Lysosomal α-1,4-glucosidase (acid maltase)	All organs	Massive increase in amount, normal structure	Glycogen accumulates in lysosomes in almost all the tissues; heart is mostly involved; enlarged liver and heart, nervous system is also afiected; death occurs at an early age due to heart failure
III Cori (limit dextrinosis, Forbe's disease)	Amylo α-1,6-glucosidase (debranching enzyme)	Muscle and liver	Increased amount, short outer branches	Branched chain glycogen accumulates; clinical manifestations are similar but milder compared to von Gierke's disease
lV Anderson's disease	Glucosyl 4-6 transferase (branching enzyme)	Most tissues	Normal amount, very long outer branches	A rare disease, glycogen with only few branches accumulate; cinhosis ol liver, impairment in liver function. Liver failure cuases death usually before age 2.
V McArdle's disease (typeVglycog	Muscle glycogen phosphorylase	Muscle	Moderately increased amount, normal structure	Muscle glycogen stores very high, not available during exercise; subiects cannot perform strenous exercise; suffer from muscle cramps; blood lactate and pyruvate do not increase after exercise; muscles may get damaged due to inadequate energy supply.
VI Htrs	Liver phosphorylase	Liver	Increased amount	Liver enlarged; liver glycogen cannot form glucose (pyruvate and lactate can be precursors for glucose); mild hypoglycemia and ketosis seen, not a very serious diseas
VII Tarui's disease	Phosphofructokinase	Skeletal muscles	Increased amount, normal structure	Muscle cramos due to exercise: blood lactate not elevated; hemolysis occurs

Type V (Muscle Phosphorylase deficiency)-McArdle's disease. It is also called McArdle's disease usually present in second or third decade with muscle cramps after exercise. Increased plasma creatine kinase activity at rest, failure of ischemic exercise to increase serum lactate concentrations while producing an exaggerated increase in ammonia, moglobinuria and diminished activity of muscle phosphorylase establish the diagnosis.

Type VI (liver phosphorylase deficiency)- Hers' disease. It manifests as hepatomegaly caused by increased deposits of normal glycogen in liver or in red or white blood cells.

Type VII (Muscle and erythrocyte phosphofructokinase deficiency)-Taruis' disease. Patients have abnormal glycogen in muscle. Exercise intolerance, unresponsiveness to glucose administration, and hemolysis (caused by decreased glycolysis in RBC) are noted clinically, producing hyperbilirubinemia, pigmenturia and reticulocytosis.

9.5. Glycoconjugates metabolism

Glycoconjugates are derivatives of carbohydrates covalently linked with other chemical species such as proteins, peptides, lipids and saccharides.

Glycoconjugates are formed in processes termed **glycosylation.** The different kinds of glycoconjugates include:

I. **Proteoglycans**: In the proteoglycans, the glucosaminoglycan moety forms the greater fraction of the molecule (tipically a proteoglycan consists of 95 % of carbohydrates) and is the main site of biological activity, providing multiple binding sites. They are found mainly in the extracellular matrix. They are major components of connective tissue.

II. **Glycoproteins**: membrane bound glycoproteins participate in a wide range of cellular phenomena, including cell recognition, cell surface antigenicity, etc. In the glycoproteins, the majority of the molecule consist of proteins; they have one or more oligosaccharides attached to a protein, and they usually are branched and do not have serial repeats, so they are rich in information, forming highly specific sites for recognition and high affinity binding by other proteins.

III. **Glycolipids**: are membrane lipids in which the hydrophilic head groups are oligosaccharides. As in glycoproteins, glycolipids act as specific sites for recognition by carbohydrate binding proteins. The four types of human RBC have different oligosaccharides (antigens) in their cell membranes. Blood groups depends on the gangliosides (a kind of sphingolipid) in the surface of the RBC.

9.5.2. Structure of glycosaminoglycans

Gycosaminoglycans (GAGs) are complex molecules made up of long chains of hetero-polysaccharides with a negative charge. One of the unique characteristics of GAGs is their ability to bind significant amounts of water, forming a gel-like matrix that makes up the body's ground substance. **The extracellular matrix (ECM),** composed of GAGs, fibrous structural proteins like collagen and elastin, and adhesive proteins like fibronectin,

forms the basis of tissue architecture. The hydrated GAGs not only serve as a flexible support for the ECM but also act as a molecular sieve, affecting the movement of substances through the ECM. These properties also make GAGs essential components in the formation of lubricating mucous secretions. GAGs were originally known as **mucopolysaccharides** due to their association with mucous secretions. The monomers of GAGs are disaccharides, consisting of an amino sugar such as glucosamine, galactosamine, mannose, xylose, fucose and a uronic acids: glucuronic acid and iduronic acid (fig. 9.8). The specific combination and sequence of these monomers determines the properties and functions of each GAG.

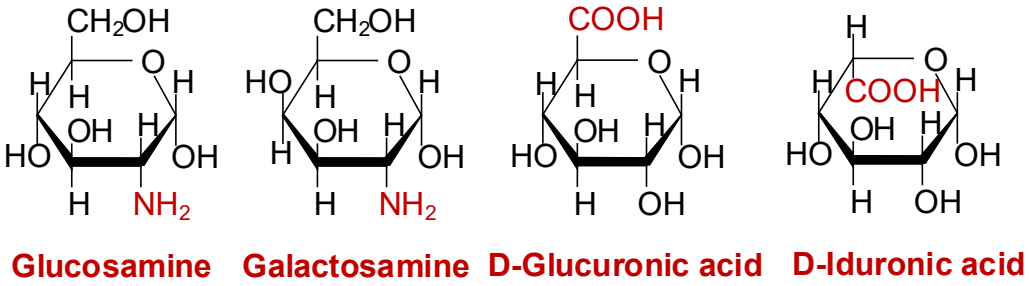


Fig. 9.8. The most common monosaccharides unit for glucosaminoglicans

The six major classes of GAGs are **chondroitin sulfate/dermatan sulfate, keratan sulfate, hyaluronic acid, heparin, heparan sulfate, and sialic acid**. These classes are differentiated based on their monomeric composition, type of glycosidic linkages, and degree and location of sulfate units.

Chondroitin sulfates (fig.9.9) are the most abundant GAGs in the body, composed of disaccharide units N-acetylgalactosamine (GalNAC) with sulfate on either C-4 or C-6, and glucuronic acid (GlcUA). Found in cartilage, tendons, ligaments, and aorta. Form proteoglycan aggregates, often aggregating noncovalently with hyaluronic acid. In cartilage, they bind collagen and hold fibers in a tight, strong network.

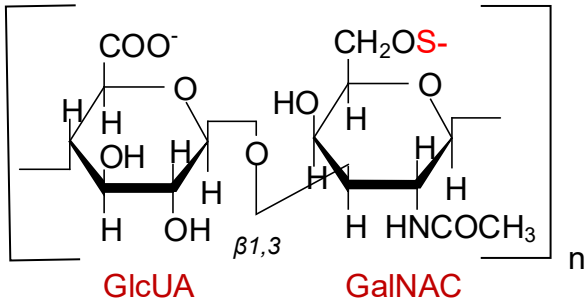


Fig. 9.9. Chondroitin 6-sulfate

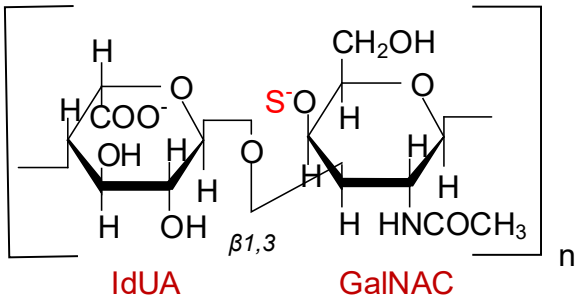


Fig. 9.10. Dermatan -sulfate

Dermatan sulfate (fig.9.10). Is made of disaccharide units N-acetylgalactosamine and L-iduronic acid (with variable amounts of glucuronic acid). Found in skin, blood vessels, and heart valves.

Keratan sulfates I and II are made of disaccharide units - N-acetylglucosamine (GlcNAC) and galactose (Gal) (no uronic acid) (fig. 9.11). Sulfate content is variable and may be present on C-6 of either sugar. Most heterogeneous GAGs because they contain additional monosaccharides such as L-fucose, N-acetylne uraminic acid, and mannose. KS II is found in loose connective tissue proteoglycan aggregates with chondroitin sulfate. KS I is found in cornea.

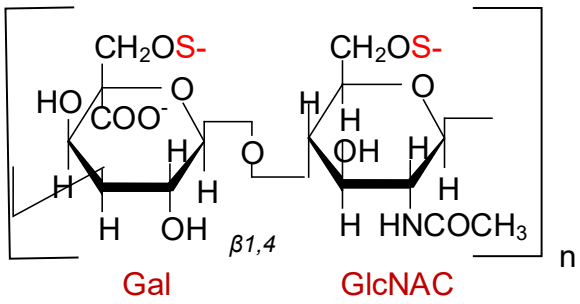


Fig. 9.11. Keratan sulfate sulfate

Heparin contains disaccharide units – glucosamine (GlcN) and glucuronic or iduronic acid (fig. 9.12). Most glucosamine residues are bound in sulfamide linkages. Sulfate is also found on C-3 or C-6 of glucosamine and C-2 of uronic acid (an average of 2.5 per disaccharide unit). α-Linkage joins the sugars. Unlike other GAGs that are extracellular compounds, heparin is an intracellular component of mast cells that line arteries, especially in liver, lungs, and skin. Serves as an anticoagulant.

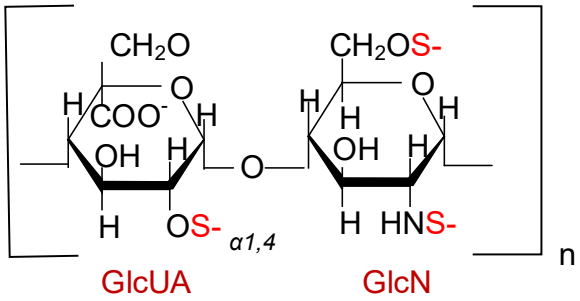


Fig. 9.12. Heparin

Heparan sulfate. Same as heparin except some glucosamines are acetylated and there are fewer sulfate groups. Extracellular GAG, found in basement membrane and as a ubiquitous component of cell surfaces.

Hyaluronic acid. Disaccharide unit of hyaluronic acid are N-acetylglucosamine and glucuronic acid (fig. 9.13). Different from other GAGs: unsulfated, not covalently attached to protein, and only GAG not limited to animal tissue, but also found in bacteria. Serves as a lubricant and shock absorber. Found in synovial fluid of joints, vitreous humor of the eye, the umbilical cord, loose connective tissue and cartilage.

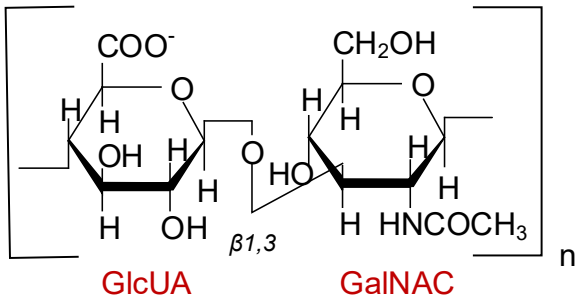


Fig. 9.13. Hyaluronic acid

9.5.3. Metabolism of glycosaminoglycans

Synthesis of GAGs is a complex process that is regulated by a variety of enzymes and proteins. The specific type of GAG produced depends on the cell type and the physiological context in which the synthesis occurs.

Synthesis of amino sugars. Amino sugars, such as glucosamine and galactosamine, are essential components of GAGs. The synthesis of these amino sugars and their subsequent incorporation into GAGs is particularly active in connective tissues. In fact, it has been estimated that as much as 20% of glucose flows through this pathway in these

tissues. Amino sugars are synthesized from glucose or other hexoses through a series of enzymatic reactions.

1. N-Acetylglucosamine (GlcNAc) and N-acetylgalactosamine (GalNAc). The monosaccharide fructose 6-phosphate is the precursor of GlcNAc, GalNAc, and the sialic acids, including N-acetyl neuraminic acid (NANA). In each of these sugars, a hydroxyl group of the precursor is replaced by an amino group donated by glutamine (fig. 9.14).

2. N-Acetylneuraminic acid: N-Acetylneuraminic acid (NANA) is a member of the family of sialic acids, each of which is acylated at a different site. The carbons and nitrogens in NANA come from N-acetyl-mannosamine and phosphoenolpyruvate (fig.9.14)

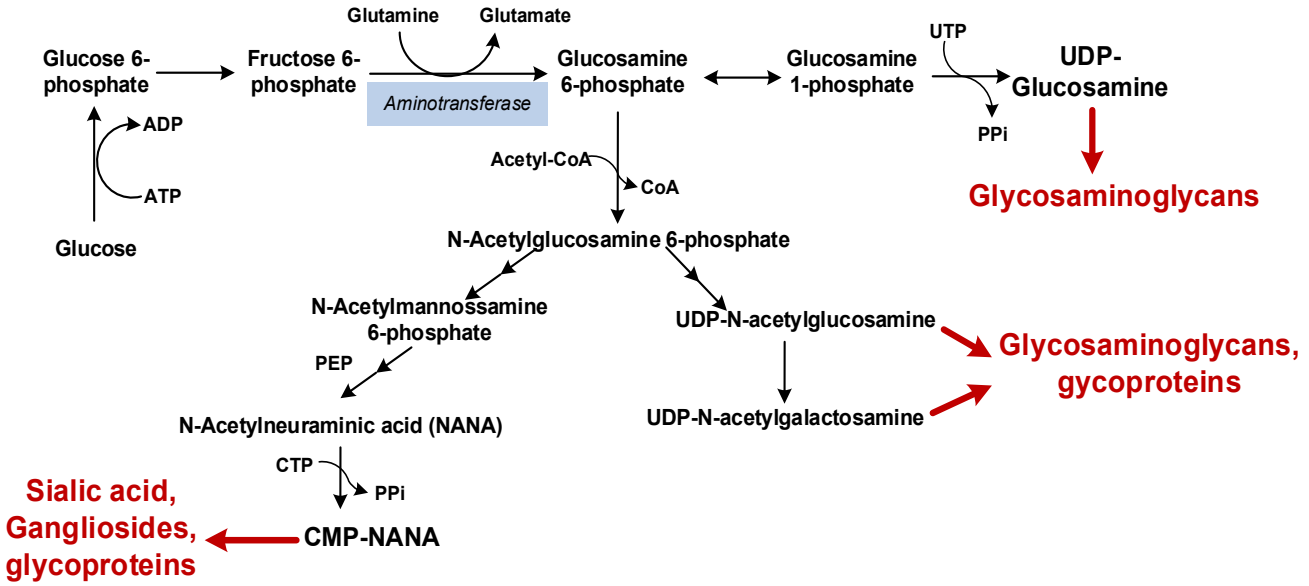


Fig. 9. 14. The biosynthesis of amino sugars can be divided into two main stages: the formation of the basic amino sugar structure and the further modification of this structure to generate the specific amino sugar required for GAG synthesis

Synthesis of acidic sugars. D-Glucuronic acid, whose structure is that of glucose with an oxidized carbon 6, and its C-5 epimer, L-iduronic acid, are essential components of glycosaminoglycans. Glucuronic acid is also required in detoxification reactions of a number of insoluble compounds. The uronic acid pathway also provides a mechanism by which dietary D-xylulose can enter the central metabolic pathways.

1. Glucuronic acid: Glucuronic acid can be obtained in small amounts from the diet. It can also be obtained from the intracellular lysosomal degradation of glycosaminoglycans, or via the uronic acid pathway.

2. L-Iduronic acid synthesis: Synthesis of L-iduronic acid residues occurs after D-glucuronic acid has been incorporated into the carbohydrate chain. Uronosyl 5-epimerase causes epimerization of the D- to the L-sugar.

Synthesis of the core protein. The core protein is synthesized on and enters the rough endoplasmic reticulum (RER). The protein is then glycosylated by bound ***glycosyltransferases*** located in the Golgi.

Synthesis of the carbohydrate chain. Carbohydrate chain formation begins by synthesis of a short linkage region on the core protein on which carbohydrate chain

synthesis will be initiated. The most common linkage region is formed by the transfer of a xylose from UDP-xylose to the hydroxyl group of a serine (or threonine) catalyzed by xylosyltransferase.

Addition of sulfate groups. Sulfation of the carbohydrate chain occurs after the monosaccharide to be sulfated has been incorporated into the growing carbohydrate chain. The source of the sulfate is 3'-phosphoadenosyl-5'-phosphosulfate (PAPS, a molecule of AMP with a sulfate group attached to the 5'-phosphate). Sulfotransferases cause the sulfation of the carbohydrate chain at specific sites.

MEDICAL IMPORTANCE

Defects in the sulfation of the growing GAG chains can result in a group of genetic disorders known as the mucopolysaccharidoses (MPS), also referred to as chondrodystrophies. These disorders are characterized by the accumulation of incompletely degraded GAGs in various tissues and organs, including the skeletal system, which leads to a wide range of clinical symptoms.

Degradation of glycosaminoglycans. GAGs are broken down in lysosomes, where hydrolytic enzymes are most effective at a pH of around 5. This low pH environment helps to protect the cell from damage in case there is a leak into the neutral cytosol. The half-life of glycosaminoglycans varies, with keratan sulfate being the exception, having a half-life of more than 120 days. Hyaluronic acid has a relatively short half-life of about 3 days, while chondroitin and dermatan sulfate have a half-life of around 10 days.

Phagocytosis of extracellular glycosaminoglycans. Because GAGs are located on the extracellular matrix or cell surface, they need to be internalized by the cell via a process called phagocytosis. During this process, the cell membrane invaginates around the GAGs, forming a vesicle that contains the GAGs. This vesicle then fuses with a lysosome, forming a single digestive vesicle where the GAGs can be efficiently degraded by the lysosomal hydrolytic enzymes.

Lysosomal degradation of glycosaminoglycans. The lysosomal degradation of GAGs involves a complex process that requires the action of many different acid hydrolases for complete digestion. Initially, the long polysaccharide chains of the GAGs are cleaved by endoglycosidases, which produce shorter oligosaccharide chains(fig. 9.15). These oligosaccharides are then further degraded sequentially from the non-reducing end of each chain, with the action of various lysosomal hydrolases. This step-by-step degradation allows for the efficient breakdown of the GAGs into their individual monosaccharide components, which can then be further metabolized by the cell.

9.6. Genetically determined disorders of glycoconjugate metabolism: mucopolysaccharidoses

Mucopolysaccharidoses (MPS) are a group of genetic disorders caused by defects in the lysosomal enzymes responsible for the degradation of GAGs. These enzymes are essential for breaking down the long chains of GAGs into smaller fragments that can be recycled or eliminated by the body. When these enzymes are deficient or absent, the incomplete degradation of GAGs leads to their accumulation in various tissues and organs, resulting in a wide range of clinical symptoms.

There are several types of MPS, each caused by a deficiency in a specific lysosomal enzyme responsible for the degradation of GAGs (fig. 9.15):

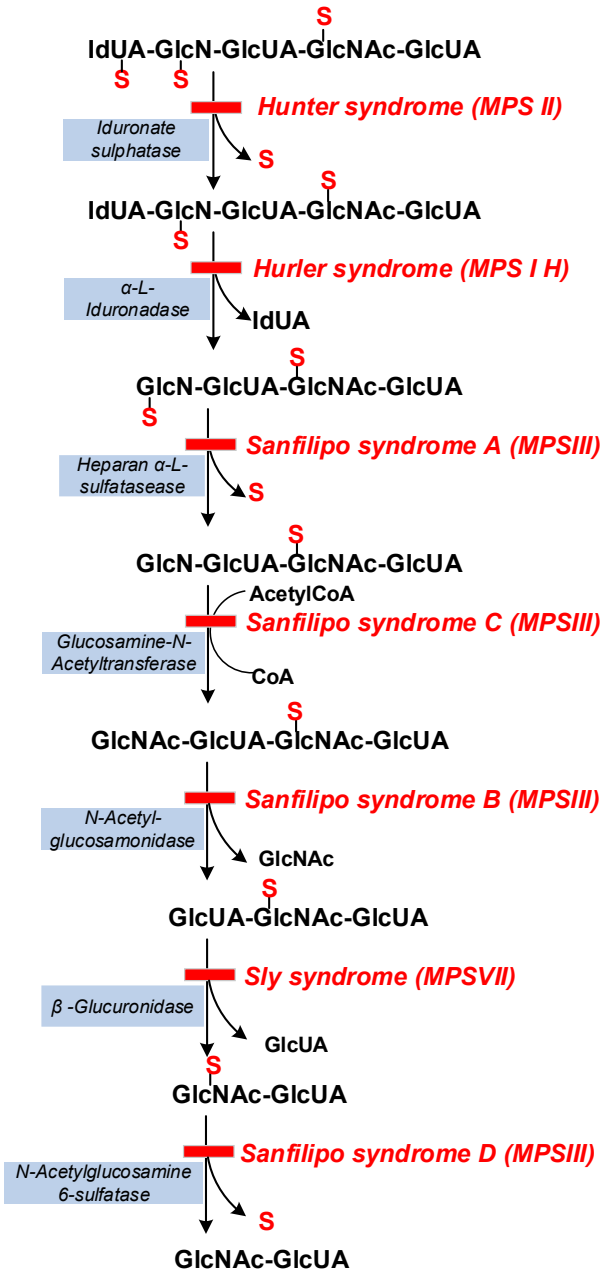


Fig. 9. 15. Degradation of the GAG heparan sulfate by lysosomal enzymes, indicating sites of enzyme deficiencies in some representative mucopolysaccharidoses

- **Hurler syndrome,** also known as MPS I-H, is caused by a deficiency in the enzyme alpha-L-iduronidase, which is responsible for the degradation of heparan sulfate and dermatan sulfate. The deficiency in this enzyme results in the accumulation of these GAGs in various tissues and organs, leading to a wide range of clinical symptoms. The clinical symptoms of Hurler syndrome can vary widely depending on the type and severity of the disorder, but commonly include skeletal abnormalities, such as short stature, joint stiffness, and skeletal deformities. Other symptoms may include enlarged organs, such as the liver and spleen, respiratory problems, heart disease, hearing and vision loss, and neurological impairment.
- **Hunter syndrome**, also known as **MPS II**, is a type of mucopolysaccharidosis caused by a deficiency in the enzyme ***iduronate-2-sulfatase***, which is responsible for the degradation of heparan sulfate and dermatan sulfate. The deficiency in this enzyme results in the accumulation of these GAGs in various tissues and organs, leading to a wide range of clinical symptoms. The clinical symptoms of Hunter syndrome can vary widely depending on the type and severity of the disorder, but commonly include skeletal abnormalities, such as short stature, joint stiffness, and skeletal deformities. Other symptoms may include enlarged organs, such as the liver and spleen, respiratory problems, heart disease, hearing and vision loss, and neurological impairment.
- **Sanfilippo syndrome**, also known as MPS III, is a type of mucopolysaccharidosis caused by a deficiency in one of four enzymes responsible for the degradation of heparan sulfate. The deficiency in these enzymes results in the accumulation of heparan sulfate in various tissues and organs, leading to a wide range of clinical symptoms. The clinical symptoms of Sanfilippo syndrome can vary widely depending on the type and severity of the disorder, but commonly include developmental delay, speech and language problems, behavioral

problems, sleep disturbances, and neurological impairment. Other symptoms may include hyperactivity, aggression, seizures, and joint stiffness.

- **Sly syndrome**, also known as MPS VII, is a type of mucopolysaccharidosis caused by a deficiency in the enzyme beta-glucuronidase, which is responsible for the degradation of dermatan sulfate and chondroitin sulfate. The deficiency in this enzyme results in the accumulation of these GAGs in various tissues and organs, leading to a wide range of clinical symptoms. The clinical symptoms of Sly syndrome can vary widely depending on the type and severity of the disorder, but commonly include skeletal abnormalities, such as short stature, joint stiffness, and skeletal deformities. Other symptoms may include enlarged organs, such as the liver and spleen, respiratory problems, heart disease, hearing and vision loss, and neurological impairment.

9.7. Gluconeogenesis, mechanisms of regulation, compartmentalization of enzymes, biological significance of the process.

Gluconeogenesis is the metabolic process by which the body synthesizes glucose from non-carbohydrate sources such as amino acids, lactate, glycerol, and propionate. **The liver** is the primary site of gluconeogenesis, accounting for approximately 90% of the process, while **the kidneys** contribute the remaining 10%. Gluconeogenesis is an important pathway for maintaining blood glucose levels during periods of fasting or low carbohydrate intake, as the body can convert these non-carbohydrate sources into glucose for use as an energy source.

Functions of gluconeogenesis:

I. Gluconeogenesis supplies the body with glucose:

1. Glucose is the only source of energy for nervous tissues, RBCs and skeletal muscles during exercises.
2. Glucose is important in maintaining adequate concentrations of intermediates of the citric acid cycle.
3. Glucose is the precursor of lactose in mammary gland.
4. Glucose is important during low carbohydrate diet or when liver glycogen is depleted (liver glycogen is depleted after 12-18 hours).

II. Gluconeogenesis clears the blood from the waste products of other tissues as lactate (produced by muscles and RBCs).

Substrates for gluconeogenesis. Gluconeogenic precursors are molecules that can be used to produce glucose through gluconeogenesis, which is the synthesis of glucose from non-carbohydrate sources. These precursors include intermediates from the glycolytic pathway and the tricarboxylic acid (TCA) cycle, as well as glycerol, lactate, and alpha-keto acids produced from the transamination of glucogenic amino acids. These precursors can be converted into glucose through a series of enzymatic reactions in the liver and kidneys, and are important for maintaining blood glucose levels during periods of fasting or low carbohydrate intake.

- **Glycerol:** Glycerol is a three-carbon molecule that is released during the breakdown of triacylglycerols in adipose tissue. It is transported in the bloodstream to the liver, where it can be used as a precursor for glucose synthesis via gluconeogenesis. To be utilized in gluconeogenesis, glycerol must first be converted to glycerol-3-phosphate, which is

catalyzed by the enzyme glycerol kinase. Glycerol-3-phosphate is then oxidized by glycerol-3-phosphate dehydrogenase to form dihydroxyacetone phosphate, an intermediate in the glycolytic pathway. From here, dihydroxyacetone phosphate can be converted to glucose via gluconeogenesis in the liver.

- **Lactate:** Lactate is a three-carbon molecule that is produced during anaerobic metabolism in exercising skeletal muscle and in cells that lack mitochondria, such as red blood cells. It can be used as a substrate for gluconeogenesis, the process of synthesizing glucose from non-carbohydrate sources. During exercise, glucose is converted to pyruvate via glycolysis, which is then converted to lactate when the availability of oxygen is limited. This lactate is released into the bloodstream and taken up by the liver. In the liver, lactate is converted back to pyruvate, which can then be used as a substrate for gluconeogenesis to produce glucose. The newly synthesized glucose can then be released into the bloodstream to be used by other tissues in the body.
- **Amino acids:** During fasting or starvation, amino acids derived from the breakdown of tissue proteins become the major sources of glucose through gluconeogenesis. Glucogenic amino acids can be converted into α-ketoacids, such as α-ketoglutarate, which can enter the TCA cycle and form oxaloacetate (OAA). OAA can then be converted to phosphoenolpyruvate (PEP), a key intermediate in gluconeogenesis. Therefore, α-ketoacids derived from the metabolism of glucogenic amino acids can serve as important precursors for gluconeogenesis during fasting.

9.7.2. Reactions unique to gluconeogenesis

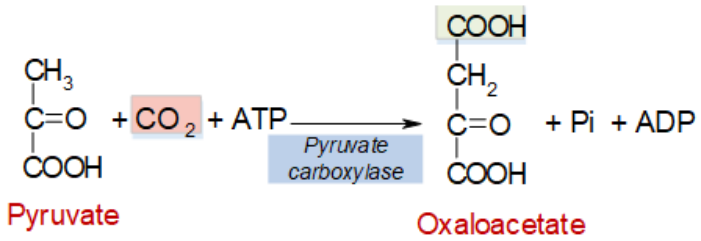
Seven glycolytic reactions are reversible and are used in the synthesis of glucose from lactate or pyruvate. However, three of the reactions are irreversible and must be circumvented by four alternate reactions that energetically favor the synthesis of glucose (table 9.2).

Table 9. 2. Irreversible reactions of glycolysis

Glycolysis	Gluconeogenesis
1. Glucokinase	1. Glucose-6-phosphatase
2. Phosphofructokinase	2. Fructose 1,6 bisphosphatase
3. Pyruvate kinase	3. Pyruvate carboxylase 4. Phosphoenolpyruvate carboxykinase

Pyruvate → Phosphoenolpyruvate reversal of the reaction catalyzed by pyruvate kinase in glycolysis involves two endothermic reactions.

Mitochondrial **pyruvate carboxylase** catalyzes the carboxylation of pyruvate to oxaloacetate, an ATP-requiring reaction in which the vitamin **biotin** is the coenzyme. Biotin binds CO_2 from bicarbonate as carboxybiotin prior to the addition of the CO_2 to pyruvate. The resultant oxaloacetate is reduced to malate, exported from the mitochondrion into the cytosol and there oxidized back to oxaloacetate.



Pyruvate carboxylase is allosterically activated by acetyl CoA. Elevated levels of acetyl CoA in mitochondria signal a metabolic state in which the increased synthesis of oxaloacetate is required. For example, this occurs during fasting, when oxaloacetate is used for the synthesis of glucose by gluconeogenesis in the liver and kidney. Conversely, at low levels of acetyl CoA, *pyruvate carboxylase* is largely inactive, and pyruvate is primarily oxidized by the *pyruvate dehydrogenase complex* to produce acetyl CoA that can be further oxidized by the TCA cycle.

In order for gluconeogenesis to continue, oxaloacetate must be converted to phosphoenolpyruvate. This conversion is catalyzed by an enzyme found in both the mitochondria and cytosol in humans. The phosphoenolpyruvate generated in the mitochondria is transported to the cytosol via a specific transporter, while that generated in the cytosol requires the transport of oxaloacetate from the mitochondria to the cytosol. However, oxaloacetate cannot directly cross the inner mitochondrial membrane and must first be reduced to malate by mitochondrial *malate dehydrogenase*. Malate can then be transported from the mitochondria to the cytosol, where it is reoxidized to oxaloacetate by cytosolic *malate dehydrogenase* as NAD^+ is reduced. This results in the production of NADH, which is used in the reduction of 1,3-BPG to glyceraldehyde 3-phosphate, a step common to both glycolysis and gluconeogenesis. Fig 9.16 illustrates this process.

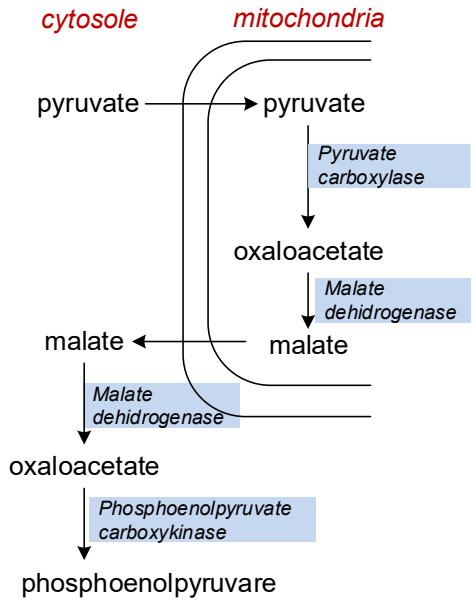
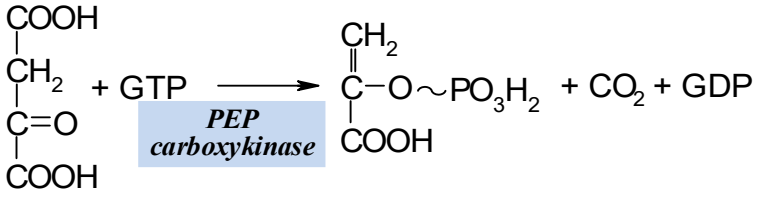


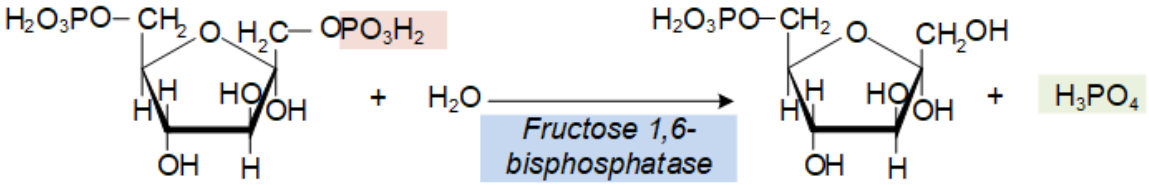
Fig. 9. 16. Transport of oxaloacetate from cytosol to mitochondria

A second enzyme, ***phosphoenolpyruvate carboxykinase***, catalyzes the decarboxylation and phosphorylation of oxaloacetate to phosphoenolpyruvate using GTP as the phosphate donor. In liver and kidney, the reaction of succinate thiokinase in the TCA cycle produces GTP (rather than ATP as in other tissues), and this GTP is used for the reaction of phosphoenolpyruvate carboxykinase, thus providing a link between citric acid cycle activity and gluconeogenesis, to prevent excessive removal of oxaloacetate for gluconeogenesis, which would impair citric acid cycle activity.



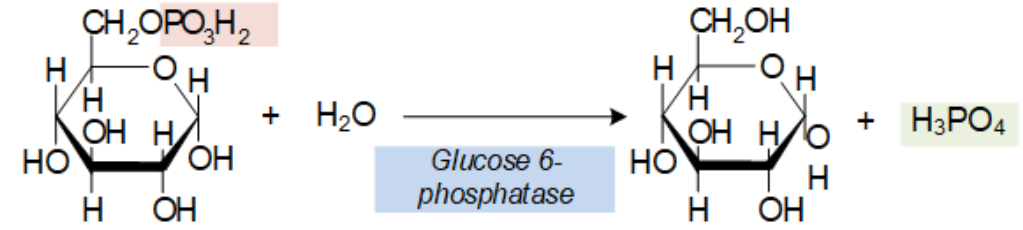
Oxaloacetate Phosphoenolpyruvate

Fructose 1,6-Bisphosphate → Fructose-6-Phosphate. The conversion of fructose 1,6-bisphosphate to fructose-6- phosphate, for the reversal of glycolysis, is catalyzed by **fructose 1,6-bisphosphatase**. Its presence determines whether a tissue is capable of synthesizing glucose (or glycogen) not only from pyruvate, but also from triose phosphates. It is present in liver, kidney, and skeletal muscle, but is probably absent from heart and smooth muscle.



Fructose-1,6-bisphospate Fructose-6-phospate

Glucose-6-Phosphate → Glucose. The conversion of glucose-6-phosphate to glucose is catalyzed by glucose-6-phosphatase. It is present in liver and kidney, but absent from muscle, which, therefore, cannot export glucose into the bloodstream.



Glucose-6-phosphate Glucose

All reactions of gluconeogenesis in comparison to glycolysis are represented on fig. 9.17.

Regulation of gluconeogenesis:

I. Hormonal regulation:

1. **Glucocorticolds** stimulate gluconeogenesis by the following mechanisms:

a) Induce the synthesis of gluconeogenesis enzymes which are: pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-bisphosphatase and glucose-6-phosphatase.

b) Glucocorticoids stimulate protein catabolism by tissues glycogenic amino acids available for gluconeogenesis.

2. **Glucagon:** stimulates gluconeogenesis by lowering the level of fructose 2,6 bisphosphate (see regulation of glycolysis).

3. **Insulin**: Inhibits gluconeogenesis. It acts as repressor (inhibitor) for synthesis of enzymes of gluconeogenesis: pyruvate carboxylase etc.

II. Allosteric regulation via Acetyl CoA and ATP:

1. Stimulate gluconeogenesis by inhibiting glycolysis (through inhibiting phosphofructokinase) and stimulate gluconeogenesis (by stimulating fructose 1,6 bisphosphatase)

2. Acetyl CoA also stimulates pyruvate (gluconeogenesis) and inhibit pyruvate (oxidation).

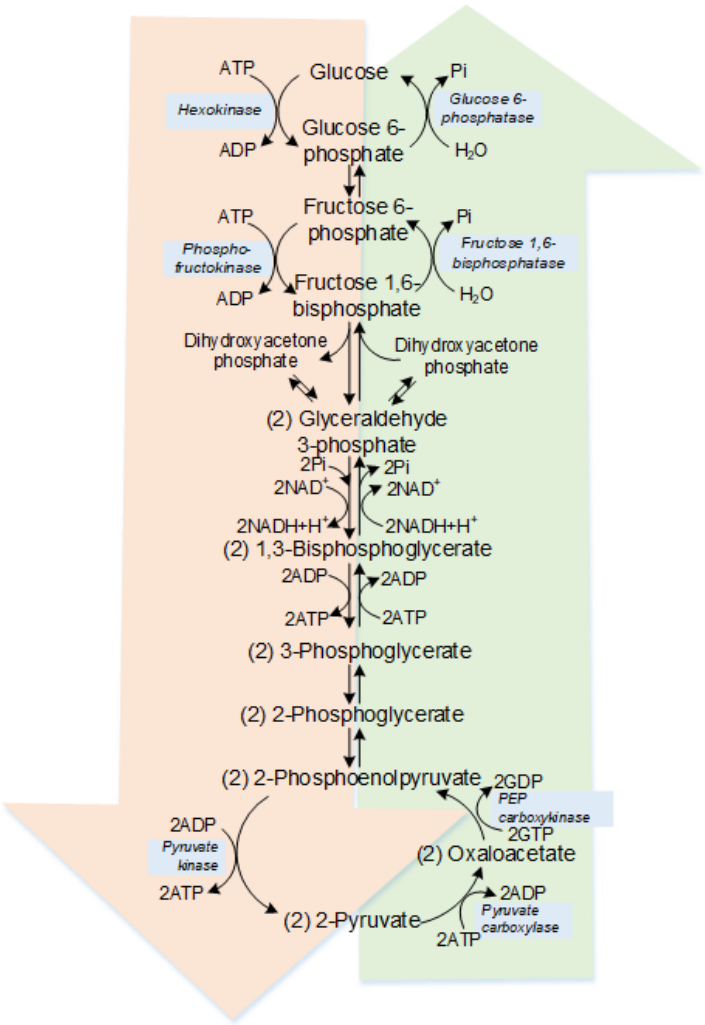


Fig. 9.17. Summary of the reactions of glycolysis and gluconeogenesis.

9.8. Relations between glycolisis and gluconeogenesis (Cori cycle). Glucose-alanine cycle

The digestible dietary carbohydrates yield glucose, galactose, and fructose that are transported to the liver via the hepatic portal vein. Galactose and fructose are readily converted to glucose in the liver (see topic 8). Glucose is formed from two groups of compounds that undergo gluconeogenesis:

(1) those which involve a direct net conversion to glucose, including most amino acids and propionate;

(2) those which are the products of the metabolism of glucose in tissues.

Thus lactate, formed by glycolysis in skeletal muscle and RBSs, is transported to the liver and kidney where it reforms glucose, which again becomes available via the circulation for oxidation in the tissues.

The Cori cycle (fig. 9.18) refers to the metabolic pathway in which lactate produced by muscle tissues during anaerobic glycolysis is transported to the liver and converted back to glucose. The glucose is then transported back to the muscle tissues and used as a source of energy in a cycle that allows for the recycling of lactate and glucose. This process is important for maintaining energy production during periods of high-intensity exercise, when the demand for ATP is greater than the supply of oxygen to the muscle tissues. The Cori cycle also helps to maintain blood glucose levels during fasting or prolonged exercise by providing a source of glucose for other organs, such as the brain, that require glucose as a fuel source.

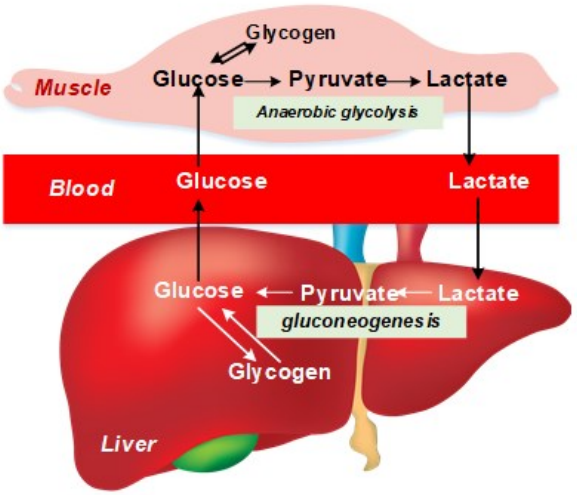


Fig. 9.18. The Cori (lactate) cycle.

The glucose-alanine cycle, also known as the Cahill cycle (fig. 9.19), is a metabolic pathway in which glucose is converted to alanine in muscle tissue and then transported to the liver, where it is converted back to glucose. The cycle begins in muscle tissue, where glucose is broken down to pyruvate through glycolysis, and then converted to alanine through a transamination reaction that involves the transfer of an amino group from an amino acid to an alpha-keto acid. The alanine is then released into the bloodstream and transported to the liver.

In the liver, the alanine is taken up by hepatocytes and converted back to pyruvate through the reverse transamination reaction. The pyruvate is then used in gluconeogenesis to produce glucose, which is released into the bloodstream and transported back to the muscle tissue, where it can be used as a source of energy.

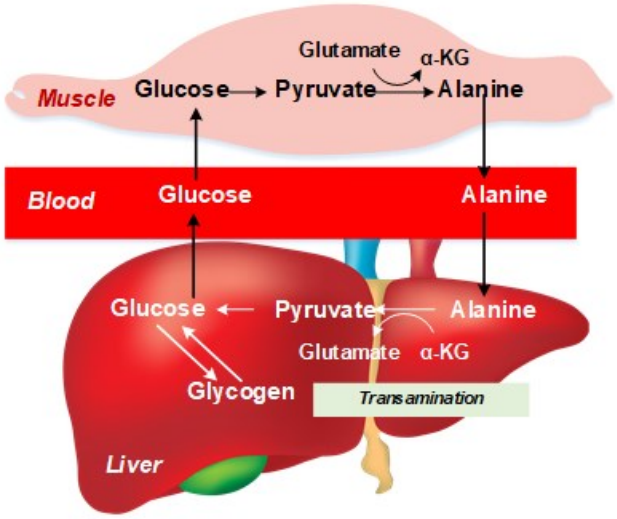


Fig. 9.19. The glucose-alanine cycle.

The glucose-alanine cycle helps to maintain glucose homeostasis in the body, particularly during times of high energy demand, such as exercise, when muscle tissue requires large amounts of glucose for energy production. The cycle also allows for the removal of excess nitrogen from the body, as the transamination reaction in muscle tissue allows for the conversion of

amino acids to alanine, which can be transported to the liver for disposal through the urea cycle.

REVIEW TEST:

№	MCQs	Answers and explanations
1.	**A newborn is found to have fasting hypoglycemia. The nursery staff begins overnight feeds by nasogastric tube because they find that the child has consistently low blood sugars. A liver biopsy and molecular studies demonstrate an absence of glycogen synthase. The normal function of this enzyme is to do which of the following?** A. Remove glucose residues one at a time from glycogen in the liver B. Remove glucose residues one at a time from glycogen in muscles C. Transfer glucose from UDP-glucose to the nonreducing end of a glycogen primer D. Hydrolyze α-1,6 bonds of glycogen E. Function as a glucosyl 4,6 transferase	**The answer is C.** Glycogen synthase is the first enzyme in the synthesis of glycogen. It transfers glucose from UDP-glucose to the nonreducing end of a glycogen primer and adds subsequent residues to the growing chain. The removal of glucose residues (answers A and B) during the catabolism of glycogen is mediated by glycogen phosphorylase, a deficiency of which results in Hers disease if in the liver and McArdle disease if in the muscle. Debranching enzyme hydrolyzes α-1,6 bonds of glycogen (answer D). Finally, deficiency of glucosyl 4-6 transferase (the branching enzyme) results in Andersen disease (answer E).
2.	**A newborn is experiencing failure to thrive. On physical examination, organomegaly is appreciated owing to accumulation of glycogen in the lysosomes of several organs, including the heart, muscle, and liver. You diagnose the condition as Pompe disease. Which one of the following biochemical deficits is seen in this disorder?** A. A deficiency of glycogenin B. Loss of α 1,6-glucosidaseactivity C. Loss of glucose 6-phosphatase activity D. Loss of muscle glycogen phosphorylase activity E. Loss of a lysosomal glucosidase activity	**The answer is E.** Pompe disease is caused by a deficiency of α-1,4-glucosidase, which prevents the release of glucose from glycogen in lysosomes, leading to lysosomal dysfunction. In contrast, McArdle syndrome is caused by a deficiency in muscle glycogen phosphorylase, which results in muscle symptoms such as weakness and cramps. A deficiency in glycogenin would result in a decrease in glycogen storage, while a deficiency in α-1,6-glucosidase would lead to an inability to release the 1,6 branch points of glycogen, as seen in Cori disease, but without lysosomal involvement. Finally, glucose 6-phosphatase deficiency, or von Gierke disease, results in a range of symptoms including hypoglycemia, hepatomegaly, hyperlipidemia, hyperuricemia, gouty arthritis, nephrolithiasis, and chronic renal failure, without lysosomal involvement.
3.	A second-year medical student decides to do research in a nutrition laboratory that is studying the effects of caffeine on cellular metabolism. Caffeine inhibits cAMP phosphodiesterase. If caffeine were added to liver cells, in the presence of glucagon, which of the following enzymes would be phosphorylated and inactivated? A. Phosphorylase kinase	**The answer is B.** Under the conditions of the experiment, cAMP levels would be elevated by glucagon treatmentand would remain elevated owing to the presence of caffeine. This leads to constant activation of proteinkinase A (PKA). PKA will phosphorylate pyruvate kinase, leading to its inactivation. Phosphorylase kinase and phosphorylase are activated by phosphorylation. PKA is not regulated by phosphorylation but by dissociation of inhibitory

	B. Pyruvate kinase C. Phosphorylase D. Protein kinaseA E. Calmodulin	subunits that bind to cAMP. Calmodulin is a calcium-binding protein that serves as a subunit of phosphorylase kinase, but is not phosphorylated by PKA.
4.	**A 28-year-old professional cyclist has been training foran opportunity torace in the Tour de France. His coach strongly suggests that he consume carbohydrates after each of his workouts to ensure that his muscle glycogen storage can endure the 28-day race. The activity of muscle glycogen synthase inresting muscles is increased by the action of which of the following?** A. Epinephrine B. Glucagon C. Insulin D. Phosphorylation E. Fastingand starvation	**The answer is C.** Glycogen synthesis occurs at times of rest and when the energy needs of the cells are being met. Of the hormones influencing the storage of glucose, insulin promotes the synthesis of energy stores through the dephosphorylation and activation of glycogen synthase. In fact, a helpful generalization is that glucagon and epinephrine typically mobilizeenergy stores through the activation of enzymes via direct phosphorylation, whereas insulin accomplishes the opposite. Fasting and starvation will not result in an increase in muscle glycogen stores.
5.	**A patient had large deposits of liver glycogen, which, after an overnight fast, contained shorter than normal branches. A defective form of which of the following could causet his abnormality?** A. Glycogen phosphorylase B. Glucagonreceptor C. Glycogenin D. Amylo-1,6-glucosidase (α-glucosidase) E. Amylo-4,6-transferase	**The answer is D.** If, after fasting, the branches were shorter than normal, phosphorylase must be functional and capable of being activated by glucagon. The branching enzyme must be normal because branches are present. The proteinglycogenin mustbe presentin order for large amounts of glycogen to be synthesized and deposited. The defect most likely is in the debranching enzyme (which contains an α-1,6-glucosidase). If the debrancher is defective, phosphorylase would break the glycogen down to within four glucose residues of the branch points, but complete degradation would not occur. Therefore, short branches would be present in the glycogen. If the short branches contain only one glucose unit, the defect is in the α-1,6 glucosidase activity of the debrancher. If they contain four glucose units, the defect is in the 4:4 transferase activity of the debrancher.
6.	**A 32-year-old woman receives anesthesia in preparation for a laparoscopic cholecystectomy. The anesthesiologist notices a subtle twitch of the masseter muscle in the jaw, followed by sinus tachycardia and an increase of the end expiratory CO_2. He immediately recognizes the early signs of malignant hyperthermia and administers dantrolene. Dantrolene is a muscle relaxant that acts specifically on skeletal muscle by interfering with the release of calcium from the sarcoplasmic reticulum. Which of the**	**The answer is D.** The regulation of glycogen differs between skeletal muscle and liver, matching their respective functions. Muscle glycogen serves as a storage of mechanical energy, while liver glycogen maintains blood glucose levels. In muscle, calcium release from the sarcoplasmic reticulum stimulates glycogen phosphorylase kinase through activation of calmodulin. This, in turn, activates glycogen phosphorylase. In the absence of calcium release, glycogen phosphorylase kinase is inactivated. Calcium does not regulate any of the other listed enzymes, which include glucokinase and phosphoglucomutase involved in glucose conversion and

	following enzymes would be affected by this action? A. Phosphoglucomutase B. Glucokinase C. Glycogen synthase D. Glycogen phosphorylasekinase E. Glucosyl 4:6 transferase	glycogen synthase, activated by phosphorylation. Glycosyl 4:6 transferase creates branches in glycogen.
7.	**A 32-year-old bodybuilder has decided to go on a diet consisting of only egg whites to ensure optimal protein for musclegrowth. After a few weeks, he notices decreased energy and is found to be hypoglycemic. A nutritionist tells the patient that the most likely has a functional biotin deficiency. Which of the following enzymes is unable tocatalyze akey stepin synthesizing glucosefrom pyruvate?** A. Pyruvatecarboxylase B. Phosphoenolpyruvate- carboxykinase C. Fructose1,6-bisphosphatase D. Glucose6-phosphatase E. Phosphoglycerate kinase	**The answer is A.** Pyruvate carboxylase uses biotin as a cofactor to catalyze the irreversible carboxylation of pyruvate to oxaloacetate. On the other hand, the enzyme PEPCK catalyzes the conversion of oxaloacetate to phosphoenolpyruvate, which is also irreversible and requires GTP as an energy source, but does not need a cofactor. The enzymes F-1,6-bisphosphatase and glucose 6-phosphatase are also used to bypass the irreversible steps of glycolysis in gluconeogenesis, but do not require biotin. Biotin is used exclusively for carboxylation reactions, and since phosphoglycerate kinase does not involve a carboxylation reaction, it does not require biotin as a cofactor.
8.	**Which one of the following occurs in an individual who is rested and has fasted for 12 hours?** A. Gluconeogenesis is the major process by which blood glucose is maintained. B. Adenylate cyclase has been inactivated in liver. C. Liverglycogen stores have been depleted. D. Glycogen phosphorylase, pyruvate kinase, and glycogen synthase are phosphorylated in the liver. E. Glycogen synthase has been activated in liver.	**The answer is D.** After 12 hours of fasting, liver glycogen stores are still substantial (liver glycogen stores arenot depleted until after about 30 hours of fasting). Glycogenolysis is stimulated by glucagon, which activates adenylatecyclase. The cAMP generated by adenylate cyclase activates proteinkinase A, which phosphorylates glycogen phosphorylase kinase, pyruvate kinase (PK), and glycogen synthase. As a result, glycogen phosphorylase is activated, whereas glycogen synthase and PK are inactivated. Gluconeogenesis does not become the major process for maintaining blood glucose until fasting has occurred for18 to20 hours.
9.	**A newborn undergoes a physical examination relevant for hepatomegaly, inguinalhernia, and deformedchest (pectuscarinatum). A family history of mucopolysaccharidosis (MPS) leads you to check enzyme activities from a sample of fibroblasts. The findings were significant for decreased activity in β-glucuronidase, which is indicative of which of the followings yndromes?** A. Hurler syndrome (MPS type I) B. Morquio syndrome (MPS type IV)	**The answer is E.** Sly syndrome is one of the few lysosomal storage disorders with clinical manifestations in utero or at birth. The signs of coarsefacial feature (gargoyle facies), mental developmental problems, and short stature can be seen in Sly syndrome as well as all the mucopolysaccharidoses. Hurler syndrome is due to a lack of a-L-iduronidase; Morquio syndrome to a lack of galactose 6-sulfatase; Hunter syndrome to a lack of iduronate sulfatase; and Sanfilippo Asyndrome to a lack of heparan sulfamidase.

	C. Hunter syndrome (MPS type II) D. Sanfilippo Asyndrome (MPS type III) E. Sly syndrome (MPS type VII)	
10	**A 3-year-old girl presents with developmental delay and growth failure. The physical examination is remarkable for coarse facial features, cranio facial abnormalities, gingival hyperplasia, prominent epicanthal fold, and macroglossia. The patient was diagnosed with I-cell diseases. Lysosomal proteins are mistargeted in this disorder. Rather than being targeted to the cell's lysosomes, lysosomal proteins in this disease are found in which of the following?** A. In the endoplasmic reticulum (ER) B. In the Golgi apparatus C. In the mitochondria D. Exported from the cell E. In the cytoplasm	**The answer is D.** If lysosomal proteins are not appropriately tagged with mannose 6-phosphate in the ER and Golgi apparatus, the proteinswill be exported from the cell. The lysosomalproteins donotcontain the appropriate targeting signals to be sent to the ER, Golgi apparatus, or mitochondria. Because these enzymes are synthesized on membrane-bound ribosomes (the rough ER), they will not be found in the cytoplasm (cytoplasmic proteins are synthesized on cytoplasmic ribosomes). Although the child's physical abnormalities are similar to other storage diseases, gingival hyperplasia is a unique clinical feature to I-cell disease.

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10. STUDIES OF MECHANISMS OF METABOLIC AND HORMONAL REGULATION OF CARBOHYDRATE METABOLISM. DIABETES MELLITUS

OBJECTIVES after studying this chapter, you should be able to:

- *Analyze the principal sources and metabolic pathways of utilization of blood glucose*
- *Explain the role of hormones in maintenance of constant glucose level in blood*
- *Explain disorders in metabolism of carbohydrates in diabetes mellitus.*
- *Analyse the biochemical tests for evaluation of diabetes mellitus*

10.1. Biochemical processes which provides the constant blood glucose level.

Monosaccharides (glucose, galactose and fructose) resulting from carbohydrate digestion are absorbed and undergo the following:

I. Uptake by liver. Glucose, galactose, and fructose are the three monosaccharides that can be derived from dietary carbohydrates. They are absorbed into the bloodstream from the small intestine and transported to the liver via the hepatic portal vein. In the liver, galactose and fructose can be converted into glucose through various enzymatic reactions, such as galactokinase and fructokinase, respectively.

II. Glucose utilization by tissues. Glucose may undergo one of the following fates:

1. Oxidation through:

a) major pathways (glycolysis and TCA) mainly for production of ATP energy, when it is needed.

b) pentose phosphate pathway: for production of pentoses and $NADPH+H^+$.

c) uronic acid pathway: for production of glucuronic and galacturonic acids. These sugar derivatives are used in detoxication and enters in the structure of glycosaminoglycans (GAGs).

2. Glucose may be used to generate the storage in the forms of:

a) glycogen: glycogenesis.

b) fat: lipogenesis.

3. Glucose and other blood sugars may undrgo the conversion to substances of biological importance:

a) Ribose, deoxyribose → RNA and DNA.

b) Glucosamine and galactosamine → mucopolysaccharides.

d) Glucuronic acid → glycosaminoglycans and mucopolysaccharides.

e) Fructose → in semen.

Glucose is essential for normal bodily functions as it serves as a source of energy and building blocks for various metabolic processes. The levels of glucose in the blood are tightly regulated in healthy individuals, with a postabsorptive or overnight fasted blood glucose concentration range of 4.5-5.5 mmol/L. While glucose concentrations increase after meals, a typical meal does not raise blood glucose beyond 8-10 mmol/L, and the body usually restores normal blood glucose levels within 2-4 hours. Exceptions to this can occur

in cases of severe exercise, prolonged fasting, certain medical conditions, or through the use of medications. However, for the average healthy adult, significant fluctuations in blood glucose levels are not a regular occurrence.

The maintenance of a stable blood glucose concentration is one of the most finely regulated of all homeostatic mechanisms, involving the liver, extrahepatic tissues, and several hormones. Liver cells are freely permeable to glucose in either direction (via the GLUT 2 transporter), whereas cells of extrahepatic tissues (apart from pancreatic β-islets) are relatively impermeable, and their unidirectional glucose transporters are regulated by insulin. As a result, uptake from the bloodstream is the rate-limiting step in the utilization of glucose in extrahepatic tissues.

Different tissues have varying levels of glucose uptake and utilization (fig. 10.1). Key tissues involved in glucose metabolism include:

- **Muscle:** Skeletal muscle is a major consumer of glucose during physical activity. It takes up glucose and uses it as a fuel source for muscle contraction and energy production.
- **Adipose tissue:** Adipocytes take up glucose and convert it into triglycerides for storage as adipose tissue (fat). During times of energy excess, glucose can be stored as fat for later use.
- **Liver:** The liver plays a central role in glucose metabolism. It can take up glucose and either store it as glycogen through glycogenesis or break down glycogen into glucose through glycogenolysis. The liver also produces glucose through gluconeogenesis when glucose levels are low.
- **Brain:** The brain relies heavily on glucose as its primary energy source. It takes up glucose from the bloodstream and metabolizes it to meet its energy demands.

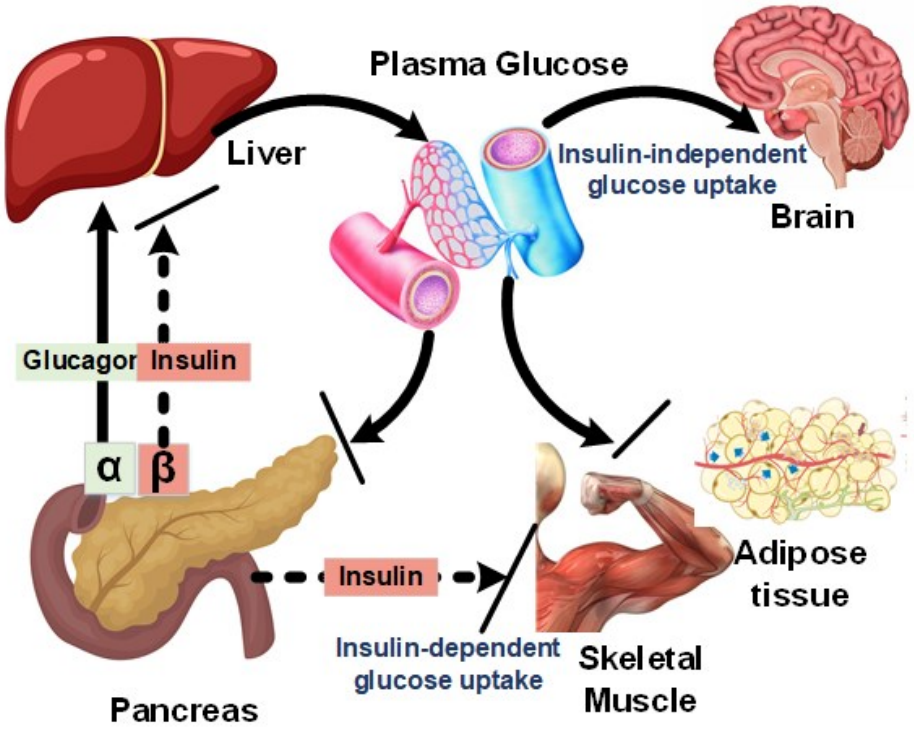


Fig.10.1. Glucose blood concentration is highly dependent on its utilization by various tissues and is also regulated by hormones.

Blood glucose levels during prolonged fasting (starvation). During starvation, the body's glucose stores become depleted, and the body begins to break down stored fat and protein to produce energy. Blood glucose levels gradually decrease, and after about 12-24 hours of fasting, glycogen stores in the liver are depleted, causing an increase in gluconeogenesis (glucose production from non-carbohydrate sources such as amino acids and glycerol). As a result, blood glucose levels may fall to as low as 2.5 mmol/L, which can lead to symptoms such as weakness, fatigue, and dizziness.

To prevent hypoglycemia, the body activates following mechanisms:

1. Changes in fuel utilization by various tissues prevent blood glucose levels from decreasing abruptly during prolonged fasting.

2. The levels of ketone bodies rise in the blood, and the brain uses ketone bodies for energy, decreasing itsutilization of blood glucose.

3 The rate of gluconeogenes is and, therefore, of urea productionby the liver decreases.

4. Muscle protein is spared. Less muscle protein is used to provide amino acids for gluconeogenesis.

Blood glucose levels during exercise. During exercise, blood glucose is maintained by essentially the same mechanisms that are used during fasting.

1. Use of endogenous fuels. As the exercising muscle contracts, ATP is used. ATP is regenerated initially from creatinephosphate. Muscle glycogen is oxidized to produce ATP. AMP activates phosphorylase b, and Ca^{2+}-calmodulin activates phosphorylase kinase. The hormone epinephrine causes the production of cAMP, which stimulates glycogen breakdown.

2. Use of fuels from the blood. As blood flow to the exercising muscle increases, blood glucose and fatty acids are taken up and oxidized by muscle. As blood glucose levels begin to decrease, the liver, by the processes of glycogenolysis and gluconeogenesis, acts to maintain blood glucose levels.

Blood glucose levels during sleep. During sleep, blood glucose levels can be affected by various factors such as the timing and composition of the last meal, physical activity prior to sleep, and any underlying medical conditions. In healthy individuals, blood glucose levels are typically maintained within a narrow range during sleep through the action of various hormones and metabolic processes.

During the early stages of sleep, blood glucose levels may decrease slightly due to reduced physical activity and the effects of insulin. However, during the later stages of sleep, particularly towards morning, blood glucose levels may start to rise as a result of several factors. These include an increase in cortisol and growth hormone secretion, which promote gluconeogenesis (the production of glucose from non-carbohydrate sources), and a decrease in insulin sensitivity.

10.2. Role of liver in carbohydrate metabolism.

The liver plays a major role in blood glucose homeostasis by maintaining a balance between the uptake and storage of glucose *via* glycogenolysis and gluconeogenesis. The liver is the primary organ for glucose metabolism. About 90% of all circulating glucose not derived directly from the diet comes from the liver. Hepatocytes take up glucose by

GLUT2 in the presence of high concentrations of glucose. In hepatocytes, glucose is phosphorylated by **glucokinase** to glucose-6-phosphate. From glucose-6-phosphate, the glucose is directed into **glycogenesis, the pentose phosphate pathway, or glycolysis.**

In response to ingestion of glucose and the resulting hyperinsulinemia and hyperglycemia, the fasting liver shifts from net output to net uptake of glucose. Healthy human adults ingesting 75 g glucose exhibited peak plasma glucose and insulin concentrations of 7.8 mmol/L and 325 pmol/L, respectively.

To elaborate on the previous response, after the ingestion of glucose, insulin secretion is stimulated and leads to the uptake of glucose by peripheral tissues, including the liver, muscle, and adipose tissue. In the liver, insulin promotes glucose uptake and metabolism, which results in decreased hepatic glucose production. Additionally, insulin stimulates glycogen synthesis, which is an important storage form of glucose in the liver. As a result, the liver shifts from a net output of glucose to a net uptake of glucose in response to hyperglycemia and hyperinsulinemia.

Glycolysis is a process by which glucose is metabolized to produce energy, and glycogenesis is the synthesis of glycogen from glucose. These pathways are opposed by gluconeogenesis, which is the synthesis of glucose from non-carbohydrate precursors, such as amino acids and lactate. The regulation of these opposing pathways is critical for the net flux of glucose to be directed in the appropriate direction.

During the postprandial state (after a meal), **glycogenolysis** is the primary source of glucose production in the liver, while gluconeogenesis becomes more important during prolonged fasting. This is because the liver glycogen stores become depleted during fasting, and the liver relies more on gluconeogenesis to maintain glucose homeostasis. The regulation of these pathways is tightly controlled by hormonal and metabolic signals to maintain normal blood glucose levels.

Glycogenolysis occurs within 2-6 hours after a meal in humans, and gluconeogenesis has a greater importance with prolonged fasting.

The rate of **gluconeogenesis** is primarily regulated by the activation of genes that encode gluconeogenic enzymes, which are controlled by glucagon, glucocorticoids, and cytokines from the interleukin-6 family. Insulin, on the other hand, decreases gluconeogenesis by suppressing the expression of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, while glucagon and glucocorticoids stimulate glucose production by inducing these genes.

Glucagon is an important regulator of hepatic glucose production during fasting, exercise, and hypoglycemia. It also limits hepatic glucose uptake. In response to a physiological rise in glucagon, hepatic glucose production is rapidly stimulated, mainly by enhancing glycogenolysis, with little or no effect on gluconeogenesis. The liver can then release glucose into the circulation. The skeletal muscle also plays a role in glucose production by releasing lactate, which can be shuttled back to the liver and used as a substrate for gluconeogenesis in a process known as the Cori cycle (see chapter 9).

10.3. Hormonal regulation of carbohydrate metabolism.

In healthy individuals, plasma glucose levels remain within a narrow range of 3.3 to 5.5 mmol/l, even during periods of feeding and fasting. This balance is maintained by the interplay between two processes:

- the release of glucose into the circulation by either absorption from the intestine or the breakdown of stored glycogen in the liver
- the uptake and metabolism of blood glucose by peripheral tissues.

The regulation of these processes is controlled by a set of **metabolic hormones** that help to maintain glucose homeostasis.

10.3.1. Insulin, its structure, mechanism of action, role in carbohydrate metabolism

lnsulin is a polypeptide hormone produced by the β-cells of islets of Langerhans of pancreas. lt has profound influence on the metabolism of carbohydrate, fat and protein. Insulin is considered as anabolic hormone, as it promotes the synthesis of glycogen, triacylglycerols and proteins.

Insulin is is synthesized as a larger pre-proinsulin protein of 11.5 kDa in the rough endoplasmic reticulum. During peptide synthesis, the endoplasmic reticulum-targeting sequence is cleaved to produce proinsulin. Proinsulin is then packaged into secretory vesicles where it undergoes further processing to become the mature insulin hormone (as shown in figure 10.2). Insulin has a half-life of approximately 5 minutes in circulation, with the liver and kidney being the primary sites of degradation. Normally, a small amount of proinsulin is secreted, accounting for about 3-5% of insulin secretion. However, during periods of high insulin release, the processing of proinsulin may be less complete, leading to a larger proportion of proinsulin being released. Although proinsulin has some activity, it is only about 10% as potent as insulin. Additionally, the **C-peptide**, which is also produced during proinsulin processing, has a longer half-life than insulin. Measuring C-peptide levels can be useful in monitoring the activity of pancreatic β-cells.

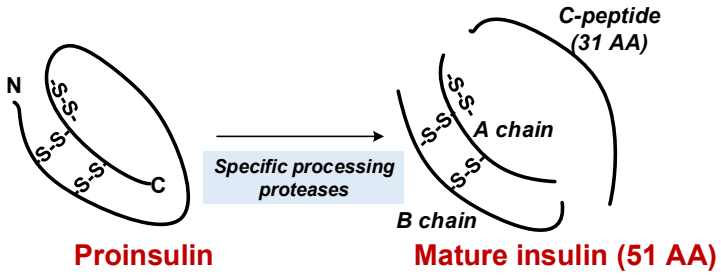


Fig. 10.2. Limited proteolysis of proinsulin generates mature insulin and Cpeptide

Insulin is released from the β-cell in response to elevated plasma glucose, mannose, and some amino acids, especially leucine (fig. 10.3). Stimulation of insulin release by glucose can be enhanced by other hormones. There are several factors that can stimulate insulin release, including:

- Glucose: The primary stimulus for insulin release is an increase in blood glucose levels. When glucose levels rise, the pancreas secretes insulin to help move the glucose into cells.
- Amino acids: Certain amino acids, particularly those found in proteins, can stimulate insulin release. This is because the breakdown of proteins into amino acids can also lead to an increase in blood glucose levels.

- Gastrointestinal hormones: Certain hormones that are released by the gastrointestinal tract, such as glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP), can stimulate insulin release.
- Neural signals: Signals from the nervous system can also stimulate insulin release. For example, the parasympathetic nervous system can stimulate insulin release by increasing blood flow to the pancreas.
- Other hormones: Certain hormones, such as cortisol and growth hormone, can also stimulate insulin release.

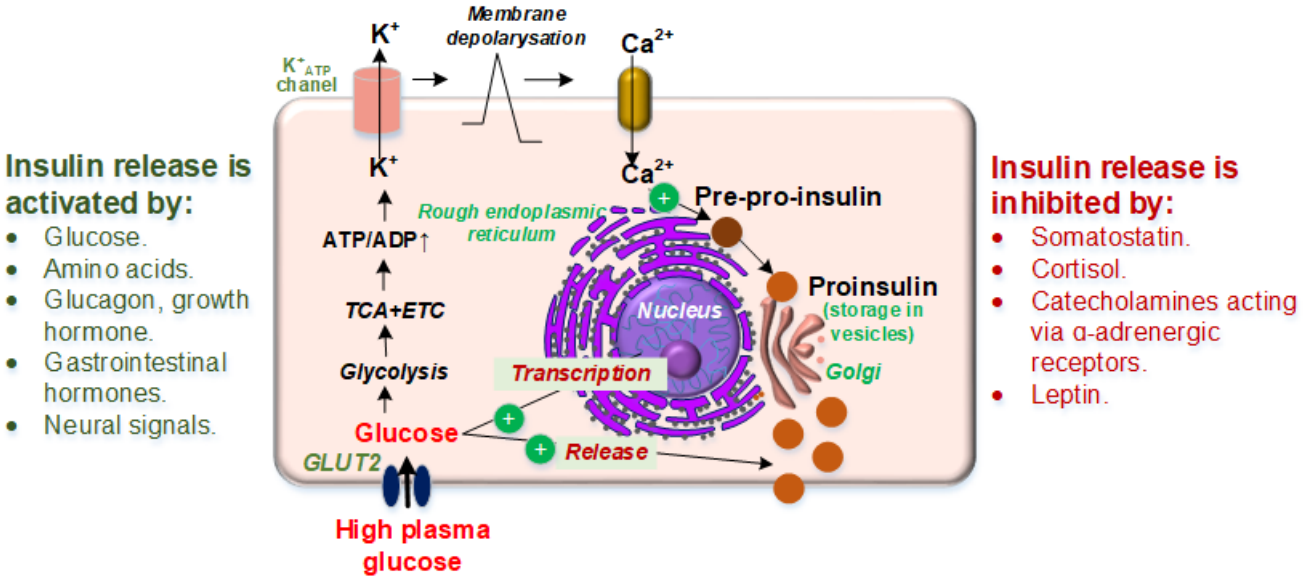


Fig. 10.3. Glucose enters β-cells through the GLUT2 transporter and undergoes glycolysis to generate ATP. This results in an increase in the ATP/ADP ratio, which leads to the closure of ATP-sensitive potassium channels, depolarization of the cell membrane, and subsequent opening of voltage-gated L-type calcium channels. The increased influx of calcium ions then triggers insulin granule fusion with the cell membrane and release of insulin into the bloodstream. Additionally, glucose stimulates the transcription of genes involved in insulin synthesis, ultimately leading to increased insulin production and release.

It's important to note that the timing and amount of insulin released in response to these stimuli can vary depending on individual factors such as age, diet, exercise habits, and overall health.

The release of insulin is suppressed by several factors, including somatostatin, cortisol, and catecholamines that act through α-adrenergic receptors. Although certain β-adrenergic agonists can have different effects on insulin release, the overall impact of catecholamine activity on insulin secretion is predominantly inhibitory in physiological conditions.

When glucose levels remain persistently high, the response of β-cells to glucose stimulation decreases, while their responsiveness to other stimuli remains unchanged. Although the exact mechanism is not fully understood, this phenomenon seems to be caused, at least partially, by a reduction in the amount of GLUT2 glucose transporter present in the β-cell membrane. This prolonged elevation of glucose levels, as observed in conditions such as hypersecretion of glucocorticoids, can deplete the β-cell insulin stores and overwhelm their capacity to synthesize additional insulin, leading to hyperglycemia.

Mechanism of insulin action. The action of insulin is facilitated through a multi-subunit glycoprotein called the insulin receptor located on the surface of cells (fig.10.4). When insulin binds to the receptor, it triggers the activation of the receptor's intrinsic ***tyrosine kinase*** activity. This activation leads to the phosphorylation of the insulin receptor and other proteins called **insulin receptor substrates (IRS),** which is believed to be necessary for insulin to have an effect. This phosphorylation process also activates various intracellular proteins, such as kinases, phosphatases, and transcription factors. One notable enzyme that is activated as a result of this cellular signaling pathway is phosphodiesterase, which reduces the levels of cellular cAMP.

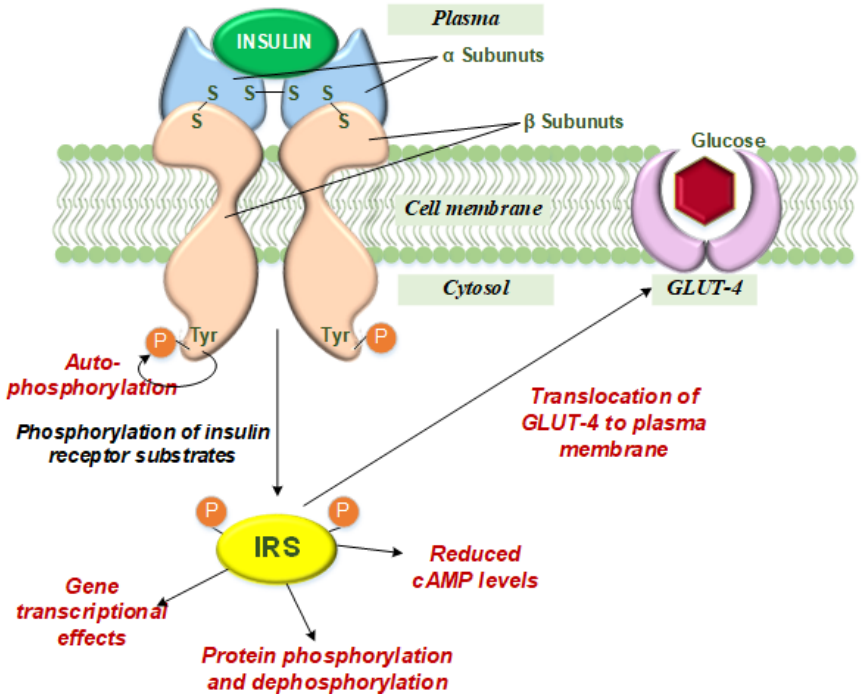


Fig. 10.4. Insulin receptor is a complex multi-subunit cell surface glycoprotein. The insulin receptor is composed of two extracellular α subunits and two transmembrane β subunits. When insulin binds to the α subunits, it causes a conformational change that activates the tyrosine kinase activity of the β subunits. The activated β subunits then phosphorylate themselves and several other intracellular proteins, which triggers a series of downstream signaling events. he key downstream effects of insulin signaling is the translocation of the glucose transporter GLUT4 to the plasma membrane of target cells, such as skeletal muscle and adipose tissue. This allows glucose uptake from the bloodstream into the cells.

Metabolic effects. Insulin plays a key role in the regulation of carbohydrate, lipid and protein metabolisms. Insulin exerts anabolic and anticatabolic influences on the body metabolism.

1. Effects on carbohydrate metabolism. Insulin influences glucose metabolism in many ways. The net effect is that insulin lowers blood glucose level (**hypoglycemic effect**) by promoting its utilization and storage and by inhibiting its production.

- **Effect on glucose uptake by tissues.** Insulin is necessary for glucose uptake by certain tissues such as skeletal, cardiac and smooth muscle, adipose tissue, leukocytes, and mammary glands. Interestingly, it is surprising to note that around 80% of glucose uptake in the body does not rely on insulin. This is because certain tissues such as the brain, kidney, erythrocytes, retina, nerve, blood vessels, and intestinal mucosa can allow glucose

to enter without the need for insulin. Although insulin is not necessary for glucose entry into hepatocytes, it does promote glucose utilization in the liver, thereby indirectly facilitating its uptake.

- **Effect on glucose utilization.** Insulin promotes the uptake of glucose into muscle and liver cells, where it stimulates the activity of several key enzymes of glycolysis, including *glucokinase, phosphofructokinase, and pyruvate kinase.* This leads to an increase in the rate of glycolysis and the production of ATP. In addition, insulin enhances glycogen production by increasing the activity of *glycogen synthase*, the enzyme responsible for glycogen synthesis. This helps to store excess glucose in the liver and muscle cells for later use.
- **Effect on glucose production.** Insulin decreases gluconeogenesis by suppressing the enzymes *pyruvate carboxylase, phosphoenol pyruvate carboxykinase and glucose 6-phosphatase*. Insulin also inhibits glycogenolysis by inactivating the enzyme glycogen phosphorylase.

2. Effects on lipid metabolism. Insulin has a net effect of decreasing the release of fatty acids from stored fat and reducing the production of ketone bodies in lipid metabolism. Adipose tissue is the most sensitive tissue to the action of insulin among all the tissues.

- **Effect on lipogenesis.** Insulin stimulates the production of triacylglycerols from glucose by increasing the availability of glycerol 3-phosphate (derived from glycolysis) and NADPH (generated from the PPP pathway). Additionally, insulin enhances the activity of *acetyl CoA carboxylase*, a critical enzyme involved in fatty acid synthesis.
- **Effect on lipolysis.** Insulin decreases the activity of *hormone-sensitive lipase* and thus reduces the release of fatty acids from stored fat in adipose tissue. Insulin also promotes the mobilization of fatty acids from liver. This is a mechanism by wich the insulin keeps the circulating free fatty acids under a constant level.
- **Effect on ketogenesis**. Insulin reduces ketogenesis by decreasing the activity of *HMG CoA synthetase*. Further, insulin promotes the utilization of acetyl CoA for oxidation (TCA cycle) and lipogenesis. Therefore, the availability of acetyl CoA for ketogenesis, in the normal circumstances, is very low.

3. Effects on protein metabolism. Insulin is an anabolic hormone. It stimulates the entry of amino acids into the cells, enhances protein synthesis and reduces protein degradation.

10.3.2. Glucagon, mechanism of its regulatory effects on carbohydrate metabolism

The other major regulatory hormone of the pancreas is **glucagon**, a 29 amino acid peptide (fig. 10.5) is synthesized as part of a precursor composed of 160 amino acid. This precursor also contains several other peptide hormones: glucagon-like peptide-1 (GLP-1), glucagon like peptide-2 (GLP-2), and glicentin-related peptide.

Release of glucagon from the α-cells is stimulated by low plasma glucose and by catecholamines and glucocorticoids. Release of glucagon is inhibited by insulin and somatostatin. Release of glucagon is also inhibited by glucose; it is not known whether this is a direct effect of glucose on the α-cell, or an indirect consequence of elevated insulin levels.

Mechanism of action. Glucagon binds to the specific receptors on the plasma membrane and acts by activating the secondary messenger cyclic AMP.

Glucagon demonstrates the opposite action to those of insulin, and therefore functions to maintain plasma glucose levels between meals. However, unlike insulin, glucagon action is probably limited to the **liver**, with limited effects in other tissues.

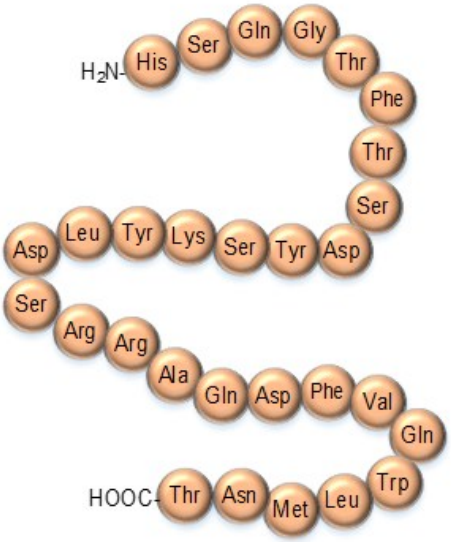


Fig. 10.5. Peptide chain of glucagon is composed of 29 amino acids

Metabolic effects of glucagon. Glucagon influences carbohydrate, lipid and protein metabolisms. In general, the effects of this hormone oppose that of insulin.

1. **Effects on carbohydrate metabolism.** Glucagon is the most potent hormone that enhances the blood glucose level (hyperglycemic). Primarily, glucagon acts on liver to cause increased synthesis of glucose (gluconeogenesis) and enhanced degradation of glycogen (glycogenolysis). The actions of glucagon are mediated through cyclic AMP.
2. **Effects on lipid metabolism.** Glucagon promotes fatty acid oxidation resulting in energy production and ketone body synthesis (ketogenesis).
3. **Effects on protein metabolism.** Glucagon increases the amino acid uptake by liver which, in turn, promotes gluconeogenesis. Thus, glucagon lowers plasma amino acids.

10.3.3. Epinephrine mechanism of its regulatory effects on carbohydrate metabolism

Epinephrine (fig. 10.6) is released by the adrenal medulla in response to hypoglycemia (as well as other stress stimuli), nd as part of the preparation for exercise. Epinephrine acts primarily on muscle, adipose, and liver tissues. The amino acid tyrosine is the precursor for the synthesis of epinephrine.

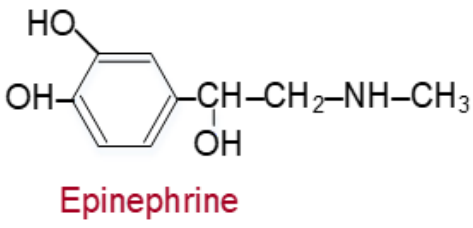


Fig.10.6. Chemical structure of epinephrine

Mechanism of action. Epinephrine and norepinephrine are hormones that act by stimulating the activity of *adenylyl cyclase*, an enzyme that converts ATP to cAMP. The mechanism is similar to that of glucagon. The increase in cAMP levels activates *hormone-sensitive lipase* through *cAMP-dependent protein kinase*, which leads to lipolysis. Processes that influence cAMP levels can affect lipolysis.

Metabolic effects of epinephrine.

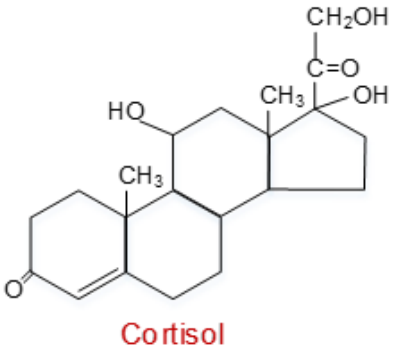
1. **Effects on carbohydrate metabolism.** Epinephrine and norepinephrine in general increase the degradation of glycogen (glycogenolysis), synthesis of glucose (gluconeogenesis) and decrease glycogen formation (glycogenesis) predominantely in muscules. The overall effect of epinephrine is to elevate blood glucose levels and make it available for the brain and other tissues to meet the emergencies.

2. Effects on lipid metabolism. Epinephrine enhances the breakdown of triacylglycerols (lipolysis) in adipose tissue. This causes increase in the free fatty acids in the circulation which are effectively utilized by the heart and muscle as fuel source.

3. Effects on physiological functions. Epinephrine increases cardiac output, blood pressure and oxygen consumption. It causes smooth muscle relaxation in bronchi, gastrointestinal tract and the blood vessels supplying skeletal muscle.

10.3.4. Glucocorticoids, their effect on carbohydrate metabolism

Glucocorticoids are 21-carbon steroids, produced mostly by zona fasciculata of adrenal glands. They affect glucose, amino acid and fat metabolism in a manner that is opposite to the action of insulin. Cortisol (also known as hydrocortisone) shown on fig. 10.7 is the most important glucocorticoid in humans. Cortisol acts on muscle, liver, and adipose tissue to supply the organism with fuel to withstand the stress.



Cortisol

Fig. 10.7. Chemichal structure of cortisol

Mechanism of action of glucocorticoids. Glucocorticoids bind to specific receptors on the target cells and bring about the action. These hormones mostly act at the transcription level and control the protein synthesis.

Metabolic effects of glucocorticoids.

1. Effects on carbohydrate metabolism. Glucocorticoids promote the synthesis of glucose (gluconeogenesis). This is brought about by increasing the substrates (particularly amino acids) and enhancing the synthesis of phosphoenolpyruvate carboxykinase, the rate limiting enzyme in gluconeogenesis. The overall influence of glucocorticoids on carbohvdrate metabolism is to increase blood glucose concentration. The biological actions of glucocorticoids generally oppose that of insulin.

2. Effects on lipid metabolism. Glucocorticoids increase the circulating free fatty acids. This is caused bv two mechanisms:

- Increased breakdown of storage triacylglycerol (lipolysis) in adipose tissue.
- Reduced utilization of plasma free fatty acids for the synthesis of triacylglycerols.

3. Effects on protein and nucleic acid metabolism. Glucocortiocoids exhibit both catabolic and anabolic effects on protein and nucleic acid metabolism. They promote transcriPtion (RNA synthesis) and protein biosynthesis in liver. These anabolic effects of glucocorticoids are caused by the stimulation of specific genes.

10.4. Characterization of hypo- and hyperglycemia, glucosuria

Hypoglycemia and hyperglycemia share similar symptoms, such as confusion, irritability, blurred vision, sweating, dizziness, and hunger, which can make it challenging to differentiate between the two conditions. It is crucial to test the patient's blood glucose level when experiencing these symptoms, as the treatment differs for each condition. The patient's recent medical history and risk factors can also aid in determining the underlying cause of the symptoms.

Hypoglycemia is a medical condition that occurs in diabetic patients when their blood glucose level falls below 4 mmol/L. When left untreated, hypoglycemia can cause a change in the patient's mental status, leading to confusion and headaches. If still left unaddressed, the patient may progress to a semi-conscious or unconscious state, which can cause brain damage. Additionally, seizures may occur as a result of hypoglycemia. Therefore, it is important to promptly treat hypoglycemia to prevent these serious consequences.

Common initial **symptoms of hypoglycemia** include:

- Cold, clammy skin.
- Weakness, faintness, tremors.
- Headache, irritability, dullness.
- Hunger, nausea.
- Tachycardia, palpitations.

These symptoms will progress to mood or behaviour changes, vision changes, slurred speech, and unsteady gait if the hypoglycemia is not properly managed.

The mammalian body has developed a well regulaied system for an efficient maintenance of blood glucose concentration. Hypoglycemia, therefore, is not commonly observed. The following three types of hypoglycemia are encountered by physicians:

- **Post-prandial hypoglycemia:** This is also called reactive hypoglycemia and is observed in subjects with an elevated insulin secretion following a meal. This causes transient hypoglycemia and is associated with mild symptoms. The patient is advised to eat frequently rather than the 3 usual meals.
- **Fasting hypoglycemia:** Low blood glucose concentration in fasting is not very common. However, fasting hypoglycemia is observed in patients with pancreatic B-cell tumor and hepatocel lular damage.
- **Hypoglycemia due to alcohol intake**: In some individuals who are starved or engaged in prolonged exercise, alcohol consumption may cause hypoglycemia. This is due to the accumulation of NADH (during the course of alcohol metabolism by alcohol dehydrogenase) which diverts the pyruvate and oxaloacetate (substrates of gluconeogenesis) to form, respectively, lactate and malate. The net effect is lhat gluconeogenesis is reduced due to alcohol consumption.
- **Hypoglycemia due to insulin overdose:** The most common complication of insulin therapy in diabetic patients is hypoglycemia. This is particularly observed in patients who are on intensive treatment regime

Hyperglycemia is a condition characterized by high blood glucose levels exceeding 7 mmol/L in a fasting state or more than 10 mmol/L two hours after eating a meal. This complication can occur in individuals with diabetes due to various reasons, including excessive consumption of food or simple sugars, inadequate insulin dosages, infection, illness, surgery, or emotional stress. Surgical patients are particularly vulnerable to hyperglycemia due to the surgical stress response. Symptoms of hyperglycemia include increased thirst (polydipsia), frequent urination (polyuria), and excessive hunger (polyphagia).

The common **symptoms of hyperglycemia** are:

- Increased urination/output (polyuria).
- Excessive thirst (polydipsia).

- Increased appetite (polyphagia), followed by lack of appetite.
- Weakness, fatigue.
- Headache

Other symptoms include glycosuria, nausea and vomiting, abdominal cramps, and progression to diabetic ketoacidosis (DKA).

Potential causes of hyperglycemia in a hospitalized patient include:

- Infection.
- Stress.
- Increased intake of calories (IV or diet).
- Decreased exercise.
- New medications or dose adjustments.

Hyperglycemia is the main symptom of all types of diabetes.

Glucosuria. Serum glucose is filtered by the glomerulus and then reabsorbed by the proximal tubules in the kidneys. However, the process of glucose reabsorption is limited and becomes saturated at high glucose concentrations. When serum glucose levels exceed 10 mmol/L, the amount of glucose in the ultrafiltrate exceeds the reabsorption capacity of the renal tubules, resulting in glucose being excreted in the urine (glycosuria).

Causes of glucosuria.

- Hyperglycemic glucosuria (e.g., pituitary pars intermedia dysfunction, Cushing's disease, and rarely diestrus)
- Renal glucosuria (normoglycemic glucosuria) due to defective renal tubular resorption resulting from tubular abnormalities or damage

10.5. Insulin dependent and noninsulin dependent forms of diabetes mellitus

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces.

There are two major clinical classes of diabetes mellitus: type I diabetes, or **insulin-dependent diabetes mellitus (IDDM)**, and type II diabetes, or **non-insulin-dependent diabetes mellitus (NIDDM)**, also called insulin-resistant diabetes.

IDDM, also known as type I diabetes or (less frequently) juvenile onset diabetes, mainly occurs in childhood (particularly between 12-15 years age). IDDM accounts for about 100 to 20 % of the known diabetics. This disease is characterized by almost total deficiency of insulin due to destruction of β-cells of pancreas. The β-cell destruction may be caused by drugs, viruses or autoimmunity. Due to certain genetic variation, the β-cells are recognized as non-self and they are destroyed by immune mediated injury. Usually, the symptoms of diabetes appear when 80-90% of the β-cells have been destroyed. The pancreas ultimately fails to secrete insulin in response to glucose ingestion. The patients of IDDM require insulin therapy.

The pathogenesis of the autoimmune beta-cell destruction involves incompletely understood interactions between susceptibility genes, autoantigens, and environmental factors.

Susceptibility genes include those within the major histocompatibility complex (MHC), which seem to regulate insulin production and processing and confer risk of

diabetes mellitus. Susceptibility genes are more common among some populations than among others and explain the higher prevalence of type 1 diabetes in some ethnic groups (Scandinavians, Sardinians).

Autoantigens include glutamic acid decarboxylase, insulin, proinsulin, insulinoma-associated protein, zinc transporter ZnT8, and other proteins in beta cells. It is thought that these proteins are exposed or released during normal beta-cell turnover or beta-cell injury (eg, due to infection), activating primarily a T cell–mediated immune response resulting in beta-cell destruction (insulitis).Glucagon-secreting alpha cells remain unharmed. Antibodies to autoantigens, which can be detected in serum, seem to be a response to (not a cause of) beta-cell destruction.

Several **viruses** (including coxsackievirus, rubella virus, cytomegalovirus, Epstein-Barr virus, and retroviruses) have been linked to the onset of type 1 diabetes. Viruses may directly infect and destroy beta cells, or they may cause beta-cell destruction indirectly by exposing autoantigens, activating autoreactive lymphocytes, mimicking molecular sequences of autoantigens that stimulate an immune response (molecular mimicry), or other mechanisms.

Diet may also be a factor. Exposure of infants to dairy products (especially cow's milk and the milk protein beta casein), high nitrates in drinking water, and low vitamin D consumption have been linked to increased risk of type 1 DM. Early (< 4 month) or late (>7 month) exposure to gluten and cereals increases islet cell autoantibody production. Mechanisms for these associations are unclear.

NIDDM, also called type II diabetes or (less frequently) adult-onset diabetes, is the most common, accounting for 80 to 90% of the diabetic population. NIDDM occurs in adults (usually above 35 years) and is less severe than IDDM. The causative factors of NIDDM include genetic and environmental. NIDDM more commonly occurs in obese individuals. Overeating coupled with underactivity leading to obesity is associated with the development of NIDDM. Obesity acts as a diabetogenic factor in genetically predisposed individuals by increasing the resistance to the action of insulin. This is oue to a decrease in insulin receptors on the insulin responsive (target) cells. The patients of NIDDM may have either normal or even increased insulin levels. lt is suggested that over-eating causes increased insulin oroduction but decreased synthesis of insulin receptors. This is based on the fact that weight reduction by diet control alone is often sufficient to correct NIDDM.

Pathogenesis is complex and incompletely understood. Hyperglycemia develops when insulin secretion can no longer compensate for insulin resistance. Although insulin resistance is characteristic in people with type NIDDM and those at risk of it, evidence also exists for beta-cell dysfunction and impaired insulin secretion, including impaired first-phase insulin secretion in response to glucose infusion, a loss of normally pulsatile insulin secretion, an increase in proinsulin secretion signaling impaired insulinprocessing, and an accumulation of islet amyloid polypeptide (a protein normally secreted with insulin). Hyperglycemia itself may impair insulin secretion, because high glucose levels desensitize beta cells, cause beta-cell dysfunction (glucose toxicity), or both. These changes typically take years to develop in the presence of insulin resistance.

The main differences between IDDM and NIDDM are given in a table 10.1.

Table. 10.1. Comparison of insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM)

Character	Insulin-dependent diabetes mellitus (IDDM)	Non-insulin dependent diabetes mellitus (N IDDM)
General		
Prevalence	10-20% of diabetic population	80-90 % of diabetic population
Age at onset	Usually childhood (<20 yrs)	Predominantly in adults (>30yrs)
Body weight	Normal or low	Obese
Genetic predisposition	Mild or moderate	Very strong
Biochemical		
Defect	Insulin deficiency due to destruction of β-cells	lmpairmenl in the production of insulin and/or resistance of target cells to insulin
Plasma insulin	Decreased or absent	Normal or increased
Auto antibodies	Frequently found	Rare
Ketosis	Very common	Rare
Acute complications	Ketoacidosis	Hyperosmolar coma
Clinical		
Duration ol symptoms	Weeks	Months to years
Diabetic complications at diagnosis	Rare	Found in 10-20% cases
Oral hypoglycemic drugs	Not useful for treatment	Suitable for treatment
Administration of insulin	Always required	Usually not necessary

10.6. Characterization of metabolic disorders in diabetes mellitus

Diabetes mellitus is associated with several metabolic alterations. Most important among them are hyperglycemia, ketoacidosis and hypertriglyceridemia.

1. Hyperglycemia. Elevation of blood glucose concentration is the hallmark of uncontrolled diabetes. Hyperglycemia is primarily due to reduced glucose uptake by tissues and its increased production via gluconeogenesis and glycogenolysis. When the blood glucose level goes beyond the renal threshold, glucose is excreted into urine (glycosuria).

One of the consequences of hyperglycaemia in human diabetes mellitus is increased metabolism of glucose by the sorbitol pathway. This involves the reduction of glucose to sorbitol catalysed by aldose reductase and the oxidation of sorbitol to fructose by sorbitol dehydrogenase. Sorbitol is not permeable to cell membranes and tends to accumulate in the cell. Conversion of glucose to sorbitol by aldose reductase requires NADPH and forms $NADP^+$ and thereby competes with other NADPH-requiring reactions. NADPH is required for the conversion of oxidized to reduced glutathione, a powerful antioxidant which protects cellular components from oxidative damage, and for fatty acid and cholesterol biosynthesis. The pentose phosphate pathway is the major source of NADPH in most tissues and its flux is generally determined by the $NADP^+$/NADPH ratio. Conversion of sorbitol to fructose is coupled to reduction of NAD^+ to NADH and this competes with glycolysis at the glyceraldehyde dehydrogenase step for NAD^+. An increase in the

$NADH/NAD^+$ ratio favours increased conversion of dihydroxyacetone phosphate to glycerol 3-phosphate, ITS accumulation leads to depletion of reduced glutathione.

2. Ketoacidosis. Increased mobilization of fatty acids results in overproduction of ketone bodies which often leads to ketoacidosis

Another characteristic metabolic change in diabetes is excessive but incomplete oxidation of fatty acids in the liver. The acetyl-CoA produced by β-oxidation cannot be completely oxidized by the citric acid cycle. Accumulation of acetyl-CoA leads to overproduction of the ketone bodies acetoacetate and β-hydroxybutyrate, which cannot be used by extrahepatic tissues as fast as they are made in the liver. In addition to β-hydroxybutyrate and acetoacetate, the blood of diabetics also contains acetone, which results from the spontaneous decarboxylation of acetoacetate. Acetone is volatile and is exhaled, and in uncontrolled diabetes, the breath has a characteristic odor sometimes mistaken for ethanol. A diabetic individual who is experiencing mental confusion due to high blood glucose is occasionally misdiagnosed as intoxicated, an error that can be fatal. The overproduction of ketone bodies, called ketosis, results in greatly increased concentrations of ketone bodies in the blood (ketonemia) and urine (ketonuria).

The ketone bodies are carboxylic acids, which ionize, releasing protons. In uncontrolled diabetes this acid production can overwhelm the capacity of the blood's bicarbonate buffering system and produce a lowering of blood pH called acidosis or, in combination with ketosis, ketoacidosis, a potentially life-threatening condition

3. Hypertriglyceridemia. Conversion of fatty acids to triacylglycerols and the secretion of VLDL and chylomicrons is comparatively higher in diabetics. Further, the activity of the enzyme lipoprotein lipase is low in diabetic patients. Consequently, the plasma levels of VLDL, chylomicrans and triacylglycerols are increased. Hypercholesterolemia is also frequently seen in diabetics.

10.7. Biochemical tests for evaluation of conditions of patients with diabetes mellitus

Most tests used in the diagnosis of diabetes measure the level of glucose in the patient's blood. Normal blood glucose levels vary among species, but individual healthy animals tend to have relatively stable glucose levels because the body's normal homeostatic mechanisms. Although excessive carbohydrate intake, strenuous exercise, stress, and various disease conditions can affect plasma glucose, daily fluctuations are usually maintained within 10% to 20% of the normal range in an individual.

Once the initial diagnosis has been established, testing in diabetic patients generally involves a blood glucose curve, urinalysis, clinical chemistry profile, and hematology profile.

Glucose testing is often the first test conducted in diagnosing suspected diabetes. Patients undergoing glucose testing must be properly fasted (8 to 12 hours) before a blood sample is taken.

The glucose tolerance test (GTT) is the most sensitive test for detecting borderline diabetes mellitus. Glucose tolerance means ability of the body to utilize glucose in the circulation. It is indicated by the nature of blood glucose curve following the administration of glucose. Thus "glucose tolerance test" is a valuable diagnostic aid in the

diagnosis of diabetes mellitus, insulin resistance, impaired beta-cell functionnand sometimes reactive hypoglycemia and acromegaly. GTT is of two types depending upon the route of glucose administration :

1. Oral Glucose Tolerance Test (OGTT)
2. Intravenous Glucose Tolerance Test (IVGTT)

OGTT is mostly preferred. IVGTT may be chosen for patients who are unable to absorb an oral dose of glucose (eg. malabsorption syndrome).

Before GTT the patient should be on balanced diet, containing normal daily requirement of carbohydrates, at least 2-3 days prior to the test, patients should avoid drugs likely to influence the blood glucose levels, at least 2 days prior to the test, patient should report to the laboratory after fasting for 12-16 hours. He/She can drink water. All samples of blood should be venous preferably. If the capillary blood from 'finger-prick' is used, all samples should be capillary blood.

Conduction of OGTT:

1. A fasting sample of venous blood is collected in a fluoride vial.
2. The bladder is emptied completely and urine is collected for qualitative test for glucose and ketone bodies.
3. The individual is given **1-1.5 grams per 1 kilogram of body weght** of glucose dissolved in water (about 250 ml).
4. A total of five specimens of venous blood are collected every 1/2 hour (30 minutes) after the oral glucose administration.
5. Glucose content of all five samples of blood are estimated by the specific methods used in laboratory. Corresponding urine samples are tested qualitatively for the presence of glucose and ketone bodies.
6. A curve is plotted by plotting time on X-axis and plasma glucose level on Y-axis, which is called **Glucose Tolerance Curve (GTC)**.

A typical **normal response (NGT)** shows following features. Initial fasting glucose within normal limits. The highest peak value is reached within 1 hour. The highest value doesnot exceed the renal threshold (9-10 mmol/l). The fasting level is again reached by 2-2.5 hours.

Response of diabetic patients. Fasting blood glucose is definitely raised above 5.5 mmol/l. The highest value is reached after 1-1.5 hours. The highest value exceeds the renal threshold. The blood glucose level doesnot return to fasting level within 2.5 hours. This is the most characteristic feature of diabetes melitus.

In individuals with impaired glucose tolerance (IGR), the fasting (6-7 mmol/l) as well as 2 hour (10-15 mmol/l) plasma glucose levels are elevated. These subjects slowly develop frank diabetes at an estimated rate of 2% per vear. Dietarv restriction and exercise are advocated for the treatment of impaired glucose tolerance.

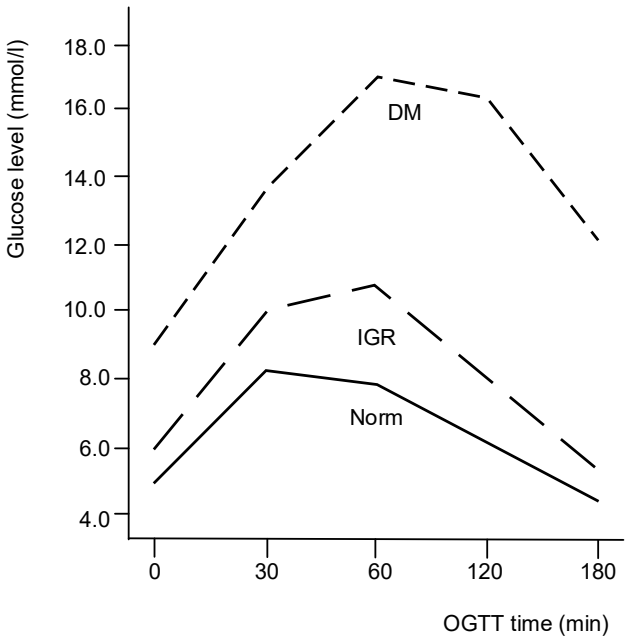


Fig. 10.8. Glucose Tolerance Curve (GTC). DM-diabetes mellitus, IGR – impaired glucose tolerance

Glycosylated Hemoglobin. Glycosylated hemoglobin, also known as HbA1c, is a form of hemoglobin that is formed when glucose molecules bind to hemoglobin in the bloodstream. The level of HbA1c in the blood is used as an indicator of the average blood glucose level over a period of 2-3 months, which is the lifespan of a red blood cell. An increased level of HbA1c indicates persistent hyperglycemia over that period of time. Since the reaction between glucose and hemoglobin is irreversible, the level of HbA1c remains a reliable indicator of long-term blood glucose control.

Ketone Bodies. In diabetes, the body may not produce enough insulin or may not use it effectively, resulting in high blood glucose levels. If the body cannot use glucose as a fuel source, it will break down fat instead, leading to the production of ketone bodies. This can occur in a condition called **diabetic ketoacidosis (DKA**), which is a medical emergency and can lead to coma or death if left untreated. DKA is more commonly seen in type 1 diabetes but can also occur in type 2 diabetes. High levels of ketone bodies in the blood can lead to a decrease in blood pH, resulting in metabolic acidosis. Symptoms of DKA include excessive thirst, frequent urination, abdominal pain, nausea, vomiting, rapid breathing, and confusion. If any of these symptoms occur, it is important to seek immediate medical attention. Ketone bodies can be detected in plasma or in urine. The ketone produced in greatest abundance in patients with ketoacidosis is β-hydroxybutyrate. However, many tests for serum and urine ketones only detect acetone and/or acetoacetate, and ketonuria often goes undetected until the patient begins to exhibit obvious clinical signs.

Fructosamine. Glucose can bind various molecules. When it binds with plasma proteins, particularly albumin, fructosamine is formed. Persistent elevation of blood glucose, as in diabetes mellitus, leads to an increase in the amount of glucose bound to serum proteins; therefore, the finding of increased fructosamine indicates persistent hyperglycemia. Because the half-life of albumin cats is 1 to 2 weeks, the fructosamine

level indicates the average serum glucose level over that period and responds more rapidly to alterations in serum glucose than glycosylated hemoglobin. Serum fructosamine may be artificially reduced in patients with hypoproteinemia.

Urine analysis in diagnistics of diabetes melitus. A complete urinalysis profile is essential for diagnosing and monitoring diabetes. Glucosuria is an early indication of poor response to therapy. Proteinuria is a common finding and is often associated with secondary infection of the urinary tract. Diabetic patients are more susceptible to bacterial and fungal infections, such as cystitis. If the patient has ketoacidosis, ketone bodies may be detected in urine.

HOMA index is used to measure insulin resistance, an early stage of type 2 diabetes that increases the risk of many chronic diseases. HOMA is a method used to assess insulin resistance and beta-cell function in individuals. It's commonly used as a tool to evaluate insulin sensitivity and pancreatic beta-cell function, both of which play a crucial role in glucose metabolism.

There are two main versions of the HOMA index:

HOMA-IR (Homeostatic Model Assessment of Insulin Resistance): This index is used to estimate insulin resistance. It's calculated using fasting insulin and fasting glucose levels in the blood. Insulin resistance refers to reduced sensitivity of cells to the effects of insulin, which can lead to elevated blood glucose levels.

HOMA-B (Homeostatic Model Assessment of Beta-cell function): This index is used to estimate pancreatic beta-cell function. It's calculated using fasting insulin and fasting glucose levels as well. Beta cells in the pancreas produce insulin, and HOMA-B helps assess their ability to respond to changes in glucose levels.

The formulas for calculating these indices are based on mathematical models that consider the interaction between insulin and glucose in a fasting state. HOMA indices are often used in research and clinical settings to gain insights into an individual's insulin sensitivity and beta-cell function, which can be helpful in diagnosing and managing conditions like type 2 diabetes.

REVIEW TEST:

№	MCQs	Answers and explanations
1.	**Which statement is true concerning the intestinal brush border membrane?** A. Amylase is only found in the brush border. B. Disaccharides cross the brushborder. C. Insulinis required for the uptake of glucose. D. Fructose requires a sodium-independent monosaccharide transporter. E. Minimal carbohydrate digestion occurs here because most occursin the mouth and stomach.	**The answer is D.** The brushborder of the jejunum is one of the mainsites of carbohydrate digestion through the action of maltase, pancreatic amylase, lactase, and other disaccharidases and oligosaccharidases. Initial digestion of carbohydrate occursin the mouth (using salivary amylase), but once the food enters the stomach, the reduction in pH inactivates salivary amylase. Once digested into monosaccharides in the intestine, absorption occurs through specific transporters that are sodium dependent (SGLT-1 for thetransport of glucose) and independent (GLUT-5 for the transport of fructose). GLUT-4, the insulin-responsive transporter, is not expressed in the intestinal epithelial cells. Once galactose, glucose, and fructose are in the intestinal mucosal cells, GLUT-2 transports these monosaccharides intotheportal circulation. Disaccharides

		are not transported across the intestinal epithelial cell membrane.
2.	**As an experiment, a high school professor convinces each of his students to put a soda cracker in their mouth and not swallow it. After several minutes, some of the students report a sweet taste. Which of the following enzymes is responsible for this phenomenon?** A. Amylase B. Sucrase C. Lactase D. Maltase E. Isomaltase	**The answer is A.** Carbohydrate digestionis initiated in the mouth through the action ofsalivary α-amylase. This enzyme will digest starch, releasing glucose residues. The salivary amylaseis rendered inactive in thestomach because of the acidic environment, being replaced by pancreatic α-amylase, which is secreted into the duodenum. The enzyme specifically cleaves the α-1,4 bonds between the glucosyl residues of starch, resulting in glucose polysaccharides ofvarying numbers of residues. The other glycosidases (sucrase, lactase, maltase, and isomaltase) are associated with enzyme complexes on the brush border of enterocytes of the intestine and would not lead to glucose production in the mouth.
3.	**A 60-year-old patient was found to have a dysfunction of main digestive enzyme of saliva. This causes the disturbance of primary hydrolysis of:** A. Carbohydrates B. Fats C. Proteins D. Cellulose E. Lactose	**The answer is A.** Saliva contains the enzyme amylase, also called ptyalin, which is capable of breaking down starch into simpler sugars such as maltose and dextrin that can be further broken down in the small intestine. About 30% starch digestion takes place in the mouth cavity.
4.	**The 13-year-old female patient having suffered from measles complains of dry mouth, thirst, body weight loss, polyuria, her glucose concentration in blood is 16 mmol/l. What disease can be suspected?** A. Type I pancreatic diabetes B. Type II pancreatic diabetes C. Diabetes insipidus D. Steroidogenic diabetes E. Glycogenosis	**The answer is A.** Type I diabetes, also known as insulin-dependent diabetes or juvenile-onset diabetes, is a chronic condition characterized by high blood glucose levels due to the destruction of insulin-producing beta cells in the pancreas. This destruction is usually caused by an autoimmune response, although other factors such as viruses may also be involved. Type I diabetes typically develops during childhood or adolescence, but can occur at any age. The symptoms of type I diabetes include increased thirst and urination, unexplained weight loss, fatigue, blurred vision, and frequent infections.
5.	**A 12-year-old boy presents with fatigue, polydipsia, polyuria, and polyphagia. A fingerstick glucose measurement shows a glucose level of 19 mmol/L in his serum. He is diagnosed with type1diabetes mellitus, a disease characterized by a deficiency of insulin. Which one of the following is most likely occurring in this patient?** A. Increased fatty acid synthesis from glucose in liver B. Decreased conversion of fatty acids to ketone bodies	**The answer is D.** A decreased insulin-to-glucagon ratio leads to a decrease in fatty acid synthesis and an increase in adipose triacylglycerol degradation, leading to fatty acid release into the circulation. The liver takes up the fatty acids, and within the mitochondria, fatty acids undergo β-oxidization. Asacetyl-CoA accumulates, the ketone bodies, acetoacetate and β-hydroxybutyrate, are formed and are released into the circulation. These ketone bodies are used to fuel theheart, brain, and muscle. Nonenzymatic decarboxylation of acetoacetate forms acetone, which can be smelled by some providers on the breath of patients in diabetic ketoacidosis. Because triglycerides are degraded under these conditions, there is

	C. Increased stores of triacylglycerol in adipose tissue D. Increased production of acetone E. Chronic pancreatitis	not an increase in triglyceride storage. Pancreatitis does not result from an inability to produce insulin.
6.	**A 33-year-old, obese man with an impressive family history of type 2 diabetes is concerned he may develop the disease as well. During a health maintenance examination, his family physician orders several laboratory tests to evaluate the patient. Which of the following results would lead to a diagnosis of diabetes?** A. A single random glucose levelof 190mg/dL B. The presence of a reducingsugar inhis urine C. A single fasting blood glucoselevelof 160 mg/dL D. A 2-hour oral glucose tolerance test with a blood glucoselevel of 210 mg/dL E. A single fasting blood glucoselevel of 110 mg/dL	**The answer is D.** Of all the test values, the one that renders a diagnosis of diabetes in a single episode is a 2-hour oral glucose tolerance test yielding a blood glucose level of 200mg/dL at the end of the test. A single random glucose level of more than 200 mg/dL (not 190 mg/dL) with symptoms of diabetes would confirm the diagnosis. Guidelines concerning fasting glucose levels indicate that to diagnose, diabetes fasting blood glucose levels of more than 126 mg/dL need to be observed on at least two occasions. Two fasting blood glucoselevels between100 and 125 mg/dL indicate impaired glucose tolerance, or what has been called prediabetes. The presence of a reducing sugar in the urine is not sufficient criteria for diabetes because patients with benign fructosuria would also be positive in such a test and not necessarily glucose intolerant (diabetic).
7.	**A 62-year-old, obese man complains of polydipsia (increased drinking), polyuria (increased urination), and fatigue. A glucosetolerance test confirms the diagnosis of diabetes. He is placed on metformin, which works by which of the following mechanisms?** A. Inhibiting hepatic gluconeogenesis B. Increasing glucagon levels C. Increasing cellular responsiveness to circulating insulin D. Stimulating the release of preformed insulin E. Replacing the need for endogenous insulin	**The answer is A.** Metformin, a biguanide, is beneficial in the treatment of type 2 diabetes because it inhibits hepatic gluconeogenesis, which is often increased in patients with type 2 diabetes. No known agent to treat diabetes directly affects the secretion of glucagon. Thiazolidinediones are used in the treatment of diabetes because they increase cellular responsiveness to insulin. Sulfonylureas stimulate the release of preformed insulin. None of these agents completely replaces the need for exogenous insulin inpatients with insulin-dependent diabetes.
8.	**A 47-year-old obese man complains of having to get out of bedt hree times a night to urinate (polyuria), being constantly thirsty (polydipsia), and eating more often (polyphagia). The patient is diagnosed with insulin resistant diabetes mellitus (type 2). If the patient's symptoms are due to a problem at the level of the glucose transporter, which one of the tissues indicated below will be most affected?** A. RBCs	**The answer is C.** Both muscle and adipose tissue rely primarily on the glucose transporter GLUT-4, which requires insulin for optimal expression onthe cell surface. The other glucose transporters are found on the cell surface in the absence of insulin secretion. These include GLUT-1,-2, -3, and-5. GLUT-1 is ubiquitously distributed in various tissues. GLUT-2 is present in liver and pancreatic β-cells. GLUT-3 is also found in the intestine with GLUT-1. Finally, GLUT-5 functions primarily as a fructose transporter.

	B. Small intestine C. Muscle D. Brain E. Liver	
9.	**The 49-year-old female patient suffering long-term from pancreatic diabetes has developed the following symptoms after administering insulin: weakness, facial pallor, palpitation, anxiety, double vision, numbness of lips and tongue apex. Glucose molar concentration in blood was 2,5 mmol/l. What complication has developed in the patient?** A. Hyperketonemic coma B. Hyperosmolar coma C. Hyperglycemic coma D. Hypoglycemic coma E. Uremic coma	**The answer is D.** Hypoglycemic coma is a serious complication that can occur in diabetic patients who receive an overdose of insulin or who do not eat enough food after taking their insulin. Insulin lowers blood glucose levels by promoting the uptake of glucose by cells and the conversion of glucose to glycogen in the liver and muscle tissue. In some cases, the administration of too much insulin can cause blood glucose levels to drop too low, resulting in hypoglycemia. Symptoms of hypoglycemic coma can include confusion, dizziness, weakness, seizures, and loss of consciousness. Treatment usually involves the administration of glucose or other carbohydrates to raise blood glucose levels. In severe cases, the patient may require hospitalization and close monitoring of blood glucose levels. Prevention of hypoglycemic coma in diabetic patients involves careful monitoring of insulin doses and regular consumption of meals to prevent low blood glucose levels.
10	**A 45-year-old woman does not have any symptoms of insulin dependent diabetes mellitus but testing on an empty stomach showed the increase of the blood glucose level (7.5 mM/l). What additional laboratory test needs to be done to substantiate the diagnosis?** A. Determination of tolerance to glucose B. Determination of tolerance to glucose on an empty stomach C. Determination of rest nitrogen level in the blood D. Determination of ketone bodies concentration in the urine E. Determination of glycosylated hemoglobin level	**The answer is B.** Glucose tolerance test is a medical test that is used to determine the body's ability to metabolize glucose and is often used to diagnose diabetes. During the test, the patient drinks a sugary drink and then blood glucose levels are measured at regular intervals to assess how well the body is able to process and clear glucose from the bloodstream. This test is commonly performed after an overnight fast on empty stomach to establish a baseline fasting blood glucose level and then repeated at intervals to assess how blood glucose levels change over time. In some cases, insulin levels may also be measured to determine how well the body is responding to glucose.

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11. CATABOLISM AND BIOSYNTHESIS OF TRIACYLGLYCEROLS AND PHOSPHOLIPIDS. INTRACELLULAR LIPOLYSIS AND MOLECULAR MECHANISMS OF ITS REGULATION

OBJECTIVES after studying this chapter, you should be able to:

- *Interpret biochemical function of simple and complex lipids in organism: their involvement in formation of structure and function of biological membranes, reserve and energetic significance, the role as precursors in biosynthesis of biologically active compounds of lipid nature.*
- *Explain the principal pathways of intracellular lipid metabolism.*
- *Describe enzymatic reactions of catabolism and biosynthesis of triacylglycerols.*
- *Interpret enzymatic reactions of synthesis of phospholipids and sphingolipids.*
- *Analyze the main pathways of lipid metabolism in human body in normal conditions and in pathology.*
- *Explain hormonal regulation of lipid metabolism.*

11.1. Classification of lipids. Biological functions of simple and complex lipids in human body

The lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, that are related more by their physical than by their chemical properties. **Lipids can be defined as compounds insoluble in water but soluble in nonpolar solvents such as ether and chloroform.**

Lipids are classified into three groups: **simple lipids, compounds lipids and derived lipids (fig. 11.1).**

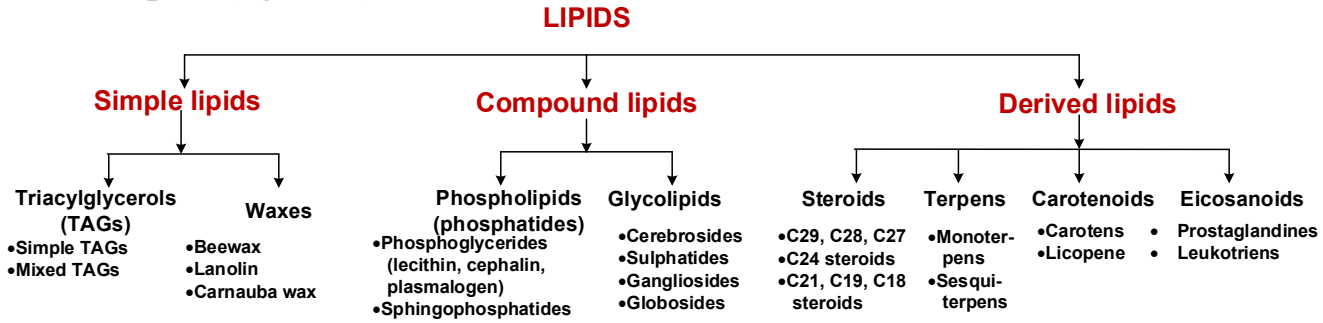

Fig. 11.1. Lipids can be classified into several categories based on their chemical structure and properties

Simple lipids include **fats and waxes**, which are esters formed by the combination of fatty acids with different alcohols:

- **Triacylglycerols (TAGs)**, also known as **triglycerides or fats**, make up approximately 90% of dietary lipids and serve as the primary form of energy storage in humans. Triacylglycerols are composed of glycerol esterified with fatty acids, such as palmitic acid and oleic acid. **Monoacylglycerols (monoglycerides),**

diacylglycerols (diglycerides), and triacylglycerols (triglycerides) consist of one, two, and three fatty acids esterified to glycerol, respectively.

- **Waxes** can be defined as esters formed by the combination of fatty acids with monohydric alcohols of higher molecular weight.

Complex lipids are characterized as esters of fatty acids that contain additional groups along with an alcohol and one or more fatty acids. They can be classified into two groups:

- **Phospholipids** consist of fatty acids, an alcohol, and a phosphoric acid residue. Phospholipids often contain nitrogen-containing bases like choline and other substituents. While glycerol is the common alcohol found in many phospholipids (known as glycerophospholipids), sphingosine, which contains an amino group, serves as the alcohol in sphingophospholipids.
- **Glycolipids,** also known as glycosphingolipids, are a class of lipids that contain a fatty acid, sphingosine (or a related sphingoid base), and a carbohydrate moiety. They are an important component of cell membranes and play various roles in cell recognition, signaling, and cellular processes.

Derived lipids include steroids, terpenes, ketone bodies, lipid-soluble vitamins and hormones.

Lipids play various important roles, some of which are listed below:

- Lipids serve as storage compounds, with triglycerides acting as the body's reserve energy.
- They are crucial components of cell membrane structure in eukaryotic cells.
- Lipids act as a source for fat-soluble vitamins such as A, D, E, and K.
- They function as electrical insulators in nerve fibers, with lipids present in the myelin sheath.
- Lipids contribute to thermoregulation, with subcutaneous fat layers providing insulation and protection against cold. Brown adipose tissue plays a role in regulating body temperature.
- Cholesterol is present in cell membranes, blood, and bile in many organisms. It helps maintain membrane fluidity by interacting with lipid complexes. Cholesterol also serves as a precursor for bile acids, Vitamin D, and steroids.
- Essential fatty acids like linoleic and linolenic acids are precursors for various biologically active substances, including prostaglandins and thromboxanes. These substances play crucial roles in pain, fever, inflammation, and blood clotting. Prostaglandins act as regulators of cellular metabolism.

11.1.1. Structure and role of fatty acids

Fatty acids are characterized as carboxylic acids with a hydrocarbon side chain. They represent the simplest form of lipids and are predominantly found in esterified form as major constituents of various lipid molecules. Additionally, fatty acids can also exist as free (unesterified) fatty acids.

In natural lipids, the majority of fatty acids have an even number of carbon atoms, typically ranging from 14 to 20 carbons. This pattern arises from the biosynthesis of fatty

acids, which involves the sequential addition of two-carbon units. The most common fatty acids include **palmitic acid (16 carbons) and stearic acid (18 carbons).**

Saturated fatty acids are characterized by the absence of double bonds (as indicated in Table 11.1), whereas unsaturated fatty acids contain one or more double bonds. Both saturated and unsaturated fatty acids are commonly found in natural lipids. Fatty acids with a single double bond are referred to as **monounsaturated,** while those with two or more double bonds are collectively known as **polyunsaturated fatty acids (PUFA)** (table 11.2).

Table 11.1. The most common saturated fatty acids

Trivial name	Systematic name	Number of carbons in chain	Formula
Lauric acid	n-dodecanoic acid	12	$CH_3(CH_2)_{10}COOH$
Myristic acid	n-tetradecanoic acid	14	$CH_3(CH_2)_{12}COOH$
Palmitic acid	n-hexadecanoic acid	16	$CH_3(CH_2)_{14}COOH$
Stearic acid	n-octadecanoic acid	18	$CH_3(CH_2)_{16}COOH$
Behenic acid	n-docosanoic acid	22	$CH_3(CH_2)_{18}COOH$
Lignoceric acid	n-tetracosanoic acid	24	$CH_3(CH_2)_0COOH$

Table 11.2. Common monounsaturated and polyunsaturated fatty acids

Trivial name	Systematic name	Number of carbons in chain	Formula
MONOUNSATURATED FATTY ACIDS			
Palmitoleic acid	cis-9-hexadecenoic acid	16	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$
Oleic acid	cis-9-octadecenoic acid	18	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
POLYUNSATURATED FATTY ACIDS			
Linoleic acid	cis-9-, cis-12-octadecadienoic acid	18	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$
Linolenic acid	cis-9-, cis-12-, cis-15-octadecatrienoic acid	18	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_4COOH$
Arachidonic acid	cis-5,8,11-eicosatetranoic acid	20	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3COOH$

The simplest form of fatty acids consists of unbranched, linear chains of CH_2 groups connected by carbon-carbon single bonds, with a terminal carboxylic acid group. When we refer to fatty acids as saturated, it means that each carbon in the molecule is bonded to the maximum possible number of hydrogen atoms. Numerous saturated fatty acids have both a common or trivial name and a chemically descriptive systematic name.

Fatty acids are named systematically based on the hydrocarbon they are derived from. Saturated fatty acids are named with the suffix -anoic (e.g., octanoic acid), while unsaturated fatty acids end with -enoic (for example, octadecanoic acid). However, fatty acids also have common names that are more commonly used.

Polyunsaturated fatty acids, which contain more than one carbon-carbon double bond, are present in relatively small quantities. The double bonds in these fatty acids are typically separated by a CH_2 group ($-CH_2-CH=CH-CH_2-CH=CH-CH_2-$), forming a regular spacing motif. This spacing is a result of the biosynthetic mechanism by which the double bonds are introduced into the hydrocarbon chain. The most common polyunsaturated fatty acids include linoleic and arachidonic acid.

Arachidonic acid (C_{20}) is particularly notable as the precursor of a family of molecules called **eicosanoids** (from Greek eikosi, meaning "twenty"), which includes prostaglandins, thromboxanes, and leukotrienes. These compounds, produced by cells under specific conditions, possess potent physiological properties, as described in the section on intracellular and extracellular messengers.

Certain essential fatty acids, such as linoleic acid (an omega-6 fatty acid) and alpha-linolenic acid (an omega-3 fatty acid), cannot be synthesized by animals and must be obtained from plant sources in the diet. These fatty acids serve as precursors for eicosanoids, thus they are referred to as essential fatty acids.

11.1.2.Triacylglycerols (TAG) their structure and properties

Triacylglycerols, also known as triglycerides, serve as the primary storage form of fatty acids in biological systems. They belong to a class of compounds that consist of glycerol, a trihydroxy alcohol with three carbon atoms, each linked to a fatty acid via an ester bond (fig. 11.2). Tristearin is an example of a typical triglyceride, which is referred to as a simple triglyceride since it contains only one type of fatty acid.

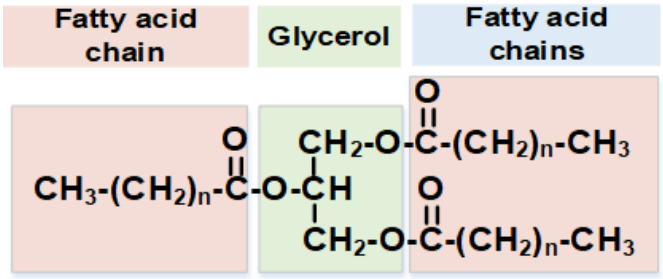

Fig. 11.2. Triacylglycerols are a type of lipid molecule composed of three fatty acid chains esterified to a glycerol backbone.

However, the majority of naturally occurring TAG molecules contain more than one type of fatty acid. In such cases, when two or more different fatty acids are present in a single molecule, it is called a **mixed TAG**. Depending on which fatty acid is bonded to the central carbon of glycerol, three different molecules are possible for any specific combination of three fatty acids.

TAGs, which are the most abundant group of lipids, primarily serve as fuel reserves in animals. Fats are predominantly found in adipose tissue, where specialized cells called

adipocytes store TAGs. These fats are stored in the form of globules dispersed throughout the cytoplasm. It is noteworthy that triacylglycerols do not serve as structural components of biological membranes.

TAGs are hydrophobic substances that are only soluble in certain organic solvents. Unlike other complex lipids, they do not possess electric charges and are thus referred to as neutral lipids. The molecular structure of studied triacylglycerols indicates that the acyl chains attached to carbon 1 and carbon 2 of glycerol, along with the glycerol itself, form a straight line. Carbon 3 extends at a right angle to this line, with its acyl chain folding over at the carboxyl carbon and aligning with the acyl chain on carbon 1.

The melting temperatures of mixed triacylglycerols are an average of the melting temperatures of their constituent fatty acids. In simple triacylglycerols, the melting temperatures increase with longer acyl chain lengths but decrease with a higher number of double bonds. Melted triacylglycerols generally exhibit a high viscosity and have an oil-like consistency. From a physiological perspective, it is crucial for stored triglycerides to remain fluid at body temperature to allow for rapid mobilization as an energy source. Liquidity is also essential for subcutaneous fat storage, as it acts as insulation without impeding the mobility of the organism and its body parts.

A few important properties of triacylglycerols, which have biochemical relevance, are discussed below.

1. **Hydrolysis**, Triacylglycerols undergo stepwise enzymatic hydrolysis to finally liberate free fatty acids and glycerol. The process of hydrolysis, catalysed by lipases is important for digestion of fat in the gastrointestinal tract and fat mobilization from the adipose tissues.

2. **Saponification**. The hydrolysis of triacylglycerols by alkali to produce glycerol and soaps is known as saponification.

3. **Rancidity**. Rancidity is the term used to represent the deterioration of fats and oils resulting in an unpleasant taste. Fats containing unsaturated fatty acids are more susceptible to rancidity. Rancidity occurs when fats and oils are exposed to air, moisture, light, bacteria etc. Hydrolytic rancidity occurs due to partial hydrolysis of triacylglycerols by bacterial enzymes. Oxidative rancidity is due to oxidation of unsaturated fatty acids. This results in the formation of unpleasant products such as dicarboxylic acids, aldehydes, ketones etc. Rancid fats and oils are unsuitable for human consumotion.

4. Lipid peroxidation in vivo. In the living cells, lipids undergo oxidation to produce peroxides and free radicals which can damage the tissue. The free radicals are believed to cause inflammatory diseases, ageing, cancer/ atherosclerosis etc. lt is fortunate that the cells possess antioxidants such as vitamin E, urate and superoxide dismutase to prevent in vivo lipid peroxidation.

11.1.3. Phospholipids: structure of main glycerophospholipids and sphingolipids, their biological role

Phospholipids are a type of complex or compound lipids that consist of phosphoric acid, fatty acids, nitrogenous bases, and alcohol. There are two classes of phospholipids:

1. **Glycerophospholipids** (or phosphoglycerides) that contain glycerol as the alcohol.

2. **Sphingophospholipids** (or sphingomyelins) that contain sphingosine as the alcohol.

Glycerophospholipids are the major lipids that occur in biological membranes. They consist of glycerol 3-phosphate esterified at its C1 and C2 with fatty acids. **Phosphatidic acid**, depicted in Figure 11.3, is the simplest form of phospholipid. It is not found in significant concentrations in tissues. Phosphatidic acid serves as an intermediate in the synthesis of triacylglycerols and other phospholipids. The various glycerophospholipids, which contain different nitrogenous bases or other groups, can be considered derivatives of phosphatidic acid. Typically, R1 in these phospholipids is an unsaturated fatty acid, while R2 is a saturated fatty acid.

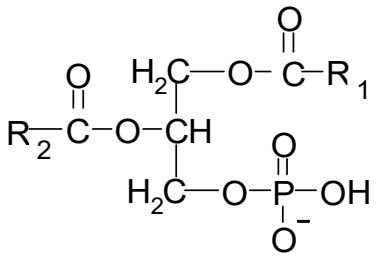

Fig. 11.3. Phosphatidic acid (PA) is a phospholipid molecule that serves as a precursor for the synthesis of other phospholipids

Lecithins, specifically **phosphatidylcholine (fig. 11.4)**, are the most abundant group of phospholipids found in cell membranes. Chemically, lecithin is composed of phosphatidic acid with **choline** as the nitrogenous base. Phosphatidylcholines serve as the storage form of choline in the body.

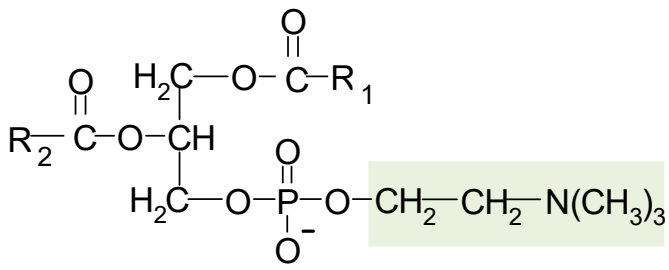

Fig. 11.4. Lecithin is a type of phospholipid that is commonly found in biological membranes and is an important component of cell membranes in various tissues.

Dipalmitoyl lecithin, a significant type of phosphatidylcholine, is found in the lungs. It functions as a surface-active agent and prevents the adherence of the lung's inner surface due to surface tension. In infants, the absence of dipalmitoyl lecithin is associated with respiratory distress syndrome, a disorder characterized by impaired lung function.

Lysolecithin is formed when one of the fatty acids is removed from lecithin, either at carbon C1 or C2.

Cephalins, specifically **phosphatidylethanolamine** (fig. 11.5), contain **ethanolamine** as the nitrogenous base. This sets them apart from lecithins, which have a different base. Phosphatidylethanolamines are present in all living cells and make up approximately 25% of all phospholipids. In human physiology, they are prominently found in nervous tissue, including the white matter of the brain, nerves, neural tissue, and the spinal cord, where they constitute around 45% of all phospholipids.

Cephalin is involved in the blood clotting cascade. It serves as a surface for the assembly and activation of coagulation factors, specifically in the intrinsic pathway of blood clotting.

Cephalin is present in various food sources, including soybeans, eggs, liver, and other animal and plant-based products. It is also commonly used as an additive in the food industry, particularly in processed foods and emulsions.

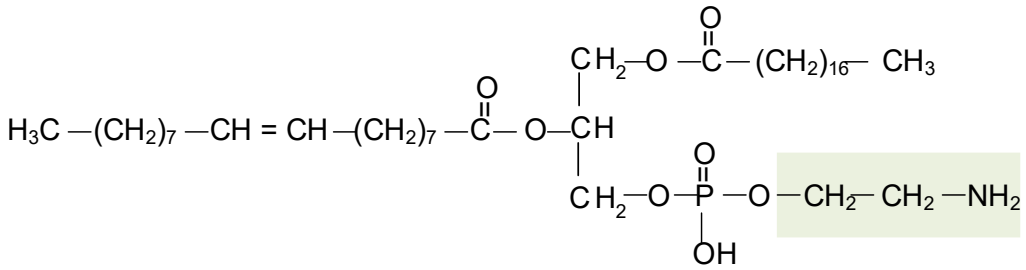

Fig. 11.5. Cephalin, also known as phosphatidylethanolamine, is a type of phospholipid found in cell membranes. It is similar in structure to lecithin, but with an ethanolamine group instead of a choline group attached to the phosphate head group.

Phosphatidylinositols (PIs) (fig. 11.6) are composed of a glycerol backbone esterified with two fatty acid and phosphoric acid, which is linked to **inositol**, a six-carbon sugar alcohol. The inositol moiety can undergo phosphorylation at different positions to generate different forms of phosphatidylinositols.

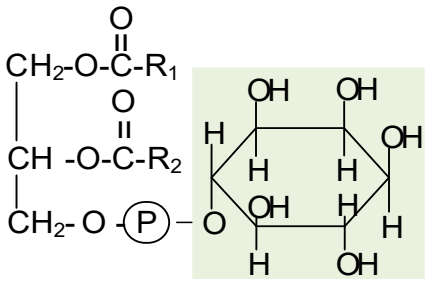

Fig. 11.6. Phosphatidylinositols are a class of phospholipids that are important components of cell membranes and play crucial roles in cellular signaling and membrane trafficking.

Phosphatidylinositols are involved in various cellular signaling pathways, including those regulated by growth factors, hormones, and neurotransmitters. Phosphorylation of specific hydroxyl groups on the inositol ring generates distinct forms of phosphatidylinositols, which act as signaling molecules by recruiting and activating specific proteins. Phosphatidylinositols are involved in processes such as cell growth, differentiation, apoptosis, membrane trafficking, and intracellular calcium signaling.

Phosphatidylinositols are involved in regulating membrane trafficking processes, such as endocytosis, exocytosis, and vesicle trafficking. Specific forms of phosphatidylinositols act as markers on membranes, facilitating the recruitment and binding of proteins involved in membrane trafficking events.

Phosphatidylserine (fig. 11.7) is a phospholipid that is found in biological membranes, particularly on the inner leaflet of the plasma membrane. It plays important roles in various cellular processes and is involved in cell signaling, membrane integrity, and protein interactions. Phosphatidylserine is composed of a glycerol backbone esterified with two fatty acid and phosphoric acid, which is linked to **serine**, an amino acid.

Phosphatidylserine is asymmetrically distributed in biological membranes, predominantly located on the inner leaflet of the plasma membrane. In healthy cells, only a small fraction of phosphatidylserine is present on the outer leaflet of the plasma membrane.

During certain cellular processes, such as apoptosis (programmed cell death), phosphatidylserine is exposed on the outer leaflet, serving as an "eat-me" signal for phagocytic cells.

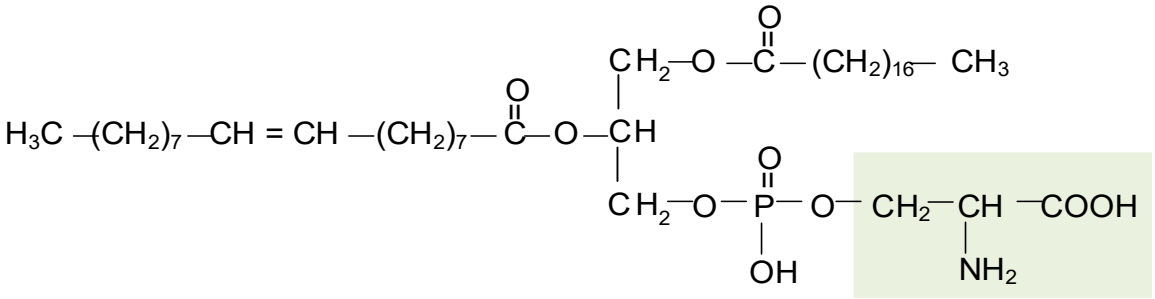

Fig. 11.7. Phosphatidylserine is a vital phospholipid that contributes to the structure, function, and signaling processes of cell membranes.

Phosphatidylserine is important for maintaining the integrity and fluidity of cell membranes. It plays a role in cell-cell interactions, cell adhesion, and membrane fusion processes. Phosphatidylserine exposure on the outer leaflet of the plasma membrane is involved in phagocytosis, where phagocytic cells recognize and engulf cells or cellular debris displaying phosphatidylserine.

Sphingophospholipids are a class of complex lipids that contain a **sphingosine** backbone instead of glycerol. They are a major component of cell membranes and play important roles in cell structure and signaling. Sphingophospholipids are composed of a sphingosine or a related sphingoid base as the backbone, which is amide-linked to a fatty acid at the amino group. The hydroxyl group of sphingosine is esterified with a phosphate group, which can further be linked to various polar head groups, such as choline, ethanolamine, serine, or inositol.

Sphingomyelins (fig. 11.8) are the most common type of sphingophospholipids. They have a phosphorylcholine or phosphoethanolamine head group linked to the phosphate group.

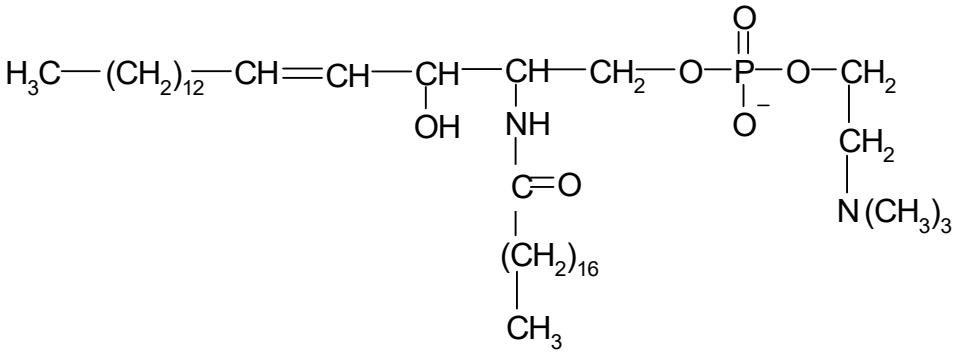

Fig. 11.8. Sphingomyelins are a type of sphingophospholipid that is widely distributed in biological membranes, particularly in the nervous system. They are a major component of myelin, the insulating sheath that surrounds nerve fibers.

Sphingophospholipids are important structural components of cell membranes, particularly in specialized membrane domains, such as lipid rafts.

They contribute to the stability, fluidity, and organization of the lipid bilayer and are involved in membrane protein localization and function.

Sphingophospholipids, particularly sphingomyelins and ceramide phosphoinositols, play crucial roles in cell signaling pathways.

Sphingomyelins can be hydrolyzed by sphingomyelinases, producing ceramides, which act as second messengers in cellular signaling, including apoptosis and stress responses.

Sphingophospholipids have diverse physiological functions, including cell signaling, cell growth, differentiation, membrane dynamics, and membrane repair.

They are particularly abundant in tissues such as the nervous system and myelin sheaths, where they contribute to the insulation and integrity of neuronal cells.

11.1.4. Structure and occurrence of main steroids.

Steroid s are a class of lipids that share a common structural feature known as the steroid nucleus - **cyclopentanoperhydrophenanthrene.**.They are derived from cholesterol and have diverse physiological functions in the body. Here are some key points about steroid lipids:

Steroid lipids have a characteristic four-ring structure known as the steroid nucleus. The rings are labeled A, B, C, and D, with varying functional groups attached to them. The basic structure of the steroid nucleus consists of three cyclohexane rings (A, B, and C) and one cyclopentane ring (D).

Cholesterol is the most well-known and abundant steroid lipid in the body (fig. 11.9). It is an essential component of cell membranes and is involved in maintaining membrane fluidity and integrity. Cholesterol serves as a precursor for the synthesis of other steroid hormones, such as cortisol, estrogen, progesterone, and testosterone.

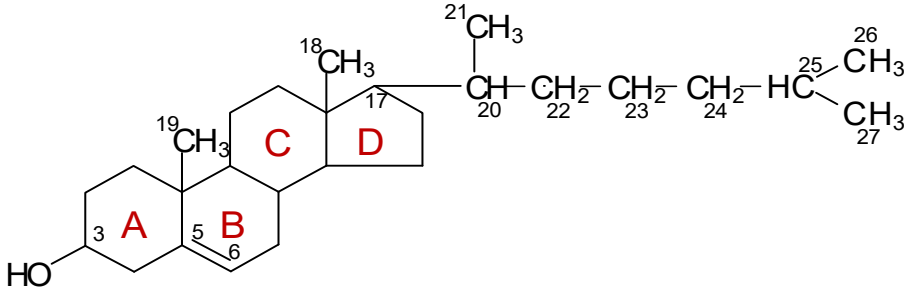

Fig. 11.9. The structure of cholesterol features a steroidal nucleus composed of four interconnected rings with a hydroxyl group at carbon-3 and a side chain derived from three isoprenoid units at carbon-17. This structure is characteristic of cholesterol and serves as the foundation for its biological functions and interactions in the body.

Steroid lipids include various steroid hormones, which act as chemical messengers in the body. These hormones regulate a wide range of physiological processes, including metabolism, growth and development, reproduction, and immune response. Examples of steroid hormones include cortisol, aldosterone, estrogen, progesterone, testosterone, and vitamin D.

In addition to their roles as hormones and membrane components, steroid lipids also have functions in lipid digestion and absorption, bile acid synthesis, and as precursors for the synthesis of other biologically active molecules.

11.2. Micelles, liposomes and biological membranes

Lipids such as triglycerides are insoluble in water due to the presence of non-polar hydrophobic hydrocarbon chains. Similarly, cholesterolesters are also insoluble in water

because of the hydrophobic nature of the steroid nucleus. On the other hand, lipids like cholesterol, phospholipids, and bile salts contain both a water-soluble polar head group and a water-insoluble non-polar tail. These molecules are referred to as **"amphipathic molecules"** because they possess two distinct types of groups. When amphipathic molecules like phospholipids are introduced into water, their polar head groups orient themselves towards the water phase while the hydrophobic tails face away from the water, typically towards the air. This arrangement leads to the formation of a **unimolecular lipid layer** at the interface between water and air.

Micelles are spherical aggregates that form when amphipathic lipids, such as phospholipids or bile salts, are present in an aqueous medium above a critical concentration (fig. 11.10). In micelles, the polar head groups of the amphipathic lipids are oriented towards the exterior, interacting with the surrounding water, while the non-polar tails are sequestered in the interior of the sphere. Bile salts, which are a type of amphipathic lipid, have the ability to form micelles in aqueous solutions. This property is essential for their function in the digestion and absorption of dietary fats. When phospholipids are present in a mixture of water and oil, such as in the case of a **lipid bilayer**, the polar head groups of the phospholipids orient towards the water phase, while the non-polar tails face the oil phase. This arrangement allows the formation of a lipid bilayer structure, which is the fundamental component of cell membranes. **Mixed micelles** are micelles composed of various types of amphipathic lipids. They form when micelles of a particular lipid combine with other lipids. During the digestion and absorption of lipids, for example, micelles of bile salts combine with products of lipid digestion to form mixed micelles, facilitating the transport and absorption of lipids in the digestive system.

Liposomes are spherical vesicles that are formed when a lipid bilayer closes on itself (fig. 11.10). They consist of an aqueous core enclosed by a lipid membrane, similar to a cell membrane. Liposomes have been extensively studied and utilized in various fields, including drug delivery and gene therapy.

Due to their unique structure, liposomes can serve as carriers or vehicles for drugs. They can encapsulate both hydrophilic (water-soluble) and hydrophobic (fat-soluble) drugs within their aqueous core or lipid bilayer, respectively. This property allows liposomes to efficiently deliver drugs to specific sites in the body.

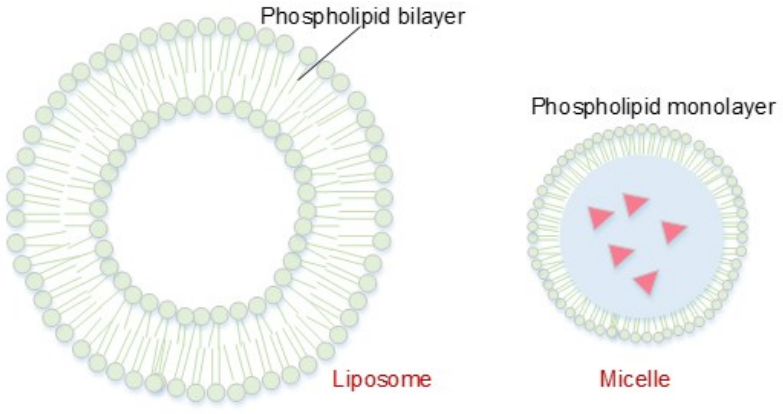

Fig. 11.10. Stucture of liposomes and micelles

Liposomes have the ability to fuse with cell membranes, which enables them to deliver drugs directly into cells. They can merge with the target cell membrane, releasing their contents into the cell's interior. This mechanism is advantageous for delivering drugs to specific cell types and enhancing the therapeutic efficacy while minimizing side effects.

In **cancer therapy**, liposomes have been used to deliver anticancer drugs selectively to cancer cells. By modifying the surface of the liposomes with targeting molecules, such as antibodies or ligands, they can specifically recognize and bind to cancer cells. This targeted drug delivery approach improves the effectiveness of chemotherapy while reducing toxicity to healthy cells.

In **gene therapy**, liposomes serve as carriers for introducing therapeutic genes into cells. The genes are encapsulated within the liposome's aqueous core or attached to its surface. Liposomes can protect the genetic material from degradation and deliver it to target cells, where the genes can be expressed and produce the desired therapeutic effect.

Biological membranes are the membranes present in biological systems. They ares are highly fluid, dynamic structures consisting of a lipid bilayer and associated proteins. **The major lipids in mammalian membranes are phospholipids, glycosphingolipids and cholesterol. Phosphatidylcholine** is generally the major phosphoglyceride by mass in the membranes of human cells. The second major class of phospholipids comprises **sphingomyelin,** a phospholipid that contains a sphingosine rather than a glycerol backbone. A fatty acid is attached by an amide linkage to the amino group of sphingosine, forming ceramide. When the primary hydroxyl group of sphingosine is esterified to phosphorylcholine, sphingomyelin is formed. As the name suggests, sphingomyelin is prominent in myelin sheaths.

The amphipathic character of phospholipids makes them form the thermodynamically the best forms such as bilayers, micelles or liposomes. Bilayers are the key structures in biological membranes. Bilayers exist as sheets wherein the hydrophobic regions of the phospholipids are sequestered from the aqueous environment, while the hydrophilic, charged portions are exposed to water. The closed bilayer provides one of the most essential properties of membranes. The lipid bilayer is impermeable to most water-soluble molecules since such charged molecules would be insoluble in the hydrophobic core of the bilayer.

Several models have been proposed to explain the ultra-structure of the plasma membrane; the most widely accepted one is **“Fluid mosaic model” (**fig.11.11) that describes the structure and organization of the cell membrane. It was proposed by **S.J. Singer and Garth L. Nicolson** in 1972 and has since become a fundamental concept in cell biology.

The key features of the fluid mosaic model are as follows:

- **Fluidity:** The cell membrane is described as a fluid structure. The lipid bilayer, which is the primary component of the membrane, consists of phospholipids with hydrophilic (water-attracting) heads and hydrophobic (water-repelling) tails. These phospholipids can move laterally within the membrane, allowing for flexibility and fluidity.
- **Lipid bilayer:** The basic structure of the cell membrane is a double layer of phospholipids, forming a lipid bilayer. The hydrophilic heads of the phospholipids face outward, interacting with the surrounding aqueous environment, while the hydrophobic tails are sandwiched between the two layers, facing inward.

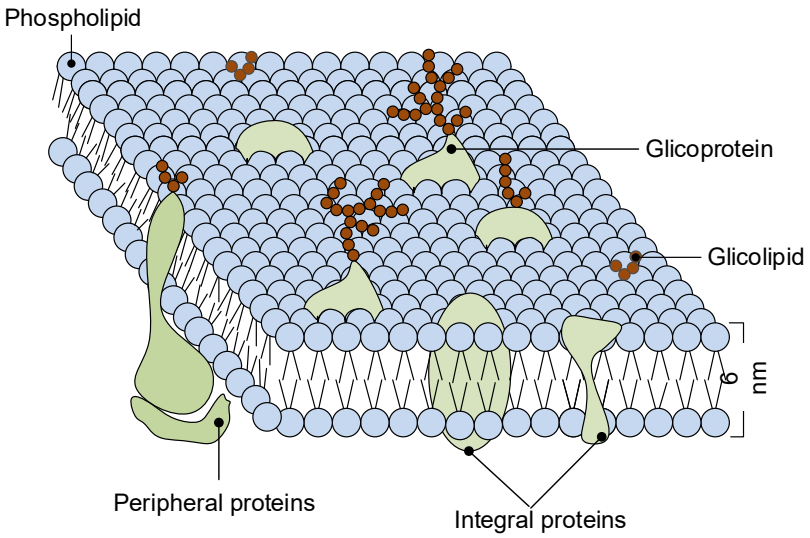

Fig. 11.11. Fluid mosaic model

• **Proteins:** The cell membrane is embedded with various proteins, including integral membrane proteins and peripheral proteins. Integral membrane proteins span the lipid bilayer, with portions exposed on both sides of the membrane. Peripheral proteins are associated with either the inner or outer surface of the membrane. These proteins are not uniformly distributed but are rather arranged in a mosaic pattern within the lipid bilayer.

- **Fluid mosaic:** The proteins in the cell membrane are not fixed in position but can move within the lipid bilayer. They exhibit a dynamic behavior, constantly changing their position and orientation. This fluid nature allows for the movement and interaction of proteins, lipids, and other molecules within the membrane.
- **Mosaic composition:** The cell membrane is composed of a variety of components, including phospholipids, cholesterol, glycolipids, and various proteins. This diverse composition creates a mosaic-like structure, with different molecules and proteins distributed unevenly throughout the membrane.

- **Membrane asymmetry:** The two leaflets of the lipid bilayer can have different compositions of lipids and proteins, resulting in membrane asymmetry. This asymmetry is important for various cellular processes, such as membrane trafficking and cell signaling.
- **Dynamic nature:** The fluid mosaic model recognizes that membranes are dynamic structures, with the lipid and protein components constantly in motion. Proteins can move laterally within the membrane (lateral diffusion), and in some cases, they can also move rotationally or undergo transverse diffusion (flip-flop) across the bilayer.

Lipids have a unique property of being amphipathic, meaning they have both hydrophilic and hydrophobic regions within their molecular structure. In an aqueous environment, such as water, lipids tend to aggregate to minimize their exposure to the surrounding water. When the concentration of amphipathic lipids reaches a critical point, they can spontaneously form **micelles**. Micelles are spherical structures where the hydrophobic tails of the lipids face inward, shielded from the water, while the hydrophilic heads are oriented outward, interacting with the surrounding water. Micelles are dynamic structures that can solubilize hydrophobic substances, such as fatty acids and fat-soluble vitamins, in their hydrophobic core, making them more soluble and accessible for transport within an aqueous environment.

In addition to micelles, amphipathic lipids, such as phospholipids, can also form bilayers. In a bilayer arrangement, two layers of amphipathic lipids align with their

hydrophilic heads facing outward and their hydrophobic tails facing inward, forming a stable lipid bilayer. This lipid bilayer is the fundamental structure of biological membranes and provides a barrier between the aqueous compartments inside and outside the cells.

Liposomes may be formed by sonicating an amphipathic lipid in an aqueous medium. They consist of spheres of lipid bilayers that enclose part of the aqueous medium. Aggregation of bile salts into micelles and liposomes and the formation of **mixed micelles** with the products of fat digestion are important in facilitating absorption of lipids from the intestine. **Liposomes are of potential clinical use** – particularly when combined with tissue-specific antibodies – as carriers of drugs in the circulation, targeted to specific organs, for example, in cancer therapy. In addition, they are used for gene transfer into vascular cells and as carriers for topical and transdermal delivery of drugs and cosmetics. Emulsions are much larger particles, formed usually by nonpolar lipids in an aqueous medium. These are stabilized by emulsifying agents such as amphipathic lipids (eg, phosphatidylcholine), which form a surface layer separating the main bulk of the nonpolar material from the aqueous phase.

11.3. Digestion of lipids.

The initial step in the breakdown of TAGs and phospholipids begins in the mouth when lipids come into contact with saliva. Subsequently, the process of chewing, combined with the presence of emulsifiers, facilitates the action of digestive enzymes. The enzyme **lingual lipase**, assisted by a small amount of phospholipid acting as an emulsifier, initiates the digestion process. These activities enhance the accessibility of fats to the digestive enzymes, resulting in the fats transforming into tiny droplets and separating from the watery components.

In the stomach, the presence of lipase is unable to hydrolyze fats due to the highly acidic nature of the gastric contents. **Gastric lipase** functions optimally at a pH of 5.5 and plays a significant role in the digestion of fats from maternal milk in newborn children, as their gastric juice has a pH of approximately 5. However, as individuals age, the pH in the stomach becomes more acidic, reaching levels of 1.5-2.0. Consequently, the majority of ingested fat is digested in the small intestine.

Since TAGs are insoluble in water, while digestive enzymes are soluble, the digestion of triacylglycerols occurs at the interfaces between lipids and water. The rate of TAG digestion is therefore dependent on the surface area of this interface, which is greatly increased by the churning peristaltic movements of the intestine in combination with the **emulsifying action of bile acids**. Bile acids, synthesized by the liver and secreted via the gallbladder into the small intestine, act as potent digestive detergents. Bile acid salts **emulsify** the dietary fats, breaking them down into smaller droplets, which increases the surface area available for pancreatic lipase to act upon.

Pancreatic lipase catalyzes the hydrolysis of triacylglycerols at their 1 and 3 positiequentially forming **2-Monoacylglycerols** and fatty acids (fig. 11.12). Like many proteins, lipase is quickly denatured at interfaces, including lipid-water interfaces. To prevent this denaturation and anchor lipase to the lipid-water interface, colipase, a pancreatic protein, forms a 1:1 complex with lipase.

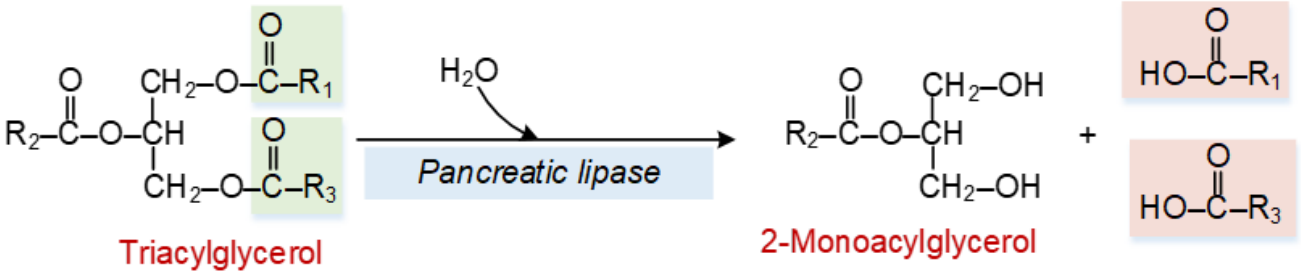

Fig. 11.12. Pancreatic lipase plays a crucial role in the digestion and absorption of dietary fats. It breaks down triglycerides into fatty acids and monoglycerides, which are then absorbed and utilized by the body.

Pancreatic lipase requires a cofactor called **co-lipase** to function optimally. Co-lipase is also produced by the pancreas and acts as an anchor, attaching pancreatic lipase to the lipid droplet surface, allowing for efficient lipolysis.

The secretion of pancreatic lipase is regulated by hormonal signals, primarily through the release of the hormone **cholecystokinin (CCK)** from the small intestine. CCK is released in response to the presence of fats and triggers the release of pancreatic enzymes, including lipase, to aid in fat digestion.

The **digestion of phospholipids**, which are a type of complex lipid, occurs primarily in the small intestine and involves the action of specific enzymes. Before phospholipids can be effectively digested, they undergo emulsification, a process similar to the emulsification of dietary fats. Once emulsified, phospholipids are acted upon by pancreatic **phospholipase enzymes**, primarily **pancreatic phospholipase A2 (PLA2).** Phospholipase A_2 hydrolytically excises the fatty acid residue at C(2) to yield the corresponding lysophosopholipids, which are also powerful detergents.

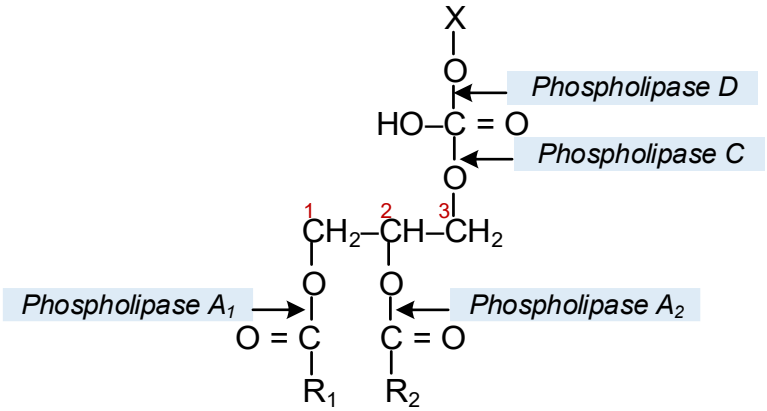

Fig. 11.13. Phospholipids degradation by phospholipases

Phospholipases A1 or A2 gives **a lysophospholipid** (lacking a fatty acid) **phospholipase C** gives a diacylglycerol **phospholipase D** gives a phosphatidate.

The fatty acids, monoacylglycerols, and diacylglycerols resulting from lipid digestion are taken up by the cells lining the small intestine, known as the intestinal mucosa. This absorption process is facilitated by bile acids. Bile acids form **mixed micelles** that encapsulate the nonpolar lipid breakdown products, allowing them to be transported across the unstirred aqueous boundary layer present at the intestinal wall. This mechanism is crucial, as it is illustrated in individuals with obstructed bile ducts who experience limited absorption of dietary lipids. Instead, these lipids are eliminated in a hydrolyzed form in the feces, resulting in a condition called **steatorrhea**.

MEDICAL IMPORTANCE

Steatorrhea is a condition characterized by the presence of abnormally high amounts of fat in the stool. It is often associated with malabsorption of dietary fats and can be indicative of an underlying digestive or absorptive disorder. Steatorrhea can be caused by various factors that interfere with the normal digestion and absorption of fats. Common causes include pancreatic disorders such as chronic pancreatitis, cystic fibrosis, pancreatic cancer, or pancreatic enzyme deficiency. Other causes include diseases affecting the small intestine, such as celiac disease, Crohn's disease, bacterial overgrowth, or surgical removal of a portion of the small intestine. Liver or gallbladder diseases, like liver cirrhosis or biliary obstruction, can also contribute to steatorrhea. The main symptom of steatorrhea is the passage of bulky, greasy, foul-smelling stools that may appear pale, float, and be difficult to flush. Other associated symptoms may include weight loss, abdominal discomfort or pain, diarrhea, and nutritional deficiencies due to malabsorption of fat-soluble vitamins (A, D, E, K) and essential fatty acids.

Bile acids not only aid in the digestion of lipids but are also essential for the absorption of lipid digestion products. Furthermore, bile acids play a critical role in the efficient absorption of lipid-soluble vitamins such as vitamins A, D, E, and K in the intestines. Without bile acids, the absorption of these vitamins would be compromised.

The fatty acids released by hydrolysis of TAGs and phospholipids are absorbed by cells in the intestinal wall, and **TAGs are resynthesized (fig. 11.14).**

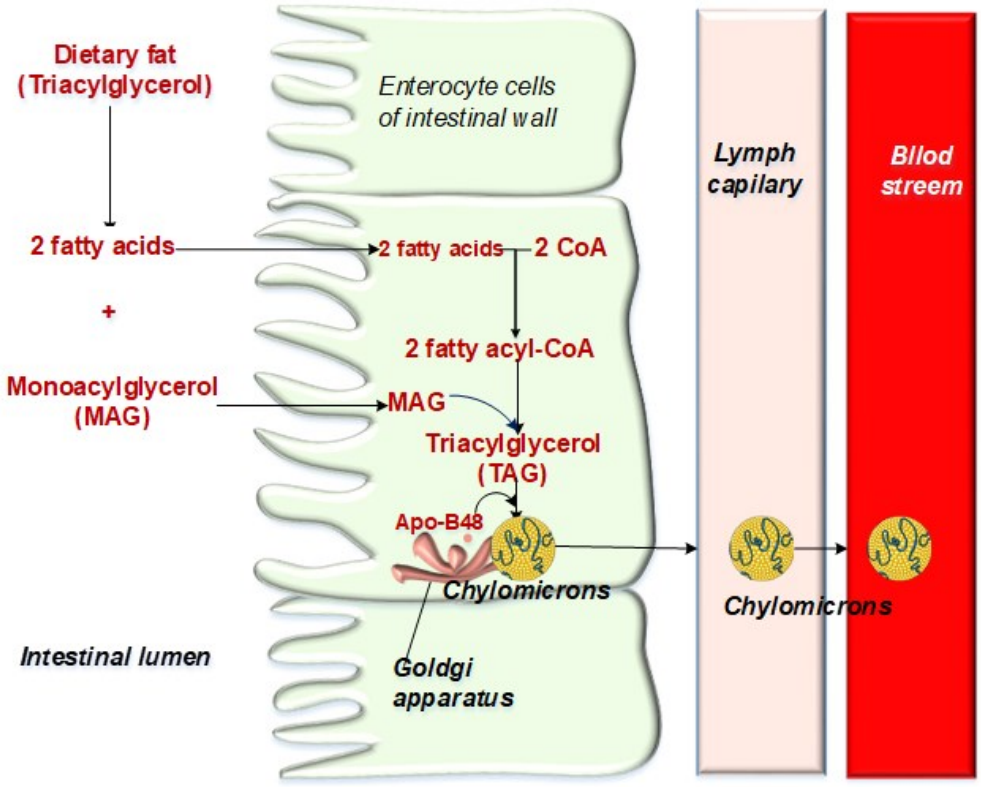

Fig. 11.14. The resynthesis of triacylglycerols (TAGs) in enterocytes - the process by which absorbed fatty acids and monoacylglycerols are reassembled into TAGs within the cells of the small intestine

After the resynthesis of TAGs in enterocytes, they are packaged into **chylomicrons** along with other lipids, such as cholesterol and phospholipids. Chylomicrons acquire apolipoproteins, particularly **apolipoprotein B-48 (apoB-48),** which is specific to chylomicrons. am: Once formed, chylomicrons are released from the enterocytes into the **lymphatic system** and eventually enter the **bloodstream**. In the bloodstream, chylomicrons circulate to deliver dietary fats to various tissues.

Lipoprotein lipase, which is present on the inner lining of blood vessels, particularly in adipose tissue and skeletal muscle, plays a key role in the metabolism of chylomicrons (fig. 11.5). Before lipoprotein lipase can act on chylomicrons, it needs to be activated. Activation of lipoprotein lipase occurs through interactions with apolipoprotein C-II (apoC-II), which is present on the surface of chylomicrons. Once activated, lipoprotein lipase hydrolyzes the triacylglycerols (TAGs) present in chylomicrons into **fatty acids and glycerol**. This enzymatic reaction occurs at the surface of blood vessel endothelial cells, where lipoprotein lipase is anchored.

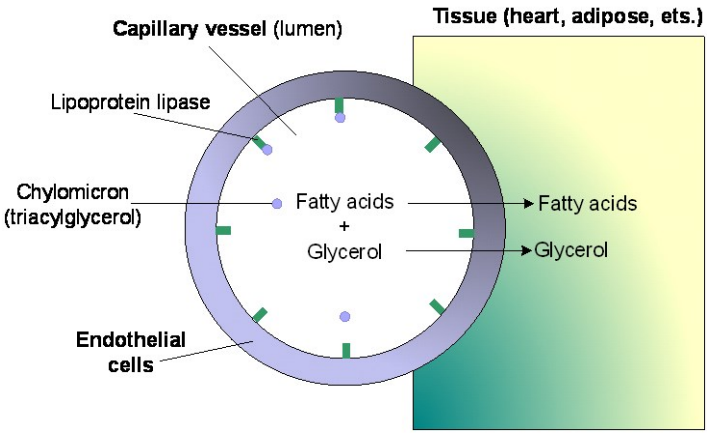

Fig. 11.15. Hydrolysis of tryacylrlycerols by lipoprotein lipase

The fatty acids that are released by lipoprotein lipase from chylomicrons can be taken up by various tissues. Adipose tissue, for example, takes up fatty acids and incorporates them into newly synthesized TAGs for storage. Skeletal muscle can also take up fatty acids for energy production or storage.

After the hydrolysis of TAGs, the chylomicrons undergo remodeling and lose a significant portion of their TAG content. The **remnants of chylomicrons**, which still contain some cholesterol and other lipids, are cleared from the bloodstream by the liver through receptor-mediated endocytosis.

11.4. Catabolism of triacylglycerols.

There are various situations in which humans utilize their energy reserves stored in adipose (fat) tissue. For instance, during periods of fasting or starvation, the mobilization of fatty acids from adipose tissue serves as a crucial energy source. Prolonged physical exertion or exercise also triggers the release of fatty acids from fat tissue. While triacylglycerols can be found in the liver, intestine, and other tissues, they are primarily stored in adipose tissue, which functions as the main depot for triacylglycerol storage. In a 70-kg human, approximately 135,000 kcal of energy is stored as triacylglycerols in adipose tissue, compared to only 450 kcal stored in the liver. **Adipocytes,** specialized cells in adipose tissue, are responsible for storing lipids. The cytoplasm of adipocytes contains vesicles that are abundant in triacylglycerols, serving as long-term energy reserves in mammals.

The breakdown (catabolism) of TAGs in adipose tissue, also known as **intracellular lipolysis**, is catalyzed by ***hormone-sensitive lipases***. The initial step involves the action of ***triacylglycerol lipase***, which removes a fatty acid from either carbon 1 or carbon 3 of the triacylglycerol, resulting in the formation of diacylglycerol. The remaining two fatty acids of the TAG molecule are then cleaved by additional lipases that specifically target diacylglycerol and monoacylglycerol (fig. 11.16).

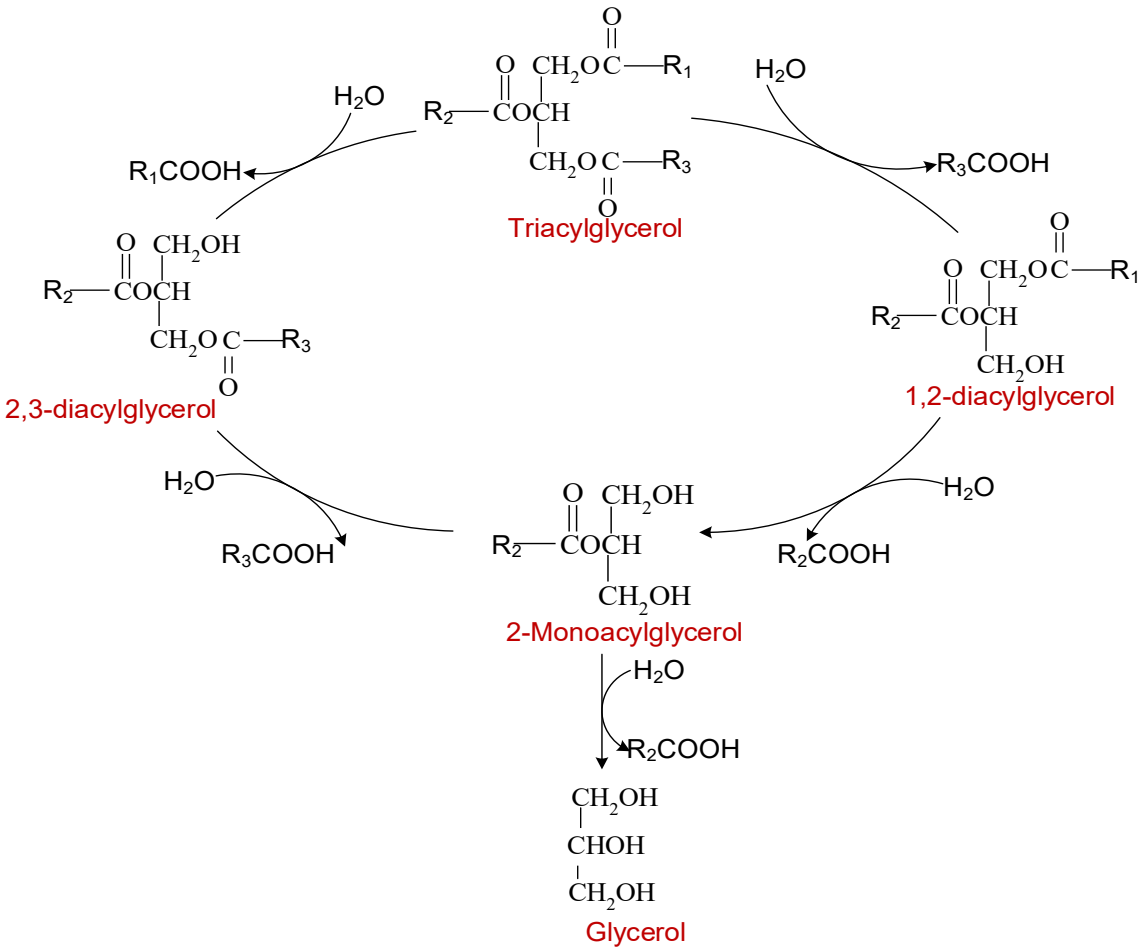

Fig. 11.16. The catabolism of triacylglycerols (TAGs) involves the breakdown of these lipid molecules into their constituent fatty acids and glycerol. This process primarily occurs during times of energy demand or fasting when stored TAGs need to be mobilized and used as a fuel source

The fatty acids released form TAGs in intracellular lipolysis can serve as an energy source for the cell. They can be utilized within the cell for various metabolic processes, including energy production through beta-oxidation, or they can be released into the bloodstream and transported to other tissues for energy production or storage. Along with fatty acids, the hydrolysis of TAGs also generates glycerol. Glycerol is released into the cytoplasm and can be further metabolized or utilized in various cellular processes.

Intracellular lipolysis in adipose tissue is regulated by hormones such as **epinephrine, norepinephrine, and glucagon** (fig. 11.17). First the hormones bind to the plasma membrane of the target cells, which stimulates synthesis of **cyclic AMP (cAMP).** cAMP activates a protein kinase that phosphorylated a key enzyme, triacylglycerol lipase. The lipase, which is active in the phosphorylated form, hydrolyzes triacylglycerol to diacyl-glycerols with release of fatty acid. This reaction is rate-limiting for the complete hydrolysis of triacylglycerols. The diacylglycerols and monoacylglycerols are rapidly hydrolysed the rest of the way to fatty acids and glycerol.

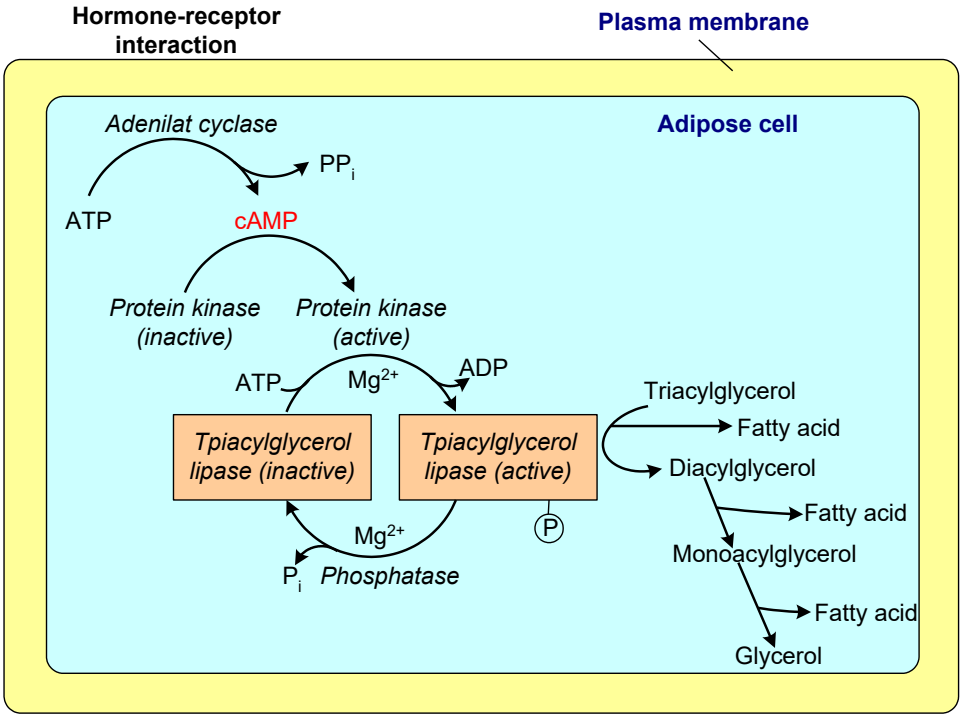

Fig. 11.16. Hormones play a crucial role in regulating intracellular lipolysis. The two primary hormones involved are epinephrine and norepinephrine, which are released from the adrenal glands and sympathetic nerve endings during stress or exercise. These hormones bind to specific receptors on the cell surface, activating intracellular signaling pathways that stimulate lipolysis. The activation of protein kinase A (PKA) is a key step in this process, as PKA phosphorylates and activates hormone-sensitive lipase, the main enzyme responsible for intracellular lipolysis.

The unesterfied (free) fatty acids move through the plasma membranes of the adipocytes into the bloodstream, where they bind to the blood plasma protein, **albumin.** The water-soluble product, glycerol, is also released into plasma and removed by the liver for glucose production. Albumin carries the fatty acids to energy defficient tissues, where fatty acids move from the plasma into the tissue; cardiac muscle utilizes fatty acids as the major oxidative source of energy for ATP synthesis and, therefore, removes other extreme, the brain does not use fatty acids as a major source of energy but depends mostly on use fatty acids as a major source of energy but depends mostly on glucose and, to a lesser extent, ketone bodies.

Insulin has the opposite effect of epinephrine and norepinephrine. It stimulates the formation of glycogen and tTAGs.

11.5. Biosynthesis of triacylglycerols and phospholipids.

Glycerol-3-phosphate serves as a precursor for the synthesis of important substances including triacylglycerols, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and cardiolipin, which is a component of mitochondrial membranes.

The synthesis of **triacylglycerols** predominantly takes place in the **liver** and **adipose tissue,** with other tissues contributing to a lesser extent. TAGs are synthesized by esterifying three fatty acids to the glycerol backbone derived from glycerol-3-phosphate. This process occurs primarily in the endoplasmic reticulum of hepatocytes and adipocytes. TAGs serve as a major form of energy storage in the body, particularly in adipose tissue,

where they are stored as lipid droplets within adipocytes. In the liver, TAG synthesis is involved in the packaging of lipids into lipoproteins for transport in the bloodstream.

Two mechanisms are involved for the synthesis of **glycerol 3-phosphate** (fig. 11.17):

1. In the **liver**, glycerol is activated by ***glycerol kinase***. This enzyme is absent in adipose tissue.
2. In both **liver and adipose tissue**, glucose serves as a precursor for glycerol 3-phosphate. Dihydroxyacetone phosphate (DHAP) produced in glycolysis is reduced by ***glycerol 3-phosphate dehydrogenase***.

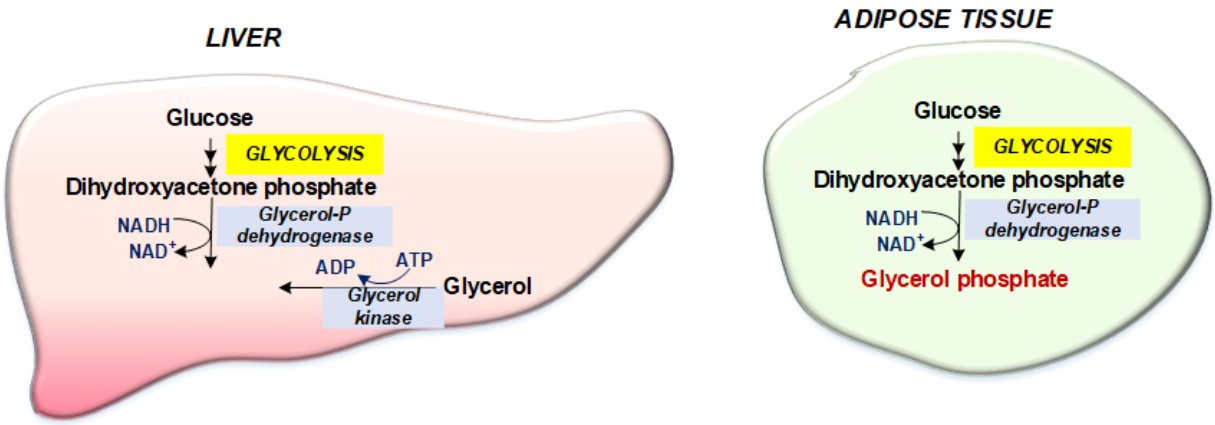

Fig. 11.17. Pathways for production of glycerol phosphate in liver and adipose tissue.

Before a fatty acid can participate in triacylglycerol synthesis, it needs to be converted to its activated form by attaching to coenzyme A (CoA). This conversion reaction is catalyzed by a group of enzymes called ***fatty acyl-CoA synthetases***, also known as ***thiokinases***.

The synthesis of a molecule of triacylglycerol (TAG) from glycerol phosphate and fatty acyl-CoA involves a series of enzymatic reactions (fig. 11.18):

- Glycerol-3-phosphate is first converted to **lysophosphatidic acid (LPA)** through the addition of a fatty acyl group from fatty acyl-CoA. This reaction is catalyzed by the enzyme ***glycerol-3-phosphate acyltransferase (GPAT)***.
- LPA is then converted to **phosphatidic acid (PA)** by the enzyme ***lysophosphatidic acid acyltransferase (LPAAT)***. This step involves the addition of another fatty acyl group from fatty acyl-CoA.
- The enzyme ***phosphatase*** cleaves off phosphate of phosphatidic acid to produce **diacylglycerol.**
- Lastly, phosphatidic acid is converted to triacylglycerol by the enzyme ***diacylglycerol acyltransferase (DGAT)***. This final step involves the addition of a third fatty acyl group from fatty acyl-CoA to the remaining hydroxyl group on the glycerol backbone.

Triacylglycerols (TAGs) have different fates in the liver and adipose tissue:

In liver: The liver synthesizes TAGs as a way to store excess fatty acids obtained from the diet or produced through de novo lipogenesis. The synthesized TAGs are packaged into **very low-density lipoproteins (VLDL)** and secreted into the bloodstream

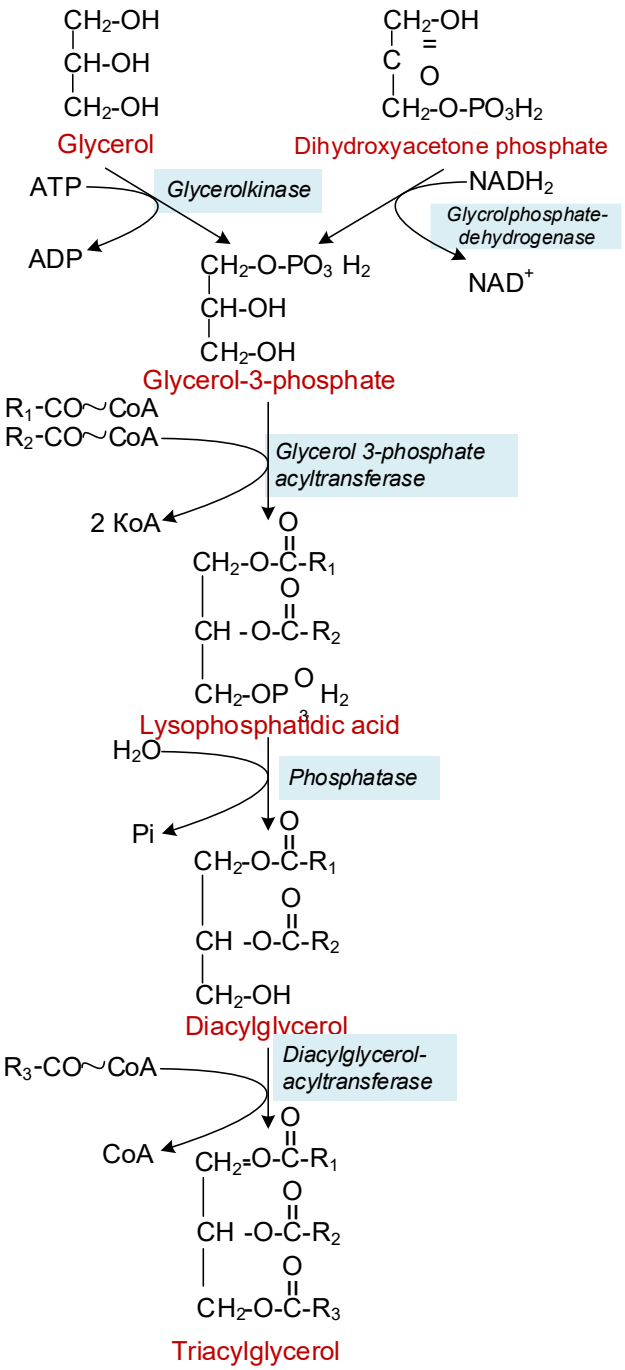

Fig.11.18. Synthesis of triacylglycerol

for transport to other tissues. The liver stores a small amount of TAGs for its own energy needs. However, the primary role of the liver is to export TAGs in the form of VLDL particles to provide energy to other tissues.

Adipose tissue is the major site of TAG synthesis and storage in the body. Excess fatty acids from the bloodstream are taken up by adipocytes and converted into TAGs through the process of esterification. These TAGs are stored within specialized lipid droplets in the cytoplasm of adipocytes. During times of energy demand, such as fasting or exercise, stored TAGs in adipose tissue undergo lipolysis. Adipose tissue constantly undergoes TAG turnover, which involves the dynamic balance between TAG synthesis and lipolysis. In times of energy surplus, TAG synthesis prevails, leading to an increase in adipose tissue mass. In contrast, during energy deficit, lipolysis predominates, resulting in the release of stored fatty acids for energy production.

Phospholipids are synthesized from phosphatidic acid and 1,2-diacylglycerol, intermediates in the production of triacylglycerol. Phospholipid synthesis occurs in the **smooth endoplasmic reticulum**.

In the biosynthesis of phosphatidylcholine and phosphatidylethanolamine, choline or ethanolamine must first be activated by phosphorylation by ATP followed by linkage to **CDP**. The resulting **CDP-choline** or **CDP ethanolamine** reacts with 1,2-diacylglycerol to form either **phosphatidylcholine** or **phosphatidylethanolamine**, respectively (fig. 11.19).

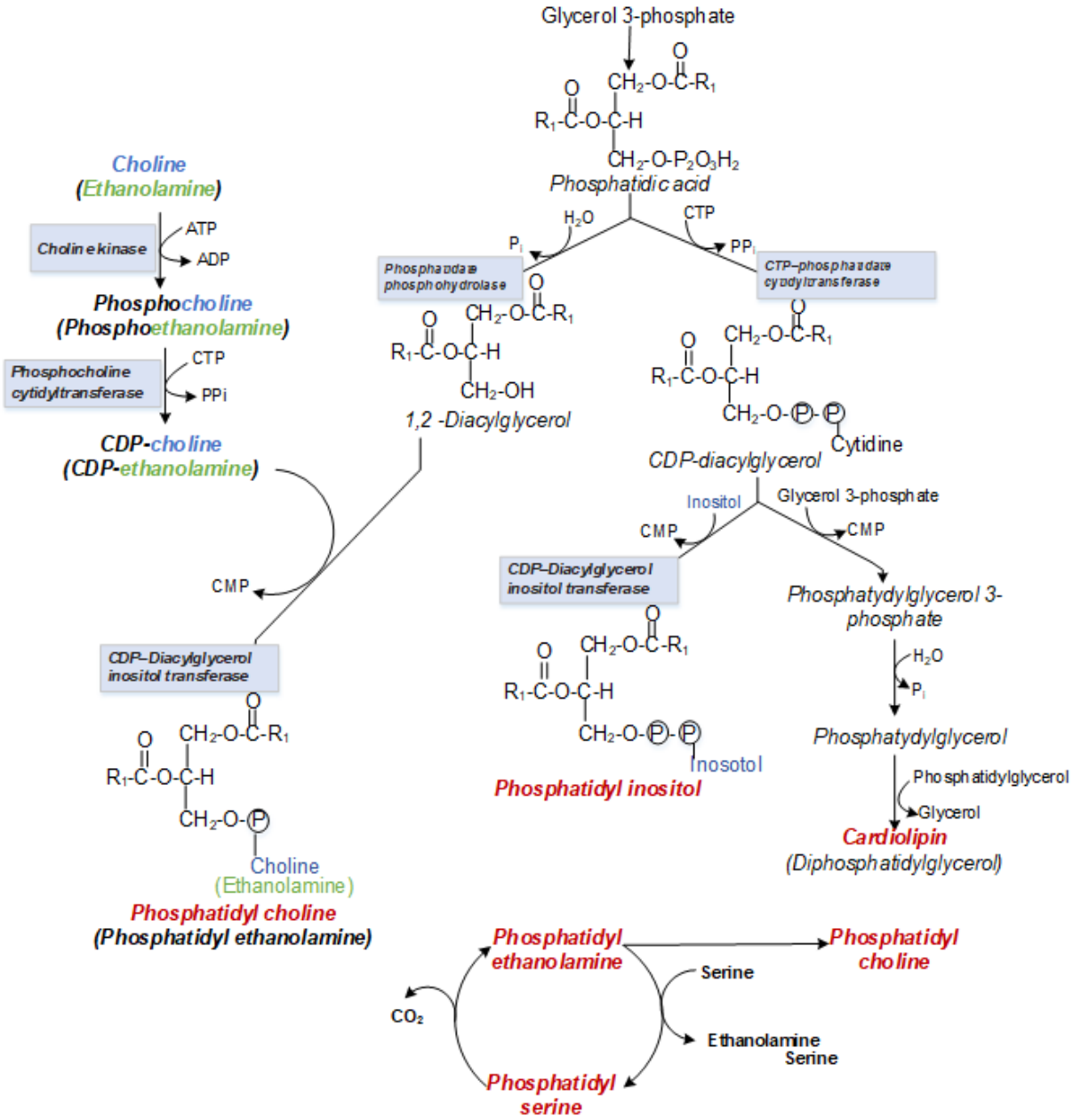

Fig. 11.19. The biosynthesis of phospholipids involves the coordinated actions of various enzymes and metabolic pathways to generate the diverse array of phospholipid molecules required for cellular membranes.

Phosphatidylserine is formed from phosphatidylethanolamine directly by reaction with serine. Phosphatidylserine may reform phosphatidylethanolamine by decarboxylation. An alternative pathway in liver enables phosphatidylethanolamine to give rise directly to phosphatidylcholine by progressive methylation of the ethanolamine residue. In spite of these sources of choline, it is considered to be an essential nutrient in many mammalian species, although this has not been established in humans.

11.6. Metabolism of sphingolipids.

Ceramide, a key component of sphingolipids, is synthesized through a series of enzymatic reactions known as the de novo synthesis pathway (fig. 11. 20):

- Formation of 3-Ketosphinganine: The first step in ceramide synthesis involves the condensation of palmitoyl-CoA, an acyl-CoA derived from fatty acids, with serine. This reaction is catalyzed by the enzyme ***serine palmitoyltransferase***, resulting in the formation of **3-ketosphinganine.**
- Reduction of 3-ketosphinganine: The 3-ketosphinganine is then reduced by ***3-ketosphinganine reductase***, converting it into **dihydroceramide**.
- Dihydroceramide is further acylated by fatty acyl-CoA molecules to form ceramide. This reaction is catalyzed by the ***enzyme dihydroceramide desaturase***, which introduces a double bond into the acyl chain of dihydroceramide.

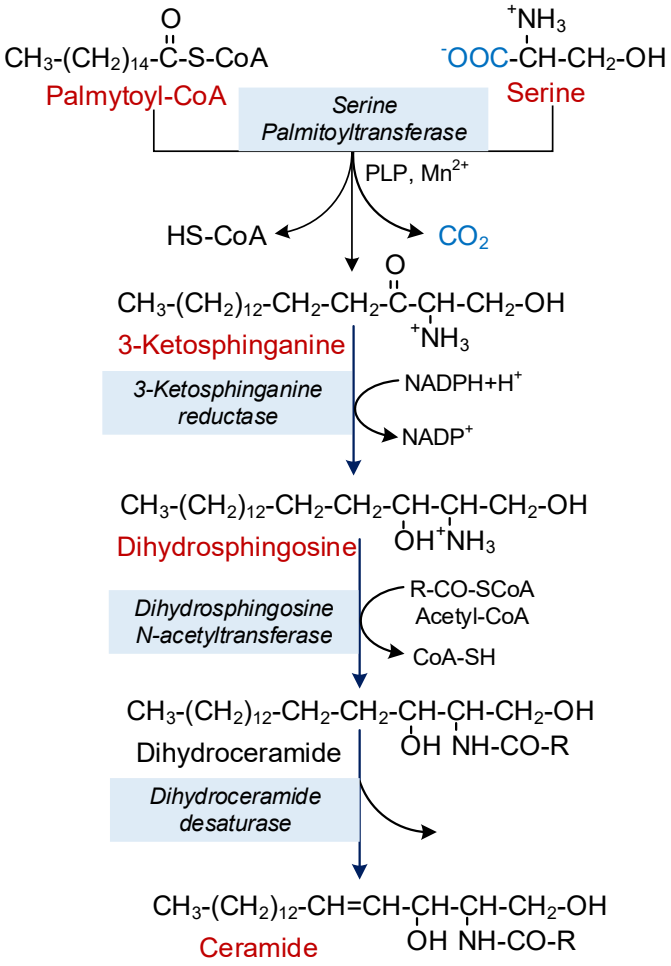

Fig. 11.20. Ceramide de novo biosynthesis

Alternatively, ceramide can also be generated through the salvage pathway, where sphingosine, a breakdown product of complex sphingolipids, is acylated by fatty acyl-CoA to form ceramide.

Ceramide serves as a central hub for the synthesis of various complex sphingolipids, including sphingomyelin, glucosylceramide, and gangliosides. It can also be further metabolized into other bioactive sphingolipids, such as sphingosine-1-phosphate (S1P), through various enzymatic reactions.

MEDICAL IMPORTANCE

Ceramides play a crucial role in maintaining the barrier function of the skin and regulating its water permeability. Specifically, ceramides with long-chain fatty acids, such as those containing 30 carbons, are major components of the stratum corneum, which is the outermost layer of the skin.

The stratum corneum acts as a barrier that prevents excessive water loss from the skin and protects it from external factors. Ceramides, along with other lipids and proteins, form a complex structure within the stratum corneum called the lipid barrier or lipid matrix. This lipid barrier helps to maintain skin hydration by reducing water loss through the skin and preventing the entry of harmful substances.

Sphingomyelins are phospholipids and are formed when ceramide reacts with phosphatidylcholine to form sphingomyelin plus diacylglycerol. This occurs mainly in the Golgi apparatus and to a lesser extent in the plasma membrane.

Cerebrosides, the simplest glycosphingolipids, include **galactosylceramide (GalCer) and glucosylceramide (GlcCer)**. GalCer is a major lipid found in myelin, while GlcCer is the primary glycosphingolipid in tissues outside the nervous system and serves as a precursor for more complex glycosphingolipids (fig.11.21). The formation of GalCer involves a reaction between ceramide and UDPGal, which is produced by the conversion of UDPGlc through epimerization. Further reactions involving **3'-phosphoadenosine 5'-phosphosulfate** (PAPS; "active sulfate") lead to the formation of sulfogalactosylceramide, sulfo(galacto)-glycerolipids, and steroid sulfates. Gangliosides, on the other hand, are synthesized from ceramide through the stepwise addition of activated sugars like **UDPGlc, UDPGal, and a sialic acid**, typically **N-acetylneuraminic acid**. This process can generate numerous gangliosides with increasing molecular weight. The Golgi apparatus houses most of the enzymes responsible for transferring sugars from nucleotide sugars (*glycosyl transferases)*. Glycosphingolipids are located in the outer layer of plasma membranes and play crucial roles in cell adhesion and recognition. Some glycosphingolipids act as antigens, such as the ABO blood group substances. Certain gangliosides also serve as receptors for bacterial toxins, including cholera toxin, which subsequently activates *adenylyl cyclase.*

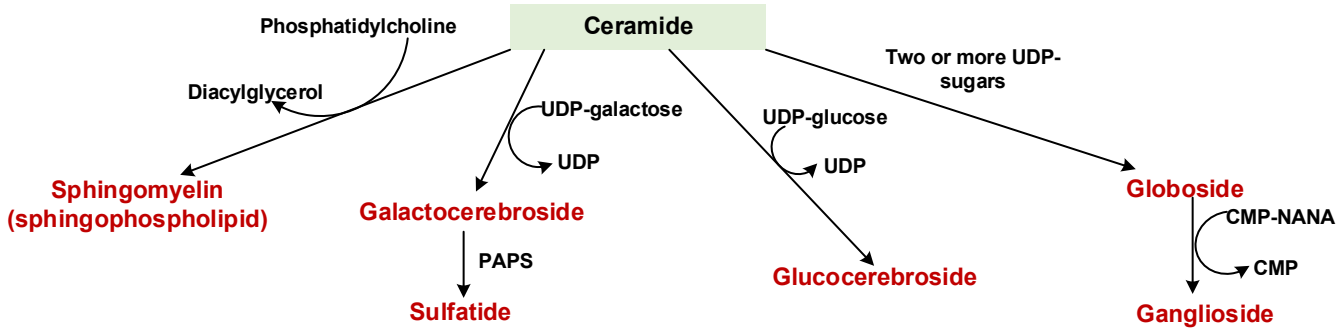

Fig. 11.21. Synthesis of sphingolipids from ceramide

The degradation of sphingolipids occurs through a series of enzymatic reactions in various cellular compartments, including the lysosomes. The process involves the breakdown of sphingolipids into their constituent components, which can be further utilized or eliminated by the cell.

- **Glycosphingolipid Degradation:** Glycosphingolipids, such as cerebrosides and gangliosides, are degraded in lysosomes by specific enzymes called *glycosidases*. Glycosidases cleave the sugar moieties (glucose, galactose, or sialic acid) from the ceramide backbone, resulting in the formation of ceramide and free sugars. Ceramide generated from glycosphingolipid degradation can then enter other metabolic pathways or be further degraded.
- **Sphingomyelin Degradation**: Sphingomyelin, a major sphingolipid found in cell membranes, is hydrolyzed by the enzyme *sphingomyelinase.* Sphingomyelinase cleaves the phosphocholine head group from sphingomyelin, yielding ceramide and

phosphorylcholine. Ceramide produced from sphingomyelin degradation can be utilized in various cellular processes or further degraded.

- **Ceramide Degradation**: Ceramide can be metabolized through different pathways, including the ceramidase pathway and the ceramide galactosyltransferase pathway. b. In the ceramidase pathway, ceramidase enzymes hydrolyze ceramide to produce sphingosine and a fatty acid. Sphingosine can be further phosphorylated to form sphingosine-1-phosphate, which is involved in various cellular signaling pathways. c. In the ceramide galactosyltransferase pathway, ceramide is converted to galactosylceramide by the enzyme ceramide galactosyltransferase. Galactosylceramide can then undergo additional modifications or be further degraded.
- **Further Degradation:** The products of sphingolipid degradation, such as ceramide, sphingosine, and fatty acids, can enter other metabolic pathways for utilization or be excreted from the cell.

The degradation of sphingolipids is tightly regulated and essential for maintaining cellular sphingolipid homeostasis. Imbalances or defects in sphingolipid degradation enzymes can lead to the accumulation of sphingolipids, which can contribute to the development of various diseases, including lysosomal storage disorders and **sphingolipid storage diseases**.

11.6.1. Genetic anomalies of sphingolipid metabolism – sphingolipidoses. Lysosomal diseases

Certain diseases are characterized by abnormal quantities of these lipids in the tissues, often in the nervous system. They may be classified into two groups: **true demyelinating diseases** and **sphingolipidoses**. **In multiple sclerosis,** which is a demyelinating disease, there is loss of both phospholipids (particularly ethanolamine plasmalogen) and of sphingolipids from white matter. Thus, the lipid composition of white matter resembles that of gray matter. The cerebrospinal fluid shows raised phospholipid levels.

The sphingolipidoses (lipid storage diseases) are a group of inherited diseases that are caused by a genetic defect in the catabolism of lipids containing sphingosine (table 11.3). They are part of a larger group of lysosomal disorders and exhibit several constant features:

- Complex lipids containing ceramide accumulate in cells, particularly neurons, causing neurodegeneration and shortening the lifespan.
- The rate of synthesis of the stored lipid is normal.
- The enzymatic defect is in the lysosomal degradation pathway of sphingolipids.
- The extent to which the activity of the affected enzyme is decreased is similar in all tissues.

There is no effective treatment for many of the diseases, although some success has been achieved with enzyme replacement therapy and bone marrow transplantation in the treatment of Gaucher and Fabry diseases. Other promising approaches are substrate deprivation therapy to inhibit the synthesis of sphingolipids and chemical chaperone therapy. Gene therapy for lysosomal disorders is also currently under investigation.

Table 11.3. Major sphyngolipidoses

Disease	Missing/defective enzyme	Major storage compound	Symptoms
Niemann-Pick disease	**Sphingo-myelinase**	**Sphingomyelins**	**Liver and spleen enlargement, mental retardation**
Farber's disease	**Ceramidase**	**Ceramide**	**Painful and progressively deformed joints, skin nodules, death within a few years**
Gaucher's disease	**β-Glucosidase**	**Glucoside**	**Liver and spleen enlargement, erosion of long bones, mental retardation in infantile form only**
Krabbe's disease	**β-Galactosidase**	**Galactocerebrosides**	**Loss of myelin, mental retardation, death by age 2**
Tay-Sachs disease	**Hexosami-nidase A**	**Galactoside GM_2**	**Mental degradation, blidness, death by age 3**
Fabry's disease	**a-Galacto-sidase**	**Ceramide Thihexoside**	**Skin rash, kidney failure, pain in lower extremities**

REVIEW TEST:

№	MCQs	Answers and explanations
1.	**Deficiency of linoleic and linolenic acids in the body leads to skin damage, hair loss, delayed wound healing, thrombocytopenia, low resistance to infections. These changes are most likely to be caused by the impaired synthesis of the following substances:** A. Catecholamines B. Interleukins C. Interferons D. Eicosanoids E. Corticosteroids	**The answer is D.** The main function of eicosanoids is immune system, state of vessels and aggregation regulation. Arachidonic acid is the main substrate for eicosanoids synthesis, but linoleic and linolenic acids also take part in this process, but after the transformation of intermediates into 20-carbon tetraenic acids.
2.	**A patient has normally coloured stool including a large amount of free fatty**	**The answer is E.** Large amount of free fatty acids seems that fats digestion is completed, so there were no problems with pancreatic

	acids. The reason for this is a disturbance of the following process: A. Lipase secretion B. Fat hydrolysis C. Biliary excretion D. Choleresis E. Fat absorption	juice and bile secretion. After digestion fatty acids and glycerol should be absorbed, the presence of them in feces means the disturbance of fat absorption.
3.	**A coprological survey revealed lightcolored feces containing drops of neutral fat. The most likely reason for this condition is the disorder of:** A. Bile inflow into the bowel B. Gastric juice acidity C. Pancreatic juice secretion D. Intestinal juice secretion E. Intestinal absorption	**The answer is A.** Light colour of feces is usually caused by deficiency of bile pigments, which come with bile. The presence of neutral fat seems that there was no lipase activation by bile acids. Bile obstruction may be the reason of this state, which decreases the bile flow into the bowel.
4.	**Due to the blockage of the common bile duct (which was radiographically confirmed), the biliary flow to the duodenum was stopped. We should expect the impairment of:** A. Protein absorption B. Fat emulsification C. Carbohydrate hydrolysis D. Secretion of hydrochloric acid E. Salivation inhibition	**The answer is B.** The main function of bile is fat emulsification and lipase activation, so the blockage of bile flow into common bile duct can cause the disturbance of these processes.
5.	**Feces of a patient contain high amount of undissociated fats and have grayish-white color. Specify the cause of this phenomenon:** A. Obturation of bile duct B. Hypoactivation of pepsin by hydrochloric acid C. Hypovitaminosis D. Enteritis E. Irritation of intestinal epithelium	**The answer is A.** Undissociated fats and grayish-white color of feces are caused by bile deficiency in small intestine, the main reason of which is obturation of bile duct.
6.	**Obesity is a common disease. The aim of its treatment is to lower content of neutral fats in the body. What hormon-sensitive enzyme is the most important for intracellular lipolysis?** A. Diacylglycerollipase B. Proteinkinase C. Adenylatekinase D. Triacylglycerollipase E. Monoacylglycerollipase	**The answer is D.** The main enzyme, which takes part in fats catabolism in cell to provide it with energy, is TAG (triacylglycerol)-lipase. The activity of TAG-lipase is regulated by hormones – glucagon, insulin and epinephrine.
7.	**Disruption of nerve fiber myelinogenesis causes neurological disorders and mental retardation. These symptoms are typical for**	**The answer is A.** Myelin is a is a lipid-rich substance that surrounds the axon of some nerve cells, forming an electrically insulating layer. Its main components are lipids and proteins, some of them are specific for myelin, for

	hereditary and acquired alterations in the metabolism of: A. Sphingolipids B. Neutral fats C. Higher fatty acids D. Cholesterol E. Phosphatidic acid	example some ceramides (galactosylceramide and glucosylceramide). Ceramides consist of sphingosine and fatty acid, so they can be called "sphingolipids". The disturbance of their synthesis can cause the decrease of myelin production.
8.	**A 2-year-old child presents with acute psychomotor retardation, vision and hearing impairment, sharp enlargement of the liver and spleen. The child is diagnosed with hereditary Niemann-Pick disease. What genetic defect is the cause of this disease?** A. Acid lipase deficiency B. Glucose 6-phosphatase deficiency C. Amylo-1,6-glucosidase deficiency D. Sphingomyelinase deficiency E. Xanthine oxidase deficiency	**The answer is D.** Nimann-Pick disease symptoms are caused by sphingomyelin accumulation, that is caused by deficiency of enzyme sphingomyelinase, which function is to breakdown sphingomyelin.
9.	**Examination of cell culture got from a patient with lysosomal pathology revealed accumulation of great quantity of lipids in the lysosomes. What of the following diseases is this disturbance typical for?** A. Tay-Sachs disease B. Gout C. Phenylketonuria D.Wilson disease E. Galactosemia	**The answer is A.** Accumulation of great quantity of lipids in the lysosomes means indicates a decreased activity of special enzymes, which take part in catabolism of these lipids. Sphingolipidoses are a group of lysosomal diseases, caused by sphingolipids catabolism enzymes deficiency and Tay-Sachs disease (deficiency of β-hexosaminidase A) is an example.
10	**Due to the blockage of the common bile duct (which was radiographically confirmed), the biliary flow to the duodenum was stopped. We should expect the impairment of:** A. Salivation inhibition B. Protein absorption C. Carbohydrate hydrolysis D. Secretion of hydrochloric acid E. Fat emulsification	**The answer is E.** The main function of bile is fat emulsification and lipase activation, so the blockage of bile flow into common bile duct can cause the disturbance of these processes.

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12. β–OXIDATION AND BIOSYNTHESIS OF FATTY ACIDS. METABOLISM OF KETONE BODIES.

OBJECTIVES after studying this chapter, you should be able to:

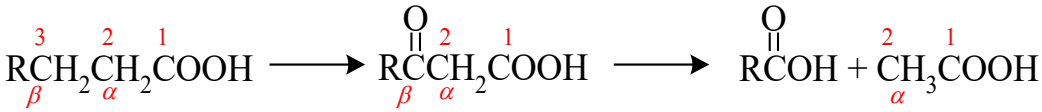
- *Describe the processes by which fatty acids are transported in the blood and activated and transported into the matrix of the mitochondria for breakdown to obtain energy.*
- *Outline the β-oxidation pathway by which fatty acids are metabolized to acetyl-CoA and explain how this leads to the production of large quantities of ATP from the reducing equivalents produced during β-oxidation and further metabolism of the acetyl-CoA via the citric acid cycle.*
- *Outline the structure of the fatty acid synthase multienzyme complex, indicating the sequence of enzymes in the two peptide chains of the homodimer.*
- *Explain how long-chain fatty acids are synthesized by the repeated condensation of two carbon units, with formation of the 16-carbon palmitate being favored in most tissues, and identify the cofactors required. Indicate the sources of reducing equivalents (NADPH) for fatty acid synthesis.*
- *Identify the three compounds termed "ketone bodies" and describe the reactions by which they are formed in liver mitochondria.*
- *Appreciate that ketone bodies are important fuels for extrahepatic tissues and indicate the conditions in which their synthesis and use are favored*

12.1. β - Oxidation of fatty acids.

Triacylglycerols (TAGs) are degraded to free fatty acids (FFAs) and glycerol in the adipose tissue and transported to other tissues (as discussed in chapter 11).

FFA also called unesterified (UFA) or nonesterified (NEFA) fatty acids are fatty acids that are in the unesterified state. In plasma, longer chain FFA are combined with albumin, and in the cell they are attached to a fatty acid binding protein, so that in fact they are never really "free." Shorter chain fatty acids are more water-soluble and exist as the unionized acid or as a fatty acid anion.

FFAs in the cells are mostly oxidized by β-oxidation. FFAs chains nearly always contain an even number of carbon atoms. Investigations into the mechanism of fatty acid catabolism began in 1904, when Fritz Knoop, reported experiments that indicated fatty acids were degraded by removal of two carbons at a time. The data indicated that carbon 3 of a fatty acid was oxidized with subsequent cleavage between carbons 2 and 3.



Carbon 2 is also known as the alpha carbon and carbon 3 as the beta carbon. Hence the term β-oxidation was coined.

The β-oxidation of fatty acids involves three stages:

l. Activation of fatty acids occurring in the cytosol.

ll. Transport of fatty acids into mitochondria.

lll. β-Oxidation proper in the mitochondrial matrix.

I. Fatty Acid Activation. In order for fatty acids to undergo oxidation, they need to undergo a process of conversion into an active form. This particular step, which is essential for the complete degradation of a fatty acid, requires energy derived from ATP. When ATP and coenzyme A are present, the enzyme ***acyl-CoA synthetase (thiokinase)*** facilitates the conversion of a fatty acid into an "**active fatty acid" or acyl-CoA.** This process involves the utilization of one high-energy phosphate and results in the formation of AMP and PPi. The PPi molecule is then hydrolyzed by inorganic pyrophosphatase, leading to the release of another high-energy phosphate. This ensures the completion of the overall reaction.

Fatty acid (FFA) + CoA + ATP ↔ Acyl-CoA + AMP + PP_i

Acyl-CoA synthetases are found in the endoplasmic reticulum, peroxisomes, and inside and on the outer membrane of mitochondria. *Acyl-CoA synthetases* exhibit substrate specificity, meaning that different isoforms of the enzyme have preferences for specific fatty acids of varying chain lengths and degrees of saturation. This specificity ensures that the appropriate fatty acids are activated and channeled into the corresponding metabolic pathways. The activity of *acyl-CoA synthetases* is tightly regulated to maintain lipid homeostasis. Their expression and activity can be modulated by various factors, such as hormonal signals, nutrient availability, and cellular energy status. For example, insulin can stimulate the expression and activity of specific isoforms of *acyl-CoA synthetases* involved in fatty acid synthesis.

Potentially confusing question concerning Acetyl CoA vs Acyl CoA. Acetyl CoA is a specific compound containing acetate bound to coenzyme A; acyl CoA is a general term used to refer to any fatty acid (acyl group) bound to coenzyme A.

II. Transport across the mitochondrial membrane. In order to facilitate the oxidation of fatty acids, the transportation of fatty acyl-CoA across the inner mitochondrial membrane is necessary since the oxidation process occurs within the mitochondrion. Direct diffusion of long-chain fatty acyl-CoA across the membrane is not possible. Instead, the acyl group of fatty acyl-CoA is first transferred to **carnitine. Carnitine** is a molecule derived from the amino acid **lysine** and is specifically involved in this transport process.

Carnitine palmitoyl transferases I and II are enzymes located on the external and internal surfaces of the inner mitochondrial membrane, respectively (fig. 12.1). These enzymes are responsible for the transfer of acyl groups. A carrier protein facilitates the translocation process, allowing acyl-carnitine to enter the mitochondrion while simultaneously transporting free carnitine in the opposite direction. This carrier protein ensures the transport of acyl-carnitine into the mitochondrial matrix while facilitating the movement of free carnitine back into the cytosol.

The transport of acyl-CoA via this mechanism involves four reactions:

- The acyl group of cytosolic acyl-CoA is transferred to carnitine, releasing CoA to the cytosolic pool.

- The resulting acyl-carnitine is transported into the mitochondrial matrix through the transport system.
- Within the mitochondrial pool, the acyl group is transferred to a CoA molecule.
- The final product, carnitine, is transported back to the cytosol, completing the cycle of acyl-CoA transport.

These reactions ensure the efficient transport of fatty acids into the mitochondria for their subsequent oxidation.

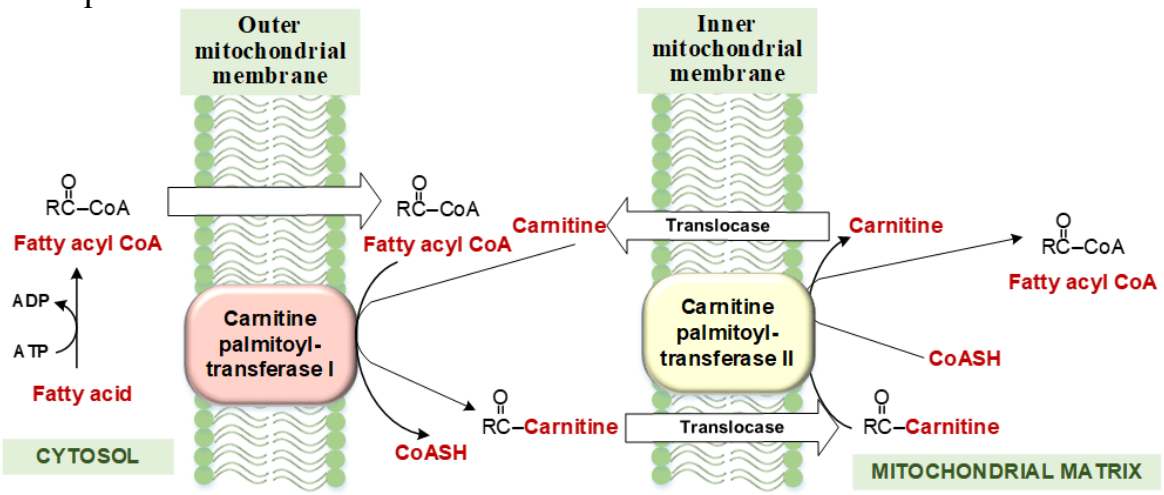


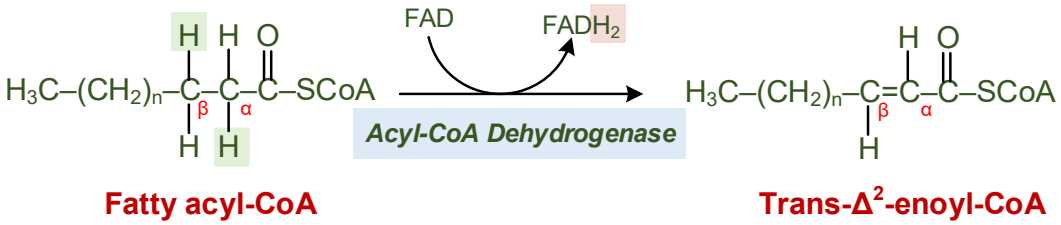
Fig. 12.1. The carnitine shuttle system and the actions of c*arnitine acyl transferases I and II* plays a crucial role in regulating fatty acid uptake and oxidation within the mitochondria.

The cell thereby maintains separate cytosolic mitochondrial pools of CoA. The mitochondrial pool functions in the oxidative degradation of pyruvate and certain amino acids as well as fatty acids, whereas the cytosolic pool supplies fatty acid biosynthesis. The cell similarly maintains separate cytosolic and mitochondrial pools of ATP and NAD^+.

III. The sequence of enzymatic reactions in β –oxidation of fatty acids.

Fatty acids undergo β-oxidation, a process that involves four sequential reactions for their breakdown:

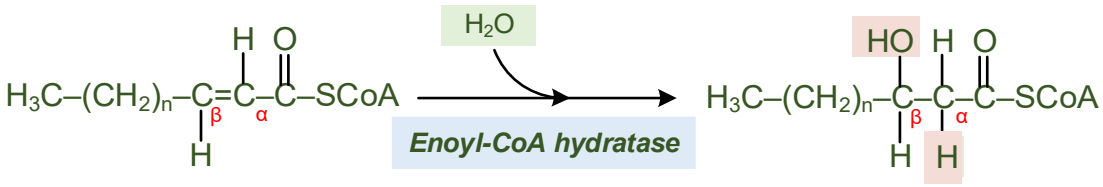
1. **Oxidation:** In the first step, a fatty acyl-CoA molecule is oxidized by an FAD-dependent enzyme ***acyl-CoA dehydrogenase***. This reaction removes a pair of hydrogen atoms from the β-carbon and forms a trans double bond between the α- and β-carbons. This results in the formation of a trans-Δ2-enoyl-CoA molecule.



MEDICAL IMPORTANCE

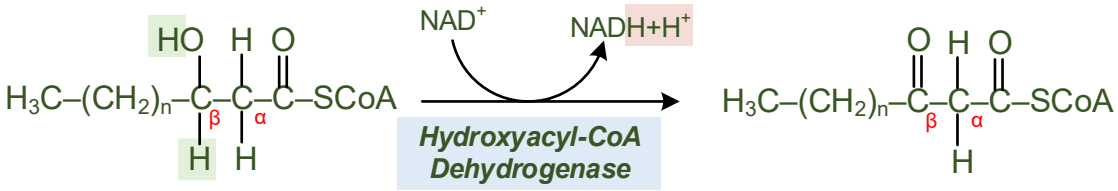
Most organisms contain multiple acyl-CoA dehydrogenase enzymes. Although each isozyme catalyzes essentially identical reactions, the isozymes differ somewhat in acyl chain-length specificity. There are different Acyl-CoA Dehydrogenases for short (4-6 C), medium (6-10 C), long and very long (12-18 C) chain fatty acids. The effect of having different isozymes is most apparent in that genetic deficiencies of specific isozymes have somewhat different physiological consequences. E.g. ***the sudden infant death syndrome (SIDS)*** *is an unexpected death of healthy infants, usually overnight. The real cause of SIDS is not known. It is now estimated that at least 10% of SIDS is due to deficiency of* ***medium chain acyl CoA dehydrogenase****. The enzyme defect has a frequency of 1 in 10,000 births and is, in fact, more prevalent than phenylketonuria*

2. **Hydration:** In the second step, the trans-Δ2-enoyl-CoA molecule undergoes hydration catalyzed by the enzyme ***enoyl-CoA hydratase***. This reaction adds a water molecule across the double bond, resulting in the formation of a **β-hydroxyacyl-CoA** molecule.



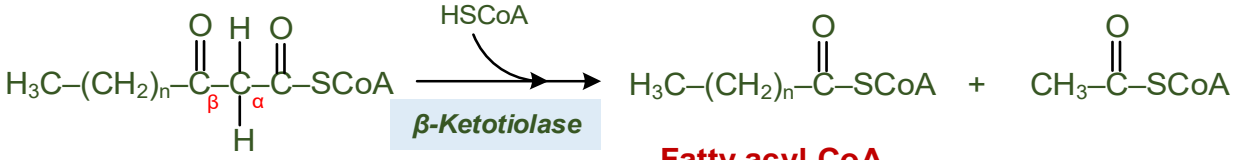
Trans-Δ²-enoyl-CoA **3-L-hydroxyacyl-CoA**

3. **Oxidation:** The β-hydroxyacyl-CoA molecule is further oxidized in the third step by the enzyme ***β-hydroxyacyl-CoA dehydrogenase***. This reaction removes a pair of hydrogen atoms from the β-carbon of the molecule, generating a keto group at the β-position. This yields the formation of a **β-ketoacyl-CoA** molecule. **NAD^+** is the electron acceptor in this reaction.



3-L-hydroxyacyl-CoA **β-Ketoacyl-CoA**

4. **Cleavage (thiolysis):** In the final step, the β-ketoacyl-CoA molecule undergoes thiolysis catalyzed by the enzyme **β-ketoacyl CoA thiolase (or simply thiolase).** This reaction involves the cleavage of the β-ketoacyl-CoA molecule into two separate molecules: an acetyl-CoA molecule and a new fatty acyl-CoA molecule, which is two carbons shorter than the original fatty acid.



β-Ketoacyl-CoA **Fatty acyl-CoA (2C shorter)** **Acetyl-CoA**

The thiolase cleavage reaction in β-oxidation is indeed inhibited by acetyl-CoA. Thiolase has the ability to catalyze the reverse reaction, which involves the condensation of two acetyl-CoA molecules to form a longer acyl-CoA molecule. When the concentration

of acetyl-CoA is high, it indicates that there is already an abundant supply of acetyl-CoA in the cell, and further production may not be necessary.

As a regulatory mechanism, the elevated levels of acetyl-CoA inhibit thiolase and the cleavage reaction. This helps prevent the excessive production of acetyl-CoA and ensures that the energy metabolism remains balanced. By inhibiting the thiolase reaction, the cell can regulate the flow of fatty acids through β-oxidation based on the energy needs and the availability of acetyl-CoA for other metabolic processes.

This feedback inhibition by acetyl-CoA helps maintain the appropriate balance between fatty acid oxidation and other energy-producing pathways in the cell.

The purpose of the first three reactions is to take an unsubstituted carbon and activate it by introducing a ketone in the β-position. The carbonyl destabilizes the carbon-carbon bond between the α and β carbons, and therefore allows the facile cleavage reaction catalyzed by thiolase to take place.

The shorter acyl-CoA (two carbons less than the original) formed in the cleavage reaction reenters β-oxidation cycle. In this way, a long-chain fatty acid with an even number of carbons may be degraded completely to acetyl-CoA (two carbon units). For example, after 7 cycles, the 16 carbons palmitate, is converted to 8 acetyl CoA molecules. Since acetyl-CoA can be oxidized to CO_2 and water via the citric acid cycle (which is also found within the mitochondria), the complete oxidation of fatty acids is achieved.

Energetic balance of β –oxidation of fatty acids The function of fatty acid oxidation is, of course, to generate metabolic energy. Each round of β oxidation produces **one NADH**, **one $FADH_2$**, and **one acetyl-CoA**. Oxidation of acetyl-CoA via the citric acid cycle generates additional $FADH_2$ and NADH, which are reoxidized through oxidative phosphorylation to form ATP. Complete oxidation of a fatty acid molecule is therefore a highly exergonic process, which yields numerous ATPs. For example, oxidation of **palmitoyl-CoA** (which has a 16 C fatty acyl group) involves **seven rounds of β-oxidation** yielding **7 $FADH_2$, 7NADH, and 8 acetyl-CoA.** Oxidation of the **8 acetyl-CoA**, in turn, yields **8GTP, 24NADH, and 8$FADH_2$**. Since oxidative phosphorylation of the **31 NADH** molecules yields **93 ATP** and that of the **15$FADH_2$** yields **30 ATPs,** subtracting the 1ATP equivalents required for fatty acyl-CoA formation, the oxidation of one palmitate molecule has a net yield of **130 ATP (table 12.1)**.

Table 12.1. Bioenergetics of palmitic acd oxidation

Stage of palmitate (C16) oxidation	**ATP**
I. β-Oxydation 7 cycles	
7 FADH2 (oxidized by ETC, each FADH2 gives 2 ATP)	14
7 NADH (oxidized by ETC, each NADH gives 3 ATP)	21
II. From 8 oxidized by citric acid cycle, each acetyl-CoA provides 12 ATP	96
Total energy from one mole of palmitoyl CoA	131
Energy used for activation	-1 (2)
Net yield oxidation of one molecule of palmitate	130 (129)

Regulation of β-oxidation. *Carnitine acyl transferase-I* (CAT-I) activity regulates fatty acid oxidation. It is inhibited by malonyl-CoA. In fed condition, more malonyl-CoA is produced. As a result, CAT-I is inhibited and fatty acid oxidation diminishes. In contrast, during fasting or starvation, malonyl-CoA concentration decreases and hence inhibition of CAT-I is relieved. As a result β-oxidation is activated. Thus, β-oxidation is regulated at entry level.

Special cases The β-oxidation pathway discussed above applies to nearly all fatty acids and their derivatives. However, some fatty acids contain **odd-numbers** of carbons or sites of **unsaturation**. These compounds require additional reactions to complete their breakdown.

Peroxisomal fatty acid oxidation refers to the process of breaking down fatty acids within peroxisomes, specialized organelles found in cells. This pathway is involved in the degradation of very long-chain fatty acids (VLCFAs) and branched-chain fatty acids.

The steps of peroxisomal fatty acid oxidation are as follows:

- **Activation:** The fatty acids are activated by forming a fatty acyl-CoA derivative with the help of specific enzymes located in the peroxisomal membrane.
- **Transport:** Fatty acyl-CoA is then transported into the peroxisome by specialized carrier proteins, such as the peroxisomal membrane protein (PMP).
- **Oxidation:** Inside the peroxisome, the fatty acyl-CoA is subjected to a series of enzymatic reactions that involve oxidation of the fatty acid chain. This includes the sequential removal of two carbon units from the acyl-CoA molecule, resulting in the production of acetyl-CoA.
- **Additional reactions:** Depending on the length and type of fatty acid being oxidized, additional reactions may occur within the peroxisome to fully metabolize the acetyl-CoA or generate other intermediates.
- **Energy production:** The acetyl-CoA generated during peroxisomal fatty acid oxidation can be further processed through the tricarboxylic acid (TCA) cycle in the mitochondria to produce ATP, providing energy for the cell.

MEDICAL IMPORTANCE

Peroxisomal fatty acid oxidation is particularly important in tissues with high fatty acid metabolism, such as the liver, kidneys, and skeletal muscle. It plays a crucial role in maintaining lipid homeostasis and energy balance within cells. Disorders affecting peroxisomal fatty acid oxidation can lead to various metabolic diseases, such as peroxisomal biogenesis disorders and X-linked adrenoleukodystrophy.

12.1.1. Disorders of fatty acids oxidation

Fatty acid oxidation is impaired in various diseases with the following manifestations:

- **Carnitine deficiency:** This condition can occur in premature infants and newborns, either due to insufficient production or loss of carnitine through urine. Inadequate carnitine leads to impaired transport of acyl-CoAs into the mitochondria, resulting in elevated levels of plasma-free fatty acids and decreased β-oxidation. Symptoms include

hypoglycemia, lipid accumulation, muscle weakness, and hypoketonemia. Oral supplementation of carnitine helps alleviate these symptoms.

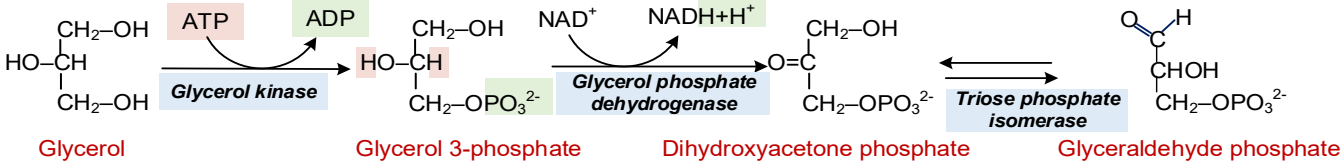
- **Carnitine acyl transferase deficiencies:**
  a) **Hepatic carnitine acyl transferase deficiency:** Deficiency of CAT-I in the liver hampers fatty acid oxidation, leading to hypoketonemia and hypoglycemia.
  b) **Muscle carnitine acyl transferase-II deficiency:** Inadequate CAT-II activity impairs fatty acid oxidation in muscle, causing symptoms like muscle weakness and myoglobinuria.
  c) **Hypoglycemic agents** (for example, glyburide, tolbutamide) used in diabetes inhibit transferases.
- **Jamaican vomiting sickness:** Consumption of unripe fruit from the akee tree results in this condition. An amino acid called hypoglycin present in the fruit inhibits or inactivates *short and medium chain acyl-CoA dehydrogenases.* Consequently, oxidation of these fatty acids is blocked, leading to hypoglycemia. However, the affected fatty acids undergo ω-oxidation, producing dicarboxylic acids that may be excreted in urine.
- **Dicarboxylic aciduria:** This disorder arises due to a deficiency of mitochondrial medium chain acyl-CoA dehydrogenase, impairing β-oxidation of these fatty acids. However, the fatty acids undergo ω-oxidation, resulting in the production of dicarboxylic acids that are excreted in urine.
- **Refsum's disease:** This inherited disease involves the blockage of α-oxidation of phytanic acid. As a result, phytanic acid accumulates in the blood and liver. Incorporation of phytanic acid into cell membranes affects their fluidity, leading to abnormalities in the skin, bones, and peripheral neuropathy. Consumption of a phytanic acid-free diet helps alleviate symptoms.
- **Zellweger's syndrome:** This rare disorder is characterized by the absence of peroxisomes in most tissues. Individuals affected by this condition are unable to metabolize very long chain fatty acids, leading to their accumulation.

12.2. Oxidation of glycerol, its bioenergetics.

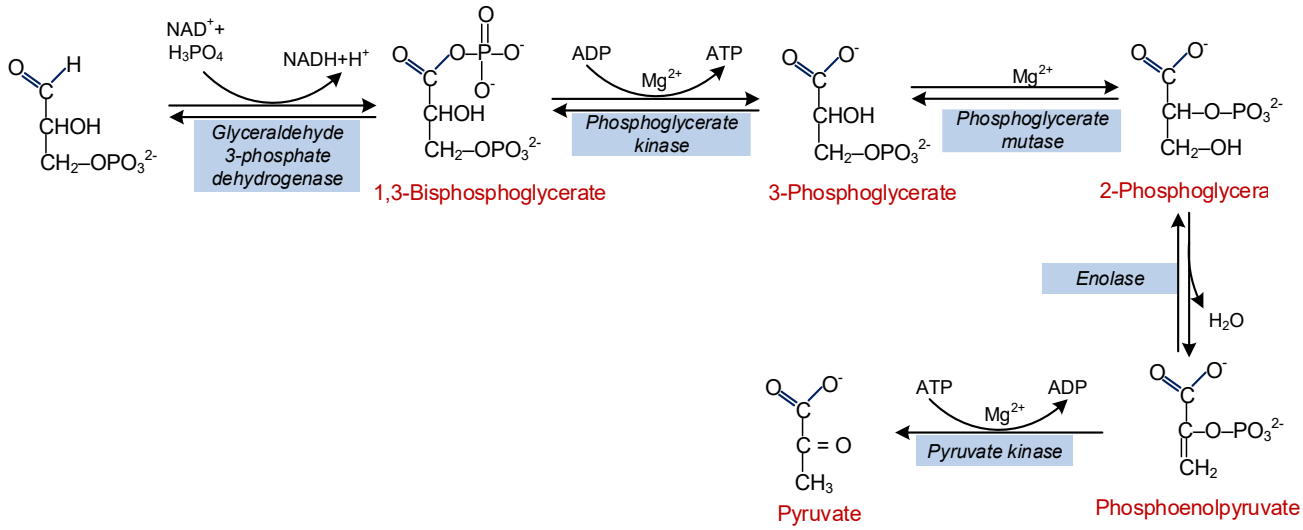
Glycerol, resulting in catabolism of TAGs is transported to the liver or kidneys.

Oxidation of glycerol is 4 stages process:

- **Stage 1:** Convertion of glycerol to the glycolytic intermediate **dihydroxyacetone phosphate (DHAP)** by the sequential actions of ***glycerol kinase*** and *glycerol-3-phosphate dehydrogenase*. DHAP is converted in **D-glyceradehyde 3-phosphate by *triosophosphate isomerase*.**



- **Stage 2:** D-glyceradehyde 3-phosphate enters the second stage of glycolysis.



- **Stage 3:** Oxidative decarboxylation of pyruvate to acetyl-CoA.
- **Stage 4:** Acetyl-CoA produced in stage 3 enters TCA cycle.

Bioenergetics of glycerol oxidation: in the 1st stage 1 ATP was used in glycerol kinase reaction and 1 $NADH+H^+$ was produced in the cytosol. 2nd stage yelds 1 $NADH+H^+$ and 2 molecules of ATP in reactions of substrate phosphorylation. 2 molecules of $NADH+H^+$ formed in cytosolic stages 1 and 2 are transferred to mitochondria using malate-aspartate shuttle (prevalent for liver and kidneys) are transferred yield 6 ATP molecules in electron transpot chain (ETC). 3rd stage takes place in mitochondria and produces 1 $NADH+H^+$. wich tranfers electrons and protons directly to the ETC and gives 3 ATP molecules. 4th stage is one round of TCA, thich energetic balance is 12 molecules of ATP.

Thus the total amaunt of ATP, produced in oxidation of glycerol is 2+6+3+12-1=**22 molecules of ATP**

12.3. Biosynthesis of long chain fatty acids.

Although fatty acid synthesis takes place in most cells, the liver is the primary site for this process. Fatty acids are synthesized when the diet lacks fat and/or contains high amounts of carbohydrates or proteins. The majority of fatty acids are produced from dietary glucose. As you may know, glucose is converted to pyruvate in the cytoplasm. Once inside the mitochondrion, pyruvate is transformed into acyl-CoA. However, acetyl-CoA cannot permeate the mitochondrial membrane and thus cannot enter the cytosol, where fatty acid synthesis occurs. The transport of acetyl-CoA into the cytosol is achieved through an indirect route (fig. 12.2)

Acetyl-CoA enters the cytosol in the form of **citrate**, which can freely cross the membrane. Citrate is formed when acetyl-CoA is converted by ***citrate synthase*** during the tricarboxylic acid (TCA) cycle. A ***tricarboxylate transporter*** in the mitochondrial membrane transports citrate into the cytosol. Acetyl-CoA is then regenerated from citrate in the cytosol through the action of ***ATP-citrate lyase***, with ATP and CoA serving as cofactors. The oxaloacetate produced in this reaction is subsequently reduced to malate by NADH-dependent cytosolic ***malate dehydrogenase.*** Cytosolic **malic enzyme** catalyzes the conversion of malate to pyruvate in an NADP+-dependent reaction. The NADPH generated in this process is utilized for fatty acid synthesis. Thus, the transport of acetyl-CoA from the mitochondria to the cytosol indirectly supplies the required NADPH for fatty acid synthesis.

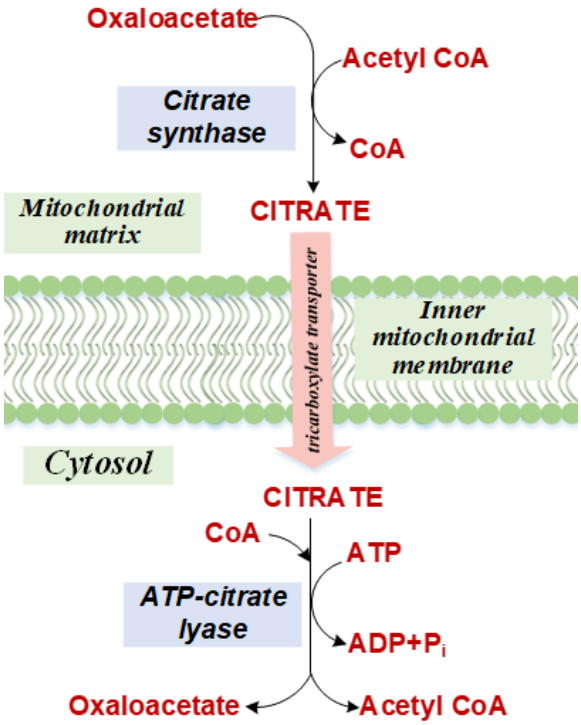


Fig. 12.2 Acetyl-CoA is delivered to cytosol from the mitochondria as citrate. When mitochondrial citrate levels are sufficiently high, citrate enters the cytoplasms, where it is cleaved to form acetyl-CoA and oxaloacetate.

Fatty acid synthesis and β-oxidation are two processes that have several differences, despite being reverse processes in many aspects:

- **Location:** Fatty acid synthesis primarily takes place in the **cytoplasm**, while β-oxidation occurs in the mitochondria.
- **Enzymes:** The enzymes involved in fatty acid synthesis differ significantly in structure from those involved in β-oxidation. In eukaryotes, most of the enzymes responsible for fatty acid synthesis are part of a complex known as ***fatty acid synthase***.
- ***Thioester linkage:*** Intermediates in fatty acid synthesis are linked through a **thioester linkage** to **acyl carrier protein (ACP),** which is a component of *fatty acid synthase*. Acyl groups are attached to both ACP and CoASH through a phosphopantetheine prosthetic group.
- **Electron carriers:** Unlike β-oxidation, which produces NADH and FADH2 as electron carriers, fatty acid synthesis consumes **NADPH**.

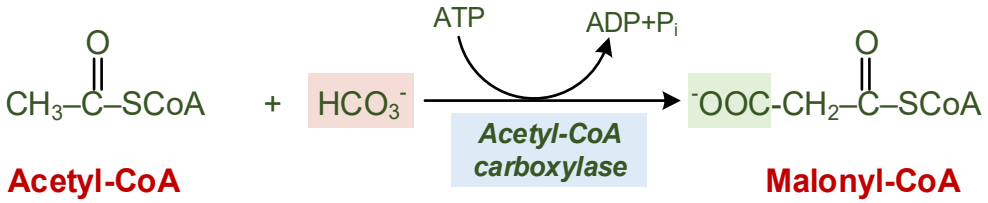
These differences highlight the distinct mechanisms and requirements of fatty acid synthesis compared to β-oxidation.

The enzymatic system responsible for synthesizing long-chain fatty acids from acetyl-CoA, malonyl-CoA, and NADPH in mammals is called ***fatty acid synthase (FAS).*** *Fatty acid synthase* is composed of **seven** component enzymes that are linked together in a large polypeptide chain. In mammals, *FAS* exists as a dimer, with each subunit being identical. The polypeptide chain of *FAS* is folded into three domains connected by flexible regions:

- Domain 1, known as the substrate entry and condensation unit, contains three enzymes: ***acetyl transacylase, malonyl transacylase, and 3-ketoacyl-ACP synthetase*** (also known as the condensing enzyme). This domain is involved in the entry of substrates and the condensation reactions.
- Domain 2, called the reduction unit, consists of the acyl carrier protein (ACP) and three enzymes: ***3-ketoacyl reductase, 3-hydroxyacyl-ACP dehydratase, and enoyl-ACP reductase.*** This domain is responsible for the reduction reactions that occur during fatty acid synthesis.
- Domain 3, known as the **palmitate release unit**, contains the thioesterase enzyme. This domain is involved in the release of the final product, palmitate.

The presence of these seven different catalytic sites on a single polypeptide chain allows for better coordination of the enzymatic activities involved in fatty acid synthesis.

The process of fatty acid synthesis begins with the carboxylation of acetyl-CoA to form **malonyl-CoA**. This carboxylation reaction, catalyzed by the enzyme ***acetyl-CoA carboxylase,*** is considered an "activating reaction" because it prepares the acetyl-CoA for condensation. Activation is necessary in fatty acid synthesis because the condensation of acyl groups is an energetically unfavorable reaction. The carboxylation of acetyl-CoA to form malonyl-CoA is the **rate-limiting step** in fatty acid synthesis.



Acetyl-CoA carboxylase consists of two subunits, each of which is bound to a **biotin** molecule. The initiation of fatty acid synthesis occurs when *acetyl-CoA carboxylase* dimers aggregate and form high molecular weight filamentous polymers. This polymerization process is triggered by an increase in cytoplasmic citrate levels. On the other hand, depolymerization of the polymers takes place when levels of malonyl-CoA or palmitoyl-CoA are high. Additionally, phosphorylation of acetyl-CoA carboxylase occurs in response to the binding of glucagon or epinephrine, leading to depolymerization. Conversely, insulin promotes the aggregation of dimers (fig. 12.3).

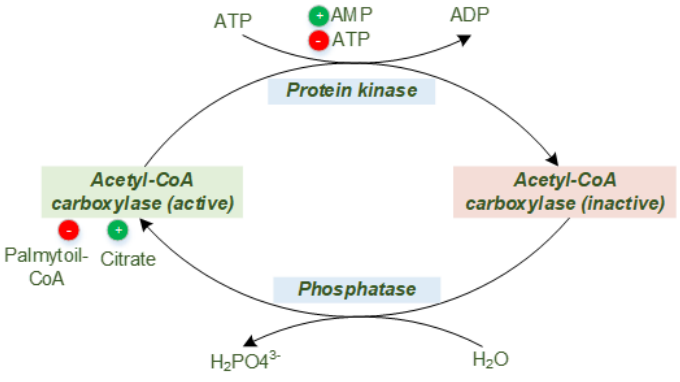


Fig. 12.3. The regulation of acetyl CoA carboxylase

Palmitoyl-CoA acts as an inhibitor of acetyl-CoA carboxylase. This mechanism of control is known as end-product inhibition since palmitoyl-CoA is the final product in fatty acid synthesis, which is initiated by acetyl-CoA carboxylase.

The remaning reactions in fatty acid synthesis take place on the ***fatty acid synthase (FAS)*** multienzyme complex. The intermediates in fatty acid synthesis are covalently linked to the **acyl carrier protein (ACP) (fig.12.4).** ACP prosthetic groups are the **thiol** of the side-chain of a **cysteine** residue and the **thiol** of **phosphopantetheine**, equivalent in structure to part of coenzymeA.

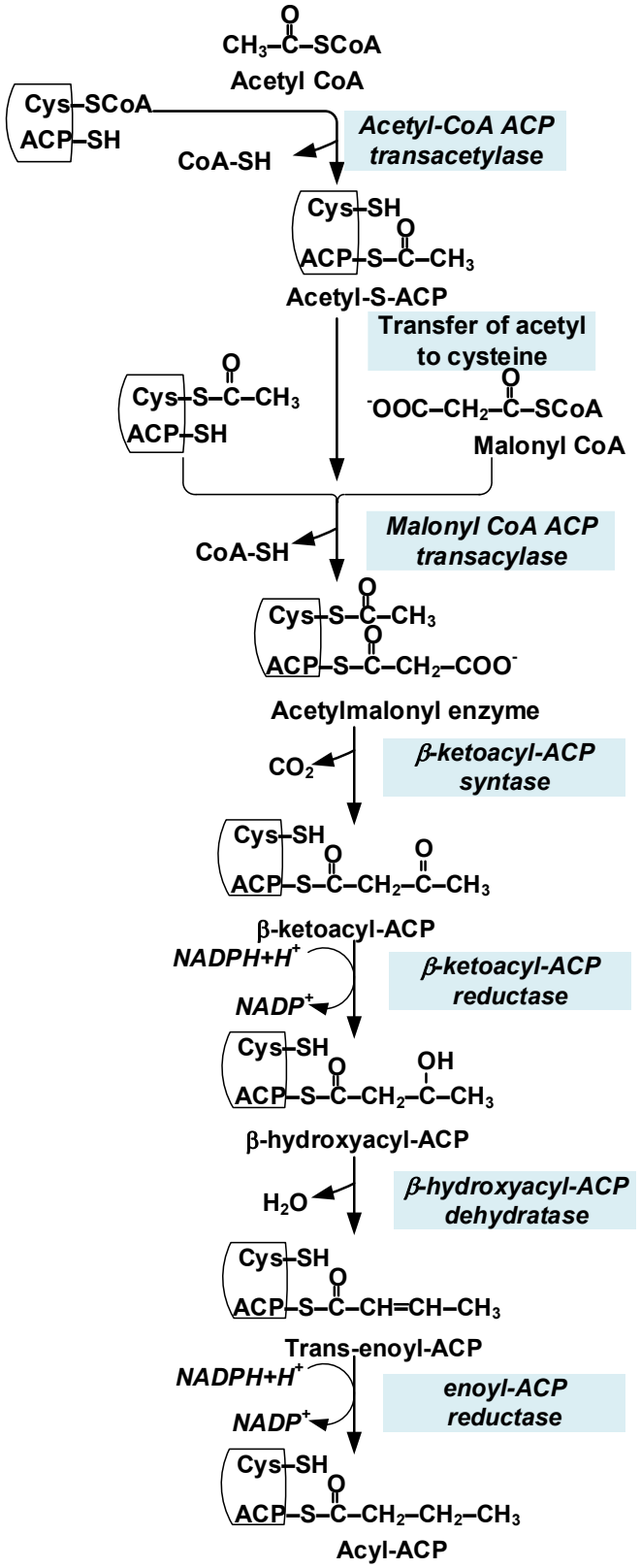


Fig. 12.4. Sequence of reactions of fatty acids synthesis

1. In the initial step of fatty acid synthesis on the acyl carrier protein (ACP) of FAS, ***acetyl-CoA transacetylase*** facilitates the transfer of the acetyl group from an acetyl-CoA molecule to the sulfhydryl (SH) group of a cysteine residue on ACP. The acetyl unit is then transferred from ACP to a cysteine residue on the enzyme, resulting in the ACP site becoming vacant.
2. Next, the enzyme ***malonyl CoA-ACP transacylase*** transfers a malonate group from malonyl-CoA to bind with ACP.
3. Following that, the acetyl unit attached to the cysteine residue is transferred to the malonyl group that is bound to ACP. During this process, the malonyl moiety loses a carbon dioxide (CO_2) molecule that was added by *acetyl-CoA carboxylase*. This reaction is catalyzed by ***β-ketoacyl ACP synthase.***
4. Subsequently, ***β-ketoacyl ACP reductase*** reduces the ketoacyl group to a hydroxyacyl group. The reducing equivalents required for this reaction are provided by NADPH.
5. The β-hydroxyacyl ACP then undergoes dehydration, which is catalyzed by ***β-hydroxyacyl ACP dehydrase***. This step involves the elimination of a water molecule and the introduction of a double bond between the α and β carbons.
6. A second NADPH-dependent reduction, catalyzed by enoyl-ACP reductase, follows to produce acyl-CoA. The four-carbon unit attached to ACP is now a butyryl group.

The carbon chain attached to ACP is transferred to cysteine residue and the reactions 2-6 are repeated 6 more times. Each time, the fatty acid chain is lengthened by a two-carbon unit (obtained from malonyl CoA). At the end of 7 cycles, the fatty acid synthesis is complete and a 16-carbon fully saturated fatty acid-namely palmitate-bound to ACP is produced (fig. 12.5).

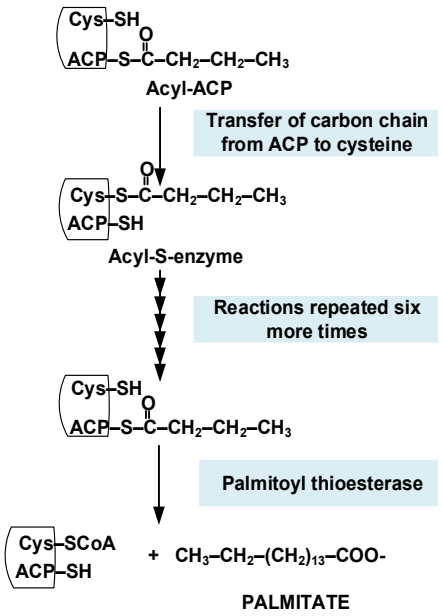


Fig. 12.5. Elongation of fatty acids and elimination of palmitate

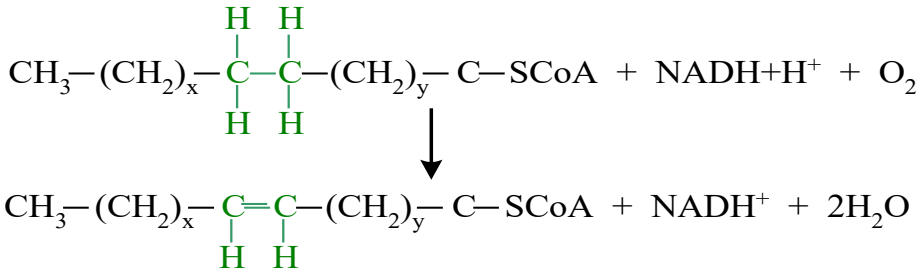
7. The enzyme ***palmitoyl thioesterase*** separates palmitate from fatty acid synthase. This completes the synthesis.

To synthesize the C16 fatty acid palmitate, a total of **7 cycles** are required in fatty acid synthesis. In these cycles, the following molecules are needed:

- **8 molecules of acetyl-CoA:** This includes one molecule of acetyl-CoA and 7 molecules of malonyl-CoA. Each cycle requires one molecule of acetyl-CoA and one molecule of malonyl-CoA.
- **14 molecules of NADPH:** There are two reductive steps in each cycle, and therefore, 14 molecules of NADPH are needed to provide reducing equivalents for these steps.
- **7 molecules of ATP:** ATP is required to generate the 7 molecules of malonyl-CoA from the 7 molecules of acetyl-CoA. Each conversion of acetyl-CoA to malonyl-CoA requires one ATP molecule.

To generate the necessary 14 molecules of NADPH, the oxidative stage of the pentose phosphate pathway in carbohydrate metabolism is involved. The pentose phosphate pathway produces NADPH as a byproduct, which can be utilized in fatty acid synthesis.

Unsaturated fatty acids are produced by **terminal desaturases.** Mammalian system contain four terminal desaturases of broad chain-length specificities designated Δ^9-, Δ^6,-Δ^5-, and Δ^4-fatty acyl-CoA desaturases. These nonheme iron-containing enzymes catalyze the general reaction:



where x is at least five and where $(CH_2)_x$ can contain one or more double bonds. The $(CH_2)_y$ portion of the substrate is always saturated. Double bonds are inserted between existing double bonds in the (CH_2)x portion of the substrate and the CoA group such that the new double bond is there three carbon atoms closer to the CoA group than the next double bond

(not conjugated to an existing double bond) and, in animals, never at positions beyond C(9).

Through a combination of elongation and desaturation reactions, various unsaturated fatty acids can be synthesized. However, due to the metabolic limitations in humans, the synthesis of linoleic acid (Δ9,12-octadecadienoic acid), which contains a Δ12 double bond, is precluded. This is because palmitic acid, the shortest fatty acid available in animals, cannot undergo desaturation at the Δ12 position.

As a result, linoleic acid cannot be synthesized in animals and must be obtained from the diet. It is classified as an essential fatty acid since it is necessary for the production of prostaglandins and other essential compounds in the body. Animals rely on dietary sources of linoleic acid to meet their requirements for this important fatty acid.

12. 4. Metabolism of ketone bodies.

Ketone bodies (**acetoacetate, β-hydroxybulyrate, and acetone (fig. 12.6)**) are made in liver when β-oxidation of fatty acids is in excess of that required by the liver. These water-soluble, energy-rich compounds are transported to other tissues for generation energy. Excess production of ketone bodies, that occurs during starvation or untreated diabetes can be harmful.

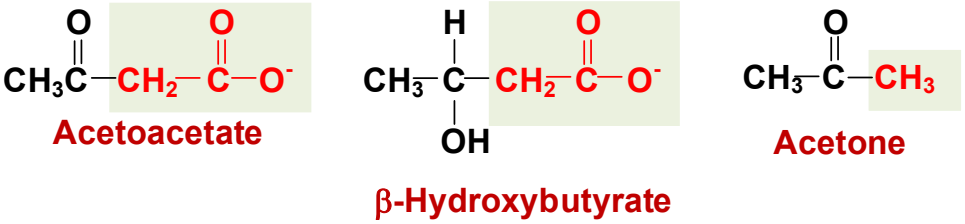


Fig. 12.6. Chemical structure of ketone bodies

Ketogenesis, the biosynthesis of ketone bodies, primarily occurs in the mitochondria of the liver. When fatty acids are broken down in the liver mitochondria, the resulting acetyl-CoA can undergo various metabolic pathways. Acetyl-CoA plays a central role in the tricarboxylic acid cycle (TCA cycle) for energy production. Additionally, acetyl-CoA can be involved in the synthesis of ketone bodies, which specifically takes place within the mitochondria.

During ketogenesis, the following reactions occur (fig. 12.7):

- In the first reaction, catalyzed by ***acetoacetyl-CoA thiolase***, two acetyl-CoA molecules condense to form **acetoacetyl-CoA**. This reaction is essentially the reverse of the last step in β-oxidation and is energetically unfavorable under normal conditions. However, when levels of acetyl-CoA rise, the equilibrium is shifted towards the synthesis of acetoacetyl-CoA.
- A third molecule of acetyl-CoA reacts with acetoacetyl-CoA, forming **β-hydroxy-β-methylglutaryl-CoA (HMG-CoA)** in a reaction catalyzed by ***HMG-CoA synthase***.
- ***HMG-CoA lyase*** catalyzes the next step, resulting in the production of **acetoacetate** and an additional molecule of acetyl-CoA.
- Acetoacetate can be further converted to **β-hydroxybutyrate** by the enzyme ***β-hydroxybutyrate dehydrogenase***, which is located on the inner membrane of the

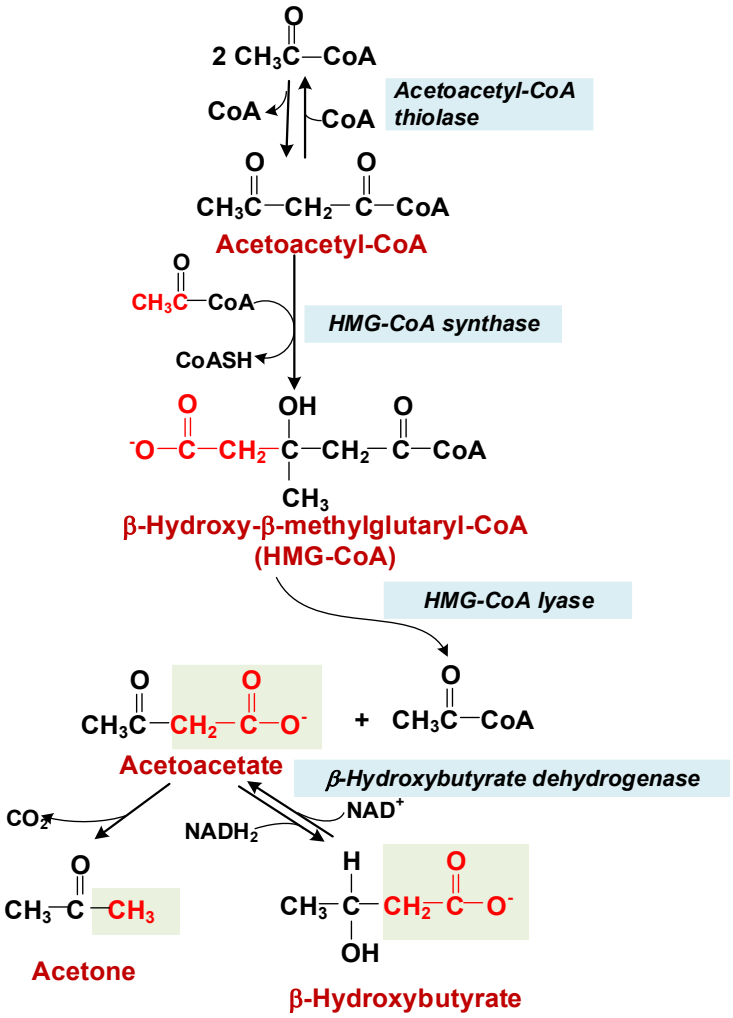


Fig. 12.7. Ketogenesis

mitochondria. While acetoacetate can also be decarboxylated to form **acetone**, this is typically of minor significance. However, individuals with uncontrolled type I diabetes may exhibit high levels of ketone bodies in their bloodstream, leading to the characteristic odor of acetone in their breath.

The increase in ketone bodies occurs when cells and tissues are deprived of glucose and rely on the mobilization of fatty acids for energy. This results in an excess production of acetyl-CoA in the liver. To utilize this surplus acetyl-CoA, the liver synthesizes ketone bodies, which can then serve as an energy source for extrahepatic tissues. However, a notable complication arises when serum levels of ketone bodies rise significantly, as it leads to a decrease in blood pH due to the increased concentration of these acids. Among other concerns, this condition can be hazardous due to the impact of lower pH on the binding of oxygen to hemoglobin.

Ketone body synthesis is primarily a liver function, since mitochondrial HMG-CoA synthase is present in large quantities only in this tissue. Acetoacetate and β-hydroxybutyrate are secreted into the blood and carried to other tissues.

Ketone bodies utilization (ketolysis) (fig. 13.8). The ketone bodies, being water-soluble, are easily transported from the liver to various tissues. The two ketone bodies-acetoacetate and β-hydroxybutyrate serve as important sources of energy for the peripheral tissues such as skeletal muscle, cardiac muscle/ renal cortex etc. The tissues which lack mitochondria (elg. erythrocytes) however, cannot utilize ketone bodies. Acetoacetate and β- hydroxybutyrate are secreted into the blood and carried to other tissues where they are converted into acetyl-CoA. The reactions of ketolysis are catalyzed by **β-hydroxybutyrate dehydrogenase** and **thiolase** are common to both the synthesis and degradation of the ketone bodies. However, the second enzyme in the sequence for degradation, **β-oxyacid-CoA transferase,** is present in all tissues but liver. Hence, ketone bodies are made in the liver and metabolized to CO_2 and energy in nonhepatic (nonliver)

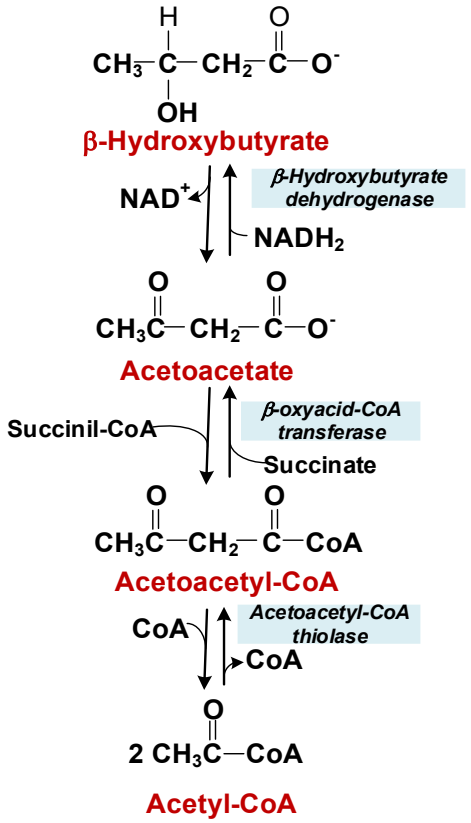


Fig. 12.8. Ketolysis

tissues. Ketone bodies can also be used to supply these tissues with acetyl-CoA for fatty acid and cholesterol biosynthesis.

In individuals with normal metabolism, the liver consistently produces ketone bodies, which are then utilized by tissues outside the liver. However, when higher-than-normal levels of ketone bodies are present in the blood or urine, it is referred to as **ketonemia (hyperketonemia)** or **ketonuria,** respectively. This overall condition is known as **ketosis.**

The basic form of ketosis occurs during periods of starvation, where there is a depletion of available carbohydrates combined with the mobilization of fatty acids. This metabolic pattern is magnified in pathological states such as diabetes mellitus. Non-pathological forms of ketosis can also occur in situations of high-fat feeding or after intense exercise during the postabsorptive state.

Acetoacetic acid and β-hydroxybutyric acid, the two primary ketone bodies, are moderately strong acids and are buffered when present in the blood or other tissues. However, their continuous excretion in large amounts gradually depletes the alkaline reserve, leading to a condition known as ketoacidosis. This can be particularly dangerous and potentially fatal in cases of uncontrolled diabetes mellitus, where ketoacidosis can develop.

REVIEW TEST:

№	MCQs	Answers and explanations
1.	**A patient with high rate of obesity was advised to use carnitine as a food additive in order to enhance "fat burning". What is the role of carnitine in the process of fat oxidation?** A. Transport of FFA (free fatty acids) from cytosol to the mitochondria B. Transport of FFA from fat depots to the tissues C. It takes part in one of reactions of FFA β-oxidation D. FFA activation E. Activation of intracellular lipolysis	**The answer is A.** Free fatty acids cannot get into mitochondrial matrix through inner membrane, that's why they are transported in complex with carnitine. Carnitine, as a transporter, provides β-oxidation of fatty acids.
2.	**1-year-old child with symptoms of muscle involvement was admitted to the hospital. Examination revealed**	**The answer is B.** The main function of carnitine is transport of fatty acids into the mitochondrial matrix. Deficiency of carnitine can

	carnitine deficiency in his muscles. What process disturbance is the biochemical basis of this pathology? A. Regulation of Ca^{2+} level in mitochondrions B. Transporting of fatty acids to mitochodrions C. Substrate phosphorylation D. Lactic acid utilization E. Actin and myosin synthesis	cause the disturbance of muscle contraction or/and the accumulation of fat, because carnitine provides β-oxidation of fatty acids.
3.	**One of the factors that cause obesity is the inhibition of fatty acids oxidation due to:** A. Excessive consumption of fatty foods B. Impaired phospholipid synthesis C. Low level of carnitine D. Choline deficiency E. Lack of carbohydrates in the diet	**The answer is C.** Free fatty acids cannot get into mitochondrial matrix through inner membrane, that's why they are transported in complex with carnitine. Low carnitine level can cause decreased β-oxidation of fatty acids, which will be accumulated in form of triacylglycerols and cause the obesity.
4.	**A sportsman was recommended to take a medication that contains carnitine in order to improve his results. What process is activated by carnitine the most?** A. Synyhesis of lipids B. Synthesis of steroid hormones C. Synthesis of ketone bodies D. Fatty acids transport to mitochondrion E. Tissue respiration	**The answer is D.** The main function of carnitine is transport of fatty acids into the mitochondrial matrix, so carnitine using can provide muscles with energy by activating of fatty acids β-oxidation.
5.	**An 18-year-old obese woman maintains a sedentary lifestyle and eats a high-fat, high carbohydrate diet. Maintenance of this diet and lifestyle has led to lipogenesis and obesity. Which of the following statements correctly describes an aspect of lipogenesis?** A. The primary source of carbons for fatty acid synthesis is glycerol B. Fatty acids are synthesized from acetyl CoA in the mitochondria. C. Fatty acid synthesis and esterification to glycerol to form triacylglycerolsoccurs primarily in muscle cells. D. The fatty acyl chain on the fatty acid synthase complex is elongated two carbons at atime. E. $NADP^+$, which is important for fatty acid synthesis, is produced by the pentosephosphate pathway.	**The answer is D.** The primary source of carbons for fatty acid synthesis is dietary carbohydrate. Fatty acids are synthesized from acetyl CoA in the hepatocyte cytosol, and esterification to glycerol to form triacylglycerols also occurs primarily in the liver. The fatty acyl chain on the fatty acid synthase complex is elongated two carbons at atime. Witheach two-carbon addition to the elongatingchain, the β-keto group is reduced in a reaction that requires NADPH. NADPH is a reducing equivalent produced by the pentose phosphate pathway and the malic enzyme. $NADP^+$ isa product of fatty acid biosynthesis, not a substrate.
6.	**An experimental animal has been given excessive amount of carbon-labeled**	**The answer is A.**

	glucose for a week. What compound can the label be found in? A. Palmitic acid B. Methionine C. Vitamin A D. Choline E. Arachidonic acid	Carbon-labeled glucose is broken down to pyruvate in aerobic oxidation of glucose pathway, then it is decarboxylated to acetyl-CoA by pyruvate dehydrogenase complex. Acetyl-CoA can be used in citric acid pathway as a source of energy (reduced NADH and FAD) or as fatty acids building material. Thats why labeled carbon of glucose can be found in palmitic acid, which is a fatty acid.
7.	**Patients who suffer from severe diabetes and don't receive insulin have metabolic acidosis. This is caused by increased concentration of the following metabolites:** A. Cholesterol B. Fatty acids C. Unsaturated fatty acids D. Triacylglycerols E. Ketone bodies	**The answer is E.** Metabolic acidosis is usually caused by accumulation of metabolites, which decrease the blood pH level. In case of severe diabetes mellitus glucose cannot get into the cells and these cells start to breakdown fatty acids by β-oxidation. As a result acetyl-CoA will be accumulated, as there is no oxaloacetate to react with. Oxaloacetate can be obtained from glucose, that's why the molecules of acetyl-CoA react with each other and form ketone bodies. The excess of ketone bodies (for example, acetoacetate) can cause metabolic acidosis, as they are acids in nature.
8.	A 6-month-old boy was admitted to the hospital after experiencing a seizure. It was discovered that he had a decreased appetite for several days prior, which was attributed to a "stomach virus". Upon admission, his blood glucose level was measured at 24 mg/dl, which is significantly lower than the normal range of 60-100 mg/dl for his age. Ketone bodies were not detected in his urine, but various dicarboxylic acids were present. A tentative diagnosis of medium-chain fatty acyl CoA dehydrogenase (MCAD) deficiency is suspected. In patients with MCAD deficiency, the underlying cause of fasting hypoglycemia is: A. Increased acetyl CoA production B. Decreased acetyl CoA production C. Decreased ability to convert acetyl CoA to glucose D. Increased conversion of acetyl CoA to acetoacetate E. Increased production of ATP and NADH	**The answer is B.** A deficiency in the oxidation of fatty acids shorter than 12 carbons in length leads to a reduced production of acetyl CoA. Acetyl CoA serves as an allosteric activator of pyruvate carboxylase, an important enzyme in gluconeogenesis. As a result, the levels of glucose in the body decrease. Acetyl CoA cannot be effectively utilized for the net synthesis of glucose. In MCAD deficiency, the production of ketone bodies, specifically acetoacetate, is also reduced. The impaired oxidation of fatty acids results in lower ATP and NADH production, both of which are essential for gluconeogenesis to occur.
9.	**39-year-old female patient with a history of diabetes was hospitalized in a precomatose state for diabetic ketoacidosis. This condition had been caused by an increase in the following metabolite level:** A. Acetoacetate B. Citrate	**The answer is A.** Metabolic acidosis typically occurs due to the buildup of certain metabolites, leading to a decrease in blood pH. In the case of severe diabetes mellitus, cells are unable to take in glucose, resulting in the breakdown of fatty acids through β-oxidation. Consequently, there is an accumulation of acetyl-CoA since there is a lack of oxaloacetate to react with it. Since oxaloacetate is derived

	C. Alpha-ketoglutarate D. Malonate E. Aspartate	from glucose, the excess acetyl-CoA molecules react with each other, giving rise to the formation of ketone bodies. An excess of ketone bodies, such as acetoacetate, can contribute to metabolic acidosis due to their acidic nature.
10	**A 12-year-old boy from Jamaica is experiencing persistent vomiting, abdominal pain, lethargy, and severe hypoglycemia. These symptoms are attributed to Jamaican vomiting syndrome, an illness triggered by the consumption of hypoglycin found in unripe ackee fruit. Hypoglycin undergoes metabolism to produce a nonmetabolizable form of carnitine, which disrupts the usual process of fatty acid oxidation. What is the primary role of carnitine?** A. Activates long-chainfatty acids inthe cytosol B. Transport of acyl groups across the inner mitochondrial membrane C. Is converted to enoyl CoA D. Is converted to β-hydroxyacyl CoA Is involved in breakdown of even-chain, but not odd-chain, fatty acids	**The answer is B.** The primary role of carnitine is to facilitate the transport of fatty acids into the mitochondria for β-oxidation, the process by which fatty acids are broken down to generate energy. Carnitine acts as a carrier molecule, forming fatty acyl carnitine in the outer mitochondrial membrane and allowing the fatty acids to cross the inner mitochondrial membrane. This transport mechanism is essential for fatty acid oxidation to occur efficiently. In the context of the presented case, the ingestion of hypoglycin leads to the formation of a nonmetabolizable form of carnitine, which interferes with normal fatty acid oxidation and contributes to the symptoms experienced by the boy with Jamaican vomiting syndrome.

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13. BIOSYNTHESIS AND BIOTRANSFORMATION OF CHOLESTEROL. LIPOPROTEINS OF BLOOD PLASMA PATHOLOGY OF LIPID METABOLISM

OBJECTIVES after studying this chapter, you should be able to:

- *Interpret stages of cholesterol biosynthesis*
- *Explain regulation of cholesterol production in human body.*
- *Analyze pathways of cholesterol biotransformation: esterification, synthesis of bile acids, steroid hormones, vitamin D_3, excretion of cholesterol from the body.*
- *Interpret pathology of lipid metabolism: atherosclerosis, diabetes mellitus, obesity, steatorrhea.*
- *Describe the processes of lipids transport with lipoproteins*

13.1. Biosynthesis of cholesterol in human body

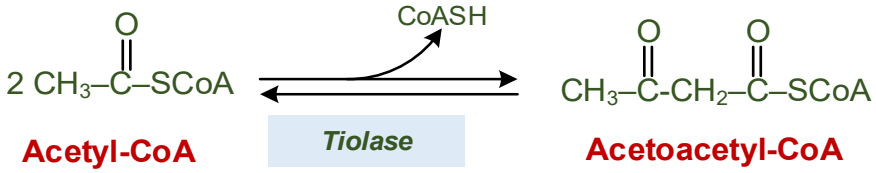
Steroids are complex hydrocarbons consisting of four interconnected rings and are more prevalent in eukaryotes compared to prokaryotes. Among steroids, cholesterol is the most prominent member and plays a vital role in the membranes of many eukaryotic cells. Furthermore, cholesterol serves as the precursor for two major classes of steroids: steroid hormones and bile acids.

Approximately half of the cholesterol found in the body is synthesized de novo, with a daily production of around 1 gram. The liver accounts for 50% of this synthesis, while the intestine contributes about 15%. Additionally, enzymes involved in cholesterol biosynthesis are present in the skin, adrenal cortex, and reproductive tissues. These tissues have the ability to produce cholesterol independently.

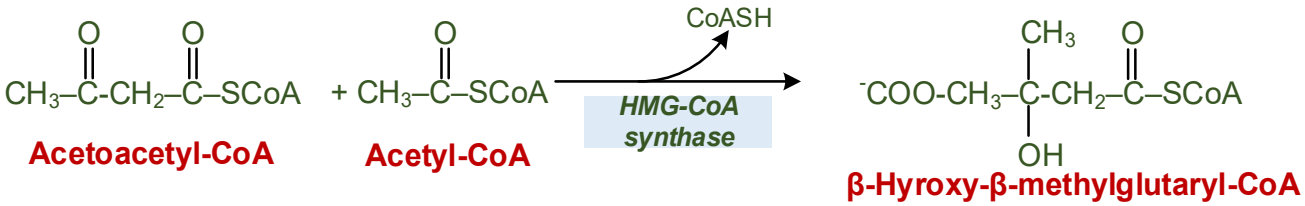
Cholesterol synthesis can be divided into five phases:

1. Formation of HMG-CoA (β-hydroxy-β-methylglutaryltobutyryl-CoA) from acetyl-CoA.
2. Conversion of HMG-CoA to mevalonate.
3. Production of isoprenoid units.
4. Formation of squalene.
5. Conversion of squalen to cholesterol.

- The first phase of cholesterol synthesis is a **cytoplasmic** process. The condensation of two acyl-CoA molecules to form acetoacetyl-CoA (β-ketobutyryl-CoA) is catalyzyd by ***thiolase:***

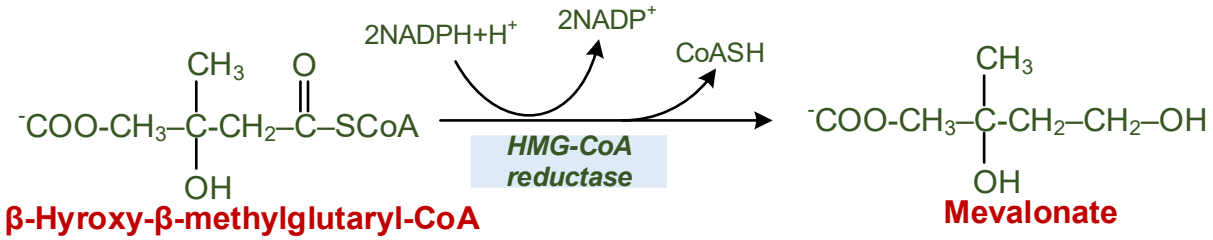


In the next reaction, acetoacetyl-CoA condenses with another acetyl-CoA to **form β-hydroxy-β-methylglutaryltobutyryl-CoA (HMG-CoA)**. This reaction is catalized by ***β-hydroxy-β-methylglutaryl-CoA synthase****:*



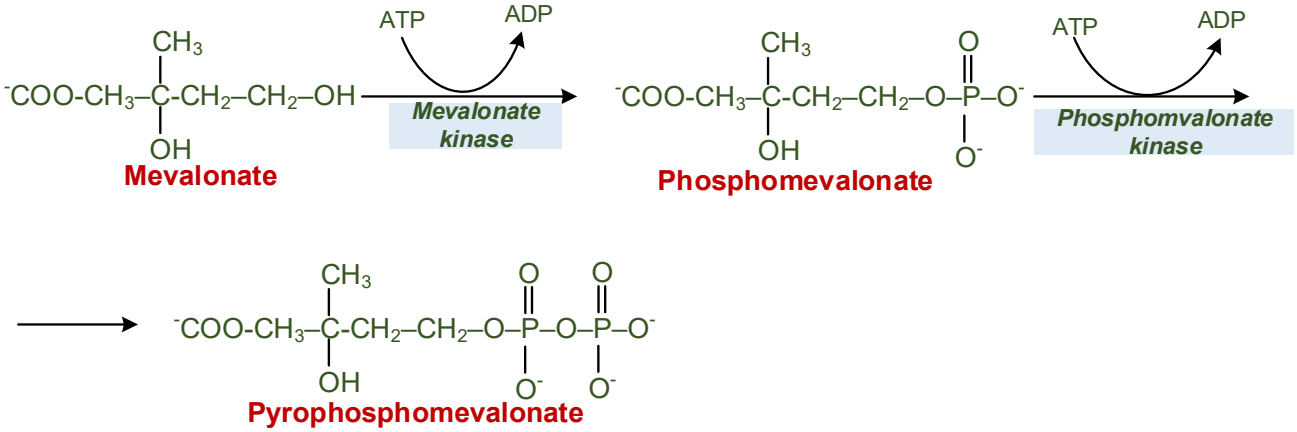
Reactions of this stage are identical to these of ketone bodies synthesis.

- The second phase of cholesterol synthesis is the reduction of HMG-CoA to mevalonate. Reacion is catalised by **HMG-CoA reductase**, NADPH is the reducing agent:

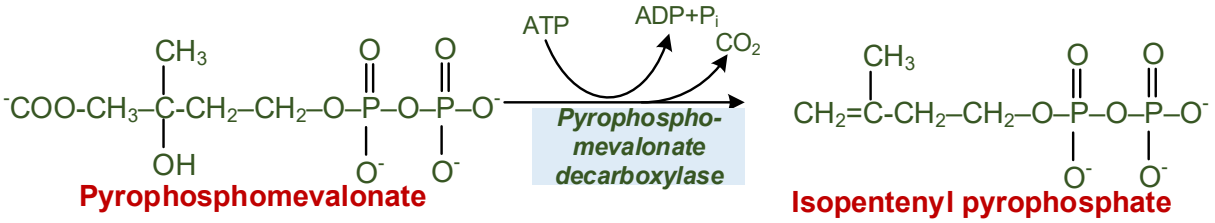


***HMG-CoA reductase* is the rate-limiting enzyme in cholesterol synthesis.** The activity of this enzyme, which is located on cytoplasmic surface of endoplasmic reticulum, is modulated by **phosphrylation-dephosphrylation**.

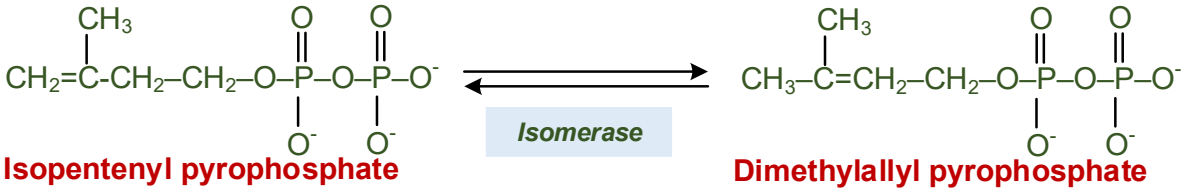
- **Production of isoprenoid units:** In the series of cytoplasmic reactions, mevalonate is converted to farnesylpyrophosphate. ***Mevalonate kinase*** catalyzes the synthesis of phosphomevalonate. A second reaction catalyzed by ***phosphomevalonate kinase*** produces 5-pyrophosphmevalonate:



5-Pyrophosphomevalonate is converted to isopentenyl pyrophosphate in a process involving a decarboxylation and dehydration:



Isopentenylpyrophosphate is next converted to its isoform 3,3-dimethylallylpyrophosphate by ***isopentenyl pyrophosphate isomerase*.**



- Geranyl pyrophosphate is generated during a condensation reaction between isopentenylpyrophosphate and dimethylallylpyrophosphate. Pyro-phosphate is also a product of this reaction and two subsequent reactions. ***Geranyl transferase*** catalyses the condensation reaction between geranylpyrophosphate and isopentenylpyrophosphate that results in the formation of farnesylpyrophosphate. Squalene is synthesized when farnesyl transferase (***squalen synthase***) (a microsomal enzyme) catalyzes the condensation of two farnesyl pyrophosphate molecules (fig. 13. 1).
- The last phase of the cholesterol synthesis begins with the binding of squalene to a specific cytoplasmic protein carrier called **srerol carrier protein**. The convertion of squalen to lanosterol occurs while the intermediates are bound to this protein. The enzyme activities that are required for the oxygen-dependent epoxide formation (***squalene monooxygenase***) and subsequent cyclization (**2,3-oxidosqualen lanosterol cyclase**) that result in lanosterol synthesis have been localized in microsomes. Squalene monooxygenase requires NADPH for activity. After its synthesis, lanosterol binds to a second carrier protein, to which it remains attached during the remaining reactions. All of the enzyme activities that catalyze the remaining 20 reactions needed to convert lanosterol to cholesterol are embedded in micrisomal membranes. In a series of transformations involving NADPH as well as some oxygen, lanosterol is converted to 7-dehydrocholesterol. This product is then reduced by NADPH to form cholesterol.

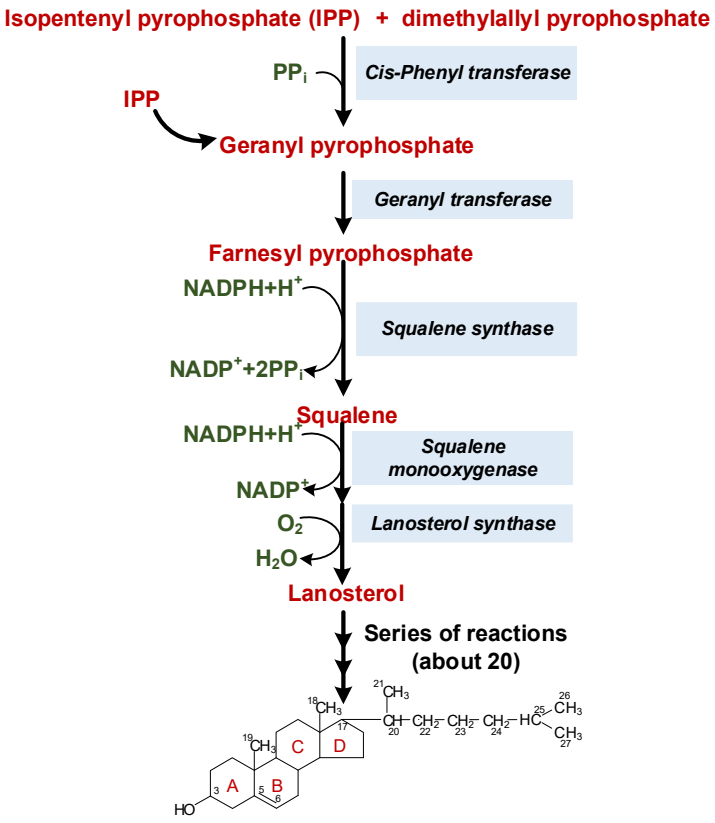


Fig.13.1. Formation of squalene (phase 4) and its conertion to cholesterol (phase 5)

The intermediates in the cholesterol biosynthetic pathway may have the diverse roles and metabolic fates:

- **Dimethylallyl pyrophosphate:** A fraction of dimethylallyl pyrophosphate, an intermediate in the cholesterol biosynthetic pathway, can be converted to HMG-CoA through the transmethyl gluconate shunt. Additionally, it is utilized in the

modification of adenosine residues in tRNA, resulting in the formation of isopentenyl adenosine.

- **Farnesyl pyrophosphate (FPP):** FPP, another intermediate in the cholesterol biosynthetic pathway, has diverse roles in the cell. It can be converted to ubiquinone (coenzyme Q), which is involved in electron transport and ATP production. FPP is also utilized in the synthesis of heme, a component of cytochrome oxidase involved in oxidative phosphorylation, and dolichol, which plays a role in protein glycosylation.
- **Squalene:** Squalene, a key intermediate in cholesterol biosynthesis, can be converted to hopane in certain bacteria and plants. Hopanoids, derived from hopane, are highly abundant biomolecules found in these organisms and are not easily biodegradable. They have been identified as precursors of petroleum products and play a role in the petroleum industry.

MEDICAL IMPORTANCE

Malaria is caused by Plasmodium falciparum, tuberculosis is caused by Mycobacterium tuberculosis, and gastritis, peptic ulcer disease, and gastric cancer are caused by Helicobacter pylori. These pathogens have the ability to synthesize cholesterol using the Mevalonate independent pathway, which involves utilizing two glycolysis intermediates: pyruvate and glyceraldehyde-3-phosphate. Current drugs have become less effective against malaria and tuberculosis due to the development of drug resistance by these pathogens. However, since the enzymes involved in the Mevalonate independent pathway are distinct from human enzymes, compounds that specifically target the enzymes of these pathogens could potentially serve as a new class of antimalarial and anti-tubercular agents.

Cholesterol synthesized within cells can undergo two primary fates: **esterification** or release as **free cholesterol**.

In the liver, intestine, and extrahepatic tissues, cholesterol can be esterified by an enzyme called ***Acyl-CoA-cholesterol acyltransferase (ACAT)***. *ACAT* catalyzes the esterification reaction, in which a fatty acid is attached to the hydroxyl group on the carbon-3 position of cholesterol. This forms a cholesterol ester, specifically a fatty acyl ester of cholesterol. The esterification of cholesterol has important implications for its **storage** and **transport.** Cholesterol esters are relatively insoluble in water and are therefore less likely to contribute to the formation of lipid droplets or interfere with cellular processes. They can be stored within cells, particularly in lipid droplets, or packaged into lipoproteins for transport through the bloodstream.

On the other hand, cholesterol can also be released as **free cholesterol**. Free cholesterol is the unesterified form of cholesterol and can be found in cell membranes, where it plays a crucial role in maintaining membrane fluidity and function. Free cholesterol can also serve as a substrate for various metabolic processes, including the synthesis of steroid hormones, vitamin D, and bile acids.

The balance between cholesterol esterification and free cholesterol levels is tightly regulated to maintain cholesterol homeostasis within cells and tissues. Disruptions in this balance can contribute to cholesterol-related disorders, such as **atherosclerosis and familial hypercholesterolemia**.

13.1.1. Regulation of cholesterol synthesis

The production of cholesterol is controlled by a key enzyme – ***HMG-CoA reductase***, which plays a crucial role as the limiting factor in this biochemical pathway. *HMG CoA reductase* is commonly found in the vicinity of the endoplasmic reticulum and is subject to diverse metabolic regulations and influences:

- **Transcriptional control:** The expression of the *HMG-CoA reductase* gene is regulated at the transcriptional level. The main regulator is a protein called **sterol regulatory element-binding protein (SREBP)**. When cellular cholesterol levels are low, SREBP is activated and translocated to the nucleus, where it binds to the sterol regulatory element (SRE) in the promoter region of the *HMG-CoA reductase* gene. This binding activates the transcription of the gene, leading to increased production of *HMG-CoA reductase*. Conversely, when cellular cholesterol levels are high, SREBP is inactivated, preventing its binding to the SRE and reducing *HMG-CoA reductase* expression.
- **Feedback control:** In terms of feedback control, the synthesis of cholesterol is regulated by a mechanism in which the end product, **cholesterol** itself, plays a crucial role. When the cellular concentration of cholesterol increases, it leads to a reduction in the synthesis of the enzyme ***HMG-CoA reductase***. This is accomplished by decreasing the **transcription** of the gene responsible for producing *HMG-CoA reductase*. Essentially, the elevated levels of cholesterol act as a feedback signal to downregulate the production of the enzyme involved in its synthesis.
- **Hormonal regulation:** *HMG-CoA reductase*, the enzyme involved in cholesterol synthesis, exists in two forms that can interconvert (fig. 13.2). The **dephosphorylated form** of *HMG-CoA reductase* is more active, while the **phosphorylated form** is less active. Hormones exert their influence on this enzyme through a series of reactions involving cAMP, similar to the control mechanism of the enzyme glycogen synthase.

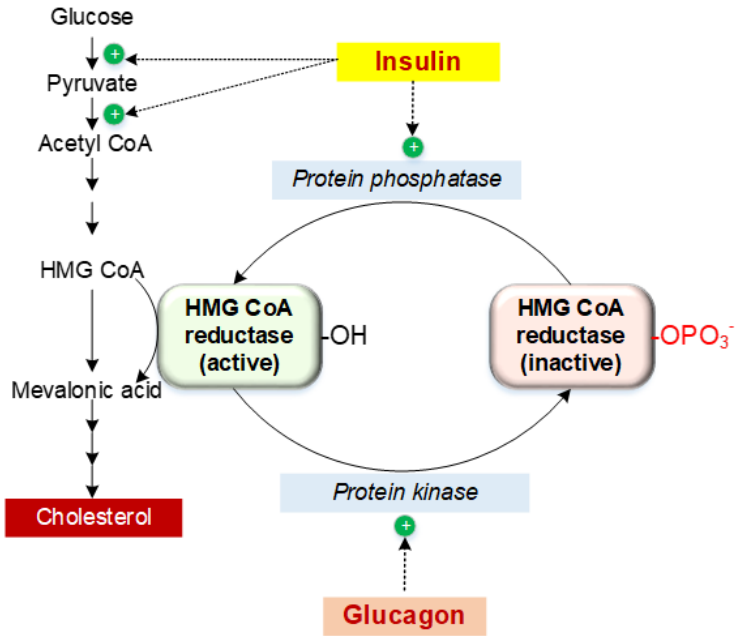


Fig. 13.2. Hormonal regulation of HMG CoA activity

The overall effect is that hormones such as **glucagon and glucocorticoids** promote the formation of the inactive, phosphorylated form of *HMG-CoA reductase*, leading to a decrease in cholesterol synthesis. On the other hand, **insulin** increases cholesterol production by enhancing the formation of the active, dephosphorylated form of *HMG-CoA reductase*. In summary, these hormones play a role in regulating cholesterol synthesis by modulating the activity of **HMG-CoA reductase** through phosphorylation and dephosphorylation processes.

Statins are a class of drugs commonly used to decrease serum cholesterol levels in patients with **hypercholesterolemia**, which is characterized by high levels of cholesterol in the blood. Statins work by inhibiting the enzyme ***HMG-CoA reductase***, which is a key enzyme involved in the synthesis of cholesterol in the body. By inhibiting *HMG-CoA reductase*, statins reduce the production of cholesterol in the liver. This leads to a decrease in the total cholesterol and low-density lipoprotein (LDL) cholesterol levels in the blood.

Lowering LDL cholesterol is particularly important because LDL cholesterol is often referred to as “bad” cholesterol. Elevated levels of LDL cholesterol are associated with an increased risk of cardiovascular diseases, including coronary artery disease, heart attack, and stroke.

In addition to lowering LDL cholesterol, statins have been shown to have other beneficial effects, such as increasing high-density lipoprotein (HDL) cholesterol levels (often referred to as "good" cholesterol), reducing inflammation, stabilizing plaques in the arteries, and improving endothelial function.

Statins are commonly prescribed along with lifestyle modifications, such as a healthy diet and regular exercise, to manage hypercholesterolemia and reduce the risk of cardiovascular events. It's important to note that statins should be prescribed and monitored by a healthcare professional, as they may have side effects and interactions with other medications.

13.2. Pathways of cholesterol catabolism (biotransformation)

Cholesterol is a complex molecule that cannot be directly broken down to carbon dioxide and water through normal metabolic pathways. Unlike other molecules, such as glucose or fatty acids, cholesterol does not undergo complete oxidation for energy production.

Cholesterol (50%) is primarily metabolized in the liver through a series of enzymatic reactions that convert it into **bile acids or bile salts** (fig. 13.3). These bile acids are then released into the intestine and eventually eliminated from the body through feces. This process is known as cholesterol catabolism.

Other 50 % of cholesterol are used as component of **cell membranes** in the synthesis of important molecules such as **steroid hormones and vitamin D**

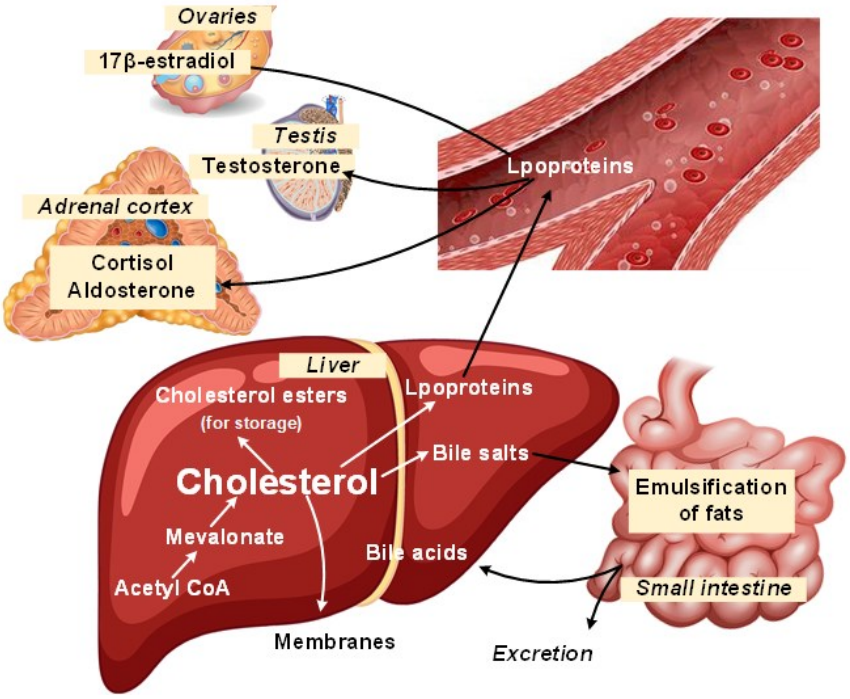


Fig. 13.3. A simplified representation of cholesterol metabolism

which all have various metabolic functions in the body.

Cholesterol in the liver is converted into **bile acids**, which are then stored in the gall bladder and released into the small intestine. These bile acids play a vital role in the emusification of lipids. The synthesis of bile acids involves a complex, multi-step process that takes place in multiple organelles. Specific hydroxyl groups are inserted at precise positions on the steroid structure, the double bond of the cholesterol B ring is reduced, and the hydrocarbon chain undergoes a three-carbon shortening, resulting in the introduction of a carboxyl group at the chain's end. The primary bile acids, commonly known as **cholic acid** (a triol) and chenodeoxycholic acid (a diol), are the most frequently produced compounds in this pathway (fig. 13.4).

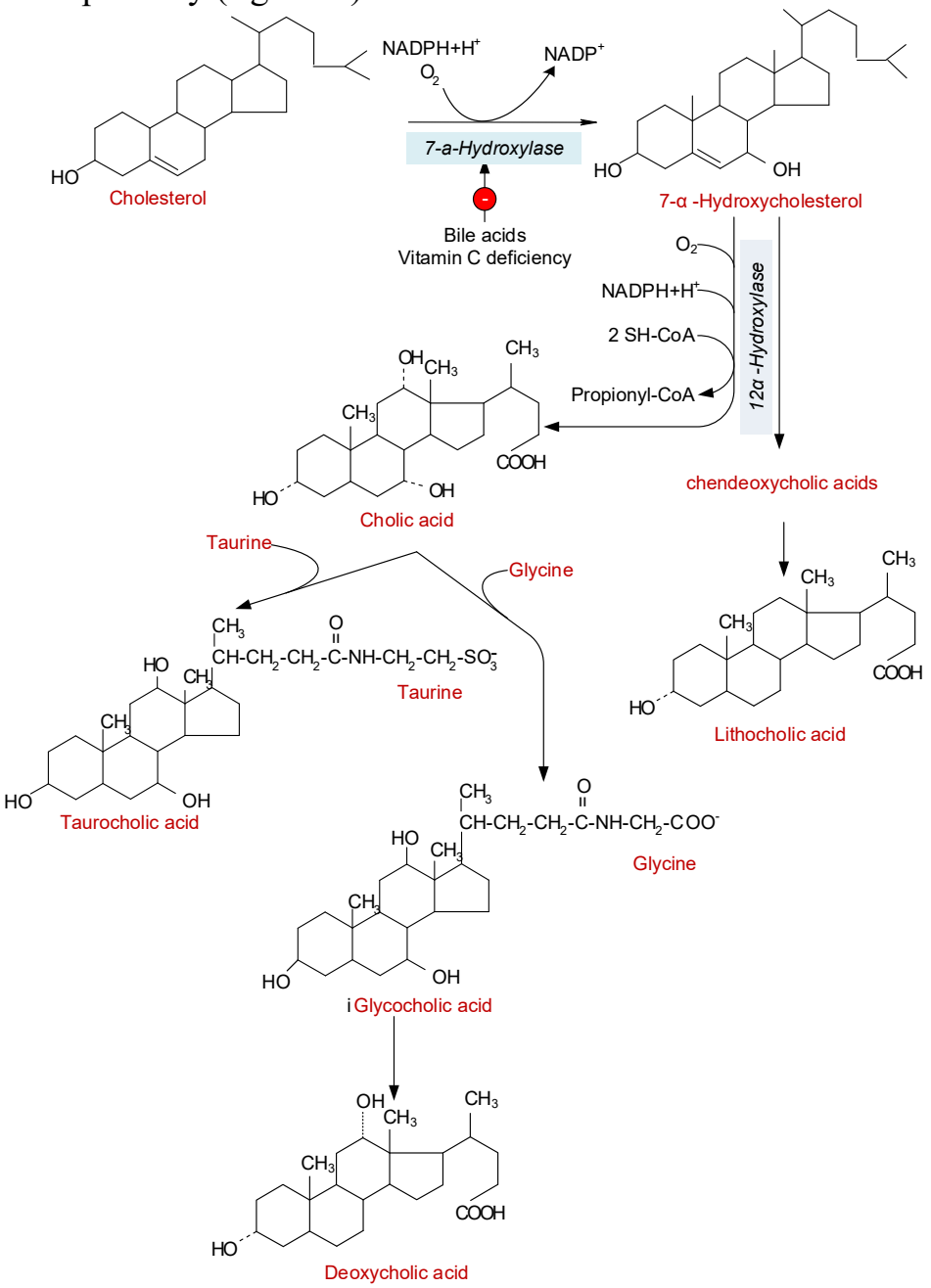


Fig. 13.4. Reactions of bile acids synthesis and their conjugation.

Before leaving the liver, primary bile acids are then conjugated with the amino acids glycine or taurine to form bile acid conjugates, specifically **glycocholic acid and**

taurocholic acid. This conjugation occurs by the action of enzymes known as ***bile acid-CoA ligases*** and ***bile acid-CoA:amino acid N-acyltransferase***. The ratio of glycine to taurine forms in bile is approximately 3:1. The addition of glycine or taurine introduces a carboxyl group with a lower pKa (in the case of glycine) or a sulfonate group (in the case of taurine). Both of these groups are fully ionized (negatively charged) at physiological pH, resulting in the formation of conjugated bile salts. Bile salts possess enhanced amphipathic properties, making them more effective detergents compared to bile acids alone. Consequently, only the conjugated forms, the bile salts, are present in bile. Individuals with genetic deficiencies in converting cholesterol to bile acids are treated by externally supplying chenodeoxycholic acid.

Bile salts and phospholipids are responsible for keeping the cholesterol in bile in a soluble state. Due to their deficiency (particularly bile salts), cholesterol crystals precipitate in the gall bladder often resulting in **cholelithiasis-cholesterol gall stone disease**. Cholelithiasis may be due to defective absorption of bile salts from the intestine, impairment in liver function, obstruction of biliary tract etc.

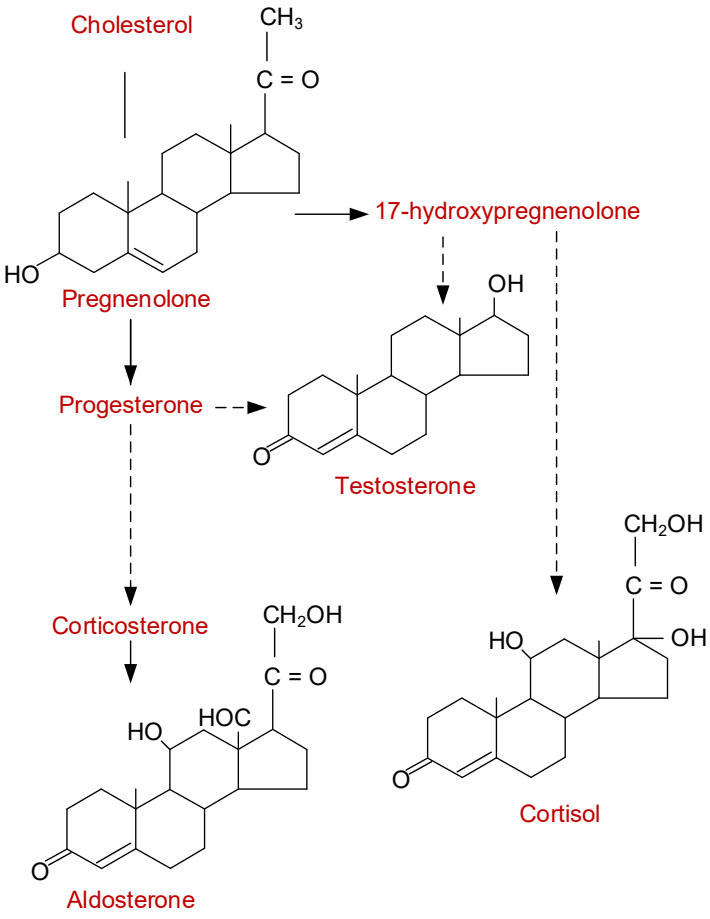


Fig. 13.5. Synthesys of steroid hormones from cholesterol

There are five classes of steroid hormones synthesized from cholesterol (as shown in fig. 13.5): **progesterone, testosterone, cortisol, aldosterone, and estradiol**. Progesterone is synthesized by the corpus luteum and placenta, while testosterone and estradiol are produced by the testes and ovaries, respectively. The adrenal cortex is responsible for the synthesis of aldosterone and cortisol. The formation of steroid hormones requires **NADPH** as an important cofactor. The majority of the biosynthetic reactions take place in the mitochondria and smooth endoplasmic reticulum. **Pregnenolone** serves as the common intermediate in the biosynthetic pathways of all five classes of steroid hormones. Enzymes involved in the synthesis of steroid hormones include ***dehydrogenases, hydroxylases, and lyases.*** A ***cholesterol desmolase***, dependent on mitochondrial cytochrome P450, converts cholesterol to pregnenolone by cleaving the side chain.

Cholesterol serves as the precursor for the synthesis of vitamin D_3, also known as **cholecalciferol** (fig. 13.6). The process begins with 7-dehydrocholesterol, an intermediate in the synthesis of cholesterol. When exposed to ultraviolet (UV) radiation from sunlight, **7-dehydrocholesterol** in the skin undergoes a photochemical reaction and is converted into cholecalciferol or vitamin D_3.

The conversion of 7-dehydrocholesterol to vitamin D3 occurs in the skin's epidermis under the influence of UV rays. Specifically, UV radiation with a wavelength of around 290-320 nanometers promotes the isomerization of 7-dehydrocholesterol, resulting in the formation of previtamin D_3. Previtamin D_3 is then thermally converted to vitamin D_3, which is the biologically active form.

After its formation, vitamin D_3 is transported to the liver and kidneys to be metabolized into its active form, **calcitriol.** This conversion occurs through a two-step process involving **hydroxylation** in the liver and subsequent hydroxylation in the kidneys.

Vitamin D_3 plays a crucial role in the regulation of calcium and phosphate metabolism, promoting their absorption from the intestines and their utilization in bone mineralization. It is essential for maintaining proper bone health and is involved in various other physiological processes, including immune function and cell growth regulation.

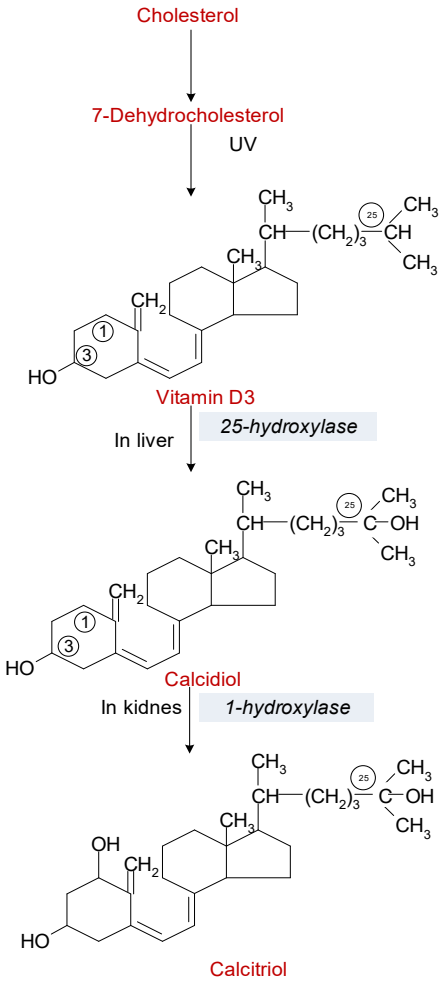


Fig. 13.6. Cholesterol serves as the precursor for the synthesis of vitamin D_3, through the process of UV-induced conversion in the skin

13.3. Lipoproteins: structure, classification, characteristics of apolipoproteins

Due to their insolubility in the watery environment of plasma, cholesterol and triacylglycerol are transported in the form of **lipoproteins**, which are spherical particles that are soluble in plasma. The various lipoprotein classes share similar structural characteristics. Each lipoprotein class consists of a neutral lipid core, which contains triacylglycerol and cholesterol esters. Surrounding this core is a layer composed of proteins, phospholipids, and cholesterol, with the polar regions oriented towards the surface of the lipoprotein and the hydrophobic portions associated with the neutral lipid core (fig. 13.7). The hydrophilic surface of the lipoprotein interacts with the water present in plasma, facilitating the solubility of the lipoprotein.

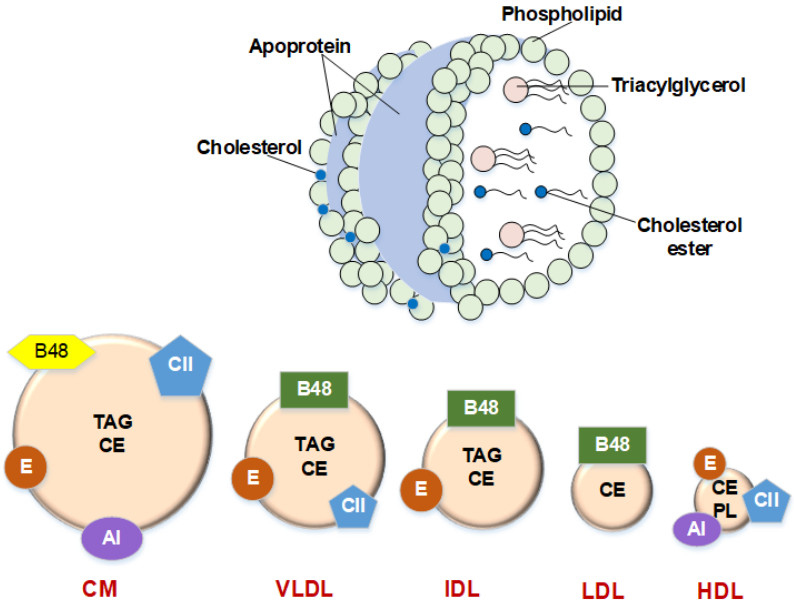


Fig. 13.7. Structure of lipoproteins and composition of different classes of lipoproteins

The lipid content and composition in human plasma vary depending on an individual's dietary habits and metabolic state. These lipids are associated with proteins in the form of lipoproteins, which are categorized into five main types based on their **density** (as shown in Table 13.1**). Chylomicrons**, which are rich in triacylglycerols, have the largest size, the highest lipid content, and the lowest percentage of protein. On the other end of the spectrum are **high-density lipoproteins (HDLs),** which are rich in cholesterol esters, have the smallest particles, the highest percentage of protein, and the lowest percentage of lipid. In between these two classes, in terms of both size and composition, are **low-density lipoproteins (LDLs)**, **intermediate-density lipoproteins (IDLs), and very-low-density lipoproteins (VLDLs)**, each with their respective lipid compositions.

Table 13.1. The five classes of lipoproteins

Class	Diameter (nm)	Source and function	Major apoliproteins
Chylomicrons (CM)	500	Intestine. Transport of *dietary* TAG	A, B48, C(I,II,III) E
Very low density lipoproteins (VLDL)	43	Liver. Transport of *endogenously* synthesised TAG	B100, C(I,II,III) , E
Low density lipoproteins (LDL)	22	Formed in circulation by partial breakdown of IDL. Delivers cholesterol to peripheral tissues	B100
High density lipoproteins (HDL)	8	Liver. Removes "used" cholesterol from tissues and takes it to liver. Donates apolipoproteins to CM and VLDL	A, C(I,II,III), D, E

The lipoproteins are associated with at least nine apolipoproteins, as well as several enzymes and a cholesterol ester transfer protein. Two major types of apolipoproteins are

present. Apo B100 and apo B48 are tightly integrated into the phospholipid monolayer. Apolipoproteins serve various functions, including:

- **Structural role:** They contribute to the overall stability and integrity of the lipoprotein structure.
- **Binding sites for receptors:** They facilitate the interaction between lipoproteins and specific receptors involved in their uptake and metabolism.
- **Activation or co-enzymes for lipid metabolism enzymes**: They act as activators or co-enzymes for enzymes involved in the metabolism and processing of lipids.

Functions of lipoproteins of different classes include:

- **Chylomicrons** have the primary function of transporting dietary triglycerides (TAG) to adipose tissues for storage as fat or to muscles where the fatty acids can be utilized for energy. They consist of the highest lipid content (99%) and the lowest protein concentration (1%) among lipoproteins. Chylomicrons are the least dense and largest in size compared to other lipoproteins.
- **Very low-density lipoproteins (VLDL)** are synthesized in the liver. They contain mainly TAG, along with a significant amount of cholesterol and cholesterol esters. The function of VLDL is to transport endogenously synthesized TAG to extrahepatic tissues for storage as fat or to muscles for energy utilization.
- **Intermediate-density lipoproteins (IDL)** are derived from VLDL. They are denser than VLDL and contain less than half the amount of triacylglycerols found in VLDL.
- **Low-density lipoproteins (LDL)** are formed from VLDL in the bloodstream. LDL has the highest content of cholesterol and cholesterol esters. Their primary role is to transport cholesterol from the liver to other tissues.
- **High-density lipoproteins (HDL)** are mostly synthesized in the liver. They have the lowest triacylglycerol content and the highest protein content among all lipoprotein particles. HDL can be further classified into three fractions (HDL 1, 2, and 3) through ultracentrifugation. HDL particles are involved in reverse cholesterol transport, transporting cholesterol from peripheral tissues back to the liver.

Thus, chylomicrons transport dietary triglycerides, VLDL transports endogenously synthesized triglycerides, LDL transports cholesterol from the liver to tissues, and HDL participates in reverse cholesterol transport, moving cholesterol from peripheral tissues to the liver.

13.4. Metabolism of lipoproteins – a general view.

Exogenous and endogenous lipid transport are two processes involved in the movement of lipids within the body.

Exogenous lipid transport is represented on the fig. 13.8 and described below:

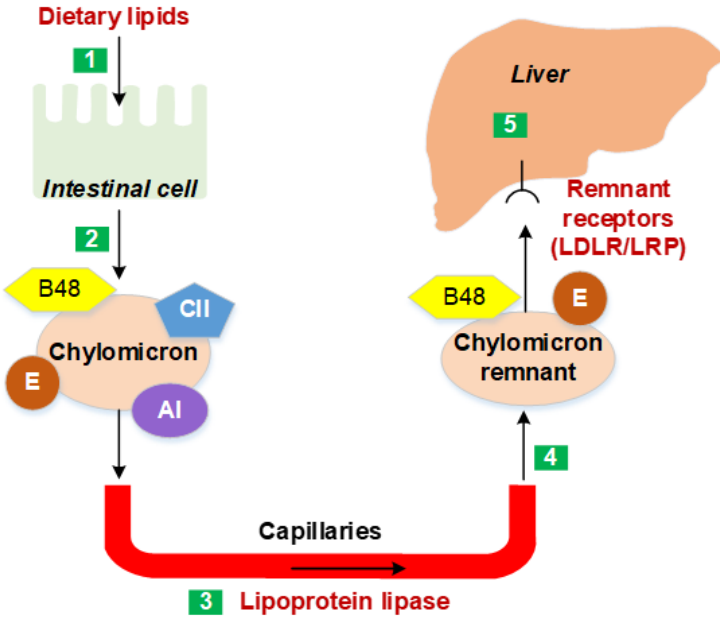


Fig. 13.8. Exogenous lipid transport. Numbers indicate

1. Chylomicrons are synthesized in the small intestine during fat absorption of dietary lipids, carrying **apo-B48** and TAGs.
2. They acquire **apo E** and **CII** from HDL.
3. Circulating through the bloodstream, chylomicrons encounter ***lipoprotein lipase (LPL)*** on the endothelial surface, facilitated by apo **CII**. *LPL* hydrolyzes TAGs, releasing free fatty acids that diffuse into local tissues.
4. As TAGs are depleted, chylomicrons undergo shrinkage, transforming into chylomicron remnants. These remnants detach from LPL and transfer apo CII back to HDL.
5. Chylomicron remnants are then captured by the liver through receptor-mediated endocytosis, utilizing liver receptors that recognize B48 and E. Within the liver, dietary cholesterol delivered by chylomicron remnants can be utilized to form VLDLs for transport to other tissues or converted into bile salts.

Endogenous lipid transport is represented on the fig. 13.8 and described below:

1. VLDL are synthesised in the liver with apo-B48. VLDL receives apo-CII and E from HDL.
2. Like chylomicrons, VLDLs travels around the circulatory system until they associate with ***LPL***. Half life of plasma VLDL is about 1-3 hours. The *LPL* hydrolyses the TAGs to liberate free fatty acids which are used by the local tissues.

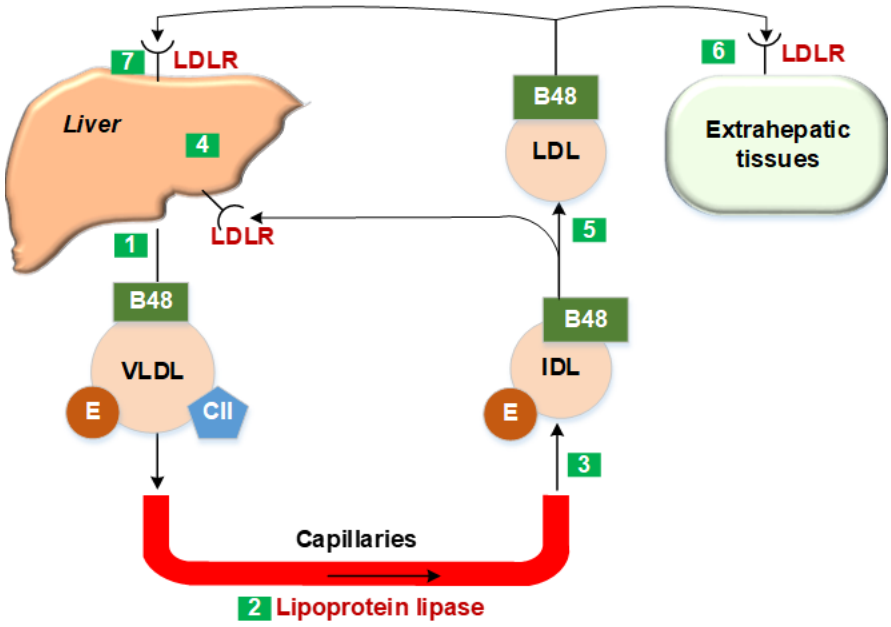


Fig. 13.9. Endogenous lipid transport. Numbers indicate stages described in the text

3. Loss of trTAGs and apo CII results in the formation of intermediate density lipoproteins (IDL) or VLDL remnants.
4. Some of the IDL are taken up by the liver. This uptake is through the apo E receptor mechanism. Liver produces LDL from IDL, for that purpose, the synthesized cholesterol combines with triglycerides, phospholipids and apoproteins to generate LDL. Apo B is the only apoprotein used for LDL formation.
5. LDL is formed from the transformation of IDL in the circulation. LDL particles are responsible for carrying cholesterol and other lipids to various tissues and organs in the body. However, the excess accumulation of LDL in the bloodstream is associated with an increased risk of cardiovascular diseases, as LDL can contribute to the formation of atherosclerotic plaques in the arteries.
6. Approximately 50% of LDL particles are removed from the circulation daily. Half of this is taken up by peripheral tissues, including cells in organs and tissues outside of the liver. In these extrahepatic tissues, LDL receptors on cell surfaces recognize and bind to LDL particles, allowing them to be internalized and metabolized by the cells. LDL are broken down by lysosomal enzymes in 5-6 minutes. Cholesterolesters and apo proteins are hydrolyzed by lysosomal hydrolases. Free cholesterol released may be reesterified. The cholesterol and other lipids carried by LDL are then utilized by the cells for various functions, including membrane synthesis and hormone production.
7. The remaining 50% of LDL particles are taken up by the liver. The liver expresses a large number of LDL receptors, allowing it to efficiently remove LDL from the bloodstream. Once taken up by the liver, LDL particles are broken down, and their cholesterol content is either stored for future use or converted into other compounds, such as bile acids or other lipoproteins.

The balance between LDL uptake by peripheral tissues and the liver helps maintain cholesterol homeostasis in the body. However, when LDL uptake is impaired or when LDL levels are excessively high, it can lead to the accumulation of LDL in the bloodstream and contribute to the development of atherosclerosis and cardiovascular diseases.

The metabolism of HDL involves several steps:

- **Synthesis**: HDL is initially synthesized by the liver (and to some extent by the intestine) as **nascent HDL**. Nascent HDL is composed of cholesterol, phospholipids, and apolipoproteins, including apo A, apo C, and apo E. It has a discoid shape.
- ***LCAT (lecithin-cholesterol acyltransferase)* activation:** Apo A-I, one of the apolipoproteins present on HDL, activates *LCAT. LCAT* catalyzes the esterification of cholesterol in HDL, converting it into cholesteryl esters. This process increases the cholesterol content of HDL.
- **Cholesterol Scavenging:** HDL, through its interaction with *LCAT*, plays a role in removing excess free cholesterol from peripheral tissues, including extrahepatic cells. Cholesteryl esters formed by *LCAT* move from the periphery to the core of the HDL particle, causing the HDL to assume a spherical shape.
- **Fate of HDL:** The exact fate of HDL in the circulation is still an area of ongoing research. However, three main fates have been identified:

a. **Cholesterol Transfer:** HDL can transfer cholesteryl esters to other lipoproteins like VLDL and LDL through the action of **cholesteryl ester transfer protein (CETP).**

Apo D component of HDL promotes the transfer of cholesteryl esters from HDL to LDL or VLDL. The cholesterol esters transferred to LDL or VLDL are then taken up by the liver.

b. Direct Uptake by the Liver: Some HDL particles are directly taken up by the liver through specific receptors, including the apo E receptor. The HDL particles are internalized by the liver cells and metabolized.

c. Conversion to HDL2: Some HDL particles are converted to HDL2, which is a larger and more cholesterol-rich form of HDL. Hepatic lipase releases free cholesterol from HDL2, which can be taken up by the liver.

The concentration of HDL in the bloodstream is inversely related to the risk of **atherosclerosis** and **coronary artery disease (CAD).** Higher levels of HDL are associated with a reduced risk of these conditions, likely due to the role of HDL in promoting the reverse cholesterol transport pathway and removing excess cholesterol from tissues.

13.5. Disorders of plasma lipoproteins.

Inherited disorders of lipoproteins can lead to **primary hyper- or hypolipoproteinemias,** which are caused by genetic defects in lipoprotein metabolism and transport. On the other hand, **secondary acquired lipoprotein disorders** can occur as a result of various diseases such as diabetes mellitus, nephrotic syndrome, atherosclerosis, and hypothyroidism. These secondary disorders often exhibit an abnormal lipoprotein pattern similar to the primary inherited conditions.

Hyperlipoproteinemias, or hyperlipidemias, refer to diseases characterized by elevated levels of one or more lipoprotein fractions. **Frederickson's classification**, based on the electrophoretic patterns of plasma lipoproteins, is widely used to understand these disorders. Different types of hyprlipoprotenemias are represented in table 13.2 and text below:

Table 13.2. Hyperlipoproteinemias types

Phenotype	Lipoproteins elevated	Serum cholesterol concentration	Serum triacylglycerol concentration	Relative frequency %
I	Chylomicrons	Normal to ↑	↑↑↑↑	<1
IIa	LDL	↑↑	Normal	10
IIb	LDL and VLDL	↑↑	↑↑	40
III	IDL	↑↑	↑↑↑	<1
IV	VLDL	Normal to ↑	↑↑	45
V	VLDL and cylomicrons	↑ to ↑↑	↑↑↑↑	6

- **Type I:** This is caused by familial ***lipoprotein lipase (LPL)*** deficiency, leading to increased levels of plasma chylomicrons and triacylglycerols.
- **Type IIa:** Also known as **hyperbetalipoproteinemia,** it results from a defect in **LDL receptors**. Secondary type IIa hyperlipoproteinemia can be seen in association with diabetes mellitus, hypothyroidism, nephrotic syndrome, and other conditions. This disorder is characterized by hypercholesterolemia.

- **Type IIb:** Both LDL and VLDL levels are elevated, along with increased plasma cholesterol and triacylglycerol levels. It is believed to be caused by overproduction of **apolipoprotein B (apo B).**
- **Type III:** Commonly known as **broad beta disease**, it is characterized by the appearance of a broad beta band corresponding to intermediate density lipoprotein (IDL) on electrophoresis.
- **Type IV:** This type is due to overproduction of endogenous triacylglycerols, resulting in elevated VLDL levels. Type IV disorder is often associated with obesity, alcoholism, diabetes mellitus, and other conditions.
- **Type V:** Both chylomicrons and VLDL are elevated in this type. It is predominantly a secondary condition associated with disorders such as obesity, diabetes, and excessive alcohol consumption.

Hypolipoproteinemias are a group of rare genetic disorders characterized by abnormally low levels of lipoproteins in the blood. Lipoproteins are responsible for transporting lipids, such as cholesterol and triglycerides, in the bloodstream. These conditions are often associated with various health complications, particularly in relation to lipid metabolism. There are several types of hypolipoproteinemia, each with its own underlying genetic defect and clinical manifestations:

- **Familial Hypobetalipoproteinemia (FHBL):** FHBL is caused by mutations in the APOB gene, which encodes for **apolipoprotein B (apoB),** a major component of low-density lipoprotein (LDL) particles. The condition is characterized by very low levels of LDL cholesterol, as well as low levels of total cholesterol and triglycerides. FHBL is usually asymptomatic, but in some cases, it can lead to malabsorption of fat-soluble vitamins and may increase the risk of fatty liver disease.
- **Abetalipoproteinemia:** Abetalipoproteinemia is a rare autosomal recessive disorder caused by mutations in the microsomal **triglyceride transfer protein (MTTP)** gene. This condition is characterized by the absence or severely reduced synthesis of apoB-containing lipoproteins, including chylomicrons, very-low-density lipoproteins (VLDL), and LDL. As a result, affected individuals have extremely low levels of cholesterol and triglycerides in their blood. Symptoms may include malabsorption of fat-soluble vitamins, neurological abnormalities, and fatty liver.
- **Hypoalphalipoproteinemia:** Hypoalphalipoproteinemia is characterized by low levels of high-density lipoprotein (HDL) cholesterol, often referred to as "good cholesterol." Multiple genetic factors can contribute to hypoalphalipoproteinemia, including mutations in genes involved in HDL metabolism, such as the ATP-binding cassette transporter A1 (ABCA1) gene. Low HDL levels are associated with an increased risk of cardiovascular disease.

13.6. Atherosclerosis.

Atherosclerosis is a complex disease characterized by the build-up of various substances, including cholesterol, in the inner walls of arteries. This build-up forms plaques that can narrow and harden the arteries over time, leading to reduced blood flow. The process of atherosclerosis is progressive and can eventually result in complete blockage of the affected arteries.

Regarding the causes of atherosclerosis, there are several factors that contribute to its development and the associated risk of **coronary heart disease (CHD).** Some of these factors include:

- **Elevated levels of low-density lipoprotein (LDL) cholesterol:** LDL cholesterol, often referred to as **"bad cholesterol",** plays a crucial role in the development of atherosclerosis. High levels of LDL cholesterol can promote the accumulation of lipids within the arterial wall, initiating the formation of plaques.
- **Decreased levels of high-density lipoprotein (HDL) cholesterol:** HDL cholesterol, known as "good cholesterol", has a protective effect against atherosclerosis. It helps remove excess cholesterol from the arterial wall and transports it back to the liver for elimination. Higher levels of HDL cholesterol are associated with a lower risk of cardiovascular diseases.
- **Gender and hormonal factors:** Women generally have higher levels of HDL cholesterol and a lower risk of heart disease compared to men, primarily due to the protective effects of estrogen. However, after menopause, when estrogen levels decrease, women's risk of atherosclerosis and CHD increases.
- **Lifestyle factors:** Unhealthy lifestyle choices, such as a diet high in saturated and trans fats, sedentary behavior, smoking, and excessive alcohol consumption, can increase the risk of atherosclerosis. Conversely, regular physical exercise, moderate alcohol intake, consumption of unsaturated fatty acids (found in vegetable and fish oils), and maintaining a healthy body weight can help increase HDL cholesterol levels and reduce the risk of CHD.
- **Genetic factors**: Certain genetic factors can contribute to an increased susceptibility to atherosclerosis. These may include genetic variations that affect lipid metabolism, inflammation, and the function of blood vessels.

The formation of plaques in the arterial wall has several stages (fig. 13.9):

- **Endothelial dysfunction:** The process begins with endothelial dysfunction, which can be triggered by various factors such as high blood pressure, smoking, and high levels of LDL cholesterol. Endothelial cells lining the inner wall of the arteries become damaged or dysfunctional, leading to an impaired ability to regulate vascular tone, inflammation, and the adherence of immune cells. In response to endothelial injury monocytes adhere to endothelial cells, move to the subendothelium (intima), and are transformed into macrophages.
- **Lipid accumulation:** In response to endothelial dysfunction, LDL cholesterol particles penetrate the damaged endothelium and accumulate within the arterial wall. LDL cholesterol undergoes oxidation, becoming **oxidized LDL (oxLDL),** which is highly inflammatory and triggers an immune response. The process of LDL oxidation involves various chemical reactions, including the interaction of LDL particles with reactive oxygen species (ROS) and free radicals.
- **Foam cell formation:** Immune cells, such as monocytes and macrophages, are attracted to the site of LDL accumulation. These immune cells engulf and attempt to digest the accumulated lipids, forming foam cells. Foam cells are lipid-laden macrophages that contribute to the early formation of fatty streaks within the arterial wall.

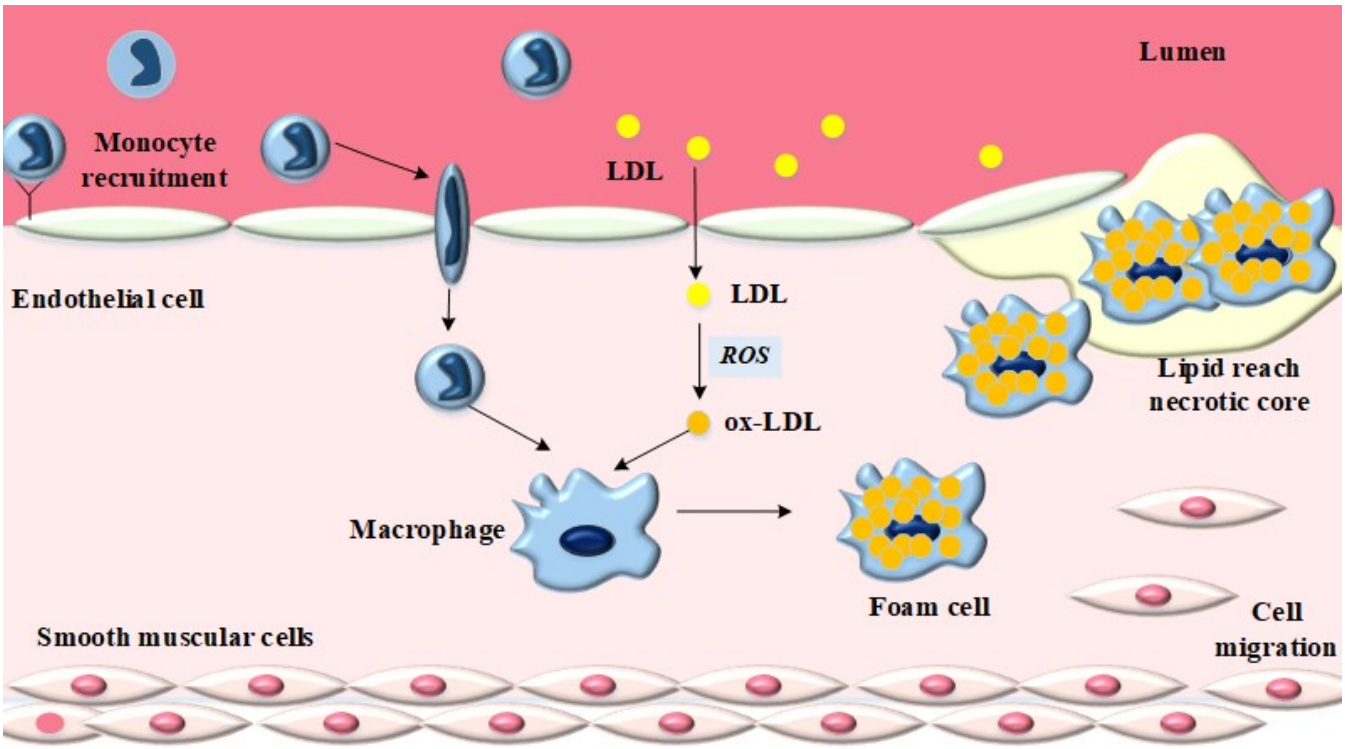


Fig. 13. The Role of lipids and lipoproteins in atherosclerosis

- **Smooth muscle cell proliferation:** The presence of oxLDL and the release of growth factors contribute to the migration and proliferation of smooth muscle cells from the arterial wall's middle layer to the inner layer. These smooth muscle cells play a critical role in the development of more advanced plaques.
- **Formation of fibrous cap:** Over time, smooth muscle cells secrete collagen and other proteins, leading to the formation of a fibrous cap covering the lipid-rich core. The fibrous cap aims to stabilize the plaque and prevent rupture.
- **Calcification:** In some cases, calcium deposits can accumulate within the fibrous cap, further contributing to plaque hardening and narrowing of the artery.
- **Plaque rupture:** Plaques with a thin and vulnerable fibrous cap are at a higher risk of rupture. Plaque rupture exposes the underlying lipid-rich core to the bloodstream, resulting in the formation of blood clots or thrombus. The blood clot can partially or completely block the artery, leading to reduced blood flow or even complete occlusion, causing tissue damage or infarction.

13.7. Fatty liver (steatosis), lipotropic factors.

For a variety of reasons, lipids - mainly as triacylglycerols can accumulate in the liver. Extensive accumulation is regarded as a pathologic condition. **Nonalcoholic fatty liver disease (NAFLD)** is the most common liver disorder worldwide. When accumulation of lipid in the liver becomes chronic, inflammatory and fibrotic changes may develop leading to **nonalcoholic steatohepatitis (NASH)**, which can progress to liver diseases including cirrhosis, hepatocarcinoma, and liver failure. Fatty livers fall into two main categories:

1. The first type is associated with raised levels of plasma free fatty acids resulting from mobilization of fat from adipose tissue or from the hydrolysis of lipoprotein triacylglycerol by lipoprotein lipase in extrahepatic tissues. The production of VLDL does not keep pace with the increasing influx and esterification of free fatty acids, allowing triacylglycerol to accumulate, which in turn causes a fatty liver. This occurs during starvation and the feeding of high-fat diets. The ability to secrete VLDL may also be impaired (e.g, in starvation). In uncontrolled diabetes mellitus, twin lamb disease, and ketosis in cattle, fatty infiltration is sufficiently severe to cause visible pallor (fatty appearance) and enlargement of the liver with possible liver dysfunction.
2. The second type of fatty liver is usually due to a metabolic block in the production of plasma lipoproteins, thus allowing triacylglycerol to accumulate.

One type of fatty liver that has been studied extensively in rats is caused by a deficiency of **choline**, which has therefore been called a **lipotropic factor**. The antibiotic puromycin, ethionine (α-amino-γ-mercaptobutyric acid), carbon tetrachloride, chloroform, phosphorus, lead, and arsenic all cause fatty liver and a marked reduction in concentration of VLDL in rat blood. Choline will not protect the organism against these agents, but appears to aid in recovery. The action of carbon tetrachloride probably involves formation of free radicals causing lipid peroxidation. Some protection against this is provided by the antioxidant action of vitamin E-supplemented diets.

Alcoholic fatty liver is the first stage in alcoholic liver disease (ALD) which is caused by alcoholism and ultimately leads to cirrhosis. The fat accumulation in the liver is caused by a combination of impaired fatty acid oxidation and increased lipogenesis, which is thought to be due to changes in the [NADH]/ [NAD^+] redox potential in the liver, and also to interference with the action of transcription factors regulating the expression of the enzymes involved in the pathways. Oxidation of ethanol by alcohol dehydrogenase leads to excess production of NADH, which competes with reducing equivalents from other substrates, including fatty acids, for the respiratory chain. This inhibits their oxidation and causes increased esterification of fatty acids to form triacylglycerol, resulting in the fatty liver. Oxidation of ethanol leads to the formation of acetaldehyde, which is oxidized by aldehyde dehydrogenase, producing acetate. The increased (NADH)/(NAD^+) ratio also causes increased (lactate)/(pyruvate), resulting in hyperlacticacidemia, which decreases excretion of uric acid, aggravating gout. Ethanol also inhibits the metabolism of some drugs, eg, barbiturates, by competing for cytochrome P450-dependent enzymes.

13.8. Obesity.

Obesity is generally defined by having a body weight that exceeds a certain percentage of fat compared to total body weight. The thresholds you mentioned, where men are considered obese if their fat weight exceeds 20% of body weight and women if it exceeds 25%, are common reference points.

Obesity typically occurs as a result of a chronic energy imbalance, where the intake of calories from food and beverages exceeds the amount of calories expended through physical activity and metabolic processes. In simpler terms, obesity is often a consequence of overeating or consuming an excess of calories.

It's important to note that the relationship between calorie consumption and fat deposition is not a fixed ratio, as individual variations in metabolism can influence weight gain. However, as a rough estimate, it is often stated that an excess consumption of approximately 7,000 calories leads to the deposition of 1 gram of fat in the body.

Lack of physical exercise or a sedentary lifestyle also contributes to obesity. When energy intake exceeds energy expenditure consistently, the excess calories are stored as fat, leading to weight gain and an increase in body fat percentage.

The biochemistry of obesity involves a complex interplay of various biological processes, hormones, and molecules that regulate appetite, energy balance, and fat metabolism. Here are some key aspects of the biochemistry of obesity:

- **Adipose tissue:** Adipose tissue, commonly known as body fat, is a critical component involved in obesity. Adipose tissue plays essential roles in maintaining lipid and glucose homeostasis. To date several types of adipose tissue have been identified, namely white, brown, and beige, that reside in various specific anatomical locations throughout the body. The cellular composition, secretome, and location of these adipose depots define their function in health and metabolic disease. In obesity, adipose tissue becomes dysfunctional, promoting a pro-inflammatory, hyperlipidemic and insulin resistant environment that contributes to type 2 diabetes mellitus (T2DM). Concurrently, similar features that result from adipose tissue dysfunction also promote cardiovascular disease by mechanisms that can be augmented by T2DM. Adipose tissue consists of adipocytes, which are specialized cells responsible for storing and releasing fat. In obesity, there is an increase in the size (hypertrophy) and number (hyperplasia) of adipocytes, leading to an overall increase in fat mass.
- **Hormones and appetite regulation:** Several hormones play a role in appetite regulation and satiety. **Leptin,** primarily produced by adipose tissue, acts as a feedback signal to the brain, indicating the body's energy stores. In obesity, there is often a state of **leptin resistance**, where the body becomes less responsive to leptin, leading to increased appetite and reduced energy expenditure. Additionally, hormones such as **ghrelin** (stimulates hunger) and **peptide YY** (suppresses appetite) also influence appetite regulation.
- **Insulin resistance:** Obesity is strongly associated with insulin resistance, where the body's cells become less responsive to the effects of insulin, a hormone involved in glucose metabolism. Insulin resistance can lead to elevated blood glucose levels, increased insulin secretion, and the accumulation of fat in tissues. This can further contribute to weight gain and metabolic complications, such as **type 2 diabetes**.
- **Inflammation and adipokines:** Adipose tissue is not merely a passive storage site for fat but also an active endocrine organ that secretes various signaling molecules called adipokines. In obesity, adipose tissue undergoes inflammation, leading to an altered profile of adipokines. Increased production of pro-inflammatory adipokines, such as **tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6),** and reduced levels of anti-inflammatory **adiponectin** can contribute to metabolic dysfunction and insulin resistance.
- **Lipid metabolism:** Obesity is characterized by an imbalance between fat storage and fat breakdown. Lipids, such as triglycerides, are taken up by adipocytes and stored as

fat droplets. Adipose tissue also releases fatty acids through lipolysis, which is the breakdown of stored fat. In obesity, there is often an increase in lipogenesis (the synthesis of new fat) and a disruption in lipolysis, leading to the accumulation of fat in adipose tissue.

- **Gut microbiota:** Emerging research suggests that the composition of the gut microbiota, the trillions of microorganisms residing in our digestive tract, may influence obesity. Imbalances in gut microbiota, known as dysbiosis, have been associated with altered energy extraction from food, inflammation, and metabolic dysregulation.

13.9. Lipid peroxidation.

Lipid peroxidation refers to the oxidative degradation of lipids, particularly polyunsaturated fatty acids (PUFAs), resulting in the formation of lipid peroxides. Lipid peroxidation includes:

1. **Formation of peroxides:** Atmospheric oxygen can react with fats, leading to the formation of peroxides. This can occur in foods during storage and processing, contributing to the rancidity of fats.
2. **In vivo lipid peroxidation:** Lipid peroxidation also occurs within the body as a result of oxidative stress. Oxidative stress can be caused by various factors, including exposure to environmental toxins, inflammation, certain diseases, and normal metabolic processes.
3. **Chain reaction:** Lipid peroxidation is a chain reaction that involves the propagation and amplification of oxidative damage. Once initiated, the process continues until terminated by antioxidants or depletion of substrate.
4. **Free radical initiation:** Free radicals, which are highly reactive molecules with unpaired electrons in their outer orbitals, initiate the lipid peroxidation chain reaction. These free radicals are often referred to as reactive oxygen species (ROS) since they contain oxygen. Examples of ROS involved in lipid peroxidation include **singlet oxygen, superoxide anion radical and hydroxyl radical.**
5. **PUFA susceptibility:** PUFAs, especially those with multiple double bonds, are particularly susceptible to lipid peroxidation. In the presence of oxygen, free radicals can extract a hydrogen atom from a methylene (-CH_2-) group of a PUFA in cell membranes, generating a PUFA free radical. This PUFA free radical can then react with oxygen to form a peroxy PUFA radical, which further reacts with other PUFAs to generate lipid peroxides and endoperoxides. Ultimately, this process leads to the production of malonic dialdehyde (MDA) and other small molecules (fig. 13.10).
6. **Assessment of lipid peroxidation:** The extent of lipid peroxidation is often assessed by measuring the levels of MDA, which is a byproduct of lipid peroxidation. MDA serves as a marker of oxidative damage and can be measured using various analytical methods.

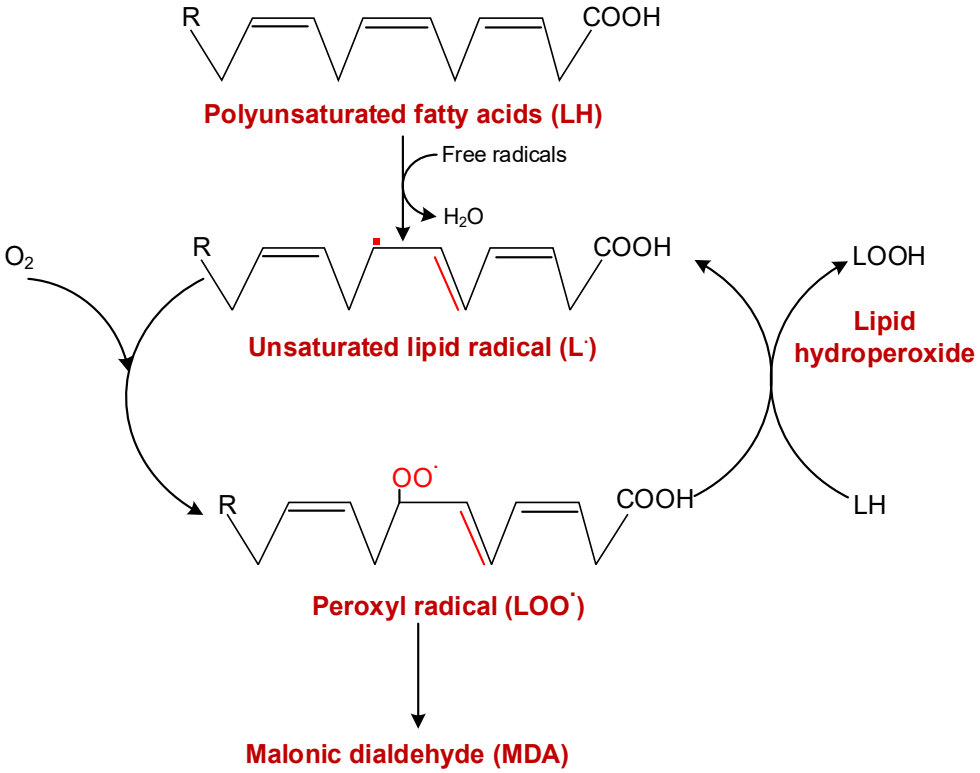


Fig. 13.10. Lipid peroxidation mechanism

REVIEW TEST:

№	MCQs	Answers and explanations
1.	A 70 year old man is ill with vascular atherosclerosis of lower extremities and coronary heart disease. Examination revealed disturbance of lipidic blood composition. The main factor of atherosclerosis pathogenesis is the excess of the following lipoproteins: A. High-density lipoproteins B. Cholesterol C. Low-density lipoproteins D. Intermediate density lipoproteins E. Chylomicrons	**The answer is C.** Atherosclerosis is a condition characterized by the buildup and accumulation of excess cholesterol in the walls of blood vessels. It is primarily caused by the presence of low-density lipoproteins (LDL) in elevated levels. The main function of LDL is to transport cholesterol from the liver to various tissues in the body. However, when there is an excessive amount of LDL in the bloodstream or if the LDL particles are modified in certain ways, they can become prone to accumulating within the inner lining of blood vessels. In atherosclerosis, LDL particles enter the vessel wall and undergo modifications, such as oxidation. These modified LDL particles trigger an inflammatory response, attracting immune cells to the site. Over time, the accumulated LDL particles, along with immune cells and other substances, form plaques within the vessel wall. These plaques can grow, leading to the narrowing and hardening of the arteries, and potentially causing complications such as heart attacks or strokes.
2.	Which of the plasma lipoproteins is best described as follows: synthesized in the intestinal mucosa, containing a high concentration of triacylglycerol and	**The answer is A.** The plasma lipoprotein that best fits the description of being synthesized in the intestinal mucosa, containing a high concentration of triacylglycerol, and responsible

	responsible for the transport of dietary lipids in the circulation? A. Chylomicrons B. High-density lipoprotein C. Intermediate density lipoprotein D. Low-density lipoprotein E. Very low density lipoprotein	for the transport of dietary lipids in the circulation is chylomicrons. Chylomicrons are large lipoprotein particles that are formed in the intestinal mucosa after the absorption of dietary fats. They are composed primarily of triacylglycerols (TGs), as well as smaller amounts of cholesterol, phospholipids, and proteins. Chylomicrons serve as the primary vehicle for transporting dietary lipids, including TGs, from the intestine to various tissues throughout the body via the bloodstream.
3.	Cholesterol content in blood serum of a 12-year-old boy is 25 mmol/l. Anamnesis states hereditary familial hypercholesterolemia caused by synthesis disruption of receptor-related proteins for: A. Chylomicrons B. High-density lipoproteins C. Low-density lipoproteins D. Very low-density lipoproteins E. Middle-density lipoproteins	**The answer is C.** If a patient has a cholesterol level in their blood that falls outside the normal range of 3.6-7.8 mmol/L, it indicates hypercholesterolemia, which is an elevated level of cholesterol in the blood. Low-density lipoproteins (LDL) are responsible for transporting cholesterol from the liver to tissues throughout the body. Therefore, a deficiency or dysfunction of LDL can contribute to hypercholesterolemia. In some cases of hypercholesterolemia, the issue lies with the receptor-related proteins that are involved in the binding and uptake of LDL by cells. These receptors, such as the LDL receptor, play a crucial role in regulating the cholesterol levels within cells by facilitating the uptake of LDL particles. However, when these receptor-related proteins are deficient or dysfunctional, the binding and uptake of LDL particles by cells are impaired. As a result, LDL particles remain in the bloodstream for longer periods, leading to an accumulation of cholesterol and increased cholesterol levels in the blood. This condition, often referred to as familial hypercholesterolemia, is typically an inherited disorder caused by mutations in the genes that encode the LDL receptors or other proteins involved in LDL metabolism. Without functioning receptors, the cells are unable to efficiently take up cholesterol from LDL particles, leading to the accumulation of LDL cholesterol in the blood.
4.	During examination of a teenager with xanthomatosis the family history of hypercholesterolemia is revealed. What transportable lipids are increased in concentration in case of such a disease? A. Low-density lipoproteins B. Chylomicrons C. Very low-density lipoproteins D. High-density lipoproteins E. Intermediate-density lipoproteins	**The answer is A.** Xanthomatosis refers to the deposition of cholesterol and/or triglycerides in the form of focal deposits called "xanthomas." These deposits can occur in various tissues, including the skin, tendons, and internal organs. Hypercholesterolemia, which is characterized by elevated levels of cholesterol in the blood, is often associated with an excess of low-density lipoproteins (LDL). When there is an imbalance in cholesterol metabolism or an underlying genetic disorder, the levels of LDL can become elevated. This increase in LDL cholesterol can lead to the development of xanthomas.

		Xanthomas are a result of the accumulation of cholesterol and/or triglycerides within cells in specific tissues. The excess LDL cholesterol in the bloodstream can infiltrate and accumulate in these tissues, causing the formation of xanthomas. The deposition of these lipids triggers an inflammatory response, leading to the appearance of yellowish nodules or plaques.
5.	Increased HDL levels decrease the risk of atherosclerosis. What is the mechanism of HDL anti-atherogenic action? A. They promote absorption of cholesterol in the intestine B. They supply tissues with cholesterol C. They are involved in the breakdown of cholesterol D. They activate the conversion of cholesterol to bile acids E. They remove cholesterol from tissues	**The answer is E.** High-density lipoproteins (HDL) play a crucial role in cholesterol metabolism and are often referred to as "good cholesterol" due to their potential anti-atherogenic effects. HDL particles are responsible for transporting cholesterol from peripheral tissues back to the liver, a process known as reverse cholesterol transport. HDL particles have several mechanisms that contribute to their anti-atherogenic properties. Firstly, they can directly remove excess cholesterol from cells in peripheral tissues, including the walls of blood vessels. This helps to prevent the buildup of cholesterol in arterial walls, reducing the risk of atherosclerosis.
6.	A patient underwent a course of treatment for atherosclerosis. Laboratory tests revealed an increase in the antiatherogenic lipoprotein fraction in the blood plasma. The treatment efficacy is confirmed by the increase in: A. LDL B. VLDL C. IDL D. HDL E. Chylomicrons	**The answer is D.** High-density lipoproteins (HDL) are a class of lipoproteins that play a crucial role in reverse cholesterol transport, a process by which excess cholesterol is removed from peripheral tissues, including the arterial wall, and transported back to the liver for excretion or recycling. HDL particles are often referred to as "good cholesterol" due to their association with a reduced risk of cardiovascular disease. The relationship between HDL and atherosclerosis is complex, but HDL particles are generally considered to have anti-atherogenic properties.
7.	67-year-old male patient consumes eggs, pork fat, butter, milk and meat. Blood test results: cholesterol - 12,3 mmol/L, total lipids - 8,2 g/L, increased low-density lipoprotein fraction (LDL). What type of hyperlipoproteinemia is observed in the patient? A. Hyperlipoproteinemia type IIa B. Hyperlipoproteinemia type I C. Hyperlipoproteinemia type IIb D. Hyperlipoproteinemia type IV E. Cholesterol, hyperlipoproteinemia	**The answer is A.** The normal value of cholesterol level in blood is 3,6-7,8 mmol/L, of total lipids – 4-8 g/L, so cholesterol level is increased, total lipids level – almost normal. The criteria of hyperlipoproteinemia type IIa are increased cholesterol and LDL levels, that's why it is a correct answer.
8.	Which of the plasma lipoproteins is best described as follows: synthesized in the liver, containing a high concentration of triacylglycerol and mainly cleared from the circulation by adipose tissue and muscle? A. Chylomicrons	**The answer is E.** The plasma lipoprotein that best fits the description of being synthesized in the liver, containing a high concentration of triacylglycerol, and mainly cleared from the circulation by adipose tissue and muscle is very low-density lipoprotein (VLDL).

	B. High-density lipoprotein C. Intermediate density lipoprotein D. Low-density lipoprotein E. Very low density lipoprotein	VLDL is a lipoprotein particle that is primarily synthesized in the liver Once synthesized, VLDL particles are released into the bloodstream. In circulation, VLDL interacts with lipoprotein lipase (LPL), an enzyme found on the surface of blood vessels. LPL hydrolyzes the TGs present in VLDL, releasing free fatty acids and glycerol. These free fatty acids are taken up by adipose tissue for storage as triacylglycerols or by muscle tissue for energy utilization.
9.	An experimantal animal that was kept on protein-free diet developed fatty liver infiltration, in particular as a result of deficiency of methylating agents. This is caused by disturbed generation of the following metabolite: A. Acetoacetate B. DOPA C. Cholesterol D. Choline E. Linoleic acid	**The answer is D.** Choline is a unique alcohol that is found in the structure of phospholipids. It is synthesized from ethanolamine through a process called methylation. Choline plays a crucial role in various physiological processes, including lipid metabolism and the formation of cell membranes. A deficiency of choline can lead to fatty liver infiltration, a condition characterized by the accumulation of fat in liver cells. Choline is necessary for the proper transport and metabolism of fats in the liver. When choline levels are insufficient, the liver is unable to effectively process and utilize fats, resulting in their accumulation and the development of fatty liver infiltration.
10	A drycleaner's worker has been found to have hepatic steatosis. This pathology can be caused by the disruption of synthesis of the following substance: A. Tristearin B. Phosphatidylcholine C. Urea D. Phosphatidic acid E. Cholic acid	**The answer is B.** Hepatic steatosis, also known as fatty liver disease, can occur in conditions where there is a deficiency in methylating factors or phospholipid components. In these situations, fatty acids are predominantly utilized for the synthesis of triacylglycerols, which leads to the accumulation of fat in the liver. A deficiency in phospholipids, specifically phosphatidylcholine, which is a phospholipid containing choline, can contribute to the development of hepatic steatosis. Phosphatidylcholine plays a vital role in maintaining normal liver function and lipid metabolism. It is involved in the synthesis and secretion of very low-density lipoproteins (VLDL), which transport triacylglycerols from the liver to other tissues. Additionally, phosphatidylcholine is required for the formation of lipoprotein particles and the transport of fats across cellular membranes. When there is a deficiency of phosphatidylcholine or impaired choline metabolism, the liver's ability to process and export fats is compromised. This can result in the accumulation of triacylglycerols within the liver cells, leading to hepatic steatosis.

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14. STUDIES ON METABOLISM OF AMINO ACIDS (DEAMINATION, TRANSAMINATION, DECARBOXYLATION)

OBJECTIVES after studying this chapter, you should be able to:

- *Describe the biochemical mechaninsm of ploteins digestion, appreciate the role of proteolytic enzyme, specify their substrate specificity*
- *Explain the general pathways of amino acids metabolism, to make an acquaintance with methods of identification of amino acid metabolites, to interpret obtained results.*
- *Characteraze diagnostical role of aspartate and alanine transaminases*
- *Explain biogenic amine generation by decarboxyation of amino acids, describe breackdown of biogenic amines*

14.1. Proteins digestion

14.1.1. Biological value of protein and nitrogen ballance

Proteins constitute 18-20% of the total body weight of a human, which means that a 70 kg individual contains 12-15 kg of protein. Unlike carbohydrates and fats, these organic compounds are not stored as reserves, but they play incredibly important roles such as plasticity, catalytic activity, regulation, protection, and energy production (1 gram yields 4.1 kilocalories), among others.

The continuous degradation and synthesis of cellular proteins, known as protein turnover, is a vital process that occurs in all living organisms. In humans, approximately 1% to 2% of the total body protein is turned over each day, with muscle protein being the primary contributor.

Proteins are large macromolecules composed of L-α-amino acids, which serve as the building blocks. There are **20 amino acids** commonly found in proteins, and they can be classified into two categories: **essential amino acids and non-essential amino acids.**

Essential amino acids are those that the body cannot synthesize and must be obtained from the diet. There are nine essential amino acids: **histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine**. On the other hand, non-essential amino acids can be synthesized by the body using various metabolic pathways.

Proteins have diverse functions within the body. They can act as enzymes, facilitating biochemical reactions, or as transporters, helping to move substances across cell membranes. Proteins also play roles as hormones, serving as chemical messengers, and they provide structural support in tissues. In fact, proteins make up approximately 80% of the dry mass of our bodies.

While the body can function without fats and carbohydrates for an extended period of time, the exclusion of proteins from the diet, even for a short period, leads to significant disruptions and, in some cases, irreversible pathological changes.

The biological value of proteins is determined by their **amino acid composition** and **digestibility**, which refers to the efficiency of breakdown in the digestive tract and

absorption rate. Thus, the higher the biological value of a protein, the closer its amino acid composition is to the average amino acid composition of the human body.

Proteins that contain all **essential amino acids** are considered to have high biological value. Examples of such proteins include egg proteins (13%), meat proteins (18-22%), fish proteins (17-22%), milk proteins (3-3.5%), and cheese proteins (20-36%). Plant proteins are considered incomplete as they lack a complete set of essential amino acids. Additionally, the grains of cereals are protected by a cellulose husk and cannot be fully digested. Among plant-based products, only soy, peas, and beans contain a significant amount of protein (26-35%), while potatoes, cabbage, carrots, and apples contain only 0.3-2.0% protein. However, by combining different plant proteins, it is possible to provide the body with a balanced mixture of amino acids. For example, legume proteins are rich in lysine but low in tryptophan, while corn proteins are low in lysine but contain sufficient amounts of tryptophan.

The protein requirements for individuals should take into account the nature of their physical activity, age, climatic conditions, physiological condition, and the presence of any illnesses. An average adult with moderate physical activity should consume **70-100 grams of protein per day**. In the case of heavy physical work, this amount should increase to 130-150 grams per day.

Nitrogen balance refers to the difference between the amount of nitrogen consumed by the body through food and the amount of nitrogen excreted from the body. Approximately 400 grams of protein are renewed in the human body daily, as they break down into amino acids and are synthesized again. Some of the amino acids generated from protein breakdown are used for biosynthesis of new proteins, with the replenishment of energy in the form of ATP, or they are converted into non-peptide substances (amines, heme, thyroxine, choline, taurine, etc.). This means that to replenish the lost amino acids during metabolism, the body must continuously receive proteins from food.

Since over 95% of the total nitrogen in the body is accounted for by proteins and free amino acids, the overall protein metabolism can be assessed through nitrogen balance (fig. 14.1). There are three types of nitrogen balance:

- **Nitrogen equilibrium:** The amount of nitrogen consumed equals the amount of nitrogen excreted. This state is characteristic of healthy adults. Nitrogen balance can be maintained even with significant fluctuations in dietary

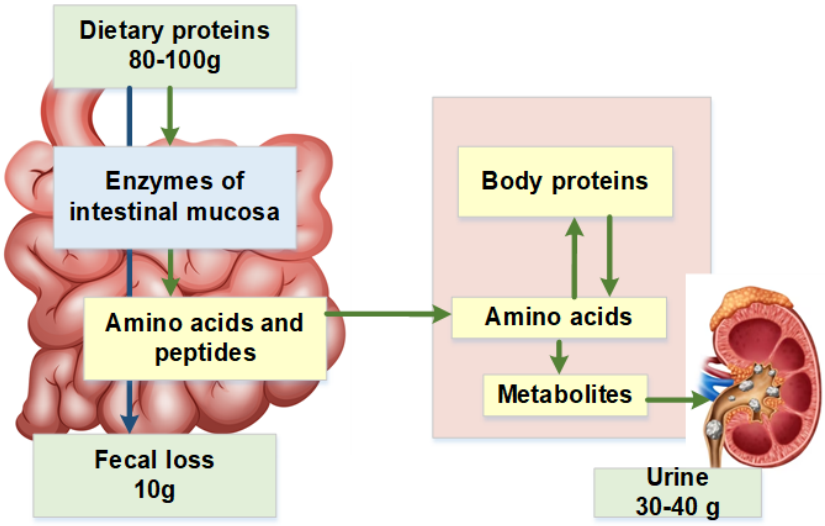


Fig. 14.1. Nitrogen ballance

protein content. The minimum amount of protein required to achieve nitrogen equilibrium is 30-50 grams, while the optimal amount is 80-100 grams.

- **Positive nitrogen balance:** The amount of nitrogen consumed exceeds the amount of nitrogen excreted from the body. This state is typical for growing organisms, pregnant women, after exhausting illnesses, and with the administration of anabolic steroids.
- **Negative nitrogen balance:** The amount of nitrogen consumed is less than the amount of nitrogen excreted from the body. This state is characteristic of older individuals, observed during debilitating illnesses, and in cases of complete or protein starvation. Exclusion of protein from the diet leads to a daily loss of 4 grams of nitrogen, equivalent to 25 grams of protein. Under conditions of complete fasting, approximately 20 grams of nitrogen are excreted daily, which means that around 125 grams of protein are degraded. Protein starvation results in a decrease in physical and mental activity, a reduction in plasma protein levels, leading to disturbances in colloid-osmotic balance, edema, decreased muscle protein synthesis, anemia, weakened cardiac activity, and impaired immunity. Prolonged and complete starvation inevitably leads to death.

Kwashiorkor is a form of severe malnutrition that primarily affects young children. It is characterized by a severe protein deficiency in the diet, which leads to a range of symptoms and complications. Kwashiorkor occurs when there is a severe lack of dietary protein, often accompanied by a calorie deficit. Protein is essential for the growth, maintenance, and repair of tissues in the body. When the body does not receive enough protein, it can lead to impaired growth and development. **The symptoms of kwashiorkor** include a swollen belly due to fluid accumulation (edema), thinning hair, skin lesions, muscle wasting, fatigue, irritability, and a weakened immune system. Edema is a hallmark sign and is often seen in the legs, feet, and face. Kwashiorkor is most commonly observed in areas where there is a lack of access to sufficient food, particularly protein-rich sources. It can occur in situations such as famine, poverty, or during times of natural disasters or conflict when food supplies are scarce. **Kwashiorkor and marasmus** are two distinct forms of severe malnutrition, but they can coexist or transition from one to another. Marasmus is characterized by overall caloric deficiency, resulting in significant weight loss, muscle wasting, and overall body emaciation. Kwashiorkor, on the other hand, typically involves a more severe protein deficiency and presents with edema. Kwashiorkor can lead to a range of complications, including increased susceptibility to infections, delayed wound healing, impaired cognitive development, and increased mortality rates, especially if left untreated. **Treatment** for kwashiorkor involves a gradual and controlled refeeding process with a nutritionally balanced diet. This includes providing foods rich in high-quality protein, essential fatty acids, vitamins, and minerals.

14.1.2. Protein digestion in the stomach

There are no specific proteolytic enzymes present in the mouth. While saliva does contain some enzymes like amylase for carbohydrate digestion, it does not contain proteases for protein digestion.

After swallowing, the bolus of food enters the stomach, where the protein digestion begins with the influence of **gastric juice**. Gastric juice is the product of the activity of

several types of cells. **Parietal cells** of the stomach wall synthesize **hydrochloric acid and intrinsic factor** (a glycoprotein that binds to vitamin B_{12}, preventing its destruction and aiding in absorption). **Chief cells** secrete **pepsinogen**, and **additional cells** secrete **mucine-containing mucus.**

In a day, a person produces 1.5-2 liters of gastric juice, with a density of 1.002 - 1.007 g/cm^3 and a pH of 1.5-2.5. The solid residue consists of organic substances (enzymes, mucins) and inorganic substances (chlorides, sulfates, phosphates, sodium bicarbonate, potassium, calcium, magnesium). The main inorganic component of gastric juice is hydrochloric acid (HCl), which can exist in a free state or bound to proteins.

Proteins entering the stomach stimulate the release of **histamine** and a group of protein hormones called gastrins, which, in turn, trigger the secretion of HCl and pepsinogen. The synthesis of HCl occurs in parietal cells and is a cyclic adenosine monophosphate (cAMP)-dependent process that is enhanced by glycogenolytic and glycolytic activity, accompanied by pyruvate production. Oxidative decarboxylation of pyruvate to acetyl-CoA and CO_2 generates NADH in the cytoplasm, which is used for the generation of H^+.

Protons are derived from H_2CO_3, which is formed in parietal cells of the stomach from CO_2 and H_2O under the influence of the enzyme ***carbonic anhydrase***. Dissociation of H_2CO_3 leads to the formation of bicarbonate, which is exchanged for Cl- in the plasma with the help of specific proteins, and protons, which enter the lumen of the stomach through active transport involving ***H^+,K^+-ATPase*** (fig. 14.2). As a result, the concentration of H^+ ions in the stomach lumen increases by 10^6 times. Cl^- ions enter the stomach lumen through chloride channels.

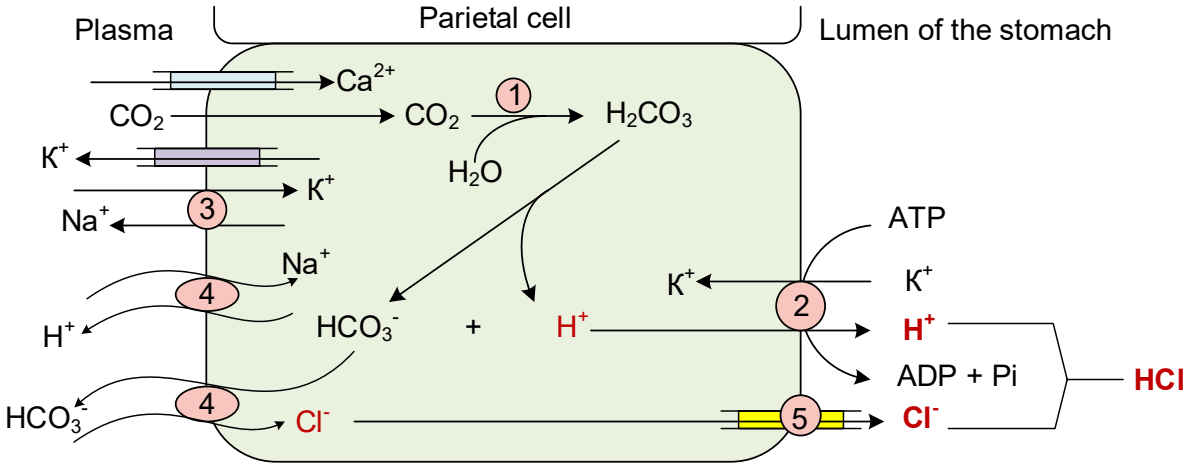


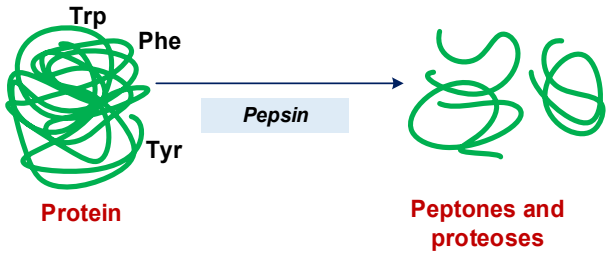
Fig. 14.2/ Scheme of hydrochloric acid secretion in the stomach: 1 - carbonic anhydrase, 2 - H^+, K^+-ATPase, 3 - Na+, K+-ATPase; 4 - exchange transport, 5 - ion channels.

The *Na^+-K^+-ATPase*, located on the basolateral surface of the parietal cell, transports K^+ ions from the blood in exchange for Na^+, while the *H^+-K^+-ATPase* also called as *proton pump* transports potassium from the primary secretion in exchange for protons.

Thus, the acid-forming function of parietal cells is carried out through **phosphorylation-dephosphorylation**, the presence of the **mitochondrial oxidative chain**, which **transports H+ ions from the matrix**, and the activity of the **H^+-K^+-ATPase** of the secretory membrane, which pumps protons out of the cell using ATP energy.

The formed hydrochloric acid creates an optimal pH for the action of proteolytic enzymes in gastric juice and causes **denaturation of proteins**, promoting their breakdown by enzymes. It participates in milk curdling, stimulates the secretion of the enzyme enterokinase by enterocytes of the duodenum, provides antibacterial properties to gastric juice, regulates the activity of the pancreas and gastric glands by inhibiting gastrin formation and stimulating the synthesis of secretin and other gastroenterointestinal hormones. It influences the motor activity of the stomach and promotes the conversion of **pepsinogen into pepsin**.

Pepsin is formed in the chief cells of the gastric mucosa in an inactive form as **pepsinogen**, a protein consisting of a single polypeptide chain with a molecular weight of 40,000 Da. Under the influence of HCl, it is converted into active pepsin (molecular weight 40,000 Da) by **limited proteolysis**, where a peptide (42 amino acid residues) is cleaved from the N-terminal end of pepsinogen, which is mainly composed of positively charged amino acids. The resulting pepsin mainly contains negatively charged amino acids and activates the rest of the pepsinogen molecules (autocatalysis). The optimum pH for the action of this enzyme is 1.5-2.5.



Fig/ 14.3. Peptones and proteoses are intermediate products formed during the process of protein digestion. They are produced through the partial hydrolysis of proteins by pepsin

The active site of pepsin contains **carboxyl groups** of two **aspartic acid** residues. In the native or denatured protein molecule, it rapidly hydrolyzes peptide bonds formed by the carboxyl groups of **aromatic amino acids (phenylalanine, tryptophan, tyrosine) (fig. 14.3)** and slowly breaks the bonds between **leucine and dicarboxylic amino acids**. Pepsin is classified as an endopeptidase because it cleaves peptide bonds within the interior of a peptide chain, as opposed to exopeptidases, which cleave bonds at the ends of the peptide chain. The action of pepsin leads to the production of shorter peptide fragments peptones and proteoses.

The gastric mucosa also contains **pepsin C (gastricsin)**, an enzyme that hydrolyzes peptide bonds of **dicarboxylic amino acids**, with an optimum pH of 3.0-3.5. The ratio of pepsin to gastricsin in gastric juice ranges from 1:2 to 1:5.

Pepsin B (gelatinase) dilutes gelatin and hydrolyzes proteins in connective tissue, but the optimum pH for its action is within the range of 5-6.

Pepsin D (chymosin, rennin) is an enzyme with a molecular weight of 40,000 Da, synthesized in the stomach of infants and young animals, specifically acting on the main milk protein, casein, by converting it into paracasein through the hydrolysis of glycopeptide. In the presence of **calcium ions**, paracasein transforms into a insoluble curd, which is further hydrolyzed by pepsin (fig 14.4). This process is particularly important for young organisms, where milk is the primary food source. In adults, the curdling of milk in the stomach occurs with the involvement of pepsin and HCl. Since all of the mentioned enzymes have a similar primary structure, it is evident that they are derived from a common precursor gene.

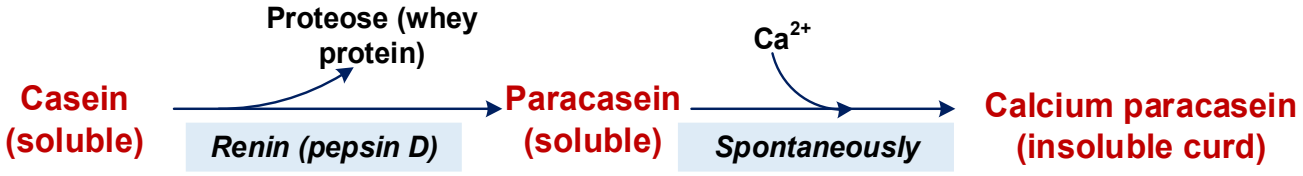


Fig. 14.4. Action of renin on milc protein casein

The secretion of pepsins is stimulated by **acetylcholine, gastrin**, and to a lesser extent, **histamine.**

14.1.3. Protein digestion in the small intestine.

The mixture of polypeptides that remain undigested in the stomach enters the small intestine, where the next stage of enzymatic protein digestion takes place in a weakly alkaline environment (pH 7-8) under the action of pancreatic and intestinal juices.

The pancreatic juice contains various enzymes (proteolytic, glycolytic, and lipolytic), including **proteolytic enzymes** such as ***trypsinogen, chymotrypsinogen, procarboxypeptidases A and B, and proelastase***. Through **limited proteolysis**, they are converted into their active forms: ***trypsin, chymotrypsin, carboxypeptidases, and elastase***, respectively.

The secretion of proteolytic enzymes in an **inactive form** has significant biological significance because the pancreatic juice also contains other enzymes (*lipase, amylase*, etc.) with a protein nature. The presence of active trypsin along with these enzymes would lead to their destruction in the pancreas. Additionally, the cells of the pancreas contain a trypsin inhibitor, which forms a strong complex with trypsin in case of premature enzyme activation.

The arrival of acidic chyme from the stomach into the duodenum stimulates the secretion of ***enterokinase (enteropeptidase)*** by the mucous membrane, which, by cleaving a hexapeptide from the N-terminus of trypsinogen, converts it into active trypsin. The action of *enterokinase* is manifested in the initial stage of *trypsinogen* activation, after which an autocatalytic mechanism is engaged, where the formed *trypsin* activates the conversion of trypsinogen into its active form (fig. 14.5)

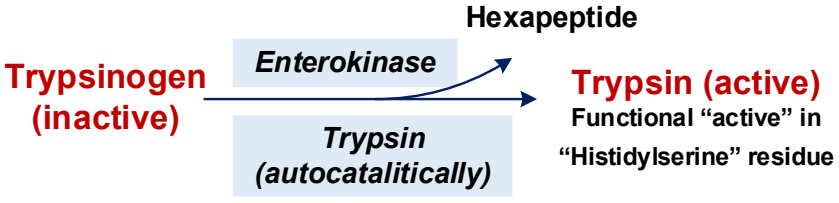


Fig. 14.5. Trypsinogen activation in small intestine

In the process of activation, the "active site" of the enzyme trypsin, which is ***histidylserine*** residue is unmasked. Trypsin belongs to the group of *serine proteases*. ***Trypsin acts*** **in an alkaline medium** pH 8 to 9 (optimum pH-7.9). *Trypsin* hydrolyzes peptide bonds formed by the carboxyl groups of **arginine and lysine,** resulting in the formation of polypeptides and a small amount of free amino acids.

Trypsin, in turn, activates other proteolytic enzymes. Under the influence of trypsin, inactive *chymotrypsinogen* is converted into several active enzymes: initially, active π (pi)-*chymotrypsin* is formed, then *delta-chymotrypsin*, and finally, the stable form of the active enzyme, *alpha-chymotrypsin*, which consists of three polypeptide chains linked by disulfide bridges.

MEDICAL IMPORTANCE

Trypsin inhibitors have several applications in medicine. Trypsin inhibitors can help reduce inflammation by inhibiting the activity of trypsin, a proteolytic enzyme involved in the inflammatory response. By blocking trypsin, these inhibitors can help alleviate symptoms associated with inflammation, such as pain and swelling. Trypsin inhibitors are sometimes used in topical formulations for wound healing. They help promote healing by preventing excessive degradation of proteins in the wound bed, allowing for proper tissue regeneration. Pancreatic enzyme replacement therapy: In some cases of pancreatic insufficiency, where the pancreas does not produce enough digestive enzymes, including trypsin, pancreatic enzyme replacement therapy (PERT) may be necessary. Trypsin inhibitors can be used in PERT formulations to protect the trypsin-sensitive enzymes, such as amylase and lipase, from premature activation and degradation in the acidic environment of the stomach. This ensures effective digestion and absorption of nutrients.: Certain types of cancers, such as pancreatic cancer, can overexpress trypsin, which contributes to tumor growth and [illegible] therapeutic agents to inhibit trypsin activ

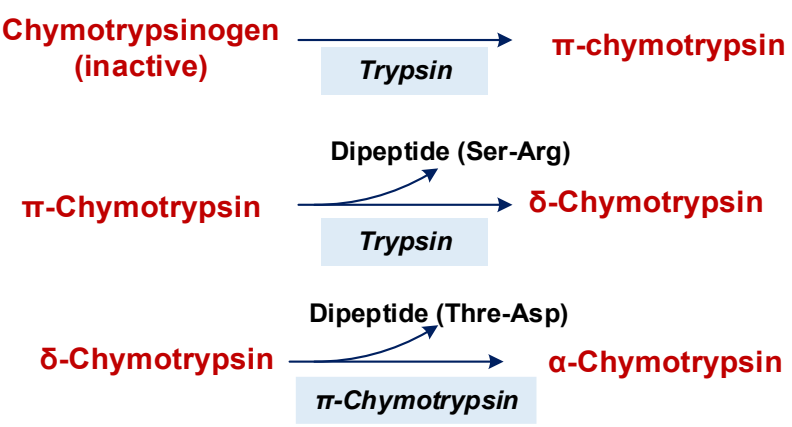


Fig. 14.6. Activation of chymotrypsinogen

Chymotrypsin hydrolyzes peptide bonds formed by the carboxyl groups of **tyrosine, phenylalanine, tryptophan**. To some extent, it can also attack peptide bonds connected with **methionine, histidine, leucine and asparagine** residues. α-*Chymotrypsin* converts the proteoses, peptones and peptides to smaller peptides and amino acids.

Carboxy peptidases belong to group of exopeptidasesm they exist as two types:

- ***Carboxy Peptidase A:*** It is a metalloenzyme, contains zinc, has three subunits, III, II and I. Trypsin converts subunit II to a proteinase and subunit III is degraded. Then both trypsin and proteinase formed from subunit II, changes subunit I of *procarboxy peptidase A* to ***active carboxy peptidase A.*** It is an ***exopeptidase*** and cannot act on peptide bonds well inside the protein molecule. The enzyme hydrolyses the terminal peptide bond connected to an end amino acids bearing free α-COOH group, particularly if the end amino acids **aromatic or aliphatic amino acid**. It liberates the end amino acids as "free" form, so that the peptide becomes shorter by one amino acid.

- ***Carboxy Peptidase B:*** It hydrolyses terminal peptide bonds, which are connected with "basic" amino acids bearing free-COOH.

Elastase: A serine protease, secreted as inactive zymogen ***proelastase,*** activated by trypsin to active ***elastase.*** *Elastase* breaks peptide bonds between residues of **neutral amino acids** and is most active against elastin.

Overall of activation and substrate specificity of the proteolytic enzymes of the pancreas is on the table 14.1.

Table 14.1. Activation and substrate specificity of pancreatic proteases

Enzymes	Activation	Substrates (specificity)	Products
Trypsin	*enteropeptidase* trypsinogen → trypsin+6 AA *trypsin* trypsinogen→trypsin+6AA (autocatalytically)	Positively charged residues: Agr,Lys	Short-chain peptides
Chymotrypsin	*trypsin* Chymotrypsinogen→chymotrypsin	Phe, Tyr, Trp	Short-chain peptides
Elastase	*trypsin* Proelastase→elastase	Small neutral residues: Ala, Gly, Ser, Val	Short-chain peptides
Carboxypeptidase A and B	*trypsin* Procarboxypeptidase→carboxypeptidase	Amino acid from C-terminus	Amino acids

The process of protein breakdown (hydrolysis) is completed with the participation of enzymes in the small intestine, whereby low-molecular-weight peptides are further hydrolyzed into amino acids (table 14.2). The small intestine also produces enzymes called peptidases, which are located on the brush border of the intestinal lining. These peptidases, such as aminopeptidases and dipeptidases, further break down small peptides into individual amino acids.

Table 14.2. Intestinal proteolytic enzymes

Aminopeptidases	
Alanine aminopeptidase Leucine aminopeptidase	Alanine and leucine are cleaved from the N-terminus of the peptide, respectively
Dipeptidases	
Glycylglycine dipeptidase	It splits a dipeptide into 2 molecules of glycine.
Prolyl dipeptidase	It catalyzes the hydrolysis of the peptide bond, in which the COOH group of proline is involved.
Proline dipeptidase	It hydrolyzes dipeptides in which the nitrogen of proline is linked by an acid-amide bond.

MEDICAL IMPORTANCE

Gluten is a mixture of proteins found in wheat and other grains such as barley and rye. It gives dough its elasticity and helps it rise. Gluten consists of two main proteins, glutenin and gliadin.

Celiac disease, also known as celiac sprue or gluten-sensitive enteropathy, is an autoimmune disorder characterized by an abnormal immune response to gluten.

In individuals with celiac disease, consuming gluten triggers an immune response that damages the lining of the small intestine. This damage hampers the absorption of nutrients from food, leading to various symptoms and potential complications.

Common symptoms of celiac disease include digestive issues such as abdominal pain, diarrhea, bloating, and weight loss. However, the condition can also manifest with non-digestive symptoms like fatigue, anemia, joint pain, skin rashes, and neurological problems.

The only effective treatment for celiac disease is strict adherence to a gluten-free diet. This involves eliminating all sources of gluten from the diet, including wheat, barley, rye, and foods made from these grains.

14.1.4. Biochemical mechanisms of amino acid absorption.

Amino acids released during protein hydrolysis are rapidly absorbed in the microvilli of the small intestine. This process can occur through **co-transport** or via the specific **γ-glutamyl cycle**.

Co-transport of amino acids across the apical membrane of enterocytes is a **secondary active** process that requires energy expenditure and is facilitated by a series of specialized carrier proteins. There are more than six different Na^+-dependent transporters located on the apical brush border, each with overlapping specificity for different types of amino acids:

- A transporter for **neutral amino acids with short carbon chains**, such as alanine, serine, and threonine.
- Another transporter specifically for amino acids with **long or branched carbon chains**, such as valine, leucine, and isoleucine.
- A third transporter that selectively transports **proline and hydroxyproline.**
- A fourth transporter that carries **acidic amino acids**.
- A fifth transporter specifically for basic **amino acids**, including lysine and arginine, as well as ornithine (an intermediate in the urea cycle) and cystine.
- An additional transporter for **imino acids**, such as proline and hydroxyproline.

These specialized transporters play a crucial role in the efficient absorption of different types of amino acids in the small intestine.

These transporters function through Na^+-dependent mechanisms of symport. Sodium ions enter the cell along with amino acids and are then pumped out of the cytoplasm through the action of Na^+-K^+-ATPase.

The γ-glutamyl cycle involves six enzymes, one of which, ***γ-glutamyltransferase***, is located in the membrane, while the others are present in the cell (figure 14.7).

Amino acid transfer is carried out by ***γ-glutamyltransferase***, with glutathione serving as a cofactor:

Amino acid + Glutathione → γ-Glutamyl-amino acid + Cysteinylglycine

The other five enzymes of the cycle are responsible for the cleavage of amino acids from γ-glutamine in the cytoplasm and the resynthesis of glutathione. The transport of one amino acid into the cell requires the expenditure of three ATP molecules.

Dipeptides and undigested proteins are absorbed through pinocytosis and hydrolyzed by lysosomal proteases within the cell.

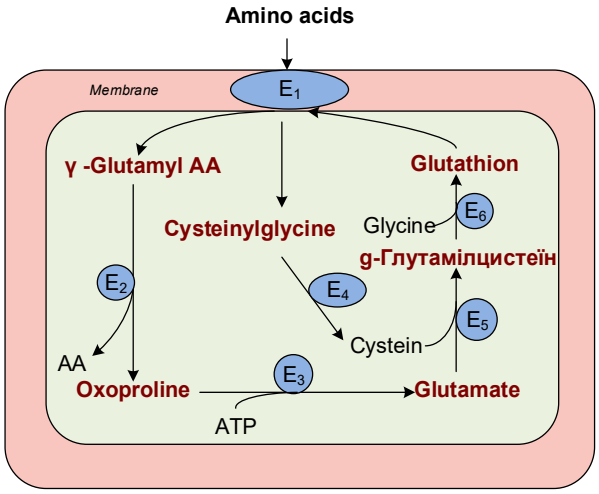


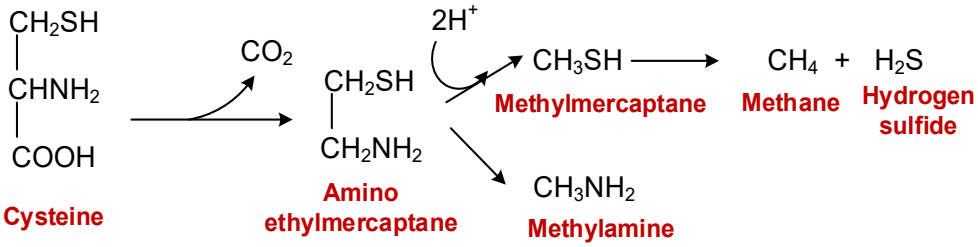
Fig. 14.7. Scheme of the γ-glutamyl cycle: E1 - γ-glutamyltransferase, E2 - γ-glutamylcyclotransferase, E3 - oxoprolinase, E4 - peptidase, E5 - γ-glutamylcysteine synthetase, E6 - glutathione synthetase

From enterocytes, amino acids enter the bloodstream through **facilitated diffusion** and reach the blood capillaries. From there, they pass through the hepatic portal system to the liver and enter the general circulation.

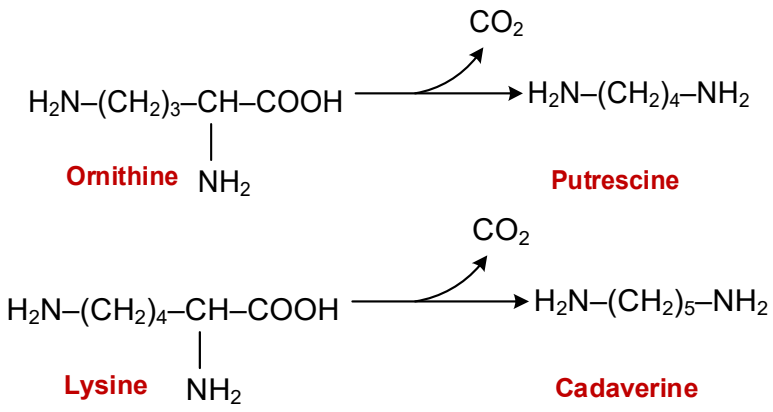
14.1.5. Intestinal putrefaction of amino acids

Some of the amino acids that were not absorbed are utilized by the microflora of the large intestine as a source of nutrition. Approximately 1% of the amino acids enter the large intestine. As is known, the intestinal microorganisms need a supply of define amino acids in the food for their development. The intestinal microflora possesses a distinct set of enzymes that differ from those found in animal tissues. These enzymes are capable of catalyzing various metabolic conversions of amino acids. As a result, conditions favorable for the formation of both toxic and non-toxic products arise in the intestine. The metabolic reactions of amino acids triggered by the activity of intestinal microorganisms are collectively referred to as **intestinal putrefaction** of amino acids.

During the breakdown of sulfur-containing amino acids such as **cysteine, cystine, and methionine**, such compounds as hydrogen sulfide, methan and methyl mercaptan are produced in the intestine.

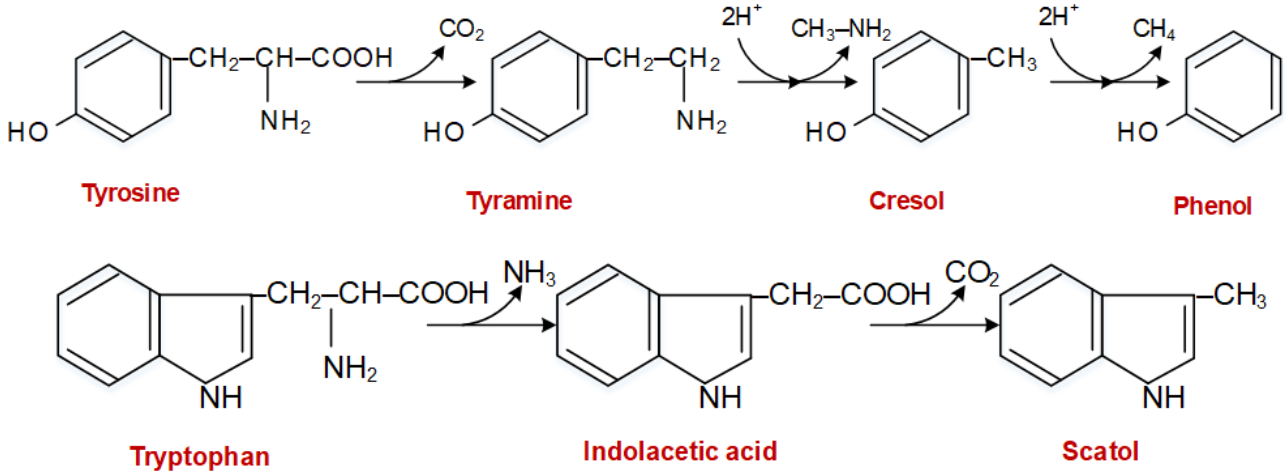


Diamino acids, specifically ornithine and lysine, undergo decarboxylation to form amines such as putrescine and cadaverine.



Similarly, aromatic amino acids like phenylalanine, tyrosine, and tryptophan can be decarboxylated by bacterial enzymes in the intestine, resulting in the formation of the corresponding amines. For example, phenylalanine can be decarboxylated to phenethylamine, tyrosine to tyramine, and tryptophan to tryptamine. These amines can have various physiological effects and can also serve as precursors for the synthesis of other important compounds in the body.

Additionally, the microbial enzymes in the intestines can gradually break down the side chains of cyclic amino acids like tyrosine and tryptophan. This breakdown process leads to the formation of toxic metabolites such as cresol and phenol from tyrosine, and skatole and indole from tryptophan.



These toxic metabolites can have detrimental effects on the body if they accumulate in high concentrations. For instance, high levels of phenol and cresol can be harmful to the liver and kidneys, while skatole and indole are associated with the characteristic odor of feces but can also have negative effects on the digestive system if present in excessive amounts.

14.2. Pathways of formation and maintenance of free amino acid pool in human body. General pathways of free amino acid turnover.

After absorption from the intestine, amino acids are carried to the liver through the portal blood circulation. In the liver, some of the amino acids are taken up by liver cells

(hepatocytes) and used for various metabolic processes, including **protein synthesis**, **energy production**, and synthesis of other **important molecules**.

The remaining amino acids that are not taken up by the liver enter the systemic circulation and are distributed throughout the body fluids, including the interstitial fluid and plasma. These amino acids are available for uptake by tissue cells in various organs and tissues.

Simultaneously, tissue proteins, both structural proteins (such as muscle proteins) and functional proteins (including plasma proteins), undergo continuous breakdown or turnover. This process is known as **protein degradation or protein catabolism**. During protein degradation, the proteins are broken down into their constituent amino acids, which are then released into the circulation.

The body's **amino acid pool** refers to the total amount of amino acids present in the body at any given time (fig. 14.8). It represents the combined pool of amino acids obtained from dietary protein intake, protein breakdown, and de novo synthesis within the body. The amino acid pool is essential for protein synthesis, the production of enzymes, hormones, and other vital molecules, as well as for energy metabolism.

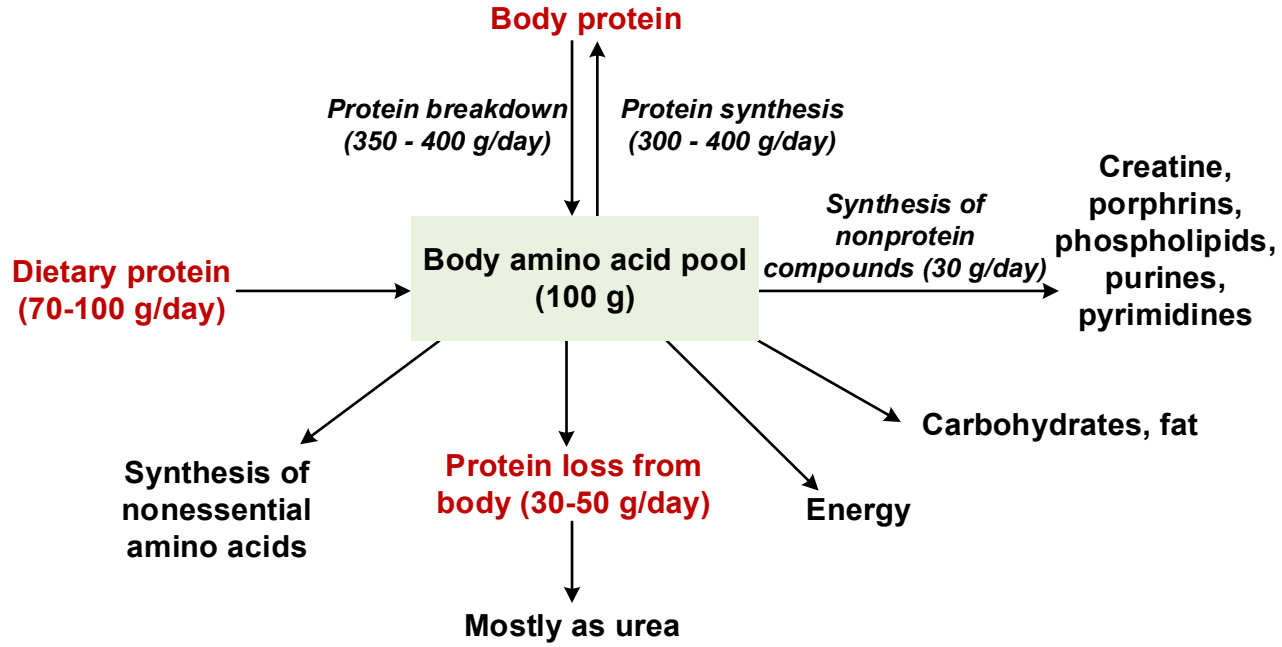


Fig. 14.8. The body's amino acid pool

The amino acid pool is not stored in a specific compartment but is distributed throughout various tissues and body fluids. The amino acid pool is dynamic and undergoes constant turnover. Amino acids are continuously utilized for protein synthesis, energy production, and other metabolic processes, and the pool is replenished through dietary intake and recycling of amino acids from protein breakdown.

The size and composition of the amino acid pool can vary depending on factors such as dietary protein intake, physiological conditions, exercise, and disease states. Maintaining a balanced and adequate amino acid pool is crucial for overall health and proper functioning of the body.

Unlike carbohydrates (stored as glycogen) and lipids (stored as triglycerides), there is no specific storage form for amino acids in the body. Excess amino acids that are not

immediately needed for protein synthesis can undergo catabolism to provide energy or be converted into glucose or fat.

Amino acids can undergo **oxidative degradation** in several metabolic circumstances:

- **Normal protein turnover:** As part of the regular synthesis and degradation of cellular proteins, some amino acids are released during protein breakdown. If these amino acids are not required for the synthesis of new proteins, they can undergo oxidative degradation to provide energy or participate in other metabolic pathways.
- **Excessive protein intake:** When the diet is rich in protein and the ingested amino acids exceed the body's requirements for protein synthesis, the surplus amino acids are catabolized. Since amino acids cannot be stored as such, the body utilizes them for energy production, converting them into glucose or fatty acids.
- **Starvation or uncontrolled diabetes mellitus**: In situations where carbohydrates are either unavailable (such as during prolonged fasting) or not properly utilized (as in uncontrolled diabetes), cellular proteins can be utilized as a source of fuel. Under these circumstances, the body breaks down its own proteins to obtain amino acids for energy production.

Under all these metabolic conditions, amino acids lose their amino groups to form α-keto acids, the "carbon skeletons" of amino acids. The α-keto acids undergo oxidation to CO_2 and H_2O or, often more importantly, provide three- and four-carbon units that can be converted by gluconeogenesis into glucose, the fuel for brain, skeletal muscle, and other tissues

14.3. General metabolic pathways of nitrogen free skeleton of amino acids in human body. Glucogenic and ketogenic amino acids.

The carbon skeletons of amino acids can undergo various fates after the removal of the amino group. The different pathways and their associated products include:

- **Oxidation via TCA cycle:** Some amino acids are converted to intermediates of the tricarboxylic acid (TCA) cycle, such as α-ketoglutarate, oxaloacetate, succinyl CoA, fumarate, and acetyl CoA (fig. 14.9). These intermediates can enter the TCA cycle and undergo oxidation to produce energy in the form of ATP (about 10-15% of body needs).
- **Synthesis of glucose:** Amino acids that form pyruvate or intermediates of the TCA cycle in the liver are considered **glucogenic**. This means that their carbon skeletons can be used for the synthesis of glucose through the gluconeogenesis. Pyruvate, oxaloacetate, and other TCA cycle intermediates can be converted to glucose, which is important for maintaining blood sugar levels and providing energy to certain tissues, such as the brain.
- **Formation of ketone bodies:** Amino acids that form acetyl CoA or acetoacetate are considered **ketogenic**. Acetyl CoA can be further metabolized to produce ketone bodies, such as acetoacetate and β-hydroxybutyrate. Ketone bodies can serve as alternative fuel sources for tissues, particularly during periods of prolonged fasting or low carbohydrate intake.

- **Synthesis of non-essential amino acids:** Some amino acids can be converted to their corresponding non-essential amino acids through various enzymatic reactions. Non-essential amino acids are those that can be synthesized by the body and are not strictly required in the diet.

Certain amino acids, such as isoleucine, tryptophan, phenylalanine, and tyrosine, can be both glucogenic and ketogenic. This means that their carbon skeletons can contribute to both glucose synthesis and the production of ketone bodies.

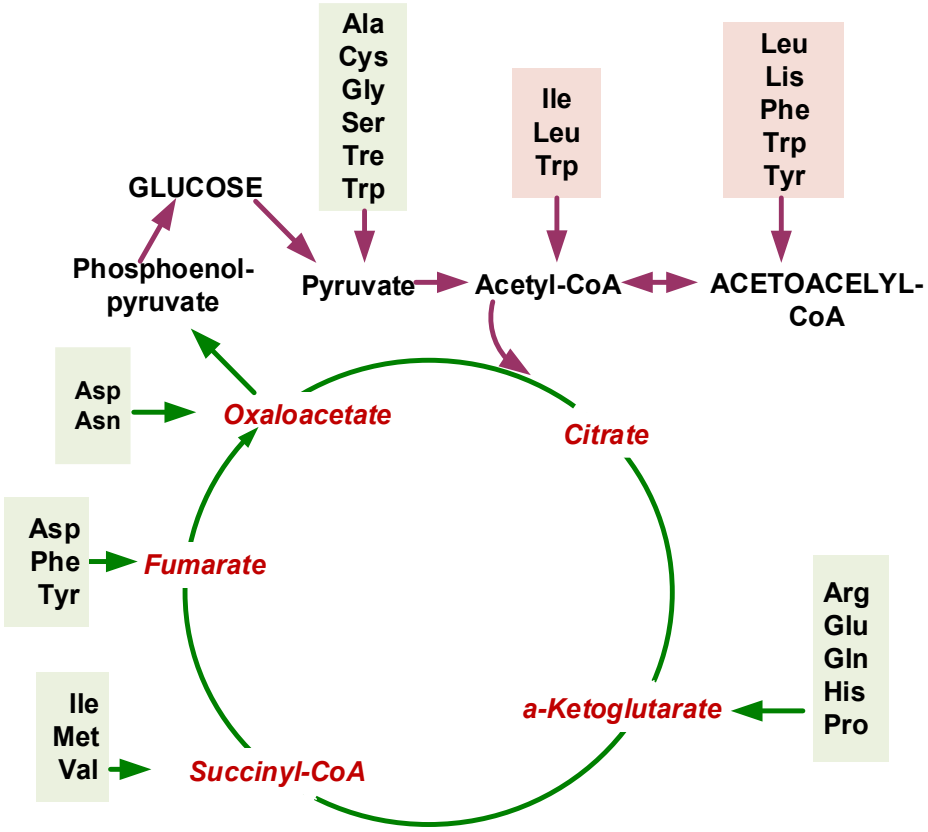


Fig. 14.9. Oxidation of amino acids in TCA cycle

Amino acids that form α-ketoglutarate:

- **Glutamate** can be deaminated by **glutamate dehydrogenase** or transaminated to form α-ketoglutarate.
- **Glutamine** is converted by **glutaminase** to glutamate with the release of its amide nitrogen as NH_4^+.
- **Proline** is oxidized so that its ring opens, forming glutamate semialdehyde, which is reduced to glutamate.
- **Arginine** is cleaved by **arginase** in the liver to form urea and ornithine. Ornithine is transaminated to glutamate semialdehyde, which is oxidized to glutamate.
- Histidine is converted to formiminoglutamate (FIGLU). The formimino group is transferred to FH4, and the remaining five carbons form glutamate.

Amino acids that form succinyl CoA. Four amino acids (**threonine, methionine, valine, and isoleucine**) are converted to propionyl CoA. Propionyl CoA is carboxylated

in a biotin-requiring reaction to formmethylmalonyl CoA. Methylmalonyl CoA is rearranged to form succinyl CoA in a reaction that requires vitamin B_{12}.

- **Threonine** is converted by a dehydratase to NH_4^+ and α-ketobutyrate, which is oxidatively decarboxylated to propionyl CoA. In a different set of reactions, threonine is converted to glycine and acetyl CoA.
- **Methionine** provides methyl groups for the synthesis of various compounds; its sulfur is incorporated into cysteine; and the remaining carbons form succinyl CoA. **Methionine and ATP** form S-adenosylmethionine (SAM), which donates a methyl group and forms homocysteine.
- **Valine and isoleucine**, two of the three branched-chain amino acids, form succinyl CoA.

Amino acids that form fumarate. Three amino acids (**phenylalanine, tyrosine, and aspartate**) are converted to fumarate:

- **Phenylalanine** is converted to **tyrosine**, which is converted to homogentisic acid. The aromatic ring is opened and cleaved, forming fumarate and acetoacetate.
- **Aspartate** is converted to fumarate through reactions of the urea cycle and the purine nucleotide cycle.

Amino acids that form oxaloacetate. Aspartate is transaminated to form oxaloacetate. **Asparagine** loses its amide nitrogen as NH_4^+, forming aspartate in a reaction catalyzed by asparaginase.

Amino acids that are converted to acetyl CoA or acetoacetate. Four amino acids (**lysine, threonine, isoleucine, and tryptophan**) can form acetyl CoA.

There are 20 amino acids from proteins that have to be degraded when they are in excess. Therefore, there are 20 pathways for this degradation. However thre are 3 most important reactions in the amino acid catabolism: the **transamination**, **deamination,** and **decarboxylation** reffered to as general pathways of amino acids metabolism (fig.14.10). Depending on the current state of metabolism, the existing amino acids are either regrouped or broken down entirely.

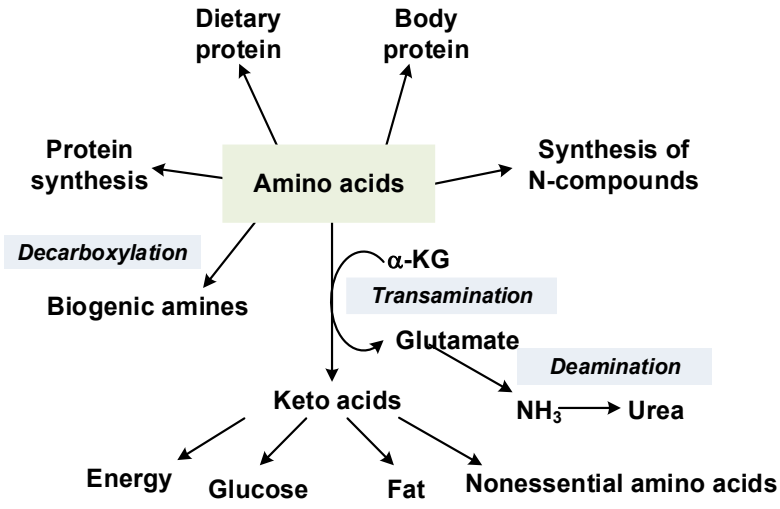


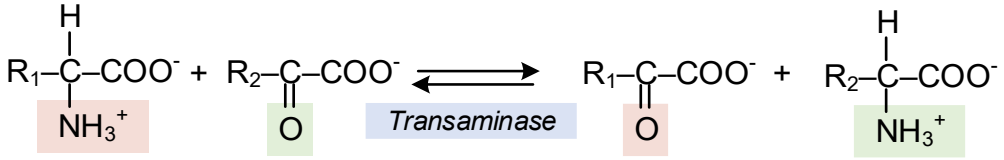
Fig. 14.10. Ttransamination, deamination, and decarboxylation as general pathways of amino acids metabolism

14.4. Transamination of amino acids.

Transamination is an important process in amino acid metabolism. Transamination reactions involve the transfer of an **amino group (-NH_2)** from an **amino acid** to a **keto acid**, resulting in the formation of a **new amino acid** and a **new keto acid**. This reaction

is catalyzed by a family of enzymes called ***transaminases or aminotransferases***. Transamination takes place principally **in liver, kidney, heart and brain**. But the enzymes are present in almost all mammalian tissues and transamination can be carried out in all tissues to some extent.

The general reaction can be represented as follows:



Amino acid 1 α-Keto acid 2 α-Keto acid 1 Amino acid 2

The amino group from amino acid 1 is transferred to the α-keto acid 2, forming a new amino acid 2 and a new α-keto acid 1. The transamination reactions are freely **reversible**, allowing the interconversion of amino acids and keto acids.

Transamination reactions play a crucial role in amino acid metabolism by facilitating the synthesis of non-essential amino acids. Non-essential amino acids can be synthesized in the body through various pathways, and transamination is one of the key steps in their biosynthesis.

It's important to note that not all amino acids participate in transamination reactions. **Lysine, threonine, proline, and hydroxyproline** are exceptions and do not undergo typical transamination. Instead, they follow alternative pathways for their catabolism and biosynthesis.

The *transaminases* require the coenzyme **pyridoxal phosphate (PLP)** as their prosthetic group, which is derived from **vitamin B_6**.

During transamination, PLP serves as a carrier of the amino group. The reaction begins with the formation of a **Schiff base** between the keto acid and the amino group of the amino acid, which is bound to the PLP cofactor. This Schiff base can undergo rearrangement, leading to the formation of an α-keto acid and an enzyme-bound **pyridoxamine phosphate (PMP).** The PMP can then be converted back to PLP through subsequent reactions, completing the catalytic cycle of the transaminase enzyme (fig. 14 11).

It's important to note that transamination reactions do not directly liberate free ammonia (NH_3). Instead, they transfer the amino group as a whole to a keto acid, resulting in the synthesis of a new amino acid and a new keto acid. The transaminase enzymes, with the help of PLP, play a crucial role in the interconversion of amino acids and the synthesis of non-essential amino acids.

Aminotransferases catalyze bimolecular **Ping-Pong reactions**. In this mechanism, the first substrate (an amino acid) binds to the active site of the enzyme and transfers its amino group to the coenzyme pyridoxal phosphate (PLP), forming a Schiff base I (aldimine) and Schiff bese II (ketamine) intermediates. The amino acid then departs from the active site as an α-keto acid. Once the first substrate is released, the second substrate (a keto acid) can bind to the enzyme, and the amino group is transferred from PLP to the keto acid, forming a new amino acid and regenerating the PLP cofactor. This Ping-Pong mechanism allows for sequential catalysis and prevents substrate interference.

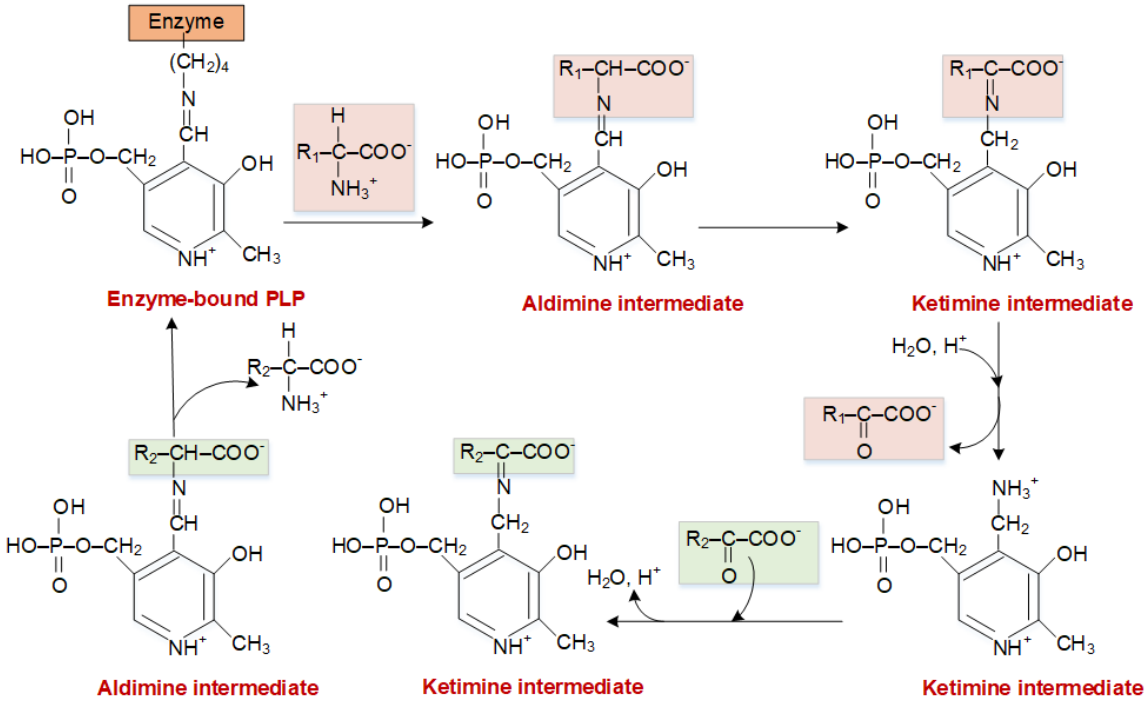
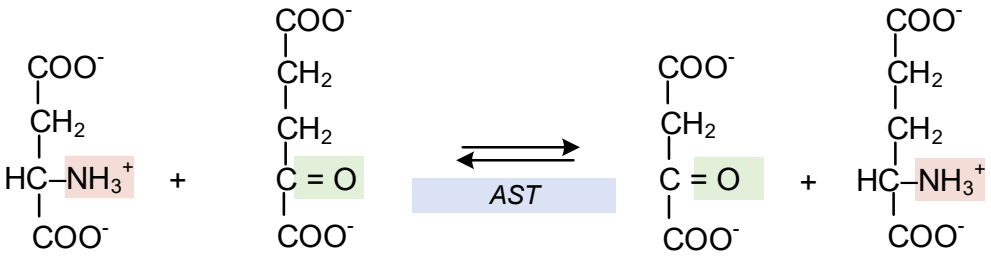


Fig. 14.11. The mechanism of transamination involves the transfer of an amino group from an amino acid to a keto acid, facilitated by the enzyme and the coenzyme pyridoxal phosphate (PLP).

α-Ketoglutarate and glutamate are often involved in transamination reactions, serving as one of the amino acid/α-ketoacid pairs.

The measurement of the **alanine aminotransferase and aspartate aminotransferase** levels in blood serum is important in some medical diagnoses.

Aspartate aminotransferase (AST) also known as glutamic-oxaloacetic transaminase (SGOT) is one of the most active enzymes in the cell. It exists in mitochondrial (predominant) and cytosolic (minor) variants, and the detailed iso-enzyme pattern is tissue-specific.



Aspartate transaminase (AST), is an enzyme found in various tissues, with the highest concentrations in the liver and heart. AST is released into the bloodstream when there is damage or injury to these tissues.

The clinical significance of AST lies in its use as a biomarker to assess liver and heart health. Elevated levels of AST in the blood can indicate liver damage, as seen in conditions such as:

- **Liver diseases:** AST levels can be increased in liver diseases like hepatitis (inflammation of the liver), cirrhosis (scarring of the liver), and liver cancer.

- **Alcohol-induced liver injury:** Chronic alcohol consumption can cause liver damage, leading to increased AST levels.
- **Drug-induced liver injury:** Certain medications, toxins, or drug overdoses can cause liver damage and result in elevated AST levels.
- **Non-alcoholic fatty liver disease (NAFLD):** NAFLD is a condition characterized by the accumulation of fat in the liver. AST levels may be elevated in more severe cases of NAFLD.

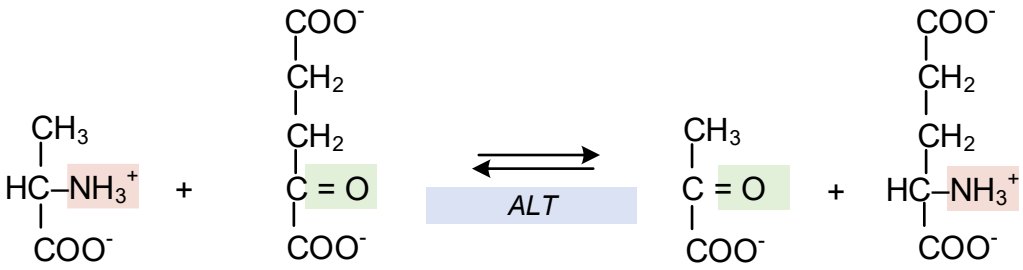
In addition to liver diseases, elevated AST levels can also be associated with heart-related conditions, such as:

- **Myocardial infarction (heart attack):** When there is damage to the heart muscle, AST is released into the bloodstream, leading to increased levels.
- **Heart failure:** In cases of congestive heart failure, where the heart is unable to pump blood effectively, AST levels can be elevated.

It's important to note that while AST is a useful marker for liver and heart health, it is not specific to these organs. Other factors, such as muscle injury or certain medications, can also cause AST levels to rise.

AST levels are typically measured along with other liver function tests, such as alanine transaminase (ALT), and in the context of a comprehensive clinical evaluation to determine the underlying cause of elevated levels and guide further diagnostic investigations or treatment decisions.

Alanine aminotransferase (ALT) also known as serum glutamic-pyruvic transaminase (SGPT), exists in mitochondrial (minor) and cytosolic (predominant) variants. The detailed iso-enzyme pattern is tissue-specific. lanine is the principal amino acid released from muscle tissue during starvation. It is an important substrate for hepatic gluconeogenesis, and alanine transamination is required for the proper maintenance of fasting blood glucose concentrations.



ALT is released into the bloodstream when there is liver damage or injury. ALT is considered a specific marker for liver function and is commonly measured alongside other liver function tests.

The clinical significance of ALT lies in its use as a biomarker to assess liver health and diagnose various liver conditions. Elevated levels of ALT in the blood can indicate liver damage or disease, including:

- **Hepatitis:** ALT levels are significantly increased in viral hepatitis (such as hepatitis A, B, or C), indicating inflammation and damage to liver cells.
- **Non-alcoholic fatty liver disease (NAFLD):** ALT levels can be elevated in cases of NAFLD, a condition characterized by the accumulation of fat in the liver.

- **Alcoholic liver disease:** Chronic alcohol consumption can lead to liver damage, causing elevated ALT levels.
- **Drug-induced liver injury:** Certain medications, toxins, or drug overdoses can cause liver damage and result in increased ALT levels.
- **Liver cirrhosis:** ALT levels may be elevated in advanced stages of liver cirrhosis, which is characterized by scarring and impaired liver function.
- **Liver cancer:** ALT levels can be increased in liver cancer, although additional tests are necessary for a definitive diagnosis.

It's important to note that ALT levels can also be influenced by factors other than liver disease. For example, strenuous physical activity, muscle injury, and certain medications can cause transient increases in ALT levels.

Serum aminotransferase assay are commonly used in the detection and evaluation of liver diseases. ALT is predominantly found in liver cells, while AST is found in various tissues including the liver, heart, and skeletal muscles.

Elevations in serum ALT levels are indicative of liver cell injury or damage, as ALT is released into the bloodstream when hepatocytes (liver cells) are damaged or undergo apoptosis (cell death). The increase in ALT activity suggests liver injury, and higher levels of ALT usually correlate with the extent of liver damage. ALT is considered a specific marker for acute hepatitis (viral or toxic), jaundice, and liver cirrhosis.

On the other hand, AST levels can also be elevated in liver diseases, but they are less specific to the liver compared to ALT. AST is found in various tissues, including the liver, and elevated AST levels indicate hepatocellular damage as well as damage to other tissues such as the heart and skeletal muscles. Therefore, AST levels alone do not provide a specific indication of liver disease. However, when AST levels are significantly higher than ALT levels, it can suggest the necrotic phase of liver cirrhosis or liver cancer.

It's important to interpret ALT and AST levels in the context of the patient's medical history, clinical symptoms, and other liver function tests to determine the underlying cause of liver dysfunction accurately. Additional diagnostic tests may be needed to confirm the specific liver disease and guide appropriate treatment decisions.

The de Ritis coefficient, also known as the AST/ALT ratio or the R value, is a calculated ratio between the levels of AST and ALT in the blood. It is derived by dividing the AST value by the ALT value. The de Ritis coefficient can provide useful information in the evaluation of liver diseases. In certain liver conditions, such as alcoholic liver disease and viral hepatitis, the de Ritis coefficient may be helpful in determining the underlying cause or assessing the severity of the disease.

The de Ritis coefficient interpretation can vary:

- **Ratio less than 1:** A de Ritis coefficient of less than 1 indicates that the ALT level is higher than the AST level. This pattern is often seen in cases of liver damage or disease primarily affecting the liver cells, such as viral hepatitis or non-alcoholic fatty liver disease (NAFLD).
- **Ratio approximately equal to 1:** A de Ritis coefficient close to 1 suggests that both AST and ALT levels are elevated to a similar extent. This can be seen in conditions such as alcoholic liver disease, where both hepatocytes and liver tissue are affected.

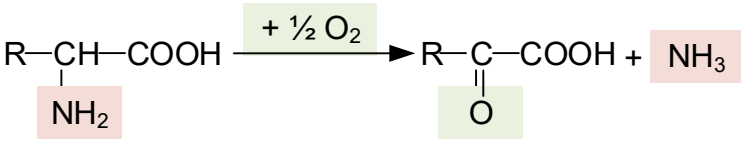
- **Ratio greater than 1:** A de Ritis coefficient greater than 1 indicates that the AST level is higher than the ALT level. This pattern may be observed in cases of severe liver damage, such as advanced cirrhosis or liver cancer, where there is significant disruption of liver tissue.

It's important to note that the de Ritis coefficient is not specific to any particular liver disease and should always be considered alongside other clinical and laboratory findings. Individual variations and other factors can also influence the ratio. Therefore, it is typically used as an additional tool rather than a definitive diagnostic marker.

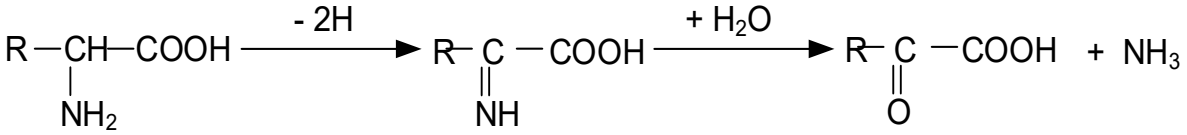
14.4. Deamination of amino acids

Deamination is a removal of **amino group** from an amino acid. Two major types of deamination of amino acids reactions are known. In all instances the amino group ($-NH_2$) is eliminated from the molecule of an amino acid to yield ammonia, fatty acids, hydroxyacids, and keto acids:

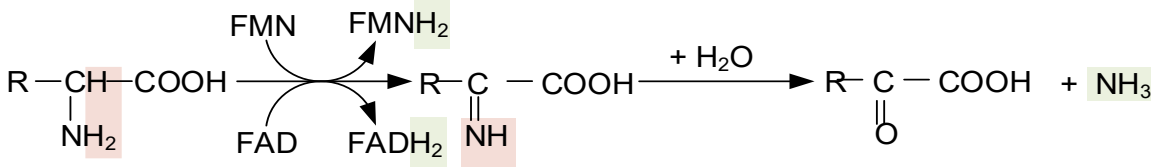
I. Oxidative deamination is the predominant type in animal tissues, plants and the majority of aerobic microorganisms.



L-Amino acid oxidase of liver and kidney convert an amino acid to an α-imino acid that decomposes to an α-keto acid with release of ammonium ion.



A point to be noted is that amino acids oxydases (L- and D-isomeric) are conjugated flavoproteins, containing **FMN** and **FAD** while D-amino acids oxidases only FAD is a prosthetic group. Schematically the reaction of the oxidative amino acids deamination catalyzed by enzymes may be represented in the following manner:



It should also be pointed out that the reduced flavin nucleotides of L- and D-amino acids oxidases can undergo a direct oxidation by molecular oxygen o produce hydrogen peroxye which than becomes decomposed to water and oxygen by the action of catalase:

$$E\text{-}FADH2(E\text{-}FMNH2) + O_2 \longrightarrow E\text{-}FAD(E\text{-}FMN) + H_2O_2$$

$$H_2O_2 \longrightarrow H_2O + ½O_2$$

A specific enzyme, **glutamate dehydrogenase (GDH),** highly active at physiological pH and capable of catalyzing the oxidative deamination of glutamate, wich plays a key role in removing nitrogen from amino acids. GDH contains NAD^+ (or $NADP^+$) as a coenzyme. The reaction proceeds through an aerobic phase of glutamic acid dehydration yielding an intermediate product (imminoglutaric acid) and through a spontaneous hydrolysis of this acid to ammonia and α-ketoglutaric acid to the scheme at the fig. 14.12.

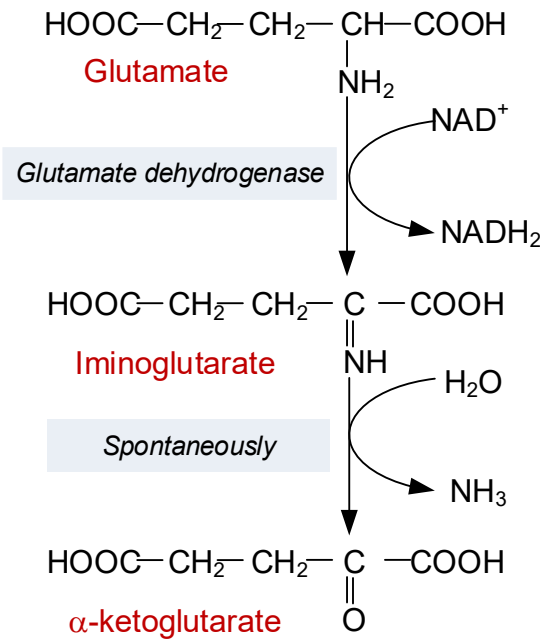


Fig. 14.12. Glutamate dehydrogenase reaction

The GDH reaction is freely reversible, and also functions in amino acid biosynthesis.

GDH is one of the best studied enzymes is **oligomeric enzyme** (MW 312 000 Da) composed of **six** subunits. In humans the activity of glutamate dehydrogenase is controlled through ADP-ribosilation. This regulation is relaxed in response to caloric restriction and low blood glucose. Under these curcumstances glutamate dehydrogenase activity is raised to increase the amount of α-ketoglutarate that is produced. The product α-ketoglutarate can be used to provide energy by being used in the citric acid cycle to ultimately produce ATP. Accomulation of excesive concentration of **ATP ar GTP** allosterically **inhibits GDH.**

MEDICAL IMPORTANCE

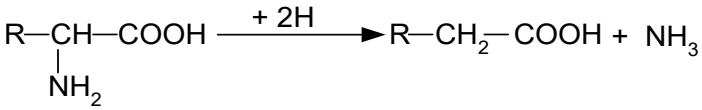
Changes in GDH activity have been observed in various liver diseases. Increased GDH activity may indicate liver cell damage, as seen in acute hepatitis, liver necrosis, or drug-induced liver injury. Elevated GDH levels have also been associated with liver tumors, such as hepatocellular carcinoma. Monitoring GDH activity can aid in the diagnosis and monitoring of liver diseases.

GDH has been utilized as a diagnostic marker for certain infections, specifically Clostridium difficile infection (CDI). GDH is a component of the C. difficile toxin detection algorithm, where GDH screening is performed initially, followed by confirmatory testing for toxin production. Elevated GDH levels in the stool can indicate the presence of C. difficile and aid in the diagnosis of CDI.

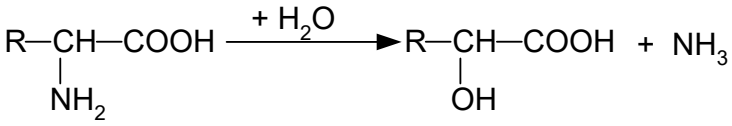
The combined action of an aminotransferase and glutamate dehydrogenase is referred to as **transdeamatination**. A few amino acids bypass the transdeamination pathway and undergo direct oxidative deamination.

II. Nonoxidative deamination of amino acids is in minor importance. Three main types of nonoxidative deamination of amino acid are described:

1. Reductive deamination:

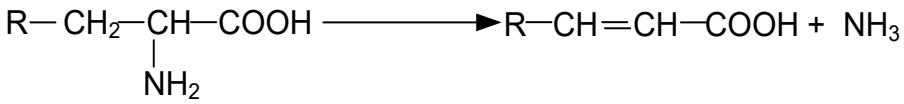


2. Hydrolytic (eliminative) deamination:



For example, the amide groups of glutamine and asparagine are released as ammonium ions by hydrolysis, ***glutaminase*** converts glutamine to glutamate ***asparaginase*** converts asparagine to aspartate.

3. Intramolecular deamination:



For example, histidine is deaminated by histidase to form NH_3 and urocanate.

Products of nonoxidative deamination are: ammonia (a common for all types of deamination), fatty acids (saturated and unsaturated) and oxyacids.

The comperative characteristics of transamination and deamination is represented in table 14.3.

Table 14.3. Comparison of transamination and deamination

TRANSAMINATION	DEAMINATION
The transfer of an amino group from one molecule to another, especially from an amino acid to a keto acid	The removal of an amino group from am amino acid or other compounds
Involves in the synthesis of nonessential amino acids	Involves in the breakdown of excess proteins
Occurs in all cells of the body	Occurs mainly in liver
Transaminases or aminotransferases catalyze transamination	Oxidases of amino acids, glutamate dehydrogenase and deaminases catalyse deamination
Results in an exchange of an amino group with a keto group	Results in the elimination of ammonia
Glutamic acid is the main form of amino acid produced in transamination reaction	Glutamic acid is the primary form of amino acid, which undergo deamination
Reversible	Irreversible

14.5. Decarboxylation of amino acids.

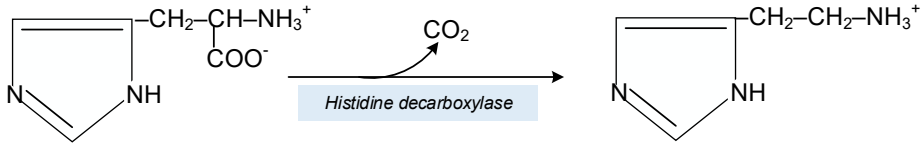
Decarboxylation is the process of removing the **carboxyl group (-COOH)** from an amino acid, resulting in the release of **carbon dioxide (CO_2).** This reaction is catalyzed by enzymes called ***decarboxylases***, and it involves the use of **pyridoxal phosphate (PLP)** as a coenzyme.

During decarboxylation, PLP forms a Schiff's base with the amino acid, which helps stabilize the α-carbanion intermediate formed after the cleavage of the bond between the carboxyl group and the α-carbon atom. This stabilization allows for the subsequent release of CO_2 and the formation of an amine.

The amines produced through decarboxylation are known as **biogenic amines**. Many of these amines, such as **histamine, serotonin, dopamine, and γ-aminobutyric acid (GABA)**, have significant physiological activity and play important roles as

hormones, neurotransmitters, or other signaling molecules in the body. Their production through decarboxylation of specific precursor amino acids is a crucial step in their biosynthesis.

Histamine is a biogenic amine that is formed by the decarboxylation of histidine. The enzyme responsible for this conversion is ***histidine decarboxylase***, which is expressed in various cells throughout the body, including gastric mucosa, neurons, parietal cells, mast cells, and basophils.



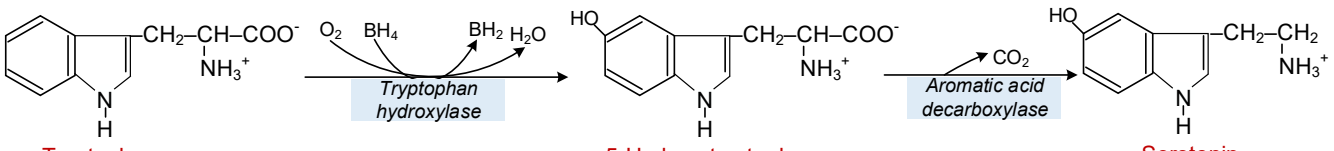
Histidine **Histamine**

Histamine plays several important roles in the body, including:

- **Allergic reactions:** Histamine is released by mast cells and basophils in response to allergens, and it triggers the classic symptoms of allergic reactions, such as itching, swelling, redness, and increased mucus production.
- **Inflammation:** Histamine is involved in the inflammatory response by causing vasodilation (widening of blood vessels) and increasing vascular permeability. This allows immune cells and molecules to reach the site of injury or infection more easily.
- **Gastric acid secretion:** Histamine stimulates the release of gastric acid from the parietal cells in the stomach. This helps in the digestion of food.
- **Neurotransmission:** In the central nervous system, histamine acts as a neurotransmitter. It is involved in regulating wakefulness, arousal, and attention. Histamine neurons in the brain send projections to various regions and play a role in maintaining normal sleep-wake cycles.
- **Blood vessel regulation:** Histamine can cause both vasodilation and vasoconstriction, depending on the specific receptors it binds to in blood vessels. It helps regulate blood flow and blood pressure.
- **Smooth muscle contraction:** Histamine can cause smooth muscle contraction in various organs, such as the airways (leading to bronchoconstriction) and the intestines (leading to increased motility).
- **Immune response:** Histamine is involved in immune responses and acts as a signaling molecule. It can attract immune cells to sites of infection or injury through its effects on blood vessels and can modulate the release of other immune mediators.

The effects of histamine are mediated through four types of histamine receptors: H_1, H_2, H_3, and H_4. These receptors are **G-protein coupled** receptors.

Serotonin, also known as **5-hydroxytryptamine (5-HT)**, is synthesized from tryptophan and stored at several sites in the body.



Tryptophan 5-Hydroxytryptophan Serotonin

Serotonin plays multiple physiological roles in the body. Some of the key functions of serotonin include:

- **Regulation of mood:** Serotonin is often referred to as the "feel-good" neurotransmitter because it is involved in regulating mood. Adequate levels of serotonin are associated with feelings of happiness and well-being. Low levels of serotonin have been linked to conditions such as depression, anxiety, and mood disorders.
- **Sleep regulation:** Serotonin is involved in the regulation of sleep-wake cycles. It helps promote sleep by facilitating the transition from wakefulness to sleep and maintaining the duration and quality of sleep.
- **Pain perception**: Serotonin is involved in modulating pain signals in the central nervous system. It can affect the perception of pain and contribute to the regulation of pain sensitivity.
- **Appetite and weight regulation:** Serotonin plays a role in regulating appetite and food intake. It helps to control feelings of hunger and satiety, and imbalances in serotonin levels have been associated with eating disorders and obesity.
- **Release of peptide hormones:** Serotonin is involved in stimulating the release of various peptide hormones from the cells of the gastrointestinal tract. These hormones include cholecystokinin (CCK), gastrin, and substance P, among others. The release of these hormones helps regulate various digestive processes, such as the secretion of digestive enzymes and gastric acid, and the contraction of smooth muscles in the gut.
- **Motility of the gastrointestinal tract:** Serotonin is essential for the regulation of gastrointestinal motility, specifically peristalsis. Peristalsis refers to the coordinated rhythmic contractions of the smooth muscles in the walls of the digestive tract that propel food and waste material through the system. Serotonin acts as a neurotransmitter in the enteric nervous system, which is the intrinsic nervous system of the gut. It helps coordinate and regulate the contraction and relaxation of the smooth muscles, enabling proper movement and transit of contents along the digestive tract.
- **Temperature regulation:** Serotonin is involved in the regulation of body temperature. It helps to maintain a stable body temperature by influencing thermoregulatory processes.
- **Blood pressure regulation:** Serotonin can affect blood vessel tone and blood pressure. It acts as a vasoconstrictor, causing blood vessels to narrow and increasing blood pressure.
- **Cognitive function:** Serotonin is involved in various aspects of cognitive function, including memory, learning, and attention. It plays a role in cognitive processes such as decision-making and emotional regulation.

MEDICAL IMPORTANCE

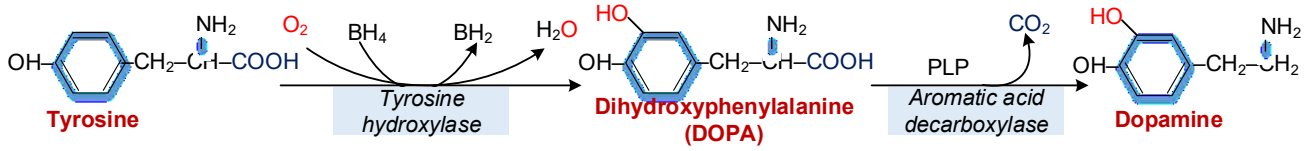
*Serotonin's role in psychiatric disorders and its modulation through medications like **selective serotonin reuptake inhibitors (SSRIs)** have been extensively studied and are widely used in the treatment of conditions such as depression, anxiety disorders, and certain mood disorders.*

Serotonin exerts its effects through various receptor subtypes, including **5-HT1A, 5-HT2B, 5-HT3, and others**. These receptors are distributed throughout the body and mediate the specific actions of serotonin in different tissues and organs.

MEDICAL IMPORTANCE

Lysergic acid diethylamide (LSD) is indeed a potent hallucinogenic drug that affects the central nervous system (CNS). It is derived from a compound found in the fungus ergot, which grows on certain grains. LSD belongs to a class of drugs known as psychedelics or hallucinogens. LSD acts primarily by interacting with serotonin receptors in the brain. Specifically, it mimics the effects of serotonin, a neurotransmitter involved in regulating mood, cognition, and perception. LSD binds to serotonin receptors, particularly the 5-HT2A receptors, and alters their activity, leading to profound changes in sensory perception, thought processes, and emotional experiences.

Dopamine, norepinephrine, epinephrine are biologically active amines (catecholaminenes). The catecholaminenes are synthesized from tyrosine.



Dopamine is primarily synthesized in specific regions of the central nervous system, including the hypothalamus, arcuate nucleus, and caudate nucleus of the brain. It is also produced in the medulla of the adrenal glands, which are part of the peripheral nervous system.

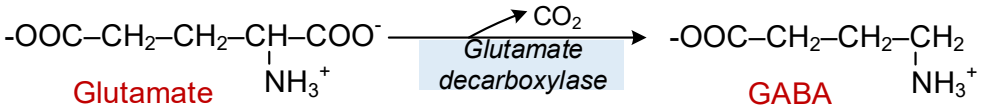
Dopamine plays a crucial role as a neurotransmitter in the central nervous system and has diverse functions throughout the body. Some of the key roles of dopamine include:

- **Regulation of Movement:** Dopamine is involved in the control of voluntary movements. It helps coordinate motor activities, muscle contractions, and movement initiation. Dysfunction in the dopamine system can lead to movement disorders such as Parkinson's disease, characterized by motor symptoms like tremors, rigidity, and bradykinesia (slowness of movement).
- **Reward and Pleasure:** Dopamine is closely associated with the brain's reward system. It is released in response to pleasurable experiences, reinforcing behaviors that are associated with rewards. Dopamine contributes to feelings of pleasure, motivation, and reinforcement of certain behaviors.
- **Mood Regulation**: Dopamine is involved in regulating mood and emotional responses. It influences feelings of happiness, well-being, and motivation. Abnormal dopamine levels or disruptions in the dopamine system have been implicated in mood disorders such as depression and bipolar disorder.
- **Cognitive Function:** Dopamine plays a role in various cognitive processes, including attention, learning, memory, and decision-making. It helps facilitate information processing in the brain and is involved in motivation and goal-directed behavior.
- **Endocrine Regulation:** Dopamine also functions as a hormone outside the central nervous system. In the periphery, it inhibits the release of prolactin from the pituitary gland, regulating lactation and reproductive functions.
- **Vasodilation and Blood Pressure Regulation:** Dopamine acts as a vasodilator, helping to widen blood vessels and improve blood flow. It also plays a role in regulating blood pressure.

Deficiency in dopamine production or dysfunction in the dopamine system can lead to neurological disorders such as **Parkinson's disease**, which is characterized by the degeneration of dopaminergic neurons in the brain. In Parkinson's disease, the loss of dopaminergic neurons in a specific region of the brain called the substantia nigra leads to a decrease in dopamine levels. This dopamine deficiency contributes to the motor symptoms associated with the disease, including tremors, rigidity, and impaired movement control.

Dopamine also plays a role in other conditions and disorders, such as schizophrenia and addiction, where there are alterations in the dopamine system. However, the specific mechanisms and imbalances in dopamine function in these conditions are more complex and multifaceted.

γ-Aminobutiric acid (GABA) is an inhibitory neurotransmitter in the central nervous system (CNS). When GABA binds to its receptor, it activates ion channels that allow the influx of chloride ions (Cl^-) into the neuron or efflux of potassium ions (K^+), hyperpolarizing the cell membrane. This hyperpolarization inhibits the firing of the neuron, reducing neuronal excitability.



By increasing the membrane permeability to chloride ions, GABA enhances inhibitory effects, making it harder for the neuron to reach the threshold for firing an action potential. This inhibitory action of GABA helps to balance and regulate the overall excitatory activity in the brain.

GABAergic transmission is involved in various physiological processes, including regulation of muscle tone, sleep, anxiety, and seizure control. GABA receptors and GABAergic signaling are targeted by several medications, such as benzodiazepines and barbiturates, which enhance GABA's inhibitory effects and have sedative, anxiolytic, and anticonvulsant properties.

Numerous pharmacologic agents (for example, benzodiazepines, topiramate, lamotrigine, and tiagabine) stimulate GABA activity in the treatment of seizures and other hyperspastic disorders.

The GABA shunt refers to a metabolic pathway that involves the conversion of glutamate to GABA. The GABA shunt pathway involves the following steps:

1. Glutamate, an amino acid, is decarboxylated by the enzyme ***glutamate decarboxylase*** to form GABA. This reaction involves the removal of a carboxyl group from glutamate.
2. GABA can undergo transamination, a process where the amino group of GABA is transferred to a keto acid. The enzyme ***GABA transaminase*** catalyzes this reaction, resulting in the formation of **succinic semialdehyde** and a keto acid.
3. Succinic semialdehyde is further metabolized to succinate by the enzyme ***succinic semialdehyde dehydrogenase***.

The GABA shunt is an alternative pathway for the metabolism of glutamate and provides a mechanism for the conversion of excess glutamate to GABA. It helps regulate the balance between excitatory and inhibitory neurotransmission in the brain. GABA acts

as an inhibitory neurotransmitter, counteracting the effects of excitatory neurotransmitters like glutamate.

Imbalances in the GABA shunt pathway have been implicated in various neurological disorders, including epilepsy, schizophrenia, and certain movement disorders. Modulation of GABAergic neurotransmission and the GABA shunt pathway is a target for pharmacological interventions aimed at treating these conditions.

Breakdown of biogenic amines. The accumulation of biogenic amines in the organism can produce an unfavorable effect on the organism physiological status and cause serious disturbances. However, the organs and tissues have at their disposal special mechanisms for neutralization of biogenic amines, the common route is an oxidative deamination leading to the formation of an aldehyde and release of ammonia:

$$\mathbf{R\text{-}CH_2\text{-}NH_2 + H_2O \rightarrow R\text{-}CHO + NH_3 + H_2O}$$

The breakdown of biogenic amines is primarily carried out by two enzymes: ***monoamine oxidase (MAO) and diamine oxidase (DAO).***

Monoamine Oxidase (MAO): MAO is an enzyme found in the outer membrane of mitochondria in various tissues, including the brain. It catalyzes the oxidative deamination of biogenic amines by removing an amino group ($-NH_2$) from the amine, resulting in the formation of an aldehyde and ammonia. This process generates hydrogen peroxide (H_2O_2) as a byproduct.

There are two isoforms of MAO: MAO-A and MAO-B. MAO-A primarily metabolizes serotonin, norepinephrine, and dopamine, while MAO-B predominantly metabolizes phenylethylamine and dopamine. The breakdown of these neurotransmitters by MAO helps regulate their levels and terminate their signaling actions in the synaptic cleft.

Inhibitors of MAO, such as monoamine oxidase inhibitors (MAOIs), are used clinically as antidepressants and have been used to treat other conditions like Parkinson's disease.

Diamine Oxidase (DAO): DAO is an enzyme found primarily in the small intestine and other tissues. It is responsible for the degradation of histamine and other biogenic amines derived from food. DAO catalyzes the oxidative deamination of these amines, converting them into aldehydes, ammonia, and hydrogen peroxide.

DAO plays a crucial role in preventing the buildup of histamine and other biogenic amines from dietary sources. Deficiency in DAO activity or inadequate levels of DAO can lead to histamine intolerance, which is associated with symptoms like headaches, flushing, gastrointestinal issues, and allergic-like reactions.

REVIEW TEST:

№	MCQs	Answers and explanations
1.	A woman resting in the countryside has been stung by a bee. Immediately after she developed pain in the stung area. In a few minutes there developed a vesicle, erythema and intense itch; later – urticaria and expiratory dyspnea. What factors	**The answer is C.** The development of expiratory dyspnea (shortness of breath during expiration) following a bee sting indicates a severe allergic reaction known as anaphylaxis. Anaphylaxis is a systemic allergic reaction that can be life-threatening if not treated promptly.

	resulted in the patient developing expiratory dyspnea? A. Lysosomal enzymes B. Hageman's factor C. Histamine D. Noradrenaline E. Adrenaline	When a person is stung by a bee, the venom injected contains various substances, including histamine. Histamine is a potent vasodilator and bronchoconstrictor. Upon release, histamine causes blood vessels to dilate, leading to increased permeability and leakage of fluid into surrounding tissues, which results in the formation of a vesicle, erythema, and swelling. In the respiratory system, histamine induces bronchoconstriction, leading to airway narrowing and difficulty breathing.
2.	Depression and emotional disturbances result from the lack of noradrenaline, serotonin, and other biogenic amines in the brain. Their content in the synapses can be increased through administration of antidepressants that inhibit the following enzyme: A. Monoamine oxidase B. Diamine oxidase C. *L*-amino acids oxidase D. *D*-amino acid oxidase E. Phenylalanine-4-monooxygenase	**The answer is A** The enzyme that can be inhibited by antidepressant medications to increase the content of noradrenaline, serotonin, and other biogenic amines in the synapses is monoamine oxidase (MAO). Monoamine oxidase is responsible for the breakdown of these neurotransmitters in the synaptic cleft, thereby reducing their availability for signaling between neurons. MAO inhibitors (MAOIs) are a class of antidepressant drugs that work by inhibiting the activity of monoamine oxidase. By blocking the enzymatic breakdown of noradrenaline, serotonin, and other biogenic amines, MAOIs increase their concentration in the synaptic cleft, allowing for enhanced neurotransmission and potentially alleviating symptoms of depression and emotional disturbances.
3.	Biogenic amines, such as histamine, serotonin, dopamine and others, are highly active substances affecting various physiological functions. What transformation process of amino acids results in biogenic amines being produced in somatic tissues? A. Oxidation B. Deamination C. Transamination D. Decarboxylation E. Reductive amination	**The answer is D** The transformation process of amino acids that results in the production of biogenic amines in somatic tissues is decarboxylation. Decarboxylation is the enzymatic removal of the carboxyl group (-COOH) from an amino acid, leading to the formation of an amine. This process is catalyzed by enzymes known as decarboxylases. Specific decarboxylases act on different amino acids to produce specific biogenic amines. For example: Decarboxylation of histidine by the enzyme histidine decarboxylase results in the production of histamine. Decarboxylation of tryptophan by the enzyme aromatic L-amino acid decarboxylase leads to the formation of serotonin. Decarboxylation of L-DOPA by the enzyme aromatic L-amino acid decarboxylase is involved in the synthesis of dopamine. These decarboxylation reactions occur in various tissues throughout the body, including the gastrointestinal tract, neurons, mast cells, and other somatic cells. The resulting biogenic amines play critical roles in regulating numerous physiological functions, including neurotransmission, hormone regulation, immune responses, and other processes.
4.	A 24-year-old patient has been administered glutamic acid to treat	**The answer is A.**

	epilepsy. Medicinal effect in this case occurs not due to glutamate itself, but due to the product of its decarboxylation: A. γ-Aminobutyric acid B. Histamine 4-monooxygenase C. Serotonin D. Dopamine E. Taurine	The product of decarboxylation of glutamic acid is gamma-aminobutyric acid (GABA). In the case of epilepsy treatment, the medicinal effect is attributed to GABA rather than glutamic acid itself. GABA is an inhibitory neurotransmitter in the central nervous system that helps regulate neuronal excitability. It works by binding to GABA receptors and reducing the activity of neurons, thereby inhibiting excessive electrical activity and preventing seizures. By administering glutamic acid, which can be decarboxylated to GABA, the levels of GABA in the brain can be increased. This increase in GABA concentrations helps to restore the balance between excitatory and inhibitory neurotransmission, thereby reducing the likelihood of seizures and providing therapeutic benefits for epilepsy patients.
5.	In recognition of hepatitis the determination of the following enzymes activity in blood has diagnostic significance: A. Creatin kinase B. Amylase C. Lactate dehydrogenase D. Aldolase E. Aminotransferases	**The answer is E.** In the recognition and diagnosis of hepatitis, the determination of the activity of certain enzymes in the blood can provide valuable diagnostic information. The enzymes commonly measured in hepatitis are aminotrasferases. Alanine aminotransferase (ALT) is primarily found in liver cells, and its levels in the blood increase when there is liver damage or inflammation. Elevated levels of ALT are indicative of liver cell injury and are commonly used as a marker of hepatitis. Aspartate aminotransferase (AST) is also found in liver cells, as well as in other organs such as the heart, muscles, and kidneys. Like ALT, increased levels of AST in the blood suggest liver cell damage. However, AST is less specific to the liver than ALT and can also be elevated in conditions affecting other organs.
6.	Production of some toxic substances in large intestines occurs due to decarboxylation of some amino acids. Indicate, what substance is produced from ornithine? A. Skatole B. Putrescine C. Indole D. Cadaverine E. Phenol	**The answer is B.** Putrescine is the substance produced from ornithine through decarboxylation in the large intestines. Putrescine is a toxic compound that is formed as part of the intestinal putrefaction process. Putrescine is considered toxic and can have adverse effects on biological systems at high concentrations.
7.	A woman has been limiting the amount of products in her diet to lose some weight. 3 months later she developed edemas and her diuresis increased. What dietary component deficiency is the cause of this? A. Minerals B. Fats C. Carbohydrates	**The answer is E.** Proteins and edema: Proteins play a crucial role in maintaining fluid balance in the body. They help regulate the movement of fluids between blood vessels and tissues. When there is a deficiency of dietary proteins, the oncotic pressure in the blood vessels decreases. This can lead to an imbalance in fluid distribution, resulting in the accumulation of fluid in the interstitial spaces and the development of edema.

	D. Vitamins E. Proteins	Increased diuresis: In response to edema and decreased oncotic pressure, the kidneys may try to compensate by increasing diuresis (increased urine production). This is an attempt to remove excess fluid from the body.
8.	A 30-year-old male patient with acute pancreatitis has been found to have a disorder of cavitary protein digestion. The reason for such condition can be the hyposynthesis and hyposecretion of the following enzyme: A. Pepsin B. Tripsin C. Lipase D. Dipeptidase E. Amylase	**The answer is B.** Acute pancreatitis is a condition characterized by inflammation of the pancreas, which can impair its normal digestive functions. Trypsin is a protease enzyme produced by the pancreas. It plays a crucial role in protein digestion by breaking down proteins into smaller peptides. Trypsin is responsible for the hydrolysis of peptide bonds in proteins, converting them into amino acids and smaller peptide fragments. In acute pancreatitis, the inflammation and damage to the pancreas can impair the synthesis and secretion of digestive enzymes, including trypsin. This can result in reduced levels of trypsin being released into the digestive system, leading to inadequate protein digestion.
9.	A patient with encephalopathy was admitted to the neurological inpatient department. There was revealed a correlation between increasing of encephalopathy and substances absorbed by the bloodstream from the intestines. What substances that are formed in the intestines can cause endotoxemia? A. Indole B. Butyrate C. Acetacetate D. Biotin E. Ornithine	**The answer is A.** Indole is a metabolite produced by certain bacteria in the intestines, primarily from the breakdown of the amino acid tryptophan. Indole production is a natural process in the gut, but excessive levels or impaired clearance can contribute to health problems. Indole has been implicated in promoting gut barrier dysfunction and inflammation. It can disrupt the integrity of the intestinal epithelial barrier, allowing bacterial products, including endotoxins, to enter the bloodstream. The presence of endotoxins in the bloodstream can contribute to endotoxemia and the development or exacerbation of encephalopathy.
10	During gastric secretory function research decrease of hydrochloric acid concentration in gastric juice was detected. What enzyme will be less active in such a condition? A. Lipase B. Amylase C. Pepsin D. Dipeptidase E. Hexokinase	**The answer is C.** Hydrochloric acid (HCl) is secreted by the parietal cells in the stomach and plays a crucial role in the process of gastric digestion. One of its primary functions is to create an acidic environment in the stomach, which is necessary for the activation and optimal function of pepsin.Pepsinogen is the inactive precursor of pepsin. When HCl is released into the stomach, it converts pepsinogen into its active form, pepsin. Pepsin is a proteolytic enzyme that helps break down proteins into smaller peptides. Pepsin is most active at an acidic pH (optimal pH range around 1.5 to 2.5). The acidic environment created by hydrochloric acid in the stomach provides the optimal pH for pepsin to function effectively. It denatures proteins, allowing pepsin to bind and cleave peptide bonds, initiating protein digestion. When there is a decrease in hydrochloric acid concentration in gastric juice, the overall acidity of the stomach environment is reduced. This decrease in acidity can negatively impact the activation and activity of

		pepsin. Without the sufficient acidic environment, pepsinogen may not be adequately converted into active pepsin, leading to a decrease in pepsin activity.

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15. DETOXIFICATION OF AMMONIA AND UREA BIOSYNTHESIS BIOSYNTHESIS OF GLUTATHION AND CREATINE

OBJECTIVES after studying this chapter, you should be able to:

- *To interpret metabolic pathways of production and neutralization of ammonia, circulatory transport of ammonia, urea biosynthesis.*
- *To analyze changes in processes of transport and neutralization of ammonia in hereditary anomalies of enzymes of ammonia turnover.*
- *To explain general metabolic pathways of nitrogen free residues of amino acids and peculiarities in transformation of aromatic and heterocyclic amino acids.*
- *Interprete reactions of creatine and glutathione generation and their medical importanc.*
- *Outline how arginine participates in the biosynthesis of creatine, nitric oxide (NO), putrescine, spermine, and spermidine.*

15.1. Pathways of ammonia production. Toxicity of ammonia and mechanisms of its detoxification.

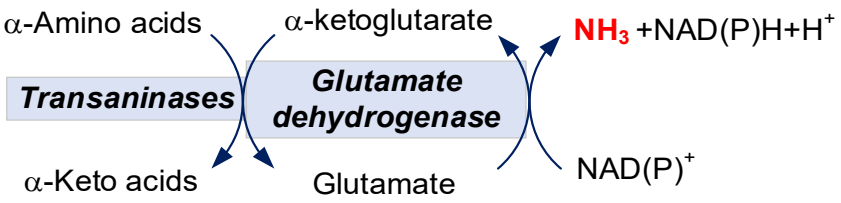
In healthy adults, nitrogen intake from dietary protein is balanced by nitrogen excretion, primarily in the form of urea. The turnover of body protein occurs at a constant rate, with approximately 1% to 2% of total body protein being degraded and resynthesized each day, mainly from muscle protein. During the catabolism of amino acids, **ammonia (NH_3)** is released as a byproduct. **Ammonia is toxic** to the body, so it is quickly converted into a less toxic form, **urea**, in the liver through a series of reactions known as the urea cycle. Urea is then excreted in the urine, allowing for the elimination of excess nitrogen.

The concentration of ammonia in the blood is normally maintained within a narrow range. In healthy individuals, the blood ammonia concentration is typically around **0.4 - 0.7 mg/L (25 - 40 µmol/L).** Elevated levels of ammonia in the blood, known as **hyperammonemia**, can occur in certain metabolic disorders or liver dysfunction and can have detrimental effects on the central nervous system.

The efficient removal of excess ammonia through the urea cycle and its excretion in urine is essential for maintaining nitrogen balance and preventing the accumulation of toxic ammonia in the body.

Besides amino acids catabolism, ammonia can be released in some pathways**. The main sources of ammonia are:**

- **Reactions of transamination and oxidative deamination of glutamate**: These reactions occur primarily in the liver and kidney.



- **Glutamine deamination catalysed by *glutaminase: Glutaminase*** is expressed and active in periportal hepatocytes, where it generates NH_3, as does glutamate dehydrogenase. *Glutaminase* is also expressed in the epithelial cells of the renal tubules, where the produced ammonia is excreted as ammonium ions. This excretion of ammonium ions is an important mechanism of renal acid-base regulation. One of the most important roles of glutaminase is found in the axonal terminals of neurons in the central nervous system.

Glutamine + H_2O → Glutamate + NH_3

- **Bacterial action in the large intestine (amino acids putrefaction)**: The ammonia produced by enteric bacteria and absorbed into portal venous blood and the ammonia produced by tissues are rapidly removed from circulation by the liver and converted to urea.
- **Deamination of biogenic amines in monoamine oxidase (MOA) or diamino oxidase (DAO)**, as shown in chapter 14.
- **Catabolism of purines and pyrimidines:** Amino groups attached to the rings of purines and pyrimidines. In the catabolism of purines and pyrimidines, amino groups attached to the rings are released as ammonia.

Ammonia is a highly toxic substance, particularly to the central nervous system. Excessive accumulation of ammonia in the body can occur in various conditions:

- **Liver disorders**, such as viral and toxic hepatitis, as well as cirrhosis, can lead to impaired liver function and compromise the ability of the liver to metabolize and eliminate ammonia effectively. This can result in elevated levels of ammonia in the blood, a condition known as hyperammonemia. The liver normally plays a crucial role in the urea cycle, which converts ammonia into urea for excretion. When liver function is compromised, ammonia accumulates, leading to potential damage to the central nervous system.
- **Impaired nitrogen-excreting function of the kidneys**, seen in acute or chronic renal failure, can also contribute to elevated ammonia levels in the blood. The kidneys play a role in the excretion of urea and other nitrogenous waste products. When kidney function is compromised, there may be reduced clearance of urea and ammonia, leading to their accumulation in the bloodstream.
- Additionally, certain genetic disorders, such as **congenital hyperammonemia**, can result from defects in the enzymes involved in the urea cycle. These inherited metabolic disorders impair the body's ability to effectively convert ammonia into urea, leading to increased ammonia levels and subsequent toxicity.

Clinically, **hyperammonemia** is characterized by profound disturbances in the function of the central nervous system and may lead to the development of a comatose state. **The toxicity** of ammonia can be explained by the following reasons:

1. High ammonia level would drive **glutamate dehydrogenase** reaction to reverse:

NH_3 + α-ketoglutarate + $NAD(P)H+H^+$ → L-glutamate + $NAD(P)^+$ + H_2O

This results in a depletion of α-ketoglutarate, an essential TCA cycle intermediate, could impair energy metabolism in the brain.

2. High concentration of ammonia would drive **glutamine synthase**:

Glutamate + ATP + NH_3 → glutamine + ADP + Pi

This would deplete glutamate – a neurotransmitter and precursor for synthesis of the neurotransmitter GABA. Excessive glutamine accumulation in CNS leads to an increase in osmotic pressure, and causing the brain edema.

3. An excessive concentration of ammonia in the blood shifts its pH to the alkaline side (**alkalosis occurs**). This, in turn, increases the affinity of hemoglobin for oxygen, which leads to tissue hypoxia, and as a result hypoenergetic state.

Different animals excrete excess nitrogen as ammonia, as uric acid, or as urea. The aqueous environment of teleostean fish, are **ammonotelic** (excrete ammonia). Birds, are **uricotelic,** address both problems by excreting nitrogen-rich uric acid. Many land animals, including humans, are **ureotelic** and excrete nontoxic, highly water-soluble **urea**. Since urea is nontoxic to humans, high blood levels in renal disease are a consequence, not a cause, of impaired renal function.

In the tissues of the human body, there are several mechanisms for **neutralizing ammonia**, which include:

1. Urea synthesis.
2. Reductive amination and/or transreamination.
3. Synthesis of asparagine and glutamine (amides of amino acids).
4. Formation of ammonium salts.

Urea is the major end product of nitrogen metabolism in humans and other mammals, and it serves as the primary disposal form for excess amino groups derived from amino acids. Approximately 90% of the nitrogen-containing components in urine are in the form of urea.

Once synthesized in the liver, urea is transported via the bloodstream to the kidneys, where it is filtered by the renal glomeruli and excreted in the urine. The kidneys play a crucial role in maintaining the balance of urea in the body by regulating its excretion.

The urea cycle and the subsequent excretion of urea in the urine are essential processes for the elimination of excess nitrogen from the body, as nitrogen must be effectively removed to prevent its toxic accumulation.

Ammonia is produced by all tissues and the main disposal is via formation of urea in liver. NH_3 needs to reach the liver because the liver contains the enzyme required to convert ammonia to urea (urea cycle enzymes). **Two mechanisms** are available in humans for the transport of ammonia from the peripheral tissues to the liver for its ultimate conversion to urea. **The first**, found in most tissues, uses **glutamine synthetase** to combine ammonia with glutamate to form **glutamine** – a nontoxic transport form of ammonia. The glutamine is transported in the blood to the liver where it is cleaved by **glutaminase** to produce glutamate and free ammonia. **The second** transport mechanism, used primarily by muscle, involves **transamination of pyruvate** (the end product of aerobic glycolysis) to form **alanine**. Alanine is transported by the blood to the liver, where

it is converted to pyruvate, again by transamination. In the liver, the pathway of gluconeogenesis can use the pyruvate to synthesize glucose, which can enter the blood and be used by muscle – a pathway called the **glucose-alanine cycle (fig. 15.1)**.

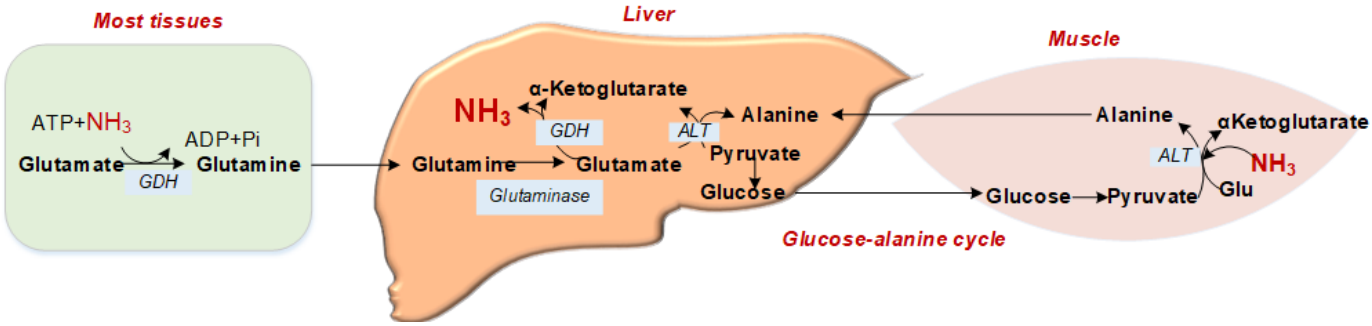


Fig. 15.1. Ammonia is transported in the bloodstream primarily in the form of glutamine and alanine

15.2. Biosynthesis of urea

The **urea cycle**, also known as the **Krebs-Henseleit cycle**, was elucidated by Hans Krebs and Kurt Henseleit in 1932. It is a series of biochemical reactions that take place in the **liver** and other tissues to convert toxic ammonia into urea for its excretion.

The synthesis of 1 mole of urea requires the utilization of 3 moles of ATP, 1 mole each of ammonium ion and aspartate, and involves the activity of five enzymes. The participating amino acids in the urea cycle are ornithine, citrulline, and argininosuccinate, which play major roles in carrying and transferring the atoms that eventually form urea.

N-acetylglutamate is an important molecule in the urea cycle as it acts as a crucial enzyme activator. Its presence is necessary for the proper functioning of the cycle. The other amino acids involved in the cycle serve as carriers or intermediates for the conversion of ammonia into urea.

The first two reactions of the urea cycle, involving the conversion of ammonia to carbamoyl phosphate and the synthesis of citrulline, take place in the mitochondria. The remaining enzymes of the cycle are located in the cytosol of the cells (fig. 15.2.)

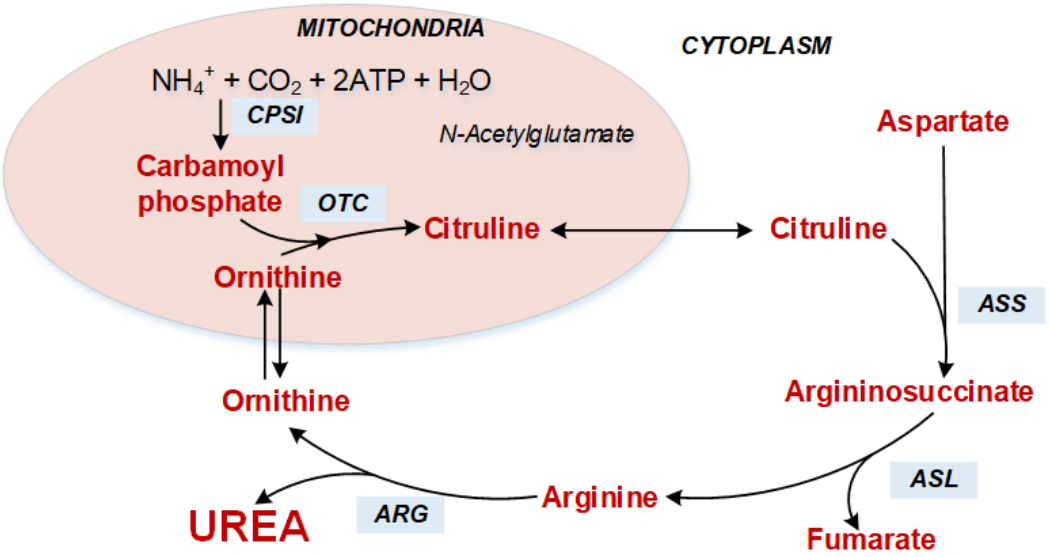
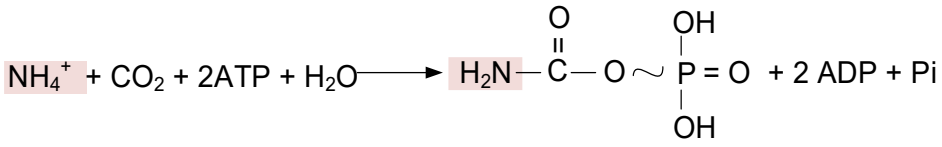


Fig. 15.2. Compartmentslisation of urea cycle reactions

- **Synthesis of carbamoyl phosphate:** The synthesis of carbamoyl phosphate, an important step in the urea cycle, involves the enzyme ***carbamoyl phosphate synthase I (CPS I)*** located in the **mitochondria**. This reaction is the first committed and rate-limiting step of the urea cycle.

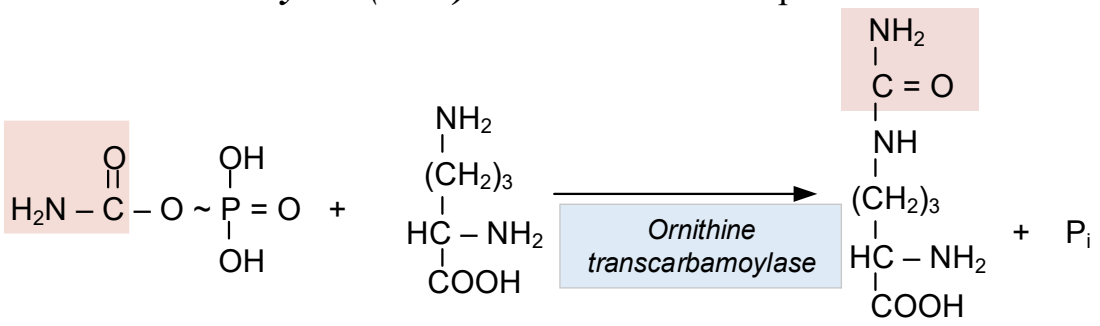


Carbamoyl phosphate

CPS I requires **N-acetylglutamate** for its activity. Another isoform of *carbamoyl phosphate synthase II (CPS II)* – involved in pyrimidine synthesis – is present in cytosol. It accepts amino group from glutamine and does not require N-acetylglutamate for its activity.

N-Acetylglutamate is synthesized from acetyl coenzyme A and glutamate by *N-acetylglutamate synthase*, in a reaction for which arginine is an activator. Therefore, the intrahepatic concentration of N-acetylglutamate increases after ingestion of a protein-rich meal, which provides both a substrate (glutamate) and the regulator of N-acetylglutamate synthesis. This leads to an increased rate of urea synthesis.

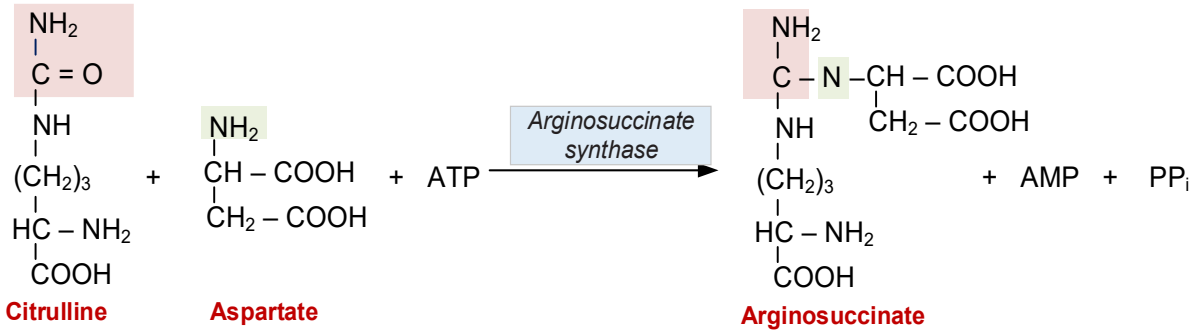
- **Formation of citrulline:** Citrulline is an intermediate in the urea cycle and is synthesized from carbamoyl phosphate and ornithine through the action of the enzyme ***ornithine transcarbamoylase (OTC).*** This reaction takes place in the mitochondria.



Carbamoyl phosphate — Ornithine — Citrulline

Ornithine and citrulline are basic amino acids that participate in the urea cycle, moving across the inner mitochondrial membrane via a **cotransporter**. They are not incorporated into cellular proteins because there are no codons for these amino acids.

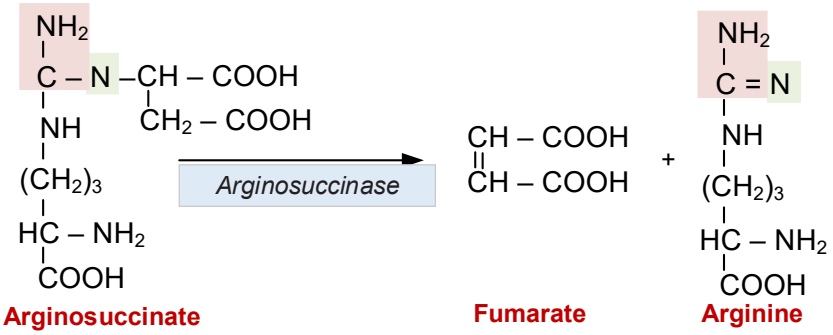
- **Synthesis of arginosuccinate:** ***Argininosuccinate synthase*** is the enzyme that catalyzes the condensation of citrulline and aspartate to form argininosuccinate in the urea cycle. This step is crucial as it incorporates the second amino group into the urea molecule.



Citrulline — Aspartate — Arginosuccinate

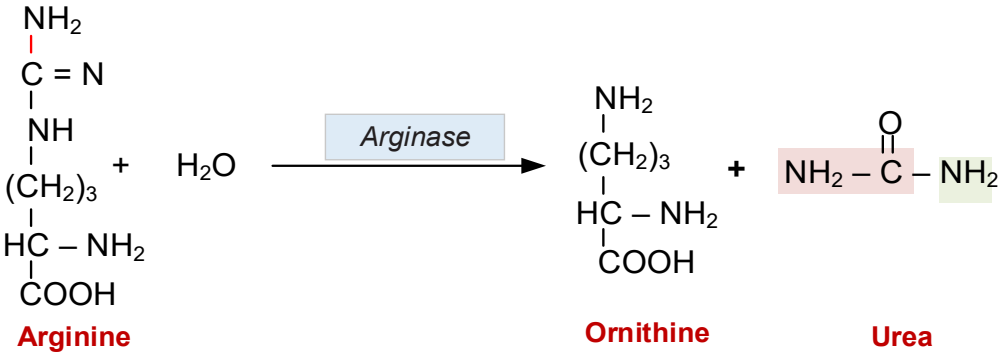
In this reaction, one molecule of citrulline, one molecule of aspartate, and one molecule of ATP are used to produce one molecule of argininosuccinate, one molecule of AMP, and one molecule of pyrophosphate (PP_i). The cleavage of ATP to AMP and PP_i provides the necessary energy for the reaction. The pyrophosphate (PP_i) is rapidly hydrolyzed by pyrophosphatase to produce two molecules of inorganic phosphate (P_i).

- **Arginosuccinase:** ***Arginosuccinase***, also known as ***argininosuccinate lyase***, is the enzyme that catalyzes the cleavage of argininosuccinate into arginine and fumarate in the urea cycle.



In this reaction, argininosuccinate is broken down into arginine and fumarate. Arginine serves as the immediate precursor for the synthesis of urea. Fumarate, on the other hand, is released as a byproduct of this reaction. Fumarate is a key intermediate in various metabolic pathways, including the tricarboxylic acid (TCA) cycle (also known as the citric acid cycle or Krebs cycle) and gluconeogenesis. It can be further metabolized in these pathways to generate energy or serve as a precursor for the synthesis of other molecules. Therefore, the cleavage of argininosuccinate by *arginosuccinase* not only produces arginine for urea synthesis but also provides a connection between the urea cycle and other metabolic pathways, such as the TCA cycle and gluconeogenesis, through the release of fumarate.

- **Formation of urea.** ***Arginase*** is the fifth and final enzyme of the urea cycle. It catalyzes the hydrolysis of arginine to yield urea and ornithine.



The production of urea in the liver is essential for the elimination of excess nitrogen from the body. Ornithine, which is generated as a byproduct of the reaction, is recycled and re-enters the mitochondria to participate in the urea cycle again.

Arginase activity is regulated by several factors. It is activated by certain metal ions, including Co^{2+} and Mn^{2+}. These metal ions serve as cofactors for the enzyme, enhancing its catalytic activity. Ornithine and lysine can competitively inhibit arginase. When these amino acids are present in high concentrations, they can bind to the active site of arginase, preventing arginine from binding and inhibiting its conversion to urea and ornithine.

While the enzymes of the urea cycle are present in various tissues, arginase is primarily found in the liver. This is why the liver is the primary site for urea synthesis. Other tissues may have the ability to synthesize arginine to some extent, but only the liver can effectively convert it to urea for excretion.

Arginine also serves as the precursor of the potent muscle relaxant nitric oxide (NO) in a Ca^{2+}-dependent reaction catalyzed by NO synthase.

Urea diffuses from the liver, and is transported in the blood to the kidneys, where it is filtered and excreted in the urine.

The capacity of the hepatic urea cycle exceeds the normal rates of ammonia generation, and the levels of serum ammonia are normally low. However, when liver function is compromised, due either to genetic defects of the urea cycle or liver disease, blood levels can rise. Such hyperammonemia is a medical emergency, because ammonia has a direct neurotoxic effect on the CNS. For example, elevated concentrations of ammonia in the blood cause the symptoms of ammonia intoxication, which include tremors, slurring of speech, somnolence, vomiting, cerebral edema, and blurring of vision. At high concentrations, ammonia can cause coma and death. The two major types of hyperammonemia are:

1. Acquired hyperammonemia: Liver disease is a common cause of hyperammonemia in adults, and may be due, for example, to viral hepatitis or to hepatotoxins such as alcohol. Cirrhosis of the liver may result in formation of collateral circulation around the liver. As a result, portal blood is shunted directly into the systemic circulation and does not have access to the liver. The conversion of ammonia to urea is, therefore, severely impaired, leading to elevated levels of ammonia.

2. Congenital hyperammonemia: Genetic deficiencies of each of the five enzymes of the urea cycle have been described, with an overall prevalence estimated to be 1:25,000 live births. Deficiency of five enzymes of urea biosynthesis corresponds to five types of congenital hyperammonemias:

- **Hyperammonemia Type I:** It is due to deficiency of enzyme **carbamoyl phosphate synthetase-I**. Mental retardation is the main symptom of this condition.
- **Hyperammonemia Type II**: *Ornithine transcarbamoylase* deficiency, which is X-linked, is the most common of these disorders, predominantly affecting males, although female carriers may become symptomatic. All of the other urea cycle disorders follow an autosomal recessive inheritance pattern. In each case, the failure to synthesize urea leads to hyperammonemia during the first weeks following birth. Historically, urea cycle defects had high morbidity (neurological manifestations) and mortality. Treatment included restriction of dietary protein in the presence of sufficient calories to prevent catabolism. Administration of compounds that bind covalently to amino acids, producing nitrogen-containing molecules that are excreted in the urine, has improved survival. For example, phenylbutyrate given orally is converted to phenylacetate. This condenses with glutamine to form phenyl - acetylglutamine, which is excreted.
- **Citrullinemia:** This disease is due to the absence of enzyme *argininosuccinate synthetase*. Hence citrulline accumulates in blood and excreted in urine.

- **Argininosuccinicaciduria:** Argininosuccinase is absent in this condition. So, argininosuccinate accumulates in blood and excreted in urine.
- **Hyperargininemia:** This condition is due to low arginase activity. Hence, arginine accumulates and excreted in urine. However some urea may be excreted in urine due to kidney arginase.

15.4. Glutathione, structure and role in metabolism of organic peroxides

Glutathione (GSH) is a tripeptide composed of three amino acids: glutamate, cysteine, and glycine (fig. 15.3). It contains a unique peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side chain.

Glutathione serves as an important antioxidant in cells, protecting them from damage caused by reactive oxygen species and other toxins, such as free radicals. It acts as a reducing agent by donating electrons to other molecules, particularly to compounds containing disulfide bonds. By doing so, glutathione helps to maintain thiol groups in a reduced state within the cytoplasm of animal cells.

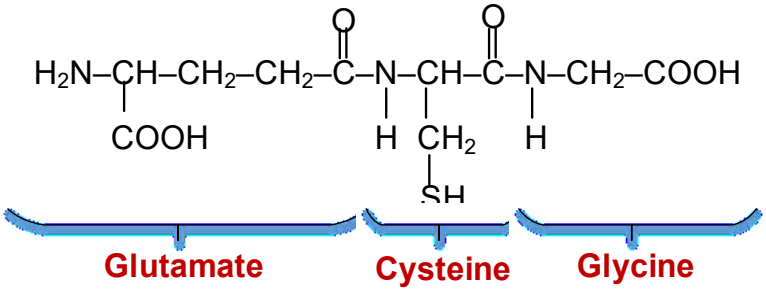


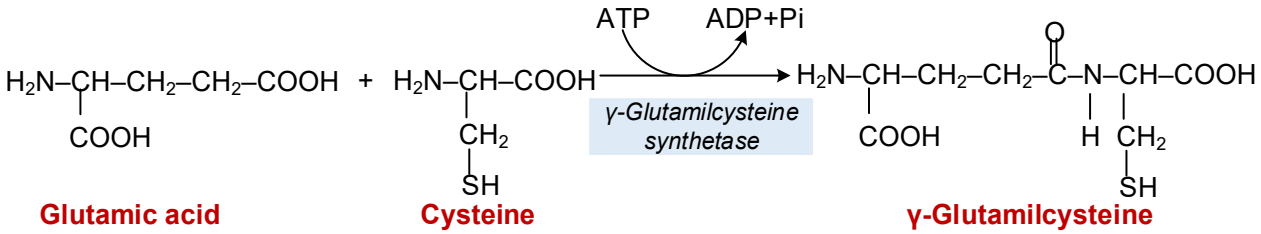
Fig. 15.3. Glutathione is a tripeptide composed of glutamate, cysteine, and glycine

The reduced form of glutathione (GSH) is the predominant form found in cells, as it serves as the major electron donor. However, under conditions of oxidative stress or when cells are exposed to toxins, the balance between reduced and **oxidized glutathione (GSSG)** can shift. GSSG is formed when glutathione donates electrons and becomes oxidized. To restore the balance, the enzyme ***glutathione reductase*** facilitates the conversion of GSSG back to GSH, ensuring that an adequate supply of reduced glutathione is available.

The ratio of reduced glutathione to oxidized glutathione within cells is often used as an indicator of cellular toxicity or oxidative stress. A lower ratio of GSH to GSSG may indicate increased oxidative damage and cellular dysfunction. Consequently, the measurement of this ratio can provide insights into the redox state and overall health of cells.

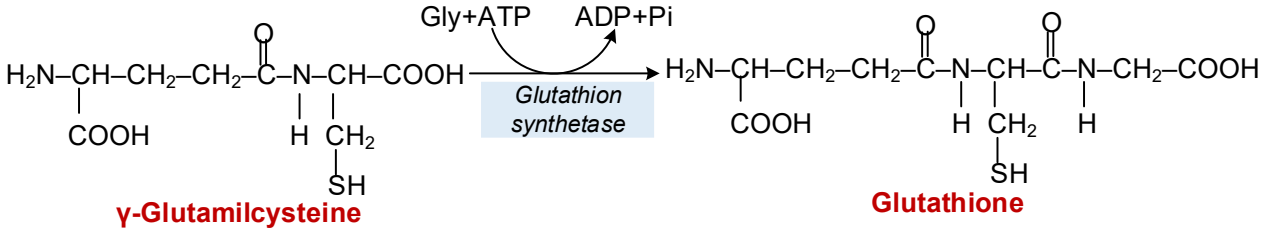
Glutathione is not an essential nutrient since it can be synthesized from the amino acids L-cysteine, L-glutamate and glycine. It is synthesized in two adenosine triphosphate-dependent steps:

- The synthesis of **gamma-glutamylcysteine** is an important step in the overall synthesis of glutathione. This process is catalyzed by the enzyme ***gamma-glutamylcysteine synthetase***, also known as ***glutamate cysteine ligase (GCL).***



Thes reaction catalyzed by *gamma-glutamylcysteine synthetase* is considered the rate-limiting step in the synthesis of glutathione. The availability and activity of this enzyme play a crucial role in determining the rate of glutathione synthesis in cells.

- The subsequent step in glutathione synthesis involves the addition of glycine to gamma-glutamylcysteine, catalyzed by the enzyme glutathione synthetase. This results in the formation of the tripeptide glutathione (gamma-glutamylcysteinylglycine), which is the biologically active form of glutathione.



While all cells in the human body are capable of synthesizing glutathione, liver glutathione synthesis has been shown to be essential.

Functions of GSH:

- **Antioxidant Activity:** Glutathione exists in a reduced state (GSH) and can donate electrons (H^+) to unstable molecules, such as reactive oxygen species (ROS), protecting cells from oxidative damage. In this process, GSH itself becomes reactive and forms glutathione disulfide (GSSG). The enzyme *glutathione reductase* helps regenerate GSH from GSSG.
- **Cellular Redox Balance:** GSH helps maintain a reduced cellular environment, which is essential for various biochemical reactions and maintaining proper cellular function.
- **Detoxification:** GSH is involved in the detoxification of various substances and xenobiotics. It serves as a substrate for *glutathione S-transferase enzymes*, which facilitate the conjugation of GSH with toxic compounds, making them more water-soluble and facilitating their excretion from the body.
- **Protection against Reactive Metabolites:** GSH can bind to reactive metabolites, such as N-acetyl-p-benzoquinone imine (NAPQI), formed during the metabolism of certain drugs or toxins. This binding prevents these reactive molecules from damaging cellular proteins and helps detoxify them. Depletion of GSH can lead to the accumulation of reactive metabolites and cell damage.
- **Leukotriene Synthesis:** GSH participates in the synthesis of leukotrienes, which are important mediators involved in inflammation and immune responses.
- **Cofactor for Glutathione Peroxidase:** Glutathione functions as a cofactor for the enzyme *glutathione peroxidase*, which helps protect cells from oxidative stress by catalyzing the breakdown of hydrogen peroxide and lipid hydroperoxides.

- **Biotransformation in the Liver:** Glutathione plays a role in the biotransformation of lipophilic toxins and waste products in the liver. It conjugates with these substances, making them more water-soluble and facilitating their elimination via bile.
- **Detoxification of Methylglyoxal:** GSH is involved in the detoxification of methylglyoxal, a toxic byproduct of metabolism. This detoxification process is carried out by the glyoxalase system.

15.5. Production of creatine and creatinine, clinical and diagnostic significance of disorders in their metabolism.

Creatine and creatinine are two closely related nitrogenous compounds that are connected with protein metabolism.

Creatine is produced from **glycine, arginine, and S-adenosylmethionine (SAM)**. Glycine combines with arginine to form ornithine and guanidinoacetate, which is methylated by SAM to form creatine (fig. 15.4).

First reaction of creatine synthesis is the formation of **guanidoacetic acid**, also called **glycocyamine**. This reaction takes place in **kidney**. Transfer of an "amidine group" from arginine to glycine is catalyzed by ***arginine-glycine transamidinase***. The process is called as **transamidination**.

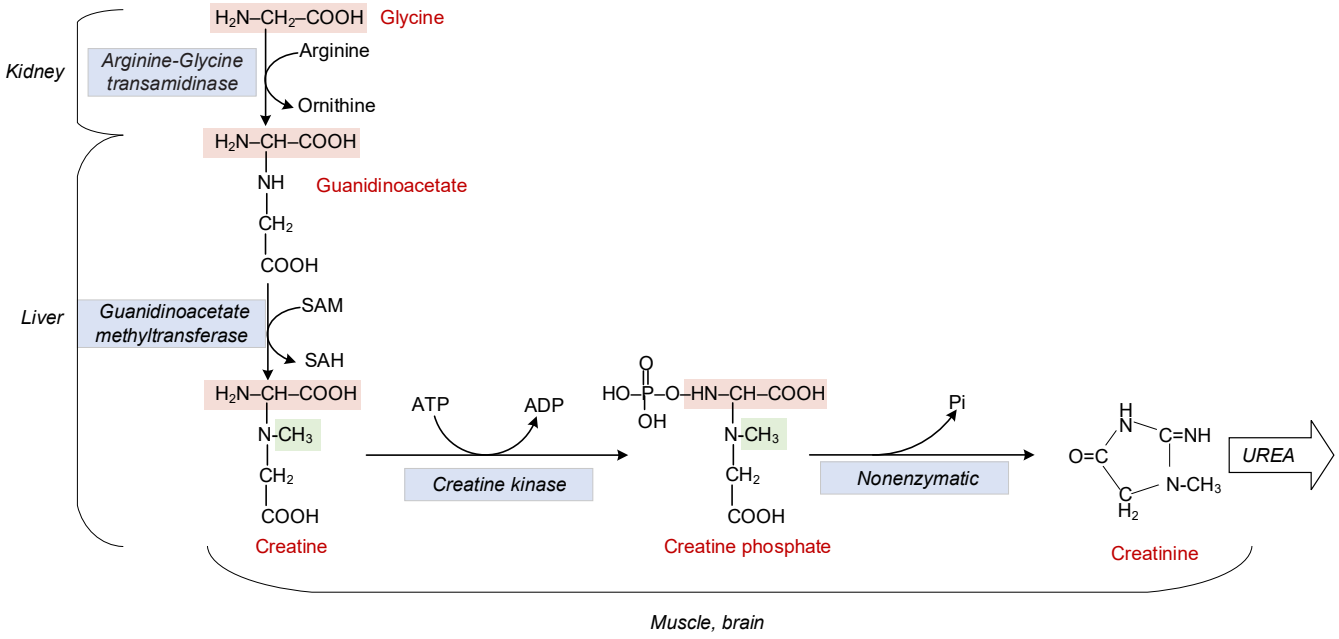


Fig. 15.4. Biosynthesis of creatine

Next reaction in creatine synthesis take plase in laver and it is catalyzed by ***guanidoacetate methylferase.*** S-adenosyl methionine (active methionine) is the "methyl" donor, for methylation. ATP is required for the synthesis which donates the phosphate group. Reaction is ***irreversible.*** Creatine travels from the liver to other tissues, where it is converted to **creatine phosphate**. **ATP** phosphorylates creatine to form **creatine phosphate** in a reaction catalyzed by ***creatine kinase (CK).*** Muscle and brain contain large amounts of creatine phosphat, it is an important molecule in energy metabolism, particularly in muscle cells. It functions as a reservoir of high-energy phosphate groups

that can be readily transferred to adenosine diphosphate (ADP) to regenerate adenosine triphosphate (ATP), the primary energy currency of cells. **Creatine phosphate spontaneously cyclizes, forming creatinine,** which is excreted by the **kidney**.

Creatinine is a waste product generated from the breakdown of creatine in muscle metabolism. It is produced at a relatively constant rate and is excreted by the kidneys into the urine. Since creatinine is not reabsorbed by the kidneys, its level in the urine can be used to estimate the glomerular filtration rate, which is a measure of kidney function.

The clearance of creatinine is a commonly used index to assess kidney function. It is calculated by measuring the concentration of creatinine in both the urine and blood plasma and comparing the two values. The clearance rate indicates how effectively the kidneys are filtering and clearing creatinine from the blood. A normal clearance index for creatinine in adults is around 120 ml/min, which represents the average rate of glomerular filtration.

The measurement of creatinine clearance is valuable in various clinical situations. It can help evaluate kidney function in potential kidney donors, assess the severity of kidney disease, monitor the effectiveness of treatments, and determine appropriate drug dosages. Changes in creatinine clearance can indicate alterations in kidney function and may guide medical decisions.

Creatine is accumulated in the blood plasma and is determined in the urine at developed **muscular dystrophy** in patients, and at old people with **hypodynamia state** (the absence of motor function for skeletal muscles).

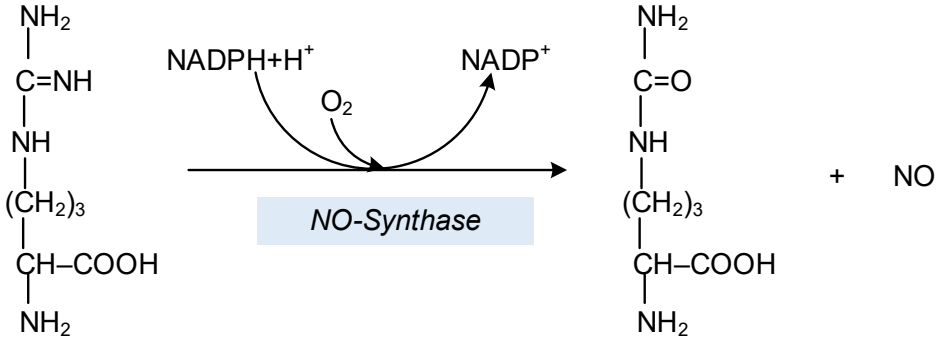
15.6. Metabolism of arginine; the biological role of nitric oxide, NO-synthase.

Arginine, as a semi-essential amino acid, has several important biological functions:

- **Creatine synthesis:** Arginine is a precursor for creatine, which plays a crucial role in energy metabolism, particularly in tissues with high energy demands such as muscle. Creatine is synthesized from arginine in a series of enzymatic reactions.
- **Synthesis of nitric oxide (NO):** Arginine serves as a substrate for the enzyme nitric oxide synthase (NOS), which converts arginine into nitric oxide. Nitric oxide is a signaling molecule involved in various physiological processes including vasodilation, neurotransmission, and immune response.
- **Polyamine synthesis:** Arginine contributes to the synthesis of polyamines, such as putrescine, spermidine, and spermine. Polyamines are involved in cell growth, differentiation, and gene expression regulation. Arginine provides ornithine, a precursor for polyamine biosynthesis.
- **GABA production:** In a minor pathway, arginine-derived ornithine can be used for the synthesis of gamma-aminobutyric acid (GABA) in the brain. GABA is an inhibitory neurotransmitter that plays a role in regulating neuronal excitability.
- **Gluconeogenesis:** Arginine can be converted into glucose through a process called gluconeogenesis. This is particularly important during periods of fasting or low carbohydrate intake when the body needs to produce glucose for energy.

- **Arginine-rich histones:** Histones, which are proteins involved in DNA packaging and gene regulation, contain a high proportion of arginine residues. Arginine-rich histones play a role in chromatin structure and gene expression.

Nitric oxide (NO) is produced from arginine by ***nitric oxide synthase (NOS***).



Arginine **Citrullin**

Three isoenzymes have been isolated and described: ***endothelial NOS (eNOS***) first was Identified first in endothelial, was found in myocardium, endocardium and platelets. In these sites, nitric oxide is constantly produced and released so as to have arterial relaxation. Activity of eNOS depends on elevated Ca^{2+} ions. ***Neuronal NOS (nNOS)*** was identified in central and peripheral neurones. Nitrogen oxide producing neurones are seen specially in cerebellum. Activity of this isoenzyme also depends on elevated Ca^{2+} ions. ***Inducible NOS (iNOS)*** was found in macrophages, responsible for their antibactericidal actions. It is independent of elevated Ca^{2+}.

Nitric oxide has a wide range of biological functions in various tissues and systems throughout the body:

- **Vasodilation:** NO is a potent vasodilator, meaning it relaxes and widens blood vessels, leading to increased blood flow and improved oxygen and nutrient delivery to tissues. This helps regulate blood pressure and maintain vascular health.
- **Smooth muscle relaxation:** NO causes relaxation of smooth muscle cells in various tissues, including the smooth muscles in the walls of blood vessels, airways, and the gastrointestinal tract. This relaxation promotes smooth muscle relaxation and helps regulate functions such as airway diameter, blood flow, and gastrointestinal motility.
- **Inhibition of platelet aggregation:** NO inhibits the aggregation and adhesion of platelets, which are involved in blood clotting. By inhibiting platelet aggregation, NO helps maintain normal blood flow and prevents the formation of excessive blood clots.
- **Regulation of blood pressure:** Through its vasodilatory effects and interactions with the cardiovascular system, NO plays a key role in regulating blood pressure. It helps maintain vascular tone and prevents excessive vasoconstriction.
- **Role in penile erection:** NO is essential for the process of penile erection. It relaxes the smooth muscles in the blood vessels of the penis, allowing increased blood flow and the engorgement necessary for an erection.

- **Neurotransmitter function:** NO acts as a neurotransmitter in the brain and the autonomic nervous system, playing a role in neuronal communication and signal transmission.

MEDICAL IMPORTANCE

Several medications that are used in the treatment of various conditions work by affecting nitric oxide (NO) metabolism. ***Viagra*** *is a medication used to treat erectile dysfunction in men. It works by inhibiting the enzyme phosphodiesterase type 5 (PDE5), which in turn increases the levels of cyclic guanosine monophosphate (cGMP) in the smooth muscle cells of the penis. Increased cGMP levels promote smooth muscle relaxation and vasodilation, allowing for increased blood flow and improved erectile function. This process is dependent on the release of NO from nerve endings in the penile tissues, which stimulates the production of cGMP.*

Glyceryl trinitrate, *also known as* ***nitroglycerin,*** *is commonly used in the treatment of angina pectoris and other cardiovascular conditions. It is a vasodilator that works by releasing NO in smooth muscle cells, including those in the blood vessels. The released NO activates guanylate cyclase, leading to increased cGMP levels and subsequent relaxation of vascular smooth muscle. This dilation of blood vessels helps relieve angina symptoms by improving blood flow to the heart.*

Sodium nitroprusside *is a medication used for the treatment of hypertensive emergencies. It acts as a potent vasodilator by releasing NO when metabolized in the body. The released NO stimulates guanylate cyclase, resulting in increased cGMP levels and relaxation of smooth muscle cells in blood vessels. This causes a decrease in peripheral resistance and a reduction in blood pressure.*

NO and inflammation The iNOS is induced by inflammatory substances like cytokines.It is the principle enzyme involved in inflammation. In macrophages, iNOS derived NO and O^{2-} form potent peroxynitrite ($ONOO^-$), which contributes to cytotoxic action of macro phages in inflammation and immune defence.

Link between NO and cyclooxygenase pathway in inflammation is established recently. In inflammatory conditions, both iNOs and COX-2 are induced. There is an NO mediated induction of COX-2 leading to increased production of pro inflammatory prostaglandins resulting in exacerbated inflammatory condition. COX-2 activation by NO contributes ischemic brain injury, cerebral ischemia and renal volume depletion. iNOS inhibitors are useful in treating such conditions due to dual inhibition of NO and PG. NO derived from iNOS is involved in promotion of chronic gut inflammation.

15.7. Polyamines synthesis and biological role.

Polyamines are organic compounds that are characterized by the presence of multiple amino groups ($-NH_2$) in their structure. **Putrescine**, **spermine** and **spermidine** are the biologically important polyamines. Spermine and spermidine were originally detected in human semen (sperms), hence they are so named. However, later they are identified in many tissues.

Polyamines are synthesized from **ornithine** and **S-adenosylmethylthio-propylamine**, which serve as donor of amine groups. ***SAM decarboxylase*** catalyzes conversion of S-Adenosylmethionine to **S-Adenosylmethylthiopropylamine (SAM decarboxylated)** (fig. 15.5).

Ornithine is formed from arginine by arginase as detailed earlier. Decarboxylation of ornithine by *ornithine decarboxylase (ODC)* generates **putrescine**. ODC has very short

half life (5 minutes) and it is site of action of many antitumor drugs. Now transfer of propylamine from S-adenosyl methyl thiopropylamine to putrescine produces **spermidine.** The reaction is catalyzed by ***spermidine synthase.*** Further transfer of propylamine from S-adenosyl methyl thiopropylamine to spermidine produces **spermine**. The reaction is catalyzed by ***spermine synthase*** (Fig. 15.5).

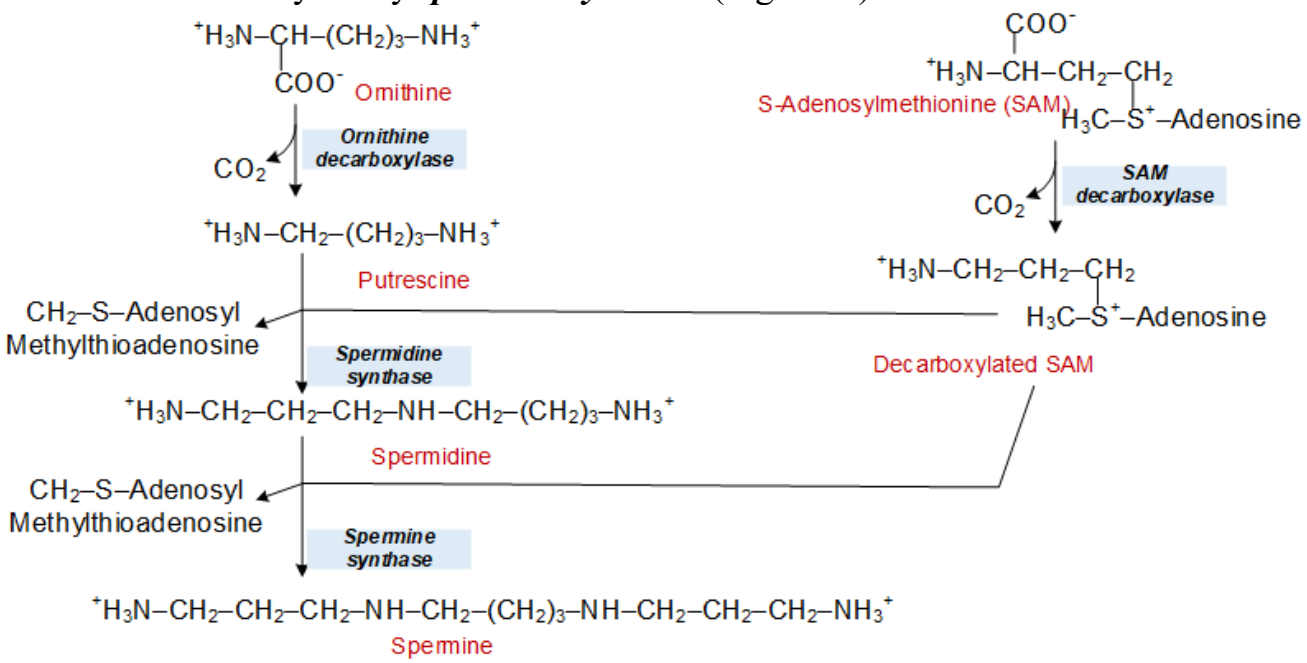


Fig. 15.5. Biosynthesis of polyamines—putrescine, spermidine and spermine

Biological importance of polyamines:

- Since polyamines are cations they are involved in the stabilization of anionic structures like DNA, ribosomes, subcellular organelle and membrane.
- They are involved in the maintenance of clover leaf structure of tRNA. They are required for packing of bacteriophage DNA.
- Polyamines are essential for cell division and growth. They are particularly important during periods of rapid growth and development, such as embryogenesis and wound healing.
- Polyamines can influence gene expression by interacting with DNA, RNA, and various proteins. They can affect transcription and translation processes, thereby modulating the synthesis of specific proteins.
- Polyamines play a role in responding to various environmental stresses, such as heat, cold, drought, and pathogens. They help protect cells and tissues from damage caused by these stresses.
- In the nervous system, polyamines are involved in regulating the activity of certain ion channels and receptors, contributing to synaptic plasticity and neurotransmission.
- Polyamines have been shown to possess antioxidant properties, helping to mitigate cellular damage caused by oxidative stress.
- Apoptosis, or programmed cell death, is a tightly regulated process crucial for maintaining tissue homeostasis. Polyamines are involved in modulating apoptosis, ensuring that the right balance between cell survival and death is maintained.

MEDICAL IMPORTANCE

1. Since polyamines are required for cell division, inhibitors of polyamine biosynthesis are used as anti cancer drugs. Most of them blocks the action of ***ornithine decarboxylase DFMO (difluoromethyl ornithine)*** *is an inhibitor of this enzyme, which is used as anti tumour agent. As such DFMO is inactive. Initial actions of ODC on this molecule produces active irreversible inhibitor, which covalently bind to active site of enzyme molecule and rendering enzyme inactive (referred as suicide inhibition). DFMO is also used to cure certain protozoal and parasitic infections. DFMO is effective against African sleeping sickness causing protozoan and pneumonia causing protozoan in AIDS patients. Malarial parasite is also susceptible to DFMO action.*

2. Excretion of polyamines in urine is increased in most of the cancers.

3. Polyamine concentration is more in cancer cells.

4. Presence of spermidine is used to identify sperm in suspected rape victims.

REVIEW TEST:

№	MCQs	Answers and explanations
1.	An unconscious patient was taken by ambulance to the hospital. On objective examination the patient was found to have no reflexes, periodical convulsions, irregular breathing. After laboratory examination the patient was diagnosed with hepatic coma. Disorders of the central nervous system develop due to the accumulation of the following metabolite: A. Glutamine B. Urea C. Ammonia D. Bilirubin E. Histamine	**The answer is C.** In the case of hepatic coma, the accumulation of the ammonia (NH_3) in the blood and brain is responsible for the development of central nervous system disorders. In a healthy liver, ammonia is converted into urea through the urea cycle and excreted in the urine. However, in liver diseases or conditions that impair liver function, such as cirrhosis or acute liver failure, the liver's ability to detoxify ammonia is compromised. As a result, ammonia accumulates in the bloodstream and crosses the blood-brain barrier, leading to elevated ammonia levels in the brain. Ammonia is highly toxic to the central nervous system and interferes with normal brain function. It affects neurotransmission, disrupts the balance of neurotransmitters, and causes oxidative stress and inflammation in the brain, contributing to the development of neurological symptoms seen in hepatic coma. The symptoms described in the unconscious patient, including the absence of reflexes, convulsions, and irregular breathing, are consistent with the effects of high ammonia levels on the central nervous system..
2.	During intensive muscle work there is a large amount of ammonia produced in the muscles. What amino acid plays the main role in the transportation of ammonia to the liver and participates in gluconeogenesis reactions? A. Alanine B. Arginine C. Lysine D. Ornithine E. Aspartate	**The answer is A.** During intensive muscle work, there is increased protein breakdown, resulting in the release of amino acids. The amino acid alanine, in particular, is produced in large quantities in the muscles. Alanine serves as a carrier of ammonia from the muscle tissue to the liver. In the liver, the alanine is involved in transamination reactions, where the amino group from alanine is transferred to alpha-ketoglutarate, forming glutamate and pyruvate. The pyruvate can then enter the gluconeogenesis pathway to be converted into glucose, while the glutamate can be further metabolized to urea in the urea cycle to eliminate

		the ammonia. This process allows the ammonia generated during intensive muscle work to be safely transported to the liver and subsequently eliminated from the body, while also providing a substrate for gluconeogenesis to maintain blood glucose levels. Therefore, option A, alanine, is the correct answer.
3.	Biochemical function of glutathion in an organism is connected with reduction and detoxification of organic peroxides. During an interaction of glutathion with hydroperoxides harmless organic alcohols are formed with subsequent further oxidation. Indicate an amino acid composing glutathion: A. Lysine B. Valine C. Glutamate D. Isoleucine E. Tryptophan	**The answer is C.** Glutathione is a tripeptide composed of three amino acids: glutamate, cysteine, and glycine. Glutamate is the amino acid specifically mentioned in the question. Glutathione plays a crucial role in the reduction and detoxification of organic peroxides, such as hydroperoxides. It acts as a reducing agent, donating electrons to these harmful compounds and converting them into harmless organic alcohols. This process is facilitated by the thiol group (-SH) of the cysteine residue in glutathione.
4.	Natural peptides can carry out various functions. What bioactive peptide is a major antioxidant and functions as a coenzyme? A. Glutathione B. Bradykinin C. Oxytocin D. Liberin E. Anserine	**The answer is A.** Glutathione is a tripeptide composed of three amino acids: glutamate, cysteine, and glycine. It plays a crucial role as an antioxidant in the body, helping to protect cells from oxidative damage caused by free radicals and reactive oxygen species. Glutathione acts as a coenzyme for various enzymes involved in cellular detoxification processes, including the detoxification of harmful compounds and the neutralization of reactive oxygen species.
5.	Many organic compounds break up in the cell into simple products. What compounds break up into ammonia, carbon dioxide, and water in the human body? A. Keto acids B. Monosaccharides C. Monohydric alcohols D. Fatty acids E. Amino acids	**The answer is E.** During the process of amino acid metabolism, amino acids are broken down in the body through various pathways, resulting in the production of ammonia (NH_3), carbon dioxide (CO_2), and water (H_2O). This breakdown occurs through processes such as oxidative deamination, decarboxylation, and other enzymatic reactions involved in amino acid catabolism. The ammonia generated from amino acid breakdown is then further processed and converted into urea in the liver through the urea cycle before being excreted by the kidneys. Carbon dioxide is produced as a byproduct of cellular respiration, and water is a common product of metabolic reactions.
6.	Main process of ammonia neutralization occurs in the liver. Arginine decomposition reaction that produces urea as a result is catalyzed with arginase. What group of enzymes does arginase belong to? A. Transferases B. Synthetases	**The answer is D.** Arginase is an enzyme that catalyzes the hydrolysis of arginine to produce urea and ornithine. Hydrolases are a group of enzymes that catalyze hydrolysis reactions, which involve the cleavage of chemical bonds by the addition of water. Arginase specifically catalyzes the hydrolysis of the peptide bond in arginine, resulting in the production of urea and ornithine.

	C. Oxidoreductases D. Hydrolases E. Isomerases	
7.	The principal end product of protein metabolism, which is excreted in the greatest quantity in human urine, is: A. Urea B. Glutamine C. Ammonium and its salts D. Uric acid E. Allantoin	**The answer is A.** Urea is the principal end product of protein metabolism in humans and is excreted in the greatest quantity in urine. It is formed in the liver through the urea cycle, which involves the conversion of ammonia into urea. Urea is highly soluble in water and is relatively non-toxic, making it an efficient and safe compound for the excretion of nitrogenous waste in urine.
8.	Ammonia is a very poisonous chemical, especially for the nervous system. What substance takes a particularly active part in the detoxification of ammonia in the brain tissue? A. Proline B. Lysine C. Glutamic acid D. Histidine E. Alanine	**The answer is C.** The substance that takes a particularly active part in the detoxification of ammonia in brain tissue is glutamic acid. Glutamic acid plays a crucial role in the brain's ammonia detoxification process. It combines with ammonia to form glutamine through the action of the enzyme glutamine synthetase. Glutamine acts as a carrier of ammonia, allowing it to be safely transported out of brain cells and into the bloodstream for further processing and elimination.
9.	Vascular endothelium is characterized by high metabolic activity and synthesizes vasoactive substances. Among these substances there is a potent vasodilator synthesized from *L*-arginine. Name this vasodilator: A. Acetylcholine B. Histamine C. Bradykinin D. Nitrogen oxide E. Adrenaline	**The answer is D.** The vasodilator synthesized from L-arginine is nitric oxide (NO). Nitric oxide is a potent vasodilator that is synthesized by the vascular endothelium from the amino acid L-arginine. It plays a crucial role in regulating vascular tone by relaxing and dilating blood vessels, thereby increasing blood flow. Nitric oxide is involved in various physiological processes, including the regulation of blood pressure, platelet aggregation, and immune function.
10	Patient presents all signs of the hepatic coma: loss of consciousness, absence of reflexes, cramps, convulsions, disorder of heart activity, recurrent (periodical) respiration. What is the cerebrotoxical substance which accumulate in blood under hepar insufficiency? A. Interleukin-1 B. Ammonia C. Autoantibody D. Necrosogenic substances E. Ketone bodies	**The answer is B.** The cerebrotoxic substance that accumulates in the blood under hepatic insufficiency and contributes to the development of hepatic coma is ammonia. In hepatic insufficiency, the liver's ability to convert and detoxify ammonia into urea is impaired. As a result, ammonia levels in the blood increase, leading to hyperammonemia. Elevated ammonia levels have toxic effects on the central nervous system, causing various neurological symptoms seen in hepatic coma, such as loss of consciousness, absence of reflexes, cramps, convulsions, and altered respiration.

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16. METABOLISM OF INDIVIDUAL AMINO ACIDS

OBJECTIVES after studying this chapter, you should be able to:

- *To discuss the role of glycine as a precursor of biologically active compounds.*
- *To explain biochemical basis in development and manifestation of genetic anomalies in metabolism of aromatic and heterocyclic amino acids, accumulation of distinct metabolic intermediates in phenylketonuria, alkaptonuria, albinism.*
- *To discuss the peculiarities of the functioning of the general pathways of metabolism of amino acids and specialized transformations of sulfur-containing amino acids and tryptophan.*
- *To be able to interpret the functioning of specialized transformations of sulfurcontaining amino acids and their disorders.*
- *To learn the pathologies of nitrogen metabolism; to explain the reasons for their occurrence.*

16. 1. Specific pathways of non-cyclic amino acids metabolism. Metabolism of glycine and serine

Glycine (Gly,) is a non-essential, optically inactive and **glycogenic** amino acid. It is the simplest amino acid, consisting of just a single hydrogen atom as its side chain. Glycine is optically inactive because it lacks a chiral center. Glycine is actively involved in the synthesis of many specialized products (heme, purines, creatine etc.) in the body, besides its incorporation into proteins, synthesis of serine and glucose and participation in one-carbon metabolism (fig. 16.1).

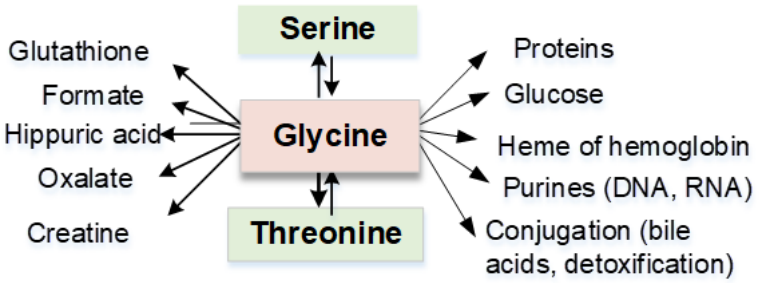
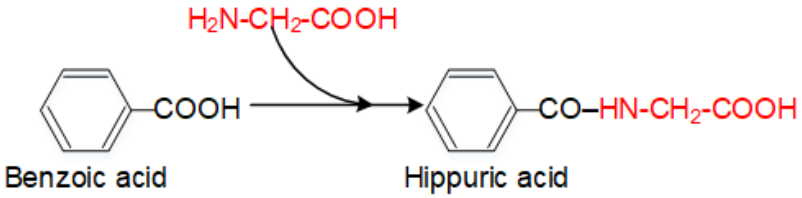


Fig. 16.1. Metabolism of glicine

Glycine plays a crucial role in the synthesis of several biologically important compounds.:

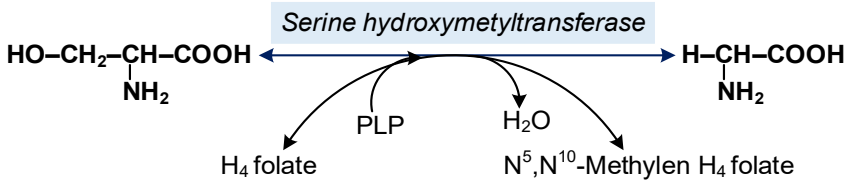
- **Collagen synthesis:** Glycine is the most abundant amino acid in collagen, a structural protein found in connective tissues. It provides the necessary structural stability and flexibility to collagen fibers.
- **Heme synthesis:** Glycine combines with succinyl CoA to form γ-aminolevulinate (ALA), which is a key intermediate in heme synthesis. Heme is a component of hemoglobin and other proteins involved in oxygen transport and storage.

- **Purine synthesis:** Glycine is involved in the synthesis of purines, which are essential components of DNA and RNA. It contributes to the carbon and nitrogen atoms in positions 4, 5, and 7 of the purine ring structure.
- **Glutathione synthesis:** Glycine is one of the three amino acids that make up glutathione, a potent antioxidant involved in cellular detoxification and protection against oxidative stress. Glycine combines with glutamate-cysteine dippeptide to form glutathione in a reaction catalyzed by the enzyme glutathione synthetase.
- **Creatine synthesis:** Glycine is involved in the formation of creatine, a nitrogenous compound found in muscle tissue. The reaction takes place in the liver and involves the combination of glycine with arginine and methionine, catalyzed by enzymes involved in the creatine biosynthesis pathway.
- **Bile acids formation:** Glycine is required for the synthesis of glycocholic acid and glycochenodeoxycholic acid, which are primary bile acids involved in the digestion and absorption of dietary fats.
- **Hippuric acid synthesis:** Glycine participates in the detoxification of benzoic acid, a common food preservative, by combining with it to form hippuric acid, which is then excreted in the urine.

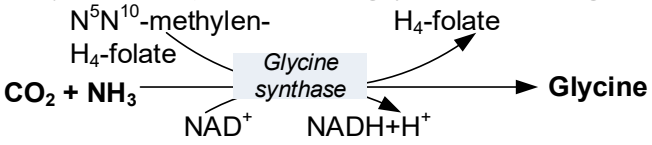


Glycine is synthesized by four mayn pathways (fig. 16.2):

1. **Glycine** can be synthesized from **serine** in a reaction catalyzed ***serine hydroxymethyltransferase.*** Tetrahydrofolate (H_4 folate) accepts the β-carbon of serine, which forms a methylene bridge between N-5 and N-10 to yield N^5,N^{10}-methylenetetrahydrofolate. The overall reaction, which is reversible, also requires **pyridoxal phosphate (PLP). Pyridoxal phosphate and tetrahydrofolate** are two cofactors required.



2. In the liver, glycine can be made by another route: in the the reversible reaction, catalyzed by ***glycine synthase*** (also called **glycine cleavage enzyme**):



3. Glycine may be formed from **glyoxalate by transamination**. Glyoxalate may arise from serine via **ethanolamine.**

4. Glycine is formed from threonine also by the action of ***serine hydroxymethyltransferase.***

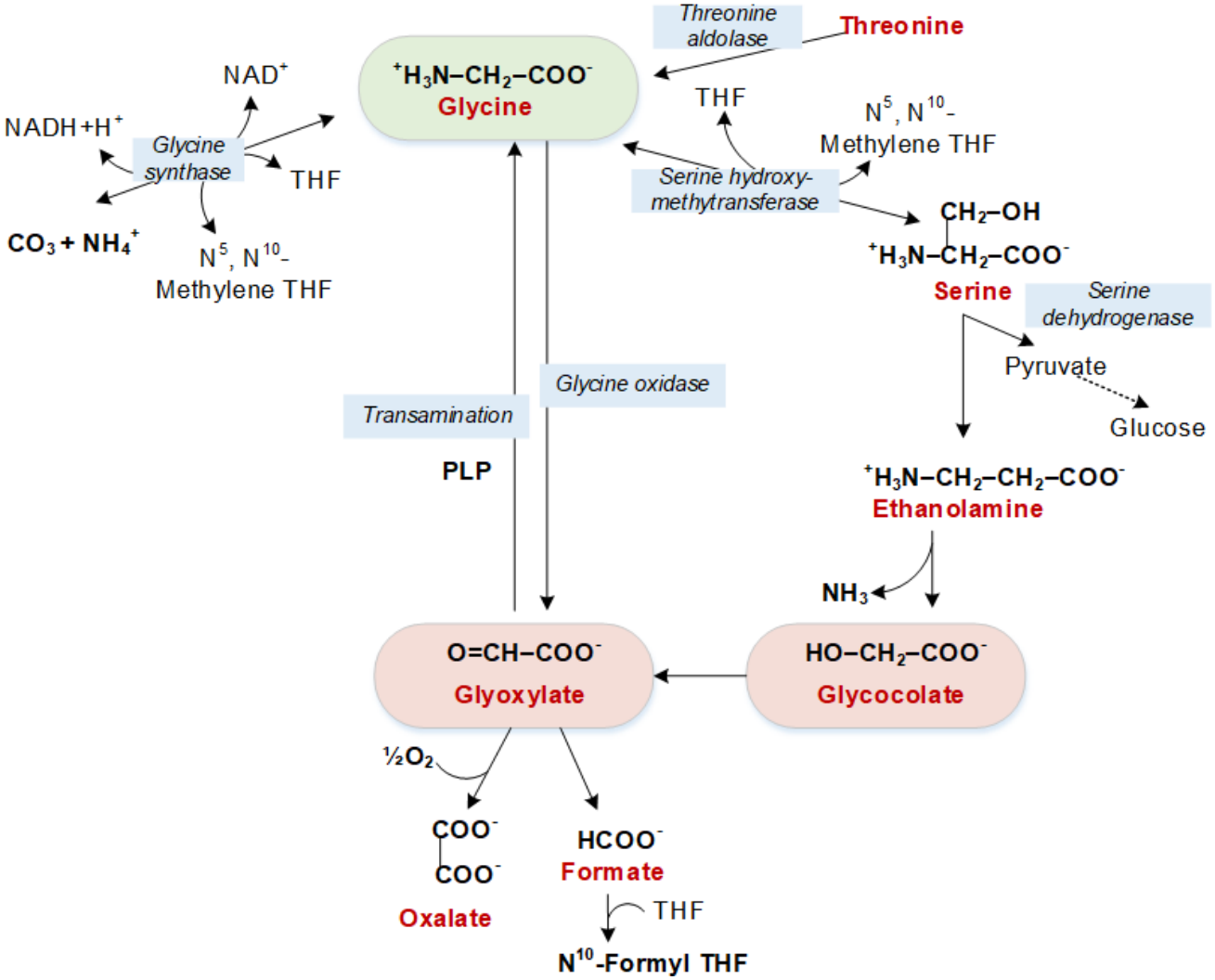


Fig. 16. 2. Metabolism of glycine

Glycine is catabolized in three following pathways (fig. 16.2):

1. Glycine cleavage pathway, in which glycine is converted to CO_2 and NH_4 by the ***glycine cleavage enzyme,*** also known as ***glycine synthase***. This pathway occurs in the mitochondria of liver cells. The reaction requires the cofactors pyridoxal phosphate (PLP), NAD^+, and tetrahydrofolate.
2. In serine pathway, glycine is converted to serine by the reversal of the ***serine hydroxymethyltransferase*** reaction. Then, ***serine dehydratase*** converts serine to pyruvate by removing ammonia. Pyruvate can be further metabolized in various ways, including conversion to glucose or oxidation in the tricarboxylic acid (TCA) cycle to produce energy in the form of acetyl-CoA.
3. In glyoxalate pathway, glycine is converted to glyoxalate by the enzyme ***D-aminoacid oxidase or glycine oxidase***, which involves deamination. Glyoxalate is then oxidatively decarboxylated to formate under normal conditions. Formate enters the one-carbon pool, which is involved in various metabolic reactions. Glyoxalate can also be further converted to oxalate, which is eventually excreted from the body.

Glycine metabolism is defective in some inherited diseases. They are due to production of defective enzymes (proteins) by defective genes. Inherited diseases of amino acid metabolism are referred as inborn errors of amino acid metabolism. Some of the known diseases of glycine metabolism are:

- **Glycinuria:** Glycinuria is a rare genetic disorder characterized by excessive urinary excretion of glycine. However, plasma glycine levels remain normal. This condition is caused by a defect in the reabsorption of glycine by the renal tubules, specifically due to a non-functional renal transporter for glycine.
- **Primary Hyperoxaluria:** Primary hyperoxaluria is a disorder characterized by the excessive excretion of oxalate in urine, regardless of dietary oxalate intake. This condition results from a block in the conversion of glyoxalate to formate, leading to the oxidation of glyoxalate to oxalate. The excess oxalate combines with calcium to form calcium oxalate crystals, which can lead to the formation of kidney stones (urolithiasis), nephrocalcinosis (calcium deposits in the kidneys), and recurrent urinary tract infections. The disease can progress to renal failure and hypertension, often resulting in death in childhood or early adulthood.
- **Non-ketotic Hyperglycinemia:** Non-ketotic hyperglycinemia is a severe and often fatal condition that presents in infancy. It is caused by a defect in the enzyme glycine synthase, which leads to the accumulation of excess glycine in the blood and urine. The characteristic symptoms include severe mental retardation and neurological abnormalities.

MEDICAL IMPORTANCE

Glycine has various uses as a medication due to its pharmacological properties. Glycine is sometimes used as a supplement to support mental health. It has been studied for its potential benefits in improving sleep quality, reducing symptoms of schizophrenia, and enhancing cognitive function. Glycine has been used in topical formulations for wound healing. It is believed to promote collagen synthesis and tissue repair, helping to accelerate the healing process. Glycine may have a role in the treatment of certain neurological disorders. It acts as an inhibitory neurotransmitter in the central nervous system and has been investigated for its potential therapeutic effects in conditions such as spasticity, epilepsy, and neuropathic pain. Glycine is commonly used as a dietary supplement for various purposes, including supporting protein synthesis, promoting muscle growth, and improving exercise performance.

Serine (Ser) is a non-essential amino acid that can be converted into glucose, making it **glycogenic**. It plays a crucial role in various cellular functions, including protein synthesis, neurotransmission, and the folate and methionine cycles. Serine is involved in the synthesis of important lipids, such as ceramides, phosphosphingolipids, and glycosphingolipids, which are abundant in cell membranes. Additionally, serine is necessary for the synthesis of glycine and sulfur-containing amino acids (fig. 16.3).

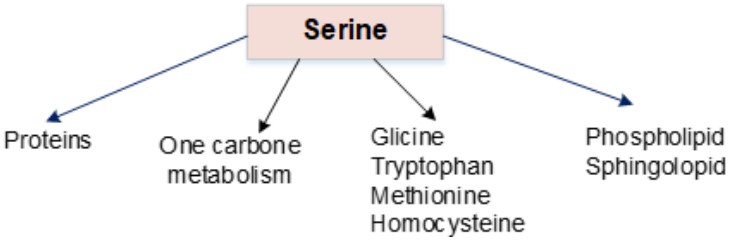


Fig. 16.3. Biosynthetic role of serine

The hydroxyl group in the side-chain of serine contributes to the polarity of proteins and serves as a key site for the

attachment of a phosphate group. This phosphorylation event regulates the function of proteins, enabling them to perform specific cellular roles effectively.

Serine can be synthesized through two pathways in the body:

1. The first pathway involves the conversion of glycine to serine, catalyzed by the enzyme ***serine hydroxymethyltransferase***. In this reaction, a one-carbon unit is transferred from 5,10-methylene-H4 folate to glycine. This pathway accounts for approximately 41% of the total glycine flux in the body.
2. The second pathway starts with **3-phosphoglycerate**, which is formed during glycolysis or gluconeogenesis. In the first reaction, 3-phosphoglycerate is oxidized by ***3-phosphoglycerate dehydrogenase*** to form 3-phosphohydroxypyruvate (fig. 16.4). Then, through a transamination reaction catalyzed by ***3-phosphoserine aminotransferase,*** 3-phosphoserine is generated. The final reaction involves the irreversible hydrolysis of 3-phosphoserine to form serine and inorganic phosphate, catalyzed by ***phosphoserine phosphatase.*** This last reaction is the rate-limiting step and is subject to feedback inhibition of L-serine synthesis. Serine synthesis from 3-phosphoglycerate is particularly high in the **brain**, especially in astrocytes, and in the kidneys. Additionally, serine biosynthetic enzymes in the liver can be activated by a protein-restricted diet or a diet rich in carbohydrates.

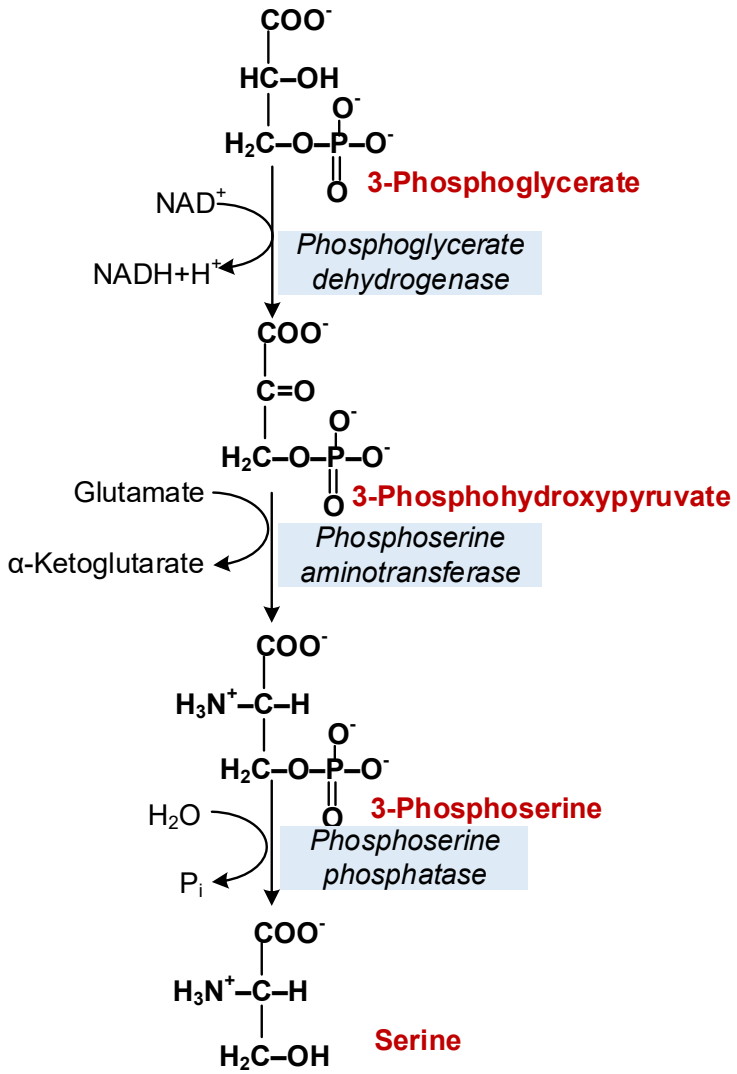


Fig. 16.4. 3-Phosphoglycerate serves a precursor for serine biosynthesis

Serine can also be degraded in the body. One major route of degradation is through its conversion to glycine. Serine can directly be converted to pyruvate by the action of ***serine dehydratase***. This pathway is activated in the liver when the protein content of the diet increases. Alternatively, serine can be converted to 2-phosphoglycerate through the actions of ***serine-pyruvate transaminase***, ***glycerate dehydrogenase***, and ***glycerate kinase***. This 2-phosphoglycerate can enter the pathways of glycolysis and gluconeogenesis, contributing to glucose production.

MEDICAL IMPORTANCE

Serine deficiency is associated with abnormal metabolism of phospholipids and sphingolipids, and impaired development and function of the nervous system and several studies have reported benefits of L-serine in therapy of neurological problems due to primary defects of serine biosynthesis, hereditary sensory neuropathy type 1, amyotrophic lateral sclerosis, and diabetes. Currently, serine and its metabolic products, specifically phosphatidylserine, are being investigated for the therapy of hyperhocysteinemia and a wide range of neurological and psychiatric disorders including Alzheimer's disease, Parkinson's disease, Huntington's disease, and schizophrenia.

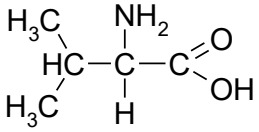
Threonine, being an essential amino acid, is not synthesized in humans but can be synthesized by bacteria from aspartate. In humans, threonine undergoes degradation through three main pathways:

1. **Conversion of threonine to propionyl-CoA**: Threonine is first converted to α-keto butyrate by the enzyme ***threonine dehydratase.*** This α-ketobutyrate is further metabolized to **propionyl-CoA** through oxidative decarboxylation by a *dehydrogenase* enzyme.
2. **Conversion of threonine to glycine and acetyl-CoA:** In this pathway, threonine is cleaved by the enzyme ***serine transhydroxymethylase*** to yield **glycine and acetaldehyde**. Unlike the conversion of serine to glycine, this cleavage reaction does not depend on tetrahydrofolate. Acetaldehyde, being toxic, is rapidly converted to acetyl-CoA after it is oxidized to acetate.
3. **Conversion of threonine to pyruvate and lactate:** ***Threonine dehydrogenase*** is responsible for converting threonine to aminoacetone through dehydrogenation and decarboxylation reactions. NAD^+ is reduced, and carbon dioxide (CO_2) is released. Aminoacetone can be further metabolized to either pyruvate or lactate via the intermediate 2-keto-propanol.

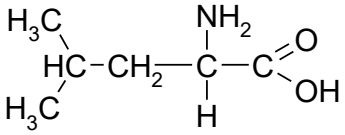
These degradation pathways allow the breakdown of threonine to produce important metabolic intermediates such as propionyl-CoA, glycine, acetyl-CoA, pyruvate, and lactate. These intermediates can be utilized for energy production or participate in various biosynthetic pathways in the body.

16.2. Peculiarities of branched-chain amino acids metabolism

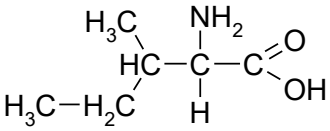
Valine, leucine and **isoleucine** are the branched chain and **essential** amino acids.



Valine (Val)



Leucine (Leu)



Isoleucine (Ile)

The catabolism of **branched-chain amino acids (BCAAs)** involves several steps (fig. 16. 5.) and leads to the production of different end products:

1. The amino groups of the three BCAAs (leucine, isoleucine, and valine) are removed through transamination, which is catalyzed by a single enzyme called **branched-chain amino acid aminotransferase**. This step requires vitamin B_6 as a cofactor.

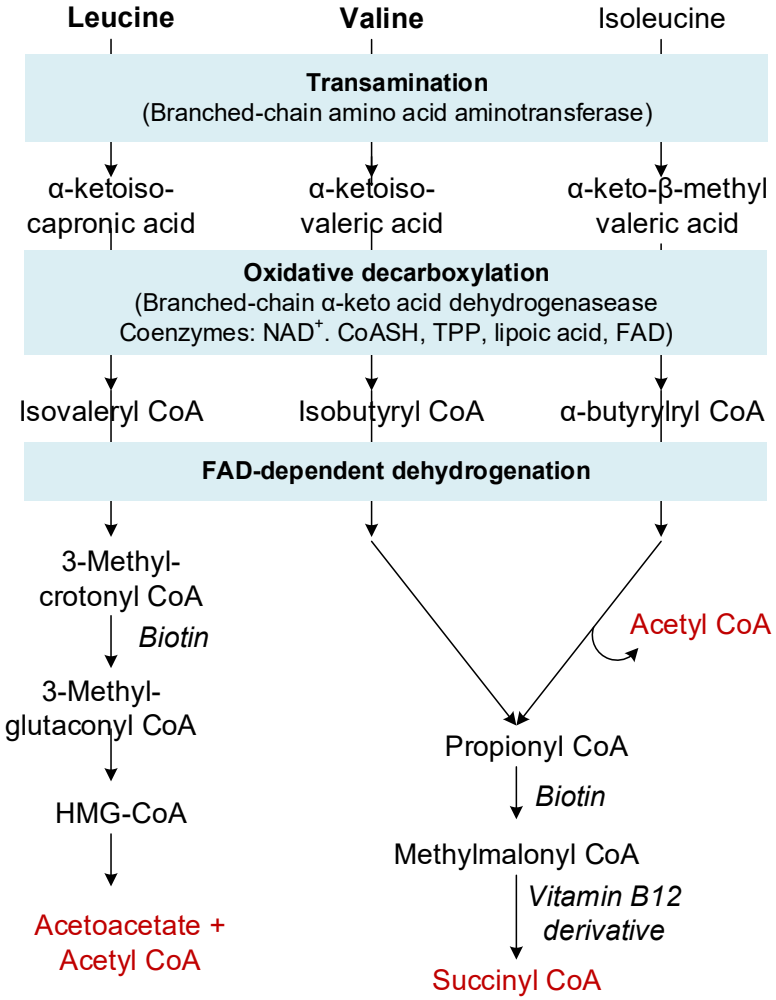


Fig. 16.5. The sceme of branched-chain amino acids

2. The α-keto acids derived from the BCAAs (**α-ketoisocaproate from leucine, α-keto-isovalerate from valine, and α-keto-methylvalerate from isoleucine**) undergo oxidative decarboxylation. This reaction is catalyzed by a multienzyme complex called ***branched-chain α-keto acid dehydrogenase complex.*** The complex utilizes coenzymes such as thiamine pyrophosphate (TPP), lipoic acid, FAD, NAD^+, and CoA. The process is similar to the conversions of pyruvate to acetyl CoA and α-ketoglutarate to succinyl CoA in other metabolic pathways. Deficiencies in branched-chain α-keto acid dehydrogenase complex can lead to the accumulation of branched-chain α-keto acids in the urine, a condition known as maple syrup urine disease.
3. The products of the previous reaction undergo **FAD-dehydrogenation**, resulting in the formation of unsaturated acyl CoA derivatives. This step is similar to FAD-linked dehydrogenation reactions.
4. The catabolism **of isoleucine yields acetyl CoA and succinyl CoA,** making it both ketogenic (producing ketone bodies) and glucogenic (yielding glucose through

gluconeogenesis). **Valine is glucogenic and produces succinyl CoA. Leucine** is predominantly ketogenic and is metabolized into **acetoacetate and acetyl CoA.**

The catabolism of branched-chain amino acids is important for energy production and the generation of key intermediates in various metabolic pathways. Any disruptions or deficiencies in the enzymes involved in this process can lead to metabolic disorders and impairments in amino acid metabolism.

Maple syrup urine disease (MSUD), also known as **leucinosis**, is a rare and serious inherited disorder that affects the catabolism of the branched-chain amino acids. The disease is caused by a deficiency of the enzyme ***branched-chain α-keto acid dehydrogenase***, which is responsible for the breakdown of these amino acids. Due to the enzyme deficiency, there is an accumulation of valine, leucine, and isoleucine, as well as their corresponding α-keto acids, in the blood. These substances are then excreted in the urine. In addition, the reduced products of the α-keto acids, known as α-hydroxy acids, also build up in the blood and are excreted in the urine.

One of the characteristic features of maple syrup urine disease is the distinct smell of the urine, resembling maple syrup or burnt sugar. This odor is due to the presence of the accumulated amino acids and their metabolites. Infants affected by the disease may initially appear normal at birth, but symptoms typically manifest during the second week of life. If left untreated, the disease can progress rapidly and lead to severe neurological problems and even death within weeks.

Intermittent branched chain ketonuria is a variant of maple syrup urine disease. In this condition, the activity of the branched-chain α-keto acid dehydrogenase enzyme is only partially impaired. As a result, affected individuals are able to utilize the branched-chain amino acids and may not experience symptoms continuously. However, they can still intermittently excrete the metabolites in their urine.

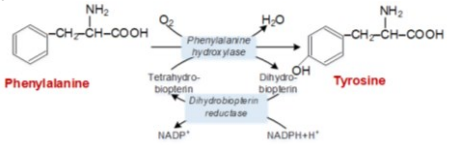
16.3. Specific pathways of metabolism of cyclic amino acids phenylalanine and tyrosine, the sequence of enzymatic reactions

Phenylalanine is an essential amino acid, meaning it must be obtained from the diet since the human body cannot synthesize it. **Tyrosine**, on the other hand, is a non-essential amino acid because it can be produced in the body through the hydroxylation of phenylalanine.

Under normal conditions, phenylalanine is primarily degraded through the conversion to tyrosine. This conversion is catalyzed by the enzyme **phenylalanine hydroxylase (phenylalanine 4-monooxygenase**, which hydroxylates phenylalanine at the para-position to produce tyrosine (also known as p-hydroxyphenylalanine). This hydroxylation reaction is irreversible and requires a specific coenzyme called **tetrahydrobiopterin,** which acts as a cofactor for the *phenylalanine hydroxylase* enzyme. Tetrahydrobiopterin (BH4) serves as an immediate hydrogen (H) donor in the reaction. Through this reaction, BH4 is oxidized to **dihydrobiopterin (BH2).**

To regenerate BH4 and maintain its availability for further phenylalanine hydroxylation, an enzyme called dihydrobiopterin reductase converts BH2 back to BH4 using NADPH as a hydrogen donor. NADPH is generated through the pentose phosphate pathway and is dependent on the activity of glucose-6-phosphate dehydrogenase.

The conversion of phenylalanine to tyrosine is an important step in phenylalanine metabolism and occurs mainly in the liver.



Due to a **defect in phenylalanine hydroxylase**, the conversion of phenylalanine to tyrosine is blocked resulting in the disorder **phenylketonuria** (PKU).

Catabolism of phenylalanine and tyrosine. The metabolism of phenylalanine and tyrosine is considered together since phenylalanine is converted to tyrosine as shown above. Catabolism of tyrosine takes place predominantly in lver. It begins with **transamination catalyzed by *tyrosine transaminase*** (fig. 16.6). **p-Hydroxyphenylpyruvatre** is a product of this reaction. Tyrosine and glucocorticoids are known to induce this *tyrosine transaminase.*

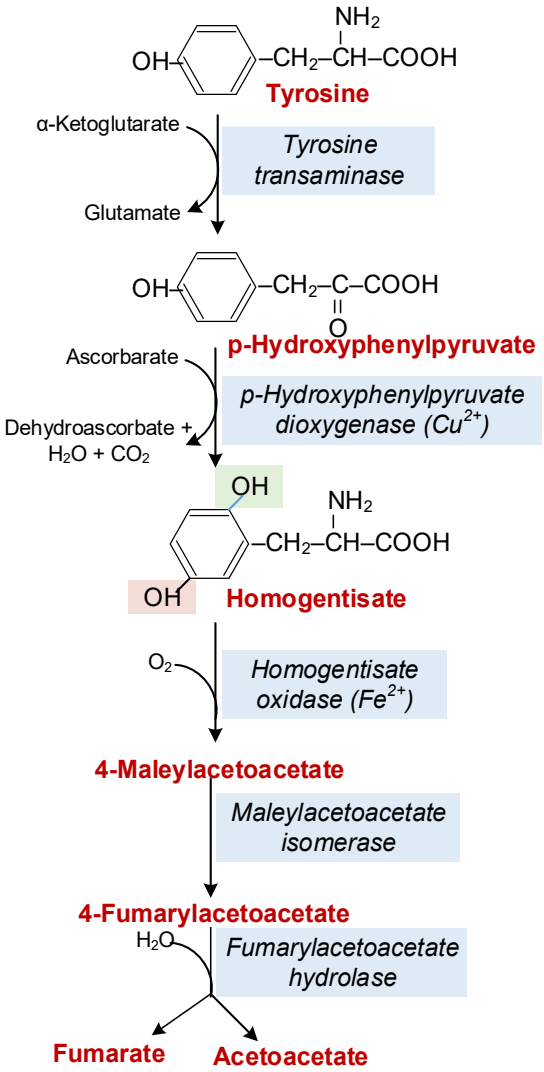


Fig.16.6. Catabolism of tyrosine in liver

p-Hydroxyphenylpyruvate hydroxylase a copper containing dioxygenase converts p-hydroxyphenylpyruvate to **homogentisic acid** in a complex reaction involving hydroxylation of benzene ring, decarboxylation and shifting of side chain. **Ascorbic acid (vitamin C)** presence isrequired for this reaction. Later benzene ring of homogentisic acid is cleaved by another dioxygenase called as ***homogentisic acid oxidase*** to form **maleylacetoacetate**. The enzyme is an iron containing metalloprotein. A glutathione dependent ***maleylacetoacetate cis-trans isomerase*** isomerize maleylacetoacetate to **fumaryl aceto acetate**. Finally fumarate and aceto acetate are formed from fumarylacetoacetate by the action of an ***hydrolase***.

Thus, four atoms of tyrosine (phenylalanine) are released as fumarate, one carbon is released as CO_2 and remaining four atoms are released as acetoacetate. Fumarate may undergo further catabolism in TCA cycle and can also serve as precursor for gluconeogenesis. Acetoacetate is a ketone body from which fat can be synthesized. Phenylalanine and tyrosine are, therefore, both glucogenic and ketogenic.

Biosynthesis of catecholamines from tyrosine. Catecholamines are a class of neurotransmitters that include **dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline).** They are derived from the dihydroxylated phenyl ring structure known as catechol.

The synthesis of catecholamines begins with the hydroxylation of the amino acid tyrosine by the enzyme ***tyrosine hydroxylase (also known as tyrosinase).*** This reaction converts tyrosine into **dihydroxyphenylalanine (DOPA)** by adding a hydroxyl group to the phenyl ring. *Tyrosine hydroxylase* is a rate-limiting enzyme and requires **tetrahydrobiopterin (BH4)** as a coenzyme, similar to phenylalanine hydroxylase. DOPA is then decarboxylated by an enzyme called **aromatic amino acid decarboxylase**, which relies on **pyridoxal phosphate (PLP)** as a cofactor. This step converts DOPA into **dopamine.** Subsequently, dopamine is hydroxylated by an enzyme called ***dopamine β-hydroxylase***, which contains copper as a cofactor. This reaction takes place in the presence of **ascorbic acid (vitamin C)** and leads to the formation of **norepinephrine (noradrenaline).** Lastly, norepinephrine can undergo methylation by the enzyme ***N-methyltransferase***, utilizing **S-adenosylmethionine (SAM)** as a methyl donor. This methylation converts norepinephrine into **epinephrine (adrenaline),** which is primarily produced in the adrenal glands.

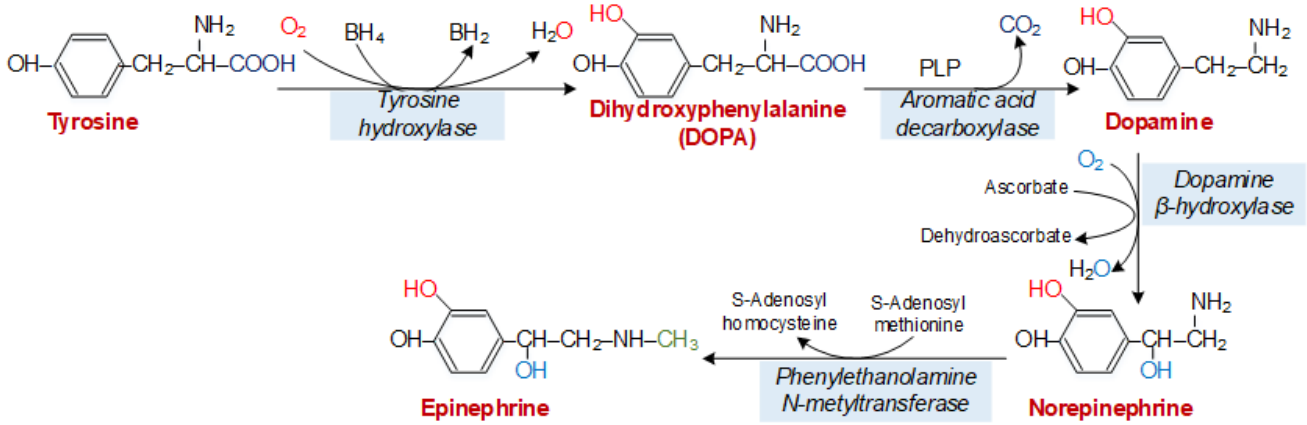


Fig. 16.7. The metabolic pathway of catecholamines biosynthesis from tyrosine

Epinephrine and norepinephrine are hormones produced by the adrenal medulla. They play crucial roles in various physiological processes. These hormones exert their effects by binding to specific receptors known as **adrenergic receptors**. There are four main types of adrenergic receptors: α1, α2, β1, and β2 receptors.

Epinephrine primarily acts on α-receptors, while norepinephrine acts on β-receptors. Activation of α-receptors by epinephrine leads to vasoconstriction (narrowing of blood vessels) and increased blood pressure. It also affects the heart, causing an increase in heart rate and contractility. Epinephrine's actions on α-receptors are involved in the body's "fight or flight" response. On the other hand, norepinephrine's actions are predominantly mediated through β-receptors. Activation of β-receptors leads to vasodilation (widening of blood vessels) and relaxation of smooth muscle in various tissues. Norepinephrine's effects on β-receptors also include increased heart rate and contractility. These actions contribute to regulating blood pressure and maintaining blood flow to different organs.

Both epinephrine and norepinephrine are involved in the sympathetic nervous system's response to stress or danger. They play important roles in mediating the physiological changes associated with the "fight or flight" response.

Dopamine is primarily known as a neurotransmitter in the brain, particularly in the substantia nigra region. It functions as both an inhibitory and excitatory neurotransmitter,

depending on the specific brain region and receptor types involved. Dopamine is involved in various neurological processes, including motor control, reward and pleasure pathways, and mood regulation.

Synthesis of melanin from tyrosine in melanocytes. Melanin is a complex pigment responsible for the coloration of various tissues in the body, including the skin, hair, and eyes. Its synthesis takes place within specialized organelles called **melanosomes,** which are predominantly found in **melanocytes** – the specialized cells that produce melanin.

There are two primary types of melanin: **eumelanin and pheomelanin**. Eumelanin is brown-black in color and is responsible for darker shades of hair, skin, and eyes. Pheomelanin, on the other hand, is a lighter pigment that ranges from yellow to red. It is responsible for the production of lighter hair colors, freckles, and the red and orange tones observed in certain skin types.

Tyrosinase a copper containing enzyme present in melanocytes initiates synthesis of different melanins by hydroxylating tyrosine to form DOPA. Dopaquinone is formed next from DOPA which is again catalyzed by **tyrosinase (fig. 16.8)**. Dopaquinone is a highly reactive molecule. In one pathway it undergo non enzymatic decarboxylation to yield 5,6-dihydroxy indole. Further action of tyrosinase on 5,6-dihydroxy indole generate indole-5,6-quinone. Polymerization of indole-5-6-quinone generates black melanin. In another branch pathway dopaquinone condenses with cysteine to form cysteinyl dopaquinone. After few steps red melanin is synthesized.

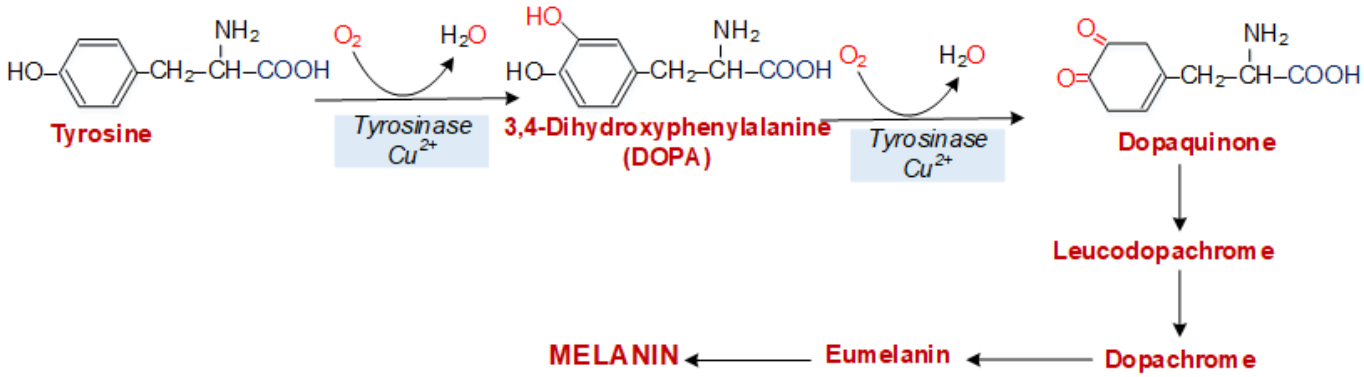


Fig. 16.8. The metabolic pathway of melanins biosynthesis from tyrosine in melanocytes

Biosynthesis of thyroid hormones from tyrosine. Thyroid hormones **thyroxine** (tetraiodothyronine) and **triiodothyronine**—are synthesized from the tyrosine residues of the protein **thyroglobulin** and activated iodine. Iodination of tyrosine ring occurs to produce mono- and diiodotyrosine from which triiodothyronine (T_3) and thyroxine (T_4) are synthesized. The protein thyroglobulin undergoes proteolytic breakdown to release the free hormones, T_3 and T_4 (fig.16.9)

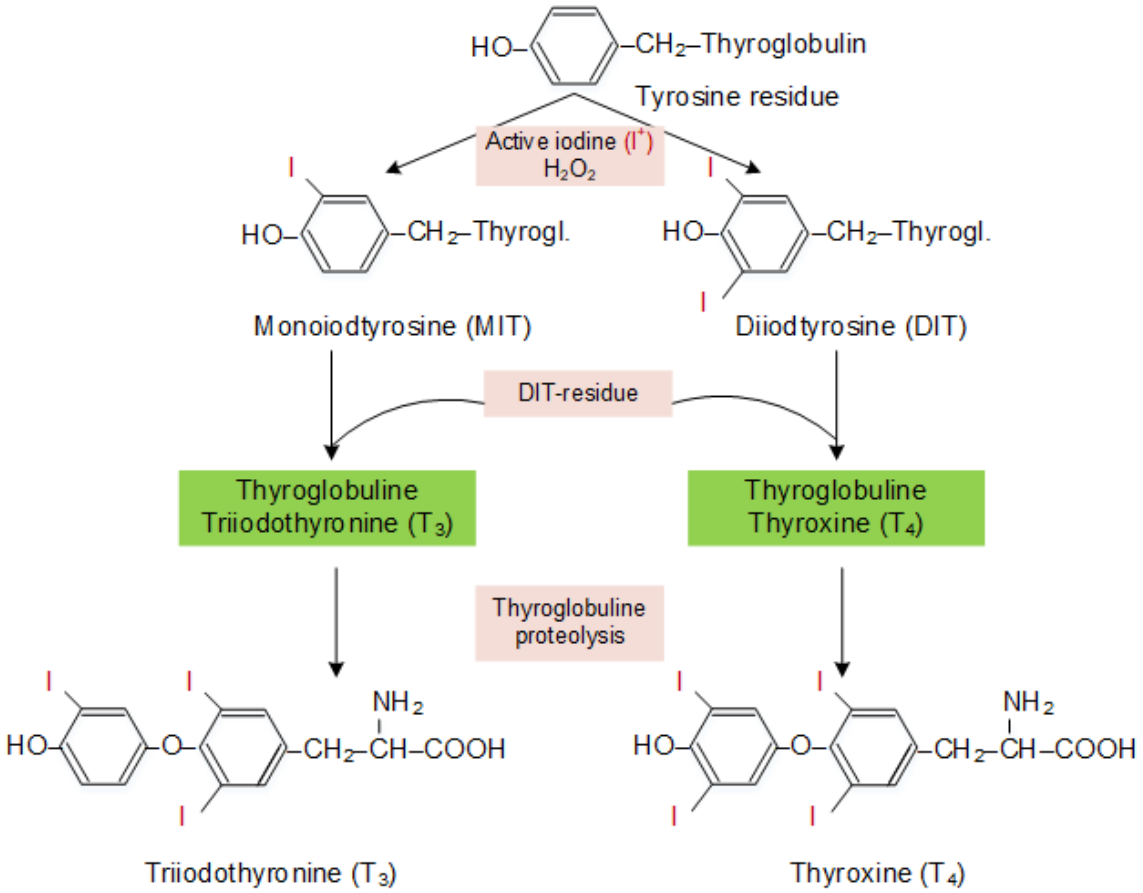


Fig. 16.9. The metabolic pathway of thyroid hormones from tyrosine

Thyroxine is a calorigenic agent. It is involved in BMR regulation. Influence of thyroxine on blood sugar level was previousely discribed. Thyroxine is a anabolic hormone. It increases protein synthesis and DNA synthesis.

16.4. Hereditary enzymopathies of phenylalanine and tyrosine metabolism – phenylketonuria, alkaptonuria, albinism

Phenyl ketonuria (PKU). PKU is the most common metabolic disorder in amino acid metabolism. It is due to lack of phenylalanine hydroxylase. So, affected individuals are unable to convert phenylalnine to tyrosine and this leads to accumulation of phenylalanine in blood. Increased blood phenylalanine is a useful index in the diagnosis of this disease. The increased blood phenylalanine level is a key diagnostic indicator of PKU. Routine screening tests measure the concentration of phenylalanine in the blood to identify individuals with the condition. If the levels are elevated, further testing is conducted to confirm the diagnosis.

In PKU, due to the metabolic disturbance, phenylalanine is redirected to alternative pathways (fig. 16.10), leading to the excessive production of several byproducts, including phenylpyruvate, phenylacetate, phenyllactate, and phenylglutamine. These metabolites are eliminated from the body through urine, where they can be detected in high concentrations in individuals with PKU.

Phenylacetate, in particular, gives the urine a distinct odor often described as "mousey." This characteristic odor can be helpful in diagnosing PKU, especially in infants who may not yet have undergone blood testing. The presence of phenylacetate in the urine can indicate the accumulation of phenylalanine and suggest the possibility of PKU.

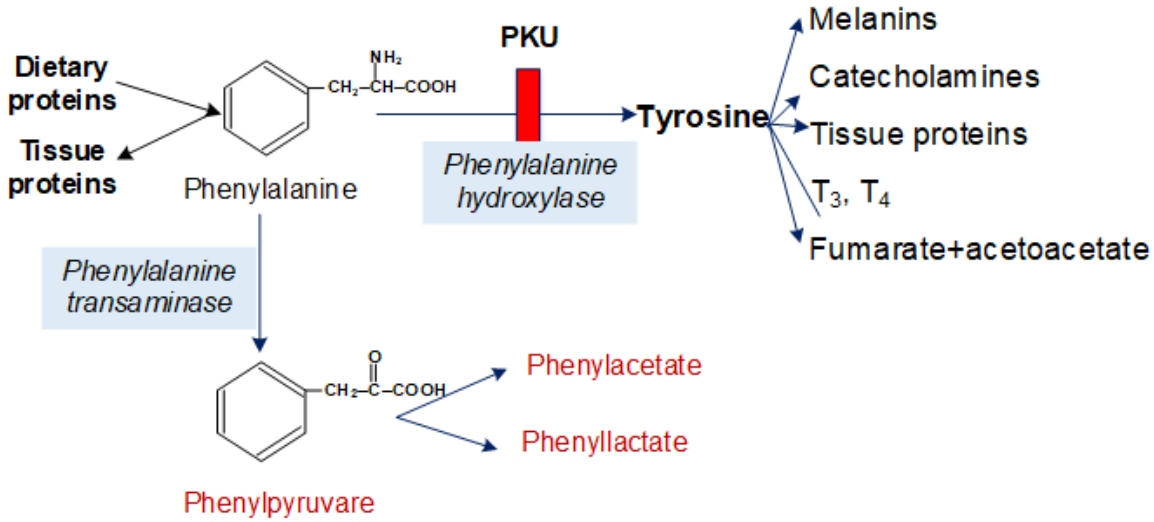


Fig. 16. 10. Transamination is an alternative pathway for phenylalanine metabolism in PKU, resulting in the production of phenylpyruvate

The disturbed metabolism of phenylalanine resulting in the increased oncentration of phenylalanine in the body causes many clinical and biochemical manisfestations. **Mental retardation**, failure to walk or talk, failure of growth, seizures and tremor are the characteristic findings in PKU. The biochemical basis of mental retardation in PKU is not well understood. There are, however, many explanations offered. Accumulation of phenylalanine in brain impairs the transport and metabolism of other aromatic amino acids (tryptophan and tyrosine). The synthesis of **serotonin** (an excitatory neurotransmitter) from tryptophan is insufficient. This is due to the competition of phenylalanine and its metabolites with tryptophan that impairs the synthesis of serotonin. Defect in **myelin** formation is observed in PKU patients. Accumulation of phenylalanine competitively inhibits tyrosinase and impairs melanin formation. The result is **hypopigmentation** that causes light skin colour, fair hair, lue eyes etc.

Tyrosinemia. This disorder—also known as **Richner-Hanhart syndrome**. It is due to defective **tyrosine transaminase**. So conversion of tyrosine to p-hydroxyphenyl pyruvate is impaired in the affected people. This leads to accumulation of tyrosine in blood. Through the alternate routes tyrosine is converted to p-hydroxyphenyl acetate and N-acetyl tyrosine and they are excreted in urine along with tyrosine. Symptoms are mental retardation, skin and eye lesions. Treatment involves feeding diet low in tyrosine.

Neonatal tyrosinemia. It is due to defective **p-hydroxyphenyl pyruvate hydroxylase**. As a result p-hydroxyphenyl pyruvate is not converted to homogentisate and it accumulates in the blood and excreted in urine either as such or after its conversion to p-hydroxyphenyl acetate. Tyrosine accumulation in blood and excretion in urine along with N-acetyl tyrosine is also observed in affected individuals. Treatment involves feeding diet low in protein.

Tyrosinosis is characterized by elevated plasma tyrosine level. Other catabolites of tyrosine are also present in excess in plasma. It may be due to defective maleylacetoacetate isomerase or fumarylacetoacetate hydrolase. Symptoms are vomiting, diarrhoea and cabbage like odor and affected infants fail to grow. If not treated death may occur within 6-8 months. Therapy involves feeding tyrosine low diet.

Alkaptonuria develops because of the deficiency of **homogentisic acid oxidase**. Deficiency of this enzyme leads to accumulations of homogentisic acid in blood and it is excreted in urine. Further the urine turns dark on standing in air. This is characteristic feature of this condition. When exposed to air homogentisic acid present in urine is oxidized to quinone by atmospheric O_2. This undergoes polymerization to produce black pigment which is responsible for the dark color that develops on standing. Symptoms in the later stage are pigmentation of connective tissue which is known as ochronosis and arthritis.

Catecholamine metabolism is defective in several diseases:

- **Pheochromocytomas:** Tumors of adrenal medulla are called as pheochromocytomas. Overproduction of catecholamines occurs in such tumors of adrenal medulla. Affected individuals suffers intermittent hypertension and this may progress into permanent hypertension. Further urine of these patients contain more of catecholamines and catabolic products of catacholamines like vanilmandelic acid.
- **Neuroblastoma:** It is another malignant condition of adrenal medulla associated with increased production of catecholamines. It is seen in children.
- **Parkinson's disease:** People over 50 years of age are affected by this disease. It is due to decreased production of dopamine in the brain. Symptoms are tremors, expressionless face and slow voluntary movements.
- **Schizophrenia:** it is another neurological disease in which disturbances in behaviour, emotions and thinking are found. Negative thinking like social withdrawl is most common in affected individuals. Circumstancial evidence indicates involvement of dopamine in this disease. Dopamine production is more in the brain. This causes excess firing of dopaminergic neurons which may be responsible for some of the symptoms.

Melanin synthesis is defective in inherited disease albinism and acquired deseases such as vitiligo and leukoderma. **Albinism** is due to defective **tyrosinase**. Affected individuals are referred as albinos. Due to lack of tyrosinase melanin formation is impaired. So pigmentation of tissues is defective. The most important function of melanin is the protection of the body from sun radiation. Lack of melanin in albinos makes them sensitive to sunlight. Increased susceptibility to **skin cancer** (carcinoma) is observed. **Vitiligo** and **leukoderma** are the important among the localized hypopigmentation disorders. Vitiligo is an acquired progressive disease with loss of pigmentation around mouth, nose, eyes and nipples. Leukoderma is comparable with vitiligo, but lack of pigmentation usually begins with hands and then spreads.

16.5. Metabolism of sulfur-containing amino acids, reactions of methylation.

The sulfur-containing amino acids are methionine, cysteine and cystine. Among these, only **methionine** is **essential**. It serves as a precursor for the synthesis of cysteine

and cystine which are, therefore, non-essential. Cysteine can spare the requirement of methionine in the diet. Cysteine and cystine are interconvertible. Cystine is found exclusively in proteins. The sulfurcontaining amino acids are almost an exclusive **dietary source of sulfur** to the body.

Metabolism of cysteine. Cysteine, besides being present in proteins, are involved in many important metabolic reactions. Cysteine, in addition to its role as a constituent of proteins, plays a vital role in various metabolic reactions. Here are some important functions of cysteine:

- **Glutathione:** Cysteine contributes to the -SH group of glutathione, a powerful antioxidant involved in cellular detoxification processes. Glutathione helps protect cells from oxidative stress and plays a crucial role in maintaining cellular redox balance.
- **Coenzyme A (CoA) synthesis:** Cysteine is required for the synthesis of Coenzyme A, a molecule that plays a central role in various metabolic pathways. CoA is involved in the oxidation of fatty acids, the production of energy from carbohydrates, and the synthesis of cholesterol, among other functions.
- **Taurine synthesis:** Cysteine is also involved in the synthesis of taurine, an amino acid-like compound that has various physiological functions, including the regulation of osmotic balance, bile acid conjugation, and antioxidant activity.
- **Cysteine proteases:** Cysteine residues in enzymes such as papain, calpains, cathepsins, and glyceraldehyde-3-phosphate dehydrogenase form the active site of these enzymes. They are known as cysteine proteases and are involved in protein degradation and various cellular processes.
- **Fatty acid synthesis:** Cysteine residues in the fatty acid synthase complex act as carriers of acyl radicals during the synthesis of fatty acids, playing a crucial role in fatty acid metabolism.
- **Sulfate source:** Cysteine serves as a source of sulfate in the body. The sulfate derived from cysteine is used for the synthesis of sulfolipids, glycosaminoglycans (components of connective tissues), proteins, and the sulfation of steroids and other organic compounds.

Cysteine is synthesized by three ways.

1. From cystine by the action of cystine reductase. NADH is the donor of hydrogen. **Cystine is the oxidized form of cysteine**.
2. In mammalian liver, cysteine is formed from **cystathionine an intermediate of methionine degradation**. Cystathionine lyase catalyzes this reaction. So the sulfur of cysteine comes from methionine.
3. In microorganisms, cysteine is synthesized from serine and H_2S. A PLP dependent cysteine synthase fixes sulfur.

Cysteine degradation. In mammals cysteine is degraded by two pathways.

1. Dioxygenase pathway (fig. 16.11) is an important metabolic pathway for the degradation of cysteine in mammals. ***Cysteine dioxygenase (CDO)*** is the enzyme responsible for catalyzing the conversion of cysteine to cysteine **sulfinic acid**. During this process, *cysteine dioxygenase* incorporates two atoms of oxygen, derived from molecular oxygen (O_2), into the cysteine molecule. The reaction also requires the presence of NAD(P)H and Fe^{2+} as cofactors. In the mammalian liver, a significant portion of cysteine

sulfinic acid formed through the action of *cysteine dioxygenase* is further metabolized into **taurine**. Taurine is an amino acid-like compound that serves various physiological functions in the body, including bile acid conjugation, osmoregulation, and antioxidant activity. The dioxygenase pathway and the conversion of cysteine to cysteine sulfinic acid and taurine are essential for maintaining cysteine homeostasis and regulating sulfur metabolism in mammals. It is worth noting that variations or disruptions in this pathway can have implications for various physiological processes and may be associated with certain diseases or conditions.

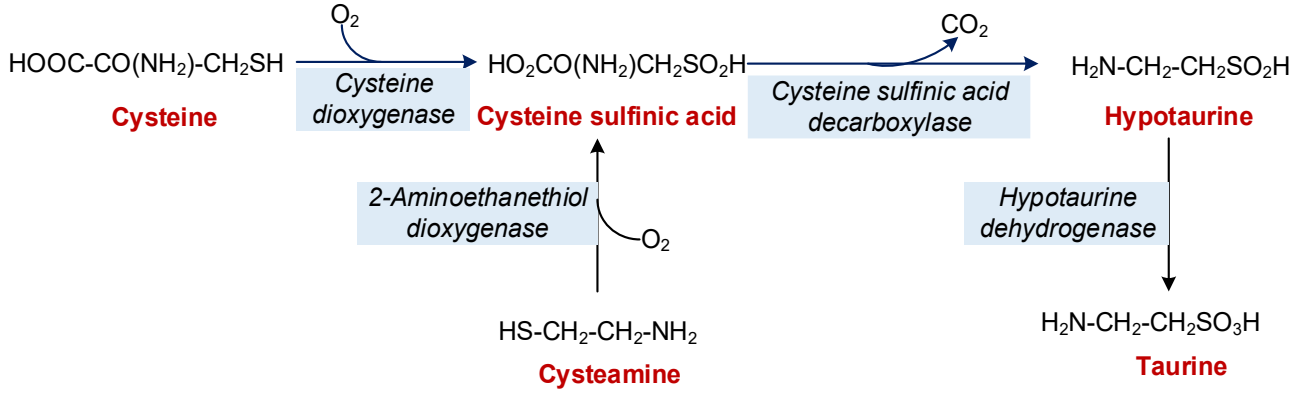


Fig. 16.11. Dioxygenase pathway of cysteine metabolism

Potentially confusing question* concerning *Cysteine and cystine. *Both are sulfur containing nonessential amino acids. Cysteine contains sulfhydryl (-SH) group; cystine is formed by condensation of two cysteine residues and contains a disulfide (-S-S-) group.*

2. Transaminase pathway. A transaminase present in mammalian liver and kidney produces **mercaptopyruvate** from cysteine by the transfer of α-amino group. The mercaptopyruvate is converted to mercaptolactate in a reaction catalyzed by dehydrogenase. The product mercaptolactate is excreted in urine. Alternately mercaptopyruvate undergoes desulfuration by several routes. In one route, **sulfur transferase** catalyzes the transfer of mercaptopyruvate sulfur to an acceptor to yield pyruvate and H_2S. In the other routes, **rhodanase** can transfer mercapto pyruvate sulfur to cyanide to form thiocyanate.

Metabolism of methionine. Methionine metabolism may be divided into three parts:

1. Utilization of methionine for transmethylation reactions.
2. Conversion of methionine to cysteine and cystine.
3. Degradation of cysteine and its conversion to specialized products.

The **transfer of methyl group** (CH_3) **from active methionine** to an acceptor is known as **transmethylation.** Methionine has to be activated to **S-adenosylmethionine** (SAM) or active methionine to donate the methyl group. The synthesis of S-adenosylmethionine occurs by the transfer of an adenosyl group from ATP to sulfur atom of methionine. This reaction is catalysed by ***methionine S-adenosyltransferase*** (fig. 16.12).

S-Adenosylmethionine is highly reactive due to the presence of a positive charge. The enzymes involved in the transfer of methyl group are collectively known as

methyltransferases. S-Adenosylmethionine transfers the methyl group to an acceptor and gets itself converted to **S-adenosylhomocysteine**. The loss of free energy in this reaction makes the methyl transfer essentially irreversible. S-Adenosylhomocysteine is hydrolysed to homocysteine and adenosine. Homocysteine can be remethylated to methionine by **N5-methyl tetrahydrofolate (THF)**. In this manner, methionine can be regenerated for reuse. It should be noted that there is no net synthesis of methionine in the S-adenosyl-methionine cycle (homocysteine, the precussor for methionine has to be derived from methionine). Hence, methionine is an essential amino acid. S-Adenosylmethionine is involved in reactions of methylation of guanodoacetate to creatine, norepinrfrine to epinephrine, ethanolamine to choline, nicotinamide to N-methylnicotinamide, acetyl serotonin to melatonin, phosphatidylethanolamine to phosphatidylcholine, serine to choline, carnosine to anserine.

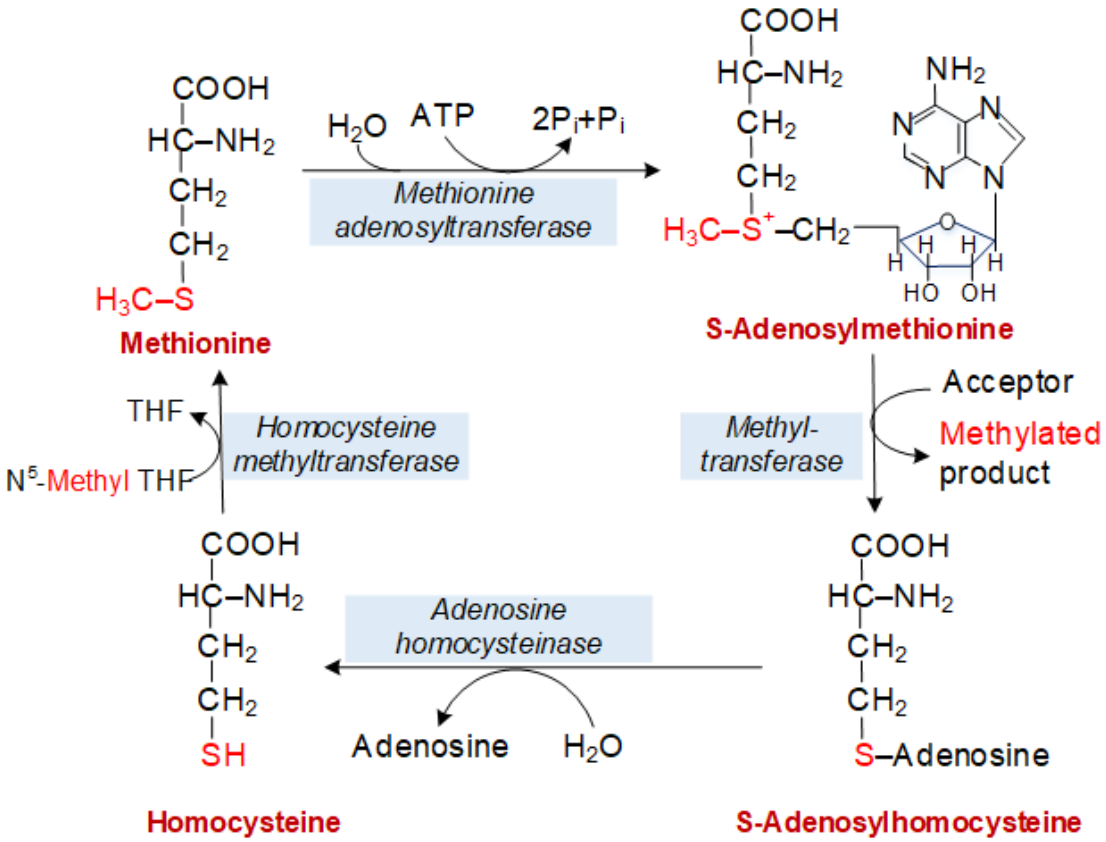


Fig. 16. 12. Methionine is involved in various transmethylation reactions, where it serves as a methyl group donor. It donates a methyl group (-CH_3) to different substrates, leading to the formation of S-adenosylmethionine (SAM). SAM is a crucial methyl donor in numerous biochemical reactions, including DNA and protein methylation, neurotransmitter synthesis, and lipid metabolism. Once SAM donates its methyl group, it is converted back to methionine through the regeneration of homocysteine.

Homocysteine formed from methionine is a precursor for the synthesis of cysteine. Homocysteine condenses with serine to form cystathionine. This reaction is catalysed by a **PLP-dependent *cystathionine β-synthase***. The enzyme ***γ-cystathioninase*** (PLP-dependent) cleaves and deaminates cystathionine to cysteine and α-ketobutyrate (fig. 16.13). The sum of the reactions catalysed by cystathionine synthase and cystathioninase is a good example of **transsulfuration** (transfer of sulfur from one compound to another).

It should be noted that only the **sulfur atom of cysteine** comes from homocysteine (originally methionine) while the **rest of the molecule is from serine**.

Cystine-lysin uria or cystinuria. This inherited disease is characterized by excretion of large amounts of cystine, lysine, arginine and ornithine in urine. It is due to renal transport defect. Since cystine is insoluble it forms stones in kidney, ureters and bladder in the affected patients.

Cystionsis. It is also an inherited but serious disease. Deposits of crystals of cysteine in the lysosomes of many tissues are found in this disease. Lysosomal dysfunction may be repsonsible for the disease. The patients may die at early age due to renal failure.

Hypermethioninemia. It is an inherited disease. It is due to defective S-adenosyl methionine synthase. This leads to accumulation of methionine in blood. Severe clinical abnormalities are not observed in this disease.

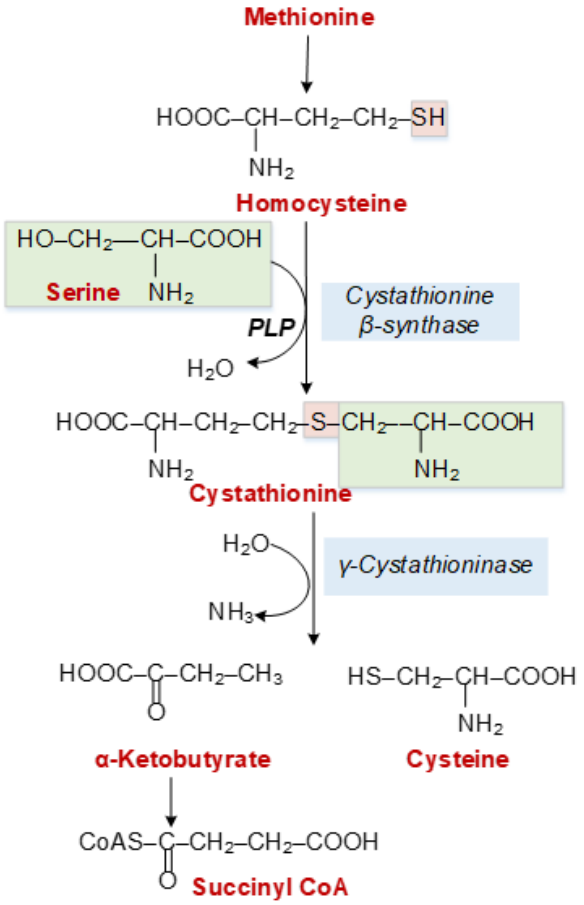


Fig. 16.13. Transsulfuration reactions

Homocystinuria and homocystinemia. Are the conditions associated with high levels of homocystine, an oxidized product of homocysteine in blood and urine. These symptoms may be due to:

- Deficiency of cystathionine synthase. Symptoms are occular abnormalities like dislocation of lens, thrombosis, mental retardation, osteoporosis etc. Block in the conversion of homocysteine of cystathionine causes accumulation of homocysteine in blood.
- Deficiency of methylene FH_4 reductase which is involved in methionine synthesis from homocysteine. This leads to accumulation of homocysteine in blood.
- Deficiency of methyl cobalamin which is required for the synthesis of methionine from homocysteine.

Failure of conversion of vit B_{12} to methyl obalamin. This also blocks conversion of homocysteine to methionine.

16. 6. Metabolism of tryptophan: kynurenine and serotonin pathways

Tryptophan is an essential amino acid that contains an indole ring and chemically it is α-amino β-indole propionic acid. Tryptophan is both glucogenic and ketogenic in nature. It serves as the precursor for various important molecules in the body, including niacin, serotonin, and melatonin:

- **Tryptophan as a precursor of niacin:** Tryptophan is converted into niacin through a series of enzymatic reactions. The conversion involves the synthesis of kynurenine,

which is further metabolized to produce niacin. Niacin is an essential vitamin that plays a vital role in energy metabolism and the synthesis of coenzymes like NAD^+ and $NADP^+$.

- **Tryptophan as a precursor of serotonin:** Tryptophan is also used as a precursor for the synthesis of serotonin, a neurotransmitter that regulates mood, appetite, sleep, and other physiological processes. Tryptophan is converted into 5-hydroxytryptophan (5-HTP) by the enzyme ***tryptophan hydroxylase***. Subsequently, 5-HTP is converted into serotonin through the action of aromatic ***L-amino acid decarboxylase***.
- **Tryptophan as a precursor of melatonin:** Melatonin, often referred to as the "sleep hormon" is synthesized from tryptophan. Tryptophan is first converted into 5-hydroxytryptophan (5-HTP), similar to the serotonin synthesis pathway. Then, 5-HTP undergoes further enzymatic reactions to form serotonin. Finally, serotonin is converted into melatonin in the pineal gland, mainly during periods of darkness. Melatonin helps regulate the sleep-wake cycle and is involved in various biological rhythms.

Catabolizm of triptophan takes place in liver. A single pathway is responsible for the degradation of tryptophan to small molecules (97%) in and synthesis of niacin (3%). One molecule of tryptophan is converted into 2 molecules of NH_3, one molecule of acetoacetyl-CoA, one molecule of acetyl-CoA, 4 molecules of CO_2 and one molecule of formate.

Sequence of reactions of kynurenine pathway of tryptophan metabolism (fig. 16.14):

1. **Tryptophan dioxygenase:** Tryptophan is initially converted to **N-formylkynurenine** by ***tryptophan dioxygenase***, which incorporates a single oxygen into each carbon of the indole ring. This enzyme is inducible and can be activated by glucocorticoids and tryptophan.
2. **Formylase:** The formyl group of N-formylkynurenine is then removed as formate through hydrolysis, catalyzed by the enzyme ***formylase***. Formate enters the one-carbon pool, and the product of this reaction is **kynurenine**.
3. **Kynurenine-3-monooxygenase:** Kynurenine is hydroxylated to form **3-hydroxykynurenine** in a reaction catalyzed by ***kynurenine-3-monooxygenase***. This reaction requires NADPH and molecular oxygen (O_2).
4. **Kynureninase:** 3-hydroxykynurenine is hydrolyzed by ***kynureninase***, a pyridoxal phosphate-dependent enzyme, to yield **3-hydroxyanthranilic acid and alanine**. Alanine is then converted to **acetyl-CoA** via pyruvate, resulting in the loss of one molecule of carbon dioxide (CO_2) and one molecule of ammonia (NH_3).
5. **Hydroxyanthranilate-3,4-dioxygenase:** The phenyl ring of 3-hydroxyanthranilic acid is opened by ***hydroxyanthranilate-3,4-dioxygenase***. This enzyme cleaves the C=C bond of the phenyl ring and incorporates an oxygen atom into each carbon, forming **2-amino-3-carboxymuconic acid semialdehyde**.
6. **Decarboxylase:** The product of the previous reaction, 2-amino-3-carboxymuconic acid semialdehyde, is converted to **2-amino muconic acid semialdehyde** by a ***decarboxylase*** enzyme, releasing one molecule of carbon dioxide (CO_2).
7. **Aldehyde dehydrogenase**: An NAD^+-dependent ***aldehyde dehydrogenase*** converts 2-amino muconic acid semialdehyde to 2**-amino muconic acid**.

8. **Amino muconic acid reductase:** Finally, 2-amino muconic acid is converted to α-keto adipic acid by amino muconic acid reductase. This reaction involves NADPH-dependent reduction and deamination. At this stage, another nitrogen from tryptophan is eliminated as ammonia (NH_3), completing the catabolism of tryptophan.

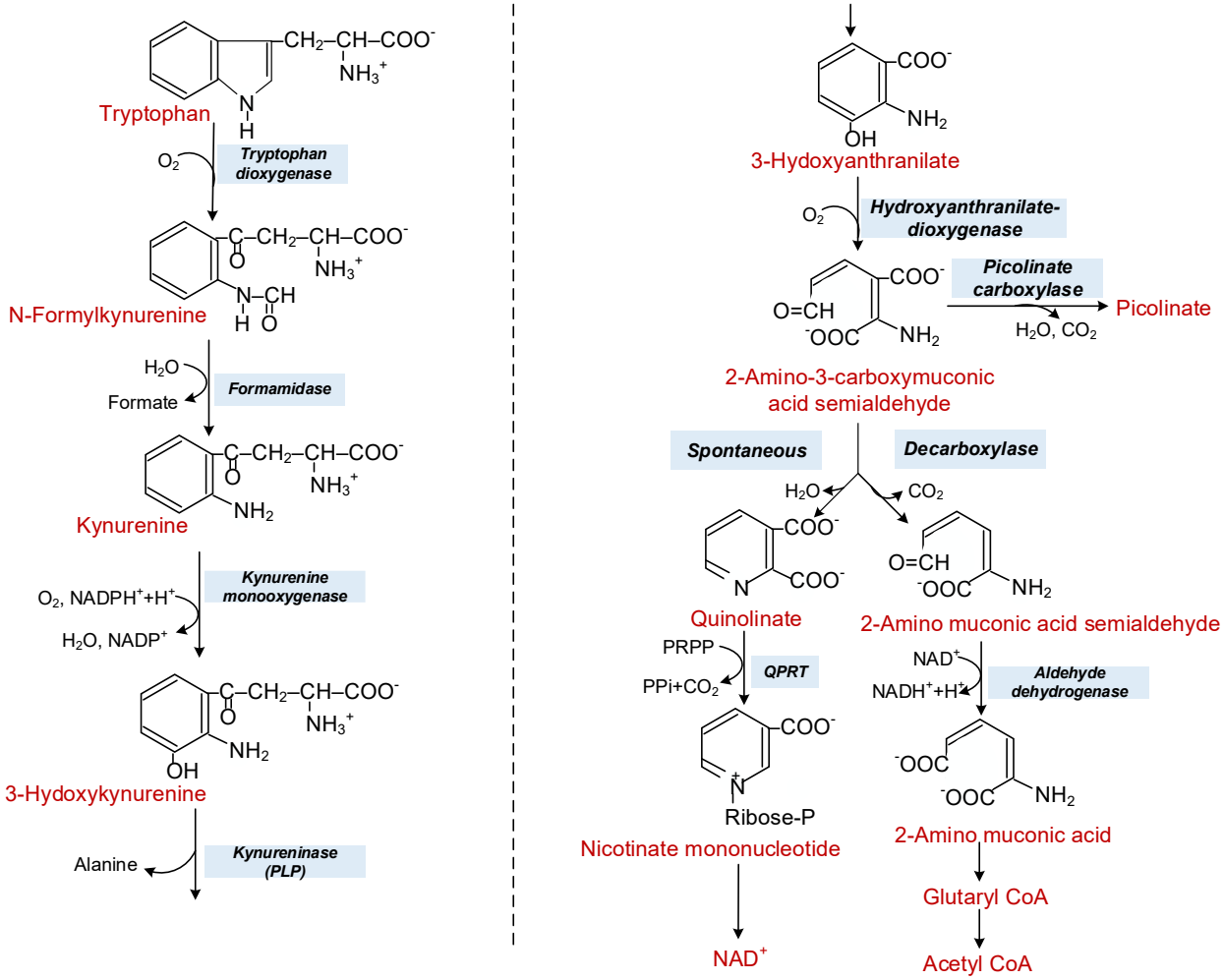


Fig. 16.14. Kynurenine pathway of tryptophan metabolism

Serotonin pathway of tryptophan metabolism (fig. 16.15):

1. **Tryptophan hydroxylase:** Tryptophan is first hydroxylated at the 5th carbon position by the enzyme ***tryptophan hydroxylase***. This enzyme requires tetrahydrobiopterin as a cofactor. The hydroxylation converts tryptophan into 5-hydroxytryptophan.
2. **Aromatic amino acid decarboxylase:** The next step involves the decarboxylation of 5-hydroxytryptophan by the enzyme aromatic amino acid decarboxylase, which is dependent on pyridoxal phosphate (PLP). This conversion results in the production of serotonin. After its release into synapses and subsequent action as a neurotransmitter, serotonin is primarily metabolized by the enzyme monoamine oxidase (MAO) (more detailes about the role of serotonine was given in the chapter 14).
3. Serotonin produced from tryptophan is acted upon by ***serotonin N-acetylase***, to give **N-acetylserotonin**. The latter undergoes methylation, S-adenosylmethionine being the methyl group donor to produce **melatonin or N-acetyl 5-methoxyserotonin**. The synthesis and secretion of melatonin from pineal gland is controlled by light.

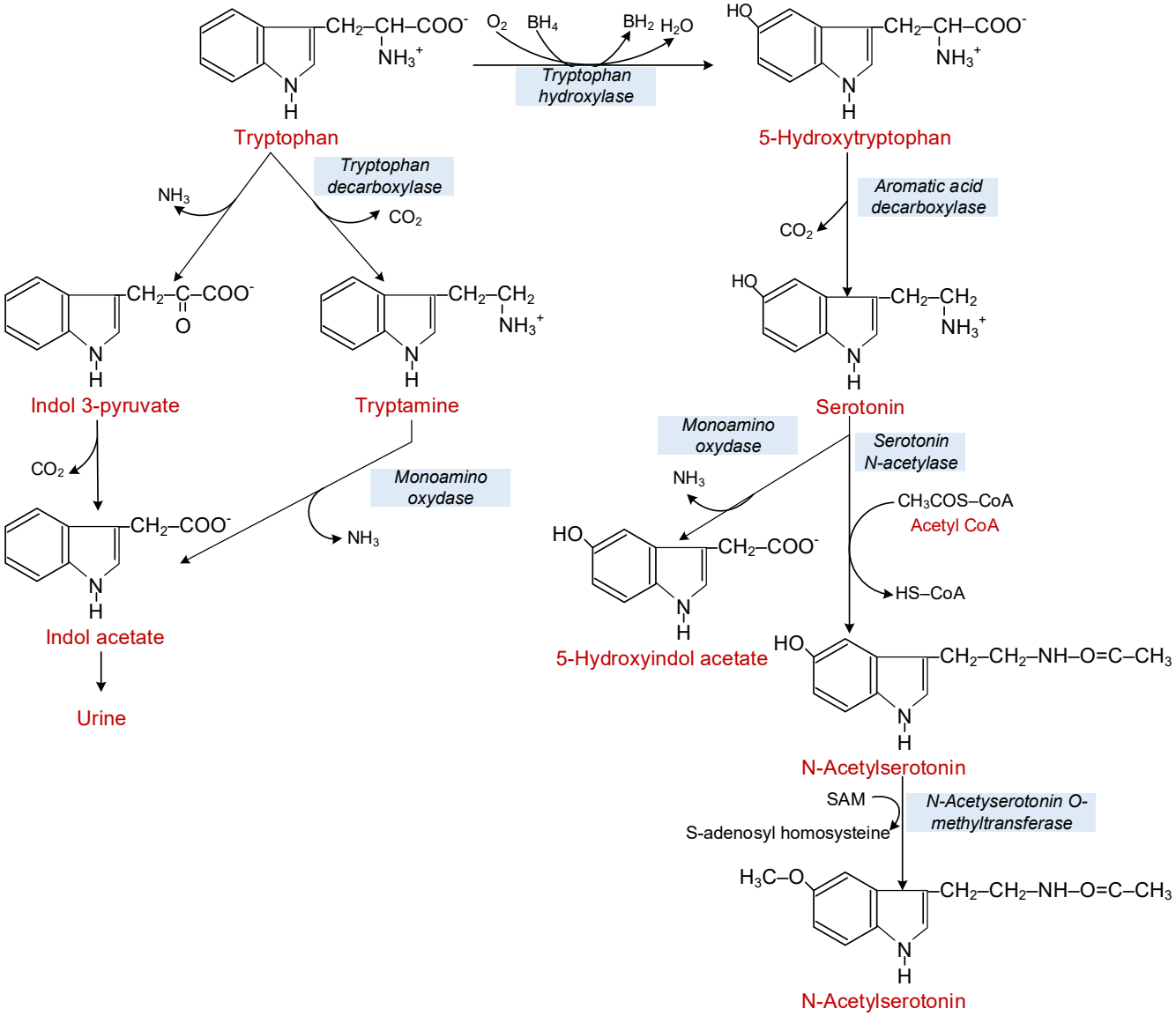


Fig. 16.15. Serotonin pathway of tryptophan metabolism

Biological functions of melatonin:

- **Regulation of circadian rhythms:** Melatonin is a key regulator of the body's circadian rhythms, which are the 24-hour cyclic processes that govern our sleep-wake cycle and other physiological functions. The production and release of melatonin are influenced by environmental light-dark cycles. In the absence of light, such as during the nighttime, the pineal gland in the brain begins to secrete melatonin, promoting drowsiness and sleep. Melatonin levels decrease during daylight hours, promoting wakefulness. This helps to synchronize our internal body clock with the external day-night cycle.
- **Inhibition of hormone production:** Melatonin has inhibitory effects on the production of certain hormones. It suppresses the release of melanocyte-stimulating hormone (MSH), which is involved in regulating skin pigmentation. Additionally, melatonin inhibits the production of adrenocorticotropic hormone (ACTH), which is produced by the pituitary gland and plays a role in stimulating the release of cortisol from the adrenal

glands. By regulating the production of these hormones, melatonin helps maintain hormonal balance in the body.

- **Inhibitory effects on ovarian function:** Melatonin has been found to have some inhibitory effects on ovarian function, particularly on the release of reproductive hormones such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH). It may play a role in regulating reproductive processes and menstrual cycles in females.
- **Neurotransmitter function:** Melatonin also functions as a neurotransmitter in the central nervous system. It interacts with specific receptors in the brain, influencing neuronal activity and neurotransmitter release. The exact mechanisms and functions of melatonin as a neurotransmitter are still being studied, but it is believed to have modulatory effects on various brain functions, including mood, cognition, and neuronal excitability.

16.6.1. Disorders of tryptophan metabolism

Tryptophan concentration in the blood (1.5 mg/100 ml) as well as in the tissues is lowest among essential amino acids (except methionine). The most common disorders of tryptophan metabolism are as follows:

- **Hartnup disease:** Hartnup disease is an inherited disorder characterized by defective tryptophan catabolism. It is caused by a deficiency of the enzyme *tryptophan dioxygenase.* As a result, tryptophan accumulates in the blood. Some of the accumulated tryptophan is diverted to other pathways, leading to the formation of **indole acetic acid**. Additionally, indole acetic acid can be conjugated with glutamine to form indole acetyl glutamine. Individuals with Hartnup disease exhibit symptoms such as mental retardation and a pellagra-like skin rash. The urine of affected individuals contains increased levels of tryptophan, indole acetic acid, and indole acetylglutamine.
- **Xanthurenic aciduria:** Xanthurenic aciduria is a condition that occurs due to vitamin B_6 deficiency. In this condition, *kynureninase*, an enzyme involved in tryptophan metabolism, is non-functional due to the lack of vitamin B_6. As a result, the conversion of 3-hydroxykynurenine to alanine and hydroxyanthranilic acid is blocked. The accumulated 3-hydroxykynurenine is diverted to another pathway, where it is converted to xanthurenic acid through transamination. Although the transaminase enzyme is also vitamin B_6 dependent, kynureninase is more sensitive to vitamin B_6 deficiency in affected individuals.
- **Malignant carcinoid (Argentaffinoma)**: Malignant carcinoid, also known as argentaffinoma, is characterized by the presence of widespread tumor cells that produce serotonin in the argentaffin tissue of the abdomen. Normally, about 1% of tryptophan is converted into serotonin. However, in malignant carcinoid, approximately 60% of tryptophan is diverted towards serotonin formation. This leads to a decreased formation of niacin from tryptophan. The symptoms of malignant carcinoid include pellagra-like rash, diarrhea, and cutaneous vasomotor episodes (flushing) due to excess serotonin. The urine of individuals with malignant carcinoid contains elevated levels of 5-hydroxyindoleacetic acid (HIAA), a metabolite of serotonin.

- **Tryptophan malabsorption syndrome:** This syndrome is characterized by impaired absorption of tryptophan from the diet due to dysfunction of the transporters responsible for its uptake in the intestines. It can result in low levels of tryptophan in the blood, leading to various symptoms such as depression, anxiety, and cognitive impairments.
- **Serotonin syndrome:** Although not strictly a disorder of tryptophan metabolism, serotonin syndrome is a condition that can occur as a result of excessive serotonin activity in the body. It can be caused by the use of certain medications or the interaction of multiple medications that increase serotonin levels. Symptoms of serotonin syndrome include agitation, confusion, rapid heartbeat, high blood pressure, dilated pupils, and in severe cases, it can be life-threatening.

REVIEW TEST:

№	MCQs	Answers and explanations
1.	A sick child presents with high content of phenyl pyruvate in urine (normally it is practically absent). Blood phenylalanine level is 350 mg/L (norm - 15 mg/L). What disease are these symptoms characteristic of? A. Tyrosinosis B. Albinism C. Phenylketonuria D. Alkaptonuria E. Gout	**The answer is C.** The symptoms described, including the high content of phenylpyruvate in urine and elevated blood phenylalanine levels, are characteristic of Phenylketonuria (PKU). Phenylketonuria is an autosomal recessive metabolic disorder caused by a deficiency of the enzyme phenylalanine hydroxylase (PAH). This enzyme is responsible for converting the amino acid phenylalanine into tyrosine. In individuals with PKU, there is a buildup of phenylalanine and its metabolic byproduct, phenylpyruvate. The elevated levels of phenylpyruvate in urine, along with the significantly increased blood phenylalanine levels, are key diagnostic markers for PKU. If left untreated, PKU can lead to severe intellectual disability and other neurological complications.
2.	In case of alkaptonuria, homogentisic acid is excreted in urine in large amounts. The development of this disease is associated with a disorder of metabolism of the following amino acid: A. Tyrosine B. Phenylalanine C. Alanine D. Methionine E. Asparagine	**The answer is A.** Alkaptonuria is a rare inherited metabolic disorder characterized by the accumulation and excretion of homogentisic acid in the urine. This condition is caused by a deficiency of the enzyme homogentisate 1,2-dioxygenase, which is responsible for breaking down homogentisic acid. Homogentisic acid is derived from the metabolism of the amino acid tyrosine. In individuals with alkaptonuria, there is a defect in the metabolism of tyrosine, leading to the accumulation of homogentisic acid. This excess homogentisic acid is then excreted in the urine, causing it to darken upon exposure to air.
3.	Laboratory examination of a child revealed increased concentration of leucine, valine, isoleucine and their ketoderivatives in blood and urine. Urine smells of maple syrup. This disease is	**The answer is C.** The disease described, characterized by increased concentrations of leucine, valine, isoleucine, and their ketoderivatives in the blood and urine, as well as the characteristic odor of maple syrup in the urine, is known as Maple Syrup Urine Disease.

	characterized by the deficit of the following enzyme: A. Glucose-6-phosphatase B. Aminotransferase C. Dehydrogenase of branched amino acids D. Phosphofructokinase E. Phosphofructomutase	Maple Syrup Urine Disease is caused by a deficiency in the enzyme branched-chain alpha-keto acid dehydrogenase (BCKDH), which is responsible for the metabolism of the branched-chain amino acids (leucine, valine, and isoleucine). The deficiency in BCKDH leads to the accumulation of these amino acids and their keto acids in the body. The name "maple syrup urine disease" comes from the distinct sweet smell of the affected individual's urine, similar to the smell of maple syrup.
4.	A man presents with signs of albinism: blonde hair, extreme photosensitivity, impaired vision. What amino acid metabolism is disrupted in the patient? A. Tyrosine B. Methionine C. Proline D. Histidine E. Valine	The answer is A. Albinism is a genetic disorder characterized by the partial or complete absence of melanin in the skin, hair, and eyes. Melanin is a pigment responsible for the coloration of these tissues. The production of melanin is dependent on the amino acid tyrosine. In individuals with albinism, there is a disruption in the metabolism of tyrosine, specifically in the enzymatic pathway responsible for converting tyrosine to melanin. This can be due to a deficiency or absence of the enzyme tyrosinase, which is necessary for the conversion of tyrosine to melanin. As a result, individuals with albinism have reduced or absent melanin production, leading to the characteristic features of the condition, such as blonde hair, extreme photosensitivity (sensitivity to light), and impaired vision. The absence of melanin in the eyes can cause various eye abnormalities, including reduced pigment in the iris, foveal hypoplasia, and nystagmus.
5.	Under the repeated action of ultraviolet rays, skin darkens because of the synthesis of melanin which protects cells from damage. The principal mechanism of this defence reaction is: A. Inhibition of phenylalanine hydroxylase B. Inhibition of tyrosinase C. Activation of homogentisate oxidase D. Inhibition of homogentisate oxidase E. Activation of tyrosinase	**The answer is E.** When UV rays penetrate the skin, they stimulate the production and release of signaling molecules that activate the expression and activity of tyrosinase. Activated tyrosinase then catalyzes the conversion of the amino acid tyrosine into melanin through a series of steps. By activating tyrosinase, more melanin is produced and deposited in the skin, leading to the darkening or tanning of the skin. This increased melanin provides a protective effect by absorbing and scattering UV radiation, helping to shield the cells from potential DNA damage. Therefore, the principal mechanism of the defense reaction that leads to skin darkening under the action of ultraviolet rays is the activation of tyrosinase.
6.	Synthesis of phospholipids is disordered under the liver fat infiltration. Indicate which of the following substances can enhance the process of methylation during phospholipids synthesis? A. Glycerol B. Ascorbic acid	**The answer is D.** Methylation is an essential process in the synthesis of phospholipids. Methylation involves the addition of a methyl group ($-CH_3$) to a molecule. In the case of phospholipid synthesis, methylation reactions are involved in the addition of methyl groups to specific positions on phosphatidylethanolamine and

	C. Glucose D. Methionine E. Citrate	phosphatidylcholine. Methionine, an essential amino acid, plays a crucial role in the methylation process. Methionine is a precursor for S-adenosylmethionine (SAM), which is the primary methyl donor in numerous biological methylation reactions, including the synthesis of phospholipids. SAM transfers its methyl group to phosphatidylethanolamine and phosphatidylcholine, catalyzed by specific enzymes called methyltransferases. This methylation reaction leads to the formation of phosphatidylcholine and phosphatidylethanolamine, which are major components of cell membranes and essential for their structure and function.
7.	Urine analysis of a 12-year-old boy reveals high concentration of all aliphatic amino acids with the highest excretion of cystine and cysteine. US of kidneys revealed kidney concrements. What is the most likely pathology? A. Cystinuria B. Alkaptonuria C. Cystitis D. Phenylketonuria E. Hartnup disease	**The answer is A.** Cystinuria is a genetic disorder characterized by the impaired reabsorption of cystine, ornithine, lysine, and arginine in the kidneys. As a result, these amino acids are excreted in high concentrations in the urine. One of the hallmarks of cystinuria is the excessive excretion of cystine and cysteine. Cystine is a dimeric form of the amino acid cysteine, and its accumulation in the urine can lead to the formation of kidney stones or kidney concrements. The high concentrations of all aliphatic amino acids, along with the specifically elevated levels of cystine and cysteine, suggest cystinuria as the most likely pathology in this case.
8.	Examination of a patient suffering from cancer of urinary bladder revealed high rate of serotonin and hydroxyanthranilic acid. It is caused by excess of the following amino acid in the organism: A. Tyrosine B. Alanine C. Histidine D. Methionine E. Tryptophan	**The answer is E.** The excess of serotonin and hydroxyanthranilic acid in a patient with cancer of the urinary bladder is caused by the excess of the amino acid tryptophan. It is an essential amino acid that serves as the precursor for the synthesis of serotonin. Serotonin is a neurotransmitter and hormone that plays a role in regulating mood, appetite, and various physiological processes. In certain cancers, including bladder cancer, there can be an increase in the metabolism of tryptophan, leading to elevated levels of serotonin. Additionally, tryptophan can be metabolized to produce hydroxyanthranilic acid, which is another metabolite that can be elevated in certain pathological conditions.
9.	Pharmaceuticals, containing mercury, arsen or other heavy metals, are inhibiting enzymes, posessing sulfhydril groups. What amino acid is used for reactivation of these enzymes? A. Aspartic acid B. Histidine C. Isoleucine D. Cysteine E. Glycine	**The answer is D.** The amino acid used for the reactivation of enzymes possessing sulfhydryl groups, which have been inhibited by pharmaceuticals containing mercury, arsenic, or other heavy metals, is cysteine. Cysteine contains a sulfhydryl (-SH) group in its side chain, which can interact with heavy metals and help to remove or chelate them from the enzyme, thereby restoring its activity. Cysteine is known for its ability to form disulfide bonds and can also act as a reducing

		agent, making it effective in reversing the inhibitory effects of heavy metals on enzymes.
10	To obese patient with risk of liver fat degeneration is recommended diet enriched with lipotropic factors. What nutritional component is the most important in diet? A. Cholesterol B. Methionine C. Vitamin C D. Glycine E. Glucose	**The answer is B.** Methionine is an essential amino acid that plays a crucial role in lipid metabolism. It acts as a lipotropic factor, aiding in the breakdown and metabolism of fats in the liver. Methionine helps in the synthesis of phospholipids, which are important components of cell membranes and lipoproteins involved in lipid transport. By increasing the intake of methionine-rich foods or supplementing with methionine, it can help support liver health, promote fat metabolism, and prevent the accumulation of fat in the liver.

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Appendix: Case Studies with Biochemical Correlations

Case 1. After consuming of numerous sweets a milkshake, a teenage individual's blood sugar level increases. In response, the liver enlists two enzymes, glucokinase (with a Km of 10.0 mM) and hexokinase (with a Km of 0.10 mM), for glucose metabolism.

- *Which of these enzymes will be more efficient in utilizing glucose, and why?*
- *What is the biological significance of the Michaelis constant?*

Case 2. A 63-year-old man arrives at the emergency department complaining of chest pain. He reports experiencing occasional chest discomfort or pressure over the past year, especially during increased physical activity. He describes the pain as pressure behind his breastbone that radiates to the left side of his neck. Unlike previous episodes, this time he was reclining and watching TV. The pain lasted around 15 minutes and then subsided. He also felt nauseated and sweaty during the episode. He hasn't had any significant medical issues and hasn't seen a doctor for several years. On examination, he appears fine, with normal vital signs. His lungs sound clear, and his heart has a regular rhythm without murmurs. An electrocardiogram (ECG) shows elevated ST segments and peaked T waves in leads II, III, and aVF. Tests reveal elevated serum creatine phosphokinase and myoglobin levels.

- *What's the most likely diagnosis?*
- *What biochemical indicators should be measured for diagnostic purposes?*

Case 3. A 45-year-old woman comes in with problems opening her eyelids and struggles to get up from a seated position. Her diagnosis is “myasthenia gravis”, a condition characterized by severe muscle fatigue due to reduced levels of acetylcholine in her muscles. She has been given a prescription for physostigmine, a medication that enhances the amount of accessible acetylcholine by inhibiting acetylcholinesterase.

- *How does physostigmine work to achieve its effects?*

Case 4. A 59-year-old man is brought to the emergency department by a family member who found him in a highly confused and disoriented state, struggling with balance, and displaying unusual, irregular eye movements. This individual has a history of heavy alcohol consumption. He has no known preexisting medical conditions and denies using any drugs other than alcohol. Upon examination, his temperature is normal, pulse is elevated at 110 beats per minute, and blood pressure is within the normal range. He appears extremely disoriented and agitated. Bilateral horizontal rapid eye movements are observed when he shifts his gaze. His walking is notably unsteady. The rest of his physical examination appears normal. The urine drug test came back negative, but his blood alcohol level is positive. The emergency room doctor administers thiamine.

- *What's the most probable diagnosis?*
- *Why is thiamine important in biochemical reactions?*

Case 5. A 34-year-old man visits his dentist due to concerns about his teeth becoming loose. During the examination, the dentist observes that the patient's gums are swollen, with a purple hue and a spongy texture. Additionally, the dentist notices multiple splinter

hemorrhages near the ends of the patient's nails, and a wound on the patient's forearm has not healed properly.

- *Which vitamin deficiency is most likely responsible for these symptoms?*

Case 6. An 18-month-old toddler is accidentally left unsupervised in the kitchen and consumes a small amount of rat poison found in the cupboard beneath the sink. The poison contains an ingredient called fluoracetate, which reacts with oxaloacetate.

- *Which bodily pathway is blocked by this poison?*
- *What is the mechanism by which fluoracetate exerts its inhibitory effect?*

Case 7. A 68-year-old woman experiencing a hypertensive crisis is being managed in the intensive care unit with intravenous nitroprusside treatment over 48 hours. Her blood pressure has been successfully brought down to normal levels. However, she complains of a burning sensation in her mouth and throat, followed by symptoms like nausea, vomiting, sweating, restlessness, and difficulty breathing. The nurse detects a sweet almond-like odor in her breath. An arterial blood gas analysis reveals a significant metabolic acidosis. A serum test indicates that a metabolite of nitroprusside, called thiocyanate, has reached toxic levels.

- *What's the probable cause of her symptoms?*
- *What's the underlying biochemical mechanism for this issue?*
- *What treatment is typically administered for this condition?*

Case 8. A 27-year-old man arrives at the emergency department displaying symptoms and signs consistent with acute appendicitis. He is promptly taken to the operating room for an emergency appendectomy. The patient is prepared for surgery and receives halothane as an inhaled anesthetic. Just two minutes after the anesthesia administration, the patient's temperature spikes dramatically, he experiences muscle rigidity, and his breathing rate increases significantly. Arterial blood gas analysis reveals metabolic acidosis, and serum electrolyte tests show elevated potassium levels. A nurse speaks with the family, who mentions that a relative who underwent surgery in the past had a similar reaction and did not survive. The doctor diagnoses the patient with malignant hyperthermia.

- *What is the underlying biochemical cause of this disease?*
- *What is the most effective treatment for this condition?*

Case 9. A 3-year-old boy is brought to the emergency department due to multiple instances of vomiting and lethargy. His pediatrician had already raised concerns about his poor growth and the potential for liver issues, in addition to recurrent bouts of vomiting and lethargy. Upon careful questioning, you note that these episodes tend to follow the consumption of specific foods, particularly those high in fructose. His blood sugar level is checked in the emergency department and is found to be unusually low.

- *What's the most likely diagnosis?*
- *What's the biochemical basis underlying the observed clinical symptoms?*
- *How is this disorder typically treated?*

Case 10. A pregnant woman with severe lactose intolerance inquires with her physician about the possibility of breastfeeding her baby, considering her inability to consume milk or dairy products.

- *What guidance should she be provided with?*

Case 11. A 12-year-old girl with a significantly enlarged abdomen presents at the outpatient department (OPD). She describes frequent episodes of weakness, sweating, and paleness that improve after eating. Her developmental milestones were delayed: she began sitting at 1 year old and walked unassisted at 2 years old. She is also not performing well in school. During the physical examination, her blood pressure, temperature, and pulse rate were all normal. However, her weight was lower than expected at 23 kg. Her liver was enlarged, firm, and descended into her pelvis. The spleen and kidneys were not detectable. Other aspects of the physical examination were within normal limits. Laboratory results showed low blood glucose, low pH, high levels of lactate, triacylglycerols, ketones, and elevated free fatty acids. Liver biopsy indicated elevated glycogen content, with normal structure. An enzyme assay on the biopsy tissue revealed very low levels of glucose-6-phosphatase.

- *What's the most likely diagnosis?*
- *What's the biochemical explanation for the observed clinical symptoms?*
- *How is this disorder typically treated?*

Case 12. A 14-year-old high school girl, highly concerned about her looks, has abstained from eating for two days to fit into a dress one size smaller than her actual size for an upcoming dance event.

- *Which organ or tissue is responsible for contributing to the glucose produced through gluconeogenesis in her body?*

Case 13. A 50-year-old woman visits your clinic, complaining about excessive thirst, fluid intake, and frequent urination. She denies experiencing any symptoms of urinary tract infection. She mentions having no prior medical issues and not having seen a doctor for many years. During the examination, you observe that she's an obese female, though she doesn't seem to be in immediate distress. Other aspects of her physical exam appear normal. The urinalysis indicates the presence of a significant amount of glucose, and a random serum blood sugar test returns a value of 18 mmol/l (324 mg/dL).

- *What's the most probable diagnosis?*
- *Which other organ systems can be affected by this condition?*
- *What's the underlying biochemical explanation for this disease?*

Case 14. A Jewish couple with roots in Eastern Europe seeks prenatal counseling at the clinic. They share that their first child passed away during early childhood, though they can't recall the specific disorder's name. However, they mention that this ailment is prevalent in their ancestral heritage. The initial child was born without evident issues, but exhibited a slightly larger head circumference than typical, along with abnormal ocular features. Additionally, the child suffered from a severe, progressively worsening

neurological condition, ultimately leading to death. The post-mortem examination was consistent with Tay-Sachs disease.

- *What kind of inheritance pattern characterizes this disorder?*
- *What's the underlying biochemical cause behind this disorder?*

Case 15. A 9-year-old boy is brought to the emergency room by his parents due to worsening nausea, vomiting, and abdominal pain over the past 2 days. The pain is centered in the upper abdomen and extends to his back. Although he's experienced similar pain episodes before, this one is notably more severe. His parents deny any signs of fever, chills, or changes in his bowel habits. In the ER, the boy doesn't have a fever but seems to be in moderate discomfort. Both his liver and spleen are visibly enlarged, and he exhibits tenderness in the upper abdomen. Additionally, small yellow-white papules are observed on his back and buttocks. Laboratory tests show elevated levels of amylase and lipase. Further inquiries reveal that the father has high triglyceride levels, and there's a history of early heart disease in the mother's family. Subsequent tests conducted after his hospitalization confirm elevated triglyceride levels and reduced lipoprotein lipase activity.

- *What is the cause of the boy's abdominal pain?*
- *What likely underlying biochemical disorder might be contributing to his symptoms?*
- *What role does lipoprotein lipase play in this scenario?*

Case 16. A teenage girl is brought to a medical center due to her ongoing complaints of excessive fatigue during participation in gym classes. Upon examination by a consulting neurologist, muscle weakness is observed in both her arms and legs. Since a clear diagnosis couldn't be established, muscle biopsies were taken for further testing. The biochemical analysis of the biopsied muscles revealed a significant elevation in the levels of triacylglycerols that were esterified with primary long-chain fatty acids. The pathology report indicated the presence of numerous lipid vacuoles in the muscle tissue.

- *What's the likely diagnosis for this case?*
- *What could be causing these symptoms?*

Case 17. A 48-year-old man visits the clinic due to concerns about his heart health. He reveals that his father passed away from a heart attack when he was 46, and his older brother experienced a heart attack at the same age but survived and is now on cholesterol-lowering medications. The patient shares that he occasionally experiences chest pain when moving around his house and finds it difficult to climb stairs due to chest pain and breathlessness. His physical examination appears normal, prompting the physician to request an electrocardiogram (ECG), exercise stress test, and blood work. The blood test results show that his cholesterol level is 350 mg/dL (considerably higher than the normal level of 200). The physician prescribes a medication aimed at the step in cholesterol biosynthesis that controls the rate of production.

- *What specific step in cholesterol metabolism is the rate-limiting one?*
- *What class of medication has been prescribed for this condition?*

Case 18. A 49-year-old woman returns to your clinic for a follow-up appointment after starting a new medication (lovastatin) to address her elevated cholesterol levels. She's currently not experiencing any issues and reports feeling well. Her repeat serum cholesterol test shows a decrease in cholesterol levels. Curious about whether she should continue taking the medication, she inquires about potential side effects and benefits. The physician explains that this medication acts by inhibiting the rate-limiting step and the crucial enzyme involved in cholesterol biosynthesis.

- *What's the mechanism of action of this medication?*

Case 19. A 1-year-old girl is brought to the pediatrician's office due to concerns about her developmental progress. The mother expresses worry that her baby isn't meeting the typical milestones for her age. Additionally, the mother notices an unusual smell in the baby's urine and observes patches of lighter skin and hair. During the examination, the child displays some muscle weakness and has a smaller than usual head size. A urine sample reveals an odor described as "mousy."

- *What's the most probable diagnosis for this case?*
- *What's the underlying biochemical reason for the hypopigmented skin and hair?*